Appendix for "Distinct heterochromatin-like domains promote transcriptional memory and silence parasitic genetic elements in bacteria"

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Appendix Figures



Appendix Figure S1: Schematic of the IPOD-HR method.

(A) Summary of the key steps in IPOD-HR: proteins are crosslinked to bound DNA with formaldehyde, cells are lysed and enzymatically treated to minimize footprints, and then subjected to a phenol-chloroform extraction. The interphase layer between the aqueous and organic phases is isolated, and DNA recovered and prepared for sequencing. Further details are in [12,13]. (B) Postprocessing of IPOD signal (log ratio of the DNA abundance in the interphase sample to a corresponding input control); values from an RNA polymerase ChIP experiment (again in the form of log ratio relative to an input control) are subtracted to yield the final IPOD-HR signal (bottom). Further details are given in [13].

WT. RDM o	lu	WT Mir	a du
Back, FPO)s	Back El	PODe
Back. EPO	pilus, GC:0009289 transposition, DNA-mediated, GC:0006313 LPS biosynthetic process, GO:0009103 cytolysis, GO:0019835 protein binding involved in protein folding, GO:0044183 response to stimulus, GO:0050896 cellular response to acid chemical, GO:0071229 self proteolysis, GO:0097264 organic phosphonate catabolic process, GO:0019700 N2-acetyl-L-omithine:2-oxoglutarate 5-aminotransferase activity, GO:0003992 sn-glycerol-3-phosphate.ubiquinone oxidoreductase activity, GO:0052590 purine nucleobase metabolic process, GO:0008144 ethanolamine catabolic process, GO:0008144	Back. El	Cell adhesion, GO:0007155 self proteolysis, GO:0097264 pilus organization, GO:0043711 cell. response to acid chemical. GO:0071229 cytolysis, GO:0019835 recombinase activity, GO:0000150 LPS biosynthetic process, GO:0009103 core prom. seq. spec. DNA binding, GO:0000987 response to silver ion, GO:0010272 translation, GO:0006412 fatty acid metabolic process, GO:0006631 flagellum-dependent motility, GO:0071973
	methyltransferase activity, GO:0008168		magnesium ion hinding. GO:0002239
	ribosome, GO:0005840		magneolarmion binaing, 00.0000201
	chemotaxis, GO:0006935	WT (2) U	Inique vs. WT. RDM alu
	ATP-binding cassette (ABC) transporter complex, GO:0043190	Back, El	PODs
WT D.S., RI Back. EPOD	DM glu pilus, GO:0009289 <u>LPS biosynthetic process. GO:0009103</u> core promoter proximal region sequence-specific DNA binding, GO:0000987 pilus organization, GO:0043711 cellular response to acid chemical. GO:0071229 N.Ndiacetylchitobiose import, GO:1902815 cytolysis. GO:0019835 translation, GO:0006412 ATP-binding cassette (ABC) transporter complex, GO:0055052 bacterial-type flagellum-dependent cell motility, GO:0071973 2 iron, 2 sulfur duster binding, GO:0051537 methylation, GO:0032259 response to heat, GO:0009408 V glu Ds transposition, DNA-mediated, GO:0006313		negative regulation of single-species biofilm formation on inanimate substrate, GO:1900232 base pairing with mRNA, GO:0000499 spermidine transmostrate ransporter activity, GO:0015606 cellular response to cell envelope stress, GO:036460 transposition, GO:0032196 cell adhesion, GO:0007155 response to cell envelope stress, GO:0036460 transposition, GO:0007155 response to acidic pH, GO:0010447 viral release from host cell, GO:0019076 lipopolysaccharide biosynthetic process, GO:0009103 programmed cell death, GO:0012501 celluar response to acid chemical, GO:0071229 DNA restriction-modification system, GO:0009307 core promoter proximal region sequence-specific DNA binding, GO:0000987 ATP-binding cassette (ABC) transporter complex, GO:0043190 ligase activity, GO:0016874 intracellular ribonucleoprotein complex, GO:0030529 anaerobic respiration, GO:0009061 response to heat, GO:0009408
	cytolysis, GO:0019835 cellular response to acid chemical, GO:0071229	representatio	n 5
	recombinase activity, GO:0000150 LPS biosynthetic process, GO:0009103 negative regulation of bacterial-type flagellum-dependent cell motility, GO:190 establishment of integrated proviral latency, GO:0075713 cell adhesion, GO:0007155 pilus organization, GO:0043711 programmed cell death, GO:012501 periplasmic side of cell outer membrane, GO:0031241 response to copper ion, GO:004688 regulation of cell division, GO:0051302 ATP-binding cassette (ABC) transporter complex, GO:0043190 chemotaxis, GO:00068035 oxidoreductase activity, acting on CH-OH group of donors, GO:0016614 NADH dehydrogenase (ubiquinone) activity, GO:0008137 oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD of intracellular ribonucleoprotein complex, GO:003529 broicene activity, acting on the aldehyde or oxo group of donors, NAD of intracellular ribonucleoprotein complex, GO:003529	02201 br NADP as acco	eptor, GO:0016620
	ligase activity, GO:0016874		
	rBNA processing CO:0006264		

Appendix Figure S2: Pathway analysis of EPODs across WT conditions.

iPAGE analysis revealed key pathways overrepresented in EPODs compared to background that remain across different growth media, harvest growth phase, and parental background (underlined). Color scale represents over- or under-representation of genes with particular GO term annotations in the EPODs vs. non-EPOD regions (background). To compare the differences among two MG1655 parental strains, we performed iPAGE specifically on EPODs that were unique in WT (2) compared to WT, shown in the bottom right.



Appendix Figure S3: Deletion of *hns* and *stpA* impacts EPODs across the genome. Density plots exhibit enrichment of H-NS binding within EPODs that is reduced upon deletion of *hns* and the double deletion of *stpA* and *hns*. Dashed lines are the median for background (grey) and EPODs (red) for each condition. (*) indicates FDR-corrected p<0.005 via permutation test (against a null hypothesis of no difference in medians).



Appendix Figure S4: Changes in protein occupancy across the genome.

The average occupancy was calculated across EPODs and background regions. The change in protein occupancy was calculated by subtracting the WT average at each region for every mutant. A gain in occupancy in the mutant is represented by a positive change in occupancy, while a loss is represented by a negative change in occupancy. Hierarchical clustering distinguished NAPs that have similar impacts on protein occupancy across the genome.



Appendix Figure S5: Memory effect of KDG exposure dissipates over time.

Shown are the replicate-level data from the competition experiments presented in FIg. 4 of the main text, demonstrating that the competitive advantage of cells pre-exposed to KDG persists for 12-24 hours of growth in glucose minimal media, and then dissipates. Colored points show the replicate-level data contributing to the analysis in Fig. 4B, with the solid black line and associated points showing the mean value across experimental replicates at each timepoint. Statistical significance is assessed in Fig. 4B.

Appendix Tables

Replicate	End of KDG Growth			After 12 hr GLU outgrowth				After 24 hr GLU outgrowth			
	Colony count	Dilution	Cells/mL	Colony count	Dilution	Cells/mL	# Doublings from KDG	Colony count	Dilution	Cells/mL #	Doublings from KDG
1	4	1.0E-05	8.0E+07	5	1.0E-05	1.0E+08	8.0	24	1.0E-05	4.8E+08	10.2
II	8	1.0E-05	1.6E+08	17	1.0E-05	3.4E+08	8.7	30	1.0E-05	6.0E+08	9.6
III	31	1.0E-01	6.2E+04	15	1.0E-02	3.0E+05	9.9	16	1.0E-05	3.2E+08	20.0

Appendix Table S1: Outgrowth of KDG-exposed cells in GLU during competition experiments.

Colony counts for cells in KDG growth experiments at the end of KDG growth and at the early stages of outgrowth in GLU media (for the competition data shown in Fig. 4B of the main text). The three stages shown here correspond to the KDG-exposed cells at the 0 day, 0.5 day, and 1.0 day timepoints. "Colony count" refers to the actually observed counts from spottings of 5 microliters of media, and are converted to inferred cells/mL for the original culture. "# Doublings from KDG" indicates the number of doublings that the cells in GLU media have undergone after the end of their exposure to KDG, and account for the changes in cell numbers, plus the 200-fold dilution of cells from the KDG condition into the GLU condition (see Methods).