applied biosystems

answers revealed

Expand your forensics workflow with the Precision ID NGS System

ThermoFisher SCIENTIFIC

Integrate NGS into your forensic DNA lab workflow

The Applied Biosystems™ Precision ID NGS System for human identification can help you solve tough cases by getting more information from your challenging samples. Now you have help moving those unknown samples from storage to investigative leads. Adopting next-generation sequencing (NGS) for forensic DNA analysis in your laboratory is simpler than ever, when you combine the Ion Chef™ System and Ion GeneStudio™ S5 series systems with optimized Applied Biosystems™ Precision ID library preparation, template preparation, and sequencing kits and forensically relevant panels. Precision ID panels are powered by Ion AmpliSeq™ technology, which applies the simplicity of PCR to targeted sequencing and enables amplification from tens to thousands of genomic regions simultaneously in a single PCR tube.

Let the evidence speak for itself. You can choose from a suite of targeted Precision ID panels for the NGS solution for your cases. Mitochondrial DNA (mtDNA) from samples recovered at mass disasters and from other unidentified remains can routinely be analyzed to assist in human identification and help establish links to related family members. Short tandem repeat (STR) panels provide high-resolution genotyping of STRs for analysis of complex mixtures. Ancestry-informative or phenotypic single-nucleotide polymorphism (SNP) analysis may help generate investigative leads when suspects are unknown. Identity-testing SNPs can associate a degraded crime scene sample to a known reference when partial results are obtained with autosomal STR analysis.

Process unsolved and missing persons cases with NGS in your lab today. With as little as 125 ng of DNA input, you can go from extracted DNA to profile in as few as 5 pipetting steps and 45 minutes of hands-on time (Figure 1).

Total hands-on time: less than 45 minutes
Total overall time: as little as 25 hours

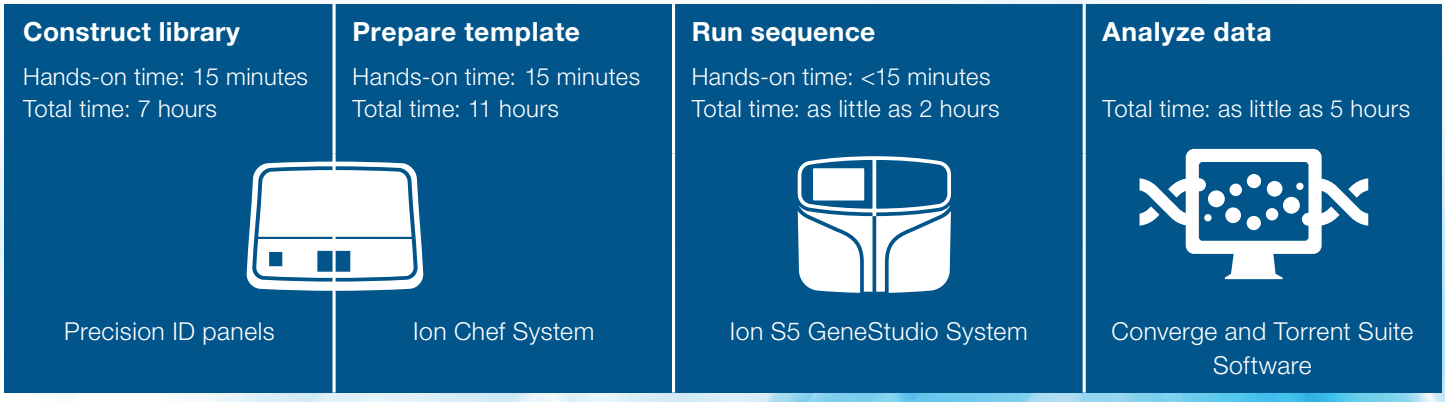


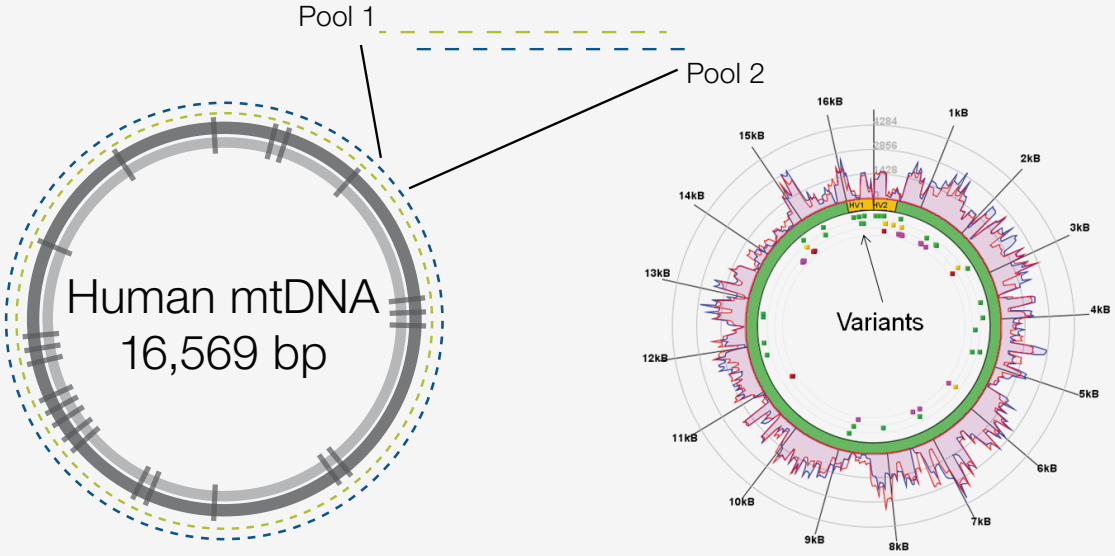
Figure 1. Precision ID workflow. Precision ID library preparation for mtDNA, SNP, and STR analysis panels can be automated using the Ion Chef System. Libraries are processed further on the Ion Chef System for emulsion PCR, enrichment, and loading onto Ion Torrent™ chips.

Panels for human identification

Analyze evidence from missing persons or remains from disaster victims

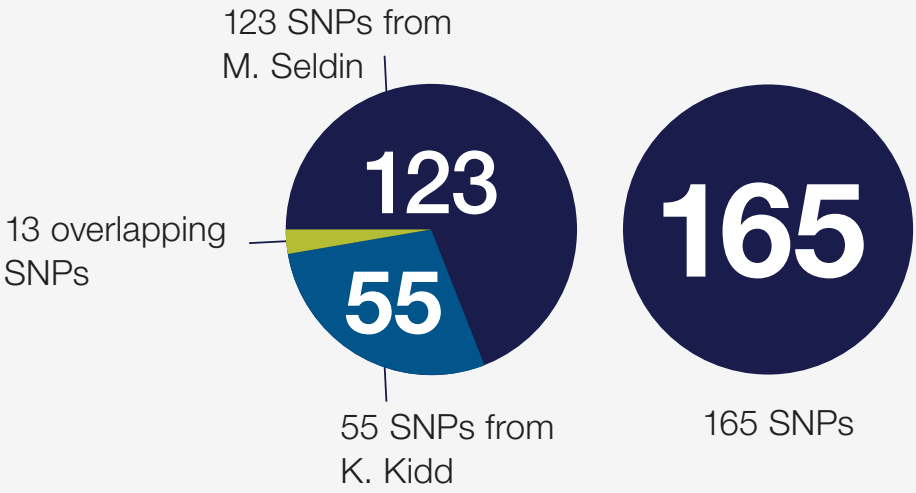
The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel is an innovative approach to mtDNA sequencing, specifically developed for forensic applications. This mtDNA tiling approach includes two primer pools of 81 small amplicons in each pool, with an average size of 163 base pairs (bp), to assist with obtaining optimal coverage data for the mitochondrial genome from highly compromised, degraded samples such as hair shafts, teeth, and bones.

The Applied Biosystems™ Precision ID mtDNA Control Region Panel is based on the same mtDNA tiling approach used in the Precision ID mtDNA Whole Genome Panel. This targeted control region panel contains two smaller sets of primer pools with 7 amplicons in each pool that span the 1.2 kb control region, which encompasses hypervariable (HV) regions I, II, and III, with the same optimal coverage for degraded forensic samples. Applied Biosystems™ Converge Software NGS Data Analysis module now gives forensic DNA laboratories the flexibility to detect variation within noncoding control region sequences or take full advantage of the genetic diversity with analysis of full mitochondrial genome (mtGenome) sequence data.



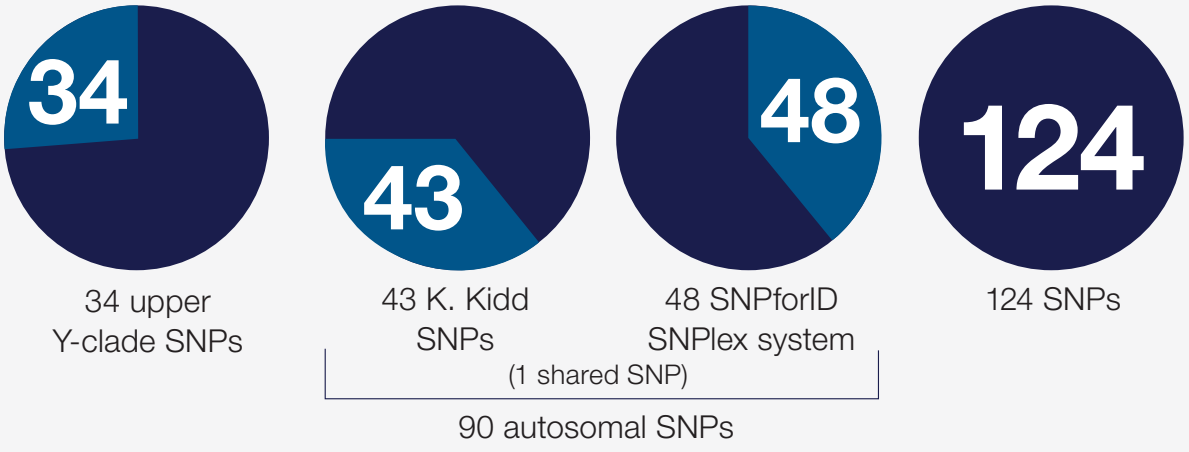
Generate more investigative leads

The Applied Biosystems™ Precision ID Ancestry Panel includes 165 autosomal markers (SNPs) that can provide you with biogeographic ancestry information and guide your investigative process. Fifty-five of these markers were selected based on a publication by Kenneth Kidd [1], and 123 markers were selected based on a publication by Michael Seldin [2]. With small amplicon sizes (<130 bp), this panel is also optimized for degraded DNA samples. Using a small sample input of as little as 125 pg of DNA, you can go from sample extraction to genotyping in less than two days using the Precision ID NGS System and the Converge Software NGS Data Analysis module.



Analyze degraded or trace DNA

The Applied Biosystems™ Precision ID Identity Panel, comprising 124 markers, provides discrimination of individuals similar to STR genotype match probabilities used by forensic analysts. This high discrimination power is achieved by using 34 upper Y-clade SNPs and 90 autosomal SNPs that have high heterozygosity and low fixation index (F_{ST}), described in publications by Kenneth Kidd [3] and the SNPforID Consortium [4,5]. Due to the shorter amplicons required for sequencing, SNPs enable high recovery of information from challenging samples—such as samples from mass disasters, low–copy number samples, or low-quality samples.



Analyze DNA mixtures

The Applied Biosystems™ Precision ID GlobalFiler™ NGS STR Panel includes 35 markers, including the same 21 autosomal STRs along with Y markers and amelogenin sex markers found in the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit, as well as 14 additional informative markers for forensic analysis. When analyzed with NGS, these markers reveal more alleles than identified with traditional CE analysis due to sequence diversity within CE alleles of the same size. Using a small sample input of as little as 125 pg of DNA (Figure 2), this targeted forensic marker panel enables you to go from sample extraction to genotyping in less than two days using the Precision ID NGS System and Converge Software with the NGS Data Analysis module. The Precision ID GlobalFiler NGS STR solution provides information about STR allele calls, STR sequence motifs, known SNPs in flanking regions, and isometric heterozygotes (alleles of the same fragment length but containing different sequences) (Figure 3). These isometric alleles can be used to help resolve complex profiles and mixtures.

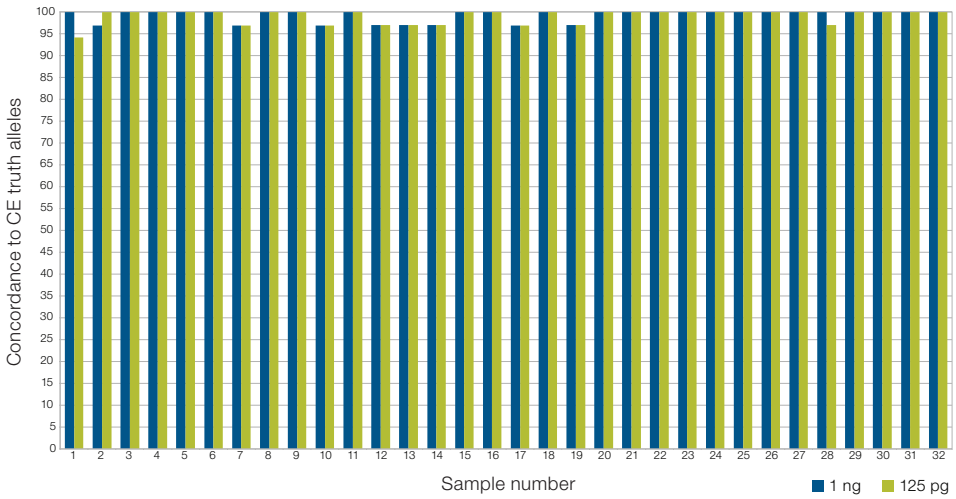


Figure 2. Concordance of NGS to CE analysis. A total of 32 samples were analyzed at 1 ng and 125 pg using the Precision ID GlobalFiler NGS STR Panel v2 and compared to genotypes obtained using the GlobalFiler PCR Amplification Kit on the Applied Biosystems™ 3500 Genetic Analyzer.

Locus	Genotype	PHR	AN	OL	PHR	BST	ME	IGV
D16S539	8, 12, 13, 8							

Allele	Status	Coverage	Sequence	Long Sequence	Validated SNP ID	QV Score
8	ABOVE_ST	3,031	[GATA]8	D16S539[CE8]-chr16-zip 86386307-86386351 [GATA]8		
12	ABOVE_ST	912	[GATA]12	D16S539[CE12]-chr16-zip 86386307-86386351 [GATA]12		
13	ABOVE_ST	4,197	[GATA]13	D16S539[CE13]-chr16-zip 86386307-86386351 [GATA]13		
8	ABOVE_ST	794	[GATA]8	D16S539[CE8]-chr16-zip 86386307-86386351 [GATA]8 86386298-G		2,895.68

AN:
Expected Allele Number: 1-2

OL:
Expected Allele(s): [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 8.3, 9.3, 11.3, 12.1, 12.2, 13.3]
Deviant(s):

Below PHR
Threshold (coverage): 0.30
Allele(s): 12

BST:

Figure 3. Detailed information on SNP in flanking region analysis of a minor contributor. The SNP is identified with the International Society for Forensic Genetics (ISFG)–recommended nomenclature.

Choose from our extensive menu or customize your own

Whether you select from our extensive menu of predesigned assays or create your own custom panel, your panel will enable high coverage and performance. Our online Ion AmpliSeq™ Designer tool puts you in the driver's seat, enabling customized designs for DNA and RNA targets. Optimized for coverage across selected genomic regions and amplicon size, our Ion AmpliSeq Designer tool uses the same powerful design engine as our predesigned panels. Community panels, such as the Ion AmpliSeq™ DNA Phenotyping Panel, are designed with input from leading forensic scientists and can be ordered on **ampliseq.com**.

Simple, reproducible automated workflows for Ion Torrent sequencing

The Ion Chef System simplifies the workflow for the Ion GeneStudio S5 series systems. It provides a reproducible walk-away solution for automated Precision ID library preparation, automated template preparation, and chip loading, with optimized Precision ID library and templating kits—giving you more time to focus on other critical lab tasks and solve cases.

Ion Chef System

- Automates library generation, equalization, and pooling for Precision ID 1- and 2-pool designs for up to 8 samples in a single instrument run
- Simplifies template preparation and chip loading using a single automated process for loading of up to two Ion Torrent™ semiconductor sequencing chips per instrument run
- Maintains a very low inter-run cross-contamination rate of <0.01%
- Helps save time and labor with just 15 minutes of hands-on time for setup prior to the start of each automated workflow (Figure 4)
- Supports all Ion Torrent semiconductor chips and sequencing chemistries
- Helps reduce sources of variability for forensic scientists of any experience level
- Enables reproducible results—minimal pipetting helps reduce experimental variability

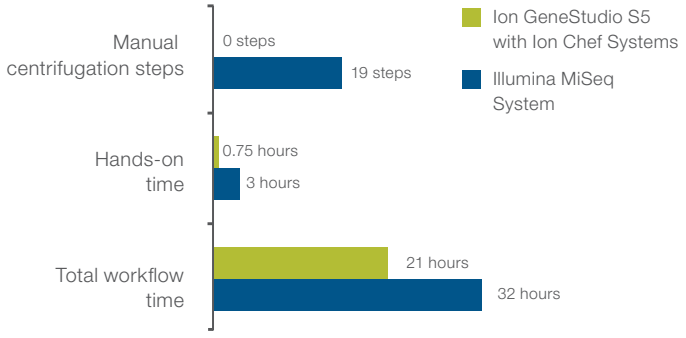


Figure 4. Comparison of the Ion Chef and Ion GeneStudio S5 Systems combination to the Illumina™ MiSeq™ System. The combination of the Ion Chef and Ion GeneStudio S5 Systems delivers data 30% faster with 75% less hands-on time. Workflow metrics were compiled from the respective product user guides.

Confidence in sequencing results

Now you can reduce day-to-day variation in manual workflows to help provide consistent results—have peace of mind and confidence in your data.

- Onboard reagent tracking—2D barcodes associated with all reagents and consumables minimize the potential for user error and help ensure every run is correctly set up (Figure 5)

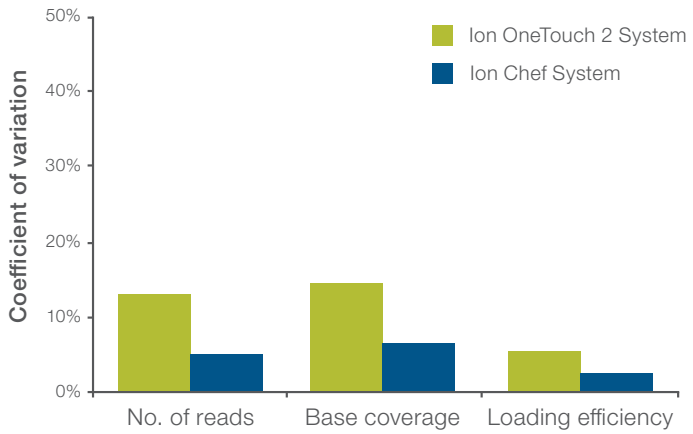


Figure 5. Comparison of reproducibility across NGS metrics for Ion AmpliSeq libraries processed (templating and chip loading) using the Ion Chef and Ion OneTouch™ 2 Systems. While both systems exhibit excellent reproducibility, the Ion Chef System demonstrates lower coefficients of variation (CV) across all metrics.

Ion GeneStudio S5 series systems

Want to sequence SNP panels on Monday, mtDNA panels on Wednesday, and STR panels on Thursday? The Ion GeneStudio S5 systems let you leverage a single benchtop instrument that scales to your applications and throughput needs.

The Ion GeneStudio S5 series systems provide the simplest DNA-to-data workflow for targeted sequencing with industry-leading speed and affordability, so you can spend less time doing repetitive lab work and more time answering the critical questions in your investigations.

Simplicity—ready, set, sequence

- Less than 15 minutes of sequencer hands-on time
- Less than 45 minutes of hands-on time for a DNA-to-data targeted sequencing workflow

Speed—because every hour counts

- As little as two hours hours for a sequencing run using the Ion S5™ Precision ID Chef & Sequencing Kit
- Go from DNA to data in less than two days

Small sample input—because every sample matters

- As little as 125 pg of DNA needed to generate human identification STR profiles

Scalability—single sequencer, multiple applications

- Multiple chip formats and read lengths to match your throughput needs (Table 1)
- Analyze mtDNA, SNP, and STR targets

Simple data analysis and storage

- Plan, monitor, and track your runs in Torrent Suite™ Software, and analyze mtDNA, SNP, and STR profiles in Converge Software

Service and support

- More than 3,700 global sales, service, and technical support specialists are available to assist you in person, by phone, or online—including our Human Identification Professional Services (HPS) team that can help you successfully navigate the verification process required to bring new technologies into your lab

Table 1. Run and analysis times for Ion GeneStudio S5 series systems.

		Ion GeneStudio S5 System			Ion GeneStudio S5 Plus System			Ion GeneStudio S5 Prime System		
		Ion 510 Chip	Ion 520 Chip	Ion 530 Chip	Ion 510 Chip	Ion 520 Chip	Ion 530 Chip	Ion 510 Chip	Ion 520 Chip	Ion 530 Chip
Reads		2–3 million	4–6 million	15–20 million	2–3 million	4–6 million	15–20 million	2–3 million	4–6 million	15–20 million
Turnaround time (sequencing + analysis)	mtDNA whole-genome panel	–	7.5 hr	10.5 hr	–	3.5 hr	5 hr	–	3 hr	4 hr
	mtDNA control regions panel	4.5 hr	7.5 hr	–	3 hr	3.5 hr	–	3 hr	3 hr	–
	Ancestry panel	4.5 hr	7.5 hr	10.5 hr	3 hr	3.5 hr	5 hr	3 hr	3 hr	4 hr
	Identity panel	4.5 hr	7.5 hr	10.5 hr	3 hr	3.5 hr	5 hr	3 hr	3 hr	4 hr
	STR panel	–	12.5 hr	22.5 hr	–	4.5 hr	5.5 hr	–	4 hr	5 hr
Samples per chip*	mtDNA whole-genome panel	–	25	32	–	25	32	–	25	32
	mtDNA control regions panel	37	56	–	37	56	–	37	56	–
	Ancestry panel	48	72	362	48	72	362	48	72	362
	Identity panel	54	81	384	54	81	384	54	81	384
	STR panel	–	16	32	–	16	32	–	16	32

* Recommendations are based on in-house determination of the number of samples that can be multiplexed while still achieving a minimum coverage of 100x for 97% of SNP amplicons for SNP panels and 100% of mtDNA amplicons. Individual lab results may vary depending on workflow used and customer requirements.

Integrated NGS analysis solutions

Converge Software for NGS analysis

Converge Software, an all-in-one modular enterprise platform from Thermo Fisher Scientific, integrates forensic DNA data management and analysis into a single software package designed to increase the efficiency of forensic DNA laboratories. With the Converge NGS Data Analysis module, laboratories are now able to examine the mitochondrial genome to identify remains when there is poor quality or no autosomal DNA available for analysis, targeted and forensically relevant SNP markers to generate investigative leads, and STR markers to help determine the number of contributors in a mixture analysis. Additionally, full auditing functionality is included for chain-of-custody requirements.

mtDNA analysis

The high copy number per cell of mtDNA is useful in the context of challenging samples that fail to produce an autosomal STR profile. Traditional Sanger sequencing using capillary electrophoresis (CE) is generally limited to the sequencing of the control region as sequencing of the entire mtGenome is time-consuming and cost-prohibitive. NGS now makes it possible to sequence the mtGenome, which increases discrimination and sensitivity. Analysis of the mtGenome can be challenging due to complex alignments, the presence of mtDNA heteroplasmy, and insertions and deletions present throughout the genome that may impact the accuracy of variant calling. NGS reads from the BAM files are first mapped to nodes in the PhyloTree database [6] and then realigned using a custom Smith–Waterman alignment algorithm that integrates PhyloTree and EMPOP [7] information into the scoring function. Variants are called with reference to the revised Cambridge Reference Sequence (rCRS). Additionally, the closest haplogroup is calculated, and variants are evaluated based on their occurrence in the haplogroup as well as other general metrics including frequency, strand bias, and coverage. Variants can be viewed in a variety of formats—a linear view (Figure 6), circular plot, or grid.

Figure 6. Linear coverage plot in Converge Software. Forward (blue) and reverse (red) coverage is shown across the entire mtGenome (bottom panel) with the ability to zoom into selected regions (top panel). Variants plotted below the coverage diagram are colored by their status (green: confirmed; yellow: possible, needs review; red: indicative of low-coverage regions).

STR analysis

With an interface similar to that of Applied Biosystems™ GeneMapper™ *ID-X* Software, you will be able to quickly evaluate sequencing data using familiar process quality values (PQVs) and flags such as allele number (AN), off-ladder allele (OL), peak height ratio (PHR), below stochastic threshold (BST), and control concordance (CC) (Figure 7). Preconfigured analysis settings are provided within the NGS Data Analysis module and may be modified by the laboratory as needed.

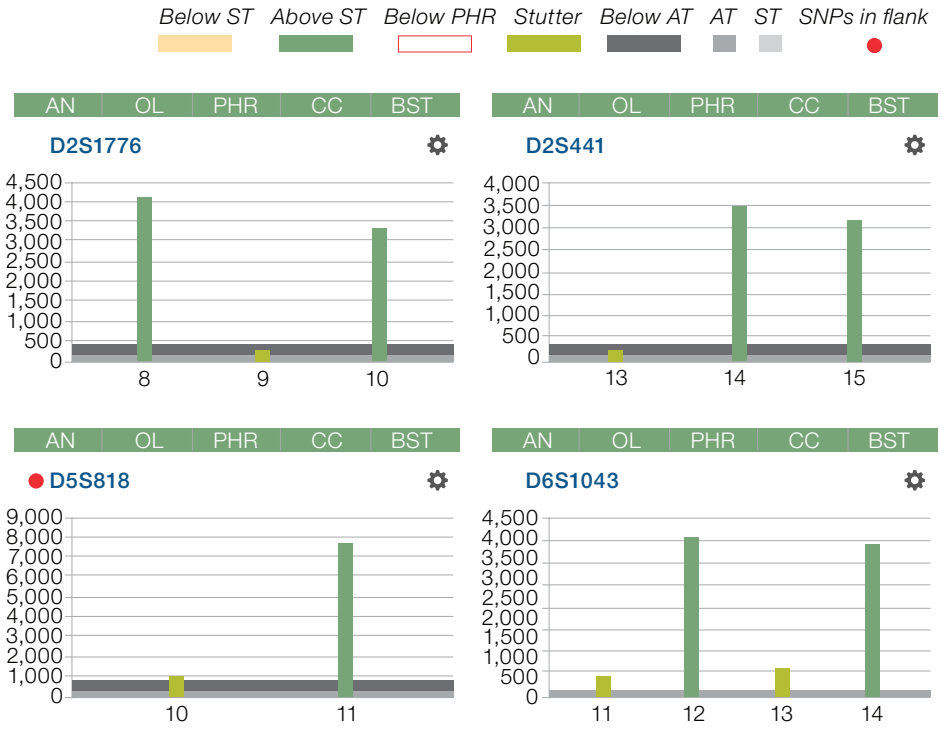


Figure 7. View of secondary NGS analysis results with Converge Software 2.0 and NGS Data Analysis module.

SNP analysis

Converge Software SNP analysis provides a variety of metrics to monitor sequencing quality, including coverage of aligned reads to a hotspot, strand bias, number of reads containing each base at the hotspot, genotype call and quality, and major allele frequency. Tertiary biogeography ancestry analysis consists of generating an estimation of admixture prediction (Figure 8) and population likelihoods (based on algorithms from Ken Kidd). Identity analysis consists of calculating random match probability (RMP) based on genotype frequencies generated from data from the 1000 Genomes Project as well as a Y-haplogroup prediction (Figure 9).

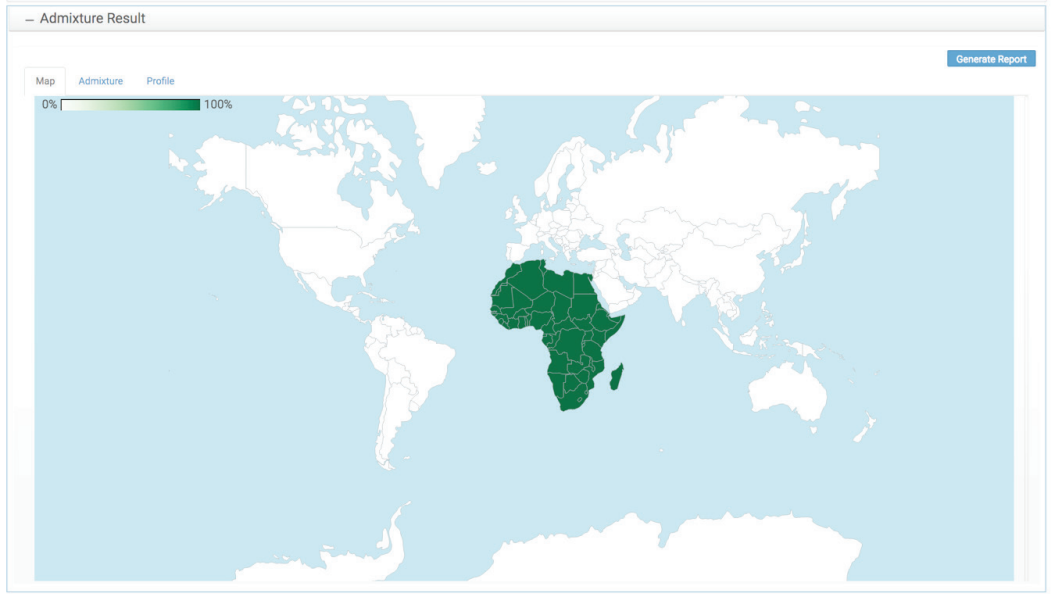


Figure 8. Graphical representation of an admixture map using the Converge SNP analysis module. The percentage of the corresponding population in the sample is displayed. The darker the color, the greater the percentage of the corresponding population in the sample. Analysts can hover over the region to display the proportion value of the corresponding population in the sample.

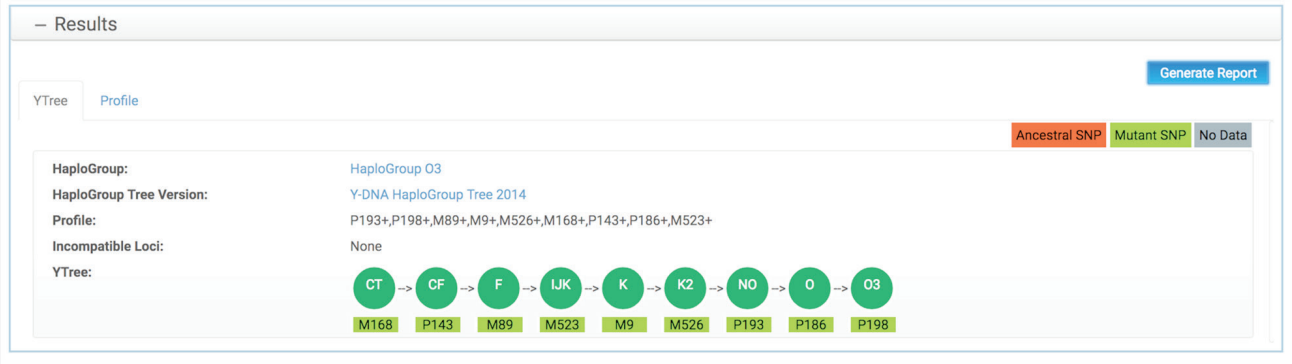


Figure 9. Y-haplogroup results from the Precision ID Identity Panel. Attributes displayed are HaploGroup, derived alleles, any markers that conflict with the reported Y lineage, and the Y clades (red: ancestral SNP; green: mutant SNP; gray: no data).

Easily compare NGS and CE profile data

As forensic laboratories begin to adopt sequence analysis of STR markers into casework applications, validation and concordance studies will be required. Using both the NGS Data Analysis module and the Case Management module of Converge Software allows for easy comparison of NGS and capillary electrophoresis profiles (Figure 10). This feature will also be useful when comparing a crime scene sample analyzed with NGS to a reference sample that has been processed using traditional methods.

Case management

Underlying the centralization of data creation, analysis, and storage to one easy-to-access location, the Case Management module of Converge Software supports case, subject, genotype profile, and laboratory data management. The module serves the needs of various users such as laboratory managers and analysts, providing intuitive data views and reports that are configurable to the needs of each user and preserved for that user's login.

Locus	Profile 1038 (Precision_ID_ GlobalFiler_NGS_STR_Panel)	Profile 1034 (GlobalFiler_Panel_v1)	Profile 1035 (NGM_Detect_Panel_v2)
AMEL	X,Y	X,Y	X,Y
CSF1PO	10,12	-	-
D10S1248	14,15	14,15	-
D12S391	21,22	21,22	21,22
D13S317	11	11	-
D16S539	12,14	14	12,14
D18S51	15	15	15
D19S433	12	12,14	12,14
D1S1656	11,16	11	11,16
D21S11	29	29	29
D22S1045	15	15	15
D2S1338	19,20	-	19,20
D2S441	10	10,14	-
D3S1358	15	15,17	15,17
D5S818	11,12	11,12	-
D7S820	9,10	-	-
D8S1179	12,13	12,13	12,13
DYS391	12	-	-
FGA	22,23	-	22,23
SE33	-	-	21.2,29.2
TH01	8,9	8,9	8,9
TPOX	8,12	-	-
Y indel	2	2	2
vWA	16	16	16
D12ATA63	12,18	-	-
D14S1434	10,13	-	-
D1S1677	13,14	-	-
D2S1776	10,11	-	-
D3S4529	13,15	-	-
D4S2408	10	-	-
D5S2800	17,18	-	-
D6S1043	17	-	-

Figure 10. Comparison of NGS and two CE profiles (imported from GeneMapper *ID-X* Software) via the Converge Case Management module. A single sample was analyzed with the Precision ID GlobalFiler NGS STR Panel v2, the GlobalFiler PCR Amplification Kit, and the Applied Biosystems™ NGM Detect™ PCR Amplification Kit. (Green = match, orange = partial match, red = not a match, gray = NA)

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Ordering information

Product	Quantity	Cat. No.
Precision ID NGS System instrumentation and software		
HID Ion Chef System	1 each	A30070
HID Ion GeneStudio S5 System	1 each	A41431
HID Ion GeneStudio S5 Plus System	1 each	A41432
HID Ion GeneStudio S5 Prime System	1 each	A41433
Panels for human identification		
Precision ID mtDNA Whole Genome Panel	96 rxns manual, 48 rxns automated	A30938
Precision ID mtDNA Control Region Panel	96 rxns manual, 48 rxns automated	A31443
Precision ID GlobalFiler NGS STR Panel v2 (for Ion GeneStudio S5 Systems)	96 rxns manual, 32 rxns automated	A33114
Precision ID Identity Panel	96 rxns manual, 32 rxns automated	A25643
Precision ID Ancestry Panel	96 rxns manual, 32 rxns automated	A25642
Precision ID Library Kit	96 rxns	A26435
Precision ID Library Kit	384 rxns	A30941
Automated library preparation		
Precision ID DL8 Kit	32 rxns	A33212
Sequencing kits and barcodes for multiplexing		
Ion S5 Precision ID Chef & Sequencing Kit (2 runs per initialization)	8 rxns	A33208
Precision ID IonCode Barcode Adapters 1–96 Kit	1 kit	A33586
Ion Xpress Barcode Adapters 1–96 Kit	3,840 rxns	4474517
IonCode Barcode Adapters 1–384 Kit	3,840 rxns	A29751
Converge Software		
Converge Software with server	1 each	A35131
Case Management and NGS Analysis modules (1 user, 3-year license)	1 each	A35987
Case Management and NGS Analysis modules (5 users, 3-year license)	1 each	A36237

References

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