



NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

**Preparation of competent cells  
January 2010**

Solutions: RF1 (500ml)  
6g RbCl  
4.95g MnCl<sub>2</sub>  
0.75g CaCl<sub>2</sub>  
1.47g potassium acetate  
75ml glycerol  
  
adjust pH to 5.8 with 1N acetic acid

RF2 (500ml)  
0.6g RbCl  
5.5g CaCl<sub>2</sub>  
1.05g MOPS  
75ml glycerol

adjust pH to 6.8 with 5N NaOH or KOH

Both solutions are stored at 4°C.

The night prior to preparing competent cells put the following into a -20°C freezer:

- 50ml Falcon tubes
- 5ml, 10ml and 25ml pipets
- autoclaved 1.5ml microfuge tubes and/or sterile 96 well plates
- 10ml tips for repeater pipet
- 200ul tips for multichannel pipet
- microfuge tube racks

Throughout the procedure, it is critical that the cells be kept as cold as possible and that they be handled as gently as possible.

*Preparation of competent cells:*

Streak cells onto an LB plate containing Streptomycin. Incubate overnight at 37°C.

Pick a single colony and use to inoculate 4-5ml LB containing Streptomycin. Incubate overnight in 37°C shaker.

The next morning, use 2x2ml saturated overnight culture to inoculate 2x200ml LB + Streptomycin in two 2L culture flasks. Incubate cultures in 37°C shaker.

Note: it is important that the cultures are well aerated. Typically, cells are grown in a volume of media 1/10<sup>th</sup> the total volume of the flask (eg. 200ml media in a 2L flask).

Check A<sub>550</sub> hourly, until A<sub>550</sub> reaches 0.45-0.55 (If you go over, start again!)

Cool flasks on ice for 15-20 minutes, preferably in a cold room.

Transfer cooled cells to 50ml Falcon tubes. (When collecting the cells, note the final volume of the culture) Pellet cells by centrifugation at 2500xg for 10 minutes. Pour off supernatant.

Gently resuspend cells in 120ml ice-cold RF1 by pipetting up and down. (Do not shake or vortex cells!) Incubate on ice for 15-20 minutes.

Pellet cells by centrifugation at 2500xg for 10 minutes. Pour off supernatant.

Gently resuspend cells in 30ml RF2. (If the total volume of culture collected was less than 400ml, adjust the volume of RF2 accordingly) Incubate on ice for 15-20 minutes.

Place microfuge tube racks on dry ice. Place tubes in racks, or lay 96 well plates on top of racks. Aliquot cells at 200ul/tube in 1.5ml microfuge tubes, or 20ul/well in 96 well plates.

Quick freeze cells in microfuge tubes by dropping closed tubes into liquid nitrogen. Cells in 96 well plates can be quick frozen on dry ice.

Store competent cells at -80°C.

*Determining transformation efficiency:*

Test transformation efficiency by combining 50ul thawed competent cells with 50pg plasmid DNA. Incubate on ice for 30 minutes.

Heat shock at 42°C for 45 seconds. Return cells to ice.

Add 200-300ul pre-warmed SOC and incubate cells in 37°C shaker for one hour.

Spread entire transformation on to an LB plate containing the appropriate antibiotic(s). Incubate plate overnight at 37°C.

For 50pg DNA, the transformation efficiency is the number of colonies multiplied by 20,000. Transformation efficiency is typically expressed as colonies/ug DNA. We routinely obtain transformation efficiencies of  $\sim 1 \times 10^8$ /ug.