Preparation of RbCl Super Competent Cells

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[Abstract] This method is used to inexpensively prepare home-made competent cells of *E. coli.* The transformation efficiency is at the high end of chemical-efficient competent cells, and close to library-efficient competent cells.

Materials and Reagents

1. 3 mM hexamine cobalt chloride
2. Tryptone
3. Yeast extract
4. NaCl
5. DMSO
6. KCl
7. $MgCl_2$
8. $MgSO_4$
9. KOH
10. $CaCl_2 \cdot 2H_2O$
11. RbCl
12. SOB or 2x YT (see Recipes)
13. MES (see Recipes)
14. TFB (50 ml) enough for 80 tubes/400 transformations. DMSO (see Recipes)

Equipment

1. Microcentrifuge

Procedure

1. Inoculate overnight culture at room temperature (RT) in 5 ml SOB or 2x YT.
2. Add overnight to 500 ml of SOB or 2x YT in 4 L flask to maximize aeration.
3. Add 36 ml of 5 M NaCl. Shake well at 30 °C.

4. Grow to OD_{600} of 0.5. About 3 h.
5. Spin cells. Drain pellet very well. Suck out extra liquid.
6. Gently resuspend in 50 ml of cold TFB. Incubate for 15 min on ice.
7. Prepare a dry ice/ethanol bath (should be thick in consistency) or liquid nitrogen.
8. While swirling cells add 1.75 ml DMSO (no need to sterilize DMSO).
9. Incubate cells for 10 min on ice and chill eppendorfs on ice.
10. Add 0.5 ml cells to each Eppendorf and quickly drop tubes into dry ice bath. Store cells at -70 °C for up to 12 months.
11. Use 100 µl for transformation. Heat shock at 42 °C for 1.5 to 2 min.

Recipes

1. SOB or 2x YT (1 L)
   Tryptone 20 g
   Yeast extract 5 g
   5 M NaCl 2 ml
   pH to 7.0 and bring up to 1 L
   Autoclave and add 2.5 ml 1 M KCl, 10 ml of 1 M $MgCl_2$, 10 ml 1 M $MgSO_4$
2. MES
   1 M MES solution made to pH 6.2 with KOH
   Filter sterilized and stored frozen
3. TFB (50 ml) enough for 80 tubes/400 transformations
   0.5 ml of 1 M MES
   0.6 g of RbCl (makes 100 mM final conc.)
   0.45 g $MnCl_2$ (45 mM)
   0.077 g $CaCl_2 \cdot 2H_2O$ (10 mM)
   0.04 g hexamine cobalt chloride (3 mM)
   Store at 4 °C
   Keeps for several months
   Use it cold

References

1. http://sinclairfs.med.harvard.edu/methods/supercompetentecoli.php

