# Purification of DNA via spin column (for DNA in solution)

**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.

DNA will not bind well to the column if the pH is much greater than 7. Therefore, it is recommended to use "PB+ buffer" (recipe below) which contains a pH indicator, instead of plain PB.

1. Figure out what volume of sample you have. If this is less than 100 µl, then make it up to 100 µl with TE. The volume you now have will be called "1 volume". Add 5 volumes of buffer PB+ to the DNA solution. e.g. if you have a 100 µl restriction digest, add 500 µl PB+.

2. If the mixture of DNA with PB+ is orange or pink in colour, add sodium acetate (3 M, pH 4.8) dropwise (e.g. in 20 µl aliquots) until it goes yellow (=acidic). Mix after each drop added.

3. 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-PB mixture onto the column. Spin at ~10,000 g for 30 sec. Discard the flow-through into culture waste.

4. If you still have more DNA-PB+ 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!

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

6. Repeat step 5.

7. Spin the column 2 min at ~15,000 x g to remove the last traces of PE, discard collection tube, and place the column part with the lid open on a clean 'Kimwipe'. Place in 60 C° oven for ~10 min to evaporate residual ethanol. Avoid touching the 'nipple' at the bottom of the column with your fingers.

8. Transfer spin column to a sterile 1.5 ml Eppi tube, and add 20-50 µl* of EB buffer 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 PB+:** 5 M guanidine HCl, 30% isopropanol, 15 µg/ml phenol red, 10 mM sodium acetate (add sodium acetate from a conc. stock, 3 M at pH 4.8). Not sterilised. Prepare in a sterile bottle or tube containing the correct amount of autoclaved RO water. Should be yellow in colour. Add more Na-Ac if not yellow.

**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.