DNA: Steps for Profile Interpretation- Step 5

Step five   *Depending on the mixture type, identify genotypes that must be present in the profile of the true contributor, if possible.*

Interpretation results   The results of the interpretation must be documented in the case file and in the report.

The following sections outline the interpretation steps for the four mixture types.

Terminology   The following terminology will be used for the interpretation of profiles and in the report:

Terminology	Explanation	Example	Included genotypes
$allele_1$, $allele_2$	Heterozygote genotype	9, 10	9, 10
$allele_1$, $allele_1$	Homozygote genotype	11, 11	11, 11
$allele_1+$	Obligate allele paired with anything	10+	10, anything
$allele_1+$, $allele_2$,…	Obligate allele paired with any of the following alleles	9+, 8, 9, 10	8, 9 9, 9 9, 10
$allele_1 \sim$	A peak in the stutter position which cannot be distinguished as an allele or stutter	10+, 11~, 12	10, 11 10, 12
[$allele_1$, $allele_2$,…]	All possible genotype combinations using the listed alleles	[10, 11]	10, 10 11, 11 10, 11

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Terminology
(continued)

{allele$_1$, allele$_2$,…}	All possible heterozygous genotype combinations using the listed alleles	{8, 9, 10}	8, 9 8, 10 9, 10
inc	Not suitable for comparison with reference profiles	inc	-
-	No signal above 50 RFU	-	-
If the terminology above does not adequately or completely describe the included genotypes, the included genotypes can be listed, separated by slashes.		10, 12/ 10, 13/ 12, 13	10, 12 10, 13 12, 13
		11, 12/ 10~, 11/ 10~, 12	11, 12 10, 11 10, 12
		8+/ 11+, 10, 11	8, anything OR 10, 11 11, 11

Type 1 mixture interpretation

A Type 1 mixture consists of DNA from two individuals and the profiles of the two individuals can be resolved into their individual components resulting in major/minor or known/unknown contributors.

The first step to interpret a Type 1 mixture is to determine the possible genotypes for each contributor. Consideration must be taken not only for genotypes which include obligate alleles and masked alleles, but also for genotypes which include potentially dropped-out alleles and alleles that may be indistinguishable from stutter (IFS).

Certain combinations of alleles create genotypes that are clearly unreasonable for the contributor in question. These genotypes may be immediately rejected by the analyst.

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Allele dropout   If an allele is detected above the stochastic threshold (225.5 RFU), the analyst may be confident that no allele dropout has occurred.

An allele that is detected below the stochastic threshold is within the stochastic range. The analyst should assume that an allele within this range may have a heterozygote partner allele that has dropped out.

EXCEPTION: If the detected alleles best explain the evidence under the assumed number of contributors, dropout does not need to be considered, even though one or more of the alleles have peak heights below the stochastic threshold.

- Example: In a 2-person mixture, the following four alleles are detected at a locus:
    - 10 allele (1000 RFU)
    - 11 allele (1000 RFU)
    - 12 allele (80 RFU)
    - 13 allele (100 RFU)
    Even though the 12 and 13 allele are below the stochastic threshold, the only explanation for a 2-person mixture is a minor contributor of 12, 13.

- Example: In a 2-person mixture, the following three alleles are detected at a locus:
    - 8 allele (120 RFU)
    - 10 allele (300 RFU)
    - 12 allele (750 RFU)
    Assume the 10, 12 genotype is attributed to a known contributor. The peak height ratio of the 10, 12 is 40%, which is below the acceptable peak height ratio threshold. This indicates that there may be another contributor sharing the 12 allele; therefore, the minor contributor may be interpreted as an 8, 12.

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Allele dropout
(continued)

Allele dropout should also be considered for the minor contributor when there are no obligate minor contributor alleles detected and, therefore, no alleles in the stochastic range.

The analyst should consider genotypes consisting of masked alleles and/or dropout alleles. Allele dropout of one or both of the minor contributor alleles may be deemed unreasonable based on donor ratio calculations. Allele dropout of the minor contributor may also be deemed unreasonable if the minor contributor's alleles are substantially represented at other loci.

An allele in a stutter position may be perceived to be above the stochastic threshold, thereby implying that no allele dropout has occurred. However, an allele in a stutter position may be higher, due to stutter contribution, than it would be otherwise. To conservatively estimate the potential contribution that a true allele may be making to a peak in the stutter position, the maximum stutter percentage of the locus is multiplied by the peak height of the parent allele to give the maximum stutter peak height. This number is then subtracted from the height of the peak in stutter position, giving a minimum peak height of the allele. The peak height generated from this calculation can then be used to evaluate possible allele dropout at the locus.

- Example: In a 2-person mixture, three alleles were detected at D8S1179 (stutter threshold: 10.32%). At first glance, all of the alleles appear to be above the stochastic threshold.
  - 10 allele (400 RFU)
  - 11 allele (3000 RFU)
  - 13 allele (3000 RFU)
    The major contributor is an 11, 13. The minor contributor has a 10 allele. To assess for dropout for the 10 allele:

  - 0.1032 * 3000 = 310 RFU (max. stutter contribution)
  - 400 – 310 = 90 RFU
    The contribution of the 10 allele could be as low as ~90 RFU; therefore, allele dropout should be considered.

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Allele dropout (continued)	Alleles near or above the stochastic threshold may also be susceptible to stochastic effects (especially samples with compromised quality or low template DNA sample). The analyst may choose to consider allele dropout for peaks above the stochastic threshold based on either of the following situations:

* if there is considerable allele dropout observed at other loci
* if the number of peaks suggest the possibility of allele sharing which may elevate the peak height above the stochastic threshold
* if there is a clearly defined peak above background, but below the detection threshold, that cannot be attributed to any artifact.

Peaks which are indistinguish-able from stutter	In mixed source samples, it is possible that an allele of the minor contributor is in a stutter position of a major contributor allele. If such an allele also happens to be of an intensity such that the peak height falls below the stutter threshold for that locus, it is possible that the true genotype of the minor contributor may be misinterpreted. A peak such as this is termed "indistinguishable from stutter". The peak could be

* stutter only
* stutter and an allele or
* an allele only.

When determining possible genotypes, peaks that are "indistinguishable from stutter" should also be included as potential alleles.

Exception: If there are two alleles which can be attributed to the minor contributor, then all other peaks in stutter positions at that locus can be designated as stutter if it is assumed that the single minor contributor has no more than two alleles.

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Profiles with limited donor ratio information

For some mixture profiles, a reliable donor ratio is difficult to obtain. This could be due to

- a low level of DNA
- contributors with a lot of allele sharing
- a profile of poor quality (degradation or inhibition)
- wide-spread donor ratios across the loci.

If a mixture such as this is determined to be resolvable at all or some loci (based on donor ratio approximations or the presence of a known contributor), the analyst may choose to simply report obligate genotypes and/or obligate alleles for each contributor, where appropriate.

- Example: In a 2-person mixture, the only locus with unshared alleles is FGA, but FGA appears to be showing signs of degradation. The donor ratio calculated does not seem to be an accurate representation of the donor ratio across the profile. Donor ratio approximations at other loci suggest the mixture is resolvable. The following alleles are detected at TPOX:
  - 6 allele (4500 RFU)
  - 9 allele (400 RFU)

The major contributor is a 6, 6. Although the 9 allele is above the stochastic threshold, the interpretation of the minor contributor may be a 9+. Alternatively, if there is no indication of allele dropout, the interpretation may include all genotypes paired with the obligate allele: 6, 9 or 9, 9.

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Determine reasonable genotypes

Using laboratory-defined thresholds, determine the genotypes that are reasonable and those that are not reasonable. Reasonable genotypes consist of one or more genotypes, one of which is present in the profile of the true contributor.

The possible genotypes identified at the beginning of the interpretation should then be examined based on the following criteria:

- donor ratio ranges
- peak height ratio thresholds
- stutter contributions.

If the genotype in question falls outside the limits of the established thresholds, the analyst may conclude that the genotype is unreasonable and remove it from the set of possible genotypes.

The criteria above are not absolute. Based on experience, the analyst may choose to retain a genotype that does not fully meet the criteria. Likewise, the analyst may choose to reject a genotype which is included only as a result of several unlikely events. These decisions must be made prior to the comparison of probative reference profiles, and they must be documented in the case file.

At times, a profile as a whole may be considered resolvable, but there may be individual loci which are irresolvable or the examination of the possible genotypes may be a complicated process. If a locus is irresolvable, the analyst may choose to do one of the following:

- mark the locus inconclusive. Consequently, the locus cannot be used for comparison with reference profiles
- include all possible genotypes for the major contributor, the minor contributor, or both.

DNA: Steps for Profile Interpretation- Step 5, Continued

Using donor ratio ranges

Each possible genotype should be tested against the appropriate donor ratio range, if applicable. When all of the alleles are above the stochastic threshold, then the 2x range should be used. If any of the alleles are below the stochastic threshold, then the 3x range should be used.

NOTE: When testing for a homozygous genotype, the RFUs of the allele should be divided by two (since there are two copies) to determine which donor ratio range to use.

If the tested genotype has a donor ratio which falls outside of the donor ratio range (and adjusting peak height ratios and stutter contributions still results in a donor ratio outside of the donor ratio range), then the analyst may eliminate it as a possible genotype.

When the genotype tested includes allele dropout (example: 10, F or F, F where F is any dropped allele), the donor ratio can be useful in determining if allele dropout is reasonable. An RFU value from 0 to 49 is used for the "dropped out" allele. If the donor ratio cannot be brought within the donor ratio range, then the tested genotype (10, F or F, F) may be considered unreasonable.

It should be noted that if the analyst determines that both alleles could have reasonably dropped out, then no other additional testing of genotypes needs to be done (since all possible genotypes would then be included). Additionally, if the analyst determines that a specific allele paired with dropout is reasonable, then no other testing needs to be done in regards to that specific allele.

- Example: 10+ includes all genotypes with at least one 10 allele.

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DNA: Steps for Profile Interpretation- Step 5, Continued

Using peak height ratio thresholds

The set of possible genotypes should also be tested to determine if they fall within acceptable peak height ratios. The following table outlines the expected peak height ratios for varying peak heights based on the laboratory's internal validation studies. It should be noted that peak height ratio thresholds are only applicable to allelic peaks that exceed the stochastic threshold.

Taller peak > 700 RFUs	Expected minimum 55% PHR
Taller peak ≤ 700 RFUs and > 400 RFUs	Expected minimum 35% PHR
Taller peak ≤ 400 RFUs and > 225 RFUs	Expected minimum 25% PHR
Taller peak ≤ 225 RFUs	No expected minimum (second allele could have dropped out)

If a tested genotype creates one or more peak height ratios which fall below the appropriate peak height ratio threshold (and adjusting stutter contributions and relative contributions of shared alleles still results in an unacceptable peak height ratio), the analyst may eliminate it from being a reasonable genotype.

- Example: In a 2-person mixture, with a major contributor and a minor contributor, the following alleles are present:
  - 12 allele (600 RFU)
  - 14 allele (550 RFU)
  - 16 allele (180 RFU)

  One of the possible genotypes for the major contributor identified in Step 4 is a 12, 12 (leaving a 14, 16 genotype for the minor contributor). The peak height ratio of the 14, 16 is 180/550 = 33%. The peak height ratio threshold (with the taller peak at 550 RFU) is 35%. Thus, the analyst may conclude that a 12, 12 genotype for the major contributor is unreasonable.

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Adjusting stutter contributions

If the genotype in question

- has thus far been excluded based on the donor ratio and/or the peak height ratio thresholds and
- contains one or more alleles in a stutter position,

then the height of the peak in stutter position should be adjusted to account for a reasonable amount of stutter contribution. The questioned genotype can then be re-tested against the donor ratio range and the peak height ratio thresholds.

- Example: In a 2-person mixture, there is a major contributor and a minor contributor. The average donor ratio is 40:1 making the 2x range 20:1 up to 80:1 and the 3x range 13.3:1 up to 120:1. The following alleles are present at D7S820:
  − 9 allele (500 RFU)
  − 10 allele (2000 RFU)
  − 12 allele (2200 RFU)
    If the tested genotype for the minor contributor is a 9, 9 (making the major contributor a 10, 12), then the donor ratio would be
  − (2000 + 2200): 500 = 8.4:1.

    And the peak height ratio for the 10, 12 would be

  − 2000 / 2200 = 91%.

    While the peak height ratio is acceptable, a donor ratio of 8.4:1 is out of the 2x range. However, the 9 allele may be inflated by stutter. The maximum stutter contribution is calculated as follows:

  − 2000 * 0.0969 (max stutter at D7S820) = 194 RFU

    After removing the maximum stutter contributions from the 9 allele, the donor ratio is

  − (2000 + 2200): 306 = 13.7 : 1

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Adjusting stutter contributions (continued)	The donor ratio is still outside the 2x range; however, since the questioned genotype is a homozygote, the height of the allele must be divided by two: − 306/2 = 153 RFU Since 153 RFU is below the stochastic threshold, a 3x donor ratio range should be applied. The calculated donor ratio does fall within the 3x range; therefore, the 9, 9 genotype may be included as a possible genotype of the minor contributor.

Type 2 mixture interpretation	A Type 2 mixture consists of DNA from two individuals, and the profiles of the two individuals *cannot* be resolved into their individual components. This type of mixture is called an irresolvable mixture, but does not imply that the profile is uninterpretable.
	
	Since this interpretation uses an unrestricted approach, peak height information and donor ratios are not used to identify specific genotypes of the two contributors. The only time that peak heights and donor ratios are used is to assess each locus for allele dropout, to consider a peak that is IFS, or to assess the Amelogenin locus. The interpretation of a Type 2 mixture results in one list of genotypes. Included in the list are the genotypes of both of the two true contributors.
	
	• Example: If three alleles are detected at TPOX (8, 9 and 10), the interpretation may lead to the following list of genotypes:
	  − 8,8
	  − 8,9
	  − 9,9
	  − 10+
	
	According to this locus, anyone that has one of these genotypes would be included as a possible contributor to the mixture.

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Type 2 mixture interpretation (continued)

For mixture profiles in which a reliable donor ratio is difficult to obtain, the analyst should be cautious when using donor ratios to assess for allele dropout and IFS peaks. The analyst may choose to mark individual loci or the entire profile inconclusive.

Each locus in a Type 2 mixture should have between one and four alleles (unless no DNA was detected at the locus).

At each locus, the genotypes of the two true contributors will be represented by one or more of the following scenarios:

- all alleles from both contributors are detected
- a portion of the alleles from the contributors are detected and a portion of the alleles have dropped out
- a portion of the alleles from the contributors are detected and a portion of the alleles are IFS.

The Type 2 mixture approach involves the inclusion of all genotypes using the detected alleles along with consideration of allele dropout and IFS peaks.

Depending on the number of alleles detected at a locus, the following is a list of interpretation steps.

Locus with four alleles

Under the assumption of two contributors, a four-allele locus ensures that all alleles are detected. The interpretation will include all heterozygote genotypes consisting of the detected alleles (regardless of the peak heights of the alleles). A peak in the stutter position can be distinguished as stutter since it is not expected that the contributors will have more than two alleles each.

Observed alleles	Interpretation	Included genotypes	
A, B, C, D	{A, B, C, D}	A, B A, C A, D	B, C B, D C, D

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Locus with three alleles

By looking at the peak height of each allele, determine which peaks (if any) could have a sister allele dropping out. Use the following chart to help with the interpretation.

Observed alleles		Interpretation	Included genotypes
Alleles greater than the stochastic threshold	**Alleles less than the stochastic threshold**		
A, B, C	none	[A, B, C]	A, A B, C A, B B, B A, C C, C
A, B	C	[A, B]/C+	A, A A, B B, B C, anything
A	B, C	A, A/B+/C+	A, A B, anything C, anything
none	A, B, C	A+/B+/C+	A, anything B, anything C, anything

The peak height of an allele in a stutter position may be overestimated due to the contribution of stutter. Potential stutter contribution must be considered when determining if an allele may have a sister allele dropping out.

If there is a peak in a stutter position (that is being filtered as stutter) and its peak height is consistent with other allelic peaks in the mixture, the analyst may either mark the locus inconclusive or include the IFS peak in the interpretation. For more information and examples about IFS peaks, see the section following Type 2 mixture interpretation.

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Locus with two alleles

The first step in interpreting a two-allele locus is to determine if one of the contributors may have both alleles dropping out. If so, then the locus is inconclusive.

- Example: In a Type 2 mixture with an average donor ratio of 3:1, the following two alleles are detected at D3S1358:
  - 14 allele (340 RFU)
  - 16 allele (280 RFU)

    To determine if one of the contributors could be completely dropping out of the mixture, calculate the donor ratio.
  - (340 + 280) : (49 + 49)
  - 6.3 : 1

    This donor ratio should be compared against the 3x range (1:1 to 9:1) since one or more of the tested alleles are below the stochastic threshold. A donor ratio of 6.3:1 does fall within the 3x range and is therefore a reasonable donor ratio for this mixture. Since one of the contributors could be completely dropping out, this locus is inconclusive.

If complete dropout of one of the contributors is not reasonable, then the analyst can be sure that at least one allele from each contributor is being detected. Refer to the following chart for the remaining interpretation steps of a two-allele locus. Consider two alleles, A and B, where F is a dropped out allele.

If	Then	Included genotypes
A, F and B, F are reasonable	A+/B+	A, anything B, anything
Only A, F reasonable	B, B/A+	B, B A, anything
Only B, F reasonable	A, A/B+	A, A B, anything
Dropout is not reasonable for either contributor	[A, B]	A, A A, B B, B

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Locus with two alleles
(continued)

- Example: In a Type 2 mixture with an average donor ratio of 3:1, the following two alleles are detected at D3S1358:
  - 14 allele (420 RFU)
  - 16 allele (100 RFU)

  Complete dropout of one of the contributors is not reasonable due to the peak height ratio of the 14 and 16 (24%). 16, F is reasonable since the 16 allele is below the stochastic threshold. To determine if 14, F is reasonable, calculate the donor ratio assuming one contributor is a 14, 16 and the other is a 14, F:

  - (195 + 100) : (225 + 49)
  - 1.1 : 1

  This donor ratio should be compared against the 3x range (1:1 to 9:1) since one or more of the tested alleles are below the stochastic threshold. A donor ratio of 1.1:1 does fall within the 3x range and is therefore a reasonable donor ratio for this mixture. The resulting interpretation should be 14+/16+.

- Example: In a Type 2 mixture with an average donor ratio of 3:1, the following two alleles are detected at D3S1358:
  - 14 allele (750 RFU)
  - 16 allele (250 RFU)

  Complete dropout of one of the contributors is not reasonable due to the peak height ratio of the 14 and 16 (33%)

  While both alleles are above the stochastic threshold, dropout should still be considered due to potential allele sharing. To test for dropout with the 14 allele, one contributor would be a 14, 16 and the other a 14, F. Since the 14 is a shared allele in this scenario, the analyst should decide if the 14 allele can be shared in a way that would reasonably explain the locus. One way to do this is to remove the maximum amount of the 14 allele that could pair with a dropped-out allele:

  - 750 - 225 = 525

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Locus with two alleles (continued)
Locus with two alleles (continued)

So, 525 RFUs would belong to the 14, 16 contributor, leaving 225 RFUs remaining for the 14, F contributor. The donor ratio would be

- $(525 + 250) : (225 + 49)$
- $2.8 : 1$

The peak height ratio of the 14, 16 would be

- $250/525 = 48\%$

A donor ratio of 2.8:1 is within the 3x range (1:1 to 9:1) and a peak height ratio of 48% is acceptable; therefore, the 14, F genotype is reasonable.

To test for dropout with the 16 allele, it is not reasonable for one contributor to be a 14, 14 and the other a 16, F because the 16 allele is above the stochastic threshold. It is also not reasonable for the 16 allele to be a shared allele due to peak height ratio imbalances. The interpretation at this locus should be 16, 16/14+.

As with the three-allele loci, loci with peaks which are IFS must be marked inconclusive or the IFS peak must be included within the interpretation. For more information and examples about IFS peaks, see the section following Type 2 mixture interpretation.
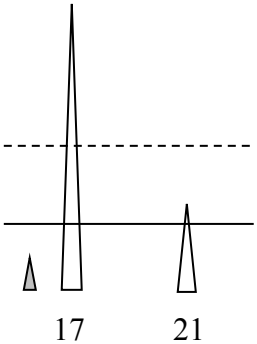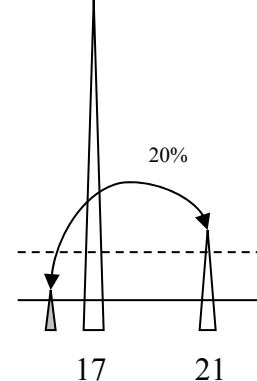

Locus with one allele

The first step in interpreting a one-allele locus is to determine if one of the contributors may have both alleles dropping out. If so, then the locus is inconclusive.

If complete dropout of one of the contributors is not reasonable, then the analyst can be sure that the two contributors share the one detected allele. It then must be determined if one or both of the contributors could have the shared allele (A) and a sister allele that is dropping out. If so, then the interpretation is A+. If dropout is not reasonable for either contributor, then the interpretation is A, A.

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Locus with one allele (continued)

- Example: In a Type 2 mixture with an average donor ratio of 2:1, the following allele is detected at D21S11:

  − 28 allele (1000 RFU)

    To test for complete dropout of one of the contributors, calculate the donor ratio.

  − 1000 : (49 + 49)
  − 10.2 : 1

    A donor ratio of 10.2:1 falls outside of the 3x range (.67:1 to 6:1); therefore, complete dropout of one of the contributors is not reasonable.

    To test for partial dropout (one contributor is a 28, 28 the other is 28, F), calculate the donor ratio.

  − (1000 − 225) : (225 + 49)
  − 2.8 : 1

    A donor ratio of 2.8:1 does fall within the 3x range; therefore, partial dropout is reasonable. The interpretation is 28+. (NOTE: this interpretation also covers the scenario where both contributors are 28, F.)

As with the two and three-allele loci, loci with peaks which are IFS must be marked inconclusive or the IFS peak must be included within the interpretation. For more information and examples about IFS peaks, see the section following Type 2 mixture interpretation.

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Examining stutter peaks in an interpretation

When interpreting a mixture, peaks in a stutter position should be examined to determine if they could be potential alleles. These stutter peaks will generally fall into one of three categories:

- The peak can be distinguished as stutter (not an allele)
- The peak is indistinguishable from stutter, but the stutter peak is already included in the interpretation at that locus
- The peak is indistinguishable from stutter, and the stutter peak is *not* included in the interpretation at that locus.
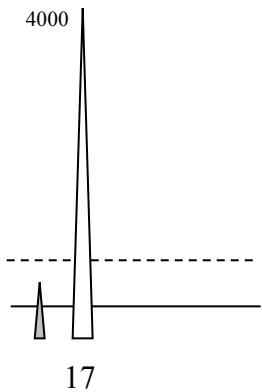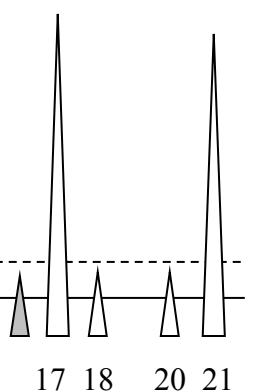
If the peak can be distinguished as stutter, then it can be ignored for interpretational purposes. The following are a few examples:

	Explanation
17 21	If the stutter peak is below the detection threshold, it can be ignored.
20% 17 21	The peak height ratio between the stutter peak and the 21 allele is 20%; therefore, the stutter peak can be distinguished as stutter and can be ignored.

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Examining stutter peaks in an interpretation (continued)

4000 ⟨peak graph with peak at 17⟩	If the contributors are well-represented across the profile (alleles out of the stochastic range), the stutter peak can be distinguished as stutter and can be ignored.
⟨peak graph with peaks at 17 18 20 21⟩	Under the assumption of two contributors, the four detected alleles represent all expected genetic information for two contributors. In this case, the stutter peak can be distinguished as stutter and can be ignored.
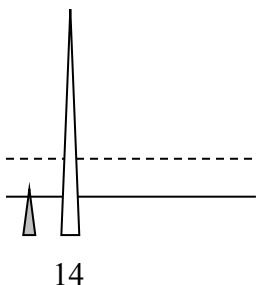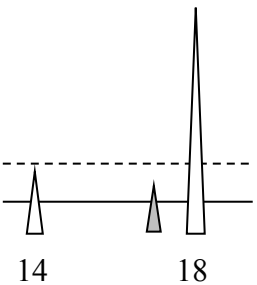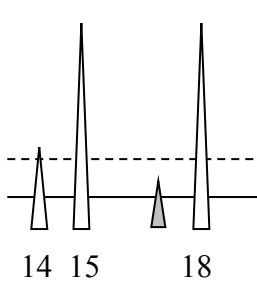
- - - - - - stochastic threshold
——— detection threshold

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Examining stutter peaks in an interpretation (continued)

If the peak is indistinguishable from stutter, but the stutter peak is already included in the interpretation at that locus, nothing further needs to be done. The following are a few examples:

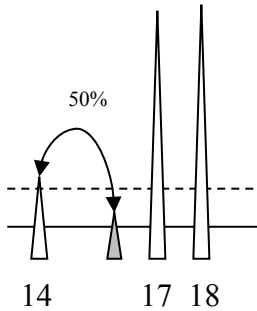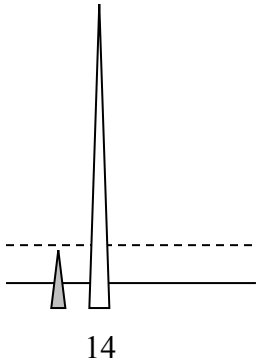|	**Explanation**
14	In a Type 2 mixture, an interpretation result of 14+ includes the 13, 14 genotype, so nothing further needs to be done. (NOTE: 13, F was tested and not reasonable.)
14 18	In a Type 1 mixture, the interpretation of the minor contributor is 14+. Since the 14, 17 genotype is already included within the interpretation, nothing further needs to be done.
14 15 18	Consider a Type 2 mixture. Even though the 14 allele is above the stochastic threshold, it is in a stutter position. Once the potential stutter contribution has been removed from the 14 allele, it is then below the stochastic threshold. The interpretation is [15, 18]/14+. Since the 14, 17 genotype is already included within the interpretation, nothing further needs to be done.

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DNA: Steps for Profile Interpretation- Step 5, Continued

Examining stutter peaks in an interpretation (continued)

If the peak is indistinguishable from stutter, and the stutter peak is *not* included in the interpretation at that locus, the analyst has three options:

• Mark the locus inconclusive
• Add to the interpretation: stutter+
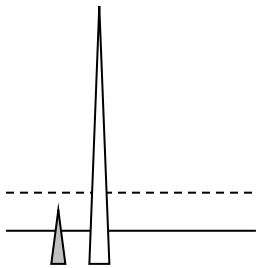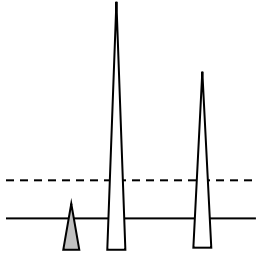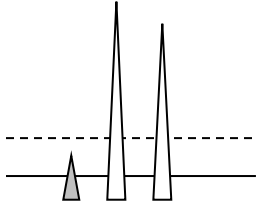• Add to the interpretation: all genotypes that may include the stutter peak
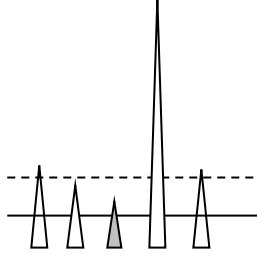
The following are a few examples:

	Explanation	Interpretation Options
50% 14 17 18	Consider a Type 2 mixture. The interpretation would be [14, 17, 18], but that does not include a potential 14, 16 genotype.	• Inconclusive • [14,17,18]/16~+ • [14,17,18]/14,16~
14	Consider a Type 2 mixture with an interpretation of 14, 14. If a 13, 14 genotype is reasonable, it is not included in the interpretation.	• Inconclusive • 14,14/13~+ • 14,14/13~, 14

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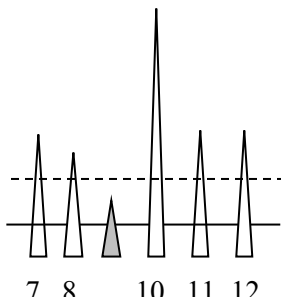
Examining stutter peaks in an interpretation (continued)

16	Consider a Type 2 mixture with an interpretation of 16+. If a 15, F genotype is reasonable, it is not included in the interpretation.	• Inconclusive • 16+/15~+
15 17	Consider a Type 2 mixture with an interpretation of [15, 17]. If a 14, 15 genotype is reasonable, it is not included in the interpretation.	• Inconclusive • [15,17]/14~+ • [15,17]/14~, 15
15 16	Consider a Type 2 mixture with an interpretation of 15+/16+. If a 14, F genotype is reasonable, it is not included in the interpretation.	• Inconclusive • 15+/16+/14~+
14 15 17 18	Consider a Type 4 mixture (assuming 3 individuals) with an interpretation of [14, 17, 18]/15+. If a 14, 16 genotype and a 16, 18 genotype are reasonable, they are not included in the interpretation.	• Inconclusive • [14,17,18]/15+/16~+ • [14,17,18]/15+/14, 16~/16~,18

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DNA: Steps for Profile Interpretation- Step 5, Continued

Examining stutter peaks in an interpretation (continued)

7 8 10 11 12	Consider a Type 4 mixture (interpreted with no assumptions of number of contributors). If the interpretation is [7, 8, 10, 11, 12], but a peak in a stutter position could reasonably pair with another allele (8, 9~), then this genotype is not included in the interpretation.	• Inconclusive

Type 3 mixture interpretation

A Type 3 mixture consists of DNA from three or more individuals and the relative peak heights allow for a major component to be separated from a minor component. Listed below are some examples of Type 3 mixtures:

- one major contributor, multiple minor contributors
- multiple major contributors, one minor contributor
- multiple major contributors, multiple minor contributors
- one major contributor, one minor contributor, one trace contributor.

The interpretation approach will vary depending on

- the total number of assumed contributors
- the assumed number of contributors within each major/minor component
- the presence of an assumed known contributor.

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DNA: Steps for Profile Interpretation- Step 5, Continued

One major, multiple minors

Interpretation of the major contributor:

Assuming one major contributor, the genotypes of the major contributor should be distinguishable from the alleles of the minor component. If there are loci in which the genotype of the major contributor is uncertain, the analyst may include a list of possible major genotypes or may mark the locus inconclusive.

The interpretation of the major contributor is a restricted approach because peak height information is utilized in the formation of the major contributor profile.

Interpretation of the minor component:

If an assumption can be made as to the number of contributors in the minor component, the analyst may provide a minor component interpretation for loci in which at least one allele from each contributor is being observed. The interpretation approach is unrestricted because the peak heights of the minor alleles are not being used to form specific genotypes, but rather a list of all genotypes created by the observed minor alleles.

- Example: Under the assumption of one major contributor and two minor contributors, the following five alleles are detected at D2S1338:

  − 18 allele (2000 RFU)
  − 20 allele (250 RFU)
  − 22 allele (2000 RFU)
  − 23 allele (150 RFU)
  − 25 allele (100 RFU)

  The 18, 22 genotype is attributed to the major contributor. Three alleles are detected from the minor component (ensuring that at least one allele from each contributor is being observed). The interpretation is 20+/23+/25+.

  NOTE: Even though the 20 allele is above the stochastic threshold (and not in a stutter position), due to the complexity of a 3-person mixture, the presence of IFS peaks, and the amount of potential allele sharing, it is recommended that the interpretation of the 20 allele be 20+.

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DNA: Steps for Profile Interpretation- Step 5, Continued

One major, multiple minors (continued)

Upon occasion, the peak heights of the contributors within the minor component may be well above the stochastic threshold across the profile, indicating that allele dropout of the minor component is not reasonable. In this situation, the interpretation of the minor component may include all genotype combinations of the observed alleles (major and minor alleles), without consideration of allele dropout. Be aware, however, that IFS peaks must also be included in the interpretation or the locus must be marked inconclusive.

- Example: Using the above example, the minor component alleles are well above the stochastic threshold across the profile. At D2S1338, the following peaks are detected:

  – IFS peak (450 RFU)
  – 18 allele (4200 RFU)
  – 20 allele (600 RFU)
  – IFS peak (150 RFU)
  – 22 allele (4000 RFU)
  – 23 allele (500 RFU)
  – 25 allele (500 RFU)

  The interpretation of the minor component may be [17~, 18, 20, 22, 23, 25]. NOTE: The peak in the 21 position can be distinguished as stutter since it would not reasonably pair with the three obligate alleles for the minor (peak height ratio too low).

With an assumed known contributor:

If the major contributor profile is consistent with an assumed known contributor, it may not aid in the interpretation of the minor component.

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One major, multiple minors (continued)

If one of the contributors to the minor component is an assumed known contributor, and the remaining minor contributor can be assumed to be from one individual, the analyst may be able to deduce a profile for the unknown minor contributor. After removing the alleles attributed to the known,

- if two remaining minor alleles (A and B) are detected, then A, B.
- if one remaining minor allele (A) below the stochastic threshold is detected, then A+.
- if one remaining minor allele (A) above the stochastic threshold is detected, then either A+ or A paired with all other detected alleles (including IFS peaks).
- if no additional minor alleles are detected, the interpretation will typically be inconclusive for the unknown minor contributor.

Multiple majors, one minor

Interpretation of the major component:

Using peak heights of the detected alleles, identify alleles that can be attributed to the major component versus alleles that are from the minor contributor. This may be possible at all or some of the loci. If there are individual loci where it is difficult to distinguish major alleles from minor alleles, the analyst may choose to do one of the following:

- mark the locus inconclusive. Consequently, the locus cannot be used for comparison with reference profiles
- include all possible genotypes for the major component.

If the major component can be assumed to be from two individuals, then the interpretation can follow the Type 2 mixture interpretation approach. Because the relative proportion of DNA from the minor contributor is significantly less than the proportion of DNA from the major component, the alleles of the minor contributor and their relative contribution to the mixture can be disregarded for the purposes of interpreting the major component.

If the major component is assumed to be from three or more individuals, then the interpretation of the major component can follow the Type 4 mixture interpretation approach. The alleles attributed to the minor contributor can be disregarded for the purposes of interpreting the major component.

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DNA: Steps for Profile Interpretation- Step 5, Continued

Multiple majors, one minor (continued)

Interpretation of the minor contributor:

As long as the minor contributor can be assumed to be from one individual, the analyst may be able to deduce a profile for the minor contributor. By examining alleles attributed to the minor,

- if two minor alleles (A and B) are detected, then A, B.
- if one minor allele (A) below the stochastic threshold is detected, then A+.
- if one minor allele (A) above the stochastic threshold is detected, then A paired with all other detected alleles (including IFS peaks). (An allele such as this may also be interpreted as A+, according to analyst discretion.)
- if no minor alleles are detected, the interpretation will typically be inconclusive for the minor contributor.

With an assumed known contributor:

If the minor contributor is assumed to be from a known contributor, it may not aid in the interpretation of the major component.

If one of the major contributors can be assumed to be from a known contributor and the major component is assumed to be from two individuals, the interpretation approach for the major component can follow the Type 1 mixture interpretation approach.

Type 4 mixture interpretation

A Type 4 mixture consists of DNA from three or more individuals, and the mixture *cannot* be resolved into its individual components.

Based on the analyst's ability to estimate the number of contributors, there are two interpretation approaches that can be used.
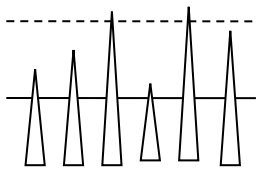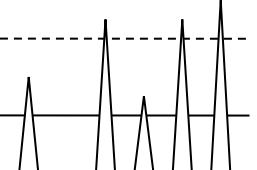
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DNA: Steps for Profile Interpretation- Step 5, Continued

If an assumption is made as to the number of contributors

If an assumption can be made as to the number of contributors, the analyst may provide an interpretation for loci in which at least one allele from each contributor is being observed. The interpretation approach is unrestricted. Peak heights of the detected alleles are not used to narrow down a list of specific genotypes, but rather a list of all genotypes created by the observed alleles. The genotypes of the true contributors should be found within this list of genotypes. Peak heights can be used, to a limited extent, to assess the locus for dropout, evaluate peaks in a stutter position, and to help identify the minimum number of alleles detected given the number of assumed contributors.
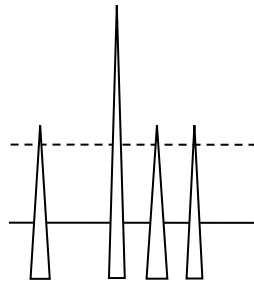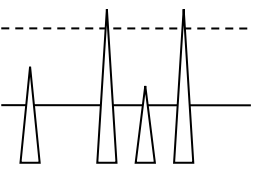
To interpret a Type 4 mixture, identify loci in which at least one allele from each contributor is detected. For those loci, identify alleles that may have a sister allele dropping out. If complete dropout of one or more contributors is possible, the locus must be marked inconclusive. Consider a Type 4 mixture with an assumed three contributors:

	Interpretation	Included genotypes	
A B C D E F (all alleles detected)	{A, B, C, D, E, F}	A, B A, C A, D A, E A, F B, C B, D B, E	B, F C, D C, E C, F D, E D, F E, F
A B C D E (at least one allele detected)	[B, D, E]/A+/C+	B, B B, D B, E D, D	D, E E, E A, anything C, anything

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If an assumption is made as to the number of contributors (continued)

* (at least one allele detected) A B C D	[A, C, D]/B+	A, A A, C A, D C, C C, D D, D B, anything
A B C D (complete dropout possible for one contributor)	inconclusive	inconclusive

* see explanation below

Allele dropout should be considered for all alleles, regardless of their peak height. Due to allele sharing, it is possible for alleles with peak heights well above the stochastic threshold to exhibit allele dropout. Consider the mixture from the table above (*) made up of the following three individuals:

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If an assumption is made as to the number of contributors (continued)

	Contributor 1: A, B Contributor 2: C, D Contributor 3: B, dropped out allele Even though allele B is the tallest allele, if (through allele sharing) it is possible for a contributor to have allele B paired with dropout, then dropout must be considered. The interpretation at this locus may be [A, C, D]/B+.
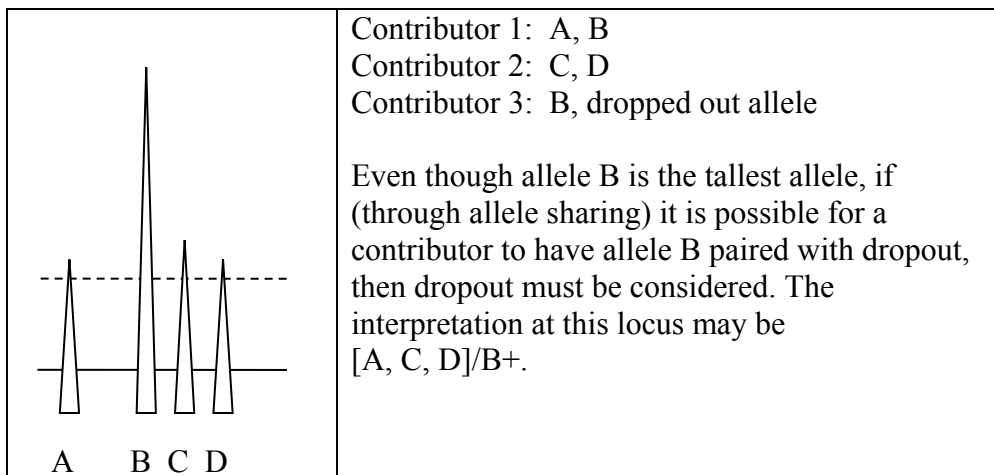
Loci with 3 or less alleles (and sometimes 4 or less alleles) will often be marked inconclusive because it may be difficult to determine if an allele from every contributor is being detected. If the analyst is certain that no dropout has occurred (all contributors well represented in the mixture), it may be possible to use loci with 3 or less alleles.

• Example: Consider a profile with an assumed three contributors and all alleles are above the stochastic threshold. At TPOX, the following two alleles are detected:

– 8 allele (3000 RFU)
– 10 allele (3500 RFU)

Because dropout is not expected for any of the contributors, the interpretation may be [8, 10].

Keep in mind, an allele in a stutter position may be perceived to be above the stochastic threshold, thereby implying that no allele dropout has occurred. However, an allele in a stutter position may be higher, due to stutter contribution, than it would be otherwise. The maximum potential stutter contribution should be subtracted from the peak, and then the peak should be evaluated for possible allele dropout.
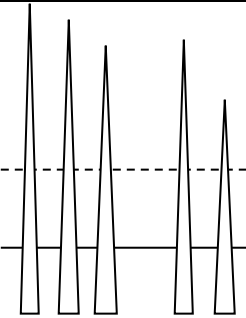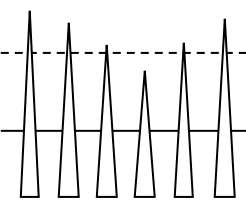
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If an assumption is made as to the number of contributors (continued)

If there is a peak in a stutter position (that is being filtered as stutter) and its peak height is consistent with other allelic peaks in the mixture, the analyst may either mark the locus inconclusive or include the IFS peak in the interpretation. For more information and examples about IFS peaks, see the section following Type 2 mixture interpretation.

If an assumption is *not* made as to the number of contributors

Each locus should be examined for indications of allele dropout. Any loci which do not show indications of allele dropout may be used in the interpretation. The interpretation should include all genotypes created from any combination of the detected alleles.

	Interpretation	Included genotypes
A B C D E	[A, B, C, D, E]	A, A B, D A, B B, E A, C C, C A, D C, D A, E C, E B, B D, D B, C D, E E, E
A B C D E F	inconclusive	inconclusive

If there are any alleles below the stochastic threshold, allele dropout may be possible; therefore, the locus should be marked inconclusive. Stutter contribution must be considered when assessing an allele in a stutter position for allele dropout.

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If an assumption is *not* made as to the number of contributors (continued)

If there are no alleles below the stochastic threshold, allele dropout may still be considered based on any of the following situations:

- if there is considerable allele dropout observed at other loci
- if the number of peaks suggest the possibility of allele sharing which may elevate the peak height above the stochastic threshold
- if there is a clearly defined peak above background, but below the detection threshold, that cannot be attributed to any artifact.

If a filtered peak in a stutter position is of similar height to other alleles detected in the mixture, then the stutter peak should be included as a potential allele in the interpretation or the locus should be marked inconclusive.

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