## The Detection and Fate of Enveloped Viruses in Water Environments

by

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## Dedication

This dissertation is dedicated to my beloved grandfather, Linsen Ye.

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### Abstract

Removing and inactivating infectious viruses in water is critical in controlling waterborne diseases. Studies on the presence of viruses in wastewater and their fate through wastewater treatment plants have focused primarily on enteric viruses, which transmit gastrointestinal diseases via water. Most enteric viruses are nonenveloped, consisting only of proteins and nucleic acids. Enveloped viruses contain an outer lipid membrane in addition to proteins and nucleic acids. Certain enveloped viruses are responsible for high-profile diseases, such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and influenza. Enveloped viruses have often been assumed to be absent from wastewater and rapidly inactivated when they are released to water. However, recent studies suggest that certain enveloped viruses can enter wastewater, and may survive in water for long periods of time. Our current state of knowledge on enveloped viruses in aquatic environments has been limited due to a lack of appropriate methods for capturing and detecting infectious enveloped viruses in water. To address the knowledge gaps, this dissertation research aims to 1) evaluate the survival, partitioning, and recovery of model enveloped viruses in wastewater, 2) characterize the reactivity of enveloped viruses with common disinfectants, and 3) develop a new method for monitoring infectious human viruses in water samples.

To evaluate virus survival and partitioning, we applied four model viruses, two enveloped and two nonenveloped, and used plaque assays to track the infectivity and partitioning of the model viruses in untreated wastewater. We simulated our experimental data with virus sorption and inactivation models to quantitatively characterize the fate of model enveloped viruses and model nonenveloped viruses. Our results suggest that model enveloped viruses can survive in wastewater, especially at cooler temperatures. We also demonstrated that a larger fraction of model enveloped viruses partitioned to the wastewater solids than nonenveloped viruses. As a result, we expect that enveloped viruses are removed to a greater extent than nonenveloped viruses during primary wastewater treatment. With the knowledge gained from the survival and partitioning experiments, we optimized an ultrafiltration method for recovering infectious enveloped viruses from wastewater. The second portion of this dissertation research characterized the reactivity of enveloped viruses in the disinfection process. The reactions in a model virus lipids, proteins, and genome were tracked as a model enveloped virus was treated with disinfectants using quantitative lipid and protein mass spectrometry, and molecular PCR techniques. We found that protein reactions drive the inactivation of the model enveloped virus by free chlorine, and genome reactions drive the inactivation of the model enveloped virus by UV<sub>254</sub>. Furthermore, our results suggest that the model enveloped virus proteins were more susceptible to oxidant attack than the proteins of a model nonenveloped virus. The final portion of this dissertation research focused on the development of an integrated cell culture-mass spectrometry (ICC-MS) method for detecting infectious human viruses in wastewater. In proof of concept experiments, reoviruses were detected in samples collected throughout a wastewater treatment plant by applying the ultrafiltration concentration method developed in the first study and the ICC-MS detection method. These results suggest that ICC-MS is a promising tool for monitoring infectious enveloped or nonenveloped viruses in water samples.

### **Chapter 1 Background**

#### 1.1 Water environments and virus transmission

Water resources are essential for every aspect of human life. However, these resources are limited, and we are increasingly reusing our water in areas with high populations and limited water sources. Maintaining high water quality as water is circled through the urban water cycle is challenging due to the introduction of pollutants to the water, such as human viruses. Waterborne viruses are responsible for spreading a number of human diseases. Enteric viruses, for example, cause infections in human gastrointestinal system and are primarily transmitted via the fecal-oral route.<sup>1-3</sup> Enteric viruses such as norovirus, coxsackievirus, echovirus, and reovirus have been frequently detected in untreated municipal wastewater with infectious concentrations ranging from 10<sup>0</sup> to 10<sup>8</sup> gene copies/L.<sup>1</sup> If the wastewater is insufficiently treated, the infectious enteric viruses in the final effluent can contaminate surface waters that are used for recreation, agriculture irrigation, or serve as drinking water sources.<sup>4-6</sup> Enteric viruses are mostly nonenveloped and thus consist of nucleic acids and protein capsids (Figure 1.1). Their diameters range in size from 20-100 nanometers. Previous water treatment research and monitoring efforts have focused primarily on removing and inactivating nonenveloped enteric viruses.

Family/Genus	Virus	Diseases	Genome type	Levels in human specimens (ref.)	Levels in untreated wastewater (ref.)
Coronaviridae/ Torovirus	Torovirus	Gastroenteritis	ss RNA		Gene positive in winter municipal wastewater ( <sup>7</sup> )
	SARS coronavirus	Respiratory illness, severe	ss RNA	30-70% gene positive last 10 days after disease onset ( <sup>8</sup> )	
Coronaviridae/ Coronavirus	MERS coronavirus	pneumonia, gastroenteritis,	ss RNA	$10^3$ gc/g stool ( <sup>9</sup> )	
	Human coronavirus	Pneumonia, bronchiolitis, gastroenteritis,	ss RNA	2.3% gene positive in stool samples ( <sup>10</sup> )	
	Avian influenza H5N1	Severe respiratory illness	ss RNA	8.6×10 <sup>2</sup> -1.5×10 <sup>6</sup> gc/mL in rectal swab samples $(^{11})$	
Orthomyxoviridae/ Influenzavirus A	Avian influenza H7N9	Severe respiratory illness	ss RNA	12/14 gene positive in stool samples ( <sup>12</sup> )	
	Seasonal influenza A virus	Respiratory illness	ss RNA	47% gene positive in stool samples, $10^4$ - $10^6$ gc/g stool ( <sup>13</sup> )	
Flaviviridae/	Zika virus	Microcephaly, Guillain-Barre syndrome	ss RNA	Gene positive in urine samples. ( <sup>14,15</sup> ) Zika-carrying mosquito eggs detected in 49% of septic tank samples ( <sup>16</sup> )	
Flavivirus	Dengue virus	Severe bleeding, shock	ss RNA	20%-80% gene positive in urine, lasting for $\sim$ 2 weeks ( <sup>17</sup> )	
	West Nile virus	Encephalitis, meningitis	ss RNA	44% gene positive in urine with acute infection $(^{18})$	
Herpesviridae/ Cytomegalovirus	Cytomegalovirus	Hearing loss, pneumonia, microencephaly, liver disease	ds DNA	Prolonged excretion in urine from children with congenital infection ( <sup>19</sup> ) Infectious cytomegaloviruses in urine isolated in MRC-5 cell lines ( <sup>20</sup> )	

 Table 1.1 Example enveloped viruses detected in human specimens and/or wastewater.

\* gc, gene copies; IU, infectious units; MPNCU, maximum probable number culture unit; PFU, plaque-forming unit; FFU, focus-forming unit.

\*\* Family and genus were based on the 2017 International Committee on Taxonomy of Viruses (ICTV), https://talk.ictvonline.org/taxonomy/

Unlike nonenveloped viruses, the presence and fate of enveloped viruses have not been broadly studied. Enveloped viruses contain a lipid membrane outside of their nucleic acids and protein capsids (Figure 1.1). Enveloped viruses are responsible for a number of high-profile diseases in humans, such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome

(MERS), and avian influenza. They are also responsible for less dangerous illnesses such as the common cold. Enveloped viruses have widely been assumed to be absent in water environments. In fact, some enveloped do enter wastewater, but methods for their detection and an understanding of their mechanistic fate is lacking. Some example enveloped viruses that can be released to wastewater are described below.



Figure 1.1 Structural illustrations of enveloped and nonenveloped viruses.

#### 1.1.1 Coronavirus

Different coronaviruses can cause both respiratory and gastrointestinal illnesses.<sup>21</sup> Some strains of human coronaviruses, such as SARS coronavirus and MERS coronavirus, are the responsible agents for epidemics of deadly acute pneumonia diseases. The overall case-fatality rate for the SARS outbreak in 2003 was 10%,<sup>22</sup> and the accumulated case-fatality rate of MERS was 35%.<sup>23</sup> Infected individuals shed SARS and MERS coronavirus genes in their stool and urine samples with high frequency (Table 1.1), and infectious SARS coronaviruses were isolated from human stool samples.<sup>24</sup> In fact, a SARS outbreak in an apartment complex in Hong Kong was attributed to the SARS coronavirus in wastewater forming aerosols when toilets were flushed.<sup>25</sup> Genome shedding was reported for other low pathogenic strains of human coronaviruses (i.e., 229E, NL63, OC43, and HKU1),<sup>10,26</sup> and infectious coronavirus HKU1 was isolated from human

stool samples (Table 1.1).<sup>21</sup> These human coronaviruses are not deadly like SARS and MERS coronaviruses, and cause seasonal outbreaks of the common cold.

#### 1.1.2 Influenza virus

Infectious avian influenza viruses (AIV) are shed in an extremely high concentrations in bird feces  $(10^9-10^{10} \text{ infectious units per day})^{27}$  and are transmitted primarily via fecal-oral route in birds.<sup>28</sup> Occasionally, humans can acquire AIV, and the accumulated AIV H5N1 case fatality rate of human infection from 2003 to 2017 was 53% as estimated by WHO.<sup>29</sup> The transmission route of AIV from poultry to human is still elusive, but several transmission routes are hypothesized, including direct contact with the infected poultry, and contact with virus-laden fecal matter or water.<sup>30,31</sup> Despite that the human-to-human transmission has rarely been reported once humans acquired AIV, infected individuals can shed AIV genes in their stool samples with high frequency (Table 1.1).<sup>11,12,32,33</sup> The concentration of avian influenza virus H5N1 genes detected in rectal swab samples ranges from  $8.6 \times 10^2$  to  $1.5 \times 10^6$  gene copies/mL.<sup>32,33</sup> Like avian influenza viruses, seasonal human influenza virus strains were detected in feces, and the concentrations were  $10^4 - 10^6$  gene copies/g of stool samples.<sup>34</sup>

#### 1.1.3 Other enveloped viruses

Zika virus is an emerging mosquito-borne human pathogenic virus, and Zika virus genes can be detected in urine specimens.<sup>15</sup> The genes of other mosquito-borne enveloped viruses such as dengue virus and West Nile virus were also widely detected in urine,<sup>17,18,35</sup> and infectious West Nile virus was isolated from the urine of infected individuals with acute infection (Table 1.1).<sup>18</sup> Alternatively, wastewater is a habitat for mosquito larvae and adults that can carry and transmit those enveloped viruses.<sup>36-38</sup>

Cytomegalovirus is carried by people of all ages, in most cases, asymptomatically, but can be a threat to those who are immunodeficiency or immunocompromised. Infectious cytomegaloviruses can be shed in the urine from infants and children who are infected at birth (Table 1.1).<sup>19,20</sup> Contacting with urine is suspected as one of transmission routes of cytomegalovirus.

Ebola virus, causing deadly hemorrhagic fever, can enter wastewater when patients shed bodily fluids that contain high levels of infectious viruses;<sup>39-41</sup> however, the environmental transmission route for Ebola diseases has been observed.

Currently available clinical and epidemiological evidence therefore suggests that water environments can, in fact, be reservoirs for enveloped viruses. This highlights the importance to expand our knowledge on the presence and fate of viruses in water beyond nonenveloped viruses to include enveloped viruses. To do this, we must first develop reliable methods for capturing and monitoring infectious enveloped viruses from water. We must also evaluate the survivability of enveloped viruses that enter municipal wastewater.

#### **1.2 Virus survival in wastewater**

To cause infection, viruses in the environment must retain their infectivity until they come into contact with the next host. The survivability of viruses is often measured by the length of time to lose 90% of their original infectivity (i.e., T<sub>90</sub> value). Enveloped viruses have often been assumed to be less stable in water, but this assumption is too simplistic. The T<sub>90</sub> values available in the

literature suggest that enveloped viruses are not necessarily more susceptible to environmental conditions than nonenveloped viruses in various water environments<sup>42</sup> (Figure 1.2). Some strains of coronavirus and avian influenza virus retain their infectivity as long as nonenveloped viruses (Figure 1.2). SARS coronavirus and human coronavirus 229E, for example, had T<sub>90</sub> greater than one day in urine and filtered wastewater samples, respectively.<sup>42</sup> For context, one day is the maximum retention time of wastewater in a common sewage system. However, the current survival studies of enveloped viruses have been less reported for raw wastewater.



**Figure 1.2** T<sub>90</sub> values of viruses in different water matrices and temperatures. Data in this figure was replotted from previous research on virus survival.<sup>42</sup>

If the viruses are able to survive in raw wastewater and then enter the wastewater treatment plants, viruses need to be removed or inactivated effectively through the treatment processes. The removal efficiency and mechanisms of nonenveloped enteric viruses in wastewater treatment plants have been reviewed in previous publications.<sup>43,44</sup> For nonenveloped viruses, the removal efficiency from wastewater depends on virus partitioning with wastewater solids in primary

treatment and the adsorption to activated sludge in secondary treatment.<sup>43</sup> Corresponding studies have not been conducted for enveloped viruses. We therefore have a limited ability to predict the fate of infectious enveloped viruses in wastewater treatment plants.

Particle interaction theories have been applied for investigating the interactions between nonenveloped viruses and solids in water. The DLVO (Derjaguin-Landau-Verwey-Overbekk) theory and the extended DLVO (XDLVO) theory can be valid to describe the forces between virus particles and solids in water, depending on the solid materials.<sup>45,46</sup> In the DLVO and XDLVO theories, virus particles present in water are modelled as colloids that carry surface charges as a result of their protein and nucleic acid compositions.<sup>47,48</sup> To quantitatively characterize virus adsorption to solids, Langmuir and Freundlich isotherm adsorption models have been successfully applied for nonenveloped viruses in water at an equilibrium state.<sup>47</sup> Grant *et al.*<sup>49</sup> integrated the isotherm adsorption model with the first-order inactivation kinetics model to describe nonenveloped virus inactivation in liquid and on solid surface. We hypothesize that the adsorption and inactivation models are still applicable for enveloped viruses, but enveloped virus particioning with wastewater solids could help in predicting enveloped virus survivability and removal efficiency through wastewater treatment processes.

#### **1.3 Virus inactivation by disinfection treatment**

Disinfection is used in both drinking water and wastewater treatment plants, and is intended to inactivate pathogenic viruses and other microorganisms. The disinfection efficacy of a number of disinfection methods has been widely reported for nonenveloped viruses,<sup>50-53</sup> whereas limited data is available for enveloped viruses. Here, we focus on reviewing virus inactivation mechanisms by

ultraviolet 254 ( $UV_{254}$ ) and free chlorine, as representative UV light disinfection and chemical oxidant disinfection, respectively.

#### 1.3.1 UV disinfection

UV is one of the most commonly applied disinfection methods. UV light can be subdivided into three regions according to wavelength, namely UVA (320-400 nm), UVB (290-320 nm), and UVC (100-290 nm). Viruses are most sensitive to UVC due to the high photoreactivity of nucleic acids in the UVC region. Low-pressure mercury lamps emit the highest UVC intensity around 254 nm; therefore, most studies on virus inactivation by UVC focus on this specific region (i.e., UV<sub>254</sub>).

Our current knowledge on virus inactivation mechanisms was established primarily with nonenveloped model viruses. A study of bacteriophage MS2, for example, suggests that the inactivation of a nonenveloped virus by  $UV_{254}$  is majorly attributed to damage in the viral genome.<sup>54</sup> Follow-up studies on nonenveloped viruses underscore the findings that the  $UV_{254}$  reactivity of viral genomes correlate to virus susceptibility to  $UV_{254}$ .<sup>55-59</sup> Two main factors determine the  $UV_{254}$  reactivity of viral genomes, namely genome size and genome types (single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA)). Other mechanisms of virus particle damage by  $UV_{254}$  can also lead to nonenveloped virus inactivation. In the MS2 model, protein damage sensitized by adjacent viral RNA sequences contributes to 20% of the observed virus inactivation,<sup>54</sup> whereas in nonenveloped dsDNA viruses, the damaged genome can be repaired in the host cell and this results in higher resistance to  $UV_{254}$ .<sup>60</sup> Compared to nonenveloped viruses, the mechanisms of enveloped virus inactivation.

#### 1.3.2 Free chlorine disinfection

Free chlorine is a strong oxidant that readily inactivates microorganisms. Free chlorine is an aqueous solution of the following chlorine species: HOCl, OCl<sup>-</sup>, Cl<sub>2</sub>(aq), and Cl<sub>2</sub>O(aq).<sup>61</sup> The primary oxidant species is the neutral molecule hypochlorous acid (HOCl). Based on the nonenveloped MS2 model, the reactions of free chlorine with virus proteins and genomes impact the ability of viruses to bind, enter, and replicate in the host cell.<sup>54</sup> The inactivation of enveloped viruses with free chlorine have only been compared to nonenveloped viruses in one study. There, the enveloped bacteriophage Phi6 and Ebola virus experienced higher levels of inactivation than nonenveloped bacteriophages MS2 and M13 in 0.5% sodium hypochlorite solution.<sup>62</sup> However, that report provided limited information on the chlorine demand of samples and other important experimental conditions; consequently, it is impossible to draw general conclusions about whether enveloped viruses are more or less susceptible to inactivation by free chlorine than nonenveloped viruses.

A bottom-up characterization of enveloped virus inactivation could help identify molecular features that drive inactivation. With this information, we would be better equipped to select and improve disinfection methods for treating enveloped viruses. This is particularly important during outbreak events, when culturing viruses to see how well disinfection are working is often not possible.

#### **1.4 Virus concentration and detection**

Monitoring infectious human viruses in water is important for environmental surveillance and water quality control. Due to the low concentrations of human viruses in wastewater and drinking water samples, concentration steps are often necessary prior to virus detection. Published virus concentration methods have nearly all been developed and optimized for nonenveloped viruses and therefore may not be effective for recovering infectious enveloped viruses from water samples. In the limited studies that attempted to recover enveloped viruses, low recoveries of infectious enveloped viruses were reported. For example, a method employing glass wool and ceramic membrane filtration combined with PEG precipitation only recovered of 0.01% to 7.89% of infectious enveloped influenza A viruses from lake water and 3.63% to 13.79% from rainwater.<sup>63</sup> A positively charged membrane filtration method recovered 1% of infectious enveloped SARS coronaviruses from sewage samples.<sup>64</sup> A reliable concentration method is therefore needed for recovering infectious enveloped viruses from water.

Once the infectious viruses have been concentrated, viruses must be detected. The traditional culture-based methods detect infectious viruses using host cell lines that are susceptible to virus infection. One major drawback of this technique is that it requires long periods of time for clear cytopathic effects to appear in the host cells, which is a sign of virus infection. Another drawback is that it is usually impossible to discern the virus strain or species responsible for the cytopathic effects observed in the cells without further testing.

To decrease this detection period, virus culturing has been integrated with polymerase chain reaction (ICC-PCR) to detect viral genomes that are formed in the culture system before cytopathic effects appear.<sup>65-68</sup> The success of ICC-PCR, however, depends on the effectiveness of primers, and PCR assay optimization can be time-consuming. Moreover, unpredictable genetic variations in viruses may result in the failure of PCR methods.<sup>69</sup> In recent years, mass spectrometry (MS) techniques have been developed to identify viruses in clinical samples.<sup>70,71</sup> In those studies, infectious viruses in clinical samples were first cultured in cells. Proteins were then extracted from

the culturing system, digested into peptide sequences, and sent to mass spectrometry for peptide detection.<sup>70,71</sup> Mass spectrometry detects the masses and sequences of peptides that are compared to those available in viral protein database. The obtained protein sequences are likely to distinguish viruses at strain levels. Integrated cell culture-MS (ICC-MS) methods hold promise for detecting infectious human viruses in water samples as they can screen for large groups of viruses at once and may help avoid tedious method optimization.

#### **1.5 Overview of dissertation chapters**

This dissertation aims to expand our current state of knowledge on the fate and detection of nonenveloped enteric viruses in wastewater and drinking water. To evaluate enveloped virus survival in wastewater and removal in treatment processes, the inactivation kinetics and solid partitioning kinetics were characterized for model viruses (Chapter 2). The initial results guided the optimization of a concentration method designed for recovering infectious enveloped viruses from wastewater (Chapter 2). To investigate enveloped virus inactivation through disinfection processes, the biomolecule reactions in a model enveloped virus were characterized following the exposure to free chlorine and  $UV_{254}$  (Chapter 3). Molecular features that contributed to the model enveloped virus inactivation by free chlorine and  $UV_{254}$  were identified and compared with a model nonenveloped virus (Chapter 3). In the final chapter, a new virus detection method using integrated cell culture-mass spectrometry (ICC-MS) was developed for monitoring infective viruses in water. A proof-of-concept application of the ICC-MS method was successfully applied to detect human viruses in wastewater (Chapter 4).

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# Chapter 2 Survivability, partitioning, and recovery of enveloped viruses in

#### untreated municipal wastewater

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#### 2.1 Introduction

Recent severe disease outbreaks caused by enveloped viruses, such as Ebola, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and avian influenza H5N1 have heightened fears of an imminent deadly viral pandemic. The major transmission routes of these viruses involved direct person-to-person contact or indirect contact with contaminated objects.<sup>1,2</sup> Human enveloped viruses are often presumed to exist in low concentrations in human excrement and undergo rapid inactivation in aqueous environments; however, several lines of evidence suggest these assumptions are not always correct. The genes of coronaviruses and avian influenzas have been detected in the feces of infected individuals,<sup>3,9</sup> and some enveloped viruses were measured in wastewater biosolid residuals.<sup>10</sup> Likewise, some enveloped viruses can survive for days to weeks in pasteurized wastewater.<sup>11-13</sup> A review of virus T<sub>90</sub> values (*i.e.* time to reach 90% inactivation) suggests that avian influenza viruses survive just as long, if not longer, than nonenveloped enteric viruses in some aqueous environments.<sup>14</sup> Based on this information, it is therefore feasible that sewage and fecal-contaminated water could serve as vectors for certain

enveloped viruses. Indeed, a SARS coronavirus outbreak in an apartment complex in Hong Kong was attributed to the transport of viruses in wastewater to the air ducts.<sup>15</sup>

The vast majority of studies on the presence and fate of viruses in human waste and municipal wastewater have focused on nonenveloped enteric viruses (e.g., adenoviruses, polioviruses, enteroviruses, noroviruses and rotaviruses).<sup>16-21</sup> These viruses replicate in human gut tissues and transmit diseases primarily via the fecal-oral route. Due to the major role of water and food in the transmission of enteric viruses, there are a number of established methods for nonenveloped enteric virus detection in complex environmental matrices. Enveloped viruses differ structurally from nonenveloped viruses due to the presence of a lipid bilayer membrane outside the viral protein capsid, which contains proteins or glycoproteins. The different functional groups on the outer surface of enveloped viruses compared to nonenveloped viruses likely impact their survival and partitioning behavior in aqueous environments.<sup>22-24</sup> Likewise, methods to concentrate and recover nonenveloped viruses. For example, lipid layers are sensitive to the detergents and organic solvents<sup>25,26</sup> that are commonly used to extract and purify nonenveloped enteric viruses.

To address the paucity of data on the fate and recovery of enveloped viruses in wastewater matrices, we studied the survival and partitioning behavior of the human enveloped virus surrogates, murine hepatitis virus (MHV) and *Pseudomonas* phage Phi6, in pasteurized and unpasteurized wastewater. We compared the inactivation kinetics and liquid-solid partitioning of the two enveloped viruses with two nonenveloped virus surrogates, *Enterobacteria* phage MS2 and T3. Furthermore, we systematically tested the effectiveness of three virus recovery methods— initially developed for using on enteric viruses—for extracting and concentrating enveloped

viruses from both liquid and solid fractions in wastewater. Finally, we proposed an optimized ultrafiltration method for detecting both enveloped and nonenveloped viruses.

#### 2.2 Materials and methods

#### 2.2.1 Wastewater samples

Wastewater samples were collected from the Ann Arbor Wastewater Treatment plant, an activated sludge treatment plant serving roughly 115,000 people with an average flow rate of 19 million gallons per day (MGD). Grab samples were collected after wastewater equalization, screening, and grit removal chambers, and just before the primary settling tanks. All samples were collected and sealed in sterile plastic bottles and then immediately transported on ice to laboratories at the University of Michigan where they were stored at 4 °C and analyzed within 24 hours. Wastewater pH, total suspended solids (TSS), volatile suspended solids (VSS), and total chemical oxygen demand (COD) were measured with standard methods.<sup>27</sup>

#### 2.2.2 Virus strains and methods

We chose to study MHV strain A59 and *Pseudomonas* phage Phi6 because they are common surrogates for human enveloped viruses (Table 2.1).<sup>11,13,28</sup> We also studied two nonenveloped *Enterobacteria* phages MS2 and T3 to allow for direct comparisons between enveloped and nonenveloped virus inactivation, partitioning, and recovery.<sup>29-31</sup>
Virus	Structure	Family/Genus	Genome	Genome	Particle Size
			Туре	Size (Kb)	(nm)
MHV	Enveloped	Coronaviridae/Coronavirus	(+) ssRNA	32	100
Phi6	Enveloped	Cystoviridae/Cystovirus	Segmented	13.5	80
			dsRNA		
MS2	Nonenveloped	Leviviridae/Levivirus	(+) ssRNA	3.6	25
T3	Nonenveloped	Podoviridae/T7-like viruses	dsDNA	38.2	$50 \times 20$ (tail)

Table 2.1 Characteristics of tested viruses.

MHV strain A59, and its supporting cell lines L2 and DBT, were kindly provided by Dr. Leibowitz's lab at Texas A&M Health Science Center College of Medicine. L2 and DBT cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% newborn calf serum, 1% L-glutamine, and 1% penicillin/streptomycin, and incubated at 37 °C with 5% CO<sub>2</sub>. MHV stocks were propagated in DBT and titered by plaque assay on L2 according to a published protocol.<sup>32</sup> After amplification, MHV stocks were centrifuged at 3,000 × g for 10 min, and then filtered through a 0.22  $\mu$ m polyethersulfone (PES) membrane (Millipore, USA), in order to remove cell debris and aggregated viruses. The MHV stocks (~10<sup>6</sup> PFU mL<sup>-1</sup>) were stored at -80 °C.

Phi6 and its bacterial host *Pseudomonas syringae* were kindly provided by Dr. Linsey Marr's lab at Virginia Tech. *P. syringae* was grown in Luria-Bertani (LB) medium containing 5 g L<sup>-1</sup> NaCl at 26 °C. To propagate Phi6 stocks, soft LB-agar (0.7% agar) layers were removed from the double-layer plates, and dissolved in 3 mL of LB medium.<sup>33</sup> The recovered viruses were purified with centrifugation at 3,000 × g for 10 min at 4 °C and filtration through 0.22  $\mu m$  PES membranes. The Phi6 stocks (~10<sup>10</sup> PFU mL<sup>-1</sup>) were stored at 4 °C.

MS2 (ATCC 15597-B1) and T3 (recovered from ATCC 11303-B4), and their corresponding *Escherichia coli* hosts ATCC 15597 and ATCC 11303, respectively, were purchased from American Type Culture Collection (ATCC). The MS2 and T3 were propagated and assayed in their *E. coli* hosts based on published methods.<sup>34,35</sup> The viruses were purified with an Econo Fast

Protein Liquid Chromatography system (Bio-Rad, USA) equipped with a HiPrep Sephacryl S-400 HR column (GE, USA). The collected viral fraction was concentrated with 100 kDa Amicon ultracentrifugal filters (Millipore, USA), and filtered through a 0.22  $\mu$ m PES membrane filter. The final MS2 and T3 stocks (~10<sup>11</sup> PFU mL<sup>-1</sup>) were stored in phosphate buffer (5 mM NaH<sub>2</sub>PO<sub>4</sub> and 10 mM NaCl, pH 7.5) at 4 °C.

#### 2.2.3 Survivability experiments

Virus surrogates were spiked into 30 mL samples of unpasteurized and pasteurized wastewater to final concentrations of  $3 \times 10^4$  PFU mL<sup>-1</sup> for MHV and  $5-8 \times 10^5$  PFU mL<sup>-1</sup> for Phi6, MS2 and T3; the lower MHV concentrations were due to the lower MHV stock concentrations. Wastewater was pasteurized by heating to 70 °C for 3 h; this treatment is consistent with previous studies involving enveloped virus survival in pasteurized wastewater.<sup>11,13</sup> Wastewater samples were quickly mixed after viruses were added, titered for the initial virus concentrations, and then incubated at 25 °C or 10 °C to mimic typical summer and winter wastewater temperatures. Aliquots of wastewater were removed at specific incubation times and infective virus concentrations were enumerated with plaque assays. The wastewater samples were diluted at least 10-fold to minimize wastewater effects on the host cells. Replicate experiments (n = 3) were conducted in wastewater samples collected on different days to incorporate potential impacts of wastewater variation on virus survivability.

#### 2.2.4 Partitioning experiments

To evaluate the kinetics and extent of virus sorption to wastewater solids, the virus surrogates were spiked into 30 mL samples of untreated wastewater and wastewater with solids removed via

centrifugation at 30,000 × g for 10 min. (i.e., solids-removed samples). This centrifugation treatment, which was previously shown to remove solids less than 0.3  $\mu m$  in diameter,<sup>36</sup> consistently removed 85–95% of the TSS in our wastewater samples (Table S2). Samples were spiked to achieve final virus concentrations of 5 × 10<sup>4</sup> PFU mL<sup>-1</sup> for MHV, and 6–8 × 10<sup>5</sup> PFU mL<sup>-1</sup> for Phi6, MS2, and T3–these were low enough to be feasible concentrations present in wastewater (< 10<sup>6</sup> PFU mL<sup>-1</sup>) and high enough that more than 99% loss could be quantified with plaque assays. The spiked samples were stirred and then incubated at 4 °C; this temperature is at the low-end of mean municipal wastewater temperatures in the U.S. (3 °C–27 °C)<sup>37</sup> and was selected to minimize virus inactivation through the duration of the experiment. At various incubation times, aliquots of the untreated and solids-removed samples were centrifuged at 30,000 × g for 10 min, and the centrates were assayed for infective viruses.

Virus inactivation and sorption kinetics in wastewater batch reactors were analyzed with an approach proposed by Grant *et al.* that accounts for virus sorption and desorption from sorbents, as well as inactivation in the liquid and solid fractions.<sup>38</sup> In our system, the solids-containing samples were the untreated wastewater influent and the solids-free samples were wastewater samples with solids removed via centrifugation. Virus inactivation in the wastewater liquid was assumed to be equal to virus inactivation in the solids-removed sample, and to follow first-order kinetics:

$$\ln C_l^* = -k_1 t \tag{1}$$

where,  $C_l^*$  is the nondimensional concentration of infective viruses measured in the solidsremoved wastewater samples  $(C_l/C_{l,0})$ , t is the incubation time in hours, and  $k_1$  (h<sup>-1</sup>) is the firstorder virus inactivation constant in the solids-removed wastewater. In a wastewater sample spiked with viruses, the nondimensional concentration of infective viruses in the wastewater liquid  $C_{l,ww}^*$  is related to the fraction of viruses inactivated in the liquid phase ( $\xi_1^*$ ), and the fraction reversibly adsorbed to wastewater solids ( $\xi_2^*$ ):

$$C_{l,ww}^* = 1 - \xi_1^* - \xi_2^* \tag{2}$$

The change of the viral fraction in the liquid and solid phases with time can be described with the following set of differential equations:

$$\frac{d\xi_1^*}{d\tau} = 1 - \xi_1^* - \xi_2^* \tag{3a}$$

$$\frac{d\xi_2^*}{d\tau} = N_b \left[ 1 - \frac{n_{sro}^*}{N_f - 1} - \xi_1^* - \xi_2^* \left( \frac{N_f}{N_f - 1} \right) + \xi_3^* \left( \frac{N_s}{N_f - 1} \right) \right]$$
(3b)

$$\frac{d\xi_3^*}{d\tau} = N_i [n_{sro}^* + \xi_2^* - N_s \xi_3^*]$$
(3c)

where,  $\xi_3^*$  is the fraction of viruses inactivated on the solid surface;  $\tau$  is the nondimensional time, equal to  $k_1t$ ;  $n_{sro}^*$  is the initial amount of viruses reversibly adsorbed to solids (assumed zero in the study);  $N_b = k_2/k_1$ , where  $k_2$  (h<sup>-1</sup>) is the rate constant for reversible virus adsorption;  $N_s = (k_3 + k_4)/k_3$ , where  $k_3$  (h<sup>-1</sup>) is the rate constant for virus inactivation at the solid surface and  $k_4$ (h<sup>-1</sup>) is the rate constant for the conversion of reversibly adsorbed viruses to an irreversibly adsorbed state;  $N_i = k_3/k_1$ ;  $N_f = [(k_2W/k_{-2}V) + 1]$ , where  $k_{-2}$  (g L<sup>-1</sup> h<sup>-1</sup>) is the rate constant for virus desorption from solid phase to liquid phase, W (g) is the mass of solids, and V (L) is the liquid volume. At time zero ( $\tau = 0$ ),  $\xi_1^* = \xi_2^* = \xi_3^* = 0$ .

The relationship between  $C_{l,ww}^*$  and incubation time t was solved from numerical simulations of the above differential equation system with the 4<sup>th</sup> order Runge-Kutta algorithm in MATLAB2015. An extensive description of the equation derivations, simplifications, and parameter calculations can be found in 38.

## 2.2.5 Virus recovery methods

Virus recovery methods were tested with wastewater that had been spiked with one enveloped virus (MHV) and one nonenveloped virus (MS2). Three approaches for separating and concentrating viruses from the liquid fraction of municipal wastewater, including polyethylene glycol (PEG) precipitation,<sup>39,40</sup> ultracentrifugation,<sup>19</sup> and ultrafiltration,<sup>18,41</sup> were selected based on their previous application in recovering viruses from wastewater. Published enteric virus methods that involved steps likely to inactivate the enveloped viruses (e.g., pH adjustment outside 6-8 range,<sup>42-44</sup> organic solvent extractions,<sup>25,26</sup> etc.) were avoided. The best-performing method for MHV and MS2 was then further validated with the enveloped virus Phi6 and nonenveloped virus T3. In the first set of experiments, MHV and MS2 were spiked in wastewater samples to final concentrations of  $8 \times 10^3$  PFU mL<sup>-1</sup> and  $5 \times 10^5$  PFU mL<sup>-1</sup>, respectively. Samples were then briefly mixed and incubated at 4 °C for one hour before they were treated with the extraction/concentration techniques; the one-hour incubation time was selected based on the results from the partitioning experiments. In each experiment, samples were concentrated  $100 \times$ , and infective viruses in the concentrates were measured with plaque assays. Virus recovery was calculated based on the following relationship:

$$Virus \ recovery \ (\%) = \frac{c_{con} \cdot V_{con}}{c_s \cdot V_s} \times 100\%$$
(4)

where  $(C_s \cdot V_s)$  equals the number of infective viruses spiked in, and  $(C_{con} \cdot V_{con})$  is the number of infective viruses measured in the concentrate.

*Polyethylene glycol (PEG) precipitation method.* Following incubation with the spiked viruses, wastewater samples (250 mL) were centrifuged at 2,500  $\times$  g for 5 min at 4 °C to remove large solids. The centrate was collected and mixed with 8% (w/v) of PEG 8000 and 0.5 M of NaCl. The mixture was incubated for 2 h at 4 °C, and then centrifuged at 10,000 g for 30 min at 4 °C. The

PEG pellet was resuspended in 2.5 mL phosphate buffered saline (PBS, pH 7.4; Life Technologies, USA) and assayed for infective viruses.

*Ultracentrifugation method*. Following incubation with the viruses, wastewater samples (60 mL) were centrifuged at 100,000 × g for 1 h at 4 °C using a Sorvall WX Ultra centrifuge (Thermo Scientific, Germany; SureSpin 630 (36 mL) rotor, P/N 79368; SureSpin swinging bucket, P/N 79388). The pellet was resuspened in 8 mL of 0.25 M glycine buffer (pH 9.5) and allowed to sit on ice for 30 minutes. After neutralizing the solution pH with 16 mL PBS, the solids were removed by centrifugation at 10,000 × g for 15 min at 4 °C. The supernatant was collected and centrifuged again at 100,000 × g for 1 h at 4 °C to pellet the viruses. The final virus pellet was dissolved in 600  $\mu$ L PBS.

*Ultrafiltration method*. Following incubation with the spiked viruses, solids in the wastewater samples (250 mL) were removed by either centrifuging at 30,000 × g for 10 min at 4 °C, or by centrifugation at 2,500 × g for 5 min at 4 °C followed by filtration through 0.22  $\mu$ m PES membrane filters. After the large solids had been removed, the samples were concentrated with Centricon centrifugal filters (Millipore, USA) to a final volume of 2.5 mL. Recoveries from centrifugal filters with 10 kDa and 100 kDa cut-offs were compared. Centrifugal filter reuse was tested by first washing used filters with 100 mL of 0.5 M NaOH and then storing the regenerated filters in 70% ethanol. The reused filters were rinsed with 100 mL of Milli-Q water prior to use.

In an attempt to recover viruses associated with wastewater solids, the solids collected in the centrifugation step prior to ultrafiltration were mixed with different elution buffers, including PBS, 0.05 M glycine buffer (pH 8.5), 0.05 M glycine buffer (pH 9.5), 0.05 M glycine buffer (pH 10.5), 3% beef extract (pH 7.5), 3% beef extract (pH 9.5), and 3% beef extract with 0.5 M sodium chloride (pH 9.5). Suspensions were set on ice for 30 min. and gently shaken every 10 min. The

solutions were centrifuged at  $10,000 \times \text{g}$  for 15 min at 4 °C and the resulting centrate was neutralized with PBS (pH 7.4), and then titered for infective viruses.

#### 2.2.6 Statistical analyses

Non-parametric t-tests were applied to two groups of experimental data to assess statistical significance. Two-tailed P values were calculated, and P < 0.05 was considered statistically significant.

# 2.3 Results and discussion

# 2.3.1 Comparison of virus survival in wastewater

Inactivation of the two enveloped viruses (MHV and Phi6) and nonenveloped virus MS2 in unpasteurized and pasteurized wastewater at 10 °C and 25 °C followed first-order kinetics (Figure 2.1; Table A-3), with inactivation proceeding faster for the enveloped viruses. In unpasteurized wastewater at 25 °C, the T<sub>90</sub> (±s.d.) values for MHV and Phi6 were 13 (±1) and 7 (±0.4) hours, respectively, and 121 (±36) hours for MS2 (Table A-3). The nonenveloped T3 virus survived much longer than the other virus surrogates with no significant decrease in infectivity observed within the 48-hour experiments for both temperatures (Figure 2.1). This is consistent with long survival times reported for tailed phages in adverse conditions.<sup>45</sup> The inactivation kinetics of the enveloped viruses were significantly (P < 0.0001) slower in wastewater at 10 °C compared to 25 °C (Figure S4), with T<sub>90</sub> (±SD) values of 36 (±5) and 28 (±2) hours for MHV and Phi6 at 10 °C, respectively (Table A-3). Like T3, MS2 inactivation was not statistically different at the two temperatures (P = 0.1813) within the tested timescale (Figure A-4).

Inactivation kinetics of the enveloped viruses MHV, Phi6, and Ebolavirus in pasteurized or gamma-irradiated wastewater have been reported previously.<sup>11-13</sup> In our experiments, the two enveloped viruses lost infectivity at a significantly slower rate in pasteurized wastewater compared to unpasteurized wastewater, except for the case of MHV at 25 °C (Figure 2.1; Table A-3). The most pronounced effect occurred with Phi6, which had a first-order inactivation rate constant (±s.d.) of 0.317 (±0.022) h<sup>-1</sup> in unpasteurized wastewater and 0.044 (±0.004) h<sup>-1</sup> in pasteurized wastewater at 25 °C. A statistically significant difference in the inactivation kinetics of the nonenveloped viruses was not observed in pasteurized wastewater and unpasteurized wastewater; this may be due to the fact that our experiments were stopped before 90% of the nonenveloped viruses were inactivated. Discrepancies in inactivation kinetics in sterilized and non-sterilized wastewater have been reported previously for nonenveloped viruses,<sup>46</sup> and may be due to bacterial extracellular enzyme activity and protozoan or metazoan predation.<sup>47,48</sup> Overall, the results suggest that unpasteurized wastewater samples should be employed for survivability tests when feasible.

Wastewater residence times in sewage systems are typically less than 24 hours. Although Phi6 and MHV had  $T_{90}$  values of 7–13 hours in unpasteurized wastewater at 25 °C, the  $T_{90}$  values increase to 28–36 hours at 10 °C. Human enveloped viruses excreted in feces may therefore reach wastewater treatment plants in an infective state, especially in cool climates. Local outbreaks and global pandemics of enveloped viruses excreted in feces or urine are therefore relevant for wastewater utilities.



**Figure 2.1** Virus survival in wastewater and pasteurized wastewater at 10 and 25 °C. Viruses were spiked into wastewater to final concentrations of  $3 \times 10^4$  PFU mL<sup>-1</sup> for MHV and 5— $8 \times 10^5$  PFU mL<sup>-1</sup> for MS2, T3 and Phi6. Error bars represent the standard deviations of replicates from wastewater samples collected on different days (n = 3). Table S3 summarizes corresponding rate constants and estimated T<sub>90</sub> values.

#### 2.3.2 Comparison of virus partitioning in wastewater.

The measured concentrations of infective MHV and Phi6 in the solids-removed wastewater samples immediately after spiking, mixing, and centrifuging, were consistently lower than the theoretical concentrations based on the amount of viruses spiked into the sample (Figure S1). Approximately 47% of the spiked MHV and 77% of the spiked Phi6 were recovered in the centrate of the solids-removed wastewater. This is compared to a nearly 100% recovery of the nonenveloped viruses MS2 and T3. Nearly all of the MHV was recovered when it was spiked into PBS and centrifuged in the same manner (Figure S1). This suggests that a fraction of the enveloped

viruses (53% MHV and 23% Phi6) were rapidly inactivated in the solids-removed wastewater. A pronounced initial decrease in infective virus concentration was previously observed when Ebola virus was added to pasteurized wastewater.<sup>12</sup> In those experiments, the number of infective Ebola viruses decreased rapidly over the first 24 hours (~2-log loss) and then stabilized at a much slower inactivation rate over the subsequent seven days. Similar biphasic inactivation kinetics have also been observed with nonenveloped viruses, which were attributed to subpopulations of viruses with varied susceptibilities to solution chemistry or temperature.<sup>38</sup> In our partitioning experiments, we chose to normalize measured concentrations in the wastewater and solids-removed wastewater samples over time to concentrations measured in solids-removed samples immediately after they were spiked with viruses, mixed, and centrifuged. We felt this approach was justified because the behaviors of the persistent subpopulations are of most interest for real wastewater systems.

MHV, Phi6, and MS2 concentrations decreased significantly over a three-day period in the solids-removed wastewater samples (Figure 2.2) and the resulting rate constants were assumed to equal virus inactivation rates in the liquid fraction of wastewater (Eq. 1,  $k_1$ ).<sup>38</sup> When the viruses were spiked in wastewater samples containing solids, the normalized MHV and Phi6 concentrations in the wastewater liquid phase (in centrate after centrifugation) decreased rapidly in the first hour, and then eventually decreased at the same rate as virus inactivation in the solids-removed sample (Figure 2.2). The MS2 concentration in the wastewater liquid phase decreased rapidly at first, and then slowed to a rate that was faster than MS2 inactivation in the solids-removed sample (Figure 2.2). No significant decay of T3 was observed in the solids-removed wastewater samples or the liquid phase of wastewater samples.



**Figure 2.2** Adsorption and inactivation kinetics and model simulations for enveloped viruses (MHV and Phi6) and nonenveloped viruses (MS2 and T3) in 4 °C wastewater. Viruses were spiked into wastewater and solids-removed wastewater samples to final concentrations of  $5 \times 10^4$  PFU mL<sup>-1</sup> for MHV, and 6—8 × 10<sup>5</sup> PFU mL<sup>-1</sup> for MS2, T3 and Phi6.  $C_l^*$  and  $C_{l,ww}^*$  are nondimensional concentrations of infective viruses in the solids-removed sample centrates and wastewater sample centrates, respectively. Both values were normalized to the initial measured virus concentration in the solids-removed sample centrates. No significant decline in T3 infectivity was observed within 36 hours. Error bars represent the range of data from duplicate experiments conducted in wastewater samples collected on different days (n = 2).

Based on these results, the MHV and Phi6 sorption kinetics can be best described by a noninstantaneous quasi-equilibrium adsorption model in which the virus sorption to wastewater solids does not occur instantaneously and the inactivation rates in the wastewater solid and liquid phases are equal (Table A-4). A similar model was used to describe bacteriophage  $\lambda$  sorption kinetics with sand.<sup>38</sup> In comparison, MS2 behavior is best described by the non-instantaneous quasi-equilibrium adsorption and surface sink model. In this model, virus inactivation is faster in the solid phase than in the liquid phase (Table A-4); a similar model was proposed for the interaction of bacteriophage MS2 and PRD1 with sediments.<sup>49</sup> Bacteriophage T3 could not be modeled due to the nonsignificant decreases in infective viruses measured over the experiment timescale.

These models predict that 26% of MHV, 22% of Phi6, and 6% of MS2 adsorbed to wastewater solids at equilibrium (Figure 2.3; Table A-4). Although the T3 virus kinetics could not be modeled, < 5% of the spiked T3 had partitioned to the wastewater solids at the end of the 36-hour experiment; this suggests that like MS2, T3 partitions overwhelmingly to the liquid fraction of wastewater (Figure 2.2). The equilibrium percentages reported here are not representative for all wastewaters because wastewater solids concentrations vary widely. It should be noted that our wastewater solid concentrations were typical for medium-strength municipal wastewaters<sup>37</sup> (Table A-1) with an average TSS value of 235 mg L<sup>-1</sup>.



**Figure 2.3** Models for adsorption and inactivation kinetics of enveloped viruses (MHV and Phi6) and nonenveloped viruses (MS2) in 4 °C wastewater.  $\xi_1^*$  represents the fraction of viruses inactivated in liquid fraction of wastewater;  $\xi_2^*$  represents the fraction of viruses reversibly adsorbed to wastewater solids;  $\xi_3^*$  represents the fraction of viruses inactivated on the solid surface.

The partitioning results for MS2 and T3 are consistent with an early observation that wastewater solids are poor at absorbing enteric viruses.<sup>50</sup> Wastewater solids tend to be negatively charged, as is MS2 (isoelectric point = 3.9). The isoelectric point for T3 has not been reported, but the similar T2 and T4 viruses have isoelectric points <  $6.^{51}$  A study on the adsorption of four nonenveloped viruses to various solid surfaces demonstrated that long-ranged electrostatic interactions and hydrophobic effects between the virus capsid proteins and the sorbent surfaces dictated adsorption, with short-ranged van der Waals and steric interactions playing less important

roles.<sup>52</sup> Similar work has not been conducted for enveloped viruses, and the impact that the surface phospholipids and various membrane proteins have on partitioning remains elusive.

Despite the poor sorption of nonenveloped enteric viruses to wastewater solids, some enteric viruses have been observed in primary settled solids in high concentrations.<sup>36,53</sup> In such cases, the viruses were likely released into wastewater within or strongly associated with fecal solids and never reached equilibrium between the liquid and solid fractions. When excreted in watery diarrhea or urine, the viruses would more likely reach equilibrium. Our results suggest that if allowed to reach equilibrium, enveloped viruses more strongly associate with wastewater solids than nonenveloped viruses. Consequently, enveloped viruses would be removed to a greater extent than nonenveloped viruses in primary wastewater treatment. More enveloped and nonenveloped viruses will need to be tested to confirm the results obtained with the two enveloped and two nonenveloped model viruses.

In addition to relaying information on virus partitioning between solid and liquid phases at equilibrium, the models also predicted the amount of time it takes for the viruses to reach equilibrium. This information is important for virus recovery experiments, where viruses are spiked into an environmental sample and then extracted and quantified with various techniques. If the spiked viruses are extracted too soon, results may be biased due to the spiked viruses in liquid phase. In water with soils and clays, nonenveloped virus adsorption is assumed to reach equilibrium within an hour.<sup>54</sup> Our models estimated that the viruses in wastewater reached 90% of equilibrium concentrations after 0.3-1.5 hours, and 99% of equilibrium concentrations after 0.4-2.9 hours (Figure 2.3; Table A-4). Based on these results, we allowed samples to equilibrate for at least one hour before extraction methods were tested.

## 2.3.3 Virus recovery from wastewater.

According to the simulation results of virus partitioning, greater than 70% of the infective model enveloped viruses were associated with wastewater liquids at equilibrium. We therefore focused primarily on the wastewater liquid fraction in our virus recovery experiments. Of the three methods we tested, the ultrafiltration method and the PEG precipitation methods involved an initial step to remove wastewater solids and then focused on recovering the viruses in the liquid phase. The ultracentrifugation method, on the other hand, involved pelleting all of the wastewater solids and colloids and then extracting the viruses from the pellet.

The enveloped MHV recoveries were consistently lower than the nonenveloped MS2 recoveries when the PEG precipitation and ultrafiltration methods were applied (Figure 2.4); this was not unexpected given that MHV partitioned to solids to a greater extent than the MS2. Low mean recoveries (< 6%) were achieved for both MS2 and MHV with the ultracentrifugation method (Figure 2.4). The ultrafiltration method resulted in significantly higher MHV recoveries than the PEG precipitation (P = 0.0065) and the ultracentrifugation (P = 0.0084) methods. MS2 recoveries with the ultrafiltration method were significantly higher than ultracentrifugation (p=0.0074), but not significantly different than PEG precipitation (P = 0.4137) method (Figure 2.4).



**Figure 2.4** Recoveries for enveloped and nonenveloped viruses from wastewater with PEG precipitation, ultracentrifugation, and optimized ultrafiltration method. Viruses were spiked into wastewater samples to final concentrations of  $8 \times 10^3$  PFU mL<sup>-1</sup> for MHV, and  $2-5 \times 10^5$  PFU mL<sup>-1</sup> for MS2, T3 and Phi6.

Additional experiments suggested that incubation with PEG caused a major drop in infective MHV. The T<sub>90</sub> for MHV in wastewater with PEG was 16 hours compared to 40 hours in wastewater without PEG (Figure A-2). The enveloped influenza viruses were previously recovered from surface waters with the PEG method,<sup>55</sup> but recoveries were very low (0.2% - 0.6%). The low recoveries for MHV and influenza with PEG may be due to disruption of their lipid bilayers.<sup>56</sup> Meanwhile, the MS2 recovery obtained here with the PEG method ( $43.1 \pm 16.8\%$ ) was comparable to the recovery of nonenveloped *Echovirus* 7 from raw wastewater ( $78.5 \pm 11.0\%$ ).<sup>57</sup> These results suggest that PEG precipitation method, which is effective at recovering infective nonenveloped viruses.

In the ultracentrifugation method, the initial centrifugation  $(100,000 \times \text{g} \text{ for 1 h})$  step did not effectively pellet bacteriophage MS2, and 63% of the spiked MS2 was detected in the centrate. Comparatively, only 1% of the spiked MHV was detected in the centrate. Previously, the ultracentrifugation method was successful at recovering rotavirus genes from raw wastewater (47% mean recovery), but the infectivity state of the recovered viruses was not tested.<sup>19</sup> Our low recovery of infective MHV viruses in the pellet may be due to virus inactivation by the large ultracentrifugation may be effective at recovering enveloped viruses genes for qPCR detection, but not appropriate when infective viruses are desired.

Additional experiments were conducted to optimize recoveries with the ultrafiltration method (description in Appendix A; Figure A-3). The optimized method involves pre-filtering 250 mL of wastewater through a 0.22  $\mu$ m PES membrane to remove solids, followed by concentration of the filtrate with 10 kDa centrifugal filters to a final volume of 2.5 mL. Using this method, we achieved mean virus recoveries of 25.1% for MHV, 18.2% for Phi6, 55.6% for MS2, and 85.5% for T3 (Figure 2.4). Ultrafiltration methods have been successfully applied for recovering nonenveloped enteric viruses from wastewater, such as polioviruses, adenoviruses, noroviruses, and enteroviruses.<sup>18,41</sup> Here, we have demonstrated that the method can also be optimized for recovering enveloped viruses. In future work, we will test hollow fiber ultrafilters and tangential flow ultrafiltration to potentially increase wastewater sample volumes that can be processed, and thus decrease the detection limits of infective enveloped viruses in wastewater.

# 2.3.4 Environmental Implications.

Our results shed light on the behavior of enveloped viruses in wastewater and provide guidance on how to recover infective enveloped viruses from raw wastewater. Although the two model enveloped viruses were more rapidly inactivated in wastewater, they did survive long enough to be of concern for wastewater treatment facilities, stormwater overflow events, and wastewater intrusion in drinking water. The results presented here will be particularly important during potential future avian influenza or coronavirus outbreaks in humans, as some strains of these viruses can be excreted in feces. Future work should examine additional enveloped viruses to elucidate the specific virus characteristics that contribute to their survival times and enhanced partitioning to solids.

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# Chapter 3 Reactivity of enveloped virus genome, proteins, and lipids with free

chlorine and UV<sub>254</sub>

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# **3.1 Introduction**

Viruses that are transmitted through direct person-to-person contact or in large respiratory droplets do not typically survive for very long outside of their host in the environment.<sup>1</sup> On the other hand, viruses that are transmitted through aerosols or by contact with water, food, and solid surfaces tend to survive longer in order to come into contact with their next host.<sup>2,3</sup> Survivability, often reported as the time necessary for 90% of a population to lose infectivity (T<sub>90</sub>), can therefore vary widely amongst different viruses, and depends on environmental conditions including temperature,<sup>4,6</sup> relative humidity,<sup>4,9</sup> UV radiation,<sup>10,12</sup> and oxidants.<sup>13,15</sup> Survivability is also impacted by virus structures. Nonenveloped viruses are generally considered more stable than enveloped viruses in the environment. For example, the T<sub>90</sub> values for nonenveloped viruses range from several hours to days.<sup>16,18</sup> In terms of susceptibility to chemical disinfectants, the enveloped Ebola and Phi6 viruses experience higher levels of inactivation than the nonenveloped MS2 and M13 viruses when exposed to 0.5% NaOC1.<sup>19</sup> Even closely related enveloped viruses can have varied

survivabilities. For example, the  $T_{90}$  of severe acute respiratory syndrome (SARS) coronavirus in serum-free culture media is 9 days, whereas the  $T_{90}$  of human coronavirus 229E is less than 1 day under the same conditions.<sup>20</sup> The mechanistic reasons for the higher susceptibility of enveloped viruses to inactivation in aqueous environments are mostly unknown.

Despite their greater susceptibility to environmental conditions, many enveloped viruses do undergo environmental transmission. SARS coronaviruses were transmitted through airflow and virus-laden fecal aerosols.<sup>21,22</sup> Human influenza viruses retain their infectivity on the nonporous surfaces, increasing their chances to infect.<sup>23,24</sup> Avian influenza viruses are shed into water, where they can survive months before being consumed by their next host.<sup>25,26</sup> Porcine reproductive and respiratory syndrome viruses can travel several kilometers via aerosols, spreading swine diseases from farm to farm.<sup>27</sup> Infectious Ebola viruses persist in patient blood, feces and urine, and can survive in liquid for several days; however, an environmental transmission route has been observed.<sup>3,28</sup>

Research on what makes enveloped viruses more or less persistent in the environment and through disinfection processes could help with predicting risks posed by newly emerging viruses that are difficult or dangerous to culture. For example, in the recent Ebola outbreak, a mechanistic understanding of enveloped virus inactivation would have helped scientists predict how long Ebola virus remained infective in wastewater, blood, and vomit.<sup>29</sup> Comprehensive inactivation mechanisms have been published for a limited number of nonenveloped viruses with disinfectants,<sup>12,15,30,31</sup> but are lacking for enveloped viruses. In general, UV radiation targets nonenveloped virus genomes, whereas chemical oxidants inactivate nonenveloped viruses by genome or protein reactions, depending on the virus and oxidant.

Recent fate and survival studies of enveloped viruses in the environment have adopted *Pseudomonas* virus Phi6 as a model enveloped virus.<sup>17,32-36</sup> Phi6 is a double stranded RNA virus. The Phi6 particle is 85 nm in diameter<sup>37</sup> and contains 11 different viral proteins.<sup>38</sup> Like influenza viruses, Phi6 is enveloped, has a segmented genome, and contains glycerophospholipids in its envelope.<sup>39,40</sup> In addition, it is the best studied virus in the family *Cystoviridae*, which includes the only bacteriophages that have a lipid outer layer. Moreover, Phi6 is easier to work with than other enveloped viruses and can be propagated to high titers.

To develop a better mechanistic understanding of enveloped virus inactivation, we employed Phi6 in an initial investigation of enveloped virus reactivity with chemical oxidants and UV radiation. We characterized the biomolecule reactions in Phi6 following exposure to free chlorine and UV<sub>254</sub>. Phi6 inactivation, genome reactions, and protein and lipid reactions were quantified with plaque assays, real-time reverse transcription polymerase chain reactions (RT-qPCR), and liquid chromatography-tandem mass spectrometry (LC-MS/MS), respectively. We compared the reaction rate constants of the Phi6 genome and proteins to those of nonenveloped viruses reported in earlier studies and measured under similar experimental conditions to elucidate the molecular features that may impact enveloped virus persistence in aqueous environments.

# 3.2 Materials and methods

## 3.2.1 Virus propagation and purification

Phi6 and its bacterial host *Pseudomonas syringae* pv. phaseolicola were kindly provided by Dr. Linsey Marr's lab at Virginia Tech. To propagate Phi6, *P. syringae* was grown in Luria-Bertani (LB) medium containing 5 g L<sup>-1</sup> NaCl at 26 °C and 180 rpm to an optical density of 0.10 at 640 nm (i.e., when the cell density was approximately  $1.8 \times 10^8$  cells mL<sup>-1</sup>). At that point, Phi6 was

added to the bacteria at a multiplicity of infection equal to 2 (i.e., ratio of Phi6 plaque-forming units (PFU) to *P. syringae* colony forming units (CFU)), and then incubated under the same conditions for 7 to 9 hours. Cells and debris were removed from the virus suspension by filtering it through 0.22  $\mu$  m polyethersulfone (PES, Millipore) membranes.

The filtered virus suspensions (~1 L) were concentrated to approximately 20 mL (i.e., ~50× concentration) in a lab-scale tangential flow filtration system (Millipore) outfitted with a 30 kDa cellulous filter. The concentrate was purified in a 10-40% (w/v) step sucrose gradient (average 65,700 × g, 1.5 h, 4 °C), then in a 40-60% (w/v) linear sucrose gradient (average 65,700 × g, 15 h, 4 °C). The phage band was collected with a needle and the buffer was exchanged for 5 mM phosphate buffer (PBS; 10 mM NaCl, pH 7.4) with a 100 kDa Amicon Ultra-15 centrifugal filter (Millipore). Virus purity was confirmed by SDS-PAGE with 8-16% TGX<sup>TM</sup> precast protein gels (Bio-Rad), according to the manufacturer's instructions (Figure B-1). The final Phi6 stocks (10<sup>12</sup> PFU mL<sup>-1</sup>) were filter-sterilized with 0.22 µm PES membranes, aliquoted, and stored at -80 °C until use.

## 3.2.2 Free chlorine and UV<sub>254</sub> experiments

Experimental virus solutions were prepared by diluting Phi6 in PBS. All free chlorine and  $UV_{254}$  experiments were conducted at room temperature. Infectious virus concentrations (PFU mL<sup>-1</sup>) were measured immediately before and after the viruses were exposed to chlorine and  $UV_{254}$  via plaque assays on LB agar plates.<sup>41</sup> Samples were stored on ice during the plaque assays. Following free chlorine and UV treatment, samples were immediately stored at -80 °C prior to nucleic acid, protein, and lipid analyses.

Free chlorine experiments. Free chlorine was prepared by diluting NaClO stock solution (Sigma-Aldrich) in PBS. At pH 7.4, HOCl and OCl- are equal in molar concentrations. Free chlorine disinfection was conducted in a modified continuous guench-flow system that was described previously for ozone reactions (Figure B-2).42 In brief, free chlorine and Phi6 solutions were continuously mixed in a PEEK micro static mixing tee (IDEX Health & Science) at flow rates of 0.125 mL min<sup>-1</sup> each to reach initial reaction conditions of 2 mg L<sup>-1</sup> free chlorine as Cl<sub>2</sub> and  $4-5 \times 10^{10}$  PFU mL<sup>-1</sup> Phi6. The reacting mixture then passed through sample loops with varied volumes to reach contact times of 0.3, 0.6, 2, 4, 8 and 11 s. The reactions were quenched with 550 mM Tris-HCl (pH 7.4) at a flow rate of 0.025 mL min<sup>-1</sup>. Control experiments demonstrated that the addition of Tris-HCl as a quenching agent for free chlorine effectively halted Phi6 inactivation (Figure B-3). Approximately 2.4 mL of the quenched samples were collected for nucleic acid, protein, and lipid analyses. After each experiment, the quench-flow system was thoroughly rinsed with chlorine-demand-free water. Free chlorine concentrations in reaction solutions were measured with the N,N-diethyl-p-phenylenediamine (DPD) ferrous titrimetric method and the DPD colorimetric method according to the standard method.<sup>43</sup> The percentage of free chlorine consumed through the experiments was kept below 20% in order to maintain pseudo-first order conditions. Negative controls for the free chlorine experiments were run in the continuous quenchflow system in the same manner as the free chlorine samples, but with PBS rather than free chlorine.

 $UV_{254}$  experiments. The UV<sub>254</sub> experimental solutions consisted of 2.4 mL of Phi6 (4-5 × 10<sup>10</sup> PFU mL<sup>-1</sup>) in PBS continuously stirred in 10 mL glass beakers. Samples were exposed to UV<sub>254</sub> in a collimated beam reactor<sup>44</sup> with 0.16 mW cm<sup>-2</sup> lamps (model G15T8, Philips) that were regularly measured with chemical actinometry.<sup>45</sup> The average UV<sub>254</sub> intensity was corrected based on the

solution absorbance at  $UV_{254}$  and the sample depth. After correcting for shielding, the  $UV_{254}$  intensity was 0.14 mW cm<sup>-2</sup>. Samples were exposed to  $UV_{254}$  for 0, 5, 15, and 25 min, which corresponded to  $UV_{254}$  doses of 0, 42, 130, 210 mJ cm<sup>-2</sup>. Negative controls for the  $UV_{254}$  experiments were prepared in the same manner as the experimental samples, but were stirred in dark to capture any background virus inactivation and biomolecule reactions.

Virus inactivation kinetics by free chlorine and  $UV_{254}$  were calculated based on the Chick-Watson model:<sup>46</sup>

$$\ln\left(\frac{C}{C_0}\right) = -kL$$

where *C* is the infectious titer (PFU mL<sup>-1</sup>),  $C_0$  is the initial infectious titer (PFU mL<sup>-1</sup>), *k* is the inactivation rate constant (L mg<sup>-1</sup> s<sup>-1</sup> or cm<sup>2</sup> mJ<sup>-1</sup>), and *D* is the free chlorine concentration (mg L<sup>-1</sup>) × contact time (s) or UV dose (mJ cm<sup>-2</sup>).

# 3.2.3 RT-qPCR assays.

Following the UV<sub>254</sub> and free chlorine reactions, the viral genomes were extracted by QIAamp viral RNA mini kits (Qiagen). The Phi6 dsRNA genome consists of three segments designated as small (S), medium (M), and large (L) based on their relative sizes. Three primer sets for the Phi6 genome were designed and tested individually, each targeting a different genome segment. The sum of the three amplicons (~1500 bp) covered approximately 10.5% of the Phi6 genome (Table B-1). The extracted viral genomes were mixed with 10 mM forward primer and 10 mM reverse primer at a ratio of 10:1.5:1.5 (v/v/v) in Tris-EDTA buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.7), and then heated at 99 °C for 5 min, and quickly chilled on ice for 5 min before mixing with RT-qPCR reagents. RT-qPCR reactions were prepared in 96-well plates (Eppendorf) and

conducted in duplicates on a Mastercycler ep RealPlex 2 system (Eppendorf) with Gotaq OneStep RT-qPCR kits (Promega). The 20- $\mu$ L RT-qPCR reactions consisted of 10  $\mu$ L 2× qPCR master mix, 0.4  $\mu$ L 50× RT mix, 5.2  $\mu$ L template-primer mixture, 4  $\mu$ L 5 M Betaine (Sigma-Aldrich), and 0.4  $\mu$ L nuclease-free water. The RT reaction was conducted at 40 °C for 15 min, followed by an initial PCR activation step at 95 °C for 10 min. The PCR reaction included 40 cycles of DNA denaturation at 95 °C for 15 s, primer annealing at 59 °C for 30 s, and extension at 72 °C for 40 s. Melting curves were conducted by increasing the temperature from 60 °C to 95 °C over 10 min. RNA standards used for the RT-qPCR calibration curves consisted of Phi6 genomes extracted from the purified stock and quantified with a Qubit Fluorometer 2.0 (ThermoFisher Scientific). The amplification efficiencies (mean ± standard deviation) of RT-qPCR reactions targeting S, M, and L genome segment were 0.82 ± 0.10, 0.81 ± 0.05, and 0.81 ± 0.07, respectively. The mean R<sup>2</sup> were ≥ 0.99. The reaction kinetics of RT-qPCR target regions were modeled with first-order reactions:

$$\ln\left(\frac{N_i}{N_{0,i}}\right) = -k_{g,i}D$$

The reactions of the whole genome were predicted by extrapolating RT-qPCR results from the  $\sim 1500$  bp covered by the three target regions:<sup>47</sup>

$$\log_{10}\left(\frac{N}{N_0}\right) = \left(\frac{\sum_{i=S,M,L} L_i}{\sum_{i=S,M,L} L_{amp,i}}\right) \sum_{i=S,M,L} \log_{10}\left(\frac{N_i}{N_{0,i}}\right)$$

where  $N_i$  is the concentration of the RT-qPCR target region *i* (copies mL<sup>-1</sup>, *i* = S, M, and L genome segment),  $N_{0,i}$  is the mean concentration of the RT-qPCR target region *i* in controls (copies mL<sup>-1</sup>),  $k_{g,i}$  is the reaction rate constant of the RT-qPCR target region *i* (L mg<sup>-1</sup> s<sup>-1</sup> or cm<sup>2</sup> mJ<sup>-1</sup>), *D* is the free chlorine concentration (mg L<sup>-1</sup>) × contact time (s) or UV dose (mJ cm<sup>-2</sup>),

 $\log_{10}\left(\frac{N}{N_0}\right)$  is the  $\log_{10}$  decay of the whole Phi6 genome,  $L_i$  is the size of the entire genome segment *i* (bp), and  $L_{amp.i}$  is the size of the RT-qPCR amplicon *i* (bp). In this extrapolation, we assumed a "single-hit inactivation model" and that the damage measured in the 10.5% of the genome by RT-qPCR was representative of damage in the whole genome.<sup>47</sup>

# 3.2.4 Peptide LC-MS/MS and quantification.

Following free chlorine or UV<sub>254</sub> treatment, virus samples were combined with equal amounts of <sup>15</sup>N-labeled Phi6 internal standards (see Appendix B for <sup>15</sup>N-metabolic labeled Phi6), and the mixture was digested with trypsin (Worthington) or chymotrypsin (Worthington) at 37 °C overnight (see Appendix B for protein digestion). The digests were then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Specifically, 20 µL aliquots of the virus protein digests were loaded on an Accucore aQ column (50 × 2.1 mm, 2.6 µm particle size, ThermoFisher Scientific) attached to an Accucore aQ Defender guard column (ThermoFisher Scientific) at a flow rate of 200  $\mu$ L min<sup>-1</sup>. The mobile phase was first maintained at 94% solution A (Milli-Q water with 0.1% formic acid) and 6% solution B (LC-MS grade methanol with 0.1% formic acid) for 3 min, and the ratio then increased linearly to 80% B over 30 min, at which point it was maintained at 80% B for 7 min, and then equilibrated at 6% B for 5 min. Full mass spectrometry (MS) scans and data-dependent tandem mass spectrometry (dd-MS<sup>2</sup>) scans were conducted with a Q Exactive Orbitrap high resolution mass spectrometer (ThermoFisher Scientific) in positive ion mode (Table B-2). Raw mass spectrometric results were searched against a customized Phi6 protein database with Mascot Distiller (2.6.2.0) on a local Mascot server. The following employed for peptide identification: search parameters were cysteine

carbamidomethylations (+ $C_2H_4ON$ ) as a fixed modification due to the reactions of iodoacetamide with intact cysteine thiol groups during the protein digestion. When searching for potential oxidation products, we set variable modifications based on oxidation products that were reported previously with LC-MS/MS systems,<sup>48</sup> namely methionine oxidations (+O, +2O), cysteine oxidations (+O, +2O, +3O), and chlorotyrosines (+Cl-H, +2Cl-2H). All searches were set with a 10 ppm mass tolerance for MS scans, and a 0.3 Da mass tolerance for MS<sup>2</sup> scans. False discovery rates of less than 1% and significant P-values of less than 0.05 were employed in each search. Peak areas of all detected peptides and their corresponding <sup>15</sup>N-labelled peptides were integrated in TraceFinder 3.2 (ThermoFisher Scientific), and the relative abundance of each peptide was calculated by the peak areas of the <sup>14</sup>N-peptide and <sup>15</sup>N-labelled peptide:<sup>49</sup>

$$P_j = \frac{PA_{14N-pep.,j}}{PA_{15N-\text{labeled } pep.,j}}$$

Where  $P_j$  is the relative abundance of peptide j;  $PA_{14N-pep,,j}$  is the peak area of the <sup>14</sup>N-peptide j;  $PA_{15N-labeled pep,,j}$  is the peak area of the corresponding <sup>15</sup>N-labelled peptide j.

Calibration curves of peptides were analyzed to determine limits of quantification (LOQ) and limits of detection (LOD) (see Appendix B for determination of peptide LOQ and LOD). If the relative abundance of a peptide replicate value was below its LOQ but above its LOD, this value was replaced by an expected number between the LOQ and LOD based on the assumption of normal distribution.<sup>50</sup> Peptide reactions were modeled with first-order reaction kinetics:

$$\ln\left(\frac{P_j}{P_{0,j}}\right) = -k_{p,j}D$$

where  $P_j$  is the relative abundance of peptide j,  $P_{0,j}$  is the mean relative abundance of peptide j in control samples,  $k_{p,j}$  is the reaction rate constant of peptide j (L mg<sup>-1</sup> s<sup>-1</sup> or cm<sup>2</sup> mJ<sup>-1</sup>), and D is the free chlorine concentration (mg L<sup>-1</sup>) × contact time (s) or UV dose (mJ cm<sup>-2</sup>).

# 3.2.5 Lipid LC-MS/MS and quantification

Phi6 lipids were extracted following a methyl-tert-butyl ether (MTBE) protocol (see Appendix B, lipid extraction).<sup>51</sup> Lipid extracts (20  $\mu$ L) were injected on an Accucore aQ column (50 × 2.1 mm, 2.6  $\mu$ m particle size) attached to an Accucore aQ Defender guard column at a flow rate of 200  $\mu$ L min<sup>-1</sup>. The column temperature was maintained at 55 °C. Mobile phases C (60% LC-MS grade acetonitrile and 40% Milli-Q water with 0.1% formic acid) and D (90% LC-MS grade isopropanol and 10% LC-MS grade acetonitrile with 0.1% formic acid) were used for lipid separations. The gradient started at 25% D for 3 min, then linearly increased to 70% D over 15 min, and then to 97% D over 3 min. It was held at 97% D for 4 min, and then decreased to 25% D over 5 min. Full MS and dd-MS<sup>2</sup> scans were operated in negative ion mode (Table B-2). Lipids were identified with LipidXplorer software<sup>52</sup> based on a Phi6 lipid database reported previously,<sup>39</sup> and peak areas were measured with TraceFinder 3.2 (ThermoFisher Scientific). Eight of the most abundant phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) compounds in the Phi6 lipid database were quantified (Figure B-4). Their relative abundances (L/L<sub>0</sub>) in the samples were calculated using calibration curves developed from Phi6 lipid extracts (Figure B-4).

#### 3.2.6 Statistical analyses

Rate constants were calculated by pooling all experimental data together and modeling the combined data with linear regressions. Analysis of covariance (ANCOVA) was applied to test whether rate constants were significantly different from zero, and whether two rate constants were significantly different from zero, and whether two rate constants were significantly different from zero, and whether two rate constants were significantly different from zero, and whether two rate constants were

constants were equal to zero or that the two rate constants were identical. Student's unpaired ttests were used to assess if mean virus inactivation levels ( $C/C_0$ ) at two chlorine contact times were significantly different. The null hypotheses were that the mean  $C/C_0$  values were the same. A null hypothesis was rejected if the P-value was less than 0.05. Statistical analyses were conducted in GraphPad Prism 7 software.

#### 3.3 Results and discussion

# 3.3.1 Phi6 inactivation by free chlorine and UV<sub>254</sub>

*Free chlorine inactivation.* Phi6 was rapidly inactivated by free chlorine (Figure 3.1), and a continuous quench-flow system was necessary to characterize disinfection while maintaining pseudo-first order conditions. The inactivation displayed significant tailing, which in other viruses has been ascribed to virus aggregation, <sup>53</sup> adsorption to particles, <sup>53,54</sup> and accumulation of oxidation products on the surface of viral particles. <sup>55</sup> In order to minimize the presence of aggregated virus particles, virus stocks were always filtered through 0.22 µm membranes (Phi6 virion is ~85 nm in diameter). Interestingly, the inactivation plateau occurred at lower doses when Phi6 stocks were stored at 4 °C after purification, suggesting that the enveloped viruses aggregated during storage (Figure B-5). Consistent inactivation curves were only possible when Phi6 stocks were purified, filtered through 0.22 µm membranes, and then stored at -80 °C until use. Even then, inactivation nearly levelled off after 4-log<sub>10</sub> inactivation by free chlorine (Figure 3.1). We modelled the first 2-log<sub>10</sub> inactivation, and obtained an inactivation rate constant equal to 4.6 ± 0.5 (mean ± standard error) L mg<sup>-1</sup>s<sup>-1</sup>. For comparison, this rate constant is approximately 30× larger than that of ssRNA MS2 under the same reaction conditions (0.17 L mg<sup>-1</sup> s<sup>-1</sup>).<sup>56</sup>



**Figure 3.1** Inactivation of Phi6 by 2 mg L<sup>-1</sup> free chlorine (FC) and UV<sub>254</sub> (UV). Data includes  $n \ge 5$  replicates for each chlorine contact time and n = 3 replicates for each UV<sub>254</sub> dose. Student's unpaired t-tests were used to determine statistical differences of Phi6 infectivity (C/C<sub>0</sub>) by free chlorine at two contact times. \*\* indicates P < 0.01, and thus that Phi6 infectivity was significantly different at the two time points; ns indicates Phi6 infectivity was not significantly different at the two time points; ns indicates Phi6 infectivity was not significantly different at the two time points (P > 0.05).

 $UV_{254}$  inactivation. Inactivation of Phi6 by  $UV_{254}$  followed first-order reactions over the entire measured 6-log<sub>10</sub> inactivation, with an inactivation rate constant of 0.067 ± 0.005 cm<sup>2</sup> mJ<sup>-1</sup>. Compared to other enveloped viruses reported in the literature, Phi6 is quite resistant to  $UV_{254}$ . For example, it is approximately 15 to 30× more resistant to  $UV_{254}$  than influenza A virus (~1 cm<sup>2</sup> mJ<sup>-1</sup>)<sup>11,57</sup> and vesicular stomatitis virus (VSV) (~2.3 cm<sup>2</sup> mJ<sup>-1</sup>).<sup>58</sup> When compared to nonenveloped viruses, which are better characterized in the literature, Phi6  $UV_{254}$  inactivation kinetics were similar to MS2 (~0.06 cm<sup>2</sup> mJ<sup>-1</sup>)<sup>30,56,59</sup> and adenovirus (~0.046 cm<sup>2</sup> mJ<sup>-1</sup>).<sup>12</sup>

## 3.3.2 Reactions in Phi6 genome

*Free chlorine reactions.* When Phi6 was treated with free chlorine up to  $6-\log_{10}$  inactivation, the reaction rate constants of the three ~500 bp regions in the genome were significantly different

from zero as detected by RT-qPCR (Figure 3.2A; Table B-3). The reaction rate constants of the three RT-qPCR regions did not differ significantly from one another following free chlorine treatment (P > 0.05 for all the three ANCOVA comparisons). We extrapolated the damage measured in the RT-qPCR regions to the entire genome in order to compare genome damage with inactivation.<sup>47</sup> We note that by measuring ~1500 bp with our RT-qPCR analysis (i.e., 10.5% of the entire Phi6 genome), we aimed to minimize the impact that specific bases and base sequences have on the reactivity of small RNA regions.<sup>47</sup> Based on the RT-qPCR extrapolation results, the fraction of viruses with damaged genomes was less than the fraction that was inactivated (Figure 3.2B). These results suggest that in Phi6, genome damage may not drive Phi6 inactivation by free chlorine. For comparison, genome damage did drive free chlorine inactivation in bacteriophage MS2.<sup>56</sup>

To directly compare the nucleic acid reactivity of two viruses with different genome sizes and types, we normalized the genome reaction rate constants of MS2 ( $0.066 \text{ Lmg}^{-1} \text{ s}^{-1}$ )<sup>56</sup> and Phi6 ( $0.26 \text{ Lmg}^{-1} \text{ s}^{-1}$ ) to the total number of bases in their genomes. This approach assumes that the genomes have the same proportion of reactive bases. In fact, MS2 and Phi6 do have similar proportions of bases that are reactive to free chlorine and UV<sub>254</sub> (Table B-4). Interestingly, the normalized MS2 ssRNA genome reaction rate constant with free chlorine ( $1.8 \times 10^{-5} \text{ Lmg}^{-1} \text{ s}^{-1}$ ) is similar to the normalized value measured here for the Phi6 dsRNA genome ( $9.4 \times 10^{-6} \text{ Lmg}^{-1} \text{ s}^{-1}$ ).

 $UV_{254}$  reactions. Statistically significant decreases in the concentrations of RT-qPCR target regions were detected following 6-log<sub>10</sub> inactivation by UV<sub>254</sub> (Figure 3.2A; Table B-3), and the reaction rate constants of the three regions were not significantly different from one another (P > 0.05 for each ANCOVA comparison). When the RT-qPCR results were extrapolated to the entire genome, the approximated reaction rate constant of the Phi6 genome (0.063 ± 0.012 cm<sup>2</sup> mJ<sup>-1</sup>) was not significantly different from the rate constant of Phi6 inactivation  $(0.067 \pm 0.005 \text{ cm}^2 \text{ mJ}^{-1})$  (P > 0.05). This suggests that genome reactions drive UV<sub>254</sub> inactivation of the enveloped virus Phi6. Although this type of analysis has not been previously reported for enveloped viruses, our finding is consistent with previous research on nonenveloped viruses.<sup>12,30,56,60</sup> A comparison with the per base reaction rate constants measured here with those reported previously suggests that the dsRNA genome of Phi6 (2.4× 10<sup>-6</sup> cm<sup>2</sup> mJ<sup>-1</sup> base<sup>-1</sup>) is more resistant to UV<sub>254</sub> than the dsDNA genome of adenovirus (11 × 10<sup>-6</sup> cm<sup>2</sup> mJ<sup>-1</sup> base<sup>-1</sup>)<sup>61</sup> and the ssRNA genome of MS2 (24 × 10<sup>-6</sup> cm<sup>2</sup> mJ<sup>-1</sup> base<sup>-1</sup>).<sup>56</sup> It is worth noting that in the case of adenovirus, the modified bases detected by PCR can be repaired by the host cell;<sup>12</sup> a similar repair mechanism has not been reported for the RNA viruses.



**Figure 3.2** Phi6 genome reactions when the viruses were reacted with 2 mg L<sup>-1</sup> free chlorine (FC) and UV<sub>254</sub> (UV). A: Reactions in three ~500 bp regions ( $N_i/N_{0,i}$ ) as measured by RT-qPCR with respect to chlorine contact time and UV<sub>254</sub> doses. Data includes  $n \ge 2$  replicates for each chlorine contact time and n = 3 replicates for each UV<sub>254</sub> dose; B: Reactions in the entire Phi6 genome
$(N/N_0)$ . This data was extrapolated from the three RT-qPCR regions presented in A, and is presented with respect to virus infectivity  $(C/C_0)$  as measured by plaque assays.

We note that using RT-qPCR to estimate genome damage misses a fraction of the RNA modifications that can be detected by mass spectrometry.<sup>44</sup> Work on the photolysis of MS2 by UV<sub>254</sub>, however, demonstrated that a single-hit inactivation model was appropriate for damage detected with RT-qPCR; in other words, every modification in MS2 RNA that causes virus inactivation can be detected by RT-qPCR.<sup>47</sup> Our ongoing work aims to better characterize the chemistry and biological impact of RNA and DNA modifications detected by reverse transcriptases, polymerases, and mass spectrometry.

#### 3.3.3 Reactions in Phi6 proteins

The Phi6 virion contains 11 distinct proteins that are assembled into three layers, including viral membrane proteins (P3, P6, P9, P10, P13), nucleocapsid proteins (P5, P8), and polymerase complex proteins (P1, P2, P4, P7) (Figure B-6).<sup>38</sup> The functions of these proteins in the Phi6 life cycle have been reviewed in previous literature and are briefly described in the SI (Figure B-6).<sup>38</sup> Our LC-MS/MS method was capable of detecting a total of 184 pairs of <sup>14</sup>N- and <sup>15</sup>N-labelled Phi6 peptides. Protein coverage was over 60% for all proteins except for P6 and P2 (Figure B-7). The repeated poor coverage of P6 and P2 was likely due to the low number of P6 and P2 protein copies in the viral particles (Figure B-7).

*Free chlorine reactions*. We tracked Phi6 protein degradation over the first  $2-\log_{10}$  Phi6 inactivation in order to model the peptide reactions with first-order kinetics. Free chlorine reacted with all Phi6 peptides with reaction rate constants ranging from 0.41 to 6.3 L mg<sup>-1</sup> s<sup>-1</sup> (Figure 3.3;

Table B-6). As expected, the most reactive peptides contained Met or Cys residues (Table B-6). Despite the similar reactivity of Met and Cys in the free amino acid form (Table B-5), the most reactive Cys-containing peptide C257-F267 in Phi6 P4 ( $2.8 \pm 0.3 \text{ Lmg}^{-1} \text{ s}^{-1}$ ) reacted slower than the most reactive Met-containing peptide D448-R463 ( $6.3 \pm 0.9 \text{ L mg}^{-1} \text{ s}^{-1}$ ) in Phi6 P1 (Table B-6). Furthermore, the rate constants of peptides containing Met varied. For example, in the Phi6 P1, the rate constant of M198-K208 ( $1.1 \pm 0.1 \text{ Lmg}^{-1} \text{ s}^{-1}$ ) was approximately 3 × smaller than that of peptide L53-Y66 ( $3.1 \pm 0.5$  L mg<sup>-1</sup> s<sup>-1</sup>) and 4 × smaller than that of peptide M209-K215 ( $4.5 \pm 0.7$ L mg<sup>-1</sup> s<sup>-1</sup>), despite the three peptides having spatially adjacent Met residues (Figure B-8). The variation in reactivity of peptides containing Met and Cys is likely related to the accessibility of free chlorine to the amino acids.<sup>30</sup> Indeed, the Cryo-EM model (PDB ID: 5muu) of Phi6 suggests that in the P1 complex, the dimethyl sulfide of M198 in M198-K208 is protected by surrounding amino acid residues, whereas the M65 in L53-Y66 and M209 in M209-K215 have higher solventaccessible surface areas (SASA, Figure B-8). It is also worth noting that oxidized Met residues were the only products in Phi6 proteins detected following 2-log<sub>10</sub> inactivation by free chlorine (Figure B-9).



**Figure 3.3** Heatplot of Phi6 protein peptide abundances following Phi6 exposure to free chlorine (FC) and  $UV_{254}$  (UV). Each row in the heatplot represents one peptide. Peptides were arranged based on their sequential order in proteins, and the undetected peptides are shown in grey. Peptide concentrations (P/P<sub>0</sub>) in this heatplot were averaged from 3 independent experiments. Detailed information of peptide sequences, reaction rate constants and standard errors are provided in Table B-6.

We initially hypothesized that the increased susceptibility of Phi6 to free chlorine inactivation compared to nonenveloped viruses, such as MS2, was due to reactions in the membrane proteins. These proteins play critical roles in the early steps of virus infection, and are located on the outermost layer of the viral particle (Figure B-6). In fact, our results showed that in Phi6, some membrane proteins (e.g., P6, P9, P10, P13) reacted slower than the nucleocapsid proteins (e.g., P8) and polymerase complex proteins (e.g., P1, P2, P4) (Figure 3.3; Table B-6). This suggests that free chlorine molecules readily penetrate the lipid membrane and react with proteins in the nucleocapsid and polymerase complex. Similar findings were reported in bacteria, where the non-dissociated HOCl molecules could penetrate the negatively-charged bacterial membrane to react with intracellular structures.<sup>62</sup>

The 8 most reactive peptides found in Phi6 proteins P3, P8, P1, P2, and P4 had rate constants that were comparable to the Phi6 inactivation rate constant (Figure 3.4; Table B-6), forming Met oxidations as the main products (Figure B-9). Consequently, one or several of these protein reactions may drive Phi6 inactivation by free chlorine. Given the protein reactivity and the Phi6 life cycle, Phi6 inactivation may be due to the direct interruption of the ability to bind host cell (P3) or to penetrate plasma membrane (P8).<sup>63,64</sup> Alternatively, damage to proteins P2, P4, and P7 may indirectly inactivate Phi6 by causing changes in the vial structure.<sup>48,64,65</sup>

Peptides in Phi6 proteins were more reactive with free chlorine than peptides in the nonenveloped MS2 proteins. The two most reactive peptides D448-R463 ( $6.3 \pm 0.9 \text{ Lmg}^{-1} \text{ s}^{-1}$ ) and I678-R697 ( $5.9 \pm 1.0 \text{ Lmg}^{-1} \text{ s}^{-1}$ ) in Phi6 were approximately 150× more reactive than the fastest reacting peptide S373-R388 in the MS2 A protein ( $0.033 \text{ Lmg}^{-1} \text{ s}^{-1}$ ).<sup>56</sup> The marked discrepancies in reactivity of Phi6 and MS2 peptides may be due to the relative solvent accessibilities of their reactive amino acids.<sup>30</sup> The average SASAs of the M456 and M680 residue in the Phi6 peptide are

95 Å<sup>2</sup> and 77 Å<sup>2</sup>, respectively, as calculated by YASARA software.<sup>66</sup> Unfortunately, the SASA cannot be estimated for the Met in the most reactive MS2 peptide due to the fact that the crystal structure of the MS2 A protein has not been resolved.



**Figure 3.4** Decay of the 8 most reactive Phi6 peptides ( $P/P_0$ ) by free chlorine with respect to virus infectivity ( $C/C_0$ ). Data below the LC-MS/MS limit of quantification is shown in grey.

 $UV_{254}$  reactions. All Phi6 peptides reacted following UV<sub>254</sub> exposure, but much less than they reacted with free chlorine at the same levels of inactivation. Peptide concentrations decreased by less than 50% following 4.2-log<sub>10</sub> Phi6 inactivation (Figure 3.3), with rate constants ranging from 0.0009 to 0.0048 cm<sup>2</sup> mJ<sup>-1</sup> (Table B-6). Similar reaction rate constants were reported for peptides in bacteriophage MS2, fr, and GA proteins with UV<sub>254</sub>.<sup>30,56</sup> Certain peptides in membrane protein P3 and RNA polymerase P2 reacted with faster kinetics, likely due to the presence of one or more UV-reactive amino acids in their sequences, including Trp (W), Tyr (Y), or Phe (F) (Table B-6).<sup>67</sup> Indirect photoreactions with nucleic acids may also play a role in the enhanced photoreactivity of certain viral peptides as reported in MS2.<sup>68</sup>

#### 3.3.4 Reactions in Phi6 lipids

Phi6 lipid membranes consist of glycerophospholipids, including phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL) compounds at a molar ratio of 58:38:4.<sup>39</sup> Here, we measured the relative abundances of the eight most prevalent PE and PG compounds in Phi6 membranes.

*Free chlorine reactions*. The PE and PG relative concentrations did not decrease significantly over 9.1-log<sub>10</sub> Phi6 inactivation by free chlorine (Figure B-10; P > 0.05 for all tests). A number of products were detected in the LC-MS spectra, including monochloramine products of PE (16:0/16:1) and PE (18:1/16:1) (Figure 3.5). The peak intensities of PE monochloramine products increased with increasing chlorine contact time, although at the highest inactivation level (i.e., 9.1-log<sub>10</sub> Phi6 inactivation), the product peak intensities were still three orders of magnitude smaller than the parent PE peaks (Figure 3.5). The low PE monochloramine product concentration within the Phi6 inactivation timeframe was not surprising given that the reported rate constant for this reaction  $(1.8 \times 10^4 \text{ M}^{-1} \text{s}^{-1})$  is three orders of magnitude lower than the rate constants for free chlorine reacting with Met and Cys residues.<sup>69</sup> Other lipid products were detected following free chlorine treatment, with peak intensities no greater than 1% of the parent compound peak intensities (Figure 3.5). The chemical compositions and structures of these products did not correspond to commonly reported lipid oxidation products, such as lipid hydroperoxides and chlorohydrins.<sup>70,71</sup>

 $UV_{254}$  reactions. Statistically significant reductions in the relative concentrations of the eight major lipid compounds were not detected following  $UV_{254}$  doses resulting in 8.5-log<sub>10</sub> Phi6 inactivation (Figure B-10; P > 0.05 for all tests). Likewise, no major products were detected in the LC-MS spectra of samples following  $UV_{254}$  treatment (Figure 3.5). Previous research on  $UV_{254}$ 

reactions with the membrane of VSV suggest that the lipid envelope do not protect VSV from inactivation.<sup>72</sup>



**Figure 3.5** Phi6 lipids data collected by LC-MS before and after free chlorine (FC) and  $UV_{254}$  (UV) treatments. Arrows identify specific lipid products [M-H]<sup>-</sup> following free chlorine treatment, including the following accurate masses (1) 775.513; (2) 779.464; (3) 710.477; (4) 684.461; (5) 846.588; (6) 820.572; (7) 804.541; (8) 778.525; (9) 748.469 (monochloramine of PE(16:0/16:1)); (10) 722.454 (monochloramine of PE(18:1/16:1)).

The lipid compositions of eukaryote enveloped viruses are more diverse than those of bacteriophage Phi6. Influenza virus membranes, for example, contain not only PE and PG, but also cholesterol and phosphatidylserine (PS).<sup>40</sup> The kinetics of these lipids with HOCl, however, are not markedly faster than the lipids in the Phi6 membrane.<sup>73,74</sup> In summary, based on our Phi6 lipid results and what has been reported for other lipids found in virus membranes, we anticipate that reactions in membrane lipids do not drive enveloped virus inactivation by free chlorine or  $UV_{254}$ .

#### 3.3.5 Environmental implications

Enveloped viruses are often assumed to be more susceptible than nonenveloped viruses to inactivation in the environment, but mechanistic descriptions of their differing inactivation mechanisms are lacking in the literature. Our preliminary work with enveloped virus Phi6 sheds light on how a model enveloped virus reacts with chemical oxidants and UV radiation. We found that Phi6 was  $30\times$  more susceptible to free chlorine inactivation than the commonly studied nonenveloped virus MS2. Our work suggests that unlike MS2, the overall Phi6 particle reactivity with free chlorine is driven more by protein reactions than by genome and lipid reactions. Free chlorine reactive MS2 peptides, specifically for Phi6 peptides that contain solvent-accessible Met and Cys residues. Consequently, the relatively high number of solvent-accessible Met and Cys residues in the Phi6 proteins may be responsible for its fast inactivation kinetics with free chlorine. In contrast to chlorine,  $UV_{254}$  inactivates Phi6 primarily by reacting with the genome. This is consistent with previous research on nonenveloped viruses. It is therefore unlikely that

enveloped viruses are more susceptible than nonenveloped viruses to direct photolysis via sunlight or UV disinfection processes.

Looking beyond this initial comparison of enveloped Phi6 with nonenveloped MS2, this work raises a number of hypotheses about virus reactivity and inactivation that can be tested with additional viruses. For example, future work should explore whether proteins in enveloped viruses are generally more susceptible to oxidants than proteins in nonenveloped viruses, and if this is the reason that enveloped viruses tend to be more susceptible to inactivation by chemical oxidants. Related to this, research should explore the link between the presence of solvent-accessible reactive amino acids in viral proteins with virus inactivation by oxidants. UVC inactivation should be tested with additional enveloped viruses that contain various genome sizes and genome types. Possible enveloped viruses to study in the future include vesicular stomatitis virus and avian influenza viruses. These are animal viruses with particle diameters and genome types that differ from Phi6 and can be propagated to stocks with high titers. This last point is important for cases in which researchers wish to apply the LC-MS/MS methods described in this study. Finally, enveloped viruses in open air may undergo reactions and inactivation mechanisms similar to viruses in water. Future studies should aim to compare aqueous virus reactivity with aerosolized virus reactivity.

Enveloped viruses are structurally diverse, ranging in size, envelope composition, genome type, and shape. These variations are likely reflected in a range of reactivities and inactivation mechanisms. That being said, the structure and composition of the Phi6 are not especially unique amongst the enveloped viruses. We are therefore confident that this study on Phi6 reactivity with free chlorine and  $UV_{254}$  will be a valuable benchmark for future studies on enveloped virus fate in disinfection processes.

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# Chapter 4 Development of an integrated cell culture-mass spectrometry method for monitoring infectious viruses in environmental samples

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#### 4.1 Introduction

Human pathogenic viruses in water are responsible for a number of waterborne human diseases. Compared to other waterborne pathogens (e.g., bacteria, protozoa), virus detection in water is especially challenging due to their small dimensions and low abundances. Sensitive methods have been developed to detect viral nucleic acid sequences, including polymerase chain reaction (PCR) and genome sequencing. These methods alone, however, are not able to differentiate infectious and noninfectious viruses. Culture-based methods, including plaque assays, detect only infective viruses. In culture-based methods, virus replication in their host cells can result in the formation of cytopathic effects (CPEs). These CPEs are often the endpoints used for detection and quantification. However, the formation of clear CPEs requires time, sometimes up to weeks. Furthermore, environmental samples can cause CPEs in the absence of viruses when materials in the samples are toxic to the cells.<sup>1,2</sup> Yet another issue with culture-based methods is that several different viruses can often infect the same cultured cell system. It is therefore often impossible to identify the viruses responsible for the cytopathic effects.

To address the issue of molecular methods detecting noninfective viruses, and the issue of culture-based methods not identifying specific viruses, methods that integrate cell cultures with polymerase chain reaction (integrated cell culture-PCR; ICC-PCR) were developed. This approach has been used to detect many different viruses in environmental samples,<sup>3-6</sup> including enteroviruses in wastewater samples. As few as one infectious virus can be detected by ICC-PCR earlier than the formation of clear CPEs.<sup>6</sup> The other advantage of ICC-PCR over culture-based method alone is that multiple viruses can be detected with a number of primer sets. Hwa Kyung Lett *et al.*<sup>7</sup> used virus specific primers to monitor adenoviruses, enteroviruses, and reoviruses in water samples with ICC-PCR methods.

Although ICC-PCR methods addressed some of the issues of culture methods and PCR methods alone, it still requires primer design and PCR assay optimization. In most ICC-PCR applications, two rounds of PCR amplifications are optimized to improve assay sensitivity and confirm positive results.<sup>8</sup> If virus strain-level identification is necessary, strain-specific primer sets are required, and it may need further characterization by sequencing of PCR products.<sup>9</sup> Another challenge associated with PCR methods is that viruses rapidly evolve, and this can occasionally cause pre-designed PCR primers to fail due to the newly evolved sequences.<sup>10</sup>

Recently-developed mass spectrometry (MS) techniques may be capable of virus detection while alleviating some of the limitations of other techniques.<sup>11</sup> MS instruments can scan peptide ions present in a sample and fragment peptide ions, ultimately making de novo peptide sequencing possible.<sup>12,13</sup> Genetic information carried on proteins can then be used for microorganism identification. For instance, in a shotgun proteomics method for bacterial identification, bacterial proteins were digested into peptides, and the peptide sequences were analyzed on a liquid chromatography tandem mass spectrometry system. The detected peptides were then compared

with sequences in protein database to identify bacteria.<sup>14,15</sup> The most obvious advantage of MS methods over PCR methods is the lack of necessary primer design and method optimization. Moreover, MS is able to detect single amino acid mutations based on the peptide fragments.<sup>16</sup> Methods that integrate cell culture and mass spectrometry (ICC-MS) have been applied for detecting viruses in clinical samples.<sup>17-19</sup> Here, the detected peptide sequences could correctly distinguish viruses at the strain-level.<sup>19</sup> To date, however, ICC-MS methods have not been applied for detecting viruses in environmental samples. Environmental matrices are often more complex than clinical samples, and virus concentrations are typically much lower.

Here, we report on an ICC-MS method for detecting infectious human viruses in environmental water samples. Sample preparation methods and MS detection protocols were developed and optimized with a model virus culture system (i.e., murine hepatitis virus and its host L2 cells). The effects of virus concentrations and the potential toxicity of wastewater samples on the culture system were evaluated. Two monkey kidney cell cultures (i.e., Vero and BSC-1 cells) were then employed to demonstrate the ICC-MS method could detect infectious viruses in samples collected throughout a full-scale wastewater treatment plant. Our results suggest that the ICC-MS method is able to detect multiple infectious viruses in wastewater, and identifies viruses at the strain-level rapidly. The ICC-MS method can be easily adopted for detecting other viruses in water samples.

#### 4.2 Materials and methods

#### 4.2.1 Wastewater samples and concentration

Municipal wastewater primary influent, effluent pre-UV disinfection, and final effluent samples were collected from autosamplers at the Ann Arbor Wastewater Treatment Plant between June and September in 2018. Samples were collected in sterile containers and transported on ice to the laboratory at the University of Michigan, Ann Arbor. Wastewater samples were concentrated with ultrafiltration methods, which have achieved higher recoveries of both enveloped and nonenveloped viruses than other virus concentration methods.<sup>20, 21</sup> In brief, wastewater samples were concentrated 50 × in volume with an REXEED 25S dialysis filter (Asahi Kasei Medical) or a Pellicon XL 30 kDa ultrafilter (Millipore). The concentrates were filter sterilized with 0.22  $\mu$ m poly(ether sulfone) (PES) membranes (Millipore) to remove bacteria contamination that can be potentially introduced to cell culture. The final concentrated wastewater samples were aliquoted and stored at -80 °C before use.

### 4.2.2 Viruses and cell lines

Murine hepatitis virus (MHV) strain A59 and its host L2 cell lines (Table C-1) were used as a model system for detection by an integrated cell culture/mass spectrometry (ICC-MS) method. L2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies 11960) supplemented with 10% newborn bovine serum (Life Technologies), 2 mM of L-glutamine (Life Technologies), 1% (v/v) of penicillin/streptomycin (Life Technologies) at 37 °C, 5% CO<sub>2</sub>. MHV was propagated in DBT cell lines according to a previously published method. Briefly, DBT cells were grown to 80% confluency, and infected by MHV at a multiplicity of infection (MOI) of 0.1 plaque forming units (PFU) per DBT cell. Infected cells were incubated in DMEM (Life Technologies 11960) with 2% newborn bovine serum at 37 °C and 5% CO<sub>2</sub> for 48 h. After incubation, cells were frozen and then thawed. Cell debris was removed by centrifuging the sample at 3,000 × g for 5 min. The supernatant was collected and filtered through a 0.22  $\mu$ m PES membrane. The final MHV stocks (~10<sup>6</sup> PFU mL<sup>-1</sup>) were aliquoted and stored at -80 °C.

Monkey kidney cells include Vero cells and BSC-1 cells (Table C-1) were used as generic cell lines for culturing human viruses in wastewater concentrates. Vero and BSC-1 cells were grown in DMEM (Life Technologies 12430) with 10% newborn bovine serum, 1% (v/v) penicillin/streptomycin at 37 °C and 5% CO<sub>2</sub>.

#### 4.2.3 Virus infection and culturing

Initial proof-of-concept experiments were conducted in the MHV-L2 culture systems. For these, MHV stock was added to DMEM with 2% newborn bovine serum to reach final concentrations of 300, 30, and 3 PFU mL<sup>-1</sup>. At the time of infection, cells were first washed with ice-cold PBS (Life Technologies 10010). 1.2 mL of each concentration was then inoculated into cells grown to ~80-90% confluency in culture plates (~23 cm<sup>2</sup>). Consequently, the total inoculated MHV was 360 PFU, 36 PFU, and 3.6 PFU per 23 cm<sup>2</sup>, and the ratio of the infectious MHV particles to cells was approximately 0.001, 0.0001 and 0.00001, respectively. The inoculated samples were incubated with cells for 1 h at 37 °C, with manually rocking every 15 min. The inoculum was then removed, and fresh DMEM containing 2% newborn bovine serum was added to sustain minimum cell growth. After culturing, protein samples were extracted (see Protein extraction) between 12 to 42 hours post infection at intervals of 6 hours.

To test the impact of wastewater components on the performance of the ICC-MS method, MHV stock was spiked into wastewater concentrates to reach the same final concentrations of 300, 30, and 3 PFU mL<sup>-1</sup>. The control experiments in the MHV-L2 culture systems were then conducted in the same procedures of cell infection. Proteins were extracted at 18 and 24 hours post infection from the cells inoculated with 360 PFU, 30 and 36 hours post infection from the cells inoculated with 3.6 PFU.

For experiments to detect viruses in wastewater samples, wastewater concentrates without further purification were directly added to cell monolayers of monkey cell culture systems, using the same procedures of virus inoculation. Cytopathic effects (CPE) were observed daily. Vero and BSC-1 cells were replenished with fresh DMEM containing 2% newborn bovine serum at the 7 days post infection, and incubated for a total of 14 days. Negative controls were inoculated with virus-free PBS rather than wastewater extract.

#### 4.2.4 Protein extraction

Proteins were collected from the cell monolayer at various times post inoculation with the MHV viruses or the wastewater extracts. Briefly, liquid culture media in the culture systems was removed, and cells were washed with ice-cold PBS. 100  $\mu$ L of Triton X-114 buffer (20 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-114, pH 8) was added to lyse cells on ice. Cell lysates vortexed and centrifuged at 3000 × g for 5 min at 4 °C to pellet nuclei. The supernatant was collected for phase separation to harvest hydrophilic proteins in the aqueous phase and amphiphilic proteins (i.e., integral proteins) in the detergent phase according to a previously published method.<sup>22</sup> The hydrophilic portion of the proteins was precipitated with 25% (v/v) trichloroacetic acid by centrifuging at 14 000 × g for 10 min at 4 °C and then washed twice with cold (-20 °C) acetone. Protein pellets were saved at -80 °C until they were protease digested.

#### 4.2.5 Protein digestion and LC-MS/MS analysis

Protein pellets were dissolved in a reducing buffer (8 M urea, 100 mM dithiothreitol, 50 mM Tris-HCl, pH 8), and denatured by boiling for 3 min. The undissolved fraction was removed by

centrifuging at 14,  $000 \times g$  for 5 min. The supernatant was transferred to a Microcon 10 kDa centrifugal filter unit (Millipore), and proteins were protease treated based on a filter-aided sample preparation protocol.<sup>23</sup> After overnight digestion with trypsin (Worthington), peptides were analyzed on a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. Specifically, peptides were separated in a Dionex UltiMate 3000 LC system (ThermoFisher Scientific) equipped with an Accucore aQ column (50 nm × 2.1 mm, 2.6 µm particle size, ThermoFisher Scientific) protected by an Accucore aQ Defender guard column (ThermoFisher Scientific). 20 µL samples were loaded onto the system at a flow rate of 200 µL min<sup>-1</sup>. The solvent gradient began at 94% solution A (Milli-Q water with 0.1% formic acid) and 6% solution B (LC-MS grade acetonitrile with 0.1% formic acid) for 3 min. Solution B was then linearly increased to 40% over 30 min, followed by a linear increase to 80% over 2 min, and then maintained at 80% B for 5 min. B was then decreased to 6% and maintained there for 5 min to equilibrate the column. Eluted peptides were analyzed with a Q Exactive Orbitrap high-resolution mass spectrometer (ThermoFisher Scientific) in positive ion mode. Settings for the full mass spectrometry (MS) scans and data-dependent tandem mass spectrometry  $(dd-MS^2)$  scans are provided in Table C-2.

#### 4.2.6 MS data analysis

Raw MS data was analyzed with MASCOT Distiller software (2.6.1.0) connected to a local server. Protein database of *Homo sapiens* (174238 sequences, released on September 14<sup>th</sup>, 2018) and *Mus musculus* (83937 sequences, released on September 14<sup>th</sup>, 2018) in the FASTA format were downloaded from the UniProtKB. The viral protein database was downloaded from the UniProtKB taxonomic divisions as uniport\_sprot\_viruses.dat (28453 sequences, modified on September 12<sup>th</sup>, 2018), and converted to FASTA format with InSilicoSpectro::Databanks modules.

All databases were uploaded to the MASCOT local server. MS results related to the L2 culture system were searched against the *Mus musculus* database and the SwissProt virus database. MS results related to Vero and BSC-1 culture systems were searched against the *Homo sapiens* database and the SwissProt virus database. Carbamidomethylation of cysteine residues was selected as a fixed modification. N-terminal acetylation and methionine oxidation were set as variable modifications. The peptide mass tolerance was set at less than 10 ppm and the fragment mass tolerance was set at less than 0.3 Da. The false discovery rate (FDR) was calculated with a decoy database, and an FDR of less than 0.01 was set for all searches. The significance of peptide sequence (p-values) was defined as less than 0.01, and the peptide expectation (E) value was set at less than 0.001. Protein identification was considered positive when at least two distinct peptides from that protein were detected.

The sequence coverage of a target protein was calculated based on the detected peptide sequences that were assigned to the protein and the length of the protein reported in database: Protein sequence coverage (× 100%) =  $\frac{\# of amino acids detected in peptides}{Total \# of amino acids in the full length of protein sequence}$ .

#### 4.3 Results and discussion

#### 4.3.1 Identification of MHV by LC-MS/MS in culture media

Proteins associated with host cells were the most identified in all samples (Data not shown). MHV nucleoproteins were positively detected at 18 hours post infection (hpi) when cells were inoculated with 360 PFU of MHV, at 24 hpi when cells were inoculated with 36 PFU, and at 36 hpi when inoculated with 3.6 PFU (Figure 4.1). Higher sequence coverage of MHV nucleoproteins was observed as the culturing period extended (Figure 4.1; Table C-3). Approximately 30%

coverage was observed for the inoculation of 360 PFU at 27 hpi and 36 PFU at 38 hpi (Figure 4.1). For the 3.6 PFU inoculation, only 12% of nucleoproteins were detected at 42 hpi. At this low MHV inoculation, if cells infection is assumed to follow Poisson probability distribution,<sup>24</sup> majority of cells may not have received an infectious MHV particle (>99.999%), thus explaining why the overall viral protein synthesis was slower. In addition to nucleoproteins, coronavirus spike glycoproteins were also detected at greater hpi in one sample (Table C-3). However, the protein sequence coverage of spike glycoproteins was lower compared to that of nucleoproteins. No viral proteins were detected in negative controls where cells were inoculated with virus-free PBS. These control experiments with the MHV model demonstrated that the ICC-MS method could detect viruses and that the time required for detection depends on the number of infectious viruses in the sample.



**Figure 4.1** Sequence coverage of MHV nucleoproteins detected by LC-MS/MS with respect to hours post infection. MHV was suspended in growth culture media. Negative controls consisted of cells infected with virus-free PBS.

Interestingly, the detected peptide sequences of MHV nucleoproteins made it possible to identify the MHV virus at the strain level. Specifically, the peptide SFVPGQENAGGR with an acetylation modification at the N-terminus was detected early, and this peptide is unique to MHV strain A59 (P03416) and MHV strain 3 (P18447) (Table C-3; Figure C-1). The nucleoproteins of other MHV strains possess peptide SFVPGQENASGR (Figure C-1) at the same position with an S residue at position 11 rather than a G residue. At higher MHV nucleoprotein coverage (i.e., at greater hpi), another unique peptide, namely LGTSDPQFPILAELAPTVGAFFFGSK, helped differentiate strain A59 and strain 3 from other strains (Table C-3; Figure C-1). However, the polymorphisms in nucleoproteins may limit the strain identification to some extent. The detected peptides were not able to distinguish strain A59 from strain 3. This is because the nucleoprotein sequences of these two strains have a similarity of 99.8%, and only vary by one amino acid residue at position 17.

# 4.3.2 Identification of MHV in concentrated wastewater samples

To test the impact of wastewater samples on the ICC-MS method, we suspended MHV in concentrated influent and effluent wastewater samples and conducted follow-up experiments. We hypothesized that if the wastewater inhibits protein synthesis, fewer viruses will be propagated in the culture system. Infectious MHV propagated and released into the liquid media was first tracked post inoculation by plaque assays. MHV propagation curves were similar when the cells were inoculated with MHV in concentrated wastewater or with MHV in growth media (Figure C-2). This suggests that virus infection was not affected by components in the concentrated wastewater samples.

The cell systems inoculated with MHV in wastewater concentrates were also analyzed with the ICC-MS method. Consistent with the results when MHV in media was added to cells, nucleoproteins of MHV strains A59 and 3 were positively identified in all samples except for the sample collected at 42 hpi of 3.6 PFU inoculation in wastewater influent (Table C-4). Approximately 30% to 40% coverage of MHV nucleoproteins was observed for the inoculation of 360 PFU at 24 hpi, 36 PFU at 36 hpi, and 3.6 PFU at 48 hpi (Figure 4.2). The sequence coverage for MHV in wastewater concentrates was also comparable to the experiments conducted in culture media. These results demonstrate that protein synthesis was not inhibited when the cells were exposed to the concentrated wastewaters.



**Figure 4.2** MHV nucleoprotein coverage detected by LC-MS/MS at hours post infection. MHV was suspended in concentrated wastewater influent (ww inf) and concentrated wastewater effluent (ww eff). Negative controls were fake infected with virus-free PBS.

Previous work suggests that wastewater can impact cell cultures.<sup>1</sup> It is possible that we avoided these issues by filtering our wastewater samples through filters with 0.22 µm pores. We did

observe bacterial contamination when the wastewater was pre-filtered through 0.45  $\mu$ m pores rather than 0.22  $\mu$ m pores (data not shown). Previous studies have conducted organic solvent extractions to remove wastewater toxicity (e.g., Freon, chloroform), but we avoided these steps due to their impact on enveloped viruses.<sup>25,26</sup>

The experiments with MHV in wastewater suggest that the ICC-MS can detect infectious viruses in wastewater samples. Here, we used the nucleoproteins for identification due to the consistent detection and high sequence coverage compared to other viral proteins (i.e., spike glycoproteins) identified in our experiments. In MHV, nucleoproteins are the most abundant proteins.<sup>27</sup> The peptides from the most abundant viral proteins are more likely to be captured earlier by MS. Work on MS-based detection of influenza A viruses in clinical samples reported the correct strains with nucleoprotein peptides when the protein sequence coverage was 30-40%.<sup>19</sup> Evolutionary analyses on the abundant viral capsid proteins of iridoviruses suggests that major capsid proteins are highly conserved but also diverse enough to distinguish close isolates.<sup>28</sup> These findings suggest that the abundant proteins such as nucleoproteins and major capsid proteins are suitable for virus identification.

#### 4.3.3 Identification of infectious viruses in wastewater samples by ICC-MS/MS method

Two monkey kidney cell lines (Vero and BSC-1) were used to detect infectious viruses in the concentrated wastewater samples by the ICC-MS method. Vero and BSC-1 cells have been used to isolate polioviruses, coxsackieviruses, echoviruses, reoviruses, and adenoviruses from sewage samples in a previous study.<sup>25</sup> Here, sewage samples were inoculated to cell cultures and incubated 10 days for the observation of cytopathic effects. Supernatants were collected from the cells with positive cytopathic effects and passaged for another two rounds of 10-day culturing for the

confirmation of positive cytopathic effects. Viruses were finally identified based on immuno-based clinical assays.<sup>25</sup>

We applied the same inoculation procedures as we did in the MHV proof-of-concept experiments to infect Vero and BSC-1 cells with concentrated wastewater samples. Throughout the 14-day culture period, cells did not show clear cytopathic effects, and no cells were observed detaching from the culture plates. The same protein extraction method that was developed in the MHV culture system was applied to extract proteins from Vero and BSC-1 cells at 14 days post infection (dpi). Control cells were inoculated with virus-free PBS.

#### Virus detection in the influent samples

In both Vero and BSC-1 culture systems, proteins associated with *homo sapiens* are the most identified (data not shown). In addition, in the Vero cell extracts, a number of reovirus proteins were detected (Table 4.1). Reovirus proteins mu-1 and sigma-3 had consistently high sequence coverage (30%-40%) from the cells inoculated with influent samples, followed by sigma-2, sigma-NS and mu-NS proteins of approximately 20% sequence overage. The coverage of lambda-1 and mu-2 varied significantly in two influent samples. Proteins lambda-2, and lambda-3 had sequence coverage less than 10%. Contrary to the Vero samples, no viral proteins were detected in the BSC-1 cell extracts.

A reovirus particle contains 8 distinct virion proteins that assemble into an outer protein capsid containing mu-1, sigma-1, sigma-3, lambda-2 proteins and an inner protein capsid containing proteins lambda-1, lambda-3, sigma-2, mu-2.<sup>29</sup> Three nonstructural proteins are involved in the virus replication cycle,<sup>30-32</sup> namely sigma-NS, mu-NS, and sigma-1s. The consistently high sequence coverage of mu-1 and sigma-3 proteins in our study suggests that these two proteins were

the most abundant in the cell culture system. Indeed, mu-1 and sigma-3 proteins are the major outer capsid proteins in reoviruses, with 600 copies in one reovirus particle (Table C-5). Based on the results in MHV control experiments, we would use the mu-1 and sigma-3 proteins for the strain-level identification of reoviruses. Protein mu-1 and sigma-3 of both type 1 and type 3 strains were identified, suggesting the coexistence of infectious reovirus type 1 and type 3 in the influent samples. Reovirus type 2 was detected in the influent samples based on peptides in protein sigma-3 (coverage = 16 % in average), but peptides from protein mu-1 were not detected. This may be because the protein mu-1 sequences of the three reovirus strains have higher similarities than the three sigma-3 sequences (Figure C-3; Figure C-4); consequently, the chances of detecting unique peptides from protein mu-1 are lower. These results suggest that reovirus type 1, type 2, and type 3 are present in the influent wastewater samples.

#### *Virus detection in the effluent samples*

In contrast to the influent samples, only three reovirus proteins were detected in the cells inoculated with the effluent pre-UV treatment (Table 4.1). Sigma-3 proteins of reovirus type 2 and type 3 were detected with sequence coverage of 7% and 15%, respectively. Mu-1 protein of reovirus type 1 was detected with sequence coverage of 6%. Although this assay is not yet quantitative, these results suggest a lower concentration of infectious reoviruses in the effluent samples than in the influent samples. Reovirus proteins were not detected in the cells infected with final effluent, suggesting that the concentrations of infectious reoviruses decreased further through the UV disinfection treatment. These negative results can be interpreted that infectious reoviruses in the final effluent was too low to capture in 1.2 mL of the wastewater concentrate samples that were used for inoculation. In this concentrate sample, 1.2 mL of the concentrate corresponded to

60 mL of the effluent, which suggests that the infectious concentrations of reoviruses in the final effluent are no higher than 16.7 infectious particles/L if single infectious reovirus can be detected by the ICC-MS method after 14-day culturing.

Contrary to the Vero culture system, negative results were observed for all effluent samples in the BSC-1 culture system. BSC-1 cells have been more applied for polioviruses surveillance in water;<sup>33</sup> polioviruses, however, are unlikely to be present in Ann Arbor wastewater samples. This result highlights the importance of the cell types used for an ICC-MS method, because only the viruses that can grow in the cell lines will be detectable by mass spectrometry. Our ongoing work is exploring the application of other cell lines in this method.

By applying the ICC-MS method, we detected infectious reovirus type 1, type 2, and type 3 in the primary influent samples, and we found peptides from reovirus type 1, type 2, and type 3 in the effluent pre-UV samples. This suggests that infectious reoviruses persist after primary and secondary treatment. As mentioned above, no infectious reoviruses were detected in the 60 mL of disinfected effluent.

Primary influent 1, 50× concentrate					
Accession number	Protein description	Sequence coverage (%)	Protein score		
P11077	mu-1, Reovirus type 1 (strain Lang)	32	1287		
P11078	mu-1, Reovirus type 3 (strain Dearing)	28	918		
P03527	sigma-3, Reovirus type 3 (strain Dearing)	24	695		
P30211	sigma-3, Reovirus type 2 (strain D5/Jones)	13	503		
P07939	sigma-3, Reovirus type 1 (strain Lang)	25	489		
P03525	sigma-2, Reovirus type 3 (strain Dearing)	25	204		
P11314	sigma-2, Reovirus type 1 (strain Lang)	26	193		
P07940	sigma-NS, Reovirus type 1 (strain Lang)	19	418		
P03526	sigma-NS, Reovirus type 3 (strain Dearing)	17	384		
P12419	mu-NS, Reovirus type 3 (strain Dearing)	17	601		

**Table 4.1** Proteins detected in extracts from Vero cells. Vero cells were inoculated with different wastewater samples, and incubated for 14 days.

O9PY83	mu-NS Reovirus type 1 (strain Lang)	13	428			
O9WAB2	lambda-1. Reovirus type 1 (strain Lang)	7	453			
P15024	lambda-1, Reovirus type 3 (strain Dearing)	7	453			
P11079	lambda-2, Reovirus type 3 (strain Dearing)	3	128			
Q00335	mu-2, Reovirus type 1 (strain Lang)	3	90			
P12418	mu-2, Reovirus type 3 (strain Dearing)	3	90			
Primary influent 2, 50× concentrate						
Accession	· · · · ·	Sequence	Protein			
number	Protein description	coverage (%)	score			
P11077	mu-1, Reovirus type 1 (strain Lang)	40	1765			
P11078	mu-1, Reovirus type 3 (strain Dearing)	36	1400			
P03527	sigma-3, Reovirus type 3 (strain Dearing)	43	1234			
P07939	sigma-3, Reovirus type 1 (strain Lang)	38	1029			
P30211	sigma-3, Reovirus type 2 (strain D5/Jones)	27	862			
Q9WAB2	lambda-1, Reovirus type 1 (strain Lang)	29	1975			
P12419	mu-NS, Reovirus type 3 (strain Dearing)	24	1165			
Q9PY83	mu-NS, Reovirus type 1 (strain Lang)	19	931			
P12418	mu-2, Reovirus type 3 (strain Dearing)	17	619			
P03526	sigma-NS, Reovirus type 3 (strain Dearing)	22	720			
P07940	sigma-NS, Reovirus type 1 (strain Lang)	24	684			
P03525	sigma-2, Reovirus type 3 (strain Dearing)	17	323			
P11314	sigma-2, Reovirus type 1 (strain Lang)	19	318			
Q91RA6	lambda-2, Reovirus type 1 (strain Lang)	9	516			
P11079	lambda-2, Reovirus type 3 (strain Dearing)	7	330			
P0CK32	lambda-3, Reovirus type 1 (strain Lang)	3	105			
Effluent pre-UV, 50× concentrate						
Accession		Sequence	Protoin			
number	Protein description	coverage	score			
number		(%)	score			
P03527	sigma-3, Reovirus type 3 (strain Dearing)	15	199			
P30211	sigma-3, Reovirus type 2 (strain D5/Jones)	7	118			
P11077	mu-1, Reovirus type 1 (strain Lang)	6	160			
Final effluent, 50× concentrate						
No viral protein hit						
Negative control, inoculated with virus-free PBS						
No viral prote	No viral protein hit					

# 4.3.4 Environmental implications

Results from this proof-of-concept work on the development of an ICC-MS method suggests that it holds promise for the detection of infectious viruses in environmental water samples. The ICC-MS method identifies viruses more directly than ICC-PCR methods, as it avoids the need of primer design and assay development for each strain detected. In our study, the sample preparation and MS detection protocols that have been developed with a mouse virus and its host cells can be

easily adopted for other virus detection in different culture systems. Moreover, the MS technique can readily identify multiple virus strains, given their sequences are present in the database. We were able to detect three reovirus strains in wastewater with Vero cells that displayed no apparent cytopathic effects, and demonstrated the virus removal/inactivation through a full-scale wastewater treatment. Compared to sequencing methods, which can also identify viruses without designing specific primers, the ICC-MS method has the advantage of needing much less data processing. In our study, the MS data analysis took less than an hour, while metagenomic sequencing data may take several days or weeks to process. Furthermore, since metagenomic methods sequence all of the DNA in a sample, much of the data recovered is not relevant. Consequently, numerous copies of a specific gene need to be present for the organism of interest to be detected. Here, we were able to detect MHV in our wastewater samples when as few as 3 infectious particles were present.

Further research will be required before this method can be broadly applied. Specifically, the ICC-MS method will need to be optimized for different cell lines so that a range of viruses can be detected. Ideally, cell lines that can detect several human viruses at once would be selected for environmental monitoring. The impact of multiple virus infection will need to be assessed as it is possible that only the fastest replicating viruses will be identified. Finally, it is worth pointing out that at this point the method is qualitative or semi-quantitative. We believe the method could be readily modified to become quantitative by developing a most-probable-number type method. Here, sequential dilutions of the wastewater are assessed simultaneously, and statistics performed on the positive/negative replicates can provide a value of the infective viruses present in the sample.

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# **Chapter 5 Significance and implications**

## 5.1 Overview

This dissertation research seeks to advance our current state of knowledge on the detection and fate of nonenveloped viruses in water environments. The outcomes help fill the knowledge gaps of treating enveloped viruses in wastewater and monitoring infectious viruses in water samples. This dissertation begins to explore the survivability and partitioning of model enveloped viruses in municipal wastewater (Chapter 2). Lab-scale experiments and computational simulations were used to quantitatively characterize the inactivation of model viruses in liquid and solids fractions of wastewater and the partitioning of model viruses to wastewater solids. The knowledge obtained from the experiments facilitated the optimization of an ultrafiltration method for concentrating infectious enveloped viruses from wastewater with high recovery rates (Chapter 2). Chapter 3 focuses on the inactivation of enveloped viruses with common disinfectants. Considering the difficulties with studying the inactivation kinetics of highly pathogenic enveloped viruses and viruses that are nonculturable, we developed a framework to understand the inactivation of enveloped viruses on a molecular basis. Virus infectivity, reactions in lipids and proteins, and reactions in nucleic acids following the treatment by common disinfectants were tracked by cell culture assays, quantitative mass spectrometry, and molecular polymerase chain reaction (PCR) techniques. Finally, Chapter 4 reports an integrated cell culture-mass spectrometry (ICC-MS) method for detecting infectious viruses. This ICC-MS method was developed and optimized with an enveloped murine coronavirus and its culture system, and was then validated by applying it to detect infectious viruses in wastewater samples collected throughout a full-scale municipal wastewater treatment plant. Major findings from this dissertation research and their implications for water quality control and viral disease control are discussed in detail below.

#### 5.2 Implications for operations of wastewater treatment plants

Our findings from the survivability of enveloped viruses underscore that enveloped viruses can persist in wastewater, especially at cooler temperatures.<sup>1-3</sup> Considering that outbreaks of certain enveloped virus diseases peak during the winter, higher concentrations of infectious enveloped viruses may be present in wastewater during winter. Further studies on enveloped virus removal throughout wastewater treatment plants should focus more on removal during conditions of cooler temperatures.

Enveloped viruses tended to partition to a greater extent to wastewater solids than nonenveloped viruses. Consequently, a larger fraction of enveloped viruses is expected to be removed by primary treatment settling. The models developed here were built based on the partitioning experiments at 4 °C. Wastewater temperatures, however, can range from 3 °C to 27 °C.<sup>4</sup> We would expect higher levels of enveloped virus sorption to solids in wastewater at higher temperatures. Another limitation to our sorption study is that when viruses are shed, any can be within fecal solids, whereas we spiked purified model enveloped viruses into the wastewater and observed their partitioning between solids and liquids. The fraction of enveloped viruses that are associated with solids at equilibrium may therefore be underestimated in our study. Given these two points, the fraction of enveloped viruses that are removed in the primary settling tank in real systems is likely to be greater than the fraction estimated in our study.
Our models also indicate that the inactivation kinetics of enveloped viruses in the liquid fraction of wastewater is similar to the inactivation kinetics of enveloped viruses sorbed on the solid surfaces, suggesting that infectious enveloped viruses may persist in sediments. The presence of enveloped virus genes in the sludge of anaerobic reactors have been reported previously.<sup>5</sup> More research regarding the fate of enveloped viruses in solids needs further investigation.

#### 5.3 Implications for predict enveloped virus reactivity with disinfectants

To understand virus susceptibility to disinfectants, culture-based infectivity assays have been used widely to track the loss of virus infectivity following disinfection treatments. However, some enveloped viruses are too dangerous to work with, and many viruses are not culturable. A molecular-based understanding of virus inactivation could help in predicting virus reactivity with disinfectants. The framework developed in our study identifies the molecular features in a model enveloped virus that drive virus inactivation by common disinfectants. Our results demonstrate that the presence of reactive amino acids in viral proteins that are easily accessible by solvents correlate with high virus reactivity with free chlorine. Genome reactions, on the other hand, drive virus inactivation by UV<sub>254</sub>. The molecular-based understanding of virus inactivation explains the discrepancies of inactivation kinetics among viruses. For example, we were able to identify that the higher resistance of nonenveloped virus MS2 to free chlorine compared to the enveloped virus Phi6 is due to the different reactivities of their proteins. The most reactive peptide in MS2 is  $150 \times$ less reactive to free chlorine than the most reactive peptide in Phi6. Before generalizations about enveloped virus mechanisms versus non-enveloped virus mechanisms are possible, similar investigations with other model enveloped and nonenveloped viruses must be conducted.

### 5.4 Implications for virus environmental surveillance

Human viruses are generally at very low concentrations in water environments. It is therefore challenging to monitor their presence and infectivity in water. In this dissertation, we optimized an ultrafiltration method for concentrating infectious viruses from water samples with high recovery rates for both enveloped and nonenveloped viruses. To follow up the concentration method, we developed a new virus detection method that integrates cell culture and mass spectrometry (ICC-MS) for detecting infectious viruses in the concentrated water samples. This ICC-MS method has a number of advantages over other currently available virus detection methods; most notably, the sample preparation and mass spectrometry protocols can be easily adopted for detecting multiple viruses at once, as long as they are propagated in the same culture system. This would not be possible for PCR-based detection methods, which require primer design and PCR assay optimization for different viruses. The ICC-MS detects the most abundant viral proteins that carry conservative but diverse genetic information suitable for virus identification. Data processing by comparing the detected peptide sequences with the sequences available in viral protein database takes less than one hour. This is an advantage over viral genome sequencing, for which data processing may take several days or weeks and requires a supercomputer.

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## Appendices

### **Appendix A. Supplementary Information for Chapter 2**

**Optimization of Ultrafiltration Method.** A higher MHV recovery was achieved when the wastewater solids removal step was carried out with filtration through 0.22  $\mu$ m PES membranes rather than centrifugation at 30,000 × g for 10 min (P = 0.0046; Figure A-3A). Employing 10 kDa ultra-filters for the concentration step resulted in higher and more consistent MHV recoveries compared to 100-kDa ultra-filters (Figure A-3B). In comparison, the specific solids-removal techniques and the 10 kDa versus 100 kDa filter sizes did not impact the MS2 recoveries. Following ultra-filter regeneration, the mean recovery of MHV decreased and the mean recovery of MS2 increased, although neither change was significant (P = 0.2444; Figure S3B).

**Recovery of MHV from Wastewater Solids.** When solids were collected from the wastewater with centrifugation at  $30,000 \times g$  for 10 min., the extraction solution consisting of pH 9.5 glycine buffer resulted in the highest recovery of MHV (3.7% of spiked MHV; Figure A-3C) from the wastewater solids; this recovery was low considering that nearly a quarter of spiked MHV were reversibly adsorbed to solids after incubating for one-hour, and therefore should have been recoverable. The limited recovery may be due to viruses losing infectivity as they are detached from the soil surface, as reported elsewhere.<sup>1</sup> MS2 recoveries were ~2% for all of the tested

extraction solutions; this was not surprising based on the low percentage (6%) of MS2 associated with the wastewater solids at equilibrium.



Figure A-1 Virus recovery immediately after viruses were spiked into samples at 4 °C (t = 0) and after 1-hour incubation at 4 °C. Here,  $N_t$  (PFU) represents the amount of infective viruses measured at time T;  $N_s$  (PFU) is the amount of infective viruses in the spiked aliquots. Bars indicate the mean recovery for each tested viruses.



**Figure A-2** Virus inactivation in 4 °C wastewater with and without the presence of PEG. Error bars represent the ranges of replicates from wastewater samples collected on different days (n = 2).







**Figure A-3** Method optimization for enveloped virus (MHV) and nonenveloped virus (MS2) in liquid and solid phases: (A) Virus recoveries in liquid fraction of wastewater following solids removal by centrifuging at 30,000 × g for 10 min at 4 °C (Cen), or by centrifugation at 2,500 × g for 5 min at 4 °C followed by 0.22  $\mu$ m filtration (Fil); (B) Ultrafiltration method tested with prefiltration and pre-centrifugation, with filter cut-off sizes of 100 kDa and 10 kDa, and with filter reuse. (C) Virus recoveries from wastewater solids collected from wastewater samples by centrifuging at 30,000 × g for 10 min at 4 °C. Tested elution buffers include PBS (pH 7.4), 0.05 M glycine buffer (0.05 M GB, pH 8.5, pH 9.5, and pH 10.5), 3% beef extract (3% BE, pH 7.5 and pH 9.5), and 3% beef extract with 0.5 M sodium chloride (3% BE + 0.5 M NaCl, pH 9.5). Bars represent the average infective virus recoveries of the replicate experiments (n≥3).

The effect of pasteurization (pww vs. ww)



Figure A-4 Statistical significance analysis of virus inactivation kinetics under different conditions.

# Table A-1 Wastewater parameters

TSS (mg L <sup>·1</sup> ) <sup>a</sup>	235 ± 97
VSS (mg L <sup>-1</sup> ) <sup>a</sup>	205 ± 85
VSS/TSS <sup>a</sup>	$0.87 \pm 0.13$
pH <sup>a</sup>	$7.63 \pm 0.25$
Total COD (mg COD L <sup>-1</sup> ) <sup>b</sup>	300-768
Background bacteriophage concentrations tested with <i>E. coli</i> ATCC 15597 (PFU mL <sup>-1</sup> ) <sup>b</sup>	800-1000

<sup>a</sup>Results from 34 wastewater samples.

<sup>b</sup>Ranges of 3 wastewater samples.

	TSS (VSS) of wastewater samples (mg L <sup>-1</sup> )	TSS (VSS) after centrifugation (mg L <sup>-1</sup> )	TSS (VSS) Removal (%)
1	327 (297)	50.0 (43.3)	85 (85)
2	213 (183)	15.0 (10.0)	95 (95)
3	237 (193)	16.7 (16.7)	93 (91)

**Table A-2** TSS and VSS removal by centrifugation at  $30,000 \times \text{g}$  for 10 min.

		Temp.	First order rate constant (h <sup>-1</sup> ) (avg±s.d.)	Estimated T <sub>90</sub> (h) (avg±s.d.)	R <sup>2</sup> (avg)
	MHV (enveloped)	25 °C	$0.142\pm0.015$	$13 \pm 1$	0.88
	will v (enveloped)	10 °C	$0.059\pm0.006$	$36 \pm 5$	0.95
	Dhi6 (anvalanad)	25 °C	$0.317\pm0.022$	$7\pm0.4$	0.99
Wastewater	Fillo (ellveloped)	10 °C	$0.091\pm0.010$	$28 \pm 2$	0.96
vv aste water	MS2 (nonenvalened)	25 °C	$0.022\pm0.006$	$121 \pm 36$	0.85
	wisz (nonenveloped)	10 °C	$0.014\pm0.003$	$175 \pm 33$	0.78
	T3 (nonenveloped)	25 °C	ng	na	ng
	15 (nonenveloped)	10 °C	11.a.	11.a.	II.a.
	MHV (enveloped)	25 °C	$0.120\pm0.037$	$19\pm 8$	0.97
		10 °C	$0.021 \pm 0.012$	$149\pm103$	0.84
	Phi6 (enveloped)	25 °C	$0.044\pm0.004$	$53\pm 8$	0.95
Pasteurized	Tino (criveroped)	10 °C	$0.017\pm0.005$	$146 \pm 43$	0.86
wastewater	MS2 (nonenvalened)	25 °C	$0.020\pm0.007$	$121 \pm 55$	0.95
	Wisz (nonenveloped)	10 °C	$0.013\pm0.006$	$212 \pm 88$	0.73
	T3 (nonenveloped)	25 °C 10 °C	n.a.	n.a.	n.a.

**Table A-3** Inactivation rates of enveloped and nonenveloped virus surrogates in unpasteurized and pasteurized wastewater.

Virus	k <sub>1</sub> (h <sup>-1</sup> ) <sup>a</sup>	k <sub>2</sub> ( <b>h</b> <sup>-1</sup> ) <sup>b</sup>	k <sub>3</sub> ( <b>h</b> <sup>-1</sup> ) <sup>c</sup>	Viruses adsorbed at equilibriu m (%)	T (90% equilibriu m, h)	T (99% equilibriu m, h)
MHV (enveloped)	0.048	2.8	0.048	26.3	0.3	0.4
Phi6 (enveloped)	0.026	0.33	0.026	22	1.5	2.9
MS2 (nonenveloped)	0.0013	0.13	0.037	6.0	1.1	2.5
T3 (nonenveloped) <sup>d</sup>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table A-4 Simulation results for virus sorption and inactivation kinetics in wastewater at 4 °C.

 $k_1$  is the virus inactivation rate constant in the liquid fraction of wastewater, equal to the virus inactivation rate constant in solids-removed wastewater;

<sup>b</sup>  $k_2$  is the rate constant for reversible adsorption from the liquid to solid phase;

 $^{c}k_{3}$  is the virus inactivation rate constant on solid surfaces. In our model, the rate constant for reversibly adsorbed viruses transitioning to irreversible adsorption ( $k_{4}$ ) was assumed to equal zero.

<sup>d</sup> No significant decline of the T3 infectivity was observed in wastewater and solids-free samples within the experimental time-scale.

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### **Appendix B. Supplementary Information for Chapter 3**

**Protein digestion.** The virus samples (2.4 mL,  $\sim 10^{11}$  PFU) were concentrated with 100 kDa Amicon Ultra-0.5 centrifugal filters (Millipore), washed three times with 50 mM Tris-HCl buffer (pH 8) and eventually collected at a final volume of approximately 40 µL. Protein concentrations were measured in a Qubit Fluorometer 2.0 with Protein Assay Kits (ThermoFisher Scientific). Total protein concentrations of 28.6  $\pm$  2.2 µg per 10<sup>11</sup> PFU were consistently measured in the concentrated virus samples. Each of the 40 µL concentrate was equally split into two portions, and the two 20  $\mu$ L samples were digested with trypsin and chymotrypsin, respectively. In brief, the virus concentrates were denatured by submerging the sealed centrifuge tube in boiling water for 5-6 min. Following the denaturing step, iodoacetamide was added to a final concentration of 10 mM in order to prevent the formation of disulfide bonds, and the sample was incubated in the dark at 37 °C for 1 h. Unreacted iodoacetamide was deactivated by adding L-cysteine to a final concentration of 16.7 mM and incubating the solution in the dark at room temperature for 30 min. Finally, calcium chloride was added to a final concentration of 1 mM and trypsin or chymotrypsin was added to achieve a protein-to-enzyme ratio of 50:1. Samples were gently vortexed and then incubated at 37 °C overnight. After incubation, samples were injected directly onto the LC-MS/MS system without further purification.

<sup>15</sup>N-Metabolic labeled Phi6. To prepare a stock of <sup>15</sup>N-labeled Phi6, the Phi6 host *P. syringae* was first cultured in <sup>15</sup>N-M9 medium (1g L<sup>-1</sup> <sup>15</sup>NH<sub>4</sub>Cl, 48 mM Na<sub>2</sub>HPO<sub>4</sub>, 22 mM KH<sub>2</sub>PO<sub>4</sub>, 8.5 mM NaCl, 2 mM MgSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>, and 0.4% (v/v) glucose, pH 7.4). <sup>14</sup>N-Phi6 was then added to <sup>13</sup>N-labeled *P. syringae* at an OD<sub>640</sub> of ~0.1 and at a MOI of 2, and incubated at 26 °C while shaking at 180 rpm. Propagation was stopped 12-17 hours after infection. The Phi6 propagation in <sup>15</sup>N-labeled *P. syringae* was repeated for two generations in <sup>15</sup>N-M9 medium to obtain a stock with over 99% <sup>15</sup>N. The virus concentration and purification techniques used for the <sup>15</sup>N-labeled Phi6 were the same as those used for the <sup>14</sup>N-Phi6. The final <sup>15</sup>N-labeled Phi6 stocks (8 × 10<sup>11</sup> PFU mL<sup>-1</sup>) were filtered through 0.22 µm PES membranes, aliquoted, and stored at -80 °C until use.

**Determination of peptide limit of quantification (LOQ) and limit of detection (LOD).** The LOQ of each Phi6 peptide measured by LC-MS/MS was determined from its calibration curve. In brief, <sup>14</sup>N-Phi6 samples were serially diluted and mixed with equal amounts of <sup>15</sup>N-labeled Phi6 to yield PFU ratios of 3:1, 1:1, 0.3:1 and 0.1:1. The mixtures were digested with trypsin and chymotrypsin (see digestion procedure above), and were analyzed with LC-MS/MS. The ratios of peak areas of the <sup>14</sup>N-peptides and <sup>15</sup>N-labeled peptides (Y) were plotted as a function of PFU ratios (X). The linear regression model and the LOQ, LOD of peptide *j* were expressed as:<sup>1</sup>

 $Y = b_i X + a_i$ 

 $LOQ_j = 10S_{a_j}/b_j$ 

 $LOD_j = 3S_{a_i}/b_j$ 

Where  $a_j$  and  $b_j$  are the intercept and slope of the linear curve, and  $S_{a_j}$  is the standard deviation of the intercept  $a_j$ .

**Lipid extraction.** For lipid analysis, virus samples (2.4 mL,  $\sim 10^{11}$  PFU) were freeze-dried (FreeZone 6, Labconco) to a final volume of 200 µL. Viral lipids were then extracted with methyl-tert-butyl ether (MTBE) as described previoulsy.<sup>2</sup> In brief, the 200 µL samples were mixed with 1.5 mL methanol and 5 mL MTBE, and were shaken at room temperature for 1 h. After the addition of 1.25 mL Milli-Q water, the mixture was centrifuged at 1000 × g for 10 min. The upper organic phase, where the lipids partitioned, was collected and dried under nitrogen gas. The dried lipids were then resuspended in 400 µL of acetonitrile/isopropanol/water (6.5:3:0.5, v/v/v) prior to lipid analysis.



**Figure B-1** SDS-PAGE of purified Phi6 stock. Electrophoresis was conducted in 8-16% TGX<sup>TM</sup> precast gels (Bio-Rad).



**Figure B-2** Lab-scale continuous quench-flow system for free chlorine treatment. The system was modified from a previous study on ozone reactions.<sup>3</sup>



**Figure B-3** Effect of Tris-HCl quenching on Phi6 inactivation. Phi6 inactivation was compared when samples sit on ice in 5 mM phosphate buffer (PBS; 10 mM NaCl, pH 7.4) added with Tris-HCl (no chlorine control) and when samples sit on ice in free chlorine solution quenched with Tris-HCl. The Phi6 inactivation was effectively quenched with Tris-HCl for up to 30 min (i.e., the time that samples sit on ice following the addition of Tris-HCl in the experiments).



**Figure B-4** Calibration curves of eight the most abundant Phi6 lipid compounds. The relative peak areas of lipids (PA/PA<sub>0</sub>) were determined by lipid LC-MS/MS method, and the relative lipid concentrations ( $L/L_0$ ) were prepared from Phi6 lipid extracts that were not exposed to free chlorine or UV<sub>254</sub>.

PE(16:0/16:1): Y=0.623X+0.382 (R<sup>2</sup>=0.990), PE(18:1/14:1): Y=0.986X+0.012 (R<sup>2</sup>=0.998), PE(18:0/16:1): Y=0.859X+0.144 (R<sup>2</sup>=0.994), PE(18:1/16:1): Y=0.662X+0.341 (R<sup>2</sup>=0.995), PG(16:1/16:0): Y=0.989X+0.009 (R<sup>2</sup>=0.994), PG(16:1/16:1): Y=1.048X-0.054 (R<sup>2</sup>=0.988), PG(16:1/18:0): Y=1.029X-0.033 (R<sup>2</sup>=0.994), PG(16:1/18:1): Y=0.993X+0.005 (R<sup>2</sup>=0.994).



**Figure B-5** The impact of storage at 4 °C for 48 hours on Phi6 resistance to free chlorine. Here, the "unstored Phi6" refers to an experiment where the stock was thawed from -80 °C and treated with free chlorine on the same day of the experiments. The "stored Phi6" refers to an experiment in which the stock was thawed from -80 °C and stored at 4 °C for 48 hours before the chlorine treatment was conducted.



**Figure B-6** Schematic of Phi6 structure.<sup>4</sup> For the early infection steps, the Phi6 viral particle binds to the pilus of *Psudomonas syringae* with spike protein P3. Then P6 initiates the virus membrane fusion with the host membrane. P5 is responsible for the penetration of the nucleocpasid and polymerase complex through the peptidoglycan layer. Finally, nucleocapsid protein P8 helps the polymerase complex continuously penetrate the cytoplasmic membrane, delivering the viral genome into the cytoplasm for replication.<sup>4</sup>



Figure B-7 Phi6 protein coverage captured with the LC-MS/MS method. Error bars represent the standard deviations of protein coverage in free chlorine and  $UV_{254}$  experiments, n=18. NA indicates information not available.



**Figure B-8** Cryo-EM structure of Phi6 protein P1 (PDB ID: 5muu) and close-up of residues Met 65, Met 198, Met 209 within P1. Sulfur atoms in Met 65, Met 198, and Met 209 are colored in red. Solvent-accessible surface areas of Met 65, Met 198, and Met 209 are identified with transparent red coloring.



**Figure B-9** Relative abundances of Met oxidation products of the fastest reacting peptides following chlorine treatments. The peak areas of the oxidized peptide ions ( $PA_{M[O]}$ ) were normalized to the peak areas of the corresponding <sup>15</sup>N-labeled peptide ions ( $PA_{15N}$ ). Unpaired student's t tests were used to identify statistical difference in the relative abundances of oxidation products at two levels of Phi6 inactivation. \*\* indicates P < 0.01 and ns indicates not significant (P > 0.05).



**Figure B-10** Relative abundances (L/L<sub>0</sub>) of eight major Phi6 lipid compounds with respect to Phi6 inactivation (C/C<sub>0</sub>) by free chlorine (FC) and UV<sub>254</sub> (UV).

Primer set ID	Genome segment	Genome segment size (kbp)	Direction	Primer sequence (5'-3')	Nucleotide position	RT-qPCR amplicon size (bp)	Ampliconsizerelativetogenomesegmentsize (%)
c	Segment	2.05	F	GCAGACCCAGCTGACTTCTT	1141-1160	400	16.0
3	S	2.95	R	AAGGCGCTATCCTTGGACAC	1639-1620	499	10.9
м	Segment	1.06	F	CCTGAGGAAACGGCTCAACT	1307-1326	470	11.6
IVI	М	4.00	R	CATAGCCAACGAACTGCTGC	1778-1759	472	11.0
T	Segment	6.27	F	GCCTACCAGCTCCACCAAAT	1510-1529	49.4	76
L	L	0.57	R	CGTACCCCATGTTGAGCAGT	1993-1974	404	1.0

 Table B-1 Phi6 primers and amplicon sizes.

Table B-2 Q Exactive settings for Phi6 protein and lipid analysis.

	Protein (positive mode)	Lipid (negative mode)
Sheath gas flow rate	24	24
Auxiliary gas flow rate	8	8
Sweep gas flow rate	1	1
Spray voltage	3 kV	2.8 kV
Capillary temp.	300 °C	250 °C
S-len RF level	50.0	50
Aux gas heater temp.	275 °C	275 °C
Column temperature	25 °C	55 °C
	Full MS settings	
Resolution	70,000	70,000
AGC target	5e5	1e6
Max IT	200 ms	200
Scan range	400-1800 m/z	400-1800 m/z
	dd-MS <sup>2</sup> settings	
Resolution	35,000	35,000
AGC target	2e5	2e5
Max IT	100 ms	100 ms
Loop count	20	5
Isolation window	1.6 Da	3 Da
NCE	30	30
Intensity threshold	2e4	2e4
Charge exclusion:	Unassigned, 1	Unassigned, 5-8, >8
Dynamic exclusion	20 sec	30 sec

**Table B-3** Reaction rate constants  $(k_g)$  of three RT-qPCR regions on S, M, and L segments measured by RT-qPCR and extrapolated rate constants of the entire Phi6 genome following free chlorine and UV<sub>254</sub> treatments. Errors represent standard errors of the reaction rate constants. ANCOVA analyses were applied to test whether the reaction rate constants were significantly different from zero. The results of ANCOVA analyses were shown in the table.

	Size of RT-qPCR	Free chlorine	UV <sub>254</sub>
	amplicon or Phi6 gnome in	$(L mg^{-1} s^{-1})$	$(cm^2 mJ^{-1})$
	base pairs		
S segment	499	$0.0070 \pm 0.0027^*$	$0.0027 \pm 0.0007^{**}$
M segment	472	$0.0127 \pm 0.0032^{***}$	$0.0014 \pm 0.0003^{***}$
L segment	484	$0.0109 \pm 0.0032^{**}$	$0.0027 \pm 0.0007^{**}$
Genome (extrapolated)	13380	$0.26 \pm 0.06$	$0.063 \pm 0.012$

All rate constants were significantly different from zero, where \* indicates P < 0.05; \*\* indicates P < 0.01; \*\*\* indicates P < 0.001.

**Table B-4** UV-reactive and chlorine-reactive bases in Phi6 and MS2 genomes, and the fraction these bases make up in the entire genome sequence.

		Bases	that are read	tive with UV	V <sub>254</sub> <sup>5</sup>	
	Genome size	Bases in genome	U	С	UU	Total reactive bases (U+C+UU) per genome base <sup>a</sup>
Phi6, dsRNA	13.4 kbp	26.8 kb	5914	7471	1120	0.54
MS2, ssRNA	3.6 kb	3.6 kb	901	909	185	0.55
		Bases tha	t are reactive	with free cl	nlorine <sup>6,7</sup>	
	Genome size	Bases in genome	U	С	А	Total reactive bases (U+C+A) per gnome base <sup>b</sup>
Phi6, dsRNA	13.4 kbp	26.8 kb	5922	7471	5922	0.72
MS2, ssRNA	3.6 kb	3.6 kb	901	909	827	0.73

<sup>a</sup> The proportion of UV-reactive bases in Phi6 and MS2 genomes is similar.

<sup>b</sup> The proportion of chlorine-reactive bases in Phi6 and MS2 genomes is similar.

D ( '		U.D.	Amino acio	ds (AA)	nata appatant	<b>M</b> -1 a-1)			T ( 1
location	Protein	Accession	$\frac{\text{(Second-of})}{\text{Met}^{a}}$ $(3.8 \times 10^{7})$	$\frac{\text{der reaction 1}}{\text{Cys}}$ (3.0×10 <sup>7</sup> )	His $(1.0 \times 10^5)$	$\frac{\text{M}^{2}\text{S}^{2}}{\text{Trp}}$ (1.1×10 <sup>4</sup> )	Lys (5.0×10 <sup>3</sup> )	Tyr (4.4×10 <sup>1</sup> )	_ Total AAs
	P3	P11129	17	9	7	17	24	22	648
	P6	P11128	0	0	0	7	10	2	168
Membrane	P9	P07581	0	0	1	1	5	1	90
	P10	P11127	0	0	0	0	4	1	42
	P13	P11130	0	0	0	0	3	2	72
Nucleo-	P8	P07579	4	0	1	0	6	6	149
capsid	P5	P07582	0	0	3	2	10	10	220
<b>^</b>	P1	P11126	19	3	18	11	25	23	769
Polymerase	P2	P11124	24	7	14	12	37	28	665
complex	P4	P11125	10	3	5	2	13	7	332
_	P7	P11123	4	0	5	3	5	3	161

Table B-5 Number of reactive amino acid residues in Phi6 proteins, and literature values for second-order rate constants of individual amino acids reaction with HOCl at pH 7.4.22 °C 8

Table B-6 Detailed information of Phi6 peptides, reaction rate constants following inactivation by UV<sub>254</sub> and free chlorine.

Data in red: value below the limit of	quantificat	ion, and was repl	aced by	an expecte	d number.			
The 8 most reactive Met-containing p	peptides w	ith free chlorine						
The most reactive Cys-containing pe	ptides with	n free chlorine						
The 8 most reactive peptides with U	V 254							
Uncovered Feplue			_					
	Р	hif Dontis	daa					
	P	по Рерис	Jes					
	Pro					Retention	DED S	Dep
pep_seq	ID	- pep_formula	z	m/z_14N	m/z_15N	time	tart	nd
YQGINEWLGGAK	P3	C61H90N16O18	2	668.338	676.313	18-19.5	3	1
LTTANGEIGAIYLSAAPPTDAAR	P3	C99H161N27O34	2	1137.092	1150.550	20.5-22	16	2
TAGWPSAIVDCADATRAKQNY	P3	00211311423020	3	025.000	037.301	17-20	47	6
QNYLWVGDNVVHIGAK	P3						65	8
LDLWGGTGDAW ACPMI DLCRAW	P3 P3	C55H75N13O17	2	595.777	602.258	22-24	85	11
ASASVTTGSLQGY	P3	C52H84N14O21	2	621.304	628.283	14-15.5	117	12
LDVEQQQF	P3	0.001070144.044		175 754	101.001	105.11	135	14
DNLNLYGDNCLDLATSSSAGR	P3 P3	C94H151N29O38S1	2	4/5./51 776.357	481.234 785.663	13.5-14	143	16
AFLEQCMGCALPEDCIFGWYVK	P3						166	18
MDWEGSAVADAYAAIR	P3	C75H112N20025S1 C39H64N12010	2	863.399	873.368	21-28 15-16 F	188	20
VQGFATVMAPWQSVGGAGYVYAR	P3	C111H163N29O30S1	3	805.735	815.375	22.8-33.5	200	22
ARVPQKGAW	P3						225	23
VHGTSGQPAYGIPM	P3 pa	C40H65N9O11S1 C62H95N17O19S1	1	880.460 707.843	889.432	20.7-21.3	234	24
TLSGFTGNMGQVASKWLM	P3						256	27
LMIVDPHVVQIL	P3	C64H109N15O16S1	2	688.902	696.379	24-25.5	276	28
RGTKSDPR	P3	C53P109IN 13U14	2	300.840	5/3.320	20.1+21	280	30
TTDVYADPK	P3	C44H68N10O17	2	505.245	510.230	8.2-9	303	31
ADPKVPASRISGPM INCT/ADDATIDATIDATIO	P3	C61H104N18O19S1	3	475.922	481.904	12-14.5	306	32
APLGGAGGPGAQGF	P3 P3						341	34
QVYPVFTW	P3	C53H70N10O12	2	520.266	525.251	21.5-23	355	36
MTDVTIEGTVTADSNGL	P3	C48H73N15O16	2	558 775	588 253	123-135	366	36
NYVWNGTALAAIEQVNAADGR	P3	C97H149N29O32	3	745.038	754.675	22-24	392	41
VTLTDSER	P3	C37H65N11O16	2	460.738	466.221	8.5-9.7	413	42
AQLASET VR QQLSVGADPLSK	P3 P3	C41H75N13O13 C53H91N15O19	2	479.788 621.838	486.268	14-15.5	421 435	42
SVGADPLSKTSIW	P3	C61H97N15O20	2	680.859	688.336	19.5-20.5	438	45
ADYDLLSQQIIEADTVK	P3	C84H136N20O31	2	961.491	971.461	21-23	456	47
NLPAVTFAQANK	P3 P3	C57H92N16O17	2	637.349	998.497 645.324	16-17.5	402	4/
AQANKAAGGQSETLW	P3	C65H102N20O23	2	766.379	776.348	13.5-15	480	49
AAGGQSETLWHQMYR	P3	C75H111N23O23S1 C98H169N29O35S1	3	578.939 782.409	586.582 792.046	15-17 23.7-24.5	485	49
WSATAGGLVVDADEQDAVIAISSGKPVK	P3	C 122H 198N 32O42	3	928.819	939.453	25-28	523	55
NSSDLPTADAVNYLFGITADDMPGIVSSQK	P3	C135H212N34O49S1	3	1042.836	1054.135	28-29	551	58
GITADDMPGIVSSQKEM EMNSEEEEGELOK	P3	C73H123N19O28S2 C69H102N16O25S1	2	889.919 794.353	899.390 802.329	18-20	586	58
LWNPR	P3		-				596	60
LVENVQNAYFLMVYAR	P3	C89H136N22O24S1	3	644.001	651.312	23-24.5	602	61
QFHSLVASSLAMAK LGVSTR	P3	C66H106N18O19S1	3	497.266	503.248	17.75-21.8	635	64
ACKESYGC	P3						641	64
SIFSSLFK	P6						1	
KKWPLLLIVAIYF	P6						27	4
APYLAGF	P6						42	4
F I GIGGIF SSIATTITPTI TSF	P6	C64H106N14O23	2	720.385	727.363	23.3-24.3	62	7
SGVGSLASTAW	P6	C45H70N12O16	2	518.259	524.240	19.1-19.8	81	g
SGFQSL	P6	0.771 (4000) 400 400 4	0	070.004	004 007	04 7 00	92	9
IAPEETAQL	P6	C5/H100N1001951	2	673.301	061.337	21.7+22	113	12
VTEIGTTVGDIAGTIIGGVAKAL	P6						122	14
ALPGWIWIAAGGLAVWALWPSSDSK	P6	C129H186N30O31 C41H66N8O8S1	3	884.804	894.774	33.1-33.8	143	16
PFPLVKQDPTSKAF	P9	C75H115N17O20	2	787.932	796.406	17.8-19.4	2	1
AFTEASER	P9	C38H59N11O15	2	455.717	461.200	7.2-8.2	14	2
APIGI EGDDAK	P9	C50H78N12O18	2	580.830 552 290	587.310 558.272	18.1-18.6	22 33	3
GDDAKHEF	P9	C39H55N11O15	2	459.701	465.184	5.9-7.0	39	4
VTRQEQAVSVVSW	P9	C65H105N19O21	2	744.894	754.366	17.2-18.5	47	5
KALANIPELA	P9 P9	C58H95N13O17 C57H95N13O13	2	623.856 585,886	630.396 592.346	26.1-26.9 24.4-25.0	60 80	7
MDNILDPLK	P10	C46H79N11O15S1	2	529.781	535.264	19.6-20.6	1	
APFSSEAAAK	P10	C43H67N11O15	2	489.748	495.231	8.5-9.4	10	1
ISTLESOLOPLVK	P10 P13	C6/H151N19O21 C65H114N16O21	2	900.074 728.425	909.545 736.400	34.8-35.8 20.6-21.0	25	4
VKLVATETPGAL	P13	C54H95N13O17	2	599.856	606.336	17.4-18.0	17	2
VAVADOL	D13						29	3
CLEEADBERLYRUB								
GLSSADRSRLYRLLR SI FOAIPK	P13	C39H68N10O13	2	443 256	448 240	135-139	34 49	4
	Init: Ind available Data in red: value below the limit of r The 8 most reactive Met-containing r The 8 most reactive Met-containing r The 8 most reactive periods with UT Uncovered Peptide Uncovered Petide Uncovered Peptide Uncovered Petide Uncovered Peptide Uncovered Peptide	na: not available Data in red: value below the limit of quantificat The S most reactive Met-containing peptides with The S most reactive Met-containing peptides with The S most reactive peptides with UV254 Uncovered Peptide P	na: not available Data in red: value below the limit of quantification, and was repl The S most reactive Med-containing peptides with free chlorine The most reactive Q-so-containing peptides with free chlorine The S most reactive Q-so-containing peptides with red chlorine The S most reactive peptides with UV254 Uncovered Peptide Philo Peptide Celefishing Philo	mit not available         protection           Data in red: value below the limit of quantification, and was replaced by The Smost reactive Med-containing peptides with free chlorine The most reactive Cys-containing peptides with free chlorine The Smost reactive peptides with UV254           Uncovered Peptide         Protection         Protection         Protection           Value Peptide         Protection         Protection         Protection         Protection           Value Peptide         Protection         Protection         Protection         Protection         Protection         Protection           Value Perturbation         Protection         Protection	Pail: Individual Schematic action, and was replaced by an expect           Data in red: value below the limit of quantification, and was replaced by an expect           The most reactive Medicon in peptides with free chlorine           The most reactive peptides with UV254           Uncovered Peptide           Philo Peptides           Philo Peptides           Philo Peptides           Philo Peptides           Promotion         2         met.344           Promotion	Data in ref. value below the limit of quantification, and was replaced by an expected number. The Smot reactive Advectoriating peptides with free chorine The most reactive peptides with three chorine The Smot reactive peptides with three chorine The Smot reactive peptides with UV2s4           Dense reactive peptides with UV2s4         Dense reactive peptides with UV2s4           Unoversed Peptide         Philo Peptides           Dense reactive peptides with UV2s4         Dense reactive peptides with UV2s4           Unoversed Peptide         Philo Peptides         2         66.3         67.3           Todsensor         Philo Peptides         2         66.3         67.3           DUNGGTOAW         Pp         Central Notor         2         67.3         77.3         77.8         78.3     <	Pail: India Valuable         Description         Description         Description           The Sinst reactive Met Containing peptides with free chloring The most reactive peptides with UV2s4         Description         Description           Uncovered Peptide         PPIC_peptides         PPIC_peptides         PPIC_peptides         Description           Proc.         PPIC_peptides         PPIC_peptides         PPIC_peptides         Description         Description           Proc.         PPIC_peptides         PPIC_peptides         2         Mol. 301         Res. 301	Pail: Ind: Valuable         Pail: Ind: Valuable

		Ch	lori	ne,	Pil	P <sub>0.j</sub>									U	V25	4, F	P <sub>i</sub> /P	0.j					
	Rep 1			Rep 2			Rep 3							Rep 1			Rep 2			Rep 3				
0	0.6	1.2	0	0.6	1.2	0	0.6	1.2	$K_{p,j}$	SE of	R^2		0	42	130	0	42	130	0	42	130	Kpj	SE of Kp.j	R'
mg s/L	mg s/L	mg s/L	mg s/L	mg s/L	mg s/L	mg s/L	mg s/L	mg s/L	L/(mg s)	K <sub>PJ</sub>			mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	mJ/cm2	cm2/mJ		
-0.024 log10	0.99 log10	2.4 log10	0.052 log10	0.84 log10	1.9 log10	-0.024 log10	1.7 log10	2.8 log10					0.11 log10	1.8 log10	4.2 log10	-0.053 log10	1.6 log10	4.2 log10	-0.039 log10	1.7 log10	4.2 log10			
1.066	0.521	0.348	0.999	0.617	0.361	0.935	0.485	0.308	0.90	0.07	0.96		1.112	1.047	0.908	0.894	0.656	0.597	0.994	0.906	0.506	0.0033	0.0013	0.47
1.252	0.575	0.374	0.944	0.725	0.467	0.804	0.541	0.372	0.75	0.11	0.87		1.208	1.095	0.990	0.865	0.721	0.593	0.928	0.844	0.489	0.0032	0.0015	0.38
1.379	0.466	0.286	0.787	0.488	0.315	0.834	0.405	0.283	0.99	0.13	0.89		1.108	1.013	0.846	0.989	0.509	0.493	0.903	0.803	0.500	0.0039	0.0016	0.44
1.124	0.529	0.368	1.004	0.658	0.338	0.872	0.482	0.326	0.89	0.08	0.95		1.150	1.098	0.949	0.857	0.697	0.619	0.993	0.842	0.499	0.0031	0.0014	0.41
1.098	0.567	0.353	0.982	0.607	0.323	0.919	0.482	0.309	1.47	0.06	0.97		1.107	1.066	0.896	0.893	0.696	0.630	0.956	0.916	0.461	0.0036	0.0014	0.50
1.289	0.124	na	0.785	0.070	na	0.926	0.070	na	4.07	0.40	0.96		1.181	1.195	0.955	0.800	0.686	0.606	1.019	0.940	0.633	0.0026	0.0014	0.32
1.067	0.396	0.414	0.682	0.642	0.378	1.0924	0.514	0.353	1.25	0.06	0.96		0.658	0.631	0.606	0.885	0.670	0.621	1.592	0.892	0.478	0.0048	0.0015	0.38
1 192	0.370	0.072	1.113	0.392	0.119	0.887	0.256	0.062	2.04	0.24	0.95		1.110	0.969	1.043	0.882	0.664	0.557	1.008	0.891	0.473	0.0033	0.0016	0.37
1.083	0.510	0.333	1.003	0.611	0.340	0.924	0.469	0.281	0.98	0.07	0.97		1.098	1.070	0.930	0.901	0.702	0.627	1.002	0.895	0.491	0.0032	0.0013	0.46
1.085	0.532	0.351	0.995	0.615	0.360	0.920	0.475	0.304	0.90	0.07	0.96		1.130	1.055	0.923	0.875	0.629	0.579	0.995	0.914	0.508	0.0033	0.0014	0.43
1.004			0.000	0.002		0.000			2.00	0.2.1			1.121			0.007			0.000			0.0004		
1.284	0.470	0.326	0.900	0.592	0.355	0.816	0.442	0.310	0.91	0.12	0.90		1.077	1.004	0.905	0.835	0.750	0.639	1.088	0.945	0.459	0.0035	0.0014	0.48
1.216	0.100	na	1.165	0.475	na	0.619	0.100	na	2.90	0.94	0.70		1.248	1.363	1.595	0.787	0.841	0.515	0.965	0.927	0.492	0.0024	0.0026	0.12
1.153	0.545	0.335	0.993	0.629	0.3/5	0.812	0.414	0.280	0.92	0.12	0.90		1.157	1.143	0.993	0.785	0.642	0.503	1.068	0.928	0.513	0.0034	0.0019	0.33
1.093	0.531	0.364	0.994	0.609	0.361	0.913	0.469	0.315	0.88	0.07	0.95		1.118	1.052	0.927	0.873	0.642	0.587	1.009	0.912	0.518	0.0032	0.0014	0.43
1.083	0.529	0.353	0.994	0.605	0.375	0.923	0.472	0.309	0.89	0.07	0.95		1.123	1.068	0.920	0.887	0.642	0.588	0.990	0.912	0.511	0.0033	0.0014	0.44
1.432	0.457	0.326	0.809	0.552	0.312	0.759	0.421	0.294	0.94	0.15	0.85		1.170	1.087	0.965	0.901	0.664	0.610	1 014	0.932	0.502	0.0031	0.0015	0.37
1.077	0.503	0.340	0.962	0.634	0.343	0.961	0.509	0.328	0.91	0.06	0.97		1.116	1.063	0.930	0.852	0.680	0.607	1.032	0.921	0.473	0.0034	0.0015	0.44
1.044	0.524	0.344	1.035	0.639	0.375	0.920	0.481	0.305	0.90	0.07	0.95		1.126	1.045	0.924	0.871	0.636	0.574	1.004	0.915	0.509	0.0033	0.0014	0.44
1.064	0.529	0.365	1.009	0.663	0.389	0.927	0.532	0.362	0.82	0.06	0.96		1.099	1.041	0.955	0.903	0.706	0.612	0.997	0.915	0.491	0.0032	0.0014	0.45
1.110	0.443	0.168	0.000			0.780	0.324	0.360	1.11	0.29	0.79		1.083	1.049	0.952	0.921	0.758	0.682	0.996	0.740	0.577	0.0024	0.0011	0.40
1.194	0.702	0.501	1.260	0.850	0.456	0.545	0.441	0.339	0.66	0.22	0.55		1.315	1.326	1.443	0.722	0.799	0.857	0.963	0.670	0.415	0.0015	0.0026	0.04
1.170	0.359	0.067	0.882	0.281	0.126	0.948	0.285	0.057	2.16	0.19	0.95		0.888	0.751	0.606	0.972	0.740	0.636	1.140	1.013	0.511	0.0042	0.0008	0.79
1.099	0.471	0.177	0.974	0.554	0.211	0.927	0.378	0.140	1.46	0.13	0.96		1.112	1.052	0.906	0.883	0.653	0.590	1.005	0.903	0.489	0.0035	0.0012	0.47
1.648	0.414	0.311	0.701 1.191	0.488 0.691	0.287	0.651	0.184 0.347	0.032	1.55 0.93	0.54	0.54		1.378	1.115	1.141 0.886	0.765	0.604	0.547	0.857	0.878	0.524 0.403	0.0026	0.0021	0.18
1.326	0.604	0.455	0.856	0.560	0.356	0.819	0.457	0.330	0.79	0.13	0.83		1.206	1.138	0.961	0.905	0.777	0.649	0.889	0.864	0.583	0.0026	0.0012	0.38
1.000	0.019	0.404	0.565	0.704	0.411	0.524	0.020	0.401	0.71	0.07	0.54		1.087	1.001	0.525	0.504	0.784	0.009	0.535	0.040	0.000	0.0025	0.0010	0.50
1.553	0.747	0.255	0.830	0.457	0.176	0.617	0.295	0.103	1.43	0.30	0.77		1.262	1.068	1.028	0.859	0.734	0.589	0.879	0.887	0.601	0.0025	0.0014	0.31
1.323	0.708	0.442	0.938	0.605	0.390	0.739	0.443	0.285	0.81	0.16	0.79		1.317	1.262	1.209	0.870	0.691	0.557	0.813	0.764	0.499	0.0027	0.0021	0.18
1.296	0.642	0.421	0.966	0.225	0.126	0.738	0.247	0.151	1.32	0.35	0.67		1.426	1.342	1.162	0.784	0.636	0.565	0.790	0.733	0.543	0.0023	0.0023	0.13
1.007	0.682	0.483	1.017	0.767	0.514	0.966	0.654	0.446	0.63	0.06	0.97		1.081	1.064	0.966	0.957	0.801	0.760	0.962	0.911	0.697	0.0018	0.0006	0.40
1.025	0.688	0.485	1.019	0.762	0.517	0.957	0.650	0.442	0.61	0.04	0.97		1.089	1.073	0.968	0.949	0.794	0.753	0.961	0.919	0.703	0.0017	0.0008	0.40
1.025	0.685	0.484	1.014	0.760	0.510	0.961	0.655	0.447	0.61	0.04	0.97		1.081	1.061	0.965	0.948	0.790	0.749	0.971	0.918	0.699	0.0017	0.0008	0.41
1.040	0.703	0.521	1.006	0.762	0.526	0.954	0.670	0.507	0.55	0.03	0.98		1.086	1.064	0.969	0.958	0.854	0.786	0.956	0.908	0.6/6	0.0017	0.0008	0.41
1.052	0.693	0.520	1.006	0.751	0.536	0.942	0.662	0.506	0.54	0.03	0.97		1.096	1.083	0.995	0.956	0.845	0.782	0.948	0.902	0.693	0.0016	0.0008	0.36
1.021	0.691	0.500	1.011	0.762	0.511	0.968	0.654	0.452	0.60	0.04	0.97		1.098	1.068	0.964	0.935	0.787	0.739	0.967	0.916	0.692	0.0018	0.0008	0.40
0.901	0.212	0.127	1.114	0.189	0.061	0.986	0.091	0.058	2.14	0.32	0.87		0.957	0.921	0.790	1.012	0.792	0.732	1.032	0.950	0.696	0.0023	0.0004	0.82
1.072	0.716	0.690	0.998	0.761	0.465	0.935	0.551	0.421	0.63	0.06	0.94		1.121	1.120	1.04/	0.925	0.781	0.754	0.935	0.917	0.762	0.0015	0.0010	0.25
1.269	0.794	0.687	0.890	0.606	0.483	0.841	0.604	0.434	0.52	0.14	0.68		1.349	1.272	1.199	0.814	0.653	0.629	0.837	0.789	0.604	0.0018	0.0020	0.10
1.330	0.809	0.712	0.870	0.602	0.500	0.800	0.588	0.451	0.49	0.15	0.60		1.366	1.266	1.087	0.731	0.605	0.489	0.903	0.882	0.682	0.0024	0.0021	0.15
1.234	0.778	0.700	0.906	0.607	0.477	0.859	0.574	0.440	0.52	0.13	0.68		1.348	1.303	1.212	0.810	0.660	0.645	0.842	0.797	0.633	0.0016	0.0020	0.08
1.277	0.852	0.750	0.926	0.662	0.499	0.797	0.564	0.450	0.48	0.15	0.58	1	1.387	1.325	1.216	0.776	0.672	0.647	0.838	0.768	0.600	0.0017	0.0021	0.08

	AAVPAIESAIAATPGLVSR	P8		-				9	27
	VSRIAAAIGSKVSPSAIL	P8						25	42
	AAVKSNPVVAGL	P8	C50H88N14O15	2	563.335	570.313	15.2-16.2	43	54
<u>s</u>	SNPVVAGLTLAQIGSTGYDAYQQLLENHPEVAEMLK	P8	C170H270N44O56S1	4	964.991	975.957	31.3-32.2	47	84
	ADEIOPDEIGNLGOVR	P8	C81H122N22O20	2	918.450	000.044	22.7+24	88	10
<u></u>	ADEIOPDEIGNI GOYREELEI VEDAAR	P8	C134H208N38O48	3	1030 839	1042 801	264-30.0	88	11
4	FVGGMSNLIR	P8	C48H80N14O13S1	2	547.295	554.273	17.6-19.0	115	12
ò	QALELDIK	P8	C41H72N10O14	2	465.269	470.253	16.0-17.2	127	13
2	GLKMQLNDMGY	P8	C54H88N14O17S2	2	635.302	642.281	18.2-19.6	137	14
Q	MQLNDMGYR	P8	C46H74N14O15S2	2	564.252	571.231	13.5-14.8	140	14
_	DSAFAVQYSLR	P5	C56H85N15O18	2	628.817	636.295	17.2-18.5	4	1
<u> </u>	ALGOK	P5	0.000 1000 1400 40	0	445.000	404 074	0.05	15	1
<u>.</u>	VRADGVVGSETR	Pb	C50H88N18O19 C20H87N12O17	3	415.890	421.8/1	8-8.5	20	3
õ	ADGVVGSETR AALDALDENOKK	P0	C39P107N13U17	2	490.740	438 559	0.7+0.3	30	3
×	AIVELOALLPK	P5	C56H99N13O15	2	597 876	604 358	23.2-25	44	5
<sup>1</sup>	AQSVGNNR	P5		-				55	6
õ	FTTAEVDSAVAR	P5	C54H87N15O20	2	633.820	641.297	13-16	65	7
Ó.	QFLIPIENF	P5	C55H81N11O14	2	560.806	566.289	24-26	89	9
Ð	VVAGGFETTVSGSF	P5						98	11
~	GLGQFNR	P5	C34H54N12O10	1	791.416	803.379	10.6-12	113	11
≅	QTWDRLRRLGR	P5						120	13
_	NLPAFEEGSAQLNASLYAIGFLYLENK	Pb	C13/H206N32O42	3	991.506	1002.140	29.9-31	131	15
Z	LENRRAT AVEASEK	PD	C39H04N12U12	2	447.246	403.227	4-0.0	104	10
_	THEIAM	P5	C30H59N9O12	2	408.200	412,100	14.5.15.5	170	17
	HNQGAPAAEQY	P5	C50H72N16O18	2	593.268	601.244	8.3-9.5	178	188
	LTSGRLVYPK	P5		_				189	19
	QSEAAVAAVAAAR	P5	C50H87N17O18	2	607.828	616.302	12.5-15.5	199	21
	NLKVKDL	P1	C37H68N10O11	2	415.261	420.245	11-17.6	3	1
	DLNGSAR	P1						8	1
	GLTQAFAIGELK	P1	C57H94N14O17	2	624.353	631.332	20-22	15	2
	KNQLSVGAL	P1	C40H72N12O13	2	465.274	471.256	14-16	26	3
	NQLSVGALQLPLQFTR	P1	C80H133N23O23	2	893.005	904.469	24-25.5	27	4
	TESASM I SELLWEVGK	P1	C80H124N18O26S1	2	893.440	902.412	26-27	43	5
	ADAGGAI SVDELVNOE	P1	C70H111N19025	2	810.908	819.382	20+22	74	00
	HOSTACNPEW	P1	C57H83N17O19S1	2	671 796	679 771	13-16	93	101
	LTAYITGSSNR	P1	C50H83N15O18	2	591.809	599.287	11.5-12.5	106	11
	AIKADAVGK	P1						117	12
	VPPTAILEQLR	P1	C56H97N15O16	2	618.869	626.346	19-21	126	136
	RTLAPSEHELF	P1						136	146
	HHITTDF	P1						147	153
	VCHVLSPLGF	P1						154	163
	ILPUAATVT TATVONEVALVOCVO	PI	0910100000000	2	905 432	004.004	22.6.24	104	101
	ALVDCVRASDI	P1	C50H87N15O18S1	2	609.811	616 789	17-18	185	196
	MLTALSSVDSK	P1	C48H86N12O18S1	2	576.302	582.284	14.5-15.5	198	208
	MLQATEK	P1	C38H63N9O10S1	2	419.728	424.214	12-14.2	209	215
	GALAPALISQHLANAATTAFER	P1	C98H159N29O30	3	741.734	751.372	23.8-28	218	239
	01/50 41/14 20014 71 00	D1	C80H133N23O26	2					
	GNEDANAVVSSVLTIEGK		0001100120020	-	916.997	928.461	31-33	242	255
	LWSPSTPK	P1	C43H66N10O12	2	916.997 458.250	928.461 463.235	31+33 11-12.1	242 260	265
	LWSPSTPK SPSTPKELDPSARL	P1 P1	C43H68N10D12 C64H108N18D23	2 3	916.997 458.250 499.935	928.461 463.235 505.917	31-33 11-12.1 14-15.5	242 260 262	255 267 275
6	UWSPSTPK SPSTPKELDPSARL RNTNGIDQL	P1 P1 P1	C43H68N10O12 C64H108N18O23 C41H71N15O16	2 3 2	916.997 458.250 499.935 515.767	928.461 463.235 505.917 523.245	31-33 11-12.1 14-15.5 11-12.5	242 260 262 276	265 265 275 284
รเ	GIVEDANAVSSVETILLER LWSPSTPK SPSTPKELDPSARL RNTNGIDQL NTNGIDQL NTNGIDQLR	P1 P1 P1 P1	C43H66N10012 C64H108N18023 C41H71N15016 C41H71N15016 C41H71N15016	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 515.767	928.461 463.235 505.917 523.245 523.244 915.209	31-33 11-12.1 14-15.5 11-12.5 10-12.1	242 260 262 276 277 299	256 267 275 284 285 285
ins	UNSPSTK SPSTPKELDPSARL RNTNGIDQL SNLLFLAYQDMVK ADJESPECE SSTUDJEJEANSELSPEC	P1 P1 P1 P1 P1	C43H66N 10012 C64H106N 18023 C41H71N 15016 C41H71N 15016 C74H117N 17021S1	2 3 2 2 2	916.997 458.250 499.935 515.767 515.767 806.924	928.461 463.235 505.917 523.245 523.244 815.398	31-33 11-12-1 14-15.5 11-12.5 10-12-1 23.5-25 25-27	242 260 262 276 277 286 204	265 265 275 284 285 295
eins	LANFDANAWUSSVLILLAR LWSPSTFK SPSTFKELDPSARL RNTNGIDOL NTNGIDOL SNLAFLWYDDMVK AEWFSDEELSSTIIEWFIEAMSEVSPFK KI RPINFTFS	P1 P1 P1 P1 P1 P1 P1	C43H68N10012 C64H108N18023 C41H71N15016 C41H71N15016 C74H117N17021S1 C153H229N31048S1 C58H98N18019	2 3 2 2 2 3 2	916.997 458.250 499.935 515.767 515.767 806.924 1101.212 681.359	928.461 463.235 505.917 523.245 523.244 815.398 1111.515 689.334	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15	242 260 262 276 277 286 304 302	255 267 284 285 295 334 345
oteins	LANF DAWAWNYSYL I LLAN LWYSPTTK SPRTTKELDPSARL RYTTNEIDOL NTTNEIDOLR SNLALFAYQDMVK ACHYFSDELESTIIEWFIEMISEVSPFK KARPINETTSY IGGTSALDHINGOPSHWWY	P1 P1 P1 P1 P1 P1 P1 P1	C43H68N10012 C64H108N18023 C41H71N15016 C41H71N15016 C74H117N15016 C74H117N17021S1 C153H229N31048S1 C58H96N18019	2 3 2 2 2 3 2	916.997 458.250 499.935 515.767 515.767 806.924 1101.212 661.359	928.461 463.235 505.917 523.245 523.244 815.398 1111.515 669.334	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15	242 260 262 276 277 286 304 332 343	265 265 275 284 285 295 335 345 345
oteins	UARTDWARVUSSVLILLAK UWSPSTIK SPSTIKELDPSARL RYTINGIDL SINULFIAVODIVK AEVIFSDELLSSTIRWFIEMSEVSPFK (ARTRIETTS IGDTSJADHINGOPSHVVVY EQDTSJADHINGOPSHVVVY	P1 P1 P1 P1 P1 P1 P1 P1	C43H68N10012 C64H108N18023 C41H71N15016 C41H71N15016 C74H117N17021S1 C153H229N31048S1 C58H96N16019 C70H97N15021	2 3 2 2 2 3 2 2 3 2	916.997 458.250 499.935 515.767 515.767 806.924 1101.212 661.359 742.856	928.461 463.235 505.917 523.245 523.244 815.398 1111.515 669.334 750.334	31-33 11-12_1 14-15_5 11-12_5 10-12_1 23.5-25 35-37 13.5-15 22-24	242 260 262 276 277 286 304 332 343 362	265 267 284 285 284 285 299 334 344 367 373
oroteins	UNICESTRY ASSULTER VALUESTRYKELDER VERTYKELDER VERTYKELDER STURFANDOMK UNICESTRYKELS ALVYSDELSSTIWPIEAMSEVSPRK ULRPINETSY DOGTSADHMAGDSHVWY EDWORKETAF	P1 P	C43H68N10012 C43H68N10012 C64H108N18023 C41H71N15016 C41H71N15016 C74H117N1702151 C153H229N3104851 C58H96N16019 C70H97N15021 C47H76N10014	2 3 2 2 2 2 3 2 2 2 3 2 2 2 2 2 2	918.997 458.250 499.935 515.767 515.767 806.924 1101.212 661.359 742.856 503.284	928,461 463,235 505,917 523,245 523,244 815,398 1111,515 689,334 750,334 508,269	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15 22-24 14.5-16.3	242 260 262 276 277 286 304 332 343 362 369	265 265 284 285 296 335 345 345 361 375 375
proteins	UNISPECTATION ON LEGA SESTEMELOPSAL RITINGIDAL NITINGIDAL SINUERSEESSESSESSESSESSESSESSESSESSESSESSESS		C43H68N10012 C64H108N10012 C64H171N15016 C74H171N15016 C74H171N15016 C74H171N15016 C58H98N16019 C70H97N15021 C70H97N15021 C70H97N15021 C70H97N15021	2 3 2 2 2 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 515.767 806.924 1101.212 661.359 742.856 503.284 458.733	928.461 463.235 505.917 523.245 523.244 815.396 1111.515 669.334 750.334 508.269 468.210	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15 22-24 14.5-16.3 1.3-26 1.3-26	242 260 262 276 304 332 343 362 369 378	265 265 284 285 296 335 345 365 375 375 385
x proteins	UNDERSTRANSISTICALER VALUESTRANSISTERIE VERSTRANSISTERIE VERSTRANSISTERIE STULATIVODUK SULATIVODUK SULATIVODUK VALVRSDELSSTRWITEAMSEVSPRK LARINETTSY EDWORKETTSY EDWORKETTSF EDWORKETTSF EDWORKETTSF EDWORKETTSF EDWORKETTSF		C43H68N10012 C6H1H7N15016 C4H1F7N15016 C4H1F7N15016 C7H1H7N15016 C7H117N15016 C7SH117N15016 C5SH98N16019 C70H97N15021 C47H7RN10014 C3SH61N15014 C5SH61N15014	2 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 515.767 806.924 1101.212 661.359 742.856 503.284 458.733 624.317 624.317	928.461 463.235 505.917 523.245 523.244 815.398 1111.515 669.334 750.334 508.269 466.210 633.296	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15 22-24 14.5-16.3 1.3-26 16.3-17.5	242 260 262 276 277 286 304 332 343 362 369 378 389 378	256 267 284 285 296 333 343 367 377 385 396
ex proteins	UNISCIPATIVA ONLI LON UNISCIPATIVA ONLI LON SISTIMELLO PARAL RINTIGIOLI NITUGIOLIR SIN LE INVOIDINK SIN LE INVOIDINK SIN LE INVOIDINK COSTANIMACORSINNYY EDVO: MATTI EDVO: MATTI PARATIVA LINISNOR FLOVERGER MATTI PARATIVASIANYK		C3H68N10012 C4H68N10012 C6H1H7N15016 C4H1F7N15016 C4H1F7N15016 C7H117N15016 C7H117N1702151 C5BH98N16019 C70H97N15021 C47h78N10014 C3SH61N15014 C3SH61N15014 C3SH61N14019 C8BH141N2102551	2 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 515.767 515.767 515.767 515.767 515.767 515.767 503.224 458.733 624.317 963.014 475.775	928.461 463.235 505.917 523.245 523.244 815.396 1111.515 669.334 750.334 508.269 468.210 631.296 973.462	31-33 11-12.1 14-155 11-12.5 10-12.1 23.5-25 35-37 13.5-15 22-24 14.5-16.3 1.3-2.6 16.3-17.5 24-25.7 6.7.7 c	242 280 262 276 304 332 343 362 369 378 386 397	255 267 284 285 296 333 343 367 377 385 396 415 422
lex proteins	UWBERTER SPETRELDERAL PRITIEDIDL INTEGIDL SPETRELDERAL SPETRELDERAL URAPIELTS URAPIELT		C3H68N10012 C3H68N10012 C6H171N15016 C4H171N15016 C7H171N15016 C7H171N15016 C7H171N1702151 C5H68N16019 C70H97N15021 C7H97N15021 C7H97N15014 C35H61N15014 C35H71N1	2 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 515.767 515.767 515.767 515.767 681.359 742.856 503.284 458.733 624.317 963.014 475.775 562.291	928.461 463.235 505.917 523.245 523.244 815.396 1111.515 669.334 750.334 750.334 750.334 750.269 468.210 631.296 973.482 468.2255 569.269	31-33 11-12.1 14-15.5 11-12.5 10-12.1 23.5-25 35-37 13.5-15 22-24 14.5-16.3 1.3-2.6 16.3-17.5 24-25.7 6.7-7.5 10-12.1	242 260 262 276 277 286 304 332 343 362 369 378 386 389 378 386 397 414 418	255 267 275 284 285 299 332 342 367 377 385 396 415 421 421
plex proteins	UNISOFTIC SOLLEGE UNISOFTIC SOLLEGE SPETRELOPSAL SPETRELOPSAL SILLESSTEWFEAMSEVSPK ALPRSELESSTEWFEAMSEVSPK EAVERSELESSTEWFEAMSEVSPK ETAFTPK ETAFTPK ETAFTPK EAVERSELESSTEWFEAMSE EXAMPSION		C34H68N 10012 C64H108N 10012 C64H171N15016 C41H71N15016 C74H1171N15016 C74H1171N15016 C74H1171N1502151 C158H98N16019 C70H97N15021 C70H97N15021 C35H68N15014 C35H68N15014 C35H68N15014 C35H68N15014 C35H68N15014 C35H68N15014 C64H178N14019 C64H1080202781	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 499.935 515.767 806.924 1101.212 661.359 742.856 503.284 458.733 624.317 963.014 475.775 562.291 825.381	928.461 463.225 505.917 523.245 523.244 815.396 1111.515 669.334 750.334 508.269 468.210 631.296 973.462 482.255 569.269 836.347	31-33 11-121 14-155 11-125 10-121 235-25 35-37 135-15 22-24 14.5-16.3 1.3-2.6 16.3-17.5 24-25.7 6.7-7.5 10-12.1 9.5-10.2	242 260 262 276 277 286 304 332 343 362 369 378 386 399 378 386 397 414 418 422	255 267 275 284 285 296 332 342 342 342 342 342 342 342 342 342
nplex proteins	UNIVERSITY ON LIGH UNIVERSITY ON LIGH PSTPTELOPSAL PSTP		C43H68N 10012 C41H71N15018 C41H71N15018 C41H71N15018 C41H71N15018 C41H71N15018 C58H28N16019 C70H97N15021 C70H97N15020 C70H97N15021 C70H	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	916.997 458.250 459.335 515.787 515.787 506.924 1101.212 661.359 742.856 503.284 458.733 624.317 963.014 475.775 562.291 825.381 638.005	928.461 463.225 505.917 523.245 815.396 1111.515 669.334 750.334 508.289 468.210 631.286 973.482 468.210 631.286 973.482 468.247 697.313	31-33 11-121 14-155 11-125 10-121 23.5-25 35-37 13.5-15 22-24 14.5-163 1.3-26 16.3-17.5 24-25.7 6.7-7.5 10-12.1 9.5-10.2 20-22	242 260 267 277 286 304 332 343 362 369 378 386 399 378 386 397 414 418 422 428	255 267 275 284 285 299 332 344 367 377 385 396 415 427 427 437 447
mplex proteins	UNISOFTIC SOLLEGE UNISOFTIC SOLLEGE SPETRELOPSAL SPETRELOPSAL SITURGIDUL INTRIGIDUR SINUFICIONS SINUFICIONS SINUFICIONS SINUFICIONS EDVERSIGNE SINUFACTOR ELOVERSIGNE SINUFACTURIS ELOVERSIGNE SINUFACTURIS SINUFACTU		C43H8N10012 C4HF7IN15018 C4HF7IN15018 C4HF7IN15018 C4HF7IN15018 C5HF8IN16019 C70H97N15021 C4HF7IN15014 C5SH6N16019 C70H97N15024 C4F7FN16014 C5SH6N145014 C5SH6N145014 C48H141N250451 C48H141N250451 C48H141N250451	2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	91618/99/ 458.250 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 515.767 542.856 503.284 458.733 624.317 963.014 475.775 862.291 825.281 829.004 859.004 859.014	508.4401 443.225 505.917 523.245 523.245 523.245 523.245 523.245 750.334 750.334 750.334 750.334 750.334 750.334 750.334 750.334 508.269 836.240 836.347 607.313 604.624	31-33 11-121 11-121 11-125 110-125 10-121 225-25 35-37 13-5-15 22-24 14-5-18.3 1.3-26 16.3-17.5 24-25.7 6.7-7.5 10-12.1 9.5-10.2 20-22 22-24	242 260 267 277 286 304 332 343 362 369 378 386 399 378 386 397 414 418 422 428 448	255 265 275 284 285 296 332 342 365 373 377 385 396 415 421 421 435 445
omplex proteins	UNIVERSITY OF AN OUTLAND		C49469110012 C414771115016 C414771115016 C414771115016 C159420411701702151 C159420410019 C704970115021 C39469116019 C39469116019 C39469116014 C3946911401420251 C4847170115012 C4847170115012 C4847170115012 C784112620151	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	91818/997 458.250 459.205 515.767 515.767 515.767 661.359 742.856 651.359 742.856 651.359 651.359 742.856 651.359 651.359 742.856 651.359 652.251 652.251 652.251 655.251 659.7314 455.367	528.4401 483.225 505.917 523.244 815.386 1111.515 689.334 508.289 466.210 631.296 466.210 631.296 466.210 631.296 466.210 631.296 836.347 687.313 604.624 501.246	31-33 11-12-1 14-15.5 11-12.5 10-12.1 23.5-25 36-37 12.5-15 22-24 14.5-16.3 1.3-2.6 16.3-15.5 22-24 14.5-16.3 1.3-2.6 16.3-15.7 5 10-12.1 20-22 24-25.7 9.5-10.2 20-22 22-24 22-25 22-24 22-25 22-25 22-25 22-25 22-25 22-25 22-25 22-25 22-25 24 22-25 24 22-25 2	242 260 267 277 286 304 332 343 369 378 386 397 414 418 428 428 448 463	255 267 286 286 299 332 342 342 342 342 342 342 342 342 342
complex proteins	UNISOPTIAL ON LEAR UNISOPTIAL OPERAL ENTINGID CL INTRIGID CL INTRI		C39469110012 C4811108118023 C481171115018 C7481171115018 C74811711170218 C784197115018 C784197115021 C784197115021 C784197115021 C7841971115021 C7841971115021 C784197111502 C784197111502 C784197111502 C784110812020751 C6871141112202451 C6871141112202451 C6871141112202451 C6871141112202451	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918(89/ 498,250 499,235 515,787 515,787 515,787 742,856 503,284 458,733 624,317 458,733 624,317 562,291 683,014 475,775 562,291 689,005 597,314 495,287 428,215	528.4401 483.225 505.917 523.244 815.398 1111.515 669.334 750.334 508.289 468.210 631.296 631.296 631.296 631.296 631.296 697.313 604.624 509.289 838.342 607.313 604.624 501.2445 501.2445 501.2445 5	31-33 11-121 11-121 11-121 11-125 11-125 35-37 135-15 22-24 14.5-16.3 1.3-26 16.3-17.5 24-25.7 6.7-7.5 10-121 9.5-10.2 20-22 22-24 8.5-9.3 8.5-9.6	242 260 267 277 286 304 332 343 369 378 386 397 414 418 428 428 448 448 463 472	255 267 267 286 299 332 342 342 342 342 342 342 342 342 342
e complex proteins	UNIVERSITY OF AN OLLEAN UNIVERSITY OF AN OLLEAN STRAELOPSAL RITINGIOL SILLE AVACIONY SILLE AVACIONY SILLE AVACIONY SILLE AVACIONY SILLE AVACIONY SILLEAN SILLE		C29499110012 C41177115018 C41177115018 C74117115018 C74117115018 C7411711700215 C1594220104851 C594980140019 C70497115021 C47170115021 C47170115021 C4817111502 C481711502 C4817111502 C4817111502 C4817111502 C4817111502 C4817111502 C481711502 C481	2 3 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2	9168.09/ 458.250 459.305 515.767 806.624 1101.212 661.359 742.856 503.284 458.733 623.317 963.014 475.775 562.291 825.381 869.005 597.314 495.267 493.215 803.437	528.441 483.225 505.917 523.244 815.398 1111.515 689.334 750.334 508.289 466.210 631.296 635.296 635.2	31-33 11-121 11-121 11-125 11-125 10-121 235-35 35-37 135-15 22-24 145-163 1-32-6 163-175 24-257 67-75 24-257 67-75 24-257 67-75 24-257 67-75 24-257 67-75 10-121 9-5102 20-22 22-24 85-89 35-56 14-18	242 260 262 276 277 286 304 332 343 362 369 378 389 377 397 397 397 397 397 397 397 397 39	255 267 286 286 296 332 342 361 377 385 396 415 421 421 421 447 447 447 447 447 447 447
se complex proteins	UNISOTIAN SOLLEAR UNISOTIAN SOLLEAR SPETRELOPARE RITINGIDAL ITINGIDAL SINUERSESSIW/FEMILEMISEVSPK LARNETSY EDVS AVELTSY EDVS AVELTSY ED		C39469N10012 C48H109N10023 C4H177N15018 C74H17N15018 C74H17N170218 C78H39N16019 C78H39N16019 C78H39N16019 C78H39N16019 C78H39N16019 C3946H115014 C3946H115014 C3946H115014202751 C48H108N14017 C48H108N14000 C48H108N1400 C48H108N140000 C48H108N140000 C48H108N140000 C48H108N1400000 C48H108N140000000000000000000000000000000000	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	91618/99/ 458.250 459.305 515.767 806.824 1101.212 661.359 742.856 503.284 458.733 824.317 963.014 458.733 824.317 963.014 458.755 822.91 859.314 485.267 438.215 803.437	528.441 483.225 505.917 523.244 815.368 1111.515 689.334 750.334 750.334 750.334 750.334 750.334 689.334 750.334 689.334 897.342 883.547 887.313 604.624 683.649 883.5401	31-33 11-121 11-121 11-121 121-121 125-25 35-37 13-5-15 22-24 13-5-16 13-26 13-26 13-26 13-26 13-26 13-27 10-121 20-22 22-24 85-9.3 5-5-6 1.4-1.8	2422 2600 2276 3042 362 362 362 362 362 362 362 362 362 36	256 265 275 284 285 299 332 342 365 373 385 396 415 422 422 422 433 443 445 445 445 445 445 455 555
ase complex proteins	LIVESTICATION CALLER LIVESTICATION CALLER SETTICLOPERAL ENTRODOL NITHODOL SALLERANCTAN SALLERA		C481608110012 C4811708118023 C411771115016 C741171115016 C741171170170215 C581628116013 C4717181105014 C4717181105014 C4717181105014 C581618114017 C581618114017 C581618114017 C581618114017 C68114182202751 C781128202645 C781128220261 C481771115012 C48171811200351 C781128220261 C48172811201501 C781128220261 C38168112015016 C3816811201501	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	91618/99/ 458.250 459.2005 515.767 808.524 1101.212 661.359 742.856 503.284 458.733 742.856 503.284 458.733 742.856 503.284 458.733 742.856 503.284 458.273 562.291 863.005 597.314 495.287 436.215 803.437	9283.4401 443.225 505.917 523.245 523.245 523.244 815.532 41111.515 669.334 750.334 750.334 750.334 750.334 973.462 462.209 631.296 734.296 744.196 744.196	31-33 11-121 11-121 11-125 11-125 10-121 225-23 36-37 13-5-15 22-24 14-5-163 13-26 163-17-5 10-12-1 9-5-102 20-22 22-24 85-93 55-6 1-4-1.8	2422 2600 2776 2777 2866 3042 343 362 3699 3977 414 418 4488 4488 4488 4488 4494 4521	255 265 275 284 285 299 332 361 373 377 385 396 415 421 421 421 421 431 445 451 574 574
ase complex proteins.	LWBSPTRA STORAGE SPETRALDORAL SPETRALDORAL SPETRALDORAL SPETRALDORAL SPETRALDORAL SPETRALDORAL SPETRALDORAL SPETRALS SPE		C-591699110012 C-68141059119023 C-4114771115018 C-4114771115018 C-411477115018 C-411477115018 C-411478115018 C-471478116014 C-55188114012 C-471478115012 C-55188114012 C-55188114012 C-55188114012 C-55188114012 C-55188114012 C-55188114012 C-551822 C-55182 C-551822 C-551822 C-551822 C-551822 C	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	916396 458.250 459.305 515.767 515.767 515.767 500.524 601.529 742.856 503.284 458.733 624.317 963.014 458.733 624.317 963.014 475.775 562.291 669.052 673.14 495.287 438.215 603.437	528.4401 453.225 505.917 522.245 522.245 522.244 815.365 1111.515 689.334 750.334 508.234 750.334 508.234 750.334 508.246 973.442 973.442 973.442 973.445 989.299 863.347 667.514 646.24 501.248 444.196 815.401	31-33 11-121 11-121 11-121 11-121 121-121 235-37 13-5-15 22-24 13-5-15 22-24 13-2-16 13-2-16 13-2-16 13-2-17 20-22 20-20	2422 260 277 286 277 286 304 343 362 343 362 343 362 399 414 418 418 422 428 448 463 472 475 488 494 528	255 267 277 284 285 295 334 367 377 385 396 415 427 427 427 447 447 447 447 447 447 457 547 547 54
erase complex proteins	UNISPETTA STEPALOPARA ENTREDORAL SETTALOPARA ENTREDOL SILLEYACOMWE ALSOUTEST SILLEYACOMWE ALSOUTEST SILLEYACOMWE ALSOUTEST SILLEYACOMWE ALSOUTEST SILLEYACOM ENTRE SILLEYACOM SILLEYACOM ENTRE SILLEYACOM SILLEYACOM ENTRE SILLEYACOM S		C-341609110012 C-6411078119023 C-411771115016 C-7411178115016 C-7411178115016 C-741178115016 C-741178115016 C-741178115014 C-751168114017 C-551168114017 C-551168114017 C-561114112020151 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-781112020051 C-7811020051 C-7811020051 C-7811020051 C-7811020051 C-7811020050 C-7811020050 C-7811000	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9103.940 458.250 459.250 515.767 515.767 806.524 661.369 742.656 503.284 458.733 653.374 458.215 889.005 <b>597.314</b> 455.251 889.005 <b>597.314</b> 455.251 889.015 <b>597.314</b> 455.251 455.251 803.437	5263.4401 453.225 505.917 523.245 523.245 523.244 815.336 915.336 903.34 750.334 750.334 750.334 750.334 973.452 973.5572 973.5572 973.5572 973.5572 973.5572 973.5572 97572 97575777777777777777777777777	31-33 11-12-1 14-155 11-12-5 10-12-1 23.5-37 13.5-15 22-24 14.5-18.3 1.3-26 10.12-1 9.5-10.2 20-22 22-24 8.5-9.3 5.5-6 1.4-1.8 16.5-18 20-22	2422 2600 2622 2776 304 3332 343 3629 378 369 378 369 378 369 378 369 378 369 378 369 378 414 418 422 448 448 463 472 472 476 494 452 452 452 452 452 452 452 452 452 45	255 267 277 284 285 295 334 367 377 385 396 415 427 427 437 447 447 447 447 447 447 447 517 526 541 564
nerase complex proteins	LWEETEN SILLEAN UNE STANSIE LEAN SPETRELIPSEAL ENTRODOL NITHOGOL N		C-6814008110012 C-6814108118023 C-411471115018 C-411471115018 C-41147115175018 C-41147115175018 C-471470115012 C-471470115012 C-471470115012 C-471470115012 C-471471115012 C-471471115012 C-471471115012 C-471471115012 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112020451 C-471411112015 C-471411112015 C-471411112015 C-471411112015 C-471411112015 C-471411112015	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9163.990 458.250 459.355 515.767 505.787 808.624 1101.212 661.352 661.352 661.3284 458.733 458.734 458.735 662.317 963.014 475.775 562.291 863.014 475.775 562.291 863.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.014 475.775 873.0174 875.775 873.0175 875.0175 8	9263.4401 443.225 505.917 523.245 815.386 815.386 815.386 81131.515 689.284 468.210 631.286 631.286 631.286 631.286 631.286 836.347 837.313 604.624 501.248 442.255 589.286 836.347 837.313 804.624 501.248 445.245 836.347 837.313 835.4211 835.4211 835.421 835.4214	31-33 11-121 11-121 14-155 11-155 11-155 11-155 10-121 22-24 13-515 13-515 22-24 13-26 13-26 13-26 13-26 13-26 13-26 13-26 14-18 18-5-18 20-22	2422 2600 2776 304 332 362 362 369 3786 362 369 3786 362 369 3786 362 369 3786 362 369 3786 362 369 3786 362 369 3786 414 416 422 426 426 426 426 426 426 426 426 42	255 267 278 286 299 332 342 367 377 386 339 342 433 447 422 433 447 447 447 447 447 547 547 5580
merase complex proteins	UNISPETTAL ORAL LEAR UNISPETTAL ORAL CANADAL SINTAGEDOLR SINTAGEDOLR SINTAGEDOLR SINTAGEDOLR SINTAGEDOLR SINTAGEN CONSTRUCTION CONSTRUC		C-591609110012 C-68141029116023 C-4114771115018 C-7441171115018 C-7441171115018 C-744117115018 C-744117115018 C-74417115018 C-74417115014 C-744771115014 C-744771115014 C-744771115014 C-744771115014 C-744771115014 C-744772115014 C-744772115014 C-744772115014 C-744772115014 C-744772115014 C-744772115014 C-744772115015 C-74477215015 C-744772150015 C-7447721500000000000000000000000000000000000	2 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9163.997 458.250 459.355 515.767 555.767 506.624 101.212 661.359 742.856 624.317 963.014 475.775 562.291 863.014 475.775 562.291 863.014 475.775 562.291 863.014 475.775 562.291 863.045 475.775 562.291 863.045 475.775 562.291 863.045 475.775 562.291 863.045 475.775 562.291 863.045 475.775 562.291 863.045 577 485.267 485.267 485.267 485.267 485.267 485.275 475.2755 475.2755 475.27555 475.2755555555555555555555555	9268.481 463.225 505.917 523.245 815.386 1111.515 669.334 750.334 750.334 750.334 750.334 604.624 501.246 805.3129 604.624 501.246 815.401 732.383 855.432 415.164	31-33 11-121 11-121 11-125 11-125 10-121 22-24 14.5-16.3 13-25 24-25.7 10-121 8.5-10.2 20-22 22-24 14.5-16.3 13-26 8.5-9.3 8.5-9.5 10-121 8.5-9.5 10-121 8.5-9.5 10-121 8.5-9.5 10-121 8.5-18 20-22 7.6-9	2422 2600 2776 304 332 343 362 369 378 3969 378 3969 378 3969 378 3969 378 3969 378 3969 378 3969 378 414 418 422 428 428 428 428 428 428 521 528 527 557 567 557 567 567 567 567 567 567 56	286 286 287 288 288 288 288 288 288 333 342 367 337 337 337 337 337 338 339 418 422 433 445 422 433 445 445 545 545 558 564 580 581
/merase complex proteins	LINESPECTATION DIL LEAR LINESPECTATION DIL LEAR PETTYALLOPARAL PETTYALLOPARAL PATTAGOLE NITAGOLE SILALJANDON SILALJ		C-591609110012 C-6911109118023 C-111711115016 C-111711115016 C-111711115016 C-111711115016 C-11171115016 C-11171115016 C-11171115016 C-11171115016 C-11171115017 C-691110911 C-691110911 C-691110912 C-69110002 C-	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9163997 458.250 459.350 515.767 555.767 568.524 1101.212 568.524 4101.212 563.284 458.733 458.735 562.291 562.291 562.291 562.291 562.291 562.291 683.005 567.314 445.267 438.215 803.437 723.391 410.677 410.677 433.740	9283.4401 4453.225 505.917 523.245 815.398 1111.515 609.334 750.345 750.345 75	31:33 11:121 14:155 10:121 12:55 13:5-15 22:24 14:5-163 13:26 10:3:175 24:257 10:121 22:24 14:5-163 13:26 10:3:175 24:257 10:121 20:22 22:24 8:593 5:56 1.4:18 10:5-18 20:22 22:24 8:593 5:56 1.4:18	2422 2600 2776 2777 2776 2786 304 3323 3602 3609 3788 362 369 3786 369 3786 444 448 4486 4486 4486 4486 4486 4521 488 4522 488 4522 488 4941 528 562 562 562 562 562 562 562 562 562 562	255 265 275 284 285 299 332 342 365 3365 3365 3365 3365 3365 3365 415 422 433 445 445 445 545 545 554 554 554 566 567 567 567 567 567 567 567 567 567
lymerase complex proteins	UNISPETTA STEPAL DEVAIL SETTAGE DEVAIL ENTRODOL STILLEDAD SILLE AVOODNY SILLEDADON SILLE		C494981002 C4147111506 C4147111506 C4147111506 C4147111506 C4147111506 C4147111506 C4147111506 C4147111507 C414711507 C414	2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9163.997 458.250 459.035 515.767 515.767 808.564 1101.212 808.564 41101.212 808.324 458.733 624.317 953.014 475.775 552.291 825.381 825.381 826.317 455.267 455.267 455.267 455.267 455.267 455.267 455.275 455.261 455.275 457.275 455.275 457.2757 457.2757 457.2757 457.2757 457.2757 457.2757 457.2757 457	908.4401 443.225 505.917 523.245 815.398 1111.515 699.239 468.210 631.286 973.442 482.225 599.289 808.347 697.343 604.624 501.246 815.401 732.253 855.432 444.196 815.401	31:33 11:12:1 14:155 11:12:5 10:12:1 22:24 13:5-16 13:5-16 13:25 10:12:1 9:5-10:2 20:22 22:24 8:5:93 5:5:6 1.4:18 10:5:16 20:22 7:6:9 4:5:6:5 20:23	2422 2420 2622 2767 2866 3042 343 3622 3699 3937 3967 3967 3967 3967 3967 3967	255 265 275 284 299 332 342 367 377 365 396 418 422 433 396 418 422 433 396 418 422 433 5396 418 422 433 545 547 554 554 554 560 560 560 560 560 560 560 560 560 560
olymerase complex proteins	LIVESSITE UNISSISTER SPETPRELOPSAL SPETPRELOPSAL SINLAJSKOON SINL			2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9163997 458.250 459.035 515.767 505.777 508.524 1101.212 508.524 458.733 504.317 563.014 475.775 563.014 475.775 563.261 475.775 563.261 475.775 563.231 668.005 567.314 455.267 438.215 803.437 723.391 446.127 410.677 433.740	9283.4401 4453.225 505.9177 523.245 815.398 8115.398 8115.398 8115.398 8115.398 8115.398 8115.398 8115.398 8115.398 8115.398 8115.302 813.298 973.482 973.482 973.482 969.347 604.624 501.248 444.196 815.401 815.401 815.401	31:33 11:121 14:155 11:125 10:121 12:23 13:515 22:24 14:5163 13:26 163:175 24:257 8:775 10:121 20:22 20:20 20:22 2	2422 262 2766 2876 304 362 362 362 362 362 362 362 362 362 362	255 265 275 284 288 299 332 342 363 342 363 343 357 365 356 415 422 433 445 445 451 545 545 556 560 611 635
Polymerase complex proteins	UNISPECTACIONAL LEAR UNISPECTACIÓN DE LEAR SETTRELLOPARLE ENTREDIDAL SITUEJOLA SITUEJOLA SITUEJOLA SITUEJOLA SITUEJOLA CONSTRUCTURA EDVORANTA EDVORANTA SITUEJOLA SITU		C494981002 C41177115036 C41177115036 C41177115036 C41177115036 C41177115036 C39498110034 C39498110034 C49498110034 C49498110034 C49498110035 C4947111505 C4947111505 C4947111505 C4947111505 C4947111505 C4947111505 C494711005 C4947111505 C494711505 C49471	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	916.8997 458.250 459.035 515.767 515.767 508.564 1101.212 665.532 742.656 503.284 458.733 624.317 953.014 475.775 552.291 825.381 829.037 438.215 803.437 445.267 438.215 803.437 445.267 438.215 803.437 445.267 433.740 845.237 445.267 433.740 845.237 445.247 445.257 445.2777 445.2777 445.2777 445.27777 445.277777777777777777777777777777777777	9283.4401 443.225 505.917 523.245 815.398 1111.515 609.334 508.229 466.210 631.226 973.482 452.225 589.229 836.347 604.624 501.246 873.43 604.624 501.246 815.401 732.263 865.432 444.196 815.401	31:33 11:121 11:121 14:155 11:125 10:121 21:52 10:121 22:24 11:55 10:121 22:24 11:55 10:121 22:24 11:55 10:121 95:102 20:22 22:24 8:59.3 5:56 11:41 20:22 10:55 11:555 11:55 1	242 242 242 242 242 242 242 242 242 242	255 265 277 268 299 333 342 366 377 377 388 399 419 42 433 442 433 442 433 442 433 445 511 522 545 568 568 600 611 639 655
Polymerase complex proteins	LIVERSTYL UNERSTYL SPETPHELOPSAL SPETPHELOPSAL SILLEJAVCDAWNEPAL		C49449041002 C41471141030 C41471141030 C41471411030 C41471411030 C41471411030 C41471411030 C414714100 C41471400 C414714000 C414714000 C414714000 C414714000 C414714000 C414714000 C414714000 C414714000 C414714000 C414714000 C4147140000 C4147140	2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918.997 498.250 499.035 515.767 515.767 515.767 515.767 500.224 1101.212 661.359 500.224 500.224 500.224 500.224 500.224 61.359 624.317 624.317 624.317 625.291 625.29	928.401 443.225 505.917 523.245 815.386 815.386 815.384 750.2344 815.385 608.334 750.2344 815.401 831.245 808.347 607.634 806.347 607.634 815.401 815.401 835.432 855.432 415.164 439.223 588.226 641.280	31:33 11:12:1 11:12:1 11:12:5 11:12:5 10:12:1 22:52:5 33:37 12:5-15 12:5-15 12:5-16 13:5-16 22:24 25:52 14:5-18 20:22 7:8-9 4.5-65 20:23 19:4-20:4	2422 2422 2767 2866 304 3342 3342 3362 3378 3366 3378 3366 3378 3367 414 418 448 448 448 4472 4475 4475 4475 4475 463 463 542 5677 601 605 638 646 658	255 265 277 278 288 299 333 342 367 377 388 399 411 422 433 441 422 433 442 433 442 455 545 558 568 568 600 611 639 644 658 668
Polymerase complex proteins	LWEETEN DUILLEN UNE STERKLIDSEN STETRELIDSEN UNT GOLD UNT			2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918.89/7 448.250 448.250 452.502 515.767 515.767 515.767 515.767 515.767 515.722 661.359 742.856 503.284 458.733 624.317 963.014 475.775 562.291 853.014 475.775 862.317 863.014 475.775 862.317 448.215 803.437 723.391 848.127 448.215 803.437 448.215 803.437 848.127 448.215 803.437 848.127 848.1	9283.4401 443.223 505.0116 523.244 523.244 505.306 1111.515 699.334 508.239 466.210 631.286 631.286 631.286 631.286 508.299 836.347 697.313 604.624 501.246 442.255 599.299 836.347 697.313 604.624 501.246 836.347 637.313 655.432 445.1104 439.223 588.226 641.280	31-33 11-12-1 11-12-1 11-12-1 11-12-1 11-12-1 11-12-1 11-12-1 35-37 13-5-15 12-24 13-5-15 12-24 13-5-15 12-24 13-5-15 12-24 13-5-16 12-22 12-24 14-5-18 12-22 7.6-9 14-5-18 12-22 19-4-20.4 15-5-17	2422 2420 2622 2767 2866 3044 332 343 3629 3788 3699 3788 3699 3788 3699 3788 3699 3788 3699 3788 4148 4422 4284 4633 4725 4888 4521 567 581 6066 6155 581 6066 6155 581	256 266 277 268 299 332 366 377 377 377 377 377 377 377 377 377
Polymerase complex proteins	LIVERSTYLESSENSE SPETTRELOPSAL SPETTRELOPSAL SPETTRELOPSAL SILLEJAVCDAVK		C444891402 C414711415016 C414714115016 C414714115016 C414714115016 C4157122411014501 C4157122411014501 C415712411014501 C4147114141141 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141501 C41471141141141141 C4147114114114114114114114114114114114114	2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918.997 498.250 498.250 515.767 515.767 515.764 1101.212 661.353 661.353 673.2856 403.274 405.775 562.291 824.317 953.014 475.775 562.291 825.381 869.005 597.314 495.267 433.215 803.437 723.391 446.127 433.216 81.317 631.317 631.317 631.317	9283.4401 443.225 505.0177 523.245 815.398 815.398 815.398 93.344 815.398 93.344 969.334 969.334 97.90.334 97.90.334 97.90.234 97.3482 482.255 599.299 8363.47 697.313 805.4524 504.624 504.624 504.624 515.401 7722.363 855.432 415.164 439.223 586.247 673.399 673.999	31:33 11:12:1 14:155 11:12:5 12:5:25 33:37 12:5:15 12:5:26 13:5:15 12:26 13:5:15 13:5:16 13:26 13:27 10:12:1 20:22 22:24 8:5:93 14:18 16:5:18 20:22 7:6:9 4:5:65 12:20 20:22 7:6:9 4:5:65 12:42:24	2422 2420 2622 2767 2866 3323 3433 3423 3423 3423 3423 3423 34	256 267 277 268 299 332 367 377 377 377 377 377 377 377 377 377
Polymerase complex proteins	LIVESPETA EVENENCE SPETARELOPERAL ENTINGICA INTIGICA UNITIGICA UNITIGICA ENTINGICA ENTINGICA ENTINE ENTITIE ENT			2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918.89/7 448.250 448.250 448.250 455.707 515.707 515.707 515.707 515.707 515.707 515.707 515.707 515.207 742.856 503.284 458.733 624.317 983.014 475.775 552.291 825.381 888.005 677.344 485.257 803.457 445.275 803.457 445.275 803.457 445.275 803.457 445.275 803.457 445.275 803.457 81.317 833.291 843.215 833.291 843.215 833.291 843.215 833.291 843.215 833.295 853.205 853.295 855.295 855.20	203.441 443.227 505.0176 502.0176 502.0176 502.2024 502.224 502.224 502.2324 502.2324 502.2324 502.235 508.299 508.299 505.429 466.210 603.224 503.246 503.247 503.246 503.247 503.245 503.247 503.245 503.247 503.245 503.247 503.245 503.247 503.245	31-33 11-12-1 11-12-1 11-12-1 11-12-1 11-12-1 11-12-1 35-37 13-5-15 12-24 14-5-16 13-17-5 10-12-1 9-5-10-2 20-22 22-24 8-5-9.3 5-5-6 1.4-1.8 10-5-16 20-22 7.8-9 4.5-6.5 20-23 19-4-20.4 155-17 20-5-16 20-5-17 20-5-1	2422 2420 2622 2767 2866 3044 332 343 3629 3786 3369 3977 414 4422 4468 4463 4472 4755 418 4464 4632 4475 5677 5871 5867 5872 5875 5876 5866 6015 6036 6056 6056 6056 6056 6056 6056 605	2265 2267 2277 228 2282 2292 3343 346 337 336 337 336 337 336 337 336 339 411 42 42 42 42 42 42 42 42 42 43 44 45 56 56 58 58 58 56 56 60 1 63 67 70 57 70 70 70 70 70 70 70 70 70 70 70 70 70
Polymerase complex proteins	UNISPETTANO DI LEAN UNISPETANO DI LEAN UNISPECTANO DI LEAN UNISPECTANO DI LEAN UNISPECTANO DI LEAN UNISPECTANO UNI			2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	918.890 448.200 448.200 448.200 448.200 458.200 515.767 700.604 1101.212 661.369 742.666 503.284 458.733 624.317 961.014 458.733 624.317 961.014 458.733 669.005 997.314 458.215 803.433 745.757 453.249 455.267 458.215 803.433 745.757 453.249 743.340 551.257 553.256 554.301 553.256 554.301	203.49(1) 203.29(5) 203.29	31-33 11-121 11-121 11-125 11-121 235-25 35-37 13-5-15 22-24 14-5-163 13-26 4-37-75 105-112 23-22 23-22 23-24 8-5-83 1-4-18 20-22 7.8-9 4-5-85 20-22 19-4-20.4 15-5-17 20-5-22 10.8-11.4 8-1-65	2422 2420 2622 2767 2866 3323 343 342 362 369 3362 362 369 337 362 369 337 44 418 422 4463 472 475 4475 463 463 463 463 666 668 668 668 668 668 668	256 266 267 277 288 299 334 36 377 377 389 341 42 42 42 43 44 44 42 42 42 43 44 44 45 51 52 54 54 55 58 60 61 61 58 63 64 67 700 770 770 770 770 770 770 770 770
Polymerase complex proteins	LWEEPTRY UNESTRY PETPYELDPARL ENTRODUC INTRODUC			2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	910 List/2012 910 Li	2023.045 2023.045 2023.045 2023.045 2023.044 815.3086 11111.515 699.334 750.334 2033.045 2033.04 401.026 973.402 2033.04 401.026 973.402 2055 2093.209 2033.41 2013.04	31-33 1 14-165 14-165 10-12-1 22-24 13-5-15 22-34 13-5-15 22-34 13-5-15 22-34 13-5-15 22-34 13-5-15 22-34 23-57 67-75 10-12-1 9-5-102 20-22 22-24 85-93 55-6 85-93 55-6 13-16 14-18 10-12-1 10-5-18 20-22 7-6-9 45-65 20-23 19-4-20-4 15-5-17 20-5-14 81-95 10-8-14 10-8-14 10-8-14 10-8-14 10-8-14 10-8-14 10-12-14 10	2422 2420 2626 2777 2866 3423 3423 3423 3423 3423 3423 3423 34	2265 2667 277 288 2892 2892 2892 2893 3333 343 366 3377 3377 3377 3383 349 411 422 433 441 422 433 442 433 442 432 432 447 456 547 554 556 566 667 667 667 667 677 697 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 70
Polymerase complex proteins	UNISPETTAL ONLIGAN UNISPETATA ONLIGAN SETTATAL OPARAL ENTRODOL NITHODOL SALUE JAYODAWA LANDAGA EDITATA EDITATA EDITATA EDITATA EDITATA EDITATA ETA SETTATA ETA SETA EDITATA ETA SETA EDITATA ETA SETA EDITATA ETA SETA EDITATA		C49449041022 C41471113020 C41471113020 C41471113020 C41471113020 C41471113020 C41471131020 C41471131020 C41471131021 C41471131021 C4147113120 C4147111	2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	91613/921 4451370 4513707 805524 805526 805526 805526 805526 805526 9055	9.08.847 9.02.261 9.02.261 9.02.261 9.02.261 9.02.261 9.02.261 9.02.262 9.03.261 9.051 9.051 9.051 9.051 9.051 9.051 9.051 9.051 9.051 9.0	31-33 11-121 11-121 11-121 11-121 125-25 22-24 125-15 125-15 22-23 125-15 125-15 22-23 125-15 125-15 125-16 12-22 22-24	2422 2420 2626 2777 2866 3433 362 3433 362 3433 362 3433 362 3433 362 3433 362 3433 362 3433 362 3433 362 4448 448 448 448 448 448 448 448 448 4	256 266 267 277 288 299 299 333 342 429 421 422 423 447 447 447 447 447 447 447 451 52 54 56 56 588 600 611 639 644 657 700 714 727 707 707 714 727 714 717 717
Polymerase complex proteins	LINESSET LINESSET PETPHELOPSAL PETPHELOPS			2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9(16), 3(2),	102.8407 202.017 202.026 1111.515 002.034 1111.515 002.034 002.040 1111.515 002.034 002.040 1111.515 002.040 0000000000	31-30 10 14-155 14-155 14-155 15-57 13-	2422 2420 2420 2420 2420 2420 2420 2420	256 265 267 288 299 288 299 288 299 288 333 341 366 337 338 399 411 42 42 433 441 42 433 441 452 548 558 568 667 669 670 700 700 700 711 721 744 747 747 747 747 747 747 747 747 74
Polymerase complex proteins	LWEETEN STULLES UNE STERKLOPSAL SETERKLOPSAL SETERKLOPSAL SETERKLOPSAL SETERKLOPSAL SETERKLOPSAL SETERKLOPSAL SETERK			2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	910.3370 2003005 515.767 515.767 800.524 601.526 601.526 601.526 601.526 601.526 601.526 601.526 601.526 601.526 601.526 601.526 601.527 601.526 601.527 601.526 601.527 601.5	Vide also 12 Vide also 12 Vi	31-31 (1) 14-155 14-125 14-155 14-125 15-15 1	2422 2420 2627 2777 2866 343 3629 3629 3629 3629 3629 3629 3629 362	2265 265 265 273 288 299 289 299 233 342 365 377 377 388 399 411 422 433 377 377 388 399 411 422 433 441 422 433 441 422 433 441 422 433 541 556 566 667 670 611 655 667 670 700 710 710 710 710 710 710 710 710 7
Polymerase complex proteins	LIVERSTYL STORENESSE SPETPHELOPSAL SPETPHELOPSAL SILLE/SVCDU/NE SILLE/SVC		C444890402 C44479041022 C4447914026 C4447914026 C4447914026 C45472941024 C4547944704 C4547944704 C4547944704 C44791402 C447914912 C4	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9 (1) (1) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2	24.44.192 25.244 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2344 25.2347 27.2462 25.2454 25.2577 25.2456 25.2454 25.2454 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.25544 25.255	31-31-11 14-155 14-155 14-125 25-25 22-34 15-15 13-55 13-26 13-	2422 2420 2620 2620 2777 2866 3322 3643 3622 3639 363 363 363 363 363 363 363 363 36	256 266 267 277 268 2277 268 2292 233 342 366 377 388 399 411 42 422 423 433 377 388 399 411 42 422 422 423 441 42 422 422 423 517 56 56 56 56 66 67 67 700 710 710 700 710 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 710 700 70
Polymerase complex proteins	LIVESPETA SPETARLOPERAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELDPSRAL PSTPAELSPETAR PSTPAE				910 Light 2014 2014 2014 2014 2014 2014 2014 2014 2014	4443302 4443302 4443302 4443302 445354 455386 455386 455386 455386 4455542 44555642 4455542 44555642 4455542 445564	31-0211 14-1025 14-1025 10-121 123-52 13-54 13-54 14-54 13-24 14-54 13-24 14-5	2422 2420 2420 2420 2420 2420 2420 2420	2265 2652 2662 2773 288 289 289 333 341 365 3373 377 377 377 377 377 377 377 377 3
Polymerase complex proteins	LIVERSTANDER LEUK LIVERSTANDER SETTRALE DARAGE RETTRALE DARAGE RETTRALE DARAGE SALLE JAVGDAVA SALLE JAVG		C444891102 C44471111050 C44471111050 C44471111050 C455224110462 C44471111050 C455224110462 C4767211051 C4767211051 C4767211051 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C48471110510 C576224100000000000000000000000000000000000	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9 (6) (3) (3) (4) (4) (4) (4) (4) (4) (4) (4) (4) (4	04.003 04.003	31-31-11 14-155 14-125 14-125 15-15 12-52 13-55 13-	2422 2400 2602 2766 2777 2777 2860 304 3629 304 3629 3788 3629 3788 3629 3788 3788 3788 3788 3788 3788 418 422 428 429 418 422 428 429 429 429 418 422 428 429 429 429 418 429 429 429 429 429 429 429 429 429 429	2266 266 266 277 288 289 333 346 367 377 377 389 411 422 433 441 442 433 441 442 433 441 442 433 441 442 433 441 445 566 568 568 568 568 568 568 569 700 710 717 722 741 752 770 714 772 741 752 770 714 772 741 752 770 774 774 774 774 774 774 774 774 774
Polymerase complex proteins	LINESPECTATION DUI LEUK LINESPECTATION DUI LEUK PETPELLOPARL RETTRUDOL INTIGIOL PETPELLOPARL DUI DUI LINESPECTATION LI			2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	948.350 948.350 949.555 951.577 806.544 806.545 806.545 806.545 806.545 806.545 902	444350 44450 4445060 44450600 4445000000000000000000000	31-31-11 14-155 14-155 14-155 14-155 15-15 13-56 14-15 13-26 14-15 13-26 14-15 13-26 14-15 13-26 14-15 13-26 14-15 13-26 14-15 13-26 14-15 13-27 14-15 14-14	2422 2420 2620 2626 2777 2777 2866 343 3629 3788 3967 3788 3967 3788 3967 3788 3967 3788 3967 414 418 4428 4483 463 3977 414 4228 4484 463 462 422 428 428 463 463 463 463 463 463 463 463 463 463	2552 2652 2652 2652 2652 2652 2652 2652
Polymerase complex proteins	LIVERSTANDER LEUK LIVERSTANDER SETTRAL DEPARL RETTRAL DEPARL RETTRAL DEPARL RETTRAL DEPARL SELLER SELLE			2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	944330 946330 946305 9515707 805304 9515707 805304 96155707 805304 96155707 805304 96155707 805304 96155707 805304 96155707 962301 963304 9634040000000000000000000000000000000000	4445.000 445.000 445.0000 445.0000 445.0000 445.0000 445.0000 445.0000 445.0000	31:011 14:105 14:105 14:105 14:105 14:105 15:012 12:021 12:021 12:023 14:05 15:02 15	2422 2420 2602 2676 2777 3042 3043 3629 3937 3937 3937 3937 3937 3937 3937 39	2852 2853 2853 2853 2853 2853 2853 2853
Polymerase complex proteins	LWEETER PERFECTORES PERFECTORES INTEGOLE INTEGOLE INTEGOLE PERFECTORE INTEGOLE INTEG				91033200 91033200 905055 915.707 800524 905055 915.707 800524 905255 900254 900255 900254 900255 900254 900254 900255 900254 900255 900254 900255 900254 900255 900555 9000555 900555 9005550 9005550 9005550	2442525 252,244 252,244 252,244 252,244 252,244 252,244 252,244 252,244 252,244 252,244 252,244 254,255 254,255 254,255 254,255 254,255 254,255 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255,277 255,264 255	311-111 11-125 20-22 20-	2422 2420 2602 2766 2777 342 343 362 343 362 343 362 343 362 343 362 343 362 362 362 362 362 362 362 362 362 36	22652 200 200 200 2000 20
Polymerase complex proteins	UNISPETIAL OSA UNISPETACIONA SETTACIONA SETTACIONA SETTACIONA SILLE ANACOMA SILLE ANACOMA			2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	9464300 9464300 945305 945377 805304 945377 805304 945377 805304 945377 940304 940373 940373 940373 94237 94257 94237 94257 947577 947577 947577 947577 947577 947577 947577 9475777 94757	0440320 0440320 0440320 0450317 042346 045360 045360 045320 045320 047346 047446 047346 04746 047466 047466 047466 047466 047466 047466 047466 047	31-011 14-105 14-105 14-105 14-105 14-105 15-02 12-02 13-05 13-05 13-05 13-05 13-05 13-05 13-05 13-05 13-02 13-05 13-02 13-05 13-02 13-05	2422 242 2460 2462 2460 2462 2460 2462 2476 2476 2476 2476 2476 247 247 24 24 24 24 24 24 24 24 24 24 24 24 24	2862 2875 2885 2885 2885 2885 2885 2885 288

1.004	0.254	0.105	1.041	0.290	0.099	0.954	0.214	0.095	1.92	0.10	0.96	1.090	1.056	0.907	0.948	0.771	0.700	0.962	0.893	0.614	0.0024	0.0009	0.52
1.034	0.441	0.203	1.028	0.580	0.228	0.938	0.444	0.209	129	0.07	0.98	1 113	1 101	0.967	0.936	0.803	0 744	0.951	0.907	0.638	0.0020	0.0009	0.40
1.070	0.188	na	1.013	0.218	na	0.918	0.077	na	3.20	0.55	0.89	1,105	1.037	0.911	0.933	0.776	0.685	0.963	0.871	0.595	0.0026	0.0009	0.52
0.965	0.400	0.168	0.996	0.556	0.198	1.038	0.435	0.185	1.41	0.07	0.98	1.076	1.058	0.916	0.978	0.853	0.750	0.946	0.881	0.595	0.0024	0.0009	0.52
1.009	0.414	0.177	1.038	0.558	0.208	0.953	0.429	0.184	1.39	0.07	0.96	1.106	1.100	0.960	0.940	0.775	0.729	0.954	0.905	0.651	0.0020	0.0009	0.40
0.951	0.517	0.248	1.191	0.752	0.254	0.857	0.470	0.203	1.20	0.13	0.92	1.185	1.187	1.040	0.917	0.711	0.760	0.897	0.887	0.642	0.0017	0.0013	0.19
1.032	0.447	0.183	1.029	0.565	0.194	0.939	0.394	0.147	1.46	0.10	0.97	1.108	1.107	0.970	0.932	0.767	0.718	0.961	0.908	0.649	0.0020	0.0010	0.38
1.0/1	0.545	0.292	1.015	0.655	0.295	0.914	0.488	0.254	1.06	0.07	0.97	1.120	1.125	1.010	0.924	0.773	0.736	0.956	0.917	0.672	0.0018	0.0010	0.31
1.034	0.207	0.067	1.080	0.372	0.008	0.904	0.257	0.084	2.25	0.13	0.96	1.075	1.003	0.005	0.937	0.782	0.690	0.970	0.893	0.600	0.0020	0.0008	0.00
0.878	0.410	0.261	1.243	0.773	0.517	0.000		0.040	0.87	0.30	0.68	0.928	0.933	0.805	0.007		0.000	1.143	1.045	0.717	0.0025	0.0008	0.73
0.913	0.329	0.254	0.962	0.395	0.243	1.125	0.371	0.306	1.10	0.14	0.89	0.873	0.847	0.764	1.050	0.588	0.599	1.076	0.911	0.711	0.0027	0.0011	0.47
1.058	0.531	0.373	1.030	0.626	0.430	0.912	0.485	0.337	0.81	0.08	0.93	1.113	1.054	0.945	0.8/7	0.697	0.648	1.010	0.956	0.654	0.0023	0.0011	0.39
1.010	0.518	0.371	1.032	0.634	0.428	0.958	0.513	0.337	0.81	0.07	0.95	1.104	1.044	0.948	0.904	0.743	0.679	0.991	0.953	0.655	0.0022	0.0010	0.43
1.000	0.519	0.372	1.033	0.631	0.432	0.967	0.505	0.341	0.81	0.08	0.94	1.090	1.052	0.951	0.927	0.737	0.680	0.983	0.949	0.658	0.0022	0.0010	0.43
1.065	0.520	0.361	0.975	0.615	0.396	0.961	0.512	0.332	0.83	0.07	0.96	1.063	1.023	0.967	0.920	0.741	0.000	0.997	0.900	0.000	0.0025	0.0010	0.44
1.036	0.446	0.308	1.011	0.606	0.396	0.953	0.487	0.320	0.90	0.09	0.93	1.097	1.009	0.911	0.917	0.709	0.662	0.967	0.922	0.651	0.0024	0.0009	0.47
4 0000	0.040	0.000	0.055	0.004	0.440	0.050	0.450	0.040	0.00	0.00	0.04	4.040	4.040	0.054	0.000	0.770	0.000	0.070	0.004	0.700	0.0040	0.0000	0.40
1.089	0.616	0.396	0.900	0.568	0.352	1.072	0.453	0.619	0.80	0.09	0.91	1.043	1.046	0.938	0.960	0.773	0.699	1.005	1.040	0.685	0.0018	0.0008	0.42
1.014	0.485	0.340	1.031	0.612	0.414	0.954	0.475	0.322	0.86	0.09	0.93	1.100	1.051	0.932	0.909	0.732	0.670	0.991	0.954	0.647	0.0023	0.0010	0.45
1.003	0.471	0.337	1.109	0.605	0.378	0.888	0.504	0.324	0.88	0.08	0.95	1.090	1.121	1.006	0.966	0.770	0.726	0.944	0.863	0.594	0.0022	0.0011	0.34
1.027	0.527	0.375	1.028	0.631	0.434	0.945	0.522	0.368	0.78	0.07	0.95	1.107	1.065	0.927	0.922	0.768	0.679	0.971	0.929	0.610	0.0025	0.0010	0.49
1.031	0.537	0.380	1.051	0.654	0.440	0.918	0.511	0.356	0.78	0.06	0.94	1.097	1.048	0.939	0.919	0.755	0.695	0.964	0.922	0.661	0.0022	0.0009	0.45
1.033	0.646	0.477	0.933	0.709	0.447	1.034	0.631	0.411	0.68	0.04	0.97	1.071	1.028	0.861	1.035	0.853	0.707	0.893	0.913	0.681	0.0023	0.0006	0.66
4.077	0.05/	0.407	4.000	0.705	0.405	0.000		0.000	0.70	0.000	0.05	4	4.00.1	0.00.	0.075	0.046	0.705	0.055	0.046	0.005	0.0047	0.0007	0.45
1.077	0.654	0.437	1.001	0.725	0.439	0.922	0.5/6	0.369	0.73	0.06	0.95	1.073	1.061	0.961	0.972	0.818	0.769	0.955	0.918	0.685	0.001/	0.0008	0.40
1.078	0.648	0.449	1.008	0.781	0.438	0.914	0.458	0.341	0.75	0.12	0.85	1.096	1.110	1.003	0.942	0.799	0.763	0.962	0.919	0.675	0.0017	0.0009	0.33
1.254	0.450	0.223	0.939	0.156	0.054	0.807	0.085	0.054	2.02	0.47	0.73	0.992	1.022	1.020	1.075	0.897	0.863	0.932	0.886	0.772	0.0010	0.0006	0.27
1.046	0.202	na	0.998	0.217	na	0.956	0.084	na	3.12	0.51	0.90	0.923	1.035	0.966	1.055	0.932	0.831	1.022	0.967	0.671	0.0017	0.0007	0.45
1.248	0.910	0.660	0.863	0.919	0.593	0.889	0.754	0.560	0.41	0.10	0.71	1.092	1.024	0.962	0.960	0.888	0.805	0.962	0.886	0.661	0.0018	0.0008	0.44
1.143	0.663	0.461	0.976	0.705	0.431	0.881	0.499	0.333	0.75	0.10	0.88	1.153	1.166	1.043	0.960	0.759	0.645	0.897	0.915	0.631	0.0022	0.0013	0.31
1.045	0.657	0.453	1.002	0.726	0.442	0.962	0.596	0.388	0.71	0.05	0.97	1.081	1.068	0.966	0.962	0.804	0.755	0.956	0.917	0.685	0.0018	0.0008	0.42
0.000	0.040	0.005	4 0000	0.005	0.405	0.074	0.404	0.000	4.00	0.40	0.07	4.040	4 000	0.055	0.074	0.700	0.070	0.070	0.000	0.044	0.0007	0.0000	0.00
1.076	0.246	0.111	0,999	0.200	0.105	0.975	0.191	0.134	1,90	0.13	0.95	1.048	1,080	0.905	0.960	0.763	0.694	0.974	0.909	0.612	0.0027	0.0008	0.63
1.042	0.559	0.279	1.005	0.620	0.288	0.953	0.459	0.227	1.11	0.08	0.97	1.079	1.067	0.950	0.961	0.806	0.749	0.960	0.924	0.671	0.0019	0.0006	0.46
1.040	0.086	na	1.009	0.121	na	0.951	0.028	na	4.52	0.73	0.91	1.073	1.041	0.939	0.972	0.817	0.748	0.955	0.915	0.661	0.0020	0.0008	0.50
1.153	0.757	0.550	1.127	0.813	0.495	0.720	0.553	0.503	0.53	0.12	0.73	1.164	1.084	1.003	0.966	0.786	0.678	0.870	0.869	0.733	0.0017	0.0010	0.29
1.008	0.627	0.431	0.995	0.674	0.414	0.996	0.580	0.370	0.75	0.04	0.98	1.064	1.063	0.980	0.977	0.810	0.747	0.939	0.885	0.651	0.0019	0.0009	0.40
1.065	0.684	0.466	0.999	0.751	0.445	0.936	0.627	0.374	0.66	0.07	0.93	1.086	1.105	0.964	0.949	0.844	0.720	0.966	0.933	0.667	0.0018	0.0008	0.35
1.070	0.653	0.481	0.978	0.763	0.467	0.952	0.625	0.445	0.64	0.05	0.96	1.084	1.093	0.963	0.953	0.846	0.803	0.962	0.922	0.681	0.0016	0.0008	0.38
1.013	0.656	0.447	1.053	0.765	0.471	0.934	0.580	0.377	0.70	0.07	0.94	1.157	1.127	1.045	0.923	0.785	0.742	0.921	0.893	0.643	0.0018	0.0011	0.26
1.125	0.573	0.308	0.983	0.680	0.315	0.892	0.469	0.234	1.05	0.10	0.94	1.082	1.100	0.990	0.981	0.882	0.804	0.937	0.879	0.696	0.0015	0.0008	0.36
0.962	0.856	0.402	1.016	0.831	0.401	1.022	0.558	0.320	0.63	0.08	0.94	1.127	1.105	1.046	0.909	0.850	0.812	0.960	0.958	0.685	0.0016	0.0010	0.33
1.061	0.655	0.445	1.053	0.707	0.442	0.947	0.577	0.426	0.69	0.07	0.96	1.157	1.061	1.056	0.938	0.839	0.735	0.905	0.870	0.637	0.0018	0.0011	0.29
1.001	0.658	0.440	1.020	0.724	0.435	0.944	0.578	0.383	0.72	0.05	0.95	1.0/6	1 100	0.996	0.946	0.813	0.768	0.950	0.910	0.674	0.0017	0.0008	0.36
1.041	0.658	0.441	0.998	0.719	0.440	0.961	0.592	0.386	0.72	0.05	0.97	1.078	1.069	0.960	0.952	0.807	0.762	0.970	0.924	0.685	0.0018	0.0008	0.43
1.103	0.640	0.408	1.004	0.722	0.404	0.893	0.522	0.323	0.81	0.09	0.93	1.062	1.091	0.949	0.967	0.853	0.753	0.951	0.884	0.673	0.0019	0.0008	0.47
0.999	0.719	0.564	1.055	0.802	0.471	0.946	0.682	0.369	0.64	0.09	0.88	1.112	1.077	0.930	0.919	0.786	0.723	0.969	0.909	0.634	0.0022	0.0009	0.46
1.0/1	0.642	0.446	1.015	0.720	0.451	0.941	0.566	0.386	0.71	0.05	0.96	1.091	1.006	0.990	0.906	0.799	0.724	0.901	0.903	0.657	0.0021	0.0008	0.38
0.994	0.588	0.359	1.096	0.767	0.414	0.910	0.509	0.304	0.86	0.10	0.91	1.099	1.075	0.954	0.965	0.809	0.751	0.946	0.921	0.678	0.0019	0.0008	0.44
1.110	0.045	na	1.066	0.036	na	0.824	0.008	na	6.25	0.93	0.92	1.061	1.037	0.871	0.977	0.763	0.707	0.962	0.878	0.633	0.0024	0.0006	0.58
1.053	0.513	0.212	1.014	0.624	0.248	0.933	0.444	0.181	1.29	0.10	0.96	1.110	1.100	1.010	0.958	0.847	0.765	0.932	0.903	0.643	0.0018	0.0009	0.35
1.056	0.681	0.484	1.016	0.829	0.522	0.928	0.597	0.300	0.63	0.08	0.90	1.073	1.085	0.968	0.965	0.827	0.767	0.972	0.928	0.688	0.0018	0.0008	0.40
1.012	0.300	0.009	0.342	0.000	0.347	1.040	0.309		0.30	0.00	0.01	1.061		0.311	0.303	0.042	0.000	0.000	0.314	0.300	0.0010		
1.064	0.615	0.423	0.969	0.698	0.436	0.927	0.551	0.367	0.75	0.06	0.95	1,094	1.113	1.033	0.961	0.817	0.771	0.945	0.923	0.707	0.0015	0.0009	0.29
1.036	0.663	0.447	1.000	0.725	0.443	0.963	0.596	0.394	0.71	0.05	0.97	1.094	1.061	0.964	0.969	0.778	0.757	0.947	0.927	0.678	0.0018	0.0006	0.39
1.053		0.513	0.995	0.735	0.445	0.952	0.620	0.430	0.64	0.05	0.96	1.127	1.126	1.043	0.967	0.832	0.750	0.917	0.869	0.613	0.0019	0.0011	0.30
4.490	0.688	0.610	1.001	0.763	0.403	0.030	0.526	0.416	0.63	0.10	0.96	1.094	1.119	1.017	0.965	0.822	0.006	0.945	0.907	0.694	0.0020	0.0010	0.38
1.130	0.688	0.519	0.991	0.710	1144.32	1.			0.10	0.00	0.00	1.000		1.017		June	J O		J. J	~~~			
1.130 1.070	0.688	0.519 0.436	0.991	0.710	0.432	0.555	0.077															0.0000	
1.130 1.070 1.059	0.688 0.674 0.642 0.688	0.519 0.436 0.499	0.991 0.978	0.710	0.432	0.963	0.642	0.464	0.61	0.04	0.96	1.083	1.074	0.922	0.973	0.845	0.731	0.945	0.879	0.630	0.0023	0.0008	0.52
1.130 1.070 1.059	0.688 0.674 0.642 0.688	0.519 0.436 0.499	0.991	0.710	0.432	0.963	0.642	0.464	0.61	0.04	0.98	1.083	1.074	0.922	0.973	0.845	0.731	0.945	0.879	0.630	0.0023	0.0008	0.52
1.130 1.070 1.059 1.064	0.688 0.674 0.642 0.688 0.295	0.519 0.436 0.499 0.049	0.991	0.747	0.432	0.963	0.642	0.464	0.61 2.63	0.04	0.98	1.063	1.074	0.922	0.973	0.845	0.731	0.945	0.879	0.630	0.0023	0.0008	0.52
1.130 1.070 1.059 1.064 1.163	0.688 0.674 0.642 0.688 0.295 0.089	0.519 0.436 0.499 0.049 na	0.991	0.710	0.470 0.050 na	0.963	0.642	0.464 0.031 na	0.61 2.63 5.90	0.04	0.96	1.083	1.074 1.062 1.069	0.922 0.933 0.910	0.973	0.845 0.795 0.813	0.731 0.718 0.742	0.945 0.961 0.977	0.879 0.904 0.918	0.630 0.654 0.665	0.0023	0.0008	0.52 0.49 0.54
1.130 1.070 1.059 1.064 1.163 1.060	0.688 0.674 0.642 0.688 0.295 0.295 0.089 0.479	0.519 0.436 0.499 0.049 na 0.230 0.212	0.991 0.978 0.997 0.960 0.992	0.710 0.747 0.353 0.016 0.529	0.470 0.050 na 0.228	0.963 0.939 0.877 0.948 0.945	0.642 0.169 0.016 0.368	0.464 0.031 na 0.184	0.61 2.63 5.90 1.29	0.04 0.19 0.95 0.09	0.96 0.96 0.91 0.97	1.083 1.086 1.061 1.083	1.074 1.062 1.069 1.074	0.922 0.933 0.910 0.922	0.973 0.963 0.962 0.973	0.845 0.795 0.813 0.845	0.731 0.718 0.742 0.731	0.945 0.961 0.977 0.945	0.879 0.904 0.918 0.879	0.630	0.0023 0.0021 0.0021 0.0023 0.0018	0.0008	0.52 0.49 0.54 0.52
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072	0.688 0.674 0.642 0.688 0.295 0.089 0.479 0.464 0.480	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238	0.991 0.978 0.997 0.960 0.992 1.000 1.001	0.710 0.747 0.353 0.016 0.529 0.516 0.540	0.432 0.470 0.050 na 0.228 0.220 0.232	0.963 0.939 0.877 0.948 0.945 0.927	0.642 0.169 0.016 0.368 0.351 0.364	0.464 0.031 na 0.184 0.170 0.201	0.61 2.63 5.90 1.29 1.34 1.25	0.04 0.19 0.95 0.09 0.09	0.98 0.96 0.91 0.97 0.97	1.083 1.086 1.061 1.083 1.083 1.083	1.074 1.062 1.069 1.074 1.071 1.072	0.922 0.933 0.910 0.922 0.964 0.969	0.973 0.963 0.962 0.973 0.963 0.963	0.845 0.795 0.813 0.845 0.805 0.831	0.731 0.718 0.742 0.731 0.754 0.778	0.945 0.951 0.977 0.945 0.954 0.925	0.879 0.904 0.918 0.879 0.922 0.904	0.630 0.654 0.665 0.630 0.686 0.656	0.0023	0.0008	0.52 0.49 0.54 0.52 0.42 0.42
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212	0.688 0.674 0.642 0.688 0.295 0.069 0.479 0.464 0.480 0.681	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.471	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948	0.747 0.353 0.016 0.529 0.516 0.540 0.722	0.470 0.050 na 0.228 0.220 0.232 0.446	0.963 0.939 0.877 0.948 0.945 0.927 0.839	0.642 0.169 0.016 0.368 0.351 0.364 0.536	0.464 0.031 na 0.184 0.170 0.201 0.394	0.61 2.63 5.90 1.29 1.34 1.25 0.68	0.04 0.19 0.95 0.09 0.09 0.09 0.09 0.10	0.98 0.96 0.91 0.97 0.97 0.97 0.88	1.083 1.086 1.061 1.083 1.083 1.083 1.109 1.137	1.074 1.062 1.069 1.074 1.071 1.062 1.132	0.922 0.933 0.910 0.922 0.964 0.960 1.055	0.973 0.963 0.962 0.973 0.963 0.965 0.965	0.845 0.796 0.813 0.845 0.805 0.831 0.883	0.731 0.718 0.742 0.731 0.754 0.778 0.822	0.945 0.951 0.977 0.945 0.954 0.925 0.897	0.879 0.904 0.918 0.879 0.922 0.904 0.886	0.630 0.654 0.685 0.630 0.686 0.656 0.730	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.20
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212 1.212	0.688 0.674 0.642 0.688 0.295 0.089 0.479 0.464 0.480 0.681 0.618	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.238 0.471 0.391	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974	0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.667	0.432 0.470 0.050 na 0.228 0.220 0.232 0.232 0.246 0.389	0.963 0.963 0.939 0.877 0.948 0.945 0.927 0.839 0.846	0.642 0.169 0.016 0.368 0.351 0.364 0.536 0.467	0.464 0.031 na 0.184 0.170 0.201 0.201 0.394 0.308	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84	0.04 0.19 0.95 0.09 0.09 0.09 0.09 0.10 0.10	0.96 0.91 0.97 0.97 0.97 0.97 0.88 0.90	1.083 1.086 1.083 1.083 1.083 1.109 1.137 1.197	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096	0.973 0.963 0.962 0.973 0.963 0.965 0.965 0.966 0.915	0.845 0.796 0.813 0.845 0.805 0.831 0.883 0.814	0.731 0.718 0.742 0.731 0.754 0.778 0.822 0.794	0.945 0.951 0.977 0.945 0.954 0.925 0.897 0.889	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865	0.630 0.654 0.685 0.630 0.686 0.656 0.730 0.718	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.20 0.12
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212 1.212 1.181 1.071	0.688 0.674 0.642 0.688 0.295 0.089 0.479 0.464 0.480 0.681 0.618 0.643	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.238 0.238 0.238 0.471 0.391 0.391	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974 0.995	0.710 0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.667 0.727	0.432 0.470 0.050 ra 0.228 0.220 0.232 0.446 0.389 0.449	0.939 0.939 0.877 0.948 0.945 0.927 0.839 0.846 0.934	0.642 0.169 0.016 0.368 0.351 0.364 0.536 0.467 0.591	0.464 0.031 na 0.184 0.170 0.201 0.394 0.308 0.424	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67	0.04 0.19 0.95 0.09 0.09 0.09 0.10 0.10 0.05	0.98 0.91 0.97 0.97 0.97 0.98 0.90 0.96	1.083 1.086 1.061 1.063 1.083 1.109 1.137 1.197	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.060	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096 0.998	0.973 0.963 0.962 0.973 0.965 0.965 0.966 0.915 0.977	0.845 0.795 0.813 0.845 0.805 0.831 0.883 0.814 0.865	0.731 0.718 0.742 0.731 0.754 0.822 0.794 0.801	0.945 0.951 0.977 0.945 0.954 0.954 0.955 0.897 0.889 0.945	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.885 0.930	0.630 0.654 0.665 0.630 0.686 0.656 0.730 0.718 0.689	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012 0.0012	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.20 0.12 0.38
1.130 1.070 1.059 1.064 1.163 1.064 1.054 1.072 1.212 1.181 1.071 1.071	0.688 0.674 0.682 0.688 0.295 0.689 0.479 0.464 0.480 0.681 0.618 0.643 0.643 0.605	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.471 0.391 0.469 0.417	0.991 0.978 0.997 0.960 0.962 1.000 1.001 0.948 0.974 0.995 0.991	0.740 0.353 0.016 0.529 0.516 0.540 0.722 0.667 0.727 0.694	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449	0.963 0.963 0.939 0.877 0.948 0.945 0.927 0.839 0.846 0.934 0.934 0.941	0.642 0.169 0.016 0.368 0.351 0.364 0.536 0.467 0.591 0.555	0.464 0.031 na 0.184 0.170 0.201 0.394 0.308 0.424 0.308	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67 0.76	0.04 0.19 0.95 0.09 0.09 0.09 0.10 0.10 0.05 0.05	0.98 0.96 0.91 0.97 0.97 0.97 0.97 0.98 0.90 0.96 0.96	1.083 1.086 1.061 1.083 1.003 1.103 1.137 1.137 1.078 1.052	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.080 1.080	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096 0.998 1.015	0.973 0.963 0.962 0.973 0.963 0.965 0.966 0.915 0.977 0.968	0.845 0.795 0.813 0.845 0.805 0.831 0.814 0.865 0.818	0.731 0.718 0.742 0.731 0.754 0.778 0.822 0.794 0.801 0.785	0.945 0.951 0.977 0.945 0.954 0.955 0.897 0.889 0.945 0.945	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865 0.930 0.930	0.630 0.654 0.665 0.630 0.686 0.730 0.718 0.689 0.700	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0018 0.0012 0.0012 0.0016 0.0015	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.20 0.12 0.38 0.31
1.130 1.070 1.064 1.163 1.060 1.054 1.072 1.212 1.181 1.071 1.068 1.064	0.688 0.674 0.642 0.688 0.295 0.069 0.479 0.464 0.480 0.681 0.618 0.643 0.643 0.605 0.605	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.471 0.391 0.469 0.417 0.391	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974 0.995 0.991 0.975	0.710 0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.667 0.727 0.6974 0.594	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449 0.4207 0.227	0.963 0.963 0.877 0.948 0.945 0.927 0.839 0.846 0.934 0.941 0.941	0.642 0.169 0.016 0.368 0.361 0.364 0.364 0.591 0.591 0.591 0.591 0.5450 0.4467	0.464 0.031 na 0.184 0.170 0.201 0.394 0.308 0.424 0.368 0.424 0.369 0.292	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67 0.76 0.95	0.04 0.19 0.95 0.09 0.09 0.10 0.10 0.05 0.06 0.09	0.96 0.91 0.97 0.97 0.97 0.98 0.90 0.96 0.96 0.93 0.00	1.083 1.086 1.081 1.083 1.083 1.108 1.137 1.197 1.078 1.082 1.106	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.060 1.098 1.101	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096 0.998 1.015 0.998	0.973 0.963 0.962 0.973 0.963 0.965 0.966 0.915 0.977 0.968 0.977	0.845 0.795 0.813 0.845 0.805 0.831 0.883 0.814 0.865 0.818 0.818	0.731 0.718 0.742 0.731 0.754 0.822 0.794 0.801 0.785 0.777	0.945 0.951 0.977 0.945 0.925 0.925 0.889 0.945 0.945 0.945 0.945	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.886 0.930 0.930 0.926 0.926	0.630 0.654 0.655 0.630 0.686 0.656 0.730 0.718 0.689 0.700 0.689	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012 0.0012 0.0016 0.0015 0.0019	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.20 0.12 0.38 0.31 0.38
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212 1.181 1.071 1.068 1.084 1.048 1.052	0.688 0.674 0.642 0.688 0.295 0.469 0.469 0.460 0.681 0.681 0.618 0.643 0.605 0.603 0.319	0.519 0.436 0.499 0.049 na 0.230 0.213 0.238 0.471 0.391 0.469 0.417 0.391 na 0.322	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974 0.995 0.991 0.995 1.000 0.995	0.740 0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.687 0.727 0.694 0.574 0.294 0.594	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449 0.420 0.232 0.449 0.420 0.234	0.963 0.963 0.939 0.877 0.948 0.945 0.927 0.839 0.846 0.934 0.941 0.941 0.951 0.951 0.951	0.642 0.169 0.016 0.368 0.351 0.364 0.536 0.467 0.591 0.555 0.440 0.149 0.375	0.464 0.031 na 0.184 0.170 0.201 0.394 0.394 0.398 0.424 0.366 0.292 na 0.227	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67 0.76 0.95 2.37 1.03	0.04 0.95 0.09 0.09 0.09 0.10 0.10 0.05 0.06 0.06 0.09 0.40 0.12	0.96 0.91 0.97 0.97 0.97 0.97 0.96 0.96 0.96 0.93 0.90 0.91	1.083 1.086 1.061 1.083 1.083 1.109 1.137 1.197 1.092 1.109 1.082 1.109 1.082	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.080 1.098 1.101 1.002 1.200	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096 0.998 1.015 0.985 0.876 0.876	0.973 0.963 0.962 0.973 0.965 0.965 0.966 0.915 0.968 0.915 0.968 0.957 0.968 0.977 0.968	0.845 0.795 0.813 0.845 0.805 0.831 0.883 0.814 0.865 0.818 0.853 0.818 0.853 0.842 0.750	0.731 0.718 0.742 0.731 0.754 0.822 0.794 0.801 0.785 0.777 0.731 0.727	0.945 0.951 0.977 0.945 0.954 0.925 0.897 0.889 0.945 0.945 0.940 0.935 0.954	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865 0.930 0.926 0.826 0.825 0.826 0.826	0.630 0.654 0.655 0.630 0.686 0.656 0.730 0.718 0.689 0.700 0.630 0.614 0.691	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0018 0.0012 0.0016 0.0015 0.0015 0.0019 0.0024	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.42 0.42 0.42 0.38 0.31 0.38 0.64 0.29
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212 1.181 1.071 1.068 1.068 1.068 1.052 0.922	0.688 0.674 0.642 0.688 0.295 0.469 0.469 0.681 0.681 0.643 0.683 0.605 0.603 0.319 <<0.129 <<0.129	0.519 0.436 0.499 0.049 na 0.230 0.213 0.233 0.471 0.391 0.469 0.417 0.391 na 0.323 na	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974 0.995 0.991 0.975 1.000 0.863	0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.687 0.727 0.694 0.574 0.594 0.594	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449 0.449 0.420 0.287 na 0.314	0.963 0.963 0.977 0.948 0.945 0.927 0.839 0.846 0.934 0.934 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.945 0.977 1.078	0.642 0.169 0.016 0.368 0.351 0.364 0.536 0.467 0.591 0.555 0.440 0.149 0.375 <<0.129	0.464 0.031 ra 0.184 0.201 0.201 0.394 0.308 0.424 0.366 0.424 0.366 0.227 ra 0.227 ra	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67 0.76 0.95 2.37 1.03 >3.41	0.04 0.95 0.09 0.09 0.09 0.09 0.10 0.10 0.05 0.06 0.09 0.40 0.12 na	0.98 0.91 0.97 0.97 0.97 0.98 0.90 0.96 0.96 0.93 0.90 0.91 153	1.083 1.086 1.061 1.083 1.083 1.083 1.109 1.077 1.077 1.079 1.076 1.089 1.047 1.116	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.080 1.098 1.101 1.002 1.209 1.033	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.066 0.998 1.015 0.965 0.965 0.965 0.965 0.918	0.973 0.963 0.962 0.973 0.965 0.965 0.965 0.965 0.977 0.968 0.977 0.988 0.977 0.888 0.977	0.845 0.795 0.813 0.845 0.805 0.831 0.883 0.814 0.865 0.818 0.853 0.818 0.853 0.845 0.818 0.8750 0.709	0.731 0.718 0.742 0.731 0.754 0.822 0.794 0.801 0.785 0.777 0.731 0.727 0.607	0.945 0.951 0.977 0.945 0.954 0.925 0.897 0.889 0.945 0.940 0.935 0.954 1.065 0.938	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865 0.930 0.926 0.895 0.895 0.889 0.880 0.880 0.922	0.630 0.654 0.685 0.636 0.686 0.730 0.718 0.689 0.700 0.630 0.614 0.630 0.622	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012 0.0015 0.0019 0.0024 0.0024 0.0024	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.42 0.42 0.38 0.31 0.38 0.64 0.28 0.50
1.130 1.070 1.059 1.064 1.163 1.060 1.054 1.072 1.212 1.181 1.071 1.068 1.048 1.052 0.922 1.047	0.688 0.674 0.642 0.688 0.295 0.069 0.479 0.464 0.480 0.681 0.643 0.661 0.643 0.605 0.603 0.349 <<0.129 0.471	0.519 0.436 0.499 0.049 na 0.200 0.213 0.223 0.471 0.391 0.469 0.417 0.391 0.469 0.417 na 0.323 na 0.323 na	0.991 0.978 0.997 0.960 0.992 1.000 1.001 0.948 0.974 0.995 0.991 0.975 1.000 0.863 0.979	0.740 0.747 0.353 0.016 0.529 0.516 0.540 0.722 0.667 0.727 0.694 0.574 0.596 0.596 0.582	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449 0.420 0.287 na 0.314 0.294	0.963 0.963 0.939 0.877 0.948 0.945 0.927 0.839 0.846 0.934 0.941 0.941 0.951 1.017 1.078 0.974	0.642 0.169 0.361 0.361 0.364 0.536 0.467 0.591 0.555 0.440 0.149 0.375 <<0.129 0.528	0.464 0.031 na 0.184 0.170 0.201 0.394 0.308 0.424 0.368 0.424 0.368 0.424 0.368 0.227 na 0.227 na 0.245	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.67 0.76 0.95 2.37 1.03 >3.41 0.86	0.04 0.19 0.09 0.09 0.09 0.10 0.10 0.05 0.06 0.09 0.09 0.40 0.12 ras 0.10	0.98 0.91 0.97 0.97 0.97 0.96 0.96 0.96 0.96 0.93 0.90 0.91 na 0.92	1.083 1.085 1.083 1.083 1.109 1.137 1.097 1.097 1.097 1.099 1.099 1.099 1.049 1.049 1.049 1.049	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.060 1.096 1.101 1.002 1.209 1.033 0.917	0.922 0.933 0.910 0.922 0.964 0.960 1.055 1.096 0.996 1.015 0.996 1.015 0.976 0.876 1.018 0.902 0.791	0.973 0.963 0.962 0.973 0.965 0.965 0.965 0.966 0.915 0.977 0.968 0.958 0.977 0.888 0.977 1.257	0.845 0.795 0.813 0.845 0.805 0.831 0.863 0.814 0.865 0.818 0.853 0.842 0.750 0.709 0.806	0.731 0.718 0.742 0.731 0.754 0.778 0.822 0.794 0.801 0.785 0.777 0.731 0.727 0.607 0.902	0.945 0.951 0.977 0.945 0.925 0.897 0.889 0.945 0.945 0.940 0.935 0.954 0.935 0.954 0.938 0.938 0.938	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.885 0.930 0.926 0.895 0.895 0.895 0.895 0.889 0.860 0.922 0.728	0.630 0.654 0.685 0.630 0.686 0.730 0.718 0.689 0.700 0.639 0.614 0.603 0.614 0.603 0.622 0.517	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012 0.0015 0.0019 0.0024 0.0028 0.0028 0.0023	0.0008	0.52 0.49 0.54 0.52 0.42 0.42 0.42 0.42 0.38 0.31 0.38 0.64 0.28 0.50 0.31
1.130 1.070 1.059 1.064 1.064 1.060 1.054 1.072 1.212 1.212 1.071 1.064 1.071 1.064 1.072 1.212 1.011 1.064 1.052 0.922 1.047 0.974	0.688 0.674 0.642 0.688 0.295 0.069 0.479 0.464 0.480 0.681 0.643 0.661 0.643 0.605 0.603 0.319 0.489 <0.129 0.471 0.465	0.519 0.436 0.499 0.230 0.213 0.238 0.471 0.391 na 0.391 na 0.340 0.340 0.340	0.991 0.978 0.960 0.960 0.962 1.000 1.001 0.942 0.974 0.965 0.991 0.975 1.000 0.863 0.979 1.040	0.740 0.353 0.016 0.529 0.516 0.540 0.722 0.687 0.727 0.684 0.727 0.684 0.574 0.594 0.596 0.582 0.519	0.432 0.470 0.050 na 0.228 0.220 0.232 0.446 0.389 0.449 0.449 0.420 0.287 na 0.314 0.294 0.294 0.294	0.963 0.963 0.939 0.877 0.948 0.945 0.927 0.839 0.846 0.934 0.941 0.941 0.951 1.017 1.078 0.974 0.996	0.642 0.169 0.368 0.351 0.364 0.536 0.467 0.591 0.555 0.440 0.149 0.375 <<0.129 0.375 <0.129 0.528 0.452	0.464 0.031 na 0.184 0.201 0.394 0.308 0.424 0.368 0.424 0.369 0.227 na 0.227 na 0.445 0.310	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.68 0.96 2.37 1.03 >3.41 0.86 0.99 >3.41	0.04 0.19 0.09 0.09 0.09 0.09 0.10 0.05 0.06 0.06 0.09 0.40 0.12 ras 0.10 0.12	0.98 0.91 0.97 0.97 0.97 0.97 0.97 0.97 0.96 0.96 0.96 0.93 0.90 0.91 ma 0.92 0.92 0.92	1.083 1.086 1.081 1.083 1.105 1.137 1.197 1.078 1.105 1.066 1.066 1.069 1.069 1.069 1.069 1.069 1.069 1.069 1.069 1.069	1.074 1.062 1.069 1.074 1.071 1.062 1.132 1.216 1.060 1.096 1.101 1.002 1.209 1.033 0.917 1.013	0.922 0.933 0.910 0.922 0.964 0.965 1.055 1.096 0.998 1.015 0.965 0.865 0.876 1.018 0.902 0.791 0.945	0.973 0.963 0.962 0.973 0.965 0.965 0.966 0.915 0.968 0.958 0.958 0.958 0.957 0.888 0.977 0.888 0.947 1.257 0.947	0.845 0.795 0.813 0.845 0.805 0.831 0.844 0.865 0.818 0.818 0.853 0.842 0.750 0.709 0.709 0.806 0.777	0.731 0.718 0.742 0.731 0.754 0.778 0.822 0.794 0.801 0.785 0.777 0.731 0.727 0.607 0.902 0.692	0.945 0.951 0.977 0.945 0.925 0.897 0.889 0.945 0.945 0.940 0.935 0.955 0.955 0.955 0.938 0.938 0.938 0.937	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865 0.930 0.926 0.895 0.885 0.885 0.885 0.885 0.885 0.880 0.922 0.728 0.939	0.630 0.654 0.665 0.630 0.686 0.730 0.718 0.689 0.700 0.630 0.630 0.630 0.614 0.603 0.622 0.517 0.599	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0016 0.0015 0.0015 0.0015 0.0015 0.0024 0.0024 0.0022 0.0024	0.0006 0.0007 0.0006 0.0006 0.0006 0.0006 0.0009 0.0012 0.0008 0.0009 0.0012 0.0009 0.0013 0.0013 0.0010	0.52 0.49 0.54 0.52 0.42 0.20 0.42 0.38 0.31 0.38 0.64 0.28 0.50 0.31 0.47
1.130 1.070 1.059 1.064 1.163 1.064 1.064 1.064 1.064 1.062 1.071 1.072 1.212 1.181 1.071 1.068 1.048 1.048 1.048 1.048 0.922 1.047 0.974	0.688 0.674 0.642 0.688 0.295 0.069 0.479 0.464 0.480 0.480 0.481 0.618 0.603 0.603 0.603 0.403 0.403 0.409 <0.129 0.471 0.485 0.494	0.519 0.436 0.499 0.230 0.213 0.238 0.471 0.391 na 0.340 0.321 0.340	0.991 0.978 0.960 0.992 1.000 1.001 0.942 0.995 0.991 0.975 1.000 0.863 0.979 1.040 1.087	0.747 0.353 0.016 0.529 0.516 0.529 0.516 0.722 0.687 0.727 0.694 0.574 0.596 0.596 0.582 0.582 0.519 0.602	0.432 0.470 0.050 rs 0.228 0.220 0.232 0.449 0.420 0.449 0.449 0.420 0.287 rs 0.314 0.285 0.344	0.963 0.963 0.877 0.948 0.945 0.927 0.846 0.934 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.941 0.974 0.974 0.976 0.974 0.976 0.974	0.642 0.169 0.016 0.368 0.351 0.364 0.591 0.555 0.440 0.199 0.375 <0.129 0.375 <0.129 0.528 0.452 0.570	0.464 0.031 ra 0.184 0.170 0.394 0.398 0.424 0.386 0.424 0.366 0.227 ra 0.445 0.310 0.324	0.61 2.63 5.90 1.29 1.34 1.25 0.68 0.84 0.84 0.67 0.76 0.95 2.37 1.03 0.96 0.99 0.89 0.89	0.04 0.19 0.95 0.09 0.09 0.10 0.10 0.10 0.06 0.09 0.40 0.40 0.40 0.10 0.06 0.09	0.96 0.91 0.97 0.97 0.97 0.97 0.96 0.96 0.96 0.96 0.93 0.90 0.91 ra 0.92 0.97 0.96	1.083 1.086 1.081 1.081 1.083 1.083 1.197 1.077 1.077 1.068 1.069 1.077 1.116 0.933 1.117	1.074 1.062 1.069 1.071 1.062 1.132 1.216 1.080 1.098 1.101 1.002 1.209 1.033 0.917 1.018 0.945	0.922 0.933 0.910 0.922 0.964 0.966 1.055 1.096 0.998 1.015 0.985 0.876 1.018 0.902 0.791 0.945 0.784	0.973 0.963 0.962 0.973 0.963 0.966 0.915 0.977 0.968 0.977 0.968 0.977 0.968 0.977 0.988 0.977 1.257 0.947 0.964	0.845 0.795 0.813 0.845 0.805 0.831 0.863 0.814 0.865 0.818 0.853 0.844 0.853 0.842 0.750 0.709 0.806 0.777 0.876	0.731 0.718 0.742 0.731 0.754 0.778 0.782 0.794 0.801 0.785 0.777 0.785 0.777 0.727 0.607 0.902 0.692 0.834	0.945 0.951 0.977 0.945 0.954 0.955 0.945 0.945 0.945 0.945 0.935 0.945 1.065 0.938 0.938 0.938 0.939	0.879 0.904 0.918 0.879 0.922 0.904 0.886 0.865 0.930 0.926 0.826 0.829 0.860 0.922 0.728 0.939 0.902	0.630 0.654 0.685 0.630 0.686 0.730 0.718 0.689 0.700 0.614 0.630 0.614 0.622 0.517 0.599 0.647	0.0023 0.0021 0.0021 0.0023 0.0018 0.0018 0.0012 0.0012 0.0015 0.0015 0.0019 0.0024 0.0028 0.0024 0.0023	0.0006 0.0007 0.0006 0.0007 0.0006 0.0006 0.0002 0.0002 0.0002 0.0009 0.0009 0.0009 0.0007 0.0013 0.0010 0.0010 0.0010	0.52 0.49 0.54 0.52 0.42 0.30 0.42 0.38 0.31 0.38 0.64 0.28 0.50 0.31 0.47 0.74

	GNGVRTNF	P2 C38H57N13O12	2	432.720	439.200	9.8-10.5	78 85	1	0.963	0.466	0.430	1.083	0.540 0.	.359   0	.954 0.	508 0.41	2 0.76	0.10	0.90	I	1.087 1	.127 0.	.946 1	.007 0	.829 (	0.691 L (	0.905	0.857	0.666	0.0022	0.0009	0.45
	TNFFGMR	P2 C39H57N11O10S1	2	436.708	442.191	16-17.5	83 89		0.896	0.390	0.157	0.935	0.409 0.	210 1	.169 0.	401 0.14	2 1.49	0.09	0.97		1.134 1	149 1	.037 0	0.914 0	.859 (	0.802	0.952	0.878	0.664	0.0015	0.0010	0.25
	HMNGFPMIPATWPLASNLK	P2					90 108																									
	RADADLADGPVSER	P2 C59H98N20O24	3	491.243	497.889	10.5-11.5	110 123		0.971	0.400	0.282	1.029	0.485 0.	107 1	197 0	215 0.20	1.10	0.10	0.97		0.956 1	.028 0.	.985	074 0	779 (	1690	1.044	0.938	0.665	0.0017	0.0011	0.36
	LMFSDLEPVPLK	P2 C65H105N13O18S1	2	694.878	701.359	21.5-23.5	134 145		1.026	0.407	0.303	1.053	0.407 0.	.303 0	0.920 0.	466 0.28	7 1.01	0.06	0.98		1.079 1	.026 0.	.961 0	0.927 0	.788 (	0.690	0.994	0.908	0.669	0.0022	0.0008	0.44
	KGSSTCIPYFSNDMGTK	P2					148 164																									
	AEEAGNLMLQGK	P2					175 186																									
	FDDAYQLHQMGGAYYVVYR	P2 C44H79N14O17	2	£28.004	646,260	14.2.15	187 205		0.957	0.428	0.275	1.022	0.620 0	260 1	120 0	290 0.26	2 1.10	0.00	0.05		1 224	204 4	125 0		910 0	1624	044	0.990	0.608	0.00222	0.0017	0.20
	TGKEVSKDR	P2 C44P178N14017	2	030.251	040.200	14.2110	218 226		0.007	0.420	0.275	1.025	0.025 0.	2.00	1.120 0.	365 0.20	2 1.10	0.08	0.55		1.224	.304 1.	.135 0	1.032 0	.012 (	1.034	1.544	0.000	0.000	0.0022	0.0017	0.20
	EYAVTGGEQGSLF	P2					232 244																									
	AASKDASRL	P2					245 253																									
	LKEQYGIDVPDGFFCER	P2 CONTRACTOR	2	704 709	712 240	22.24	253 269		1.045	0.265	0.059	1.010	0.338 0	010	045 0	195 0.01	2.00	0.20	0.02		1.054	101 1	000 1	001 0	717 (	1701	0.045	0.979	0.654	0.0017	0.0011	0.26
	YAYTEHHTTR	P2 C54P1154N2502552	3	704.700	713.340	2.3*24	299 308		1.040	0.200	0.000	1.010	0.336 0.	.015	1.545 0.	100 0.01	5 3.00	0.30	0.55		1.004	. 101 1.	.008 1				3.545	0.070	0.004	0.0017	0.0011	0.20
	LNKEEKVK	P2					309 316																									
	EWSLCVATDVSDHDTFWPGWLR	P2					317 338																									
	DEICDELLNMGYAPWWVK	P2 C103H151N23O28S2 P2 C39H64N8O12	3	/41.692	/49.003	27-29	339 356		1.072	0.135	0.051	0.866	0.516 0	342	1.063 0.	469 0.31	3 2.54	0.41	0.90		1299 0	.594 U. 287 1	072 0	1999 0	853 0	1,766	1.091	1.024	0.581	0.0031	0.0011	0.53
	VGAPAPEQGHTL	P2 C51H81N15O17	2	588.804	596.281	11.2-12.5	368 379		1.030	0.506	0.348	1.020	0.588 0.	.324 0	.950 0.	515 0.34	9 0.90	0.05	0.96		1.076 1	.057 0.	.970 0	0.969 0	.847 (	0.733	0.954	0.908	0.646	0.0021	0.0008	0.47
	LGDPSNPDLEVGL	P2 C57H92N14O22	2	663.333	670.311	19.8-19.9	381 393		0.968	0.504	0.339	1.054	0.735 0.	.326 0	0.958 0.	506 0.32	3 0.92	0.08	0.95		1.107 1	.186 1.	.009 0	0.970 0	.833 (	0.707 (	0.923	0.999	0.606	0.0023	0.0011	0.37
	SSGQGATDLMGTLL	P2 C55H95N15O22S1	2	675.832	683.309	22.4-23.5	393 406		1.063	0.454	0.283	0.911	0.542 0.	.272 1	1.026 0.	515 0.32	3 1.02	0.06	0.96		1.090 1	.060 0.	.883 0	0.938 0	.812 (	0.728 (	0.972	0.878	0.596	0.0025	0.0008	0.56
	DMPSACR	P2 P2					407 423																									
	FLDSYWQGHEEIR	P2 C77H106N20O23	3	560.599	567.245	17-19.8	436 448		1.134	0.487	0.302	1.168	0.592 0.	290 0	.696 0.	385 0.08	3 1.35	0.30	0.74		1.045 1	.122 0.	.914 0	.992 0	.816 (	0.697	0.963	0.823	0.515	0.0030	0.0012	0.48
õ	QISKSDDAILGWTK	P2					449 462																									
. <u> </u>	ALVGGHRLFEMLK	P2 042H68N1001261	2	400 220	479 221	07.115	465 477		0.071	0.606	0.269	1.071	0.662 0	201 0	0.059 0	448 0.24	7 1.00	0.06	0.08		1 102 -	102 1	028	004 0	924 0	1750	1.012	0.064	0.608	0.0016	0.0011	0.21
Ð	EHGGAF	P2 04210041001231	2	400.230	473.221	8.7*11.5	492 496		0.571	0.000	0.200	1.0/1	0.365 0.	201 0	1.506 0.	440 0.24	.7 1.00	0.00	0.56		1.103	. 103 1.	.030 0		.031 0	.150	1.013	0.504	0.050	0.0010	0.0011	0.21
Ħ	LGDILLY	P2 C39H63N7O11	1	806.466	813.445	20-22	498 504		0.997	0.506	0.314	1.068	0.566 0.	276 0	0.915 0.	466 0.35	0 0.97	0.07	0.96		1.050 1	.054 1.	.014 0	.969 0	.836 (	0.737 (	0.961	0.965	0.631	0.0020	0.0009	0.41
2	DSRREPGSAIF	P2		017.404	0077-404	17.5.40	505 515		4.400	0.400	0.470	4.074	0.407.0	~ ~ ~				0.00	0.07		4 407				705 0			0.700	0.500	0.0000	0.0044	0.00
ā	RPEPGLAWASMK	P2 C30P162N10012S1	-	847.434	607.404	17.5-19	545 556		1.123	0.406	0.170	1.0/1	0.407 0.	210 0		360 0.17	6 1.39	0.09	0.97		1.137	.238 0.	900 0	1.962 0	.765 (	1.096	1.902	0.739	0.539	0.0026	0.0014	0.33
	DTYGACPIYSDVLEAIER	P2					557 574																									
S.	CWWNAFGESYR	P2 C68H86N18O18S1	2	738.312	746.785	20.5-22	575 585		0.809	0.124	0.037	1.191	0.280 0.	.115			2.26	0.45	0.86		1.304 1	.268 1.	.068 0	0.843 0	.629 (	0.421 (	0.853	0.842	0.396	0.0044	0.0024	0.32
<u>_</u>	AYREDMLKR DTI ELSP	P2 C34H60N10O14	2	417 222	422.206	11.4-12	595 601		1.045	0.445	0.302	1.016	0.503 0	251 0	1939 0	376 0.25	8 1.09	0.09	0.95		1.067 1	065 0	916 0	1960 O	781 0	1703	1073	0.909	0.645	0.0023	0.0008	0.52
Q	QAGLAELTPIDLEVLADPNKLQYK	P2 C119H195N29O38	3	880.481	890.119	25.55-25.7	609 632		0.971	0.548	0.316	1.029	0.467 0.	218		510 0.25	1.11	0.12	0.96		0.993 1	.173 0.	.969 0	.809 0	.812 0	0.777	1.198	0.880	0.595	0.0020	0.0013	0.26
F	ADPNKLQY	P2 C42H65N11O14	2	474.743	480.227	10.5-11	624 631		1.055	0.482	0.339	0.984	0.581 0.	.312 0	0.961 0.	484 0.33	6 0.93	0.06	0.97		1.097 1	.054 0.	.933 0	0.943 0	.844 (	0.671 (	0.960	0.850	0.547	0.0028	0.0010	0.51
5	WTEADVSANIHEVLMHGVSVEK	P2					633 654																									
ĸ	PIV/TOAHIDR	P2 P4 C55H93N17O16	3	416 907	422 556	115-135	2 12		1.134	0.673	0.438	0.998	0.732 0	432 (	1867 0	532 0.39	4 0.72	0.08	0.92		1 117 1	106 0	972 0	0 040	770 (	1633	1942	0.902	0.669	0.0023	0.0010	0.41
0	VGIAADLLDASPVSLQVLGRPTAINTVVIK	P4 C136H236N36O41	3	1010.924	1022.888	21-29	13 42		1.009	0.655	0.499	0.953	0.794 0.	458 1	1.038 0.	648 0.41	6 0.65	0.06	0.95		1.106 1	132 1	.032 0	.882 0	822 0	0.822	1.013	0.869	0.612	0.0017	0.0011	0.24
Ð	TYIAAVMELASK	P4 C58H97N13O18S1	2	648.847	655.327	22.5-23.5	43 54		1.207	0.596	0.251	1.039	0.687 0.	.267 0	0.755 0.	391 0.16	0 1.24	0.18	0.87		1.115 1	.159 1.	.064 0	0.974 0	.852 (	0.809	0.911	0.861	0.692	0.0013	0.0010	0.20
S	QGGSLAGVDIRPSVLLK	P4 C75H132N22O23	3	570.668	577.980	19-21	55 71		1.061	0.670	0.477	0.979	0.742 0.	.473 0	0.959 0.	622 0.44 577 0.36	8 0.64	0.04	0.97		1.097 1	.089 1.	.038 0	0.934 0	.738 (	0.706 0	0.968	0.870	0.672	0.0018	0.0011	0.28
, a	DTAIFTKPK	P4 040H75N13014	2	013.001	520.262	17-15	72 80		1.070	0.024	0.410	1.000	0.062 0.	.301 0		5// 0.36	0.75	0.00	0.56		1.107	.110 1.	.000 0	.9/3 0	.037 0	.142	1.520	0.000	0.045	0.0015	0.0010	0.30
5	SADVESDVDVLDTGIYSVPGLAR	P4 C102H164N26O39	3	793.396	802.036	24-26	83 105		1.011	0.592	0.367	0.993	0.649 0.	.372 0	0.997 0.	561 0.32	9 0.86	0.04	0.99		1.087 1	.129 1.	.037 0	.957 0	.814 (	0.753 (	0.956	0.913	0.663	0.0017	0.0010	0.31
¥	KPVTHR	P4					106 111																									
⊢	GATGSGKSITI	P4 C41H74N12O16	2	496 275	502 257	11-13.2	12 125		1.048	0.478	0.279	1.015	0.684 0	349 0	937 0	481 0.27	0 101	0.09	0.94		1 100 1	096 1	005 0	1972 0	858 (	767	927	0.899	0.649	0.0018	0.0009	0.36
>	SITLNEK	P4 C34H61N9O13	2	402.727	407.213	9.3-10.5	133 139		1.047	0.615	0.387	1.011	0.713 0.	.388 0	0.941 0.	569 0.34	5 0.82	0.05	0.97		1.108 1	.117 1.	.013 0	0.938 0	.785 0	0.747	0.954	0.913	0.668	0.0018	0.0010	0.31
	NEKLRPDVL	P4 C47H82N14O15	2	542.311	549.291	12-17.5	137 145		1.060	0.626	0.406	1.118	0.801 0.	.422 0	0.822 0.	543 0.35	6 0.77	0.10	0.89		1.146 1	.157 1.	.052 0	0.892 0	.776 (	0.726	0.962	0.948	0.622	0.0020	0.0012	0.27
ັ	IRWGEVAEAY DELDTAVHISTI DEMI	P4 C55H80N14O16	2	597.301	604.279	17.4-19	146 155		1.045	0.699	0.437	1.011	0.975 0.	.510 0	1.943 0.	570 0.35	0.70	0.13	0.82		1.094 1	.097 0.	.965 0	1.972 0	.863 (	2.7/1	1.935	0.933	0.625	0.0021	0.0009	0.45
	LIVCIGLGALGF	P4					171 182																									
	NVAVDSVRPL	P4 C46H80N14O15	2	535.304	542.282	14-16	183 192		1.065	0.603	0.406	1.006	0.743 0.	.421 0	0.929 0.	584 0.39	G 0.75	0.06	0.96		1.101 1	.104 1.	.016 0	0.961 0	.835 (	0.765 (	0.938	0.891	0.648	0.0018	0.0010	0.34
	KGAASAGGIVAVFY	P4 C61H95N15O17	2	655.859	663.336	20.95-24	197 210		4.070	0.000	0.404	1.104	0.771 0.	.452 0	0.896 0.	587 0.49	2 0.62	0.10	0.90		1.144 1	.212 1.	.061 0	0.993 0	.995 0	0.775	0.863	0.814	0.600	0.0019	0.0013	0.24
	DCSVVM	P4 C51H63N11017	2	301.800	007.289	20.0-29.0	211 220 224 229		1.072	0.630	0.424	0.999	0.717 0.	.416 U	1.929 0.	603 0.36	0.74	0.05	0.97		1.142	.117 0.	.966 U	1.945 U	.614 (	1.129	1913	0.865	0.627	0.0019	0.0010	0.34
	VVNPMVDAEKIEY	P4 C67H107N15O22S1	2	753.879	761.356	19-20	230 242		1.048	0.219	0.037	1.012	0.326 0.	.041 0	.940 0.	139 0.01	9 2.89	0.23	0.96		1.080 1	.069 0.	.958 0	.978 0	.844 (	0.767	0.942	0.903	0.644	0.0020	0.0008	0.44
	IEYVFGQVMASTVGAILCADGNVSR	P4 C115H185N31O37S2	3	886.440	896.409	29.55-31.5	240 264		1.144	0.437	0.149	0.944	0.271 0.	.051 0	.912 0.	115 0.08	0 2.05	0.33	0.85		1.052 1	.089 1.	.040 0	0.961 0	.856 (	0.836	0.987	0.904	0.714	0.0012	0.0008	0.26
	CADGNVSRTMF	P4 C50H80N16O18S2	2	629.271	636.748	13.6-14.5	257 267 268 275		0.990	0.185	0.023	1.064	0.345 0.	.069 (	0.947 0.	169 0.02	3 2.83	0.28	0.94		1.061 1	.002 0.	.911 0	0.946 0	.715 (	0.685 (	0.994	0.928	0.590	0.0026	0.0010	0.50
	IFNGAAPLAADTHMPSMDRPTSMK	P4					274 297																									
	AADTHMPSMDRPTSM	P4 C65H106N20O24S3	3	549.902	556.549	13.4-14.6	282 296		1.026	< 0.06	na	1.051	< 0.06	na (	).922 <1	0.06 na	>4.69	na	na		1.080 1	.007 0.	.820 0	0.962 0	.800 0	0.654 (	0.958	0.872	0.547	0.0032	0.0008	0.69
	ALDHTSIASVAPLER	P4 C68H114N20O23	3	527.286	533.932	15-18	298 312		1.051	0.599	0.379	1.010	0.702 0.	.389 0	0.939 0.	566 0.33	7 0.83	0.05	0.97		1.096 1	.044 0.	.952 0	0.965 0	.894 0	0.784 (	0.950	0.916	0.650	0.0019	0.0008	0.48
	NSAPR	P4 C32H53N11017	2	432.000	438.171	2.0-4.4	313 320		1.025	0.596	0.375	1.020	0.695 0.	.397 0	1900 0.	5/2 0.34	.7 0.62	0.05	0.96		1.066	.065 0.	.960 U	1.960 0	.796 (	1.753	7901	0.914	0.009	0.0018	0.0009	0.36
	TLYLVPPLDSADK	P7 C66H106N14O21	2	716.390	723.368	20-22	2 14		0.857	0.243	0.108	1.047	0.362 0.	.102 1	.095 0.	287 0.12	6 1.82	0.11	0.96		0.967 0	.940 0.	726 0	.965 0	780 0	0.704	1.057	0.973	0.588	0.0032	0.0006	0.81
	ELPALASK	P7 C37H65N9O12	2	414.745	419.231	11.6-12.1	15 22		1.003	0.279	0.127	1.042	0.376 0.	.137 0	0.965 0.	273 0.13	7 1.68	0.09	0.98		1.100 1	.073 0.	.915 0	0.930 0	.752 (	0.682 (	0.970	0.901	0.613	0.0025	0.0010	0.49
	I HEI WPHI SGGOIVIAAI	P7					23 32																									
	NANNLAIL	P7 C36H63N11O12	2	421.740	427.224	18.2-19.5	51 58		0.923	0.333	0.151	0.926	0.509 0.	.171 1	.152 0.	384 0.19	6 1.47	0.10	0.97		1.037 1	.004 0.	.816 0	0.964 0	.814 (	0.737	1.000	0.914	0.577	0.0028	0.0007	0.69
	HMSTLLVELPVAVMAVPGASYR	P7					61 82																									
	SDWNMIAHALPSEDWITLSNK	P7	2	000 244	674 240	125.14	83 103		0.077	0.265	0.110	1.021	0.391 0	120 0	062 0	265 0.12	0 175	0.10	0.08		1.097	067 0	000 0	0.010 0	740 0	1057	007	0.900	0.672	0.0020	0.0010	0.64
	ANDTVQGEKRSGAEPLSPNVY	P7 C94H150N28C35	2	744.701	074.319 754.007	12.5-14	107 119		0.945	0.200	0.110	1.039	0.381 0.	.120 0	1.902 U.	205 0.12 286 0.13	9 1.75	0.10	0.98		1.058 1	.032 N	.826 0		.146 (	0.644		0.895	0.546	0.0029	0.0008	0.68
	SGAEPLSPNVYTDALSR	P7 C76H121N21O28	2	888.942	899.410	18.4-19.8	121 137		0.962	0.270	0.116	1.073	0.388 0.	128 0	.965 0.	274 0.13	3 1.73	0.10	0.98		1.077	.050 0.	.849 0	0.929 0	.756 0	0.638	0.994	0.913	0.590	0.0030	0.0009	0.62
	LGIATAHAIPVEPEQPFDVDEVSA	P7 C112H173N27O38	3	835.755	844.728	21.9-24	138 161		0.968	0.261	0.119	1.036	0.403 0.	.127 0	.996 0.	286 0.14	4 1.70	0.11	0.97	ļ	1.061 1	.042 0.	.863 0	0 939 0	.759 (	0.631	1.001	0.920	0.578	0.0030	0.0009	0.61

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## Appendix C. Supplementary Information for Chapter 4

SP	P03416	NCAP_CVMA5	MSFVPGQENAGGRSSSVNRAGNGILKKTTWADQTERGPNNQNRGRRNQPKQTATTQPNSG	60
SP	P18447	NCAP CVM3	MSFVPGQENAGGRSSSGNRAGNGILKKTTWADQTERGPNNQNRGRRNQPKQTATTQPNSG	60
SP	P03417	NCAP CVMJH	MSFVPGOENAGSRSSSGNRAGNGILKKTTWADOTERGLNNONRGRKNOPKOTATTOPNSG	60
CD	OBBABE	NCAD CUM2		56
DF	Q3F130	NCAF_CVM2	Mar VFGQENAGSKSSSSGNRAGNGTLKRTTWADQTERGNRGRRAGHFRQTATTQFNAG	50
SP	P18446	NCAP_CVM1	MSFVPGQENAGSRSSSVNRAGNGILKKTTWADQTERGPNNQNRGRRNQPKQTATTQPNSG	60
SP	P18448	NCAP_CVMS	MSFVPGQENAGSRSSSGSRSGNGILKKTTWADQTERAGNNGNRGRRNQPKQTATTQPNSG	60
SP	Q83360	NCAP CVMDV	MSFVPGQENAGSRSSSGNRAGNGILKKTTWADQTERGPNNQNRGRRNQPKQTATTQSNSG	60
		-	***************************************	
CD	02416	NOAD OTHAE	CUMUNITY CHECCT COCKERENCE & ECOCUPITANCE DA CEOROVERUND DE EVENDOCOOR	120
SP	P03416	NCAP_CVMA5	SVVPHYSWFSGITQFQKGKEFQFAEGQGVPIANGIPASEQKGYWYRHNRRSFKTPDGQQK	120
SP	P18447	NCAP_CVM3	SVVPHYSWFSGITQFQKGKEFQFAEGQGVPIANGIPASEQKGYWYRHNRRSFKTPDGQQK	120
SP	P03417	NCAP_CVMJH	SVVPHYSWFSGITQFQKGKEFQFAQGQGVPIANGIPASQQKGYWYRHNRRSFKTPDGQQK	120
SP	Q9PY96	NCAP_CVM2	SVVPHYSWFSGITQFQKGKEFQFAQGQGVPIASGIPASEQKGYWYRHNRRSFKTPDGQHK	116
SP	P18446	NCAP CVM1	SVVPHYSWFSGITOFOKGKEFOFAOGOGVPIANGIPASEOKGYWYRHNRRSFKTPDGOOK	120
SP	P18448	NCAP CUMS	SWUPHY SWESCITOFOKCKEFOFUCCOCUPIANCIPASEOKCYWY PHNPPSFKTPDCOOK	120
CD	093360	NCAD CUMDU	CUMPLING SOLL ST STORE ST ST STORE ST	120
BP	003300	NCAP_CVMDV	SVVPHISWESGIIGE QEGEEF QEADGQGVPIANGIPASEQEGIWIERNERSEETEPDGQQE	120
			***************************************	
SP	P03416	NCAP CVMA5	<b>OLLPRWYFYYLGTGPHAGASYGDSIEGVFWVANSOADTNTRSDIVERDPSSHEAIPTRFA</b>	180
SP	P18447	NCAP CVM3	OLL PRWYFYYL GTGPHAGASYGDSTEGVFWVANSOADTNTRSDTVER	180
CD	D02417	NCAD CUMTH		100
or	F03417	NCAF_CVMOH	QUERKWITTINGIGFIKGAEIGDDIEGVVWVASQQAEIKISADIVEKDFSSHEAIFIKFA	100
SP	Qabrae	NCAP_CVM2	QLLPRWYFYYLGTGPHAGAEYGDDIEGVVWVASQQADTKTTADVVERDPSSHEAIPTRFA	1/6
SP	P18446	NCAP_CVM1	QLLPRWYFYYLGTGPHAGAEYGDDIDGVVWVASQQADTKTTADIVERDPSSHEAIPTRFA	180
SP	P18448	NCAP CVMS	QLLPRWYFYYLGTGPHAGAEYGDDIEGVVWVASQQADTKTTADIVERDPSSHEAIPTRFA	180
SP	083360	NCAP CVMDV	OLL PRWY FYYL GTGPHAGATYGDSTEGVFWVANSOADTNTRSDTVER DPSSHEAT PTRFA	180
21	2000000		***************************************	100
SP	P03416	NCAP_CVMA5	PGTVLPQGFYVEGSGRSAPASRSGSRSQSRGPNNRARSSSNQRQPASTVKPDMAEEIAAL	240
SP	P18447	NCAP_CVM3	<b>PGTVLPQGFYVEGSGR</b> SAPASRSGSRSQSRGPNNRARSSSNQRQPASTVKPDMAEEIAAL	240
SP	P03417	NCAP CVMJH	PGTVLPOGFYVEGSGRSAPASRSGSRPOSRGPNNRARSSSNOROPASTVKPDMAEEIAAL	240
SP	09PY96	NCAP CVM2	PGTVLPOGFYVEGSGRSAPASRSGSRSOSRGPNNRARSSSNOROPASAVKPDMAEEIAAL	236
CD	D19446	NCAD CUM1		240
or	P10440	NCAF_CVM1	PGIVLEQGEIVEGSGRSAFASKSGSRSQSRGFNNRARSSSNQRQFASIVR DMALLIAAL	240
SP	P18448	NCAP_CVMS	PGTVLPQGFYVEGSGRSAPASRSGSRSQSRGPNNRARSSSNQRQPASTVKPDMAEEIAAL	240
SP	Q83360	NCAP_CVMDV	PGTVLPQGFYVEGSGRSAPASRSGSRSQSRGSNNRARSSSNQRQPASTVKPDMAEEIAAL	240
SP	P03416	NCAP CUMAS	VT. AKT.GKDAGODKOVTKOSAKEVBOKTT.NKDBOKDTDNKOCDVOOCFGKDGDNONFGGSE	300
SP	P03416	NCAP_CVMA5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark>	300
SP SP	P03416 P18447	NCAP_CVMA5 NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark>	300 300
SP SP SP	P03416 P18447 P03417	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <u>GPNQNFGGSE</u> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <u>GPNQNFGGPE</u>	300 300 300
SP SP SP	P03416 P18447 P03417 Q9PY96	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQXILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <del>GPNQNFGGPE</del> VLAKLGKDAGQPKQVTKQSAKEVRQKILTKPRQKRTPNKQCPVQQCFGKR <del>GPNQNFGGSE</del>	300 300 300 296
SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQXILNKPRQKRTPNKQCPVQCFGKRGFNQNFGGSE	300 300 300 296 300
SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <mark>GPNQNFGGSE</mark> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <del>GPNQNFGGSE</del> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <del>GPNQNFGGSE</del> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKR <del>GPNQNFGGSE</del> VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKR <del>GPNQNFGGSE</del> VLAKLGKDAGOPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPCVQCFGKR <del>GPNQNFGGSE</del>	300 300 300 296 300 300
SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGFNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGFNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGFNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGFNQNFGGSE	300 300 300 296 300 300
SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1 NCAP_CVMS NCAP_CVMS	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGPE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGPE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQFFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE	300 300 300 296 300 300 300
SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTSQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTSQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTSQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTSQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE	300 300 296 300 300 300 300
SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM2 NCAP_CVM1 NCAP_CVMS NCAP_CVMDV	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGPE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE	300 300 296 300 300 300
SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTFQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE	300 300 296 300 300 300 300
SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3H NCAP_CVM2 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM55 NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTFGSKLELVKKNSGGADEPTKDVYELQVSGAVRFDS	300 300 296 300 300 300 300 300
SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417	NCAP_CVMA5 NCAP_CVM3 NCAP_CVMJH NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVNQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDDQPFDILAELAPTVGAPFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS	300 300 296 300 300 300 300 360 360 360
SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417 P03417	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPFQVTRQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTFGSAKELVRQKILNKPRQKTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPFTLAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS	300 300 296 300 300 300 300 360 360 360
SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9Py96 P18446 Q83360 P18448 Q83360 P03416 P18447 P03417 Q9Py96	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM8 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM45 NCAP_CVM45	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS VLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS	300 300 296 300 300 300 300 360 360 360 356
SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9PY96 P18446	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTFLAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS	300 300 296 300 300 300 360 360 356 356 360
SP SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9Y96 P18446 P18448	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS	300 300 296 300 300 300 360 360 360 356 360 356 360
SP SP SP SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P18447 P03416 P18447 P18446 P18446 P18448 Q83360	NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM8 NCAP_CVM8 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKFRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGATRFDS	300 300 296 300 300 300 360 360 356 360 360 360 360
SP SP SP SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9PY96 P18446 P18448 Q9S360	NCAP_CVMA5 NCAP_CVMJ NCAP_CVMJ NCAP_CVM2 NCAP_CVM2 NCAP_CVMS NCAP_CVMA5 NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS	300 300 296 300 300 300 360 360 356 360 360 360 360
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SP SP SP SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9Py96 P18448 Q83360 P03416 P18447 P03416 P18447 P03416 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDDAGPFQVTRQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDDAGPFQVTRQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDDQPFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAVRFDS MLKUGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAVRFDS MLKDGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAVRFDS MLKDGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAVRFDS MLKDGTSDQFFGSKGFGSKGFGSKGFGSKGFGSKGFGSKGFGSKGFGS	300 300 296 300 300 300 360 360 360 360 360 360
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9PY96 P18448 Q83360 P03416	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM2	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRPDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS MLKUGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS MLKUGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS MLKUGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS MLKUGTSDPQFPILAELAPTGAFFFGSKLEUVKNSGGADEPTKDVYELLYSGAIRFDS	300 300 296 300 300 300 360 360 356 360 356 360 360 360
SP SP SP SP SP SP SP SP SP SP SP SP SP	P03416 P18447 P03417 Q9PY96 P18448 Q83360 P03416 P18447 P03417 Q9PY96 P18448 Q83360 P03416 P18448	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM8 NCAP_CVM8 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM4	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTLAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRPDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELQYSGAIRFDS MLKGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELYSGSAIRFSS MLKDGTSDPQFPILAELAPTGAFFFGSKLEVKNSGGVCDFYKDVYELYSGS MLKGTSDPQFFJCAKFSV MLKGTSDPQFFJCAKFFKGKGKCKDEVDNVSVAKPKSSV	300 300 296 300 300 300 360 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 Q9PY96 P18448 Q83360 P18447 P18447 P18447 P18447 P184446 P184446 P184446 P184446 P184447 P184447 P18447 P18447 P18447 P18447	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAVRPDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRPDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRPDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS VTMTTTPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV	300 300 300 300 300 300 300 360 360 360
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 Q9PY96 P18448 Q83360 P03416 P18448 Q83360 P18446 P18448 Q9PY96 P18446 P18448 Q9PY96 P18446 P18447 P03416 P18447 P03416	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM8 NCAP_CVM8 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKIGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLEVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLEVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFSS TLPGFFTMKVLNENLNAYQK-DGGADVVSPKPQRKGRRQAGEKKDEVDNVSVAKPKSSV TLPGFFTMKVLNENLNAYQDQJGGDVVSPKPQRKGRRQAGEKKDEVDNVSVAKPKSSV	300 300 296 300 300 300 300 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 Q9PY96 P18448 Q83360 P18447 P03416 P18447 P18448 Q83360 P18446 P18448 Q83360 P13416 P18446 P18446 P18446 P18447 P03417 Q9PY96 P18446	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM1 NCAP_CVM1	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTVGAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLEVNXNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLEVNXNSGGADEPTKDVYELYSGAIRFDS TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDQGGSDVVSPKPQRKGTKQKLKGEVDNVSVAKPKSV	300 300 296 300 300 300 300 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 Q9PY96 P18448 Q83360 P03416 P18448 Q83360 P18446 P18448 Q9PY96 P18446 P18448 Q83360 P18444 P03416 P18444 P03416 P18446 P18446 P18446	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGSSE VLAKLGKDAGPFDILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLEVKNSGGADEPTKDVYELQYSGAIRFSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDQAGSVDVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDQAGSVDVSPKPQRKGTKQKAQKACVDVNSVAKPKSSV TLPGFFTIMKVLNENLNAYQDQAGSVDVSPKPQRKGTKQKAQXEKDEVDNVSVAKPKSSV	300 300 296 300 300 300 300 300 360 356 360 360 360 360 360 419 420 419
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SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 O9PY96 P18446 P18448 Q83360 P03416 P18447 P18446 P18448 Q83360 P03416 P18448 P03416 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLEVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLEVKNSGGADEPTKDVYELYSYSAFFSSV TLPGFFTIMKVLNENLNAYQR-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDAGGADVVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDAGGADVVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDAGGVDVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDAGGVDVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQDAGGVDVSPKPQRKGTKQKAQKEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNAGGVDVSPKPQRKGTKQKALGEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNAGGVDVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV	300 300 296 300 300 300 300 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 C9PY96 P18446 P18446 P18448 Q83360 P03416 P18447 P03417 Q9PY96 P18448 Q83360 P03416 P18444 P184448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM4 NCAP_CVM4 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQUTAGSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQUTAGSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTBGAFFGSKLEVNKNSGGADEVTNDVSVKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRAQAKENEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQKAQEKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQKALGEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQKALGEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFETIMKVLNENLNAYQNDQGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV	300 300 300 300 300 300 360 360 360 360
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 OPPY96 P18446 P18448 Q83360 P03416 P18447 P18448 Q83360 P18446 P18448 Q83360 P03416 P18448 Q97Y96 P18446 P18446 P18448 Q83360	NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM4 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM4	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFPILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTAGAFFFGSKLELVKKNSGGADEPTKDVYELYSKARFKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNQAGGADVVSPKPQRKGTKQKAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQN-QAGGADVVSPKPQRKGTKQAQEKKDEVDNVSVAKPKSSV	300 300 296 300 300 300 300 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 Q9Py96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9Py96 P18448 Q83360 P03416 P18446 P18448 Q83360 P18448 Q8360 P18448 Q8360	NCAP_CVMA5           NCAP_CVM3           NCAP_CVM1           NCAP_CVM2           NCAP_CVM3           NCAP_CVM4	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFQTLAELAPTVGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTMTT VLGFSTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRVGACEKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRVGACEKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRVGACEKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRVGACEKDEVDNVSVAKPKSSV TLPGFSTIMKVLNENLNAYQN_GGGDVVSPKPQRKGRVGACEKDEVDNVSVAKPKSSV	300 300 300 300 300 300 360 360 360 360
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SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 G9FY96 P18446 P18448 Q83360 P03416 P18447 Q9FY96 P18448 Q83360 P03416 P18446 P18448 Q8360 P18448 Q8360 P184448 Q8360	NCAP_CVMA5           NCAP_CVM3           NCAP_CVM1           NCAP_CVM2           NCAP_CVM2           NCAP_CVM3           NCAP_CVM4           NCAP_CVM4           NCAP_CVM5           NCAP_CVM3	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGFE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPFDILAELAPTGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTMT VLCGTSDPQFFILAELAPTBSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTMT VLCGTSDPQFFILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTMT VLCGTSDPQFFILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS VTMTMT VLCGTSDPQFFILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELLYSGAIRFDS VTMTMT VLCGTSDPQFFILAELAPTBGAFFGSKLELVKKNSGGADEPTKDVYELYSGXAFKSSV TLPGFFTIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGGDVVSPKPQRKGTKQKALGEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGADVVSPKPQRKGTKQALGKEVDVNSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGGDVVSPKPQRKGTKQALKGEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGGDVVSPKPQRKGTKQALGKEVDVNSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGADVVSPKPQRKGTKQALGEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGGDVVSPKPQRKGTKQALGEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGSDVVSPKPQRKGTKQALGEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGGDVVSPKPQRKGTKQALGEKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGEDDVSVFVQRKGTVSALFGTVSLAKPKSV TLPGFFTIMKVLNENLNAYQNDGGDDVVSPKPQRKGTYQALGENDSVSVAKPKSSV TLPGFFTIMKVLNENLNAYQNDGGEDDVSVFVGAKFSSV TLPGFFTIMKVLNENL	300 300 300 300 300 300 360 356 360 356 360 360 360 360 419 419 420 419 420
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03416 P18448 Q83360 P03416 P18448 Q83360 P03416 P18448 Q83360 P03416	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM2 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM1 NCAP_CVM5 NCAP_	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDDQPFJLAELAPTVGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS TLPGFFINKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQNDOGGADVVSPKPQRKGTKQKALKGEVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQAQEKKDEVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQAQEKKDEVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEDVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKEVDVNSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPQRKGTKQALKDEVDNVSVAKPKSSV TLPGFFINKVLNENLNAYQN-DGGADVVSPKPGRGRQAQEKKDEVDNSVAKPKSSV TLPGFFINKVLNENLNAYCM-DGGADVVSPKPGRGRQAQEKKDVNSVAKPKSSV TLPGFFINKVLNENLNAYCM-DGGADVVSPKPGRGTKGTKDAQA	300 300 296 300 300 300 360 360 360 360 360 360 36
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 Q9Py96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9Py96 P18448 Q83360 P03416 P18448 Q83360 P03416 P18444 P18448 Q8360 P184448 Q8360	NCAP_CVMA5           NCAP_CVM3           NCAP_CVM1           NCAP_CVM2           NCAP_CVM2           NCAP_CVM3           NCAP_CVM45           NCAP_CVM3           NCAP_CVM45           NCAP_CVM45	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDDQPFILAELAPTGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTTT VFGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKRGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGDDVVSPKPQRKRGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGGDDVVSPKPQRKRGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGGDDVVSPKPQRKRGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGGDDVVSPKPQRKRGRQAPEKSDVSVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGGDDVVSPKPQRKRGRQAPEKSDVSVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGGDDVVSPKPQRKGRVQAPEKSDVSVSVKPKSSV TLPGFET	300 300 296 300 300 300 360 360 360 360 360 360 36
SP	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03416 P18448 Q83360 P03416 P18448 Q83360 P18446 P18448 Q83360 P18446 P184447 P03416 P18446 P184447 P03416 P18446 P18447 P03416 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P18447 P18446 P1846 P186 P186 P186 P186 P186 P186 P186 P18	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM3 NCAP_CVM3 NCAP_CVM1 NCAP_CVM3 NCAP_CVM5 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM2 NCAP_CVM3 NCAP_	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDDPFTILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS NLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS NLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELYSGAIRFDS VLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEFTKDVYELYSGAIRFDS VLKUGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEFTKDVYELYSGAIRFDS VLKUGTSDPQFFILAELAPTSAFFFGSKLEVKNNSGGADEVSKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQN-DGGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQN-DGGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQN-DGGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-DGGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA-GGADVVSPKPQRKGRTQACKKDEVDNVSVAKPKSSV TLPGFFTIMKVLNENLNAYQA	300 300 300 300 300 300 360 360 360 360
SP         SP           SP         SP      SP         SP	P03416 P18447 Q9Py96 P18446 P18448 Q83360 P03416 P18447 P03417 Q9Py96 P18448 Q83360 P03416 P18447 P03417 P03417 Q9Py96 P18448 Q83360 P03416 P18447 P03416 P18446 P18448 Q9Py96 P18448	NCAP_CVMA5           NCAP_CVM3           NCAP_CVM1           NCAP_CVM2           NCAP_CVM1           NCAP_CVM3           NCAP_CVM45           NCAP_CVM45           NCAP_CVM45           NCAP_CVM1           NCAP_CVM45           NCAP_CVM45           NCAP_CVM41	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDDQPFILAELAPTGAFFFGSKLELVKNSGGADEPTKDVYELQYSGAVRPDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS MLKLGTSDPQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTMTTT VFGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDQAGSDDVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRAQAEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNAYQDAGGADVVSPKPQRKGRAQAEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLNA	3000 3000 296 3000 3000 3600 3600 3600 3600 3600 4199 4200 419 4200 419 4200
SP SP SP SP SP SP SP SP SP SP SP SP SP S	P03416 P18447 P03417 Q9PY96 P18446 P18448 Q83360 P03416 P18447 P03416 P18448 Q83360 P03416 P18448 Q83360 P03416 P18448 Q83360 P18446 P18444 P18448 Q83360	NCAP_CVMA5 NCAP_CVM3 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM5 NCAP_CVM3 NCAP_CVM2 NCAP_CVM2 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM1 NCAP_CVM5 NCAP_CVM3 NCAP_	VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQNFGGSE VLAKLGKDAGQPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDAGPKQVTKQSAKEVRQKILNKPRQKRTPNKQCPVQCFGKRGPNQNFGGSE VLAKLGKDDQFFILAELAPTGAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAVRFDS MLKLGTSDPQFFILAELAPTSAFFFGSKLELVKKNSGGADEPTKDVYELQYSGAIRFDS VTTATT VLFQFETIMKVLNENLANYQK-DGGADVVSPKPQRKGRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQN-DGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQN-DGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDQAGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDQAGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDQAGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV TLPGFETIMKVLNENLANYQDDGGADVVSPKPQRKGRRQAQEKKDEVDNVSVAKPKSSV	300 300 296 300 300 300 360 350 360 356 360 360 419 420 419 420 420

**Figure C-1** Multiple sequence alignment of MHV nucleoproteins for seven MHV strains on SwissProt, including MHV-A59 (P03146), MHV-3 (P18447), MHV-JHM (P03417), MHV-2 (Q9PY96), MHV-1 (P18446), and MHV-S (P18448), and MHV-DVIM (Q83360). All detected peptides by LC-MS/MS were highlighted.



**Figure C-2** MHV propagation curves when the L2 culture system inoculated with MHV suspended in various aqueous environments, including media, concentrated wastewater influent (ww inf), and concentrated wastewater effluent (ww eff).

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SP|P03527|SIGM3 REOVD MEVCLPNGHQVVDLINNAFEGRVSIYSAQEGWDKTISAQPDMMVCGGAVVCMHCLGVVGS 60
SP P07939 SIGM3 REOVL MEVCLPNGHQIVDLINNAFEGRVSIYSAQEGWDKTISAQPDMMVCGGAVVCMHCLGVVGS 60
SP|P30211|SIGM3_REOVJ MEVCLPNGHQIVDWINNAFEGRVSIYSAQQGWDKTISAQPDMMVCGGAVVCMHCLGVVGS 60
                   \texttt{SP} \texttt{|P03527|SIGM3_REOVD LQRKLKHLPHHRCNQQIRHQDYVDVQFADRVTAHWKRGMLSFVAQMHEMMNDVSPDDLDR 120}
SP|P07939|SIGM3_REOVL LQRKLKHLPHHRCNQQIRHQDYVDVQFADRVTAHWKRGMLSFVAQMHAMMNDVSPEDLDR 120
SP|P30211|SIGM3_REOVJ LQRKLKHLPHHKCNQQLRQQDYVDVQFADRVTAHWKRGMLSFVSQMHAIMNDVTPEELER 120
                   SP|P03527|SIGM3 REOVD VRTEGGSLVELNWLQVDPNSMFRSIHSSWTDPLQVVDDLDTKLDQYWTALNLMIDSSDLI 180
SP|P07939|SIGM3_REOVL VRTEGGSLVELNWLQVDPNSMFRSIHSSWTDPLQVVDDLDTKLDQYWTALNLMIDSSDLV 180
SP|P30211|SIGM3_REOVJ VRTDGGSLAELNWLQVDPGSMFRSIHSSWTDPLQVVEDLDTQLDRYWTALNLMIDSSDLV 180
                   \texttt{SP} \texttt{P03527} \texttt{SIGM3\_REOVD} \texttt{PNFMMRDPSHAFNGVKLGGDARQTQFSRTFDSRSSLEWGVMVYDYSELEHDPSKGRAYRK \texttt{240}
SP|P07939|SIGM3_REOVL PNFMMRDPSHAFNGVRLEGDARQTQFSRTFDSRSSLEWGVMVYDYSELEHDPSKGRAYRK 240
SP|P30211|SIGM3_REOVJ PNFMMRDPSHAFNGVKLEGEARQTQFSRTFDSRSNLEWGVMIYDYSELERDPLKGRAYRK 240
                   SP|P03527|SIGM3 REOVD ELVTPARDFGHFGLSHYSRATTPILGKMPAVFSGMLTGNCKMYPFIKGTAKLKTVRKLVE 300
SP|P07939|SIGM3 REOVL ELVTPARDFGHFGLSHYSRATTPILGKMPAVFSGMLTGNCKMYPFIKGTAKLKTVRKLVD 300
SP|P30211|SIGM3 REOVJ EVVTPARDFGHFGLSHYSRATTPILGKMPAVFSGMLTGNCKMYPFIKGTAKLRTVKKLVD 300
                   SP|P03527|SIGM3_REOVD AVNHAWGVEKIRYALGPGGMTGWYNRTMQQAPIVLTPAALTMFPDTIKFGDLNYPVMIGD 360
SP|P07939|SIGM3_REOVL SVNHAWGVEKIRYALGPGGMTGWYDRTMQQAPIVLTPAALTMFSDTTKFGDLDYPVMIGD 360
SP|P30211|SIGM3_REOVJ AVNHTWGSEKIRYALGPGGMTGWYNRTMQQAPIVLTPAALTMFPDMTKFGDLQYPIMIGD 360
                          • * * * • * *
SP|P03527|SIGM3_REOVD PMILG 365
SP|P07939|SIGM3_REOVL PMILG 365
SP P30211 SIGM3_REOVJ PAVLG 365
                   * :**
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Figure C-3 Multiple sequence alignment of reovirus sigma-3 proteins. Sequence similarity: 88.9%.

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SP|P11078|MU1 REOVD MGNASSIVQTINVTGDGNVFKPSAETSSTAVPSLSLSPGMLNPGGVPWIAVGDETSVTSP 60
SP P11077 MU1 REOVL MGNASSIVQTINVTGDGNVFKPSAETSSTAVPSLSLSPGMLNPGGVPWIAIGDETSVTSP 60
SP P12397 MU1_REOVJ MGNASSIVQTINVTGDGNVFKPSAETSSTAVPSLSLSPGMLNPGGVPWIAIGDETSVTSP 60
SP|P11078|MU1 REOVD GALRRMTSKDIPETAIINTDNSSGAVPSESALVPYIDEPLVVVTEHAITNFTKAEMALEF 120
SP P11077 MU1_REOVL GALRRMTSKDIPETAIINTDNSSGAVPSESALVPYNDEPLVVVTEHAIANFTKAEMALEF 120
SP P12397 MU1 REOVJ GALRRMTSKDIPETAIINTDNSSGAVPSESALVPYNDEPLVVVTEHAIANFTKAEMALEF 120
                  ******
SP|P11078|MU1 REOVD NREFLDKMRVLSVSPKYSDLLTYVDCYVGVSARQALNNFQKQVPVITPTRQTMYVDSIQA 180
SP P11077 MU1_REOVL NREFLDKLRVLSVSPKYSDLLTYVDCYVGVSARQALNNFQKQVPVITPTRQTMYVDSIQA 180
SP P12397 MU1 REOVJ NREFLDKLRVLSVSPKYSDLLTYVDCYVGVSARQALNNFQKQVPVITPTRQTMYVDSIQA 180
                  SP|P11078|MU1 REOVD ALKALEKWEIDLRVAQTLLPTNVPIGEVSCPMQSVVKLLDDQLPDDSLIRRYPKEAAVAL 240
SP|P11077|MU1 REOVL ALKALEKWEIDLRVAOTLLPTNVPIGEVSCPMOSVVKLLDDOLPDDSLIRRYPKEAAVAL 240
SP P12397 MU1_REOVJ ALKALEKWEIDLRVAQTLLPTNVPIGEVSCPMQSVVKLLDDQLPDDSLIRRYPKEAAVAL 240
                  ***************
SP|P11078|MU1 REOVD AKRNGGIOWMDVSEGTVMNEAVNAVAASALAPSASAPPLEEKSKLTEOAMDLVTAAEPEI 300
SP P11077 MUL REOVE AKRNGGTOWMDVSEGTVMNEAVNAVAASALAPSASAPPLEEKSKLTEOAMDLVTAAEPET 300
SP P12397 MU1_REOVJ AKRNGGIQWMDVSEGTVMNEAVNAVAASALAPSASAPPLEEKSKLTEQAMDLVTAAEPEI 300
                  SP|P11078|MU1 REOVD IASLAPVPAPVFAIPPKPADYNVRTLRIDEATWLRMIPKSMNTPFOIOVTDNTGTNWHLN 360
SP P11077 MU1_REOVL IASLVPVPAPVFAIPPKPADYNVRTLKIDEATWLRMIPKTMGTPFQIQVTDNTGTNWHLN 360
SP P12397 MU1_REOVJ IASLVPVPAPVFAIPPKPADYNVRTLKIDEATWLRMIPKTMNTPFQIQVTDNTGTSWHMN 360
                  SP P11078 MILL REOVE LEGGTRVVNLDOIAPMREVLDLGGKSYKETSWDPNGKKVGEIVFOSKIPFELWTAASOIG 420
SP P11077 MU1_REOVL LRGGTRVVNLDQIAPMRFVLDLGGKSYKETSWDPNGKKVGFIVFQSKIPFELWTAASQIG 420
SP P12397 MUI REOVJ LRGGTRVVNLDQIAPMRFVLDLGGKSYKETSWDPNGKKVGFIVFOSKIPFELWTAASOIG 420
                  SP|P11078|MU1_REOVD QATVVNYVQLYAEDSSFTAQSIIATTSLAYNYEPEQLNKTDPEMNYYLLATFIDSAAITP 480
SP P11077 MU1 REOVL OATVVNYVOLYAEDSSFTAOSIIATTSLAYNYEPEOLNKTDPEMNYYLLATFIDSAAITP 480
\texttt{SP} \texttt{P12397} \texttt{MU1}_\texttt{REOVJ} \texttt{QATVVNYVQLYAEDSSFTAQSIIATTSLAYNYEPEQLNKTDPEMNYYLLAAFIDSAAIST 480}
SP|P11078|MU1_REOVD TNMTQPDVWDALLTMSPLSAGEVTVKGAVVSEVVPADLIGSYTPESLNASLPNDAARCMI 540
SP P11077 MU1_REOVL TNMTQPDVWDALLTMSPLSAGEVTVKGAVVSEVVPAELIGSYTPESLNASLPNDAARCMI 540
SP|P12397|MU1_REOVJ SNMTQPDVWDALLTMSPLSAGEVTVKGAVVSEVIPADLVGSYTPESLNASLPNDAARCMI 540
                  \texttt{SP} \,|\, \texttt{P11078} \,|\, \texttt{MU1}\_\texttt{REOVD} \,\, \texttt{DRASKIAEAIKIDDDAGPDEYSPNSVPIQGQLAISQLETGYGVRIFNPKGILSKIASRAM} \,\, \texttt{600}
SP|P11077|MU1_REOVL DRASKIAEAIKIDDDAGPDEYSPNSVPIQGQLAISQLETGYGVRIFNPKGILSKIASRAM 600
SP P12397 MU1 REOVJ DRASKIAEAIKIDDDAGPDEYSPNSVPIQGQLAISQLETGYGVRIFNPKGILSKIASRAM 600
SP|P11078|MU1 REOVD QAFIGDPSTIITQAAPVLSDKNNWIALAQGVKTSLRTKSLSAGVKTAVSKLSSSESIQNW 660
SP P11077 MU1_REOVL QAFIGDPSTIITQAAPVLSDKNNWIALAQGVKTSLRTKSLSAGVKTAVSKLSSSESIQNW 660
SP P12397 MU1_REOVJ QAFIGDPSTIITQAAPVLSDKNNWIALAQGVKTSLRTKSLSAGVKTAVSKLSSSESIQSW 660
                  *****
SP|P11078|MU1 REOVD TQGFLDKVSAHFPAPKPDCPTSGDSGESSNRRVKRDSYAGVVKRGYTR 708
SP P11077 MU1 REOVL TQGFLDKVSTHFPAPKPDCPTNGDGSEPSARRVKRDSYAGVVKRGYTR 708
SP P12397 MU1_REOVJ TQGFLDKVSTHFPAPKPDCPQSGDSGDGSARRLKRDSYAGVVKRGYTR 708
```

Figure C-4 Multiple sequence alignment of reovirus mu-1 proteins. Sequence similarity: 96.2%.
Cell	Cell type	Source	Example viruses that can be	Citations
line	• 1		cultured	
L2	Mouse lung	Dr. Julian Leibowitz's lab at Texas A&M	murine hepatitis virus	
	epithelial cells	Health Science Center College of Medicine		
DBT	Mouse lung	Dr. Julian Leibowitz's lab at Texas A&M	murine hepatitis virus	
	epithelial cells	Health Science Center College of Medicine		
Vero	Monkey	Dr. Michael J. Imperiale's lab, University of	poliovirus, coxsackie virus,	1,2
	kidney	Michigan	echovirus, reovirus,	
	epithelial cells		adenovirus, picornavirus	
			simian virus 40	
BSC-1	Monkey	Dr. Michael J. Imperiale's lab, University of	poliovirus, coxsackie virus,	1,3,4
	kidney	Michigan	echovirus, reovirus,	
	epithelial cells	-	hepatitis A virus, simian	
			virus 40	
Virus				
Murine	hepatitis virus	Dr. Julian Leibowitz's lab at Texas A&M		
strain A59		Health Science Center College of Medicine		

Table C-1 Cell lines and viruses used in this study.

## Table C-2 MS instrument settings for ICC-MS proteomics analysis.

Peptides (positive mode)	
Sheath gas flow rate	24
Auxiliary gas flow rate	8
Sweep gas flow rate	1
Spray voltage	3 kV
Capillary temp.	300 °C
S-len RF level	50.0
Aux gas heater temp.	275 °C
Column temperature	25 °C
Full MS settings	
Resolution	70,000
AGC target	5e5
Max IT	200 ms
Scan range	400-1800 m/z
dd-MS <sup>2</sup> settings	
Resolution	35,000
AGC target	2e5
Max IT	100 ms
Loop count	20
Isolation window	1.6 Da
NCE	30
Intensity threshold	2e4
Charge exclusion:	Unassigned, 1
Dynamic exclusion	20 sec

**Table C-3** Viral peptides detected by LC-MS/MS in MHV control experiments, where MHV was added to culture media. Nucleoproteins of MHV strains A59 and 3 were confidently identified. Peptides that differentiate strains are highlighted. A protein score greater than 76 was considered significant identification.

	Hours post infection	Identified Protein	Detected peptides	Protein sequence coverage	Protein score
	12 h		N.A.	N.A.	N.A.
300 PFU/mL, 1.2 mL inoculum	18 h	Nucleoprotein (MHV-A59, MHV-3)	TTWADQTER DPSSHEAIPTR <b>SFVPGQENAGGR</b> FAPGTVLPQGFYVEGSGR	9%	120
	26 h	Nucleoprotein (MHV-A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK GPNQNFGGSEMLK DVYELQYSGAVR KDEVDNVSVAKPK FAPGTVLPQGFYVEGSGR LGTSDPQFPILAELAPTVGAFFFGSK	25%	509
		Spike glycoprotein (Coronavirus)	SAIEDLLFDK	0.7%	76
	26 h	Nucleoprotein (MHV-A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR LGTSDPQFPILAELAPTVGAFFFGSK	28%	469
	18 h		N.A.	N.A.	N.A.
	24 h	Nucleoprotein (MHV-A59, MHV-3)	TTWADQTER SFVPGQENAGGR FAPGTVLPQGFVEGSGR LGTSDPQFPILAELAPTVGAFFFGSK	12%	77
	30 h	Nucleoprotein (MHV-A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR FAPGTVLPQGFYVEGSGR	9%	113
30 PFU/mL, 1.2 mL inoculum	37 h	Nucleoprotein (MHV-A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR FAPGTVLPQGFYVEGSGR	11%	242
	37 h	Nucleoprotein (MHV-A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR KDEVDNVSVAKPK FDSTLPGFETIMK RGPNQNFGGSEMLK FAPGTVLPQGFVVEGSGR QPASTVKPDMAEEIAALVLAK LGTSDPQFPILAELAPTVGAFFFGSK	33%	593
	30 h		N.A.	N.A.	N.A.
	36 h	Nucleoprotein (MHV-A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR	2%	40
3 PFU/mL, 1.2 mL inoculum	42 h	Nucleoprotein (MHV-A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK GPNQNFGGSEMLK FAPGTVLPQGFYVEGSGR	12%	263

			Wastewater influent		
	Hours post infection	Identified Protein	Detected peptides	Protein sequence coverage	Protein score
300 PFU/mL, 1.2 mL inoculum	18 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR GPNQNFGGSEMLK FAPGTVLPQGFYVEGSGR	12%	249
	24 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR DEVDNVSVAKPK VLNENLNAYQK DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR KDEVDNVSVAKPK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR QPASTVKPDMAEEIAALVLAK EFQFAEGQGVPIANGIPASEQK LGTSDPQFPILAELAPTVGAFFFGSK	40%	640
		Spike glycoprotein (MHV)	SAIEDLLFDK FGAISASLQEILTR	2%	98
	30 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK GPNQNFGGSEMLK DVYELQYSGAVR FAPGTVLPQGFYVEGSGR LGTSDPQFPILAELAPTVGAFFFGSK	25%	645
30 PFU/mL, 1.2 mL inoculum	36 h	Nucleoprotein (MHV- A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR DEVDNVSVAKPK VLNENLNAYQK DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR KDEVDNVSVAKPK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR QPASTVKPDMAEEIAALVLAK EFQFAEGQGVPIANGIPASEQK EFQFAEGQGVPIANGIPASEQK LGTSDPQFPILAELAPTVGAFFFGSK	40%	761
		Spike glycoprotein (MHV)	CFGSISVDK SAIEDLLFDK SVPSPLNWER YDLYGITGQGEILTR FGAISASLQEILTR VANLPACNIEEWLTAR	5%	239
	42 h	Nucleoprotein (MHV- A59, MHV-3, MHV-S, RCV-NJ)	DPSSHEAIPTR VLNENLNAYQK GPNQNFGGSEMLK FAPGTVLPQGFYVEGSGR	9%	168
3 PFU/mL, 1.2 mL inoculum	48 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR FAPGTVLPQGFYVEGSGR QPASTVKPDMAEEIAALVLAK EFQFAEGQGVPIANGIPASEQK LGTSDPOFPILAELAPTVGAFFFGSK	35%	642
		Spike glycoprotein	SAIEDI I EDK	0.7%	88
		(coronavirus)	OURDERDIK		

## Table C-4 Viral peptides detected by LC-MS/MS in MHV wastewater experiments.

## Table C-4 Continued.

Wastewater effluent					
	Hours post infection	Identified Protein	Detected peptides	Protein sequence coverage	Protein score
300 PFU/mL, 1.2 mL inoculum	18 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK GPNQNFGGSEMLK FAPGTVLPQGFYVEGSGR	14%	386
	24 h	Nucleoprotein (MHV- A59, MHV-3)	DPSSHEAIPTR SFVPGQENAGGR VLNENLNAYQK DGGADVVSPKPQR GPNQNFGGSEMLK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR	20%	355
	30 h	Nucleoprotein (MHV- A59, MHV-3)	ELTPEDR DPSSHEAIPTR SFVPGQENAGGR FAPGTVLPQGFYVEGSGR	9%	265
30 PFU/mL, 1.2 mL inoculum	36 h	Nucleoprotein (MHV- A59, MHV-3)	TTWADQTER DPSSHEAIPTR SFVPQENAGGR DGGADVVSPKPQR GPNQNFGGSEMLK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR	17%	577
	42 h	Nucleoprotein (MHV- A59, MHV-3)	DPSSHEAIPTR VLNENLNAYQK GPNQNFGGSEMLK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR LGTSDPQFPILAELAPTVGAFFFGSK	20%	210
3 PFU/mL, 1.2 mL inoculum	48 h	Nucleoprotein (MHV- A59, MHV-3)	ELTPEDR TTWADQTER DPSSHEAIPTR SFVPGQENAGGR DEVDNVSVAKPK DGGADVVSPKPQR GPNQNFGGSEMLK DVYELQYSGAVR KDEVDNVSVAKPK FDSTLPGFETIMK FAPGTVLPQGFYVEGSGR EFQFAEGQGVPIANGIPASEQK LGTSDPQFPILAELAPTVGAFFFGSK	36%	740
		Spike glycoprotein (MHV)	SAIEDLLFDK SVPSPLNWER FGAISASLQEILTR	2%	205

**Table C-5** Proteins in reovirus virion.

Location	Protein	Molecule copies in
		reovirus virion <sup>5</sup>
Outer capsid	mu-1	600
	sigma-3	600
	lambda-2	60
	sigma-1	36-48
Inner capsid (core)	sigma-2	150
	lambda-1	120
	lambda-3	12
	mu-2	12

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