Competent Cell Protocol (2021 English Version)

Before starting to make the competent cells, remember to send an email to inform lab's members in advance that you need to use shaker for long time (DAY2~DAY4).

DAY 1

• E.coli plating (17:00)

↓Plant E.coli on the LB plate without antibiotics (stored at the refrigerator behind clean bench).x2

*Keep clean!!! Use disposable bacteria spreader under the clean bench. Slowly push the spreader out of the bag. Be careful not to touch the top of the spreader.

↓Incubate at 37℃, overnight.

DAY 2

• Check the size of colonies (10:00)

If it grows well, wrap the plate by parafilm and preserve it at 4℃.

• Make culture medium (13:00~)

↓Add following solutions(use disposable pipettes) into 2L baffled cell culture flask:

SOB medium 420ml (cold room)

2M Mg+ 4.2ml (cold room)

↓Add 2ml of medium into each 15ml tube. x4 (3 for pre-culture, 1 for blank)

• Pre-culture

↓Use P200 tip to pick colony, then insert the top of tip into 15ml tube containing 2ml of medium, pipetting. x3

↓Incubate at 37℃, 180~200rpm, 2~4 H.

*Pay attention to the turbidity, be careful not to be too cloudy.

*From next step, every operations must be performed on ice!!!

• Main culture (on ice) (15:00~)

↓If you can see the cell suspension of E.coli, select 1 best tube then use disposable pipette to move the E.coli into 2L flask.

(Compare the turbidity, choose the middle one. Preserve remain samples at 4℃, in cold room)

↓Incubate at 16℃, 60~90 rpm (start at 70rpm)

DAY 3

• Continue main culture

Detect absorbance OD600 at night (on ice) (17:00)

Add 2ml of medium into cuvette .(use disposable pipette stored at -80℃)

At this moment, OD600 would be around 0.03.

Adjust shaking speed(60~90 rpm) and temperature(16~20℃).

Tomorrow we need to collect the competent cells at around 0.3~0.5 OD600.

To Be Continued→

(Use Purex to wash cuvette, then rinse the cuvette by tap water and deionized water.
Use disposable pipette to add 2ml of blank into cuvette as control. Wrap this blank cuvette by parafilm to prevent evaporation. After absorbance detection, preserve blank cuvette at 4℃, so that it can be used next time.
When the detection finished, rinse the cuvettes by tap water and deionized water, then put it into 50ml tube containing diluted Purex.)

DAY 4

• Detect OD600 (on ice)

Detect competent cells' absorbance in the morning.
If OD600 is low, adjust shaking speed and temperature again. (we collect competent cells at 0.3~0.5 OD600)
If OD600 has already arrived 0.3~0.5, put the 2L flask on ice then preserve it at 4℃ in cold room.

• Collect competent cell (on ice) (It takes at least 2H, please arrange your schedule.)

↓Pre-cool the centrifuge (the one near professor's room).
↓Separate culture medium into 50ml tube. We can get 8 tubes in total.
Add remaining medium into last 2 tubes to make balance for centrifuge.
↓Centrifuge (3500rpm, 7min, 4℃)
↓Pour supernatant into 2L flask.
↓Collect 4 tubes of E.coli into 1 tube with 10ml of transformation buffer. (2 tubes in total)
(use disposable pipettes)
↓Add additional 23ml transformation buffer into each tube. (33ml/tube in total)
(use disposable pipette)
↓Centrifuge (3500rpm, 5min, 4℃)
↓Pour supernatant into 2L flask.
↓Collect 2 tubes' E.coli into 1 tube with 8ml of transformation buffer. (use disposable pipette)
↓Drop 602ul of DMSO slowly into the tube, while shaking the tube. (on ice)
*After 1 drop, shake the tube well, then add the next drop. Otherwise, competent cells will die!!!

At least 3 people are required to start next step.
↓Use cut tip to add 20 of competent cells into 1.5ml tubes.
↓Close the caps and put the tubes into liquid nitrogen immediately.
↓Collect 1.5ml tubes into paper box, then preserve it at -80℃.

*During centrifuge, prepare 3~5 boxes of ice, metal racks, 1.5ml tubes(stored at -80℃), stainless steel cage, competent cells' paper boxes, and styrofoam polystyrene box containing liquid nitrogen(from the tank with orange cap, which is used for storing bacteria).