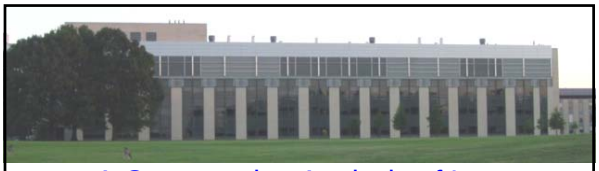

A Comparative Analysis of Low Template (LT) DNA Testing: How Low Can you Go?

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Outline of Topics to Discuss

- Introduction to Low Template (LT) DNA
- Technical Aspects of LT-DNA testing
  - Challenges and limitations with LT-DNA testing
  - Approaches to genotyping low template DNA
  - LT-DNA data and Peak Height Ratios (PHR)
- Future studies with LT-DNA testing
- Summary and conclusions

Some Definitions of Low Template (LT) DNA

- Working with **<100 pg genomic DNA**
- Considered to be data below stochastic threshold level where PCR amplification is not as reliable (determined by each laboratory; typically 150-250 RFUs)
- Enhancing the sensitivity of detection (increasing PCR cycles, PCR product clean-up, increasing CE injection/voltage)
- Having too few copies of DNA template to ensure reliable PCR amplification (allelic or full locus drop-out)
- Can often be the minor component of mixture samples consisting of low level DNA template amounts

Challenges of LT-DNA Testing

Gill, P. (2001) *Croatian Med. J.* 42(3): 229-232

- Increased chance for contamination (want a sterile lab environment to reduce staff contamination)
- Data interpretation is more complicated (due to stochastic variation during PCR amplification):
  - Heterozygote peak imbalance
  - Allele drop-out
  - Allele drop-in
  - Increased stutter products

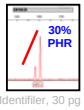
LT-DNA profiles should be interpreted with careful guidelines

Stochastic (Random) Effects with Low DNA

When Combined with Higher Sensitivity Techniques

Loss of True Signal (***False Negative***)

Heterozygote Peak Imbalance



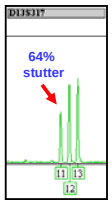
Identifiler, 30 pg DNA, 31 cycles

Allelic **Drop-out**

Identifiler, 30 pg DNA, 31 cycles

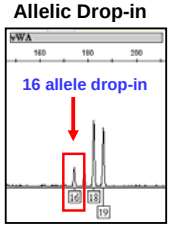
Gain of False Signal (***False Positive***)

Higher Stutter



Identifiler, 10 pg DNA, 31 cycles

Allelic Drop-in



Identifiler, 10 pg DNA, 31 cycles

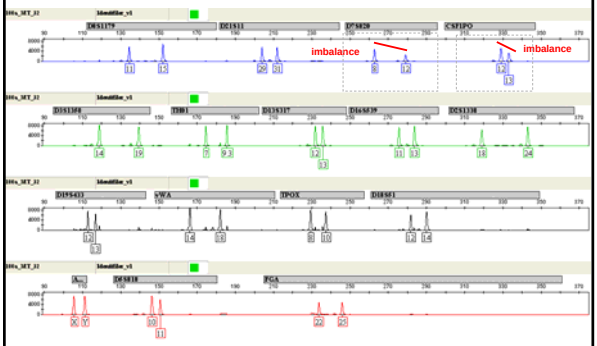
Suggestions for Optimal Results with LT-DNA

- Typically at least 2 – 3 PCR amplifications from the same DNA extract are performed to obtain consensus profiles
- An allele cannot be scored (considered real) unless it is present at least twice in replicate samples
- Extremely sterile environment is required for PCR setup to avoid contamination from laboratory personnel or other sources

Experimental Design to Study LT-DNA Issues

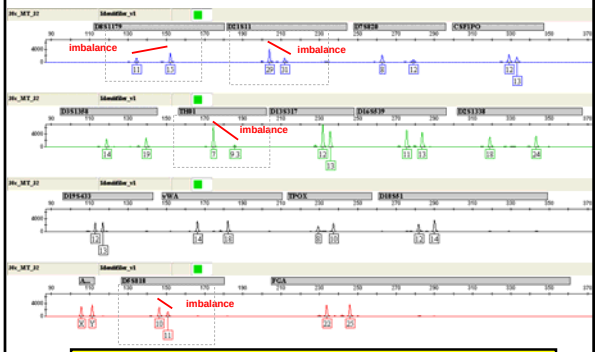
- Pristine DNA Samples
  - 2 single-source samples
  - **heterozygous for all loci tested** (permits peak height ratio studies)
- **Low DNA Template Amounts**
  - Dilutions made after DNA quantitation against NIST SRM 2372
  - **100 pg, 30 pg, and 10 pg** (1 ng tested for comparison purposes)
- Replicates
  - **5 separate PCR reactions** for each sample
- STR Multiplex Kits
  - **Identifiler Plus and PowerPlex 16 HS** (half-reactions)
- **Increased Cycle Number**
  - Identifiler Plus (**29 cycles and 32 cycles**; 28 for 1 ng)
  - PowerPlex 16 HS (**31 cycles and 34 cycles**; 30 for 1 ng)

Identifiler Plus, 100 pg @ 32 cycles, ½ Reaction



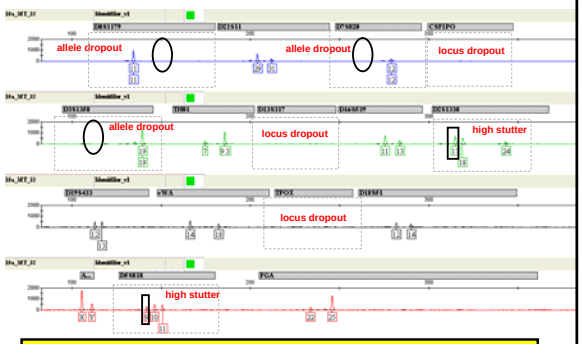
*No drop-out, slight peak height imbalance, full profiles in all replicates

Identifiler Plus, 30 pg @ 32 cycles, ½ Reaction



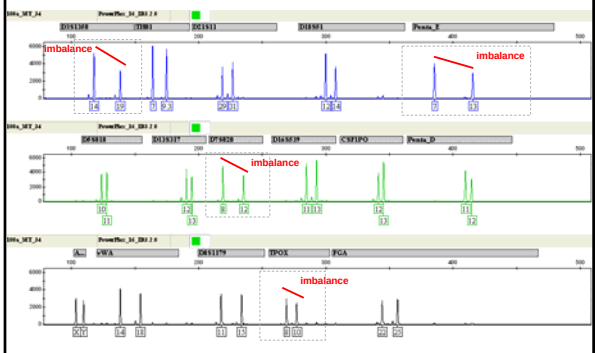
*No allelic drop-out in replicates, significant peak height imbalance

Identifiler Plus, 10 pg @ 32 cycles, ½ Reaction



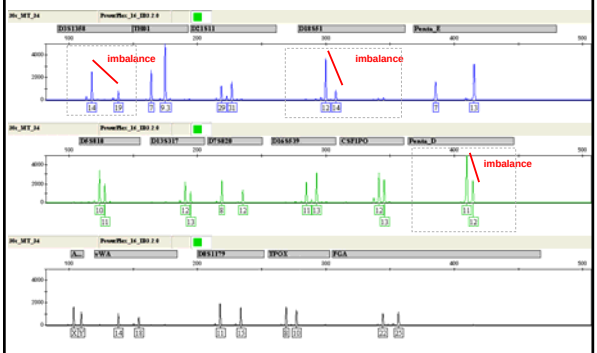
*Significant allelic drop-out in replicates, high stutter and allelic drop-in

PowerPlex 16 HS, 100 pg @ 34 cycles, ½ Reaction



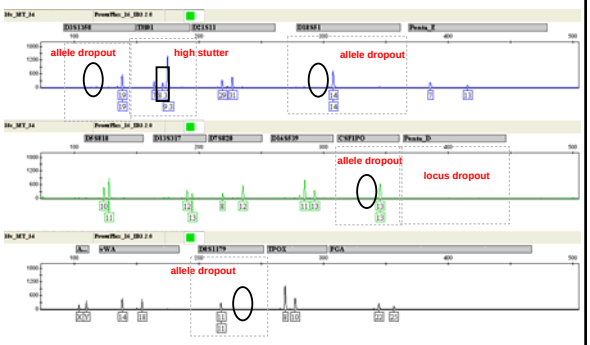
*No drop-out, slight peak height imbalance, full profiles in all replicates

PowerPlex 16 HS, 30 pg @ 34 cycles, ½ Reaction



*No allelic drop-out in replicates, significant peak height imbalance

PowerPlex 16 HS, 10 pg @ 34 cycles, ½ Reaction

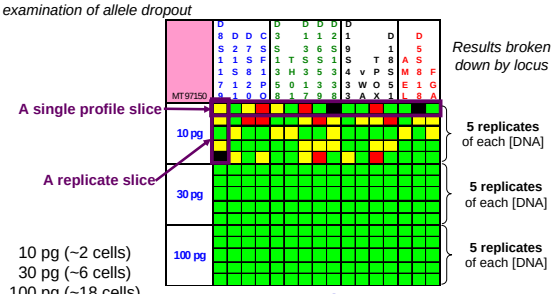


*Significant allelic drop-out in replicates, high stutter and allelic drop-in

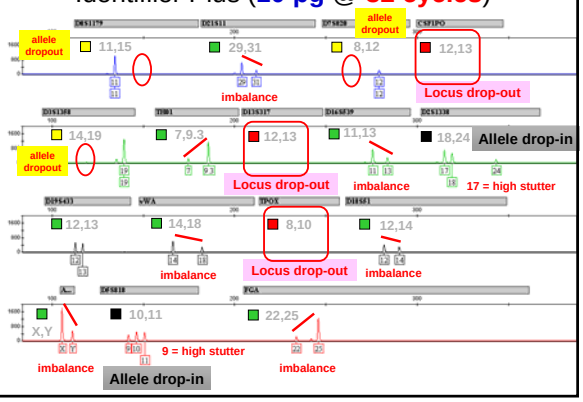
Sensitivity Comparison

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in

Tested sample is heterozygous (possesses 2 alleles) at every locus, which permits an examination of allele dropout



Identifiler Plus (10 pg @ 32 cycles)

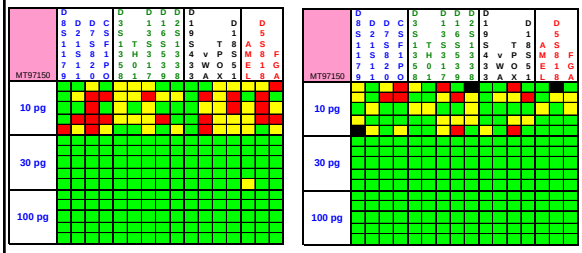


Impact of Three More PCR Cycles

Identifiler Plus

Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in

*Full type = both alleles above 50 RFU (does not account for peak imbalance)



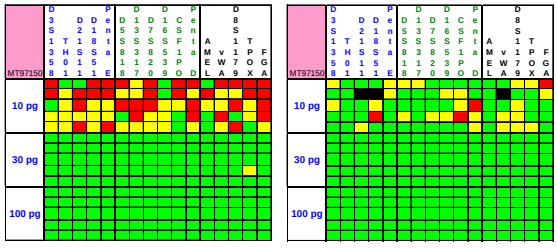
33% vs. 53% full profiles

38% improvement with 3 extra cycles

Sensitivity & Performance

PowerPlex 16 HS

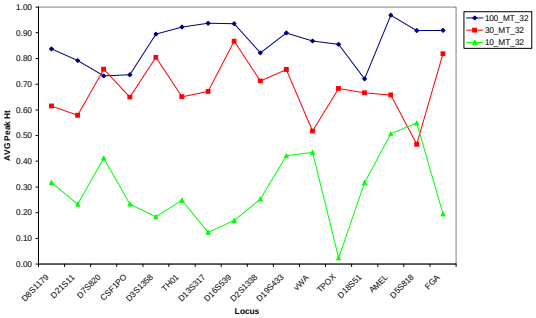
Green = full (correct) type
Yellow = allele dropout
Red = locus dropout
Black = drop-in



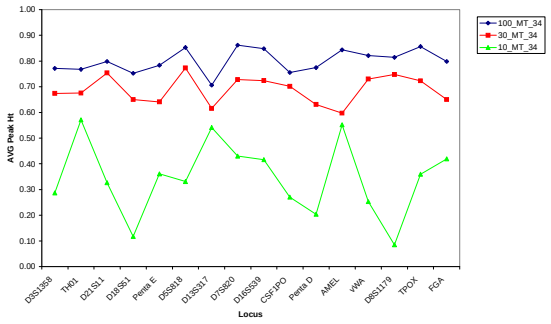
16% vs. 60% full profiles

73% improvement with 3 extra cycles

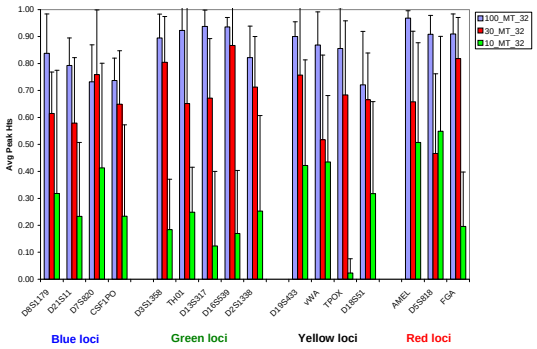
Peak Height Ratio: Identifiler Plus, 32 cyc



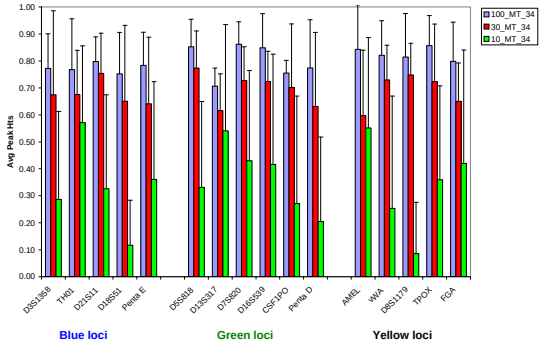
Peak Height Ratio: PP16HS, 34 cyc



Peak Height Ratio: Identifiler Plus, 32 cyc



Peak Height Ratio: PP16HS, 34 cyc

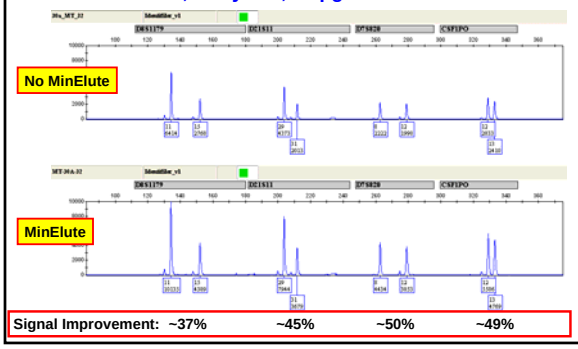


Additional Methods of LT-DNA Testing and Future Studies at NIST

- Signal enhancing techniques
  - MinElute PCR purification kit (Qiagen) for salt removal in final product
  - Increasing CE injection voltage and time
  - Reduced volume PCR (concentrates amplicon)
- Degraded DNA studies
- LT-DNA mixture studies

MinElute PCR Purification Kit

Identifiler Plus, 32 cycles, 30 pg



New Section of STRBase on LT-DNA

- Recently launched webpage
  - **http://www.cstl.nist.gov/biotech/strbase/LTDNA.htm**
  - Low-template DNA = LT-DNA (not LCN!)
- The LT-DNA section includes:
  - **Presentations from past LT-DNA talks and workshops**
  - **Validation data from our sensitivity studies** to illustrate problems and consensus profile solution to low levels of DNA testing
  - **Literature listing of pertinent articles** to help explain the issues involved in this topic

Conclusions

- LT-DNA testing involves enhancing detection sensitivity usually through increasing the number of PCR cycles when amplifying DNA with conventional STR kits.
- The results with pristine full heterozygous samples demonstrate that replicate testing can produce reliable information with single source samples at low levels of DNA when consensus profiles are created.
- Identifiler Plus with 32 cycles and PowerPlex 16 HS with 34 cycles were comparable in performance with low-level DNA analysis.
- With 3 extra cycles, there was better recovery at 10 pg of DNA using both kits including less allelic and full locus drop-out. However, there is a greater potential for allele drop-in or high stutter.

Acknowledgments

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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

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