ø29 polymerase WGA

The end of large scale sample preparation?

Whole Genome Amplification

PCR: only one specific genomic sequence is the target

WGA: amplify all DNA sequences in a sample (sample might be a clone or a genome)

Permit multiple PCR analysis on very small samples –Guthrie spots or single cells (Genomiphi)

Rationale

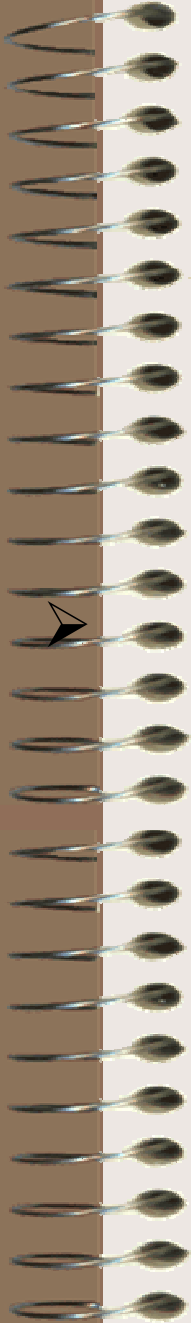Make large amounts of DNA from limited starting quantities.

Template DNA should be high molecular weight (>2kbp).

Uses DNA polymerase from ø29 (*B. subtilis* 'phage) that is proofreading and highly processive.

The final product is double standed with an average size of over 10kbp. Over 100,000 fold amplification is claimed, with the retention of original allele distribution.

Famous last words?

In summary, our results show that ϕ29MDA DNA provides a highly accurate and comprehensive representation of the unamplified **genome**, suitable for high resolution genetic analysis, including SNP genotyping, gene copy number detection and direct sequencing. Thus, it is now reasonable to expect that such technologies may be widely applied on a **genome** scale to primary clinical samples.

Diagnostically useful WGA must fulfil 2 criteria:

1. Increase the total amount of DNA sequences by several orders of magnitude

2. Unbiased amplification (all of the sequences in a sample should be amplified to the same extent)

This presentation

- Review the process

- Summarise Dean *et al* findings

- Put in context of other recent papers

- Show WGA results from Leeds

- Make case for QA of WGA

- Conclude with view on current state of play and ways forward for WGA

Strand Displacement Amplification (SDA) using ø29 polymerase

SDA is isothermic (all reaction stages are carried out at the same temperature)

ø29 has unique biochemical properties, proofreading, high processivity and strand displacement activities

Random modified hexamer primers anneal to the template DNA at multiple sites

As synthesis proceeds, strand displacement of complementary DNA generates new single stranded DNA serving as additional templates

Random Sequence Hexamers

DNA sample

Denature and Hybridize

Add dNTPs and φ29 DNA Polymerase Incubate at 30°C
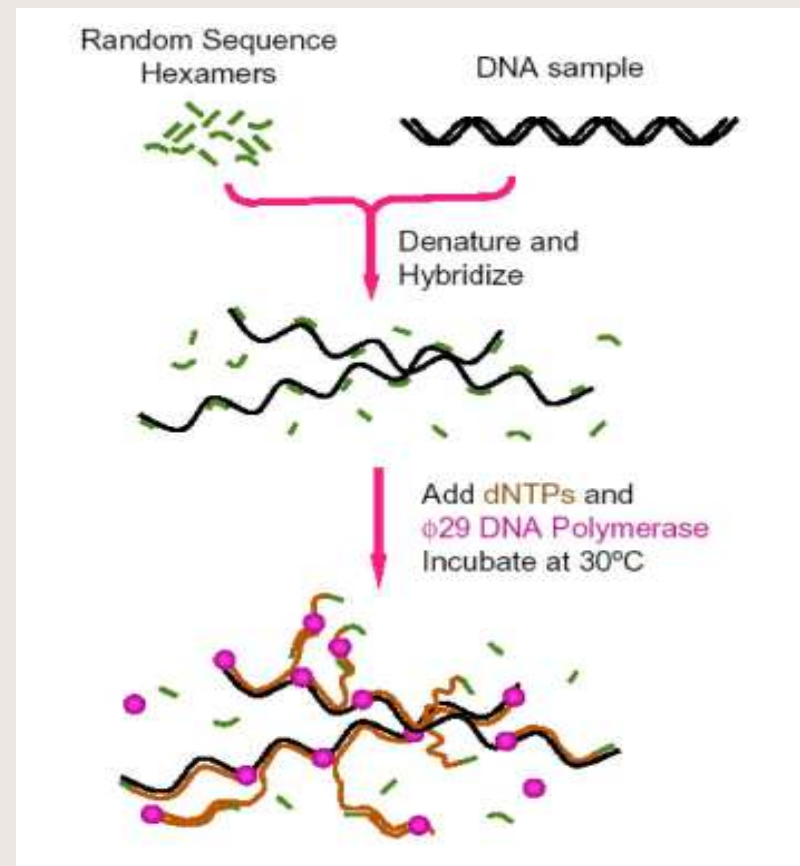
Universal gDNA Amplification

Multiple Displacement Amplification

Genomic DNA
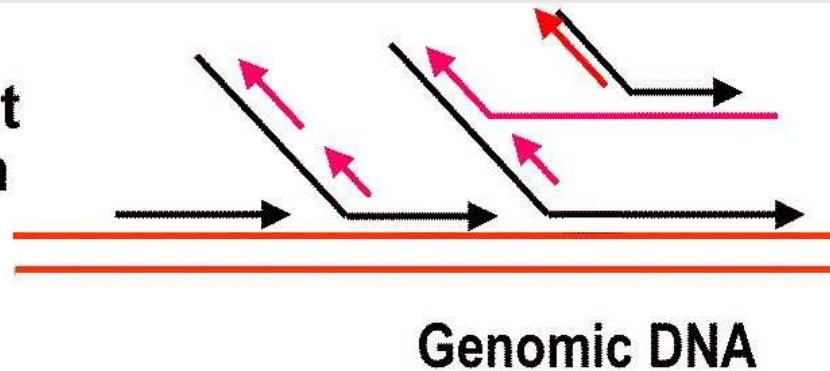
What makes it work?
1. ER Random Hexamers (5'-NpNpNpNpsNpsN-3')
2. Phi29 DNA Polymerase
   High fidelity, 3'-5' exonuclease proofreading
   Strand Displacing
3. Isothermal Reaction (30°C)

Comprehensive human genome amplification using multiple displacement amplification

Frank B. Dean*, Seiyu Hosono*, Linhua Fang, Xiaohong Wu, A. Fawad Faruqi, Patricia Bray-Ward, Zhenyu Sun, Qiuling Zong, Yuefen Du, Jing Du, Mark Driscoll, Wanmin Song, Stephen F. Kingsmore, Michael Egholm, and Roger S. Lasken

Molecular Staging, Inc., 300 George Street, Suite 701, New Haven, CT 06511

Quantitative PCR Analysis.

- TaqMan analysis used the Applied Biosystems 7700 with 1 µg of amplified DNA as template. Three replicate TaqMan assays were averaged for each of the eight loci for each DNA amplification reaction.

Amplification bias between two loci is the ratio between the two locus representation values. The maximal amplification bias for a group of loci is the ratio between the locus representations for the two loci with the highest and the lowest locus representations.

Bias Study

0.3 – 300 ng
Genomic DNA

No amplification   DOP   PEP   MDA

TaqMan analysis for copy number of 8 loci
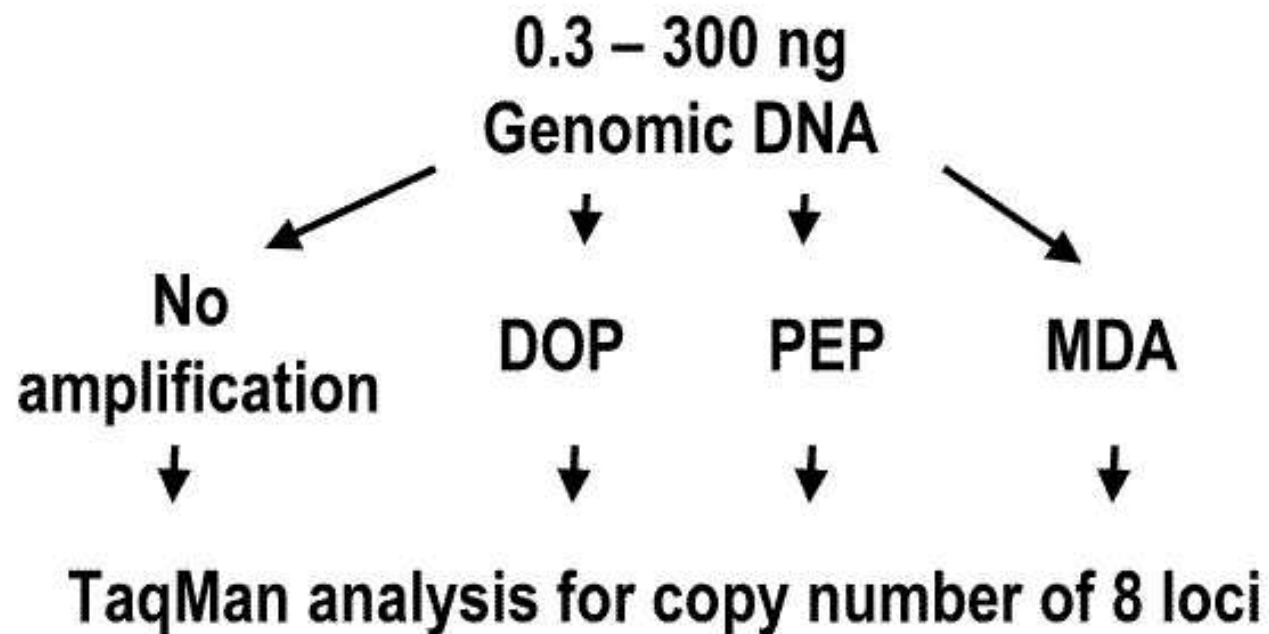
DOP: Degenerate Oligonucleotide Priming
PEP: Primer Extension Pre-amplification

TaqMan Quantification of 8 Loci
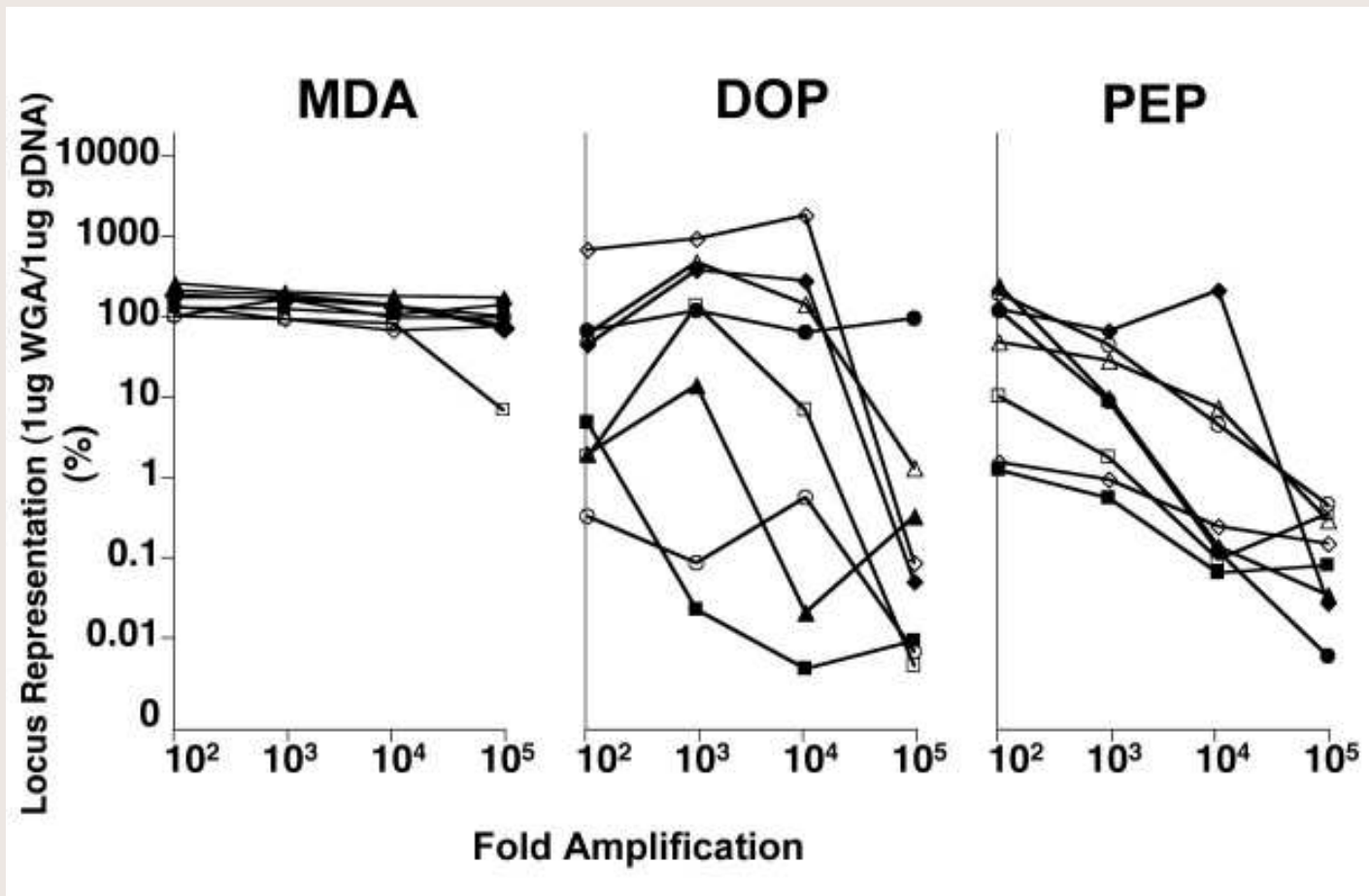
Note the log scale!

Southern blotting

RFLP Analysis on 10,000X DNA

gDNA WGA
5.8 kb
5.2 kb
3.0 kb
1.0 kb

Probe for the Thyro-globulin gene (8q24)

AB BB BB AB BB AB
3.8 kb
2.1 kb
1.1 kb

D13S2 (13q22)
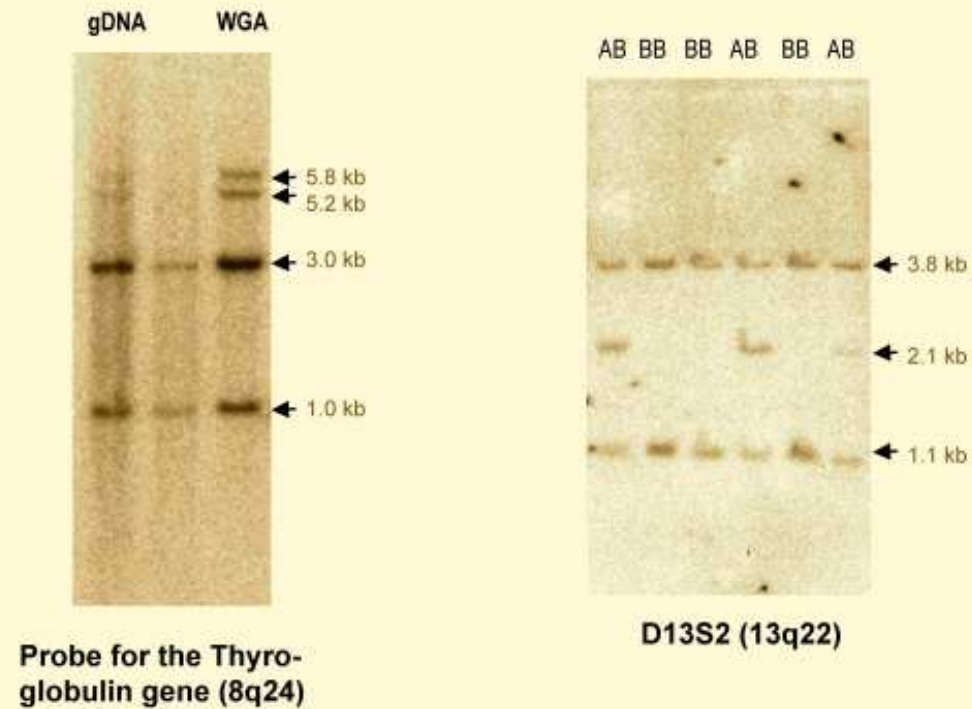
46 Loci Bias Study on 44 Patients

Recent publications

Recent publications

Recent publications

Figure 3. Rare homozygous sequence variations in φ29MDA samples. DNA sequence traces for unamplified (upper) and φ29MDA (lower) samples showing a homozygous 2 bp (CT) insertion and a homozygous C→T substitution (white arrows) detected by NQS analysis. All samples were non-denatured.

Summary of literature

Study	Source	Assay Method	Finding	Conclusion
Lage et al Genome Res 2003	Cell line DNA BT474, "1000 cells, 500 cells"	Array CGH	Detected large gene dosage increases in tumour cell lines. >80% concordance with 1000 cells, but only 50% concordance with 500 cells	Preferred *Bst* pol: possibly capable of template switching
Lovmar et al NAR 2003	3ng DNA	Array capture of minisequencing products	34 SNPs concordant in both directions, 10 concordant in 1 dirn only (=10 fails out of 88)	Better than PEP.
Rook et al Am J Pathol 2004	LCM, 5ng DNA	TaqMan and STR	Relative copy number range 0.46-1.42. Saw LOH, suggested pooling WGAs.	Pooling WGA products increased SNP calling accuracy
Barker et al Genome Res 2004	WGA: 10ng Omniplex: 100ng	Primer extension/ligation Illumina	>99% genotyping concordance, >90% within a factor of 2 of the mean	Immortalisation of DNA in vitro
Mai et al J Clin Pathol 2004	1ng DNA or 1ul whole blood	Sequencing	22/22 concordant	"Suitable for routine clinical testing"
Paez et al NAR 2004	10ng DNA	Affymetrix 10K chip; Xba digest and single primer PCR	89% SNP call rate, but 92% if denatured prior to WGA. Consistent signal loss in 14 genomic regions. No obvious features at these regions. 80% copy number concordance.	May be more underamplified regions seen on higher density chips
Wong et al NAR 2004	10ng	LOH by Affymetrix 10K, ABI linkage mappng set of STRs	Copy number correlation (r^2) 0.77	Can identify regions of LOH using WGA products on 10K chips

Genomiphi products look similar

- High molecular weight products

- May include DNA in -ve control!

- Variation in complexity?

- How can we tell if it is representative?
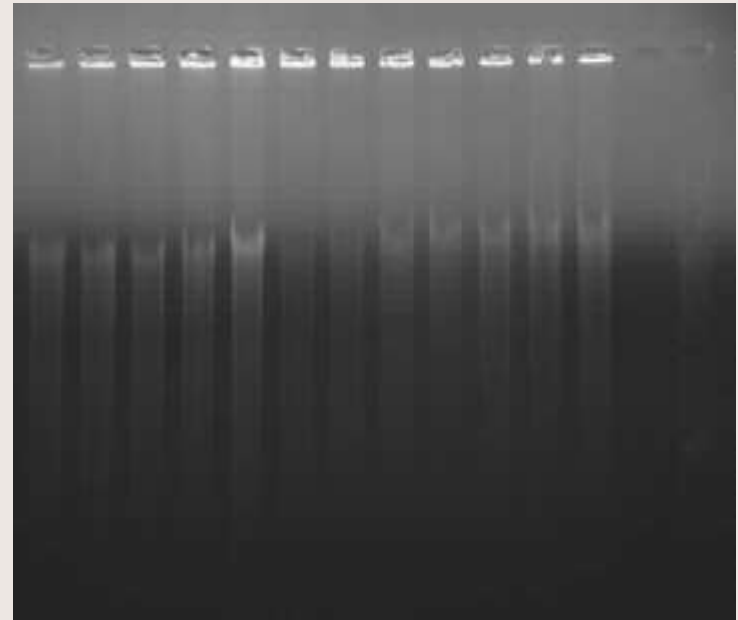
MLPA analysis of WGA products

MLPA carried out on 3 aneuploidy: trisomy 21, XXY & XXYY and 2 controls

+21

XXY

XXYY

Normal control

Normal control
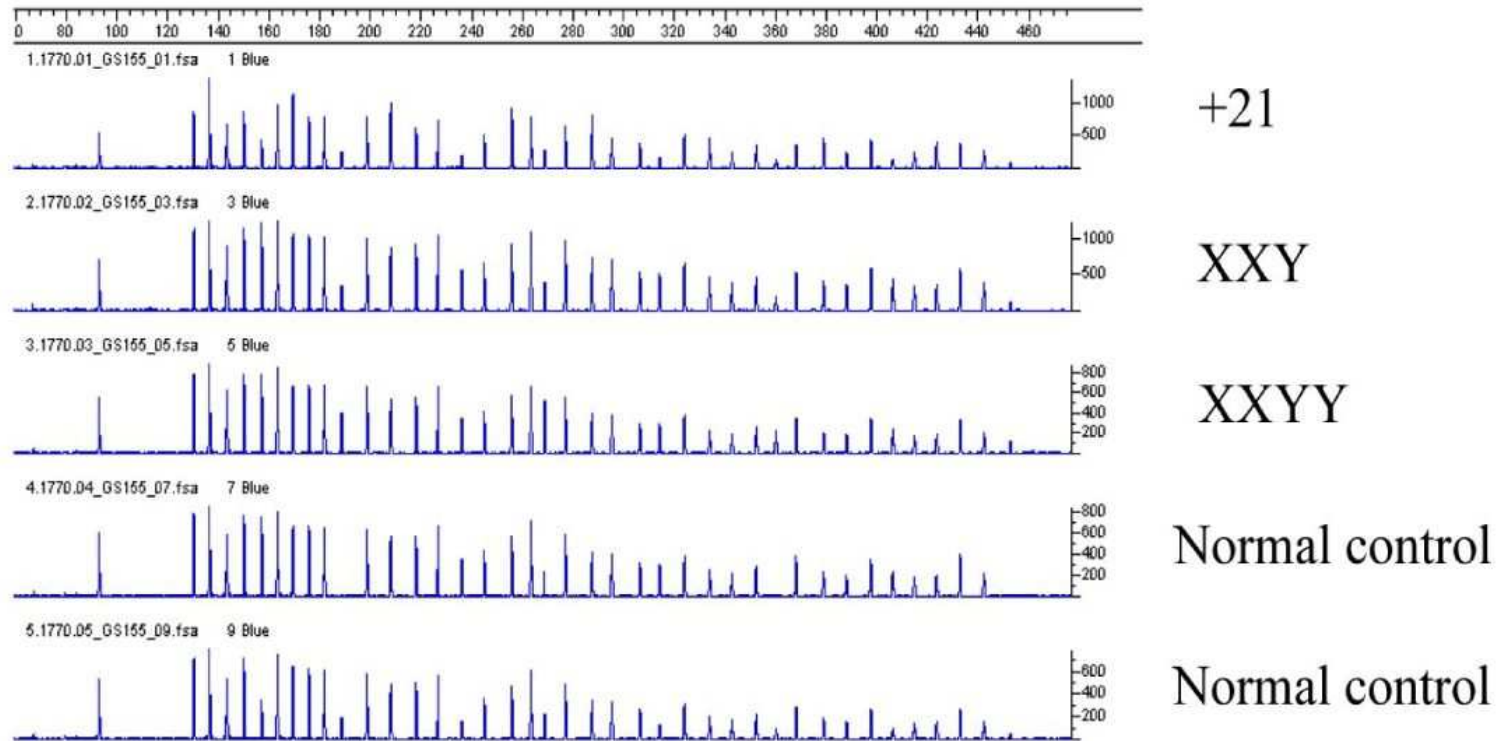
MLPA results on templates
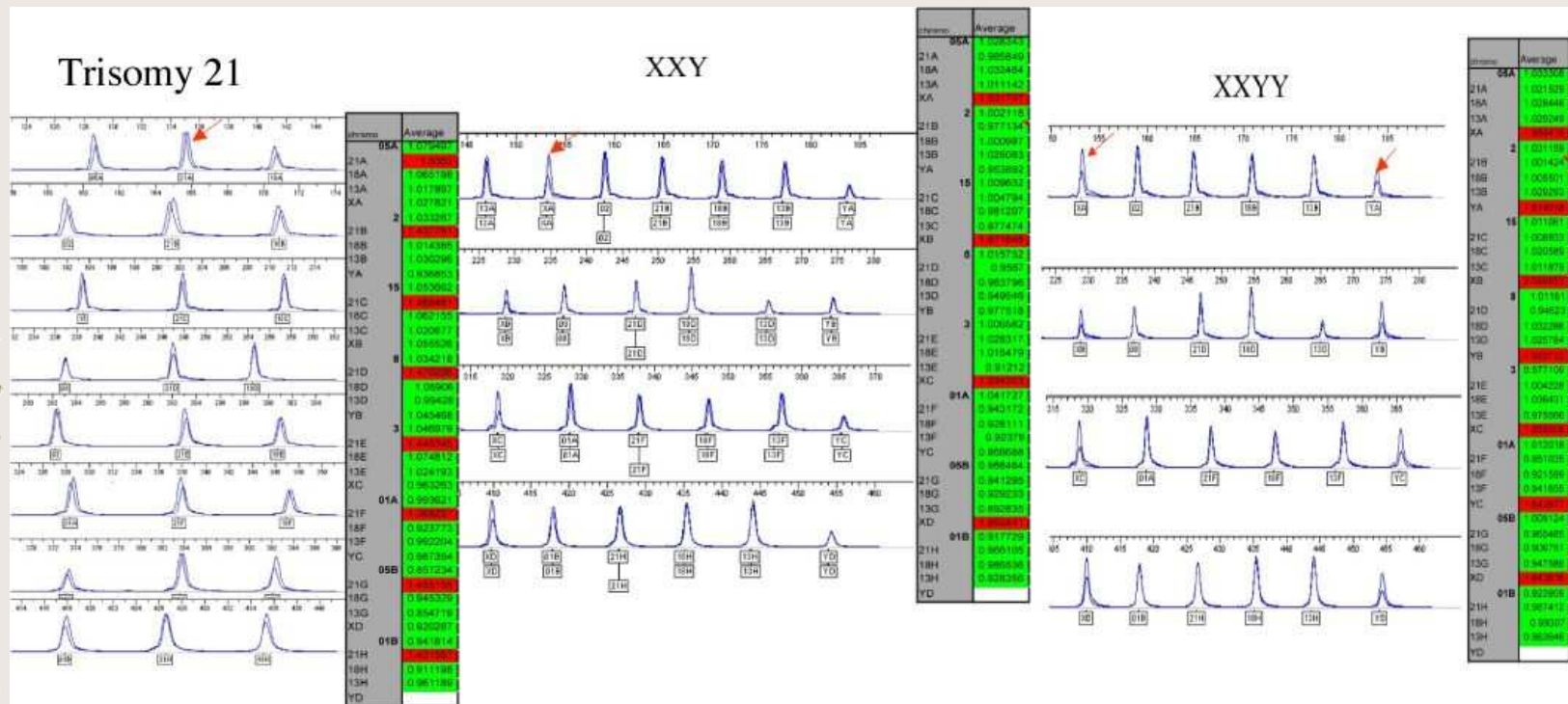
MLPA dosage templates available here:
http://leedsdna.info/downloads.htm

Sample dilutions do not yield MLPA products beyond 1/10 (50 ng)
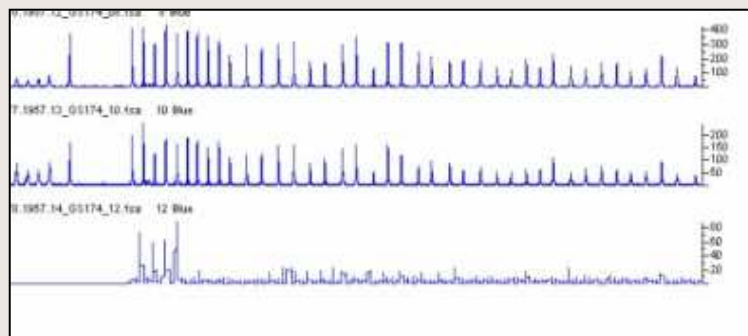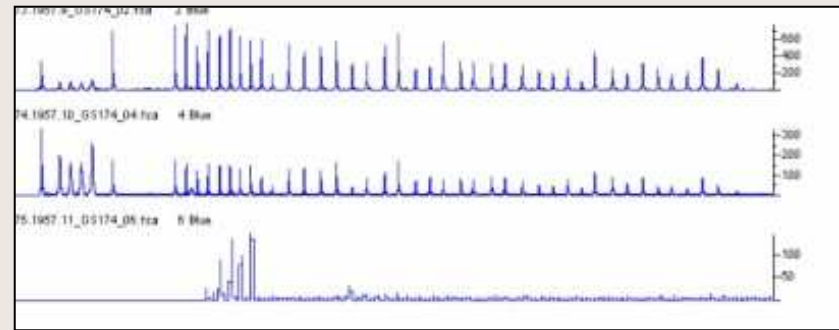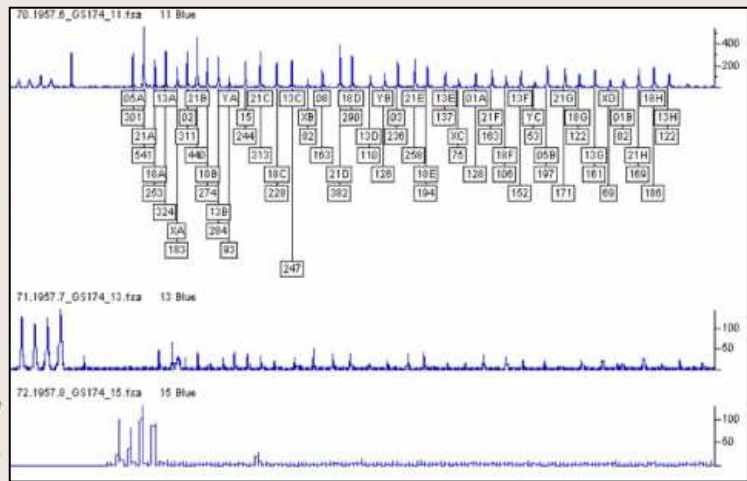
WGA using native or denatured template
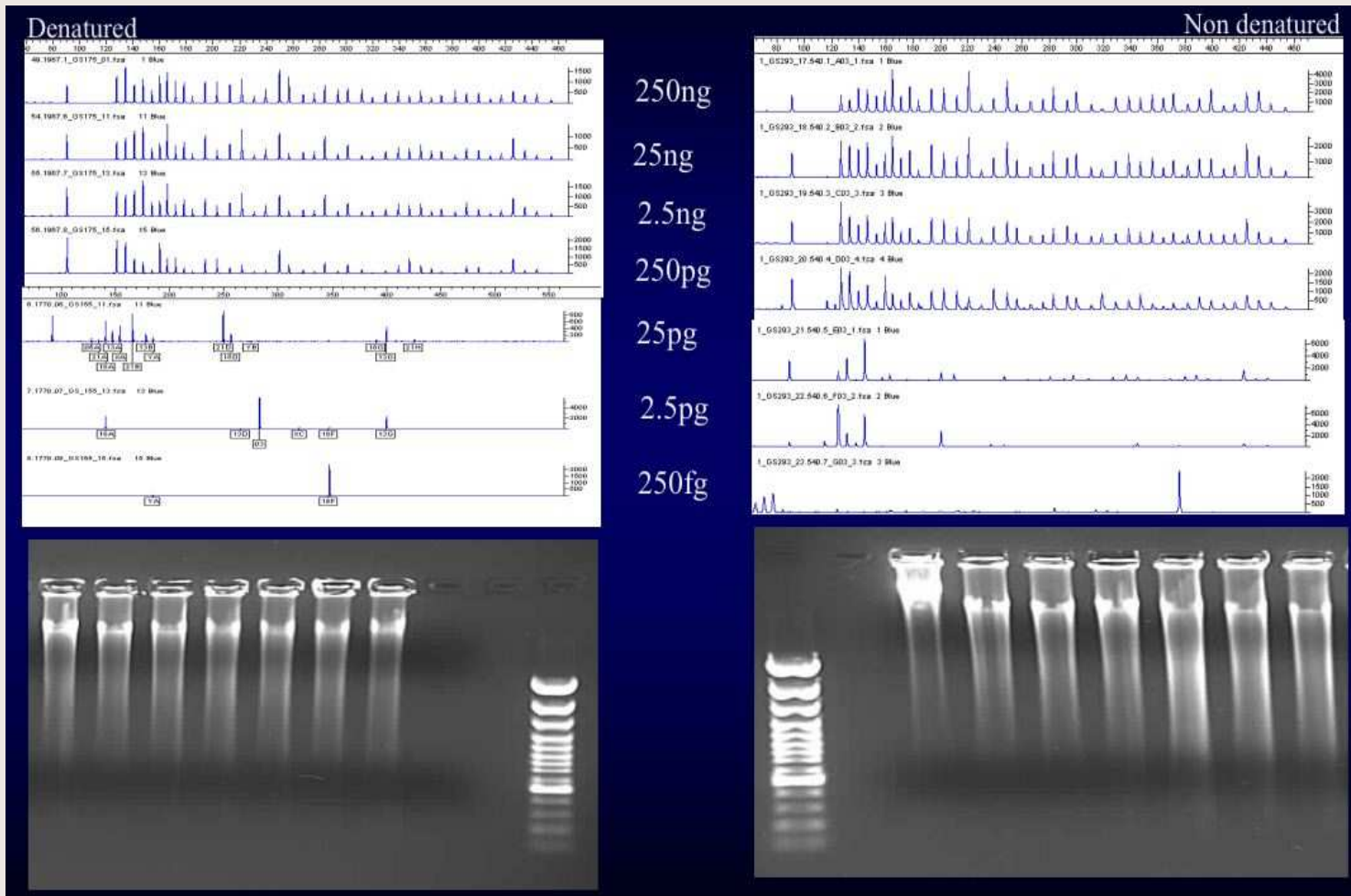
Trisomy 21 WGA MLPA
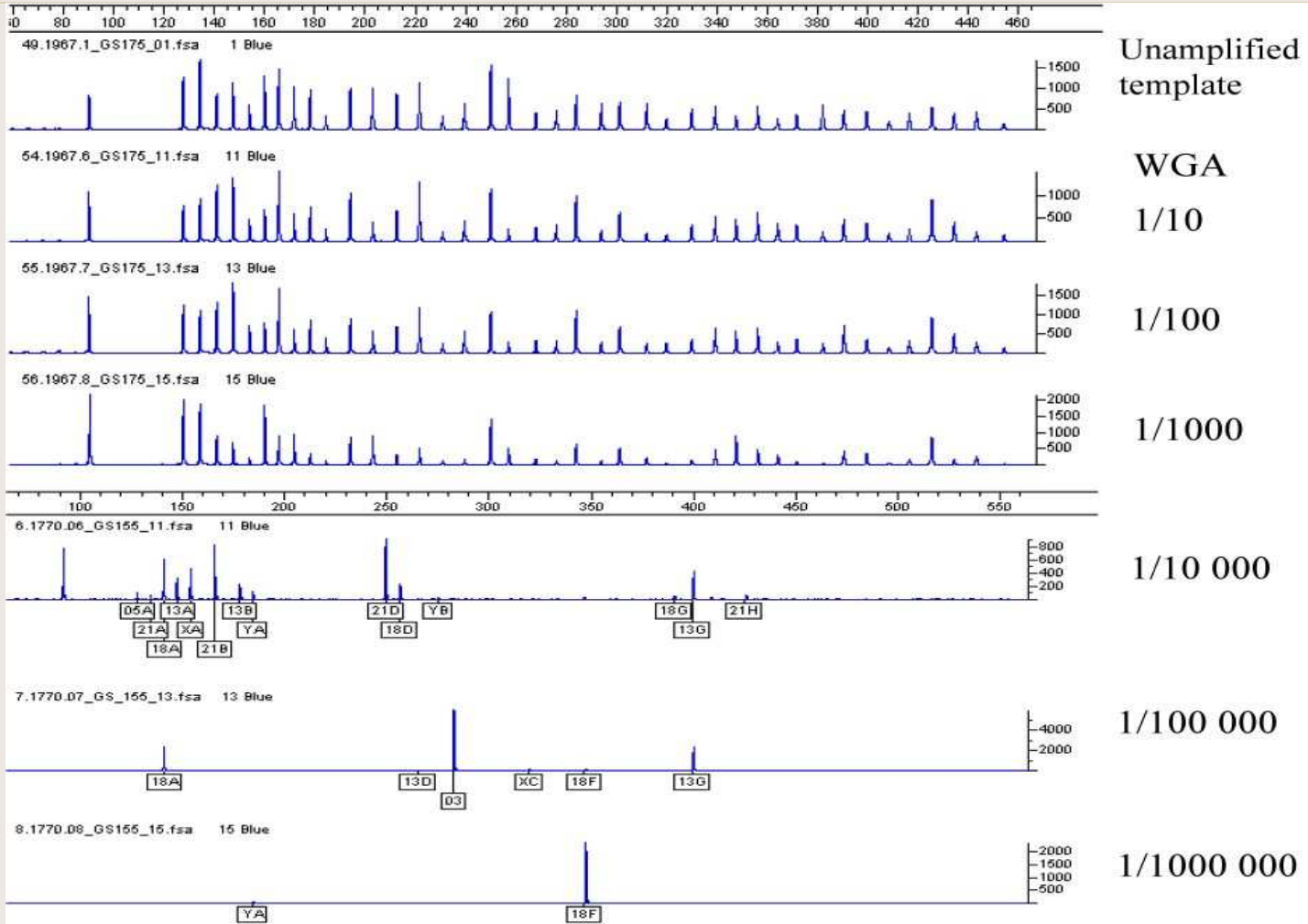
XXY
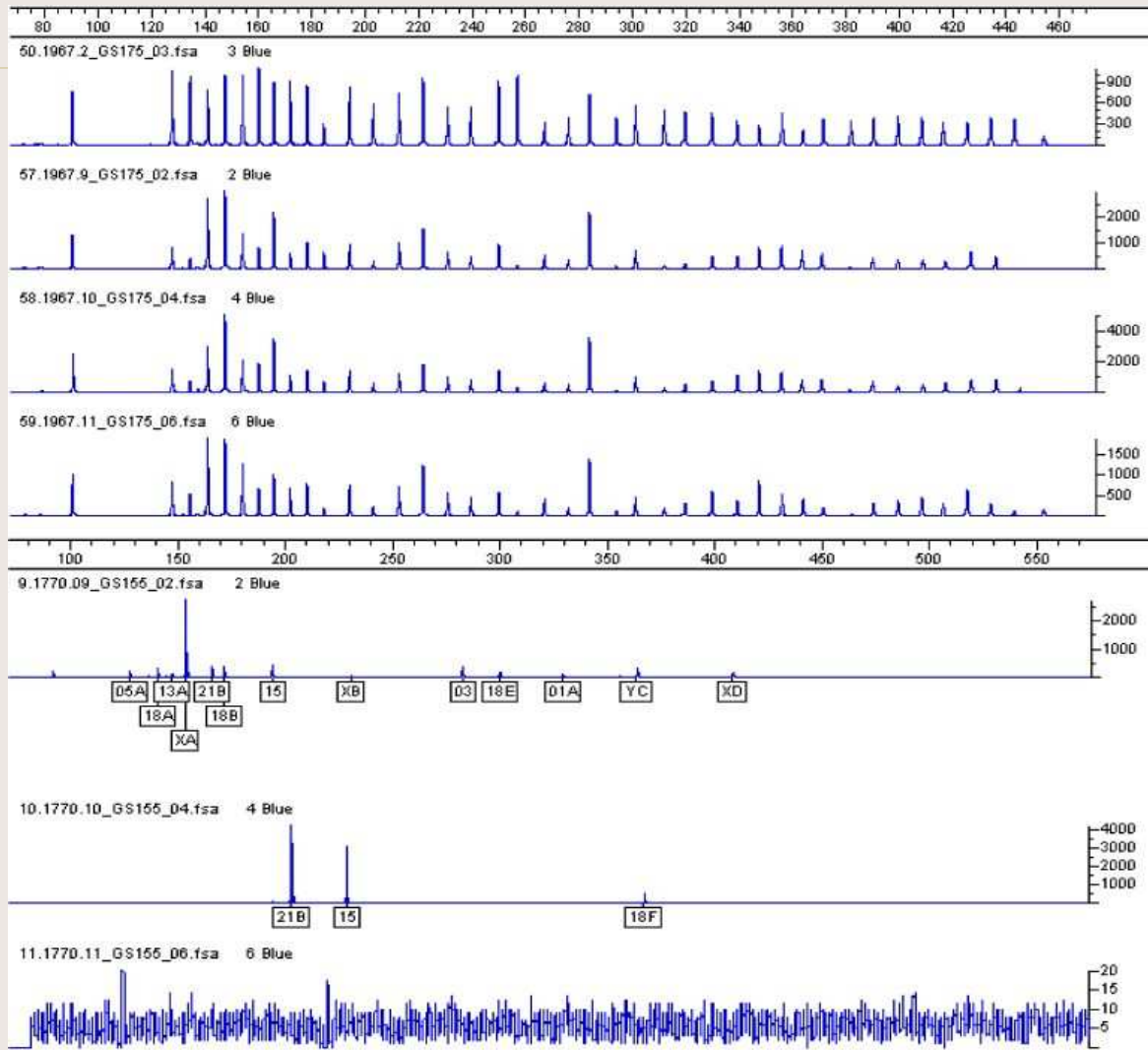
Dosage quotients of MLPA products denatured WGA template

Trisomy 21   XXY   XXYY

Dosage quotients of MLPA products
non denatured WGA template

Trisomy 21   XXY   XXYY

Ethanol Cleanup before MLPA: Trisomy
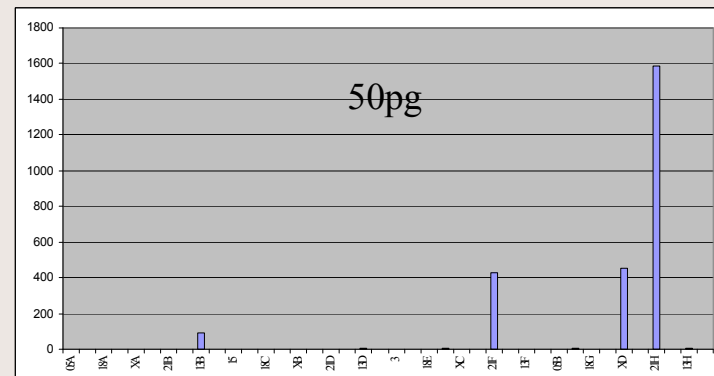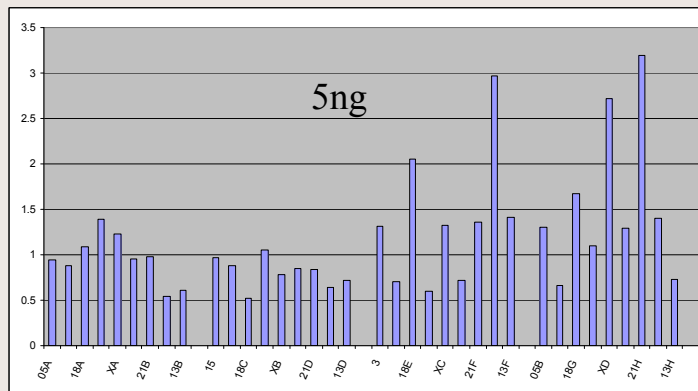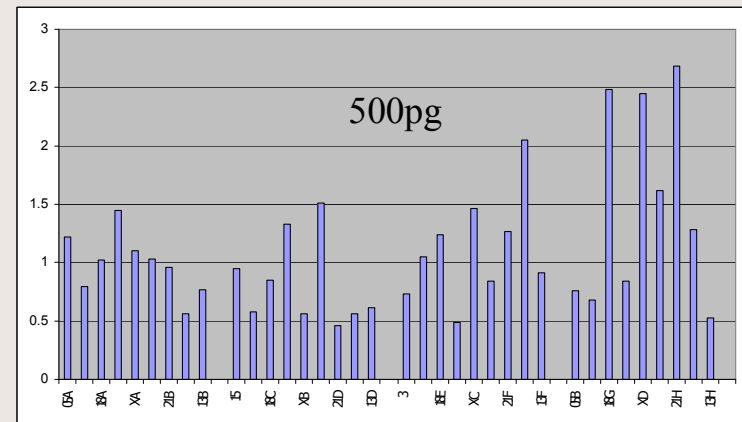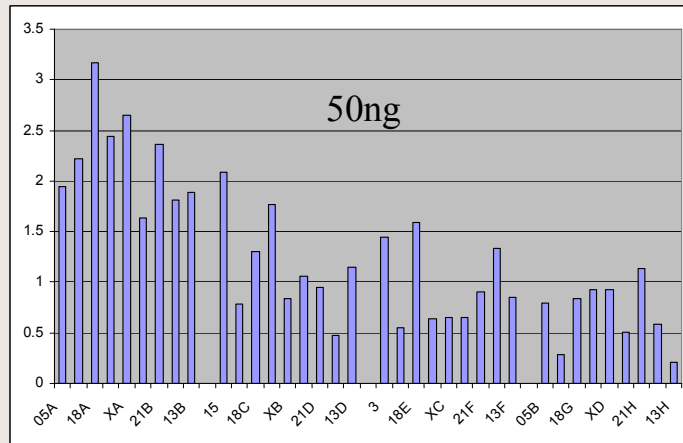
Ethanol Cleanup before MLPA: *MSH2 & MLH1*
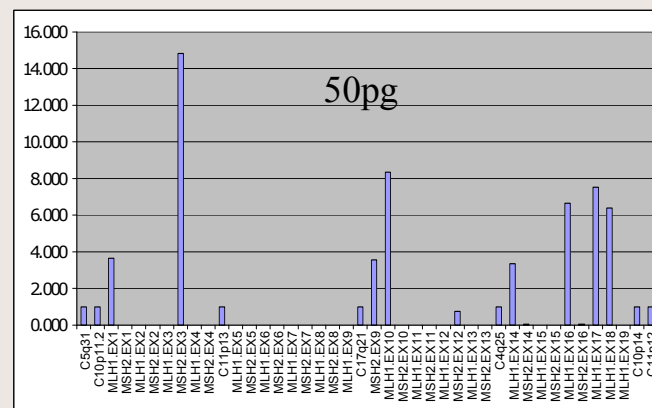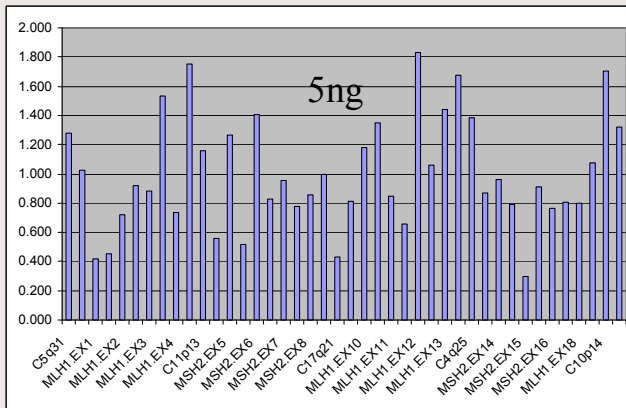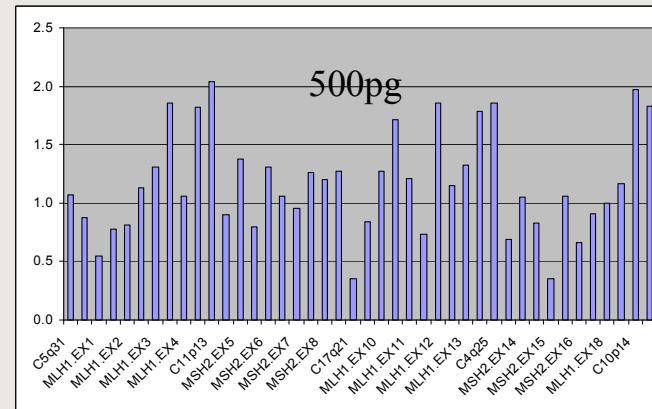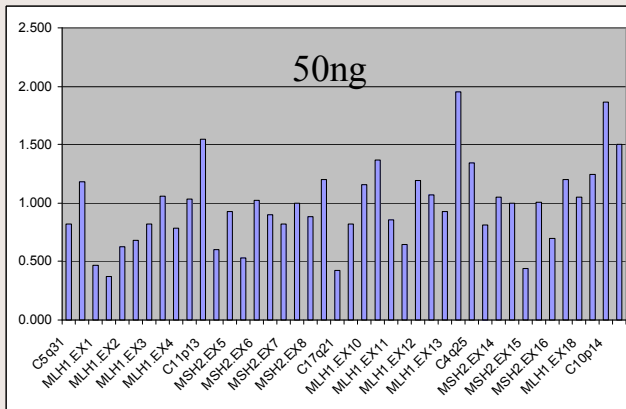
WGA then PCR for sequencing templates
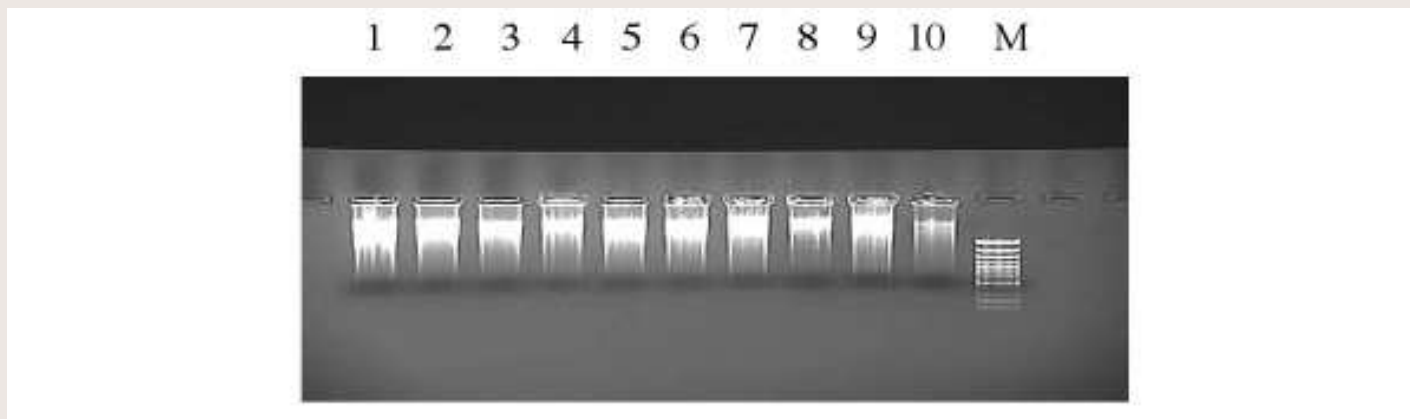
- 1-3 c.332C>T (P67L): 10^{-1}, 10^{-2}, 10^{-3}
- 4-6 c.394delTT: 10^{-1}, 10^{-2}, 10^{-3}
- 7-9 Wild type: 10^{-1}, 10^{-2}, 10^{-3}
- 10 -ve

394delTT 1/10 *vs* genomic
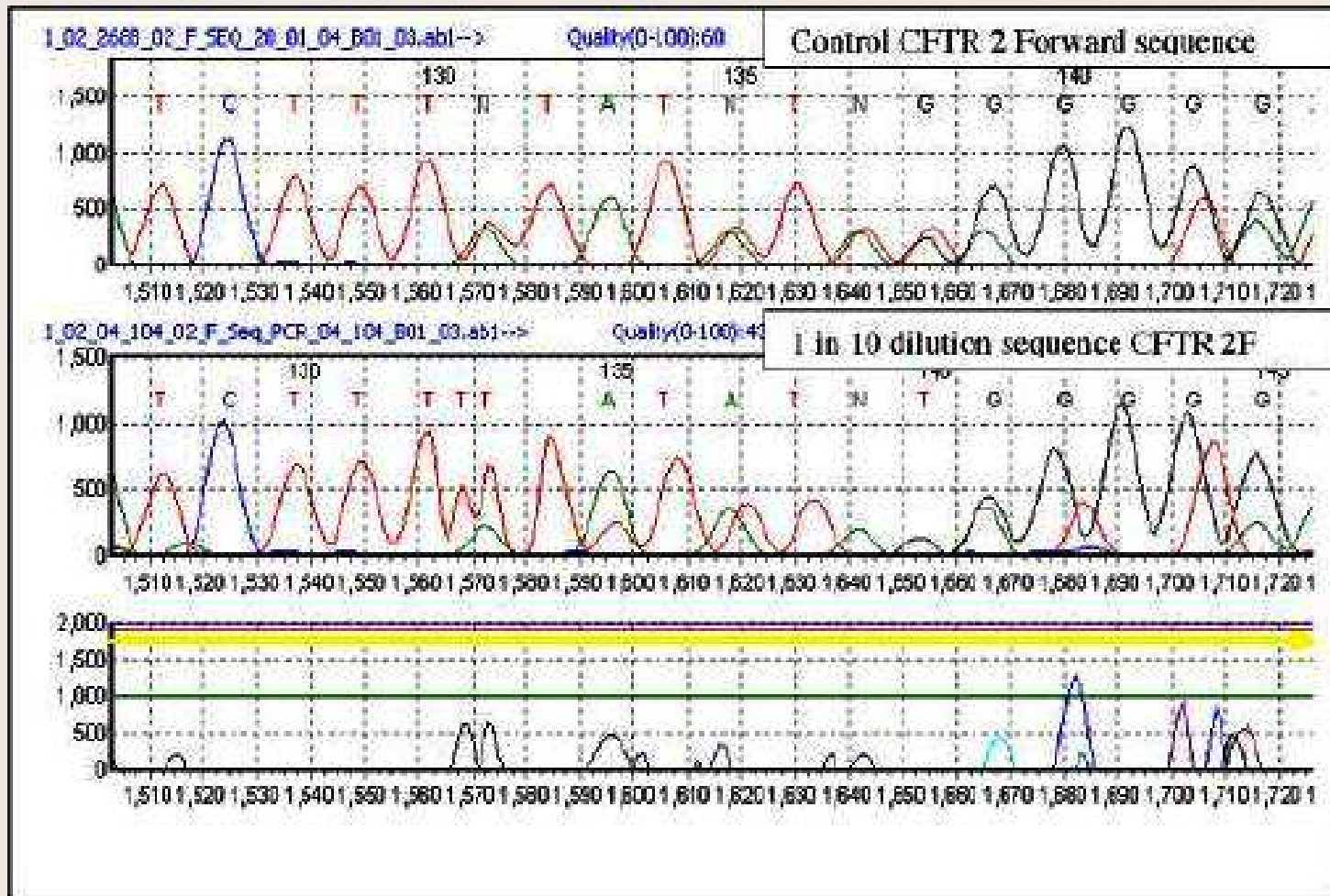
394delTT : 1/100 *vs* genomic
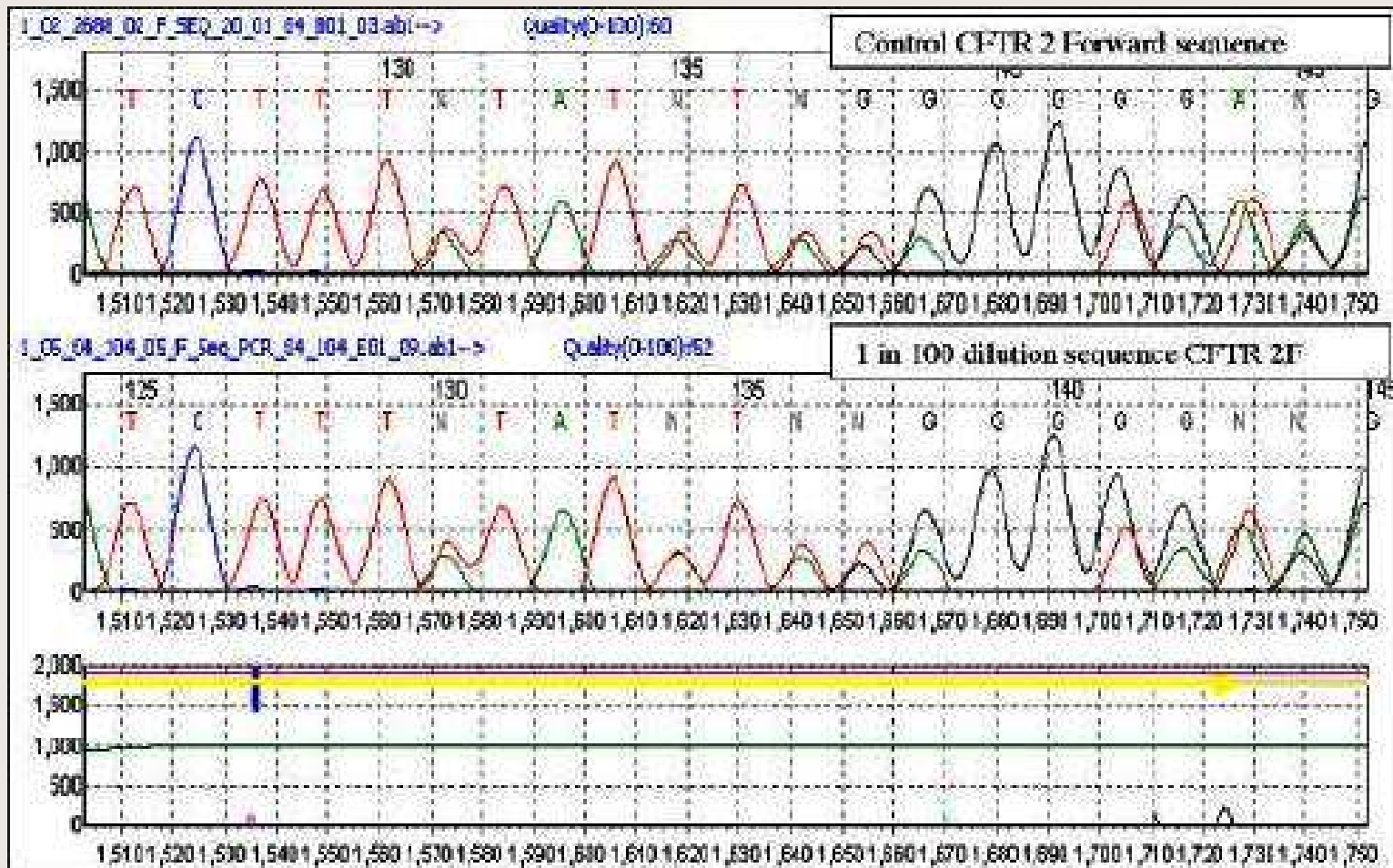
394delTT 1/1000 *vs* genomic
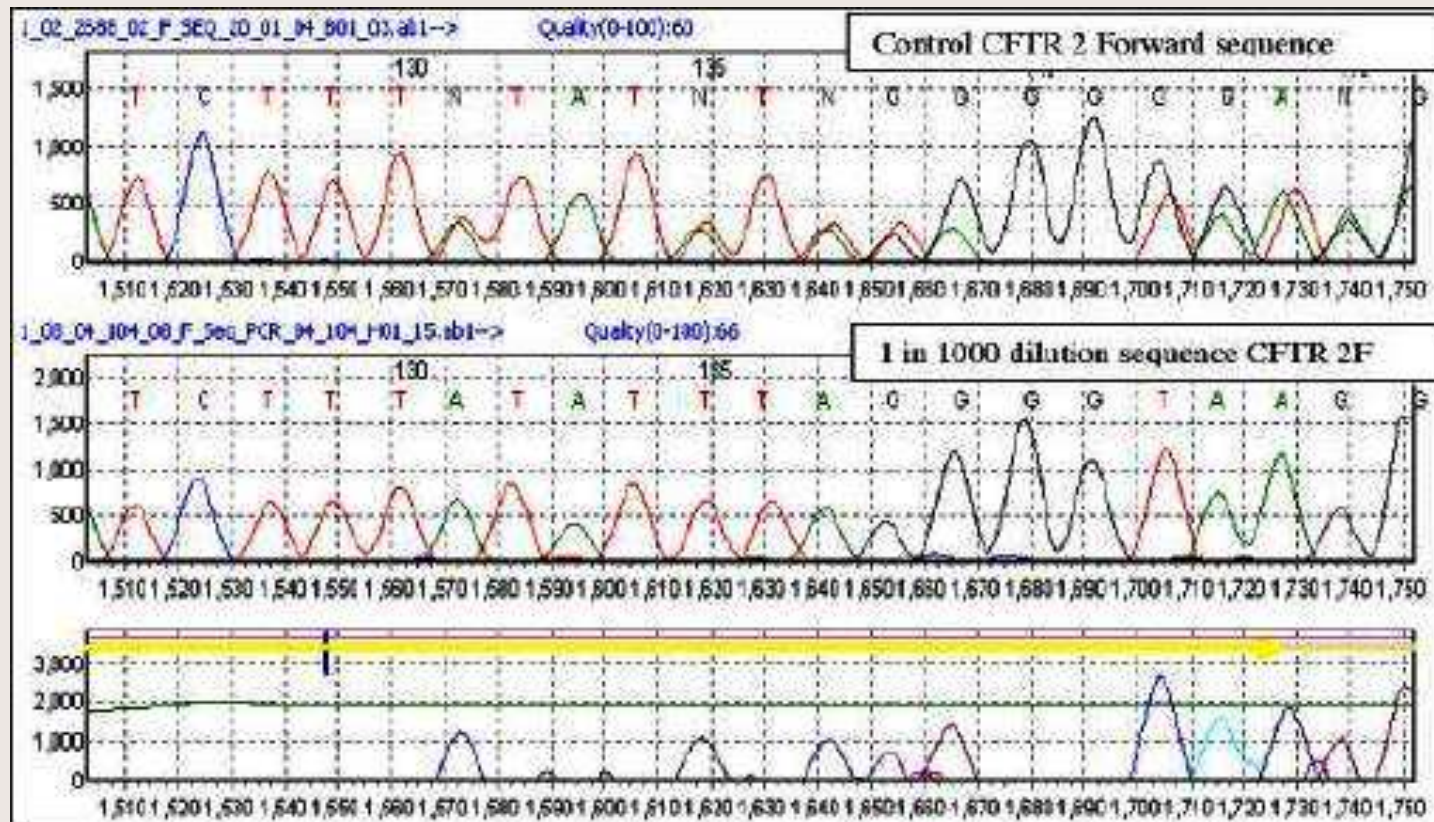
332C>T 1/10 *vs* genomic
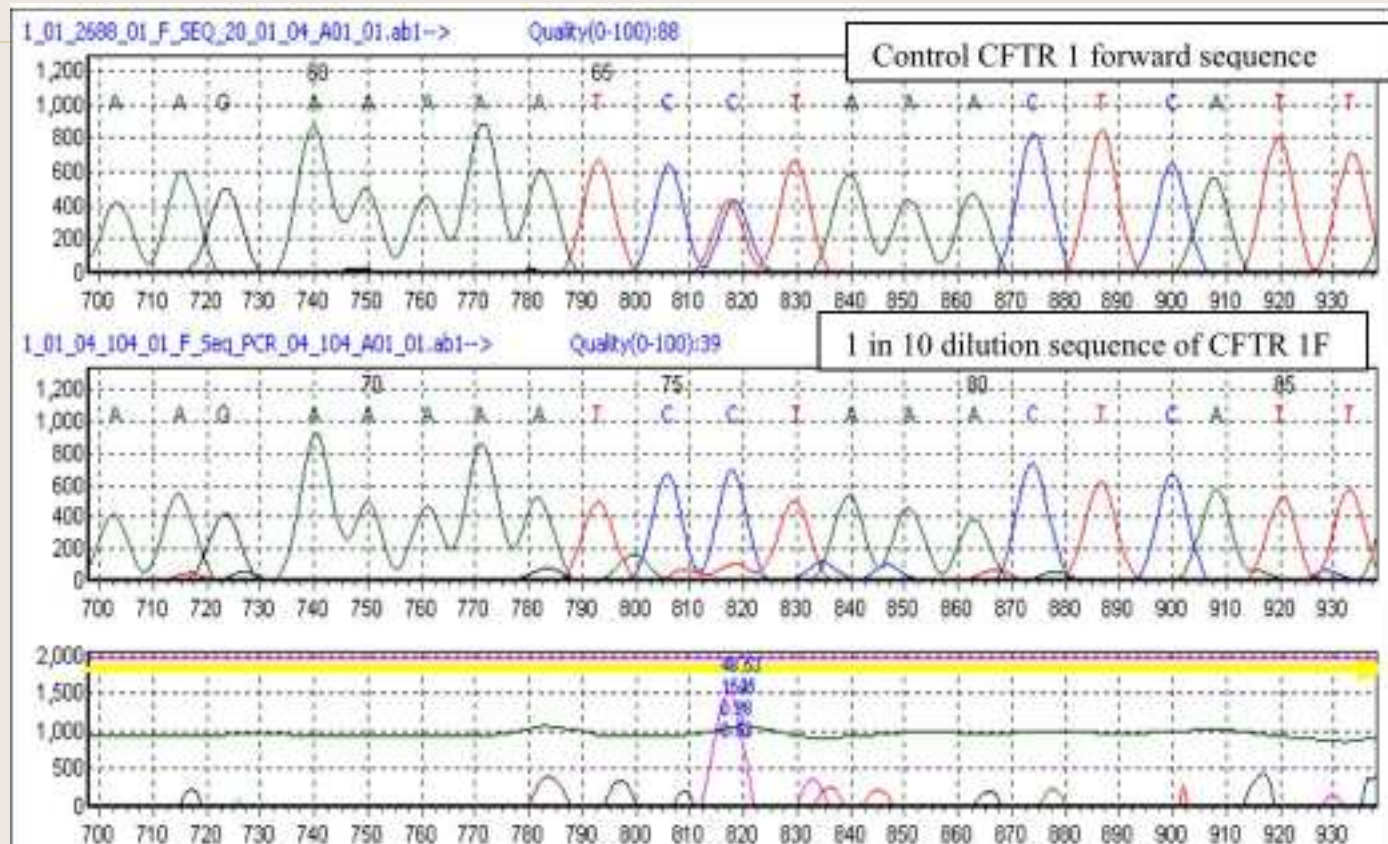
332C>T 1/100 *vs* genomic
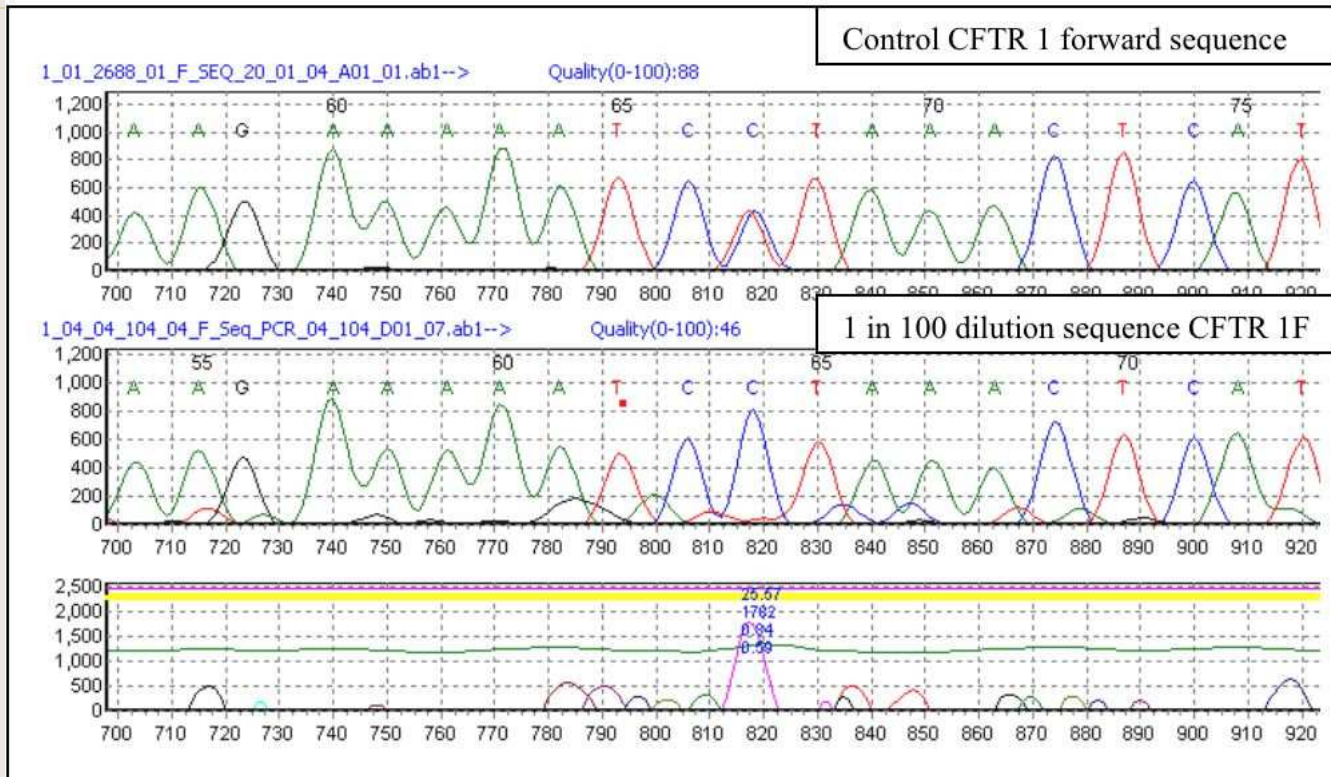
Multilocus finger printing of WGA products

Peak Height Ratios of WGA products

- Even 500ng starting DNA showed some loss of allele ratios, but at 50ng dosage ratios were lost. However the results were not as bad as with MLPA

Fingerprinting of WGA products from Blood/Tumour pairs
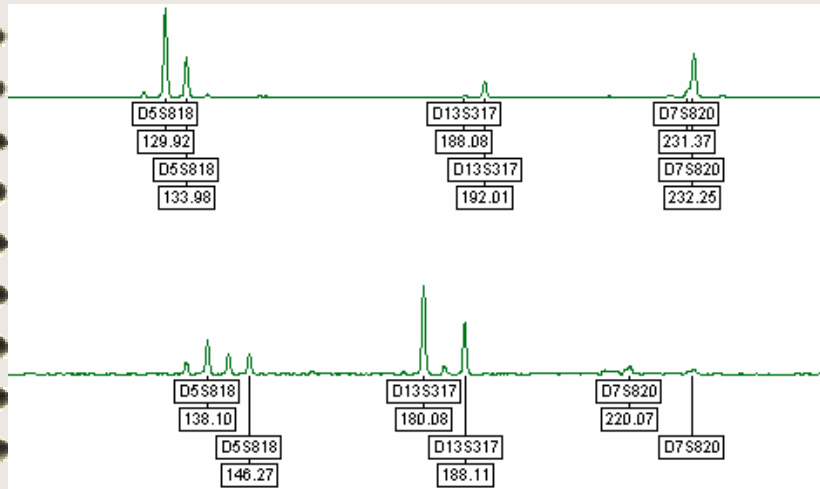
<-Tumour

<-Tumour (msat instability?)

Blood->

Blood->

Summary

- As with literature reports, we found that allele ratios were not preserved to diagnostic accuracy
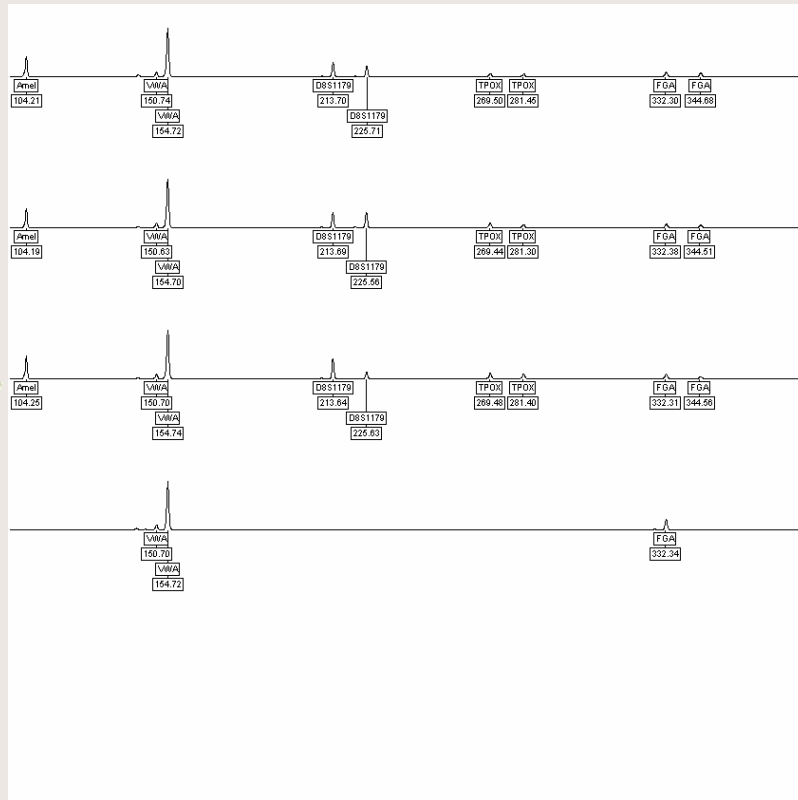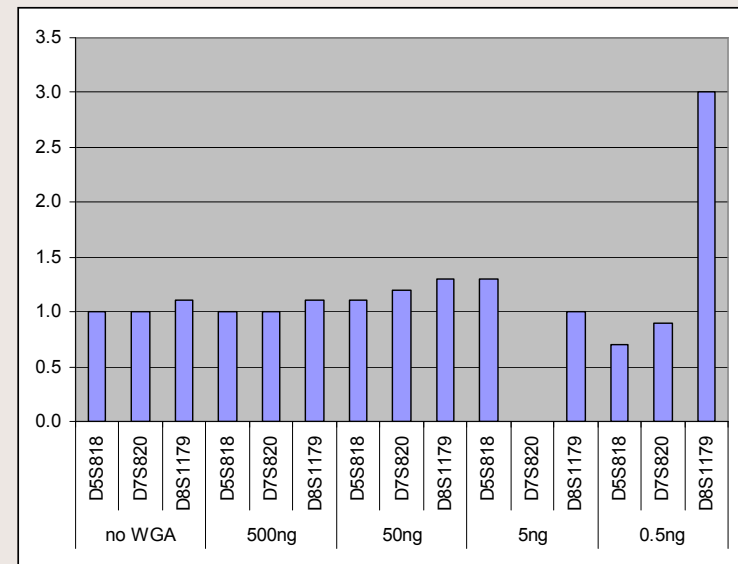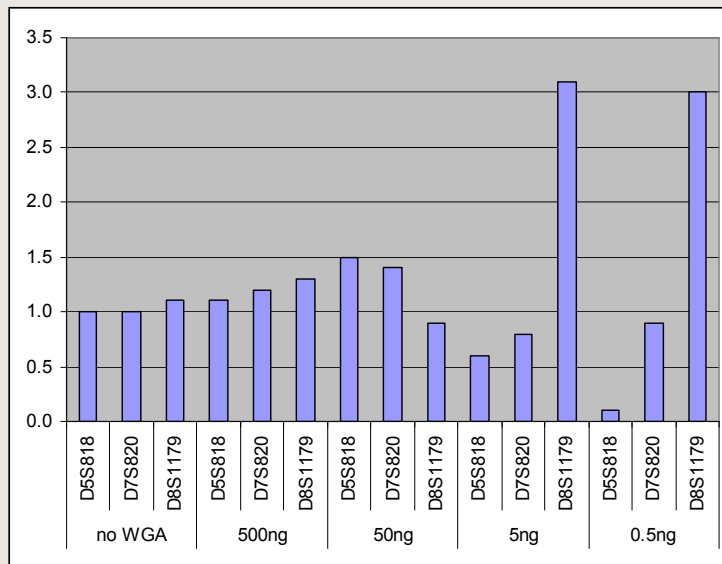- MLPA assays were especially vulnerable
- Although native DNA seemed to produce better WGA yields, the MLPA dosage measurements were worse
- All assays became inaccurate at low starting quantities.

Limits of WGA

- Minimum starting quality and quality 500-50ng?
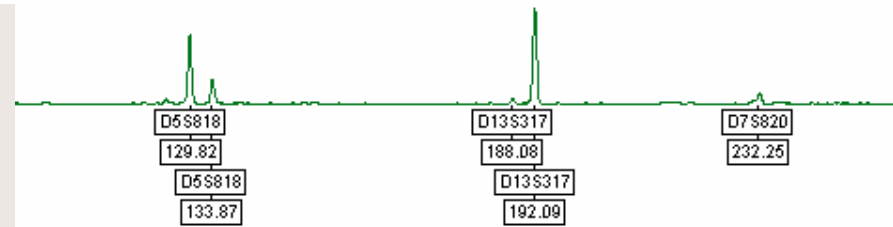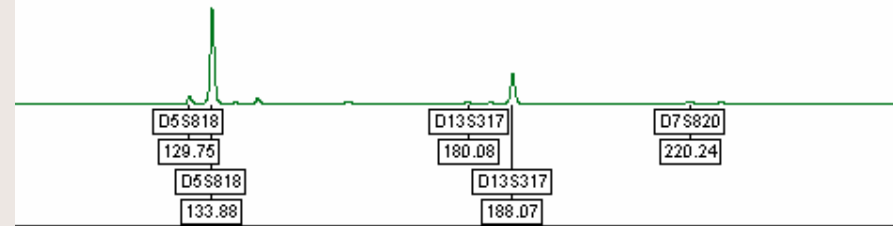- Can WGA products be reamplified?
- Alternatives to ø29 e.g. *Bst*
- Too early to give up on blood extractions, but Genomiphi or other WGA methods could play a useful role
- QA of WGA product: may be technique dependent

Acknowledgements

- Shahnaz Bibi (Trisomy MLPA)
- Rebecca White (Sequencing)
- Shabnam Qamer (Multilocus MLPA)
- DNA Lab, Leeds: MLPA data analysis & review of data