national diagnostics

SequaGel® *MD*

MUTATION DETECTION

- ***Point Mutation Analysis***
- ***SSCP Analysis***
- ***Heteroduplex Analysis***

SequaGel MD permits minor mutational differences in DNA sequences to be detected as a high resolution relative mobility (R_f) shift. SequaGel MD is a proprietary formulation, supplied as a 2X stock, designed to resolve such sequence related differences by SSCP (Single Strand Conformational Polymorphism)[5,7] and Heteroduplex Analysis[4].

DNA mutations, or sequence modifications, are readily associated with specific disease states[1]. Since a variation of a single nucleotide in a sequence may indicate a significant genetic anomaly, an extremely sensitive method to analyze these mutations is necessary.

Two principle methods of analyzing conformational differences have been developed. National Diagnostics' SequaGel MD can be applied to both of these methods as follows:

SSCP Analysis has been demonstrated to be effective in detecting polymorphisms of single base differences[3,6]. In brief, the method involves:

1. Amplification of a discrete sequence of DNA by PCR*.
2. Denaturation (separation) of the double stranded PCR product.
3. Snap cooling of the denatured DNA to maximize self-annealing of individual strands.
4. Analysis of the differences in relative mobility (R_f) of the single strands by electrophoresis in National Diagnostics' SequaGel MD. See figure below.

SequaGel MD is designed to run SSCP samples in the 100-300 nucleotide range at a 0.5X concentration. Small fragments may be better resolved by using higher concentration (0.75X) gels. Larger fragments may be better resolved by running lower concentration (0.4X) gels.

Heteroduplex Analysis is an effective method of sequence mutation analysis which compares double stranded heteroduplex chain formation to a standard homoduplex. In brief, the method involves:

1. Amplification of a discrete sequence of DNA by PCR.
2. Denaturation of a mixture of homoduplex control fragment with homoduplex test sample fragment.
3. Reannealing of the mixture of strands creating homoduplex and heteroduplex strands.
4. Analysis of the differences in relative mobility (R_f) of the resulting double strands by electrophoresis in National Diagnostics' SequaGel MD. See figure below.

SequaGel MD is designed to run heteroduplex analysis on DNA fragments up to 900 bases at a 1X concentration. The proprietary formulation of National Diagnostics' SequaGel *MD* results in clear, sharp bands, and a larger shift in mobility of the heteroduplex relative to the homoduplex.

FIGURE 1: SSCP ANALYSIS

Wild Type Control *Mutant Sample* Wild Type+*Mutant*

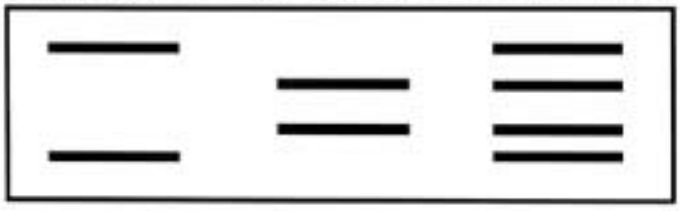

FIGURE 2: HETERODUPLEX ANALYSIS

Wild Type Control *Mutant Sample* Wild Type+*Mutant*

*Heteroduplexes

*The PCR process is covered by U.S. patents owned by Hoffmann-LaRoche Inc.