ABSTRACT

Human mitochondrial variants have been important in forensic analysis, disease association studies, and human evolution. Due to the large number of variable positions in the mitochondrial genome, sequencing is the method of choice for accurately interrogating the sequence variability. Presented here is a new and simple PCR/sequencing workflow integrated with data analysis and variant reporting for resequencing of mitochondrial DNA. The new resequencing workflow with newly designed sequencing primers improves 5’ sequence resolution, increases throughput, and reduces hands-on time. The novel sequencing primer chemistry produces high quality bases from base 1 on POP-7™ polymer previously only could be resolved on the slower POP-6™ polymer. The new primer chemistry and workflow also eliminate the need for a separate PCR clean-up step. These improvements reduce the entire workflow from PCR to finished sequence data to under 5 hours, compared to 8 hours for the standard workflow. The sequencing results were analyzed with Variant Reporter® Software. The use of quality control metrics, including the use of Quality Values for DNA trace score, enables validating variants with high confidence. Examples will demonstrate this improved mitochondrial resequencing workflow for variant detection.

RESULTS

Figure 2. BigDye® Direct workflow enhancement for CE platforms

- **Standard PCR/sequencing workflow:** Five hands-on steps in 8 process hours.
- **BigDye Direct® workflow:** Four hands-on steps in 5 process hours.

The existing PCR/sequencing workflow requires more than 8 hours of process time and 5 hands-on steps to complete an experiment; in contrast, the new workflow takes less than 5 hours and 4 steps, producing sequence reads ~40% faster and with less hands-on time. In addition, the new sequencing primers enable electrophoretic run times more than 50% faster to generate up to 700 high quality bases from a 3500 Genetic Analyzer.

Figure 3. Newly designed sequencing primer improves 5’ resolution

Figure 4. Variant Discovery

Three mitochondrial specimens (CEPH1347-02, 9947A and CHR) were tested on the 3130xl Genetic Analyzer. CEPH gDNA has a T>C variant at position 16,189 on RSA001250252 (left). CEPH gDNA has a C>T variant at position 482 on RSA0201525241 (right).

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Figure 5. Quality and Variant Reports

Samples can be imported to the project and automatically assigned to specimens and amplicons. There are various reports in Variant Reporter® Software to summarize quality assessments, nucleotide and amino acid changes, genotypes across specimens and variant confidence score in either graphic or table formats. Figure 5 shows examples of quality summary and the variant table of these three specimens.

CONCLUSIONS

As demonstrated here for mitochondrial genome resequencing, a workflow is fully optimized with ready to use PCR and sequencing reagents, PCR and sequencing primers, and data analysis by using Variant Reporter® Software. BigDye® Direct Cycle Sequencing kit is streamlined, making it simple, fast, cost-effective and high quality resequencing workflow. The newly designed M13 sequencing primers provide the advantages of the speed of POP-7™ polymer electrophoresis and eliminates the separate post PCR clean-up by combining it with the cycle sequencing step. Variant Reporter® Software offers easy project setup, navigation, filtering, quality scoring of traces and specimens, variant review, and reporting.

REFERENCES


TRADEMARKS/LICENSING

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