

CS509 - Project 2 - Extra Credit Tasks

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Extra Credit Task E1 - Interpretation of top genes

The tables below summarize our top five DEGs for each effect according to either the padj value or the log-fold change value. Common genes between our results and those discovered by the authors of the original project are highlighted in blue (Han et al 2022).

Gene.Id	Name	Details	Paper Mention
ENSMUSG00000069045.12	Ddx3y	Y-linked	participating in known sex-determination processes
ENSMUSG00000086503.5	Xist	X-inactivation	participating in known sex-determination processes
ENSMUSG00000068457.15	Uty	Y-linked	
ENSMUSG00000056673.15	Kdm5d	Y Chromosome	
ENSMUSG00000000787.13	Ddx3x	X Chromosome	

Figure 1: Top 5 DEGs for Sex Effect by Padj Value

Gene.Id	Name	Details	Paper Mention
ENSMUSG00000030862.14	Cpxm2	carboxypeptidase X	negative sex-by-age interactions
ENSMUSG00000029330.9	Cds1	CDP-diacylglycerol synthase 1	
ENSMUSG00000076612.9	Ighg2c	immunoglobulin	
ENSMUSG00000055489.9	Ano5	enable chloride channel activity	
ENSMUSG00000020333.18	Acsl6	acyl-CoA synthetase	

Figure 2: Top 5 DEGs for Aging Effect by Padj Value

Gene.Id	Name	Details	Paper Mention
ENSMUSG00000022797.17	Tfrc	transferrin receptor	negative sex-by-age interaction
ENSMUSG00000030862.14	Cpxm2	carboxypeptidase X	negative sex-by-age interactions
ENSMUSG00000030889.15	Vwa3a	von Willebrand factor	
ENSMUSG00000040283.15	Btln9	butyrophilin-like 9	
ENSMUSG00000020333.18	Acsl6	acyl-CoA synthetase	

Figure 3: Top 5 DEGs for Age-Sex Interaction Effect by Padj Value

Gene.Id	Name	Abs (logfoldch)	Details	Paper Mention
ENSMUSG00000086503.5	Xist	9.27405	X-inactivation	participating in known sex-determination processes
ENSMUSG00000069049.12	Eif2s3y	9.60106	Y-linked	participating in known sex-determination processes
ENSMUSG00000056673.15	Kdm5d	10.20324	Y Chromosome	
ENSMUSG00000068457.15	Uty	10.26482	Y-linked	
ENSMUSG00000069045.12	Ddx3y	10.60469	Y-linked	participating in known sex-determination processes

Figure 4: Top 5 DEGs for Sex Effect by Log-fold Change Value

Gene.Id	Name	abs(logfoldch)	Details	Paper Mention
ENSMUSG00000030046.7	Bmp10	4.12404	bone morphogenetic protein 10	
ENSMUSG00000035186.7	Ubd	4.19087	ubiquitin D	
ENSMUSG00000031495.9	Cd209d	4.32299	CD209d antigen	aging
ENSMUSG00000079190.4	-	5.33078		
ENSMUSG00000045967.12	Gpr158	5.59113	G protein-coupled receptor	

Figure 5: Top 5 DEGs for Aging Effect by Log-fold Change Value

Gene.Id	Name	abs(logfoldch)	Details	Paper Mention
ENSMUSG00000022797.17	Tfrc	1.76148	transferrin receptor	negative sex-by-age interactions
ENSMUSG00000050359.8	Sprr1a	2.3341	small proline rich protein 1A	induced in aging more highly in males than in females
ENSMUSG00000055333.15	Fat2	2.77873	calcium ion binding	
ENSMUSG00000039913.13	Pak5	2.94676	learning or memory	
ENSMUSG00000045967.12	Gpr158	3.8482	G protein-coupled receptor	

Figure 6: Top 5 DEGs for Sex-Age Interaction Effect by Log-fold Change Value

E1.1 Comparison of Top DEGs to Paper

When we compare our top DEGs for each effect to the original project paper, we find that our results are consistent with the findings of the author. For each of the three effects, whether by padj or log-fold change, we had at least 1 top gene in common that was mentioned specifically in the paper.

E1.2 Biological Interpretation

In order to determine a wider overview of the biological processes that our top DEGs are involved in, we determine the Gene Ontology (GO) terms using the Generic Gene Ontology Term Mapper (Lewis-Sigler Institute for Integrative Genomics, Princeton University). The top 5 DEGs sorted by padj value and log-fold change value were combined in order to capture more terms.

AGE

When we combine the top 5 DEGs by Age Effect by padj and logfold change values, the gene ontology (GO) terms include cell differentiation, anatomical structure development, defense response, immune response, signaling, transport, and metabolic processes. This is consistent with the paper, where they say “that sex-differential genes appear to primarily cluster around metabolic pathways” (Han et al 2022).

It is also important to consider that many genes function in multiple pathways, for example, Bmp10 (bone morphogenetic protein-10) sounds out of place in the heart. In fact, it has been found to be critical for cardiomyocyte proliferation (Sun et al 2014), a process that is necessary for cardiac repair after stress or injury and likely becomes less robust as age increases.

Both the original author’s results and ours confirm *Cd209d* as a DEG in older hearts, which functions as a receptor on macrophages and dendritic cells to recognize infectious agents. Another study shows that many of the DEGs in aging hearts are related to immune reactions and are often upregulated due to high protein turnover due to cellular damage (Bartling et al 2019).

Gpr158 was found to be upregulated in older hearts, which is interesting as members of the PAK family have been implicated in many age-related diseases (Amirthalingam et al 2021). Gpr158 represents a g-coupled protein receptor that is involved in pathways related to age-related memory loss (Kosmidis et al 2018). *Cds1* is an enzyme that typically functions downstream of g-coupled proteins, and has been found to be critical to metabolism pathways in the mitochondria, which are very dense in heart tissue.

Ighg2c (immunoglobulin heavy constant gamma 2C) function in antigen binding activity, upstream of antibacterial response pathways.

Ano5 enables chloride channel activity and is associated with skeletal muscle. According to ‘Alliance of Genome Resources’, “One type of homozygous KO causes abnormalities in skeletal muscle mitochondria and impairs muscle regeneration and repair, leading to exercise intolerance [provided by MGI curators].”

GO Terms from the biological_process Ontology			
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
cell differentiation (GO:0030154)	Acs16, Bmp10, Cds1, Ubd	4 of 9 genes, 44.44%	4802 of 21078 annotated genes, 22.78%
anatomical structure development (GO:0048856)	Acs16, Bmp10, Gpr158, Ubd	4 of 9 genes, 44.44%	6561 of 21078 annotated genes, 31.13%
defense response to other organism (GO:0098542)	Cd209d, Ighg2c, Ubd	3 of 9 genes, 33.33%	1343 of 21078 annotated genes, 6.37%
immune system process (GO:0002376)	Cd209d, Ighg2c, Ubd	3 of 9 genes, 33.33%	3126 of 21078 annotated genes, 14.83%
signaling (GO:0023052)	Bmp10, Gpr158, Ubd	3 of 9 genes, 33.33%	7189 of 21078 annotated genes, 34.11%
transmembrane transport (GO:0055085)	Acs16, Ano5	2 of 9 genes, 22.22%	1426 of 21078 annotated genes, 6.77%
lipid metabolic process (GO:0006629)	Acs16, Cds1	2 of 9 genes, 22.22%	1448 of 21078 annotated genes, 6.87%
sulfur compound metabolic process (GO:0006790)	Acs16	1 of 9 genes, 11.11%	303 of 21078 annotated genes, 1.44%
mitotic cell cycle (GO:0000278)	Ubd	1 of 9 genes, 11.11%	881 of 21078 annotated genes, 4.18%
muscle system process (GO:0003012)	Bmp10	1 of 9 genes, 11.11%	452 of 21078 annotated genes, 2.14%
regulation of DNA-templated transcription (GO:0006355)	Bmp10	1 of 9 genes, 11.11%	3290 of 21078 annotated genes, 15.61%
vesicle-mediated transport (GO:0016192)	Cd209d	1 of 9 genes, 11.11%	1567 of 21078 annotated genes, 7.43%
cell motility (GO:0048870)	Bmp10	1 of 9 genes, 11.11%	1873 of 21078 annotated genes, 8.89%
nervous system process (GO:0050877)	Gpr158	1 of 9 genes, 11.11%	2465 of 21078 annotated genes, 11.69%
protein localization to plasma membrane (GO:0072659)	Gpr158	1 of 9 genes, 11.11%	318 of 21078 annotated genes, 1.51%
cell junction organization (GO:0034330)	Gpr158	1 of 9 genes, 11.11%	852 of 21078 annotated genes, 4.04%
protein catabolic process (GO:0030163)	Ubd	1 of 9 genes, 11.11%	1028 of 21078 annotated genes, 4.88%
carbohydrate derivative metabolic process (GO:1901135)	Acs16	1 of 9 genes, 11.11%	1019 of 21078 annotated genes, 4.83%
cell adhesion (GO:0007155)	Bmp10	1 of 9 genes, 11.11%	1551 of 21078 annotated genes, 7.36%
programmed cell death (GO:0012501)	Ubd	1 of 9 genes, 11.11%	2164 of 21078 annotated genes, 10.27%
circulatory system process (GO:0003013)	Bmp10	1 of 9 genes, 11.11%	585 of 21078 annotated genes, 2.78%
protein maturation (GO:0051604)	Cpxm2	1 of 9 genes, 11.11%	526 of 21078 annotated genes, 2.50%
nucleobase-containing small molecule metabolic process (GO:0055086)	Acs16	1 of 9 genes, 11.11%	574 of 21078 annotated genes, 2.72%
cytoskeleton organization (GO:0007010)	Bmp10	1 of 9 genes, 11.11%	1498 of 21078 annotated genes, 7.11%

Figure 7: GO Terms for Age Effect (Top Padj and Logfold Change)

SEX

As expected, all of the DEGs determined for the effect of sex are linked to either the X or Y chromosome. The gene ontology (GO) terms include reproductive process, cell differentiation, chromatin organization, regulation of DNA-templated transcription, immune processes, etc. These results indicate that the regulation of transcription differs between the sexes, which subsequently affects cell signaling pathways. There may be differences in the immune system reactions as well, which complicates the study of aging-related diseases.

Ddx3x and *Ddx3y* are DEAD-box RNA helicases encoded by the X and Y chromosomes, respectively. They also seem to be involved in stress response, as they are components of RNA-protein complexes (stress granules) that indicate changes in mRNA metabolism, such as translational repression (Shen et al 2022). According to the GO term analysis, they are also involved in immune response. These underlying differences in metabolism and immune response highlight fundamental differences of how the sexes differ in their susceptibility to disease and aging.

Xist is a long non-coding RNA that regulates X chromosome inactivation. Since females receive two XX chromosomes and males only receive one, the dosage is compensated by transcriptionally silencing one of the female's X chromosomes. Many diseases are associated with X chromosome silencing errors including autoimmune thyroid diseases, bipolar disorder and depression (Chabchoub et al 2009, Ji et al 2015).

Uty (ubiquitously transcribed tetratricopeptide repeat containing, Y-linked) appears to be involved in muscle system processes and anatomical structure development according to the GO terms. One study shows that this gene in males actually helps protect them from the progression of pulmonary hypertension (Cunningham et al 2022).

In relation to heart disease, there are many sex-related differences in the diagnosis and treatment of disease. One study found that “heart failure disproportionately contributes to coronary heart disease mortality in women, potentially due to undiagnosed ischaemic heart disease in women. The strength of the association with cardiovascular risk factors differ by sex.” (Snyder et al 2014). There are also differences in treatments for cardiac failure, where “evidence suggests that optimal survival in women occurs with lower doses of blockers, angiotensin receptor blockers, and angiotensin converting enzyme inhibitors than in men” (Santema et al 2019).

GO Terms from the biological_process Ontology			
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
reproductive process (GO:0022414)	<i>Ddx3x, Ddx3y, Xist</i>	3 of 6 genes, 50.00%	1685 of 21078 annotated genes, 7.99%
cell differentiation (GO:0030154)	<i>Ddx3x, Ddx3y, Xist</i>	3 of 6 genes, 50.00%	4802 of 21078 annotated genes, 22.78%
chromatin organization (GO:0006325)	<i>Kdm5d, Uty, Xist</i>	3 of 6 genes, 50.00%	673 of 21078 annotated genes, 3.19%
regulation of DNA-templated transcription (GO:0006355)	<i>Ddx3x, Kdm5d</i>	2 of 6 genes, 33.33%	3290 of 21078 annotated genes, 15.61%
immune system process (GO:0002376)	<i>Ddx3x, Kdm5d</i>	2 of 6 genes, 33.33%	3126 of 21078 annotated genes, 14.83%
signalling (GO:0023052)	<i>Ddx3x, Kdm5d</i>	2 of 6 genes, 33.33%	7189 of 21078 annotated genes, 34.11%
protein-containing complex assembly (GO:0065003)	<i>Ddx3x, Eif2s3y</i>	2 of 6 genes, 33.33%	1535 of 21078 annotated genes, 7.28%
anatomical structure development (GO:0048856)	<i>Uty, Xist</i>	2 of 6 genes, 33.33%	6561 of 21078 annotated genes, 31.13%
mitotic cell cycle (GO:0000278)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	881 of 21078 annotated genes, 4.18%
chromosome segregation (GO:0007059)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	421 of 21078 annotated genes, 2.00%
muscle system process (GO:0003012)	<i>Uty</i>	1 of 6 genes, 16.67%	452 of 21078 annotated genes, 2.14%
defense response to other organism (GO:0098542)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	1343 of 21078 annotated genes, 6.37%
ribosome biogenesis (GO:0042254)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	321 of 21078 annotated genes, 1.52%
cytoplasmic translation (GO:0002181)	<i>Eif2s3y</i>	1 of 6 genes, 16.67%	149 of 21078 annotated genes, 0.71%
regulatory ncRNA-mediated gene silencing (GO:0031047)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	215 of 21078 annotated genes, 1.02%
inflammatory response (GO:0006954)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	858 of 21078 annotated genes, 4.07%
programmed cell death (GO:0012501)	<i>Ddx3x</i>	1 of 6 genes, 16.67%	2164 of 21078 annotated genes, 10.27%
circulatory system process (GO:0003013)	<i>Uty</i>	1 of 6 genes, 16.67%	585 of 21078 annotated genes, 2.78%

Figure 8: Go Terms for Sex Effect (Top Padj and Logfold Changes)

AGE-SEX INTERACTION

Our model explores not only the effects of age and sex on cardiac gene expression but also the possible interactions of age and sex. Due to the underlying differences between the transcriptomes of the two sexes, the expression of genes related to aging seems to be affected by sex.

The gene ontology (GO) terms include signaling, anatomical structure development, cell differentiation, immune system process, nervous system processes cell adhesion, programmed cell death, etc. Many of these genes were also found within the ‘age’ or ‘sex’ effect and were previously discussed, so here I will focus on those genes that have not yet been covered.

Btnl9 (butyrophilin-like 9) is a T-cell receptor that has been shown to be a negative regulator of immune activation. In terms of heart disease, a study of Samoan subjects shows that a

stop-mutation in *Btnl9* was associated low levels of “good” cholesterol and high levels of high triglycerides (Carleson et al 2022).

Interestingly, the cardiac gene *Tfrc* promotes iron uptake and is critical to heart function. In a recent paper, the researchers found that this gene also participates in immune processes by promoting macrophage infiltration (Pan et al 2023). This example demonstrates the hidden complexity of gene function and interactions, which may not be apparent without more research into specific genes.

GO Terms from the biological_process Ontology			
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
signalling (GO:0023052)	<i>Btnl9, Gpr158, Pak5, Tfrc</i>	4 of 8 genes, 50.00%	7189 of 21078 annotated genes, 34.11%
anatomical structure development (GO:0048856)	<i>Acsf6, Gpr158, Sprr1a, Tfrc</i>	4 of 8 genes, 50.00%	6561 of 21078 annotated genes, 31.13%
cell differentiation (GO:0030154)	<i>Acsf6, Sprr1a, Tfrc</i>	3 of 8 genes, 37.50%	4802 of 21078 annotated genes, 22.78%
immune system process (GO:0002376)	<i>Btnl9, Tfrc</i>	2 of 8 genes, 25.00%	3126 of 21078 annotated genes, 14.83%
nervous system process (GO:0050877)	<i>Gpr158, Pak5</i>	2 of 8 genes, 25.00%	2465 of 21078 annotated genes, 11.69%
cell adhesion (GO:0007155)	<i>Fat2, Tfrc</i>	2 of 8 genes, 25.00%	1551 of 21078 annotated genes, 7.36%
programmed cell death (GO:0012501)	<i>Pak5, Tfrc</i>	2 of 8 genes, 25.00%	2164 of 21078 annotated genes, 10.27%
sulfur compound metabolic process (GO:0006790)	<i>Acsf6</i>	1 of 8 genes, 12.50%	303 of 21078 annotated genes, 1.44%
regulation of DNA-templated transcription (GO:0006355)	<i>Tfrc</i>	1 of 8 genes, 12.50%	3290 of 21078 annotated genes, 15.61%
vesicle-mediated transport (GO:0016192)	<i>Tfrc</i>	1 of 8 genes, 12.50%	1567 of 21078 annotated genes, 7.43%
cell motility (GO:0048870)	<i>Fat2</i>	1 of 8 genes, 12.50%	1873 of 21078 annotated genes, 8.89%
transmembrane transport (GO:0055085)	<i>Acsf6</i>	1 of 8 genes, 12.50%	1426 of 21078 annotated genes, 6.77%
protein localization to plasma membrane (GO:0072659)	<i>Gpr158</i>	1 of 8 genes, 12.50%	318 of 21078 annotated genes, 1.51%
cell junction organization (GO:0034330)	<i>Gpr158</i>	1 of 8 genes, 12.50%	852 of 21078 annotated genes, 4.04%
protein-containing complex assembly (GO:0065003)	<i>Tfrc</i>	1 of 8 genes, 12.50%	1535 of 21078 annotated genes, 7.28%
mitochondrion organization (GO:0007005)	<i>Tfrc</i>	1 of 8 genes, 12.50%	581 of 21078 annotated genes, 2.76%
carbohydrate derivative metabolic process (GO:1901135)	<i>Acsf6</i>	1 of 8 genes, 12.50%	1019 of 21078 annotated genes, 4.83%
nucleobase-containing small molecule metabolic process (GO:0055086)	<i>Acsf6</i>	1 of 8 genes, 12.50%	574 of 21078 annotated genes, 2.72%
protein maturation (GO:0051604)	<i>Coxm2</i>	1 of 8 genes, 12.50%	526 of 21078 annotated genes, 2.50%
cytoskeleton organization (GO:0007010)	<i>Pak5</i>	1 of 8 genes, 12.50%	1498 of 21078 annotated genes, 7.11%
DNA recombination (GO:0006310)	<i>Tfrc</i>	1 of 8 genes, 12.50%	335 of 21078 annotated genes, 1.59%
lipid metabolic process (GO:0006629)	<i>Acsf6</i>	1 of 8 genes, 12.50%	1448 of 21078 annotated genes, 6.87%

Figure 9: GO Terms for Age-Sex Interaction (Top Padj and Log-Fold Change)

Extra Credit Task E2 - Differential Splicing Analysis

```
suppressWarnings({ suppressMessages({
  if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
  BiocManager::install("DEXSeq")

  library("DEXSeq")
})})
```

```
suppressWarnings({ suppressMessages({
  count_files <- c("dexseq_counts_SRR19123213_1.txt",
                  "dexseq_counts_SRR19123214_1.txt",
```



```

        "dexseq_counts_SRR19123215_1.txt",
        "dexseq_counts_SRR19123216_1.txt",
        "dexseq_counts_SRR19123217_1.txt",
        "dexseq_counts_SRR19123218_1.txt",
        "dexseq_counts_SRR19123219_1.txt",
        "dexseq_counts_SRR19123220_1.txt",
        "dexseq_counts_SRR19123221_1.txt",
        "dexseq_counts_SRR19123222_1.txt",
        "dexseq_counts_SRR19123223_1.txt",
        "dexseq_counts_SRR19123224_1.txt")

counts = list.files("/kaggle/input/mouse-reference-genome-read-counts", pattern=".txt$", f
annotation_file <- "dexseq.annotation.gff"
}))

```

Age as Condition

```

# Sample data frame with age as condition
sample_data_age <- data.frame(
  row.names = c("SRR19123213", "SRR19123214", "SRR19123215", "SRR19123216", "SRR19123217",
  condition = c("20months", "20months", "20months", "20months", "20months", "20months",
                "4months", "4months", "4months", "4months", "4months", "4months"),
  sex = c("Male", "Male", "Male", "Female", "Female", "Female",
          "Male", "Male", "Male", "Female", "Female", "Female"),
  stringsAsFactors = TRUE
)

```

```
sample_data_age
```

	condition	sex
SRR19123213	20months	Male
SRR19123214	20months	Male
SRR19123215	20months	Male
SRR19123216	20months	Female
SRR19123217	20months	Female
SRR19123218	20months	Female
SRR19123219	4months	Male
SRR19123220	4months	Male
SRR19123221	4months	Male
SRR19123222	4months	Female

SRR19123223 4months Female
SRR19123224 4months Female

```
suppressWarnings({ suppressMessages({  
  dxd_age <- DEXSeqDataSetFromHTSeq(count_files,  
                                   sample_data_age,  
                                   design = ~sample + exon + condition + condition:exon,  
                                   flattenedfile=annotation_file)  
}))})
```

```
dxd_age <- estimateSizeFactors(dxd_age)  
dxd_age <- estimateDispersions(dxd_age)
```

```
dxd_age <- testForDEU(dxd_age)
```

```
dxd_age <- estimateExonFoldChanges(dxd_age, fitExpToVar="condition")
```

Fit for gene/exon ENSMUSG000000074218.4 threw the next warning(s): Too much damping - converg

```
results_age <- DEXSeq(dxd_age)
```

Warning in MulticoreParam(workers = 1): MulticoreParam() not supported on
Windows, use SnowParam()

Fit for gene/exon ENSMUSG000000074218.4 threw the next warning(s): Too much damping - converg

```
head(results_age)
```

LRT p-value: full vs reduced

DataFrame with 6 rows and 16 columns

	groupID	featureID	exonBaseMean
	<character>	<character>	<numeric>
ENSMUSG000000000001.5:E001	ENSMUSG000000000001.5	E001	1562.845
ENSMUSG000000000001.5:E002	ENSMUSG000000000001.5	E002	282.580
ENSMUSG000000000001.5:E003	ENSMUSG000000000001.5	E003	216.765

ENSMUSG000000000001.5:E004	ENSMUSG000000000001.5	E004	203.348
ENSMUSG000000000001.5:E005	ENSMUSG000000000001.5	E005	183.888
ENSMUSG000000000001.5:E006	ENSMUSG000000000001.5	E006	227.077
	dispersion	stat	pvalue
	<numeric>	<numeric>	<numeric>
ENSMUSG000000000001.5:E001	0.00231691	0.78121402	0.376770
ENSMUSG000000000001.5:E002	0.00274410	0.31236251	0.576234
ENSMUSG000000000001.5:E003	0.00289297	0.13127477	0.717114
ENSMUSG000000000001.5:E004	0.00245552	0.04544665	0.831185
ENSMUSG000000000001.5:E005	0.00720102	0.38957545	0.532523
ENSMUSG000000000001.5:E006	0.00328350	0.00564288	0.940120
	padj	X20months	
	<numeric>	<numeric>	
ENSMUSG000000000001.5:E001	0.900461	47.7533	
ENSMUSG000000000001.5:E002	0.947336	24.7806	
ENSMUSG000000000001.5:E003	0.970188	21.7553	
ENSMUSG000000000001.5:E004	0.985122	21.3485	
ENSMUSG000000000001.5:E005	0.938713	20.6063	
ENSMUSG000000000001.5:E006	0.996453	22.3230	
	X4months	log2fold_4months_20months	X20months.1
	<numeric>		<numeric>
ENSMUSG000000000001.5:E001	48.1628	0.0369102	47.7533
ENSMUSG000000000001.5:E002	24.5279	-0.0337580	24.7806
ENSMUSG000000000001.5:E003	22.0045	0.0365528	21.7553
ENSMUSG000000000001.5:E004	21.2674	-0.0121376	21.3485
ENSMUSG000000000001.5:E005	20.1635	-0.0688023	20.6063
ENSMUSG000000000001.5:E006	22.4161	0.0134187	22.3230
	X4months.1	log2fold_4months_20months.1	
	<numeric>		<numeric>
ENSMUSG000000000001.5:E001	48.1628	0.0369102	
ENSMUSG000000000001.5:E002	24.5279	-0.0337580	
ENSMUSG000000000001.5:E003	22.0045	0.0365528	
ENSMUSG000000000001.5:E004	21.2674	-0.0121376	
ENSMUSG000000000001.5:E005	20.1635	-0.0688023	
ENSMUSG000000000001.5:E006	22.4161	0.0134187	
	genomicData	countData	
	<GRanges>	<matrix>	
ENSMUSG000000000001.5:E001	chr3:108014596-108016632:-	1708:1549:1205:...	
ENSMUSG000000000001.5:E002	chr3:108016719-108016928:-	311:256:213:...	
ENSMUSG000000000001.5:E003	chr3:108019251-108019404:-	233:213:182:...	
ENSMUSG000000000001.5:E004	chr3:108019789-108019918:-	250:190:163:...	
ENSMUSG000000000001.5:E005	chr3:108023079-108023207:-	224:185:120:...	
ENSMUSG000000000001.5:E006	chr3:108025617-108025774:-	218:238:185:...	
	transcripts		
	<list>		
ENSMUSG000000000001.5:E001	ENSMUST000000000001.5		
ENSMUSG000000000001.5:E002	ENSMUST000000000001.5		
ENSMUSG000000000001.5:E003	ENSMUST000000000001.5		
ENSMUSG000000000001.5:E004	ENSMUST000000000001.5		
ENSMUSG000000000001.5:E005	ENSMUST000000000001.5		
ENSMUSG000000000001.5:E006	ENSMUST000000000001.5		

```
# Extract significant genes for each factor
significant_aging <- results_age[which(!is.na(results_age$padj) & results_age$padj < 0.05)]

# Get the counts
n_significant_aging <- nrow(significant_aging)

# Print the results
cat("Number of significant genes for Aging effect:", n_significant_aging, "\n")
```

Number of significant genes for Aging effect: 146

```
top_age <- head(significant_aging[order(significant_aging$padj), ], 5)
top_age
```

LRT p-value: full vs reduced

DataFrame with 5 rows and 16 columns

	groupID	featureID	exonBaseMean	
	<character>	<character>	<numeric>	
ENSMUSG00000005371.16:E027	ENSMUSG00000005371.16	E027	54.3254	
ENSMUSG000000028796.18:E011	ENSMUSG000000028796.18	E011	235.7523	
ENSMUSG00000005371.16:E026	ENSMUSG00000005371.16	E026	95.3709	
ENSMUSG000000110279.2:E002	ENSMUSG000000110279.2	E002	8776.4410	
ENSMUSG000000023353.15:E001	ENSMUSG000000023353.15	E001	74.5237	
	dispersion	stat	pvalue	padj
	<numeric>	<numeric>	<numeric>	<numeric>
ENSMUSG00000005371.16:E027	0.02625119	82.0257	1.34330e-19	2.86809e-14
ENSMUSG000000028796.18:E011	0.02052946	59.4189	1.27437e-14	1.36046e-09
ENSMUSG00000005371.16:E026	0.01946756	58.0127	2.60425e-14	1.39009e-09
ENSMUSG000000110279.2:E002	0.00347529	58.2270	2.33548e-14	1.39009e-09
ENSMUSG000000023353.15:E001	0.02229010	53.5518	2.51864e-13	1.07551e-08
	X20months	X4months	log2fold_4months_20months	
	<numeric>	<numeric>		<numeric>
ENSMUSG00000005371.16:E027	6.83875	14.4022		2.204110
ENSMUSG000000028796.18:E011	17.27685	26.2896		1.343807
ENSMUSG00000005371.16:E026	11.05400	17.8251		1.445511
ENSMUSG000000110279.2:E002	78.88413	76.6833		-0.175532
ENSMUSG000000023353.15:E001	9.67395	15.9598		1.499727
	X20months.1	X4months.1	log2fold_4months_20months.1	

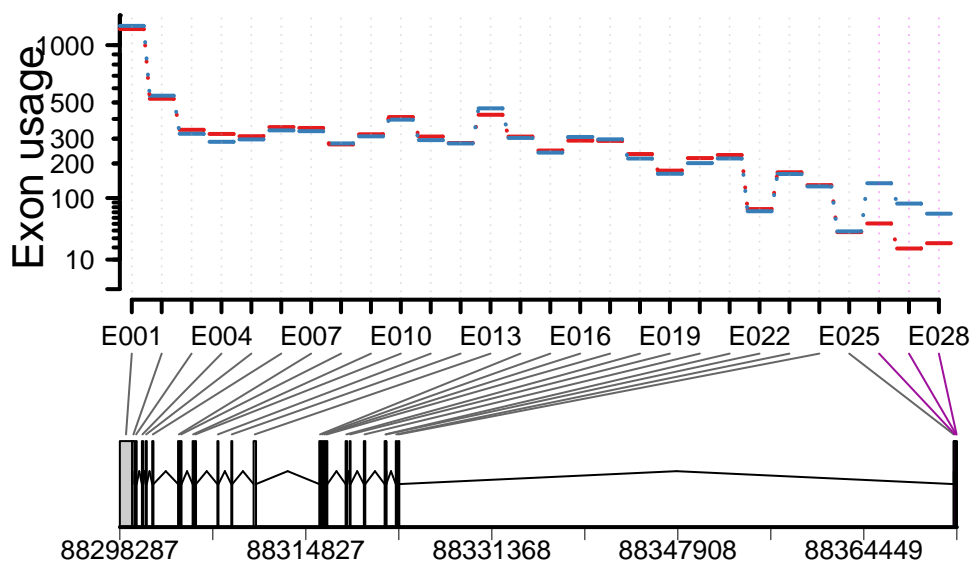
	<numeric>	<numeric>	<numeric>
ENSMUSG00000005371.16:E027	6.83875	14.4022	2.204110
ENSMUSG000000028796.18:E011	17.27685	26.2896	1.343807
ENSMUSG00000005371.16:E026	11.05400	17.8251	1.445511
ENSMUSG000000110279.2:E002	78.88413	76.6833	-0.175532
ENSMUSG000000023353.15:E001	9.67395	15.9598	1.499727
	genomicData		countData
	<GRanges>		<matrix>
ENSMUSG00000005371.16:E027	chr17:88372646-88372671:-		13:27:12:...
ENSMUSG000000028796.18:E011	chr4:128621378-128621663:+		113:136:97:...
ENSMUSG00000005371.16:E026	chr17:88372457-88372645:-		39:76:30:...
ENSMUSG000000110279.2:E002	chr7:44772487-44772644:+	8242:8503:8190:...	
ENSMUSG000000023353.15:E001	chr5:24657175-24657204:+		27:51:34:...
	transcripts		
	<list>		
ENSMUSG00000005371.16:E027	ENSMUST00000005504.15,ENSMUST00000235112.2		
ENSMUSG000000028796.18:E011	ENSMUST00000106079.10		
ENSMUSG00000005371.16:E026	ENSMUST00000005504.15		
ENSMUSG000000110279.2:E002	ENSMUST00000209340.2		
ENSMUSG000000023353.15:E001	ENSMUST00000024123.9		

```

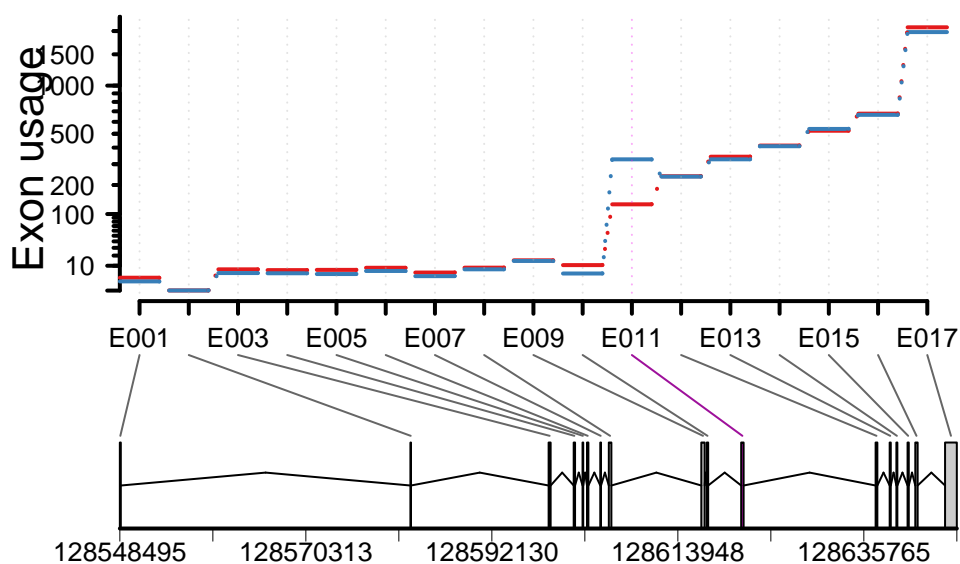
for (id in unique(top_age$groupID)){
  plotDEXSeq(results_age, id, legend=TRUE, expression=FALSE, splicing=TRUE, cex.axis=1.2)
}

```

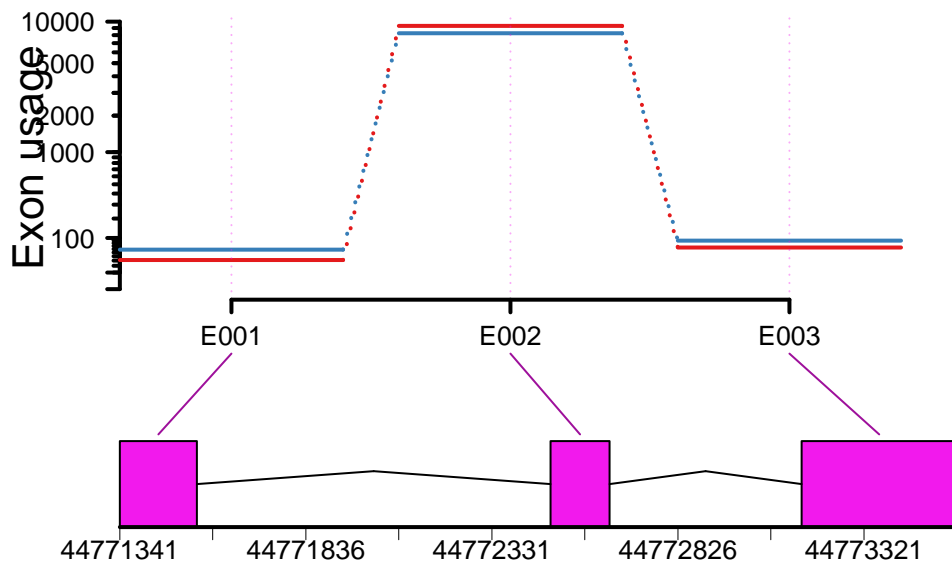
ENSMUSG00000005371.16 20months 4months



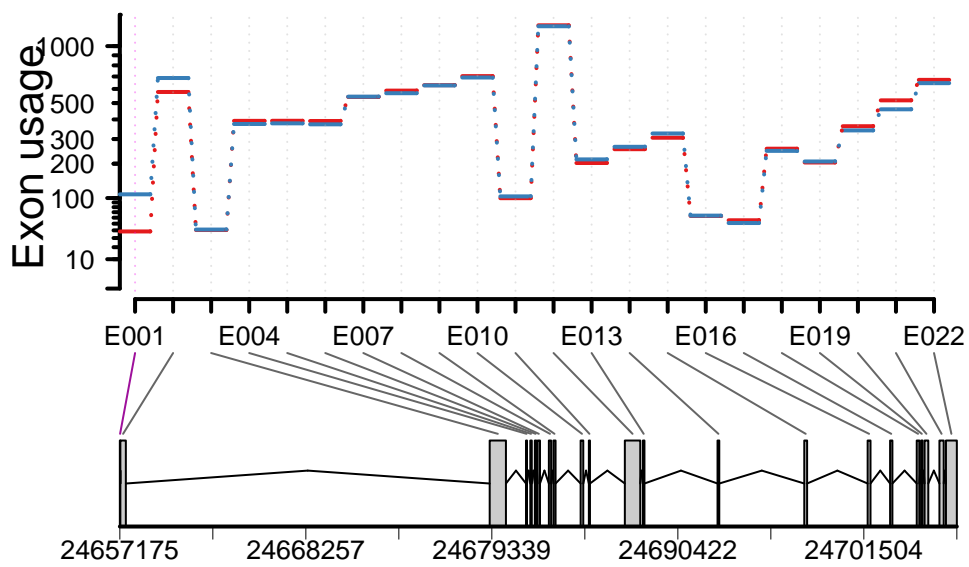
ENSMUSG000000028796.18 20months 4months



ENSMUSG00000110279.2 ^{20months} ^{4months}



ENSMUSG00000023353.15 ^{20months} ^{4months}



`top_age$transcripts`

```
[[1]]  
[1] "ENSMUST00000005504.15" "ENSMUST00000235112.2"
```

```
[[2]]  
[1] "ENSMUST00000106079.10"
```

```
[[3]]  
[1] "ENSMUST00000005504.15"
```

```
[[4]]  
[1] "ENSMUST00000209340.2"
```

```
[[5]]  
[1] "ENSMUST00000024123.9"
```

Sex as Condition

```
# Sample data frame with age as condition  
sample_data_sex <- data.frame(  
  row.names = c("SRR19123213", "SRR19123214", "SRR19123215", "SRR19123216", "SRR19123217",  
  age = c("20months", "20months", "20months", "20months", "20months", "20months",  
    "4months", "4months", "4months", "4months", "4months", "4months"),  
  condition = c("Male", "Male", "Male", "Female", "Female", "Female",  
    "Male", "Male", "Male", "Female", "Female", "Female"),  
  stringsAsFactors = TRUE  
)
```

```
sample_data_sex
```

	age	condition
SRR19123213	20months	Male
SRR19123214	20months	Male
SRR19123215	20months	Male
SRR19123216	20months	Female
SRR19123217	20months	Female
SRR19123218	20months	Female
SRR19123219	4months	Male
SRR19123220	4months	Male
SRR19123221	4months	Male
SRR19123222	4months	Female
SRR19123223	4months	Female

SRR19123224 4months Female

```
suppressWarnings({ suppressMessages({
dxd_sex <- DEXSeqDataSetFromHTSeq(count_files,
                                sample_data_sex,
                                design = ~sample + exon + condition + condition:exon,
                                flattenedfile=annotation_file)
}}})
```

```
dxd_sex <- estimateSizeFactors(dxd_sex)
dxd_sex <- estimateDispersions(dxd_sex)
dxd_sex <- testForDEU(dxd_sex)
dxd_sex <- estimateExonFoldChanges(dxd_sex, fitExpToVar="condition")
results_sex <- DEXSeq(dxd_sex)
```

Warning in MulticoreParam(workers = 1): MulticoreParam() not supported on Windows, use SnowParam()

```
head(results_sex)
```

LRT p-value: full vs reduced

DataFrame with 6 rows and 16 columns

	groupID	featureID	exonBaseMean			
	<character>	<character>	<numeric>			
ENSMUSG000000000001.5:E001	ENSMUSG000000000001.5	E001	1562.845			
ENSMUSG000000000001.5:E002	ENSMUSG000000000001.5	E002	282.580			
ENSMUSG000000000001.5:E003	ENSMUSG000000000001.5	E003	216.765			
ENSMUSG000000000001.5:E004	ENSMUSG000000000001.5	E004	203.348			
ENSMUSG000000000001.5:E005	ENSMUSG000000000001.5	E005	183.888			
ENSMUSG000000000001.5:E006	ENSMUSG000000000001.5	E006	227.077			
	dispersion	stat	pvalue	padj	Female	
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>	
ENSMUSG000000000001.5:E001	0.00254650	0.0439054	0.8340297	1.000000	47.1472	
ENSMUSG000000000001.5:E002	0.00161597	6.0953175	0.0135541	0.979597	24.9651	
ENSMUSG000000000001.5:E003	0.00279176	0.4157342	0.5190733	1.000000	21.8599	
ENSMUSG000000000001.5:E004	0.00243621	0.4909722	0.4834940	1.000000	20.9400	
ENSMUSG000000000001.5:E005	0.00739771	0.3469423	0.5558491	1.000000	20.0232	
ENSMUSG000000000001.5:E006	0.00306538	0.8081767	0.3686598	1.000000	21.9331	

	Male	log2fold_Male_Female	Female.1	Male.1
	<numeric>	<numeric>	<numeric>	<numeric>
ENSMUSG000000000001.5:E001	47.0579	-0.00834051	47.1472	47.0579
ENSMUSG000000000001.5:E002	23.8844	-0.14712639	24.9651	23.8844
ENSMUSG000000000001.5:E003	21.5565	-0.04521351	21.8599	21.5565
ENSMUSG000000000001.5:E004	21.3640	0.06448938	20.9400	21.3640
ENSMUSG000000000001.5:E005	20.4676	0.07002259	20.0232	20.4676
ENSMUSG000000000001.5:E006	22.4566	0.07665677	21.9331	22.4566

	log2fold_Male_Female.1	genomicData
	<numeric>	<GRanges>
ENSMUSG000000000001.5:E001	-0.00834051	chr3:108014596-108016632:-
ENSMUSG000000000001.5:E002	-0.14712639	chr3:108016719-108016928:-
ENSMUSG000000000001.5:E003	-0.04521351	chr3:108019251-108019404:-
ENSMUSG000000000001.5:E004	0.06448938	chr3:108019789-108019918:-
ENSMUSG000000000001.5:E005	0.07002259	chr3:108023079-108023207:-
ENSMUSG000000000001.5:E006	0.07665677	chr3:108025617-108025774:-

	countData	transcripts
	<matrix>	<list>
ENSMUSG000000000001.5:E001	1708:1549:1205:...	ENSMUST000000000001.5
ENSMUSG000000000001.5:E002	311:256:213:...	ENSMUST000000000001.5
ENSMUSG000000000001.5:E003	233:213:182:...	ENSMUST000000000001.5
ENSMUSG000000000001.5:E004	250:190:163:...	ENSMUST000000000001.5
ENSMUSG000000000001.5:E005	224:185:120:...	ENSMUST000000000001.5
ENSMUSG000000000001.5:E006	218:238:185:...	ENSMUST000000000001.5

```
# Extract significant genes for each factor
significant_sex <- results_sex[which(!is.na(results_sex$padj) & results_sex$padj < 0.05),

# Get the counts
n_significant_sex <- nrow(significant_sex)

# Print the results
cat("Number of significant genes for Sex effect:", n_significant_sex, "\n")
```

Number of significant genes for Sex effect: 38

```
top_sex <- head(significant_sex[order(significant_sex$padj), ], 5)
top_sex
```

LRT p-value: full vs reduced

DataFrame with 5 rows and 16 columns

	groupID <character>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	ENSMUSG00000098078.2..
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	ENSMUSG000002075825.1..
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	ENSMUSG00000098078.2..
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	ENSMUSG000002075825.1..
ENSMUSG00000035183.15:E009	ENSMUSG00000035183.15
	featureID <character>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	E016
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	E003
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	E015
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	E011
ENSMUSG00000035183.15:E009	E009
	exonBaseMean <numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	418.469
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	11561.495
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	276.831
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	5509.787
ENSMUSG00000035183.15:E009	319.859
	dispersion <numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	0.00261221
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	0.00796475
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	0.00385842
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	0.07476335
ENSMUSG00000035183.15:E009	0.01009141
	stat <numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	151.0178
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	145.1600
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	67.2156
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	55.1517
ENSMUSG00000035183.15:E009	52.2997
	pvalue <numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	1.03874e-34
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	1.98144e-33
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	2.43386e-16

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	1.11576e-13
ENSMUSG00000035183.15:E009	4.76449e-13
	padj
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	2.35593e-29
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	2.24702e-28
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	1.84005e-11
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	6.32655e-09
ENSMUSG00000035183.15:E009	2.16124e-08
	Female
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	18.2135
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	39.3732
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	15.0072
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	29.4834
ENSMUSG00000035183.15:E009	23.7546
	Male
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	14.7775
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	35.6209
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	12.9711
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	50.8191
ENSMUSG00000035183.15:E009	27.8870
	log2fold_Male_Female
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	-0.645605
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	-0.388585
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	-0.442092
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	2.169142
ENSMUSG00000035183.15:E009	0.541024
	Female.1
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	18.2135
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	39.3732
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	15.0072
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	29.4834
ENSMUSG00000035183.15:E009	23.7546
	Male.1
	<numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	14.7775
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	35.6209
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015	12.9711
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011	50.8191


```

ENSMUSG00000035183.15:E009                                27.8870
                                                            log2fold_Male_Female.1
                                                            <numeric>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016 -0.645605
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003 -0.388585
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015 -0.442092
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011  2.169142
ENSMUSG00000035183.15:E009                                0.541024
                                                            genomicData
                                                            <GRanges>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016 chrX:102525312-102526012
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003 chrX:102504892-102511642
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015 chrX:102524881-102525311
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011 chrX:102517322-102526860
ENSMUSG00000035183.15:E009                                chr2:124929884-124930597
                                                            countData
                                                            <matrix>
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016  9:19:20:...
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003  9:17:27:...
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015  1:11:16:...
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011 20:70:124:...
ENSMUSG00000035183.15:E009                                333:264:223:...

ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016 ENSI
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E015 ENSMUST00000211291.2,ENSI
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E011
ENSMUSG00000035183.15:E009

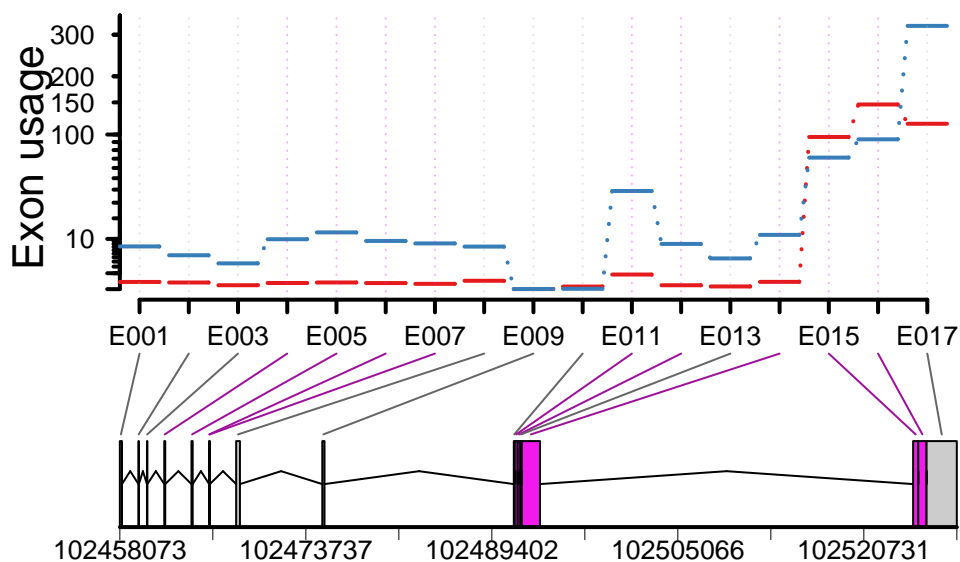
```

```

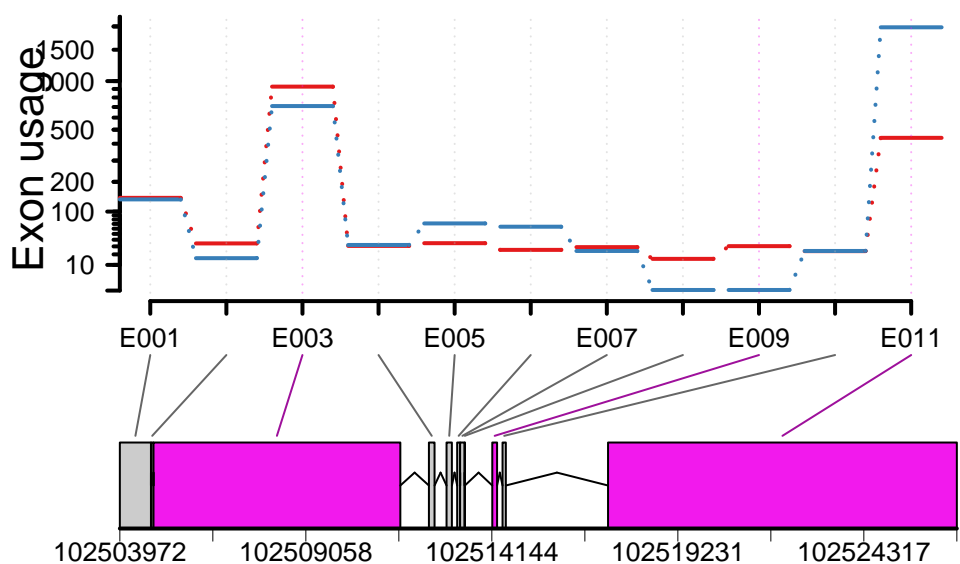
for (id in unique(top_sex$groupID)){
  plotDEXSeq(results_sex, id, legend=TRUE, expression=FALSE, splicing=TRUE, cex.axis=1.2)
}

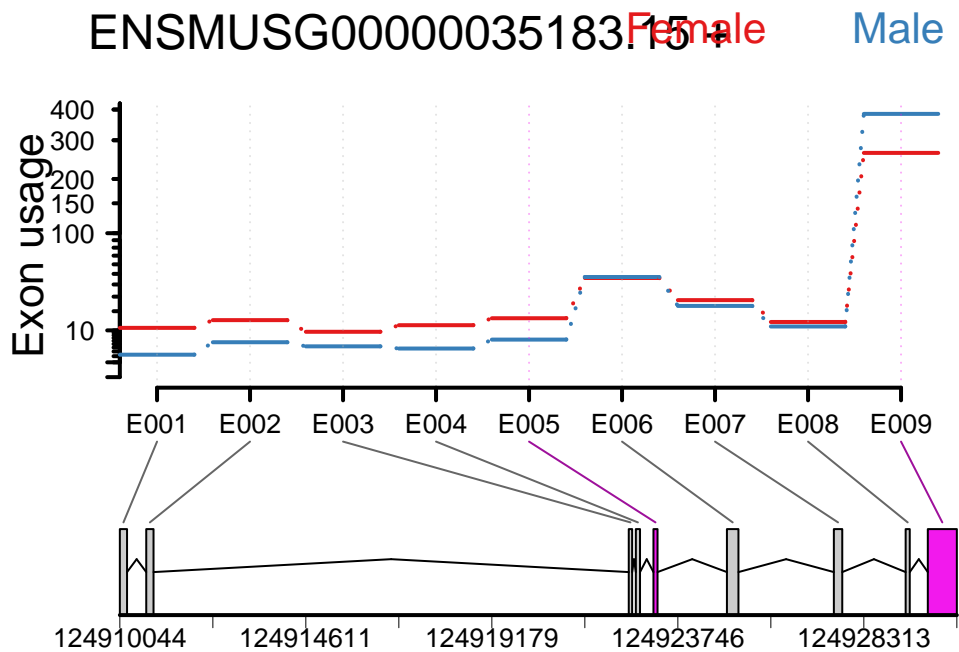
```

3G00000098078.2+ENSMUSG00000031329.5+EI



3G00002075825.1+ENSMUSG00002076282.1+EI





```
top_sex$transcripts
```

```
[[1]]
```

```
[1] "ENSMUST00000211291.2" "ENSMUST00000152916.2"
```

```
[[2]]
```

```
[1] "ENSMUST00000127786.4"
```

```
[[3]]
```

```
[1] "ENSMUST00000211291.2" "ENSMUST00000152916.2" "ENSMUST00000182089.2"
```

```
[[4]]
```

```
[1] "ENSMUST00000127786.4"
```

```
[[5]]
```

```
[1] "ENSMUST00000070353.4"
```

Age:Sex Interaction as Condition

```
# Condition based on age:sex interaction
sample_data_interaction <- data.frame(
  row.names = c("SRR19123213", "SRR19123214", "SRR19123215", "SRR19123216", "SRR19123217",
  condition = c("20months.Male", "20months.Male", "20months.Male",
                "20months.Female", "20months.Female", "20months.Female",
                "4months.Male", "4months.Male", "4months.Male",
                "4months.Female", "4months.Female", "4months.Female"),
  stringsAsFactors = TRUE
)
```

```
sample_data_interaction
```

	condition
SRR19123213	20months.Male
SRR19123214	20months.Male
SRR19123215	20months.Male
SRR19123216	20months.Female
SRR19123217	20months.Female
SRR19123218	20months.Female
SRR19123219	4months.Male
SRR19123220	4months.Male
SRR19123221	4months.Male
SRR19123222	4months.Female
SRR19123223	4months.Female
SRR19123224	4months.Female

```
suppressWarnings({ suppressMessages({
dxd_interaction <- DEXSeqDataSetFromHTSeq(count_files,
                                          sample_data_interaction,
                                          design = ~sample + exon + condition + condition:exon,
                                          flattenedfile=annotation_file)
}}})
```

```
dxd_interaction <- estimateSizeFactors(dxd_interaction)
dxd_interaction <- estimateDispersions(dxd_interaction)
dxd_interaction <- testForDEU(dxd_interaction)
dxd_interaction <- estimateExonFoldChanges(dxd_interaction, fitExpToVar="condition")
results_interaction <- DEXSeq(dxd_interaction)
```

Warning in MulticoreParam(workers = 1): MulticoreParam() not supported on Windows, use SnowParam()

```
head(results_interaction)
```

LRT p-value: full vs reduced

DataFrame with 6 rows and 24 columns

	groupID	featureID	exonBaseMean	
	<character>	<character>	<numeric>	
ENSMUSG000000000001.5:E001	ENSMUSG000000000001.5	E001	1562.845	
ENSMUSG000000000001.5:E002	ENSMUSG000000000001.5	E002	282.580	
ENSMUSG000000000001.5:E003	ENSMUSG000000000001.5	E003	216.765	
ENSMUSG000000000001.5:E004	ENSMUSG000000000001.5	E004	203.348	
ENSMUSG000000000001.5:E005	ENSMUSG000000000001.5	E005	183.888	
ENSMUSG000000000001.5:E006	ENSMUSG000000000001.5	E006	227.077	
	dispersion	stat	pvalue	padj
	<numeric>	<numeric>	<numeric>	<numeric>
ENSMUSG000000000001.5:E001	0.00223322	2.539376	0.4682170	0.842439
ENSMUSG000000000001.5:E002	0.00164998	6.507483	0.0893679	0.455200
ENSMUSG000000000001.5:E003	0.00212189	4.053096	0.2557875	0.690387
ENSMUSG000000000001.5:E004	0.00276124	0.594692	0.8976464	0.982810
ENSMUSG000000000001.5:E005	0.00697409	2.857067	0.4141916	0.811442
ENSMUSG000000000001.5:E006	0.00316023	1.940026	0.5849474	0.896176
	X20months.Female	X20months.Male	X4months.Female	
	<numeric>	<numeric>	<numeric>	
ENSMUSG000000000001.5:E001	49.4073	49.8543	50.4244	
ENSMUSG000000000001.5:E002	25.5312	24.6317	25.5869	
ENSMUSG000000000001.5:E003	21.6537	22.3349	22.9566	
ENSMUSG000000000001.5:E004	21.2490	21.9014	21.3977	
ENSMUSG000000000001.5:E005	21.0113	20.5659	19.6309	
ENSMUSG000000000001.5:E006	22.5770	22.5518	22.1134	
	X4months.Male	log2fold_20months.Male_20months.Female		
	<numeric>	<numeric>		
ENSMUSG000000000001.5:E001	49.7271	0.03694598		
ENSMUSG000000000001.5:E002	24.1796	-0.11535845		
ENSMUSG000000000001.5:E003	21.5781	0.09728721		
ENSMUSG000000000001.5:E004	21.6025	0.09469972		
ENSMUSG000000000001.5:E005	21.0424	-0.06672769		
ENSMUSG000000000001.5:E006	23.2128	-0.00351897		

	log2fold_4months.Female_20months.Female
	<numeric>
ENSMUSG00000000001.5:E001	0.08385985
ENSMUSG00000000001.5:E002	0.00703558
ENSMUSG00000000001.5:E003	0.18393590
ENSMUSG00000000001.5:E004	0.02180027
ENSMUSG00000000001.5:E005	-0.21094118
ENSMUSG00000000001.5:E006	-0.06532753

	log2fold_4months.Male_20months.Female
	<numeric>
ENSMUSG00000000001.5:E001	0.02644528
ENSMUSG00000000001.5:E002	-0.17462035
ENSMUSG00000000001.5:E003	-0.01095912
ENSMUSG00000000001.5:E004	0.05161190
ENSMUSG00000000001.5:E005	0.00460103
ENSMUSG00000000001.5:E006	0.08782605

	X20months.Female.1	X20months.Male.1	X4months.Female.1
	<numeric>	<numeric>	<numeric>
ENSMUSG00000000001.5:E001	49.4073	49.8543	50.4244
ENSMUSG00000000001.5:E002	25.5312	24.6317	25.5869
ENSMUSG00000000001.5:E003	21.6537	22.3349	22.9566
ENSMUSG00000000001.5:E004	21.2490	21.9014	21.3977
ENSMUSG00000000001.5:E005	21.0113	20.5659	19.6309
ENSMUSG00000000001.5:E006	22.5770	22.5518	22.1134

	X4months.Male.1
	<numeric>
ENSMUSG00000000001.5:E001	49.7271
ENSMUSG00000000001.5:E002	24.1796
ENSMUSG00000000001.5:E003	21.5781
ENSMUSG00000000001.5:E004	21.6025
ENSMUSG00000000001.5:E005	21.0424
ENSMUSG00000000001.5:E006	23.2128

	log2fold_20months.Male_20months.Female.1
	<numeric>
ENSMUSG00000000001.5:E001	0.03694598
ENSMUSG00000000001.5:E002	-0.11535845
ENSMUSG00000000001.5:E003	0.09728721
ENSMUSG00000000001.5:E004	0.09469972
ENSMUSG00000000001.5:E005	-0.06672769
ENSMUSG00000000001.5:E006	-0.00351897

	log2fold_4months.Female_20months.Female.1
	<numeric>
ENSMUSG00000000001.5:E001	0.08385985

ENSMUSG000000000001.5:E002	0.00703558
ENSMUSG000000000001.5:E003	0.18393590
ENSMUSG000000000001.5:E004	0.02180027
ENSMUSG000000000001.5:E005	-0.21094118
ENSMUSG000000000001.5:E006	-0.06532753

log2fold_4months.Male_20months.Female.1

<numeric>

ENSMUSG000000000001.5:E001	0.02644528
ENSMUSG000000000001.5:E002	-0.17462035
ENSMUSG000000000001.5:E003	-0.01095912
ENSMUSG000000000001.5:E004	0.05161190
ENSMUSG000000000001.5:E005	0.00460103
ENSMUSG000000000001.5:E006	0.08782605

genomicData

countData

<GRanges>

<matrix>

ENSMUSG000000000001.5:E001	chr3:108014596-108016632:-	1708:1549:1205:...
ENSMUSG000000000001.5:E002	chr3:108016719-108016928:-	311:256:213:...
ENSMUSG000000000001.5:E003	chr3:108019251-108019404:-	233:213:182:...
ENSMUSG000000000001.5:E004	chr3:108019789-108019918:-	250:190:163:...
ENSMUSG000000000001.5:E005	chr3:108023079-108023207:-	224:185:120:...
ENSMUSG000000000001.5:E006	chr3:108025617-108025774:-	218:238:185:...

transcripts

<list>

ENSMUSG000000000001.5:E001	ENSMUST000000000001.5
ENSMUSG000000000001.5:E002	ENSMUST000000000001.5
ENSMUSG000000000001.5:E003	ENSMUST000000000001.5
ENSMUSG000000000001.5:E004	ENSMUST000000000001.5
ENSMUSG000000000001.5:E005	ENSMUST000000000001.5
ENSMUSG000000000001.5:E006	ENSMUST000000000001.5

```
# Extract significant genes for each factor
```

```
significant_interaction <- results_interaction[which(!is.na(results_interaction$padj) & re
```

```
# Get the counts
```

```
n_significant_interaction <- nrow(significant_interaction)
```

```
# Print the results
```

```
cat("Number of significant genes for Age:Sex interaction effect:", n_significant_interacti
```

Number of significant genes for Age:Sex interaction effect: 4228

```
top_interaction <- head(significant_interaction[order(significant_interaction$padj), ], 5)
top_interaction
```

LRT p-value: full vs reduced

DataFrame with 5 rows and 24 columns

	groupID <character>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	ENSMUSG000002075825.1..
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	ENSMUSG00000098078.2..
ENSMUSG00000005371.16:E027	ENSMUSG00000005371.16
ENSMUSG000000059149.18:E007	ENSMUSG000000059149.18
ENSMUSG00000031765.9:E002	ENSMUSG00000031765.9
	featureID <character>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	E003
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	E016
ENSMUSG00000005371.16:E027	E027
ENSMUSG000000059149.18:E007	E007
ENSMUSG00000031765.9:E002	E002
	exonBaseMean <numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	11561.4946
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	418.4691
ENSMUSG00000005371.16:E027	54.3254
ENSMUSG000000059149.18:E007	68.6941
ENSMUSG00000031765.9:E002	1132.4847
	dispersion <numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	0.00222232
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	0.00280761
ENSMUSG00000005371.16:E027	0.02035375
ENSMUSG000000059149.18:E007	0.00521630
ENSMUSG00000031765.9:E002	0.00242670
	stat <numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	229.2198
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	148.8992
ENSMUSG00000005371.16:E027	100.9955
ENSMUSG000000059149.18:E007	101.0473
ENSMUSG00000031765.9:E002	98.8864

	pvalue
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	2.03937e-49
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	4.55217e-32
ENSMUSG00000005371.16:E027	9.49351e-22
ENSMUSG000000059149.18:E007	9.25312e-22
ENSMUSG00000031765.9:E002	2.69731e-21
	padj
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	3.50191e-44
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	3.90838e-27
ENSMUSG00000005371.16:E027	4.07545e-17
ENSMUSG000000059149.18:E007	4.07545e-17
ENSMUSG00000031765.9:E002	9.26337e-17
	X20months.Female
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	39.5711
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	18.1804
ENSMUSG00000005371.16:E027	6.9557
ENSMUSG000000059149.18:E007	14.6804
ENSMUSG00000031765.9:E002	45.3292
	X20months.Male
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	36.28605
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	15.64126
ENSMUSG00000005371.16:E027	6.74496
ENSMUSG000000059149.18:E007	14.99735
ENSMUSG00000031765.9:E002	45.04181
	X4months.Female
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	39.84587
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	18.20588
ENSMUSG00000005371.16:E027	13.01039
ENSMUSG000000059149.18:E007	7.89868
ENSMUSG00000031765.9:E002	38.73479
	X4months.Male
	<numeric>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	38.2085
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	14.6560
ENSMUSG00000005371.16:E027	15.6991
ENSMUSG000000059149.18:E007	11.5666
ENSMUSG00000031765.9:E002	47.9293
	log2fold_20months.Male_2

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

log2fold_4months.Female_1

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

log2fold_4months.Male_20

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

X20months.Female.1

<numeric>

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

39.5711
 18.1804
 6.9557
 14.6804
 45.3292

X20months.Male.1

<numeric>

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

36.28605
 15.64126
 6.74496
 14.99735
 45.04181

X4months.Female.1

<numeric>

ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003
 ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016
 ENSMUSG00000005371.16:E027
 ENSMUSG00000059149.18:E007
 ENSMUSG00000031765.9:E002

39.84587
 18.20588
 13.01039
 7.89868
 38.73479

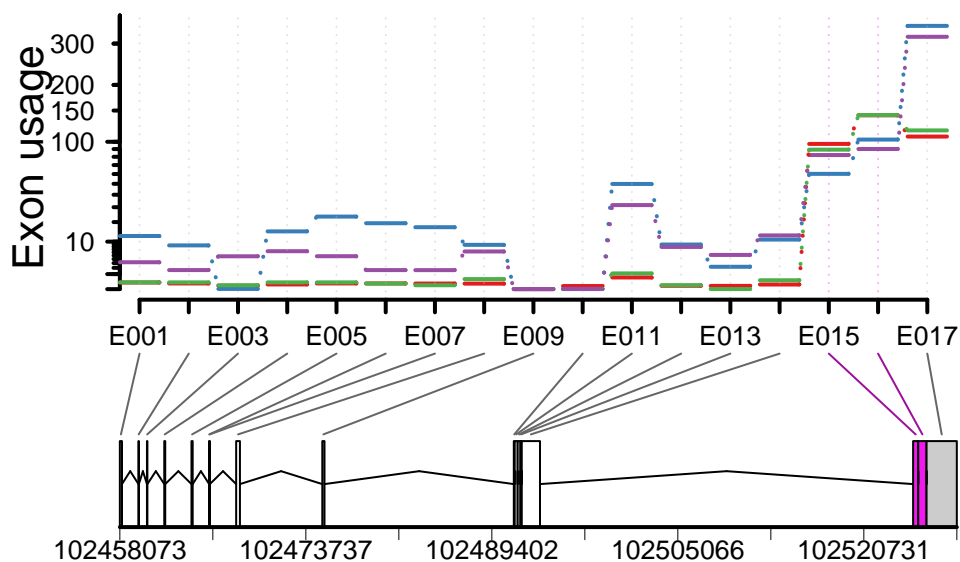
X4months.Male.1

<numeric>

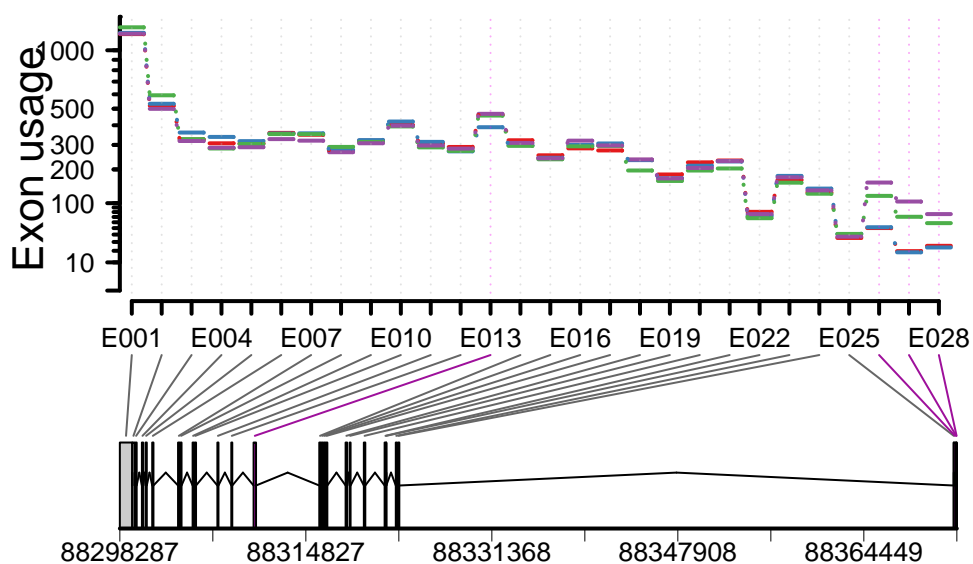
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	38.2085
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	14.6560
ENSMUSG00000005371.16:E027	15.6991
ENSMUSG00000059149.18:E007	11.5666
ENSMUSG00000031765.9:E002	47.9293
	log2fold_20months.Male_20
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	
ENSMUSG00000005371.16:E027	
ENSMUSG00000059149.18:E007	
ENSMUSG00000031765.9:E002	
	log2fold_4months.Female_20
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	
ENSMUSG00000005371.16:E027	
ENSMUSG00000059149.18:E007	
ENSMUSG00000031765.9:E002	
	log2fold_4months.Male_20
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	
ENSMUSG00000005371.16:E027	
ENSMUSG00000059149.18:E007	
ENSMUSG00000031765.9:E002	
	genomicData
	<GRanges>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	chrX:102504892-102511642
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	chrX:102525312-102526012
ENSMUSG00000005371.16:E027	chr17:88372646-88372671
ENSMUSG00000059149.18:E007	chr1:131966251-131966654
ENSMUSG00000031765.9:E002	chr8:94905839-94905868
	countData
	<matrix>
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	9:17:27:...
ENSMUSG00000098078.2+ENSMUSG00000031329.5+ENSMUSG00000085715.3:E016	9:19:20:...
ENSMUSG00000005371.16:E027	13:27:12:...
ENSMUSG00000059149.18:E007	119:95:78:...
ENSMUSG00000031765.9:E002	1312:1223:1311:...
ENSMUSG000002075825.1+ENSMUSG000002076282.1+ENSMUSG00000086503.5:E003	ENSMUSG00000005371.16:E027

```
for (id in unique(top_interaction$groupID)){
  plotDEXSeq(results_interaction, id, legend=TRUE, expression=FALSE, splicing=TRUE, cex.
}
```

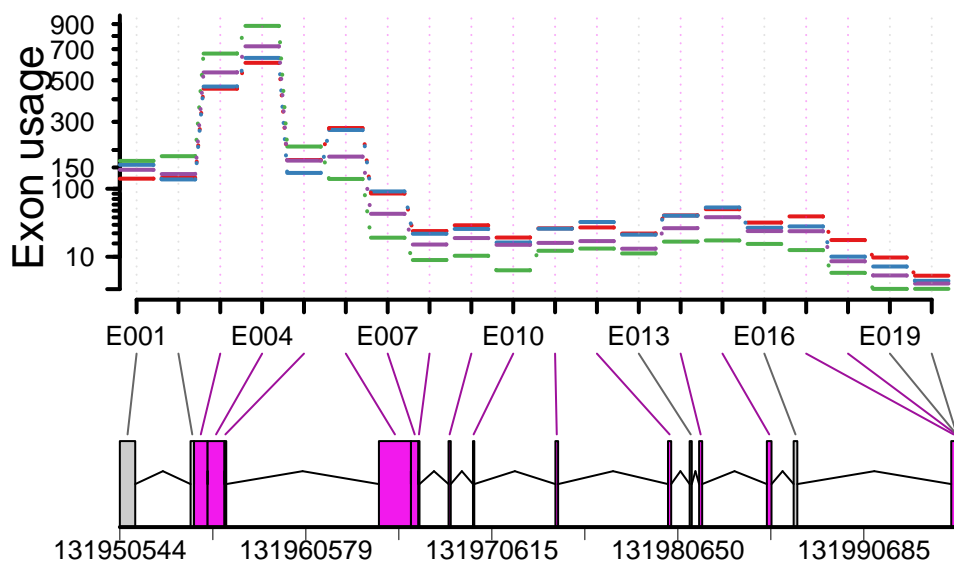

ENSMUSG00000098078.2+ENSMUSG000000001329.5+EI



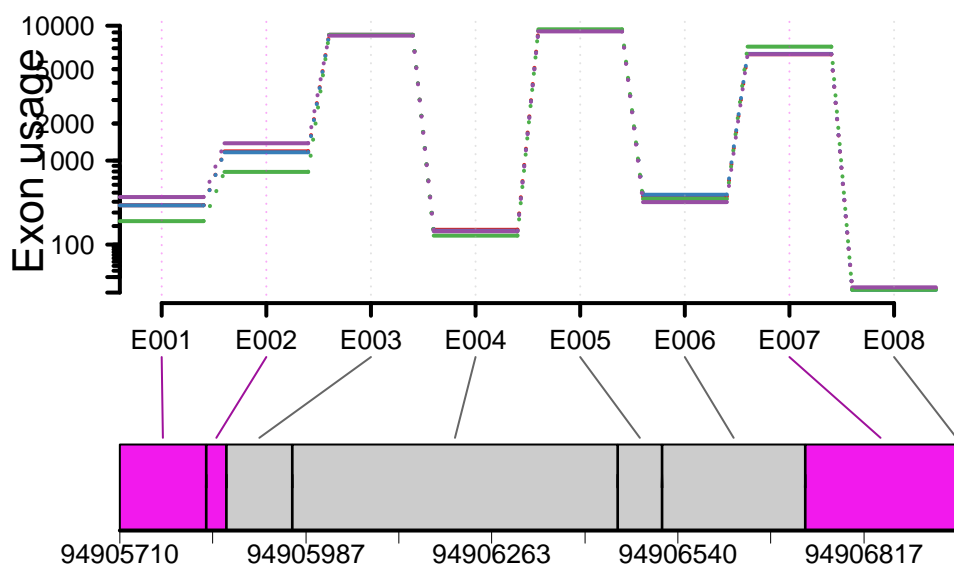
ENSMUSG00000002537.1+ENSMUSG000000001329.5+EI



ENSMUSG000000039149.16 Female Male



ENSMUSG000000031763.16 Female Male



```
top_interaction$transcripts
```

```

[[1]]
[1] "ENSMUST00000127786.4"

[[2]]
[1] "ENSMUST00000211291.2" "ENSMUST00000152916.2"

[[3]]
[1] "ENSMUST00000005504.15" "ENSMUST00000235112.2"

[[4]]
[1] "ENSMUST00000159038.8" "ENSMUST00000046658.10"

[[5]]
[1] "ENSMUST00000034215.8" "ENSMUST00000212291.2"

```

Extra Credit Task E2 - Summary

AGE

The total number of significant genes for the aging effect was 146 and we show splicing plots for the top genes. Overall, where differences in splicing are observed between the groups, the older mice typically have less coverage over these exons.

Fbxo11 (ENSMUSG00000005371.16) shows differential splicing at the E025-E028 region, where there are fewer transcripts detected in the older mice. Fbxo11 functions in the E3 ubiquitin-protein ligase complex, which downstream acts to regulate B-cell differentiation and termination, TGF- β signaling, macrophage cell death, and inflammation. (E012)

Phc2 (ENSMUSG00000028796.18) is predicted to enable chromatin and histone binding activity Phc2-KO mouse fibroblasts exhibit premature senescence and adults display defects along the axial skeleton. Older mice show fewer reads at E012.

T(brachyury, T-box transcription factor T, ENSMUSG00000110279.2) functions as an RNA polymerase II-specific DNA-binding transcription factor and is critical to embryonic development, including the circulatory system. E001 and E003 show lower levels in the older mice, but higher reads at E002.

Agap 3(ENSMUSG00000023353.15) functions in synapse potentiation as part of a complex that strengthens synapse connections, reinforcing learning and memory. Older mice show lower reads at E001-E002).

SEX

The total number of significant genes for the aging effect was 36.

X (inactive) specific transcripts (ENSMUST00000211291.2 and ENSMUST00000127786.4) works in the inactivation of the paternal X-chromosome. Males appear to have higher exon usage over a majority of the transcript.

Slc24a5 (ENSMUSG00000035183.15) enables calcium ion transmembrane transporter activity. Through E001-E005, we see higher exon usage in females, but at E009 it is higher in males.

SEX:AGE INTERACTION

Samples are labeled according to color as follows:

Color	Age (months)	Sex
Red	20	Female
Blue	20	Male
Green	4	Female
Purple	4	Male

Number of significant genes for Age:Sex interaction effect was found to be 4228.

Gm55585 (ENSMUST00002075825.1) is a miscellaneous RNA generated from the same region as the Xist gene which is involved in X-chromosome inactivation. Despite age, you see that exon usage in females is more similar to each other than in males, which is particularly evident at E011.

Gm26992 (ENSMUSG00000098078.2) is the anti-sense strand of the Xist gene (Tsix) and appears to have higher exon usage among males across most of the transcript despite age.

Fbxo11 (ENSMUSG00000005371.16) appears again in this site with variation at site E013 and E025-28. The latter region specifically highlights that males and females of the same age group have similar slice patterns, with younger mice displaying more exon coverage than older mice.

Mfsd4a (ENSMUSG00000059149.18) functions in glucose transmembrane transport. Exon usage here varies greatly across the sex and age groups, although the young female seems to be more divergent from the other groups. In the literature, I found that this gene is "down-regulated in nasopharyngeal carcinoma (NPC) and whether it is associated with malignant progression and poor prognosis of NPC" according to one study (Yang et al 2022). Due to the interaction of sex and age, this may lead to questions about sex-bias in treatment of disease and how progression may differ between these groups.

SUMMARY

For the effect of Age, we saw the top differential splicing in genes that were associated with immune function, structural development, and synapse reinforcement. In general, older mice show lower exon coverage. This may serve as an example of aging on the molecular scale, where processes are not as robust in younger animals.

For the effect of sex, we see top variants such as the gene for X-chromosome inactivation, which appears to have more exon coverage than in females. This may be due to the complex regulation of this gene to prevent X-inactivation in a male, as they only have one X chromosome.

The interaction of age and sex is complex and tends to show a mixture of the biological processes we have seen in the individual groups such as: X-inactivation (Xist and Tsix), immune function, and transmembrane transport.

From this project, we have learned that disease and aging do not progress identically between males and females. The underlying difference in genetic makeup, sex chromosomes, lends itself to a basal difference in gene expression that shapes subsequent signaling pathways. To add to the complexity of the system, multiple transcript variants can be produced from a single gene. A greater understanding of differentially spliced genes between the sexes can lead to better understanding of those small biological differences that can affect the progression of aging or disease.

References

Han, Yu, Sara A Wennersten, Julianna M Wright, R W Ludwig, Edward Lau, and Maggie P Y Lam. 2022. “Proteogenomics Reveals Sex-Biased Aging Genes and Coordinated Splicing in Cardiac Aging.” *Am J Physiol Heart Circ Physiol* 323 (3): H538–58. <https://doi.org/10.1152/ajpheart.00244.2022>.

Shen H, Yanas A, Owens MC, Zhang C, Fritsch C, Fare CM, Copley KE, Shorter J, Goldman YE, Liu KF. Sexually dimorphic RNA helicases DDX3X and DDX3Y differentially regulate RNA metabolism through phase separation. *Mol Cell*. 2022 Jul 21;82(14):2588-2603.e9. doi: 10.1016/j.molcel.2022.04.022. Epub 2022 May 18. PMID: 35588748; PMCID: PMC9308757.

Cunningham CM, Li M, Ruffenach G, Doshi M, Aryan L, Hong J, et al. Y-chromosome gene, *uty*, protects against pulmonary hypertension by reducing proinflammatory chemokines. *Am J Respir Crit Care Med*. 2022;206(2):186–96.

Pan Y, Yang J, Dai J, Xu X, Zhou X, Mao W. TFRC in cardiomyocytes promotes macrophage infiltration and activation during the process of heart failure through regulating Ccl2 expression mediated by hypoxia inducible factor-1. *Immun Inflamm Dis*. 2023 Aug;11(8):e835. doi: 10.1002/iid3.835. PMID: 37647427; PMCID: PMC10461419. <https://pubmed.ncbi.nlm.nih.gov/37647427/>

Kosmidis S, Polyzos A, Harvey L, Youssef M, Denny CA, Dranovsky A, Kandel ER. RbAp48 Protein Is a Critical Component of GPR158/OCN Signaling and Ameliorates Age-Related Memory Loss. *Cell Rep*. 2018 Oct 23;25(4):959-973.e6. doi: 10.1016/j.celrep.2018.09.077. PMID: 30355501; PMCID: PMC7725275.

Chabchoub G., Uz E., Maalej A., et al. Analysis of skewed X-chromosome inactivation in females with rheumatoid arthritis and autoimmune thyroid diseases. *Arthritis Research & Therapy* . 2009;11(4):p. R106. doi: 10.1186/ar2759.

Ji B., Higa K. K., Kelsoe J. R., Zhou X. Over-expression of XIST, the master gene for X chromosome inactivation, in females with major affective disorders. *EBioMedicine* . 2015;2(8):909–918. doi: 10.1016/j.ebiom.2015.06.012.

Carlson JC, Krishnan M, Rosenthal SL, Russell EM, Zhang JZ, Hawley NL, Moors J, Cheng H, Dalbeth N, de Zoysa JR, Watson H, Qasim M, Murphy R, Naseri T, Reupena MS, Viali S, Stamp LK, Tuitele J, Kershaw EE, Dekka R, McGarvey ST, Merriman TR, Weeks DE, Minster RL. A stop-gain variant in *BTNL9* is associated with atherogenic lipid profiles. *HGG Adv.* 2022 Oct 12;4(1):100155. doi: 10.1016/j.xhgg.2022.100155. PMID: 36340932; PMCID: PMC9630829.

Yang, H., Qin, G., Luo, Z. *et al.* MFSD4A inhibits the malignant progression of nasopharyngeal carcinoma by targeting EPHA2. *Cell Death Dis* **13**, 332 (2022). <https://doi.org/10.1038/s41419-022-04793-x>