Protocol for PCR purification from gel
Using a microcentrifuge

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.

Notes: • 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 10,000 x *g* (~13,000 rpm) 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, sharp scalpel.

Minimize the size of the gel slice by removing extra agarose. Minimize exposure to UV light and wear protective clothes and equipment.

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

For example, add 300 µl 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. (you can use the same column)

3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing 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 µl 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.

5. Add 1 gel volume of isopropanol to the sample and mix (if your gene is less than 500 bp or larger than 4000 bp.

For example, if the agarose gel slice is 100 mg, add 100 µl 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. Do not centrifuge the sample at this stage.

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 µl. For sample volumes of more than 800 µl, 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. (Optional): Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.

This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.

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

Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE, before centrifuging.

11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 10,000 x *g* (~13,000 rpm).

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 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the QIAquick membrane and centrifuge the column for 1 min at maximum speed. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick 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 QIAquick membrane for complete elution of bound DNA. The average elute volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl. 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 (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.