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Some of the Problems Associated with LCN (Low Copy Number) Testing

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Some of the problems associated with LCN (Low Copy Number) testing

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What is LCN?

- DNA profiling performed at or beneath the stochastic threshold
- Typically less than 0.5 ng of DNA template
- Typically involves modifications of the testing methodology (e.g. increased polymerase; additional rounds of amplification; skipping quantitation)
- Consensus profiles
Quantitating and Amplifying DNA

♦ Determine the quantity of DNA in samples to be amplified. See Chapter 4 for more details on DNA quantitation.

♦ Amplify DNA samples using the AmpFLSTR SGM Plus kit reagents (see Chapter 5). The recommended range of input DNA is 1.0–2.5 ng.

Note A useful initial experiment is to amplify a range of input DNA for each of several samples in order to establish the range of input DNA (as determined by your laboratory’s quantitation system) that provides optimal results. For example, amplify 0.5, 1.0, 1.5, 2.0, 2.5 ng, and 5.0 ng of input DNA for each sample.
“The PCR amplification parameters have been optimized to produce similar peak heights within and between loci. The peak height generated at a locus for a heterozygous individual should be similar between the two alleles. The kit is also designed to generate similar peak heights between loci labeled with the same dye so that each locus will have approximately the same sensitivity.”

What is LCN?

- DNA profiling performed at or beneath the stochastic threshold
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- Consensus profiles
Stochastic effects

• Ultimately due to poor statistical sampling of underlying template
• The four horsemen of stochasticism
  – Exaggerated stutter
  – Exaggerated peak height imbalance (0 to 100%)
  – Allelic drop-out (extreme peak height imbalance)
  – Allelic drop-in (contamination)
Stochastic sampling effects

Copies of allele 1

Copies of allele 2

True amount

What might be sampled by the PCR

Resulting electropherogram

Allele imbalance

OR

Extreme allele imbalance

Allele dropout
Stochastic effects

• Ultimately due to poor statistical sampling of underlying template

• The four horsemen of stochasticism
  – Exaggerated stutter (up to 50%)
  – Exaggerated peak height imbalance (0 to 100%)
  – Allelic drop-out (extreme peak height imbalance)
  – Allelic drop-in (contamination)
Comparison of STR Kit Amplification SOP with LCN Using the Same DNA Donor

Input DNA

SOP
1 ng

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

PHR = 87%

50 μL PCR

Allele Drop Out

LCN
8 pg

PHR = 50%

5 μL PCR

Allele Drop In

Heterozygote Allele Imbalance
Impact of DNA Amount into Multiplex PCR

**DNA amount** (log scale)

- 100 ng
- 10 ng
- 1 ng
- 0.1 ng
- 0.01 ng

**High levels of DNA create interpretation challenges (more artifacts to review)**

- Off-scale peaks
- Split peaks (+/-A)
- Locus-to-locus imbalance

**Too much DNA**

**STR Kits Work Best in This Range**

- 2.0 ng
- 0.5 ng

**Well-balanced STR multiplex**

**Too little DNA**

- Heterozygote peak imbalance
- Allele drop-out
- Locus-to-locus imbalance

**Stochastic effects when amplifying low levels of DNA can produce allele dropout**
How helpful is quantitation?

- Optimum amount of template: 0.5 to 2.0 ng
- 6 to 7 pg of DNA in each diploid human cell
- In a mixed sample containing 0.5 ng of template, less than 0.5 ng comes from each contributor
Assume sample is a **1:1 mixture** of two sources:

<table>
<thead>
<tr>
<th>Amount of DNA</th>
<th>Total Cells in sample</th>
<th>~ # of cells from each component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ng</td>
<td>152</td>
<td>76</td>
</tr>
<tr>
<td>0.5 ng</td>
<td>76</td>
<td>38</td>
</tr>
<tr>
<td>0.25 ng</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>0.125 ng</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>0.0625 ng</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Amount of DNA</th>
<th>~ # of cells from major component</th>
<th>~ # of cells from minor component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ng</td>
<td>137</td>
<td>15</td>
</tr>
<tr>
<td>0.5ng</td>
<td>68</td>
<td>8</td>
</tr>
<tr>
<td>0.25ng</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>0.125ng</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>0.0625ng</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

Robin Cotton, AAFS 2003 LCN Workshop
“Are we already doing low copy number (LCN) DNA analysis?”
Consensus profiles

• Alleles are not reported unless they are seen in at least two runs

• Considering two runs serves as a safeguard against allelic drop-in (contamination)

• Considering three or more runs begins to safeguard against drop-out

• If a sample is being split four or more times, shouldn’t conventional tests be done?
### Consensus profiles

<table>
<thead>
<tr>
<th>Runs used to make consensus</th>
<th>D3</th>
<th>vWA</th>
<th>D16</th>
<th>D2</th>
<th>D8</th>
<th>D21</th>
<th>D18</th>
<th>D19</th>
<th>THO1</th>
<th>FGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+2+3</td>
<td>16</td>
<td>17</td>
<td>10</td>
<td>13</td>
<td>20</td>
<td>10</td>
<td>13</td>
<td>28</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>1+2</td>
<td>16</td>
<td>17</td>
<td>13</td>
<td>20</td>
<td>10</td>
<td>13</td>
<td>30</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>1+3</td>
<td>16</td>
<td>17</td>
<td>13</td>
<td>20</td>
<td>10</td>
<td>13</td>
<td>30</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>2+3</td>
<td>16</td>
<td>17</td>
<td>10</td>
<td>13</td>
<td>20</td>
<td>10</td>
<td>13</td>
<td>28</td>
<td>30</td>
<td>13</td>
</tr>
</tbody>
</table>

Consensus profiles include runs used to make consensus, with data for each run across various markers.
What minimum peak height thresholds should be used for LCN?

- “Conservative” thresholds established during validation studies
- Eliminate noise (even at the cost of eliminating signal)
- Can arbitrarily remove legitimate signal
- Contributions to noise vary over time (e.g. polymer and capillary age/condition)
- Analytical chemists use LOD and LOQ
Signal measurements

Measured signal (In Volts/RFUS/etc)

Saturation

$\mu_b + 10\sigma_b$

$\mu_b + 3\sigma_b$

$\mu_b$

0

Quantification limit

Detection limit

Mean background Signal
Opportunities to measure baseline
Two reference samples in a 1:10 ratio (male:female). Three different thresholds are shown: 150 RFU (red); LOQ at 77 RFU (blue); and LOD at 29 RFU (green). Taken from Gilder et al., 2007, *J. For. Sci.* 52:97-101.
Questions?

Click on the questions tab on your screen, type in your question (and name if you wish) and hit send.