Analysis of DNA from Post-blast Fragments for Identification and Determination of Ancestry

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ABSTRACT

Improvised explosive devices (IEDs), such as pipe bombs, are weapons used to cause bodily harm or death, property damage, and/or cause fear. Tannerite® (Tannerite® Sports, LLC) is a brand of exploding targets intended to be used by licensed gun enthusiasts, but has been identified as a potential material for abuse as an explosive in pipe bombs. The ability to identify a suspect that may have touched or constructed the explosive device is critical. DNA analysis via short tandem repeats (STRs) is the conventional method for DNA-based identification but alternate genetic markers such as insertions/null polymorphisms (INNULs) and single nucleotide polymorphisms (SNPs) may be better suited to recover DNA from challenging samples, or be able to provide additional genetic information.

In this study, we created 10 identical polyvinyl chloride (PVC) pipe bombs, each 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 end of the wire (simulated finger prick). The bombs were detonated with the binary explosive Tannerite® using double-base smokeless powder to initiate the reaction.

INTRODUCTION

Pipe bombs are the most common type of IED [1-3]. After an explosion, several analyses, including DNA analysis, may be performed in an attempt to identify a suspect. However, it is likely that only trace amounts of DNA will be recovered. In addition, the DNA may also be degraded from heat produced by the explosion, further complicating the ability to generate good quality DNA profiles.

While STR markers are commonly used for DNA analysis, they can be relatively long (up to 450 base pairs). Therefore, the longer markers (> 250 bp) are most susceptible to PCR failure when DNA degrades [4]. The use of alternative molecular markers, such as INNULs and SNPs, may overcome difficulties associated with low-template and degraded DNA as they both have the potential to yield smaller amplicons (> 200 bp) [5,6].

New developments in DNA technology may also overcome some difficulties associated with typing low-template and degraded DNA samples. Massively parallel sequencing (MPS) is an alternative approach to capillary electrophoresis methods, and may provide more information from each sample for both human identification (HID), ancestry prediction, and investigative purposes [7].

RESULTS

The genotyping success of 25 post-blast samples using the GlobalFiler® Amplification Kit and the INNUL multiplex was evaluated using the number of correct alleles detected and the resulting Random Match Probability (RMP) values. In addition, the comparative success of genotyping 4 post-blast touch samples with the HID-Ion AmpliSeq™ Identity Panel was also examined. All 6 blood samples recovered from the copper wires generated complete STR profiles, but resulted in variable ancestry prediction success via MPS.

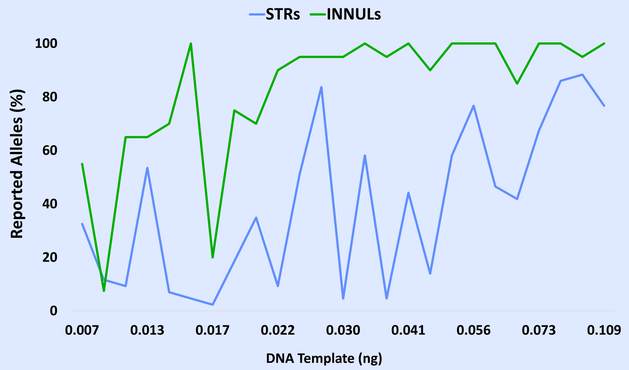


Figure 1. Comparative percentage of alleles recovered for 25 post-blast samples using STR and INNUL analyses with DNA input ranging from 0.01 to 0.2 ng.

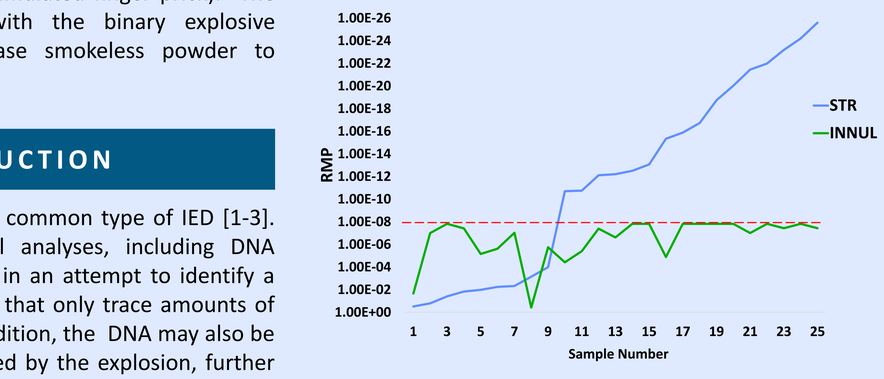


Figure 2. Comparative RMP calculations of STRs and INNULs for 25 post-blast sample. Samples are in order of increasing STR alleles. Dotted red line indicates the RMP for a complete INNUL profile.

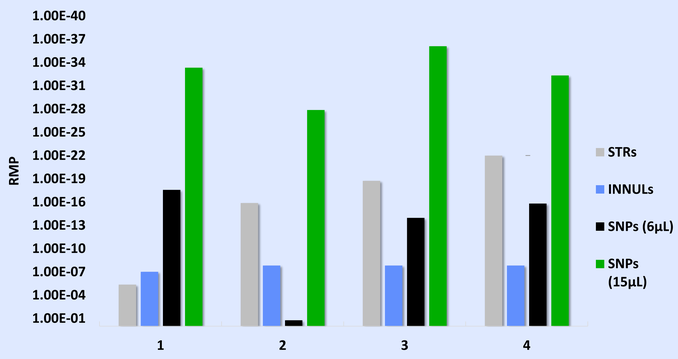
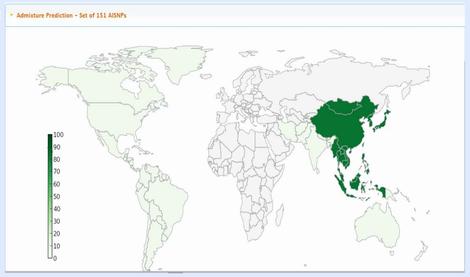


Figure 3. Comparative RMP calculations of STRs, INNULs, and SNPs via MPS for 4 post-blast samples.

(a)

Ancestry	Admixture Prediction	Confidence	Reported Alleles (%)	DNA (ng)
Asian	East Asian	Low	83	0.1
	East Asian	Low	53	0.05
Caucasian	European	Low	15	0.38
	European	Low	35	0.27
African - American	INC	INC	1	0.11
	African	Low	8	0.34

(b)



(c)

Population	Percentage
America	5
East Asia	85
Oceania	5
Africa	0
Europe	0
South Asia	5
Southwest Asia	0

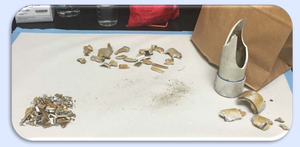
Figure 4. (a) Percentage of correct ancestry informative SNP markers called and the ancestry prediction for each sample (N = 6). (b) Geographical representation of admixture prediction for one Asian blood sample. (c) Percentage of populations predicted to be admixed within one Asian blood sample.

DISCUSSION & CONCLUSIONS

- The majority of touch samples recovered from the pipe bombs generated partial STR profiles (Fig. 1). In addition, stochastic effects such as heterozygote peak height imbalance and allelic drop-out were frequently observed, highlighting the difficulties of recovering DNA and generating reliable STR profiles from low-template samples.
- The InnoTyper™ 21 Kit was more sensitive and generated more complete genetic profiles than STR analysis, and resulted in a higher power of discrimination for some LT-DNA samples. However, STRs became more discriminatory when more than 14 STR alleles were reported (Fig. 2). INNULs are therefore an ideal adjunct for STR analysis with low-template and/or degraded DNA samples.
- The samples from post-blast fragments showed variable success when analyzed via MPS using the recommended 6 µL of neat DNA. However, concentrating the same volume of DNA extract used for STR and INNUL analysis (15 µL) to 6 µL resulted in more complete and more discriminatory SNP profiles (Fig. 3).
- The HID-Ion AmpliSeq™ Ancestry Panel was able to accurately predict the ancestry for 5 of 6 blood samples recovered from the wires attached to detonated IEDs. Although the ancestry prediction was accurately called, the confidence was low (Fig. 4).

MATERIALS AND METHODS

- An epithelial cell suspension was created from buccal swabs and the number of cells counted using a hemocytometer.
- PVC pipes (N = 10) were prepared (20 cm in length).
- Insulated copper wire (8 cm segments), 0.5 cm of insulation was stripped from each end and spiked with 10 µL of blood from 1 of 3 sources (Asian, Caucasian, or African-American).



- Cell suspension (20 µL) was placed onto the shafts and end caps of sterilized pipe bombs (11 spots).
- Pipe bombs were filled using 113 g of Tannerite® binary powder (Tannerite® Sports LLC) and 29 g of wrapped double-base smokeless powder and detonated.
- DNA was collected using swabs (wetted with 2% SDS) and extracted using the QIAamp® DNA Mini Kit.
- STRs were amplified using the GlobalFiler® PCR Amplification Kit (ThermoFisher Scientific). Separation and detection of amplified products was performed on a 3500 Series Genetic Analyzer. An analytical threshold of 175 RFU was applied.
- INNULs were amplified using the InnoTyper™ 21 Kit (InnoGenomics Technologies, LLC).
- HID and ancestry SNP analysis was performed using the HID-Ion AmpliSeq™ Identity and Ancestry Panels (v2). Library amplification and chip loading was performed on the Ion Chef and sequenced on the Ion Torrent PGM (ThermoFisher Scientific).

REFERENCES

1. Berti A, Barni F, Virgili A, Colozza C, Maiorino F, Tocca M. The recovery of DNA profiles from saliva and touch evidences after postal bomb explosion. Forensic Science International: Genetics Supplement Series. 2011;3(1):e471-e2.
2. Phetpeng S, Kitpipit T, Thanakiatkrai P. Systematic study for DNA recovery and profiling from common IED substrates: From laboratory to casework. Forensic Science International: Genetics. 2015;17:7.
3. Bille TW, Cromartie C, Farr M. Effects of Cyanoacrylate Fuming, Time After Recovery, and Location of Biological Material on the Recovery and Analysis of DNA from Post-Blast Pipe Bomb Fragments. J Forensic Sci. 2009;54(5):8.
4. Horsman-Hall KM, Orihuela Y, Karczynski SL, Davis AL, Ban JD, Greenspoon SA. Development of STR profiles from firearms and fired cartridge cases. Forensic Science International: Genetics. 2009;3(4):242-50.
5. Romanini C, Catelli ML, Borosky A, Pereira R, Romero M, Salado Puerto M, et al. Typing short amplicon binary polymorphisms: supplementary SNP and Indel genetic information in the analysis of highly degraded skeletal remains. Forensic Sci Int Genet. 2012;469-76.
6. LaRue B, Sinha S, Montgomery AH, Thompson R, Klaskala L, Ge J, et al. INNULs: A Novel Design Amplification Strategy for Retrotransposable Elements for Studying Population Variation. Human Heredity. 2012;74:9.
7. Børsting C, Morling N. Next generation sequencing and its applications in forensic genetics. Forensic Science International: Genetics. 2015;18:78-89.

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