Transforming *E.*Coli, isolating plasmid and making glycerol stocks

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Sean Weise

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Materials:
DH5α Competent *E. coli* strain, -80°C
Precision H_2O bath at 42°C
Sterile 2 ml microfuge tubes
Plasmid (5 ng / µl)
LB plates with antibiotic

LB liquid

sterile cryoviles
sterile 80% glycerol
LN_2

QIAprep Spin Miniprep Kit (Quiagen, 27104)
P1 buffer from above kit, 4°C
1.5 ml microfuge tubes sterile, with top cut off
0.5 ml sterile microfuge tubes

Methods:
Transforming and selecting E.Coli with your plasmid
1. Thaw DH5α *E. coli* 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 in bath to warm up
3. In a 1.5 ml microfuge tube, mix 50 µl competent cells with 2 - 4 µl plasmid (10 ng DNA), treat gently don't vortex
4. Incubate on ice for 0.5 hr
5. Heat shock by putting in floating rack in water bath at 42°C for 20 s
6. Immediately put back on ice for 2 minutes
7. Add 450 µl of prewarmed 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

Growing up lots of E.coli with plasmid
1. Prepare a small 50 ml sterile glass flask with 5 ml of LB and antibiotic (same concentration as on plates)
2. Take 1 colony from plate with 200 µl pipette tip and place in flask
   Grow up 2 cultures from 2 separate colonies per plate, in case one doesn't work
3. Incubate overnight at 37°C, 200 rpm on shaker

Glycerol Stock
1. To make glycerol stock prepare a sterile cryovile by adding 800 µl of 80% sterile glycerol
2. To the cryovile add 1 ml of E.Coli liquid culture (try to catch culture in exponential phase of growth)
3. Snap freeze in LN_2

Mini Prep to isolate plasmid
If using Miniprep kit for first time (i.e. a new miniprep box)
1. Add the RNase A solution to buffer P1
2. Add lysis blue to buffer P1
3. Check box on buffer P1 that RNase A has been added, and label bottle top that lysis blue has been added
4. Place date on bottle (**Only good for 6 months**)
5. Add ethanol to buffer PE, will say on bottle how much ethanol to add
6. Label buffer PE that ethanol has been added

1. Place 2 ml of E.Coli liquid culture into a 2 ml microfuge Tube and centrifuge for 10 min

2. Discard supernatant in biohazard waste and resuspend pellet in 250 µl P1 buffer (in 4°C) break up pellet with pipette tip
   ** P1 buffer is good for 6 months so watch date on bottle **
3. Add 250 µl of P2 cell lysis buffer, mix by inverting until liquid is all blue
   ** Do not allow to remain in P2 buffer for more then 5 minutes **
4. Add 350 µl of buffer N3, mix by inverting until liquid is clear and SDS precipitate forms
5. Centrifuge at max speed for 10 minutes, white pellet will from this is genomic DNA and junk
6. Pipette supernatant onto spin column and discard tube with pellet in biohazard waste
7. Centrifuge for at max speed for 1 minute, plasmid will stick to membrane
   Don't need to wash is PB buffer after this step when using DH5α E. coli
   If needed add 0.5 ml PB buffer centrifuge 1 min discard flow through
   - This removes trace nuclease activity with using EnAt strains
8. Discard flow through in biohazard waste and add 750 µl of PE buffer
   ** Make sure ethanol has been added to the PE buffer **
9. Centrifuge for 1 minute and discard flow through
10. Centrifuge again for 1 minute to remove residual liquid and discard flow through
11. Transfer spin column to a new sterile 1.5 ml tube with top cut off
12. Add 30 – 50 µl EB or DNase RNase free H_2O to center of membrane, let stand for 1 minute
13. Centrifuge for one minute, this is your purified plasmid

Check plasmid quality, concentration on spectrophotometer
1. Use nano-drop spectrophotometer, choose nucleic acids
2. Blank with EB buffer
3. 230 nm = polyphenolics, 260 = DNA, 280 = protein
   260/280 > 1.8
   230/260 > 2

Reagents for Growing E.Coli:

LB Plates, 200 ml
In 200 ml dH_2O
+ 5 g LB Broth (Difco, Luria-Bertani)
+ 3 g Agar (Fishger, BP 1423-500)
Autoclave for 20 min, swirl immediately, allow to cool to touch
Pour plates (≈ 35 ml) label, store at 4°C

LB Liquid, 200 ml
In 200 ml dH_2O
+ 5 g LB Broth (Difco, Luria-Bertani)
Autoclave for 20 min

80% Glycerol, 80 ml
In 16 ml dH_2O
+ 64 ml Glycerol
Autoclave for 20 min

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

10 mg/ml Antibiotic, 10 ml
In 10 ml of sterile dH_2O
+ 0.1g Antibiotic
Filter sterilize using 0.2 - 0.45 μm syringe filter
Aliquot into sterile 1.5 ml microfuge tubes and store at -20°C

* light sensitive: store stock solutions and plates in the dark