

# Functional analysis of RNA-seq data : answers to questions

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# Question 1

- Use “Functional Annotation Chart” tool

## Annotation Summary Results

[Help and Tool Manual](#)

**Current Gene List:**  
siMitfvssiLuc\_upgenes\_lfc1\_padj005

**793 DAVID IDs**

**Current Background: Homo sapiens**

**Check Defaults**



Clear All

- Disease** (2 selected)
- Functional\_Annotations** (6 selected)
- Gene\_Ontology** (3 selected)
- General\_Annotations** (0 selected)
- Interactions** (1 selected)
- Literature** (0 selected)
- Pathways** (3 selected)
- Protein\_Domains** (4 selected)
- Tissue\_Expression** (0 selected)

\*\*\*Red annotation categories denote DAVID defined defaults\*\*\*

## Combined View for Selected Annotation

Functional Annotation Clustering

Functional Annotation Chart

Functional Annotation Table

# Question 1

- 10 most significantly enriched functional annotation terms among the over-expressed genes annotations
- Number of genes annotated with each of these terms
- Most enriched GO term

Category	Term	RT	Genes	Count	%	P-Value	Benjaminf
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine	RT		256	32,3	1,1E-19	3,5E-16
UP_KW_BIOLOGICAL_PROCESS	<a href="#">Cell adhesion</a>	RT		55	6,9	3,2E-16	3,3E-14
UP_KW_PTM	<a href="#">Glycoprotein</a>	RT		269	33,9	7,5E-15	2,4E-13
GOTERM_BP_DIRECT	<a href="#">cell adhesion</a>	RT		56	7,1	8,5E-13	2,8E-9
UP_KW_DOMAIN	<a href="#">Signal</a>	RT		236	29,8	2,5E-12	7,2E-11
GOTERM_CC_DIRECT	<a href="#">extracellular matrix</a>	RT		35	4,4	1,1E-11	2,9E-9
GOTERM_CC_DIRECT	<a href="#">extracellular region</a>	RT		131	16,5	1,2E-11	2,9E-9
GOTERM_MF_DIRECT	<a href="#">extracellular matrix structural constituent</a>	RT		25	3,2	8,5E-11	7,1E-8
GOTERM_BP_DIRECT	<a href="#">extracellular matrix organization</a>	RT		33	4,2	3,3E-10	5,5E-7
UP_KW_CELLULAR_COMPONENT	<a href="#">Extracellular matrix</a>	RT		35	4,4	4,2E-10	1,8E-8

- To view these genes click on :

OFFICIAL_GENE_SYMBOL	GENE NAME
ADAM23	<a href="#">ADAM metallopeptidase domain 23(ADAM23)</a>
CCL2	<a href="#">C-C motif chemokine ligand 2(CCL2)</a>
CD24	<a href="#">CD24 molecule(CD24)</a>
CD36	<a href="#">CD36 molecule(CD36)</a>
CASS4	<a href="#">Cas scaffold protein family member 4(CASS4)</a>
EDIL3	<a href="#">EGF like repeats and discoidin domains 3(EDIL3)</a>
EPHA3	<a href="#">EPH receptor A3(EPHA3)</a>
FREM2	<a href="#">FRAS1 related extracellular matrix 2(FREM2)</a>
KITLG	<a href="#">KIT ligand(KITLG)</a>

# Question 2

- Use “Functional Annotation Table” tool

**Annotation Summary Results** [Help and Tool Manual](#)

**Current Gene List:**  
siMitfvssiLuc\_upgenes\_lfc1\_padj005

**Current Background:** Homo sapiens

**732 DAVID IDs**

**Check Defaults**

- Disease** (1 selected)
- Functional\_Categories** (3 selected)
- Gene\_Ontology** (3 selected)
- General\_Annotations** (0 selected)
- Literature** (0 selected)
- Main\_Accessions** (0 selected)
- Pathways** (3 selected)
- Protein\_Domains** (3 selected)
- Protein\_Interactions** (0 selected)
- Tissue\_Expression** (0 selected)

\*\*\*Red annotation categories denote DAVID defined defaults\*\*\*

**Combined View for Selected Annotation**

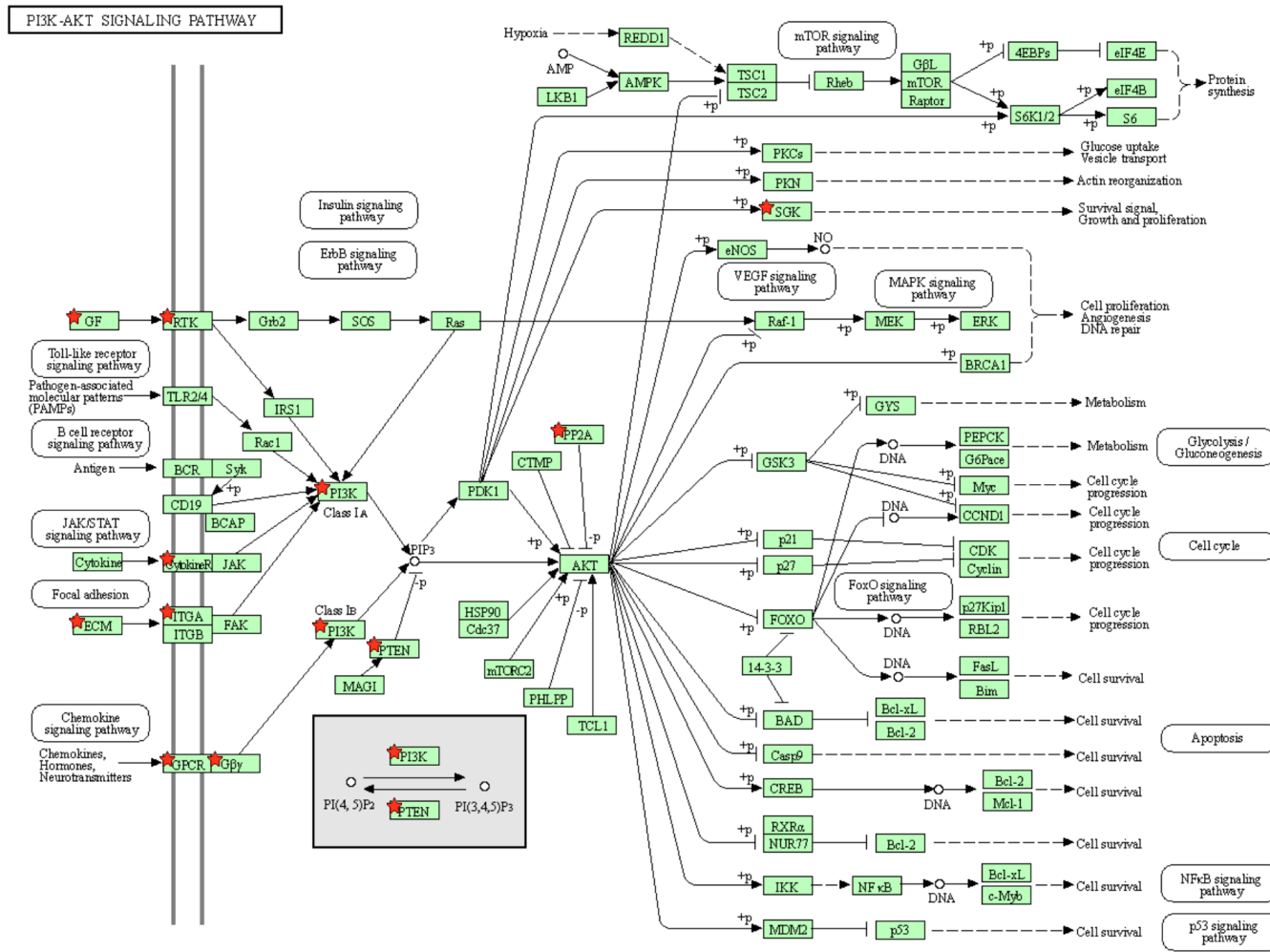
# Question 2

- Search for *KITLG* in the page to see all associated annotations

KITLG	KIT ligand(KITLG)	Related Genes	Homo sapiens
BBID	124.GH-IGF1_thymopoiesis,		
BIOCARTA	<a href="#">Regulation of BAD phosphorylation</a> , <a href="#">Erythrocyte Differentiation Pathway</a> , <a href="#">IL 17 Signaling Pathway</a> , <a href="#">CDK Regulation of DNA Replication</a> , <a href="#">Melanocyte Development and Pigmentation Pathway</a> ,		
GOTERM_BP_DIRECT	<a href="#">MAPK cascade</a> , <a href="#">ovarian follicle development</a> , <a href="#">neural crest cell migration</a> , <a href="#">positive regulation of leukocyte migration</a> , <a href="#">positive regulation of myeloid leukocyte differentiation</a> , <a href="#">cell adhesion</a> , <a href="#">positive regulation of cell proliferation</a> , <a href="#">male gonad development</a> , <a href="#">negative regulation of mast cell apoptotic process</a> , <a href="#">embryonic hemopoiesis</a> , <a href="#">ectopic germ cell programmed cell death</a> , <a href="#">positive regulation of MAP kinase activity</a> , <a href="#">positive regulation of melanocyte differentiation</a> , <a href="#">positive regulation of Ras protein signal transduction</a> , <a href="#">positive regulation of peptidyl-tyrosine phosphorylation</a> , <a href="#">positive regulation of protein kinase B signaling</a> , <a href="#">positive regulation of mast cell proliferation</a> , <a href="#">extrinsic apoptotic signaling pathway in absence of ligand</a> , <a href="#">positive regulation of hematopoietic progenitor cell differentiation</a> , <a href="#">positive regulation of hematopoietic stem cell proliferation</a> ,		
GOTERM_CC_DIRECT	<a href="#">extracellular region</a> , <a href="#">extracellular space</a> , <a href="#">cytoplasm</a> , <a href="#">cytoskeleton</a> , <a href="#">plasma membrane</a> , <a href="#">membrane</a> , <a href="#">integral component of membrane</a> , <a href="#">lamellipodium</a> , <a href="#">filopodium</a> ,		
GOTERM_MF_DIRECT	<a href="#">cytokine activity</a> , <a href="#">stem cell factor receptor binding</a> , <a href="#">protein binding</a> , <a href="#">growth factor activity</a> ,		
INTERPRO	<a href="#">Stem cell factor</a> , <a href="#">Four-helical cytokine-like</a> , <a href="#">core</a> ,		
KEGG_PATHWAY	<a href="#">MAPK signaling pathway</a> , <a href="#">Ras signaling pathway</a> , <a href="#">Rap1 signaling pathway</a> , <a href="#">Phospholipase D signaling pathway</a> , <a href="#">PI3K-Akt signaling pathway</a> , <a href="#">Hematopoietic cell lineage</a> , <a href="#">Melanogenesis</a> , <a href="#">Pathways in cancer</a> ,		
OMIM_DISEASE	<a href="#">Hyperpigmentation with or without hypopigmentation</a> , <a href="#">Skin/hair/eye pigmentation 7</a> , <a href="#">blond/brown hair</a> , <a href="#">Deafness, autosomal dominant 69</a> , <a href="#">unilateral or asymmetric</a> ,		
PIR_SUPERFAMILY	<a href="#">Kit ligand precursor/stem cell factor</a> ,		
UP_KW_BIOLOGICAL_PROCESS	<a href="#">Cell adhesion</a> ,		
UP_KW_CELLULAR_COMPONENT	<a href="#">Cytoskeleton</a> , <a href="#">Membrane</a> , <a href="#">Cytoplasm</a> , <a href="#">Secreted</a> , <a href="#">Cell projection</a> , <a href="#">Cell membrane</a> ,		
UP_KW_DISEASE	<a href="#">Deafness</a> , <a href="#">Disease variant</a> , <a href="#">Non-syndromic deafness</a> ,		
UP_KW_DOMAIN	<a href="#">Coiled coil</a> , <a href="#">Signal</a> , <a href="#">Transmembrane</a> , <a href="#">Transmembrane helix</a> ,		
UP_KW_MOLECULAR_FUNCTION	<a href="#">Growth factor</a> ,		
UP_KW_PTM	<a href="#">Glycoprotein</a> , <a href="#">Disulfide bond</a> ,		
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine, CARBOHYD:N-linked (GlcNAc...) asparagine; partial, CARBOHYD:O-linked (GalNAc...) serine, CARBOHYD:O-linked (GalNAc...) threonine, REGION:Disordered, SITE:Not glycosylated, TOPO_DOM:Cytoplasmic, TOPO_DOM:Extracellular, TRANSMEM:Helical,		

- Click on “PI3K-Akt signalling pathway” to see all genes from this pathway

# Question 2



04151 7/7/21  
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Genes from your list are highlighted with a red star

# Question 3

1. Download list genes in PI3K-Akt signalling pathway from DAVID :  
Click on “Show all list genes” on the bottom of the page representing PI3K-AKT signalling pathway



then right click on Download File (top right) and save link target on disk

## Gene Report

[Help and Manual](#)

[Download File](#)

pi3k\_akt\_signalling\_genes.txt




# Question 3

## 2. Import `pi3k_akt_signalling_genes.txt` file on Galaxy




Download from web or upload from disk

Regular Composite Collection Rule-based

You added 1 file(s) to the queue. Add more files or click 'Start' to proceed.

Name	Size	Type	Genome	Settings	Status
 pi3k_akt_signalling	593.7 KB	tabular	unspecified (?)		0% 

Type (set all): Auto-detect Genome (set all): unspecified (?)

 Choose local files  Choose remote files  Paste/Fetch data **Start** Pause Reset Close



# Question 3

3. Join `siMitfvssiLuc.up.annot.txt` with `pi3k_akt_signalling_genes.txt` on their common column (Gene name)

**Join two Datasets** side by side on a specified field (Galaxy Version 2.1.3) ☆ ▼

**Join**

28: siMitfvssiLuc.up.annot.txt ▼

**using column**

Column: 29 ▼

**with**

31: pi3k\_akt\_signalling\_genes.txt ▼

**and column**

Column: 1 ▼

**Keep lines of first input that do not join with second input**

No ▼

**Keep lines of first input that are incomplete**

No ▼

**Fill empty columns**

No ▼

**Keep the header lines**

Yes ▼

**Email notification**

Send an email notification when the job completes.

# Question 3

4. Use the **Cut** tool to retain only columns of interest (Gene name, norm.siLuc2, norm.siLuc3, norm.siMitf3, norm.siMitf4)

 **Cut** columns from a table (Galaxy Version 1.0.2)

**Cut columns**

c29,c6-c9

**Delimited by**

Tab

**From**



32: Join two Datasets on data 31 and data 28

**Email notification**



Send an email notification when the job completes.

Gene name	norm.siLuc2	norm.siLuc3	norm.siMitf3	norm.siMitf4
LAMC1	18515	19104	50030	53404
LAMA4	474	507	2261	2286
PTEN	1651	1603	4900	4571
LAMB1	2137	2136	4690	4760
LAMB2	3919	3750	7696	8207
KITLG	391	428	1121	1093
PIK3R3	507	438	1091	1114
FGF12	69	72	339	354
COL6A1	341	336	796	919
FGFR3	52	37	252	229
ITGA3	105	131	333	381
ITGA1	62	61	223	257
EFNA1	165	131	351	331
VTN	8	4	65	55
GNG2	101	127	243	235
COL6A3	43	27	98	129
VEGFD	57	50	137	138
CHRM2	2	1	36	29
FGF1	1	0	39	36
LPAR2	40	38	102	97
SGK2	7	9	46	36
FGFR2	30	24	79	74
IL7R	14	11	54	38
FGF7	0	1	21	13
COL5A2	16	26	48	63
PPP2R2B	7	5	24	26
PDGFRA	9	6	28	23
PIK3R5	4	5	12	27




5. Download this file and change file extension to txt

→ [siMitfvssiLuc\\_up\\_pi3k\\_akt.txt](#)

# Question 3

## 6. Heatmap using <http://www.heatmapper.ca/expression/>


**Data Format**

Select Data File. 

Example File

Upload File

Upload Multiple Files

Browse... siMitfvssiLuc\_up\_pi 

Scale Type: Row

Colour Brightness:  (range: -50 to 50)

Number of Shades:  (range: 3 to 100)

Colour Scheme: Custom

Low Colour: #0016DB Middle Colour: #FFFFFF High Colour: #FFFF00

Missing Data Colour: #000000

Clustering Method: Average Linkage

Distance Measurement Method: Pearson

