B151 Forensic DNA Typing of Envelopes and the Dye Quenching Phenomenon

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After attending this presentation, attendees will understand novel characteristics from envelopes when performing DNA typing procedures, and learn of means to troubleshoot problematic samples for DNA typing and analysis.

This presentation will impact the forensic community and/or humanity by allowing the forensic DNA community to troubleshoot problematic samples when it comes to envelope samples, and continue to keep lines of communication open within the community.

Introduction: With the recent expansion of the scope of DNA casework in forensic science, the processing of envelopes to elucidate a DNA profile has become a much more frequent endeavor. Envelopes sealed from licking can provide a low yield DNA sample that results in a full STR profile. Cuttings from the adhesive strip portion of the envelope are extracted, quantified, amplified, and analyzed using capillary electrophoresis. In a few isolated cases, the amplification products of the samples when electrophoresed on either the slab gel (377) or capillary (3100/3130) technology, the size standard became quenched. That phenomenon either produces low relative fluorescent units of the size standard or the entire sizing standards is absent in the data.

The purpose of this study was to try to determine the source of the size standard inhibition, to characterize the phenomenon, and to explore ways to obtain a full STR profile from problem samples.

Fingerprinting reagents can decrease the amount of DNA recovered. In a study done by members of the California Criminalistics Institute and the Latent Print Unit, they concluded that the majority of fingerprinting reagents (generally decreasing in DNA yield) did not inhibit the ability to obtain an STR profile from a bloody fingerprint in all test cases but one. It was found that a combination of “Stickyside” powder reagent and “Un-du” reagent was the only test scenario that gave no results.[2]

Ninhydrin (Triketohydrindane hydrate) is a chemical used to detect fingerprints on porous surfaces such as paper. Ninhydrin can be applied by dipping, brushing, or spraying the substrate. Ninhydrin reacts with free amines left over from proteins that are present in fingerprints, developing from a colorless liquid to a red/purple print only when exposed to high heat and humidity. The ninhydrin crystal is dissolved in HFE-7100, a CFC replacement consisting of a mixture of methyl nonafluoroisobutyl ether and methyl nonafluorobutyl ether. Un-du Adhesive Remover is a product used in fingerprinting to dissolve adhesive portions of envelopes and stamps in order to search for fingerprints underneath sealed surfaces. The primary ingredient in Un- du is the chemical heptane, $H_3C(CH_2)_5CH_3$.

Materials and Methods: Five extraction sets were run, a total of 23 envelopes were used and 53 total cuttings/samples were created. Chemical treatment, envelope type, envelope processing technique and incubation time were varied throughout extraction sets. Envelopes were treated with ninhydrin and Un-du. Steam development was withheld from some ninhydrin treated envelopes. Two cuttings were taken from the adhesive strip of each envelope. Cuttings were approximately 1cm x 2cm. Some of these cuttings were then teased apart to reveal the adhesive material of the envelope while other cuttings were not teased apart. All cuttings were processed separately. Standard Connecticut State Forensic Laboratory protocol was used for DNA extraction, quantification, amplification, and analysis. Three different envelopes were used during the experiments: plain white envelopes, state forensic lab addressed envelopes, and plain cream colored envelopes.

QIAquick PCR Purification: PCR product samples known to exhibit size standard quenching were cleaned using a Qiagen, QIAquick PCR Purification kit. This purification procedure is designed to remove impurities such as nucleotides, enzymes, mineral oil, salts, agarose, ethidium bromide, and primers. Binding buffer is added directly to the PCR sample, nucleic acids bind to the membrane in high-salt conditions, impurities are washed through the membrane, and pure DNA is eluted with a provided low-salt buffer or water.

NaOH treatment: Pre-PCR extracted DNA samples known to exhibit size standard quenching once amplified were cleaned using standard NaOH treatment protocol.[1] This protocol is designed to remove various compounds that intercalate into double-stranded DNA and inhibit taq polymerase. This method involves the denaturation and washing of the DNA sample using NaOH in Microcon-100 filtration units. This protocol has been found to be effective in removing inhibitors found in many substrates that are encountered with forensic evidentiary samples. However, as much as 50% of the DNA may be lost and therefore is not recommended for low yield DNA samples.

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Results and Discussion:
Extraction Set #1: Plain White Envelopes, Adhesive strip not teased apart, all envelopes licked

Envelope	Cutting	Treatment	DNA Yield	Size Standard	Profile
1	1	Un-du		Good	full
	2			Good	full
2	1	Ninhydrin, steam		Good	full
	2			Good	full
3	1	Nin+Un-du, steam	0.02 ng/ul	Good	partial (13 loci)
	2		0.00 ng/ul	Quenched	none
4	1	No chemicals		Good	full
	2			Good	partial (13 loci)

Extraction set 1 explored the effect of Un-du and ninhydrin on size standards and ability to obtain a full STR profile. All envelopes were plain white, adhesive strips were not teased apart, and all envelopes were licked. One cutting taken from an envelope treated with ninhydrin and Un-du with steam treatment was found to exhibit size standard quenching. In addition no profile was attained from this sample. Partial profiles were obtained from two other cuttings; however neither of these samples exhibited any kind of size standard quenching.

Extraction Set #2: State Envelopes, Adhesive strip teased apart, all envelopes licked

Envelope	Cutting	Treatment	DNA Yield	Size Standard	Profile
1	1	Un-du	0.02 ng/ul	quenched	none
	2		0.02 ng/ul	quenched	none
2	1	Ninhydrin, steam	0.02 ng/ul	quenched	none
	2		0.06 ng/ul	partially quenched	3 loci
3	1	Nin+Un-du, steam	0.02 ng/ul	quenched	none
	2		0.06 ng/ul	quenched	none

Extraction set 2 was done to further explore the effect of the fingerprinting chemicals on size standard quenching. State addressed envelopes were used, the adhesive strip was teased apart and all envelopes were licked. All cuttings were either fully or partially quenched. One cutting from the ninhydrin treated envelope exhibited partial quenching and yielded a partial profile. Only D8S1179, TPOX and Amelogenin loci had peak heights over calling threshold (50 RFU) for this sample.

Extraction Set #3: Plain White Envelopes, Adhesive strip not teased apart, all envelopes licked.

Envelope	Cutting	Treatment	DNA Yield	Size Standard	Profile
1	1	Un-du	0.12 ng/ul	good	full
	2		0.14 ng/ul	good	full
2	1	Ninhydrin, no steam	0.16 ng/ul	some quenching	full
	2		0.10 ng/ul	good	full
3	1	Ninhydrin, steam	0.12 ng/ul	good	full
	2		0.12 ng/ul	good	full
4	1	Nin+Un-du, no steam	0.26 ng/ul	good	full
	2		0.16 ng/ul	good	full
5	1	Nin+Un-du, steam	0.04 ng/ul	good	full
	2		0.08 ng/ul	good	full
6	1	untreated	0.24 ng/ul	good	full
	2		0.22 ng/ul	good	full

Extraction set 3 was done to further explore the effect of ninhydrin and Un-du on size standard quenching. Plain white envelopes were used, the adhesive strip was not teased apart and all envelopes were licked. Steam treatment, which is a necessary step during the ninhydrin treatment, was withheld from some of the envelopes. Sample 2-1 showed a subtle quenching pattern but still yielded a full profile. All other samples yielded a good size standard and full profile. DNA yield was consistent for all samples tested.

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Extraction Set #4: Plain White, State, Plain Cream, Adhesive strip processing varied, Untreated with fingerprinting chemicals.

Envelope	Cutting	Envelope Type	Adhesive Strip	DNA Yield	Size Standard	Profile
1	1	plain white	teased apart	0.02 ng/ul	good	full
	2		not teased apart	0.00 ng/ul	good	full
2	1	plain white	teased apart	0.04 ng/ul	good	full
	2		not teased apart	0.16 ng/ul	good	full
3	1	state	teased apart	0.08 ng/ul	good	none
	2		not teased apart	0.06 ng/ul	good	full
4	1	state	teased apart	0.08 ng/ul	good	full
	2		not teased apart	0.08 ng/ul	good	full
5	1	plain cream	teased apart	0.10 ng/ul	good	none
	2		not teased apart	0.04 ng/ul	good	full
6	1	plain cream	teased apart	0.18ng/ul	good	full
	2		not teased apart	0.04 ng/ul	good	full
7	1	plain white	Un-licked		good	none
8	1	state	Un-licked		good	none
9	1	plain cream	Un-licked		good	none

Extraction set #4 explores the possibility that the envelope type and processing method may result in size standard quenching. All three types of envelopes were used for this experiment and samples were processed by either teasing apart the adhesive strip or maintaining the strip as is. None of these envelopes were treated with fingerprinting chemicals.

None of the samples exhibited size standard quenching. DNA yield was fairly consistent with the exception of two samples that yielded no profile at all, despite relatively high DNA yield.

Extraction Set #5: Cuttings taken from previous “problem” envelopes (around original cutting), extraction incubation time varied.

Envelope	Cutting	Incubation Time	DNA Yield	Size Standard	Profile
Set2, Envelope #1	1	1 hr	0.04 ng/ul	quenched	none
	2	18 hr	0.16 ng/ul	good	full
Set2, Envelope #2	1	1 hr	0.18 ng/ul	partially quenched	partial (11 loci)
	2	18 hr	0.10 ng/ul	partially quenched	partial (7 loci)
Set2, Envelope #3	1	1 hr	0.02 ng/ul	quenched	none
	2	18 hr	0.20 ng/ul	good	full
Set1, Envelope #3	1	18 hr	0.04 ng/ul	good	full
	2	1 hr	0.00 ng/ul	quenched	none

Extraction set 5 was done to determine if cuttings taken from surrounding regions of previous “problem” samples would also result in size standard quenching. Cuttings of about 1cm^2 were taken from both sides of a “problem” cutting and combined for testing. Set 1, envelope 3, cutting 1 was included as a sample that did not contain quenching in the original cutting, yet was on the same envelope as a “problem cutting.” Incubation time during extraction was included as another variable. Many of the cuttings yielded very similar results to their corresponding “problem” cuttings with a few exceptions. Envelope 1, cutting 2 resulted in a good size standard and full STR profile. Envelope 2 yielded two partially quenched samples that both yielded partial profiles. Cutting two from the third envelope from set 2 yielded a good size standard and full profile. The sample that was not taken from a “problem area” did not exhibit any kind of size standard quenching. In general, the long (18 hour) incubation time resulted in samples that did not exhibit quenching. Two out of three cuttings taken from “problem” areas and incubated 18 hours were found to exhibit no size standard quenching. The third cutting was only partially quenched. While this could be coincidence, further exploration of incubation time might be useful. In general this evidence suggests that the source of the size standard quenching is not homogeneous across the adhesive strip of “problem envelopes.”

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Extraction set 6 attempted to answer the question of whether or not the adhesive strip is causing the quenching. In addition this experiment explored the effects of the fingerprinting chemicals. One state addressed envelope was used for this experiment. Adhesive strip cuttings were teased apart for one of the two adhesive strip cuttings for each chemical condition. Initially, the adhesive strip was licked and the envelope sealed. The entire back side (seal side) was then licked. Four cuttings were then taken, two of the adhesive strip and two from non-adhesive portions of the licked envelope. After these cuttings were taken the back of the envelope was treated with ninhydrin and four more cuttings were taken as before. Next, the back of the envelope was treated with Un-du. Finally, four more cuttings were taken; two from the adhesive strip and two from non-adhesive portions of the chemically treated envelope surface.

Extraction Set #6: State envelope, adhesive strip not teased apart, cuttings taken from licked adhesive stripand licked non-adhesive surface, all samples incubated for 1 hour.

Cutting	Surface	Treatment	Adhesive Strip	DNA Yield	Size Standard	Profile
1	Adhesive	none	teased apart	2.04 ng/ul	Good	Full
2	Adhesive	none	not teased	.64 ng/ul	Good	Full
3	Non-Adhesive	none	N/A	1.18 ng/ul	Good	Full
4	Non-Adhesive	none	N/A	.38 ng/ul	Good	Full
5	Adhesive	Ninhydrin	teased apart	.18 ng/ul	Good	Full
6	Adhesive	Ninhydrin	not teased	.22 ng/ul	Good	Full
7	Non-Adhesive	Ninhydrin	N/A	.14 ng/ul	Quenched	none
8	Non-Adhesive	Ninhydrin	N/A	.14 ng/ul	Quenched	none
9	Adhesive	Un-du (w/ Nin)	teased apart	.12 ng/ul	Good	Full
10	Adhesive	Un-du (w/ Nin)	not teased	.12 ng/ul	Good	Full
11	Non-Adhesive	Un-du (w/ Nin)	N/A	.14 ng/ul	Partially	none
12	Non-Adhesive	Un-du (w/ Nin)	N/A	.12 ng/ul	Quenched	none

Extraction set six demonstrated that non-adhesive cuttings could also result in quenching. Of the four partially or fully quenched samples all were cuttings taken from non-adhesive strip areas of the envelope. All cuttings taken from the adhesive strip resulted in a normal size standard. Fingerprinting chemical treatment resulted in far lower DNA yield than untreated cuttings. The average DNA yield for untreated cuttings was 1.06 ng/ul, while the average DNA yield for cuttings taken after ninhydrin treatment was 0.15 ng/ul. In addition, only after treatment with ninhydrin was any quenching noted.

The QIAquick PCR Purification procedure was successful in eliminating size standard quenching in all samples processed. Resulting samples yielded partial STR profiles. In general, samples showed expected alleles with peak heights ranging from 100-500 RFU at the following 5 loci: Amelogenin, D8S1179, TH01, vWA, TPOX. Expected allele peak heights ranging from 10-50 RFU were detectable at the following 5 loci: D21S11, D16S539, D2S1338, D19S433, D5S818. No peak heights were detectable at the following 6 loci: D3S1358, D13S317, D7S820, CSF1PO, D18S51, FGA. The pattern of peak heights and loci dropout does not correlate to the DNA fragment size of the loci.

NaOH treatment was unsuccessful in removing size standard quenching in all three test samples. The QIAquick PCR Purification procedure was then performed on these samples. Once again the procedure was successful in getting rid of size standard quenching phenomena, however once cleaned there was no STR profile at all. The lack of STR profile might be explained by the loss of DNA characteristic of the NaOH treatment. The fact that the treatment was unsuccessful in removing the quenching suggests that the compound of interest does not intercalate exclusively into double stranded DNA.

Only envelopes treated with the fingerprinting chemicals were found to exhibit any kind of size standard quenching. It seems likely that the fingerprinting chemicals increase the chances of finding this phenomenon. There was no definitive pattern to the size standard quenching based on other variables induced. Cuttings from the same envelope may not exhibit the same result. Extraction set 6 showed that the adhesive strip is likely not the source of the quenching.

It seems likely that the fingerprinting chemicals are causing the size standard quenching phenomena. In addition the fingerprinting chemicals appear to generally decrease DNA yield. Chemical analysis of extracted DNA may be necessary to determine the chemicals responsible for the size standard quenching. An alternate method for envelope processing may be necessary. It may be advisable for future cases to take cuttings of envelopes for DNA before exposing the envelope to fingerprinting chemicals.

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References:

[1] Bourke MT, Scherczinger CA, Ladd C, LeeHC. NaOH treatment to neutralize inhibitors of Taq polymerase. J Forensic Sci 1999;44(5):1046-1050. [2] Terry Spear and Neda Khoshkebari, California Criminalistics Institute, CA DOJ; Jeanne Clark and Michael Murphy, Latent Print Unit, CA DOJ. "Summary of Experiments Investigating the Impact of Fingerprint Processing and Fingerprint Reagents on PCR-based DNA Typing Profiles".

DNA, Envelopes, Dye Quenching

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