

R / Bioconductor for Epigenomic Analysis

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R and *Bioconductor*

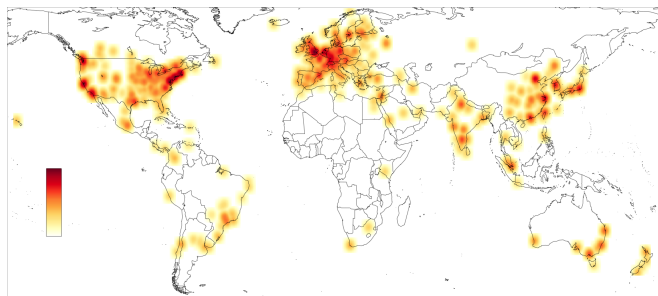
R, <http://r-project.org>

- ▶ Open-source, statistical programming language; widely used in academia, finance, pharma, ...
- ▶ Core language, 'base' and > 5000 contributed packages
- ▶ Interactive sessions, scripts, packages

Bioconductor, <http://bioconductor.org>

- ▶ Analysis and comprehension of high-throughput genomic data
- ▶ Themes: rigorous statistical analysis; reproducible work flows; integrative analysis
- ▶ > 12 years old, 825 packages
- ▶ Courses, conferences (package developers: travel scholarships available!), mailing list, ...

Bioconductor



Trailing 12 month statistics

- ▶ 1702 PubMedCentral full-text citations
- ▶ 9.5M package downloads to 242,000 distinct IP addresses
- ▶ 1M sessions from 400k visitors to web site
- ▶ Annual conferences; courses; active mailing list; ...

Epigenomics

1. Methylation
 - ▶ Illumina 450k arrays
 - ▶ Whole genome and restricted representation bisulfite sequencing
2. ChIP-Seq
3. Integration & visualization
 - ▶ Data types, e.g., expression
 - ▶ Consortium resources via *AnnotationHub*
4. A key data structure: *GRanges*

Find relevant packages using [BiocViews](#), in addition to standard scholarly approaches.

Methylation: selected packages

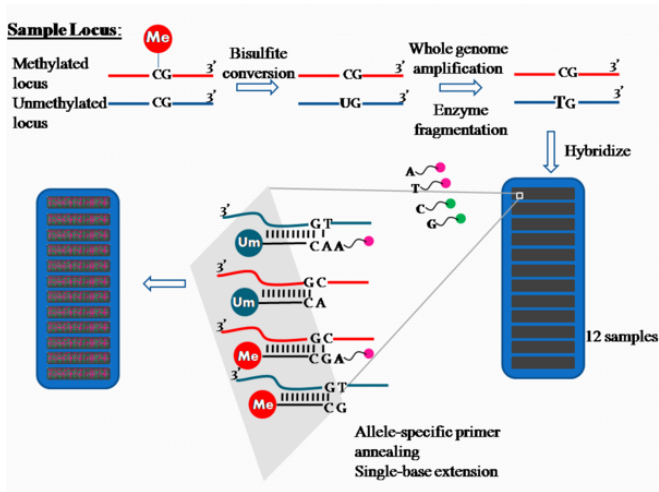
450k arrays

- ▶ *minfi* – pre-processing, differential methylation
- ▶ *ChAMP* – comprehensive work flow

Bisulfite sequencing

- ▶ *bsseq* – whole genome
- ▶ *BiSeq* – restricted representation

Methylation: Illumina 450k arrays



[http:](http://en.wikipedia.org/wiki/Illumina_Methylation_Assay)

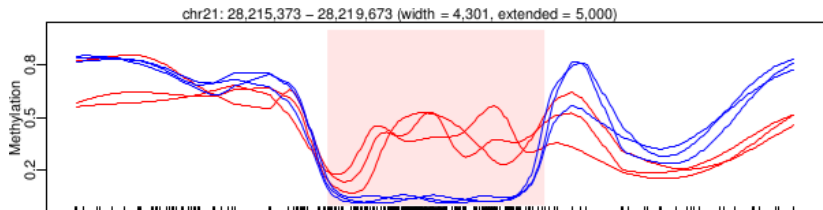
[//en.wikipedia.org/wiki/Illumina_Methylation_Assay](http://en.wikipedia.org/wiki/Illumina_Methylation_Assay)

Methylation: *minfi*

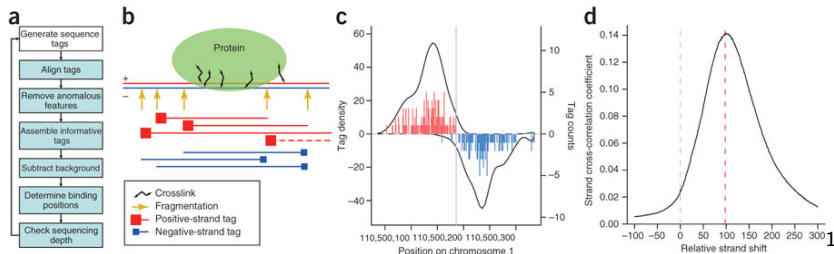
Work flow steps and representative functions

1. Data input: `read.450k.exp()`
2. Quality assessment: `densityPlot()`
3. Pre-processing, e.g., background correction, normalization: `preprocessIllumina()`
4. Differentially methylated probes: `dmpFinder()`
5. Differentially methylated regions: `bumphunter()`

See the [vignette](#) for additional detail.

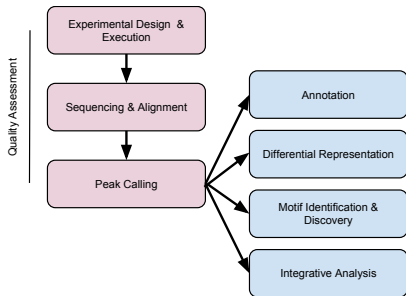


ChIP-seq: peak calling



- ▶ Chromatin immunoprecipitation, followed by sequencing to determine location of proteins bound to DNA
- ▶ Useful for locating transcription factor binding sites, histone modifications, ...

ChIP-seq work flow



Analysis overview: Bailey et al. (2013)

- ▶ Annotation: what genes are my peaks near?
- ▶ Differential representation: which peaks are over- or under-represented in treatment 1, compared to treatment 2?
- ▶ Motif identification (peaks over known motifs?) and discovery
- ▶ Integrative analysis, e.g., association of regulatory elements and expression

ChIP-seq quality assessment: *ChIPQC*

Inputs: BAM files (raw data) and BED files (called peaks)

```
experiment <- ChIPQC(samples)
ChIPQCreport(experiment)
```

Output: HTML report — <http://starkhome.com/ChIPQC/Reports/tamoxifen/ChIPQC.html>

ChIP-seq annotation: *ChIPpeakAnno*, *ChIPseeker*

Inputs

- ▶ Peaks: e.g., from `rtracklayer::import()` BED files
- ▶ Annotation: gene boundaries or queries to *biomaRt*

```
library(ChIPpeakAnno)
## ...
annotated <- annotatePeakInBatch(peaks,
  AnnotationData=annotation)
```

Output: *RangedData* with annotations about near-by peaks.

ChIP-seq differential representation: *DiffBind*

Inputs: called peaks and raw BED or BAM files

```
library(DiffBind)
tamoxifen = dba(sampleSheet="tamoxifen.csv")
tamoxifen = dba.count(tamoxifen)
tamoxifen = dba.contrast(tamoxifen,
  categories=DBA_CONDITION)
tamoxifen = dba.analyze(tamoxifen)
tamoxifen.DB = dba.report(tamoxifen)
```

Outputs: diagnostics, visualizations, and 'top table' of differentially expressed regions.

Integration & visualization

- ▶ Combining multiple data types
 - ▶ *Rcade*, *Repitools*: ChIP / expression
- ▶ Import / export from common formats (BED, WIG, ...)
 - ▶ *rtracklayer* `import()`, `export()`
- ▶ *AnnotationHub*: accessing large-scale resources, e.g., ENCODE tracks
- ▶ Visualization

Integration & visualization: *AnnotationHub*

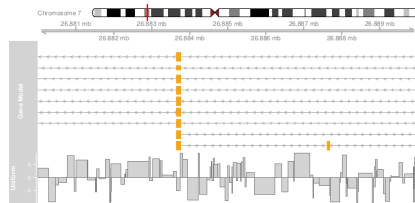
```
> library(AnnotationHub)
> hub = AnnotationHub()
> hub
class: AnnotationHub
length: 10780
filters: none
hubUrl: http://annotationhub.bioconductor.org/ah
snapshotVersion: 2.14/1.4.0; snapshotDate: 2014-05-15
hubCache: /home/mtmorgan/.AnnotationHub
> hub$<tab>
hub$dbSNP.organisms.human_9606.VCF. ... [302]
hub$haemcode.blood. ... [899]
hub$ensembl.release. ... [2611]
hub$inparanoid8.Orthologs.hom. ... [265]
hub$goldenpath. ... [6699]
hub$refnet. ... [4]
```

Integration & visualization

Gviz

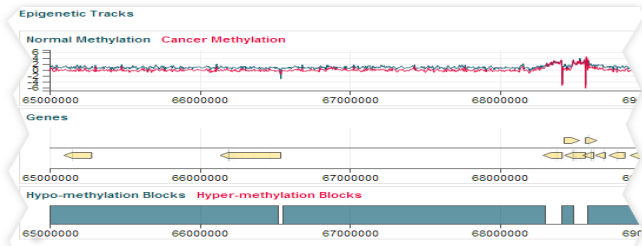
- ▶ Static track-like visualizations
- ▶ Data panels

ggbio
epivizr



Integration & visualization

Gviz
ggbio
epivizr



- ▶ Genome browser with tight communication to *R* / *Bioconductor*
- ▶ Flexible *interactive*, *representation* and *computation*, e.g., 'brushing'

Genomic ranges for data integration

- ▶ Chromosome, start, end, strand define a *genomic range*
- ▶ Data (reads, CpG islands, peaks, ...) are genomic ranges
- ▶ Annotations (exons, genes, binding sites, ...) are genomic ranges

```
> gr = exons(TxDb.Hsapiens.UCSC.hg19.knownGene); gr
```

```
GRanges with 289969 ranges and 1 metadata column:
```

	seqnames	ranges	strand	exon_id
	<Rle>	<IRanges>	<Rle>	<integer>
[1]	chr1	[11874, 12227]	+	1
[2]	chr1	[12595, 12721]	+	2
[3]	chr1	[12613, 12721]	+	3
...
[289967]	chrY	[59358329, 59359508]	-	277748
[289968]	chrY	[59360007, 59360115]	-	277749
[289969]	chrY	[59360501, 59360854]	-	277750

```
seqlengths:
```

chr1	chr2 ...	chrUn_g1000249
249250621	243199373 ...	38502

GRanges

```
length(gr); gr[1:5]  
seqnames(gr)  
start(gr)  
end(gr)  
width(gr)  
strand(gr)
```

DataFrame

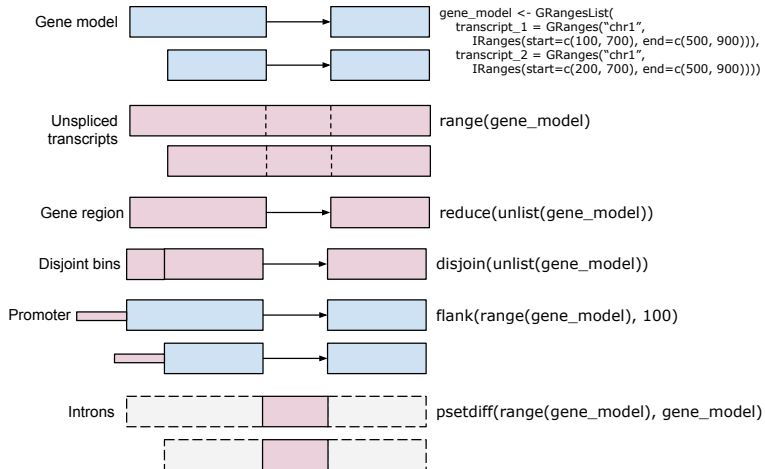
```
mcols(gr)  
gr$exon_id
```

Seqinfo

```
seqlevels(gr)  
seqlengths(gr)  
genome(gr)
```

GenomicRanges, *GenomicAlignments* packages

Operating on genomic ranges





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People

- ▶ Seattle Bioconductor team: Sonali Arora, Marc Carlson, Nate Hayden, Valerie Obenchain, Hervé Pagès, Dan Tenenbaum
- ▶ Vincent Carey, Robert Gentleman, Rafael Irizarry, Sean Davis, Kasper Hansen, Michael Lawrence, Levi Waldron
- ▶ International community of *Bioconductor* developers and users

References I

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- P. V. Kharchenko, M. Y. Tolstorukov, and P. J. Park. Design and analysis of CHIP-seq experiments for DNA-binding proteins. *Nat. Biotechnol.*, 26(12):1351–1359, Dec 2008.