Repeat Expansion Analysis in GeneMarker® software: Streamlined workflow for custom or commercial chemistries of tri- and hexa- nucleotide repeat data, including Huntington's Disease (HTT), Amyotrophic Lateral Sclerosis/Frontotemporal Dementia (ALS, C9ORF72) and Dystrophia Myotonica Protein Kinase (DMPK)

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Introduction

Expansions of simple sequence repeats, mainly but not limited to tri-nucleotide repeats, are responsible for over 40 human diseases.[1] In general, an increasing number of repeats results in more severe phenotype and the number of repeats increase (expand) as the disease gene is inherited. [2]

GeneMarker is a user-friendly tool for rapid and accurate genotyping of repeat expansion data (Figure 1). The new linked Repeat Expansion Application which

- avoids the potentially error prone step of data transfer.
- provides a straight forward user interface to lock in analysis templates that conform to laboratories' standard operating procedures.
- performs the repetitive calculations for converting fragment size to repeat length (Figures 2 and 3).
- print or save final reports with customized header (Figure 4).

Procedure

1. Import raw data files, make size and allele calls and select Applications – Repeat Expansion Analysis (no need to export sized data to a second analysis software).

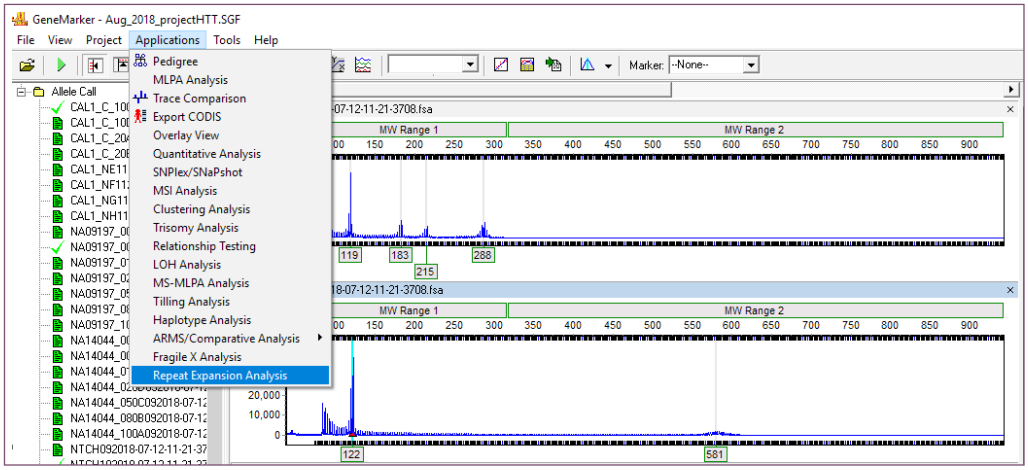


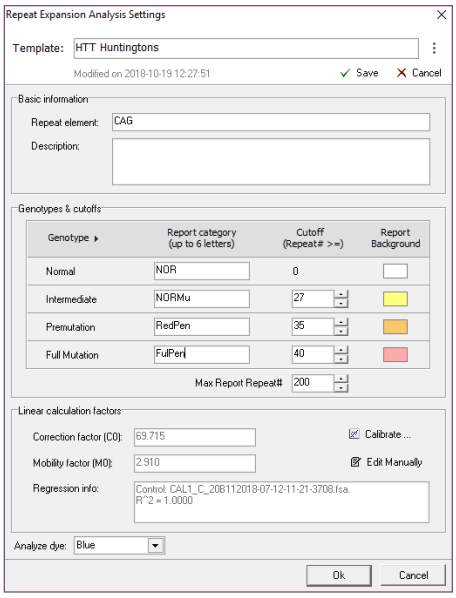
Figure 1: Link directly to the Repeat Expansion Analysis application from the sized data.

2. Select from a list of analysis templates, or create/modify existing templates

Figure 2.
Select a template or create one by entering a descriptive template title (Huntingtons, C9orf72, DMPK….)

Enter the appropriate Report category, Repeat Cutoff values and if desired, Highlighting color.

Calculate the C0 and M0 or enter values from lab validation studies.



3. Review the results

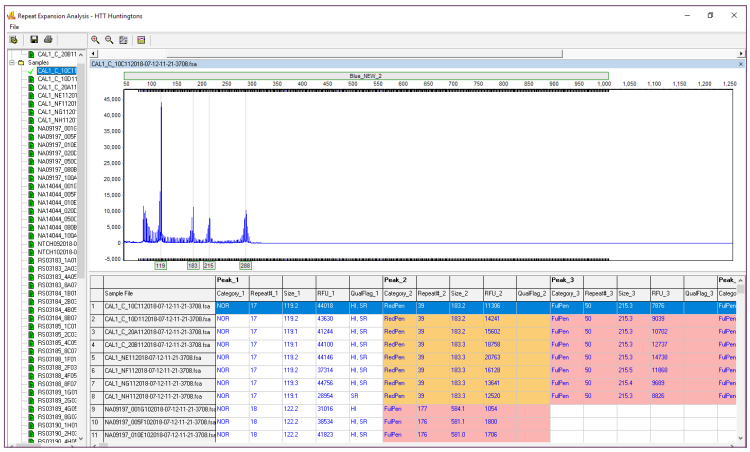
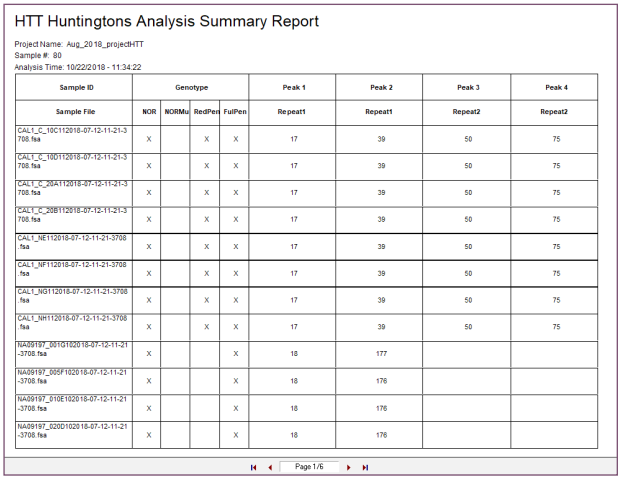


Figure 3: Review results in the application. If background shading was specified in the template, cells in that range will have the designated color. If shading was not specified, there will not be any shading in report table cells. The analyst can select which of the columns to include in the report table.

4. Save/Print Summary and Individual Sample Reports



HTT Huntingtons Analysis Summary Report

Project Name: Aug_2018_projectHTT
Sample #: 80
Analysis Time: 10/22/2018 - 11:34:22

Sample ID	Genotype				Peak 1	Peak 2	Peak 3	Peak 4
Sample File	NOR	NORMu	RedPen	FuPen	Repeat1	Repeat1	Repeat2	Repeat2
CAL1_C_10C112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_C_10D112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_C_20A112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_C_20B112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_NE112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_NF112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_NG112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
CAL1_NH112018-07-12-11-21-3708.fsa	X		X	X	17	39	50	75
NA09197_001G102018-07-12-11-21-3708.fsa	X			X	18	177		
NA09197_005F102018-07-12-11-21-3708.fsa	X			X	18	176		
NA09197_010E102018-07-12-11-21-3708.fsa	X			X	18	176		
NA09197_020D102018-07-12-11-21-3708.fsa	X			X	18	176		

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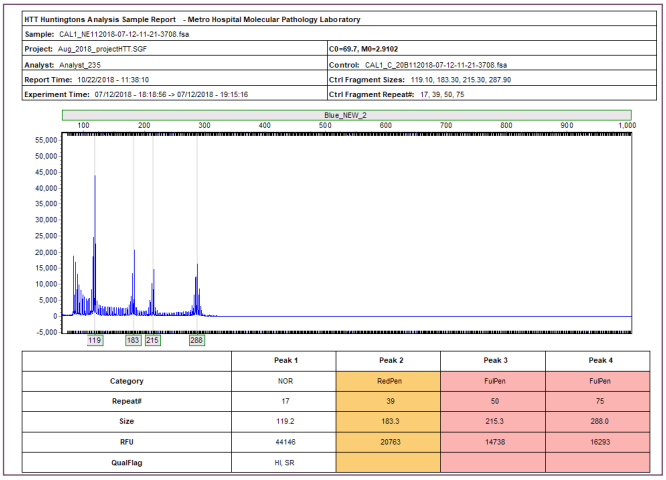

HTT Huntingtons Analysis Sample Report - Metro Hospital Molecular Pathology Laboratory

Sample: CAL1_NE112018-07-12-11-21-3708.fsa	
Project: Aug_2018_projectHTT.SQF	C0=69.7, M0=2.9102
Analyst: Analyst_235	Control: CAL1_C_20B112018-07-12-11-21-3708.fsa
Report Time: 10/22/2018 - 11:38:10	Ctrl Fragment Sizes: 119.10, 183.30, 215.30, 287.90
Experiment Time: 07/12/2018 - 18:18:56 -> 07/12/2018 - 19:15:16	Ctrl Fragment Repeat#: 17, 39, 50, 75

	Peak 1	Peak 2	Peak 3	Peak 4
Category	NOR	RedPen	FuPen	FuPen
Repeat#	17	39	50	75
Size	119.2	183.3	215.3	288.0
RFU	44146	20763	14738	16293
QualFlag	HI, SR			

Figure 4: Select the desired reports to print or save for electronic records. Reports are named using the lab specified template name. The summary report provides a quick overview of the project results; listing each sample, x in the cell that corresponds to the peak range(s) for the sample and the calculated repeat number of each fragment.

Conclusion

The Repeat Expansion Application provides a user-friendly tool to streamline data analysis, customizable templates for different chemistries and reporting flexibility. GeneMarker software is compatible with data files from all major capillary electrophoresis systems (ABI PRISM®, Beckman-Coulter™ and MegaBACE™), and Windows® 7 – 10 operating systems.

References

1. Repeat expansion diseases. Handbook Clin Neurol. 2018;147:105-123. Paulson H.
2. A Brief History of Triplet Repeat Diseases. Helen Budworth and Cynthia T. McMurray Methods Mol Biol. 2013; 1010: 3–17.

Acknowledgements

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