

This protocol works best with fragments excised from 1% gels, I have extracted from gels up to 3% with a longer incubation time for dissolving the agarose.

Gel Extraction Buffers and supplies

Buffer QG (Gel Solubilization Buffer)
5.5M guanidine thiocyanate (GuSCN), 20 mM Tris-HCl, pH 6.6

Buffer PE (Wash Buffer)
20 mL NaCl, 2 mM Tris-HCl, pH 7.5 {final concentration after adding ethanol} with 80% EtOH

Buffer EB (Elution Buffer)
10 mM Tris-HCl pH 8.5

Econospin Prepacked Silica Membrane Spin Column Cat. NO 1920-250 (\$95.00/250) (1/sample)

1.5 ml microcentrifuge tube (2/ sample)

isopropanol

Gel Extraction Protocol (QIAquick gel extraction Kit Protocol)

- 1. Excise the DNA fragment with a sterilized tip**
- 2. Weigh the gel slice. Add 3 volumes of Buffer QG to 1 volume of gel** (I used the back of 200ul tips for gel excision and assumed the weight was about 150mg and therefore used 450ul of Buffer QG)
- 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 incubation.** (This works well for ~1% agarose gels, for thicker gels, increase incubation time).
- 4. Add 1 gel volume isopropanol to the sample and mix.**
Following above protocol, this would mean to add 150ul isopropanol. This step increases the yield of DNA fragments <500 bp and >4kb. For fragments between 500 and 4kb, addition of isopropanol has no effect on yield. Do NOT centrifuge the sample at this stage.
- 5. Place a spin column in a provided 2 ml collection tube.**
- 6. To bind DNA apply the sample to the column and centrifuge for 1 min.**
The maximum volume of the column reservoir is 800ul. For sample volumes of more than 800 ul, simply load and spin again.
- 7. Discard flow-through and place Qiaquick column back in the same collection tube.**
- 8. Recommended: Add 0.5ml of Buffer QG to column and centrifuge for 1 min.**
This step will remove all traces of agarose. It is only required when the DNA will be used for direct sequencing, in vitro transcription, or microinjection.
- 9. To wash, add 0.75 ml of Buffer PE to column and centrifuge for 1 min.**
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 and before centrifuging.
- 10. Discard the flow through and centrifuge the column for an additional 1 min at 10,000 x g.** Residual ethanol from Buffer PE will not be completely removed unless the flow through is discarded before this additional centrifugation.
- 11. Place column in a clean 1.5 ml microcentrifuge tube.**
- 12. To elute DNA, add 30 ul of Buffer EB or water to the center of the membrane, let the column stand for 1 min and then centrifuge for 1 min.** (50 ul may also be used but I prefer to have a more concentrated sample).
EB or water may be used for this but do not use TE for samples that will be used for direct sequencing because the EDTA may inhibit subsequent enzymatic reactions.

Sequencing reactions

3 ul of gel extracted DNA
2 ul of Big Dye
3 ul of Big Dye buffer
20 pmole primer
Bring to 20 ul with water

Direct sequence program

1. 95°C 2:30

2. 96°C 0:25
3. 55°C 0:20
4. 62°C 5:00
5. go to 2 for 49 cycles
6. Hold 12°C