

ERC *Drosophila melanogaster*

15/09/2015

Outline

Overview of RNA-seq samples

Wing compartments

Decomposing the variation of gene expression across time and space

- Gene sets with high variation across space

- Gene sets with high variation across time

Differential gene expression - EdgeR

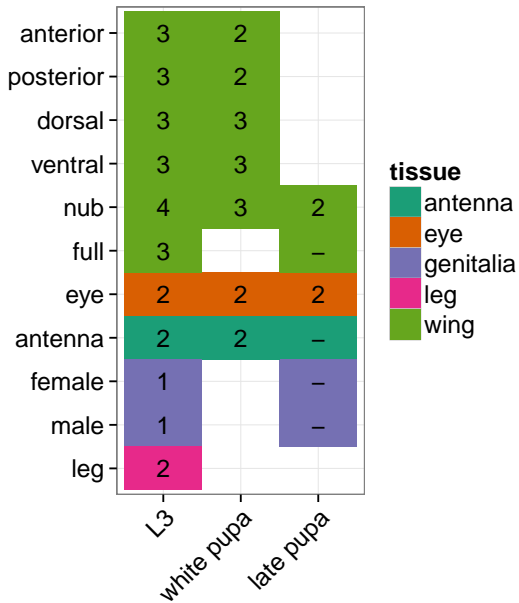
- Space-specific genes - pairwise comparison of wing compartments

Outline

Overview of RNA-seq samples

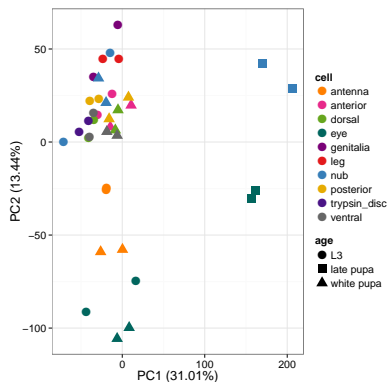
Wing compartments

Overview of processed RNA-seq samples

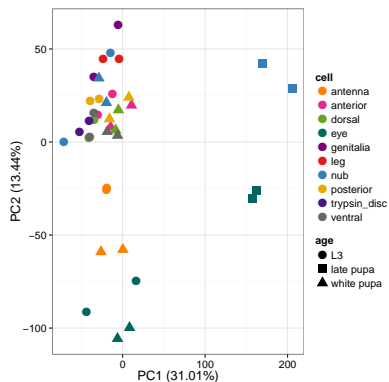


PCA - selected replicates

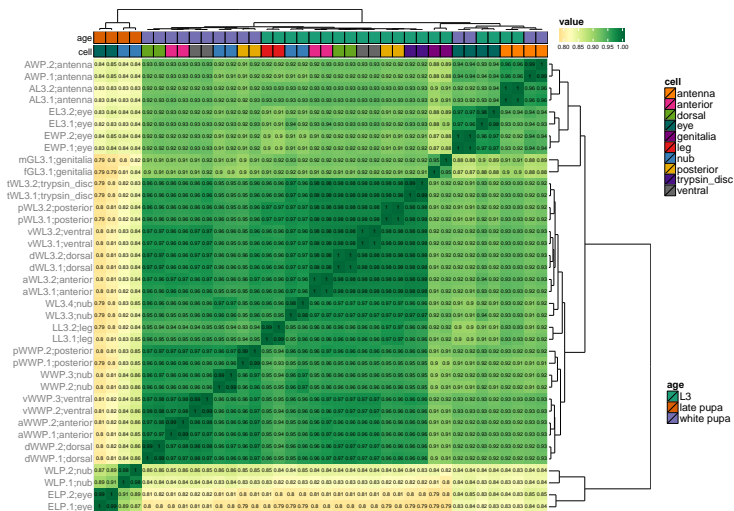
17158 genes



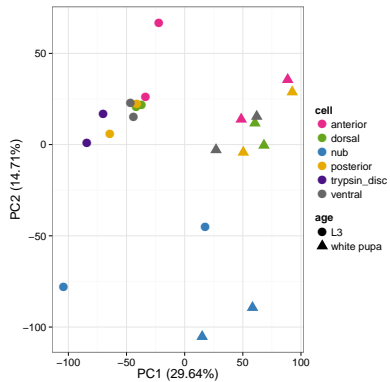
13920 protein-coding genes



Clustering by gene expression



PCA wing compartments



Outline

Overview of RNA-seq samples

Wing compartments

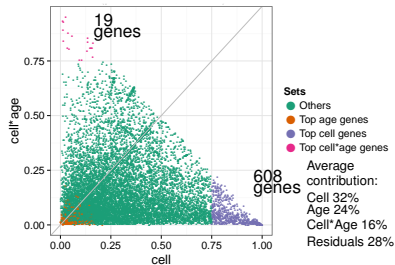
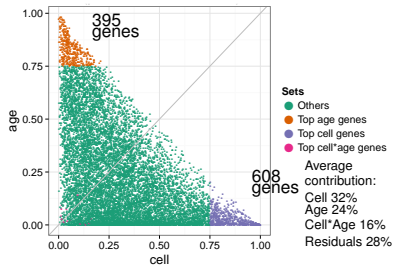
Decomposing the variation of gene expression across time and space

Gene sets with high variation across space

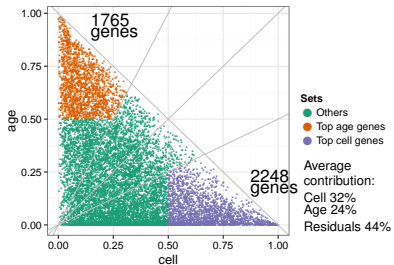
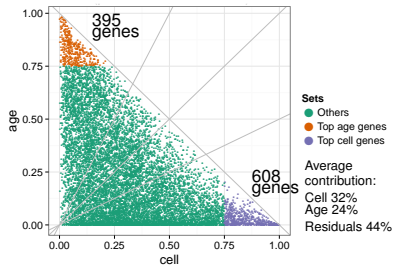
Gene sets with high variation across time

Differential gene expression - EdgeR

Decomposing the variation of gene expression across time and space



Decomposing the variation of gene expression across time and space



Outline

Overview of RNA-seq samples

Wing compartments

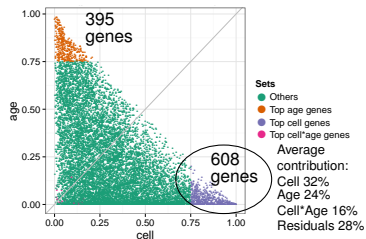
Decomposing the variation of gene expression across time and space

Gene sets with high variation across space

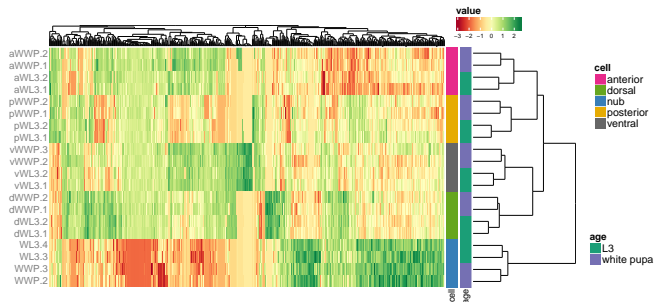
Gene sets with high variation across time

Differential gene expression - EdgeR

Gene sets with high variation across space

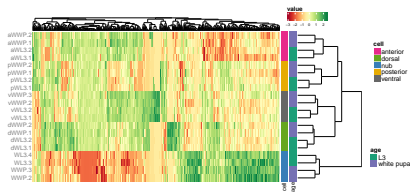


Biotype	
mRNA	
ncRNA	
GO terms	
GO terms (wing, imaginal disc, development)	
No GO terms	



Decomposing the variation of gene expression across space

topCellGenes = 608 genes

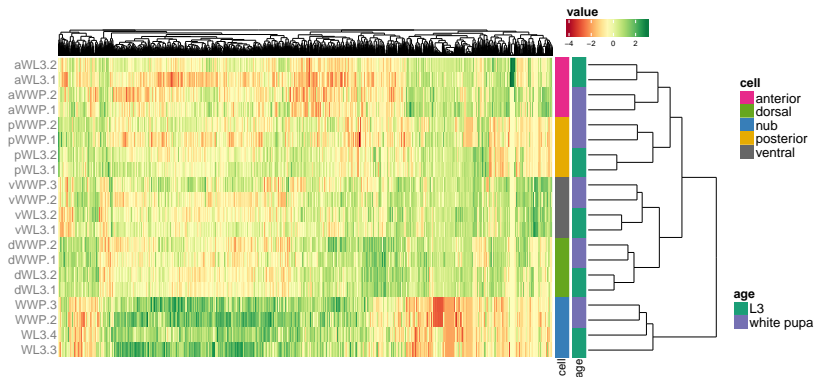


KEGGID	Pvalue	Term
-	-	-

Pvalue	BP
2e-13	tube development
2e-10	tissue morphogenesis
2e-10	digestive system development
3e-10	epithelial tube morphogenesis
2e-09	regulation of transcription, DNA-templated
2e-09	regulation of RNA biosynthetic process
2e-09	appendage morphogenesis
3e-09	regulation of biosynthetic process
8e-09	nucleobase-containing compound biosynthetic process
...	
9e-09	imaginal disc-derived appendage development
3e-07	imaginal disc pattern formation
3e-06	wing disc development
9e-06	imaginal disc-derived wing morphogenesis
2e-05	imaginal disc-derived leg segmentation
3e-05	wing disc anterior/posterior pattern formation
Pvalue	CC
3e-05	mitochondrial membrane part
Pvalue	MF
1e-15	sequence-specific DNA binding transcription factor activity
1e-07	sequence-specific DNA binding RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity
1e-06	oxidoreductase activity
5e-05	fibroblast growth factor receptor binding

Gene sets with high variation across space

topCellGenes = 2248 genes



Outline

Overview of RNA-seq samples

Wing compartments

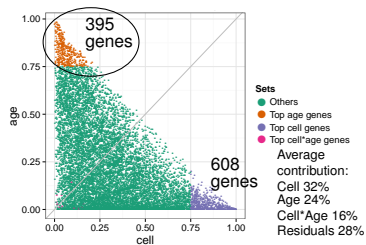
Decomposing the variation of gene expression across time and space

Gene sets with high variation across space

Gene sets with high variation across time

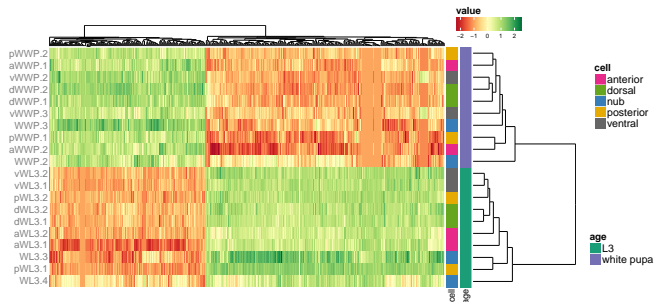
Differential gene expression - EdgeR

Gene sets with high variation across time



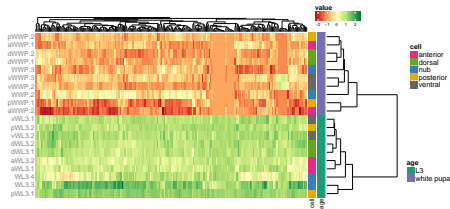
Biotype	Cluster1	Cluster2
mRNA	235	153
ncRNA	4	3

GO terms	217	141
GO terms (wing, imaginal disc, development)	39	35
No GO terms	22	15



Variance decomposition

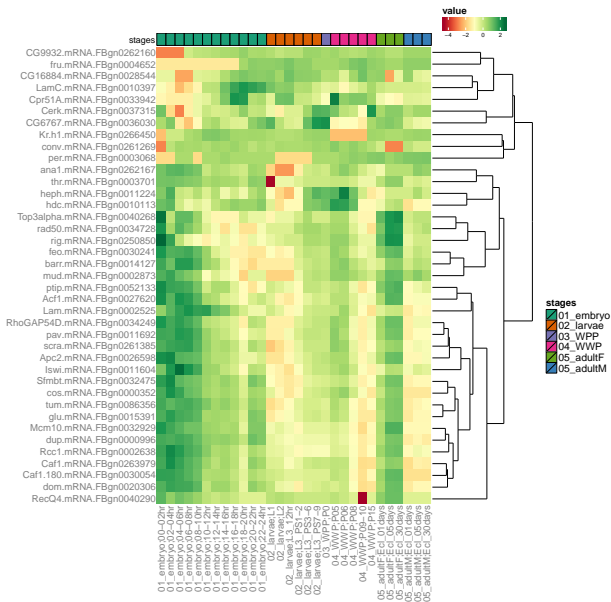
topAgeGenes = 395 genes, cluster 1 = 239 genes



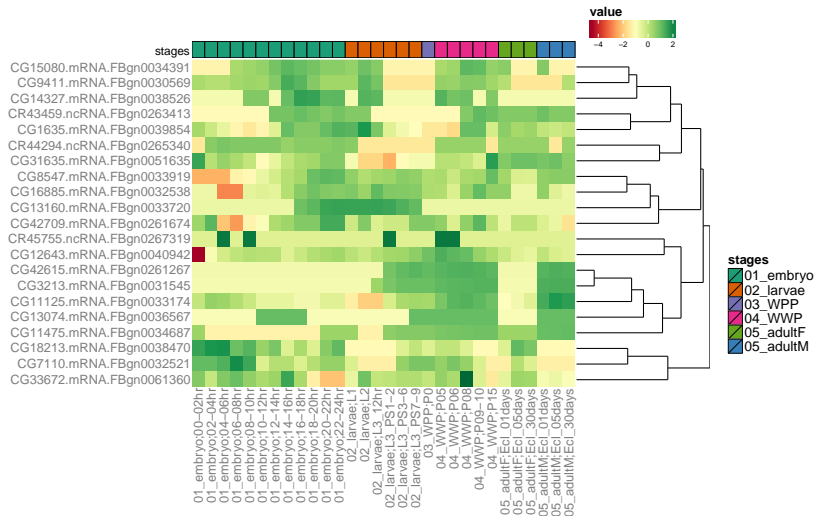
KEGGID	Pvalue	Term
03030	8.8323e-12	DNA replication
03430	5.4271e-10	Mismatch repair
03420	3.5912e-05	Nucleotide excision repair

Pvalue	BP
4e-21	cell cycle
9e-16	DNA conformation change
6e-15	sister chromatid segregation
1e-14	nucleobase-containing compound metabolic process
1e-12	DNA replication initiation
2e-12	DNA-dependent DNA replication
2e-12	nitrogen compound metabolic process
1e-11	microtubule cytoskeleton organization
1e-11	mitotic chromosome condensation
Pvalue	CC
1e-17	nucleus
5e-15	nuclear lumen
6e-15	intracellular
7e-13	chromosome
7e-13	MCM complex
8e-12	organelle lumen
1e-11	membrane-bounded organelle
1e-11	cell
3e-11	nuclear pre-replicative complex
Pvalue	MF
7e-12	chromatin binding
5e-11	microtubule binding
5e-11	pyrophosphatase activity
8e-11	hydrolase activity, acting on acid anhydrides
1e-10	3'-5' DNA helicase activity
2e-10	macromolecular complex binding
2e-10	adenyl nucleotide binding
3e-10	DNA binding
7e-10	ATP binding

modEncode for cluster1 - GO selected terms

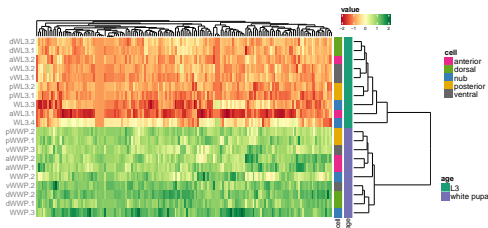


modEncode for cluster1 - no GO terms



Variance decomposition

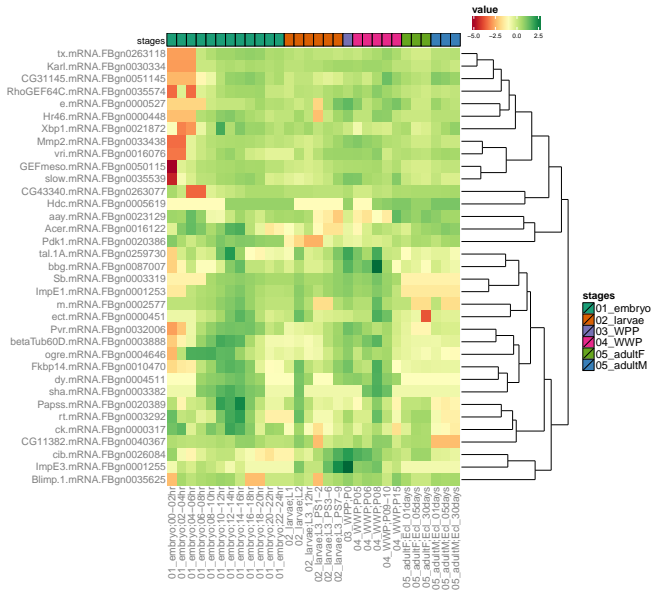
topAgeGenes = 395 genes, cluster 2 = 156 genes



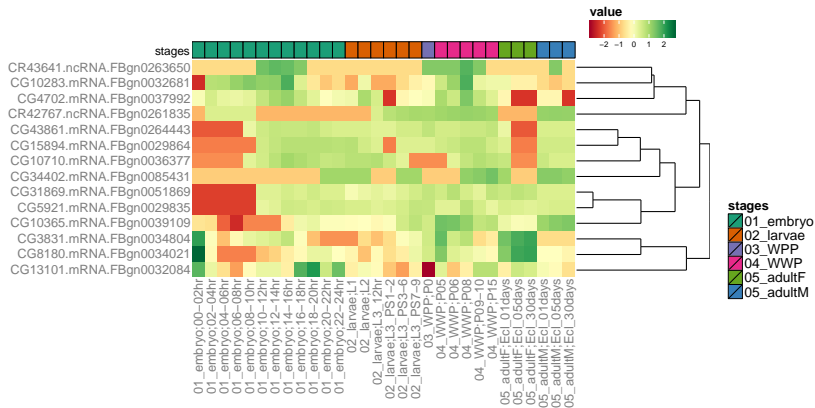
KEGGID	Pvalue	Term
00260	0.0003	Glycine, serine and threonine metabolism

Pvalue	BP
7e-08	post-embryonic organ development
2e-07	tube morphogenesis
7e-07	instar larval or pupal development
1e-06	ecdysteroid metabolic process
2e-06	imaginal disc morphogenesis
3e-06	morphogenesis of an epithelium
8e-06	imaginal disc-derived wing morphogenesis
1e-05	post-embryonic morphogenesis
1e-05	appendage morphogenesis
Pvalue	CC
7e-05	endoplasmic reticulum
8e-05	Sec61 translocon complex
1e-04	cell periphery
2e-04	membrane
3e-04	rough endoplasmic reticulum membrane
Pvalue	MF
5e-07	choline dehydrogenase activity
4e-05	flavin adenine dinucleotide binding
2e-04	Rho guanyl-nucleotide exchange factor activity

modEncode for cluster2 - GO selected terms

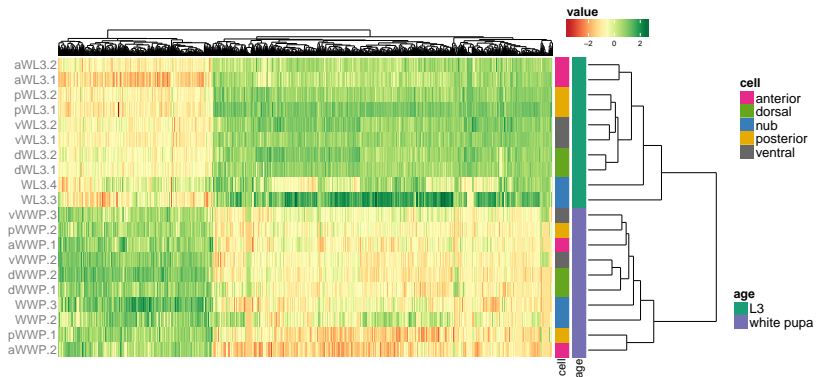


modEncode for cluster2 - no GO terms



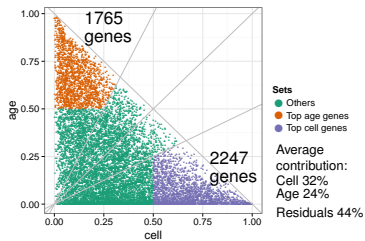
Gene sets with high variation across time

topAgeGenes = 1765 genes

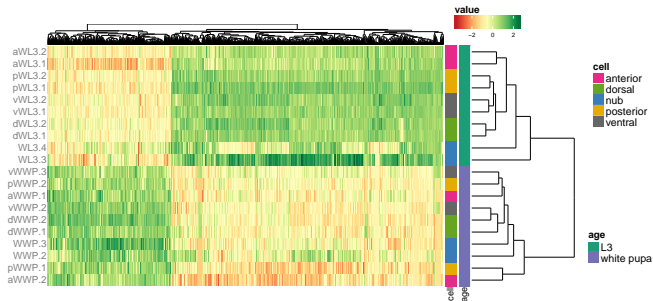


Gene sets with high variation across time

topAgeGenes = 1765 genes

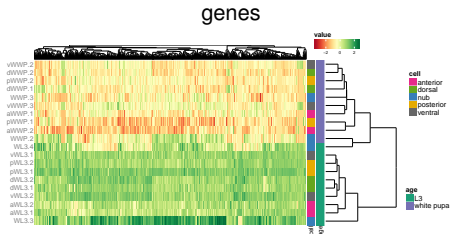


Biotype	Cluster1	Cluster2
mRNA		
ncRNA		
GO terms		
GO terms (wing, imaginal disc, development)		
No GO terms		



Gene sets with high variation across time

topAgeGenes = 1765 genes, cluster 1 = 1214 genes



KEGGID	Pvalue	Term
3030	2.3e-21	DNA replication
03008	6.9e-12	Ribosome biogenesis in eukaryotes
03430	3.3e-10	Mismatch repair
03420	1.4e-09	Nucleotide excision repair
00240	1.3e-05	Pyrimidine metabolism
03013	1.4e-05	RNA transport

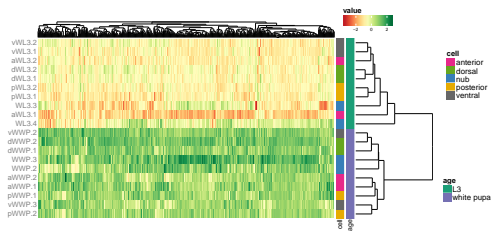
Pvalue	BP
5e-57	nucleic acid metabolic process
6e-52	heterocycle metabolic process
2e-50	cellular aromatic compound metabolic process
1e-47	cellular nitrogen compound metabolic process
2e-47	organic cyclic compound metabolic process
1e-32	cellular process
4e-30	neurogenesis
3e-26	cellular developmental process
1e-23	cellular response to stress
Pvalue	Term
1e-24	DNA binding
2e-23	adenyl nucleotide binding
5e-23	ATP binding
1e-22	purine NTP-dependent helicase activity
4e-21	ribonucleoside binding
5e-20	purine nucleoside binding
5e-20	purine ribonucleotide binding
2e-19	nucleotide binding
9e-17	pyrophosphatase activity
Pvalue	Term
2e-46	intracellular organelle
1e-40	intracellular
2e-39	nuclear lumen
8e-39	organelle lumen
5e-38	membrane-bounded organelle
7e-33	non-membrane-bounded organelle
2e-26	cell
1e-24	chromosome
2e-22	nucleus

modEncode for cluster1 - GO selected terms

modEncode for cluster1 - no GO terms

Gene sets with high variation across time

topAgeGenes = 1765 genes, cluster 2 = 551 genes



KEGGID	Pvalue	Term
03060	7.3e-09	Protein export
04141	1.6e-08	Protein processing in endoplasmic reticulum

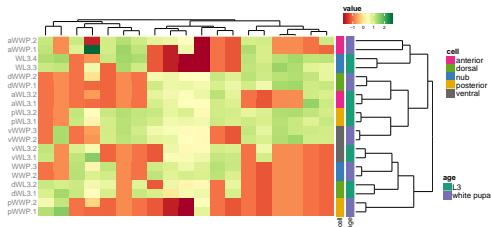
Pvalue	BP
3e-10	protein targeting to membrane
3e-10	post-embryonic organ development
6e-10	instar larval or pupal development
6e-10	imaginal disc morphogenesis
6e-10	single-multicellular organism process
8e-10	post-embryonic morphogenesis
1e-09	morphogenesis of an epithelium
4e-09	establishment of protein localization to endoplasmic reticulum
5e-09	developmental process
Pvalue	Term
2e-08	choline dehydrogenase activity
6e-06	transporter activity
5e-05	signal sequence binding
7e-05	organophosphate ester transmembrane transporter activity
Pvalue	Term
4e-11	rough endoplasmic reticulum membrane
2e-10	endoplasmic reticulum part
8e-09	apical part of cell
1e-07	Sec61 translocon complex
3e-07	endoplasmic reticulum
3e-07	nuclear outer membrane-endoplasmic reticulum membrane network
4e-07	bounding membrane of organelle
5e-07	Golgi apparatus
1e-06	integral component of membrane

modEncode for cluster2 - GO selected terms

modEncode for cluster2 - no GO terms

Gene sets with high variation across time and space

topCell*AgeGenes = 19 genes



Pvalue	BP
0.002	fatty acid metabolic process
0.002	cuticle hydrocarbon biosynthetic process
Pvalue	MF
8e-04	phytanoyl-CoA dioxygenase activity

KEGGID	Pvalue	Term
-	-	-

Outline

Overview of RNA-seq samples

Wing compartments

Decomposing the variation of gene expression across time and space

Differential gene expression - EdgeR

Space-specific genes - pairwise comparison of wing compartments

EdgeR - design matrix

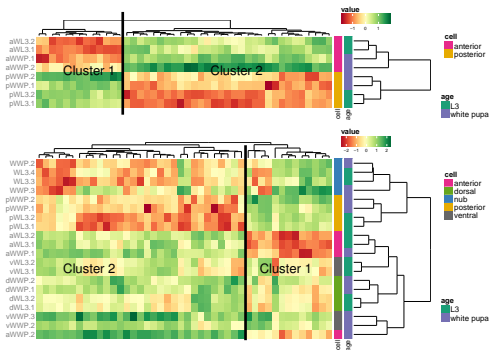
allCompartments	A/P
anterior.L3	anterior.L3
anterior.white pupa	anterior.white pupa
dorsal.L3	posterior.L3
dorsal.white pupa	posterior.white pupa
nub.L3	
nub.white pupa	
posterior.L3	
posterior.white pupa	
ventral.L3	
ventral.white pupa	

Contrasts			space
anterior.L3	vs	posterior.L3	1
anterior.white pupa	vs	posterior.white pupa	1
anterior.L3	vs	anterior.white pupa	0
posterior.L3	vs	posterior.white pupa	0
anterior.L3	vs	posterior.white pupa	?
anterior.white pupa	vs	posterior.L3	?

Contrasts			space
anterior.L3	vs	posterior.L3	1
anterior.white pupa	vs	posterior.white pupa	1
anterior.L3	vs	anterior.white pupa	0
posterior.L3	vs	posterior.white pupa	0
anterior.L3	vs	posterior.white pupa	?
anterior.white pupa	vs	posterior.L3	?

Contrasts			space			
anterior.L3	vs	posterior.L3	1	1	1	1
anterior.WP	vs	posterior.WP	1	1	1	1
anterior.L3	vs	anterior.WP	0	0	0	0
posterior.L3	vs	posterior.WP	0	0	0	0
anterior.L3	vs	posterior.WP	0	1	0	1
anterior.WP	vs	posterior.L3	0	0	1	1
number of genes			0	2	9	33

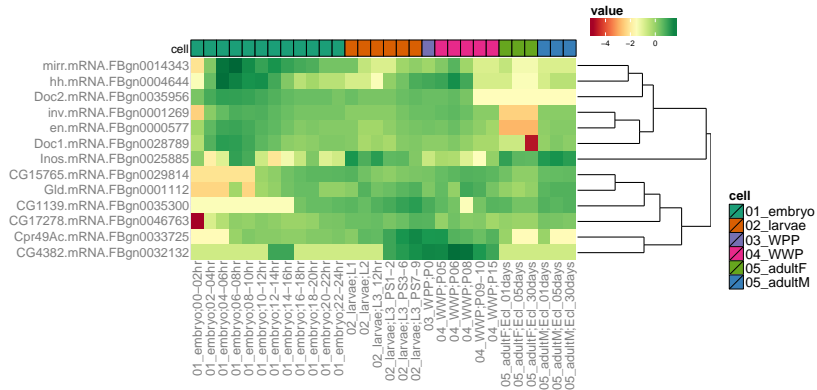
anterior vs posterior - space-specific genes = 44



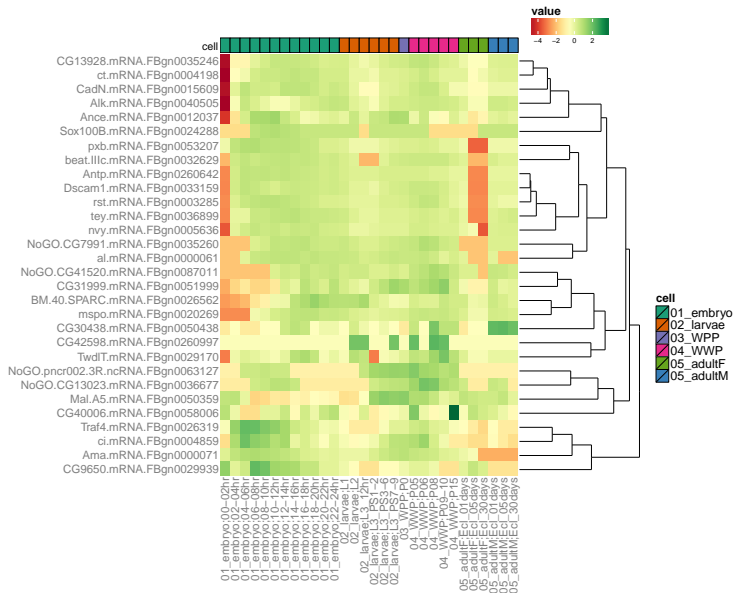
Biotype	Cluster1	Cluster2
mRNA	13	30
ncRNA	0	1
GO terms	13	27
GO terms (wing, anterior, posterior, imaginal disc, development)	9	14

Pvalue	Term BP - Cluster1
2e-07	wing disc anterior/posterior pattern formation
1e-06	anterior commissure morphogenesis
1e-06	forebrain development
8e-06	anterior/posterior lineage restriction, imaginal disc
2e-05	compartment pattern specification
2e-05	analia development
2e-05	central nervous system neuron axonogenesis
3e-05	imaginal disc-derived wing vein specification
3e-05	central nervous system neuron differentiation
Pvalue	Term BP - Cluster2
2e-07	cell adhesion
4e-07	cell morphogenesis involved in differentiation
2e-06	multicellular organismal development
5e-06	generation of neurons
6e-06	neuron development
1e-05	neuron projection guidance
2e-05	cell projection morphogenesis
3e-05	axonogenesis
4e-05	cellular component morphogenesis

modEncode for cluster1



modEncode for cluster2



Outline

Overview of RNA-seq samples

Wing compartments

Decomposing the variation of gene expression across time and space

Differential gene expression - EdgeR

Space-specific genes - pairwise comparison of wing compartments

EdgeR - design matrix

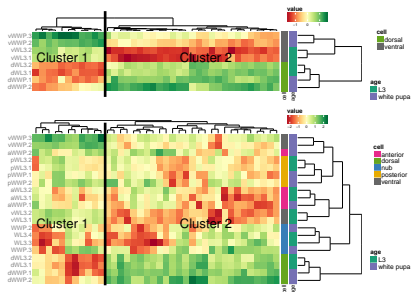
allCompartments	D/V
anterior.L3	dorsal.L3
anterior.white pupa	dorsal.white pupa
dorsal.L3	ventral.L3
dorsal.white pupa	ventral.white pupa
nub.L3	
nub.white pupa	
posterior.L3	
posterior.white pupa	
ventral.L3	
ventral.white pupa	

Contrasts			space
dorsal.L3	vs	ventral.L3	1
dorsal.white pupa	vs	ventral.white pupa	1
dorsal.L3	vs	dorsal.white pupa	0
ventral.L3	vs	ventral.white pupa	0
dorsal.L3	vs	ventral.white pupa	?
dorsal.white pupa	vs	ventral.L3	?

Contrasts			space
dorsal.L3	vs	ventral.L3	1
dorsal.white pupa	vs	ventral.white pupa	1
dorsal.L3	vs	dorsal.white pupa	0
ventral.L3	vs	ventral.white pupa	0
dorsal.L3	vs	ventral.white pupa	?
dorsal.white pupa	vs	ventral.L3	?

Contrasts			space			
dorsal.L3	vs	ventral.L3	1	1	1	1
dorsal.WP	vs	ventral.WP	1	1	1	1
dorsal.L3	vs	dorsal.WP	0	0	0	0
ventral.L3	vs	ventral.WP	0	0	0	0
dorsal.L3	vs	ventral.WP	0	1	0	1
dorsal.WP	vs	ventral.L3	0	0	1	1
number of genes			0	4	3	31

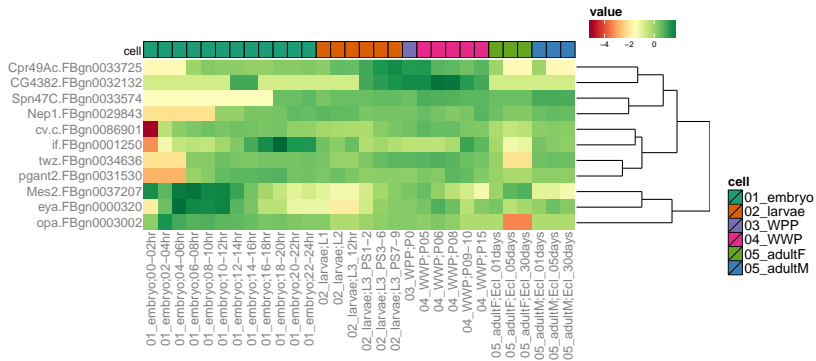
dorsal vs ventral - space-specific genes = 38



Biotype	Cluster1	Cluster2
mRNA	11	25
ncRNA	0	2
GO terms	11	21
GO terms (wing, dorsal, ventral, imaginal disc, development)	6	9

Pvalue	Term BP - Cluster1
4e-06	midgut development
7e-05	maintenance of epithelial integrity, open tracheal system
1e-04	embryo development
1e-04	cell motility
1e-04	digestive system development
3e-04	imaginal disc development
4e-04	post-embryonic development
7e-04	tissue homeostasis
7e-04	eye-antennal disc morphogenesis
Pvalue	Term BP - Cluster2
3e-06	myoblast migration
3e-06	fibroblast growth factor receptor signaling pathway
3e-06	response to fibroblast growth factor
6e-05	regulation of crystal cell differentiation
7e-05	cellular response to growth factor stimulus
1e-04	larval visceral muscle development
2e-04	glial cell proliferation
3e-04	larval somatic muscle development
5e-04	enzyme linked receptor protein signaling pathway

modEncode for cluster1



modEncode for cluster2

