Development, Characterization and Performance of New MiniSTR Loci for Typing Degraded Samples

Michael Coble, Becky Hill, Peter Vallone, and John Butler
May 04, 2006

Current Areas of NIST Research Effort

- Resources for “Challenging Samples”
- Standard Reference Materials (SRM 2391 DNA Profiling Standard)
- Information on New Loci (SNPs, Y-Chromosome, new STRs)
- Standard Information Resources (STRBase website, training materials/review articles, validation standardization)
- Allele Sequencing and Interlaboratory Studies (Real-time qPCR, mixture interpretation)

Highly Degraded DNA

PowerPlex 16 Result on Aged Blood Stain
(15 years at room temperature storage)

A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples.


http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Why Go Beyond the CODIS Loci?

(1) Large Allele Ranges (e.g. FGA)

(2) “Unclean” Flanking Sequences (e.g. D7S820)

Why go beyond CODIS loci?

“STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association.”

“To achieve this purpose, either new STRs could be developed, or alternatively, existing STRs could be supplemented with a SNP panel.”


Commercial STR Kit Loci Positions (including CODIS 13 STRs)

Positions determined along May 2004 Human Genome Reference Sequence (NCBI Build 35)

Locations of Focus for New miniSTR Loci (relative to CODIS 13 STRs)

Characterization of New miniSTR Loci

“Computer Work”

- Candidate STR marker selection
- Pull-down sequence data from the web
- Identify Chromosome Location
- Search for PCR Primers
- Test primers for Multiplex-ability

“Laboratory Work”

- Test Markers on Repetitive samples
- Sequence Homologous to eliminate false reads
- Build Movers for Genotyping
- Candidate Allele Ladders

Candidate STR marker selection

Reconstruction of Human Evolutionary Tree Using Polygenic Autosomal Microsatellites

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Characterization of New miniSTR Loci

Rosenberg et al. 2002 – 1062 samples; 377 STRs; diverse populations

Focus on:
- High Heterozygosity
- Small # of Alleles
- Tetranucleotide Repeats

Identification of PCR Primers

Drop in sequence from GenBank

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

PCR Primer Design

9 GATA repeats

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
PCR Primer Design

13 GGAA Repeats

102 bp Amplicon

Problematic Markers

D9S324 miniSTR primers

Alternative reverse (larger product)

"False Homozygote"

Initial Testing Results with Potential miniSTR Loci

Miniplex "NC01"

NC01 Allelic Ladders

We have just completed our final pass.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm


Coble – New miniSTR loci
Mid-Atlantic Association of Forensic Scientists (Richmond, VA)
MiniSTR Assay Sensitivity (D10S1248)

28 cycles – 1U Taq
20 pg
100 pg
50 pg
20 pg
10 pg
5 pg

32 cycles – 2U Taq

Sensitivity - Degraded DNA from an OU Bone Sample

Loss of larger alleles

Sensitivity - Degraded DNA from an OU Bone Sample

Amelogenin
D3
D5
vWA
TH01
D13
D21
D8
TPOX
D7

10 pg/µL (30 pg input DNA), 32 cycles, 2U Taq

EDNAP Exercise on Degraded DNA

Conducted in the Fall of 2004

MiniSTR primer mixes and allelic ladders were provided by NIST

MiniSTR performance on degraded DNA samples

Individual 2
Blood Stain – 2 Weeks
Allelic drop out at D16 and FGA
Failure at D18

SGM+
32 cycles

Global Impact of NC miniSTRs

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Global Impact of MiniSTRs

100s of bones are tested each week with miniSTRs to help in the re-association of remains.

Global Impact of NC miniSTRs

FSI (2006) 156(2): 242-244

...recommended that existing multiplexes are re-engineered to enable small amplicon detection, and that three new mini-STR loci with alleles <130 bp (D10S1248, D14S1434 and D22S1045) are adopted as universal. This will increase the number of European standard Interpol loci from 7 to 10.

(D14 has been replaced with D2S441 from NC02)
Conclusions

- MiniSTRs will have a critical role in future forensic DNA investigations (archived samples – post-conviction testing, skeletal remains in missing persons cases, mass disasters).
- Additional markers not linked to the CODIS loci will be helpful for cases involving paternity disputes, or complex criminal investigations (incest).

Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

New contact information:
michael.coble@afip.osd.mil
(301) 319-0268

The opinions and assertions contained herein are solely those of the author and are not to be construed as official or as views of the U.S. Department of Commerce, the National Institutes of Justice, the U.S. Department of Defense, or the U.S. Department of the Army.