**Electro-Competent Cell Preparation**

This protocol has been demonstrated effective for the preparation of electro-competent cells of *E. coli* and *P. putida* for (co-)transformation of purified plasmids. However, cloning efficiency cells should be used for all cloning activities.

1. Grow cells in 2x5 mL culture tubes at 30oC, over night (for approximately 16 h).
   1. TIP: inoculate your cultures as the last thing you do in the evening and begin the competent cell preparation first thing the next morning.
2. Pellet cells at 5000xg for 5 min at 4oC.
3. Pour off supernatant from both tubes. Re-suspend one pellet in 10% ice-cold glycerol solution (sterile). Transfer suspension to other tube to resuspend the second pellet (i.e., combine both pellets in one suspension in a single tube).
   1. TIP: keep your cells as cold as possible for as long as possible (i.e., on ice whenever they not being handled).
4. Repeat Steps 2&3 a total of 3-4 times.
5. Re-suspend final pellet in residual or fresh glycerol solution (a total of ~150 microliters).
6. Aliquot cell solution into sterile Eppendorf tubes at desired volume (typically 20 microliters) and promptly move to -80oC freezer for storage.

\*\* Greater competency can be obtained with younger cultures and a greater number of washes.