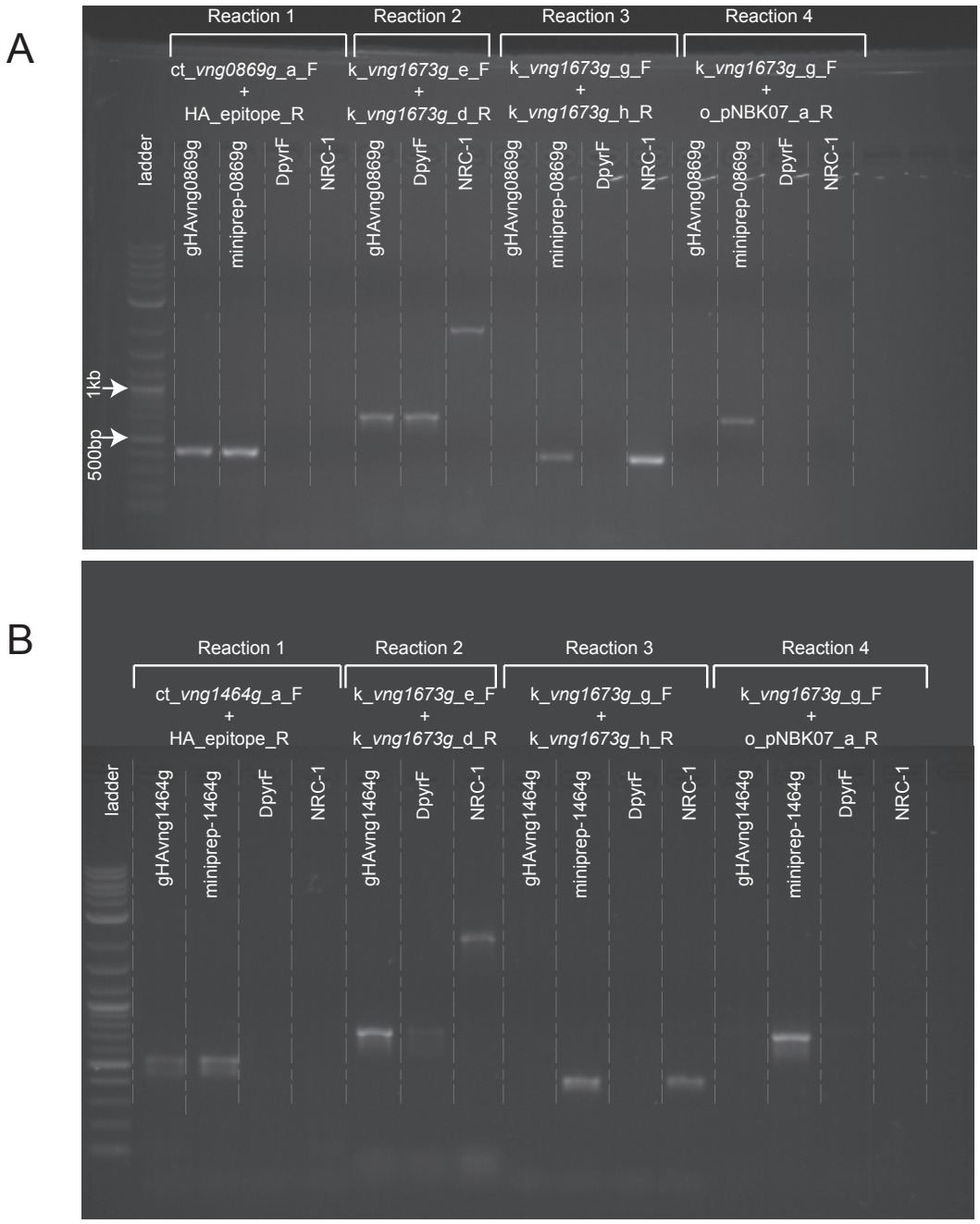
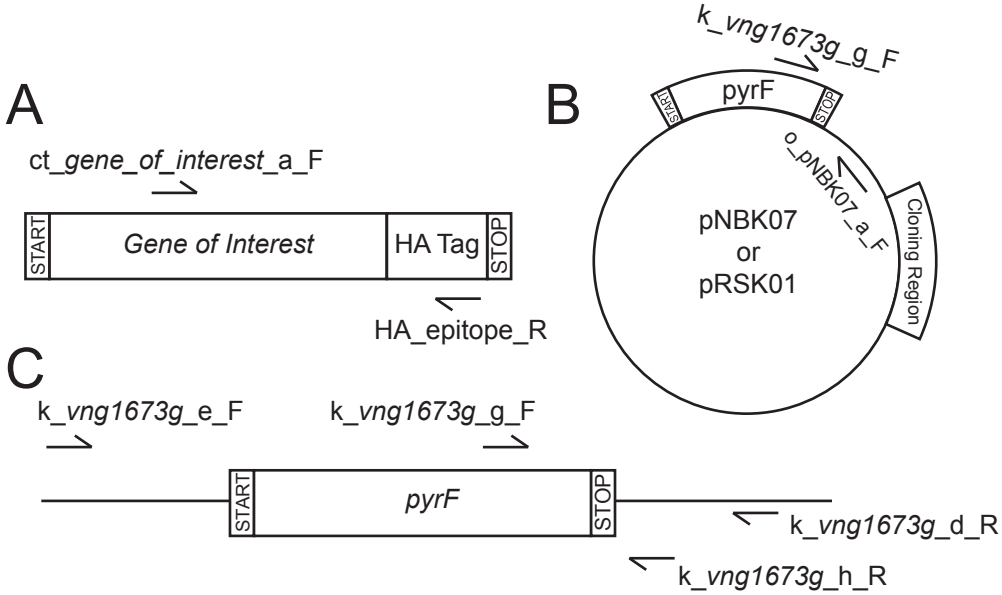
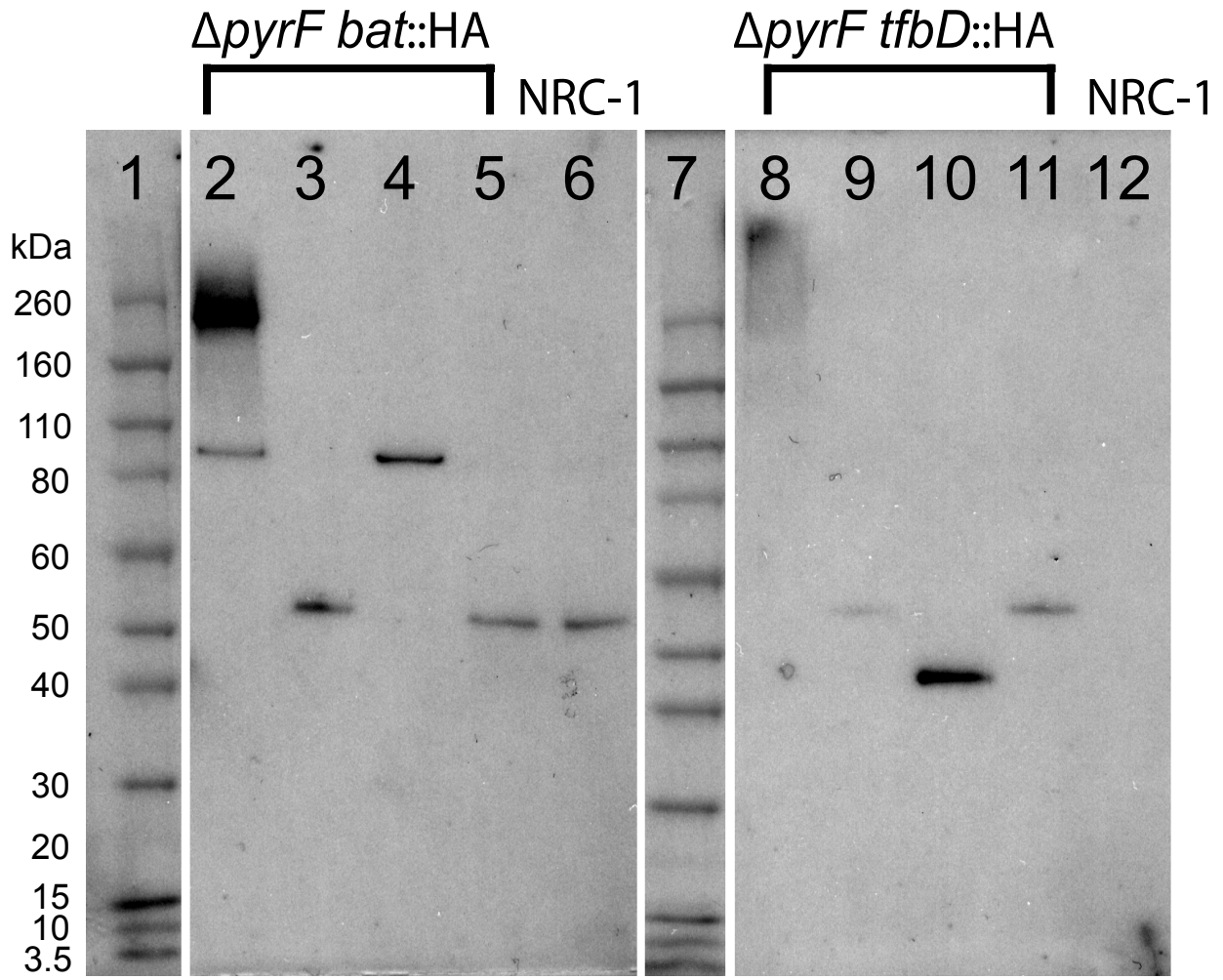


Supplementary Figure 1. PCR verifications of C-terminal HA epitope tagging of chromosomally integrated **(A)** *tfbD* target gene and **(B)** *bat* target gene. Primers are described in Supplemental Figure 2 and sequences provided in Supplemental Table 1. Reactions 1-4 are described below and were run on the successful recombinant strain a mini-prepped sample of the recombinant plasmid used to prepare the strain, the Δ*pyrF* parent strain and the wild-type *Hb.* NRC-1 strain. ***Reaction 1:*** PCR primers ct_gene_of_interest_a_F and HA_epitope_R are used to amplify a fragment of the target gene that includes the HA-epitope. This verifies either chromosomal tagging or the presence of residual tagging vector. ***Reaction 2:*** PCR primers k_vng167g3_e_F and k_vng1673g_d_R that flank the chromosomally encoded *pyrF* gene are used to amplify across the *pyrF* locus. This reaction should yield a 2050bp product when *Hb.* NRC-1 genomic DNA is used as template and a 712bp when *Hb.* NRC-1 Δ*pyrF* strain genomic DNA is used as template. This reaction verifies that the absence of *pyrF* gene reintegration into the *Hb.* NRC-1 Δ*pyrF* chromosome. ***Reaction 3:*** PCR primers k_vng1673g_g_F and k_vng1673g_h_R are used to amplify a region 465bp upstream of the pyrF stop codon to 70bp down stream of the *pyrF* stop codon. This final reaction should yield no product in the *Hb.* NRC-1 Δ*pyrF* strain and its derivatives. A 535bp product is formed in *Hb.* NRC-1 and the pNBK07 and pRSK01 plasmids. This reaction confirms that the plasmid is absent and that the pyrF gene has not reintegrated in Hb. NRC-1 Δ*pyrF* background. ***Reaction 4:*** PCR primers k_vng1673g_g_F and o_pNBK07_a_R are used to amplify a segment of the pNBK07 or pRSK01 encoded *pyrF* gene. The absence of product, in combination a product when the same PCR primers are used with in plasmid containing control reaction, further confirms loss of the plasmid.



ct_*gene_of_interest*_a_F: 500bp upstream from stop codon of gene of interest
HA_epitope_R: HA-tag specific primer
k_*vng1673g*_d_R: 500bp downstream from the *pyrF* stop codon
k_*vng1673g*_e_F: 750bp upstream from the *pyrF* start codon
k_*vng1673g*_g_F: 465bp upstream from the *pyrF* stop codon
k_*vng1673g*_h_R: 70bp downstream from the *pyrF* stop codon
o_pNBK07_a: 219bp downstream from the *pyrF* stop codon

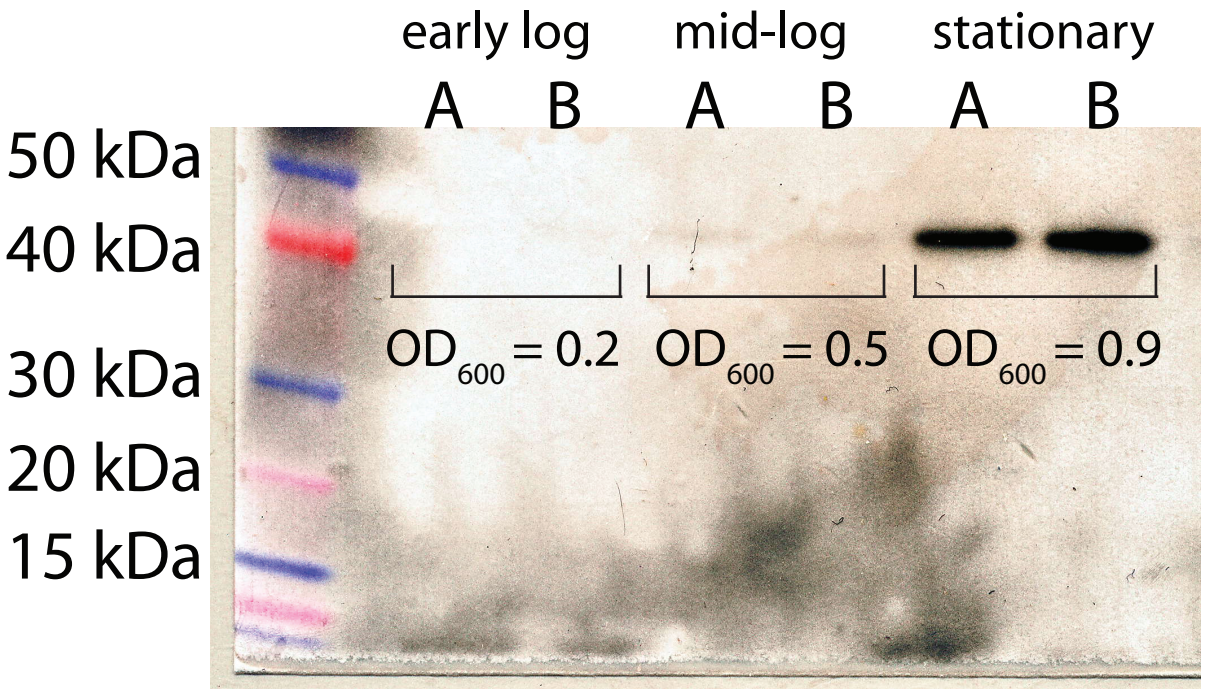
Supplementary Figure 2. A-C. Schematic of the binding locations of the primers used in this study for screening and construction of strains. In the vicinity of the **A)** gene of interest or target gene **B)** plasmid **C)** *pyrF* locus in the chromosome. Primer sequences are provided in Supplemental Information.



Supplementary Figure 3. Western blot of ChIP and IP reactions from Δ*pyrF bat*::HA strain (2-5), and Δ*pyrF tfbD*::HA strain (8-11). Lanes 1 and 7 contain Invitrogen Novex Sharp Pre-Stained Protein Standards (LC5800). ChIP reactions (2, 8) and unfixed IP reactions (4, 10) produce significant enrichment of the target protein which could not be observed in the whole cell extract, either fixed (3, 9) or unfixed (5, 11). The *Halobacterium salinarum NRC-1* strain without any introduced HA-epitopes was found to have no IP-enriched proteins (12), though a band was detected in the unfixed whole cell extract (6). This band likely results from a native protein with an epitope similar to that of HA. This band was observed in all other whole cell extracts (3,5,9,11); however we note that the IP reaction was of sufficient stringency to remove this artifact as this band is not detected in any of the ChIP or IP samples. Contents of each lane are summarized again below:

1. Invitrogen Novex Sharp Pre-Stained Protein Standards (LC5800)
2. Δ*pyrF bat*::HA strain ChIP
3. Δ*pyrF bat*::HA strain formaldehyde-fixed Whole Cell Extract (WCE)
4. Δ*pyrF bat*::HA strain IP
5. Δ*pyrF bat*::HA strain unfixed WCE
6. *Hb.* NRC-1 strain unfixed WCE

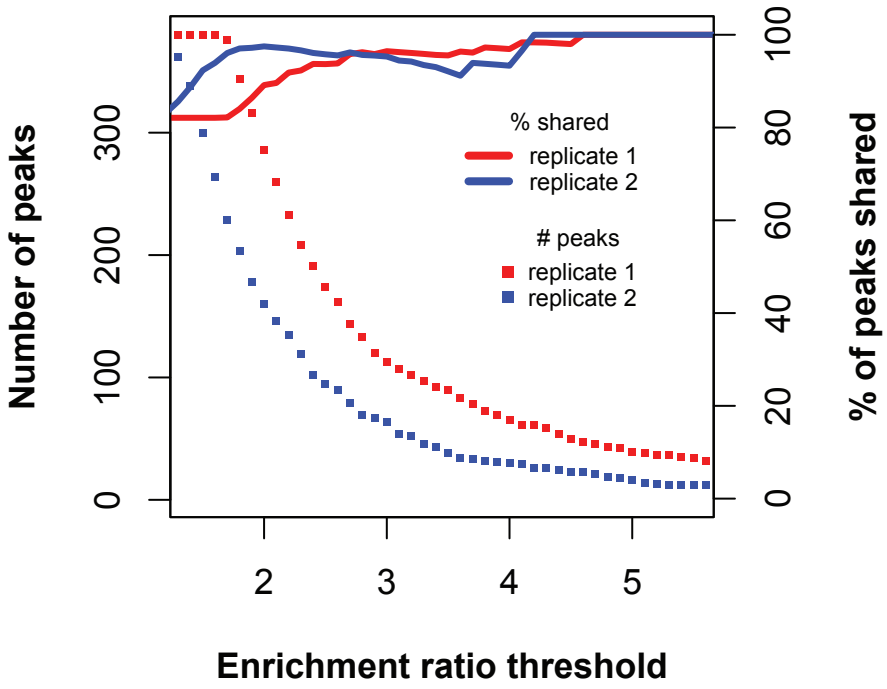
7. Invitrogen Novex Sharp Pre-Stained Protein Standards (LC5800)
8. Δ*pyrF tfbD*::HA strain ChIP
9. Δ*pyrF tfbD*::HA strain formaldehyde-fixed Whole Cell Extract (WCE)
10. Δ*pyrF tfbD*::HA strain IP
11. Δ*pyrF tfbD*::HA strain unfixed WCE
12. *Hb.* NRC-1 strain IP



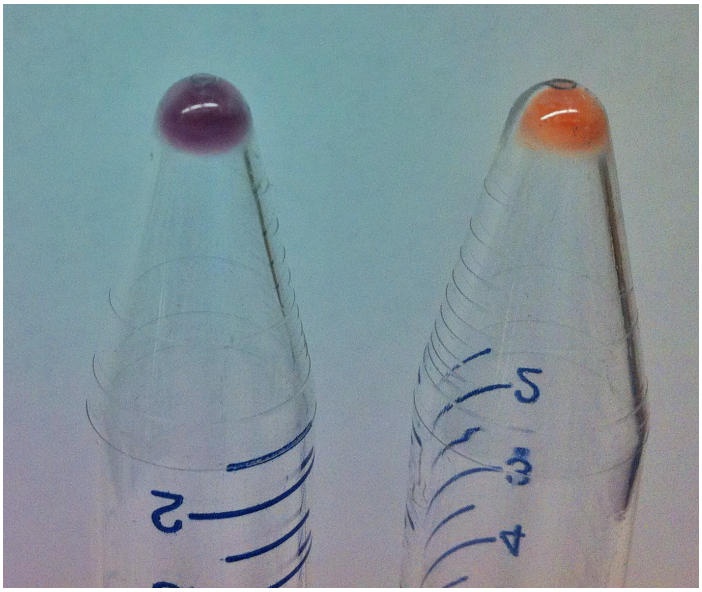
Supplementary Figure 4. The chromosomally integrated epitope tagged target gene *tfbD*::HA is conditionally expressed in the recombinant strain Δ*pyrF tfbD*::HA. The increase in abundance of the TfbD protein in stationary phase (OD_{600}= 0.9) is consistent with the accumulation of *tfbD* transcript previously documented for wild-type strains (Facciotti et al. 2007).

Figure shows a Western blot of two biological replicate cultures A and B (two flasks grown side by side). $3.5x10^8$ cells were harvested at each time point and immunoprecipated as described in Materials and Methods. Immunoprecipated sample was mixed with 6x Loading Buffer and loaded directly onto PAGE gel. Protein was transferred to PVDF membrane, blocked with 0.5% casein and probed with HA-HRP conjugated antibody. Membrane was exposed to radiography paper for 20 mins (shown above). Increasing exposure to 9 hours (not shown) did not increase the intensity of the faint bands seen at 20 minutes. Negative controls (IPs run without antibody and whole cell samples that are not shown) displayed no visible bands. Quantitative measurements of the bands in the image above (shown in the table below) were calculated using ImageJ Software in reference to OD_{600} 0.90, replicate A and are shown on a scale of 0 to 100.

OD_{600}	Replicate	Intensity
0.23	A	Not detected
0.23	B	0.5
0.55	A	4.7
0.55	B	5.9
0.90	A	83.8
0.90	B	100



Supplementary Figure 5. For each biological replicate of the TfbD ChIP-seq experiment, the full peak lists were examined at increasingly stringent different enrichment ratios. Each of these increasing stringency datasets was examined for the number of predicted binding sites (squares, left y-axis) and the percentage of predicted binding sites that were shared with the full dataset of the biological replicate (solid lines, right axis).



SD20 with plasmid expressed Bat-cHA **SD20**

Supplementary Figure 6. Bat protein with a c-terminal HA epitope does not impede the function of the protein. SD20 is a bat knockout strain with light pink color (on right) due the absence of bacteriorhodopsin production (46). When SD20 is transformed with a plasmid expressing cHA-tagged Bat, the cells become purple from bacteriorhodposin production induced by the engineered Bat protein (at left). Cell pellets are from 12 mL of stationary phase cultures.

Supplementary Table 1. Oligonucleotides used in this study.

Oligo Name	**Sequence 5’ to 3’**
ct_vng1464g_a_F	CCCAGGCGGTCGTCGACACC
ct_vng1464g_b_R	TCACGCGTAGTCCGGGACGTCGTACGGGTACGCCTCCTCGAAGAACGCGCCGAC
ct_vng1464g_c_F	GCGTACCCGTACGACGTCCCGGACTACGCGTGACGCGGGCCACACGCCGGCTGT
ct_vng1464g_d_R	CCAACTCCACACCACTCATCC
ct_vng0869g_a_F	GCCGCCGCGAGGCAGGCAAA
ct_vng0869g_b_F	GCGTACCCGTACGACGTCCCGGACTACGCGTAACCCATATTCGGGGCGTC
ct_vng0869g_c_R	TTACGCGTAGTCCGGGACGTCGTACGGGTACGCCGCTTCCACGCCGGGTTCA
ct_vng0869g_d-2_R	CGGCGAATACCACTACCAGG
o_pNBK07_a_R	GAGGGTACCAGAGCTCACCTAGGTA
k_vng1673g_d_R	GGTCCCGGGACCGCCCCCCG
k_vng1673g_e_F	GGGGACGTGGCCGCGACGTT
k_vng1673g_g_F	ATCACCGTCAACCCGTACCTC
k_vng1673g_h_R	CCGTGTTCGCCCGCGAAGATG
HA_epitope_R	GCGTAGTCCGGGACGTCGTACGG
m13F	GTAAAACGACGGCCAG
m13R	CAGGAAACAGCTATGAC
pNBK07 F	GGCGCGACAGATCGAGCAGA
pNBK07 R	GGGATGTGCTGCAAGGCGAT
ccdB500F	CTACCTATTCATATACGATA
ccdB500R	TTCACCATAATGAAATAAGA

Supplementary Table 2. Strains and plasmids used in this study

Name	Reference	Purpose
Hb. salinarum NRC-1	Gift from Ford Doolittle	Wild type strain
Δ*pyrF*	17	Uracil auxotroph
tfbD::HAΔ*pyrF*	This study	Uracil auxotroph, tfbD with C-terminal HA epiope fusion
bat::HAΔ*pyrF*	This study	Uracil auxotroph, bat with C-terminal HA epiope fusion
pNBK07	17, gift from N. Baliga	5-FOA counter selection, mevinolin resistance, can be used for targeted gene deletion
pRSK01	This study	Modification of pNBK07 for Gateway ligation-independent cloning of segments of DNA suitable for chromosomal modification by homologous recombination

Supplementary Table 3. Quantities of different solutions used for the scaled down ChIP reactions with few numbers of cells.

# of cells harvested	**Lysis Buffer (mL)**	**Size of Tube (mL)**	**DYNABEADS added (µL)**	**PBS/BSA to Resuspend DYNABEADS (µL)**	**ANTIBODY (µg)**
1.75E+10	1.6	15	30	30	1
8.75E+09	0.8	15	25	15	0.5
3.50E+09	0.3	1.5	10	6	0.2
1.75E+09	0.3	1.5	10	6	0.2
3.50E+08	0.3	1.5	10	6	0.2

Supplementary Table 4. Multiplexing sequence barcodes used in this study. Barcode sequence was at the 3' end of all sequence reads.

Name	**sequence**
barcode 1	TGCGTT
barcode 2	TAACTT
barcode 3	ACGGCT
barcode 4	GAGCCT
barcode 5	AAGAAT
barcode 6	ACCTGG
barcode 7	CTCTTG
barcode 8	TCTTAG
barcode 9	CTAATC
barcode 10	GGATGC
barcode 11	GCTGGC
barcode 12	GGTACC