

MA2C Manual

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1 Installation

- **REQUIREMENT: You need Java Runtime Environment (JRE) 5.0 or higher.**
You can download it from <http://java.sun.com/>.
- Download the package MA2C.zip and uncompress it using your favorite method.
- Windows: Double-click on MA2C\dist\MA2C.bat to launch the program. By default, the script allocates 600 MB of memory; you can change this amount by editing the script with a text editor.
- Linux, Unix or Mac: change directory to MA2C/dist/ and execute the command
`java -Xmx600m -jar MA2C.jar`

On Mac, double-clicking on MA2C.jar should also start the program automatically; but if you want to pre-allocate memory, then use the command above.

2 Loading Files



- Upon launching the program, click on the “Run” button to display the main interaction panel.

- Click on the “SampleKey” button to select a `SampleKey.txt` file that came with your NimbleGen CD. Ideally, the data structure should be

`SampleKey.txt, DesignFiles/*.ndf,*.pos, PairData/*.txt`

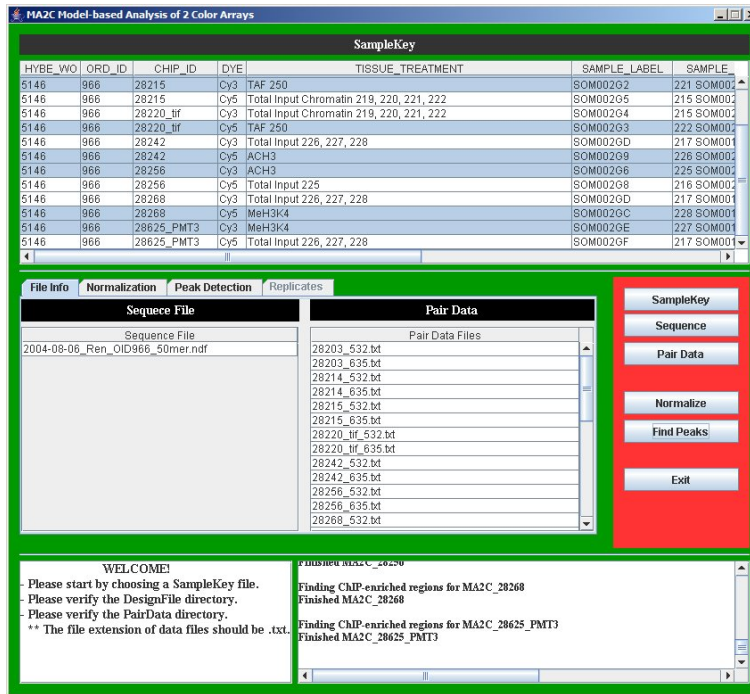
If your data structure follows this pattern, then MA2C will automatically look for sequence (`.ndf`, `.pos`) files and pair data (`.txt`) files and display the information on the GUI.

Some CD’s arrive at your door with a quite rebellious disposition, so if some files or directories don’t load properly, then check their names!

- For some reason, if the sequence and pair data folder names do not follow the above convention, then you can select the folders manually via the “Sequence” and “Pair Data” buttons. (But, we recommend that you instead change the folder names to conform to the above convention.)
- **NOTE:** The entries in `SampleKey.txt` are tab-delimited and **MUST** contain the `CHIP_ID`, `DESIGN_ID` and `DYE` information corresponding to each experiment. If necessary, you can create or modify this file using a text editor.
- **NOTE:** It is important that all data files in `PairData/` have the `.txt` extension.
- An example of `SampleKey.txt`:

CHIP_ID	DESIGN_ID	DYE	SAMPLE_DESCRIPTION	...
1111	9876	Cy3	ER Input1	...
1111	9876	Cy5	ER IP1	...
2222	9876	Cy3	ER Input2	...
2222	9876	Cy5	ER IP2	...

Table 1: Example `SampleKey.txt`



3 Analysis

3.1 Normalization

- In the SampleKey table, Ctrl-left click to select the IP DYE for each experiment you want to analyze. In the example given in Table 1, one should thus select only the lines written in blue. **It is important that you select only the IP channel.** MA2C will automatically find and use the correct Input channel based on your selection of IP channel.
- Choose a normalization method in the “Normalization” tab and click on the “Normalize” button.
- MA2C will generate the following files:
 1. A .tpmap file will be generated in the sequence directory, and for each experiment, MA2C- $\{\text{CHIP_ID}\}$ _raw.txt and MA2C- $\{\text{CHIP_ID}\}$ _normalized.txt will be created in the pair data directory.
 2. A new directory called MA2C_Output will be created in the same parent directory as SampleKey.txt.
 3. A text log file and a .pdf probe histogram file for the run will be written to MA2C_Output.

3.2 Peak Detection

- After normalization, check the “Peak Detection” tab for available options, and click on the “Find Peaks” button. You don’t need to renormalize the data each time you want to find peaks using different options.

- MA2C will generate the following files in MA2C_Output:

1. MA2C_\${CHIP_ID}.bed, MA2C_\${CHIP_ID}.gff, MA2C_\${CHIP_ID}.xls, MA2C_\${CHIP_ID}.pdf, and MA2C_\${CHIP_ID}_FDRtable.txt for peak locations and quality control.

In MA2C_\${CHIP_ID}.xls, the column “Peak_Pos” contains the location of peak with respect to the peak starting position; the peak location is computed to be the average between the geometric center and the peak summit. In some cases, two enriched regions may overlap and cause problems with some genome browsers; in MA2C_\${CHIP_ID}.bed and MA2C_\${CHIP_ID}.gff, we resolve this issue by truncating the overlapping left-edge, if necessary. MA2C_\${CHIP_ID}.xls contains the untruncated coordinates for further processing.

2. MA2C_\${CHIP_ID}.ratio.bar containing normalized probe ratios.
 3. MA2C_\${CHIP_ID}.MA2Cscore.bar containing MA2C scores.
- You can load the .bed and .bar files into Affymetrix’s Integrated Genome Browser (IGB).

3.3 Histogram Panels

- Left-click and drag to zoom in.
- Right-click and choose “Save as” to save the image as a PNG file.

4 Diagnostic Tools

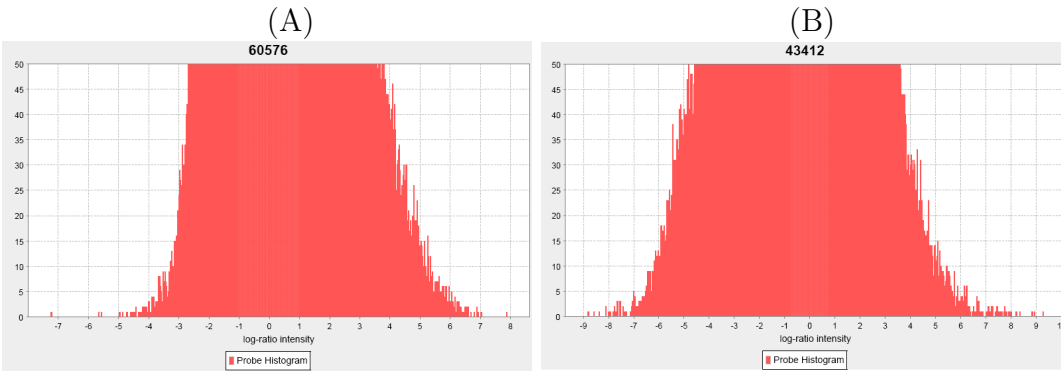


Figure 1: (A) Good probe level enrichment is indicated by a heavy right tail. (B) A heavy left tail signals potential problems, possibly related to poor antibody performance.

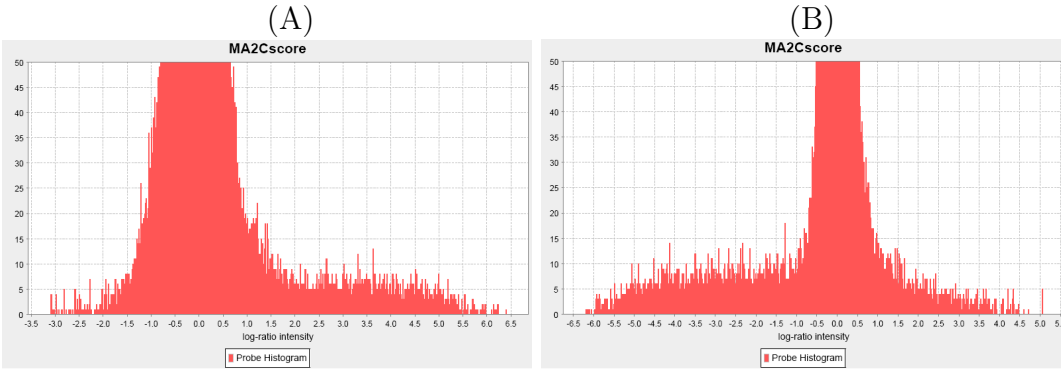


Figure 2: (A) The presence of many enriched peaks is indicated by a heavy right tail in the MA2Cscore histogram. (B) A heavy left tail indicates significant negative peaks and suggests that the experiment has probably failed.

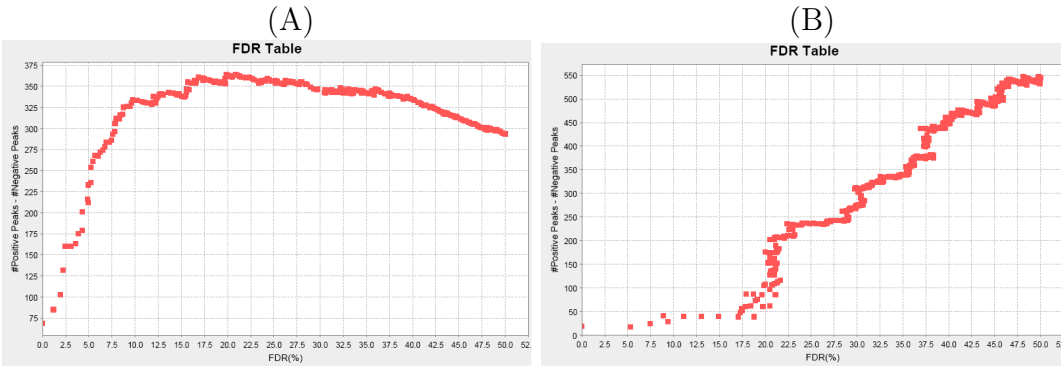


Figure 3: (A) An example of good FDR curve. (B) An example of bad FDR curve.

5 Agilent Data

To convert Agilent data to a format compatible with MA2C:

- Create a `.ndf` file in `DesignFiles/` containing: `DESIGN_ID`, `SEQ_ID`, `PROBE_ID`, `PROBE_SEQUENCE`, `POSITION`, `X`, `Y`, where `X` and `Y` are array coordinates and `POSITION` genomic coordinate.
- Create a `.pos` file in `DesignFiles/` containing: `CHROMOSOME`, `PROBE_ID`, `SEQ_ID`, `POSITION`
- Create `IMAGE_ID.txt` files in `PairData/` containing: `IMAGE_ID`, `X`, `Y`, `PM`. `PM` is the background subtracted hybridization intensity, such as `F635 Median – B635 Median` for `Cy5`. `IMAGE_ID` should be of the form `12345_532` and `12345_635`, for `Cy3` and `Cy5`, respectively

6 Contact Information

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