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.

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

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. 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.

3. 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!

<<Can take off glasses and gloves now >>

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

5. Repeat step 4.

6. 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.

7. 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. Not sterilised.

Prepare in a sterile bottle or tube containing the correct amount of autoclaved RO water.

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.