Preparation of competent bacteria - Inoue method

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

Day 2:

1. Pick a single colony to inoculate 25 mL LB medium; shake at 37˚C for 6-8 hr
2. 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)
3. Shake at 15˚C O/N

Day 3:

1. Monitor OD_{600} until it reaches 0.5, then chill the culture in ice water for 10 min
2. Spin out cells at 5,000 rpm for 10 min at 4˚C
3. Decant and invert centrifuge tube onto paper towel for 2 min
4. Gently resuspend cell pellet in 80 mL ice-cold Inoue buffer
5. Repeat steps 2 and 3
6. Gently resuspend cell pellet in 20 mL ice-cold Inoue buffer and transfer volume to 50-mL disposable capped tube
7. Add 1.5 mL DMSO; mix by inversion; incubate in ice water bath for 10 min
8. Dispense aliquots into chilled eppi tubes (on ice), then snap-freeze in liquid N_2
9. Store aliquots at –80˚C

Inoue buffer	for 200 mL Inoue buffer:
55 mM $MnCl_2$	2.17 g $MnCl_2$•$4H_2O$
15 mM $CaCl_2$	0.44 g $CaCl_2$•$2H_2O$
250 mM KCl	3.73 g KCl
10 mM PIPES, pH 6.7	4 mL 0.5M PIPES, pH 6.7
	Bring volume to 200 mL with H2O

0.5 M PIPES, pH 6.7
15.1 g PIPES dissolved in 80 mL H2O
pH to 6.7 using 10 M KOH
Bring volume to 100 mL with H2O