



# Analyze mtDNA and STR/Y-STRs Simultaneously



# **Mitochondrial DNA Analysis**

- ✓ Whole Genome or HV1/HV2 and Control Region Analysis
- ✓ Unique Alignment Technology<sup>1,2</sup> Motif Consensus
- ✓ Forensic Nomenclature
- Easy uploads to EMPOP<sup>3</sup>

## **STR Analysis**

- ✓ Autosomal & Y-STR
- ✓ Forensic Nomenclature
- ✓ Genotype & SNP Reporting

# Validated

Easy-to-Use Windows® Interface

**Compatible with major Chemistries & Platforms** 

## **Audit Trail & User Control**

**Comprehensive Reporting Options** 





## **Mitochondrial DNA Analysis**

GeneMarkerHTS software provides a validated, streamlined workflow for forensic casework and medical research of mitochondrial DNA data analysis from massively parallel sequencing (MPS) systems such as the Illumina<sup>®</sup> and Ion Torrent<sup>®</sup> platforms; in an easy to use Windows<sup>®</sup> operating system with password protected user rights and administrative controls/ audit trail. Developed in collaboration with leading laboratories, GeneMarkerHTS software provides rapid analysis of multiple samples using consensus alignment or a unique motif alignment technology that automates the recommendations of *DNA Commission of the International Society for Forensic Genetics: Revised and extended guidelines for mitochondrial DNA typing*. Using forensic motif alignment provides recognition and proper assignment of motifs and INDELs consistent with phylogenetic and forensic considerations.

### Analysis results include:

- Consensus sequence, Variants, SNPs, Indels
- Depth of coverage graphics
- Major variant report for haplogroup determination, Import to EMPOP<sup>3</sup> (ENDAP mtDNA Population Database)
- Consensus sequence aligned to reference (IUPAC nomenclature) o Whole mtDNA genome, spanning the origin
  Spacified energy of interact, such as control region, UV(1, UV(2))
- o Specified areas of interest, such as control region, HV1, HV2
- Read pile-up (with depth and direction indicators)
- Compare multiple samples in single view
  - o Synchronized view, scroll and zoom of multiple samples
  - o Comparison viewer, table with sample-to-sample and and variant composition



#### Global and zoom example of a whole mtDNA genome alignment:



Global (left) and zoom example (right) of a whole mtDNA genome alignment: The Global View shows the depth of coverage; forward read coverage in blue and reverse read coverage in red, Reference sequence and Consensus Sequence are above the pile-up of the reads, linked result table is located under the read pileup. The large black rectangle in the pileup (left) is the magnified region (right).

### Rapid Analysis and Synchronized Viewing of Multiple Samples

GeneMarkerHTS software provides results in minutes; for example, 30 MiSeq whole mtDNA chromosome data files with 10,000 average depth of coverage were aligned in 90 minutes (3 minutes per sample). In a more extreme example, 200 whole mtDNA chromosome data files with 10,000 average depth of coverage aligned in 16 hours.



Example of synchronized viewing of two samples, E1a and H12a: all open samples are synchronized when the analyst clicks on a position or variant in the table or zooms/scrolls in the pile-up. Up to four samples can be easily reviewed on most monitors, with larger monitors accommodating up to 8 open samples for synchronized viewing and review.

## Time saving tools

### Motif alignment reduces manual edits for forensic alignmemt<sup>1,2</sup>

GeneMarkerHTS software has an extensive, preloaded forensic motif file as well as a motif editor to assist labs in adding new motifs. Motif alignment technology automates the recommendations of the DNA Commission of the International Society for Forensic Genetics and decreases analyst intervention.



### **Comparison Viewer and Reporting:**

Compare results between samples of a project after alignment, or samples selected from previous projects in the database.

### Variety of reports:

- Consensus sequence
- Variant reports SNPs, insertions and deletions
- Haplotype, heteroplasmy
- Report compatible for import into EMPOP<sup>3</sup>

#### The software includes:

- Audit trail capability
- User management
- Customizable viewing and reporting to protect privacy of potential health information (PHI) sequences
- Comparison Capabilities

**Comparison Viewer** is a viewing tool to compare analysis results of multiple samples. Use the comparison viewer for:

- 1. Sample to sample comparison (above)
- 2. As well as a variant comparison of all the samples in the project at the same time (below)

G Compa	arison View	er							-			<
Comparison F	Filter Setting	s Major to	Major Ma	jor to Mind	or Minor	r to Minor						
Sample to	Sample Co	mparison		Propor	rtion of Sh	ared Varian	nts: None			All	Save As	
	G2a	3a_ 2a3aH	1n57( H1a	3a1_ 1a	3a 1R 30b 2	H1b5_	Hin	5_ R30	b2_ т2	07a1_	2b7a1H1b	1
G2a3a_	22	/22 22/	22 3,	/6	3/6	3/6	3/5	i 5/	7 7	/15	7/15	
G2a3aH1n57	03 22	/22 22/	22 4,	/6	4/6	4/6	5/5	5 5/	7 7	/15	7/15	
H1a3a1_	3/	22 3/2	22 6	/6	6/6	4/6	4/5	i 3/	7 3	/15	3/15	
H1a3a1R30b	27 5/	22 5/2	2 6	/6	6/6	4/6	4/5	i 7/	7 5	/15	5/15	
H1b5_	3/	22 3/2	2 4	/6	4/6	6/6	4/5	i 3/	7 3	/15	3/15	,
Column: O	Both 🖲 Ma	jor 🔿 Minor						Ro	w: 🖲 Both	<u>О</u> М	> ajor ○ Mir	or
	· ·	Column's N	lajor) / (Co	lumn's N	(lajor)				rcentage	~		
Variant Cor	nparison					Major All	ele 📃 Mii	nor Allele	Low Cove	rage	Save As	
	G2a3a_	2a3aH1n570	H1a3a1_	1a3a1R3	30b: H1	b5_	H1n5_	R30b2_	T2b7a1_	. Г <b>2</b> Ь	7a 1H 1b 570	1
G3010A	0%	23%	99%	64%		9%	99%	0%	0%		25%	
G3010G	99%	76%	0%	35%	5 (	)%	0%	100%	100%		74%	
A4769G	99%	99%	99%	99%	9	9%	99%	99%	99%		99%	
A4833A	0%	34%	99%	100%	6 9	9%	99%	99%	99%	Τ	99%	
A4833G	99%	65%	0%	0%	C	)%	0%	0%	0%		0%	
40174	00%	00%	00%	00%	10	v09/	00%	009/	0%		25%	1

## Autosomal and Y-STR Genotypes and SNPs

High-throughput sequencing data for forensic applications, database or casework, can be analyzed by selecting a builtin panel or by loading a panel for custom chemistries. Autosomal and Y-STR analysis includes conventional forensic nomenclature. **Contact info@softgenetics.com for a copy of the concordance study.** GeneMarkerHTS software capabilities include simultaneous analysis and reporting of mtDNA and STR chemistries.

Review and save reports at different levels of details -- no need to scroll through pages of results to locate the area(s) of interest.

- Overview Assignment of reads to different analysis categories (Autosomal, CHR Y, ...)
- Category Assignment of reads among the loci in category
- Locus- frequency and identified names of sequences in the selected locus
- Sequence detailed quantitative information for the selected sequence along with annotation information

G MainWindow									
All	Sample Info Name: C:/Users/soft/Desktop/GMHTS_Test/ISHI17poster/STR-AutoMitoY\2800M.bam Date: Tue Sep 26 10:29:32 2017								
	Category:		Locus:						
	Amelogenin	*	Amelogenin	•	chrY 🔻				
	Overview Categ	jory Locus	Sequence						
	Category	Count	Percent	Forward Cou	unt Reverse Count				
	Amelogenin	2891	1.2%	1503	1388				
	Autosomal STR	68789	27.7%	33669	35120				
	ChrY STR	35749	14.4%	18226	17523				
	Mitochondrial	70265	28.3%	29322	40943				
	Unsorted	70831	28.5%	70831	0				

The Overview results are initially displayed allowing a comprehensive review of the results to start. The results can be broken down by selecting a category and locus from the dropdown lists.

SF1PO	Sample Info Name: C:/Users/soft/Desktop/GMHTS_Test/ISHI17poster/STR-AutoMitoY\2800M.bam Date: Tue Sep 26 10:29:32 2017								
	Category: Locus:								
	Autosomal STR	•	CSF1PO	▼ 12	12 •				
	Overview Cat	egory Locus	Sequence						
	Name	Count	Percent	Forward Count	Reverse Count				
	12	3329	89.8%	1783	1546				
	11	295	8.0%	143	152				
	13	35	0.9%	17	18				
	10	21	0.6%	13	8				
	12	18	0.5%	7	11				
	15	11	0.3%	6	5				
	15	11	0.3%	6	5				

The Locus tab displays results for each allele in the selected locus.

## **Visualization of Isoalleles**

**Isoallele:** A locus that appears homozygous in length-based measurements (such as CE), but is heterozygous by sequence. High throughput sequencing provides allele calls and sequence variants. *This depth of information has applications in identification of individuals and relatives in single source samples and the potential for improved assignment of alleles to contributors during analysis of mixtures.* 



## User management and control of access rights is managed by the laboratory administrator

Per	mission Activity Project	Activity User A	ctivity			
	Date	Name	Event	Comment		1
1	Tue Jan 10 16:54:38 2017	Michael	Logout			
2	Tue Jan 10 16:54:57 2017		Login	success		
3	Tue Jan 10 16:55:42 2017	test	Edit User	success		
4	Tue Jan 10 16:56:03 2017	test	Logout			
5	Tue Jan 10 16:56:11 2017	labtec	Login	invalid		
6	Tue Jan 10 16:56:15 2017	LabTec	Login	invalid		
7	Tue Jan 10 16:56:17 2017	LabTec	Login	disabled		
8	Tue Jan 10 16:56:31 2017	test	Login	success		
9	Tue Jan 10 16:58:28 2017	test	Logout			

The database provides password protected access rights, audit trail and allows users to upload/download projects with the initial analysis parameters and upload/download changes to analysis parameters of subsequent analysts. The database provides a record of all analysis parameters and activities on a data set.

Minimum Recommended processing hardware:

64 bit Windows OS 12 GB RAM 2.4GHz Dual Quad Core Processor



<sup>1</sup> Parsons et al. 2014 Forensic Science International: Genetics. 13:134-42

<sup>2</sup> Holland, Pack and McElhoe. 2017 Forensic Science International: Genetics. 28:90-98

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<sup>&</sup>lt;sup>3</sup> European DNA Profiling Group (EDNAP) ENDAP mtDNA Population Database https://empop.online/

Thank you to Promega Corporation, Madison, WI, USA for providing Autosomal and Y-STR data.



Software PowerTools for Forensic Analysis

SoftGenetics Oakwood Centre 100 Oakwood Avenue Suite 350 State College PA 16803 USA 1-888-791-1270 info@softgenetics.com www.softgenetics.com

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