F. Electrophoresis

1	Preparation	① take a glass bottle; ② measure 30mL 1 × TAE buffer into the bottle; ③ weighing 0.3g agarose added to the bottle; ④ use microwave oven heating about 1min to make the liquid boil; ⑤ add 3μl of Genecolor, shake the bottle to mix up; ⑥ remove the plastic box, put in the plastic sheet, insert the comb, pour the gel solution into the box; ⑦ at room temperature conditions, standing for more than 20min to make the gel solution to curdle;
2	Electrophoresis	① remove the plastic sheet, and put into the TAE buffer in electrophoresis instrument; ②remove the marker from 4 ℃ refrigerator, extract 3μL to inject into the first hole; ③ extract plasmid to inject into the PCR tube, add 1/5 plasmid's volume of 6 × DNA loading Buffer, mix up and inject into the gel hole, make sure the number was recorded; ④ open the electrophoresis device, adjust voltage to 110V, running 35min.
3	Check the Results	①turn off the electrophoresis instrument, remove the plastic sheet; ② use E-Gel™ Imager System to get the results; ③ analysis and save the image; ⑤ turn off the device; ⑥ throw the gel need not recycle into the trash, clean the plastic box and plastic sheet, put back in the drawer;
4	Calculation	Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the

		volume. For example, 100 mg of gel is equivalent to a 100 µl volume. Transfer the gel slice into a 1.5 ml microfuge tube.
5	Melting the Gel	Add a 3x sample volume of Buffer DE-A. Resuspend the gel in Buffer DE-A by vortexing. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). Heat at 40°C if low-melt agarose gel is used. Intermittently vortexing (every 2-3 minutes) will accelerate gel solubilization.
6	Combination	Add 0.5x Buffer DE-A volume of Buffer DE-B, mix. Attach the vacuum manifold to a vacuum source. Position a Miniprep column securely into one of the complementary fittings. Transfer the binding mix from Step 4 to the Miniprep column(s). Switch on the vacuum source and adjust the negative pressure to -25-30 inches Hg. Continue to apply vacuum until no liquid remains in the Miniprep column.
7	Wash	Pipette 500 µl of Buffer W1 into the Miniprep column(s). Draw all liquid through the column(s).
8	Remove Salt	Pipette 700 µl of Buffer W2 along the wall of the Miniprep column(s) to wash off all residual Buffer W1. Draw all liquid through the column(s) . Repeat this wash step with a second 700 µl aliquot of Buffer W2. Transfer the Miniprep column into a 2 ml microfuge tube (provided) and centrifuge at 12,000xg for 1 minute to purge residual Buffer W2 from the binding membrane.
9	Collection	Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.