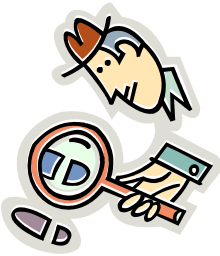
Mitchell M. Holland, Ph.D.
Associate Professor, Biochem & MolBio
Former Director, Forensic Science Program
Eberly College of Science
Penn State University, University Park, PA



Crash Course in MPS mtDNA Analysis: Survey of Approaches

24 September 2018

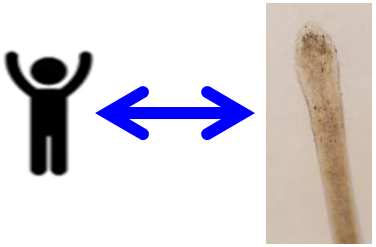
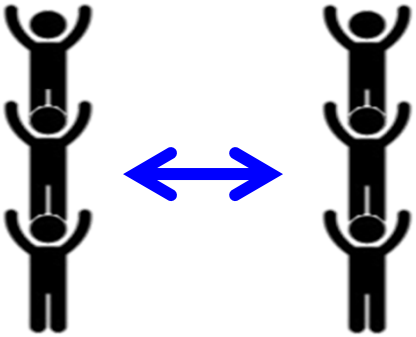
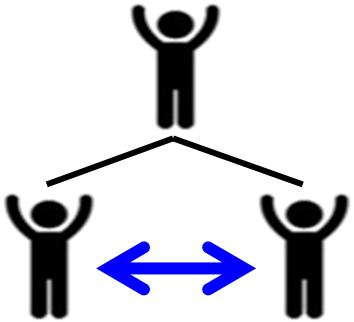
Next Generation Sequencing (NGS) of mtDNA

… a Massively Parallel Sequencing (MPS) Approach to mtDNA Analysis

A recent search of the literature identified >25,000 published articles on MPS applications

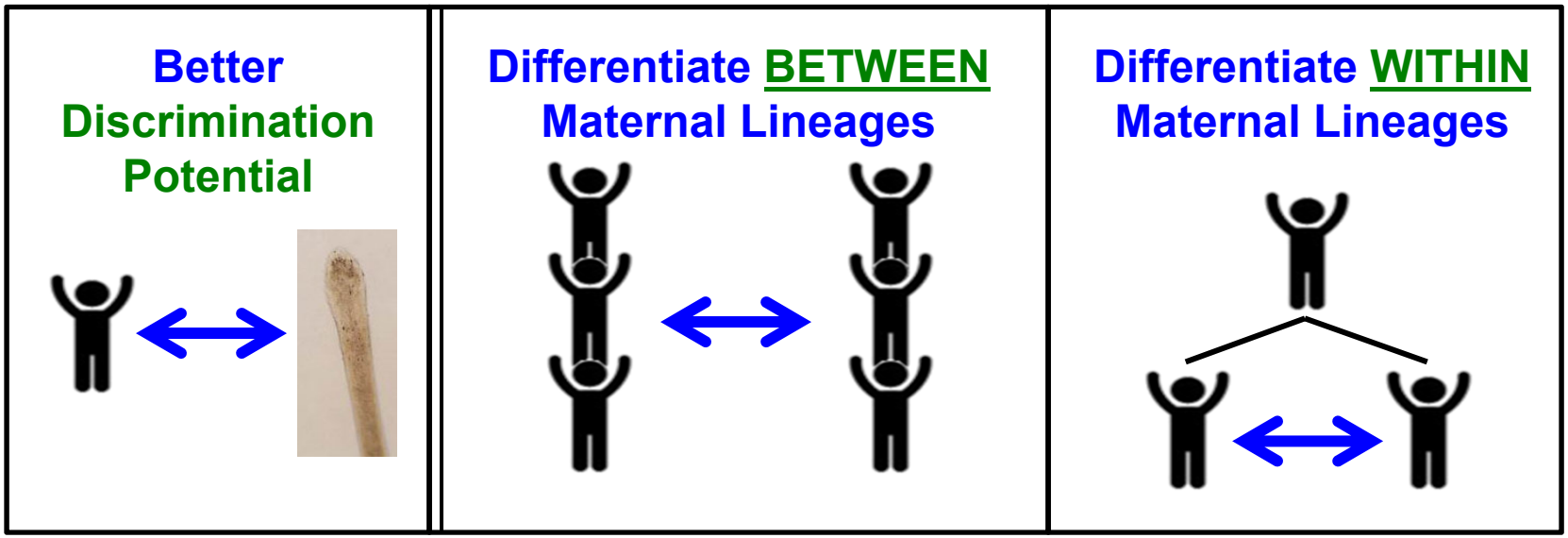
>675 publications on MPS mtDNA
77 publications on forensic MPS mtDNA

For the broader forensic community to move to routine mtDNA analysis, greater discrimination potential is desirable

Better Discrimination Potential	Differentiate BETWEEN Maternal Lineages	Differentiate WITHIN Maternal Lineages
		

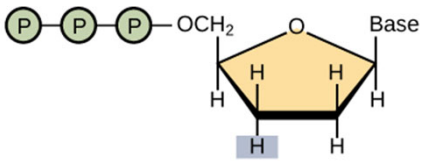
MPS mtDNA analysis will allow us to accomplish these goals

For the broader forensic community to move to routine mtDNA analysis, greater discrimination potential is desirable



… through heteroplasmy and whole mtgenome analysis

Given that the forensic community is moving to MPS methods for STR and SNP analysis, it makes sense to consider MPS mtDNA analysis … in fact, perhaps starting with mtDNA analysis is the best approach



Dideoxynucleotide (ddNTP)

(STS) Sanger Type

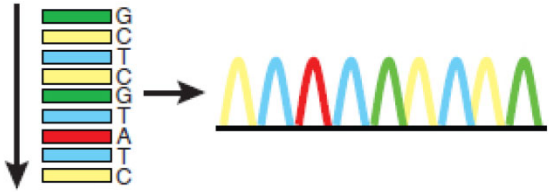
Cycle sequencing

3'-... GACTAGATACGAGCGTGA...-5' (template)
5'-... CTGAT (primer)

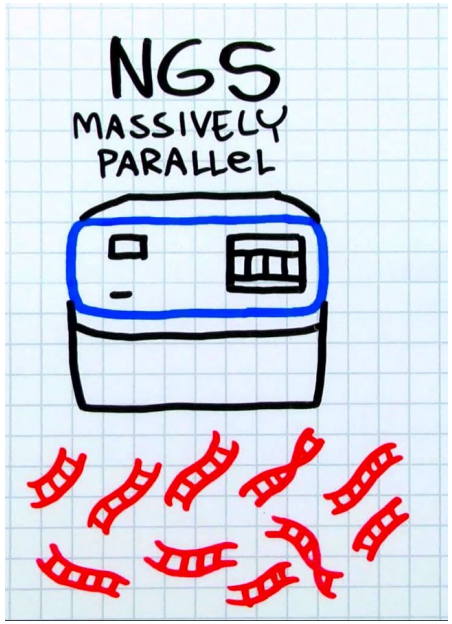
...CTGATC
...CTGATCT
...CTGATCTA
...CTGATCTAT
...CTGATCTATG
...CTGATCTATGC
...CTGATCTATGCT
...CTGATCTATGCTC
...CTGATCTATGCTCG

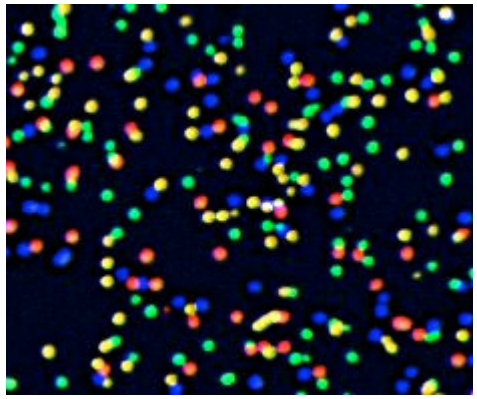
Polymerase
dNTPs
Labeled ddNTPs

Electrophorsesis
(1 read/capillary)



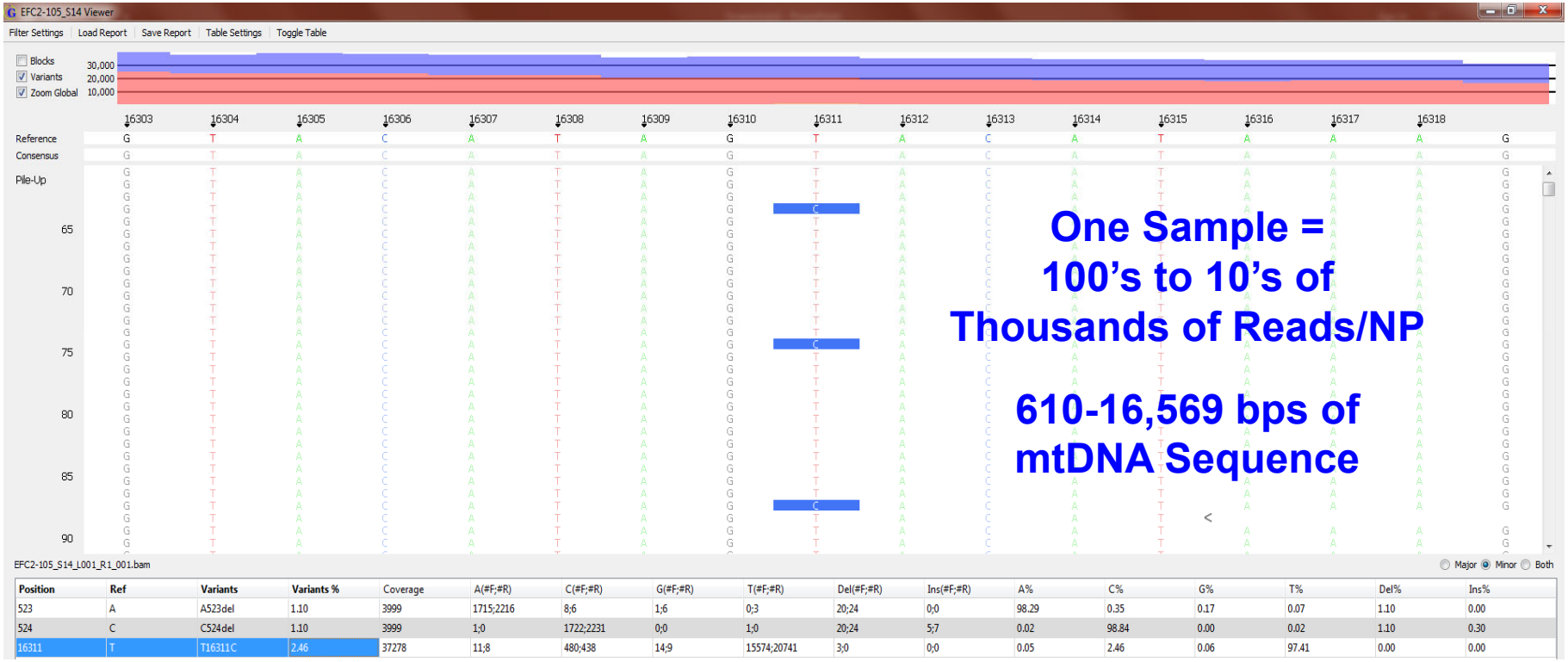
One Amplicon/Sample
= One Read/NP





Up to 160+ Amplicons/Sample =
100's to 10's of Thousands of Reads/NP

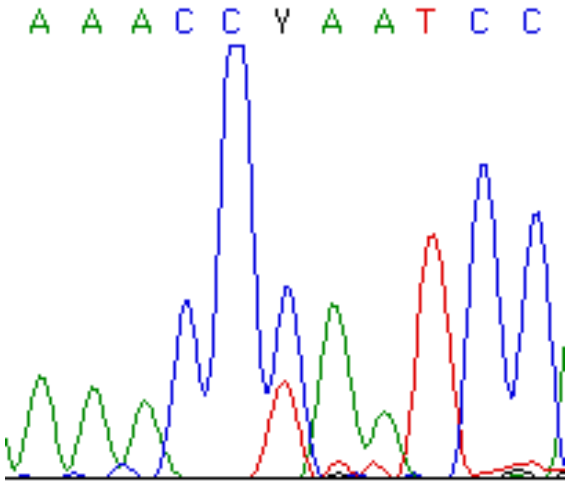
NP = nucleotide position



Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%
523	A	A523del	1.10	3999	1715;2216	8;6	1;6	0;3	20;24	0;0	98.29	0.35	0.17	0.07	1.10	0.00
524	C	C524del	1.10	3999	1;0	1722;2231	0;0	1;0	20;24	5;7	0.02	98.84	0.00	0.02	1.10	0.30
16311	T	T16311C	2.46	37278	11;8	480;438	14;9	15574;20741	3;0	0;0	0.05	2.46	0.06	97.41	0.00	0.00

One Amplicon/Sample = One Read/NP

100-450 bps of mtDNA Sequence



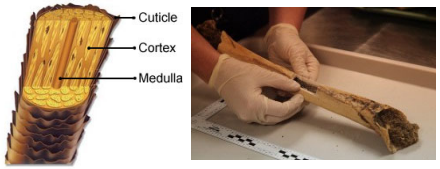
Can resolve heteroplasmy … and often times resolve mixtures

Holland et al., FSIG 2017

Workflow

MPS

DNA Extraction



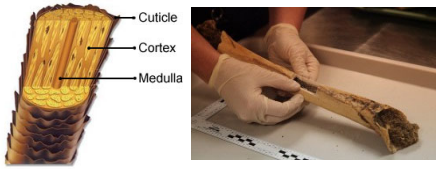
DNA extraction is the same, but in many instances the amount of extract needed for amplification is reduced

- **Amplification of mtDNA**
- **Sample Preparation**
- **Sequencing**
- **Data Analysis**

Workflow

MPS

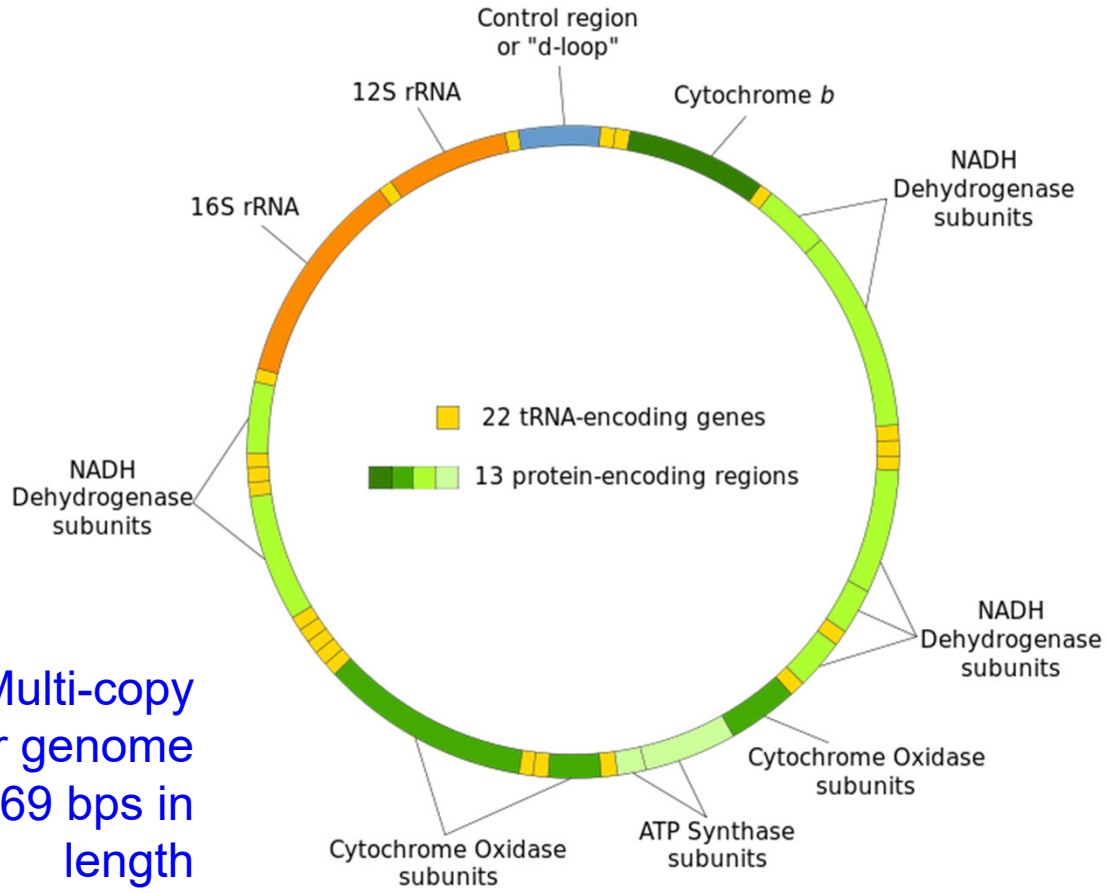
DNA Extraction



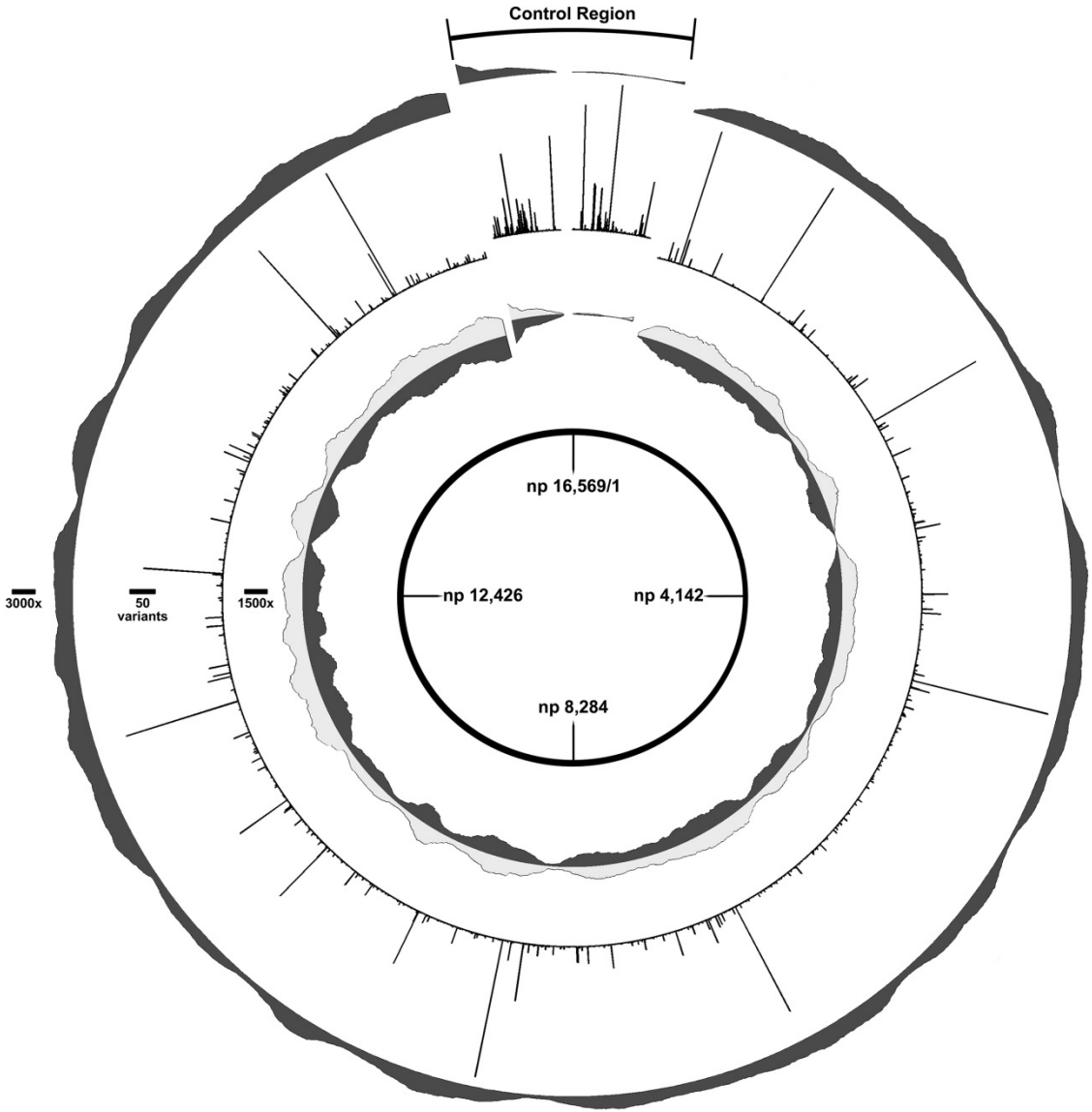
DNA extraction is the same, but in many instances the amount of extract needed for amplification is reduced

- **Amplification of mtDNA**
- **Library Preparation**
- **Sequencing**
- **Data Analysis**

1122 bps = control region (6.8%)
16024-16569 & 1-576



Multi-copy number genome of 16569 bps in length

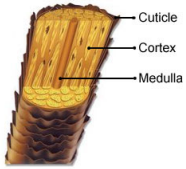


While there is a greater density of active SNP sites in the CR, there is considerable discrimination potential in the coding region

King et al., FSIG 2014

MPS Workflow

DNA Extraction



Amplification of mtDNA

Library Preparation

Sequencing

Data Analysis

Amplification Approaches

mtDNA D-loop Protocol (HVRs)

Traditional 4 separate amplification reactions across HV1/HV2

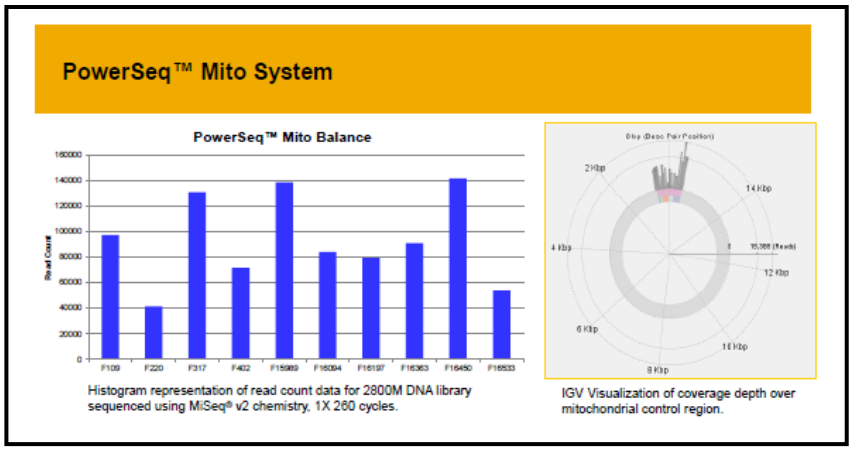
NexteraXT library preparation

Int J Legal Med
DOI 10.1007/s00414-017-1530-9

ORIGINAL ARTICLE

MPS analysis of the mtDNA hypervariable regions on the MiSeq with improved enrichment

Mitchell M. Holland[1] · Laura A. Wilson[2] · Sarah Copeland[3] · Gloria Dimick[3] · Charity A. Holland[1] · Robert Bever[2] · Jennifer A. McElhoe[1]

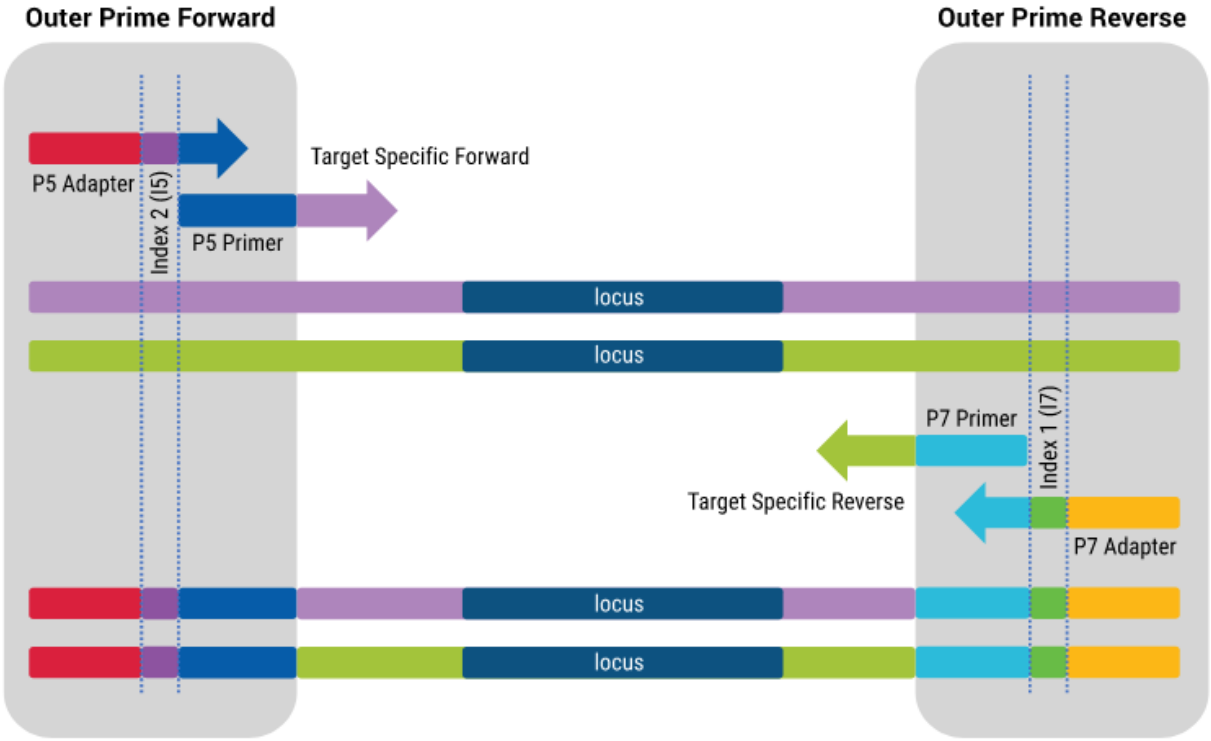


PowerSeq™ CRM Nested System

10-plex (multiplex) amplification across the entire control region

Streamlined library preparation due to incorporation of adaptors & indices into the amplicon

PowerSeq™ CRM Nested



Whole mtGenome Sequencing

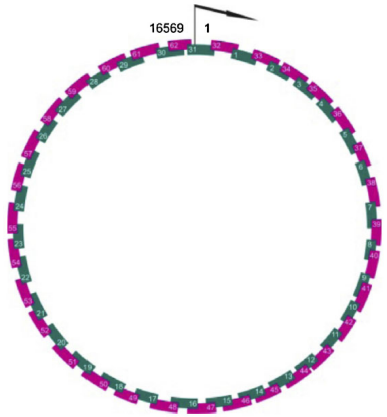
Electrophoresis 2018, *0*, 1–10

Vania Pereira
Antonio Longobardi
Claus Børsting

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Research Article

Sequencing of mitochondrial genomes using the Precision ID mtDNA Whole Genome Panel



International Journal of Legal Medicine (2018) 132:1049–1056
https://doi.org/10.1007/s00414-017-1755-7

ORIGINAL ARTICLE

Assessment of mitochondrial DNA heteroplasmy detected on commercial panel using MPS system with artificial mixture samples

Sohee Cho[1] · Moon Young Kim[2] · Ji Hyun Lee[2] · Soong Deok Lee[1,2]

Forensic Science International: Genetics 15 (2015) 8–15

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Original Research Paper

Massively parallel sequencing of complete mitochondrial genomes from hair shaft samples

Walther Parson[a,b,*], Gabriela Huber[a], Lilliana Moreno[c], Maria-Bernadette Madel[a], Michael D. Brandhagen[c], Simone Nagl[a], Catarina Xavier[a], Mayra Eduardoff[a], Thomas C. Callaghan[c], Jodi A. Irwin[c,*]

[a] Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria
[b] Penn State Eberly College of Science, University Park, PA, USA
[c] FBI Laboratory, Quantico, VA, USA

Forensic Science International: Genetics 36 (2018) 213–224

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Evaluation of the precision ID mtDNA whole genome panel on two massively parallel sequencing systems

August E. Woerner[a,b,*,1], Angie Ambers[a,b,*,1], Frank R. Wendt[a], Jonathan L. King[a], Rodrigo Soares Moura-Neto[c], Rosane Silva[d], Bruce Budowle[a,b,e]

[a] Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[b] Department of Microbiology, Immunology and Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[c] Laboratório de Biologia Molecular Forense, Instituto de Biologia Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[d] Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio, de Janeiro, Rio de Janeiro, RJ, Brazil
[e] Center of Excellence in Genomic Medicine (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

Forensic Science International: Genetics 35 (2018) 21–25

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Evaluation of the precision ID whole MtDNA genome panel for forensic analyses

Christina Strobl[a], Mayra Eduardoff[a], Magdalena M. Bus[b], Marie Allen[b], Walther Parson[a,c,*]

[a] Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria
[b] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
[c] Forensic Science Program, The Pennsylvania State University, University Park, PA, USA

Table 1
Overview of forensic and ancient samples tested with the early access version of the Precision ID Whole MtDNA Genome Panel (TFS) and Massively Parallel Sequencing (MPS) using the Ion Personal Genome Machine (PGM).

Sample						Quantity	Volume	Sequencing result	
Info	#	Type	Age	Tissue	Extraction method	[mtGE/µl]	MT [µl]	Mitogenome (MPS)	CR (STS)
Mock samples									
Hair shaft 1	1	forensic	6 yrs old	Hair shaft	Qiagen EZ1 DNA Investigator	983	5	full	full
Hair shaft 2	2	forensic	6 yrs old	Hair shaft	Qiagen EZ1 DNA Investigator	1.129	6	full	full
Forensic samples									
Case 1									
Hair shaft	3	forensic	recent	Hair	Qiagen EZ1 DNA Investigator	220	10	full	CR partial
Hair end	4	forensic	recent	Hair	Qiagen EZ1 DNA Investigator	94	12	full	CR partial
Case 2									
Swab	5	forensic	recent	Swab from floor	QIAamp DNA Investigator	115	6	full	CR partial
Victim	6	reference	recent	Reference sample	QIAamp DNA Investigator	2000	2	full	full
Suspect	7	reference	recent	Reference sample	QIAamp DNA Investigator	2000	2	full	full
Ancient solid tissue samples									
FA10003T01a	8	aDNA	~1 kyrs	molar	PCI	4.548	9	full	CR partial
FA10005T01a	9	aDNA	~1 kyrs	molar	PCI	1.082	12	partial	CR partial
FA10006T01a	10	aDNA	~1 kyrs	molar	PCI	566	18	partial	CR partial
FA10007T01a	11	aDNA	~1 kyrs	pre-molar	PCI	448	18	partial	CR partial
FA10010T01a	12	aDNA	~1 kyrs	molar	PCI	7.835	7	partial	CR partial
FA10011T01a	13	aDNA	~1 kyrs	molar	PCI	1.086	12	partial	CR partial
FA10012T01a	14	aDNA	~1 kyrs	femur	PCI	528	18	full	CR partial
FA10014T01a	15	aDNA	~1 kyrs	molar	PCI	1.665	7	partial	CR partial

mtGE/µl … mitochondrial DNA genome equivalents per microliter; MT … mitotiling; STS … Sanger-type Sequencing; CR … control region, PCI … Phenol Chloroform Isoamylalcohol.

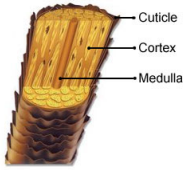
Amplification Approaches & Kits Available

- ThermoFisher
  - Precision ID mtDNA Control Region Panel (2 multiplexes)
  - Precision ID mtDNA Whole Genome Panel (2 multiplexes of 81 amplicons averaging 161 bps)
- Verogen
  - Human D-loop protocol (HV1/HV2, 4 amplicons)
  - Human mtDNA Genome protocol (databanking)
- Promega
  - PowerSeq CRM (control region, 1 multiplex, 144-237 bps)
  - PowerSeq WGM (mtgenome, in development, 1 multiplex of 161 amplicons averaging 167 bps)

Custom approaches are still viable

MPS Workflow

DNA Extraction



Amplification of mtDNA

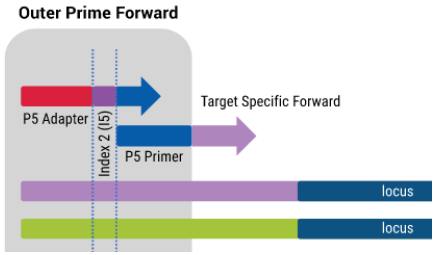
Library Preparation

Sequencing

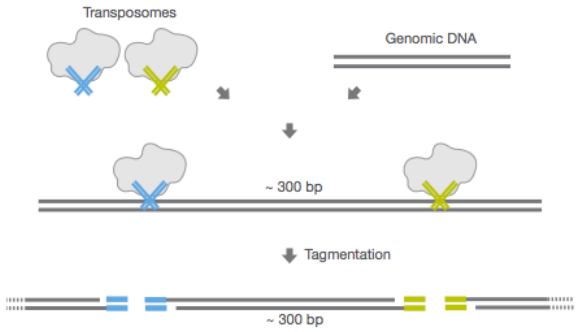
Data Analysis

Library Preparation

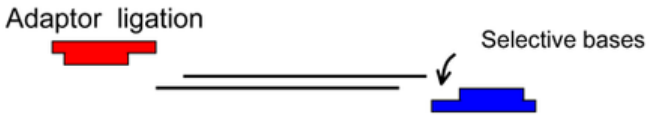
Adaptor Integration



Tagmentation



Adaptor Ligation



Shearing

Covaris

DNA Shearing Quick Guide:

M220 Focused-ultrasonicator and Holder XTU

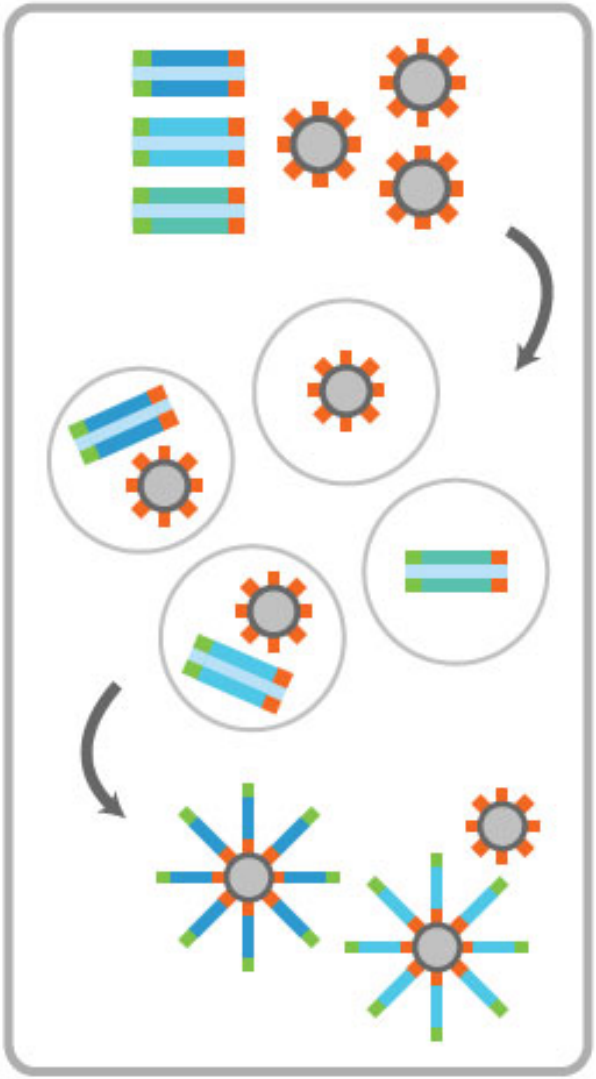
This Quick Guide provides DNA Shearing protocols for using Holder XTU with the Covaris M220 Focused-ultrasonicator. Holder XTU should be used with microTUBE-15 AFA Beads Screw-Cap and requires a specific insert for 15 µl sample volume.

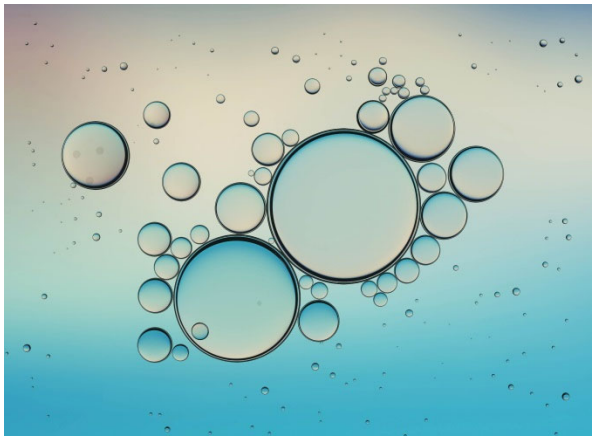
15 µL sample volume
microTUBE-15 AFA Beads Screw-Cap - from 150 to 550 bp

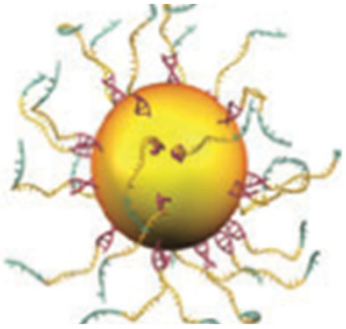
Target BP (Peak)	150	250	350	550
Peak Incident Power (W)	30	30	30	30
Duty Factor	20%	20%	20%	20%
Cycles per Burst	50	50	50	50
Treatment Time (s)	250	80	42	23
Temperature (°C)	20	20	20	20
Sample volume (µl)	15	15	15	15

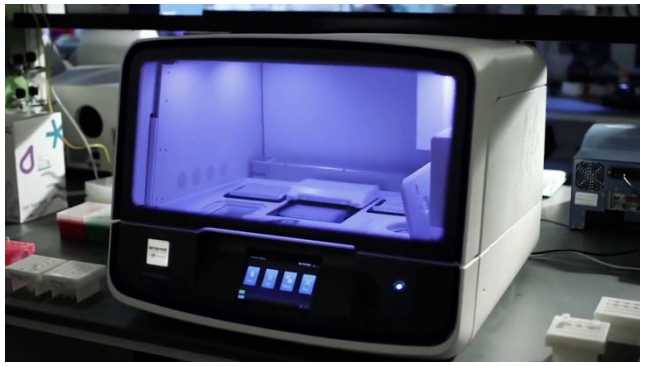
Ion Chef

Emulsion PCR





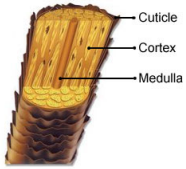




Ion Chef
Prior to being loaded
on the Ion S5

MPS Workflow

DNA Extraction



Amplification of mtDNA

Library Preparation

Sequencing

Data Analysis

MPS Instruments

Current Players

MiSeq FGx

https://verogen.com/

https://www.illumina.com

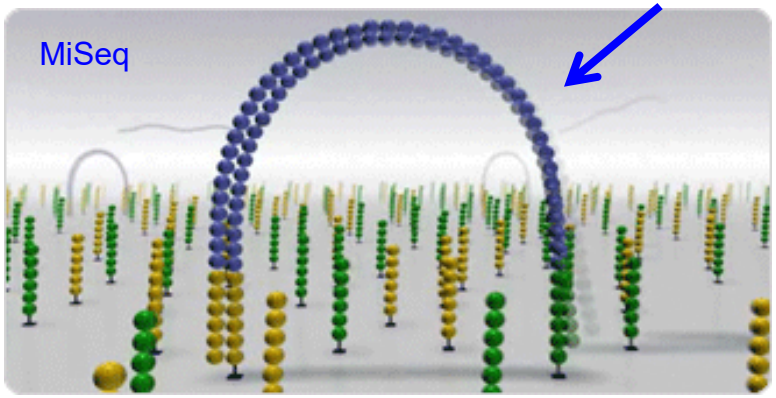
Run the FGx in Research Mode

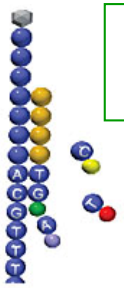
Ion S5

https://www.thermofisher.com

Illumina MiSeq

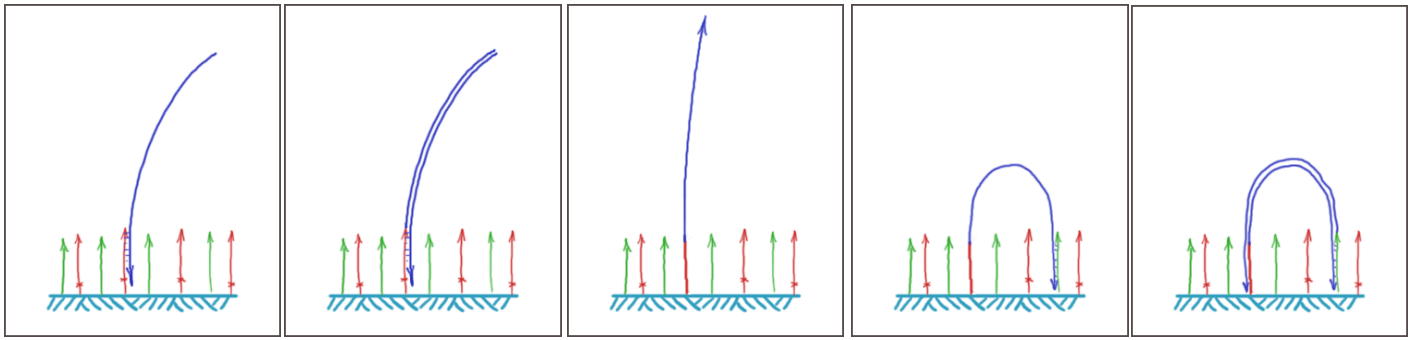
Bridge Amplification
"Cluster" Generation

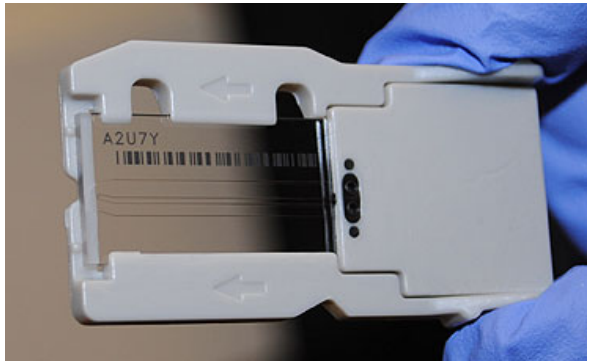




Sequencing By Synthesis
Reversible Terminators

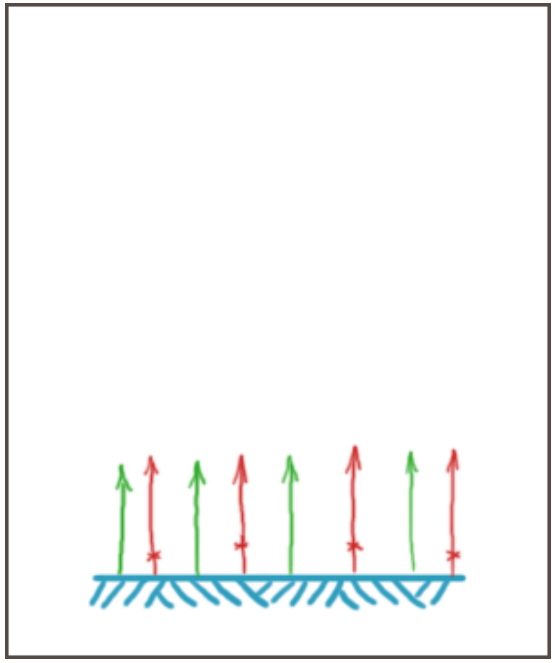
***YouTube* video: "Intro to Sequencing by Synthesis: Industry-leading Data Quality"**

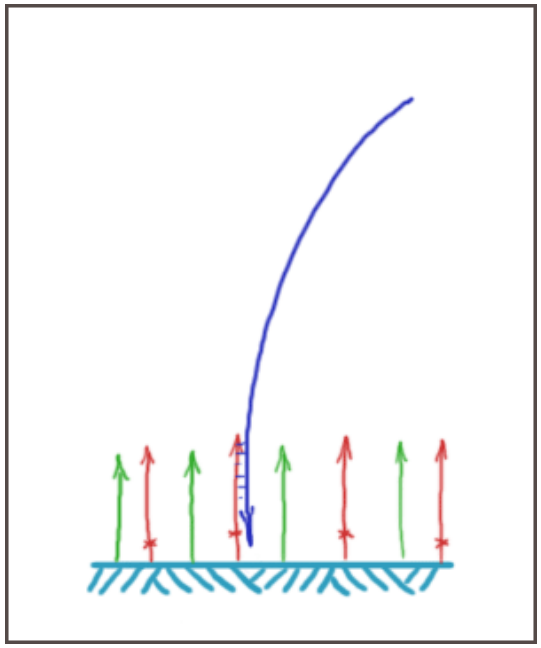


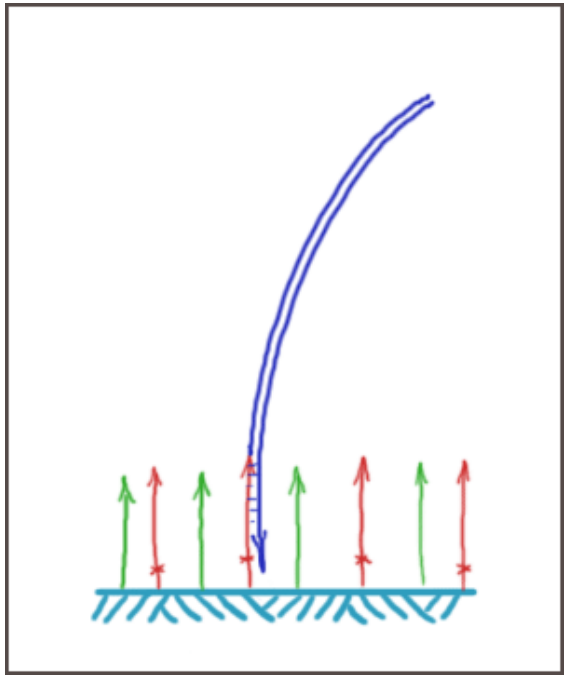


Bridge amplification happens on the flow cell to generate “clusters” of DNA templates for DNA sequencing

Bridge Amplification (BA)

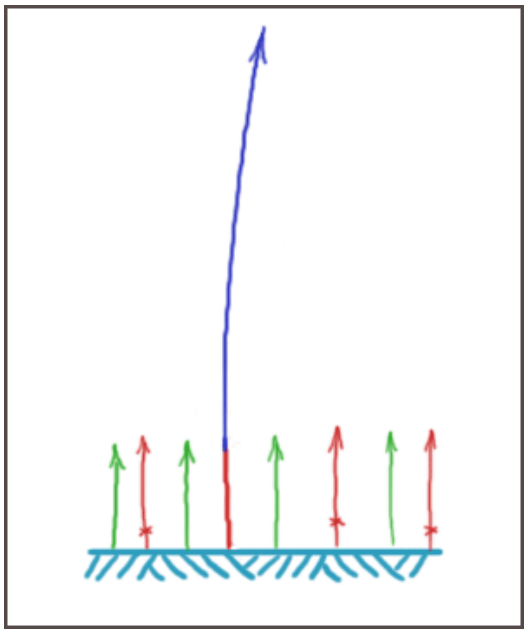


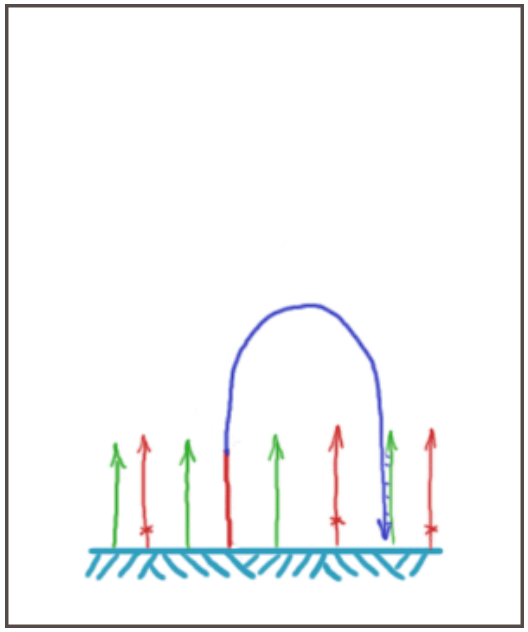


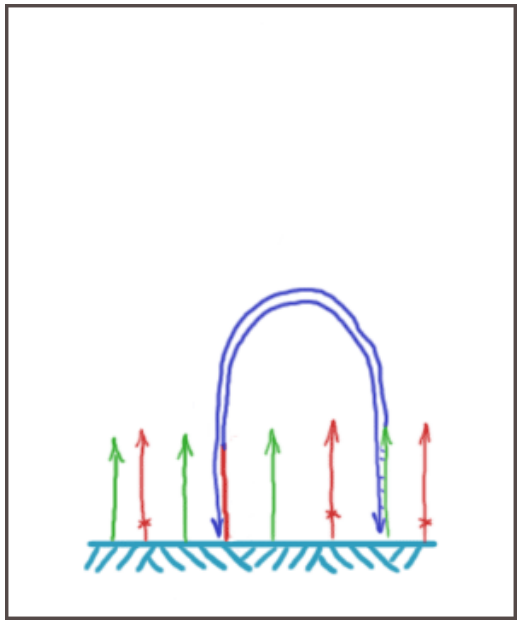


A single copy of ssDNA attaches to the **lawn** via P5 or P7 to a complimentary oligomer, and BA makes a copy of the fragment

Bridge Amplification

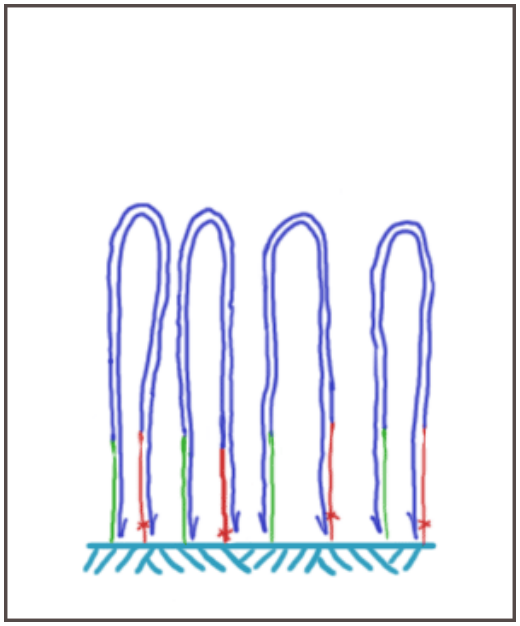


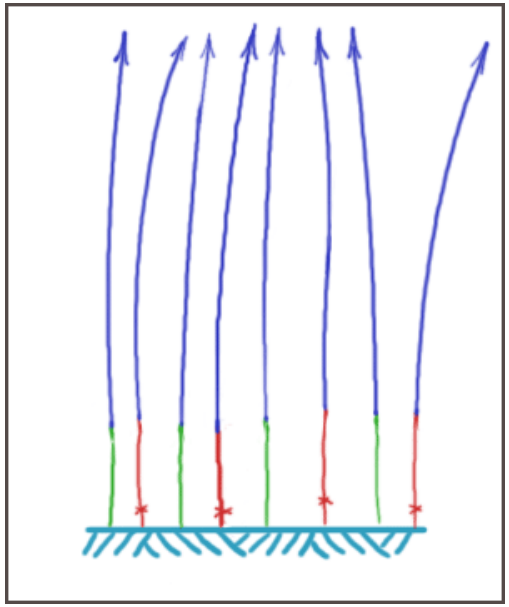


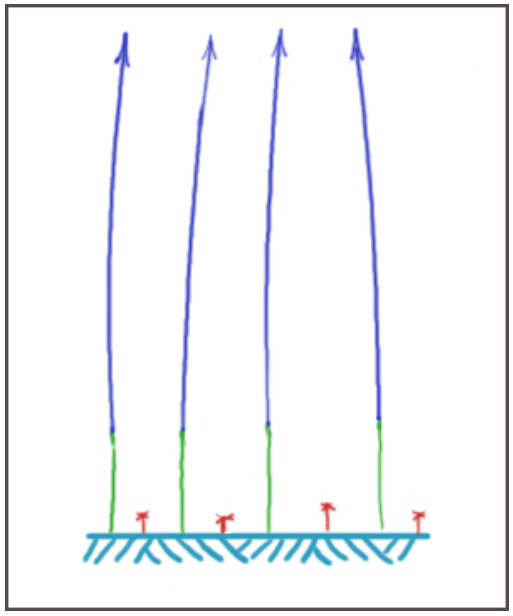


That copy is now covalently bound to the lawn, will bridge over and bind to the other complimentary oligomer (P5 or P7), and allow for repeated amplification and **cluster** formation

Bridge Amplification



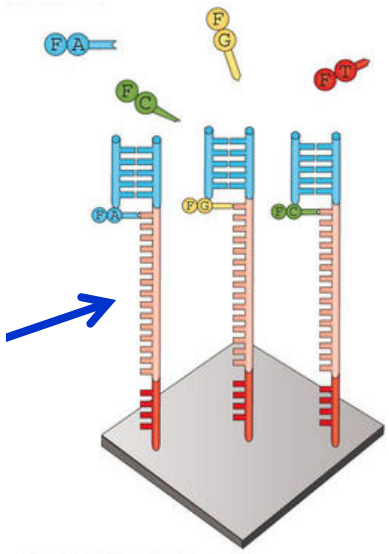




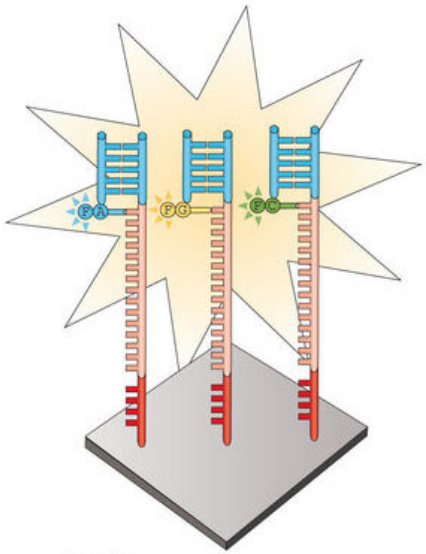
The P5 or P7 strands are selected for by cleaving off the other strands from the lawn, leaving behind a cluster of DNA strands in the same orientation

Reverse Terminator Sequencing

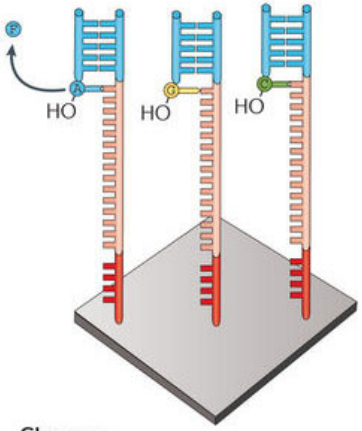
Represents a cluster



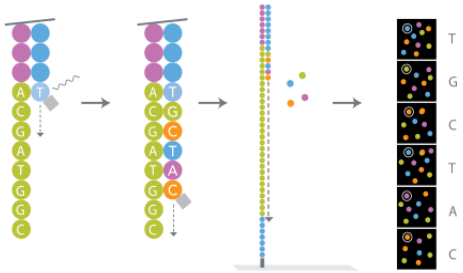
Nucleotide addition
Fluorophore-labelled, terminally blocked nucleotides hybridize to complementary base. Each cluster on a slide can incorporate a different base.



Imaging
Slides are imaged with either two or four laser channels. Each cluster emits a colour corresponding to the base incorporated during this cycle.

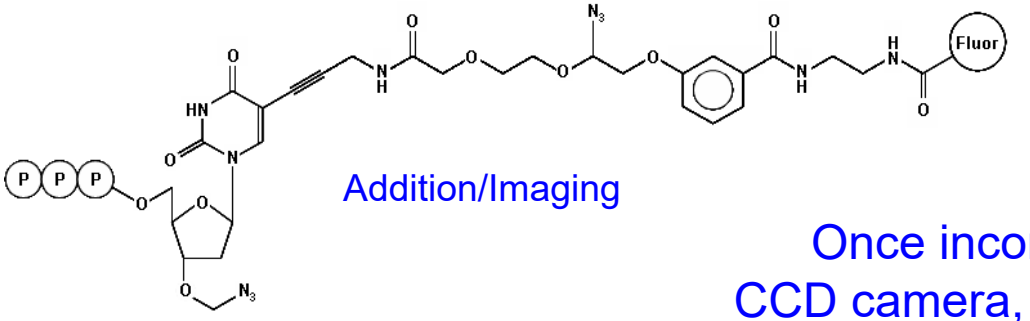


Cleavage
Fluorophores are cleaved and washed from flow cells and the 3′-OH group is regenerated. A new cycle begins with the addition of new nucleotides.



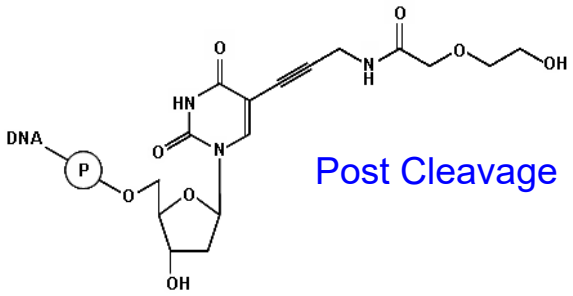
Goodwin et al., Nat Genet Review 2016

Reversible Terminators

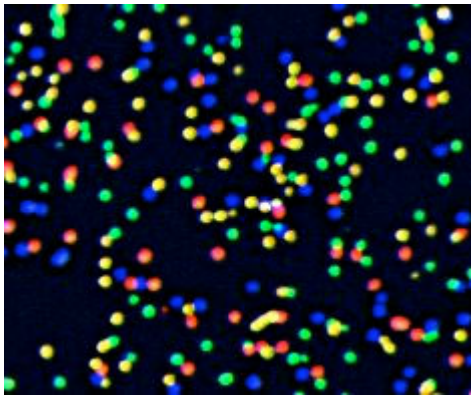


Addition/Imaging

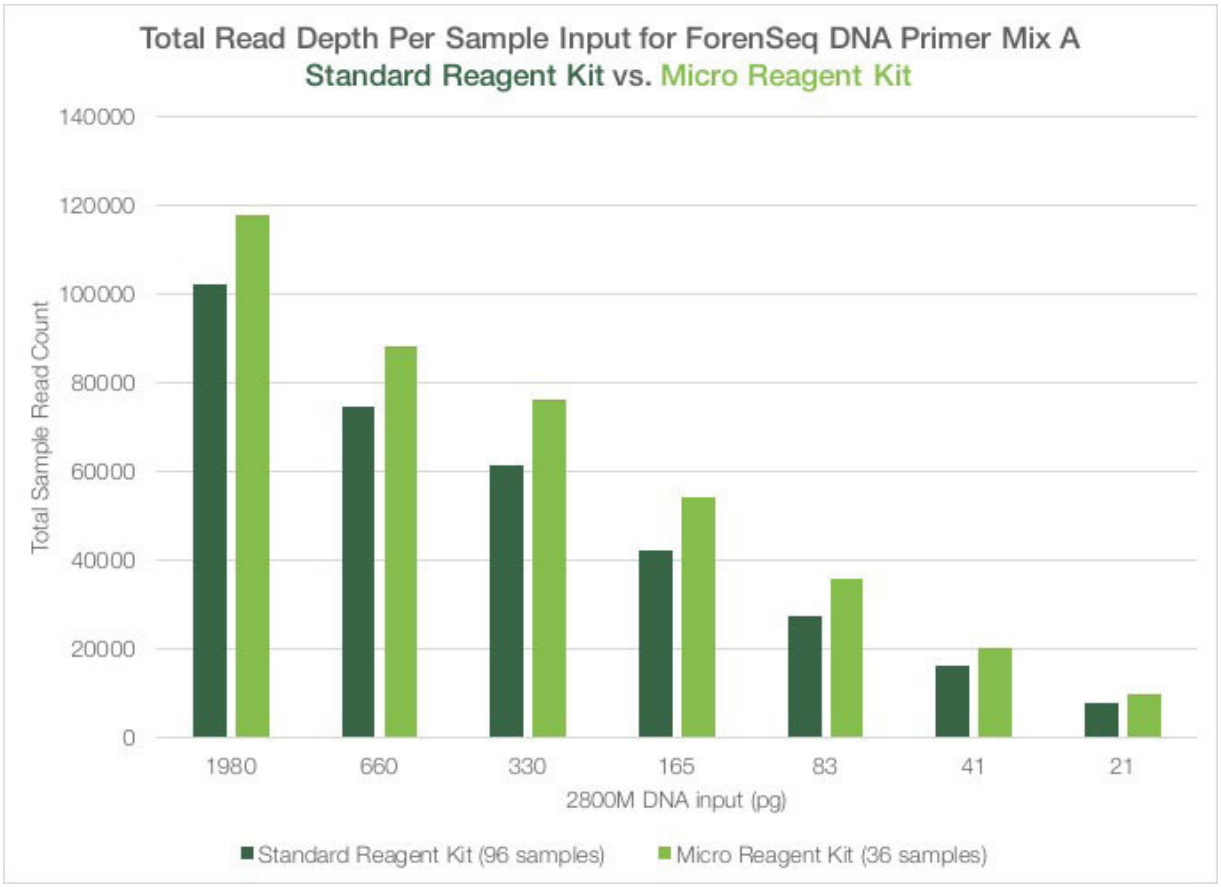
Once incorporated and read by the CCD camera, the dye is removed from the terminator along with the protective group on the 3’-hydroxyl group



Post Cleavage



Flow Cell Capacity

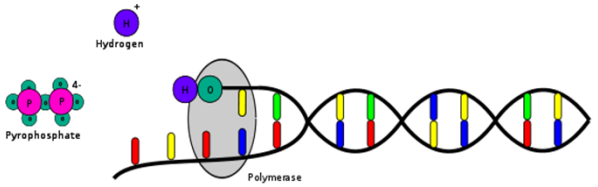


Standard v. Micro

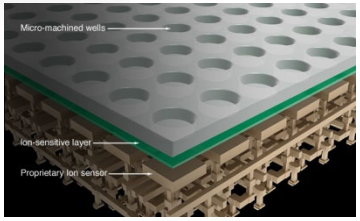
~1/3 the capacity,
but the same read output

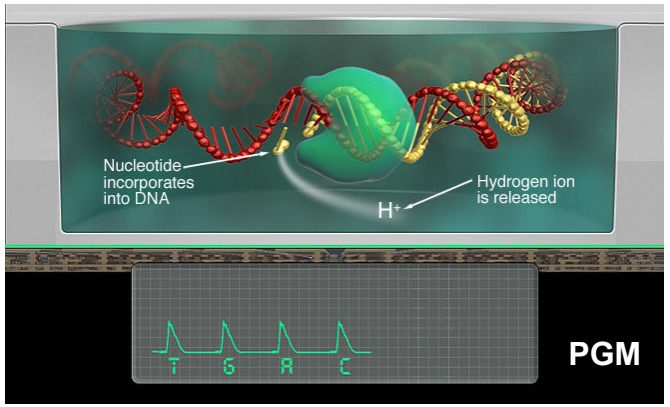
YouTube video: “Ion Torrent™ next-gen sequencing technology”

Ion S5



Hydrogen and pyrophosphate are released.



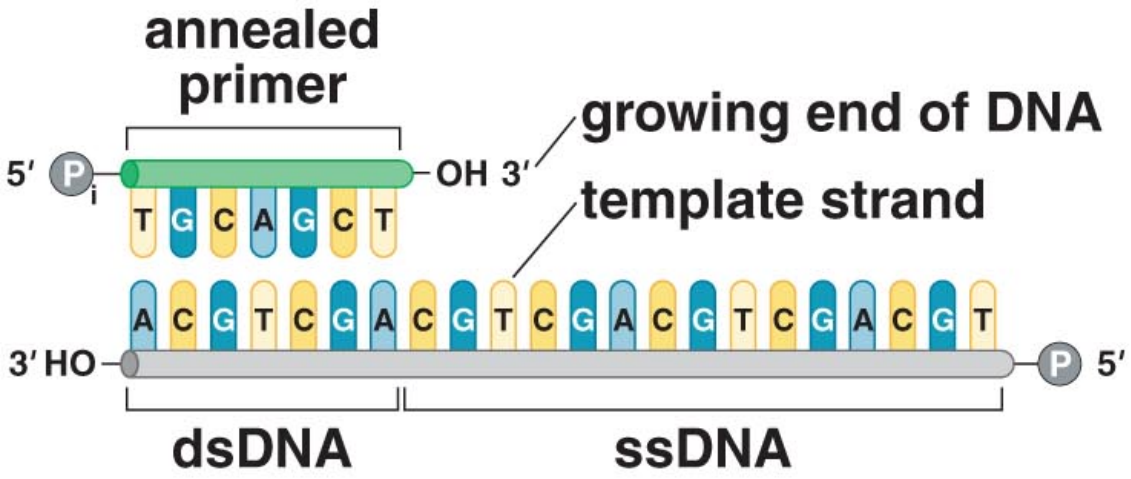


Sequencing By Synthesis

Solid-State pH Meter

DNA Synthesis

- New synthesis requires a double stranded region of DNA (dsDNA) next to a single stranded region (ssDNA) = **Primer:Template Junction**

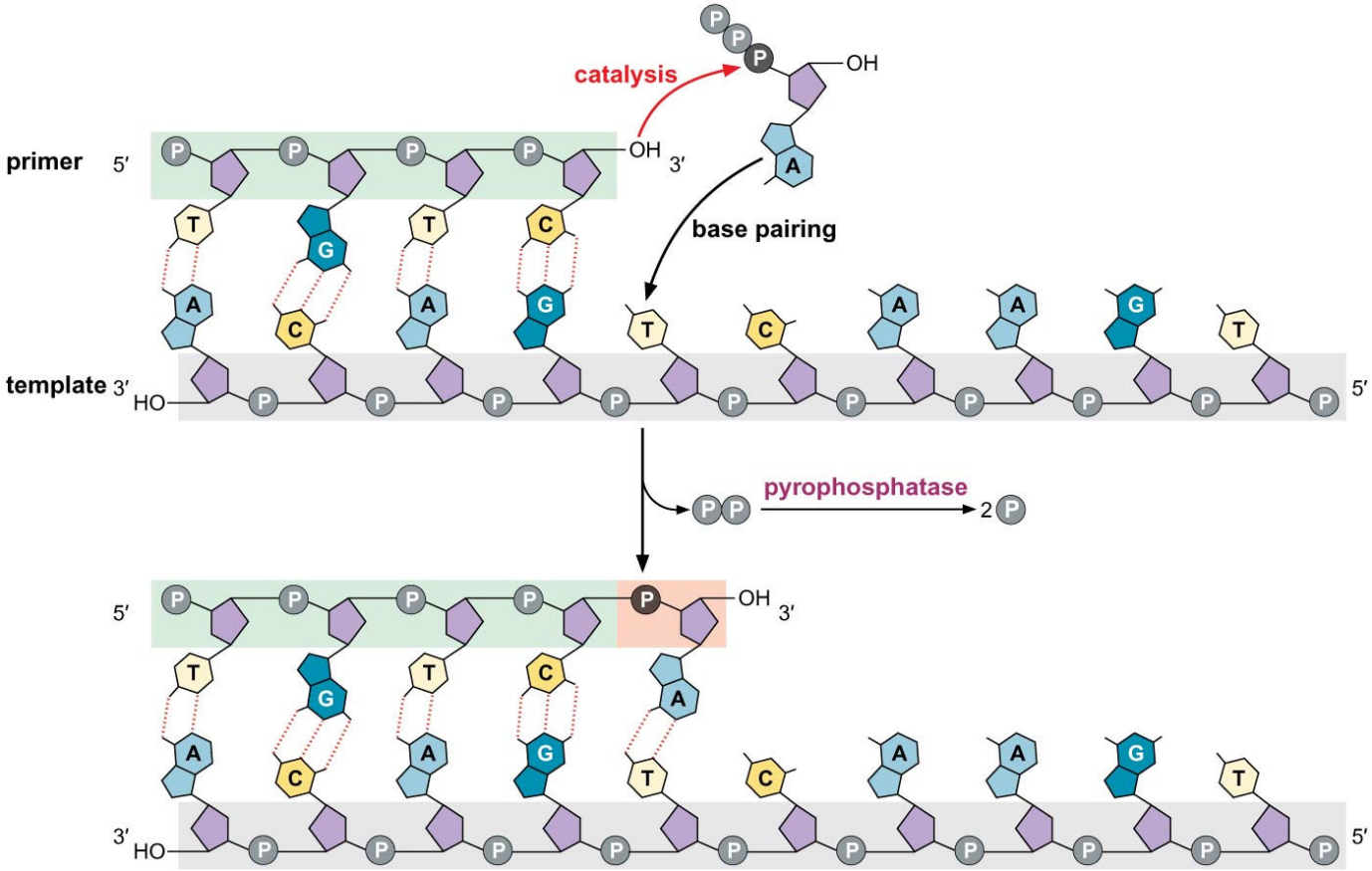


Watson et al., MolBio of the Gene

DNA Synthesis

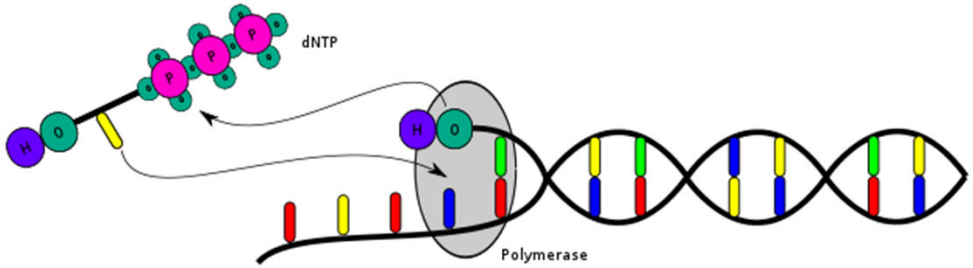
- Initiation of synthesis involves an S_N2 (Substitution Nucleophilic Bimolecular) reaction where;
  - The hydroxyl (OH) group at the 3'-end of the primer is the **nucleophile** that attacks the α-phosphate group of the incoming dNTP
  - The **leaving group** is PP_i

Mechanism of Synthesis



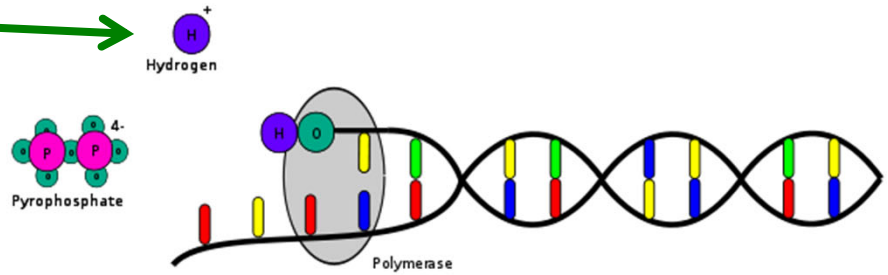
Watson et al., MolBio of the Gene

Measuring H^+ or PP_i Production

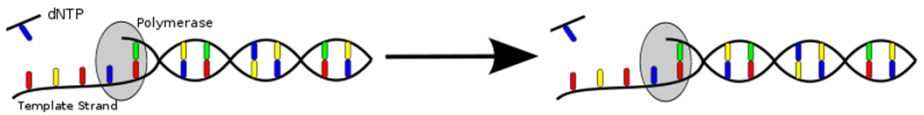


Polymerase integrates a nucleotide.

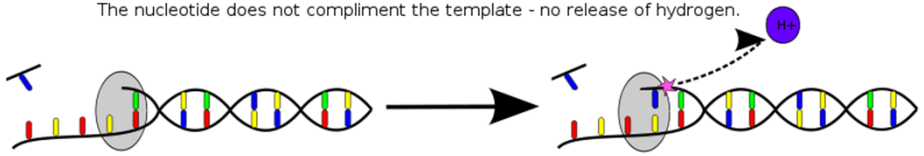
Hydrogen Ion



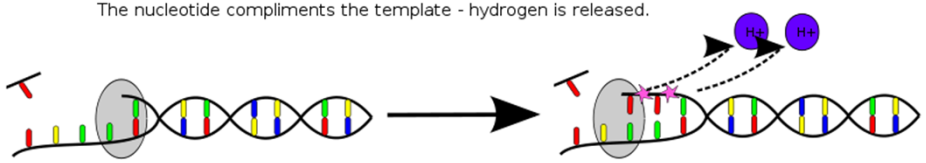
Hydrogen and pyrophosphate are released.



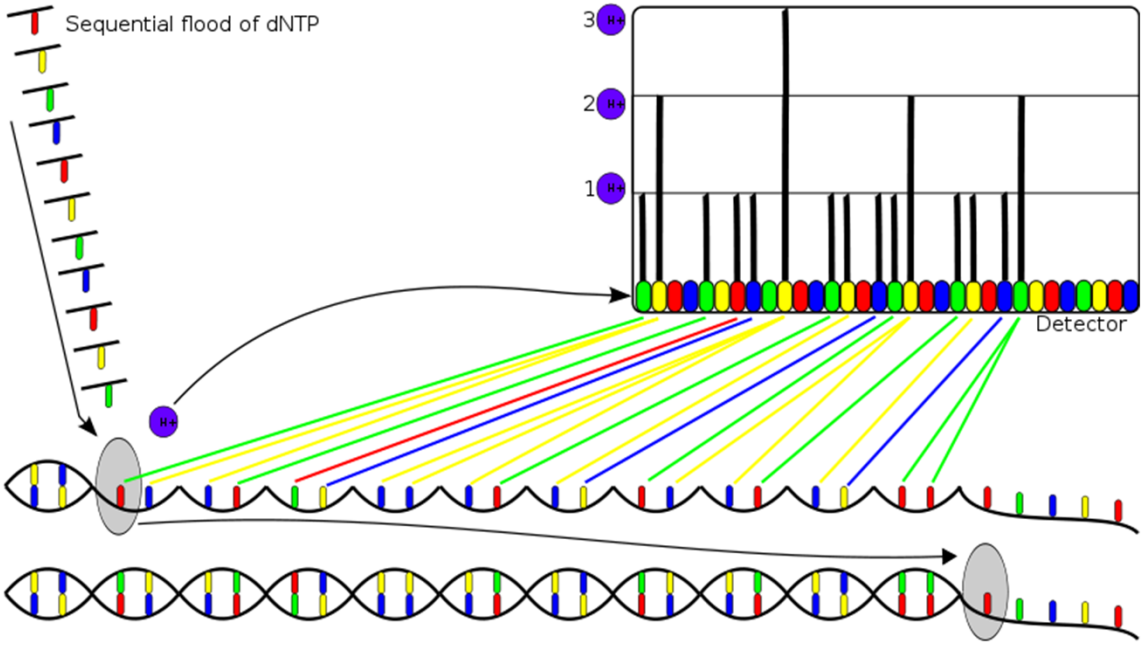
The nucleotide does not compliment the template - no release of hydrogen.

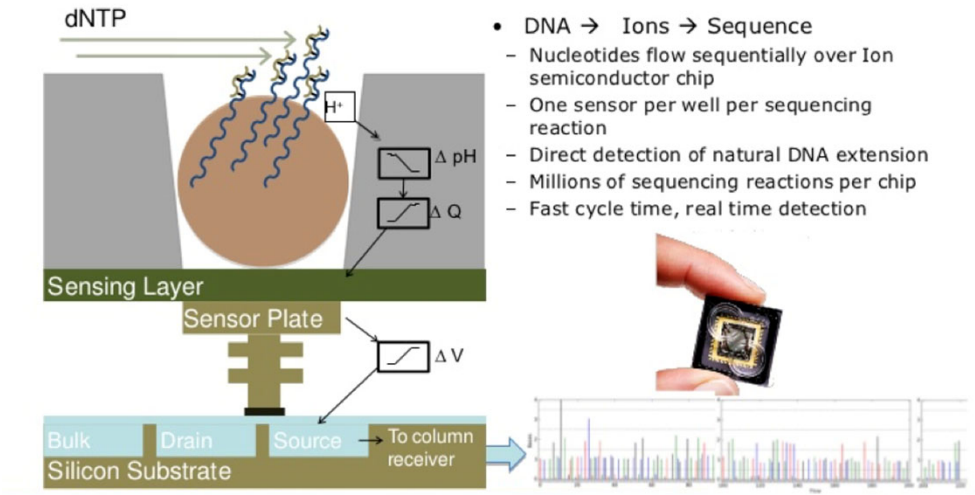


The nucleotide compliments the template - hydrogen is released.

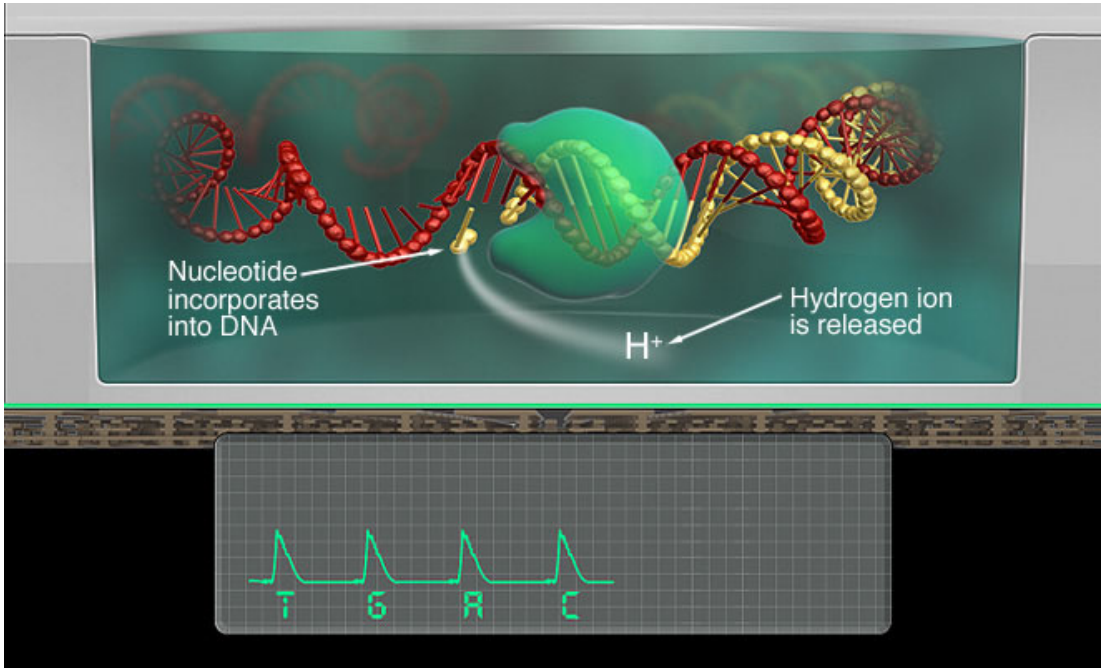


The nucleotide compliments several bases in a row - multiple hydrogen ions are released.





- DNA → Ions → Sequence
  - Nucleotides flow sequentially over Ion semiconductor chip
  - One sensor per well per sequencing reaction
  - Direct detection of natural DNA extension
  - Millions of sequencing reactions per chip
  - Fast cycle time, real time detection



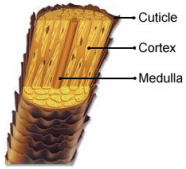
Chip Specifications

Chip type	Number of reads	Read length (output*)	Ion GeneStudio™ S5 System	Ion GeneStudio™ S5 Plus System	Ion GeneStudio™ S5 Prime System
			Turnaround time (sequencing run** plus analysis time)		
Ion 510 Chip	2–3 million	200 bp (0.3–0.5 Gb)	4.5 hr	3 hr	3 hr
		400 bp (0.6–1 Gb)	10.5 hr	5 hr	5 hr
Ion 520 Chip	4–6 million	200 bp (0.6–1 Gb)	7.5 hr	3.5 hr	3 hr
		400 bp (1.2–2 Gb)	12 hr	5.5 hr	5.5 hr
	3–4 million	600 bp (0.5–1.5 Gb)	12 hr	5.5 hr	5.5 hr
Ion 530 Chip	15–20 million	200 bp (3–4 Gb)	10.5 hr	5 hr	4 hr
		400 bp (6–8 Gb)	21.5 hr	8 hr	6.5 hr
	9–12 million	600 bp (1.5–4.5 Gb)	21 hr	8 hr	7 hr
Ion 540 Chip	60–80 million	200 bp (10–15 Gb)	19 hr	10 hr	6.5 hr
		200 bp (20–30 Gb) 2 runs in 1 day	NA	20 hr	10 hr†
Ion 550 Chip	100–130 million	200 bp (20–25 Gb)	NA	11.5 hr	8.5 hr
		200 bp (40–50 Gb) 2 runs in 1 day	NA	NA	12 hr†

* Expected output with >99% aligned or measured accuracy. Output dependent on read length and application.
** Sequencing run times are between 2.5 and 4 hr.
† Analysis of first run occurs concurrently with the second sequencing run.

MPS Workflow

DNA Extraction



Amplification of mtDNA

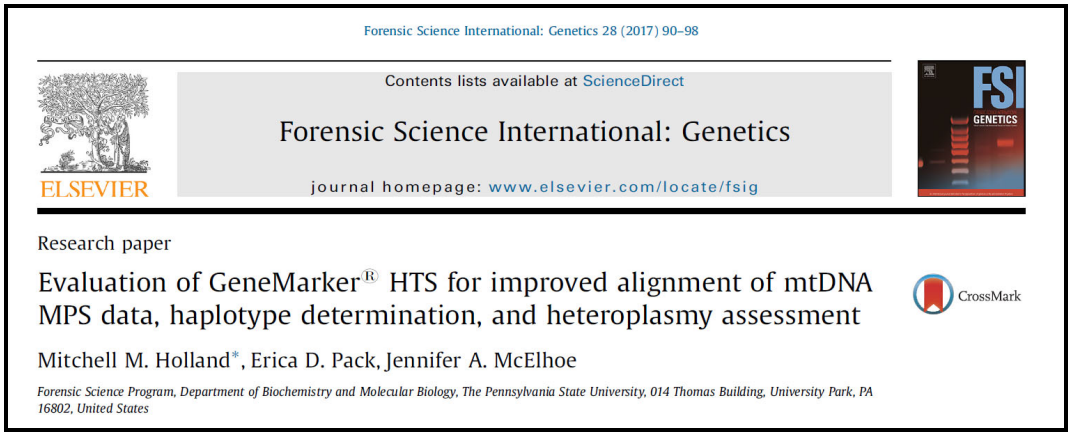
Library Preparation

Sequencing

Data Analysis

Software Development

In collaboration with SoftGenetics, Inc.



Forensic Science International: Genetics 28 (2017) 90–98

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Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Evaluation of GeneMarker® HTS for improved alignment of mtDNA MPS data, haplotype determination, and heteroplasmy assessment

Mitchell M. Holland*, Erica D. Pack, Jennifer A. McElhoe

Forensic Science Program, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, 014 Thomas Building, University Park, PA 16802, United States

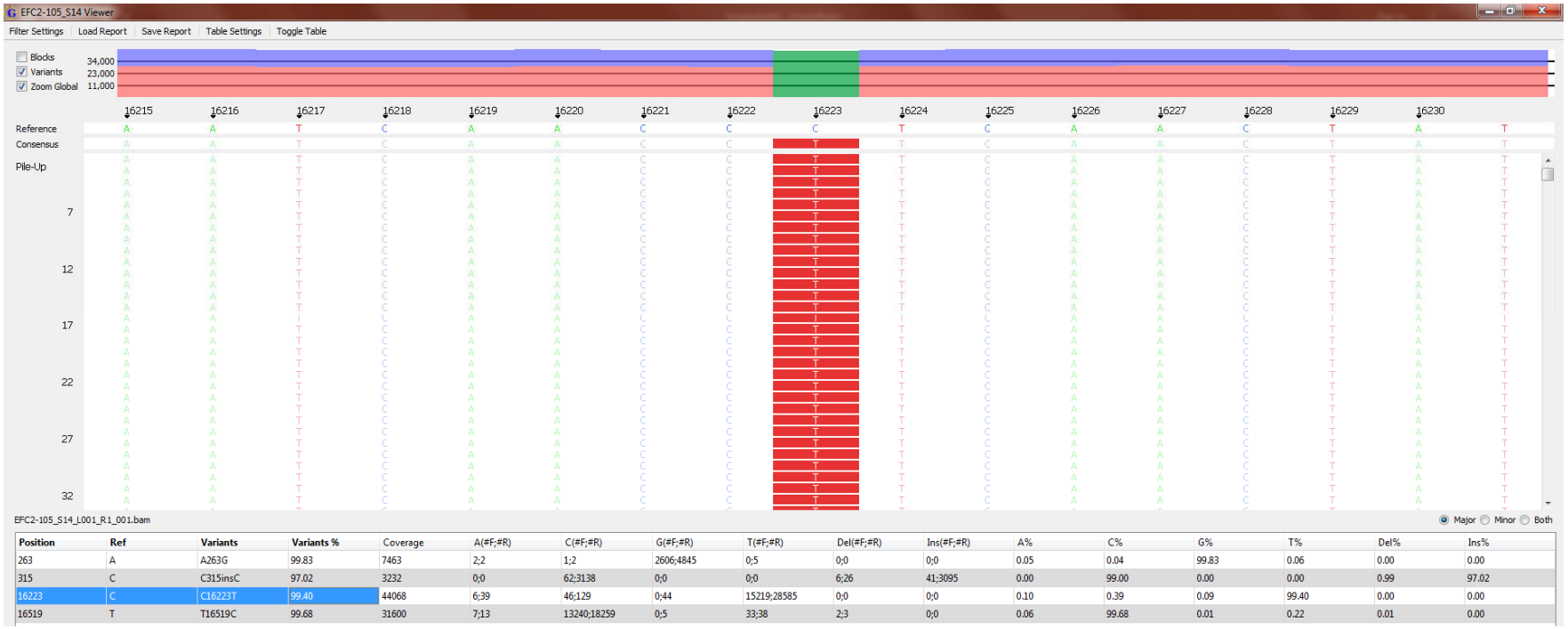
MPS data from 500 individuals used to assess the software; control region data.

*Correct mtDNA haplotypes and assessment of heteroplasmic variants with **minimal manual interpretation**. Numerous user-defined parameters for filtering the data that address the interests of researchers and practitioners. Multiple options for viewing and navigating through the data, and reporting the findings.*

NOTE: no vested interest, no conflict of interest

Software Development

In collaboration with SoftGenetics, Inc.

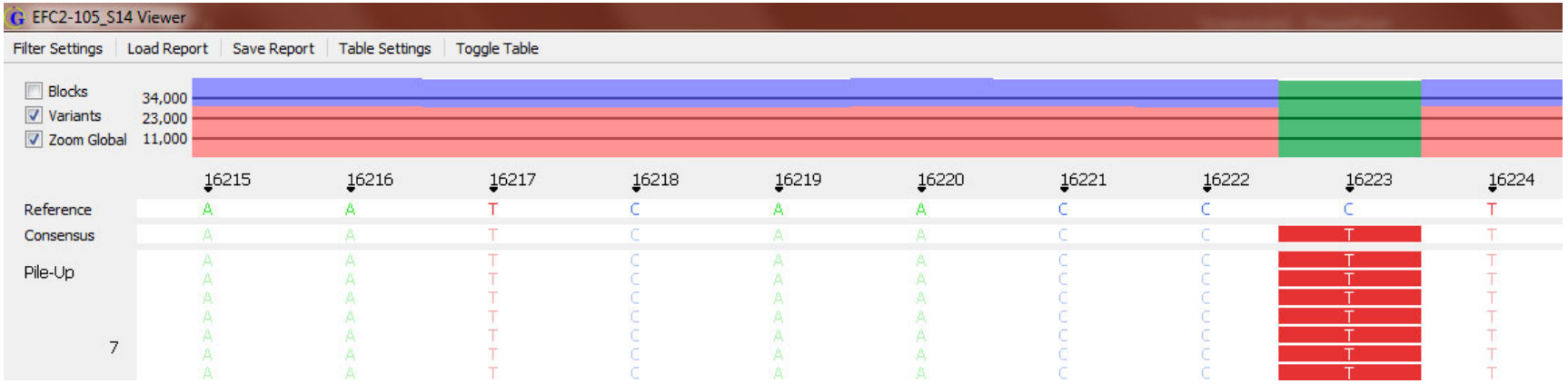


Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%
263	A	A263G	99.83	7463	2;2	1;2	2606;4845	0;5	0;0	0;0	0.05	0.04	99.83	0.06	0.00	0.00
315	C	C315insC	97.02	3232	0;0	62;3138	0;0	0;0	6;26	41;3095	0.00	99.00	0.00	0.00	0.99	97.02
16223	C	C16223T	99.40	44068	6;39	46;129	0;44	15219;28585	0;0	0;0	0.10	0.39	0.09	99.40	0.00	0.00
16519	T	T16519C	99.68	31600	7;13	13240;18259	0;5	33;38	2;3	0;0	0.06	99.68	0.01	0.22	0.01	0.00

GeneMarker®HTS
Forensic mtDNA Analysis Software
©SoftGenetics, LLC

Generates an exportable consensus haplotype with phylogenetically correct SNP and INDEL calls using a customizable motif-based alignment algorithm

GeneMarker HTS



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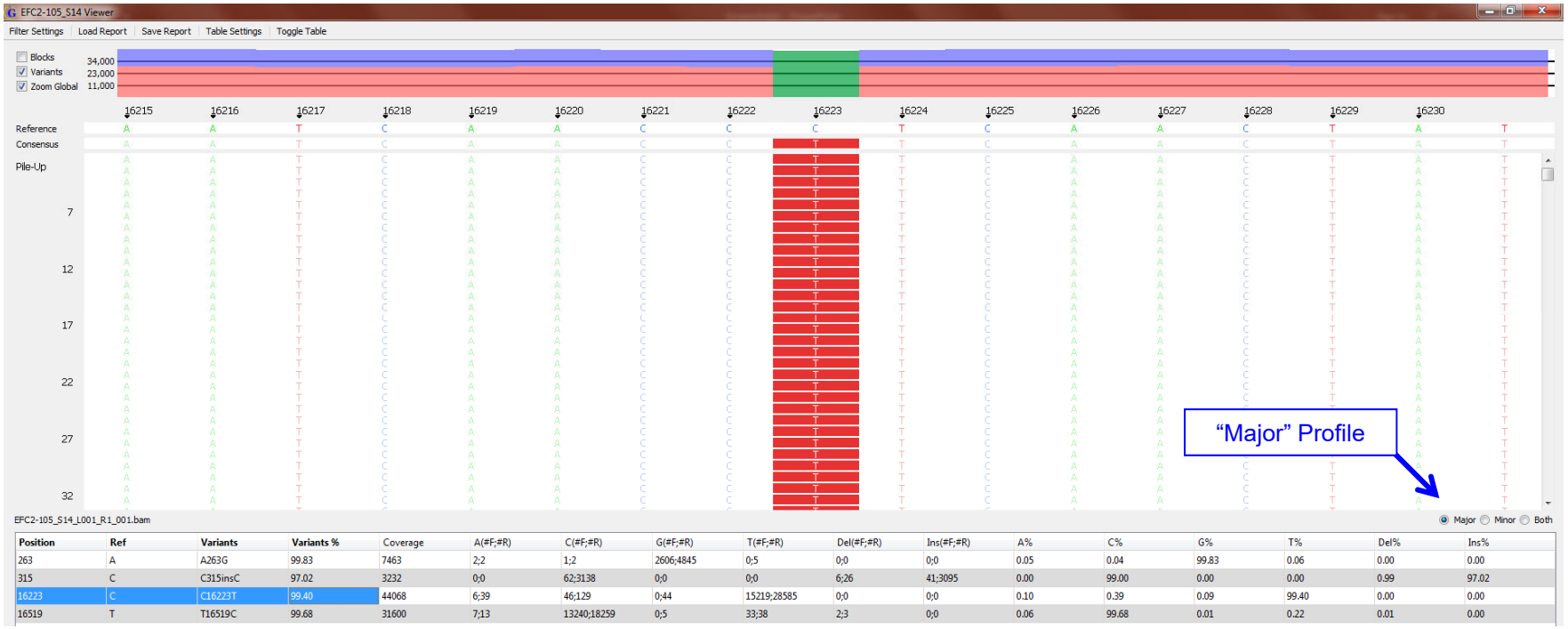

Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%
263	A	A263G	99.83	7463	2;2	1;2	2606;4845	0;5	0;0	0;0	0.05	0.04	99.83	0.06	0.00	0.00
315	C	C315insC	97.02	3232	0;0	62;3138	0;0	0;0	6;26	41;3095	0.00	99.00	0.00	0.00	0.99	97.02
16223	C	C16223T	99.40	44068	6;39	46;129	0;44	15219;28585	0;0	0;0	0.10	0.39	0.09	99.40	0.00	0.00
16519	T	T16519C	99.68	31600	7;13	13240;18259	0;5	33;38	2;3	0;0	0.06	99.68	0.01	0.22	0.01	0.00

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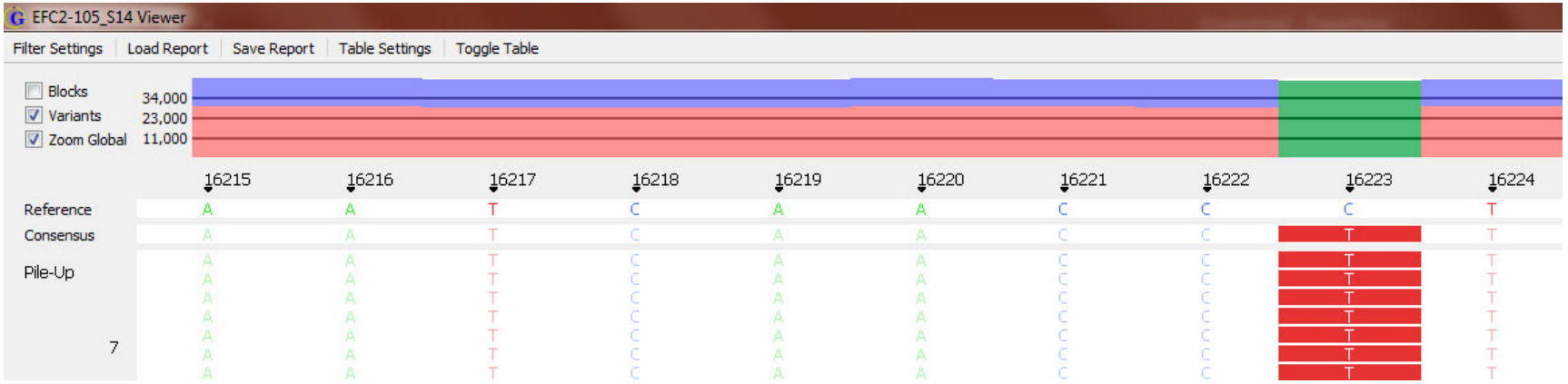
GeneMarker HTS

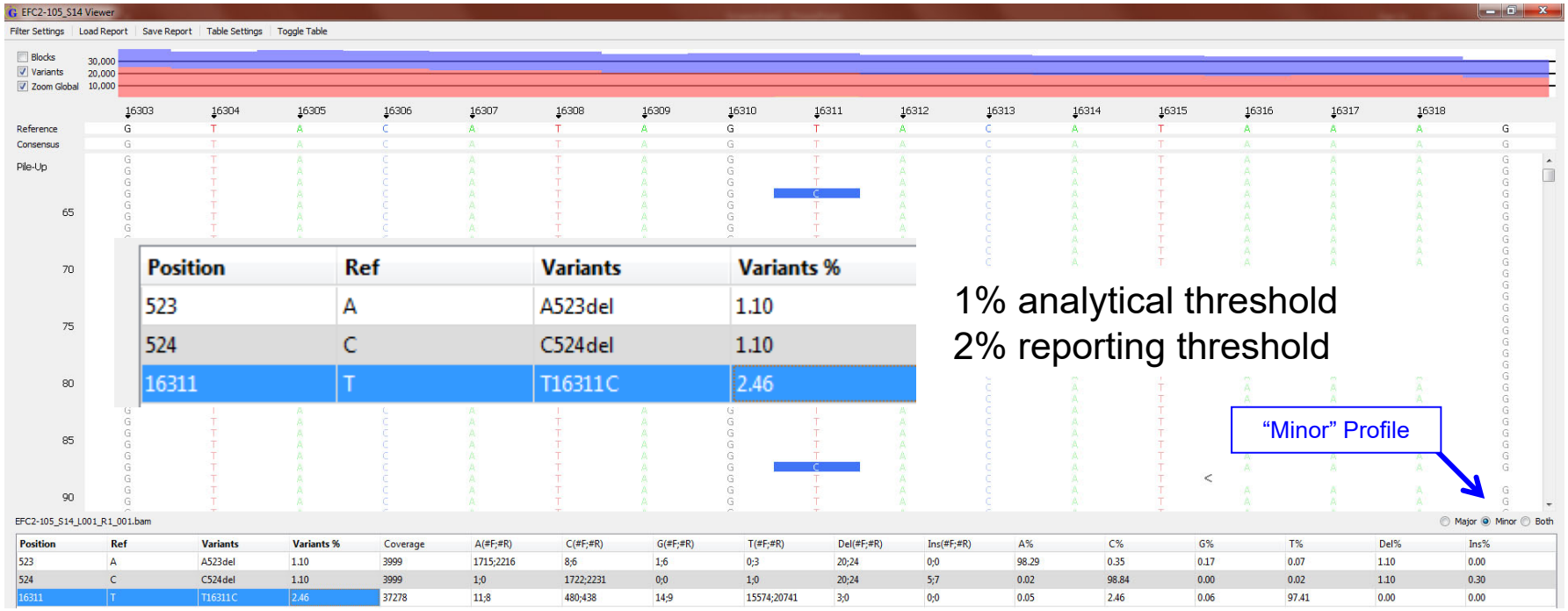


Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)
263	A	A263G	99.83	7463	2;2	1;2	2606;4845	0;5	0;0	0;0
315	C	C315insC	97.02	3232	0;0	62;3138	0;0	0;0	6;26	41;3095
16223	C	C16223T	99.40	44068	6;39	46;129	0;44	15219;28585	0;0	0;0
16519	T	T16519C	99.68	31600	7;13	13240;18259	0;5	33;38	2;3	0;0

GeneMarker® HTS
Forensic mtDNA Analysis Software
©SoftGenetics, LLC

Generates an exportable consensus haplotype with phylogenetically correct SNP and INDEL calls using a customizable motif-based alignment algorithm

GeneMarker HTS



Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%
523	A	A523del	1.10	3999	1715;2216	8;6	1;6	0;3	20;24	0;0	98.29	0.35	0.17	0.07	1.10	0.00
524	C	C524del	1.10	3999	1;0	1722;2231	0;0	1;0	20;24	5;7	0.02	98.84	0.00	0.02	1.10	0.30
16311	T	T16311C	2.46	37278	11;8	480;438	14;9	15574;20741	3;0	0;0	0.05	2.46	0.06	97.41	0.00	0.00

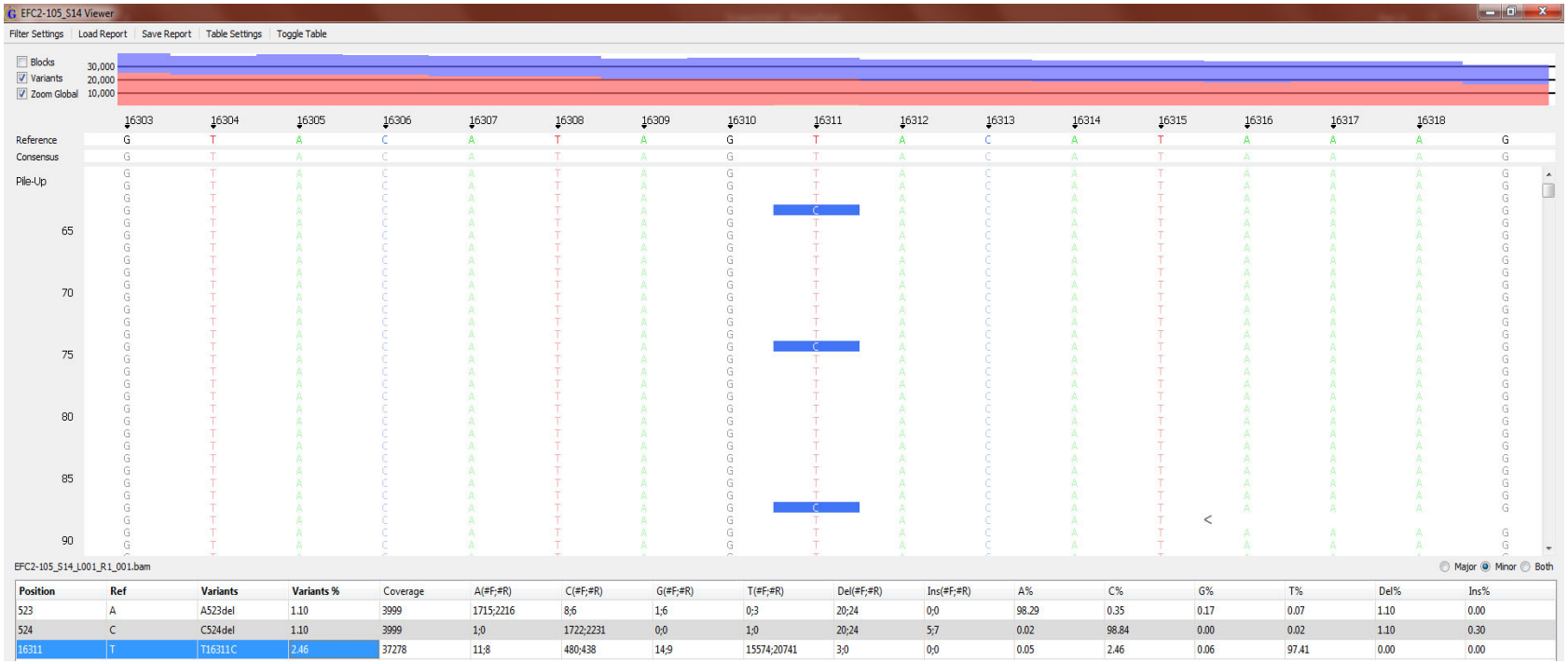
GeneMarker®HTS
Forensic mtDNA Analysis Software
©SoftGenetics, LLC

Provides a list of minor sequence variants that can be assessed as potential heteroplasmic positions

Filtering the data:

2000+ read coverage per nucleotide position

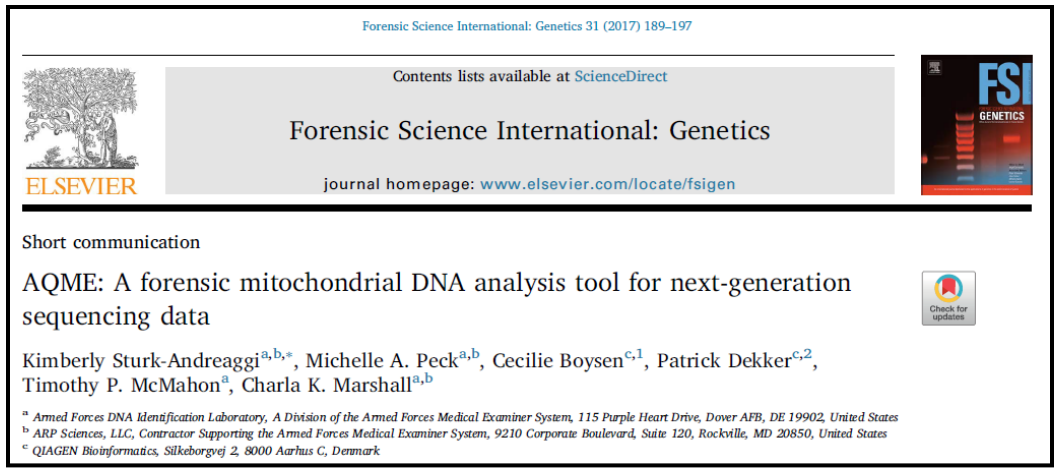
40+ reads of the minor variant (≥2%) for reporting consistent read balance (#F;#R)



Position	Ref	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%
523	A	A523del	1.10	3999	1715;2216	8;6	1;6	0;3	20;24	0;0	98.29	0.35	0.17	0.07	1.10	0.00
524	C	C524del	1.10	3999	1;0	1722;2231	0;0	1;0	20;24	5;7	0.02	98.84	0.00	0.02	1.10	0.30
16311	T	T16311C	2.46	37278	11;8	480;438	14;9	15574;20741	3;0	0;0	0.05	2.46	0.06	97.41	0.00	0.00

Software Development

In collaboration with Qiagen



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journal homepage: www.elsevier.com/locate/fsigen

Short communication

AQME: A forensic mitochondrial DNA analysis tool for next-generation sequencing data

Kimberly Sturk-Andreaggi[a,b,*], Michelle A. Peck[a,b], Cecilie Boysen[c,1], Patrick Dekker[c,2], Timothy P. McMahon[a], Charla K. Marshall[a,b]

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GxNGS

AQME =
AFDIL-Qiagen
mtDNA Expert

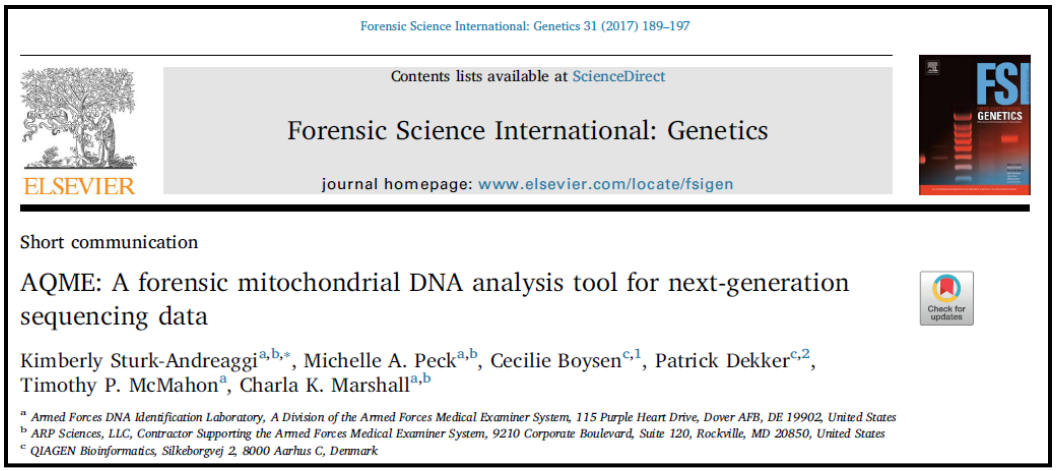
A custom toolkit for use in the CLC Genomics Workbench.

Generates an editable mtDNA profile that employs forensic conventions and includes the interpretation range required for mtDNA data reporting; integrates an mtDNA haplogroup estimate into the analysis workflow without the use of an external tool; and generates configurable export files and an audit trail to assist the analyst during review.

NOTE: no vested interest, no conflict of interest

Software Development

In collaboration with Qiagen



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Short communication

AQME: A forensic mitochondrial DNA analysis tool for next-generation sequencing data

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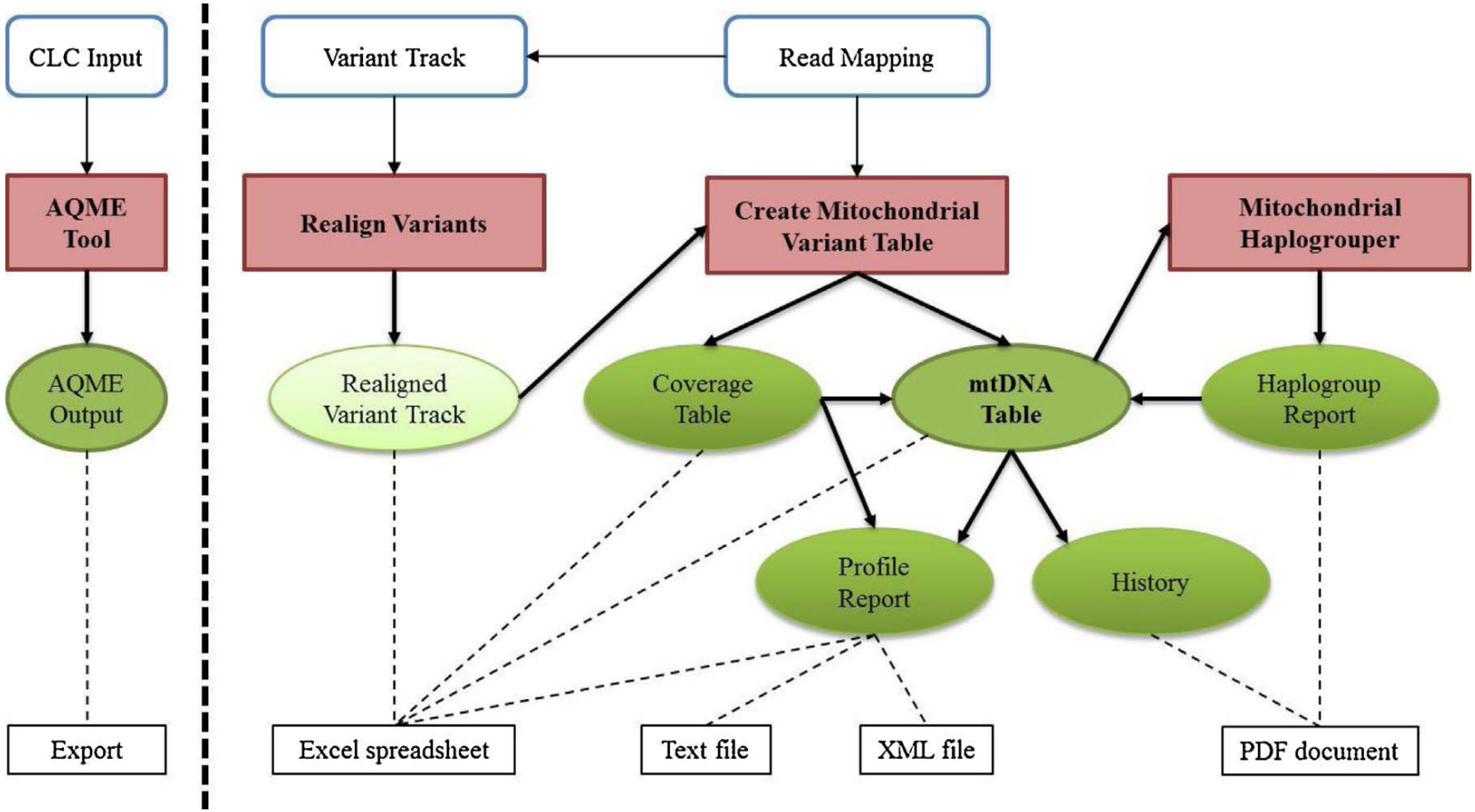
Analyzed mtgenome data from 21 samples of varying quality and preparations.

A total of 211 tool edits were automatically applied to 130 of the 698 total variants reported in an effort to adhere to forensic nomenclature. Additional manual edits were required for three samples, with AQME reporting accurate haplogroups for 18 of the 19 samples analyzed; due to partial mtgenome data.

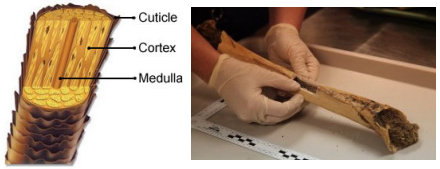
NOTE: no vested interest, no conflict of interest

AQME


CLC Input
Variant Track
Read Mapping
AQME Tool
Realign Variants
Create Mitochondrial Variant Table
Mitochondrial Haplogrouper
AQME Output
Realigned Variant Track
Coverage Table
mtDNA Table
Haplogroup Report
Profile Report
History
Export
Excel spreadsheet
Text file
XML file
PDF document

Take Home Message

DNA Extraction



Amplification of mtDNA

Library Preparation

Sequencing

Data Analysis

The Future is Now for MPS mtDNA Analysis