

ERC *Drosophila melanogaster*

06/08/2015

Outline

Introduction

Overview of RNA-seq samples

Wing compartments - resolution of analysis all together

- Differential gene expression - EdgeR

- Variance decomposition

Space-specific genes - pairwise comparison of wing compartments

- Anterior vs posterior

- Dorsal vs ventral

Nubbin vs all other wing compartments

Time-specific genes

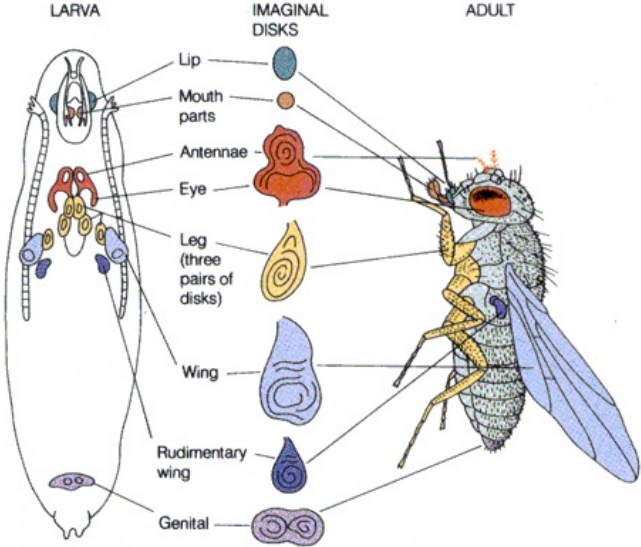
Space and time

- Variance decomposition

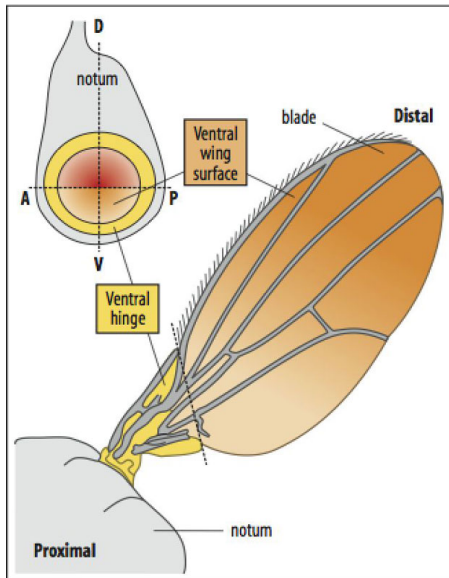
Hedgehog signalling

Ibrahim

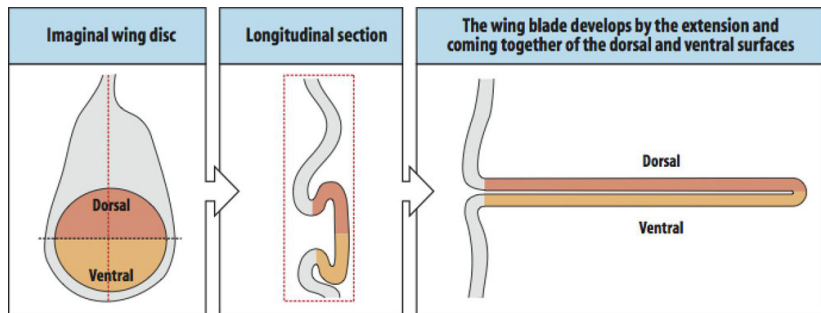
Imaginal discs



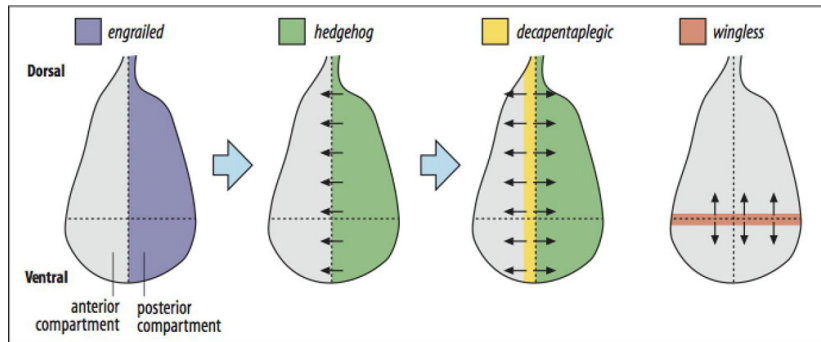
Wing imaginal disc



Wing imaginal disc



Wing A/P and D/V boundaries



Considerations

- ▶ data highly similar
- ▶ all wing compartments together - no resolution
 - ▶ EdgeR - impossible to identify genes over expressed in a compartment-specific manner
 - ▶ Decomposing the variation of gene expression across time and space
- ▶ anterior / posterior / L3 / WP genes
 - ▶ EdgeR (A/P matrix vs all wing compartments)
 - ▶ Decomposing the variation of gene expression across time and space

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Wing compartments - resolution of analysis all together

Space-specific genes - pairwise comparison of wing compartments

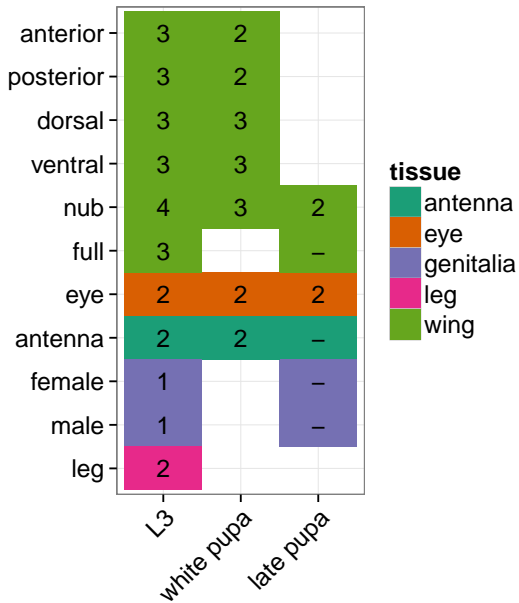
Nubbin vs all other wing compartments

Time-specific genes

Space and time

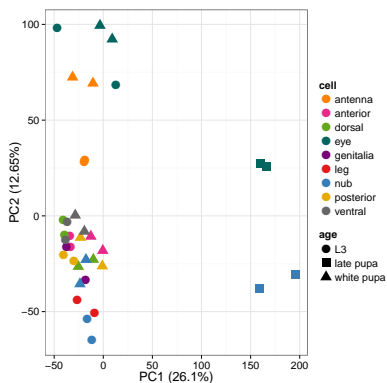
Hedgehog signalling

Overview of processed RNA-seq samples

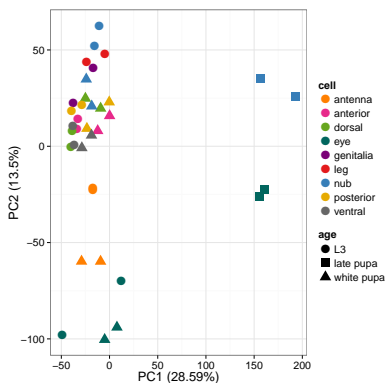


PCA all samples

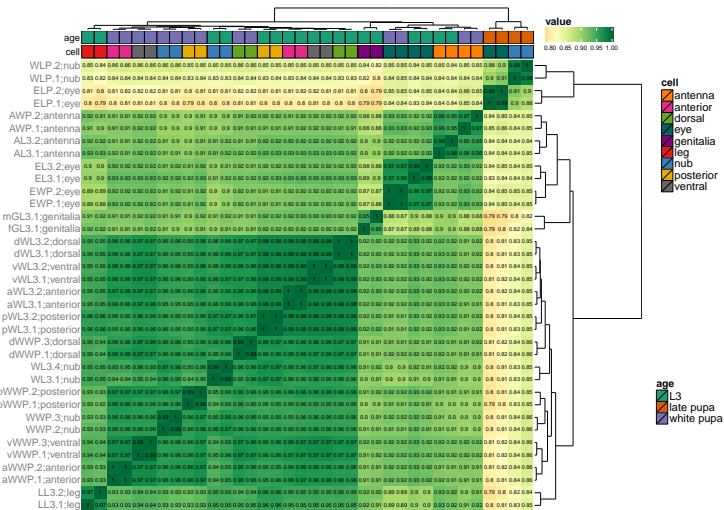
17092 genes



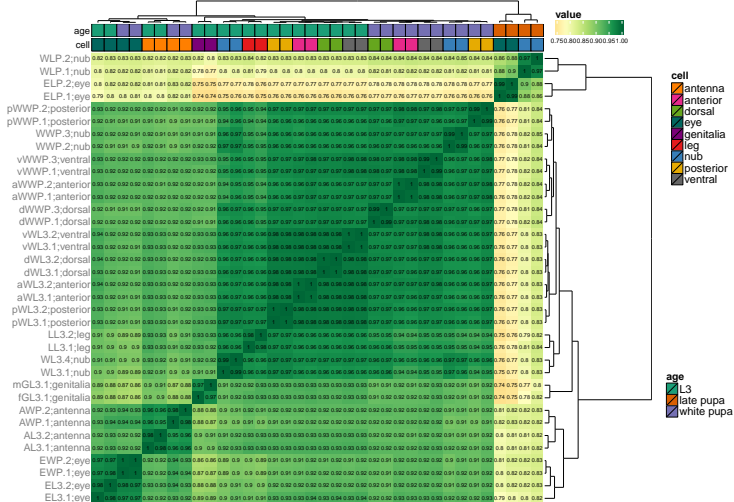
13854 protein-coding genes



Clustering by gene expression

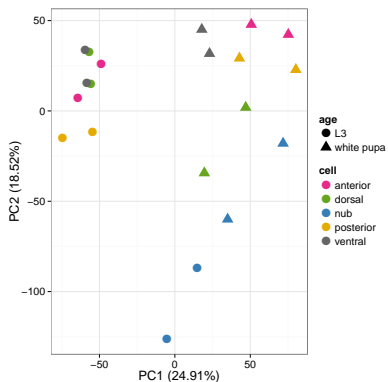


Clustering by gene expression - protein-coding

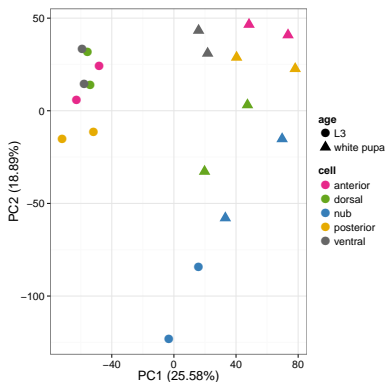


PCA wing compartments

17092 genes

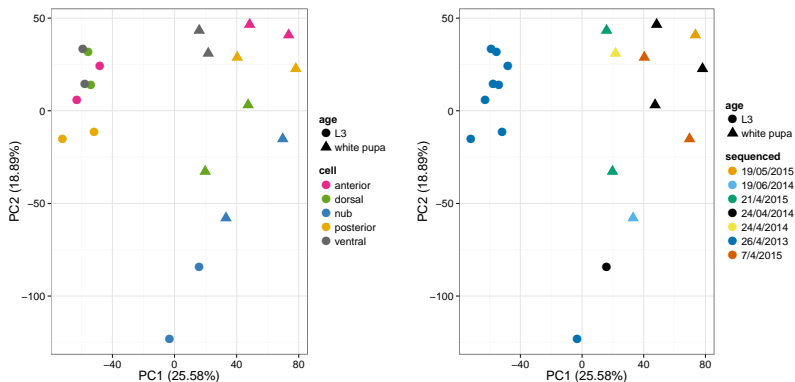


13854 protein-coding genes

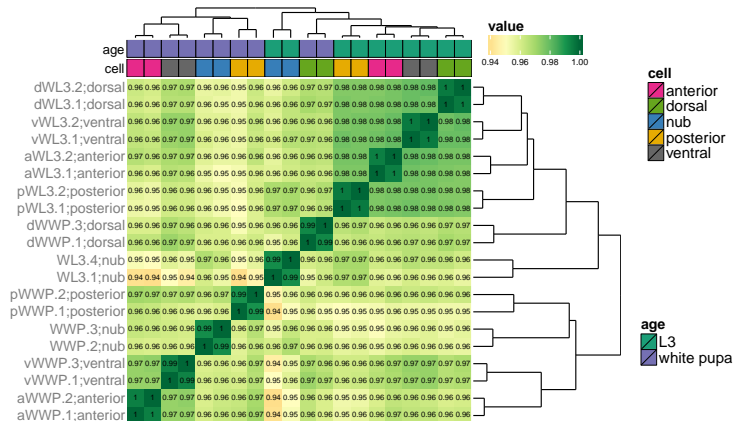


PCA wing compartments

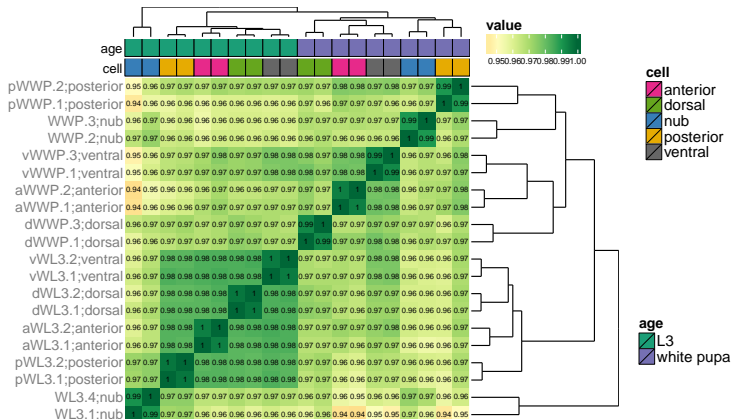
13854 protein-coding genes



clustering by gene expression - wing compartments



clustering by gene expression - wing compartments - protein-coding



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Wing compartments - resolution of analysis all together

Differential gene expression - EdgeR

Variance decomposition

Space-specific genes - pairwise comparison of wing compartments

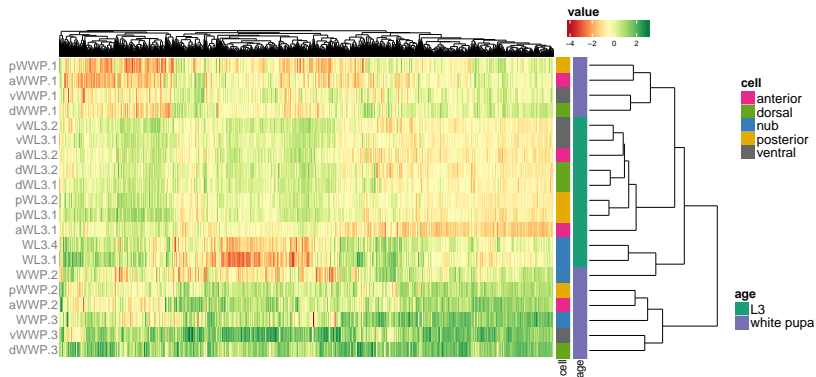
Nubbin vs all other wing compartments

Time-specific genes

Space and time

EdgeR allCompartments - general design

4166 differentially expressed genes



factor cell*age as two-way anova 2:10

(Intercept)

cellnub

cellventral

celldorsal:agewhite pupa

cellposterior:agewhite pupa

celldorsal

cellposterior

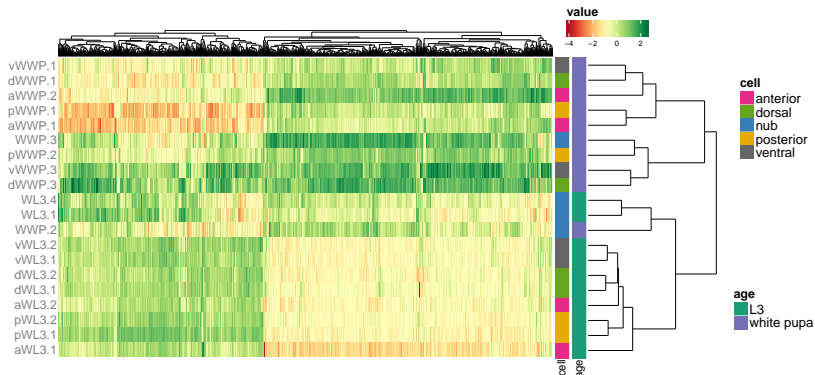
agewhite pupa

cellnub:agewhite pupa

cellventral:agewhite pupa

EdgeR allCompartments - general design: age contrast

1264 differentially expressed genes



factor cell*age coefficient for the comparison 6

(Intercept)	celldorsal
cellnub	cellposterior
cellventral	agewhite pupa
celldorsal:agewhite pupa	cellnub:agewhite pupa
cellposterior:agewhite pupa	cellventral:agewhite pupa

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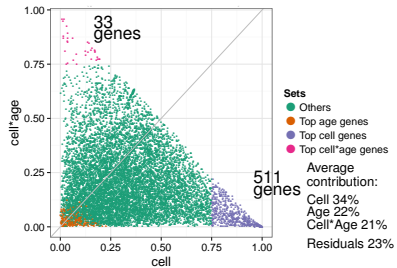
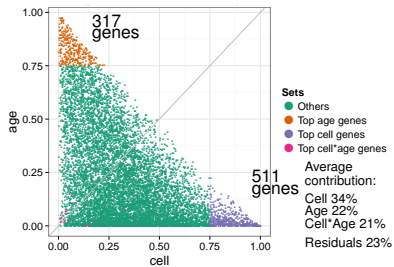
Space-specific genes - pairwise comparison of wing compartments

Nubbin vs all other wing compartments

Time-specific genes

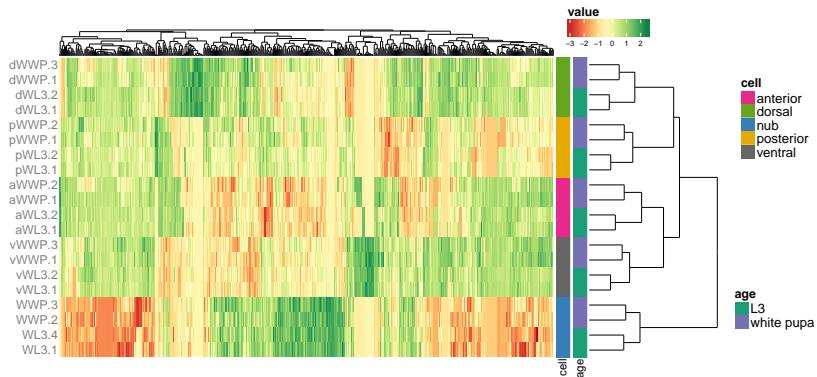
Space and time

Variance decomposition



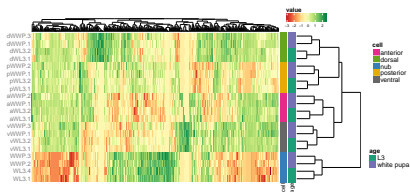
Variance decomposition

topCellGenes = 511 genes



Variance decomposition

topCellGenes = 511 genes

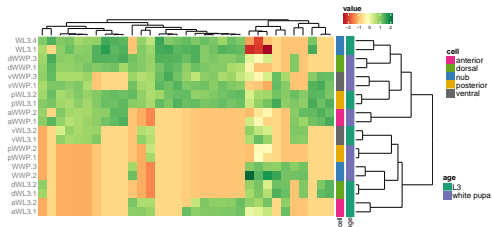


KEGGID	Pvalue	Term
-	-	-

Pvalue	BP
3e-15	tissue morphogenesis
3e-12	digestive system development
7e-12	pattern specification process
7e-12	regulation of transcription, DNA-templated
7e-12	regulation of RNA biosynthetic process
2e-11	epithelial tube morphogenesis
2e-11	sensory organ development
5e-11	developmental process
1e-10	urogenital system development
Pvalue	Term
Pvalue	Term
3e-10	sequence-specific DNA binding transcription factor activity
3e-10	sequence-specific DNA binding
9e-07	RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity
9e-07	sequence-specific DNA binding RNA polymerase II
6e-06	transcription factor activity
4e-05	metal ion binding
5e-05	fibroblast growth factor receptor binding
5e-05	enhancer sequence-specific DNA binding
6e-05	ion binding
1e-04	transcription regulatory region DNA binding

Variance decomposition

topCell*AgeGenes = 33 genes

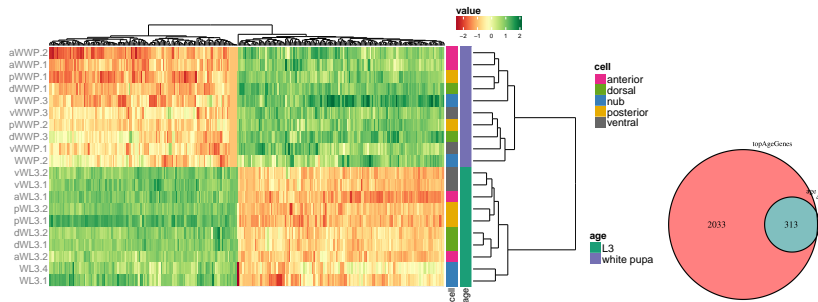


Pvalue	BP
5e-04	synaptic vesicle docking
5e-04	exocytosis
8e-04	vesicle docking
1e-03	synaptic vesicle transport
Pvalue	CC
Pvalue	MF
0.001	SNARE binding
0.001	SNAP receptor activity

KEGGID	Pvalue	Term
-	-	-

Variance decomposition

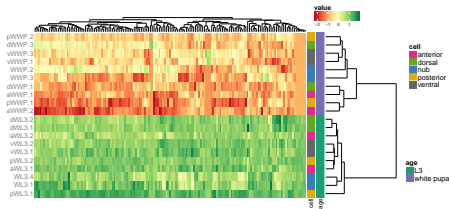
topAgeGenes = 317 genes



Variance decomposition

topAgeGenes = 317 genes, cluster 1 = 151

genes

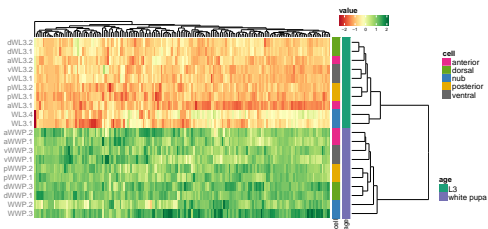


KEGGID	Pvalue	Term
03030	4.1e-13	DNA replication
03430	5.4e-06	Mismatch repair
03440	8.0e-05	Homologous recombination

Pvalue	BP
8e-14	cell cycle
8e-13	organelle fission
2e-11	DNA conformation change
2e-11	microtubule cytoskeleton organization
4e-11	DNA-dependent DNA replication
3e-10	DNA replication initiation
5e-10	DNA repair
1e-09	cellular component organization
6e-09	pre-replicative complex assembly involved in nuclear cell cycle DNA replication
Pvalue	CC
4e-14	MCM complex
5e-11	intracellular non-membrane-bounded organelle
3e-10	nuclear pre-replicative complex
6e-10	nucleus
7e-08	microtubule
8e-08	replication fork
1e-07	intracellular part
2e-07	organelle lumen
6e-07	nuclear chromosome
Pvalue	MF
6e-10	3'-5' DNA helicase activity
1e-09	microtubule binding
2e-09	pyrophosphatase activity
2e-09	hydrolase activity, acting on acid anhydrides
5e-09	chromatin binding
5e-09	macromolecular complex binding
1e-08	adenyl nucleotide binding
4e-08	helicase activity
4e-08	nucleotide binding

Variance decomposition

topAgeGenes = 317 genes, cluster 2 = 166 genes



Pvalue	BP
3e-06	instar larval or pupal development
3e-06	imaginal disc morphogenesis
4e-06	post-embryonic organ development
4e-06	post-embryonic morphogenesis
1e-05	tube morphogenesis
2e-05	single-organism process
4e-05	ecdysteroid metabolic process
6e-05	wing disc development
8e-05	imaginal disc-derived wing morphogenesis
Pvalue	CC
4e-08	endoplasmic reticulum
1e-05	rough endoplasmic reticulum membrane
2e-05	plasma membrane
6e-05	membrane region
1e-04	Sec61 translocon complex
2e-04	cytoplasm
2e-04	lipid particle
Pvalue	MF
6e-05	choline dehydrogenase activity

KEGGID	Pvalue	Term
04141	0.000155	Protein processing in endoplasmic reticulum

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Wing compartments - resolution of analysis all together

Space-specific genes - pairwise comparison of wing compartments

Anterior vs posterior

Dorsal vs ventral

Nubbin vs all other wing compartments

Time-specific genes

Space and time

EdgeR - design matrix

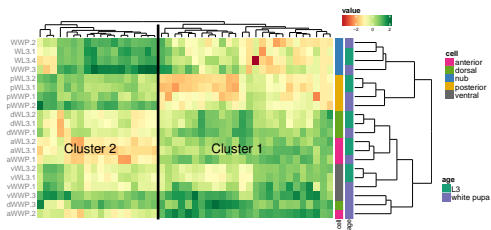
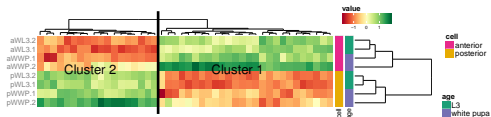
allCompartments	A/P
anterior.L3	anterior.L3
anterior.white pupa	anterior.white pupa
dorsal.L3	posterior.L3
dorsal.white pupa	posterior.white pupa
nub.L3	
nub.white pupa	
posterior.L3	
posterior.white pupa	
ventral.L3	
ventral.white pupa	

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	6	36

anterior vs posterior - space-specific genes = 44



Pvalue	Term - Cluster1
1e-06	sensory organ development
1e-06	digestive tract development
2e-06	cell morphogenesis involved in differentiation
5e-06	neuron differentiation
6e-06	cell projection morphogenesis
9e-06	developmental process
1e-05	single-multicellular organism process

Pvalue	Term - cluster2
1e-08	regulation of transcription, DNA-templated
1e-08	regulation of RNA biosynthetic process
3e-08	tissue development
5e-08	regulation of nucleobase-containing compound metabolic process
7e-08	regulation of biosynthetic process
3e-06	wing disc development
3e-05	imaginal disc-derived appendage development
7e-05	imaginal disc morphogenesis
1e-04	post-embryonic organ development
2e-04	wing disc anterior/posterior pattern formation
2e-04	post-embryonic morphogenesis
4e-04	instar larval or pupal development
2e-03	posterior compartment specification
2e-03	determination of wing disc primordium

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Space-specific genes - pairwise comparison of wing compartments

Anterior vs posterior

Dorsal vs ventral

Nubbin vs all other wing compartments

Time-specific genes

Space and time

EdgeR - design matrix

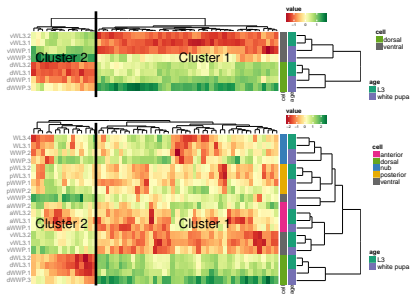
allCompartments	D/V
anterior.L3	dorsal.L3
anterior.white pupa	dorsal.white pupa
dorsal.L3	ventral.L3
dorsal.white pupa	ventral.white pupa
nub.L3	
nub.white pupa	
posterior.L3	
posterior.white pupa	
ventral.L3	
ventral.white pupa	

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	3	6	48

dorsal vs ventral - space-specific genes = 57



Pvalue	Term - Cluster1
1e-06	eye-antennal disc development
2e-06	tube morphogenesis
9e-06	myoblast migration
1e-05	tissue development
2e-05	organ morphogenesis
2e-05	fibroblast growth factor receptor signaling pathway
2e-05	response to fibroblast growth factor
4e-05	post-embryonic organ development
6e-05	learning or memory
Pvalue	Term
Pvalue	Term
3e-08	fibroblast growth factor receptor binding
3e-05	receptor binding
3e-04	sequence-specific DNA binding transcription factor activity
Pvalue	Term Cluster2
8e-06	digestive system development
9e-06	midgut development
1e-04	maintenance of epithelial integrity, open tracheal system
5e-04	tube morphogenesis
9e-04	morphogenesis of an epithelium
1e-03	instar larval or pupal development
1e-03	tissue homeostasis
1e-03	epithelial cell migration, open tracheal system
1e-03	muscle attachment
Pvalue	Term
9e-04	basolateral plasma membrane
3e-03	sarcolemma
Pvalue	Term

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Space-specific genes - pairwise comparison of wing compartments

Nubbin vs all other wing compartments

Time-specific genes

Space and time

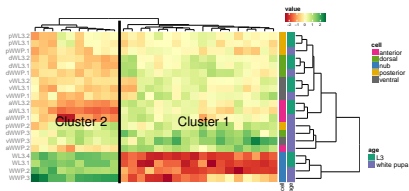
Hedgehog signalling

EdgeR - design matrix and contrasts

allCompartments
anterior.L3
anterior.white pupa
dorsal.L3
dorsal.white pupa
nub.L3
nub.white pupa
posterior.L3
posterior.white pupa
ventral.L3
ventral.white pupa

Contrasts			space
nub.L3	vs	anterior.L3	1
nub.L3	vs	dorsal.L3	1
nub.L3	vs	posterior.L3	1
nub.L3	vs	ventral.L3	1
nub.WP	vs	anterior.WP	1
nub.WP	vs	dorsal.WP	1
nub.WP	vs	posterior.WP	1
nub.WP	vs	ventral.WP	1
nub.L3	vs	nub.WP	0
anterior.L3	vs	anterior.WP	0
dorsal.L3	vs	dorsal.WP	0
posterior.L3	vs	posterior.WP	0
ventral.L3	vs	ventral.WP	0
number of genes			28

dorsal vs ventral - space-specific genes = 28



Pvalue	Term - cluster1
3e-07	aromatic compound biosynthetic process
3e-07	heterocycle biosynthetic process
3e-07	cellular nitrogen compound biosynthetic process
4e-07	organic cyclic compound biosynthetic process
1e-06	system development
1e-06	sensory organ development
2e-06	negative regulation of transcription
4e-06	compound eye development
6e-06	Malpighian tubule stellate cell differentiation
Pvalue	Term
8e-05	nucleus
Pvalue	Term
2e-04	sequence-specific DNA binding transcription factor activity
1e-03	protein tyrosine phosphatase activity, metal-dependent
2e-03	sequence-specific DNA binding RNA polymerase II
Pvalue	Term - cluster2
2e-05	ganglion mother cell fate determination
2e-04	cell fate determination
3e-04	regulation of nucleic acid-templated transcription
3e-04	regulation of transcription from RNA polymerase II promoter
4e-04	transcription, DNA-templated
4e-04	RNA biosynthetic process
4e-04	ventral cord development
4e-04	regulation of RNA metabolic process
5e-04	regulation of cellular macromolecule biosynthetic process
Pvalue	Term
Pvalue	Term
6e-06	sequence-specific DNA binding transcription factor activity
5e-04	positive regulation of transcription
7e-04	sequence-specific DNA binding
8e-04	inositol-3-phosphate synthase activity
3e-03	nucleic acid binding
4e-03	AT DNA binding

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Nubbin vs all other wing compartments

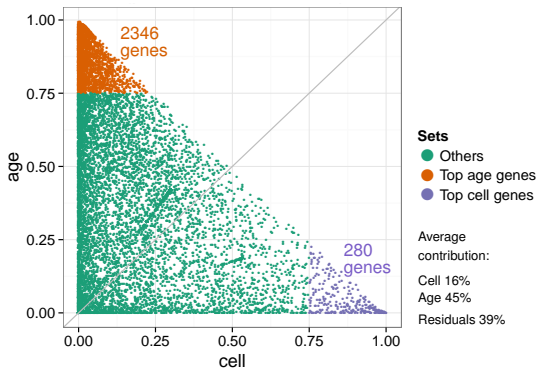
Time-specific genes

Space and time

Variance decomposition

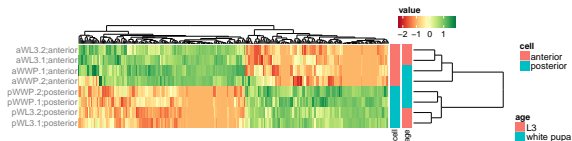
Hedgehog signalling

Variance decomposition

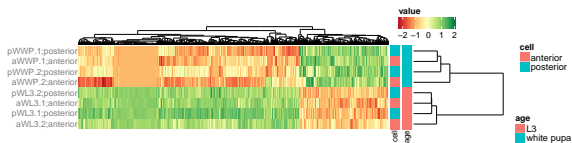


Variance decomposition

topCellGenes = 280 genes

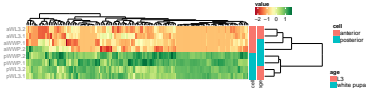


topAgeGenes = 2346 genes

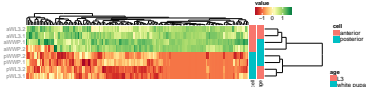


Variance decomposition

topCellGenes = 280 genes, cluster 1
= 129 genes



topCellGenes = 280 genes, cluster 2
= 151 genes



Cluster1 KEGGID	Pvalue	Term
Cluster2 KEGGID	Pvalue	Term

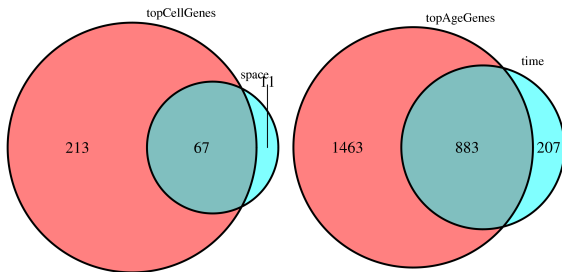
Cluster1 Pvalue	Term BP
4e-05	compartment pattern specification
6e-05	wing disc development
1e-04	anterior commissure morphogenesis
1e-04	positive regulation of gene expression
1e-04	imaginal disc lineage restriction
1e-04	imaginal disc-derived wing vein specification
2e-04	wing disc anterior/posterior pattern formation
2e-04	positive regulation of RNA metabolic process
2e-04	appendage morphogenesis
2e-04	post-embryonic organ morphogenesis
2e-04	instar larval or pupal development

Pvalue	Term
Pvalue	Term
1e-04	sequence-specific DNA binding RNA polymerase II transcription factor activity
3e-04	sequence-specific DNA binding transcription factor activity
4e-04	sequence-specific DNA binding

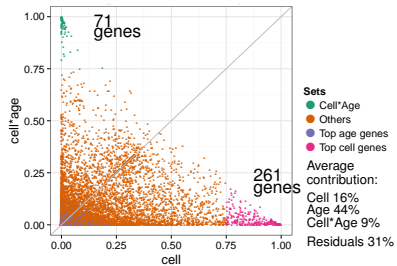
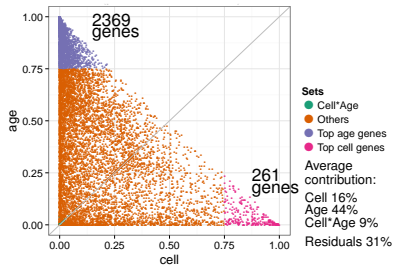
Cluster2 Pvalue	Term
1e-06	post-embryonic organ development
5e-06	imaginal disc-derived appendage development
1e-05	post-embryonic development
5e-05	tissue development
6e-05	post-embryonic appendage morphogenesis
6e-05	cell part morphogenesis
9e-05	imaginal disc morphogenesis
1e-04	cell surface receptor signaling pathway
3e-04	organ morphogenesis
3e-04	cell morphogenesis

Pvalue	Term
Pvalue	Term
2e-07	sequence-specific DNA binding transcription factor activity
6e-05	transmembrane receptor protein tyrosine kinase activity
2e-04	transcription regulatory region sequence-specific DNA binding

Variance decomposition VS EdgeR

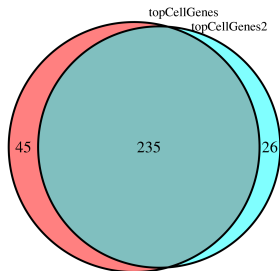


Variance decomposition

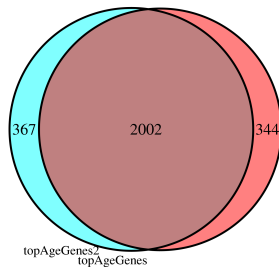


Variance decomposition

topCellGenes = 280 vs 261 genes

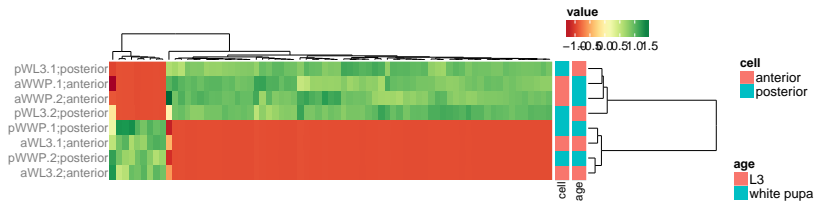


topAgeGenes = 2346 vs 2369 genes



Variance decomposition

topCellAgeGenes = 71 genes



Variance decomposition

topCellAgeGenes, cluster1 = 63 genes



topCellAgeGenes, cluster2 = 10 genes

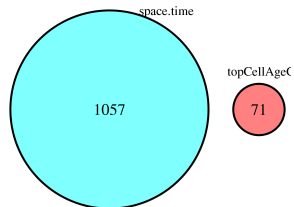


cluster1 - Pvalue	Term BP
Pvalue	Term CC
Pvalue	Term MF
KEGG Pvalue	Term

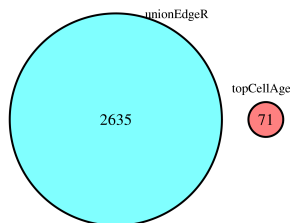
Cluster2 - Pvalue	Term BP
7e-04	response to silver ion
1e-03	detoxification of cadmium ion
2e-03	response to zinc ion
2e-03	response to mercury ion
2e-03	stress response to metal ion
3e-03	response to copper ion
Pvalue	Term CC
0.005	ciliary basal body
Pvalue	Term MF
0.005	RNA 7-methylguanosine cap binding
KEGG Pvalue	Term

Variance decomposition

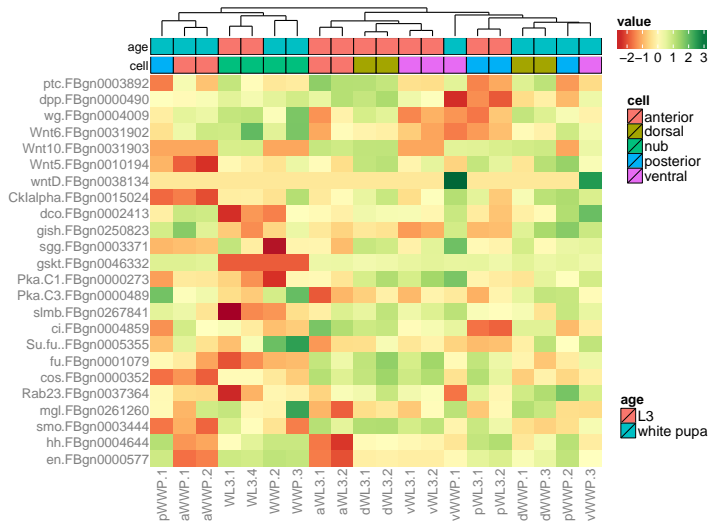
topCellAgeGenes vs space.time EdgeR



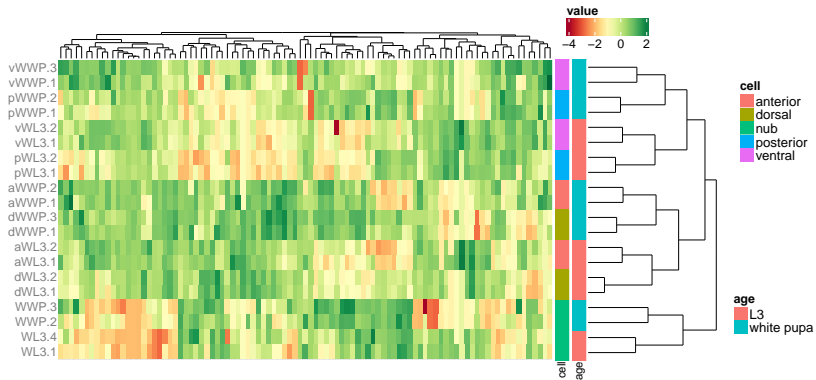
topCellAgeGenes vs all differentially
expressed genes EdgeR



Hedgehog signalling KEGG



Ibrahim



Ibrahim

