

# Hanahan Competent Cell Protocol

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**\*\*USE STERILE TECHNIQUE AT ALL TIMES**

**\*\*AFTER STEP 6, WORK ON ICE IN THE COLD ROOM TO INCREASE THE QUALITY OF THE FINAL CELL PREPARATION.**

**\*\*PROTOCOL PRODUCES ABOUT 160 X 250ul ALIQUOTS OF COMPETENT CELLS. PLACE SUFFICIENT 1.7ml MICROCENTRIFUGE TUBES AT -80 DEGREES C. AT THE BEGINNING OF THE DAY FOR USE IN STEP #15.**

- 1) Streak out frozen stock of XL1-Blue MRF' bacteria on an LB-tet plate. Grow overnight at 37 degrees C.
- 2) Inoculate a single colony into a 250 ml flask containing:

20 ml of SOC

20ul of 12.5 mg/ml tetracycline

- 3) Grow overnight in a shaker at 37 degrees C.
- 4) The next day set up two 2 liter flasks containing 250 ml 2XYT. Into each flask, pipet 2.5 ml of overnight culture.
- 5) Grow with shaking until culture reaches an OD600 of ~0.5.
- 6) Set up two centrifuge bottles on wet ice and transfer culture to bottles by pouring.
- 7) Pellet bacteria by spinning in Sorvall centrifuge in GSA rotor; 5000 r.p.m., 4 degrees C., 10 minutes.
- 8) Working in the cold room, with cells on ice as much as possible: Pour out supernatant, then use pipet to remove all residual fluid.
- 9) Resuspend each pellet gently in 83 ml RF1. Use 10 ml pipet to gently pipet up and down until all clumps have been resuspended.
- 10) Incubate on wet ice in cold room for 1 hour.
- 11) Pellet bacteria by spinning in Sorvall centrifuge in GSA rotor; 5000 r.p.m., 4 degrees C., 10 minutes.
- 12) Working in the cold room, with cells on ice as much as possible: Pour out supernatant, then use pipet to remove all residual fluid.
- 13) Resuspend each pellet gently in 20 ml of RF2. Use 10 ml pipet to gently pipet up and down until all clumps have been resuspended.
- 14) Incubate on wet ice in cold room for 15 minutes.
- 15) Take microcentrifuge tubes out of -80 degree C freezer and at least 160 tubes with open lids in an ice bucket containing wet ice. Dispense 100 ul of cell suspension into each tube. If possible, work with a second person, one person dispensing the cells, and the second person sealing tubes as they are filled. Once tubes are sealed, drop them in a Dewar flask containing liquid nitrogen. Once all 40 ml of competent cells are dispensed into microcentrifuge tubes, freeze all remaining tubes in liquid nitrogen. Collect tubes and store in a paper freezer box with no dividers.
- 16) Measure the efficiency of the competent cells by transforming a standard amount of commercial plasmid. Express efficiency as colony forming units / microgram of DNA [cfu/ug]. If you transform 0.1 ng of plasmid DNA and obtain 500 colonies, the efficiency of the cells is  $5 \times 10^6$  cfu/ug.

## SOLUTIONS

### RF1

		<u>FW</u>	<u>PER LITER</u>
100mM	Rubidium Chloride	120.9	12.1 g
50mM	Manganese Chloride	197.9	9.895 g
30mM	Potassium Acetate	98.14	2.944 g
10mM	Calcium Chloride	147.0	1.47 g
15% w/v	Glycerol		150 g

Adjust pH to 5.8 and bring volume to 1 liter.  
Sterilize by filtration.

### RF2

		<u>FW</u>	<u>PER 500ML</u>
10mM	MOPS	209.3	1.05 g
10mM	Rubidium Chloride	120.9	0.6 g
75mM	Calcium Chloride	147.0	5.51 g
15% w/v	Glycerol		75 g

Bring volume to 500 ml.  
Sterilize by filtration.