

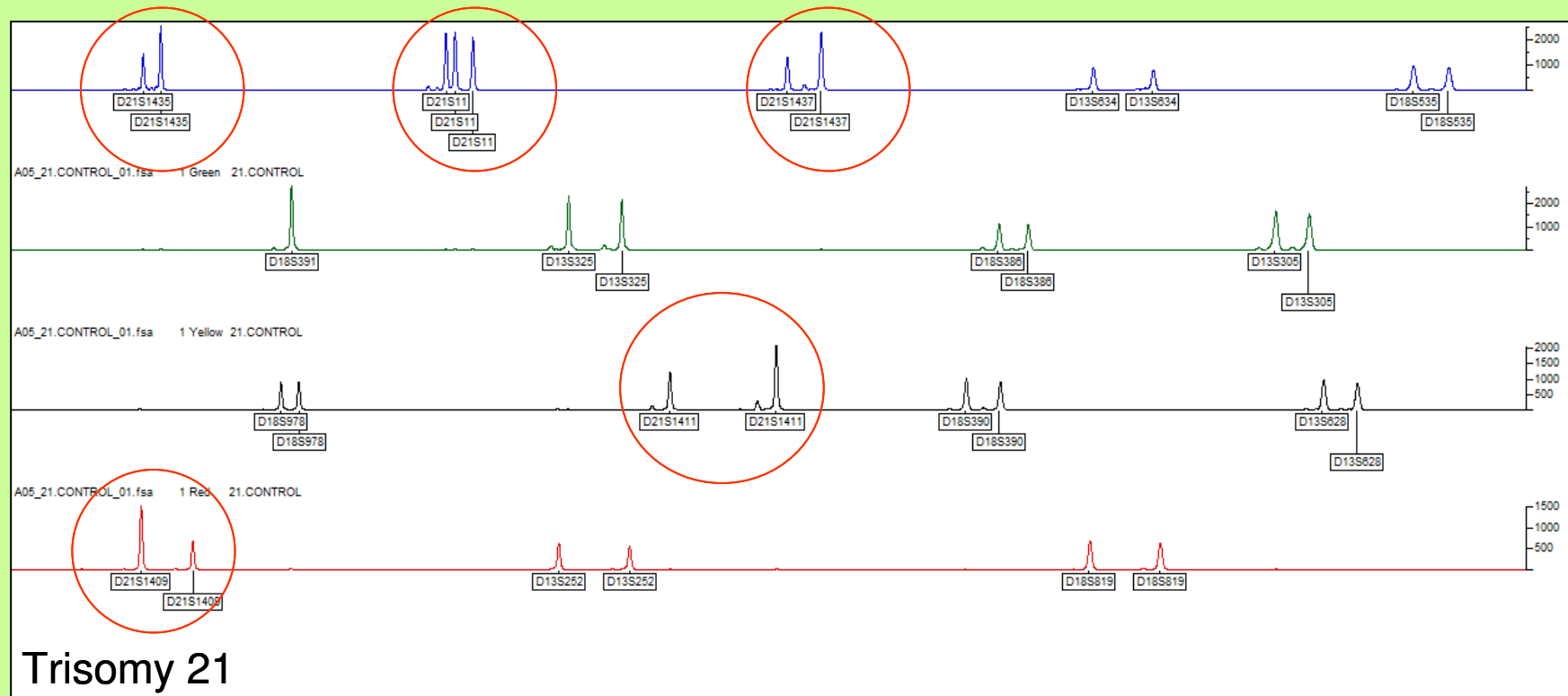
The use of paralogous sequences for the improved diagnosis of X chromosome imbalance by QF-PCR

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QF-PCR

- Rapid, robust, efficient technique for diagnosis of aneuploidy in prenatal samples
- Involves semi-quantitative analysis of microsatellite markers



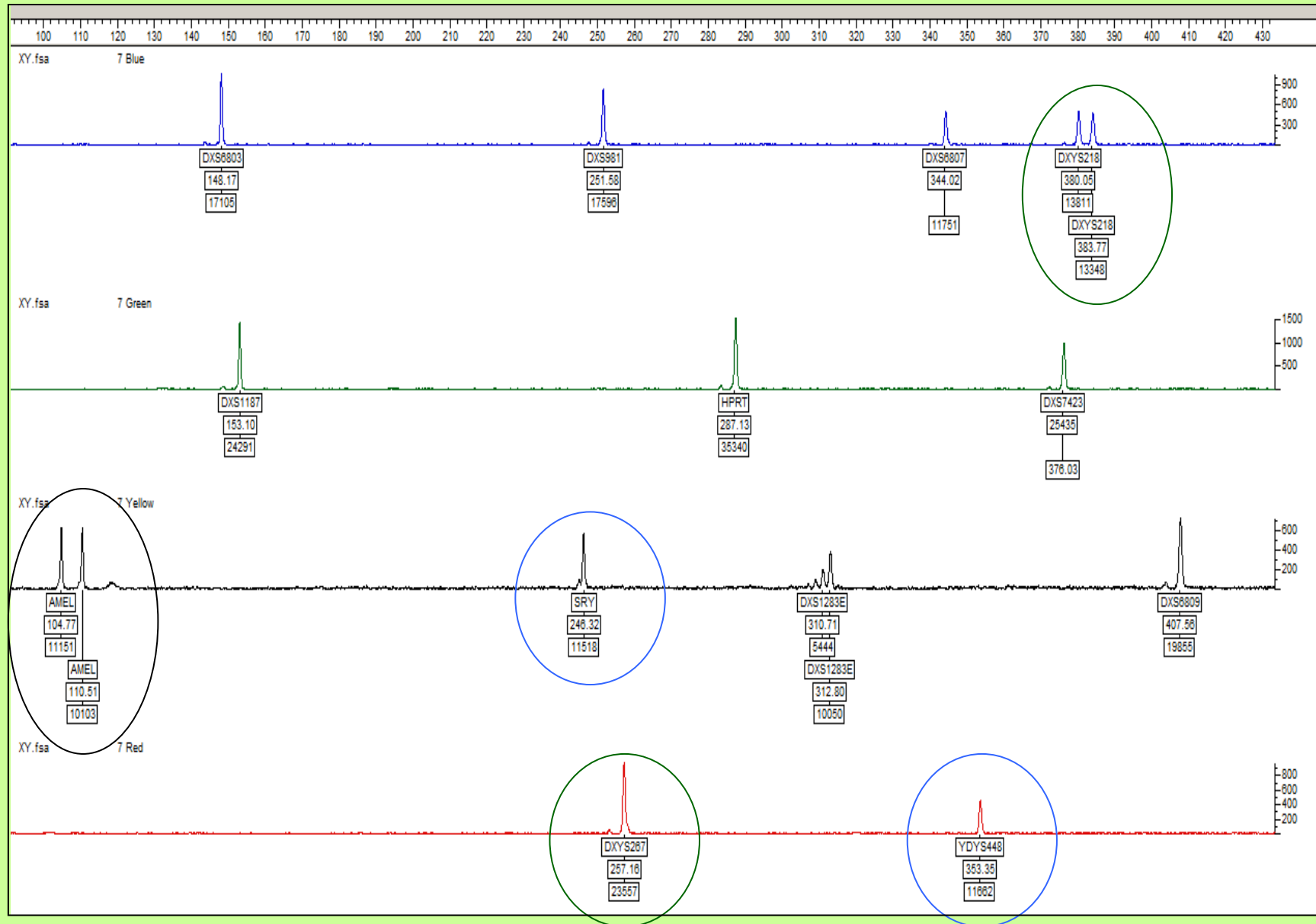
Use of QF-PCR at Guys

- Used to diagnose aneuploidies for chromosomes 13, 18, 21, X and Y
- >40,000 prenatals tested
 - ~ 26,500 AF
 - ~ 14,000 CVS
- 12% targeted for sex chromosome testing

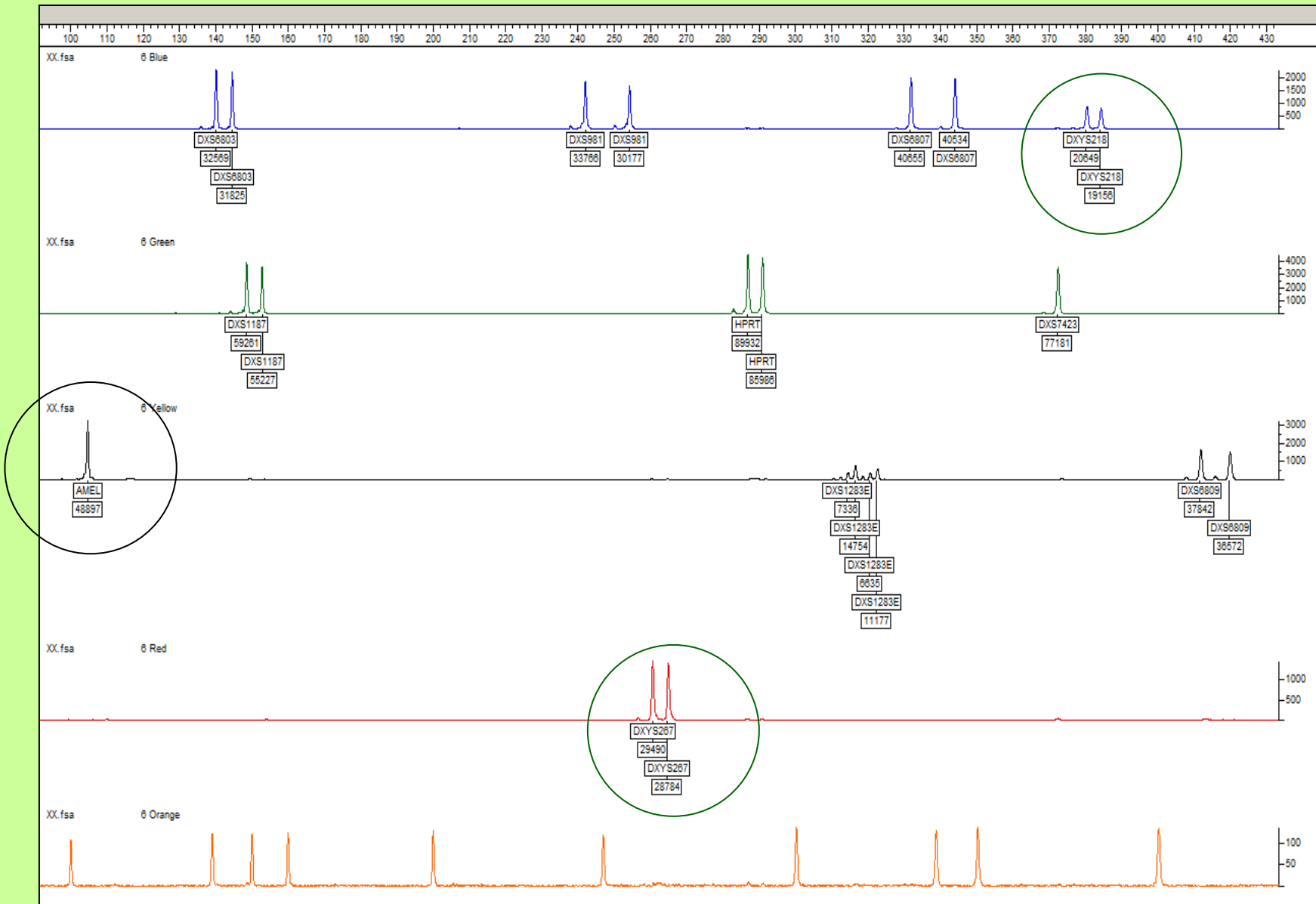
Sex chromosome assay

- Prenatals with u/s abnormalities indicative of Turner syndrome
- Original assay
 - Amelogenin (X and Y)
 - Two Y chromosome-specific markers
 - Two sex chromosome polymorphic markers (X and Y)
 - Eight X chromosome-specific polymorphic markers

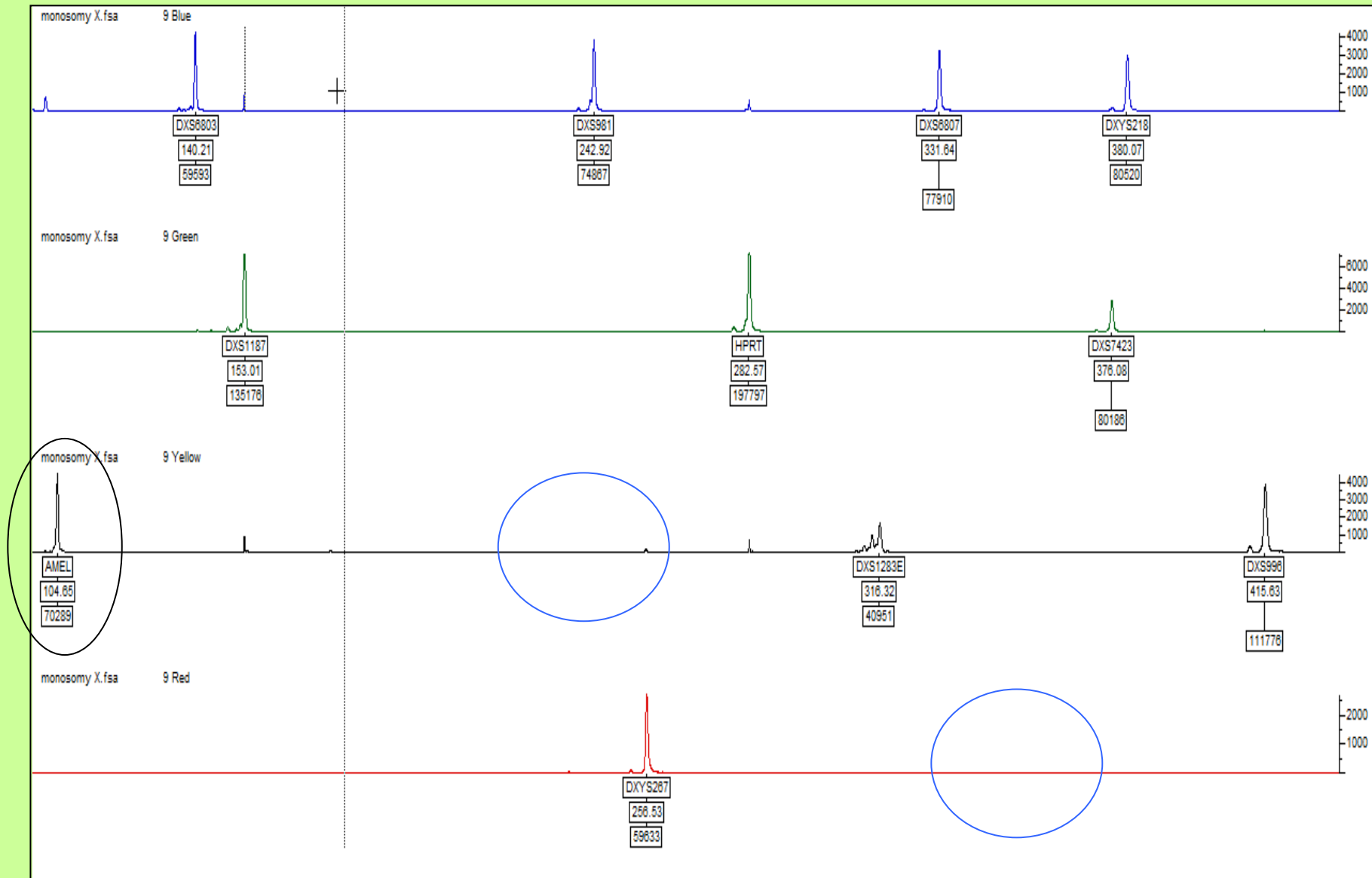
Normal male XY



Normal female XX



Original QF-PCR assay - Monosomy X

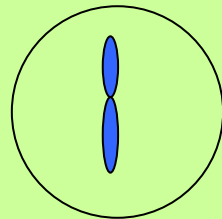


Limitation of polymorphic markers to diagnose monosomy X

- All polymorphic markers exhibit one allele
- Two possible interpretations:

Monosomy X

chromosome

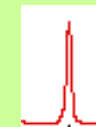
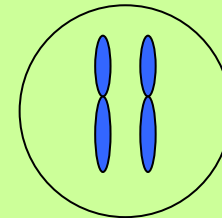


peak



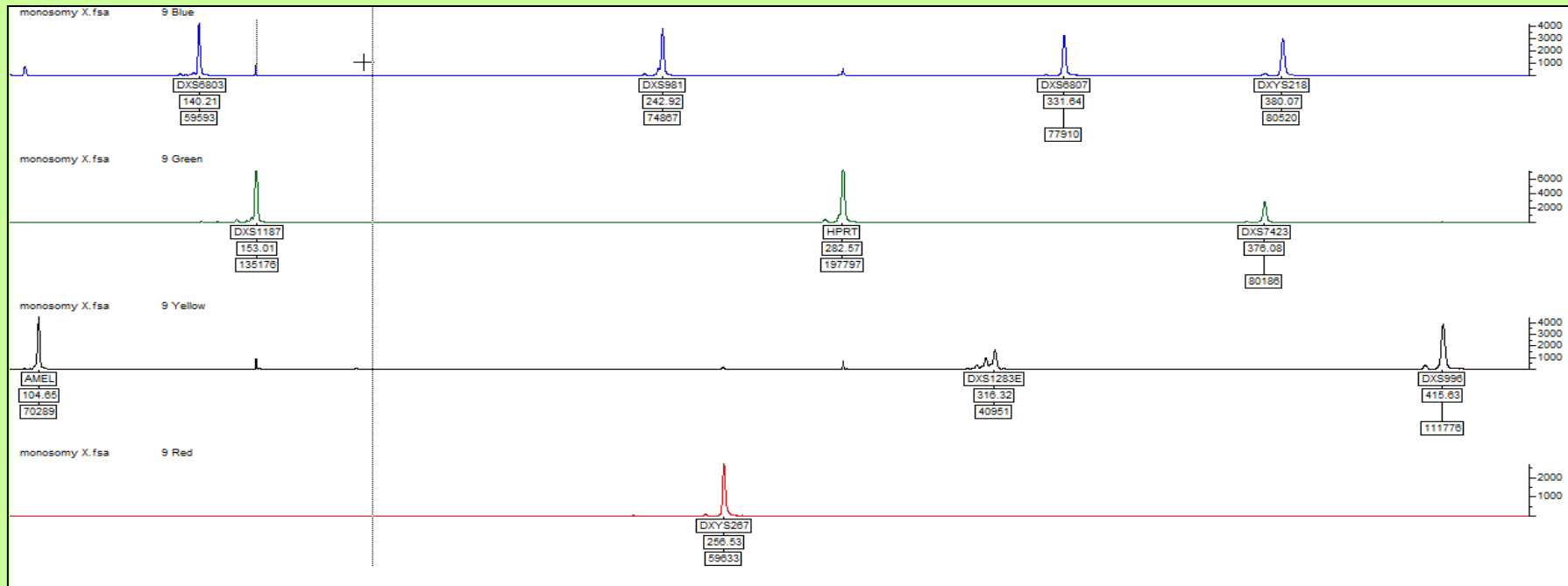
Hemizygous
One copy

Normal female



Homozygous
Two copies, same allele

Original QF-PCR assay - monosomy X trace



Bayesian probability of being XX (assume 0.8 heterozygosity)

Referral

general

cystic hygroma

10 polymorphic markers

1:66,686

1:5,967,881

6 polymorphic markers

1:107

1:9,549

4 polymorphic markers

1:4

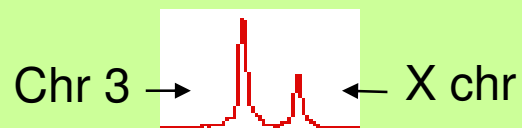
1:382

More likely to be XX in a consanguineous family

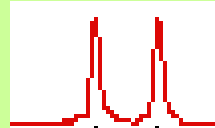
Addition of a paralogous marker

- Duplicated sequences that have diverged over time
- Present on different chromosomes
- Can be used to determine relative sequence copy number
- **TAF9L**- Xq21.1 and 3p24.2
 - Deutsch et al, J Med Genet, 2004
- Both sequences are amplified using the same primer pair
- Length differences allow products to be separated and quantified
- Can calculate the relative dosage of the X chromosome to chromosome 3

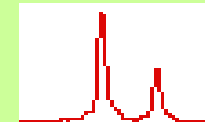
TAF9L traces



Male
2:1



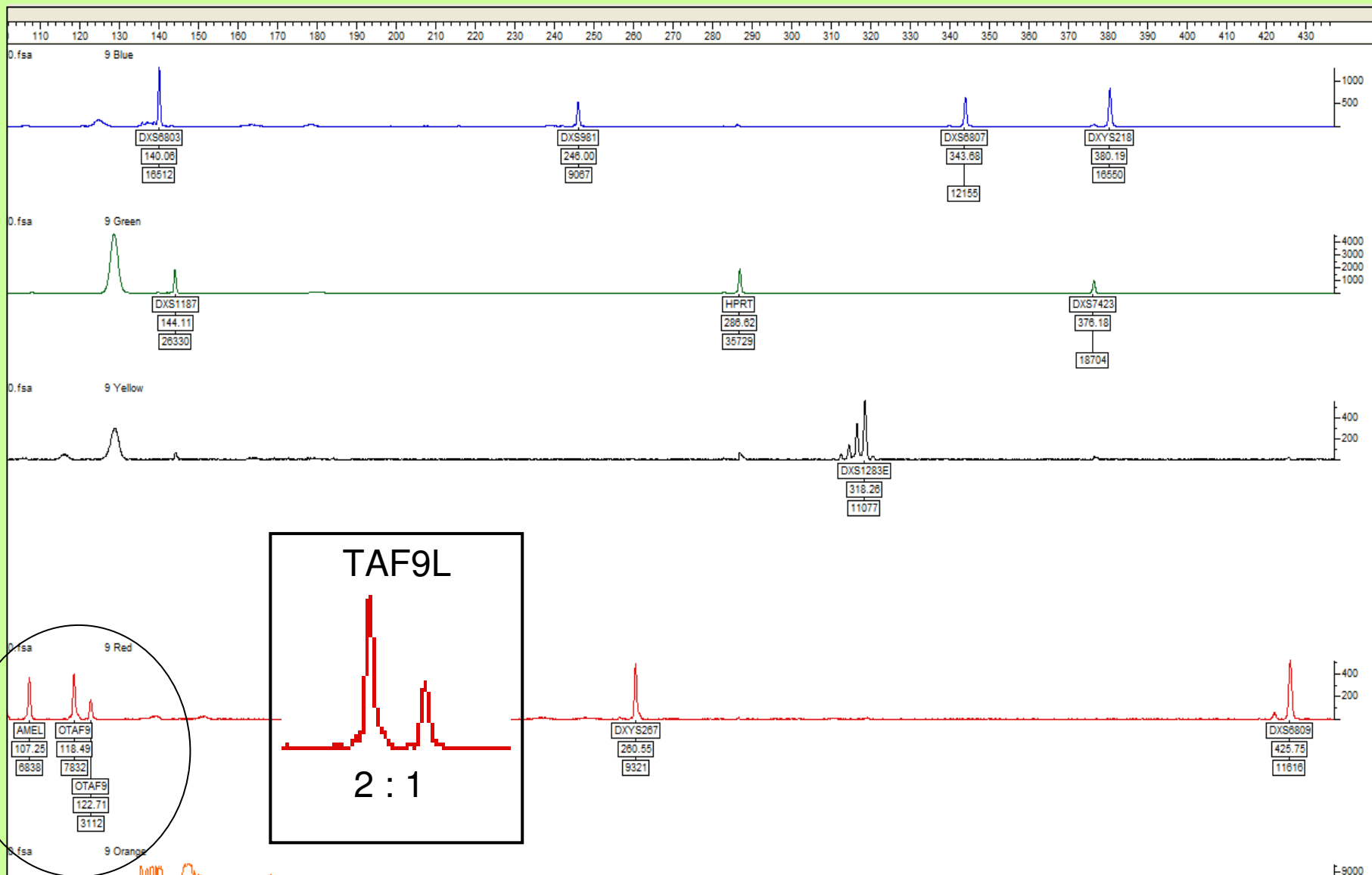
Female
2:2



Monosomy X
2:1

- Use in multiplex with other X and Y markers
- Possibility of SNPs and CNVs

Monosomy X



Use of TAF9L at Guy's (Sept 2008)

1146 prenatal samples targeted for sex chromosome testing

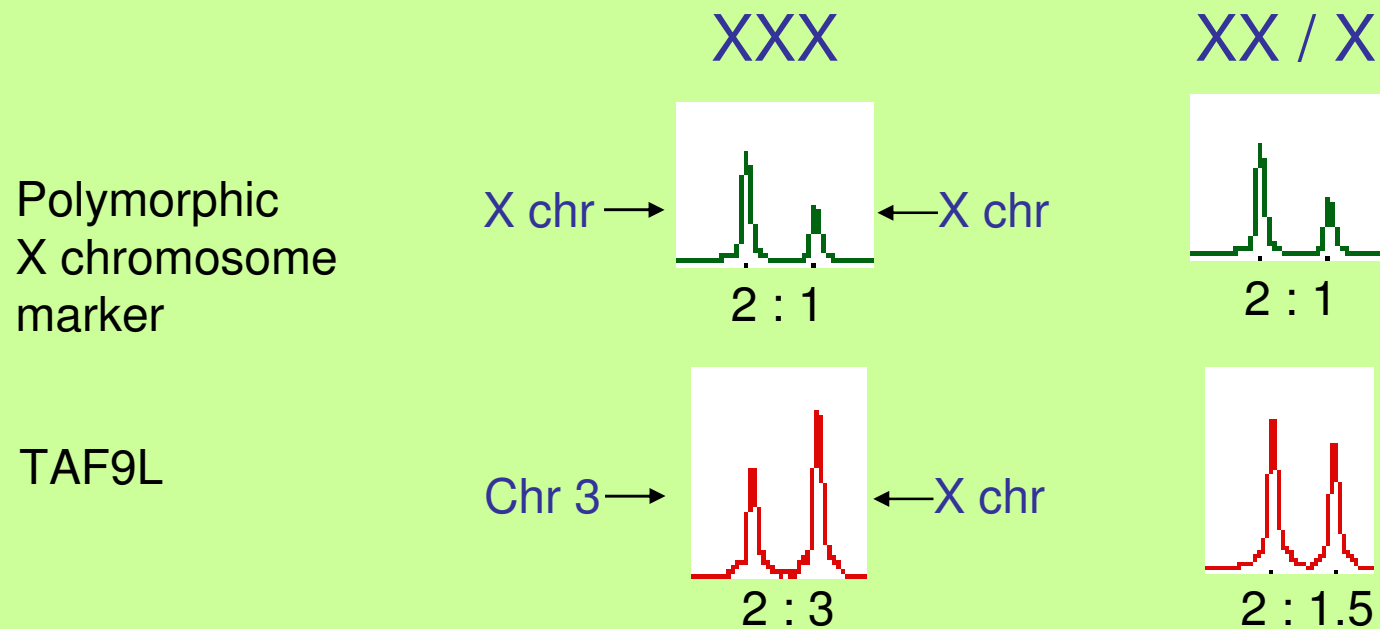
- 93 monosomy X (8%)

919 tissue samples

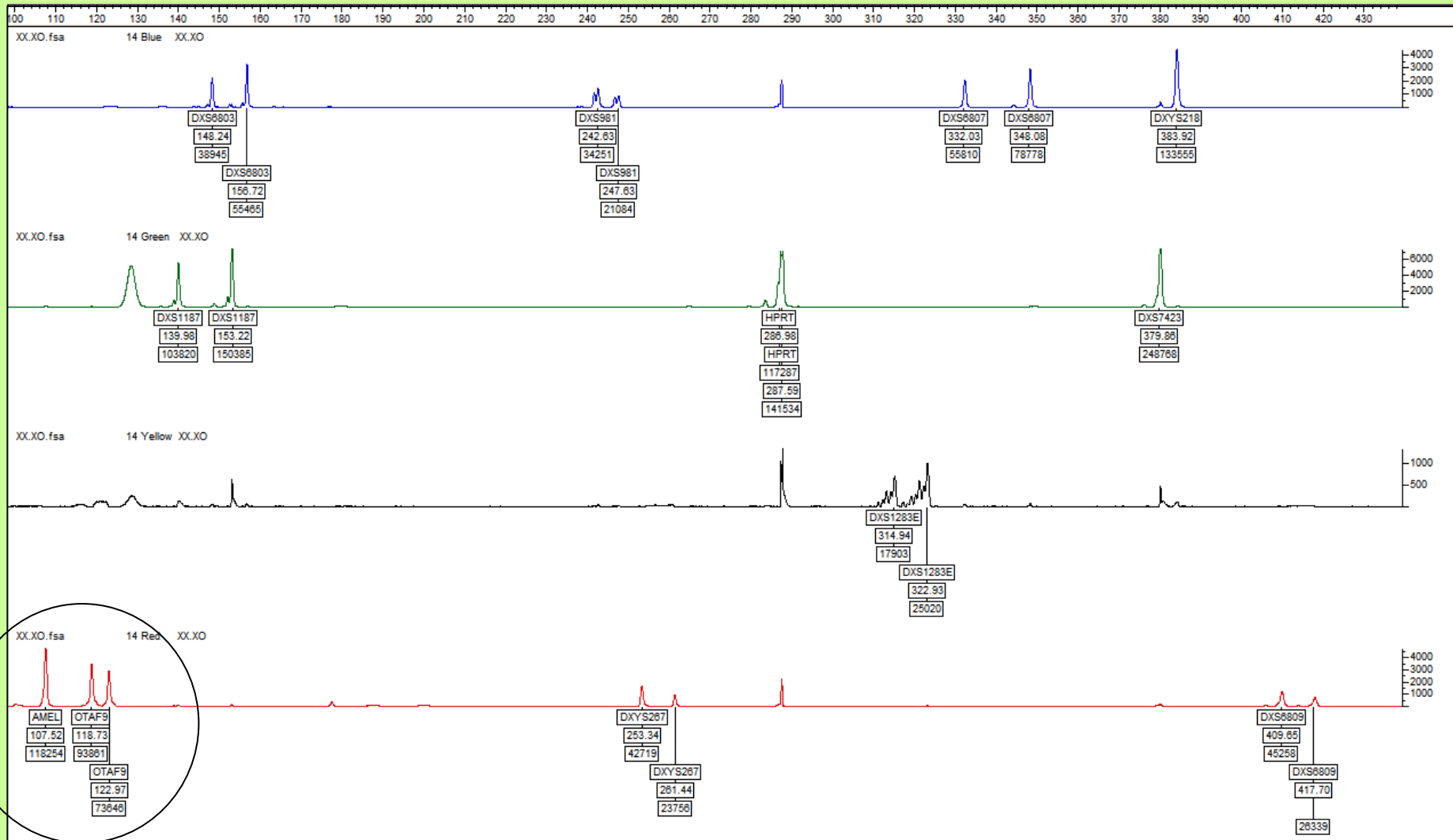
- 26 monosomy X (3%)

Additional use of TAF9L

- Distinguish between XXX and XX/X mosaicism

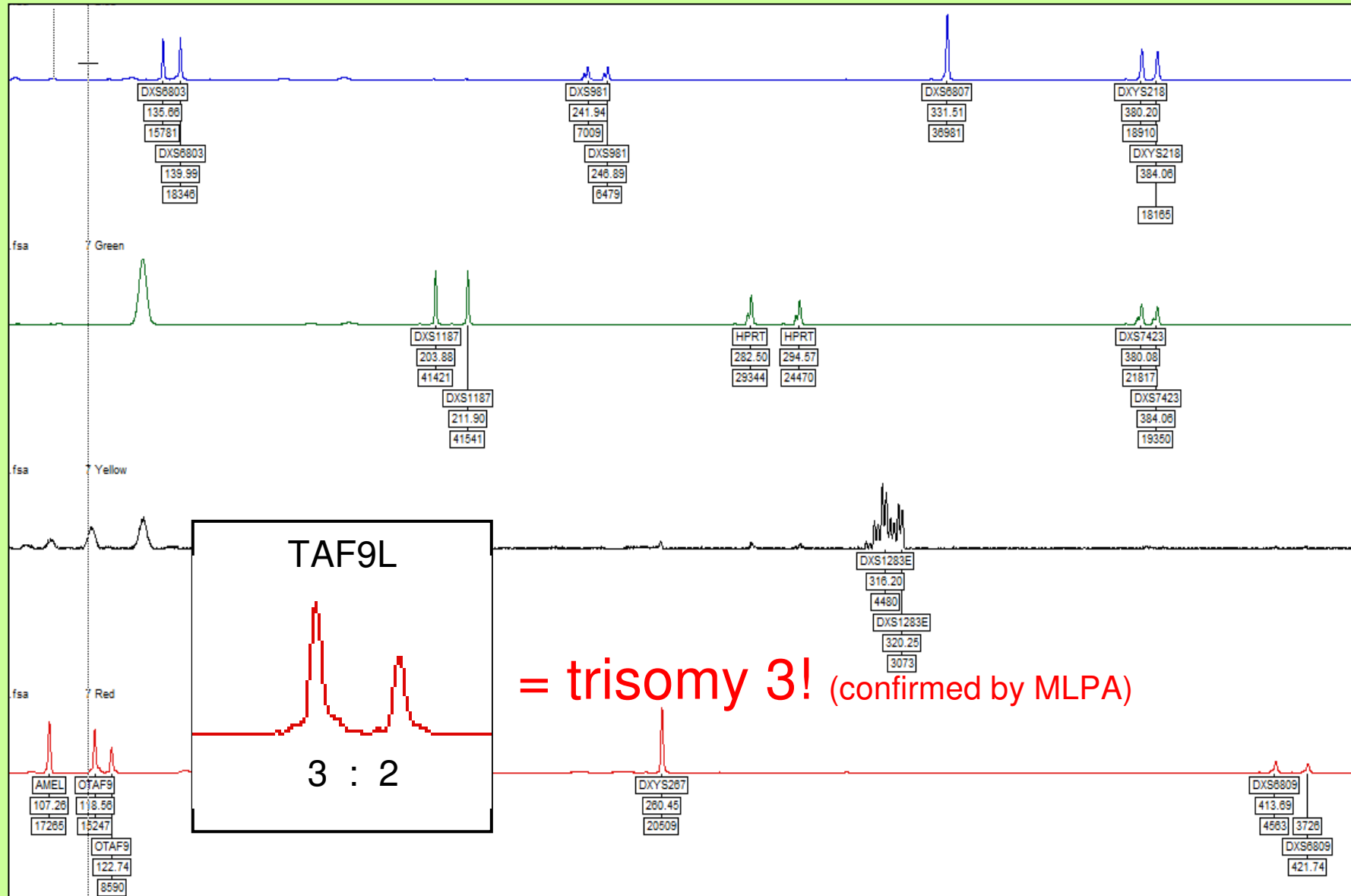


XX/X mosaicism



- Aids interpretation of other sex chromosome abnormalities

Tissue sample



- Draft updated BPGs recommend inclusion of an X chromosome counting marker such as TAF9L
- Primer sequences available (kathy.mann1@nhs.net)