Reagents.

Electrophoresis buffer (50x): 2M Tris-acetate, pH 8.5, 100 mM EDTA

KqGreen safe DNA dye (20,000x in water) (Shanghai Keqing Biotech)

DS™5000 molecular weight marker (Dongsheng Biotech)

The sizes of the bands (base pairs) in the molecular weight marker sample are:

5,000

3,000

2,000

1,500

1,000 (index strip)

750

500

250

100

Gel-lysis: guanidinium thiocyanate 3M, potassium acetate 0.375M, pH 5.0

Buffer W2

Elution buffer

A. Preparation of the gel.

1. 2.0g agarose was made up to 150mL with electrophoresis buffer.
2. The solution was heated in a microwave until it turned clear.
3. After the solution had cooled to about 50-60°C, 6uL of a concentrated stock of KqGreen safe DNA dye was added.
4. The mixture was gently swirled and poured into the gel tray containing the comb.
5. After the gel had set (approx 30 minutes) the comb was taken out.
6. The tape sealing the ends of the gel tray was removed and the gel tray placed in position in the tank.

B. Loading the samples and electrophoresis.

1. Add sufficient electrophoresis buffer to completely cover the gel by about 2 mm.
2. Using an automatic pipette, add 50uL of each DNA sample or the molecular weight marker to separate wells. Use a fresh tip each time.
3. When all samples have been loaded, connect the leads to the power supply, making sure that the lead connected to the negative (cathode) is nearest the wells, and run at 160V for 15 minutes.
4. After electrophoresis is complete, switch off the power pack. Take out the gel tray; take the gel to the UV gel documentation system, scan and save the picture. Analyze the positions of the bands in the different lanes.
5. Take the gel to the UV illuminator, cut the bright band out and put each band in a 1.5mL centrifuge tube.
6. Add 700uL Gel-lysis into each tube; put them into 60°C water bath for 20-30min until it is dissolved.
7. After the solution is cooled down to room temperature, pour the solution into GeneClean column with 2mL collection tube and centrifuge for 1min at 12,000rpm. After it finished,

pour the solution back to the column and centrifuge for another 1min at 12,000rpm.
8. Discard the solution, add 700uL Buffer W2 and centrifuge for 1min at 12,000rpm. After it finished, pour the solution back to the column and centrifuge for another 1min at 12,000rpm.
9. Discard the solution, centrifuge again at 12,000rpm for 2min.
10. Move the GeneClean column into 1.5 mL centrifuge tube, pipette 30uL elution buffer into the column, on the center of silica membrane.
11. Keep it still for 5mins, then centrifuge at 12,000 for 1min to collect the purified DNA.