Functional analysis
of RNA-seq data :
answers to questions

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Question 1

- Use "Functional Annotation Chart" tool

Question 1

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

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
☐	UP_KEYWORDS	Glycoprotein	RT		200	0,2	6,8E-17	2,5E-14
☐	UP_SEQ_FEATURE	glycosylation site:N-linked (GlcNAc...)	RT		187	0,2	3,6E-15	6,4E-12
☐	UP_SEQ_FEATURE	signal peptide	RT		154	0,2	1,5E-13	1,3E-10
☐	UP_KEYWORDS	Cell adhesion	RT		43	0,1	4,0E-12	7,3E-10
☐	UP_KEYWORDS	Signal	RT		173	0,2	7,9E-12	9,7E-10
☐	GOTERM_BP_DIRECT	cell adhesion	RT		40	0,0	4,7E-10	1,2E-6
☐	UP_KEYWORDS	Secreted	RT		92	0,1	1,7E-8	1,6E-6
☐	UP_SEQ_FEATURE	disulfide bond	RT		123	0,2	2,6E-8	1,5E-5
☐	UP_KEYWORDS	Disulfide bond	RT		138	0,2	2,8E-8	2,1E-6
☐	INTERPRO	Immunoglobulin I-set	RT		18	0,0	2,2E-7	2,1E-4

- To view these genes click on :

200 record(s)   📥 Download File

OFFICIAL_GENE_SYMBOL	GENE NAME	Related Genes	Species
HTR2B	5-hydroxytryptamine receptor 2B(HTR2B)	RG	Homo sapiens
ADAM23	ADAM metallopeptidase domain 23(ADAM23)	RG	Homo sapiens
ADAMTS18	ADAM metallopeptidase with thrombospondin type 1 motif 18(ADAMTS18)	RG	Homo sapiens
ART3	ADP-ribosyltransferase 3(ART3)	RG	Homo sapiens
ALG9	ALG9, alpha-1,2-mannosyltransferase(ALG9)	RG	Homo sapiens

Question 2

- Use "Functional Annotation Clustering" tool

Question 2

- First identified cluster :

- Click here to visualize members of this cluster

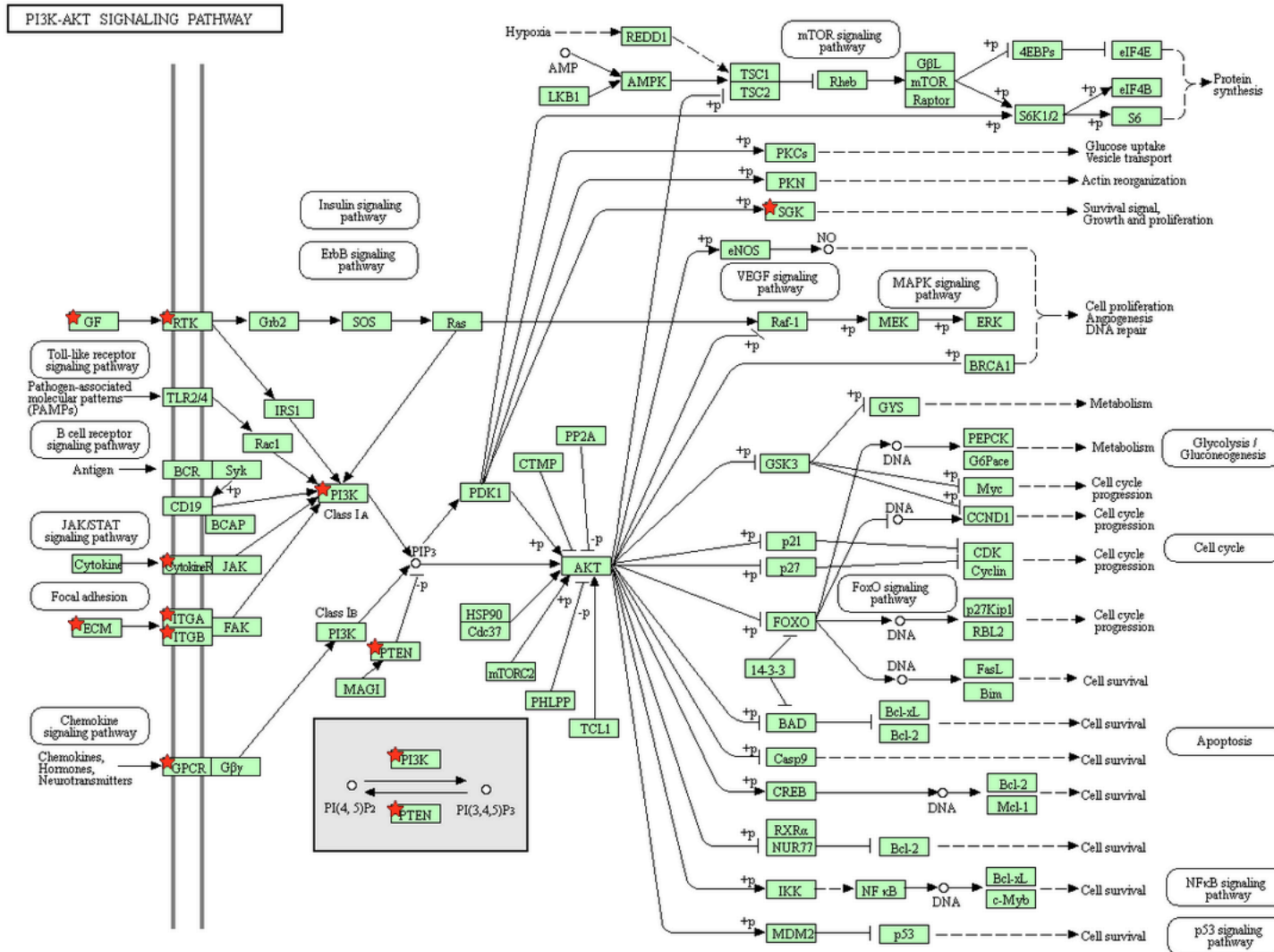# Question 2

Question 3

- Use "Functional Annotation Table" tool

Question 3

- 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	Regulation of BAD phosphorylation, Erythrocyte Differentiation Pathway, IL 17 Signaling Pathway, CDK Regulation of DNA Replication, Melanocyte Development and Pigmentation Pathway,		
GOTERM_BP_DIRECT	MAPK cascade, ovarian follicle development, neural crest cell migration, positive regulation of leukocyte migration, positive regulation of myeloid leukocyte differentiation, cell adhesion, signal transduction, cell proliferation, male gonad development, regulation of phosphatidylinositol 3-kinase signaling, negative regulation of mast cell apoptotic process, embryonic hemopoiesis, ectopic germ cell programmed cell death, positive regulation of MAP kinase activity, positive regulation of GTPase activity, positive regulation of melanocyte differentiation, positive regulation of DNA replication, positive regulation of Ras protein signal transduction, phosphatidylinositol phosphorylation, phosphatidylinositol-mediated signaling, positive regulation of peptidyl-tyrosine phosphorylation, positive regulation of mast cell proliferation, extrinsic apoptotic signaling pathway in absence of ligand, positive regulation of hematopoietic stem cell proliferation,		
GOTERM_CC_DIRECT	extracellular region, extracellular space, cytoplasm, cytoskeleton, plasma membrane, membrane, integral component of membrane, lamellipodium, filopodium,		
GOTERM_MF_DIRECT	Ras guanyl-nucleotide exchange factor activity, cytokine activity, stem cell factor receptor binding, protein binding, growth factor activity, phosphatidylinositol-4,5-bisphosphate 3-kinase activity,		
INTERPRO	Stem cell factor, Four-helical cytokine-like, core, Four-helical cytokine, core,		
KEGG_PATHWAY	Ras signaling pathway, Rap1 signaling pathway, PI3K-Akt signaling pathway, Hematopoietic cell lineage, Melanogenesis, Pathways in cancer,		
OMIM_DISEASE	Hyperpigmentation with or without hypopigmentation, Skin/hair/eye pigmentation 7, blond/brown hair, Deafness, congenital, unilateral or asymmetric,		
PIR_SUPERFAMILY	Kit ligand precursor/stem cell factor,		
UP_KEYWORDS	3D-structure, Alternative splicing, Cell adhesion, Cell membrane, Cell projection, Complete proteome, Cytoplasm, Cytoskeleton, Deafness, Direct protein sequencing, Disease mutation, Disulfide bond, Glycoprotein, Growth factor, Membrane, Non-syndromic deafness, Polymorphism, Proteomics identification, Reference proteome, Secreted, Signal, Transmembrane, Transmembrane helix,		
UP_SEQ_FEATURE	chain:Kit ligand, disulfide bond, glycosylation site:N-linked (GlcNAc...), glycosylation site:N-linked (GlcNAc...); partial, glycosylation site:O-linked (GalNAc...), helix, sequence conflict, sequence variant, signal peptide, site:Not glycosylated, splice variant, strand, topological domain:Cytoplasmic, topological domain:Extracellular, transmembrane region, turn,		

- Click on "PI3K-Akt signalling pathway" to see all genes from this pathway
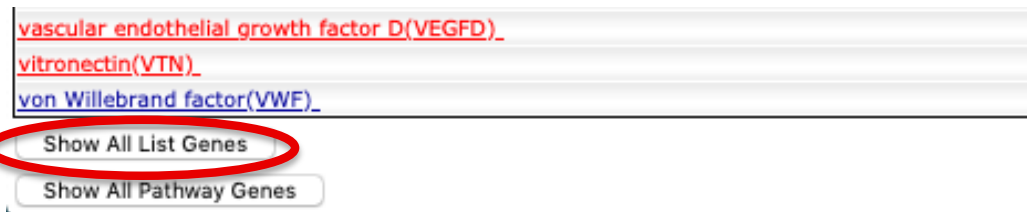
Question 3

PI3K-AKT SIGNALING PATHWAY

Genes from your list are highlighted with a red star

Question 4

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
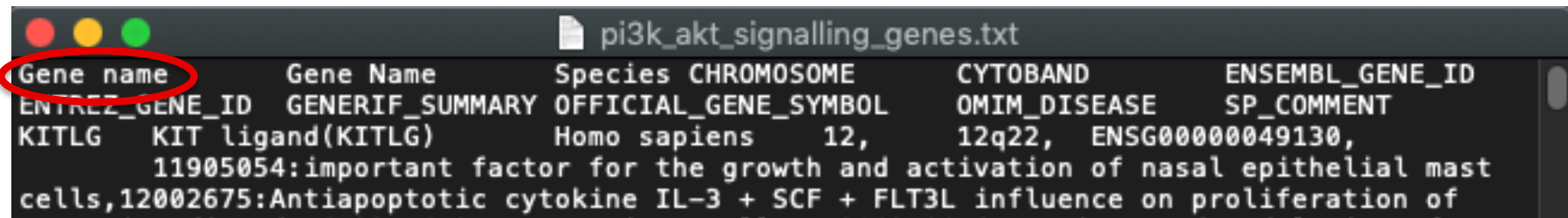
   vascular endothelial growth factor D(VEGFD)
   vitronectin(VTN)
   von Willebrand factor(VWF)

   Show All List Genes
   Show All Pathway Genes

   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

   List Id: KITLG   KIT ligand(KITLG)   Related Genes   Homo sapiens

2. Rename "ID" column to "Gene name" in pi3k_akt_signalling_genes.txt file

   pi3k_akt_signalling_genes.txt
   Gene name   Gene Name   Species CHROMOSOME   CYTOBAND   ENSEMBL_GENE_ID
   ENTREZ_GENE_ID GENERIF_SUMMARY OFFICIAL_GENE_SYMBOL OMIM_DISEASE   SP_COMMENT
   KITLG   KIT ligand(KITLG)   Homo sapiens   12,   12q22,   ENSG00000049130,
        11905054:important factor for the growth and activation of nasal epithelial mast
   cells,12002675:Antiapoptotic cytokine IL-3 + SCF + FLT3L influence on proliferation of

Question 4

3. Import pi3k_akt_signalling_genes.txt file on GalaxEast

Question 4

4. Join siMitfvssiLuc.up.annot.txt with pi3k_akt_signalling_genes.txt on common column (Gene name)

Join two Datasets side by side on a specified field (Galaxy Version 2.0.2)   ▼ Options

Join

90: siMitfvssiLuc.up.annot.txt   ▼

using column

Column: 28   ▼

with

104: 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   ▼

✔ Execute

Question 4

5. Use "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) ▾ Options

Cut columns

c28,c6−c9

Delimited by

Tab ▾

From

105: Join two Datasets on data 104 and data 90

✔ Execute

Gene name	norm.siLuc2	norm.siLuc3	norm.siMitf3	norm.siMitf4
LAMC1	18551	19142	49967	53248
LAMA4	476	510	2259	2277
PTEN	1646	1603	4886	4557
LAMB1	2112	2099	4581	4658
LAMB2	3928	3758	7688	8185
KITLG	392	429	1120	1090
FGF12	66	68	336	350
PIK3R3	509	439	1090	1111
COL6A1	341	337	796	917
FGFR3	52	37	252	228
ITGA1	62	61	223	256
ITGA3	106	131	333	380
EFNA1	166	136	361	336
FGF1	1	0	39	36
VTN	10	4	66	54
COL6A3	43	27	98	129
CHRM2	2	1	36	29
VEGFD	57	50	137	138
LPAR2	40	38	102	96
FGFR2	30	23	77	71
SGK2	7	9	43	32
IL7R	14	11	54	38

6. Download this file
and change file extension to txt

→ siMitfvssiLuc_up_pi3k_akt.txt

Question 4

7. Heatmap using http://www.heatmapper.ca/expression/