Protocol name: QIAquick Gel Extraction Kit & QIAquick PCR & Gel Cleanup Kit

Materials:
When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.
For all protocols
- Ethanol (96â€“100%)*
- Microcentrifuge
- 1.5 or 2 ml microcentrifuge tubes
- 3 M sodium acetate, pH 5.0, may be necessary for PCR purification and gel extraction protocols
- Optional: Distilled water or TE buffer (10 mM TrisÂ·Cl, 1 mM EDTA, pH 8.0) for elution of DNA

auxilary information about the protocol:
Notes before starting
This protocol is for the purification of up to lO pg DNA (70 bp to 10 kb).
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The yellow color of Buffer QG indicates a pH s7.5. DNA adsorption to the membrane is only efficient at pH s7.5.
Add ethanol (96-100%) to Buffer PE before use (see botle label for volume).
Isopropanol (100%) and a heating block or water bath at 50Â°C are required.
All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional table-top microcentrifuge.

Protocol steps:

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to l volume gel (100 mg gel ~l00 pl). The maximum amount of gel per spin column is 400 mg.
For >2% agarose gels, add Ã³ volumes Buffer QG
3. Incubate at 50Â°C for l0 min (or until the gel slice has completely dissolved). Vortex the tube every 2-3 min to help dissolve gel. 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 1O pl 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.
4. Add l gel volume isopropanol to the sample and mix.
5. Place a QIAquick spin column in a provided 2 ml collection tube or into a vacuum manifold. To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min or apply vacuum to the manifold until all the samples have passed through the column. Discard flow-through and place the QlAquick column back into the same tube.
For sample volumes >800 pl, load and spin/apply vacuum again.
6. If DNA will subsequently be used for sequencing, in vitro transcription, electroparation or microinjection, add 500 l Buffer QG to the QIAquick column and centrifuge for 1 min or apply vacuum.
Discard flow-through and place the QlAquick column back into the same tube.
7. To wash, add 750 ul Buffer PE to QIAquick column and centrifuge for l min or apply vacuum. Discard flowthrough and place the QlAquick column back into the same tube.
Note: If the DNA will be Used for salt-sensitive applications (e.g., sequencing, blunt eleepora ended ligation) let the column stand 2-5 min after addition of Buffer PE.
Centrifuge the QlAquick column in the provided 2 ml collection tube for l min to removeresidual wash buffer.
8. Spin once more to remove residual ethanol
9. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

10. To elute DNA, add 50 pl Butter EB (TO mM Tris-Cl, pH8.5) or water to the center of the QIAquick membrane and centrituge the column for 1 min. For increased DNA concentration, add 30 pl Buffer EB to the center of the QlAquick membrane, let the RFTro
column stand for 1 min, and then centrifuge for 1 min. After the addition of Buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 min can increase the yield of purified DNA.
11. If purified DNA is to be analyzed on a gel, add l volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.