

Heat Shock Transformation

1. Thaw competent cells on wet ice. Place 15 ml polypropylene tubes (Falcon2059)^a on ice.
2. Gently mix cells, then aliquot 100 μ l competent cells^b into chilled tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath before returning them to -70°C freezer.
4. Add 5 μ l of ligation reaction mixture (1~10 ng DNA) to the competent cells and mix by gently tapping the tube.
5. Incubate cells on ice for 30 min.
6. Heat-shock 45 seconds in a 37°C water bath^c, do not shake.
7. Place on ice for 2 min.
8. Add 0.9 ml of RT SOC (LB is just fine) medium^d.
9. Shake at 225 rpm at 37°C for 1 hour^e.
10. Put LB (with proper antibiotic) plates in 37°C for about 30min^f.
11. Plate 50~100 μ l of cells.
12. Incubate overnight at 37°C.

Note:

- a. 1.5 ml microfuge tube can be used instead.
- b. For most ligation, 50 μ l subcloning competent cells are sufficient.
- c. This is different from most of the older protocol, which uses 42°C.
- d. Use 450 μ l of SOC for 50 μ l cells.
- e. For ampicillin resistant colonies, this step can be skipped.
- f. This step is only necessary if the plates are too wet.