Bacterial culture

Sterile technique

1. Always work around a flame
2. Flame the mouth and cap of any bottle, flask or tube upon uncapping and recapping
3. Sterilize metal instruments between uses by dipping in 100% ethanol and flaming

LB liquid media (1 L)

1. Dissolve 25 g LB powder per 1 l DI H2O
2. Autoclave media immediately (115 C for 30 min)
3. Add desired antibiotic upon cooling if required
4. Store at RT or 4 C

2X TY liquid media (1 L)

1. Dissolve 31 g 2X TY powder per 1 L DI H2O
2. Autoclave media immediately (115 for 30 min)
3. Store at 4 C

LB plates (1 L)

1. Dissolve 25 g LB powder and 15 g agar per 1 L DI H2O
2. Autoclave media immediately (115 C for 30 min)
3. Add desired antibiotic upon cooling if required
4. Pour plates 1/3 full when media is cool to the touch, in the hood with sash lowered and flame on
5. Allow plates to dry uncovered in the hood
6. Store plates in sleeves at 4 C upside down

Beginning a monoclonal culture from a glycerol stock

1. Streak LB + antibiotic plate with a glycerol stock stab
2. Incubate plate O/N at 37 C
3. Pick an individual colony from plate and inoculate 2 ml LB + antibiotic media
4. Incubate culture O/N at 37C

Freezing down a glycerol stock of cells

1. Pick an individual colony from a plate and inoculate 2 ml LB + antibiotic media
2. Incubate at 37 C until OD 600 = 0.5
3. Combine 0.8 ml culture + 0.2 ml 80% glycerol in 2 ml cryotube and mix
4. Snap freeze samples in an ethanol-dry ice bath
5. Store glycerol stock at -80 C

Transformation of chemically competent cells

1. Thaw cells and incubate transformant DNA on ice
2. Combine 50 ul thawed cells with ~1 ug DNA and mix gently
3. Incubate samples on ice 20 min
4. Heat shock cells in a 42 C H2O bath for 1 min
5. Incubate samples on ice 5 min
6. Recover cells in 1 ml 2X TY media, shaking at 37 C for 1 hr
7. Plate 20 ul < V < 200 ul culture
8. Incubate plates O/N at 37 C

DNA work

Plasmid prep from culture

1. Pick an individual colony from a plate and inoculate 2 ml LB + antibiotic media
2. Incubate culture O/N at 37 C
3. Spin cells down at 10K RPM for 2 min at RT and remove S/N
4. Re-suspend cells in 150 ul of miniprep solution 1 by vortexing vigorously
5. Incubate samples on ice for 5 min
6. Make miniprep solution 2:

Reagent	vol / 1 ml (ul)	[final]
10 X NaOH	20	0.2 X
10 % SDS	100	1%
H2O	880	-
Total volume	1 ml	-

7. Add 200 ul miniprep solution 2 to samples and mix carefully by inverting
8. Incubate samples on ice for 5 min
9. Lyse cells with 150ul miniprep solution 3, add and immediately vortex vigorously
10. Incubate on ice 5 min
11. Spin cell debris down at 10K RPM for 5 min at RT
12. Moved S/N to new tube
13. Precipitated plasmid DNA with 1 ml ice cold 100% etOH
14. Incubated samples on ice for 5 min
15. Spun down plasmid at 10K RPM for 10 min at RT and removed S/N
16. Washed plasmid with 0.5 ml 70% etOH, added, vortexed, pulsed down plasmid, and removed S/N
17. Air dried pellet at 37 C for 20 min
18. Resuspended plasmids in 50 ul <= volume <= 200 ul TE buffer + RNASEA at 37 C for 30 min
19. Quantitate DNA via spectrometry:
    a. Dilute DNA as appropriate in H2O (1 <= DF <= 1/100)
    b. Add 1 to 100 ul sample to 394-well-plate well and bring total volume to desired level with water
    c. Include a blank where the sample is plain TE buffer
    d. Read absorbances at A260 and A280
    e. Calculate [DNA] (ng/ul) = DF * A260 * 50

Miniprep solution 1

Reagent	1 L	[final]
Glucose (MW=180.16)	9 g	50 mM
Tris HCl (MW=157.56)	3.9 g	25 mM
EDTA (MW=372.24)	3.7 g	10 mM
H2O	→ 1 L	-
Total volume	1 L	-

Miniprep solution 3

Reagent	1 L
5 M KAc (MW=98.15)	600 ml (5 M sln) or 88.34 g (powder)
Glacial acetic acid	115
H2O	→ 1 L
Total volume	1 L

(autoclave solutions 1 and 3 and store at 4 C)

Polymerase chain reaction

1. Combine the following on ice:

Reagent		1X (vol in ul)	[final]
10X Buffer	5		1X
10 mM dNTP		1	0.2 mM
50 mM MgSO4		2	2 mM
10 uM F primer		1	0.2 uM
10 uM R primer		1	0.2 uM
H2O		vol → 50	-
DNA		vol for < 1 ug	-
Taq polymerase		0.2	1 U
Total volume		50 ul	-

2. Thermocycle:

a.	Initial denaturation:	94 C	2 min	
b.	Denaturation:	94 C	30 sec	(repeat 25-35
c.	Annealing:	~55 C	30 sec	cycles of
d.	Extension:	68 C	1 min / kb amplicon	steps b-d)
e.	Final:	68 C	5 min	
f.	Hold:	12 C	forever	

DNA purification

1. Separate products by size on an agarose gel
    a. 0.8 < gel density < 1.5
    b. Run at 100V for 40 min at RT
    c. Visualize DNA under UV light
2. Purify DNA (Qiagen Kit)
    a. Cut DNA band out of gel with a clean razor blade
    b. Dissolve gel in 3X volume of GQ buffer at 50 C for ~10 min
    c. Precipitate DNA with 1X volume of isopropanol
    d. Apply sample to quick spin column and spin DNA into column at 10K RPM for 1 min at RT
    e. Remove F/T
    f. Dissolve residual gel by spinning 0.75 ml GQ buffer through column at 10K RPM for 1 min at RT
    g. Remove F/T
    h. Wash DNA by spinning 0.5 ml PE buffer through column at 10K RPM for 1 min at RT
    i. Remove F/T
    j. Spin residual liquid out of column at 10K RPM for 1 min at RT
    k. Elute DNA with 50 ul EB buffer; apply buffer to column, incubate at RT for 2 min and spin DNA into a fresh epi tube at 10K RPM for 1 min at RT
    l. Quantitate DNA via spectrometry:
        i. Dilute DNA as appropriate in H2O (1 <= DF <= 1/100)
        ii. Add 1 to 100 ul sample to 394-well-plate well and bring total volume to desired level with water
        iii. Include a blank where the sample is plain TE buffer
        iv. Read absorbances at A260 and A280
        v. Calculate [DNA] (ng/ul) = DF * A260 * 50

Restriction digest

1. Combine on ice:

Reagent	1X (vol in ul)	[final]
10X buffer	5	1X
BSA	0.5	1X
H2O	→ 50	-
DNA	1 < ug < 10	-
Restriction enzyme	<1	1 U
Total volume	50 ul	-

2. Incubate at 37 C for 2-20 hrs
3. Heat inactivate enzyme at 65 C for 15 min

Double restriction digest

1. Combine on ice:

Reagent	1X (vol in ul)	[final]
10X buffer	5	1X
BSA	0.5	1X
H2O	→ 50	-
DNA	1 < ug < 10	-
Restriction enzyme 1	<1	1 U
Restriction enzyme 2	<1	1 U
Total volume	50 ul	-

2. Incubate at 37 C for 2-20 hrs
3. Heat inactivate enzyme at 65 C for 15 min

Vector dephosphorylation

1. Combine on ice:

Reagent	1X (vol in ul)	[final]
10X buffer	5	1X
H2O	→ 50	-
DNA	1 < ug < 10	-
Antart Ptase	<1	1 U
Total volume	50 ul	-

2. Incubate at 37 C for 30 min
3. Heat inactivate enzyme at 65 C for 15 min
4. Purify DNA:
    a. Add 5 volumes PB1 buffer to sample and mix
    b. Apply sample to quick spin column and spin DNA into column at 10K RPM for 1 min at RT
    c. Remove F/T
    d. Wash DNA by spinning 0.75 ml PE buffer through column at 10K RPM for 1 min at RT
    e. Remove F/T
    f. Spin residual liquid out of column at 10K RPM for 1 min at RT
    g. Elute DNA with 50 ul EB buffer; apply buffer to column, incubate at RT for 2 min and spin DNA into a fresh epi tube at 10K RPM for 1 min at RT
    h. Quantitate DNA via spectrometry:
        i. Dilute DNA as appropriate in H2O (1 <= DF <= 1/100)
        ii. Add 1 to 100 ul sample to 394-well-plate well and bring total volume to desired level with water
        iii. Include a blank where the sample is plain TE buffer
        iv. Read absorbances at A260 and A280
        v. Calculate [DNA] (ng/ul) = DF * A260 * 50

Ligation

1. Combine on ice:

Reagent	1X (vol in ul)	[final]
10X buffer	2	1X
H2O	→ 20	-
Vector DNA	10 < ng < 100	-
Insert DNA	30 < ng < 300	-
T4 DNA ligase	<1	1 U
Total volume	20 ul	-

2. Incubate at 16 C (up to RT) for 10 min
3. Heat inactivate enzyme at 65 C for 15 min