

Galaxy Limma-voom DEG Analysis Tutorial

Mentor: Ayse

PPT prep: Fatma

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Getting Started

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ChIP-seq

RNA-seq

Slamdunk - streamlining SLAM-seq analysis with ultra-high sensitivity

Trinotate functional transcript annotation

limma Perform differential expression with limma-voom or limma-trend

edgeR Perform differential expression of count data

featureCounts Measure gene expression in RNA-Seq experiments from SAM or BAM files

StringTie merge transcripts

limma Perform differential expression with limma-voom or limma-trend (Galaxy Version 3.48.0+galaxy2)

Differential Expression Method

limma-voom

Select the limma-voom or limma-trend method. See Help section below for more information. Default: limma-voom

Apply voom with sample quality weights?

☐ No

Apply weights if outliers are present (voomWithQualityWeights). Default: False.

Count Files or Matrix?

Single Count Matrix

You can choose to input either separate count files (one per sample) or a single count matrix

Count Matrix

1: Erb1KO_vs_control.txt

Input factor information from file?

No

1. Önce araçlar panelinden seçim yapacağız

2. RNA-seq verisi analizi olacak

3. Diferensiyel gen ifadesi analizi için «limma» paketini kullanacağız

4. Daha özelse ise «limma-voom»


5. Hazır «raw count matrix» verisi yüklüyoruz kontrol ve mutant verisi tek dosyada





<https://usegalaxy.org/>

Yüklediğimiz
örnek «count»
verisinin içeriği

Erb1KO_vs_control - Notepad					
File Edit Format View Help					
GeneID	Control_1	Control_2	Erb1KO_1	Erb1KO_2	
1/2-SBSRNA4	12	18	38	23	
A1BG	199	184	98	105	
A1BG-AS1	279	318	285	203	
A1CF	0	0	0	0	
A2LD1	163	126	129	134	
A2M	3	0	2	2	
A2ML1	8	3	4	6	
A2MP1	0	1	1	0	
A4GALT	198	188	133	106	
A4GNT	1	0	0	0	
AA06	0	0	0	0	
AAA1	0	1	0	0	
AAAS	2832	2516	2577	1836	
AACS	5643	5115	5002	3388	
AACSP1	4	3	6	1	
AADAC	0	3	0	0	
AADACL2	0	0	0	0	
AADACL3	0	0	0	0	
AADACL4	0	0	0	0	
AADAT	440	291	582	382	
AAGAB	5027	4671	5684	3762	
AAK1	2520	2183	3090	2113	
AAMP	7871	7612	7315	5995	
AANAT	5	1	0	4	
AARS	89996	74123	98451	72790	
AARF	1000	000	1007	784	

Verinin orijinal kaynağı: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104296>

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StringTie merge transcripts

Filter lowly expressed genes?

Yes

Treat genes with very low expression as unexpressed and filter out. See the Filter Low Counts section below for more information. Default: No

Filter on CPM or Count values?

CPM

It is slightly better to base the filtering on count-per-million (CPM) rather than the raw count values so as to avoid favoring genes expressed in samples sequenced to a higher depth.

Minimum CPM

4.0

Treat genes with CPM below this value as unexpressed and filter out. See the Filter Low Counts section below for more information.

Minimum Samples

0

Filter out all genes that do not meet the Minimum CPM in at least this many samples. See the Filter Low Counts section below for more information.

Output Options

Sütunları
kategorize
ediyoruz

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Mapping

Variant Calling

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StringTie merge transcripts

StringTie transcript assembly and quantification

You can choose to input the factor and group information for the samples from a file or manually enter below. NOTE: Please only use letters, numbers or underscores (case sensitive), the group names MUST not contain hyphens.

Factor

1: Factor

Factor Name

tr

Name of experiment factor of interest (e.g. Genotype). One factor must be entered and there must be two or more groups per factor. Additional factors (e.g. Batch) can be entered using the Insert Factor button below, see Help section below. NOTE: Please only use letters, numbers or underscores.

Groups

WT,WT,Mut,Mut

Enter the group names for the samples separated with commas e.g. WT,WT,WT,Mut,Mut,Mut. The order of the names must match the order of the samples in the

+ Insert Factor

Use Gene Annotations?

No


If you provide an annotation file, annotations will be added to the table(s) of differential expression results to provide descriptions for each gene, and used to label the top genes in the Volcano plot. Interactive Glimma Volcano and MD plots will also be generated. See Help section below.





Input Contrast information from file?

No

You can choose to input the contrast information for the samples from a file or manually enter below. NOTE: Please only use letters, numbers or underscores (case sensitive), the group names MUST not contain hyphens. Use a hyphen to separate the groups you want to compare, as shown in the Help section below.

Contrast

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GENOMIC FILE MANIPULATION

FASTA/FASTQ

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Contrast

1: Contrast

Contrast of Interest

MuT-WT

Names of two groups to compare separated by a hyphen e.g. Mut-WT. If the order is Mut-WT the fold changes in the results will be up/down in Mut relative to WT. If you have more than one contrast enter each separately using the Insert Contrast button below. For differences between contrasts use e.g. (Mut1-Ctrl1)-(Mut2-Ctrl2). For more

Normalizasyon yapabilmesi için bir karşılaştırma seçeneği olarak mutant (MuT) vs. Wild-tye(WT)

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Measure gene expression in RNA-Seq experiments from SAM or BAM files

Output Options

Additional Plots

Select/Unselect all

☒ Glimma Interactive Plots

☐ Density Plots (if filtering)

☐ CpmsVsCounts Plots (if filtering on cpms)

☐ Box Plots (if normalising)

☐ MDS Extra (Dims 2vs3 and 3vs4)

☐ MD Plots for individual samples

☒ Heatmaps (top DE genes)

☐ Stripcharts (top DE genes)

Select additional plots to output in the report

Output Filtered Counts Table?

No

Output a file containing the raw filtered counts if Filter Low Counts is selected. Default: No

Output Normalised Counts Table?

No

Output a file containing the normalised counts, these are in log2 counts per million (logCPM). Default: No

History

search datasets

Unnamed history

1 shown

467.81 KB


1: Erb1KO_vs_control.txt





Bize sonuç olarak vereceği verileri seçebiliyoruz. Hangi veri görselleştirme tekniği vs.

11/6/2022

MednOmics

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GENOMIC FILE MANIPULATION

+

 Insert Contrast


Filter Low Counts

Output Options

Advanced Options

✓


 Execute





 What it does

Given a matrix of counts (e.g. from featureCounts) and optional information about the genes, this tool performs differential expression (DE) using the [limma](#) Bioconductor package and produces plots and tables useful in DE analysis. Interactive Glimma plots and tables can also be generated and links to the Glimma plots will be provided in the report. See an example workflow [here](#).

In the [limma](#) approach to RNA-seq, read counts are converted to log2-counts-per-million (logCPM) and the mean-variance relationship is modelled either with precision weights or with an empirical Bayes prior trend. The precision weights approach is called "voom" and the prior trend approach is called "limma-trend". For more information, see the Help section below.

 Sonra da analizi başlatıyoruz

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FASTA/FASTQ

 Executed **limma** and successfully added 1 job to the queue.

The tool uses this input:

- 1: Erb1KO_vs_control.txt

It produces 3 outputs:

- 7: limma on data 1: Report
- 8: limma on data 1: Filtered counts
- 9: limma on data 1: Normalised counts

You can check the status of queued jobs and view the resulting data by refreshing the History panel. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

search datasets

Unnamed history

5 shown, 4 deleted

467.81 KB

9: limma on data 1: Normalised counts

8: limma on data 1: Filtered counts

7: limma on data 1: Report

6: limma on data 1: DE tables

1: Erb1KO_vs_control.txt

İstedğimiz analiz dosyalarını «history» sekmesi altında bulabiliriz



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GeneID	Control_1	Control_2	Erb1KO_1	Erb1KO_2
1/2-SBSRNA4	-1.71373293125231	-0.990099544369114	-0.174361808336457	-0.37755321100273
A1BG	2.28265581519531	2.32792409606233	1.18090347042502	1.78895712602682
A1BG-AS1	2.76911535181615	3.11559665225824	2.71619858633148	2.73674292158588
A1CF	-6.35758912102704	-6.19955290999806	-6.44114834903136	-5.93214206268037
A2LD1	1.9955770447104	1.78344066469625	1.5756599386552	2.13932029987626
A2M	-3.55023419896944	-6.19955290999806	-4.119220254144	-3.61021396779301
A2ML1	-2.2701262797767	-3.39219798794046	-3.27122334758905	-2.23170234453928
A2MP1	-6.35758912102704	-4.61459040927691	-4.8561858483102	-5.93214206268037
A4GALT	2.27540607611592	2.3588678032706	1.6195475826562	1.80256755754547
A4GNT	-4.77262662030588	-6.19955290999806	-6.44114834903136	-5.93214206268037
AA06	-6.35758912102704	-6.19955290999806	-6.44114834903136	-5.93214206268037
AAA1	-6.35758912102704	-4.61459040927691	-6.44114834903136	-5.93214206268037
AAAS	6.11027111968084	6.09764997249884	5.89060836322411	5.91060111805129
AACS	7.104785338056	7.12110663365051	6.84728519776175	6.79428899343387
AACSP1	-3.18766411958473	-3.39219798794046	-2.74070863089027	-4.34717956195921
AADAC	-6.35758912102704	-3.39219798794046	-6.44114834903136	-5.93214206268037
AADACL2	-6.35758912102704	-6.19955290999806	-6.44114834903136	-5.93214206268037
AADACL3	-6.35758912102704	-6.19955290999806	-6.44114834903136	-5.93214206268037

History

Unnamed history

5 shown, 4 deleted

14.51 MB

9: limma on data 1: Normalised counts

8: limma on data 1: Filtered counts

7: limma on data 1: Report

6: limma on data 1: DE tables a list with 1 item

1: Erb1KO_vs_control.txt

«view data» ile
normalize
edilmiş veriler
solda

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GENOMIC FILE MANIPULATION

GeneID	logFC	AveExpr	t	P.Value	adj.P.Val	B
IFI44L	4.03815421260305	7.08186076878113	42.4769679825325	6.67007841335006e-08	0.00142063027624071	8.38698181300211
IFITM1	2.83218298199832	6.36688504284607	36.8511449509934	1.40997796903354e-07	0.00142063027624071	7.92980445119413
IFIT1	3.64060883686041	8.06029417133901	35.0532359983159	1.8348074861039e-07	0.00142063027624071	7.9225571308528
PARP10	2.0072791910943	6.58592075819304	30.867627642187	2.87127460185266e-07	0.00153794107096402	7.58930147075325
IFIT5	1.60448779865671	5.59834772456855	25.2702962123123	3.83383263356464e-07	0.00153794107096402	7.38433149309041
PLSCR1	1.96813616317087	6.76043965188571	29.7391065016783	4.35726653878736e-07	0.00153794107096402	7.25842968062622
USP18	2.05087671012132	4.80256677092994	26.8998092720577	4.63474577955404e-07	0.00153794107096402	6.97934323546266
SLC7A11	1.43551676236645	7.28645926138254	24.7929134186485	5.63668473616585e-07	0.00163661141314575	7.15024418701542
HERC6	3.04392678130893	5.16909226649151	27.1914933210593	6.97555085229945e-07	0.00169196372397968	6.60273034823496
TMEM140	1.99319775060762	2.94927260153154	21.8538769027014	8.81480981191383e-07	0.00169196372397968	5.95387338095875
TRIM21	1.84320236420599	3.84817797161202	21.4891736635137	9.70601817441643e-07	0.00169196372397968	6.28498264541762
PSMB9	2.02316589080236	3.44927823620169	21.4684394266964	9.75977834396654e-07	0.00169196372397968	6.12081242428185
TSPAN1	-1.23475420065549	6.59601612565389	-21.3109807149201	1.01796185264974e-06	0.00169196372397968	6.60594845724621
ISG15	3.1037334311636	7.63712561634435	25.293726127474	1.01978182089356e-06	0.00169196372397968	6.59599797259664
APOL6	2.16583318380289	4.4181167716944	23.8761144111936	1.37998293216371e-06	0.00212365342448422	6.00821007810136
TAP2	1.7405983293079	6.83524418763369	23.5300787391241	1.4897239057559e-06	0.00212365342448422	6.25920230444849

History

< Back to Unnamed history

limma on data 1: DE tables

a list with 1 item

limma-voom_MuT-WT

23,229 lines

Format: tabular, database: hg19, track: 15

1	2	3	4
GeneID	logFC	AveExpr	t
IFI44L	4.03815421260305	7.08186076878113	42.476967
IFITM1	2.83218298199832	6.36688504284607	36.851144
IFIT1	3.64060883686041	8.06029417133901	35.053235
PARP10	2.0072791910943	6.58592075819304	30.867627

«view data» ile
differensiyel veria analizi
sonuç dosyası

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FASTQ Quality Control

SAM/BAM

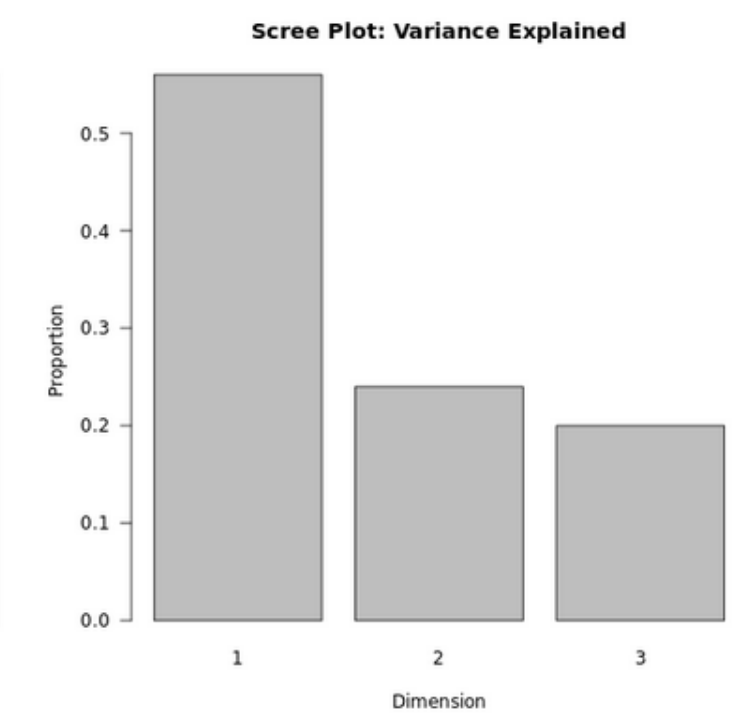
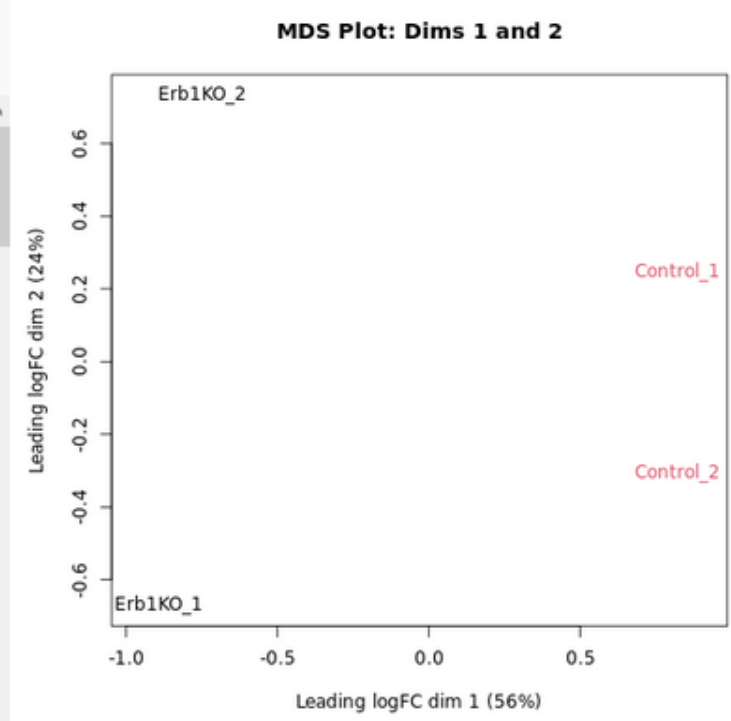
BED

VCF/BCF

Nanopore

Limma Analysis Output:

Links to PDF copies of plots are in 'Plots' section below



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1: Erb1KO_vs_control.txt

View data

«view data» ile veri analizinin detayı sonuç grafikleri

Hepsi ve daha fazlası, Galaxy ile mümkün.
Kodlama bilmeden yapılabilecek hızlı
standardize edilmiş hızlı analizler için faydalı
olabilir

Standardize edilmemiş analizler için siz yine de bilen biyoenformatikçilere önce danışın.