The *Ranges Suite

Use cases and examples from high-throughput sequencing

Michael Lawrence

Genentech

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Outline

Sequencing Approaches

Source	Genome, transcriptome, synthetic
Enrichment	WGS, ChIP, PCR, poly-A RNA, exome capture, etc

General Process

1. QA on raw instrument output, see *ShortRead*
2. Usually, external alignment of data, i.e., gSNAP
3. Import of alignments and/or sequences into R
4. Analysis of sequences, alignments and enrichment patterns

The *Ranges Packages

IRanges

Base of the sequence analysis infrastructure in Bioconductor

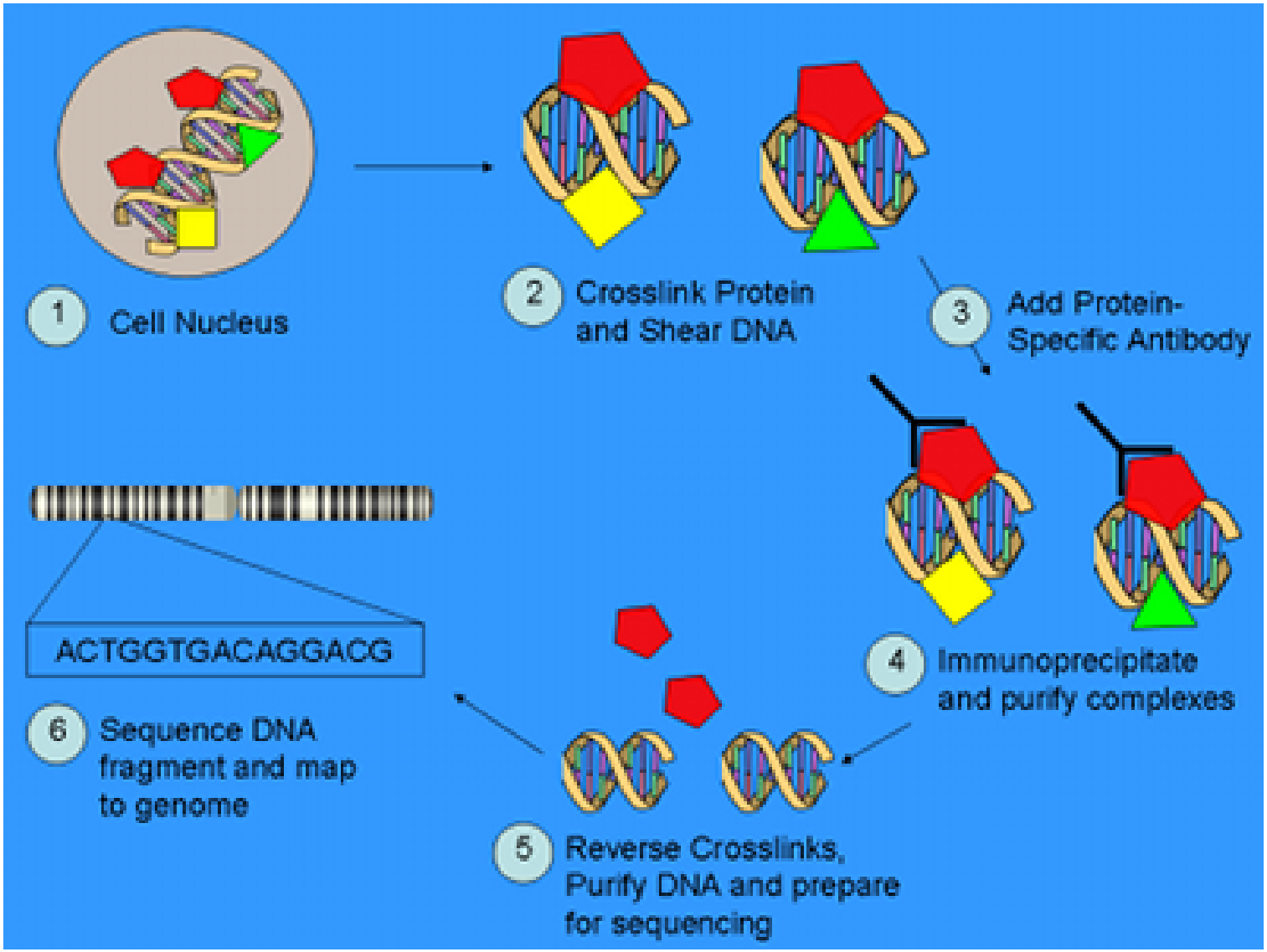
- Data structures for interval datasets and genome-scale vectors
- Routines for finding regions of enrichment and overlap between features

GenomicRanges

Extension of *IRanges* for genomic (biological) datasets, including sequence annotations and experimental measurements

Outline

ChIP-seq Protocol



ChIP-seq Questions

- Where are the peaks?
- Do the peaks tend to fall in a certain genomic context, e.g., promoters or conserved regions?
- How do the peaks correspond to and inform TF motif analyses?
- Is there a relationship between binding and expression?

Workflow Overview

- ShortRead QA report on reads
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- Calculate coverage
- Estimate peak cutoff and call peaks
- Various peak-level analyses

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Example Data: `data(cstest)`

- Solexa sequencing of CTCF and GFP (control) ChIP in mouse
- Aligned with MAQ
- One lane each, subset to three chromosomes

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Representing Read Alignments

Read alignments are intervals on stranded sequences

Sequence Name	Start	End	Strand	metadata...
chr10	3012936	3012959	+	
chr10	3012941	3012964	+	
chr10	3012944	3012967	+	

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chr10	3012941	3012964	+	
chr10	3012944	3012967	+	

All genomic data fits this basic format

Genomic Datasets in R

The *GRanges* Class

A *Vector* of genomic intervals, with metadata

- Constructor: `GRanges(seqnames, ranges, strand, ...)`
- `seqnames(x)`: sequence name
- `start(x)`, `end(x)`, `width(x)`: interval information
- `strand(x)`: strand (+/-/*)
- `values(x)`: a *DataFrame* of metadata columns, like score or gene
- `seqinfo(x)`: a *Seqinfo* with information about the sequences

Group multiple *GRanges* in a *GRangesList*

Loading the CTCF Data

```
> library(chipseq)
> data(cstest)
> names(cstest)

[1] "ctcf" "gfp"

> head(cstest$ctcf, 1)

GRanges with 1 range and 0 elementMetadata values
    seqnames              ranges strand |
       <Rle>           <IRanges>  <Rle> |
[1]    chr10 [3012936, 3012959]      + |

seqlengths
        chr1  chr1_random ...         chrM
   197195432       1231697 ...        16299
```

GRanges Excercises

1. Count the number of reads on each chromosome
2. Count the number of reads on each strand
3. Sort the reads by start position

Peak-level Analyses

- Summarize peaks
- Intersect with genomic annotations
- Visualization

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Estimating Fragment Length

```
> fraglen <-
+   estimate.mean.fraglen(cstest$ctcf,
+                         method = "correlation",
+                         seqLen = 35)

> fraglen

chr10 chr11 chr12
  265   265   255

> median(fraglen)

[1] 265
```

Resizing the Reads

```
> ctcf.ext <- resize(cstest$ctcf,
+                    width = median(fraglen))
> head(ctcf.ext, 3)

GRanges with 3 ranges and 0 elementMetadata values
    seqnames               ranges strand |
       <Rle>            <IRanges>  <Rle> |
[1]    chr10 [3012936, 3013200]      + |
[2]    chr10 [3012941, 3013205]      + |
[3]    chr10 [3012944, 3013208]      + |

seqlengths
      chr1  chr1_random ...        chrM
 197195432      1231697 ...       16299
```

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Representing Coverage

- As often the case with genomic data, coverage contains long runs of identical values
- Could use a *GRanges*, with a range for each run
- More efficient is a Run-Length Encoding (RLE)
- Already an *rle* class in R, but lacks functionality

A Better Run Length Encoding in R

The *Rle* Class

A *Vector* that run-length encodes any atomic vector type, e.g., *logical*, *integer*, *character*, etc.

- Constructor: `Rle(x)`
- Usually treated like any other R vector
- `runLength`, `runValue`: get the lengths and values

Multiple chromosomes fit into an *RleList*

Calculating the Coverage

```
> cov.ctcf <- coverage(ctcf.ext)
> cov.ctcf$chr10

'integer' Rle of length 129993255 with 289551 runs
  Lengths: 3012734      97 ...     265    6212
  Values :       0       1 ...       1       0
```

Rle Excercise

Calculate how many elements were saved through the run-length encoding vs. an ordinary vector

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Calling Peaks

- Finding a peak cutoff is a complex problem
- Many peak detection methods rely on "island" summaries
- An island is a contiguous region with depth $>= 1$
- Analyzing the islands, or any set of enriched regions, requires combining the coverage with the ranges of interest

Combining a Vector with Ranges

The *Views* Class

A *Vector* of views, by overlaying a set of *Ranges* on a subject *Vector*

The *RleViews* Class

A *Views* subclass with an *Rle* subject; useful for coverage

The *RleViewsList* Class

A *(Views)List* of *RleViews*; useful for coverage over multiple chromosomes

Slicing the Coverage into Islands

The `slice` Function

```
> islands <- slice(cov.ctcf, lower = 1)
> head(islands$chr10, 3)

Views on a 129993255-length Rle subject

views:
      start     end width
[1] 3012735 3013335   601 [1 1 1 1 1 1 1 1 ...]
[2] 3018464 3018728   265 [1 1 1 1 1 1 1 1 ...]
[3] 3020766 3021030   265 [1 1 1 1 1 1 1 1 ...]
```

Calling the Peaks

Assume we ended up choosing a cutoff of 8.

```
> peak_viewsList <- slice(cov.ctcf, lower = 8)
> peak_rangesList <- ranges(peak_viewsList)
> peaks <- as(peak_rangesList, "GRanges")
> head(peaks, 3)

GRanges with 3 ranges and 0 elementMetadata values
    seqnames              ranges strand |
       <Rle>           <IRanges>  <Rle> |
[1]    chr10 [3012955, 3013200]      * |
[2]    chr10 [3234798, 3234896]      * |
[3]    chr10 [3269945, 3270362]      * |

seqlengths
        chr1  chr1_random ...          chrM
          NA           NA ...            NA
```

Island Calling Excercise

Generate a similar *GRanges* for the GFP lane

Calling Peaks in Four Lines

```
> findPeaks <- function(reads) {
+   fraglen <- estimate.mean.fraglen(reads,
+                                    method = "correlation",
+                                    seqLen = 35)
+   reads_ext <- resize(reads, fraglen)
+   cov <- coverage(reads_ext)
+   slice(cov, lower = 8)
+ }
```

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Summarizing Coverage by Peaks

Some statistics of interest:

- Maximal coverage under peak
- Total coverage under peak
- Summit interval

Summarizing *RleViews(List)* of Peaks

Maximal coverage under peak

```
> values(peaks)$max <-
+   unlist(viewMaxs(peak_viewsList))
```

Sum of coverage under peak

```
> values(peaks)$sum <-
+   unlist(viewSums(peak_viewsList))
```

Find summits

```
> values(peaks)$summits <-
+   unlist(viewRangeMaxs(peak_viewsList))
```

Calling a `view*` summary function on a *RleViewsList* returns a *List*, which we `unlist`.

Peak Annotation

- Genomic context (promoters, exons, etc)
- Motif hits
- Conservation
- ...

Representing Transcript Models

- Many ways to represent transcript models as ranges:
  - Transcripts
  - Exons, introns
  - CDS, UTRs
- Reference annotations, so prefer persistent storage

Transcript Models in R

The *TxDb* Class

Reference to SQLite DB with transcript information

- `transcripts(x)`: whole transcript ranges
- `exons(x)`: exon ranges
- `cds(x)`: coding exon ranges
- `*By(x)` variants: *GRangesList* object, grouping by transcript, gene
- `*ByOverlaps(x)` variants: annotations overlapping query ranges

For common organisms/models, *TxDb** packages available

Obtaining the Mouse Transcripts

Find regions 500 bp upstream, 200 downstream of TSS

```
> library(TxDb.Mmusculus.UCSC.mm9.knownGene)
> mm9_tx <- transcripts(Mmusculus_UCSC_mm9_knownGene_TxDb,
+                       columns = "gene_id")
```

Fixing up the gene_id Column

- Sometimes possible for transcript to belong to multiple genes
- Not the case for our mouse genes
- Need to coerce the *CharacterList* to *character*

```
> gene_id <- values(mm9_tx)$gene_id
> all(elementLengths(gene_id) <= 1)

[1] TRUE

> flat_gene_id <- character(length(mm9_tx))
> flat_gene_id[elementLengths(gene_id) == 1] <-
+   unlist(gene_id)
> values(mm9_tx)$gene_id <- flat_gene_id
```

Obtaining the Mouse Promoters

Find regions 500 bp upstream, 200 downstream of TSS

```
> promoters <- resize(flank(mm9_tx, 500), 700)
```

Finding Peaks that Overlap the Promoters

%in%: any overlap?

```
> values(peaks)$in_promoter <- peaks %in% promoters
> table(values(peaks)$in_promoter)

FALSE  TRUE
 5622   391
```

match: find index of first overlap

```
> values(peaks)$in_promoter_of <-
+   values(promoters)$gene_id[match(peaks, promoters)]
> head(subset(values(peaks), in_promoter)$in_promoter_of, 3)

[1] "270685" "67844"  ""
```

Peak Annotation Exercises

1. Find the peaks in the region 10kb upstream of the TSS
2. Find the peaks in the introns (i.e., in transcript, but not exons)

Comparing Peaks Across Samples

```
> ctcf_peaks <- findPeaks(cstest$ctcf)
> gfp_peaks <- findPeaks(cstest$gfp)

> peak_summary <- diffPeakSummary(ctcf_peaks, gfp_peaks)
> colnames(peak_summary)

[1] "comb.max" "sums1"    "sums2"    "maxs1"
[5] "maxs2"
```

Creating a *SummarizedExperiment*

The range-aware version of *ExpressionSet*

```
> peak_summary_gr <- as(ranges(peak_summary), "GRanges")
> max_matrix <- with(peak_summary, cbind(maxs1, maxs2))
> SummarizedExperiment(max_matrix, rowData = peak_summary_gr)

class: SummarizedExperiment
dim: 6021 2
assays(1): ''
rownames: NULL
rowData values names(0):
colnames(2): maxs1 maxs2
colData names(0):
```

Outline

RNA-seq Questions

- Which genes, exons, alleles, isoforms, etc are expressed, and which are differentially expressed?
- Are there any expressed variants/editing?
- Are there any novel splicing events?

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Data from *leeBamViews*

- Four samples from a Yeast RNA-seq experiment
- Two wildtype, two RLP mutants
- Alignments in *leeBamViews* for positions 800000 to 900000 on chromosome XIII
- Stored as BAM files

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Representing SAM/BAM in R

Could store SAM alignments in a *GRanges*, but common enough for a formal representation

The *GappedAlignments* Class

A *Vector* of alignments with SAM-specific fields

- Load with `readGappedAlignments(file)`
- Access `cigar`, `qpos`, etc.
- Often acts like *GRanges*, with `start(x)`, `coverage(x)`, etc.

Loading a BAM File

```
> library(leeBamViews)
> bams <- dir(system.file("bam", package="leeBamViews"),
+             full = TRUE, pattern = "bam$")
> reads_ga <- readGappedAlignments(bams[1])
> head(reads_ga, 1)

GappedAlignments of length 1
      rname strand cigar qwidth  start    end
[1] Scchr13      -   36M     36 799975 800010
    width ngap
[1]    36    0

seqlengths
 Scchr01 Scchr02 Scchr03 ... Scchr16  Scmito
  230208  813178  316617 ...  948062   85779
```

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Representing Ranges with Gaps

- Read alignments may contain gaps, e.g., cross a splice junction
- Multiple ranges per read
- Need to group ranges by read
- Use *GRangesList*, with one *GRanges* per read

Creating a *GRangesList* from *GappedAlignments*

```
> reads_grl <- grglist(reads_ga)
```

Creating a *GRangesList* from *GappedAlignments*

```
> reads_grl <- grglist(reads_ga)
```

Note

This groups by read, not by read pair. Pair grouping currently takes a little more work.

Preparing Some Transcript Models

Reads were not mapped to UCSC sacCer2 assembly, so we use some annotations of expressed regions from *leeBamViews*

```
> data(leeUnn)
> leeUnn <-
+   subset(leeUnn, lengthWithoutMask > 0 & !is.na(chr))
> leeUnn$strand <- c("-", "*", "+")[leeUnn$strand + 2]
> sc2_tx <- with(leeUnn,
+                GRanges(sprintf("Scchr%02d", chr),
+                        IRanges(start, end), strand))
> seqlevels(sc2_tx)[length(seqlevels(sc2_tx))] <- "Scmito"
```

Count Reads in Exons, by Transcript

Using countOverlaps

```
> values(sc2_tx)$counts <-
+   countOverlaps(sc2_tx, reads_grl, ignore.strand = TRUE)
```

These counts could then be passed to *DEseq* or *edgeR*

Find Transcript Hits for Each Read

Using findOverlaps

```
> ol <- findOverlaps(reads_grl, sc2_tx,
+                    ignore.strand = TRUE)
> reads_factor <- factor(queryHits(ol),
+                        seq_len(length(reads_grl)))
> values(reads_grl)$tx_hits <-
+   split(subjectHits(ol), reads_factor)
> head(table(elementLengths(values(reads_grl)$exon_hits)))

integer(0)
```

Outline

Further Analysis

ChIP-seq Annotation *ChIPpeakAnno*, *chipseq*

RNA-seq DE *edgeR*, *DEseq*, *DEXseq*