Mitochondrial DNA Sequencing on the iSeq™ 100 Sequencing System

Uncover a comprehensive picture of the mitochondrial genome.

Highlights

- **Cost-Efficient Mitochondrial Sequencing**
  Rapid, affordable analysis of the entire mitochondrial genome
- **Simple, DNA-to-Data Solution**
  Supported workflow from library prep to data analysis
- **Exceptional Data Accuracy**
  Highly accurate data for the detection of rare variants and transcripts compared to qPCR or Sanger sequencing

Introduction

The mitochondrial genome comprises all of the DNA located within mitochondria, organelles important for energy conversion and other cellular functions. Mitochondrial DNA (mtDNA) is organized as a circular, double-stranded DNA molecule that is ~16,600 base pairs in length and codes for 37 genes. In humans (and most animals), mitochondria and mtDNA are maternally inherited during sexual reproduction.[1,2]

Mitochondrial diseases are a heterogeneous group of disorders caused by mitochondrial dysfunction, resulting from inherited or somatic mutations in mtDNA or nuclear genes that code for mitochondrial components. Genetic defects in mtDNA include point mutations, deletions, and copy number variations (CNVs).[2] Studying mutations associated with mitochondrial diseases remains challenging due to phenotypic variability and genetic heterogeneity among individuals. Adding to this complexity is the heteroplasmic nature of mtDNA mutations, which is similar to genetic mosaicism, but on a subcellular, organelle level.

Figure 1: The iSeq 100 System—The iSeq 100 System harnesses the power of NGS in the most affordable, compact benchtop sequencing system in the Illumina portfolio.

Conventional analysis of the mitochondrial genome often starts with PCR-based screens for common point mutations and large deletions. If negative, Sanger sequencing of the entire mitochondrial genome is used to attempt to identify less common variants. However, conventional Sanger sequencing is not sensitive enough to detect low-level heteroplasmy and is not reliable in quantifying the level of heteroplasmy.[3] Mitochondrial genome sequencing with next-generation sequencing (NGS) technology address these challenges, enabling comprehensive detection and analysis of mtDNA mutations.

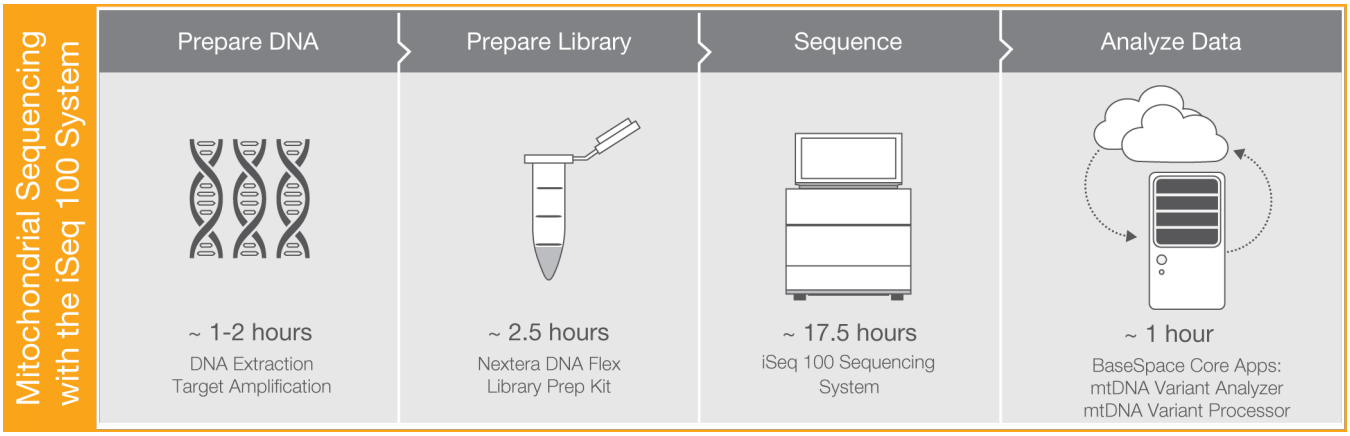

Figure 2: Mitochondrial Sequencing Workflow—Mitochondrial sequencing on the iSeq 100 System is part of a streamlined, comprehensive NGS workflow that includes Nextera DNA Flex library preparation, sequencing, and data analysis.

Mitochondrial sequencing with NGS enables:

- Reliable detection of common and rare mtDNA point mutations and deletions
- Rapid, cost-effect analysis of the entire mitochondrial genome
- Accurate, sensitive measurement of heteroplasmy

The latest innovation in NGS is poised to advance mitochondrial disease research. The compact iSeq 100 System (Figure 1) combines complementary metal-oxide–semiconductor (CMOS) technology with proven Illumina sequencing by synthesis (SBS) chemistry to deliver high-accuracy data with fast time to results. The iSeq 100 System generates 1.2 Gb of data per run in under 18 hours and delivers the high resolution and analytical sensitivity needed for detection of rare variants and transcripts.

Simple, Integrated Workflow

Mitochondrial sequencing on the iSeq 100 System is part of an integrated NGS workflow that includes library preparation with the Nextera™ DNA Flex Library Preparation Kit, proven Illumina sequencing, and push-button data analysis in BaseSpace™ Sequence Hub (Figure 2). The entire workflow proceeds from DNA to data in less than 24 hours.

Library Preparation

The mitochondrial sequencing workflow begins with PCR amplification using specific primers to enrich the mitochondrial genome (Table 1). The generated amplicons are input directly into the Nextera DNA Flex protocol, which uses bead-linked transposomes (BLTs) to mediate On-Bead Tagmentation, simultaneous DNA fragmentation and tagging of Illumina sequencing primers (Figure 3). The Nextera DNA Flex Library Preparation Kit offers optimized library prep in a fast and simple workflow.[4]

Table 1: Primer Sequences for mtDNA Enrichment

Name	Sequence
MTL-F1	5′- AAA GCA CAT ACC AAG GCC AC -3′
MTL-F2	5′- TAT CCG CCA TCC CAT ACA TT -3′
MTL-R1	5′- TTG GCT CTC CTT GCA AAG TT -3′
MTL-R2	5′- AAT GTT GAG CCG TAG ATG CC -3′

Sequencing on the iSeq 100 System

After preparation, libraries are loaded into a prefilled reagent cartridge for sequencing on the iSeq 100 System. Starting a run on the iSeq 100 System is as easy as load and go with less than five minutes of setup. The iSeq 100 System integrates clonal amplification, sequencing, and data analysis into a single instrument. The intuitive user interface provides guidance through every step of the run setup and run initiation processes, allowing researchers to perform various sequencing applications with minimal user training and minimal set up time.

The iSeq 100 System harnesses proven Illumina SBS chemistry, used to generate more than 90% of the world's sequencing data.[5] Illumina SBS chemistry is used in all Illumina sequencing systems, enabling researchers to compare data across systems and scale their studies to higher throughput systems.

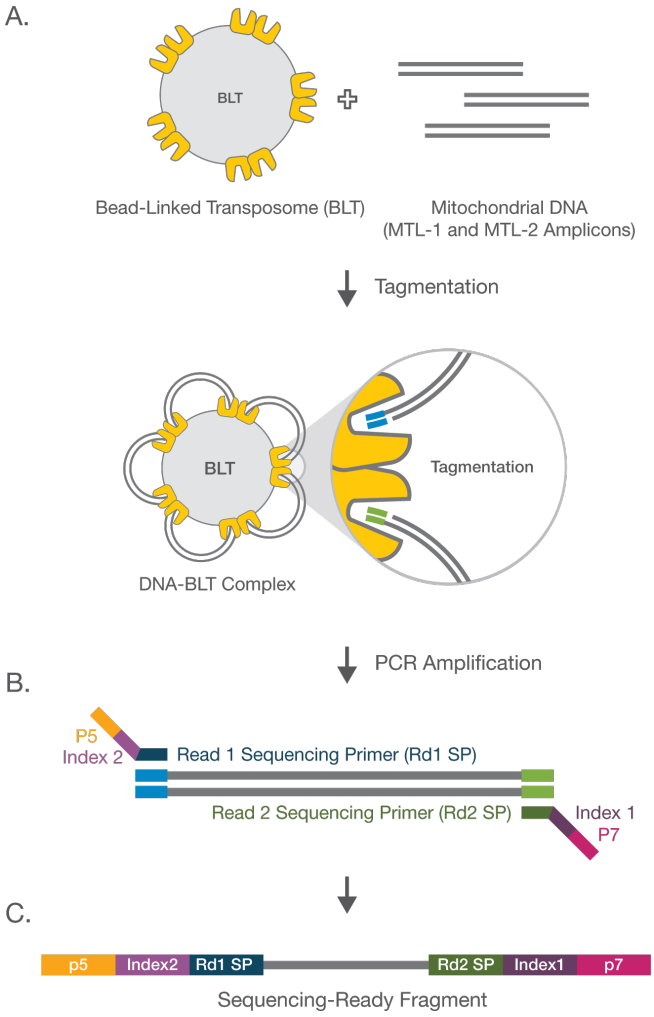

Figure 3: Nextera On-Bead Tagmentation Chemistry—(A) BLTs mediate tagmentation. (B) Reduced-cycle PCR amplifies sequencing ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

Easy, Flexible Data Analysis

The iSeq 100 System offers several data analysis options, including onboard and cloud-based data analysis. The Local Run Manager software, a fully integrated onboard analysis software, features modular architecture to support current and future assays. Local Run Manager software supports planning sequencing runs, tracking libraries and runs with audit trails, and integration with onboard data analysis modules.

Alternatively, sequence data can be instantly transferred, analyzed, and stored securely in BaseSpace Sequence Hub, the Illumina genomics computing environment. BaseSpace Sequence Hub

features a rich ecosystem of commercial and open-source apps for downstream data analysis. Two new apps in BaseSpace Sequence Hub enable variant analysis and easy visualization of mtDNA sequence data (Table 2).

Table 2: Mitochondrial Sequencing BaseSpace Apps

BaseSpace App	Description
mtDNA Variant Processor	The mtDNA Variant Processor app enables streamlined variant analysis of d-loop and whole mtDNA sequence data.
mtDNA Variant Analyzer	The mtDNA Variant Analyzer app enables simplified visualization of mtDNA sequence data.

Experimental Methods and Results

To demonstrate the exceptional performance of the iSeq 100 System as part of a mitochondrial sequencing solution, mtDNA sequencing data generated on the iSeq 100 System was compared against data generated on the MiniSeq™ and MiSeq™ Systems.

Methods

Library Preparation

Two mtDNA amplicons (MTL-1 and MTL-2) were generated by PCR amplification with specific primers to enrich for the mitochondrial genome (Table 1) using NA12878 human genomic DNA as input. Libraries were prepared from the MTL-1 and MTL-2 amplicons using the Nextera DNA Flex Library Preparation Kit, with 1 ng of input for each amplicon. Prepared libraries were normalized and pooled.

Sequencing and Data Analysis

Prepared and pooled libraries were run at a read length of 2 × 151 bp on the iSeq 100 System, MiniSeq System, and MiSeq System, all subsampled to 200,000 paired-end reads. Sequencing results were analyzed using mtDNA apps in BaseSpace Sequence Hub. Initial processing and variant calling was done in the mtDNA Variant Processor app, and coverage plots were generated with the mtDNA Variant Analyzer app.

Exceptional Coverage of the Mitochondrial Genome

Analysis of sequence data for the entire mitchondiral genome shows similar uniformity of coverage when comparing data from the iSeq 100 System against either the MiniSeq System (Figure 4A) or the MiSeq System (Figure 4B). Focusing on the D-loop region, a ~1kb noncoding promoter region in mtDNA that is a hotspot for genetic alterations,[6] shows that the iSeq 100 System provides as good or better coverage, compared to the MiniSeq or MiSeq Systems (Figure 4A and 4B, dashed gray box).

Highly Concordant Variant Calling

The reproducibility of variant calling was compared between the iSeq 100 System and the MiniSeq and MiSeq Systems. 18 variants were identified with most calls at 0%. Variant calling on the iSeq 100 System was highly concordant with both the MiniSeq System (Figure 5A) and the MiSeq System (Figure 5B).

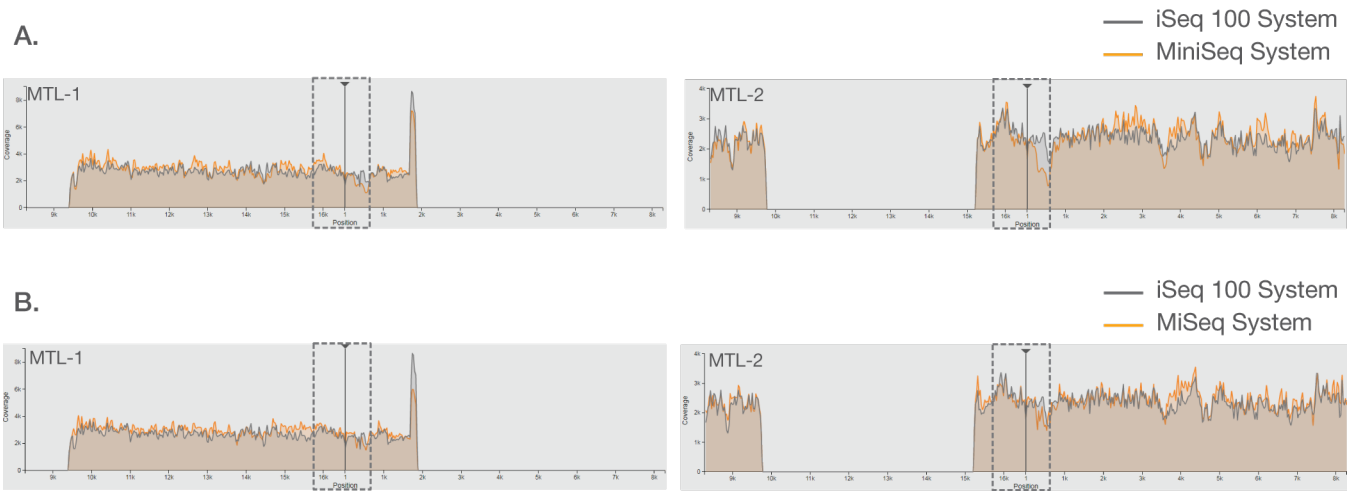

Figure 4: Mitochondrial Sequencing on the iSeq 100 System Results in Similar Uniformity of Coverage Compared to Desktop Sequencers—The iSeq 100 System provides similar uniformity of coverage of the entire mitochondrial genome when compared against the (A) MiniSeq System or (B) MiSeq System. The highly variable D-loop region is outlined with a dashed gray box.

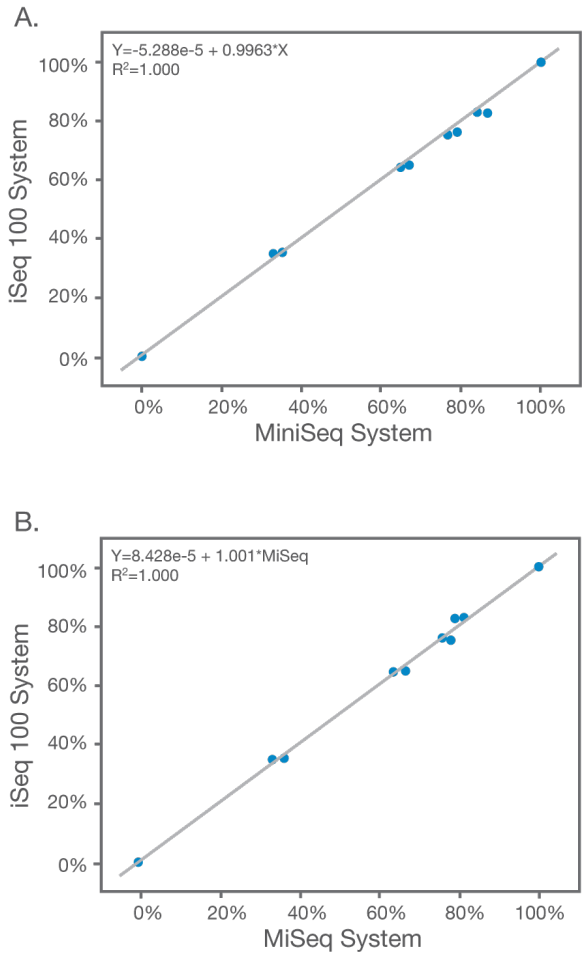

Figure 5: Variant Calling on the iSeq 100 System is Highly Concordant with Desktop Sequencers—Calling of variants in mtDNA was highly concordant between the iSeq 100 System and the (A) MiniSeq System and (B) MiSeq System.

Summary

The iSeq 100 System is part of a fully supported solution for mitochondrial sequencing from simple library preparation with the Nextera DNA Flex Library Preparation Kit to sequencing and user-friendly data analysis. The iSeq 100 System delivers the same data quality as larger desktop sequencers in a smaller footprint with faster run times, making it an ideal, cost-effective solution for small-scale mitochondrial sequencing applications.

Ordering Information

Library Prep	Catalog No.
Nextera DNA Flex Library Prep Kit (24 samples)	20018704
Nextera DNA Flex Library Prep Kit (96 samples)	20018705
Nextera DNA CD Indexes (24 indexes, 24 samples)	20018707
Nextera DNA CD Indexes (96 indexes, 96 samples)	20018708
Sequencing System	**Catalog No.**
iSeq 100 System	20021532
Sequencing Reagents	**Catalog No.**
iSeq i1 Reagents (300 cycles single kit)	20021533
iSeq i1 Reagents (300 cycles quad kit)	20021534

Learn More

To learn more about the iSeq 100 System, visit www.illumina.com/systems/sequencing-platforms/iseq.html

To learn more about mitochondrial sequencing, visit www.illumina.com/techniques/sequencing/dna-sequencing/targeted-resequencing/mitochondrial-sequencing.html

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