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Upload Data

App Name

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Data Importation

Upload Data

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Quality Control

Grouping of Samples

Sample Grouping

Statistical Analysis

DEG

Functional Analysis

Functional

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Input Type

Processed Expression Data (CSV/txt File)

Processed Expression Data (CSV/txt)

Browse... No file selected

Metadata (CSV)

Browse... No file selected

Select the feature you wish to analyze further

Load Data

@STEM-Away

Input Type:

- Processed Expression Data (CSV/txt file)
- Raw Affymetrix Data (.tar file containing CEL files)
- Raw Illumina Data (.tar file containing IDAT files)
- GEO Accession Number

Processed Expression Data (csv/txt): The selected GSE datasets from the GEO database.

Metadata: Input the simplified csv file that contains columns for sample IDs, cancer/normal identifications, and sample groups. There are a maximum of 2 sample groups.

Selecting Features: By uploading the metadata CSV file, the names of the columns will be extracted and put into this dropdown list. You will now be able to select which feature you would like to analyze further, such as comparing cancer vs. normal groups or any other condition of interest.

Instructions for Processed Expression Data and Acquiring Metadata:

1. Select and download expression data from the GEO database. Explore the GEO database [here](#).

Example of downloading expression data from GEO:

Supplementary file	Size	Download	File type/resource
GSE8671_RAW.tar	558.0 Mb	(http)(custom)	TAR (of CEL, CHP)

Raw data provided as supplementary file
Processed data included within Sample table
Processed data provided as supplementary file

Custom GSE8671_RAW.tar archive:

Supplementary file	File size
<input checked="" type="checkbox"/> GSM215051.CEL.gz	5.0 Mb
<input type="checkbox"/> GSM215051.CHP.gz	6.5 Mb
<input checked="" type="checkbox"/> GSM215052.CEL.gz	4.5 Mb
<input type="checkbox"/> GSM215052.CHP.gz	6.3 Mb

- Unzip the .tar file(s).
 - For Windows users, you may need to download [WinRAR](#) (preferably x64).
 - For Mac users, double-click the .tar folder.
- Acquire metadata by following the steps below. Metadata is important as this data will be used in batch correction and limma analysis (a method of statistical analysis on the significance of differential gene expression (DGE)).

STEP 1: Download SERIES MATRIX FILES txt from GEO and unzip.

Download family	Format
SOFT formatted family file(s)	SOFT ?
MINiML formatted family file(s)	MINiML ?
Series Matrix File(s)	TXT ?

Once you unzip the txt file, you should see something like this:

```
GSE18105_series_matrix.txt
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!Series_geo_accession "GSE18105"
!Series_status "Public on Feb 04 2010"
!Series_submission_date "Sep 14 2009"
!Series_last_update_date "Mar 25 2019"
!Series_submission_id "20162577"
!Series_summary "Distant metastasis is the major causes of death in colorectal cancer (CRC) patients. In order to identify genes influencing the prognosis of patients with CRC, we compared gene expression in primary tumors with and without distant metastasis using an oligonucleotide microarray. We also examined the expression of the candidate gene in 100 CRC patients by quantitative real-time reverse transcription PCR and studied the relationship between its expression and the prognosis of patients with CRC. As a result, we identified MUC12 as a candidate gene involved in metastasis processes by microarray analysis. Quantitative real-time reverse transcription PCR showed that MUC12 expression was significantly lower in cancer tissues than in adjacent normal tissues (P < 0.001). In stage II and stage III CRC, patients with low expression showed worse disease-free survival (P = 0.038). Multivariate analysis disclosed that MUC12 expression status was an independent prognostic factor in stage II and stage III CRC (relative risk, 9.532; 95% confidence interval, 2.303-41.905; P = 0.002). This study revealed the prognostic value of MUC12 expression in CRC patients. Moreover, our result suggests MUC12 expression is a possible candidate gene for assessing postoperative adjuvant therapy for CRC patients."
!Series_overall_design "Total of 111 microarray datasets (77 for LCM samples, and 17 pairs for homogenized samples from tumor and adjacent tissues) were normalized using robust multi-array average (RMA) method under R 2.6.2 statistical software together with BioConductor package, as described previously. Then, the gene expression levels were log2-transformed, and 62 control probe sets were removed for further analysis. In order to identify a set of genes associated with development of metastatic recurrence, we performed Wilcoxon rank-sum test for gene expression differences of 54,633 probe sets between recurrence and non-recurrence groups. Similarly, Wilcoxon signed-rank test was conducted to select genes which showed significant expression difference between tumor and adjacent tissue. Then, we selected a set of genes that satisfied both of above two criteria."
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!Series_contributor "Kaoru, Higashihara"
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!Series_contact_institute "Fujitendo University"
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!Sample_cell "patient 480, cancer, LCM" "patient 481, cancer, LCM" "patient 482, cancer, LCM" "patient 483, cancer, LCM" "patient 484, cancer, LCM" "patient 487, cancer, LCM"
!Sample_accession "GSM4494778" "GSM4494779" "GSM4494780" "GSM4494781" "GSM4494782" "GSM4494783"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 490, cancer, LCM" "patient 491, cancer, LCM" "patient 492, cancer, LCM" "patient 493, cancer, LCM" "patient 494, cancer, LCM" "patient 497, cancer, LCM"
!Sample_accession "GSM4494784" "GSM4494785" "GSM4494786" "GSM4494787" "GSM4494788" "GSM4494789"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 500, cancer, LCM" "patient 501, cancer, LCM" "patient 502, cancer, LCM" "patient 503, cancer, LCM" "patient 504, cancer, LCM" "patient 507, cancer, LCM"
!Sample_accession "GSM4494790" "GSM4494791" "GSM4494792" "GSM4494793" "GSM4494794" "GSM4494795"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 510, cancer, LCM" "patient 511, cancer, LCM" "patient 512, cancer, LCM" "patient 513, cancer, LCM" "patient 514, cancer, LCM" "patient 517, cancer, LCM"
!Sample_accession "GSM4494796" "GSM4494797" "GSM4494798" "GSM4494799" "GSM4494800" "GSM4494801"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 520, cancer, LCM" "patient 521, cancer, LCM" "patient 522, cancer, LCM" "patient 523, cancer, LCM" "patient 524, cancer, LCM" "patient 527, cancer, LCM"
!Sample_accession "GSM4494802" "GSM4494803" "GSM4494804" "GSM4494805" "GSM4494806" "GSM4494807"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 530, cancer, LCM" "patient 531, cancer, LCM" "patient 532, cancer, LCM" "patient 533, cancer, LCM" "patient 534, cancer, LCM" "patient 537, cancer, LCM"
!Sample_accession "GSM4494808" "GSM4494809" "GSM4494810" "GSM4494811" "GSM4494812" "GSM4494813"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 540, cancer, LCM" "patient 541, cancer, LCM" "patient 542, cancer, LCM" "patient 543, cancer, LCM" "patient 544, cancer, LCM" "patient 547, cancer, LCM"
!Sample_accession "GSM4494814" "GSM4494815" "GSM4494816" "GSM4494817" "GSM4494818" "GSM4494819"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 550, cancer, LCM" "patient 551, cancer, LCM" "patient 552, cancer, LCM" "patient 553, cancer, LCM" "patient 554, cancer, LCM" "patient 557, cancer, LCM"
!Sample_accession "GSM4494820" "GSM4494821" "GSM4494822" "GSM4494823" "GSM4494824" "GSM4494825"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 560, cancer, LCM" "patient 561, cancer, LCM" "patient 562, cancer, LCM" "patient 563, cancer, LCM" "patient 564, cancer, LCM" "patient 567, cancer, LCM"
!Sample_accession "GSM4494826" "GSM4494827" "GSM4494828" "GSM4494829" "GSM4494830" "GSM4494831"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 570, cancer, LCM" "patient 571, cancer, LCM" "patient 572, cancer, LCM" "patient 573, cancer, LCM" "patient 574, cancer, LCM" "patient 577, cancer, LCM"
!Sample_accession "GSM4494832" "GSM4494833" "GSM4494834" "GSM4494835" "GSM4494836" "GSM4494837"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 580, cancer, LCM" "patient 581, cancer, LCM" "patient 582, cancer, LCM" "patient 583, cancer, LCM" "patient 584, cancer, LCM" "patient 587, cancer, LCM"
!Sample_accession "GSM4494838" "GSM4494839" "GSM4494840" "GSM4494841" "GSM4494842" "GSM4494843"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 590, cancer, LCM" "patient 591, cancer, LCM" "patient 592, cancer, LCM" "patient 593, cancer, LCM" "patient 594, cancer, LCM" "patient 597, cancer, LCM"
!Sample_accession "GSM4494844" "GSM4494845" "GSM4494846" "GSM4494847" "GSM4494848" "GSM4494849"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 600, cancer, LCM" "patient 601, cancer, LCM" "patient 602, cancer, LCM" "patient 603, cancer, LCM" "patient 604, cancer, LCM" "patient 607, cancer, LCM"
!Sample_accession "GSM4494850" "GSM4494851" "GSM4494852" "GSM4494853" "GSM4494854" "GSM4494855"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 610, cancer, LCM" "patient 611, cancer, LCM" "patient 612, cancer, LCM" "patient 613, cancer, LCM" "patient 614, cancer, LCM" "patient 617, cancer, LCM"
!Sample_accession "GSM4494856" "GSM4494857" "GSM4494858" "GSM4494859" "GSM4494860" "GSM4494861"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM" "LCM"
!Sample_cell "patient 620, cancer, LCM" "patient 621, cancer, LCM" "patient 622, cancer, LCM" "patient 623, cancer, LCM" "patient 624, cancer, LCM" "patient 627, cancer, LCM"
!Sample_accession "GSM4494862" "GSM4494863" "GSM4494864" "GSM4494865" "GSM4494866" "GSM4494867"
!Sample_status "N" "N" "N" "N" "N" "N"
!Sample_type "LCM" "LCM" "LCM" "LCM" "LCM
```

Quality Control:

QC:

App Name

Transcripto

→ Introduction

Data Importation

Upload Data

Quality Control

Quality Control

QC

Normalization

Batch Correction

Find Potential Outliers

Choose a QC visualization method before normalization.

NUSE

Visualize Data

QC Visualization Methods (Un-Normalized): Select visualization method: NUSE or RLE. Click on “Visualize Data” to visualize the un-normalized gene set into a box plot.

Normalization:

App Name

Transcripto

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Quality Control

QC

Normalization

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Find Potential Outliers

Which normalization method do you want to use?

RMA

GCRMA

MASS

Begin Normalization

Normalization Methods: Select one of the normalization methods: RMA, GCRMA, or MASS. Click “Begin Normalization” to normalize your data.

Batch Correction:

App Name

Transcripto

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Quality Control

QC

Normalization

Batch Correction

Find Potential Outliers

If samples come from different batches, specify which metadata feature indicates the batch each sample belongs to.

Perform Batch Correction

Choose a QC visualization method after normalization.

Boxplot

Generate Plot

Metadata Feature (only if there are different batches): Specify which metadata feature corresponds with which batch. Click “Perform Batch Correction” to begin batch correction.

QC Visualization Method (Normalized): Select visualization method: Boxplot or PCA. Click on “Generate Plot” to visualize the normalized gene set into a box plot.

Find Potential Outliers:

The screenshot shows a software interface with a sidebar on the left and a main panel on the right. The sidebar has a green header 'App Name' and a menu icon. Below it are sections: 'Introduction' with a right arrow, 'Data Importation' with an upload icon, 'Quality Control' with a dropdown arrow, and 'Find Potential Outliers' with a magnifying glass icon. The main panel has a header 'Transcrip' and a menu icon. Below it is a section 'Outlier Detection Method' with a dropdown menu showing 'KS'. At the bottom of the main panel are two buttons: 'Find Potential Outliers' and 'Show Updated List of Samples'.

Outlier Detection Method: Select the desired method of outlier identification: KS, SUM, or Upper quartile.

Find Potential Outliers: Click to find outliers.

Show Updated List of Samples: Updated list of samples will exclude outliers found using the “Find Potential Outliers” button.