Sieving Agarose Gel Electrophoresis

Sieving agarose is a specially treated type of agarose designed to be used at high concentrations. Sieving agarose gels are poured and run like conventional agarose gels, but resolve small DNA fragments like nondenaturing polyacrylamide gels.

Materials

Low gelling/melting temperature sieving agarose
TAE buffer, pH 8 (*APPENDIX 2*)
1 M NaCl
10 mg/ml tRNA
Buffered phenol at room temperature (*UNIT 2.1*)
Ethanol or isopropanol
TE buffer, pH 8 (*APPENDIX 2*)

Additional reagents and equipment for agarose gel electrophoresis (*UNIT 2.5*)

1. Melt 2% to 4% low gelling/melting temperature sieving agarose in TAE buffer. Pour gel of desired size for ordinary agarose gel apparatus.
2. Load sample and run gel as for an ordinary agarose gel (*UNIT 2.5*). Bromphenol Blue will migrate at ~50 bp for a 2% gel.
3. For isolation of fragment follow steps 2 to 9 of alternate protocol for low gelling/melting temperature agarose (*UNIT 2.6*).

COMMENTARY

Background Information

The unique pore size of sieving agarose allows separation of much smaller fragments than ordinary agarose. From 2 to 4% sieving agarose gels separate in the range of 10 to 1000 bp and provide an alternative to polyacrylamide gels. Although the bands on the sieving gels are somewhat more diffuse and the resolution is slightly poorer, sieving gels are easier and faster to pour and run, making them useful for a variety of applications, such as checking ligation of linker monomers into ladders (*UNIT 3.16*). Like low gelling/melting temperature agarose, sieving agarose is used to purify DNA fragments because of its unique properties of depolymerizing at 65°C and remaining liquid at 29°C. However, DNA fragments prepared from polyacrylamide gels are generally cleaner and can be more reproducibly used as substrates for a variety of enzymes after gel purification.

Critical Parameters

Low gelling/melting temperature agarose does not have the integrity of regular agarose, and sieving agarose is even more fragile than the common low gelling/melting temperature agaroses. The manufacturers recommend that you do not use agarose concentrations of less than 2%. Extreme care should be taken when pulling the combs out of the gel, as wells tear easily. Because low gelling/melting temperature agarose depolymerizes at 65°C, maintain a low voltage (6 to 7 V/cm of gel) to keep the gel from heating too much. In 4% gels, using a TAE buffer system, the Bromphenol Blue marker migrates at ~50 bp.

Anticipated Results

Yields are similar to conventional low gelling/melting temperature agarose (≥ 70%) as long as fragments are < 1000 bp. These gels resolve best below 500 bp.

Time Considerations

The procedure is short, especially for minigels, and can be completed in 2 hr. After the addition of ethanol to the extracted fragment, the protocol can be stopped for as long as desired.

Key Reference

Sieving agarose was developed by FMC Marine Colloids. Literature describing the use and properties of sieving agarose is available from FMC.

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