PEG/DMSO competent cells and transformation

From Simon Boulton's lab.

Preparation of PEG/DMSO competent cells

1) Streak the required strain on an LB plate and incubate overnight at 37ºC.
2) Inoculate a single colony into 5 ml LB and shake overnight at 37ºC.
3) Use this 5 ml LB overnight culture to inoculate 500 ml of LB. Shake at 37ºC until an OD600 of 0.5-0.6 is reached.
4) Spin cells for 5 min at 2000 rpm, 4ºC.
5) Gently resuspend cells in 25 ml of ice cold TSB buffer.
6) Incubate on ice for 10 minutes.
7) Aliquot, flash freeze in liquid nitrogen and store at -80ºC.

TSB buffer
LB pH 6.1 (with concentrated HCl)
10% PEG-3350
5% DMSO
10 mM $MgCl_2$
10 mM $MGSO_4$

Filter sterilize and store at 4ºC.

Transformation of PEG/DMSO competent cells.

1) Thaw cells on ice.
2) Mix DNA, 5xKCM and dH20 to a final volume of 100 µl at 1xKCM final concentration (this reaction and cells added can be scaled down or up).
3) Add an equal amount of cells, mix and incubate on ice for 20 minutes.
4) Incubate at room temperature for 10 minutes.
5) Add 1 ml of SOC or LB and shake for 1 hr at 37ºC. Plate on LB agar+appropriate antibiotic(s).

5xKCM
500 mM KCl
150 mM $CaCl_2$
250 mM $MgCl_2$

Filter sterilize and store at room temperature.