Applications of Novel PCR Technologies that Provide Enhanced Molecular Characterization of the Fragile X Gene

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Fragile X associated disorders (FXD) impact a broad population and age range.

The Wheeler Family, ***Time*** Magazine, June 26, 2008

Grandfather, Gary:	**89 repeats, FX-TAS**
Mother, Cari:	**24/85**
Son, Max:	**336 repeats, FXS**

- What are the risk factors for expansion? For FX-TAS? For FX-POI?
- How can we simplify interpretation of results and resolve tough technical cases?
- Are there molecular markers of response to therapy?

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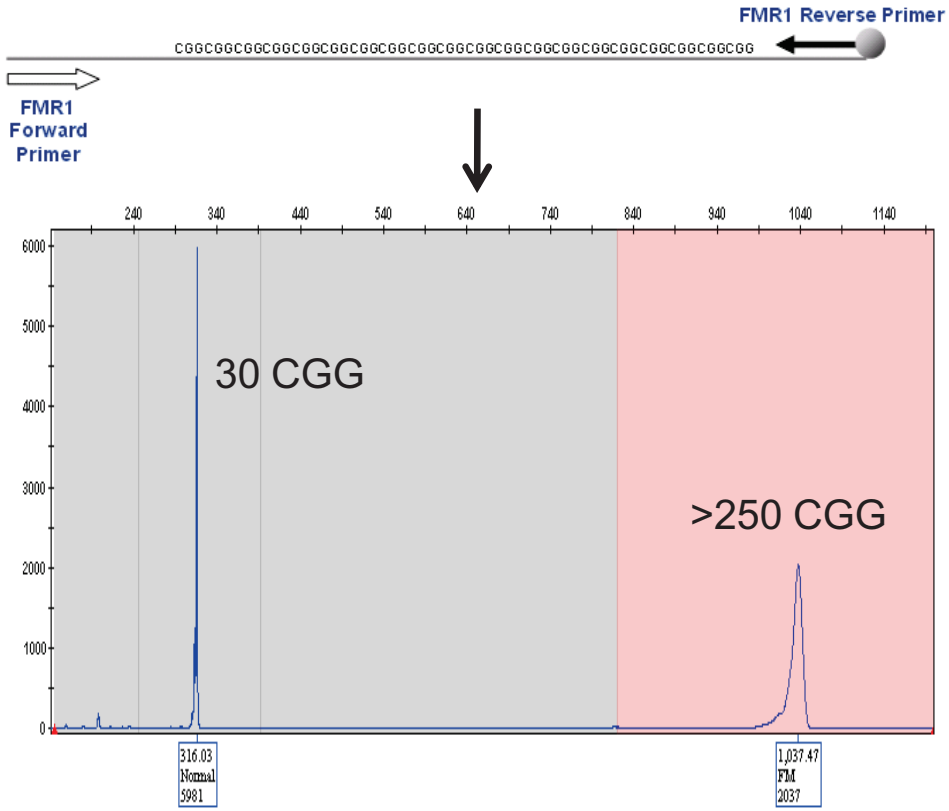
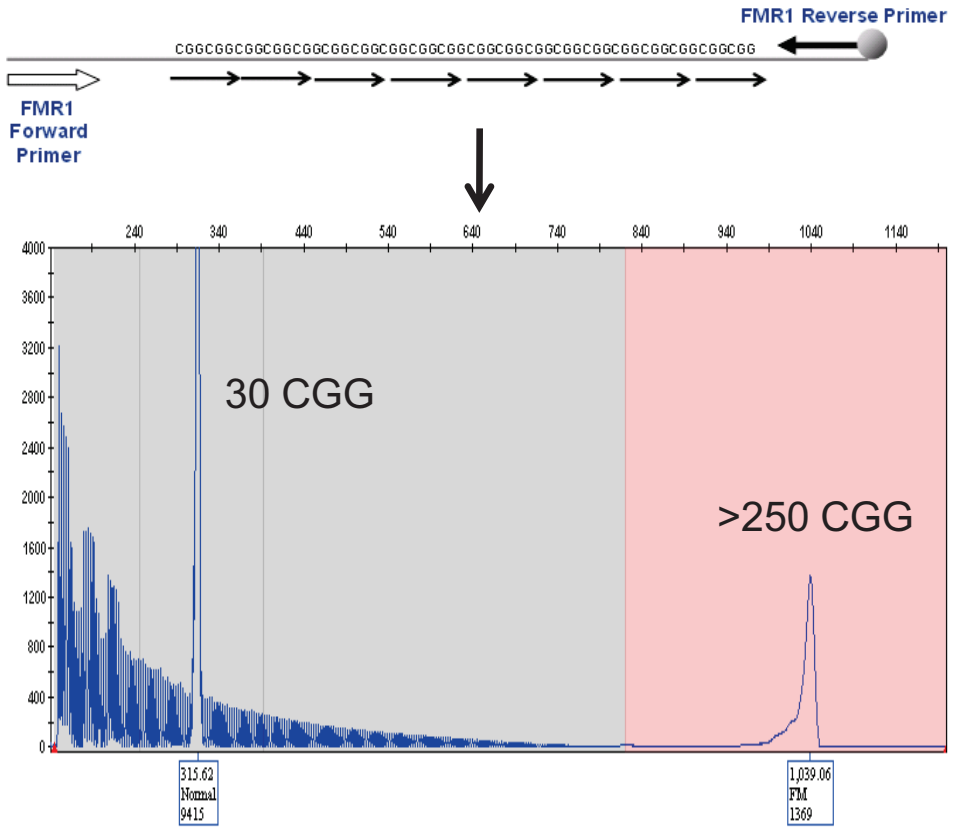
AmplideX™ *FMR1* PCR reagents quantify repeats and identify expanded alleles in all samples.

Gene-specific *FMR1* PCR



Clin.Chem., Mar 2010

CGG Repeat Primed *FMR1* PCR



J. Molec. Diagn. Sep 2010

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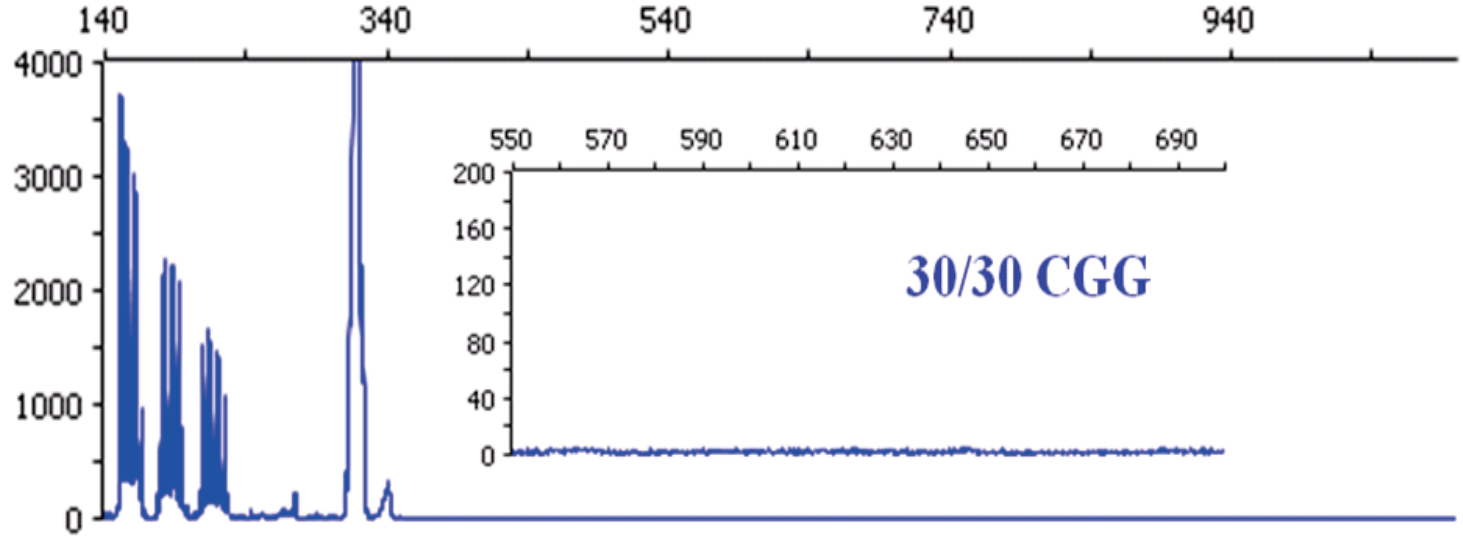
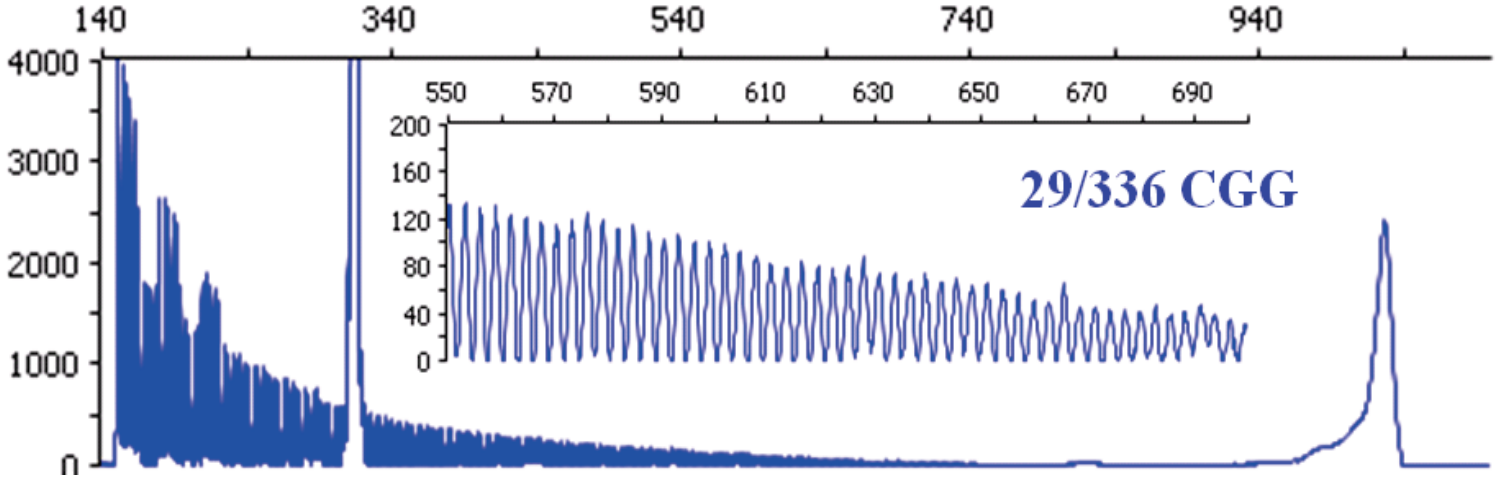
AmplideX™ *FMR1* PCR reagents provide an unmistakable signature of expanded alleles and resolution of zygosity.

Homozygous



Heterozygous



This capability significantly reduces the need for Southern blot analysis.

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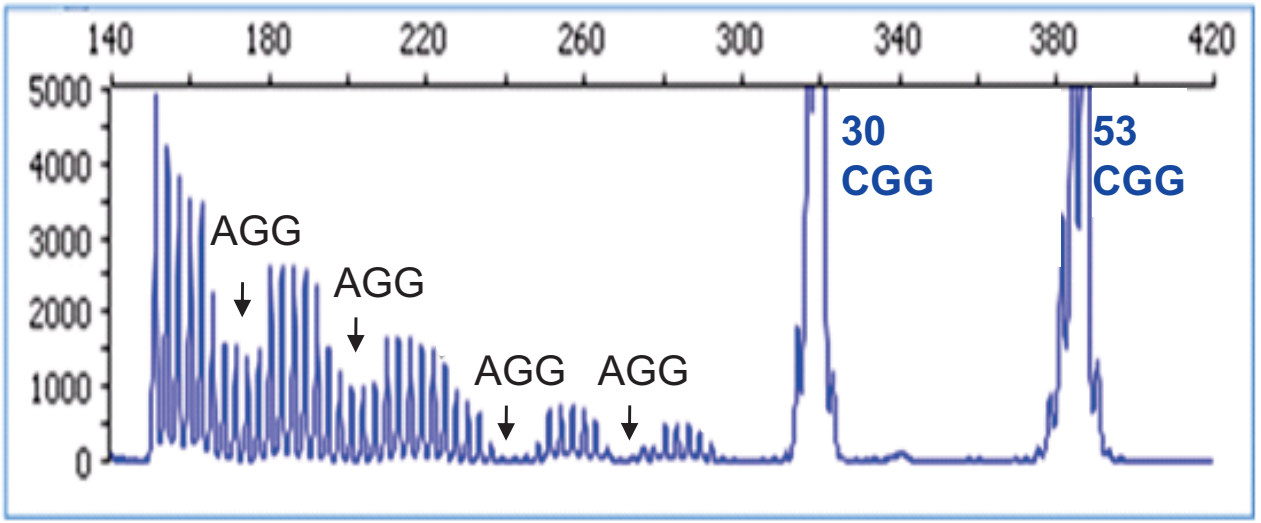
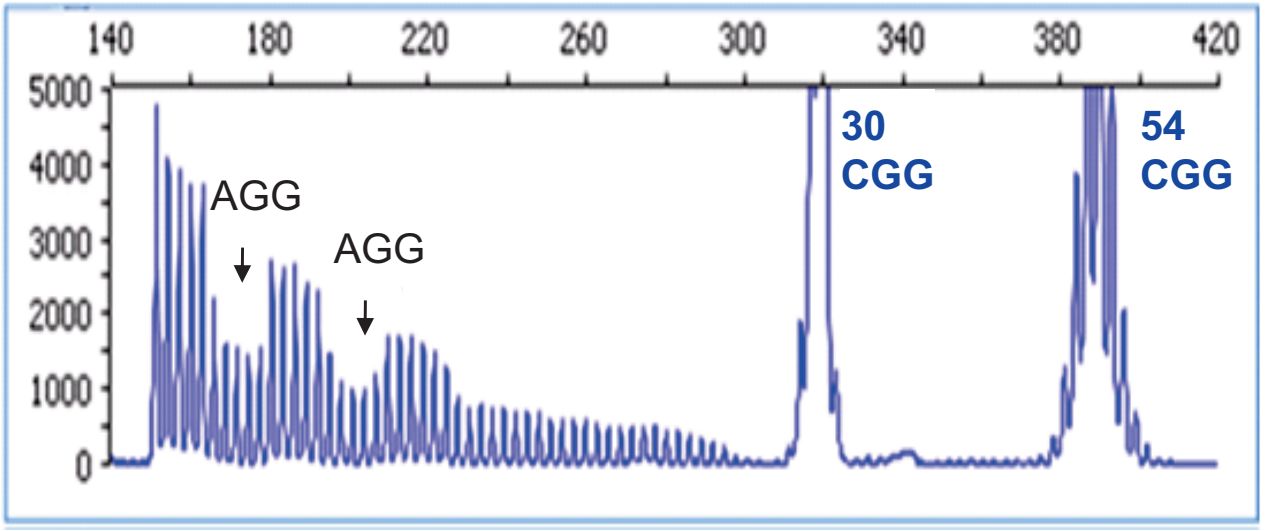
AGG mapping yields information about the potential risk for expansion.





Note that the number of CGG repeat peaks provides an unbiased, extremely accurate determination of the number of allele triplet repeats.

Is 54 CGG without AGGs at a higher risk for expansion than 54 CGG with AGGs?

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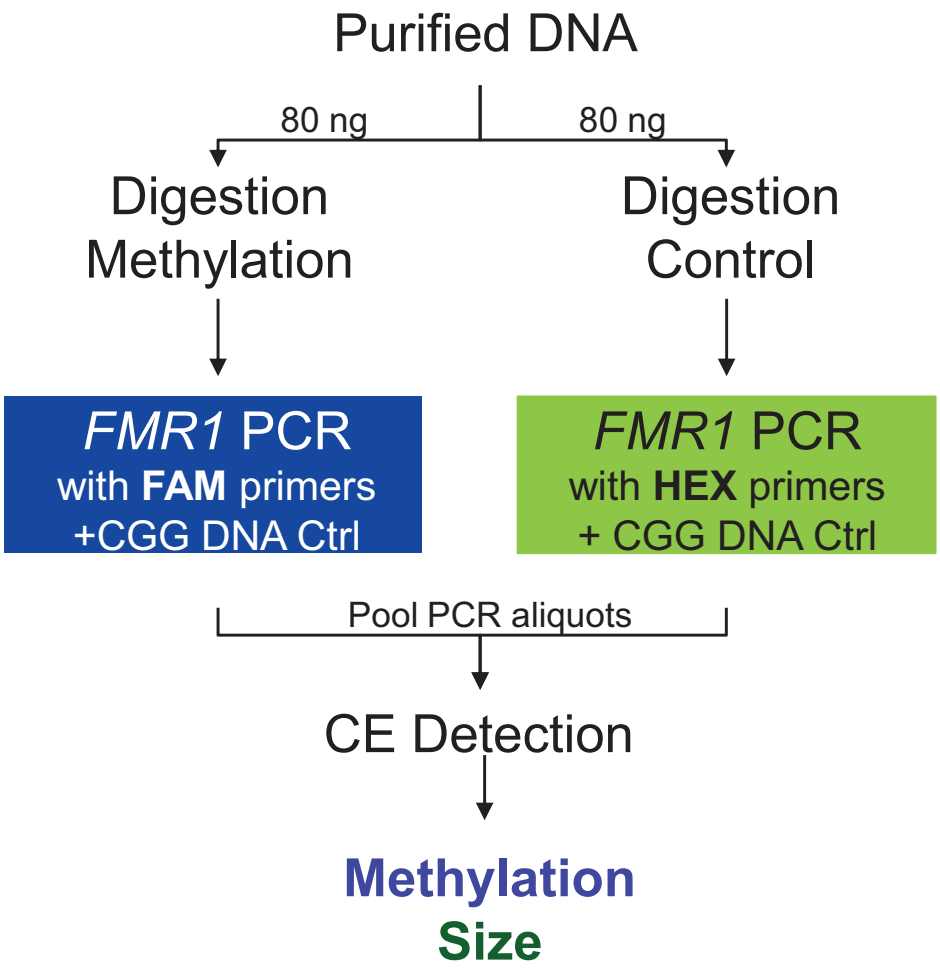
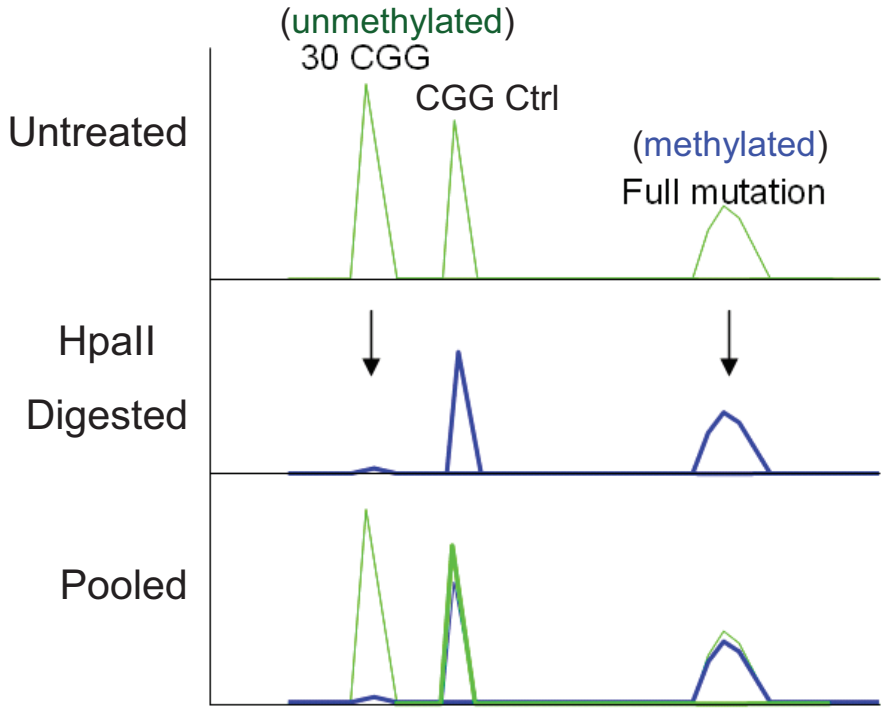
Amplidex™ *FMR1* Methylation PCR reagents and CE analysis determines methylation status for all alleles.





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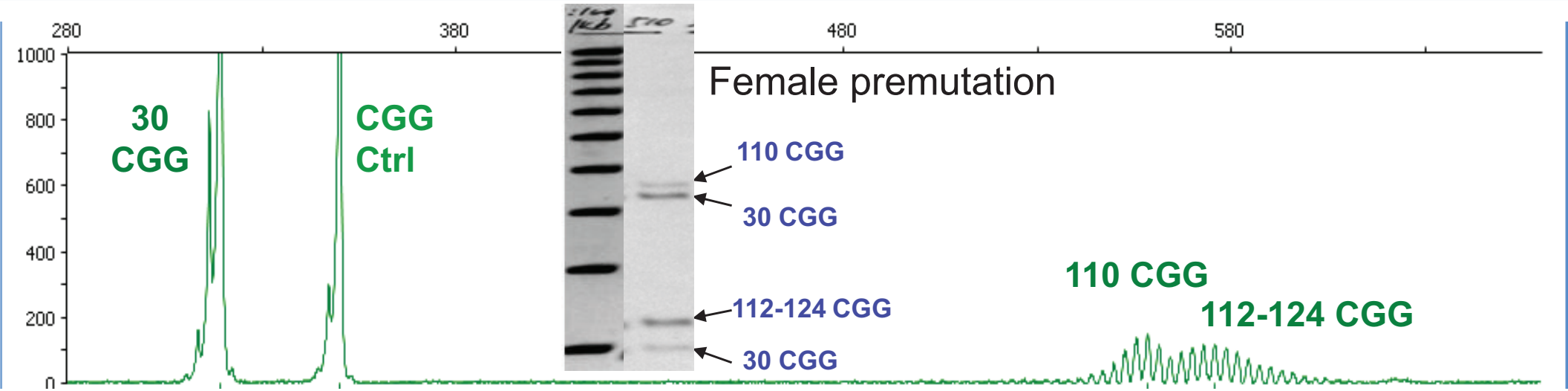
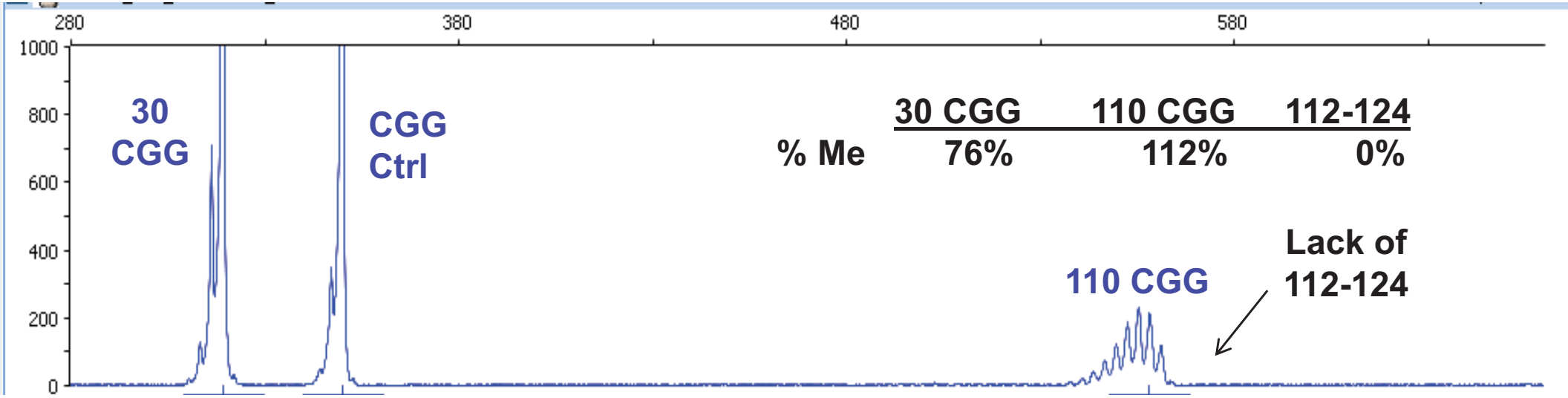
Methylation PCR Reveals Unexpected Allele-specific Methylation Patterns that cannot be Detected by Southern Blotting





What appears to be partial methylation of a single allele (~110 CGG) is actually completely skewed methylation of two alleles similar in size.

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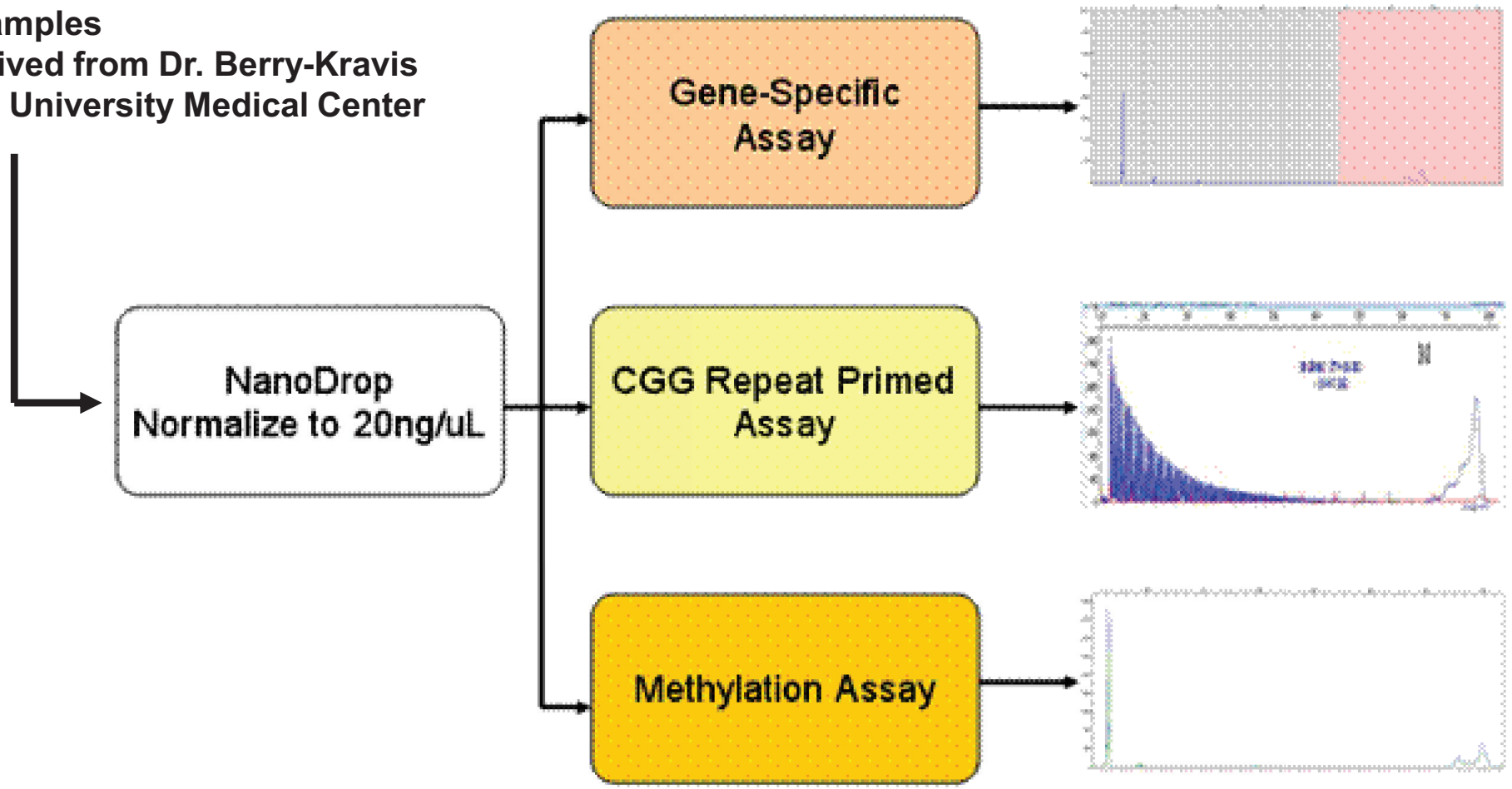
The workflow for 41 clinical samples combined results for each *FMR1* PCR method.

41 Samples
Received from Dr. Berry-Kravis
Rush University Medical Center



After amplification, each sample was analyzed on CE (3130*xl*, POP-7, 36 cm capillary) to enable accurate fragment sizing, AGG mapping, and methylation status.

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The pilot study results revealed concordance with previous methodologies and additional molecular information.

- Based upon Asuragen PCR analyses, the 41 specimens were correctly categorized using each PCR method.
- All full mutation alleles were detected.
- All alleles up to 200 CGG were accurately sized by CE.

CGG Repeats	Indication	Gene-specific	CGG Repeat	mPCR
>200	Full mutation	20	20	20
55-200	Premutation	10	10	10
45-54	Intermediate	10	10	10
<45	Normal	1	1	1
Total		**41**	**41**	**41**

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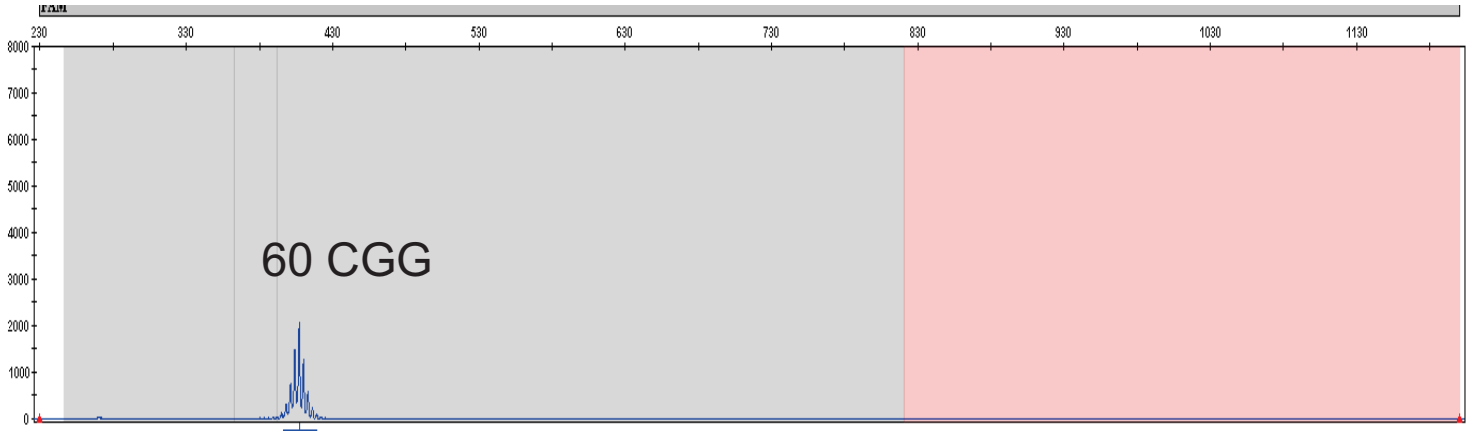
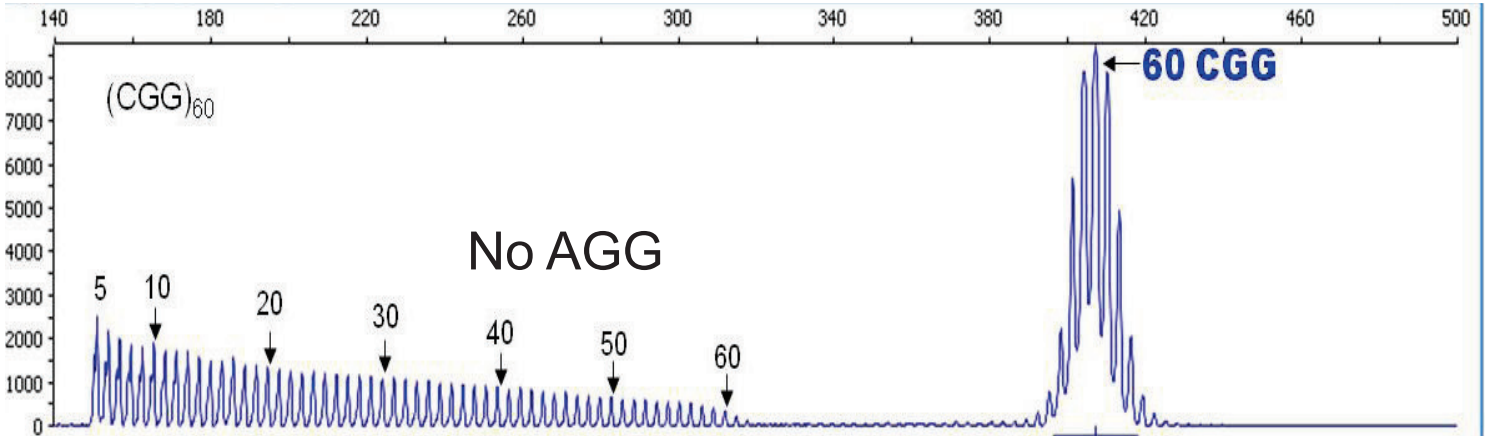
Case Study 1

Phenotype: Male sample with 60 CGG. Allele expanded to 78 CGG in daughter of this father, and then to a full mutation in grandson.

g.s. PCR



CGG RP PCR



Outcomes: CGG PCR can highlight consecutive CGG and flag samples of questionable stability.

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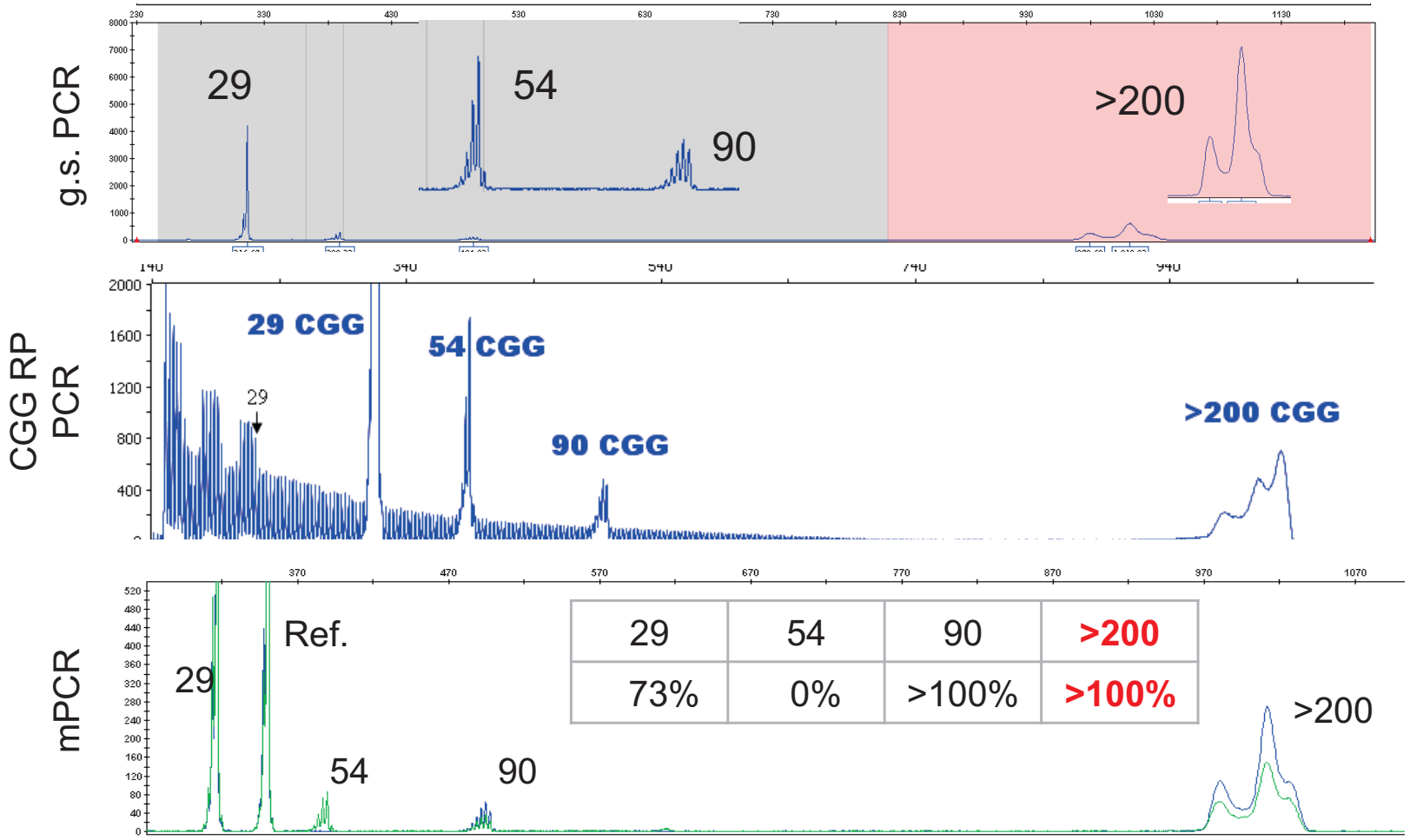
Case Study 2

Phenotype: Low functioning female, 65 IQ, referred for fragile X testing.



29	54	90	>200
73%	0%	>100%	>100%

Outcomes: Phenotype consistent with genotype. Expanded CGG repeat detection and assessment of methylation without Southern blot.

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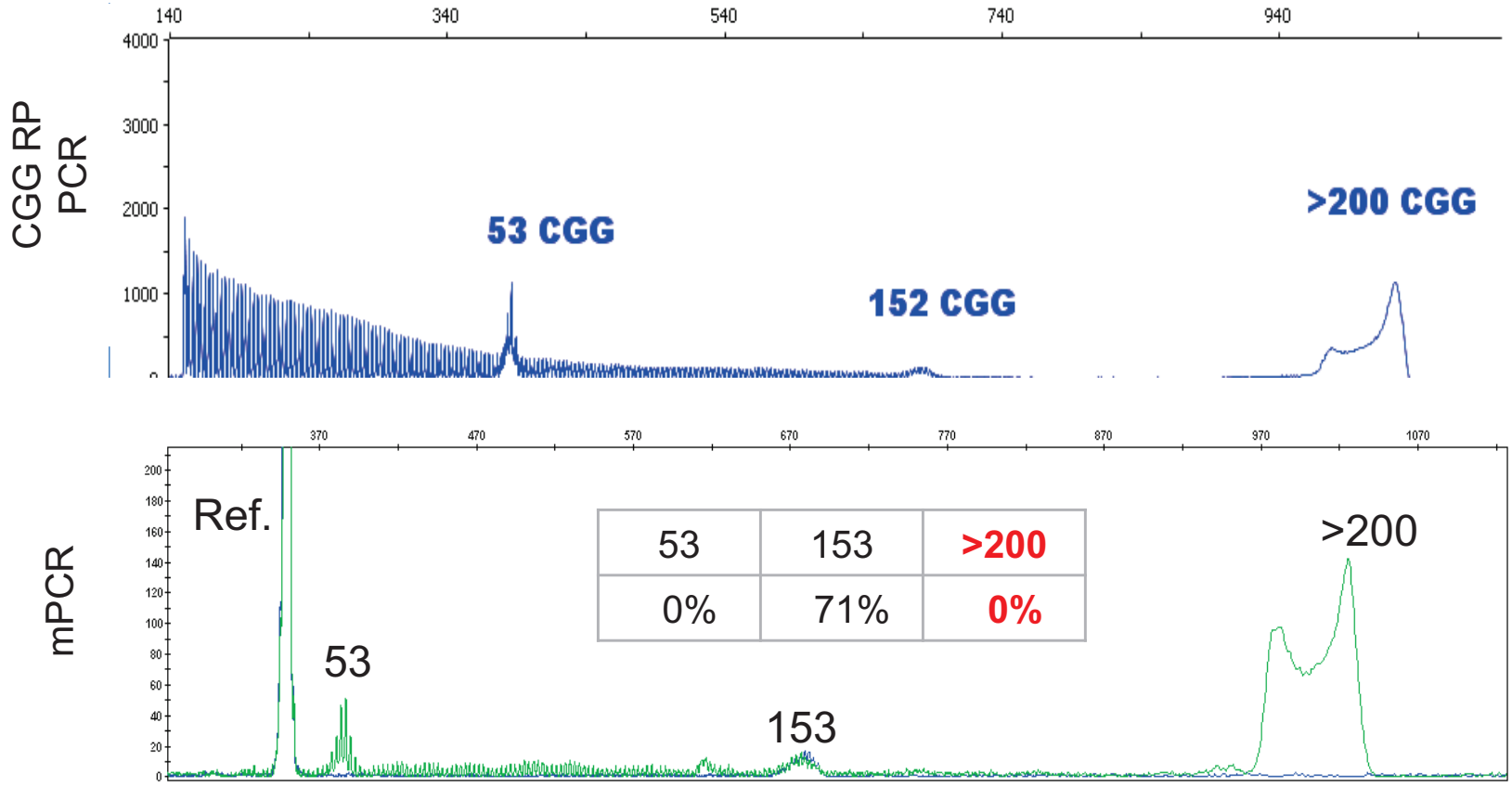
Case Study 3

Phenotype: Male in his 50s. Normal cognitive function, mild anxiety. Referred from sister who has FXS. Daughter has a premutation allele.



Outcomes: Phenotype consistent with genotype. mPCR was able to assess full dynamic range of size, relative abundance and methylation status of each allele.

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Acknowledgments and Collaborators

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