

Preparation of Genomic Overexpression Library

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[Abstract] This protocol is used to identify/select for *Escherichia coli* genes that, when overexpressed in *Vibrio cholerae* Hsp33 (hsI_O gene) deletion mutant, protect against oxidative heat stress and, by extension, against HOCl-mediated protein damage. In the referenced publication, I found that *V. cholerae* mutant lacking Hsp33 (in addition to its established role in HOCl stress) has severe temperature sensitive phenotypes on MacConkey plates (this phenotype is not found in *E. coli* Δ hsI_O mutant). I constructed genomic expression libraries from *E. coli* and transformed the libraries into *V. cholerae* Δ hsI_O mutant. I then searched for complementing *E. coli* proteins that rescue the temperature sensitive phenotype of *V. cholerae* Δ hsI_O mutant on MacConkey plate. This protocol might work for searching complementation of other gene deletion mutants that have a good selective phenotype.

Materials and Reagents

1. Bacteria donor (*E. coli* MG1655 wild-type or the Δ hsI_O deletion)
2. Bacteria recipient (*Vibrio cholerae* O395 Δ hsI_O deletion)
3. GenElute Bacterial Genomic DNA kit (Sigma-Aldrich)
4. Plasmid pBR322 (New England BioLab, linearized with *Bam*H1)
5. Plasmid pET11a (New England BioLab, linearized with *Bam*H1)
6. *Bam*H1 (New England Biolabs)
7. *Bfu*CI (New England Biolabs)
8. T4 DNA ligase (New England Biolabs)
9. Gel Extraction Kit (QIAGEN)
10. Ultracompetent XL10-Gold cells (Stratagene)
11. Wizard Plus SV Minipreps kit (Promega Corporation)
12. MacConkey plates (lab-made)
13. LB medium
14. TAE buffer

Equipment

1. Gel electrophoresis apparatus
2. Incubator for bacteria

Procedure

1. Prepare genomic DNA from the donor *E. coli* strains (MG1655 wild-type or the Δ hslO deletion) from 5 ml overnight culture (in LB medium) using the GenElute Bacterial Genomic DNA kit following the manufacturer's instruction.
2. Partially digest the genomic DNA (2 μ g) with 0.05-0.2 units of BfuCI in a total volume of 50 μ l for 10 min at 37 °C. Then heat inactivate the enzyme.
3. Analyze digested products on 1% agarose gels (in TAE buffer) and make sure that fragment sizes ranging between 1 and 8 kb.
4. Gel purify fragmented genomic DNA with Gel Extraction Kit (some researchers prefer to separate DNA fragments into bins, for example 1-3 kb, 3-5 kb and 5-8 kb).
5. Ligate the DNA fragments (approximately 50-80 ng/ μ l) into linearized *pBR322/BamH1* or *pET11a/BamH1* plasmid vectors (approximately 80-100 ng/ μ l) using T4 DNA ligase overnight at 15 °C following the manufacturer's instruction.
6. To propagate overexpressing plasmids library, transform 1-5 μ l of ligation results into ultracompetent XL10-Gold cells following the manufacturer's protocol.
7. Count colony forming units (CFU) of transformants the next day and calculate the size of the libraries (1 x 10⁵ CFU/ml of cell or more is good library size).
8. Combine the transformants by scraping off all colonies from the library and purify the total plasmids using Wizard Plus SV Minipreps kit.
9. Transform 1-3 μ l of the *E. coli* genomic library plasmids into recipient *Vibrio cholerae* Δ hslO deletion.
10. Plate the transformants on MacConkey agar and incubate for 24 h at 43 °C to select for clones that rescue the temperature sensitive phenotype of the *V. cholerae* Δ hslO mutant strain (this mutant cannot grow on MacConkey plate at 43 °C).
11. To ensure the growth phenotype, re-streak transformants that formed healthy looking colonies on MacConkey plates and grew for 24 h at 43 °C.
12. To eliminate the possibility of mutations in the strain background, purify and re-transform plasmids (that rescued phenotype) of transformants into *V. cholerae* Δ hslO mutant.
13. To identify inserted *E. coli* gene(s), purify the plasmid and send for DNA sequencing.

Acknowledgments

The protocol described here is adapted from one reported previously (Wholey *et al.*, 2012).

References

1. Wholey, W. Y. and Jakob, U. (2012). [Hsp33 confers bleach resistance by protecting elongation factor Tu against oxidative degradation in *Vibrio cholerae*](#). *Mol Microbiol* 83(5): 981-991.