

Cyber-TPlus Manual

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Cyber-TP: Analysis of Differential Gene Expression Based on Bayesian Estimation of Variance

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Web page: <http://www.australianprostatecentre.org/research/software/cybertplus>

1. Installation

1.1. Java Requirement

J-Circos requires Java 7 or later. Instructions to download and install Java Platform (Java SE Development Kit) are available from

<http://www.oracle.com/technetwork/java/javase/downloads/index.html>

1.2. Command Line start

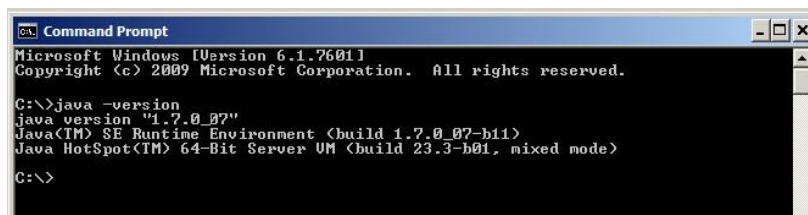
- a. J-Circos source files can be downloaded as a zip archive from:
<http://www.australianprostatecentre.org/research/software/cybertplus>
- b. Download the zip file into a folder – e.g. C:\cyber_tp (Windows), or /home/xxx/cyber_tp (Linux, MacOS).
- c. Once the files are extracted from the zip archive, a new folder “cyber_tp” will appear. The folder contains four files:
 - **cyber-TP.bat**, is a batch file, which runs in the Windows environment. A shortcut can be created on the desktop to allow cyber-TPlus to run by simply clicking the shortcut.
 - **cyber-TP.sh**, is a bash file for linux and MacOS.
 - **cybertp.jar**, stores Java classes of cyber-TPlus. This is the main file to run this tool.

And two subdirectories:

- **Lib folder**, contains all Java libraries, used by cyber-TPlus.
- **Data**. Under the data folder you will find:
 - **aglient_44k.txt** (used for microarray data): aglient 44k human microarray probe list, including probe name and its corresponding transcript and gene names. If you use other aglient chips you need to download probe file from http://www.chem.agilent.com/cag/bsp/gene_lists.asp. For other brand microarray chips, please download their probe file accordingly.
 - **refseq_hg19.tx** and **refseq_mm10.tx** (used for RNAseq data): human transcript annotation files. The annotation file is in UCSC transcript format. You can download other annotation files from <http://genome.ucsc.edu/cgi-bin/hgTables?command=start>
 - **demo** subdirectory: there are 7 demo files. **ctrl1.txt**, **ctrl2.txt**, **ctrl3.txt**, **expr1.txt**, **expr2.txt**, **expr3.txt** are for demonstrating microarray. **RNAseq_example.txt** for demonstrating RNAseq.

1.3.1 Start cyber-TPlus

- a. First ensure that Java is executable, as shown in Figure 1. If not, you need to edit the PATH system environment variable.



```
Microsoft Windows [Version 6.1.7601]
Copyright (c) 2009 Microsoft Corporation. All rights reserved.

C:\>java -version
java version "1.7.0_07"
Java(TM) SE Runtime Environment (build 1.7.0_07-b11)
Java HotSpot(TM) 64-Bit Server VM (build 23.3-b01, mixed mode)

C:\>
```

Figure 1 test whether java is installed

- b. In Windows, double click on the cyber-TP.bat to start cyber-TPlus. You can also start cyber_TPlus manually from the command line.
- c. In Linux or MacOS, change sh file executable: >chmod +x cyber-TP.sh and type cyber-TP.sh.

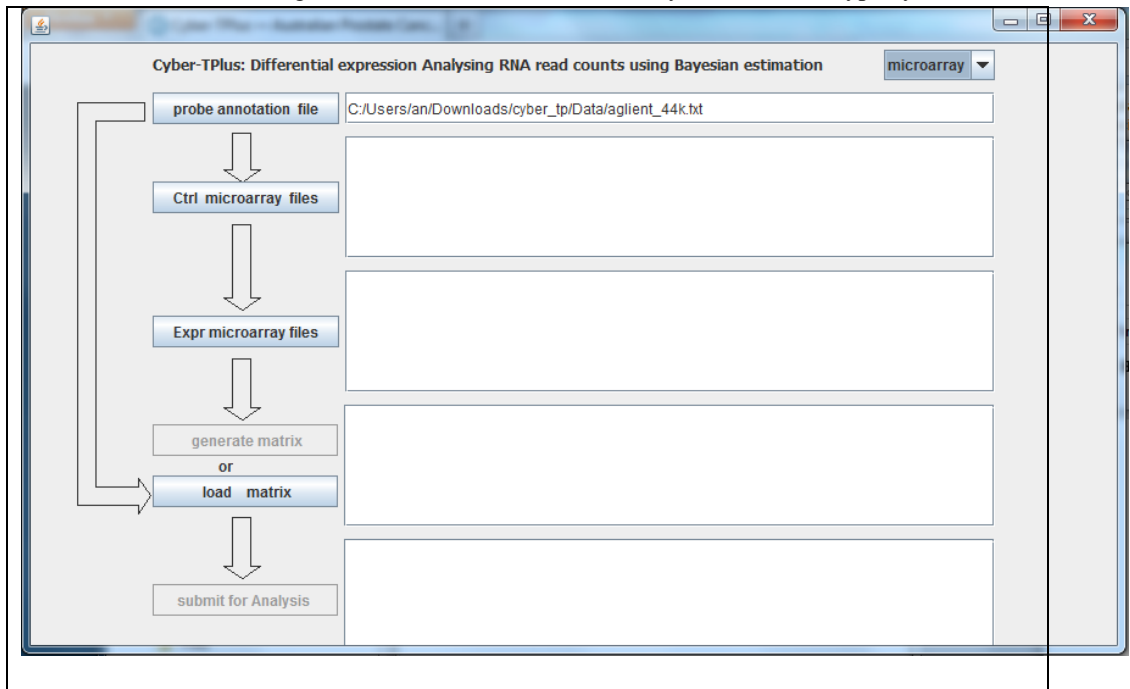


Figure 2 the initial interface of Cyber-TPlus.

1.3.2 Microarray data

Click “ctrl microarray files”, select microarray data files for control as shown in Figure 3. The same procedure for uploading experimental microarray data files.

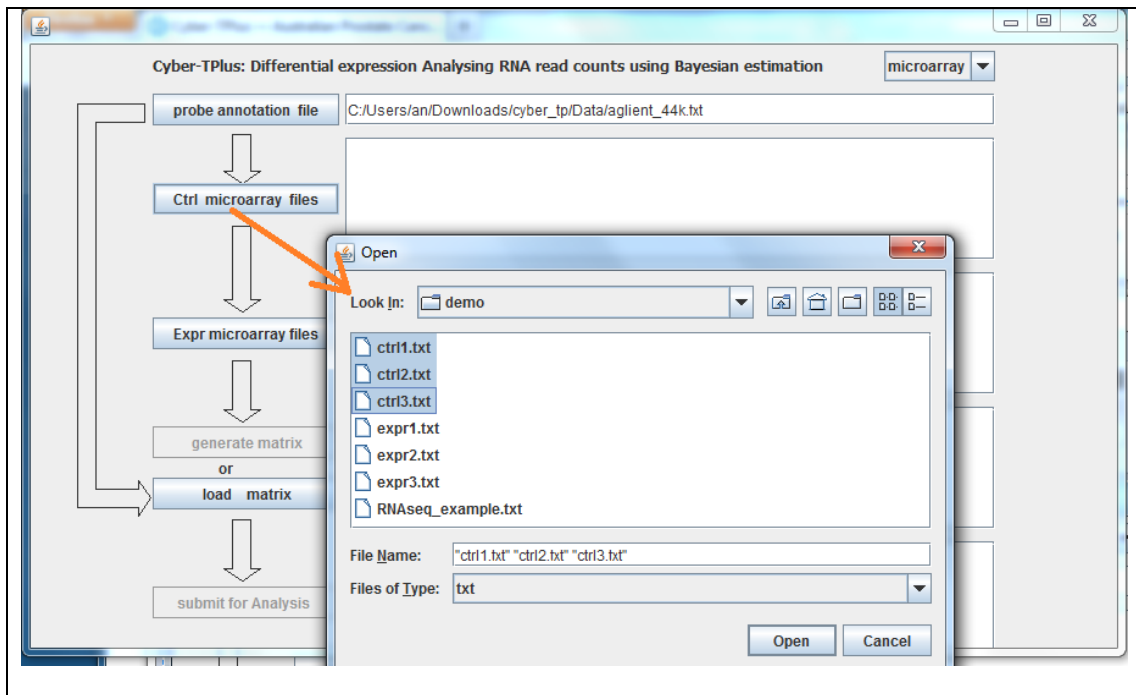


Figure 3 upload microarray data file

To generate raw expression level table for analysis, click “generate matrix” as **Figure 4**. In the working directory, a file called “generated_table.txt”, which summaries all raw expression levels into a table, is generated.

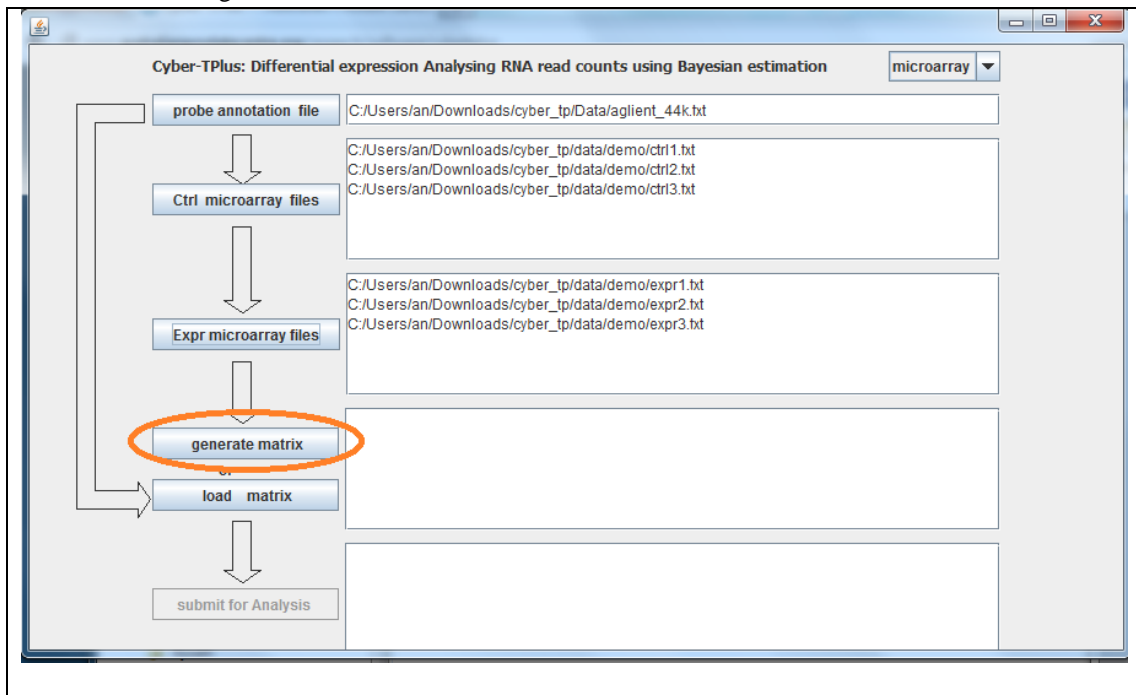


Figure 4 generate raw microarray expression level.

To analyse expression data, click “submit for Analysis” as shown in **Figure 5**. Two files “normalized_data.txt” and “result.cyber-tplus.txt”, which stores the normalized expression level and result of analysis respectively, are generated.

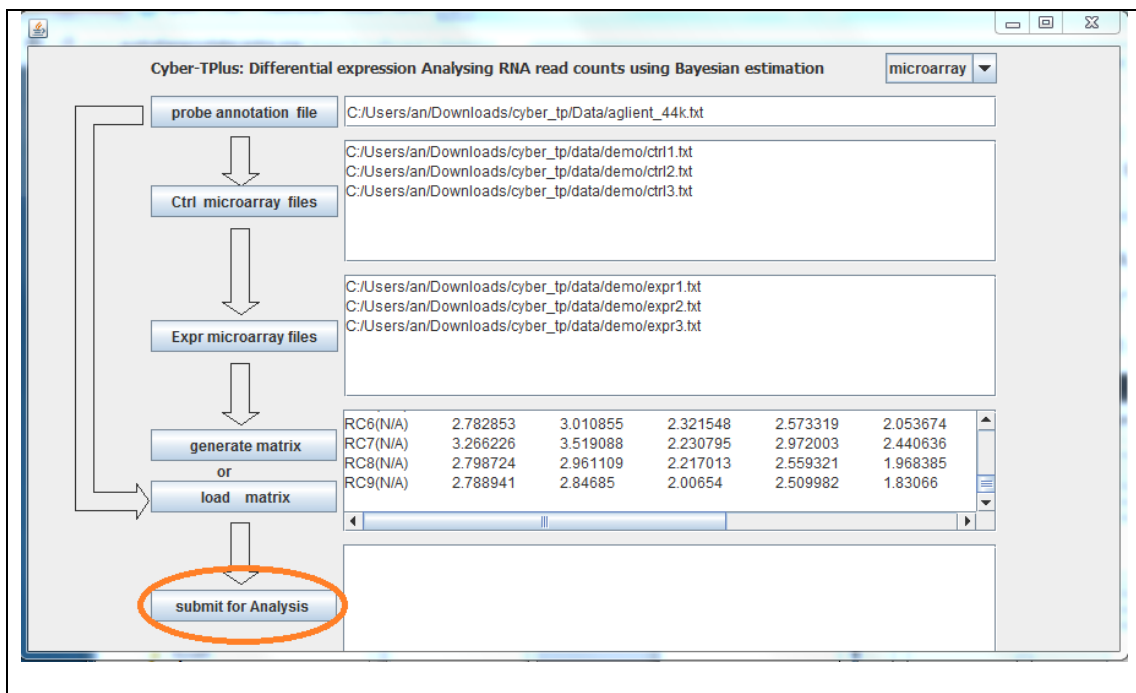


Figure 5 Differential analysis of expression data

1.3.3 RNAseq data

Select RNASeq item from the pull-down menu on the up-right corner. The default transcript annotation file is human refseq hg19 assembly. You can download other transcript annotation from from <http://genome.ucsc.edu/cgi-bin/hgTables?command=start>

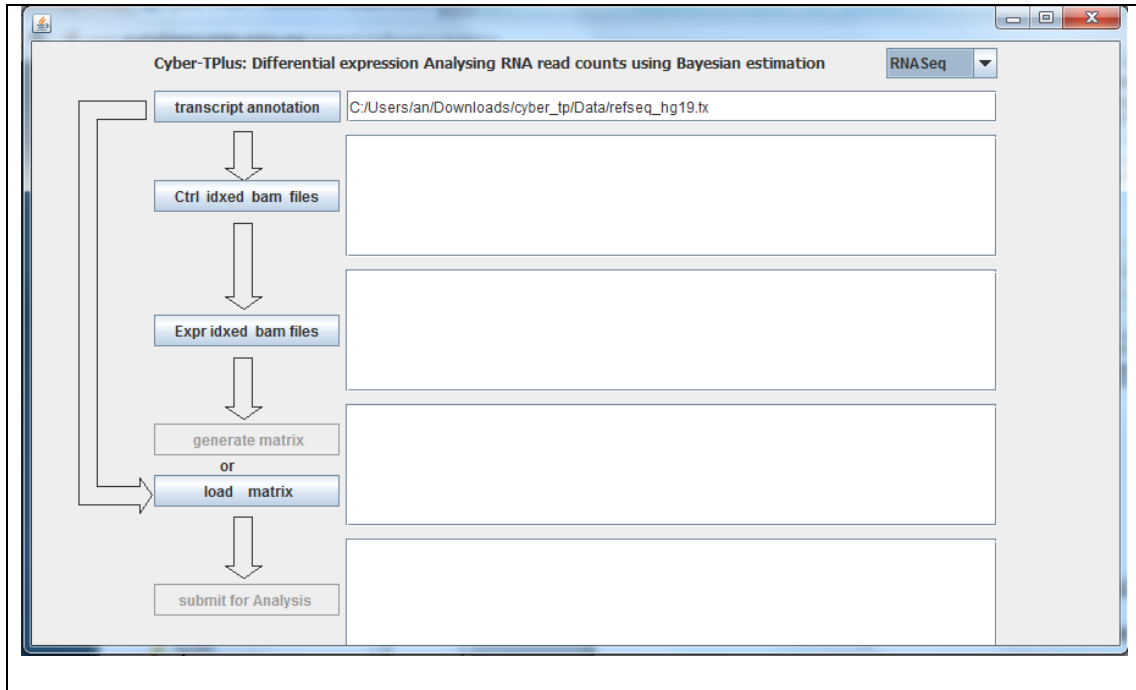


Figure 6 initial interface for RNAseq.

You can upload bam files for control and experiment as did for microarray and then click “generate matrix” to get read counts for all transcripts. You can also upload a pre-generated matrix count table as shown in Figure 7.

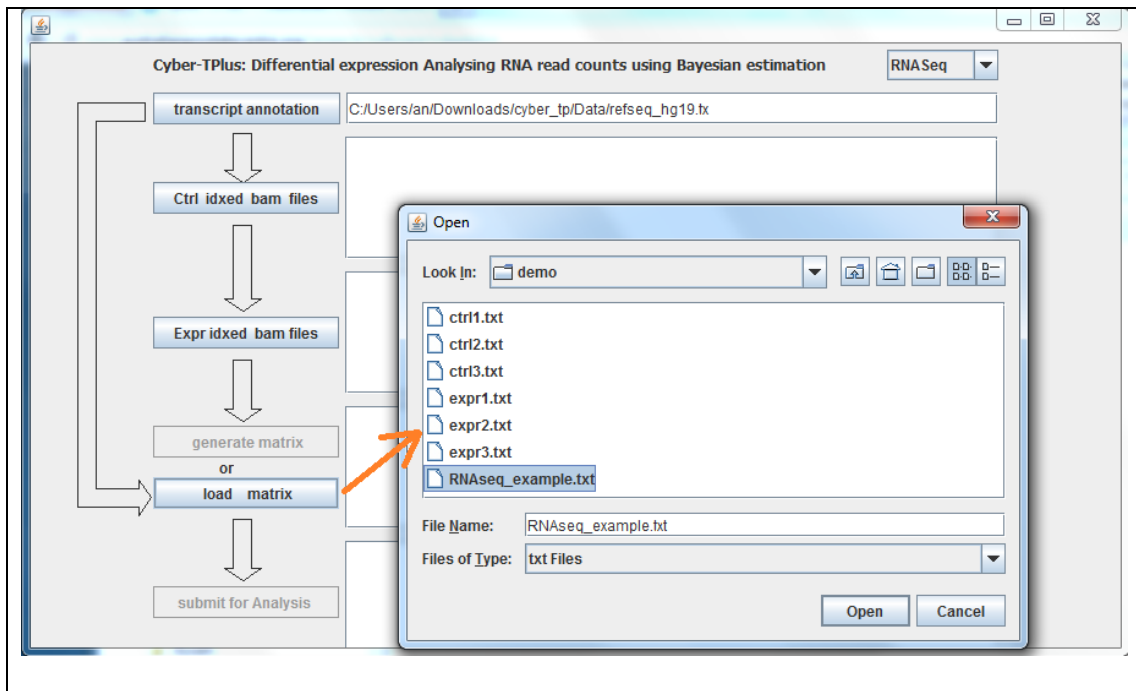


Figure 7 upload pre-generated read counts table

To analyse the RNAseq read counts data, click “submit for analysis” as shown in Figure 8. As microarray data analysis, two files “normalized_data.txt” and “result.cyber-tplus.txt” are generated.

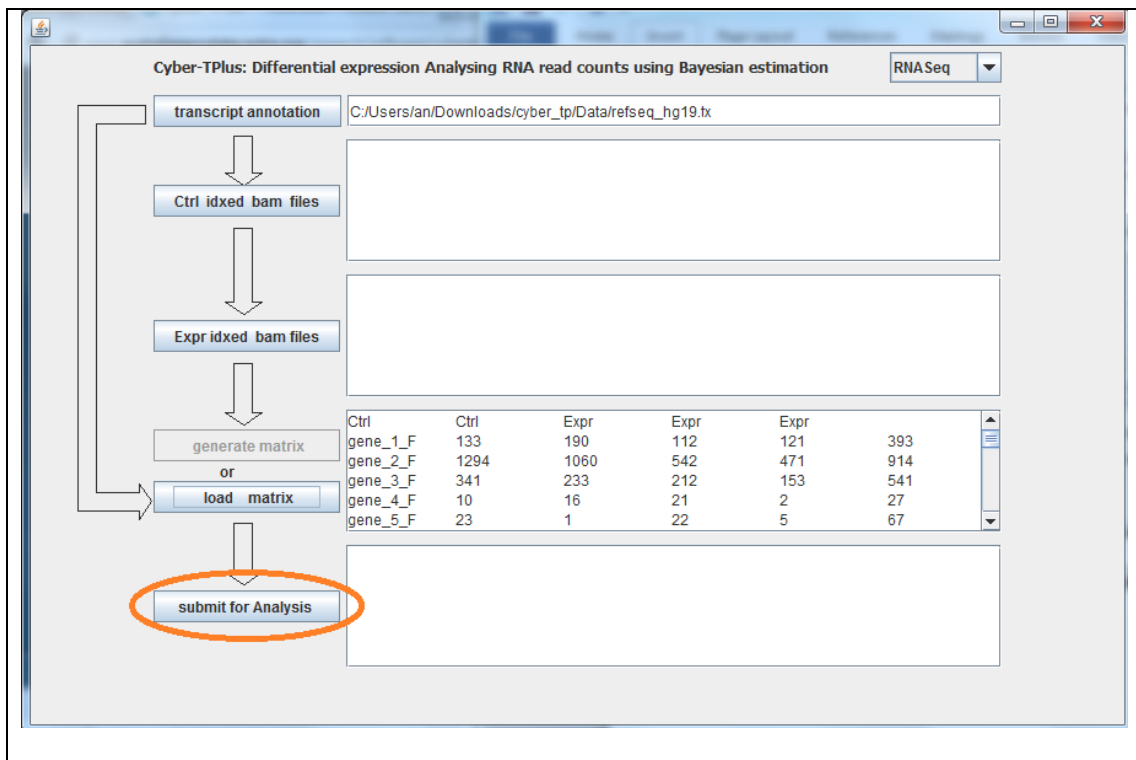


Figure 8 analyse RNAseq data