

Analysis of copy number change using quantitative real-time PCR

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arrayCGH follow-up studies

- ★ FISH or arrayCGH depending on: Size
Loss or Gain
BAC availability

time-consuming, expensive

- ★ Alternative technique for follow-up:

qPCR

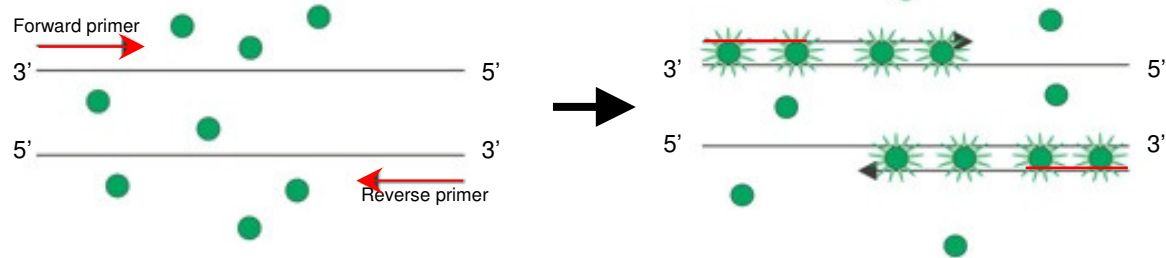
$\Delta\Delta\text{Ct}$ method

StepOnePlus™ real-time PCR machine (ABI)

qPCR chemistries

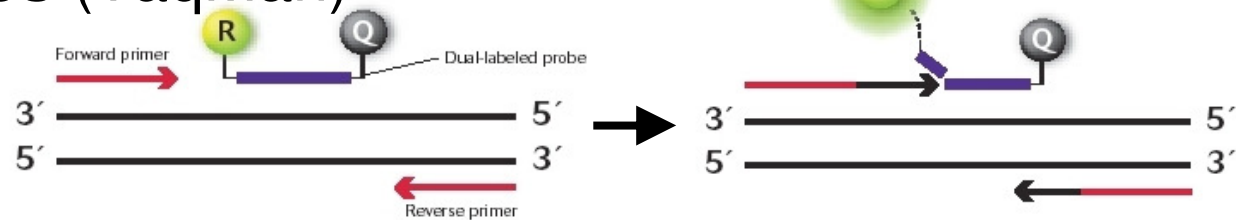
★ SYBR Green

Inexpensive
Flexibility



★ Hydrolysis Probes (TaqMan)

Specificity



★ UPL (Universal ProbeLibrary) (Roche)

Set of 165 8-9bp long hydrolysis probes

99% coverage of the human transcriptome

★ No significant difference between results obtained using the SYBR Green and the UPL chemistries.

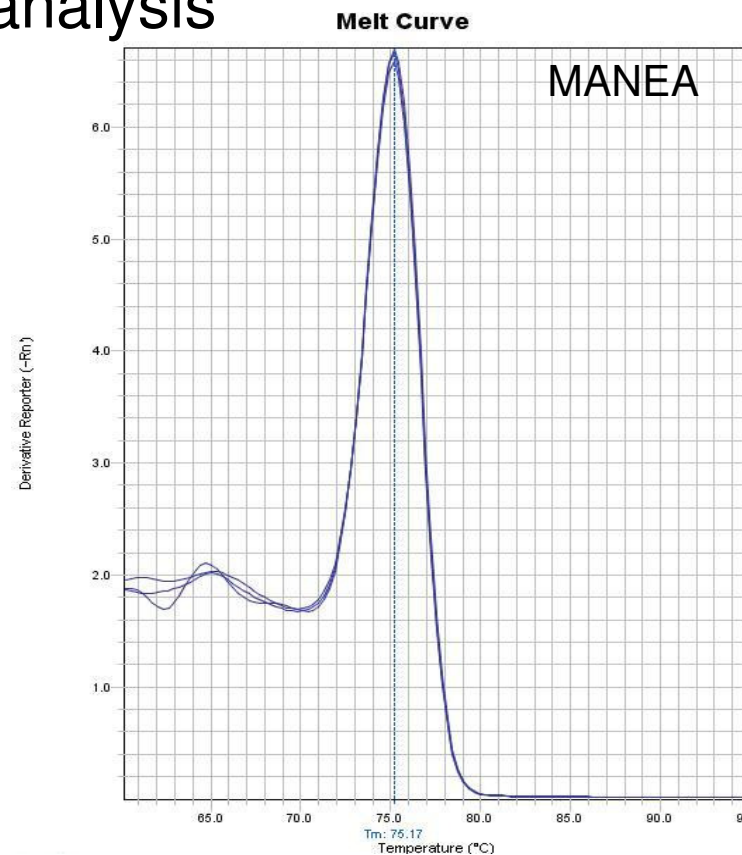
Primer Design

- ★ length: ~20bp
 - T_m: 59°C
 - GC content: 40-60%
 - amplicon size: 70-150bp
- ★ SNP and CNV check
- ★ UCSC *in silico* PCR
- ★ amplicon secondary structure check (mfold)

Primer validation and optimization

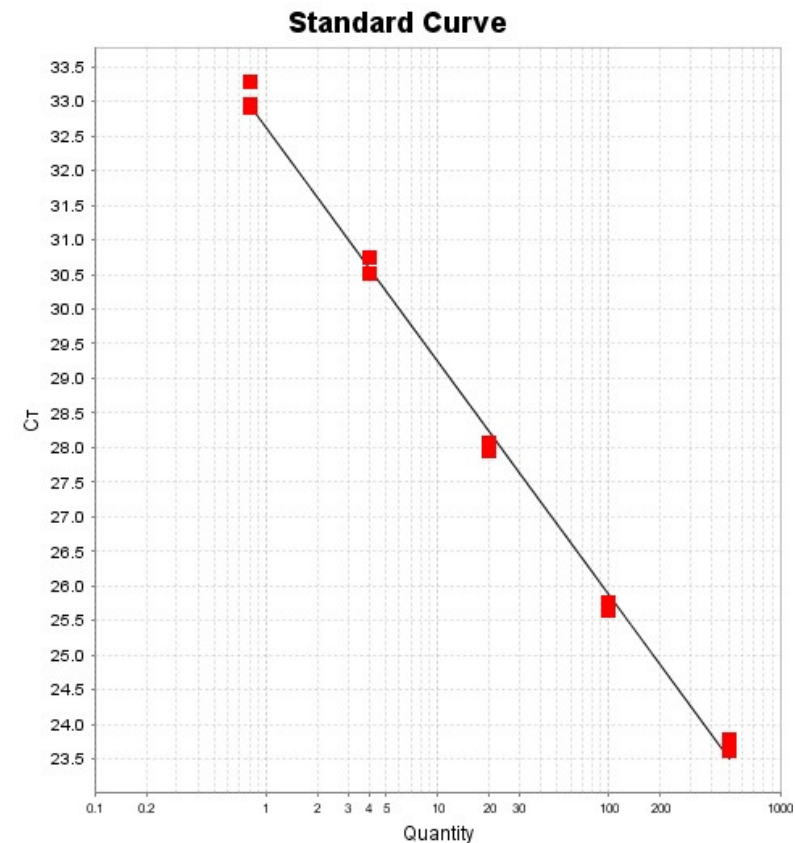
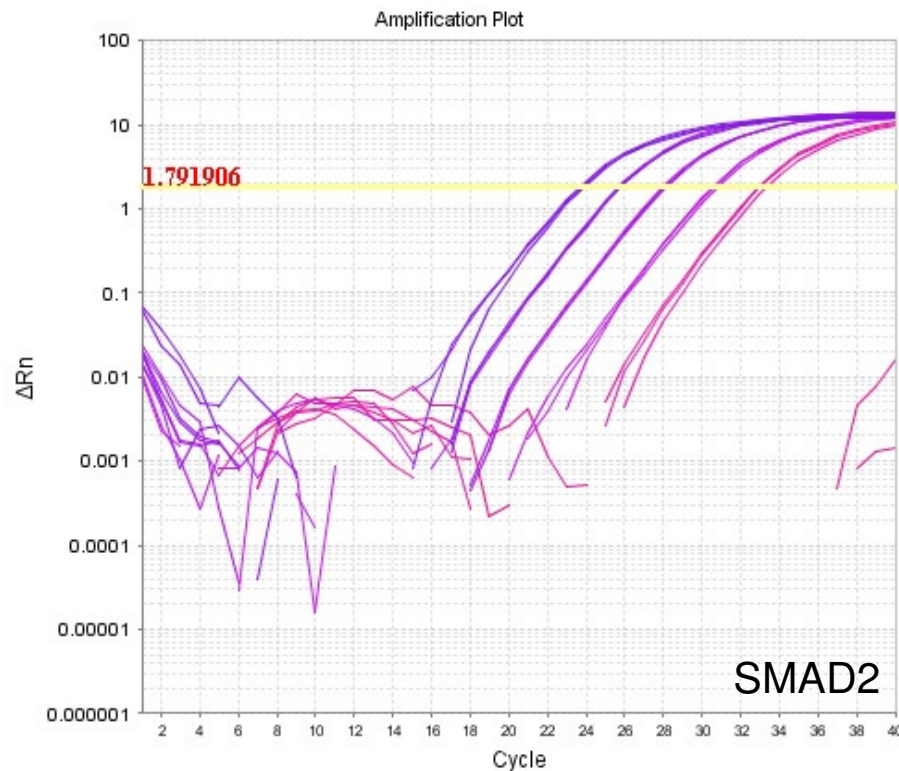
★ Primer concentration for SYBR Green Chemistry:
400nM/400nM (Forward/Reverse)

★ Melt curve analysis



Primer validation and optimization

- ★ Efficiency optimal efficiency: 100% (90-110% acceptable)
5x5 serial dilutions of normal gDNA, $R^2 > 0.99$, slope: -3.32



slope: -3.374, R^2 : 0.997, Eff%: 97.872

Normal Range

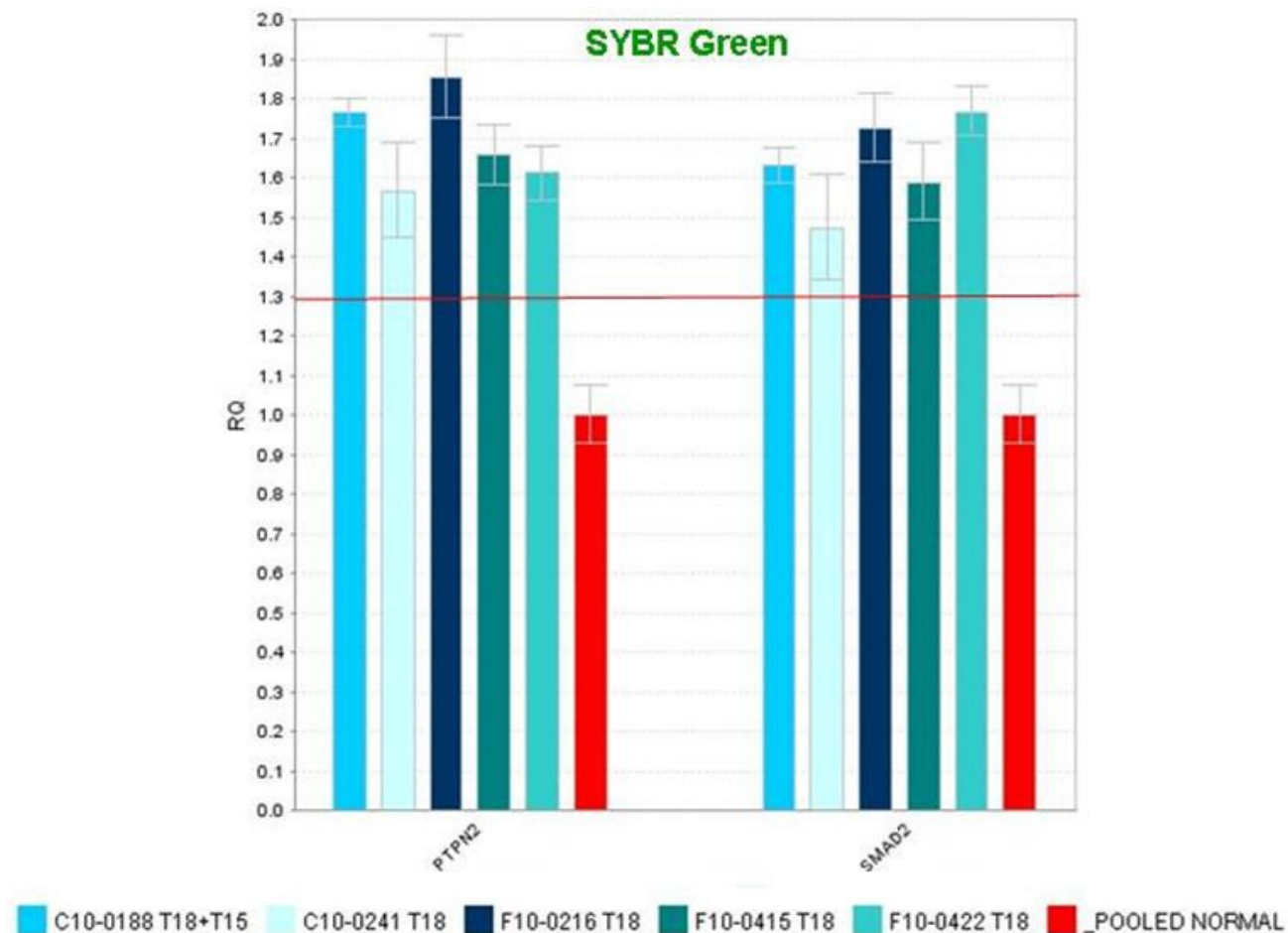
- ★ 24 cases normal on arrayCGH
- ★ ACTBL2, MANEA and TEAD1 used as multiple endogenous controls and ACTBL2-S as test assay
- ★ RQ range: 0.77 - 1.33
- ★ assuming a normal distribution, 95.4% of the RQ data is included within 2SD (0.23) from the average (1.01)
 $1.01 \pm 0.23 \Rightarrow 0.78 - 1.24$
- ★ Majority of results (72%) between 0.9-1.1

Prenatal

- ★ T13 (CDC16, IPO5)
4 prenatal samples (2 AFs, 2 CVSs) tested
- ★ T15 (UBE3A, SNRPN)
1 prenatal sample (1 CVS) tested
- ★ T18 (PTPN2, SMAD2)
5 prenatal samples (3 AFs, 2 CVSs) tested
- ★ T21 (STCH, C21orf59)
5 prenatal samples (2 AFs, 3 CVSs) tested

Prenatal Trisomies

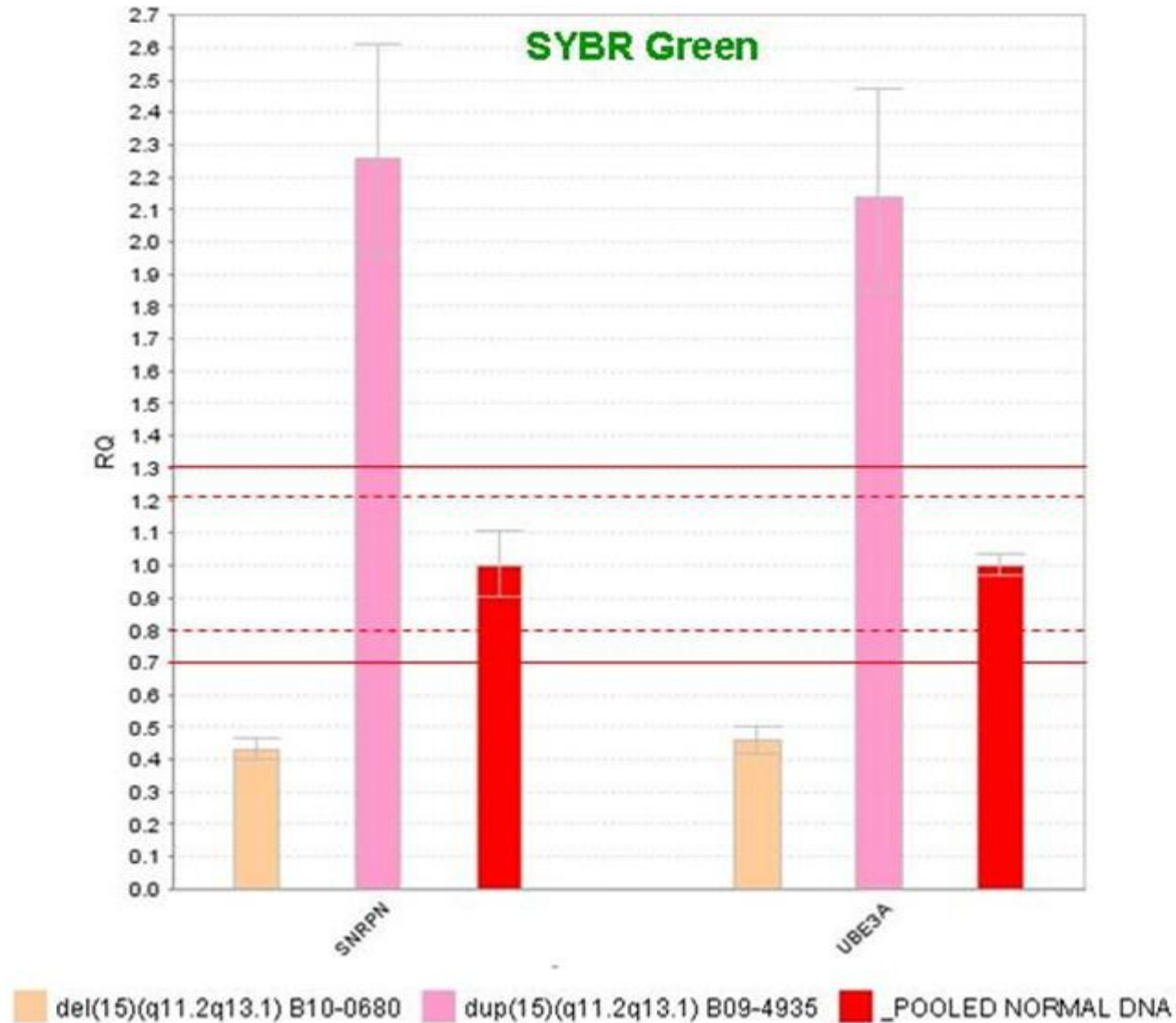
- ★ The trisomy was picked-up in all known abnormal samples tested.



Postnatal

- ★ 5 cases presented on arrayCGH with common syndromes (PWS/AS and VCSF regions microdeletions and microduplications)
- ★ 25 family studies (proband with unknown significance copy number change on arrayCGH, father and mother)
- ★ 9 cases presented on arrayCGH with other abnormalities (e.g. +i18p, del18p, r18, T21, Turners)

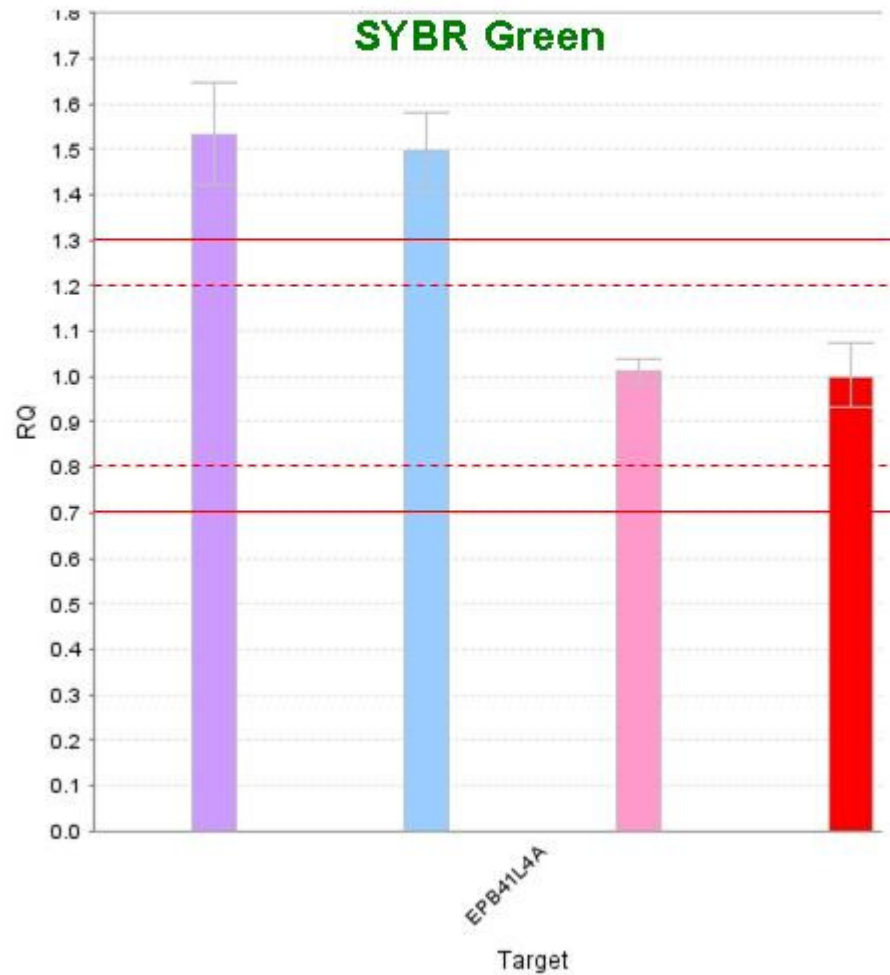
PWS/AS region micro-deletion/duplication



Family studies

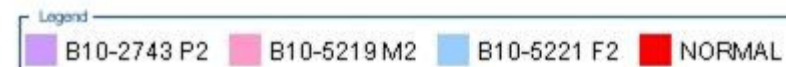
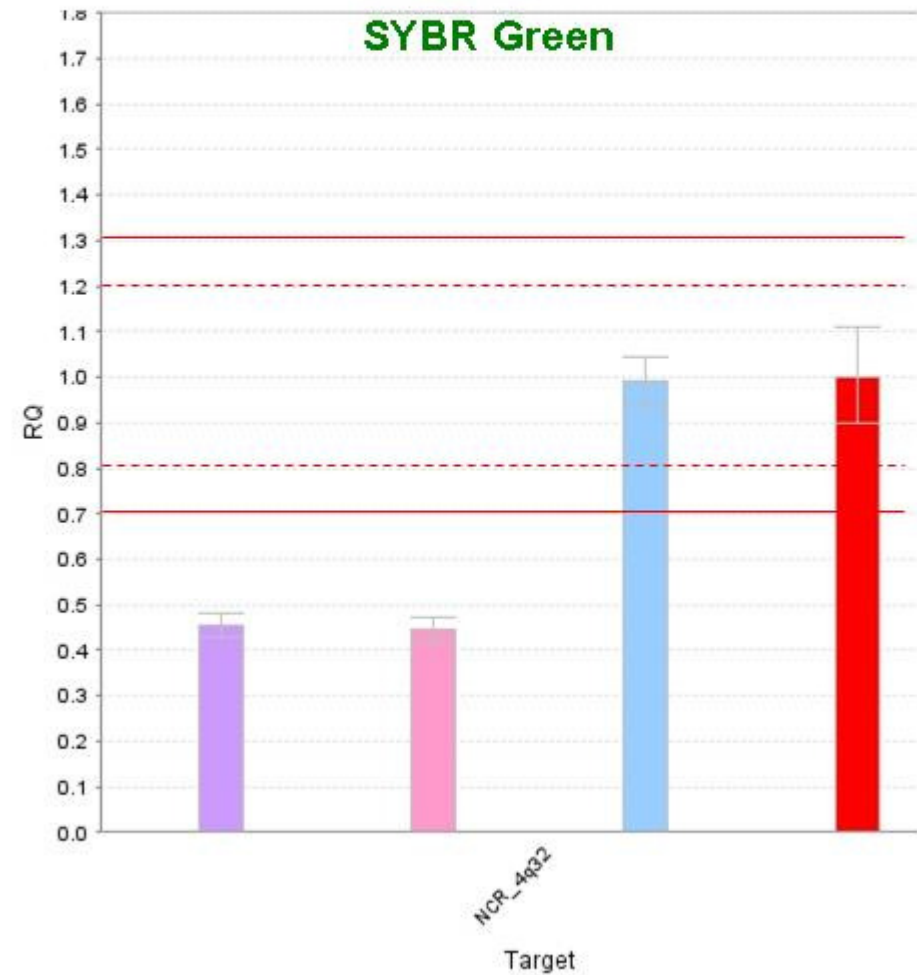
Family1

arr 5q22.1q22.2(111,306,880-112,566,440)x3



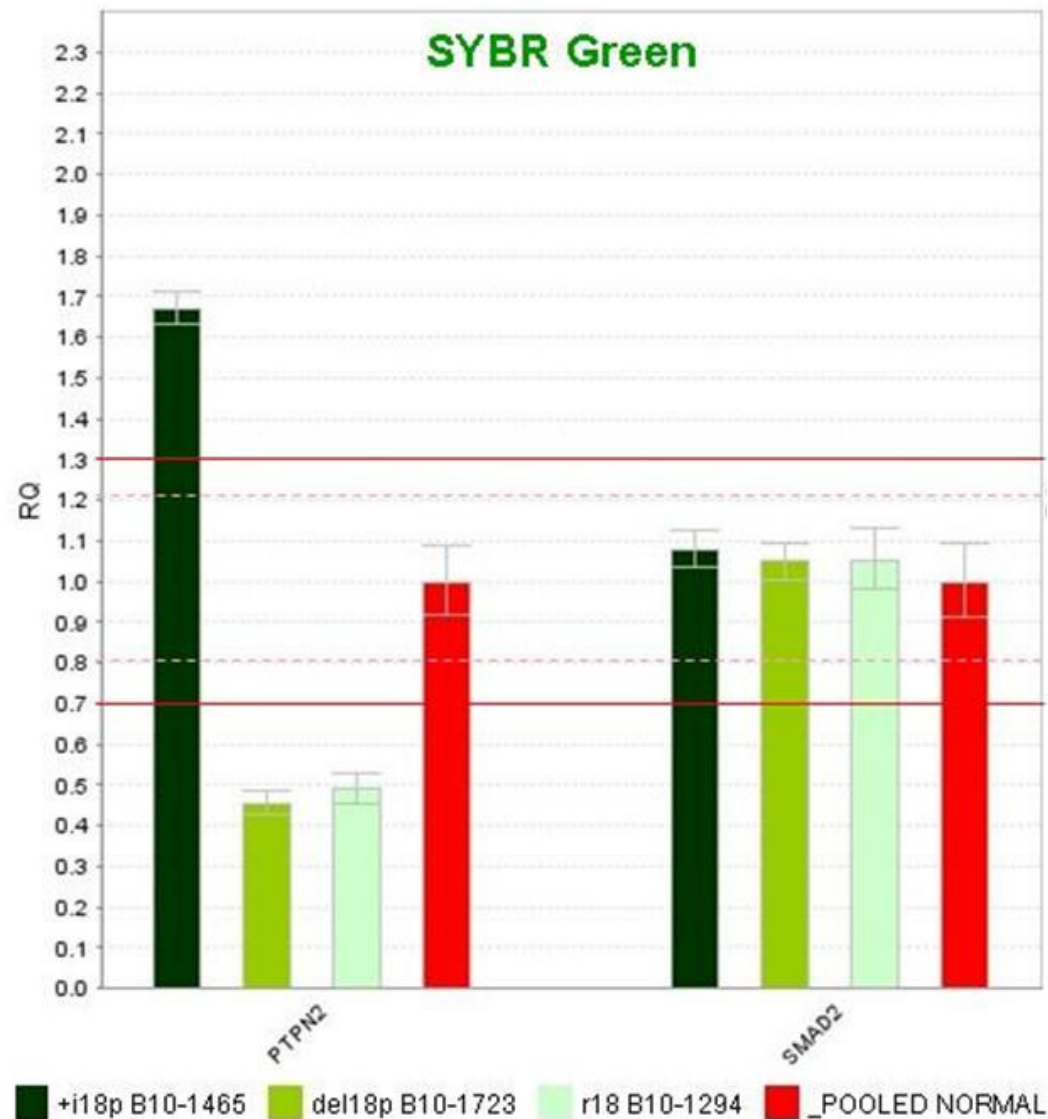
Family2

arr 4q32.3(167,687,942-167,856,796)x1



chr.18 abnormalities

★ PTPN2 - 18p11.21 and SMAD2 - 18q21.1 assays

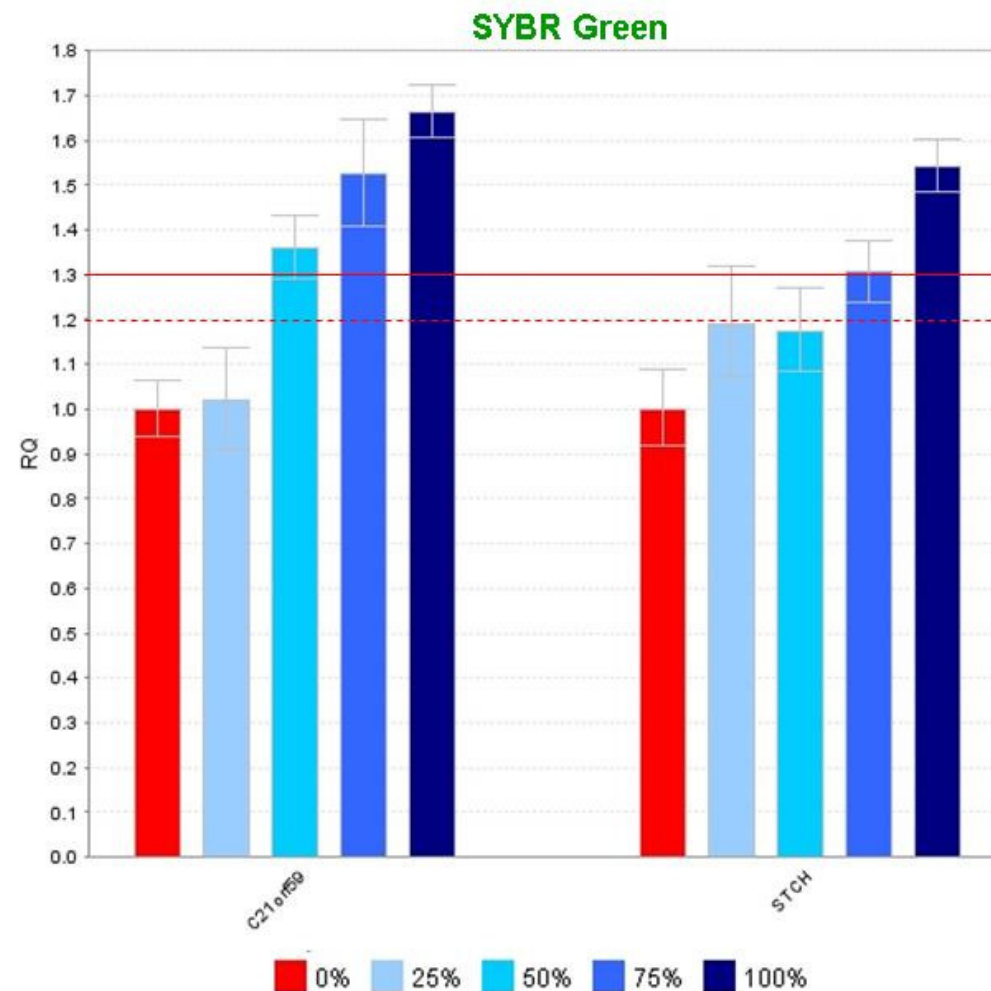


Postnatal

- ★ qPCR confirmed and was consistent with the microarray and/or FISH result in all unique cases and family studies.

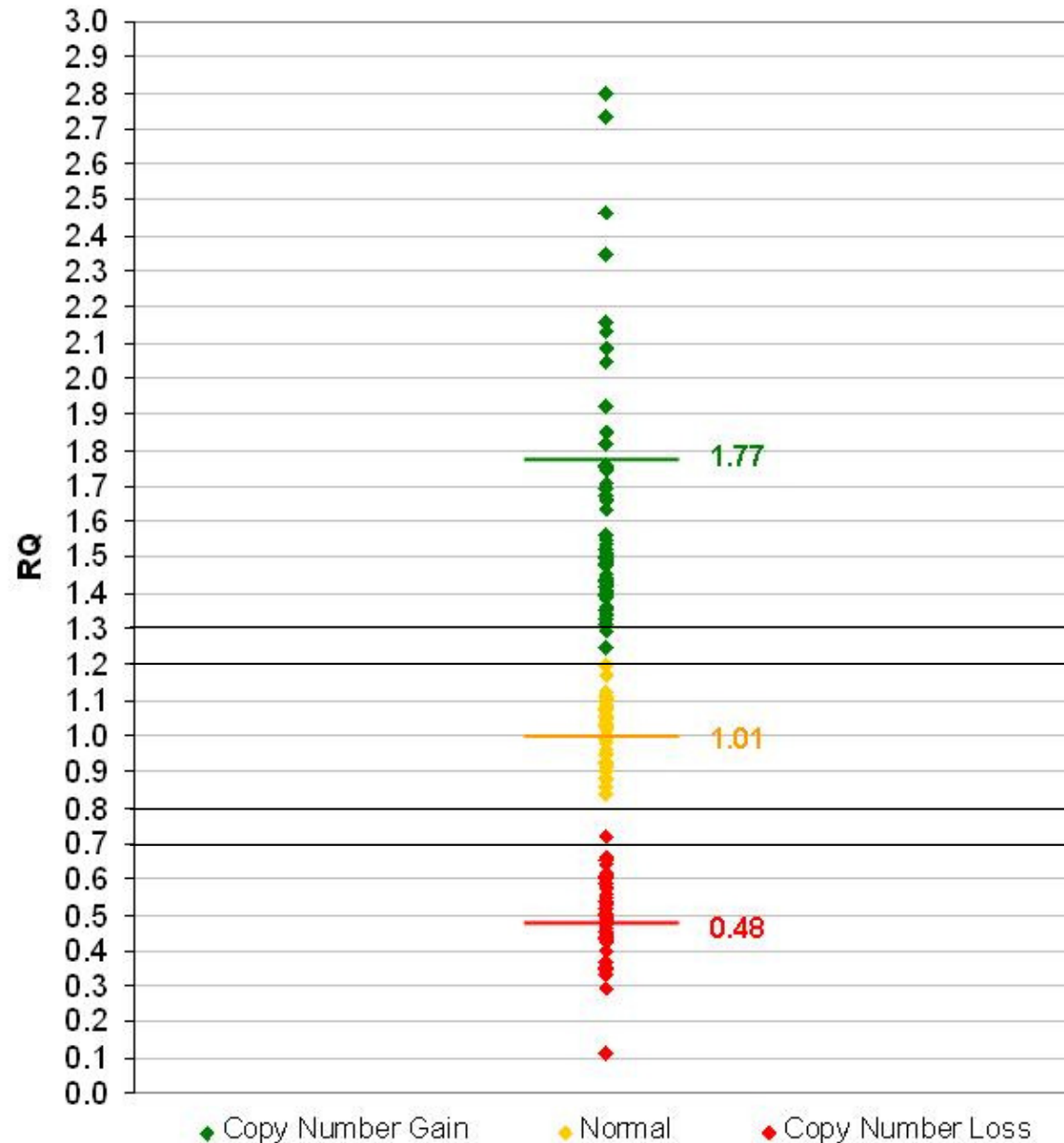
Mosaicism

- ★ Different levels of T21 mosaicism (0%, 25%, 50%, 75%, 100%) were made by mixing different amounts of a normal and a T21 sample.



Abnormal Range

Relative Quantification



Proposed RQ ranges:

normal	0.8-1.2
loss	<0.7
gain	>1.3
inconclusive	0.7-0.8 and 1.2-1.3

=> 2% of samples
would need a repeat
qPCR test

Conclusions

- ★ qPCR is an accurate and robust technique for the detection of copy number changes

- ★ Proposed RQ ranges:

normal	0.8-1.2
copy number loss	<0.7
copy number gain	>1.3
inconclusive	0.7-0.8 and 1.2-1.3

Conclusions

★ Cost-effective

Test	Indicative cost
1 family - 1 assay	£30
1 family - 2 assay	£38
2 families - 1 assay / family	£50

★ For more accurate and reliable results

- triplicates
- at least two endogenous controls assays (ACTBL2, MANEA, TEAD1)
- two reference DNA samples (Normal, Pooled) and
- more than one assays (depending on the size of the locus of interest)

should be used

Acknowledgements

All North East Thames Regional
Genetics Laboratory Staff

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QUESTIONS?