**DNA Mixture Interpretation**:
Principles and Practice in Component Deconvolution and Statistical Analysis

# Real Case Example –
## Importance of Properly Stating Your Conclusions

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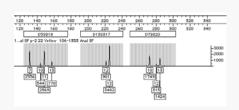- **Case Example (adjudicated 2006 case)**
  - Sexual assault case
  - Semen on anal swab
  - Differential extraction with clear sperm fraction separation
  - DNA profile c/w two semen contributors

- **Importance of Conclusions**
  - Inclusions (frequency calculation provided)
  - Exclusion
  - Inconclusive

- **Complex Case Experiment**
  - Transition from major profile to inclusion to inconclusive

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**CASE EXAMPLE – Sperm Fraction; Profiler Plus**

**Prepare a mixture analysis worksheet**

Mixture Analysis Worksheet – Profiler Plus

## Mixture Calculations

- Can do peak height ratio (PHR) calculations to determine which allele pair combinations meet expectations.

- The expectations for allele pairs are based on parameters determined in a lab's validation studies. For example we expect allele pairs to be within a range of equal to 70%.

## Profiler Plus Calculations

D3 – 4 peaks  14, 16, 17, 19

- 16,17 PHR @  91% - meets the expectation of allele pairing

- 17,19  PHR @  40% - does not meet the expectation of allele pairing

## Mixture Calculations (cont'd)

- Expectations for shared peaks are based on the difference between contributor proportions.

- Estimates of contributor proportions are based on information considered from non-shared alleles at the same locus and at other loci across the entire profile.

## Profiler Plus Calculations

D21 – 3 peaks (one shared) 29, 30, 31.2

- 30,30 is not probable as the peak height is too low ( 9% of the smaller of the two stronger peaks and the overall minor contributor proportion is about 30% or just under 33% which corresponds to a ratio of 1 part to 3 parts)
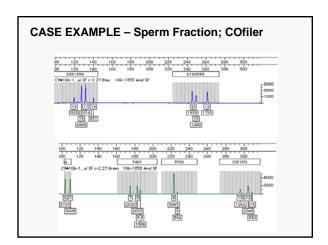
D21 – 3 peaks (one shared) 29, 30, 31.2 (continued)

- Unshared 29,31.2 is possible with a peak height ratio of 82% **however** if allele 29 is shared and the expected shared portion (30 @ 455 rfu) is subtracted away the PHR works out to 98%**.**

- The virtual PHR percentage of 98% for the major in combination with the virtual peak height ratio of 100% for the minor component supports a **29,31.2 major and a 29,30 minor**.

## Profiler Plus Calculations

FGA – 3 peaks (none shared) 20, 21, 22

- 22,22 is probable as the peak height is within minor contributor proportion expectations (33% of the smaller of the two stronger peaks and the overall minor contributor proportion is about 30%).

- 20,21 is probable as the PHR percentage of 71% is within expectations.

**CASE EXAMPLE – Sperm Fraction; COfiler**





**Add to mixture analysis worksheet**

**Mixture Analysis Worksheet – COfiler**

Add in reference sample genotypes last

- **Mixture Analysis Worksheet – Profiler Plus +**

- **Mixture Analysis Worksheet – COfiler +**

A distinct quantitative difference allowing unambiguous deduction of major contributor.

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**Conclusions**

- The DNA typing profile obtained from the anal swab (item#) is mixed in origin consistent with originating from three individuals.

  – a. The male component is mixed in origin consistent with originating from two individuals. The DNA profile of the major component matches that of the reference sample from suspect (item #). The estimated probability of selecting an unrelated individual at random from the U.S. population with a matching profile is 1 in 620 quadrillion (note; most conservative reported out).

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Source attribution example

- Specimen No. _____ reacts as a mixture. Within a reasonable degree of scientific certainty, _____ is identified as the source of the major/minor DNA profile obtained.

## Conclusions (cont'd)

– b. "Victim's boyfriend name" is included as a possible contributor to the male component. Based on the U.S. population, it is estimated that 1 in 8.9 million individuals is a potential contributor to this profile. (note: most conservation estimate reported for IP)

– c. The female component matches the DNA profile from the victim. (Does a calculation need to be reported from victim on an intimate swab? Do you need to compare to the victim's reference?)

## Alternative IP example

• Based on the loci which include all of the alleles from <SUSPECT>, the number of people who cannot be excluded as having contributed to this mixture is approximately:

– 1 in __ of the African American population
– 1 in __ of the Caucasian population
– 1 in __ of the Hispanic population

**Useful wording for the reporting of an incomplete profile.**

## Exclusion or Inconclusive

• Due to the nature of the DNA profile obtained from Specimen No. ___, it can be used only for exclusionary purposes.

For Example:
• Mr "A" can be excluded.
• Inconclusive for Mr "B". (he could not be excluded and no stat given so inconclusive)

## Complex Mixtures

- Robbery case (DNA profile matching victim's blood on stolen money)
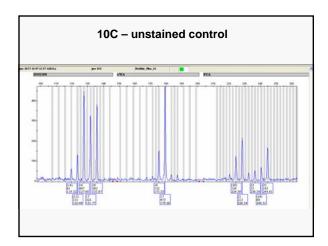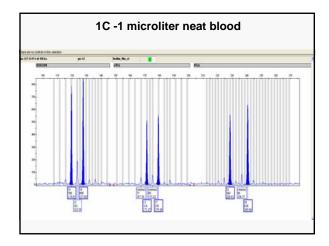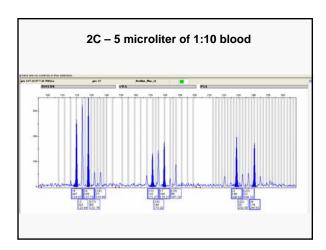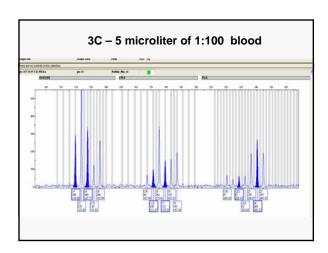


(Court pending)

## Blood on money experiment

- Dilutions of blood on circulated dollar bill
- Swabbing versus cutting
- Transition from major profile to inconclusive on complex background to blend in with background



**10C – unstained control**

**1C -1 microliter neat blood**



**2C – 5 microliter of 1:10 blood**



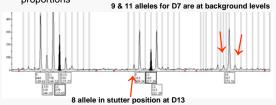**3C – 5 microliter of 1:100  blood**

## Summary: For all mixture cases

- Prepare a mixture analysis worksheet in table format for all loci and include:
  - the alleles above threshold (quantitative/match/true allele),
  - peaks above detection threshold,
  - the corresponding peak heights and
  - add the reference genotypes after determining the major and minor contributions
- Have wording example guidelines for conclusions to report matches, inclusions and their corresponding statistics.
- Peer technical review is important in process

## Summary: For difficult cases

- base exclusions on what should be present from the reference genotype considering the observed contributor proportions

**9 & 11 alleles for D7 are at background levels**



**8 allele in stutter position at D13**

**Reference Genotype  D5 – 12,12; D13 – 8,11; D7 – 9,11**

**Exclude as a substantial contributor.**

**(Trace profile present but not suitable for comparison)**

## Difficult cases continued

- base inclusions on what is substantially present in the profile.



**Reference Genotype  D8 – 12,14; D21 – 28,29; D18 – 15,16**

**Reference genotype alleles at reportable levels in evidence profile.**

## Difficult cases continued

- use inconclusive statements whenever the data does not meet reporting expectations.



**Reference Genotype D8 – 12,14; D21 – 28,29; D18 – 15,16**

**Comparison of reference to DNA profile is inconclusive (too weak – components at LCN levels).**



**Thank you for your attention**

**Questions?**

# Mixture Analysis Worksheet – Profiler Plus

| Locus | | | Allele Call | Base Pair | Peak Height | <150 rfu | Calculations | |
|---|---|---|---|---|---|---|---|---|
| D3 | | | 14<br>16<br>17<br>19 | | 1114<br>3054<br>3329<br>1240 | - | **14,19 / 16,17**<br>3054/3329 = 91%<br>1114/1240 = 89%<br>1240/3054 = 40% | |
| vWA | | | 16<br>17<br>18 | | 827<br>3105<br>2767 | - | **16,17 / 17,18**<br>3105-827 = 2278<br>2278/2767 = 82%<br>827/2767 = 36% | **16,16 / 17,18**<br>2767/3105 = 89%<br>827/2 = 413.5<br>413.5/2767 = 14% |
| FGA | | | 20<br>21<br>22 | | 2681<br>1911<br>1290 | - | **22,22 / 20,21**<br>1911/2681 = 71%<br>1290/2 = 645<br>645/1911 = 33% | **20,22 / 20,21**<br>2681-1290 = 1391<br>1391/1911 = 72%<br>1290/1911 = 67% |
| Amelo. | | | X<br>Y | | 4770<br>4254 | - | **X,Y**<br>4254/4770 = 89% | **X,X / X,Y**<br>4770-4254 = 516<br>516/2 = 258<br>258/4254 = 6% |
| D8 | | | 10<br>11<br>13<br>14 | | 2798<br>628<br>769<br>2979 | - | **11,13 / 10,14**<br>628/769 = 81%<br>2798/2979 = 93%<br>769/2798 = 27% | |
| D21 | | | 29<br>30<br>31.2 | | 2800<br>455<br>2321 | - | **29,30 / 29,31.2**<br>2800 - 455 = 2345<br>2321/2345 = 98%<br>455/2321 = 19% | **30,30 / 29,31.2**<br>2321/2800 = 82%<br>455/2 = 227.5<br>227.5/2321 = 9% |
| D18 | | | 12<br>15<br>16<br>22 | | 617<br>619<br>2199<br>1880 | - | **12,15 /16,22**<br>617/619 = 99%<br>1880/2199 = 85%<br>619/1880= 32% | |
| D5 | | | 7<br>10<br>11<br>13 | | 2556<br>544<br>2569<br>770 | - | **10,13 / 7,11**<br>544/770 = 76%<br>or 2556/2569= 99%<br>770/2556 = 30% | |
| D13 | | | 12<br>13 | | 901<br>3482 | - | **12,12 / 13,13**<br>901/3482 = 25% | **12,13 / 13,13**<br>3482-901 = 2581<br>2581/2 = 1290.5<br>901/1290.5 = 69% |
| D7 | | | 10<br>12<br>13 | | 1749<br>315<br>1424 | - | **10,12 / 10,13**<br>1749-315 = 1434<br>1424/1434 = 99%<br>315/1424 = 22% | **12,12 / 10,13**<br>1424/1749 = 81%<br>315/2 = 157.5<br>157.5/1424 = 11% |

Comments:

blue = putative major alleles
black = minor alleles

Revised XXX

Reviewed by _____

## Mixture Analysis Worksheet – Profiler Plus +

| Locus | Friend | Accused | Allele Call | Base Pair | Peak Height | <150 rfu | Calculations | |
|-------|--------|---------|-------------|-----------|-------------|----------|--------------|---|
| D3 | 14<br><br><br>19 | 16<br>17 | 14<br>16<br>17<br>19 | | 1114<br>3054<br>3329<br>1240 | - | **14,19 / 16,17**<br>3054/3329 = 91%<br>1114/1240 = 89%<br>1240/3054 = 40% | |
| vWA | 16<br>17 | 17<br>18 | 16<br>17<br>18 | | 827<br>3105<br>2767 | - | **16,17 / 17,18**<br>3105-827 = 2278<br>2278/2767 = 82%<br>827/2767 = 36% | **16,16 / 17,18**<br>2767/3105 = 89%<br>827/2 = 413.5<br>413.5/2767 = 14% |
| FGA | <br><br>22 | 20<br>21 | 20<br>21<br>22 | | 2681<br>1911<br>1290 | - | **22,22 / 20,21**<br>1911/2681 = 71%<br>1290/2 = 645<br>645/1911 = 33% | **20,22 / 20,21**<br>2681-1290 = 1391<br>1391/1911 = 72%<br>1290/1911 = 67% |
| Amelo. | X<br>Y | X<br>Y | X<br>Y | | 4770<br>4254 | - | **X,Y**<br>4254/4770 = 89% | **X,X / X,Y**<br>4770-4254 = 516<br>516/2 = 258<br>258/4254 = 6% |
| D8 | 11<br>13 | 10<br><br><br>14 | 10<br>11<br>13<br>14 | | 2798<br>628<br>769<br>2979 | - | **11,13 / 10,14**<br>628/769 = 81%<br>2798/2979 = 93%<br>769/2798 = 27% | |
| D21 | 29<br>30 | 29<br><br>31.2 | 29<br>30<br>31.2 | | 2800<br>455<br>2321 | - | **29,30 / 29,31.2**<br>2800 - 455 = 2345<br>2321/2345 = 98%<br>455/2321 = 19% | **30,30 / 29,31.2**<br>2321/2800 = 82%<br>455/2 = 227.5<br>227.5/2321 = 9% |
| D18 | 12<br>15 | 16<br>22 | 12<br>15<br>16<br>22 | | 617<br>619<br>2199<br>1880 | - | **12,15 /16,22**<br>617/619 = 99%<br>1880/2199 = 85%<br>619/1880= 32% | |
| D5 | 10<br><br>13 | 7<br><br>11 | 7<br>10<br>11<br>13 | | 2556<br>544<br>2569<br>770 | - | **10,13 / 7,11**<br>544/770 = 76%<br>or 2556/2569= 99%<br>770/2556 = 30% | |
| D13 | 12 | 13 | 12<br>13 | | 901<br>3482 | - | **12,12 / 13,13**<br>901/3482 = 25% | **12,13 / 13,13**<br>3482-901 = 2581<br>2581/2 = 1290.5<br>901/1290.5 = 69% |
| D7 | 10<br>12 | 10<br><br>13 | 10<br>12<br>13 | | 1749<br>315<br>1424 | - | **10,12 / 10,13**<br>1749-315 = 1434<br>1424/1434 = 99%<br>315/1424 = 22% | **12,12 / 10,13**<br>1424/1749 = 81%<br>315/2 = 157.5<br>157.5/1424 = 11% |

Comments:

blue = putative major alleles
black = minor alleles

Revised XXX                                                          Reviewed by _____

## Mixture Analysis Worksheet – COfiler

| Locus | | | Allele Call | Base Pair | Peak Height | <150 rfu | Calculations |
|---|---|---|---|---|---|---|---|
| D3 | | | 14<br>16<br>17<br>19 | | 923<br>2805<br>3016<br>831 | - | **14,19 / 16,17**<br>2805/3016 = 93%<br>831/923 = 90%<br>923/2805 = 32% |
| D16 | | | 9<br>10<br>13 | | 1939<br>1280<br>1793 | - | **10,10 /9,13**  **9,9 /10,13**  **13,13 /9,10**<br>1793/1939=92% 1280/1793=71% 1280/1939=66%<br>1280/2= 640    1939/2= 969.5    1793/2=896.5<br>640/1793= 35%  969.5/1280=75%  896.5/1280=70% |
| Amelo. | | | X<br>Y | | 3729<br>3605 | - | **X,Y**<br>3605/3729 = 96% |
| TH01 | | | 7<br>9<br>9.3 | | 2020<br>2023<br>1006 | - | **9.3,9.3 / 7,9**<br>2020/2023 = 99%<br>1006/2 = 503<br>503/2020 = 24% |
| TPOX | | | 8<br>9 | | 5065<br>542 | - | **9,9 / 8,8**      **8,9 / 8,8**<br>542/5065 = 10%    5065 – 542 = 4523<br>                 4532/2 = 2261.5<br>                 542/2261.5 = 23% |
| CSF | | | 10<br>12<br>13 | | 1262<br>2048<br>332 | - | **12,13 / 10,12**      **13,13 / 10,12**<br>2048 - 322 = 1716    1262/2048 = 61%<br>1262/1716 = 73%       332/2 = 162<br>332/1262 = 26%        166/1262 = 13% |
| D7 | | | 10<br>12<br>13 | | 1500<br>383<br>1330 | - | **10,12 / 10,13**      **12,12 / 10,13**<br>1500-383 = 1117    1330/1500 = 88%<br>1117/1330 = 83%     383/2 = 191.5<br>383/1117 = 34%      191.5/1330 = 14% |

Comments:

blue = putative major alleles
black = minor alleles

## Mixture Analysis Worksheet – COfiler +

| Locus | Boyfriend | Susp | Allele Call | Base Pair | Peak Height | <150 rfu | Calculations |
|-------|-----------|------|-------------|-----------|-------------|----------|--------------|
| D3 | 14<br><br><br>19 | 16<br>17 | 14<br>16<br>17<br>19 | | 923<br>2805<br>3016<br>831 | - | **14,19 / 16,17**<br>2805/3016 = 93%<br>831/923 = 90%<br>923/2805 = 32% |
| D16 | 10 | 9<br><br>13 | 9<br>10<br>13 | | 1939<br>1280<br>1793 | - | **10,10 /9,13   9,9 /10,13   13,13 /9,10**<br>1793/1939=92% 1280/1793=71% 1280/1939=66%<br>1280/2= 640   1939/2= 969.5   1793/2=896.5<br>640/1793= 35% 969.5/1280=75% 896.5/1280=70% |
| Amelo. | X<br>Y | X<br>Y | X<br>Y | | 3729<br>3605 | - | **X,Y**<br>3605/3729 = 96% |
| TH01 | <br><br>9.3 | 7<br>9 | 7<br>9<br>9.3 | | 2020<br>2023<br>1006 | - | **9.3,9.3 / 7,9**<br>2020/2023 = 99%<br>1006/2 = 503<br>503/2020 = 24% |
| TPOX | 9 | 8 | 8<br>9 | | 5065<br>542 | - | **9,9 / 8,8**        **8,9 / 8,8**<br>542/5065 = 10%    5065 – 542 = 4523<br>                          4532/2 = 2261.5<br>                          542/2261.5 = 23% |
| CSF | <br><br>13 | 10<br>12 | 10<br>12<br>13 | | 1262<br>2048<br>332 | - | **12,13 / 10,12        13,13 / 10,12**<br>2048 - 322 = 1716    1262/2048 = 61%<br>1262/1716 = 73%       332/2 = 162<br>332/1262 = 26%        166/1262 = 13% |
| D7 | 10<br>12 | 10<br><br>13 | 10<br>12<br>13 | | 1500<br>383<br>1330 | - | **10,12 / 10,13        12,12 / 10,13**<br>1500-383 = 1117     1330/1500 = 88%<br>1117/1330 = 83%      383/2 = 191.5<br>383/1117 = 34%       191.5/1330 = 14% |

Comments:

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