



## HRM Analysis Procedures

HRM analysis: Follow this step-by-step procedure for HRM analysis

1. Export the melt curve analysis file from the database
2. Open the file titled *melt\_curve\_data.csv*
3. Data from all wells are exported together in this file. You will see 5 columns labeled as such: channel, well\_num, temperature, normalized\_data, and derivative\_data
4. Highlight and copy the temperature and normalized\_data values of **each well** across the complete temperature range for which the melt curve was performed (figure 1). Do not copy the column names. Paste these values into a new excel sheet under columns A and B (figure 2).

	A	B	C	D	E	F
1	channel	well_num	temperature	normalized_data	derivative_data	
2	1	1	59.996	140075.443	140.704	
3	1	1	60.172	140005.427	491.497	
4	1	1	60.349	139926.784	871.112	
5	1	1	60.525	139730.13	1428.942	
6	1	1	60.701	139402.846	1919.53	
7	1	1	60.878	139025.925	2322.111	
8	1	1	61.054	138606.277	2636.542	
9	1	1	61.23	138084.627	2909.107	
10	1	1	61.406	137558.115	3171.419	
11	1	1	61.583	136993.959	3317.525	
12	1	1	61.759	136363.625	3611.251	
13	1	1	61.935	135742.013	3674.427	
14	1	1	62.112	135028.024	3674.78	
15	1	1	62.288	134440.009	3669.546	
16	1	1	62.464	133781.917	3672.821	
17	1	1	62.64	133108.279	3605.126	
18	1	1	62.817	132482.219	3594.526	
19	1	1	62.993	131913.164	3672.058	
20	1	1	63.169	131175.652	3798.034	
21	1	1	63.346	130520.666	3775.186	
22	1	1	63.522	129871.437	3736.221	
23	1	1	63.698	129227.715	3723.235	
24	1	1	63.875	128517.376	3642.827	
25	1	1	64.051	127938.954	3583.395	
26	1	1	64.227	127304.865	3723.035	
27	1	1	64.403	126631.108	3769.922	
28	1	1	64.58	125897.855	3620.691	

Figure 1

	A	B	C	D	E	F
1	59.996	140075.443				
2	60.172	140005.427				
3	60.349	139926.784				
4	60.525	139730.13				
5	60.701	139402.846				
6	60.878	139025.925				
7	61.054	138606.277				
8	61.23	138084.627				
9	61.406	137558.115				
10	61.583	136993.959				
11	61.759	136363.625				
12	61.935	135742.013				
13	62.112	135028.024				
14	62.288	134440.009				
15	62.464	133781.917				
16	62.64	133108.279				
17	62.817	132482.219				
18	62.993	131913.164				
19	63.169	131175.652				
20	63.346	130520.666				
21	63.522	129871.437				
22	63.698	129227.715				
23	63.875	128517.376				
24	64.051	127938.954				
25	64.227	127304.865				
26	64.403	126631.108				
27	64.58	125897.855				

Figure 2

Save the file as **Tab Delimited Text (txt)**. Do this for each well and save as a new file every time.

If you run 16 reactions, you should have 16 separate txt files saved. If you run 8 reactions, you should have 8 separate txt files saved.

5. Go to this url: <https://www.dna.utah.edu/ua/uanalyze.html>
6. From the pull-down menu on the left side that displays *Auto-Detect*, select *Generic* for file type. Choose the files and upload all tab delimited txt files for controls and samples (figure 3).

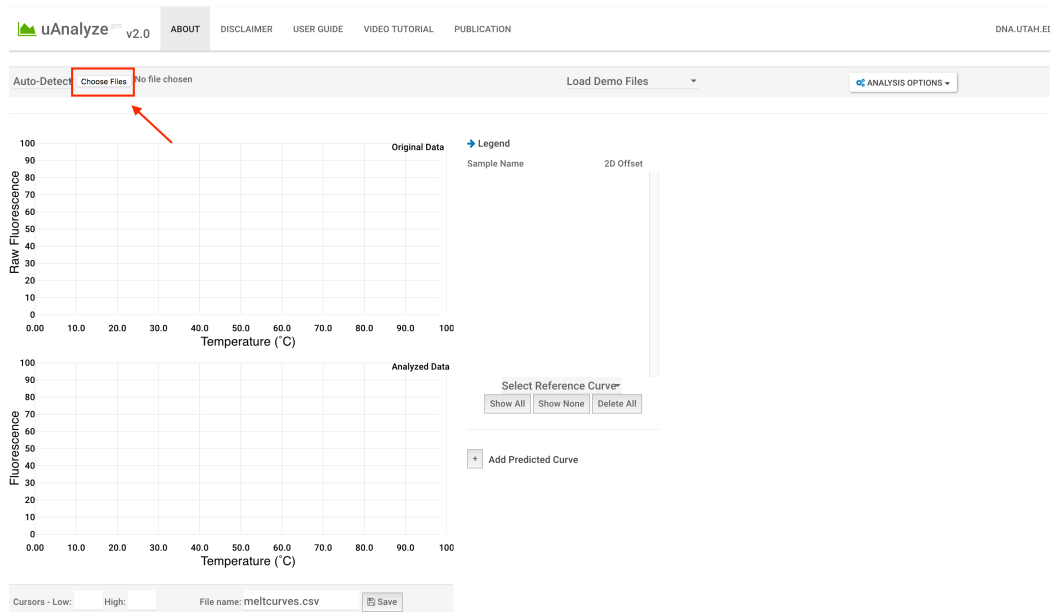


Figure 3

7. Once the files are chosen, the plot will populate as displayed in figure 4 below. You may deselect wells to view as necessary using the legend on the right side.

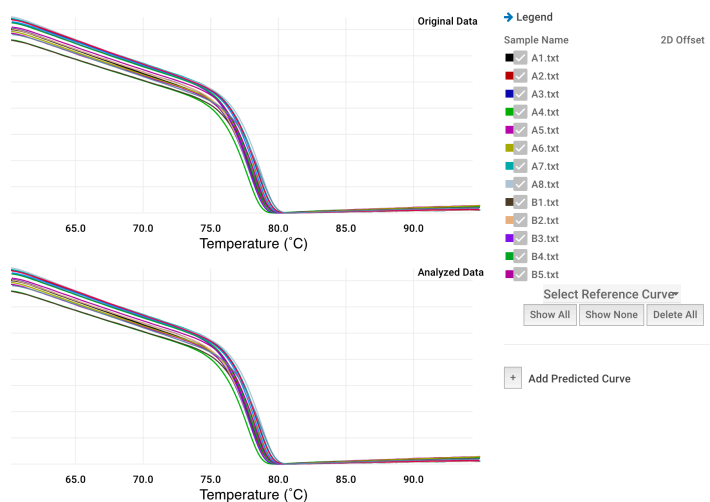


Figure 4

8. Select an area (gray shaded region) for HRM analysis as shown in figure 5. This area is the region of active melt. Click on the *Analysis Options* dropdown box on the top right and select *Baseline* for normalization (figure 6). This normalizes the curves in the region of active melt from 0% to 100% helicity. The normalized curves are displayed as the *Analyzed Data* plot. Distinct shapes are obtained for each sample.

Once you select the *Save* option near the bottom, the file will be saved in a .csv format.

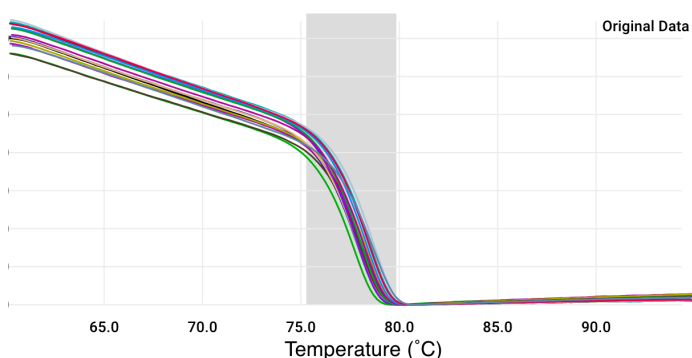


Figure 5

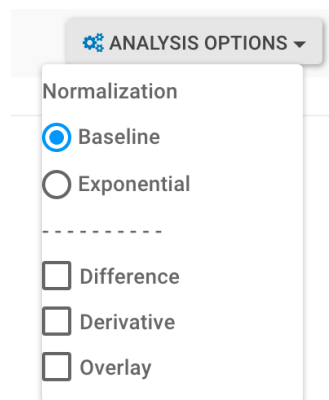
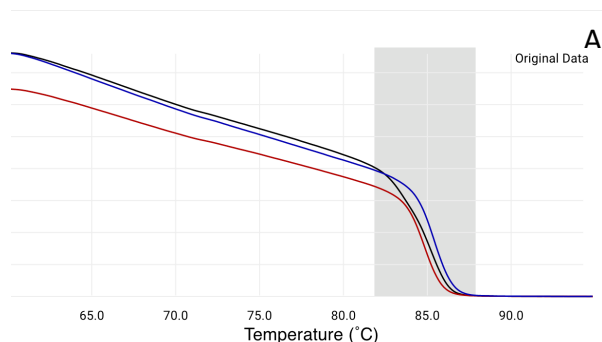
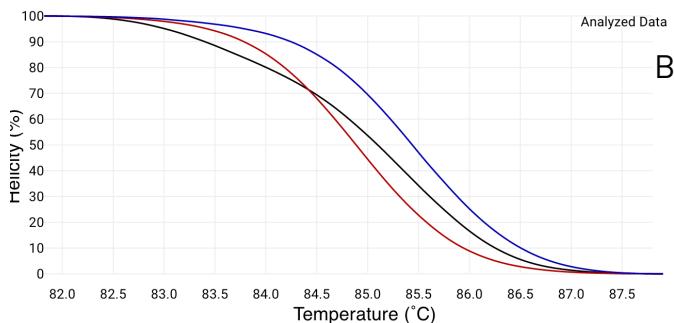


Figure 6

The figures below illustrate an example of HRM analysis using the uAnalyze software. Figure A shows the fluorescence data of melt curves in response to temperature increase. The gray shaded area is the region of active melt selected by the user. Figure B shows the normalized curve in the active melt regions. The curves that are displayed in Figures A and B belong to three different genotypes of the human ACTN3 gene: wild type (blue), mutant (red), and heterozygous (black).



A



B