Forensics: Human Identity Testing in the Applied Genetics Group

Workshop to Identify Standards Needed to Support Pathogen Identification via Next-Generation Sequencing (SPIN)
October 20-21, 2014

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Applied Genetics Group

Advancing technology and traceability through quality genetic measurements to aid work in Forensic and Clinical Genetics

A core competency of our group is the application of nucleic acid-based methods
PCR – Genotyping – Sequencing – Real-time PCR – Digital PCR – DNA based SRMs

Forensic Genetics Clinical Genetics

Recent focus areas: update of SRM2391c, digital PCR, & next generation sequencing

Steps in Forensic DNA Analysis

Usually 1-2 day process (a minimum of ~8 hours)

DNA Extraction DNA Quantitation

Multiplex PCR Amplification

Target region (short tandem repeat)

STRA Typing

Technology

Biology

Genetics

Statistics Calculated
DNA Database search
Paternity test
Reference sample enrolled

Applied Use of Information

Blood stain Buccal swab
Sample Collection 
& Storage

~3.5 h

1.5 h

1.5 h

1.5 h

1.5 h

~2 days

2 repeats

5 repeats

7 repeats

9 repeats

11 repeats

12 repeats

13 repeats

The number of consecutive repeat units can vary between people

The frequencies of these length-based alleles are known in the various population groups (published in the literature)

Short Tandem Repeat (STR) Markers

Length-based polymorphism present in the human genome

TCCCAAGCTTCTTCTCCTCCCTAGATCAATACACAGACAG
GTGAGATGAGATGAGATGAGATGAGATGAGATGAGATA
GATAGATGATAGATAGATAGATAGATAGATAGATAGATA
ATGCTTACAGATGAGC

= 12 GATA repeats (“12” is reported)
Identifiers (Applied Biosystems) 15 STR Loci Kit

Information is tied together with multiplex PCR and data analysis

The frequencies of the STR alleles are known in a population
The allele frequencies are independent and can be multiplied (product rule)

The Random Match Probability (RMP) is over 1 in 800 trillion for unrelated individuals

This test contains the 13 FBI core loci (NDIS)

Applications of Human Identity Testing

- Forensic cases: matching suspect with evidence
- Kinship determination
- Missing persons investigations
- Military DNA “dog tag”
- Convicted felon DNA databases
- Mass disasters: putting pieces back together
- Historical investigations
- Genetic genealogy

Human Identity Testing with DNA

- Always testing human DNA (one species)
- The majority of the identification tests are performed with a core set of short tandem repeat markers (STRs) 13 → 20
  - Mitochondrial DNA (high copy number, maternally inherited)
  - Selected SNPs (identity, biogeographical ancestry, phenotype)
- Currently the workflow is very similar in all DNA testing labs
  - Extraction, qPCR quantification, multiplex PCR kit, separation and detection (capillary electrophoresis)
  - Performed with very similar commercial reagents and instrumentation (no in house or home brew assays are used – validation is important)

FBI DNA Advisory Board Quality Assurance Standards for Forensic DNA Testing Laboratories Oct 1, 1998

A lab must follow the QAS to attain accreditation

- Scope
- Definitions
- Quality Assurance Program
- Organization and Management
- Personnel
- Facilities
- Validation
- Analytical Procedures
- Equipment and Calibration Maintenance
- Reports
- Review
- Proficiency Testing
- Corrective Action
- Audits
- Safety
- Outsourcing
http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas_testlabs

Standard Reference Material 2391c: PCR-Based DNA Profiling Standard

- Components A through D are DNA extracts in liquid form
- Components E and F are cells spotted on 903 paper or FTA paper
- Certified values are for STR alleles based on length polymorphisms observed using capillary electrophoresis

NIST Nucleic acid-based standards

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<th>SRM</th>
<th>NIST DNA-based SRMs</th>
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<tr>
<td>2366</td>
<td>Cytomegalovirus (CMV) for DNA Measurements</td>
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<td>2393</td>
<td>CAG Repeat Length Mutation in Huntington’s Disease</td>
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<td>2374</td>
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<td>Heteroplasmy Mitochondrial DNA Mutation Detection Std</td>
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Candidates currently under characterization

- BK Virus 1
- HER2 Copy Number Measurement
- Pathlength Standard for Nanoliter Spectrophotometers
- Genome in a Bottle (NA 12878)

- extracted genomic DNA (human)
- extracted genomic DNA (viral in BAC)
- extracted DNA (plasmid)
- PCR products
- cell lines on paper substrate
- Uracil and Tryptophan solutions

Purpose of an Interlaboratory Study

Interlaboratory studies (ILS) are a way for multiple laboratories to compare results and demonstrate that the methods or instrument platforms used in one’s own laboratory are reproducible in another laboratory.
Impact Examples

miniSTRs

Smaller PCR product size (<125 bp)
Utility: typing degraded samples

World Trade Center – Phase I Summary

Profiler Plus – Partial Profile
Degradation or Inhibition

Larger PCR products fail

12,392 Bone samples processed
3,405 Full profiles (13 STR loci)
2,143 High partial profiles (>7 STR loci)
2,670 Low partial profiles (<7 STR loci)
4,174 No loci

Over 6800 profiles miniSTRs are helping here

Final 20% of WTC victims identified were based on a miniSTR technique pioneered at NIST

Rapid PCR

- Up until 2008 PCR amplification times required approximately 3 hours
- Utilizing new (faster) DNA polymerases and rapid PCR thermal cyclers we demonstrated results in 36 minutes
- Enabling faster commercial STR typing kits (37 min) and fully integrated ‘Rapid DNA’ typing instruments (swab to profile in 90 minutes)

New STR Loci

- The U.S. DNA database is expanding their core STR loci from 13 to 20
- 3 of the new candidates come from research performed at NIST (D2S441, D10S1248, D22S1045)
- NIST is providing the allele frequencies for U.S. populations

Emerging: Next-generation sequencing

Characterization of forensic SRMs with NGS technologies

Samples

Markers

Platforms

- SRM 2391c
- STR
- MiSeq

- SRM 2382/2520 (mtDNA)
- mtDNA
- PGM

- NIST Population Samples
- SNP

Acknowledgements

- Stable funding (NIJ, NIST, FBI)
- Great team of people (past and present)
- Forensic DNA typing built on a foundation of science, QAS, and reference materials to ensure quality measurements

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