KCM COMPETENT CELLS PREPARATION

MATERIALS:

- Bacteria of choice
- TSB Media

PROCEDURE:

- Plate bacteria from frozen stock (with adequate antibiotics if it contains any resistance gene)
- Pick a single colony and grow cells overnight in 5 mL of LB media (+ antibiotic if needed)
- Next morning inoculate 200 mL of LB media (+ antibiotic if needed) with 1 mL of overnight culture and grow them to OD 600 nm = 0.3-0.6
- Spin down 5 min at 3000 g and 4 ^{o}C
- Re-suspend in 1/20 volume of TSB at 4 ^{o}C
- Incubate cells on ice 10 min and add glycerol to make final concentration 10%
- Aliquot in individual Eppendorf tubes (50 µL of cells), freeze in liquid nitrogen or dry ice
- Store at -80C

Recipe to prepare TSB:

LB Broth (pH to 6.1 with 6N HCl)	800 mL
PEG 8000	100 g
1M MgSO4	10 mL
1M MgCl2	10 mL
DMSO (dimethyl sulfoxide)	50 mL
Distilled water	Upto 1 L

Special instructions:

DMSO should be treated as a toxic reagent even though it is not toxic in itself. It is a transporter, which will act to transport any chemicals through your skin. Label bottles with a hazardous warning label.

The cells are ready to be transformed, See 'Transformation Protocol'. Calculate the transformation efficiency to check the efficacy of chemo-competency.