TRANSFORMATION

ITO Kei, 1993 October

1. thaw **100 ul competent cell**.
   on ice 10 min.
2. add **5 ul** ligated DNA (<5% total volume)
   mix gently
3. **on ice 30-60 min.**
4. prepare 37°C shaker and 42°C water bath
   prepare SOC (SOB + 1/100 vol. $MgSO_4$/$MgCl_2$ + 1/100 vol. Glucose)
   :400ul per 100ul competent cell
   place SOB plates at 37°C
5. heat shock **42°C, 90 sec**. *precisely!*
6. **on ice 2 min.**
7. add **400 ul SOC**.
8. shake **37°C, 60 min.**
9. innoculate **100ul cell culture** to a plate.
10. cfg. **4000 rpm. 10 min.**
11. discard 300ul medium, resuspend the cell in **100 ul medium**.
12. innoculate the culture to another plate.
13. **37°C, <16 hrs.**

MAKING COMPETENT CELLS

1. with a platinum wire, scrape the surface of stock cell kept at **-70°C**.
2. innoculate onto **an SOB** plate
3. **37°C, O/N**
4. pick 1 colony per 10 ml culture and suspend them in **1 ml SOB**.
5. add to **100 ml SOB** in 1 l flasc.
6. **37°C, 1-1.5 hr.** (until OD_{550} = 0.35)
7. **on ice, 15 min.**
8. cfg. **4°C, 3000 rpm, 15 min.**
9. discard supernatant
   remove the remaining medium with a pipette.
10. resuspend the cells in **30 ml RF1**
11. **on ice, 15 min.**
    *place a metal block at **-80°C**.*
12. cfg. **4°C, 3000 rpm, 15 min.**
13. discard supernatant
    remove the remaining solution with a pipette.
14. resuspend the cells in **8 ml RF2**
15. **on ice, 15 min.**
16. aliquot **200 ul** per microtube.
17. put the tubes in the metal block at **-80°C** to freeze them quickly.
    or better, freeze them with **liquid N_2**.
18. store the tubes at **-80°C**.