Gel DNA Recovery

Joshua Timmons

Abstract

This is a protocol for high yield recovery of pure DNA from agarose gels, using GeneJET Gel Extraction and DNA Cleanup Micro Kit

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Guidelines

The GeneJET Gel Extraction and DNA Cleanup Micro Kit is based on the ability of DNA to bindÂ to silica membranes in the presence of chaotropic salts. DNA adsorbs to the silica membraneÂ while contaminants pass through the column. Alternatively, after electrophoresis to separateÂ the DNA fragments, the band(s) of interest is excised from an agarose gel and dissolved inÂ Extraction Buffer, then mixed with ethanol and loaded on DNA Purification Micro Column.Â Impurities are subsequently removed from the silica membrane by the addition of the PrewashÂ Buffer and Wash Buffer, and the pure DNA is effectively eluted with Elution Buffer. The purifiedÂ DNA is used for a wide variety of downstream applications.

Before start

Add 35 ml 100% ethanol to the **Wash Buffer** concentrate. Add 2.5 ml 100% ethanol to the **Prewash Buffer** concentrate.

Protocol

Step 1.
Excise up to 200 mg gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a 1.5 mL tube.

Step 2.
Add 200 µL of Extraction Buffer. Mix thoroughly by pippeting.

Step 3.
Incubate the gel mixture at 50-58°C for 10 minutes or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved

DURATION
00:10:00

Step 4.
Add 200 µL of ethanol (96-100%) and mix by pipetting.

Step 5.
Transfer the mixture to the DNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for 1 minute at 14,000 × g. Discard the flow-through. Place the DNA Purification Micro Column back into the collection tube.

DURATION
00:01:00

Step 6.
Add 200 µL of Prewash Buffer (supplemented with ethanol, see p. 3) to the DNA Purification Micro Column and centrifuge for 1 minute at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube.

AMOUNT
200 µl Additional info:

DURATION
00:01:00

Step 7.
Add 700 µL of Wash Buffer (supplemented with ethanol, see p. 3) to the DNA Purification Micro Column and centrifuge for 1 minute at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube.

DURATION
00:01:00

Step 8.
Repeat step 7

AMOUNT
200 µl Additional info:

DURATION
00:01:00

Step 9.
Centrifuge the empty DNA Purification Micro Column for an additional 1 minute at 14,000 × g to completely remove residual Wash Buffer.

DURATION
00:01:00

Step 10.
Transfer the DNA Purification Micro Column into a clean 1.8 mL microcentrifuge tube

AMOUNT
6 µl Additional info:

Step 11.
Add 10 µL of Elution Buffer to the DNA Purification Micro Column. Centrifuge for 1 minute at 14,000 × g to elute DNA.

DURATION
00:01:00