

False positive MLPA deletion cases resulting from variable quality DNA

Helen Stuart

All Wales Molecular Genetics laboratory

Cardiff



MLPA service in Cardiff

Disorder (gene)	Current MRC Holland kit number
Breast cancer (<i>BRCA1</i>)	P002-C1
Breast cancer (<i>BRCA2</i>)	P045-B2
DMD+BMD (<i>DMD</i>)	P034-A2 and P035-A2
Ectodermal dysplasia (<i>EDA+EDAR+EDARADD</i>)	P183-B1
FAP (<i>APC</i>)	P043-B1
HNPCC/Lynch (<i>MLH1+MSH2</i>)	P003-B1
HNPCC/Lynch (<i>MSH6</i>)	P072-B1
Lissencephaly (<i>LIS1+DCX</i>)	P061-B2
LONG QT (<i>KCNQ1+KCNH2</i>)	P114-A2
Neurofibromatosis (<i>NF1</i>)	P081-B1 and P082-B1 and P122-B
Optic atrophy (<i>OPA1</i>)	P229-B1
Parkinson (<i>PARK2</i>)	P051-B1
Pitt Hopkins (<i>TCF4</i>)	P075-A1
Polycystic kidney disease (<i>PKHD1</i>)	P341-B1 and P342-B1
RETT+LUBS XLMR (<i>MECP2</i>)	P015-E1 (previously -D2 and -C2)
RETT-like (<i>CDKL5</i>)	P189-B1
RETT (congenital) (<i>FOXG1</i>)	P075-A1
SMA (<i>SMN1</i>)	P021-A1
TSC (<i>TSC1</i>)	P124-B1
TSC (<i>TSC2</i>)	P046-B2

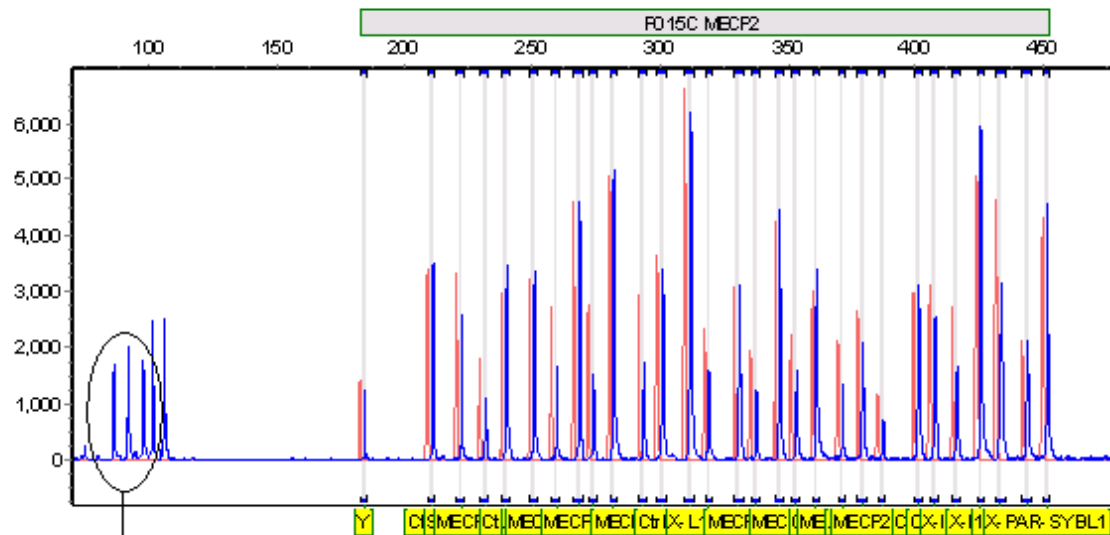


1 - *MECP2* patient referrals

- 3 males referred for diagnostic Rett syndrome or LUBS XLMR testing
- All referrals came from the same UKGTN lab
- DNA - Puregene
- Standard testing initiated:
 - *MECP2* sequencing and MLPA for Rett referrals
 - MLPA only for LUBS XLMR referrals
- Sequencing: normal
- MLPA all 3 patients (P015-C2): mosaicism for a deletion of Xq28 including *MECP2* gene.

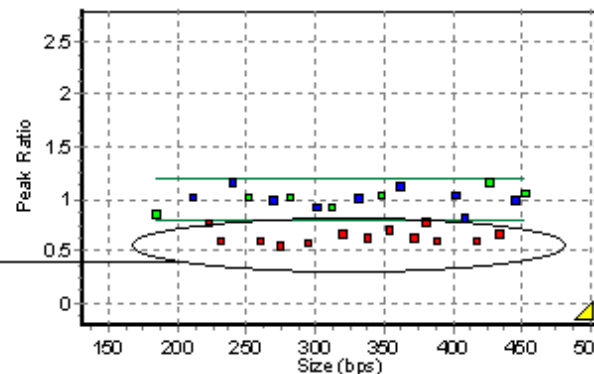
Example of mosaic MLPA result

13_0_9M4053_VWN_10.fsa - Dye: Blue



D fragments indicate efficient denaturation

0.5 < Dosage values < 0.8



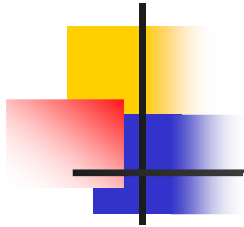
	Probe Name	Bin Size	13_0_9M4053_VWN
1	11q	282.1	1.024
2	12p	311.7	0.936
3	17p13	433.7	0.681
4	18q21	347.0	1.054
5	Ctrl01	210.9	1.027
6	Ctrl02	240.0	1.158
7	Ctrl03	268.3	1.001
8	Ctrl04	300.9	0.935
9	Ctrl05	331.2	1.008
10	Ctrl06	361.6	1.130
11	Ctrl07	401.4	1.041
12	Ctrl08	407.7	0.824
13	Ctrl09	444.0	0.989
14	MECF2 ex1a	293.7	0.589
15	MECF2 ex1b	387.2	0.616
16	MECF2 ex2	371.6	0.649
17	MECF2 ex3	353.5	0.702
18	MECF2 ex4a	337.6	0.638
19	MECF2 ex4b	231.5	0.613
20	MECF2 ex4c	274.0	0.560
21	MECF2 ex4d	259.4	0.617
22	SLC6A8	222.2	0.782
23	X-IDH3G	379.6	0.791
24	X-IRAK1	416.5	0.609
25	X-L1CAM	319.3	0.680
26	X-PAR-SYBL1	451.8	1.059
27	X-PARI	250.9	1.030
28	X-PARI	425.8	1.171
29	Y	184.5	0.861



Re-analysis of *MECP2* samples

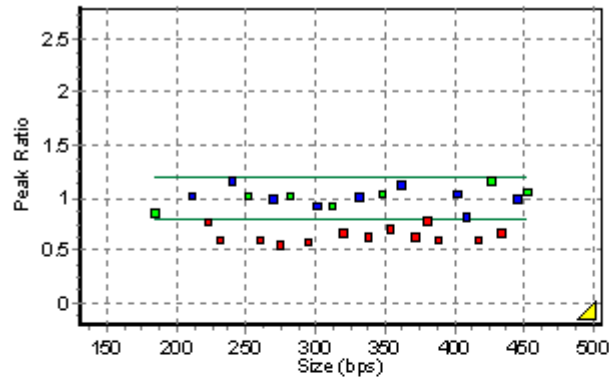
- Original and repeat samples using P015-C2 and P015-D2
- Mosaic Xq28 deletion results not reproducible
- Result dependent on:
 - DNA sample
 - MLPA kit version

Comparison of mosaic and normal *MECP2* MLPA results



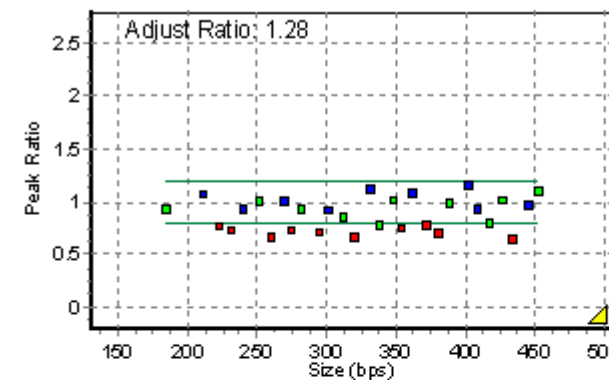
Patient 1 - sample 1 (Puregene)

Lab ref: 9M4053 09-2820



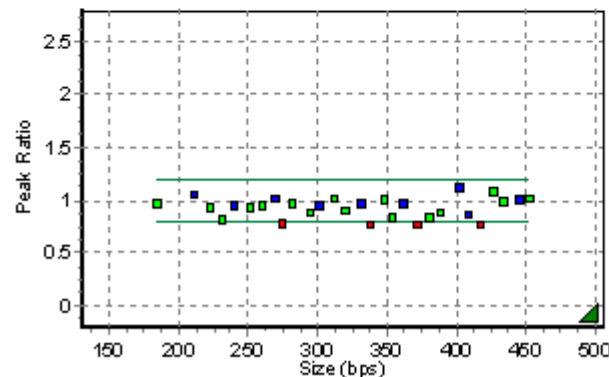
Patient 2 - sample 2 (Autopure), kit P015-C2

Lab ref: 10M2405 10-1263



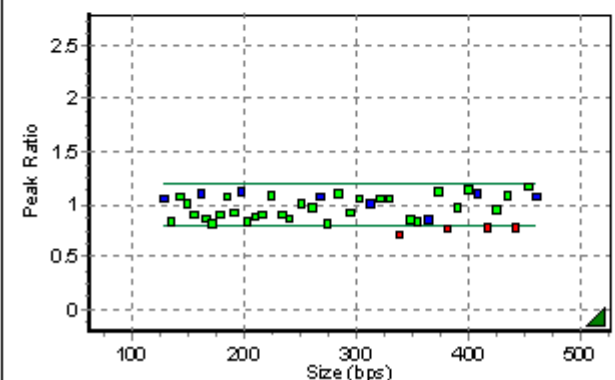
Patient 1 - sample 2 (Puregene)

Lab ref: 10M0539 10-760



Patient 2 - sample 2 (Autopure), kit P015-D2

Lab ref: 10M2405 10-1920



SAMPLE- SPECIFIC DIFFERENCES

KIT-SPECIFIC DIFFERENCES



MECP2 results summary

- Inconsistent presence of mosaicism for a deletion of Xq28 including *MECP2* gene.
- Attributed to:
 - Sample-specific differences
 - Kit differences
- Related to differences in:
 - Sample purity
 - Sensitivity of probes
 - Low 260:230 of ~ 1.5



2 - *TSC2* patient referrals

- 4 patients referred for diagnostic TS testing
- 3 referrals came from the same UKGTN lab
- DNA - Genecatcher magnetic bead
- 1 international referral
- Standard testing initiated:
 - *TSC1* & *TSC2* sequencing and *TSC2* MLPA
- MLPA all 4 patients: large heterozygous *TSC2* deletions extending to include exon 46 of *PKD1*. Including 1 patient with an apparent whole *TSC2* gene deletion.

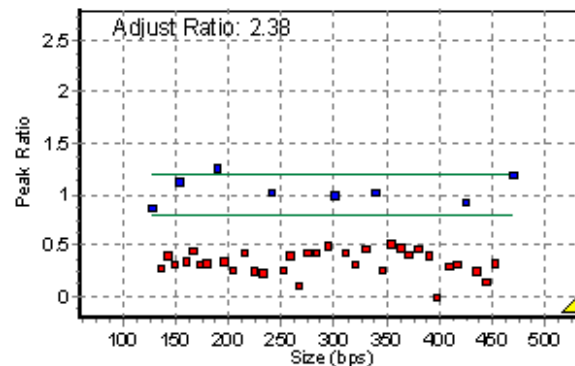


Re-analysis of *TSC2* samples

- Unusual results
 - Heterozygous deletion dosage values not at 0.5
 - Genotype did not fit with phenotype:
PKD1 deletion = renal involvement
- Repeat MLPAs using ethanol ppt DNA
 - All results normal

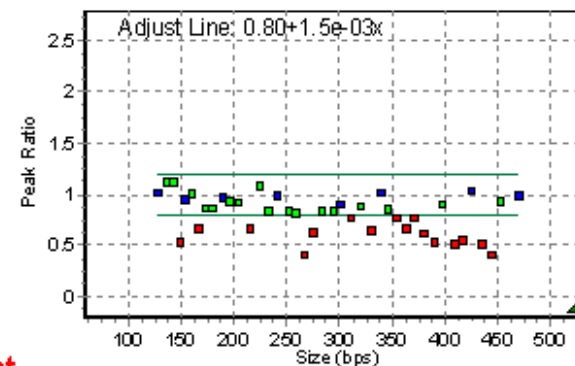
Comparison of deletion and normal *TSC2* MLPA results

Patient 1 - original DNA



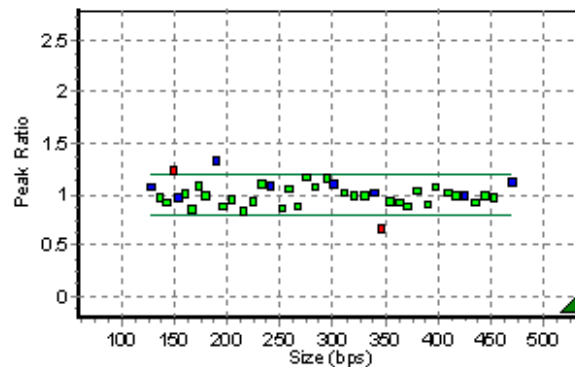
Absence of 88nt and 96nt D fragments

Patient 2 - original DNA

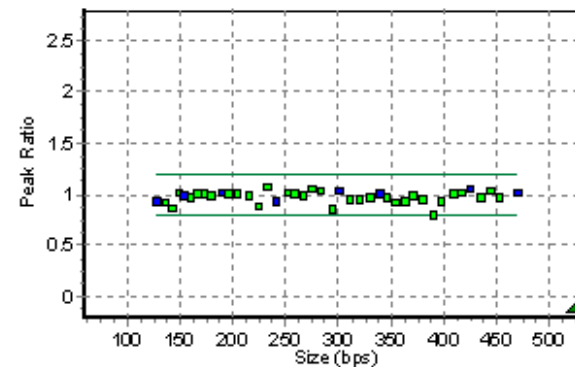


Reduction in 96nt D fragment

Patient 1 - ppt DNA



Patient 2 - ppt DNA



Conclusion: Inefficient DNA denaturation of original DNA sample, reflected by reduction in height of control D fragments, can cause false positive results. These can be rectified by using ppt DNA.



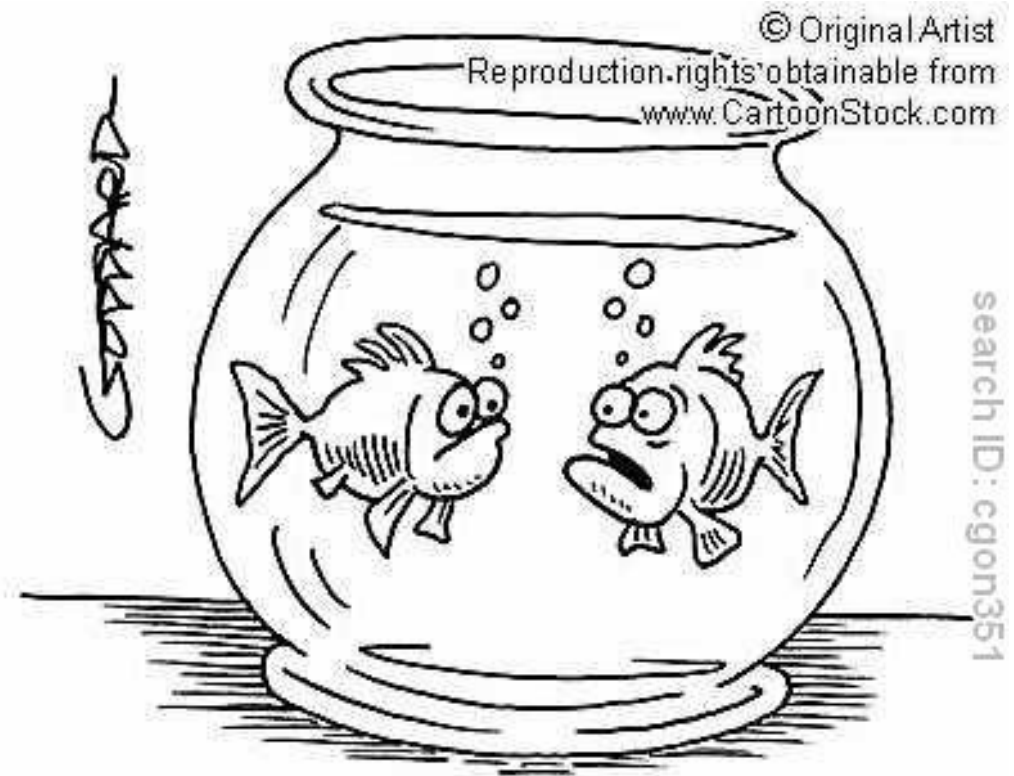
Conclusions

- Quality control fragments in MLPA kit must be checked
 - Q fragments – not visible
 - D fragments – 88 and 96nt not $< 1/3$ 92nt
- Sample purity is essential
 - 260:230 $\sim 2.0-2.2$
 - 260:280 ~ 1.8
- Ethanol precipitate DNA if necessary

With thanks to...

- Vicky Newsway
- Laz Lazarou
- Rachel Butler

- Everyone for listening!



"Pardon? Oh, sorry, I thought you were talking to someone else..."