Gel DNA Extraction

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ABSTRACT

This protocol details how to extract DNA from agarose gel using Thermo Scientific GeneJET Gel Extraction Kit. This is suitable for gels run in either TAE or TBE buffer, and for DNA fragments from 25 bp to 20 kb. The extraction is performed using spin columns.

ATTACHMENTS

genejet_gel_extraction_thermo.pdf

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PROTOCOL CITATION

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KEYWORDS

DNA extraction, gel electrophoresis, GeneJET

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MATERIALS TEXT

GeneJET Gel Extraction Kit
1.5 mL centrifuge tube(s)
[May need] [M]**3 Molarity (M)** sodium acetate, pH**5.2**

SAFETY WARNINGS

When performing gel excision, use necessary precautions to avoid tissue damage due to UV radiation exposure. If a proper shield is not in place, wear a full face covering made of a UV protective material, such as polycarbonate.

DISCLAIMER:

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This protocol is not novel or created by the authors. This protocol is adapted from the one provided by the GeneJET Gel Extraction kit manufacturer, Thermo Scientific. The original protocol can be found in the attached document.

BEFORE STARTING

Pre-heat heat block to **55 °C** .

Gel Electrophoresis

1 Perform Gel Electrophoresis on your DNA sample to separate the DNA bands.

The protocol recommends **1 % w/v** for TBE/agarose gel and **1.2 % w/v** for TAE/agarose gel.
Recommended well depth is 1 mm / 1 µL sample.
Other recommendations can be found in the original protocol, such as recommended voltage.

2 Stain gel for visualization.

Excise Gel Bands

3 Pre-weigh and mark 1.5 mL centrifuge tubes for each DNA band.

4 Place gel in transilluminator, and using a clean scalpel or blade, closely excise the desired DNA band(s).

To avoid damage to the transilluminator surface, use a blunted blade.
Each extraction column can process up to **1 g** 1 agarose gel.

5 Weigh the tubes with gel, and determine gel mass.

Bind DNA to Column 1m

6 Add **100 µl** Binding Buffer per **100 mg** gel to centrifuge tube with gel (1:1 volume:weight).

For gels with greater than **2 % w/v** agarose, use 2:1 volume:weight.

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7 Incubate gel mixture at **55 °C** until gel is completely dissolved. Agitate by inversion occasionally to improve mixing. Ensure that gel is completely dissolved before proceeding.

8 Briefly vortex gel mixture.

9 Check color of solution. A yellow color is desired. If color is orange or violet, add **10 µl** of **3 Molarity (M)** sodium acetate, **5.2** . Mix well.

10
- If DNA fragment length is ≤500 bp, add 1 gel volume of **100 % volume** isopropanol to gel solution. E.g. **100 µl** isopropanol to **100 mg** gel in **100 µl** binding buffer. Mix well.
- If DNA fragment length is >10 kb, add 1 gel volume water to gel solution. Mix well.

11 Transfer up to **800 µl** gel solution to extraction column.

If gel solution volume exceeds **800 µl** , add solution to column in stages; centrifuge for 1 min each time, discarding flow

12 Centrifuge at **14000 x g** for **00:01:00** . 1m

Always make a counter-balance when centrifuging. This is best achieved by weighing the tube and using water to make another tube of equal weight. Place same-weight pairs on opposite sides of centrifuge.

A trick for getting the weight right quickly is to place tube with gel on balance, tare it, add empty tube to balance. Add **1 µl** per **1 mg** difference.

13 Discard flow through, place column back in same collection tube.

Wash Column 2m

14 Add **700 µl** wash buffer to purification column.

15 Centrifuge at **14000 x g** for **00:01:00** . Discard flow through. Place column back in same collection tube. 1m

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Be sure to make a new counter balance for this step and all later steps since the weights will change.

16 Centrifuge empty column at **14000 x g** for an additional **00:01:00** to remove any residual wash buffer. This step is not optional. 1m

Elute DNA

17 Transfer purification column to clean 1.5 mL centrifuge tube.

18 Add **50 µl** of Elution Buffer to the center of the column membrane.

An elution volume between 20-50 µL can be used. Do not use less that **10 µl** elution volume.

19 Discard purification column. Store DNA at **-20 °C** .

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