DNA Electrophoresis & Gel Extraction

Aim

Separate DNA on an agarose gel.

Equipment

- Electrophoresis tank
- Power supply
- TAE 50X
- DNA ladder
- Scalpel
- UV table + Gel Doc (Biorad)
- Eppendorf tubes
- Pipette, cones
- EB*
- De-ionized water

DNA Electrophoresis & Gel Extraction

Protocol

1. Prepare an electrophoresis gel (see Agarose Gel Preparation Protocol).
2. Pour 500 ml of TAE 1X in the electrophoresis tank.
3. Remove comb delicately.
4. Load the wells with the digested DNA solutions, undigested DNA as negative control (30-45 µl per well) and the ladder.

Gel lay out:

Lane number	1	2	3	4	5	6	7	8	9	10
Volume µl										
Sample Name										

5. At the start, set the voltage at 75 V for 10 min.
6. Afterwards, set the voltage at 150 V (80 mA) for 1h30 approximately.
7. Place gel in EB* bath (de-ionized H2O + 3 drops of EB) for 15 min.
8. Wash gel in de-ionized water bath for 5 min.
9. Check by UV imaging the presence of expected DNA fragments. And check that the size of the DNA sequence to extract is correct. Save image.
10. Weigh microcentrifuge tubes.
11. Cut gel with clean scalpel to extract DNA fragment and place it in microcentrifuge tube previously weighed.
12. Check with UV imaging that the gel was correctly cut. Save image.
13. Weigh microcentrifuge tubes containing the gel.

DNA Electrophoresis & Gel Extraction

Gel Extraction QIAGEN kit (QIAquick Gel Extraction Kit):

DNA fragment	Tube (g)	Tube + gel (g)	Gel (g)	Gel (mg)	Buffer QG (µl) =3 x Gel	Isopropanol (µl) =1 x Gel

1. 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.
2. Incubate at 50 °C for 10 min (or until the gel slice has completely dissolved). Vortex the tube every 2-3 min 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.
3. Add 1 gel volume isopropanol to the sample and mix.
4. 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 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.
5. To wash, add 750 µl buffer PE to QIAquick column and centrifuge for 1 min at 16 100 g. Discard flow-through and place the QIAquick column back into the same tube.
6. Centrifuge the QIAquick column in the provided 2 ml collection tube again for 1 min at 16 100 g to remove residual wash buffer.
7. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

DNA Electrophoresis & Gel Extraction

.....

8. To elute DNA, add 50 µl buffer EB (Tris-Cl 10 mM, pH 8.5) or water or TE0.1* to the center of the QIAquick membrane, let the column stand for 5 minutes, and the centrifuge for 1 minute at 16 100 g.
9. Measure DNA concentrations by Nanodrop or UV5 spectrophotometer.

Sample of: …….. Concentrations (ng/µl)	Average concentration (ng/µl)
Measure 1 :	
Measure 2 :	
Measure 3 :	

DNA Electrophoresis & Gel Extraction

Gel extraction NEB kit (Monarch DNA Gel Extraction Kit #T1020):

1. Excise the DNA fragment from the agarose gel, taking care to trim excess agarose. Transfer to a 1.5 ml microcentrifuge tube and weigh the gel slice. Minimize exposure to UV light.

DNA fragment	Tube (g)	Tube + gel (g)	Gel (g)	Gel (µg)	Gel dissolving buffer (µl) =4 x Gel	Water (µl) =1.5 x Gel

2. Add 4 volumes of Gel Dissolving Buffer to the gel slice.
3. Incubate the sample between 37-55°C (typically 50°C), vortexing periodically until the gel slice is completely dissolved (generally 5-10 min). For DNA fragments >8 kB, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA.
4. Insert column into collection tube and load sample onto the column. Spin for 1 min at 16 000 g, then discard flow-through.
5. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 min at 16 000 g. Discard flow-through.
6. Repeat step 5, and spin for 1 min at 16 000 g twice.
7. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 min at 16 000 g.
8. Add 50 µl of DNA elution buffer or nuclease-free water (pH 7-8.5) to the center of the matrix. Wait for 5 min, and spin for 1 min at 16 000 g, to elute DNA.
9. Measure DNA concentrations by Nanodrop or UV5 spectrophotometer.

DNA Electrophoresis & Gel Extraction

Sample of: …….. Concentrations (ng/µl)	Average concentration (ng/µl)
Measurement 1 :	
Measurement 2 :	
Measurement 3 :	

*EB : Ethidium Bromide (Wear gloves and dispose in a carcinogenic waste container), TE0.1 (Tris-Cl, 10 mM, pH 8.0, EDTA, 0.1 mM, pH 8.0