RetroPrep
Gel Extraction Kit

6645 Nancy Ridge Dr.
San Diego, CA 92121
Tel: 800-retrogen (738-7643) Fax: 858-455-7987
Sales@retrogen.com www.retrogen.com

RetroPrep
Gel Extraction Kit

Contents

I. Description

II. Buffers and Solutions

III. Materials to Be Supplied by the User

IV. Protocol

I. Description

The Gel extraction system is designed for fast cleanup of DNA fragments from enzymatic reactions and agarose gels. The Retrogen extraction columns contain a silica-gel membrane for binding of up to 10 µg DNA in high-salt buffer and elution in low-salt buffer. Impurities are washed away, and pure DNA is eluted in a small volume of water, ready for use in any subsequent application. The purification procedure removes nucleotides, enzymes, mineral oil, salts, agarose, polyacrylamide, ethidium bromide, dyes, detergents and other impurities from DNA samples. Retrogen silica-membrane technology eliminates the problems and inconvenience associated with loose resins and slurries. Binding buffers promote selective adsorption of DNA molecules within the size range of 70 bp to 10 kb.

Features and benefits

- Efficient removal of contaminants
- Up to 95% recovery of ready-to-use DNA
- DNA cleanup in 3 simple steps: just bind, wash, and elute
- No resins, no phenol, no alcohol precipitation

Retrogen, Inc.

RetroPrep
Gel Extraction Kit

DNA Recovery

The purity and yield of DNA following cleanup is illustrated in Figure 1 below. DNA recovery from agarose gel is typically 60-90%. Up to 10µg DNA in a maximum of 400mg agarose can be processed per gel extraction column.

Figure 1

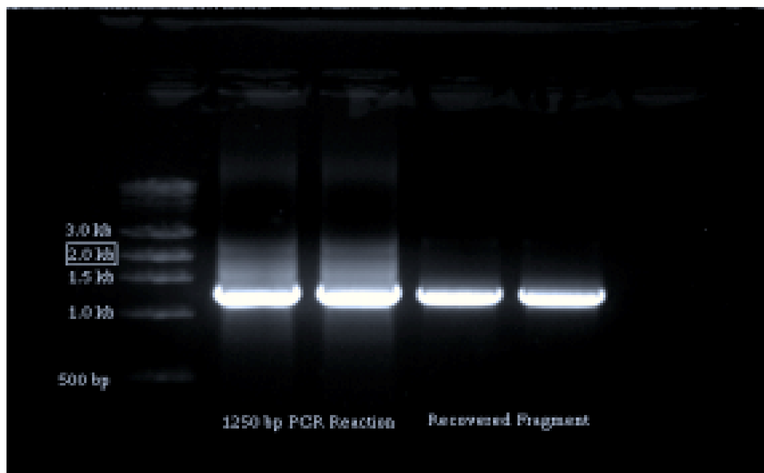


Figure 1 PCR reaction of a 1.2kb fragment shown on the left. The 1.2kb band was excised from the gel, eluted from the column in 35 µl of water and rerun on agarose gel. Recovered DNA fragment is shown on the right. M is a 1kb DNA ladder.

II. Buffers and Solutions

1. Agarose Solubilizing Solution

   Guanidine Thiocyanate
   pH Indicator

2. Column Wash Solution (100 mL buffer concentrate)

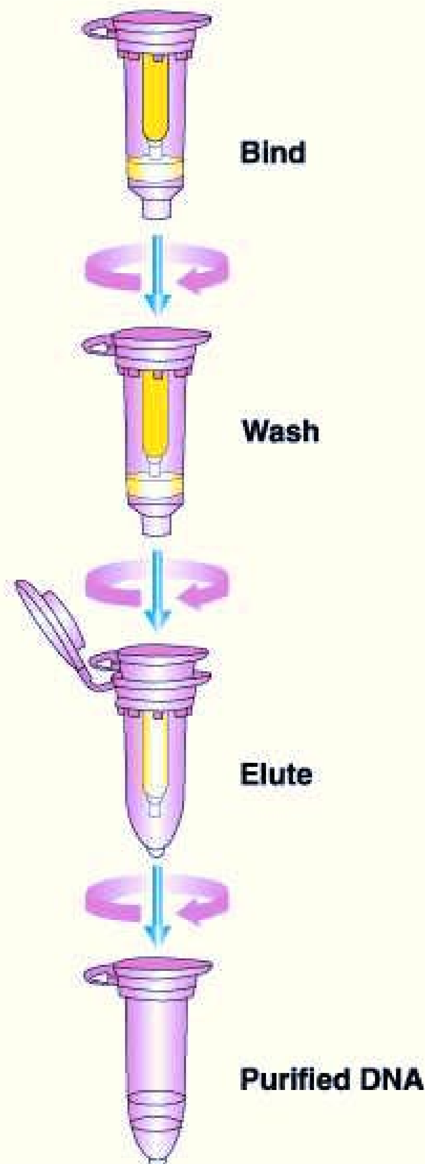
   Potassium acetate
   Tris-HCl, pH 7.5

Note: Add 170ml of 95% ethanol before use for a total volume of 270 mL.

Retrogen, Inc.

RetroPrep
Gel Extraction Kit

PCReaction or Solubilized Gel Slice



RetroPrep
Gel Extraction Kit

IV. Agarose Gel Extraction Protocol

1. Using a clean razor blade, cut out the slice of agarose from the gel which contains the band of interest. Cut as close to the band as possible to minimize the amount of agarose. Maximum size of the gel slice is 400mg per column.

2. Place the gel slice in a 1.5ml microcentrifuge tube. Add 500-600µl of Agarose Solubilizing Solution. Incubate at 40- 60°C until completely dissolved. Mix the contents by inverting the tube several times to hasten the solubilization.

3. After the gel has completely dissolved, check the color of the solution. A yellow color (similar to the original solution) is at the correct pH for DNA binding. If the solution is orange or violet, add 10µl of 3M Sodium Acetate pH 5.2 to bring the solution back to the proper pH range.

   Place a spin column in a 2 ml collection tube.

4. Apply up to 700µl of the agarose solution from step 3 to the column either by decanting or pipetting.

5. Centrifuge at 14,000 x g for 1 minute at room temperature. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.

6. If there is more than 700µl of solution from step 3, repeat steps 4 and 5 until all of the solution has been applied to the column.

7. Add an additional 200µl of Agarose Solubilizing Solution to the column to remove all traces of agarose. (optional)

8. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.

9. Add 700µl of Column Wash Solution (to which 95% Ethanol has been added, See Section II,2).

10. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.

11. Centrifuge at 14,000 x g for a full 2 minutes at room temperature. Remove the spin column from the collection tube and discard the flowthrough. This step is necessary to remove all traces of ethanol from the column membrane.

12. Transfer the spin column to a sterile 1.5ml microcentrifuge tube being careful not to transfer any of the Column Wash Solution with the spin column. To elute DNA, add 25-50µl of nuclease free water to the center of the membrane. Let stand for 1 minute. Centrifuge for 1 minute