Purification of DNA via spin column (for DNA in a gel slice)

GENERAL NOTES:

This protocol is good for restriction fragments, small digested plasmids, and PCR products. It is NOT good for genomic or chromosomal DNA. Use the FastPrep reagents or CTAB-phenol type prep instead for genomic DNA. Digested plasmids work well up to about 5 kb, but the yield quickly falls off past this point. Generally, plasmids don't purify as well as PCR products on these columns.

Gel-purification: Don't expose the DNA to any UV light and don't use borate-containing buffers in the gel. Even minimal exposure to short-wave UV light will greatly damage DNA, and make it hard to ligate later. Long-wave UV (e.g. hand-held lamp) is less damaging than short-wave UV (transilluminator), but best to avoid UV altogether. Borate can cause problems for later enzyme steps.

NOTES ON GEL EXTRACTION USING GEL-GREEN (recommended)

1. If you need to purify DNA from an agarose gel, use TAE-agarose (don't use TBE!). Put Gel-Green (1 µl) in the gel. Load plenty of DNA so it is easy to visualize.

2. Run gel as usual, then visualize under blue illumination (NOT UV!) in a dark room or box. There is a blue light in the lab for this purpose and orange-tinted glasses also help. Cut out your band(s) of interest with a clean, sharp scalpel blade, and put them into an Eppi tube.

3. Weigh the tube containing gel slice and subtract weight of empty Eppi tube to figure out the weight of gel. Proceed to 'main protocol' below.

NOTES ON GEL EXTRACTION USING GEL-RED or ETHIDIUM (not recommended)

1. If you need to purify DNA from an agarose gel, use TAE-agarose (don't use TBE!). For this version of the protocol, you can use GelRed or ethidium bromide, either in-gel or post stain.

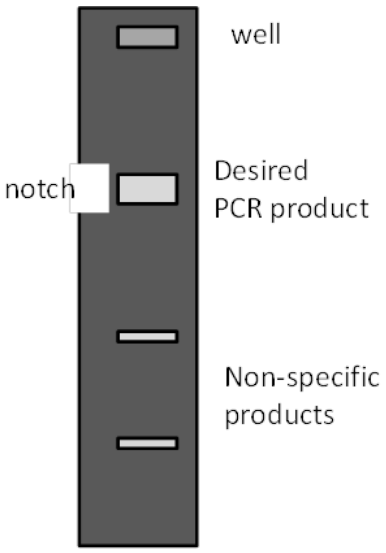
2. Run one lane with a small amount of the sample (e.g. 5 µl; 'test lane'), then leave a space, then load the rest of the lanes with the remaining sample (e.g. 6 x 50 µl).

3. After the gel has run (and stained if necc), slice off the 'test lane' and view on an old-fashioned UV transilluminator where you can cut the agarose while the UV light is on (CAUTION! wear full face shield!).

   === Do not expose the DNA that you want to use for cloning to UV light! Only expose the test lane.===

4. Make a notch in the agarose corresponding to your position and width of your band of interest (see pic for an example →)

5. Line up test gel slice with main part of gel, cut out across the other lanes in line with your notch.



6. Weigh the tube containing gel slice and subtract weight of empty Eppi tube to figure out the weight of gel. Proceed to 'main protocol' below.

MAIN PROTOCOL :

<<put on gloves + safety glasses. QG contains guanidine which is somewhat toxic >>

1. If your weight of agarose is less than 100 mg (=100 µl), then make it up to 100 µl with TE. The volume you now have will be called "1 volume". Then add "3.5 volumes" of QG buffer to your gel slice. eg. if you have 100 mg of agarose, add 350 µl of QG.

2. Slice up or mash the agarose in an Eppi (<300 mg agarose) or a McCartney bottle (>300 mg). This will speed dissolution. Melt the agarose with heating (60°C for 5 min is usually enough, mix occasionally), then when it's all dissolved, allow to cool to room temp.

3. Add "1.5 volumes" of isopropanol to the mixture. e.g. for 100 mg agarose, use 150 µl isopropanol.

4. Place a silica-based spin column (e.g. 'Econospin') into its 2 ml catch tube (if it isn't already set up that way). Load up to 750 µl of the DNA / QG / isopropanol mixture onto the column. Spin at ~10,000 g for 30 sec. Discard the flow-through into culture waste.

5. If you still have more DNA /QG / isopropanol mixture left, repeat the previous step until all of the mixture has been put thru the column. The columns will hold a total of ~10 µg DNA, which is a lot!

<<Can take off glasses and gloves now >>

6. Add 750 µl of buffer PE to the column, spin ~10,000 g for 30 sec, discard flow-through.

7. Repeat step 6.

8. Spin again for 30 sec to remove all traces of PE from the column. Discard both the flow-through and catch tube, and transfer the spin column onto a clean Kimwipe. Leave the column lid open. Transfer Kimwipe to 60°C oven, and allow to dry for 10 min.

9. Transfer spin column to a sterile 1.5 ml Eppi tube, and add 20-50 µl* of EB buffer (5 mM Tris, pH 8) to the centre of the spin column – ie on the membrane, not the walls of tube. Allow to sit for 2 min. Spin at ~10,000 g for 1 min, retain Eppi tube with DNA solution in EB, discard spin column.

* Usually we want high concentration rather than high yield, so use 20 µl. If max. yield is important, or if you have lots of DNA, use 50 µl. Note that you lose approx 5 µl EB during the procedure.

DNA PURIFICATION SOLUTIONS (SPIN COLUMN) – based on recipes at openwetware.org

Buffer QG: 5M guanidine HCl. Not sterilized.
Prepare by adding GuHCl to autoclaved RO water in a sterile bottle/tube.

Buffer PE: 10 mM Tris-HCl, pH 8, 80% ethanol. Not sterilised.

Add appropriate vol. of autoclaved 1 M Tris-HCl to the correct amount of autoclaved RO water in a sterile bottle or tube, then add 100% ethanol to give 80% final conc.

Buffer EB: 10 mM Tris-HCl, pH 8. Autoclaved.

Add appropriate vol. of autoclaved 1 M Tris-HCl to the correct amount of autoclaved RO water in a sterile bottle or tube.