**Rubidium Chloride Competent Cells (Competence and Transformation)**

**Materials**

Reagents

- Filter sterilized 50 mL chilled RF1 (33mL would be used):
    - 0.605g 100 mM RbCl
    - 0.495g 50 mM MnCl2•4H2O
    - 0.147g 30 mM Potassium acetate
    - 0.074g 10 mM CaCl2•2H2O
    - 7.5g or 6 mL 15% m/v glycerol
- Filter sterilized 50 mL chilled RF2 (4 mL would be used)
    - 0.105g 10 mM MOPS
    - 0.06g 10 mM RbCl
    - 0.55g 75 mM CaCl2•2H2O
    - 7.5 g or 6 mL 15% m/v glycerol
- Sterilized 100 mL LB
- MilliQ water
- 0.2M Acetic acid

Equipment

- Sterilized 250 mL centrifuge bottles
- Sterilized 1.5 mL microfuge tubes (at least 50)
- 250mL flask
- Syringe filters

**Procedure**

How to use Syringe Filters

Always work in a sterile environment (near a Bunsen burner).

1. Screw filter onto end of syringe
2. Pull out stopper (plunger) and fill with reagent (RF1 or RF2)
3. Place plunger back in and slowly push down to expel liquid into a new labelled sterile container
4. Single use only

Making RF1 Solution

This protocol makes 50 mL RF1 solution.

1. Mix 0.605g 100 mM RbCl, 0.495g 50mM MnCl2•4H2O, 0.147g 30mM Potassium acetate, 0.074g 10 mM CaCl2•2H2O, and 7.5g or 6mL 15% m/v glycerol.
2. Add sterile MilliQ up to ~45 mL.
3. Adjust final pH to 5.8 using 0.2M acetic acid (~400 µL for 33 mL).
4. Add additional sterile MilliQ to reach final volume of 50mL.
5. Filter sterilize.

Note: use glacial acetic acid [1.049g/cm3 / 60.05g/mol = 17.47M] to make 0.2M acetic acid.

Making RF2 Solution

6. Mix 0.105g 10 mM MOPS, 0.06g 10mM RbCl, 0.55g 75mM CaCl2•2H2O, and 7.5 g or 6 mL 15% m/v glycerol.
7. Add sterile MilliQ up to ~45mL.
8. Adjust final pH to 6.8 using 1M NaOH (~200µL for 30mL).
9. Add additional sterile MilliQ to reach final volume of 50mL.
10. Filter sterilize.

Day 1

1. Streak DH5α from frozen glycerol stock on the LB plate.
2. Incubate at 37 °C overnight.
3. Prepare sterilized LB.

Day 2

1. Pick up a single colony from the LB plate.
2. Inoculate to 3 mL sterilized LB.
3. Incubate at 37°C overnight.
4. Put RF1, RF2, centrifuge tube, and Eppendorf tubes into the 4°C refrigerator.

Day 3

1. Put RF1, RF2, centrifuge tube, and Eppendorf tubes on ice.
2. Inoculate 1 mL of overnight culture to 99 mL of LB in 250mL flask.
3. Monitor OD600 from initial until 0.2-0.6. (0.4-0.55 optimum).
4. Transfer culture to centrifuge bottle and chill on ice 10-15 min.
5. Pellet cells by centrifugation at 2700 g (4200 rpm in an F14 6x250y rotor) for 10 min at 4°C.
    a. Pre-chill centrifuge (0 rpm, 4°C)
6. Decant liquid and stand the bottle in an inverted position for < 1 min.
7. Resuspend in 1/3 original volume (33 mL) chilled RF1 buffer gently (do not vortex).
    a. Into 2 tubes, 16.5 ml in each
8. Optimally, resuspend using a 25mL disposable pipette (RbCl will permanently stain glass pipettes).
9. Continue mixing until cells are evenly resuspended and no clumps are visible.
10. Incubate on ice for 15 min.
11. Pellet cells by centrifugation at 580g (1950 rpm in an F14 6x250y rotor) for 15 min at 4°C.
12. Decant liquid and gently resuspend in 1/25 original volume (4 mL) chilled RF2 buffer.
13. Incubate on ice for 15 min.
14. Aliquot 100µL into each chilled 1.5mL Eppendorf tube and freeze on dry ice (or ice).
15. Store at -80 °C.

**Acknowledgements**

Protocol adapted from: http://openwetware.org/wiki/RbCl_competent_cell