DNA gel extraction

Buffers:

QG

- 5.5 M guanidine thiocyanate (GuSCN)
- 20 mM Tris-HCl, pH 6.6 (25°C)

PE

- 10 mM Tris pH 7.5
- 80% ethanol

Protocol

1. Run a DNA gel
2. Using the geldoc, visualize the gel and using a new blade cut around the band you would like to extract
3. Weigh the gel fragment by weighing an empty tube and then adding the gel
4. Add 3 times the weight of the gel in QG buffer (e.g. for a gel piece that weighs 120 mg, add 360 µl of QG)
5. Incubate at 50°C for 10 minutes, inverting every 2-3 minutes
6. Add the weight of the gel in isopropanol (e.g. 120 µl for a 120 mg gel piece)
7. Transfer to a spin column and centrifuge at top speed for 1 min, discard waste
8. Add 500 µl QG buffer, centrifuge 10000g, 1 min and discard waste
9. Add 750 µl PE buffer, centrifuge 10000g, 1 min and discard waste
10. To dry the spin column centrifuge 10000g, 2 min
11. Transfer the spin column to a properly labeled eppendorf
12. Apply 35-50 µl of either nuclease-free water, EB, or TE buffer
13. Elute by centrifuging 10000g, 1 min
14. Measure concentration using the nanodrop, blanking with the same buffer used for elution