

# EPICENTRE'S Customer Focus

## FailSafe™ PCR System SequiTherm EXCEL™ II DNA Sequencing Kit - LC

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Molecular diagnostics, the accurate diagnosis of disease based on the patient's specific genetic information, is becoming more widely used as a tool to both determine the cause of an illness and develop an appropriate therapy. Horizon Molecular Medicine, in Atlanta, Georgia, was established to "provide metabolic and molecular genetic testing and consultation for a variety of inherited disorders affecting children and adults." Dr. Frances Kendall directs the Metabolic Laboratory and Dr. John Shoffner directs the Molecular Laboratory.

Due to Dr. Shoffner's expertise in mitochondrial diseases,<sup>1</sup> Horizon Molecular Medicine offers both the complex biochemical enzyme analysis in muscle tissue and the mitochondrial DNA sequence analysis necessary to diagnose oxidative phosphorylation disorders, which result in decreased ATP production. The molecular lab also does nuclear DNA testing for Angelman syndrome, Huntington's (also called Huntington's) syndrome, Fragile X, Medium Chain Acyl CoA dehydrogenase (MCAD) deficiency, Muscular dystrophies, and Prader-Willi syndrome. Horizon's website, [www.horizonmedicine.com](http://www.horizonmedicine.com), details the diseases, symptoms, and required tests for diagnosis.

*"The FailSafe PCR System and SequiTherm EXCEL II DNA Sequencing Kit – LC, therefore, play pivotal roles in maintaining turn-around-times of results, a very important function for Horizon Molecular Medicine."*

Dr. David Calnek joined Horizon Molecular Medicine in January 2004 as the Research Laboratory Manager. Typically he receives specimens from clin-



Members of Horizon Molecular Medicine's Molecular Diagnostic Lab include, from top to bottom: Tanya Frolov, Jan Gurr, David Calnek, Will Reid, Jeff Dale, Genevieve Niedziela, Kevin Bennett, Edward Kritchevski, Audra Aliberti, and Matthew Phillips.

ics in the Atlanta area or from referral labs outside of the area, which don't have the available molecular diagnostic resources. David outlined the process of mitochondrial DNA sequence analysis for us.

Initially, the 16.6-kb human mitochondrial DNA is amplified in 31 PCR reactions using EPICENTRE's FailSafe™ PCR System. David has found that Buffer D usually works for this amplification and starts with that buffer. If Buffer D is unsuccessful, he uses the FailSafe™ PreMix Selection Kit and a gradient thermocycler to vary reaction temperatures over a range of 20°C to determine the optimum reactions conditions.

After PCR results are confirmed on a gel, the PCR products are cleaned up and sequenced using EPICENTRE's SequiTherm EXCEL™ II DNA Sequencing Kit – LC and a LI-COR DNA Analysis System. After running 3 gels, with 11 samples each, the results are aligned to confirm the sequence and analyzed for polymorphisms and mutations.

According to David, "By utilizing EPICENTRE's FailSafe PCR System, we are able to obtain robust amplified PCR products through rapid optimization of

PCR conditions of newly designed DNA primers. These PCR products maintain excellent sample integrity for the numerous diagnostic applications we provide at Horizon Molecular Medicine including DNA sequencing, point mutation and RFLP analysis, and DNA probe generation. The FailSafe PCR System and SequiTherm EXCEL II DNA Sequencing Kit – LC, therefore, play pivotal roles in maintaining turn-around-times of results, a very important function for Horizon Molecular Medicine."

### Reference

1. Shoffner, J. M. (2000) *Neurol. Clin.* **18**(1),105

SEE ASK FRANK ON PAGE 8 AND  
PRODUCT DATA SHEET ON PAGE 9

FOR MORE INFORMATION ON  
SEQUITHERM EXCEL™ II DNA  
SEQUENCING KITS.

info