NEAFS QIAGEN DNA Workshop

Introduction to LCN (Low-Copy Number) – Implications for DNA Typing

John M. Butler, PhD
NIST

November 2, 2006
NEAFS QIAGEN Workshop
(Rye Brook, NY)

Northeastern Association of Forensic Scientists

QIAGEN

NIST and NIJ Disclaimer

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

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

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.

MAAFS DNA Workshop

Introduction to Low Copy Number (LCN) DNA Testing Issues

Workshop conducted with Theresa Caragine (NYC OCME) and Peter Gill (FSS)

John M. Butler, PhD

john.butler@nist.gov
NIST

Richmond, VA
May 3, 2006

Some Definitions of Low-Copy Number (LCN)

- Work with **<100 pg genomic DNA** (~15-17 diploid copies of nuclear DNA markers such as STRs)
- Below stochastic threshold level where PCR amplification is not as reliable (determined by each laboratory; typically 150-250 pg)
- Enhancing sensitivity of detection (34 cycles instead of 28 cycles)
- Too few copies of DNA template to ensure reliable PCR amplification
- Other terms for LCN:
  - Low-level DNA
  - Trace DNA
  - Touch DNA

LCN is dependent on the amount of DNA present NOT the number of PCR cycles performed; LCN conditions may exist with 28 or 34 cycles

LCN is not a "new" technique…

- 1996 – Taberlet *et al.* describe "reliable genotyping of samples with very low DNA quantities using PCR"
- 1997 - single cell STR analysis reported
- 1999 – Forensic Science Service begins LCN casework in UK (as an alternative to mtDNA)
- 2001 – Budowle and FBI co-authors urge caution with using LCN

Amounts of DNA Required

1985-1995	**RFLP/VNTRs**	**50 ng – 1000 ng**
↓		
1991-present (kits since 1996)	**PCR/STRs**	**0.5 – 2 ng**
↓		
1999-present	**LCN/STRs**	**<0.1 ng**

LCN extends the range of samples that may be attempted with DNA testing

Why attempt LCN? …

- Improved success rates with high sensitivity DNA testing vs. standard procedures
- Volume crime samples (burglary)
- Bone samples to provide improved matching statistics over mtDNA analysis

Early LCN Literature

- **Early work on touched objects and single cells**:
  - van Oorschot, R. A. and Jones, M. K. (1997) DNA fingerprints from fingerprints. *Nature*. 387(6635): 767
  - Findlay, I., Taylor, A., Quirke, P., Frazier, R., and Urquhart, A. (1997) DNA fingerprinting from single cells. *Nature*. 389(6651): 555-556
- **Application to routine forensic casework was pioneered by the Forensic Science Service:**
  - Gill, P., Whitaker, J., Flaxman, C., Brown, N., and Buckleton, J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 112(1): 17-40
  - Whitaker, J. P., Cotton, E. A., and Gill, P. (2001) A comparison of the characteristics of profiles produced with the AMPFlSTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. *Forensic Sci. Int.* 123(2-3): 215-223
  - Gill, P. (2001) Application of low copy number DNA profiling. *Croatian Medical Journal* 42(3): 229-32

Other Useful LCN Articles (1)

- Budowle, B., Hobson, D.L., Smerick, J.B., Smith, J.A.L. (2001) Low copy number – consideration and caution. *Proceedings of the Twelfth International Symposium on Human Identification*. Available at http://www.promega.com/geneticidproc/ussymp12proc/contents/budowle.pdf.
- Buckleton, J. and Gill, P. (2005) Low copy number. Chapter 8 in *Forensic DNA Evidence Interpretation* (Eds. J. Buckleton, C.M. Triggs, S.J. Walsh) CRC Press: Boca Raton, FL, pp. 275-297.
- Gill, P. (2002) Role of short tandem repeat DNA in forensic casework in the UK--past, present, and future perspectives. *BioTechniques* 32(2): 366-385.
- Kloosterman, A.D. and Kersbergen, P. (2003) Efficacy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci. *J. Soc. Biol.* 197(4): 351-359.
- Lowe, A., Murray, C., Whitaker, J., Tully, G., and Gill, P. (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci. Int.* 129(1): 25-34.
- Rutty, G. N., Hopwood, A., and Tucker, V. (2003) The effectiveness of protective clothing in the reduction of potential DNA contamination of the scene of crime. *Int. J. Legal Med.* 117(3): 170-174.

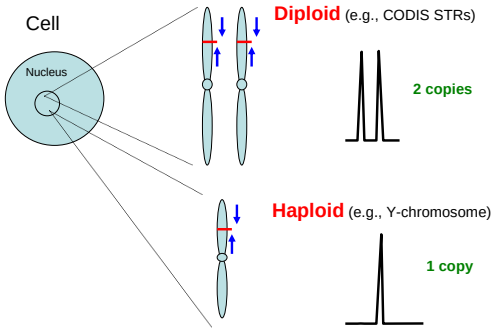
Other Useful LCN Articles (2)

- Schneider, P.M., Balogh, K., Naveran, N., Bogus, M., Bender, K., Lareu, M., Carracedo, A. (2004) Whole genome amplification – the solution for a common problem in forensic casework? *Progress in Forensic Genetics 10 – International Congress Series* 1261: 24-26.
- Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., and Bouvet, J. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res*. 24: 3189-3194.
- Van Oorschot, R.A.H., Phelan, D.G., Furlong, S., Scarfo, G.M., Holding, N.L., Cummins, M.J. Are you collecting all available DNA from touched objects? *Progress in Forensic Genetics 9 – International Congress Series* 1239: 803-807.
- Walsh, P. S., Erlich, H. A., and Higuchi, R. (1992) Preferential PCR amplification of alleles: Mechanisms and solutions. *PCR Meth. Appl.* 1: 241-250.
- Wickenheiser, R. A. (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J. Forensic Sci.* 47(3): 442-450.

AAFS 2003 (Chicago) Workshop on LCN

DNA quantity in samples

Diploid vs. Haploid



Calculation of the Quantity of DNA in a Cell

1. **Molecular Weight of a DNA Base Pair = 618 g/mol**

   A = 313 g/mol; T = 304 g/mol; A-T base pairs = 617 g/mol
   G = 329 g/mol; C = 289 g/mol; G-C base pairs = 618 g/mol

2. **Molecular Weight of DNA = 1.98 x10^{12} g/mol**

   There are **3.2 billion base pairs** in a haploid cell ~3.2 x 10^9 bp
   (~3.2 x 10^9 bp) x (618 g/mol/bp) = 1.98 x 10^{12} g/mol

3. **Quantity of DNA in a Haploid Cell = 3 picograms**

   1 mole = 6.02 x 10^{23} molecules
   (1.98 x 10^{12} g/mol) x (1 mole/6.02 x 10^{23} molecules)
   = 3.3 x 10^{-12} g = 3.3 picograms (pg)
   A diploid human cell contains ~6.6 pg genomic DNA

4. **One ng of human DNA comes from ~152 diploid cells**

   1 ng genomic DNA (1000 pg)/6.6pg/cell = **~303 copies of each locus**
   (2 per 152 diploid genomes)

Adapted from D.N.A. Box 3.3, J.M. Butler (2005) Forensic DNA Typing, 2nd Edition (Elsevier Academic Press), p. 56

At the 2003 AAFS LCN Workshop (Chicago,IL), **Robin Cotton** from Orchid Cellmark presented a talk entitled "Are we already doing low copy number (LCN) DNA analysis?"

Where does low copy number start?

<100 pg template DNA

(Butler, 2001, Fregeau & Fourney 1993, Kimpton *et al* 1994)

Amount of DNA	~ # of cells
1 ng	152
0.5 ng	76
0.25 ng	38
0.125 ng	19
0.0625 ng	10

Values for # of cells adjusted to reflect updated DNA quantitation numbers

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Assume sample is from a **single source**:

Amount of DNA	Total Cells in sample	~ # of copies of each allele if het.
1 ng	152	152
0.5 ng	76	76
0.25 ng	38	38
0.125 ng	19	19
0.0625 ng	10	10

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Assume sample is a **1:1 mixture** of two sources:

Amount of DNA	Total Cells in sample	~ # of cells from each component
1 ng	152	76
0.5 ng	76	38
0.25 ng	38	19
0.125 ng	19	10
0.0625 ng	10	5

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Assume sample is a **1:3 mixture** of two sources:

Amount of DNA	~ # of cells from **major component**	~ # of cells from **minor component**
1 ng	114	38
0.5 ng	57	19
0.25 ng	28	10
0.125 ng	14	5
0.0625 ng	7	2

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Assume sample is a **1:9 mixture** of two sources:

Amount of DNA	~ # of cells from **major component**	~ # of cells from **minor component**
1ng	137	15
0.5ng	68	8
0.25ng	34	4
0.125ng	17	2
0.0625ng	9	1

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

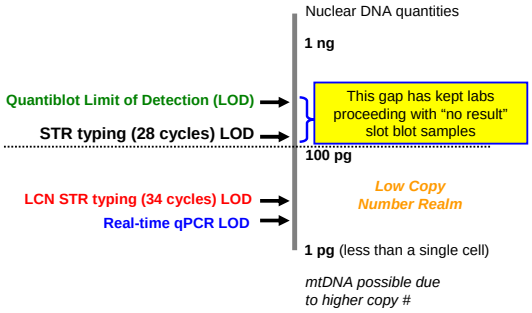
Low copy number situations exist in many samples

- In a 1:1 mixture, each DNA source is at LCN when the total amount of DNA in the amplification reaction is ~ 0.125 ng.
- In a 1:9 mixture, the minor component could be at LCN **even when the total amount of DNA in the amplification is 1 ng**.

Two different amplifications would be useful with a 1:9 mixture situation:
Normal level of total DNA (e.g., 1 ng) so that major component is on-scale
High level of total DNA (e.g., 5 ng) so that minor (e.g., ~500 pg) is out of LCN realm – yes, the major component will be off-scale…

Robin Cotton, AAFS 2003 LCN Workshop
"Are we already doing low copy number (LCN) DNA analysis?"

Difference in DNA Quantitation Capability vs. STR Typing Sensitivity

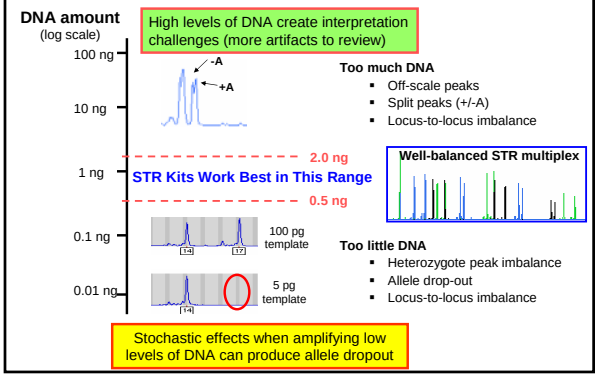


Proceeding with Testing when "No DNA" Detected

If the qPCR results indicate that there is no detectable DNA, will you stop testing or will you proceed with attempting STR typing?

- The practice of proceeding even with a "no result" Quantiblot was **because the STR typing assay was more sensitive than the quantification method**.
- **What types of experiments might be done to satisfy you that "no result" from a qPCR assay is truly "no DNA"?**

Impact of DNA Amount into Multiplex PCR Reaction
We generally aim for 0.5-2 ng



Stochastic PCR amplification

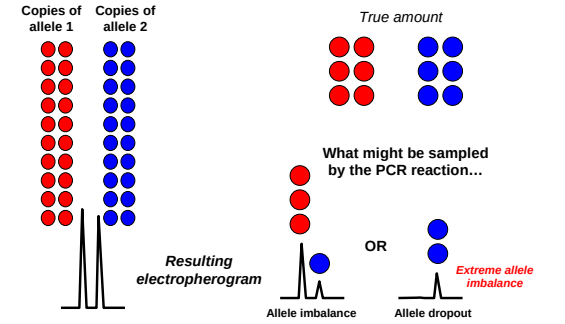
Stochastic = random selection

Stochastic Fluctuation Effects

- Unequal sampling of the two alleles present in a heterozygous individual can occur when low levels of input DNA are used (**results in allele drop-out**)
- PCR reactions **with <100 pg** (~17 diploid copies)
- Walsh *et al.* (1992) – propose avoiding stochastic effect by adjusting the number of PCR cycles in an assay so that the sensitivity limit is around 20 or more copies of target DNA (i.e., a full profile is obtained with ~125 pg)

Walsh PS, Erlich HA, Higuchi R. Preferential PCR amplification of alleles: Mechanisms and solutions. *PCR Meth Appl* 1992; 1:241-250.

Stochastic Statistical Sampling

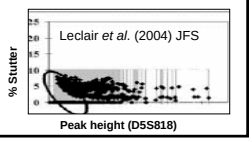


>20 copies per allele

6 copies copies per allele **(LCN)**

Stochastic Effect

- Sometimes called "preferential amplification" – not really a correct term since either allele may be amplified if the other drops-out…not related to allele size
- Stutter product amounts may go up…
  - If in an early cycle of PCR, the stutter product is amplified more (due to sampling effect)
- Contaminating DNA can also be amplified giving rise to allele "drop-in" or a mixture



qPCR Assays Are Also Impacted by Stochastic Sampling in the LCN Region

Note the larger spread in these dilution series points for the LCN samples (16 pg) because of stochastic sampling

Quantifiler Kits User's Manual, p. 6-17

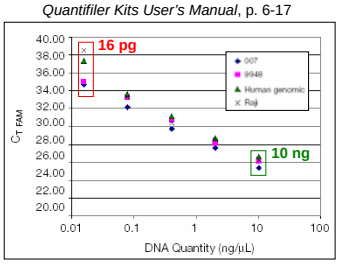


Figure 6-4 Sensitivity using the Quantifiler Y kit

Remember that DNA quantitation assays are also impacted by stochastic problems and may not be extremely reproducible on the low end, i.e., <100 pg…

Challenge with Being Able to Go Lower In DNA Quantitation Measurements

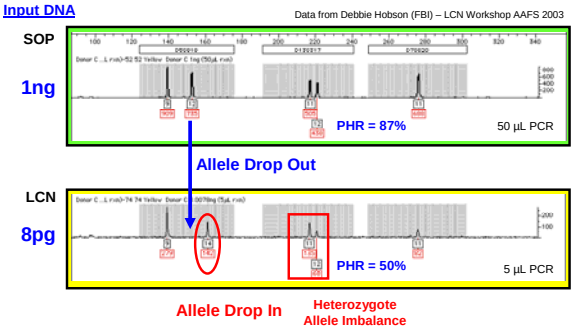
- Multi-copy marker (e.g., Alu assay) will be better than a single copy (e.g., Quantifiler) with qPCR of low quantity DNA samples
- qPCR enables measurement of lower amounts of DNA but…
- Going into the low copy number realm introduces new challenges
  - Interpretation of mixtures
  - Defining thresholds for different dyes and amplification systems
  - Defining the difference between investigative data and reliable "court-worthy" data

Comparison of STR Kit Amplification SOP with LCN

Using the Same DNA Donor

Input DNA

Data from Debbie Hobson (FBI) – LCN Workshop AAFS 2003

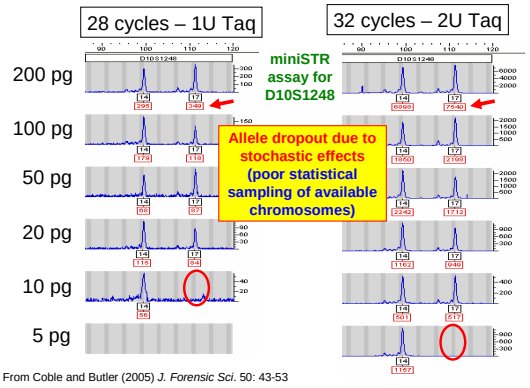


Balance of Assay Sensitivity and Potential for Stochastic Effects

- **One of the ways that assays can be made more sensitive is by increasing the number of PCR amplification cycles**
- Optimal cycle number will depend on desired assay sensitivity
- The number of PCR cycles was set to 28 for ABI STR kits to limit their sensitivity for generating full profiles to ~125 pg or 20 cells
- Sensitivity is a combination of fluorescent dye characteristics (relative to the instrument and laser excitation used) and PCR amplification conditions such as primer concentration and amount of polymerase used

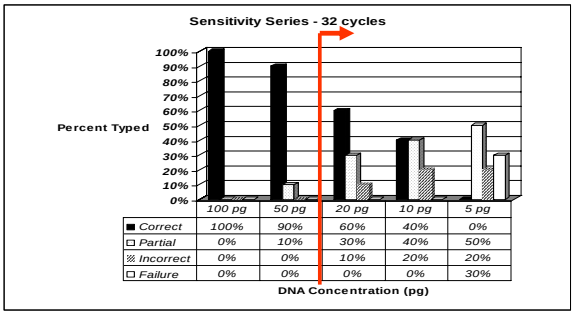
Note that Promega STR kits use higher numbers of cycles to generate roughly equivalent sensitivity to ABI kits because they have less efficient dye labels and lower primer and polymerase concentrations

Higher Sensitivity with More Polymerase and Cycle Numbers



From Coble and Butler (2005) *J. Forensic Sci.* 50: 43-53

Problems with Obtaining Correct Allele Calls at Low DNA Levels



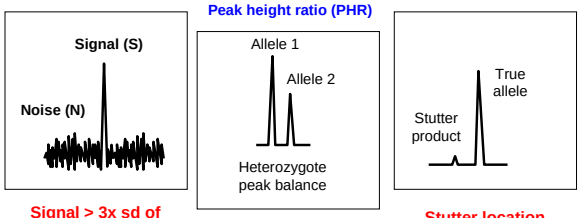
	100 pg	50 pg	20 pg	10 pg	5 pg
Correct	100%	90%	60%	40%	0%
Partial	0%	10%	30%	40%	50%
Incorrect	0%	0%	10%	20%	20%
Failure	0%	0%	0%	0%	30%

Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50: 43-53

What is a true peak (allele)?

GeneScan function *Genotyper function*

Peak detection threshold **Peak height ratio (PHR)** **Stutter percentage**



Signal > 3x sd of noise

PHR consistent with single source Typically above 60%

Stutter location above 15%

Threshold Settings for the ABI 310/3100

Detection Limit: 3x the standard deviation of the noise.
Estimated using **2x peak to peak noise**. (approximately 35 - 50 RFUs)

Limit of Quantitation: 10x the standard deviation of the noise
Estimated using **7x peak to peak noise** (150-200 RFUs)
Below this point estimates of peak area or height are unreliable.

Dynamic Range: The range of sample quantities that can be analyzed from the lowest to the highest **(linear range is also important)**

Stochastic Threshold: Level of quantifiable DNA below which peaks can show severe imbalance (peak height ratios below 60%) Approximately 150 -200 RFUs. Enhanced stutter also occurs at these signal levels.

The Scientific Reasoning behind the Concept of an Analytical Threshold (limit of detection)

- This is fundamentally an issue of reliability
- **For a peak intensity three times the standard deviation of the noise there is a limited chance that such a signal is the result of a random fluctuation**
- This is because 99.7 percent of all noise signals fall below this value (from the definition of a Gaussian curve)
- **Below this point the very real possibility exists that what you think is a peak is simply a statistical fluctuation in the baseline noise**.

Sensitivity

- Limit of detection (**LOD**) – "the lowest content that can be measured with reasonable statistical certainty."
- Limit of quantitative measurement (**LOQ**) – "the lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test."
- How low can you go?

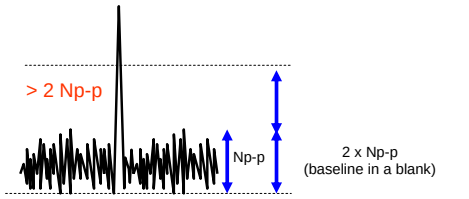
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 43; available at http://www.eurachem.ul.pt/guides/valid.pdf

Limit of Detection (LOD)

- Typically 3 times the signal-to-noise (based on standard deviation of the noise) or 2x Np-p

Is this peak real?

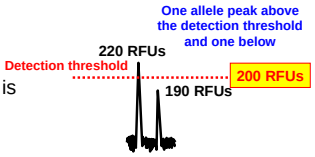
*Yes, it is a peak but you cannot rely on it for concentration determinations as it is **not >10 S/N***



Types of Results at Low Signal Intensity
(Stochastic amplification potential)

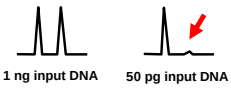
Straddle Data

- Only one allele in a pair is above the laboratory stochastic threshold



Allelic Drop-out

- one or more sets of alleles do not amplify



TWGDAM validation of AmpFlSTR Blue
Wallin *et al.* (1998) *J. Forensic Sci.* 43(4): 854-870

- Minimum cycle # (27-30 cycles examined)
- **Amplification adjusted to 28 cycles so that quantities of DNA below 35pg gave very low peaks or no peaks** (below the analytical threshold!)
- 35 pg is approx 5 cells
- (but is 35pg the analytical threshold?) Determining this value might be a useful goal of a validation study

TWGDAM validation of AmpFlSTR Blue
Wallin *et al.* (1998) *J. Forensic Sci.* 43(4): 854-870

Determination of Minimum Sample

- Goal: avoid situations where peak imbalance results in only one detectable allele from a heterozygous pair.
- Perform serial dilution (1ng- 8pg) of 2 control samples which were heterozygous at all 3 loci
  - **Samples above 125pg had peak height RFUs above 150**
  - **Below 125pg peak heights were not significantly above background**
  - **At 31 pg peaks were very low or undetectable**
- **"Peaks below 150 RFU should be interpreted with caution"** **Why? Noise and stochastic fluctuation!**

How to determine the stochastic threshold

- Examine intensity and peak height ratio of 5 samples at three different low concentrations (e.g., 60, 75, and 125 pg)
- Observe variation in peak height ratio and peak intensity
- The stochastic threshold is the point at which this variation begins a rapid increase (change in slope of line relating std dev vs concentration)
- This can also be defined as the concentration at which a set percentage of peak height ratio values fall below 60%

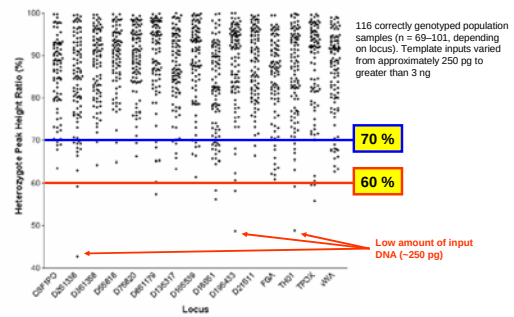
TWGDAM validation of AmpFlSTR Blue PCR

Wallin et al.JFS, 1998 43(4) 854-870

- In approximately 80 heterozygous loci in population samples:
  - Average peak height ratio was 92% for each locus – D3, vWA, FGA
  - Standard deviation was 7%
- Thus 99.7% of all samples should show a peak height ratio (PHR) above 71%
- Those that have a PHR of <70% may result from mixtures, low [DNA], inhibition, degradation or poor primer binding

Heterozygote Peak Height Ratios

Identifiler STR Kit Developmental Validation



116 correctly genotyped population samples (n = 69–101, depending on locus). Template inputs varied from approximately 250 pg to greater than 3 ng

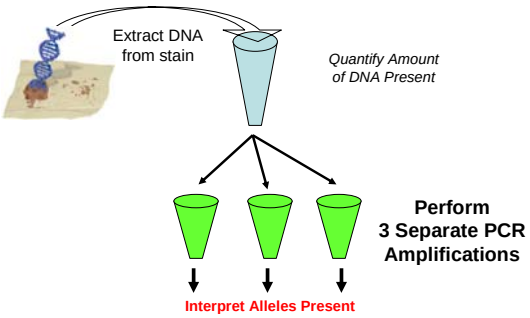
Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

Peak height ratios

Moretti et al., JFS 2001, 46(3) 647-660

- PP + Cofiler gave PHR >88% n= 230+ samples with a lower range PHR (-3sd) of 59%
- Suggest using 59% as a guide
- 2% of single source samples were below this value
- Many validation studies focus on 1ng input DNA. What happens with lower amounts?

Typical LCN Procedure



Develop a Consensus Profile

(based on replicate consistent results)

New Interpretation Rules Required for LCN

ELSEVIER

Forensic Science International 112 (2000) 17–40

Forensic Science International

www.elsevier.com/locate/forsciint

An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA

Peter Gill[a,*], Jonathan Whitaker[a], Christine Flaxman[a], Nick Brown[a], John Buckleton[b]

[a]Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK
[b]ESR, Private Bag 92021, Auckland, New Zealand

Received 9 December 1999; received in revised form 12 February 2000; accepted 13 February 2000

Suggestions to Optimal Results with LCN

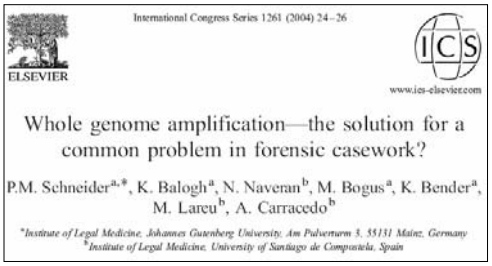
- At least two* PCR amplifications from the same DNA extract (if enough DNA is present to do more than 4-5 amplifications, then most likely a single aliquot would be run under standard STR typing conditions)
- An allele cannot be scored (considered real) unless it is present at least twice in replicate samples
- Extremely sterile environment is required for PCR setup to avoid contamination from laboratory personnel or other sources

*five is better; results are investigative

Whole genome amplification – is it a solution to LCN?

Whole Genome Amplification will also be subject to stochastic sampling when starting with LCN DNA templates

See Schneider *et al.* (2004) *Progress in Forensic Genetics 10*, ICS 1261: 24-26



International Congress Series 1261 (2004) 24–26

ELSEVIER

ICS

www.ics-elsevier.com

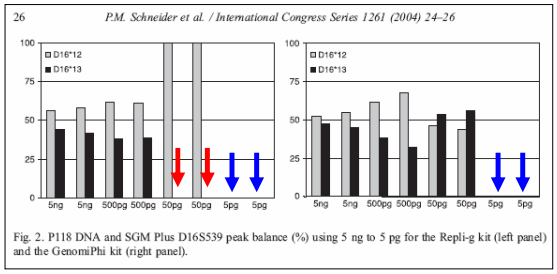
Whole genome amplification—the solution for a common problem in forensic casework?

P.M. Schneider[a,*], K. Balogh[a], N. Naveran[b], M. Bogus[a], K. Bender[a], M. Lareu[b], A. Carracedo[b]

[a]Institute of Legal Medicine, Johannes Gutenberg University, Am Pulverturm 3, 55131 Mainz, Germany
[b]Institute of Legal Medicine, University of Santiago de Compostela, Spain

Allele Dropout Seen with WGA at LCN Amounts of 50 pg and 5 pg

Schneider *et al.* (2004) *Progress in Forensic Genetics 10*, ICS 1261: 24-26



26 P.M. Schneider et al. / International Congress Series 1261 (2004) 24–26

Fig. 2. P118 DNA and SGM Plus D16S539 peak balance (%) using 5 ng to 5 pg for the Repli-g kit (left panel) and the GenomiPhi kit (right panel).

Other methods for higher sensitivity and signal enhancements

Improving Sensitivity

- Improved recovery of biological material and DNA extraction
- Longer injection on CE
- Salt removal from CE sample – enhances electrokinetic injection
- Reduced volume PCR – concentrates amplicon
- Increase number of cycles in PCR and/or TaqGold concentration
- Use miniSTRs – shorter amplicons amplify better
- Use mtDNA – higher copy number per cell

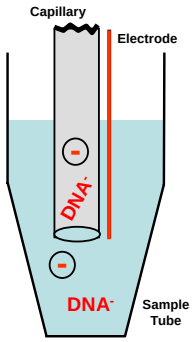
Modifications in DNA Analysis Process to Improve LCN Success Rates

- **Collection** – better swabs for DNA recovery
- **DNA Extraction** – into smaller volumes
- **DNA Quantitation** – qPCR helps with low DNA amounts
- **PCR Amplification** – increased number of cycles
- **CE Detection** – longer electrokinetic injection; more sensitive fluorescent dyes
- **Interpretation** – composite profile from replicate analyses with at least duplicate results for each reported locus
- **Match** – is it even relevant to the case?

Signal Enhancements

- Higher PCR cycles
- Lower PCR volume (problems with inhibitors)
- Brighter fluorescent dyes
- Longer CE injection
  - 10 s @ 3 kV = 30
  - 5 s @ 2 kV = 10
- Microcon cleanup to remove salts that interfere with electrokinetic injection

Electrokinetic Injection Process



Amount of DNA injected is inversely proportional to the ionic strength of the solution

Salty samples result in poor injections

Sample Conductivity Impacts Amount Injected

$$[DNA_{inj}] = \frac{Et(\pi r^2)(\mu_{ep} + \mu_{eof})[DNA_{sample}](\lambda_{buffer})}{\lambda_{sample}}$$

$[DNA_{inj}]$ is the amount of sample injected

E is the electric field applied

t is the injection time

r is the radius of the capillary

μ_{ep} is the mobility of the sample molecules

μ_{eof} is the electroosmotic mobility

$[DNA_{sample}]$ is the concentration of DNA in the sample

λ_{buffer} is the buffer conductivity

λ_{sample} is the sample conductivity

Cl- ions and other buffer ions present in PCR reaction contribute to the sample conductivity and thus will compete with DNA for injection onto the capillary

Butler *et al.* (2004) Electrophoresis 25: 1397-1412

LCN Summary

- LCN often defined as <100-200 pg input DNA
- Typically involves increasing the number of PCR cycles when performing multiplex PCR to amplify DNA with conventional STR kits (e.g., 34 cycles instead of 28 cycles)
- Enables lower amounts of DNA to be detected with STR markers but is prone to contamination
- Cautious data interpretation rules must be adopted as allele drop-out and drop-in may occur due to stochastic amplification effects

Is LCN Effort Worthwhile?
Thoughts to Consider…

- Success rates are often low
- Requires dedicated "clean" facilities and extreme care to avoid limit contamination
- Complex interpretation procedure – requires more experienced analysts to do
- Significance of a DNA match?? – intelligence information but likely not to be probative due to unknown time when sample may have been deposited…

miniSTRs and LCN

- miniSTR assays are typically more sensitive than conventional STR kits currently in use
- Labs will start "pushing the envelope" in order to try and get a result with more sensitive assays including future miniSTR assays and kits
- Labs may move into the LCN realm without realizing it or adopting the careful LCN interpretation rules such as replicate analyses with duplicate results prior to reporting alleles

The Wisdom of Obi Wan Kenobi

http://www.starwars.com/kids/explore/lore/img/news20000902_1.jpg

Just before entering the Mos Eisley spaceport in Episode IV, Ben (Obi Wan) Kenobi warned Luke Skywalker, "You will never find a more wretched hive of scum and villainy…

WE MUST BE CAUTIOUS!"

DNA Profiles are An Investigative Tool

Finding DNA indicates contact.

Lack of a DNA profile is inconclusive.

LCN is analogous to a bigger, more powerful magnifying glass

Theresa Caragine (AAFS 2003 LCN Workshop)

Thank you for your attention…

Questions?

http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov
301-975-4049

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm