Project Title:

Improved Nucleic Acid Recovery From Trace and Degraded Samples Using Affinity Purification

Award Recipient Organization:

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Project Summary

Goals and Objectives

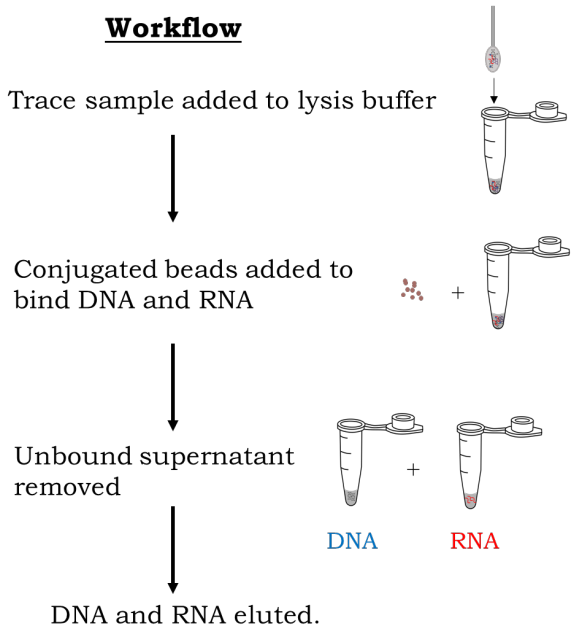
The objective of the project is to develop a novel trace sample nucleic acid recovery approach, including new methods and reagents, that maximizes DNA yield of intact and degraded DNA. This enables development of non-destructive/minimally destructive methods for evidentiary sample collection. Further, this represents a tool and method that can separate multiple analytes from typical trace and forensic samples that are limited in yield and/or have degraded DNA, while not reducing the efficiency of downstream forensic methods.

Maximum recovery of trace DNA is critical to downstream DNA profiling success. Since the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories, Standard 9.4 states that there is a requirement to quantify DNA in forensic casework samples prior to STR typing, and because very low amounts of recovered DNA produce profiles with very low levels of interpretability, extraction methods that maximize yield are critical. Commercially available kits for nucleic acid recovery can lose trace DNA during extraction and purification due to multiple wash steps and buffer exchanges. Commercially available DNA isolation kits are also destructive to RNA, proteins, and metabolites, because they are designed with the sole intent of isolating DNA. There is an emerging interest in analyzing molecules beyond DNA. For example, RNA can help identify tissue type and post-mortem interval, genetically variant peptides (GVPs) complement incomplete DNA profiles from touch samples, and metabolites can provide insight into toxicology.

Research Questions:

1. Can we develop a novel capture agent that co-binds DNA and RNA?
2. Can the capture agent co-elute DNA and RNA?
3. Can the capture agent differentially elute DNA and RNA?
4. Can we develop a protocol that captures and elutes DNA and RNA from trace blood, saliva, touch and semen samples?
5. What is the impact of environmental exposure on DNA recovery?

Summary of project design and methods:



Highlights

- Beads bind DNA and RNA with >95 % efficiency.
- Beads bind highly degraded DNA (~150 bp) with >95 % efficiency.
- All-in-one method increases recovery since sample is not divided into separate extractions.
- Analytes are compatible with downstream processing.
- Protocol is compatible with automation.

Figure 1: Depiction of novel workflow

Chemicals and beads: Magnetic SpeedBeads (Cytiva) were conjugated with the nucleic acid binding protein using a proprietary method that controlled the binding density. The beads were stored wet in TTNA buffer (100 mM Tris, 150 mM NaCl, 7.7 mM NaN3, 0.1% (vol) Tween 20, pH 7.5) at 4 degrees C. Prior to use, the beads were washed twice to remove excess sodium azide and detergent. Lysis buffer contained 0.4% sodium dodecyl sulfate (SDS, Invitrogen 15553-035) and/or 5-40mM DL-Dithiothreitol (DTT, Promega P117A), and SDS sequestration buffer contained alpha-cyclodextrin powder (Sigma C4642) resuspended to 50 mg/ml in nuclease free water.

DNA and RNA processing and quantification: Genomic DNA (>50kb; Promega G1521) was used to develop binding and elution protocols. The DNA was sheared to 4-10 kb (~6 kb average size) using a Covaris g-tube according to manufacturer's protocol and to 40-200 bp (average ~150 bp) using a Covaris M220 focused ultrasonicator. The size of the DNA was confirmed by agarose gel electrophoresis after shearing. DNA sizes bound and eluted were assessed by agarose gel electrophoresis. Quant-It PicoGreen (ThermoFisher P7589) and Quantifiler Trio (Applied Biosystems 4482910) was used for DNA quantification. Human Spleen Total RNA (Takara 636525) was used to develop RNA binding and elution protocols. Quant-It RiboGreen (ThermoFisher R11491) was used for RNA quantification. To separately quantify RNA after differential elution, the samples were treated with DNase I in 1x DNase buffer for 30 minutes at 37 degrees C. Optionally, DNA samples were treated with RNase A. Samples were read on a Spectromax M5.

Nucleic acid binding and elution: 170 – 300 ug of beads (wet weight) were added to the sample and incubated for 30 minutes at room temperature. The tubes were then placed on Dynamag to

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collect beads for approximately 30 seconds. While on the magnet, the tubes were slowly twisted to ensure all beads were bound to the magnet. The supernatant containing unbound protein and metabolites was collected. The tubes were removed from the magnet, then 50 ul of elution buffer was added and incubated for up to 30 minutes at room temperature. The tubes were again placed on the magnet for approximately 30 seconds, and the eluted nucleic acid was recovered. Binding buffer was 50mM Tris pH8, 150 mM NaCl, 1mM EDTA and trace detergent. Elution buffer was 0.5M to 0.8M NaCl.

STR: Up to 500 pg of DNA was analyzed by Powerplex fusion 6C kit (DC2705, Promega) and or half-reaction GlobalfilerTM (4476135, ThermoFisher) and was analyzed by capillary electrophoresis using the ABI3500 genetic analyzer.

RT-PCR: After body fluid elution, mRNA was reverse transcribed using a Lunascript kit, amplified with 2x platinum mastermix using fluorescently labeled primers for body fluid specific transcripts below (as defined in [38]) and the B-actin housekeeping gene, then analyzed by capillary electrophoresis. For blood, a 103 bp sequence from the ALAS2 gene was analyzed using (ALAS2Fwd (VIC) CTG CAC CAG AAG GAC TCA GCC; ALAS2Rev TAA ATC TCG CAC CCT GGC AGG ATC) and a 151 bp sequence from the CD93 gene (CD93Fwd (6FAM) ACC AGT ACA GTC CGA CAC; CD93Rev TTG CTA AGA TTC CAG TCC AG). For saliva, a 134 bp sequence from the HTN3 gene was analyzed using (HTN3Fwd (VIC) GCA AAG AGA CAT CAT GGG TA; HTN3Rev GCC AGT CAA ACC TCC ATA ATC). For touch samples, a 99 bp sequence from the LCE1C gene was analyzed using (LCE1CFwd (NED)TGT GAC CCC GCT CCT GAA TCC G; LCE1CRev CTT GGG AGG GCA CTT GGG GGT G). For semen, a 121 bp sequence from the SEMG1 gene was analyzed using (SEMG1Fwd (6FAM) GGA AGA TGA CAG TGA TCG T and SEMG1Rev CAA CTG ACA CCT TGA TAT TGG). B-actin housekeeping gene primers amplify a 75 bp sequence (ActBFwd (6FAM) TGA CCC AGA TCA TGT TTG AG and ActBRev CGT ACA GGG ATA GCA CAG).

Blood isolation protocol: 1 ul of blood was deposited on glass, polypropylene or brass surfaces. A Copan FLOQ swab (519CS01) was pre-wet with 5 ul of 0.4% SDS in 10 mM Tris HCl pH 8 and 1mM EDTA. The swab was immersed in 300 ul of 0.4% SDS in 10 mM Tris HCl pH 8 and 1mM EDTA for 30 minutes at room temperature with 1400 rpm agitation. The swab and excess liquid was placed in a DNA IQ spin basket (Promega, V1225) and spun for 5 min at 7000xg. Liquid that passed through was transferred to a clean low bind tube. Alpha-cyclodextrin (ACD, Sigma C4642) was added to achieve a 1:1 molar ratio of ACD:SDS and incubated for 10 minutes at room temperature. The sample was brought up to 500 ul total volume using 4.2x concentrated binding buffer, such that the final buffer concentration was 50mM Tris HCl, pH 8, 150mM NaCl, 1mM EDTA, trace detergent. Beads were then added to the sample and incubated for 30 minutes at room temperature, following the standard binding and elution protocol.

Saliva isolation protocol: Saliva samples were obtained from volunteer donors using an IRB approved protocol for collection of passive drool into a 50 ml conical tube. The donors did not eat or drink for 30 minutes prior to sample collection. Saliva was spiked into lysis buffer or spotted onto surfaces within 3 hours of collection. 1 ul of saliva was used to test trace sample recovery. A Copan FLOQ swab was pre-wet with 5 ul of 0.4% SDS in 10 mM Tris HCl pH 8 and 1mM EDTA. The swab was immersed in 300 ul of 0.4% SDS in 10 mM Tris HCl pH 8 and 1mM EDTA for 30

minutes at room temperature with 1400 rpm agitation. The swab and excess liquid was placed in a spin basket and spun for 5 min at 7000xg. Liquid that passed through was transferred to a clean low bind tube. ACD was added to achieve a 1:1 molar ratio of ACD:SDS and incubated for 10 minutes at room temperature. The sample was brought up to 500 ul total volume using 4.2x concentrated binding buffer, such that the final buffer concentration was 50mM Tris HCl, pH 8, 150mM NaCl, 1mM EDTA, trace detergent. Beads were then added to the sample and incubated for 30 minutes at room temperature, following the standard binding and elution protocol.

Semen isolation protocol: Semen was purchased from Innovative Research (#IRHUSMS1ML). Up to 2 ul of semen was lysed in 20 ul of 0.4% SDS, 10mM Tris, 1mM EDTA, 40mM DTT and placed in a thermomixer at 45C for 30 min at 900rpm. 2.5µL of lysate from step 1 was added to the binding reaction with 70µg of washed beads in TNT up to 500µL (50mM Tris, 150mM NaCl, 1mM EDTA, trace detergent). Sample was vortexed and briefly centrifuged, then placed in a thermomixer at room temperature at 900 rpm for 30min. Beads were then added to the sample and incubated for 30 minutes at room temperature, following the standard binding and elution protocol.

Touch sample isolation protocol: Touch samples from volunteer donors were deposited on glass, polypropylene, or brass using an IRB approved protocol. Volunteers were asked to wash their hands with soap and water, then air dry their hands for 2 minutes. Donors then rubbed their hands and fingers together vigorously and touched their nose and forehead to apply sebum onto their fingers. Donors then touched the surfaces firmly while rolling their fingers and thumb on the surface (using a process similar to fingerprints taken by police). A Copan FLOQ swab was pre-wet with 20 ul of 0.01% SDS in 10 mM Tris HCl pH 8 and 1mM EDTA. The swab was immersed in 80 ul of 5-10 mM DTT in 10 mM Tris HCl pH 8 and 1mM EDTA to for 30 minutes at 45 degrees C, with 1400 rpm agitation. The swab and excess liquid was placed in a spin basket and spun for 5 min at 7000xg. Liquid that passed through was transferred to a clean low bind tube. The sample was brought to 500 ul total volume in 1.3x concentrated binding buffer, such that the final buffer concentration comes to 1x concentration (50mM Tris HCl, pH 8, 150mM NaCl, 1mM EDTA, trace detergent). Beads were then added to the sample and incubated for 30 minutes at room temperature, following the standard binding and elution protocol.

Summary of results

Trace DNA binding and elution: In order to establish protocols for binding and elution of DNA, >50kb genomic DNA was sheared to 4 to 10 kb size as this represents the DNA size that frequently is found in forensic environmental samples. These experiments established a robust and reproducible protocol for binding and elution (depicted in figure 1) consisting of up to a 30 minute room temperature incubation of beads in 50 to 500 ul of binding buffer, then use of a Dynamag magnet to collect the nucleic acid bound beads for approximately 30 seconds. Subsequently, the supernatant containing unbound protein and metabolites is collected and then the nucleic acid is eluted off the bead using 0.5 to 1.2 M NaCl at room temperature for up to 30 minutes.

The recovery efficiency of 10 to 100 ng input DNA starting amounts was similar to 500 pg trace DNA recovery. Comparing to PrepFiler beads (Figure 2), we found comparable or better elution, and more consistent performance, from our prototype beads (>90% elution at input amounts as low as 500 pg DNA) than from the standard PrepFiler protocol (70-80% elution, more inconsistent). The initial and eluted DNA was run on an agarose gel to assess any bias in

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binding/elution of specific sizes of DNA, and no bias was found; DNA as small as 25 bp, and as large as the limit of detection of the agarose gel (>15kb) efficiently bound and eluted. Thus, we confirm that our prototype bead formulation performs as well as a well-validated commercial kit.

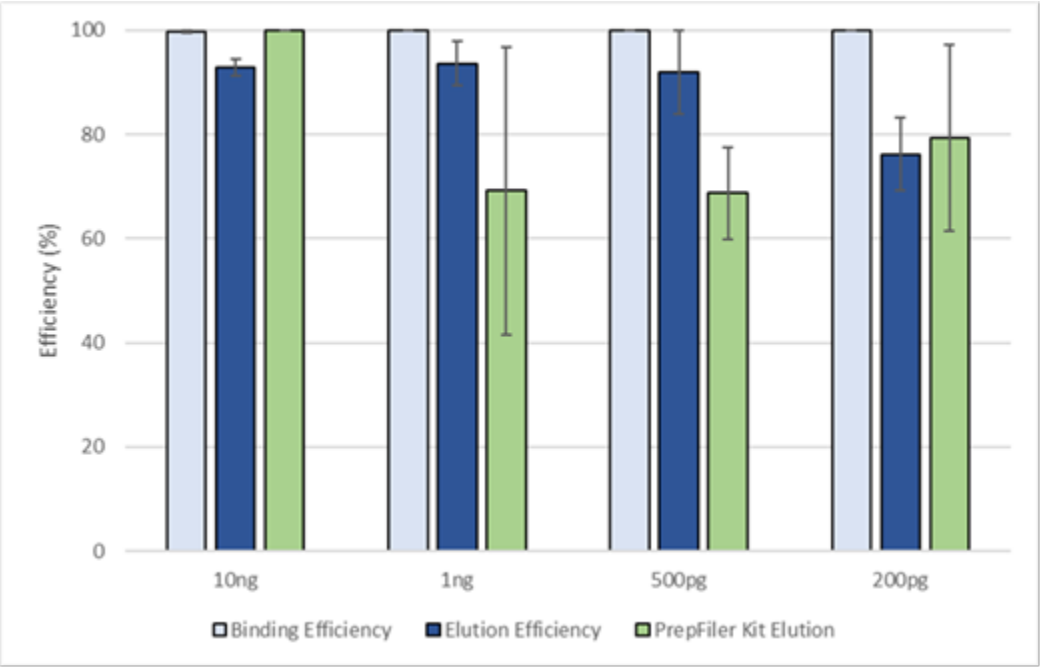

Figure 2: DNA binding and elution efficiency

Heavily degraded DNA binding and elution: Highly degraded genomic DNA samples, sheared using a Covaris M220 sonication device to an average size of 150 bp (range 25-500 bp), were generated to serve as a surrogate for heavily environmentally damaged/aged forensic samples. The Quantifiler Trio degradation index value was ~5.5 for the sheared DNA. Our beads were compared to the commercial kit PrepFiler for binding and elution of heavily degraded DNA (Figure 3). Using 0.8M NaCl as the elution buffer, our prototype beads performed similar to PrepFiler at degraded DNA concentrations down to 200 pg with recoveries of 60%-80%.

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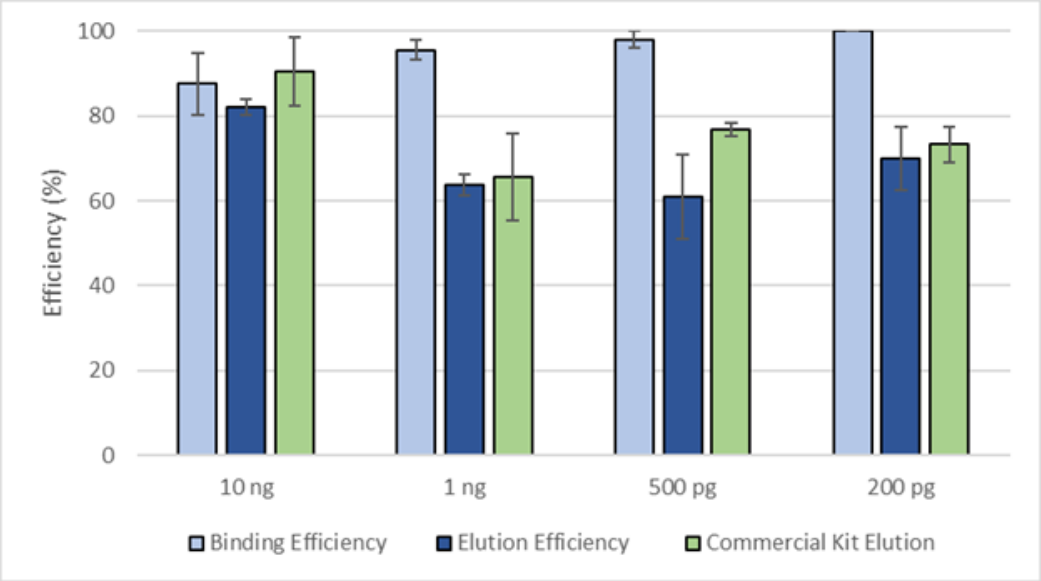

Figure 3: Degraded DNA binding and elution efficiency

All sizes of DNA efficiently bind and elute: DNA was sheared to 20-800 bp in size to mimic severely damaged DNA caused by environmental conditions and aging. The DNA was denatured to single stands by heating to 95 degrees C or kept double stranded, then protein conjugated beads or non-conjugated negative control beads were added. All sizes of DNA bound efficiently, as depicted by the lack of DNA in the supernatant and presence of all sizes of DNA in the eluted fraction including 20-100 bp and 20-100 nucleotide fragments (Figure 4). This is a significant finding because recovery of very small DNA fragments using traditional ion exchange resins and silica beads is inefficient.

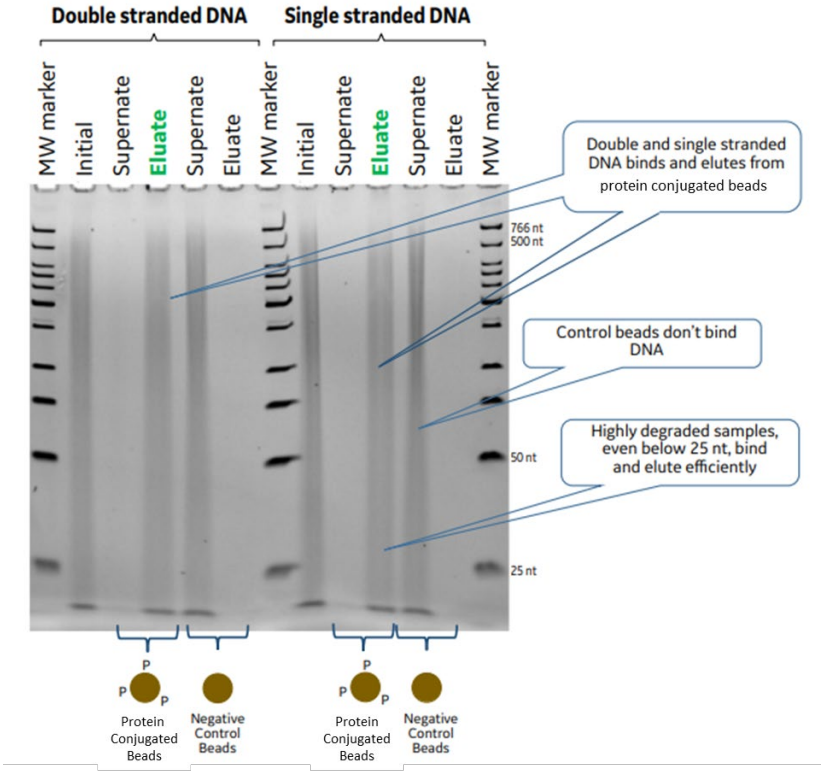

Figure 4: Efficient binding and elution of highly degraded DNA

RNA binding and elution: To develop protocols for RNA binding and elution, 2.5 ng to 100 ng of human spleen total RNA was used. Efficient RNA binding and elution was achieved using the

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same binding buffer and 0.8M NaCl elution buffer. The eluted RNA was quantified using the Quant-itTM RiboGreen RNA assay providing consistent very high levels of binding (> 95%) and elution (> 89%), with most samples eluting at >95% efficiency in 0.8M NaCl (Figure 5). Thus, the nucleic acid binding protein beads recover both DNA and RNA using the same buffers and binding/elution conditions.

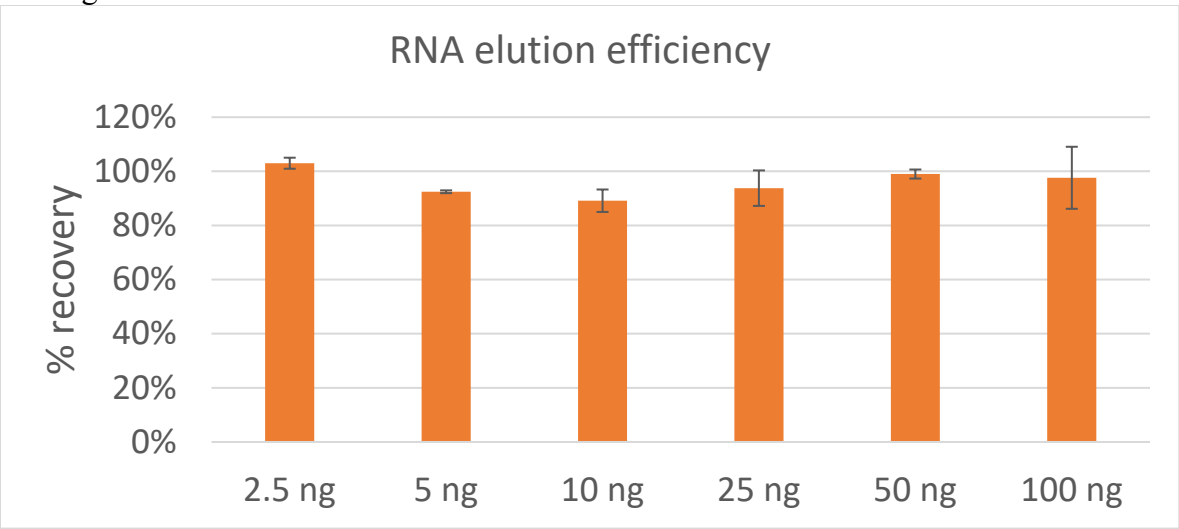

Figure 5: RNA binding and elution efficiency

DNA and RNA co-binding and co-elution: Subsequently, co-binding and co-elution of 4 - 10 kb genomic DNA and total RNA within the same input sample was assessed using a single binding and elution condition. Samples tested included a mix of DNA and RNA at 1:1, 5:1 and 1:5 ratios (comprising 20 to 100 ng input amounts). To eliminate DNA/RNA cross-detection using PicoGreen and RiboGreen, eluted samples were digested with 1U of RNase A prior to DNA quantification, or 1U of DNase I prior to RNA quantification. Mixed DNA and RNA samples were recovered at similar efficiency as the individual nucleic acid control samples (Figure 6). Thus, these data confirm that DNA and RNA can be co-bound and co-eluted from the same sample with recoveries consistent with prior single molecule inputs.

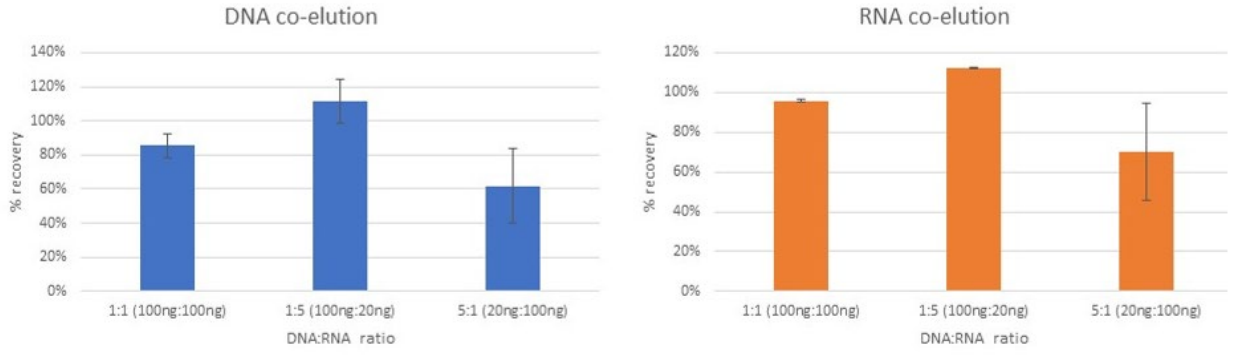

Figure 6: DNA and RNA co-binding and co-elution

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Differential DNA and RNA elution: While co-elution of DNA and RNA may be sufficient for many forensic applications, differential recovery of DNA and RNA may be advantageous in other conditions, particularly in situations where trace samples would need to be split into two fractions each containing half the initial amount of DNA and RNA. Modulating the salt concentrations in elution buffers promoted stepwise elution of DNA followed by RNA, but the differential samples were not pure. The greatest differential between DNA and RNA elution was observed at 0.5M NaCl vs 0.8M NaCl. 0.5M NaCl was sufficient to elute ~80% of the bound DNA, with minor RNA contamination (about 5% of the total RNA) (Table 1). Likewise, 0.8M contained about 95% of the RNA with ~10-15% of the total DNA eluting in the 0.8M NaCl fraction. Thus, a trade-off of differential elution is reduced total yield relative to co-elution. However, differential elution separates and isolates >80% of each nucleic acid type into two separate fractions compared to 50% of each nucleic acid type per fraction if the sample was simply split into two. Future studies may identify elution conditions that more fully separate pure DNA and pure RNA with minimal cross-contamination.

Table 1. Differential elution of DNA and RNA as a function of buffer salt concentration.

	DNA Recovery	RNA Recovery
Elution 1 in 0.5M NaCl	88%	5%
Elution 2 in 0.8M NaCl	12%	95%
Total Yield	~80 ng	~100 ng

DNA and RNA recovery from bodily fluids: The bodily fluids blood, saliva, semen and touch samples were deposited on glass, polypropylene and brass, and tested for recovery of DNA and RNA leveraging the protocols developed above. Samples were collected on Copan FLOQ swabs. Blood and saliva were lysed in buffers containing 0.4% SDS with or without 5mM DTT. Semen was lysed in buffers containing 0.4% SDS and 40mM DTT. Touch samples were lysed in buffers containing 5 to 10 mM DTT. The SDS tolerance of the nucleic acid binding protocol is ~0.0025% to 0.005% final concentration, therefore a method to sequester SDS was developed using alpha-cyclodextrin (ACD). ACD is a cyclic oligosaccharide that has specificity to SDS, sequestering it in solution and restoring function to SDS sensitive enzymatic reactions such as PCR [16]. ACD effectively neutralizes 0.4% SDS lysis buffers at a 1:1 molar ratio, and enables DNA and RNA binding to the magnetic beads.

The nucleic acid binding protein conjugated lacks cysteine amino acids, and therefore should be relatively resistant to reducing agents. Reducing agents help facilitate lysis of certain case type samples with cell membranes that contain an abundance of crosslinked proteins (such as cornified epithelial cells and semen). Efficient DNA and RNA binding was achieved at 2 mM DTT concentrations, and in some samples efficient nucleic acid recovery was obtained from samples containing up to 10 mM DTT.

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As depicted in Figure 7, high DNA and RNA recovery was obtained from blood, saliva and semen. Recovery from touch samples were on par with expected results. From 1 ul of blood, ~15-20 ng DNA was obtained from glass and polypropylene surfaces, and ~9 ng DNA from brass. From 1 ul of saliva ~15 ng DNA was recovered from glass and ~2.5-5 ng DNA from polypropylene and brass. From 2 ul of semen on glass, ~18 ng DNA was recovered. This recovery is in-line with published reports that estimate the DNA content to be 14.6 ng/µL [17]. Touch samples from thumbprints on glass and polypropylene provided 500 pg to 2.6 ng DNA. The high yield is likely from the isolation protocol which may have collected loose dead cells from the forehead. Minute DNA recovery (~30 pg) was obtained from touch samples deposited onto brass. Thus, we confirm compatibility of the new bead protocols with multiple types of bodily samples recovered from multiple surfaces.

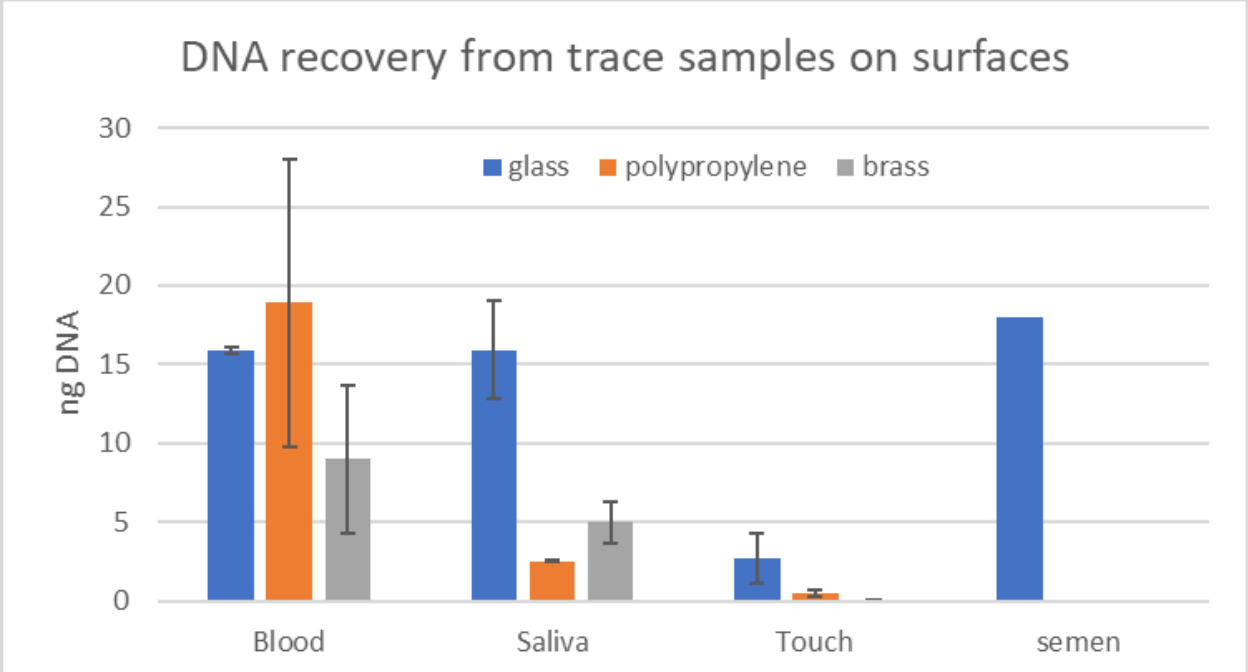

Figure 7: DNA recovery from trace (~1 ul) bodily fluids on various surfaces

To assess co-recovery of DNA and RNA from case type samples, the DNA content was quantified using Quantifiler Trio and RNA was quantified using RiboGreen. Figure 8 shows RNA yields of on average 9.4 ng from 1 ul of blood, 6.8 ng from 1 ul of saliva, and 2.8 ng from thumb touch samples. The high amount of RNA in the touch samples was likely due to the collection protocol which uses forehead and nose as a sebum source which may also collect live epithelial cells. In addition, differential DNA and RNA recovery was confirmed on blood and saliva samples, in which 0.5M NaCl elution recovered ~90% of the total DNA and >80% of the RNA was present in the 0.8M elution fractions (Figure 9).

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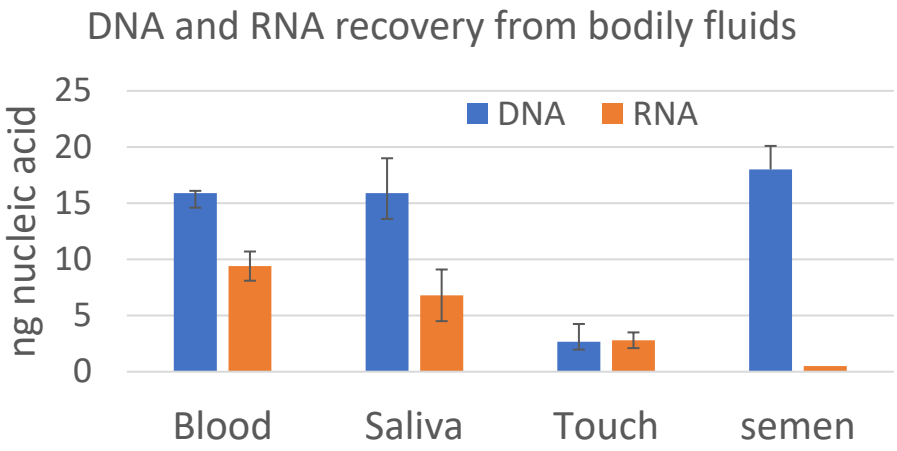

Figure 8: DNA and RNA recovery from trace (~1 ul) bodily fluids

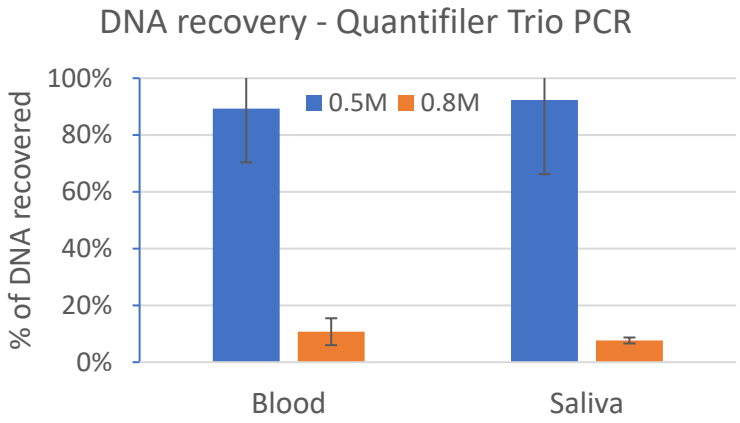

Figure 9: Differential DNA recovery from bodily fluids

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RT-PCR: Bodily fluid derived nucleic acid isolates was evaluated by Taqman RT-PCR for tissue specific gene expression, using primers and probes described in [18]. Figure 10 shows specific peaks for the saliva marker HTN3 (40 cycles PCR) and the semen marker SEMG1 (30 cycles PCR), confirming the presence of co-eluted RNA from these bodily fluids. These results demonstrate a process for simultaneous human identification and confirmation of the body fluid source from the same eluted sample.

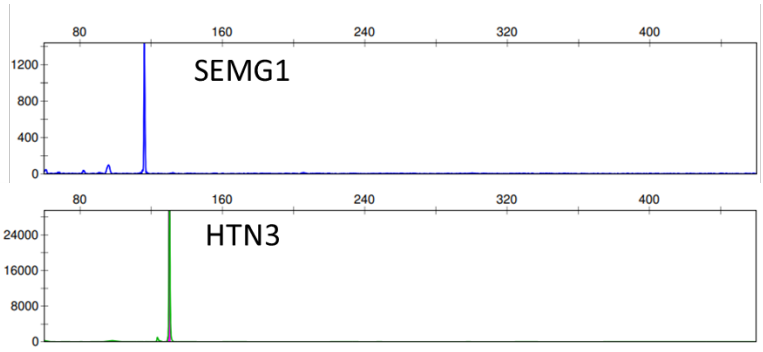

Figure 10. cDNA amplification demonstrating body fluid specific RNA recovery from nucleic acid binding beads

Beads bind environmentally damaged UV exposed DNA: 1 ul of blood was spotted on glass slides and allowed to air dry. The samples were then exposed to UV-B radiation from a laboratory transilluminator device for 2 minutes, 30 minutes, 4 hours or 16 hours. After the exposure, samples were stored for 1-4 days prior to recovery using FLOQ swabs and nucleic acid binding beads as described above. DNA spotted on the slide and exposed similarly were used as controls. DNA content was analyzed by Quantifiler Trio and PicoGreen. The untreated control and 2 minute UV exposure provided ~15 ng DNA recovery, and as expected, prolonged exposure of blood and naked DNA to UV-B (30 minutes or longer) interfered with PCR, giving Quantifiler Trio yields of <1 ng (Figure 11a). The degradation index on the naked DNA was 5.5 after 30 minutes of UV, while SA and LA quantified as 0 at the higher exposure times, indicating very severe degradation. In blood samples, the DNA was more protected, with DI or 1.5 after 30 minutes, ~4 after 4 hours and ~7 after 16 hours. We used PicoGreen to confirm DNA content as DNA damage induced by UV-B does not interfere with intercalation into DNA.

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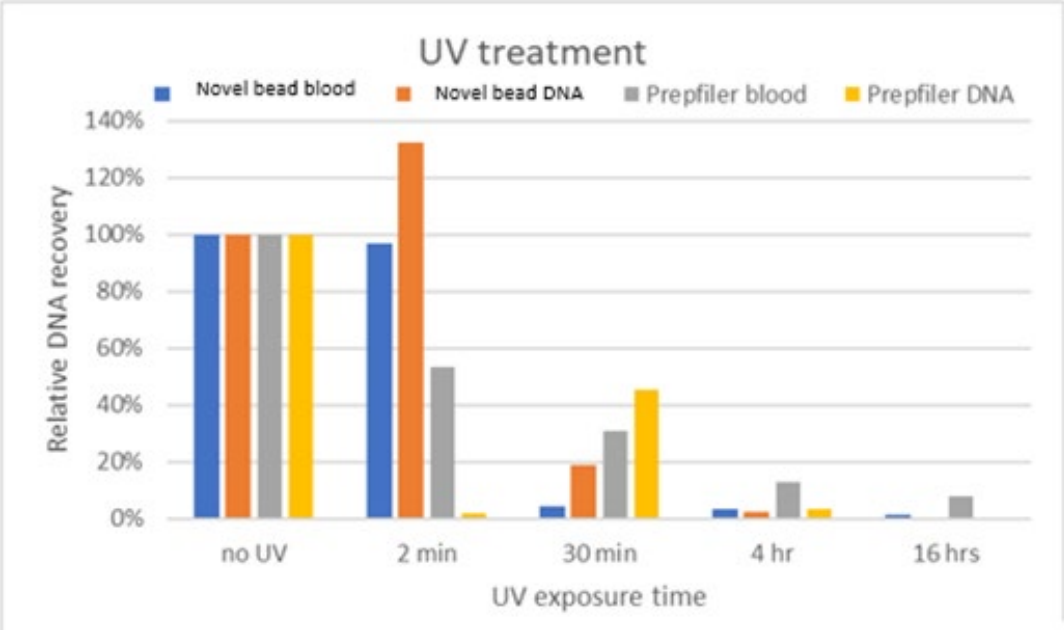

Figure 11a. DNA recovery after UV treatment quantified by Quantifiler Trio

Figure 11b shows PicoGreen results from the nucleic acid bead collected samples, confirming that we successfully extracted the DNA from UV irradiated samples, but the Quantifiler Trio method was negatively impacted by the presence of thymine dimers after UV treatment.

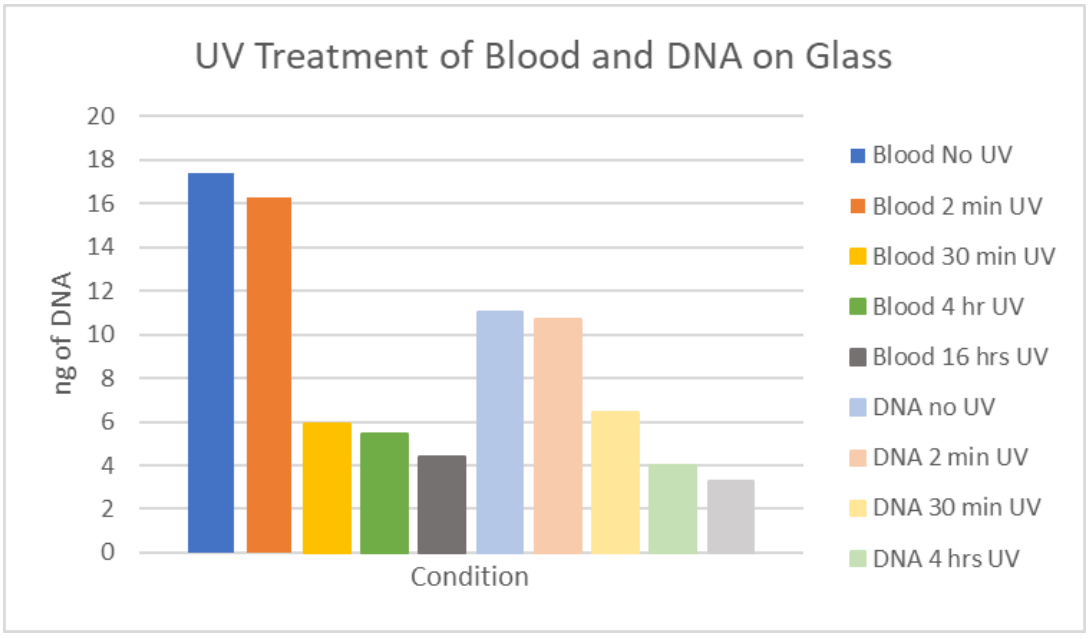

Figure 11b. DNA recovery after UV treatment quantified by PicoGreen

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STR analysis on DNA isolated from a semen sample: DNA and RNA was co-extracted from semen samples. In these samples, the RNA underwent cDNA synthesis and was analyzed by RT-PCR using SEMG1 specific primer/probes. Concurrently, 0.5 ng of DNA was amplified using GlobalfilerTM (half-reaction) and short tandem repeats were analyzed by capillary electrophoresis using the ABI3500 genetic analyzer, confirming that a full profile was obtained. These data importantly present an example in which a bodily fluid was lysed, nucleic acid recovery was performed using the new beads, and both DNA and RNA from the same sample were successfully used in commonly applied forensic assays for individualization (STR profiling) and body fluid identification (cDNA amplification).

Applicability to criminal justice:

Trace biological samples are increasingly being analyzed in forensic testing laboratories, creating a need to maximize the amount of DNA recovered from such samples even when the DNA is degraded. In addition, analytes other than DNA are proving critical in casework analysis. RNA can provide insight into the post-mortem interval (PMI) and body fluid identification. For example, mRNA is important in sexual assault casework to detect the presence of semen (on victim) or vaginal secretion (on suspect). Despite the need for a multi analyte approach becoming very apparent, there are no commercially available multi analyte recovery kits for trace biological samples. Here, we describe a novel reagent and protocol that provides highly efficient recovery of trace amounts of DNA and RNA.

Trace DNA samples make up an increasing proportion of DNA casework in the USA, and a significant number of cases go unsolved due to insufficient DNA yield. For example, a recent review on property crime casework by the New York State Police Forensic Investigation Center (NYSP FIC) revealed that DNA evidence from such samples was insufficient in 50% of cases [4]. Likewise, the West Virginia State Police Forensic Laboratory communicated that samples with very low DNA quantities (0.001-0.010 ng/μL) have been found to result in very low rates of interpretability (0-11 %) using their current interpretation protocols [5]. Therefore, accessibility of reagents that maximize recovery of trace amounts of DNA is essential for successful DNA profiling of more casework samples.

In addition to property crimes, other types of casework reliant on recovering trace DNA include those involving firearms. Evidence from property and firearm related crimes comprise a significant proportion of a crime laboratory's caseload e.g., so far in 2023, 27 % and 12 % of the NYSP FIC's casework reports involved property and firearms [4]. Further, New York City has recently announced the first DNA Gun Crimes Unit which will perform DNA profiling of evidence from gun crimes in ≤ 30 days [9]. Recovering DNA from fired ammunition is known to be problematic since the firing process results in gases, high temperature, and high pressure [10]. Further, brass, mostly used for ammunition, consists of copper and zinc, and is a difficult surface to obtain DNA from since the DNA is thought to undergo oxidative damage upon interaction with copper. Although incorporation of additives in the rinsing and swabbing buffer used for collection such as BSA and a 3 amino acid peptide (GGH) which bind copper was found to improve DNA recovery, only 40 % of casework samples resulted in at least 50 pg total DNA [11]. Yet another area of forensic investigation reliant on successful trace DNA recovery is that of illicit drug manufacture

and trading. Studies using mock trials and volunteers have shown that external surfaces of Zip-lock bags frequently used to package illicit drugs [12] and the exterior surface of capsules and tablets of MDMA or Ecstasy can be sampled for DNA [13].

Methods used for purification of trace DNA from crime scene samples include silica-membrane-based columns for manual processing or silica-based magnetic particles for automated workflows. Solid phase column-based methods can result in loss of up to 70 % of the DNA due to inefficient binding to the silica membrane after adding the lysate. The DNA that is lost has been found in the flow-through which is normally discarded [6, 7]. Losses up to 40 % have also been observed using silica beads and extraction robots [8]. Severely degraded DNA often encountered in forensic casework can also be difficult to recover. Silica based solid phase extraction columns often have a cut-off for DNA fragment size and will not effectively bind fragments below that length. Thus, silica bead-based methods are considered limited in their capacity to bind trace amounts of highly degraded DNA [14]. However, degraded DNA can yield useful profiles e.g., the CE-based Globalfiler™ kit has 10 STR loci within the mini-STR size range (<220 bp) [1] and the Massively Parallel Sequencing (MPS) based ForenSeq™ DNA Signature prep kit has been observed to successfully amplify 90 % of the 20 CODIS loci in highly degraded samples (~200 bp) [15]. Taken together, any improvement in the yield of DNA from property, firearm, and drug related crime evidence will make a significant difference in rates of successful DNA profiling.

While the target for STR genotyping and human individualization is clearly DNA, there is a growing need for analysis of multiple analytes in forensic casework. mRNA profiling is proving to be one of the best methods for the identification of body fluids which is critically important in sexual assault casework and in associating a body fluid to a DNA profile via cSNPs. There is currently no validated multianalyte recovery method available for forensic samples. Qiagen's AllPrep® kit is a multianalyte kit that can extract DNA and RNA using spin columns, but it is designed for tissue and cultured cells i.e., high amounts of biological material. DNA recovery rates using our prototype method show equivalence to a widely used commercial kit (Prepfiler™).

PRODUCTS:

- List of all scholarly products (with DOIs or other durable links), including:
    - Peer reviewed publications
        - A manuscript for peer-reviewed publication is currently in progress.
    - Book chapters, theses, conference proceedings, etc.- none
    - Technologies developed (patents, prototypes, etc.)-
        - GE HealthCare has decided not to patent this technology.
    - Software, databases, other products- none
    - Archived research data
        - Data is archived on FigShare 10.6084/m9.figshare.25928188

- List of all dissemination activities (with DOIs or other durable links, if available), including:
    - Conference presentations
        - We have presented poster presentations on our preliminary findings at 2022 ISHI, 2023 ISHI, the graduate student symposium associated with 2023 ISHI, and at the 2023 NIJ Research and Development symposium. We also gave an oral presentation at the 2024 NIJ Research and Development symposium.
    - Webinars, workshops- none
    - General press, podcasts, and other media- none

REFERENCES

[1] Ludeman MJ, Zhong C, Mulero JJ, Lagacé RE, Hennessy LK, Short ML, Wang DY (2018) Developmental validation of GlobalFiler™ PCR amplification kit: a 6-dye multiplex assay designed for amplification of casework samples. International Journal of Legal Medicine 132: 1555-1573.

[2] Giovanelli A, Garrido RG, Rocha A, Hessab T (2022) Touch DNA recovery from vehicle surfaces using different swabs. Journal of Forensic Science 67:701-711.

[3] Fullár A, Kutnyánszky V, Leiner N (2020) Identification of burglars using foil impressioning based on tool marks and DNA evidence. Forensic Science International 316:110524.

[4] Fitzpatrick M (2023) NY State Police Forensic Investigation Center, personal communication.

[5] Haynes J (2023) West Virginia State Police, Biology Unit Supervisor, personal communication.

[6] Menchhoff SI, Delacruz MT, Hytinen ME, Cox JO, Miller MT, Cruz TD (2020) DNA purification cell lysis and wash step modifications for low-template DNA sample processing. Journal of Forensic Science 65, 597-600.

[7] Katevatis C, Fan A, Klapperich, CM (2017) Low concentration DNA extraction and recovery using a silica solid phase. PLoS ONE 12(5): e0176848, 2017.

[8] Romsos EL, Vallone PM (2019) Estimation of extraction efficiency by droplet digital PCR. Forensic Science International: Genetics Supplement Series 7, 515-517.

[9] Taylor M (2022) NYC launches nation's first DNA gun crimes unit. Forensic magazine. Available at https://www.forensicmag.com/587857-NYC-Launches-Nation-s-First-DNA-GunCrimes-Unit/?catid=26395. Accessed 3/16/2023.

[10] Elwick E, Gauthier Q, Rink S, Cropper E, Kavlick MF (2022) Recovery of DNA from fired and unfired cartridge casings: comparison of two DNA collection methods. Forensic Science International: Genetics 59:102726.

[11] Bille TW, Fahrig G, Weitz SM, Peiffer GA (2020) An improved process for the collection and DNA analysis of fired cartridge cases. Forensic Science International: Genetics 46: 102238.

[12] Fonneløp AE, Faria S, Shanthan G, Gill P (2022) Who packed the drugs? Application of Bayesian networks to address questions of DNA transfer, persistence, and recovery from plastic bags and tape. Genes 13: 18.

[13] Griffin A, Kikbride KP, Henry J, Painter B, Linacre A (2021) DNA on drugs! A preliminary investigation of DNA deposition during the handling of illicit drug capsules. Forensic Science International:Genetics 54: 102559.

[14] Rubenstein L (2020) Comparing two automated methods of DNA extraction from degraded skeletal remains. Forensic Medicine and Anatomy Research 9, 24-30.

[15] Sharma V, van der Plaat D, Liu Y, Wurmbach E (2020) Analyzing degraded DNA and challenging samples using the ForenSeq™ DNA Signature Prep kit. Science & Justice 60, 243- 252.

[16] Nakanishi, et.al. (2022) Plant and Cell Physiology, Volume 63: 8, 2022, Pages 1025–1028

[17] Xavier MJ, et.al. (2018) PLOS One 13:e0195003

[18] M. van den Berge, et.al. (2016) Prevalence of human cell material: DNA and RNA profiling of public and private objects and after activity scenarios Forensic Sci. Int. Genet., 21, 81-89.