INTELLIGENCE SNP DATA FROM LATENT DNA USING MASSIVE PARALLEL SEQUENCING

Presentation by Adrian Linacre

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Acknowledgements

Touch DNA

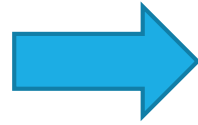Touching an item for only a few seconds can leave very little DNA

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?
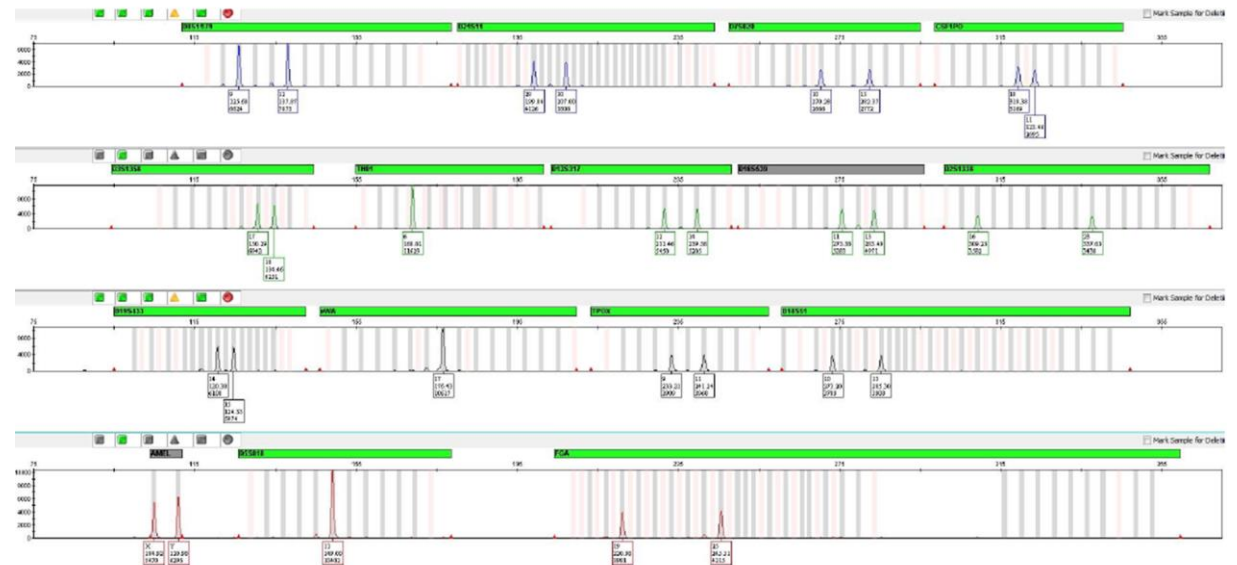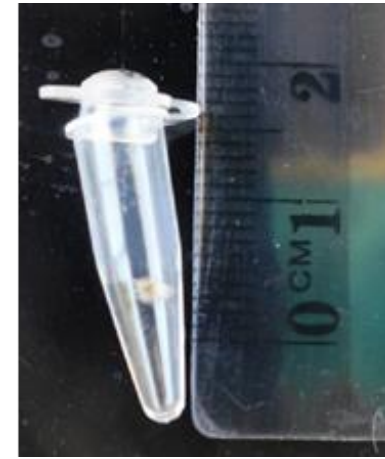
STR Profile from zip-lock bag using direct PCR

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

Touch Samples (N=60)

- 5 individuals

  - 4 of known shedder status

- 4 substrates per individual

  - glass slide, fuse, zip-lock bag, wire

- Each individual/substrate combination done in triplicate

- Each substrate was touched for 15 seconds

1 x reference sample per individual

1x negative control included per SNP panel

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

Two SNP panels tested using Precision ID Library Kit

Ion Chef System

Ion Torrent PGM

1) Phenotype – HIrisPlex System

The HIrisPlex System

	Gene	SNP	Allele	No. of Alleles			
1	MC1R	rs312262906	A	0	1	2	NA
2	MC1R	rs11547464	A	0	1	2	**NA**
3	MC1R	rs885479	T	0	1	2	NA
4	MC1R	rs1805008	T	0	1	2	NA
5	MC1R	rs1805005	T	0	1	2	NA
6	MC1R	rs1805006	A	0	1	2	NA
7	MC1R	rs1805007	T	0	**1**	2	NA
8	TUBB3	rs1805009	C	0	**1**	2	NA
9	MC1R	rs201326893	A	0	**1**	2	NA
10	MC1R	rs2228479	A	0	1	2	NA
11	MC1R	rs1110400	C	0	1	2	NA
12	SLC45A2	rs28777	C	0	1	**2**	NA
13	SLC45A2	rs16891982	C	0	1	2	NA
14	KITLG	rs12821256	G	0	1	2	NA
15	LOC105374875	rs4959270	A	0	**1**	2	NA
16	IRF4	rs12203592	T	0	1	2	NA
17	TYR	rs1042602	T	0	1	2	NA
18	OCA2	rs1800407	A	0	1	**2**	NA
19	SLC24A4	rs2402130	G	0	**1**	2	NA
20	HERC2	rs12913832	T	0	1	2	NA
21	PIGU	rs2378249	C	0	1	2	NA
22	LOC105370627	rs12896399	T	0	**1**	2	NA
23	TYR	rs1393350	T	0	1	2	NA
24	TYRP1	rs683	G	**0**	1	2	NA

Display Predicted Phenotype   Download Predicted Phenotype

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] ✉

Hair & Eye colour phenotype

Prediction result

Hair	Dark	Light		
	0.839	0.161		
Black	Brown	Red	Blond
0.631	0.309	0.001	0.059

Eye	Blue	Int.	Brown
	0.058	0.183	**0.759**

2) Biogeographic Ancestry

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

HID SNP Genotyper Report

Sample: Sample 7	Admixture Prediction - Set of 151 AISNPs	Barcode: IonXpress_007

Population Name	Percentage
Europe	90.0
Oceania	0.0
East Asia	0.0
Africa	0.0
South Asia	0.0
America	0.0
Southwest Asia	10.0

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

ORIGINAL ARTICLE

Assessment of the Precision ID Ancestry panel

Muna Al-Asfi[1] · Dennis McNevin[1] · Bhavik Mehta[1] · Daniel Power[2] · Michelle E. Gahan[1] · Runa Daniel[3]

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 sequences)

Precision ID Ancestry Panel

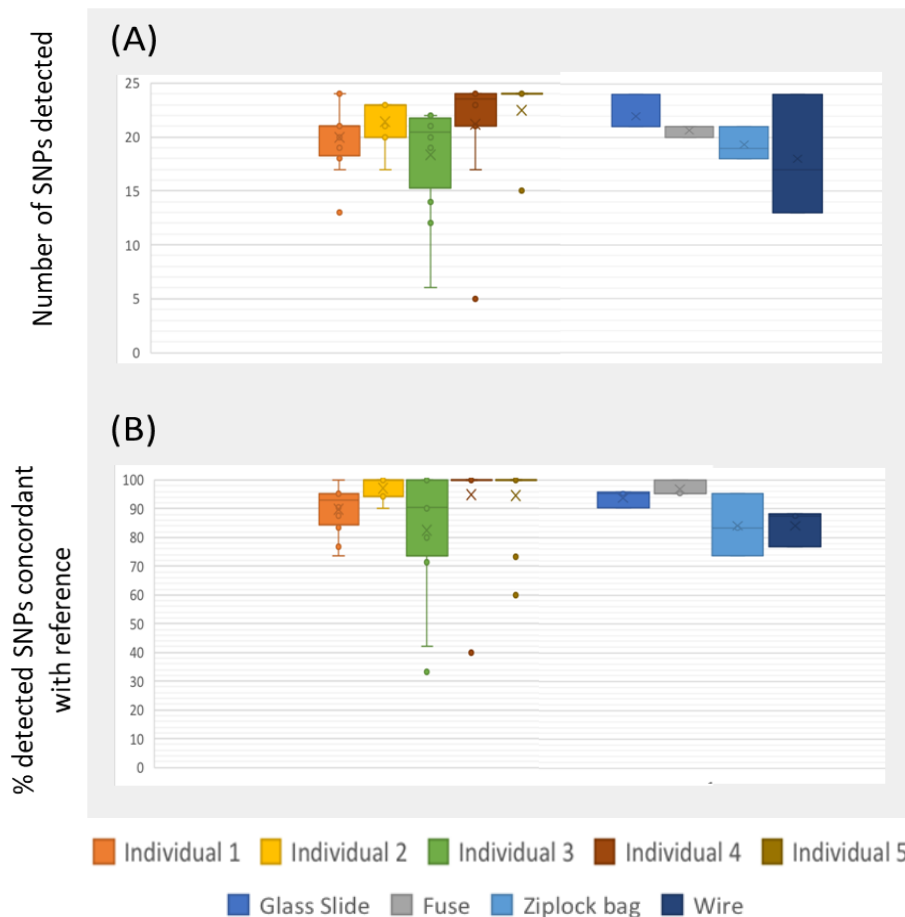|	# Sequences obtained	# Samples that produced a genotype
Reference Samples	238,277 – 344,388	5/5
Touch Samples	30,251 -1,420,719	52/60*
Negative Control	1,106	0

* 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	0

Individual	Glass Slide			Fuse			Ziplock			Wire		
	A	B	C	A	B	C	A	B	C	A	B	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

(A)

Number of SNPs detected

(B)

% detected SNPs concordant with reference

Individual 1 Individual 2 Individual 3 Individual 4 Individual 5

Glass Slide Fuse Ziplock bag Wire

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
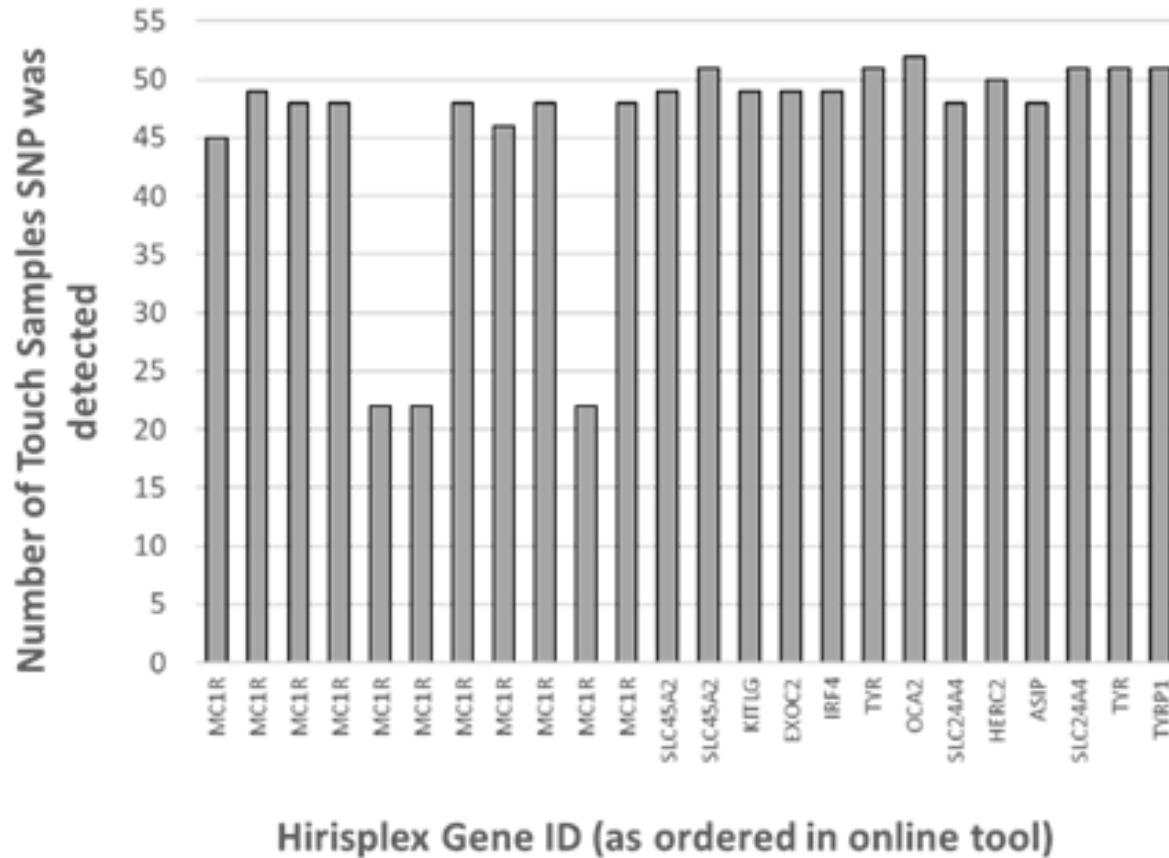
- 24/24 SNPs were detected in all reference samples

- 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
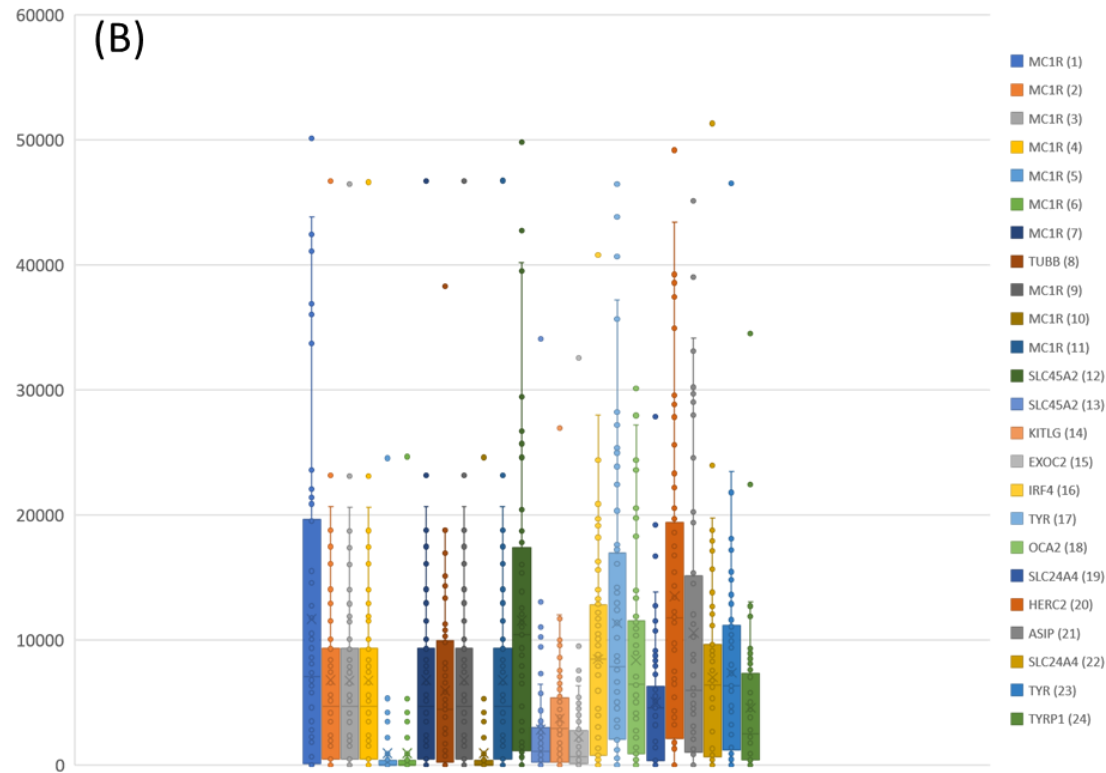
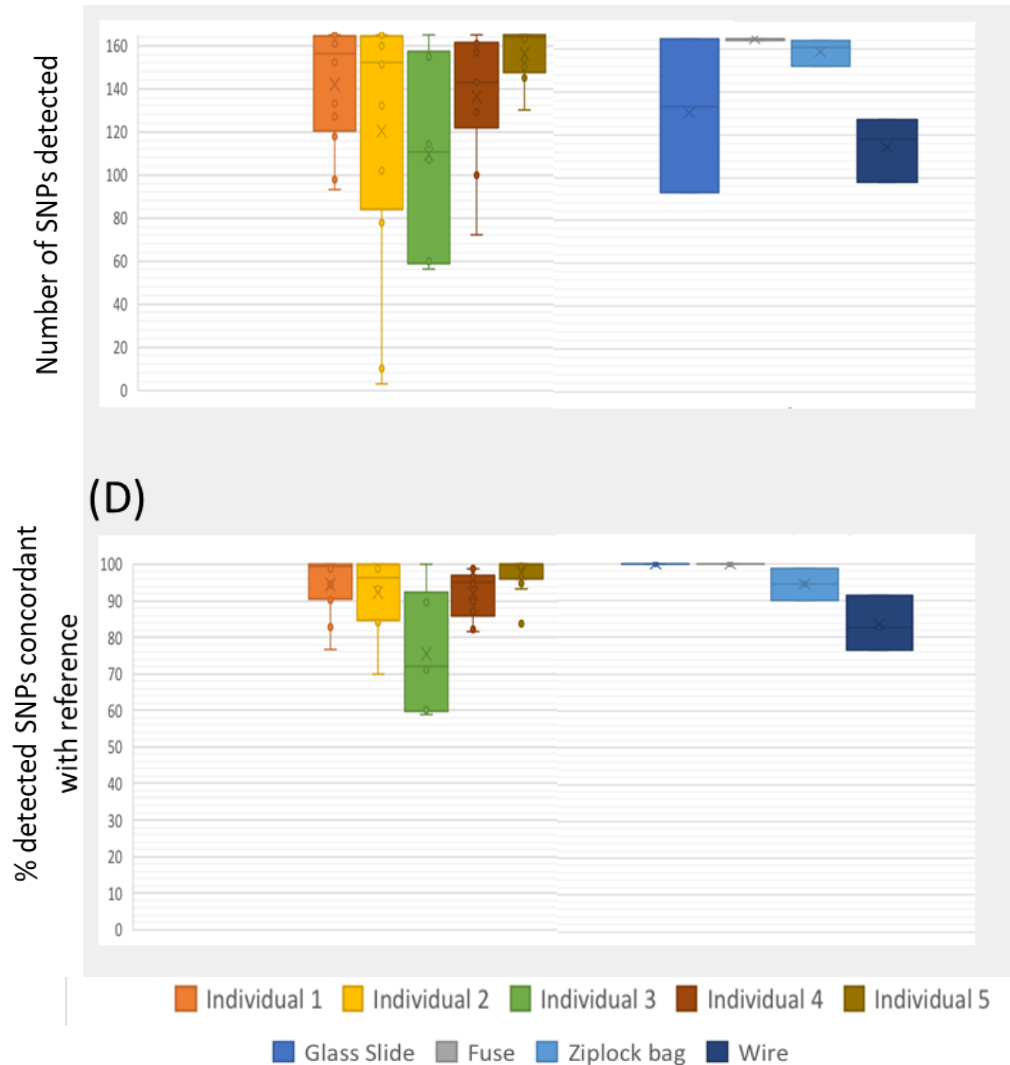- 3 MC1R SNPs are under-represented
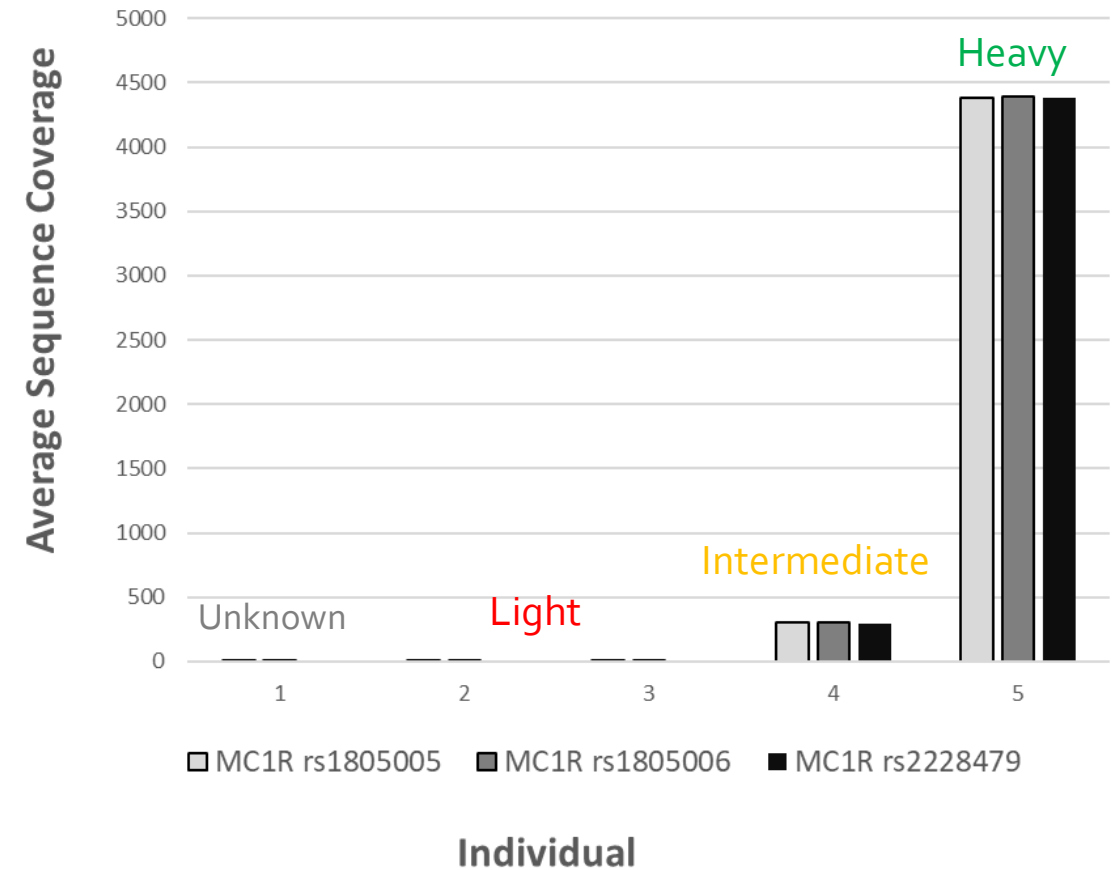
- Coverage of these 3 SNPs was related to shedder status
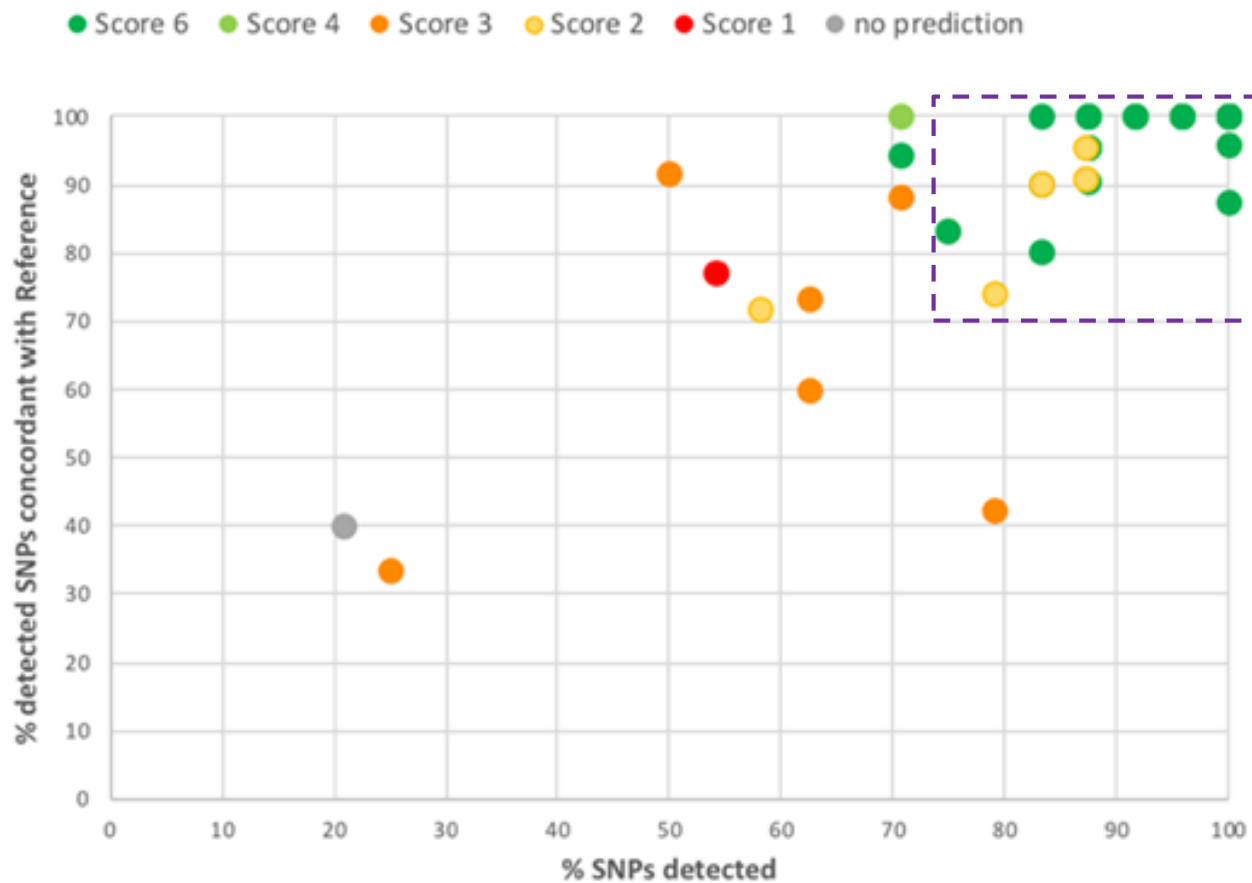
Legend: Score 6, Score 4, Score 3, Score 2, Score 1, no prediction

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	0

- >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

	Glass Slide			Fuse			Ziplock Bag			Wire		
Individual	A	B	C	A	B	C	A	B	C	A	B	C
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
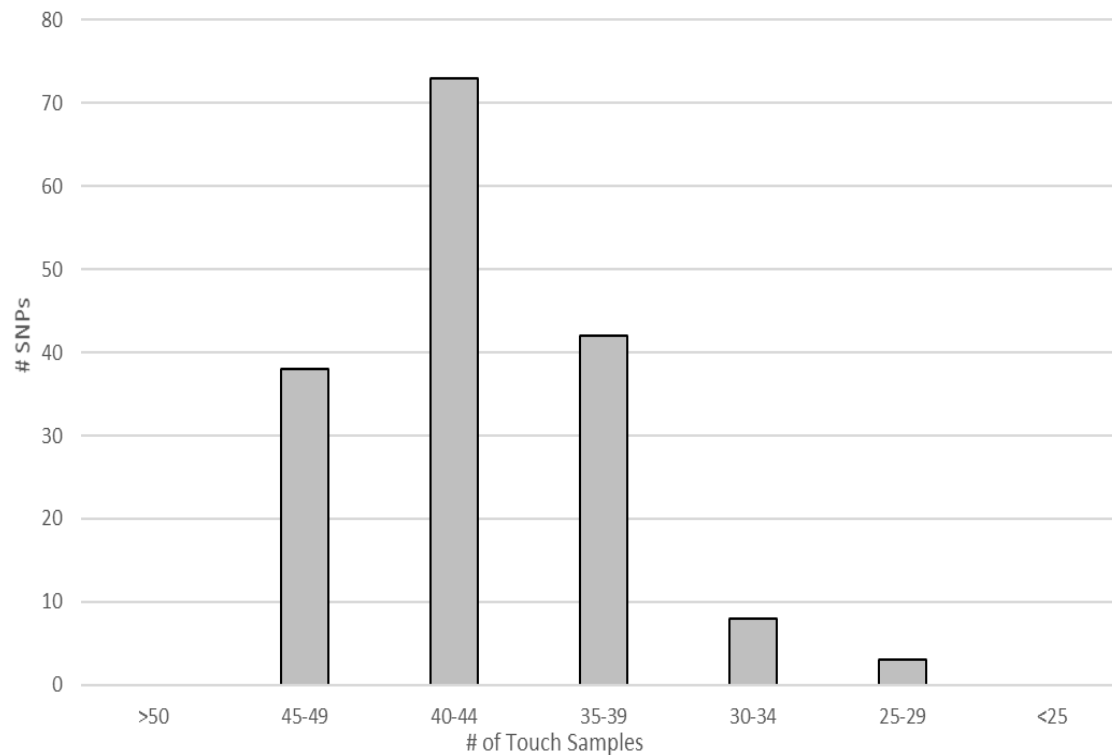
Fig: Frequency of SNPs detected in 52 touch samples

- 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

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

Acknowledgements & Disclaimer