

Figure 1. Methodology

We have taken “**Prostate cancer - comparison of androgen-dependent and -independent micro dissected primary tumor**” GSE2443 (20 samples) and processed .CEL files with WB-DEGS.

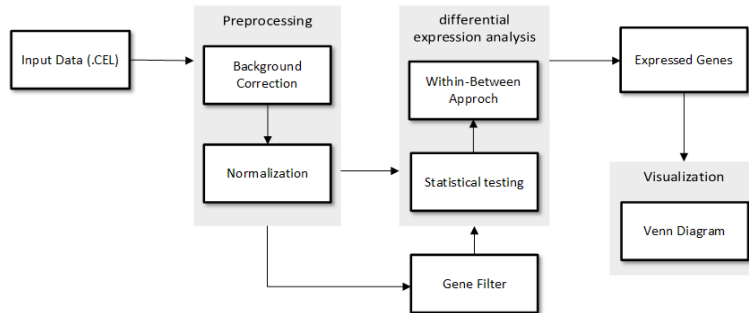


Figure 2. WB-DEGS workflow.

After installation of latest version of R, we installed the necessary packages from CRAN and Bioconductor:

from Cran:

```
install.packages("shiny")
install.packages("VennDiagram")
```

from Bioconductor:

```
source("http://bioconductor.org/biocLite.R")
biocLite("affy")
biocLite("affyPLM")
biocLite("limma")
biocLite("siggenes")
biocLite("twilight")
biocLite("genefilter")
```

Every Shiny app has the same structure: two R scripts saved together in a directory. At a minimum, a Shiny app has `ui.R` and `server.R` files.

We can run WB-DEGS opening `ui.R` or `server.R` or by following code:

```
library(shiny)
runApp("WB_Degs")
```

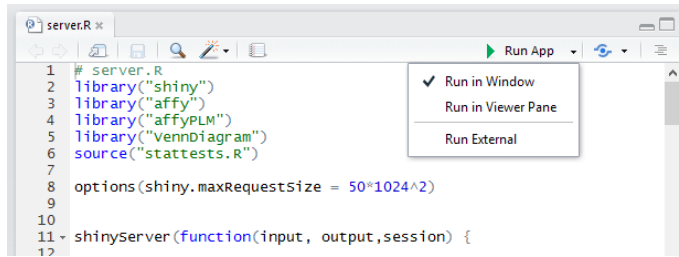


Figure 3. Running WB-DEGS

Within-Between Differential Expression Genes

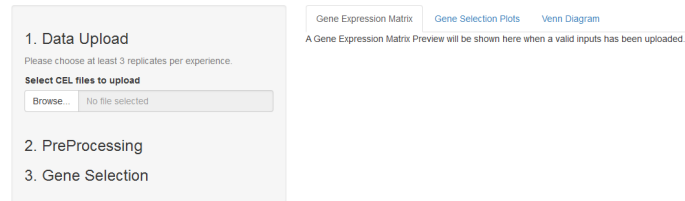


Figure 4. WB-DEGS UI window

We uploaded the selected .CEL files of GSE2443 in Data Upload Option (Figure 5), then, we used RMA as background correction method with Quantiles as normalization method (Figure 6 & 7). After the preprocessing of data is done, we divided the samples into two groups: (1) Group 1: test group, (2) Group 2 : control group based on the curated sample data from the cited paper from GEO [Best CJ et. Al.] (Figure 8). In the final step, we applied statistical analysis for estimation of local and global false discovery rate (FDR) and mapping the overexpressed and under expressed genes (Figure 9):

1. Simple Statistical Test (t-test)
2. Linear Models
3. Twilight
4. Significance Analysis of Microarray (SAM)

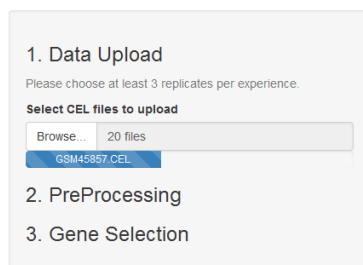


Figure 5. Uploading the. CEL files

1. Data Upload

Please choose at least 3 replicates per experience.

Select CEL files to upload

Browse... 20 files

Upload complete

2. PreProcessing

Please costumize your preprocessing method

Background Correction Method :

Choose a Method

Choose a Method

RMA

MAS

Figure 6. Background Correction Method

Background Correction Method :

RMA

Normalization Method :

Choose a Method

Choose a Method

Quantiles

Constant

Loess

Qspline

Figure 7. Normalization Methods

3. Gene Selection

☐ Filter Genes

filter out genes with a small variance across samples.

Select Group 1 entries

GSM45730.CEL GSM45855.CEL GSM45854.CEL

GSM45853.CEL GSM45852.CEL GSM45851.CEL

GSM45850.CEL GSM45849.CEL GSM45848.CEL

GSM45847.CEL

Select Group 2 entries

GSM45865.CEL GSM45864.CEL GSM45863.CEL

GSM45862.CEL GSM45861.CEL GSM45860.CEL

GSM45859.CEL GSM45858.CEL GSM45857.CEL

GSM45856.CEL

Figure 8. Group 1: control group, Group 2: test group. Gene Selection

Statistical Analysis :

Choose a Method

Choose a Method

Simple Statistical Test

Twilight

Linear Models

Significance Analysis of Microarray

Figure 9. Statistical Analysis (t-test, twilight, linear, SAM).