Human mtDNA Analysis in BaseSpace

Mitochondrial DNA (mtDNA) analysis enables forensic laboratories to extract genetic data from small biological samples, found in less than ideal condition. Mitochondria in humans cell contain about 1,000 copies of mtDNA. The ease-of-use of next-generation sequencing (NGS) and Nextera XT enables labs to speed up their workflow, reducing time and labor spent on prep, and generate deeper coverage data compared to Sanger sequencing at 1X coverage/rx.

The updated Illumina mtDNA demonstrated protocols provide a complete mitochondrial DNA solution – from targeted library prep and sequencing to bioinformatics analysis and report generation in BaseSpace – to help investigators draw conclusions in a straightforward and intuitive workflow.

The two new mtDNA apps in BaseSpace allow for variant analysis and easy visualization of mitochondrial sequence data. This workflow can analyze any part of the full circular genome, without any origin dead zone, using quality and coverage thresholds customized by the user (Figure 1).

Figure 1: mtDNA Analysis Workflow. Sequence data is streamed into BaseSpace. Initial processing is performed using the mtDNA Variant Processor app and the results are stored in your BaseSpace project. Use mtDNA Variant Analyzer to visualize the data and generate a downloadable Excel report.

mtDNA Variant Processor

The mtDNA Variant Processor app processes FASTQ files directly from your MiSeq or MiSeq FGx instrument and generates VCF files. Easily set up your analysis and configure thresholds on the input form before launching the app (Figure 2).

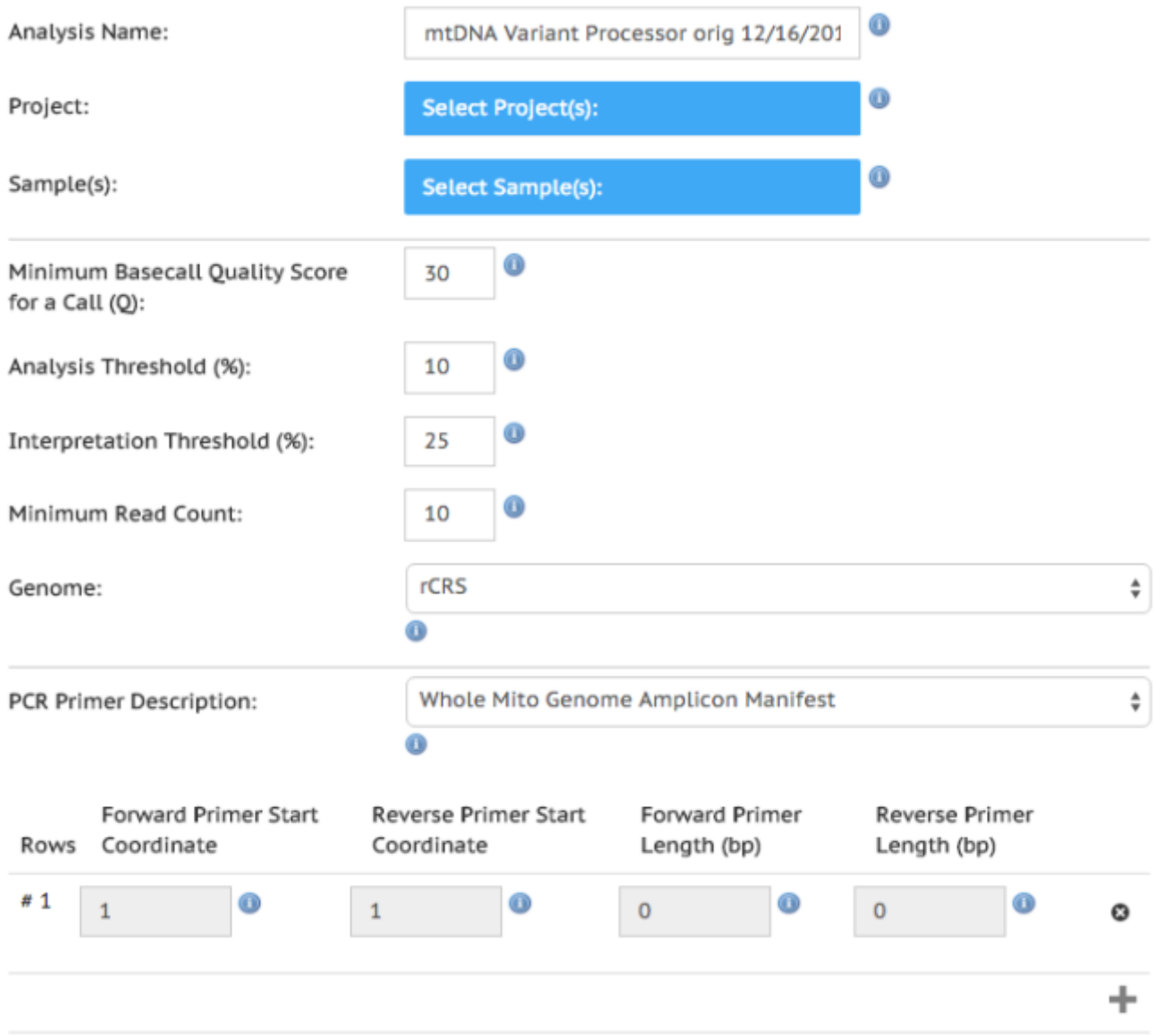

Figure 2: The mtDNA Variant Processor input form. The form allows you to select input samples and adjust the analysis parameters for Minimum Basecall Quality Score, Analysis Threshold, Interpretation Threshold, Minimum Read Count, and choose the Reference Genome and PCR Primer Description. Optionally, you can define a custom manifest of targeted regions by entering forward and reverse primer start coordinates and lengths.

mtDNA Variant Analyzer

The mtDNA Variant Analyzer app consumes the VCFs output by the mtDNA Variant Processor app. the VCF output is then displayed in this web-based app for visualization and optional report generation in an Excel format.

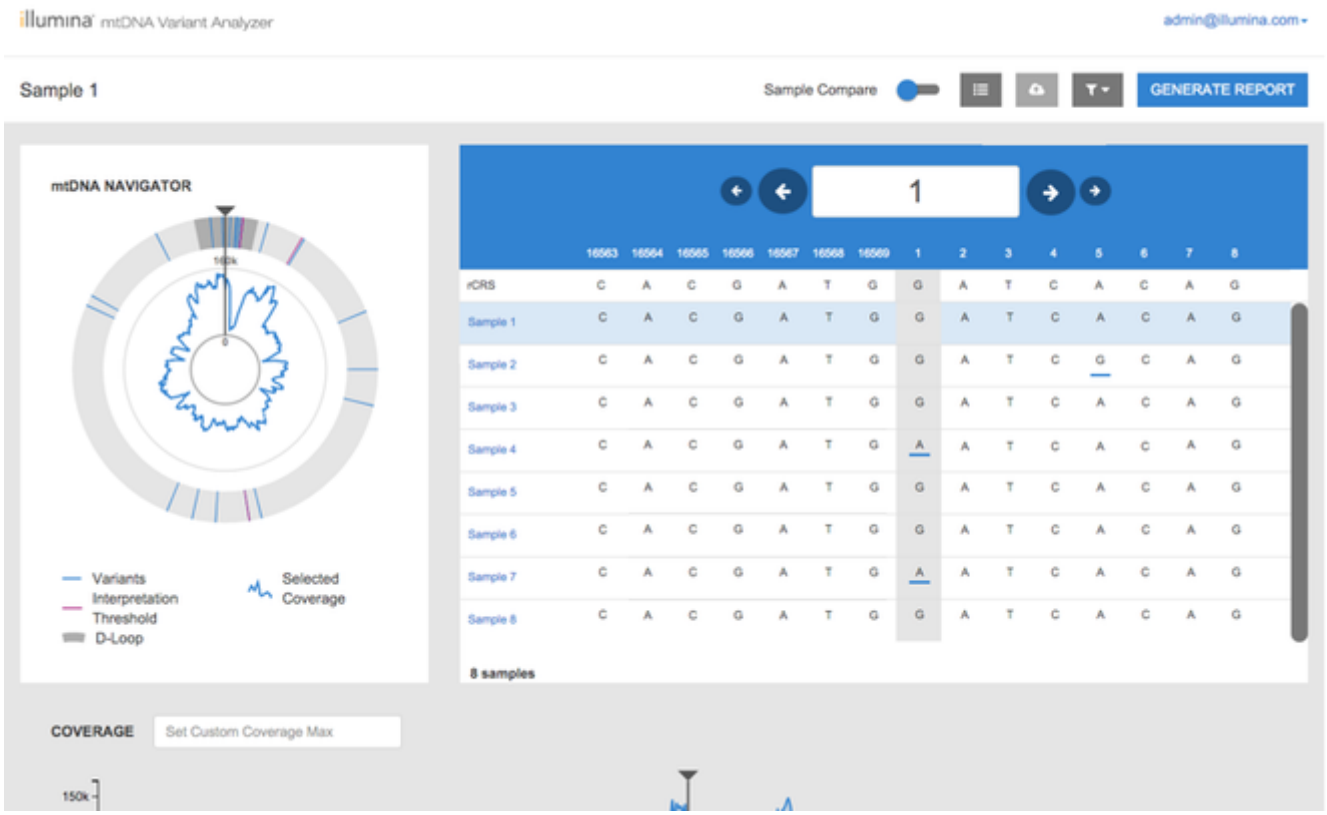

Figure 3: The mtDNA Variant Analyzer app

Explore the apps for yourself in BaseSpace:

1. Log in to your BaseSpace account
2. Retrieve the example datasets in BaseSpace
3. Launch the mtDNA Variant Processor app using the example datasets as input. Configure the analysis settings and continue to kick off the analysis.
4. View and interact with your results using the mtDNA Variant Analyzer web app. Download your reports using the Generate Report button.