A. Preparation of competent Cells

**Note: All glassware was rinsed with pure water. Sterile filtration units used in preparing solution were pre-rinsed with pure water.

1. Pick 12x 2-3 mm diameter colonies off a freshly streaked SOB agar plate and disperse in 1 ml SOB medium by vortexing. (Use one colony per 10 ml of culture medium). The cells are best streaked from a frozen stock or a fresh stab about 16-20 h prior to initiating liquid growth.
2. Inoculate the cells into a 2-liter Erlenmeyer flask containing 120 ml of SOC medium.
3. Incubate at 37°C shaker with 250 r.p.m. until the cell density is 4-7 $X10^7$ (OD_{550} of 0.35-0.60) viable cells/ml.
4. Collect the culture into 4x 30 ml in 50ml Falcon (2070) polypropylene centrifuge tubes and chill on ice for 10-15 min.
5. Pellet the cells by centrifugation at 750-1000xg for 12-15 min at 4°C. Drain the cell pellet thoroughly by inverting the tubes on paper towels, and rapping to remove any liquid. A micropipette can be used to draw off recalcitrant drops.
6. Resuspend the cell pellet by moderate vortexing in 10 ml of RFI (1/3 of the culture) and pool into one 50 ml tube. Incubate the cells on ice for 15 min (for DH1 and JM101) to 2 h (for HB101).
7. Pellet cells as in step 5.
8. Resuspend the cells in 9.6 ml of RF2 (1/12.5 of the original volume). Incubate the cells on ice for 15 min.
9. Distribute 200 μl of aliquot into chilled 1.5 ml microcentrifuge tubes (48x tubes).
10. Freeze aliquots in a dry ice/alcohol bath or in liquid nitrogen, then place at –70°C.

B. Use of Frozen Competent cells

1. Remove tubes from the freezer and thaw in air at room temperature until the cell suspension is just liquid. Place the tubes on ice.
2. Add the DNA solution in a volume of <20 μl. Swirl the tube to mix the DNA evenly with the cells.
3. Incubate the tubes on ice for 10-60 min.
4. Heat shock the cells by placing the tubes in a 42°C water bath for 90 sec, and then chill by returning tubes immediately to 0°C (crushed ice).
5. Add 80 μl of SOC medium and incubate at 37°C with moderate agitation for 30-60 min.

C. Solutions

RF1

Compound	Amount/liter	Final concentration
RbCl	12 g	100 mM
$MnCl_2$ $4H_2O$	9.9 g	50 mM
Potassium acetate	30 ml of 1 M (pH7.5)	30 mM
$CaCl_2.2H_2O$	1.5 g	10 mM
Glycerol	150 g	15% (w/V)

Adjust the pH to 5.8 with 0.2 M acetic acid. Sterilize by filtration through a pre-rinsed 0.22 u membrane.

RF2

Compound	Amount/liter	Final concentration
MOPS	20 ml of 0.5 M (pH6.8)	10 mM
RbCl	1.2 g	10 mM
$CaCl_2.2H_2O$	11 g	75 mM
Glycerol	150 g	15% (w/v)

Adjust pH to final pH6.8 with NaOH (as necessary) and sterilize by filtration through pre-rinsed 0.22 um memebrane.

SOB medium (1 liter)

Compound	Amount/liter	Final concentration
Trypton	20 g	2%
Yeast Extract	5 g	0.5%
NaCl	0.584 g	10 mM

Add 10 ml of a 250 mM solution of KCl (1.86 g of KCl in 100 ml of H_2O).
Adjust the pH of the medium to 7.0 with 5 N NaOH (about 0.2 ml).
Adjust voume to 1 liter and autoclave.

Just before use, add 5 ml of 2 M $MgCl_2$ (19 g of $MgCl_2$ to 100 ml H_2O, and autoclave)

SOC medium (SOB + 20 mM glucose)
Add 20 ml of sterile1M glucose (18 g of glucose in 100 ml solution, and filter) to 1 liter SOB.