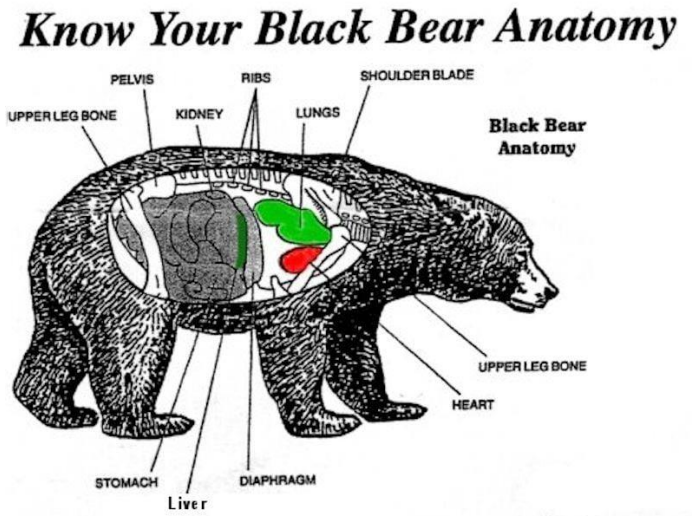
Magda Kmiecik

Differentially Expressed Genes in American Black Bear (*Ursus americanus*) Kidneys in Relation to Cardiac Function

Original Paper



- Purpose: Identify genes in bear kidneys pre and post-hibernation to aid in human targeted therapies for Chronic Kidney Disease (CKD)
- Methods: Bear kidneys homogenized using Trizol from which an aliquot was used for RNA isolation using miRNeasy Mini kit (Qiagen). RNA sequencing prep was performed with Illumina TruSeq and sequencing was done on Illumina NextSeq 2500 lanes.
- Results: 169 differentially expressed protein-coding genes were identified. 101 genes were downregulated and 68 genes were upregulated post-hibernation (spring).
- Conclusion: Upregulation of three cytokine suppression genes were of note which contradict kidney regeneration or recovery. Further analysis needs to be done to understand the effects of these changes in the kidneys.

Chronic Kidney Disease

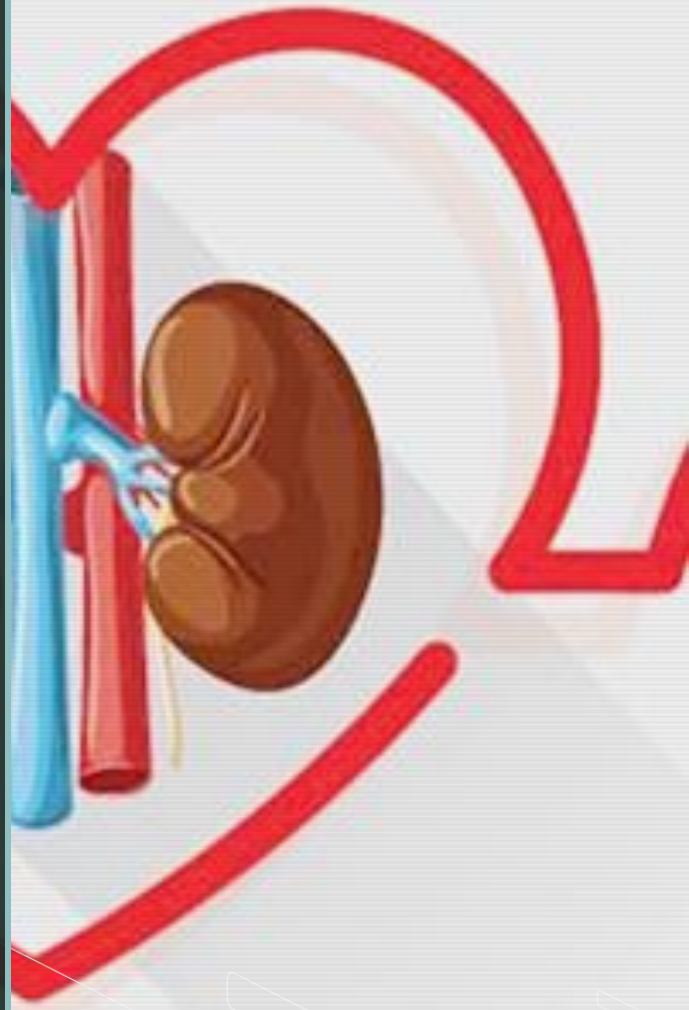
- Progressive loss of kidney functionality
- In 2021, CKD was said to affect 15% of adults in the United States (37 million people)
  - 9/10 adults do not know they have CKD, symptoms develop at aggressive stages of this disease
- More common in older people (65+)
- Slightly more common in women
- Might not notice any symptoms
  - Weight loss
  - Shortness of breath
  - Muscle cramps
  - Blood in urine
- Test for creatinine level in blood and protein level in urine
- CKD tends to worsen and can potentially lead to kidney failure (need transplant). However, it can also be treated depending on how advanced the disease is

Research Question

Due to the strong relationship between the kidney and the heart, which differentially expressed genes in bear kidneys are related to cardiac pathways?

Relationship between Kidney and Heart

- The heart pumps oxygen rich blood throughout the body, the kidneys filter that blood for waste and excess water
- A significant link between the heart and the kidney is blood pressure
  - Increased blood pressure can damage blood vessels in the kidney, but also cause your heart to work at an abnormal strength that it cannot maintain long term
  - Damaged kidneys cannot filter out wastes and excesses like water and salt. An accumulation of wastes has the potential to increase blood pressure and without proper functionality this can further damage the kidneys and heart.
- Therefore, lowered kidney function could potentially link to genes that regulate blood pressure
- Kidney and heart disease are commonly a result of one another



Hibernation in American Black Bears

- Stage 1 - **Hibernation**:
  - Lowering of metabolic and heart rates as well as oxygen consumption
  - Do not eat, drink or defecate
- Stage 2 - **Emergence**:
  - 2-3 weeks after emerging from den where bodily functions start to return back to normal
- Stage 3 - **Normal**:
  - Full normal function, from spring to midsummer/fall
- Stage 4 - **Hyperphagia**:
  - Hyperphagia which means that the bear is consuming more food and water in preparation for hibernating (fattening).
- Stage 5 - **Transition to Hibernation**:
  - Metabolic processes start to change, less food consumed.
  - Rest for 22 hours a day, very lethargic

Experimental Design

Original

Samples
- 12 total bears
  - 6 fall (3F, 3M)
  - 6 spring (3F, 3M)

NGS QC Toolkit
- Quality check and filtering for next generation sequencing data of Illumina technology

STAR
- Aligned for paired end reads
- Soft masked genome
- --sjdbOverhang 75
- --quantMode GeneCounts
- --twopassMode Basic

DESeq2
- DE analysis
- DE = FDR < 0.05

Project

Samples
- 10 total bears
  - 5 fall (3F, 2M)
  - 5 spring (3F, 2M)

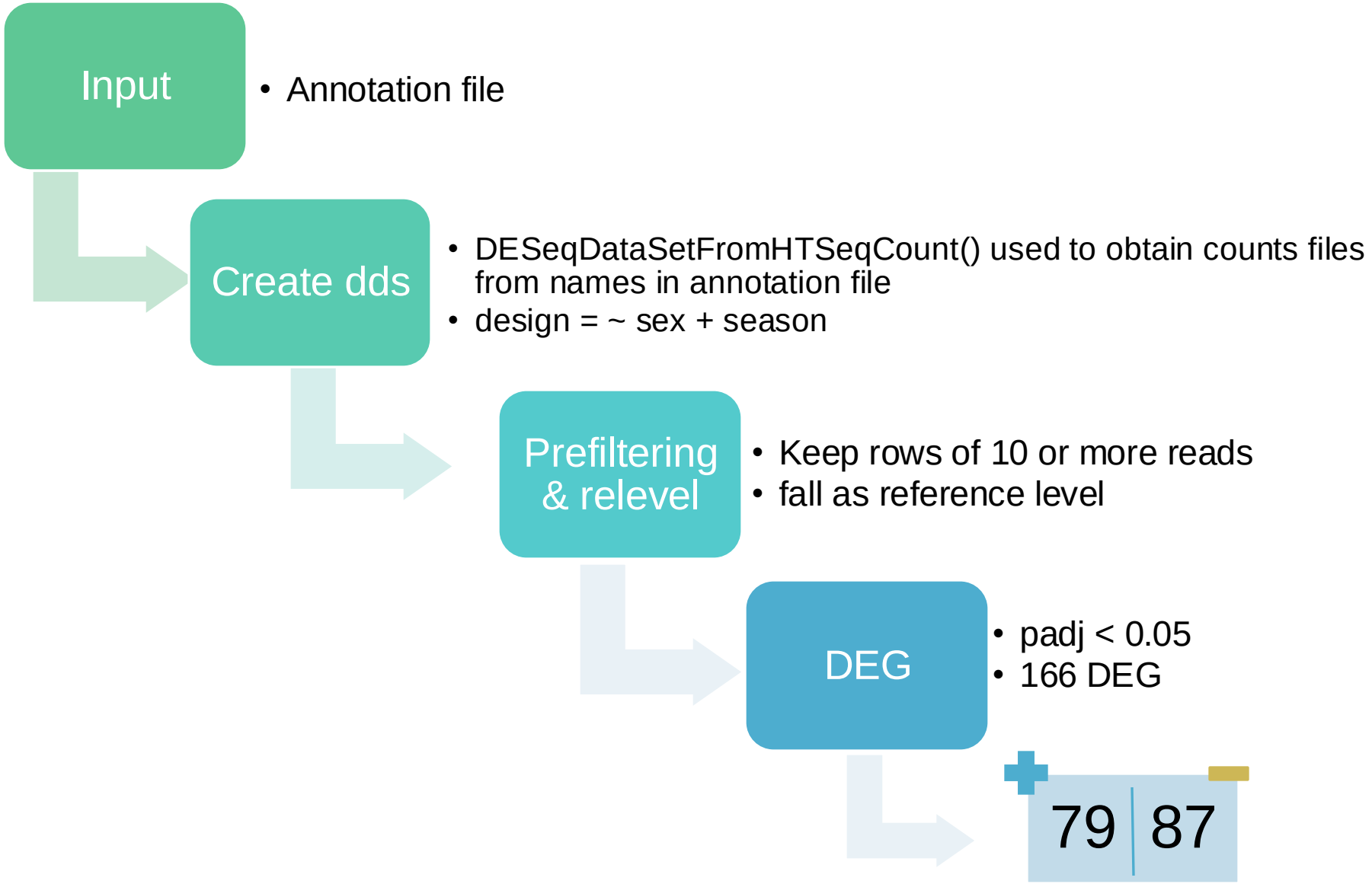
FASTQC
- Download samples
- Quality check of fastq files

HISAT2
- Aligned for paired end reads
- Toplevel genome

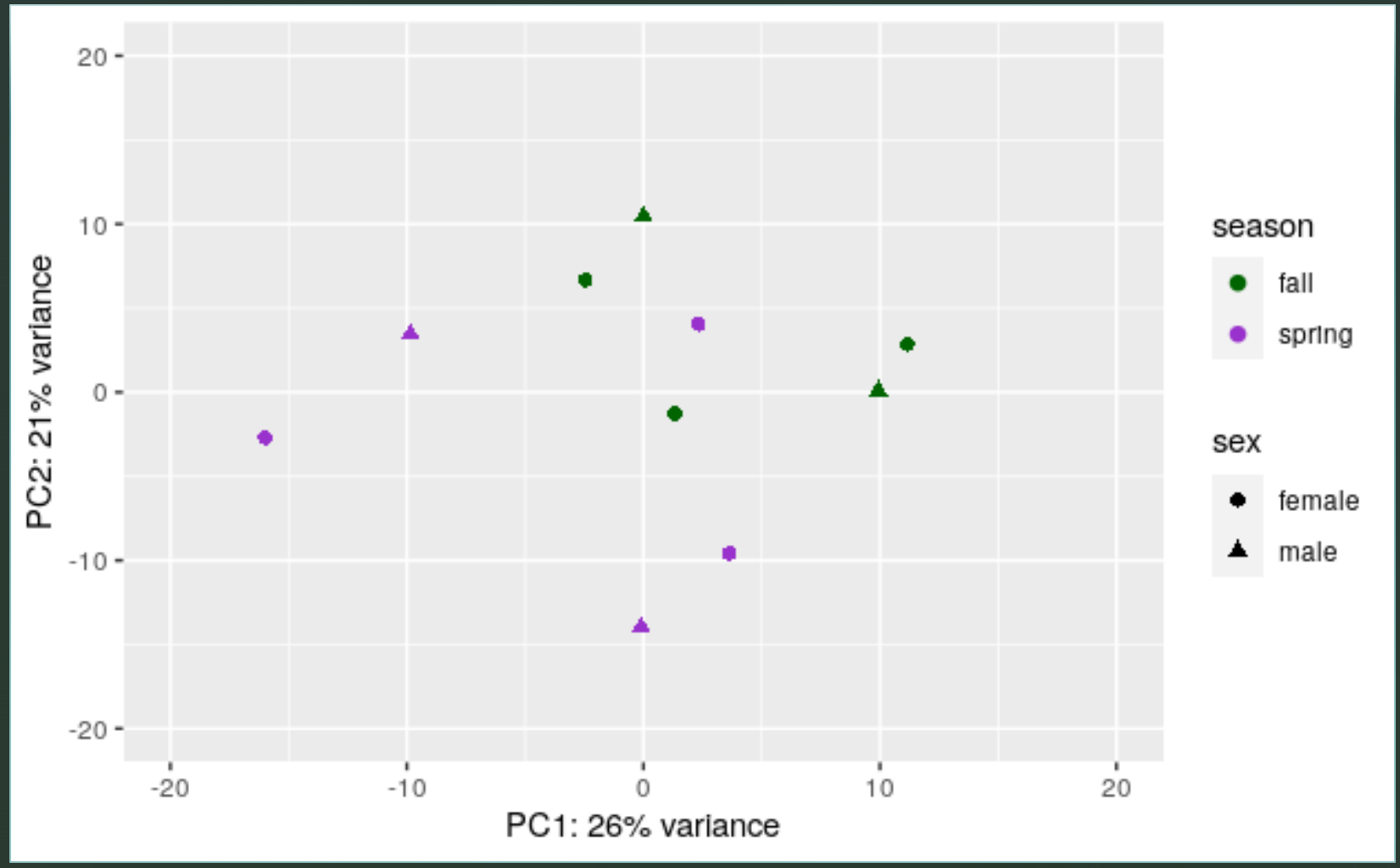
HTSeq
- Create counts files (.bam)

DESeq2
- DE analysis
- DE = FDR < 0.05

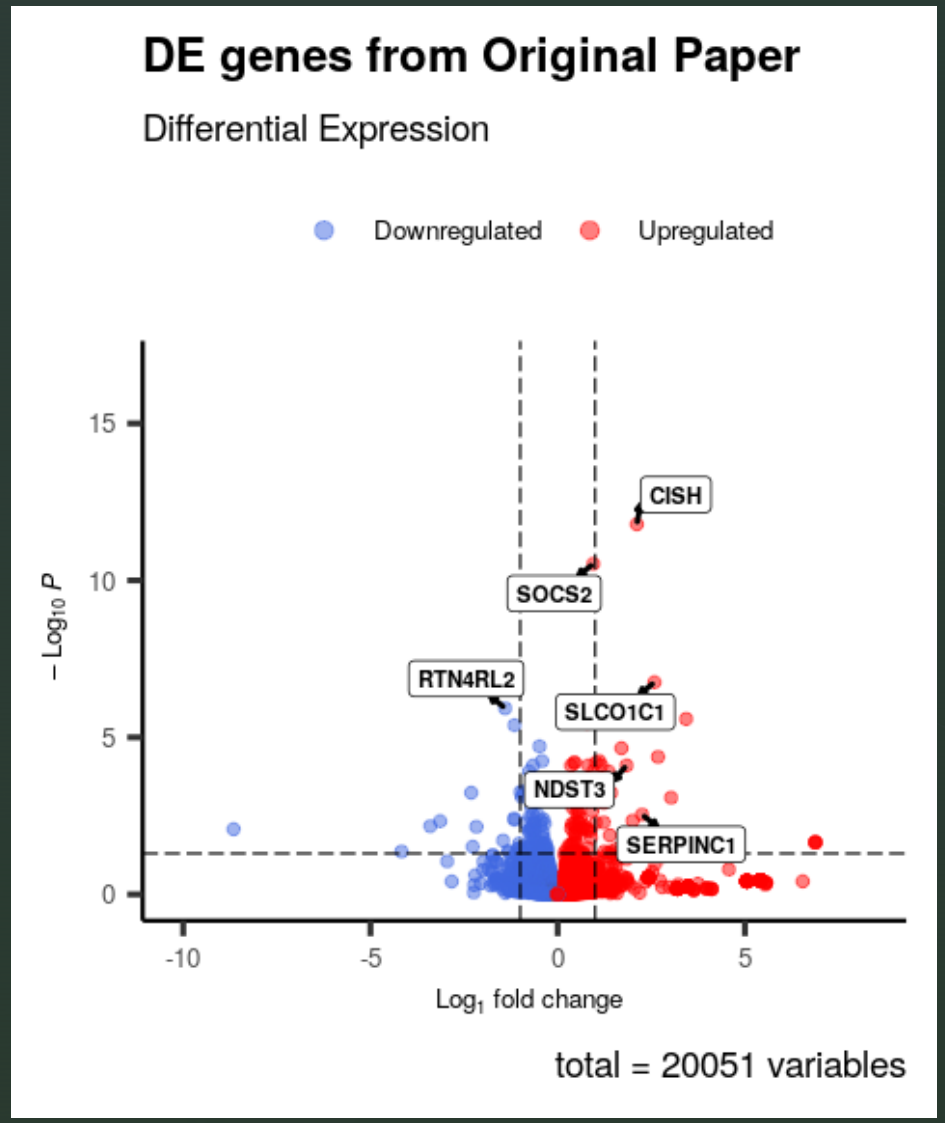
Description of DE Design

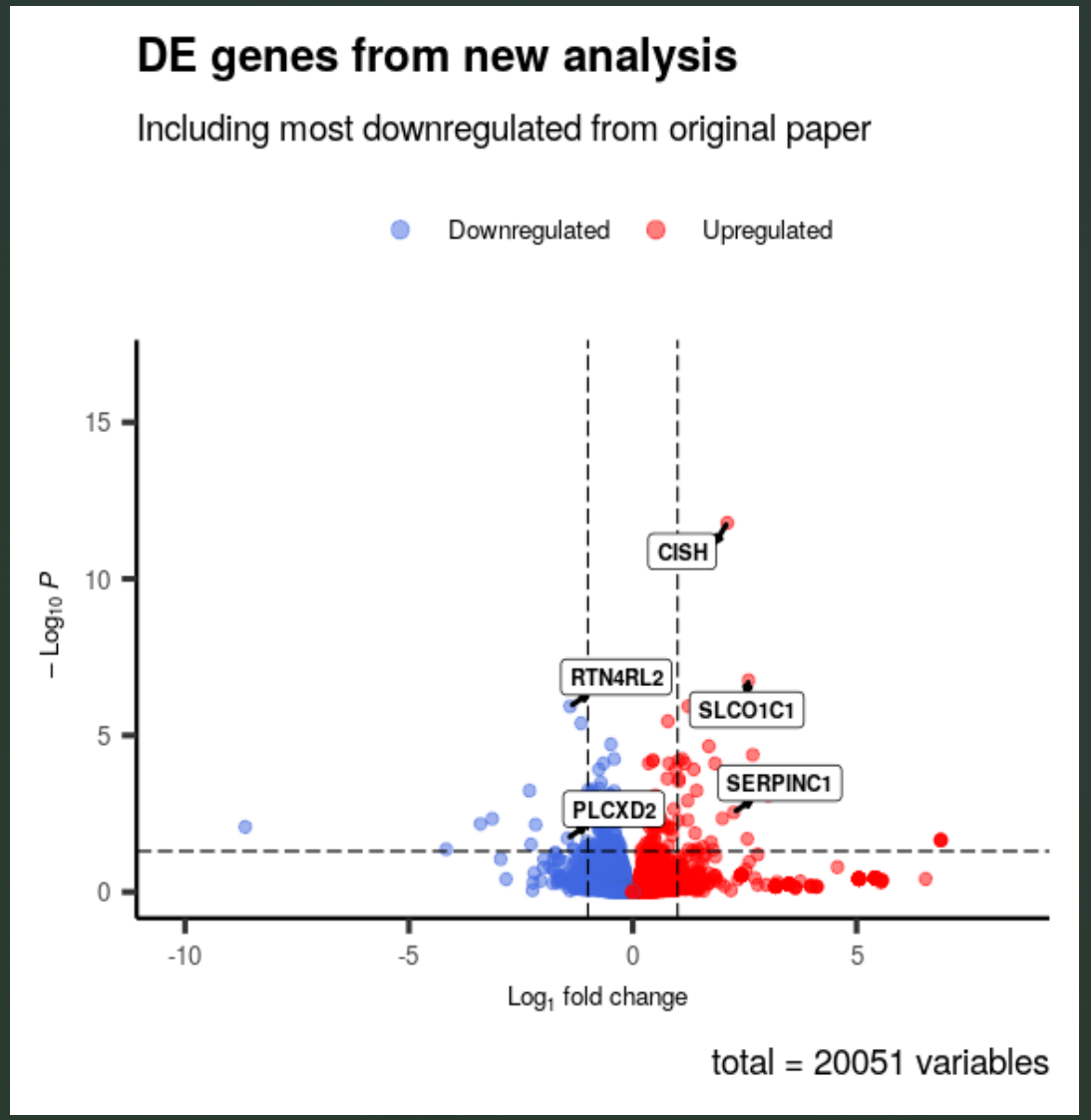


PCA Plot

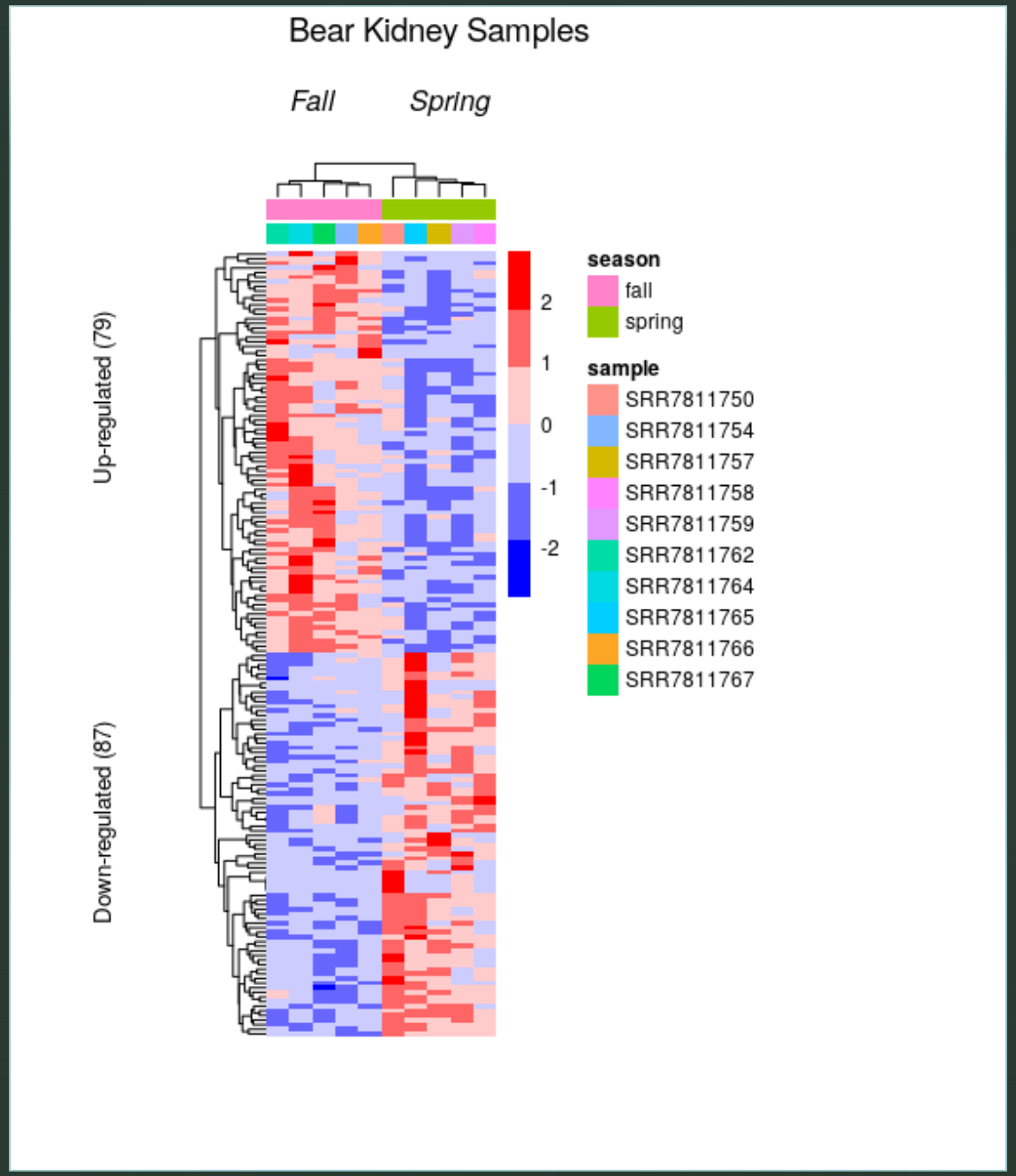

20
10
0
-10
-20
PC2: 21% variance
-20
-10
0
10
20
PC1: 26% variance
season
fall
spring
sex
female
male

Volcano plots



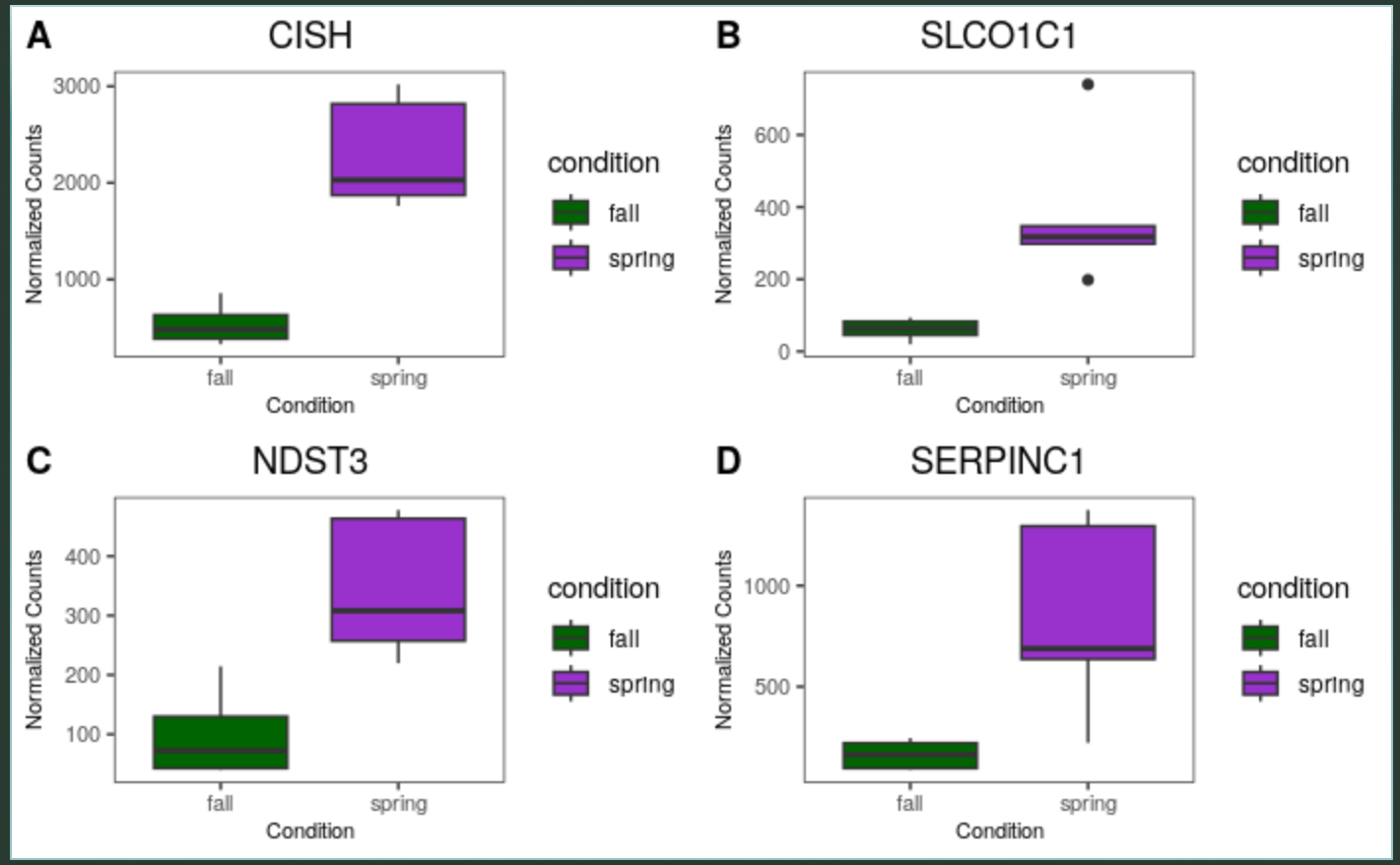


Heatmap


Bear Kidney Samples
Fall
Spring
Up-regulated (79)
Down-regulated (87)
2
1
0
-1
-2
season
fall
spring
sample
SRR7811750
SRR7811754
SRR7811757
SRR7811758
SRR7811759
SRR7811762
SRR7811764
SRR7811765
SRR7811766
SRR7811767

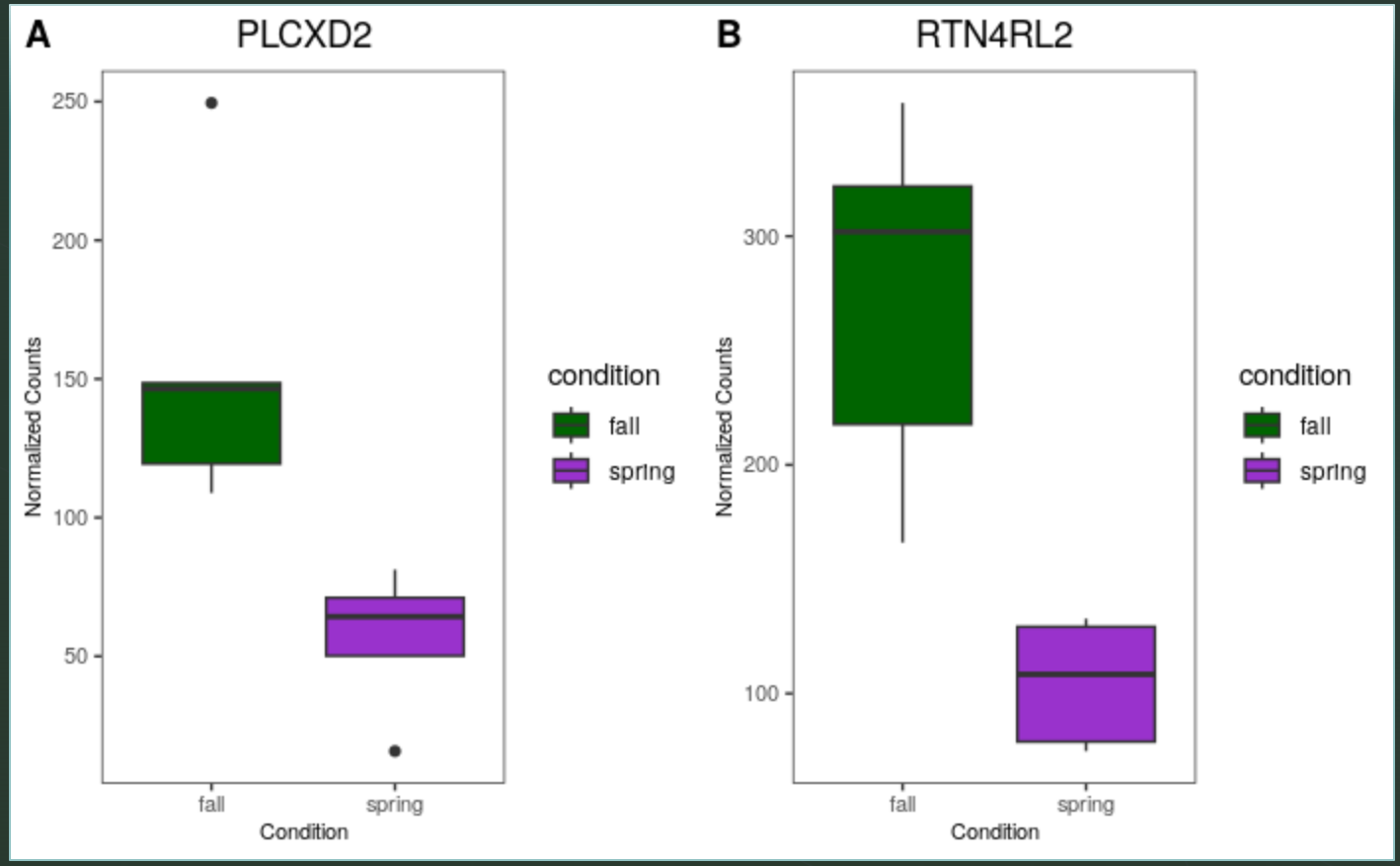
Box Plots for Upregulated Genes

- CISH: cytokine suppression
- SLCO1C1: Na uptake thyroid
- NDST3: heparin sulfate
- SERPINC1: inhibits thrombin



Box Plots for Downregulated Genes

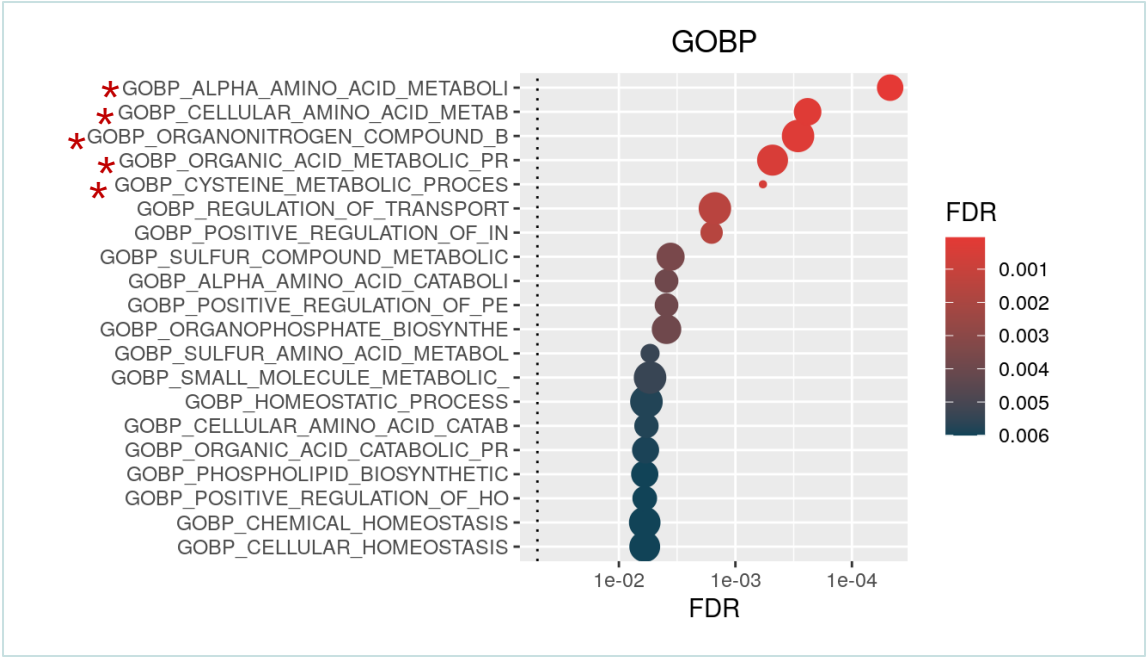
- PLCXD2: lipid catabolism
- RTN4RL2: signaling receptor pathway

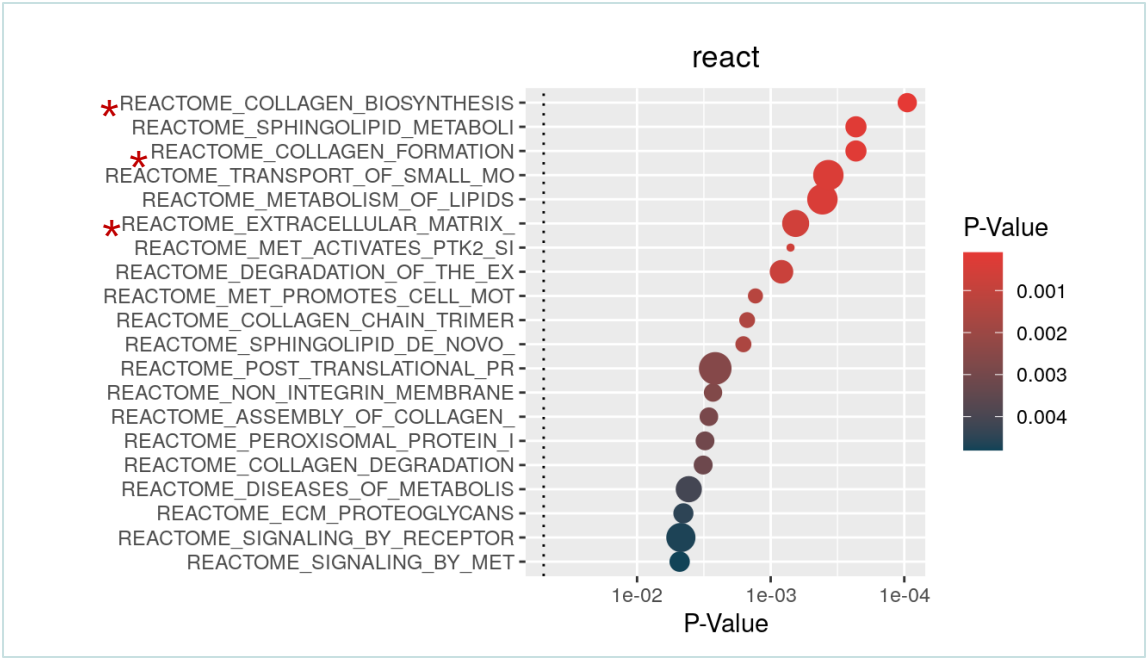


Gene Ontology Pathway

Upregulated: AGXT

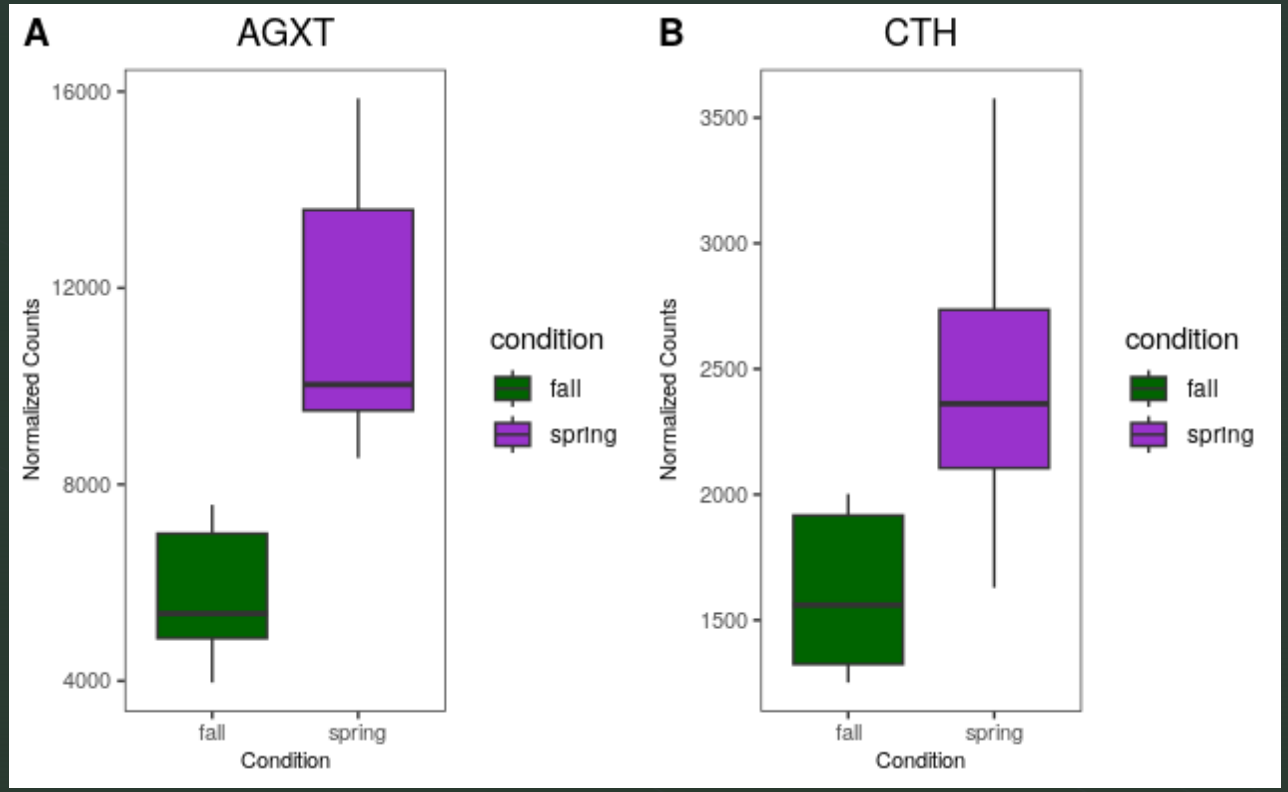
Downregulated: PLOD3





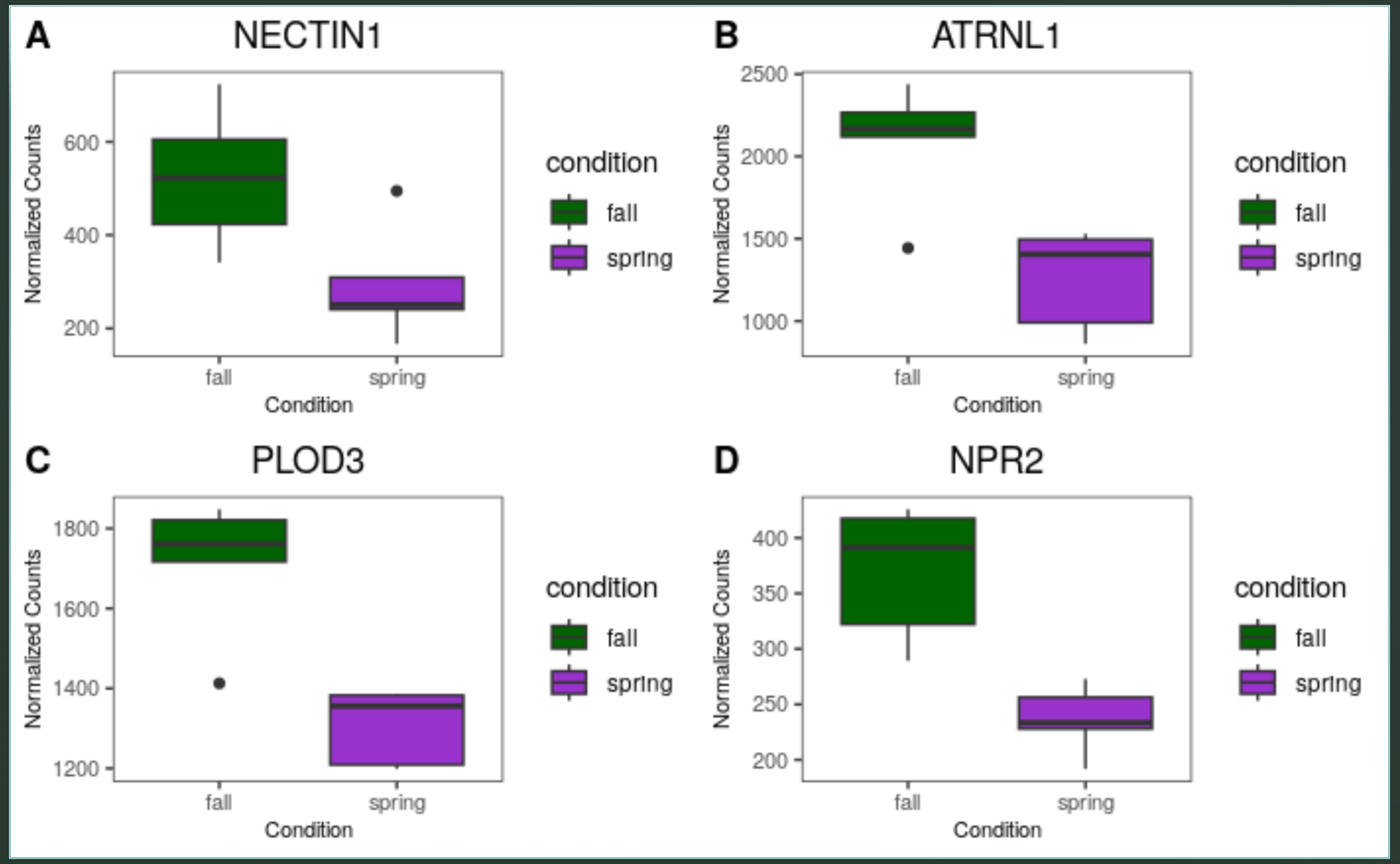
Box Plots for Upregulated Pathway Genes

- AGXT: alanine-glyoxylate transaminase
- CTH: cytoplasmic enzyme Met -> Cys

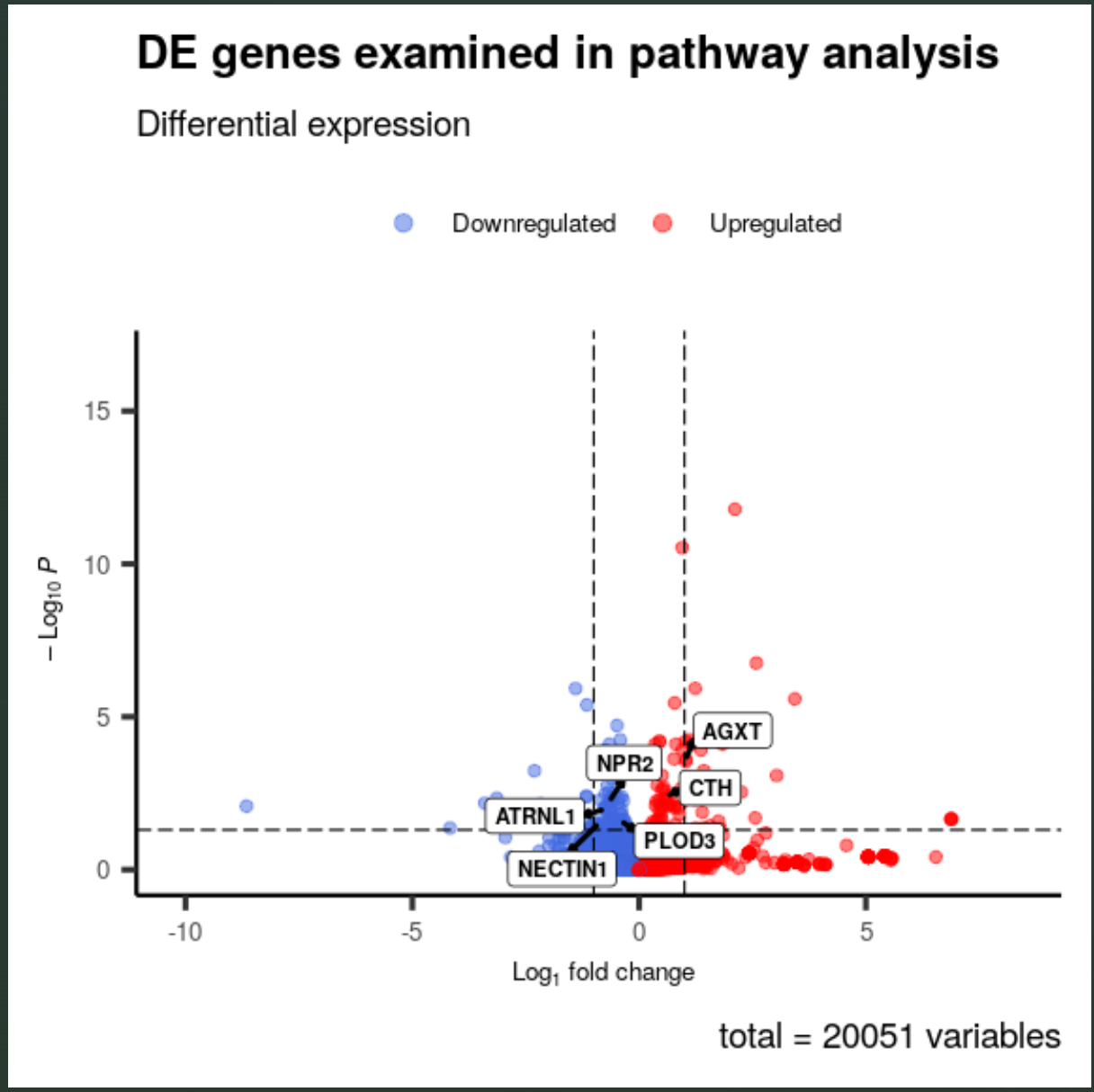


Box Plots for Downregulated Pathway Genes

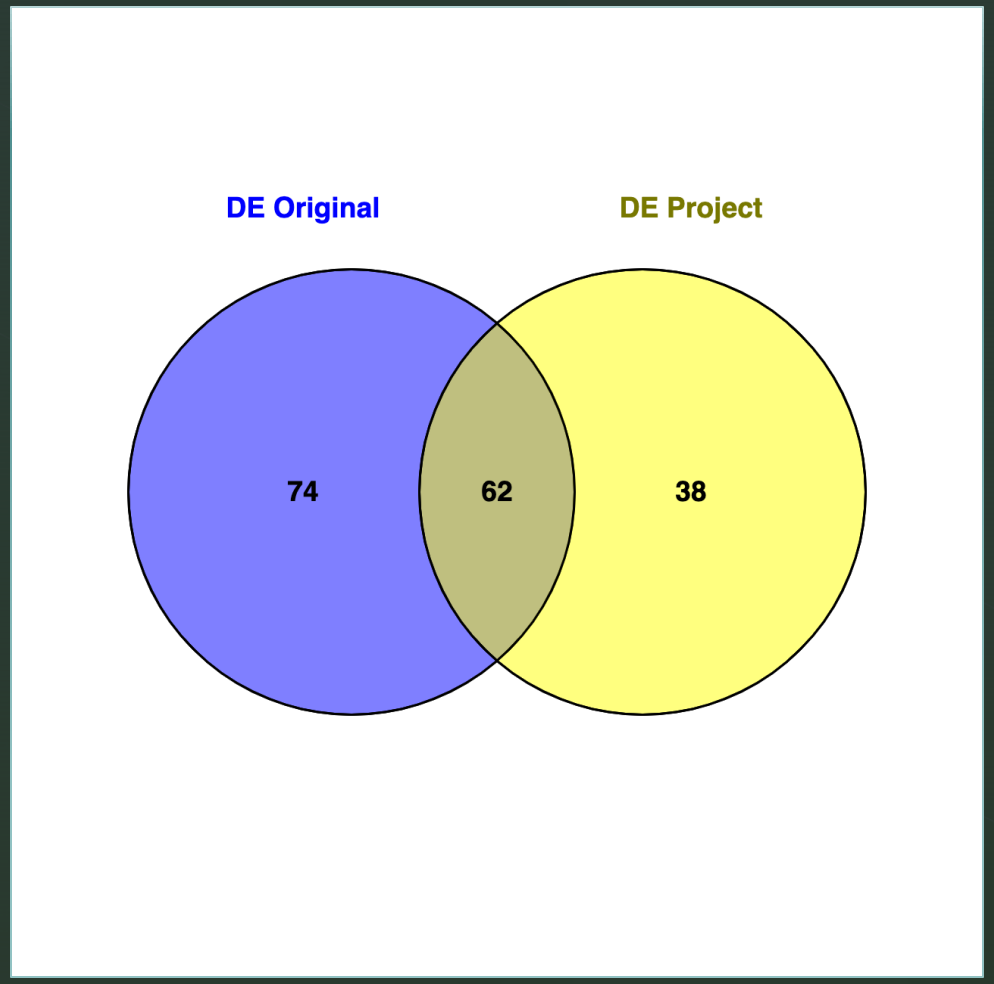
- NECTIN1: adhesion protein
- ATRNL1: carbohydrate binding
- PLOD3: hydroxylation in lysyl residues in collagen
- NPR2: natriuretic peptides



Volcano Plot



Venn Diagram between DE genes from original and current analysis

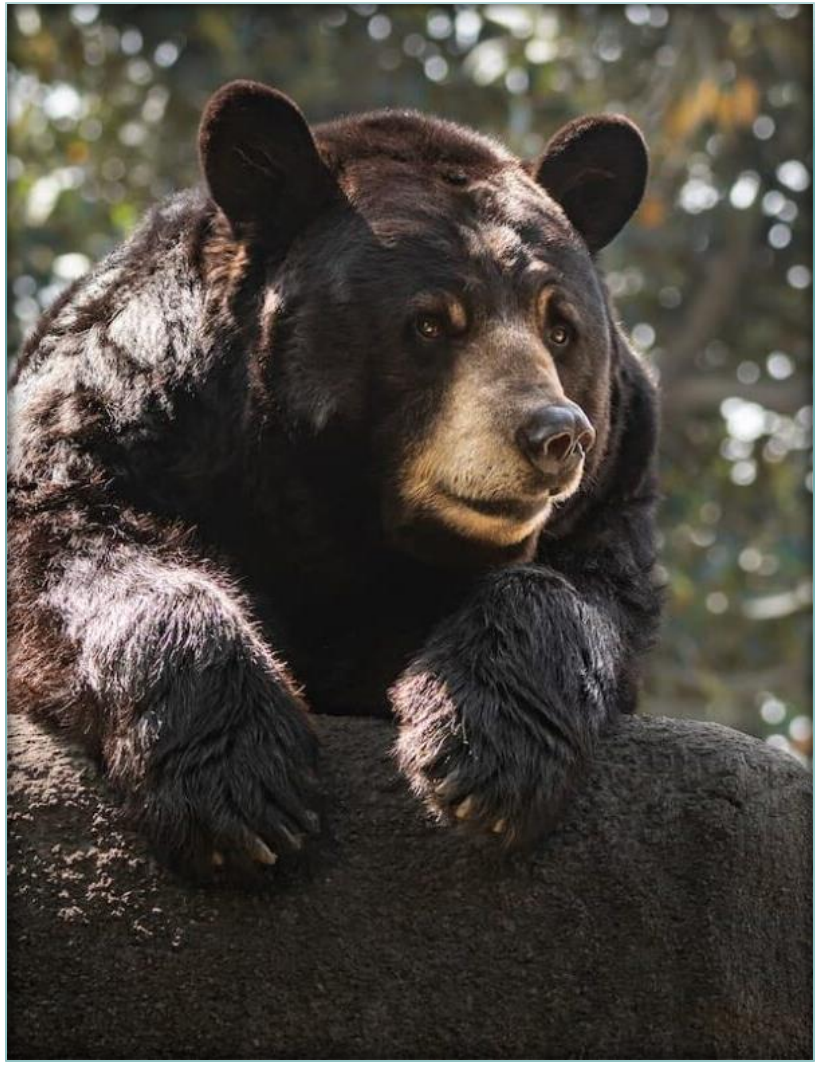


Discrepancies

- Age of bears not taken into account
- Lack of information about mother bears
- Time frame in sample collection post-hibernation (spring)
- Food availability in the north east could have affected activity levels
- Use of different aligners and genomes

Summary

- Significant differences in number of DEGs
  - Original: 169 – 68 up, 101 down
  - Project: 166 – 79 up, 87 down
- Analysis in wildlife requires navigation through different organisms
- No direct cardiac pathways associated with DEGs in kidney post-hibernation
- Several metabolic processes were connected to kidney genes, particularly regarding amino acids



Conclusions

- Differences in fall and spring bear expression profiles is minimal
  - Could be caused by several factors with data collection and analysis
- Authors next steps
  - Conduct more studies to see how the DEGs identified play a role in the kidney
- Further studies concerning the manipulation of amino acids with effect on kidney functionality could be performed due to large pathway presence

References

Center for Disease Control and Prevention. (2022). Chronic Kidney Disease Basics. CDC. https://www.cdc.gov/kidneydisease/basics.html#print

Genestack. (2016). How to…choose a reference genome? Genestack. https://genestack.com/blog/2016/07/12/choosing-a-reference-genome/

National Institute of Diabetes and Digestive and Kidney Diseases. (2016). Heart Disease & Kidney Disease. National Institute of Health. https://www.niddk.nih.gov/health-information/kidney-disease/heart-disease

NCBI. ATRNL1. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/26033

NCBI. AGXT. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2023 – [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/11611

NCBI. CTH. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/1491

NCBI. NECTIN1. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/5818

NCBI. NPR2 (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/4882

NCBI. PLCXD2. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=257068

NCBI. PLOD3. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/8985

NCBI. RTN4RL2. (2023). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2023 – [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/349667

NCBI. SERPINC1. (2023) Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2023 – [cited 2023 April 27]. https://www.ncbi.nlm.nih.gov/gene/462

North American Bear Center. (2023). 5 Stages of Activity and Hibernation. North American Bear Center. https://bear.org/5-stages-of-activity-and-hibernation/

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html

Patel RK, Jain M (2012). NGS QC Toolkit: A toolkit for quality control of next generation sequencing data. PLoS ONE 7(2): e30619. https://github.com/mjain-lab/NGSQCToolkit

Srivastava, A., Kumar Sarsani, V., Fiddes, I., Sheehan, S. M., Seger, R. L., Barter, M. E., Neptune-Bear, S., Lindqvist, C., & Korstanje, R. (2019). Genome assembly and gene expression in the American black bear provides new insights into the renal response to hibernation. *DNA research : an international journal for rapid publication of reports on genes and genomes*, *26*(1), 37–44. https://doi.org/10.1093/dnares/dsy036

The University of Edinburgh Natural History Collections. (2007). Natural History Collections: Ursidae. The University of Edinburgh. http://www.nhc.ed.ac.uk/index.php?page=493.172.289.2

Thank you