Aneuploïdies comunes i el seu diagnòstic per QF-PCR

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TIPUS D'ANOMALIES CROMOSÒMIQUES

1. Alteracions numèriques:
   - Aneuploïdies
   - Mosaïcisme

2. Alteracions estructurals:
   - Translocacions recíproques
   - Translocacions robertsonianes
   - Inversions
   - Delecions
   - Duplicacions
   - Cromosomes marcadors
   - CNV

Introducció

1. Anomalies del autosomes

   Síndrome de Down (trisomia 21)

   Síndrome de Edwards (trisomia 18)

   Síndrome de Patau (trisomia 13)

2. Anomalies dels cromosomes sexuals

   Síndrome de Turner (45,X)

   Síndrome de Klinefelter (47,XXY)

   47,XXX

   47,XYY

Prevalença de les Cromosomopaties (Jacobs i Hassold,1995; Clusellas,2000)

Cromosomopatia	Avortaments	Nounats morts	Naixement	DPrenatal
45,X	8,6	0,3	0,005	0,1
47,XXY	0,2	0,2	0,05	0,01
47,XXX	0,1	0,2	0,05	0,06
47,XYY	-	-	0,05	0,03
47, +21	2,3	1,1	0,12	0,75
47, +18	1,1	1,2	0,01	0,22
47, +13	1,1	0,3	0,005	0,05

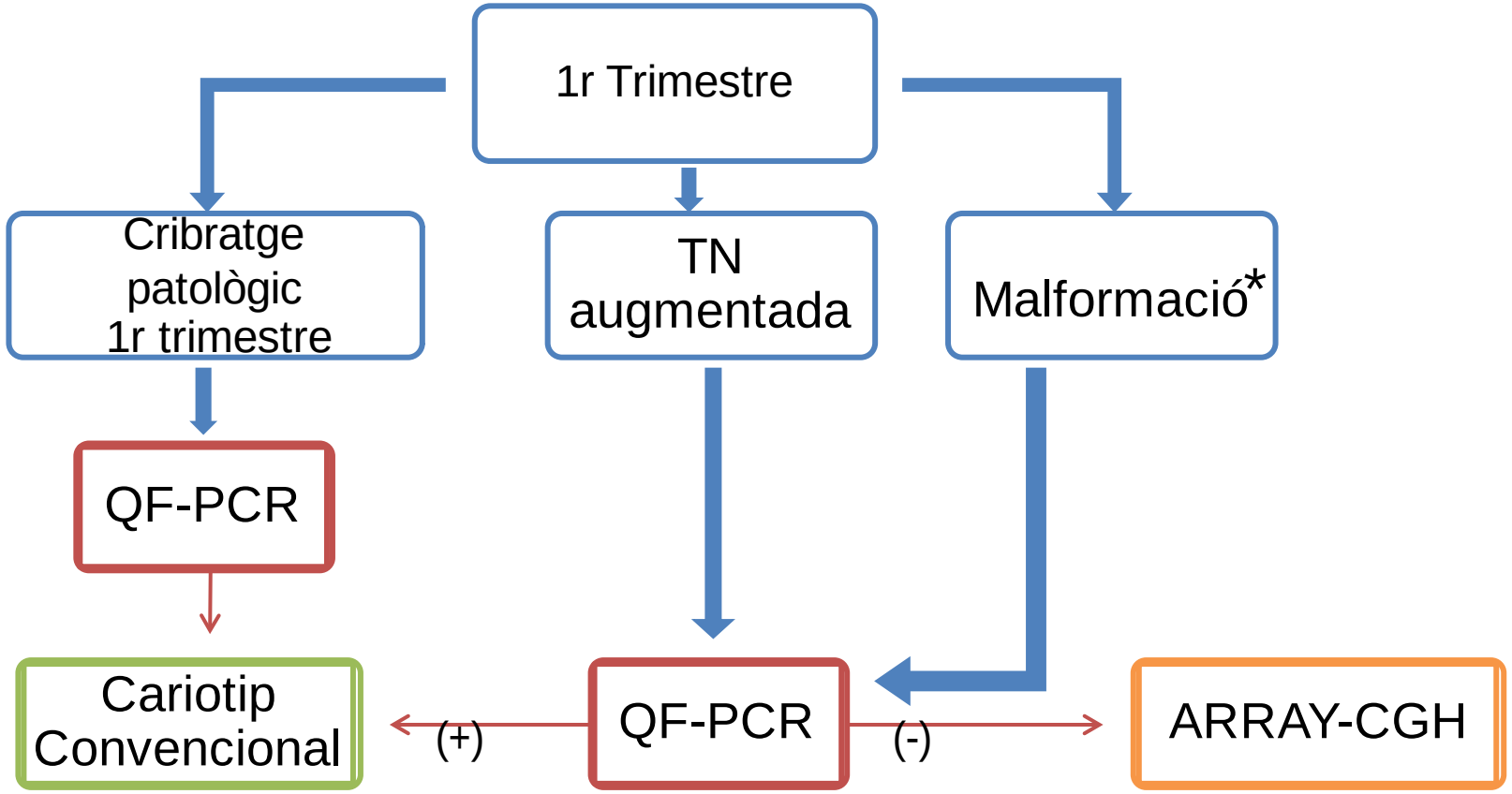
Quantitative Fluorescent Polymerase Chain Reaction: Aplicació clínica

- Detecció d'aneuploïdies
- Corionicitat

QF-PCR: INDICACIONS

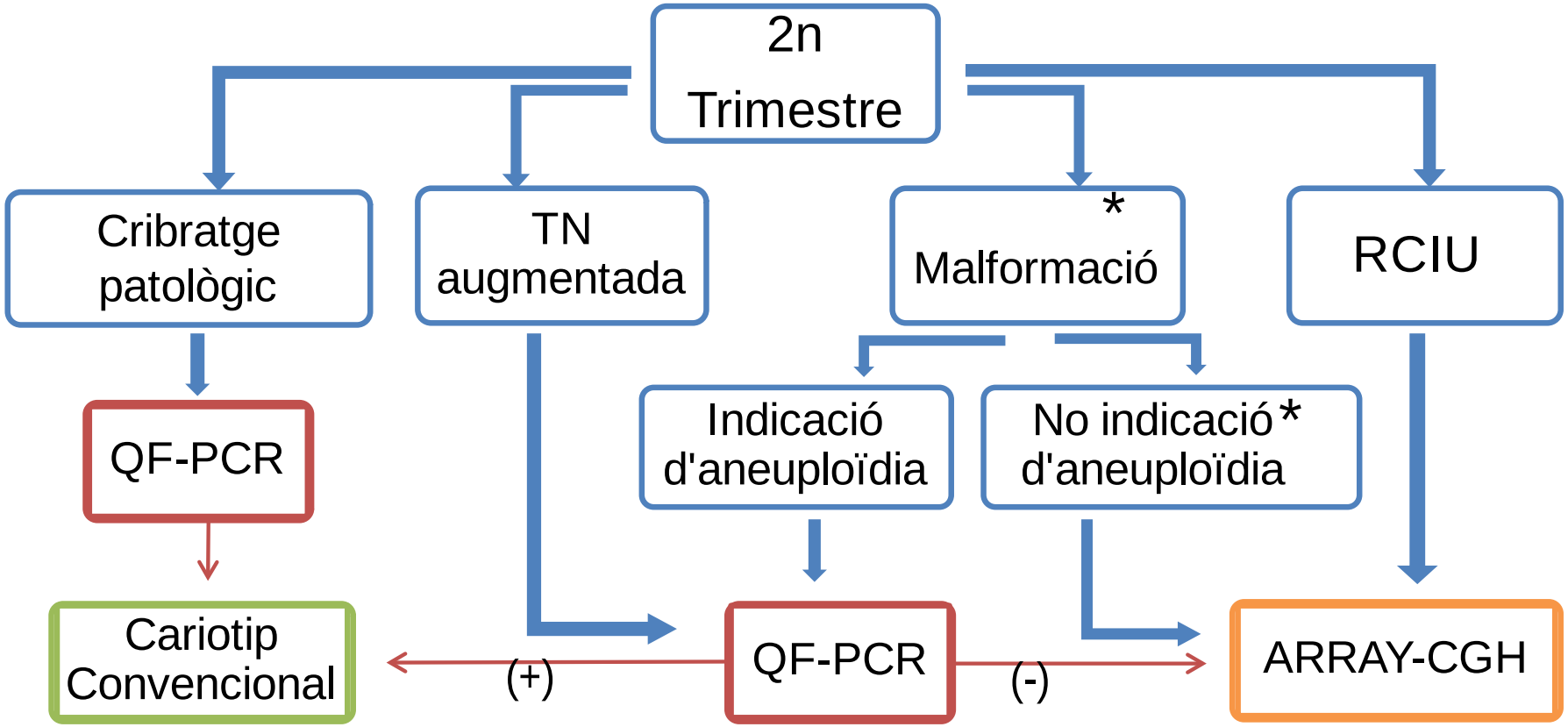
1. Cribatge prenatal bioquímic combinat d'alt risc
2. TN augmentada
3. Altres marcadors ecogràfics, malformacions
4. EMA, història familiar ...

QF-PCR: ALGORITME



* A valorar per la Unitat de Genètica Clínica

QF-PCR: ALGORITME



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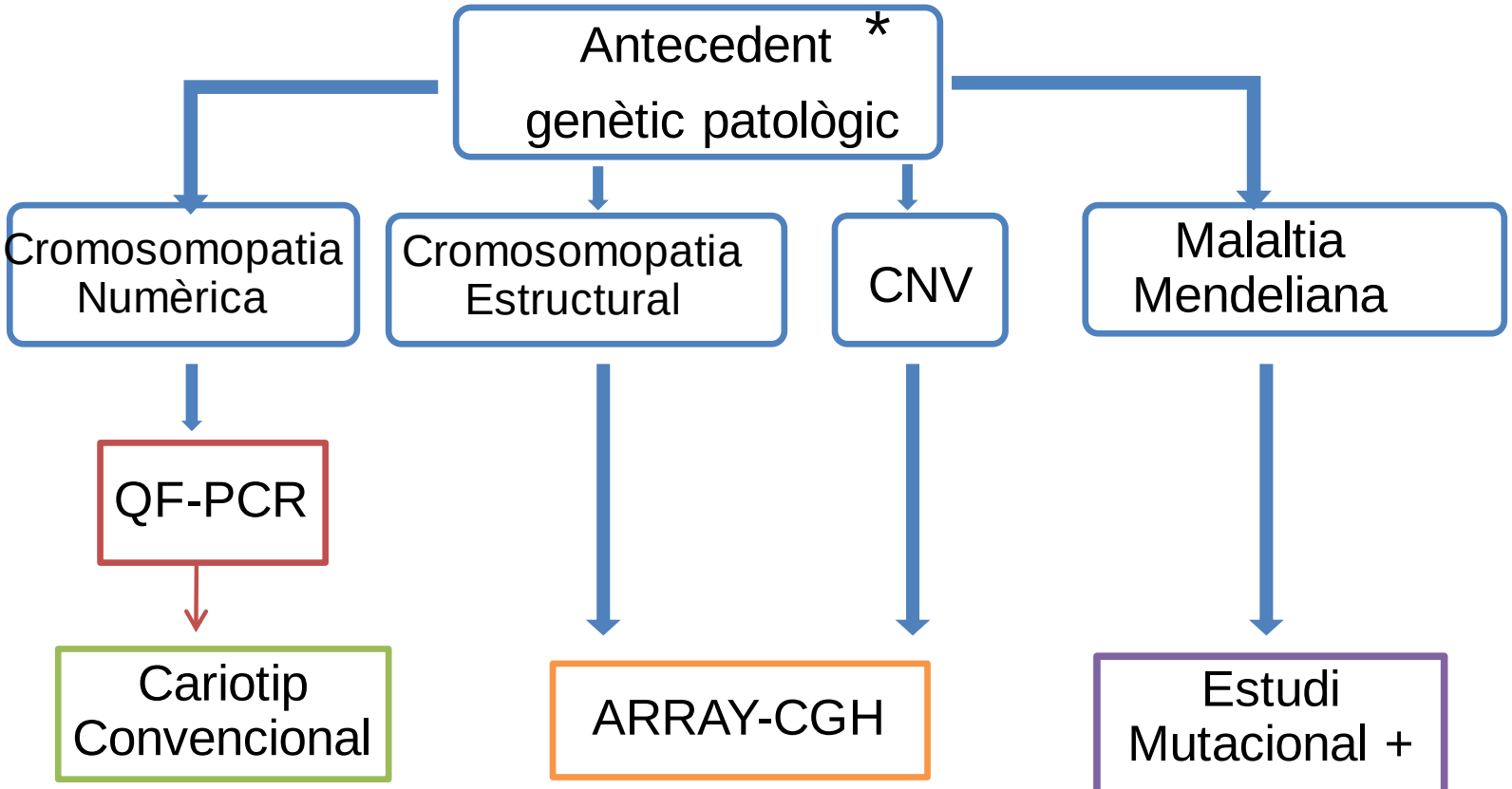
QF-PCR: ALGORITME



* A valorar per la Unitat de Genètica Clínica

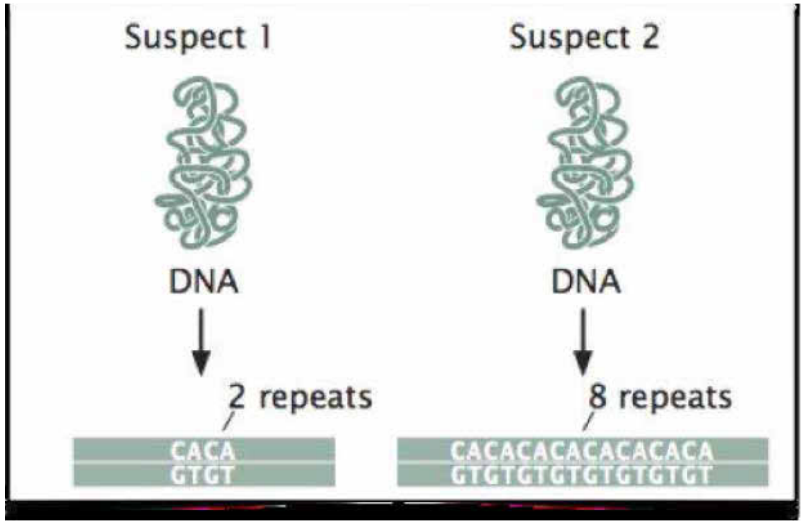
Quantitative Fluorescent Polymerase Chain Reaction

- ADN de cèl·lules sense cultivar
- PCR multiplex (26 marcadors)
- **NOMES aneuploïdies 13, 18, 21, X i Y**: alteracions estructurals ??
- Resultat en 24-48 h
- Automatitzable (fiable, reproduïble, gran volum)

QF-PCR: Metodologia

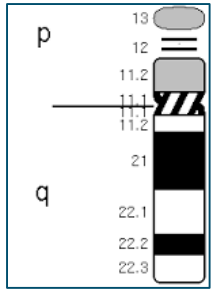
STRs (short tandem repeat):

- ü Fragment ADN 1-6 nucleòtids
- ü Es repeteixen de forma consecutiva
- ü Molt polimòrfics (nº repeticions)

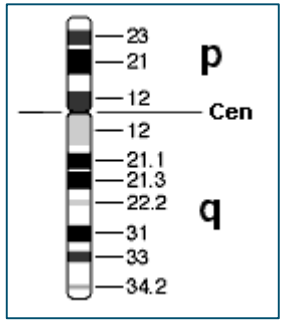


La variació del nº de repeticions crea diferents AL.LELS que es diferencien entre sí per la longitud total del fragment

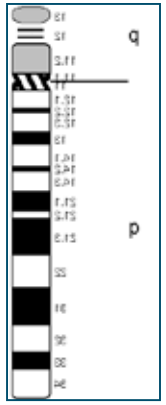
QF-PCR: Metodologia



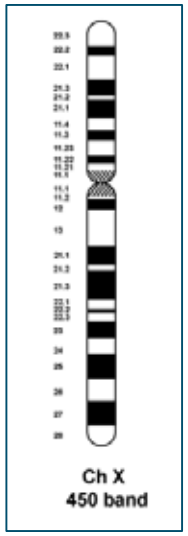
21B D21S111	21q21.1
21I D21S1437	21q21.1
21A D21S1435	21q21.3
21H D21S1442	21q21.3
21D D21S1444	21q22.13
21C D21S1411	21q.23.3



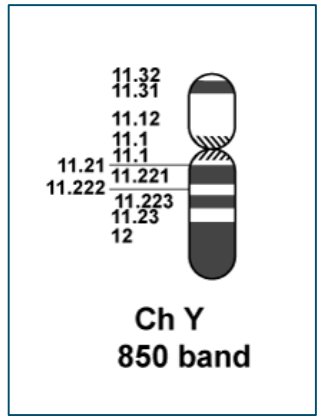
18J D18S976	18p11.31
18M GATA178F11	18p11.32
18B D18S978	18q12.3
18C D18S535	18q12.3
18D D18S386	18q22.1



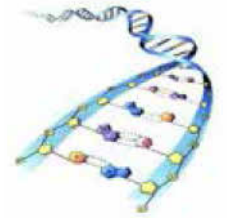
13A D13S742	13q12.12
13D D13S305	13q13.3
13K D13S1492	13q21.1
13BD13S634	13q21.32
13C D13S628	13q31.3

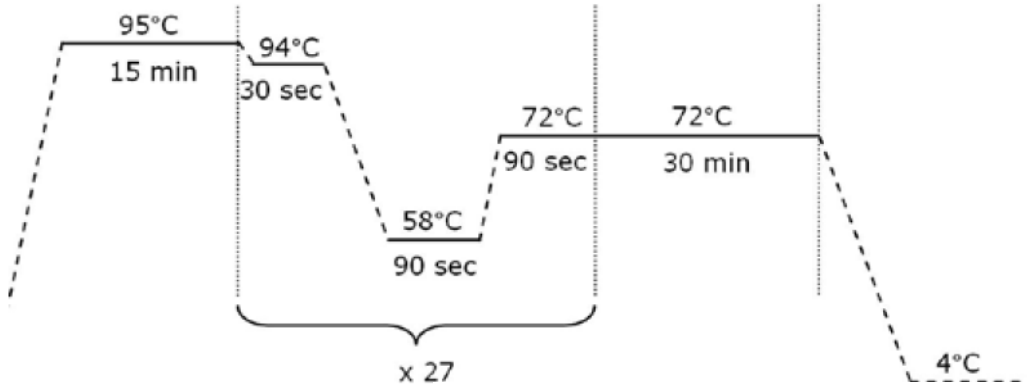


XY3 DXYS218	Xp22.33/Yp11.32
XY2 DXYS267	Xq21.3/Yp11.31
ZFYX	Yp11.31/Xp22.11
X3 XHPRT	Xq26.2q26.3
X2 DXS981	Xq13.1
X9 DXS2390	Xq27.1q27.2
SRY SRY	Yp11.31
T1	7q34/Xq13
T3	3p24.2/Xq21.1
AMELXY	Xp22.2/Yp11.2



QF-PCR: Metodologia



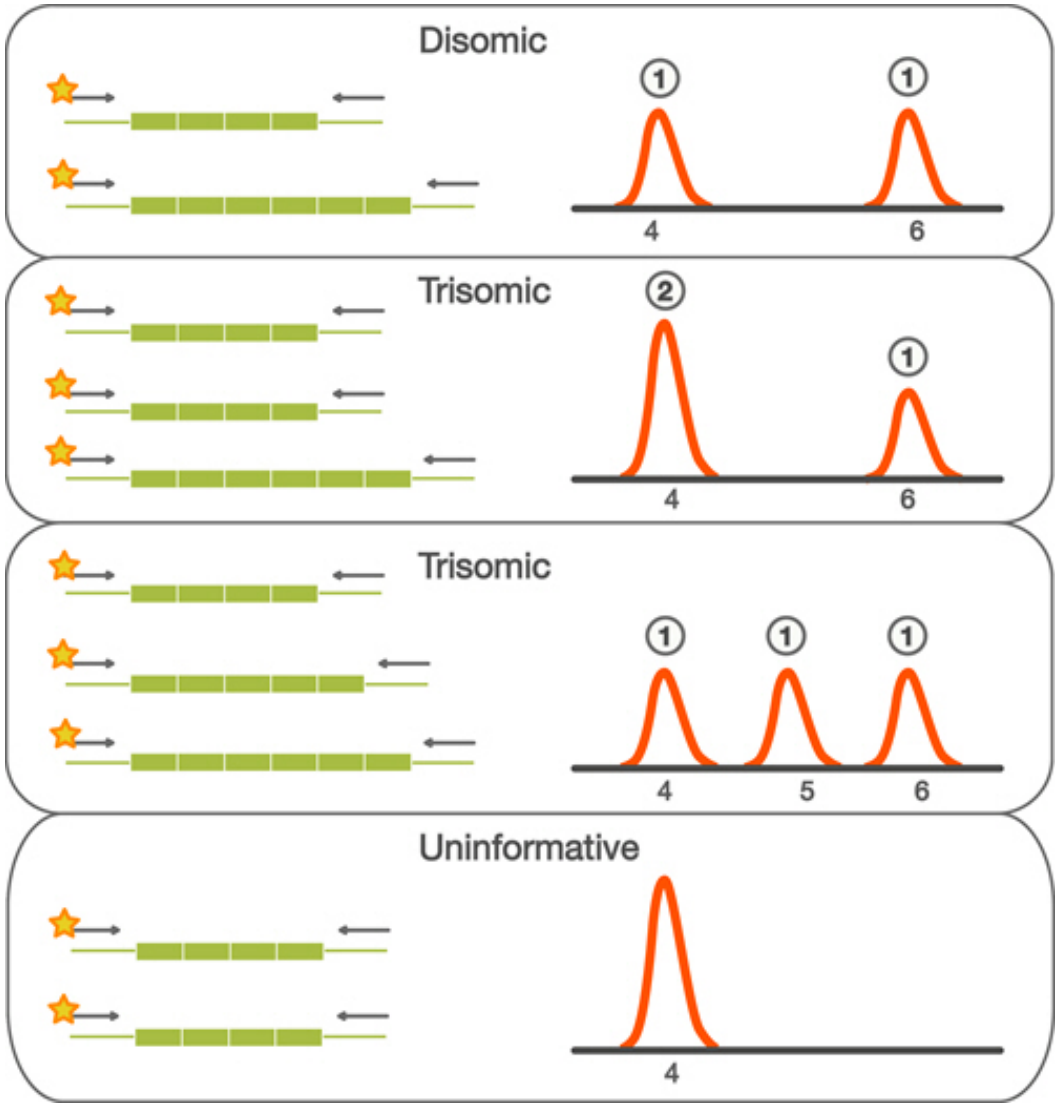


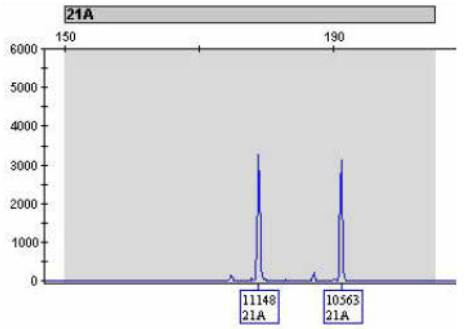
30′

3 h

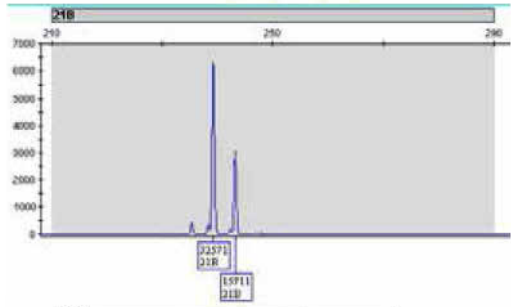
1/2 h/ 4 mostres

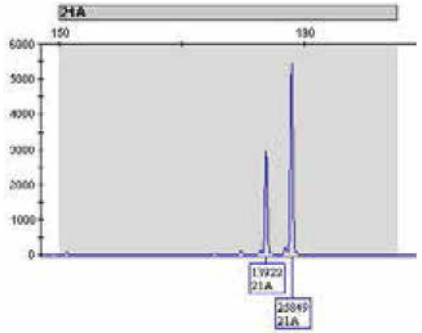
QF-PCR: Resultats





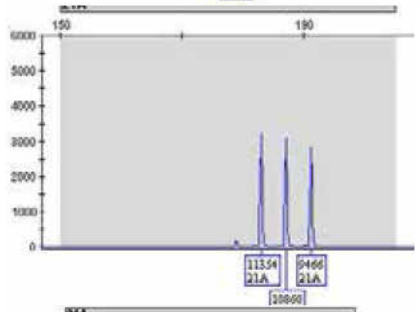
Rati 1:1



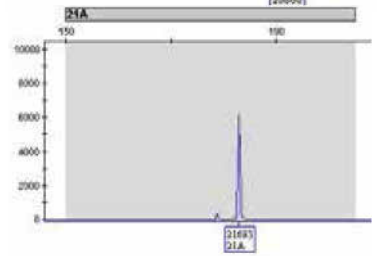


Rati 2:1

Rati 1:2



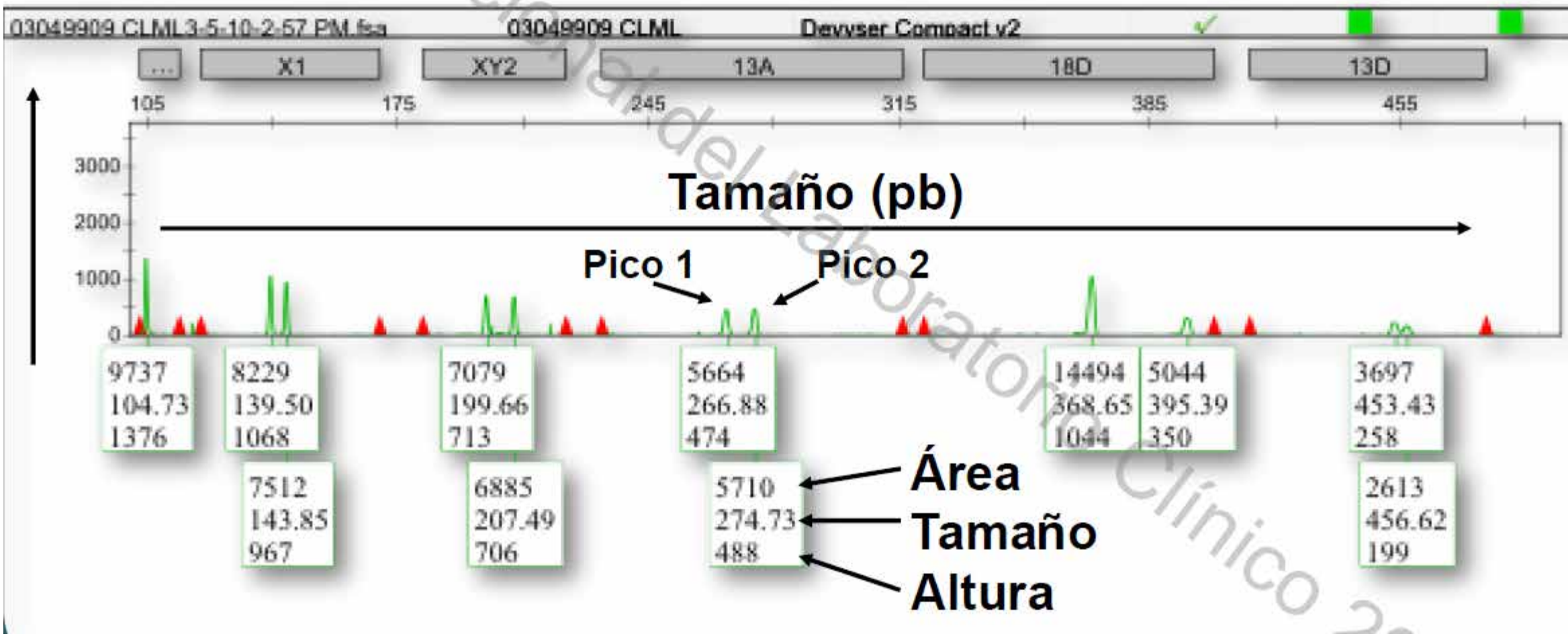
Rati 1:1:1



No informatiu o homozigot o monosomia

QF-PCR: Resultats

Intensidad señal



QF-PCR: Resultats

Marcadors amb dos (2) pics de al·lels

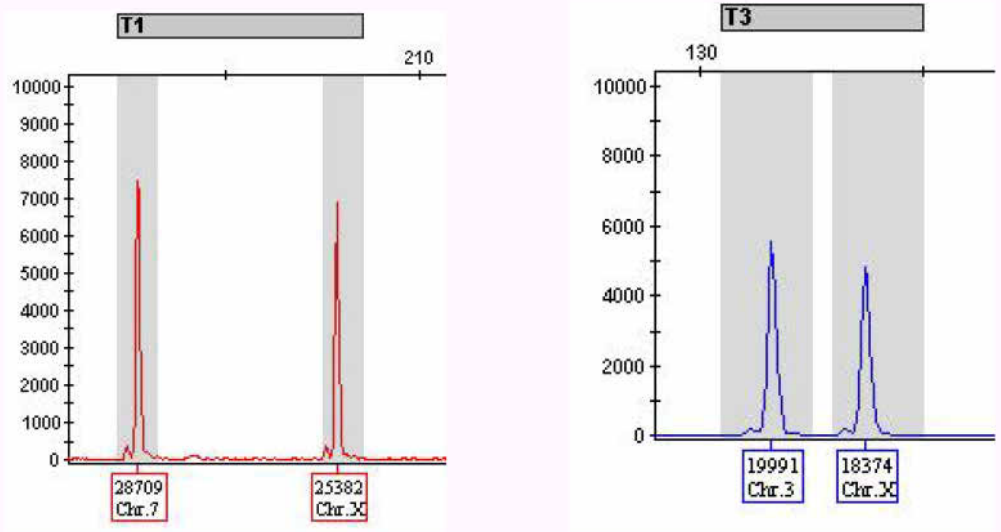
Relació	1:2	No concloent	1:1	No concloent	2:1
RC1 <24pb	<0,65	0,65-0,74	0,75-1,44	1,45-1,75	>1,75
RC2 ≥ 24pb	<0,65	0,65-0,74	0,75-1,54	1,55-1,75	>1,75

Marcadors amb tres (3) pics de al·lels

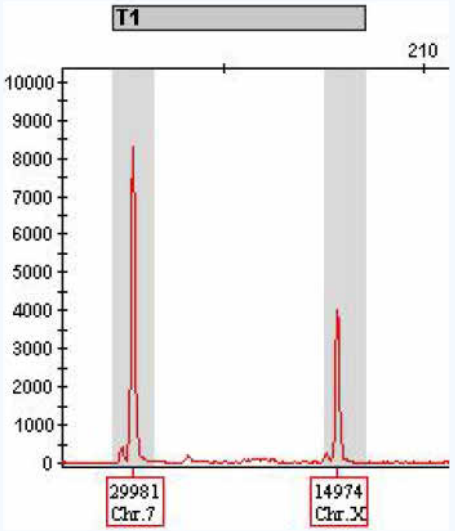
Relació	No concloent	1:1:1	No concloent
RC1 <24pb	<0,74	0,75-1,44	>1,45
RC2 ≥ 24pb	<0,74	0,75-1,54	>1,55

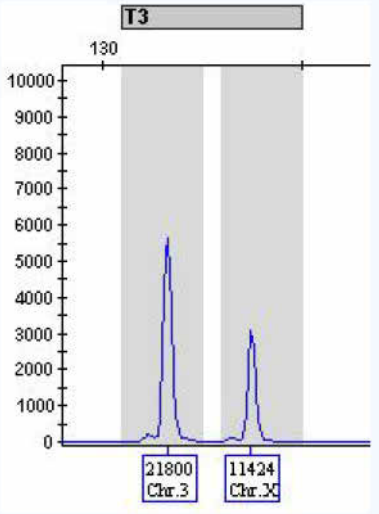
QF-PCR: Resultats. Interpretació sexe fetal

Femella normal, marcadors T1 T3 (àrea rati 1:1).



Mascle normal, marcadors T1 T3 (àrea rati 1:1).





Marcadors no polimòrfics

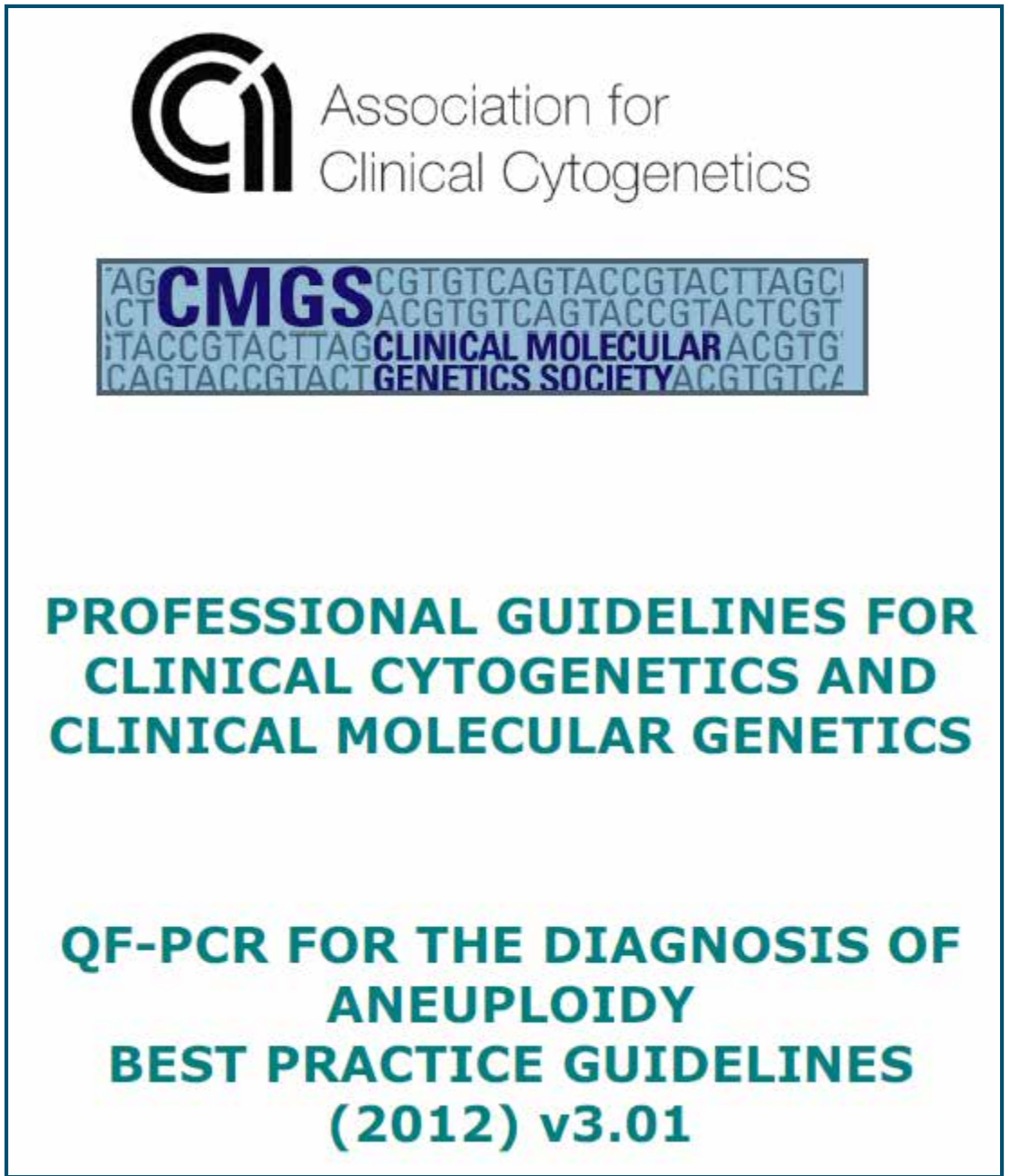
Els marcadors AMELXY i SRY amplifiquen seqüències no polimòrfiques del cromosoma X (AMELX) i del cromosoma Y (AMELY i SRY) i es poden utilitzar per determinar la presència o l'absència del cromosoma Y. AMELXY pot utilitzar-se para avaluar el número relatiu de cromosomes X i Y.

Marcadors XY pseudoatosòmics

Els marcadors XY2 i XY3 són marcadors STR polimòrfics presents en els cromosomes X i Y.

El marcador ZFYX és un marcador no polimòrfic (no-STR) present en els cromosomes X i Y.

QF-PCR: Interpretació Resultats



A minimum of 4 markers should be tested for each autosome and X chromosome…… A minimum of two Y specific sequences is recommended to determine sex chromosome status which should include AMEL or similar to determine the ratio of X to Y sequences. In addition the use of an X chromosome counting marker is recommended to determine the number of X chromosomes in the absence of a Y chromosome; the TAF9 sequences present on 3p24.2 and Xq21.1 are amplified using the same pair of primers. They can be separated according to length and then compared to assess X chromosome copy number.

6.2.1.1 The Normal Range

The normal range for allele ratios should not exceed 0.8-1.4. However, for alleles separated by more than 24 bp an allele ratio of up to 1.5 is acceptable and can beconsidered to fall within the normal range.

6.2.1.2 The Abnormal (triallelic) Ranges

Three alleles are evident by three peaks in a 1:1:1 ratio or two alleles in 2:1/1:2 ratios.Values between 0.45 and 0.65 and between 1.8 and 2.4 define the triallelic range for alleles where two of the three alleles are of the same size.

6.2.2.1 Normal Results

At least two informative marker results consistent with a normal biallelic pattern (with all other markers uninformative) are required to interpret a result as normal. It is acceptable to report cases where only a single marker shows a normal diallelic pattern, all

6.2.2.2 Abnormal Results (non-mosaic trisomy)

To interpret a result as abnormal, at least two informative marker results consistent with a triallelic genotype are required, with all other markers uninformative. It is unacceptable to interpret a result as abnormal if this is shown by only one marker

http://www.inesss.qc.ca/fileadmin/doc/INESSS/Analyse_biomedicale/Avril_2014/Detection_aneuploidies_chromosomes_13-18-21-X_Y_par_QF-PCR.pdf. It was translated into English by the Canadian Agency for Drugs and Technologies in Health (CADTH) with INESSS's permission. INESSS assumes no responsibility with regard to the quality or accuracy of the translation.

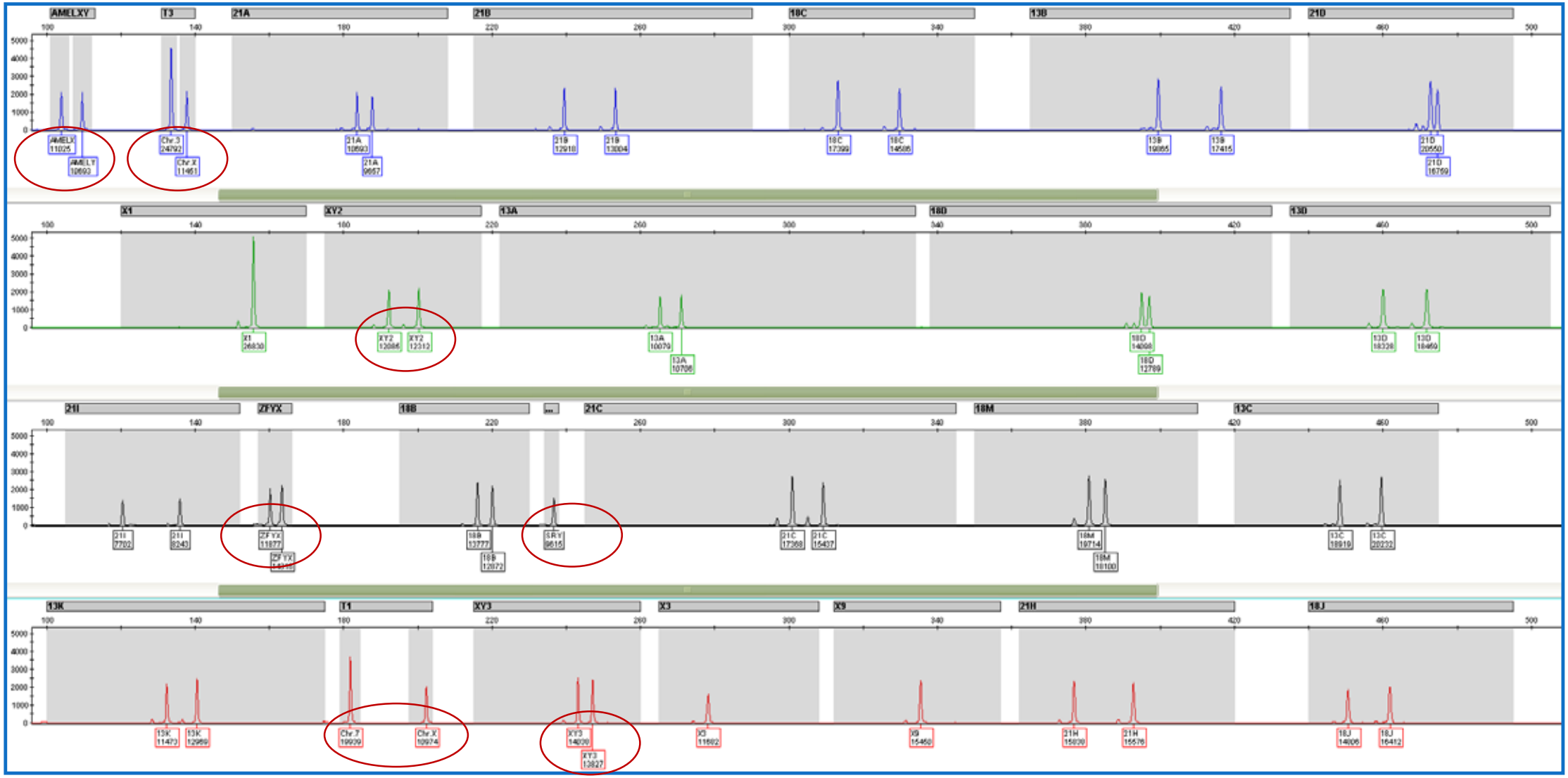
Table 1: Comp

STUDY	CHARACTERISTICS	CLINICAL VALIDITY				
		SE % (n/N)	SP % (n/N)	PPV %	NPV %	ACCURACY %
Moftah et al., 2013 Germany	163 samples: 115 aneuploidy 13, 18, and 21 5 unbalanced rearrangements on chromosomes 18 and 21 43 controls with normal karyotype Chromosomes 13, 18, and 21	94.2 (113/120)	100 (43/43)	100	86	96
Papoulidis et al., 2012 Greece	13,500 samples between 2006 and 2010 in two centres Chromosomes 13, 18, 21, X, and Y	75.3 (214/284)	99.5 (13,180/13,250)	75.3	99.5	99
Cirigliano et al., 2009 Spain and Italy	43,000 consecutive samples between 1999 and 2008 in 2 centres 37,544 AF, 4,687 CVS; 178 fetal blood; 591 fetal tissue after abortion Chromosomes 13, 18, 21, X, and Y	92.3 (1,608/1,741)	99.6 (41,019/41,178)	91	99.7	99.3
Speevak et al., 2011 Canada	4,176 samples of AF in one year Chromosomes 13, 18, 21, X, and Y	94.6 331/350	NA	100	NA	NA
Badenas et al., 2010 Spain	7,679 samples between 2004 and 2008 from 3 hospitals 1,243 CVS; 6,436 AF Chromosomes 13, 18, 21, X, and Y	NA	NA	NA	NA	98.75
Baig et al., 2010 Singapore	1,000 samples in only one centre 978 AF; 14 CVS; 8 fetal blood Chromosomes 13, 18, 21, X, and Y	100 63/63	100	NA	NA	NA
Kagan et al., 2007 UK	3,854 samples between 1998 and 2005 185 trisomies 13, 18, and 21; 4 mosaicisms; 14 abnormalities related to XY; 17 triploidy; 17 others Chromosomes 13, 18, 21, X, and Y	92.4 219/237	100 3,617/3,617	100	99.5	99.5

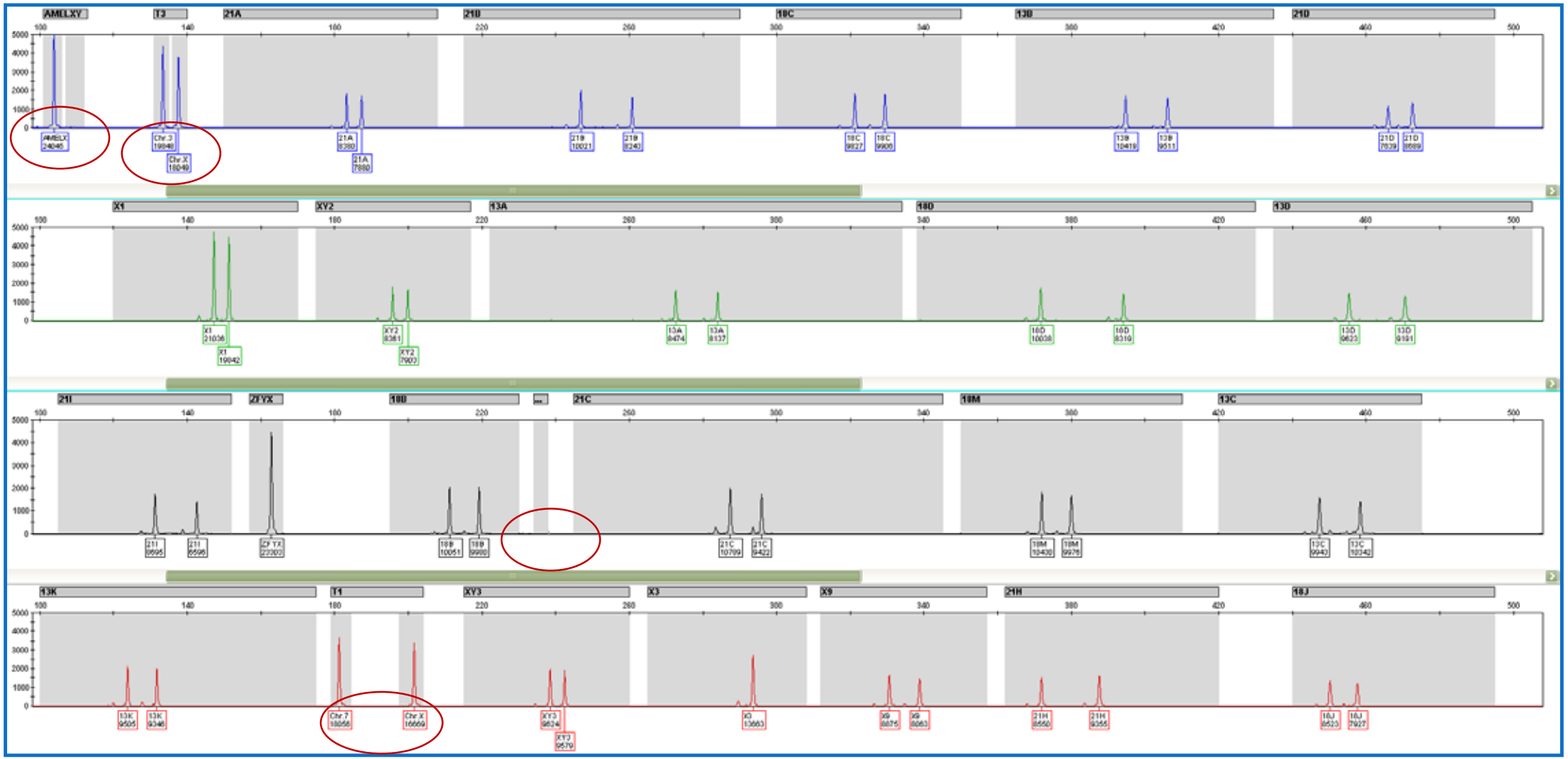
Abbreviations: AF = amniotic fluid; CVS = chorionic villus sampling; NA = not available; NPV = negative predictive value; PPV = positive predictive value; SE = sensitivity; SP = specificity; UK = United Kingdom

XY NORMAL

All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.
The presence of AMELY and SRY is consistent with male gender.
The X chromosome counting markers (T1 and T3) demonstrate a 2:1 marker ratio and all the X chromosome STR markers are uninformative, in line with the expected dosage of X chromosomes in a normal male.
The 1:1 marker ratio of the informative pseudoautosomal XY chromosome STR markers (XY2 and XY3) and the non-polymorphic XY markers (AMELXY and ZFYX) confirms a normal male sex chromosomal dosage.

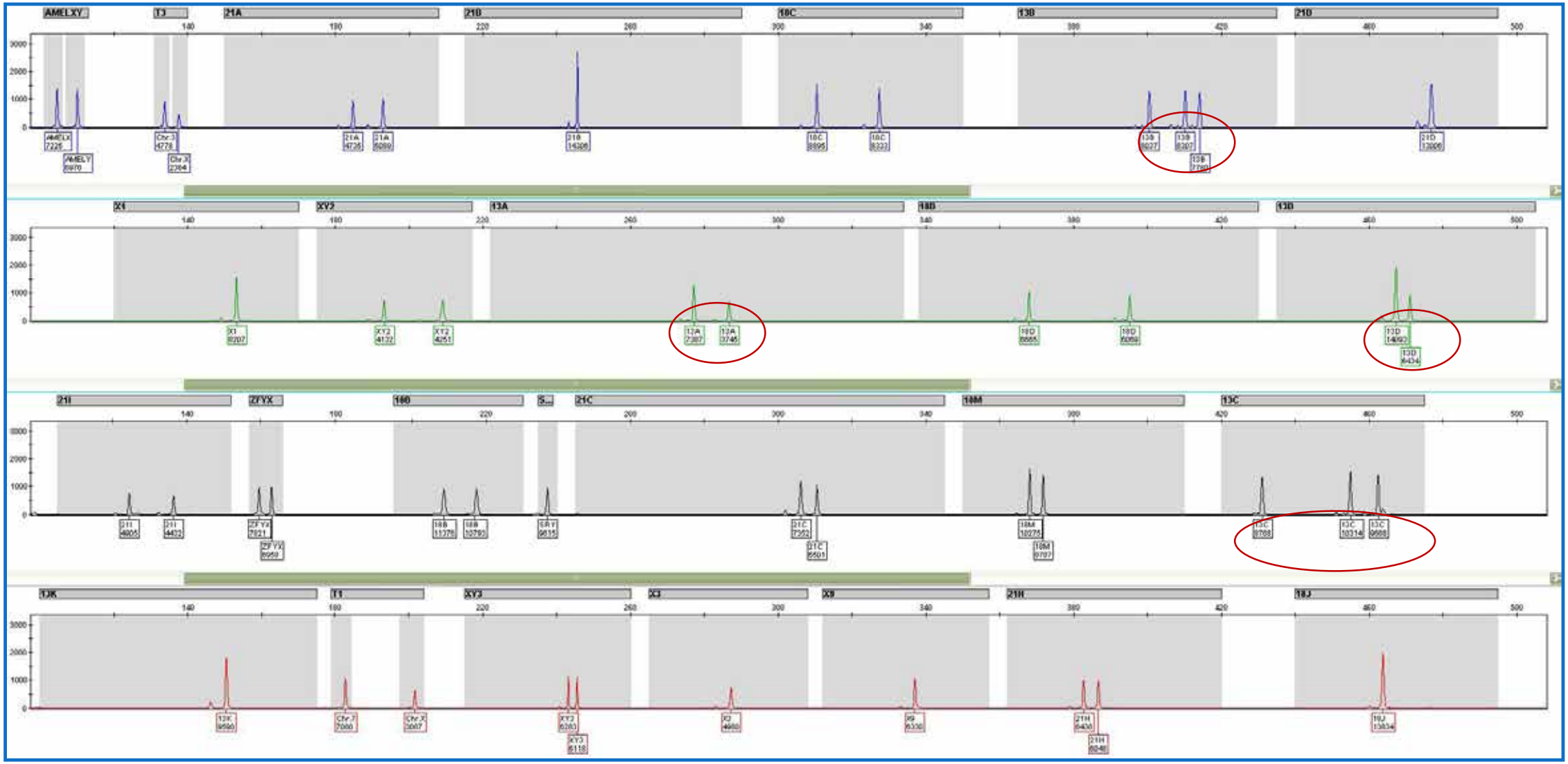


XX normal All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.
The presence of AMELX and the absence of AMELY and SRY is consistent with female gender.
The X chromosome counting markers (T1 and T3) demonstrate a 1:1 marker ratio, in line with the dosage of two X chromosomes.
The presence of informative X chromosomal STR markers with normal marker ratios confirms the dosage of two X chromosomes.
The presence of informative pseudoautosomal STR markers (XY2 and XY3) with normal marker ratios confirms the dosage of two sex chromosomes



Patau Syndrome, Trisomy 13

All informative autosomal STR markers on chromosome 13 demonstrate abnormal 1:2, 2:1 or 1:1:1 marker ratios consistent with trisomy 13.



Edwards Syndrome, Trisomy 18

All informative autosomal STR markers on chromosome 18 demonstrate abnormal 1:2, 2:1 or 1:1:1 marker ratios consistent with trisomy 18.

Down Syndrome, Trisomy 21

All informative autosomal STR markers on chromosome 21 demonstrate abnormal 1:2, 2:1 or 1:1:1 marker ratios consistent with trisomy 21.

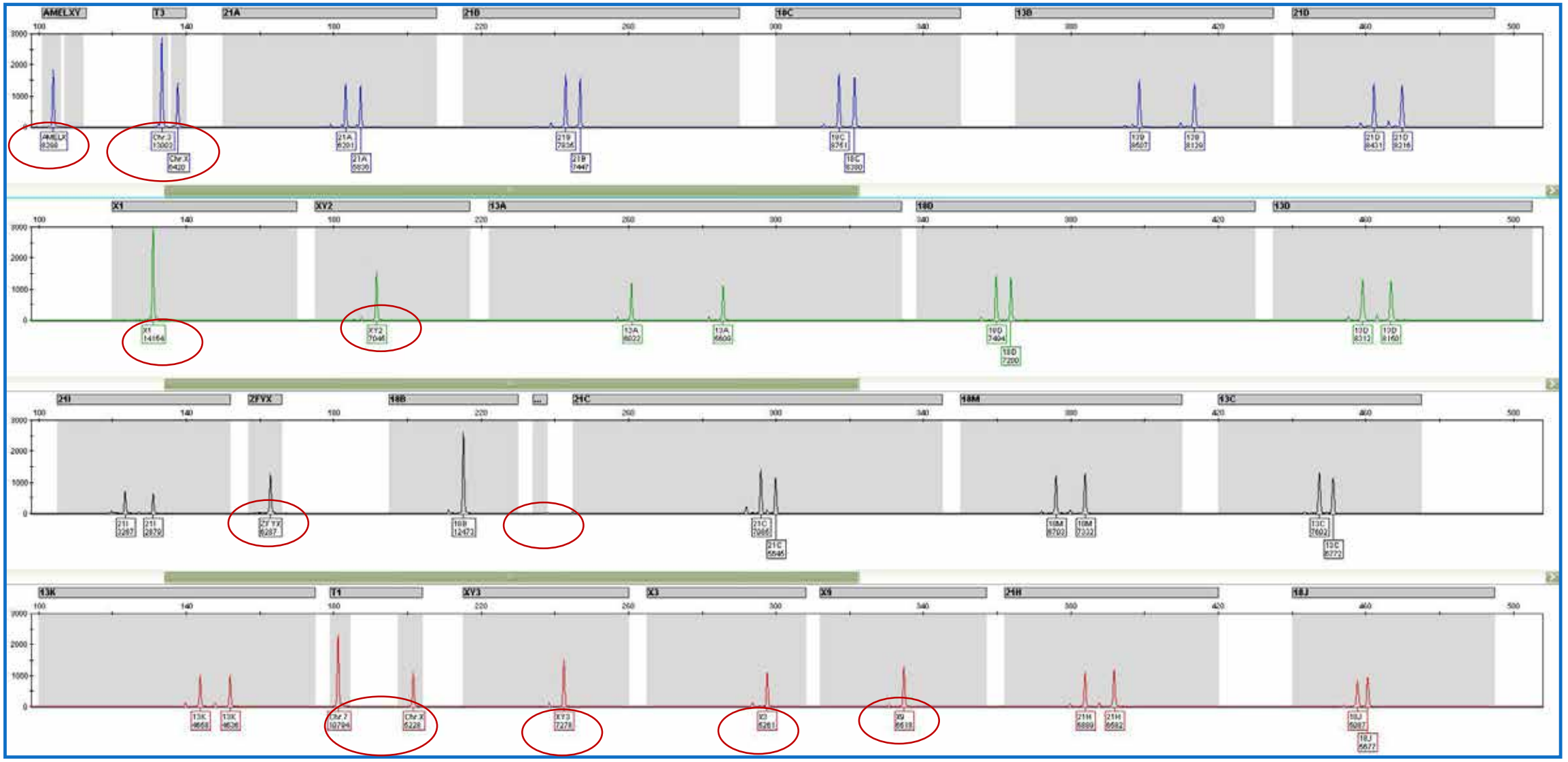
Turner Syndrome (45, X)

All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.

The presence of AMELX and the absence of AMELY and SRY confirm female gender.

The X chromosome counting markers (T1 and T3) demonstrate an abnormal female 2:1 marker ratio consistent with the dosage of a single X chromosome.

All X and XY STR markers are uninformative (X1, X3, X9, XY2, XY3), consistent with the dosage of a single X chromosome.



Klinefelter Syndrome (47, XXY)

All informative autosomal STR markers demonstrate a normal 1:1 marker ratio.

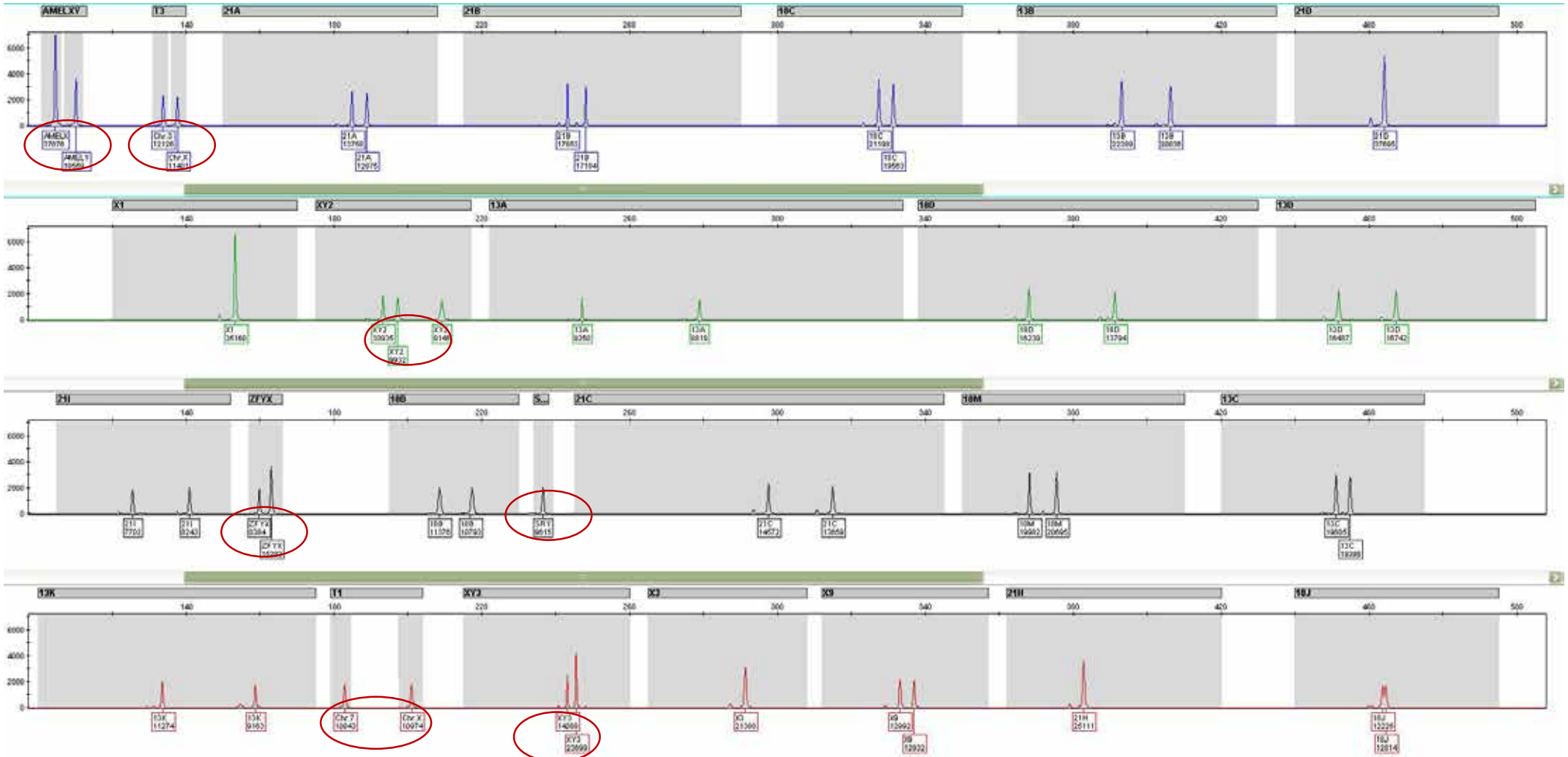
The AMELXY marker demonstrates an abnormal AMELX to AMELY (2:1) marker ratio and pseudoautosomal markers are trisomic (XY2 and XY3).

The X chromosome counting markers (T1 and T3) demonstrate an abnormal male 1:1 marker ratio consistent with the dosage of two X chromosomes.

The presence of an informative X chromosomal STR marker confirms the dosage of two X chromosomes.

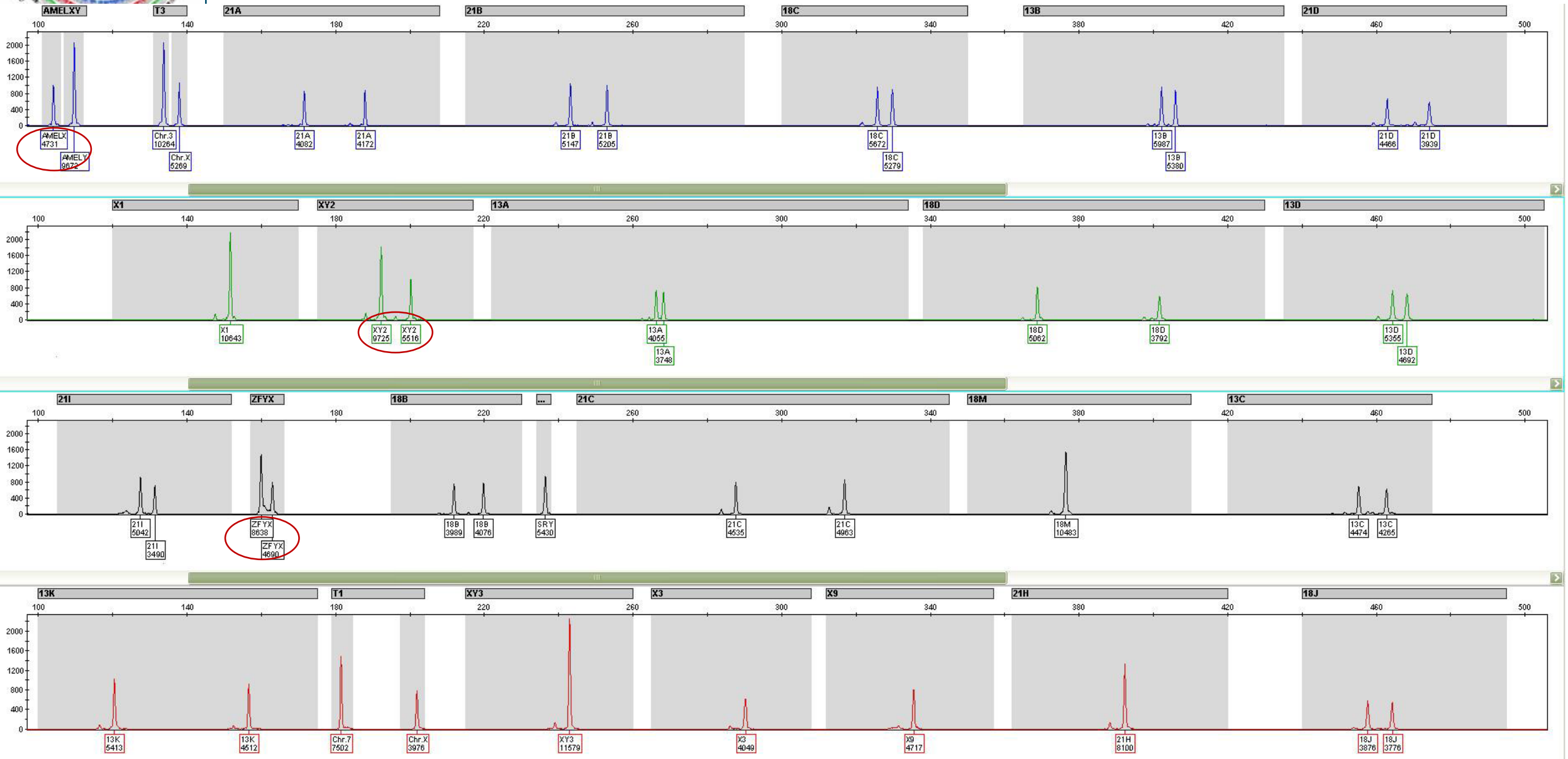
AMELY and SRY confirm the presence of a Y chromosome.

The abnormal ZFYX marker ratio (1:2) confirms the dosage of three sex chromosomes.



47, XYY Syndrome

Informative STR markers on all autosomal chromosomes demonstrate a normal 1:1 marker ratio. Markers AMELXY, XY2 and ZFYX demonstrate abnormal (1:2 and 2:1) marker ratios consistent with the dosage of two Y chromosomes



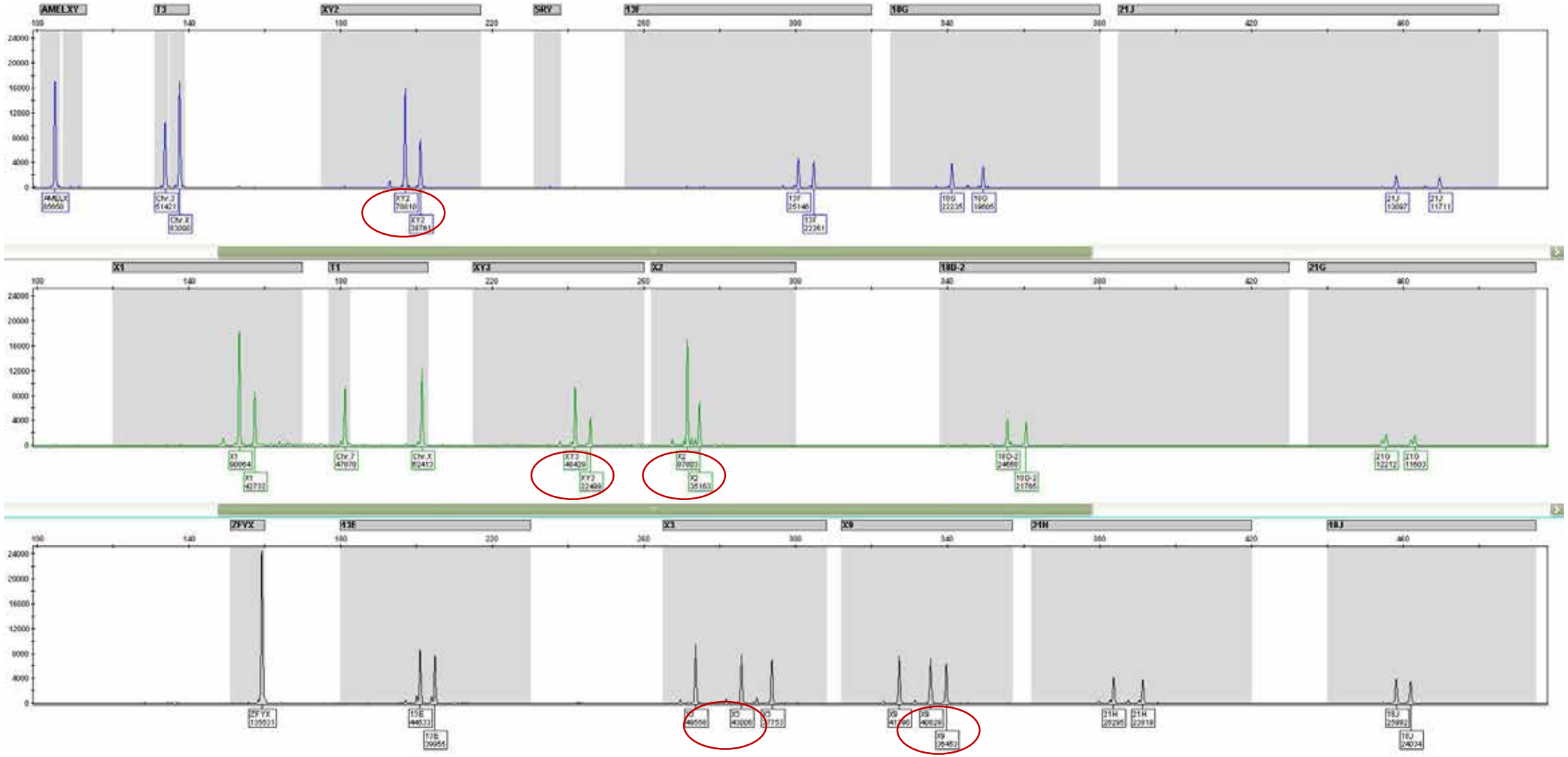
Triple X Syndrome (47, XXX)

Informative STR markers on all autosomal chromosomes demonstrate a normal 1:1 marker ratio.

Informative pseudoautosomal STR markers (XY2 and XY3) demonstrate an abnormal (2:1) marker ratio.

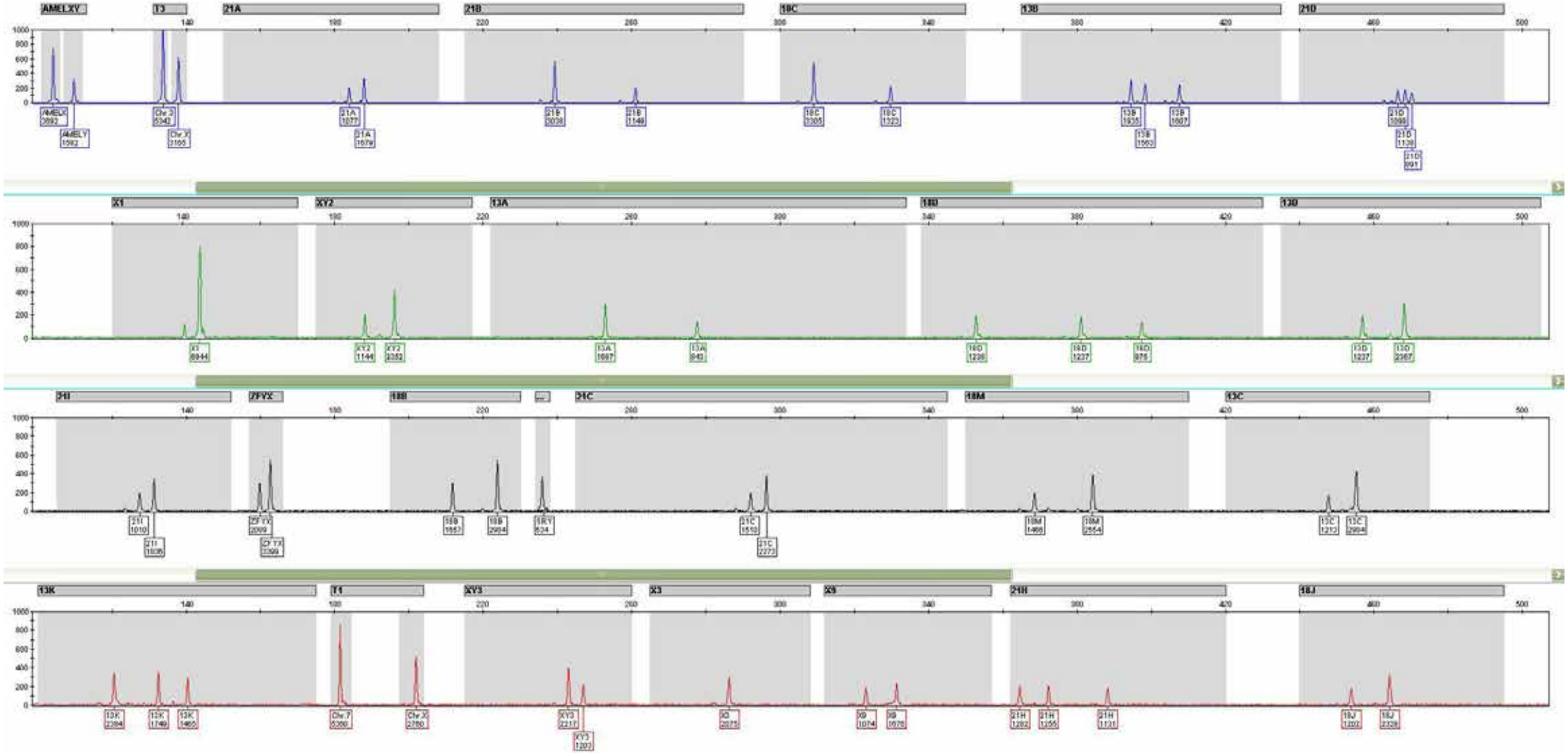
Informative X chromosomal STR markers (X1, X2, X3, X9) demonstrate abnormal (1:2, 2:1 or 1:1:1) marker ratios, consistent with the dosage of three X chromosomes.

The abnormal marker ratio (1:2) of the X chromosome counting markers (T1 and T3) is consistent with an abnormal female X chromosome dosage.



Triploidy

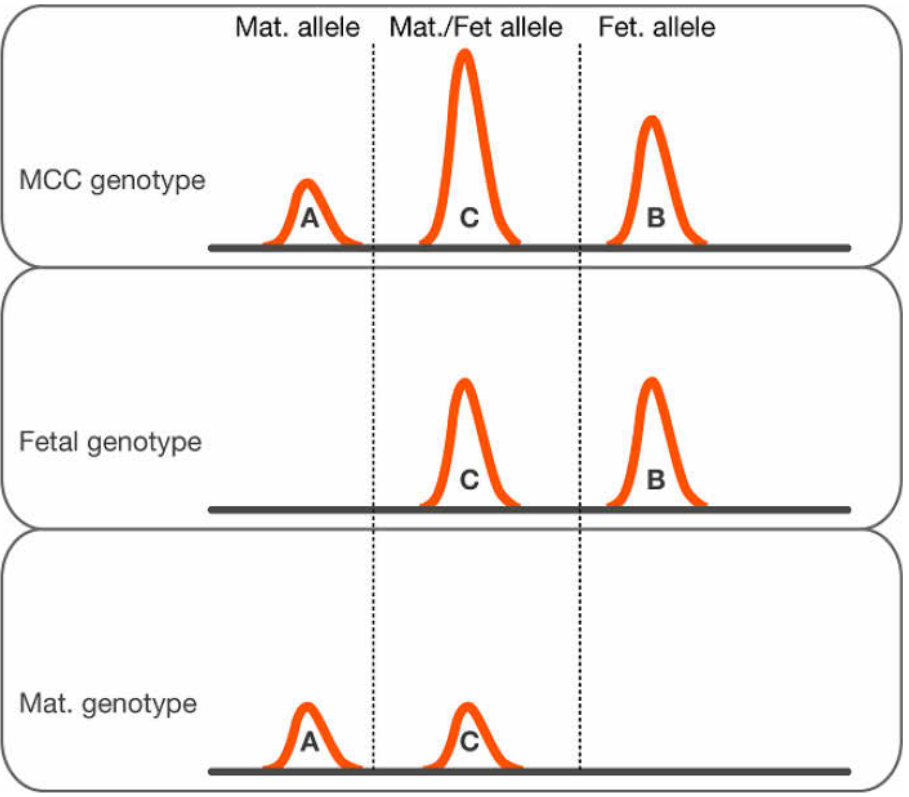
All informative STR markers on chromosomes 13, 18, 21, X and Y demonstrate abnormal 1:2, 2:1 or 1:1:1 marker ratios consistent with triploidy

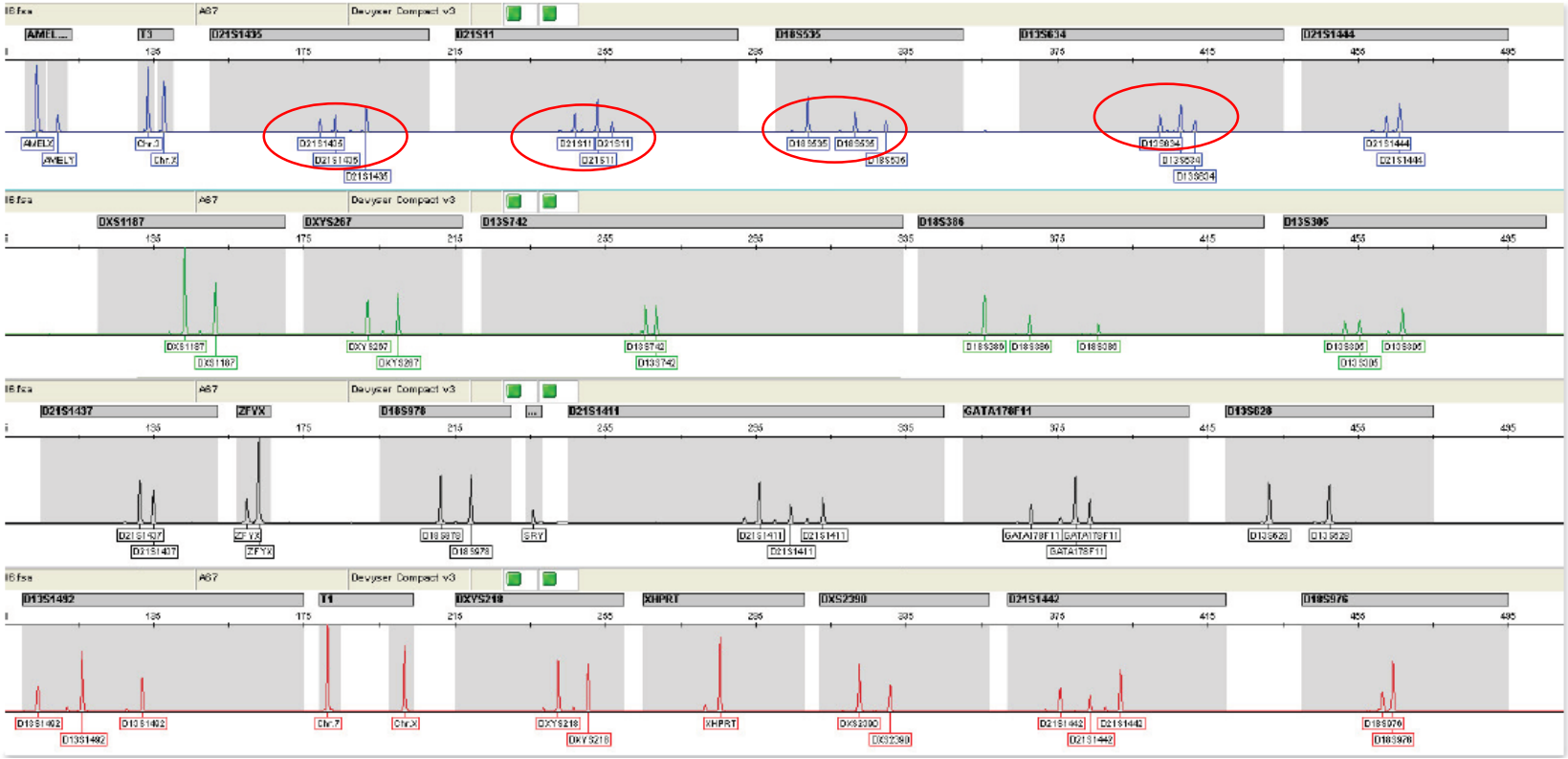


Contaminació materna

Líquid amniòtic hemàtic

Material matern en vellossitat corial





Mosaicisme

Gestant de 28 anys

TN 3.3 mm

1/47 Sd Down

1/3739 Sd Edwards

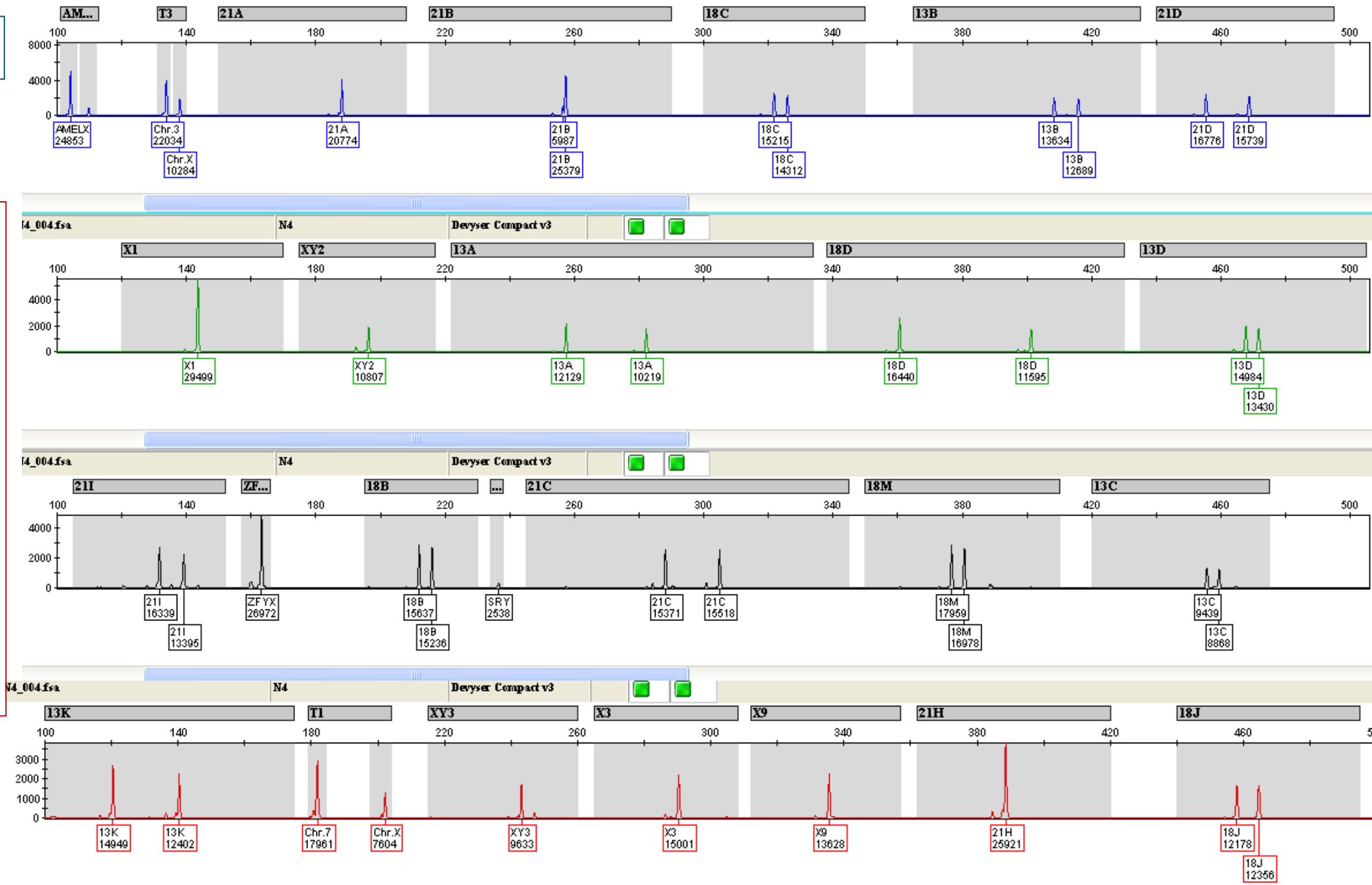
0/0/1/0

Biòpsia còrion:

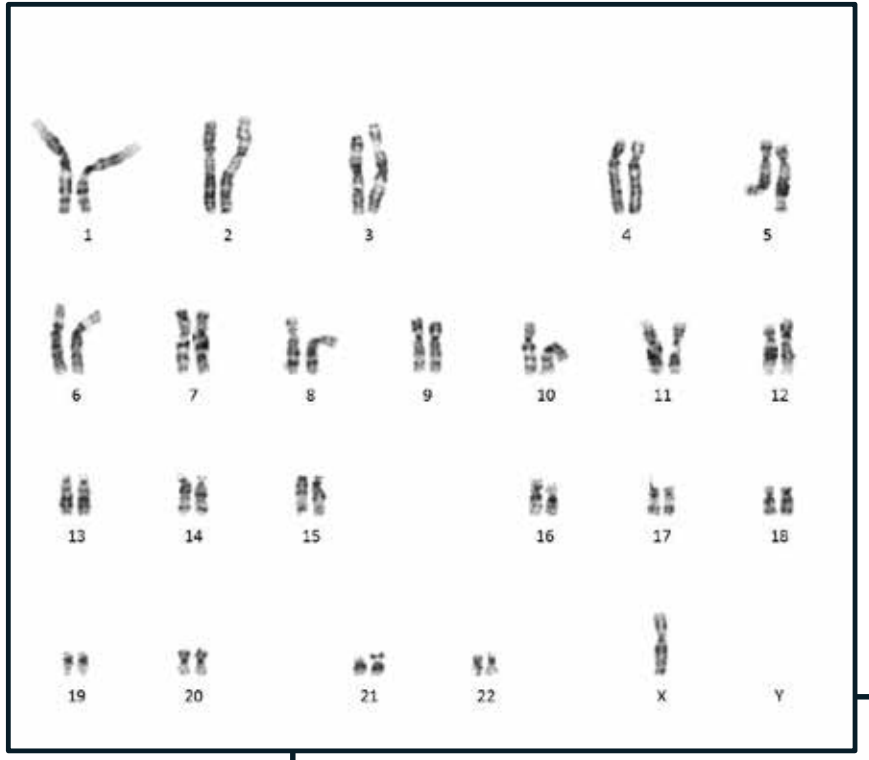
QF-PCR

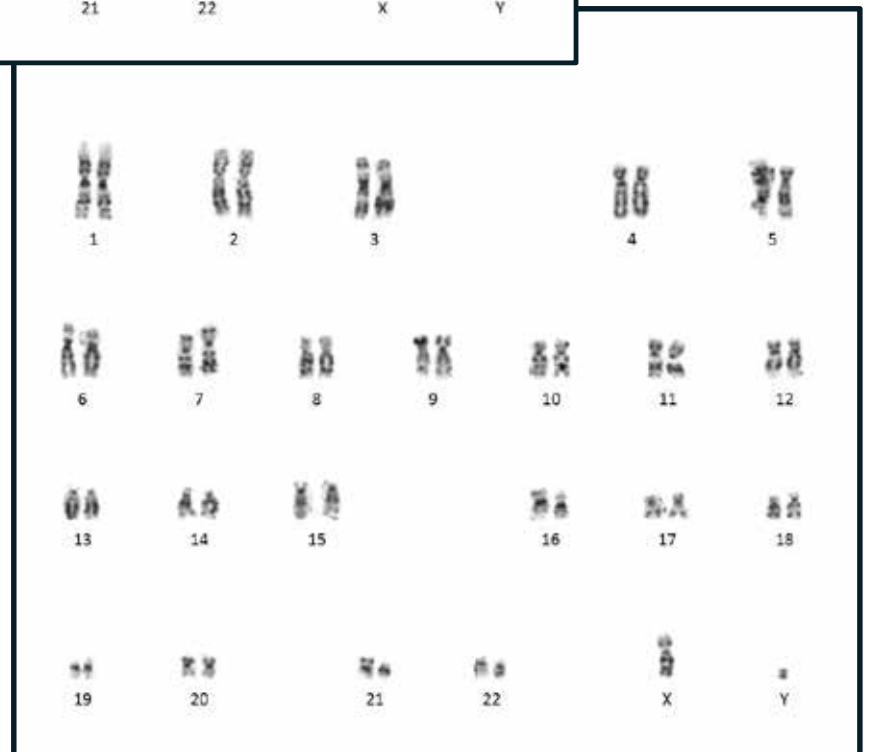
arrayCGH

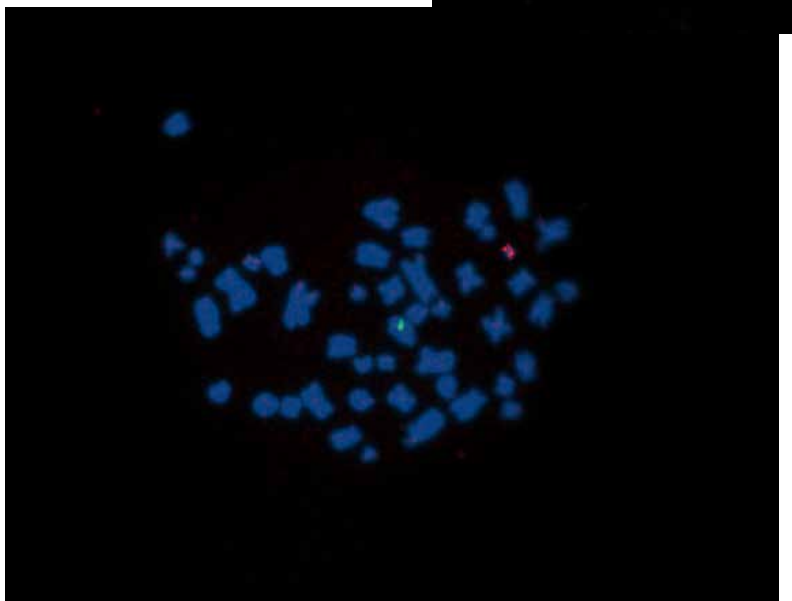
16w sexe fetal nena



45,X[73]/46,X, i(Y)(p10).ish(Y)(SRY++, DYZ3-)[37]





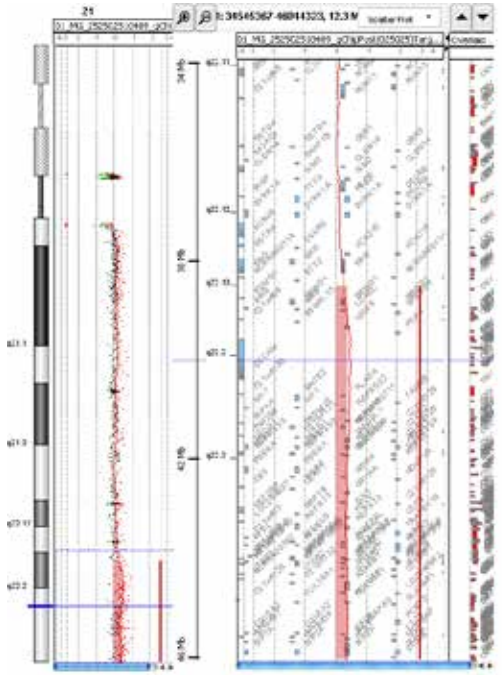


Gestant 43 anys.

12 w TN 4,3 mm i absència d'os nasal.

TS 1/5 (Down) i 1/45 (Edwards).

Biopsia corion: QF-PCR i aCGH.

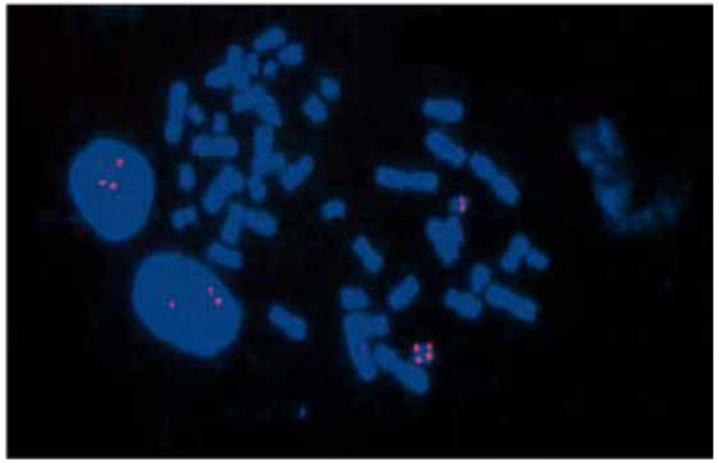


Duplicació de 7.67Mb

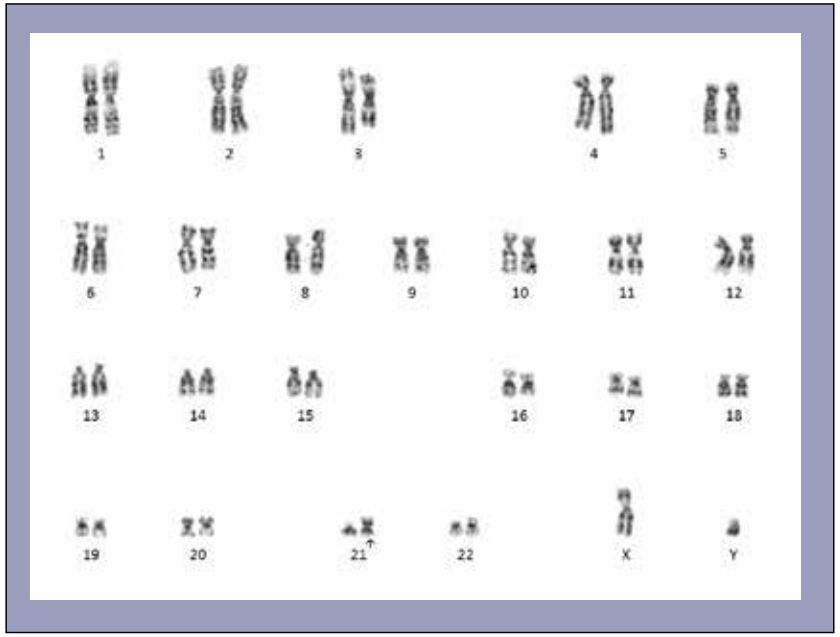
21q22.2q22.3 (>100genes referència).

No inclou regió crítica Sd. Down

QF-PCR:
Relació 2:1 en 2 marcadors compatible amb duplicació 21q22.3.

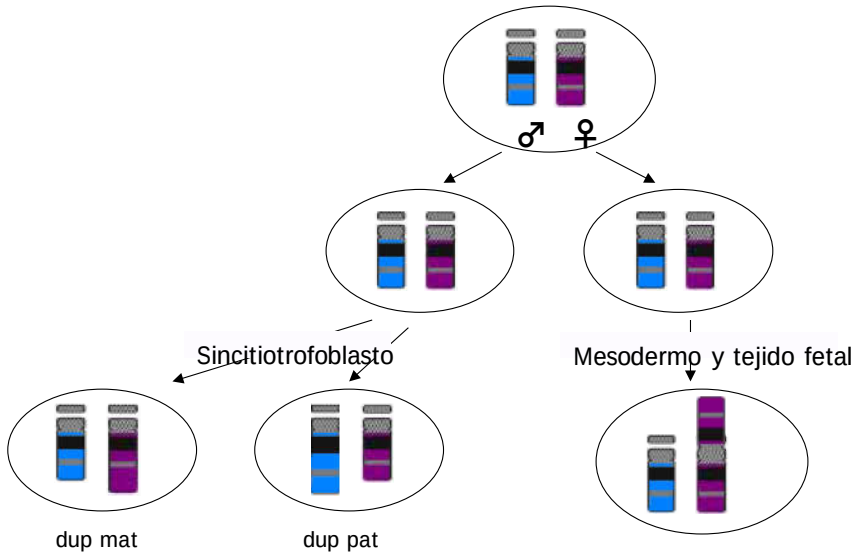


FISH cultiu llarg VC



Cariotip fetal post ILE

Origen postcigòtic



QF-PCR: application, overview and review of the literature

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	CVS	LA
Contaminació materna	0.25%	2.1-6 %
Discrepàncies (mosaicisme)*	<1/10.000	Poc reportat (mes freqüents)
No resultat (no informatiu)	<1/2000	

*

1. Qualitat/quantitat de la mostra
2. Estratègia en el processament de la mostra
3. Expertesa en l'anàlisi

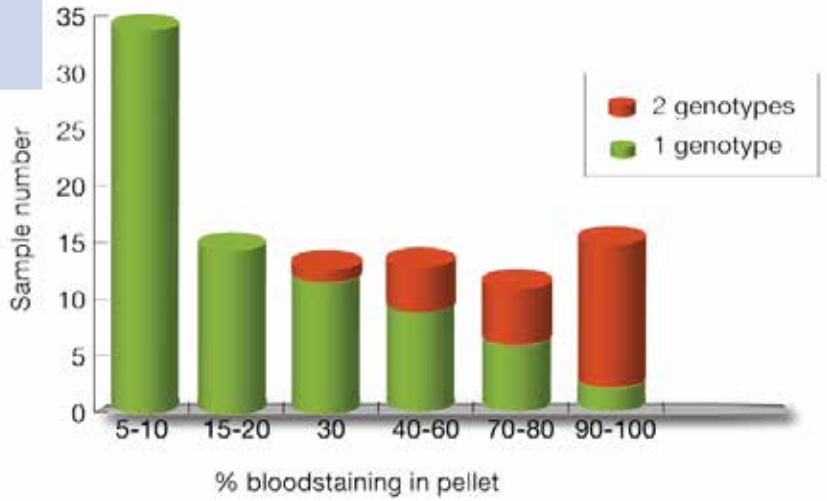

Figure 3. Number of blood-stained AF samples plotted against the degree of blood-staining in the cell pellet following centrifugation. Green columns show samples with a single genotype following QF-PCR testing of uncultured material whilst orange columns show samples with two genotypes (Stojilkovic-Mikic, 2005).