

EPICENTRE'S Customer Focus

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I have been incorporating genomics into my professional and academic work since around 1996. Currently, I work for IBT Reference Laboratory in Kansas City, KS and focus on immunology, spending most of my time designing assays that can be used in allergy determination, medicinal clinical trials, and basic research. Although I work on a variety of projects, in several different species, I will focus on the continually expanding field of single nucleotide polymorphisms (SNPs) and my collaborations with EPICENTRE.

I have used EPICENTRE as a source for molecular biology reagents after becoming frustrated with large, non-specialized vendors. Recently, I used a FailSafe™ Real-Time PCR System to develop a reliable PCR protocol to amplify genomic DNA and determine SNPs within the interleukin-4 (IL-4) receptor gene.

PCR can produce poor results in sections of DNA template with high GC content. This is particularly problematic in SNP evaluation, where it is necessary to observe multiple melt-curve peaks. The multiple peaks generated in the melt-curve analyses can easily overlap if the PCR reaction is not fully optimized, making evaluation of the different alleles difficult. The current protocol for analyzing SNPs uses hybridization probes.

EPICENTRE does not currently offer a product for the capillary tube PCR system that does not contain SYBR® Green I, which is incompatible with hybridization probes. However, Fred Hyde, one of EPICENTRE's technical service scientists, has been willing to work with me and sent me a custom "beta-test" kit which is essentially the FailSafe™ Real-Time PCR Capillary PreMix Selection Kit without the SYBR® Green I. This allowed me to efficiently optimize many SNP reactions so that a panel of SNPs, within a particular gene, could be evaluated using multiplex PCR reactions.

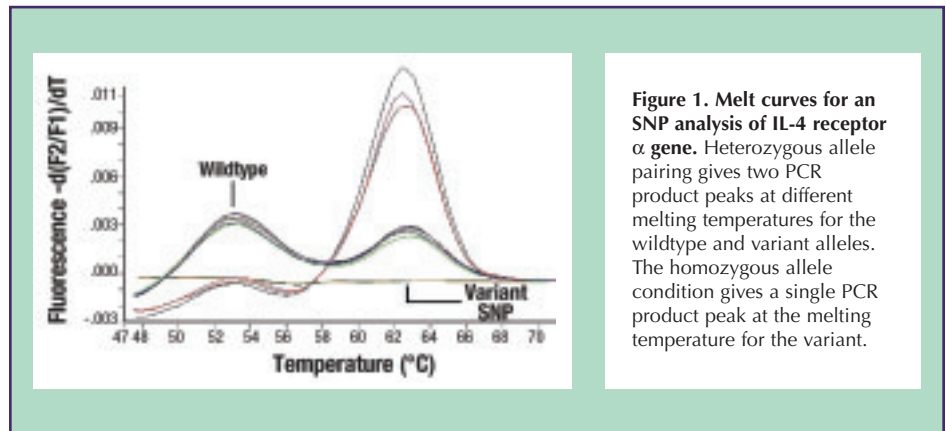


Figure 1. Melt curves for an SNP analysis of IL-4 receptor α gene. Heterozygous allele pairing gives two PCR product peaks at different melting temperatures for the wildtype and variant alleles. The homozygous allele condition gives a single PCR product peak at the melting temperature for the variant.

Figure 1 shows melt curves for an SNP analysis of the recently optimized IL-4 receptor α gene. The graph shows several DNA samples displaying either a heterozygous allele pairing (two PCR product peaks with different melting temperatures) or a homozygous allele condition (single PCR product peak) with the presence of a variant nucleotide.

In addition to SNP analyses, I am also exploring mRNA expression in allergy-mediating cells, such as basophils, in order to measure the *in vivo* expression potential for different forms of allergies. For gene expression I use non-capillary based real-time PCR and use the FailSafe™ Real-Time PCR System for primer development.

The goal of most of my assays is to transfer them to our commercial lab, which requires an easily standardized methodology. Establishing a consistent assay is important because, due to the technical complexity of our analyses, it can be difficult to train scientists and technicians who are new to PCR and gene quantification. With FailSafe I am able to quickly optimize a PCR reaction and then transfer the technology within our company, while maintaining robust, stable, and quantifiable results.

www.epicentre.com/pcr.asp

FailSafe™ Real-Time PCR Capillary PreMix Selection Kit

FSRC3832 32 Reactions

Contents:

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8 FailSafe™ Real-Time PCR Capillary
2X PreMixes

FailSafe™ Real-Time PCR Capillary System

FSRC3896 96 Reactions
FSRC38384 4 X 96 Reactions

Contents:

FailSafe™ PCR Enzyme Mix
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