

**INVESTIGATION OF DNA PROFILING METHODS FOR FORENSIC  
EXAMINATION OF SOIL EVIDENCE.**

By

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# Table of Contents

SUMMARY.....	4
DECLARATION.....	6
ACKNOWLEDGEMENTS.....	7
ABBREVIATIONS.....	8
<b>1 INTRODUCTION AND BACKGROUND.....</b>	<b>9</b>
1.1 CURRENT FORENSIC TECHNIQUES FOR SOIL ANALYSIS ARE MOSTLY PHYSICAL.....	10
1.2 A BIOLOGICAL APPROACH TO FORENSIC SOIL ANALYSIS.....	10
1.2.1 DNA Based Analysis of Soil Community DNA.....	12
1.2.2 Profiling the Soil Community Metagenome.....	14
1.3 MICROARRAYS.....	19
1.3.1 Phylogenetic Arrays (PAs).....	22
1.3.2 Functional Gene Arrays (FGAs).....	24
1.3.3 Community Genome Arrays (CGAs).....	25
1.3.4 Random Oligonucleotide Arrays (ROAs).....	26
1.4 REQUIREMENTS FOR AN IDEAL ANALYTICAL TECHNIQUE FOR FORENSIC SOIL EXAMINATION.....	26
1.4.1 Reproducibility of Analysis.....	27
1.4.2 Discrimination Between Soils.....	27
1.4.3 Dealing with Environmental Variables.....	27
1.4.4 Suitable for Minute Sample Sizes.....	28
1.4.5 Practical Considerations Regarding Forensic Soil Samples.....	29
1.5 DNA EXTRACTION METHODS.....	29
1.5.1 Indirect Extraction of DNA from Soil.....	30
1.5.2 Direct Extraction of DNA from Soil.....	30
1.5.3 Comparing Direct and Indirect Methods of DNA Extraction from Soils.....	30
1.6 DNA PURIFICATION METHODS.....	31
1.6.1 Blocking the Influence of Soil-Borne Inhibitors on Downstream Processes.....	32
1.7 INTRODUCTION TO THE RESEARCH AND FINDINGS ENCOMPASSED IN THIS THESIS.....	33
<b>2 MATERIALS AND METHODS.....</b>	<b>35</b>
2.1 CHEMICALS.....	35
2.2 ENZYMES.....	35
2.3 OLIGONUCLEOTIDE PRIMERS.....	35
2.4 COLLECTION OF SOILS AND EXTRACTION OF DNA.....	37
2.4.1 Lysis and Extraction of DNA from Soil Organisms.....	37
2.4.2 Lysis and Extraction of Human DNA from Whole Blood.....	41
2.5 POLYMERASE CHAIN REACTION.....	42
2.5.1 Hardware and Devices.....	42
2.5.2 Amplification of 16S-rDNA Loci from Soil-Borne Bacterial DNA.....	42
2.5.3 Amplification of Arbitrary DNA Sequence.....	43
2.5.4 Real-Time Amplification of Arbitrary DNA Sequence.....	44
2.6 GENERATION AND ANALYSIS OF LENGTH POLYMORPHISM FINGERPRINTS.....	44
2.6.1 Terminal Restriction Fragment Length Polymorphism Fingerprinting.....	44
2.6.2 Arbitrarily Amplified DNA Length Polymorphism (AADLP) Fingerprinting.....	45
2.6.3 Analysis of Length Polymorphism Data.....	45
2.7 LOW RESOLUTION, CONVENTIONAL AGAROSE GEL ELECTROPHORESIS, SOUTHERN TRANSFER AND HYBRIDISATION.....	46
2.8 MICROARRAY TECHNIQUES.....	48
2.8.1 Microarray Preparation and Printing.....	48
2.8.2 Quality Control of Microarray Printing.....	49
2.8.3 Labelling and Hybridisation of DNA profiles.....	49
2.8.4 Analysis of Microarray Data.....	50
2.9 CLONING OF ARBITRARILY AMPLIFIED DNA PROFILE SEQUENCES.....	50
2.9.1 Ligation Reactions.....	50
2.9.2 Transformation of Ligated Vectors into Competent <i>E. coli</i> JM109.....	50
2.9.3 Isolation of Plasmid DNA from Recombinant Hosts.....	51
2.9.4 Sequencing Reactions.....	52

<b>3 RESULTS AND DISCUSSION.....</b>	<b>53</b>
<b>3.1 TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (TRFLP) ANALYSIS..</b>	<b>53</b>
3.1.1 Soil Profile Comparisons.....	53
3.1.2 Technical Variation of TRFLP Profiles.....	57
3.1.3 Temporal Variation of TRFLP Profiles.....	59
3.1.4 Conclusions for the TRFLP Technique.....	62
3.1.5 A Soil TRFLP Profile Database is Not Currently Feasible.....	62
<b>3.2 ARBITRARILY AMPLIFIED DNA PROFILES.....</b>	<b>63</b>
3.2.1 Electrophoresis and Southern Analysis.....	63
3.2.2 Length Polymorphism Comparisons of Arbitrarily Amplified Soil DNA Profiles..	67
<b>3.3 MICROARRAY ANALYSIS.....</b>	<b>70</b>
3.3.1 General Principles of the Array.....	70
3.3.2 Monitoring the Quality of Microarray Probes.....	70
3.3.3 Soil Discrimination via Sequence similarity of DNA profiles.....	72
3.3.4 Technical Sources of Arbitrarily Amplified DNA Profile Variation.....	79
3.3.5 Environmental Variables that may affect Profiles.....	85
3.3.6 Conclusions for Arbitrarily Amplified DNA Profiles.....	92
<b>3.4 THE MOLECULAR MECHANISMS OF THE ARBITRARY AMPLIFICATION.....</b>	<b>93</b>
3.4.1 Amplification Efficiency and Sensitivity of Various Primers.....	94
3.4.2 Amplification of Trace Amounts of DNA from PCR Reagents.....	102
3.4.3 The Effect of Successive Rounds of PCR on Profile Composition.....	104
3.4.4 DNA Profiles of Different Humans and Organisms.....	107
3.4.5 The Degree of Overlap of Amplification Products using Different but Related Primers.....	109
<b>4 DISCUSSION.....</b>	<b>125</b>
<b>4.1 COMPARISON OF TRFLP AND ARBITRARILY AMPLIFIED DNA LENGTH POLYMORPHISM (AADLP) ANALYSES.....</b>	<b>125</b>
4.1.1 Similarity Index Comparisons.....	125
4.1.2 Reproducibility Comparisons.....	125
4.1.3 Significance of Discriminatory Power.....	126
4.1.4 Fundamental Differences of Analysis.....	126
4.1.5 Potential for Improvement.....	127
4.1.6 Other Banding Based DNA Technologies that may be suited to Forensic Soil Evidence.....	127
<b>4.2 COMPARISON OF MICROARRAY AND SOUTHERN MEMBRANE SEQUENCE SIMILARITY ANALYSIS OF ARBITRARILY AMPLIFIED DNA PROFILES.....</b>	<b>131</b>
<b>4.3 COMPARISON OF LENGTH POLYMORPHISM AND SEQUENCE SIMILARITY ANALYSIS OF ARBITRARILY AMPLIFIED DNA PROFILES.....</b>	<b>132</b>
4.3.1 Comparison of Similarity Index and Relative Fluorescence Unit Values.....	132
4.3.2 Reproducibility Comparisons.....	133
4.3.3 Significance of Discriminatory Power.....	133
4.3.4 Fundamental Differences of Analysis.....	134
4.3.5 Other Sequence Hybridisation Based Methods that may be suited to Forensic Soil Evidence.....	135
<b>4.4 COMPARISON OF TRFLP AND SEQUENCE HYBRIDISATION ANALYSIS OF SOIL SAMPLES TAKEN AT DIFFERENT TIMES FROM THE SAME LOCATION.....</b>	<b>137</b>
<b>5 CONCLUSIONS.....</b>	<b>139</b>
<b>5.1 FUTURE DIRECTIONS FOR BIOLOGICAL PROFILING OF SOIL EVIDENCE.....</b>	<b>140</b>
5.1.1 Improving the Profiling Techniques.....	140
5.1.2 Practical Issues that may Arise in Forensic Casework Need Addressing.....	141
<b>REFERENCES.....</b>	<b>143</b>
<b>APPENDIX CD.....</b>	<b>BACK COVER INSERT</b>

## Summary

In this thesis, an investigation of the potential of two DNA based profiling techniques for the analysis of forensic soil evidence is presented. These profiling techniques were:

- The previously described technique, Terminal Restriction Fragment Length Polymorphism Analysis (TRFLP) of 16S DNA (Chpt 3.1) and
- A semi-novel profiling technique, Arbitrarily Amplified DNA (AAD) profiling (Chpts 3.2 and 3.3), analysed by both:
  - *conventional* length polymorphism of DNA fingerprints (AADLP) (Chpt 3.2) and
  - *a completely novel method*, DNA sequence similarity (AADSS) (Chpts 3.2 and 3.3), which was investigated using
    - ⌚ Southern Hybridisation (Chpt 3.2) and
    - ⌚ Microarray Technology (Chpt 3.3).

These methods were successful at distinguishing samples of soil to varying degrees. TRFLP analysis was capable of generating low but significant differences in similarity statistics between replicate and distinct soil profiles, while both AAD analyses (AADLP and AADSS) generated large and significant differences in similarity statistics between replicate and distinct soil profiles. The potential for significant differences between similarity statistics to be generated enables classification of soil samples as either having common origins (matching, if there is no significant difference) or not (excluded from matching, if there is a significant difference). The affect of several technical, environmental and practical variables on the biological profiles generated using these techniques was investigated further. These variables included sampling and processing of soils (assessed with TRFLP, AADLP and AADSS), time *in situ* (TRFLP and AADSS), time *ex situ* under a number of storage conditions, as well as spatial variations of microbial communities over small distances (AADSS only).

The most capable method for distinguishing soils of different origin was AADSS, closely followed by AADLP, with TRFLP obtaining only marginal success relative to the other two methods. The preferred format of the AADSS technique is the microarray technology as it is capable of generating a good deal more data from soil DNA profiles than Southern hybridisation. However, as both are capable of distinguishing soils, the low cost Southern hybridisation technique may provide a suitable entry point technology for many forensic laboratories.

The molecular mechanism of the arbitrary amplification profiling system (AAD) was investigated (Chpt 3.4), allowing insight into the way these profiles are generated and potential ways to control the process in order to optimise profiles for various purposes. Many potential improvements and developments are suggested which may further enhance the utility of the techniques presented in this thesis.

The findings presented in this thesis demonstrate the potential for biological profiling of soil communities as a relatively simple, high resolution, objective tool that permits stringent statistical analysis, is not reliant on expert interpretation and is complementary to existing strategies for the forensic examination of soil evidence.

## **Declaration**

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

James M Waters  
September 2004

I believe that this thesis is properly presented, conforms to the specifications for a thesis and is sufficient to be, *prima facie*, worthy of examination.

Leigh A Burgoyne  
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## Abbreviations

A <sub>600</sub>	absorbancy at 600nm
AFLP	alternate fragment length polymorphism
AP-PCR	arbitrarily primed polymerase chain reaction
Bp	base-pair(s)
BSA	bovine serum albumin
cDNA	copied <i>or</i> cloned deoxyribonucleic acid
CTAB	hexadecyltrimethylammonium bromide
CV	coefficient of variance
DGGE	denaturation gradient gel electrophoresis
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
A	adenine
C	cytosine
G	guanine
T	thymine
dNTP	deoxynucleoside triphosphate
dsDNA	double stranded deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FAM	6-carboxyfluorescein
Kbp	kilobase pairs
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid
RAPD	random amplified polymorphic DNA
rDNA	ribosomal deoxyribonucleic acid
RFLP	restriction fragment length polymorphism
RFU	relative fluorescence unit
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SD	standard deviation
SDS	sodium dodecyl sulphate <i>or</i> sodium lauryl sulphate
SMIPS	structurally mediated inter-primer selectivity
SI	similarity index
TAE	Tris acetate and EDTA
Taq	<i>Thermus aquaticus</i>
TGGE	temperature gradient gel electrophoresis
TRF	terminal restriction fragment
TRFLP	terminal restriction fragment length polymorphism
UV	ultraviolet