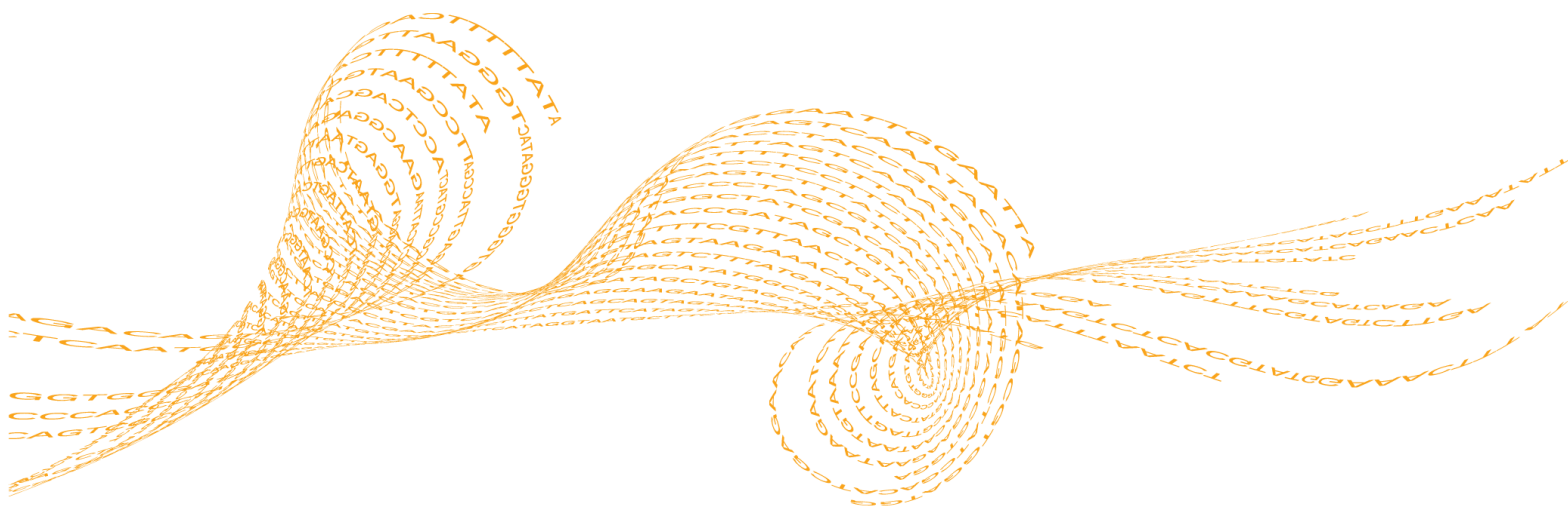


mtDNA Variant Analyzer v1.0

BaseSpace App Guide

For Research Use Only. Not for use in diagnostic procedures.

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Introduction

Using the analysis results from the mtDNA Variant Processor App, the BaseSpace® mtDNA Variant Analyzer v1.0 App produces the analysis for mitochondrial DNA (mtDNA) sequencing data. The app can analyze any part of the full circular genome, without any origin dead zone, using quality and coverage thresholds customized by the user. The app also generates analysis reports in Excel file format.

Compatible Data

The Variant Call Format (VCF) output files from the mtDNA Variant Processor App are compatible with the mtDNA Variant Analyzer App.

Reference Genomes


The genome for alignment is the Revised Cambridge Reference Sequence (rCRS), a mtDNA genome used by the Forensic Genomics community.

Log In to BaseSpace

- 1 Navigate to BaseSpace at basespace.illumina.com, enter your user name and password, and click **Login**.
- 2 Click the **Apps** tab.
- 3 In Categories, click **Targeted Sequencing**, and then click **mtDNA Variant Analyzer**.
- 4 From the drop-down list, select **version 1.0.0**, and then click **Launch** to open the app. The permission request dialog box appears.
- 5 From the dialog box, click **Accept**.
By default, this dialog box appears the first time you launch the app. After you click **Accept**, the BaseSpace Projects page appears.
For additional training on BaseSpace, see the BaseSpace support page at support.illumina.com/sequencing/sequencing_software/basespace/training.html

BaseSpace Projects Page Functions

You can perform the following functions while in the BaseSpace Projects page:

- ▶ To search for a project, enter the project name in the `search project name` field.
- ▶ To search for samples, enter a sample name in the `search sample name` field.
- ▶ To remove a sample, click the selected sample again or click .
- ▶ To select all samples, click the **Select All Visible** link.
- ▶ To remove all samples, click **Clear all**.

Select Samples From a Project or Multiple Projects

- 1 In the BaseSpace Projects page, select the project you want to analyze.
- 2 Select the samples you want to analyze.
The app supports a maximum of 96 samples. The right-side pane shows the selected samples for analysis.
- 3 [Optional] To add samples from a different project, click **BaseSpace Projects** to return to the BaseSpace projects page.
Repeat step 1 and 2.
- 4 When you have completed selecting the samples, click **Submit**.
By default, the first time you select a sample, a dialog box requesting for access to samples in the project appears.
- 5 From the dialog box, click **Accept**
The app reloads the selected samples. Any unsaved changes to samples are not loaded to the analysis. When analysis is complete, the app shows the sample analysis in the Reference Genome Compare View, mtDNA Navigator, and Coverage Plot.

Analysis Output




The mtDNA Variant Analyzer App uses VCF and BAM output files from the mtDNA Variant Processor App to produce the following analysis output:

- ▶ Reference Genome Compare View
- ▶ Sample Compare View
- ▶ mtDNA Navigator
- ▶ Coverage Plot
- ▶ Aggregate Analysis Report
- ▶ Sample Analysis Reports

Status Icons

The mtDNA Variant Analyzer App provides the following status icons.

Table 1 Status Icon Table

Icon	Description
	In the BaseSpace Projects page, this icon at the top-right of the sample indicates that the sample is modified.
	In the Reference Genome Compare View, this blue icon next to the sample indicates that the sample is modified. In the Sample Compare View, the icon color is orange.
	In the Reference Genome Compare View, this blue icon next to the sample name indicates that the sample changes are unsaved. In the Sample Compare View, the icon color is orange.

Reference Genome Compare View

The Reference Genome Compare View has a blue background and lets you compare the reference genome (rCRS) with the selected samples. You can view the following information in the view pane:

- ▶ Reference genome
- ▶ Selected samples for comparison
- ▶ Call of a position for each sample
- ▶ Variant indicator (blue line under the call)
- ▶ Interpretation threshold indicator (purple line under the call)
- ▶ User modified call indicator (asterisk sign next to the call)
- ▶ Total number of samples






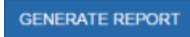
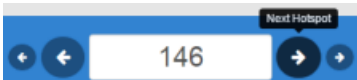
Any changes you make are immediately updated to the following areas:

- ▶ Reference Genome Compare View
- ▶ mtDNA Navigator
- ▶ Coverage Plot

When you select a sample (it is highlighted), the mtDNA Navigator and Coverage Plot immediately update the position and coverage for that sample.

Use these tools to refine your analysis. When you make changes to the Reference Genome Compare View, the changes are also reflected in the mtDNA Navigator and Coverage Plot.

Table 2 Reference Genome Compare View Tools Table

Tools	Description
	Sample Compare —To make a selected sample a reference and compare it to the rest of the samples, highlight the sample by selecting it, and then drag the blue dot to the other side until the background is orange. Use this tool to find differences between samples or to find identical samples.
	Sample Selection —To add more samples from the same project or a different project, use this tool to return to the BaseSpace projects page.
	Save —When you modify a base call, use this tool to save changes to BaseSpace. The icon is activated when you make a change. Click Sample Selection to view samples that are modified.
	Filter —Use this tool to filter samples for D-Loop, variants, interpretation threshold, and user modified. See <i>Filter Options</i> on page 8.
	Call for a Position —Use this tool to view call details. See <i>View Call Details</i> on page 9.
	Generate Report —Use this tool to export sample analysis data in Excel file format.
	Next Hotspot —A hotspot is any variant, interpretation threshold indicator, or user modified position for the selected sample. Use the large arrow to get to the next hotspot. Next Page —Use the small arrow to get to the next 15 positions.

Filter Options

You can select one or more filters per analysis.

Table 3 Reference Genome Compare Filter Options Table

Filter	Description
D-Loop	This option constrains the view range positions from 16024 to 576. The constraint affects the Reference Genome Compare View, mtDNA Navigator, and Coverage Plot.
Variants	This option filters the positions with variants for each sample.
Interpretation Threshold	This option filters the positions with interpretation threshold indicators for each sample.
User Modified	This option filters the positions that are modified.

Sample Compare View

The Sample Compare View has an orange background and lets you compare a specific sample with the selected samples. The sample located at the top of the list is the reference sample. You can view the following information in the view pane:

- ▶ Reference sample
- ▶ Selected samples for comparison
- ▶ Call of a position for each sample
- ▶ Differences indicator (orange line under the call)

- ▶ Interpretation threshold indicator (purple line under the call)
- ▶ User modified call indicator (asterisk sign next to the call)
- ▶ Total number of samples




Any changes you make are immediately updated to the following areas:

- ▶ Sample Compare View
- ▶ mtDNA Navigator
- ▶ Coverage Plot

When you select a sample (it is highlighted), the mtDNA Navigator and Coverage Plot immediately update the position and coverage for that sample and the reference sample.

The tools for tools for the Reference Genome Compare View and Sample Compare View are the same, with the exception of the following differences. See *Reference Genome Compare View* on page 7.

Table 4 Sample Compare View Tools Table

Tools	Description
	Sample Compare —To return to the Reference Genome Compare View, drag the orange dot to the other side until the background is blue.
	Filter —Use this tool to filter samples for D-Loop, differences and zero differences, interpretation threshold, and user modified. See <i>Filter Options</i> on page 9.
	Sample Navigation —Use this tool change the sample you want to compare. Click the arrow to move the selected sample to the top of the list.


Filter Options

You can select one or more filters per analysis.

Table 5 Sample Compare Filter Options Table

Filter	Description
D-Loop	This option constrains the view range positions from 16024 to 576. The constraint affects the Sample Compare View, mtDNA Navigator, and Coverage Plot.
Differences	This option filters the positions that are different from the reference sample.
Interpretation Threshold	This option filters the positions with interpretation threshold indicators for each sample.
User Modified	This option filters the positions that are modified.
Zero Differences	This option filters samples that are not different from the reference sample.

View Call Details

- 1 Click a specific call position  to view the Detailed Call View.
- 2 [Optional] From the Original Call field, expand the drop-down arrow to select a different call.

Detailed Call View

Table 6 Detailed Call View Table

Call Detail	Description
Reference Sample Name	The reference sample name.
Sample Name	The sample name for the result.
Call	The call for the position.
Paired-End Depth	The maximum depth of either the forward or reverse reads.
Score	Score for the call.
Percentages	The percentages are stacked and shown in relation to the thresholds.
it	The location where the interpretation threshold resides for the sample.
at	The location where the analytical threshold resides for the sample.
Original Call	The original call. To change the call, expand the drop-down arrow and select a call from the list.

mtDNA Navigator

The following are the mtDNA Navigator features:

- ▶ The pointer indicates the position in the Reference Genome Compare View or Sample Compare View.
- ▶ To view genomic variants, interpretation threshold, D-Loop positions, and selected coverage, move the pointer around the circle plot.
- ▶ The mtDNA Navigator zooms in to show more details of the D-Loop area when you select the D-Loop filter option in the Reference Genome Compare View or Sample Compare View.
- ▶ The mtDNA Navigator shows coverage for the selected sample in relation to the visual representation of the genome. In Sample Compare View, the app shows the overlapped coverage of the reference sample and selected sample.

Any changes you make are immediately updated in the following areas:

- ▶ mtDNA Navigator
- ▶ Reference Genome Compare View or Sample Compare View
- ▶ Coverage Plot

Coverage Plot

The following are the Coverage Plot features:

- ▶ The pointer indicates the position in the Reference Genome Compare View or Sample Compare View.
- ▶ To zoom in and out, enter a value in the Coverage field.

- ▶ When you are in Reference Genome Compare View, the blue line in the coverage plot represents the coverage for the selected sample.
- ▶ When you are in Sample Compare View, the gray line in the coverage plot represents the coverage for the reference sample and the orange line represents the coverage for the selected sample.

Any changes you make are immediately updated in the following areas:

- ▶ Coverage Plot
- ▶ Reference Genome Compare View or Sample Compare View
- ▶ mtDNA Navigator

Aggregate Analysis Report

The mtDNA Variant Analyzer App generates an aggregated summary of all the analyzed samples in an Excel file.

Table 7 Summary Table

Statistic	Definition
Sample	The sample name.
Single Nucleotide Variants (SNVs)	The number of positions where the call for the sample differs from the reference genome (rCRS).
Insertions	The number of one or more nucleotides inserted in the sample genome.
Deletions	The number of one or more nucleotides deleted from the sample genome.
Manually Changed Calls	The changed call when it is modified, which is presented as [position] [original call] -> [new call].
Variants	The position and call for calls that differ from the reference genome.

Sample Analysis Reports

In addition to the aggregated summary of all the analyzed samples, the mtDNA Variant Analyzer App generates a summary of read count and variant statistics for each sample in the same Excel file.

Table 8 Sample Analysis Table

Statistic	Definition
Sample	The sample name.
Single Nucleotide Variants	The number of positions where the call for the sample differs from the reference genome (rCRS).
Insertions	The number of one or more nucleotides inserted in the sample genome.
Deletions	The number of one or more nucleotides deleted from the sample genome.

Statistic	Definition
Manually Changed Calls	The changed call when it is modified, which is presented as [position] [original call] -> [new call].
Variant	The position and call for calls that differ from the reference genome.
Reference	The base position in the reference genome (rCRS). When there is an insertion, the reference base reports a plus (+) sign.
Score	The score for the call.
Paired-End Depth	The maximum depth of either the forward or reverse reads.
Reads A, C, G, T	The number of forward and reverse reads for the A, C, G, or T bases at the call position.
Percent A, C, G, T	The percentage of forward and reverse reads out of the total reads for the A, C, G, or T bases at the call position.
Reads Del	The number of forward and reverse reads for a deletion at the call position.
Percent Del	The percentage of forward and reverse reads out of the total reads for a deletion at the call position.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 9 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 10 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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