DNA transformation and cell culture

General solutions

20% (wt/vol) Glucose solution
for 500 ml solution add
100 g D-Glucose

fill to 500 ml with DI H_2O
mix thoroughly and filter sterilize

50% glycerol (vol %)
For 500 mL solution add
250 mL DI H_2O
250 mL Glycerol (Enzyme Grade)
mix thoroughly and filter sterilize

Antibiotic stock solutions

50 mg/mL Ampicillin stock
for 10 ml solution add
0.5 g Ampicillin salt
9.5 mL H_2O
Syringe filter to sterilize
Store at -20°C

34 mg/mL Chloramphenicol stock
for 10 mL solution add
0.34g Chloramphenicol
Fill to 10 mL with 100% ethanol
Syringe filter to sterilize
Store at -20°C

10 mg/ml Kan Stock
For 10 ml solution add
100 mg Kan
Fill to 10 ml with H_2O
Filter sterilize and store at -20°C

Cell Culture media

LB (Luria-Bertani) media
for 1L solution add

10 g tryptone	1% wt/v
5 g yeast extract	0.5% wt/v
10 g NaCl	1% wt/v

Fill to 1 L with DI H_2O
Autoclave at 40 min sterilization - wet setting
For test tubes add 11 ml LB each
For midipreps add 100 ml in 500 ml flask

SB (Super-Broth) media
for 1L solution add

32 g tryptone	3.2 % wt/v
20 g yeast extract	2.0 % wt/v
5 g NaCl	0.5 % wt/v

Fill to 1 L with DI H_2O
pH to 7.5
Autoclave 40 min sterilization, wet cycle

LB-Agar Plate media

for 1L solution add	Final Conc.
10 g tryptone	1% wt/v
5 g yeast extract	0.5 % wt/v
10 g NaCl	1% wt/v
15 g Agar	15% wt/v

Fill to 1 L with DI H_2O or 900 mL if glucose will be added
Autoclave 40 min sterilization, wet cycle
Antibiotics next column

Add (optional)	Final Conc.
100 ml 20% Glucose	2% wt/vol
Antibiotic	
Ampicillin	100 µg/mL
Chloramphenicol	50 µg/mL
Tetracycline	50 µg/ml
Kan	50 µg/ml

Transformation solutions

1 M $MgCl_2$
in 200 mL bottle add
20.39 g $MgCl_2 \cdot 6H_2O$
fill to 100 ml with DI H_2O
Autoclave to sterilize

1 M $MgSO_4$
in 200 mL bottle add
24.6 g $MgSO_4 \cdot 7H_2O$
fill to 100 mL with DI H_2O
mix and autoclave to sterilize

SOB solution

For 200 ml of solution add	final conc.
4g tryptone	2% wt/vol
1g yeast extract	0.5% wt/vol
0.117g NaCl	0.0585%
0.057g KCl	0.0285%

mix and autoclave to sterilize

SOC solution

For 10 ml solution add	Final conc.
10 mL SOB solution	
180 µL 20% Glucose	0.36% w/v
100 µL 1 M $MgSO_4$	1 mM
100 µL 1 M $MgCl_2$	1 mM

Do not store for longer then 2 days

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Tranforming DNA

Follow companies instructions for cells

General protocol - Electroporation

1. Make SOC solution and chill cuvettes in ice
2. Make controls
    a. Positive control – 1 ng pure DNA vector compatible with cell line to determine CFU of cells
    b. Negative controls – For ligations – usually unligated, digested vector or dephosphorylated vector that has been incubated with ligase – should not form colonies
3. Add DNA to be transformed to 0.5 ml microcentrifuge tube and chill on ice.
4. Thaw electrocompetent cells on ice
5. Set up Biorad Gene Pulser II or MicroPulser systems
    a. Micropulser
        i. set to Ec1 for 0.1 cm cuvettes (1.8kV)
        ii. Ec2 for 0.2 cm cuvettes (2.5kV)
    b. Genepulser system:
        i. Capacitance – 25 μF
        ii. Resistance – 200 ohms
        iii. Voltage - depends on electrocompetent cells used and cuvette
6. Add cells to DNA and mix gently, put back on ice (should sit on ice for 45 sec but no longer than 1 minute)
7. Draw 1 ml of SOC into pipette and set aside
8. Dry cuvettes and remove lid
9. Add cell/DNA mix to cuvette and tap to ensure that cell mixture is at bottom of cuvette and replace cap.
10. Slide into pulser until cuvette makes contact with electrodes. Press pulse buttons (both red buttons must be pressed at the same time for Gene pulser). When complete you will hear a beep. (time constant should be around 4.5-5.2)
11. Immediately remove cap and add 1 ml of SOC to cuvette and mix.
12. Remove SOC-cell mix and place in a sterile microcentrifuge tube or 15ml tube. Incubate 1-1.5 hrs at 37°C (optional – can put in shaker)
13. Plate on LB-Agar plates with appropriate antibiotics and grow overnight at 37°C.