Competent Cell generation and transformation

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$CaCl_2$ Competent Cell Protocol Using FM5/Alper Cells

Methods:

Growing E.Coli from Glycerol Stocks to be used as Competent Cells

1. Thaw Line Q E.coli aliquot at room temperature.
2. In a 50mL flask with 5mL LB liquid (Spectinomycin 50µg/mL), add the Line Q aliquot.
3. Put on shaker (150 rpm) at room temperature overnight.
4. In AM, dilute by mixing 100µL of the liquid culture with 1.9mL LB liquid (Spectinomycin 50µg/mL) in a 2mL microfuge tube. Mix thoroughly by pipetting up and down.
5. Add 50µL of the dilution to LB (Spectinomycin 50µg/mL) plates.
   Make up at least two plates in case one does not grow. You can store extra plates at 4°C.
6. Incubate plates at 37°C overnight.
7. In AM, pick a single colony and use pipette tip to transfer to a 5mL LB (Spectinomycin 50µg/mL) media in a 50mL flask. Make up at least two flasks from plate colonies in case one does not grow.
8. Put cultures on shaker (200 rpm) at 37°C overnight.

Creating Competent Cells

All following operations should be under sterile conditions. Cells should be maintained on ice.

1. Transfer 450µL from the preferred overnight culture into 30mL LB (Spectinomycin 50µg/mL) in a 250 mL flask. Allow cells to grow at 37°C (200 rpm) until OD600= 0.6 (~2-3 hours). Once culture reaches 0.400, check OD every 15 minutes. Dilute culture if reaches OD greater than 0.600.
2. Incubate on ice for 30 minutes.
3. Transfer cells to a 50mL sterile falcon tube and centrifuge cells at 1700g for 10 minutes at 4°C.
4. Pour off media and re-suspend cells in 30mL ice-cold 0.1M $CaCl_2$ and centrifuge at 1700g for 10 minutes at 4°C.
5. While waiting, label 1.5mL microfuge tubes to be used for aliquots and place on dry ice to ensure that cells will freeze immediately.
6. Pour off media from falcon tube and re-suspend cells in 1.5mL ice-cold 0.1M $CaCl_2$ (10% glycerol).
7. Aliquot 100µL with a wide mouthed pipette into sterile 1.5mL microfuge tubes (from dry ice).
8. Store at -80°C until use.

Transforming and selecting E.Coli with your plasmid

1. Thaw cells on ice, must be kept on ice cannot come to room temp!
2. Turn on Precision H_2O bath to reach 42°C, put LB or SOC in bath to warm up
3. In a 1.5 ml microfuge tube, mix 50 µl competent cells with ≈ 10 - 200ng DNA, treat gently don't vortex
4. Incubate on ice for 30 minutes
5. Heat shock by putting in floating rack in water bath at 42°C for 40 s
6. Immediately put back on ice for 2 minutes
7. Add 450 ml of room temperature SOC or LB
8. Incubate tubes for 1 hour at 37°C, 225 rpm on shaker (can be slower)
9. Put » 80 - 150 µl of E. coli on LB agar plates with antibiotic
   You can save the extra E. coli at 4°C overnight in case your plates don't work
10. Incubate the plates over night at 37°C

Transforming E. coli, CIBT protocol

1. Thaw aliquots on ice until liquid.
2. Add 1-2µL of circular plasmid or all of a ligation reaction of DNA. Gently mix them.
3. Incubate for 30 mins on ice.

4. Heat shock for 1.5 mins @ 42°C. Put back on ice.
5. Add 900 µL of LB to tubes. Incubate @ 37°C for 45-60 mins.
6. Plate 100-200 µL of the cells on plates with selection agent.

LB (Spectinomycin 50µg/mL) Plates, 200 mL
In 200 mL dH_2O
+5 g LB Broth (Difco, Luria-Bertani)
+3 g Agar (Fishger, BP 1423-500)
Autoclave for 25 minutes, swirl immediately, allow to cool to touch (~50°C)
+1 mL Spectinomycin
Pour plates (~35mL/plate), label, and store at 4°C

LB Liquid, 200 mL
In 200 mL dH_2O
+5 g LB Broth (Difco, Luria-Bertani)
Autoclave for 25 minutes.

10 mg/ml Antibiotic, 10 mL
In 10 mL autoclaved dH_2O
+ 0.1 g Antibiotic
Filter Sterilize using 0.2 – 0.45 µ syringe filter
Aliquot into sterile 1.5 mL microfuge tubes and store at -20°C

Antibiotic	Stock (mg/ml)	Working (µg/ml)	µl of stock in 5 ml	µl of stock in 20 ml	µl of stock in 40 ml	ml of stock in 200 ml	ml of stock in 500 ml	ml of stock in 1 L
Ampicillin	10	50	25	100	200	1	2.5	5
Chloramphenicol (methanol)	10	20	10	40	80	0.4	1	2
Kanamycin	10	25	12.5	50	100	0.5	1.25	2.5
Rifampicin* (methanol)	30	150	25	100	200	1	2.5	5
Spectinomycin	10	100	50	200	400	2	5	10
Streptomycin	10	30	15	60	120	0.6	1.5	3

Note: If using multiple antibiotics you can cut the concentration of each by half
* Rifampicin is light sensitive cover with aluminum foil

For $CaCl_2$ Competent Cell Protocol
Spectinomycin required for 50µg/mL concentration from 10mg/mL stock in differing amounts of LB

LB (mL)	Spec (µL) needed to make 50µg/mL from 10mg/mL stock
1.9	9.5
5	25
30	150
200	1000

150 mL $CaCl_2$ (0.1 M)
In 150 mL dH_2O
+1.67 g $CaCl_2$
Autoclave 25 minutes. Store at 4°C.

150 mL $CaCl_2$ (0.1 M) in 10% Glycerol
In 135 mL dH_2O
+15 mL glycerol
+1.67 g $CaCl_2$
Autoclave 25 minutes. Store at 4°C.