

# FRIS

## Informal seminar

# 84

2016.

9/28  
WED

10:30  
▼  
12:00

学際科学フロンティア研究所では  
研究者間の交流を活発化し  
また、新しい学際領域を創成する場として  
インフォーマル・セミナーを  
定期的に開催しています。  
コーヒー片手に気楽にご参加ください。



第84回 学際科学フロンティア研究所インフォーマルセミナー

## Comprehensive *E.coli* single-gene knockout mutant libraries

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Gene-knockout is a widely used genetic technique to study gene function. Genotype-phenotype relationships have been identified individually by much effort of researchers in the manner. *Escherichia coli* is one of the most well studied bacteria of which most of gene functions are annotated. However, prediction by using genome-scale metabolic network models of *E.coli* [1], still shows false negative (model had predicted no-growth but colonies of a gene knockout showed normal growth). These discrepancies between predicted and observed cell growth show the existence of unknown enzymes or unknown pathways. In order to find such unknown function in the cell, genome-scale screening will be required. Our group has established two comprehensive collections of single-gene deletion mutants of *E.coli*; Keio collection and Aska deletion. We also have developed a time-course monitoring system (Colony-live) to monitor the growth of colonies on agar plates [2] and performed detection of essential genes on minimal media using Keio collection. Aska deletion library contains single gene-deletion mutants carrying synthetic barcode sequences that can be used as identifier of deleted genes. By using Aska deletion, population dynamics in the mixed culture of all mutants was observed by deep sequencing. Our team also established a method to construct double-knockout (DKO) mutants comprehensively via conjugal transfer between Keio strains and ASKA strains, and observed their growth [3]. Our libraries are freely available for academic users. I would like to introduce current achievements by using our libraries.

1. Orth JD, Conrad TM, Na J, Lerman JA, Nam H, Feist AM, Palsson BO: A comprehensive genome-scale reconstruction of *Escherichia coli* metabolism--2011. *Mol Syst Biol* 2011, 7:535.
2. Takeuchi R, Tamura T, Nakayashiki T, Tanaka Y, Muto A, Wanner BL, Mori H: Colony-live--a high-throughput method for measuring microbial colony growth kinetics--reveals diverse growth effects of gene knockouts in *Escherichia coli*. *BMC Microbiol* 2014, 14:171
3. Typas A, Nichols RJ, Siegle DA, Shales M, Collins SR, Lim B, Braberg H, Yamamoto N, Takeuchi R, Wanner BL et al: High-throughput, quantitative analyses of genetic interactions in *E. coli*. *Nat Methods* 2008, 5(9):781-787.

