Top 10 competent cells

Preparation of Competent Cells: Modified RbCl Method
This rubidium chloride protocol gives better transformation efficiencies than the $CaCl_2$ procedure for most strains. The procedure is an adaptation of one described in the QIAexpressionist.

Materials to Be Supplied by the User
- LB medium and plates with appropriate antibiotics
- TFB1, ice-cold
- TFB2, ice-cold
- dry ice/isopropanol bath

TFB1
30mM potassium acetate
10mM $CaCl_2$
50mM $MnCl_2$
100mM RbCl
15% glycerol
Adjust pH to 5.8 with 1M acetic acid. Filter-sterilize (0.45μM) and store at room temperature.

TFB2
10-mM MOPS or PIPES (pH 6.5)
75mM $CaCl_2$
10mM RbCl
15% glycerol
Adjust the pH to 6.5 with 1M KOH. Filter-sterilize (0.45μM) and store at room temperature.

1. Inoculate a single colony from an LB plate into 2.5ml of LB medium + antibiotics. Incubate overnight at 37°C with shaking (approximately 225rpm).

2. Subculture the overnight culture 1:100 by inoculating 1ml into 100ml of pre-warmed LB + antibiotics. Grow the cells in a 250ml flask until the OD_{600} reaches 0.4 - 0.6 (usually 5 - 6 hours, but the time may vary).

3. Cool the culture on ice for 5 min, and transfer the culture to a sterile, round-bottom centrifuge tube.

3. Pellet the cells by centrifugation at low speed (4,000 x *g* for 5 minutes at 4°C) and discard the supernatant. Always keep the cells on ice.

4. Gently resuspend the cell pellet in ice-cold TFB1 (30 mL for a 100 ml culture). Combine the resuspended cells in one bottle. For the remaining steps, keep the cells on ice and chill all pipettes, tubes and flasks.

5. Incubate the resuspended cells on ice for 90 minutes at 4°C.

6. Pellet the cells by centrifugation at 4,000 x *g* for 5 minutes at 4°C. Discard the supernatant carefully, and always keep the cells on ice.

7. Gently resuspend the cells in 4ml of ice-cold TFB2.

8. Incubate the cells on ice for 15 - 60 minutes, then aliquot 100μl/tube for storage at -80°C. Quick-freeze the tubes in a dry ice/isopropanol bath or liquid nitrogen.