Preparation of competent bacteria - Inoue method

Day 1:

1. Streak bacteria from frozen stock onto LB agar medium; incubate O/N at 37˚C

Day 2:

2. Pick a single colony to inoculate 25 mL LB medium; shake at 37˚C for 6-8 hr

3. Use starter culture to inoculate 3 250-mL LB medium (in 1-L flasks) *e.g., use 10 mL, 5 mL, and 3 mL (or 8 mL, 4 mL, and 2 mL)

4. Shake at 20˚C O/N

Day 3:

5. Monitor OD_{600} until it reaches 0.5, then chill the culture in ice water for 10 min

6. Spin out cells at 5,000 rpm for 10 min at 4˚C

7. Decant and invert centrifuge tube onto paper towel for 2 min

8. Resuspend cell pellet in 80 mL ice-cold Inoue buffer

9. Repeat steps 6 and 7

10. Resuspend cell pellet in 20 mL ice-cold Inoue buffer and transfer volume to 50-mL disposable capped tube (e.g., 50-mL Falcon tube)

11. Add 1.5 mL DMSO; mix by inversion; incubate in ice water bath for 10 min

12. Dispense 210-µL aliquots into chilled eppi tubes (on ice); snap-freeze in liquid nitrogen.

13. Store at –80˚C.

Typical competence is ~$1x10^6$ colonies per µg DNA after transformation

Inoue Buffer	***for 200 mL:***	**0.5M Pipes, pH 6.7**
55 mM $MnCl_2$	***2.17 g $MnCl_2•4H_2O$***	**15.1 g Pipes dissolved in 80 mL H2O**
15 mM $CaCl_2$	***0.44 g $CaCl_2•2H_2O$***	**pH to 6.7 using 10M KOH**
250 mM KCl	***3.73 g KCl***	**Bring volume to 100 mL with H2O**
10 mM PIPES, pH 6.7	***4 mL 0.5M PIPES, pH 6.7***	