Implementing a DNA sequencing workflow for forensic human identification in South Africa

Donna-Lee Martin, Laura Heathfield, PhD

Division of Forensic Medicine & Toxicology | Department of Pathology | Faculty of Health Sciences | University of Cape Town | Laura.royle@uct.ac.za

Introduction

- In South Africa, a staggering number of bodies remain unidentified.
- DNA profiling is crucial for identification when **visual identification is impossible** due to **decomposition** or **burns** [1]**.**
- Traditional DNA profiling methods using capillary electrophoresis (CE) provide limited success **for challenging forensic samples** from decomposed or burnt remains.
- **Massively parallel sequencing (MPS)** is able to overcome these limitations, as well as **improve discriminatory power**, but implementation requires the following: [2]
  - **A** **Large-scale sequence-based population data to facilitate statistical DNA profile interpretation.**
  - **B** **A workflow that is internally validated against the manufacturer's criteria of acceptance.**
- To lower the cost associated with large-scale databasing, an **optimised direct-PCR approach** for crude swab lysates was investigated. This project will aim to provide the basis for **implementation** of the **MiSeq FGx™ workflow.**

Workflow Overview

Figure 1. The MiSeq FGx™ workflow. The ForenSeq™ DNA Signature Prep kit is used to prepare libraries for sequencing on the MiSeq FGx sequencer. The analysis of data is automated and integrated into the workflow.

Experimental Approach

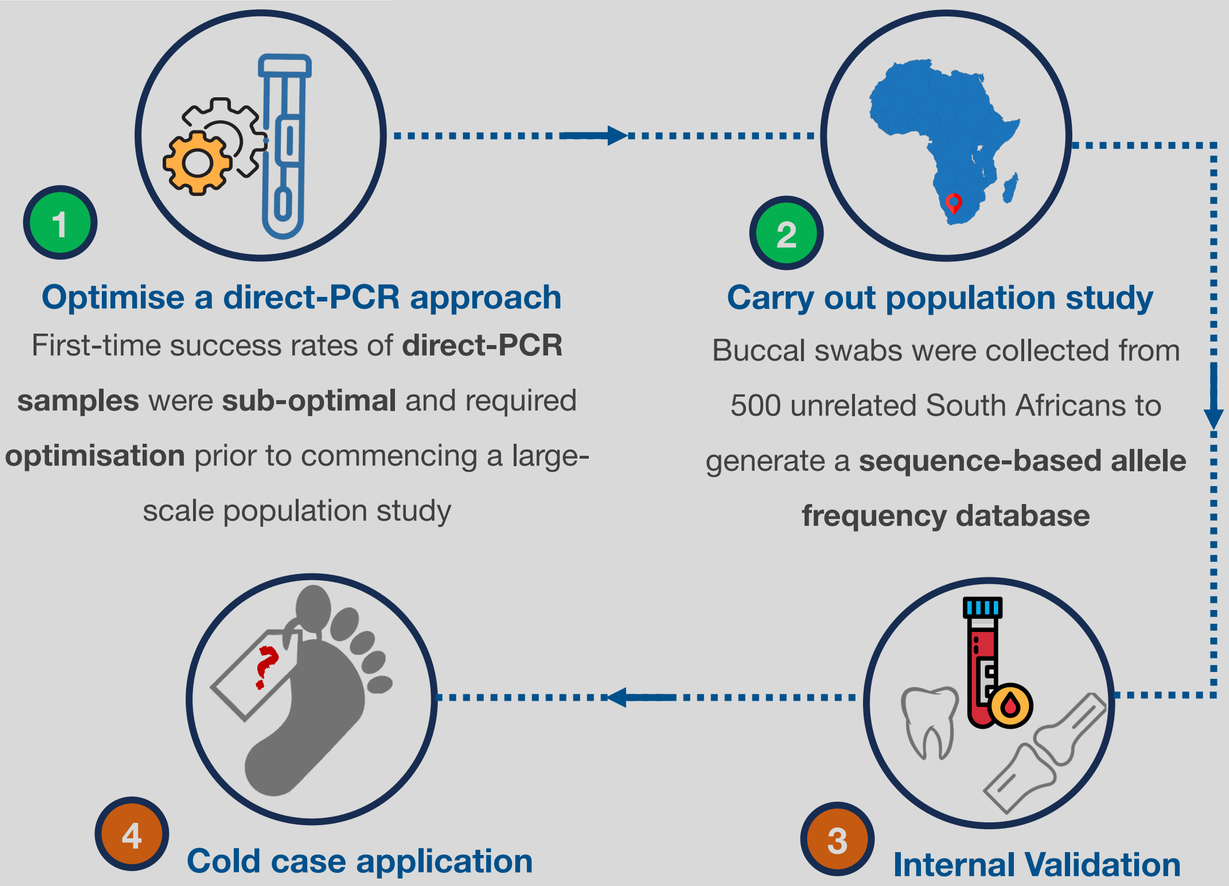

Figure 2. Experimental approach for implementation of the MiSeq FGx™ workflow.

Preliminary Results

1 An optimised direct-PCR approach

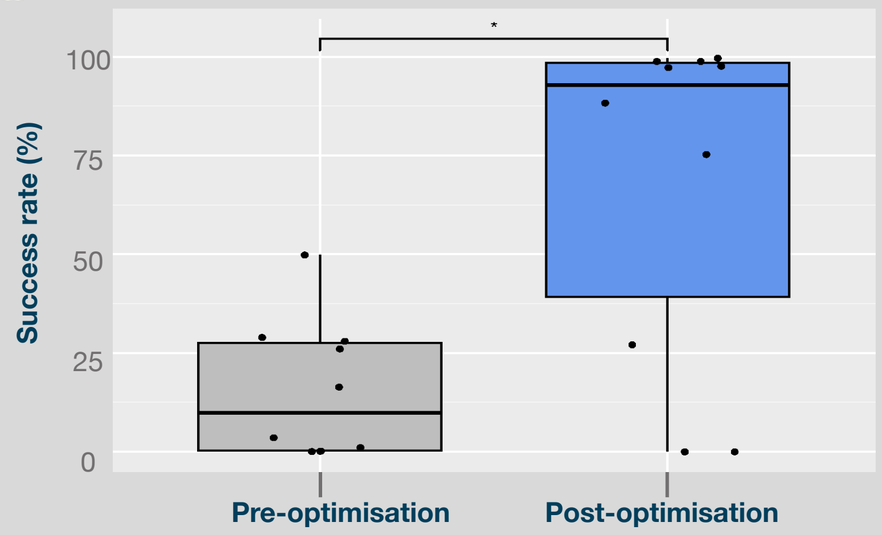

Figure 3. A box plot illustrating a significant increase in success rate of sequence-based DNA profiles generated for direct-PCR samples after optimisation ($p < 0.05$).

2 Population study

Preliminary results from the **sequence-based population study** allowed for the comparison of **length and sequence-based alleles** for **autosomal STR markers.**

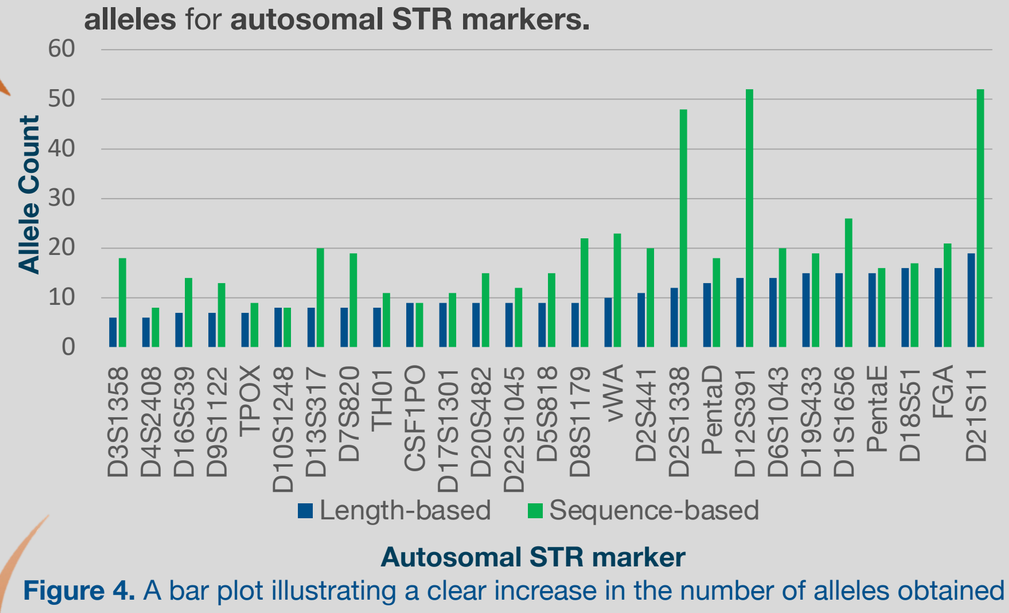

Figure 4. A bar plot illustrating a clear increase in the number of alleles obtained through sequence-data (MPS), compared to length-based data (CE). This plot is based on a subset of 150 individuals from the South African population.

Discussion and ongoing work

- This project represents the **first forensic sequence-based** data for the **South African population.**
- An **optimised** and **streamlined approach** for processing **direct-PCR** samples with the **MiSeq FGx™ workflow** was achieved.
- Preliminary results from sequence-based population data showed that **sequence-based DNA profiles** capture a **large amount of variation** for **improved discriminatory power.**
- Further experiments towards implementation will involve the internal validation of the MiSeq FGx™ workflow for reference and casework samples.

References

[1] Reid KM, Martin LJ, Heathfield LJ. Bodies without names: a retrospective review of unidentified decedents at Salt River Mortuary, Cape Town, South Africa, 2010–2017. *South African Med J.* 2020;110 (3): 223-228.

[2] Borsting, C and Morling N. Next generation sequencing and its applications in forensic genetics. *Forensic Sci. Int. Genet.* 2015;18: 78–89.

National Research Foundation

MOLECULAR FORENSICS RESEARCH GROUP