

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



www.tourisminbarcelona.com

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HIDS, Barcelona, Spain 2016

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



"All those in favour of accepting more robots?"

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

crime laboratory
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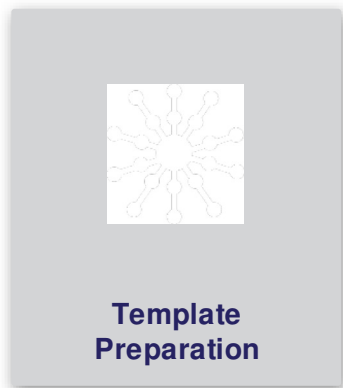


"All those in favour of accepting more robots?"

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!



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?



Library Preparation

Manual preparation



Template Preparation

Ion AmpliSeq™ (qPCR Quantitation)
Publication Number: 54547
This quick reference is for ready-to-use, Custom Preparation User Guide.

PCR amplification

1. Select a PCR system

PCR System	Stage	Temp	Time
7900 HT System	Hold	95°C	2 min
7900 HT Fast System	Hold	95°C	20 sec
7900 HT Fast System	Cycle (40 cycles)	95°C	1 sec
7900 HT Fast System	Cycle (40 cycles)	60°C	20 sec

2. Prepare reaction mixtures. For each sample, control, and standard, combine 20 µL of 2X TagMax™ MasterMix and 2 µL of 20X Ion TagMax™ Assay and mix thoroughly. Dispense 11 µL aliquots into the wells of a PCR plate.

3. Add 9 µL of the diluted (1:100) Ion AmpliSeq™ library or 9 µL of each control dilution to each well (two wells per sample as noted below), for a total reaction volume of 20 µL.

4. Program your real-time instrument as follows:

- Enter the concentrations of the control library standards.
- Use ROX™ Reference Dye as the passive reference dye.
- Select a reaction volume of 20 µL.
- Select FAM™ dye as the TagMax™ probe reporter.
- The Ion Library TagMax™ qPCR Mix can be used on a variety of Life Technologies instruments, as listed below. The best cycling program was developed using the StepOne™ System in Fast mode.

Optional Combine amplicon libraries

There are multiple resources and strategies for combining Ion AmpliSeq™ libraries, as described in Chapter 2, "Strategies for combining Ion AmpliSeq™ libraries" in the Ion AmpliSeq™ Library Preparation User Guide (Pub. no. MAN0006700).

Store libraries

Libraries may be stored at 4-8°C for up to 3 months. For longer term storage, store at -20°C.

Template preparation

Template preparation documentation is available on the Ion AmpliSeq™ website at <http://ionampliseq.lifetech.com>. Under the Protocols menu, select the Prepare Template link for your sequencer.

5. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ library by multiplying the concentration determined with qPCR by 100.

6. Based on the calculated library concentration, determine the dilution that results in a concentration of 100 pM. For example:

- The undiluted library concentration is 100 pM.
- The library dilution factor is 100 pM / 100 pM = 1.
- Therefore, 1 µL of library mixed with 9 µL of Low TE (1:1 dilution) yields ~100 pM.

7. Dilute library to ~100 pM as described and proceed to combining libraries or template preparation.

8. Place the plate in the magnetic rack for at least 2 minutes. Prepare a 100-fold dilution by removing 5 µL of supernatant and combine with 495 µL of Nuclease-free water for quantification.

Quantify the unamplified library by qPCR and dilute

Determine the concentration of each Ion AmpliSeq™ library by qPCR with the Ion Library Quantitation Kit using the steps below. Each sample, standard, and negative control should be analyzed in duplicate 20-µL reactions.

Unamplified libraries typically have yields of 100-500 pM.

1. Prepare three 10-fold serial dilutions of the E. coli DNA200 Ion Control Library (~40 pM, from the Ion Library Quantitation Kit) at 4.0 pM, 0.40 pM, and 0.040 pM. Mark these as standards and use these concentrations in the qPCR instrument software.

2. Prepare reaction mixtures. For each sample, control, and standard, combine 20 µL of 2X TagMax™ MasterMix and 2 µL of 20X Ion TagMax™ Assay and mix thoroughly. Dispense 11 µL aliquots into the wells of a PCR plate.

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- The Ion Library TagMax™ qPCR Mix can be used on a variety of Life Technologies instruments, as listed below. The best cycling program was developed using the StepOne™ System in Fast mode.

Real-Time PCR Systems

PCR System	Stage	Temp	Time
7900 HT System	Hold	95°C	2 min
7900 HT Fast System	Hold	95°C	20 sec
7900 HT Fast System	Cycle (40 cycles)	95°C	1 sec
7900 HT Fast System	Cycle (40 cycles)	60°C	20 sec

Component

Component	Volume
Switch Solution (yellow cap)	4 µL
Diluted barcode adapter mix (for barcoded libraries) or Ion AmpliSeq™ Adapters (green cap, for non-barcoded)	2 µL
Total volume (includes 22 µL of digested amplicons)	28 µL

3. Add 2 µL of DNA Ligase to each well.

4. Seal the plate with MicroAmp® adhesive film, vortex thoroughly, and spin down to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times prior to sealing the plate.

5. Place a MicroAmp® Compression Pad on the plate, load program.

Temperature

Temperature	Time
22°C	30 min
22°C	10 min
95°C	Hold (for up to 1 hour)

STOPPING POINT Samples may be stored at -20°C.

IMPORTANT! The only Ion OneTouch™ ES Do not use reactions with the template preparation.

1. Prepare the Ion OneTouch™ ES

2. Load a new tip in the Tip Arm.

3. Place cover tip in the Tip Arm. Remove the Tip Arm from the reader and align the cover tip of the Tip Arm with the Tip. Keeping the Tip in the Tip Arm, gently press the Tip Arm down onto the cover tip until the Tip Arm meets the Tip. Hold the Tip Arm in the Tip Arm for 1 second to ensure proper installation of the tip. Lift the Tip Arm at angle.

4. Load a new tip in the Tip Arm.

5. Place cover tip in the Tip Arm. Remove the Tip Arm from the reader and align the cover tip of the Tip Arm with the Tip. Keeping the Tip in the Tip Arm, gently press the Tip Arm down onto the cover tip until the Tip Arm meets the Tip. Hold the Tip Arm in the Tip Arm for 1 second to ensure proper installation of the tip. Lift the Tip Arm at angle.

6. Prepare reagents then fill the 8-well.

7. Perform the run

8. Confirm that a new tip and opened 8.2-µL PCR tube have been loaded and that the 8-well step is correctly loaded. Ensure that Well 1 (SP sample) is the leftmost well and that the 8-well step is pushed to the far right position within the slot.

9. Pipet the contents of Well 2 up and down to resuspend the beads before starting the run. Do not introduce bubbles into the solution.

10. If necessary, turn ON the Ion OneTouch™ ES and wait for the instrument to initialize. The screen displays "Idle". The Tip Arm performs a series of initialization movements and returns to the home position (~5 seconds).

11. Press Start/Stop. The screen displays "Run" during the run. The run takes ~35 minutes.

IMPORTANT! Remove the stretched 8.2-µL PCR tube after the end of the run. Evaporation and prolonged exposure to the Multi-ON solution can cause SP and DNA damage. Do not leave the stretched 8.2-µL PCR tube in the Multi-ON solution overnight.

Notes: If necessary to stop a run, press Start/Stop. The instrument completes the current step, then stops the run and displays "Idle". Press Start/Stop again to return the Tip Arm to the home position. It is not possible to restart (resume you left off) after stopping a run.

2.2 – templating, sequencing ES - enrichment

Prepare reagents then fill the 8-well

1. Prepare Multi-ON Solution

2. Prepare Ion AmpliSeq™ Library

3. Fill the 8-well

4. Perform the run

5. Confirm that a new tip and opened 8.2-µL PCR tube have been loaded and that the 8-well step is correctly loaded. Ensure that Well 1 (SP sample) is the leftmost well and that the 8-well step is pushed to the far right position within the slot.

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Manually load chip (with foam?!)

Action 1

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

Pipette samples*

* Assume use of
multichannel pipette

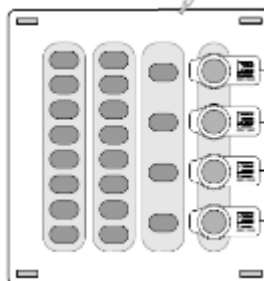
	1	2	3	4	5	6	7	8	9	10	11	12
A	●	○	○	○	○	○	○	○	○	○	○	○
B	●	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	●	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

96-well plate with IonCode™
Barcode Adapters

Action 2

Add Precision ID Panel to Reagents Cartridge

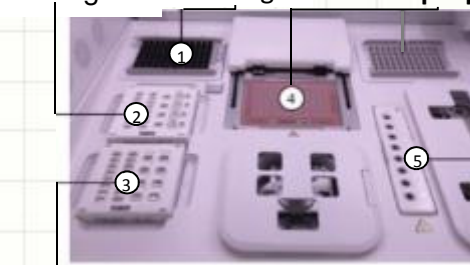
Pipette primers



Reagents Cartridge

Action 3

Snap 5 cartridge components onto Ion Chef deck

Reagents
CartridgeTips
cartridge96-well
sample plateSolutions
CartridgeEnrichment
Cartridge

~7.5 hrs

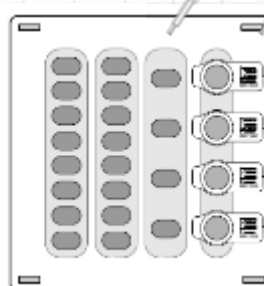
Action 1

Plan Run in Ion Torrent Browser

Action 2

Add Libraries to Reagents Cartridge

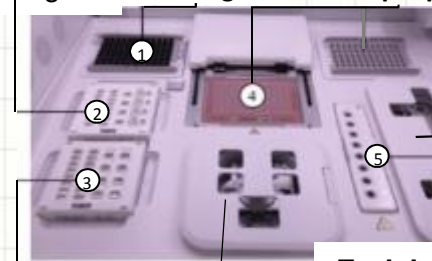
Pipette libraries



Reagents Cartridge

Action 3

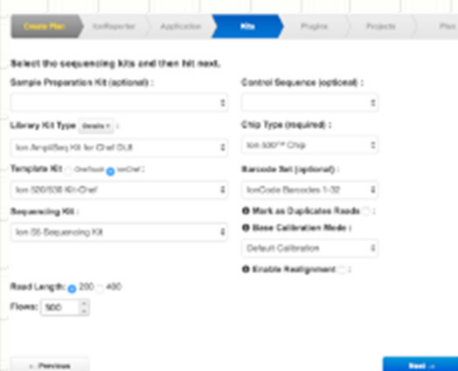
Snap cartridge components onto Ion Chef deck

Reagents
CartridgeTips
cartridge96-well
sample plateSolutions
Cartridge

Chips

Enrichment
CartridgeDip trays,
centrifuge
lids,
swinging
buckets

~13.5 hrs



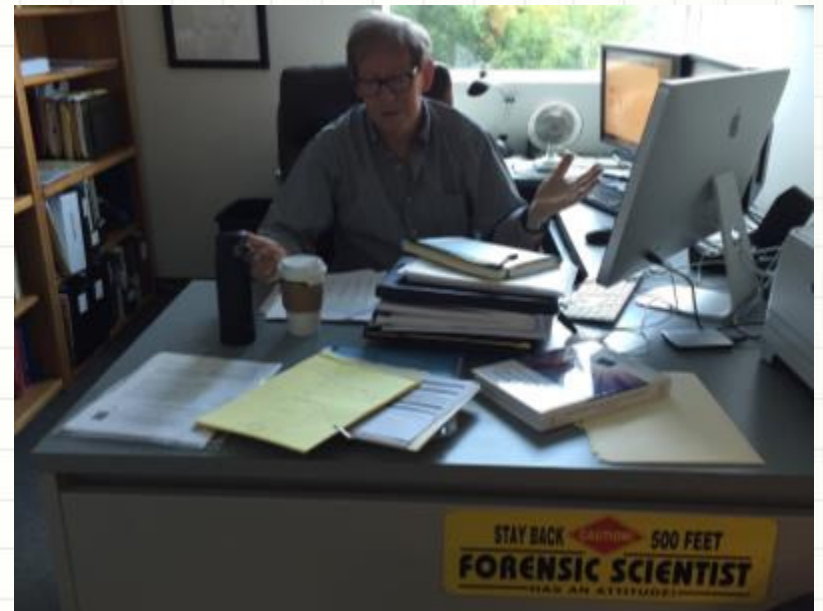
While the *Chef* is working....

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



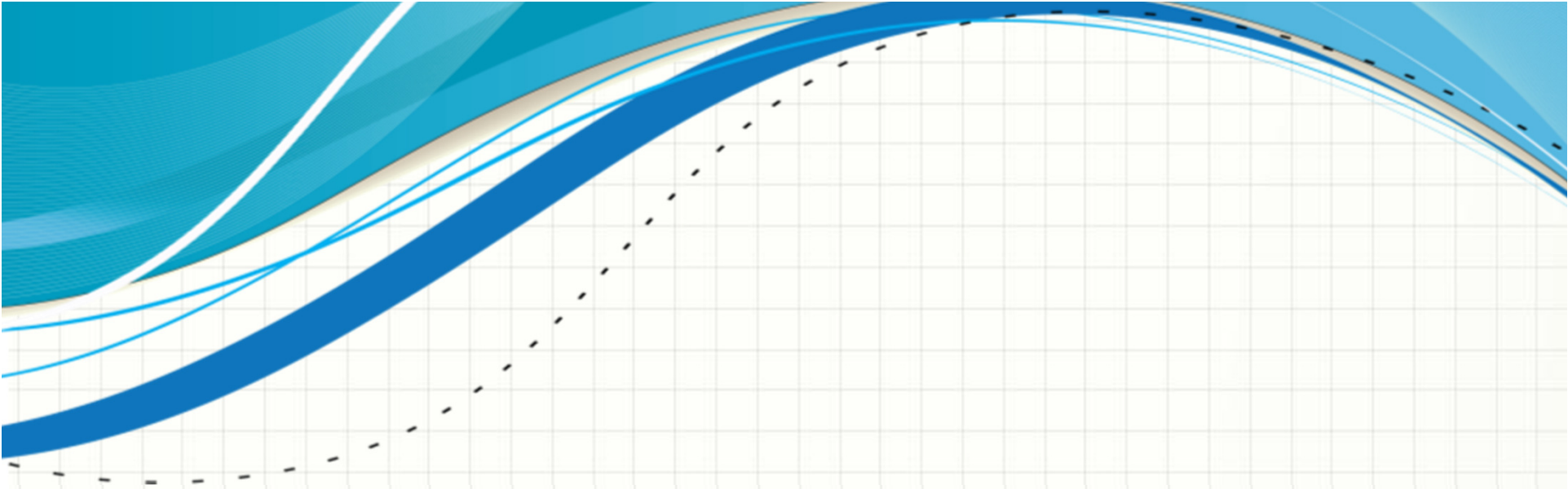
- Hands-on time in the lab
- Work piles up!!!
- No time for a break!

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

HID Genotyper Result

Run ID:	SPV21	Analysis Settings:	
Experiment Name:	R_2016_04_20_08_16_06_user_55-00280-10-GlobalFile_Mixture_ID_NGS_Panel-Concordance	Platform File:	
Results Name:	Auto_user_55-00280-10-GlobalFile_Mixture_ID_NGS_Panel-Concordance_M5	Target File:	
Notes:	TSS v5.0 / HID Genotyper v1.0 Analysis	Run Results:	
Batch ID:	Batch-1002		

Sample Data					
Sample Name	Barcode ID	Download	Upload Status	Description	
S1-M	IonCode_0101		Completed	Successful	
S2-M	IonCode_0103		Completed	Successful	
S2-F	IonCode_0102		Completed	Successful	
S4-F	IonCode_0105		Completed	Successful	
S4-F	IonCode_0104		Completed	Successful	
S3-M	IonCode_0107		Completed	Successful	
S3-M	IonCode_0106		Completed	Successful	
S1-F	IonCode_0108		Completed	Successful	

Data exported directly into CONVERGE software

Batch-1002

Batch Title:

R_2016_04_20_08_16_06_user_55-00280-10-GlobalFile_Mixture_ID_NGS_Panel-Concordance

Batch External ID:

29

Batch Type:

NGS Genotype analysis

Materials & Method:

TSS v5.0 / HID Genotyper v1.0 Analysis

Status:

In Progress

Priority:

Normal

Creation Date:

Apr 29-2016 6:19:27 PM

Last Modified Date:

Apr 27-2016 12:52:25 PM

Created By:

Eric Hanson

Batch Description:

Auto_user_55-00280-10-GlobalFile_Mixture_ID_NGS_Panel-Concordance_M5

Modified By:

Eric Hanson

Owner:

Eric Hanson

Batch Start Date:

Batch End Date:

Total Samples:

8

Ready Samples:

0

Completed Samples:

8

Incomplete Samples:

0

Batch Comments:

Sample Setup

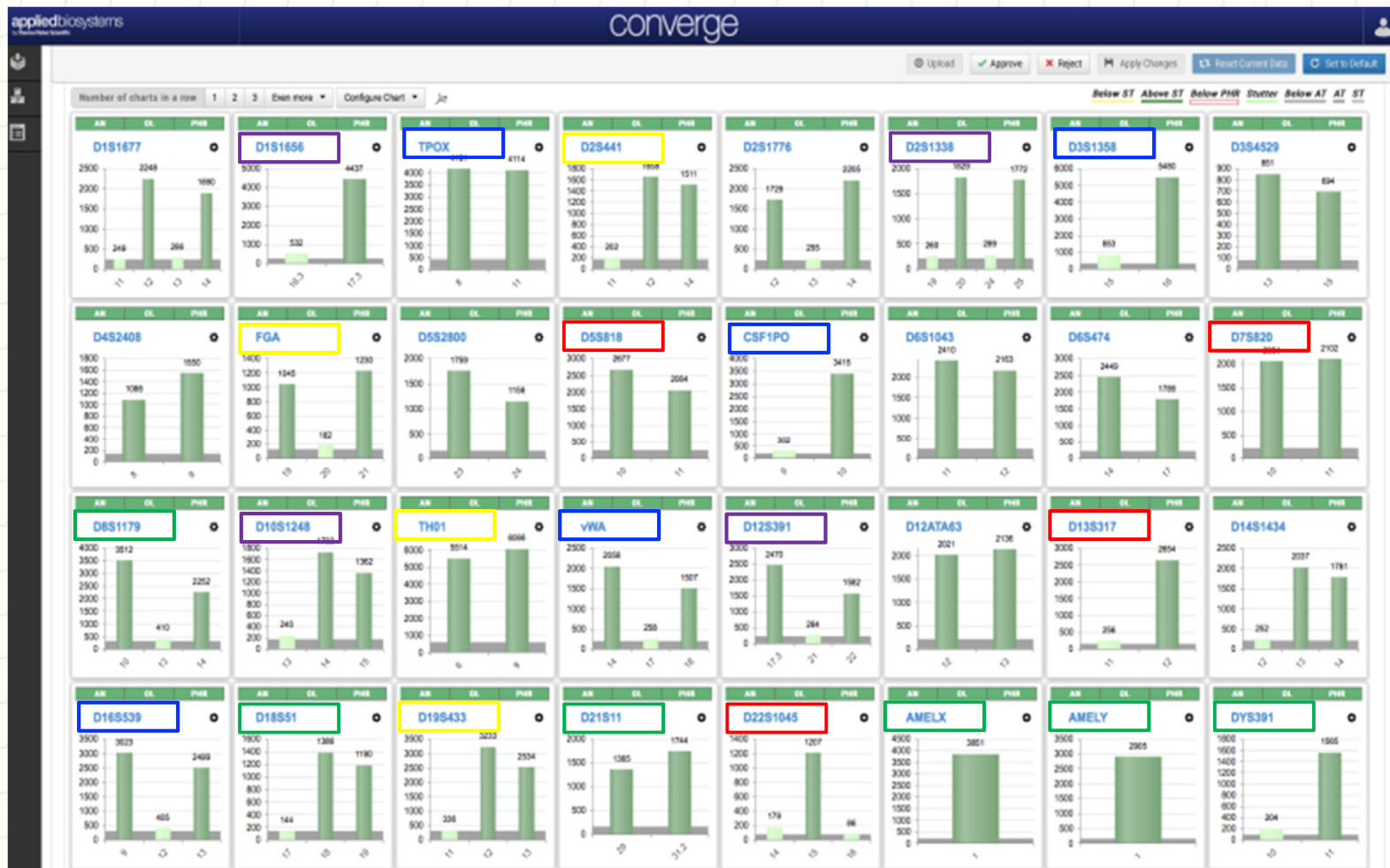
Sample ID	Sample Name	Analysis Settings	Case ID	Action
S-1028.1	IonCode_E107 : S9-M	Default Analysis Settings		
S-1028.1	IonCode_E108 : S10-M	Default Analysis Settings		
S-1022.1	IonCode_E102 : S2-F	Default Analysis Settings		
S-1023.1	IonCode_E101 : S1-M	Default Analysis Settings		
S-1024.1	IonCode_E104 : S4-F	Default Analysis Settings		
S-1025.1	IonCode_E103 : S3-M	Default Analysis Settings		
S-1026.1	IonCode_E105 : S5-F	Default Analysis Settings		
S-1027.1	IonCode_E106 : S8-M	Default Analysis Settings		

Results

Result ID	Sample Name	Sample Type	Batch Processing Status	COQ at Sample Level	COQ at Profile Level STR SNP MH	Secondary Analysis Review STR SNP MH	Action
Result-S-1028.1	IonCode_0107 : S9-M	Sample	Complete			N/A	
Result-S-1028.1	IonCode_0108 : S10-M	Sample	Complete			N/A	
Result-S-1022.1	IonCode_0102 : S2-F	Sample	Complete			N/A	
Result-S-1023.1	IonCode_0101 : S1-M	Sample	Complete			N/A	
Result-S-1024.1	IonCode_0104 : S4-F	Sample	Complete			N/A	
Result-S-1025.1	IonCode_0103 : S3-M	Sample	Complete			N/A	
Result-S-1026.1	IonCode_0105 : S5-F	Sample	Complete			N/A	

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 <i>D22S1045</i>	6637 <i>TH01</i>
	125	96,096	3314	924 <i>D22S1045</i>	6758 <i>TH01</i>
S3 Male	250	88,812	3063	651 <i>D22S1045</i>	5630 <i>TH01</i>
	125	113,218	3904	915 <i>D22S1045</i>	7200 <i>TPOX</i>
S4 Female	250	100,085	3451	731 <i>D22S1045</i>	7963 <i>TPOX</i>
	125	111,909	3859	864 <i>D22S1045</i>	9291 <i>TPOX</i>
S9 Male	250	75,510	2604	127 <i>D19S433</i>	5665 <i>TH01</i>
	125	101,648	3505	817 <i>D22S1045</i>	7243 <i>TH01</i>

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	blue
C18	Dried chewing gum	0.8	0.200	0.0670	15	1.01	117	
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

systems

converge

Upload Approve Reject Apply Changes Reset Current Data Set to Default

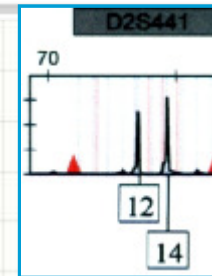
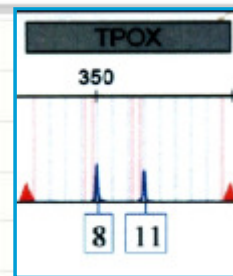
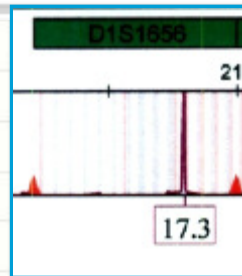


Chewing gum (~4-5 days old)

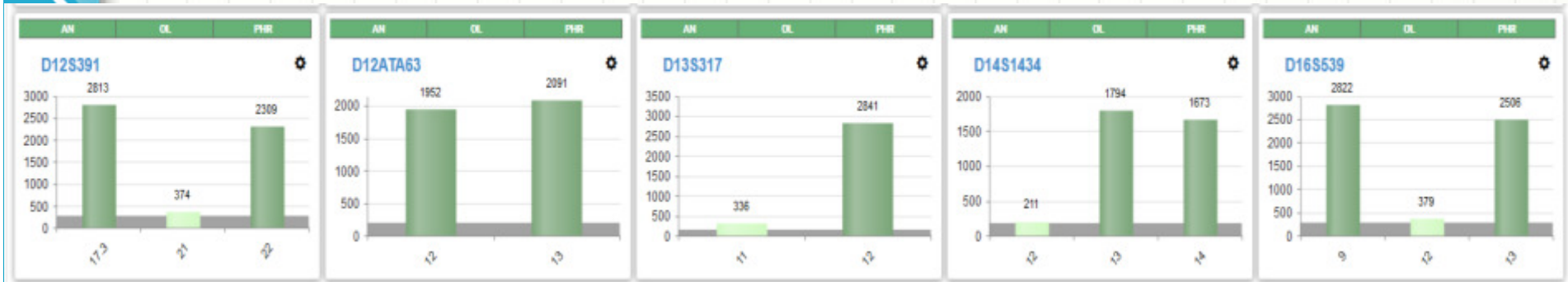
Full profiles:
STR, SNP, MH



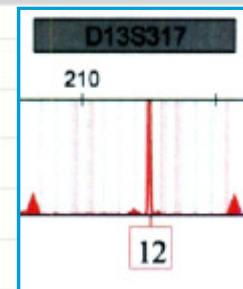
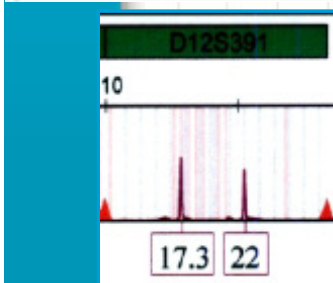
New STR



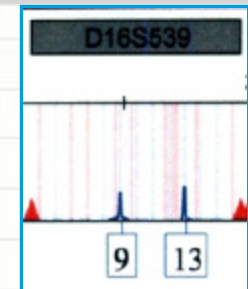
New STR



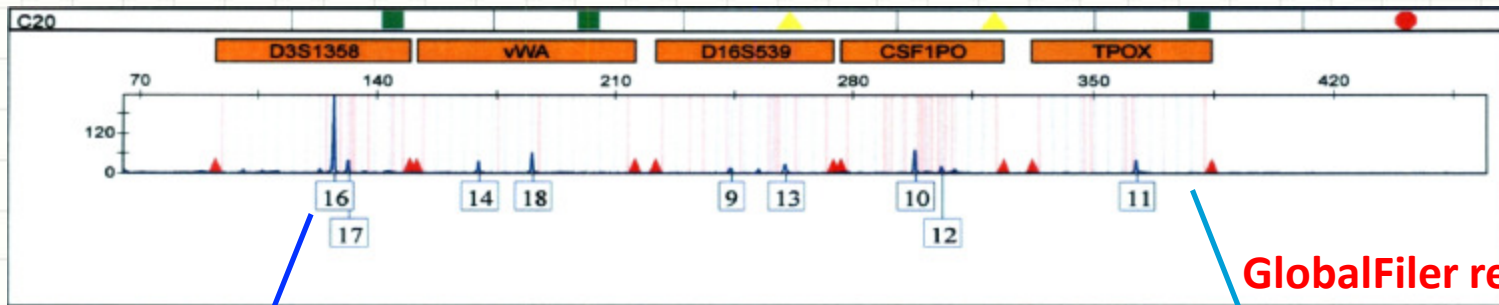
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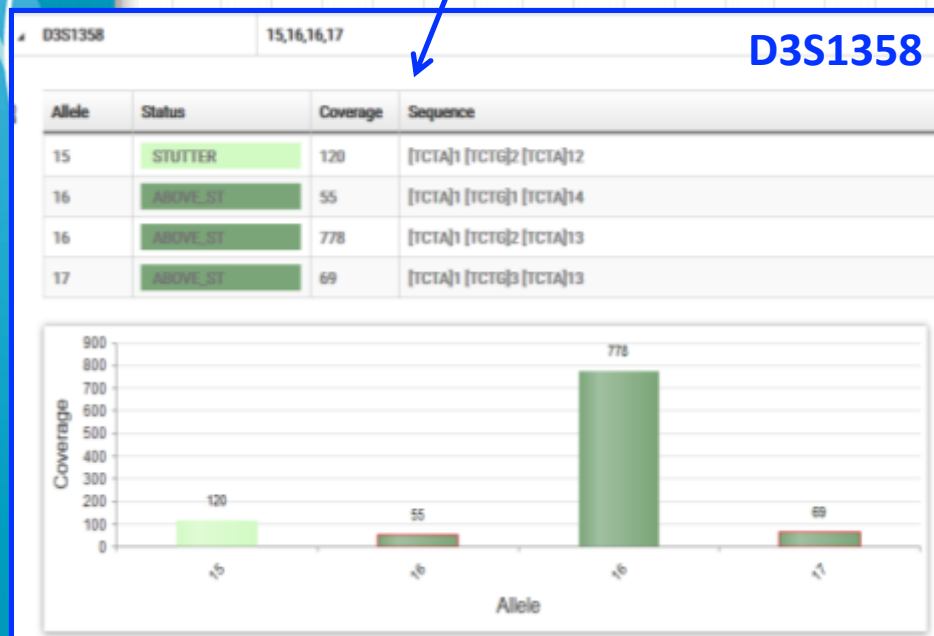
New STR



'Spit' on pavement



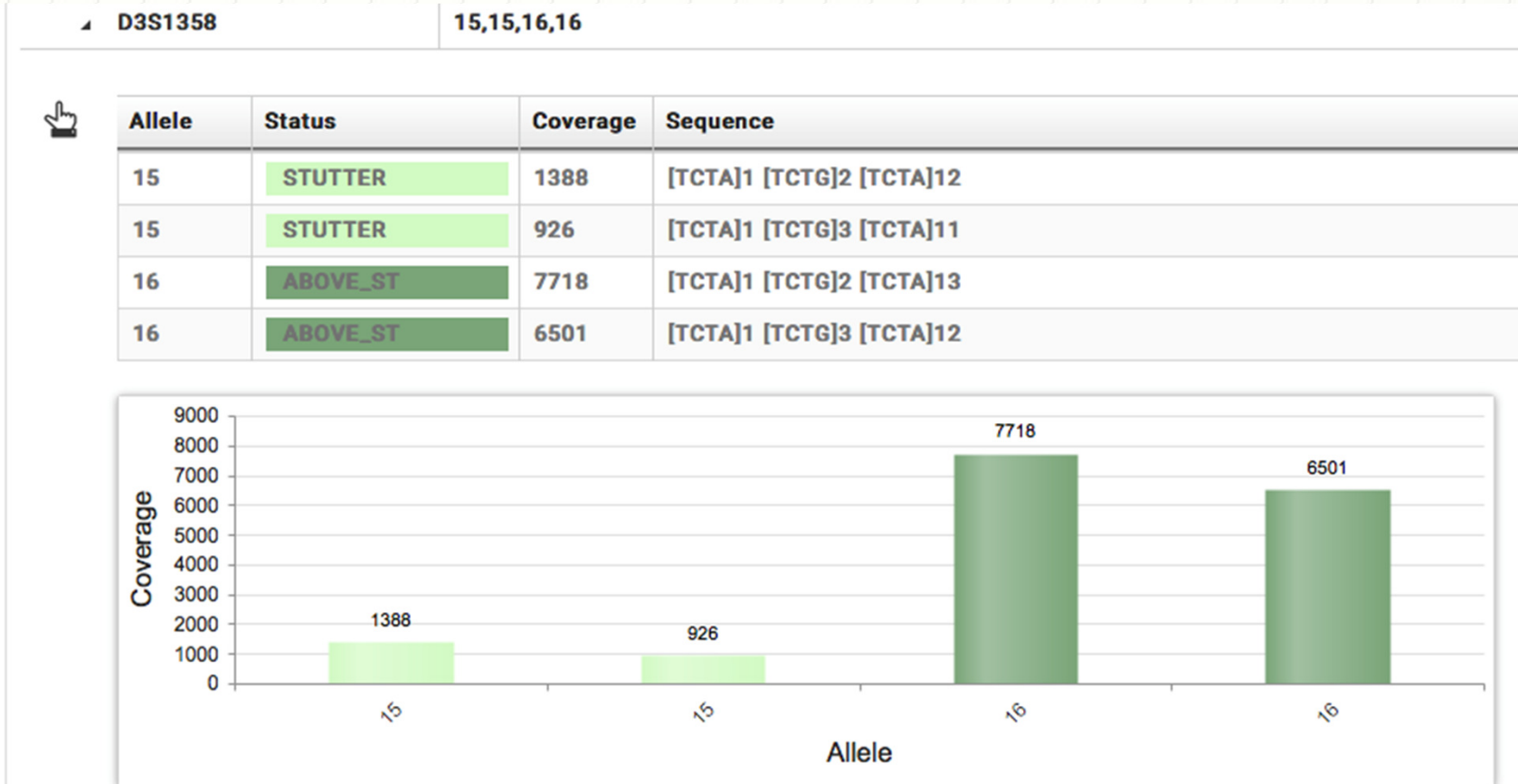
GlobalFiler results



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

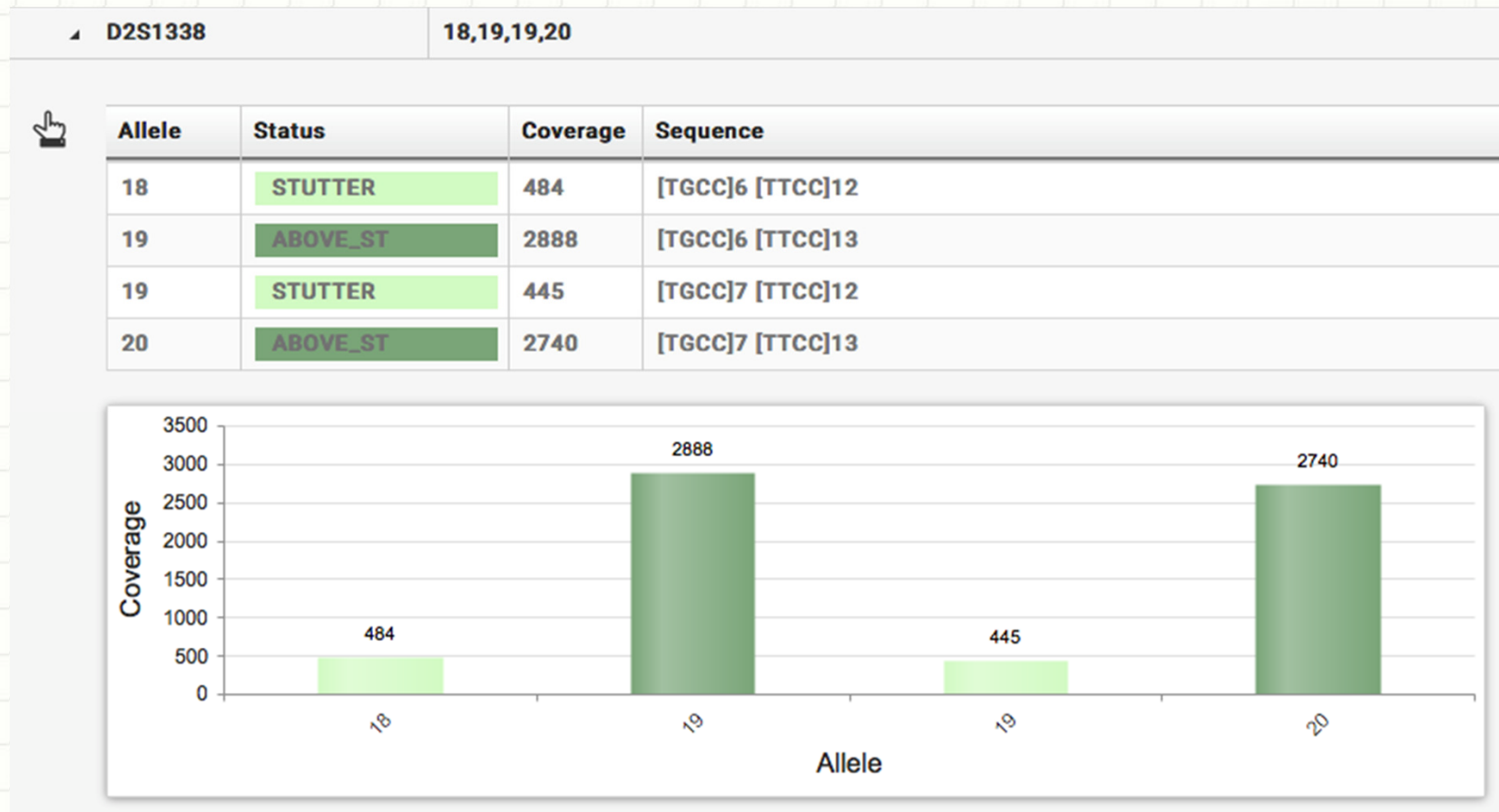
- NGS – '8' allele not detected with GlobalFiler result

Extra Discrimination – Sequence Level



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

Extra Discrimination – Sequence Level



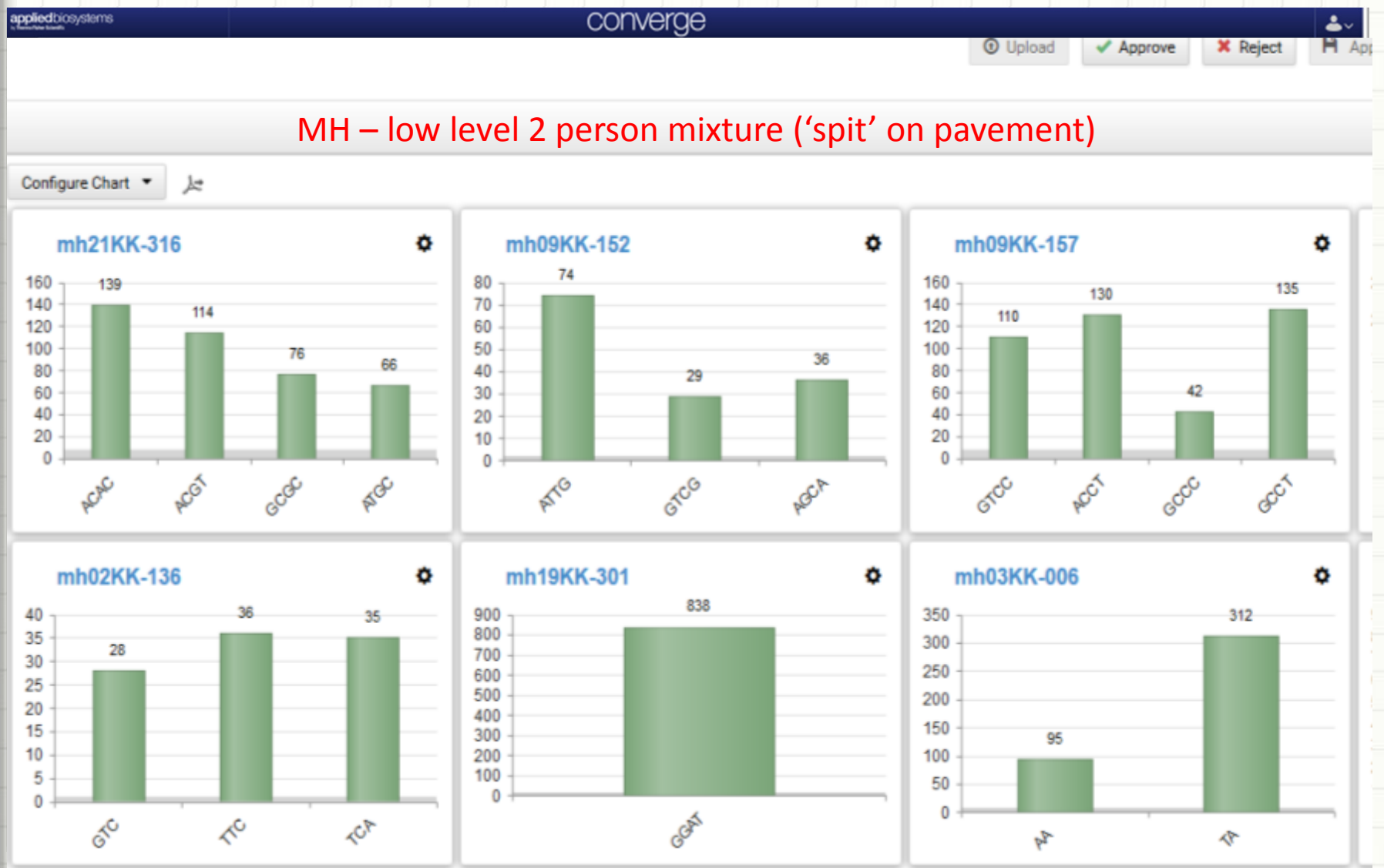
'19' stutter (from 20 allele) different
sequence than main '19' allele

Additional Identity Information - SNPs

converge									
appliedbiosystems									
Upload Approve Reject Apply Changes Save Current Data Set to Default Calculate MA									
Position	Locus	Genotype	QC	Coverage	Allele Freq	Coverage%	HIV		
chr1:105717631	rs4847034	AA	●	4529					
chr1:160786670	rs560681	AG	●	5146					
chr1:242936797	rs1413212	CC	●	4386					
chr2:114974	rs876726	CT	●	3261					
chr2:10085722	rs1109037	AG	●	4895					
chr3:32417644	rs4364205	GT	●	7843					
chr4:190318060	rs1876055	CG	●	4822					
chr5:2879395	rs717302	GG	●	6359					
chr5:174778678	rs251934	AG	●	5862					
chr5:178698725	rs336882	GG	●	6208					
chr6:12099954	rs13218443	AG	●	7537					
chr6:152897786	rs214955	TT	●	740					
chr7:4310365	rs4955448	CT	●	6418					
chr7:155990813	rs737681	CT	●	4719					
chr8:136839229	rs4286409	CC	●	5025					
chr9:1823774	rs1015250	GG	●	3602					
chr9:126881448	rs1463729	CT	●	6277					
chr9:128968063	rs1366288	CT	●	7425					
chr10:3374178	rs735155	CT	●	6900					
chr10:17193346	rs3780962	AG	●	4130					
chr10:132698419	rs964481	CT	●	6305					
chr11:115287176	rs10488719	CC	●	1742					
chr11:134647546	rs2076048	AA	●	3822					
chr12:106328254	rs2111980	CC	●	3521					
chr12:130761656	rs10773768	AG	●	6997					
chr13:20901724	rs1335873	AT	●	5267					
chr13:100338223	rs1058083	GG	●	6805					
chr14:25850832	rs1454361	TT	●	5927					
chr15:24571796	rs2016276	CT	●	4081					
chr17:2019263	rs965077	AG	●	7696					
chr17:80765788	rs2292972	CT	●	6761					
chr18:1127986	rs1489232	AA	●	1018					
chr18:9749879	rs9951171	GG	●	3171					
chr19:28463337	rs719366	GG	●	2267					
chr21:16685558	rs722098	AG	●	4570					
chr21:28608163	rs2830795	AA	●	5567					
chr21:28679687	rs2831700	AG	●	4571					

RMP Range	
RMP Range: 9.43907e-23 to 5.7380845e-18	
More	جزء
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?

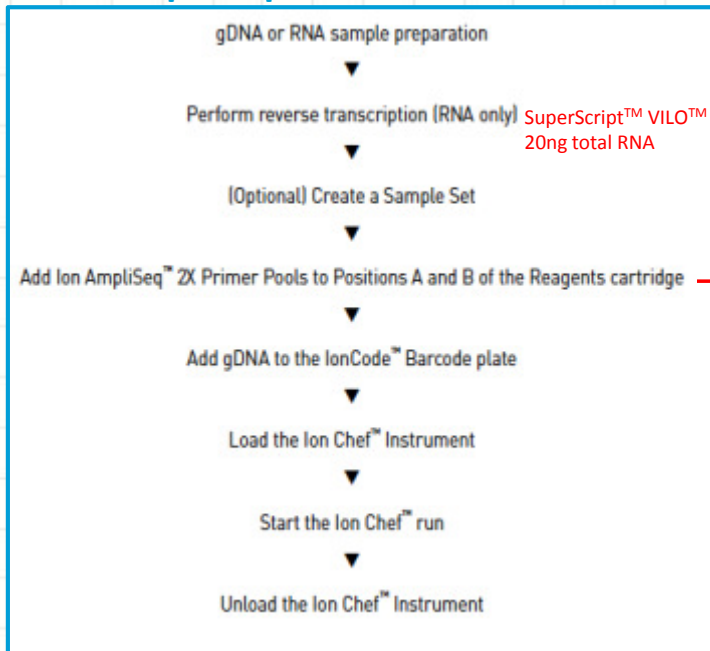
activity = what?

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)?

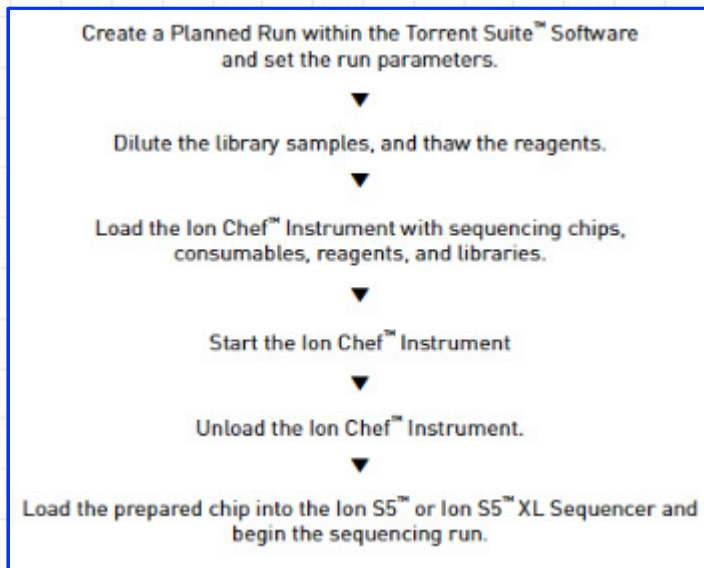
Methods

Ion AmpliSeq™ Kit for Chef DL8 Kit



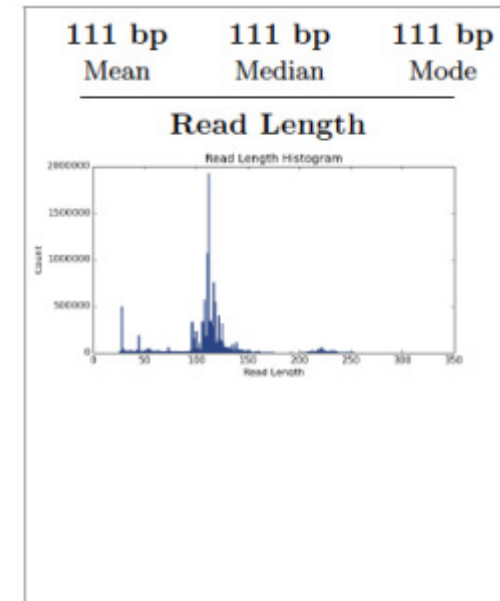
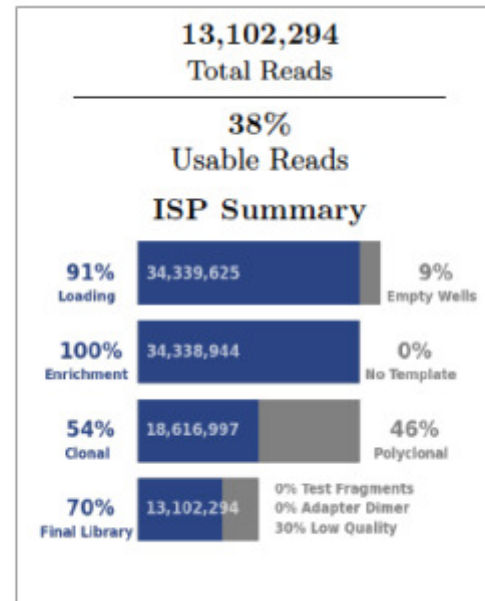
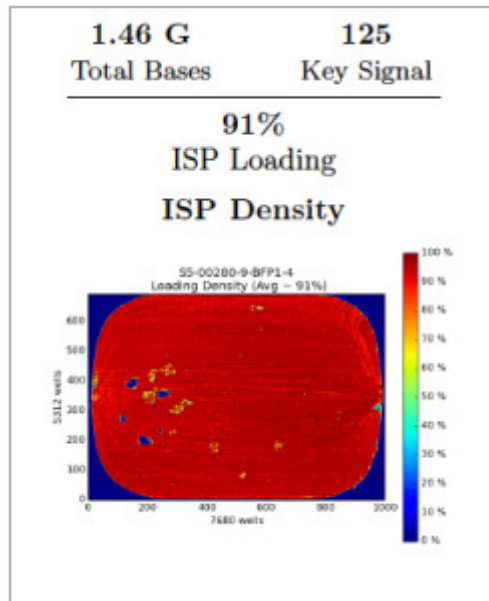
Custom Ion
AmpliSeq™
Primer Pool

Ion 520/530 Kit-Chef



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		

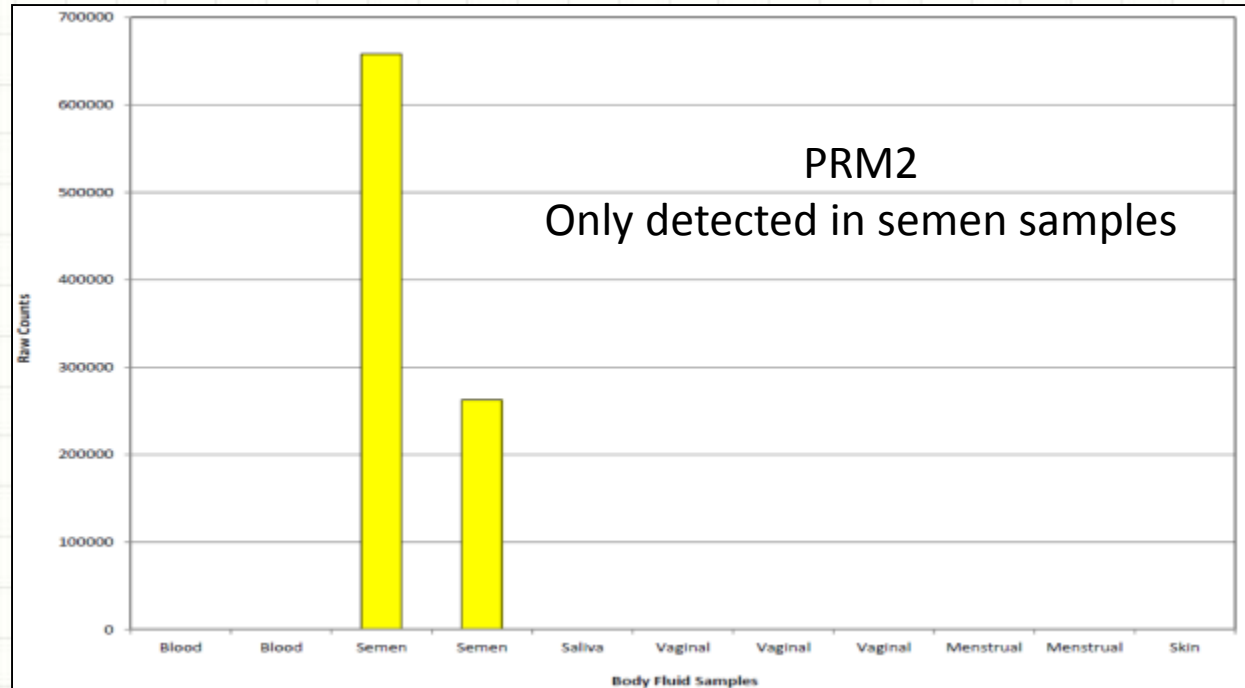
Run Data – Ion Chef™ System- Ion S5™ System



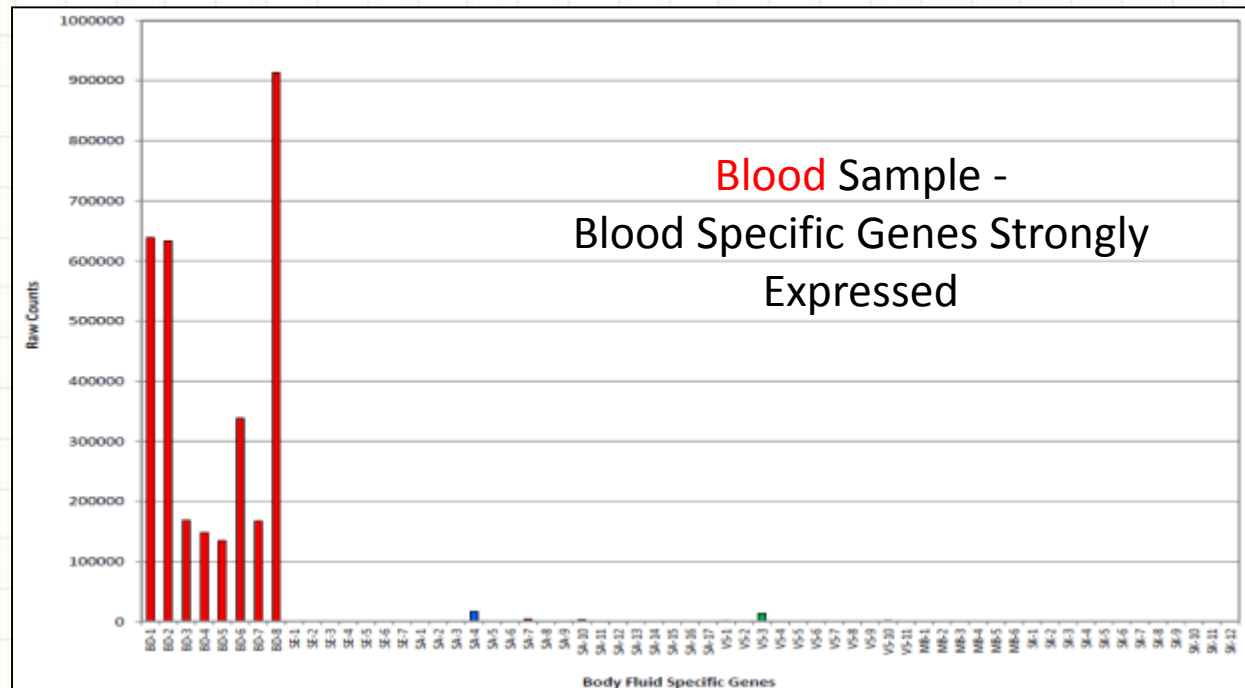
Barcode Name	Sample	Bases	$\geq Q20$	Reads	Mean Read Length
No barcode	none	60,999,878	51,458,510	526,263	116 bp
IonCode_0125	b7477	190,686,921	173,885,771	1,652,575	115 bp
IonCode_0126	SE22	226,174,742	205,968,784	2,033,923	111 bp
IonCode_0127	se19	110,666,506	101,744,622	1,083,866	102 bp
IonCode_0128	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

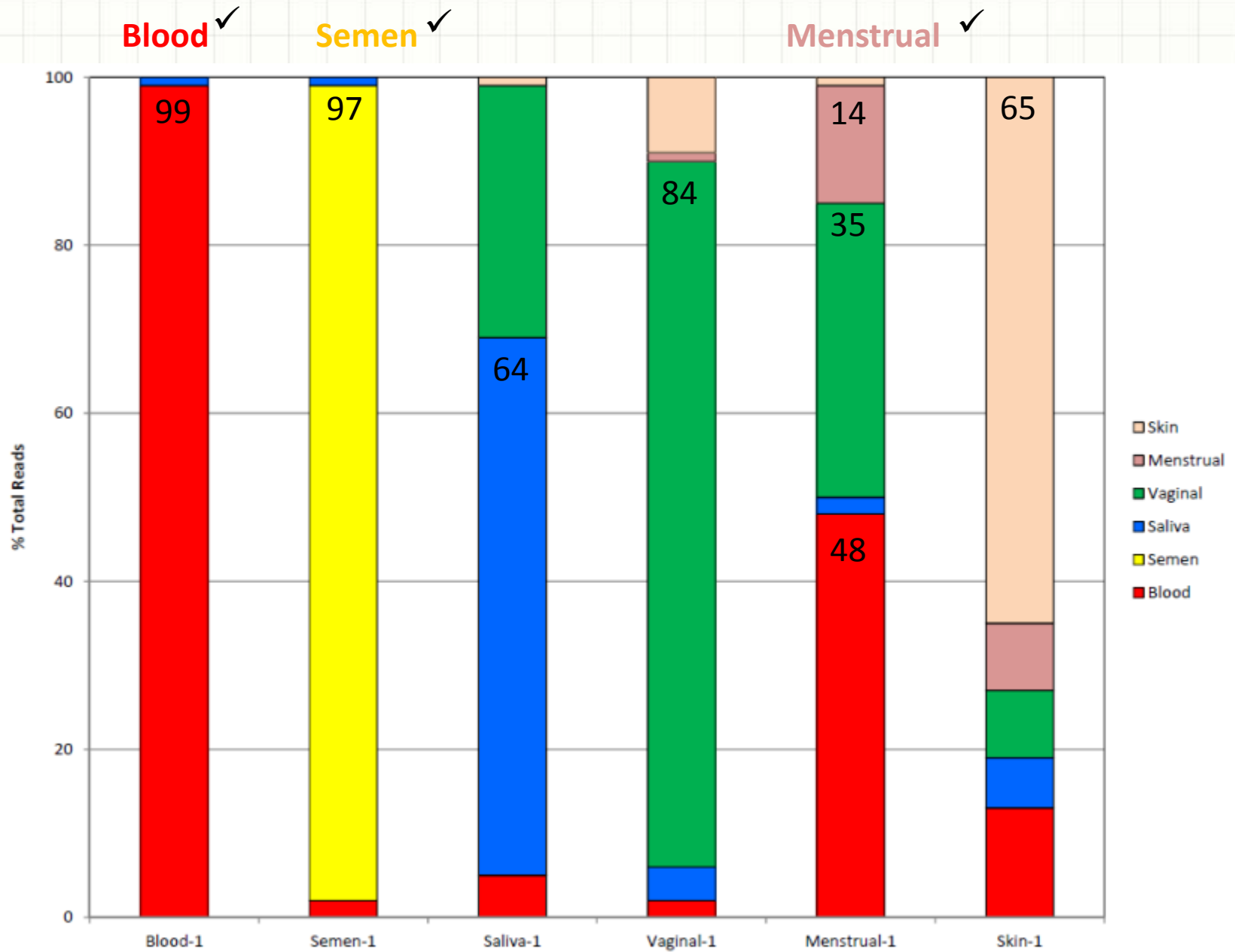
Specificity

By
Gene



By
Sample





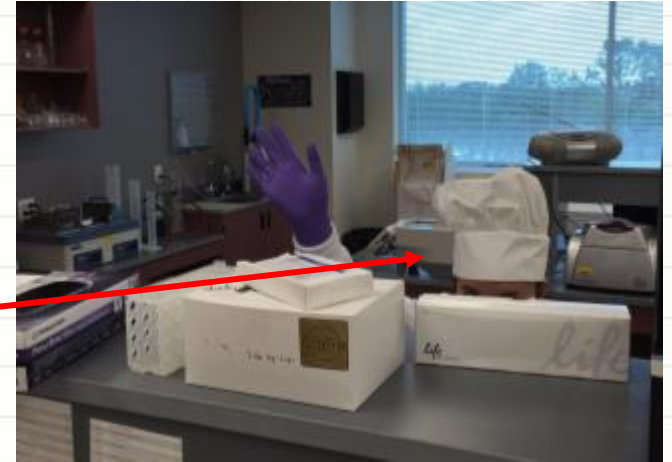
Saliva: ~64%, Vaginal: ~63-84%, Skin: ~65% → genes removed in new primer pool to improve specificity

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





*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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Speaker was provided travel and hotel support by Thermo Fisher Scientific for this presentation, but no remuneration