Faculty of Health and Medical Sciences

Validation of the HID-Ion Ampliseq Identity Panel for relationship testing and reflections on the experience with MPS in real case work

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Topics

- Short introduction to the HID-Ion Ampliseq™ Identity Panel (New name: Precision ID Identity Panel)
- Results from the validation study*
- Implementation of and experiences with an MPS based kit in forensic genetic case work

* Buchard et al., Electrophoresis (2016), in press

HID-Ion Ampliseq Identity Panel

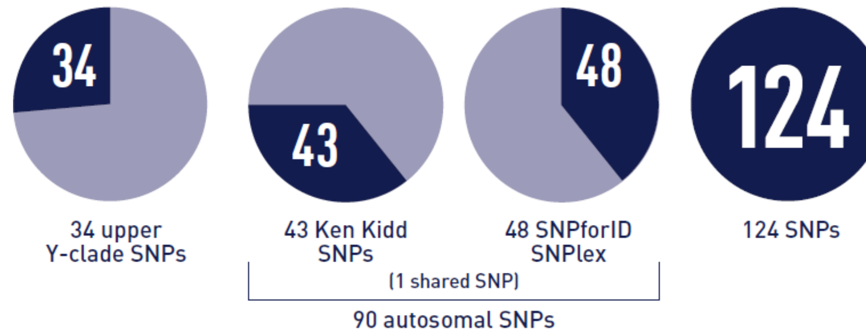
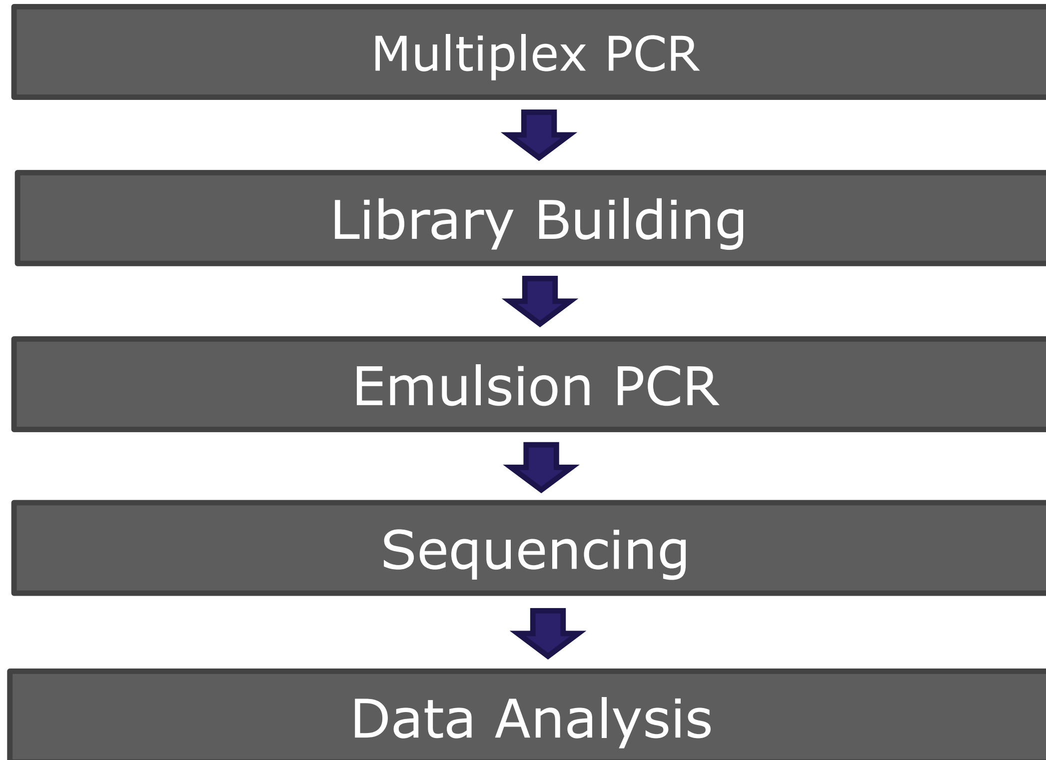


Table 1. HID-Ion AmpliSeq™ Identity Panel specifications.

Targets	124 single-nucleotide polymorphisms (SNPs)
Amplicon length	Average of 132 bp for the 90 autosomal SNPs from Ken Kidd[2] and the SNPforID Consortium[3] Average of 141 bp for the 34 upper Y-clade SNPs[1]
Primer pool size	124 primer pairs in 1 tube
Input DNA recommended	1 ng
Time to results	<24 hours (sample to result)
Sample multiplexing (observed performance)	Assuming 80% chip loading and 60% usable chip, users can expect the following capacities: **Ion 314™ Chip: 8 samples** **Ion 316™ Chip: 38 samples** **Ion 318™ Chip: 77 samples**

Workflow



Validation

Samples:

- 100 Danes (50 females, 50 males) typed in duplicates

- Samples were previously typed with an ISO 17025 validated SNaPshot assay*

Material:

- FTA-card punches

- DNA extractions (from FTA-cards and blood)

* Børsting et al., FSI genet. 4 (2009) 34-42

Locus balance (autosomal SNPs)

Locus Balance

10.00
4.00
3.00
2.00
1.50
1.00
0.50
0.20
0.10
0.05
0.03

rs2342747
rs12997453
rs993934
rs729172
rs727811
rs1109037
rs722290
rs10776839
rs10488710
rs1015250
rs6444724
rs2149555
rs876724
rs719366
rs9951171
rs907100
rs4288409
rs1979255
rs964681
rs1528460
rs826472
rs733164
rs917118
rs1493232
rs2046361
rs1886510
rs2111980
rs3780962
rs740910
rs321198
rs4847034
rs891700
rs2056277
rs159606
rs2076848
rs1357617
rs987640
rs576261
rs1736442
rs740598
rs1523537
rs7520386
rs7704770
rs7041158
rs1355366
rs1413212
rs737681
rs430046
rs4530059
rs717302
rs2292972
rs445251
rs251934
rs1382387
rs1005533
rs735155
rs1360288
rs10495407
rs2016276
rs6811238
rs914165
rs9905977
rs2269355
rs1463729
rs6955448
rs2831700
rs873196
rs1490413
rs1028528
rs10773760
rs338882
rs354439
rs560681
rs1872575
rs13218440
rs10092491
rs938283
rs1498553
rs1454361
rs1024116
rs1058083
rs1821380
rs901398
rs1335873
rs2040411
rs722098
rs4364205
rs1031825
rs221956
rs2830795

Calculated as the number of reads for a locus divided by the average number of reads per locus

Heterozygote balance

Calculated as the number of reads for one nucleotide divided by the number of reads for the other nucleotide in the order A, C, G and T

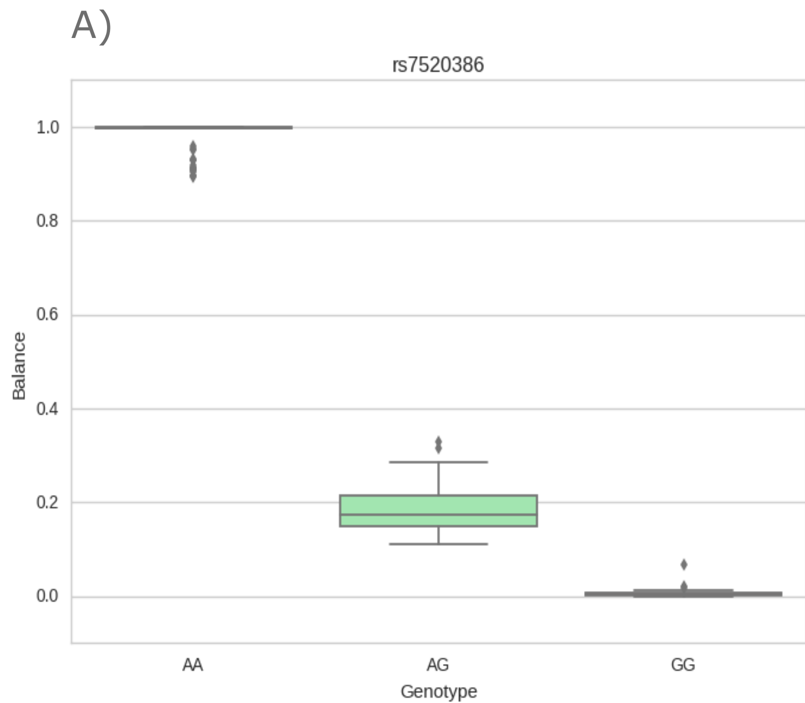
Level of noise

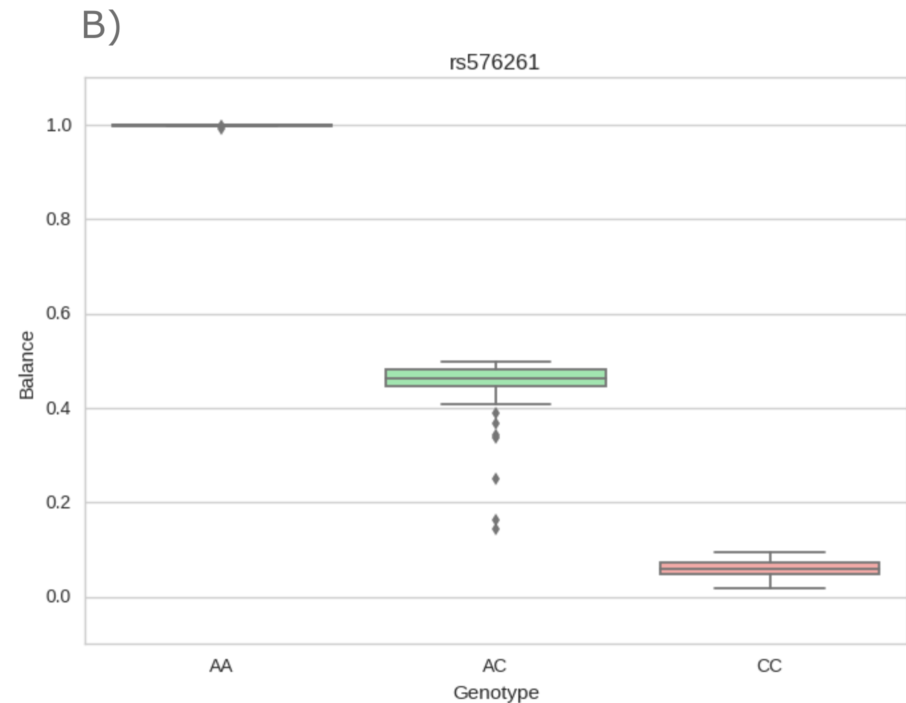
Calculated as the numbers of reads that were different from the genotype divided by the numbers of reads that made up the genotype

Concordance

- All 100 samples were typed in duplicates
- 13 dis-concordances were found in the two loci rs576261 (one sample) and rs7520386 (twelve samples)
- Undetermined allele calls (NN) were observed in rs2269355 and rs4288409 in one of the duplicate typings

Fraction of reads for the SNPs A) rs7520386 and B) rs576261





- Heterozygote balance was calculated as the least frequent nucleotide divided by the total number of reads for both nucleotides

Concordance with the ISO 17025 validated SNaPshot kit (39 SNPs)

- Full concordance was obtained except for one SNP (rs2040411) in one sample
- The SNP rs2040411 was typed as homozygous (A/A) with the HID-Ion Ampliseq™ Identity Panel and heterozygous (A/G) using the PCR-SBE-CE assay
- Allele frequencies of the autosomal SNPs not included in the SNaPshot kit were compared to published data from Danes. No significant difference was found

Case work data analysis

- A custom made Python script named SNPonPGM was developed for analysis of the csv files

- The script highlighted all SNPs in a sample that did not fulfill one of the following criteria:

    - Minimum sequencing depth of 200

    - Maximum noise level of 3 %

    - Heterozygote balance of at least 0.3

    - Genotypes called as “NN” by the HID_SNP_Genotyper plugin

Number of samples sequenced per chip

- In this work, a total of 16 and 32 samples were sequenced per 316™ or 318™ chip, respectively. This is less than half the number of samples recommended for this assay by the manufacturer

- We chose to run fewer samples per chip in order to generate a minimum coverage of approximately 200 reads per autosomal SNP

Mixture study

- Two 2-person mixtures were constructed in mixture ratios of 1:1, 1:3, 1:6, 1:12, 1:24 and 1:48, respectively. Sample A and B (both females) were mixed, and sample C and D (both males) were mixed. Each mixture was typed twice
- The mixtures were sequenced, and the results were analysed with the SNPonPGM script to mimic a real case scenario

Mixture study

DNA mixtures	Number of SNPs with warning for heterozygote imbalance					Number of SNPs with warning for high noise				
	AB(1)	AB(2)	CD(1)	CD(2)	average	AB(1)	AB(2)	CD(1)	CD(2)	average
1:1	18	15	8	11	13	0	1	2	1	1
1:3	27	27	15	15	21	2	2	1	1	1.5
1:6	11	10	0	1	5.5	23	25	17	15	20
1:12	0	1	0	0	0.25	32	33	14	15	23.5
1:24	0	0	0	1	0.25	10	13	4	8	8.75
1:48	0	0	0	0	0	4	5	2	1	3

- The average number of warnings for heterozygote imbalance was 0.85 (±0.15) for the 100 single source samples
- The average number of warnings for elevated noise was 1.3 (±0.15) for the 100 single source samples
- Mixtures with almost equal amounts of DNA from the major and minor contributor can be identified by the high number of warnings for heterozygote imbalance, whereas mixtures with relatively little DNA from the minor contributor can be identified by the number of warnings for elevated noise

How does the script work in real life?

- Makes it easy for the analyst to identify the typing result that require further scrutiny
- The criteria function as guidelines
- As the trained analysts gain more experience, they interpret and use the criteria with more flexibility
- At present, it is common to accept a genotyping result with e.g. 120 reads if the heterozygote balance is close to one and there are very few noise reads

SNPonPGM – simplifies the work of the reporting officer

- Compare profiles from duplicate typings
- Generate consensus profiles from duplicate typings
- Collect approved genotyping data for all typed samples and individuals in a case
- Identify inconsistencies in relationship cases with first degree relatives
- Export the genotypes of all individuals in the case to a text file that may be used for import into various softwares for likelihood calculations and for the final report

Final remarks

- Works very well – produces high quality data
- Easy workflow for the reporting officers
- Very expensive: with our setup the reagent cost of typing one sample is ~93€ (the price for typing a sample with the PCR-SBE-CE assay was ~8.5€)
- Tested using half reaction volumes on the AIMs kit. This works fine both for reference samples and the tested trace samples (adjusted price will be ~65€)
- Investigating levels of contamination

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