SOP: Competent cell preparation

By

Richard Yu

Research fellow

Centre for Marine Environmental Research and Innovative Technology (MERIT)
Department of Biology and Chemistry
City University of Hong Kong
(Email: bhrichyu@cityu.edu.hk)

Day 1

Procedure:

1. Streak out the DH5α frozen stock (stored in –80°C freezer) onto a LB plate (without antibiotics). Incubate overnight (16–20 hours) at 37°C.
2. Autoclave 200 ml (in 250-ml blue-cap bottle) and 500 ml (in a 1-L Erlenmeyer flask) ψ broth for Day 2 experiment.

Day 2

Procedure:

1. Transfer 25 ml autoclaved ψ broth (from the 250-ml blue-cap bottle) to a sterile 100-ml Erlenmeyer flask and inoculate with a single DH5α colony on the plate. Grow overnight (no more than 16 hours) in a 37°C shaker (250 rpm).
2. For Day 3 experiment, place the 1-L flask containing 500 ml autoclaved ψ broth in a 37°C incubator.

Day 3

Things to prepare:

1. Switch on the UV-VIS spectrophotometer (Shimadzu UV-1206) and warm it up for at least 30 min.
2. Switch on the Beckman Avanti J-25I centrifuge and maintain it at 4°C.
3. Chill two 500-ml centrifuge bottles on ice.
4. Pre-cool 200 pcs autoclaved 1.5-ml eppendorf tubes.
5. Get 1–2 liters of liquid nitrogen.

Procedure:

1. Take out 1 ml ψ broth from the 1-L flask and save it (the blank for OD measurement).
2. Transfer the 25 ml overnight culture to the 1-L flask. Swirl the flask to mix and measure the initial OD_{550} of the culture.
3. Incubate the culture in a 37°C shaker (250 rpm).
4. Assume that the bacteria will double every 20 min, estimate the shortest time required for the culture to reach OD_{550} = 0.44 (it takes approx. 45 min to 1 h). Thereafter, measure OD_{550} every 10–20 min.
5. When the culture OD_{550} reaches 0.44, transfer the flask on an ice-bath (Note: It is important to keep the cells at 4°C (or on ice) for the remainder of the procedure).
6. Split the culture into two equal parts by pouring ~250 ml of the culture into each chilled 500-ml centrifuge bottle. Measure and balance the weights of the bottles using a digital balance.
7. Pellet the cells at 5,000 rpm at 4°C for 10 min using the Beckman Avanti J-25I centrifuge.
8. Decant the supernatant (into the emptied 1-L Erlenmeyer flask) and resuspend thoroughly the cell pellet (on ice) in 2 ml chilled TfbI using a 1000-µl filter tip. Add additional 98 ml TfbI to each bottle and mix by gentle swirling.
9. Hold the content on ice for 5 min.
10. Pour the contents together. Centrifuge the cells at 4,000 rpm at 4°C for 10 min.
11. Decant the supernatant and resuspend thoroughly the cells in 4 ml chilled TfbII using a 1000-µl filter tip. Add additional 16 ml TfbII to the cells and mix by gentle swirling.
12. Hold the content on ice for 15 min.
13. Aliquot 100 µl (use a 1000-µl tip) into each pre-cooled 1.5-ml eppendorf tube.
14. Quickly freeze the cells in nitrogen liquid prior to storage in a –80°C.

Determination of Transformation Efficiency of Competent Cells

Things to prepare:

1. Set a water bath at 42°C.
2. Take out 4 LB agar/amp plates from 4°C refrigerator and prewarm in a 37°C air-incubator.

Procedure:

1. Serially dilute a plasmid [e.g. pGEM 3Z(f+)] stock to 20 pg/µl and 2 pg/µl. Below is an example for such a serial dilution:

   pGEM 3Z(f+) stock = 0.2 g/L = 0.2 µg/µl = 200 ng/µl = 200 000 pg/µl

   Dilution A:
   1 µl pGEM 3Z(f+) stock (use a P2.5 autopipette and a P10 tip to dispense)
   + 99 µl water → 2000 pg/µl

Dilution B:
10 µl Dilution A + 90 µl water → 200 pg/µl
Dilution C:
10 µl Dilution B + 90 µl water → **20 pg/µl**
Dilution D:
10 µl Dilution C + 90 µl water → **2 pg/µl**

2. Remove 4 tubes of frozen competent cells from –70°C freezer and place them on ice until just thawed (about 5 mins). Label them as **100 pg (Replicate 1)**, **100 pg (Replicate 2)**, **10 pg (Replicate 1)** and **10 pg (Replicate 2)**.
3. Add 5 µl **Dilution C** to the 100 pg (Replicate 1) and 100 pg (Replicate 2) tubes and 5 µl **Dilution D** to the 10 pg (Replicate 1) and 10 pg (Replicate 2).
4. Gently flick the tube, or use a pipette tip to stir, to mix and place the tubes on ice for 20 minutes. AVOID PIPETTING UP AND DOWN TO MIX, AS THE CELLS ARE VERY VERY FRAGILE.
5. Heat shock the cells for 50 seconds in a water bath at exactly 42°C.
6. Immediately return the tubes to ice for 2 minutes.
7. Add 1000 µl of room temperature SOC or LB medium to the tubes.
8. Incubate for 1.5 hours in a bath water at 37°C with or without shaking (ca. 150 rpm).
9. Centrifuge the cells at 3000 rpm for 10 mins using a microcentrifuge. Decant the supernatant and resuspend the cells in about 50 µl LB broth.
10. Plate them onto LB agar/amp plates using a hockey stick.
11. Incubate the plates overnight (16 hours) at 37°C. Calculate the average transformation efficiency based on the colony counts on the plates. This should be at 1×10^6–1×10^7 cfu/µg plasmid DNA (i.e. 100–1000 cfu on the 100 pg plates and 10-100 cfu on the 10 pg plates)

Appendix

ψ broth:

	500 ml	200 ml	Final conc.
Tryptone	10g	4 g	2%
Yeast extract	2.6 g	1.04 g	0.5%
$MgSO_4$	2.4 g	0.96 g	20 mM
NaCl	0.3 g	0.12 g	10 mM
KCl	0.94	0.376 g	25 mM

Autoclave at 121°C for 15 min.

TfbI buffer (per 200 ml):

	Amount	Final conc.
KAc	**0.59 g**	**30 mM**
$CaCl_2$	**0.29 g**	**10 mM**

(1) Stir to dissolve and adjust pH to 5.8 with acetic acid (only a few drops).

$MnCl_2$	**1.98 g**	**50 mM**
$RbCl_2$	**2.42 g**	**100 mM**

(2) Stir to dissolve.

Glycerol	**30 ml**	**15%**

(3) Make the final volume to 200 ml with deionized H_2O.
(4) Filter sterile the buffer using a 50-ml syringe attached to a 0.45-µm syringe filter.
(5) Collect the filtrate with a sterile 250-ml CC bottle
(6) Store the buffer at 4°C.

TfbII buffer (per 200 ml):

	Amount	Final conc.
MOPS	**0.462 g**	**10 mM**
$CaCl_2$	**2.36 g**	**75 mM**
$RbCl_2$	**0.24 g**	**10 mM**

(1) Stir to dissolve and adjust pH to 6.5 with KOH (only a few drops).

Glycerol	**30 ml**	**15%**

(3) Make the final volume to 200 ml with deionized H_2O.
(4) Filter sterile the buffer using a 50-ml syringe attached to a 0.45-µm syringe filter.
(5) Collect the filtrate with a sterile 250-ml CC bottle
(6) Store the buffer at 4°C.