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7.13 Experimental Microbial Genetics

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QIAquick Gel Extraction Kit Protocol

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. Read through the QIAquick® Spin Handbook posted at the 7.13 Stellar website for in depth information about this protocol.

Notes:

- The yellow color of Buffer QG indicates a pH ≤ 7.5 .
- Add ethanol (96–100%, check cap to see if it has already been added) to Buffer PE before use (see bottle label for volume).
- Isopropanol (100%) and a heating block at 50°C are required.
- All centrifugation steps are carried out at 13,000 rpm ($\sim 17,900 \times g$) in a conventional table-top microcentrifuge.
- 3 M sodium acetate, pH 5.0, may be necessary.

1. Excise the DNA fragment from the agarose gel with a clean, razor.

Minimize the size of the gel slice by removing extra agarose.

2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 μ l).

For example, add 300 μ l of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.

3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by inverting the tube every 2–3 min during the incubation.

IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

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 μ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤ 7.5 . Buffer QG contains a pH indicator, which is yellow at pH ≤ 7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding. Note: if your DNA is running with the bromophenol blue or xylene cyanol FF in the loading dye the QG buffer may turn blue, but probably no need to adjust the pH in this case.

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

For example, if the agarose gel slice is 100 mg, add 100 μ l isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield.

6. Place a QIAquick spin column in a provided 2 ml collection tube.

7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.

The maximum volume of the column reservoir is 800 μ l. For sample volumes of more than 800 μ l, simply load and spin again.

8. Discard flow-through and place QIAquick column back in the same collection tube.

Collection tubes are re-used to reduce plastic waste.

9. Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.

This step will remove all traces of agarose.

10. To wash, add 0.75 ml of Buffer PE to QIAquick column, leave for 2-5mins and centrifuge for 1 min.

11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min

at 13,000 rpm (~17,900 x g).

IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

12. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

13. To elute DNA, add 30-50 μ l H₂O (depending on desired concentration of sample, generally inserts for ligation should be concentrated (elute in 30 μ l)) to the center of the QIAquick membrane, wait 1min, and then centrifuge the column for 1 min.

IMPORTANT: Ensure that the water is dispensed directly onto the QIAquick membrane for complete elution of bound DNA (but don't touch the membrane with your tip). The average eluate volume is 48 μ l from 50 μ l elution buffer volume, and 28 μ l from 30 μ l. 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.