

Quick Start Guide

RiboCAT Instructions

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. If you already have a project saved in a “.ribocat” file, this can be opened here as well.
- 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

<i>Dye 1</i>	Blue Dyes
<i>Dye 2</i>	Green Dyes
<i>Dye 3</i>	Yellow Dyes
<i>Dye 4</i>	Red Dyes
<i>Dye 5</i>	Orange Dyes

- *Optional:* Press the “Clear Data” button to clear the data on the “DataImport” sheet.
- *Optional:* Press the “Clear RiboCAT” button to clear the data from the entire project. Be sure to only use this function after data has been exported using the “Export Data” function on “ScaleNorm” sheet.

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.
 - 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 by pressing the *Pick Size Standards* button.
 - In the case of messy size standard traces, some peaks in the size standard list may be picked manually and optimized to the local maximum by pressing the *Optimize Manually Picked Size Standards* button.

- The size standard list can be altered to accommodate other size standard sets, or to eliminate peaks that are frequently missed algorithmically.

- 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.

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. Peak Picking

- 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.
- 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.
- 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.

RiboDOG Instructions

I. Data Summarization

- Open an empty RiboDOG file, select a sequence file, and enter the number of primers used in experiments. Additionally, select the desired orientation of the data from the drop down menu.
- Select the RiboCAT files for each primer, and then press the *Import Data* button.

II. Trace Visualizer

- To open the Trace Visualizer, click the “Open Trace Visualizer” button on the first page.
- The “Visualize” tab allows users to select traces from different replicates of the same primer to compare them.
- The “Edit” tab allows users to see how the addition or subtraction of a peak would affect the Gaussian fitting by pressing the “Update Preview” button. It also allows the users to incorporate these changes by pressing the “Incorporate Alterations” button.
 - *Note:* The Trace Visualizer is the only function of RiboCAT or RiboDOG that does not work identically on Mac and PC operating systems. See Advanced User Manual for more details.

III. Data Export

- To generate a .shape file simply return to the RiboDOG home page, and click the “Generate RNA Structure File” button after analysis of the summarized data is complete.

