

ngsplot: Hands-on

Goal: Run ngsplot tool to generate plots (average profile and heatmap) to show/find enrichment of interest.

Datasets for the hands-on exercises are from ngsplot website and/or ENCODE project.

Setup Files Needed

Create symbolic links (symlinks) to the needed files,

```
ln -s /nfs/BaRC_Public/Hot_Topics/ngsplot_Apr2014/demo/* .
```

Hands-on Exercises

I. List ngsplot available genomes installed

```
ngsplotdb -y list
```

II. Average profile plots and heatmaps

a. H3K4me3 enrichment near TSS

```
bsub ngs.plot.r -G hg19 -R tss -C hesc.H3k4me3.1M.bam -O  
hesc.H3k4me3vsInp.tss -T H3K4me3 -L 3000 -FL 300
```

➤ Note: you can view the output (pdf) files from the *tak* command line as well
eg. `evince myFile.pdf`

b. H3K27me3 distribution across the gene body

```
bsub ngs.plot.r -G hg19 -R genebody -C  
wgEncodeSydhHistoneK562bH3k27me3bUcdAlnRep1.bam -O  
hesc.k27.genebody -D ensembl -FL 300
```

c. Multiple plots on the same graph with subsets of gene lists

```
bsub ngs.plot.r -G hg19 -R genebody -C config.hesc.k4.txt -O  
hesc.k4.genebody -D ensembl -FL 300
```

➤ Note: here a config (text) file is used with the “-C” option.

d. TF binding site (TFBS) near the TSS

```
bsub ngs.plot.r -G hg19 -R tss -C config.erythropoiesis.txt -O  
gata2_eryth.tss -D ensembl -L 1000 -FL 200
```

➤ Gene list from PMID: 21270440

e. H3K4me3 in custom repeats regions

```
bsub ngs.plot.r -G hg19 -C hesc.H3k4me3.1M.bam -O  
hesc.H3k4me3vsInp.Repeats -T H3K4me3 -L 3000 -FL 300 -R bed -E  
UCSC_RepeatMaskerRepeats.bed
```

f. Small RNA-Seq coverage

```
bsub ngs.plot.r -G hg19 -R genebody -C  
GSM605626_hg19_wgEncodeCshlShortRnaSeqProstateCellTotalAln.bam -O  
ShortRNASeq_Prostate.genebody -T SmallRNASeq -L 1000 -FL 200
```