Purification of DNA in 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.

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.

1. Use TAE- or LAB- buffer agarose to make the gel (don't use TBE). Put thiazole orange (TO) or Gel Green in the gel. Use more dye than is normally required, to help see the band of interest e.g. add 10 µl of TO stock (15 mg/ml in DMSO) to a 50 ml gel. Load plenty of DNA (>>200 ng) so it is easy to visualize. Use new buffer for the gel, the bottles of used buffer may have unknown DNA or protein floating in them that will contaminate your extraction.

2. In the case of a restriction digest, it is a good idea to do this in a large-ish volume (e.g. 100 µl) to dilute any impurities in the DNA, and then column-purify the digest before loading on the gel. Purifying the digest first will allow you to squeeze all your DNA into one or two wells, minimising the amount you'll need to process but also tends to give sharper bands. It's best to leave an empty lane between each different sample (if there are multiples), and to avoid the outer lanes, as they are the most likely to warp as the gel runs.

3. 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. If possible, cut off any excess gel to get a smaller slice.

Now proceed to step 4 in either of the protocols below. The 'no reagent' method is recommended.

'No reagent' method

4. If you are only extracting one band, cut a similar sized piece of empty gel to use as a balance. Place each slice in fresh (labeled!) spin column in a sterile 1.5mL tube and spin for 1 minute at max speed (~15,000 g) . You should see most of the liquid from the gel slice has collected in the 1.5mL tube but a small amount will still be to one side of the spin column.

5. Rotate the column 180° in the 1.5mL tube and spin for another minute. You can check the column and squeezed out liquid with the blue torch to see if there is any fluorescence from DNA still in the remaining gel in the column. If there is, repeat the spinning and rotating steps a couple more times and/or add some fresh TE (50 µL) to the gel slice and spin again. There should be almost no visible fluorescence in the column and a faint greenish glow in the liquid in the bottom of the tube.

6. Discard the column and get a new one. Estimate the volume eluted liquid from the gel slice by pipetting and clean up the with a standard column purification, eluting with 20-25uL of EB or TE and check the concentration with the Nanodrop.

Spin column reagents method

4. Weigh the tube containing gel slice and subtract weight of empty Eppi tube to figure out the weight of gel. 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.

5. 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.

6. Add “1.5 volumes” of isopropanol to the mixture. e.g. for 100 mg agarose, use 150 µl isopropanol.

7. 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.

8. 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!

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

10. Repeat step 6.

11. 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: 5.5 M guanidine thiocyanate (GuSCN). 20 mM Tris HCl pH 6.6.

Prepare by dissolving GuSCN in autoclaved Tris HCl. The GuSCN will take up a large fraction of the total volume, so begin with less volume, adjust to final volume with Tris at end.

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.