Advanced Topics in Forensic DNA Analysis

qPCR and Low-Copy Number DNA Testing

New Jersey State Police
Training Workshop

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Earlier Workshops on These Topics

- **qPCR workshop by Vallone and Orrego** (July 2006) – slides available on STRBase
  - **http://www.cstl.nist.gov/biotech/strbase/qPCRworkshop.htm**
- **LCN workshop by Butler, Caragine, and Gill** (May 2006) – Butler slides available on STRBase
  - **http://www.cstl.nist.gov/biotech/strbase/training.htm**

qPCR Workshop Materials

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

For workshop held at NFSTC on July 26-27, 2006 by Peter Vallone (NIST) and Cristian Orrego (CA DOJ)

Handouts for PowerPoint presentations:

- Introduction
- Quantitation Using PCR
- Instrumentation
- Signals and Probes

Selected Forensic qPCR Assays:

- VT Alu SYBR Green and Quantifiler
- CA DOJ nuclear/mtDNA duplex and CA DOJ degradation triplex

- Maintenance, Validation, etc.
- Data Analysis and Troubleshooting
- qPCR Analysis Software for ABI 7000 & 7500

Purpose of Human-Specific DNA Quantitation

- All sources of DNA are extracted when biological evidence from a crime scene is processed to isolate the DNA present.
- Thus, non-human DNA such as bacterial, fungal, plant, or animal material may also be present in the total DNA recovered from the sample along with the relevant human DNA of interest.
- For this reason, the DNA Advisory Board **(DAB) Standard 9.3 requires human-specific DNA quantitation** so that appropriate levels of human DNA can be included in the subsequent PCR amplification.
- **Multiplex STR typing works best with a fairly narrow range of human DNA** – typically 0.5 to 2.0 ng of input DNA works best with commercial STR kits.

Higher quality data saves time and money

Why Do We Care About Quantitating DNA?

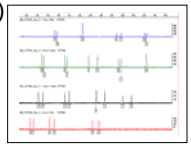
- If we can confidently determine the amount of DNA in an extract we can then ask questions:
  - Will mitochondrial sequencing be required (skip STR analysis)
  - Should we use a miniSTR assay?
  - Should we use low copy number LCN methods for STRs?
  - Re-extract the sample?
  - If problems occur in the STR typing process we can have confidence that the DNA template is not the source (CE, cycler, kit)

qPCR

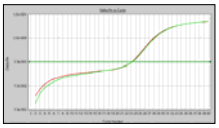
- qPCR is a recently developed technique
  - Developed by Higuchi in 1993
  - Used a modified thermal cycler with a UV detector and a CCD camera
  - Ethidium bromide was used as intercalating reporter As [dsDNA] increased fluorescence increased
- First paper on qPCR:
  - Higuchi, R.; Fockler, C.; Dollinger, G.; Watson, R. “Kinetic PCR analysis: real-time monitoring of DNA amplification reactions” Biotechnology (N Y). 1993 Sep;11(9):1026-30

PCR/qPCR What is the Difference?

- In the PCR the products are analyzed after the cycling is completed (static)
  - gel, CE, UV, fluorimeter
  - End point assay



- qPCR the products are monitored as the PCR is occurring (dynamic)
  - Once per thermal cycle
  - Fluorescence is measured
  - Kinetics of the system



Why Real Time qPCR?

Advantages

- The availability of commercial qPCR kits (labs are beginning to switch over to this method)
- Higher throughput and reduced user intervention
  - Automated set up
  - Simple data analysis
  - Experimental data rapidly analyzed in software; interpolating into the calibration curve
- qPCR will be sensitive to the same inhibitors as faced in a traditional STR test (both PCR based)

Why Real Time qPCR?

Advantages

- No post PCR manipulation (reduced contamination issues)
- High sensitivity (down to a single copy number ?)
- Large dynamic range: ~30 pg to 100 ng
- Assays are target specific (autosomal, mito, Y) and can be multiplexed – to a degree…

Why Real Time qPCR?

Challenges

- qPCR is subject to inhibition
  - internal PCR controls (IPC) can help
- qPCR quantitation precision suffers at low copy numbers (below 30 pg by a factor of 2)
- When working below 100 pg qPCR is still subject to variability and uncertainty

Why Real Time qPCR?

Challenges

- qPCR quantitates specific target sequences, it does not quantify "DNA"
  - In highly degraded samples, assays that amplify short target sequences will detect and measure more DNA than assays that amplify long target sequences (relevant to STR typing)
- Accurate qPCR quantitation assumes that each unknown sample is amplified at the same efficiency as the Calibrant sample in the dilution series
- Results are relative to the Calibrant (these can vary)

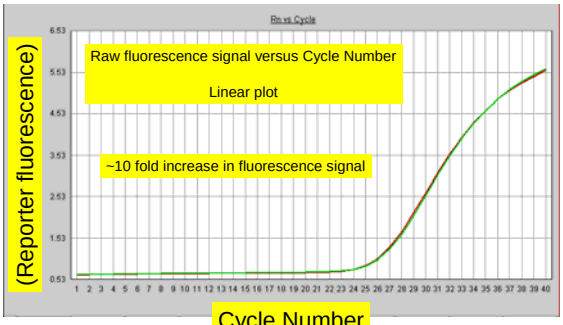
PCR Amplification

- 4 phases of PCR amplification
  - Lag (doubling, but not detected)
  - Exponential (doubling)
  - Linear (less than doubling)
  - Plateau (little change)

Efficiency is dropping < 100%

- The exponential phase is where we make our qPCR measurements

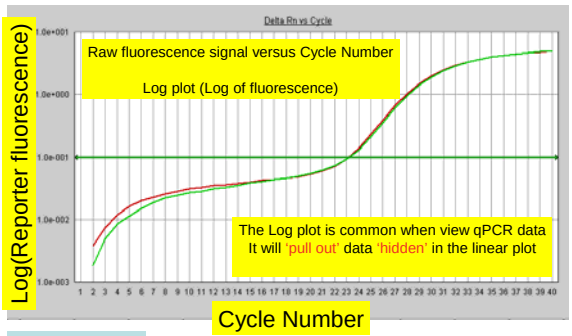
qPCR Real Time Curves



Y scale 0.5 to 5.5

Quantifiler Data

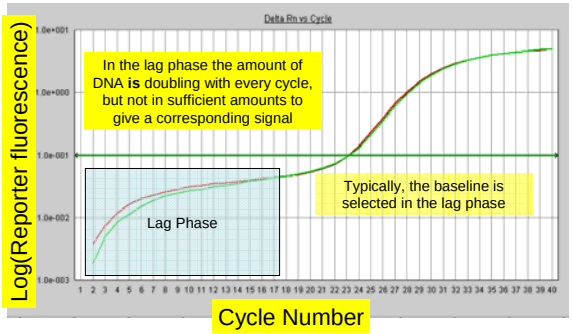
qPCR Real Time Curves



Y scale 0.001 to 10

Quantifiler Data

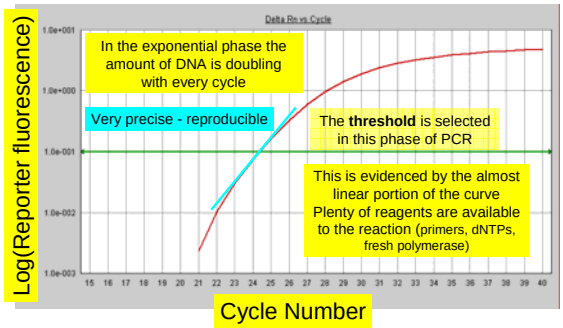
Lag Phase



Cycles ~ 1 - 20

Quantifiler Data

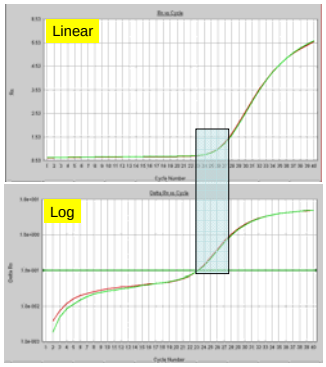
Exponential Phase



Cycles ~ 20 - 27

Quantifiler Data

qPCR Real Time Curves



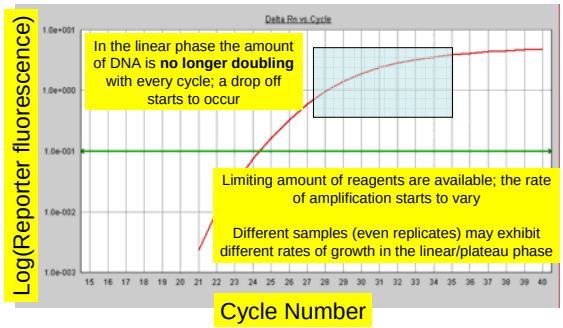
These plots are on the same X scale (Cycle Number)

The log plot is useful to see where the amount of DNA is doubling with cycle number

This can not be readily observed in the linear plot

Quantifiler Data

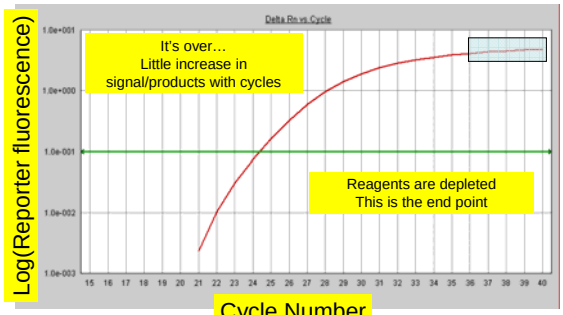
Linear Phase



Cycles ~ 27 - 35

Quantifiler Data

Plateau Phase

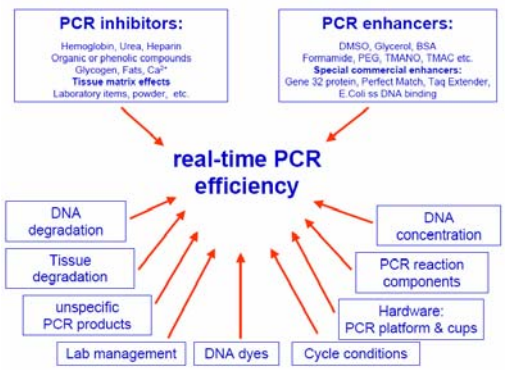


Cycles ~ 36 - end

Quantifiler Data

PCR Efficiency

- How is the PCR progressing?
- Is the PCR running at maximum efficiency?
- Is there some factor (environmental) inhibiting the reaction?
- Are we at the optimal annealing-extension temperatures (during assay development)?
- Are the unknowns amplifying with the same E as the Calibrants?



http://www.gene-quantification.info/ Michael W. Pfaffl: pfaffl@wzw.tun.de

PCR Efficiency

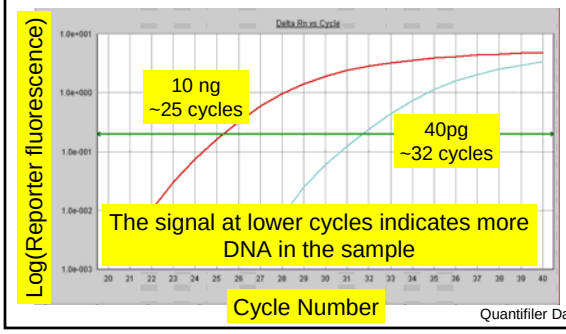
- Taking our previous relationship 2^N
- The efficiency of the PCR can be represented as:
- $X_N = X_0 (1 + E)^N$
  - X_N predicted copies
  - X_0 starting copy number
  - E efficiency (0 to 1)
  - N number of cycles

PCR Efficiency

- Starting with 100 copies and 100% and 28 cycles
  $X_N = 100(1 + 1)^{28}$
  2.68×10^{10} copies
- 90%
  $X_N = 100(1 + 0.9)^{28}$
  6.38×10^9 copies
- 80%
  $X_N = 100(1 + 0.8)^{28}$
  1.40×10^9 copies

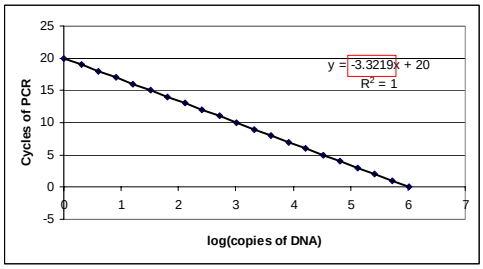
PCR Efficiency

- When applied to qPCR the relationship is the inverse (the signal at lower cycles indicates more DNA in the sample)



PCR Efficiency

- When applied to qPCR the relationship is the inverse (the signal at lower cycles indicates more DNA in the sample).
- The line has a negative slope



PCR Efficiency

- A optimal reaction should be between 90% to 110% slope = -3.58 to -3.10
- The slope may vary even more when looking at more complex (multiplex) qPCR assays; multiplex probes, targets, copies etc

Importance of the Calibrant!

- Things to keep in mind about Calibrants
- The Calibrant is usually a pristine well-characterized DNA sample
  - Not extracted
  - Not subjected to the same environment as your unknown(s)
  - Will not contain inhibitors, Ca^{++} etc
  - May be from a cell line or mixed source sample
  - **May exhibit lot-to-lot variation (monitor this)**

Varying the C_T Value

- Relative small changes in C_T result in large variations in concentration

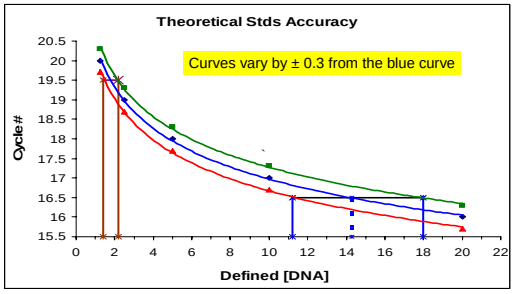
m	b	CT	[DNA]	%	delta
-3.3219	26	25.1	1.87	6.70	0.13
-3.3219	26	25	2.00		
-3.3219	26	24.9	2.14	6.70	-0.14
-3.3219	26	20.1	59.72	6.70	4.29
-3.3219	26	20	64.00		
-3.3219	26	19.9	68.60	6.70	-4.59

± 0.1 C_T

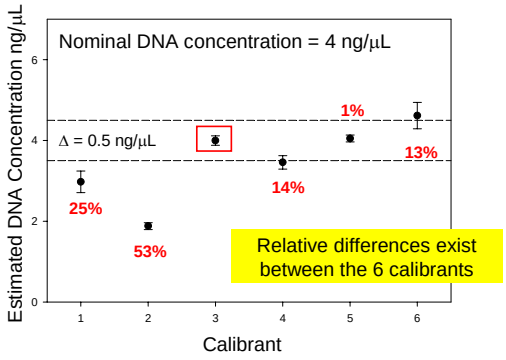
m	b	CT	[DNA]	%	delta
-3.3219	26	25.3	1.62	18.77	0.38
-3.3219	26	25	2.00		
-3.3219	26	24.7	2.46	18.77	-0.46
-3.3219	26	20.3	51.99	18.77	12.02
-3.3219	26	20	64.00		
-3.3219	26	19.7	78.80	18.77	-14.79

± 0.3 C_T

Importance of the Calibrant!



Differences Between Calibrants



NIST Lessons Learned from Real Time-qPCR Assays

Using ABI 7500 (early work with ABI 7000 and some Roche LightCycler)

- Results are RELATIVE to standards used
- Single source and mixed source samples with same UV concentrations differ with RT-qPCR assays
- Need to keep instrument clean to avoid background fluorescence problems
- Assay reagent costs:
  - Quantifiler: $2.46/sample (only permits 2 µL/sample)
  - SYBR Green: $0.80/sample (up to 10 µL/sample)
  - QuantiBlot: $0.54/sample (5 µL/sample)

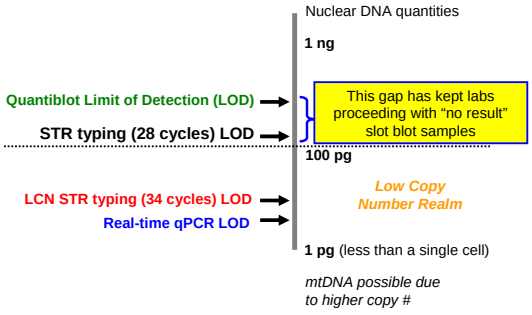
http://www.cstl.nist.gov/biotech/strbase/DNAquant.htm

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"?

Difference in DNA Quantitation Capability vs. STR Typing Sensitivity



DNA Quantitation Summary

- RT-qPCR is a homogeneous PCR based method that enables human specific quantification
  - Is easily automated, provides electronic storage of data
  - SYBR green or targeted probes can be used
- Results give quantity of amplifiable DNA – not necessarily overall quantity
  - PCR inhibition can be detected
  - Multiplexing can be used
- Big advantages are speed and dynamic range
- Commercial kits are now available

MAAFS DNA Workshop

Introduction to Low Copy Number (LCN) DNA Testing Issues

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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

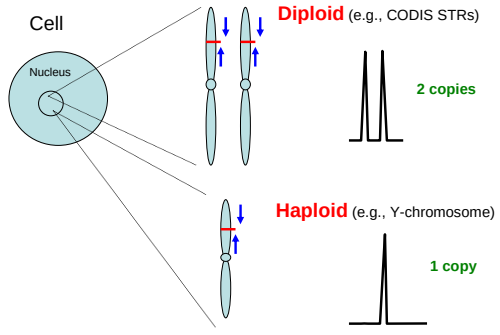
LCN is dependent on the amount of DNA present NOT the number of PCR cycles performed; LCN conditions may exist with 28 or 34 cycles

Low-Copy Number (LCN) Work

- **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 AMPFISTR 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

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

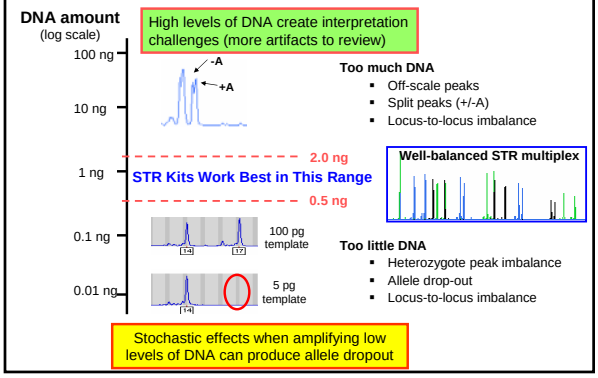
- In a 1:1 mixture, each DNA source is at LCN when the total amount of DNA in the amplification reaction is ~ 0.125 ng.
- In a 1:9 mixture, the minor component could be at LCN **even when the total amount of DNA in the amplification is 1 ng**.

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?"

Impact of DNA Amount into Multiplex PCR Reaction

We generally aim for 0.5-2 ng



Stochastic PCR amplification

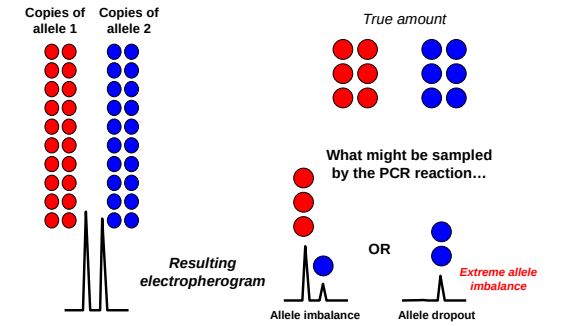
Stochastic = random selection

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

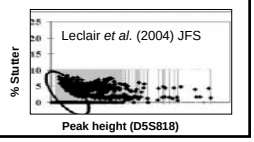


>20 copies per allele

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

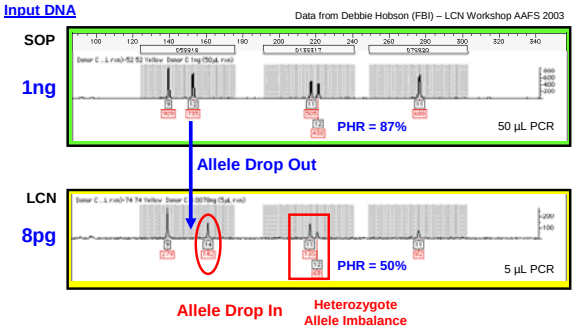
Stochastic Effect

- 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



Comparison of STR Kit Amplification SOP with LCN

Using the Same DNA Donor

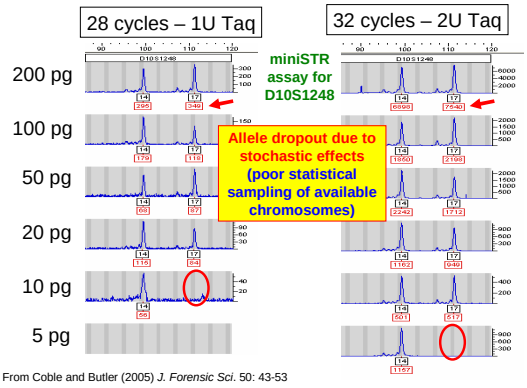


Balance of Assay Sensitivity and Potential for Stochastic Effects

- **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

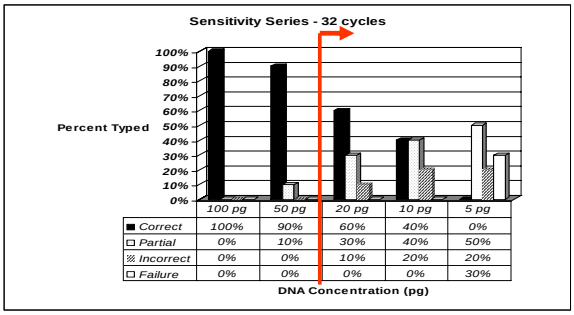
Note that Promega STR kits use higher numbers of cycles to generate roughly equivalent sensitivity to ABI kits because they have less efficient dye labels and lower primer and polymerase concentrations

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



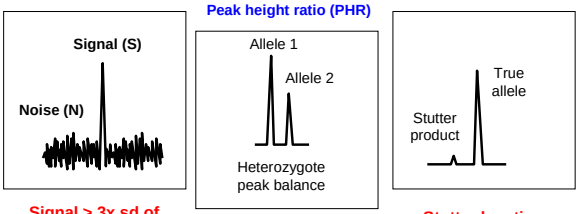
	100 pg	50 pg	20 pg	10 pg	5 pg
Correct	100%	90%	60%	40%	0%
Partial	0%	10%	30%	40%	50%
Incorrect	0%	0%	10%	20%	20%
Failure	0%	0%	0%	0%	30%

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**.

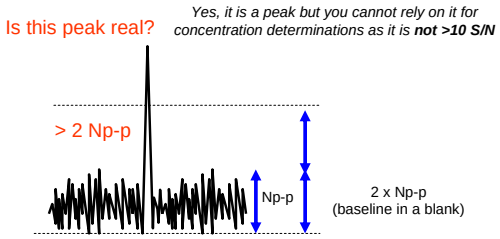
Sensitivity

- 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)

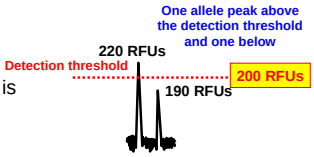
- Typically 3 times the signal-to-noise (based on standard deviation of the noise) or 2x Np-p



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

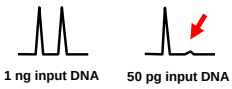
Straddle Data

- 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!**

Sensitivity of Detection

Moretti et al, JFS, 2001, 46(3), 661-676

- Different 310 instruments have different sensitivities; determination of stochastic threshold should be performed following in-house studies
  - Variations in quantitation systems
  - Variations in amplification systems
  - Variations in instrument sensitivity
- Peaks with heights below the threshold should be interpreted with caution
  - Caution should be used before modification of
    - Amplification cycles
    - Electrophoretic conditions

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%

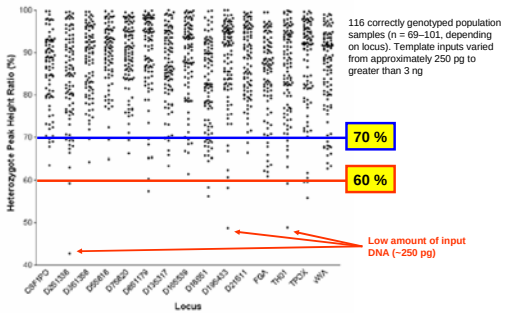
TWGDAM validation of AmpFlSTR BluePCR

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



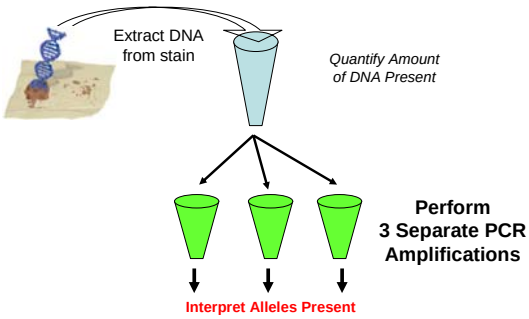
Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

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



Interpret Alleles Present

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

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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 typically viewed as investigative

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?

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!"

Thank you for your attention…

Questions?

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