Protocol 1-2: DNA Gel Extraction

General Protocol

1) Perform agarose gel electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. It is strongly recommended, however, that fresh TAE buffer or TBE buffer be used as running buffer. Do not re-use running buffer as its pH will increase and reduce yields.
2) When adequate separation of bands has occurred, carefully excise the DNAfragment of interest using a wide, clean scalpel.
3) Determine the approximate volume of the gel slice by weighing it in a clean 1.5 ml microfuge tube. Assuming a density of 1 g/ml of gel, the volume of gel is derived as follows: A gel slice of mass 0.3 g will have a volume of 0.3 ml. Add equal volume of Binding Buffer (XP2). Incubate the mixture at 55oC- 60oC for 7 min or until the gel has completely melted. Mix by shaking or inverting the tube every 2-3 minutes. Centrifuge the tube briefly to collect all the liquid to the bottom of the tube.
4) Apply up to 700 :l of the DNA/agarose solution to a HiBind® DNA spincolumn assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at 8,000-10,000 x g for 1 min at room temperature. Discard the liquid. Re-use the collection tube in Steps 5-8. For volumes greater than 700 :l, load the column and centrifuge successively, 700 :l at a time. Each HiBind® spin-column has a total capacity of 25-30 :g DNA.
5) Discard liquid and add 300:l Binding Buffer. Centrifuge at 10,000 x g for 1 minutes.
6) Add 700 :l of SPW Buffer diluted with absolute ethanol into the column and wait 2-3 minutes. Centrifuge at 10,000 x g for 1 min at room temperature to wash the sample.
7) Optional: Discard liquid and repeat step 6 with another 700 :l SPW Buffer.
8) Discard liquid and, re-using the collection tube, centrifuge the empty column

Tips

1) For DNA fragment less than 500bp, add 1 sample volume of isopropanol after the addition of Binding Buffer (XP2).
2) Monitor the pH of the Gel/Binding Buffer mixture after the gel completely dissolves. DNA yield will significantly decrease when pH > 8.0. If the color of the mixture become orange or red, Add 5 :l of 5M sodium acetate, pH 5.2, to bring the pH down. After this adjustment, the color of the gel/Binding Buffer mixture should be light yellow.
3) Perform this second wash step for any salt sensitive downstream applications.

Reference

1) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.
2) Robert F. Weaver. Molecular Biology, McGrawHill, 4th edition, 2007
3) E.Z.N.A Gel Extraction Kit: http://www.genex.cl/stock/EZNAD250101.pdf