Poster J-4

Systematic Analysis of Phenotypes of E. coli Single Gene Deletion Mutants

Authors:
Hirotada Mori (*Nara Institute of Science and Technology*)
Hiroko Matsui (*Mitsubishi space Co. Ltd.*)
Hiroaki Gobara (*Nara Institute of Science and Technology*)
Shigeki Tanishima (*Mitsubishi space Co. Ltd.*)
Sivasundaram Suharnan (*Axio Helix Co. Ltd.*)
Shigehiko Kanaya (*Nara Institute of Science and Technology*)
Barry L. Wanner (*Purdue University*)

Short Abstract: Complete single gene knock out library of E. coli was established and applied for systematic functional network analysis using BIOLOG system. Data mining has been performed by statistical data processing followed by clustering based on Graph theory. The initial target genes were glycolysis and TCA cycle enzyme genes.

Long Abstract:
We recently created a library of precisely defined, single-gene deletions of all non-essential genes in Escherichia coli K-12. ORF coding regions were replaced with a kanamycin cassette flanked by FLP recognition target (FRT) sites by using a one-step method (Datsenko and Wanner, 2000) for inactivation of chromosomal genes and primers designed to create in-frame deletions upon excision of the resistance cassette. Of 4288 genes targeted, mutants were obtained for 3985. To alleviate problems encountered in high-throughput studies, we saved two independent mutants for every deleted gene (Baba et al., 2006). With the goal towards defining the function of all E. coli genes, we have begun systematic screening of these mutants using phenotype microarray (PM) technology (Bochner et al., 2001) (Biolog, Inc., http://www.biolog.com/main.html), an integrated system of cellular assays, instrumentation, and bioinformatics software for high-throughput screening of cells. PM uses Biolog's patented redox chemistry, employing cell respiration as a universal reporter under a set of 1920 growth conditions. For threshold determination and computation of a coefficient of variance, we ran ten replicas for our standard wild-type E. coli K-12 BW25113, recording respiration at 15-minute intervals over a 24-hour period. Due to low intensity, 46 conditions were eliminated from further analysis, leaving 1,874. We are now using several means for clustering these huge data sets. Results from testing 200 mutants related to central metabolism (TCA cycle and glycolysis) will be presented.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L. and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knock-out mutants – the Keio collection. Mol. Sys. Biol., doi:10.1038/msb4100050.
Bochner, B.R., Gadzinski, P. and Panomitros, E. (2001) Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. Genome Res, 11, 1246-1255.

Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A, 97, 6640-6645.