**Conjugation/deletion in *Flavobacterium psychrophilum* (Modified on 4/20/24)**

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**Refer to (1)**

1. Streak *E. coli* on LB and *F. psychrophilum* on TYES from the freezer (-80oC). *F. psychrophilum* is incubated at 18oC for 3 days (or until enough growth is seen).
2. Lawn inoculate *F. psychrophilum* on fresh TYES plates using the 3-day old culture. Incubate the plates at 18oC for 1.5-2 d before the cells are harvested for conjugation.
3. Inoculate *E. coli* into LB broth with appropriate antibiotics the day before conjugation starts. Grow *E. coli* overnight at 37oC (~16 h).
4. Scrape cells of *F. psychrophilum* offthe TYES plate using cell lifters and suspend the cells in 10 ml of TYES.

Note: We typically use the amount of cells from one TYES plate and 5 ml of *E. coli* for each deletion experiment. For shuttle vectors (pCP11 etc.), less cells can be used.

1. Collect cells by centrifugation at 4000 x g and 20oC for 10 min.
2. Wash *E. coli* and *F. psychrophilum* cells once with equal volume of TYES.
3. Re-suspend the cells in a small volume of TYES and measure the OD600 if needed.
4. Mix *E. coli* with *F. psychrophilum* at a cell ratio of 1:1 and centrifuge at 4000 x g for 10 min.
5. Re-suspend the mixture in ~50-100 µl of TYES.
6. Transfer all the cells to a TYES plate on one spot.
7. Let the plate dry at 18oC and incubate at 18oC for 2 days.
8. Suspend the cells in 1 ml of TYES (perform serial dilution if needed, typically 100, 10-1) and plate them on TYES plates (100 µl / plate) with antibiotics (20 µg/ml erythromycin).
9. Incubate the plates at 18oC for 7 days or until colonies are seen (can be longer than 7 days). Just don’t discard the old plates before you see something.
10. **The next steps are for the 2nd recombination of pYT313 (2) based plasmids to make deletions.**
11. Streak erythromycin resistant colonies on TYES plates (with erythromycin) for purification.
12. Streak a single colony on a fresh plate (with erythromycin) and incubate until enough growth is seen.
13. Inoculate enough cells (one single colony may not work well) into 5 ml of TYES broth without antibiotics (in a test tube). The rest of the cells on the plate can be frozen in glycerol stocks.
14. Incubate the test tube at 18oC with shaking for 24 h (can be extended if there is not enough growth).
15. Serial dilution (typically 100, 10-1, 10-2, 10-3) and plate the cells onto TYES plates with 2.5% sucrose (2.5% works better for CSF259-93).
16. Incubate the plates at 20oC for 7 days or until single colonies are observed.
17. Patch the same single colonies on both sucrose and erythromycin plates.
18. Colony PCR to screen the deletion mutants from the erythromycin-non-growing colonies.
19. Purify the sucrose resistant mutants (erythromycin sensitive) by streaking and confirm the deletions again using PCR.

References

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).

2. Y. Zhu *et al.*, Genetic analyses unravel the crucial role of a horizontally acquired alginate lyase for brown algal biomass degradation by *Zobellia galactanivorans*. *Environmental microbiology* **19**, 2164-2181 (2017).