GCB 2012 Satellite Workshop on Systems Biology of Ageing,
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Cross-species analysis of age-related transcriptome data

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Leibniz Institute
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Hans Knöll Institute

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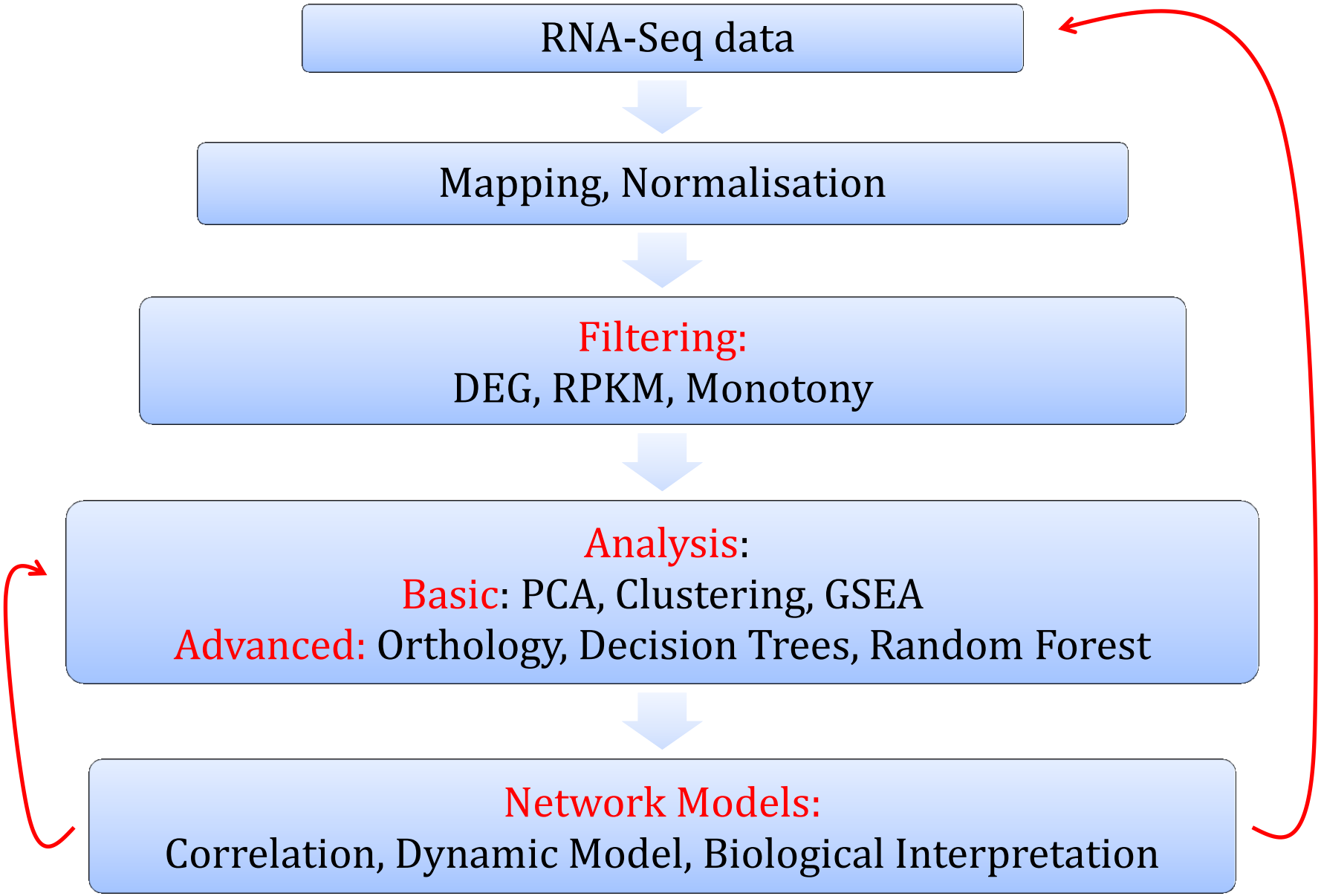
Multi-species approach

	Scientific name	Common name	Lifetime
	*Caenorhabditis elegans*	Worm	2-3 weeks
	*Nothobranchius furzeri*	Killifish	3 months
	*Danio rerio*	Zebrafish	30 months
	*Mus musculus*	Mouse	36-48 months

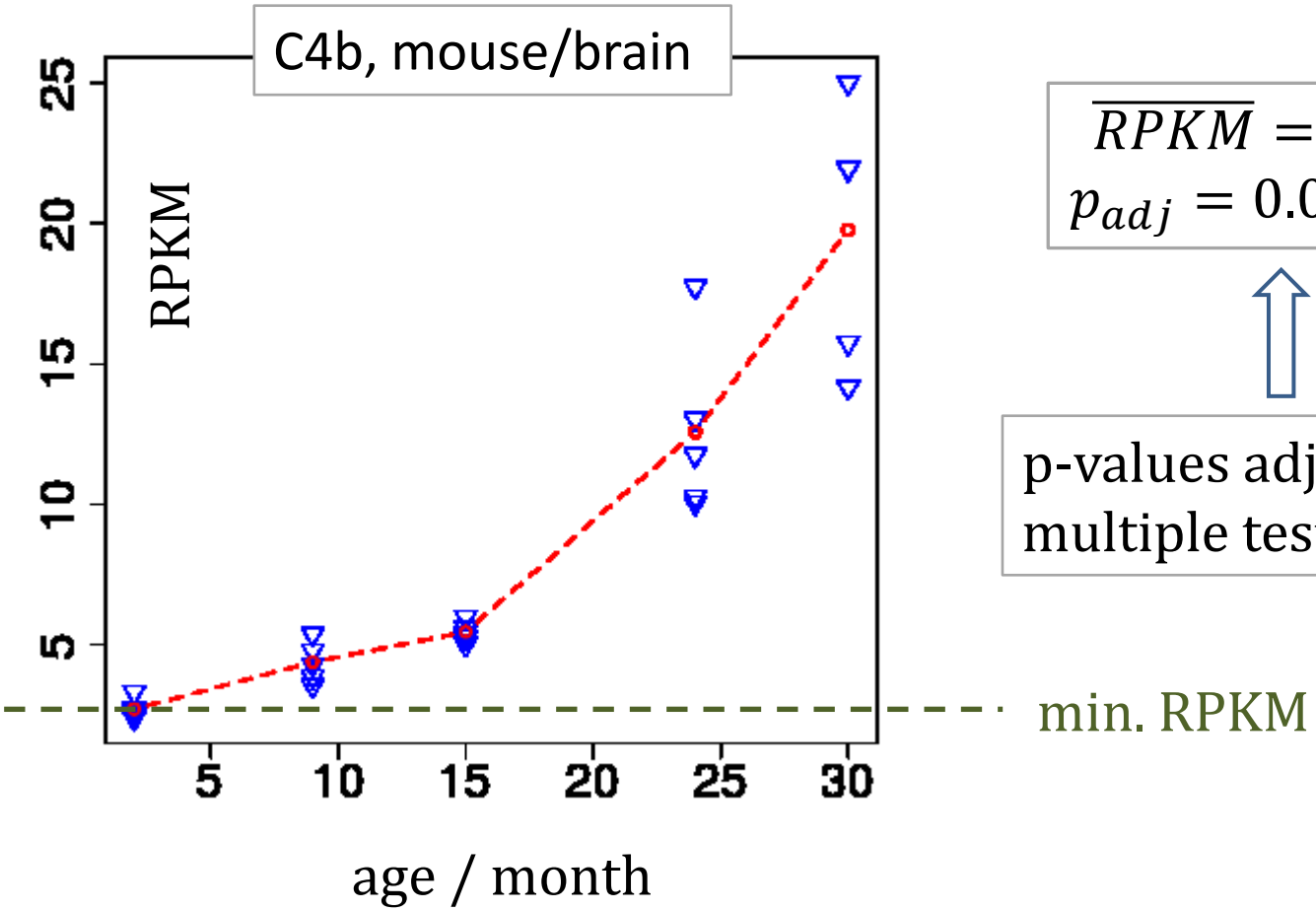
- 4 - 5 age levels
- 2 - 5 replicates at each level

Data flow


RNA-Seq data
Mapping, Normalisation
Filtering:
DEG, RPKM, Monotony
Analysis:
Basic: PCA, Clustering, GSEA
Advanced: Orthology, Decision Trees, Random Forest
Network Models:
Correlation, Dynamic Model, Biological Interpretation

Filtering: 1) DEG, 2) RPKM

1. DEG: Differentially Expressed Genes (over age)
   - edgeR, DESeq, baySeq (NegBin, overdispersion)
2. RPKM: Reads per Kb of exon model per Million mapped reads

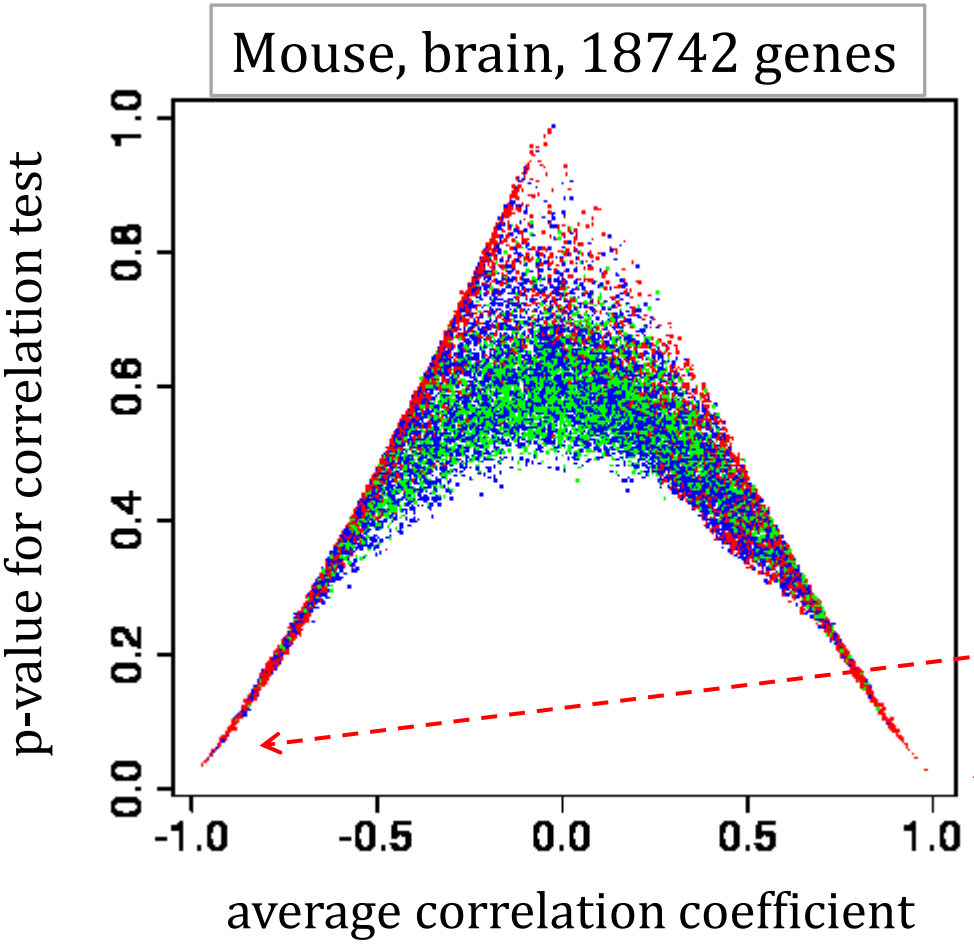


$\overline{RPKM} = 8.98$
$p_{adj} = 0.0012$

p-values adjusted for multiple testing (FDR)

Filtering: 3) Monotony with age

- Genes changing monotonically with age are interesting
- Spearman rank correlation to prototype (Spearman's ρ)
- permutation test to calculate p-values



- 18742 genes
- $|RPKM| \geq 1$
- $FDR \leq 0.05$

genes with a strongly monotonic slope

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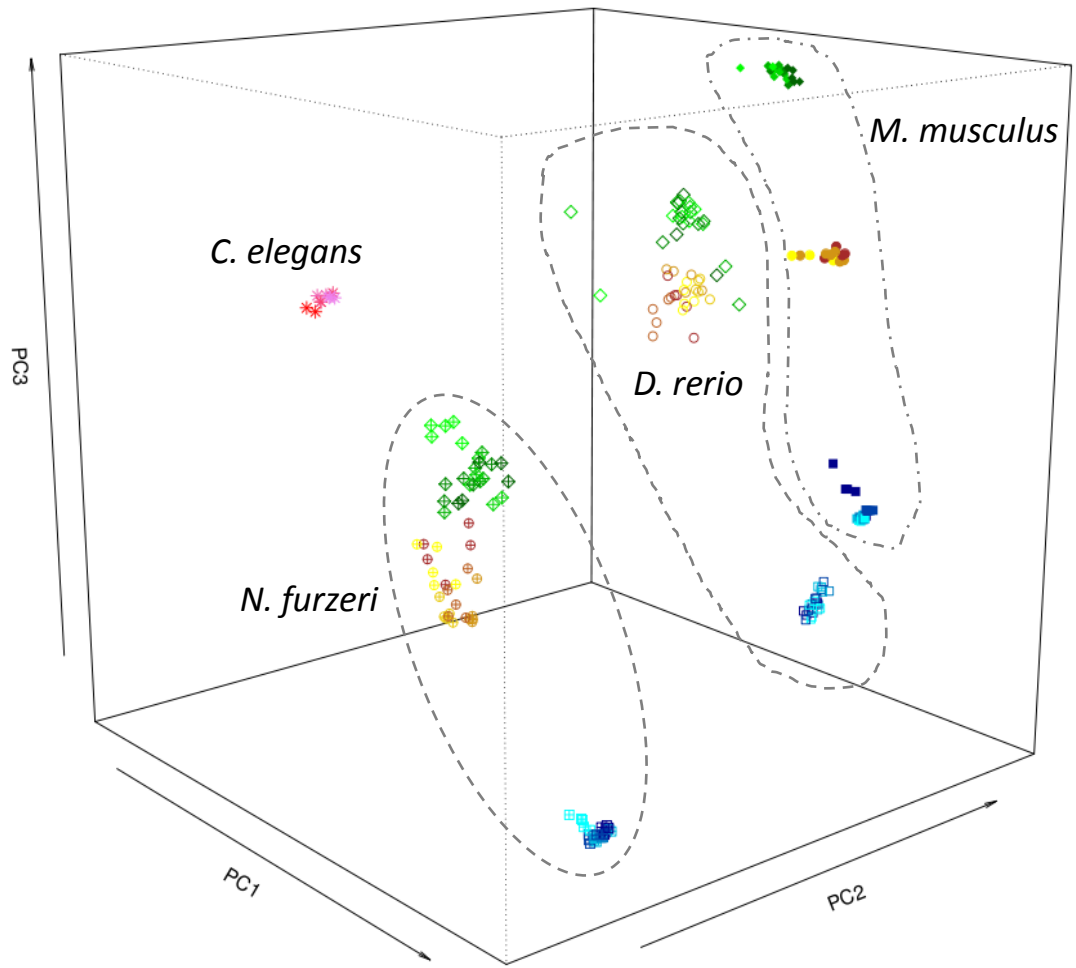
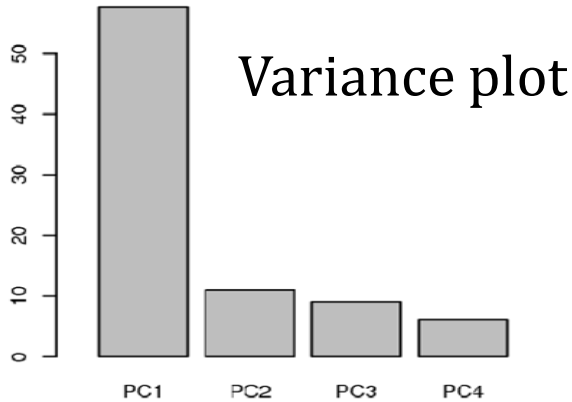
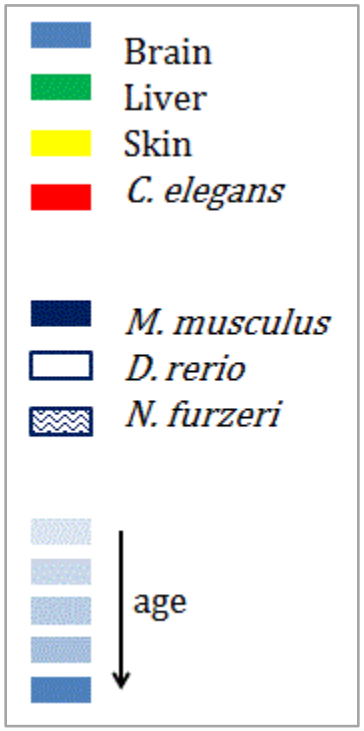
Principal Component Analysis (PCA)

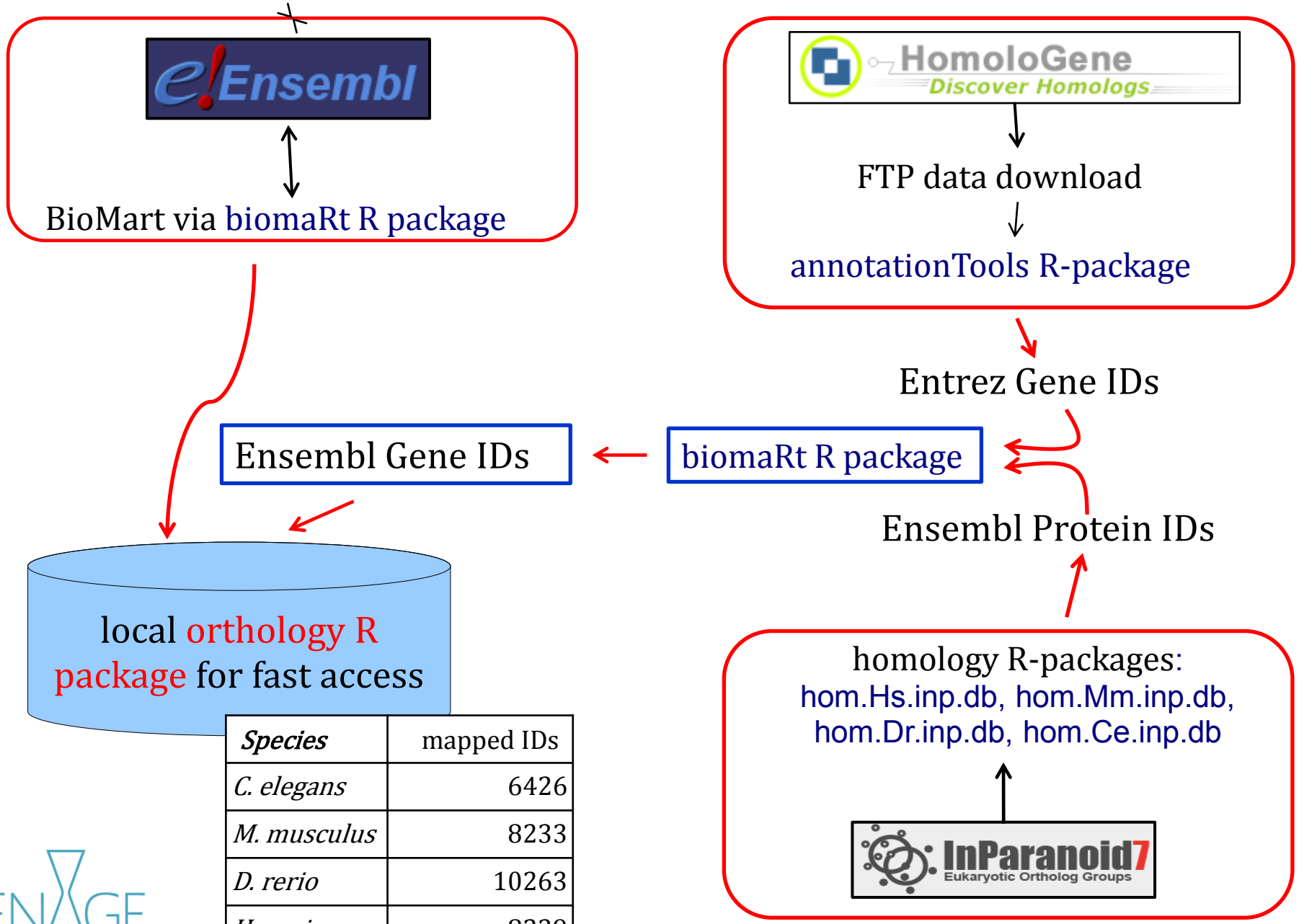
Principal components 1, 2 & 3







Orthology: New, integrated R-package



Species	mapped IDs
C. elegans	6426
M. musculus	8233
D. rerio	10263
H. sapiens	8239

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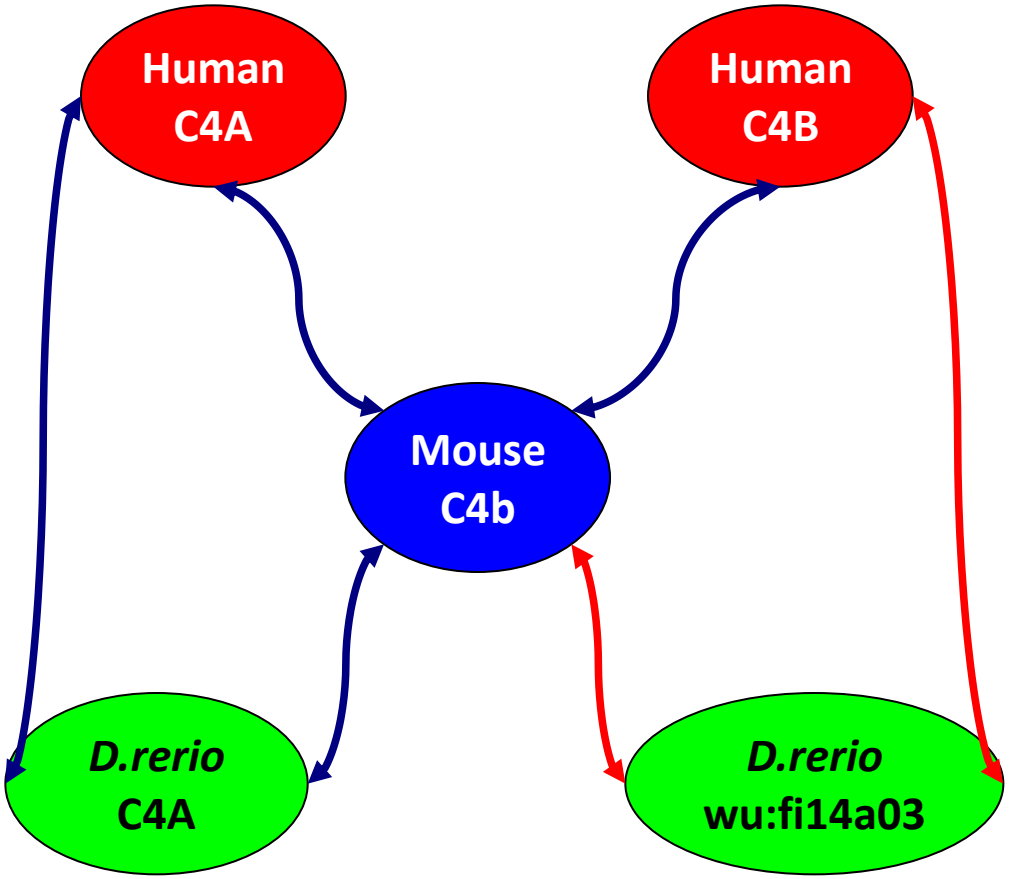
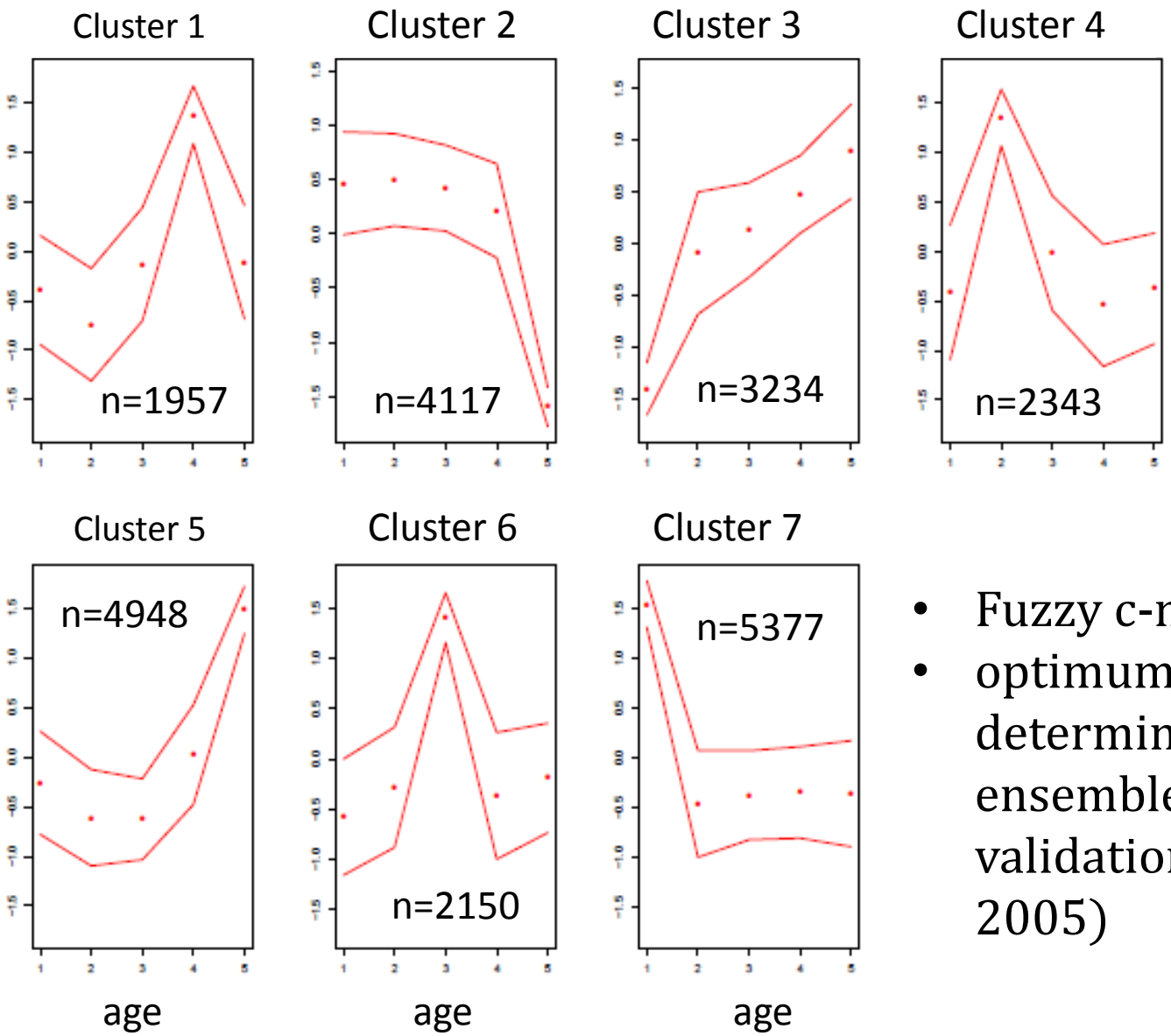
Orthology



color of the arrows indicates database of origin

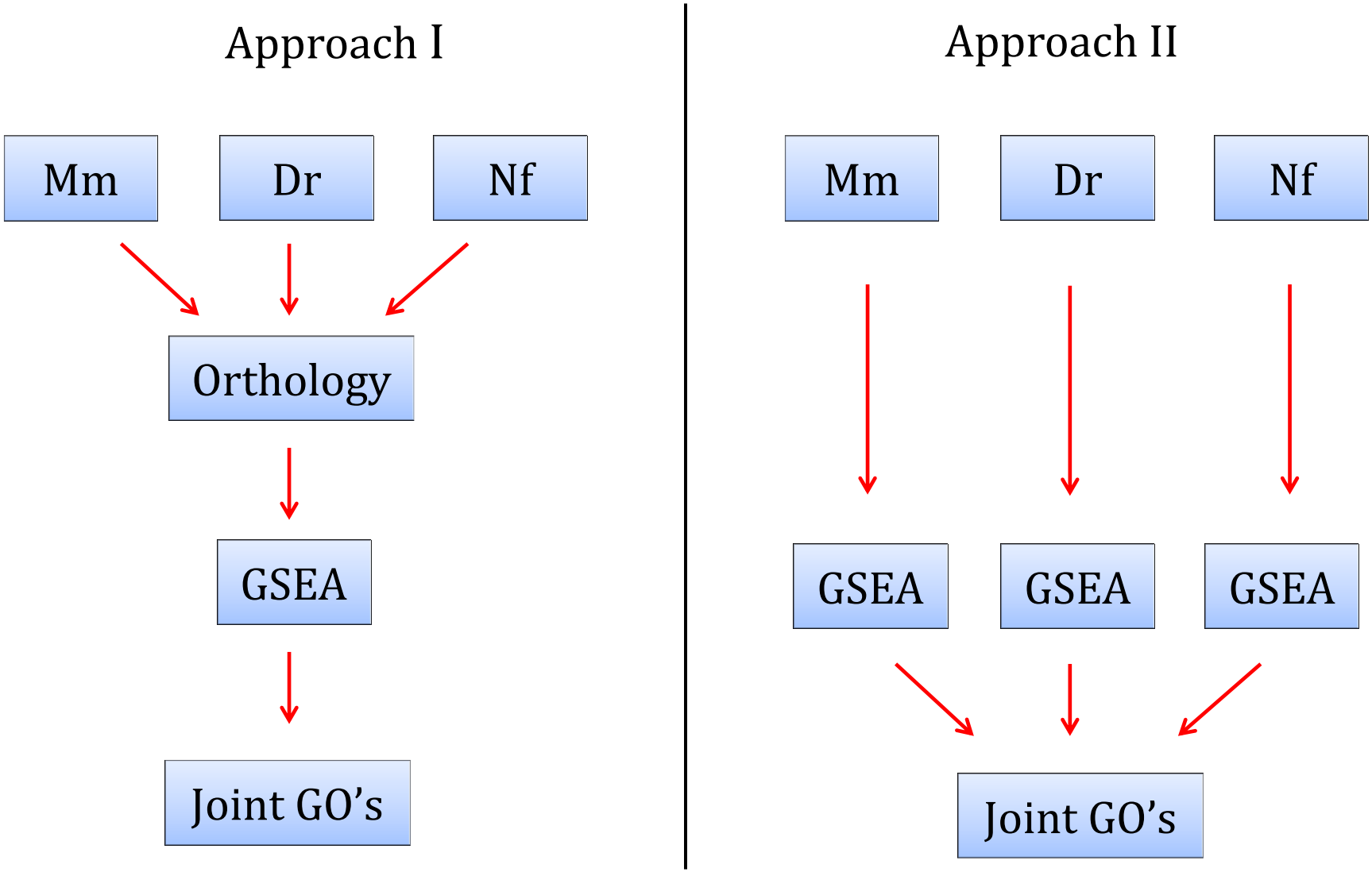
Clustering (across species)

Union of Species: *M. musculus, D. rerio, N. furzeri, C. elegans*



- Fuzzy c-means clustering
- optimum no. of clusters determined using an ensemble of cluster validation indices (Guthke 2005)

Gene Set Enrichment Analysis (GSEA)



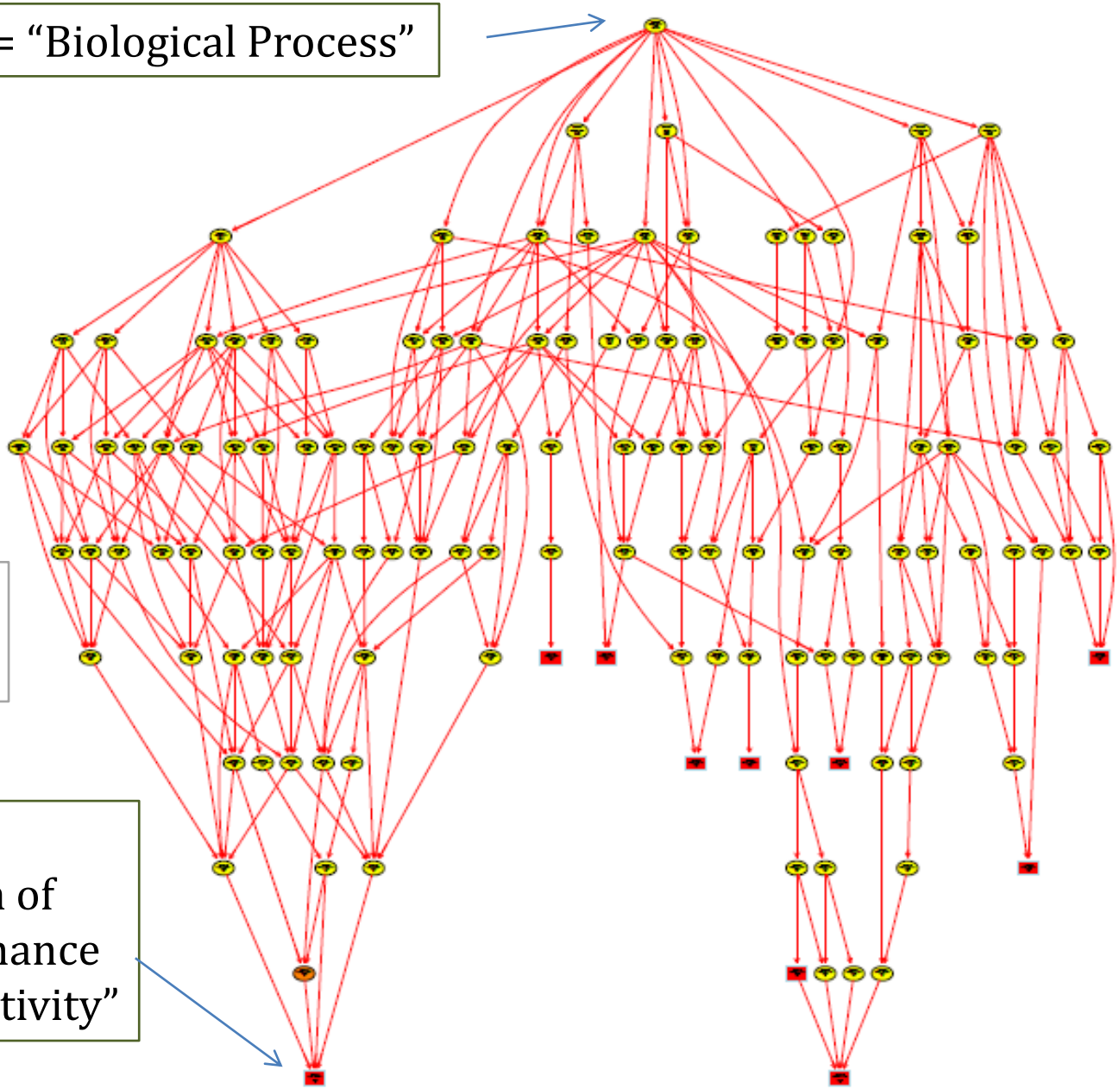
GSEA: Graphical Representation

GO:0008150 = "Biological Process"

Mouse, brain:
176 up-genes
$FDR \leq 0.05$
$\overline{RPKM} \geq 3$
$\bar{\rho} \geq 0.75$

KS-Test with elimination

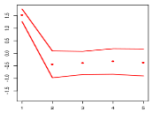
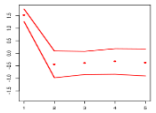
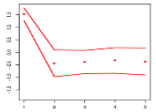
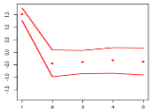
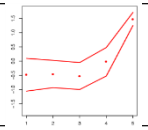
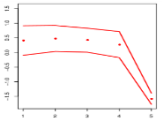
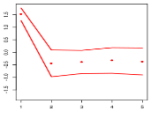
GO:0032211 = "down-regulation of telomere maintenance via telomerase activity"



GSEA: Table of Enriched GO's & Pathways

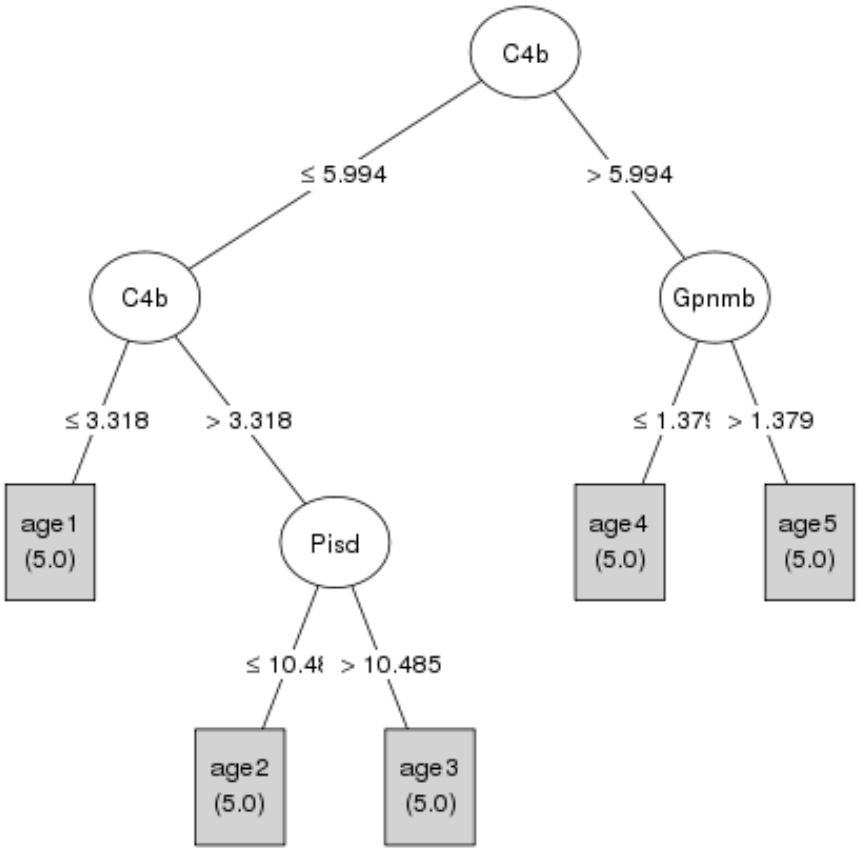
Clustering → GSEA → Intersection

3 species: mouse + 2 fishes, > 200 enriched GO's

Tissue	GO-ID	Description	Cluster
brain	GO:0007399	nervous system development	
brain	GO:0007017	microtubule-based process	
brain	GO:0007169	transmembrane receptor protein tyrosine ...	
brain	KEGG:04512	ECM-receptor interaction	
skin	GO:0042113	B cell activation	
skin	GO:0031012	Extracellular matrix	
liver	GO:0000278	Mitotic cell cycle	

Supervised Machine Learning: Decision Trees

- Classifier, can be used when the number of variables (genes) is higher than the number of observations (transcriptome data sets)
- pinpoints genes that are informative with regard to some attribute, e.g. age, tissue, or species.



Mouse, brain
$|\bar{\rho}| \geq 0.85$
no FDR or RPKM-filter

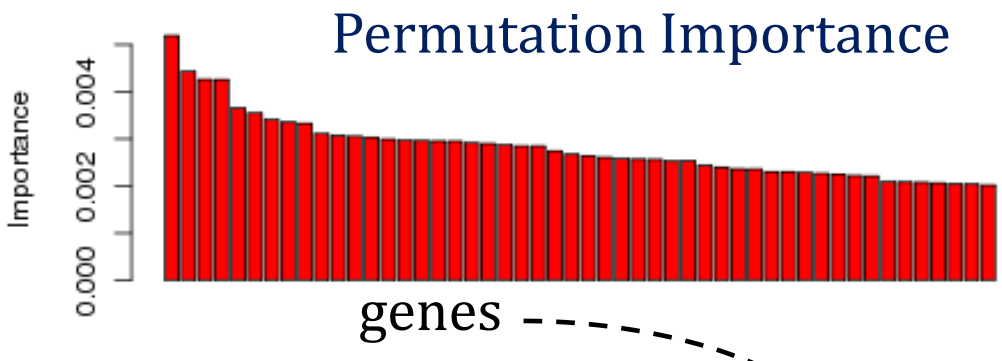
C4b	Complement component 4B
GpnmB	Glycoproteine (transmembrane)
Pisd	Phosphatidylserine decarboxylase

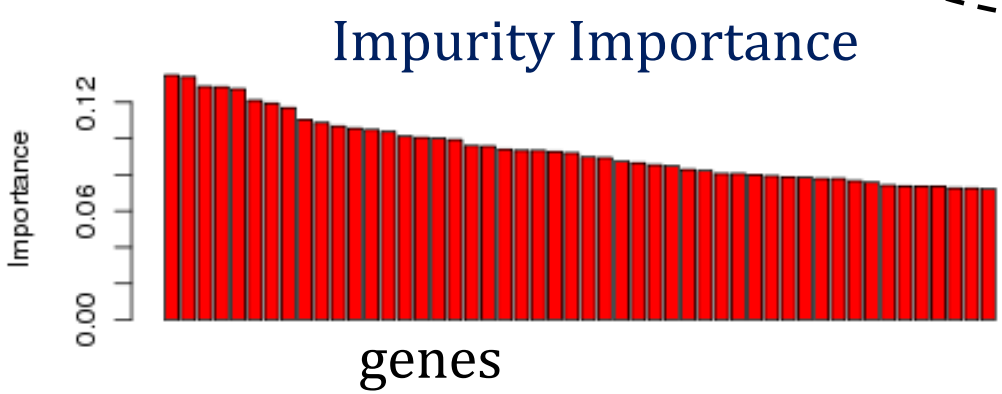
- C4.5 algorithm (Quinlan 1993).
- Overfitting reduced by pruning

Random Forest: Variable Importance

- Ensemble classifier, builds many Decision Trees (Breiman, Cutler)
- random exclusion of a part of variables (and samples) in each tree
- **Variable importance:** measures explanatory power of a variable (gene)

same data as in previous slide



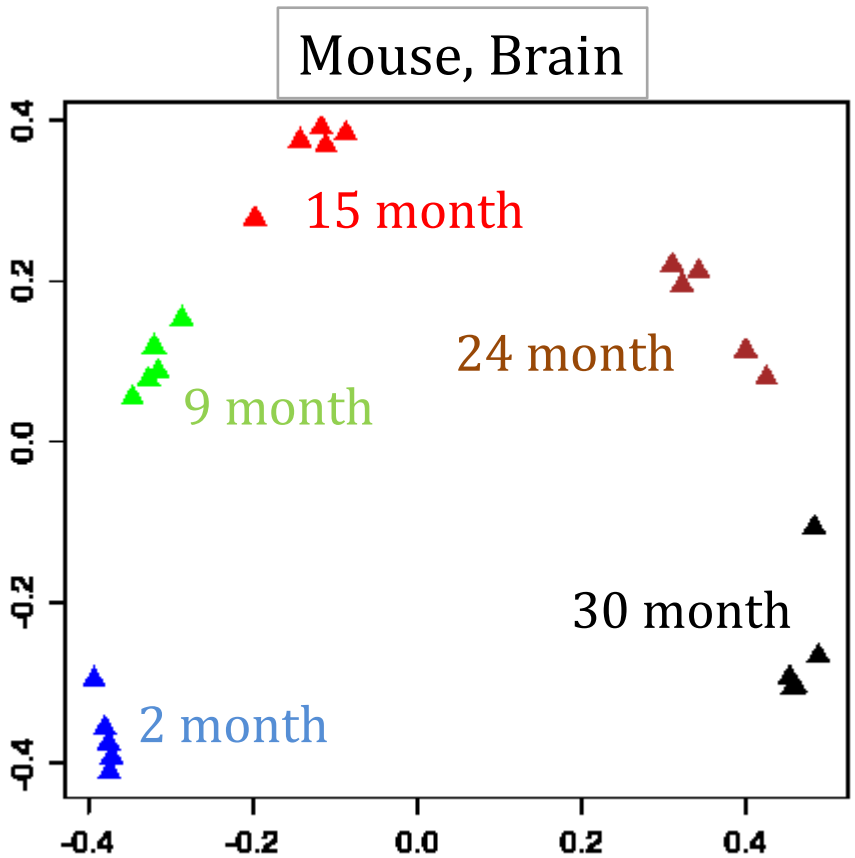


Putative biomarkers:

Apc
C4b
Pisd-ps1
ing4
depdc1a
Pcdh20
Pisd
Plekhb1
Epha3
Tmem167
Pcdhb9
Gpnmb
Prkci

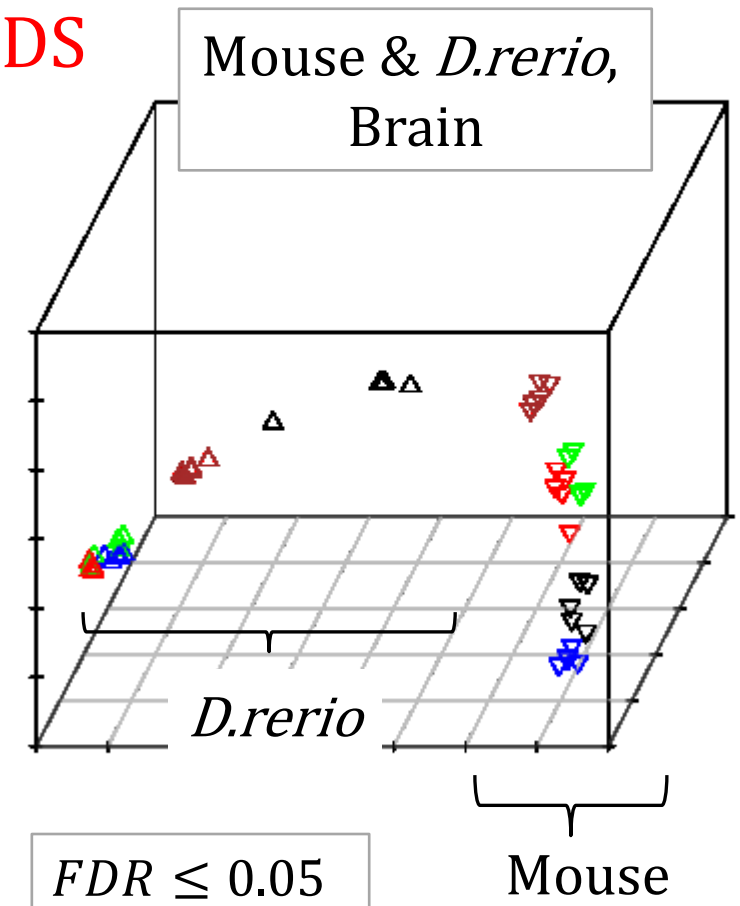
Random Forest: Sample Proximity

- A RF-classifier estimates the pairwise sample proximity (SP)
- samples are "close" if they end up in the same leaf frequently



$|\bar{\rho}| \geq 0.85$
no FDR or RPKM-filter

MDS



$FDR \leq 0.05$
$|RPKM| \geq 1$
$|\bar{\rho}| \geq 0.3$

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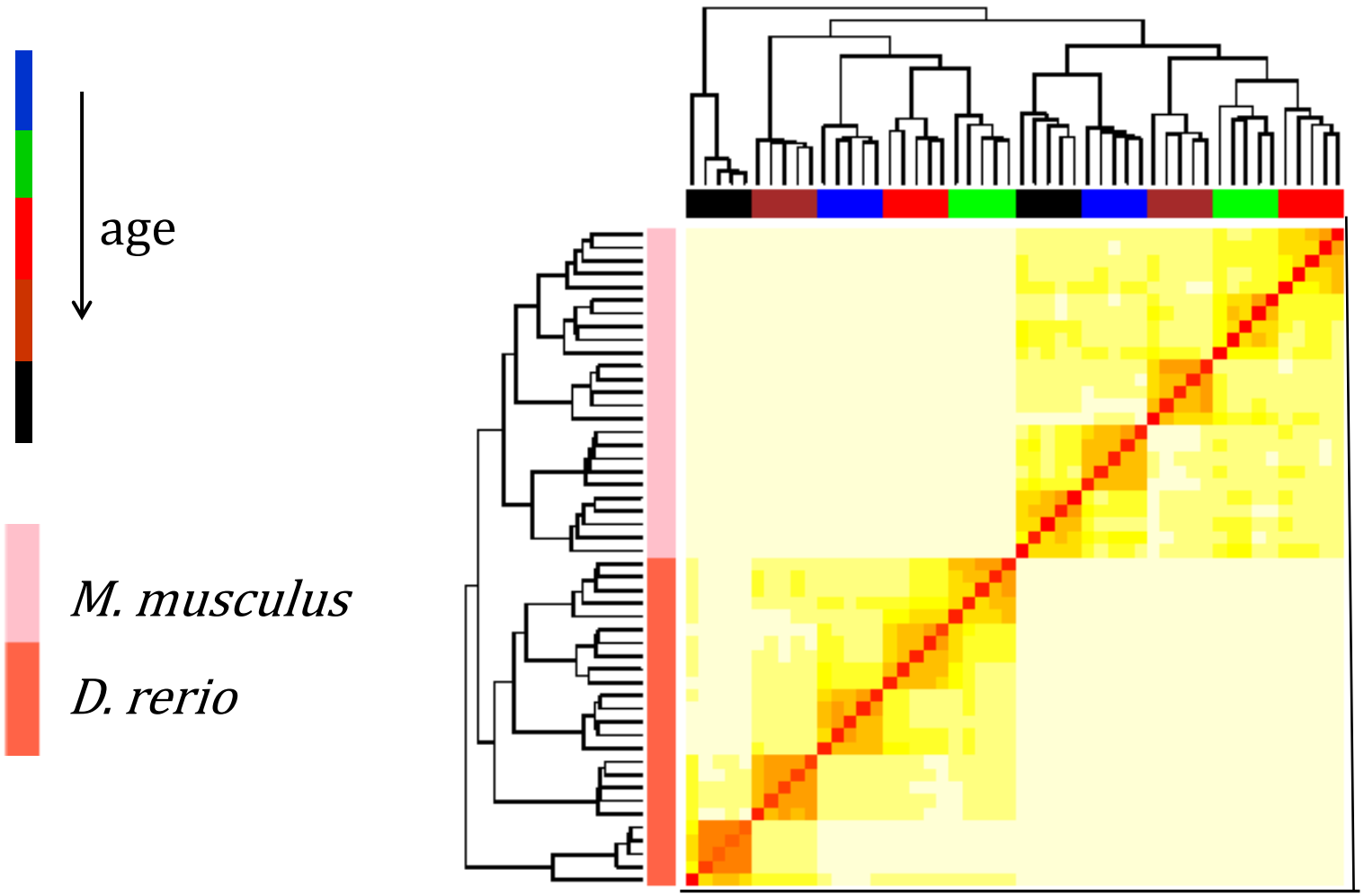
Random Forest: Hierarchical clustering

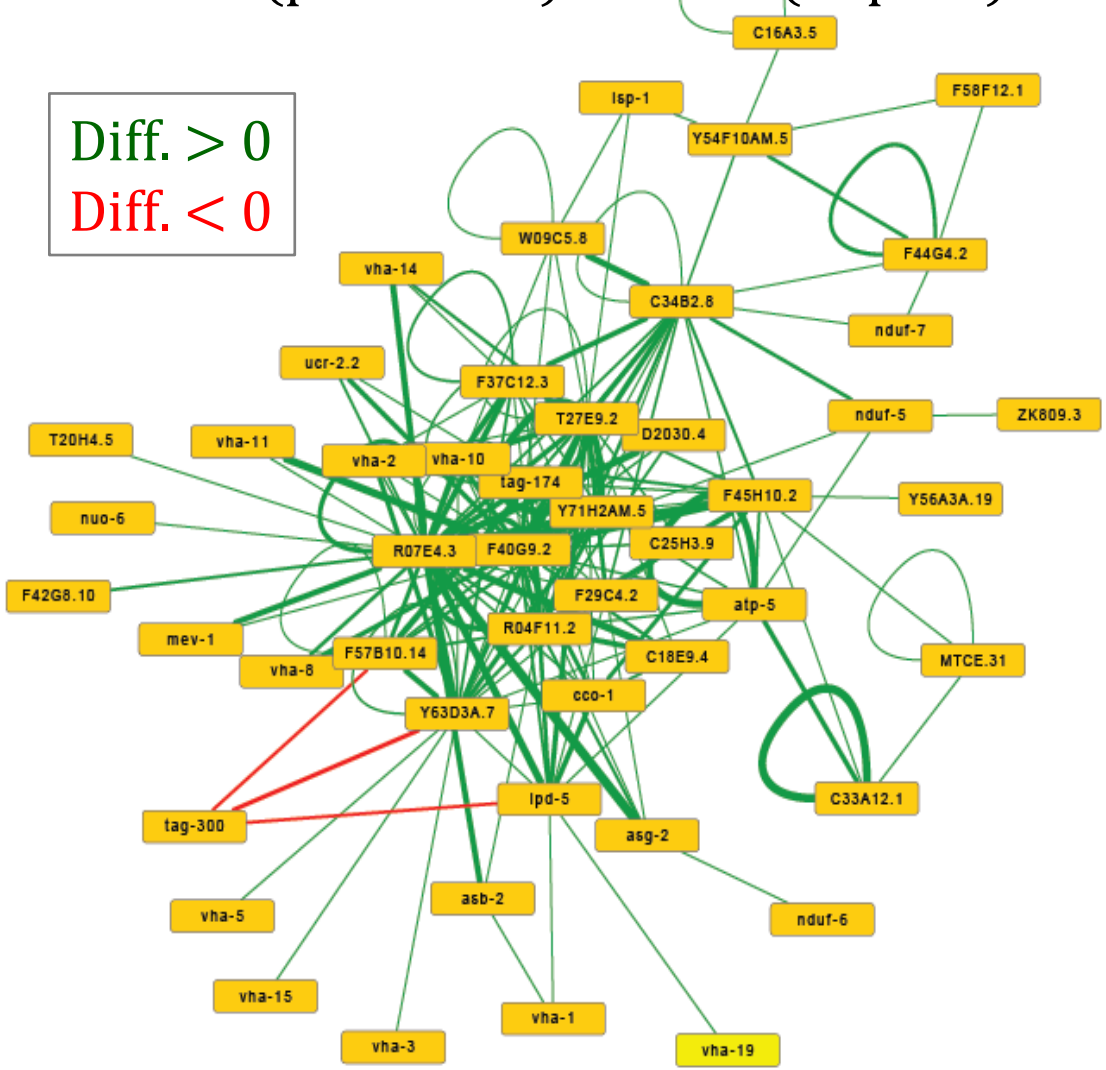
- Hierarchical clustering for *M. musculus* and *D. rerio* based on proximity from Random Forest



Correlation Networks

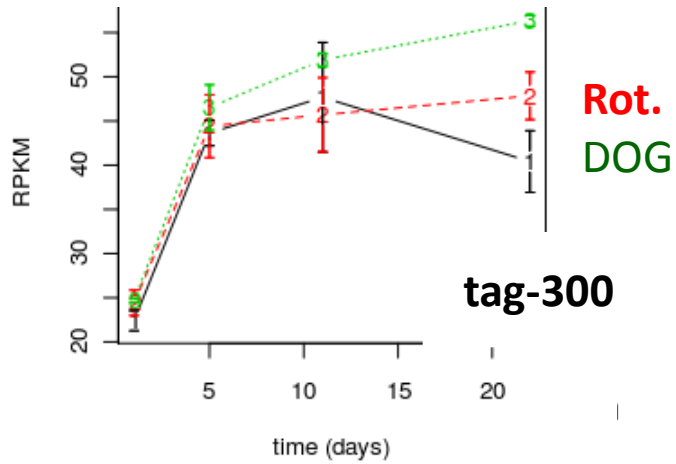
Difference network: *C. elegans*
KEGG-pathway "Oxidative Phosphorylation"
Rotenone (perturbed) - DMSO (unpert.)

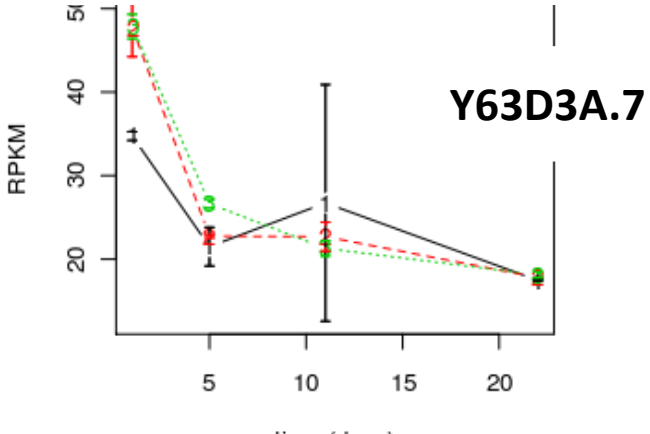
Diff. > 0
Diff. < 0



tag-300/Y110A7A.12 encodes an ortholog of subunit B of the cytoplasmic (V1) domain of vacuolar proton-translocating ATPase

Y63D3A.7 encodes the C. elegans ortholog of the NDUFA2/B8 subunit of the mitochondrial NADH dehydrogenase (ubiquinone) complex (complex I)





Pathways with significantly changed co-expression

In the difference network:

- count fraction of connected genes in a pathway/GO-term
- count fraction of connected genes in the whole network

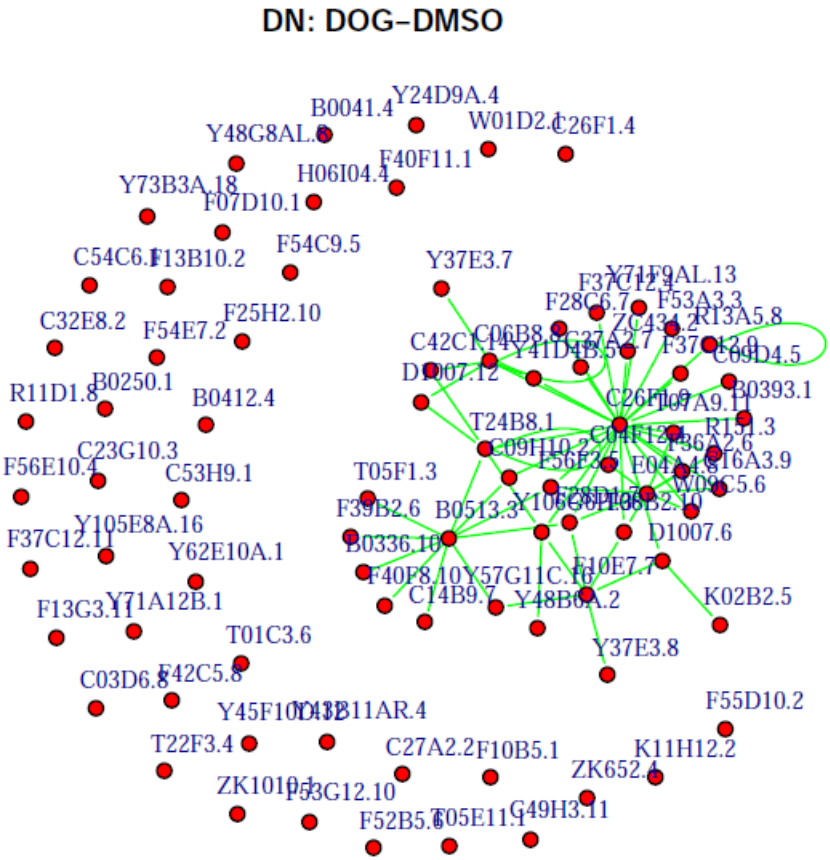
➢ pathways with disproportionately many connected genes

Fisher's Exact Test

fisher.test

Do this for:

- 2881 GO terms and
- 125 KEGG-pathways



Pathways with significantly changed co-expression

- KEGG-pathways whose co-expression is affected most by the perturbation (DOG, glucose restriction):

KEGG-ID	Term	p.adj	Genes	… connected
3010	Ribosome	0.0000	83	41
1100	Metabolic pathways	0.0000	596	214
190	Oxidative phosphorylation	0.0000	100	39
3018	RNA degradation	0.0159	37	9
350	Tyrosine metabolism	0.0396	19	3

Outlook

- Inclusion of human cell lines into the analysis (orthology)
- Perturbed ageing for more species (currently Ce, Mm, Hs only)
- Dynamic models of hormesis connected to ageing
  - mTOR
  - preliminary results achieved
- Identification of relevant biomarkers for ageing, and of targets to support healthy ageing
- Wet-lab validation

Jena Centre for Systems Biology of Ageing

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