DESCRIPTION DATA FILE

1. GENERAL INFORMATION.

Title: *LeuO and H-NS Data Set*

Reference:

Shimada T, Bridier A, Briandet R, Ishihama A. Novel roles of LeuO in transcription regulation of E. coli genome: antagonistic interplay with the universal silencer H-NS. Mol Microbiol. 2011 Oct;82(2):378-97.
PMID: 21883529

Contact person for this data set:

Questions concerning the content of the data set that are raised by users of RegulonDB will be forwarded to this person. We would appreciate receiving a copy of the response to the user, so we can keep track of taking care of user requests.

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2. DATA SET DESCRIPTION.

Summary:

LeuO Data Set.

LeuO, the regulator of the leucine biosynthesis operon of *Escherichia coli*, is involved in the regulation of as-yet-unspecified genes that affect the stress response and pathogenesis expression. LeuO is involved in an antagonistic interplay with the universal silencer H-NS. Genomic SELEX screening was performed to identify the whole set of LeuO and H-NS regulation targets. A total of 140 LeuO-binding peaks (183 regulatory interactions) were identified by SELEX methodology, of which as many as 133 (95%) were found to contain the binding site of H-NS. In addition, a DNA microarray assay was carried out to identify genes affected by deletion or overexpression of the *leuO* gene. A total of 35 regulatory interactions were found via SELEX as well as microarray analysis. Based on the behavior of the regulated genes in the microarray analysis, from which it was determined that LeuO acts as an activator for 18 of them, 13 as a repressor and 4 as a dual function; we deduced that LeuO acts as a dual regulator. In addition, these experiments supported

the concept that LeuO is a dual global regulator, which is consistent with the knowledge in RegulonDB indicating that it is a DNA-binding transcriptional dual regulator of transcription of the *Escherichia coli* genome.

We generated two tables, one of them with all 140 LeuO peaks (183 regulatory interactions) that were identified by SELEX, and the other one with 35 LeuO regulatory interactions that were identified by SELEX as well as by microarray analysis.

H-NS Data Set

The H-NS protein, for "Histone-like Nucleoid Structuring protein," is a nucleoid-associated multifunctional protein that is capable of condensing and supercoiling DNA. H-NS engages in an antagonistic interplay with LeuO, the regulator of the leucine biosynthesis operon. Genomic SELEX screening was performed to identify the whole set of LeuO and H-NS regulation targets. A total of 987 H-NS-binding peaks (1,060 regulatory interactions) were identified via the SELEX methodology. In addition, a DNA microarray assay was carried out to identify genes affected by deletion or overexpression of the *hns* gene. A total of 77 regulatory interactions were found by SELEX as well as microarray analysis. Based on the behavior of the regulated genes in the microarray assay, where H-NS acted as an activator for 21 of them and as a repressor for the other 56 genes, we deduce that H-NS acts as a dual regulator. The results here support the antagonistic interplay between H-NS and LeuO.

We have generated two tables, one of them with all 987 N-NS peaks (1,060 regulatory interactions) identified by SELEX and the other one with 77 H-NS regulatory interactions identified by SELEX analysis as well as by microarray analysis.

Experiment:

The following information is essential to facilitate curation, availability, and classification of your data set within RegulonDB:

- Version of *E. coli* ´s genome used in the experiment: *Escherichia coli* K-12 W3110
- Sequence identifier: Purified His-tagged LeuO and H-NS were mixed with a collection of *E. coli* genome fragments of 200–300 bp in length, and LeuO-bound DNA fragments were affinity purified for the identification of LeuO recognition sequences.
- Experimental conditions: Cells were grown in LB medium at 28ºC or 37ºC under aeration with constant shaking at 140 rpm. Cell growth was monitored by measuring the turbidity at 600 nm.

- Number of technical replicates: A cutoff level of 3.0 was used for the SELEX-chip pattern.

Methods:

"The genomic SELEX method was carried out as previously described (Shimada et al., 2005). A mixture of DNA fragments of the *E. coli* K-12 W3110 genome was prepared, after sonication of purified genome DNA, and cloned into a multicopy plasmid, pBR322. In each SELEX screening, the DNA mixture was regenerated by PCR. For SELEX screening, 5 pmol of the mixture of DNA fragments and 10 pmol of His-tagged transcription factors were mixed in a binding buffer (10 mM Tris-HCl, pH 7.8 at 4°C, 3 mM magnesium acetate, 150 mM NaCl, and 1.25 mg/ml bovine serum albumin) and incubated for 30 min at 37°C. For the competition assay, H-NS was added and incubated for 20 min, then His-tagged LeuO was added and incubated for an additional 20 min. The DNA-transcription factor mixture was applied to a Ni-NTA column, and after washing out unbound DNA with the binding buffer containing 10 mM imidazole, DNA-protein complexes were eluted with an elution buffer containing 200 mM imidazole. DNA fragments recovered from the complexes were PCR amplified. For SELEX-chip analysis, PCR-amplified products of the isolated DNA-protein complexes and original DNA library were labeled with Cy3 and Cy5 and then combined. The fluorescent-labeled DNA mixtures were hybridized to a DNA microarray consisting of 43,450 species of 60-bp-long DNA probe, which were designed to cover the entire *E. coli* genome at 105-bp intervals (Oxford Gene Technology, Oxford, UK). The fluorescence intensity of the test sample at each probe was normalized to that of the corresponding peak of the original library. After normalization of each pattern, the Cy5/Cy3 ratio was measured and plotted along the *E. coli* genome."