Gel Extraction

PROTOCOLS IGEM FZU-China

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Biological Engineering

Fuzhou University

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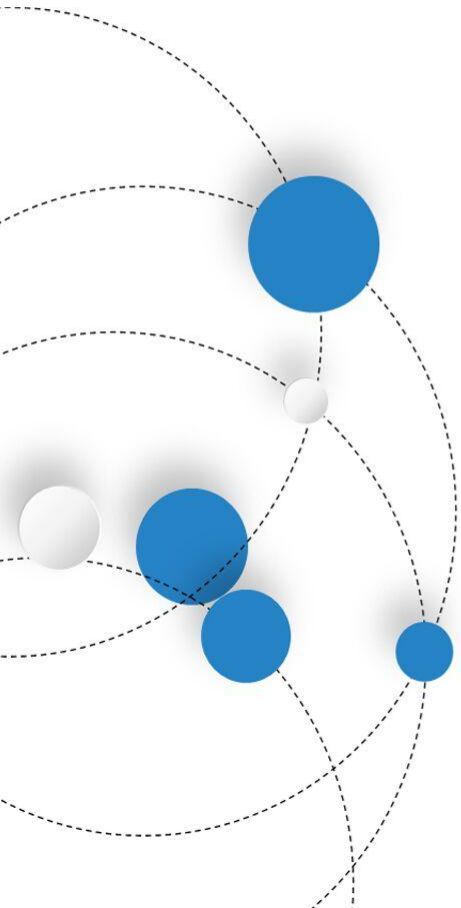

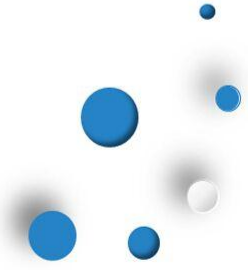

Content

1 Gel Extraction

Estimated bench time: 1 hour
Estimated total time: 1 hour
Purpose: Isolate a desired DNA fragment from an agarose gel based on the size of this fragment.
It is essential to use gloves to protect your DNA fragments from DNase activity

1.1 Materials

- Agarose gel containing the DNA of interest
- Scalpel
- Blue light source
- QIAGEN Gel Extraction Kit
- 1.5 mL Eppendorf tubes
- Milli-Q

1.2 Setup & protocol

- Place the gel on the blue light source to check which bands need to be extracted.
- Cut out the correct bands with a scalpel. Do this as quickly as possible, as the blue light can result in mutations.
- Put the gel slice in 1.5 mL Eppendorf tube and weigh it. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg = 100µL).
- Incubate the sample at 50 °C for 10 minutes until the gel slice has completely dissolved. To help dissolve the gel, vortex the tube every 2-3 minutes during incubation. In addition, incubate a 1.5 mL Eppendorf tube of Milli-Q water under the same conditions.
- Check that the color of the mixture is yellow. If the color of the mixture is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
- Add 1 gel volume of isopropanol to the sample and mix well
- Place a QIAquick spin column in a provided 2 mL collection tube.
- To bind DNA, apply the sample to the QIAquick column and then centrifuge for 1 minute.
- Discard flow-through and place QIAquick column back into the same collection tube.
- (Optional) Add 0.5 mL buffer QC to the QIAquick column and centrifuge for 1 minute. This is only required if the DNA will be used for direct sequencing, in vitro transcription or microinjection.
- To wash, add 0.75 mL of Buffer PE to the QIAquick column. Let it stand for 2-5 minutes and centrifuge for 1 minute. Discard flow-through.
- Add 0.5 mL of buffer PE to the QIAquick column and centrifuge for 1 minute. Discard flow-through and repeat this step
- Centrifuge the QIAquick column for an additional 2 minutes.
- Place the QIAquick column into a clean 1.5 mL Eppendorf tube.
- Add 30 µL of Milli-Q water (which was incubated at 50 °C) to the center of the QIAquick membrane. Let the column stand for 5 minutes and then centrifuge for 2 minutes.
- Measure the DNA concentration using NanoDrop. See our NanoDrop protocol for more information.