Date: 20170712
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Miniprep on transformed Bacteria DH5α pET43.1a.C162

Aim: retrieve amplified plasmids from colony of transformed bacteria
We need a pET backbone. We will obtain it from an existing vector in the lab pET43.1aC162

Equipment

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

Transformed Bacteria
- DH5α pET43.1a-C162

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 16100 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 16100 xg 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 (10 mM TrisCl, pH 8.5) or deionized water 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 UV5 spectrophotometer. 3 measures of 3 µl each. Cf UV5 protocol.

Measure the DNA concentrations using the UV5 machine.

UV5 use for concentration analysis:

1. Buffer: deionized water or $TE_{0.1}$ 1X
2. Start the program iGEM_DNA (2µl)
3. Clean the lens with deionized 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. Analyze the solution
6. Clean the lens with a tissue and deionized sterile water
7. Vortex the solution to analyze
8. Add delicately **3 µl** of the solution to analyze 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 analyze
12. Don't forget to clean the machine when finished!

Sample: pET43.1a-C162 colony 1 concentration (ng/µl)	Average concentration
	65.3 µg/ml

Sample: pET43.1a-C162 colony 2 concentration (ng/µl)	Average concentration
	56.3 µg/ml

Sample: pET43.1a-C162 colony 3 concentration (ng/µl)	Average concentration
	55.6 µg/ml

Sample: pET43.1a-C162 colony 4 concentration (ng/µl)	Average concentration
	62.0 µg/ml

Date: 2017/07/12
Operators: Ersin, Gabriel, Alexis

DNA Digestion pET43.1a.C162

Aim: Digest DNA plasmids using restriction enzymes to retrieve specific DNA sequences.
Equipment:

- Restriction enzymes stored at – 20°C
- Plasmid to digest stored at – 20°C
- 10X NEB buffer CutSmart stored at – 20°C
- Sterile water
- Water-bath at 37°C
- Dry-heater for incubation at 65°C
- Timer
- Pipet p10, p20, p200 & associated cones
- Gel loading dye 6X, stored at -20°C

Plasmid digested:
- pET 43.1a.C162
Restriction enzymes:
- Xba I
- BamH I

Protocol:
DNA plasmid to digest concentration:

15.15 ng	1 µl
1000 ng	66 µl
500 ng	33 µl
50 ng	3.3 µl

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

For 500 ng of DNA sequence to digest: volume= 33 µl
Volume of restriction enzyme needed:

Quantity of DNA to digest	Volume of restriction enzyme
1 µg	1 µl
0.5 µg	0.5 µl

Mix for a total volume of 50µl

DNA: pET 43.1a.C162, 500 ng	33 µl
Restriction enzyme:BamHI	1 µl
Restriction enzyme:XbaI	1 µl
10X NEBuffer	5 µl
Deionized water	10 µl
Total Rxn Volume	50 µl

Mix for a total volume of 50 µl of negative control

DNA: pET 43.1a.C162, 50 ng	3.3 µl
10X NEB buffer	5 µl
Deionized water	41.7 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 75 ml electrophoresis gel:

Gel Electrophoresis 100 ml	
Deionized H_2O	98 ml
TAE 50X	2 ml
Agarose 0.7%	0.7 g

Electrophoresis Solution mix

Deionized H_20	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 agarose has dissolved 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 (TAE 1X or 0.5X) in tank
13. Remove comb delicately
14. Load the wells with the digested DNA solutions and the negative control (30-45 µl per well) and the ladder

Gel lay out:

Lane	1	2	3	4	5	6	7	8	9	10
Volume	40 µl		40 µl	……. µl	……. µl	……. µl	……. µl	……. µl	5 µl	……. µl
	Digested pET43.1a		Negative Control						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 H_20 + 3 drops of EB) for 10 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 fragment to extract is correct. Save image → Presence of plasmid and C162 fragment.
20. Weigh microcentrifuge tubes.

Tube 1	0.996 g
Tube 2	1.013 g

21. Cut gel to extract DNA fragment, visible with lighting (312 nm) and place it in microcentrifuge tube previously weighed.
22. Check with UV imaging that the gel was correctly cut. Save image.
23. Store Over Night (O/N) at 4°C

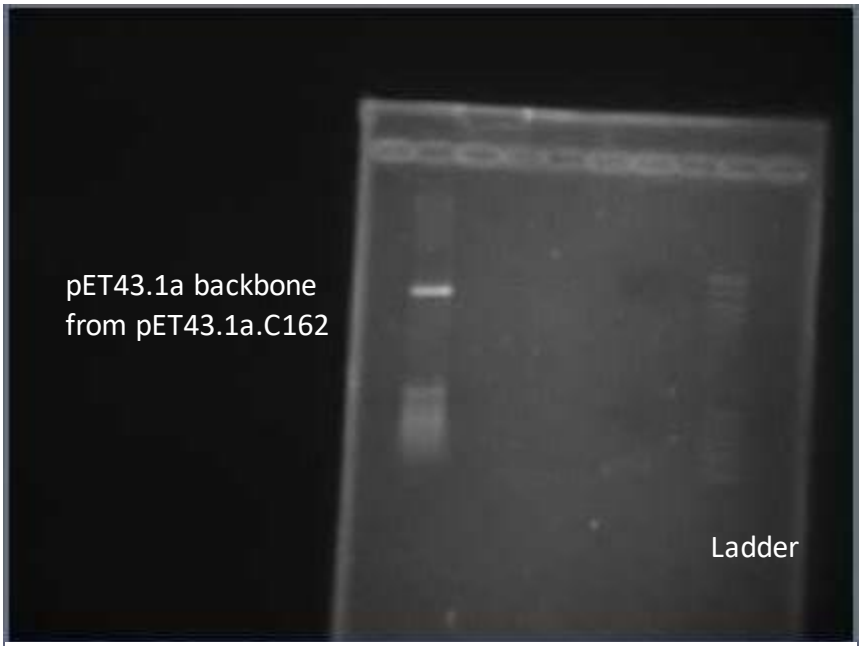

Figure 1: Electrophoresis gel of pET43.1a.C162 before cut

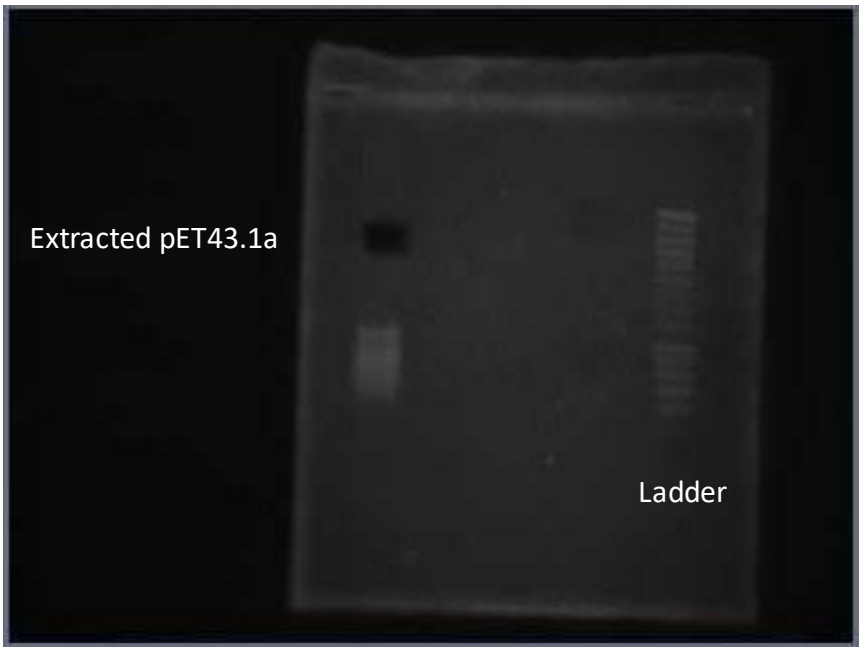

Figure 2: Electrophoresis gel of pET43.1a.C162 before cut