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(54) Title: APPARATUS AND METHOD FOR COLLECTING TOUCH DNA

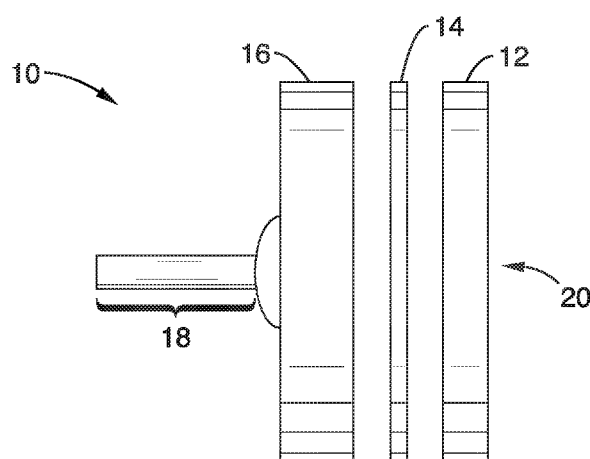


FIG. 1

(57) Abstract: Devices and methods for the collection and storage of low copy number DNA such as touch DNA for later analysis are provided. The collector has a collector matrix with a non-reactive, non-porous backing. The matrix is impregnated with a chemical composition capable of lysing cells and denaturing proteins. Nucleic acids are deposited directly on the collector matrix and stabilized for long term storage eliminating the need to transfer the DNA that can decrease the overall DNA yield. The collector can be used to collect touch DNA over large surface areas before drying out or becoming saturating with a material as compared to traditional touch DNA collection methods.

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APPARATUS AND METHOD FOR COLLECTING TOUCH DNA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. provisional
5 patent application serial number 62/394,241 filed on September 14, 2016,
incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

10 **[0002]** Not Applicable

INCORPORATION-BY-REFERENCE OF COMPUTER PROGRAM APPENDIX

15 **[0003]** Not Applicable

BACKGROUND

[0004] 1. Technical Field

[0005] The technology of this disclosure pertains generally to forensic
20 nucleic acid material collection devices and fabrication methods, and more
particularly to a device for collecting, purifying, and storing nucleic acids
from low copy DNA sources for later identification.

2. Background Discussion

[0006] DNA is the genetic blueprint of an individual and each individual's
25 DNA is unique. Like fingerprints, the analysis of DNA is a highly
discriminating method for identification. Consequently, the analysis of DNA
has proven to be a powerful tool for solving crimes.

[0007] Forensic DNA samples are typically collected from blood, saliva,
semen, and other bodily fluids as well as from bones, hair and skin found at
30 a crime scene. DNA evidence is collected at a crime scene to accurately
detect and identify suspects as well as rule out suspects and to establish
other facts related to the crime. DNA crime scene evidence may be
presented at trial to assist a jury in their determination of the guilt or

innocence of the accused.

[0008] However, the collected specimen must yield enough DNA for analysis to allow the identification of a sufficient number of genetic features to permit DNA matching with an alleged suspect with a high degree of confidence. This has been made possible with the development of powerful amplification methods. These methods substantially increase the number of copies of extremely small sample amounts that are often undetectable without amplification. Polymerase chain reaction (PCR) is the amplification method that is commonly used in forensic detection settings. The small DNA sample is used as a replication template for creating large numbers of copies that can be easily detected and evaluated by conventional DNA analysis techniques. However, the effectiveness and reproducibility of the PCR amplification is dependent may be greatly influenced by the purity and quantity of the sample DNA template. Samples may not only have contaminating DNA but they may also have molecules that inhibit PCR amplification or damage the sample. Therefore, precise collection and the removal or deactivation of inhibitors is important to performing successful PCR amplifications.

[0009] One type of DNA forensic evidence that is desirable can be collected from fingerprints or from surfaces that have been contacted by a potential suspect. DNA fingerprints from fingerprints is called "Touch DNA" and typically yields very small amounts of low copy number (LCN) DNA, often from only one or two cells. Touch DNA may be present in limited quantities at a crime scene, and high recovery is critical in order to allow DNA typing. In many cases, touch DNA samples only yield a partial profile that is frequently attributed to the low shedding status of a donor. Low yields can also be explained by ineffective collection methods that leave a portion of deposited DNA behind.

[0010] Forensic analysis of touch DNA evidence was first described in 1997 by Van Oorschot and Jones. Since then, the topic of touch DNA has become of particular interest to researchers in the field of Forensic Science. Now this type of evidence is frequently used in criminal cases worldwide

and the number of cases solved solely by virtue of touch DNA has grown dramatically. Each year approximately 800 thousand vehicles are stolen in the United States and an estimated 4.9 million vehicles are stolen worldwide. Many of the stolen vehicles are used to transport illegal substances or are involved in other types of crimes. In many cases, touch DNA collected from the steering wheel is the only evidence that can link a perpetrator to the crime (e.g. most recent driver to the carjacking).

[0011] Despite the widespread use of touch DNA evidence, there is no standardized touch DNA collection protocol. The collection methods used for low copy DNA specimens by many labs were optimized for high copy number DNA evidence. Not all techniques that are used for blood, sperm and saliva DNA collection are effective for collecting touch DNA. Since most of the time bodily fluids are present in abundance at the crime scene, there is no need to collect DNA from the entire specimen. Touch DNA, on the other hand, is present in limited quantities and therefore high recovery is critical.

[0012] Most forensic laboratories use cotton swabs to collect touch DNA using a double swab technique. Tape lifting using water soluble tape is an alternative method that is used to collect touch DNA evidence. Collection swabs are typically kept in sterile containers or bags before use and returned to the sterile container after collection to limit contamination. The container is usually marked with information such as the time, date, location and the identity of the collector to establish a chain of custody for the sample.

[0013] Conventional touch DNA collection devices are designed to collect touch DNA over a small surface area. The swab, for example, has a small surface area that becomes saturated with extraneous material very quickly. Once saturated, the swab can smear or spread additional and already-collected material over a large area rather than collecting more material. However, at a crime scene, the location of the deposited touch DNA is typically unknown and can only be approximated. Therefore, there is a need for a method that can effectively collect small deposits of touch DNA

scattered over a large surface area. There is also a need for a DNA collection device for efficient and reliable recovery of forensic low copy number DNA from fingerprints.

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BRIEF SUMMARY

[0014] The present technology generally provides a biological material collector apparatus that is designed to speed up and increase the efficiency of the touch DNA collection process and similar acquisitions of low copy DNA. The device is a sensitive crime scene collection tool for maximum recovery of trace forensic DNA evidence that has been left at a crime scene for subsequent DNA PCR analysis.

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[0015] The apparatus provides for the efficient collection, storage, and purification of nucleic acids, such as DNA or RNA, from biological samples such as cells for subsequent genetic characterization. The nucleic acids can be released from the collector after collection or storage and then amplified by conventional amplification methods such as polymerase chain reaction and evaluated. For, example DNA analysis of collected trace DNA can be obtained by generating small tandem repeat (STR) profiles.

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[0016] In general terms, the collector has a preferably flexible base with an optional handle or coupling for reversibly attaching a handle to the base. The base of the collector may be made from a flexible foam square or may be formed from a rigid material. A collector matrix sheet with a liquid impermeable membrane on the back side may be mounted to the base or directly to the impermeable membrane. In various embodiments, the backing membrane may comprise cellophane or other material that prevents liquid from permeating through. In other embodiments, the collector matrix and backing membrane are not joined with a base. The collector has a larger surface area than double swabs and can cover larger surface areas before drying out and/or saturating with a material, when compared to traditional touch DNA collection methods (e.g., double swabbing).

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[0017] The collector matrix layer is preferably an absorbent layer that can

be made from a variety of materials that may be woven fabrics, nonwoven materials or foams. Preferred materials include such as cotton, nylon, cellulose-based materials such as blotter or filter paper as well as paper/cloth blend materials and microcellular foams and similar materials.

Preferred materials do not bind nucleic acids irreversibly.

[0018] The absorbent collector matrix is preferably impregnated with a chemical composition that lyses cells and denatures proteins and separates the nucleic acids. When cells, for example, are collected by the collection matrix they are lysed and the nucleic acids are immobilized and stabilized within the collector matrix. The DNA is effectively deposited directly on to the collector matrix eliminating the need to transfer the DNA to a separate storage material that often decreases the overall DNA yield. The chemical composition can include components that can inhibit microbial growth that can damage the DNA and pollute the identification schemes. In one preferred embodiment, the chemical composition comprises a mixture of a weak base; a chelating agent; an anionic detergent; and optionally a uric acid or urate salt. Particularly preferred embodiments include a weak base of tris(hydroxymethyl)methane, a chelating agent comprising ethylenediaminetetraacetic acid (EDTA) and a detergent comprising sodium dodecyl sulfate (SDS).

[0019] Further aspects of the technology described herein will be brought out in the following portions of the specification, wherein the detailed description is for the purpose of fully disclosing preferred embodiments of the technology without placing limitations thereon.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0020] The technology described herein will be more fully understood by reference to the following drawings which are for illustrative purposes only:

[0021] FIG. 1 is a schematic exploded side view of a collector according to one embodiment of the technology.

[0022] FIG. 2A is a front view of a steering wheel structure and sampling of

water-soluble tape lifting or double swabbing collection techniques that were used to collect touch DNA from the surface of the steering wheel and then compared.

5 **[0023]** FIG. 2B is a front view of a steering wheel structure and sampling of FTA paper scraping or double swabbing collection techniques that were used to collect touch DNA from the surface of the steering wheel and compared.

10 **[0024]** FIG. 3 is a graph of% STR Allele Recovery of Last Driver's Profile for the steering wheels of eight cars that were compared based on how well the swab or tape collection technique recovered the last driver's profile.

15 **[0025]** FIG. 4 is a graph of% STR Allele Recovery of Last Driver's Profile for the steering wheels of eight cars that were compared based on how well the swab or FTA pad collection technique recovered the last driver's profile.

20 **[0026]** FIG. 5 is a functional block diagram a method for using the collector apparatus for the collection and storage of low copy number DNA and analysis for forensic investigation and evidence.

DETAILED DESCRIPTION

25 **[0027]** Referring more specifically to the drawings, for illustrative purposes, embodiments of devices and methods of collection and storage of low copy number DNA for later analysis are generally shown. Several embodiments of the technology are described generally in FIG. 1 and FIG. 5 to illustrate the characteristics and functionality of the apparatus and system. It will be appreciated that the methods may vary as to the specific steps and sequence and the systems and apparatus may vary as to structural details without departing from the basic concepts as disclosed herein. The method steps are merely exemplary of the order that these steps may occur. The steps may occur in any order that is desired, such that it still performs the goals of the claimed technology.

30 **[0028]** Generally, the apparatus for low copy nucleic acid collection is described in the context of forensic touch DNA collections from a surface such as a steering wheel to illustrate the technology. Although the methods

are demonstrated with touch DNA collections, the apparatus and methods can be adapted and applied to other conventional collection settings.

[0029] Turning now to FIG. 1, one embodiment of a collector 10 that is particularly suited for collecting small quantities of material over a comparatively large collection area is shown schematically and not to scale. The collector 10 shown in the exploded view of FIG. 1 has a collector sheet 12 that is mounted to a liquid impermeable membrane 14. The membrane 14 is coupled to a base 16 that has a handle 18 to allow the user to avoid touching and contaminating the collector sheet 12 during use.

[0030] The handle 18 can be a hard plastic member such as a disk that is mounted with a generally perpendicular orientation to the face 20 of the collector sheet 12. The user can pinch the handle 18 with the first finger and thumb during use. In another embodiment, the handle 18 is a shaft of a variety of lengths that further isolates the user from the collection surface 20 of collector sheet 12 during use. In another embodiment, the handle 18 is a loop of flexible material that can receive one or two fingers therein to facilitate use. In a further embodiment, the base 16 has a coupling that permits the collector 10 to reversibly couple the base 16 to a telescoping handle 18, for example.

[0031] The base 16 is preferably made from a flexible and resilient material such as foam to allow flexibility in the collector to adapt to rough or non-planer surfaces including cylindrical or rounded surfaces. In another embodiment, the base 16 is made from a rigid material making the collector 10 particularly suited for collections from smooth planar surfaces. Samples are typically collected from surfaces such as metal, plastic, concrete, vinyl, wood, glass, and even some fabrics.

[0032] The membrane 14 separating the collector sheet 12 from the base 16 is preferably liquid impermeable so that biological material that is collected by the collector sheet 12 is isolated and contained by the collector sheet 12. In one embodiment, the membrane 14 is made from a non-reactive and non-porous material such as cellophane. In another embodiment, the membrane is made from a hydrophobic material. The

membrane 14 prevents liquid from permeating through to the base and increases the DNA collection yield by preventing loss of collected DNA material from the collector sheet 12.

[0033] In one embodiment, an apparatus for collecting touch DNA from a surface comprises an "collector matrix" (a matrix comprising cotton, a paper/cloth blend material or any type of cellulose-based material, or filter material preferably contacted with a chemical composition that lyses cells and denatures proteins), and one or more sides of the collector matrix are contacted with a non-reactive, non-porous backing membrane 14.

[0034] The collector sheet 12 is preferably absorbent and porous and capable of absorbing or adsorbing fluids and biological materials such as touch DNA to the collector sheet 12 material. Suitable materials for the collector sheet 12 should not irreversibly bind nucleic acids (i.e. through covalent or ionic bond formation) and preferably should be substantially hydrophilic or capable of being made hydrophilic. The porous material of the collector sheet 12 may be a paper/cloth blend, cotton fibers, filter material, blotter paper, absorbent foam or a cellulose-based material, for example. The porous material of the collector sheet 12 may also be woven or non-woven or be made of porous polymer based materials such as nylon or polyester etc.

[0035] A chemical composition capable of lysing cells and denaturing proteins is preferably disposed on a collecting surface and within the pores of the absorbent collecting sheet 12. Cells and proteins of biological samples that are collected at the collecting surface 20 of the absorbent collector sheet 12 are lysed and denatured on contact by the chemical composition and absorbed by the absorbent sheet 12 and the nucleic acids remain intact are immobilized and stabilized within the matrix. In one embodiment, the chemical composition is composed of a weak base; a chelating agent an anionic detergent; and optionally a uric acid or urate salt. In one preferred embodiment, the weak base is tris(hydroxymethyl)methane (THYM) or Tris(hydroxymethyl)aminomethane, the chelating agent is ethylenediaminetetraacetic acid (EDTA), and the detergent is sodium

dodecyl sulfate (SDS).

[0036] At the time of collection, the surface 20 of collecting sheet 12 may be primed with an optional collection solution such as a few drops (1-8) of deionized water or other solvent to dampen the collector sheet 12.

5 Buffered saline, lysis buffer, SDS are suitable alternatives to water. In some settings a dampened collection sheet 12 increased the efficiency of the collector in the acquisition of small numbers of cells and/or amounts of nucleic acids.

[0037] The collectors 10 may be kept in sterile containers that can be sealed and avoid contamination before and after use. The containers can be marked with sample collection information such as the date, time and place of the sample collection as well as the name of the person taking the sample.

[0038] The stabilized nucleic acids in the absorbent collector sheet 12 may be stored with the collector for a period of time before processing or the collecting sheet 12 may be separated and removed from the collector body and stored separately. In one embodiment, only a portion of the collecting sheet 12 is processed and a second portion is stored for archival purposes and later processing if there is some question about the results from the first portion.

[0039] Collectors 10 and containers may be individually packaged or they may be assembled into self-contained sampling kits. Sampling kits may include instructions, labels, log, collection solution, and a number of collectors and storage containers, for example. The kit can be used to safely transport the collected samples without leakage from the site for further analysis. The sealed container of the kit that stores the used collector can also serve as a long term storage container.

[0040] The collectors 10 can be used with high sensitivity PCR DNA testing protocols for amplification. The collectors can also be used with a method for collecting and preserving nucleic acids from trace forensic biological samples in one step because the collection matrix also serves as a sample preservation and storage matrix.

[0041] For example, the collectors provide a method of acquiring and analyzing trace DNA from a fingerprint or trace cells. Referring now to FIG. 5, one method 100 for the collection and identification of forensic touch DNA is shown. At block 110, low copy number (LCN) DNA is acquired in an analyzable amount with the use of the collector apparatus and placed and sealed in a container. At block 120, the sample that has been stabilized the DNA in the collector and sealed in the container can be transported and preserved without contamination. The stabilized DNA in the collector and container can be stored for long periods of time if necessary before analysis. The sample can also be divided and portions analyzed and other portions stored to replicate results if necessary.

[0042] At block 130 of FIG. 5, the trace DNA is released from the collector for analysis. The DNA samples may be further purified from the cell constituents upon release. Because of the small quantities of nucleic acids that are present in the sample, the released DNA is preferably amplified and analyzing using a high sensitivity PCR DNA testing protocol in the step at block 140 of FIG. 5.

[0043] The amplified DNA is now in sufficient quantities to reliably analyze unique markers and other characteristics of the DNA to identify its origins. Accordingly, at block 150, multiple loci of the DNA are identified for comparison with DNA marker entries in a crime database or to processed DNA samples from a specific suspect.

[0044] The technology described herein may be better understood with reference to the accompanying examples, which are intended for purposes of illustration only and should not be construed as in any sense limiting the scope of the technology described herein as defined in the claims appended hereto.

[0045] Example 1

[0046] In order to demonstrate the operational principles of the apparatus and collection methods, a collector similar to that shown in FIG. 1 was prepared and evaluated. The collector had a collector sheet of a 3 cm x 3 cm cotton fabric that had been soaked in a mixture of chemicals that lyses

cells and denatures proteins. One side of the collector fabric was covered with a non-reactive, non-porous cellophane backing that prevented liquid from permeating through the collector fabric. The collector fabric was then attached to a foam base of a 2.8 cm x 2.8 cm flexible foam square that contained a handle located in the middle of the square for a better grip.

[0047] Before the start of the collection process, in one embodiment the prepared collector fabric was connected to the applicator and 4 drops of deionized water was administered to the surface of the fabric. Then, the collector was used to scrape or rub the surface of the fabric against the surface of the steering wheel 22 in a circular motion as shown in FIG. 2B. Fingers could be used to apply light pressure to the foam part of the collector for better collection results or to go over curvy areas.

[0048] Accordingly, the collector described herein has a larger surface area and can cover larger surface areas before drying out and/or saturating with a material, when compared to traditional touch DNA collection methods (e.g., double swabbing). The collector has all the advantages of FTA cards (no extraction process, long term DNA storage), plus it allows one to deposit a DNA directly on an storage fabric that eliminates the need to transfer DNA that in turn can decrease the overall DNA yield. The collector in this example used a modified version of FTA paper (wet FTA paper easily tears apart on a rough surface); that is, filter paper has been replaced by a cotton fabric that is firmer and does not break apart easily. It was found that FTA paper collected higher yields of touch DNA compared to standard DNA collection method.

[0049] Example 2

[0050] To better understand the capabilities of the apparatus and collection methods, three different DNA collection techniques for touch DNA collection were tested and compared. Conventional double swabbing collection with a circular motion was directly compared to tape lifting collection as shown in FIG. 2A and FTA paper scraping methods with the collector as shown in FIG. 2B for collecting touch DNA deposited on steering wheels 22 of vehicles under circumstances that closely resemble a real life situation. The

steering wheel is an ideal surface for touch DNA collection as it is relatively smooth and prone to a long contact with the driver's hands. In order to replicate a real life situation, a blinded study was performed with little or no information about the drivers, their shedding status, hygiene routine, and driving schedule. In order to maximize DNA recovery, DNA was collected over the entire area of each half of the steering wheel 22. A total of 70 cars were selected to participate in this study. All of the selected cars were in fully operational conditions; all were driven for 2 minutes to 60 minutes before the touch DNA collection. The steering wheels of the cars were selected for hard plastic, relative cleanliness and absence of wheel covers.

[0051] Car owners were asked to provide a reference cheek swab and answer a questionnaire. 35 cars were selected to compare the "gold standard" double swabbing method to tape lifting method, and another 35 cars were selected for double swabbing vs. FTA paper scraping method comparison.

[0052] As shown in FIG. 2A, one half of a steering wheel 22 was sampled using a double swabbing technique with two swabs 24 and the other side of a steering wheel 22 was sampled tape 26 applied over the surface of the wheel. Similarly, one half of a steering wheel 22 was sampled using a double swabbing technique with swabs 28, while the other side of a steering wheel was sampled using the FTA paper scraping technique with the collector 30 as shown in FIG. 2B. Swabs 24, 28 in both evaluations were moistened with de-ionized water before the sample collection. Each Whatman WB120205 FTA Classic Card was cut into four pieces; each piece was used to scrape one side of a steering wheel. Four drops of de-ionized water were applied to an FTA card before the sample collection. A 6 cm piece of 3MTM Water-Soluble Wave Solder Tape 5414 was used to collect DNA from one side of the steering wheel.

[0053] Steering wheel collection in both comparisons randomly alternated sides in order to minimize the possibility that the difference in DNA yield was due to a difference in shedding status between the left and right hands or bias in driving hand.

[0054] DNA was thereafter extracted from the collected samples from the swabs, buccal swabs, collector and tape using commercial DNA extraction kits. Then, real-time qPCR analysis using the Promega® PlexorHY Human Quantitation kit following the manufacturer's procedure was used to
5 determine the amount of DNA present in each sample. Each sample was quantified two times to increase the accuracy of the estimated DNA amount; if a CT difference greater than 0.5 was observed between the replicates then the quantitative analysis was repeated.

[0055] Thirty-two samples were selected from both studies for STR
10 analysis; eight pairs were selected from the double swabbing vs. tape lifting study and eight pairs were selected from the double swabbing vs. FTA paper scraping study. AmpFLSTR Identifiler™ kit was used to generate a driver's profile from the 32 selected samples and the 16 corresponding buccal swabs. Results were analyzed for mixed profiles. Results from STR
15 analysis were used to assess the difference between the quantifiable results versus typable results. DNA collection methods were compared based on how well they recovered the STR profile of the most recent driver by counting the number of observed alleles.

[0056] Detectable quantities of touch DNA were recovered from each of the
20 70 steering wheels sampled independent of collection method and randomized for collecting from the right or the left side for a total of 140 samples.

[0057] Statistical tests were used to determine if there was a difference in DNA yields for the double swabbing versus tape lifting comparison. The
25 data were $\log_{10}$ transformed in order to improve normality of variables. A Wilcoxon Signed Rank test was used to determine if the differences in DNA yield between swabbing versus tape lifting were statistically significant and no significant difference in yield was observed ($p = 0.21$).

[0058] The data from the double swabbing versus FTA paper scraping
30 study were also $\log_{10}$ transformed. A Wilcoxon Signed Rank test was used to determine if the differences in DNA yield between swabbing versus FTA scraping were statistically significant. The difference in yield between the

FTA scraping and the double swabbing was statistically significant with FTA paper scraping method yielding higher amounts of touch DNA ($p=0.017<0.05$).

[0059] Thirty-two samples were selected for STR analysis from both studies from samples that contained at least 1 ng of DNA. The effectiveness of the DNA collection methods were compared based on how well they recovered the last driver's STR profile by comparing the number of observed alleles from the steering wheel profile to the expected drivers' profile (% STR allele recovery). For the double swabbing versus tape lifting study, the mean % STR allele recovery was higher for double swabbing (90.8%) when compared to tape lifting (59.4%) and shown in FIG. 3.

[0060] For the double swabbing versus FTA paper scraping study, the mean % STR allele recovery of the DNA was slightly higher for double swabbing (99.4%) than for FTA paper scraping (91.1%) as seen in FIG. 4. A full STR profile was observed for 21 out of the 32 samples (65.6%); only four of the 21 full profiles were from a single source. No profile was generated for two of the 32 samples and both samples were collected using tape. Nine samples (28.1%) yielded only partial profiles and five of these samples generated searchable partial profiles (containing STR alleles at $\geq$ ten CODIS Core Loci). A mixed STR profile containing two or more contributors was observed for 28 samples or 87.5%. For all car steering wheels that were sampled, the primary driver was the last driver and for 30 samples out of 32 the driver was also the major DNA contributor.

[0061] The results show that each of the three DNA collection methods was able to recover detectable quantities of DNA. However, not all methods were equally effective in collecting touch DNA samples. It was also observed that steering wheels can retain DNA of the recent drivers even if vehicles are only driven for a short period of time (two minutes to one hour).

[0062] The FTA paper scraping method was shown to be the most effective for collecting DNA from steering wheels followed by double swabbing and tape lifting methods. The double swabbing method collected slightly higher amounts of touch DNA compared to tape lifting; however, the difference

was not statistically significant.

[0063] DNA yields using the double swabbing collection method were most highly dispersed, ranging from 0.08 ng to 883.75 ng. The swab has a relatively small surface area and it is a very tedious and time consuming process to collect from the entire surface of the steering wheel with a swab. Since there was no visual indication as to whether or not the area was covered by swabbing, certain areas could have been covered twice while others missed. Using a second wet swab immediately following the first one increased the DNA yield and was prone to the same limitations as the first swab collection.

[0064] Touch DNA yields collected by tape lifting were not as widely dispersed when compared to double swabbing, and ranged between 0.206 ng to 82.33 ng. The smaller range can be explained by the tape getting saturated with materials, therefore reducing its ability to collect touch DNA. Since there is a great variability for the hand position of the driver, it is not always possible to correctly estimate areas where the steering wheel was handled. Due to a large surface area, the tape was able to cover the entire steering wheel. Unfortunately the tape was not effective in collecting DNA from small grooves located on certain steering wheels.

[0065] The FTA paper scraping method with the collector was able to collect more touch DNA from the surface of a steering wheel as compared to the double swabbing method. The FTA paper was larger when compared to swabs and could cover a greater surface area before getting saturated. The FTA paper did not dry as fast as swabs and still appeared wet at the end of the collection process. The effectiveness of FTA paper remained approximately the same throughout the entire collection process, which allowed it to cover large surface areas in one attempt. Unfortunately, wet FTA paper easily tears apart on rough surfaces which may have contributed to lower yields for some of the samples. Despite this issue, FTA paper still can be used to collect DNA from flat and smooth surfaces such as mirrors, table tops, shelves and floors. This method could be useful in situations when the exact location of touch DNA is unknown or small

quantities of touch DNA are dispersed throughout the surface.

[0066] The goal of the STR analysis was to assess the difference between quantifiable and typable results among the three collection methods. The results show that the double swabbing method is more likely to recover the last driver's profile when compared to the tape lifting method and that the FTA paper scraping method recovers similar completeness of the profile as the double swabbing method (FIG. 3-4).

[0067] From the description herein, it will be appreciated that that the present disclosure encompasses multiple embodiments which include, but are not limited to, the following:

[0068] 1. A method for collecting nucleic acids from trace forensic biological samples, the method comprising: (a) contacting a surface with a collector to acquire trace nucleic acids, the collector comprising: (i) a collection matrix; (ii) a chemical composition that lyses cells and denatures proteins wherein the chemical composition is contacted to the collection matrix; and (iii) a non-porous, non-reactive backing wherein the backing is contacted with one or more surfaces of the collection matrix; (iv) wherein trace nucleic acids are freed from cells or organisms on contact with the chemical composition; and (b) preserving the acquired trace nucleic acids in the collection matrix of the collector for a period of time prior to analysis.

[0069] 2. The method of any preceding or following embodiment, further comprising: releasing the acquired trace nucleic acids from the collection matrix of the collector for analysis.

[0070] 3. The method of any preceding or following embodiment, further comprising: dampening the collection matrix with a fluid prior to contacting the surface, the fluid selected from the group of fluids consisting of water, buffered saline, lysis buffer, and sodium dodecyl sulfate (SDS).

[0071] 4. The method of any preceding or following embodiment, wherein the collection matrix is a material selected from the group of materials consisting of blotting paper, filter paper, cotton fibers, nylon fibers, polyester fibers, and a blend of paper and cloth fibers.

[0072] 5. The method of any preceding or following embodiment, wherein

the chemical composition comprises a mixture of a weak base; a chelating agent; and an anionic detergent.

[0073] 6. The method of any preceding or following embodiment, the chemical composition further comprising: a uric acid or a urate salt.

5 **[0074]** 7. The method of any preceding or following embodiment, wherein the backing of the collector matrix comprises cellophane.

[0075] 8. A method for identifying nucleic acid origins from trace forensic biological samples, the method comprising: (a) contacting a surface with a collector to acquire trace nucleic acids, the collector comprising: (i) a
10 collection matrix with an inert, impermeable backing layer with an inner surface and an outer surface and an absorbent layer mounted to the inner surface of the backing layer; and (ii) a chemical composition that lyses cells and denatures proteins-contacted to the absorbent layer of the collection matrix; (iii) wherein trace nucleic acids are freed from cells or organisms on
15 contact with the chemical composition; (b) releasing acquired trace nucleic acids from the absorbent layer of the collection matrix of the collector; (c) amplifying purified released nucleic acids; (d) analyzing amplified nucleic acids for identifiable markers; and (e) comparing identified nucleic acid markers with nucleic acids from a specific suspect or from a crime database
20 of nucleic acids to identify a match.

[0076] 9. The method of any preceding or following embodiment, further comprising: dampening the absorbent layer of the collection matrix with a fluid prior to contacting the surface, the fluid selected from the group of fluids consisting of water, buffered saline, lysis buffer, and sodium dodecyl
25 sulfate (SDS).

[0077] 10. The method of any preceding or following embodiment, further comprising: sealing the collector in a container for a period of time after collection to preserve the collected sample on the absorbent layer of the collector matrix free from contamination.

30 **[0078]** 11. An apparatus for collection of nucleic acids from a surface, comprising: (a) an inert, impermeable backing layer with an inner surface and an outer surface; (b) an absorbent layer mounted to the inner surface

of the backing layer; and (c) a chemical composition capable of lysing cells and denaturing proteins disposed on a collecting surface of the absorbent layer; (d) wherein cells and proteins of biological samples collected at the collecting surface of the absorbent layer are lysed and denatured by the chemical composition; and (e) wherein nucleic acids from the collected samples are absorbed by the absorbent layer remain intact.

[0079] 12. The apparatus of any preceding or following embodiment, further comprising at least one handle coupled to the impermeable backing layer.

[0080] 13. The apparatus of any preceding or following embodiment, further comprising: a planar base mounted to the impermeable backing layer; and at least one handle coupled to the planar base.

[0081] 14. The apparatus of any preceding or following embodiment, wherein the absorbent layer is at least one material selected from the group of materials consisting of a cotton material, a paper/cloth blend material, a cellulose-based material and a filter material.

[0082] 15. The apparatus of any preceding or following embodiment, wherein the absorbent layer of the collection matrix is a woven material selected from the group of materials consisting of nylon, polyester, cotton, cellulose, and a blend of paper and cloth fibers.

[0083] 16. The apparatus of any preceding or following embodiment, wherein the absorbent layer comprises an absorbent foam.

[0084] 17. The apparatus of any preceding or following embodiment, wherein the backing layer comprises cellophane.

[0085] 18. The apparatus of any preceding or following embodiment, wherein the chemical composition comprises: (a) a weak base; (b) a chelating agent; (c) an anionic detergent; and (d) a uric acid or urate salt.

[0086] 19. The apparatus of any preceding or following embodiment, wherein the weak base comprises tris(hydroxymethyl)methane.

[0087] 20. The apparatus of any preceding or following embodiment, wherein the chelating agent comprises ethylenediaminetetraacetic acid (EDTA).

[0088] 21. The apparatus of any preceding or following embodiment, wherein the detergent comprises sodium dodecyl sulfate (SDS).

[0089] Although the description herein contains many details, these should not be construed as limiting the scope of the disclosure but as merely providing illustrations of some of the presently preferred embodiments. Therefore, it will be appreciated that the scope of the disclosure fully encompasses other embodiments which may become obvious to those skilled in the art.

[0090] In the claims, reference to an element in the singular is not intended to mean "one and only one" unless explicitly so stated, but rather "one or more." All structural, chemical, and functional equivalents to the elements of the disclosed embodiments that are known to those of ordinary skill in the art are expressly incorporated herein by reference and are intended to be encompassed by the present claims. Furthermore, no element, component, or method step in the present disclosure is intended to be dedicated to the public regardless of whether the element, component, or method step is explicitly recited in the claims. No claim element herein is to be construed as a "means plus function" element unless the element is expressly recited using the phrase "means for". No claim element herein is to be construed as a "step plus function" element unless the element is expressly recited using the phrase "step for".

CLAIMS

What is claimed is:

5

1. A method for collecting nucleic acids from trace forensic biological samples, the method comprising:

(a) contacting a surface with a collector to acquire trace nucleic acids, the collector comprising:

10

(i) a collection matrix;

(ii) a chemical composition that lyses cells and denatures proteins wherein said chemical composition is contacted to said collection matrix; and

15

(iii) a non-porous, non-reactive backing wherein said backing is contacted with one or more surfaces of said collection matrix;

(iv) wherein trace nucleic acids are freed from cells or organisms on contact with the chemical composition; and

(b) preserving the acquired trace nucleic acids in the collection matrix of the collector for a period of time prior to analysis.

20

2. The method of claim 1, further comprising:

releasing the acquired trace nucleic acids from the collection matrix of the collector for analysis.

25

3. The method of claim 1, further comprising:

dampening the collection matrix with a fluid prior to contacting the surface, said fluid selected from the group of fluids consisting of water, buffered saline, lysis buffer, and sodium dodecyl sulfate (SDS).

30

4. The method of claim 1, wherein said collection matrix is a material selected from the group of materials consisting of blotting paper, filter paper, cotton fibers, nylon fibers, polyester fibers, and a blend of paper and cloth fibers.

5. The method of claim 1, wherein said chemical composition comprises a mixture of a weak base; a chelating agent; and an anionic detergent.

6. The method of claim 5, said chemical composition further comprising: a uric acid or a urate salt.

7. The method of claim 1, wherein said backing of said collector matrix comprises cellophane.

8. A method for identifying nucleic acid origins from trace forensic biological samples, the method comprising:

(a) contacting a surface with a collector to acquire trace nucleic acids, the collector comprising:

(i) a collection matrix with an inert, impermeable backing layer with an inner surface and an outer surface and an absorbent layer mounted to the inner surface of the backing layer; and

(ii) a chemical composition that lyses cells and denatures proteins-contacted to the absorbent layer of the collection matrix;

(iii) wherein trace nucleic acids are freed from cells or organisms on contact with the chemical composition;

(b) releasing acquired trace nucleic acids from the absorbent layer of the collection matrix of the collector;

(c) amplifying purified released nucleic acids;

(d) analyzing amplified nucleic acids for identifiable markers; and

(e) comparing identified nucleic acid markers with nucleic acids from a specific suspect or from a crime database of nucleic acids to identify a match.

9. The method of claim 8, further comprising:

dampening the absorbent layer of the collection matrix with a fluid prior to contacting the surface, said fluid selected from the group of fluids consisting of water, buffered saline, lysis buffer, and sodium dodecyl sulfate (SDS).

10. The method of claim 8, further comprising:
sealing the collector in a container for a period of time after collection to preserve the collected sample on the absorbent layer of the collector matrix free from contamination.

5

11. An apparatus for collection of nucleic acids from a surface, comprising:

(a) an inert, impermeable backing layer with an inner surface and an outer surface;

10 (b) an absorbent layer mounted to the inner surface of the backing layer; and

(c) a chemical composition capable of lysing cells and denaturing proteins disposed on a collecting surface of the absorbent layer;

15 (d) wherein cells and proteins of biological samples collected at the collecting surface of the absorbent layer are lysed and denatured by the chemical composition; and

(e) wherein nucleic acids from the collected samples are absorbed by the absorbent layer remain intact.

20 12. The apparatus of claim 11, further comprising at least one handle coupled to the impermeable backing layer.

13. The apparatus of claim 11, further comprising:

a planar base mounted to the impermeable backing layer; and

25 at least one handle coupled to the planar base.

14. The apparatus of claim 11, wherein said absorbent layer is at least one material selected from the group of materials consisting of a cotton material, a paper/cloth blend material, a cellulose-based material and a filter material.

30

15. The apparatus of claim 11, wherein said absorbent layer of said collection matrix is a woven material selected from the group of materials

consisting of nylon, polyester, cotton, cellulose, and a blend of paper and cloth fibers.

16. The apparatus of claim 11, wherein said absorbent layer comprises
5 an absorbent foam.

17. The apparatus of claim 11, wherein said backing layer comprises cellophane.

10 18. The apparatus of claim 11, wherein said chemical composition comprises:

- (a) a weak base;
- (b) a chelating agent;
- (c) an anionic detergent; and
- 15 (d) a uric acid or urate salt.

19. The apparatus of claim 18, wherein said weak base comprises tris(hydroxymethyl)methane.

20 20. The apparatus of claim 18, wherein said chelating agent comprises ethylenediaminetetraacetic acid (EDTA).

21. The apparatus of claim 18, wherein said detergent comprises sodium dodecyl sulfate (SDS).

1/5

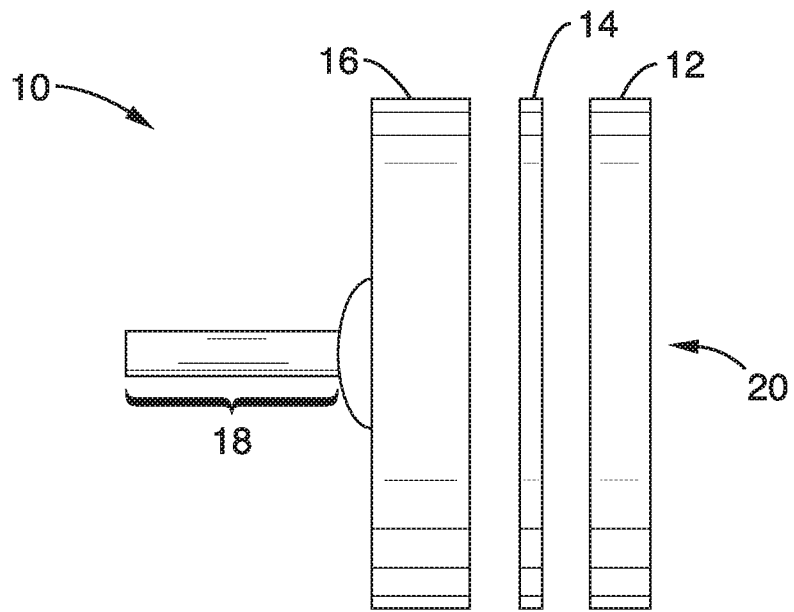


FIG. 1

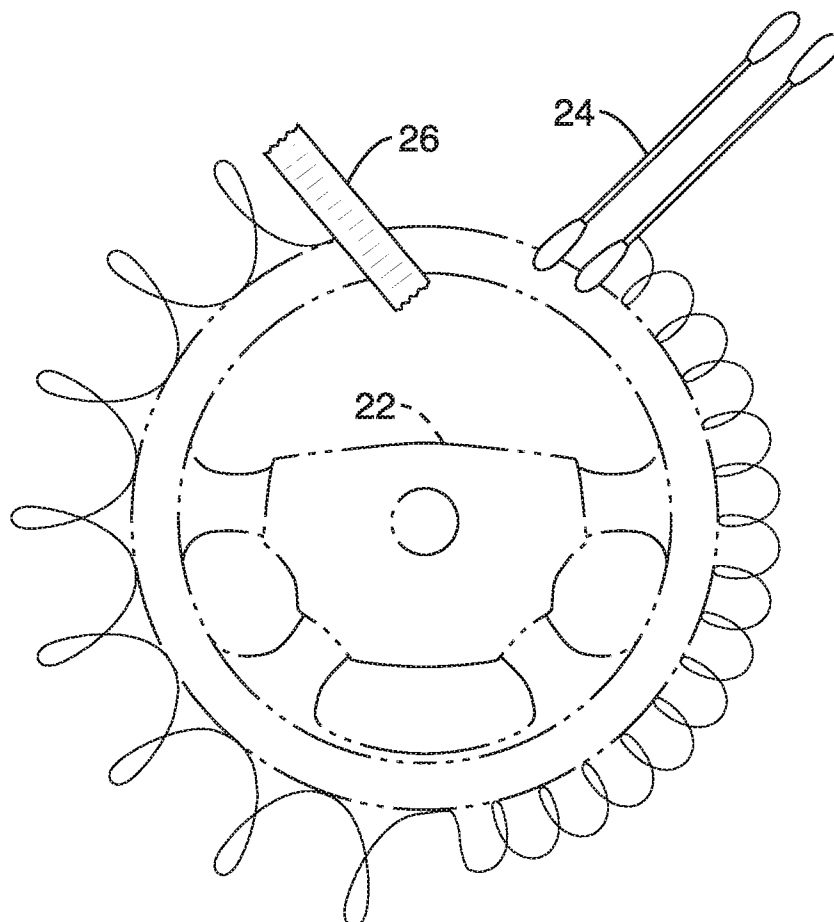


FIG. 2A

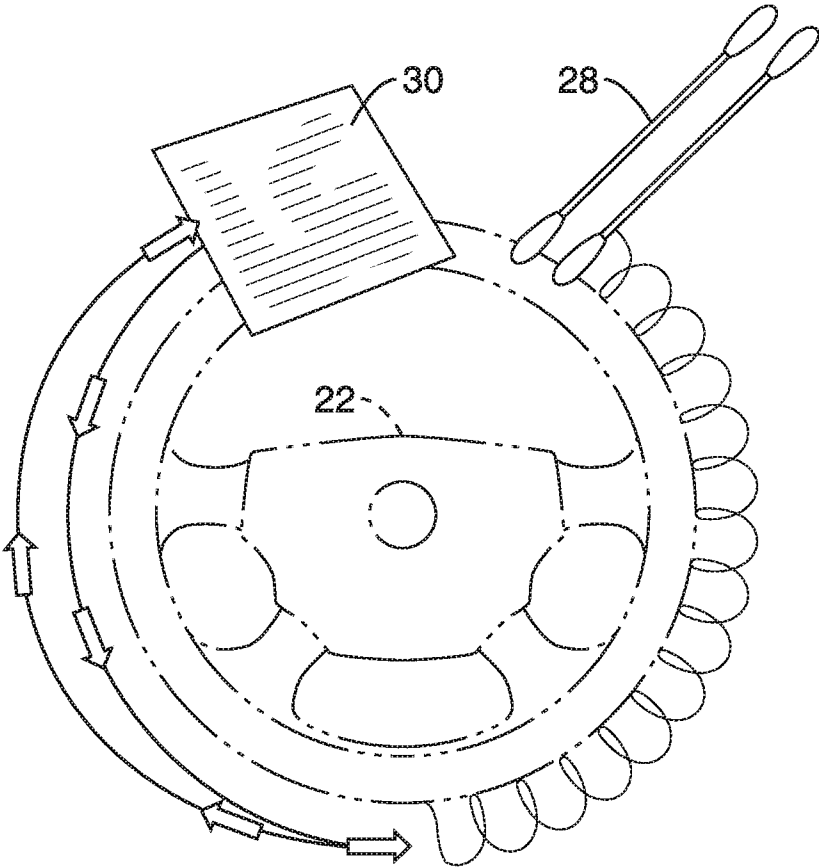
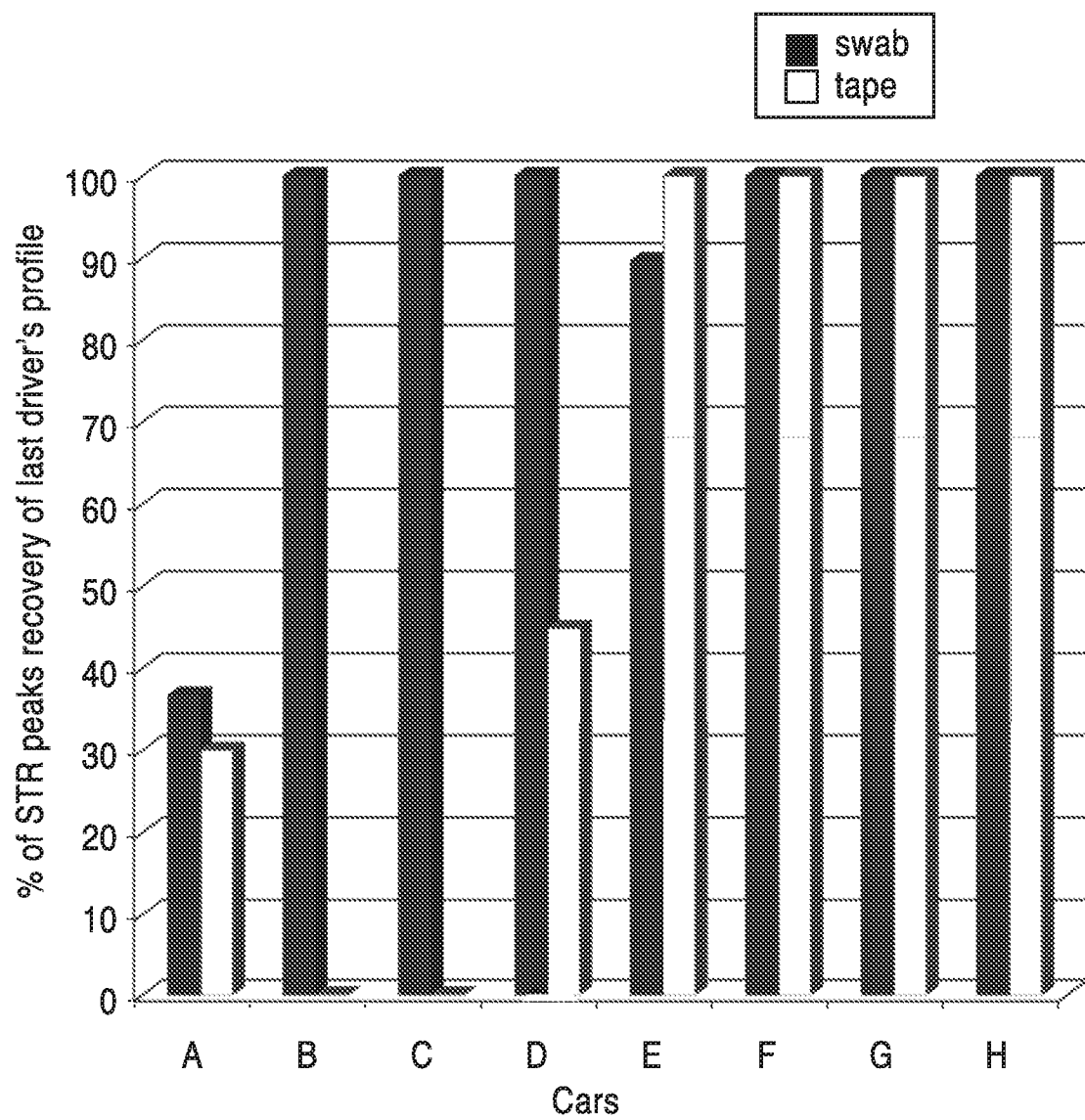
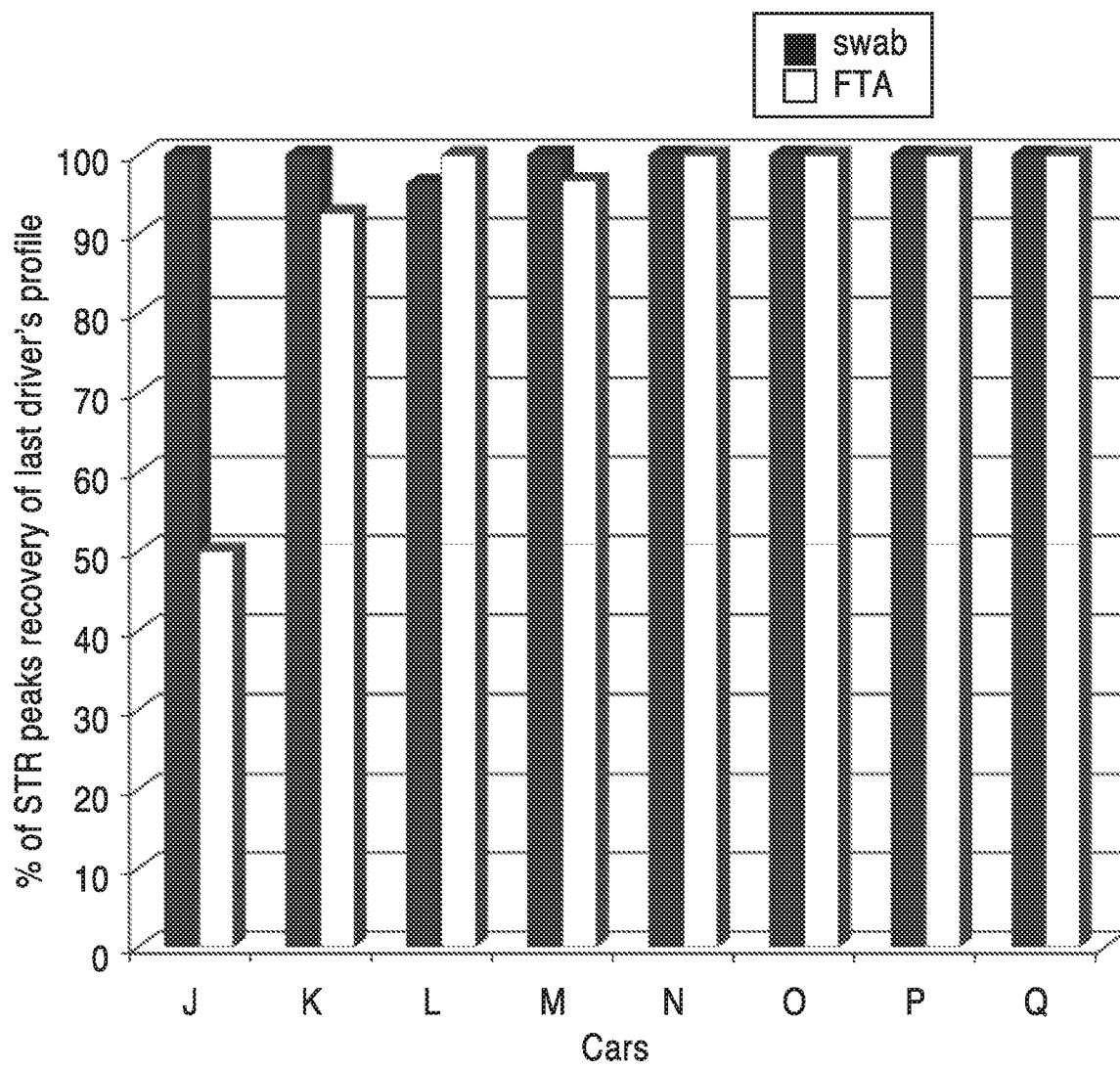
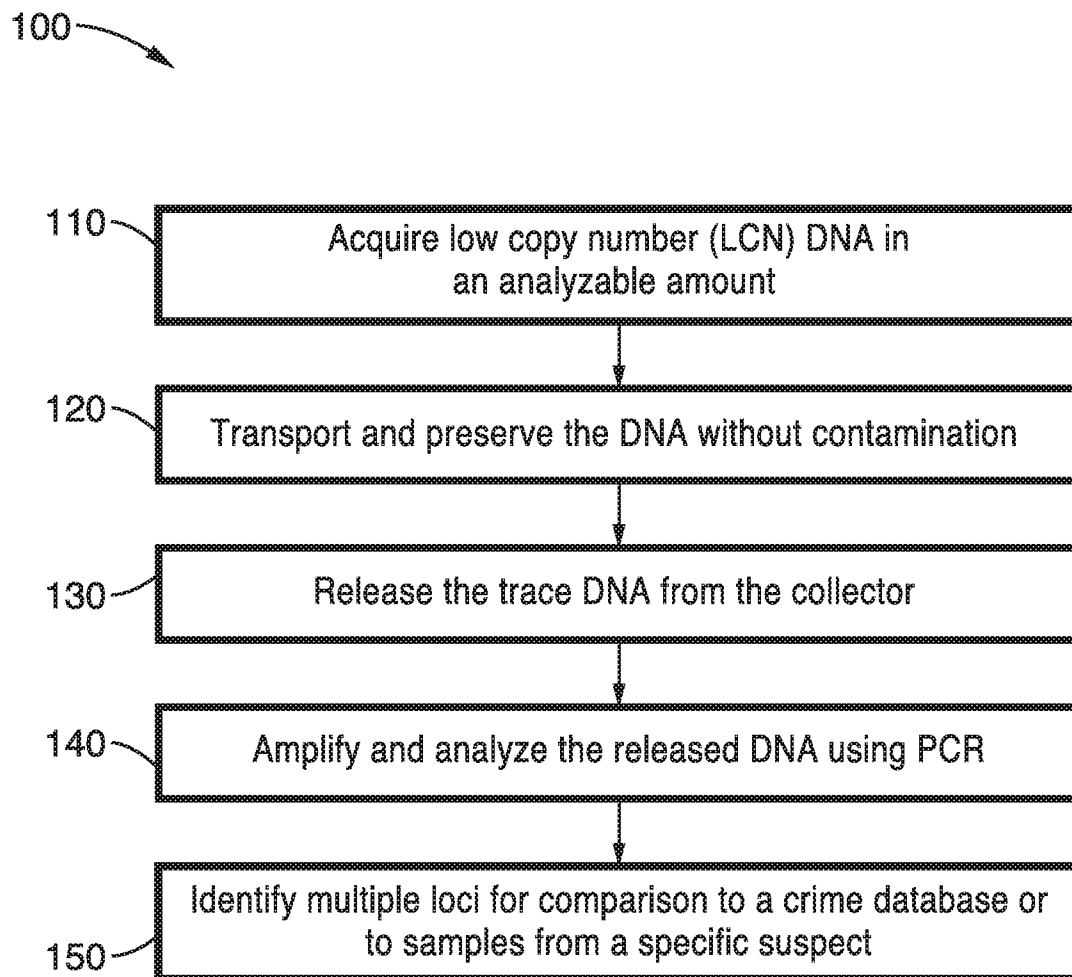


FIG. 2B

**FIG. 3**

**FIG. 4**

**FIG. 5**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/51650

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - G01N 1/02, B65D 81/00 (2017.01)
 CPC - G01N 2001/028, A61B 10/0045, G01N 1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2014/0272983 A1 (GE HEALTHCARE UK LIMITED) 18 September 2014 (18.09.2014) Abstract; Claim 1; Claim 14; Claim 15; Claim 18; Claim 19; para [0001]; para [0009]; para [0039]; para [0047]; para [0048]	1-7 ----- 8-10
Y	US 2016/0047720 A1 (DIOMICS CORPORATION) 18 February 2016 (18.02.2016) para [0040]; para [0056]	8-10

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 November 2017

Date of mailing of the international search report

18 JAN 2018

Name and mailing address of the ISA/US

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 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/51650

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not solinked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-10, directed to a method of collecting and identifying nucleic acid origins from trace forensic biological samples.

Group II: Claims 11-21, directed to an apparatus for collection of nucleic acids from a surface.

-----Please see continuation in first extra sheet-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/51650

Continuation of Box No III. Observations where unity of invention is lacking.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Groups II does not require method steps of contacting a surface with a collector to acquire trace nucleic acids and preserving the acquired trace nucleic acids in the collection matrix, as required by group I.

Group I does not require an apparatus comprising an inert backing and an absorbent layer, as required by group II.

Common Technical Features

The common technical feature shared by Groups I and II comprising collecting nucleic acids from a surface comprising

- (a) an inert, impermeable backing layer with an inner surface and an outer surface;
- (b) an absorbent layer mounted to the inner surface of the backing layer; wherein nucleic acids from the collected samples are absorbed by the absorbent layer
- (c) a chemical composition capable of lysing cells and denaturing proteins disposed on a collecting surface of the absorbent layer;
- (d) wherein cells and proteins of biological samples collected at the collecting surface of the absorbent layer are lysed and denatured by the chemical composition; and nucleic acids remain intact.

However, this shared technical feature does not represent a contribution over prior art in view of US 2003/0113906 A1 to Sangha et al., (hereinafter 'Sangha') and US 2014/0272983 A1 to GE Healthcare UK Limited (hereinafter 'GE'). Sangha teaches a means for collection of nucleic acids from a surface comprising

(a) an inert, impermeable backing layer with an inner surface and an outer surface and (b) an absorbent layer mounted to the inner surface of the backing layer; wherein nucleic acids from the collected samples are absorbed by the absorbent layer (para [0040] 'A device for collection of DNA-containing materials is provided', para [0026] '[an] embodiment of the present invention having an absorbent collection substrate underneath a cover sheet and a protective backing behind the collection substrate'); wherein nucleic acids from the collected samples are absorbed by the absorbent layer (para [0057] '[the] collection substrate ... can be an absorbent material such as one of the previously identified cellulose absorbent papers. Collection substrate ... can be used in a wet or dry fashion to collect a skin surface sample of DNA containing material ... can be inserted into the mouth of an individual for collection of DNA containing material from the mouth'), a chemical composition capable of lysing cells disposed on a collecting surface of the absorbent layer; wherein cells by the chemical composition; and nucleic acids remain intact [e.g. are not disabled] (para [0041] 'the absorbent material of the collecting substrate or the collection area on the subject may be pre-wetted with a solution which does not disable specimen DNA or interfere with the DNA analysis. Examples of suitable wetting agents are cell-lysing detergents'). Sangha does not expressly teach that the chemical composition of (c) and (d) denatures proteins, however, GE teaches protein denaturants in solutions used to collect and preserve DNA or RNA (para [0001] "The invention provides methods and kits which can be used to collect cell samples containing nucleic acids by reducing the use of chemicals that may interfere with the polymerase chain reaction. The invention has applications in the long-term storage, recovery and further processing of nucleic acids and is particularly useful in genotyping, diagnostics and forensics applications", para [0007] "a solid medium for storing a sample of genetic material and subsequent analysis; the solid medium comprising a protein denaturing agent", para [0024] "a solid matrix which can be used to store and lyse biological samples by contacting a solid support embedded with a surfactant and a chaotropic salt.", Chaotropic salts are widely known in protein art as denaturants of proteins.). It would have been obvious to an artisan of ordinary skill to include a protein denaturant such as a chaotropic agent, because said agent would aid in preservation of nucleic acids by inactivating proteins such as nucleases or proteases that could degrade nucleic acid or inactivate enzymes used to analyze the nucleic acid samples.

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.