Combined Extraction Method for Mitochondrial DNA and Proteins from Hair for Human Identification

How to get the most out of your sample

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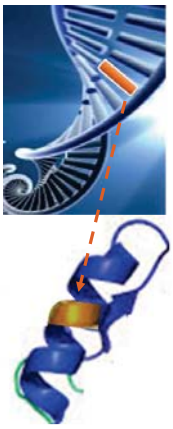
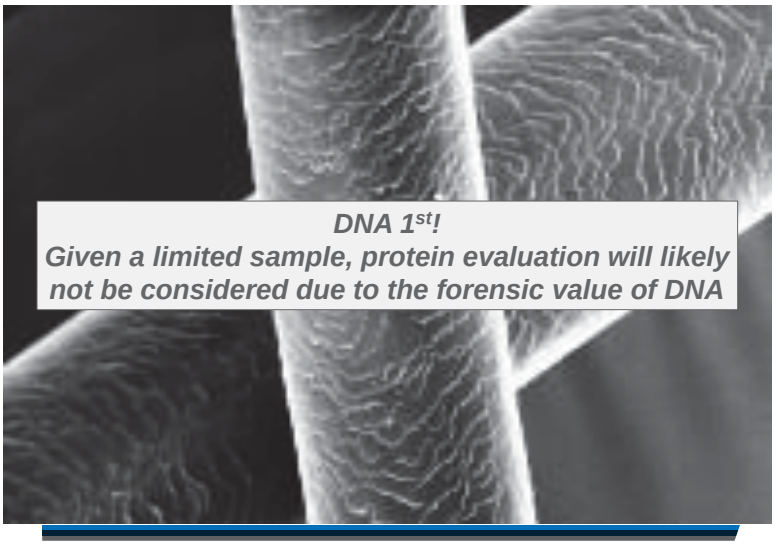
nsSNPs lead to SAPs/ GVPs

- **Discriminatory power: 1 in 12,500***
- ***Can mtDNA and protein signatures provide complete discrimination?***



*Parker et al 2016.



DNA 1st!
Given a limited sample, protein evaluation will likely not be considered due to the forensic value of DNA

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Hair: DNA "needle" in a protein "haystack"

- *Hair is ~95% protein by mass*
- *Proteins can be more stable than DNA*
- *Nuclear DNA from hair is difficult to recover*
- *State of the art limited to mtDNA (limited) and microscopy (subjective)*

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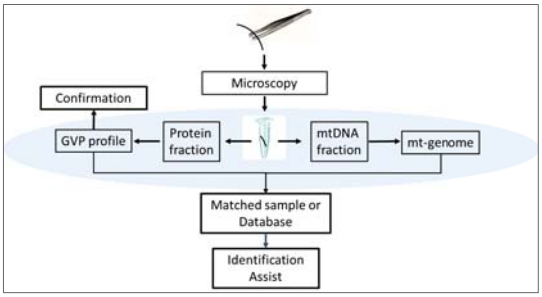
Goal
Develop a workflow that enables the analysis of proteins from hair samples without sacrificing DNA analysis



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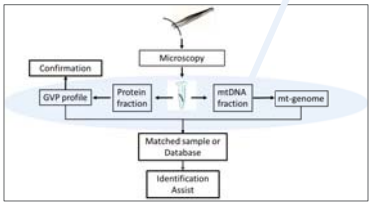
1. *Optimize protein protocol for ease-of-use and sensitivity*
2. *Ensure compatibility with mtDNA forensic sample processing*
3. *Extract and analyze DNA and protein from the same sample*



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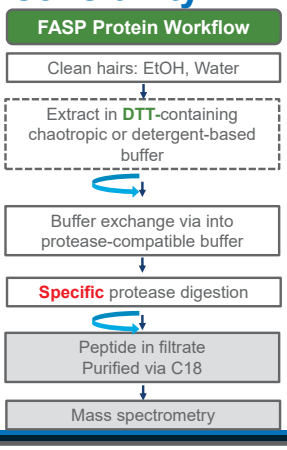
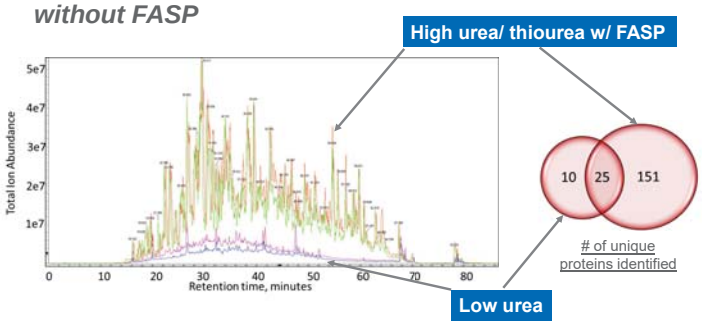
1. Optimize protein protocol for ease-of-use and sensitivity



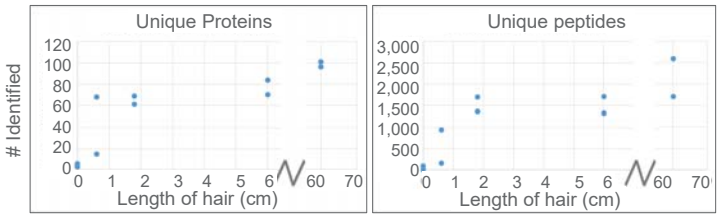
1. Optimize

- *High urea/ thiourea extraction buffer paired with FASP found to be superior to SDS/FASP or low urea/thiourea without FASP*



1. Optimize

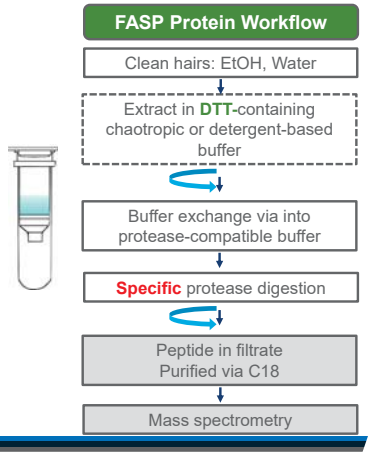
- *Rough working limit of >0.6 cm of hair established to identify unique peptides and proteins*



1. Optimize

- *Rapid trypsin + lysC (45 minutes, 70 ºC) can be used in place of traditional trypsin + lysC (O/N, RT)*

Gold standard spin-column mtDNA workflows differ from spin-column protein workflows



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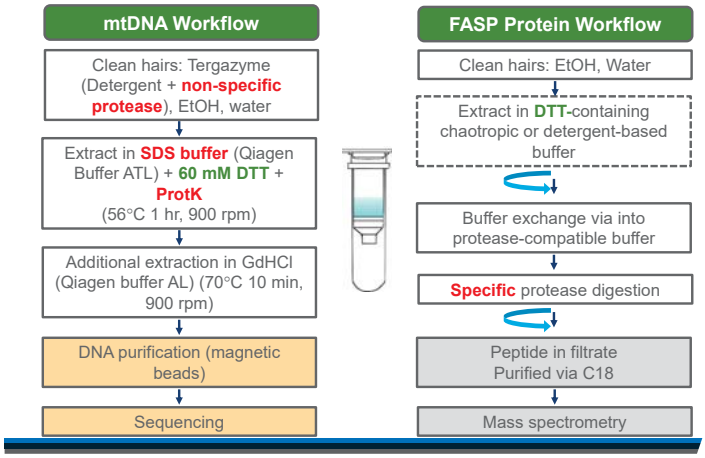

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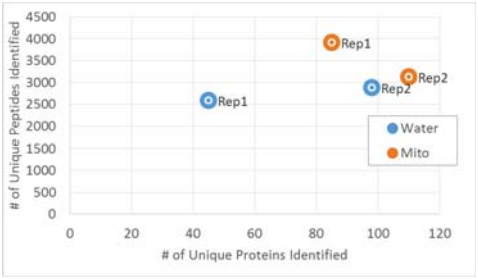
2. Ensure compatibility with mtDNA forensic sample processing

- ***Rigorous washing protocols do not impact peptide yields***



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2. Ensure compatibility

- ***Rigorous washing procedures reduces background contamination***

Sample	# Unique Proteins	# Unique Peptides
EtOH, Water washing	8	50
LC/MS system blank	2	2
Tergazyme, EtOH, water	**2**	**2**

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3. Extract and analyze DNA and protein from the same sample: Truth Data

- ***Haplotypes and “Saplotypes” from 2 hair donors***

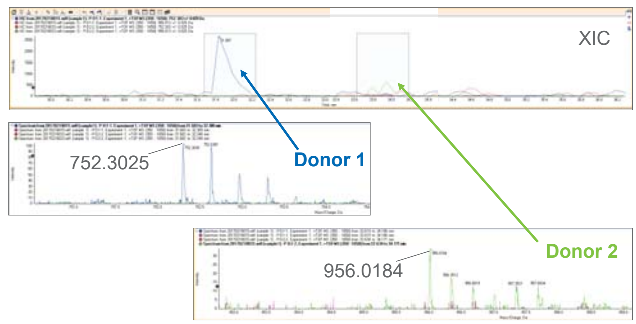
Position	Donor 1	Donor 2 (new)
	Haplotype	Haplotype
73	None	G
152	None	C
195	None	C
263	G	G
709	None	A
750	G	G
1438	G	G
1888	None	A
1896	C	N/A
2706	None	G
3010	A	None
3795	T	None
4216	None	C
4769	G	G
4917	None	G
7028	None	T
8697	None	A
8860	G	G
9899	None	C
10463	None	C
11251	None	G
11719	None	A
12633	None	A
13368	None	A
14766	None	T
14905	None	A
15047	A	None
15326	G	G
15452	None	A
15607	None	G
15928	None	A
16126	None	C
16163	None	G
16186	None	T
16189	C	C
16294	None	T
16519	C	C
Haplogroup	H1ab	T1a1

GN	peptide	Donor 1	Donor 2	Associated rs#
JUP	SAIVHLINYQDDAELAT[R/h]	SAIVHLINYQDDAELATR	Neither identified	rs41283425
JUP	NEGTATYAAA[V/i]LFR	NEGTATYAAAVLFR	Neither identified	rs143043662
KRT31	DN[A/v]ELENLIR	DNAELENLIR	DNAELENLIR	rs6503627
KRT31	SQYE[A/v]LVETNR	SQYEALVETNR	SQYEALVETNR	rs112544857
KRT31	QNQEYQ[V/m]LLDVR	QNQEYQVLLDVR	QNQEYQVLLDVR	rs146247884
KRT32	LEGEIN[T/m]YR	Both identified	Both identified	rs2071563
KRT32	CQYEAMVEAN[R/h]R	CQYEAMVEANRR	Neither identified	rs72830046
KRT33A	QVVSSSEQLQSYQ[A/v]EIIELR	QVVSSSEQLQSYQAEIIELR	QVVSSSEQLQSYQAEIIELR	rs12937519
KRT33B	T[L/V]NALEIELQAQHNLR	Both identified	Both identified	rs11448884
KRT34	SQYEALVE[I/t]NR	Both identified	Both identified	rs2239710
KRT35	VSAMYSSS[S/p]CKLPSLSPVAR	VSAMYSSSSCKLPSLSPVAR	VSAMYSSSSCKLPSLSPVAR	rs743686
KRT35	YETEVSLR	YETEVSLR	Neither identified	rs138303882
KRT35	TN[C/y]S[P/a]RPICVPCPGGRF	TNCSPRPICVPCPGGRF	TNCSPRPICVPCPGGRF	rs2071601, rs12451652
KRT81	LYEEEI[L/r]ILQSHISDTSVVVK	ILQSHISDTSVVVK	Both identified	rs6580873
KRT81	GLTGGFGSHSVC[G/r]GFR	GLTGGFGSHSVCGGFR	Both identified	rs2071588
KRT83	DLNMDC[I/m]VAEIK	DLNMDCmVAEIK	DLNMDCIVAEIK	rs2852464
KRT83	LEAAVAQSEQQ[G/s]EAALSDAR	Both identified	LEAAVAQSEQQGEAALSDAR	rs140635030
KRT83	L[L/Q]EGEE[H/Q]R	All variants identified	All variants identified	
KRT83	[V/I]LQSHISDTSVVVK	Both identified	Both identified	
KRT85	EAECVEA[D/n]SGR	EAECVEADSGR	EAECVEADSGR	rs112554450
KRT86	EEINELN[R/c]MIQR	EEINELNR	EEINELNR	rs139895699
KRTAP3-2	[S/g]CSVPTGPATTICSSDK	Both identified	Both identified	
S100A3	SLACLCLYC[H/q]EYFK	SLACLCLYCHEYFK	SLACLCLYCHEYFK	rs41265164
KRT86	EEINELN[R/c]MIQR	EEINELNR	EEINELNR	
KRT86	L[Q/p]FYQNR	LQFYQNR	LQFYQNR	rs58717266
KRT31	LNVEVDAAPTVDLN[Q/R]VLNETR	Both identified	Both identified	
KRT85	DVD[C/g]AYLR	DVDCAYLR	DVDCAYLR	rs61740813
KRT33B	DNAEL[E/k]NLIR	DNAELENLIR	DNAELENLIR	
KRT33B	LNVEVDAAPAVDLN[Q/R]VLNETR	LNVEVDAAPAVDLNQVLNETR	LNVEVDAAPAVDLNQVLNETR	
KRT33B	[D/Y]SLENTLTESEAR	Both identified	Both identified	
KRT33B	EVEQWFATQTE[E/k]LNK	EVEQWFATQTEELNK	EVEQWFATQTEELNK	rs12450621
KRT39	QNQEYEIL[L/m]DVK	QNQEYEILLDVK	QNQEYEILLDVK	rs17843023
KRT32	MVVN[I/t]DNAK	MVVNIDNAK	MVVNIDNAK	rs2071560
KRT32	LNIEVD[A/t]APPVDLTR	LNIEVDAAPPVDLTR	LNIEVDAAPPVDLTR	rs146792525
KRT32	[T/C]VNTLEIELQAQHSLR	TVNTLEIELQAQHSLR	TVNTLEIELQAQHSLR	
KRT32	DSLEN[T/m]LTESEAR	DSLENTLTESEAR	DSLENTLTESEAR	rs16966929
KRT32	QNQEYQVLLD[V/i]R	QNQEYQVLLDVR	QNQEYQVLLDVR	rs149524933
KRT82	ELDVD[G/s]IIAEIK	ELDVDGIIAEIK	ELDVDGIIAEIK	rs61730589
KRTAP2-1	TGSCCGSTLSSLSYGGGCCQ[P/r]CCCR	TGSCCGSTLSSLSYGGGCCQr	TGSCCGSTLSSLSYGGGCCQPCCCR	rs9635728
PKP1	AVQYLSSQDE[K/n]YQAIGAYYIQHTCF(	AVQYLSSQDEK	Neither identified	
LGALS3	I[Q/H]VLVEPDHFK	IQVLVEPDHFK	IQVLVEPDHFK	

GVP: Donor 1 versus Donor 2

- ***KRTAP3-2 SAP Evidence***

TGSCCGSTLSSLSYGGGCCQr: 752.3030 m/z
TGSCCGSTFSSLSYGGGCCQPCCCR: 956.0127 m/z



3. DNA and protein from One sample

- ***Can Rapid Trypsin can be substituted for Prot K in mtDNA workflow?***

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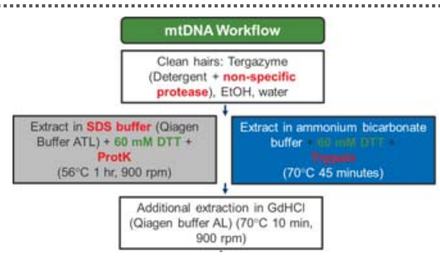
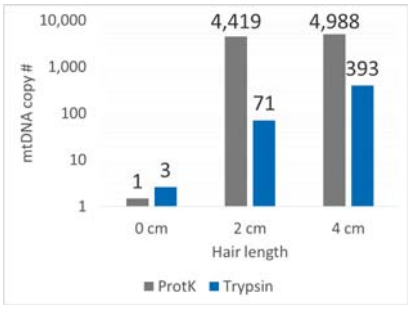


3. DNA and protein from One sample

- *Rapid Trypsin can substitute for ProtK but mtDNA but yields are lower*



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3. DNA and protein from One sample

- *Mito profiles (Precision ID mtDNA whole genome panel) can be obtained from trypsinized hair*

Sample	Donor	Enzyme	Hair length	# Mapped Reads	Haplogroup prediction* (Missing, private mutations)
BN1	#2	ProtK	4 cm	1,575,760	T1a1 (0, 2)
BN2	#2	ProtK	2 cm	1,767,023	T1a1 (0, 0)
BT1	#2	Trypsin	4 cm	1,454,222	T1a1 (0, 1)
BT2	#2	Trypsin	2 cm	1,135,412	T1a1 (0, 1)
B (saliva)	#2	ProtK	Saliva	1,738,314	**T1a1 (0, 1)**
2800M**	2800M	N/A	N/A	1,949,741	**H1c+T152C (0/0)**
NC	None	ProtK	N/A	54,491	--
TC	None	Trypsin	N/A	56,140	--

*https://empop.online/
**Corroborated by: http://forensic.yonsei.ac.kr/presentation/113.pdf ; https://www.promega.com/resources/profiles-in-dna/2016/analyzing-data-from-next-generation-sequencers-using-the-powerseq-automitoy-system/

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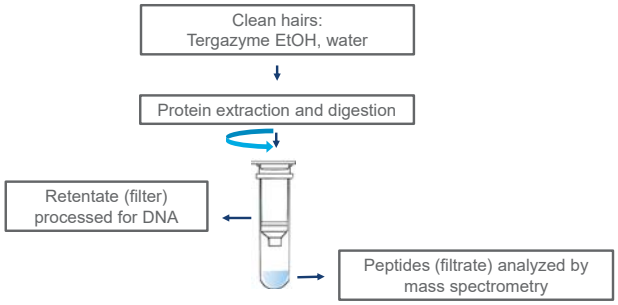
3. DNA and protein from One sample

- *Can we extract proteins first then DNA without compromising DNA yields?*



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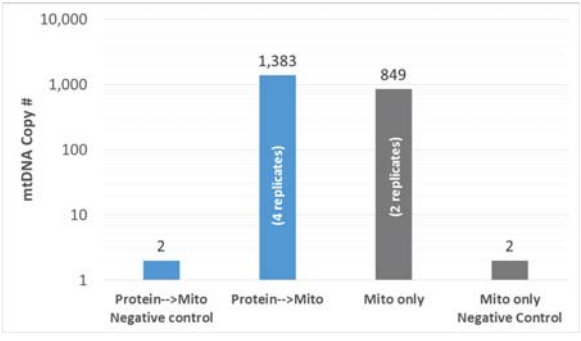
3. DNA and protein from One sample

- *mtDNA can be recovered following protein digestion*



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Summary and Next Steps

- Summary
  - ✓Developed easy spin column-based format with rapid digestion
  - ✓Working limit approaching ~0.6 cm of hair
  - ✓Rigorous washing procedures reduces background contamination without impacting peptide yields
  - ✓Whole mito genome profiles can be obtained from trypsinized hair
  - ✓mtDNA can be recovered following protein digestion
- Next steps
  - Proteomic and mtDNA profiles of mito DNA following protein digestion
  - Sensitivity analysis, validation
  - Other relevant samples (e.g., touch)

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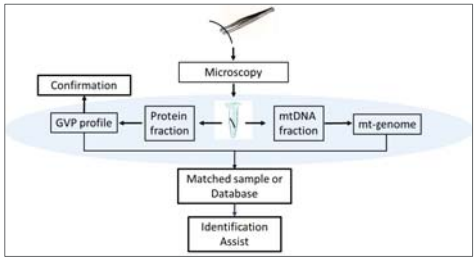
GVP Path to forensic laboratory implementation



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It can be done

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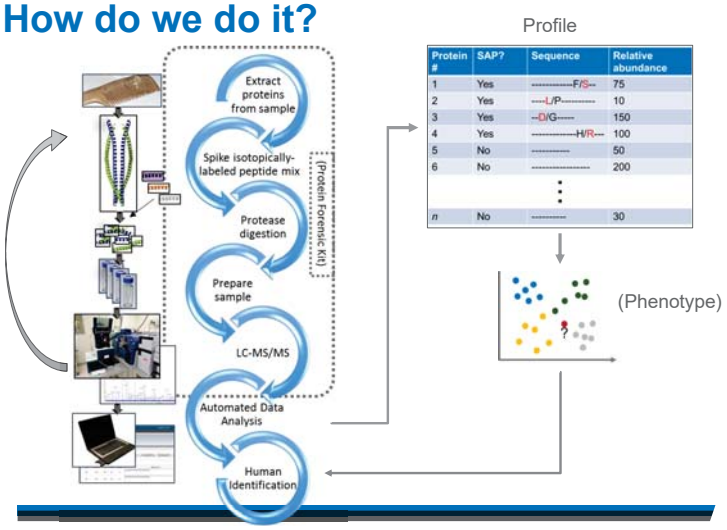
Backup slides

Can combined mtDNA-protein profiles be used to…

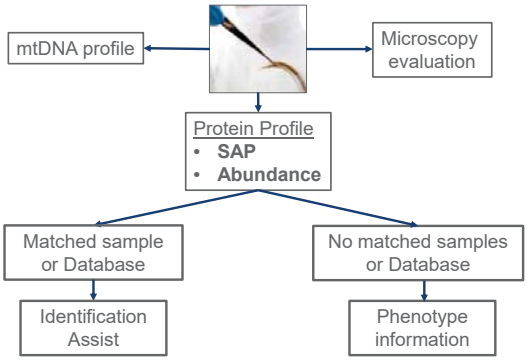
- *Discriminate one human from another?*
- *Distinguish ethnicity?*
- *Distinguish head hair from different body sites?*
- *Provide other identifying information?*
  - *Animal vs. human hair?*
  - *True hair color/ has hair been dyed/ bleached?*
  - *Exposure?*
  - *Other phenotypes?*

		Saliva				BN2				BT2		
Position		Variant	Frequency	Quality		Variant	Frequency	Quality		Variant	Frequency	Quality
73		G	100	37427.9		G	100	36669.1		G	100	36951.2
152		C	100	37697.9		C	100	37742.6		C	100	37515.7
195		C	100	37342.6		C	100	37484.8		C	100	37127.9
263		G	100	37769.1		G	100	37667.6		G	100	37051.6
311		C	99.9	14816.8		NO VARIANT CALLED				C	100	14878.4
709		A	100	37032.7		A	100	36486.8		A	95.1	32924.5
750		G	100	36453.5		G	100	37576.8		G	100	36047.4
1438		G	100	36388		G	100	37631.1		G	100	37600.1
1888		A	100	37666.1		A	96.3	34089.9		A	97.2	34928.3
2706		G	100	37382.9		G	100	35800.3		G	100	35649.7
4216		C	100	37181.3		C	100	35083.6		C	100	35475.2
4769		G	100	37866.8		G	100	37804.8		G	100	29467.4
4917		G	100	36673		G	100	36256.7		G	100	30627.2
7028		T	100	36871.2		T	100	36406.4		T	95.1	33025.5
8697		A	100	7671.85		A	100	9902.94		A	97.5	3479.31
8860		G	100	37747.9		G	100	37779.3		G	100	32373.7
9899		C	100	37660.2		C	100	35758.9		C	92.5	30794.8
10463		C	100	37243.4		C	100	29705.6		C	97	20907
11251		G	100	37007.7		G	100	35878		G	91.5	24316.8
11719		A	100	36965.1		A	100	36002.6		A	100	35998.9
12633		A	100	36970		A	100	36748.1		A	100	35306.8
13368		A	100	36800.1		A	100	35810.8		A	97	34718
14766		T	100	37534.2		T	100	35713.1		T	100	37662.9
14905		A	100	37729.3		A	100	36560.1		A	97.2	23896.4
15326		G	100	37592.8		G	100	37565.5		G	100	37748.2
15452		A	100	37060.4		A	100	34983.3		A	100	35915.8
15607		G	100	37223.4		G	100	36613.2		G	96.8	34545.7
15928		A	100	37052.9		A	100	36960.1		A	96.8	34572.4
16126		C	100	37177.5		C	100	37021.9		C	100	37271.4
16163		G	100	35235.6		G	100	35282.5		G	100	35870.7
16186		T	71.3	17318.3		T	71.8	17638.2		T	71.2	17309
16189		C	100	36132.2		C	100	36495.2		C	100	36907
16294		T	100	36586.2		T	100	36881.3		T	100	35892.7
16519		C	100	37431		C	100	37550.7		C	100	37492.4
16642		G	100	37660.1		G	100	36308		G	100	37645.8

How do we do it?

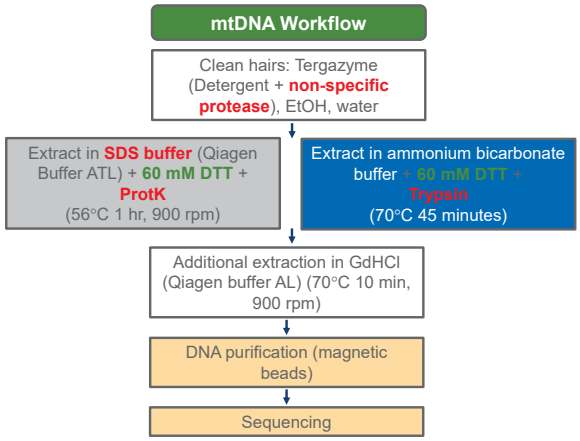


Using protein sequence *and* abundance provides unique and complementary information



Why success can be achieved: Key challenges can be overcome

- Sample preparation optimization
  - Currently investigating SDS vs urea-based extraction, detection limits, QC measures, degradation/ inhibition studies
  - Integrated sample processing
  - Mass spectrometry detection limits are improving
- Methodology can be standardized and independently verified in a manner similar to DNA-based forensics
  - Evaluated four NGS platforms, and based on sponsor criteria, down-selected one for implementing STR genotyping of human DNA samples
  - Currently evaluating a number of different RapidDNA and nanopore-based sequencing devices for field-based human identification applications



Step 3: Extract and analyze DNA and protein from the same sample

- *Mito profiles can be obtained from trypsinized hair*

Sample	Donor	Enzyme	Hair length	# Mapped Reads	Haplogroup prediction* (no mismatches)
BN1	#2	ProtK	4 cm	1,575,760	T1a1/ T1a1'3
BN2	#2	ProtK	2 cm	1,767,023	**T1a1/** T1a1'3
BT1	#2	Trypsin	4 cm	1,454,222	T1a1/ T1a1'3
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B (saliva)	#2	ProtK	Saliva	1,738,314	T1a1/ T1a1'3
2800M**	2800M	N/A	N/A	1,949,741	**H1c(T152C)**/ H1c/ H1(T152C)
NC	None	ProtK	N/A	54,491	H1c2/ H1c(T152C)/ H1c/ H1(T152C)/ H1(T16189C)
TC	None	Trypsin	N/A	56,140	H1c2/ H1aj/ H1c

*https://dna.jameslick.com/mthap/
**Corroborated by: http://forensic.yonsei.ac.kr/presentation/113.pdf ; https://www.promega.com/resources/profiles-in-dna/2016/analyzing-data-from-next-generation-sequencers-using-the-powerseq-automitoy-system/

Step 1: Optimize protein protocol for ease-of-use and sensitivity

- *Filter passivation*

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