Question) How to feed pre-selected miRNA list and mRNA list into MMIA.
See below the message.

-----Original Message-----
From: XXXXXXX
Sent: XXXXXXXX
To: XXXXX
Subject: MMIA-help

Dear Dr. XXXXX,
I wonder whether the MMIA webserver is capable of using processed data files for miRNA and mRNA.
I am having trouble using the MMIA web server, using the files attached. I truly appreciate if could help me use these data to perform MMIA analysis.

Error message:
miRNA list processed : 2 % completed 0 min 1 sec left ======Step 1/4 finished======
======Step (Optional) finished====== Check your mRNA file format : rc_code -3.1.

Thank you in advance,
Best,
XXXXXXXX

Answer)

1. Client's data

- miRNA file (miR_UnstimAll5AzvsNo.txt) : the file contains up-regulated microRNAs from their own differential expression analysis.
- mRNA file (Genes5AzUnstimDwn.txt) : the file contains down-regulated mRNA genes (Entrez id) from their own differential expression analysis.

Let's assume that you have two nominal groups (e.g., group1 and group2). Your miRNAs in file "miR_UnstimAll5AzvsNo.txt" are up-regulated in group2 compared to group1. Your mRNAs in file "Genes5AzUnstimDwn.txt" are down-regulated in group2 compared to group1.

The two files are stored into two different sheets in a same file "Genes5AzUnstimDwn.xls".

2. How to feed the pre-selected/pre-processed miRNA list or mRNA list.

1) Preparation of up-regulated miRNA gene list. Your miRNA file has already only up-regulated miRNA names and cut the names elsewhere for the future pasting into step 1 in MMIA input webpage.

hsa-miR-28-3p
hsa-miR-32
hsa-miR-34c-3p
hsa-miR-103
hsa-miR-125a-3p
hsa-miR-129-3p
hsa-miR-132
hsa-miR-135b
hsa-miR-138
hsa-miR-140-3p
hsa-miR-145
hsa-miR-146b-5p
hsa-miR-155
hsa-miR-187
hsa-miR-192
hsa-miR-193a-5p
hsa-miR-199a-5p
hsa-miR-200b
hsa-miR-204
hsa-miR-224
hsa-miR-296-5p
hsa-miR-297
hsa-miR-299-5p
hsa-miR-340

2) Converting mRNA fold change data to our SIP format. You can also refer to the section "Data format" in our documentation webpage. See the attached excel file "Genes5AzUnstimDwn.xls". We assume that gene identifiers are NCBI Entrez id. You already have your own down-regulated mRNA list in group2 compared to group1. You can make fake expressions for group1 and group2.

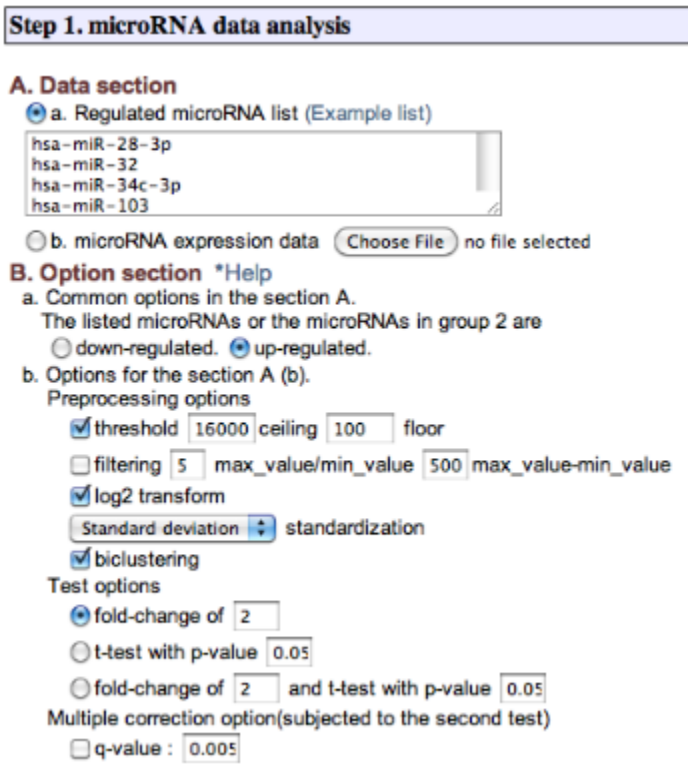
2-1) In the excel file, sheet "Genes5AzUnstimDwn.txt" has your original mRNA fold-changes. The actual fold-change data is useless for your case. In sheet "Genes5AzUnstimDwn.sip", nominal expression data was generated***. All values in group1 are 1s and all values in group2 0.5 (1/2)s. For step 3 in MMIA webpage, the***

fold-change cut-off option can be set to any value greater than 1 and less than 2 (e.g., 1.5). (ß The bold italic sentence is important.)

2-2) The sheet "Genes5AzUnstimDwn.sip" was saved as tab-delimited format by using menu "File > Save As > Format > Tab Delimited Text (.txt)" in the excel program. If you use Mac, you would remember that Mac uses a different end of line character in their text files from Windows or Linux/Unix (More details in section "FAQ" in MMIA documentation webpage).

3. Running MMIA by using the materials from the previous section.

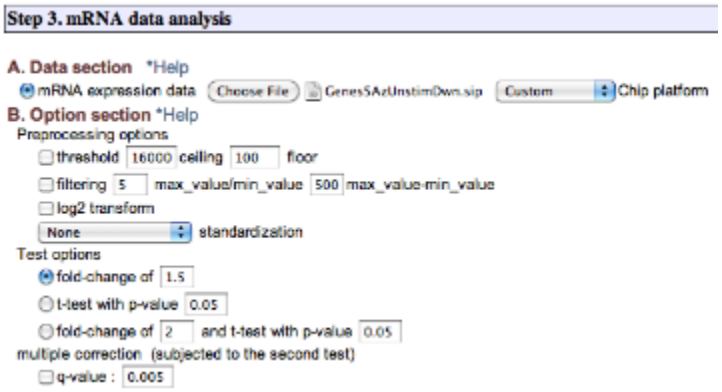
3-1) Step in MMIA web page.
Select options carefully as shown in Figure 1. You paste the up-regulated miRNA list into the text box in section A. a.
You don't need to care about section B.b since the section is only good for a microRNA expression SIP file format (section A.b).



[Figure 1. Step 1 in MMIA input webpage]

3-2) Step 2 in MMIA web page.
You can skip the step. By default, TargetScan 5.1 algorithm is used to identify mRNA targets for the up-regulated miRNAs. The available miRNA names in TargetScan 5.1 are in http://www.targetscan.org.

3-3) Step 3 in MMIA web page.
Options are carefully selected as shown in Figure 2. ***As mentioned earlier, the fold-change cut off is set to 1.5.***



[Figure 2. Step 3 in MMIA web page]

3-4) Step 4 in MMIA web page
You can skip the step. By default, every analysis is done in the step.

Now, everything is set. Press “Submit” button.
You see the result.