Qiagen MinElute Gel Extraction

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Abstract

MinElute columns can also be used on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus or QIAvac 6S with Luer Adapters). The following protocol is designed to extract and purify DNA of 70 bp to 4 kb from standard or low-melt agarose gels in TAE or TBE buffer using vacuum-driven processing resulting in high end-concentrations of DNA. Up to 400 mg agarose can be processed per MinElute column.

Citation: Michael Crone Qiagen MinElute Gel Extraction. **protocols.io**
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Before start

The yellow color of Buffer QG indicates a pH ≤7.5.

Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).

Isopropanol (100%) and a heating block or water bath at 50°C are required.

All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional table-top microcentrifuge.

Protocol

Excision of Fragment

Step 1.
Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

Solubilisation

Step 2.
Weigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg 100 µl). For >2% agarose gels, add 6 volumes Buffer QG.

REAGENTS
Buffer QG by Contributed by users

Solubilisation

Step 3.
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 gel.

DURATION
00:10:00

Solubilisation

Step 4.

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 color of the mixture will turn yellow.

AMOUNT

10 µl Additional info:

REAGENTS

Sodium acetate View by P212121

Solubilisation

Step 5.

Add 1 gel volume of isopropanol to the sample and mix.

Binding

Step 6.

Place a QIAquick spin column into a provided 2 ml collection tube.

Binding

Step 7.

To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 µl, load and spin/apply vacuum again.

DURATION

00:01:00

Wash

Step 8.

If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 0.5 ml Buffer QG to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back into the same tube.

DURATION

00:01:00

Wash

Step 9.

To wash, add 0.75 ml Buffer PE to QIAquick column and centrifuge for 1 min.

Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2–5 min after addition of Buffer PE.

DURATION

00:01:00

Wash

Step 10.

Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min at 17,900 x g (13,000 rpm) to remove residual wash buffer.

Elution

Step 11.

Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

Elution

Step 12.

To elute DNA, add 10 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9 µl from 10 µl elution buffer volume.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

DURATION

00:02:00

Analysis

Step 13.

If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel. Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.