Forensic Science International: Genetics 28 (2017) 195–202

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

Analysis of DNA from post-blast pipe bomb fragments for identification and determination of ancestry

Esiri Tasker[a], Bobby LaRue[b,1], Charity Beherec[a], David Gangitano[a], Sheree Hughes-Stamm[a,*]

[a] Department of Forensic Science, Sam Houston State University, Huntsville, TX, United States
[b] Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, Fort Worth, TX, United States

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 8 February 2017
Accepted 28 February 2017
Available online 1 March 2017

Keywords:
Forensic science
DNA typing
Improvised explosive devices
Short tandem repeats
Insertion/null polymorphisms
Single nucleotide polymorphisms
Massively parallel sequencing

ABSTRACT

Improvised explosive devices (IEDs) such as pipe bombs are weapons used to detrimentally affect people and communities. A readily accessible brand of exploding targets called Tannerite® has been identified as a potential material for abuse as an explosive in pipe bombs. The ability to recover and genotype DNA from such weapons may be vital in the effort to identify suspects associated with these devices. While it is possible to recover DNA from post-blast fragments using short tandem repeat markers (STRs), genotyping success can be negatively affected by low quantities of DNA, degradation, and/or PCR inhibitors. Alternative markers such as insertion/null (INNULs) and single nucleotide polymorphisms (SNPs) are bi-allelic genetic markers that are shorter genomic targets than STRs for amplification, which are more likely to resist degradation.

In this study, we constructed pipe bombs that were spiked with known amounts of biological material to: 1) recover "touch" DNA from the surface of the device, and 2) recover traces of blood from the ends of wires (simulated finger prick). The bombs were detonated with the binary explosive Tannerite® using double-base smokeless powder to initiate the reaction.

DNA extracted from the post-blast fragments was quantified with the Quantifiler® Trio DNA Quantification Kit. STR analysis was conducted using the GlobalFiler® Amplification Kit, INNULs were amplified using an early-access version of the InnoTyper™ 21 Kit, and SNP analysis via massively parallel sequencing (MPS) was performed using the HID-Ion Ampliseq™ Identity and Ancestry panels using the Ion Chef and Ion PGM sequencing system.

The results of this study showed that INNUL markers resulted in the most complete genetic profiles when compared to STR and SNP profiles. The random match probabilities calculated for samples using INNULs were lower than with STRs when less than 14 STR alleles were reported. These results suggest that INNUL analysis may be well suited for low-template and/or degraded DNA samples, and may be used to supplement incomplete or failed STR analysis. Human identification using SNP analysis via MPS showed variable success with low-level post-blast samples in this study (<150 pg). While neat DNA samples (6 μL input as recommended) resulted in <50% of SNP calls, samples that were concentrated from 15 μL to 6 μL (15 μL was added for STR and INNUL typing) resulted in more complete SNP profiles. Five out of six blood samples recovered from the wires attached to the pipe-bombs resulted in the correct ancestry predictions.

1. Introduction

Improvised explosive devices (IEDs) are weapons commonly used in crimes and acts of terror to cause bodily harm or death, property damage, or instill fear in communities [1–6]. There are several forms of IEDs, but pipe bombs are the most commonly used

* Corresponding author at: Department of Forensic Science, Sam Houston State University, 1003 Bowers Blvd Huntsville, TX, United States.
E-mail address: sxh039@shsu.edu (S. Hughes-Stamm).
[1] Current address: Department of Forensic Science, Sam Houston State University, 77340, 1003 Bowers Blvd., Huntsville, TX, 3202, United States.

http://dx.doi.org/10.1016/j.fsigen.2017.02.016
1872-4973/

devices due to the free availability of materials, such as explosive powder, and ease of assembly [1,2].

Ammonium nitrate and aluminum powder are commonly used to manufacture homemade explosives [3,4]. While materials such as smokeless gunpowder are more commonly used in pipe bombs [5], newer commercial products such as Tannerite® (Tannerite® Sports LLC, Pleasant Hill, OR) may also warrant concern. Tannerite® is a patented brand of binary reactive targets comprised of ammonium nitrate and aluminum powder, and it is marketed as shot indicators for licensed gun enthusiasts. Due to the wide accessibility and no purchasing restrictions for Tannerite® within the United States, these exploding targets have recently been identified as a potential explosive powder for use as IEDs [6].

When an item such as a pipe bomb is touched by a suspect (during assembly or placement), the amount of DNA transferred from the individual to the device varies and can greatly affect the quality of a resulting genetic profile [7–11]. Typically, only a few cells are transferred and trace quantities of DNA or low template DNA (LT-DNA) are recovered from touched items, such as mobile phones and firearms [2,7,12–15]. Low-template and/or degraded DNA samples often produce incomplete and/or poor quality genetic profiles due to stochastic effects such as allele and/or locus drop-out, allele drop-in, and peak height imbalance, or no amplification [7,16–20].

While several studies have examined various ways to improve the recovery and genotyping of low-template and degraded DNA samples [12,16,17,19,21–24], few have focused specifically on recovering DNA from IED post-blast debris [1,25,26]. Furthermore, the studies that have assessed DNA recovery from post-blast fragments only focused on using standard DNA collection and genotyping methods [1,25–27].

Several methods have been suggested to improve LT-DNA analysis including increasing the number of PCR cycles, increasing the injection time during capillary electrophoresis, and reducing the PCR reaction volume [20]. However, these methods may exaggerate stochastic effects and result in increased stutter peaks, peak height imbalance, and drop-in alleles [19]. Another approach to retrieving more genetic information from challenging DNA samples such as those recovered from IEDs is to employ alternative molecular markers such as insertion/null (INNUL) or single nucleotide polymorphisms (SNPs). INNULs are retrotransposable elements consisting of long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) [28,29]. INNULs are bi-allelic markers that do not possess stutter peaks and have low mutation rates. Due to their abundance in the human genome and the ability to generate small amplicons, they are potentially advantageous when amplifying LT-DNA and degraded samples. The InnoTyper® 21 kit (InnoGenomics Technologies) used in this study is a commercially available small amplicon (60–125 bp) DNA typing system that has demonstrated some success with forensic samples [30–34]. SNPs are single base variations in the genome that also enable shorter amplicons (<150 bp) [35], no stutter, and lower mutation rates compared to STRs [36]. As with INNULs, these characteristics make SNPs well suited for use with challenging samples, such as low-template and degraded DNA [37,38]. Furthermore, SNPs analyzed via massively parallel sequencing (MPS) has also shown some success with retrieving information from challenging DNA samples [39–41].

The aim of this study was to compare the success of genotyping DNA recovered from post-blast pipe bomb fragments using standard short tandem repeats (STR), and alternate markers such as INNULs, and SNPs via MPS analyses for human identification (HID) and ancestry.

2. Materials and methods

2.1. Biological samples

Buccal swabs from a single male Caucasian donor were collected. Blood added to the wires was collected from three ethnically different individuals (Asian, Caucasian, and African-American). All samples were obtained in accordance with Sam Houston State University Institutional Review Board Guidelines (#2016-09-31948).

2.1.1. Epithelial cell suspension

A method for creating a homogeneous cell suspension from buccal swabs was adapted from a previous study examining the success of DNA recovery from post-blast bomb fragments [1]. This method was chosen to ensure a controlled number of epithelial cells was added to each device. Briefly, buccal cells were collected from one donor using three cotton swabs, with each swab being placed in a 1.5 mL tube with 1 mL of 1 X phosphate buffered saline (PBS) (pH 7.4) and briefly vortexed. The swabs were removed and centrifuged (12,400 × *g*) for 3 min to pellet the cells. The supernatant was removed, 1 mL of nuclease-free water was added, and vortex and centrifugation steps were repeated to wash the cells. The cells were finally re-suspended in 1 mL 1 X PBS solution. This process was followed in parallel for all three buccal swabs. Finally, the cell suspensions were then combined into one tube. Cells were stained by adding 15 μL of 1% Methylene Blue (Kordon®, LLC, Hayward, CA) to 15 μL of cell suspension and counted with a hemocytometer (Gizmo Supply Co., Fountain Valley, CA) under a Leica DM 750P compound light microscope (Leica Microsystems, Wetzlar, Germany) at 100× magnification using a standard cell counting method [42]. Three cell counts were performed and averaged to estimate the total concentration of cells in suspension. The same cell suspension was used to spike all pipe bombs used in this study.

2.2. Pipe bomb preparation

Polyvinyl chloride (PVC) pipes (60 mm diameter) were cut to 20 cm lengths and a hole (1 cm diameter) was drilled in the center of one end cap (N = 13). The pipes and end caps were washed with Alconox® detergent (Alconox, Inc. White Plains, NY), soaked in a 15% bleach solution for 30 min, wiped with 70% ethanol, and UV-treated in a cross-linker (UVP, LLC., Upland, CA) for 10 min, rotating each pipe after 5 min. The pipe bomb casings were partially assembled by applying several coats of Oatey® Purple Primer (Oatey®, Cleveland, Ohio) and Fast Set Heavy Duty Gray PVC Cement (Oatey®) to one end of the pipe and on the inside of the intact end cap. The end cap was twisted onto the pipe end and held in place for 30 s. Four circles were engraved on each end cap and three on the pipe shaft to denote where the epithelial cells would be spiked (Suppl. Fig. 1). These engraved circles assist with post-blast identification of cells' original location. A 20 μL aliquot of cell suspension (approx. 42 cells/μL) was added to each circle and left to dry in a sterile hood overnight.

2.3. Wire preparation

Insulated copper wire was cut into 8 cm segments and cleaned with 15% bleach, 70% ethanol, and then treated in the UV cross-linker for 10 min. The ends of the wires (approx. 5 mm) were stripped with sterile pliers and spiked with 10 μL of neat blood from one of three sources of blood, and left in a sterile hood to dry overnight.