Preparation of ultra-competent *E. coli* cells for transformation

Making Competent Cells according to D. Hanahan et al. "DNA Cloning Techniques".
This technique is a little bit more involved than the $CaCl_2$ method but I have found that it yeilds cells that store well and are at higher competency. If you are looking for a very simple method, use the $CaCl_2$ method as described in Maniatis or in Current Protocols.

Things to do ahead of time (recipes for some solutions are provided at the end).

1. Prepare 200 ml of sterile SOB media in a 1 liter flask. The instructions for preparing this are provided on page A.3 of the third volume of the Maniatis method book or a slightly different recipe is provided at the end. The sterile 2M $MgCl_2$ solution should be made by diluting the concentrated lab stock with water. $MgCl_2$ readily absorbs moisture from the atmosphere so we generally dissolve the entire bottle of salt at a concentration of 4 M. Also autoclave a test tube for use in growing a 3 ml starter culture.
2. Prepare sterile RF1 solution. You will need 67 ml of this. RF1 consists of 100 mM KCl, 50 mM $MnCl_2$, 30 mM Potassium acetate, 10 mM $CaCl_2$, 15% glycerol. (Note that you are to use Manganese Chloride and not Magnesium Chloride in RF1.) The solution is adjusted to pH 5.8 with 10 % glacial acetic acid (do not use the concentrated acid stock as it is very easy to overshoot the pH adjustment). Filter sterilize this solution through a pre-rinsed 0.22 micron filter and store at 4^oC. Prerinsing is done by passing water through the filter (discard this wash). It removes wetting agents often incorporated into the filter that might inhibit formation of competent cells.
3. Prepare sterile RF2 solution. You will need 16 ml of this. RF2 consists of 10 mM MOPS, 10 mM KCl, 75 mM $CaCl_2$, 15% glycerol. The solution is adjusted to pH 6.8 with 100 mM NaOH and filter sterilized through a pre-rinsed 0.22 micron filter. Store at 4^oC.
4. Thoroughly clean six 40 ml tubes. Scrub with soapy water and then rinse with copious amounts of water. Finally, rinse and sterilize the tubes with ethanol (the 95% stuff in a squirt bottle will do). It is important that the tubes be free of soap since this reduces the competency of the cells. It is also important that the tubes be free of contaminating DNA as this can lead to transformation of the cells. Drain off the excess ethanol, air dry, cover the tops with foil and set them aside until needed.

Preparing competent cells.

1. Transfer 3 ml of sterile SOB from the 1 liter flask to a sterile test tube and inoculate with the desired bacteria. Grow up an overnight culture by shaking at 37^oC. Also transfer 1 ml of SOB to a test tube. This will be used for the blank on the spectrophotometer when you a tracking the growth of the culture.
2. The next day, use 1 ml of the overnight to inoculate the SOB that remains in the 1 liter flask. Incubate at 37^oC with shaking. After 2 hours, start checking the OD550. When the OD550 is between 0.35 and 0.6 for a recA- strain (or between 0.2 and 0.4 for a recA+ strain), chill the cells on ice. Note, for recA- strains, the OD range corresponds to 5-9 x 10^7 viable cells/ml.
3. Transfer the cells to chilled 40 ml tubes and collect cells by centrifuging at 3000 rpm for 15 minutes at 4^oC in an SS34 rotor.
4. Pour off the supernatant and remove residual supernatant with a pipette.
5. Resuspend the cells in a total volume of 67 ml of RF1. Use gentle vortexing to resuspend the cells. Combine the suspensions of cells into two tubes and incubate the cells on ice. If the cells are DH5alpha, incubate for 15 minutes. If the cells are HB101, incubate for 2 hours. Other cells should probably incubated for 30 minutes although the optimizing the time would require a systematic analysis.
6. Collect the cells by centrifuging at 3000 rpm for 15 minutes at 4^oC in an SS34 rotor. Remove as much supernatant as possible and resuspend the cells in 16 ml of RF2. Incubate the cells on ice for 15 minutes and then dispense in 400 ul aliquots. Flash freeze in liquid nitrogen or a dry ice/ethanol bath. Store cells at -75^oC.

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Transforming cells.

1. Add DNA to 100 ul of competent cells.
2. Incubate on ice for 30 minutes.
3. Heat shock for 2 minutes at 42°C.
4. Add 900 ul of LB and incubate for 1 hour at 37°C.
5. Briefly spin down the cells and remove all but 100 ul of media.
6. Resuspend the cells and plate on media containing the appropriate antibiotic.

Recipes.

RF1		**RF2**	
Component (final concentration)	Amount for 150 ml	Component (final concentration)	Amount for 50 ml
100 mM KCl (74.55 mw)	1.12 g	10 mM MOPS pH 6.8 (209.3 mw)	0.105 g
50 mM $MnCl_2$-$4H_2O$ (197.91 mw)	1.48 g	10 mM KCl (74.55 mw)	0.037 g
30 mM potassium acetate (98.14 mw)	0.44 g	75 mM $CaCl_2$-$2H_2O$ (147.02 mw)	0.55 g
10 mM $CaCl_2$-$2H_2O$ (147.02 mw)	0.22 g	15 % glycerol	7.5 ml
15% glycerol	22.5 ml	Adjust to pH 6.8 with 1 M NaOH and filter sterilize as with RF1.	
Adjust to pH 5.8 with 10% glacial acetic acid, and filter sterilize through a pre-rinsed 0.22 micron membrane.			

SOB media	Final concentration	Amount per 100 mls
Bactotryptone	2%	2 g
Yeast Extract	0.5%	0.5 g
NaCl	10 mM	1 ml of 1M stock
KCl	2.5 mM	250 ul of 1 M stock
Adjust pH to 7.0 and autoclave.		
Then add:		
$MgCl_2$	10 mM	1 ml of 2M sterile stock
$MgSO_4$	10 mM	1 ml of 2M sterile stock

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