**DH10 Competent Cells**

*Preparation competent cells DH10bac:*

CaCl$_2$ solution: 60mM CaCl$_2$, 15%(v/v) glycerol, 10mM PIPES, pH adjusted to 7.0 with NaOH. Filter sterilize.

**For 300 ml of CaCl$_2$ solution:**

- 30ml PIPES of 10mM stock
- 45ml of Glycerol 100% stock
- 36ml of CaCl$_2$ 500mM stock

Fill up to 300ml final volume with distilled water.

Filter and put it in ice or refrigerator until you use it.

You can start culture from one single colony after streaking on plate no antibiotics, but you can also use a loop of cells from the frozen stock.

Culture ON 4ml in LB+Kan + tetracycline (in 100% water).

(Remember to put a sterile LB flask at 37 degrees over night!)

Early in the morning inoculate 400ml LB Kan+ tetracycline with the 4ml culture grown over night.

Grow 37°C agitating till OD reaches OD600nm= 06-08

Pellet cells 8000 rpm 10 min 4°C

(From now on make sure that all the tubes where the cells have to be are prechilled in ice!)

Wash cells with 100 ml of ice cold CaCl$_2$ 60 mM solution.

(Don’t worry if you see small pellets of cells not resuspending)

Let sit 10'-30' in ice, pellet cells 10 min 4000 rpm 4°C

Resuspend carefully in 4 ml CaCl$_2$ + 15% glycerol

Aliquot in prechilled and sterile tubes in 250 ul aliquots and freeze them at -80 right away (while in ice)

Check with 1 µL of miniprep the pFastbac to test if the cells are competent.

*Calcium chloride protocol for DH10 competent cells CA 9-5-14.pdf*