SNP Information Values for NIST SRM 2391c

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Over the past decade, panels of single nucleotide polymorphism (SNP) markers have been developed for forensic applications such as estimating biogeographical ancestry[1,2], phenotype predictions[4], as well as one to one matching[5,6]. However, several barriers to implementation have existed, including: 1) low-throughput genotyping methodologies, 2) unfamiliarity with interpreting ancestry/phenotype predictions, and 3) existence of large databases containing STR profiles. Next generation sequencing (NGS) technologies address the first barrier by enabling co-analysis of hundreds of forensically-relevant SNPs, in stand-alone assays or alongside STR loci. To support the implementation of SNPs in forensic DNA analysis, NIST has examined the single-source components of NIST SRM 2391c PCR-based DNA Profiling Standard using the HID-Ion Ampliseq Identity Panel (now marketed as Precision ID Identity Panel) and the HID-Ion Ampliseq Ancestry Panel (now marketed as the Precision ID Ancestry Panel) on the Ion Torrent Personal Genome Machine (PGM) and the Illumina ForenSeq DNA Signature kit on the Illumina MiSeq FGx. In total, over 300 SNP markers were typed with 43 % of loci being represented in more than one multiplex. Genotype data has been evaluated for concordance across replicates and/or platforms, and will be released as "information" values supplemental to the 2391c Certificate of Analysis. This analysis serves as a model for inclusion of forensically-relevant SNP genotypes in future versions of the 2391 series reference material.

Next Generation Sequencing Platforms

Two platforms with commercial SNP genotyping panels intended for forensic DNA analysis were used:

Credit: www.lifetechnologies.com

Ion Torrent PGM

HID-Ion AmpliSeq Identity Panel
90 Identity SNPs [5,6]
34 Lineage SNPs [3]

HID-Ion AmpliSeq Ancestry Panel
165 Ancestry SNPs [1,2]

Credit: www.illumina.com

Illumina MiSeq FGx

ForenSeq Signature Assay Kit Primer Mix B

94 Identity SNPs [5,6]

56 Ancestry SNPs [2]

22 Phenotype SNPs [4]

Five single-source components of NIST SRM 2391c (two female and three male) were sequenced as follows:

PGM:
- HID-Ion AmpliSeq Identity & Ancestry Panels
- DNA input ≈ 1 ng
- Three replicate amplifications per sample
- Standard library protocol for PGM HID SNP Panels
- ISP templating & chip loading performed on Ion Chef
- 30 samples + negative control in barcoded library pool
- Sequenced each panel on one Ion 318 Chip

MiSeq FGx:
- ForenSeq Primer Mix B
- DNA input ≈ 1 ng
- Two replicate amplifications per sample
- Standard library protocol for ForenSeq
- 16 samples + negative control in barcoded library pool
- Sequenced on one FGx run

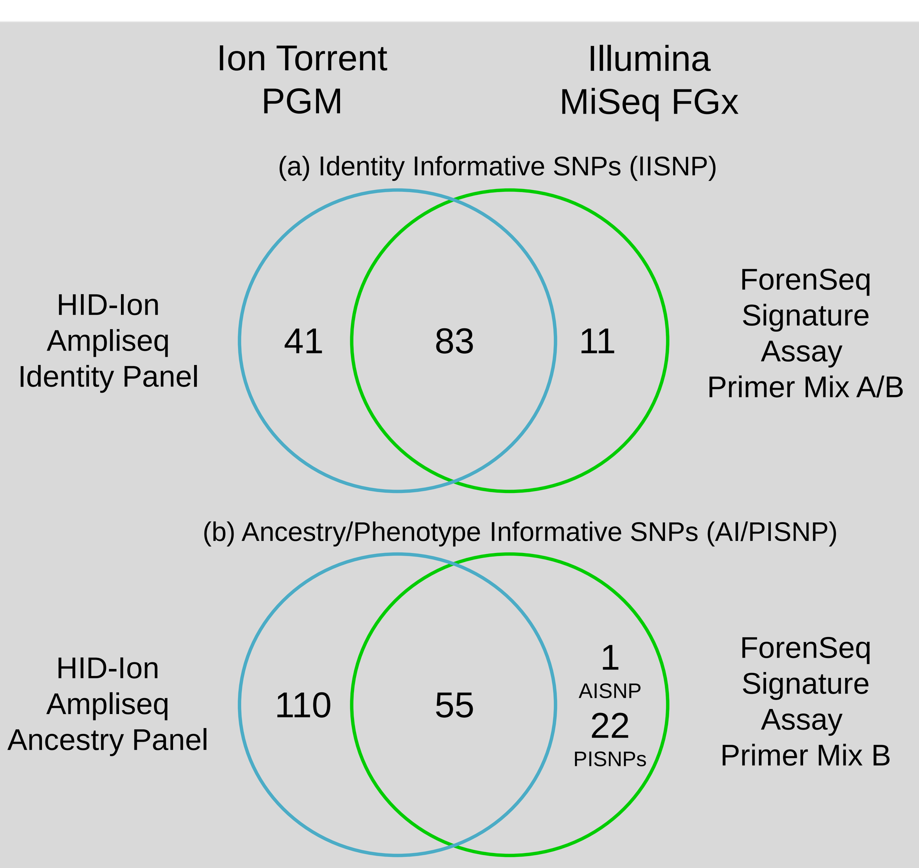

Figure 1: Venn diagrams of the SNP loci in HID-Ion AmpliSeq and Illumina ForenSeq multiplexes with (a) overlapping identity informative markers (IISNP) and (b) ancestry/phenotype informative markers (AISNP/PISNP). There are 139 loci in common (out of a total 323) between the two platforms.

NIST Data Quality Descriptors

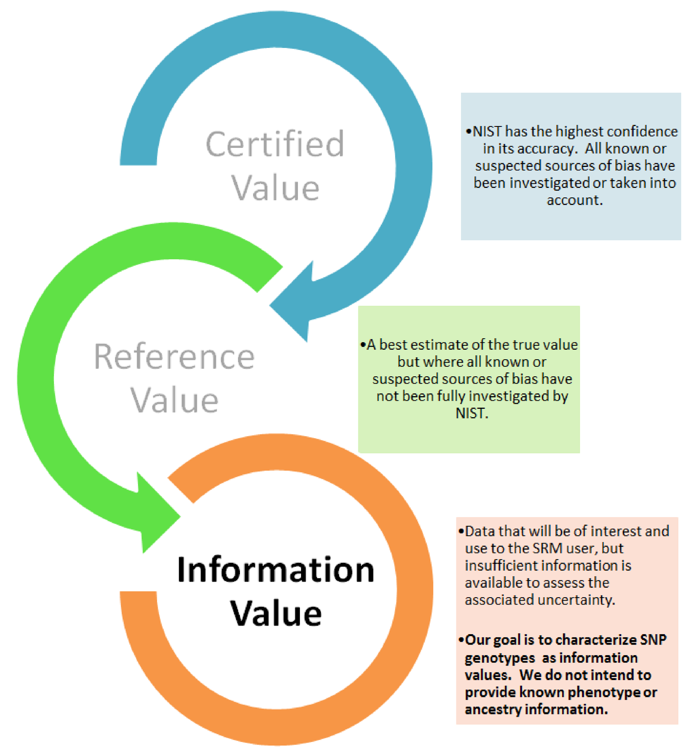

Table 1a. Information values for SRM 2391c components A, B, C, E, and F, with average coverage and coefficient of variance indicators.

SNP	Chr	SNP Category[a]	ForenSeq Primer Mix B on MiSeq FGx: 2391c Component A	2391c Component B	2391c Component C	2391c Component E	2391c Component F	Average Coverage	CV	HID-Ion AmpliSeq Identity Panel on PGM: 2391c Component A	2391c Component B	2391c Component C	2391c Component E	2391c Component F	Average Coverage	CV
rs1294331 ǂ	1	I	GG	GG	GG	AG	GG	pink	black							
rs13182883	5	I	AA	AA	AG	AG	AG	grey	black							
rs1336071 ǂ	6	I	AA	AG	AG	GG	GG	pink	black							
rs2107612	12	I	AA	GG	AG	AA	AG	black	black							
rs2399332 ǂ	3	I	AC	AA	AC	AC	CC	grey	black							
rs279844 *	4	I	AA	TT	AA	AT	AT	grey	black							
rs2920816 ǂ	12	I	CT	CT	TT	TT	CT	pink	black							
rs4606077	8	I	CC	CT	CC	CC	CC	grey	grey							
rs763869 ǂ	8	I	CT	CT	CC	CT	CT	black	black							
rs8037429	15	I	CT	CC	CC	CC	CT	black	pink							
rs8078417	17	I	CC	TT	CC	CT	CC	black	black							
rs1005533	20	I	GG	GG	GG	AA	AA	black	black	GG	GG	GG	AA	AA	black	black
rs10092491	8	I	CT	TT	CC	CT	CC	grey	black	CT	TT	CC	CT	CC	black	black
rs1015250	9	I	GG	GG	GG	GG	GG	grey	black	GG	GG	GG	GG	GG	grey	black
rs1024116 ǂ	18	I	AG	AA	GG	AG	AA	black	black	CT	TT	CC	CT	TT	black	black
rs1028528	22	I	GG	AA	AG	AA	AG	black	pink	GG	AA	AG	AA	AG	black	black
rs1031825	20	I	AC	AC	CC	CC	AA	red	black	AC	AC	CC	CC	AA	black	black
rs10488710 ǂ*	11	I	GG	CC	CG	CG	GG	pink	black	CC	GG	CG	CG	CC	grey	black
rs10495407	1	I	GG	AA	AG	GG	AA	black	black	GG	AA	AG	GG	AA	black	black
rs1058083	13	I	GG	AG	GG	GG	GG	black	pink	GG	AG	GG	GG	GG	black	black
rs10773760	12	I	AG	AG	AG	GG	AG	black	grey	AG	AG	AG	GG	AG	black	black
rs10776839	9	I	GG	GG	GG	GT	GT	black	grey	GG	GG	GG	GT	GT	grey	black
rs1109037	2	I	AG	GG	GG	AA	GG	black	black	AG	GG	GG	AA	GG	black	grey
rs12997453	2	I	AG	AG	GG	GG	GG	grey	black	AG	AG	GG	GG	GG	grey	black
rs13218440	6	I	AG	GG	AG	GG	GG	grey	black	AG	GG	AG	GG	GG	black	black
rs1335873 ǂ*	13	I	AT	AA	TT	TT	TT	black	grey	AT	TT	AA	AA	AA	black	black
rs1355366 ǂ	3	I	AA	AA	AA	AG	AG	grey	black	TT	TT	TT	CT	CT	black	black
rs1357617 ǂ*	3	I	AA	AA	AA	TT	AT	grey	black	TT	TT	TT	AA	AT	black	black
rs1360288	9	I	CT	CC	CC	TT	CT	black	black	CT	CC	CC	TT	CT	black	black
rs1382387 ǂ	16	I	GG	TT	TT	TT	TT	black	grey	CC	AA	AA	AA	AA	black	black
rs1413212 ǂ	1	I	GG	GG	GG	GG	GG	grey	pink	CC	CC	CC	CC	CC	black	black
rs1454361 ǂ*	14	I	AT	AA	TT	AA	TT	black	black	AT	TT	AA	TT	AA	black	black
rs1463729 ǂ	9	I	AA	AA	GG	AG	AG	black	pink	TT	TT	CC	CT	CT	black	black
rs1490413	1	I	AA	AG	GG	AG	GG	black	grey	AA	AG	GG	AG	GG	black	black
rs1493232	18	I	AA	CA	CC	AA	AC	grey	grey	AA	AC	CC	AA	AC	black	black
rs1498553	11	I	CC	CC	CT	CT	CT	grey	grey	CC	CC	CT	CT	CT	black	black
rs1523537	20	I	TT	CT	CC	CC	TT	black	black	TT	CT	CC	CC	TT	black	black
rs1528460	15	I	TT	CT	CT	TT	CT	pink	black	TT	CT	CT	TT	CT	black	black
rs159606	5	I	AA	AG	GG	AA	AG	grey	black	AA	AG	GG	AA	AG	black	black
rs1736442 ǂ	18	I	AG	AG	GG	GG	AA	red	black	CT	CT	CC	CC	TT	black	black
rs1821380 ǂ*	15	I	CG	CC	CC	CG	CG	black	black	CG	GG	GG	CG	CG	black	black
rs1886510 ǂ	13	I	CT	CC	CC	CT	CT	black	black	AG	GG	GG	AG	AG	black	black
rs1979255 ǂ*	4	I	CG	CC	CG	CG	CG	black	grey	CG	GG	CG	CG	CG	black	black
rs2040411	22	I	AG	AA	AG	AG	AA	black	pink	AG	AA	AG	AG	AA	black	black
rs2046361 ǂ*	4	I	AT	AT	AT	AT	AA	black	black	AT	AT	AT	AT	TT	black	black
rs2056277	8	I	CC	CC	CC	CT	CC	grey	grey	CC	CC	CC	CT	CC	black	black
rs2076848 ǂ*	11	I	AA	TT	AT	TT	AT	grey	black	TT	AA	AT	AA	AT	black	black
rs2111980 ǂ	12	I	AA	AG	AA	AG	AG	black	grey	TT	CT	TT	CT	CT	black	black
rs214955 ǂ	6	I	GG	AG	AG	GG	GG	grey	black	CC	CT	CT	CC	CC	black	grey
rs221956	21	I	CT	TT	CT	CC	CT	black	grey	CT	TT	CT	CC	CT	black	black
rs2269355 *	12	I	CG	GG	GG	CC	CG	black	pink	CG	GG	GG	CC	CG	black	black
rs2342747	16	I	AG	GG	AA	AA	GG	grey	grey	AG	GG	AA	AA	GG	grey	black
rs251934 ǂ	5	I	TT	TT	CT	CT	CT	black	black	AA	AA	AG	AG	AG	black	black
rs2830795	21	I	AA	GG	AA	AA	AG	grey	black	AA	GG	AA	AA	AG	black	black
rs2831700	21	I	AG	AG	AG	AA	AA	grey	grey	AG	AG	AG	AA	AA	black	black
rs321198	7	I	CT	CC	CT	TT	CT	grey	black	CT	CC	CT	TT	CT	black	black
rs338882 ǂ	5	I	TT	CC	CT	CT	CT	grey	black	AA	GG	AG	AG	AG	black	black
rs354439 ǂ*	13	I	AT	AT	AA	TT	AT	grey	black	AT	AT	TT	AA	AT	black	black
rs3780962 ǂ	10	I	CT	CT	CT	CT	CT	grey	grey	AG	AG	AG	AG	AG	black	black
rs430046	16	I	CT	CT	CC	TT	CT	black	black	CT	CT	CC	TT	CT	black	black
rs4364205	3	I	GT	GT	GG	GG	TT	black	black	GT	GT	GG	GG	TT	black	black
rs445251 ǂ*	20	I	CG	GG	CC	CC	CG	grey	black	CG	CC	GG	GG	CG	black	black
rs4530059	14	I	GG	AG	AA	GG	GG	black	black	GG	AG	AA	GG	GG	black	grey
rs560681	1	I	AG	AA	GG	AA	GG	black	black	AG	AA	GG	AA	GG	black	black
rs576261	19	I	AC	AC	AC	AA	AA	black	pink	AC	AC	AC	AA	AA	black	grey
rs6444724	3	I	CT	CT	CT	CC	CT	black	black	CT	CT	CT	CC	CT	black	black
rs6811238	4	I	TT	GT	GG	GT	GG	grey	black	TT	GT	GG	GT	GG	black	black
rs6955448	7	I	CC	CC	CC	CT	CT	black	black	CC	CC	CC	CT	CT	black	black
rs7041158	9	I	CC	CC	CC	CC	CT	pink	black	CC	CC	CC	CC	CT	black	black
rs717302	5	I	AG	AA	AA	GG	GG	black	black	AG	AA	AA	GG	GG	black	black
rs719366 ǂ	19	I	TT	CT	CT	TT	CT	pink	black	AA	AG	AG	AA	AG	black	black
rs722098	21	I	AA	AA	AG	AA	AG	black	black	AA	AA	AG	AA	AG	black	black
rs722290 ǂ*	14	I	CG	GG	CC	CG	GG	black	black	CG	CC	GG	CG	CC	black	black
rs727811 ǂ	6	I	CC	CC	AA	AC	AA	grey	black	GG	GG	TT	GT	TT	black	black
rs729172 ǂ	16	I	AC	AA	CC	CC	AC	grey	grey	GT	TT	GG	GG	GT	black	black
rs733164	22	I	AG	GG	GG	AG	AG	black	black	AG	GG	GG	AG	AG	black	black
rs735155 ǂ	10	I	AG	GG	AG	GG	GG	black	black	CT	CC	CT	CC	CC	black	black
rs737681	7	I	CT	CT	CC	CT	CT	black	grey	CT	CT	CC	CT	CT	black	black
rs740598	10	I	AG	AA	AA	AA	GG	grey	black	AG	AA	AA	AA	GG	black	black
rs740910	17	I	AA	AG	AA	AG	AG	black	black	AA	AG	AA	AG	AG	black	black
rs826472	10	I	CC	CT	CC	CT	CT	grey	black	CC	CT	CC	CT	CT	black	black
rs873196	14	I	CT	TT	CT	CT	CT	black	grey	CT	TT	CT	CT	CT	black	grey
rs876724	2	I	CT	CT	CC	CT	CC	black	black	CT	CT	CC	CT	CC	black	black
rs891700	1	I	AG	AA	AA	AG	AG	black	black	AG	AA	AA	AG	AG	black	black
rs901398	11	I	TT	TT	CT	TT	CC	grey	grey	TT	TT	CT	TT	CC	black	black
rs907100 *	2	I	CG	CG	CG	CG	CC	grey	black	CG	CG	CG	CG	CC	black	black
rs914165	21	I	AA	GG	AG	GG	GG	grey	black	AA	GG	AG	GG	GG	black	black
rs917118	7	I	CT	CT	CC	CC	CC	black	grey	CT	CT	CC	CC	CC	black	black
rs938283	17	I	TT	CT	TT	TT	TT	black	grey	TT	CT	TT	TT	TT	black	black
rs964681	10	I	CT	TT	CT	TT	TT	black	grey	CT	TT	CT	TT	TT	black	black
rs987640 *	22	I	AT	TT	AT	TT	AA	black	black	AT	TT	AT	TT	AA	black	black
rs9905977	17	I	AG	GG	AG	GG	AG	black	black	AG	GG	AG	GG	AG	black	black
rs993934 ǂ	2	I	CC	CT	TT	TT	CT	black	black	GG	AG	AA	AA	AG	grey	black
rs9951171	18	I	AG	GG	GG	AG	AG	black	black	AG	GG	GG	AG	AG	black	black
rs1872575	3	I								AG	GG	AG	AG	AA	black	black
rs2016276	15	I								TT	TT	TT	TT	TT	black	black
rs2292972	17	I								CC	CT	TT	CT	CT	black	grey
rs4288409	8	I								AA	AC	AC	CC	AA	black	black
rs4847034	1	I								GG	GG	AG	AG	GG	black	black
rs7520386	1	I								AG	AG	GG	AG	GG	black	grey
rs7704770	5	I								AG	GG	GG	AG	AG	black	black
L298	Y	L								-	T	T	-	T	grey	black
M479	Y	L								-	C	C	-	C	grey	black
P202	Y	L								-	T	T	-	T	grey	black
P256	Y	L								-	G	G	-	G	grey	black
rs13447443	Y	L								-	A	A	-	A	black	black
rs16980426	Y	L								-	T	T	-	T	black	black
rs16981290	Y	L								-	C	C	-	C	grey	black
rs17222573	Y	L								-	A	A	-	A	black	black
rs17250845	Y	L								-	C	G	-	G	grey	black
rs17269816	Y	L								-	C	C	-	C	grey	black
rs17306671	Y	L								-	A	T	-	A	black	black
rs17842518	Y	L								-	G	G	-	G	black	black
rs20320	Y	L								-	G	G	-	G	grey	black
rs2032595	Y	L								-	T	T	-	T	grey	black
rs2032599	Y	L								-	T	T	-	T	grey	black
rs2032602	Y	L								-	T	T	-	T	grey	black
rs2032624	Y	L								-	A	A	-	A	grey	black
rs2032631	Y	L								-	G	G	-	G	grey	black
rs2032636	Y	L								-	G	G	-	G	grey	black
rs2032652	Y	L								-	T	T	-	T	black	black
rs2032658	Y	L								-	A	A	-	A	grey	black
rs2032673	Y	L								-	T	T	-	T	grey	black
rs2033003	Y	L								-	A	C	-	A	grey	black
rs2319818	Y	L								-	G	G	-	A	grey	black
rs2534636	Y	L								-	C	C	-	C	grey	black
rs35284970	Y	L								-	C	C	-	C	grey	black
rs3848982	Y	L								-	C	C	-	C	grey	black
rs3900	Y	L								-	C	G	-	C	black	black
rs3911	Y	L								-	A	A	-	A	grey	black
rs4141886	Y	L								-	A	A	-	A	grey	black
rs8179021	Y	L								-	C	C	-	C	grey	black
rs9341278	Y	L								-	G	G	-	G	grey	black
rs9786139	Y	L								-	G	G	-	G	grey	black
rs9786184	Y	L								-	C	C	-	C	black	black

Legend

	Average Coverage	CV
black	>1000X	<25%
grey	300-999X	25-49%
pink	100-299X	50-74%
red	<100X	>75%

ǂ Locus reported on reverse strand in ForenSeq
* Locus is CG or AT SNP
[a] SNP Categories: I=IISNP, A=AISNP, L=LISNP, P=PISNP

Table 1b. Information values for SRM 2391c components A, B, C, E, and F, with average coverage and coefficient of variance indicators.

SNP	Chromosome	SNP Category[a]	ForenSeq Primer Mix B on MiSeq FGx: 2391c Component A	2391c Component B	2391c Component C	2391c Component E	2391c Component F	Average	CV	HID-Ion AmpliSeq Ancestry Panel on PGM: 2391c Component A	2391c Component B	2391c Component C	2391c Component E	2391c Component F	Average Coverage	CV
rs1042602	11	P	AA	CC	CC	CC	AA	black	black							
rs1110400	16	P	TT	TT	TT	TT	TT	black	grey							
rs11547464	16	P	GG	GG	GG	GG	GG	black	grey							
rs12203592	6	P	CT	CT	CC	CC	CC	grey	grey							
rs12821256	12	P	TT	TT	TT	TT	CT	grey	black							
rs12896399	14	P	GG	GG	GT	GT	GT	black	pink							
rs1393350	11	P	GG	GG	GG	AG	GG	black	black							
rs1800407 ǂ	15	P	GG	GG	GG	AG	GG	black	grey							
rs1805005	16	P	GG	GT	GG	GG	GG	black	black							
rs1805006	16	P	CC	CC	CC	CC	CC	black	black							
rs1805007	16	P	CC	CC	CC	CT	CC	black	grey							
rs1805008	16	P	CC	CC	CC	CC	CC	black	grey							
rs1805009	16	P	GG	GG	GG	GG	GG	black	black							
rs201326893_Y152OCH	16	P	CC	CC	CC	CC	CC	black	grey							
rs2228479	16	P	GG	GG	GG	GG	GG	black	black							
rs2378249	20	P	AA	AA	AA	AA	AG	grey	black							
rs2402130	14	P	AG	AG	AA	AA	AG	black	grey							
rs28777	5	P	AA	AC	AC	AC	AA	black	pink							
rs312262906_N29insA	16	P	CC	CC	CC	CC	CC	black	black							
rs4959270	6	P	AC	AA	AA	CC	CC	black	black							
rs683	9	P	AC	CC	CC	CC	AC	grey	black							
rs885479	16	P	GG	GG	GG	GG	GG	black	grey							
rs1919550 *	3	A	AA	AA	AA	AA	AA	black	black							
rs10497191	2	A	CC	CC	CC	CT	CC	grey	black	CC	CC	CC	CT	CC	black	black
rs1079597 ǂ	11	A	GG	AA	GG	GG	GG	grey	black	CC	TT	CC	CC	CC	black	black
rs11652805	17	A	TT	CT	CT	TT	TT	black	black	TT	CT	CT	TT	TT	grey	black
rs1229984 ǂ	4	A	GG	AG	AG	GG	GG	black	black	CC	CT	CT	CC	CC	grey	black
rs12439433	15	A	AA	AG	AA	AA	AA	black	black	AA	AG	AA	AA	AA	pink	black
rs12498138	3	A	GG	GG	GG	GG	GG	black	black	GG	GG	GG	GG	GG	grey	grey
rs12913832	15	A-P	GG	AG	AA	AG	AG	black	black	GG	AG	AA	AG	AG	grey	black
rs1426654	15	A	AA	AA	GG	AA	AA	grey	pink	AA	AA	GG	AA	AA	grey	black
rs1462906	8	A	CT	CC	CC	CC	CC	grey	grey	CT	CC	CC	CC	CC	grey	black
rs1572018 ǂ	13	A	GG	GG	AA	GG	GG	pink	black	CC	CC	TT	CC	CC	grey	black
rs16891982 *	5	A-P	GG	CG	CC	CG	GG	grey	black	GG	CG	CC	CG	GG	grey	black
rs174570	11	A	CC	CT	TT	CT	CC	black	black	CC	CT	TT	CT	CC	grey	black
rs17642714 *	17	A	AT	AA	AA	AA	AA	black	grey	AT	AA	AA	AA	AA	black	grey
rs1800414 ǂ	15	A	AA	AA	AA	AA	AA	black	black	TT	TT	TT	TT	TT	black	black
rs1834619	2	A	GG	AG	AG	GG	GG	grey	grey	GG	AG	AG	GG	GG	grey	black
rs1871534 ǂ*	8	A	CC	CC	CC	CC	CC	black	red	GG	GG	GG	GG	GG	grey	black
rs1876482 ǂ	2	A	CC	CC	TT	CC	CC	black	black	GG	GG	AA	GG	GG	grey	black
rs192655	6	A	AA	AA	AA	AG	AA	black	grey	AA	AA	AA	AG	AA	grey	black
rs200354	14	A	GT	GT	TT	GG	GG	black	black	GT	GT	TT	GG	GG	black	grey
rs2024566	22	A	AG	AA	AA	AA	AA	black	grey	AG	AA	AA	AA	AA	grey	black
rs2042762 ǂ	18	A	AA	AG	AA	AA	AA	black	black	TT	CT	TT	TT	TT	grey	grey
rs2166624	13	A	GG	AA	GG	AG	GG	black	pink	GG	AA	GG	AG	GG	grey	black
rs2196051 ǂ	8	A	CT	CT	CC	TT	TT	black	black	AG	AG	GG	AA	AA	grey	black
rs2238151	12	A	CT	CC	TT	CT	CT	grey	black	CT	CC	TT	CT	CT	black	black
rs2593595 ǂ	17	A	TT	TT	TT	CT	TT	black	grey	AA	AA	AA	AG	AA	grey	black
rs260690	2	A	AA	AA	AC	AC	AA	black	black	AA	AA	AC	AC	AA	grey	grey
rs2814778 ǂ	1	A	AA	AA	AA	AA	AA	black	grey	TT	TT	TT	TT	TT	grey	black
rs310644 ǂ	20	A	AA	AA	GG	AA	AA	pink	black	TT	TT	CC	TT	TT	grey	black
rs3737576 ǂ	1	A	AA	GG	AA	AA	AA	grey	grey	TT	CC	TT	TT	TT	black	black
rs3811801 ǂ	4	A	CC	CC	CC	CC	CC	grey	black	GG	GG	GG	GG	GG	grey	black
rs3814134 ǂ	9	A	TT	TT	TT	TT	TT	black	red	AA	AA	AA	AA	AA	black	black
rs3823159	6	A	AA	AG	AG	AA	AA	pink	black	AA	AG	AG	AA	AA	grey	black
rs3827760 ǂ	2	A	TT	TT	CT	TT	TT	black	grey	AA	AA	AG	AA	AA	grey	black
rs3916235 ǂ	18	A	GG	GG	GG	GG	GG	pink	black	CC	CC	CC	CC	CC	grey	black
rs4411548 ǂ	17	A	GG	GG	GG	GG	AA	black	black	CC	CC	CC	CC	TT	grey	black
rs4471745	17	A	GG	GG	AA	GG	AG	grey	pink	GG	GG	AA	GG	AG	black	black
rs459920	16	A	CT	CT	TT	CT	CT	black	pink	CT	CT	TT	CT	CT	black	black
rs4833103	4	A	AC	AC	CC	AC	AC	grey	grey	AC	AC	CC	AC	AC	grey	black
rs4891825	18	A	AA	AA	AA	AA	AG	black	black	AA	AA	AA	AA	AG	black	black
rs4918664	10	A	AA	AG	GG	AA	AA	black	black	AA	GG	GG	AA	AA	grey	grey
rs671	12	A	GG	GG	GG	GG	GG	black	black	GG	GG	GG	GG	GG	grey	black
rs6754311	2	A	TT	CT	CC	CT	TT	grey	black	TT	CT	CC	CT	TT	grey	black
rs6990312	8	A	GT	GG	TT	GG	GT	grey	black	GT	GG	TT	GG	GT	grey	grey
rs7226659	18	A	GG	GT	TT	GG	GG	grey	black	GG	GT	TT	GG	GG	grey	black
rs7251928	19	A	AA	CC	AC	AA	AA	grey	black	AA	AC	AC	AA	AA	grey	grey
rs7326934 *	13	A	GG	GG	CG	GG	GG	grey	black	GG	GG	CG	GG	GG	grey	black
rs735480	15	A	TT	TT	CC	TT	TT	black	grey	TT	TT	CC	TT	TT	grey	black
rs7554936	1	A	CT	TT	TT	CT	TT	grey	grey	CT	TT	TT	CT	TT	grey	black
rs7657799	4	A	TT	TT	TT	TT	TT	black	black	TT	TT	TT	TT	TT	grey	black
rs7722456	5	A	TT	TT	CC	TT	TT	black	black	TT	TT	CC	TT	TT	grey	black
rs798443	2	A	AA	AA	AA	AG	AA	black	black	AA	AA	AA	AG	AA	grey	black
rs7997709	13	A	TT	CC	TT	CT	TT	black	grey	TT	CC	TT	CT	TT	grey	black
rs870347 ǂ	5	A	TT	TT	TT	TT	TT	grey	grey	AA	AA	AA	AA	AA	grey	black
rs917115	7	A	CT	CC	CC	TT	TT	black	grey	CT	CC	CC	TT	TT	grey	black
rs9522149	13	A	TT	TT	TT	CC	TT	black	black	TT	TT	TT	CC	TT	grey	black
rs10007810	4	A								GG	AG	AG	GG	GG	grey	black
rs10108270	8	A								AC	AC	CC	AC	CC	black	black
rs10236187	7	A								AA	CC	AA	AA	AA	grey	black
rs1040045	6	A								AG	AA	AA	AG	AA	grey	black
rs1040404	1	A								AG	AG	AG	AG	AG	grey	black
rs10496971	2	A								TT	TT	GG	TT	TT	grey	black
rs10510228	3	A								TT	TT	TT	TT	TT	grey	black
rs10512572	17	A								GG	AG	AG	GG	GG	grey	black
rs10513300	9	A								CT	TT	CT	TT	TT	black	black
rs10839880	11	A								TT	TT	CT	CT	CT	grey	black
rs10954737	7	A								TT	CT	TT	TT	TT	grey	black
rs11227699	11	A								AA	AG	GG	GG	GG	grey	red
rs12130799	1	A								AA	AA	AA	AA	AA	grey	black
rs12544346	8	A								AA	AG	GG	GG	AG	black	black
rs12629908	3	A								GG	AG	AG	GG	GG	grey	black
rs12657828	5	A								AA	GG	AA	AG	AA	grey	black
rs1296819	22	A								CC	CC	AC	CC	AC	pink	black
rs1325502	1	A								GG	GG	AA	AG	AG	black	black
rs13400937	2	A								TT	TT	GT	TT	GT	grey	black
rs1369093	4	A								TT	TT	CT	TT	TT	grey	black
rs1407434	1	A								GG	AG	GG	GG	GG	grey	grey
rs1471939	8	A								CT	CT	CT	TT	CT	grey	black
rs1500127	5	A								CT	CT	CT	CC	CT	grey	black
rs1503767	12	A								TT	TT	GT	GT	TT	grey	black
rs1513056	12	A								GG	AA	AA	AG	GG	grey	black
rs1513181	3	A								AG	GG	GG	GG	AG	black	black
rs1569175	2	A								CC	CC	CC	CC	CC	grey	black
rs1760921	14	A								TT	CT	CT	TT	TT	grey	black
rs1837606	11	A								CT	CT	CT	CT	CC	black	black
rs1871428	6	A								AG	AG	AA	AG	GG	grey	black
rs1879488	17	A								CC	CC	CC	AC	CC	grey	black
rs1950993	14	A								GT	TT	TT	GT	TT	grey	black
rs2001907	8	A								CC	CC	CC	CC	CC	grey	black
rs2030763	3	A								GG	AG	GG	GG	GG	grey	black
rs2033111	17	A								AA	AG	AA	AG	AG	grey	black
rs2070586	12	A								GG	GG	AG	AA	AA	grey	black
rs2073821	9	A								CC	TT	CC	CC	CC	black	black
rs2125345	17	A								CC	TT	TT	CC	CT	black	black
rs214678	12	A								TT	TT	TT	TT	TT	grey	black
rs2306040	9	A								TT	TT	TT	TT	TT	grey	black
rs2330442	7	A								GG	AA	AA	AA	GG	grey	black
rs2357442	14	A								AA	AA	AA	AC	AA	grey	black
rs2416791	12	A								GG	GG	GG	AG	GG	grey	black
rs2504853	6	A								CT	CC	CC	TT	TT	grey	black
rs2532060	19	A								CT	CC	CC	CC	CC	black	black
rs2627037	2	A								GG	AG	GG	GG	GG	grey	black
rs2702414	4	A								GG	GG	GG	GG	GG	grey	black
rs2835370	21	A								TT	TT	TT	TT	TT	black	black
rs2899826	15	A								AA	GG	GG	AA	AA	black	black
rs2946788	11	A								GT	GT	GT	GG	TT	black	black
rs2966849	16	A								GG	GG	GG	GG	GG	grey	grey
rs2986742	1	A								CC	TT	TT	TT	TT	grey	grey
rs3118378	1	A								AA	GG	AA	AG	AA	grey	black
rs316598	5	A								TT	TT	CT	TT	CT	black	black
rs316873	1	A								CT	CT	TT	CC	CC	grey	black
rs32314	7	A								CT	CT	CC	TT	CT	grey	black
rs37369	5	A								CC	CT	CT	CT	CC	grey	black
rs3745099	19	A								AA	AG	AG	AA	AA	black	black
rs3784230	14	A								AA	AG	AA	AA	AA	grey	grey
rs3793451	9	A								CT	CC	CC	CT	CC	black	black
rs3793791	10	A								TT	TT	CT	TT	TT	black	black
rs385194	4	A								GG	AG	AG	AA	GG	grey	black
rs3907047	20	A								TT	CT	TT	TT	TT	grey	black
rs3943253	8	A								AA	AG	AA	AA	AG	grey	black
rs4458655	6	A								TT	CT	CT	CT	TT	grey	black
rs4463276	6	A								GG	AA	AA	AA	AG	black	black
rs4666200	2	A								AG	AG	AA	AA	AG	grey	black
rs4670767	2	A								GT	GG	GT	GT	GG	grey	black
rs4717865	7	A								GG	GG	GG	GG	GG	grey	black
rs4746136	10	A								AA	AA	AG	AG	GG	grey	black
rs4781011	16	A								GG	GT	TT	GG	GT	black	black
rs4798812	18	A								GG	AG	GG	AG	AG	grey	black
rs4821004	22	A								CT	CT	CT	CC	CT	black	black
rs4880436	10	A								CC	CC	CC	CC	CC	grey	black
rs4908343	1	A								AG	AA	GG	AA	AA	black	black
rs4918842	10	A								TT	TT	CC	TT	CT	black	black
rs4951629	1	A								TT	CT	TT	TT	TT	black	black
rs4955316	3	A								GT	GT	TT	TT	GT	black	black
rs4984913	16	A								AA	AA	GG	AG	GG	black	grey
rs5768007	22	A								CC	CT	CC	CC	CC	grey	black
rs6104567	20	A								TT	GG	TT	GT	GT	grey	black
rs6422347	5	A								TT	CT	TT	TT	TT	grey	black
rs6451722	5	A								GG	GG	AG	AG	GG	black	black
rs6464211	7	A								CC	CT	CT	CC	CC	grey	black
rs647325	1	A								AA	AA	AG	AA	AG	grey	black
rs6541030	1	A								GG	GG	GG	GG	GG	grey	black
rs6548616	3	A								TT	CT	CT	TT	CC	grey	black
rs6556352	5	A								CC	CT	CT	CT	CT	grey	black
rs705308	7	A								CC	AC	AC	CC	CC	black	black
rs7238445	18	A								AG	GG	GG	GG	AG	grey	black
rs731257	7	A								GG	AA	AG	GG	GG	grey	black
rs734873	3	A								GG	GG	GG	GG	GG	grey	black
rs7421394	2	A								AA	AG	GG	AG	AA	black	black
rs772262	12	A								GG	GG	GG	GG	GG	grey	black
rs7745461	6	A								AG	AG	GG	GG	GG	grey	black
rs7803075	7	A								AG	AA	AA	GG	AG	grey	black
rs7844723	8	A								CT	CT	CC	CC	TT	grey	black
rs8021730	14	A								GG	GG	GT	GT	GG	grey	black
rs8035124	15	A								AA	CC	AC	AA	AA	black	black
rs8113143	19	A								AC	AC	AC	AC	AC	black	black
rs818386	16	A								CC	TT	CC	CC	CC	black	black
rs874299	18	A								CT	CT	TT	CT	CT	grey	black
rs881728	18	A								CC	CC	AC	AC	CC	grey	black
rs9291090	4	A								AA	AA	AA	AA	AA	grey	black
rs9319336	13	A								TT	CT	CT	TT	TT	grey	black
rs946918	14	A								GG	GT	GG	GT	GT	black	grey
rs948028	11	A								AA	AC	AA	AA	AA	grey	black
rs9530435	13	A								CT	CC	CC	TT	CC	black	black
rs9809104	3	A								TT	CT	TT	TT	TT	grey	grey
rs9845457	3	A								AG	GG	AA	AG	AG	grey	grey

Results:

Tables 1a and **1b** contain information values for II/LISNP and AI/PISNP loci, respectively. Many loci are denoted as reported on the reverse strand in ForenSeq and forward strand in HID-Ion AmpliSeq panels (referred to hereafter as AmpliSeq), and this is reflected in the genotype data shown at overlapping loci. In addition, loci which consist of AT or CG SNPs are denoted. It is particularly challenging to compare results across assays at these loci because strand orientation is not obvious.

The average coverage and coefficient of variation (CV, defined as the standard deviation divided by the average coverage) both appear more variable in ForenSeq than in AmpliSeq. This could be attributable to the increased number of amplicons in ForenSeq, totaling 231 compared to 165 in the AmpliSeq Ancestry panel and 124 in the AmpliSeq Identity panel. This is consistent with the observation of improved average coverage and lowering of CV in the smaller AmpliSeq Identity panel compared to the Ancestry panel.

Two SNP loci are reproducibly discordant between platforms, rs4918664 and rs7251928, both in Component B (**Table 1b**, in red). For these discordant calls, factors such as strand bias and coverage balance are evaluated.

At rs7251928 in Component B, ForenSeq reports a CC with high coverage and the data contain no "A" allele calls. In addition, the other genotype possibilities AA and AC are observed, and behave as expected. For the same sample at this locus, AmpliSeq reports an AC with moderate coverage. The allele coverage is imbalanced at 14% "A" and 86% "C" on average. There is also negative strand bias for the "A" allele. From the IGV view in **Figure 2**, there appears to be an alignment issue affecting the negative strand sequences which results in either a deletion or an "A" call. The AC heterozygote observed in Component C AmpliSeq data appears balanced. This information suggests that the ForenSeq CC is the correct genotype call, and that AmpliSeq on the PGM may consistently mistype CC homozygotes at this locus.

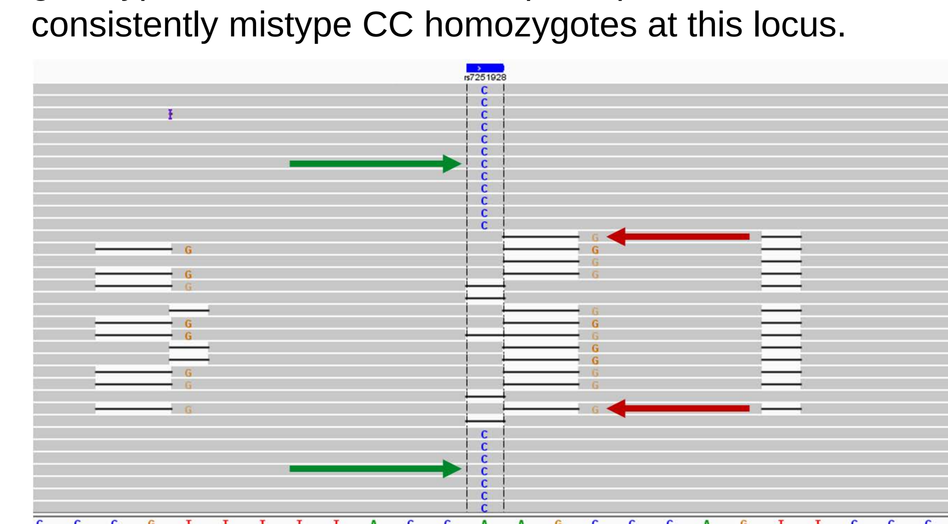

Figure 2. AmpliSeq IGV screen view for rs7251928 in Component B. Red arrows bracket 16 negative strand sequences; green arrows denote forward strand sequences.

At rs4918664 in Component B, ForenSeq reports an AG with high coverage, albeit relatively low 68% balance favoring the "G" allele. For the same sample at this locus, AmpliSeq reports a GG but a QC filter is flagged due to 5% "A" allele calls. The AmpliSeq coverage is lower compared to other samples at this locus, including other heterozygous samples which are concordant between AmpliSeq and ForenSeq (data not shown). This information indicates a potential sample-specific issue, such as a polymorphism in the primer binding region which reduces the efficiency of amplifying the "A" allele.

Discussion and Future Directions:

These information values are expected to be of use to the forensic community in evaluating and validating NGS SNP assays. However, demonstrating consistency with these information values does not guarantee that all genotypes will be correctly determined. Firstly, this is due to the fact that not all possible genotypes are represented among these five samples, and some discordances are genotype specific. **Figure 3** shows the coverage of possible genotypes among these five samples. In addition, sample-specific polymorphisms in primer binding regions can affect genotyping results in an unpredictable manner. Ongoing work with NIST population samples aims to address these gaps.

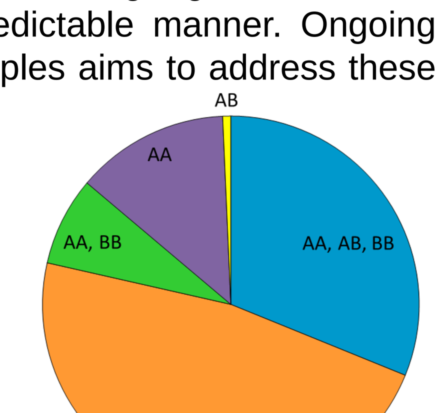

Figure 3. Relative genotype coverage of the 289 autosomal SNPs in the five components of SRM 2391c. Biallelic SNPs have three possible states, represented here as AA, AB, and BB. 31% of the 289 loci demonstrate all three states across the five samples, while 55% of loci demonstrate two states (either AA,BB or AA,AB). 14% of loci only demonstrate one state across the five samples (either AA or AB).

These information values will be appended to the SRM 2391c Certificate of Analysis and this analysis will serve as a model for inclusion of forensically-relevant SNP genotypes in future versions of the 2391 series reference material.

Poster download:
http://www.cstl.nist.gov/biotech/strbasepub_pres/GettingsGRC2016poster.pdf

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Financial disclosure: This work was supported by funding from the FBI Laboratory and Biometrics Center of Excellence: Forensic DNA Typing as a Biometric tool.

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