Shyam Biswal : MeDIP on 2.1M Nimblegen Array

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Analysis

Data from Shyam Biswal, used to compare the differential methylation regions of H460 Knocking cell line and H460 parent cell line.

Data Description

Data was generated from two color Nimblegen microarrays. The raw Nimblegen data was in the form of `.tif` images and the corresponding array design files were given for building the annotation package `100929_HG19_Deluxe_Prom_Meth_HX1` with `pdInfoBuilder`.

Array images were processed with DEVA-v1.2 (Nimblegen software for automated feature extraction and data analysis). The TIF files were processed and converted to `.xys` files for analysis. The TIF files were also processed with DEVA using the DNA methylation work flow to identify peaks and generate a result for each sample.

Preliminary Assessments

Analysis using CHARM

Initial array quality assessment was done using `charm` and the `.xys` files.

Array quality scores were generated with `charm::qcReport`, and data quality was checked using the *Enriched* channel. Four of the six arrays show a large standard deviation in the signal strenth and seem to have a problem in hybridization. `charm::pmQuality`, provides the array signal quality score. 3 of the samples have a signal strength above the cutoff(70).

`Refer: qcReport.pdf`

Charm fails to find DMRs both while taking into account surrogate variables(SV) and not while not accounting for SV's. This might be because the image quality is poor after hybridization with a lot of variability in 3 out of the 6 images. The case control being used is the H460_parent vs H460_knockin. No other annotation is recorded in the experimental metrics `.xlsx` file given.

Analysis using Bumphunter

1. Run 1

Initially ran `bumphunter` on the data, with cutoff value 1.0, this failed to find any bumps. The sensitivity of the cutoff was not enough to catch any DMRs in the sample set. Bumphunter is better used for large sample sets, for better performance.

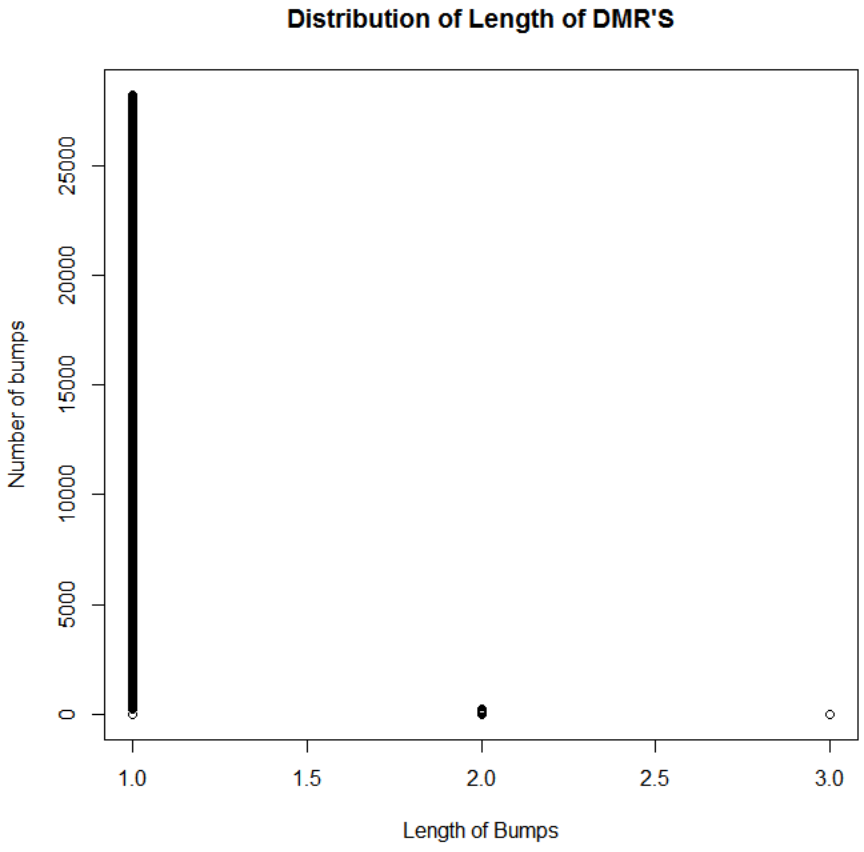
2. Run 2

   Bumphunter, with the inbuilt argument to pickCutoff was run. The cutoff chosen by bumphunter was at 0.41, bumps found 28206. But just like other nimblegen data sets, it is unable to match genes, as the number of bumps of length greater than 4 is 0.

   bumps.rda file attached. The distribution of bumps found, is shown below. Most of them are single CpG sites of length 1. This does not work for a significant analysis.

```
>  table(bumps$L)

 1       2     3
27981   222     3
```




```
Argument description for bumphunter,

cutoff:
```

```
A numeric value. Values of the estimate of the genomic profile above the cutoff or
below the negative of the cutoff will be used as candidate regions. It is possible to
give two separate values (upper and lower bounds). If one value is given, the lower
bound is minus the value.

pickCutoff:

Should bumphunter attempt to pick a cutoff using the permutation
distribution?
```

Analysis using Peaks files from DEVA

This analysis uses the Peaks files generated from DEVA-v1.2, using the standard Nimblegen algorithms to identify peaks which coincide with methylated regions.

Sequential intersections, based on the number of peaks identified in each sample, was done in decreasing order for each experimental status. This step results in all the genes within each sample which are intersecting.

We find the common genes between H460_knocking and H460_parent", and remove these genes from the intersected lists. We then order them by distance from the transcription start site(TSS).

```
NOTE: Files are attached as knockin_genes_distFromTSS.csv and
parent_genes_distFromTSS.csv.  The distance is ordered in decreasing order of
since the Max distance is a smaller positive number.
```

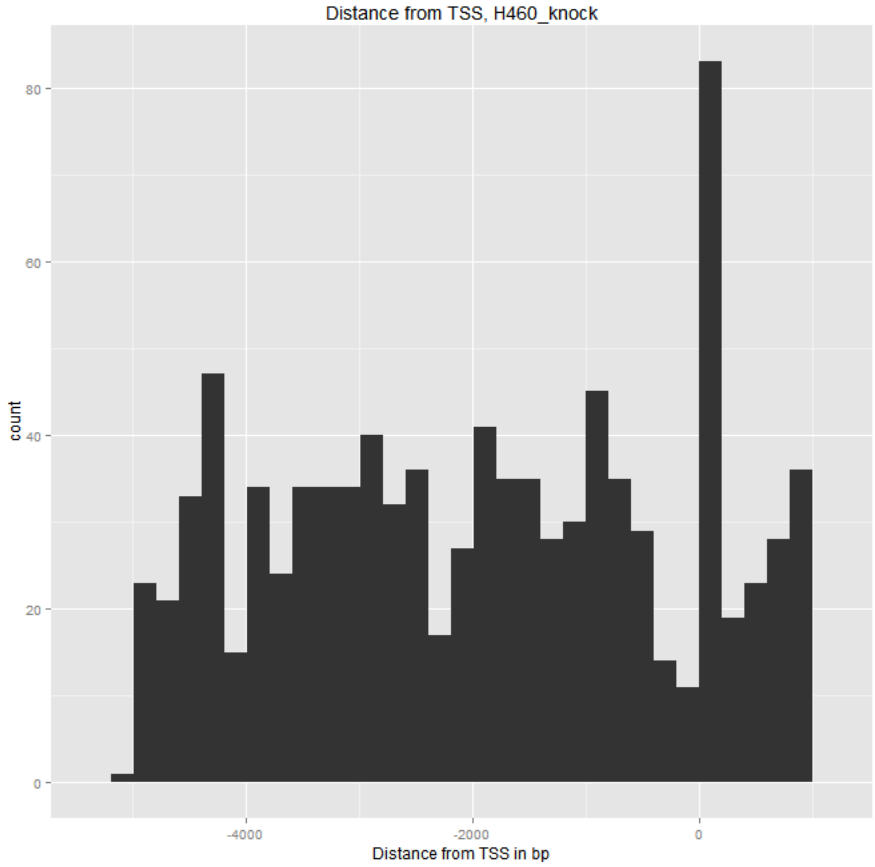
The only features left in the result are transcription start sites, other possible feature is CpG Island (column name is FEATURE TRACK). The distribution of the distance from the TSS is also shown for both status types. The window for the Distance from the TSS is measured by -5000 to +5000 , so window size is 10,000 bp (column name is SHORTEST_DISTANCE_FROM_FEATURE_TO_DATA_POINT).

H460_knocking

```
> table(annot_knock$FEATURE_TRACK)

transcription_start_site
                     944

> summary(annot_knock$SHORTEST_DISTANCE_FROM_FEATURE_TO_DATA_POINT)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
-4998.0 -3363.0 -1916.0 -1956.0  -569.8   995.0
```

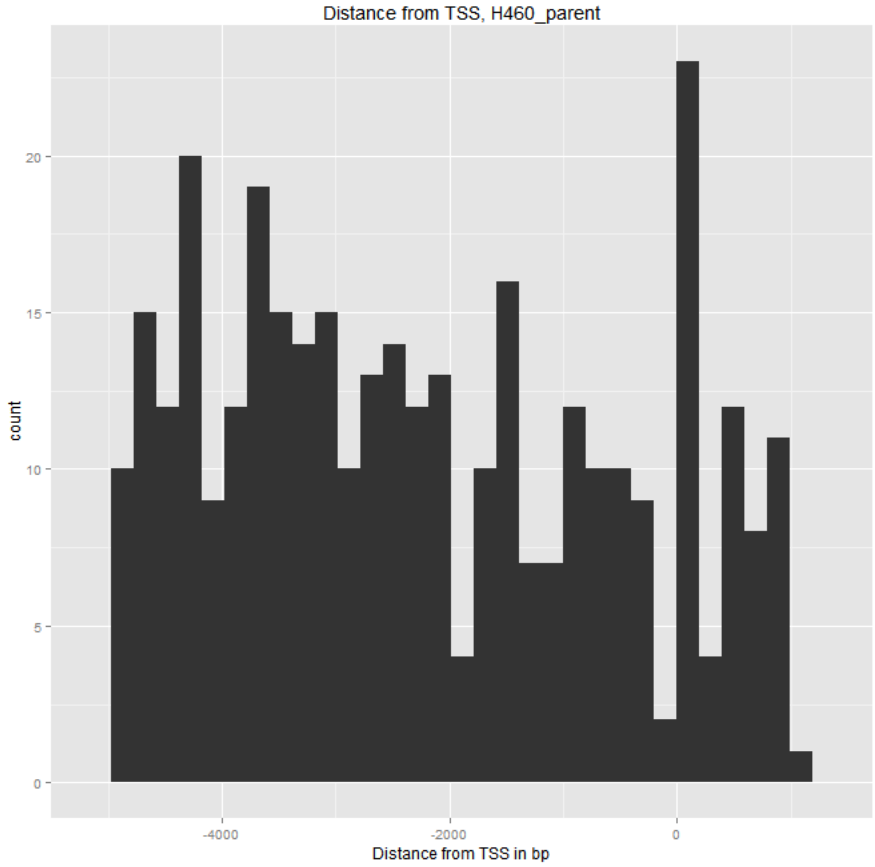


H460_parent

```
> table(annot_parent$FEATURE_TRACK)

transcription_start_site
                     349

> summary(annot_parent$SHORTEST_DISTANCE_FROM_FEATURE_TO_DATA_POINT)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  -4975   -3669   -2474   -2223    -718     997
```



Algorithms and R-packages

List of R-packages used for analysis:

1. Charm
2. Bioconductor
3. BiocGenerics
4. RCircos

Extracting the percentage methylation values from the raw data was done using `charm::methp`, where the default arguments were used to normalize. The normalization methods included were spatial normalization(to correct for spatial artifacts), background subtraction(to estimate and remove the background signal before computing the log-ratios), loess within sample normalization and quantile between sample normalization.

These percentage methylation values are on a logit scale.

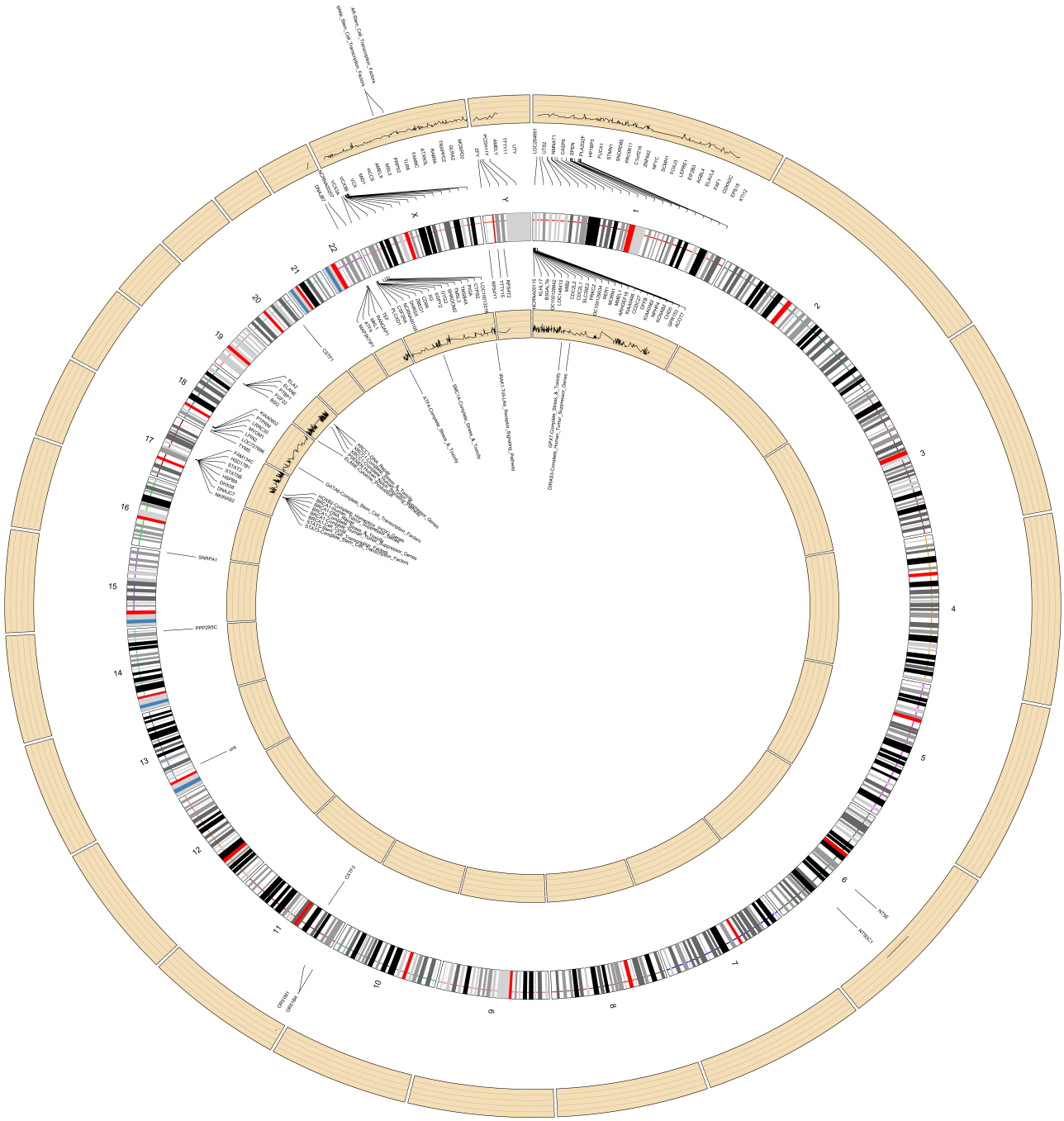
A regression based DMR-finding after correcting for batch effects pipeline was used in this analysis. Removing batch effects and using surrogate variables in finding DMRs (`refer sva-package`) have been shown to reduce dependence on unknown noise in the data set.

Comparisons

Plots and Results

Circos plot was made using RCircos, the inner track refers to the H460 knocking genes and the outer track refers to the H460 Parent genes.

As it can be seen from the circos plot, very few chromosomes contribute to the methylated regions, including the sex chromosomes. The plot next to the gene names correspond to the peak values. It is easy to infer from this plot where there is a high frequency of methylated regions.



References

1. Aryee MJ et al., Accurate genome-scale percentage DNA methylation estimates from microarray data, Biostatistics (2011) 12(2): 197-210

2. Seth Falcon, Benilton Carvalho with contributions by Vince Carey, Matt Settles and Kristof

de Beuf. pdInfoBuilder: Platform Design Information Package Builder. R package version 1.24.0.

3. Rafael A. Irizarry, Martin Aryee, Hector Corrada Bravo, Kasper D. Hansen and Harris A. Jaffee (). bumphunter: Bump Hunter. R package version 1.0.0.