HealthSupport
Queensland

# Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis

Robert Morgan, Emma Caunt, February 2015

## Introduction

The aim of these guidelines is to assist in the assessment of the number of contributors for mixed DNA profiles obtained using the PowerPlex® 21 system. These guidelines should be used in conjunction with the training and experience of the scientist. There may be features within the DNA profile other than those detailed in this document that may inform the number of contributors. If the scientist observes information/behaviours within the DNA profile that override these guidelines, it is acceptable for these observations to be used in the determination of the number of contributors. There are also certain reworks that may be required, for example for quality reasons, before a reasonable assessment of the number of contributors can be made and these should be performed separately to the guidelines provided. Background information has also been presented in this document as this has been considered in the development of the recommendations provided. References to stochastic effects relate to peaks which may drop out or be imbalanced.

## Background

Bright et al [1] showed that the assumption of an increased number of contributors to a mixed profile does not affect the likelihood ratio (LR) assigned to the stronger contributor to the profile. However, the assumption of an increased number of contributors may significantly decrease the LR assigned to known low level contributors. This is definitely the case if all of the alleles in the low level component of the profile match those of the known contributor under the assumption of two contributors. Consider however a mixed profile that consists of three contributors (with two of those contributors being in the low level component), due to the stochastic effects of low level profiles, the low level contribution appears to only have originated from one person. In this case, a known contributor to the mixture could be excluded from the low level contribution if alleles from both low level contributors are displayed in the profile. Additionally, the low level contribution could be considered to be an unknown profile and be loaded to NCIDD possibly leading to adventitious links. This has been demonstrated in constructed mixtures where known contributors were not related [2].

It is noted that the true number of contributors to a casework mixture can never be known with certainty. If a three contributor casework mixture (with two low level contributions) was incorrectly assessed as having originated from two contributors, then the risk is that a true contributor to the low level contribution would be excluded. Exclusion is a statement of certainty rather than a LR favouring non-contribution and false exclusion is not a desirable outcome. Conversely, if a two contributor casework mixture was incorrectly assessed as having originated from three contributors, then there is an increased chance of a non-contributor being falsely ‘included’. An incorrect assessment of this type is most likely to occur with a mixture with a stronger contribution and one/two low level contributions, with a non-contributor being falsely ‘included’ in the low level contribution. In this instance, it is expected that the non-contributor would be a poor fit to the profile and would produce a LR favouring non-contribution or a LR approaching 1.

## Initial assessment

Before assessing the number of contributors to a mixed DNA profile, a general overview of how the profile is behaving should be obtained. The following things should be looked for in profiles generated using the PowerPlex®21 system:

- Preferential Amplification (PA) – this appears as an arc shape in the blue and/or red dyes (i.e. increasing peak heights from D3 to D6 before decreasing towards Penta E) and indicates too much input DNA in the amplification [3]. This pattern has the potential to mask the true number of contributors and the sample should be reworked by decreasing the amount of input DNA.
- Degradation – this is expected to occur in some samples and is displayed as decreasing peak heights moving from left to right across a profile in accordance with an increase in fragment size [3]. This pattern has the potential to misinform the number of contributors to a profile. Degradation is not usually able to be rectified by reworking, but should be factored into the interpretation.
- Bad baseline – this can be displayed in different ways, but is often due to excessive pull-up. Bad baseline has the potential to mask any low level contributions. Samples displaying bad baseline should be reworked according to the cause e.g. too much input DNA.

## Stutter

Before assessing how many contributors there may be to a profile, the scientist should examine the profile for the presence of artefacts. Kit specific artefacts and -2 and +1 repeat stutters should be removed at plate reading stage. Some -2 and +1 repeat stutter peaks may still be present at interpretation stage for reasons including peaks above one threshold but below a combined threshold, or they fall below the limit of reporting.

In-house stutter thresholds determined during the validation of the PowerPlex®21 system are provided as a guideline to assist the scientist to determine the number of contributors. They are calculated using the mean peak height + 3 standard deviations meaning that, for each threshold there are outliers. Some of the thresholds have been calculated using very few data points (see Appendix 4) and therefore it would be expected that stutter peaks above the threshold may be observed.

The stutter threshold should be used to inform the scientist of the expected height of stutter peaks at a particular locus, however an allele that falls slightly above threshold may not necessarily be indicating an additional contributor e.g. with a stutter threshold of 14.6%, a peak does not necessarily become an allele at 14.7%. It is appropriate for a scientist to use their experience in conjunction with the thresholds to determine whether a peak should be considered to be a stutter or an allele.

Please refer to Appendix 4 for information regarding the designation of -2, -1 and +1 rpt stutter peaks and guidelines for combining thresholds.

## Limited information

In some instances there may be very little information within the low level contribution of the profile and further investigation may not assist the scientist to provide meaningful interpretation of this contribution. Regardless of how many contributors there are to this low level component, interpretation will be limited. If the low level contribution, where it is expected that the impact of stochastic variation is heightened, consists of four or fewer alleles (including sub-threshold peaks) it is acceptable to consider that there is

only one contributor to this low level component[1]. It is at the discretion of the scientist whether to rework the sample or not. There may be scenarios where this number is not appropriate, however for the majority of profiles in this category this approach is considered suitable and is favoured over increasing the number of contributors to this limited component.

The reasoning behind using a cut-off of four alleles uses experience from Profiler Plus interpretations, an understanding of LRs that are generated by components with limited information as well as the success of reworking samples. It has also been developed based on the understanding of the risks of false inclusion and exclusion.

When considering a profile interpreted using a binary approach, such as with Profiler Plus, scientists within Forensic DNA Analysis would generally consider a minor profile to be unsuitable for meaningful interpretation if approximately four, or less, alleles are obtained. This is due to the increased probability of more than one person having these four alleles in their DNA profile. With a PP21 profile and a continuous interpretation approach, if a person matched all four alleles in a low level contribution, it is likely that the LR at best would provide low support for contribution.

When dealing with so few alleles, any interpretation of this information will be limited. It is likely that reworking the profile will not provide any additional information to either inform the number of contributors or to add significant weighting to the interpretation. It is therefore suggested that reworking a sample such as this will not be beneficial and that the profile should be interpreted as it stands.

The limitation of interpreting these low level components as single source is that a person could be falsely excluded if the alleles do originate from two different people. However, as previously mentioned it is expected that any LR generated under the assumption of an increased number of contributors would provide limited information.

## Sub-threshold peaks

Forensic DNA Analysis has validated a limit of reporting for the purpose of confidently distinguishing true allelic peaks from background noise [4]. This means that only those peaks above the limit of reporting can be used in the statistical analysis of DNA profiles. It is noted however that there is a chance that peaks below this level could be from DNA and the closer these peaks are to the limit of reporting, the more likely they are to be from DNA. Where there is a low level contribution to the profile it is expected that these sub-threshold peaks could interfere with the interpretation of the allelic peaks above the limit of reporting and therefore should be considered in the determination of the number of contributors. The results of the testing have demonstrated that it is appropriate to use sub-threshold peaks during the interpretation of a DNA profile. Following on from this, if these sub-threshold peaks are used in the determination of the number of contributors it is expected that they would also be used for exclusionary purposes.

Note: Sub-threshold peaks should only be considered if they are distinct from baseline, above the LOD, below the LOR and not potential stutter peaks.

## Conditioning

Before interpreting a profile, the scientist should assess from the case circumstances/sample type whether it is reasonable to assume the presence of DNA from a person. If the presence of DNA from a person can be assumed and there is information within the profile supporting this assumption, then the

---

[1] Unless three or more of these alleles are at one locus, in which case there is evidence that there is more than one contributor to the low level component.

profile can be conditioned. There is no minimum number of alleles required before this assumption can be made.

If the profile has one or more contributors in the stochastic range, then the presence of one or more 'matching' alleles in that contribution is enough to assume the presence of DNA from the conditioned reference sample.

If the low level contribution can be accounted for by the conditioned reference sample in its entirety, then the low level contribution can be assumed to have originated from one contributor (the conditioned reference sample).

If the low level contribution can only be partly accounted for (one or more matching alleles) by the conditioned reference sample, then the number of contributors should be adjusted to allow for the alleles, in addition to the conditioned contribution, that are present.

Given sub-threshold peaks are considered in the assessment of the number of contributors these must also be consistent with the conditioned contribution where a low level component is assumed to be single source. If this is not the case, there is no need to add a contributor to account for these sub-threshold peaks, rather, the profile can be reported as having possible sub-threshold peaks not interfering with the interpretation.

## Reproducibility

When a profile has a low level contributor, it is expected that this contributor will be subjected to stochastic effects. If there are two low level contributors to the mixture, then both of those contributors will be subjected to these stochastic effects. What could potentially happen, and has been observed [2], is that different peaks from each contributor could be displayed in the profile; however other peaks have dropped out. This could make the profile appear as though there is only one low level contributor rather than two.

If the profile has been reworked such that there are, for example three amplifications, then these three profiles should be considered together. When considered together, the following observations may be made:

1. The profiles have different alleles (either above or below threshold) in the low level contribution, however it appears that overall there is only one low level contributor.
2. The profiles have the same alleles (either above or below threshold) in the low level contribution and it appears that there is only one low level contributor.
3. There is additional information in subsequent amplifications and it is evident that there are two low level contributors.

If the observation is consistent with option 1, then it may be the case that there are two low level contributors, however the stochastic effects are such that different alleles are appearing in the different amplifications. In this instance, there is no certainty that there is only one contributor to the low level contribution and a contributor should be added.

If the observation is consistent with option 2, then the fact that the low level contribution is reproducible should give confidence to the notion that there is only one contributor to the low level component.

If the observation is consistent with option 3, then the profile should be considered as a three person mixture (two low level contributors).

Thee amplifications is the suggested requirement for profiles with a suspected single source low level component because testing has demonstrated this increases the opportunity to identify where peaks are reproducible [2].

## Guidelines

**1. Initial assessment of number of contributors**

This should involve an assessment of the number of peaks (including those which are sub-threshold), the height of the peaks and the presence of stutters and other kit specific artefacts to identify the *minimum* number of potential contributors to the mixed DNA profile.

**2. Stochastic range**[2]

This should involve assessing whether any one potential contribution may be subject to stochastic effects. This may be indicated by the presence of peaks less than ~300RFU or by a quantitation value which has resulted in an input of any one potential contribution to be less than ~150pg. If there is no contribution falling within the stochastic range further investigation may not be required and interpretation can continue based on the original assessment of the minimum number of contributors (see Appendix 2 for further information).

**3. Conditioning**

Where the presence of a reference sample can be assumed this should be conducted before further investigation into the potential number of contributors. If this assumed contributor can account for the low level contribution in its entirety then any stochastic variation can be accepted. In this case no further investigation is required and interpretation may continue based on the original assessment of the minimum number of contributors. If this assumed contributor can account for only part of the low level contribution, it is reasonable to increase the original assessment of the number of contributors to account for this assumed contribution.

**4. Allelic Imbalance (AI)**

Whilst there is no defined AI threshold for casework profiles, it is reasonable to see imbalance as low as 40% [4]. If there is imbalance below this figure at one or more loci, this sample should be reworked to confirm the ratio of these peaks as this imbalance could indicate an additional contributor to the low level contribution. If there is one imbalance between 40%-60% this may be accepted without further investigation, however if imbalance within this range is present at more than one locus the sample should also be reworked to confirm the ratio of these peaks. Imbalance, or variation in peaks heights, between loci within the same dye should also be considered, and imbalance between loci within the same dye not consistent with the expected degradation should be investigated further by reworking the sample[3].

**5. Degradation**

Degradation is characterised as a decrease in peak heights moving from left to right across a profile as the molecular weight increases. Laboratory observations [3] have shown that some variation can be seen however larger peaks at larger fragments may be an indication of more than one contributor to a low level component. If this is observed, the sample should be reworked to assist the scientist to determine whether this pattern is reproducible or due to expected variation.

---

[2] The stochastic range has been developed by the project officers based on observations made in the PowerPlex 21 validation [4].

[3] AI is only confirmed if that same pattern is observed across runs, i.e. 14 (1000rfu) and 16(400rfu) in one run and 14 (1200rfu and 16 (480rfu) in a second run = AI confirmed; if the AI flips to 14 (400rfu) and 16 (1000rfu) in the second run or if the partner drops out this is an assessment of the stochastic effects within the profile.

**6. Ratios**

It is noted that the peak heights may differ between dye lanes, however the approximate ratio of the mixture should hold across the profile [5]. This means that if the mixture appears to have a stronger contribution, a mid range contribution and a low level contribution in the blue dye, then this should also be represented in the other dyes. If the green dye then displays a stronger contribution and two low level contributions, this may indicate that there are four contributors to the mixture. If there is uncertainty regarding the mixture ratio, a rework should be performed to confirm, or otherwise, how the profile is behaving.

**7. Reworking**

The aim of the rework should be to confirm how a profile is behaving, assess the reproducibility of a component(s) for which the number of contributors is unclear or to potentially provide additional information in the form of additional peaks. Where more extract can be included in the amplification without overloading or increasing baseline noise, this should be done. For mixed DNA profiles the input can be increased above 0.5ng where it is suitable to do so based on the peak heights and the complexity of the profile [3]. In other cases where an increase in template is not possible a repeat amplification is sufficient. Where reworks have been performed, the minimum number of contributors may need to be reassessed based on the reproducibility of peaks or additional information that may have been obtained. It is recommended that, where a contribution in the stochastic range is thought to be single source, two reworks are performed so that final assessment can be made with a total of three amplifications. Depending on whether the input template is being increased or kept the same[4], these reworks may be ordered at the same time. It is also recommended that no more than three amplifications are performed for the determination of the number of contributors, unless there is an issue with one or more of these runs, since more amplifications may increase the complexity of the interpretation.

**8. Reproducibility[5]**

This should be used to confirm a single source contribution within the stochastic range and the following guidelines have been developed to address this. The level of reproducibility will depend on the rework that was ordered, that is, if there is a change in input template it is possible that the number of peaks and peak heights will differ between amps and potentially make the reproducibility assessment more difficult. For single source contributions within the stochastic range:

a. Labelled peaks – it is expected that not all peaks within the stochastic range will be reproduced due to the nature of DNA profiling, however a single source contribution in the stochastic range with greater than 12 labelled alleles should have 80% of alleles reproduced twice out of a possible three runs [2] (see Appendix 3 for further information). If this reproducibility is not achieved the number of contributors should be increased by one. If there is a suspected single source contribution within the stochastic range with less than 12 labelled alleles a lower level of reproducibility may be expected. This has been demonstrated to be around 60% but may fall as low as 30%, therefore whether this contribution can be called single source should be at the discretion of the scientist in conjunction with other factors.
b. Peak heights – it is expected that peak heights within the stochastic range will fluctuate due to the nature of DNA profiling, however the ratio should remain consistent across runs. When there is a change in the ratio at one or more loci it may be appropriate to increase the number of contributors by one.
c. Allelic imbalance – if any AI less than 40% is confirmed at one or more loci then increasing the number of contributors by one should be considered.
d. Degradation – it is expected that peak heights will decrease when moving from left to right and if any apparently significant deviation from this is confirmed on rework the number of contributors should be increased by one.

---

[4] Where the input template is changed it may be appropriate to order one rework at a time as there is a chance the sample may become overloaded.

[5] Reproducibility is defined as the number of reproduced peaks divided by the total number of peaks in this contribution.

## Reference comparisons

Following the determination of the number of contributors to a mixture, reference samples (if available) can be compared. Unless conditioning, it is important that reference samples are not compared until an assessment of the number of contributors has been completed.

Although the LRs are calculated separately for each reference sample in the case, the manual comparison should include a check of all reference samples together, particularly for strong profiles with low mixture ratios. For example, if the casework sample gives a two person mixture and both reference samples could have contributed, it is important to check that both reference samples could have contributed together. If they could not then multiple scenarios are possible:

- Person 1 and an unknown (not person 2) have contributed
- Person 2 and an unknown (not person 1) have contributed
- Neither person 1 nor person 2 have contributed
- Person 1 and person 2 have contributed but the profile actually has more than two contributors

It is not possible for the scientist to determine which of these scenarios is more likely leaving only two options for reporting the result to the court. The first option is to present all of the scenarios to the court and let the court decide which scenario is more likely; the second option is to concede that the number of contributors cannot be determined and that the profile is unsuitable for meaningful interpretation. It is considered that the second option is the most appropriate approach. Taylor et al [6] acknowledge that presenting these options to a court places the decision with the court and that if it is not possible for an expert to distinguish between these scenarios then it is asking a lot for the court to do so. If this scenario is encountered the number of contributors should not be increased to allow for person 1 and person 2 to have contributed together without further investigation into the reassessment of the number of contributors including reworks.

This approach may not be relevant for all mixture types. If a profile has contributors in the stochastic range there may only be limited information within the profile with which to make an assessment regarding whether or not a person could be a potential contributor. These assessments would generally result in a LR closer to 1. It is important to note that there is little difference between two different values which only slightly fall either side of 1 and that it is not expected that it is the role of the scientist to determine the level of support for either proposition where a figure falls in this range. It is expected in these instances that the LR provided would speak for itself in terms of the level of support or fit to the profile for that reference comparison.

When the reference sample(s) is compared, it may be that the reference sample is a poor fit to the profile under the assumed number of contributors, however increasing the number of contributors will make the reference sample a good fit to the profile. It is not acceptable to increase the number of contributors based on the fit of the reference sample. If this situation is encountered, then the scientist may decide to rework the sample to reinforce, or otherwise, the assessment of the number of contributors. Should the rework support the change in the number of contributors (without the influence of the reference sample), then this may occur. Should the rework back up the original assessment of the number of contributors, then this assessment should stand. If the scientist considers that the fit of the reference sample to the profile is not intuitively correct then the scientist has the option of deeming the profile unsuitable for interpretation.

## References

[1] J. Bright, J.M. Curran, J.S. Buckleton, The effect of uncertainty in the number of contributors to mixed DNA profiles on profile interpretation, Forensic Sci. Int.: Genet. 12 (2014) 208-214

[2] E. Caunt, R. Morgan, J. Howes, C. Allen, Development of guidelines for the determination of number of contributors to a PowerPlex®21 profile 2015

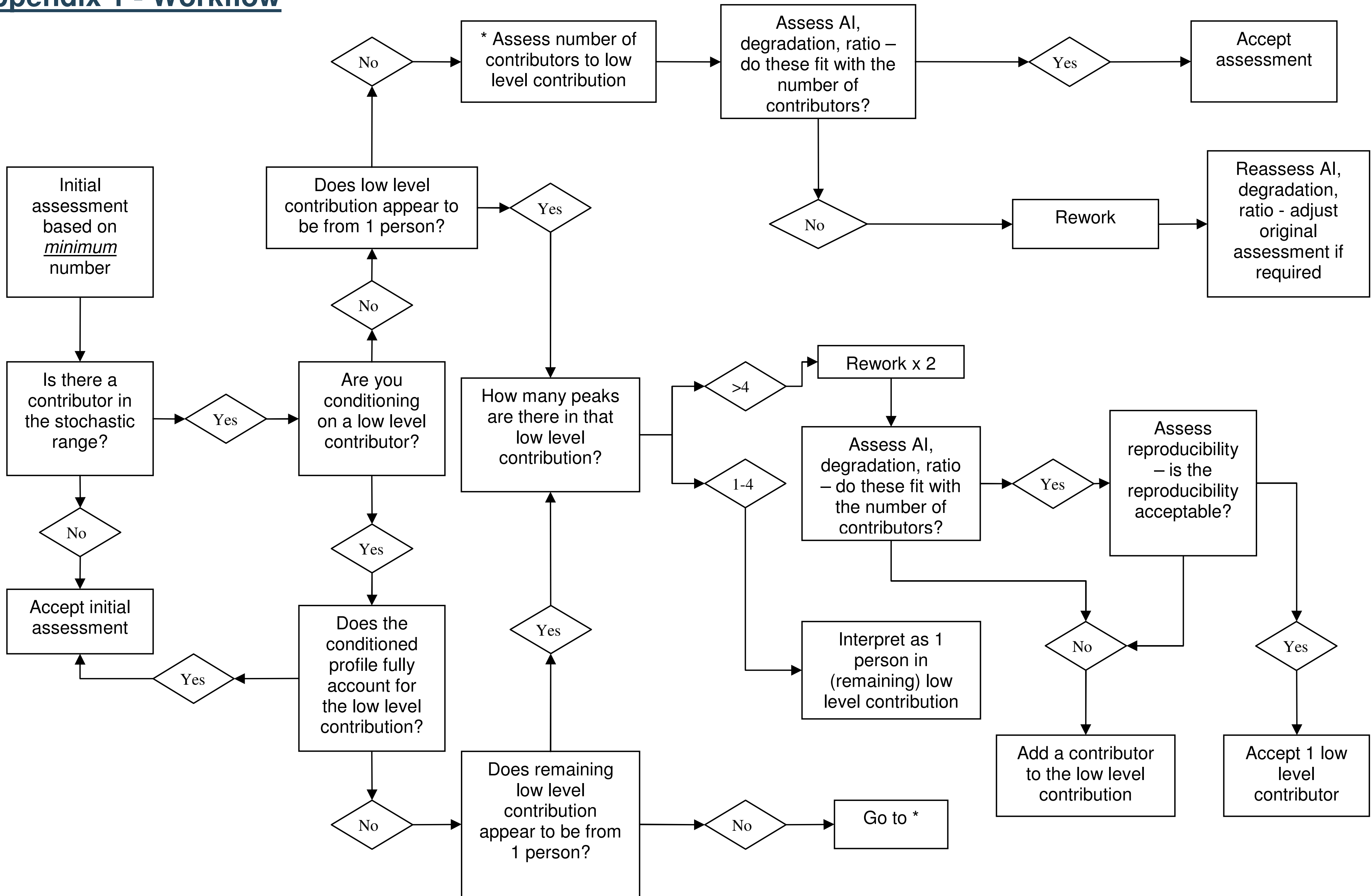
[3] Powerpoint presentation, PowerPlex 21 Observations, October 2014 (located in G:\ForBiol\AAA Forensic Reporting & Intel\AAA_Reporting guidelines\PP21 and STRmix™ case mgt)

[4] T. Nurthen, M. Mathieson, C. Allen, PowerPlex 21 – Amplification of extracted DNA validation v2.0 2013

[5] T.M. Clayton, J.P.Whitaker, R. Sparkes, P. Gill, Analysis and interpretation of mixed forensic stains using DNA STR profiling, Forensic Sci. Int. 91 (1998) 55-70

[6] D. Taylor, J. Bright, J. Buckleton, Interpreting forensic DNA profiling evidence without specifying the number of contributors, Forensic Sci. Int.: Genet. 13 (2014) 269-280

## Appendix 1 - Workflow



# Appendix 2 – Determining contributors within the stochastic range

A contribution within the stochastic range is defined as one with peaks heights less than 300RFU and/or an input of less than 150pg. It may be difficult to determine whether all of the peaks of a contribution that is potentially in the stochastic range are below 300RFU due to the possibility of sharing with other allelic peaks or stutters. If all of the peaks associated with one (or more) low level contribution(s) are below 300RFU then that contribution is clearly in the stochastic range. However, if some of the peaks are below 300RFU and some of the peaks are above 300RFU it may be necessary to use the following additional information to assist the determination of whether this contribution falls within the stochastic range:

- Quantitation value and approximate input template of contribution
- The proportion of peaks above and below 300RFU
- For any peaks below 300RFU, how much below 300RFU they are, e.g. 298RFU vs 56RFU

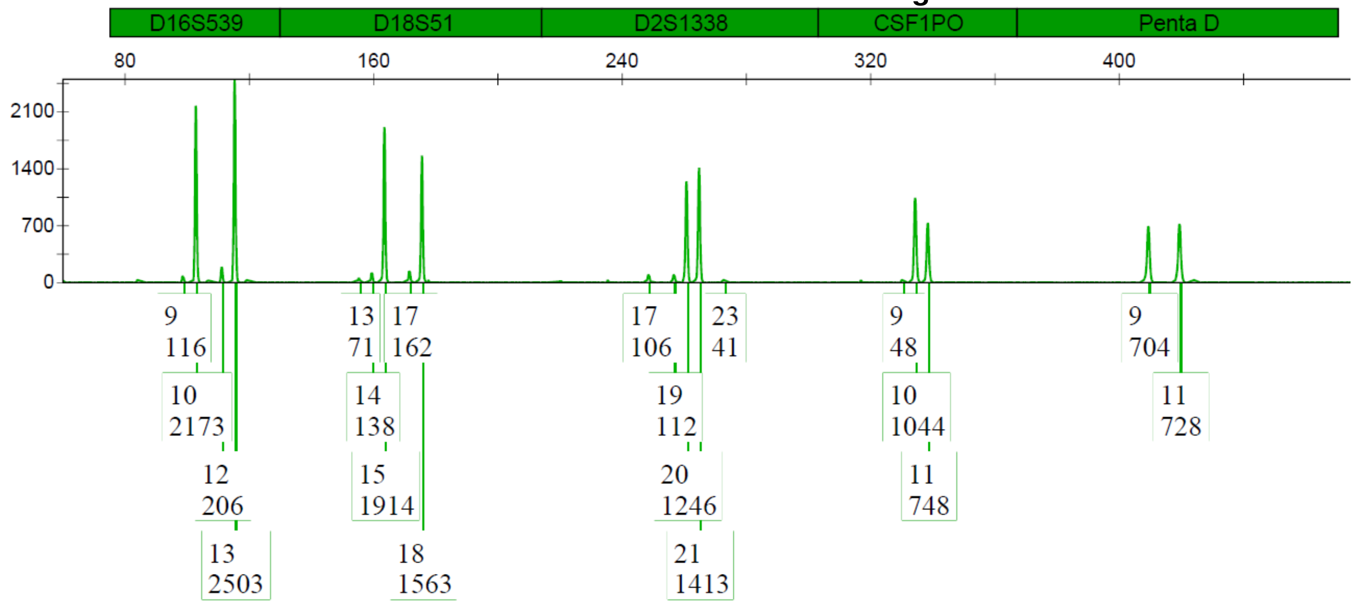
When determining the approximate theoretical input of a contribution the total input template and the approximate proportion of the questioned contribution in the profile should be used. It is important to note that the values of 300RFU and 150pg are approximate and that a contribution at 299RFU and/or 149pg is not much different from a contribution at 301RFU and/or 151pg.

*Multiple contributors in the stochastic range*

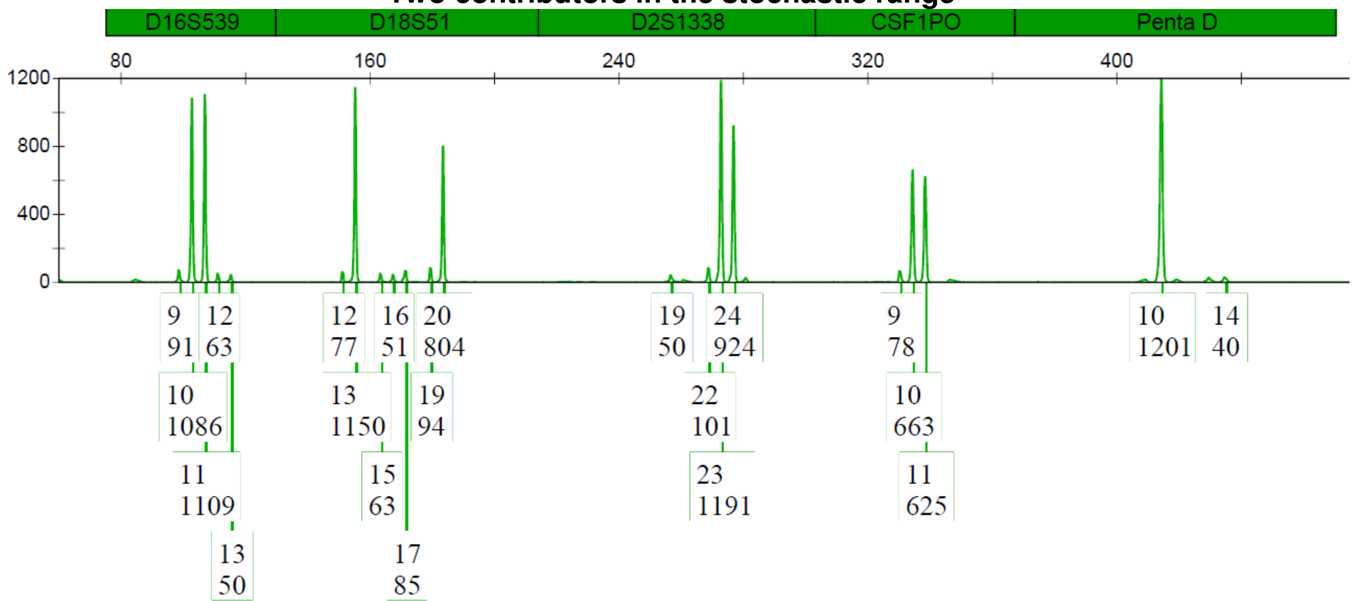
The contributors to a mixture can range in ratio from distinct strong contributions and low level contributions to all strong contributions or all low level contributions. If the profile has more than one low level contribution that falls either within or close to the stochastic range the risk of false exclusion and/or uploading a false profile to NCIDD decreases. In this instance the STRmix™ deconvolution should be flexible enough (in most situations) to allow the LR relating to the low level contribution to speak for itself, i.e. reference samples with a poor fit will produce a low LR and reference samples with a good fit will produce a higher LR. Unless there are a lot of labelled alleles at a locus, exclusion is unlikely (as allele,NR and NR,NR combinations will be considered) and false exclusion more unlikely still. With this type of profile it is not expected that a profile for upload to NCIDD will be deconvoluted. Therefore, profiles with more than one low level contribution are not required to have as thorough an investigation as those with only one low level contribution.

## Examples

**One contributor in the stochastic range**




**Two contributors in the stochastic range**



# Appendix 3 – Reproducibility calculation

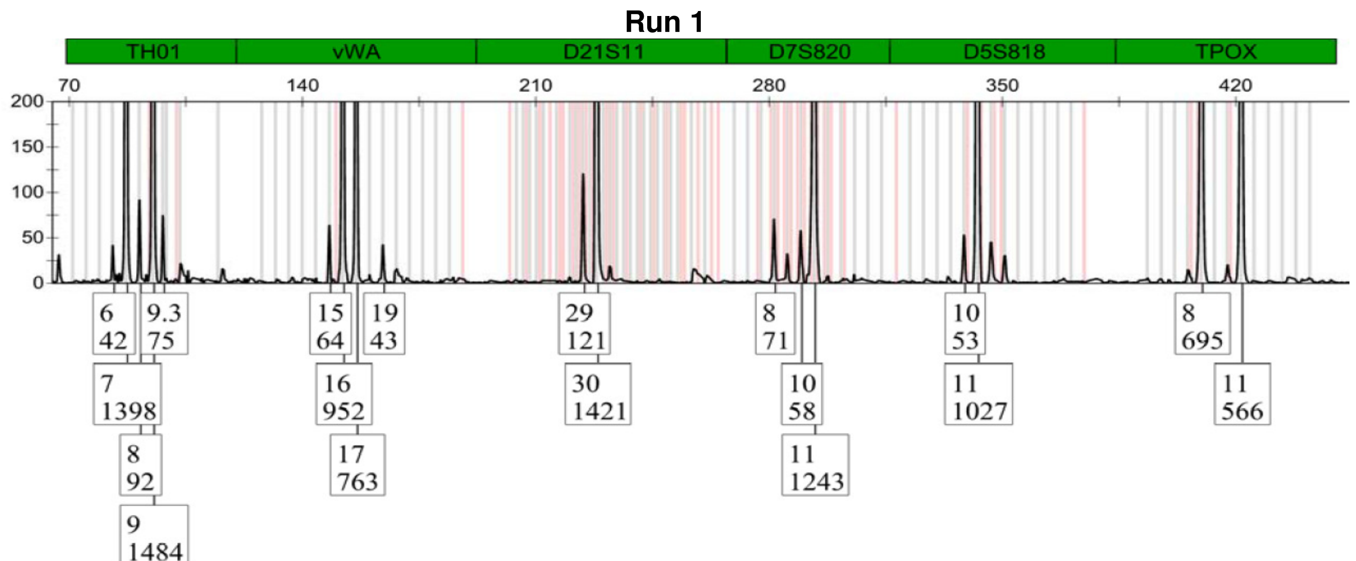
Reproducibility is only used to confirm a single source component in the stochastic range. It is not possible to determine the reproducibility for a mixed component due to the increase in variability and the inability to determine which alleles belong to a single contributor. To determine whether a profile falls into the <12 or >12 allele category only labelled peaks should be included (this would be the total number of different labelled peaks across the three runs). This number has been selected as it is the upload to NCIDD threshold. Once the category has been selected, sub-threshold peaks may then be considered.

To determine whether a peak is reproduced peaks above and below threshold are considered. If any peak above or below threshold is only seen once across all of the three runs this peak is considered not reproduced. If a peak is seen above or below threshold two or three times across the three runs this peak is considered reproduced. To determine the percentage reproducibility the number of reproduced alleles (both above and below threshold) should be divided by the total number of different alleles seen (reproduced and not reproduced both above and below threshold) and then multiply by 100.

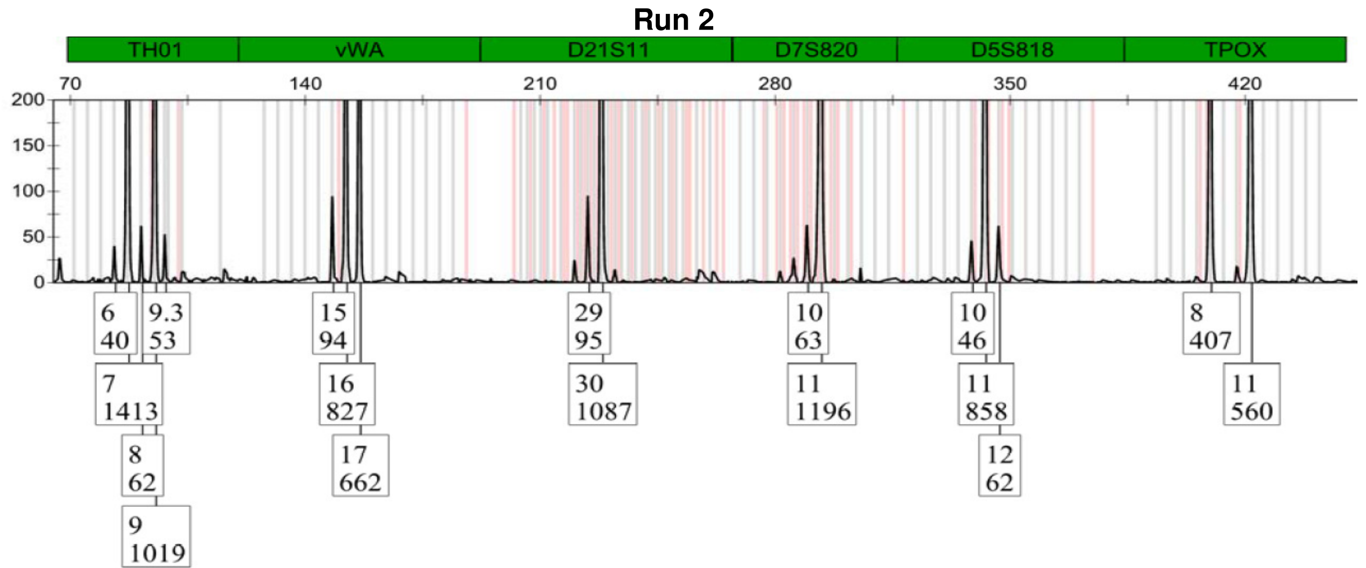
Three runs are required to determine reproducibility due to the stochastic nature of low level DNA.

**Example reproducibility calculation**

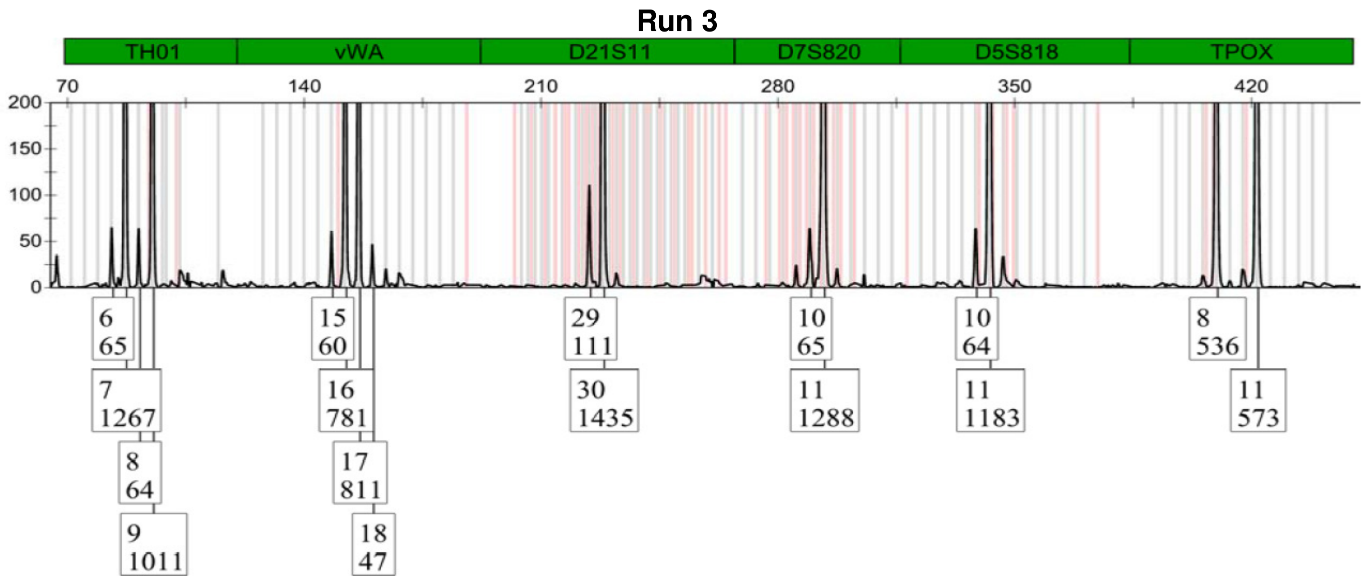
The diagrams below show three runs of the same profile. The table details the alleles observed in the stochastic range (including sub-threshold alleles above the LOD shown in brackets) for each of the three runs. The final column shows whether the allele is reproduced.



**Run 2**




**Run 3**




| Run 1 | Run 2 | Run 3 | Reproduced |
|---|---|---|---|
| TH01 9.3 | TH01 9.3 | | Yes |
| | | vWA 18 | No |
| vWA 19 | | (vWA 19) | Yes |
| D7 8 | | | No |
| | D5 12 | | No |
| (D5 13) | | | No |

A total of six alleles were observed in the stochastic range, of these two were reproduced. The reproducibility is therefore 2/6 = 33%.

Note: If a peak falls in a stutter position and is considered part of the low level contribution as it is above the stutter threshold it can be included in the reproducibility calculation.

# Appendix 4 – Stutter

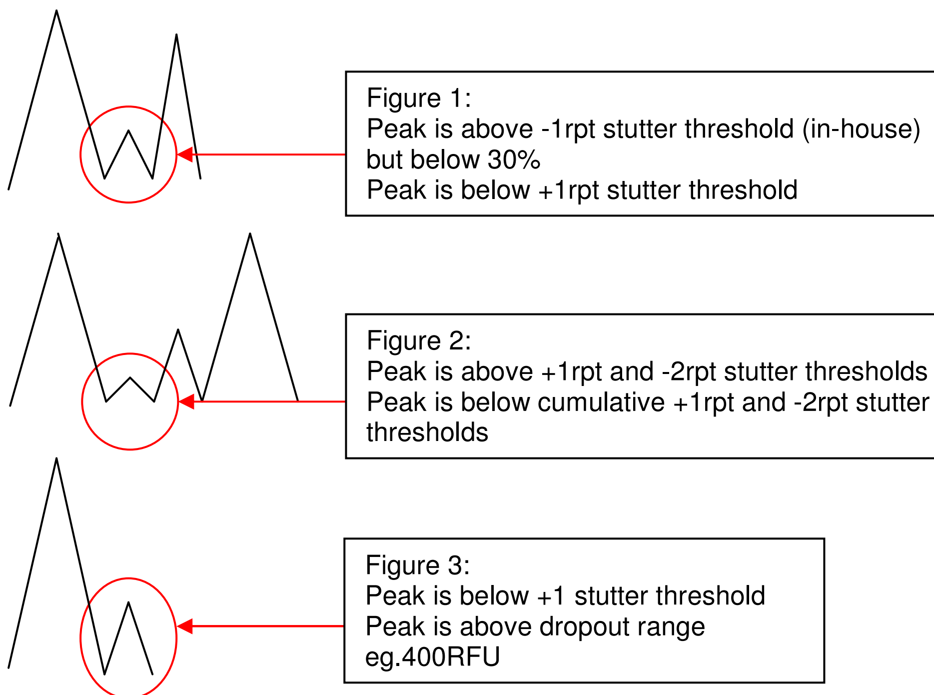
Forensic DNA Analysis has implemented locus specific stutter thresholds based on data from the validation of the PowerPlex®21 kit. These stutter thresholds are calculated by comparing the height of the stutter peak to the height of the allele peak. The -1 repeat stutter thresholds are used during case management for the determination of number of contributors, whilst -2 repeat and +1 repeat stutter thresholds are used at plate reading to determine which peaks should be removed before the profile is suitable for interpretation. Table 1 shows the stutter thresholds which have been implemented (% calculated using mean + 3 standard deviations) as well as the number of times each of the stutter peaks were seen (allele count column).

| Locus | Stutter -2 repeats (%) | Allele Count | Stutter -1 repeat (%) | Allele Count | Stutter +1 repeat (%) | Allele Count |
|---|---|---|---|---|---|---|
| D3S1358 | 2.3 | 147 | 14.2 | 310 | 4.3 | 60 |
| D1S1656 | 4.3 | 132 | 17.2 | 371 | 6.7 | 128 |
| D6S1043 | 2.7 | 33 | 12.0 | 366 | 7.4 | 84 |
| D13S317 | 3.4 | 14 | 11.8 | 200 | 7.4 | 39 |
| Penta E | 2.1 | 4 | 10.7 | 184 | 1.7 | 2 |
| D16S539 | 2.1 | 108 | 12.1 | 270 | 4.3 | 118 |
| D18S51 | 2.8 | 133 | 16.0 | 422 | 4.9 | 119 |
| D2S1338 | 2.9 | 106 | 14.9 | 372 | 6.5 | 12 |
| CSF1PO | 3.7 | 29 | 13.7 | 190 | 4.4 | 54 |
| Penta D | 0 | 0 | 8.2 | 86 | 8.8 | 8 |
| TH01 | 2.6 | 39 | 8.7 | 243 | 2.1 | 22 |
| vWA | 3.5 | 54 | 15.2 | 278 | 5.6 | 52 |
| D21S11 | 4.0 | 58 | 14.1 | 322 | 7.1 | 120 |
| D7S820 | 3.4 | 15 | 11.4 | 252 | 5.8 | 60 |
| D5S818 | 2.3 | 20 | 12.0 | 214 | 5.6 | 51 |
| TPOX | 2.7 | 2 | 9.0 | 164 | 6.3 | 4 |
| D8S1179 | 2.6 | 54 | 13.2 | 315 | 5.5 | 62 |
| D12S391 | 2.8 | 146 | 18.8 | 376 | 5.3 | 45 |
| D19S433 | 3.2 | 32 | 12.8 | 210 | 7.1 | 8 |
| FGA | 3.1 | 48 | 13.8 | 280 | 5.9 | 40 |

Table 1 – Forensic DNA Analysis Casework Stutter Thresholds

As STRmix™ cannot model -2 repeat and +1 repeat stutter peaks, a binary approach is applied in that those peaks which are equal to or less than the threshold are removed before the profile is interpreted. It should be noted that where this has resulted in a large peak being removed, STRmix™ users need to check the deconvolution to ensure the removal of this artefact is covered by the consideration of dropout of this allele if required based on the rest of the profile. Where there is a -2 repeat or +1 repeat stutter peak over threshold which may result in an increase in the number of contributors reworking to confirm that this peak is not stutter may also be performed.

As STRmix™ can model -1 repeat stutter, and does so up to a value of 30% of the main peak, these thresholds are only used to determine number of contributors and the % value is only used as a guideline. Where there is a peak below the +1 repeat stutter threshold but also below the -1 repeat stutter threshold, this peak is left labelled to be modelled by STRmix™ as -1 repeat stutter. There are a few scenarios encountered in casework for which guidelines have had to be developed:




In Figure 1 the highlighted peak is above the -1 repeat stutter threshold but is below the +1 repeat stutter threshold. In this example the peak would be removed at plate reading stage because if it was left on STRmix™ may model this peak as -1 repeat stutter but it is above the in-house stutter threshold and therefore may lead to the number of contributors being increased. The deconvolution would be checked to ensure the removal of this artefact is covered by the consideration of drop-out of this allele if required based on the rest of the profile.

In Figure 2 the highlighted peak is above the +1 repeat and -2 repeat stutter thresholds. In this example the peak would not be removed at plate reading stage but the reporting scientist may consider adding the two thresholds to remove the peak if it doesn't fit the rest of the profile. There are no strict guidelines around when this approach can be used however it would not be used often. The reporting scientist may also choose to rework the sample to aid in their decision making.

In Figure 3 the highlighted peak is below the +1 repeat stutter threshold but is above the RFU range at which we would reasonably expect STRmix™ to model dropout. As a result, a reference sample which may have contributed to the profile and has the allele that falls in that position may be excluded simply because of the height of the main allele. If dropout is not modelled even after increasing the number of iterations this sample would be reworked to see if the peak falls above the threshold on another run.