Protocol for preparation of chemically competent E.coli cells (rubidium chloride)

NOTES:

Use excellent aseptic technique at all times. All materials must be sterile.

Protocol can be scaled up or down as required. 100mL of *E. coli* culture produces about 40 x 220 µL aliquots of competent cells. Each individual aliquot of cells is sufficient for 4 transformations (4 x 50µl, with a bit left over as a safety margin).

The competent cells can also be used fresh, without making frozen aliquots, this actually works even better, but it is tedious to do over and over, thus we typically make frozen stocks. These freezer stocks don't survive multiple freeze/thaw cycles well, so throw out unused cells if there are leftovers after your transformation experiment.

After step 6, work on ice and in the cold room to increase the quality of the final cell prep. Keep RF1 and RF2 solutions in cold room/fridge, so they are always ready to use. At the beginning of the day, place centrifuge bottles in cold room or in the -20°C freezer, ready to use.

PROTOCOL:

1) Streak *E. coli* strain (e.g. JM109, TOP10, DH5α) from glycerol stock or other source onto plain LB agar (no antibiotics). Incubate overnight at 37°C.

2) Check that culture looks pure. Inoculate a 5mL plain LB broth with growth (several colonies) from the LB plate. Incubate overnight (16-24 h) at 37°C, with shaking

3) Aseptically inoculate 100mL plain LB broth (in a 500mL Schott bottle or Erlenmeyer flask) with 3 ml of overnight culture; this should give an initial OD_{600} of ~0.05. (check this to be sure)

4) Grow cells at 37°C with shaking, until the culture reaches OD_{600} of ~0.5 (anywhere from 0.3-0.7 is OK, but try to get close to 0.5). This should take 2-3 hours, so check the OD_{600} every 15 minutes or so after the first hour of incubation.

5) Aseptically transfer (pour) the cells into sterile centrifuge tubes or bottles (one 250 ml Sorvall bottle or 2 x 50 ml Falcon tubes) and balance these, either by transferring culture aseptically between the tubes/bottles or by adding sterile water or LB to the lighter tube/bottle.

6) Centrifuge at 4000 rpm (~3000 g in Centaur/Centurion machine) at 4°C for 10 minutes. Note: centrifuge needs to be cold. Turn on and set temperature beforehand. You can spin faster, up to say 7000 g, but above this point, faster is not better, and cell pellets will be hard to resuspend.

7) Working in the cold room with the cells on ice, pour off the supernatant into culture waste (don't let the centrifuge tube/bottle actually touch the edge of the culture waste bottle). Try to remove as much of the liquid as possible – give it a shake / tap to assist this.

8) Resuspend the pellet gently in 33mL RF1 solution by vortexing and/or shaking the tube/bottle. Its OK to be rough with the cells at this stage in the process, but you shouldn't need to shake or vortex for more than 10 sec or so.

9) Incubate on ice for 1 hour, then pellet the bacteria again at 4000rpm, 4°C for 10 minutes.

10) Working in the cold room, pour off the supernatant into culture waste. As before, try to remove as much of the residual liquid as possible.

11) Resuspend the pellet in 8mL RF2 solution by vortexing or shaking. At this stage, the cells have become more fragile due to the RF1 treatment, so its important not to shake/vortex any longer than ~10 sec (this shouldn't be necessary).

12) Incubate on ice for 15 minutes. While the cells are incubating, set up all your Eppi tubes (~40) on ice with the lids open, so they are pre-chilled, and ready to receive cells. Label these tubes on top with the strain name before putting them on ice (labelling becomes difficult with cold and wet tubes!). Be careful to only push the tubes only about 2/3 of the way into the ice. If they are pushed in too far (right up to the lip of the tube), you risk getting ice or melted ice (not sterile!) into your cell aliquots

13) Working quickly (but still carefully!), aliquot 220 µL of cell suspension into the pre-chilled Eppi tubes. Once dispensed, close tube lids tightly, and collect all tubes into a bag/box with a clear and prominent label and store it immediately in the -80°C freezer. It's a good idea to label both the outside of the bag/box AND place a label written on paper inside.

14) Test the transformation efficiency of the freshly-prepared competent cells using a known amount of a plasmid standard (see protocol for heat shock transformation).

15) Streak out a loopful of the cells onto plain LB medium (using either a sample of a frozen aliquot or some residual cells remaining in the large centrifuge tube/bottle), and incubate at 30°C for three days to allow any of the common types of contaminants (e.g. *Staphylococcus*) to grow. This is to check the purity of the cell stock. The streak-plate should look completely uniform, with colonies of only one type (E.coli), and no heterogeneity in the initial patch or the streaklines which would indicate a mixture of bacteria is present.

COMPETENT CELL SOLUTIONS:

RF1 solution				**RF2 solution**		
Chemical	**Amount**	**Final conc.**		**Chemical**	**Amount**	**Final Conc.**
ROW*	200 ml**	na		ROW*	100 ml**	na
RbCl	2.4 g	100mM		MOPS	0.2 g	10mM
$MnCl_2$	2.0 g	50mM		RbCl	0.1 g	10mM
K Acetate	0.6 g	30mM		$CaCl_2$	1.0 g	75mM
$CaCl_2$	0.3 g	10mM		Glycerol	15 g	15% (w/v)
Glycerol	30 g	15% (w/v)				

* ROW = reverse osmosis water

** Add all the ingredients to 100 ml ROW, then make up to final volume in a measuring cylinder

For RF1: adjust pH to 5.8 with conc. acetic acid. pH will change very quickly and only requires ~ 10µL of acetic acid. Sterilize by filtration into an autoclaved media bottle.

For RF2: adjust pH to 6.8 with NaOH or HCl, as appropriate. Sterilize by filtration into an autoclaved media bottle.