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Unparalleled Tool for Mitochondrial DNA Analysis - NEXTflex™ mtDNA-Seq Kit

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INTRODUCTION

The NEXTflex™ mtDNA-Seq Kit uses a unique, highly specific protocol for the isolation of mitochondrial DNA (mtDNA) from primary or transformed cells, coupled with a rapid library preparation protocol for next generation sequencing (NGS). The novel mtDNA isolation method incorporates a selective enzymatic digestion of nuclear DNA (nDNA) resulting in a ~ 100 - 350 fold enrichment of mtDNA. The NEXTflex mtDNA-Seq Kit presents a powerful tool for mtDNA-Seq analysis, empowering mitochondrial disease research, analysis of genetic variation, and forensics.

The Importance of mtDNA in the Study of Human Diseases

mtDNA is one of the most useful markers for studying population genetics and evolutionary biology, due to its high mutation rate, lack of recombination, endosymbiotic origin, and maternal inheritance (1). Mutations in mtDNA are of clinical importance for a number of metabolic and genetic disorders including diabetes, Leber's hereditary optic neuropathy, and deafness (2). Somatic mutations in mtDNA have been increasingly observed in primary human cancers (3-5). As a single cell can contain many mitochondria with multiple copies of mtDNA, wild-type and mutant mtDNA can co-exist in a state called heteroplasmy. Heteroplasmy is observed in cancer cells and in many metabolic diseases associated with mtDNA (6, 7).

Challenges in mtDNA Isolation

Currently, mtDNA is examined by the sequencing of PCR amplified hypervariable regions (the D-loop of mtDNA) or by whole genome sequencing. Reduced coverage and complexity limitations in these protocols make them impractical for accurate determination of heteroplasmy, and do not allow for proper separation of the mtDNA sequence from nuclear mitochondrial DNA (Numt). Furthermore, PCR amplification of hypervariable regions can introduce sequence artifacts, off-target amplification, and uneven coverage of the entire mtDNA sequence. Isolation of mtDNA from nDNA prior to library construction offers a more robust solution for mtDNA analysis (5, 8).

Traditionally, mtDNA isolation procedures require extraction using ultra-high-speed centrifugation. This process is tedious, requiring a CsCl gradient and a specialized centrifuge. There are several kits on the market for isolation of mitochondria. These protocols involve a preliminary step of lysing cells to release organelles, the physical separation followed by extraction of mtDNA from organelles using enzymes to inactivate DNases and other proteins. mtDNA obtained by these methods still contains high levels of nDNA contamination. The NEXTflex mtDNA-Seq Kit isolates mtDNA directly from total cellular DNA, and enriches it by orders of magnitude.

RESULTS

The NEXTflex™ mtDNA-Seq Kit Provides an Improved Solution for mtDNA Sequencing

The novel NEXTflex mtDNA-Seq Kit's approach for purifying circular mtDNA involves the enzymatic depletion of linear nDNA, followed by mtDNA shearing and library construction (Figure 1).

In the following experiment, mtDNA was purified from gDNA isolated from human blood and human A549 cells. To determine the efficiency of mtDNA isolation, end-point PCR was performed with nDNA and mtDNA primers (Figure 2). As expected, in the undigested sample (top panel) bands specific to both nDNA and mtDNA appear, whereas the sample of mtDNA isolated using the NEXTflex mtDNA-Seq Kit (bottom panel) shows a clear loss of nDNA and retention of intact mtDNA. These results demonstrate the selective digestion of nDNA and preservation of mtDNA.

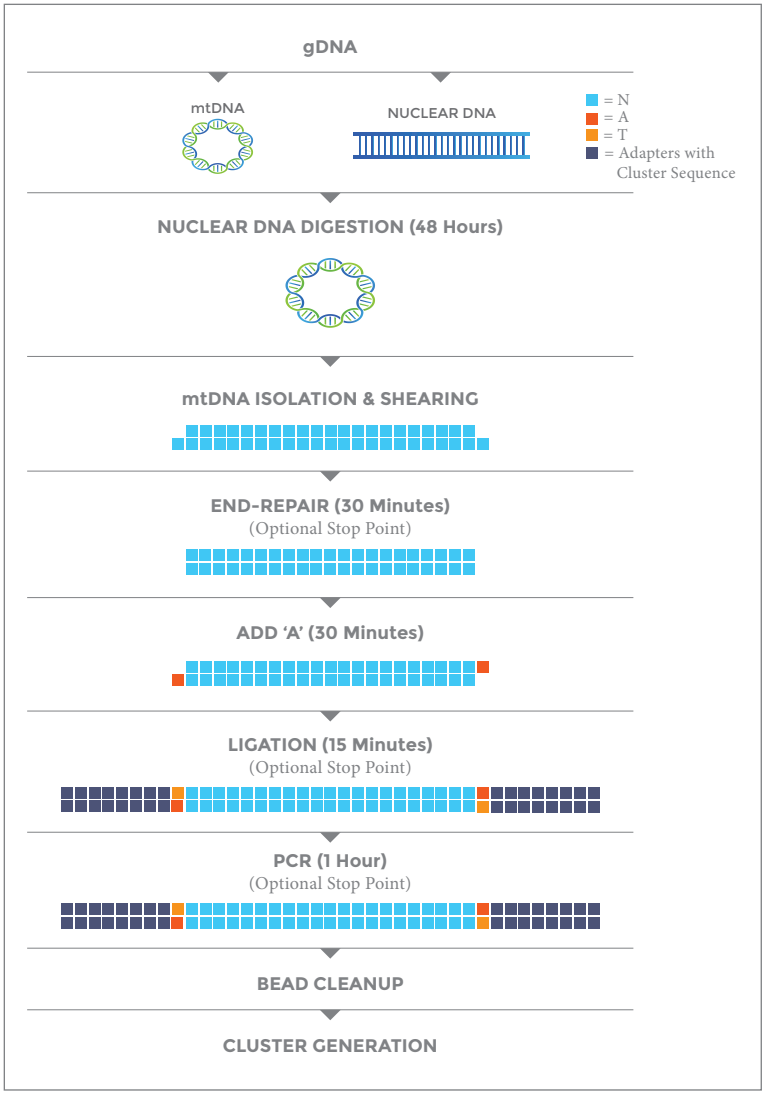

Figure 1. Sample flow chart of the NEXTflex™ mtDNA-Seq Kit with approximate times necessary for each step.

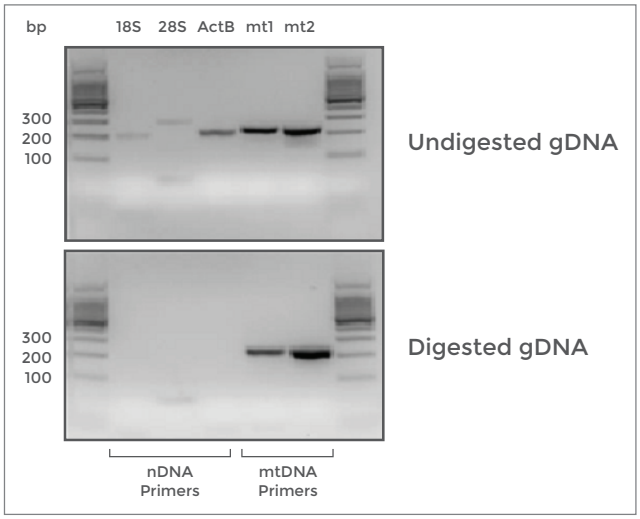

Figure 2. PCR amplification of mtDNA isolated from human blood sample (4 µg gDNA) using the NEXTflex mtDNA-Seq Kit. PCR products of gDNA using three primers sets for nDNA genes (18S, 28S, β-actin) and two sets specific to mtDNA (mt1, mt2) were run on a 2% agarose gel. Prior to enzymatic digestion, both nDNA and mtDNA bands are visible (top panel). After digestion, nDNA bands disappear, leaving mtDNA intact (bottom panel). A 100 bp ready-to-load ladder was used. The PCR product sizes are as follows: 18S – 210 bp, 28S – 280 bp, β-actin – 210 bp, mt1 – 217 bp, and mt2 – 212 bp.

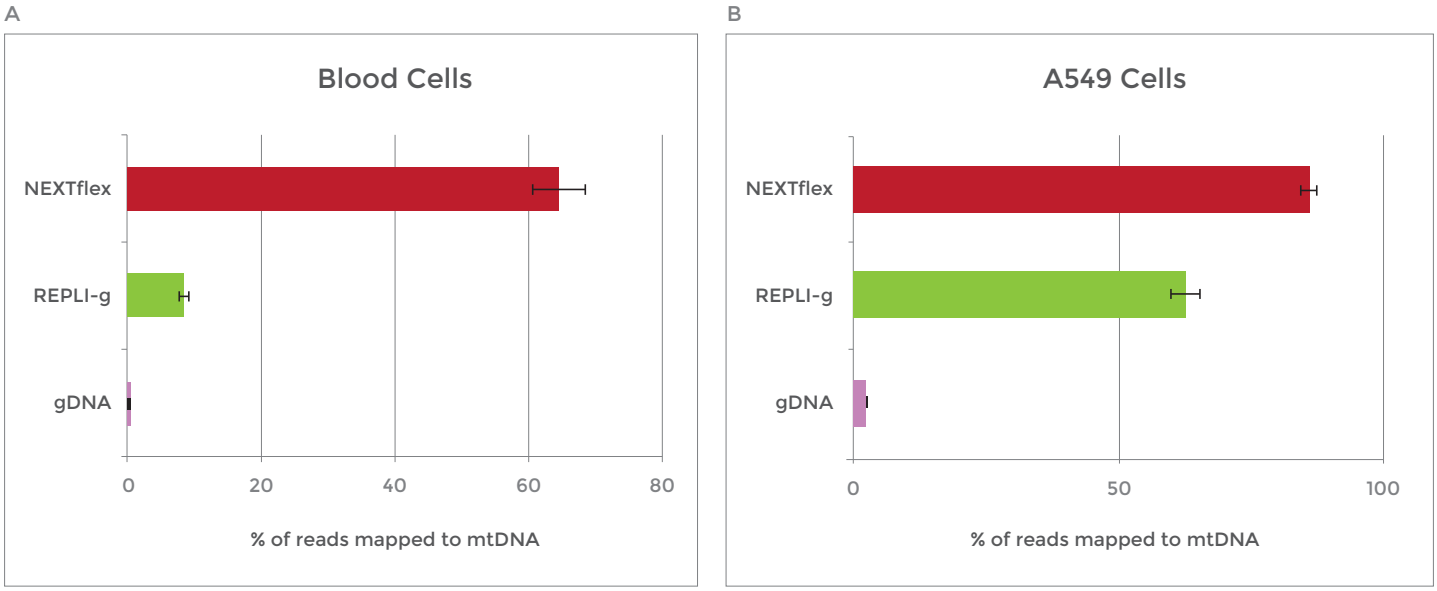

Figure 4. Enrichment of mtDNA reads in samples prepared using the NEXTflex mtDNA-Seq Kit. Triplicate libraries were made from mtDNA isolated using the NEXTflex mtDNA-Seq Kit (red), REPLI-g (green) or untreated gDNA (purple). mtDNA was isolated from 4 µg blood (A) or A549 (B) gDNA. The bars represent mean and standard deviation.

CONCLUSIONS

The NEXTflex mtDNA-Seq Kit offers a novel and effective method for the isolation and sequencing of mtDNA. The efficient isolation of mtDNA from nDNA eliminates the need for whole genome amplification. The NEXTflex mtDNA-Seq Kit reduces amplification bias and produces a greater number of uniquely mapped mtDNA reads compared to other commercially available kits. Moreover, the superior enrichment of mtDNA reduces the number of sequencing reads needed per sample and hence the cost of sequencing mtDNA. The NEXTflex mtDNA-Seq Kit is an ideal tool for next-generation sequencing-based mtDNA analysis.

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