21st International Symposium on Human Identification
Mixture Interpretation Workshop:
Principles, Protocols, and Practice
October 11, 2010 – San Antonio, TX

Mixture Principles & Reporting Basics

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Outline for Mixture Principles

- **GUIDELINES**
  - SWGDAM Guidelines – All Guidelines relating to interpretation of mixed samples are relevant
- **PRINCIPLES**
  - Review of general principles for mixtures
- **PROTOCOLS**
  - Laboratory analysis protocols are developed from validation studies and principles
- **PRACTICE**
  - Later today in the final workshop section with mixtures.

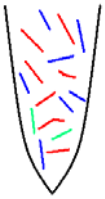
Outline for Reporting Basics

- **GUIDELINES**
  - SWGDAM Guidelines 3.4.3, 3.4.3.1, 3.5.2, 3.6, 3.6.1, 3.6.2, 3.6.2.1, 3.6.2.2, 3.6.3, 3.6.4, 3.6.5, 3.6.6
- **PRINCIPLES**
  - General principles of reporting.
- **PROTOCOLS**
  - None.
- **PRACTICE**
  - Some practice questions, and later today with mixture examples.

PRINCIPLES

The DNA mixture in a *single extracted* sample is constant

- Number of contributors is constant
- Ratio of the DNA from the 2 (or more) contributors is a constant value at all loci
- No change upon re-amplification

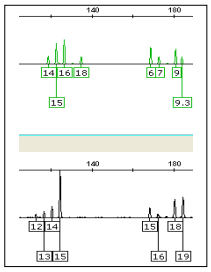


PRINCIPLES

The DNA mixture in a *single extracted* sample is constant

Variation across loci or across kits suggests other scientific or technical issues

- Degradation
- Inhibition
- Stochastic effects
- Allele shares
- Related contributors
- More than 2 contributors
- Kit differences

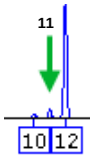


PRINCIPLES

Mixture Ratios (e.g.,1:9, 1:19)

The more disparate the amount of DNA from each contributor:

- Artifacts vs. true alleles of minor contributor?
- Pull-up more prevalent
- Number of minor contributors?
- More alleles "missing" from minor contributor(s)
  - As stutter peaks
  - Below analytical threshold
  - Shared with major contributor(s)

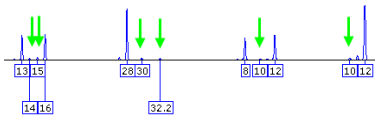


PRINCIPLES

Mixture Ratios (e.g.,1:9, 1:19)

The more disparate the amount of DNA from each contributor:

- Major vs. minor profiles more distinct
- Obligate alleles from a minor contributor distinguishable, especially if there are only two sources
- Peak Height Ratios useful

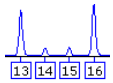


PRINCIPLES

Mixture Ratios (e.g.,1:9, 1:19)

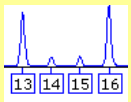
The more disparate the amount of DNA from each contributor:

- Including/excluding someone as the major (or minor) contributor becomes easier
- Restricted genotypes possible
- Single-source statistical frequencies for the major contributor and in rare situations, the minor contributor more likely



Amplifying more DNA with a 1:19 ratio…

1. May improve the minor contributor profile
2. May increase the number of artifacts
3. Unlikely to affect the major contributor profile
4. All of the above
5. None of the above
6. What does 1:19 mean?

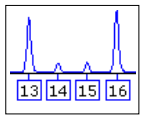


PRINCIPLES

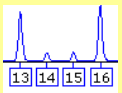
Mixture Ratios (e.g.,1:9, 1:19)

- Decreasing the amount of DNA amplified:
  - ⇄ Major Profile
  - ↓ Minor Profile
- Increasing the amount of DNA amplified:
  - ↑ ? Minor Profile, but ↑ artifacts
  - ⇄ Major Profile

⇄ = little or no significant change



To improve the profile from a minor contributor, you could…



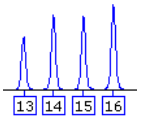
1. Amplify 10 times more DNA
2. Extract the remaining ½ of the sample
3. Inject product for 50 seconds
4. Go below the analytic threshold by 20 RFU
5. All of the above
6. None of the above
7. Who cares about the minor contributor?

PRINCIPLES

Mixture Ratios (e.g.,1:1, 1:2)

The more similar the amount of DNA from each contributor:

- All alleles more likely observed
- Distinguishing true alleles vs. artifacts easier as the amount of DNA amplified and injected approaches optimal ranges
- Minimal number of contributors more likely correct

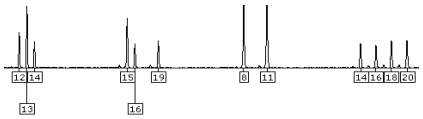


PRINCIPLES

Mixture Ratios (e.g.,1:1, 1:2)

The more similar the amount of DNA from each contributor:

- Distinguishing the profiles of a major vs. minor contributor(s) becomes more unlikely
- Peak Height Ratios not as helpful
- Unrestricted and restricted genotypes



PRINCIPLES

Mixture Ratios (e.g.,1:1, 1:2)

The more similar the amount of DNA from each contributor:

- Including/excluding someone as the major or minor contributor becomes unlikely
- Including someone as a possible contributor becomes more likely, especially as number of alleles increases
- Single-source statistical frequencies rare

PRINCIPLES

Mixture Ratios (e.g.,1:1, 1:2)

The more similar the amount of DNA from each contributor:

- Excluding someone as a possible contributor becomes more unlikely
- Risk of including an individual who is NOT a true contributor to the DNA mixture increases as the number of contributors and alleles increase

PRINCIPLES

Mixture Ratios (e.g.,1:1, 1:2)

The more similar the amount of DNA from each contributor:

↓ Amount of DNA, ↑ Inconclusive due to stochastic issues

PRINCIPLES

Principles of Mixtures
Mixture Ratios (e.g., 1:1, 1:2)

- Decreasing the amount of DNA amplified:
  - May lose some alleles
- Increasing the amount of DNA amplified:
  - May gain some alleles

If the alleles from a minor contributor are of interest, you could try to get a better profile by…

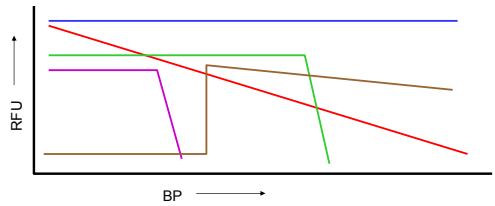
1. Extract another cutting from a different area of the sample
2. Extract another sample from the case
3. Try Y STRs if the minor contributor is male
4. All of the above
5. None of the above
6. Who cares about the minor contributor?

PRINCIPLES

Allele Detection and Ratios

Degradation and/or inhibition of amplification

- **Do NOT necessarily occur evenly across a locus and/or the profile**

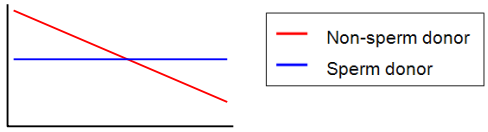


PRINCIPLES

Allele Detection and Ratios

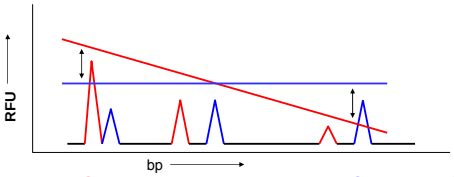
Degradation and/or inhibition of amplification

- **Do NOT need to occur equally for the DNA from each of the contributors in a mixture (e.g., sample with sperm where non-sperm DNA degrades more/drops off faster than DNA from sperm)**



PRINCIPLES

Allele Detection and Ratios

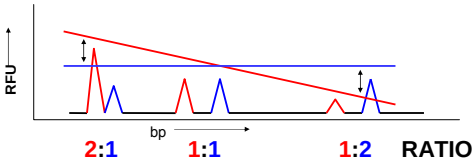


2:1 1:1 1:2 RATIO

Ratio of two DNAs *seem to* vary across the profile

PRINCIPLES New Slide

Allele Detection and Ratios



2:1 1:1 1:2 RATIO

Risk of associating wrong alleles in a genotype when considering restricted genotypes

Risk of associating wrong genotypes in composite profile for major/minor contributors

PRINCIPLES

Considerations

When interpreting data from possible or obvious mixed DNA profiles, consider the following:

- **triallelic pattern/trisomy**
- **elevated stutter**
- **primer mutations**

But remember, they are:

- **Rare, except under certain circumstances**
- **Only at one locus in a profile**

PRINCIPLES

Principles of Mixtures

More than 2 alleles at 2 or more loci is most likely due to a mixture of DNA from at least 2 individuals.

The presence of peak height imbalance at 2 or more loci may or may not be due to the presence of a mixture, particularly if the peak heights of the alleles are near or below the stochastic threshold.

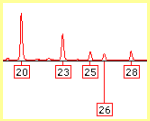
PRINCIPLES

Data Analysis Summary

- Alleles to interpret (Analytical threshold, stutter % cut off)
- Loci that can be interpreted vs. inconclusive (Stochastic threshold)
- Mixed DNA sample?
- Degradation/inhibition likely? (PHR)
- # of minimum contributors
- Major/minor contributor? (PHR)
- Restricted Genotypes

When deciding which alleles to report and interpret from a mixture of unknown origin, it is a very good idea to frequently refer to the known standards for comparison.
This statement is…

1. **Absolutely True**
2. **Absolutely False**



GUIDELINE

Reporting Guidelines
Results First

SWGDAM Interpretation Guideline 3.6.1:

The laboratory MUST establish guidelines to ensure that, to the extent possible, DNA typing results from evidentiary samples are interpreted BEFORE comparison with any known samples, other than those of assumed contributors.

GUIDELINE

Reporting Guidelines
Conclusions

SWGDAM Interpretation Guideline 3.6:

The following determinations can be made upon comparison of evidentiary and known DNA typing results (and between evidentiary samples):

- **The known individual cannot be excluded (i.e., is included) as a possible contributor to the DNA obtained from an evidentiary item.**

GUIDELINE

Reporting Guidelines
Conclusions

SWGDAM Interpretation Guideline 3.6 (cont.):

- **The known individual is excluded as a possible contributor.**
- **The DNA typing results are inconclusive/ uninterpretable.**
- **The DNA typing results from multiple evidentiary items are consistent or inconsistent with originating from a common source(s).**

GUIDELINE

Reporting Guidelines
Conclusions

SWGDAM Interpretation Guideline 3.6.3:

The laboratory MUST establish guidelines for inclusionary, exclusionary and inconclusive/uninterpretable conclusions based on comparisons of DNA typing results from known samples and both single-source and mixed evidentiary samples.

The term "included as a contributor" means….

1. The included person is the source of the DNA
2. The same thing as "cannot be excluded as a contributor"
3. The person is not a source of the DNA
4. The DNA is contaminated

PRINCIPLE

Inclusion vs. Exclusion

- Included as a source
- Included as a possible source
- Cannot be excluded as a (possible) source

These all mean the same thing!

Only EXCLUDED means excluded

Precision in wording is very important!

GUIDELINE

Reporting Guidelines
Partial Profiles

SWGDAM Interpretation Guideline 3.6.2:

DNA typing results may not be obtained at all loci for a given evidentiary sample (e.g., due to DNA degradation, inhibition of amplification and/or low-template quantity); a partial profile thus results.

GUIDELINE

Reporting Guidelines
Partial Profiles

SWGDAM Interpretation Guideline 3.6.2.2:

The laboratory should establish guidelines for inclusions and exclusions when a known individual's DNA profile is not fully observed in the evidentiary profile.

GUIDELINE

Reporting Guidelines
Partial Profiles

SWGDAM Interpretation Guideline 3.6.2.1:

For partial profiles, the determination of which alleles/loci are suitable for comparison and statistical analysis should be made prior to comparison to the known profiles.

PRINCIPLE

Reporting Guidelines
Partial Profiles

Analyze with same principles as full profiles, but with awareness of possible:

- ↑ Stochastic effects
- ↑ Degradation issues (e.g., missing alleles, loci)
- ↑ Imprecision for # of contributors
- ↑ Inconclusive loci
- ↓ Ability to exclude falsely-accused individual

GUIDELINE

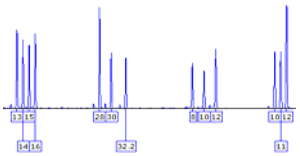
Reporting Guidelines
Mixture Inclusions

SWGDAM Interpretation Guideline 3.6.4:

For mixtures for which two or more individuals cannot be excluded as potential contributors, the laboratory may establish guidelines for assessing whether all of the DNA typing results obtained from the mixed sample are accounted for by the multiple known samples.

PRINCIPLE

Reporting Guidelines
Two Inclusions



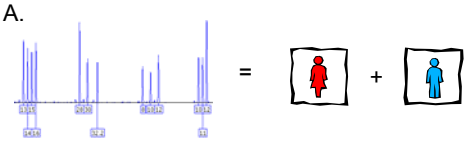
Person 1  **Included**

Person 2  **Included**

PRINCIPLE

Reporting Guidelines
Two Inclusions – Two Possibilities

A.

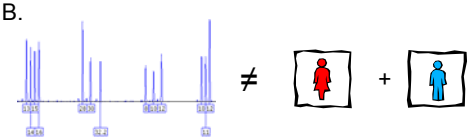


The results are consistent with a mixture of DNA from the two individuals.

PRINCIPLE

Reporting Guidelines
Two Inclusions – Two Possibilities

B.

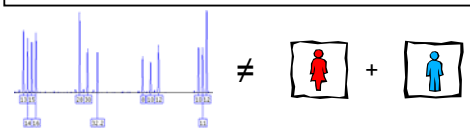


The results are NOT consistent with both of the individuals being contributors together in the sample.

IMPORTANT TO REPORT BECAUSE…

PRINCIPLE

Reporting Guidelines
Two Inclusions – ~~Two~~ Four Possibilities



Could be:

1)  +  OR

2)  +  OR

3)  + 

If there are alleles in the profile that cannot be from any of the known references tested, they should …

1. **Not be reported - unimportant**
2. **Be reported – might be important**
3. **Can wait and just be told to the jury in court**
4. **Should not be revealed to opposing expert**
5. **Be ignored – probably from the husband**
6. **Be ignored – probably from the analyst**

PRINCIPLE

Reporting Unaccounted for Alleles

Report:

Results from an additional unknown individual are present in the mixture of DNA.

Can state if major or minor contributor, if appropriate.

Can state if male or female contributor, if appropriate.

PRINCIPLE

Reporting Guidelines
Inconclusive/No comparison

SWGDAM Interpretation Guideline 3.6.6:

The laboratory should establish guidelines for identifying DNA typing results for which comparisons of evidentiary and known samples are not made (at a minimum, to include inconclusive/uninterpretable results).

PRINCIPLE

Inconclusive Results/Conclusions

Inconclusive = data are not suitable for reporting "inclusion" or "exclusion"

↑ As # of contributors ↑ & as peak heights ↓

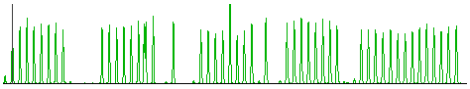
Helpful to state in the report why the data are INCONCLUSIVE

PRINCIPLES

Multiple Contributors

When there are multiple contributors to a mixture:

- **HIGH number of alleles**
- **"inclusion" is meaningless – everyone is included**
- **Inconclusive due to too much data**



PRINCIPLES

Inconclusive Reports

Report states:

Due to the high number of DNA contributors, no conclusion can be made regarding this sample.

Due to an insufficient amount of DNA, no conclusions can be made regarding this sample.

GUIDELINE

Reporting Guidelines
Assumptions/Documentation

SWGDAM Interpretation Guideline 3.5.2:

The laboratory should define and document what, if any, assumptions are used in a particular mixture deconvolution.

GUIDELINE

Reporting Guidelines
Assumptions/Documentation

SWGDAM Interpretation Guideline 3.6.5:

Because assumptions regarding the origin of evidence or the number of contributors to a mixture can impact comparisons, the laboratory should establish guidelines for documenting any assumptions that are made when formulating conclusions.

PRINCIPLE

Reporting Assumptions

All assumptions used MUST be stated

- **Number of contributors**
- **Assumed contributor used to deduce obligate alleles for another contributor**
- **Evidence assumptions (e.g., worn by or obtained from the body of)**
- **"If" – Any other statement preceded by "If" used in reaching the conclusion**

All assumptions used to form a conclusion should be documented in…

1. **The case file**
2. **The report**
3. **Both the case file and the report**
4. **Your desk on a paper towel note**
5. **The QC manager's office**
6. **Pencil on the evidence envelop**

PRINCIPLE

Reporting Multiple Conclusions

Different conclusions may result from using different assumptions.

If 2 contributors:  **EXCLUDED**

BUT

If 3 contributors:  **INCLUDED**
INCONCLUSIVE

REPORT ALL CONCLUSIONS!

PRINCIPLE

Thorough documentation

Important for:

- **Testing at a later date – by you or someone else**
- **Court testimony – you or someone else**
- **Discovery**

GUIDELINE

Reporting Guidelines
Multiple Samples/Amps/Profiles

SWGDAM Interpretation Guideline 3.4.3

Where multiple amplifications and/or injections are generated for a given sample extract, the laboratory should establish guidelines for determining which results are used for comparisons and statistical calculations.

GUIDELINE

Reporting Guidelines
Composite Profiles

SWGDAM Interpretation Guideline 3.4.3.1: (highlights)

1. **Establish guidelines for generation of composite results**
2. **Separate extracts pooled BEFORE PCR, is NOT a composite profile**
3. **Data from separate extracts/different locations should NOT be combined for interpretation**

PRINCIPLE

Reporting Multiple Cuttings/Extracts

Different extractions or cuttings of a sample

- **May not contain the "same" DNA**
  - **Different # of contributors**
  - **Different mixture ratios**
  - **Different contributors**
- **May need to treat each sample cutting/extraction separately in the report**
- **Use caution with samples that might not be the same (e.g., vaginal swabs)**

One DNA extract is amplified several times with the same kit. Is it OK to combine the results for interpretation?

1. **Yes, generally**
2. **Never**
3. **With permission from Technical Leader**
4. **Yes, if the Magic 8 Ball says so**
5. **Only on Fridays**

GUIDELINES

Reporting Guidelines
Old Cases

Guidelines not intended to be applied retroactively, however, GOOD PRACTICE to always review data, report and conclusions prior to:

- **Additional testing**
- **Re-testing**
- **Discussing case with attorney or law enforcement**
- **Court testimony**
- **Providing discovery**