

Note: the bioinformatics scripts conclude shell and R scripts which are given in directories named “shell_scripts” and “R_scripts” respectively. R custom functions used in R scripts are listed in directory named “R_functions”. Please source the related functions before performing R script. In this text, shell scripts, R scripts, R custom functions and figure names are marked by green, orange, blue and red color respectively to facilitate understanding. In addition to, the provided scripts here may require additional steps and modifications to perform well on different platforms.

1.analysis of RNA-seq data for *atino80-5* mutant at white light

1)trim adapters, map reads to genome, sort bam files, get bigwig files and align reads to genes

sh **RNAseq_paired_stranded.sh**

2)get differentially expressed genes comparing mutant with wild-type

Rscript **RNAseq_DEG_atino80_WL.r**

2.overlap between AtINO80-regulated and light-induced genes for **Figure 3A-3C**

1)use R to get venn diagram and p value for two sets of genes, analyze GO functions for overlap genes

source("**OverlapPlot_function.r**")

OverlapPlot("light_induced_genes.xls","WLino80_diff.xls","light_induced","atino80_diff","atino80-diff and light_induced")

2)use R to get bubble plot for GO analysis result

source("**GOBubblePlot_function.r**")

BubblePlot("GO_terms_WL.txt",c(0,50),c(1,18),c(1,3,6,9))

3.analysis of RNA-seq data for *atino80-6* mutant at dark

1)trim adapters, map reads to genome, sort bam files, get bigwig files and align reads to genes

sh **RNAseq_paired_unstranded.sh**

2)get differentially expressed genes comparing mutant with wild-type

Rscript **RNAseq_DEG_atino80_dark.r**

4. overlap between AtINO80-regulated and dark-induced genes for **Figure 3D-3F**

1)use R to get venn diagram and p value for two sets of genes, analyze GO functions for overlap genes

source("**OverlapPlot_function.r**")

OverlapPlot("dark_induced_genes.xls","Darkino80_diff.xls","dark_induced","atino80_diff","atino80-diff and dark_induced")

2)use R to get bubble figure for GO analysis result

source("**GOBubblePlot_function.r**")

BubblePlot("GO_terms_Dark.txt",c(0,20),c(1,14),c(0,3,6))

5.analysis of ChIP-seq data for *atino80-5* mutant at white light

1)map reads to genome, sort bam files, remove duplicates, get bigwig files and bed files

sh **ChIPseq_paired_nocut.sh**

2) get fragment size for **Figure S8**

sh **FragmentSize.sh**

3) find enriched regions (peaks) of histone variants for H2A.Z and H3 (a routine method considering input as background control)

sh **PeaksSicer.sh**

4) find overlap peaks between two replicates by R

```
source("PeakOverlap_function.r")
```

```
Peakover("sig_WT1_H2AZ_repeat1.bed", "sig_WT1_H2AZ_repeat2.bed", "WT1_repeat1",  
WT1_repeat2", "WT1_H2AZ_repeats_overlap_peaks")
```

```
Peakover("sig_ino80_H2AZ_repeat1.bed", "sig_ino80_H2AZ_repeat2.bed", "ino80_repeat1",  
"ino80_repeat2", "ino80_H2AZ_repeats_overlap_peaks")
```

```
Peakover("sig_WT1_H3_repeat1.bed", "sig_WT1_H3_repeat2.bed", "WT1_repeat1", "WT1_r  
peat2", "WT1_H3_repeats_overlap_peaks")
```

```
Peakover("sig_ino80_H3_repeat1.bed", "sig_ino80_H3_repeat2.bed", "ino80_repeat1", "ino8  
0_repeat2", "ino80_H3_repeats_overlap_peaks")
```

5) get merged bam files, bigwig files and bed files

sh **merge.sh**

6. analysis of published ChIP-seq data (GSE108450)

1) download data from NCBI, map reads to genome, sort bam files, remove duplicates and get bigwig files and bed files

sh **ChIPseq_single_nocut.sh**

2) find enriched regions (peaks) of H2A.Z (a routine method considering input as background control)

sh **PeakSicer_GSE108450.sh**

3) find overlap peaks between two replicates by R

```
source("PeakOverlap_function.r")
```

```
Peakover("sig_SRR6412321.bed", "sig_SRR6412322.bed", "WT1_repeat1", "WT1_repeat2", "  
WT1_H2AZ_repeats_overlap_peaks")
```

7. compare H2A.Z peaks in our ChIP-seq data with published data for **Figure S5C**

```
source("PeakOverlapTest_function.r")
```

```
Peakover("WT1_H2AZ_repeats_overlap_peaks_nochr.bed", "WT1_H2AZ_repeats_overlap_  
peaks_nochr.bed", "our", "published", "WTH2AZ_enriched_overlap_peaks_our_published")
```

8. fragment size for **Figure S5B**

sh **FragmentSize.sh**

9. normalized reads density of histone variants (H2AZ and H3) across genes for **Figure 4A-4B and 5A-5C**

1) get matrix of normalized reads density on gene body, around TSS and TES

sh **matrix.sh**

2) get matrix for heatmap by R

Rscript **HeatmapMatrix.r**

```
89 3)plot heatmap
90   sh HeatmapPlot.sh
91 4)plot average density profile by R
92   Rscript PlotProfile.r
93
```

10.differentially enriched regions of histone modifications (H2A.Z and H3; a routine method considering input as background control) for **Figure 4C-4E** and **Figure S6.**

1)get fold change of histone variants (H2AZ and H3) on enrichment regions (peaks) in mutant and wild-type

```
97   sh PeaksSicerDiff.sh
```

2)find differentially enriched regions by R

```
101 source("DiffPeak_function.r")
102 DiffPeak1("H2AZ","FCH2AZ_merge_nochr.summary",1.2,0.05)
103 DiffPeak1("H3","FCH3_merge_nochr.summary",1.2,0.05)
```

3)annotate differentially enriched regions by R

```
105 source("PeakAnnotate_function.r")
106 Peakanno("H2AZ_sigPeakdown.xls","H2AZ_downpeak")
107 Peakanno("H2AZ_sigPeakup.xls","H2AZ_uppeak")
108 Peakanno("H3_sigPeakdown.xls","H3_downpeak")
109 Peakanno("H3_sigPeakup.xls","H3_uppeak")
```

4)plot venn diagram and perform GO analysis for the overlap between H2A.Z, H3 diff-peaks and AtINO80-regulated genes

```
112 source("VennPlot1_function.r")
113 VennPlot1("H2AZ_diffpeak_annoOvgene_genelist.xls","WLino80_diff.xls","H2AZ_diff","
114 atino80-diff","H2AZdiff_and_atino80diff")
115 VennPlot1("H3_diffpeak_annoOvgene_genelist.xls","WLino80_diff.xls","H3_diff","atino80
116 -diff","H3diff_and_atino80diff")
```

5)plot venn diagram for the overlap between H2A.Z diff-peaks and H3 diff-peaks

```
118 source("VennPlot2_function.r")
119 VennPlot2("H2AZ_diffpeak_annoOvgene_genelist.xls","H3_diffpeak_annoOvgene_genelist
120 .xls","H2AZ_diff","H3_diff","H2AZdiff_and_H3diff")
121
```

11.differentially enriched regions of H2A.Z/H3 (considering H3 as background control) for **Figure 4F**

1)find differentially enriched regions of H2A.Z considering H3 as background control

```
125   Rscript DiffBind_H2AZvsH3.r
```

2)plot MAplot by R

```
127   Rscript MAplot.r
```

3)annotate differentially enriched regions

```
129 source("PeakAnnotate_function.r")
130 Peakanno("H2AZ_H3_Down_nochr.xls","H2AZ:H3_downpeak")
131 Peakanno("H2AZ_H3_Up_nochr.xls","H2AZ:H3_uppeak")
132
```

**12.enrichment analysis of H2AZ/H3 differentially enriched regions on DEGs in
atino80-5 for Figure 4G and 4H**

```
source("EnrichmentAnalyze_function.r")  
EnrichAna("H2AZ:H3_downpeak_annoOvgene_genelist.xls","WLino80_up.xls","downH2  
AZ:H3","up-regulated genes","H2AZ:H3down_genes_with_upregulated")  
EnrichAna("H2AZ:H3_downpeak_annoOvgene_genelist.xls","WLino80_down.xls","down  
H2AZ:H3","down-regulated genes","H2AZ:H3down_genes_with_downregulated")  
EnrichAna("H2AZ:H3_uppeak_annoOvgene_genelist.xls","WLino80_up.xls","upH2AZ:H3  
","up-regulated genes","H2AZ:H3up_genes_with_upregulated")  
EnrichAna("H2AZ:H3_uppeak_annoOvgene_genelist.xls","WLino80_down.xls","upH2AZ:  
H3","down-regulated genes","H2AZ:H3up_genes_with_downregulated")
```