Fragile X Application in GeneMarker® Software: Linked Calculation of Triplet CGG Repeats and Percent Methylation

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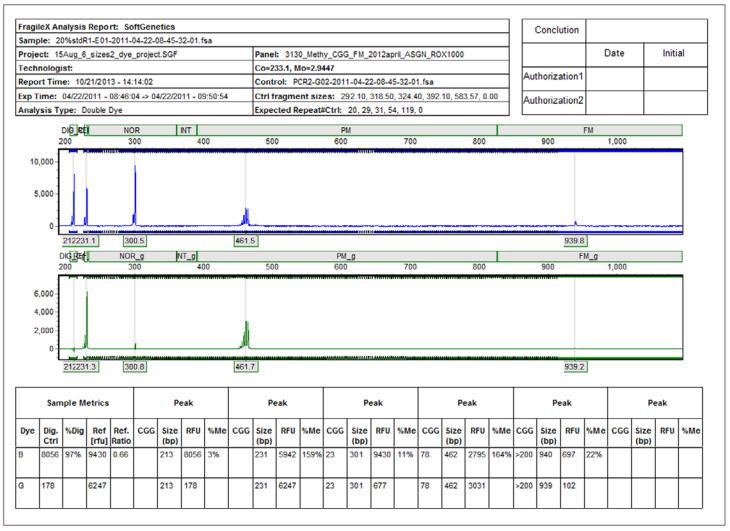
Introduction

GeneMarker software is an excellent tool for the analysis of DNA fragment data from fragile X syndrome (FXS) and associated disorders. Fragile X syndrome (FXS) is caused by expansions of a CGG triplet repeat in the FMR1 gene on the X chromosome; similar to trinucleotide expansions associated with other diseases such as Huntington's disease[1] and myotonic dystrophy. In studies of FXS, the number of repeats and methylation status of the gene are associated with a constellation of disorders that impact a broad range of ages and populations. In the United States, repeat lengths less than 44 CGG are considered normal and at low risk for expansion, repeats between 45-54 CGG are intermediate, 55-200 CGG are considered premutation and alleles greater than 200 CGG are considered full mutation. Premutation alleles are associated with increasing risk of expansion in their children and ovarian insufficiency (FXPOI) in women and tremor and ataxia disorders (FXTAS) in older males. Approximately 1.5 million people in the US are at risk for premutation associated disorders. Fully expanded alleles are typically hypermethylated, resulting in the fragile X phenotype through inactivation of the FMR1 gene. However, the degree of methylation and mosaicism can impact the behavioral and cognitive capabilities of people with FXS. Thus accurate determination of repeat length and methylation status are important to characterizing FMR1 associated disorders.[2] Recent advances in PCR methods for the FMR1 gene have allowed researchers to directly determine repeat length and methylation status using a two-color PCR approach following restriction digestion.[3]

GeneMarker is a user-friendly tool for rapid and accurate analysis of single dye or two dye data associated with FXS. The linked Fragile X application avoids the potentially error prone step of data transfer, automatically coordinates edits in Control and Digestion channel for two-dye projects and performs the repetitive calculations for converting fragment size to repeat length (Figures 1 and 2) and calculating percent methylation (Figure 1).

Figure 1: Final sample report from two –dye methylation chemistry includes: Header with details from user management and analysis parameters, Conclusion/Authorization box, Electropherogram and report table. The report table contains the sample metrics for the digestion control and reference fragment, the calculated number of triplet repeats, size of fragment in base pairs, peak height and calculated percent methylation for each fragment.



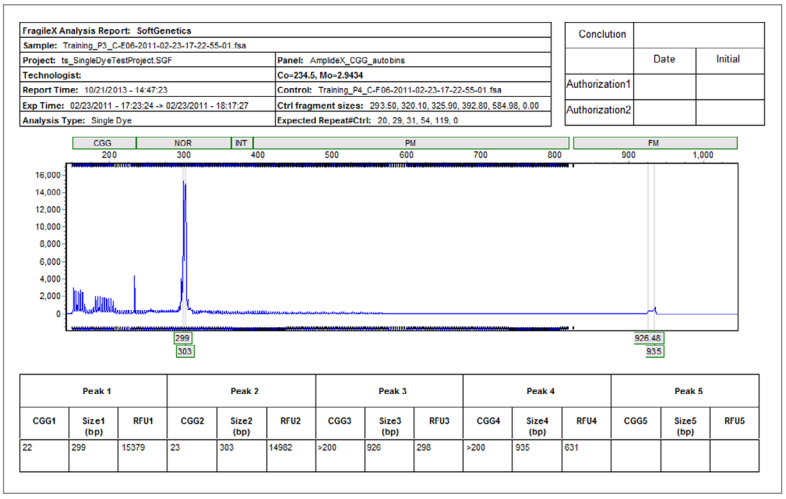


Figure 2: Final sample report from a single dye chemistry to calculate the number of CGG repeats for each fragment, including a similar Header, Conclusion/Authorization box, Electropherogram and Report Table with the calculated number of triplet repeats, size in bp and peak height for each fragment.

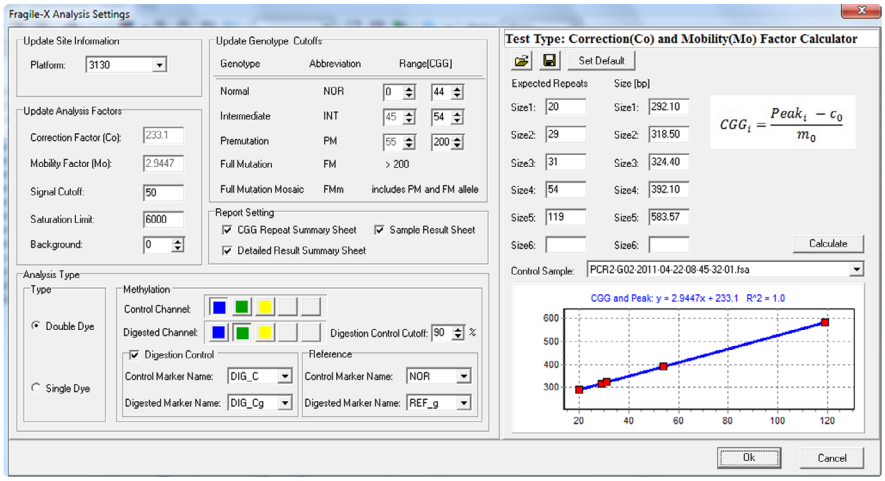


Figure 3: Parameters for Fragile X analysis are easily customized for single dye or two dye projects. Control samples with known repeat sizes can be selected from the drop-down menu; or a .txt file can be imported with the expected repeat and bp size for the application. Co and Mo factors are automatically calculated when the control sample is selected.

Job ID: 15Aug_6_sizes2_dye_project
Operator:
Data Processed: 10/21/2013 - 14:34:07
Sample: 11

Signal>Saturation Limit(FAM)
Signal<Signal Cutoff
Missing/Mismatched Peak Information
Dig Ctrl.<Digestion Control Cutoff
* Missing Dig.Ctrl information and/or invalid Size ranges.

ID	Sample Metrics		Allele Ranges Detected				Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6		Peak 7	
Sample File	%Dig	Ref. Ratio	Nor	Int	PM	FM	CGG1	%Me1	CGG2	%Me2	CGG3	%Me3	CGG4	%Me4	CGG5	%Me5	CGG6	%Me6	CGG7	%Me7
0%stdR1-F01-2011-04-22-08-45-32-01.fsa	93%	0.68						7%		100%	23	13%	78	100%	>200	0%				
100%stdR1-A01-2011-04-22-08-45-32-01.fsa	92%	0.57						8%		100%	23	15%	78	100%	>200	100%				
20%stdR1-E01-2011-04-22-08-45-32-01.fsa	97%	0.66						3%		100%	23	11%	78	100%	>200	22%				
40%stdR1-D01-2011-04-22-08-45-32-01.fsa	92%	0.62						8%		100%	23	20%	77	100%	82	1%	>200	83%		
60%stdR1-C01-2011-04-22-08-45-32-01.fsa	91%	0.63						9%		100%	23	11%	78	100%	82	0%	82	0%	>200	100%
80%stdR1-B01-2011-04-22-08-45-32-01.fsa	93%	0.76						7%		100%	23	19%	78	100%	>200	100%				
80%stdR2-B02-2011-04-22-08-45-32-01.fsa	93%	0.65						7%		100%	23	16%	78	100%	>200	100%				
NTCR1-H01-2011-04-22-08-45-32-01.fsa	100%	*						0%		0%										
NTCR2-H02-2011-04-22-08-45-32-01.fsa	100%	*						0%		0%										
PCR1-G01-2011-04-22-08-45-32-01.fsa	70%	0.54						30%		100%	20	18%	29	38%	54	12%	119	7%		
PCR2-G02-2011-04-22-08-45-32-01.fsa	**%	0					20	NAN	29	NAN	31	NAN	54	NAN	119	NAN	199	NAN		

Figure 4: Project Summary report example; color coded for fragments that did not meet specified parameters.

Discussion

Analysis of Fragile X and other triplet repeat fragment data is challenging due to the variable migration of large fragments. GeneMarker solves the sizing problem with a linked Fragile X analysis application and unique size calling algorithms capable of addressing this migration variability. The user-friendly interface enables researchers to review/edit allele calls, enter comments, and select from a variety of report formats. User management provides an audit trail and password protected control of access rights. The user friendly Fragile X application has an extensive settings dialog box which provides the flexibility to meet the specific needs of each laboratory (Figure 3). A summary report (Figure 4) with color coding of peaks that did not meet specified parameters can be printed or saved for each project. The software is compatible with data files from all major capillary electrophoresis systems (ABI PRISM®, Beckman-Coulter™ and MegaBACE™), and Windows® XP, Vista, 7 and 8 operating systems.

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References

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