**Chapter 9 Gene Mutations**

Point Mutations

* Gene mutations involving one or few base pairs
* Not detectable by the cytogenetic method
* Detected at the DNA sequence level
* Point mutations do not always have phenotypic effect

Types of Mutation Detection Methods

* Hybridization based
* Sequencing (polymerization) based
* Cleavage based

Hybridization-Based Methods

* SSCP
* ASO
* Melt curves
* Array technology

Single-Strand Conformation Polymorphism

* Scans several hundred base pairs
* Based on intra-strand folding
  + Single strands will fold based on sequence.
  + One base difference will affect folding.
* Folded single strands (conformers) can be resolved by size and shape.
* Strict temperature requirements
* Procedure
  + Amplify region to be scanned using PCR
  + Denature and dilute the PCR products
  + Separate conformers by PAGE or CGE
  + Analyze results by comparison to reference normal control (+)
  + Detect PAGE bands by silver staining

Allele-Specific Oligomer Hybridization (ASO)

* Three specimens spotted on duplicate membranes
* One membrane exposed to probe complementary to the normal sequence (+ probe)
* One membrane exposed to probe complementary to the mutant sequence (m probe)
* Chromogenic probe detection
  + 1: normal (+/+)
  + 2: heterozygous (m/+)
  + m: heterozygous mutant control
  + +: normal control
  + N: negative control

Melt Curve Analysis

* Based on sequence effect on Tm
* Can be performed with or without probes
* Requires double-strand DNA–specific dyes
  + Ethidium bromide
  + SyBrGreen
* Also performed with fluorescence resonance energy transfer (FRET) probes
* Double-stranded DNA–specific dye (SyBrGreen) will fluoresce when bound to DNA.
* Denaturation of DNA to single strands will result in loss of fluorescence.
* Every sequence has a characteristic Tm.
* Melt curve Tm indicates which sequence is present.
* Detection instrument software may convert the melt curve to a derivative of fluorescence (speed of drop vs. temperature).

Array Technology

* Reverse dot blot methods
* Used to investigate multiple genomic sites simultaneously
* Unlabeled probes are bound to substrate.
* Specimen DNA is labeled and hybridized to immobilized probes.

High-Density Oligonucleotide Arrays

* Interrogate thousands of genes simultaneously
* Requires a new array for each sample
* Unlabeled probes are synthesized on the substrate.
* Test DNA is fragmented before hybridization.
* Short fragments will bind specifically to complementary sequences on the array.
* Tiling (overlapping probe sequences) is used to blanket detection of nucleotide changes in the sample.
* Fluorescent signal indicates which sample hybridized DNA to probe.
* Fluorescence is detected, normalized, and averaged by array readers and software.

Sequencing (Polymerization)-Based Methods

* Sequence-specifc PCR (SSP-PCR)
* Allelic discrimination
* Direct sequencing
* PCR primer extension requires that the 3′ base of the primer is complementary to the template.
* Primer design is used to detect mutations in DNA.
* Generation of PCR product indicates the presence of mutation or polymorphism in the template.

Allelic Discrimination

* Uses fluorescently labeled probes
* Similar to TaqMan technology
* Generates “color” signal for mutant or normal sequence
* Performed on real-time PCR instruments
* Probe complementary to normal sequence labeled with FAM fluorescent dye
* Probe complementary to normal sequence labeled with VIC fluorescent dye
* Signals are detected and analyzed by the instrument software.
* Multiple samples are analyzed simultaneously.

Cleavage-Based Methods

* Restriction fragment length polymorphism (RFLP)
* Nuclease cleavage
* Invader (Hologic)

Restriction Fragment Length Polymorphism (RFLP)

* Restriction enzyme site recognition detects presence of sequence changes.

e.g., G->A change creates *EcoR1* site:

Heteroduplex Analysis with Single-Strand-Specific Nucleases

* Uses nucleases that cut single-stranded bubbles in heteroduplexes.
* Region of interest is amplified by PCR.
* PCR product is denatured and renatured with or without added normal PCR product.
* Renatured duplexes are digested with nuclease; e.g., S1 nuclease.
* Products are observed by gel electrophoresis.

Invader Technology

* Mutation detection with proprietary Cleavase enzyme
* Sample is mixed with probes and enzyme.
* Enzyme cleavage of probe-test sample hybrid will yield fluorescent signal.
* Signal will only occur if probe and test sample sequence are complementary.

Summary

* Mutations and polymorphisms are changes in the DNA sequence.
* DNA sequence changes have varying effects on the phenotype.
* Molecular detection of mutations include hybridization-, sequence-, or cleavage-based methods.