Annotation

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What is ‘Annotation’?

- Genes – classification schemes (e.g., Entrez, Ensembl), pathway membership, . . .
- Genomes – reference genomes; exons, transcripts, coding sequence; coding consequences
- System / network biology – pathways, biochemical reactions, . . .
- ‘Consortium’ resources, TCGA, ENCODE, dbSNP, GTEx, . . .

Other defintions (not covered here)

- SNP (and similar) consequences (*VariantAnnotation*, *VariantFiltering*, *ensemblVEP*)
- Assign function to novel sequences
- . . .

Bioconductor Annotation Resources – Packages

Model organism annotation packages

- *org.** – gene names and pathways
- *TxDb.** – gene models
- *BSgenome.** – whole-genome sequences

org.* packages

The ‘select’ interface:

- Discovery: `keytypes`, `columns`, `keys`
- Retrieval: `select`

```r
library(org.Hs.eg.db)
keytypes(org.Hs.eg.db)
columns(org.Hs.eg.db)
egid <-
  select(org.Hs.eg.db, "BRCA1", "ENTREZID", "SYMBOL")
```

*org.** pacakges – Under the hood…

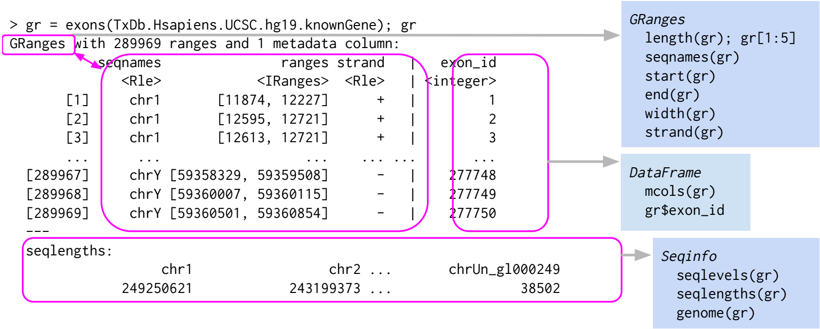
SQL (sqlite) data bases

- `org.Hs.eg_dbconn()` to query using *RSQLite* package
- `org.Hs.eg_dbfile()` to discover location and query outside *R*.

Background: Genomic Ranges

- Defined by chromosome, start, end, strand
  - *Bioconductor*: 1-based, closed interval
  - *GRanges*: Vector of genomic ranges
  - *GRangesList*: List, each element of which is a genomic range
- Describe data
  - *GRanges*: SNP locations, ungapped read alignments, ChIP peaks, copy number changes, ...
  - *GRangesList*: gapped or paired-end alignments, ...
- Describe annotations
  - *GRanges*: genes, exons, ...
  - *GRangesList*: transcripts, ...

Genomic Ranges: *GRanges*



```
> 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_gl000249
              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)
```

Genomic Ranges: *GRangesList*

```
> grl = exonsBy(TxDb.Hsapiens.UCSC.hg19.knownGene, ”tx”, use.names=TRUE); grl
GRangesList of length 82960:
$uc001aaa.3
GRanges with 3 ranges and 3 metadata columns:
      seqnames           ranges strand |   exon_id   exon_name exon_rank
         <Rle>        <IRanges>  <Rle> | <integer> <character> <integer>
  [1]     chr1 [11874, 12227]        + |         1        <NA>         1
  [2]     chr1 [12613, 12721]        + |         3        <NA>         2
  [3]     chr1 [13221, 14409]        + |         5        <NA>         3

$uc010nxq.1
GRanges with 3 ranges and 3 metadata columns:
      seqnames           ranges strand | exon_id exon_name exon_rank
  [1]     chr1 [11874, 12227]        + |       1      <NA>         1
  [2]     chr1 [12595, 12721]        + |       2      <NA>         2
  [3]     chr1 [13403, 14409]        + |       6      <NA>         3

$uc010nxr.1
GRanges with 3 ranges and 3 metadata columns:
      seqnames           ranges strand | exon_id exon_name exon_rank
  [1]     chr1 [11874, 12227]        + |       1      <NA>         1
  [2]     chr1 [12646, 12697]        + |       4      <NA>         2
  [3]     chr1 [13221, 14409]        + |       5      <NA>         3

...
<82957 more elements>
---
seqlengths:
                 chr1                  chr2 ...        chrUn_gl000249
            249250621             243199373 ...                 38502
```

```
GRangesList
  (list of GRanges)
  length(grl)
  grl[1:3]
  shift(grl, 1)
  range(grl)
```

```
GRanges
  grl[[2]]
  grl[[“uc010nxq.1”]]
```

```
Two kinds of fun!
  introns =
    psetdiff(range(grl), grl)

  grr = unlist(grl)
  ## transform grr, then...
  grl = relist(grr, grl)
```

‘flesh’ ‘skeleton’

Genomic Ranges: Range-Based Operations

- Within range: "I have a *GRangesList* instance *exByTx* of exons within transcripts. They use a 0-based, 1/2-open convention. I want them 1-based and closed."

```r
resize(shift(exByTx, 1), width(exByTx) - 1)
```

- Between ranges within instance: "I have a *GRanges* instance `reads` representing aligned reads. I want coverage."

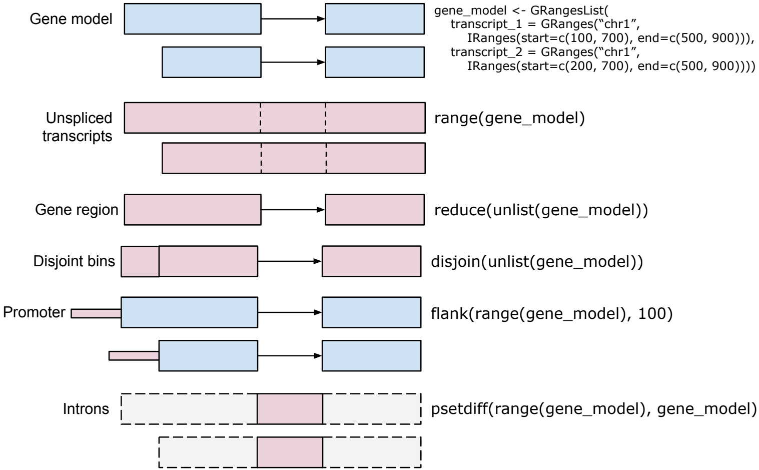
```r
coverage(reads)
```

- Between instances: "How many reads overlap each gene?"

```r
countOverlaps(exByTx, reads)
```

(Better: `GenomicAlignments::summarizeOverlaps` on the underlying BAM files)

Genomic Ranges: Range-Based Operations



Gene model

```
gene_model <- GRangesList(
    transcript_1 = GRanges(“chr1”,
        IRanges(start=c(100, 700), end=c(500, 900))),
    transcript_2 = GRanges(“chr1”,
        IRanges(start=c(200, 700), end=c(500, 900))))
```

Unspliced transcripts

```
range(gene_model)
```

Gene region

```
reduce(unlist(gene_model))
```

Disjoint bins

```
disjoin(unlist(gene_model))
```

Promoter

```
flank(range(gene_model), 100)
```

Introns

```
psetdiff(range(gene_model), gene_model)
```

*TxDb.** packages

- Gene models for common model organsisms / genome builds / known gene schemes
- Supports the 'select' interface (`keytypes`, `columns`, `keys`, `select`)
- 'Easy' to build custom packages when gene model exist

Retrieving genomic ranges

- `transcripts`, `exons`, `cds`,
- `transcriptsBy` , `exonsBy`, `cdsBy` – group by gene, transcirpt, etc.

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
cdsByTx <- cdsBy(txdb, "tx")
```

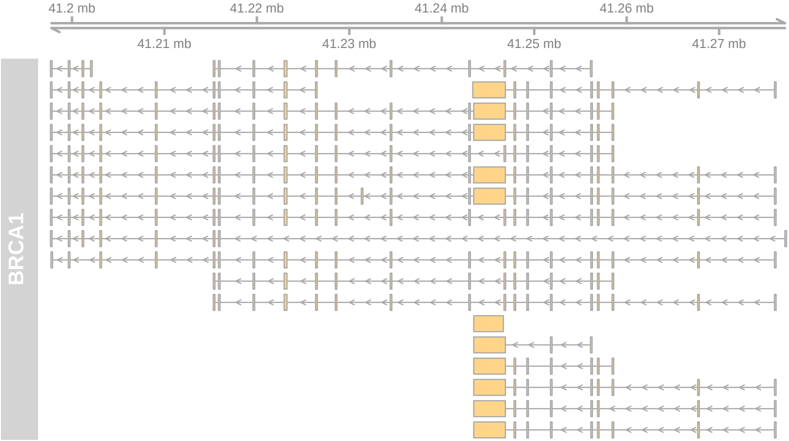
Example: Visualize BRCA1 Transcripts

```r
library(org.Hs.eg.db)
eid <- select(org.Hs.eg.db, "BRCA1", "ENTREZID",
  "SYMBOL")[["ENTREZID"]]

library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
txid <- select(txdb, eid, "TXNAME", "GENEID")[["TXNAME"]]
cds <- cdsBy(txdb, by="tx", use.names=TRUE)
brca1cds <- cds[names(cds) %in% txid]

library(Gviz)
tx <- rep(names(brca1cds), elementLengths(brca1cds))
id <- unlist(brca1cds)$cds_id
grt <- GeneRegionTrack(brca1cds, name="BRCA1",
  gene="BRCA1", feature=tx, transcript=tx, id=tx, exon=id)
plotTracks(list(GenomeAxisTrack(), grt))
```

Example: Visualize BRCA1 Transcripts



*BSgenome.** Packages: Whole-Genome Sequences

- ‘Masks’ when available, e.g., repeat regions
- Load chromosomes, range-based queries: `getSeq`, `extactTranscriptSeqs`

```
library(BSgenome.Hsapiens.UCSC.hg19)
extractTranscriptSeqs(Hsapiens, brca1cds)

##   A DNAStringSet instance of length 20
##      width seq                         names
##  [1]  2280 ATGGATTTATCTG...AGCCACTACTGA uc010whl.2
##  [2]  5379 ATGAGCCTACAAG...AGCCACTACTGA uc002icp.4
##  [3]   522 ATGGATGCTGAGT...AGCCACTACTGA uc010whm.2
##  ...   ... ...
## [18]  3954 ATGCTGAAACTTC...GATTCAAACTTA uc010cyz.2
## [19]  4017 ATGGATTTATCTG...GATTCAAACTTA uc010cza.2
## [20]  3207 ATGAATGTAGAAA...GATTCAAACTTA uc010wht.1
```

Bioconductor Annotation Resources – Web-based

Rich web resources

- *biomaRt* (`http://biomart.org`), *rtracklayer* (UCSC genome browser)
- *ArrayExpress*, *GEOquery*, *SRAdb*
- *PSICQUIC*, *KEGGREST*, *uniprot.ws*, . . .
- *AnnotationHub*

biomaRt

- `http://biomart.org`
- Drill-down discovery: `listMarts`, `listDatasets`, `listFilters`, `listAttributes`
- Retrieval: `getBM`

```r
library(biomaRt)
ensembl <-                                ## discover & use
   useMart("ensembl", dataset="hsapiens_gene_ensembl")
head(listFilters(ensembl), 3)
myFilter <- "chromosome_name"
myValues <- c("21", "22")
myAttributes <- c("ensembl_gene_id","chromosome_name")
res <-
    getBM(attributes=myAttributes, filters=myFilter,
          values=myValues, mart=ensembl)
```

PSICQUIC

- **P**rotemics **S**tandard **I**nitiative **C**ommon **QU**ery **I**nterfa**C**e
- Programmatic access to molecular interaction data bases.
- https://code.google.com/p/psicquic/

```
library(PSICQUIC)
## Query web service for available providers
psicquic <- PSICQUIC()
providers(psicquic)         # 25 available providers
## interactions between TP53 and MYC
tbl <-
    interactions(psicquic, c("TP53", "MYC"), "9606")
nrow(tbl)                   # 7 interactions
```

See the package vignette for additional detail.

AnnotationHub

- Large-scale genome resources, lightly curated for easy access from *R*.
- Supports tab-completion, `metadata` discovery, selection and filtering.

```
library(AnnotationHub)
hub <- AnnotationHub()
hub        ## 10511 resources
```

AnnotationHub: Example

- Evoln'arily conserved enhancer SNPs near genes on chr17

Resources

- SNPs from dbGAP
- Enhancers from ENCODE ChromHMM
- Conservation track, from UCSC

Steps

1. Retrieve enhancers, SNPs from *AnnotationHub*, gene coordinates from *TxDb.**; harmonize chromosome and genome names
2. Download (large!) conservation track as BED file from UCSC, query for chr17 using *rtracklayer*
3. `subsetByOverlaps` SNPs and enhancers
4. Annotate enhancer SNPs with evolutionary conservation score
5. Find `nearest` and `distanceToNearest` genes to each SNP

Conclusions

Rich annotation resources

- Model organism and custom *org.**, *TxDb.**, *BSgenome.** packages
- Web-based access to public (e.g., *biomaRt* and *Bioconductor*-specific (e.g., *AnnotationHub*) resources

Facile manipulation of genomic ranges

- Many data munging and research questions very easy to answer
- Integrative analysis across data types

Resources

Additional resources

- Annotation,
  VariantAnnotation and other work flows
- AnnotationDbi, AnnotationHub and other package landing pages, including links to vignettes.
- Previous course material, including an Annotation walk-through from *useR!* 2014.

Bioc2014 Annual Conference[1], July 30 – August 1, Boston

[1] `https://register.bioconductor.org/BioC2014/`

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