Courtagen
DIAGNOSTICS LABORATORY

Courtagen's mtSEEK®

Mitochondrial Disease

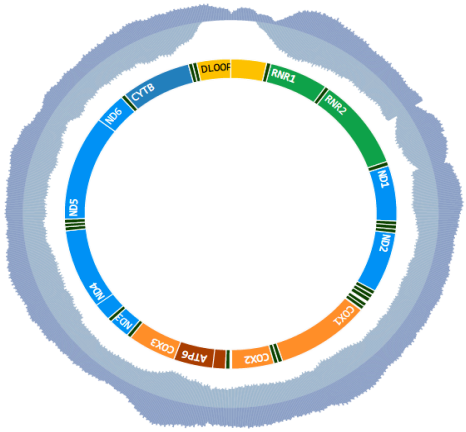
Technical Note: Mitochondrial Genome (mtDNA) Sequencing with Deletion Detection and Heteroplasmy Analysis

Introduction

Mitochondrial disorders are very diverse conditions and can affect one or multiple systems of the body. Mitochondrial dysfunction depletes cells of energy, causing cell damage and even cell death. Diseases of the mitochondria appear to cause the most damage to cells of the brain, heart, liver, skeletal muscles, kidney and the endocrine and respiratory systems.

Highly Accurate Sequencing

Genomic DNA extracted from the sample submitted is amplified using independent primer sets, enriching the mitochondrial genome into two PCR pools, which are subsequently barcoded into two libraries. Each library is then sequenced to over 5,000x coverage by 250bp paired-end Next Generation Sequencing. Results are mapped to the revised Cambridge Reference Sequence (rCRS) using Courtagen's proprietary Ziphyr® bioinformatics pipeline. Concordant variants detected in both pools of DNA were considered valid and confirmed, with any discordant results of clinical significance confirmed using di-deoxy (Sanger) sequencing. Sequencing techniques used by Courtagen allow for rapid generation of a gigabase of sequence per patient.



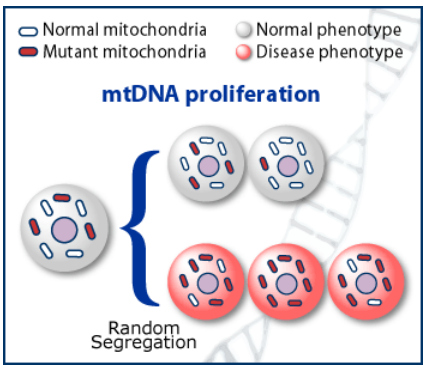
Coverage Map from a patient sample: In the diagram above, the darker outer circle is from a pool of 2 ~9kb fragments, while the inner circle represents a single ~16kb LR-PCR amplicon. The diagram illustrates evenness of coverage over the entire deeply sequenced mitochondrial genome. In this example, average coverage was ~15,000x with a minimum coverage greater than 5,000x for each amplicon.

Deletion Detection

Large mitochondrial DNA rearrangements that arise are often *de novo* and are almost always heteroplasmic. Courtagen's mtSEEK® test detects deletions of greater than 500 bases with heteroplasmy sensitivity as low as 5% for greater than 98% of deletions listed in mitomap.org.

Heteroplasmy Detection

Heteroplasmy controls are run with each patient sample as a routine quality control measure. This allows the laboratory to ensure run to run consistency. Two NIST control DNA samples are mixed at defined ratios (90%:10%, 95%:5%, 98%:2%, 99%:1%). Samples are assessed for 32 known heteroplasmy locations and reported with each sequencing run.

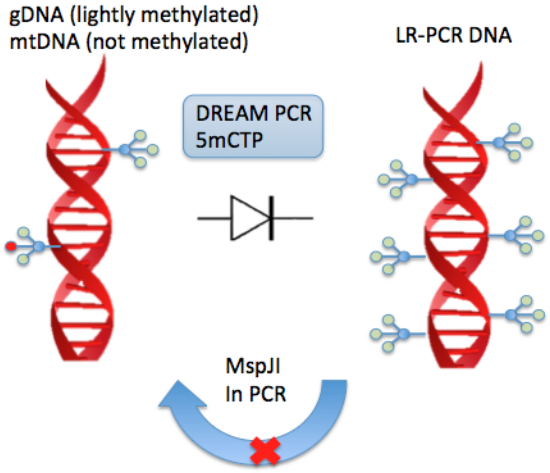


Pseudogene Avoidance

Long Range PCR has been shown to significantly reduce regions of nuclear mitochondrial DNA homologs (NUMTs) captured compared to other methods widely used in the field such as amplification with several shorter range PCR primers and in solution hybridization capture technologies[1,2]. Furthermore, primers used by Courtagen for amplification have been rigorously tested to ensure no off target amplification of NUMTs.

Contamination Control

Courtagen's proprietary decontamination ready encoded amplification (D.R.E.A.M.) PCR results in exceptional laboratory decontamination, which goes above and beyond separating pre and post-PCR laboratories with physical partitions.[3] D.R.E.A.M. PCR enzymatically obliterates post-PCR amplified products from contaminating a different patient's sample in the laboratory.



Mitochondrial Tests Available from Courtagen

mtSEEK® Mitochondrial Genome (mtDNA) Sequencing with Deletion Detection and Heteroplasmy Analysis

nucSEEK® Comprehensive Sequence Analysis of the Nuclear Mitochondrial Exome

Contact Us

Phone: 877.395.7608 | Fax: 617.892.7192 | Email: genomics@courtagen.com. | www.courtagen.com

References

1. Li, M. *et al*. Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. *Am. J. Hum. Genet*. **87,** 237–249 (2010).

2. Cui, H. *et al*. Comprehensive next-generation sequence analyses of the entire mitochondrial genome reveal new insights into the molecular diagnosis of mitochondrial DNA disorders. *Genet. Med*. **15,** 388–394 (2013).

3. McKernan, K. J., Spangler, J., Helbert, Y., Zhang, L. & Tadigotla, V. DREAMing of a patent-free human genome for clinical sequencing. *Nat. Biotechnol*. **31,** 884–887 (2013).