SUPPLEMENTARY METHODS

Microarray design

Microarray probes for the custom-designed Agilent microarray were designed by computing an approximate melting temperature of each of 115,991 oligos in the set of equally spaced 60-mer sequences, 40 bps apart, in the E. coli genome (NC_000913.2). The melting temperature was computed as:

$$Tm = 64.9 + (0.6833 \times (\text{Number of Guanines} + \text{Number of Cytosines} - 16.4))$$

Oligos were then sorted by melting temperature and the lower quartile, 28,997 probes, removed. The remaining 86,994 oligos had Tm's ranging from 86.9 °C to 72.8 °C. The hybridization potential (Hp) of each of these oligo was then computed against every forward and reverse strand 60-mer in the genome by counting of pairs of matching bases:

$$Hp = ((\text{A/A pairs} + \text{T/T pairs}) \times 2) + ((\text{G/G pairs} + \text{C/C pairs}) \times 3)$$

The ten highest values for each oligo were saved and the distribution of the range of differences between the maximal Hp and the 9 smaller values was examined. Subsequently, in order to limit the amount of cross hybridization, probes with a minimum Hp difference of 62 between the top two probes were selected. This resulted in a set of 43,284 oligos with a maximal Hp difference of 72 between its target and its most likely competitive cross-hybridization locus. This set of probes covered 2,597,040 base pairs or 56% of the genome with the largest gap between two probes being 6,720 base pairs and the average gap being 105 bp. Note that the largest gaps are due to long, repetitive sequence, such as ribosomal RNA loci. The microarray probeset is listed in Supplementary Table 2.

Chromatin Immunoprecipitation (ChIP)/qPCR and ChIP-chip

ChIP and ChIP-chip methods were based on an earlier study (21). 40 ml *E. coli* cells expressing C-terminally TAP-tagged AraC (for ChIP-chip; SAC003; Table 1), or C-terminally FLAG-tagged AraC (for ChIP/qPCR; AMD187; Table 1) were grown in LB or LB + 0.2% arabinose at

37 °C to an OD_{600} of 0.6-0.8. For *S. enterica*, 40 ml cells expressing C-terminally FLAG-tagged AraC (CB005; Table 3) were grown in LB + 0.2% arabinose at 37 °C to an OD_{600} of 0.6-0.8. Cells were crosslinked for 20 minutes with formaldehyde (1% final concentration), pelleted by centrifugation and washed once with Tris-buffered saline (TBS). Cell pellets were resuspended in 1 ml FA lysis buffer (50 mM Hepes-KOH, pH 7, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS) with 2 mg/ml lysozyme and incubated at 37 °C for 30 minutes. Samples were then chilled and sonicated for 30 minutes in a Bioruptor sonicator (Diagenode) with 30 s on/30 s off pulsing at maximum amplitude. Samples were pelleted in a microcentrifuge to remove debris and supernatants ("chromatin") were saved, 1 ml FA lysis buffer was added, and samples were stored indefinitely at -20 °C. For each immunoprecipitation (IP), 500 µl chromatin was incubated with 300 µl FA lysis buffer, 20 µl Protein A Sepharose slurry (50%) in TBS and either 1 µl anti-β (RNA polymerase subunit) antibody (NeoClone) or 2 µl M2 anti-FLAG antibody (Sigma) for 90 minutes at room temperature with gentle mixing on a Labquake Rotisserie Rotator (Thermo Scientific). For ChIP of AraC-TAP, Protein A sepharose and antibody was replaced with IgG sepharose. Beads were then pelleted at 1,500 x g in a microcentrifuge for 1 minute. The supernatant was removed and the beads were resuspended in 750 µl FA lysis buffer and transferred to a Spin-X column (Corning). Beads were then incubated for 3 minutes with gentle mixing on a rotisserie rotator before being pelleted at 1,500 x g in a microcentrifuge for 1 minute. Equivalent washes were performed with FA lysis buffer, high salt FA lysis buffer (50 mM Hepes-KOH, pH 7, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS), ChIP wash buffer (10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 1 mM EDTA, 0.5% Nonidet-P40, 0.5% sodium deoxycholate) and TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). After the TE wash, beads were transferred to a fresh Spin-X column and eluted with 100 µl ChIP elution buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 1% SDS) for 10 minutes at 65 °C with occasional agitation. Eluted samples were centrifuged at 1,500 x g in a microcentrifuge for 1 minute. Supernatants were decrosslinked by boiling for 10 minutes and cleaned up using a PCR purification kit (Qiagen). For all ChIP/qPCR and ChIP-chip experiments, 20 µl chromatin was decrosslinked by boiling for 10 minutes and cleaned up using a PCR purification kit (Qiagen). This sample served as the "input" control.

For qPCR, ChIP and input samples were analyzed using an ABI 7500 Fast real time PCR machine, as described previously (22). Enrichment of ChIP samples was calculated relative to a control region within the transcriptionally silent *bglB* gene (for *E. coli*), or a region upstream of *sinR* (*S. enterica*) that is not predicted to bind AraC, and normalised to input DNA. Occupancy units represent background-subtracted fold-enrichment. Oligonucleotides used for real time PCR with *E. coli* samples were JW125 + JW126 (*bglB*), JW393 + JW394 (*ydeN*), JW071 + JW072 (*araB* upstream region), JW073 + JW074 (*araE* upstream region), JW075 + JW076 (*araF*), JW389 + JW390 (*ytfQ*), JW1312 + JW1313 (*dcp*), JW1416 + JW1417 (*ygeA*), JW1398 + JW1399 (*araE* ORF), JW1450 + JW1451 (*polB*), and JW1404 + JW1405 (*araA*). Oligonucleotides used for real time PCR with *S. enterica* samples were JW2412 + JW2413 (*sinR*), JW3114 + JW3115 (*araB*), JW3116 + JW3117 (*araE*), JW3118 + JW3119 (*araJ*), JW3824 + JW3825 (*sseD*), and JW4471 + JW4472 (*araT*).

For ChIP-chip, ChIP samples from 8 independent cultures grown in the presence of arabinose were combined and ethanol precipitated to reduce volume. 8 samples were required to generate sufficient DNA for dye labeling. These samples were labeled with Cy3 dye as described previously (23). 100 ng input DNA was labeled with Cy5 dye. Dye-labeled samples were combined and hybridised to the custom-designed Agilent microarrays in a hybridisation oven (Agilent) according to the manufacturer's instructions. Microarray slides were washed and scanned using an Agilent scanner and Agilent software was used to determine Cy3/Cy5 ratios for all probes. Any probes with a score of <1,000 pixels for either Cy3 or Cy5 were removed as these are likely to indicate regions deleted in the strain used. We selected an arbitrary cut-off for Cy3/Cy5 ratio of two standard deviations above the mean to select putative AraC-bound regions. ChIP-chip raw data are listed in Supplementary Table 3.

ChIP-seq

The ChIP-seq procedure was identical to the ChIP procedure up to and including the 90 min incubation of chromatin samples with Protein A Sepharose and antibody. All further wash steps involved incubation of samples with the wash solution for 3 min with gentle mixing on a rotisserie rotator before pelleting beads at 1,500 x g in a microcentrifuge for 1 min. Following the 90 min incubation, beads were washed twice with FA lysis buffer and twice with 10 mM

Tris-HCl, pH 7.5. Beads were resuspended in 100 µL 1X Quick Blunting Buffer (NEB) containing dNTPs (concentration specified by NEB Quick Blunting kit) and 1 µL Quick Blunting enzyme mix (NEB), and incubated at room temperature for 30 min with gentle mixing. Beads were washed twice with FA lysis buffer and twice with 10 mM Tris-HCl, pH 8.0. Beads were resuspended in 100 µL Buffer 2 (NEB) containing 2 mM dATP and 2 µL Klenow DNA polymerase (NEB), and incubated for 30 min at 37 °C with gentle mixing. Beads were washed twice with FA lysis buffer and twice with 10 mM Tris-HCl, pH 7.5. Barcoded adapter oligonucleotides JW2870 + JW2881 or JW2876 + JW2887 (two different barcoded pairs) were annealed by boiling a mixture of 100 µM each in 10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, and cooling slowly. The adapter oligonucleotide mix was then diluted 10-fold in water. Beads were resuspended in 1X DNA Quick Ligase buffer (NEB), 1 µL adapter oligonucleotide mix and 2 µL Quick DNA Ligase (NEB), and incubated at room temperature for 15 min with gentle mixing. Beads were washed twice with FA lysis buffer, and once each with high salt FA lysis buffer, ChIP wash buffer and TE. After the TE wash, beads were transferred to a fresh Spin-X column and eluted with 100 µl ChIP elution for 10 minutes at 65 °C with occasional agitation. Samples were decrosslinked by boiling for 10 minutes. DNA was extracted with phenol/chlorofom/isoamyl alcohol, ethanol precipitated, and resuspended in 11 µL H_2O. 1 µL DNA was used for real time PCR amplification with oligonucleotides JW1169 + JW2327 using an ABI 7500 Fast real time PCR machine. The number of cycles required to reach a Delta Rn score of 0.1 was recorded ("X"). 8 µL of the remaining DNA was amplified using conventional PCR with oligonucleotides JW1169 + JW2327 for X+3 cycles with an annealing temperature of 60 °C. PCR products were purified using Ampure XP magnetic beads (Ampure) and resuspended in 20 µL H_2O. Purified PCR products between 200 bp and 600 bp were gel purified from an 8% non-denaturing polyacrylamide gel, eluted overnight in 0.4 M NaCl, and ethanol precipitated to generate the final ChIP-seq libraries. Libraries were resuspended in 10 µL H_2O and quantified using a Qubit fluorimeter (Invitrogen). Libraries were sequenced using a HiSeq 2000 sequencer (Illumina; University at Buffalo Next Generation Sequencing Core Facility). Sequence reads were aligned to non-repetitive sequences in the *S. enterica* subsp. *enterica* serovar Typhimurium 14028s genome using the CLC Genomics Workbench and overall coverage was determined using custom Python scripts. AraC-bound regions were called for any genomic coordinate with >200 sequence reads mapping to both strands. ChIP-seq peaks were

identified as the position with the most number of mapped sequence reads within each AraC-bound region.

Motif identification

An AraC binding site motif was identified using MEME (24) using default settings, with sequences taken from 200 bp upstream to 200 bp downstream of the peak probes for each AraC-bound genomic region.