K-097

E. coli-Synthetic Genetic Arrays (E-SGA): Deducing Functional Relationships Genome-Wide by Systematic Epistatic Double Mutant Deletion Analysis

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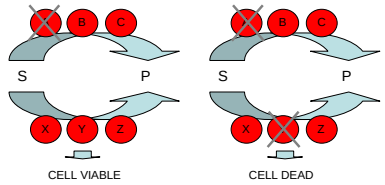
Functional Interactions by Synthetic Sickness and Lethality

Genetic interactions, which exist between genes encoding proteins involved in parallel pathways or processes, provide information regarding functional redundancy within the cell and help understand the basis of suppressor mutations and synthetic lethality. We also are developing a procedure to screen for functional interactions in *E. coli* on a genome wide scale based on the natural genetic system of conjugation. A null mutation in a gene whose genetic interactions are to be screened ("query") is constructed in a High Frequency Recombination (Hfr) strain and combined in a pair wise manner with all 4000 deletion array strains and the viability of the strains bearing both mutations assayed. Utilizing a small number of query and array strains, we have demonstrated proof of principle that mutations can be transferred from query to array strains via conjugation.

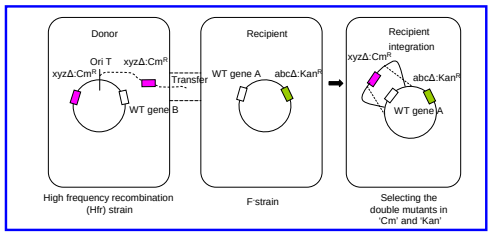
A Simple Case of Synthetic Sickness and Lethality



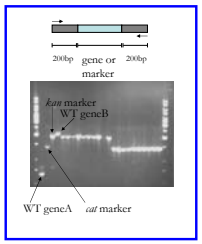
- Two isolated parallel pathways which both perform an essential conversion S to P
- Gene A should have a functional interaction with X, Y and Z but not B or C

The simplest case of a functional interaction involving two redundant parallel pathways which perform an essential process. Here, a mutation(s) in a single pathway would not result in cell mortality, or perhaps even sickness due to the redundant pathway. Mutations in both pathways would however result in a "synthetic" lethality phenotype.

Hfr Dependent Transfer of Mutations



Query mutations whose genetic interactions are to be screened are first created in the E. coli Hfr strain (the "donor" strain). Genes are replaced using the method of (Datsenko and Wanner, PNAS 2000) and are marked with a Chloramphenicol cassette. These query strains are then conjugated against F- "recipient" strains from the KEIO collection which contain single gene deletions mutations marked with a Kanamycin cassette. During conjugation, the Hfr chromosome is transferred from the donor strain into the recipient strain via the sex pilus, allowing the opportunity for recombination to take place. Recipient strains in which the wild type copy of geneA has been replaced with the mutant copy from the donor strain are selected for by selection of Cm^R and Kan^R cells.



Colonies for all strains were isolated and genomic DNA prepared. PCR amplification of loci directly from genomic DNA was used to verify the mutations. PCR primers were designed to bind to DNA 200 bp upstream and 200 bp downstream of the target gene. Strains in which the target had been replaced by the Cm^R or Kan^R cassettes resulted in a product of 1400 bp and 1900 bp respectively. In the example shown opposite, all double mutant conjugants are shown to contain both antibiotic marker cassettes at the correct loci. This indicates that mutations can be transferred from one strain to another via conjugation in an efficient manner.

Acknowlegdements

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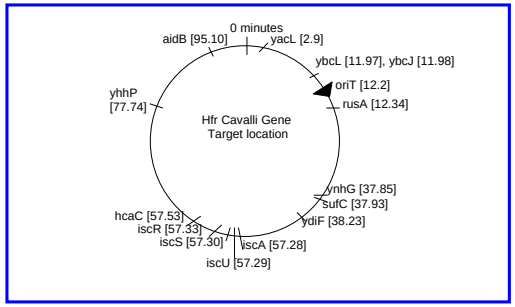
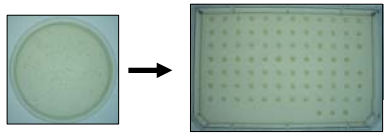
Organisation of Markers on the *E. coli* Hfr Cavalli Chromosome



Hfr Cavalli has its origin of transfer (oriT) present at 12.2 minutes on the chromosome and is oriented such that *ybcJ*, a gene present at 11.98 minutes on the chromosomal map would be transferred early in the conjugation and loci such as *rusA*, present at 12.34 minutes on the circular map would be among the last genes to be transferred.

In order to demonstrate proof-of-principle that this conjugation based approach could indeed be used to generate double mutants in a high throughput manner, we transitioned into a pin grid array based assay wherein the donor strain and recipient are both pinned onto the same spot and left to undergo mating.

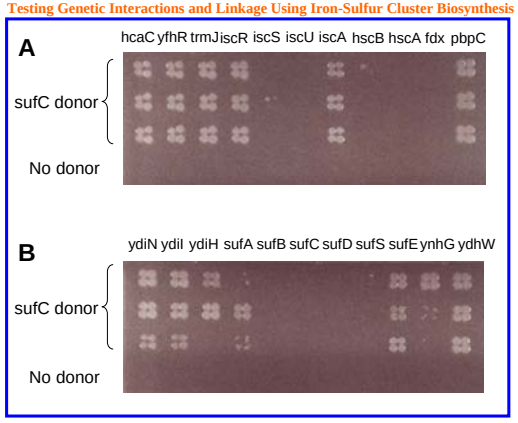


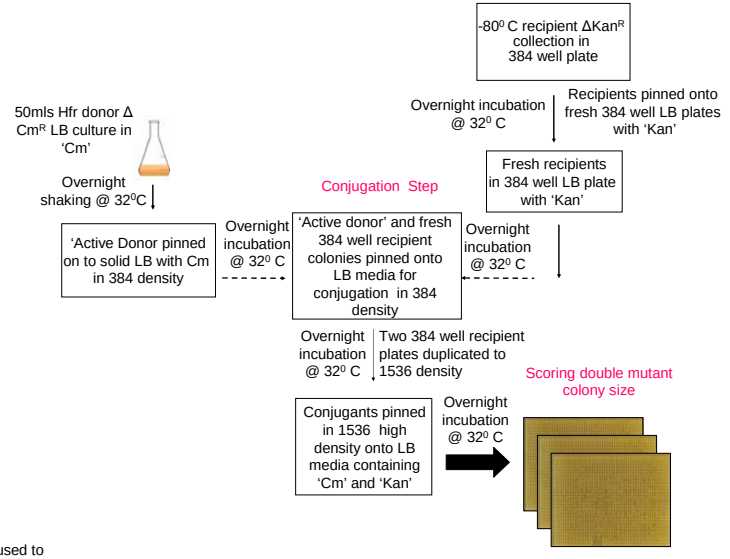
Low Throughput – each conjugation serially diluted to obtain colonies

High Throughput – each spot represents a different conjugation

E. coli contains at least two systems for the biosynthesis of iron-sulfur clusters. The Isc system encoded by in the iscRSUA-hscBA-fdx operon and the Suf system encoded in the sufABCDSE region have been demonstrated to display synthetic lethal relationships between certain gene pairs. We decided to test these observations using out system. As can be seen from panel A, using a sufC donor strain we performed conjugations in triplicate with the various recipient mutant from the KEIO collection listed above and assayed for growth on Cm/Kan plates. The sufC donor displayed synthetic a synthetic lethal relationship with iscS, iscU, hscA, hscB and fdx, but not iscA. We further tested the "linkage" effect which would result in false positive synthetic effects when the two loci in question are very close together. In panel B, we can see that it is not possible to obtain mutants of sufC in a sufB, sufD or sufS strain in addition to sufC itself. Other genes such as sufA appear to display partial effects.

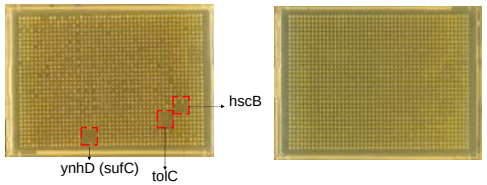
Testing Genetic Interactions and Linkage Using Iron-Sulfur Cluster Biosynthesis





The process of work flow used in the construction of large scale double mutants are shown above. The overnight liquid-Cm donor is pinned onto fresh LB-Cm plates in 384 density. Simultaneously, the overnight recipient deletion strains marked with KanR marker is pinned onto fresh LB-Kan plates in 384 density. On the next day, the overnight recipient KanR and donor Cm R deletion colonies are overlaid and subjected to overnight conjugation at 32°C. Finally, the double mutant colony sizes are scored after the overnight conjugants are selected for their outgrowth in two rounds of Kan and Cm drug selection.

16 x 96 xyzΔ : Kan^R recipient strains

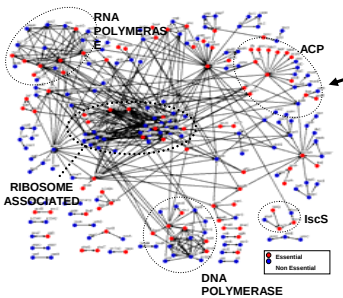


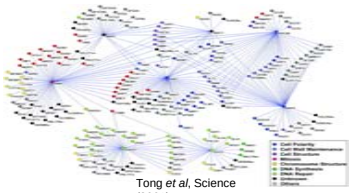
Selection of double mutants with chloromphenicol (Cm) and kanamycin (Kan)

96 recipient Δ mutant selection with kanamycin (Kan)

To demonstrate the ability of our system in a high density pin grid array format, we chose iscUΔCmR as a donor query mutant and conjugated with one of the recipient Δ mutant plate containing the deletion mutant ynhD ΔKanR. The conjugants in 96 density were pinned 16 times and scored for the double mutant colonies in two rounds of Cm and Kan drug selection. As projected, the iscUΔCmR donor Δ mutant showed synthetic sick interactions to ynhD and with others such as hscB, tolC etc (right hand panel). The figure on the left hand panel is the control plate where 96 single recipient Δ mutant strain were pinned 16 times onto the LB-Kan plates.

Summary





Tong *et al*, Science (2001)

Integration of both physical (left) and functional (above) interaction data, along with other high-throughput data types such as phenomics, metabolomics and transcriptomics, will increase our ability to predict function and also provide higher quality annotations than any single data type alone.