Modified Prep-A-Gene DNA Isolation from Agarose Gel

Procedures

1. Run approximate 2-3 μg of digested DNA on an agarose gel to separate the DNA fragments.

2. Acquire DNA gel image for documentation, cut the DNA band of interest (as little gel as possible without losing the DNA) under the preparative wavelength and rinse the gel twice with distilled water. Cut the gel into small pieces.

3. Put the gel pieces into a pre-weighed 1.5-ml tube and measure the weight of the gel.

4. Add Prep-A-Gene Binding Buffer to the gel (2.5 ml/g).

5. Incubate at 65°C for 5 min and vortex to completely dissolve the gel. Vortex Prep-A-Gene Matrix very well.

6. Add Prep-A-Gene Matrix (5 μl/μg DNA) to the dissolved gel mixture, gently mix and incubate at room temperature for 10 min.

7. Spin for 30 sec and gently pour off the supernatant and keep the matrix.

8. Add 500 μl of Prep-A-Gene Binding Buffer, vortex and spin for 30 sec and then remove the supernatant.

9. Add 500 μl of Prep-A-Gene Wash Buffer to the matrix, vortex and spin for 30 sec and then remove the supernatant. Repeat this step two more times.

10. Add 40 μl of Prep-A-Gene Elution Buffer, incubate at 65°C for 10 min, spin for 30 sec. Transfer the supernatant to a fresh tube. Repeat the elution one more time and combine the supernatants.

11. To the combined supernatant add 8 μl of 3 M NaAc and 180 μl of 100% ethanol, vortex for 20 seconds and spin for 20 min at room temperature.

12. Drain the supernatant and add 500 μl of 70% ethanol. Incubate on ice for 5 min and spin for 5 min. Decant the supernatant and speed vacuum dry the sample for about 4 min.

13. Resuspend the DNA pellet in 10 μl 1X TE.