The Copenhagen Forensic Genetic Summer School
Advanced Topics in STR DNA Analysis
June 27-28, 2012

# Concordance Testing Comparing STR Multiplex Kits

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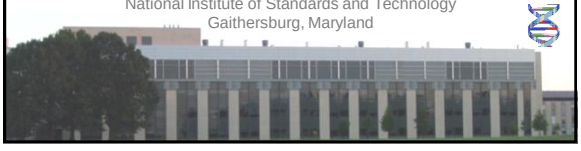


## Outline of Topics to Discuss

- Introduction and importance of concordance testing
- NIST role in concordance testing
- Concordance results with various STR multiplex kits
- Variant allele sequencing
- Summary and conclusions

## Why are concordance studies important?

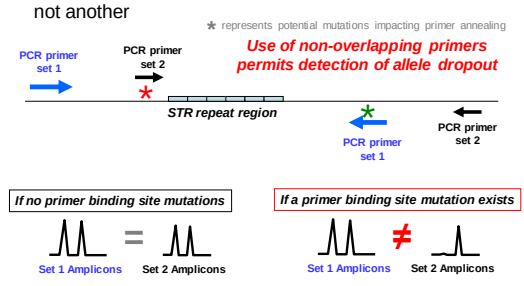
## Importance of Concordance Testing

- There are a variety of commercial STR multiplex kits with different configurations of STR markers
  - Different primer sequences are used to amplify the same markers
  - Discordant results can impact DNA databases
- Detection of primer binding site mutations that cause **null alleles**, or allele drop-out
  - Can only be determined with concordance testing and DNA sequencing
- Concordance with NIST reference materials
  - Important to test with all new STR typing kits

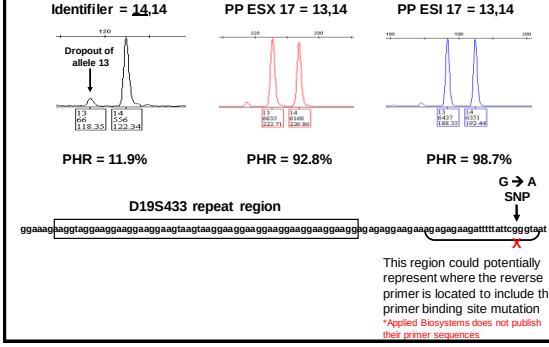
Hill, C.R., Kline, M.C., Duewer, D.L., Butler, J.M. (2010) Strategies for concordance testing. *Profiles in DNA (Promega)*, 13(1).

## Purpose of Concordance Studies

When different primer sets are utilized, there is a concern that allele dropout may occur due to primer binding site mutations that impact one set of primers but not another




## Example Primer Binding Site Mutation that Causes a Null Allele



## To Avoid Overlapping PCR Product Size Ranges with STR Loci in the Same Dye Channel

- Applied Biosystems (Strategy 1)
  - **Maintains primer sequences** (except MiniFiler & NGM kits)
  - Utilizes mobility modifiers or additional dyes, no primer redesign is necessary
  - Enables comparison to legacy data with earlier kits but null alleles may go undetected with the potential for incorrect genotypes within data sets
- Promega Corporation (Strategy 2)
  - Moves primer sequences to change PCR product size ranges
  - Primer redesign can be difficult, but can be moved from primer-binding-site mutations
  - **Requires concordance studies to check for potential allele dropout**

## Why is NIST involved in concordance studies?

## Purpose of Concordance Studies

1. To test SRM 2391b/c (PCR-based DNA Profiling Standard) components with all new STR multiplex kits and verify results against certified reference values
2. To gain a better understanding of primer binding site mutations that cause null alleles

# What are the NIST strategies for concordance testing?

## STR Kit Concordance Testing

*Profiles in DNA* Article Published April 2010

Article Type: Feature

Volume 13 No. 1, April 2010

**Strategies for Concordance Testing**

Carolyn R. Hill, Margaret C. Kline, David L. Duewer and John M. Butler
National Institute of Standards and Technology, Biochemical Science Division, Gaithersburg, Maryland, USA

*Concordance evaluations are important to conduct to determine if there are any allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial short tandem repeat (STR) multiplex kits with different configurations of STR markers available to the forensic community. The placement of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allele dropout may occur due to primer-binding-site mutations that affect one set of primers but not another.*

http://www.promega.com/profiles/1301/1301_08.html

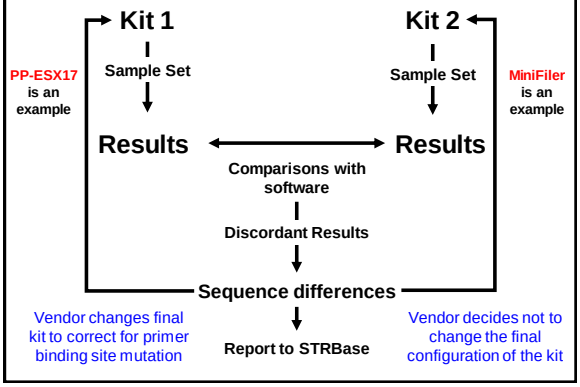
## The 4 "S's" of Concordance

- NIST Standard Samples
  - Run same samples with multiple kits to compare results
- Concordance Software
  - Allows comparison of data sets using NIST developed software

    http://www.cstl.nist.gov/biotech/strbase/software.htm
- DNA Sequencing
  - To validate and determine the exact cause for the null allele
- STRBase website
  - To report verified null alleles and discordant results to the forensic community

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

## NIST Concordance Testing Steps




## What concordance studies have been completed thus far?

## Applied Biosystems AmpFℓSTR Kits

- Identifiler
- **MiniFiler**
- Profiler Plus
- SGM Plus
- NGM
- NGM SElect

Hill, C.R., Kline, M.C., Mulero, J.J., Lagace, R.E., Chang, C.-W., Hennessy, L.K., Butler, J.M. (2007) Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. *J. Forensic Sci.* 52(4): 870-873.

## Promega PowerPlex Systems

- PowerPlex 16
- **PowerPlex ESX 17**
- **PowerPlex ESI 17**
- PowerPlex ESI 17 Pro
- PowerPlex 18D (rapid and direct kit)
- PowerPlex 21


Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems

## Qiagen Investigator HID Kits

- ESSplex
- ESSplex Plus
- ESSplex SE
- ESSplex SE Plus (SE33 only)
- Hexaplex ESS
- IDplex

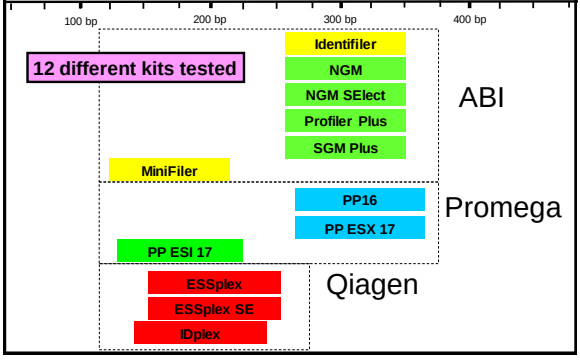
## What samples are used at NIST to perform concordance testing?

## NIST Sample Set (>1450 Samples)

- **NIST U.S. population samples**
  - 260 African American, 260 Caucasian, 140 Hispanic, 3 Asian
- **U.S. father/son paired samples**
  - ~**100 fathers/100 sons for each group**: 200 African American, 200 Caucasian, 200 Hispanic, 200 Asian
- **NIST SRM 2391b**, PCR-based DNA Profiling Standard (highly characterized)
  - 10 genomic DNA samples, 2 cell line samples
  - Includes 9947A and 9948
- **NIST SRM 2391c**, PCR-based DNA Profiling Standard
  - 4 genomic DNA (one mixture)
  - 2 cell lines (903 and FTA paper)

## What are the results from the completed concordance studies?

## D18S51 Concordance Checking



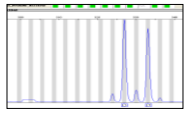
## Primer Set Compared

| Marker | # of Sets | Marker | # of Sets |
|---|---|---|---|
| Amelogenin | 13 | D2S441 | 9 |
| D18S51 | 12 | D19S433 | 9 |
| D21S11 | 12 | D1S1656 | 7 |
| FGA | 12 | D12S391 | 7 |
| D3S1358 | 11 | SE33 | 5 |
| TH01 | 11 | D5S818 | 4 |
| D16S539 | 11 | D7S820 | 4 |
| vWA | 11 | D13S317 | 4 |
| D8S1179 | 11 | TPOX | 3 |
| D2S1338 | 10 | CSF1PO | 4 |
| D10S1248 | 9 | Penta D | 1 |
| D22S1045 | 9 | Penta E | 1 |

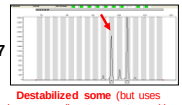
## D22S1045 Discordance

ESX 17 (prototype) = 17,17

ESI 17 (prototype) = 15,17



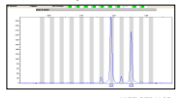
NIST NC01 = 15,17



Destabilized some (but uses lower annealing temperature with fewer amplicons in multiplex)

ESX 17 (final) = 15,17

NIST 23plex = 15,17

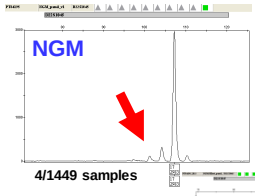


NIST PT84195

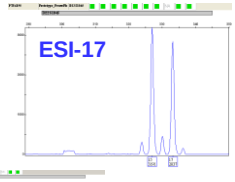
G→T 15 bp upstream impacting forward primer binding with ESX17

*Promega added additional primer to correct issue*

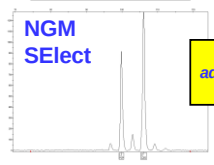
## D22S1045 Null Allele




4/1449 samples



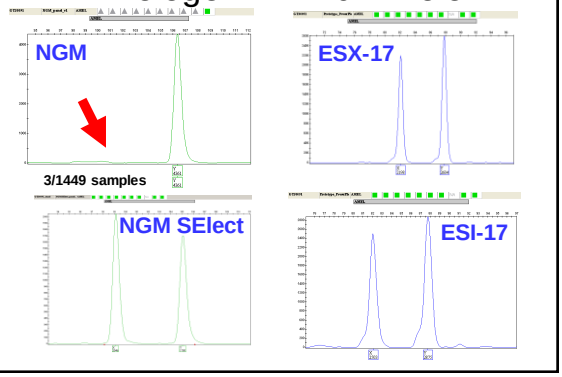

Correct type (15,17)



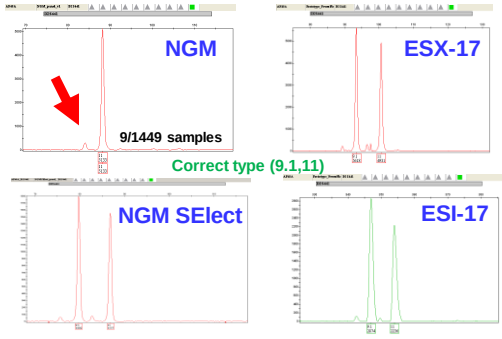

*ABI added an additional primer to correct issue*

G→T 15 bp upstream impacting forward primer binding with NGM

## Amelogenin X Null Allele

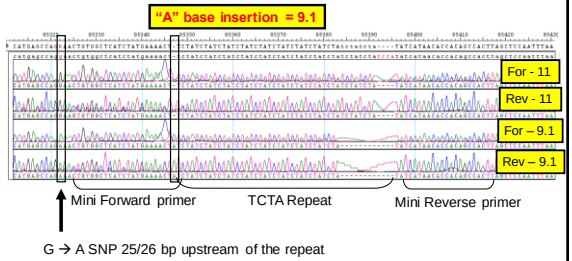



## D2S441 Null Allele




**8/9 null alleles were from Asian samples**

## D2S441 Sequencing




True Genotype = 9.1,11

NGM Genotype = 11,11

## Primer Changes with ABI Kits

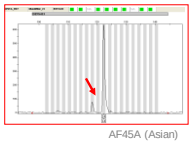
| AmpFℓSTR® Kit | Primer Set Configuration | |
|---|---|---|
| | STR Primers | Amelogenin |
| Profiler® Kit | Identical primer sequences for all common loci | Identical Amelogenin primer sequences |
| Profiler Plus® Kit | | |
| COfiler® Kit | | |
| SGM Plus® Kit | | |
| Identifiler® Kit | Inclusion of one additional primer for D8S1179 | |
| Profiler Plus® *ID* Kit | | |
| SEfiler Plus™ Kit | | |
| NGM™ Kit | | Amelogenin primers redesigned |
| NGM SElect™ Kit | SE33 primer sequences redesigned | |
| MiniFiler™ Kit | All primers redesigned | |

**D2S441 and D22S1045 have an additional primer in NGM and NGM SElect**

*Table 4 from "Development of the AmpFℓSTR NGM SElect Kit: New Sequence Discoveries and Implications for Genotype Concordance", Forensic News (January 2011)*

## D19S433 Discordance

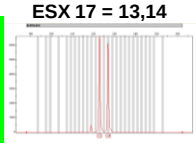
**Identifiler & NGM = 14,14**



AF45A (Asian)

**Allele 13** was missing in **two different Asian samples** with ABI primers = 2/2886 = **0.07% discordance**

**ESX 17 = 13,14**



**ESI 17 = 13,14**

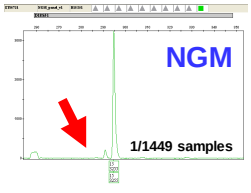
Frequencies [for] the silent allele were determined to be 0.0114 in 176 people from Shizuoka (Honshu) and 0.0128 in 156 people from Okinawa

Natsuko Mizuno,[1] D.V.M.; Tetsushi Kitayama,[1] M.Sc.; Koji Fujii,[1] Ph.D.; Hiroshi Nakahara,[1] D.V.M.; Kimuko Yoshida,[1] Ph.D.; Kazumasa Sekiguchi,[1] Ph.D.; Naoto Yonezawa,[1] Ph.D.; Minoru Nakano,[1] Ph.D.; and Kentaro Kasai,[1] Ph.D.

A D19S433 Primer Binding Site Mutation and the Frequency in Japanese of the Silent Allele It Causes

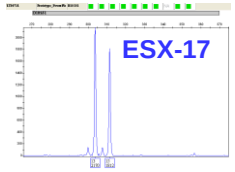
**T→A 8 bp downstream impacting reverse primer binding with Identifiler (and thus SGM Plus)**

## D18S51 Null Allele

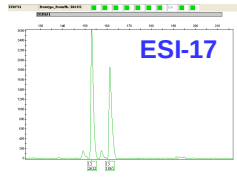


**NGM**

**1/1449 samples**



**ESX-17**

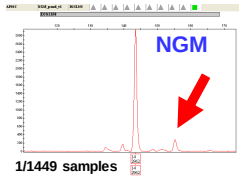


**ESI-17**

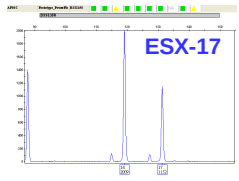
**Correct type (13,15)**

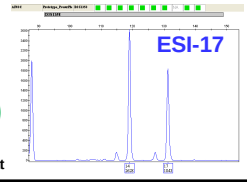
**C→T SNP 172 bp downstream from repeat**

## D3S1358 Null Allele



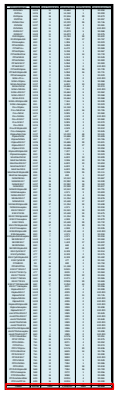

1/1449 samples




**Correct type (14,17)**

G → C SNP 11 bp downstream from repeat



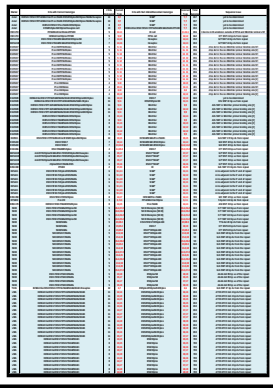
## Completed Concordance Studies

| Kits compared | Samples | Loci Compared | Comparisons | # Differences | Concordance (%) |
|---|---|---|---|---|---|
| 128 | 114144 | 1245 | 1,104,031 | 1224 | 99.889 |

**1,104,031 allele comparisons**
**1,224 total differences**
**99.89% concordance**

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

## Final Concordance Results



- All up-to-date results can be found on STRBase:
  - ISFG poster (Vienna, Austria), 8/31-9/2, 2011, "Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set"
  - Promega ISHI (National Harbor, MD), 10/4-10/5, 2011, "Concordance Testing Comparing STR Multiplex Kits with a Standard Data Set"

## Primer Binding Site Mutations Causing Allele Dropout, Not Corrected by ABI

**From >1400 U.S. population samples tested:**

- **D18S51** – 1 difference (Hispanic); loss of allele 13 with ID/NGM/ProPlus/SGM+ while ESX/ESI showed full 13,15 type
- **D3S1358** – 1 difference (Caucasian); loss of allele 17 with ID/ProPlus/SGM+/NGM while ESX/ESI showed full 14,17 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
- **D8S1179** – 1 difference (Asian); loss of allele 15 with ProPlus/SGM+ while ID/NGM/ESX/ESI showed full 14,15 type

**http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm**

## Was there complete concordance with SRM 2391b and SRM 2391c?

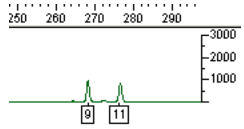
## SRM 2391b/2391c PCR-Based Profiling Standard

- The first set of samples run with new STR multiplex kits is SRM 2391b/SRM 2391c
- All new kits tested have been completely concordant with the certified values of all markers for each component for SRM 2391b and 2391c
- One exception for SRM 2391b: MiniFiler
  - Genomic 8 with D16S539

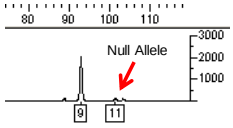
## SRM 2391b Genomic 8 with D16S539

*All allele calls with MiniFiler for CSF1PO, D7S820, D13S317, D18S51, D21S11, FGA, and D16S539 (with the exception noted below)* ***match previously certified values.***

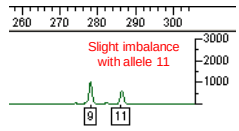
Identifiler




MiniFiler



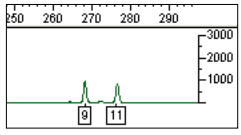

PowerPlex 16



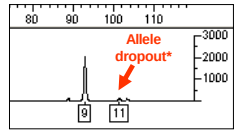

**Due to primer binding site mutation*

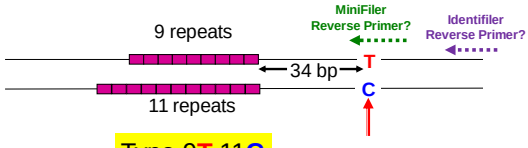
## SRM 2391b Genomic 8 with D16S539

Identifiler




MiniFiler

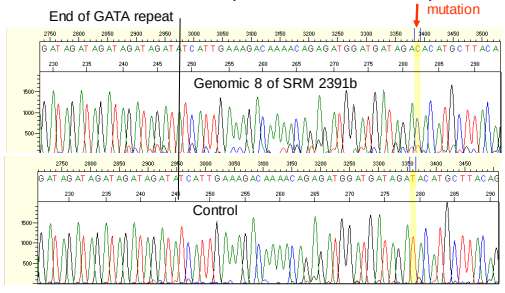



Type 9T,11C

## D16S539 SRM 2391b Genomic 8

T→C mutation 34 bp downstream of the repeat




Position of the T→C probably affects the reverse primer of Minifiler and is the 3rd base found the 5'end of the Reverse PP16 primer. This could explain the imbalance of the allele seen when using PP16.

# Summary & Final Thoughts

## Conclusions

- Concordance testing is valuable when different sets of primers are used to amplify the same markers
- Null alleles, variant alleles and discordant results are reported on STRBase:
  - http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm
  - http://www.cstl.nist.gov/biotech/strbase/STRseq.htm
- NIST plays an important role in concordance testing to aid the community
  - SRM 2391b/c concordance
  - Several null alleles have been fixed before the final release of new STR multiplex kits

## Acknowledgments

**NIST Funding: Interagency Agreement 2008-DN-R-121 between the National Institute of Justice and NIST Office of Law Enforcement Standards**

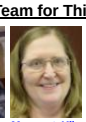
**NIST Disclaimer**: Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

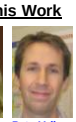
**Points of view are mine** and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

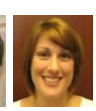
**NIST Team for This Work**


John Butler


Dave Duewer


Margaret Kline


Pete Vallone


Kristen O'Connor

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# Thank you for your attention

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