Date: 20170804
Operators: Alexis, Paul

Miniprep on transformed Bacteria DH5α pET43.1a-E2

Aim: retrieve amplified plasmids from colony of transformed bacteria
Equipment:

- Pipette p20, p200 + associated cones (p200/20), Pipet p10, p1000 + paired cones
- Plastic graduated pipette (10 ml or 20 ml)
- Electric propipet
- QIAGEN kit: QIAprep Spin Miniprep Kit

Transformed Bacteria:

- DH5α pET43.1a-E2 colony 1 & 2

Miniprep : QIAGEN kit: QIAprep Spin Miniprep Kit

1. Pellet 1-5 ml bacterial overnight culture by centrifugation at 3500 x g for 3 minutes at room temperature (15-20°C)
2. Resuspend pelleted bacterial cells in 250 µl buffer P1 and transfer to a microcentrifuge tube (1.5 Eppendorf tubes)
3. Add 250 µl buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 minutes.
4. Add 350 µl buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.
5. Centrifuge for 10 minutes at 16 100 x g in a table-top microcentrifuge.
6. Apply the 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. Centrifuge for 60 seconds at 16 100 x g and discard flow-through.
7. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centrifuge for 60 seconds at 16 100 x g and discard the flow-through. Place the QIAprep 2.0 spin column back in the collection tube.
8. Centrifuge for 1 minute at 16 100 x g to remove residual wash buffer.
9. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl buffer EB (Tris-Cl 10 mM, pH 8.5) to the center of the of the QIAprep 2.0 spin column, let stand for 5 minutes, and centrifuge for 1 minute at 16 100 x g.
10. Measure the DNA concentration using the Nanodrop machine. 3 measures of 3 µl each.

UV5 use for concentration analysis:

1. Buffer: EB (Tris-Cl 10 mM, pH 8.5)
2. Start the program iGEM_DNA (2 µl)
3. Clean the lens with sterile water on a tissue
4. Add **3 µl** of solution Buffer on the lens (no bubbles, no overflow of the solution outside the lens)
5. Analyse the solution

6. Clean the lens with a tissue and sterile water
7. Vortex the solution to analyse
8. Add delicately **3 µl** of the solution to analyse on the lens without bubbles or spilling around the lens
9. Measure the concentration of your sample
10. Clean the lens with a tissue and sterile water
11. Repeat steps 6 to 10 three times per sample to analyse
12. Don't forget to clean the machine when finished!

Sample: pET43.1a-E2 col 1.1 concentration (ng/µl)	Average concentration
509.44	509.25 ng/µl
509.05	

Sample: pET43.1a-E2 col 1.2 concentration (ng/µl)	Average concentration
439.03	437.66 ng/µl
436.28	

Sample: pET43.1a-E2 col 1.3 concentration (ng/µl)	Average concentration
410.42	410.55 ng/µl
410.68	

Sample: pET43.1a-E2 col 2.1 concentration (ng/µl)	Average concentration
15.96	22 ng/µl
29.18	

Sample: pET43.1a-E2 col 2.2 concentration (ng/µl)	Average concentration
22.10	22 ng/µl
22.25	

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DNA pET43.1a-E2 Digestion X-B

Aim: Digest DNA plasmids using restriction enzymes to verify the correct ligation specific DNA sequence: insert E2

Equipment:

- Restriction enzymes stored at – 20°C XbaI-BamHI
- Plasmid to digest stored at – 20°C pET43.1a-E2
- 10X NEB buffer CutSmart stored at – 20°C
- Deionized water
- Water-bath at 37°C
- Heater block for incubation at 65°C
- Timer
- Pipet p10, p20, p200 & associated cones
- Gel loading dye 6X

Plasmid digested:

- pET43.1a-E2

Restriction enzymes:

- XbaI
- BamHI

Protocol:

DNA plasmid to digest concentration:
Concentration tube 1: pET43.1a-E2 colony 1.1

509.25 ng	1µl
1.0185 µg = 1018.5 ng	2 µl
500 ng	1 µl

Plasmid + DNA sequence	Plasmid	DNA sequence
…… bp	…… bp	…… bp

Volume of restriction enzyme needed:

Quantity of DNA to digest	Volume of restriction enzyme
1 µg	1 µl
1 µg	1 µl

- Mix for a total volume of 50 µl of negative control pET43.1a-E2 colony 1.1

- DNA: pET43.1a-E2 colony 1.1	1 µl =500 ng
10X NEBuffer	5 µl
Deionized water	44.0 µl
Total Rxn Volume	50 µl

- Tube col1 Digestion BamHI-XbaI Mix for a total volume of 50 µl pET43.1a-E2 colony 1.1

DNA: pET43.1a-E2 colony 1.1	2 µl = 1 000 ng
Restriction enzyme: BamHI	1 µl
Restriction enzyme: XbaI	1 µl
10X NEB buffer	5 µl
Deionized water	41 µl
Total Rxn Volume	50 µl

1. Mix gently by pipetting up and down 4-6 times
2. Microcentrifuge briefly 3 seconds
3. Incubate at 37°C for 1 hour
4. Stop reaction by heat inactivation: incubate at 65°C for 20 minutes. This step is only for specific restriction enzymes (XbaI)
5. Stop reaction by adding 10 µl of 6X gel loading dye to the 50 µl reaction. Mix by pipetting up and down and microcentrifuge briefly (3 seconds)

6. Prepare a 75ml electrophoresis gel :

Gel Electrophoresis 75 ml	
Deionized H_2O	73.5 ml
TAE 50X	1.5 ml
Agarose 0.7%	0.525 g

Electrophoresis Solution mix

Deionized H_2O	495 ml
TAE 50X	5 ml

7. Mix the TAE 50X and water first, then pour the solution in an Erlenmeyer and add the agarose
8. Microwave until the solution is transparent and mix every 30 seconds
9. Once the solution is clear cool the Erlenmeyer using tap water
10. Add the comb for the wells in the tank
11. Pour the gel solution in the electrophoresis tank, avoid leaks, and wait for the gel to solidify
12. Once the gel is set, pour the 500 ml electrophoresis solution in tank
13. Remove comb delicately
14. Load the wells with the digested DNA solutions and the negative control (30-45 µl per) and the ladder

Gel 1 lay out:

Lane	1	2	3	4	5	6	7	8	9	10
Vol(µl)	50	50	50			50	50	50	5	
			Col 1.1 ctrl -			Col 1.1 X-B			Smart Ladder	

15. Start the voltage at 75 Volts for 10 min
16. Set voltage at 150 Volts (80 mA) for 1h30 approximately
17. Place gel in EB bath (deionized H20 + 3 drops of EB) for 15 minutes
18. Wash gel in deionized water bath for 5 minutes
19. Check by UV imaging the presence of 2 digested DNA fragments. And check that the size of the DNA sequence to extract is correct. Save image.
20. Weigh microcentrifuge tubes.
21. Cut gel to extract DNA fragment and place it in microcentrifuge tube previously weighed.
22. Check with UV imaging that the gel was correctly cut. Save image.
23. Weigh microcentrifuge tubes containing the gel.

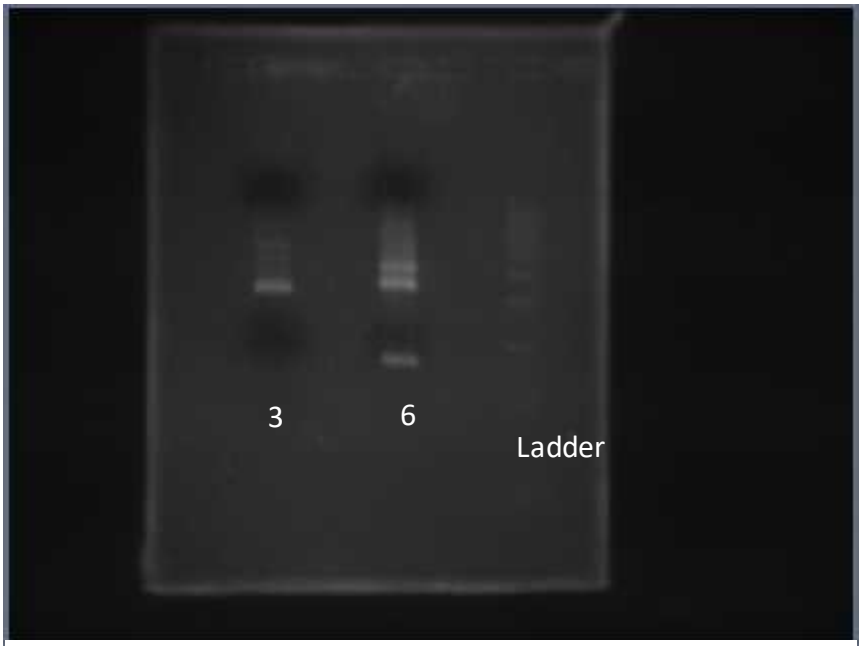

Figure 1: Electrophoresis gel of pET43.1a-E2