

A spiral-bound notebook with a light beige, textured cover and a dark brown border. The spiral binding is on the left side.

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.

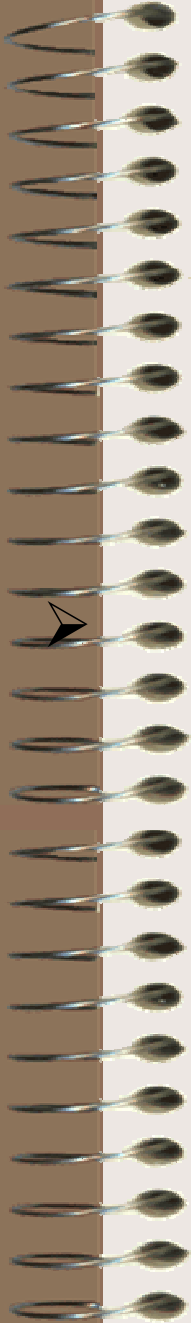
Template DNA should be high molecular weight (>2kbp).

Uses DNA polymerase from $\phi 29$ (*B. subtilis* 'phage) that is proofreading and highly processive.

The final product is double stranded 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 ϕ 29MDA 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

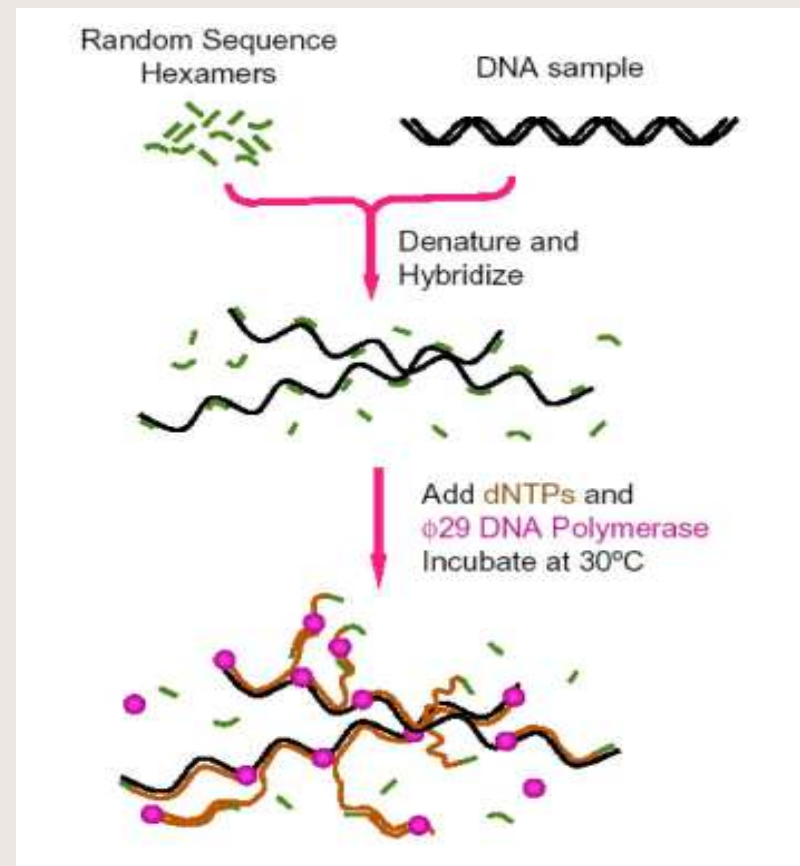
Strand Displacement Amplification (SDA) using ϕ 29 polymerase

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

ϕ 29 has unique biochemical properties, proofreading, high processivity and strand displacement activities

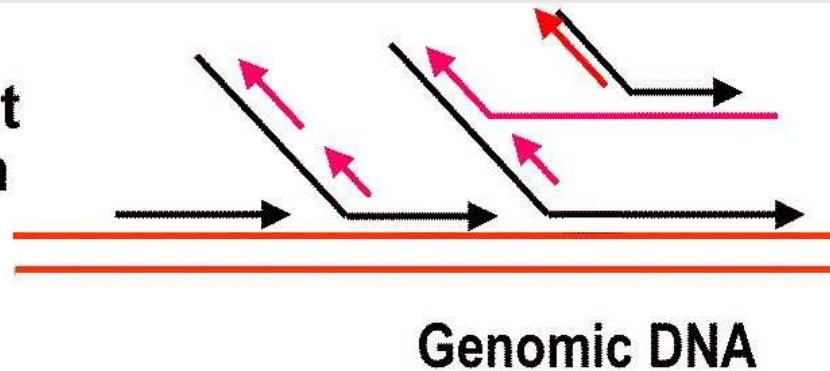
Random modified hexamer primers anneal to the template DNA at multiple sites

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



Universal gDNA Amplification

Multiple Displacement Amplification



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

- PNAS 2002 99 (8) 5261–5266

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

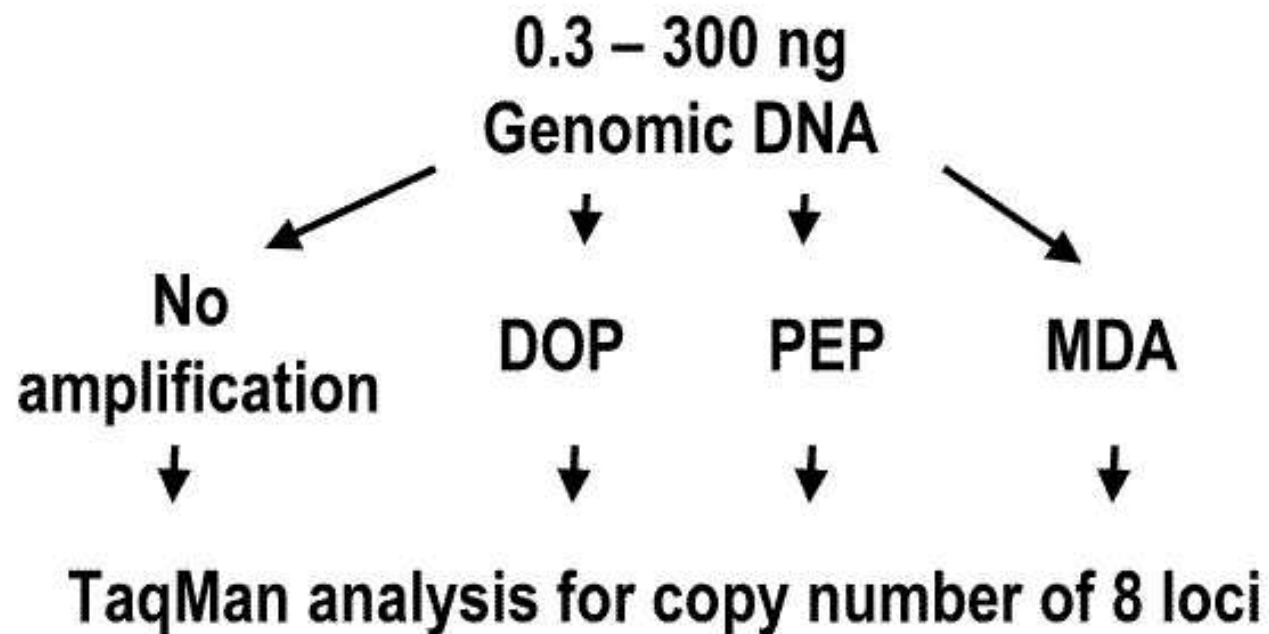
Molecular Staging, Inc., 300 George Street, Suite 701,
New Haven, CT 06511

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.

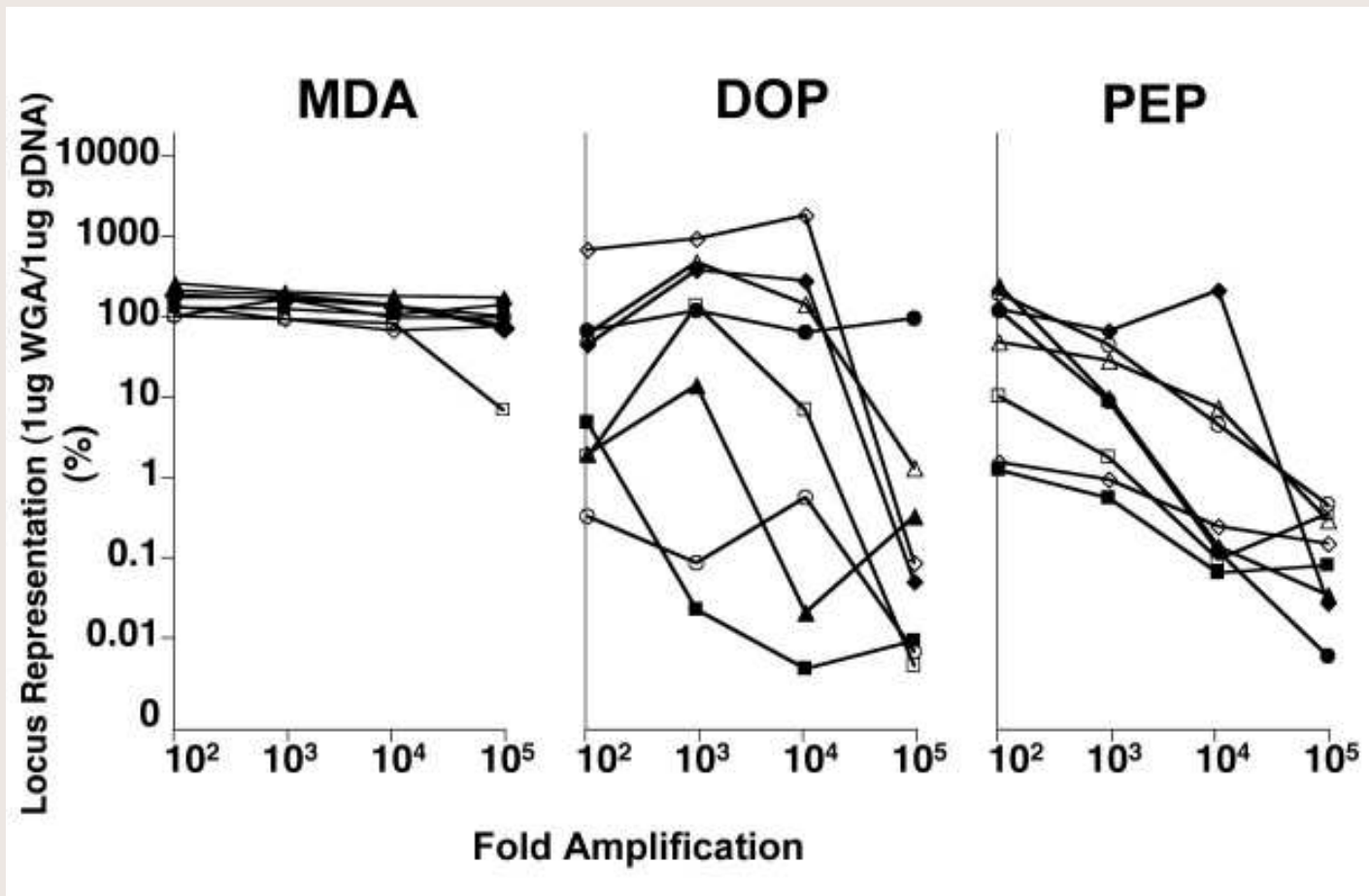
Bias Study



DOP: Degenerate Oligonucleotide Priming

PEP: Primer Extension Pre-amplification

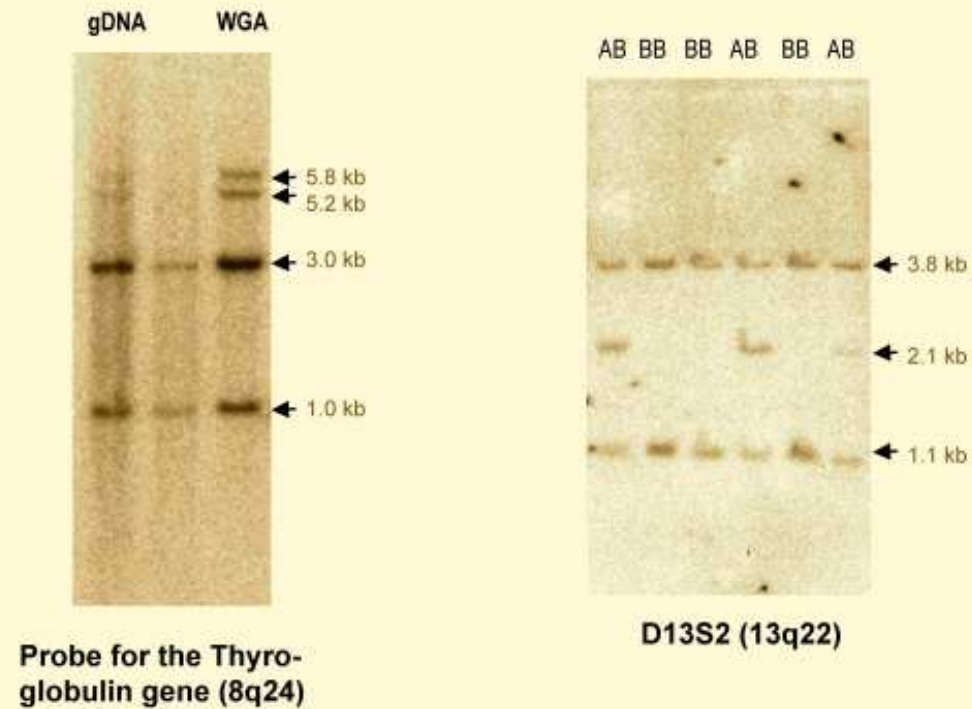
TaqMan Quantification of 8 Loci



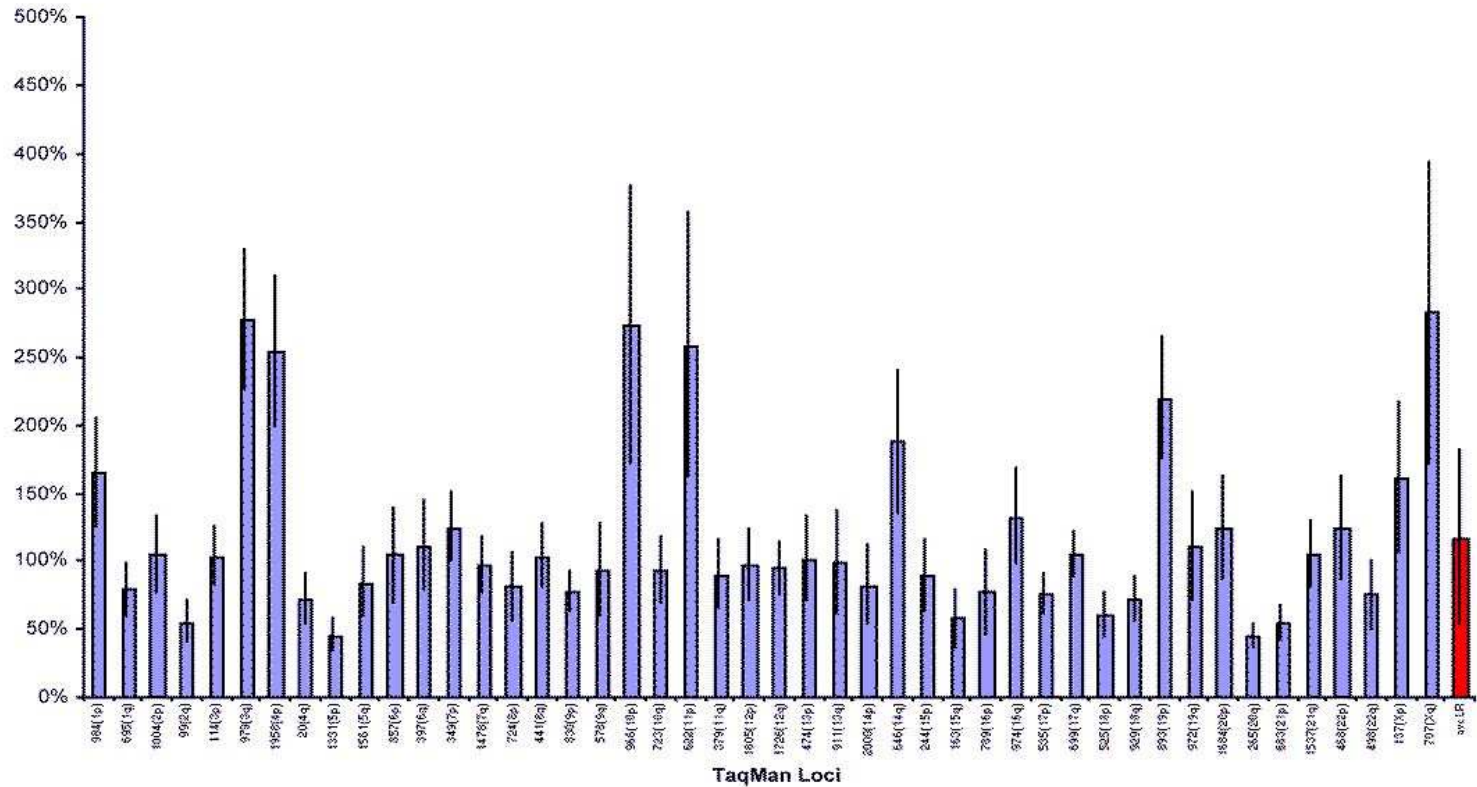
Note the log scale!

Southern blotting

RFLP Analysis on 10,000X DNA



46 Loci Bias Study on 44 Patients



Recent publications

Nucleic Acids Research, 2003, Vol. 31, No. 21 e129
 © 2003 Oxford University Press

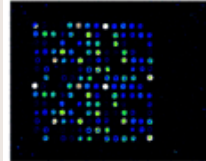
Quantitative evaluation by minisequencing and microarray multiplexed SNP genotyping of **whole genome amplification**

Nucleic Acids Research, 2004, Vol. 32, No. 9 e66
 © 2004 Oxford University Press

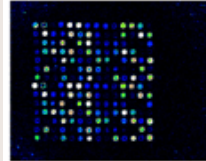
Lovisa Lovmar, Mona Fredriksson, Ulrik

Molecular Medicine, Department of
 Sweden

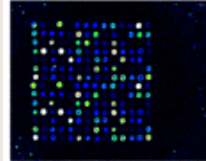
ddATP-Texas Red



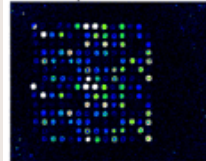
ddCTP-Tamra



ddGTP-R110



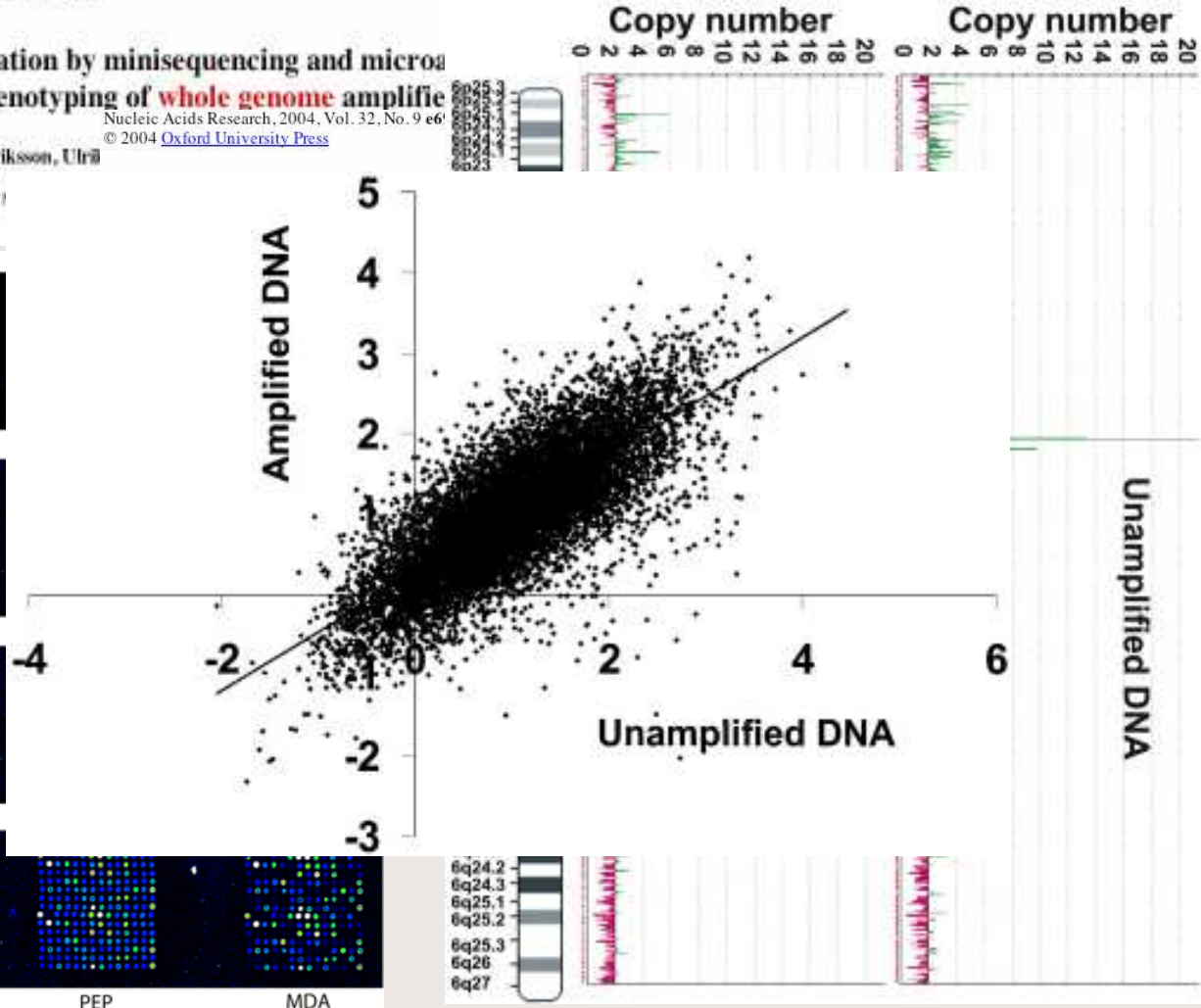
ddUTP-Cy5



Genomic DNA

PEP

MDA



Recent publications

The American Journal of
PATHOLOGY

HOME HELP FEEDBACK SU

Institution: Cancer Re

(American Journal of Patho
© 2004 American Society fo

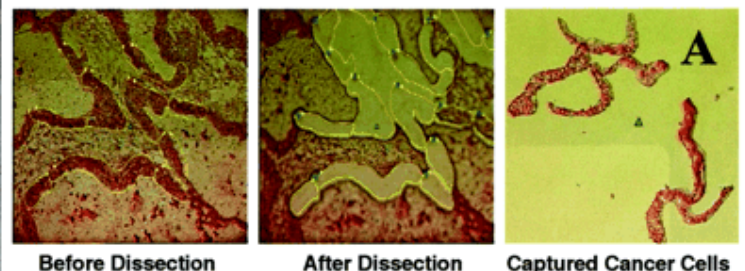
Technical Advanc

Whole Genome
High-Through
Genotyping

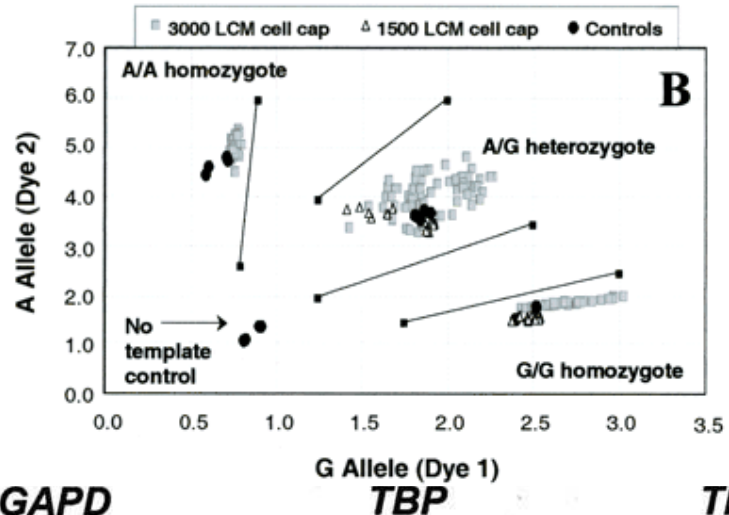
Martha S. Rook, Scott
From Nuvelo Incorporated.

Santa Cruz Biotechnology
RESEARCH ANTIBODIES 2003

Microdissected Tissue for
Tandem Repeat



Before Dissection After Dissection Captured Cancer Cells



B

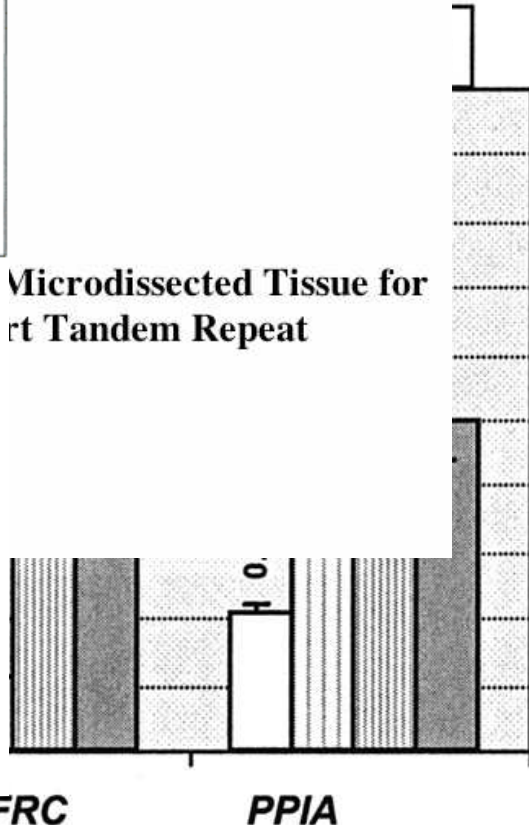
Legend: 3000 LCM cell cap (square), 1500 LCM cell cap (triangle), Controls (circle)

Y-axis: A Allele (Dye 2) (0.0 to 7.0)

X-axis: G Allele (Dye 1) (0.0 to 3.5)

Labels: A/A homozygote, A/G heterozygote, G/G homozygote, No template control

Genes: **GAPD**, **TBP**



TFRC PPIA

Rel 0.0, 0.2, 0.4, 0.6, 0.8, 1.0

Recent publications

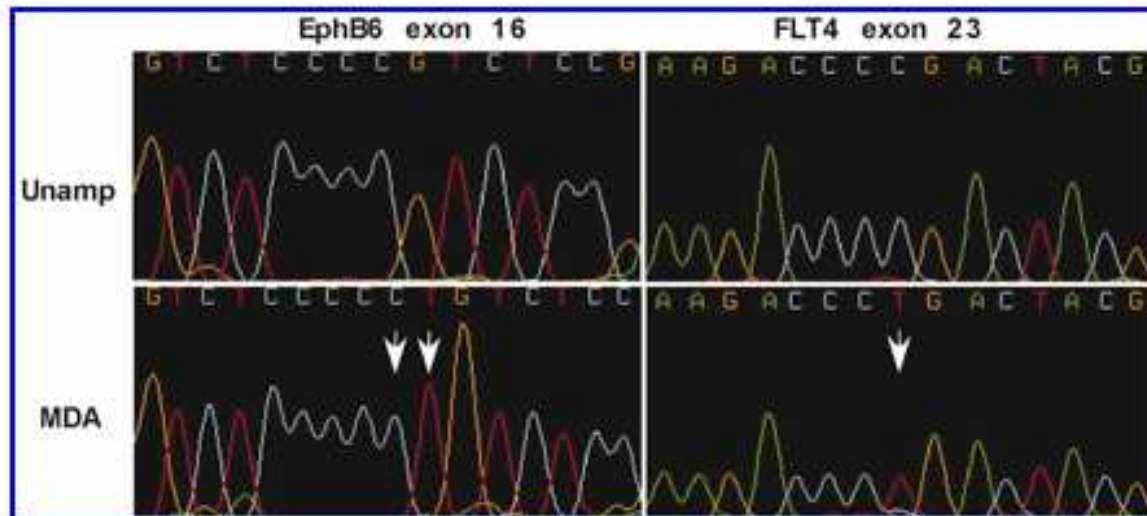


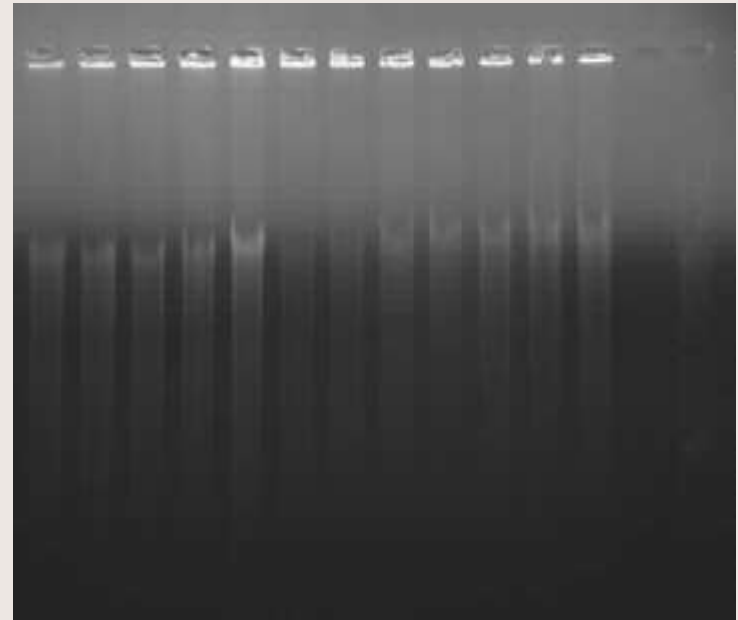
Figure 3. Rare homozygous sequence variations in ϕ 29MDA samples. DNA sequence traces for unamplified (upper) and ϕ 29MDA (lower) samples showing a homozygous 2 bp (CT) insertion and a homozygous C→T substitution (white arrows) detected by NQS analysis. All samples were non-denatured.

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 <i>Bst</i> 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 |

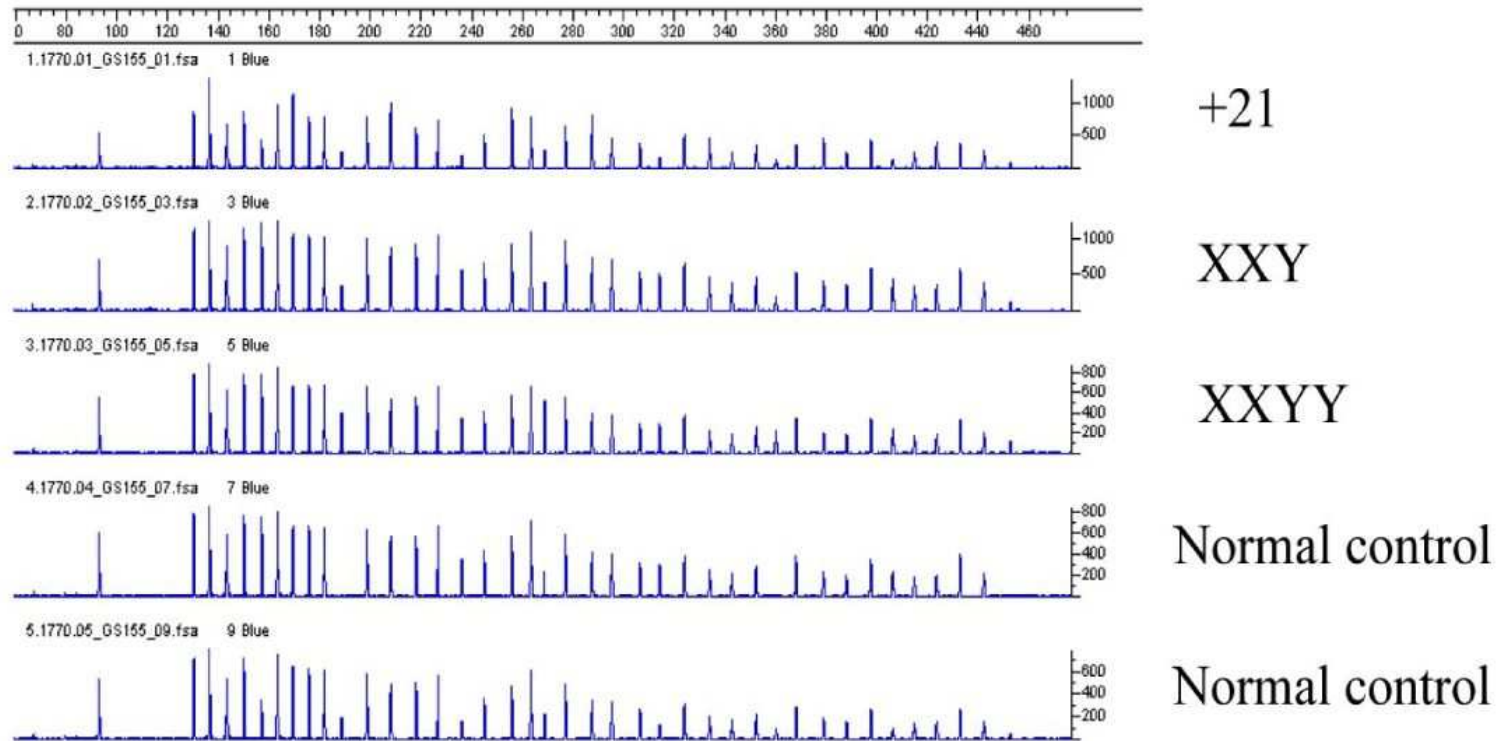
Genomiphi products look similar

- High molecular weight products
- May include DNA in -ve control!
- Variation in complexity?
- How can we tell if it is representative?

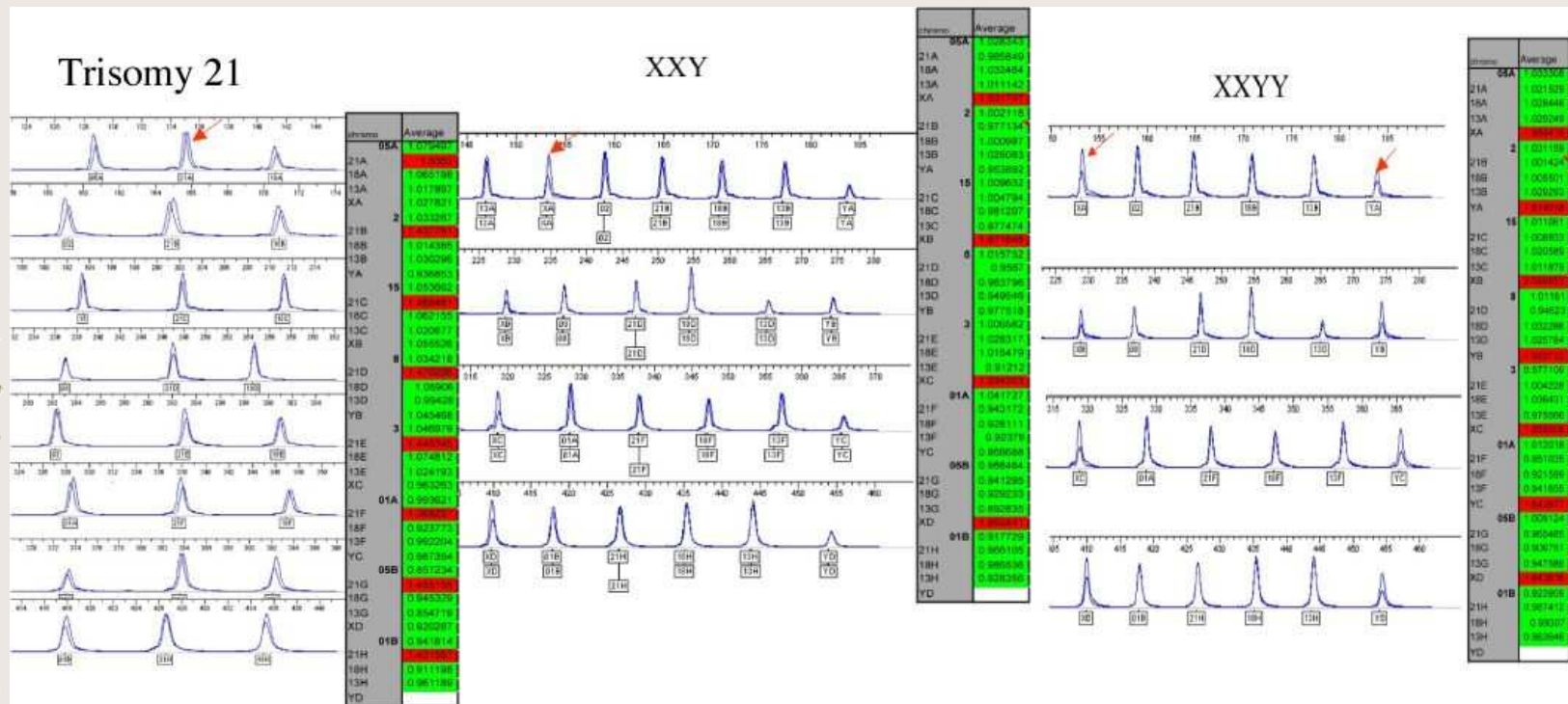


MLPA analysis of WGA products

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

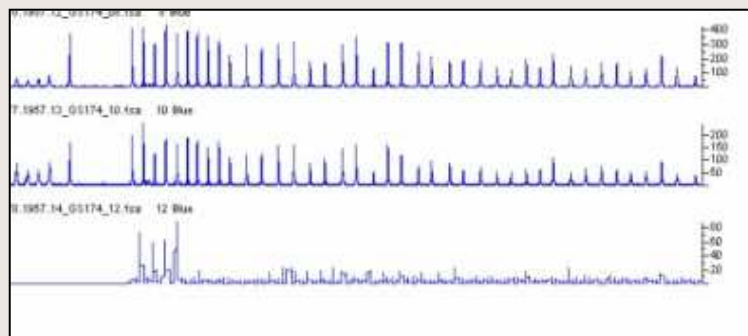
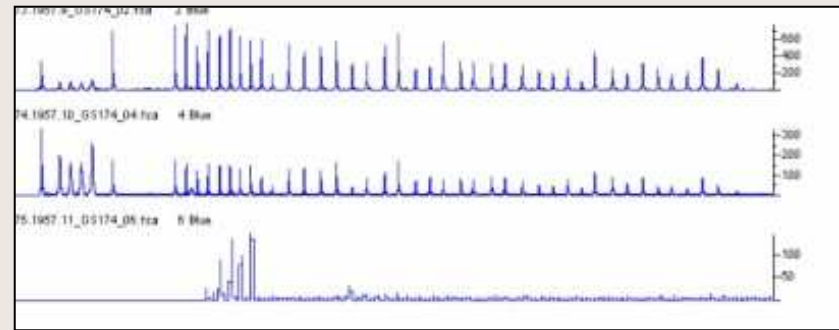
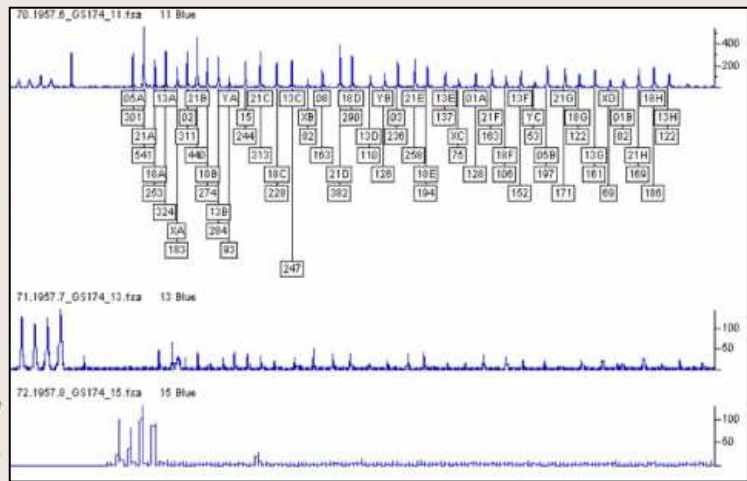


MLPA results on templates

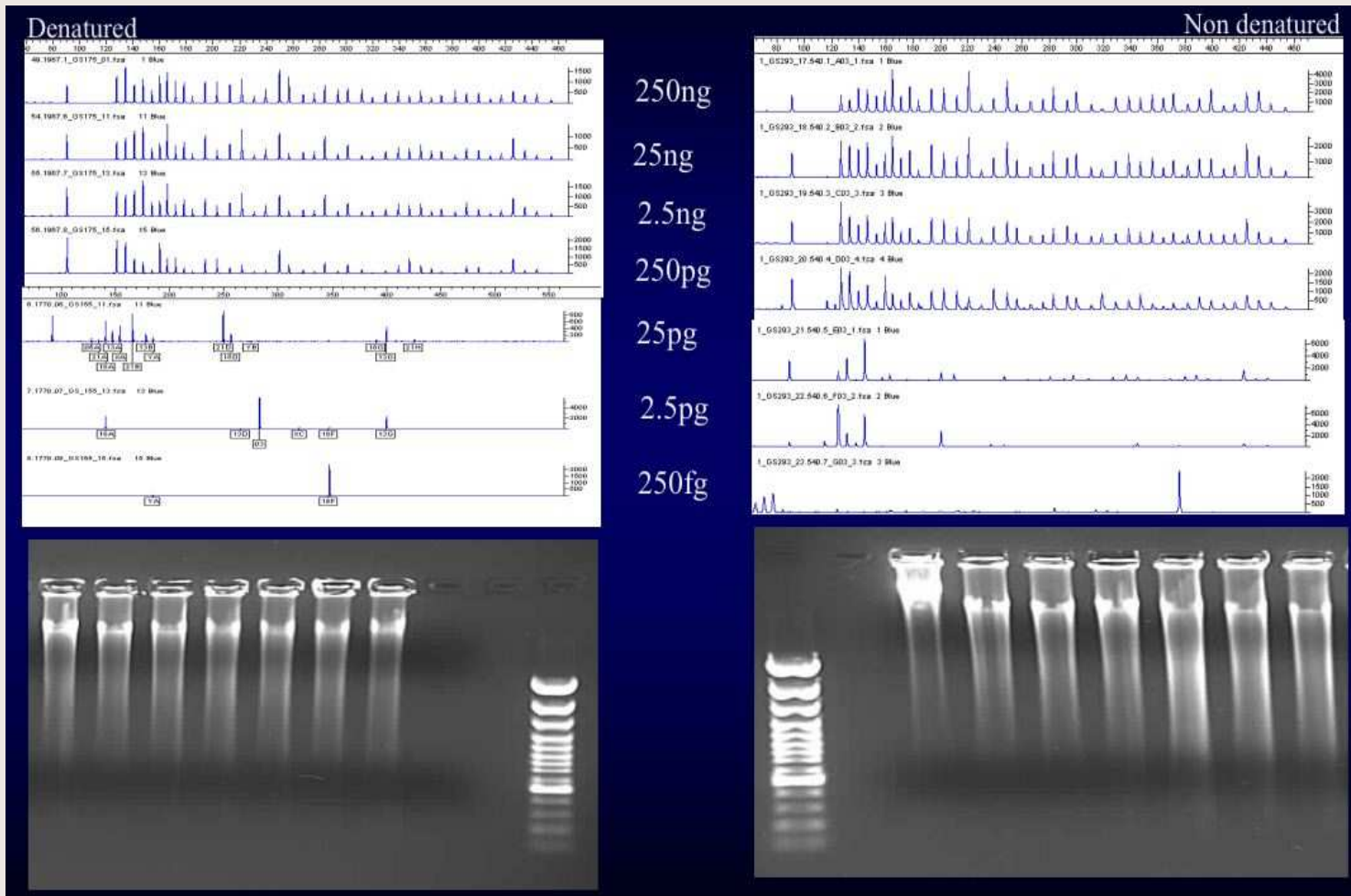


MLPA dosage templates available here:
<http://leedsdna.info/downloads.htm>

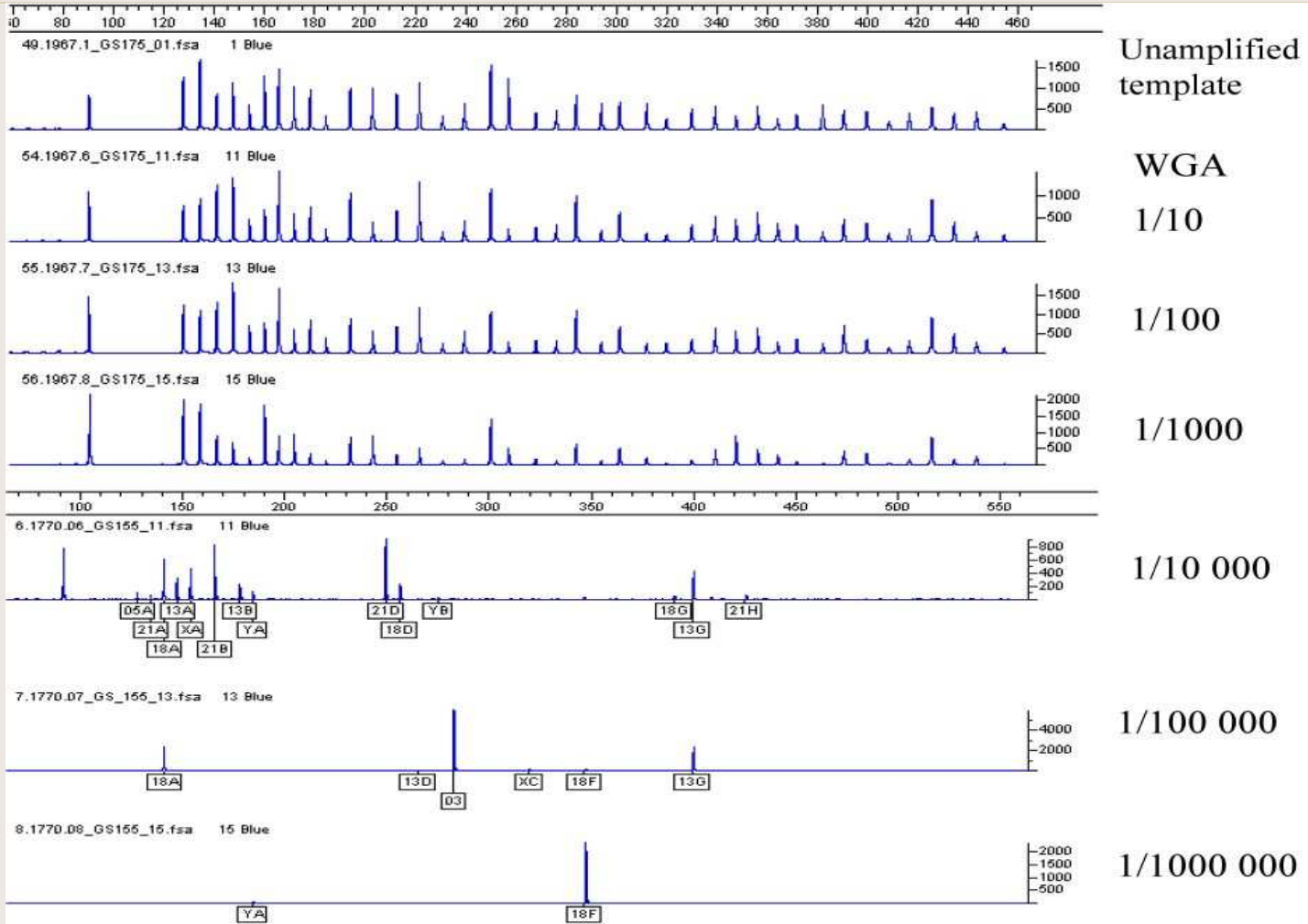
Sample dilutions do not yield MLPA products beyond 1/10 (50 ng)



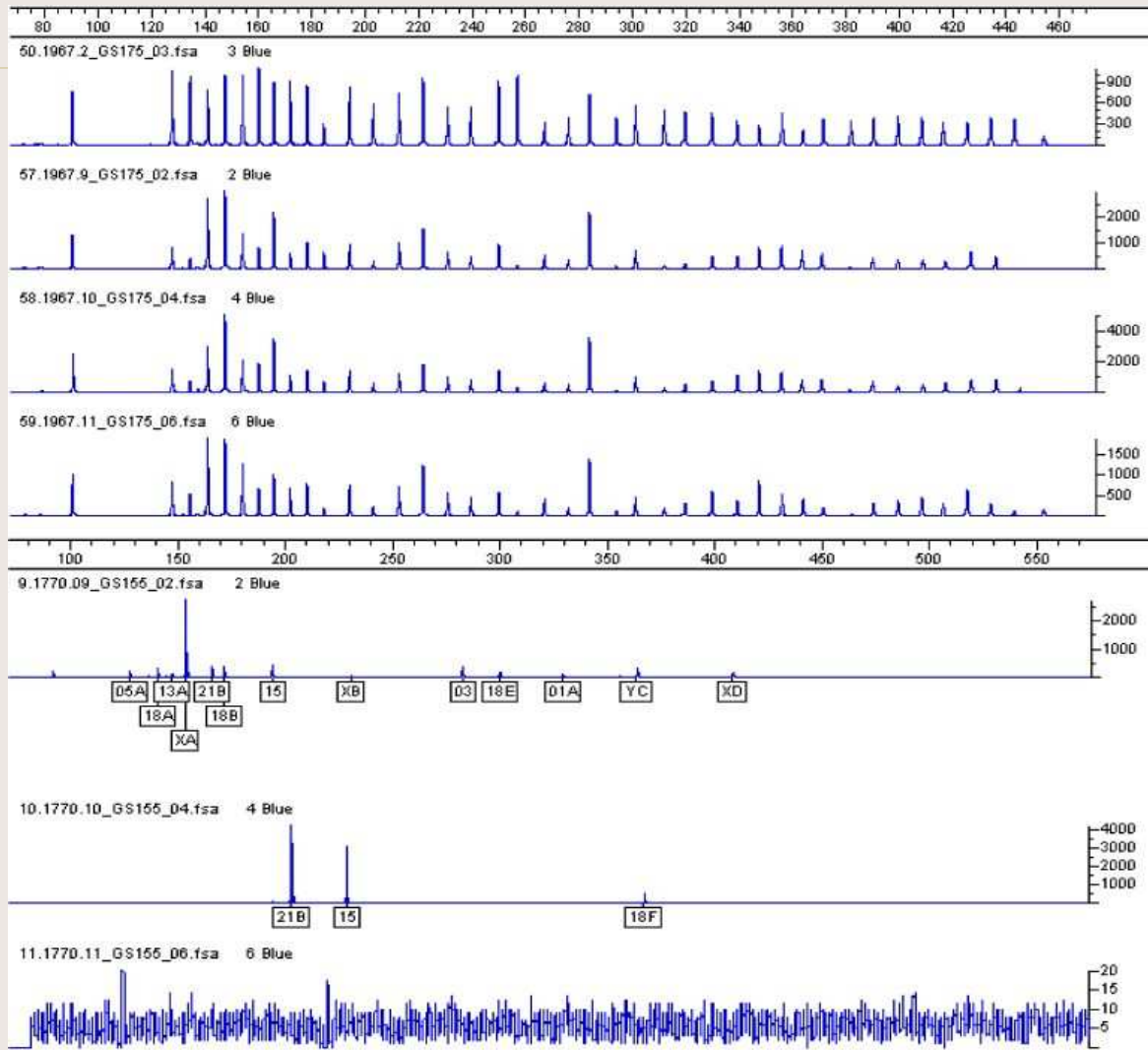
WGA using native or denatured template



Trisomy 21 WGA MLPA



XXY



Template

WGA
1/10

1/100

1/1000

1/10 000

1/100 000

1/1000 000

Dosage quotients of MLPA products denatured WGA template

Trisomy 21

| chromo | Original #21 Average | 1/10 DG +21 Average | 1/50 DG +21 Average | 1/1000 DG +21 Average |
|--------|-------------------------|------------------------|------------------------|--------------------------|
| 05A | 1.079497 | 1.149571 | 0.81615726 | 0.300983449 |
| 21A | 1.03552 | 0.927458 | 0.26474305 | 28.37521245 |
| 18A | 1.065198 | 0.738284 | 0.64947819 | 0.802761972 |
| 13A | 1.017897 | 0.848495 | 0.85717551 | 0.900831892 |
| XA | 1.027821 | 0.728219 | 0.85850947 | 7.415168837 |
| 2 | 1.033267 | 0.920861 | 0.93048972 | 5.671110107 |
| 21B | 4.57732 | 1.029168 | 1.03795466 | 3.300182035 |
| 18B | 1.014385 | 1.17038 | 1.07909052 | 0.978349373 |
| 13B | 1.030296 | 0.84621 | 1.01524203 | 1.102501706 |
| YA | 0.936853 | 0.529671 | 0.87909288 | 0.304038980 |
| 15 | 1.053662 | 1.351065 | 0.86370537 | 0.978706253 |
| 21C | 0.88489 | 0.982375 | 0.8893656 | #DIV/0! |
| 18C | 1.062155 | 0.921353 | 0.85608701 | 0.00834441 |
| 13C | 1.020677 | 1.117667 | 0.80677928 | 5.235824113 |
| XB | 1.055526 | 0.88773 | 1.05470577 | 0.51881529 |
| 8 | 1.034218 | 1.112727 | 1.29188222 | 0.531076028 |
| 21D | 1.470238 | 0.91731 | 0.9048483 | 0.30095738 |
| 18D | 1.05906 | 0.540226 | 0.40718601 | 18.10392126 |
| 13D | 0.99428 | 0.910028 | 0.93662322 | 0.368618664 |
| YB | 1.045468 | 1.201 | 0.85933737 | 1.187434664 |
| 3 | 1.048979 | 0.615371 | 0.64326030 | 1.47106654 |
| 21E | 1.480345 | 0.462495 | 1.1306551 | 1.475938963 |
| 18E | 1.074812 | 1.206282 | 1.11889772 | 2.809910878 |
| 13E | 1.024193 | 1.833588 | 1.73265804 | 0.492525839 |
| XC | 0.963263 | 2.418898 | 1.58175209 | #DIV/0! |
| 01A | 0.993621 | 0.857399 | 0.85909853 | 0.4148117438 |
| 21F | 0.85270 | 1.488925 | 0.68881882 | 2.051272367 |
| 18F | 0.923773 | 0.726906 | 0.80663024 | 1.548462607 |
| 13F | 0.992204 | 1.049631 | 0.91893393 | 1.60458892 |
| YC | 0.987354 | 0.960805 | 0.81556028 | 0.517584853 |
| 05B | 0.857234 | 1.141083 | 1.20280942 | #DIV/0! |
| 21G | 1.458155 | 2.383874 | 4.14062442 | #DIV/0! |
| 18G | 0.945329 | 1.490118 | 1.80188228 | 1.898479368 |
| 13G | 0.854719 | 1.05871 | 1.81847896 | 4.700278586 |
| XD | 0.920287 | 1.317882 | 0.73435609 | 1.424248348 |
| 01B | 0.941814 | 1.355182 | 1.11910938 | 1.486083412 |
| 21H | 1.481597 | 1.91741 | 1.8799336 | 1.43385344 |
| 18H | 0.911198 | 1.737525 | 1.81054769 | 1.54958046 |
| 13H | 0.961189 | 1.482552 | 1.89768277 | 1.935065728 |
| YD | 0 | 0 | 0 | 0 |

XXY

| chromo | Original XXY Average | 1/50 DG XXY Average | 1/100 DG XXY Average | 1/1000 DG XXY Average |
|--------|-------------------------|------------------------|-------------------------|--------------------------|
| 05A | 1.028343 | 1.006920794 | 1.160927916 | 1.48995855 |
| 21A | 0.985849 | 0.886698145 | 1.159683247 | 4.228335981 |
| 18A | 1.032484 | 0.9090277 | 0.747767226 | 0.978561167 |
| 13A | 1.011142 | 1.13519267 | 1.042299111 | 1.097294865 |
| XA | 1.007797 | 0.88993989 | 1.223592564 | 7.489148003 |
| 2 | 1.002118 | 0.867714859 | 1.129145305 | 1.428345072 |
| 21B | 0.977134 | 1.148718064 | 1.126107525 | 2.486398726 |
| 18B | 1.009987 | 0.960527452 | 1.063827937 | 0.816993363 |
| 13B | 1.026083 | 0.918170219 | 0.878249996 | 1.583348799 |
| YA | 0.953892 | 1.030023614 | 0.839012105 | 0.27541939 |
| 15 | 1.006632 | 1.021716272 | 0.710501395 | 0.487645982 |
| 21C | 1.004794 | 1.093523878 | 1.883770889 | #DIV/0! |
| 18C | 0.981207 | 1.132470088 | 0.823861418 | 3.781002557 |
| 13C | 0.977474 | 1.096399618 | 0.803270198 | 8.896963906 |
| XB | 0.971829 | 0.87939982 | 2.106314129 | 1.371022182 |
| 8 | 1.015752 | 0.910347877 | 0.912284977 | 0.781422205 |
| 21D | 0.9587 | 1.052635492 | 0.938472779 | 1.888282843 |
| 18D | 0.963796 | 0.810913257 | 0.588658229 | 1.589279236 |
| 13D | 0.949546 | 1.190011475 | 0.906090922 | 0.483305089 |
| YB | 0.977518 | 0.909533964 | 0.876539688 | 1.004616609 |
| 3 | 1.006582 | 1.091078879 | 1.113229179 | 0.890789939 |
| 21E | 1.028317 | 0.92660115 | 0.770981783 | 1.716899318 |
| 18E | 1.016479 | 1.118389241 | 0.842306187 | 1.588984974 |
| 13E | 0.91212 | 1.075622775 | 1.933923528 | 0.308590807 |
| XC | 1.854325 | 2.402396004 | 2.578973803 | #DIV/0! |
| 01A | 1.041727 | 0.851660589 | 0.889290245 | 0.951833575 |
| 21F | 0.943172 | 1.060888601 | 1.382124531 | 0.900232681 |
| 18F | 0.928111 | 1.03163549 | 1.008926195 | 0.847103278 |
| 13F | 0.92376 | 1.147208591 | 0.988985976 | 1.033093453 |
| YC | 0.866688 | 1.147007498 | 1.165561765 | 0.374323899 |
| 05B | 0.988484 | 1.488817517 | 1.37825883 | #DIV/0! |
| 21G | 0.941295 | 1.132952923 | 1.430788028 | #DIV/0! |
| 18G | 0.929233 | 1.094918647 | 0.940777596 | 0.784706679 |
| 13G | 0.892385 | 0.97707095 | 1.110626473 | 2.437599011 |
| XD | 1.892841 | 0 | 0 | 0.804189091 |
| 01B | 0.917729 | 0 | 0 | 0.537298333 |
| 21H | 0.966105 | 0 | 0 | 0.610650859 |
| 18H | 0.986536 | 0 | 0 | 1.441217188 |
| 13H | 0.928356 | 0 | 0 | 0.427531858 |
| YD | 0 | 0 | 0 | 0 |

XXYY

| chromo | Original XXYY Average | 1/50 DG XXYY Average | 1/100 DG XXYY Average | 1/1000 DG XXYY Average |
|--------|--------------------------|-------------------------|--------------------------|---------------------------|
| 05A | 1.033305 | 1.132881745 | 0.759881981 | 0.333333333 |
| 21A | 1.021529 | 0.923284285 | 0.734659494 | 2.89583021 |
| 18A | 1.028448 | 0.982727272 | 0.711901688 | 0.89583021 |
| 13A | 1.020249 | 0.789978125 | 0.811399999 | 0.761993058 |
| XA | 0.988585 | 0.889999999 | 0.889999999 | 0.889999999 |
| 2 | 1.031159 | 0.843821235 | 0.891018425 | 1.416600777 |
| 21B | 1.021424 | 1.089760796 | 0.892481044 | 2.58302228 |
| 18B | 1.005501 | 1.043888848 | 0.718574248 | 0.752888114 |
| 13B | 1.024093 | 0.865168594 | 1.150887687 | 1.150887687 |
| YA | 0.931278 | 0.899728 | 0.899728 | 0.895884669 |
| 15 | 1.211051 | 1.041221585 | 0.759881981 | 0.333333333 |
| 21C | 1.006635 | 1.19184211 | 0.899728 | #DIV/0! |
| 18C | 1.020589 | 1.087031929 | 0.909842032 | 1.10209420 |
| 13C | 1.011679 | 0.899728 | 1.000721234 | 1.000721234 |
| XB | 0.971829 | 0.87939982 | 0.899728 | 1.09447422 |
| 8 | 1.01181 | 0.85531811 | 0.899728 | 0.899728 |
| 21D | 0.94603 | 1.189048475 | 1.164076184 | 1.164076184 |
| 18D | 1.032089 | 0.899728 | 0.899728 | 1.164076184 |
| 13D | 1.025784 | 1.036218883 | 0.835674031 | 1.100000000 |
| YB | 0.977518 | 0.909533964 | 0.876539688 | 0.876539688 |
| 3 | 0.977109 | 0.7816204 | 0.898803827 | 0.898803827 |
| 21E | 1.004228 | 0.900000000 | 0.899728 | 1.000000000 |
| 18E | 1.030431 | 1.121838799 | 1.051180779 | 1.100000000 |
| 13E | 0.975086 | 0.899728 | 0.899728 | 1.100000000 |
| XC | 0.958325 | 0.899728 | 0.899728 | 0.899728 |
| 01A | 1.012016 | 0.863647022 | 1.059037981 | 0.781626526 |
| 21F | 0.951038 | 0.994658948 | 0.9799625 | 0.961753225 |
| 18F | 0.921589 | 0.87717038 | 0.91487018 | 0.89583021 |
| 13F | 0.941685 | 1.188071215 | 0.988982889 | 0.946822028 |
| YC | 0.866688 | 1.088999999 | 0.899728 | 0.885786771 |
| 05B | 1.009134 | 1.009134 | 0.899728 | 0.899728 |
| 21G | 0.965485 | 1.28881888 | 0.899728 | #DIV/0! |
| 18G | 0.909751 | 1.009134 | 0.899728 | 0.891000000 |
| 13G | 0.847585 | 1.037235595 | 0.899728 | 2.89583021 |
| XD | 0.892385 | 1.109825277 | 1.109825277 | 2.71839934 |
| 01B | 0.925900 | 0.911888888 | 0.879000047 | 0.89583021 |
| 21H | 0.997412 | 0.997412 | 0.962000168 | 1.000000000 |
| 18H | 0.989307 | 1.009134 | 0.899728 | 0.89583021 |
| 13H | 0.953646 | 1.117020222 | 0.899728 | 1.161269308 |
| YD | 0 | 0 | 0 | 0 |

Dosage quotients of MLPA products non denatured WGA template

Trisomy 21

| chromo | Neat NDG +21 Average | 1/10 NDG +21 Average | 1/100 NDG +21 Average | 1/1000 NDG +21 Average |
|--------|-------------------------|-------------------------|--------------------------|---------------------------|
| 05A | 0.74866417 | 0.79230977 | 1.52932543 | 1.277174974 |
| 21A | 1.15839866 | 1.53657389 | 1.94468827 | 2.246764386 |
| 18A | 1.23785908 | 0.74114321 | 1.58826622 | 1.588941073 |
| 13A | 0.86485179 | 0.66211286 | 0.5311301 | 0.398903053 |
| XA | 1.3420977 | 0.82993704 | 0.98581818 | 8.367370045 |
| 2 | 1.09932241 | 0.68362311 | 1.1697428 | 1.436161784 |
| 21B | 1.92267136 | 1.18773066 | 1.19020164 | 1.591158501 |
| 18B | 0.98962851 | 0.84021573 | 0.97927276 | 0.862054777 |
| 13B | 1.29482973 | 1.02966668 | 0.88681298 | 0.668513266 |
| YA | 1.78208713 | 1.10327796 | 1.19178911 | 0.998367914 |
| 15 | 0.96819646 | 0.87920063 | 0.75399953 | 2.247652808 |
| 21C | 2.16639973 | 1.29072992 | 1.15420972 | 1.909490227 |
| 18C | 0.94614881 | 0.72089682 | 0.80120199 | 2.050633895 |
| 13C | 1.35223486 | 1.13795157 | 1.00817867 | 0.72966379 |
| XB | 1.04731662 | 0.72365219 | 0.92930275 | 0.454367882 |
| 8 | 0.85766693 | 0.72190057 | 1.73275887 | 0.935029462 |
| 21D | 1.97225205 | 1.58688487 | 1.44286306 | 1.596782615 |
| 18D | 0.53804761 | 1.00822094 | 1.58046546 | 0.949017286 |
| 13D | 1.34316749 | 1.17450573 | 1.13191893 | 1.236463483 |
| YB | 1.39387925 | 1.06756112 | 1.11389533 | 1.230230438 |
| 3 | 1.12932094 | 1.06257152 | 1.21307901 | 1.339457883 |
| 21E | 1.16116058 | 1.42927397 | 1.60889206 | 1.266138757 |
| 18E | 1.03489476 | 0.98015218 | 0.72180113 | 0.70363458 |
| 13E | 0.87593754 | 1.21429964 | 1.27923975 | 1.105156114 |
| XC | 0.53984027 | 0.97029684 | 0.87284559 | 2.260627182 |
| 01A | 1.14973567 | 1.36603689 | 0.90892365 | 1.336304552 |
| 21F | 1.98820906 | 2.24416071 | 1.18941888 | 0.867108085 |
| 18F | 1.16008004 | 1.57387556 | 1.01008908 | 3.753790146 |
| 13F | 1.25902736 | 1.71938191 | 0.71520698 | 0.478953261 |
| YC | 1.84311407 | 1.78683619 | 1.39896487 | 0.517695012 |
| 05B | 1.73451291 | 1.73938837 | 0.80444133 | 0.583659966 |
| 21G | 1.17613097 | 2.50631446 | 1.26379466 | 0.610139162 |
| 18G | 0.99350819 | 1.63175012 | 1.08250659 | 1.051847781 |
| 13G | 1.50185015 | 1.68584975 | 0.67488822 | 0.731580342 |
| XD | 0.96786642 | 1.50056433 | 1.05241374 | 3.943195004 |
| 01B | 0.93192223 | 1.93020986 | 0.79413123 | 0.555949908 |
| 21H | 1.28729488 | 3.67889934 | 1.19329134 | 0.904303651 |
| 18H | 1.21707986 | 2.68925291 | 0.89866257 | 2.700243445 |
| 13H | 1.15532681 | 2.84404478 | 1.42107856 | 0.78816057 |
| YD | 0.9959794 | 2.85332177 | 1.09322827 | 2.872891215 |

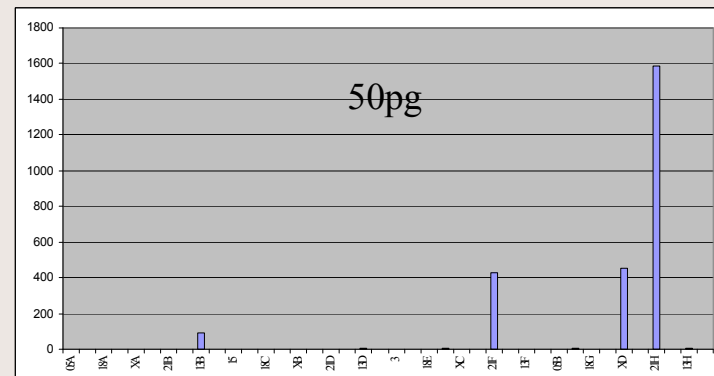
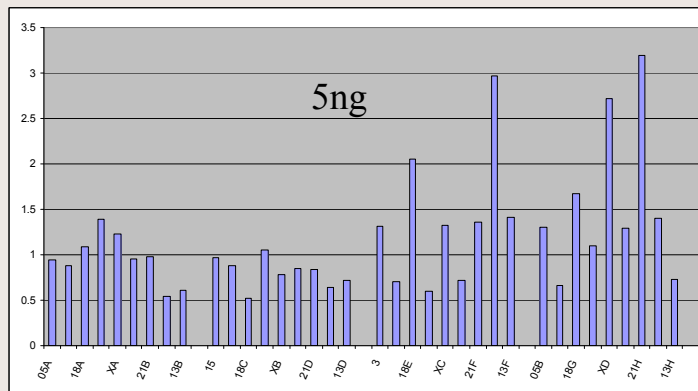
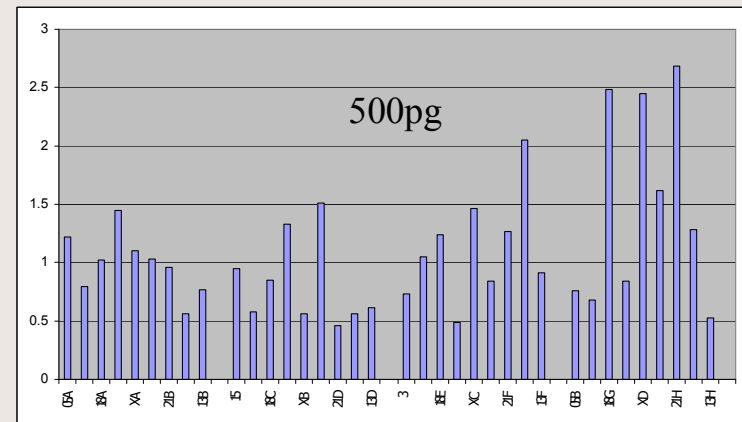
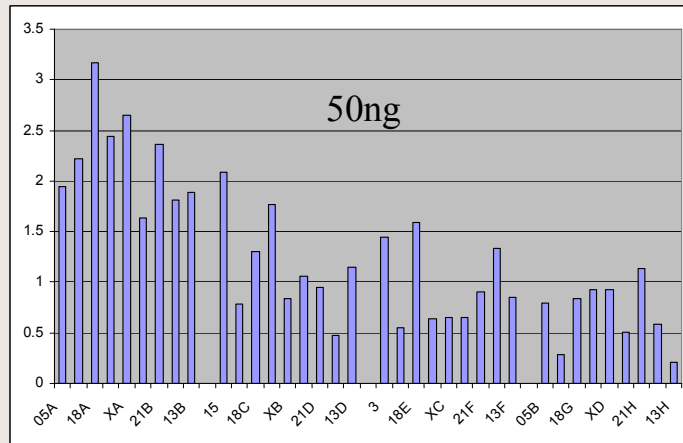
XXY

| chromo | Neat NDG XXY Average | 1/10 NDG XXY Average | 1/100 NDG XXY Average | 1/1000 NDG XXY Average |
|--------|-------------------------|-------------------------|--------------------------|---------------------------|
| 05A | 0.607719535 | 1.021526945 | 4.570320143 | 1.560445819 |
| 21A | 0.643578313 | 1.072482566 | 4.909801907 | 2.063397708 |
| 18A | 1.805229907 | 0.487748903 | 3.241058424 | 1.135422921 |
| 13A | 0.909225829 | 0.97782368 | 2.730848215 | 0.5767330394 |
| XA | 2.59893539 | 1.26768788 | 8.63232588 | 26.31733782 |
| 2 | 1.03155903 | 0.71900436 | 4.946914876 | 3.856406998 |
| 21B | 1.69015121 | 0.699530980 | 2.805578486 | 1.589744599 |
| 18B | 0.986291435 | 0.770354509 | 2.891811582 | 5.750367288 |
| 13B | 1.141911547 | 0.709009471 | 1.582230301 | 0.244784503 |
| YA | 1.923682148 | 1.055866693 | 3.963579283 | 2.375689852 |
| 15 | 1.06229202 | 0.747850956 | 1.604106514 | 0.835283059 |
| 21C | 1.120962661 | 1.376701272 | 3.010186776 | 3.044519942 |
| 18C | 0.984464955 | 0.899173621 | 3.430639138 | 0.444796958 |
| 13C | 1.500894954 | 0.757731351 | 1.570269448 | 0.962335995 |
| XB | 2.188427677 | 2.313502026 | 8.776708105 | 3.339378578 |
| 8 | 0.918175516 | 0.805464552 | 5.482795891 | 1.007331507 |
| 21D | 1.222041361 | 0.81644612 | 1.137879829 | 0.755731037 |
| 18D | 0.472723868 | 1.453215642 | 4.482473208 | 1.757379106 |
| 13D | 1.5385132 | 0.87302104 | 1.180555163 | 1.232958506 |
| YB | 1.408865371 | 0.901445857 | 1.688934005 | 1.097751292 |
| 3 | 1.262434965 | 0.941810117 | 1.743708282 | 0.421385753 |
| 21E | 0.697046018 | 1.255596178 | 4.713448018 | 6.238916377 |
| 18E | 1.506993158 | 0.760721516 | 1.012258407 | 0.182737808 |
| 13E | 0.828668647 | 0.995242768 | 1.115150815 | 0.7885108 |
| XC | 1.065325341 | 3.672036795 | 8.509489351 | 2.009833538 |
| 01A | 1.333039923 | 1.020725291 | 1.003480041 | 1.068578646 |
| 21F | 1.078879018 | 1.284714889 | 0.978434164 | 1.014366839 |
| 18F | 1.515727524 | 1.537941084 | 0.427635273 | 4.342507431 |
| 13F | 1.631162298 | 1.193835606 | 0.354531757 | 0.142633374 |
| YC | 1.969817517 | 1.2888889 | 0.817425087 | 0.794563847 |
| 05B | 1.380286637 | 1.786810410 | 0.509343331 | 1.801048378 |
| 21G | 0.787354964 | 1.726505588 | 0.740621443 | 1.183451478 |
| 18G | 1.319714576 | 1.11372017 | 0.602943179 | 0.298398191 |
| 13G | 1.78898866 | 1.54098167 | 0.296754439 | 0.881391351 |
| XD | 2.540006204 | 2.766514771 | 0.83905611 | 6.849128794 |
| 01B | 0.973652268 | 2.220053428 | 0.290073567 | 1.988085039 |
| 21H | 1.340742358 | 2.507812519 | 0 | 2.471865098 |
| 18H | 1.53589194 | 1.830522407 | 0 | 2.448729489 |
| 13H | 1.10534306 | 1.673992311 | 0 | 0.46861639 |
| YD | 1.329152627 | 2.04781979 | 0 | 0.997111541 |

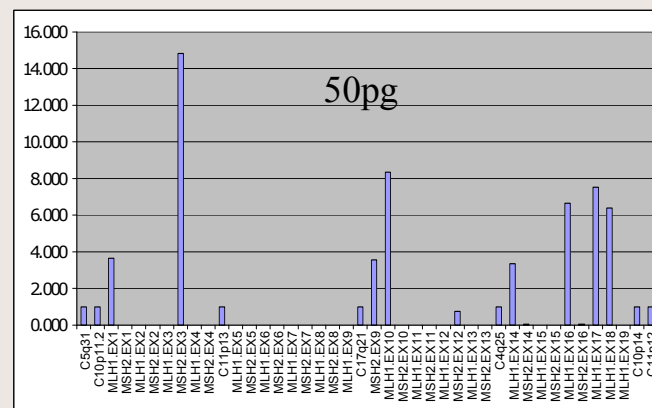
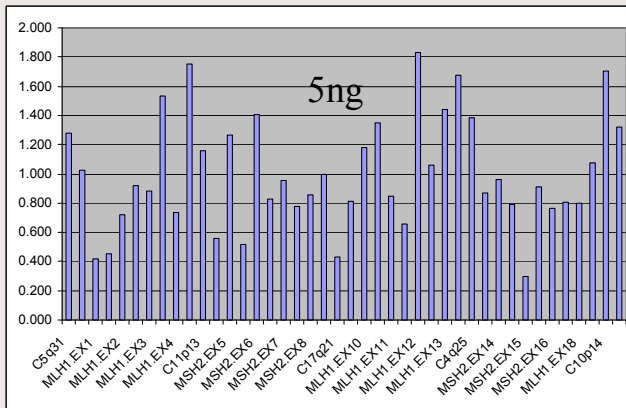
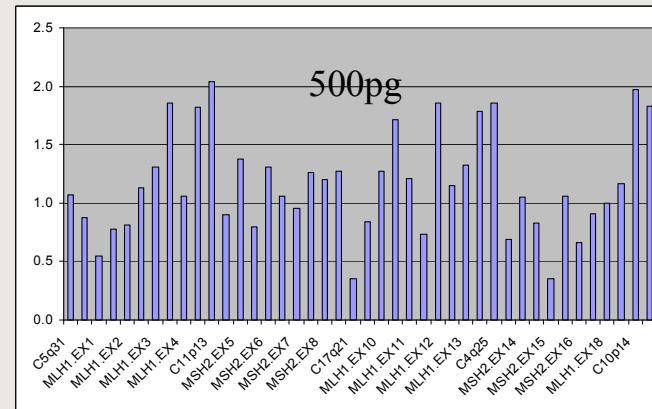
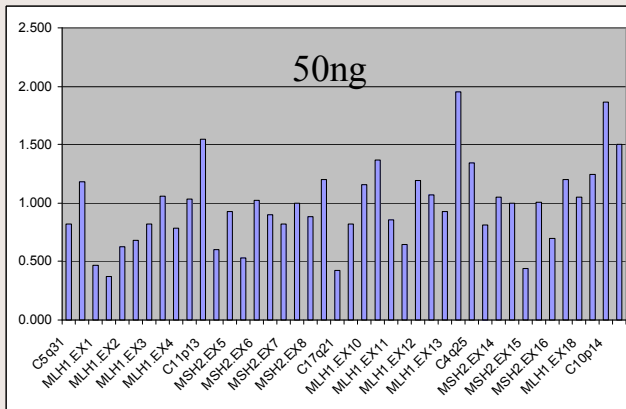
XXYY

| chromo | Neat NDG XXYY Average | 1/10 NDG XXYY Average | 1/100 NDG XXYY Average | 1/1000 NDG XXYY Average |
|--------|--------------------------|--------------------------|---------------------------|----------------------------|
| 05A | 1.001287544 | 0.758923887 | 1.128012501 | 0.961599959 |
| 21A | 1.188628486 | 0.96767647 | 0.587600974 | 1.298021592 |
| 18A | 0.991961886 | 0.51110271 | 0.77072566 | 1.00002933 |
| 13A | 1.018343286 | 0.659053886 | 0.632261949 | 0.76897546 |
| XA | 1.842392343 | 1.994952071 | 2.291704369 | 7.11333078 |
| 2 | 1.139542951 | 0.882748747 | 1.118941181 | 1.978899072 |
| 21B | 0.962150921 | 0.828154166 | 0.819826787 | 4.220319506 |
| 18B | 1.031416087 | 0.789190663 | 1.247607634 | 0.654451748 |
| 13B | 0.963126091 | 0.882350004 | 1.117635731 | 0.573801411 |
| YA | 1.858257331 | 1.568837478 | 1.84128791 | 1.541471351 |
| 15 | 0.920355292 | 0.730282429 | 0.7304192374 | 2.966455308 |
| 21C | 1.20775734 | 0.78112505 | 0.461124631 | 0.608974879 |
| 18C | 0.975289028 | 0.734435289 | 0.68891522 | 4.410009989 |
| 13C | 0.839759016 | 0.815037198 | 0.718307699 | 2.390935245 |
| XB | 1.751985741 | 1.171068593 | 1.381570098 | 2.499914732 |
| 8 | 0.994247512 | 0.91037631 | 1.363628915 | 2.63849817 |
| 21D | 0.997159019 | 0.888942023 | 0.831287572 | 0.760170397 |
| 18D | 1.328791708 | 1.222210398 | 2.584188231 | 3.996709886 |
| 13D | 1.05780868 | 0.826320865 | 0.708358908 | 3.748489121 |
| YB | 3.0251758 | 1.585040723 | 2.270969414 | 3.333758169 |
| 3 | 0.890573294 | 0.912013743 | 1.054068811 | 1.054115194 |
| 21E | 1.156284273 | 1.088574519 | 0.827229642 | 5.961908055 |
| 18E | 1.073281142 | 1.075556633 | 0.838569059 | 0.811983454 |
| 13E | 1.16033154 | 1.435885149 | 0.994830075 | 2.079615769 |
| XC | 2.74319170 | 3.308229118 | 1.773803056 | 2.599833746 |
| 01A | 0.987303044 | 1.297096561 | 1.030705479 | 0.94544913 |
| 21F | 1.065921908 | 1.202103839 | 0.750233652 | 0.467662773 |
| 18F | 0.982957014 | 1.484894192 | 0.748052641 | 1.934300014 |
| 13F | 0.994139803 | 1.394390768 | 0.654390803 | 0.727978467 |
| YC | 1.88805178 | 1.691017482 | 1.587470187 | 1.473029836 |
| 05B | 0.946545313 | 1.942234023 | 0.749332831 | 0.554508799 |
| 21G | 1.097783415 | 1.840126097 | 0.697286093 | 0.25228771 |
| 18G | 1.075671072 | 1.398648406 | 0.877734268 | 1.72840577 |
| 13G | 0.969429624 | 1.216331444 | 0.332000841 | 0 |
| XD | 2.461985396 | 2.910108492 | 1.232705129 | 4.090766822 |
| 01B | 1.191593329 | 2.130998314 | 1.21426281 | 0.467808642 |
| 21H | 1.03676624 | 2.239782094 | 0.852271981 | 1.040984012 |
| 18H | 0.967504535 | 2.081398868 | 0.741786573 | 1.450782986 |
| 13H | 1.25123647 | 2.742378998 | 1.931123268 | 0.723246353 |
| YD | 1.740013942 | 3.739979924 | 1.687449083 | 0 |

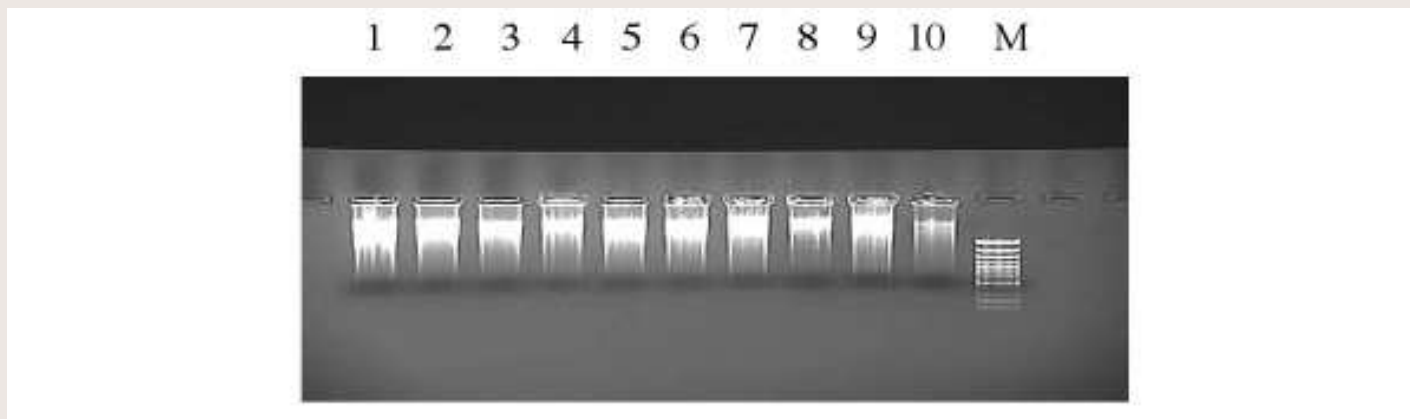
Ethanol Cleanup before MLPA: Trisomy



Ethanol Cleanup before MLPA: *MSH2* & *MLH1*

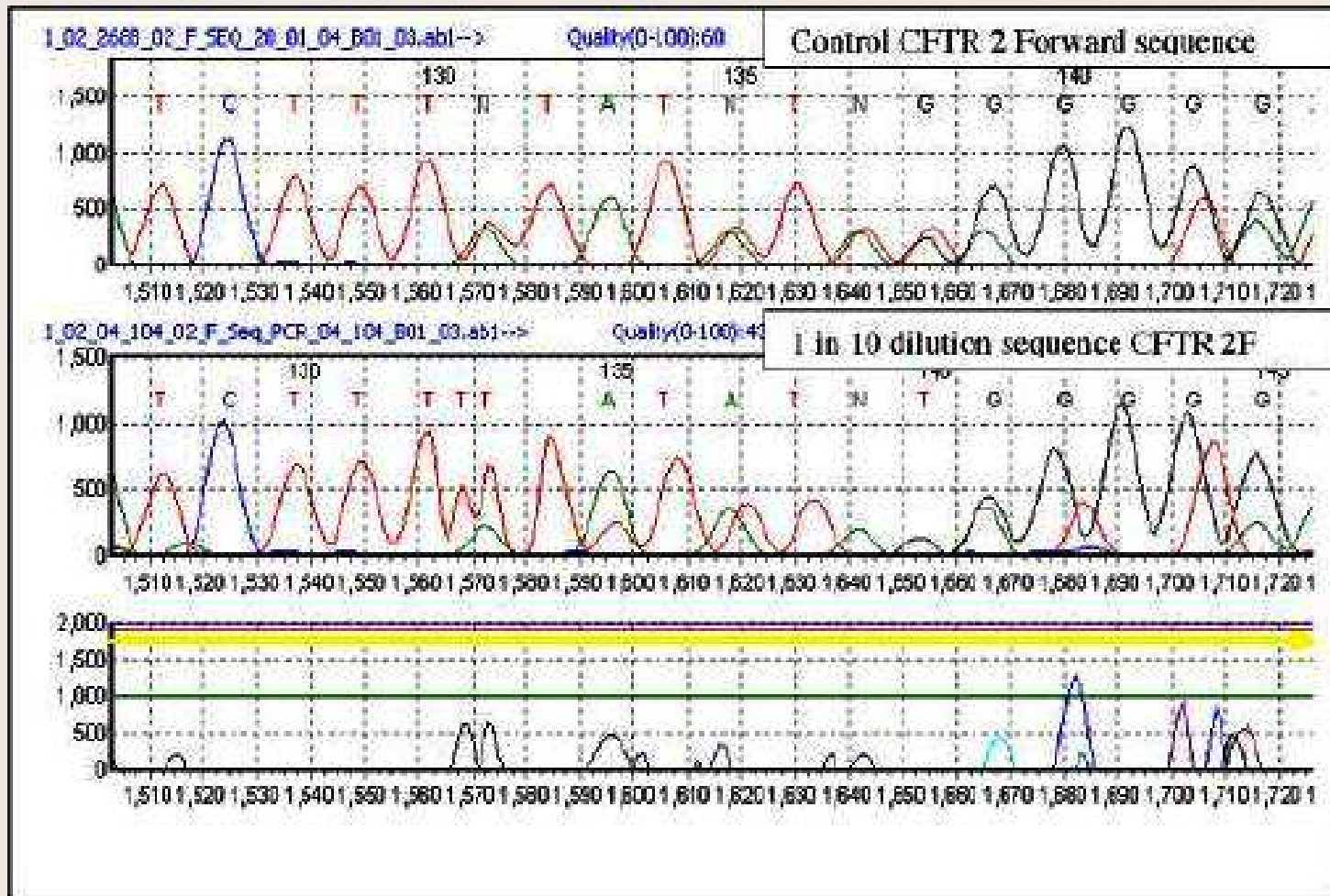


WGA then PCR for sequencing templates

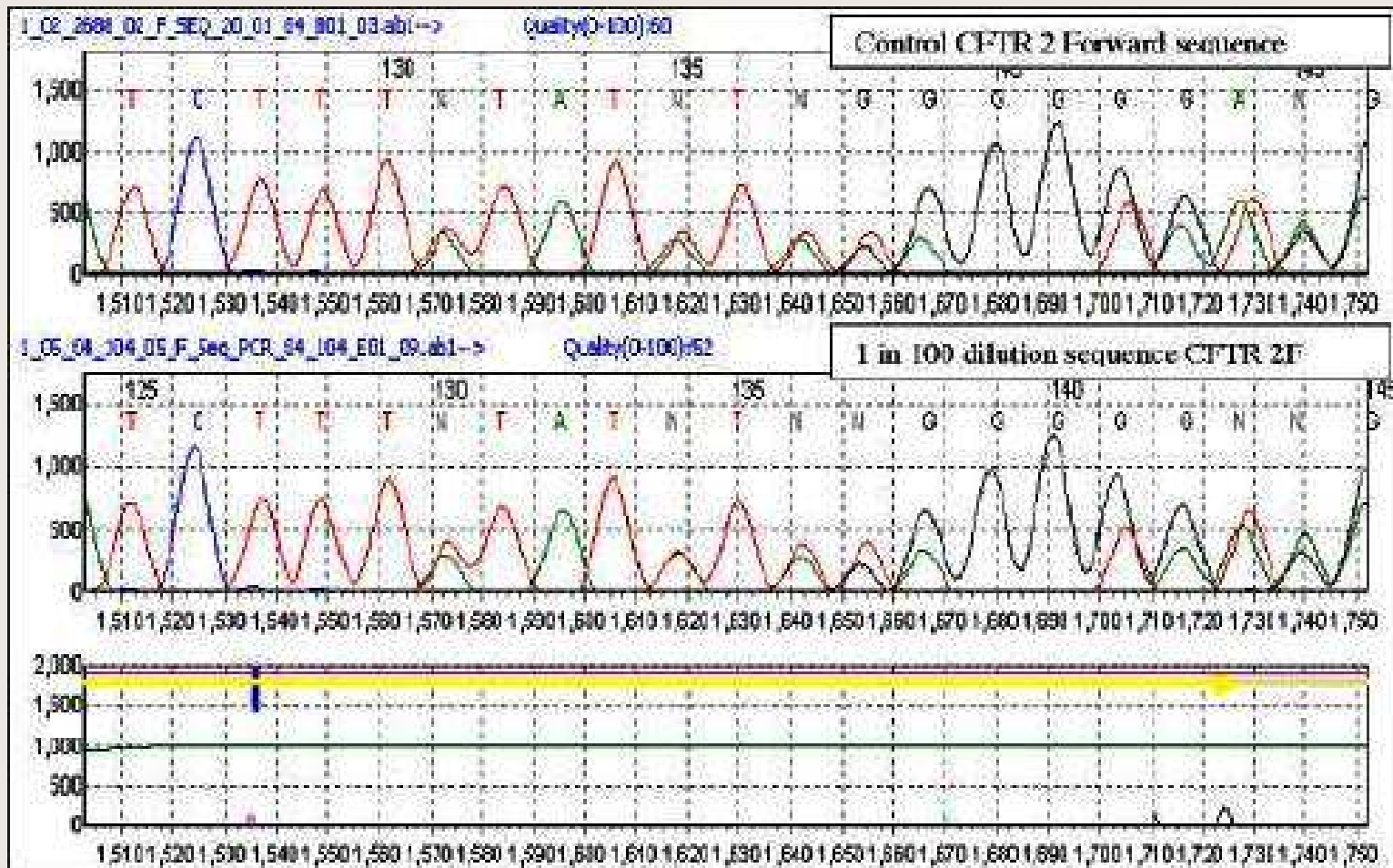


- 1-3 c.332C>T (P67L): 10^{-1} , 10^{-2} , 10^{-3}
- 4-6 c.394delTT: 10^{-1} , 10^{-2} , 10^{-3}
- 7-9 Wild type: 10^{-1} , 10^{-2} , 10^{-3}
- 10 -ve

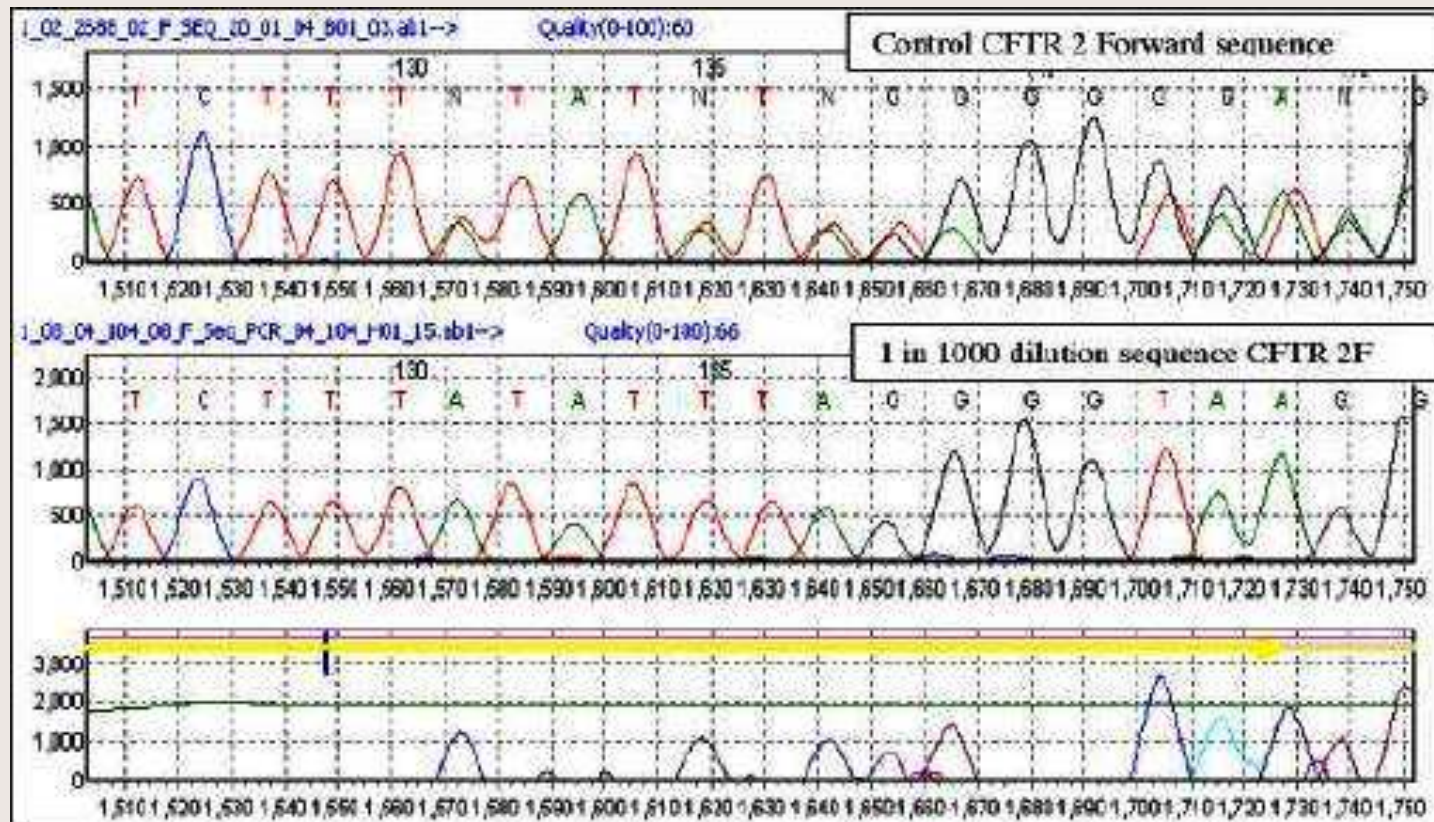
394delTT 1/10 vs genomic



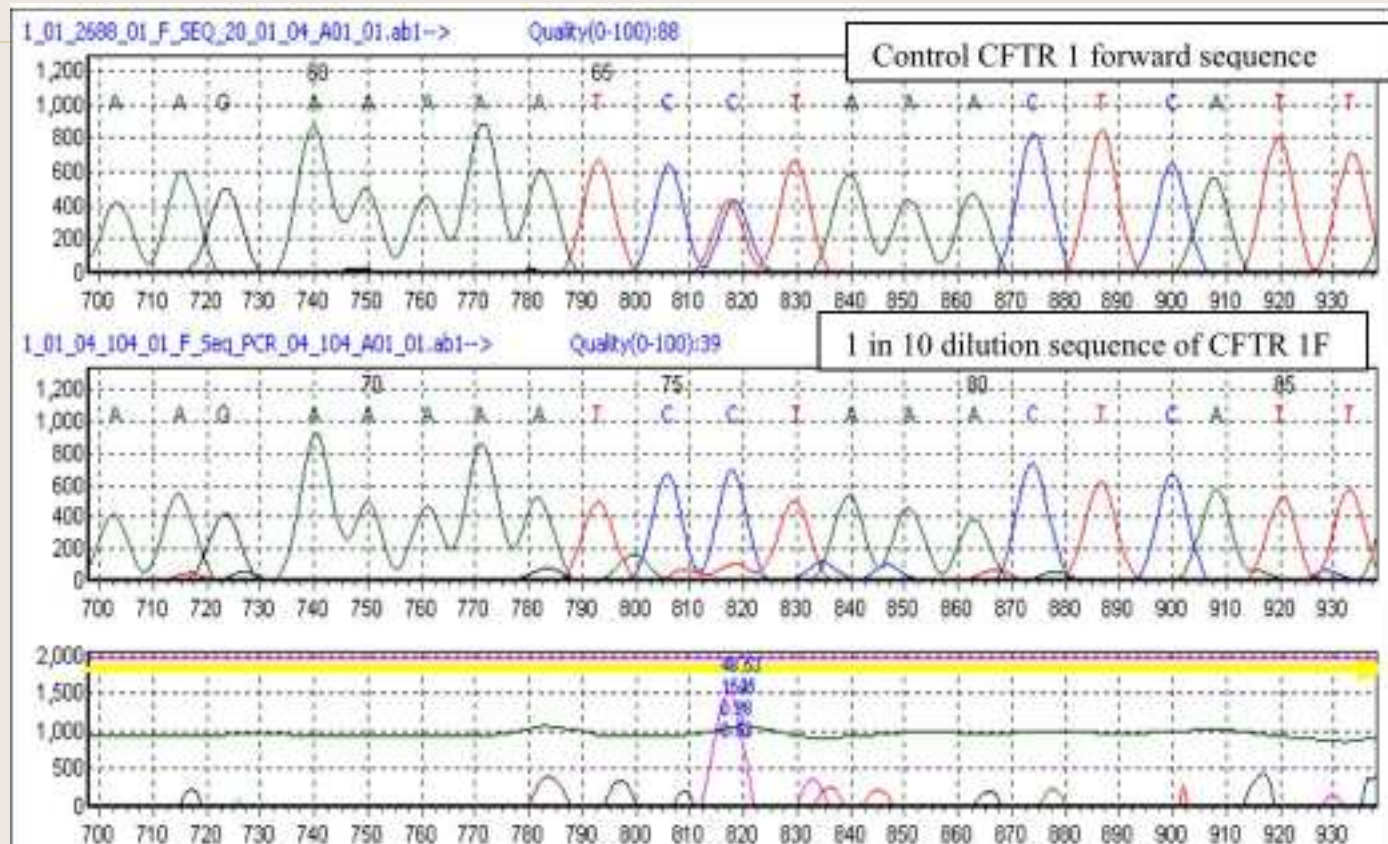
394delTT : 1/100 vs genomic



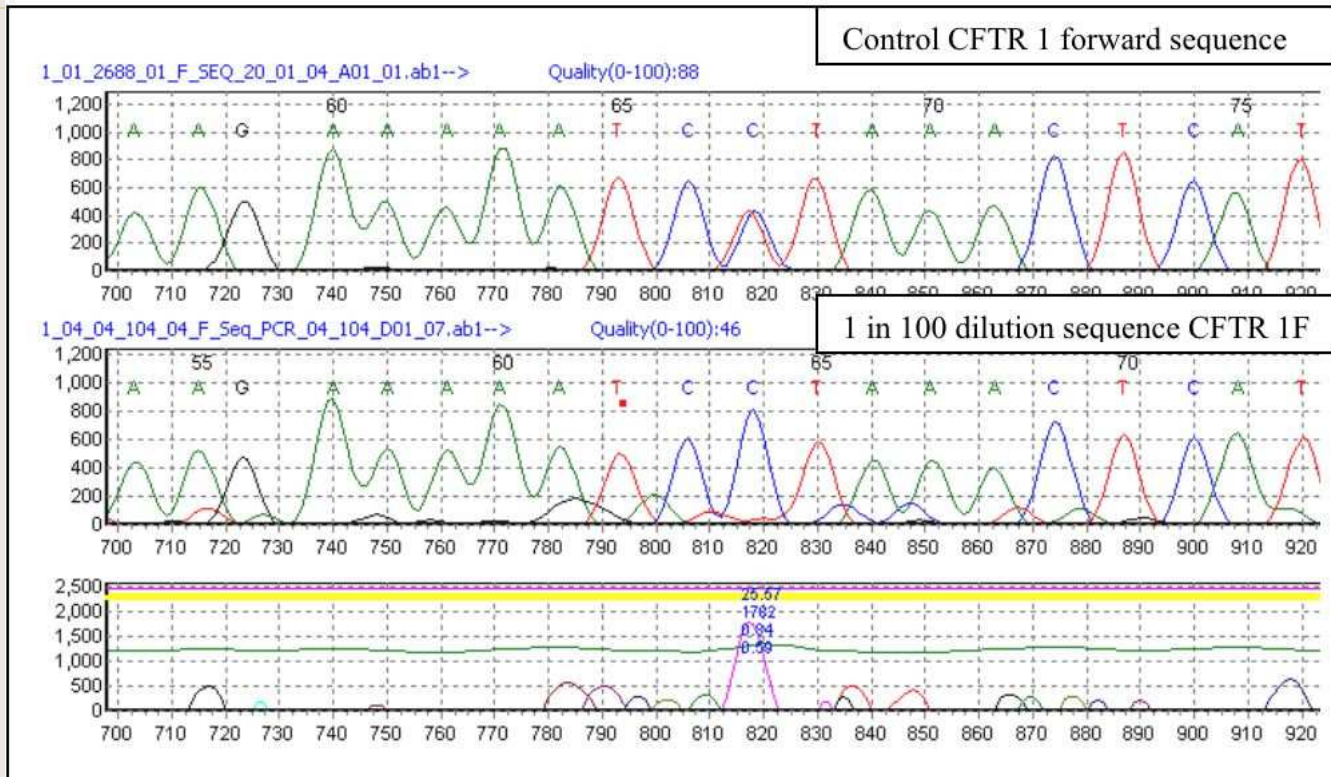
394delTT 1/1000 vs genomic



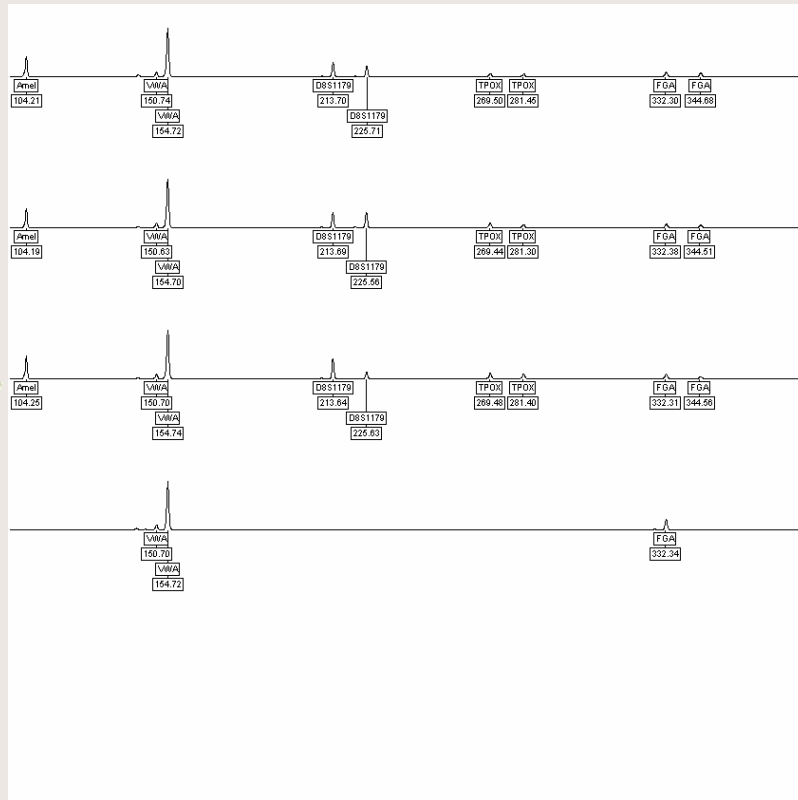
332C>T 1/10 vs genomic



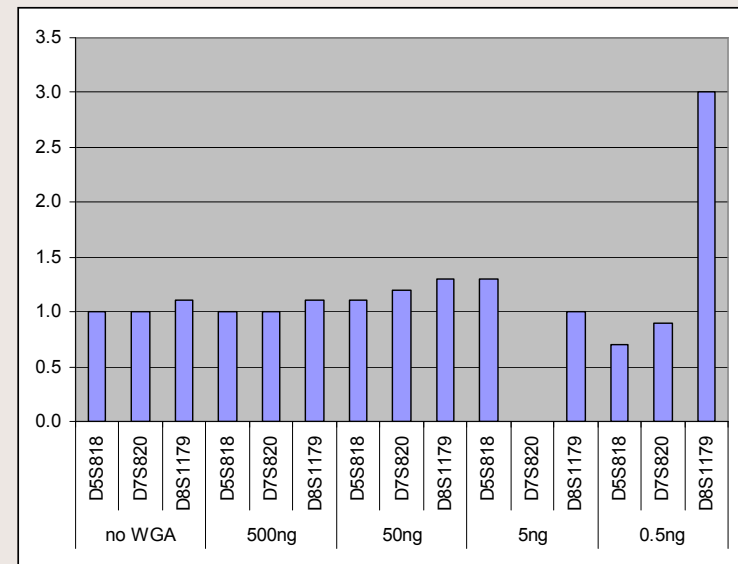
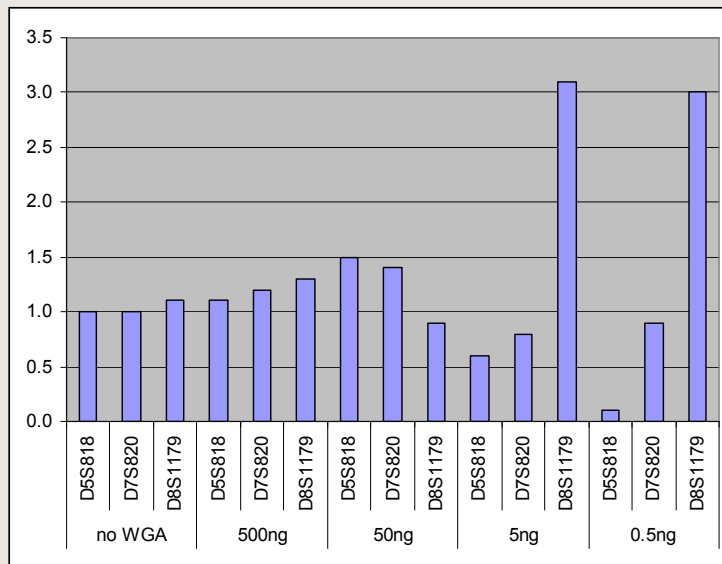
332C>T 1/100 vs genomic



Multilocus finger printing of WGA products

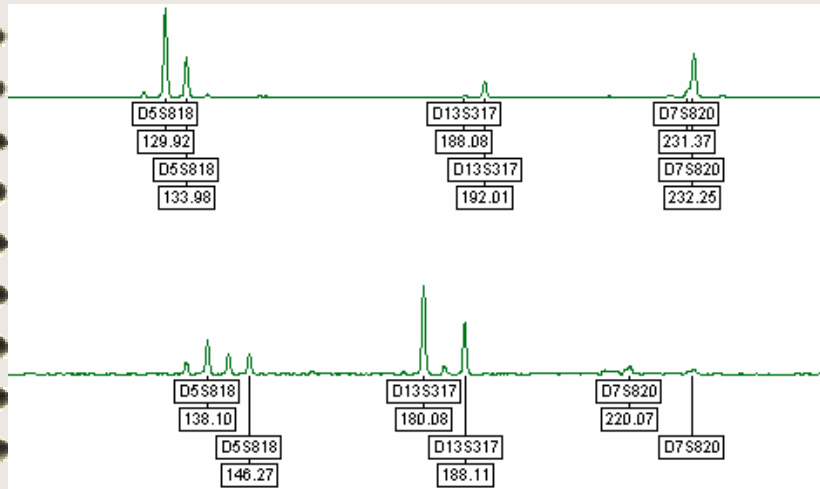


Peak Height Ratios of WGA products



- 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

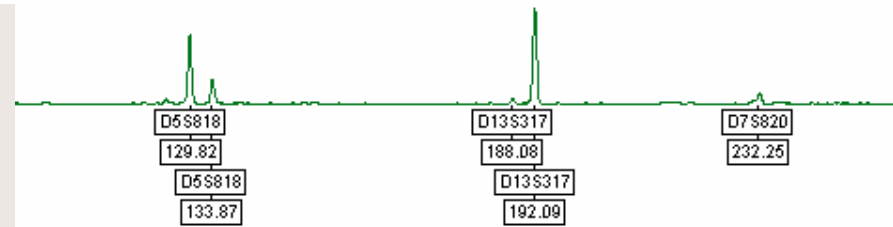
Fingerprinting of WGA products from Blood/Tumour pairs



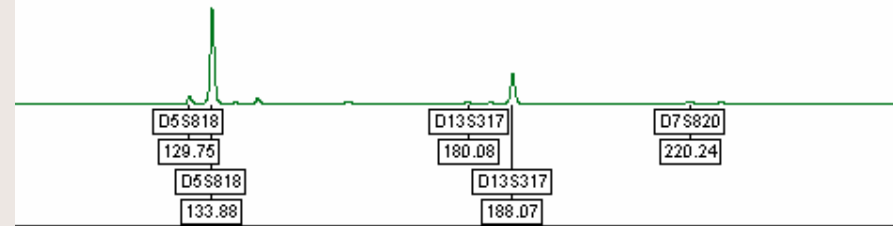
<-Tumour

<-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 quantity 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