USER GUIDE

applied biosystems® by life technologies™

Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits

INSTRUCTIONS FOR USE

Publication Part Number 4454039 Rev. C
Revision Date July 2011

life technologies™

The results obtained from these or any other diagnostic kits should be used and interpreted only in the context of the overall clinical picture. Life Technologies cannot accept responsibility for any clinical decisions that are made.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

NOTICE TO PURCHASER: Not for sale in U.S.A.

These products are sold for use by the end-user only and may not be re-sold, distributed or re-packaged.

LIMITED USE LABEL LICENSE: No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

CE IVD

THE *TRUESCIENCE*™ ANEUPLOIDY KIT IS SUPPLIED FOR PROFESSIONAL IN VITRO DIAGNOSTIC TESTING. THIS PRODUCT DOES NOT PROVIDE A LICENSE TO PERFORM PCR UNDER PATENTS OWNED BY ANY THIRD PARTY INCLUDING HOFFMAN-LA ROCHE (HOFFMANN-LA ROCHE LTD, DIAGNOSTICS, CH-4070 BASEL, SWITZERLAND) AND ROCHE MOLECULAR SYSTEMS, INC (ROCHE MOLECULAR SYSTEMS, INC., 1145 ATLANTIC AVENUE, ALAMEDA, CALIFORNIA 94501)

Life Technologies™ Ltd.

7 Kingsland Grange

Woolston, Warrington, WA1 4SR, United Kingdom

www.appliedbiosystems.com/aneuploidy

TrueScience™ Aneuploidy STR Kits are manufactured for Life Technologies Ltd. and also manufactured as Elucigene® QST*R® kits by Gen Probe Life Sciences, UK within quality systems accredited to ISO9001:2008 and ISO13485:2003

TRADEMARKS: The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. ELUCIGENE and QST*R are registered trademarks of Gen-Probe Life Sciences Ltd.

Customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the RespiFinder products.

© 2011 Life Technologies Corporation. All rights reserved.

Publication Part Number 4454039 Rev. C
July 2011

Contents

About this guide

Purpose of the guide

The *Applied Biosystems® TrueScience™* Aneuploidy STR Kits Protocol provides reference information for preparation of the sample and interpretation and analysis of the data.

Safety information

For general safety information, see this section and Appendix D, "Safety" on page 57. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Life Technologies user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – There are specific warnings or precautions associated with the device that do not appear on the label.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The SDSs for any chemicals supplied by Life Technologies are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 61.

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies or Ambion contact the chemical manufacturer.

Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits

This section covers:

Description of the product

The *TrueScience*™ Aneuploidy Kits are multiplexed DNA-based assays for the rapid prenatal determination of aneuploidy status for the three most common viable autosomal trisomies and the sex chromosomes X and Y. *TrueScience*™ Aneuploidy Kits are available in the formats listed below. For more information on the kits in the *TrueScience*™ Aneuploidy Kit range please visit: **www.appliedbiosystems.com/aneuploidy**

Product	Number of tests	Product code
TrueScience™ Aneuploidy STR-Plus Kit	50	4453756
	10	4453757
TrueScience™ Aneuploidy STR-XY Kit	50	4453601
	10	4453755
TrueScience™ Aneuploidy STR-13 Kit	10	4453760
TrueScience™ Aneuploidy STR-18 Kit	10	4453759
TrueScience™ Aneuploidy STR-21 Kit	10	4453758

Intended use

Kit	Intended Use
STR Plus	For the routine in vitro quantitative diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). The kit also includes X and Y chromosome markers and the TAF9L marker for the determination of sex status.
STR-XY	For routine in vitro quantitative diagnosis for the analysis of sex chromosome status including the common aneuploidies of Klinefelter syndrome and Turner syndrome.
STR-13 **STR-18** **STR-21**	Supplemental kits containing additional autosomal markers, to be used in conjunction with STR-Plus, for the routine quantitative in vitro diagnosis of the three most common viable autosomal trisomies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome).

The method employed by the TrueScience™ Aneuploidy kits is the Qf-PCR (Quantitative fluorescence-Polymerase Chain Reaction) technique.

The TrueScience™ Aneuploidy kits are intended to be used on DNA extracted from either chorionic villus (CV) samples or amniotic fluid (AF) samples.

The intended target population is pregnant women who have been assessed as being at 'high risk' of carrying an affected fetus, by either biochemical or ultrasound diagnostic procedures, or assessed to be 'at risk' due to either previous family history or maternal age.

The device is intended to be used in conjunction with other diagnostic procedures to support or discount the proposed clinical diagnosis.

The device is for Professional Use Only within a molecular or cytogenetics laboratory environment.

Principle of the procedure

The method employed by *TrueScience*™ Aneuploidy Kits uses the Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) technique. Using PCR amplification, fluorescent dye labeled primers target highly polymorphic regions of DNA sequence called short tandem repeats (STRs) that are located on the chromosomes of interest. Each targeted STR marker is specific to the chromosome on which it is located, thus the copy number of the STR marker can be diagnostic of the copy number of the chromosome. Informative STR markers have been selected that exhibit a high heterogeneity so that copy number can be easily determined.

A normal diploid sample has the normal complement of two of each of the somatic chromosomes, thus two alleles of a chromosome specific STR are determined by the QF-PCR technique as two peaks in a 1:1 ratio. The observation of an extra STR allele as either a three-peak pattern in a 1:1:1 ratio or a two-peak pattern in 2:1 peak ratio is diagnostic of the presence of an additional sequence which in turn may represent an additional chromosome, as in the case of a trisomy.

Amplified products of the QF-PCR technique are analyzed quantitatively on a capillary Genetic Analyzer to determine the copy number of the analyzed STR markers.

Product information

Contents and storage

Contents

Each of the *TrueScience*™ Aneuploidy Kits includes:

- *TrueScience*™ Aneuploidy Kit Reaction Mix containing markers described in the table below, to amplify short tandem repeat (STR) markers. The primer also contains DNA polymerase and deoxynucleotide triphosphates in buffer.
- DNA Control (one 50-µL vial, concentration: 1 ng/µL). The control DNA is diploid for the markers detected by *TrueScience*™ Aneuploidy Kits.
- 0.2-mL PCR vials: 50 vials for 50-test kits or 10 vials for 10-test kits.
- Product insert with safety information.

For details on kit contents, see "Ordering Information" on page 55. More information on the kits is available at: **www.appliedbiosystems.com/aneuploidy**

Storage

When you open the kit, we recommend that you perform these steps to minimize freeze-thaw cycles:

1. Thaw the *TrueScience*™ Aneuploidy Kit Reaction Mix and mix it thoroughly.
2. Pipet the reaction mix in 10-µL aliquots into 0.2-mL PCR vials.
3. Store the vials at –20 °C.

Make sure to thaw and mix vial contents thoroughly before use.

Materials and equipment required

Use of the Aneuploidy Kit requires materials such as a thermal cycler, a genetic analysis system, and standard laboratory equipment, which are not provided. For more information about materials required, see "Materials and equipment not included" on page 56.

Workflow

Before you begin: one-time procedures (See page 12)

Before each run (See page 12)

Collect and store samples (See page 12)

Extract DNA (See page 12)

Procedure (See page 14)

Amplify DNA (See page 14)

Set up samples for capillary electrophoresis (See page 15)

Analyze data (See page 15)

Interpret results† (See page 17)

† **IMPORTANT!** The results obtained from these or any other diagnostic kits should be used and interpreted only in the context of the overall clinical picture. Life Technologies cannot accept responsibility for any clinical decisions that are made. For more information, see "Limitations and disclaimer" on page 53. 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 **http://www.cmgs.org.uk**

Before you begin: one-time procedures

Before using the *TrueScience*™ Aneuploidy Kits, set up the genetic analysis instrument for fragment analysis. For the Applied Biosystems 3500 or 3130 Genetic Analyzer, refer to the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038).

Before each run

Collect and store samples

Use Chorionic Villus (CV) or Amniotic Fluid (AF) samples. Reports have indicated that some sample collection devices degrade certain analytes and can interfere with some assays. Make sure that you use your sample collection device according to the manufacturer's instructions and that your laboratory conducts internal validation studies to ensure that both sample collection devices and DNA preparation methods are compatible with this test.

Amniotic fluid (AF)

Use approximately 1 to 2 mL of amniotic fluid.

Chorionic Villus (CV)

Carefully clean CV samples according to your laboratory protocol to remove any adhering maternal decidua. Be sure to test cells from more than one region of the sample and that the sample includes cells from the mesenchyme core. This ensures that the aneuploidy test result is obtained from the same population of cells used for karyotype analysis.

Extract DNA

The *TrueScience*™ Aneuploidy Kits are validated for use with the InstaGene matrix method of DNA extraction. Using this method, you can perform extraction in a single tube. Prior to using the results in diagnosis, your laboratory should validate, according to your laboratory procedures, any alternative extraction methods and sample types used.

The InstaGene method of DNA extraction is described below.

Prepare the sample

1. Resuspend the InstaGene matrix on a magnetic stirrer and stir at a medium speed for at least 5 minutes.
2. Centrifuge the sample (AF or CV) at 12,000x*g* for 1 minute to pellet the cells.
3. Remove the samples from the centrifuge and visually check the pellet for bloodstaining. Record the percentage of bloodstaining, if any.
4. Carefully remove and discard the supernatant from the pellet, ensuring that the pellet is undisturbed. Leave approximately 10 to 20 µl of supernatant in the tube to re-suspend the pellet.

5. Thoroughly mix the sample by vortexing.

6. If >50% bloodstaining is observed proceed to step 7. If <50% blood-staining is observed proceed to "Perform the extraction".

7. Wash the cells:
   a. Add 200 µL of sterile deionized water to the cell pellet. Thoroughly mix by vortexing.
   b. Centrifuge at 12,000x*g* for 1 minute.
   c. Remove the supernatant leaving 10 to 20 µL of supernatant behind to resuspend the pellet.

   Note: This additional washing step helps to lyse the red blood cells and remove haem that could inhibit PCR.

Perform the extraction

1. Add 200 µL of InstaGene matrix to the samples using a pipette tip with a large bore, such as 1000 µL.

   Note: To optimize the extraction protocol, you can adjust the amount of InstaGene matrix (Chelex-100 resin) that you add. For example, you can use 100 µL of InstaGene matrix for small (barely visible) AF cell pellets, or 300 µL of the matrix for pellets large enough to cover the base of the tube. You can also adjust the amount of matrix to the quantity of CV samples. Record the amount of InstaGene matrix added to each sample.

2. Thoroughly mix the samples by vortexing and incubate the samples at 100 °C for 8 minutes using a hot block or water bath.

3. Thoroughly mix the samples again by vortexing at high speed for 10 seconds.

4. Centrifuge the samples at 12,000x*g* for 3 minutes.

   Note: The supernatant contains the extracted DNA.

5. Proceed to PCR setup or store the extracted DNA at –20 °C until required.

Recommended DNA concentration

Under optimal PCR conditions and using the appropriate genetic analysis settings, acceptable results are consistently obtained with input DNA amounts of 1.25 to 10 ng.

Genetic analysis settings can be modified to suit the amount of amplicon produced during the PCR reaction, which can vary due to the amount of input genomic DNA added. Less amplicon can be applied to the column for analysis by reducing time of injection. Conversely, more amplicon can be applied to the column for analysis by increasing either time or voltage of injection. Previously amplified samples can be re-injected multiple times for re-analysis.

Procedure

Amplify DNA

Where samples have been extracted using the InstaGene method, we recommend using undiluted supernatant directly in the PCR reaction.

Combine DNA and Reaction Mix

1. Thaw sufficient vials of pre-aliquoted *TrueScience*™ Aneuploidy Kit Reaction Mix for the number of samples and controls to be run (10 µL for 10 tests) and centrifuge the vials at 12,000x*g* for 10 seconds.

IMPORTANT! To minimise the risk of contamination, steps 2 to 4 must be carried out in an area free from DNA. Steps should also be taken to avoid contamination with PCR product.
Include a negative control (water) in each PCR run.Your laboratory procedure may also include other controls, such as a positive normal DNA control (supplied) and positive trisomy DNA control (not supplied).

---

2. Using separate pipette tips, add 2.5 µL of test DNA to a sample vial containing 10 µL of *TrueScience*™ Aneuploidy Kit Reaction Mix pipette up and down to mix. Repeat for all of the samples to test.

   Do not add DNA to the PCR vial for the negative control. Instead, add 2.5 µL of sterile distilled water.

   Note: Make sure that you do not contaminate the PCR reaction with any InstaGene resin.

3. Briefly centrifuge the vials until all liquid is at the bottom of each vial.

Run PCR

1. Place all vials firmly in the thermal cycler block.

2. Program the thermal cycler as shown in the table below.

Stage	Temp	Time (mm:ss)
Enzyme activation	95 °C	15:00
Cycling: 26 cycles	95 °C	00:30
	59 °C	01:30
	72 °C	01:30
Final extension	72 °C	30:00
Hold	4 °C	≤7 days†

† When PCR is finished, samples may be stored at 2 to 8 °C for ≤ 7 days before analysis by capillary electrophoresis.

3. Start the PCR reaction.

Set up samples for capillary electrophoresis

Note: When performing capillary electrophoresis, make sure that the genetic analysis instrument is validated with this test. Use the electrophoresis equipment according to the manufacturer's instructions.

We recommend using the following sample setup for a run on an Applied Biosystems 3130 or 3500 Series Genetic Analyzer. For more information on setup of these Genetic Analyzers, refer to the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038).

IMPORTANT! If you include reactions from different kits in the same autoanalysis plate, you must group the reactions in the plate by kit, so that the kit reactions are injected in separate injections.

Combine size standard, formamide, and PCR product

1. Combine 6.85 µL of size standard with 250 µL of Hi-Di™ Formamide (sufficient mix for 16 wells, plus overage) and mix thoroughly. On a 96-well optical plate, pipet 15 µL of the mix into the required number of wells.
2. Pipette 3 µL of sample PCR product into the size standard mix already pipetted into the plate in step 1. Use the pipet to mix.
3. Seal the plate.

Incubate

Denature the PCR product by heating the plate in a thermal cycler set to:

- 94 °C for 3 minutes
- 4 °C for 30 seconds

Run sample plates

1. Centrifuge the plate at 1,000x*g* for 10 seconds to remove any bubbles in the wells.
2. Load the plate into the Genetic Analyzer.

Analyze data

It is recommended that each laboratory develop 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: **http://www.cmgs.org.uk**.

PCR products are observed as a 5 dye labeled system using filter set G5. Filter set G5 detects the 6-FAM™ (blue) dye, VIC® (green) dye, NED™ (yellow) dye, and PET® (red) dye labeled fragments plus the Size Standard marker labelled with LIZ® (orange) on an electropherogram in the GeneMapper® Software.

For more information on analysis with GeneMapper® Software, refer to the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038).

IMPORTANT! Different combinations of instrument, polymer, and size standard may cause the size calling to vary slightly. During validation of the kit, users should check that the default bin settings result in accurate peak labelling and adjust if necessary. In case of any difficulty, please contact Technical Support for advice.

Interpret results

IMPORTANT! Before continuing, read "Limitations and disclaimer" on page 53.

General analysis guidelines for all *TrueScience*™ Aneuploidy Kits

Sample results criteria

- The negative control should show no sharp peaks within the read range of 100 to 510 bp.
- The normal control should show the expected results. (See "Examples of results" on page 26).
- Electropherograms should not show:
  - "Pull-up" (excessive bleed-through between dye colors). Dilute and re-inject the sample.
  - "Electrophoretic spikes" (sharp peaks present in more than one dye). Check for bubbles in the polymer and re-inject.
- For each marker, there must be at least 1 peak (except for Y chromosome markers, which will not be present in normal females).
- For 3130 Series instruments, peaks should be on-scale and >50 rfu. For 3500 Series instruments, peaks should be on-scale and >175 rfu.

Assess peak area ratios

Perform complete analysis of chromosome copy number status by comparing peak area ratios. Assess peak ratios A1/A2, where A1 is the peak area of the shorter fragment and A2 is the peak area of the longer fragment. The resulting ratio is diagnostic of locus copy number.

- Heterozygous di-allelic (that is, two alleles) markers should have a ratio of 0.8 to 1.4 (or 0.8 to 1.5 for two alleles separated by more than 24 bp in size). Any values within this range are referred to as having a ratio of 1:1.
- Ratios may be outside this range due to a number of factors, such as:
  - Whole chromosome trisomy
  - Partial chromosome trisomy (including sub-microscopic duplications)
  - Mosaicism
  - Contaminating second genotype (for example, 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.

Refer to "Supplementary Information" on page 25 for examples of typical profiles for many of the factors in this list.

Criteria for abnormal results

To interpret a result as abnormal at least two informative markers are required, with all other markers being uninformative. If required, follow-up testing with the single chromosome kits may provide sufficient information for interpretation.

Trisomy is determined by either:

- Two peaks of uneven height, due to one of the peaks representing two alleles that are common to one or both parents. In this case the ratio between the two peaks is classified as 2:1 or 1:2, such that A1/A2 is approximately 1.8 to 2.4 when the peak representing the shorter allele is greater in area than the peak representing the longer allele, or where A1/A2 is approximately 0.45 to 0.65 when the peak representing the shorter allele is smaller in area than the peak representing the longer allele.

  or

- Three peaks of comparable height. The ratio of the peaks is classified as 1:1:1 and their values fall within the normal range of 0.8 to 1.4 (although for alleles separated by more than 24 bp 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 listed in "Assess peak area ratios".

Criteria for normal results

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.

Criteria for inconclusive results

A result should be considered inconclusive under these circumstances:

- Peak area ratios between the normal and abnormal ranges are classified as inconclusive. Inconclusive results may be resolved by using the single chromosome kits.
- If both normal and abnormal allele patterns are obtained for a single chromosome, then we recommend follow-up studies to identify the reason for the discrepant results prior to reaching any conclusions.
- In rare cases allele size ranges for markers may overlap. If you suspect this has occurred, analysis with the single chromosome kits may resolve it.

Aneuploidy XY markers (STR-Plus and STR-XY kits)

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

Non-polymorphic X and Y markers

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.

The TAF9L marker is an invariant paralogous marker with sequences on chromosomes 3 and X. The chromosome-3-specific peak (116 bp, 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 (121 bp peak). Analyzed 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.

Polymorphic STR markers

The DXYS267 and DXYS218 polymorphic STR markers are present on both the X and Y chromosomes and represent the total number of sex chromosomes. For informative male results it is not possible to determine which allele represents the X or Y chromosome.

X-specific markers

Informative X-specific markers DXS981, DXS1187, XHPRT, DXS6807, DXS7423, DXS6803, and DXS6809, represent the number of X chromosomes.

Y-specific marker SRY

The Y-specific marker, SRY, produces a single peak in normal males and does not amplify in normal females.

Y-specific marker DYS448

In most cases the Y-specific marker, DYS448, produces a single peak in normal males and does not amplify in normal females. However, it is reported 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 every 171,000 females is 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.

Troubleshooting

Observed Issue	Possible Cause	Corrective Action
Decrease in sample volume after PCR	Evaporation of sample	Prepare new samples and new primer mix, carefully seal the PCR plates, then repeat the PCR. If the problem persists, use a different plate seal product.
Off-scale peaks present after primer peaks: Allele peaks too high	Excess DNA in sample	Prepare a new sample solution using diluted PCR product, then repeat the PCR.
Off-scale peaks present after primer peaks: Size standard peaks too high	Excess size standard in sample	Prepare a new sample using less size standard, then repeat the electrophoresis run.
Pull-up or pull-down peaks	Wrong dye set	Confirm what dye set was used for the run and, if necessary, repeat the run with the correct dye set.
	Spectral calibration is old	Repeat the spectral calibration and re-run the samples.
No sizing data (size quality fails)	Incorrect size standard or size standard not selected	In the GeneMapper® analysis software, ensure that the size standard settings are correct (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)).
	Too much size standard or PCR product	Prepare capillary electrophoresis samples with less PCR product or size standard, then repeat the electrophoresis run.
	No size standard in well(s)	Prepare a new capillary electrophoresis sample, then repeat the electrophoresis run.
Size standard peak(s) identified incorrectly	Size Match Editor settings need to be edited	In the GeneMapper® analysis software, in the Size Match Editor, manually edit peaks (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)).

Observed Issue	Possible Cause	Corrective Action
Cut-off size standard peaks	Run was too short	On the Genetic Analyzer instrument, set a longer run time (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)). Repeat the PCR.
Size standard peaks not present or too low	Various causes (old array, old polymer, no current, leak, salt or sample DNA competing out the SS)	Prepare a new sample, then repeat the electrophoresis run.
Too many peaks (noisy data) appearing at initial 25 to 50 bp	Analysis range begins with fragments that are too short	In the GeneMapper® analysis software, set the Analysis Range to begin at >70 bp, then repeat the electrophoresis run. Make sure the size standard definition matches the analysis range.
Low or no size standard peaks and low or no allele peaks	Low size standard concentration	Repeat the electrophoresis run using more size standard.
	Volume in well is less than 10 µL	Repeat the capillary electrophoresis, using more sample.
	Bubble(s) at the bottom of well(s)	Spin the plate, then repeat the electrophoresis run.
	Injection problem	Repeat the injection, then repeat the electrophoresis run.
	Tubing blocked due to impurities in buffer, outdated buffer, insufficient/no buffer in sample, or bubbles in polymer	In the EPT (Electrophoresis Power and Temperature) window of the genetic analyzer, note the current value for the run. Low values may indicate the presence of bubbles in the polymer. Add new buffer and remove bubbles from the column if necessary, then repeat the electrophoresis run.
	Capillary array is outdated or defective	Repeat the electrophoresis run using a new capillary array.
Low or no size standard peaks, but high or normal allele peaks	Insufficient or no size standard in electrophoresis sample(s)	Prepare a new sample of PCR product for electrophoresis (see "Set up samples for capillary electrophoresis" on page 15) and repeat the run.
	Excess electrophoresis sample and/or salts compete with size standard	Dilute the sample of PCR product, add size standard, and repeat the electrophoresis run.

Observed Issue	Possible Cause	Corrective Action
Low or no allele peaks, but high or normal size standard peaks	Insufficient DNA for PCR amplification	Repeat the PCR with more DNA.
	PCR inhibition	1. Purify or re-extract the DNA. 2. Dilute the DNA to dilute inhibitors. 3. Repeat the PCR.
	Non-homogenous DNA used for PCR	Homogenize the DNA, then repeat the PCR.
	PCR reagents contain impurities or are outdated	Repeat the run using unexpired reagents that are stored properly.
	Primer dilution error	Prepare new primer dilutions from stock solution and repeat the PCR.
	Incorrect thermocycling parameters	Set the PCR parameters as shown in step 2 on page 14, then repeat the PCR.
	Primer design error	Review primer design and resynthesize primers if necessary.
No high molecular weight allele peaks and no size standard peaks	Ineffective electrophoresis	Repeat the electrophoresis run using new buffer, polymer, and array.
No allele peaks	Inhibition of DNA or inhibition of PCR	Determine the quality of DNA in the PCR sample. If necessary, repeat the PCR using less DNA.
	Incorrect thermocycling parameters	Set the PCR parameters as shown in step 2 on page 14, then repeat the PCR.
	Degraded DNA	Determine the quality of the DNA. If necessary, purify or re-extract the DNA. Repeat the electrophoresis.

Observed Issue	Possible Cause	Corrective Action
Extra allele peaks and/or allele peaks in wrong positions	Contamination by other sample	Repeat the PCR using negative control
	Incorrect analysis settings	In the GeneMapper® software, in the **S** sample tab of the project window, verify that the panel selected matches the product used (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)).
		In the GeneMapper® software, verify that the appropriate bin file with the correct bin sizes is selected in the **Allele** tab of the Analysis Method being used (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)).
	Incorrect sizing analysis	Review the sizing to make sure the correct size standard peaks are identified (see the *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide* (PN 4454038)).
Spikes (tall narrow peaks in all 4 colors)	Urea crystals in polymer	Repeat the electrophoresis run, using non-outdated polymer that is stored correctly.
	Bubbles in polymer	Eliminate bubbles in all of the polymers used, then repeat the electrophoresis run.
Split peaks that differ by 1bp	Partial addition of adenosine to PCR products	Repeat the PCR using a final extension time of 30 to 90 minutes.

Supplementary Information

IMPORTANT! Before continuing, read "Limitations and disclaimer" on page 53.

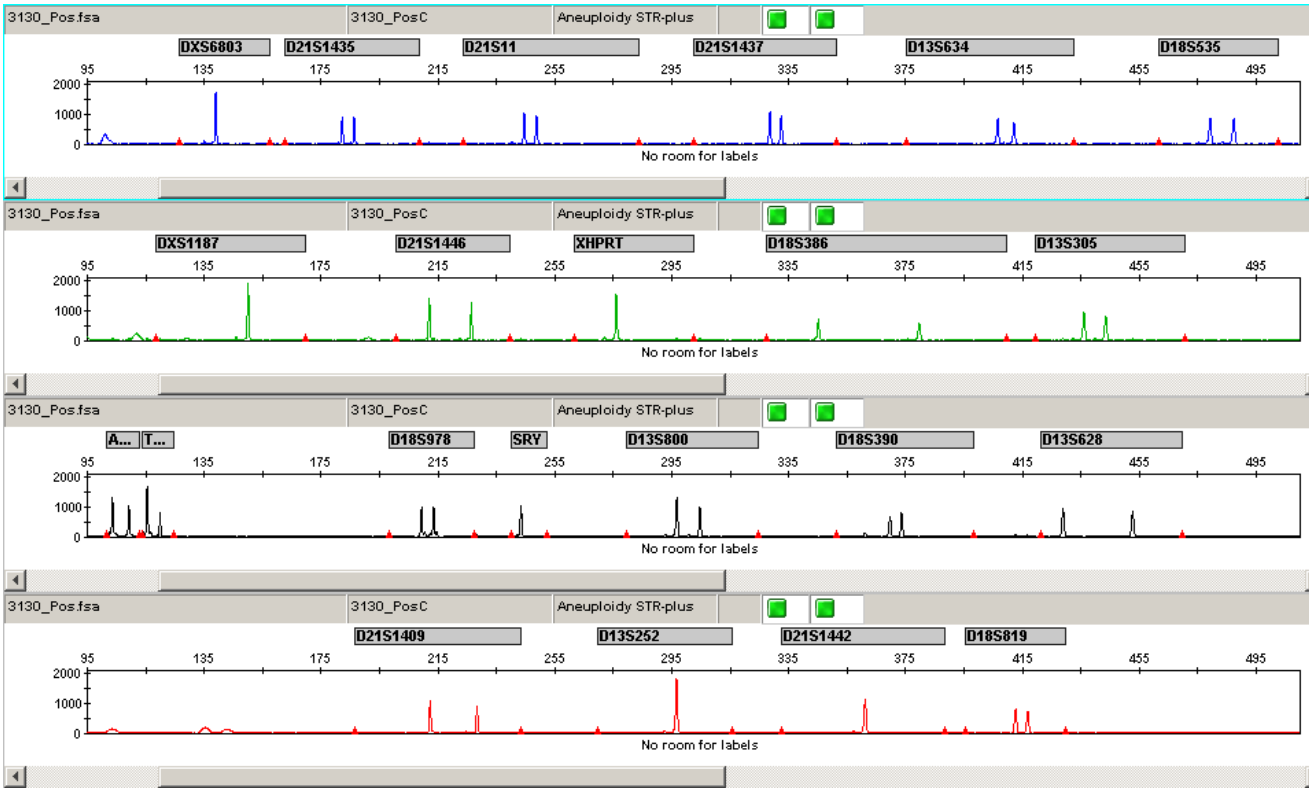
This section covers:

Note: Examples provided here were made with Elucigene QST*R aneuploidy kits. These products are equivalent in form and function to the Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits.

Examples of results

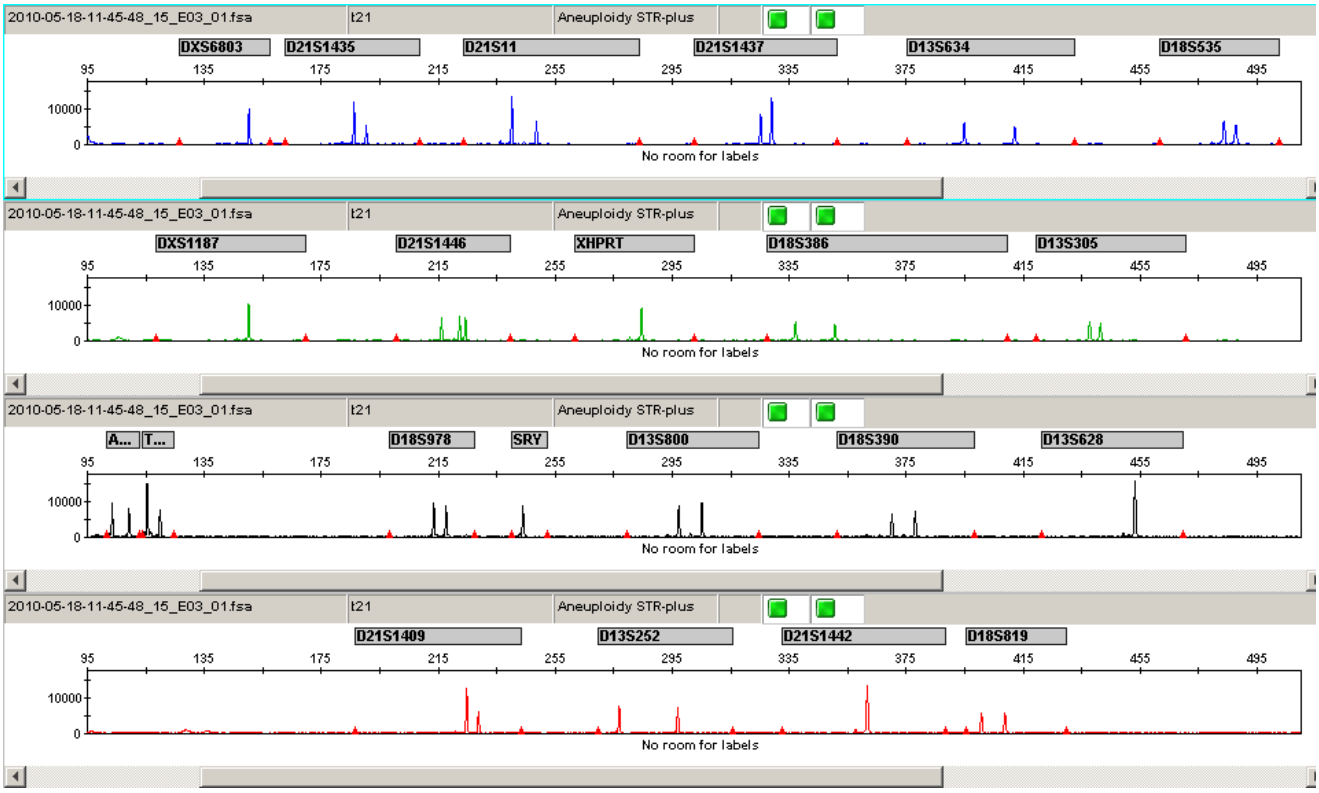
Normal XY profile

Figure 1 Normal male electropherogram. (This example was made using the STR-Plus product.)



Whole Chromosome Trisomy

Figure 2 Trisomy 21 profile. (This example was made using the STR-Plus product.)



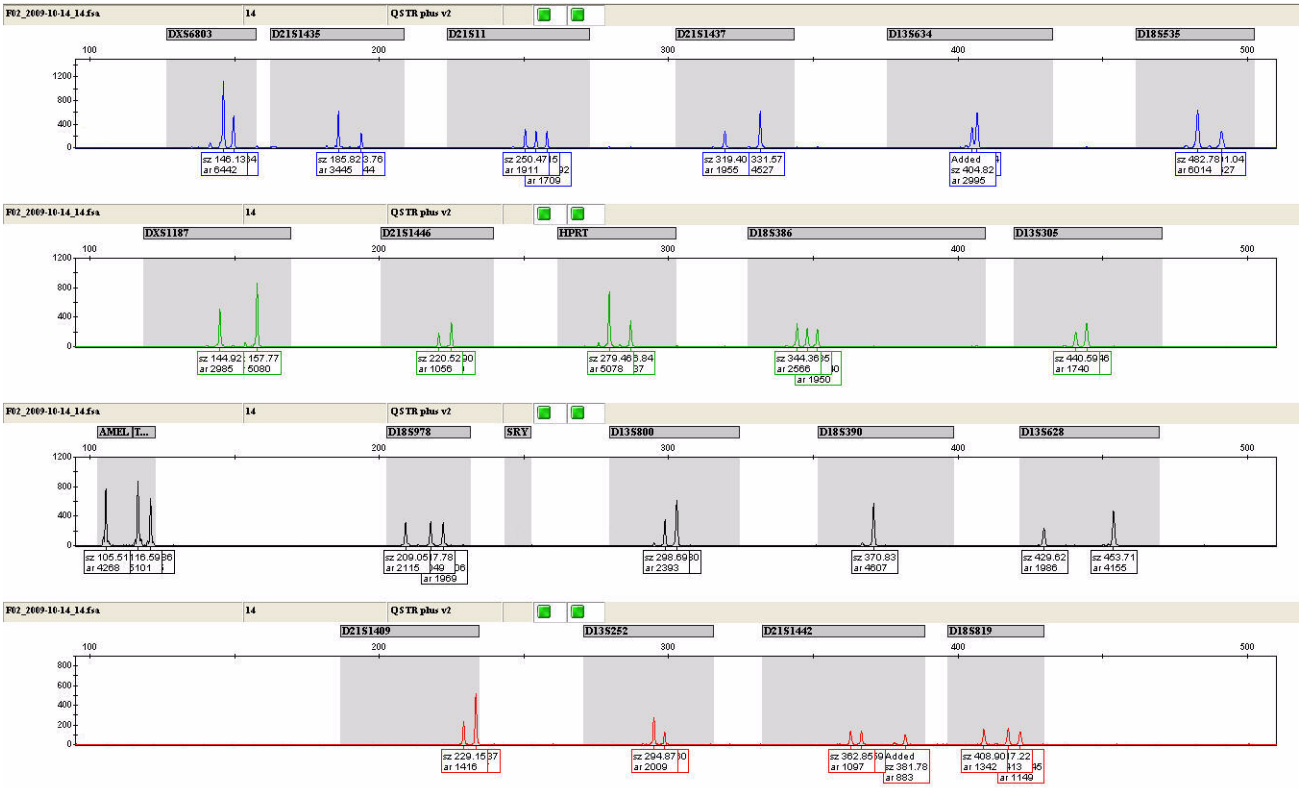
Note: Informative markers for individual chromosomes demonstrate a deviation from the expected normal 1:1 ratio. This can either result in a 2:1 or 1:2 ratio for di-allelic markers or a 1:1:1 ratio where markers demonstrate three alleles. A dialleleic trisomy result demonstrates two peaks of uneven height 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 24 bp 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 nondisjunction 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.

Triploidy

The profiles in Figure 3 demonstrate trisomy at all informative loci, on all chromosomes tested.

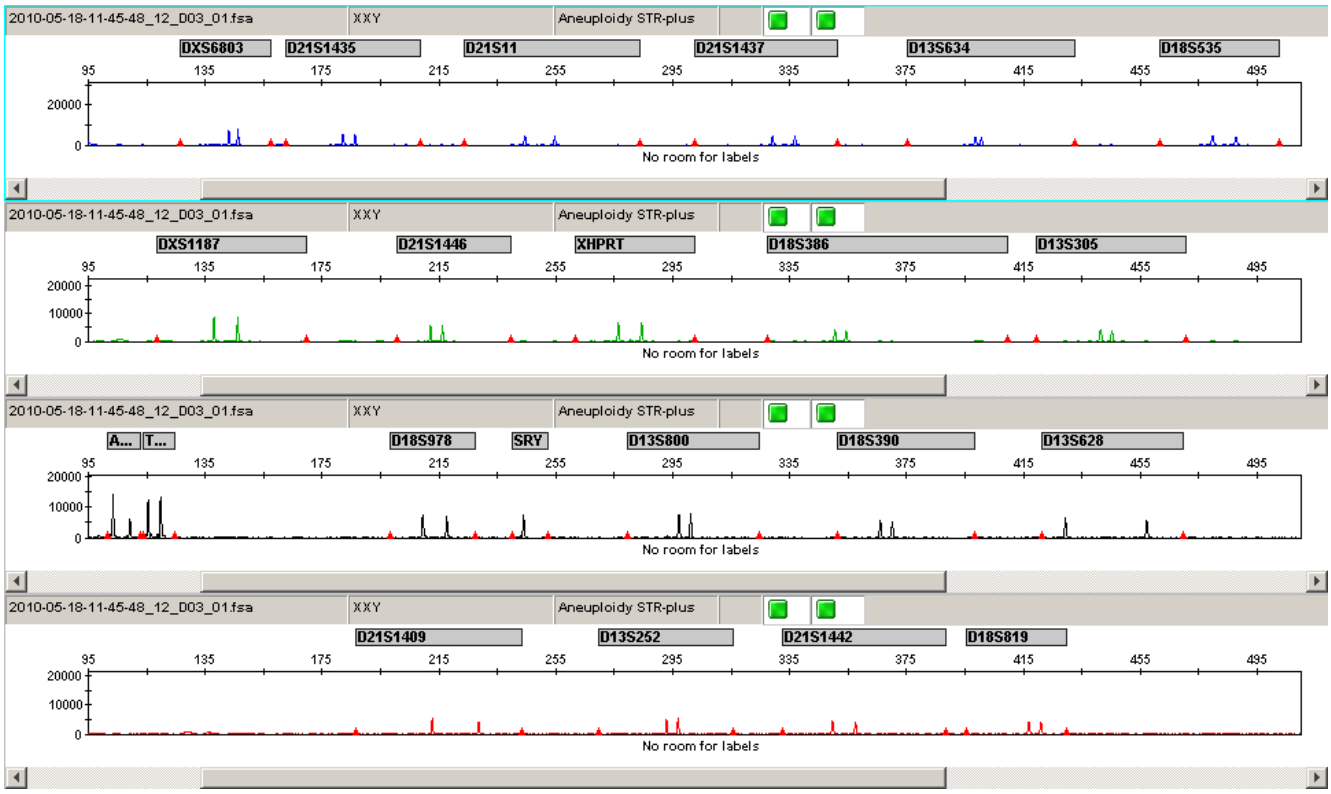
Figure 3 Triploidy profile. (This example was made using the QST*R*$_{plus}$v2 product.)



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.

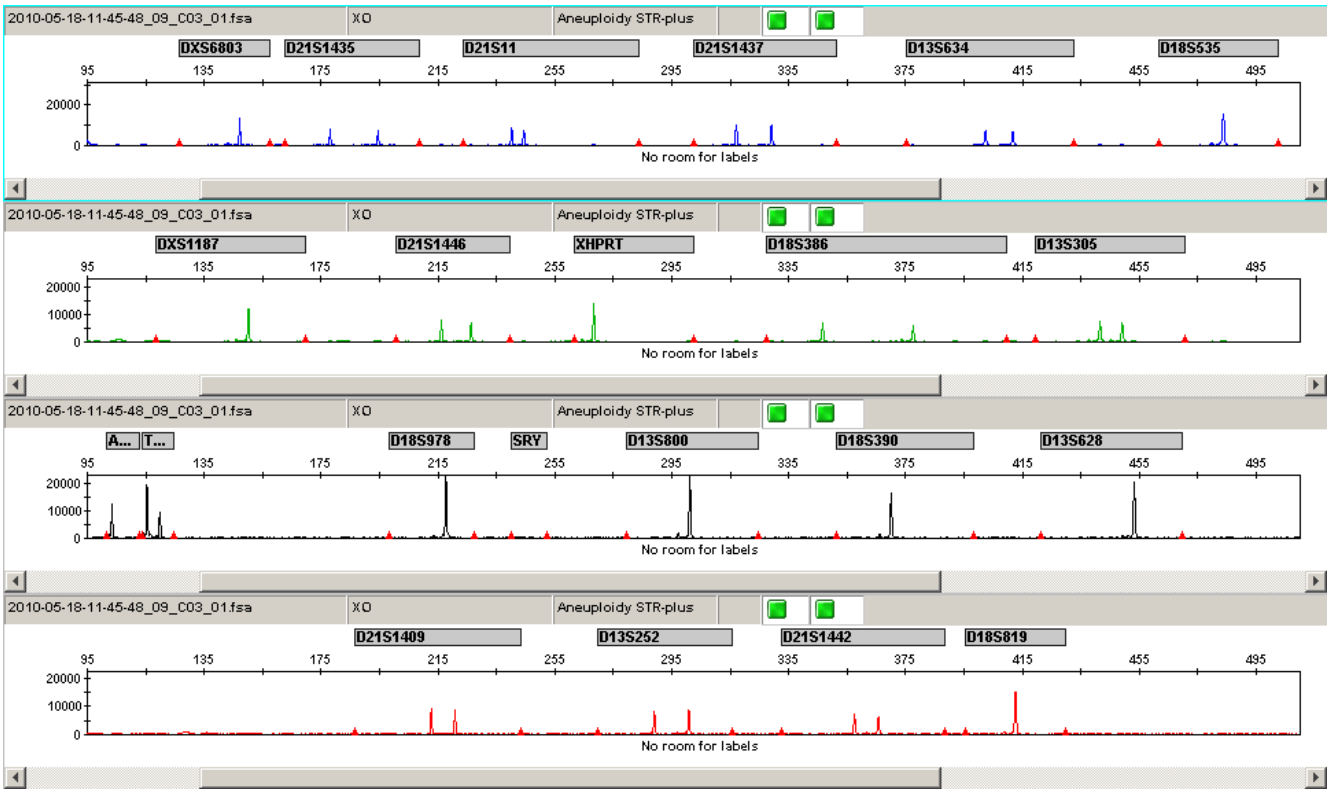
Figure 4 Example of classic 47,XXY Klinefelter profile. (This example was made using the STR-Plus product.)



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,XO) karyotype.

Figure 5 Example of classic 45,X Turner profile. (This example was made using the STR-Plus product.)

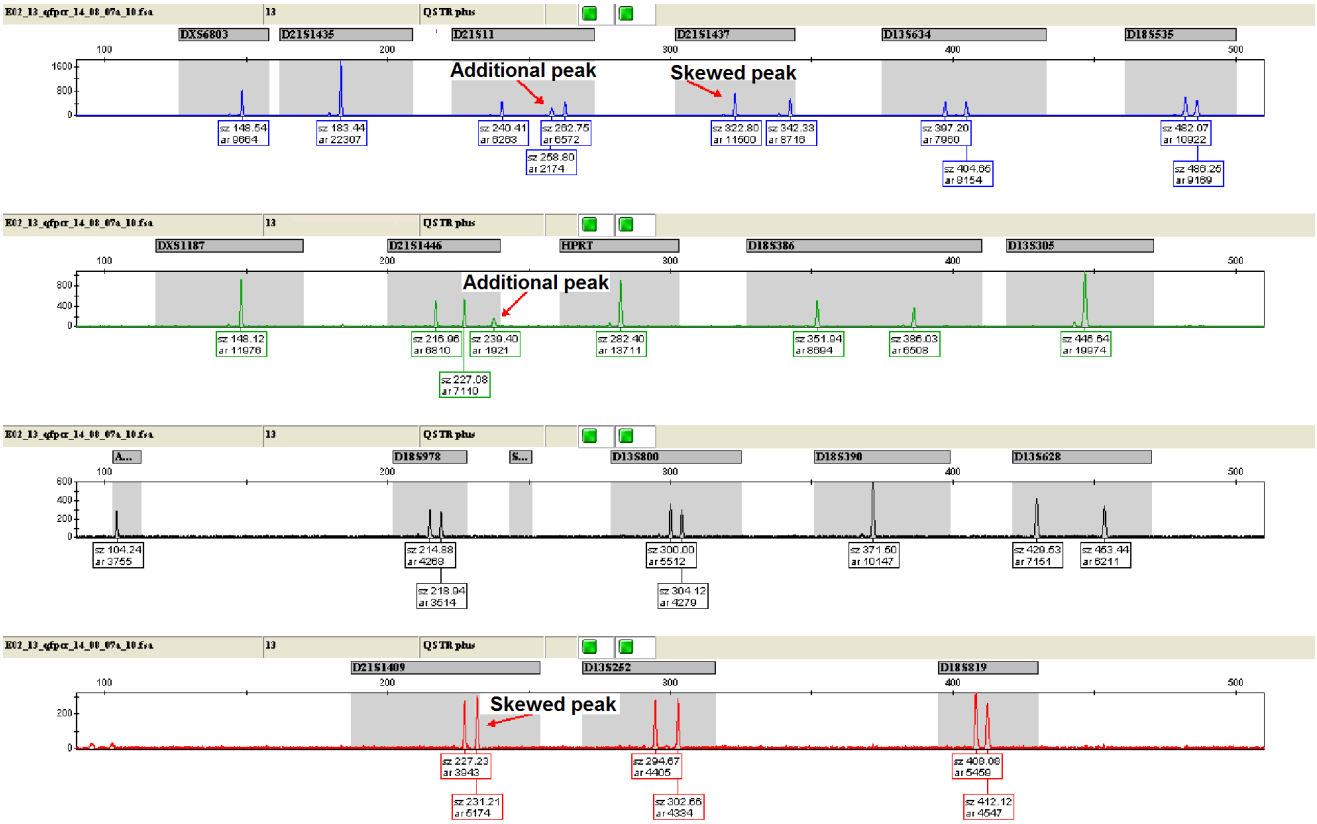


Note: Approximately 1 in every 171,000 normal females demonstrate a homozygous profile for all X markers by chance. Consanguinity increases the risk of a homozygous genotype in a normal female.

Mosaicism

All informative markers on a single chromosome show skewed allele ratios and/or a minor third allele peak. Care should be taken to distinguish this result from maternal cell contamination. In an optimized system, a normal or abnormal cell line can be detected if present at a level of >20% of the total cell population. This example was made using the QST*R*plus product.

Figure 6 Mosaic trisomy 21 (approximately 25-30%)



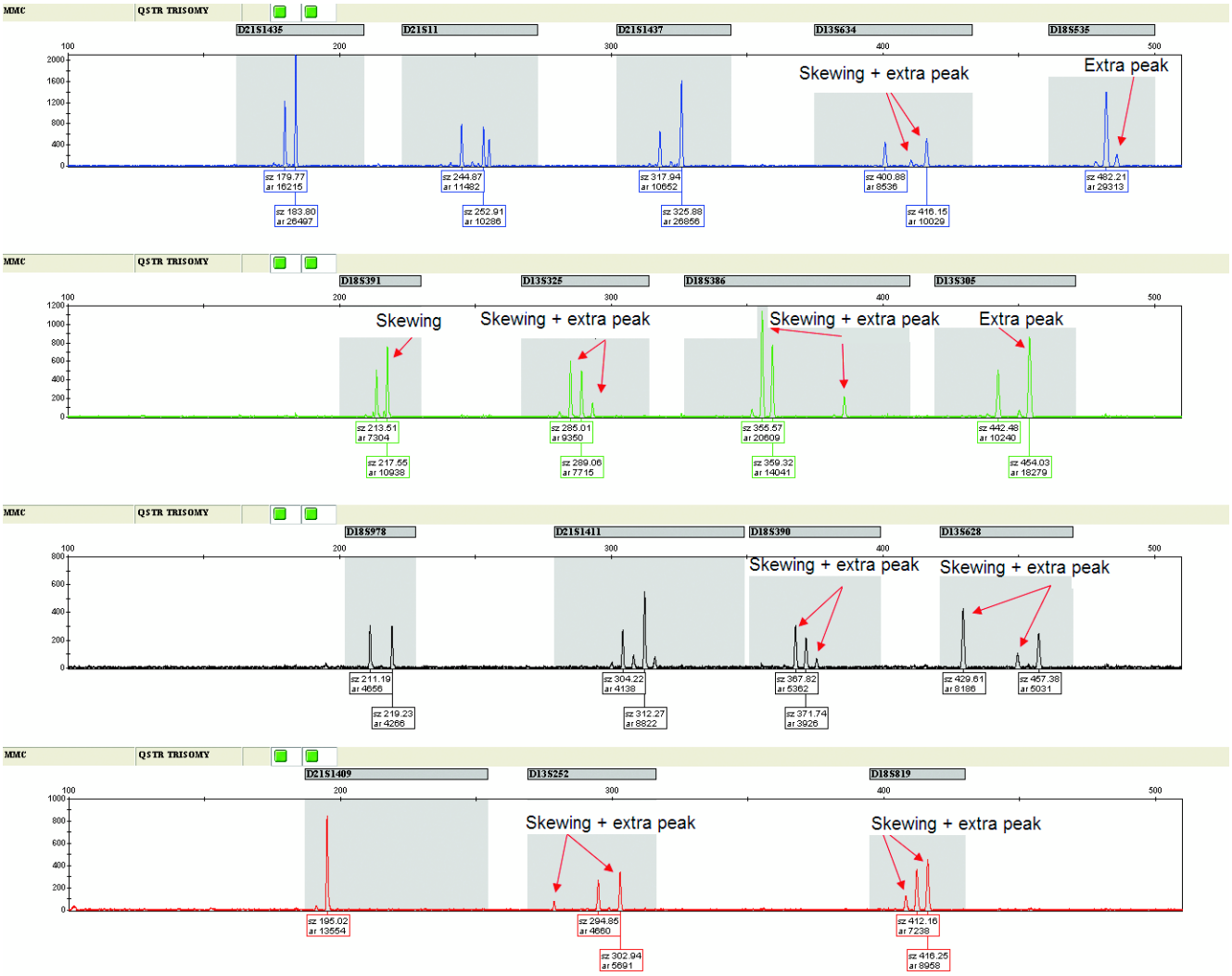
Note: Testing a small aliquot of the digested/chopped villi prepared for cell culture set-up increases the likelihood of detecting the predominant cell lines in the whole CVS sample. Testing a small section of a single villus may result in a majority cell line present in the CVS sample not being represented by the *TrueScience*™ Aneuploidy Kit result.

Confined placental mosaicism (CPM) may result in a discrepancy between the *TrueScience*™ Aneuploidy Kit result and the true fetal karyotype.

Maternal Cell Contamination (MCC)

The profiles in Figure 7 demonstrate the presence of two genotypes where one allele is shared between the fetus and mother, and therefore no four-allele results (which may represent twin, chimera, or external sample contamination) are observed. 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 is the shared allele. Maternal cell contamination 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 is in the inconclusive range, then using the profile to assess the chromosome status is not recommended.

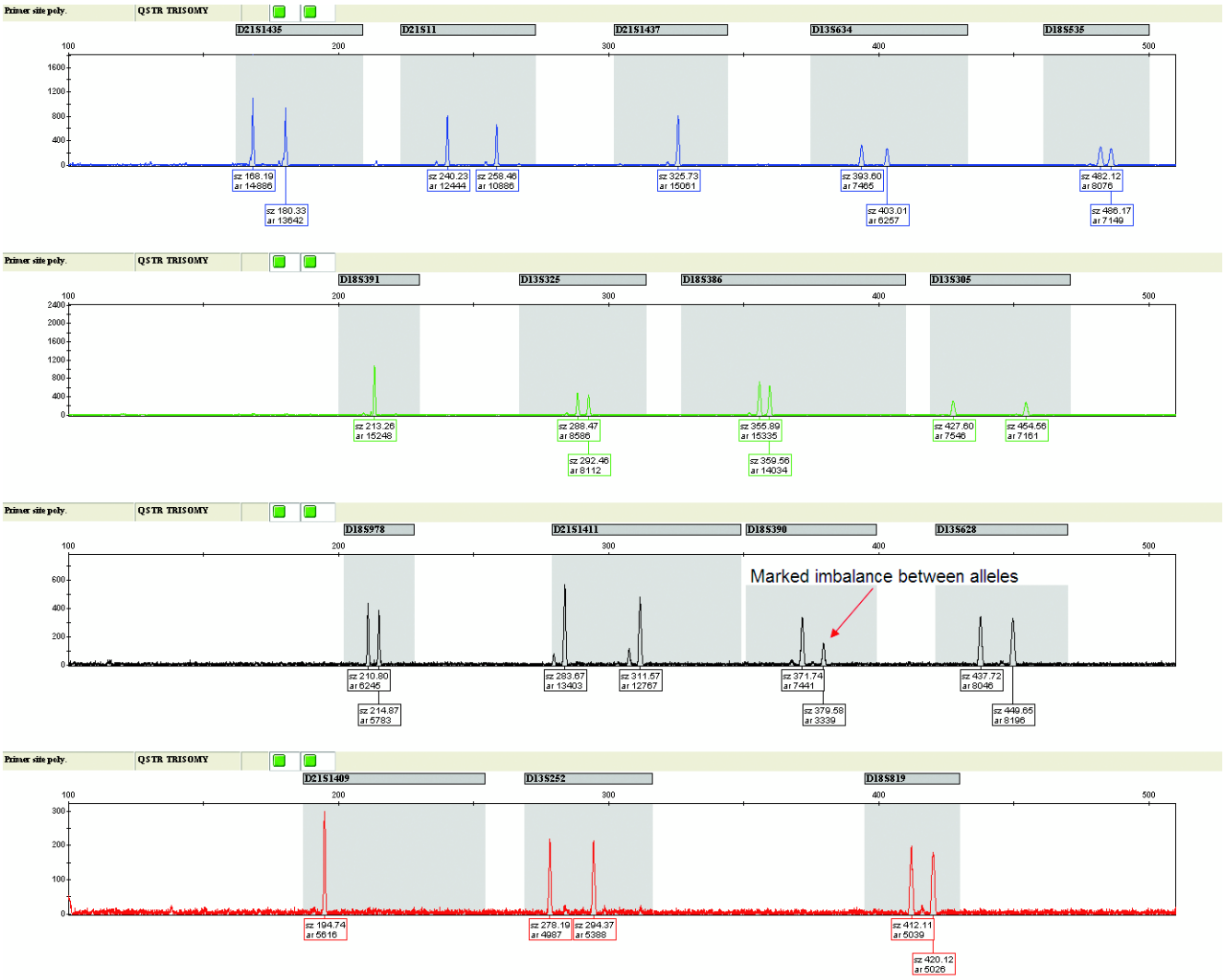
Figure 7 Trisomy 21 with maternal cell contamination. (This example was made using the QST*R product.)



Primer Binding Site Polymorphism (PSP)

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 demonstrated by an additional peak at a reduced height, which can result in skewed, inconclusive, or apparent 1:2:2:1 allele ratios.

Figure 8 Primer binding site polymorphism in D18S390. (This example was made using the QST*R product.)

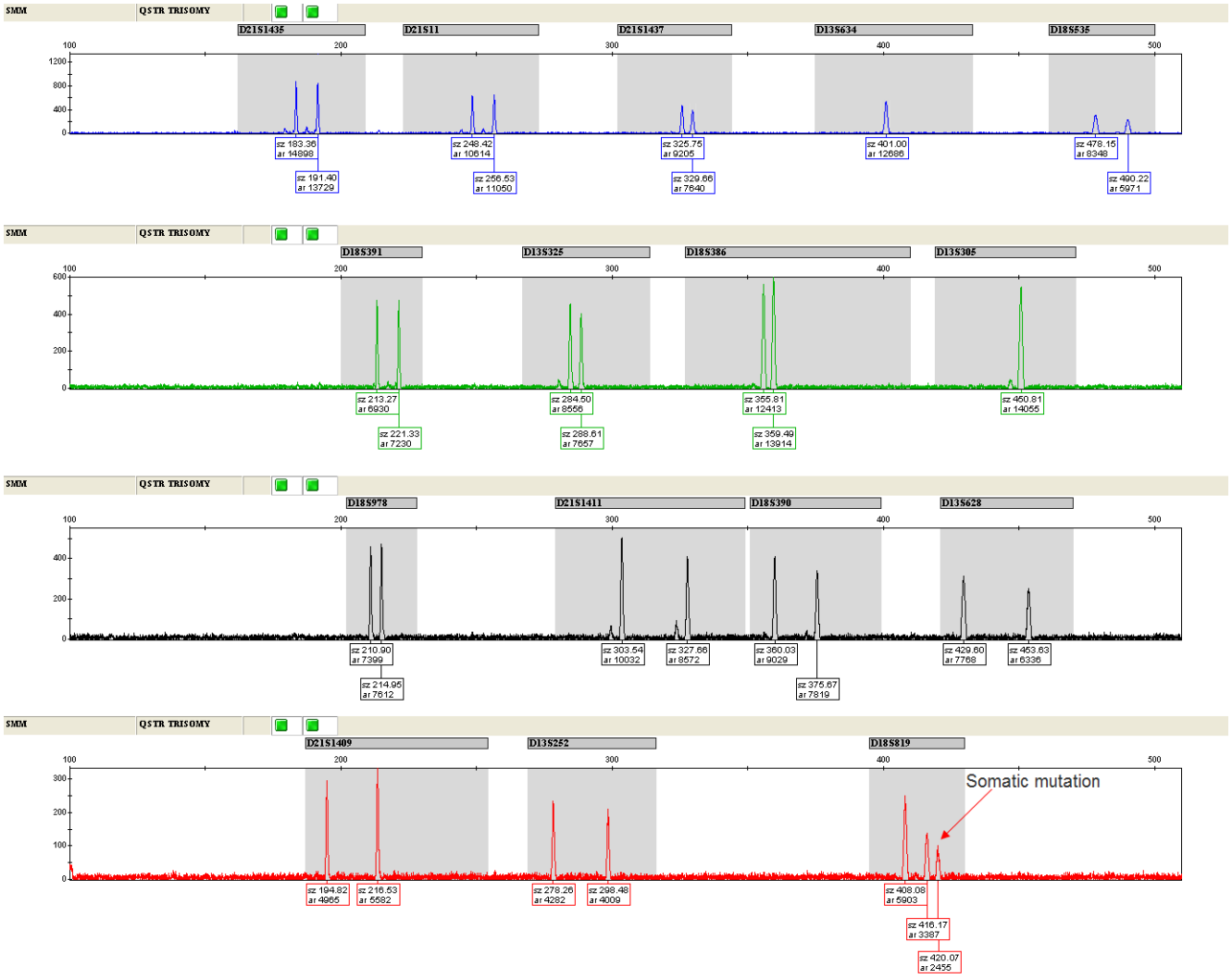


Note: Primer binding site polymorphisms can often be distinguished by repeating the assay at a significantly lower annealing temperature (for example, 4 °C lower). This allows more permissive annealing of the primer and may result in the restoration of the expected profile/peak size. If this is the case,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

Somatic Microsatellite Mutation (SMM)

Somatic microsatellite mutation (SMM) is the generation of a novel allele at a single locus, probably due to mitotic replication error. It is evident on a profile when present in a subpopulation of cells (mosaic), as either 3 alleles where A + B = C (A and B are the reduced height peaks) or persistent skewed di-allelic ratios. It is more frequently observed in CVS; only one of the two villi may be affected.

Figure 9 Somatic microsatellite mutation in D18S819. (This example was made using the QST*R product.)

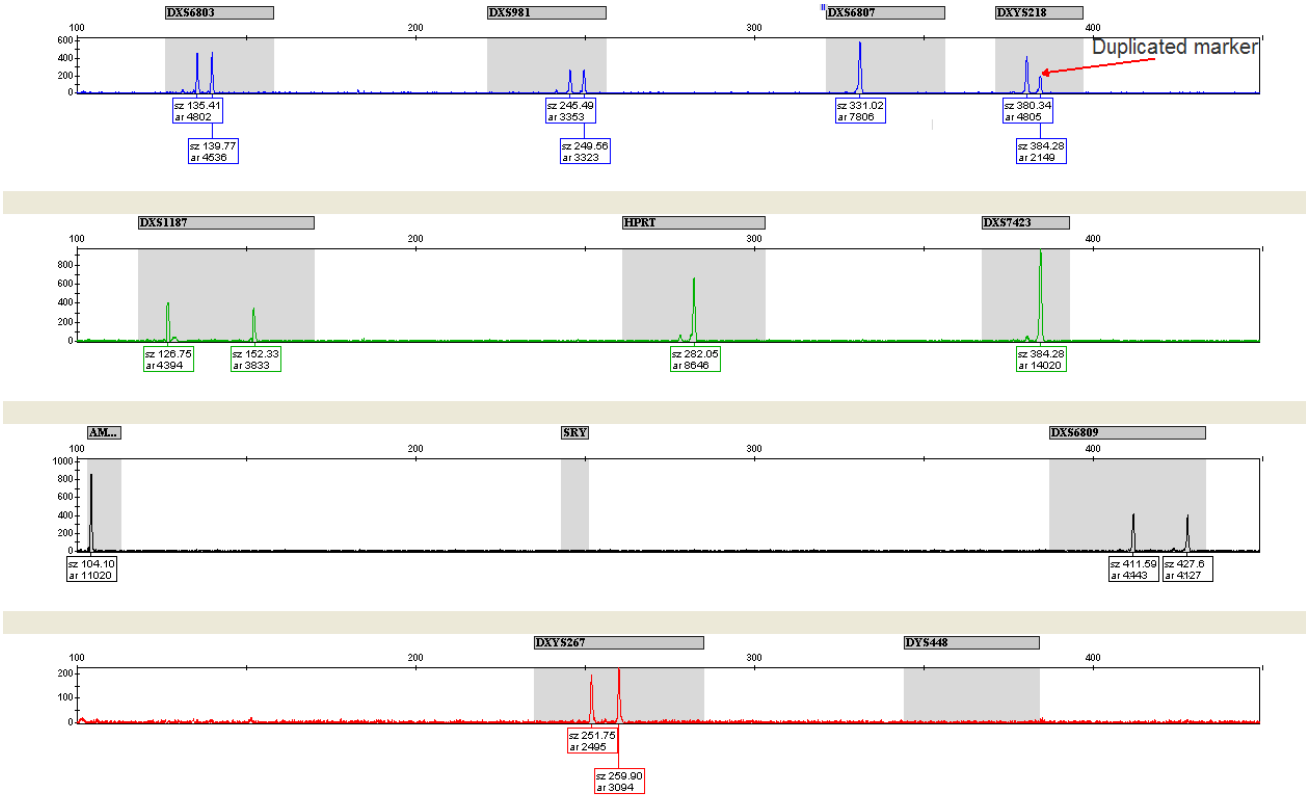


Note: SMMs are mosaic. Testing different cell populations (other villi or cultured cells) can help to classify results as SMMs and distinguish them from sub-microscopic duplications (see"Sub-microscopic Duplication" on page 35) if they appear as a 2:1/1:2 ratio.

Sub-microscopic Duplication

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

Figure 10 Sub-microscopic duplication in DXYS218. (This example was made using the QST*R-XY product.)



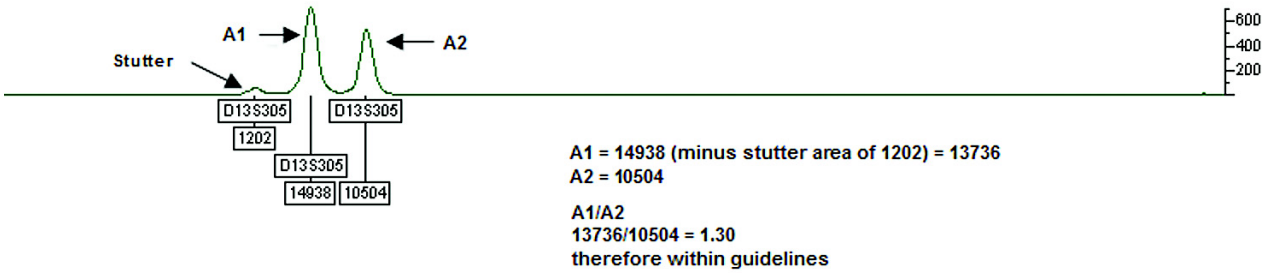
Skewing

Skewing can be defined as any significant variation from a 1:1 allele ratio. A number of mechanisms may cause skewed allele ratios (for example Mosaicism, Low input DNA and Contamination from external source).

Stutter

PCR conditions that are not optimized can increase stutter formation. As PCR product accumulates, the shorter stutter peak may be preferentially amplified, resulting in a reduction in the amplification of the full allele. Although stutter peaks are representative of the sample being tested, they are not typically included in the analysis.

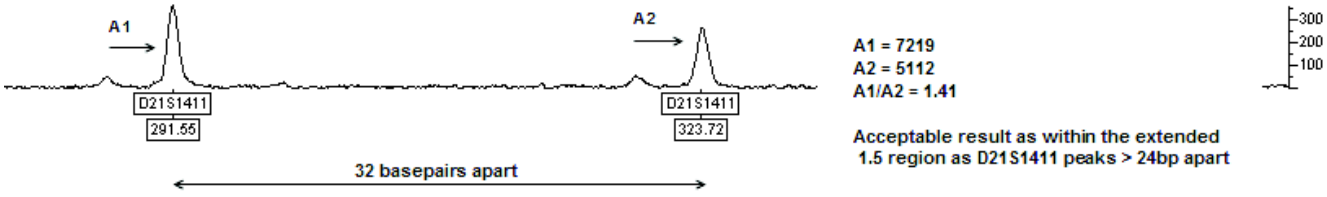
Figure 11 Stuttering in d13S305



Large difference in size between two alleles

PCR and electrophoretic injection are competitive reactions. In instances where allele fragment lengths are separated by a large number of repeats, the shorter allele can be preferentially amplified, leading to an imbalance between the two alleles.

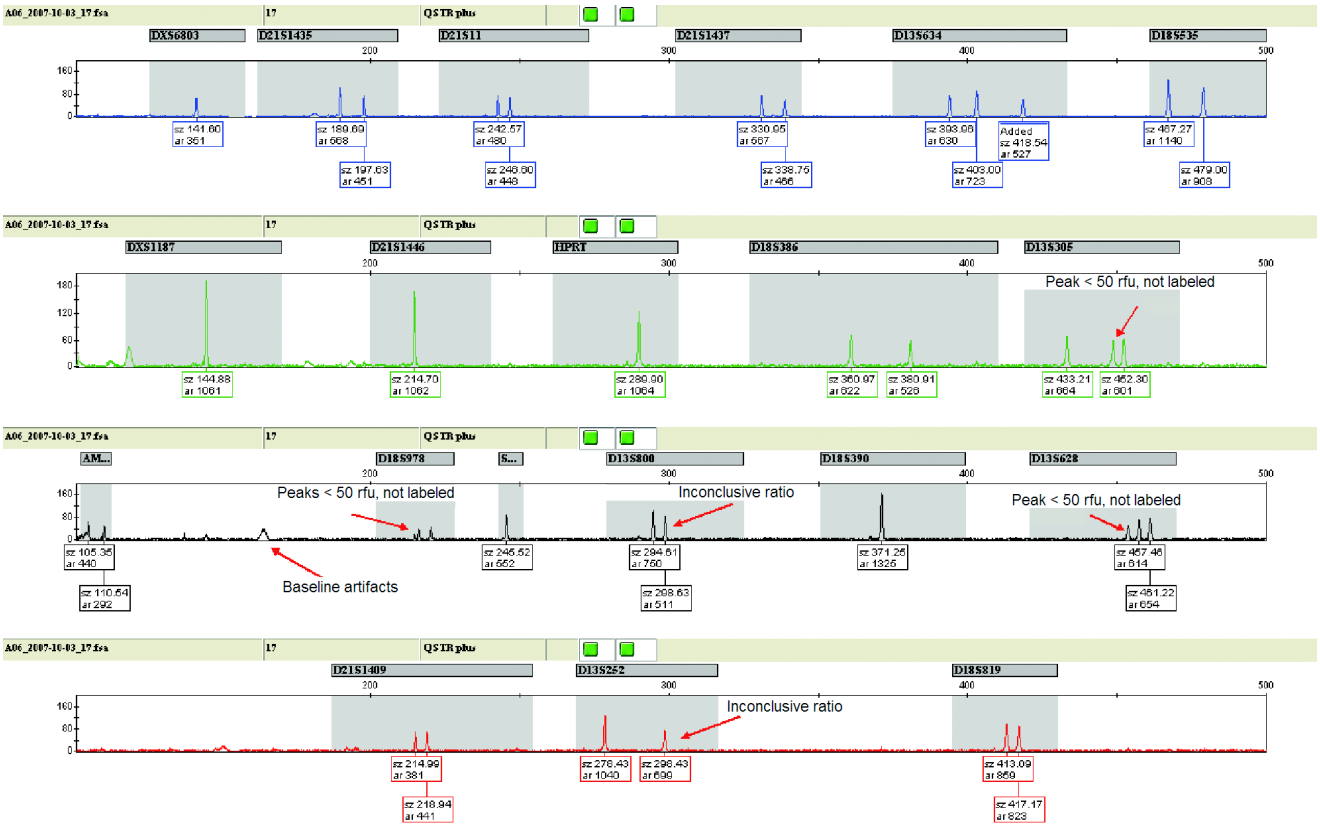
Figure 12 Large interval between alleles in d21S1411



Low input DNA

A 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, DNA should be re-extracted and re-suspended in a lower volume. Low input DNA can also result in very small peaks that fail to label (that is, peak height is below threshold set for labelling a peak), so results should be reviewed with extra care.

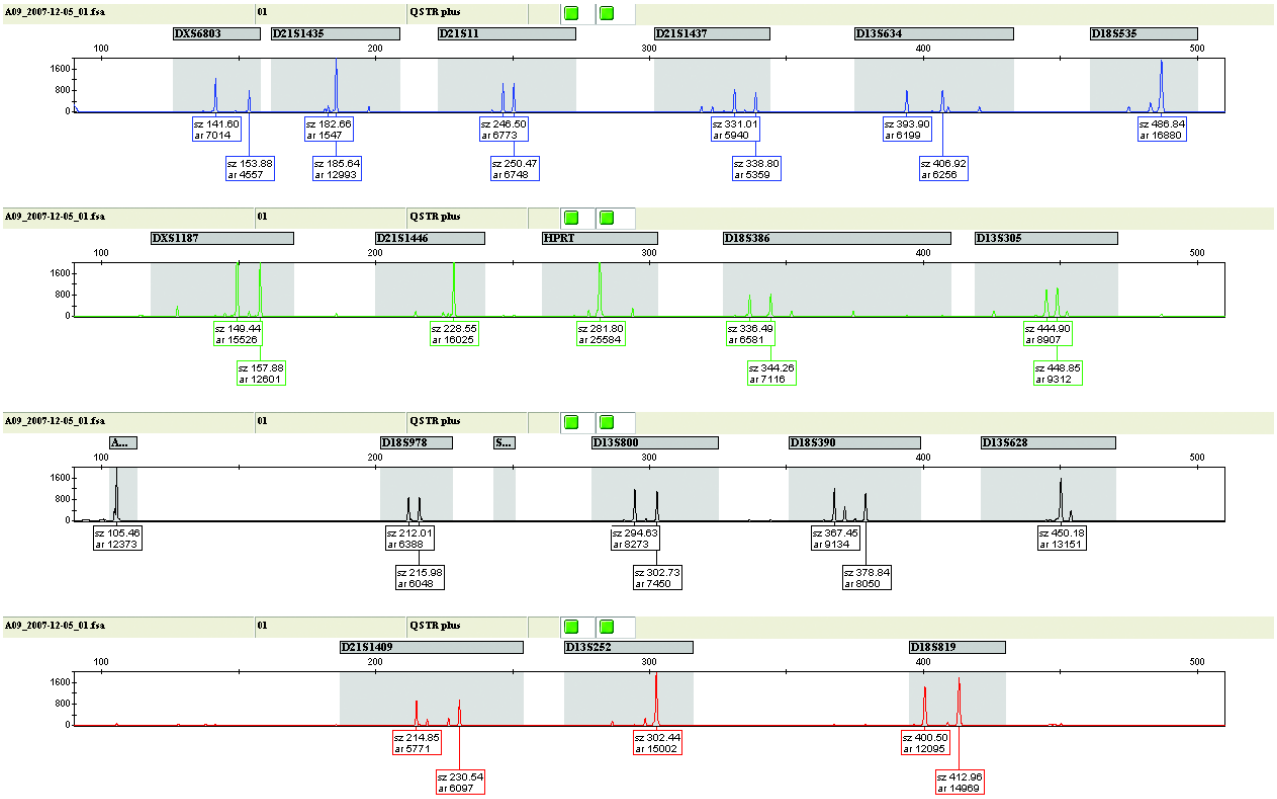
Figure 13 Normal XY with low input DNA. (This example was made using the QST*R*plus* product.)



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 14 Example of external contamination. (This example was made using the QST*R*plus product.)

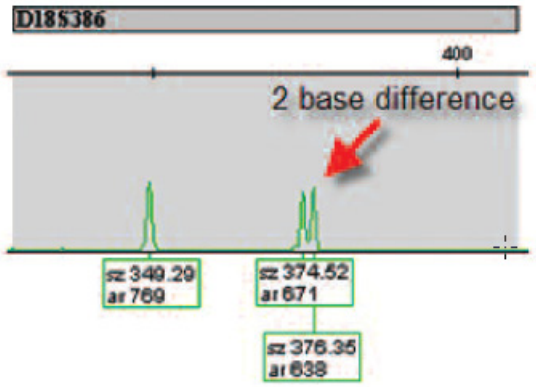


Note: The profile above is an example of confirmed external contamination of the amniotic fluid sample. An apparent contaminating profile may also be due to the presence of a dizygotic twin or may represent a chimera. A chimera is an individual with two cell lines derived from two separate zygotes. This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

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. For example, a 9.3 allele indicates 9 full repeats and a 3-base microvariant. Such a variant is 1 bp shorter than a 10 allele.

Figure 15 Example of 2 bp microvariant in d18S386



Note: According to the Best Practice Guidelines, markers demonstrating an apparent 1-bp separation should not be used because peak splitting can also result in a peak being separated into 2 peaks, which are 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 show imbalance. In profiles where peak splitting is present, it usually affects more than one marker.

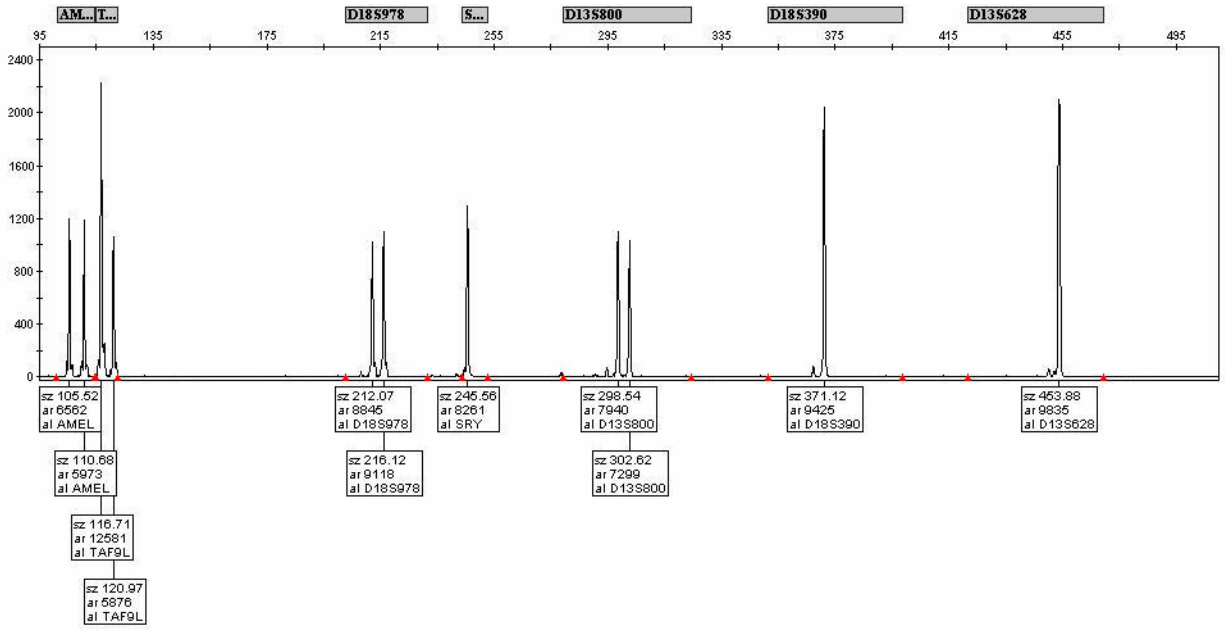
Interpretation of TAF9L marker in conjunction with Amelogenin and SRY

The TAF9L marker has been included as a quantitative marker to assist in determining the number of X chromosomes present. The TAF9L marker 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.

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

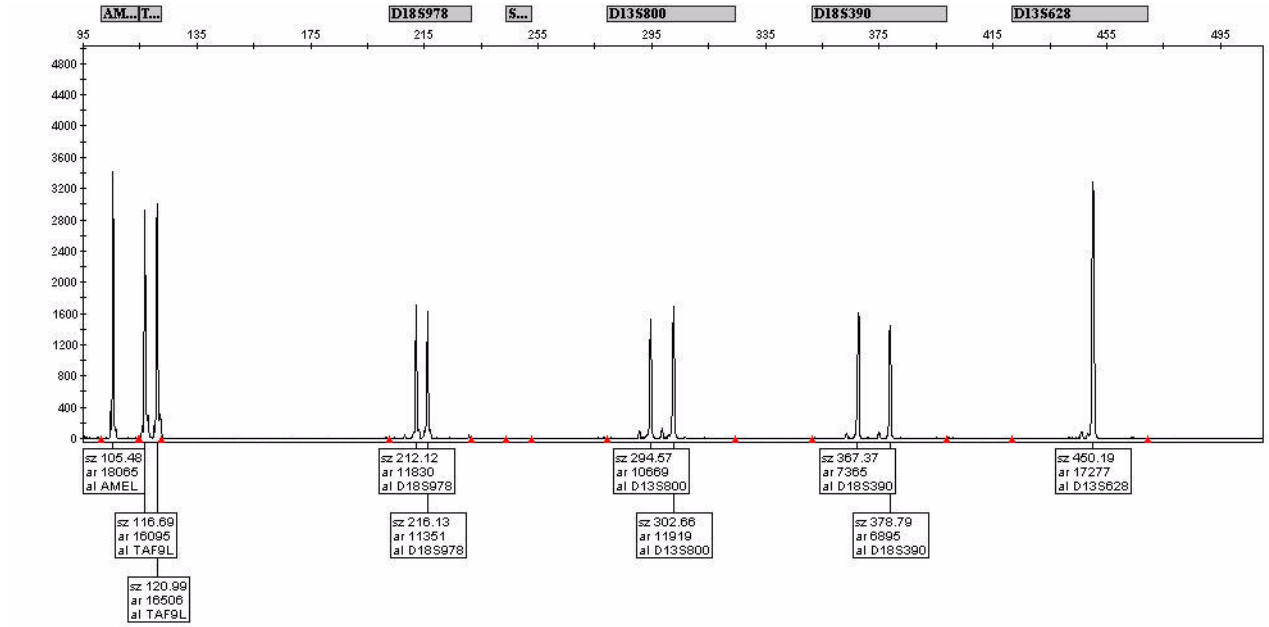
Figure 16 Normal XY male



Results consistent with XY sex chromosome complement are indicated by:

- **Amelogenin** – 1:1 ratio, consistent with equal number of X and Y chromosomes
- **SRY present** – Consistent with presence of Y chromosome. SRY is similar in height to AM X/AM Y and TAF9L X peaks
- **TAF9L** – 2:1 ratio, consistent with 2 copies of chromosome 3 and 1 copy of X chromosome

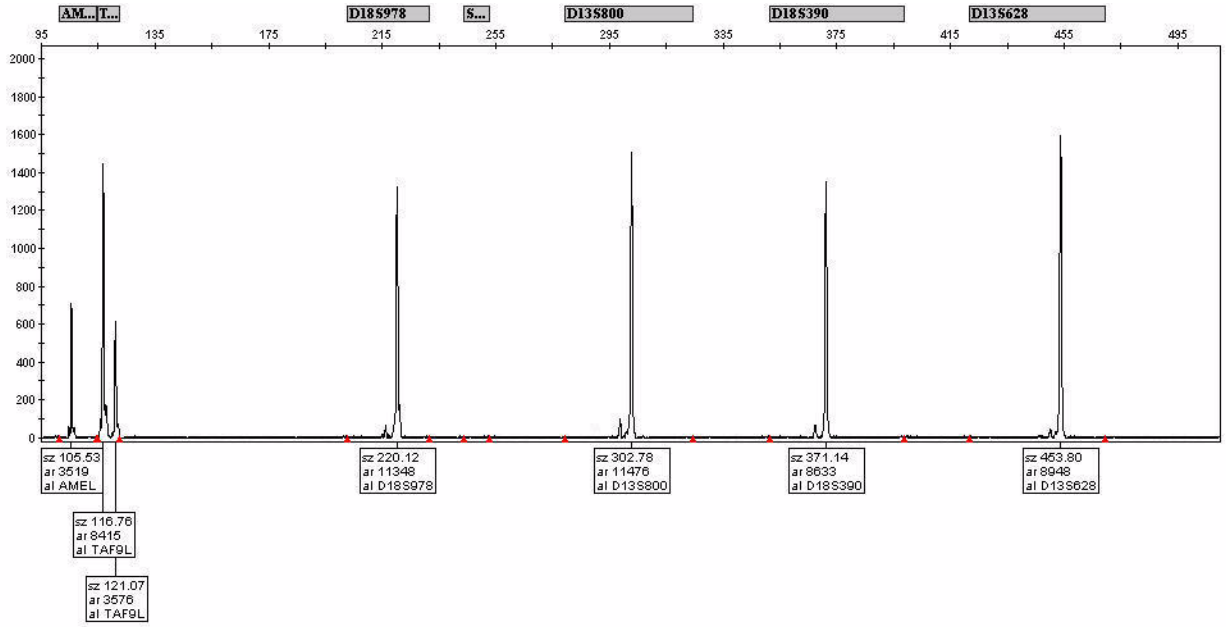
Figure 17 Normal XX female



Results consistent with XX sex chromosome complement are indicated by:

- **Amelogenin** – Single peak for X chromosome, consistent with presence of X chromosome only
- **SRY absent** – Consistent with absence of Y chromosome
- **TAF9L** – 1:1 ratio, consistent with 2 copies of chromosome 3 and 2 copies of X chromosome

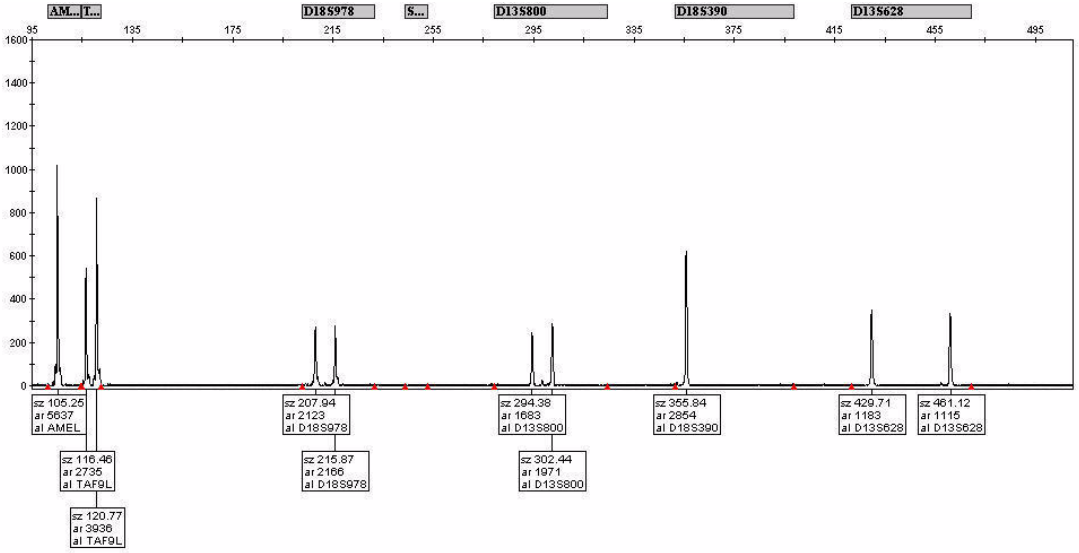
Figure 18 X0 female



Results consistent with X0 sex chromosome complement are indicated by:

- **Amelogenin** – Single peak for X chromosome, consistent with presence of X chromosome only
- **SRY absent** – Consistent with absence of Y chromosome
- **TAF9L** – 2:1 ratio, consistent with 2 copies of chromosome 3 and 1 copy of X chromosome

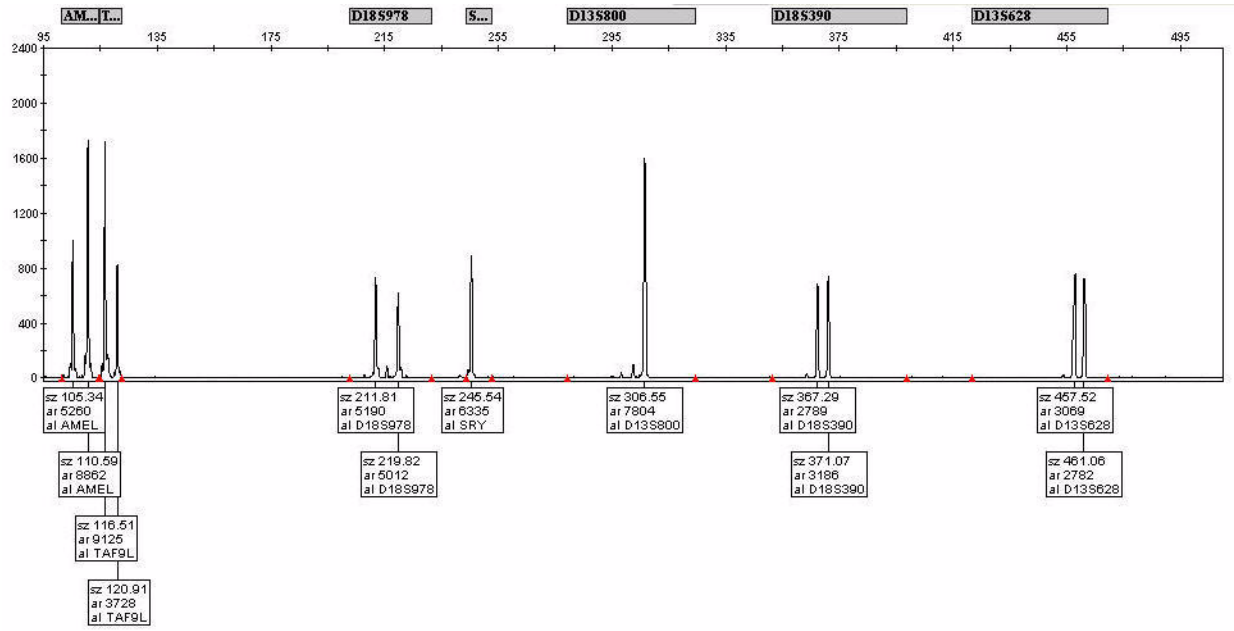
Figure 19 XXX female



Results consistent with XXX sex chromosome complement are indicated by:

- **Amelogenin** – Single peak for X chromosome, consistent with presence of X chromosome only
- **SRY absent** – Consistent with absence of Y chromosome
- **TAF9L** – 2:3 ratio, consistent with 2 copies of chromosome 3 and 3 copies of X chromosome

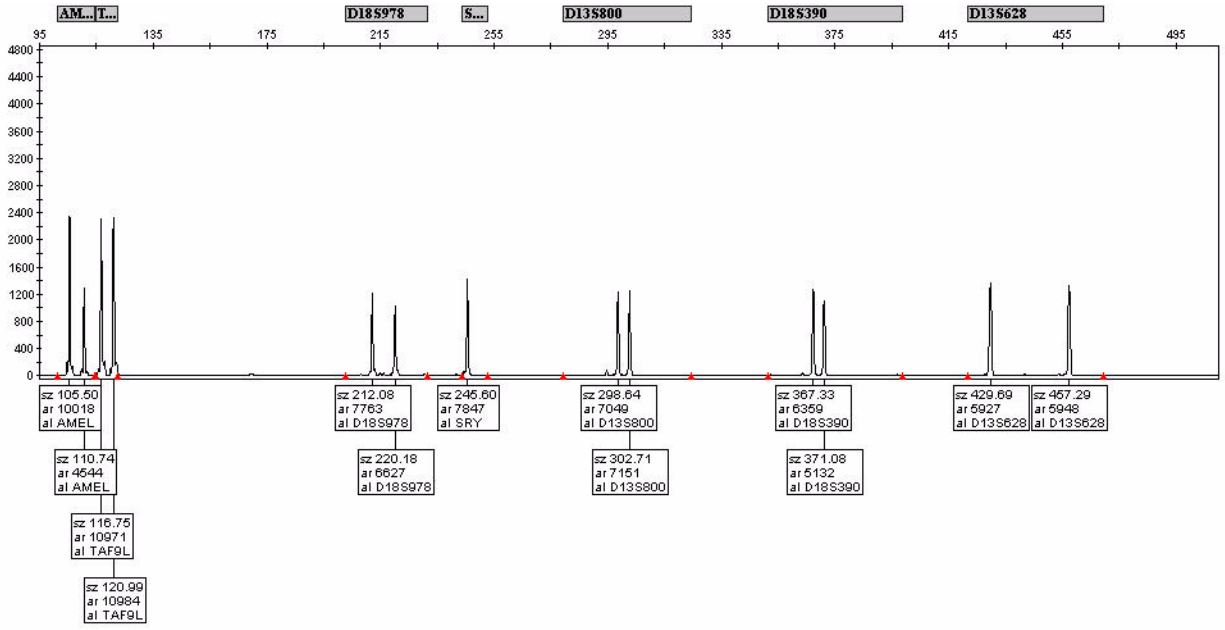
Figure 20 XYY male



Results consistent with XYY sex chromosome complement are indicated by:

- **Amelogenin** – 1:2 ratio, consistent with presence of one copy of X chromosome and 2 copies of Y chromosome
- **SRY present** – 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

Figure 21 XXY male



Results consistent with XXY sex chromosome complement are indicated by:

- **Amelogenin** – 2:1 ratio, consistent with presence of 2 copies of X chromosome and 1 copy of Y chromosome
- **SRY present** – Consistent with presence of Y chromosome. SRY is similar in height to AM X/AM Y and TAF9L X peaks
- **TAF9L** – 1:1 ratio, consistent with 2 copies of chromosome 3 and 2 copies of X chromosome

Markers in the *TrueScience*™ Aneuploidy Kits

Note: The NED™ dye used in the kits is identified spectrally as a yellow dye. It is conventionally displayed in black type for clarity.

Table 1 Markers in *TrueScience*™ Aneuploidy STR-Plus Kit

Marker	Location	Observed Heterozygosity	Allele Size Range	Marker Dye Color
D13S252	13q12.2	0.74	274-311 bp	red
D13S305	13q13.3	0.79	424-466 bp	green
D13S634	13q21.33	0.84	380-428 bp	blue
D13S800	13q22.1	0.72	284-320 bp	yellow
D13S628	13q31.1	0.75	426-465 bp	yellow
D18S819	18q11.2	0.73	400-425 bp	red
D18S535	18q12.3	0.77	466-498 bp	blue
D18S978	18q12.3	0.71	207-223 bp	yellow
D18S386	18q22.1	0.92	332-405 bp	green
D18S390	18q22.3	0.69	356-394 bp	yellow
D21S11	21q21.1	0.82	228-279 bp	blue
D21S1437	21q21.1	0.76	307-347 bp	blue
D21S1409	21q21.2	0.74	191-239 bp	red
D21S1442	21q21.3	0.85	332-389 bp	red
D21S1435	21q21.3	0.74	167-204 bp	blue
D21S1446	21q22.3	0.76	205-235 bp	green
AMEL	Xp22.22/Yp11.2	-	104/110 bp	yellow
TAF9L	Xq21.1/3p24.2	-	116/121 bp	yellow
DXS6803	Xq21.31	0.86	131-153 bp	blue
XHPRT	Xq26.2	0.72	266-298 bp	green
DXS1187	Xq26.2	0.73	123-165 bp	green
SRY	Yp11.31	-	248 bp	yellow

Table 2 Markers in *TrueScience*™ Aneuploidy STR-XY Kit

Marker	Location	Observed Heterozygosity	Allele Size Range	Marker Dye Color
DXYS218	Xp22.32/ Yp11.3	0.74	376-392 bp	blue
AMEL	Xp22.22/ Yp11.2	-	104/110 bp	yellow
DXYS267	Xq21.31/ Yp11.31	0.75	240-280 bp	red
DXS6807	Xp22.3	0.66	326-351 bp	blue
DXS981	Xq13.1	0.73	226-260 bp	blue
DXS6803	Xq21.31	0.86	131-153 bp	blue
DXS6809	Xq21.33	0.78	392-436 bp	yellow
DXS1187	Xq26.2	0.73	123-165 bp	green
XHPRT	Xq26.2	0.72	266-298 bp	green
DXS7423	Xq28	0.67	360-388 bp	green
SRY	Yp11.31	-	248 bp	yellow
DYS448	Yq11.223	-	349-372 bp	red

Table 3 Markers in *TrueScience*™ Aneuploidy STR-13 Kit

Marker	Location	Observed Heterozygosity	Allele Size Range	Marker Dye Color
D13S252	13q12.2	0.74	274-311 bp	red
D13S305	13q13.3	0.79	424-466 bp	green
D13S325	13q14.11	0.80	272-309 bp	green
D13S634	13q21.33	0.84	380-428 bp	blue
D13S800	13q22.1	0.72	284-320 bp	yellow
D13S628	13q31.1	0.75	426-465 bp	yellow
D13S762	13q31.3	0.75	302-331 bp	blue
D13S797	13q33.2	0.77	178-250 bp	blue

Table 4 Markers in *TrueScience*™ Aneuploidy STR-18 Kit

Marker	Location	Observed Heterozygosity	Allele Size Range	Marker Dye Color
D18S391	18p11.31	0.70	205-225 bp	green
D18S1002	18q11.2	0.76	337-365 bp	blue
D18S819	18q11.2	0.73	400-425 bp	red
D18S847	18q12.1	0.71	204-232 bp	blue
D18S535	18q12.3	0.77	466-498 bp	blue
D18S978	18q12.3	0.71	207-223 bp	yellow
D18S977	18q21.31	0.70	248-285 bp	red
D18S386	18q22.1	0.92	332-405 bp	green
D18S390	18q22.3	0.69	356-394 bp	yellow

Table 5 Markers in *TrueScience*™ Aneuploidy STR-21 Kit

Marker	Location	Observed Heterozygosity	Allele Size Range	Marker Dye Color
D21S11	21q21.1	0.82	228-279 bp	blue
D21S1437	21q21.1	0.76	307-347 bp	blue
D21S1409	21q21.2	0.74	191-239 bp	red
D21S1442	21q21.3	0.85	290-349 bp	green
D21S1435	21q21.3	0.74	167-204 bp	blue
D21S1411	21q22.3	0.83	283-344 bp	yellow
D21S1446	21q22.3	0.76	205-235 bp	green

Dyes in the *TrueScience*™ Aneuploidy Kits

The NED™ dye used in the kits is identified spectrally as a yellow dye. It is conventionally displayed in black type for clarity.

Table 6 *TrueScience*™ Aneuploidy STR-Plus Kit

6-FAM™ (blue)	VIC® (green)	NED™ (yellow)	PET® (red)
DXS6803	DXS1187	AMEL	D21S1409
D21S1435	D21S1446	TAF9L	D13S252
D21S11	XHPRT	D18S978	D21S1442
D21S1437	D18S386	SRY	D18S819
D13S634	D13S305	D13S800	
D18S535		D18S390	
		D13S628	

Table 7 *TrueScience*™ Aneuploidy STR-XY Kit

6-FAM™ (blue)	VIC® (green)	NED™ (yellow)	PET® (red)
DXS6803	DXS1187	AMEL	DXYS267
DXS981	XHPRT	SRY	DYS448
DXS6807	DXS7423	DXS6809	
DXYS218			

Table 8 *TrueScience*™ Aneuploidy STR-13 Kit

6-FAM™ (blue)	VIC® (green)	NED™ (yellow)	PET® (red)
D13S797	D13S325	D13S800	D13S252
D13S762	D13S305	D13S628	
D13S634			

Table 9 *TrueScience*™ Aneuploidy STR-18 Kit

6-FAM™ (blue)	VIC® (green)	NED™ (yellow)	PET® (red)
D18S847	D18S391	D18S978	D18S977
D18S1002	D18S386	D18S390	D18S9819
D18S535			

Table 10 *TrueScience*™ Aneuploidy STR-21 Kit

6-FAM™ (blue)	VIC® (green)	NED™ (yellow)	PET® (red)
D21S1435	D21S1446	D21S1411	D21S1409
D21S11	D21S1442		
D21S1437			

Internal validation

Table 11 Internal validation results

Product	Sample Number	Results	Comments	Concordance with karyotyping†
TrueScience™ Aneuploidy STR-Plus Kit	98 samples tested	22 normal XY 17 normal XX 12 trisomy 21 XY 7 trisomy 21 XX 9 trisomy 18 XY 8 trisomy 18 XX 2 trisomy 13 XY 4 trisomy 13 XX 8 normal X0 1 normal/XYY 1 triploid (for all chromosomes tested)	1 sample gave an uninformative result. 6 samples failed to give analyzable results due to poor sample quality.	100% with results previously obtained by an alternative established method
TrueScience™ Aneuploidy STR-XY Kit	321 tested 315 produced analyzable results	160 normal male 147 normal female 3 monosomy X 2 XXY 2 XYY 1 XXX	6 samples failed to give an interpretable result despite repeat amplification.	100%
TrueScience™ Aneuploidy STR-13 Kit	152 tested	144 normal 2 trisomy 13	6 samples failed to produce an interpretable result despite repeat amplification.	100%
TrueScience™ Aneuploidy STR-18 Kit	152 samples tested	143 normal 4 trisomy 18	5 samples failed to produce an interpretable result despite repeat amplification.	100%

Product	Sample Number	Results	Comments	Concordance with karyotyping†
TrueScience™ Aneuploidy STR-21 Kit	152 samples	148 normal 2 trisomy 21	2 samples failed to produce an interpretable result despite repeat amplification.	100%

† All analyzable samples showed 100% concordance with results previously obtained by karyotyping

Note: All internal validation was carried out on the corresponding Eucigene QST*R product.

Limitations and disclaimer

Limitations

The results obtained from these or any other diagnostic kits should be used and interpreted only in the context of the overall clinical picture. Life Technologies cannot accept responsibility for any clinical decisions that are made.

This test is designed to detect specific chromosomal trisomies and sex chromosome aneuploidies as detailed in the instructions for use. It may not detect structural rearrangements involving the chromosomes tested and will not detect abnormalities in any other chromosomes. Mosaicism for the chromosomes tested may not be detected. An aneuploidy test result can only be directly applied to the tissue tested and may not represent the fetal karyotype. Maternal cell contamination (MCC) and confined placental mosaicism (CPM) may result in discrepancies between the *TrueScience*™ Aneuploidy Kit results and karyotype results.

Heterozygosities of the markers used in this kit were derived from a random set of samples submitted for routine analysis from a predominantly Northern European Caucasian population. Any calculations using these heterozygosities strictly apply only to the population from which the samples were taken. A small study using locally derived samples may be carried out as part of a validation study to establish heterozygosities in the population to be tested. It is not expected that population variation will significantly alter the overall informativeness of the assay.

Disclaimer

We recommend that each laboratory develop 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:

http://www.cmgs.org.uk

We do not represent this guide as a comprehensive summary of all possible outcomes from using the Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits. This guide is intended for use solely as an aid to memory. It is not for use 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.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Ordering Information

How to order

To locate your nearest Life Technologies representative, go to:

www.appliedbiosystems.com/aneuploidy

Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits

TrueScience™ Aneuploidy Kits are available in the formats listed below. More information on the kits is available at **www.appliedbiosystems.com/aneuploidy**.

Table 12 Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits

Product	Description	PCR tube color	Reaction mix	Part number
TrueScience™ Aneuploidy STR-Plus Kit	Markers for detection of: • Trisomy 13 (Patau syndrome) • Trisomy 18 (Edwards syndrome) • Trisomy 21 (Down syndrome) • XY-related aneuploidy (Klinefelter syndrome, Turner syndrome)	Clear	2 tubes containing 250 µL each (sufficient for 50 tests)	4453756
			1 tube containing 100 µL (sufficient for 10 tests)	4453757
TrueScience™ Aneuploidy STR-XY Kit	Markers for detection of sex chromosome status including the common aneuploidies: • Klinefelter syndrome • Turner syndrome	Pink	2 tubes containing 250 µL each (sufficient for 50 tests)	4453601
			1 tube containing 100 µL (sufficient for 10 tests)	4453755
TrueScience™ Aneuploidy STR-13 Kit	Supplemental kits for each chromosome containing additional autosomal markers. For extended chromosome testing when the standard test does not provide a definitive result, or to provide additional information.	Green	1 tube containing 100 µL (sufficient for 10 tests)	4453760
TrueScience™ Aneuploidy STR-18 Kit		Purple	1 tube containing 100 µL (sufficient for 10 tests)	4453759
TrueScience™ Aneuploidy STR-21 Kit		Yellow	1 tube containing 100 µL (sufficient for 10 tests)	4453758

Materials and equipment not included

Table 13 Materials required

Item	Source
Laboratory consumables	
Pipette tips	MLS†
Gloves	MLS
Sterile, deionized water	MLS
Laboratory equipment	
Precision pipettes (preferably positive displacement pipettes): • 1 set for pre-amplification • 1 set for post-amplification handling	MLS
Protective clothing	MLS
Vortex mixer	MLS
Microcentrifuge	MLS
96-well microtiter plate centrifuge	MLS
DNA extraction and preparation	
InstaGene™ Matrix	Bio-Rad Laboratories, Cat No 732-6030
PCR amplification	
96-well GeneAmp® PCR System 9700	Applied Biosystems PN N8050200
Veriti® 96-Well Thermal Cycler	Applied Biosystems PN 4375786
Capillary electrophoresis	
GeneScan™ 600 LIZ® Size Standard v2.0	Applied Biosystems PN 4408399
dS-33 (dye set G5) matrix standard	Applied Biosystems PN 4345833
POP-6™ Polymer or POP-7™ Polymer	Applied Biosystems PN 4316357 Applied Biosystems PN 4352759
10x Genetic Analyzer Buffer	Applied Biosystems PN 402824
Hi-Di™ formamide	Applied Biosystems PN 4311320
Applied Biosystems 3500 Series Genetic Analyzer or Applied Biosystems 3130 Series Genetic Analyzer	Contact your Life Technologies representative or go to **www.appliedbiosystems.com**
Analysis	
GeneMapper® Software v4.1 or GeneMapper® Software v4.0	Applied Biosystems PN 4366925 Applied Biosystems PN 4440915

† Major Leading Supplier. For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Safety

This appendix covers:

Warnings and precautions

1. For professional *in vitro* diagnostic use only.

2. The DNA Control provided in the kits has been independently tested and found to be negative for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Human Immunodeficiency Virus (HIV) 1 and 2.

3. Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that HBV, HCV, HIV or other infectious agents are absent.

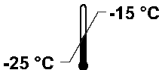
4. Handling of samples and test components, their use, storage and disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation.

5. Store all components between –15 °C and –25 °C.

6. In line with current good laboratory practice, laboratories should process their own internal QC samples of known type in each assay, so that the validity of the procedure can be assessed.

Symbols used on labels and packaging

The following table describes symbols that may be displayed on Life Technologies instruments, consumables, or reagents.

Symbol	Description
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	CAUTION There are specific warnings or precautions associated with the device that are not found on the label. Consult the user documentation for further information.
	MANUFACTURER Manufacturer's name and the address.
	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF TEMPERATURE
	USE BY Do not use after the year, month, or day shown.
	CATALOGUE NUMBER Manufacturer's catalogue number.
	BATCH CODE Manufacturer's batch code, lot number, or batch number.

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 61.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Life Technologies is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Life Technologies instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: **http://www.cdc.gov/biosafety/publications/index.htm**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

General safety alerts for all chemicals

Avoid contact with skin, eyes, or clothing. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Documentation and Support

Related documentation

The following documents are available online at: **www.appliedbiosystems.com/aneuploidy**

Document	Part number	Description
Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Analysis User Guide	*4454038*	Provides step-by-step procedures for setting up the Data Collection Software and GeneMapper® Software and for performing data analysis.

Note: To open the user documentation available online, use the Adobe Acrobat Reader software available from **www.adobe.com**

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Life Technologies web site, you can:

- Access worldwide telephone and fax numbers to contact Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Life Technologies user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Glossary

AMNIOTIC FLUID

Definition: A colorless 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: 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 composed 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 Dill 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 Vejerslev).

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.

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 optimized 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 of the duplication. 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 (for example, 4 °C 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 not be used to assess chromosome status as amplification may be incomplete.
SOMATIC MICROSATELLITE POLYMORPHISM	Definition: A post-fertilization 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:

- Delayed digyny, by incorporation of the second polar body into one blastomere nucleus of a diploid zygote, and
- Delayed dispermy, similarly, by incorporation of a second sperm pronucleus into one blastomere nucleus of a diploid zygote.

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

TRISOMY

Definition: Three copies of an individual chromosome (for example, 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.

Bibliography

1. Hahnemann, J.M., Vejerslev, L.O. 1997. 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*, 17(9): 801-820.

2. Kalousek, D.K., Dill, F.J. 1983. Chromosomal mosaicism confined to the placenta in human conceptions. *Science* 221(4611):665-667.

3. Mansfield, E. S. 1993. Diagnosis of Down Syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms. *Human Molecular Genetics* 2(1):43-50.

4. Mann, K., Fox S.P., Abbs S.J., et al. 2001. Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. *The Lancet* 358(9287):1057-1061.

5. Mann, K., Donaghue, C., Fox, S.P., Docherty Z., Mackie Ogilvie, C. 2004. Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. *European Journal of Human Genetics* 12(11):907-915.

6. Mackie Ogilvie, C., Donaghue, C., Fox, S.P., Docherty, Z., Mann, K. 2005. Rapid prenatal diagnosis of aneuploidy using Quantitative Fluorescence-PCR (QF-PCR). *Journal of Histochemistry and Cytochemistry* 53(3):285-288.

7. Badenas, C., et al. 2010. Assessment of QF-PCR as the first approach in prenatal diagnosis. *Journal of Molecular Diagnostics* 12(6):828-834.

Index

T

U

W

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

life technologies™