Freeze and Squeeze Gel Extraction– Rahul Patharkar

Note: you must use TAE gels if you plan to do cloning directly without further purification like phenol:chloroform extraction.

1. Put a filter pipet tip inside a 1.5 mL tube (P200 filter tips work if you cut some of the pointy part off so that it can fit in a 1.5 mL tube. Some P10 filter tips will fit inside without cutting but have a smaller reservoir for gel pieces).
2. Cut your gel fragment out and put it into the top of the filter tip.
3. Freeze the tube at -80°C for 5 minutes (-20°C for 5 minutes also works and may actually give better yield; the gel piece needs to freeze).
4. Immediately spin the tube at 10,000-16,000xg for 3 minutes at room temperature (the spin has to begin while the gel is frozen).
5. Discard the pipet tip. The DNA is now ready for ligations and quantification by ethidium bromide plates or Qubit Quantitation (spectrophotometric quantification will not work due to the presence of ethidium bromide; phenol:chloroform extraction will remove the ethidium bromide).
6. Optionally you can precipitate the DNA or phenol:chloroform extract the DNA and then precipitate it (it is recommended to add linear polyacrylamide as a carrier for the precipitation so that you will have a pellet you can see).

Notes:

1. To maximize yield, do not fill wells of the agarose gel all the way full. Wells on an agarose gel are typically raised a little above the rest of the gel. Therefore, DNA at the top of the well may not stay in the gel during electrophoresis.
2. Sanger sequencing works better if you further purify by phenol:chloroform extraction.

A video protocol can be found here: https://rahulpatharkar.000webhostapp.com/?p=298