

Test Dataset for Analysis with the HRM Diversity Assay Analysis Tool (DivMelt)

Summary

The three files contained in the directory along with this document constitute a test dataset for the HRM Diversity Assay Analysis Tool (DivMelt). These high resolution melting (HRM) data were generated from artificial diverse DNA populations that were created by mixing mutagenized and wildtype HIV-derived plasmids. The plasmids used to generate this dataset were the subject of an article that is currently in press.

Cousins MM, Donnell D, Eshleman SH (2012) Impact of mutation type and amplicon characteristics on genetic diversity measures generated using a high resolution melting diversity assay. *Journal of Molecular Diagnostics*. In Press.

Preparation of files

The HRM diversity assay was run as previously described (Cousins et al., 2011, PLoS ONE, 6(11):e27211), and a 96-well plate was imaged with the LightScanner instrument (BioFire Diagnostics, Salt Lake City, UT). Following analysis with the LightScanner, melt data were exported from the LightScanner Instrument and Analysis Software package as .ABT, .FLO, and .TEM files. The .ABT file contains sample name information used by DivMelt. The .FLO file contains melting data (fluorescence and temperature data) used by DivMelt. The .TEM file contains temperature data and is not used by DivMelt but is included here for completeness.

DivMelt features illustrated by the test dataset

The analysis of this dataset will illustrate several key features of DivMelt. The data were selected to provide the opportunity to observe package functionality in multiple situations. A detailed description of the test dataset is provided in the summary table given on pages 2-3 of this document.

1. The dataset contains data from 4 assay runs (wells on the 96-well plate) that were identified as marginal amplification failures by DivMelt. These assay runs are identified by the LASSO regression model that is contained within DivMelt.
2. Data from no template controls (NTC) were also included. The software tool automatically screens out wells labeled with "NTC", and these wells are not analyzed.
3. Each sample assayed in this test dataset was assayed in duplicate. This allows for comparisons to be made between duplicate assay runs as a measure of reproducibility.
4. The assay was performed on samples that were known to have certain diversity features. Clear increases in melting peak width are noted when mutations are introduced into amplicon pools (when shorter amplicons are examined). More significant mutations cause greater increases in peak width (9 bp insertion > 3 bp insertion > single base change > 1 bp deletion).
5. The examination of longer amplicons allows observation of more complex melting peak characteristics and melting domains. DivMelt functions in these contexts, but increases in peak width due to the presence of mutations are less easily observed when longer amplicons are assayed.
6. Analysis of this data with different user-specified analysis protocols demonstrates the functionality of the various DivMelt analysis options.

Test Data Summary Table. Samples analyzed to generate the test dataset.

Well #	Designation	Mixture Status	Mutation Type	Amplicon Length
1,9	WT_100bp_POL	Pure WT	N/A	100 bp
2,10	WT+AtoG(50:50)_100bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	100 bp
3,11	WT+AtoC(50:50)_100bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	100 bp
4,12	WT+AtoT(50:50)_100bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	100 bp
5,13	WT+del1bp(50:50)_100bp_POL	50% WT + 50% mutant	1 bp deletion	100 bp
6,14	WT+ins3bp(50:50)_100bp_POL	50% WT + 50% mutant	3 bp insertion	100 bp
7,15	WT+ins9bp(50:50)_100bp_POL	50% WT + 50% mutant	9 bp insertion	100 bp
8,16	NTC			
17,25	WT_200bp_POL	Pure WT	N/A	200 bp
18,26	WT+AtoG(50:50)_200bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	200 bp
19,27	WT+AtoC(50:50)_200bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	200 bp
20,28	WT+AtoT(50:50)_200bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	200 bp
21,29	WT+del1bp(50:50)_200bp_POL	50% WT + 50% mutant	1 bp deletion	200 bp
22,30	WT+ins3bp(50:50)_200bp_POL	50% WT + 50% mutant	3 bp insertion	200 bp
23,31	WT+ins9bp(50:50)_200bp_POL	50% WT + 50% mutant	9 bp insertion	200 bp
24,32	NTC			
33,41	WT_300bp_POL	Pure WT	N/A	300 bp
34,42	WT+AtoG(50:50)_300bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	300 bp
35,43	WT+AtoC(50:50)_300bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	300 bp
36,44	WT+AtoT(50:50)_300bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	300 bp
37,45	WT+del1bp(50:50)_300bp_POL	50% WT + 50% mutant	1 bp deletion	300 bp
38,46	WT+ins3bp(50:50)_300bp_POL	50% WT + 50% mutant	3 bp insertion	300 bp
39,47	WT+ins9bp(50:50)_300bp_POL	50% WT + 50% mutant	9 bp insertion	300 bp
40,48	NTC			
49,57	WT_400bp_POL	Pure WT	N/A	400 bp
50,58	WT+AtoG(50:50)_400bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	400 bp
51,59	WT+AtoC(50:50)_400bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	400 bp
52,60	WT+AtoT(50:50)_400bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	400 bp
53,61	WT+del1bp(50:50)_400bp_POL	50% WT + 50% mutant	1 bp deletion	400 bp
54,62	WT+ins3bp(50:50)_400bp_POL	50% WT + 50% mutant	3 bp insertion	400 bp
55,63	WT+ins9bp(50:50)_400bp_POL	50% WT + 50% mutant	9 bp insertion	400 bp
56,64	NTC			
65,73	WT_600bp_POL	Pure WT	N/A	600 bp
66,74	WT+AtoG(50:50)_600bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	600 bp
67,75	WT+AtoC(50:50)_600bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	600 bp
68,76	WT+AtoT(50:50)_600bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	600 bp
69,77	WT+del1bp(50:50)_600bp_POL	50% WT + 50% mutant	1 bp deletion	600 bp
70,78	WT+ins3bp(50:50)_600bp_POL	50% WT + 50% mutant	3 bp insertion	600 bp
71,79	WT+ins9bp(50:50)_600bp_POL	50% WT + 50% mutant	9 bp insertion	600 bp
72,80	NTC			

Test Data Summary Table Continued. Samples analyzed to generate test dataset.

Well #	Designation	Mixture Status	Mutation Type	Amplicon Length
81,89	WT_800bp_POL	Pure WT	N/A	800 bp
82,90	WT+AtoG(50:50)_800bp_POL	50% WT + 50% mutant	SBC (WT+AtoG)	800 bp
83,91	WT+AtoC(50:50)_800bp_POL	50% WT + 50% mutant	SBC (WT+AtoC)	800 bp
84,92	WT+AtoT(50:50)_800bp_POL	50% WT + 50% mutant	SBC (WT+AtoT)	800 bp
85,93	WT+del1bp(50:50)_800bp_POL	50% WT + 50% mutant	1 bp deletion	800 bp
86,94	WT+ins3bp(50:50)_800bp_POL	50% WT + 50% mutant	3 bp insertion	800 bp
87,95	WT+ins9bp(50:50)_800bp_POL	50% WT + 50% mutant	9 bp insertion	800 bp
88,96	NTC			

Abbreviations: Well # – well number on the 96-well plate; WT – wild type; SBC – single base change; bp – base pair; NTC – no template control; AtoG – a base that was “A” in the WT plasmid was changed to “G”; AtoC – a base that was “A” in the WT plasmid was changed to “C”; AtoT – a base that was “A” in the WT plasmid was changed to “T”