An Engaging Lesson Model for Biological Evidence Collection Training for DNA

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Abstract: According to the Locard's Exchange Principle, "every contact leaves a trace." The study of touch DNA further explores this principle: when a person comes in contact with any surface they will leave residual evidence behind. Touch DNA is a common form of evidence. Factors thought to affect the transfer and recovery of touch DNA include duration of contact, surface type, genetic "shedder status," environmental factors (e.g. heat, humidity), bacterial action, DNA degradation rate, pressure applied to surface, and recovery method (e.g. swabbing, cutting, tape lifts). Here a college-level lesson uses cell biology concepts with a set of touch DNA exercises to serve as an example of experimental design and training on DNA contamination and touch DNA in forensic science. This lesson model describes student learning activities. Students collect data to authenticate beliefs on DNA transfer and translate the information for biological evidence collection strategies and classroom discussion. The activities are divided into two categories: (1) microscopy with cytology and (2) human identification by DNA. Both categories are relevant to biological evidence collection training and identification methods. This lesson model can be useful for training workshops and forensic science, cell biology and basic biology college courses.

Keywords: biological evidence, DNA, apoptosis, cytology, forensic science

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Introduction

Touch DNA is a reference to easily transferred DNA molecules by touch (1, 2). The transferred DNA can be from the primary depositor or can be from a secondary transfer mechanism through contact with others and objects in the environment (3). Although there are many scientific forensic publications addressing touch DNA, rarely do students easily conceptualize what DNA looks like on a surface. The DNA molecule itself and frequently associated epithelial or skin cells are microscopic, clear, invisible and, therefore, theoretical for most student discussions on DNA contamination.

There are numerous traditional cytological stains that can be used to visualize cell types in standard biological training including nuclear fast red (NFR) (3 - 6), May-Grunwald giemsa (MGG) (7, 8), papanicolaou stain (PAP) (9), and trypan blue (TB) to name just a few (10, 11). Simple laboratory microscopy can be used to visualize stained cells as a low cost exercise in training for touch DNA on different surfaces and for DNA contamination exercises. The magnification can be simple from 40X to 400X total magnification with standard compound light microscopes using glass slides and cellular staining techniques. Even greater detail can be visualized with phase contrast and scanning electron microscopy. Epithelial cells from touch DNA are interesting to study since the skin is a large, constantly rejuvenating organ that grows from an active internal cell layer that undergoes programmed cell death (apoptosis) as the cells are pushed to the surface (Figure 1) (12).

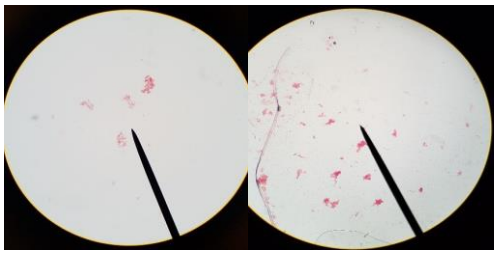

Figure 1 Nuclear fast red stained cells (keratinocytes) collected by cotton swab (left) and tape lift (right).

The epidermis (outer layer of the skin) has a human DNA component from living cells and free DNA from apoptotic cells; in addition, the human microbiome contains microbial DNA from approximately one thousand species from an estimated nineteen phyla (https://www.britannica.com/science/human-microbiome).

The forensic applications for this set of exercises are many: (a) visualization of the concept of DNA deposit,

DNA transfer, contamination and the number of DNA containing cells (keratinocytes) versus the number of non-DNA cells (corneocytes), (b) evaluation of finger marks for DNA from robbery and burglary from window glass, (c) plastic analysis for DNA for drug packaging and (d) DNA from a struggle transferred to hair and other crime scene scenarios (13 – 15).

When considering forensic education, many terms such as touch DNA are mentioned but a true understanding may not be achieved until the concept can be visualized. Even in courtroom testimony, touch DNA is testified to simply because a full understanding of how this process works has not been met. For example, how is it that an individual can be identified on video but no detectable DNA from the donor is left behind on a weapon? When analyzing this situation, multiple possibilities exist: (a) incorrect sampling of the weapon, (b) insufficient recovery of DNA for detection, (c) rapid DNA degradation, (d) use of gloves or a perspiration barrier that prevents deposition and (e) true exclusion as the DNA source. Foundational scientific disciplines such as cell biology, cytology, and microscopy are helpful aids for improving student understanding of biological evidence collection concepts and DNA deposition.

The purpose of this study is to collect and analyze touch DNA transferred from a person to a surface. Specifically, this study will test for the quality and quantity of the touch DNA from various surfaces for comparison. It will also illustrate why forensic scientists are concerned over DNA contamination based on the ease of recovering cells from thumbprints, thumb surfaces and hair shafts and surfaces.

Goals

(a) To visualize epithelial cells to improve sampling for evidence collection
(b) To conceptualize the quantity and source of DNA transferred to any surface by touch; and the necessity for wearing personal protective equipment (PPE) to reduce contamination
(c) To improve general scientific understanding of touch DNA and the deposition and recovery process
(d) To better understand cell biology and programmed cell death by differential staining to identify from which cells touch DNA originates

Methods

Supplies and Equipment

- Glass microscope slides (AmScope SKU: BS-144P-200S-22)
- Universal transparent sheets (OfficeSupply product no. UNV21013)
- Slide warmer (LabScientific model no. XH-2004)
- Nuclear fast red (Kernechrot) stain [preparation: dissolve 25g of aluminum sulfate in 500ml distilled water; add 0.5g nuclear fast red and heat gently to dissolve; cool, filter and add a few grains of thymol as a preservative (Sigma N8002 or N3020)]
- Plastic weigh boats for staining (Fisher Scientific cat. no. 13-735-741)
- Cotton tipped applicators (ULINE model. no. S-21102)
- Tape (Scotch brand)
- Kim wipes (Thomas Scientific cat. no. 34256)
- Black indelible marker (ULINE cat. no. H-286BL)
- Quantifiler human DNA quantification kit (ThermoFisher Scientific cat. no. 4343895)
- AmpFLSTR Identifiler Plus PCR Amplification kit (ThermoFisher Scientific cat. no. 4427368)
- Compound light microscope (Fisher Scientific cat. no. S23871; Swift compound microscope with integrated 10 in. tablet)
- NanoDrop One/One^{C} microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific cat. no. ND-ONE-W)
- ABI 7000 Sequence Detection System (ThermoFisher Scientific)
- Substrates (Home Depot)

Sample Collection and Processing

Experiment #1: Collection of DNA from Hair Surfaces. This experiment is designed to assess DNA recovery rates by allele counts and comparison to buccal reference samples of the student. All participants in this study were selected with no bias for age, gender or race. Donors were seated and the student investigator would place a hand on the participant's head hair for a period not exceeding 30 seconds. Swabs pre-moistened with sterile deionized water were then gently brushed along the hair on the right side of the participants' head, with care taken to avoid contact with the scalp. Pre-cut sections of Scotch® brand shipping tape were then gently placed on the left side of the participant's head and lifted between 4-12 times with care taken to avoid touching the scalp and avoid tearing participant's hair.

Right hand thumb reference samples were collected as controls by swabbing the surface of the student thumb with a moistened cotton swab 15 minutes before and after contact with the surface. Buccal reference samples were collected as controls with sterile cotton swabs by students from themselves as known comparisons for the training experiment. Negative controls included processing of reagents without DNA template.

Microscopy: Thumb print samples were created by placing a thumb on a clean glass slide with 30 seconds of pressure, heat fixing on a slide warmer for 5 minutes,

staining with nuclear fast red cytological stain (www.sigmaaldrich.com) for 5 minutes at room temperature, rinsing the slide gently with distilled water at a 30 degree angle until most of the stain was removed into a plastic disposable dish. Standard compound light microscopy was performed and photographs taken at 100-400x magnification.

DNA Methods: DNA was extracted using a QIAamp DNA Investigator kit per manufacturer instructions (www.qiagen.com). Two microliters of the DNA extract per sample was quantified for the total DNA concentration (ng/ul) per equipment manufacturer instructions for the NanoDrop™ One/One^{C}UV spectrophotometer (www.themofisher.com). The recovered DNA represents both human DNA and microbiome DNA. Human specific DNA was quantified using a Quantifiler human DNA quantification kit per manufacturer instructions (www.thermofisher.com) and compared to the UV spectrophotometric values. Human DNA profiles were generated using an AmpF*l*STR Identifiler Plus PCR amplification kit per manufacturer instructions (www.thermofisher.com). DNA fragments were separated and detected on a 3130XL Genetic Analyzer per manufacturer instructions and analyzed using GeneMarker HID software (SoftGenetics LLC, State College, PA).

Experiment #2: Collection of DNA from Various Touched Surfaces. This experiment was designed to assess the quality and quantity of touch DNA. A thumb was placed on a 1 square inch area of each clean surface (e.g. ceramic, wood, metal, plastic, carpet, paper, glass, cardboard and napkin) for approximately 10-30 seconds and rubbed back and forth with medium pressure. Between collections from each surface, 15 minutes was allowed to replenish DNA on the surface of the thumb. DNA was collected after replenishment from the thumb with a moist cotton swab. A sterile wet cotton swab was used to collect touch DNA from each surface. The cotton part of the swab was removed and placed in a 1.5 mL collection tube with a sterile blade and DNA extracted. DNA was extracted using a QIAamp DNA Investigator kit per manufacturer instructions (www.qiagen.com). Each sample was then quantified for total DNA by UV spectrophotometry to assess for quantity and evaluated by the A 260/280 ratio for purity. A ratio of approximately 1.8 is considered pure DNA. Two microliters of the DNA extract per sample was quantified for the total DNA concentration (ng/ul) per equipment manufacturer instructions for the NanoDrop™ One/One^{C}UV spectrophotometer (www.themofisher.com). The recovered DNA represents both human DNA and microbiome DNA.

Hazards and Safety Precautions

Any hazards and safety precautions are typically handled through the standard University and Occupational Safety and Health Administration (OSHA) safety regulations. Material safety data sheets (MSDS) are available for chemical safety and exposure information from chemical suppliers. Proper laboratory safety guidelines should reduce or eliminate any safety issues for handling chemicals, handling glass slides, operating laboratory equipment and for waste disposal.

Results

When examined with a microscope it was possible to determine that neither freshly cleaned hair nor a recently washed hand could be expected to be completely free of surface cells (living or dead) or cellular debris even when an effort was made to minimize contact with any surfaces that might release cells. For both the swab and tape samples the presence of living and dead cells was apparent with all samples; at least a small number of visible clear corneocytes and some DNA containing keratinocytes were consistently recovered. In our experiment, swabbing was a significantly better biological evidence collection method for human genotyping for DNA profiles (TABLE 1). On average, 22 alleles were recovered from the triplicate swabs; 3 alleles were recovered from the triplicate tape samples. This represents a seven fold increase in DNA recovery by use of the swab method. Although sufficient numbers of DNA staining cells were observed by microscopy, we hypothesize that the adhesive in the tape samples had an inhibitory effect on allele recovery.

TABLE 1 Allele counts for hair shaft samples

Sample	No. Alleles Recovered
#1 Swab	20
#2 Swab	27
#3 Swab	19
#1 Tape lift	1
#2 Tape lift	5
#3 Tape lift	3
Positive	30 possible from 15 loci
Negative	0 detected

Many alleles originated from the hair donor but some samples detected additional alleles in common with the touch DNA source and others from unknown sources in the environment (TABLE 2).

TABLE 2 Allele sourcing from hair shaft swab samples

Sample	Hair Donor	Touch Donor	Unknown Donor
#1 Swab	13	1	1

#2 Swab	24	1	2
#3 Swab	5	4	10
Positive	30 possible from 15 loci		
Negative	0 detected		

UV spectrophotometry results indicated the greatest recovery of total DNA in our study (student results may vary) from paper, wood and metal (TABLE 3).

TABLE 3 Total DNA recovered from various substrates by UV spectrophotometry

Sample	Quantity (pg/2ul)	Quality (A260/A280)
Paper	13.2	2.50
Wood	16.8	2.02
Metal	60.6	1.55
Glass	0.2	0.26
Plastic	1.7	1.52
Ceramic	1.7	3.10
Cardboard	2.0	3.56
Swab of hair	5.6	1.81
Tape lift, hair	3.0	3.98
Positive control	23.8 (buccal swab)	2.51
Negative control	9.6 (clean swab)	1.96
Negative control	0.0 (water)	0.08

Some surfaces had no detectable DNA using this nonspecific DNA detection method (e.g. napkin, carpet). Other surfaces that had detectable but small amounts of DNA included glass, ceramic, and cardboard. The average recovery of human DNA from seven randomly sampled thumbs prior to touch of a substrate was 3.79pg (range 1.1-7.3pg). Surfaces of the thumbs after touching a substrate and allowing for 15 minutes of replenishment had a range of 1.0-2.5pg of human-specific DNA indicating either residual DNA on the thumb surface after touch or a rapid replenishment of DNA on the skin surface.

Discussion and Conclusion

Training goals are met for forensic education in this sample lesson plan for forensic biology in several ways. The microscopy technique serves as a refresher for a basic but useful technique for screening and visualizing cellular material. Typically, students are surprised (79% of students surveyed) by the large quantity of pink staining cells present from just a single thumbprint and it serves as a reminder for careful collection procedures as it is fairly easy to contaminate biological evidence with cells shed from the collector's skin; an estimated 40,000 cells daily. Even after touching a surface, there is sufficient replenished or residual DNA retained on the student thumb surface that a second consecutive touch to a surface can leave detectable DNA without wearing protective latex or nitrile gloves. The DNA recovered from the touched hair samples is primarily from shed epithelial cells from the scalp of the donor. However, if the collection is carried through the human identification process along with a reference buccal swab from the student, it becomes apparent that DNA from the touch DNA donor can often be detected from the hair strand along with stray DNA alleles from exposure to the general environment. The results from this experiment can facilitate discussion on primary deposit, secondary transfer, and allelic "drop-in" events and emphasize the need for caution in interpreting human genotyping results from a variety of criminal casework scenarios (e.g. strangulation, physical and sexual assaults, etc.). Last but not least, the inherent variability of recovering touch DNA from different surfaces can be investigated as a classroom exercise to establish trends in cell adherence to such surfaces as metal, glass, plastic and ceramic.

Our student generated data and other published scientific studies support the inherent variability of touch DNA recovery and cite the wide range of environmental and genetic factors that contribute to the general inability to predict DNA quantity and quality for recovery from different surfaces at crime scenes. Each crime scene has inherently different features that can contribute to the unpredictability; only in the largest sense can one predict contributing factors such as extended contact time, perspiration, friction, and repeated handling etc. that have a positive impact on the expectation of a forensic scientist to recover DNA. Items that are commonly processed in forensic laboratories for touch DNA include firearms, tools, knives, ligatures, clothing, and cell phones to name a few. One would anticipate some differences in student results from different sections of the same course for this reason and also between individual students.

The two experiments described here can be limited to microscopy and ultra violet spectroscopy to fit into a single three hour laboratory training period. If consecutive weeks are available to the student, the DNA processing aspect of qPCR, PCR amplification for genotyping, capillary electrophoresis and software analysis can be incorporated into the course syllabus. Additional features can be built into this general experimental lesson model to have the student investigate the following: (1) impact of different lengths of contact time for touch DNA transfer to a substrate, (2) genetic variability between donors, (3) effect of hand washing, (4) effect of varying pressure on a surface, etc. to vary the training focus.

If the lesson plan is restricted to microscopy and UV spectroscopy, cell counts can be performed for each field of view with microscopy to estimate the percentages of keratinocytes to corneocytes. Students can calculate an estimate of the number of DNA containing cells that might be shed from 1 square inch of their skin surface as an additional self-discovery exercise.

A survey of twenty-eight University of New Haven students who performed the microscopy experiment generated the following results: 17.9% considered the exercise to train on DNA contamination, 39.3% considered the exercise informative on DNA transfer, 17.8%

considered the exercise a "shedder" status exercise, and 25% indicated the exercise showed one could obtain DNA from a thumb print. One hundred percent of the students surveyed indicated the microscopy exercise was effective as a training tool for DNA transfer and contamination in forensic science. Eighty-nine percent of surveyed students indicated the microscopy exercise was useful to learn about skin differentiation processes. The implications for professional practice and training of forensic scientists and crime scene personnel are clear; a visual assessment of the quantity of DNA containing cells aids in reinforcing the need for proper protective laboratory and crime scene gear to avoid DNA contamination of the biological evidence.

Acknowledgements

Thank you to University of New Haven for funding our research on touch DNA for this publication.

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