Analysis, Interpretation, and Reporting of Y-STR Data

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1 INTRODUCTION

Y-chromosome Short Tandem Repeat (Y-STR) examinations are generally conducted for lineage comparisons, in support of Missing Persons and Intelligence cases, or on samples that have a high ratio of female to male DNA.

Y-STR profiles are considered haplotypes, so all conclusions regarding comparisons apply to the person of interest (POI) and the paternal lineage of the POI. In addition, unrelated individuals may exhibit the same Y-STR typing results.

2 SCOPE

These procedures apply to DNA personnel who verify and interpret nuclear DNA typing results obtained from the PowerPlex® Y23 System using the GeneMapper™ ID-X (GMIDX) DNA typing software for forensic analysis and the Y-Chromosome Haplotype Reference Database for statistical calculations.

3 EQUIPMENT

- GeneMapper™ ID-X software (Applied Biosystems, version 1.6 or higher)
- STACS™ Casework (STACS) Software (Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies, version 5.0 or higher)
- Y-Chromosome Haplotype Reference Database (YHRD), current release, www.yhrd.org (Institute of Legal Medicine, Charite – University Medicine Berlin)

4 STANDARDS AND CONTROLS

Raw data for the electrophoretic runs of samples or controls displaying no typing results must be reviewed for the presence of a primer peak. If no primer peak is observed, the sample must be reinjected or reprepared to verify that amplicon was added to the capillary electrophoresis (CE) plate.

4.1 Verification of WEN Internal Lane Standard 500 Y23 (WEN ILS 500 Y23)

A. For Y23 data, which is sized using the Local Southern Method, verify that the 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, and 475 base pair (bp) fragments are captured and have been assigned the correct size values for each sample, control, and allelic ladder.
B. If all of the WEN ILS 500 Y23 fragments for a given injection do not meet these specifications, a different injection of the sample that displays the correct size values for all of the WEN ILS 500 Y23 fragments must be used for interpretation of the entire DNA profile, which may require that the sample be reprocessed.

4.2 Verification of Allelic Ladders

A. Any allelic ladder used for genotyping must:
   1. exhibit the correct allele designations (see Table 1) and
   2. yield the correct typing results when used to genotype the positive amplification control.

B. If any sample(s) requires reinjection the appropriate ladder must be included in the reinjection set.

Locus[1]	Known Size Range (bp)[2]	Alleles Present in Ladder[3]	Color
DYS576	97–145	11-23	Blue
DYS389I	147–179	9-17	Blue
DYS448	196–256	14-24	Blue
DYS389II	259–303	24-35	Blue
DYS19	312–352	9-19	Blue
DYS391	86–130	5-16	Green
DYS481	139-184	17-32	Green
DYS549	198-238	7-17	Green
DYS533	245-285	7-17	Green
DYS438	293-343	6-16	Green
DYS437	344-380	11-18	Green
DYS570	90-150	10-25	Yellow
DYS635	150-202	15-28	Yellow
DYS390	207-255	17-29	Yellow
DYS439	263-307	6-17	Yellow
DYS392	314-362	4-20	Yellow
DYS643	368-423	6-17	Yellow
DYS393	101-145	7-18	Red
DYS458	159-215	10-24	Red
DYS385a/b	223-307	7-28	Red
DYS456	316-364	11-23	Red
Y-GATA-H4	374-414	8-18	Red

Table 1 – Y23 Allelic Ladder Specifications

[1] Y23 haplotypes consist of tetranucleotide repeats with the exception of the trinucleotide repeats DYS392 and DYS481, pentanucleotide repeats DYS438 and DYS643, and hexanucleotide repeat DYS448.

[2] Sizes in base pairs are approximate due to electrophoretic variation and are based on plus-A addition. These sizes are published in the *PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers.* (Promega Corporation).

[3] Ranges of alleles (i.e., 13-18) include only integers (i.e., 13, 14, 15, …, 18).

4.3 Positive Amplification Control (i.e., 2800M)

A. One positive control must be processed for amplification in parallel with each set or batch of samples.
B. If any sample(s) requires repreparation, the positive control and the appropriate ladder must also be reprepared.
C. If a set of samples has multiple injections of the positive control, at least one injection must exhibit all the expected allelic peaks ≥ the dye specific analytical thresholds (ATs), and must not exhibit any extraneous allelic peaks, with the exception of drop-in peaks, which will be assessed according to section 5.2 (Drop-in Assessment).
D. A positive control with a non-allelic peak(s) (e.g., stutter, spike, pull-up) may be interpreted.
E. See Table 2 for the expected positive control typing results using Y23:

Locus	2800M Control
DYS576	18
DYS389I	14
DYS448	19
DYS389II	31
DYS19	14
DYS391	10
DYS481	22
DYS549	13
DYS533	12
DYS438	9
DYS437	14
DYS570	17
DYS635	21
DYS390	24
DYS439	12
DYS392	13
DYS643	10
DYS393	13
DYS458	17
DYS385a/b	13, 16
DYS456	17
Y-GATA-H4	11

Table 2 – Expected Y23 STR Typing Results for the 2800M Positive Control

F. Per the Promega technical manual, increased forward stutter (i.e., n+4 and n+8) can be observed at DYS389I and DYS389II loci in the cell-line derived 2800M positive control DNA. This increased forward stutter ratio is inherent to this cell line DNA and

is not seen in amplification of DNA from human blood, body fluid or other human-derived samples.

G. Refer to the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570) for guidance if the positive amplification control does not exhibit the expected results.

4.4 Negative Amplification Control

Refer to the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570) for guidance on the evaluation of the negative amplification control. If drop-in peaks are present in the negative amplification control, samples amplified in parallel with this control should be assessed as described in section 5.2 (Drop-in Assessment).

4.5 Extraction Control (i.e., reagent blank)

Refer to the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570) for guidance on the evaluation of the extraction control. If drop-in peaks are present in the extraction control, samples extracted in parallel with this control should be assessed as described in section 5.2 (Drop-in Assessment).

5 PROCEDURE

5.1 DNA Profile Determination

5.1.1 Computer Assisted Allele Designations

A. The GMIDX software analyzes the data generated by the CE instruments and generates electropherogram data to be evaluated and interpreted.
    1. The analysis method settings for Y23 data are represented in Appendix A.
        i. The analysis method "PowerPlex_Y23" applies the dye-specific ATs in section 5.4.
        ii. The marker-specific stutter used by the analysis method is contained in the panel. Panel “PowerPlex_Y23” applies the stutter percentages in section 5.1.1.1.1.
    2. Electronic printouts of the GMIDX data will display the panel used.

B. A pink box surrounding a data point label indicates that the software has identified a data point as an artifact. The GMIDX software uses the terms “spike” and “OMR” (Outside Marker Range) to represent a variety of DNA artifacts. Peak labels may be edited according to this procedure.

C. Peaks interpreted as non-allelic may be deleted within GMIDX and will appear on the electropherogram with a single strikeout.

5.1.1.1 Identification of Peaks of Non-Genetic (Non-Allelic) Origin

A. Before the Y-STR typing results from a sample can be used for comparison purposes, it is necessary to identify any non-genetic peaks that do not represent allelic Y-STRs. These non-genetic peaks may be undesired PCR products (e.g., stutter, minus-A, and non-specific product), analytical artifacts (e.g., spikes and raised baseline),

instrumental limitations (e.g., matrix failure), or be introduced into the process (e.g., dissociated primer dye and non-specific peaks).[4]

B. Reproducible non-genetic peaks (e.g., stutter, non-template dependent nucleotide addition, dissociated dye, matrix failure, non-specific product) may be interpreted.[5] Non-reproducible non-genetic peaks (e.g., spikes and raised baseline) must be evaluated as specified.

C. Refer to the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570) for information regarding excessive DNA template and off-scale samples, raised sample baseline, non-template-dependent nucleotide addition, matrix failure resulting in pull-up, spikes, and dissociated primer dyes.

5.1.1.1.1 Stutter

A. The kit-specific stutter percentage guidelines provided in Table 3 are estimates (Average + 5 Standard Deviations) of the maximum expected stutter values at each locus in the Y23 amplification kit.

B. These values are expressed as a percentage relative to the source allelic peak height (i.e., % Stutter). Stutter peaks have been observed up to +/- 3 repeat units away from the source allelic peaks in both the absence and presence of intermediate stutter peaks.

C. If such atypical stutter peaks are due to excessive amounts of template DNA, the sample may be reamplified with less template DNA or reinjected for less time.

[4] The GMIDX software applies filtering to the sized Y-STR allelic data, which removes labels from peaks at any locus that meet the FBI-defined sizing and relative peak height criteria for stutter and/or minus-A.

[5] For purposes of interpreting DNA typing results, a peak need only be identified as being of non-genetic origin.

Locus	-3 Repeat Units	-2 Repeat Units	-1 Repeat Units	-0.5 Repeat Units	+0.5 Repeat Units	+1 Repeat Units	+2 Repeat Units
DYS576	2.1*	6.8 (11.1)	24.7 (66.5)			9.2 (11.7)	1.2*
DYS389I		4.5 (4.1)	13.0 (24.2)			15.8 (10.4)	
DYS448		6.2 (6.4)	9.2 (23.4)			9.6 (4.6)	
DYS389II		3.0 (2.5)	19.1 (24.5)			12.1 (10.1)	
DYS19		1.3*	14.8 (12.3)	11.5 (13.6)	4.6 (6.3)	4.4 (3.5)	
DYS391		5.4 (3.3)	16.1 (35.7)			5.2 (4.0)	
DYS481	4.7 (3.7)	8.7 (10.6)	36.0 (36.1)			9.3 (20.6)	2.6 (1.7)
DYS549		2.8 (3.5)	15.5 (21.0)			9.0 (20.9)	
DYS533		3.1 (2.1)	14.3 (20.8)			4.8 (6.1)	
DYS438		2.1*	7.3 (10.1)			10.1 (9.7)	
DYS437		3.2 (2.2)	10.1 (9.9)			5.6 (4.2)	
DYS570		6.2 (6.6)	19.3 (28.0)			3.4 (3.6)	
DYS635		5.3 (4.9)	16.7 (21.6)			9.1 (14.2)	
DYS390		5.8 (5.8)	18.8 (25.6)			4.6 (3.6)	
DYS439		6.8 (4.1)	16.6 (21.5)			13.0 (22.6)	
DYS392		4.0 (4.1)	21.8 (22.3)			14.7 (16.7)	3.6 (2.0)
DYS643		4.2 (2.2)	10.5 (24.3)			2.5 (1.7)	
DYS393		6.2 (10.9)	20.1 (28.2)			6.8 (16.0)	
DYS458		4.9 (7.0)	21.1 (24.8)			5.8 (4.2)	
DYS385a/b		3.5 (3.5)	24.9 (34.6)			8.5 (6.9)	
DYS456	1.0*	5.7 (7.2)	20.1 (24.7)			5.8 (9.2)	4.4 (2.4)
Y-GATA-H4		8.6 (7.1)	20.6 (41.1)			14.3 (19.3)	

Values in parentheses are the maximum stutter percentages observed in validation.
*These values are from a single observation of stutter at that location in validation, which is used as the maximum expected stutter percentage.
Note: Y23 haplotypes consist of tetranucleotide repeats with the exception of the trinucleotide repeats DYS392 and DYS481, pentanucleotide repeats DYS438 and DYS643, and hexanucleotide repeat DYS448.

Table 3 – Maximum Expected Y23 STR Loci Stutter Percentages

5.1.1.1.2 Non-Specific Peaks

A. An N-2 peak is frequently observed at the DYS19 locus as an off-ladder (OL) allele. A "stutter" filter accounts for this generally reproducible artifact.
B. Previously observed DNA dependent and DNA independent artifacts are listed in Appendix C.
C. Additional peaks of non-genetic origin are described in the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570).

5.1.1.1.3 Off-Ladder (OL) Alleles

A. If an allele fails to size within a defined allele category (e.g., a bin or a virtual bin), it must be assigned a size using the following criteria.
B. Any sample containing an OL allele may be re-injected.
C. An OL allele may be a microvariant that sizes between two ladder alleles. For example, if an OL allele occurs between the 12 and 13 ladder alleles and is approximately 1 bp larger than the 12 allele, it is designated as 12.1; 2 bp larger is designated 12.2; and so on.
D. If an OL allele does not fall within the size range of any locus-specific ladder, which includes the flanking virtual bins, it should be associated with one of the two loci between which it falls.
    1. For single-source samples, if the OL allele is flanked by a locus with one peak and a locus with no peaks (with the exception of DYS385 a/b), the OL allele is assigned to the latter locus.
    2. Generally, if both loci between which an OL allele falls each display a single allele, the OL allele may be assigned to the locus closest in size.[6]
    3. If determination of the locus assignment is not possible, both loci that flank the OL allele must be deemed inconclusive for matching/statistical purposes.
    4. If the OL allele is smaller in size than the smallest respective virtual bin, or larger in size than the largest respective virtual bin, the number of repeats in the allele should be estimated.
    5. When loci are closely spaced on the x-axis of an electropherogram, an above or below OL allele may be observed within the size range of a flanking locus.

5.2 Drop-in Assessment

Drop-in is defined as the observation of 1 or 2 unexplained peaks in a profile present at ≤350 RFU. These peaks may be reproducible during reinjection, but are not reproducible if the sample is reamplified. Peaks may be designated as drop-in independent of size (bp) or bin/OL status of the peak within the profile. A single-source profile with an identified drop-in peak(s) should have no other indication of contamination in the sample (i.e., additional peaks in non-stutter positions <AT, see section 5.3 (Contamination Assessment)).

A. Profiles or control samples (positive amplification control, negative amplification control, reagent blank) that are suspected to contain a drop-in peak(s) should be re-injected or re-prepared; however, the reinjection should be assessed for the same peak(s) present <AT.
    1. If the peak is not reproducible, it may be interpreted as an artifact. The injection without the peak may be used for interpretation.
    2. If the peak is present, above or below the AT, the profile should be treated as containing drop-in, and generally, when of similar data quality, the injection containing the drop-in peak will be used for interpretation.

[6] To facilitate the interpretation of OL alleles, the Examiner may consult a listing of such alleles recorded at https://strbase.nist.gov/.

3. Generally, if 2 drop-in peaks occur, they are at different loci in a sample. If 2 drop-in peaks are suspected at one locus, consult with the Technical Leader (TL) for guidance.
4. The same drop-in peak should not be observed in more than one control (see section 5.3 (Contamination Assessment).

B. If a peak(s) consistent with drop-in occurs in a control sample, and other controls display the same peak, the controls and samples should be assessed for contamination (see section 5.3). If other controls do not display the same peak(s), the peak may be considered a drop-in peak(s).
    1. If a drop-in peak(s) is present in a control sample, the following rules for interpretation apply:
        i. The drop-in peak(s) will be noted on the interpretation sheet.
        ii. For any sample(s) processed in parallel with the control(s), any locus (loci) where the drop-in occurred will not be used for matching/statistical purposes, regardless of whether the sample contains the same peak as is present as drop-in in the control.
            a. The locus will not be used for searching in YHRD.
            b. The locus will be noted as not used for interpretation for each affected sample on the interpretation sheet.
        iii. If the only results for a sample are at the locus (loci) that is affected by drop-in for the corresponding controls, the sample will be reported as not suitable for matching/statistical purposes. Refer to the report wording section 7.2 (Other Reportable DNA Typing Results).

C. If a suspected drop-in peak(s) is present in a sample and not in any associated controls, that locus will not be used for matching/statistical purposes for the affected sample and any sample processed in parallel with the affected sample.

5.3 Contamination Assessment

Refer to the appropriate DNA procedure for the interpretation of nuclear DNA typing results (i.e., BIO-570) for guidance on the evaluation of contamination in samples or controls. Peaks in controls that are not consistent with drop-in must be evaluated for contamination.

5.4 Application of Peak Height Thresholds to Allelic Peaks

A. The Analytical Thresholds (ATs) for Y23 are:
    i. Blue: 100 RFU
    ii. Green: 125 RFU
    iii. Yellow: 135 RFU
    iv. Red: 135 RFU

B. At all loci other than DYS385a/b, any allelic peak detected at or above the AT may be used for matching/statistical purposes.

C. The Stochastic Threshold (ST) is 950 RFU and is an empirically determined parameter established specifically for the DYS385a/b locus. The ST is used to evaluate potential allelic drop-out in a profile.

D. If no allele is detected at a locus, a null allele may be declared at that locus if the allelic peaks at all other loci are at or above the ST. If more than one locus is suspected to be null, consult the TL for guidance. (Also see Appendix B)

5.5 Interpretation of Y-STR Typing Results

DNA typing results from evidentiary samples must be interpreted before the comparison to any known samples. This interpretation will be documented in the case file.

When there are multiple amplifications and/or injections for a given sample extract, generally the one that provides the most information will be used for reporting. Data where saturation interferes with interpretation may require that an alternative amplification/injection is used.

5.5.1 Peak Height Ratio Assessment

For loci that contain more than one allelic peak, peak height ratios can be used to associate two alleles to a common source (i.e., duplication) or to establish the presence of a DNA mixture. Peak height ratio assessments are generally not used in the interpretation of knowns and items that are expected to have originated from a single source, such as bones and alternate knowns. The alternate known profile may be obtained as a single-source or as a major contributor typing result.

Peak height ratios (PHR) are calculated by dividing the peak height of the allele with the lower RFU value by the peak height of the allele with the higher RFU value, expressed as a percentage.

5.5.2 Determination of the Number of Contributors to Y-STR Typing Results

A. A profile is generally considered to have originated from a single male individual if one allele (other than DYS385a/b) is present at all loci for which typing results were obtained.
    1. The Y-STR typing results at DYS385a/b may be used for matching/statistical purposes if:
        i. two alleles are detected (above or below the ST)
        ii. if one allele is detected ≥ST.
    2. If only one allelic peak is detected <ST at DYS385a/b, the locus may be used for exclusionary purposes (considering the potential for drop out) but may not be used for matching/statistical purposes.
B. A profile is generally considered to have originated from more than one male individual if two or more alleles are present at two or more loci, other than DYS385a/b. The classification of any DNA profile as a mixture must be based on an evaluation of the DNA profile in its entirety.
C. Peaks that exceed the expected stutter percentages must be evaluated considering both the maximum expected stutter percentages and the maximum stutter percentages observed in validation. (Refer to Table 3)
    1. A peak significantly above the expected stutter percentage is more likely to be allelic. However, when peaks are <2500 RFU, excessive stutter was

observed in validation at levels significantly above the stutter thresholds. When only one peak in stutter position is present in these lower level samples, regardless of locus size, this peak may be considered a stutter peak and should not increase the NOC.

2. Other apparent peaks <AT in non-stutter positions suggest that the peak is potentially allelic. Peaks in stutter positions <AT may be stochastically amplified and should not be solely relied upon to determine the NOC.
3. If the sample is a reference sample and expected to be single source, then these peaks can confidently be called stutter if there is no other evidence of contamination.
4. For apparent single source samples, a peak in a stutter position that exceeds the expected stutter percentage may be interpreted as a stutter peak for purposes of determining the number of contributors to the sample. Generally, this interpretation is limited to two instances; if a sample is suspected to be single source and has more than two instances of elevated stutter, consult the TL for guidance.
5. For mixed samples, a peak in stutter position that exceeds the expected stutter percentage may be interpreted as a stutter peak for purposes of determining the number of contributors to the sample.

D. A peak(s) that meets the basic criteria for drop-in (i.e., 1 or 2 unexplained peaks in a profile present at ≤350 RFU, see 5.2) must be evaluated considering:
   1. A peak that conforms to excess stutter expectations has more potential to be stutter.
   2. A peak at a small (<200 bp) locus where possible minor contributor types are expected has more potential to be allelic.
   3. If the peak is inconsistent (e.g., RFU) with other (minor) contributor peaks, it has more potential to be drop-in.
   4. Other apparent peaks <AT in non-stutter positions suggest that the peak is potentially allelic.
   5. If the sample is a reference sample and expected to be single source, then the peak may be interpreted as drop-in if there is no other evidence of contamination. Generally, the consideration of drop-in during NOC determination should be limited to one peak.

E. Duplications
   1. If two alleles are present at any locus other than DYS385a/b, a duplication event may have occurred. To be declared a duplication, the alleles should have a PHR generally consistent with the following expectations:

Height of taller peak	PHR expectation
≥10,000 RFU	≥60%
2500 to <10,000 RFU	≥50%
950 to <2500 RFU	≥30%

      i. When the taller peak is <950 RFU, there is no PHR expectation as both peaks are in the stochastic range.

ii. Other explanations for these peaks (e.g., stutter or drop-in) may be more likely as duplications are expected in ≤1% of samples tested.
iii. If a sample is suspected to be a duplication but does not meet these expectations, consult the TL for guidance.

2. Because a duplicated allele is typically one repeat unit larger or smaller than the other allele, the presence of two alleles at a given locus that differ in size by more than one repeat unit is generally indicative of a mixed profile.
3. The proximity of certain loci along the Y-chromosome allows for the simultaneous duplication of alleles at multiple loci. Generally, loci that are less than 1 Mb apart could potentially be duplicated together.[7] See Appendix B for the relative positioning and distance (i.e., Mb) of the Y23 loci on the Y-chromosome.
4. A profile in which three allelic peaks are observed at a single locus, but in which no other typing results indicate the presence of a mixture, may be concluded to be a single-source profile possessing a tri-allelic locus after consulting with the TL.
5. The presence of more than one allele at DYS438 and/or Y GATA H4 is generally indicative of a mixed profile.

F. The number of contributors to a mixture should be based upon greatest number of alleles detected per locus, and, because of the potential for duplication, should generally be observed at two or more loci (other than DYS385a/b). This is the initial estimate of the number of contributors to the sample.
G. Using the loci with the largest number of alleles, assess the ratio of contributors. Evaluate peak height imbalance and account for allele sharing to determine if the number of contributors should be increased.
H. Apply the general pattern of number of contributors and mixture ratio across the profile to determine if other loci are consistent with this pattern or if the number of contributors should be increased or decreased by one. Loci with more alleles will be the most informative for this assessment. Additionally, apparent peaks <AT in non-stutter positions may also be considered, especially for low level samples.

5.5.3 Deduced Single-Source Profiles by Separation of Expected Typing Results

A. For mixed typing results, when the presence of an individual's DNA in the sample can be reasonably expected, the Y-STR typing results from the assumed contributor should be separated from the other mixture results to facilitate identification of the foreign alleles.
B. If sharing of alleles among the conditional male known profile and an additional male is suspected at a locus, separation of each individual's alleles may not be possible at that locus.
C. This approach can also be used when another known male is expected to have contributed biological material to the mixed profile (e.g., consensual male). If more than one known male contributor is expected, each individual's alleles should be subtracted from the profile.

[7] A Y-STR haplotype exhibiting duplication at loci DYS437, DYS439 and DYS389I/II has been observed (Butler, 2005).

D. This approach can also be applied to evidentiary items from which DNA is isolated by means of a differential extraction. In such situations, the single-source or major contributor typing results from one fraction may be used as a conditional known profile(s) applied to the complementary fraction.
E. Mixtures comprised of three or more individuals generally may only be used for exclusionary purposes. If an apparent distinguishable mixture remains after subtraction of the assumed contributor(s), consult with the TL for additional guidance.

5.5.4 Deduced Single-Source Profiles from Distinguishable Mixtures

A. A distinguishable mixture is a Y-STR typing result from a sample for which alleles can be attributed to individual major/minor male donors. In order to determine major/minor contributors to a mixture, every locus must be distinguishable except for DYS385a/b.
    1. When a mixed profile contains at least one allele above the AT at every locus (i.e., full profile), the following should be applied:
        i. The PHR of alleles at all loci that exhibit two alleles (with the exception of DYS385a/b) must be <60% in order to assign a major contributor. When all loci meet this requirement, the allele displaying the greater peak height at each locus may be attributed to the major contributor.
        ii. If only one allele is detected at a given locus, that allele may be attributed to the major contributor at all loci, except DYS385a/b, which must meet the below criteria.
        iii. For DYS385a/b, when the sample is a mixture of only two males:
            a. The two tallest alleles may be attributed to the major contributor and may be used for matching/statistical purposes if:
                1. The two tallest alleles are ≥ST and
                2. The two tallest alleles have a PHR ≥60% and
                3. The third tallest allele is <60% of the second tallest allele (or if there is no third peak).
            b. The single tallest allele may be attributed to the major contributor and may be used for matching/statistical purposes if:
                1. The tallest allele is ≥ST and
                2. The second tallest allele, if present, is ≤20% of the tallest allele.
                3. There may be a third allele, or there may be no minor alleles present.
            c. The two shortest alleles may be attributed to the minor contributor and used for matching/statistical purposes when:
                1. If there are 4 alleles:
                    (i) The two tallest alleles are ≥ST and
                    (ii) The two tallest alleles have a PHR ≥60% and

(iii) The third tallest allele is <60% of the second tallest allele.

2. If there are 3 alleles (i.e., the single tallest allele is attributed to the major), the two shortest alleles can be attributed to the minor if:
   (i) The tallest allele is ≥ST and
   (ii) Both shortest alleles are ≤20% of the tallest allele.
3. If there are 2 alleles, the minor contributor type cannot be determined.

d. If the above criteria are not met, the major and/or minor contributor alleles may only be used for exclusionary purposes if appropriate but may not be used for matching/statistical purposes.

2. When a mixed profile fails to produce alleles above the AT at one or more loci (i.e., partial profile), the following should be applied:
   i. The PHR at all loci that exhibit two alleles (with the exception of DYS385a/b) must be <25% in order to assign a major contributor. When all loci meet this requirement, the allele displaying the greater peak height at each locus may be attributed to the major contributor.
      a. DYS385a/b may be used for exclusionary purposes if appropriate but may not be used for matching/statistical purposes.
   ii. If only one allele is detected at a given locus, that allele may be attributed to the major contributor at all loci except DYS385a/b.

B. Mixtures comprised of three or more individuals generally may only be used for exclusionary purposes. If a major contributor can be discerned from a mixture comprised of three or more individuals, consult the TL for guidance.
C. Due to the possibility that the minor contributor's alleles may be either shared by the major contributor (and thus masked) or potentially not detectable (i.e., < dye-specific AT), determination of the minor contributor profile may not be possible at some loci.
D. Generally, a multi-locus, mixed sample that contains one or more true minor contributors can be expected to display at least one allelic peak in a non-stutter position.

5.5.5 Interpretation of Y-STR Typing Results for Indistinguishable Mixtures

An indistinguishable mixture is a Y-STR typing result from a sample for which alleles cannot be attributed to individual donors. Indistinguishable mixtures may be used for exclusionary purposes only.

5.6 Reporting Y-STR Results and Conclusions

The results and/or conclusions for specimens subjected to DNA analysis will generally be reported in narrative form with tables for statistical information, when applicable.

A. Only single-source or deduced single-source (from separation of expected typing results or distinguishable mixtures) Y-STR profiles may be used for matching or statistical purposes.
B. For mixtures, the number of contributors must be determined and recorded on the interpretation sheet.
    1. For an indistinguishable mixture comparison, an exclusion is declared when the types of a known reference item cannot be included in the types present at the corresponding loci of the mixture profile, considering the number of contributors and the potential for dropout.
    2. If an exclusion of a known reference cannot be declared for an indistinguishable mixture, no conclusion regarding the known reference sample can be reported.

5.6.1 Direct Comparisons

A. An exclusion is declared when two single source or deduced single source profiles are different at one or more loci.
B. An exclusion for a direct mixture comparison requires differences at one or more loci.
C. An inclusion is declared when profiles are the same at all loci for which interpretable DNA typing results were obtained.
D. Each DNA association or inclusion must be clearly and properly qualified with either a statistic or a qualitative statement. A qualitative statement not based on a statistical calculation should be limited to situations in which the presence of an individual's DNA on an item is reasonably expected. The provenance of the sample must be established in the case record when statistics are not calculated.
    1. The Y- Chromosome Haplotype Reference Database (YHRD) calculates 95% Upper Confidence Interval (95% UCI) values using several general United States population groups: African American, Asian, Caucasian, Hispanic, and Native American. The Eskimo Aleut population may also be calculated. For all samples, the LRs for African American, Caucasian, and Hispanic are considered for reporting. For cases submitted from Native American land, the Native American LR is also considered. For all cases submitted from Alaska, the Native American LR and the Eskimo Aleut LR are also considered.[8]
    2. The single lowest LR value across all populations considered is determined separately for masked and for transient searches (see Calculations). The higher of these LR values is reported. The LR is generally truncated to two significant digits for reporting. However, if this value is less than 10, it is truncated to one significant digit for reporting.

[8] Inupiat and Yupik populations are contained within the Eskimo Aleut metapopulation, while the Athabaskan population is contained within the Native American subpopulation. Therefore, both Eskimo Aleut and Native American subpopulations are considered.

For example, if the 95% UCI LR values from YHRD are:

Population	Masked Search	Transient Search
African American	1000	2300
Caucasian	1200	2800
Hispanic	1100	2000

The lowest Masked Search LR is 1000, and the lowest Transient Search LR is 2000. The higher of these values, 2000, is reported.

3. If one of the compared profiles was developed with the Yfiler™ kit, then only a single masked search with the Y17 dataset is needed. The single lowest LR value across all populations is reported.
4. The magnitude of the LR relates to the degree of support provided by the evidence under the tested hypotheses and assumptions. A qualitative statement will be reported based on Table 4.

LR	Qualitative Equivalent
≥1,000,000	Very strong support for Inclusion
10,000 to <1,000,000	Strong support for Inclusion
100 to <10,000	Moderate support for Inclusion
2 to <100	Limited support for Inclusion
1	Uninformative
>1/100 to 1/2	Limited support for exclusion
≤1/100	Exclusion

Table 4 – Qualitative Equivalent Scale for Y-STR Likelihood Ratios

5.6.2 Lineage Comparisons[9]

A. A statement should be included to define the relationships of the submitted reference samples to each other and, if appropriate, the named person of interest or missing person.
B. Profiles from different sources (e.g., different individuals, or an unknown sample compared to the paternal relative of a person of interest) that are the same at all loci for which interpretable DNA typing results were obtained are consistent with originating from the same paternal lineage. Statistics will be provided following guidance for direct comparison inclusions above.
C. When differences are observed, they will be counted based on the total number of repeat unit differences (i.e., step differences) across all loci.
    1. For each locus that is different, each step counts as one difference.
    2. "1 difference" is defined as a one-step difference at a single locus.
    3. "2 differences" is defined as either:

[9] For lineage comparisons, differences between two items may occur due to (a) mutation among relatives in the same paternal lineage or (b) different paternal lineages. The Y23 amplification kit includes two named rapidly mutating loci: DYS570 and DYS576.

i. a one-step difference at each of two loci or
ii. a two-step difference at one locus

4. "3 differences" is defined as either:
   i. A one-step difference at each of three loci
   ii. A two-step difference at a locus and a one-step difference at another locus or
   iii. A three-step difference at a single locus

D. A visual exclusion is declared when three differences are observed. Differences that could be explained by allelic dropout and/or drop-in as applicable cannot be the basis or contribute to the basis for an exclusion.

E. A paternal lineage comparison is declared inconclusive when the profiles from putative male relatives have one or two differences, considering the potential for dropout and/or drop-in as applicable.

F. Based on the structure of a Y-STR locus, a single mutation can cause differences at one or more loci when comparing results from individuals in the same paternal lineage. For example, at DYS389I & II, a single mutation may result in differences at both the DYS389I and the DYS389II loci. Similarly, a single mutation may result in an apparent duplication at DYS437 as well as an apparent null at DYS448. These differences alone cannot be the basis of a paternal lineage exclusion, and such comparisons are generally reported as inconclusive.

6 CALCULATIONS

6.1 Statistics Using the Y- Chromosome Haplotype Reference Database (YHRD)

A. The primary database used for estimating the haplotype frequency of a single Y-STR profile is the Y-Chromosome Haplotype Reference Database (YHRD; https://yhrd.org), which is used to provide Likelihood Ratios from frequency estimates for African American, Caucasian, Hispanic, Native American, and Eskimo Aleut population groups, when applicable.

B. If the compared profiles have results at different loci (e.g., partial profiles), only loci that are shared between the profiles should be searched in YHRD.

C. If both of the compared profiles were developed using the Y23 kit, likelihood ratios will be calculated using a masked search with the Y23 dataset, and a transient search with the larger Y17 dataset.[10] The more informative search result will be reported.

D. If one of the compared profiles was developed using the Yfiler™ kit, only a single masked search with the Y17 dataset is needed. Note: There may be differences in the user interface images depicted that do not affect the statistical estimates calculated.

[10] A "masked search" for a Y23 profile in YHRD is a search with the Y23 dataset and the Y23 kit. This searches only profiles in the database with complete Y23 profiles. A "transient search" for a Y23 profile is a search with the Yfiler (Y17) dataset and the Y23 kit. This searches any profiles that contain all Y17 data and additionally uses any loci in the queried profile beyond those of the dataset to exclude non-matches.

6.1.1 Performing the YHRD Search

Go to the YHRD website (http://yhrd.org). Select the "Estimate Frequency" tab. Whenever possible, for profiles where electropherograms reflect the searched profile, choose the "Use your … GeneMapper® ID/ID-X…" option to import Y-STR profiles directly from an exported GMIDX file.

Alternatively, profiles can be manually entered by selecting "Manually enter the haplotype…" (Figure 1).

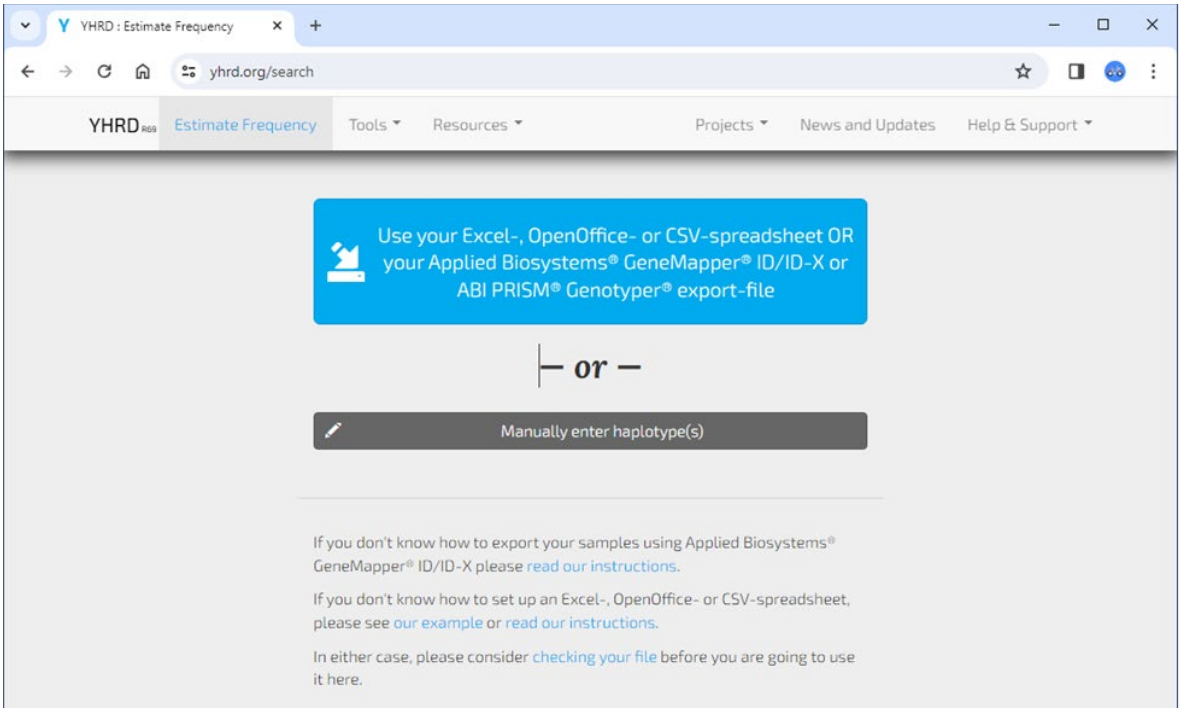

Figure 1 – YHRD Search the Database Page

A. To perform a masked search, choose the "PowerPlexY23" kit, and the "Y23" dataset, then select "Search". (Figure 2)

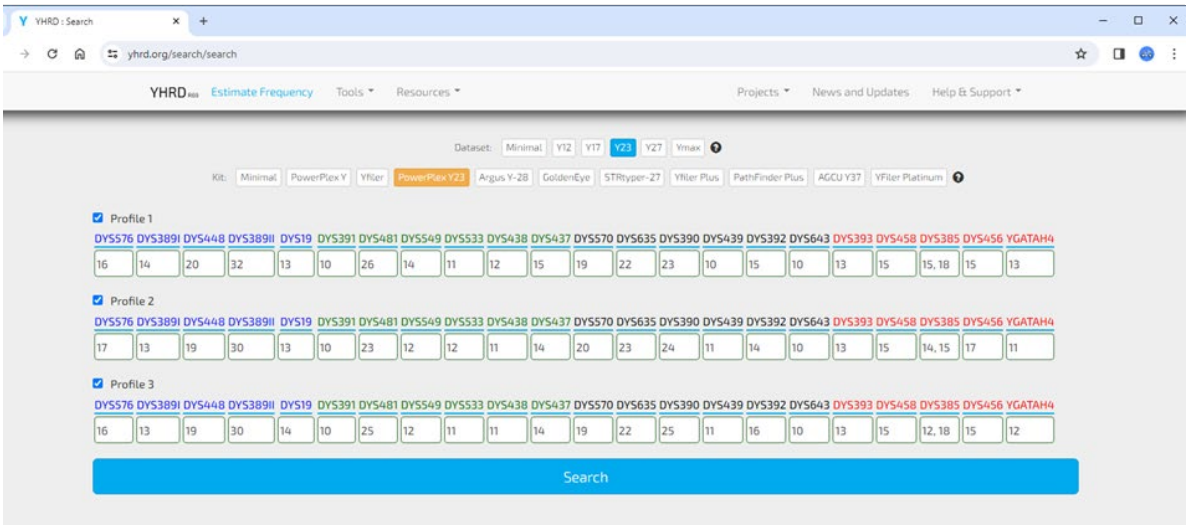

Figure 2 – YHRD Y23 Masked Search

B. To perform a transient search, choose the "PowerPlex Y23" kit and the "Y17" Dataset, then select "Search." (Figure 3)

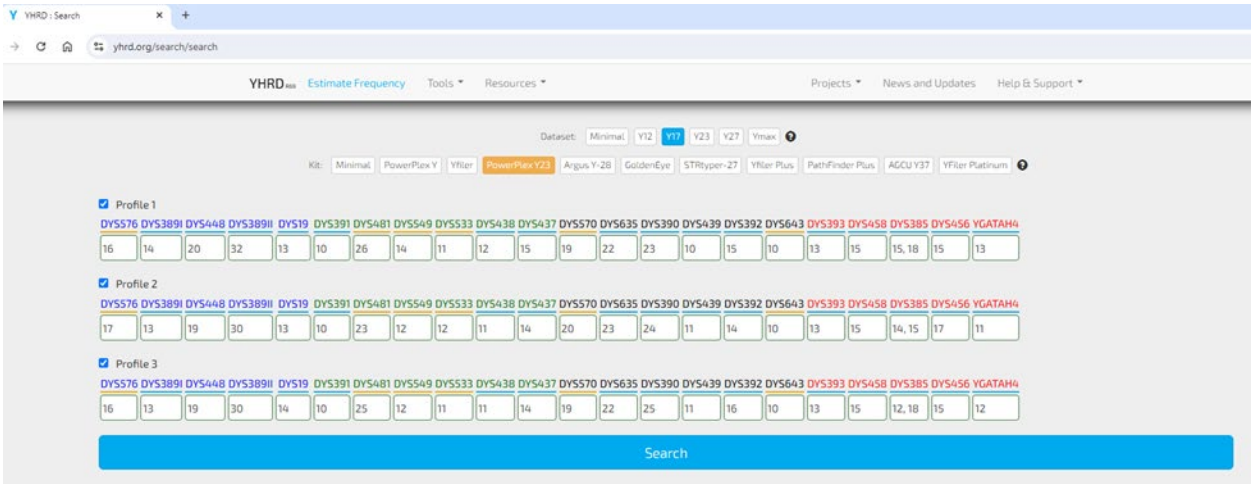

Figure 3 – YHRD Y23 Transient Search

C. For each search, select "Add feature to this report", and choose "National Database (with Subpopulations, 2014 SWGDAM-compliant)". Note that YHRD will only search against the database haplotypes that contain the same or more loci than were entered.

D. The number of observations of the searched haplotype is listed for the African American, Asian, Caucasian, Hispanic, and Native American United States subpopulations.

E. If appropriate, add the Eskimo Aleut subpopulation (see 5.6.1 D.1) by selecting "Add feature to this report" and choosing "Metapopulation". Change the default "–Admixed – U.S. Caucasian American" metapopulation in the report to "Eskimo Aleut". Change the default two-sided 95% CI calculation to "95% Upper Confidence Interval (UCI)".

F. For instances in which the haplotype has been observed in the database ($x > 0$), YHRD calculates a one-sided 95% confidence interval using the Clopper and Pearson formula:[11]

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=\alpha$$

where k = 0,1,2,3, … x observations; n = database size; and x = number of observations of the haplotype in the database. When $\alpha = 0.05$, solving for p_0 yields the population frequency of the upper bound of the 95% upper confidence limit.

[11] The Clopper and Pearson formula calculates the exact confidence interval. The exact confidence interval is a cumulative binomial distribution for all values from 0 to x matches given a sample of size *n* and frequency *p*. The listed formula is for the upper limit of a one-tailed confidence interval.

This value is the reported "95% UCI" in YHRD and is the profile probability: the probability of observing the haplotype after adjusting for sampling uncertainty.

G. For instances in which the haplotype has not been observed in the database, the following formula (derived with x = 0 in the Clopper and Pearson formula) is used to estimate the upper bound of the 95% confidence limit, p_0:

$$p_0 = 1 - \alpha^{1/n}$$

where α = 0.05 or 5% and n = database size. For example, if a particular haplotype is not observed in a database of 2000 haplotypes, then the 95% upper confidence limit is estimated to be $1 - (0.05)^{1/2000} = 0.0014977$ or 1 in every 778 haplotypes. Note that when α = 0.05, p_0 is very close to 3/n (e.g., 3/2000 = 0.0015).

H. The likelihood ratio (LR) of the upper bound frequency estimate describes how much more likely the results are if the matching profiles are from the same source or the same paternal lineage rather than if one profile is from an unknown, unrelated individual or lineage. The LR should be calculated:

$$\mathrm{LR} = \frac{1}{95\%\ UCI}$$

YHRD provides the 95% UCI expressed as 1 in X. The LR is the reciprocal of the 95% UCI, which simplifies to LR = X.

7 SUGGESTED REPORTING LANGUAGE

The results and/or conclusions for specimens subjected to DNA analysis will generally be reported in narrative form. The formatting and administrative information required in a report are described in the appropriate FBI Laboratory Operations Manual practices and the DNA Procedures Manual. For guidance on reporting language for Introductory Statements, Amplification Kit Used, Comparisons to Previously Reported Results, Alternate Reference Samples, Elimination Samples, and Differentially Extracted Samples refer to the appropriate interpretation protocols of the DNA Procedures Manual (i.e., BIO-570).

7.1 Report Wording Examples

See section 7.3 for endnote language, denoted as A-G in these examples.

7.1.1 Direct Comparisons

7.1.1.1 Match and Exclusion

The Y-STR typing results from item 1 were interpreted as originating from two individuals. The major contributor profile from item 1 is 1,100 times more likely if JAMES is the major contributor than if an unknown, unrelated male is the major contributor.[A]

Person of Interest (POI)	Likelihood Ratio (LR)[B]	Level of Support[C]
JAMES	1,100	Moderate support for **Inclusion**

The following individuals are excluded as potential contributors to the Y-STR typing results obtained from item 1:[A]

- JONES
- WHITE

7.1.1.2 Uninformative

The Y-STR typing results from item 1 were interpreted as originating from one individual. The Y-STR typing results from item 1 are equally likely if JAMES is the contributor than if an unknown, unrelated male is the contributor.[D]

7.1.2 Lineage Comparisons

7.1.2.1 Paternal Relative as Alternate Reference

Information provided by [person, agency] identifies BROWN as the biological father of JAMES GARCIA. Based on the Y-STR typing results obtained from Item 2 and BROWN, item 2 could have originated from GARCIA.[A] These results are 310 times more likely if item 2 is from GARCIA than if item 2 is from an unknown, unrelated paternal lineage.

Likelihood Ratio (LR)[B]	Level of Support[C]
310	Moderate support for **Inclusion**

7.1.2.2 Paternal Relatedness – Inclusion

Information provided by [person, agency] identifies JOHNSON as the potential biological brother of MILLER. Based on the Y-STR typing results from JOHNSON and MILLER, these individuals could be biological brothers.[A] These results are 960 times more likely if JOHNSON and MILLER are paternal relatives than if JOHNSON is from an unknown paternal lineage, unrelated to MILLER.

Likelihood Ratio (LR)[B]	Level of Support[C]
960	Moderate support for Inclusion

7.1.2.3 Paternal Relatedness – Exclusion

Information provided by [person, agency] identifies JOHNSON as the potential biological brother of MILLER. Based on the Y-STR typing results, JOHNSON is excluded as a biological brother of MILLER.[A]

7.1.2.4 Paternal Relatedness – Inconclusive

Information provided by [person, agency] identifies SPARKS as the potential biological father of MILLER. Based on the Y-STR typing results, no conclusion as to the possible biological relationship between MILLER and SPARKS can be made.[E]

7.1.3 Assumed Contributors (e.g., intimate sample or consensual partner)

7.1.3.1 No DNA Unlike

The Y-STR typing results for item 3 indicate the presence of a single male individual, and no Y-STR typing results unlike JONES were obtained from item 3.

WHITE is excluded as a potential contributor to the Y-STR typing results obtained from item 3.[A]

7.1.3.2 Match to Deduced Profile

The Y-STR typing results from item 3 were interpreted as originating from two individuals, one of whom is JONES. The Y-STR typing results unlike JONES are 230 times more likely if WHITE is the contributor than if an unknown, unrelated male is the contributor.[A]

Person of Interest (POI)	Likelihood Ratio (LR)[B]	Level of Support[C]
WHITE	230	Moderate support for **Inclusion**

BROWN is excluded as a potential contributor to the Y-STR typing results obtained from item 3.[A]

7.1.4 Indistinguishable Mixture Comparisons

7.1.4.1 Unsub

The Y-STR typing results for item 1 indicate the presence of DNA from two male individuals. Because these mixture results cannot be attributed to individual contributors, they are not suitable for matching purposes; however, they may be used for exclusionary purposes.

7.1.4.2 Indistinguishable comparison

The Y-STR typing results for item 1 indicate the presence of DNA from three male individuals. Because these mixture results cannot be attributed to individual contributors, they are not suitable for matching purposes; however, they may be used for exclusionary purposes. SMITH is excluded as a potential contributor of the DNA obtained from item 1.[A]

No conclusion can be provided for JONES.

7.2 Other Reportable DNA Typing Results

A. When no reference sample is provided for comparison, the results should be reported as follows:

   The Y-STR typing results for item 1 indicate a single male individual and are suitable for comparison purposes.

   The Y-STR typing results for item 1 indicate the presence of DNA from three male individuals and are suitable for comparison purposes.

B. For samples for which insufficient DNA is recovered for DNA typing, this information should be reported as follows:

No Y-STR typing results[F] were obtained from item 1; therefore, no comparisons could be made to SMITH.

C. If the only locus (loci) in an evidentiary profile is deemed unsuitable for analysis because an associated control contained a drop-in peak at that locus (loci), it will generally be reported as follows:

Male DNA was obtained from item 1. The results for item 1 are not suitable for comparisons.[G]

D. Y-STR typing results may be obtained that are suitable for entry into the Combined DNA Index System (CODIS) or other appropriate database. Note that the rapidly mutating loci DYS570 and DYS576 are not suitable for entry into CODIS. Refer to the DNA procedure for reporting STR data (i.e., BIO-570) for suggested reporting language.

7.3 Associated Endnotes for Reporting Language

An endnote describing the Y23 kit and limitations will be added to the report when the Y23 kit is used, for example:

DNA analysis was performed using the Quantifiler™ Trio DNA Quantification Kit for the quantitation of human DNA and the PowerPlex® Y23 System for the DNA typing of short tandem repeats (STRs). The Y-STR loci are located on the male Y-chromosome and are transmitted through a paternal lineage from father to son. Barring mutation, all males in the same paternal lineage have the same Y-STR typing results. A paternal lineage consists of those male relatives to whom the same Y-chromosome has been transmitted from a common ancestor. Additionally, unrelated males may share the same Y-STR profile.

Endnotes referenced above are generally as follows:

[A] Barring mutation, any male relative within the same paternal lineage has the same Y-STR profile and would also be expected to be included/excluded as a potential contributor.

[B] The likelihood ratio is a statistical approach that compares the probabilities of observing the DNA results under two alternative propositions. Calculations were performed using the African American, Caucasian, and Hispanic populations in the Y Chromosome Haplotype Reference Database (release xxx) using full and reduced locus searches, when applicable. The lowest calculated population likelihood ratio is reported for the most informative search.

[C] These likelihood ratio ranges provide the following support for Y-STR conclusions:

Likelihood Ratios:	Qualitative Equivalent:
≥ 1,000,000	Very strong support for Inclusion
10,000 to < 1,000,000	Strong support for Inclusion
100 to < 10,000	Moderate support for Inclusion
2 to < 100	Limited support for Inclusion
1	Uninformative
> 1/100 to 1/2	Limited support for Exclusion
≤ 1/100	Exclusion

[D] This conclusion is drawn when the likelihood ratio is equal to 1; this comparison is uninformative.

[E] A paternal lineage comparison is declared inconclusive when profiles are found to differ at one or two loci, considering the potential that allele dropout occurred.

[F] Insufficient DNA quality and/or quantity can affect the ability to generate a DNA typing result.

[G] Drop-in is a rare event where one or two low level results are present that do not originate from the evidence. A drop-in peak was observed in an associated control at the locus (loci) for which DNA typing results were obtained; therefore, the results obtained from this item are not suitable for comparisons.

8 LIMITATIONS

A. These procedures do not exhaust the possible list of the results that may be encountered by the Examiner. For those results not specifically described, conclusions should be drawn using the procedures given for the results above that are similar in concept and/or origin.
B. It is sometimes necessary to consume a sample in its entirety to ensure that the best attempt possible is made to obtain DNA typing results for comparison purposes. Should the total consumption of a sample be required, an Examiner should obtain and record permission from the contributing agency or other responsible office prior to testing.
C. A paternal lineage consists of those male relatives to whom the same Y-chromosome has been transmitted from a common ancestor. Barring mutation, all male relatives within the same paternal lineage have the same Y-STR profile. Attribution of the Y-STR typing results to a single individual, to the exclusion of relatives in the paternal lineage, is not possible based on Y-chromosome loci. Additionally, unrelated individuals may exhibit the same Y-STR profile; therefore, attribution of an individual to a paternal lineage is not possible based on Y-chromosome loci.

9 REFERENCES

ANSI/ASB Standard 040, Standard for Forensic DNA Interpretation and Comparison Protocols, First Edition, 2019. Available at nist.gov/osac/osac-registry.

Butler, J.M., Decker, A.E., Kline, M.C., and Vallone, P.M. Chromosomal duplications along the Y-Chromosome and their potential impact on Y-STR interpretation. *J. Forensic Sci*. (2005) 50:853-859.

Clopper, C., Pearson, E. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika* (1934) 26:404-413.

Promega. PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual, P/N 9FB160, Madison, WI.

Willuweit S. and Roewer L. The new Y Chromosome Haplotype Reference Database. *Forensic Sci. Int. Genet.* (2015) 15:43-8

10 REVISION HISTORY

Revision	Issued	Changes
00	02/04/2022	Reformatted DNA 215-7 into new template and assigned new Doc ID. Reworded for clarity and to add requirements to document the interpretation and report the number of contributors.
01	09/04/2024	Revisions to replace Yfiler with Y23

11 APPENDIX A: GMIDX ANALYSIS SETTINGS

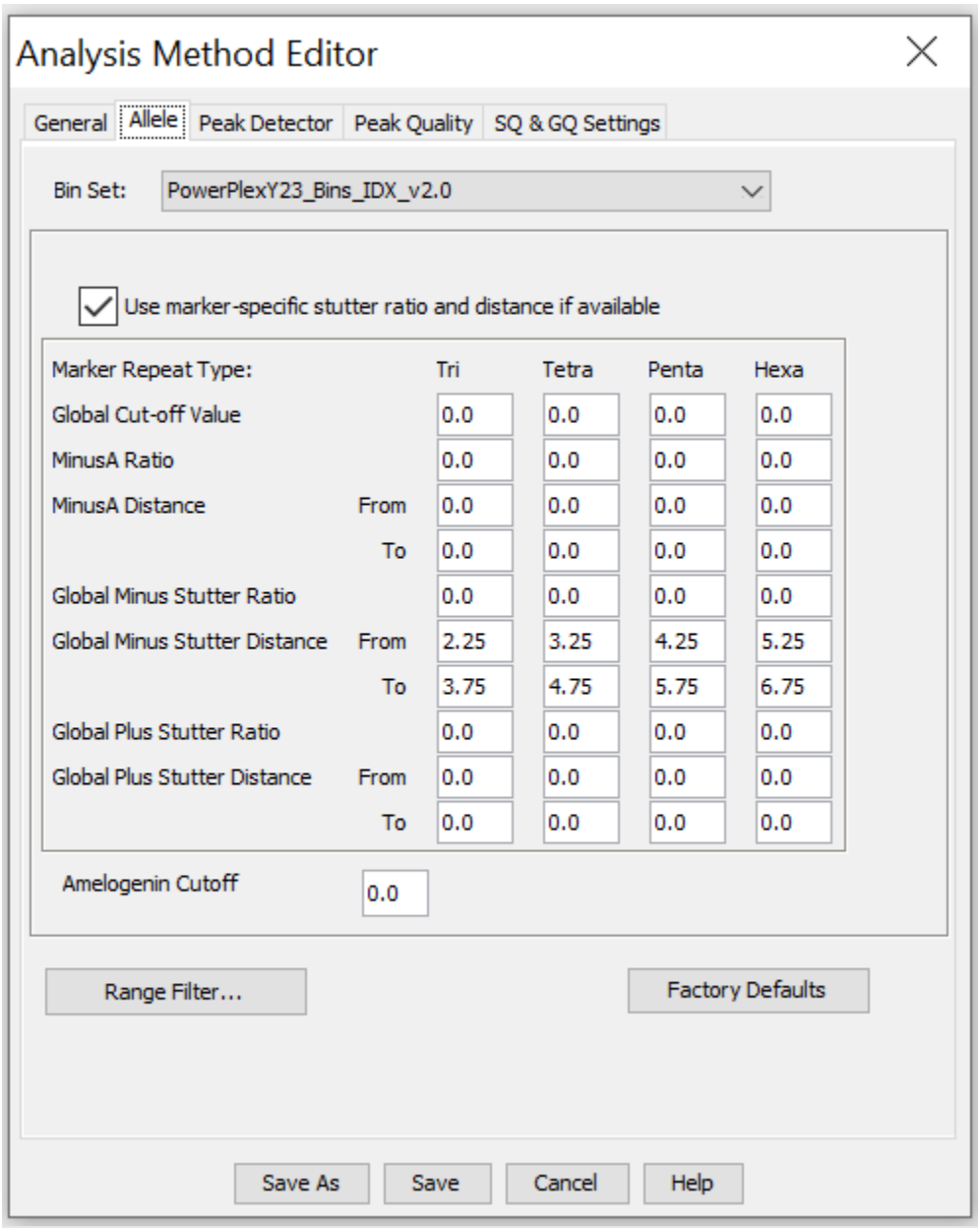

Figure 1 - Allele Tab for Y23 Analysis Method "PowerPlex_Y23"

Appendix A: GMIDX Analysis Settings (cont.)

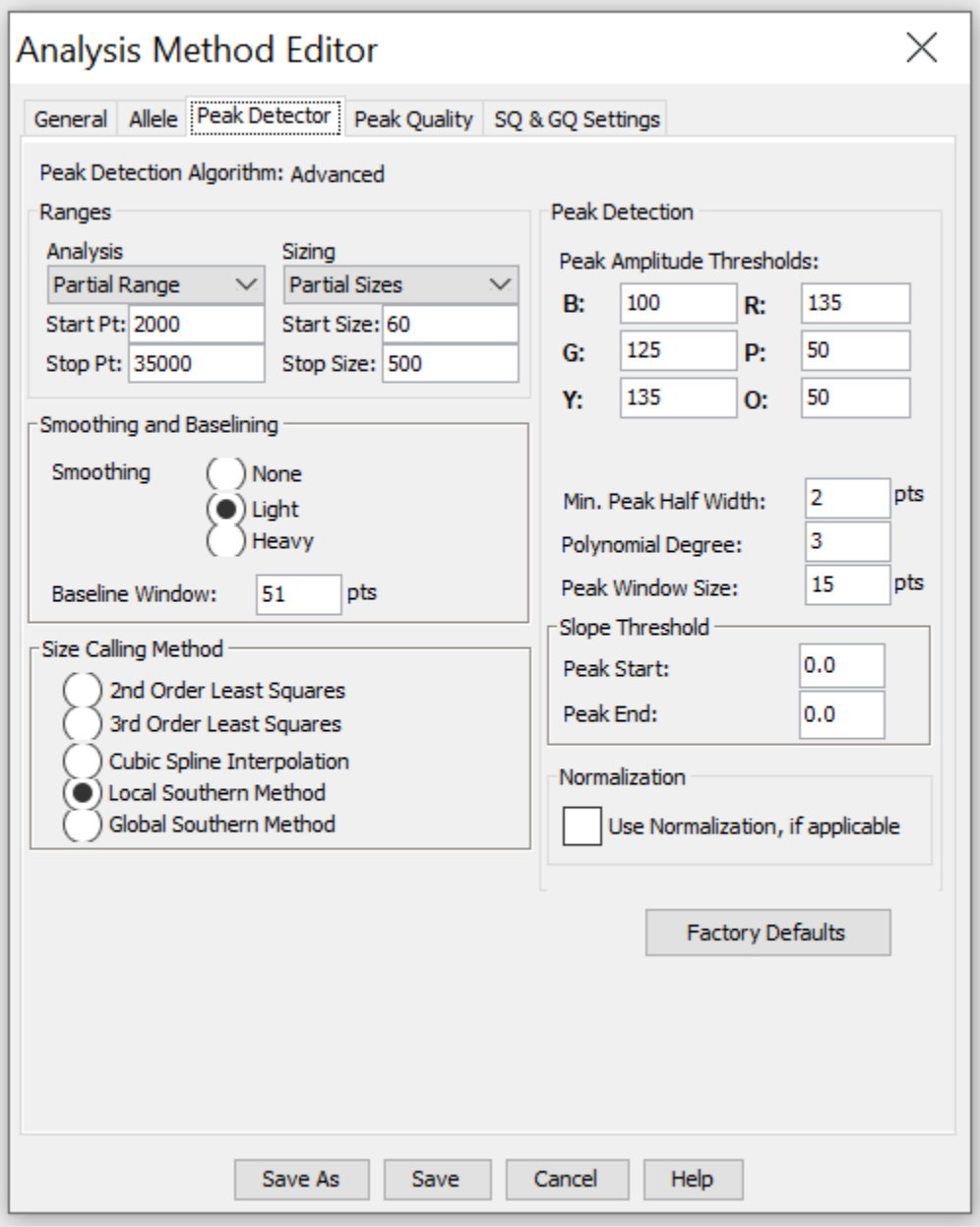

Figure 2 - Peak Detector Tab for Y23 Analysis Method "PowerPlex_Y23"

NOTE: Analysis “Start Pt” and “Stop Pt” may be adjusted as needed.

12 APPENDIX B: MAP OF THE Y-CHROMOSOME

Relative positions of 23 Y-STR loci available in the PowerPlex® Y23 System. The six new loci are shown in bold font. PAR1 and PAR2 are pseudo-autosomal regions on the tips of the Y chromosome that recombine with the X chromosome. The shaded region around AMEL Y can sometimes be deleted, causing loci such as DYS458 to be missing from an otherwise full Y-STR profile. DYS391 is located a sufficient distance away to avoid deletions affecting AMEL Y.

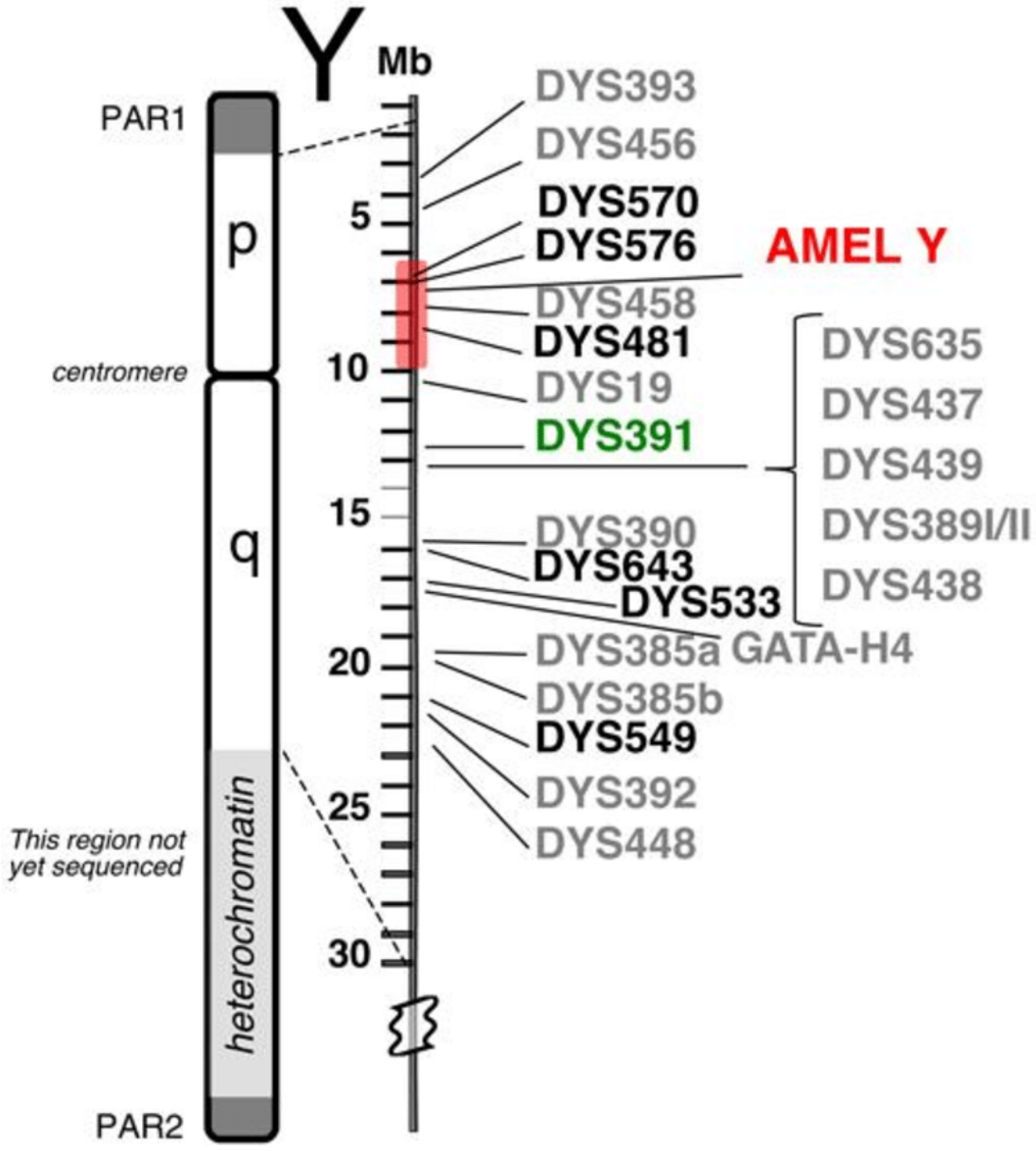

Figure and legend from: Butler et al. 2012, https://www.promega.com/resources/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups/#ArticleReferencesId-6b67909c-6b2d-4130-ad3b-89c47026a36b

13 APPENDIX C: ARTIFACTS IDENTIFIED BY PROMEGA OR OBSERVED IN FBI VALIDATION

DNA Dependent Artifacts				
Dye color	**Locus**	**Size**	**Observed in validation data?**	**Notes**
Blue	DYS448	n-7 to n-15	Yes	1, 2
Blue	DYS19	n-2; n+2	Yes: captured in stutter filters	3
Green	DY391	~96 bases	Yes	3, 4
Green	DYS481	~163 bases	Yes	3
Green	DYS549	~187 bases	Yes	3
Green	DYS533	~253 bases	Yes	3
Green	DYS533	~272 bases	No	3
Yellow	DYS635	~159 bases	Yes	3
Yellow	DYS439	~283 bases	Yes	3, 4
Yellow	DYS643	~428 bases	Yes	3
Yellow	DYS643	~441 bases	Yes	3
Red	DYS458	~201 bases	Yes	3

*DNA Independent Artifacts**			
Dye color	**Size**	**Observed in validation data?**	**Notes**
Blue	~60-65 bases	Yes	5
Blue	~58-63 base	Yes	5
Blue	~80-84 bases	Yes	
Blue	~136-147 bases	Yes	6
Green	~61-67 bases	Yes	
Green	~69-73 bases	Yes	
Green	~136-144 bases	Yes	6

*DNA independent artifact sizes may vary depending on environmental conditions in the laboratory.

Notes:

1 – These variably sized peaks on the Applied Biosystems® 3130 and 3500 Genetic Analyzers may represent double-stranded DNA derived from the DYS448 amplicon. Double-stranded DNA is known to migrate faster than single-stranded DNA on capillary electrophoresis (CE) instruments.

2 – The low-level, DNA-dependent artifact is noticeable only with high input

3 – Artifact is observed more often with samples that contain relatively higher levels of female DNA.

4 – Artifact was observed in FBI Validation data but is not in Promega's list of artifacts.

5 – The signal strength of these artifacts increases with storage of the amplification plate at 4°C.

6 – Artifact may appear as a dye blob or a peak in sample reaction and negative control reaction.

Promega information from the *PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* available at http://www.promega.com/protocols/