Solutions for preparation of chemically competent *E.coli* cells

φA medium: 5 g/l yeast extract
20 g/l tryptone
5 g/l $MgSO_4 . 7H_2O$

Adjust pH to 7.6 with KOH

14 g/l agar

250 ml makes 10 plates which should be lots

φB medium: as above without agar (you need 1.5 l for 60 ml of competent cells)

TfbI:		for 500 ml:	
	30 mM KAc		1.48 g
	100 mM KCl		3.73 g
	10 mM $CaCl_2$		0.74 g
	50 mM $MnCl_2$		4.95 g
	15% (v/v) Glycerol		75 ml

Adjust pH to 5.8 with acetic acid

0.22 μm Filter (do not autoclave) and store at 4°C

TfbII:		for 500 ml:	
	10 mM Pipes		1.51 g
	75 mM $CaCl_2$		5.51 g
	10 mM KCl		0.373 g
	15% (v/v) Glycerol		75 ml

Adjust pH to 6.5 with KOH

0.22 μm Filter (do not autoclave) and store at 4°C

Preparation of chemically competent *E.coli* cells (400x 100μl aliquots)

Day 1: - Streak bacteria on φA plate to generate single colonies
- Leave O/N at 37°C, together with at least 75 ml φB

Day 2: - Put 3x 10-20 colonies in three 100 ml flasks with 25 ml φB each
- Shake at 37°C until OD_{550} is 0.30 (± 2-3 hours, depending on inoculation)
- Pre-heat three 2L flasks containing 480 ml of φB by shaking at 37°C
- Combine pre-cultures and inoculate each 2L flask with 24 ml of culture

Remember: You want the *E.coli* to grow as fast as possible
The doubling time of *E.coli* is ± 20 min

Take <u>all</u> 2L flasks out of the incubator when you take a sample for the spec (otherwise one flask will have higher OD)

- Shake at 37°C until OD_{550} is 0.28 (± 2 hours, depending on inoculation)
- Put 2L flasks on ice for 10 min to arrest the *E. coli* growth
- From now on keep everything as cold as possible and work on ice!
- Spin down the cultures in sterile bottles at 4°C (6,000 rpm for 5 min) (This is ± 5856 x *g* for a GSA rotor)
- Remove SN (supernatant) and resuspend each pellet (derived of 250 ml culture) in 100 ml of cold TfbI by pipeting up and down
- Put on ice for 5 min and spin at 6,000 rpm for 5 min
- Remove SN and resuspend each pellet in 10 ml TfbII by pipeting up and down
- Combine the four 10 ml TfbII cell suspensions in a 50 ml screw cap tube on ice and mix thoroughly
- Put on ice for 15 min
- Aliquot 100 µl per sterile ependorf tube (occasionally mix the TfbII cell suspension)
- Place on dry ice and then store at -80°C

Transformation of chemically competent bacteria

- To 100 µl of competent cells add up to 6 ng of DNA and mix thoroughly
- Put on ice for 5 min
- Heat shock for 90 sec at 42°C
- Put on ice for 2 min
- Add 300 µl 2YT
- Put at 37°C for 1 hour, no shaking required
- Plate on 2YT plates containing the appropriate antibiotic
- Put plates at 37°C O/N

<u>2YT</u>: 16 g/l Tryptone
10 g/l Yeast extract
5 g/l NaCl

For 20 plates: Add 10 g of agar per 500 ml media

A stir bar will help cool down the media after autoclaving and allow for the mixing of the antibiotic (0.5 ml of a 1000x (100 mg/ml) Amp stock) without causing bubbles that interfere with the pouring of the plates.