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Gel Extraction QIAGEN kit: QIAquick Gel Extraction Kit: pET 43.1a

Aim: Extract pET43 DNA backbone from gel slices after digestion and gel electrophoresis

Equipment:

- QIAquick Gel Extraction Kit
- Pipette p10, p20, p200, p1000 and associated cones
- Microcentrifuge Eppendorf tubes
- Table-top centrifuge
- Gel containing DNA to extract
- Heater block set at 53°C
- Vortex

Protocol:

1. Weigh microcentrifuge tubes containing the gel.

DNA fragment	Tube (g)	Tube + gel (g)	Gel (g)	Gel (mg)	3 x Gel = Buffer QG (µl)	1 x Gel = Isopropanol (µl)
Tube 1	0.998 g	1.307 g	0.309 g	309 mg	927 µl	309 µl
Tube 2	1.004 g	1.276 g	0.272 g	272 mg → 320 mg	960 µl	320 µl
Tube 3	1.015 g	1.462 g	0.447 g	447 mg → 395 mg	1.185 µl	395 µl
Tube 4	1.003 g	1.348 g	0.345 g	345 mg	1.035 µl	345 µl

We added part of tube 3 into tube 2, because tube 3 was too full.

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel (done 2017-07-12)
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. Tube 1 is separated in 2 tubes of 630 µl, in each tube we add 157 µl of isopropanol.
5. Place a QIAquick spin column in a provided 2 ml collection tube. All for tubes were divided in two and transferred in 2 columns each.
   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 (10 mM 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. Pool the 4 tubes in one microcentrifuge Eppendorf tube. Measure DNA concentrations using the Nanodrop machine.

Sample of: pET43.1a tube 1 Concentrations (ng/µl)	Average concentration (ng/µl) of: pET43.1a tube 1
73.1	69.5
66.2	
69.1	

Sample of: pET43.1a tube 2 Concentrations (ng/µl)	Average concentration (ng/µl) of: pET43.1a tube 2
68.5	66.9
72.2	
60.0	

Sample of: pET43.1a tube 3 Concentrations (ng/µl)	Average concentration (ng/µl) of: pET43.1a tube 3
67.1	65.8
64.4	
66.1	

Sample of: pET43.1a tube 4 Concentrations (ng/µl)	Average concentration (ng/µl) of: pET43.1a tube 4
43.3	45.0
32.8	
58.8	