Single Step Protein Extraction from Trace Amount Human Hair for Genetically Variant Peptide Detection

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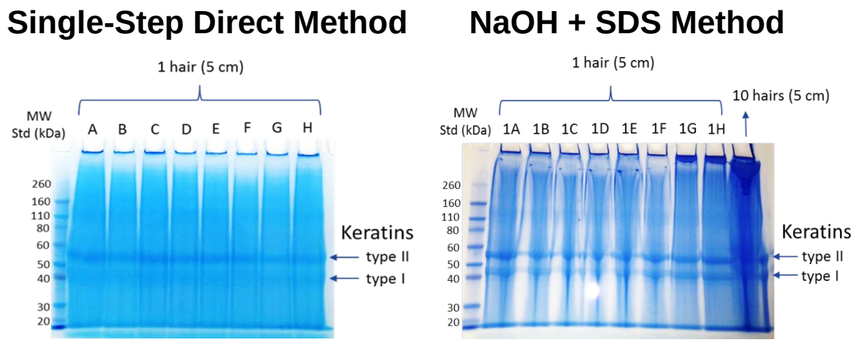
Introduction

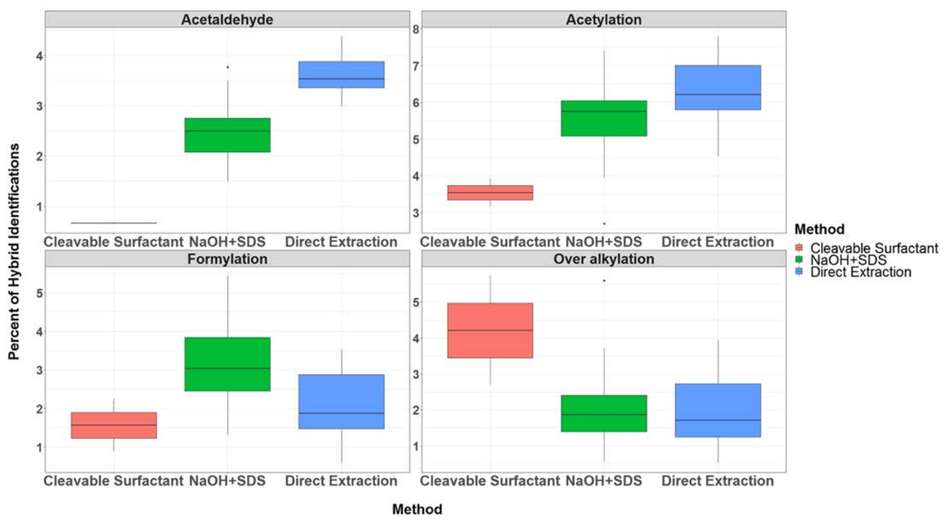
Recent reports have demonstrated that genetically variant peptides (GVPs) derived from human hair proteins can be used to differentiate individuals of different biogeographic origin (PMID:27603779 DOI:10.1371/journal.pone.0160653). We report a single-step direct extraction method ('Direct' Method) for hair proteins that is significantly more sensitive than two previously published methods (PMID:27603779 DOI:10.1371/journal.pone.0160653 and PMID: 27741315 DOI:10.1371/journal.pone.0164993). It is also simple to implement and gives reproducible results. Together with the construction of a hair specific peptide mass-spectral library including previously reported GVPs, it provides a means of reliable analyses of human hair proteome which can utilize trace amount forensic samples.

Result – Test A. *Comparison of Reproducibility*



Test A: The two gel images compare the reproducibility of single-step Direct method and NaOH+SDS method using 5 cm-long hair shaft samples from the same individual donor across 8 replicates (Left: A to H; Right: 1A to 1H). A MW standard was loaded in the first lane. Note that the NaOH+SDS gel includes a 9th lane for which the extraction from ten 5cm-long hair shaft samples was included as a reference. The major bands that correspond to type I and type II hair cuticular keratins were labeled.

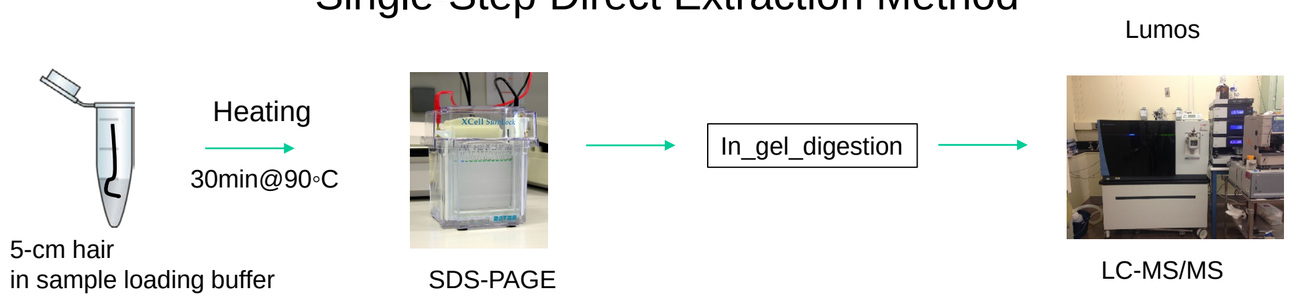
Result – Test B. *Comparison of Artefacts*



Test B: Comparison of experimentally introduced artifactual modifications among three methods using our recently developed hybrid search (PMID:28367633 DOI:10.1021/acs.jproteome.6b00988): Cleavable Surfactant method (red), NaOH+SDS method (green) and single-step Direct method (blue). The compared experimentally introduced artifactual modifications chosen as examples are: acetaldehyde (upper left), acetylation (upper right), formylation (lower left) and over alkylation (lower right).

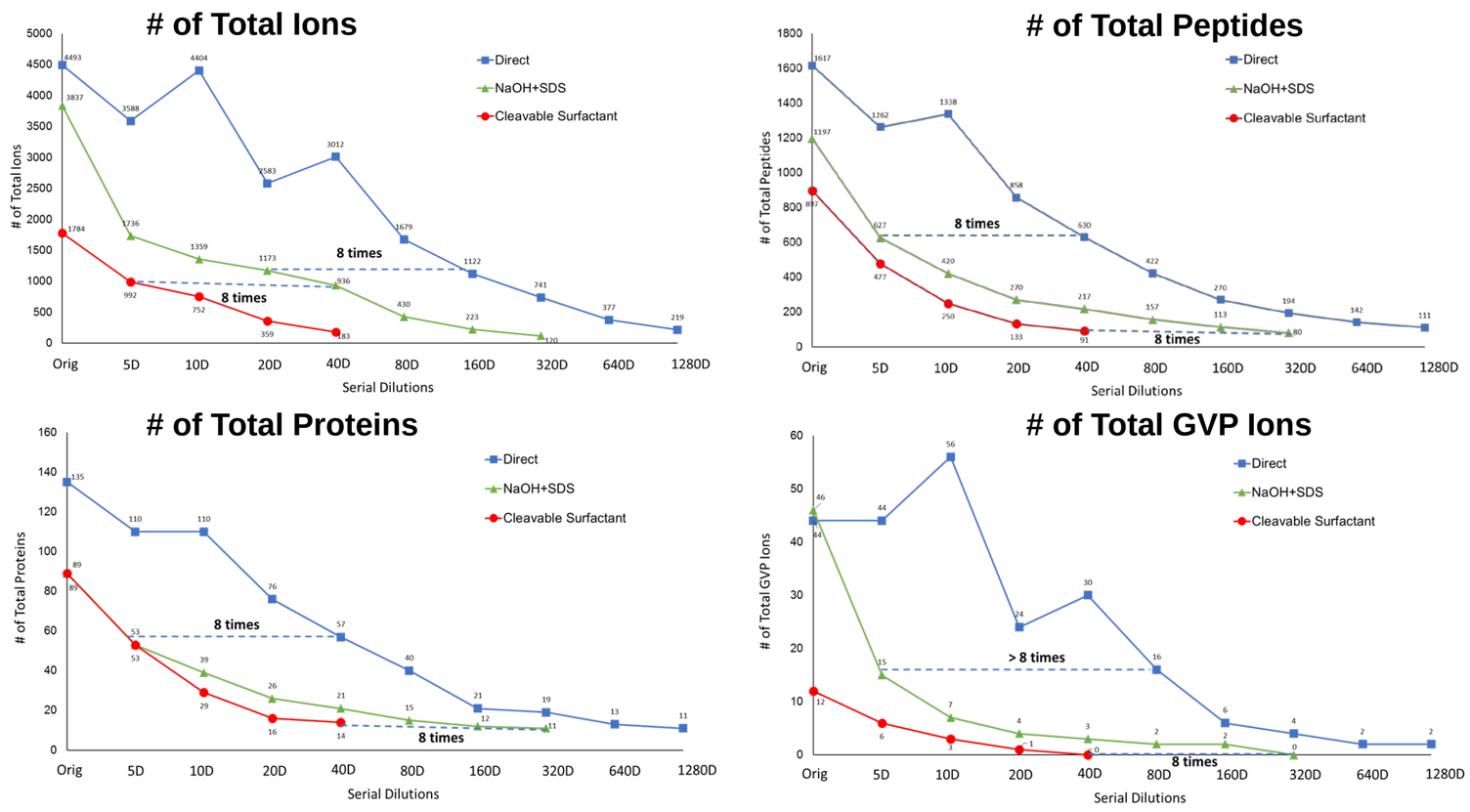
Methods

- Samples were commercially obtained. Five cm-long hair shaft sample was combined with 50 µl of the commercial sample loading buffer and 50 mM dithiothreitol (DTT). After heating at 90◦C for 30 min, extracted hair proteins were loaded onto a protein gel and then separated by size. We call this single-step protein extraction by heating a single hair shaft in sample loading buffer the 'Direct' method. After gel staining, in-gel-digestion was performed and extracted peptides were injected to a mass spectrometer for LC-MS/MS analyses.



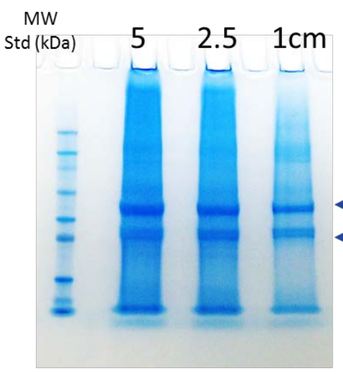
- We compared this single-step Direct extraction method to two previously published methods: 1) NaOH with SDS repeated extraction ('NaOH + SDS') method (PMID:27741315 DOI:10.1371/journal.pone.0164993); 2) Cleavable Surfactant (ProteaseMax) based method (PMID:27603779 DOI:10.1371/journal.pone.0160653)
- Using the raw data files generated from the study, a hair specific peptide mass-spectral library was constructed. It has a sequence coverage of hair cuticular keratins of about 70% and includes 14 previously reported GVPs.

Result – Test C. *Comparison of Sensitivity*



Test C: The sensitivity of all three methods were measured by comparing multiple metrics across a dilution series from 5D to 1280D: (upper left) the total number of ions; (upper right) the total number of peptides; (lower left) total number of proteins; (lower right) total number of published GVP ions detected in mass spectral data from 5 cm-long hair shaft sample derived proteins that were extracted using the Cleavable Surfactant method (red), NaOH+SDS method (green) or the Direct method (blue). Actual data has been labeled on the points of each dilution series.

Result – Test D. *Reduction of Hair Length*



In-gel digestion →

Hair Length	GVP Ions	Proteome		Hair Keratins	
		Proteins	Peptides	Proteins	Peptides
5 cm	44	135	1617	15	620
2.5 cm	40	86	1203	14	523
1 cm	39	78	1149	14	486

from fraction 6

Test D: This gel image (left) shows the separation of hair proteins from 5, 2.5, and 1 cm-long hair shaft samples from the same individual donor. A MW standard was loaded in the first lane. Bands for type I and type II hair cuticular keratins were labeled. The table (right) summarizes protein, peptide, and GVP ion identifications from 5, 2.5, and 1 cm-long hair shaft samples.

Summary & Conclusions

- **Our results show that single-step Direct method is the most sensitive and reliable method among the three tested methods.**
- **This finding was confirmed by determining that differences in the results of three tested methods are not due to experimentally introduced artefacts including modifications.**
- **The construction of a human hair specific peptide mass spectral library including previously reported GVPs provides a means of reliable identification of the human hair proteome, adding another layer of sensitivity to our method, for quick and accurate peptide identification and GVP detection.**