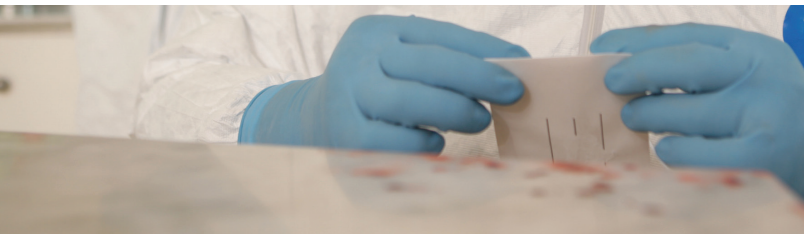

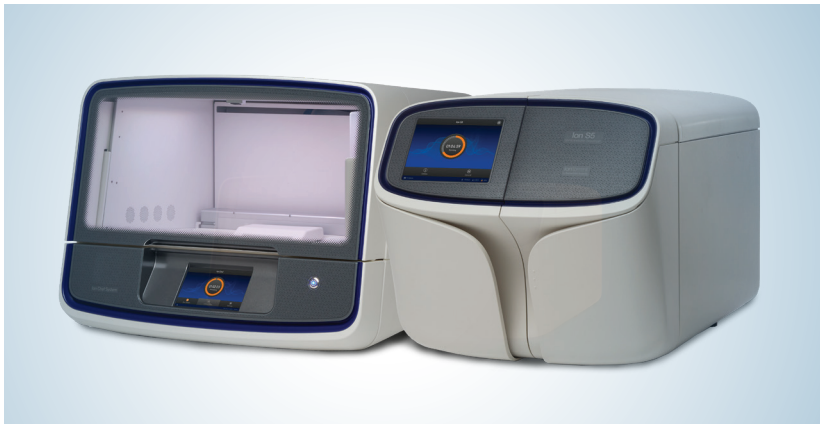

Precision ID GlobalFiler NGS STR Panel

Analyze DNA mixtures more efficiently

Analyze mixtures

The Applied Biosystems™ Precision ID Next-Generation Sequencing (NGS) System for human identification (HID) can help you solve tough cases by getting more information from your challenging samples. You can now confidently integrate NGS into your forensic DNA analysis workflow with the Applied Biosystems™ Precision ID GlobalFiler™ NGS Short Tandem Repeat (STR) Panel. With >99% genotype concordance to traditional capillary electrophoresis (CE) technologies (Table 1) and >99% sensitivity with as little as 125 pg of DNA input (Figure 1), you can quickly and easily analyze these commonly used forensic markers in less than 2 days.

Table 1. Performance of the Precision ID GlobalFiler NGS STR Panel v2 compared to CE analysis. Genotypes were obtained using Applied Biosystems™ Converge™ Software 2.0 and NGS Data Analysis v1.0 module using a 5% threshold, and were compared with matched results generated using traditional CE analysis using the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit.

Performance criteria	Data
Unique samples	568
Total CE truth markers	14,987
NGS false negatives	51
NGS false positives	65
Overall CE-NGS concordance	99.23%
Sensitivity	99.66%

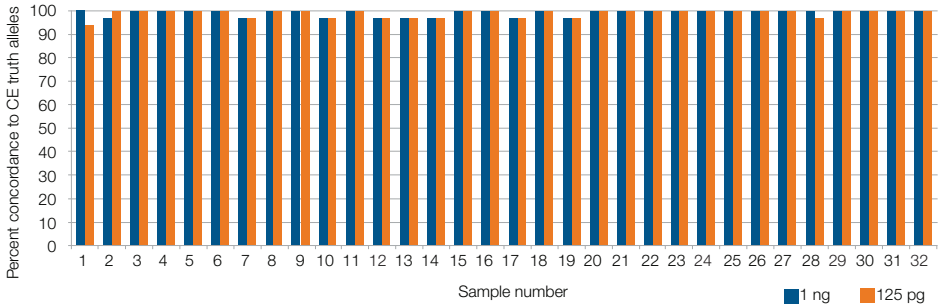

Figure 1. Concordance of NGS to CE analysis. 32 samples were analyzed at 1 ng and 125 pg using the Precision ID GlobalFiler NGS STR Panel v2 and compared to genotypes obtained using the GlobalFiler PCR Amplification kit on the Applied Biosystems™ 3500 Genetic Analyzer.

Simplicity

- As little as 125 pg of input DNA
- Interpret STR sequence variants and single-nucleotide polymorphisms (SNPs) in flanking regions with Converge Software and the NGS Data Analysis module
- Fully optimized Precision ID reagents and sequencing kits for STR panels

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Scalability and flexibility

- Multiple chip formats to meet your throughput needs (Table 2)
- Multiplex up to 32 samples for efficient sample processing

Speed

- Sequencing run times as short as 2 hours
- Sample to data in less than 2 days

Table 2. Sample throughput for the Precision ID GlobalFiler NGS STR Panels using the Ion GeneStudio™ S5™ System.*

Precision ID GlobalFiler NGS STR Panel v2 on Ion GeneStudio S5 System*	
Ion 520™ Chip	**Ion 530™ Chip**
16	32

* Minimum 500x coverage using the Precision ID GlobalFiler NGS STR Panel v2 for the Ion GeneStudio S5 System.

The 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 GlobalFiler PCR Amplification Kit, as well as 14 additional informative markers for forensic analysis. When analyzed with NGS, these markers reveal 254 more alleles (74%) than identified with traditional CE analysis due to sequence diversity within CE alleles of the same size (Table 3). These isometric alleles can be used to help resolve complex profiles and mixtures.

Table 3. Precision ID GlobalFiler NGS STR Panel markers and allele number comparison with CE from analysis of 320 population samples.

STR	Repeat structure	Source	Chr	CE alleles	NGS alleles	NGS% of CE alleles
TPOX	AATG	CODIS*	2	8	9	113
D3S1358	TCTA/TCTG	CODIS	3	9	21	233
FGA	CTTT/TTCC	CODIS	4	22	30	136
CSF1PO	AGAT	CODIS	5	8	9	113
D5S818	AGAT	CODIS	5	8	10	125
D7S820	GATA	CODIS	7	7	7	100
D8S1179	TCTA/TCTG	CODIS	8	10	26	260
TH01	TCAT	CODIS	11	7	7	100
vWA	TCTA/TCTG	CODIS	12	10	26	260
D13S317	TATC	CODIS	13	9	9	100
D16S539	GATA	CODIS	16	7	8	114
D18S51	AGAA	CODIS	18	15	19	127
D21S11	TCTA/TCTG	CODIS	21	17	55	324
AMEL-x	NA	Sex determination	X	ND**	ND	ND
AMEL-y	NA	Sex determination	Y	ND	ND	ND
rs2032678	NA	Sex determination	Y	ND	ND	ND
SRY	NA	Sex determination	Y	ND	ND	ND
D1S1656	TAGA	Expanded CODIS	1	15	27	180
D2S441	TCTA/TCAA	Expanded CODIS	2	8	14	175
D2S1338	TGCC/TTCC	Expanded CODIS	2	15	56	373
D10S1248	GGAA	Expanded CODIS	10	10	12	120
D12S391	AGAT/AGAC	Expanded CODIS	12	19	69	363
D19S433	AAGG/TAGG	Expanded CODIS	19	17	19	112
D22S1045	ATT	Expanded CODIS	22	10	10	100
DYS391	TCTA	Expanded CODIS	Y	ND	ND	ND
D1S1677	TTCC	MPS†	1	9	11	122
D2S1776	AGAT	MPS	2	9	9	100
D3S4529	ATCT	MPS	3	8	12	150
D4S2408	TCT	MPS	4	7	8	114
D5S2800	GATA/GATT	MPS	5	9	15	167
D6S474	GATA/GACA	MPS	6	7	11	157
D6S1043	AGAT/AGAC	MPS	6	15	26	173
D12ATA63	TAA/CAA	MPS	12	10	15	150
D14S1434	CTGT/CTAT	MPS	14	8	11	138
PentaD	AAAGA	Other STR	21	13	14	108
PentaE	AAAGA	Other STR	15	17	22	129
		Total alleles		**343**	**597**	**174**

* CODIS: Combined DNA Index System.
** ND: Not detected.
† MPS: Massively parellel sequencing (non-CODIS).

Integrated data analysis

Converge Software, a modular enterprise platform, was designed to increase the efficiency of forensic DNA laboratories by integrating forensic DNA data management and data analysis in a single software package. Converge Software offers streamlined solutions for NGS analysis of STRs, comparisons of CE to NGS for STR profiles, SNP marker analysis, mitochondrial genome analysis, and forensic case management.

The Converge NGS Data Analysis module is required to analyze profiles from the Precision ID GlobalFiler NGS STR Panel v2. NGS data analysis functionality includes information on STR allele calls, STR sequence motifs, known SNPs in flanking regions, and isometric heterozygotes (alleles of the same fragment length but containing different sequences). 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 2). User-defined and default analysis settings are provided in the NGS module for flexible data interpretation. Additionally, full auditing functionality is included for chain-of-custody requirements.

Sequence analysis of STR alleles with the Precision ID GlobalFiler NGS STR Panel v2 provides additional discriminatory information from populations of mixed alleles such as isometric heterozygotes (Figure 3) and SNPs, which may be present in the flanking regions. These additional sources of allelic diversity may be useful in mixture analysis as well as complex kinship interpretation.

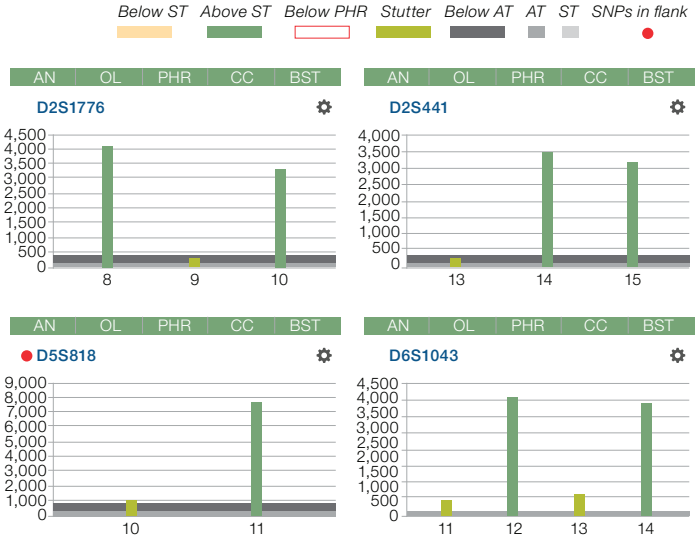

Figure 2. View of Converge Software's NGS secondary analysis results.

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.

applied biosystems

As forensic laboratories begin to adopt sequence analysis of STR markers into casework applications, demonstrating concordance with STR data generated by CE may be required. Using both the NGS Data Analysis module and the Case Management module of Converge Software allows for easy comparison of NGS and CE profiles (Figure 4). This comparison feature will be needed when comparing a crime scene sample analyzed with NGS to a reference sample processed by traditional methods.

The Precision ID GlobalFiler NGS STR Panel v2 is specifically designed for sequencing on the Ion GeneStudio S5 Systems. Library preparation can be performed manually, on an automated platform, or on the Ion Chef™ System with optimized template preparation.

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 4. 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)

Ordering information

Product	Quantity	Cat. No.
Precision ID GlobalFiler NGS STR Panel v2	96 reactions (manual)	A33114
	32 reactions (automated with Ion Chef System)	
Precision ID Library Kit	96 reactions	A26435
Precision ID Library Kit	384 reactions	A30941
Precision ID DL8 Kit	32 reactions	A33212
Precision ID IonCode Barcode Adapters 1–96 Kit	1 kit	A33586
Ion S5 Precision ID Chef & Sequencing Kit (2 runs per initialization)	8 reactions	A33208
Ion 530 Chip Kit	8 chips	A27764
Ion 520 Chip Kit	8 chips	A27762
Converge Software and Server	1 each	A35131
Case Management and NGS Data Analysis License, 1 user	3-year license	A35987
Case Management and NGS Data Analysis License, 5 users	3-year license	A36237
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

Publications

1. Müller P et al. (2018) Systematic evaluation of the early access Applied Biosystems Precision ID GlobalFiler mixture ID and GlobaFiler NGS STR panels for the Ion S5 System. *Forensic Sci Int Genet* 36:95–103.
2. Alonso A, Barrio PA, Müller P, et al. (2018) Current state-of-art of STR sequencing in forensic genetics. *Electrophoresis* doi:10.1002/elps.201800030
3. Wang Z et al. (2017) Massively parallel sequencing of 32 forensic markers using the Precision ID GlobalFiler NGS STR Panel and the Ion PGM System. *Forensic Sci Int Genet* 31:126–134.

Find out more at **thermofisher.com/hid-ngs**

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