

## 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<sub>600</sub> 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<sub>2</sub>
9. Store aliquots at -80°C

Inoue buffer	for 200 mL Inoue buffer:	0.5 M PIPES, pH 6.7
55 mM MnCl <sub>2</sub>	2.17 g MnCl <sub>2</sub> •4H <sub>2</sub> O	15.1 g PIPES dissolved in 80 mL H <sub>2</sub> O
15 mM CaCl <sub>2</sub>	0.44 g CaCl <sub>2</sub> •2H <sub>2</sub> O	pH to 6.7 using 10 M KOH
250 mM KCl	3.73 g KCl	Bring volume to 100 mL with H <sub>2</sub> O
10 mM PIPES, pH 6.7	4 mL 0.5M PIPES, pH 6.7	
	Bring volume to 200 mL with H <sub>2</sub> O	