Merging regions

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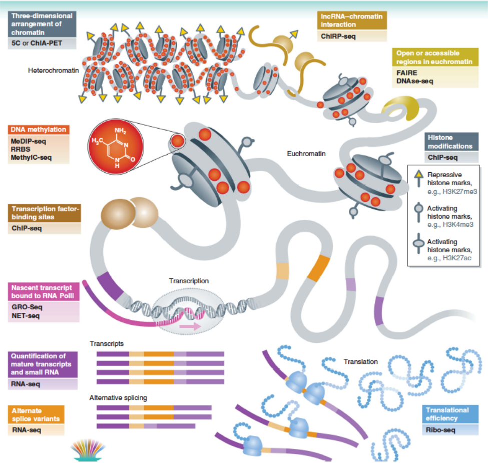
High-Throughput Genomics Panorama[1]


Three-dimensional arrangement of chromatin
5C or ChIA-PET
Heterochromatin
lncRNA–chromatin interaction
ChIRP-seq
Open or accessible regions in euchromatin
FAIRE
DNAse-seq
DNA methylation
MeDIP-seq
RRBS
MethylC-seq
NH_2
H_3C
N
N
H
O
Euchromatin
Histone modifications
ChIP-seq
Repressive histone marks, e.g., H3K27me3
Activating histone marks, e.g., H3K4me3
Activating histone marks, e.g., H3K27ac
Transcription factor-binding sites
ChIP-seq
Transcription
Nascent transcript bound to RNA PolII
GRO-Seq
NET-seq
Transcripts
Quantification of mature transcripts and small RNA
RNA-seq
Alternative splicing
Alternate splice variants
RNA-seq
Translation
Translational efficiency
Ribo-seq

[1] Wendy Weijia Soon, Manoj Hariharan, and Michael P. Snyder. “High-throughput sequencing for biology and medicine”. In: *Molecular Systems Biology* 9.1 (). UR
http://www.nature.com/msb/journal/v9/n1/fig_tab/msb201261_F2.html (visited on 03/05/2013).

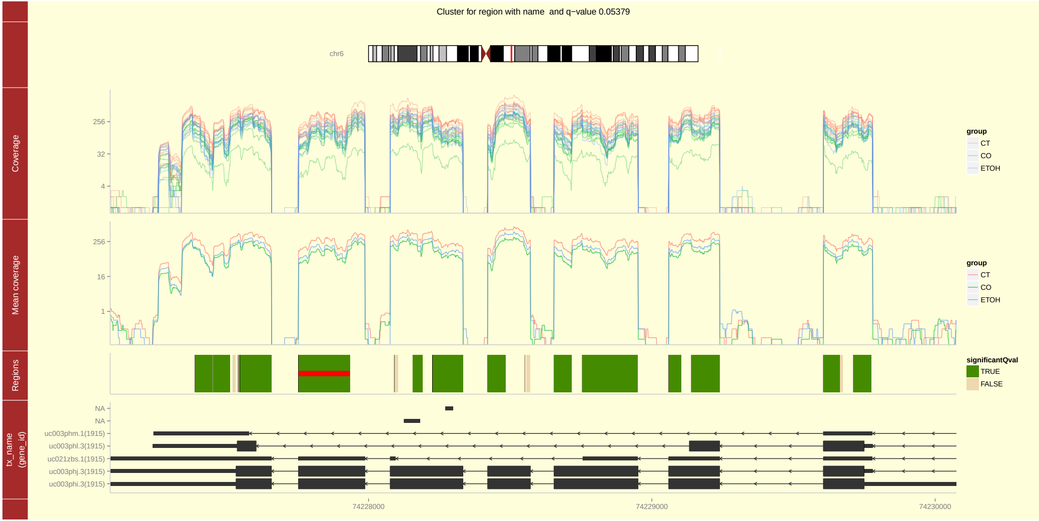
What is common?

- Measurements along the genome (sometimes summarized)
- Two or more groups of samples
- Typical question: are there differences between the groups?
    - → Find the candidate regions.

Issue: regions might be highly fragmented.
Why?

1. Biological reasons: regions correspond to two exons (intron is the cause).
2. Measurement not ideal: coverage dips.

Example region cluster (by distance)



Question

Are two adjacent regions *similar*?

1. Can we *link* them?
2. Are regions overlapping the same exon more frequently *linked*?

Translating framework

- What is measured?
  - Coverage $=: Y$
  - Transformed: $\log_2(Y + 32)$
- Individual (cluster of measurements) → sample
- Repeated visits → individual base pairs (from a given chromosome)
  - Note that the data is correlated!

Consider a region pair:

1. region1: first region
2. regionM: middle part
3. region2: second region

Proposed method

Model for sample i:

$$\log_2(Y_{ijk} + 32) = \alpha + \beta_1 \text{sampleDepth}_i + \boldsymbol{\beta}_2 \text{group}_j + \boldsymbol{\beta}_3 \text{region}_k + \epsilon$$

Using *region1* as the reference, we want to test $\boldsymbol{\beta}_3\,(\text{region}_2) = 0$.

Data sets used

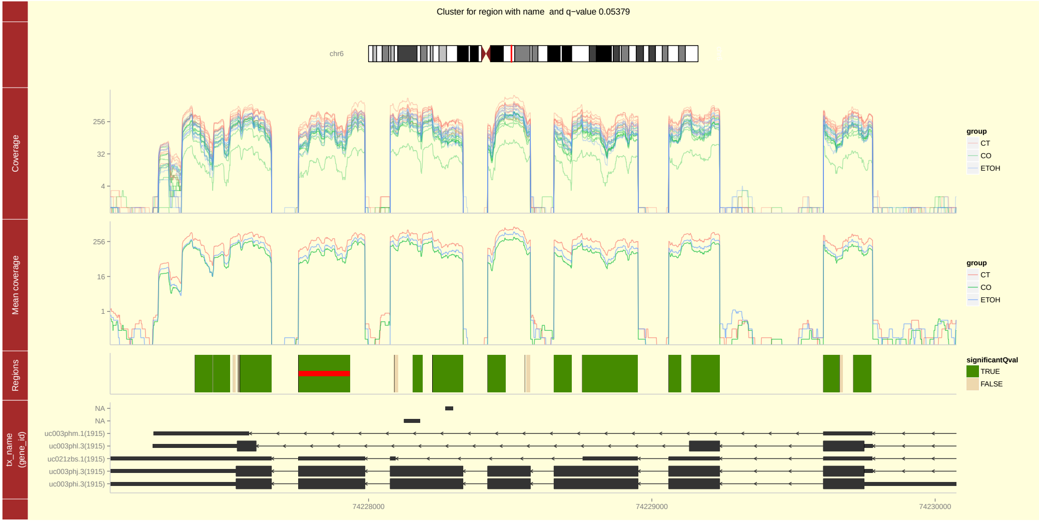
- **derHippo**: RNA-seq brain hippocampus study
- 25 samples
- 3 groups: CO, ETOH and CT

chr6 890 pairs
300 ($\sim$ 33.7%) with regions 1 & 2 having a width greater than 1, region M $<$ 250

chr22 573 pairs
187 ($\sim$ 32.6%) passing the filtering

Example:
chr 6, chose the largest cluster, then the pair starting with the largest region from the cluster.

Example region cluster



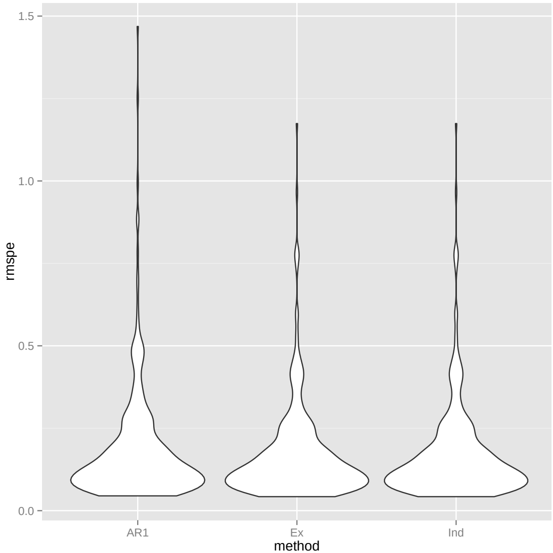
Data

```
pairs[i2, ]

##       start1     end1    startM      endM    start2      end2 cluster
## 552 74227546 74227657 74227658 74227752 74227753 74227934     168
##     width1 widthM width2 widthNoM
## 552    112     95    182      294

dim(covdata[[i2]])

## [1] 9725    6

head(covdata[[i2]], n = 1)

##   base  region sample coverage sampleDepth group
## 1    1 region1    C01    7.401       28.25    C0
```

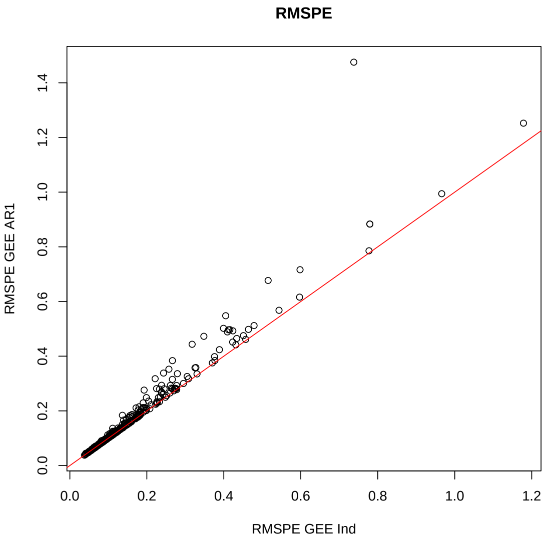
GEE - AR1

```
##
## Call:
## geeglm(formula = coverage ~ sampleDepth + group + region, family = gaussian,
##     data = covdata[[i2]], id = sample, corstr = "ar1")
##
##  Coefficients:
##                Estimate   Std.err   Wald Pr(>|W|)
## (Intercept)   -11.02816   3.69521   8.91   0.0028 **
## sampleDepth     0.66137   0.12836  26.55  2.6e-07 ***
## groupCO        -0.76057   0.10828  49.34  2.2e-12 ***
## groupETOH      -0.33737   0.10847   9.67   0.0019 **
## regionregionM  -1.79501   0.10960 268.25  < 2e-16 ***
## regionregion2  -0.11747   0.00614 366.09  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Estimated Scale Parameters:
##             Estimate Std.err
## (Intercept)    0.458  0.0224
##
## Correlation: Structure = ar1  Link = identity
##
## Estimated Correlation Parameters:
##       Estimate Std.err
## alpha    0.971 0.00691
## Number of clusters:   25   Maximum cluster size: 389
```

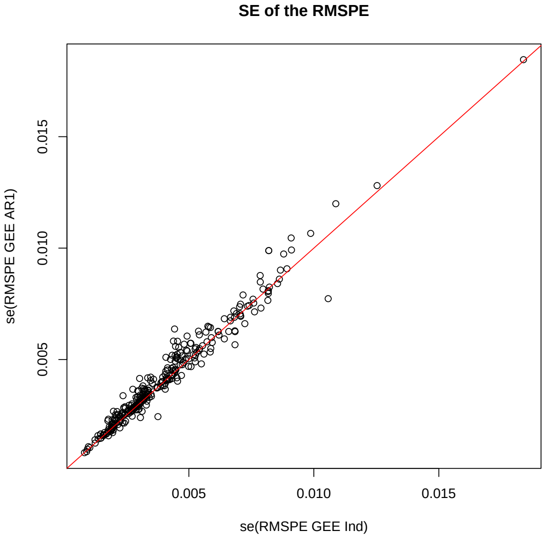
Root mean squared prediction error (RMSPE) chr6



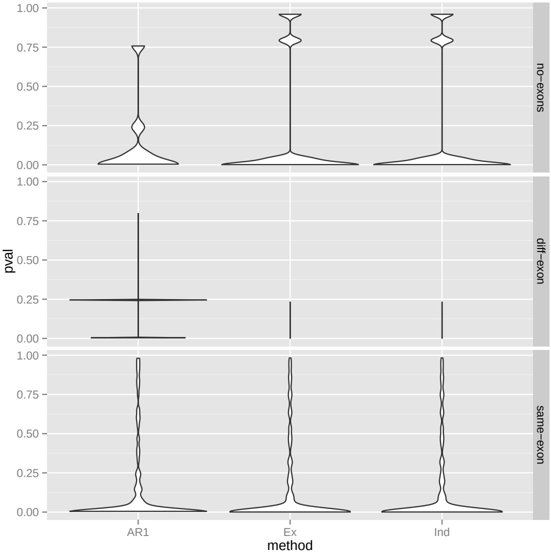
RMSPE chr6: GEE AR1 vs GEE Ind



SE RMSPE chr6: GEE AR1 vs GEE Ind



P-values by exon status: chr6



Test result by exon status

- Adjust for multiple testing by using q-value < 0.10

BothAccept	Ar1Accept	IndAccept	BothReject	chr	ExonStatus
0	1	2	7	chr6	no-exons
0	1	0	13	chr6	diff-exon
19	24	23	210	chr6	same-exon
0	4	1	11	chr22	no-exons
0	0	0	2	chr22	diff-exon
5	15	14	135	chr22	same-exon

Conclusions

- With longer region pairs, fitting GEE takes a significant amount of time.
- GEE with Independence working correlation had lower RMSPE.
- For pairs sharing an exon, 11-20% were linked.

References

- **Project** code and results: `https://github.com/lcolladotor/756final_code`
- A. Frazee, S. Sabunciyan, K. D. Hansen, R. A. Irizarry, and J. T. Leek (2013). Differential expression analysis of rna-seq data at single base resolution, Biostatistics, *recently accepted*.
- L. Collado-Torres, A. Frazee, M. Love, R. A. Irizarry, A. E. Jaffe, J. T. Leek (2013). derfinder: Software for annotation-agnostic RNA-seq differential expression analysis. Manuscript in preparation.
- `derfinder` package `https://github.com/lcolladotor/derfinder`

Thank you!