

# BMI Final Project Supplementary File

Five Guys

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## Workflow Diagram

Please see methods section for detailed overview of the project workflow.

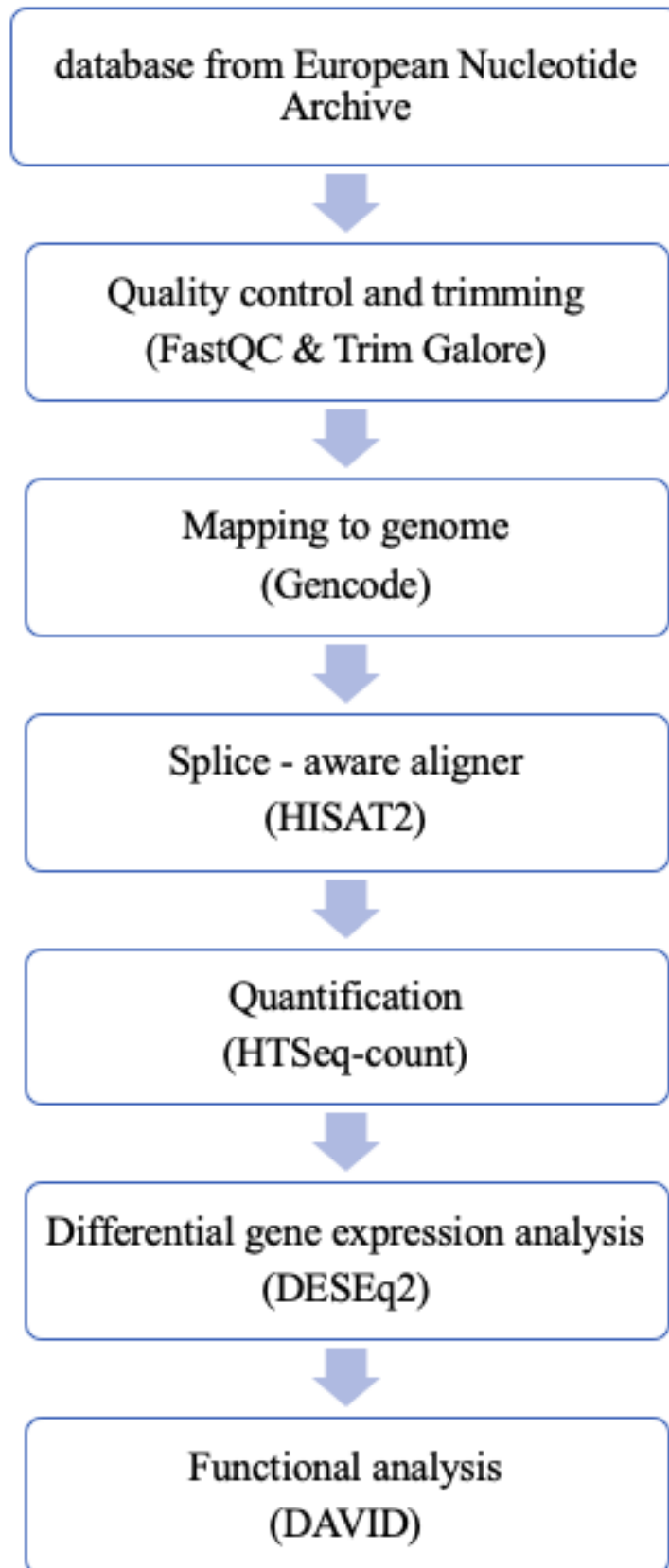
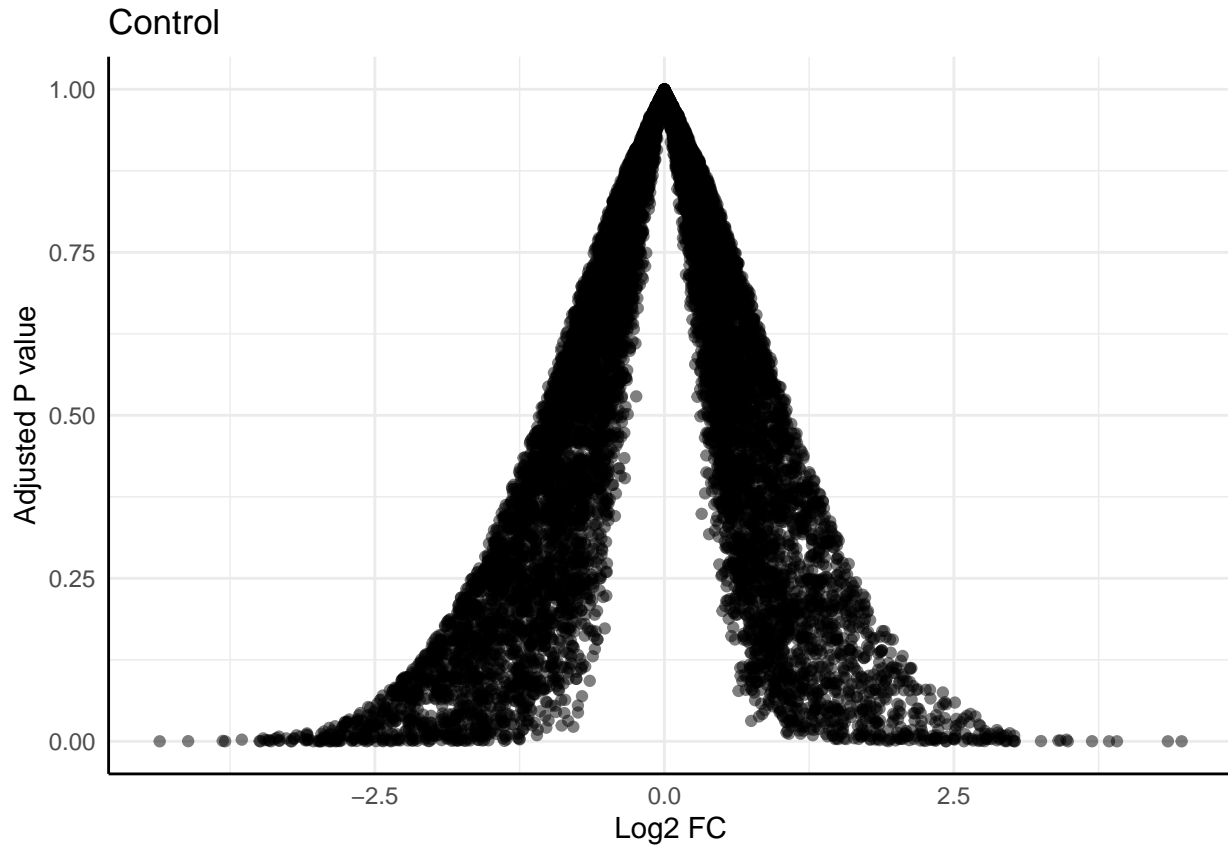


Figure 1: Project workflow

```
# Cases vs Controls R
```

## Volcano Plot

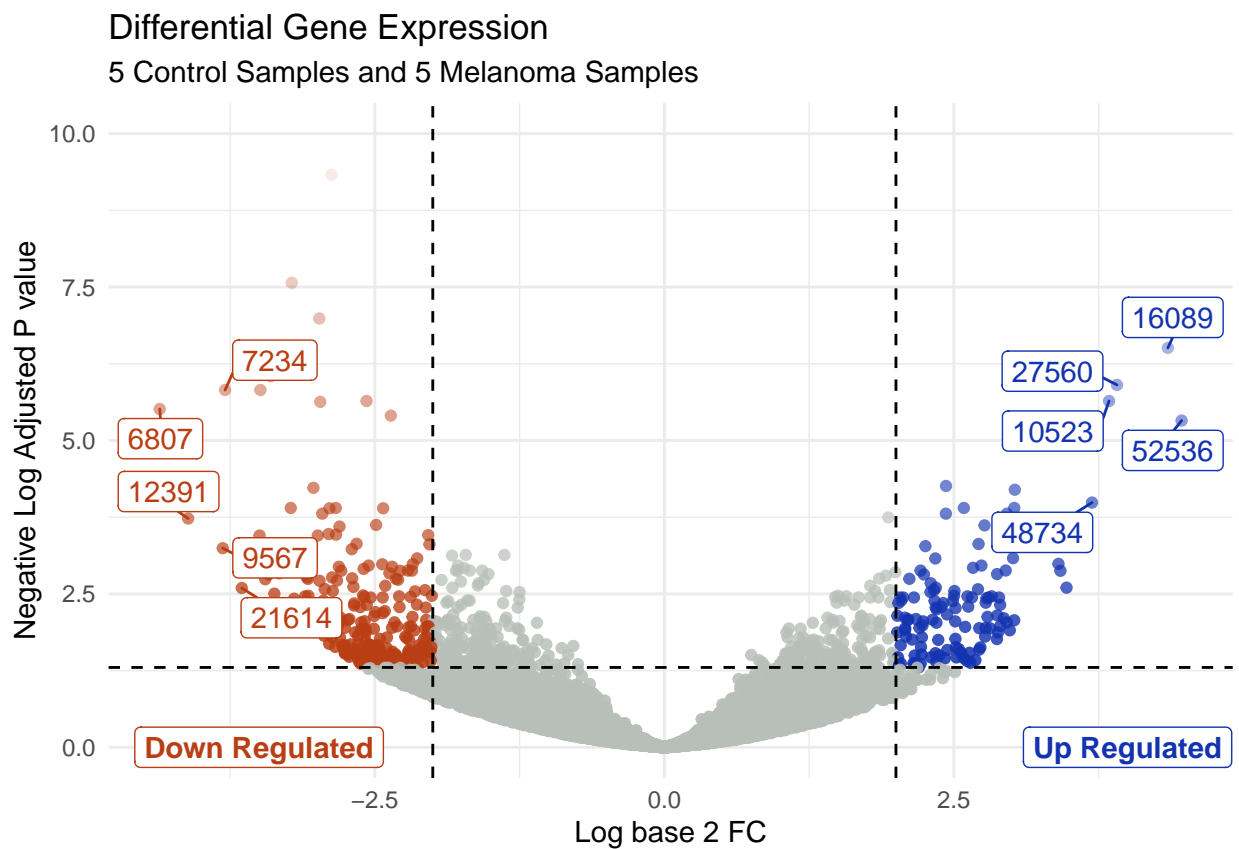
```
ggplot(data=control, aes(x=log2fc, y=padj)) +  
  geom_jitter(alpha=0.5)+  
  ggtitle("Control")+  
  xlab("Log2 FC")+  
  ylab("Adjusted P value")+  
  ggsave("case_v_con_volcano.png", width=10, height=6)
```



## Up vs Down Regulated Plot

```
tab_con<-control%>%  
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",  
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "lowdiff")))%>%  
  mutate(abslog2fc = abs(log2fc))%>%  
  filter(newdiff!="lowdiff")%>%  
  group_by(newdiff)%>%  
  slice_max(n=5, order_by=abslog2fc)%>%  
  select(Position=X1, geneID, idlabel, log2fc, padj, direction=newdiff)  
  
control%>%  
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",  
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "Low-diff")))%>%  
  mutate(newidlabel=ifelse(idlabel %in% tab_con$idlabel, idlabel, NA))%>%
```

```
ggplot(aes(x=log2fc, y=-log10(padj), col=newdiff, label=newidlabel)) +
  geom_point(aes(alpha=log10(padj)), show.legend=F) +
  geom_vline(xintercept=c(-2, 2), col="black", linetype = "dashed") +
  geom_hline(yintercept=-log10(0.05), col="black", linetype = "dashed")+
  annotate("label", x = -3.5, y = 0, label = "Down Regulated", color="#ba3e14",fontface = "bold")+
  annotate("label", x = 4, y = 0, label = "Up Regulated", color="#1333b0",fontface = "bold")+
  ggrepel::geom_label_repel(min.segment.length = unit(0, 'lines'), show.legend=F)+
  scale_color_manual(values=c("#ba3e14", "#b8bfb8", "#1333b0"))+
  labs(title="Differential Gene Expression", subtitle="5 Control Samples and 5 Melanoma Samples")+
  ylab("Negative Log Adjusted P value")+
  xlab("Log base 2 FC")+
  ylim(0,10)+
  theme_minimal()
```



```
ggsave("case_v_con.png", width=10, height=6)
```

## Tabular Views

```
tab_con<-control%>%
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "lowdiff")))%>%
  mutate(abslog2fc = abs(log2fc))%>%
  filter(newdiff!="lowdiff")%>%
  group_by(newdiff)%>%
  slice_max(n=5, order_by=abslog2fc)%>%
  select(Position=X1, geneID, idlabel, log2fc, padj, direction=newdiff)
```

```
tab_con%>%
  filter(direction=="Down")
```

Position	geneID	idlabel	log2fc	padj	direction
12	ENSG00000133872.14	6807	-4.358153	0.0000031	Down
30	ENSG00000168497.5	12391	-4.112331	0.0001870	Down
45	ENSG00000152558.15	9567	-3.813681	0.0005698	Down
8	ENSG00000136156.15	7234	-3.793525	0.0000015	Down
109	ENSG00000210194.1	21614	-3.649254	0.0025375	Down

```
tab_con%>%
  filter(direction=="Up")
```

Position	geneID	idlabel	log2fc	padj	direction
14	ENSG00000270090.5	52536	4.467033	0.0000047	Up
4	ENSG00000185818.8	16089	4.348400	0.0000003	Up
6	ENSG00000226869.6	27560	3.908796	0.0000012	Up
9	ENSG00000160678.12	10523	3.839950	0.0000023	Up
19	ENSG00000261857.7	48734	3.693108	0.0001029	Up

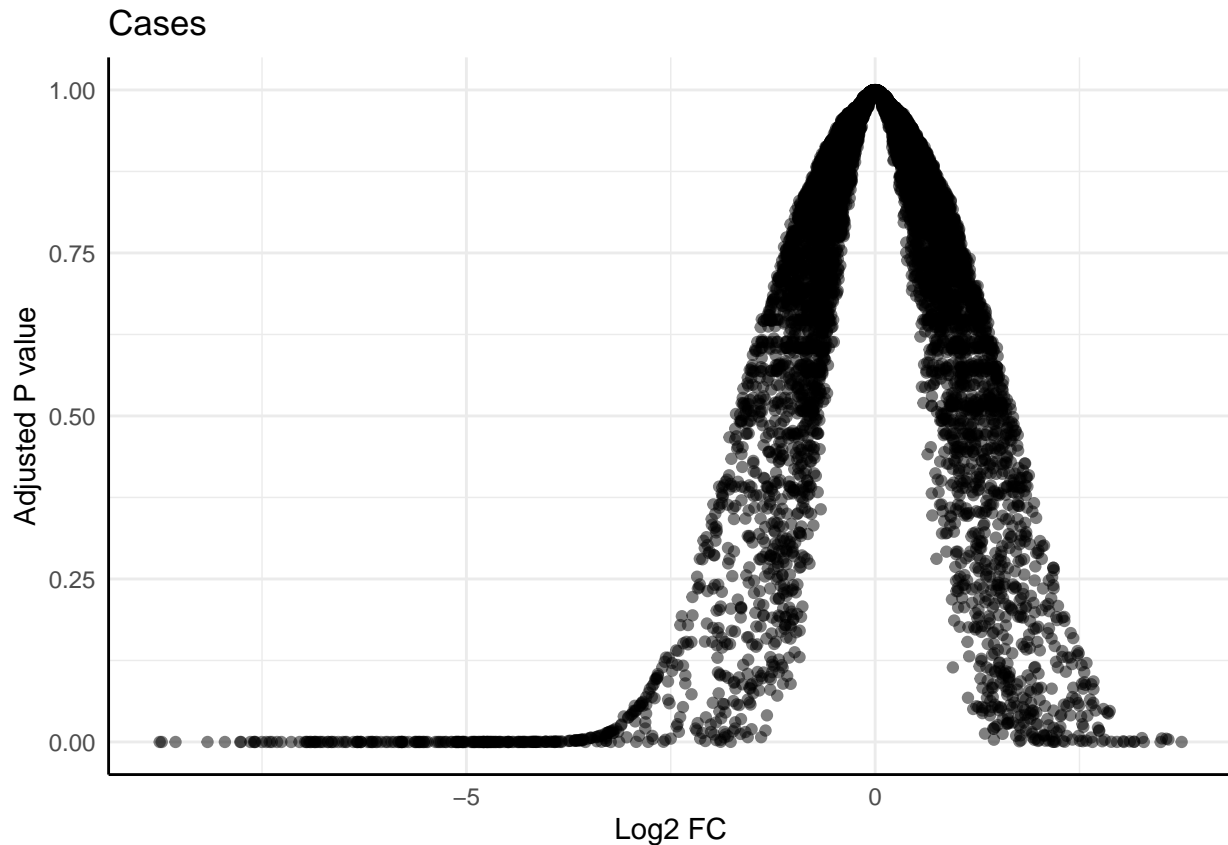
```
control%>%
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "lowdiff")))%>%
  mutate(abslog2fc = abs(log2fc))%>%
  dplyr::group_by(newdiff)%>%
  dplyr::summarize(count=n())%>%
  tidyr::pivot_wider(names_from=newdiff, values_from=count)%>%
  select(Down, `No Significant Change`=lowdiff, Up)
```

Down	No Significant Change	Up
236	11845	119

## Good vs Bad Prognosis R

### Volcano Plot

```
ggplot(data=cases, aes(x=log2fc, y=padj)) +
  geom_jitter(alpha=0.5)+
  ggtitle("Cases")+
  xlab("Log2 FC")+
  ylab("Adjusted P value")+
  ggsave("bad_v_good_volcano.png", width=10, height=6)
```



## Up vs Down Regulated Plot

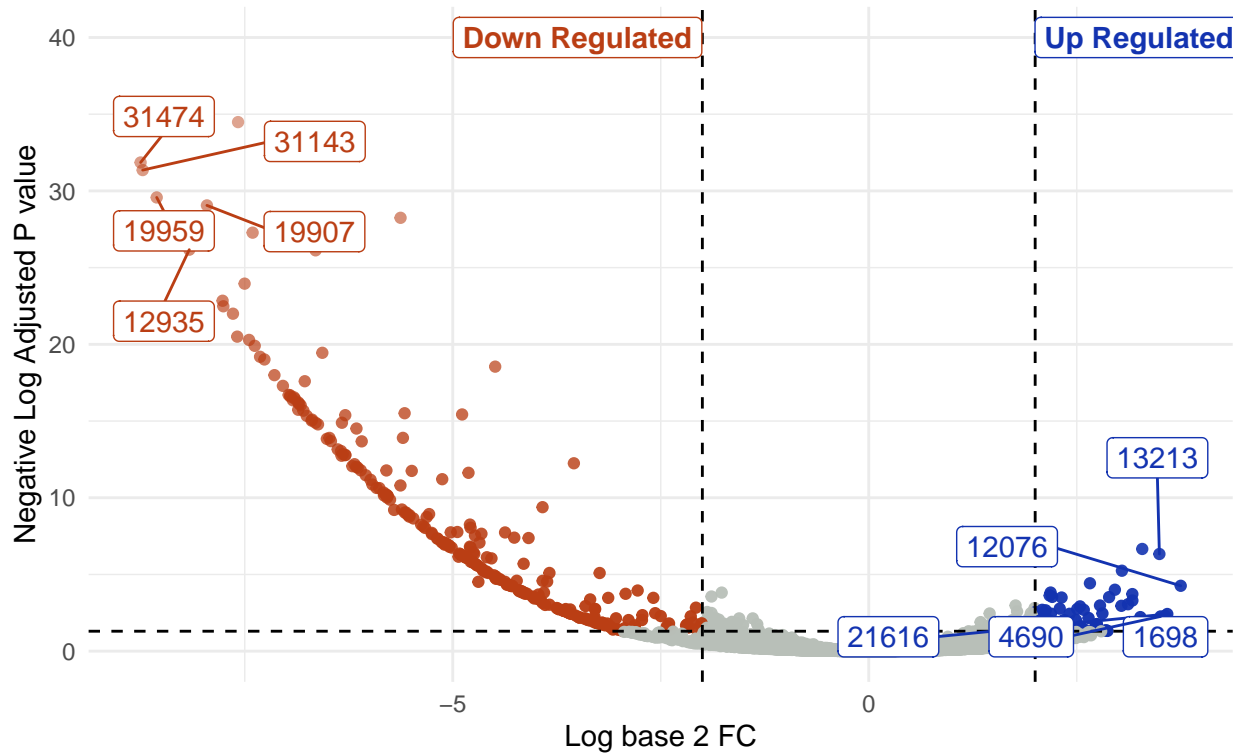
```
tab_case<-cases%>%
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "lowdiff")))%>%
  mutate(abslog2fc = abs(log2fc))%>%
  filter(newdiff!="lowdiff")%>%
  group_by(newdiff)%>%
  slice_max(n=5, order_by=abslog2fc)%>%
  select(Position=X1, geneID, idlabel, log2fc, padj, direction=newdiff)

cases%>%
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",
                           ifelse((log2fc < -2 & padj < 0.05), "Down", "Low-diff")))%>%
  mutate(newidlabel=ifelse(idlabel %in% tab_case$idlabel, idlabel, NA))%>%
  ggplot(aes(x=log2fc, y=-log10(padj), col=newdiff, label=newidlabel)) +
  geom_point(aes(alpha=log10(padj)), show.legend=F) +
  geom_vline(xintercept=c(-2, 2), col="black", linetype = "dashed") +
  geom_hline(yintercept=-log10(0.05), col="black", linetype = "dashed")+
  annotate("label", x = -3.5, y = 40, label = "Down Regulated", color="#ba3e14", fontface = "bold")+
  annotate("label", x = 3.25, y = 40, label = "Up Regulated", color="#1333b0", fontface = "bold")+
  ggrepel::geom_label_repel(min.segment.length = unit(0, 'lines'), box.padding=0.65, show.legend=F)+
  scale_color_manual(values=c("#ba3e14", "#b8bfb8", "#1333b0"))+
  labs(title="Differential Gene Expression in Melanoma Patients", subtitle="Bad Prognosis vs Good Prognosis")+
  ylab("Negative Log Adjusted P value")+
  xlab("Log base 2 FC")
```

```
ylim(0,40)+
theme_minimal()+
ggsave("bad_v_good.png", width=10, height=6)
```

## Differential Gene Expression in Melanoma Patients

Bad Prognosis vs Good Prognosis



## Tabular Views

```
tab_case%>%
  filter(direction=="Down")
```

Position	geneID	idlabel	log2fc	padj	direction
3	ENSG00000231925.12	31474	-8.751542	0	Down
4	ENSG00000231500.7	31143	-8.726711	0	Down
5	ENSG00000204592.9	19959	-8.558053	0	Down
9	ENSG00000170889.14	12935	-8.166531	0	Down
6	ENSG00000204469.13	19907	-7.954339	0	Down

```
tab_case%>%
  filter(direction=="Up")
```

Position	geneID	idlabel	log2fc	padj	direction
167	ENSG00000167286.10	12076	3.749711	0.0000548	Up
248	ENSG00000210196.2	21616	3.587957	0.0037827	Up
251	ENSG00000086548.9	1698	3.561389	0.0049815	Up
255	ENSG00000117228.10	4690	3.508025	0.0054362	Up

Position	geneID	idlabel	log2fc	padj	direction
125	ENSG00000172005.11	13213	3.493104	0.0000005	Up

```
cases%>%
  mutate(newdiff = ifelse((log2fc > 2 & padj < 0.05), "Up",
                          ifelse((log2fc < -2 & padj < 0.05), "Down", "lowdiff")))%>%
  mutate(abslog2fc = abs(log2fc))%>%
  dplyr::group_by(newdiff)%>%
  dplyr::summarize(count=n())%>%
  tidyr::pivot_wider(names_from=newdiff, values_from=count)%>%
  select(Down, `No Significant Change`=lowdiff, Up)
```

Down	No Significant Change	Up
267	9872	59