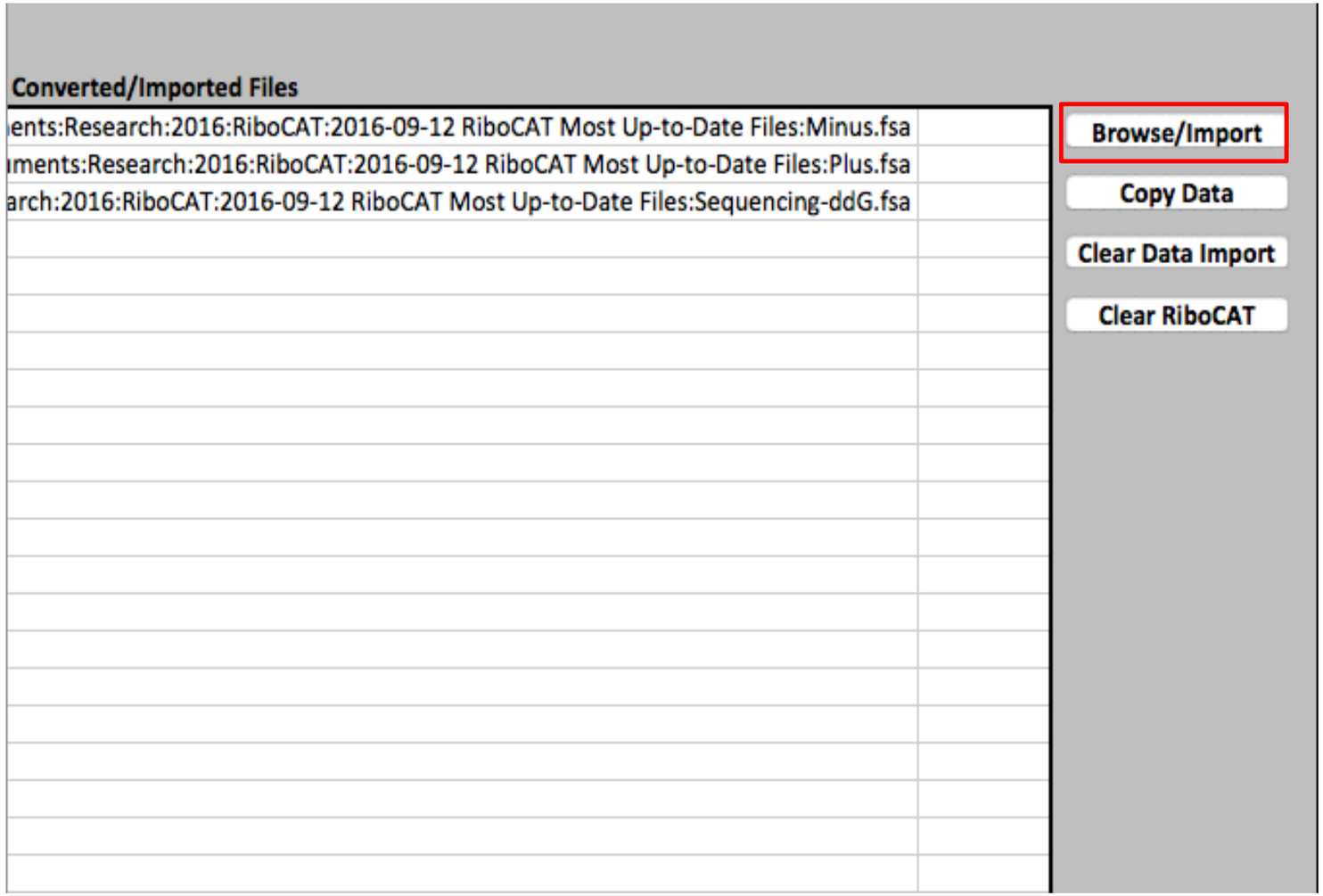
RiboCAT Quick Start Guide

I. Data Import

- Press the *Browse/Import* button, select a sequencing, minus, and plus FSA file, and press open. The data will then appear to the right of the buttons.

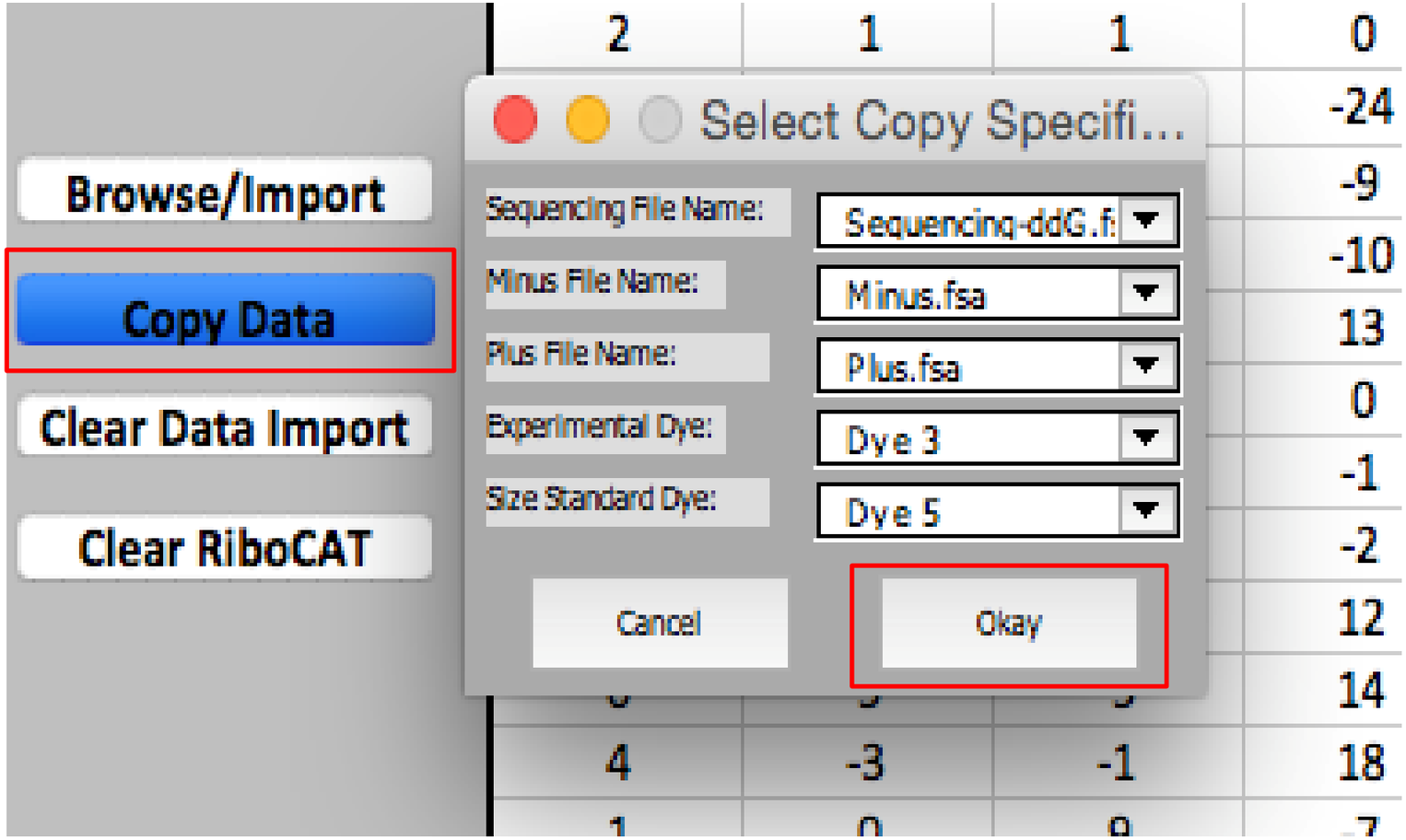


I. Data Import

- To transfer the data to the “StdAlign” sheet, press the “Copy Data” button. This should pull up a user form. Select the file name that corresponds to each trace and the correct dyes used in experiments (See dye table). Then press “Okay.” The data should now be copied to the “StdAlign” sheet under the “Size Standard Raw Data” and “Experimental Raw Data” headers.

Dye Table

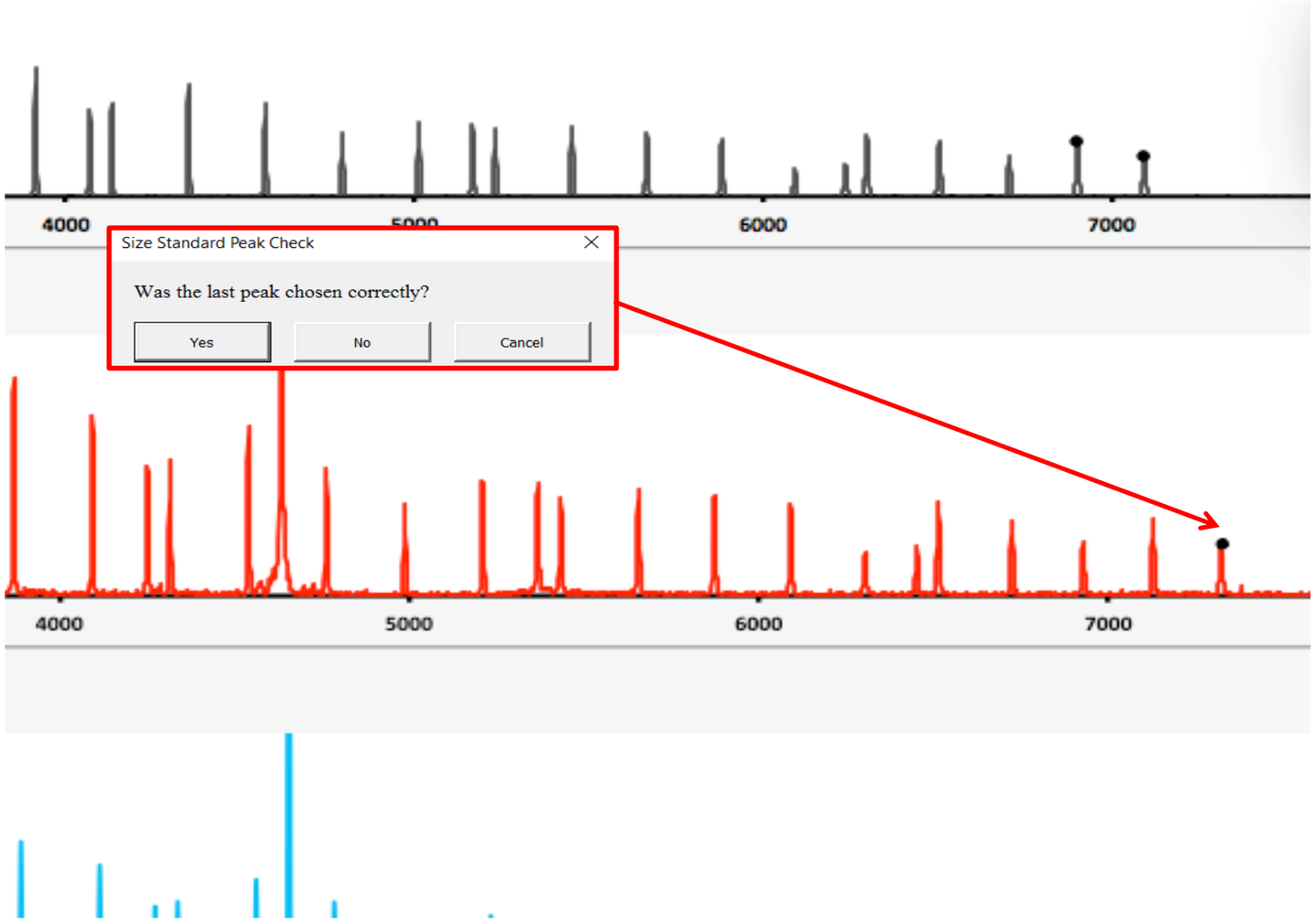
Dye 1	Blue Dyes
Dye 2	Green Dyes
Dye 3	Yellow Dyes
Dye 4	Red Dyes
Dye 5	Orange Dyes



II. Signal Alignment

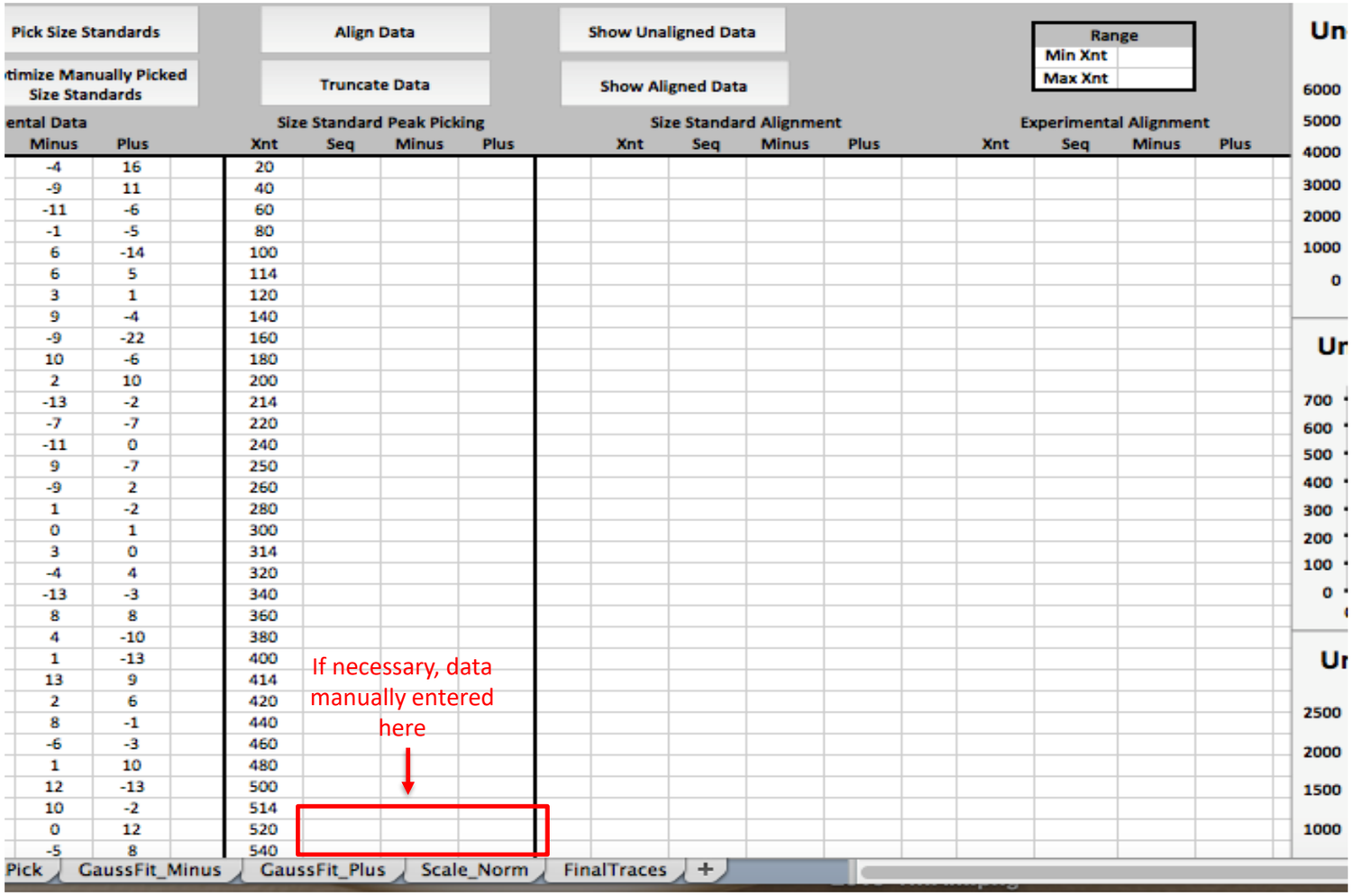
- Press the Pick Size Standards button to complete the size standard picking process.

- This will attempt to pick the peaks by first ensuring the largest two size standards from the list are picked correctly. Click “No” if the peak in question is incorrect. The program will then attempt to identify the next most likely peak.



II. Signal Alignment

- If the program is incapable of identifying the largest two peaks, these can be entered manually, and the remaining peaks should be able to be identified programmatically.



II. Signal Alignment

- When confident in the size standard peak assignments, select the *Align Data* button to align the Size Standard and Experimental data.

- Finally, check the quality of the data and in the bottom right charts, select an X_{nt} analysis range, and enter it into the "Range" table above the experimental alignment. Then press the *Truncate Data* button.

Pick Size Standards

Optimize Manually Picked Size Standards

Align Data

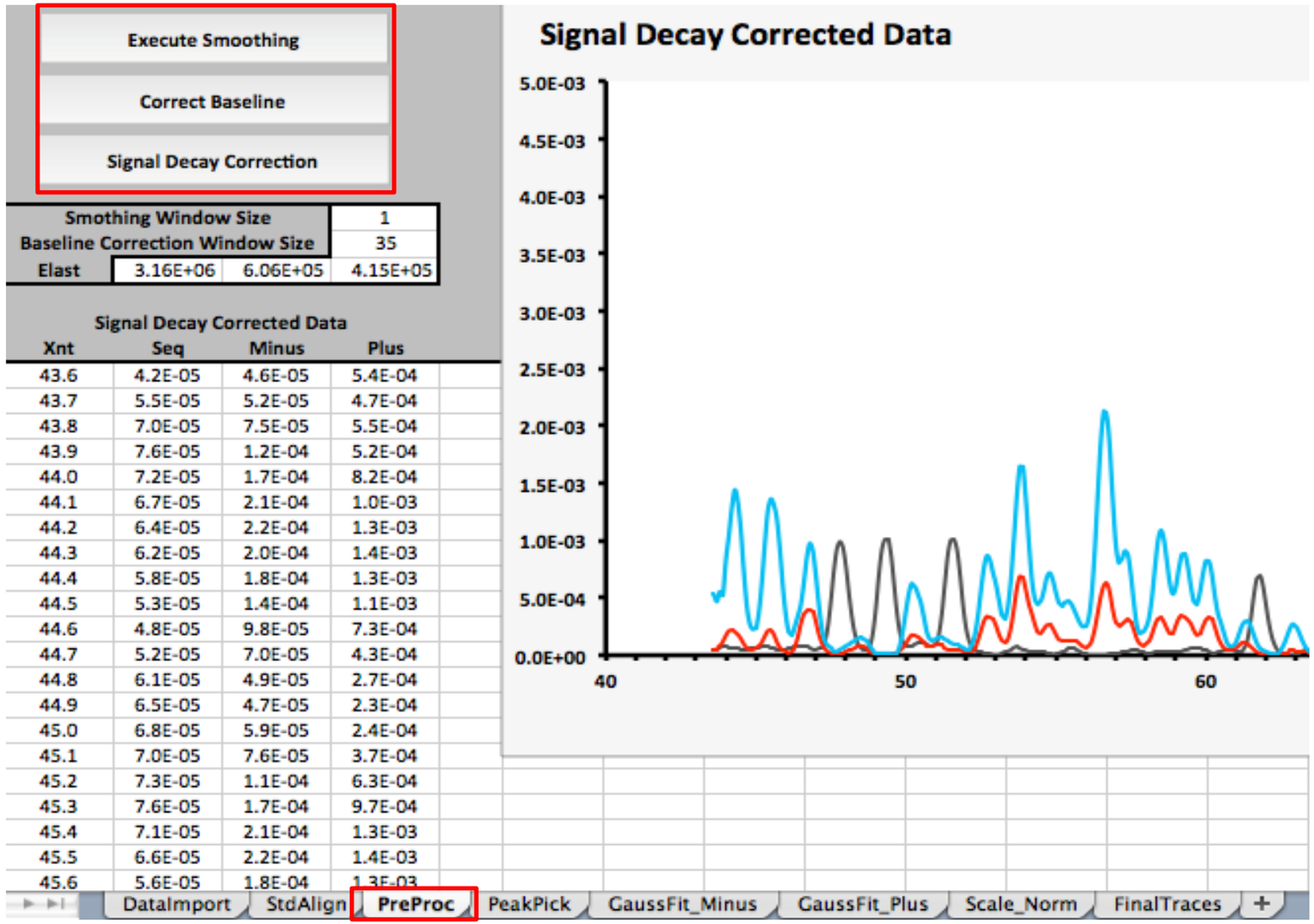
Truncate Data

Range	
Min Xnt	
Max Xnt	

Size Standard Peak Picking				Size Standard Alignment				Experimental Alignment			
Xnt	Seq	Minus	Plus	Xnt	Seq	Minus	Plus	Xnt	Seq	Minus	Plus
20	1076	1183	1168	10	34	39	89	10	2154	594	1782
40	1211	1325	1310	10.1	34	39	89	10.1	2154	594	1885
60	1386	1507	1494	10.2	41	39	89	10.2	1926	594	1885
80	1579	1707	1694	10.3	41	35	89	10.3	1926	652	1885
100	1779	1913	1902	10.4	41	35	86	10.4	1926	652	2133
114	1919	2058	2047	10.5	38	41	86	10.5	1833	648	2133
120	1982	2123	2113	10.6	38	41	91	10.6	1833	648	2294
140	2194	2341	2332	10.7	41	41	91	10.7	1812	648	2294
160	2402	2554	2546	10.8	41	38	102	10.8	1812	669	2388
180	2613	2770	2764	10.9	41	38	102	10.9	1812	669	2388
200	2825	2986	2982	11	33	39	102	11	1753	616	2388
214	2975	3140	3137	11.1	33	39	86	11.1	1753	616	2386
220	3039	3205	3202	11.2	33	39	86	11.2	1753	616	2386
240	3257	3426	3425	11.3	43	23	89	11.3	1770	567	2227
250	3363	3534	3533	11.4	43	23	89	11.4	1770	567	2227
260	3473	3646	3646	11.5	59	29	89	11.5	1814	532	2227
280	3691	3869	3870	11.6	59	29	80	11.6	1814	532	1965
300	3912	4094	4096	11.7	59	32	80	11.7	1814	488	1965
314	4067	4251	4255	11.8	38	32	90	11.8	1772	488	1773
320	4131	4316	4320	11.9	38	32	90	11.9	1772	488	1773

III. Preprocessing

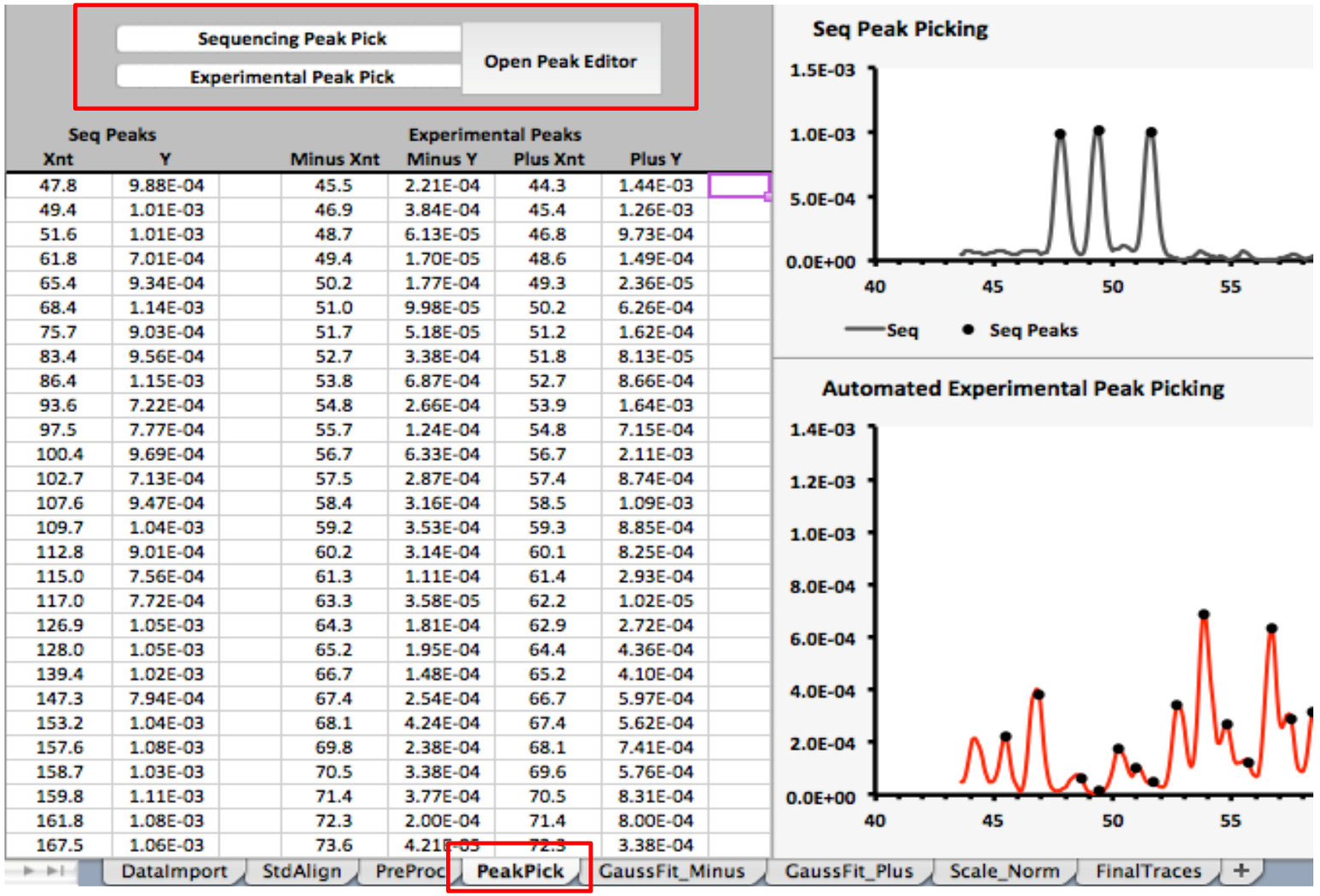
- On the "PreProc" sheet, *Execute Smoothing*, *Correct Baseline*, and apply the *Signal Decay Direction* in that order. The smoothing and baseline correction windows can be adjusted as desired



IV. Pick Peaking

- Press the *Sequencing Peak Pick* and *Experimental Peak Pick* buttons.

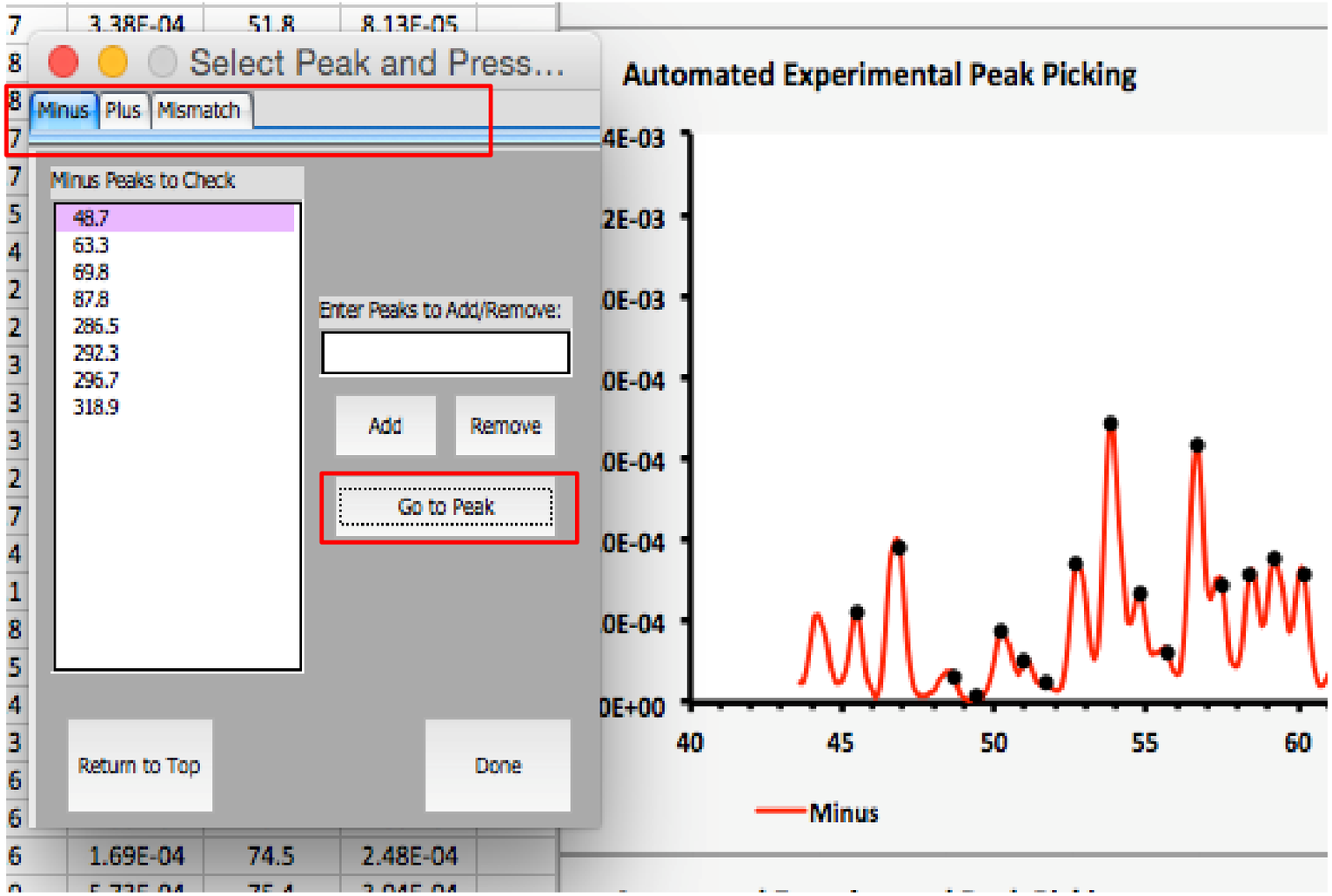
- RiboCAT will automatically run checks to inform the user of erroneously picked peaks. To view these checks and edit the peak list, press the “Open Peak Editor” button.



IV. Pick Peaking

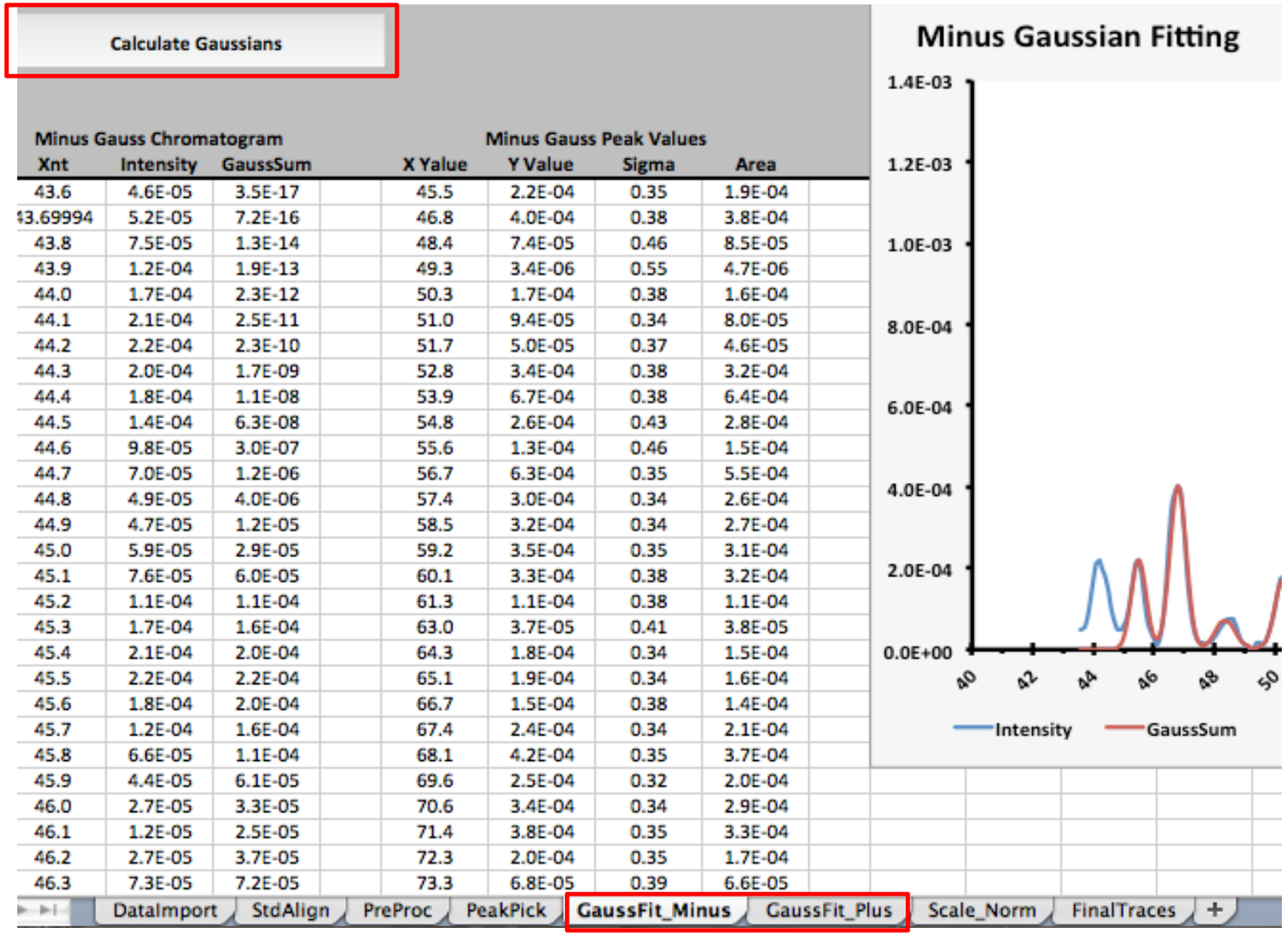
- The “Minus” and “Plus” pages of the Peak Editor contain lists of peaks that have been identified as either too close together or too far apart. The “Mismatch” page alerts the user of peaks that have been identified in one trace, but not in the other.

- To view the peaks to change, the user can click on a peak in the list and select “Go to Peak.” This will center the screen on the peak of interest. The user may then decide whether to add or remove peaks from the region. (Note: all warnings on the Mismatch page should be alleviated before proceeding to Gaussian fitting, but the same is not true for the other two pages).



V. Gaussian Fitting

- On the “GaussFit_Minus” and “GaussFit_Plus” sheets press the *Calculate Gaussians* buttons.
- Assess the quality of the Gaussian fittings for both traces and select a continuous X_{nt} range corresponding to quality fitting.
- Enter this into the Min/Max X_{nt} table on the “Scale_Norm” sheet.



VI. Reactivity Calculation and Sequence Alignment

- On the "Scale_Norm" sheet, press the *Scaling* and *Normalization* buttons to complete the reactivity calculations.

- Make sure to create a sequence file that is formatted as a text file containing only the RNA sequence on the first line with no spaces.

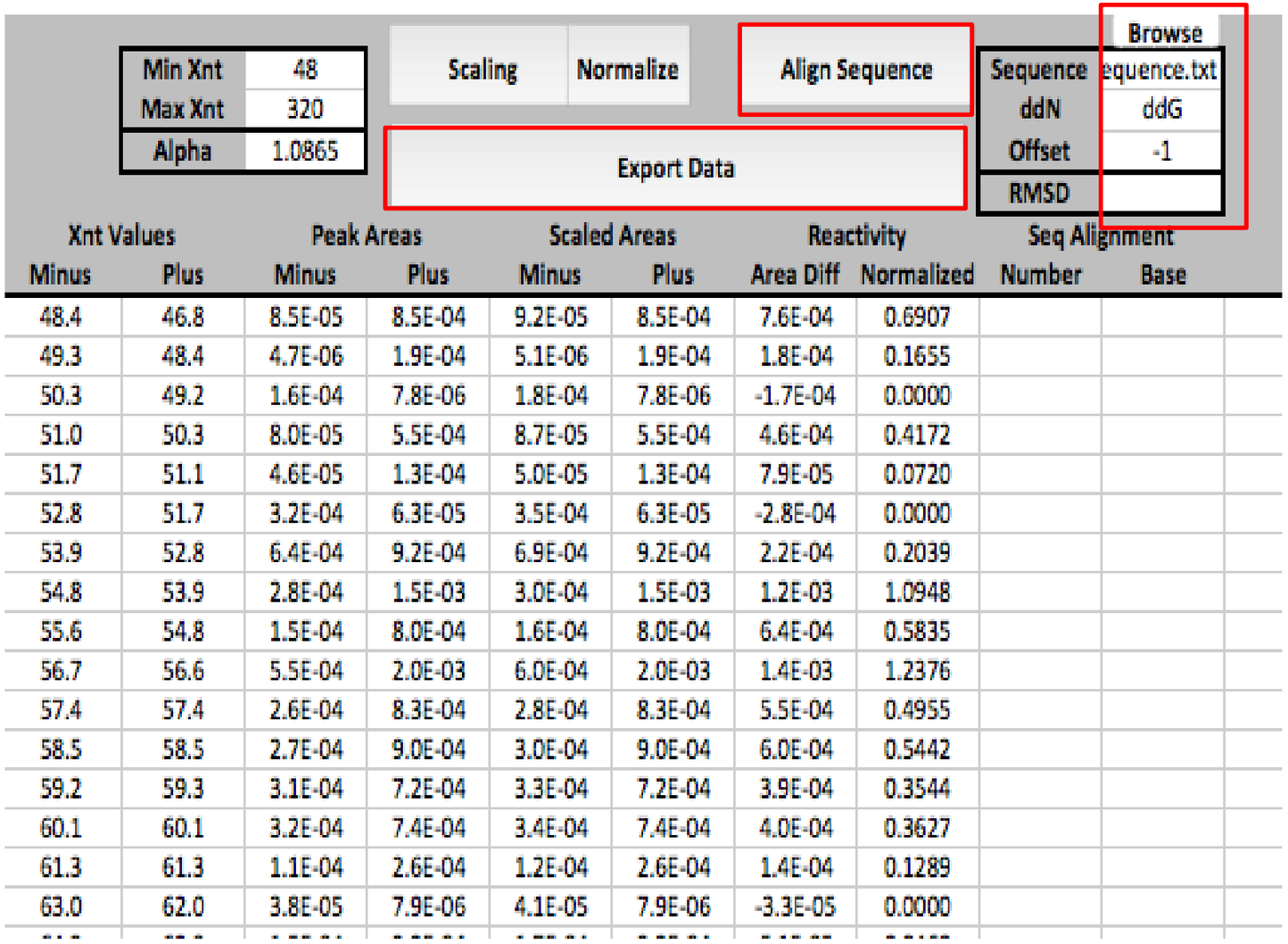
Min Xnt	48	Scaling	Normalize	Align Sequence	Browse
Max Xnt	320			Sequence	
Alpha	1.0865	Export Data		ddN	ddG
				Offset	-1
				RMSD	

Xnt Values		Peak Areas		Scaled Areas		Reactivity		Seq Alignment	
Minus	Plus	Minus	Plus	Minus	Plus	Area Diff	Normalized	Number	Base
48.4	46.8	8.5E-05	8.5E-04	9.2E-05	8.5E-04	7.6E-04	0.6907		
49.3	48.4	4.7E-06	1.9E-04	5.1E-06	1.9E-04	1.8E-04	0.1655		
50.3	49.2	1.6E-04	7.8E-06	1.8E-04	7.8E-06	-1.7E-04	0.0000		
51.0	50.3	8.0E-05	5.5E-04	8.7E-05	5.5E-04	4.6E-04	0.4172		
51.7	51.1	4.6E-05	1.3E-04	5.0E-05	1.3E-04	7.9E-05	0.0720		
52.8	51.7	3.2E-04	6.3E-05	3.5E-04	6.3E-05	-2.8E-04	0.0000		
53.9	52.8	6.4E-04	9.2E-04	6.9E-04	9.2E-04	2.2E-04	0.2039		
54.8	53.9	2.8E-04	1.5E-03	3.0E-04	1.5E-03	1.2E-03	1.0948		
55.6	54.8	1.5E-04	8.0E-04	1.6E-04	8.0E-04	6.4E-04	0.5835		
56.7	56.6	5.5E-04	2.0E-03	6.0E-04	2.0E-03	1.4E-03	1.2376		
57.4	57.4	2.6E-04	8.3E-04	2.8E-04	8.3E-04	5.5E-04	0.4955		
58.5	58.5	2.7E-04	9.0E-04	3.0E-04	9.0E-04	6.0E-04	0.5442		
59.2	59.3	3.1E-04	7.2E-04	3.3E-04	7.2E-04	3.9E-04	0.3544		
60.1	60.1	3.2E-04	7.4E-04	3.4E-04	7.4E-04	4.0E-04	0.3627		
61.3	61.3	1.1E-04	2.6E-04	1.2E-04	2.6E-04	1.4E-04	0.1289		
63.0	62.0	3.8E-05	7.9E-06	4.1E-05	7.9E-06	-3.3E-05	0.0000		
64.3	62.9	1.5E-04	2.2E-04	1.7E-04	2.2E-04	5.1E-05	0.0460		
65.1	64.3	1.6E-04	4.3E-04	1.8E-04	4.3E-04	2.5E-04	0.2280		
66.7	65.2	1.4E-04	3.9E-04	1.5E-04	3.9E-04	2.4E-04	0.2159		
67.4	66.7	2.1E-04	5.7E-04	2.3E-04	5.7E-04	3.4E-04	0.3102		
68.1	67.5	3.7E-04	4.3E-04	4.0E-04	4.3E-04	3.5E-05	0.0318		
69.6	68.0	2.0E-04	5.8E-04	2.2E-04	5.8E-04	3.6E-04	0.3227		
70.6	69.6	2.9E-04	4.5E-04	3.1E-04	4.5E-04	1.3E-04	0.1215		
71.4	70.5	3.3E-04	6.7E-04	3.6E-04	6.7E-04	3.1E-04	0.2794		
72.3	71.4	1.7E-04	6.6E-04	1.9E-04	6.6E-04	4.7E-04	0.4274		

VI. Reactivity Calculation and Sequence Alignment

- Press the Browse *button* next to the "Seq. File" cell to find and open the sequence file corresponding to the RNA being analyzed. Enter the ddN used in the sequencing trace, and chose an offset value. (For most probing methods including SHAPE the offset should be -1). Then press *Align Sequence*.

- Press the "Export Data" button to generate a comma-separated file containing all of the data from your RiboCAT analysis. This file is a more memory efficient form of storage for your RiboCAT data, and is necessary for use in RiboDOG.



Min Xnt	48
Max Xnt	320
Alpha	1.0865

Scaling | Normalize | Align Sequence | Export Data

Sequence	Browse... equence.txt
ddN	ddG
Offset	-1
RMSD	

Xnt Values		Peak Areas		Scaled Areas		Reactivity		Seq Alignment	
Minus	Plus	Minus	Plus	Minus	Plus	Area Diff	Normalized	Number	Base
48.4	46.8	8.5E-05	8.5E-04	9.2E-05	8.5E-04	7.6E-04	0.6907		
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50.3	49.2	1.6E-04	7.8E-06	1.8E-04	7.8E-06	-1.7E-04	0.0000		
51.0	50.3	8.0E-05	5.5E-04	8.7E-05	5.5E-04	4.6E-04	0.4172		
51.7	51.1	4.6E-05	1.3E-04	5.0E-05	1.3E-04	7.9E-05	0.0720		
52.8	51.7	3.2E-04	6.3E-05	3.5E-04	6.3E-05	-2.8E-04	0.0000		
53.9	52.8	6.4E-04	9.2E-04	6.9E-04	9.2E-04	2.2E-04	0.2039		
54.8	53.9	2.8E-04	1.5E-03	3.0E-04	1.5E-03	1.2E-03	1.0948		
55.6	54.8	1.5E-04	8.0E-04	1.6E-04	8.0E-04	6.4E-04	0.5835		
56.7	56.6	5.5E-04	2.0E-03	6.0E-04	2.0E-03	1.4E-03	1.2376		
57.4	57.4	2.6E-04	8.3E-04	2.8E-04	8.3E-04	5.5E-04	0.4955		
58.5	58.5	2.7E-04	9.0E-04	3.0E-04	9.0E-04	6.0E-04	0.5442		
59.2	59.3	3.1E-04	7.2E-04	3.3E-04	7.2E-04	3.9E-04	0.3544		
60.1	60.1	3.2E-04	7.4E-04	3.4E-04	7.4E-04	4.0E-04	0.3627		
61.3	61.3	1.1E-04	2.6E-04	1.2E-04	2.6E-04	1.4E-04	0.1289		
63.0	62.0	3.8E-05	7.9E-06	4.1E-05	7.9E-06	-3.3E-05	0.0000		