




Elucigene
QST**Rplusv2*

Guide to Interpretation

A green-tinted microscopic image of a cell or tissue structure, showing a central circular opening and surrounding cellular details, serving as a background for the title.

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Elucigene QST[®]R Guide to Interpretation of Results

It is recommended that each laboratory develops its own interpretation and reporting procedures and criteria. Best practice guidelines for QF-PCR have been documented by the UK's Clinical Molecular Genetics Society and Association of Clinical Cytogeneticists and are available for reference at:

www.cmgs.org.uk

DISCLAIMER

Gen-Probe Life Sciences Ltd. does not represent this guide as a comprehensive summary of all possible outcomes using the QST[®]R kits and it is intended to be used solely as an *aide memoire*. It must **not** be used in any clinical interpretation of the results of the assay. Laboratories must interpret the results of the assay in accordance with their own locally developed procedures. The UK's ACC/CMGS QF-PCR Best Practice Guidelines makes recommendations regarding the interpretation of results obtained.

GENERAL GUIDELINES FOR ALL QST[®]R KITS

1. The negative control should show no sharp peaks within the read range of 100 to 510bp.
2. The positive control must show the expected results and all peaks must meet the criteria below.
3. For analysis of DNA samples at least 1 peak should be observed for each marker tested (the exception being Y chromosome markers in female patient samples).
4. The acceptable range for marker peaks is between 50 and 6000 relative fluorescent units (rfus). Peak heights falling outside this range must not be analysed.
5. Electropherograms of poor quality due to excessive bleed-through between dye colours (also known as 'pull-up') or 'electrophoretic spikes' (sharp peaks present in more than one dye) should not be interpreted. The PCR products should be re-injected and re-analysed.
6. Analysis is performed by assessment of peak ratios (A1/A2), where A1 is the peak area of the shorter length fragment and A2 is the peak area of the longer length fragment. The resulting ratio is diagnostic of locus copy number.

INTERPRETATION

For disomic chromosomes heterozygous markers should show two peaks with similar heights. A complete analysis of chromosome copy number status is performed by comparison of peak area ratios.

1. Heterozygous di-allelic (i.e. two alleles) markers should fall within a ratio window of 0.8 to 1.4. However, for two alleles separated by more than 24bp in size a ratio of up to 1.5 is acceptable. Any values falling within this region are referred to as having a ratio of 1:1. If the ratio balance falls out of this window then it may be due to a number of factors, including but not limited to:-

- Whole chromosome trisomy
- Partial chromosome trisomy (including sub-microscopic duplications)
- Mosaicism
- Contaminating second genotype (e.g. maternal, twin, external)
- Stutters causing skewing
- Preferential amplification of one allele causing skewing
- Primer site polymorphisms
- Somatic microsatellite mutations

Homozygous markers are uninformative since a ratio cannot be determined.

2. To interpret a result as abnormal (i.e. trisomy present), at least two informative markers consistent with a tri-allelic genotype are required with all other markers being uninformative. It is not recommended to interpret a result as abnormal based on information from only one marker. If required, follow-up testing with the single chromosome kits (i.e. Elucigene QST*R-13, Elucigene QST*R-18, Elucigene QST*R-21 and Elucigene QST*R-XYv2) may provide sufficient information for interpretation.

Trisomy is determined by either:-

2.1. Two peaks of uneven height due to one of the peaks representing two alleles which are common to one or both parents. In this case the ratio between the two peaks will be classed as 2:1 or 1:2 such that A1/A2 will give a result in the region of 1.8 to 2.4 when the peak representing the shorter length allele is greater in area than the peak representing the longer length allele, or where A1/A2 will give a result in the region of 0.45 to 0.65 when the peak representing the shorter length allele is smaller in area than the peak representing the longer length allele.

2.2. Three peaks of comparable height present. The ratio of the peaks will be classed as 1:1:1 and their values fall within the normal range of 0.8 – 1.4 (although for alleles separated by more than 24bp an allele ratio of up to 1.5 is acceptable). If this does not occur then it may be due to one of the factors mentioned in paragraph 1.

3. To interpret a result as normal, at least two informative markers consistent with a di-allelic genotype are required with all other markers being uninformative. A normal result indicates the normal complement of two for the chromosomes tested.

4. Peak area ratios that fall between the normal and abnormal ranges are classed as inconclusive. Inconclusive results may be resolved by using the single chromosome QST[®]R kits.
5. If both normal and abnormal allele patterns are obtained for a single chromosome then it is recommended that follow-up studies are carried out to identify the reason for the discrepant results prior to any conclusions being reached.
6. In rare cases allele size ranges for markers may overlap. If this is suspected, analysis with the single chromosome QST[®]R kits may resolve this.

SPECIFIC TO QST[®]R-XYv2 AND THE SEX CHROMOSOME MARKERS IN QST[®]RPLUSv2

1. The AMEL marker amplifies non-polymorphic sequences on the X (104 bp) and Y (110 bp) chromosomes and can be used to determine the presence or absence of a Y chromosome and represents the relative amount of X to Y sequence.

Note: amplification failure due to mutation of the AMEL-Y sequence has been reported.

2. TAF9L is an invariant paralogous marker with sequences on chromosomes 3 and X. The chromosome 3 specific peak (116bp, representing 2 copies of chromosome 3) can therefore be used as a reference peak to assist in the determination of the number of X chromosomes present (121bp peak). Analysed in combination with Amelogenin and the other sex chromosomes markers, it is particularly useful in the diagnosis of sex chromosome aneuploidy, for example Turner syndrome. In a normal female the markers should fall within a ratio window of 0.8 to 1.4. In a normal male or monosomy X the markers will give a ratio ≥ 1.8 . Examples of results can be found on pages 32-38.
3. The DXYS267 and DXYS218 polymorphic STR markers are present on both the X and Y chromosomes and represents the total number of sex chromosomes. For informative male results it is not possible to determine which allele represents the X or Y chromosome.
4. Informative X-specific markers DXS981, DXS1187, XHPRT, DXS6807, DXS7423, DXS6803 and DXS6809 represent the number of X chromosomes.
5. The Y-specific marker, SRY, will give a single peak in normal males and will not amplify in normal females.
6. The Y-specific marker, DYS448, in most cases will give a single peak in normal males and will not amplify in normal females. However, it has been noted that on rare occasions, this marker can demonstrate a heritable di-allelic pattern (sub-microscopic duplication followed by replication slippage) or show no amplification (null allele).

Note: a result exhibiting no amplification for Y specific markers and homozygous for all other markers is not necessarily diagnostic of Turner syndrome. Approximately 1 in 171,000 females will be homozygous for all 7 X specific polymorphic markers. This gives a Bayesian probability of approximately 1 in 1400 that a profile homozygous for all X specific markers represents a true monosomy X genotype rather than a normal homozygous female.

Examples

1. *WHOLE CHROMOSOME TRISOMY*

Informative markers for individual chromosomes demonstrate a deviation from the expected normal 1:1 ratio. This can either result in a 2:1 or 1:2 ratio for di-allelic markers or a 1:1:1 ratio where markers demonstrate three alleles. A diallelic trisomy result demonstrates two peaks of uneven height due to one of the peaks representing two alleles which are common to one or both parents. In this case the ratio between the two peaks will be classed as 2:1 or 1:2 such that A1/A2 will give a result in the region of 1.8 to 2.4 when the peak representing the shorter length allele is greater in area than the peak representing the longer length allele, or where A1/A2 will give a result in the region of 0.45 to 0.65 when the peak representing the shorter length allele is smaller in area than the peak representing the longer length allele. A triallelic result will demonstrate three peaks of comparable height present. The ratio of the peaks will be classed as 1:1:1 and their values fall within the normal range of 0.8 – 1.4 (although for alleles separated by more than 24bp an allele ratio of up to 1.5 is acceptable).

The presence of a 3 allele result indicates that the trisomy cell line originated from a meiotic non-disjunction event whereas the absence of any three allele result in informative loci indicates that the trisomy most likely arose as a result of a mitotic non-disjunction event.

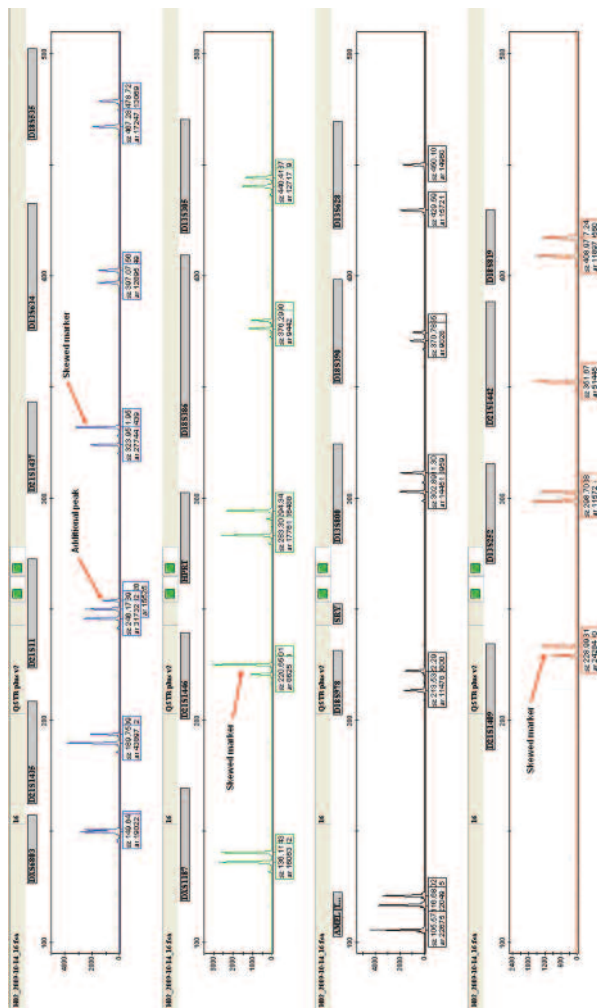
2. *TRIPLOIDY*

Profiles demonstrate trisomy at all informative loci, on all chromosomes tested.

3. MOSAICISM

All informative markers on a single chromosome show skewed allele ratios and/or a minor third allele peak. Care should be taken when distinguishing this result from maternal cell contamination. In an optimised system, a normal or abnormal cell line can be detected if present at a level of >20% of the total cell population.

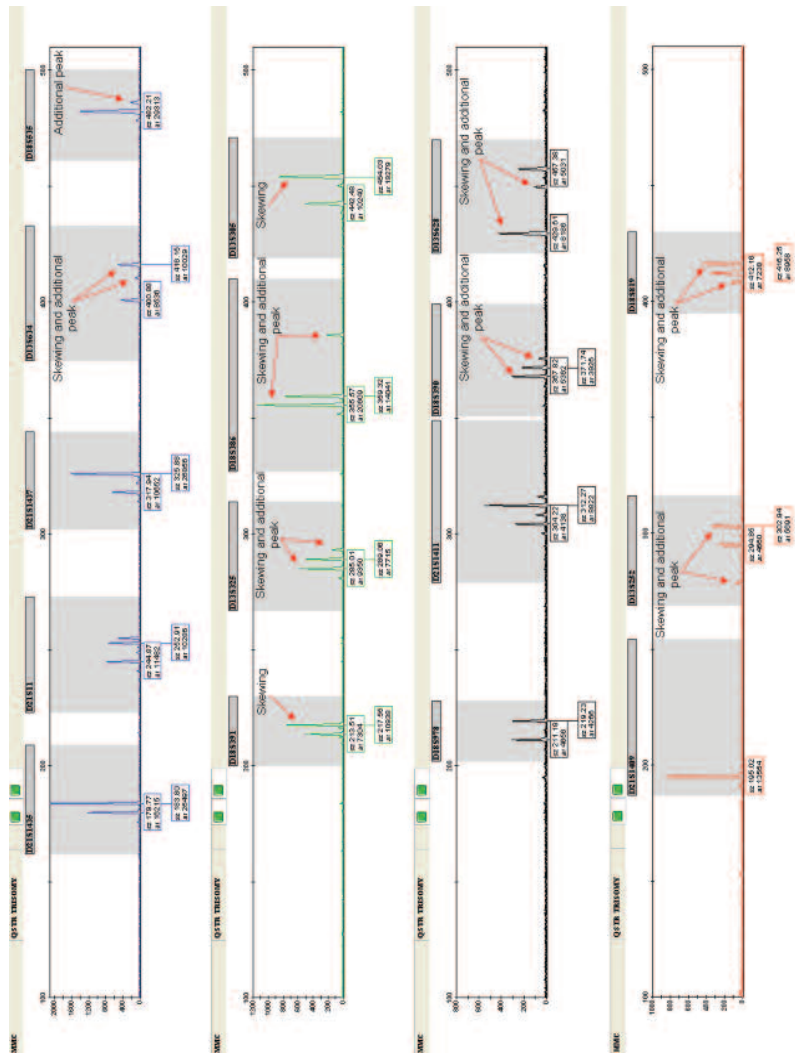
Figure 3. Mosaic trisomy 21 (approximately 25-30%)



4. MATERNAL CELL CONTAMINATION (MCC)

Profiles demonstrate the presence of two genotypes where one allele is shared between the fetus and mother, and therefore no 4 allele results are observed (such results may represent twin, chimera or external sample contamination). Where three alleles are present, a typical pattern seen is $A + B = C$ where A is the maternal allele, B is the fetal allele and C represents the shared allele. MCC is usually accompanied by some level of blood-staining in an amniotic fluid sample. MCC samples are still interpretable if the fetal genotype is present at a high level relative to the maternal genotype. If one or more of the allele ratios falls within the inconclusive range, then it is recommended that the profile is not used to assess the chromosome status.

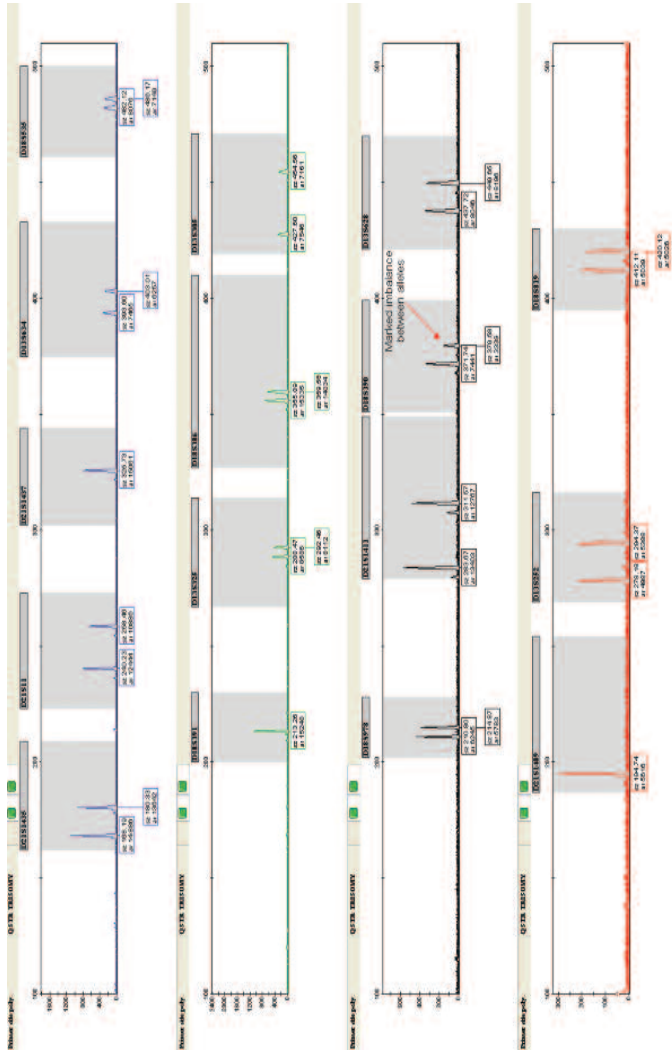
Figure 4. Trisomy 21 with maternal cell contamination (QSTR)



5. PRIMER BINDING SITE POLYMORPHISM (PSP)

Can cause partial or complete allelic drop-out. In the case of complete drop-out in a normal sample, the profile shows apparent homozygosity for an individual marker. In the case of a trisomic chromosome, the profile may show apparent disomy. Partial drop-out is evident as an additional peak at a reduced height which can result in skewed, inconclusive or apparent 1:2/2:1 allele ratios.

Figure 5. Primer binding site polymorphism in D18S390 (QSTR)

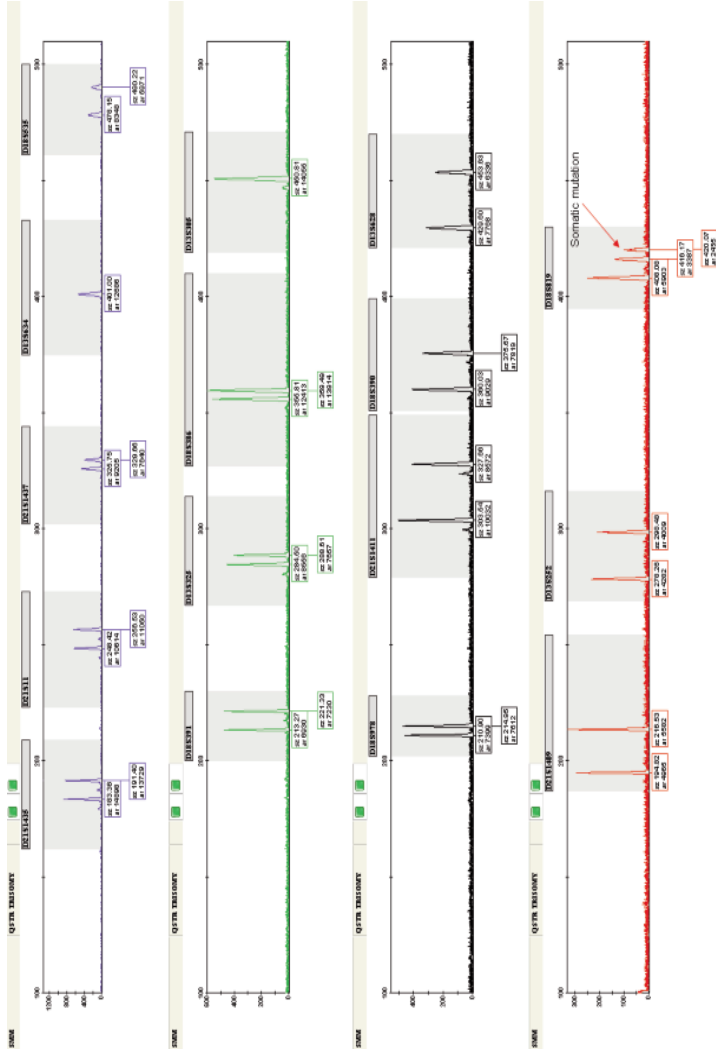


Note: Primer binding site polymorphisms can often be distinguished by repeating the assay at a significantly reduced annealing temperature (eg. 4 degrees lower). This allows more permissive annealing of the primer and may result in the restoration of the expected profile/peak size. If this is the case, it is recommended that the marker is not used to assess chromosome status as amplification may be incomplete.

6. SOMATIC MICROSATELLITE MUTATION (SMM)

Generation of a novel allele at a single locus, probably by mitotic replication error. Evident on a profile when present in a subpopulation of cells (mosaic), either 3 alleles where $A+B=C$ (A and B are the reduced height peaks) or persistent skewed di-allelic ratios. More frequently observed in CVS; only one of the two villi may be affected.

Figure 6. Somatic microsatellite mutation in D18S819 (QST*R)



Note: SMMs are mosaic. Testing different cell populations (other villi or cultured cells) can aid their classification as SMMs and distinguish them from SMDs (see below) if they appear as a 2:1/1:2 ratio.

7. SUB-MICROSCOPIC DUPLICATION

Occurs as a result of uneven cross-over during cell division. It results in trisomy in one chromosome specific marker. Markers can demonstrate either a 1:2, 2:1 or 1:1:1 ratio.

Figure 7. Sub-microscopic duplication in DXYS218 (IST-R:XY)



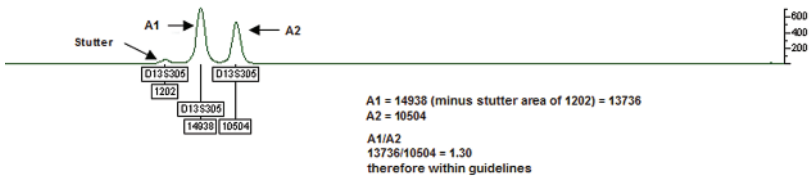
8. SKEWING

Skewing can be defined as any significant variation from a 1:1 allele ratio. In addition to the examples given below, a number of other mechanisms may give rise to skewed allele ratios (see examples of Mosaicism, Low Input DNA and External Contamination).

a. STUTTER

If PCR conditions are not optimised, this may lead to increased stutter formation. As PCR product accumulates, the shorter stutter peak may be preferentially amplified resulting in a reduction of the full size allelic product. Although stutter peaks are representative of the sample being tested, they are not typically included in the analysis.

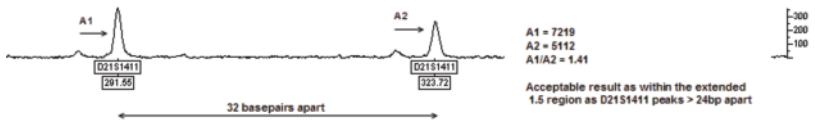
Figure 8a. Stutter in D13S305



b. LARGE DIFFERENCE IN SIZE BETWEEN TWO ALLELES

PCR is a competitive reaction. Where alleles are separated by a large number of repeats, the shorter allele can be preferentially amplified, leading to an imbalance between the two alleles.

Figure 8b. Large interval between alleles in D21S1411



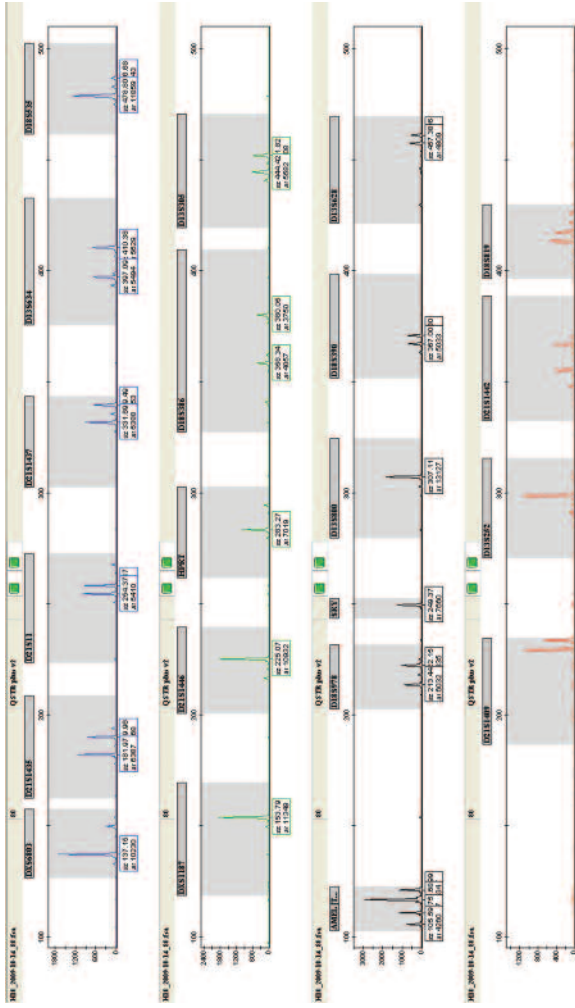
9. LOW INPUT DNA

Reduced amount of input DNA or poor quality DNA can result in preferential amplification of one allele resulting in skewing and/or uneven amplification of markers. If sufficient sample is available, it can be re-extracted and re-suspended in a lower volume. Low input DNA can also result in very small peaks that fail to label (ie. peak height is below threshold set for labelling a peak), extra care should be taken when reviewing results.

10. CONTAMINATION FROM EXTERNAL SOURCE

This is evident as two genotypes, where some markers may demonstrate four different allele peaks. These may be in a 1:1:1:1 ratio if both cell lines are present in equal quantities, or two minor and two major peaks if cell lines are present in unequal quantities. Depending upon the level of contamination some markers may exhibit some degree of skewing. Four allele systems may also occur as a result of a di-zygotic twin pregnancy or from chimerism.

Figure 10. Example of external contamination

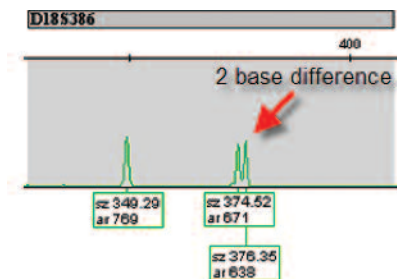


Note: The above profile is a confirmed example of external contamination of the amniotic fluid sample. An apparent contaminating profile may also be due to the presence of a dizygotic twin or may represent a chimera. A chimera is an individual with two cell lines derived from two separate zygotes. This can be confirmed by parental studies.

11. MICROVARIANT

Short tandem repeats occasionally demonstrate incomplete repeat sequences. In these cases, peaks are present that are not separated by a multiple of four bases (in the case of a tetranucleotide repeat). The interrupted repeat unit can be 1, 2 or 3 bases long. Microvariants are commonly designated by the number of full repeats and the size of the incomplete repeat eg. a 9.3 allele indicates 9 full repeats and a 3 base microvariant. This would size 1 base shorter than a 10 allele.

Figure 11. Example of 2 bp microvariant in D18S386



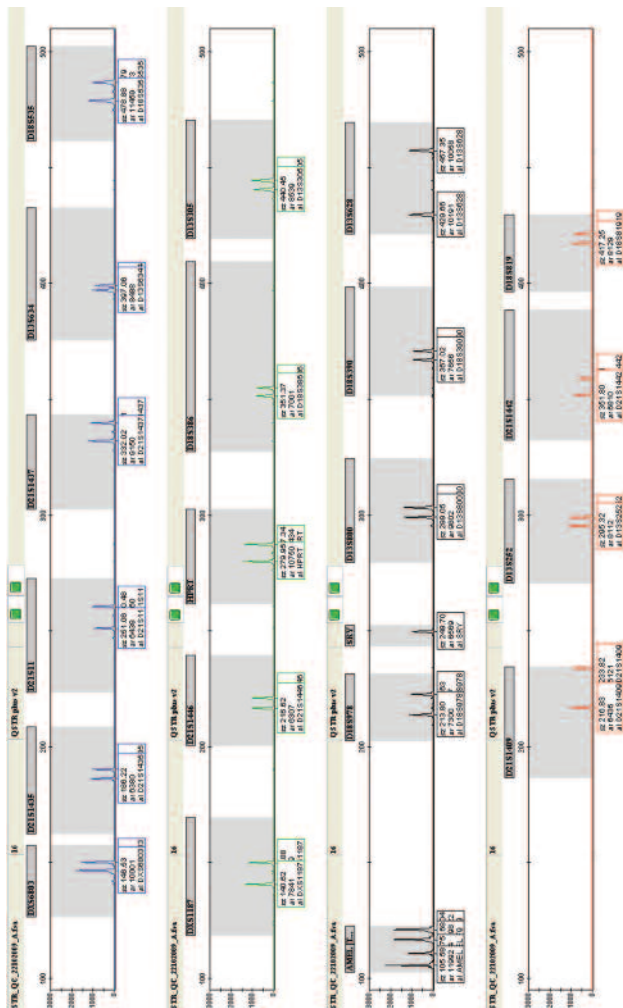
Note: According to the Best Practice Guidelines markers demonstrating an apparent one bp separation should not be used as 'peak splitting' is a phenomenon that can also result in a peak being separated by one base. This results from incomplete addition of a terminal 'A' base that is characteristic of the polymerases used in PCR and is usually due to sub-optimal PCR conditions. In many cases, the two 'peaks' will usually show imbalance. In profiles where peak splitting is present, it will usually affect more than one marker.

Sex chromosomes

12. KLINEFELTER SYNDROME

The most common form of Klinefelter syndrome is caused by the presence of an extra X chromosome in a male resulting in a 47,XXY karyotype.

Figure 12. Example of classic 47,XXY Klinefelter profile (QST-Rplusv2)



13. TURNER SYNDROME

The most common form of Turner syndrome is caused by the absence of one X chromosome in a female resulting in a 45,X (alternatively 45,X0) karyotype.

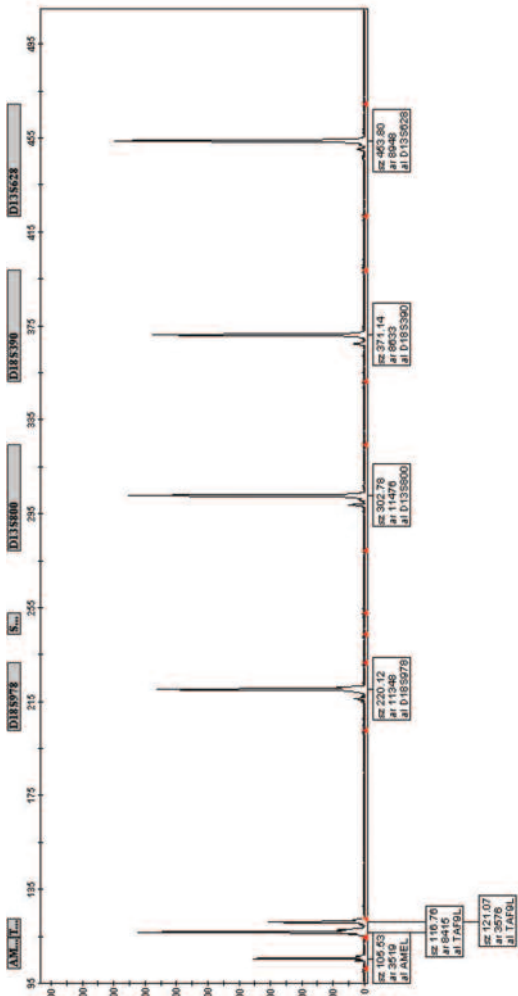
Interpretation of the TAF9L marker in conjunction with Amelogenin and SRY

The TAF9L marker has been included as a quantitative marker to assist in determining the number of X chromosomes present. TAF9L is a paralogous marker with sequences on both chromosome 3 and chromosome X.

The peak resulting from amplification of the sequence on chromosome 3 is expected to represent a normal diploid chromosome complement except in the case of triploidy (or other rare aneuploidy). Using this peak as a reference, it is possible to compare it with that amplified from the sequence on the X chromosome and thereby determine the number of X chromosomes present.

Note: All sex chromosome marker information should be assessed together when drawing conclusions over the copy numbers of the sex chromosome markers.

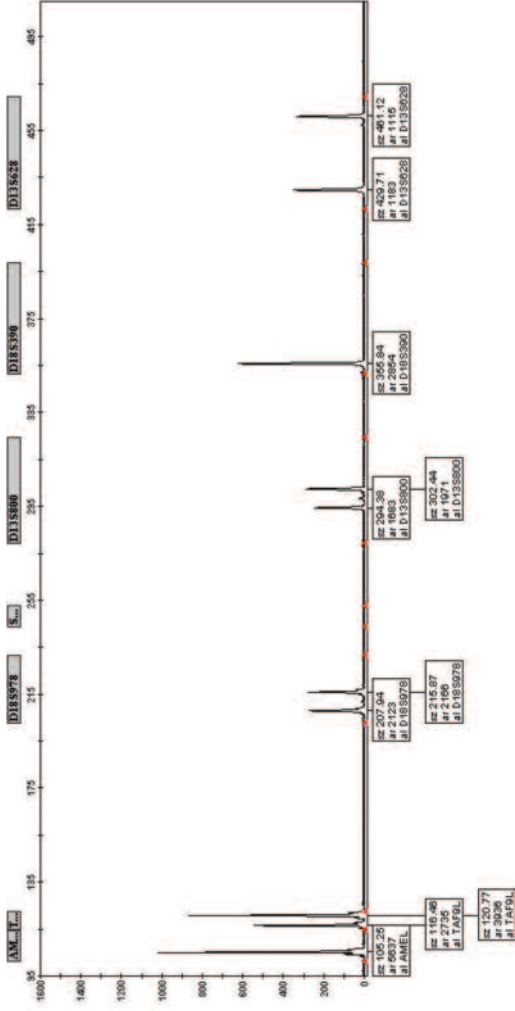
Figure 16: XO - Female



- Amelogenin: single peak for X chromosome – consistent with presence of X chromosome only
- Absence of SRY
- TAF9L: 2:1 ratio – consistent with 2 copies of chromosome 3 and 1 copy of X chromosome

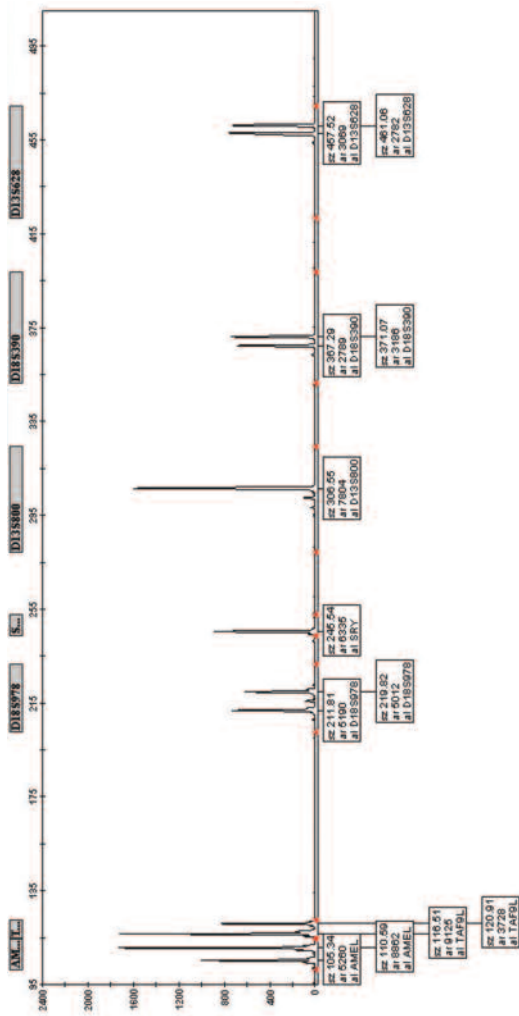
Conclusion: Result is consistent with XO sex chromosome complement

Figure 17: XXX Female



- Amelogenin: single peak for X chromosome – consistent with presence of X chromosome only
 - Absence of SRY
 - TAF9L: 2:3 ratio – consistent with 2 copies of chromosome 3 and 3 copies of X chromosome
- Conclusion:** Result is consistent with XXX sex chromosome complement

Figure 18: XYY - Male



- Amelogenin: 1:2 ratio - consistent with presence of one copy of X chromosome and 2 copies of Y chromosome
- Presence of SRY - consistent with presence of Y chromosome. - SRY is approximately twice the height of AM X/AM Y and TAF X peaks
- TAF9L: 2:1 ratio - consistent with 2 copies of chromosome 3 and 1 copy of X chromosome

Conclusion: Result is consistent with XYY sex chromosome complement

Glossary of Terms

AMNIOTIC FLUID

Definition: a colourless liquid that surrounds and protects the fetus inside the amniotic sac within the uterus. Amniocentesis is typically carried out at 14-18 weeks gestation.

ANEUPLOIDY

Definition: any deviation from the standard chromosome complement.

Effect: a deviation from the expected 1:1 ratio at informative loci.

CHORIONIC VILLUS

Definition: a finger-like projection on the surface of the chorion which is part of the placenta and contains the amnion. It is comprised of two types of cell, the outer trophoblast layer and the inner mesenchyme core. Chorionic villus (CV) biopsy can typically be carried out at 10-12 weeks gestation.

CONFINED PLACENTAL MOSAICISM

Definition: when chromosomally abnormal cells are found in the placenta but not in the fetus.

Confined placental mosaicism (CPM) represents a discrepancy between the chromosomal makeup of the cells in the placenta and the cells in the fetus and was first described by Kalousek and Dill¹ in 1983. CPM is diagnosed when a discrepancy occurs between the results of a CVS test and the result of a subsequent prenatal test, such as amniocentesis or fetal blood sampling, such that either a normal or abnormal cell line detected in the CVS is not identified in the subsequent test. CPM is detected in approximately 1-2% of ongoing pregnancies that are studied by CV biopsy. Most commonly when CPM is found it represents a trisomic cell line in the placenta and a normal diploid chromosome complement in the fetus. However, the fetus is involved in approximately 10% of cases, Hahnemann and Vejerslev².

Effect: A trisomy result can be obtained from an individual villus or both villi tested. However, sampling two or more villi from different regions of the chorion can increase the likelihood of identifying placental mosaicism.

References:

1. Kalousek DK, Dill FJ. Chromosomal mosaicism confined to the placenta in human conceptions. *Science* 1983. 221(4611): 665-667.
2. Hahnemann JM, Vejerslev LO Accuracy of cytogenetic findings on chorionic villus sampling (CVS) – diagnostic consequences of CVS mosaicism and nonmosaic discrepancy in centres contributing to EUCROMIC 1986-1992. *Prenatal Diagnosis* (1997), 17(9): 801-20.

MATERNAL CELL CONTAMINATION

Definition: profiles where there is evidence of the maternal genotype, either present with the fetal genotype (at a low or high level), or present as a single maternal genotype (fetal genotype too low to be detected).

Maternal cell contamination (MCC) can occur in amniotic fluid samples where there is bleeding into the amnion, either spontaneously (brownish AF/old blood) or as a result of the sampling procedure. Blood staining can be readily evident in the amniotic fluid when the fluid is spun down as a red layer in the cell pellet. It is worth noting that blood present in the fluid may be fetal in origin and a maternal genotype may not be detected.

MCC can also occur in chorionic villus sampling where maternal decidua tissue is attached to the villi and not removed at the clean-up stage.

Effect: profiles demonstrate the presence of two genotypes where one allele is shared between the fetus and mother, and therefore no 4 allele results are observed (such results may represent twin, chimera or external sample contamination). Where three alleles are present, a typical pattern seen is $A + B = C$ where A is the maternal allele, B is the fetal allele and C represents the shared allele. MCC is usually accompanied by some level of blood-staining in an amniotic fluid sample. MCC samples are still interpretable if the fetal genotype is present at a high level relative to the maternal genotype. If one of more of the allele ratios falls within the inconclusive range, then it is recommended that the profile is not used to assess the chromosome status.

MESENCHYMAL CORE

Definition: the inner cells of each villus, also known as the villus stroma. The cells which become the villus stroma are more closely related to the cells which become the embryo, based on early embryonic development.

MOSAICISM

Definition: two or more cell lines, with a different genetic or chromosomal constitution.

Effect: all informative markers on a single chromosome show skewed allele ratios and/or a minor third allele peak. Care should be taken when distinguishing this result from maternal cell contamination. In an optimised system, a normal or abnormal cell line can be detected if present at a level of >20% of the total cell population. The presence of a 3 allele result indicates that the trisomy cell line originated from a meiotic non-disjunction event.

POLYMORPHIC SUB-MICROSCOPIC DUPLICATION

Definition: a duplication of part of a chromosome, not readily detectable by conventional karyotyping. Inheritance studies can be carried out to assess the clinical significance.

Effect: a single marker showing a trisomy profile with all other informative markers on that chromosome showing a normal profile.

PRIMER BINDING SITE POLYMORPHISM

Definition: a polymorphism on the template DNA strand where a PCR primer anneals.

Effect: allelic drop-out. In the case of complete drop-out, the profile shows apparent homozygosity for an individual marker. In the case of a trisomic chromosome, the profile may show apparent disomy. Partial drop-out is evident as an additional peak at a reduced height which can result in skewed, inconclusive or apparent 1:2/2:1 allele ratios.

Note: Primer site polymorphisms can be distinguished by repeating the assay at a significantly reduced annealing temperature (eg. 4 degrees lower). This allows more permissive annealing of the primer and may result in the restoration of the expected profile/peak size. If this is the case, it is recommended that the marker is not used to assess chromosome status as amplification may be incomplete.

SOMATIC MICROSATELLITE POLYMORPHISM

Definition: a post-fertilisation event resulting in a change in the allele repeat length, probably caused by a replication error. This may be present in a proportion of cells or all cells tested.

Effect: generation of a novel allele at a single locus, probably by mitotic replication error. Evident on a profile when present in a subpopulation of cells (mosaic), either 3 alleles where $A+B=C$ (A and B are the reduced height peaks) or persistent skewed di-allelic ratios. More frequently observed in CVS; only one of the two villi may be affected.

TRIPLOIDY

Definition: a type of aneuploidy where three sets of chromosomes are present

Triploidy can also be present in mosaic form but this is extremely rare. Two different mechanisms of origin for these apparent mosaics have been described: (1) delayed digyny, by incorporation of the second polar body into one blastomere nucleus of a diploid zygote, and (2) delayed dispermy, similarly, by incorporation of a second sperm pronucleus into one one blastomere nucleus of a diploid zygote.

Effect: for non-mosaic triploidy, profiles demonstrate trisomy at all informative loci.

TRISOMY

Definition: where there are three copies of an individual chromosome, eg. 13, 18 or 21

Effect: all informative markers for an individual chromosome demonstrate a deviation from the expected normal 1:1 ratio resulting in a 2:1/1:2 ratio for diallelic markers or a 1:1:1 ratio where markers demonstrate three alleles.

TROPHOBLAST

Definition: the trophoblast cells are the outer layer of the chorionic villi. Other non-fetal cells become the villus stroma or mesenchymal core. The trophoblast cells are destined to become part of the placenta and are responsible for the implantation of the embryo to the uterine wall. The mesenchyme cells are closer in cell lineage to the fetus than the trophoblast cells.