DNA Extraction from Agarose Gels with the Ultrafree™-DA Centrifugal Filter Device

The Ultrafree-DA device is designed to recover 100 to 10,000 bp DNA from agarose gel slices in one 10-minute spin. It consists of a pre-assembled sample filter cup with agarose Gel Nebulizer, and a microcentrifuge vial. The device utilizes gel compression to extract DNA from the agarose. Centrifugal force collapses the gel structure, drives the agarose through a small orifice in the Gel Nebulizer and the resultant gel slurry is sprayed into the sample filter cup. As the agarose is compressed at 5,000xg, DNA is extruded from the gel's pores. The gel matrix is retained by the microporous membrane, and the DNA passes freely through the membrane. DNA can then be recovered in the filtrate vial.

DNA prepared with this device requires no further purification for most applications, including cloning and radioisotopic or fluorescent DNA sequencing. Since agarose gel electrophoresis has high resolving power, the small and large non-specific amplification products that frequently interfere with cloning and sequencing after PCR (polymerase chain reaction) are completely removed from the product.

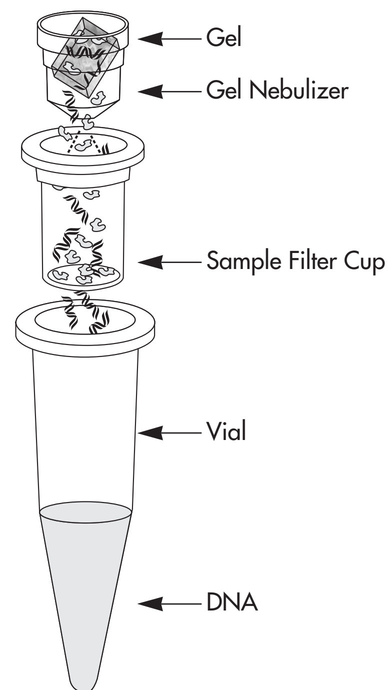
Materials

- Microcentrifuge
- Pre-assembled Ultrafree-DA centrifugal filter device
- Modified TAE* electrophoresis buffer (40 mm Tris-acetate, pH 8.0, 0.1 mm Na_2EDTA)
- SeaKem™ LE agarose (FMC BioProducts; Rockland, ME) or equivalent
- Long-wavelength UV lamp
- Scalpel or razor blade

* Modified TAE rather than TBE is recommended for the following reasons: (1) TBE buffer strongly inhibits DNA sequencing reactions while modified TAE buffer does not. (2) Modified TAE has 0.1 mm Na_2EDTA while regular TAE has 1.0 mm Na_2EDTA. The EDTA level at 0.1 mm Na_2EDTA will not interfere with the magnesium concentration in sequencing reactions and other downstream enzymatic treatments, many of which are dependent on magnesium.

Procedure

1. Electrophorese 30 µL of PCR product or other DNA through a <1.25% ordinary agarose gel, prepared in modified TAE buffer with ethidium bromide (0.5 µg/ml).
2. Locate band of interest with a long wavelength UV lamp or transilluminator. With a razor blade or scalpel, cut out the slice of agarose (<100 µL or 100 mg) containing the band of interest. Trim any excess agarose away from band.



3. Place gel slice into Gel Nebulizer sample cup assembly and seal device with the cap attached to vial.

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4. Spin at 5,000xg for 10 minutes. Centrifugation forces the agarose through the Gel Nebulizer, converting it to a fine slurry that is captured by the sample filter cup. Extruded DNA in electrophoresis buffer passes through the microporous membrane in the sample filter cup and collects in the filtrate vial.
5. DNA in the filtrate is now ready for sequencing or cloning without further purification. After discarding the filter cup and Gel Nebulizer sample cup assembly, the DNA can be stored in the capped filtrate vial.

Typical DNA Recoveries

Gel Compression is a quick and easy technique for recovering DNA from an agarose gel slice. The Ultrafree-DA includes a Gel Nebulizer that allows the addition of an intact gel slice to the Nebulizer/Sample Cup stack. There is no need to manually cut up the gel slice since gel disintegration is automatic. With Ultrafree-DA the gel is completely macerated by the Nebulizer, thus recoveries of DNA are typically higher than when this component is not incorporated.

Typical DNA Recoveries

	Percent DNA Recovered	
DNA size (bp)	Intact Gel	Gel Nebulizer Disrupted Gel
100	74	78
400	39	ND
700	43	71
1000	55	77
2027	ND	47
4361	14	35
9416	ND	32
23130	ND	29

ND = Not detectable.

Table 1. Typical DNA recoveries from agarose gels: Effect of gel disruption.

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