EVALUATION OF THE PRECISION ID SYSTEM FOR TARGETED SEQUENCING OF DNA AND RNA MARKERS FOR HUMAN IDENTITY AND BODY FLUID IDENTIFICATION

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Next Generation Sequencing

- NGS is set to revolutionize forensic biology
  - Enabling the recovery of more identifying and intelligence information
    - From a wide variety of different single, mixed source and degraded samples
- It will be several years before NGS totally supplants current CE technology
  - However, labs can TODAY begin to use NGS for certain cases
- Significant advancements in:
  - Automation of library and template preparation and sequencing
  - Forensic sequencing kits
  - Software solutions for downstream genotyping analysis
- Ion Chef™ System and Ion S5™ System
  - DNA analysis for human identification
  - RNA analysis for body fluid identification

crime laboratory trades union meeting

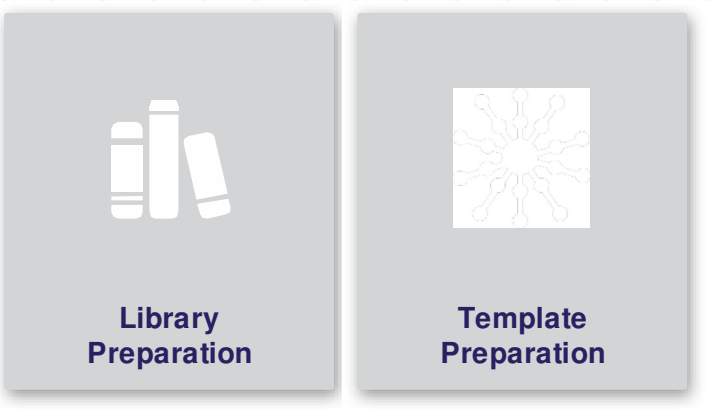
Evaluation of the EA Precision ID GlobalFiler™ Mixture ID Panel, Ion S5™ System and Ion Chef™ System

crime laboratory trades union meeting

Evaluation of the EA Precision ID GlobalFiler™ Mixture ID Panel, Ion S5 System and Ion Chef System

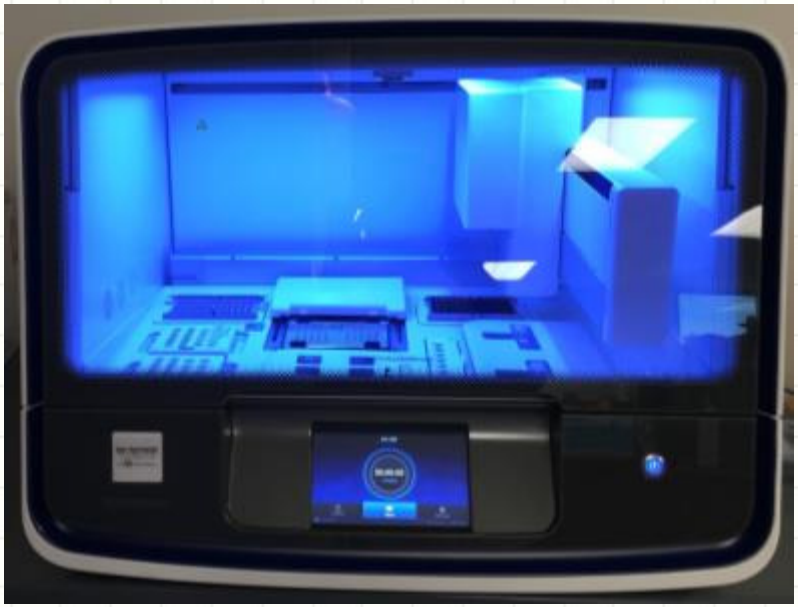
Ion Chef™ System

- Automates library, template preparation, & chip loading!



Running Complete Oh no!!

Which Chef would you prefer?

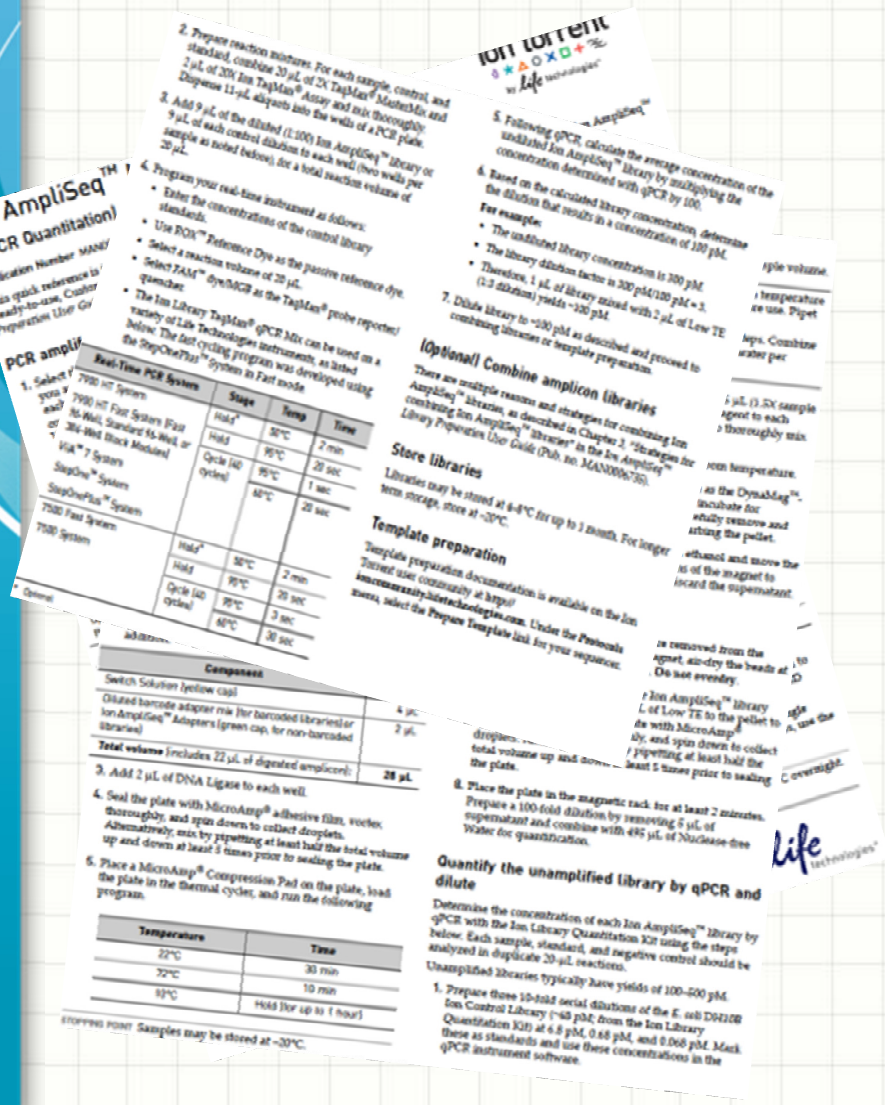


- Automated
- ~15 min or less prep time
- Fewer errors!

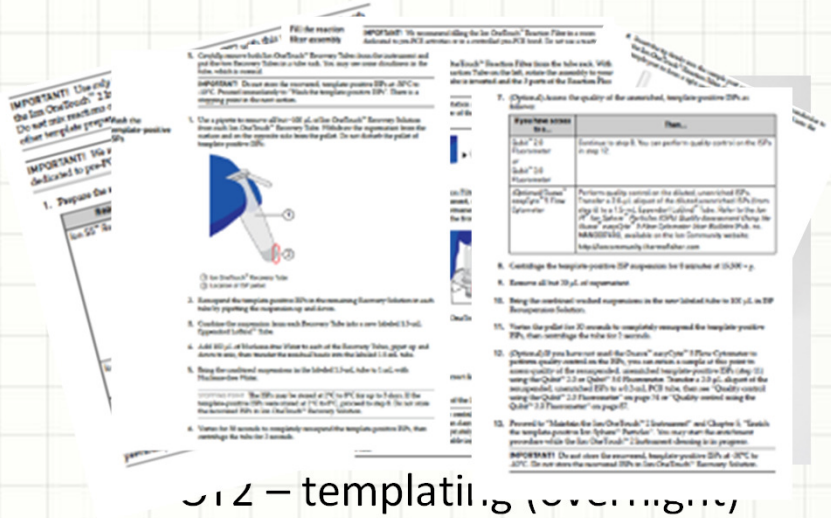
- Manual/Hands-on
- Hundreds of steps – days to complete
- Error-prone?

Manual preparation

Library Preparation

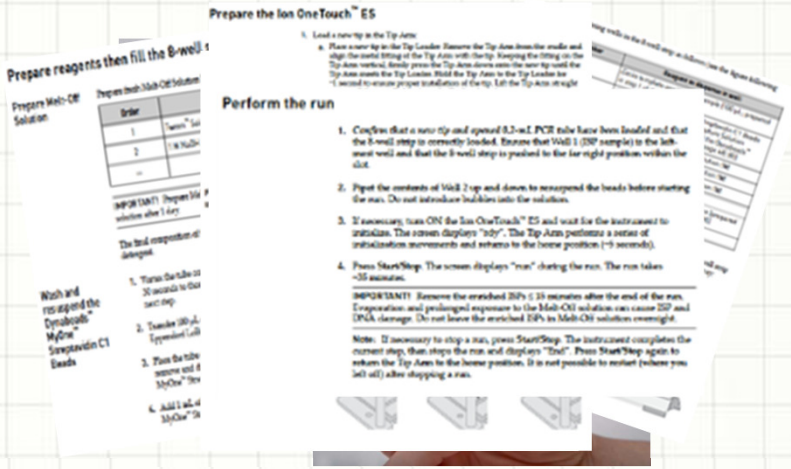


Template Preparation



OT2 – templating (overnight)

ES - enrichment



Manually load chip (with foam?!)

Library

Action 1
Add *8 samples (**1ng**/sample) to 96-well plate*

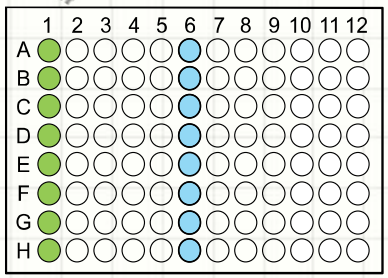
Action 2
Add *Precision ID Panel to Reagents Cartridge*

Action 3
Snap *5 cartridge components onto Ion Chef deck*

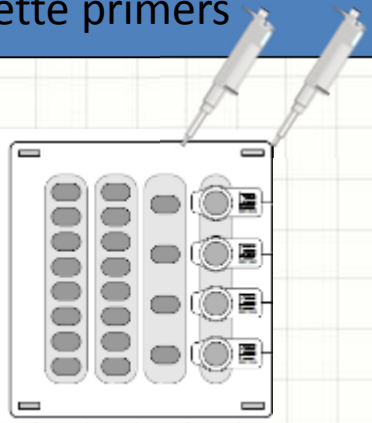
Pipette samples*

Pipette primers

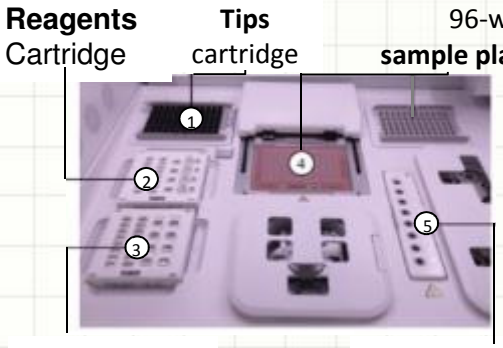
** Assume use of multichannel pipette*



96-well plate with IonCode™ Barcode Adapters



Reagents Cartridge



Reagents Cartridge

Tips cartridge

96-well **sample plate**

Solutions Cartridge

Enrichment Cartridge

~7.5 hrs

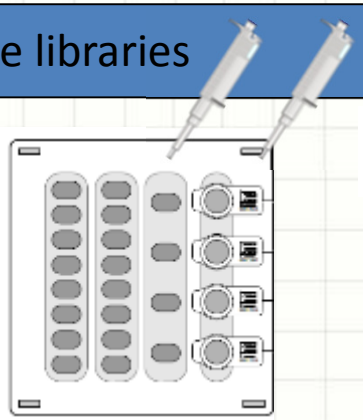
Template

Action 1
Plan *Run in Ion Torrent Broweser*

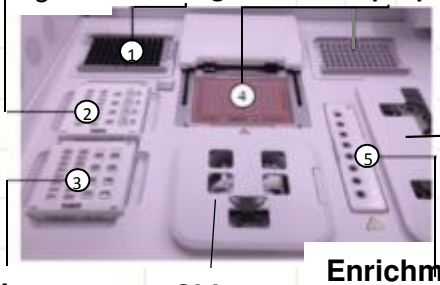
Action 2
Add *Libraries to Reagents Cartridge*

Action 3
Snap *cartridge components onto Ion Chef deck*

Pipette libraries



Reagents Cartridge



Reagents Cartridge

Tips cartridge

96-well **sample plate**

Dip trays, centrifuge lids, swinging buckets

Solutions Cartridge

Chips

Enrichment Cartridge

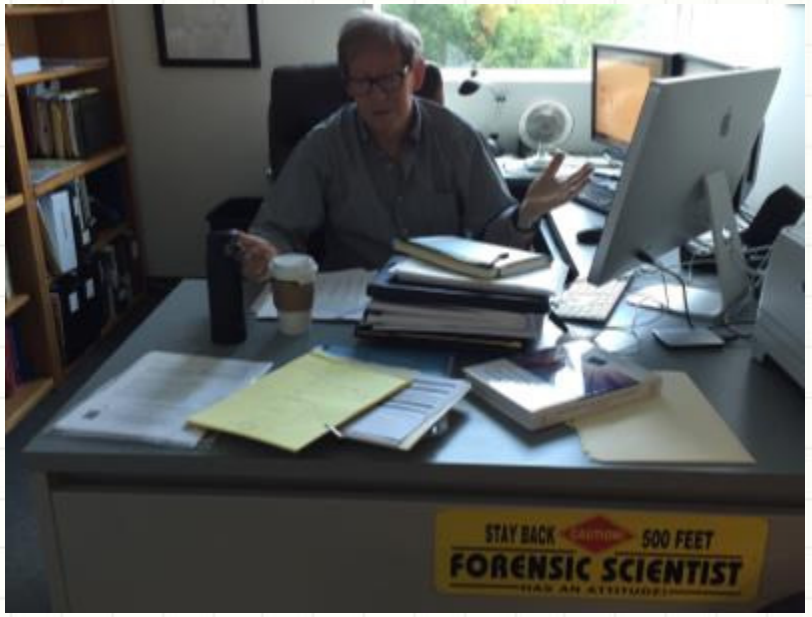
~13.5 hrs

While the Chef is working….

With the Ion Chef System

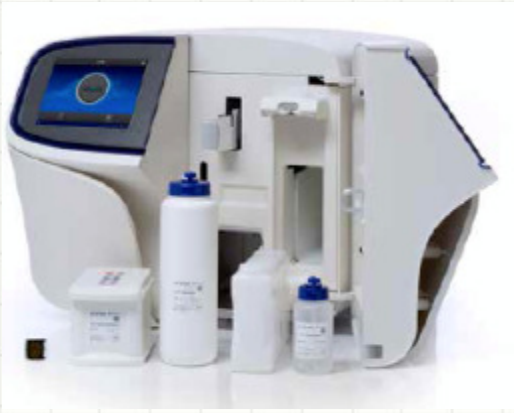
- Hands-off time while the chef is running
- Work does not pile up
- Time for a break!

With the 'Manual' Chef



- Hands-on time in the lab
- Work piles up!!!
- No time for a break!

Ion S5™ System



- Load-and-go reagents
- Straightforward user interface
- Less than 15 min of hands-on time

		Ion 520 Chip	Ion 530 Chip	Ion 540 Chip
Reads		3–5 million	15–20 million	60–80 million
Output*	200 bp	0.6–1 Gb	3–4 Gb	10–15 Gb
	400 bp	1.2–2 Gb	6–8 Gb	—
Run times	200 bp	2.5 hr	2.5 hr	2.5 hr
	400 bp	4 hr	4 hr	—
Analysis time†	200 bp	5 hr	8 hr	16.5 hr
	400 bp	8 hr	17.5 hr	—

Early access Precision ID GlobalFiler™ Mixture ID Panel

Ion Chef™ System – Ion S5™ System
Converge™ Software

EA Precision ID GlobalFiler™ Mixture ID Panel

Type of Target	Number of Targets
Autosomal STRs	29
Y-STRs	1
Autosomal SNPs	42
Y-SNPs	2
Indels	2 (Amel and Y indel)
Microhaplotypes	36 clusters of 2 to 4 SNPs

113 targets in the human genome in a single reaction!!

Early Access Ion Chef and Ion S5 reagents

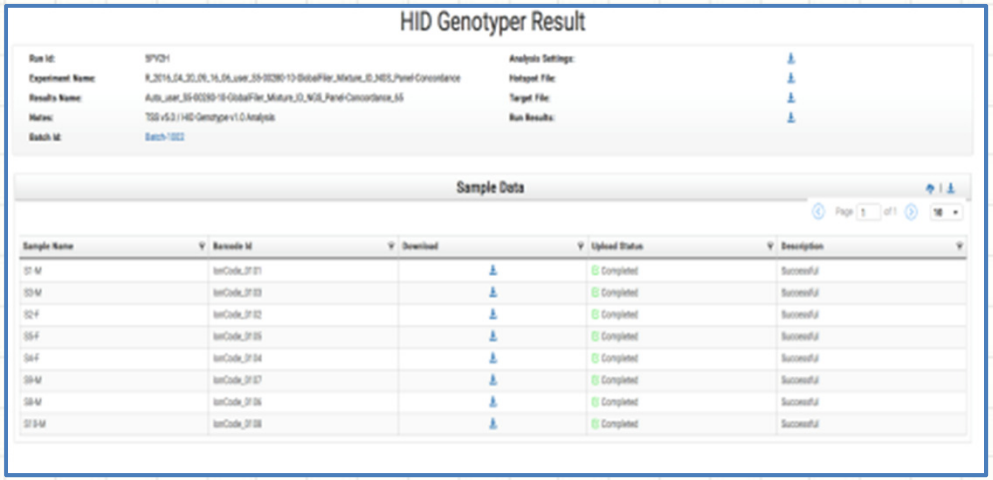
Ion 530 Chip

Designed to work on challenging samples that perform poorly in CE systems

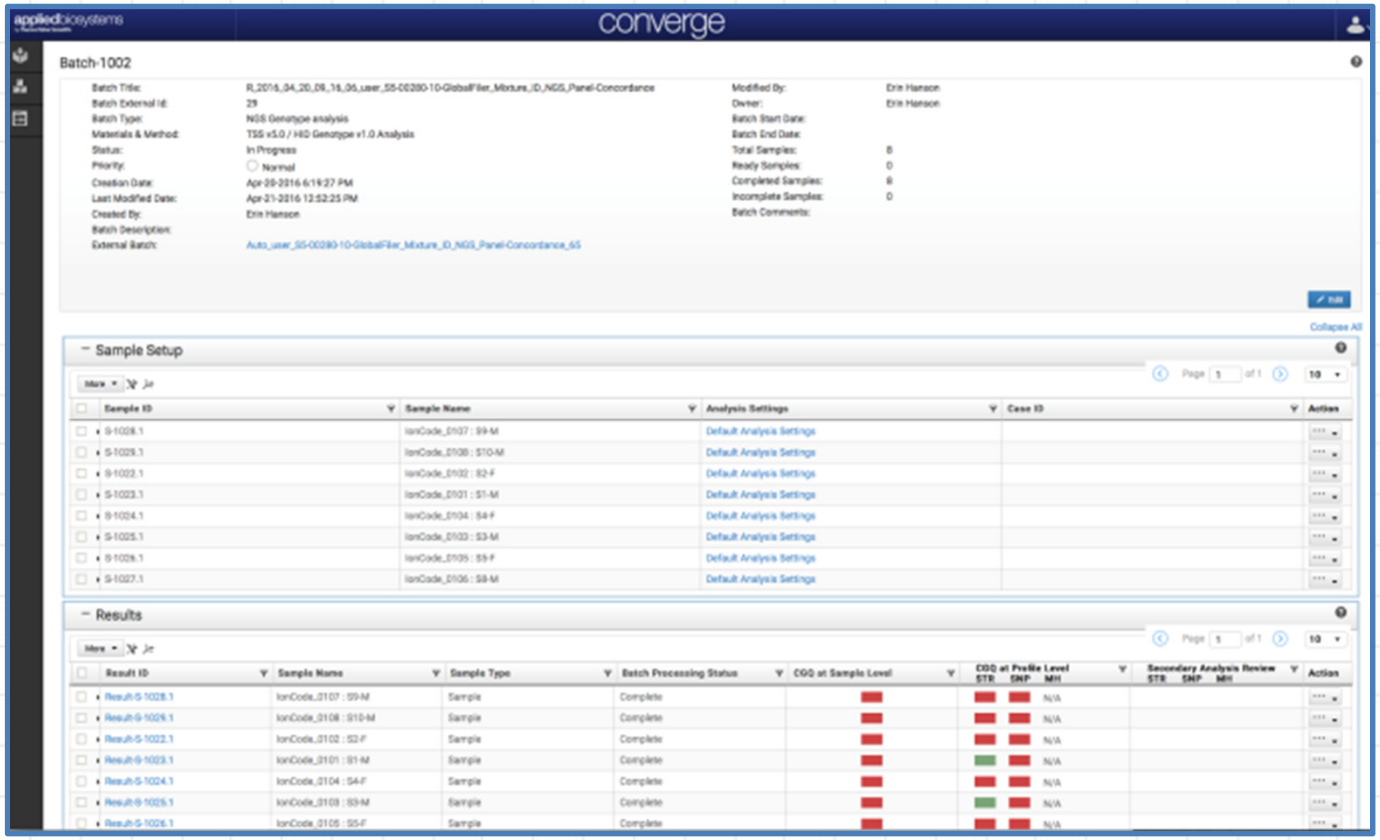
Testing & Evaluation Studies

Study	# Samples	Library quant (pM)	Chip loading	# Reads	Total Bases
Concordance	8	50	63%	4.1 M	541 M
Sensitivity (250pg, 125pg)	8	47	77%	4.0M	520 M
Casework samples	16	116 79	60%	10.7M	1.22 G
	8	in progress			

- Mixture studies to be completed next:
  - Male/female
  - Male/female multiple contributor

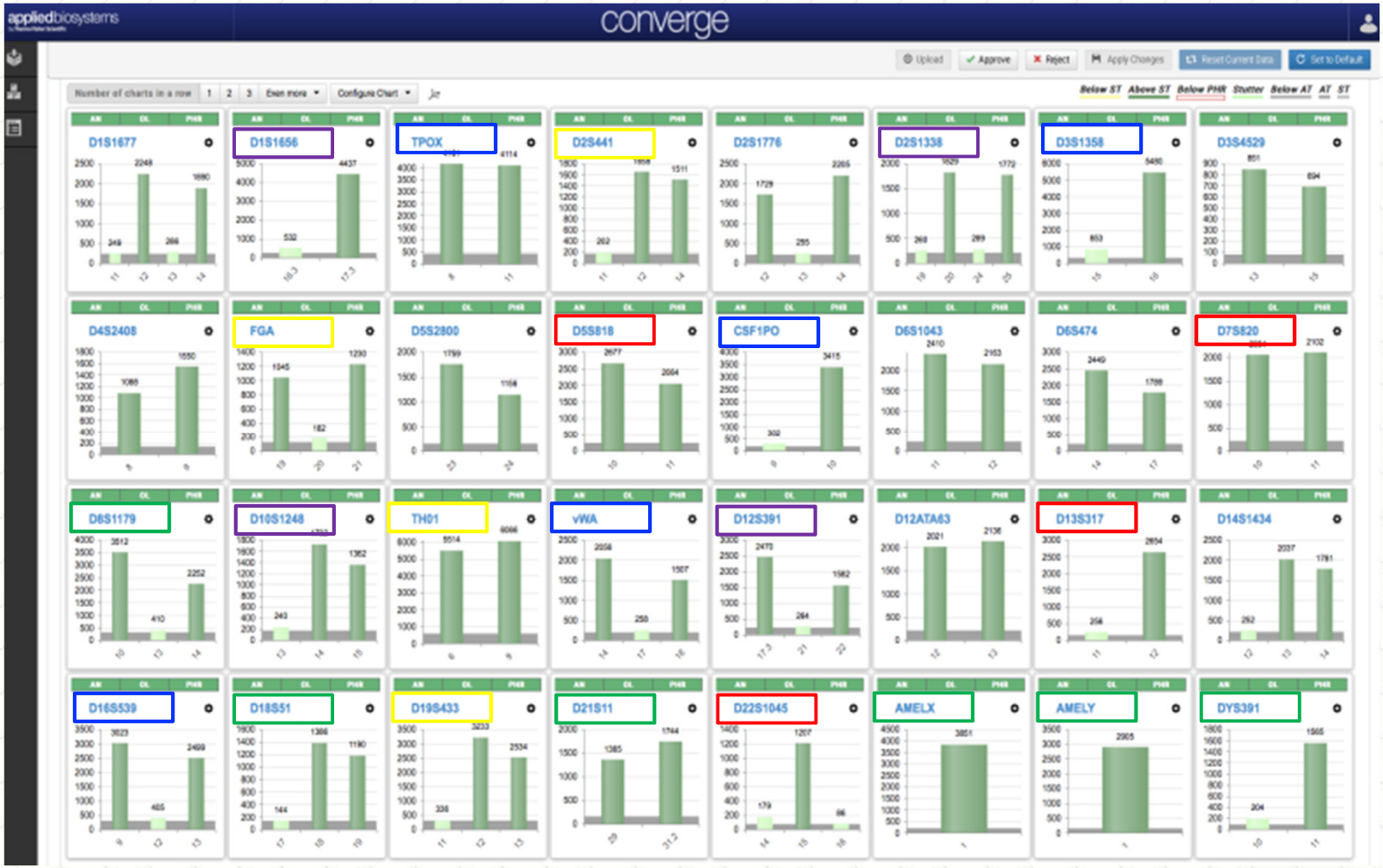


Data exported directly into CONVERGE software



Concordance

- 8 single source samples
  - 5 male, 3 female
  - Compared to CE GlobalFiler™ results
- All GlobalFiler™ loci concordant with NGS panel!



D3S1358	vWA	D16S539	CSF1PO	TPOX	Indel	AMEL	D8S1179	D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045	D5S818	D13S317	D7S820	D10S1248	D1S1656	D12S391	D2S1338
16	14,18	9,13	10	8,11	2	X,Y	10,14	29,31.2	18,19	11	12,14	12,13	6,9	19,21	15	10,11	12	10,11	14,15	17.3	17.3,22	20,25

Sensitivity – Autosomal STRs

Sample	Input (pg)	STRs			
		Total Reads	Avg. Reads (Per Locus)	Min Reads (Locus)	Max Reads (Locus)
S2 Female	250	97,680	3368	893 *D22S1045*	6637 *TH01*
	125	96,096	3314	924 *D22S1045*	6758 *TH01*
S3 Male	250	88,812	3063	651 *D22S1045*	5630 *TH01*
	125	113,218	3904	915 *D22S1045*	7200 *TPOX*
S4 Female	250	100,085	3451	731 *D22S1045*	7963 *TPOX*
	125	111,909	3859	864 *D22S1045*	9291 *TPOX*
S9 Male	250	75,510	2604	127 *D19S433*	5665 *TH01*
	125	101,648	3505	817 *D22S1045*	7243 *TH01*

Total reads = main alleles only (doesn't include stutter)

16 Casework Samples

Sample	Description	Deg. Index	Trio S. Auto (ng/ul)	conc	input vol (ul)	input (ng)	Barcode	
C10	Blood - polyester - room temp 1 year	1.0	0.978	0.0685	15	1.03	109	yellow
C11	Blood (Male) - 37oC 6 months	0.6	15.518	0.0670	15	1.01	110	
C12	Blood (Male) - 37oC 1 year	0.8	17.005	0.0670	15	1.01	111	
C13	Saliva (Female) - 37oC 6 months	1.2	6.285	0.0670	15	1.01	112	
C14	Saliva (Female) - 37oC 1 year	1.4	9.416	0.0670	15	1.01	113	
C15	Blood (Female) - Car Trunk 2 weeks	0.9	3.200	0.0670	15	1.01	114	
C16	Blood (Male) - Car Back Seat 1 week	0.7	4.054	0.0670	15	1.01	115	
C17	Blood (Female) - Car Back Seat 1 week	0.6	0.871	0.0670	15	1.01	116	
C18	Dried chewing gum	0.8	0.200	0.0670	15	1.01	117	blue
C19	Drink can (perrier) - mouth area	0.8	0.348	0.0696	15	1.04	118	
C20	Spit on pavement (dried)	2.6	0.008	0.0075	15	0.11	119	
C22	Menstrual blood	0.6	14.463	0.0670	15	1.01	120	
C23	Vasectomized male - semen	0.7	0.112	0.0659	15	0.99	121	
C7.2	Semen on denim - 56oC 1 month	0.8	0.743	0.0675	15	1.01	122	
C7.3	Vaginal swab - 56oC 6 months	1.6	20.549	0.0670	15	1.01	123	
C8.3	Vaginal swab - 37oC 2 years	2.3	33.468	0.0670	15	1.01	125	

Libraries combined to run 16 samples on one Ion 530 Chip

6 blood
5 saliva
2 semen
1 menstrual blood
2 vaginal

9 unique donors
- some samples from the same donor

- Full STR profiles from 15/16 samples
- 'Spit' on pavement – partial profile; low level mixture
  (a lot of information still obtained!)

Blood – Car Back Seat 1 week!

Full profiles:
STR, SNP, MH

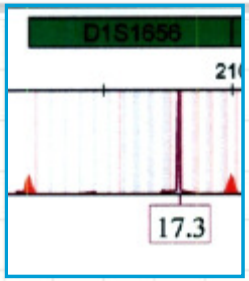
Vaginal swab – 37°C 2 years!

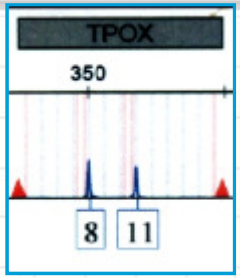
Full profiles:
STR, SNP, MH

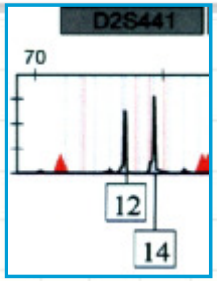
Chewing gum (~4-5 days old)

Full profiles:
STR, SNP, MH

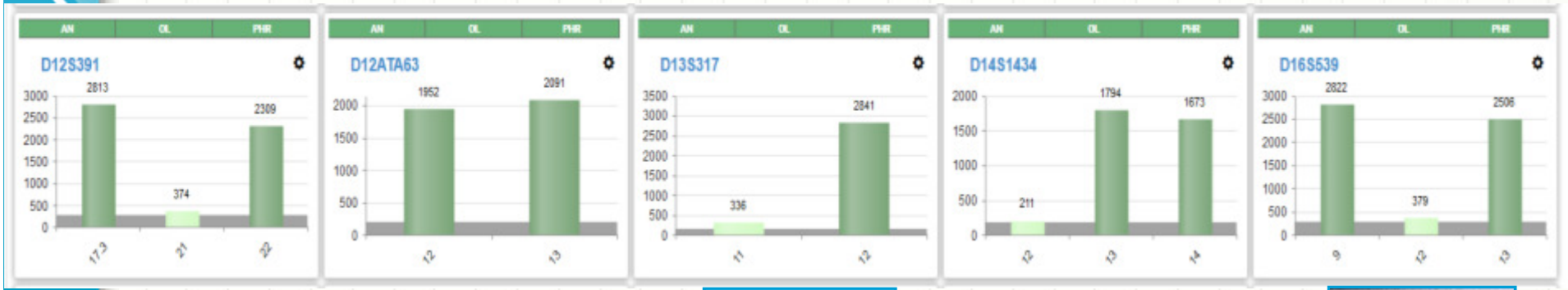
New STR

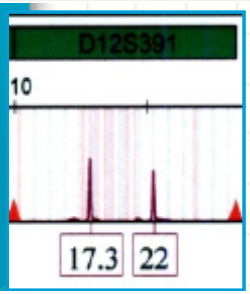




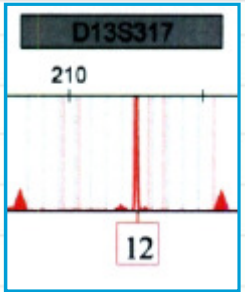


New STR

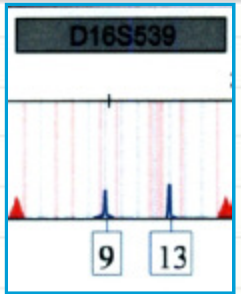




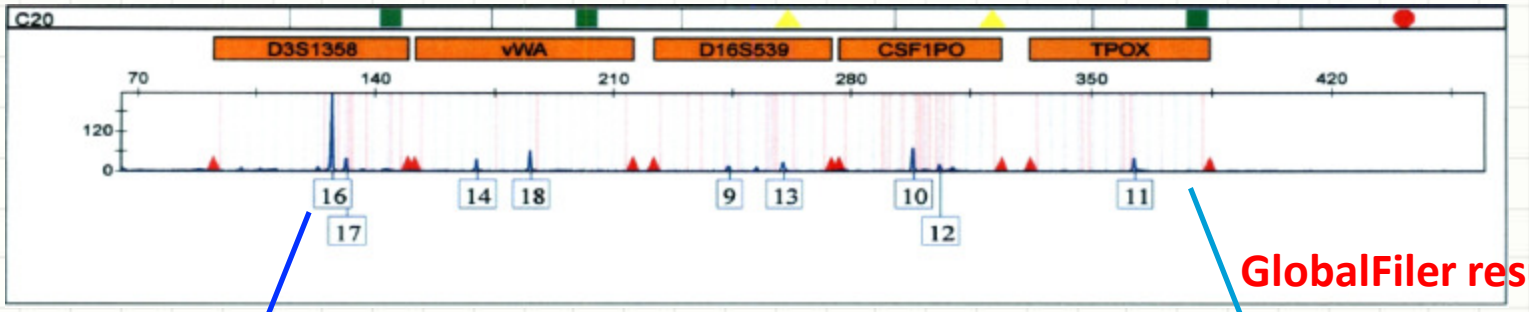
New STR



New STR



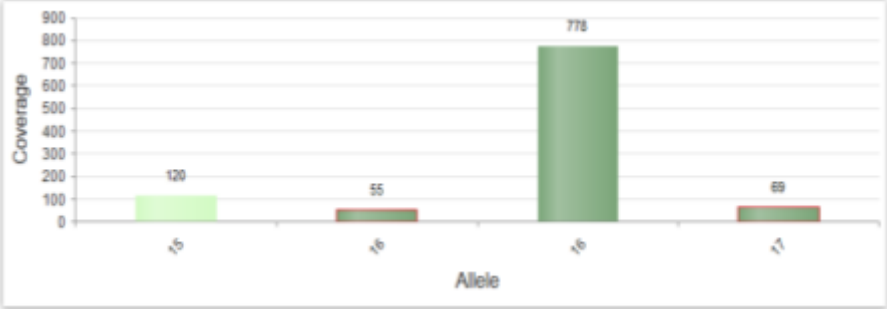
‘Spit’ on pavement



GlobalFiler results

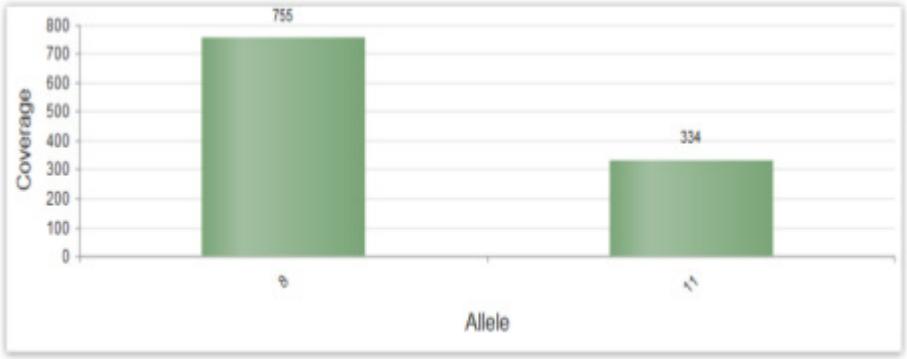
D3S1358

D3S1358	15,16,16,17		
Allele	Status	Coverage	Sequence
15	STUTTER	120	[TCTA]1 [TCTG]2 [TCTA]12
16	ABOVE_ST	55	[TCTA]1 [TCTG]1 [TCTA]14
16	ABOVE_ST	778	[TCTA]1 [TCTG]2 [TCTA]13
17	ABOVE_ST	69	[TCTA]1 [TCTG]3 [TCTA]13



TPOX

TPOX	8,11		
Allele	Status	Coverage	Sequence
8	ABOVE_ST	755	[AATG]8
11	ABOVE_ST	334	[AATG]11



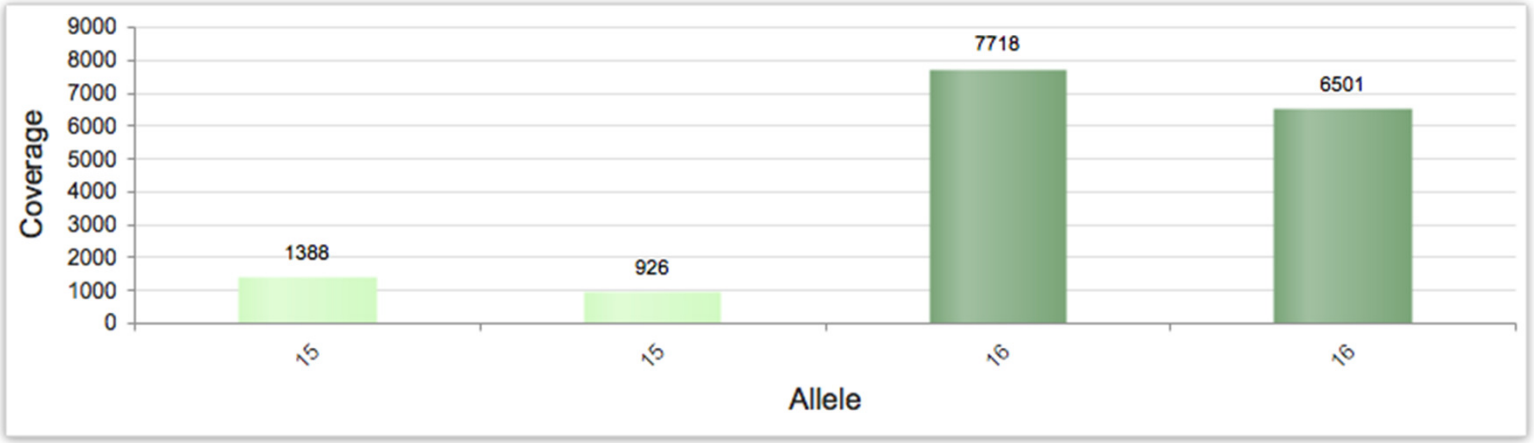
- 16, 17 identified in both systems
- NGS – additional ‘16’ (second donor?)

- NGS – ‘8’ allele not detected with GlobalFiler result

Extra Discrimination – Sequence Level

D3S1358	15,15,16,16

Allele	Status	Coverage	Sequence
15	STUTTER	1388	[TCTA]1 [TCTG]2 [TCTA]12
15	STUTTER	926	[TCTA]1 [TCTG]3 [TCTA]11
16	ABOVE_ST	7718	[TCTA]1 [TCTG]2 [TCTA]13
16	ABOVE_ST	6501	[TCTA]1 [TCTG]3 [TCTA]12

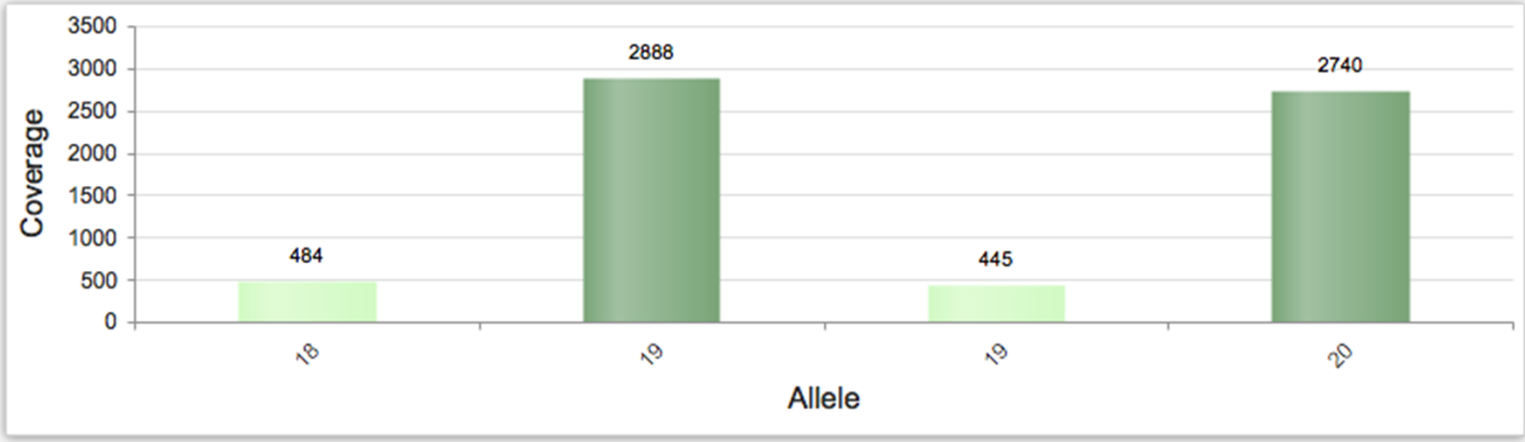


Hetero-homozygous (as Rob calls it)
Compound heterozygous (as the rest of the World calls it)

Extra Discrimination – Sequence Level

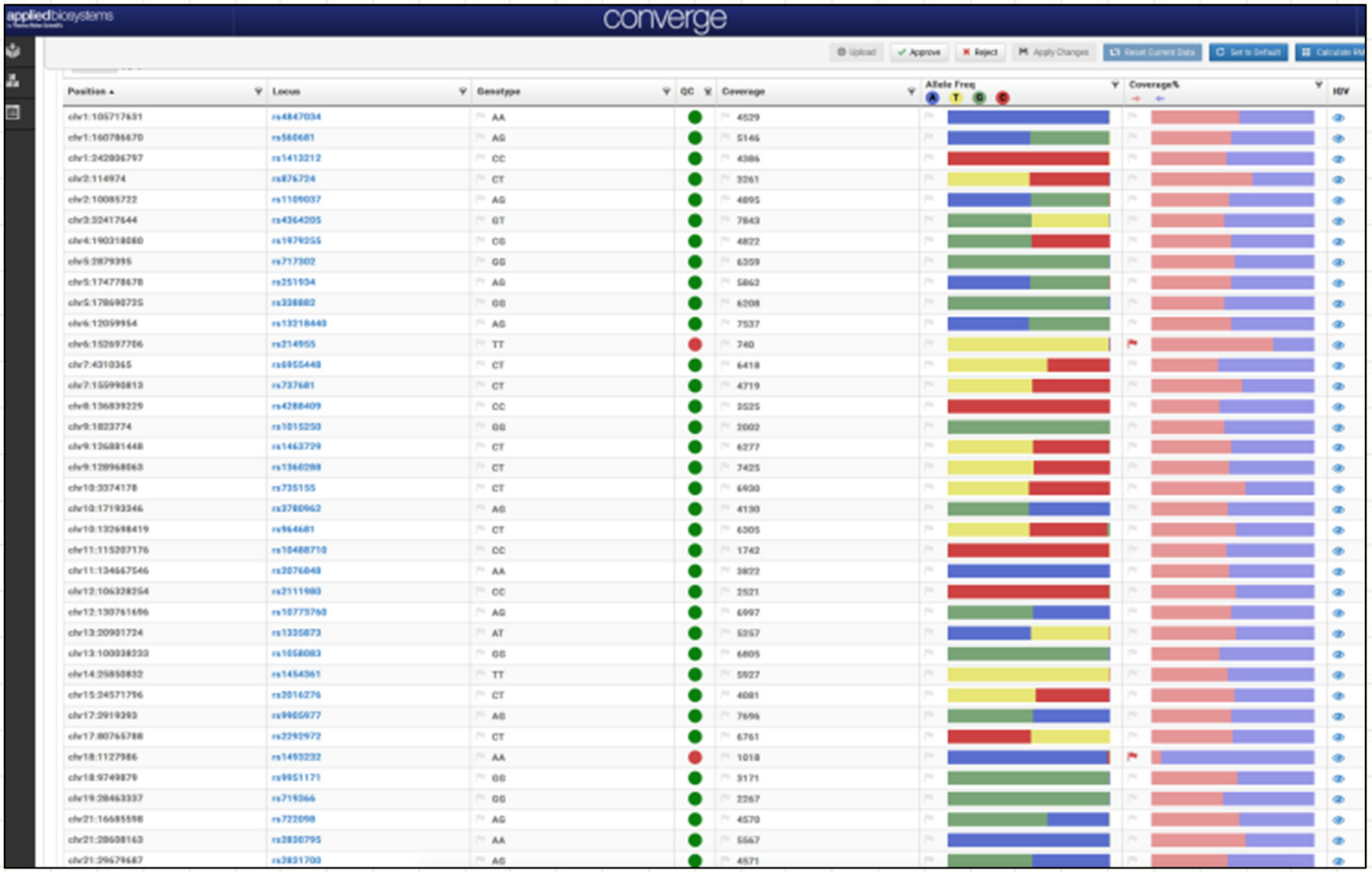
D2S1338	18,19,19,20

Allele	Status	Coverage	Sequence
18	STUTTER	484	[TGCC]6 [TTCC]12
19	ABOVE_ST	2888	[TGCC]6 [TTCC]13
19	STUTTER	445	[TGCC]7 [TTCC]12
20	ABOVE_ST	2740	[TGCC]7 [TTCC]13



‘19’ stutter (from 20 allele) different sequence than main ‘19’ allele

Additional Identity Information - SNPs



RMP Range

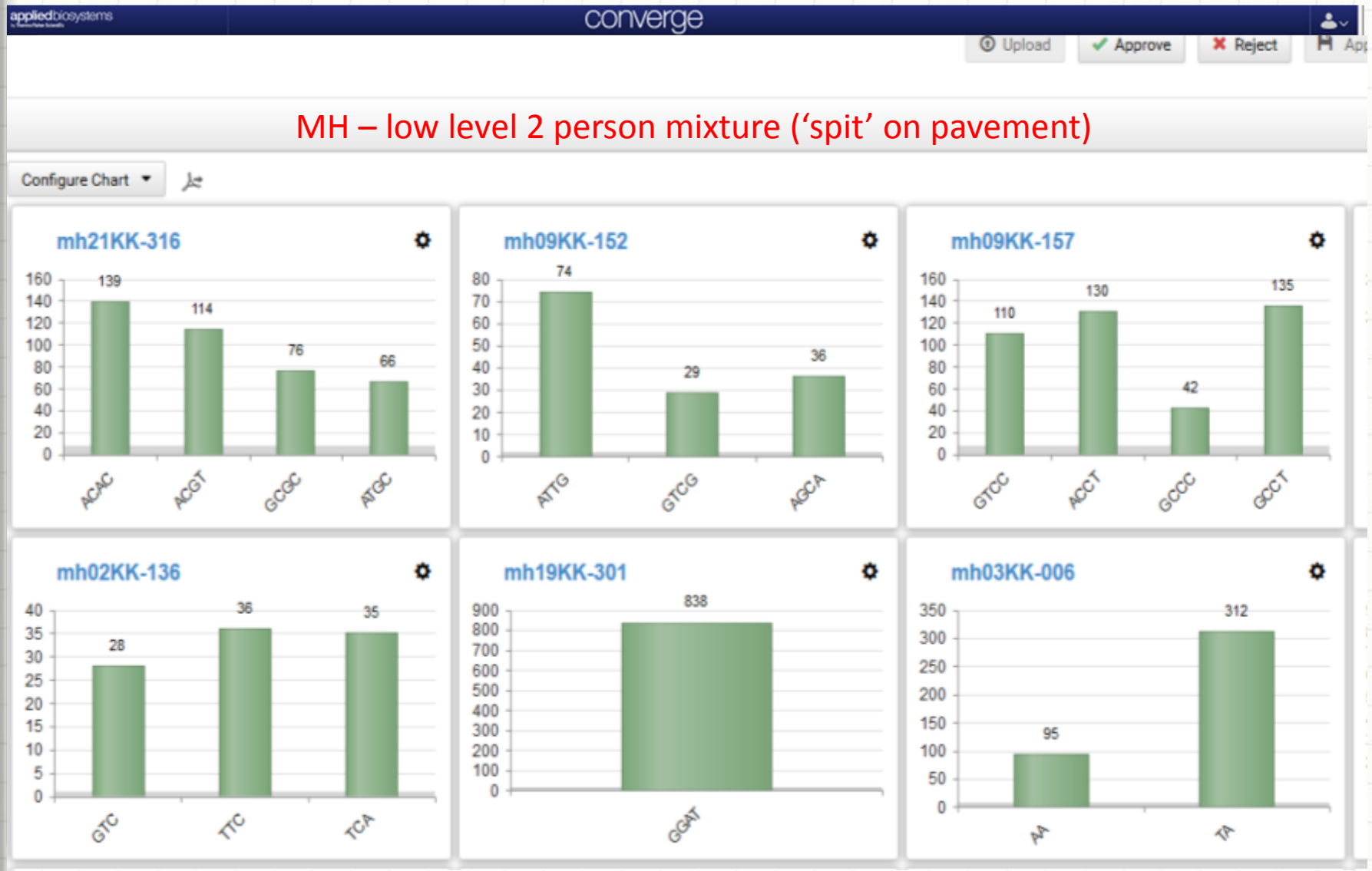
RMP Range: 9.43907e-23 to 5.7380845e-18

More

Population	rmp
Europe	5.7380845e-18
America	8.683099e-19
South Asia	6.217617e-19
East Asia	1.868273e-21
Africa	9.43907e-23

Cancel

Additional information - MH



Will be especially used in mixture studies to help in determining # of donors!

TARGETED RNA EXPRESSION FOR BODY FLUID IDENTIFICATION

ION CHEF™ SYSTEM– ION S5™ SYSTEM

Collaboration with University of Zurich

why

?

irrelevance?

activity = what?

Body Fluid/Tissue Source ID

- Whole Transcriptome versus Targeted RNA re-sequencing
- Digital gene expression (DGE)-counts how many copies of a transcript in a sample
  - Facilitates quantitative approaches
- Can detect mixtures
- After cDNA formation, same process as gDNA
- Multiplex sequence analysis-multiple genes in multiple samples (and RNA + DNA co-analysis?)
- Associate a body fluid biomarker with a DNA profile (i.e. the donor)?

Methods

Ion AmpliSeq™ Kit for Chef DL8 Kit

gDNA or RNA sample preparation

▼

Perform reverse transcription (RNA only) SuperScript™ VILO™ 20ng total RNA

▼

(Optional) Create a Sample Set

▼

Add Ion AmpliSeq™ 2X Primer Pools to Positions A and B of the Reagents cartridge

▼

Add gDNA to the IonCode™ Barcode plate

▼

Load the Ion Chef™ Instrument

▼

Start the Ion Chef™ run

▼

Unload the Ion Chef™ Instrument

Ion 520/530 Kit-Chef

Create a Planned Run within the Torrent Suite™ Software and set the run parameters.

▼

Dilute the library samples, and thaw the reagents.

▼

Load the Ion Chef™ Instrument with sequencing chips, consumables, reagents, and libraries.

▼

Start the Ion Chef™ Instrument

▼

Unload the Ion Chef™ Instrument.

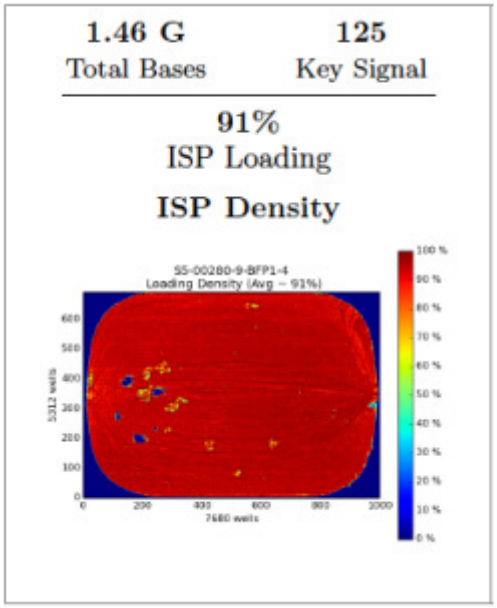
▼

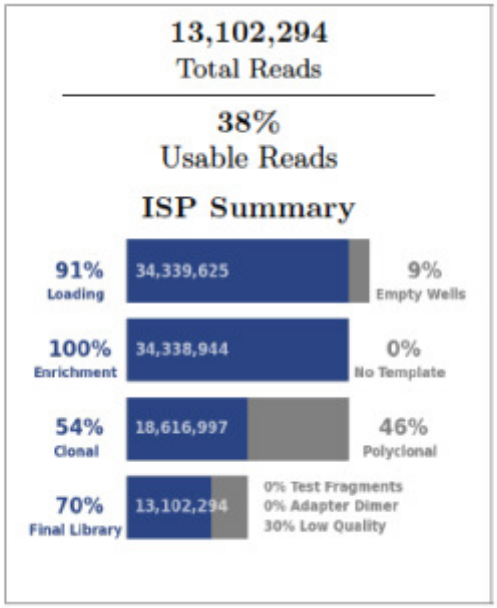
Load the prepared chip into the Ion S5™ or Ion S5™ XL Sequencer and begin the sequencing run.

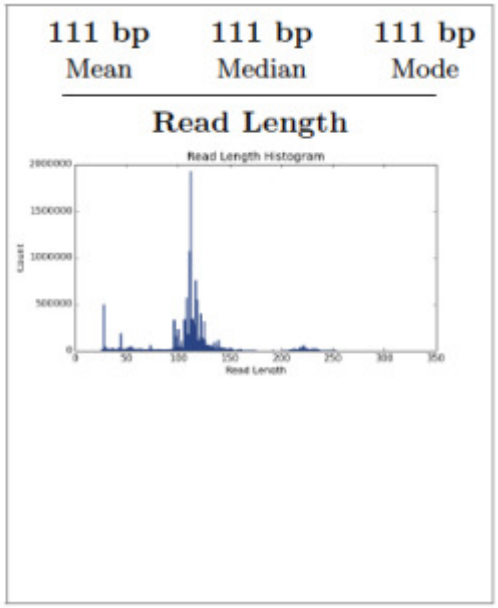
Custom Ion AmpliSeq™ Primer Pool

Body fluid	Gene	BFP1 (61plex)	BFP2 (37plex)
Blood	Blood-1		
	Blood-2		
	Blood-3		
	Blood-4		
	Blood-5		
	Blood-6		
	Blood-7		
	Blood-8		
Semen	Semen-1		
	Semen-2		
	Semen-3		
	Semen-4		
	Semen-5		
	Semen-6		
	Semen-7		
Saliva	Saliva-1		
	Saliva-2		
	Saliva-3		
	Saliva-4		
	Saliva-5		
	Saliva-6		
	Saliva-7		
	Saliva-8		
	Saliva-9		
	Saliva-10		
	Saliva-11		
	Saliva-12		
	Saliva-13		
	Saliva-14		
	Saliva-15		
	Saliva-16		
	Saliva-17		
Vaginal	Vaginal-1		
	Vaginal-2		
	Vaginal-3		
	Vaginal-4		
	Vaginal-5		
	Vaginal-6		
	Vaginal-7		
	Vaginal-8		
	Vaginal-9		
	Vaginal-10		
	Vaginal-11		
Menstrual	Menstrual-1		
	Menstrual-2		
	Menstrual-3		
	Menstrual-4		
	Menstrual-5		
	Menstrual-6		
Skin	Skin-1		
	Skin-2		
	Skin-3		
	Skin-4		
	Skin-5		
	Skin-6		
	Skin-7		
	Skin-8		
	Skin-8		
	Skin-9		
	Skin-10		
	Skin-11		

Run Data – Ion Chef™ System- Ion S5™ System



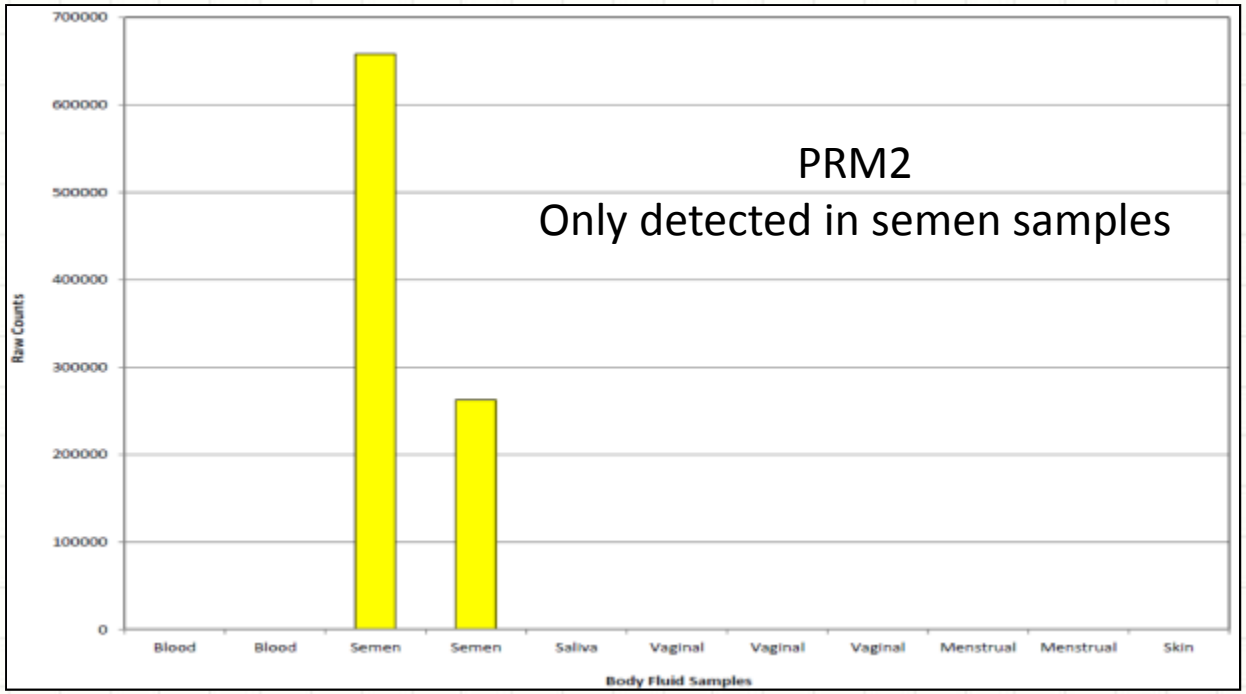




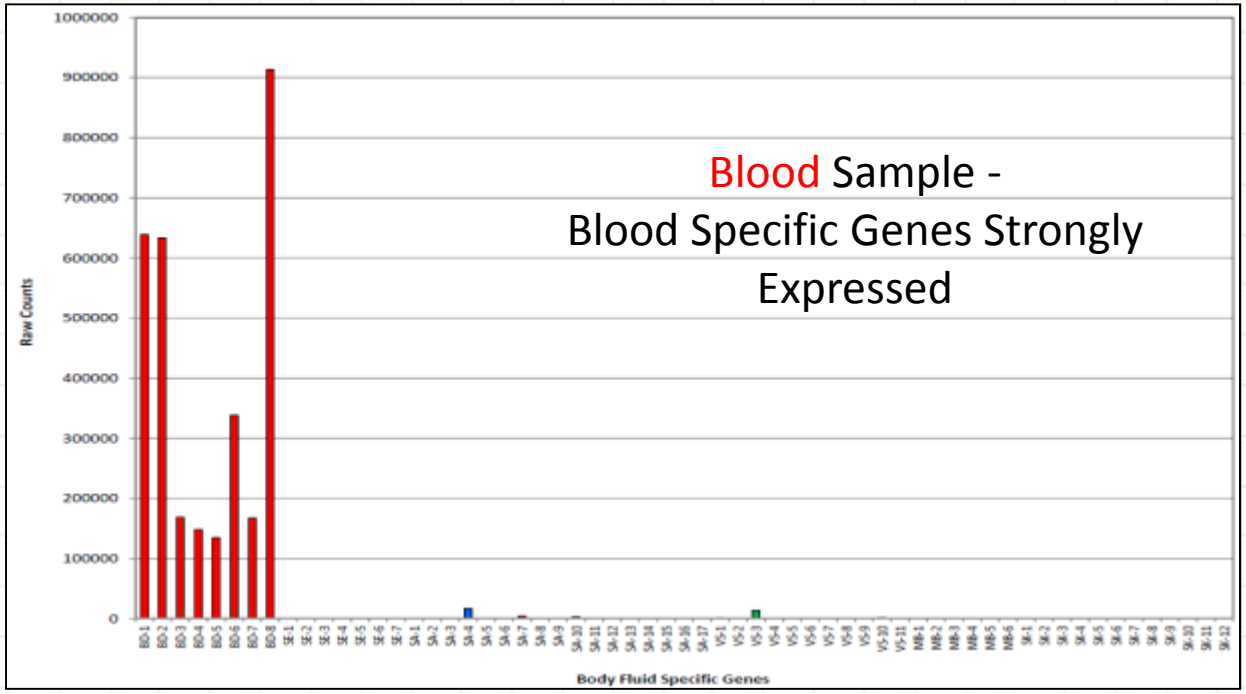
Barcode Name	Sample	Bases	≥ Q20	Reads	Mean Read Length
No barcode	none	60,999,878	51,458,510	526,263	116 bp
IonCode_0125	b7477	190,686,921	173,885,771	1,652,575	115 bp
IonCode_0126	SE22	226,174,742	205,968,784	2,033,923	111 bp
IonCode_0127	se19	110,666,506	101,744,622	1,083,866	102 bp
IonCode_0128	sa60	9,642,507	8,941,508	306,716	31 bp
IonCode_0129	sa61	154,341	138,406	2,369	65 bp
IonCode_0130	vs4	329,417,165	295,697,836	2,732,934	121 bp
IonCode_0131	vs2	152,613,820	138,022,817	1,494,241	102 bp
IonCode_0132	mb2	377,486,810	342,462,596	3,267,700	116 bp

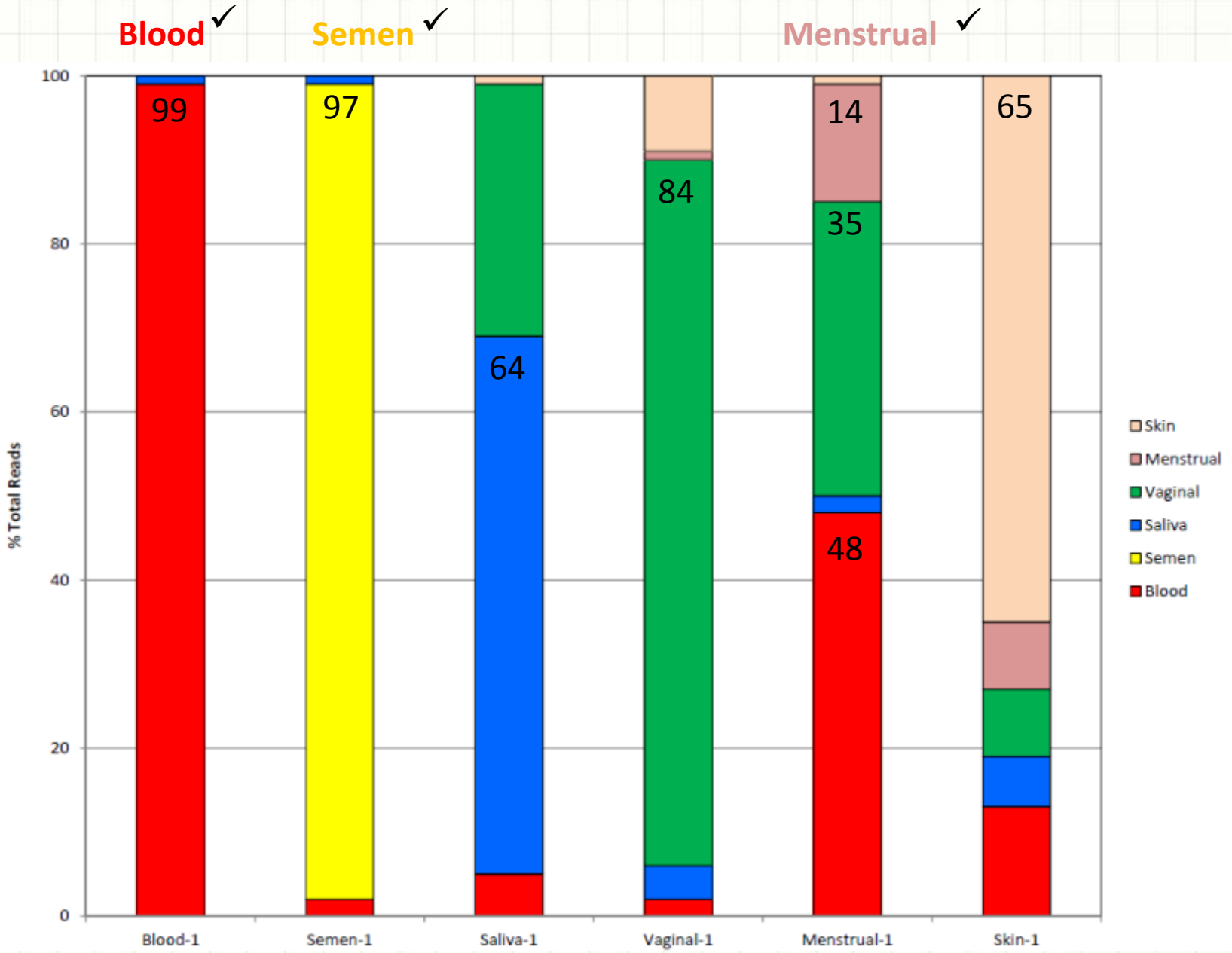
Specificity

By Gene



By Sample





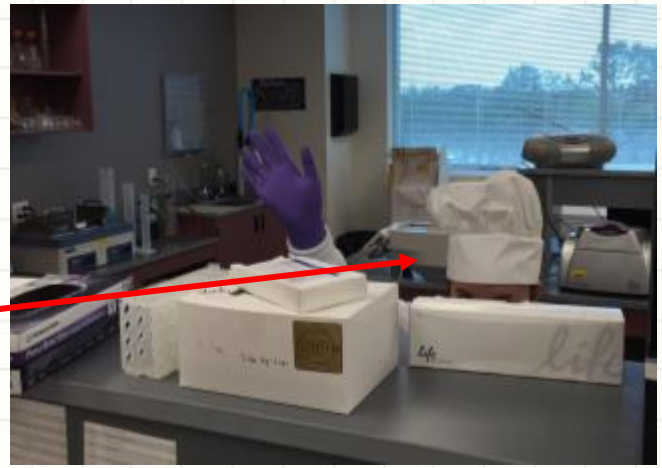
Saliva: ~64%, Vaginal: ~63-84%, Skin: ~65% → genes removed in new primer pool to improve specificity

Summary

- Autosomal NGS STR profiles concordant with CE methods easily obtained with few minutes hands-on effort using the automated Ion Chef ™ System/Ion S5 ™ System/Precision ID GlobalFiler ™ Mixture ID Panel/Converge™ Software/1 ng DNA from casework type samples
  - apart from 29 aSTRs, the genotypes of 42 aSNPs and 36 MH's also obtained
  - RMP from the aSNPs are typically 10^{-17} -10^{-23}
  - Increased variation at STR loci detected and can be used
    - 'Hetero-homozygotes'
    - Stutter peak distinguished from allele peak when indistinguishable by CE
- Modular RNA-based body Fluid ID system being developed to be compatible for the automated Ion Chef™ System and Ion S5 System shows early promise
- Next step: MIXTURES here we come!

Acknowledgements

- Dr. Erin Hanson (Co-Chef)
  (who hates having her picture taken)



- The amazing Thermo Fisher Scientific team!
  - Sheri, Rob, Joe, Matt, Shelly
  - Sharada, Jie, Ravi, Narsi & the SW team!
  - Nicolette, Tanya

- Body fluid
  - Cordula Haas, Sabrina Ingold (University of Zurich)
  - Funding: NIJ, EUROFORGEN


RY TORTUGAS 70 MI.
MIAMI 132 MI
HONOLULU 4788 MI
FRANCISCO 2550 mi
HABANA 105 MI
NASSAU 281MI
NEW ORLEANS 630 MI
Thank you for your attention!

The Precision ID GlobalFiler Mixture ID Panel is in early access development and not available for purchase. The content provided herein may relate to products that have not been officially released and is subject to change without notice.

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