R / *Bioconductor* for Integrative Genomic Analysis

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Abstract – *Bioconductor* is a collection of almost 1000 packages for the analysis & comprehension of high-throughput genomic data. This general talk starts with a description of *Bioconductor* principles and their translation to software. We then discuss particular challenges and solutions for applying *R* to large-scale data, and illustrate approaches using the GenomicRanges infrastructure. The presentation concludes with interesting challenges of data integration and analysis facing *R*'s use in emerging areas of genomics and medicine.

Outline: *R* / *Bioconductor* for Integrative Analysis

1. The *Bioconductor* project
2. High-throughput sequencing
3. Genomic Ranges
4. Large data
5. Data integration

Bioconductor

Goal Analysis and comprehension of high-throughput genomic data

Focus

- Sequencing; RNA-Seq, ChIP-Seq, Variants, ...
- Expression and other microarrays; flow cytometry; proteomics, imaging

Themes

- Contributions from 'core' members and (primarily academic) user community
- Based on the *R* programming language – statistics, visualization, interoperability
- Reproducible – data structures, scripts, *vignettes*, packages
- Interoperable – formal classes, dependencies on 'core' packages
- Open source / open development

Why *Bioconductor*?

A *community* of users and developers.

- Extensive & interoperable
- Statistical (volume, technology, experimental design, population samples)
- Reproducible: long-term, multi-participant science
- Leading edge: embrace novel technologies and analysis
- Accessible: affordable, transparent, usable (e.g., vignettes & man pages)

Huber et al., Orchestrating high-throughput genomic analysis with *Bioconductor*. *Nature Methods*: soon!

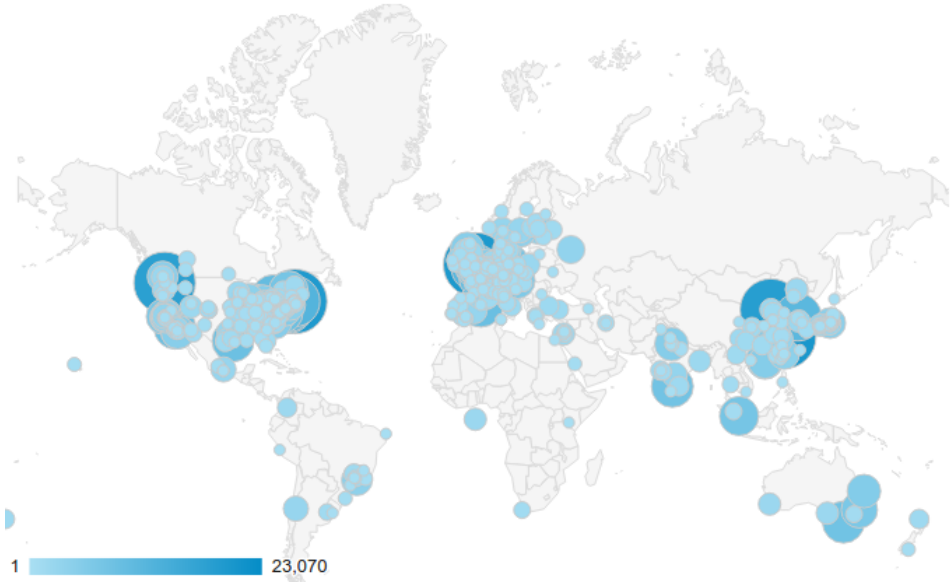
Why *Bioconductor*?

More than a software archive.

- Build on relevant software, e.g.,
  - *GenomicRanges* for efficient interoperability; *ExpressionSet* / *SummarizedExperiment* for genetic / phenotypic integration...
  - I/O via *rtracklayer*, *Rsamtools*, *illuminaio*, ...
  - Resource access via *biomaRt*, *GEOquery*, ...
- Commit to long-term support
  - e.g., *affy* in use 10 years after introduction.
  - Comprehensive documentation coupled with traditional scientific publications
  - Engage users via support forum, foster productive collaborations
- Enable transitions
  - User to developer
  - Student to professional

Driving principle: analysis & comprehension of high-throughput genomic data

Project status (December, 2014)



2014 web site visitors, by city

- 320,000 unique IP address package downloads / year
- 1,300 support site contributors / year, 8,200 visitors / month
- 10,500 PubMed Central mentions of 'Bioconductor';
  $\approx$ 22,000 citations to *Bioconductor* packages
- At least 12 of 15 initial TCGA publications
- Funding from US NIH & NSF, and (soon!) EC

High-Throughput Sequencing (HTS)

Questions

- Which genes are differentially expressed in cancer versus normal tissue?
- Which transcription factors are regulating gene expression?
- What single nucleotide polymorhpisms are present in a population / associated with a disease?

Sample sizes

- Designed experiments – e.g., 10's or 100's of samples
- Cohorts – e.g., 100's or 1000's of patients
- Populations – 1000's - 10000's of individuals

Attributes

- 10,000's of genes
- Millions of variants

HTS: Differential Expression Analysis

E.g., Gene differential expression

- Human genome: 22 autosomes, 2 sex chromosomes; 3 billion nucleotides of DNA
- DNA transcribed to mRNA, mRNA translated to proteins
- A 'gene': known ranges on the genome that encode proteins
- Roughly, highly expressed genes produce more mRNA

Protocol

- Isolate mRNA from tissue, reverse-transcribe to cDNA
- Fragment and then sequence cDNA – 10M - 100M fragments
- Align sequenced fragments to reference genome
- Summarize (count) aligned fragments in each gene

HTS: Differential Expression Analysis

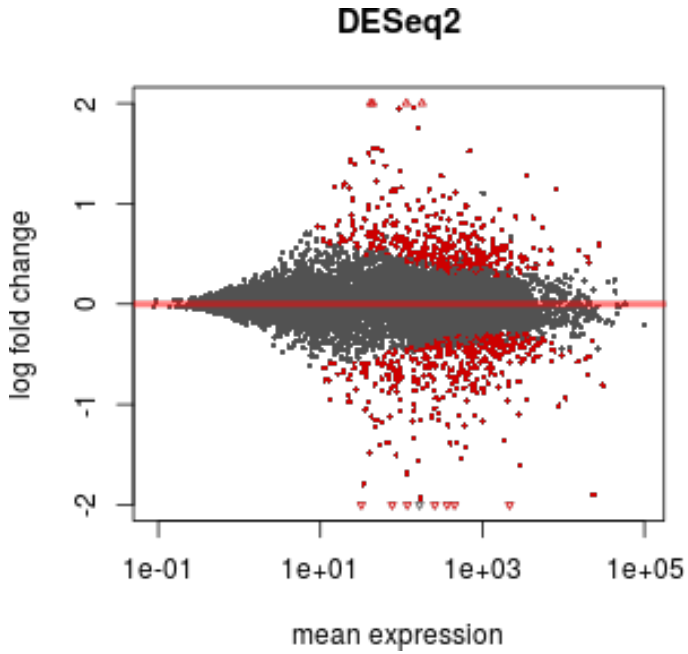
Summarized data

$$\begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1n} \\ x_{21} & x_{22} & \cdots & x_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ x_{p1} & x_{p2} & \cdots & x_{pn} \end{bmatrix}$$

- Array of counts of reads aligned to p genes in n samples.

Task

- Fit a linear model to each row, `Count ~ Treatment`



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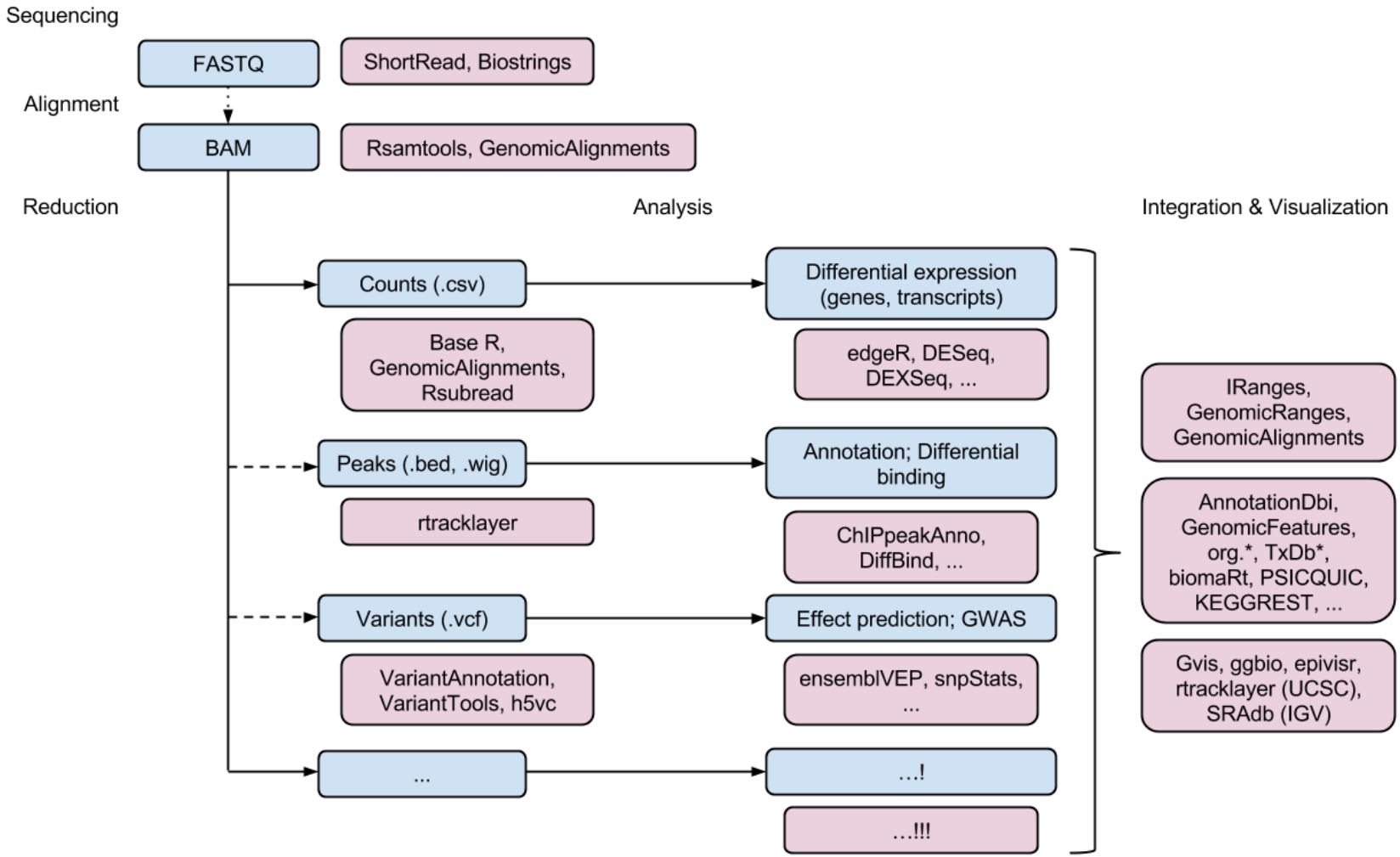
Challenges

- $p >> n$
- Filtering (?)
- Sample normalization – techincal variation between columns
- Negative binomial error model
- Shared experimental design – *moderated* test statistics
- Batch effects

HTS: Package Ecosystem



Genomic Ranges

A central concept

- Chromosome, start, end, strand provide coordinates specifying where in the genome a range occurs
- Describes *data*, e.g., aligned reads, and *annotation*, e.g., locations of genes

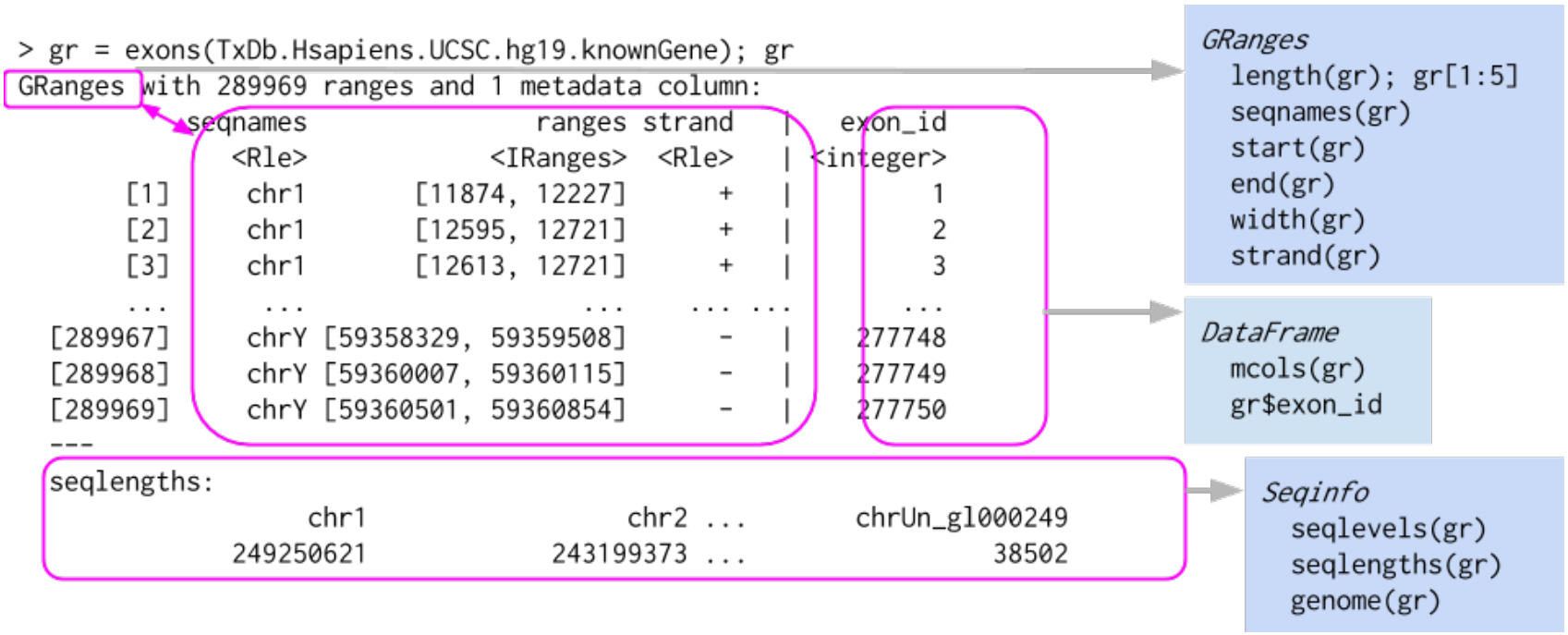
Many useful operations are based on genomic ranges

- E.g., reduction of aligned reads to a matrix of counts represents a simple tally of the number of overlaps between genomic ranges describing aligned reads and genomic ranges described gene locations.

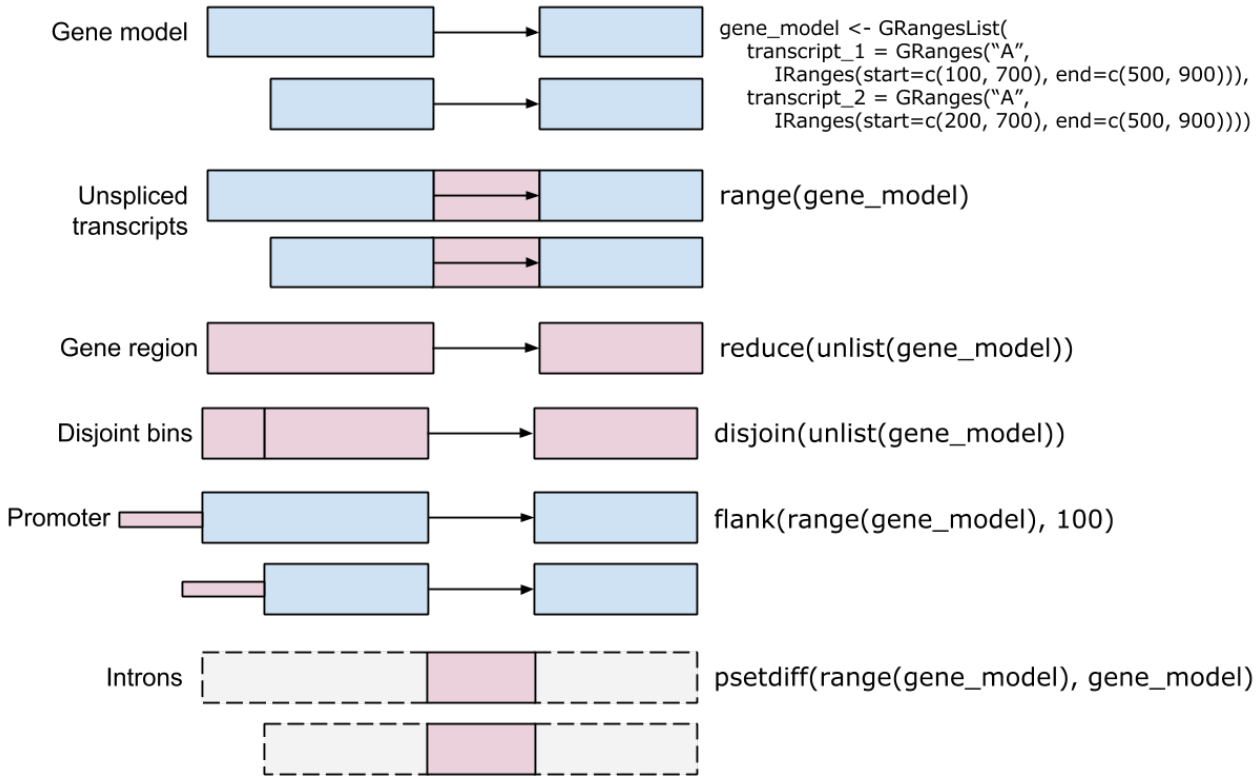
Software

- *GenomicRanges*, *GenomicAlignments*, *GenomicFeatures*
- *GRanges*, *GRangesList*

Genomic Ranges: *GRanges*



Genomic Ranges



A useful summary table of genomic ranges operations is in PLOS Computational Biology 10.1371/journal.pcbi.1003118.

Genomic Ranges

Building blocks for range-based data structures

- *GAlignments*, *GAlignmentsList* (*GenomicAlignments*)
- *SummarizedExperiment* (*GenomicRanges*)
- *VCF* (*VariantAnnotation*)

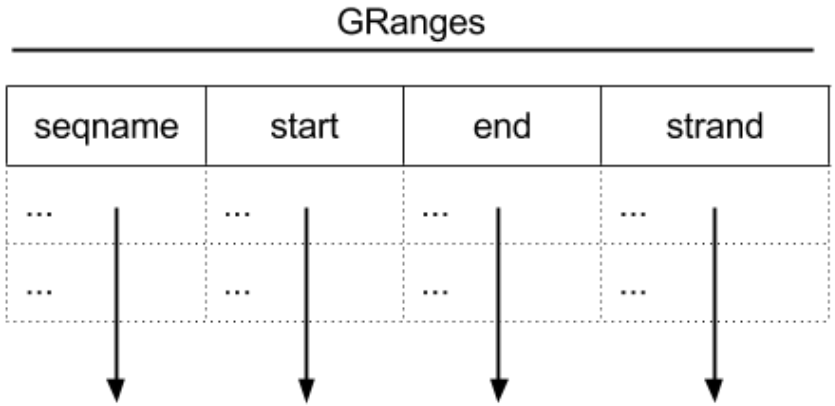
Example

- What genic regions (coding, intron, 5' or 3' UTR, promoter, ...) do SNPs occur in?

```
library(VariantAnnotation)
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
param <- ScanVcfParam(info=NA, geno=NA)
vcf <- readVcf("my.vcf", "hg19", param)
locateVariants(vcf, TxDb.Hsapiens.UCSC.hg19.knownGene)
```

Genomic Ranges: *GRanges* Implementation

- Recall: *R* works well on vectors; object creation is expensive
- *GRanges* class models *columns* of data; one class instance for millions of ranges.
- Vector-like *API* – `length`, `[`, `[[` returns number and subset of ranges
- *DataFrame metadata* associated wtih ranges



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"]]
```

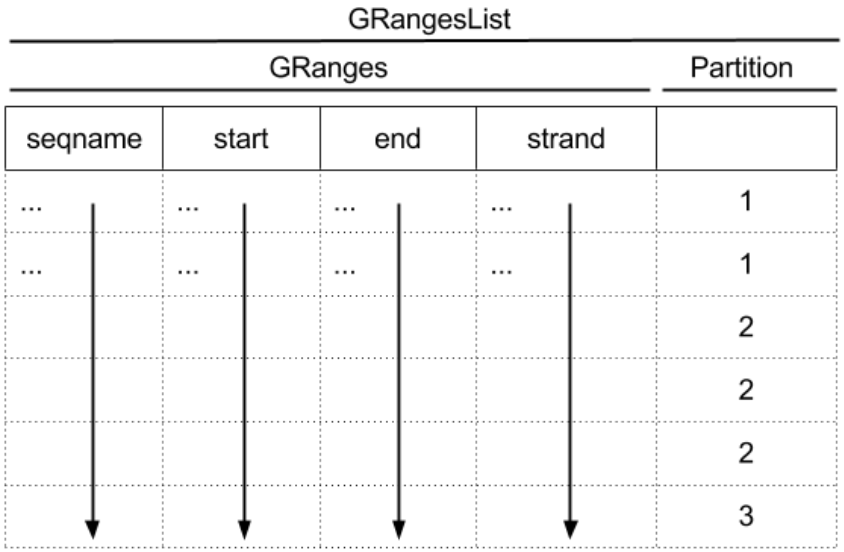
```
Two kinds of fun!
  introns =
    psetdiff(range(grl), grl)

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

'flesh' 'skeleton'

Genomic Ranges: *GRangesList* Implementation

- List-like where each element of the list is must be a *GRanges*
- Implementation: a single *GRanges* instance, and a *partitioning* describing how ranges are grouped into list elements
- Only two objects
- Some operations can be very fast – unlist, transform, relist.



Strategies for Large Data

Memory management

- Restrict input to relevant 'columns', e.g., `readGAlignments` inputs only columns necessary to describe geometery of alignment.
- Select relevant rows, e.g., `ScanBamParam(which=...)`
- Iterate: read in and operate on successive chunks – e.g., `open(BamFile(..., yieldSize=1e7)); reduceByYield(...)`

Speed

- Efficient *R* code – 10-100× speed-up
- Parallel evaluation – 2-10× speed-up
- Often implies memory management
- *BiocParallel*, *GenomicFiles*

Integrative Analysis: Annotation

- Gene identifiers (e.g., *org.Hs.eg.db*) and models (e.g., *TxDb.Hsapiens.UCSC.hg19.knownGene*
- Web-based resources (e.g., *biomaRt*, *KEGGREST*, *UniProt.ws*)
- Whole-genome annotations via *AnnotationHub*, e.g., Ensembl, UCSC, *ad hoc*

Integrative Analysis: *AnnotationHub*

File-based resources, e.g., UCSC *liftOver* files

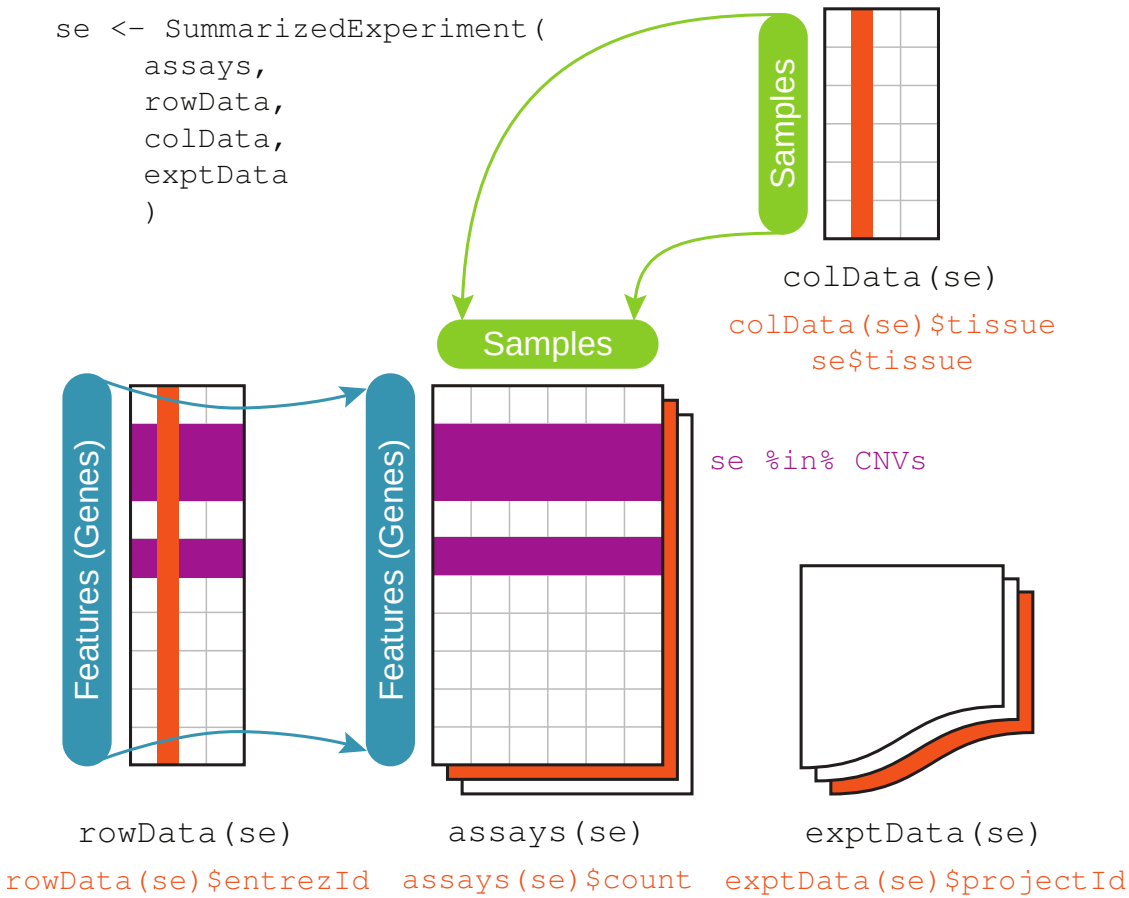
```
## hg19SNPs <- GRanges(...)
library(AnnotationHub)
hub <- AnnotationHub()
chain <- query(hub, 'hg19ToHg38')[[1]]
hg38SNPs <- liftOver(hg19SNPs, chain)
```

Annotation-style resources, e.g., grasp2

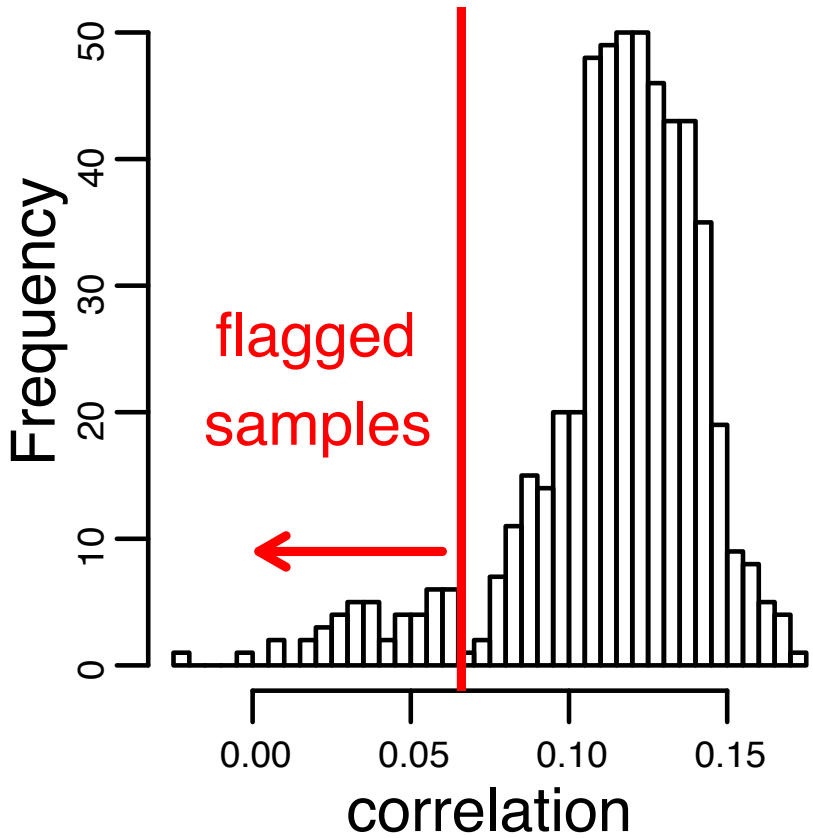
```
library(grasp2db) # Annotation package,
                  # 6 Gb AnnotationHub resource
d <- GRASP2()     # dplyr instance
hispanic <- tbl(d, "count") %>%
    filter(Population=="Hispanic")
semi_join(tbl(d, "variant"), hispanic)
```

Integrative Analysis: *SummarizedExperiment / ExpressionSet*

- Co-ordinate subsetting of 'data' and row (e.g., genomic location) or column (e.g., sample treatment) metadata



Integrative Analysis: Diverse Data Types



TCGA Ovarian gene expression / copy number correlation

- Co-ordinated management of diverse data types
- In-memory and on-disk
- e.g., Identifier / genomic ranges conversion, `x[i, , ]`
- e.g., `j = complete.cases(x$mRNA, x$miRNA); x[, j, ]`
- e.g., data type selection, `x[, , c("mRNA", "miRNA")]`
- Curated collections of public integrated data sets

Future events

- Computational Statistics for Genome Biology (CSAMA), 15-19 June, Brixen / Bressanone, Italy
- *useR!*, 1-3 July, Aalborg, Denmark
- BioC 2015, 20 - 22 July, Seattle, WA USA

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