DATE : 20170725
Operators: Karima, 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:
E2 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)
E2 XbaI-BamHI Col 1	0.9884	1.2951	0.3067	306.7	920.1	306.7
E2 XbaI-BamHI Col 2	1.0045	1.3278	0.3233	323.3	969.9	323.3
E2 XbaI-BamHI Col 3	1.0039	1.1833	0.1791	179.1	537.3	179.1
E2 XbaI-BamHI Col 4	1.0037	1.1070	0.1033	103.3	309.9	103.3

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 (Tris-Cl 10 mM, 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 Spectrophotometer.

Sample of: E2 XbaI-BamHI Col 1 Concentrations (ng/µl)	Average concentration (ng/µl) of: E2 XbaI-BamHI Col 1…….
64.2	48.9
11.8	
70.7	

Sample of: E2 XbaI-BamHI Col 2 Concentrations (ng/µl)	Average concentration (ng/µl) of: E2 XbaI-BamHI Col 2
65.78	64.4
58.1	
69.3	

Sample of: E2 XbaI-BamHI Col 3 Concentrations (ng/µl)	Average concentration (ng/µl) of: E2 XbaI-BamHI Col 3.
71.0	69.2
67.5	
69.1	

Sample of: E2 XbaI-BamHI Col 4 Concentrations (ng/µl)	Average concentration (ng/µl) of: E2 XbaI-BamHI Col 4.
67.4	68.1
62.8	
73.9	

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Ligation of plasmid with DNA insert

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, 7275 kb (stored at -20°C) (dp: Dephosphorylated XbaI-BamHI fragment)
- Insert DNA = E2 X-B, 964 kb (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, 7275 kb
Insert DNA: E2 X-B, 964 kb

M(Insert DNA : E2 X-B) = 650 x 964 = 626 600 g/mol
n(Insert DNA : E2 X-B) = 0.060 pmol
m(Insert DNA : E2 X-B) = n x M = 3.76 x 10^{-8} g = 37.6 ng

Concentration of Insert DNA : E2 X-B col 3	
69.2 ng	1 µl
37.6 ng	0.6 µl

Concentration of Insert DNA : E2 X-B col 4	
68.1 ng	1 µl
37.6 ng	0.6 µl

M(Vector DNA dp : pET43.1A dp X-B) = 650 x 7 275. = 4 728 750 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 = 7.29 x 10^{-8} g = 73 ng

Concentration of Vector DNA : pET43.1A dp X-B	
25 ng	1 µl
73 ng	2.9 = 3 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 kb)	3 µl = 0.020 pmol
Insert DNA (E2 X-B col 3 kb)	0.6 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 13.4 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 kb)	3 µl = 0.020 pmol

Insert DNA (E2 X-B col 4 kb)	0.6 µl = 0.060 pmol
Nuclease-free water	To 20 µl = 13.4 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: 20170725
Operators: Juliette, Alexis

Liquid Culture for Miniprep on transformed Bacteria DH5α pEXA128-E1-2

AIM: liquid culture of transformed bacteria for miniprep

Equipment

- Petri Dish with LB agar media + antibiotics CARB 50 µg/ml or CM
- LB broth sterilized by Bunsen burner
- Antibiotics: Carbenicillin 50 mg/ml (CARB 50 mg/ml stored at -20°C)
- 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 pEXA128-E1-2

Protocol:

1. In 50ml sterile Falcon tubes (or Erlenmeyer previously autoclaved and sterilized by Bunsen Burner (use aluminium 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 pEXA128-E1-2 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 blurring 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 pEXA128-E2 col1, col2, col3, col4

Date: 20170725

Operators : Karima, Juliette, Alexis

Single Tube Transformation Protocol

Before You Start

Estimated bench time: 1 hour
Estimated total time: 2 hours (plus 14-18 hour incubation)

When transforming competent cells, both timing and temperature are very important. Use a lab timer, follow the incubation temperatures closely, and keep materials on ice when required.

- **Read through the entire protocol before starting!**

Materials

- Resuspended DNA to be transformed
- 10 pg/µl Positive transformation control DNA (e.g. pSB1C3 w/ BBa_J04450, RFP on high-copy chloramphenicol resistant plasmid. Located in the Competent Cell Test Kit.)
- Competent Cells (50 µl per sample)
- 1.5 ml Microtubes
- SOC Media (950 µl per sample)
- Petri plates w/ LB agar and antibiotic (2 per sample)

Equipment

- Floating Foam Tube Rack
- Ice & ice bucket
- Lab Timer
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Pipettes and Tips (10 µl, 20 µl, 200 µl recommended)
- Microcentrifuge

Method

1. Resuspend DNA in selected wells in the Distribution Kit with 10 µl dH_20. Pipet up and down several times, let sit for a few minutes. Resuspension will be red from cresol red dye.

Selected wells of Plate 6 :

2. Label 1.5 ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 1.5 ml tubes (one tube for each transformation, including your control) in a floating foam tube rack.
3. **Thaw competent cells on ice:** This may take 10-15 min for a 260 µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.
4. **Pipette 50 µl of competent cells into 1.5ml tube:** 50 µl in a 1.5 ml tube per transformation. Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice. Don't forget a 1.5 ml tube for your control.
5. **Pipette 1 µl of resuspended DNA into 1.5 ml tube:** Pipette from well into appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.
6. **Pipette 1 µl of control DNA into 2 ml tube:** Pipette 1 µl of 10 pg/µl control into your control transformation. Gently pipette up and down a few times. Keep all tubes on ice.

7. **Close 1.5 ml tubes, incubate on ice for 30 min:** Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
8. **Heat shock tubes at 42°C for 45 sec:** 1.5ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.
9. **Incubate on ice for 5 min:** Return transformation tubes to ice bucket.
10. **Pipette 950 µl SOC media to each transformation:** SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.
11. **Incubate at 37°C for 1 hours, shaking at 180 rpm**
12. **Pipette 100 µl of each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.
13. **Spin down cells at 6800 x g for 3mins and discard 800 µl of the supernatant. Resuspend the cells in the remaining 100 µl, and pipette each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This increases the chance of getting colonies from lower concentration DNA samples.
14. **Incubate transformations overnight (14-18 hr) at 37°C:** Incubate the plates upside down (agar side up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.
15. **Pick single colonies:** Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep.

➔ Did not work