# Ultra-competent cells

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## Materials

1. TB solution: dissolve 0.3024g of PIPES, 0.2205g of calcium chloride dehydrate, 1.8638g of potassium chloride in about 90ml $H_2O$ and then using potassium hydroxide solution to adjust pH to 6.7 . Add $H_2O$ to make final volume of 100ml. Dissolve 1.0885g of manganese chloride tetrahydrate , inverting mix thoroughly. The solution should now turn to tawny; it's ok that there's very little undissolved solid at the bottom. Pass the solution through 0.45 filter into a sterilized bottle (the solution should now be transparent again)and store at 4℃.
2. SOB medium: weigh 1g of yeast extract , 4g of tryptone , 0.117g of chloride sodium , 0.037g of potassium chloride , 0.4g of magnesium chloride , 0.492g of magnesium sulfate heptahydrate to 200ml $H_2O$ in a 1000ml flask conical flask , sterilize and store at 4℃.
3. LB dish and medium
4. DMSO
5. Liquid nitrogen

## Methods

### Day one:

(It's better to start this step at 20:00) Pick up DH5α monoclone to 1ml LB medium , 37℃ , 250rpm shaker, overnight.(9-12 hrs)

## Day two:

1. Nanodrop: 600nm OD between 0.09-0.12.
2. 8000 x g spin down, discard 900ul supernatant and resuspend in remaining liquid. Pipet up and transfer resuspending liquid to 200ml SOB medium(in 1000ml conical flask). 19℃ 250 rpm shaker, overnight. (26-30hrs)

## Day three:

Carfully make sure the 600nm OD between 0.05-0.06.

**Begin with this step , all manipulation should be executed at 4℃**

1. Then transfer the medium into 4 x 50ml conical tube, let it still on ice for 10min.
2. Centrifuge at 2500 x g , 10min, 4 ℃
3. Discard supernatant, wash the pellet in 10ml TB solution. Let it still on ice for 10 min.
4. Centrifuge at 2500 x g , 10min, 4 ℃
5. Discard supernatant completely, resuspend in 2.5ml TB solution. Adding 188ul DMSO(to make a final concentration 7%). Pipet mix thoroughly and let it sill for 10min.
6. Aliquot to appropriate volume in 1.5ml EP tube (usually 20ul for plasmid transformation and 50 ul for reaction products). Carefully throw the tubes in liquid nitrogen to fast-freezing.
7. Scoop up the tubes from liquid nitrogen carefully with strainer, place on ice and pack in box. Store at -80℃.