**Dr. Yongtao Zhu Fall 2022**

**Co-transformation for pre-methylation of the deletion constructs**

1. Label one tube of *E. coli* S17-1 lambda pir competent cells (kept on ice) with plasmid names.
2. Set the heating block at 42oC.
3. Add 2 µl of deletion construct and 2 µl of the methylation helper plasmid pSS05 (1) (centrifuged briefly before use) to the competent cells. Do not pipette but can stir gently to mix the cells with DNA. Keep the tubes on ice for 30 mins.
4. Heat shock: Transfer the tube to the 42oC heating block and incubate exactly 90 seconds.
5. Immediately place the tube on ice for 3-5 mins.
6. Add 1 ml of LB broth to the tube and incubate at 37oC for 1 hour.
7. Sterilize a glass hockey stick by using flame and let it cool.
8. Labe 1 LB agar (with 100 µg/ml ampicillin and 10 µg/ml chloramphenicol) plate with the plasmid names, and the date.
9. Transfer 100 µl of the cells onto the plate. Save the leftover cells in fridge (4oC).
10. Spread the cells evenly on the agar using the sterile hockey stick.
11. Incubate the plates (inverted) at 37oC for no more than 24 hours and transfer them to 4oC.

Reference

1. S. Sloboda *et al.*, Methylation of foreign DNA overcomes the restriction barrier of *Flavobacterium psychrophilum* and allows efficient genetic manipulation. *Applied and environmental microbiology* **Under Revision** (2024).