

## FEASIBILITY OF CONDUCTING PCR-BASED DNA ANALYSIS AT THE CRIME SCENE

**Eduardo Ribeiro Paradelo**<sup>1,2</sup>, **Debra Glidewell**<sup>1</sup>, **Felipe Konotop**<sup>1,2</sup>, **Elizeu Fagundes de Carvalho**<sup>2</sup> and **Cecelia Crouse**<sup>1</sup>.

<sup>1</sup>-Palm Beach County Sheriff's Office, Crime Laboratory, West Palm Beach, Florida

<sup>2</sup>-Universidade do Estado do Rio de Janeiro (UERJ) – Laboratório de Diagnóstico por DNA andar – IBRAG – DBB

### **Abstract**

Collection of crime scene evidence in a manner appropriate for DNA analysis is currently being addressed by law enforcement in Brazil. In the case of a catastrophic accident or an especially violent crime involving many individuals, it would be very beneficial to have the DNA analysts involved in the collection of evidence at the crime scene. In addition, PCR DNA profiling of evidentiary sample in the field would have many advantages including prioritization of samples for laboratory analysis, identification of probative samples, maintaining the chain of custody and additional investigative leads forming.

### **Introduction**

Law Enforcement personnel must make critical judgments regarding the method and types of evidence collected at crime scenes. In many cases, this process is straightforward. In cases where many individuals have sustained catastrophic lethal injuries, the amount of biological evidence collected may become overwhelming. Although it is extremely important to collect as much evidence as possible, the sometimes unwieldy question is “Which pieces of biological evidence will offer the most immediate probative information?” The purpose of these studies is to determine if it is possible using Field PCR DNA testing to:

- a. Prioritize samples for further laboratory DNA analysis.
- b. Provide immediate determination of probative samples
- c. Maintain chain-of-custody
- d. Provide immediate investigative leads

As a result, protocols for field analysis of biological evidences using the Mobile Molecular Laboratory Model-0150 (MML) from MJ Research (Waltham, MA) were developed. The procedures include four basic steps: 1) DNA extraction, 2) amplification, 3) gel electrophoresis of amplified products and 4) visualization of the amplified products for interpretation. The DNA profile from biological materials “read-out” system is an “STR pattern” equivalent to a post-amp gel. The goal is to use minimal reagents, the least amount of manipulation and minimal run time. Genotypes cannot be ascertained by the methods employed here.

## Material and Methods

### DNA Field Extraction:

Blood from a known individual was deposited on the surface of glass, carpeting, tile floor, a cotton shirt, blue jeans, soil, and a countertop. In addition six standards from known individuals were used to validate the protocols described below. The following DNA extraction kits were evaluated: Capture Column Kit and DNA Isolation Kit (Gentra, Minneapolis MN), SV RNA (Promega, Madison Wisconsin), alkaline lysis (1) and Lyse-N-Go (Pierce, Roxford, IL). All tests were conducted at room temperature or using the heating block at 65<sup>0</sup>C or 75<sup>0</sup>C.

The Perkin Elmer Human Identification QuantiBlot System was used to determine the amount of DNA extracted from each protocol. Quantitation would not be conducted in field PCR testing.

### DNA Field Amplification:

Locus-specific short tandem repeat (STR) primer pairs were evaluated for use in multiplex reactions including primer sets for Penta B, Penta C, Penta D, Penta E, CSF1PO, D16S539, D13S317, D8S1179, D7S820, D5S818 and amelogenin loci. Multiplex System I consisted of the primers from D16S539, Amelogenin and D13S317. Multiplex System II consisted of primer sets from CSF1PO, Penta E and D5S818 *loci*. In order to optimize the amplification reaction for use in the field, the following DNA polymerase enzymes were evaluated for amplification of both Multiplex System I and System II: AmpliTaq™ Gold, AmpliTaq™ (Perkin Elmer, Foster City, CA), TaqPlus® Long and *Pfu Turbo*® (Stratagene, Austin, TX).

PCR reactions were conducted in the PTC-100 thermocycler from MJ Research (Figure 1, Waltham, MA). The cycling program selected for optimum amplification of both the System I and System II STR multiplexes is: 95<sup>0</sup>C 11 minutes and 96<sup>0</sup>C 1 minute for hot-start, 95<sup>0</sup>C 15 seconds and 60<sup>0</sup>C 35 seconds for 30 cycles followed by a 5 minutes of 60<sup>0</sup>C extension.

### Electrophoresis:

Amplified products were electrophoresed on a variety of gel matrixes to determine the optimum separation of the STR fragments. The following gel-types were tested:

<i>Gel /Company</i>	<i>Concentration Composition</i>	<i>Buffer Type</i>	<i>Gel Thickness</i>	<i>Number of Wells</i>
6% Ultra PAGE TBE Landscape 16 EMBITEC	6% / PAG	1X TBE	3 mm	
10% Ultra PAGE TBE Landscape 16 EMBITEC	10% PAGE	1X TBE	3mm	
4% Agarose TBE Landscape Gel 12	4% agarose	1X TBE	6mm	

## EMBITEC

Spreadex™ EL 1200 MINI-GEL 8-12	NR / Spreadex	1X TAE	3mm
<hr/>			
Elchrom Scientific			
4% Nusieve Gel / FMC 8	4% / Nusieve	1X TBE	5mm

NR-not revealed by manufacturer

Manually poured gels using Gel Shooters (Continental, San Diego, CA) with 1X TBE buffer were also evaluated. Different electrophoretic apparatus using voltages ranging from 3V/cm to 21V/cm were tested. The utilization of either SYBR gold (Molecular Probes, Eugene, OR), GelStar Nucleic Acid Gel Stain (FMC, Rockland, ME) or ethidium bromide were evaluated to determine optimum visualization of the amplified STR alleles. Polaroid pictures using 667 film were used to record the data taken of the amplified samples.

## **Results**

The results of our study indicate the following:

1. From the tested extraction methods both SV RNA (Promega) and alkaline lysis procedure (1) were able to release DNA in appropriate concentration (1-10 ng) and quality for PCR amplification from fresh liquid blood, surface of glass, carpeting, tile floor, cotton shirt, blue jeans, soil, and countertop. The extraction time was approximately 15 minutes for fresh blood and up to 30 minutes for dried blood stains.
2. Excess of Guanidine Thiocyanate present in SV RNA inhibited the amplification reaction.
3. The Multiplex System I which includes D16S539, amelogenin and D13S317 and Multiplex System II, which consists of CSF1PO, Penta E and D5S818 showed the best results for field analysis. The Penta E (figure 2) was the most discriminative STR marker among those evaluated. Figure 2 shows known bloodstain standards extracted using the alkaline lysis procedure and amplified using the System II in the MML thermal cycler. Embitec 6% Ultra PAGE TBE Landscape (a) and in Hitachi's 4.5% R-3 Disposable polyacrylamide Gels (b) were used for visualization of the amplified fragments. Figure 2 shows the discrimination of six samples in the Penta E *locus*. 1. homozygote sample; 2. Alleles with 20 bp difference; 3. Alleles with 20 bp difference; 4. homozygote sample; 5. homozygote sample; 6. Alleles with 5 bp difference. This protocol would be feasible in the field for determining STR allele pattern differences between samples.
4. It is possible to obtain STR profile and sex determination of the sample with 1.0 ng to 20 ng of DNA although quantification of the DNA extracts would not be conducted in the field, quantification was conducted for these studies to determine the efficacy of the extraction protocol.

5. The Stratagene's polymerase enzymes TaqPlus<sup>®</sup> Long and *Pfu* Turbo<sup>®</sup> require longer extension times to obtain positive results. The amplification reactions using AmpliTaq<sup>™</sup> showed increased amplification artifacts. AmpliTaq<sup>™</sup> Gold produced robust results without amplification artifacts and shorter extensions times could be used (5 minutes instead of 10-30 minutes)
6. It was determined that bovine serum albumin (BSA) is a critical component of the enzyme and should be used at 0.8ug/ul.
7. The final PCR program (MML I) using AmpliTaq<sup>™</sup> Gold in a PTC-100 thermocycler (MJ Research) used: 95° C for 15 seconds to denaturation, 60°C for 35 seconds to annealing and extension (30 times), with a 60°C for 5 minutes for final extension time.
8. Reduction of PCR buffer (GoldStar) concentration to a 0.4X improved the amplification of D16S539 in the multiplex System I and CSF1PO in the System II. The larger molecular weight fragments were not amplified as efficiently as the smaller molecular weight fragments when 1X PCR GoldStar concentrations were used.
9. Loading 16 samples in an Embitec 6% polyacrylamide gel at 9V/cm for 15 minutes provides adequate separation of System I and System II multiplexes. Four base pairs differences can be observed after 25 minutes of electrophoresis.
10. Samples can be discriminated even when related people are involved.
11. Gels post-stained with GelStar Nucleic Acid Gel Stain (FMC) are the easiest to analyze.

### **Discussion and Conclusions:**

One of the most common problems faced by forensic science laboratories is the high number of samples to be analyzed per case. These studies show that field PCR can discriminate between samples using the MML system and thus allow for the prioritization of samples in the laboratory setting. Homozygous and heterozygous pattern, sex determination and direct comparison among samples can be observed (Figure 1). Discrimination of 4 base pairs difference between alleles allows differentiation of samples even from related people, which in catastrophic accidents is not an uncommon occurrence. The determination of STR patterns in the field may protect the chain of custody of evidence since the analysts will have control of the samples. The entire protocol from extraction through STR pattern identification is approximately 1 hour and 15 minutes for 25 samples. Currently, non-human DNA and mixture samples are being studied. Mock scenes simulating catastrophic situations will be conducted in collaboration with Palm Beach County Sheriff's Office crime scene personal.

The most important advantage to having field DNA analysis for catastrophic scenes is the identification of samples that should be prioritized for laboratory testing. The major disadvantages of the field-testing as described here are the amount of time it takes to analyze the samples, the number of steps involved and the amount of equipment necessary. It is hoped that in the future hand held devices will be available for research and development of field DNA testing.

## References

1. Klintshar, M and Neuhuber, F. Evaluation of an Alkaline Lysis for the Extraction of DNA from Whole Blood and Forensic Stains for STR Analysis. J. Forensic Sci. 2000; 45(3): 669-673.

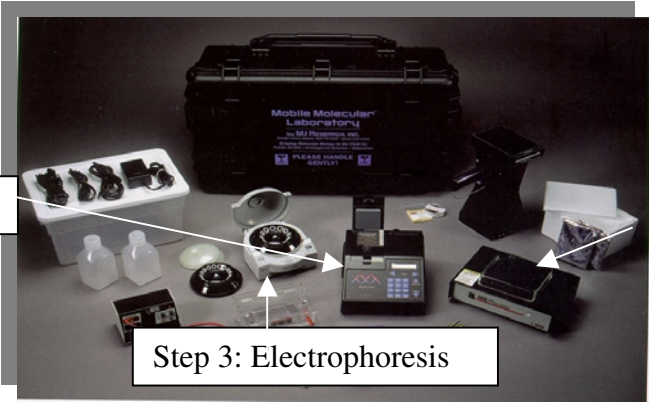
## Acknowledgments

We would like to thank Promega Corporation, Palm Beach County Sheriff's Office, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Universidade do Estado do Rio de Janeiro, and Florida International University.

Figure 1: The Mobile Molecular Laboratory equipment.

Step 1: Extraction (Room Temperature)

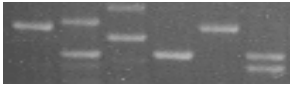
Step 2: PCR



Step 4: Allele visualization

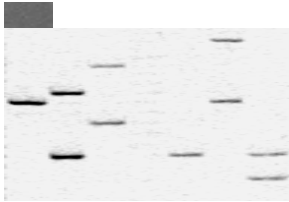
**Figure 2:** Comparison of electrophoresis of amplified System II in a 6% Ultra PAGE TBE Landscape (Embitec) and a R-3 Disposable Polyacrylamide Gels (Hitachi genetic System).

a) 1 2 3 4 5 6



6% Ultra PAGE  
TBE Landscape -  
Penta E locus

b) 1 2 3 4 5 6



4.5% R-3 Disposable Gels –  
Penta E locus