Peak Height Ratios

Charlotte J. Word

Outline for Peak Height Ratios

- GUIDELINES
  - SWGDAM Guideline 3.3
  - Related SWGDAM Guidelines 3.4.2, 3.5.1, 3.5.2, 3.5.4, 3.5.5, 3.5.6, 3.5.8 (covered later in workshop)

- PRINCIPLES
  - Calculation, Causes, Examples

- PROTOCOLS
  - Approaches to data collection and calculating
  - Review of data

- PRACTICE
  - How is this topic applied?

Peak Height Ratio

SWGDAM Interpretation Guidelines 3.3:

Intra-locus peak height ratios (PHR) are calculated for a given locus by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage.

What is PHR?

Theory: The two alleles for an individual who is heterozygous at a single locus should:

- Have equal amounts in the genome
- Amplify equally
- Inject equally
- Have peak heights that are ~equal
- Value must be ≤100%

All things being perfect these two peaks are expected to have the same height” (Buckleton, 2009)

This does NOT apply to loci that are homozygous or to inter-locus ratios.

PHR is not to be confused with stutter percentage cutoff values.

http://www.cstl.nist.gov/biotech/strbase/training.htm
Module 6: Peak Height Ratios

**PRINCIPLES**

**Peak Height Ratio Variation**

<table>
<thead>
<tr>
<th>DNA1</th>
<th>DNA2</th>
<th>DNA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>71%</td>
<td>98%</td>
<td>79%</td>
</tr>
</tbody>
</table>

The heights of the peaks will vary from sample-to-sample, even for the same DNA sample amplified in parallel.

**Causes of Peak Height Imbalance**

1. **Single-source samples**
   - Low Template DNA (LT DNA)
   - Inhibited
   - Degraded
   - Preferential amplification

2. **Mixture of DNA from 2 or more contributors is present**

**Causes of Peak Height Imbalance**

**Single Source Samples**

- LT DNA and stochastic effects
  - Elevated Stutter – artifact, not true allele
  - Unequal sampling of true alleles – the two alleles are not sampled and amplified equally

- Preferential/differential amplification
  - any situation where one allele of a heterozygous pair is amplified more often than the second allele at that locus
  - resulting in one allele with a peak height higher than the peak height of the other allele

- Mutation in the primer binding site of one allele resulting in two peaks of different heights or a single peak with 1/4 the height of a homozygous peak at another locus (with allele drop-out)
- One allele amplicon much longer than other allele amplicon – more common at loci with many alleles and with loci at the right side (i.e., higher bp length) of the profile (e.g., FGA, D18 in Profiler Plus, ID; FGA, Penta E in PP16)

http://www.cstl.nist.gov/biotech/strbase/training.htm
Causes of Peak Height Imbalance

**Mixture**

2. Mixture of DNA from 2 or more contributors is present

If the PHR is less than what was observed in validation studies, then there is a strong probability that the sample contains a mixture of DNA from 2 or more contributors, especially if sufficient amount of DNA was amplified and other indications of a mixture are observed.

**Causes of Peak Height Imbalance**

**Imbalance in the amount of DNA available to amplify**
- different amounts of alleles
  - LT, mix
- degradation

**Imbalance in amplification**
- longer alleles
- inhibition
- primer mutation

**How calculate Peak Height Ratios?**

From Validation Studies
- Sensitivity Study at different amounts of DNA
- Non-probative single-source samples with good quality profiles amplified with different amounts of DNA (or at least with different peak height ranges)
- Perform for each kit validated as PHRs may vary for the same locus amplified with different kits

From Casework and Training samples
- Known standards and single-source samples with good quality profiles amplified with different amounts of DNA (or at least with different peak height ranges)
- Database samples (as long as same procedures being used for casework)

**How calculate Peak Height Ratios?**

Use sufficient number (N=100-500) and variety of samples to get representative data from each locus, especially for loci with a wide range of alleles and long amplicons (e.g., FGA, D18).
**How calculate Peak Height Ratios?**

- **Export data from GeneMapper ID, etc. into Excel table**
- **Calculate PHR (Low RFU peak/High RFU Peak x 100%) for each locus for each sample**
  - In Excel
  - NIST worksheet

**Peak Height Ratio Data**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele 1 - Allele 2 Size Difference, bp</th>
<th>Peak Height Ratio</th>
</tr>
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<tbody>
<tr>
<td>D15S17</td>
<td>2</td>
<td>0.931</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.932</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.943</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.950</td>
</tr>
<tr>
<td>D19S432</td>
<td>2</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.985</td>
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**Peaks Variation Across Loci**

- **Range of PHRs is observed within a locus**
  - Minimum vs. Maximum %
  - Mean and Median
  - Alleles further apart tend to have lower PHR
- **Ranges of PHRs vary across loci**

**PowerPlex 16 data kindly provided by NIST**

http://www.cstl.nist.gov/biotech/strbase/training.htm
Using the data in the previous slide, what single peak height ratio would you select to use?
1. 20%
2. 50%
3. 60%
4. 75%
5. 90%
6. I don’t use PHRs

PHR Requirements

SWGDAM Interpretation Guideline 3.3.1

The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples. Different PHR expectations can be applied to individual loci (e.g., 70% for D3S1358, 65% for vWA, etc.); alternatively, a single PHR expectation can be applied to multiple loci (e.g., 60%).
Peak Height Ratio

Most peaks >1500 RFU
Most peaks
All peaks

Larger Peak Height, RFU

Based on the data in the previous slide, would it be reasonable to use two PHRs – one for alleles with peaks <1500 RFU and one for alleles with peaks ≥1500 RFU?

1. Yes
2. No
3. Need to think about this more
4. Need more data

How calculate Peak Height Ratio?

- Develop SOP interpretation guidelines using these data for making decisions regarding presence of mixtures, degradation, possible inhibition for each kit.
- Use data in conjunction with other validation studies to establish a stochastic threshold and develop mixture interpretation SOPs.

Peak Height Ratio

SWGDAM Interpretation Guideline 3.3.2

PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.

Peak Height Imbalance

When assuming that a mixture of DNA from only 2 contributors is present, the Peak Height Ratio may aid in the interpretation of the profile data when used to pair heterozygous alleles

What can be said about this profile?

1. Mixture
2. Not a mixture
3. Not enough information
4. Not sure

http://www.cstl.nist.gov/biotech/strbase/training.htm
Module 6: Peak Height Ratios

Peak Height Ratio
Determine if mixture
Could be:
1) Single-source profile from an individual who is heterozygous at that locus

11 12

Peak Height Ratio
Determine if mixture
Could be:
2) 1:1 mixture of two people who are both homozygous, with each having one of the two alleles

11,11 12,12

Peak Height Ratio
Determine if mixture
Could be:
3) Any ratio of mixture of two people who are both heterozygous for the same two alleles at that single locus

11 12

Peak Height Ratio
Determine if mixture
Correct answers are
# 3 Not enough information
or # 4 Not Sure

Need more information from other loci (if available)

Peak Height Ratio
What can be said about this profile?

Correct answers are
#3 Not enough information
or #4 Not Sure

Need more information from other loci (if available)

http://www.cstl.nist.gov/biotech/strbase/training.htm
What could NOT be the cause of the observed peak height imbalance?

1. Elevated stutter
2. Degradation
3. Differential amplification
4. Primer mutation
5. Mixture

Peak Height Ratio
Determine if mixture

Could be:
- Single-source sample from individual who is heterozygous at that locus, but:
  - Primer mutation (uncommon)
  - Degraded
  - Differential Amplification
  - NOT elevated stutter (wrong position)
  - Probably NOT LT DNA (good peak heights)

Peak Height Ratio
Determine if mixture

Could be:
- Mixture of two individuals:
  - Both homozygous
    - ~1:2 mixture of 19,19 + 15,15 (common)
  - One homozygous and one heterozygous
    - ~1:3 mixture of 15,15 + 15,19 (common)

What information should NOT be used to decide if this is a mixed DNA sample?

1. Validation studies & PHRs
2. Other loci in the profile
3. Profile from known standard(s) to compare
4. All of the above

Peak Height Imbalance

- Stutter (+4)
- Stochastic
- Preferential Amp → Degradation
- Mix
  - Heterozygous + Homozygous
  - Homozygous + Homozygous

# 3 Profile from known standard(s) to compare should NOT be included as information to consider

MUST interpret data and make conclusions WITHOUT reference to standards

http://www.cstl.nist.gov/biotech/strbase/training.htm
Based on PHR and assuming 2 contributors, what genotypes are present?

1. 11, 13 + 12, 12
2. 11, 12 + 12, 13
3. 11, 12 + 13, 13
4. 11, 11 + 12, 12
5. Can’t tell
6. 12, 13 and 11 is a stutter peak
7. 11, 12 and 13 is a +4 stutter peak

Peak Height Ratio Goals

Understanding of:
- Calculation of Peak Height Ratio
- Causes of peak imbalance
- Why Peak Height Ratios vary
- How to collect data for establishing PHR value
- How to analyze data for establishing PHR value
- Introduction – how to use PHR in mixtures (more later)