eSGA: E. coli Synthetic Genetic Array analysis

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Method Article

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Abstract

Introduction

Physical and functional interactions define the molecular organization of the cell. Genetic interactions, or epistasis, tend to occur between gene products involved in parallel pathways or interlinked biological processes. High-throughput experimental systems to examine genetic interactions on a genome-wide scale have been devised for _Saccharomyces cerevisiae_, _Schizosaccharomyces pombe_, _Caenorhabditis elegans_, and _Drosophila melanogaster_, but have not been reported previously for prokaryotes. Here, we describe the development of a quantitative screening procedure for monitoring bacterial genetic interactions based on conjugation of _Escherichia coli_ deletion or hypomorphic strains to create double mutants on a genome-wide scale. The patterns of synthetic sickness and synthetic lethality \(aggravating genetic interactions) we observe for certain double mutant combinations provide information about functional relationships and redundancy between pathways and enable bacterial gene products to be grouped into functional modules.

Reagents

1. Hfr Cavalli \(E. coli Genetic Stock Centre, New Haven, CT) 2. The KEIO recipient strain collection \(Baba et al., 2006) 3. The phage-lamda Red system \(Donald L. Court, National Cancer Institute, Frederick, MD)\ (Yu et al., 2000) 4. Standard Luria Bertani \(LB) agar media 5. 34 µg/ml chloramphenicol \(Sigma), 50 µg/ml kanamycin \(Sigma) and 100 µg/ml ampicillin 6. Qiaquick PCR purification kit \(Qiagen) 7. Electroporator \(Bio-Rad) 8. 15% glycerol 9. Cryovial tubes 10.Rectangular dishes \(Singer Instruments, UK) 11.96 deep well plates 12.RoToR-HDA benchtop robot \(Singer Instruments, UK) 13.96-well long pins \(Singer Instruments, UK) 14.384-well short pads \(Singer Instruments, UK) 15.1536-well short pads \ (Singer Instruments, UK) 16.Incubator \(Fisher Scientific) 17.Canon Powershot A640 \(10 Megapixels) digital camera

Equipment

RoToR-HDA benchtop robot \(Singer Instruments) to automate double mutant construction

Procedure

A. Construction of query deletion mutants in an Hfr Cavalli donor strain: A temperature-inducible λ Red cassette in strain DY3303 marked with AmpR \(resistance to ampicillin) is moved by P1 transduction into Hfr Cavalli prior to the construction of query deletion mutation. Four critical steps are deemed necessary in the construction of query gene deletion mutation in Hfr Cavalli: a) Preparation and generation of linear DNA cassette 1. Amplify the CmR cassette \(resistance to chloramphenicol) from the plasmid pKD33 with forward \(5’-AGATTGCAGCATTACACGTCTT-3’) and reverse \(5’-GGCTGACATGGGAATTAGC-3’) primers. 2. Purify the amplified linear DNA using a Qiagen purification kit.

3. Design the linear DNA cassette to replace the target gene intended for deletion with CmR marker. 4. Generate the cassette by PCR using 20-nt forward \(5’ GCGTGTTACGGTGAAAACCT-3’) and reverse \(5’-TCGTCGTGGTATTCACTCCA- 3’) primers homologous to the 3’ end of the CmR cassette, and 45-nt at 5’ end that are homologous to the sequences flanking the target gene intended for deletion. 5. The generated linear cassette is used as template for all subsequent PCRs. b) Electroporation and mutant selection 1. Grow Hfr Cavalli strain carrying λ Red cassette in 50 ml LB medium with ampicillin at 32 ºC to an OD of ~0.4 to 0.6 at 600 nm. 2. Mix two microliter of the amplified PCR product of CmR cassette with 50 µl of the competent cells. 3. Electroporate the amplified PCR product of CmR cassette and the competent cells in an ice-cold 0.2 cm cuvette at 2.5KV with 25µF and 200 ohms. 4. Add immediately 1 ml SOC medium, to allow entry of the linear CmR cassette. 3. Incubate the cells at 32 ºC for at least 1 hr with shaking at 220 rpm. 4. About 100 µl of the electroporated cell mixture is spread onto an agar LB plate containing 12.5 µg/ml of chloramphenicol. 5. Incubate the spread plates at 32 ºC overnight 6. Pick the positive clones and confirm the recombinants. c) PCR confirmation of recombinants 1. Knock out mutant is confirmed by isolating genomic DNA from two random recombinant clones. 2. The DNA is amplified with two sets of knock out confirmation primers: The first set consists of 20-nt flanking primer located 200 base pairs upstream of the deleted region and one internal primer complementary to the CmR cassette. The second set involves the same upstream probe, paired with another 20-nt flanking primer located 200 base pairs downstream of the deletion to verify the correct mutation. 3. The amplified PCR products are run on a 1% agarose gel electrophoresis to confirm the correct product size of the amplicon. d) Storage of confirmed query deletion mutants 1. Two randomly selected and successfully confirmed recombinant clones for each query deletion mutants is stored in cryovials containing 1.5 ml LB medium supplemented with 12.5 µg/ml chloramphenicol and 15% glycerol. **B. Construction of double mutants in E. coli using an automated strain handling arraying procedure** A six-day replica plating method is used to construct double mutants in a high throughput manner. 1) First day, the frozen glycerol stock cultures of query deletion mutant created in an Hfr strain with the target gene replacement with CmR is grown overnight at 32 ºC in rich LB liquid media supplemented with 34 µg/ml of chloramphenicol prior to pinning the cultures at 384 density. 2) Second day, the overnight culture of the query deletion mutant, and the frozen glycerol stock culture from an ordered array of recipient deletion mutant, marked with KanR \(resistance to kanamycin), is pinned onto a LB plate supplemented with 34 µg/ml chloramphenicol and 50 µg/ml kanamycin, respectively at 384 density \(24 column x 16 row) using four 96-well long pins. 3) Third day, conjugation is carried out onto a LB plate by pinning the overnight query and recipient deletion mutants at a 384 density using 384-well solid pins. 4) Fourth day, conjugants are subjected to first round of selection onto a LB plate supplemented with chloramphenicol and kanamycin antibiotics. On this day, two 384 colony density conjugation plates are combined in duplicate onto a double drug plate to create 1,536 colony density plates. 5) Fifth day, the colonies from the first double drug selection plate is pinned onto a second double drug selection plate. 6) Sixth day, the colonies from second double drug plate is photographed using a Canon Powershot A640 \(10 Megapixels) digital camera. The captured images were saved as jpeg files and growth phenotype of the double mutants is quantitatively assayed using an in-house automated image processing system originally devised for yeast \(Collins et al., 2006). In each step of the above process, the plates are incubated for 24 hrs at 32 ºC.

Timing

Single query gene deletion knock out - 3 days Confirmation of gene deletion knock out - 1 day Double mutant construction - 6 days

Anticipated Results

After conjugation, and selection for double mutants, synthetic genetic interactions can be identified when specific combinations of mutations shows cell death \(synthetic lethality) or retard growth \(synthetic sickness).

References

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