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Operators: Paul, Ersin, Diane, Alexis

Digestion of plasmids pET43.1a.C162

Aim: Digest DNA plasmids using restriction enzymes to retrieve pET43.1a backbone DNA.
Equipment:

- Restriction enzymes stored at – 20°C
- Plasmid to digest stored at – 20°C
- 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, stored at -20°C

Plasmid digested:
- pET 43.1a.C162
Restriction enzymes:
- XbaI
- Bam HI
We will use only the DNA from tubes 2 & 3 because tube 1 did not produce good results. See electrophoresis done the 13th of July 2017. Tube 4 will be kept as backup.
Concentrations of tube 1 containing pET43.1a.C162

55 ng	1 µl
500 ng	9.1 µl
50 ng	0.9 µl

Concentrations of tube 2 containing pET43.1a.C162

55 ng	1 µl
500 ng	9.1 µl
50 ng	0.9 µl

For 500 ng of DNA sequence to digest: volume= 9.1 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 of negative control Tube 1.1

DNA: pET 43.1a.C162, 500 ng	9.1 µl
10X NEB buffer	5 µl
Sterile water	35.9 µl
Total Rxn Volume	50 µl

Tube 2.1 Digestion BamHI Mix for a total volume of 50µl

DNA: pET 43.1a.C162, 500 ng	9.1 µl
Restriction enzyme:BamHI	1 µl
10X NEB buffer	5 µl
deionized water	34.9 µl
Total Rxn Volume	50 µl

Tube 3.1 Digestion XbaI Mix for a total volume of 50µl

DNA: pET 43.1a.C162, 500 ng	9.1 µl

Restriction enzyme:XbaI	1 µl
10X NEB buffer	5 µl
Deionized water	34.9 µl
Total Rxn Volume	50 µl

Tube4.1 Digestion BamHI-XbaI Mix for a total volume of 50µl

DNA: pET 43.1a.C162, 500 ng	9.1 µl
Restriction enzyme:BamHI	1 µl
Restriction enzyme:XbaI	1 µl
10X NEB b uffer	5 µl
Deionized water	33.9 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 100 ml	
Deionized H_2O	98 ml
TAE 50X	2 ml
Agarose 0.7%	0.7 g

Electrophoresis Solution mix

H_20 sterile	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 dry
12. Once the gel is dry, 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 well) and the ladder

Gel lay out:

Lane	1	2	3	4	5	6	7	8	9	10
	40 µl	40 µl	40 µl	40 µl	40 µl	40 µl	40 µl	40 µl	40µl	5 µl
	Negative Control	1.1 Negative Control	1.2 Negative Control	2.1 BamHI	2.2 BamHI	3.1 XbaI	3.2 XbaI	4.1 XbaI BamHI	4.2 XbaI BamHI	Smart Ladder

Note :Well 1 & 6 spilled

15. Start the voltage at 75 Volts for 10 min
16. Set voltage at 150 Volts (80mA) for 1h30 approximately
17. Place gel in EB bath (deionized H_20 + 3 drops of EB) for 20 minutes
18. Wash gel in deionized water bath for 10 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 → Presence of plasmid and C162 fragment.
20. Weigh microcentrifuge tubes.

Tube 1	0.998 g
Tube 2	1.004 g
Tube 3	1.015 g
Tube 4	1.003 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

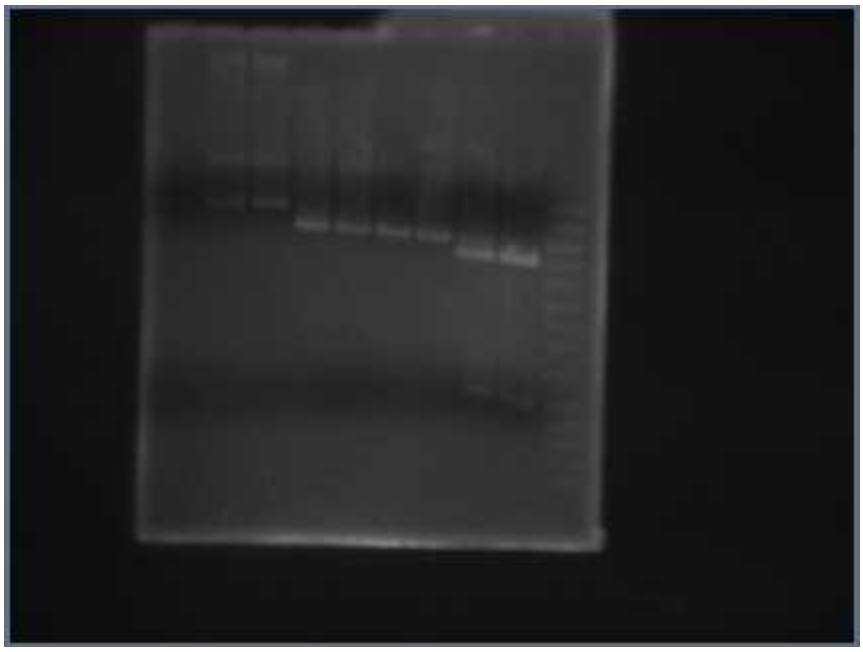

Figure 1: Electrophoresis gel of pET43.1a.C162 before cut

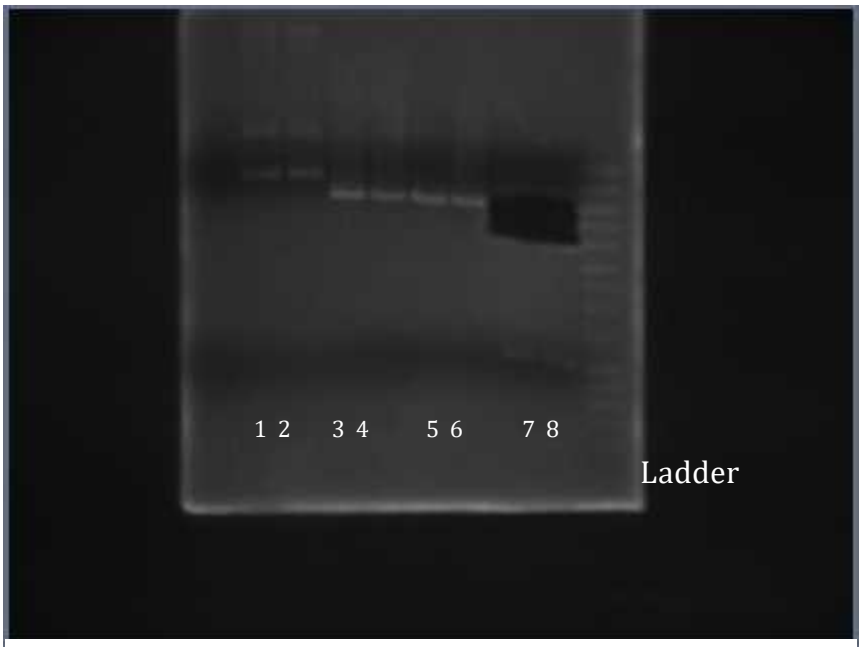

Figure 2: Electrophoresis gel of pET43.1a.C162 after cut