THE HANAHAN METHOD FOR COMPETENT CELL PREPARATION

REAGENTS REQUIRED:

- SOB Medium
- CCMB Buffer

PROCEDURE:

- Add 400 µL of secondary culture of OD 0.3 to 100 mL SOB media.
- Incubate on ice for 15 minutes. Pre-chill two 50 mL falcon tubes and eppendorf tubes on ice.
- Centrifuge at 3000 g at 4°C for 10 minutes.
- Discard the supernatant.
- Add 16 mL of CCMB-80 buffer to each falcon tube and incubate on ice for 20 minutes.
- Discard the supernatant.
- Add 2 mL of CCMB-80 buffer to each falcon tube.
- Mix the contents of both the tubes into one falcon tube.
- Incubate on ice for 20 minutes.
- Aliquot the cells in 200 µL batches to pre-chilled eppendorf tubes.
- Store in -80°C