Recovery of DNA from LM Agarose Gels with Agarase

Protocol

This protocol is for the Recovery of DNA from LM Agarose Gels with Agarase

1. Perform electrophoresis of DNA in low melting point (LM) agarose (#R0801) gel prepared in TAE (#B49), 0.5X TBE, TBE (#B52) or TPE buffer. Stain the gel with ethidium bromide.
2. Cut out the desired DNA band from the agarose gel with a clean scalpel under UV light*. Cut out only as much agarose as it is necessary. (The bottom of the excised agarose is free of DNA and should be removed.)
3. Determine the weight of the slice. To facilitate melting, cut gel slices larger than 200 mg into smaller pieces.
4. Incubate the tube at 70°C for approx. 10 min. Ensure that the agarose is completely melted.
5. Transfer the tube to a 42°C water bath and equilibrate for 5 min.
6. Add 1 u of Agarase (#EO0461) per 100 mg (approx. 100 µl) of molten 1% low melting agarose. Increase the amount of enzyme proportionally for higher percentage agarose, gently mix and incubate at 42°C for 30 min.
7. Add ammonium acetate** to a 2.5 M final concentration, chill on ice for 5 min.
8. Centrifuge at 10,000 rpm for 10 min to pellet undigested carbohydrates. Transfer the supernatant to a clean tube.
9. Add 2.5 volumes of ethanol or 0.8 volume of isopropanol, mix gently and incubate at room temperature for 1 h. If DNA fragments are smaller than 500 bp or if the DNA concentration is lower than 0.05 µg/ml, incubate at room temperature for 2 h.
10. Centrifuge at 10,000 rpm for 15 min, remove supernatant and dry the pellet. Resuspend the pellet in TE or another appropriate buffer for subsequent manipulation.

Note

** For subcloning of gel-purified DNA fragments, care should be taken to avoid DNA damage with UV light. Minimize the UV exposure to a few seconds or keep the gel on glass or plastic plate during UV illumination. Alternatively, visible dyes can be included in standard agarose gels to visualize DNA bands in ambient light (1, 2).*

*** Ammonium acetate is recommended rather than other salts to avoid co-precipitation of oligosaccharides with DNA.*

- The procedure typically recovers 90% of DNA from the gel.
- For evaluation of DNA yield use DNA ladders/markers, which are ideal for in-gel DNA quantification.
- T4 polynucleotide kinase is inhibited by ammonium ions, therefore use 1 M Sodium Acetate, (0.3 M final concentration) if T4 polynucleotide kinase will be used in downstream applications.
- Large DNA fragments (>30 kb) require delicate handling to avoid mechanical shearing. After digestion with agarase (step 6), centrifuge at maximum speed for 10 min to pellet undigested carbohydrates. Remove oligosaccharides and agarase by dialysis or carry out subsequent manipulations with DNA in the digested agarose solution.

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References

1. Rand, K.N., Crystal Violet can be used to Visualize DNA Bands during Gel Electrophoresis and to Improve Cloning Efficiency, *Elsevier Trends Journals Technical Tips Online*, T40022, 1996.
2. Adkins, S., Burmeister, M., Visualization of DNA in agarose gels and educational demonstrations, *Anal. Biochem.*, 240 (1), 17-23, 1996.

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