How *Bioconductor* advances science and contributes to *R*

Martin Morgan
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slides

When I talk about *R*...

```
1 + 2
x = rnorm(100)
hist(x)

y = x + rnorm(100)

df = data.frame(x, y); plot(y ~ x, df)
fit = lm(y ~ x, df)
anova(fit); abline(fit)

library(ggplot2)

ggplot(df, aes(x, y)) + geom_point()
```

Old-fashioned calculator
Statistical programming language
Interactive (immediate) visualization

Expressive, vectorized

Objects for coordinating data
Computable results
Interface (not implementation)

Domain-specific understanding

Personality

Domain-specific understanding

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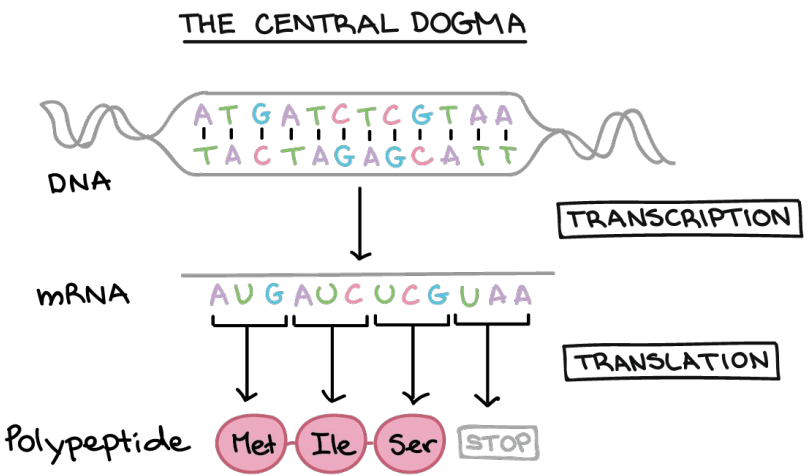
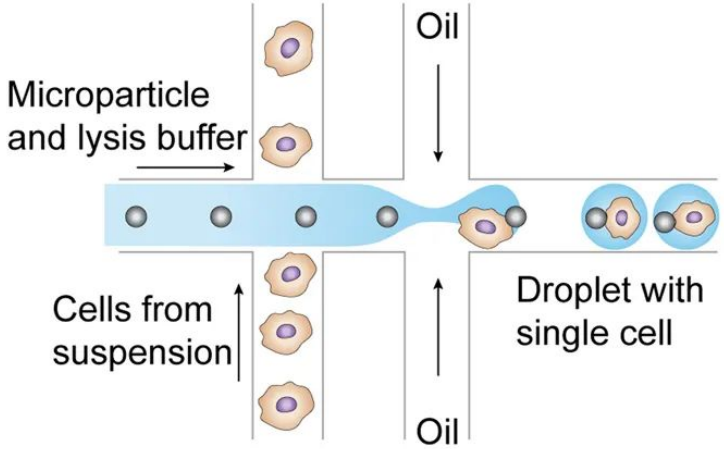
High-throughput genomics, e.g., single-cell RNA-seq



https://cdn.kastatic.org/ka-perseus-images/2b597889d05bc601803a3b4d9ec5ccd5e7b8d3af.png All Khan Academy content is available for free at www.khanacademy.org

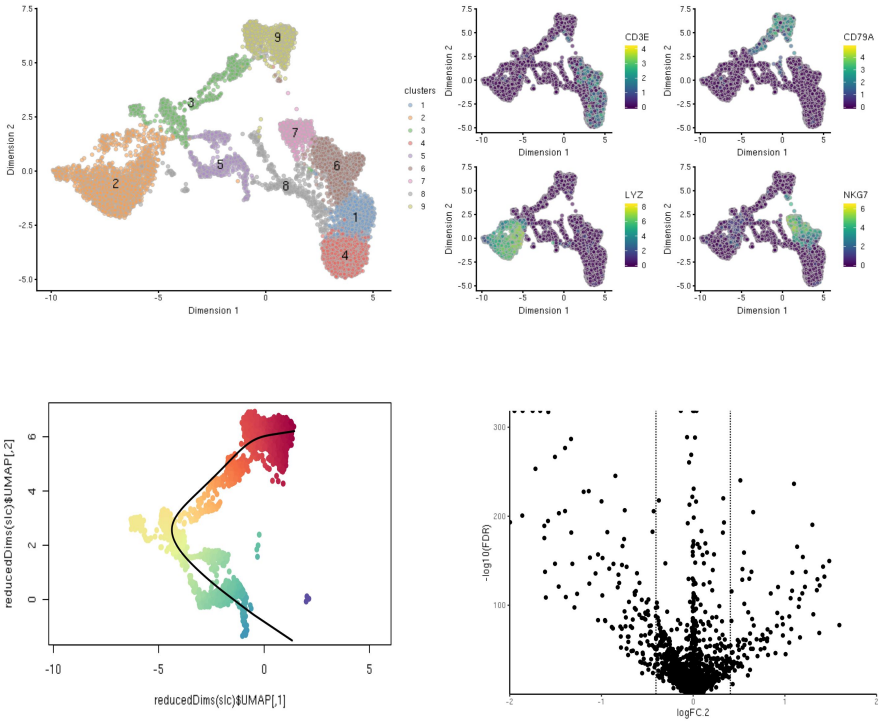


- Isolate individual cells
- Associate each cell with bar-coded beads
- Sequence bar-coded cDNA
- Hwang et al., 2018
  https://doi.org/10.1038/s12276-018-0071-8

Many statistical challenges & research questions

```
> counts      # 20k gene x 50k+ cell matrix
       GATAGGAG-1 GCGGCTTC-1 GGAATCGC-1 ...
ENSG001       679        448        873 ...
ENGS002         0          0          0 ...
ENGS003       467        515        621 ...
...
```

- Clustering & cell type classification*
- Gene expression*
- Cell trajectories*
- Data integration
- Differential expression*
- Annotation, gene set analysis, ...



Hicks, Amezquita, et al., https://osca.bioconductor.org

Data

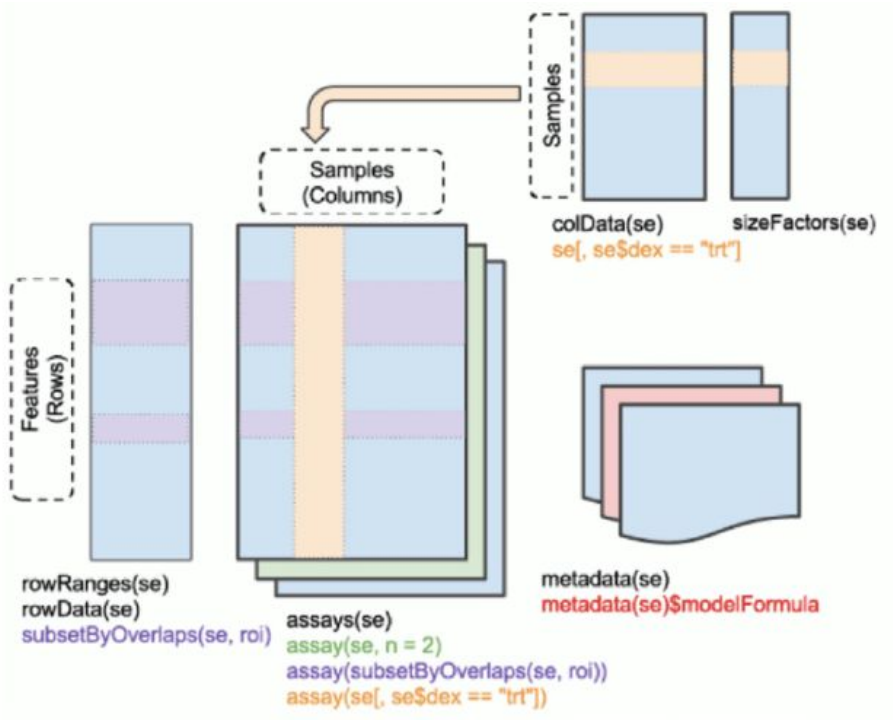
Very large primary data

- DNA sequence 'reads' (from mRNA)
- Reads aligned to a reference genome

Reduced for analysis

- E.g., matrix of read counts
- 'Genes' (e.g., 20k) x 'Samples' (e.g., 50k)
- Largest to date: ~28,000 genes x 1.3 million cells

Annotations on both genes and samples



Analysis

Inherently statistical

- Designed experiments
- Technology & artifacts -- batch effects, library size, sparsity, distribution, ...
- Usually: large P (genes) small N (samples)
    - E.g., 28k genes, 1.3 million cells, but only two (!) mice

Comparatively ‘big’

- Primary data is very large
- Even after reduction, e.g., single cell experiments, data can be 'big'
- Strategies for managing memory and throughput

Comprehension

Reproducibility

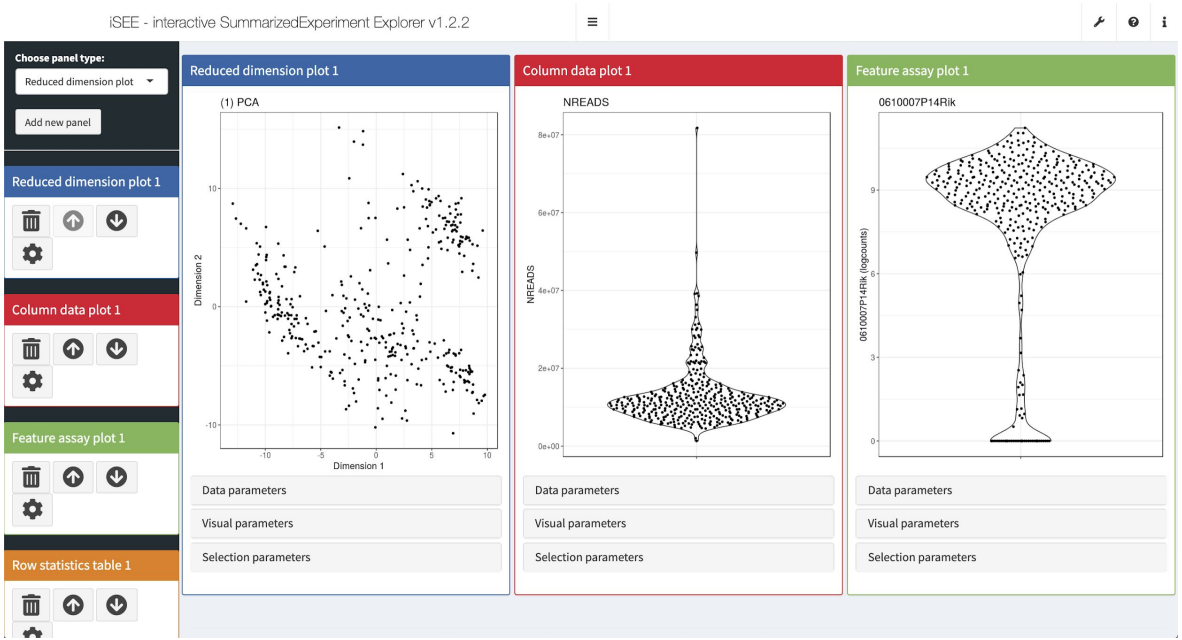
- During the analysis

Annotation

- Gene ids,pathways, drug targets, ...

Communication

- E.g., visual summaries, reports, slides, interactive apps, ...



iSEE package: https://bioconductor.org/packages/iSEE
blog: https://bit.ly/2UhNTP4

Bioconductor -- Statistical analysis and comprehension of high-throughput genomic data

Single-cell and bulk sequence data; expression, methylation, SNP and other microarrays; flow cytometry; proteomics; …

Funding from the US NIH, EU, Chan-Zuckerberg and other sources support a small professional team of developers

Real scientific progress from the broader developer & user community!

[Stickers!]

Personality

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About *Bioconductor* -- https://bioconductor.org

Established 2001 by R. Gentleman & colleagues

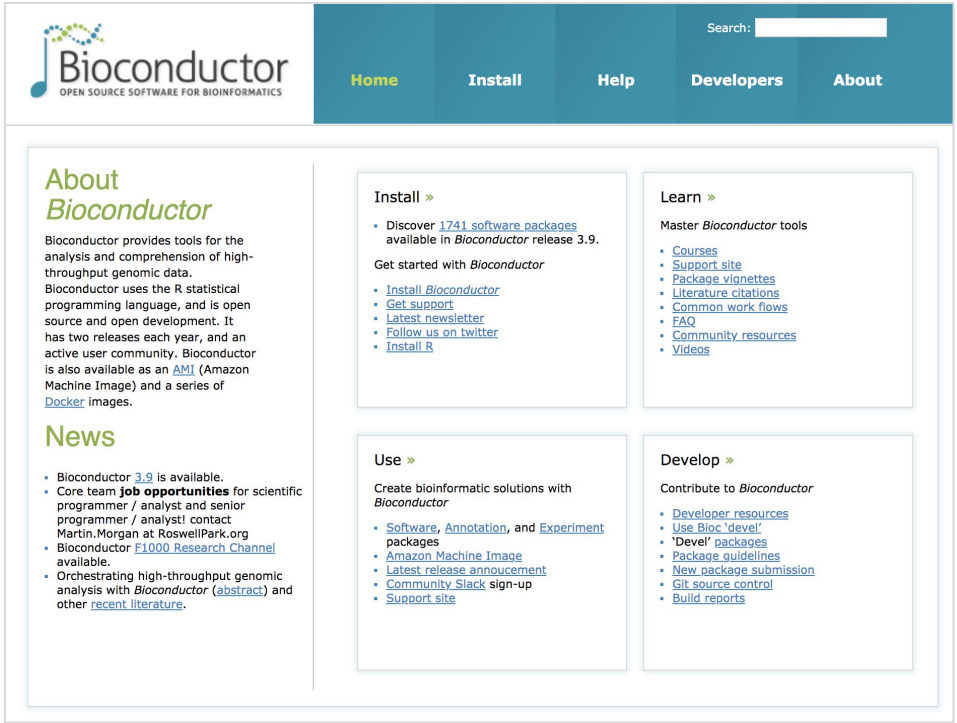
- Now 1750 *R* software packages

Users

- ½ million unique IP downloads last year
- 30,000 PubMedCentral citations

Contributors & developers

- More than 1200 maintainers worldwide

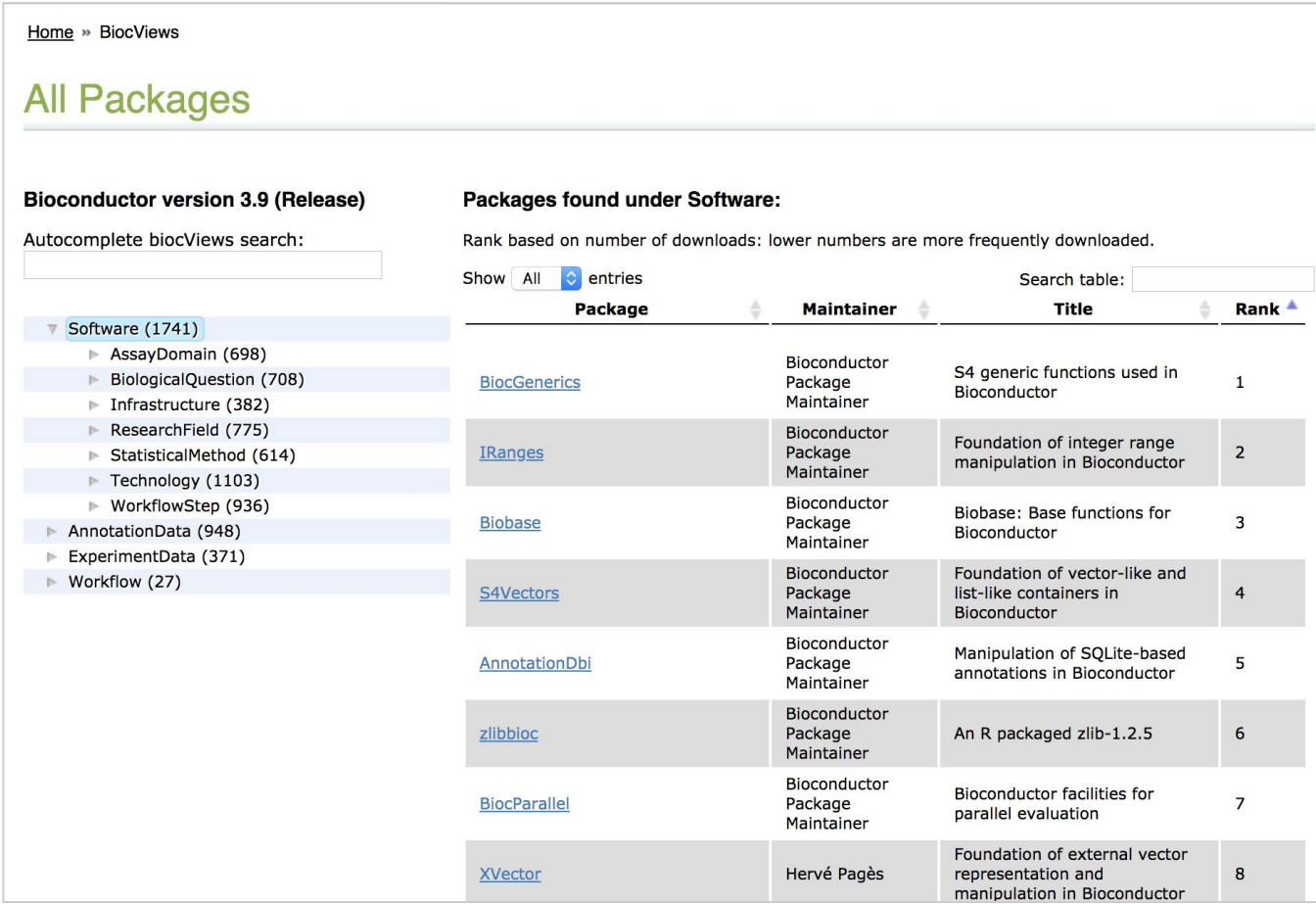


Packages

Analytic software packages

Additional types of packages

- Annotation: biological context
- Experiment data: reproducible examples & case studies
- Workflow: end-to-end documentation



Home » BiocViews

All Packages

Bioconductor version 3.9 (Release)

Autocomplete biocViews search:

- Software (1741)
  - AssayDomain (698)
  - BiologicalQuestion (708)
  - Infrastructure (382)
  - ResearchField (775)
  - StatisticalMethod (614)
  - Technology (1103)
  - WorkflowStep (936)
- AnnotationData (948)
- ExperimentData (371)
- Workflow (27)

Packages found under Software:

Rank based on number of downloads: lower numbers are more frequently downloaded.

Show All entries Search table:

Package	Maintainer	Title	Rank
BiocGenerics	Bioconductor Package Maintainer	S4 generic functions used in Bioconductor	1
IRanges	Bioconductor Package Maintainer	Foundation of integer range manipulation in Bioconductor	2
Biobase	Bioconductor Package Maintainer	Biobase: Base functions for Bioconductor	3
S4Vectors	Bioconductor Package Maintainer	Foundation of vector-like and list-like containers in Bioconductor	4
AnnotationDbi	Bioconductor Package Maintainer	Manipulation of SQLite-based annotations in Bioconductor	5
zlibbioc	Bioconductor Package Maintainer	An R packaged zlib-1.2.5	6
BiocParallel	Bioconductor Package Maintainer	Bioconductor facilities for parallel evaluation	7
XVector	Hervé Pagès	Foundation of external vector representation and manipulation in Bioconductor	8

Package landing pages

Home » Bioconductor 3.9 » Software Packages » DESeq2

DESeq2

platforms all | rank 25 / 1741 | posts 360 / 1 / 2 / 50 | in Bioc 6 years
build ok | updated before release | dependencies 109

DOI: 10.18129/B9.bioc.DESeq2

Differential gene expression analysis based on the negative binomial distribution

- Badges, citations, DOI, …
- Installation instructions
- Vignettes

Bioconductor version: Release (3.9)

Estimate variance-mean dependence in count data from high-throughput sequencing assays and test for differential expression based on a model using the negative binomial distribution.

Author: Michael Love, Simon Anders, Wolfgang Huber

Maintainer: Michael Love <michaelisaiahlove at gmail.com>

Citation (from within R, enter `citation("DESeq2")`):

Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome Biology*, **15**, 550. doi: 10.1186/s13059-014-0550-8.

Installation

To install this package, start R (version "3.6") and enter:

```
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("DESeq2")
```

For older versions of R, please refer to the appropriate Bioconductor release.

Documentation

To view documentation for the version of this package installed in your system, start R and enter:

```
browseVignettes("DESeq2")
```

HTML	R Script	Analyzing RNA-seq data with DESeq2
PDF		Reference Manual
Text		NEWS

Support

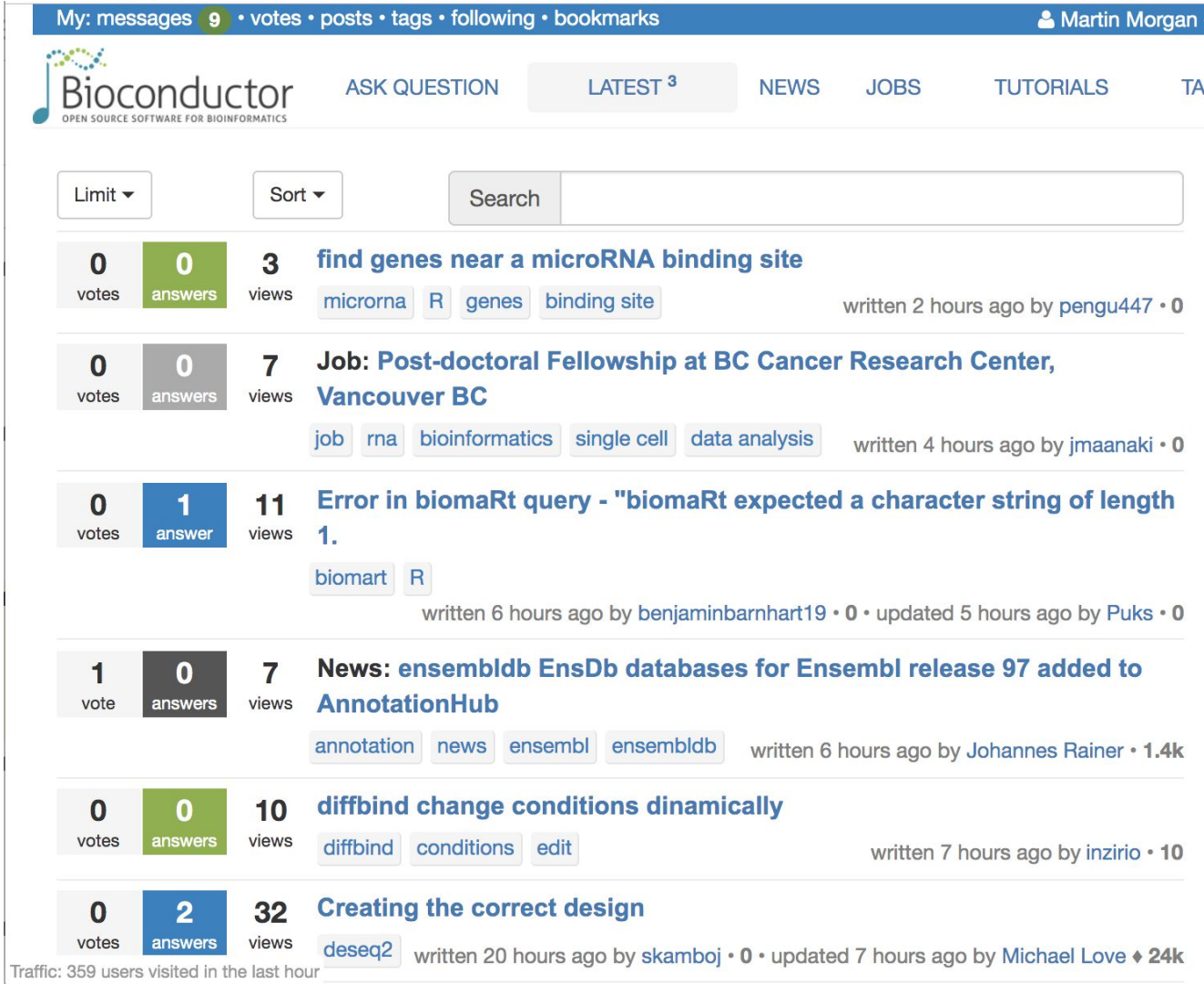
Users

- StackOverflow-style [support site]

Users / Developers

- Community [slack]

Maintainer [mailing list]



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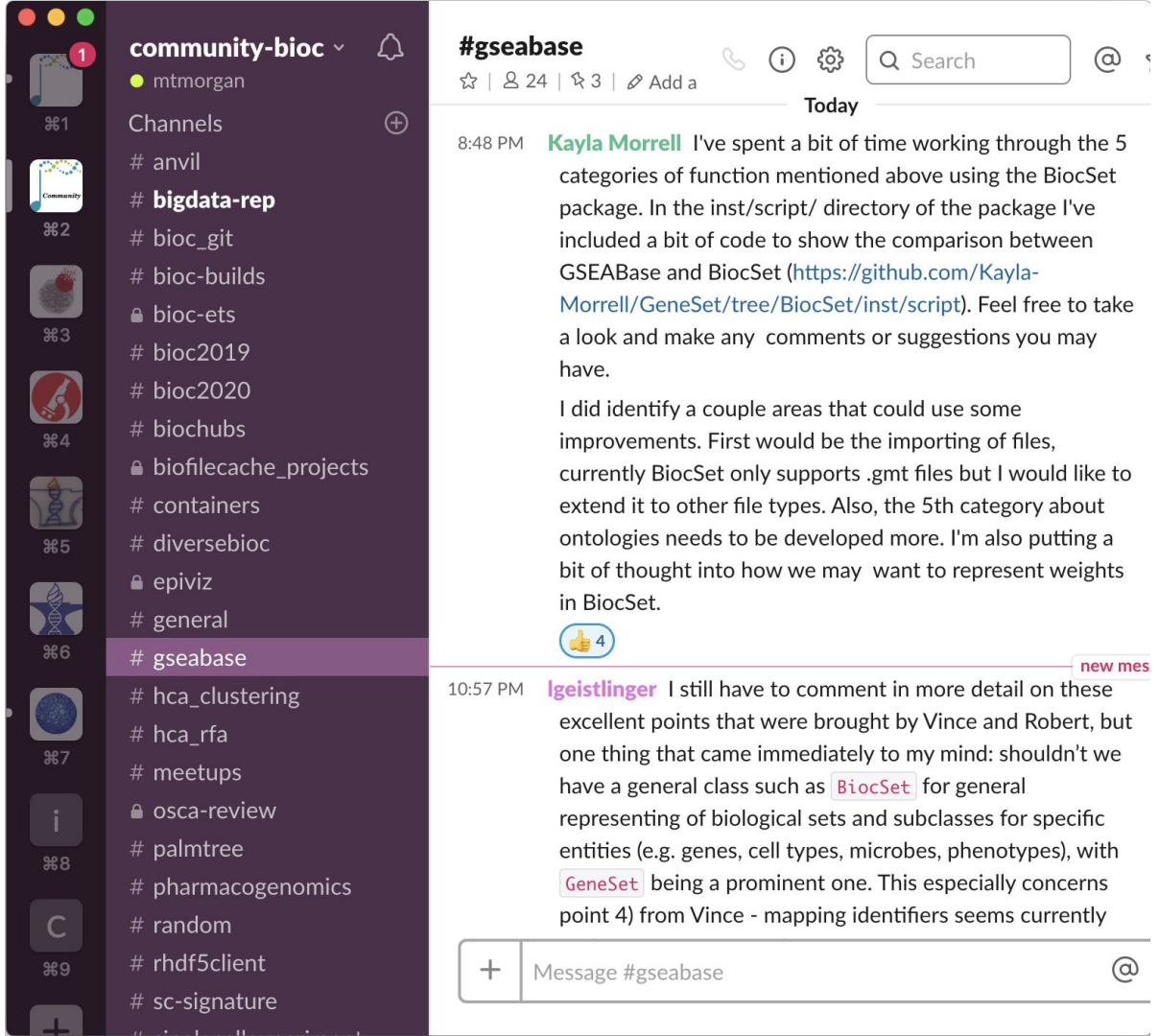


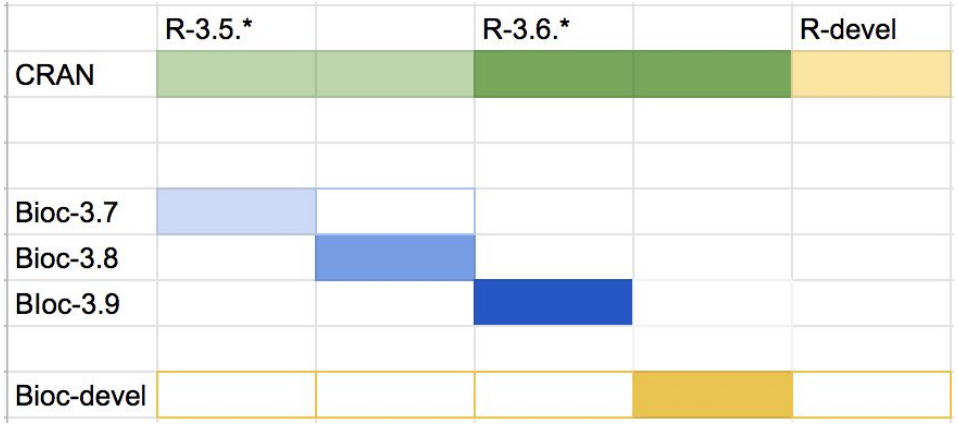
Releases & repositories

Releases

- A stable ‘release’ branch for users
- A ‘devel’ branch for new packages & features
- Twice-yearly: devel becomes release

BiocManager

- CRAN package for installing correct versions of *Bioconductor* packages
- Also installs CRAN, github packages




```
## install.packages("BiocManager") # CRAN
BiocManager::install(pkgs) # Bioc / CRAN / github
```

Packages

Version control

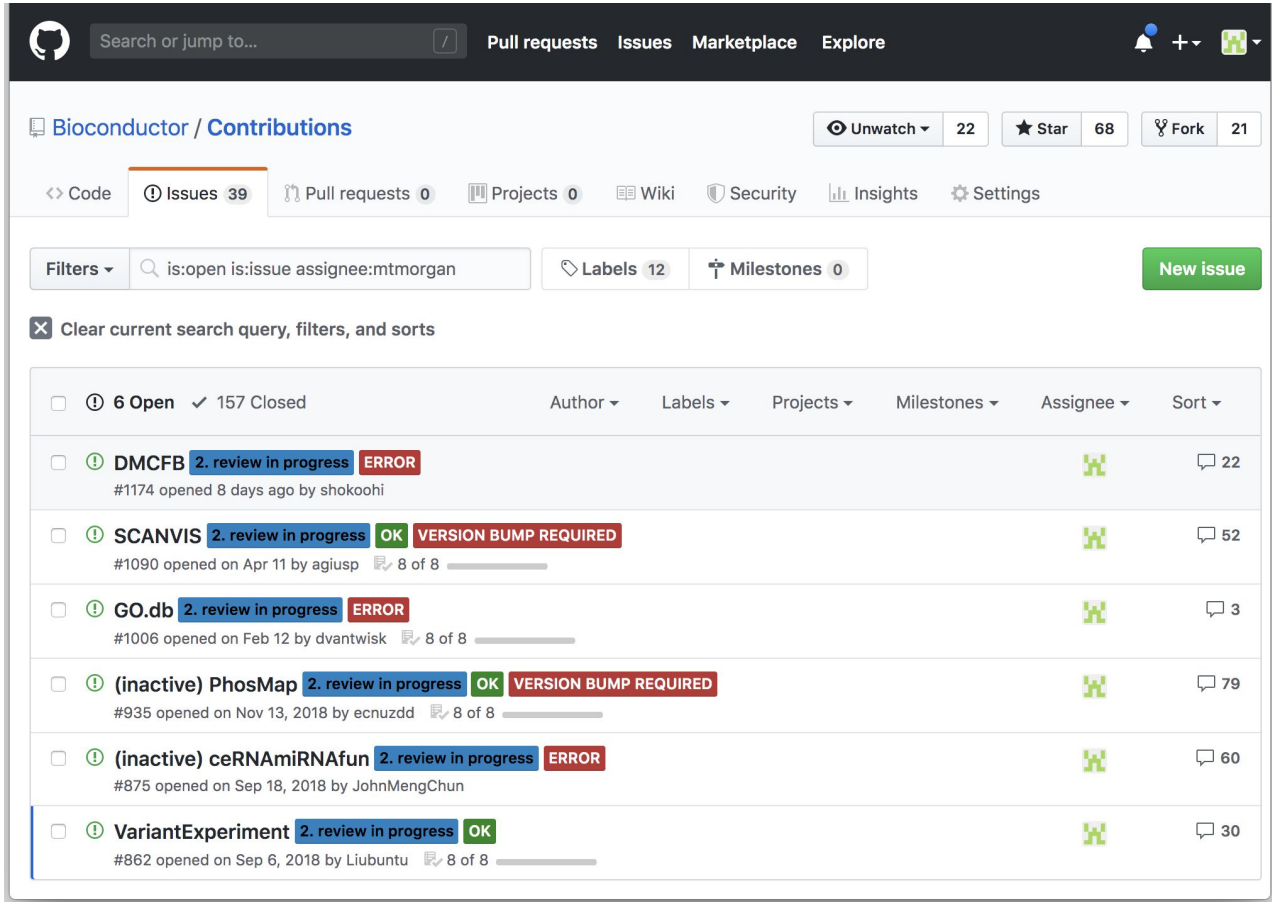
- Our own git

Nightly builds

- Cross-platform ‘integration’ tests

New packages

- Public review via github issues
- help technical quality



Personality

Broad global user base

Supportive community

- For users and developers

Extensive, tested, maintained, and supported software

Stable release branch / flexible & innovative devel branch

Large scientific and professional impact

Objects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Personality

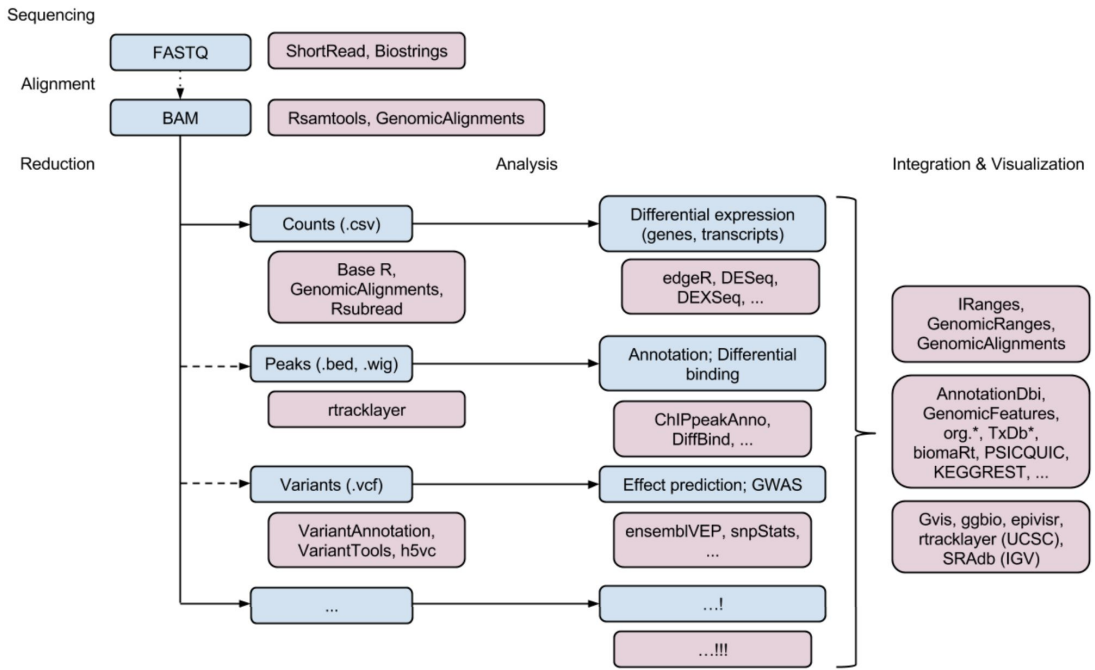
We want interoperable & robust software

Interoperable

- Exploratory: apply different approaches to the same data
- Mature: apply the same approach to different data

Robust

- Data validation
- Easy management of complicated data
- Tested through use



Why (S4) classes & methods?

```
> upstream2k
A DNAStringSet instance of length 26454
      width seq
  [1]  2000 GTTGGTGGCCCACCAGTGC...AGTTTACCGGTTGCACGGT
  [2]  2000 TTATTTATGTAGGCGCCCG...ACGGAAAGTCATCCTCGAT
  [3]  2000 TTATTTATGTAGGCGCCCG...ACGGAAAGTCATCCTCGAT
  ...

> reverseComplement(upstream2k)
A DNAStringSet instance of length 26454
      width seq
  [1]  2000 ACCGTGCAACCGGTAAACT...GCACTGGTGGGCCACCAAC
  [2]  2000 ATCGAGGATGACTTTCCGT...CGGGCGCCTACATAAATAA
  [3]  2000 ATCGAGGATGACTTTCCGT...CGGGCGCCTACATAAATAA
  ...
```

For the user

- Validation, e.g., specific nucleotide 'letters' in the DNA alphabet
- Domain-specific methods, e.g., `reverseComplement()`
- Friendly 'interface'

For the developer

- Efficient 'implementation' to store many large strings

Major *Bioconductor* classes

Vector-like representations

- `DNAStringSet`
- **`GRanges`** (genomic ranges)

Experimental results -- relational constraints

- `SummarizedExperiment`

S4Vectors

- `Vector`
- `List`
- `DataFrame`
- `...`

```
> exons
GRanges object with 515283 ranges and 1 metadata column:
    seqnames            ranges strand |        gene
       <Rle>         <IRanges>  <Rle> | <character>
[1]    chr12 56118262-56118318      + |       84872
[2]     chr7 29126856-29126939      + |   101928168
[3]    chr15 60479178-60479398      + |   101928784
...
-------
seqinfo: 25 sequences (1 circular) from hg38 genome
```

- Required and optional information
- One-based, closed-intervals
- Provenance -- what genome is this?
- Looks two-dimensional, but actually a `Vector`

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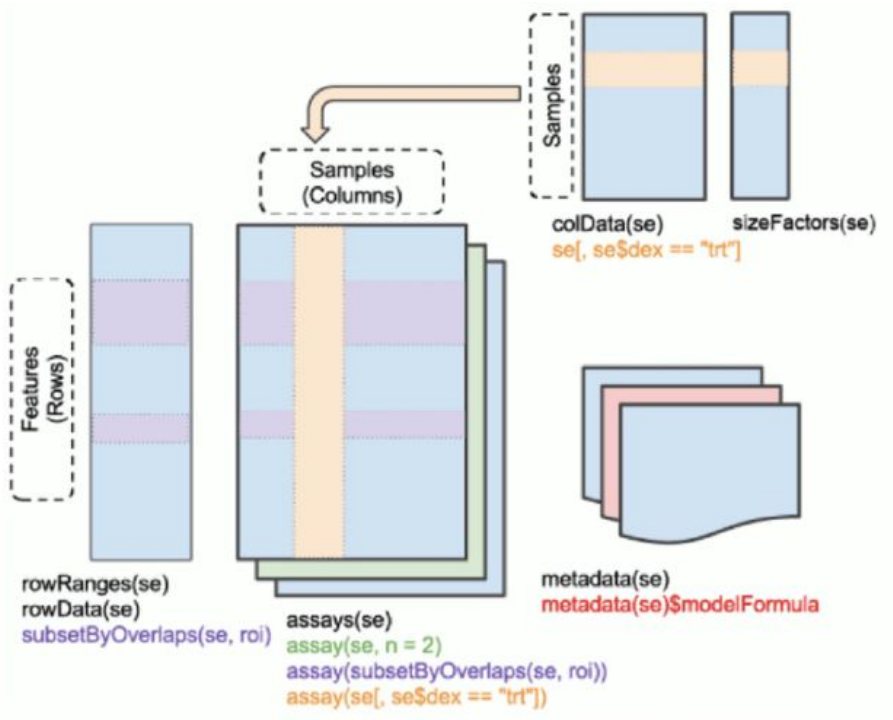
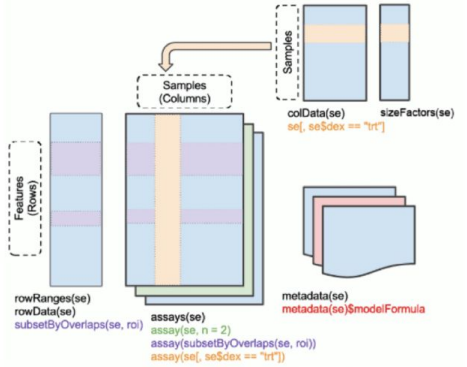


SummarizedExperiment

```
> counts     # gene x sample matrix
      SRR1039508 SRR1039509 SRR1039512 ...
ENSG001      679        448        873 ...
ENGS002        0          0          0 ...
ENGS003      467        515        621 ...
...

> samples    # data.frame/tibble/DataFrame
             cell     dex   ...
            <fct>   <fct>
SRR1039508  N61311  untrt  ...
SRR1039509  N61311  trt    ...
SRR1039512  N052611 untrt  ...
...
```




```
se = SummarizedExperiment(
    counts,
    colData = samples
)

## ‘library size’ of each sample
colSums(assay(se))

## subset counts and samples to contain
## only “untrt” samples
untrt = se[, se$dex == “untrt”]
```

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S4Vectors

- `Vector`
- `List`
- `DataFrame`
- `...`

What is a `Vector`? (approximately...)

- Anything that implements `length()`, `` `[` ``, `` `[<-` `` and `names()`

What is a `List`?

- Anything that implements `Vector` plus `lengths()`, `` `[[` ``, `` `[[<-` ``

What is a `DataFrame` *column*?

- Anything that implements `Vector`, or an atomic `vector` or `list`

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S4Vectors

- `Vector`
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```
> dna = DNAStringSet(c("AACTG", "CCCATG"))
> gr = GRanges(c("chr1:21-30", "chr2:55-59"))

> is(dna, "Vector")
[1] TRUE
> is(gr, "Vector")
[1] TRUE

> df = DataFrame(id = 1:2, gr, dna)
> df
DataFrame with 2 rows and 3 columns
         id         gr            dna
  <integer>  <GRanges> <DNAStringSet>
1         1 chr1:21-30          AACTG
2         2 chr2:55-59         CCCATG
```

Objects

Encourage interoperability between packages

Provide robust validation

Enable easy management of complex relational data

Separate

- 'interface' experienced by the user
- 'implementation' available to the developer

Reproduce & communicate

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

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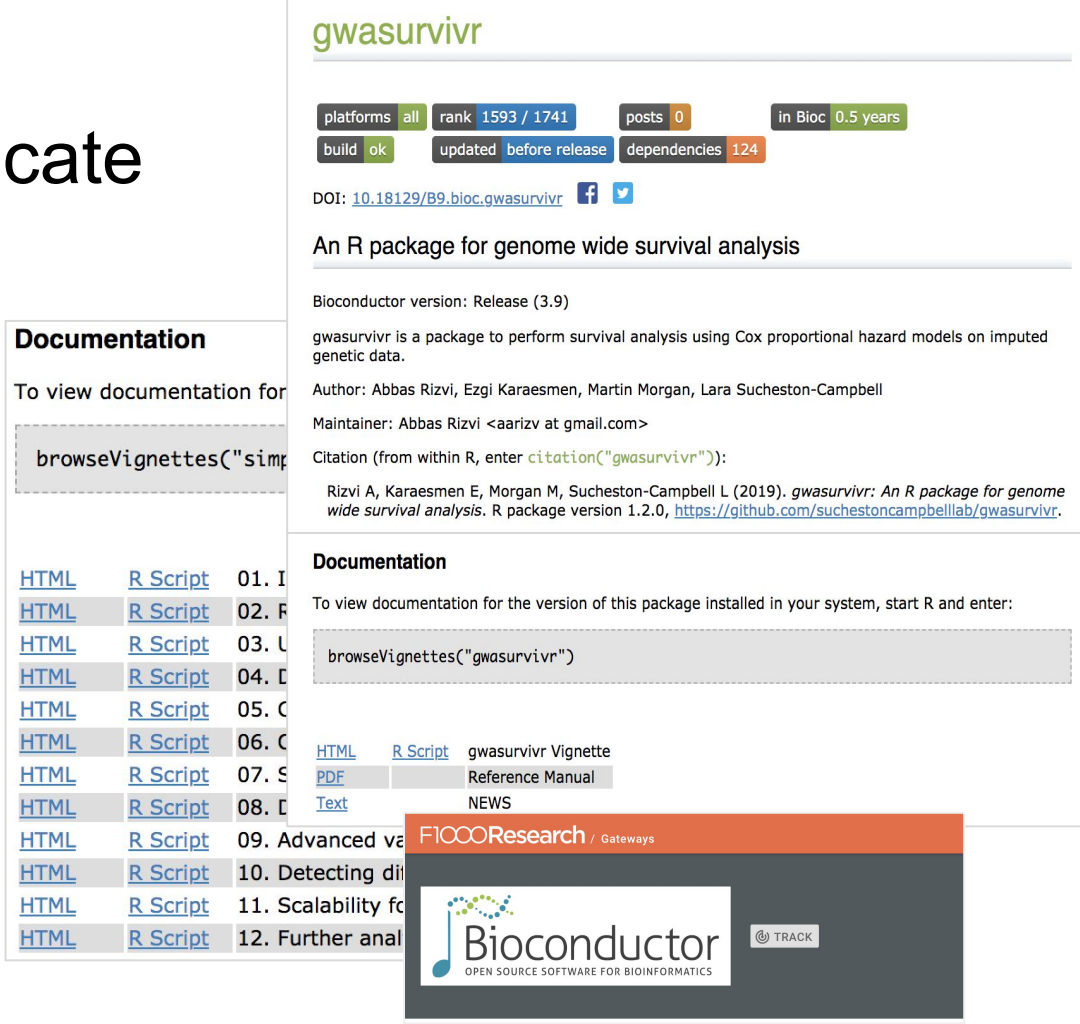
Reproduce & communicate

Integrative documentation

- Extensive, computable vignettes
  - Required of all *Bioconductor* packages
- Comprehensive workflows
- Workshop compendia (e.g., BioC2019) & community-developed material (e.g., osca)

Paths to academic recognition

- Individual packages, DOI, and citations
- F1000 Research / *Bioconductor* channel



Challenges & opportunities

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Large data representation, e.g., single-cell data

Illusions

- Show and update ‘corners’ of data
- Delay full update until needed

On-disk representation

- Only row & column indexes in memory

Chunk-wise parallel processing

- Read blocks of memory, perform calculation
- Often a reduction, e.g., `colSums()`, that fits in memory

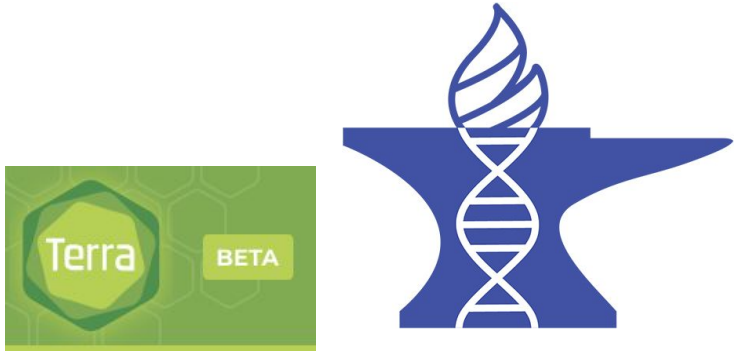
```
> tenx = TENxBrainData::TENxBrainData()
> dim(tenx) # on-disk SummarizedExperiment
[1]   27998 1306127

> log(1 + assay(tenx))          # illusion

> cidx = sample(ncol(tenx), 200)
> tenx_subset = tenx[, cidx]
> sum(assay(tenx_subset) == 0) /
    prod(dim(tenx_subset))
[1] 0.9289764                   # sparse!

> colSums(assay(tenx_subset)))  # realized
```

The AnVIL cloud https://anvilproject.org



US NHGRI initiative

- Genomic Data Commons -- large ‘consortium’ data
- Terra -- Cloud-based computation
- *Python*, *R* / *Bioconductor*, *Galaxy*, and other tools

Benefits

- Simple -- e.g., containerized *Bioconductor*
- Fast -- cloud-based data access
- Scalable, e.g., configured *BiocParallel*
- Secure

Alternative paradigms in *R*

Tidy data

- Long-form `tibble` / `data.frame`

Bioconductor data

- Generalized `Vector`
  - `DNAStringSet`, `GRanges`
- Structured, efficient matrix
  - `assay()` data of `SummarizedExperiment`
- Relational
  - `SummarizedExperiment`
  - Row & column names serve as unique keys for joins

```
> assay(airway) %>% ... %>%
    ## ...: matrix -> tidy tibble
    group_by(sample) %>%
    summarize(lib_size = sum(value))

> assay(airway) %>%
    colSums() %>%
    enframe("sample", "lib_size")

# A tibble: 8 x 2
  sample     lib_size
  <fct>         <int>
1 SRR1039508 20637971
2 SRR1039509 18809481
...
```

Challenges & opportunities

Scalability

- Memory management & delayed evaluation

Cloud-based computation

- Containerization, e.g., reliable binary installations
- Fast data access
- Scalable

Working well with other parts of the evolving *R* & broader ecosystems

- tidyverse, python, cloud, ...

I talked about...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4. Reproduce & communicate

5. Challenges & opportunities

3. S4 objects & interdependent packages

1. High-throughput genomics

2. The *Bioconductor* ecosystem

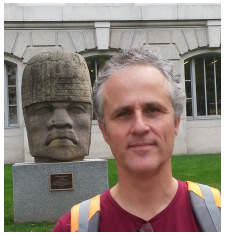
Conclusions and acknowledgements

Bioconductor core team & close collaborators

- Funded by US National Institutes of Health, European Union, Chan-Zuckerberg Initiative ...

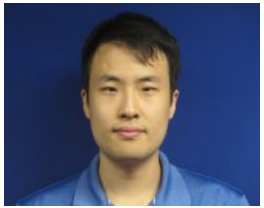
World-wide community of users & developers

Technical and scientific advisory boards







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