Date: 20170803
Operators: Ersin, Diane

Gel Extraction QIAGEN kit: QIAquick Gel Extraction Kit:

Equipment:

- Gel Extraction Kit
- Pipette p10, p20, p200, p1000 and paired cones
- Microcentrifuge Eppendorf tubes (1.5 ml)
- Isopropanol

Digested Insert extracted:
E1-1 & E3 XbaI-BamHI

Protocol:

DNA fragment	Tube (g)	Tube + gel (g)	Gel (g)	Gel (mg)	3 x Gel = Buffer QG (µl)	1 x Gel = Isopropanol (µl)
E1-1 XbaI-BamHI Col 1	1.0015	1.2187	0.2102	210.2	631	210.2
E1-1 XbaI-BamHI Col 2	0.9963	1.1706	0.1743	174.3	523	174.3
E1-1 XbaI-BamHI Col 3	0.9960	1.1833	0.1827	182.7	548	182.7
E1-1 XbaI-BamHI Col 4	0.9995	1.1070	0.1384	138.4	415	138.4
E3 XbaI-BamHI Col 1	1.0063	1.2951	0.1903	190.3	571	190.3
E3 XbaI-BamHI Col 2	0.9983	1.3278	0.1648	164.8	494	164.8
E3 XbaI-BamHI Col 3	1.0002	1.1833	0.1406	140.6	422	140.6
E3 XbaI-BamHI Col 4	1.0051	1.1070	0.1437	143.7	431	143.7

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel
2. Weigh the gel slice in a colorless tube. Add 3 volumes of buffer QG to 1 volume gel (100 mg gel = 100 µl). The maximum amount of gel per spin column is 400 mg.
3. Incubate at 50 °C for 10 minutes (or until the gel slice has completely dissolved). Vortex the tube every 2-3 minutes to help dissolve the gel. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to buffer

QG without dissolved agarose). If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.

4. Add 1 gel volume isopropanol to the sample and mix.
5. Place a QIAquick spin column in a provided 2 ml collection tube. To bind DNA, apply the sample to the QIAquick column and centrifuge at 16 100 x g for 1 min or until all the samples have passed through the column. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of > 750 µl, load and spin again.
6. To wash, add 750 µl Buffer PE to QIAquick column and centrifuge for 1 min at 16 100 x g. Discard flow-through and place the QIAquick column back into the same tube.
7. Centrifuge the QIAquick column in the provided 2 ml collection tube again for 1 min at 16 100 x g to remove residual wash buffer.
8. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
9. To elute DNA, add 50 µl Buffer EB (10mM Tris-Cl, pH 8.5) or water to the center of the QIAquick membrane, let the column stand for 5 minutes, and the centrifuge for 1 minute at 16 100 x g.
10. Measure DNA concentrations using the UV5 machine.

Sample of: E3 XbaI-BamHI Col 1 Concentrations (ng/µl)	Average concentration (ng/µl) of: E3 XbaI-BamHI Col 1
8.29	8.86
10.49	
7.80	

Sample of: E3 XbaI-BamHI Col 2 Concentrations (ng/µl)	Average concentration (ng/µl) of: E3 XbaI-BamHI Col 2
4.72	6.12
6.18	
7.46	

Sample of: E3 XbaI-BamHI Col 3 Concentrations (ng/µl)	Average concentration (ng/µl) of: E3 XbaI-BamHI Col 3.
6.12	6.39
5.78	
7.28	

Sample of: E3XbaI-BamHI Col 4 Concentrations (ng/µl)	Average concentration (ng/µl) of: E3 XbaI-BamHI Col 4.
4.82	5.11
4.88	
5.62	

Sample of: E1-1 XbaI-BamHI Col 1 Concentrations (ng/µl)	Average concentration (ng/µl) of: E1-1 XbaI-BamHI Col 1
5.46	7.67
8.30	

9.26	

Sample of: E1-1 XbaI-BamHI Col 2 Concentrations (ng/µl)	Average concentration (ng/µl) of: E1-1 XbaI-BamHI Col 2
5.75	7.74
8.66	
8.82	

Sample of: E1-1 XbaI-BamHI Col 3 Concentrations (ng/µl)	Average concentration (ng/µl) of: E1-1 XbaI-BamHI Col 3.
8.44	8.20
7.88	
8.29	

Sample of: E1-1 XbaI-BamHI Col 4 Concentrations (ng/µl)	Average concentration (ng/µl) of: E1-1 XbaI-BamHI Col 4
3.84	4.07
3.66	
4.70	

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Ligation of plasmid pET43.1a with DNA inserts E3 col 1 & E1-1 col 3

Equipment:

- T4 DNA Ligase Reaction Buffer 10X (stored at -20°C)
- T4 DNA Ligase (stored at -20°C)
- Vector DNA = pET43.1A (dp) X-B, 5 790 bp (stored at -20°C) (dp:dephosphorylated)
- Insert DNA = E1-2 col 2 X-B, 769 bp & E2 col 2 X-B, 964 bp (stored at -20°C)
- Nuclease-free water
- Pipette p10, p20, p200, p1000 and associated cones
- Microcentrifuge Eppendorf tubes (1.5 ml)

Vector DNA: pET43.1a dp X-B, 5 790 bp & pET43.1a X-B, 5 790 bp

Insert DNA: E3 colony 1 X-B, 1 108 bp
M(Insert DNA : E3 X-B) = 650 x 1 108 = 720 200 g/mol
n(Insert DNA : E3 X-B) = 0.060 pmol
m(Insert DNA : E3 X-B) = n x M = 4.32 x 10^{-8} g = 43.21 ng

Concentration of Insert DNA : E3 X-B col 1 ratio 1:3	
8.86 ng	1 µl
43.21 ng	4.87 = 4.9 µl

Insert DNA: E1-1 colony 3 X-B, 1 534 bp
M(Insert DNA : E1-1 X-B) = 650 x 1 534 = 997 100 g/mol
n(Insert DNA : E1-1 X-B) = 0.060 pmol
m(Insert DNA : E1-1 X-B) = n x M = 5.98 x 10^{-8} g = 59.836 ng

Concentration of Insert DNA : E1-1 X-B col 3 ratio 1:3	
8.20 ng	1 µl
59.836 ng	7.3 µl

Vector DNA: pET43.1A dp X-B, 5 790 bp
M(Vector DNA dp : pET43.1A dp X-B) = 650 x 5 790 = 3 763 500 g/mol
n(Vector DNA dp : pET43.1A dp X-B) = 0.020 pmol
m(Vector DNA dp : pET43.1A dp X-B) = n x M = 7.527 x 10^{-8} g = 75.27 ng

Concentration of Vector DNA : pET43.1A dp X-B	
11.36 ng	1 µl
75.27 ng	6.63 µl

Vector DNA: pET43.1a X-B, 5 790 bp
M(Vector DNA dp : pET43.1a X-B) = 650 x 5 790 = 3 763 500 g/mol
n(Vector DNA dp : pET43.1a X-B) = 0.020 pmol
m(Vector DNA dp : pET43.1a X-B) = n x M = 7.527 x 10^{-8} g = 75.27 ng

Concentration of Vector DNA : pET43.1a X-B	
7.035 ng	1 µl
75.27 ng	10.7 µl

Protocol:

1) Set up the following reaction in a microcentrifuge tube on ice.

Mix for a 20 µl reaction, ratio 1:3

Components	20 µl Reaction
T4 DNA Ligase Reaction Buffer (10X)	2 µl
Vector DNA (pET43.1a dp X-B)	6.63 µl = 0.020 pmol
Insert DNA (E1-1 X-B col 3)	7.3 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 3.1 µl
T4 DNA Ligase	1 µl

Mix for a 20 µl reaction, ratio 1:3

Components	20 µl Reaction
T4 DNA Ligase Reaction Buffer (10X)	2 µl
Vector DNA (pET43.1a X-B)	10 µl = 0.020 pmol
Insert DNA (E1-1 X-B col 3)	7.3 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 0 µl
T4 DNA Ligase	1 µl

Mix for a 20 µl reaction, ratio 1:3

Components	20 µl Reaction
T4 DNA Ligase Reaction Buffer (10X)	2 µl
Vector DNA (pET43.1a dp X-B)	6.63 µl = 0.020 pmol
Insert DNA (E3 X-B col 1)	4.9 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 5.5 µl
T4 DNA Ligase	1 µl

Mix for a 20 µl reaction, ratio 1:3

Components	20 µl Reaction
T4 DNA Ligase Reaction Buffer (10X)	2 µl
Vector DNA (pET43.1a X-B)	10.7 µl = 0.020 pmol
Insert DNA (E3 X-B col 1)	4.9 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 1.5 µl
T4 DNA Ligase	1 µl

Mix for a 20 µl reaction, ratio 1:0

Components	20 µl Reaction
T4 DNA Ligase Reaction Buffer (10X)	2 µl
Vector DNA (pET43.1a dp X-B)	6.63 µl = 0.020 pmol
Nuclease-free water	To 20 µl = 10.4 µl
T4 DNA Ligase	1 µl

The T4 DNA Ligase Reaction Buffer should be thawed and resuspended at room temperature.
T4 DNA Ligase should be added last.

2) Gently mix the reaction by pipetting up and down and microcentrifuge briefly.
3) For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes
4) For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours.
5) Heat inactivate at 65°C for 10 minutes
6) Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

Date: 20170803
Operators: Diane, Alexis

Liquid Culture for Miniprep on transformed Bacteria DH5α pET43.1a-E2

AIM: liquid culture of transformed bacteria for miniprep

Equipment:

- Petri Dish with LB agar media + antibiotics CARB 50µg/mL or CM
- LB broth sterilized by Bunsen burner
- Antibiotics: Carbenicillin 50mg/ml (CARB 50mg/ml stored at -20°C) or Chloramphenicol (CM 25 mg/ml)
- Sterile Erlenmeyer or Falcon of 50 ml
- Inoculator = inoculation loop of 1 µl
- Pipette p200 + associated cones (p200/20), Pipet p10 + paired cones
- Plastic graduated pipette (10 ml or 20 ml)
- Electric propipet

Transformed Bacteria:

- DH5α pET43.1a-E2

Protocol:

1. In 50ml sterile Falcon tubes (or Erlenmeyer previously autoclaved and sterilized by Bunsen Burner (use aluminum as lid to cover the Erlenmeyer)) we add 15 ml of LB broth and 15 µl of antibiotic: CARB (50 mg/ml)
2. Mix by pipetting up and down 6 times
3. Using an inoculation loop of 1 µl, touch a colony of transformed bacteria: DH5α pET43.1a-E2 on the petri dish. Immerse and dip the inoculation loop in the liquid media and stir.
4. On a new petri dish LB/CARB spread the rest of the bacterial colony (zig-zag movement)
5. Place the liquid culture in the incubator at 37°C for 14 hours at 150 rpm. Maintain the lids on top using tape but do not close the tubes.
6. After 7 hours we observe a turbidity of the solution, which proves the presence of bacteria in the media.
7. Place the petri dish in the incubator at 37°C for 14 hours and then stored a 4°C.

After 14 hours:

8. In contained in Erlenmeyer the liquid cultures are transferred in falcon tubes of 15 or 50 ml
9. The tubes are centrifuged (don't forget to balance the machine and use the adaptor) at 5°C for 10 minutes at 3 600 - 4 500 x g
10. We observe a solid pellet composed of cells. Discard the supernatant and the rest of media is removed using a pipette p1000 (beware not to pipette the pellet)
11. The Pellet is stored at -20°C & named: DH5α pET43.1a-E2 col1, col2

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Protocol for Bacteria Transformation of DH5α with pET43.1a-E1-1 and pET43.1a-E3:

Aim: Transform competent bacteria with plasmids
Equipment:

- 500 µl tube of competent Bacteria (competent cells stored at -80°C) : DH5α
- Plasmid to transform pET43.1a-E2
- 1.5 ml Eppendorf tubes
- P1000, P200, P10 pipettes + paired cones
- Petri Dish with LB agar/CARB for Carbenicillin (an equivalent of amp, resistant to temperature) → Petri Dish (LB/CARB)
- 42°C Water-bath
- Incubator 37°C with or without a stirrer/agitator
- Sterile rake/scraper/comb
- Timer

Plasmid transformed

- pET43.1a dp-E1-1 ligation ratio 1:3
- pET43.1a-E1-1 ligation ratio 1:3
- pET43.1a dp-E3 ligation ratio 1:3
- pET43.1a-E3 ligation ratio 1:3
- pET43.1a dp ligation ratio 1:0

Protocol:
Competent cells are extremely sensitive**; all manipulations must take place on ice**;
You must **operate in the vicinity of the Bunsen burner** when manipulating bacterial cultures.
Name the tubes with the transformed cells: Cell type/name, plasmid (vector and composition) initials of the operator: First Name/Last Name.

1. Split the tube of competent cells: DH5α in aliquots of 50 µl, in 1.5 Eppendorf tubes placed on ice.
2. Mix by Gently tapping the bottom of the tubes with soft end of your finger
3. Add 5 µl of ligated plasmid pET43.1a dp-E1-1 ligation ratio 1:3 to transform in 50 µl of a DH5α aliquot
4. Add 5 µl of ligated plasmid pET43.1a- E1-1 ligation ratio 1:3 to transform in 50 µl of a DH5α aliquot
5. Add 5 µl of ligated plasmid pET43.1a dp-E3 ligation ratio 1:3 to transform in 50 µl of a DH5α aliquot
6. Add 5 µl of ligated plasmid pET43.1a-E3 ligation ratio 1:3 to transform in 50 µl of a DH5α aliquot
7. Add 5 µl of ligated plasmid pET43.1a dp ligation ratio 1:0 to transform in 50 µl of a DH5α aliquot
8. Mix by gently tapping the bottom of the tubes with soft end of your finger.
9. Let it rest for 30 minutes on ice.

In the meantime check the water-bath at 42°C, and place the SOC media at 37°C for heating

10. Put the tubes in the floats
11. Place the floats in the 42°C water-bath for 40 seconds, after 38 seconds open the water-bath's lid as to remove the floats after 40 secs precisely, remove the floats quickly.
12. Place the tubes on ice for 3 minutes
13. Add 650 µl of SOC media per tube
14. Incubate and mix the tubes at 150 rpm at 37°C for 40 minutes on a holder placed on it's side and containing the attached (with tape) tubes.

In the meantime place the LB/AMP petri dishes at 37°C and name the dishes.

15. Generate 2 Petri dishes with each tube: one dish containing 200 µl and the other 500 µl.
16. Spread the bacteria using an inoculator (2 rotations)
17. Wait for the dishes to dry (5 min maximum)
18. Store the dishes upside down in the incubator at 37°C for 16 hours (O/N)