EDNAP and 33rd ENFSI DNA WG Meeting
Sept 27-29, 2010 – Kiev, Ukraine

NIST Update

Slides provided by John M. Butler and Kristen Lewis O'Connor

NIST Human Identity Project Team

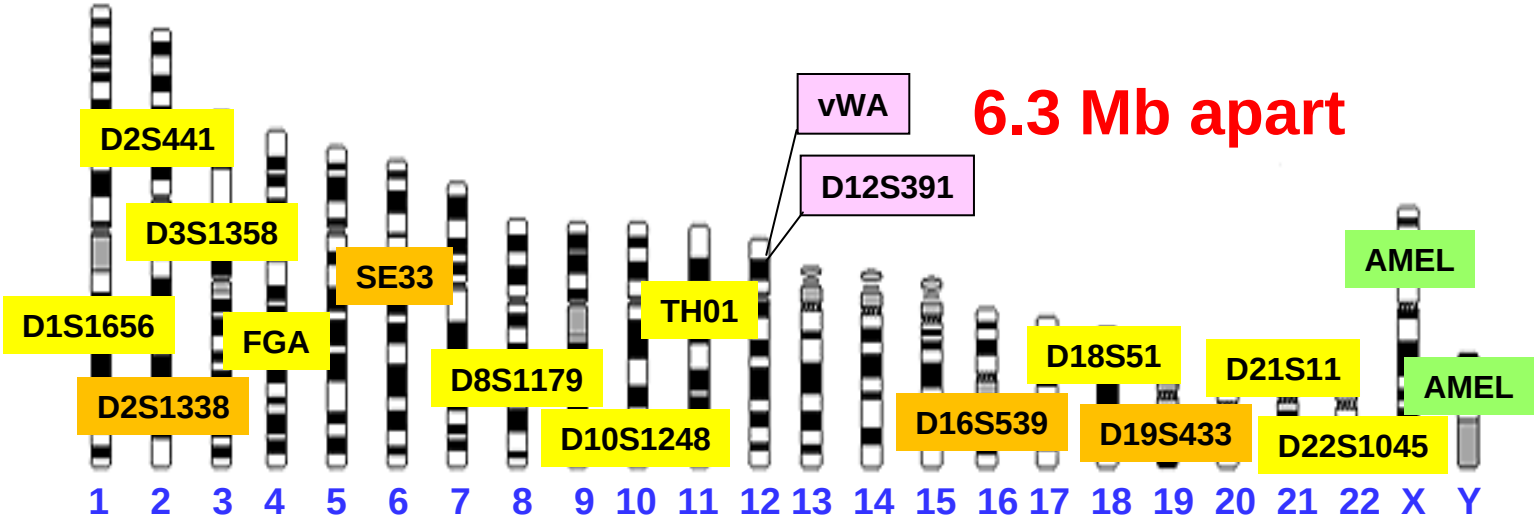
National Institute of Standards and Technology
Gaithersburg, Maryland USA

Topics to Address

- Linkage issues with D12S391 and vWA
- Concordance studies with ESX/ESI and NGM kits
- Upcoming mixture workshop

Chromosomal Positions for the European Standard Set

and Other Common STR Markers Used



European Standard Set + D16S539, D2S1338, D19S433, SE33

Are vWA and D12S391 (6.3 Mb apart) independent?

Should vWA and D12S391 be used with the product rule for match probability calculations?

Research Design

- NIST U.S. population samples
  - 254 African American, 261 Caucasian, 139 Hispanic
- U.S. father/son samples
  - 178 African American, 198 Caucasian, 190 Hispanic, 198 Asian
- Previously genotyped with PowerPlex® ESI/ESX 17
- Father/son genotypes phased to identify paternally transmitted alleles
- Tests for Hardy-Weinberg equilibrium and linkage disequilibrium in population samples
- Test for linkage in father/son samples

C.R. Hill *et al*., Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.03.014.

Linkage Disequilibrium between D12S391 and vWA

- Population samples
  - No significant departure from HWE for D12S391 or vWA
  - No significant linkage disequilibrium detected between the loci
  - Consistent with results from seven worldwide populations

    C. Phillips *et al.*, Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STRs using the CEPH human genome diversity panel, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.02.003.
- Paternity samples with known allelic phase
  - **Significant linkage** between D12S391 and vWA
  - **Non-random association of alleles** at D12S391 and vWA
- We surmise that linkage disequilibrium is present in unrelated population samples but is more difficult to detect due to less power
  - Unknown allelic phase
  - Large number of possible haplotypes

K.L. O'Connor, et al., Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.09.003

Match Probability Calculations

For casework analysis that involves unrelated or related individuals, we recommend:

- Single-locus genotype probabilities of D12S391 and vWA **should not** be multiplied to determine the match probability
- Possible solutions:
  1. Choose only one locus for match probability calculations
  2. Use haplotype frequencies of D12S391/vWA diplotype
     - A diplotype consists of two haplotypes, which are phased multilocus genotypes
     - Haplotype frequencies are generally rarer than the allele frequencies of a single locus
     - Allows for consideration of genotype data from both loci without statistical bias

K.L. O'Connor, et al., Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.09.003

G Model
FSIGEN 643 1–3

ARTICLE IN PRESS

Forensic Science International: Genetics xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short communication

Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples☆

Kristen Lewis O'Connor*, Carolyn R. Hill, Peter M. Vallone, John M. Butler

Biochemical Science Division, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8312, United States

K.L. O'Connor, et al., Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.09.003

- Haplotype frequencies of D12S391/vWA diplotypes from U.S. paternity samples are provided in the supplementary table
- Formulas are included to use the haplotype approach with unphased alleles

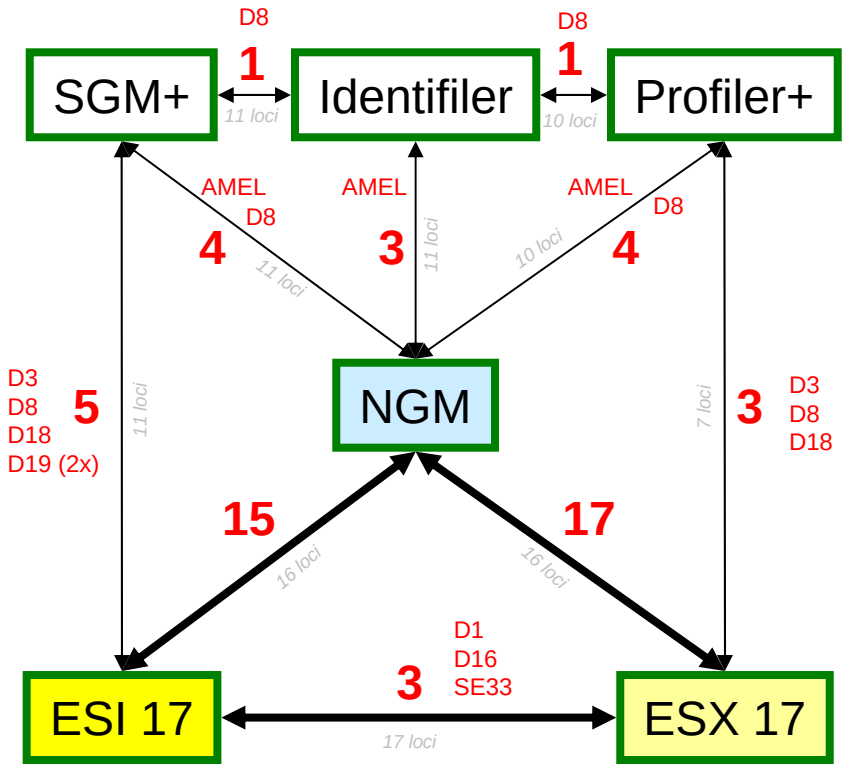
Kit Concordance Comparisons

Kits compared	Samples	Loci compared	Comparisons	# Differences	Concordance (%)
SGM-ID	1436	11	15,796	1	99.994%
ID-ProPlus	1427	10	14,270	1	99.993%
SGM-NGM	1436	11	15,796	4	99.975%
ID-NGM	1449	11	15,939	3	99.981%
ProPlus-NGM	1427	10	14,270	4	99.972%
SGM-ESI	1436	11	15,796	5	99.968%
ProPlus-ESX	1427	7	9,989	3	99.970%
ESI-NGM	1449	16	23,184	15	99.935%
ESX-NGM	1449	16	23,184	17	99.927%
ESI-ESX	1455	17	24,735	3	99.988%
		TOTAL	**172,959**	**56**	**99.970%**

Kits (except Identifiler) were kindly provided by Promega and Applied Biosystems for concordance testing performed at NIST

Concordance Testing Summary

Number of Discordant Results Observed



17 differences with NGM and ESX
D1
D3
D16
D18
D19 (2x)
D22 (4x)
D2S441 (7x)

ABI to add degenerate primers to fix in future kits?

No null alleles were detected with the ESI kit

From the >1400 U.S. population samples tested, with the STR loci that overlap between kits, the results are as follows:

Across 172,959 comparisons, there were only 56 differences

TH01 – no differences
FGA – no differences
vWA – no differences
D2S1338 – no differences
D10S1248 – no differences
D12S391 – no differences
D21S11 – no differences
D1S1656 – 1 difference (African); loss of allele 14 with ESX while ESI/NGM showed full 14,15.3 type
D3S1358 – 1 difference (Caucasian); loss of allele 17 with ID/ProPlus/SGM+/NGM while ESX/ESI showed full 14,17 type
D8S1179 – 1 difference (Asian); loss of allele 15 with ProPlus/SGM+ while ID/NGM/ESX/ESI showed full 14,15 type
D16S539 – 1 difference (Hispanic); loss of allele 13 with ESX while ESI/ID/NGM/ProPlus/SGM+ showed full 12,13 type
D18S51 – 1 difference (Hispanic); loss of allele 13 with ID/NGM/ProPlus/SGM+ while ESX/ESI showed full 13,15 type
D19S433 – 2 differences (Asian); loss of allele 13 with ID/NGM/SGM+ while ESX/ESI showed full 13,14 or 13,14.2 type
D22S1045 – 4 differences (3 Africans & 1 Hispanic); loss of allele 15 with NGM while ESX/ESI showed full 15,16 or 15,17 types
D2S441 – 7 differences (Asian); loss of allele 9.1 with NGM while ESX/ESI showed full 9.1,10 or 9.1,11 or 9.1,12 types
SE33 – 6 differences (African); loss of 24.2, 25.2, 26.2, 27.2 in SE33 monoplex and 3 bp deletion in ESX while fine with ESI
Amelogenin – 3 differences (1 Hispanic & 2 Caucasians); loss of allele X with NGM while ESX/ESI/ID/ProPlus/SGM+ showed full X,Y

99.97% concordance observed

See also Hill, C.R., et al. (2010) Strategies for concordance testing. *Profiles in DNA (Promega)*, 13(1). Available at http://www.promega.com/profiles/1301/1301_08.html

Upcoming Mixture Workshop

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

MIXTURE INTERPRETATION:
Principles, Protocols, and Practice

21st International Symposium on Human Identification
October 11, 2010 (San Antonio, TX)

~200 page handout

Presenters

John M. Butler, PhD	NIST, Applied Genetics Group
Michael D. Coble, PhD	NIST, Applied Genetics Group
Robin W. Cotton, PhD	Boston University, Biomedical Forensic Sciences
Catherine M. Grgicak, PhD	Boston University, Biomedical Forensic Sciences
Charlotte J. Word, PhD	Consultant

Supported by funding from the National Institute of Justice

- Audience participation planned with TurningPoint technology clickers
- Will discuss topics **in the context of the recently released SWGDAM Guidelines** using **8 teaching modules & 3 worked examples**:
  - Setting Analytical Thresholds
  - Determining & Dealing with Stutter
  - Amp Variation & Stochastic Effects
  - Peak Height Ratios
  - Estimating the Number of Contributors
  - Calculating & Using Mixture Ratios
  - Statistical Approaches
  - Mixture Principles & Reporting Basics
- The workshop is already full (200 people) but **slide handouts will be available after the meeting on the STRBase training section**

NIST Human Identity Project Teams
within the Applied Genetics Group

Forensic DNA Team

Data Analysis Support

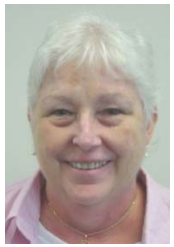
DNA Biometrics Team


John Butler


Mike Coble


Becky Hill


Margaret Kline


Jan Redman


Dave Duewer


Pete Vallone


Erica Butts


Kristen Lewis O'Connor

Funding from the **National Institute of Justice (NIJ)** through NIST Office of Law Enforcement Standards

Funding from the **FBI S&T Branch** through NIST Information Access Division

Workshops & Textbooks

Concordance & LT-DNA

SRM Support

Rapid PCR & Biometrics

Kinship Analysis

Mixtures, mtDNA & Y

Variant alleles & Cell Line ID

Software Tools & Data Analysis

DNA Extraction Efficiency

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

john.butler@nist.gov

001-301-975-4049