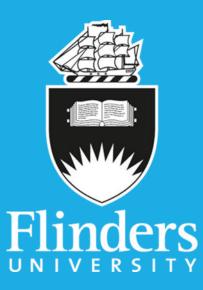
INTELLIGENCE SNP DATA FROM LATENT DNA USING MASSIVE PARALLEL SEQUENCING



Presentation by Adrian Linacre

Flinders University

June 2019

Acknowledgements

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Belinda Martin, Flinders University: sample preparation

Jennifer Young, Flinders University: MPS preparation & data analyses

Dan Power, Thermo Fisher Scientific: ION Torrent operation & data analyses

Touch DNA

Touching an item for only a few seconds can leave very little DNA

The DNA deposited by contact is invisible and at trace levels

The amount deposited by a very brief contact, or deposited in the past and degraded by bacterial growth, may no longer be suitable for standard DNA profiling.



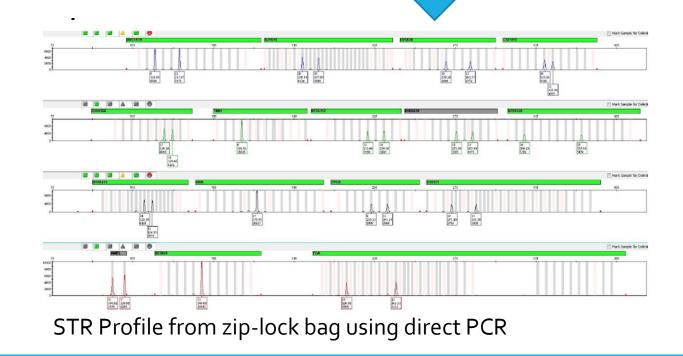








Can we generate SNP data from touch DNA using massive parallel sequencing?



Experiment 1: Can we generate MPS SNP data from touch DNA?

Touch Samples (N=6o)

- 5 individuals
 - 4 of known shedder status
- 4 substrates per individual
 - glass slide, fuse, zip-lock bag, wire

Individual	Shedder Status
1	Unknown
2	Light
3	Light
4	Intermediate
5	Heavy

- Each individual/substrate combination done in triplicate
- Each substrate was touched for 15 seconds

1 x reference sample per individual1x negative control included per SNP panel

Two SNP panels tested using Precision ID Library Kit



1) Phenotype – HIrisPlex System

The HirisPlex System

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10						19)
100		200	200			The second
A						A MAR
Gen	e <i>e 2011</i> 0 5	SNP	Allele	No). (of Allele:
1 MC		rs312262906	Α			2 NA
2 MC	IR	rs11547464	Α	0	1	2 NA
3 MC1	1R	rs885479	Т	0	1	2 NA
4 MC:	IR	rs1805008	Т	0	1	2 NA
5 MC	IR	rs1805005	Т	0	1	2 NA
6 MC	IR III	rs1805006	Α	0	1	2 NA
7 MC	IR	rs1805007	Т	0	1	2 NA
8 TUE		rs1805009	С	0	1	2 NA
9 MC		rs201326893	A	0	1	2 NA
10 MC	41.7 m (rs2228479	A	0	1	2 NA
11 MC	and the second	rs1110400	С	0	1	2 NA
12 SLC		rs28777	С	1 martine	1	2 NA
13 SLC		rs16891982	С	0	1	2 NA
14 KITI		rs12821256	G	0	1	2 NA
	105374875		A	0	1	2 NA
16 IRF		rs12203592	T	0	1	2 NA
17 TYR		rs1042602	Т	0	1	2 NA
18 OC/		rs1800407	A	-	1	2 NA
19 SLC		rs2402130	G	0	1	2 NA
20 HEF		rs12913832	Т	0	1	2 NA
21 PIG	7 /	rs2378249	C	-	1	2 NA
	105370627	and the second	Ţ	0	1	2 NA
23 TYR		rs1393350	Т	0	1	2 NA
24 TYR	(P1	rs683	G	0	1	2 NA



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The HIrisPlex system for simultaneous prediction of hair and eye colour from DNA

Susan Walsh ^a, Fan Liu ^a, Andreas Wollstein ^a, Leda Kovatsi ^b, Arwin Ralf ^a, Agnieszka Kosiniak-Kamysz ^c, Wojciech Branicki ^{d, e}, Manfred Kayser ^a A 🖾

Hair & Eye colour phenotype



Hain	Dark	Light	1
Hair	0.839	0.161	1
Black	Brown	Red	Blond
0.631	0.309	0.001	0.059
Eye	Blue	Int.	Brown
Lyc	0.058	0.183	0.759

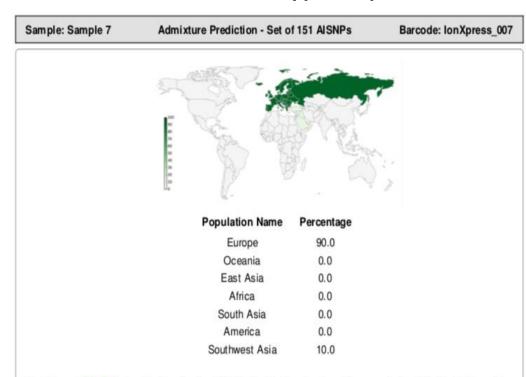
Prediction result

Display Predicted Phenotype

Download Predicted Phenotype

2) Biogeographic Ancestry

Thermofisher Scientific Precision ID Ancestry Panel - 165 autosomal markers (SNPs)



HID SNP Genotyper Report

Confidence: HIGH. Log-likelihood value: 88.98. The likelihood value of the sample is within the 95% confidence interval obtained from 10,000 random samples with the same admixture proportions as the test sample

applied biosystems



International Journal of Legal Medicine https://doi.org/10.1007/s00414-018-1785-9

ORIGINAL ARTICLE

CrossMark

Assessment of the Precision ID Ancestry panel

Muna Al-Asfi¹ • Dennis McNevin¹ • Bhavik Mehta¹ • Daniel Power² • Michelle E. Gahan¹ • Runa Daniel³

Sequencing Results

HIrisplex System

	# Sequences obtained	# Samples that produced a genotype
Reference Samples	268,033 - 354,761	5/5
Touch Samples	3,334-1,211,631	54/60*
Negative Control	730	0
* 6 touch samples failed to sequence (or < 60 s	aquancas)	

* 6 touch samples failed to sequence (or <60 sequences)

Precision ID Ancestry Panel

	# Sequences obtained	# Samples that produced a genotype
Reference Samples	238,277 – 344,388	5/5
Touch Samples	30,251 -1,420,719	52/60*
Negative Control	1,106	0

* 8 samples failed to sequence

Eye Colour Predictions

- 51/54 touch samples correctly predicted eye colour
- One zip-lock bag sample (individual 1) and two wire samples (individual 1 and individual 3) predicted blue eyes instead of brown.
- HERC2 SNPs (required for eye colour prediction) was non-concordant with reference
- 2 other samples were non-concordant for HERC2 but still generated correct predictions

	Reference	Touch Sample
Individual 1 - ziplock bag	CT (Brown)	CC (Blue)
Individual 1 - wire	CT (Brown)	CC (Blue)
Individual 3 - wire	TT (Brown)	CC (Blue)
Individual 3 -	TT (Brown)	CT (Brown)
Individual 3 -	TT (Brown)	CT (Brown)

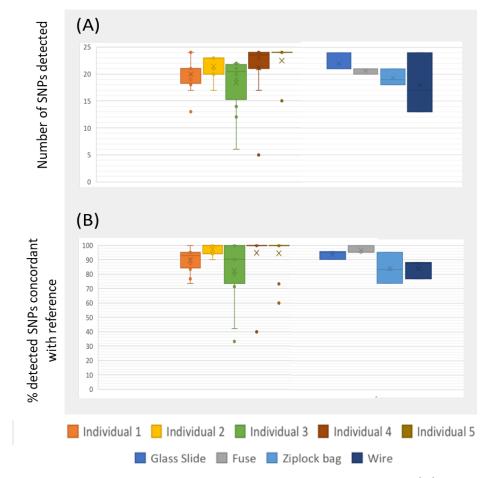
Hair Colour Predictions

- 40/54 touch samples correctly predicted both hair colour and shade
- No samples generated incorrect colour and shade predictions
- 6 had correct colour but no shade (category 3)
- 6 had correct shade but incorrect colour (e.g. black hair instead of brown)
- Individuals 4 (Intermediate) and 5 (Heavy) produced more accurate predictions than individual 1, 2 and 3
- Wire samples generated the poorest hair colour prediction results

Correct Colour and Shade	6
Correct Colour - no shade	5
Correct Colour - incorrect shade	4
Correct Shade - no colour	3
Correct Shade - incorrect colour	2
Incorrect Shade - no colour	1
Incorrect Shade and colour	

	Gla	Glass Slide		Fuse		use Ziplock				Wir	e	
Individual	Α	В	С	А	В	С	А	В	С	Α	В	C
1	6	6	6	6	2	6	6	6	2	6	1	3
2	6	6	6	6						6	6	6
3	6	2	2	6	3	6	6	3	6	3	2	2
4	6	6	6	6	6	6	6	6	6	6	4	
5	3	6	6	6	6	6	6	6	6	6	3	6

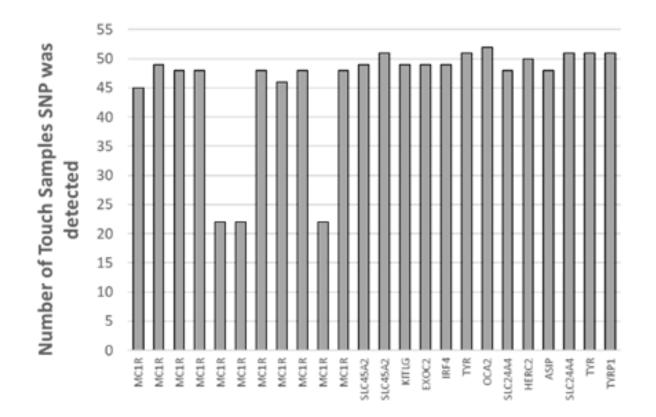
HIrisplex SNP Detection and Concordance



Number of SNPs detected per individual and per substrate (A) and the percent of detected SNPs concordant with the reference per individual and per substrate (B) for the Hirisplex panel

- 29/54 touch samples had 20 or more SNPs detected, all of which were concordant with the reference
- Individual 4 (intermediate) and 5 (heavy shedder) produced best results
- Individual 3 (light shedder) had lowest success and was most variable across samples
- Fuse and glass slide showed the highest SNP detection and concordance.
- Wire showed the lowest and least consistent results

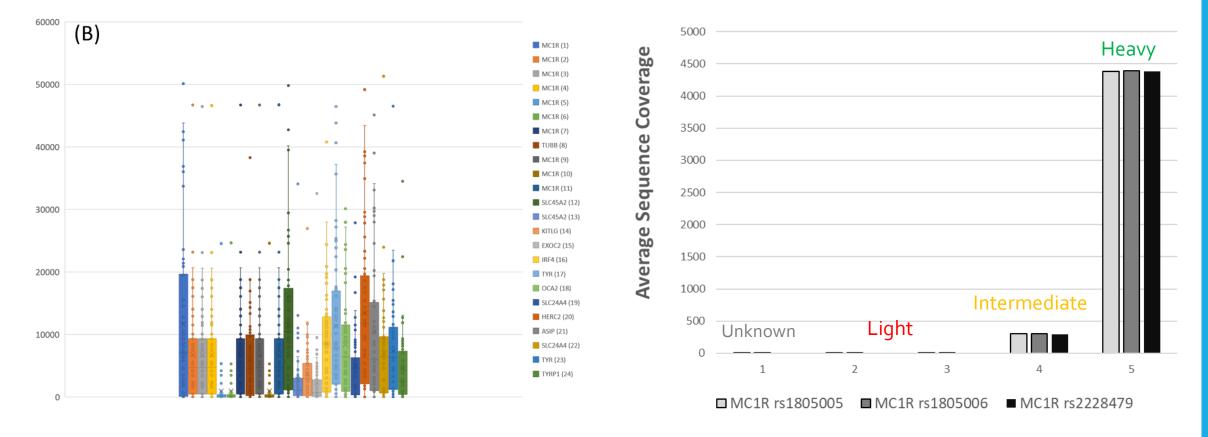
Frequency of each SNP detected in touch samples



Hirisplex Gene ID (as ordered in online tool)

- 24/24 SNPs were detected in all reference samples
- No SNP was successfully detected in all 55 touch samples
- 21 SNPs were successfully detected in 45 or more touch samples.
- 3 SNPs were only detected in 22/54 touch samples
- These 3 SNPs are important for hair colour identification

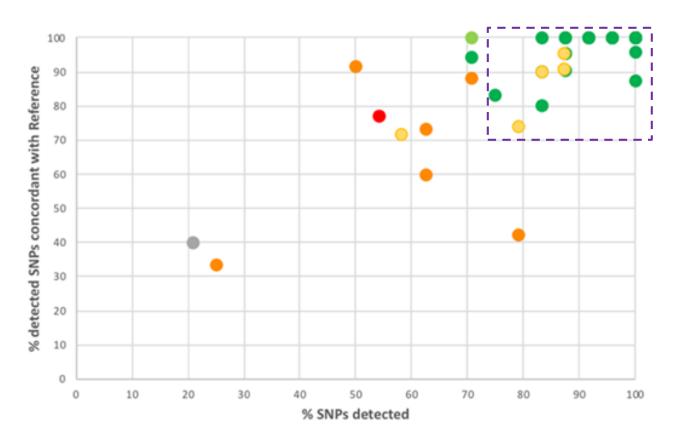
Under-representation of SNPs in touch samples



Individual

• 3 MC1R SNPs are under-represented

• Coverage of these 3 SNPs was related to shedder status



● Score 6 ● Score 4 ● Score 3 ● Score 2 ● Score 1 ● no prediction

Correct Colour and Shade	6
Correct Colour - no shade	5
Correct Colour - incorrect shade	4
Correct Shade - no colour	3
Correct Shade - incorrect colour	2
Incorrect Shade - no colour	1
Incorrect Shade and colour	

- >75% of SNPs detected with >70% concordance
- However, hair and eye colour prediction rely on the detection of specific SNPs within that 75%

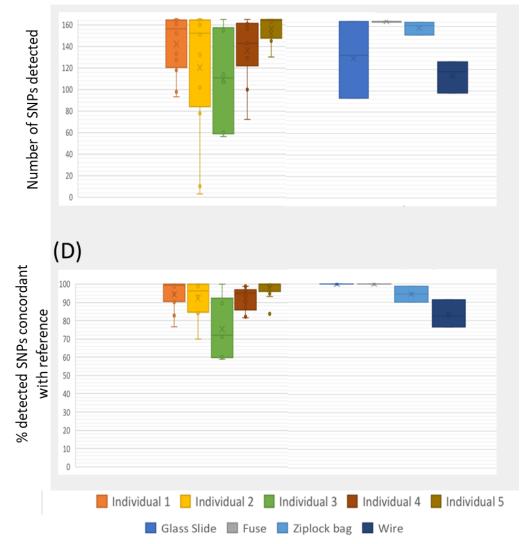
Ancestry Predictions

- 90% of the touch samples generated an accurate ancestry assignment at the population level (European, Oceanian, East Asian, African, South Asian, Southwest Asian and American).
- 42% were associated with a high confidence assignment
- 48% were associated with a low confidence assignment
- No touch samples generated an incorrect prediction with high confidence
- 10% generated an incorrect assignment with low confidence

Correct Ancestry with High confidence	4
Correct Ancestry with Low confidence	3
Incorrect Ancestry with Low Confidence	2
Incorrect Ancestry with High Confidence	1

	Gla	ass S	lide		Fuse		Zip	olock B	ag			
Individual	Α	В	С	Α	В	С	Α	В	С	Α	В	С
1	4	3	2	4	4	4	4	4	4	3	3	3
2	4	4	4	3	4	4	3	2	3	3	4	4
3							4	3	2	2	2	4
4			4	4	3	3	4	4	4	4	3	3
5	3	3	3	3	3	3	3	3	3	3	3	3

SNP Detection and Concordance



- Individual 5 (heavy shedder) detected the highest #of SNPs with highest concordance
- Individual 2 and 3 (light shedders) produced lowest (and most variable) # of SNPs with low concordance
- The wire generated the lowest SNP detection and least concordance
- The glass slide had variable SNP detection but high concordance

Frequency of SNP detection in touch samples

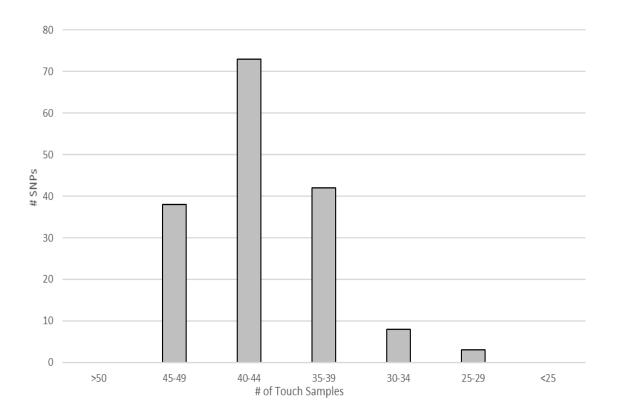
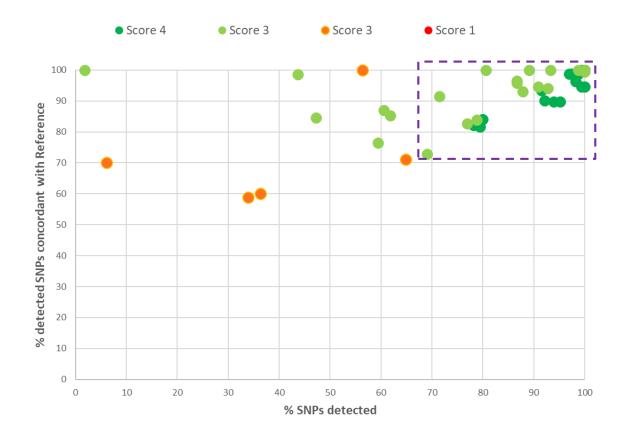


Fig: Frequency of SNPs detected in 52 touch samples

- All 165 SNPs were detected in all five reference samples
- No specific SNPs were under-represented across touch samples
- 38 SNPs were detected in 45/52 touch samples
- 73 SNPs were detected in 40/52 touch samples
- All SNPs were detected in a minimum of 28/52 touch samples



Correct Ancestry with High confidence	4
Correct Ancestry with Low confidence	3
Incorrect Ancestry with Low Confidence	2
Incorrect Ancestry with High Confidence	1

- A minimum SNP detection of 70% always generated an accurate ancestry assignment
- A minimum SNP detection of 70% was accompanied with >70% concordance
- Confidence level associated with these assignments was not solely related to %SNPs detected and concordance

Correlation between SNP detection and ancestry prediction scores

Conclusions

- Touch DNA can generate data using the Thermo Fisher Scientific forensic MPS panels
- Shedder status does have an effect
- Confidence in phenotypic prediction is affected by 3 key SNPs
- 90% of the touch samples generated an accurate ancestry assignment
- Touch DNA inevitably has trace amounts of DNA
 - using direct PCR can aid in SNP typing

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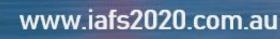


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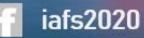
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