



Positive Findings in Clinical Mitochondrial Genome Testing by NGS

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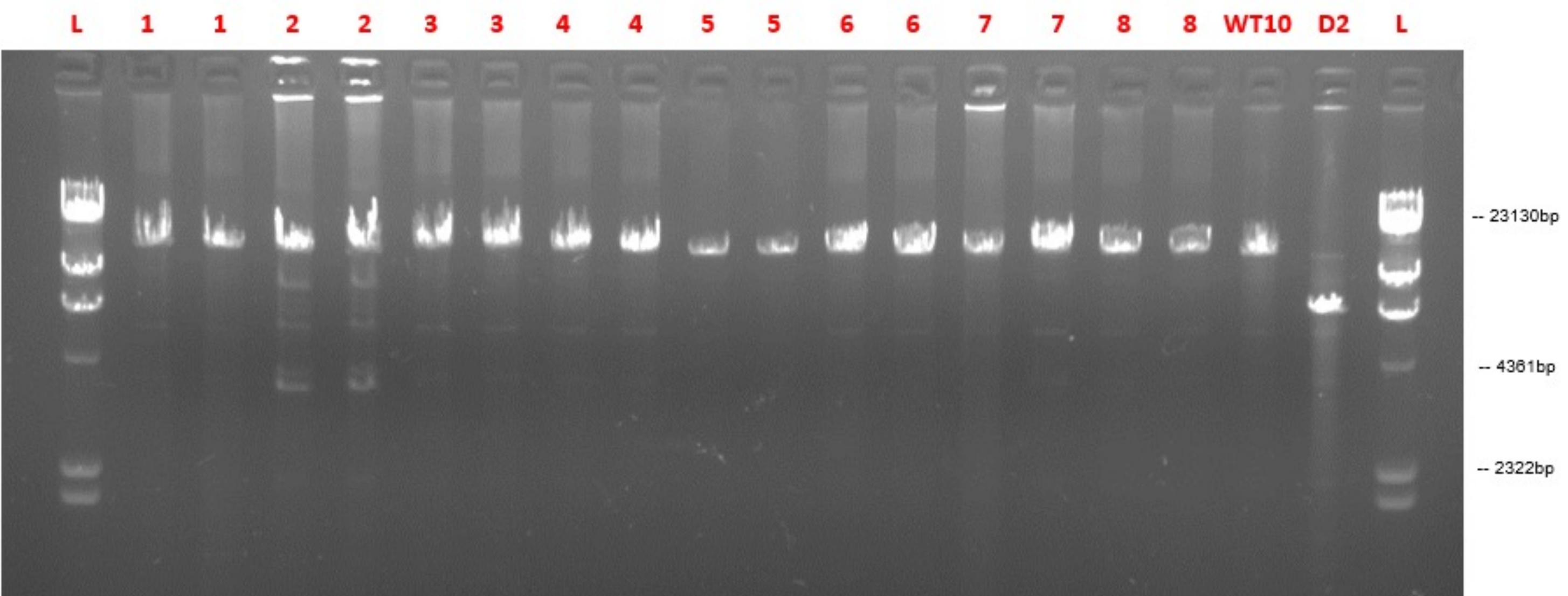
BACKGROUND

Genetic disorders lead to a wide spectrum of human disease. In addition to the nuclear-encoded DNA, maternally inherited mitochondrial DNA plays an important role in human health. The mitochondrial genome is a 16569bp double helix loop and encodes 37 genes. Pathogenic variants on the mitochondrial DNA are associated with human diseases including mitochondria myopathy, maternally inherited diabetes mellitus and deafness (MIDD), Leber's hereditary optic neuropathy (LHON), Leigh syndrome and many

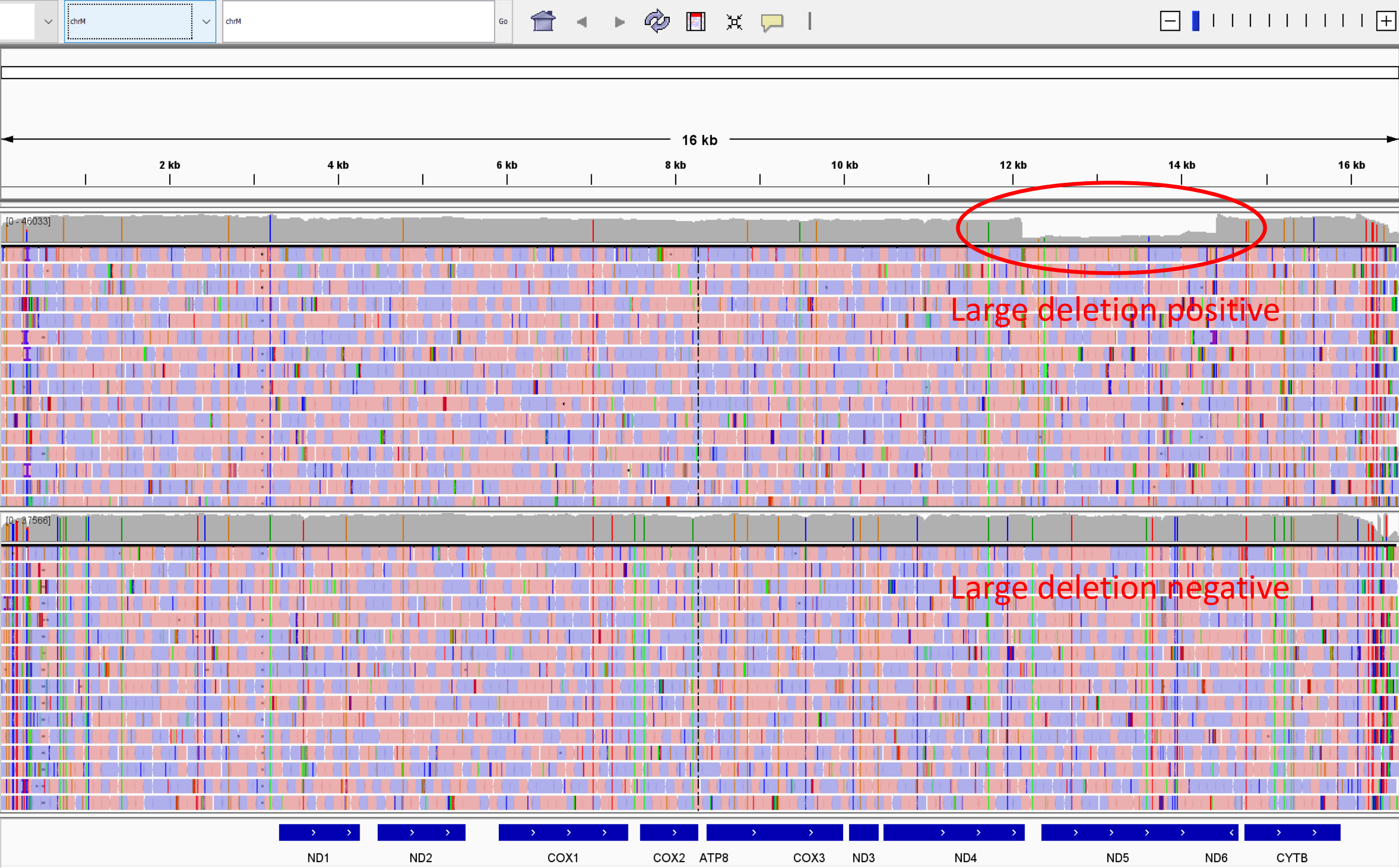
METHODS

Baylor Genetics has established a robust platform to sequence the whole mitochondrial genome using Next Generation Sequencing (NGS) technology. Long-range PCR is used to amplify the mitochondrial DNA and exclude nuclear DNA. NGS is applied to sequence the samples in a high throughput manner. NGS data are processed to call single nucleotide variants (SNV) and large deletions in the mitochondrial genome. Variants can be confidently called at heteroplasmic levels as low as 1.5%. We retrospectively analyzed positive findings from cases tested consecutively but without selection based on clinical indications.

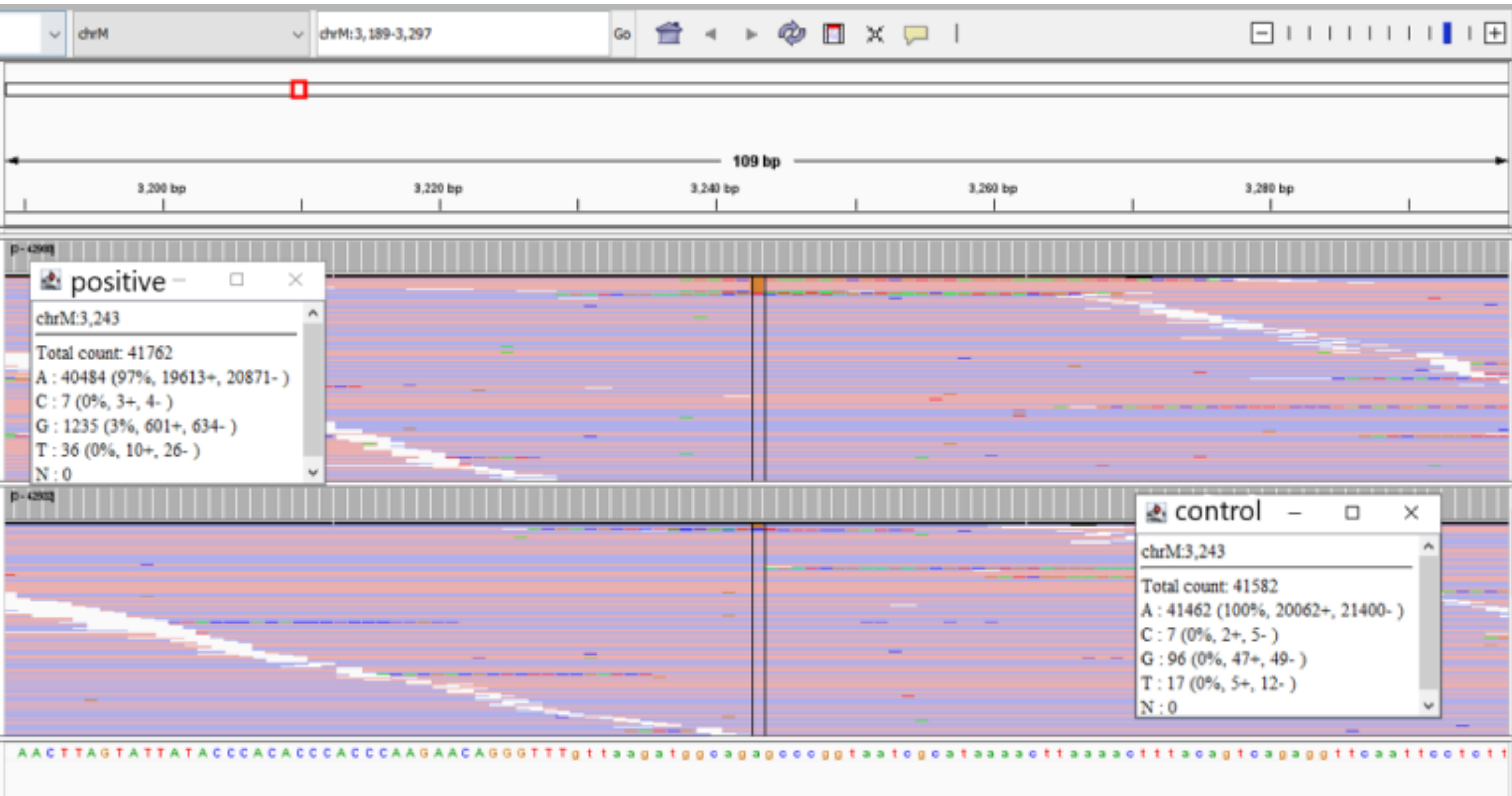
Example of Long-range PCR



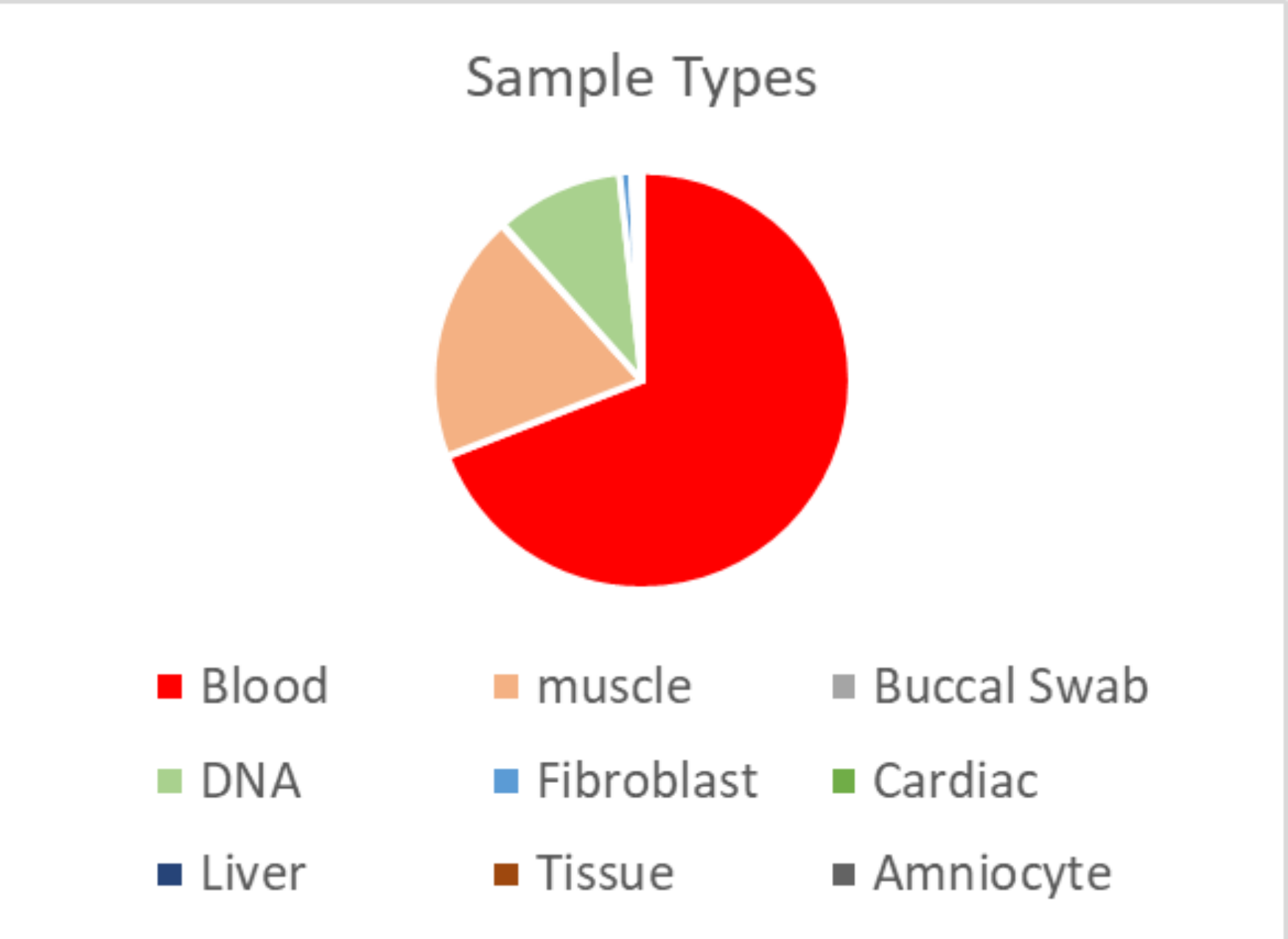
Example of Large Deletion



Example of single nucleotide variant



Sample types distribution



Diagnosis rate

	number of cases	rate %
SNV	709	7.20
Large Deletion	600	6.09
SNV/Deletion	27	0.27
Negative	8571	86.99
Total	9853	100.00

RESULTS

We detected pathogenic or likely pathogenic SNV in 709 cases and large deletions of mitochondrial genomic regions in 600 cases. 27 cases were positive for both SNV mutations and large deletions. The majority of samples were extracted from blood. Other sample types were urine, bone marrow, fibroblasts, amniocytes, buccal swabs, previously extracted DNA and affected tissues, including liver, skeletal muscle and others. The ages of test subjects ranged from prenatal to 89-year-old adults. The pathogenic variant m.3243A>G in the tRNA Leucine gene associated with MELAS syndrome was the most prevalent mutation, which was detected 186 times. It was heteroplasmic in all cases, ranging from 1.5% to 94.9%.

CONCLUSIONS

NGS based mitochondrial genome sequencing is highly robust and efficient. Our test is highly sensitive, which allow us to call variants as low as 1.5% heteroplasmy with confidence. Amplicon based sequencing excluded the noise from the nuclear DNA, thus enhanced specificity. This test has highly competitive TAT with low cost. We are proud to develop this gold standard platform that serve as molecular diagnosis for human mitochondrial genome disorders.

References

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