21st International Symposium on Human Identification
Mixture Interpretation Workshop:
Principles, Protocols, and Practice
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Peak Height Ratios

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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?

GUIDELINE

Peak Height Ratio

SWGDAM Interpretation Guidelines 3.3:

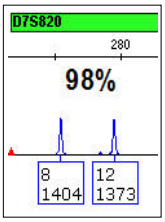
Intra-locus peak height ratios (PHR) are calculated for a given locus by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage.

PRINCIPLES

Peak Height Ratio
What is PHR?

At each locus:

$$\frac{\text{Peak A RFU (lower RFU peak)}}{\text{Peak B RFU (higher RFU peak)}} \times 100 = \text{PHR\%}$$



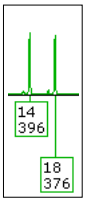
$$\frac{1373 \text{ RFU}}{1404 \text{ RFU}} \times 100 = 98\%$$

PRINCIPLES

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%**



PRINCIPLES

Peak Height Ratio
What is PHR?

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

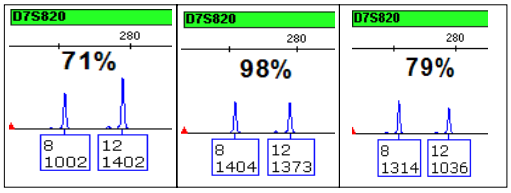
This does NOT apply to loci that are homozygous or to inter-locus ratios.

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

Buckleton, J. (2009). Validation issues around DNA typing of low level DNA. *Forensic Science International: Genetics, 3*, 255-260.

PRINCIPLES

Peak Height Ratio **Variation**

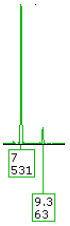


The heights of the peaks will vary from sample-to-sample, even for the same DNA sample amplified in parallel

PRINCIPLES

Causes of Peak Height Imbalance

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

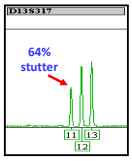


2. **Mixture of DNA from 2 or more contributors is present**

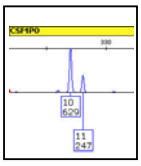
PRINCIPLES

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**



12,13



0.25 ng

PRINCIPLES

Causes of Peak Height Imbalance
Single Source Samples

- **Inhibited – generally a locus shows inhibition relative to another locus, but may see altered peak heights within a locus**
- **Degraded – the longer allele has a lower RFU peak in a heterozygous pair**

PRINCIPLES

Causes of Peak Height Imbalance
Single Source Samples

- **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**

PRINCIPLES

Causes of Peak Height Imbalance
Single Source Samples

Preferential/differential amplification

- **Mutation in the primer binding site of one allele resulting in two peaks of different heights or a single peak with ½ 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)**

PRINCIPLES

Causes of Peak Height Imbalance
Mixture

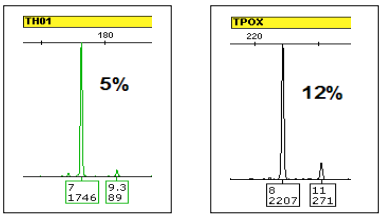
2. **Mixture of DNA from 2 or more contributors is present**

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

PRINCIPLES

Causes of Peak Height Imbalance
Mixture

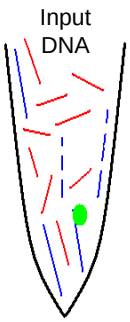
Mixture of DNA from 2 or more contributors is present



9:1 ratio

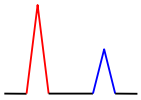
PRINCIPLES

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**



PROTOCOLS

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**

PROTOCOLS

How calculate Peak Height Ratios?

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)**

PROTOCOLS

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).**

PROTOCOLS

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

PROTOCOLS

How calculate Peak Height Ratios?

- Determine range of PHRs at various amplification input DNA amounts or different RFU heights for each locus
  - Minimum and Maximum of PHR
- Calculate average and/or median

PROTOCOLS

Peak Height Ratio Data

Locus	Δbp	#	Mean		Median		Percentiles	
			X	s(X)	X	s(X)	Min	Max
D13S317	4	103	0.913	0.082	0.930	0.079	0.637	1.000
	8	49	0.879	0.083	0.900	0.091	0.652	0.998
	12	24	0.867	0.079	0.874	0.084	0.639	0.979
	16	20	0.855	0.080	0.847	0.070	0.696	0.997
	20	11	0.828	0.069	0.822	0.067	0.742	0.959
D18S51	4	63	0.878	0.097	0.900	0.100	0.554	0.998
	8	49	0.894	0.100	0.905	0.112	0.704	0.998
	12	44	0.866	0.104	0.876	0.116	0.583	0.997
	16	27	0.872	0.107	0.895	0.119	0.574	0.995
	20	22	0.807	0.100	0.796	0.112	0.644	0.963
	28	10	0.795	0.115	0.785	0.138	0.641	0.936
D8S1179	4	105	0.884	0.082	0.886	0.079	0.683	0.997
	8	61	0.895	0.090	0.908	0.085	0.714	0.990
	12	26	0.857	0.105	0.898	0.099	0.485	1.000
	16	14	0.886	0.088	0.891	0.094	0.620	0.999

PowerPlex 16 data kindly provided by NIST

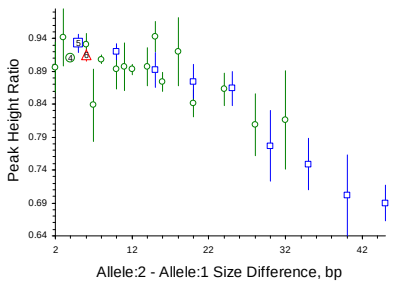
PROTOCOLS

Peak Height Ratio Observations

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

PROTOCOLS

Peak Height Ratio Data

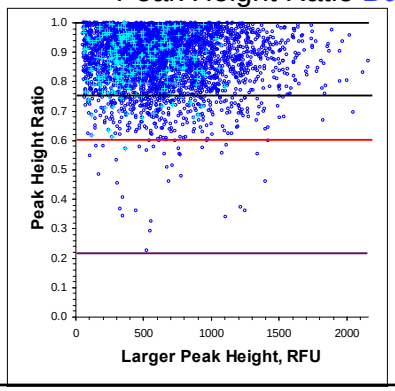


PowerPlex 16 data kindly provided by NIST

>8000 alleles

PROTOCOLS

Peak Height Ratio Data



Most peaks >1500 RFU

Most peaks

All peaks

Power Plex 16 data kindly provided by NIST

>8000 alleles

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

GUIDELINE

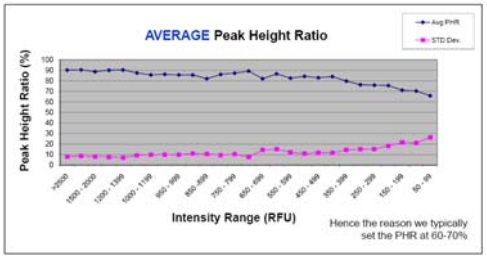
PHR Requirements

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%).

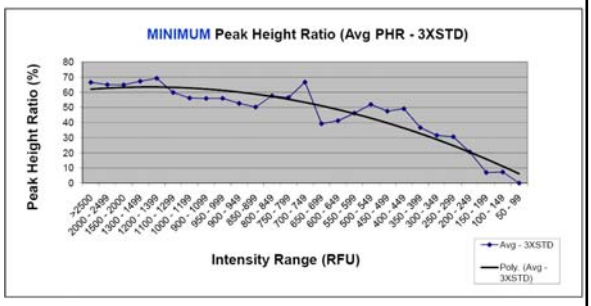
PROTOCOLS

Peak Height Ratio **Data**



Data courtesy of the AFT, Todd Bille

Peak Height Ratio **Data**



Data courtesy of the AFT, Todd Bille

PROTOCOLS

Peak Height Ratio **Observations**

Peak Height Ratios become more varied and tend to have a lower value as the amount of input DNA decreases

↓ **DNA** ↓ **PHR**

GUIDELINE

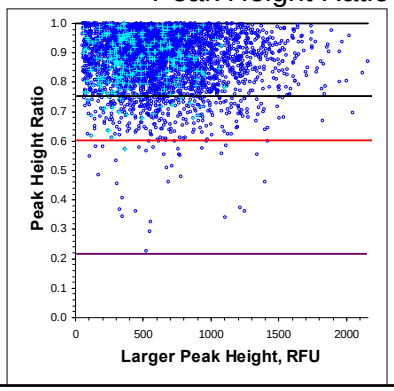
Peak Height Ratio **PHR Can Vary**

SWGDAM Interpretation Guideline 3.3.1.1

The laboratory may evaluate PHRs at various DNA template levels (e.g., dilution series of DNA). It is noted that different PHR expectations at different peak height ranges may be established.

PROTOCOLS

Peak Height Ratio



Most peaks >1500 RFU

Most peaks

All peaks

Power Plex 16 data kindly provided by NIST

>8000 alleles

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

PROTOCOLS

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.**

GUIDELINE

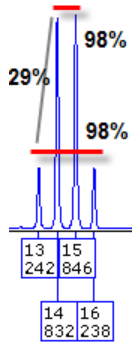
Peak Height Ratio

SWGDAM Interpretation Guideline 3.3.2

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

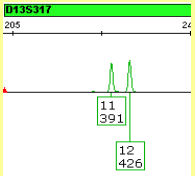
PRINCIPLES

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

Peak Height Ratio
What can be said about this profile?



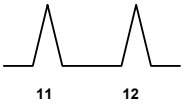
1. Mixture
2. Not a mixture
3. Not enough information
4. Not sure

PRACTICE

Peak Height Ratio
Determine if mixture

Could be:

1) Single-source profile from an individual who is heterozygous at that locus

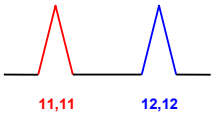


PRACTICE

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

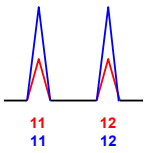


PRACTICE

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



PRACTICE

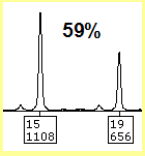
Peak Height Ratio
Determine if mixture

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

Need more information from other loci (if available)

PRACTICE

Peak Height Ratio
What can be said about this profile?



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

PRACTICE

Peak Height Ratio
Determine if mixture

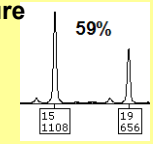
Correct answers are
#3 Not enough information
or #4 Not Sure

Need more information from other loci (if available)

PRACTICE

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**



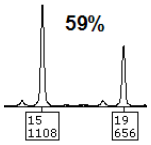
PRACTICE

Peak Height Ratio
Determine if mixture

Could be:

Single-source sample from individual who is heterozygous at that locus, but:

- **Primer mutation (uncommon)**
  - **try another kit**
- **Degraded**
- **Differential Amplification**

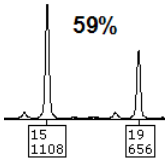


- **NOT elevated stutter (wrong position)**
- **Probably NOT LT DNA (good peak heights)**

PRACTICE

Peak Height Ratio
Determine if mixture

Could be:

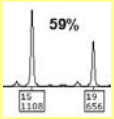


Mixture of two individuals:

- **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)**

PRACTICE

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**

PRACTICE

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

 # 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

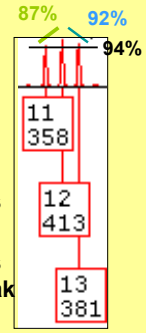
PRACTICE

Peak Height Imbalance

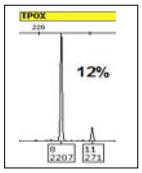
- **Stutter → (+4) X**
- **Stochastic →**
- **Preferential Amp →**
  X Degradation →
- **Mix →**
  - **Heterozyous + Homozyous**
  - **Homozyous + Homozyous**

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)