Visualization of Reads (IGV)

The Integrative Genomics Viewer (IGV) is a high-performance viewer that efficiently handles large heterogeneous data sets [13]. We are going to use this tool to visualize aligned reads on genome and the differential methylated sites.

Visualization of Aligned Reads

Upload BAM and its index (.bai) file to IGV web application and zoom in to see the aligned reads.

Visualization of Methylation Sites

1. Install IGV

```
# Java of version 11 is required.
$ java -version

# Download and install from
http://software.broadinstitute.org/software/igv/download
$ wget https://data.broadinstitute.org/igv/projects/downloads/2.9/IGV_2.9.2.zip
$ unzip IGV_2.9.2.zip
$ cd IGV_2.9.2
$ nohup bash igv.sh

# Verify installation
$ igvtools version
```

2. Generate TDF

```bash
#!/bin/bash
Data="/path/to/homo_result"
Output="/path/to/homo_igv"

for s in SRR5978827 SRR5978828 SRR5978829 SRR5978834 SRR5978835 SRR5978836
SRR5978869 SRR5978870 SRR5978871 SRR5179446 SRR5179447 SRR5179448
do
igvtools count --strands read -z 5 -w 10 -e 0 $Data/${s}_sorted.bam
$Output/${s}.tdf homo_genome.fa
wait
done
```

```bash
#!/bin/bash
Data="/path/to/mm10_result"
Output="/path/to/mm10_igv"

for s in SRR866997 SRR866998 SRR866999 SRR867000 SRR867001 SRR867002 SRR866991
SRR866992 SRR866993 SRR866994 SRR866995 SRR866996
do
igvtools count --strands read -z 5 -w 10 -e 0 $Data/${s}_sorted.bam
$Output/${s}.tdf mm10_genome.fa
wait
done
```

3. Run IGV

The generated TDF files and BED file can then be visualized using IGV browser. As clearly shown in the figure below zooming in a region of an antisense gene , the exomePeak2 has the ability to distinguish methylation sites on specific strands.

p22.2　　p21.3　p21.2　　　p15.3　　p15.1　　p14.2　　　p13　　p12.2　　p11.2　q11.1　　　q

000 bp　　　　　27,211,000 bp　　　　27,212,000 bp　　　　27,213,000 bp　　　27,214

SRR5978827(input)
reads from + fragment
[0 - 15]

SRR5978827(input)
reads from - fragment
[0 - 15]

SRR5978828(input)
reads from + fragment
[0 - 15]

SRR5978828(input)
reads from - fragment
[0 - 15]

SRR5978834(IP)
reads from + fragment
[0 - 15]

SRR5978834(IP)
reads from - fragment
[0 - 15]

SRR5978835(IP)
reads from + fragment
[0 - 15]

SRR5978835(IP)
reads from - fragment
[0 - 15]

Mod_neg.bed

peak_239

Mod_pos.bed

peak_292

RefSeq Genes

HOXA10-AS