SSCP Analysis

Single stranded DNA can adopt multiple conformations under non-denaturing conditions. In the absence of a complementary strand, DNA will anneal short internal complementary sequences, forming a complex "knot." A DNA molecule follows a complex path of folding to reach its final form, influenced by the solution environment and temperature. The path consists of a series of annealing steps, each stabilizing and to some extent directing the next. Minor alterations in the sequence of the DNA will disrupt the annealing process and result in a different final shape. The compactness of these structures will determine how fast the single stranded DNA migrates through a non-denaturing gel. Such differences in electrophoretic mobility between nearly identical strands serve as the basis for the technique known as single strand conformation polymorphism (SSCP) analysis, and like heteroduplex analysis, it is a rapid method for mutational screening.

SSCP (Single Strand Conformational Polymorphism) Analysis. Single point mutations can cause major differences in the folded form of single stranded DNA. These differences can be detected as differences in electrophoretic mobility.

Samples are denatured with heat and then rapidly cooled. Rapid cooling favors self-annealing, because insufficient time is allowed for complementary strands to collide and orient for duplex formation. The renatured samples are analyzed on a gel opposite the control DNA. As with Heteroduplex Analysis, all fragments must be the same length. Mutant samples will show a mobility different from the control DNA. The gel matrix used must be optimized for the resolution of DNA conformers of the same length. Various combinations of Acrylamide and Bis-Acrylamide are mentioned in the literature, at ratios from 29 to 1 to 50 to 1, and at percentages from 4 to 8. National Diagnostics' SequaGel MD is optimized to provide superior results in both Heteroduplex and SSCP Analysis.

SSCP Analysis

GEL PREPARATION:

1. Preparation of Working Solutions:

   To cast a 0.4 mm thick gel (>40 cm vertical gel recommended) combine the following in an Erlenmeyer flask:

   25 ml SequaGel MD
   6 ml 10X TBE

   Fill to 100 ml with deionized water and mix thoroughly.

   Prepare 0.6 X running buffer by diluting 60 ml of 10X TBE stock to 1 L with deionized water.

2. Casting the gel:

   Add the following to the solution and swirl gently:

   40 µl TEMED
   400 µl freshly prepared 10%
Using standard acrylamide procedures, pour the gel solution into the cassette, insert the comb (inverted if using a sharkstooth comb), and allow polymerization at room temperature for a minimum of 60 minutes. Attach the gel cassette to the electrophoresis apparatus.

SAMPLE PREPARATION:

1. PCR amplification:

   *PCR conditions should be optimized for desired PCR product before SSCP analysis is undertaken. It is recommended that the minimum number of PCR cycles be used on a purified, salt-free template, and that reagent and primer concentrations be optimized. If radiolabeling is going to be utilized instead of silver staining, end-labeled primers may be used, or an α-32P dNTP may be included in the PCR amplification.*

2. After PCR thermal cycling, add EDTA to a final concentration of 5 mM (1 µl of 0.5 M EDTA per 100 µl reaction) to inactivate the Taq DNA Polymerase.

ELECTROPHORESIS:

1. Rinse the gel wells with running buffer. The sharkstooth comb should be reinserted so that it just touches the surface of the gel, and 1 to 3 µl of the sample should be loaded.

2. Run the gel at a constant power of 6-8 Watts for 14 hours.

3. If the DNA was radiolabeled, transfer the gel to Whatman 3MM filter paper, place on a flat surface, and cover with plastic wrap. Dry the gel and, using standard technique, expose to X-ray film. Silver staining may be used for detection. Ethidium Bromide is not effective at detecting single stranded DNA.

NEXT TOPIC: Agarose Gel Electrophoresis of DNA & RNA

Products Related to this Discussion:

**SequaGel MD**
Gel solution designed for the detection of minor mutational differences in SSCP analysis and heteroduplex analysis.

**Ammonium Persulfate - ULTRA PURE**
Exceeds ACS standards. Low absorbed water results in consistent initiation.

**TBE Buffer (10X)**
Formulated with 18 MegOhm water. 0.2 micron filtration. The most stable TBE on the market.

**TEMED - ULTRA PURE**
Fractionally distilled to remove all trace metals and amine impurities. Stored under nitrogen.