

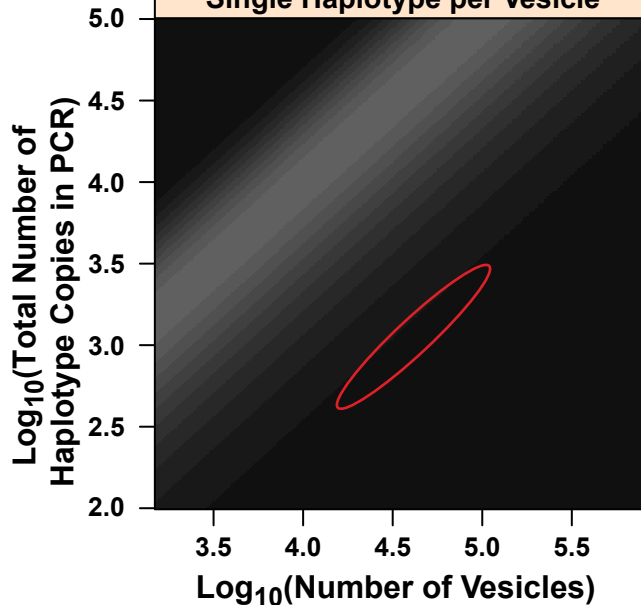
1 **Figure S1.**

2 Theoretical probability that a single vesicle contains either zero or one copy of a specific  
3 haplotype as a function of the total number of vesicles and the total number of copies of that  
4 haplotype. All calculations assume a Poisson distribution with mean equal to the average number  
5 of haplotype copies per vesicle. The number of copies of each haplotype is determined by  
6 genome size, DNA concentration, and total amount of DNA as outlined in the text. **(a)**  
7 Probability that an individual vesicle contains a single copy of a haplotype. **(b)** Probability that a  
8 vesicle contains zero template molecules for a specific locus. In order to reliably obtain  
9 haplotype sequences, vesicles must not contain multiple initial DNA template molecules. Thus,  
10 the number of DNA template molecules and vesicle size were chosen such that most vesicles  
11 would be empty and very few vesicles would contain multiple DNA templates. The red ovals  
12 highlight this targeted region for single-molecule sequencing. Using too little DNA, or too many  
13 (small) vesicles, results in no DNA template molecules in almost all vesicles and low PCR  
14 reagent levels per vesicle.

15

16 **Figure S2.**

17 Haplotype sequences of a diploid individual generated using conventional PCR **(a)** and our  
18 modified single-molecule sequencing approach **(b-m)**. Heterozygous sites are highlighted in  
19 yellow. There are a total of 32 potential haplotypes consistent with these five segregating sites.  
20 Sequencing of 12 fluorescent vesicles revealed only two haplotypes with no discrepancies. We  
21 recovered five sequences consistent with one haplotype **(red, b-f)** and seven consistent with the  
22 other haplotype **(blue, g-m)**. Two chromatograms show evidence of containing multiple initial  
23 DNA template molecules **(k and m)**.

**(a)****Single Haplotype per Vesicle****(b)****Zero Haplotype Copies per Vesicle**