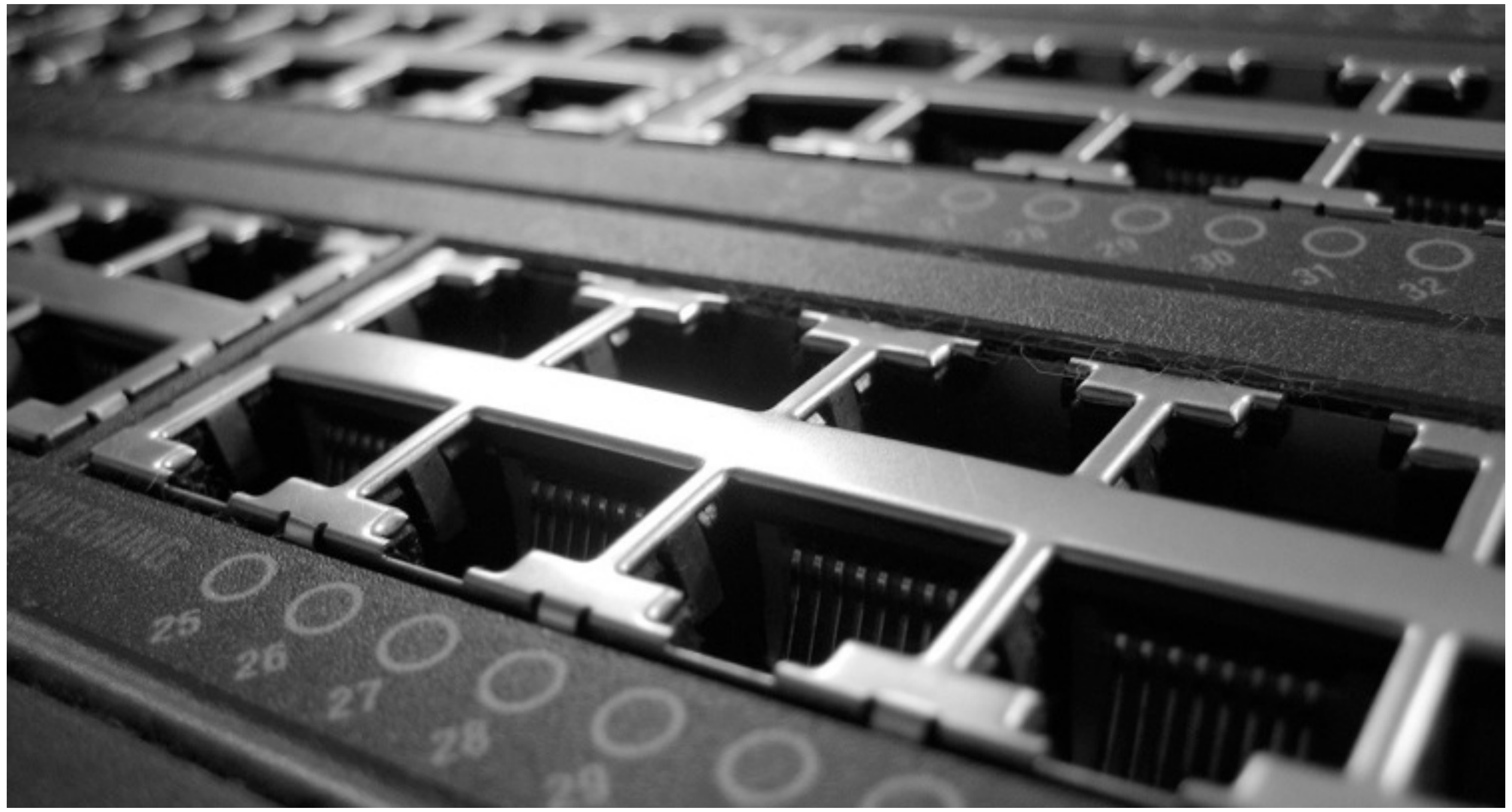
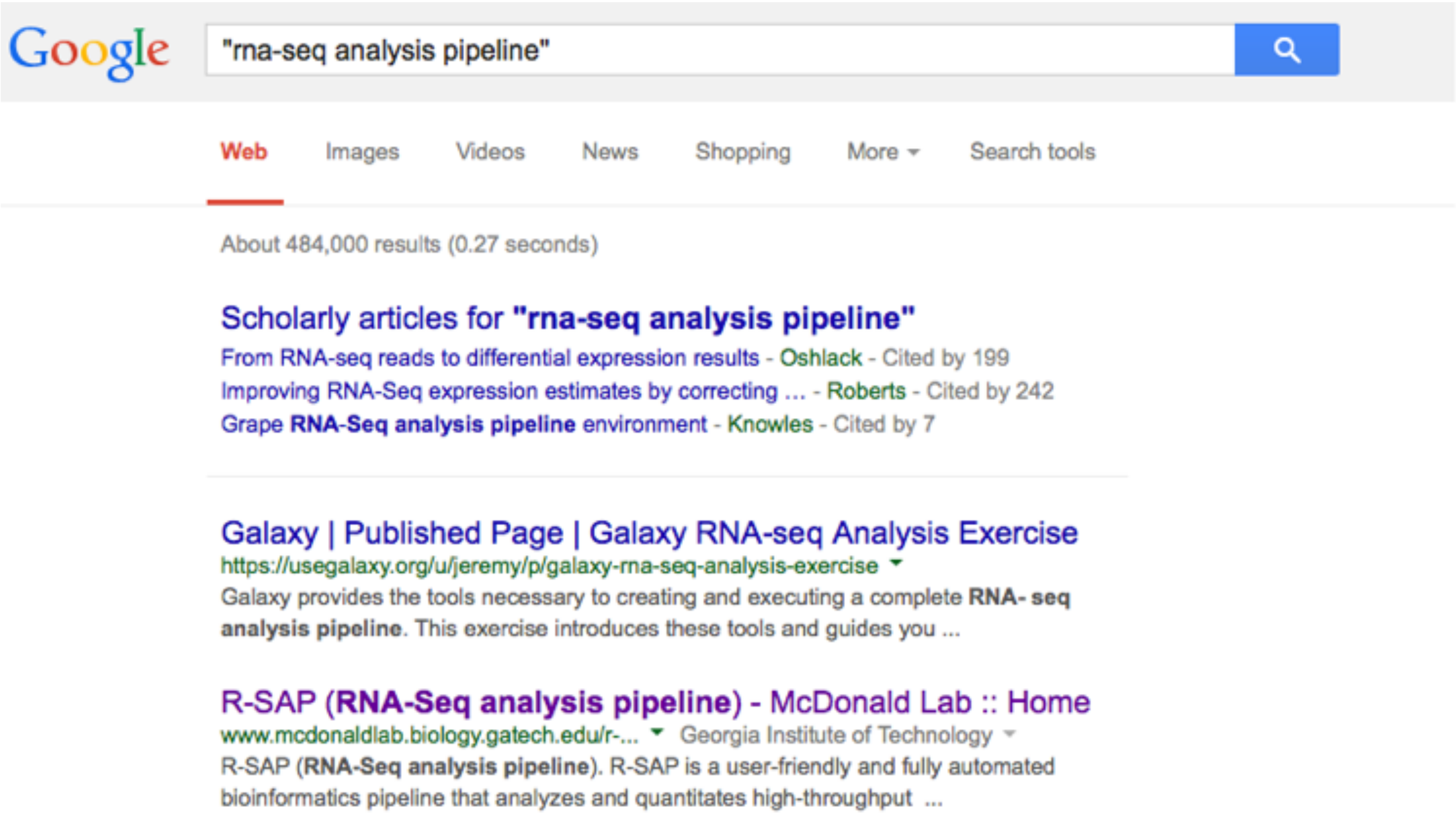


Automated RNA-seq Differential Expression Validation

Center for Health Bioinformatics, Harvard School of Public Health



"rna-seq analysis pipeline"

About 484,000 results (0.27 seconds)

Scholarly articles for **"rna-seq analysis pipeline"**

From RNA-seq reads to differential expression results - Oshlack - Cited by 199

Improving RNA-Seq expression estimates by correcting … - Roberts - Cited by 242

Grape **RNA-Seq analysis pipeline** environment - Knowles - Cited by 7

Galaxy | Published Page | Galaxy RNA-seq Analysis Exercise

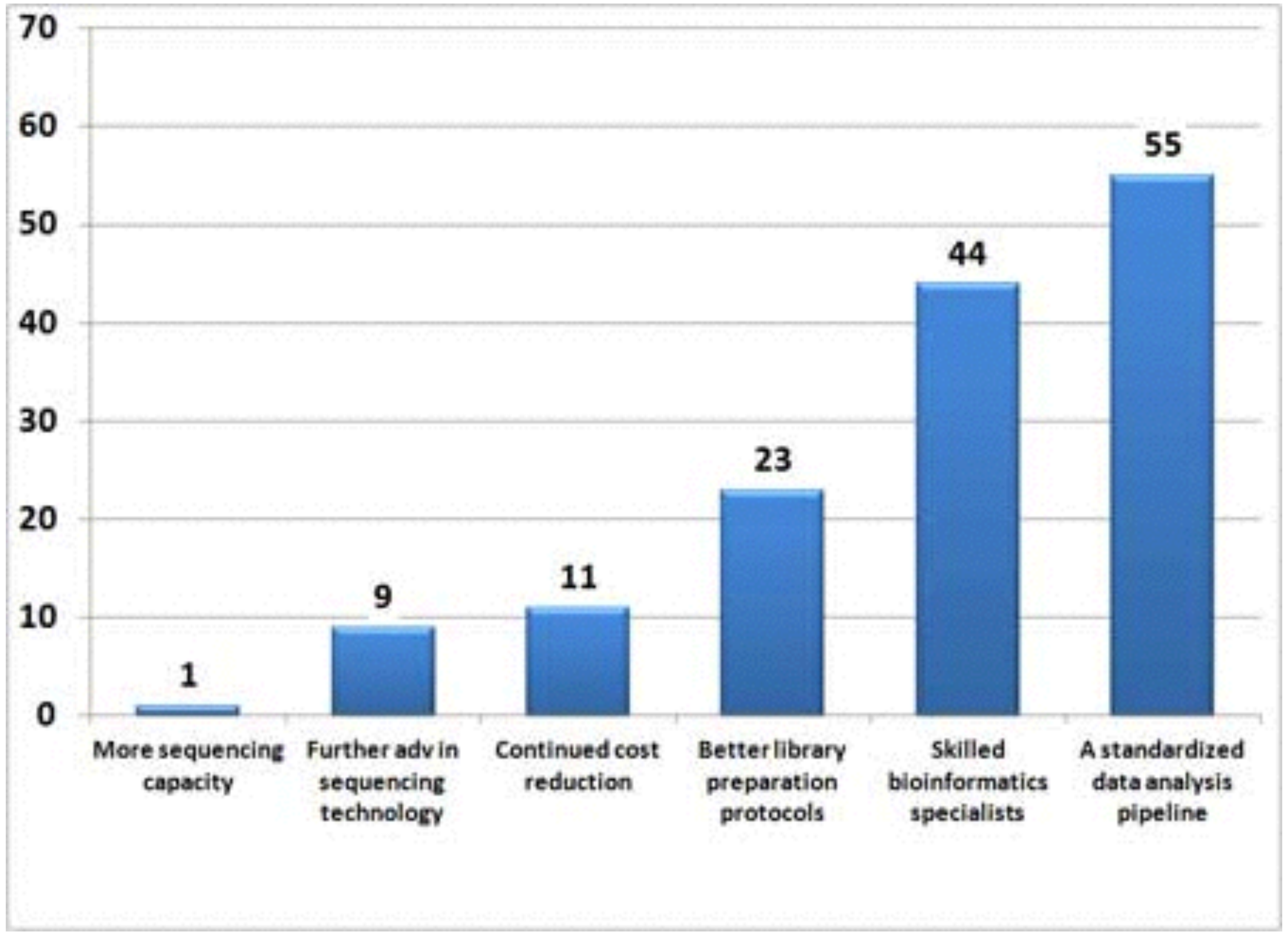
https://usegalaxy.org/u/jeremy/p/galaxy-rna-seq-analysis-exercise

Galaxy provides the tools necessary to creating and executing a complete **RNA- seq analysis pipeline**. This exercise introduces these tools and guides you ...

R-SAP (**RNA-Seq analysis pipeline**) - McDonald Lab :: Home

www.mcdonaldlab.biology.gatech.edu/r-... Georgia Institute of Technology

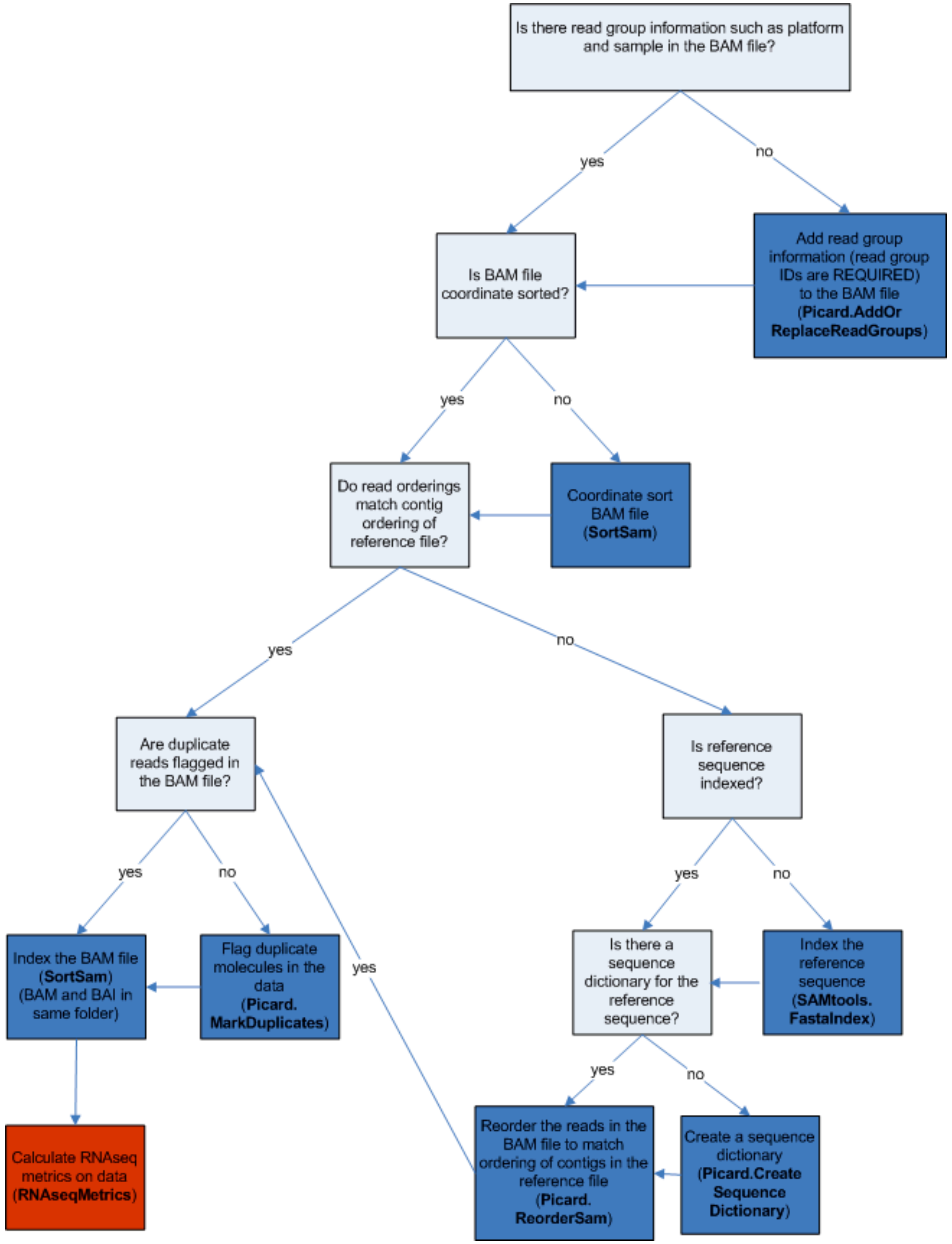
R-SAP (**RNA-Seq analysis pipeline**). R-SAP is a user-friendly and fully automated bioinformatics pipeline that analyzes and quantitates high-throughput ...



Pipeline proliferation

Complexity

- Installation
  - Third party tools
  - Bizarre environments
- Choices
  - Tools, parameters
- Data
- Glue



Is there read group information such as platform and sample in the BAM file?

Add read group information (read group IDs are REQUIRED) to the BAM file (**Picard.AddOrReplaceReadGroups**)

Is BAM file coordinate sorted?

Coordinate sort BAM file (**SortSam**)

Do read orderings match contig ordering of reference file?

Are duplicate reads flagged in the BAM file?

Is reference sequence indexed?

Index the BAM file (**SortSam**) (BAM and BAI in same folder)

Flag duplicate molecules in the data (**Picard.MarkDuplicates**)

Is there a sequence dictionary for the reference sequence?

Index the reference sequence (**SAMtools.FastaIndex**)

Calculate RNAseq metrics on data (**RNAseqMetrics**)

Reorder the reads in the BAM file to match ordering of contigs in the reference file (**Picard.ReorderSam**)

Create a sequence dictionary (**Picard.CreateSequenceDictionary**)

Development goals of bcbio-nextgen

- Community developed and driven
- Scalable
- Easy to install. Easy to use and extend.
- Well-documented
- Quantifiable

Cloud BioLinux

Anaconda

Homebrew

The missing package manager for OS X

Installation

Tools

compatible

versioned

no sudo, no problem

sandboxed

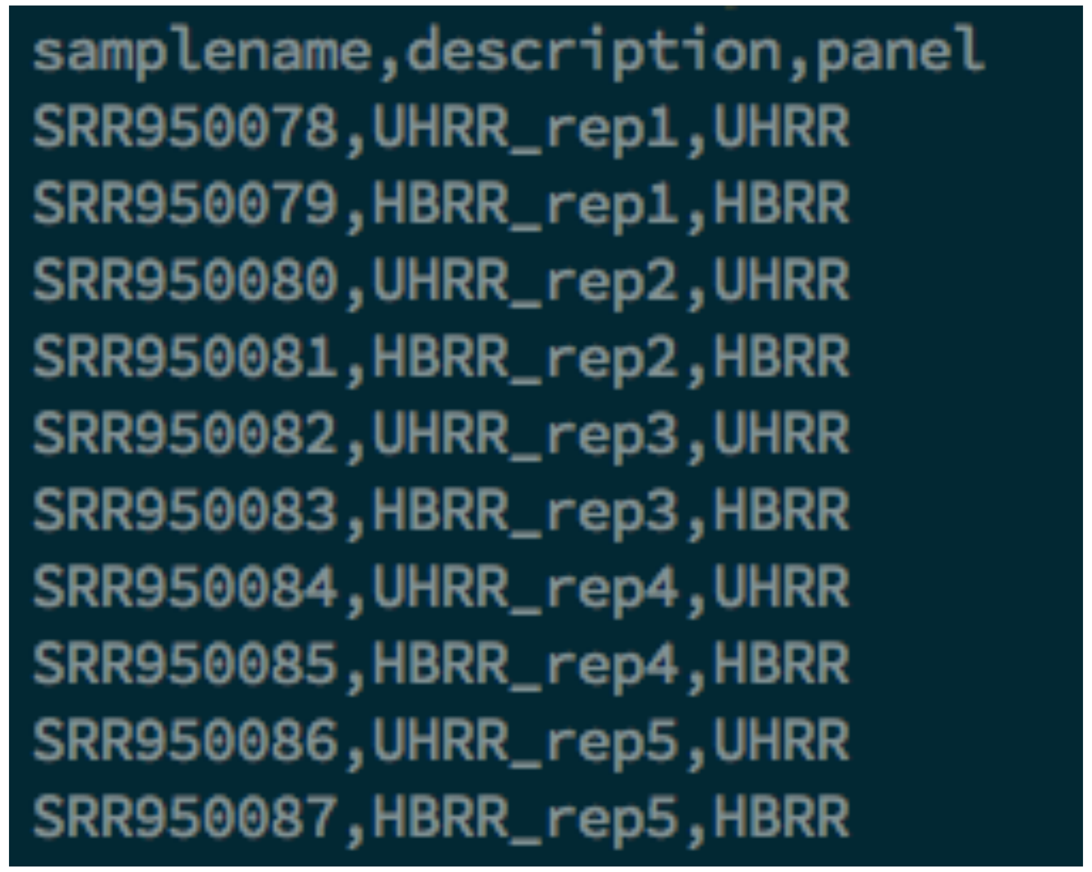
Data

coherent

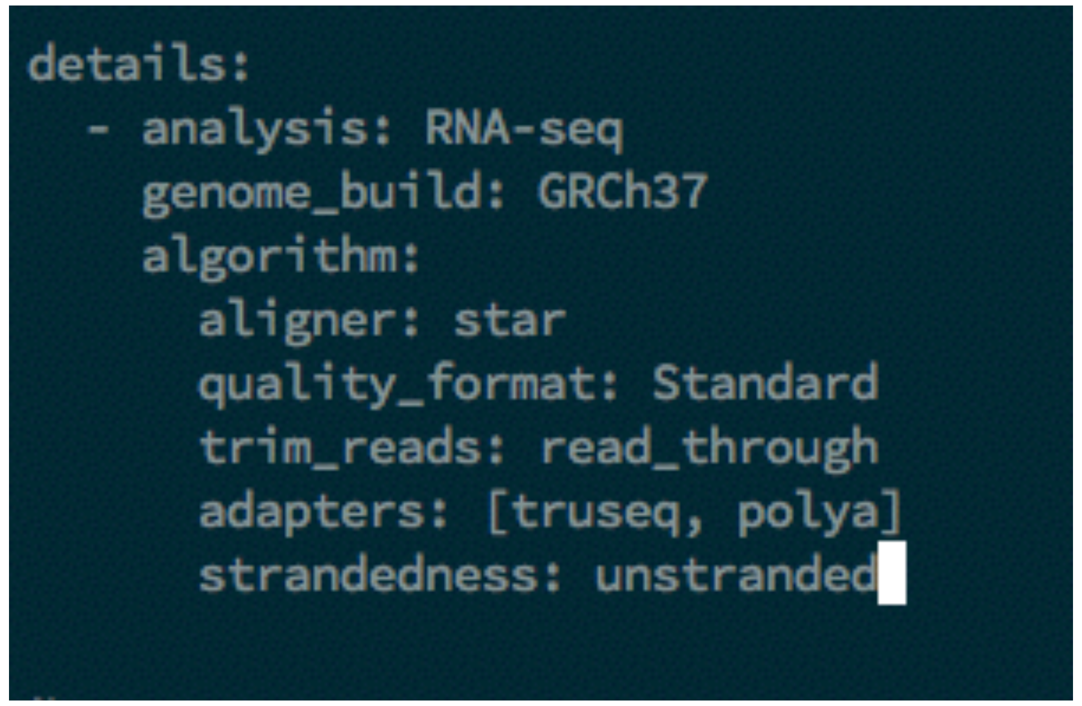
versioned

Ease of use

- Tools come pre-configured
- Analysis involves
  - Putting FASTQ/BAM files in a directory
  - Creating a CSV metadata file describing the samples
  - Editing a small configuration file

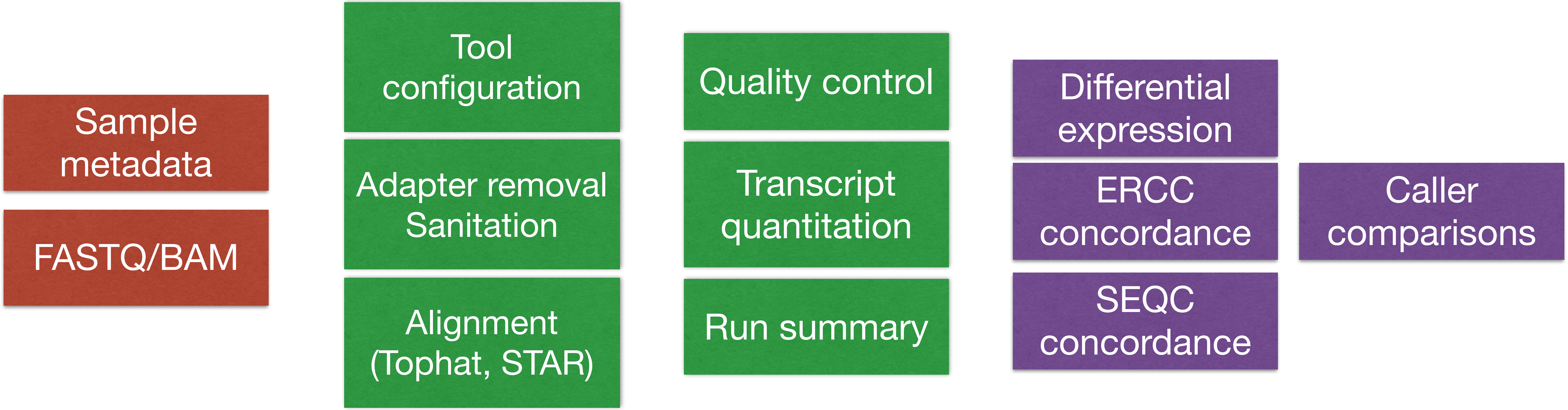


```
samplename,description,panel
SRR950078,UHRR_rep1,UHRR
SRR950079,HBRR_rep1,HBRR
SRR950080,UHRR_rep2,UHRR
SRR950081,HBRR_rep2,HBRR
SRR950082,UHRR_rep3,UHRR
SRR950083,HBRR_rep3,HBRR
SRR950084,UHRR_rep4,UHRR
SRR950085,HBRR_rep4,HBRR
SRR950086,UHRR_rep5,UHRR
SRR950087,HBRR_rep5,HBRR
```



```
details:
  - analysis: RNA-seq
    genome_build: GRCh37
    algorithm:
      aligner: star
      quality_format: Standard
      trim_reads: read_through
      adapters: [truseq, polya]
      strandedness: unstranded
```

RNA-seq pipeline overview



Sample metadata

FASTQ/BAM

Tool configuration

Adapter removal Sanitation

Alignment (Tophat, STAR)

Quality control

Transcript quantitation

Run summary

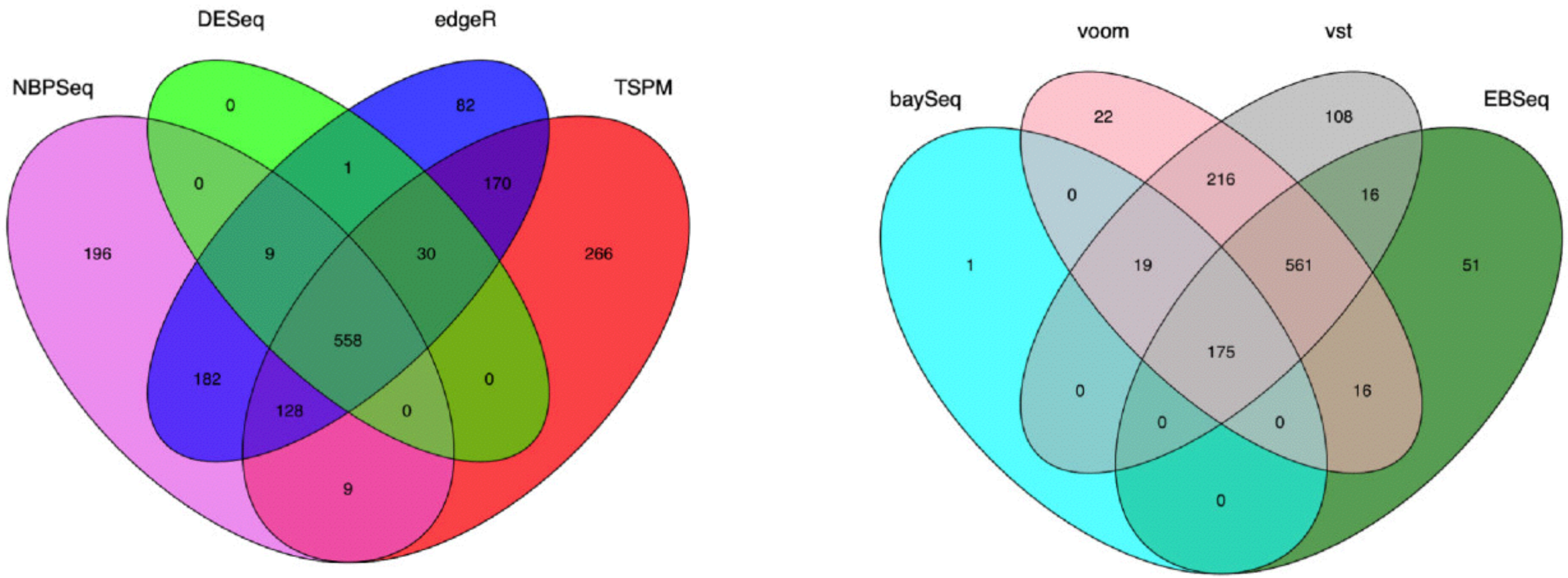
Differential expression

ERCC concordance

SEQC concordance

Caller comparisons

🟥 **User supplied** 🟩 **bcbio-nextgen** 🟪 **bcbio.rnaseq**



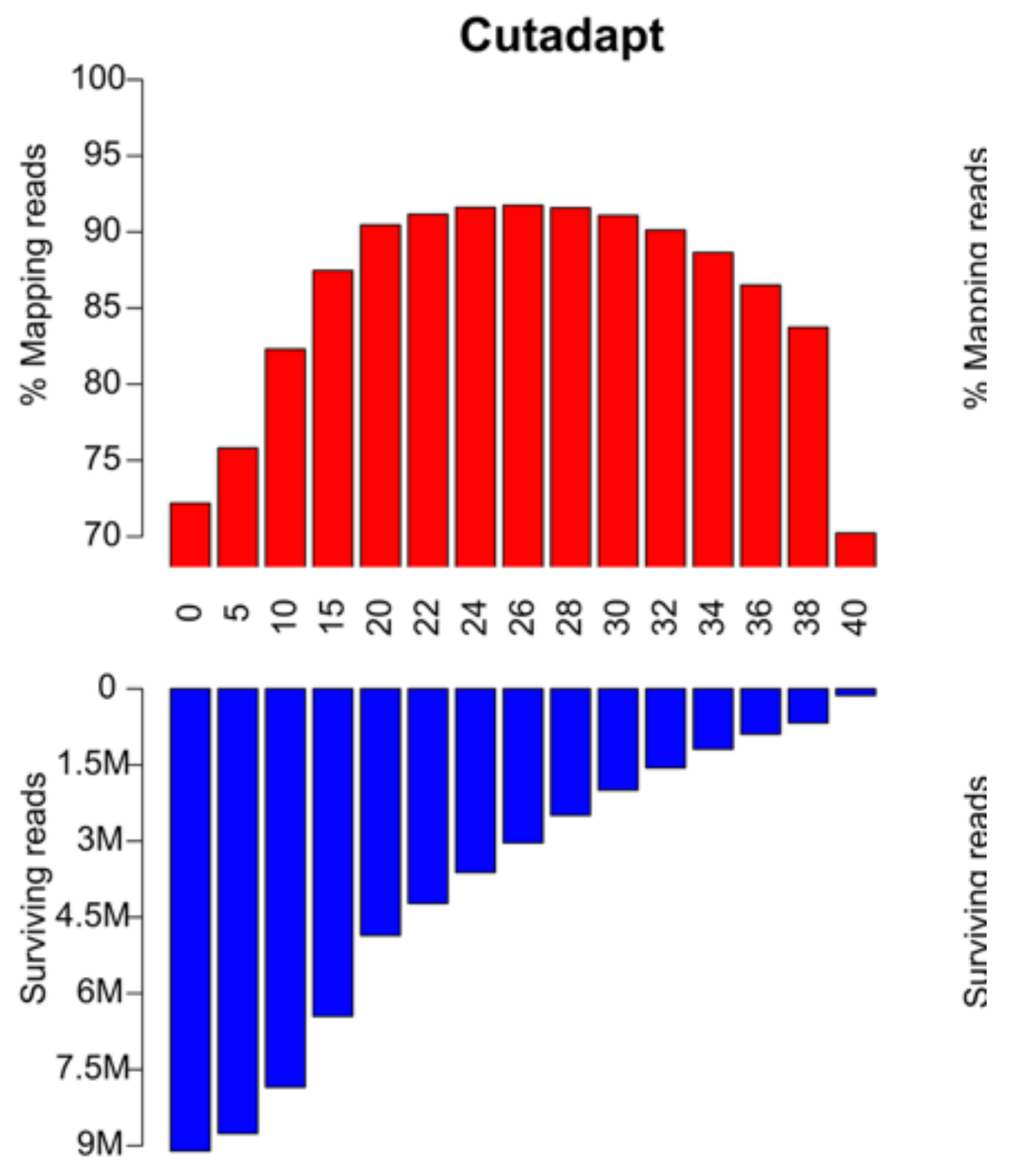
A comparison of methods for differential expression analysis of RNA-seq data
Charlotte Soneson[1*] and **Mauro Delorenzi**[12]

Varying DE calls between methods

Simulation

- SEQC data set not a great set
- Count based simulation
    - More complicated models
    - Model biological variability
- Which algorithm is best?
- Plug in and go

Is trimming beneficial in RNA-seq?



An Extensive Evaluation of Read Trimming Effects on Illumina NGS Data Analysis

Cristian Del Fabbro, Simone Scalabrin, Michele Morgante, Federico M. Giorgi

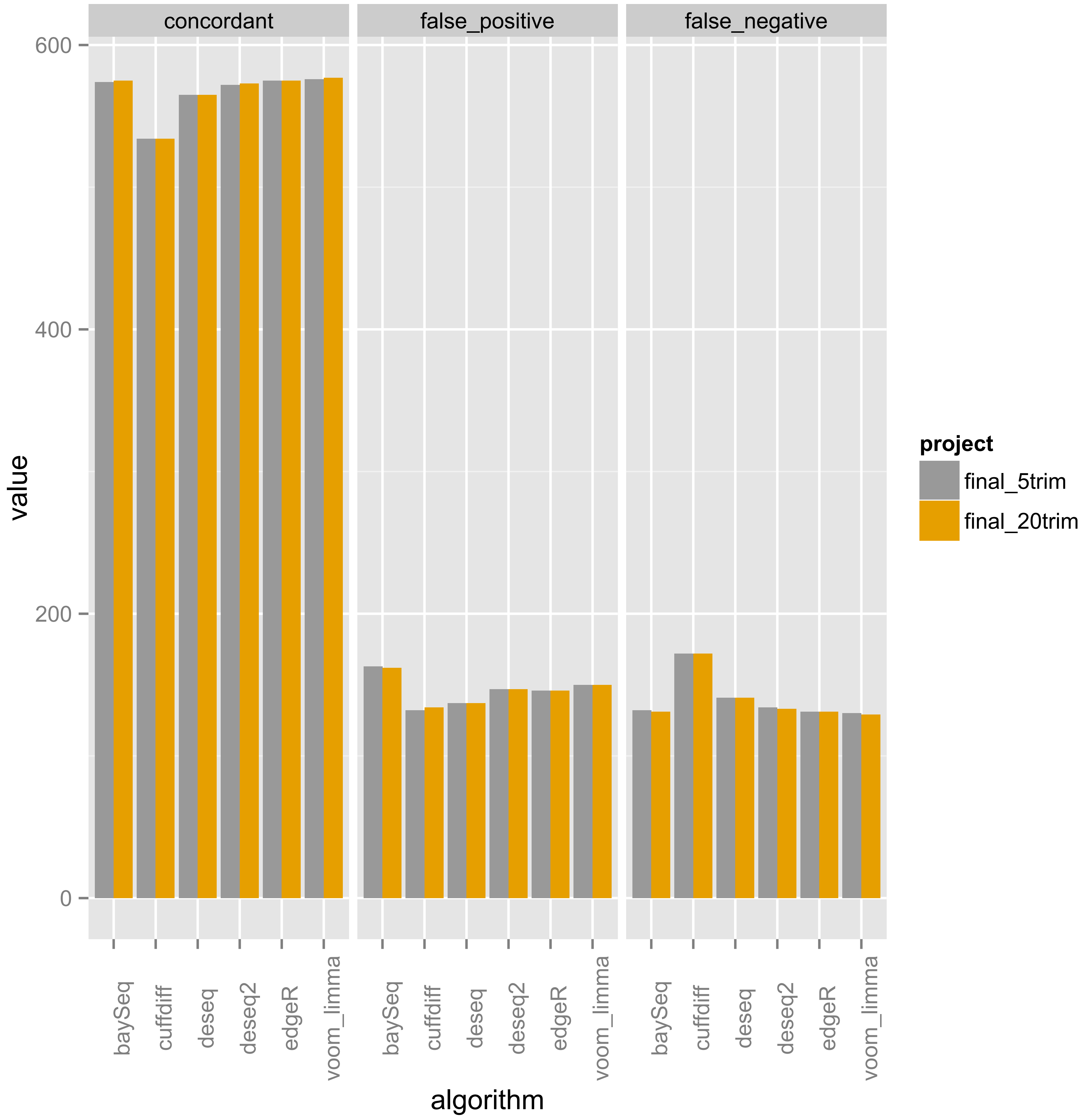
Published: December 23, 2013 • DOI: 10.1371/journal.pone.0085024

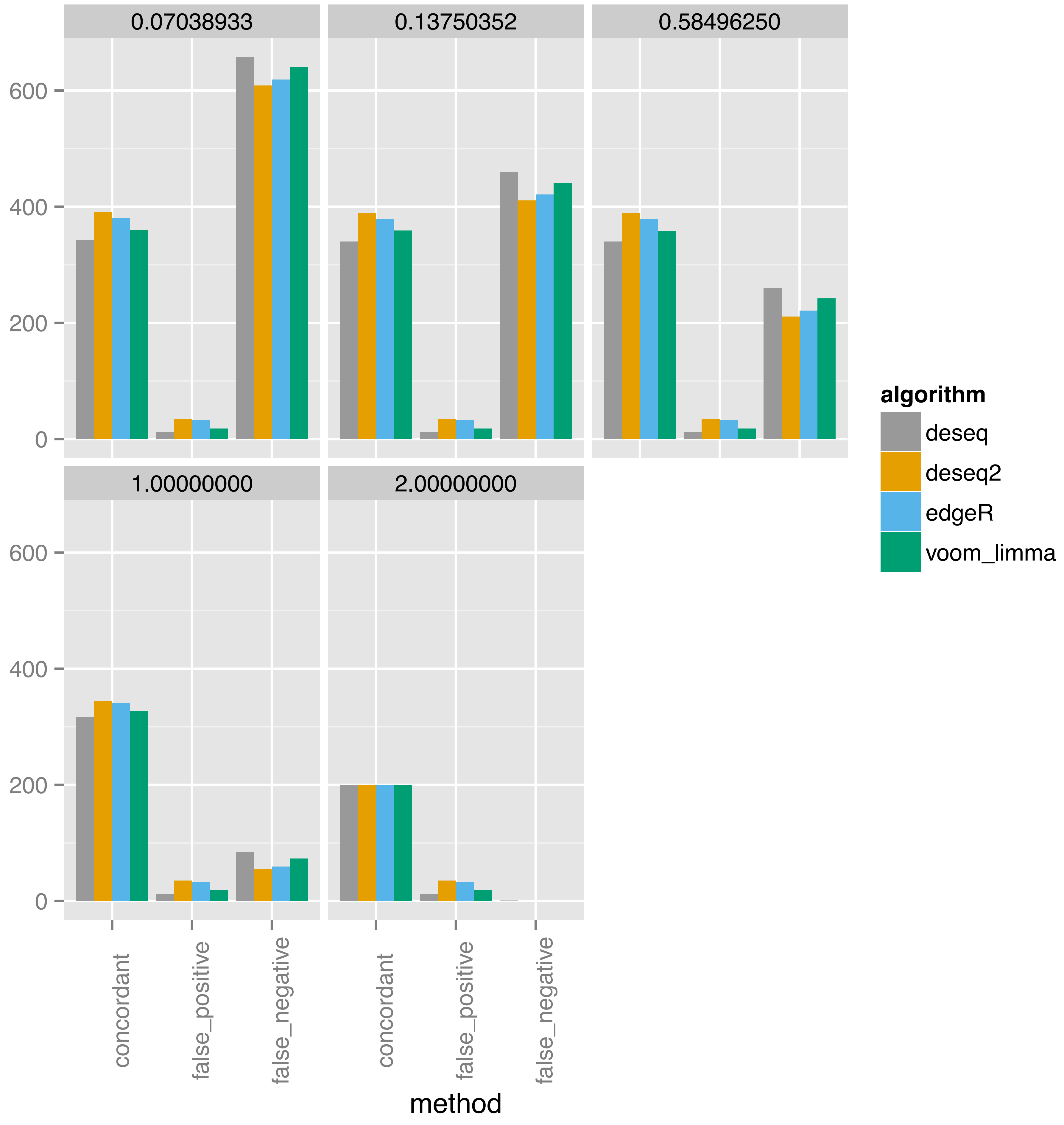
Concordance

concordant/false positive/false negative

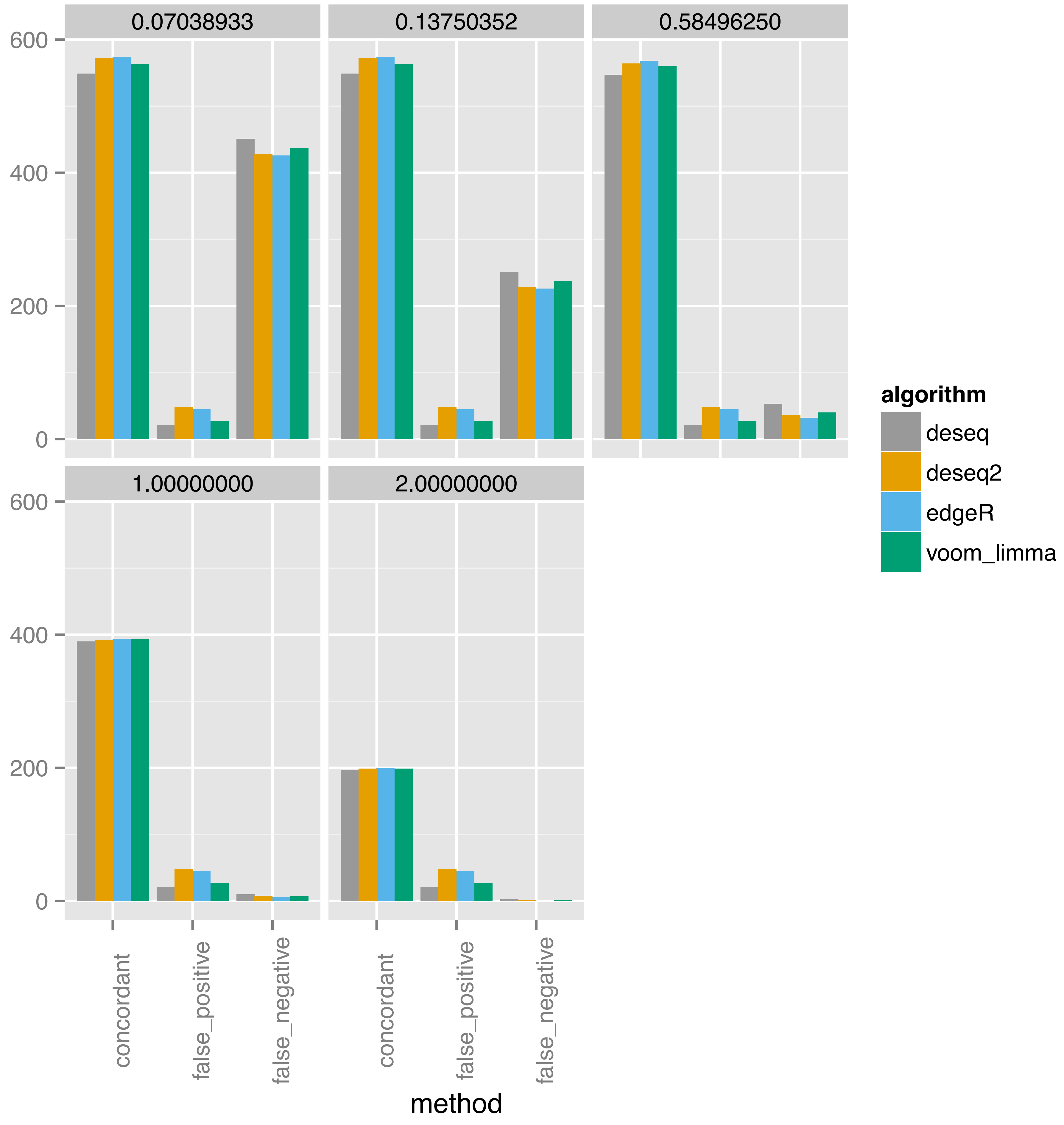
Jaccard index

Fold change





3 replicates, 100M



15 replicates, 20M

Get, install, develop

Get

```
wget https://raw.github.com/chapmanb/bcbio-nextgen/master/scripts/bcbio_nextgen_install.py
```

Install

```
python bcbio_nextgen_install.py /usr/local/share/bcbio-nextgen —tooldir=/usr/local
```

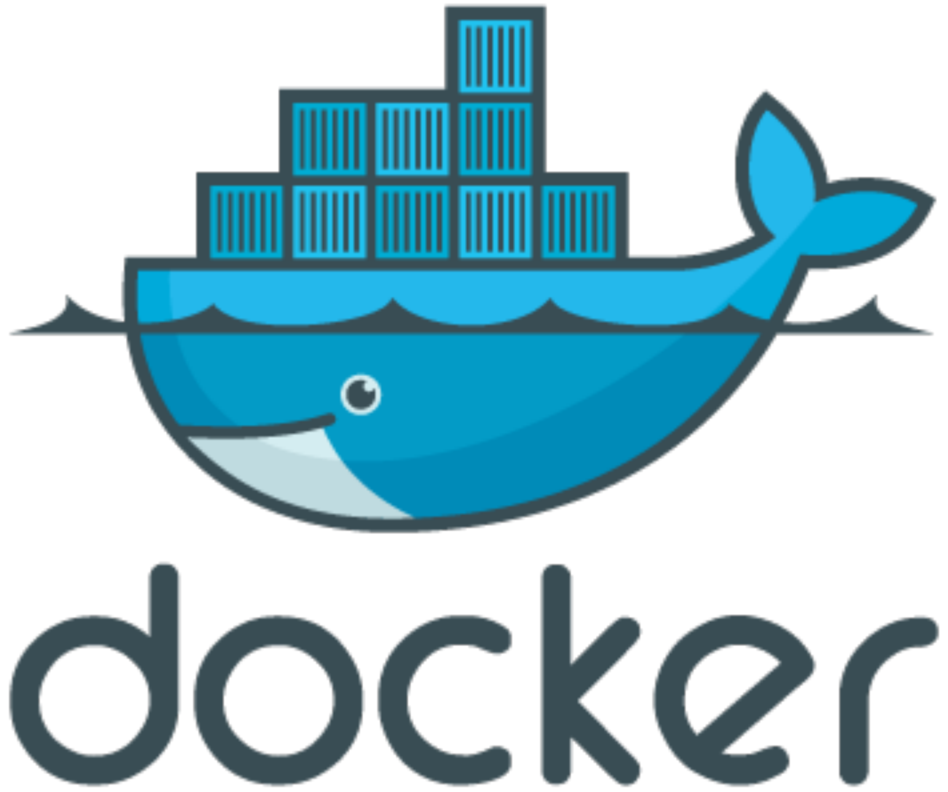
Develop

https://github.com/chapmanb/bcbio-nextgen (Python)

https://github.com/roryk/bcbio.rnaseq (Clojure, R)

Current target environment

- Cluster scheduler
  - Torque
  - SLURM
  - SGE
  - LSF
- Shared filesystem
  - NSF
  - Lustre
- Local temporary disk
  - SSD



Virtualization and reproducibility

Differential expression callers

edgeR

DESeq

DESeq2

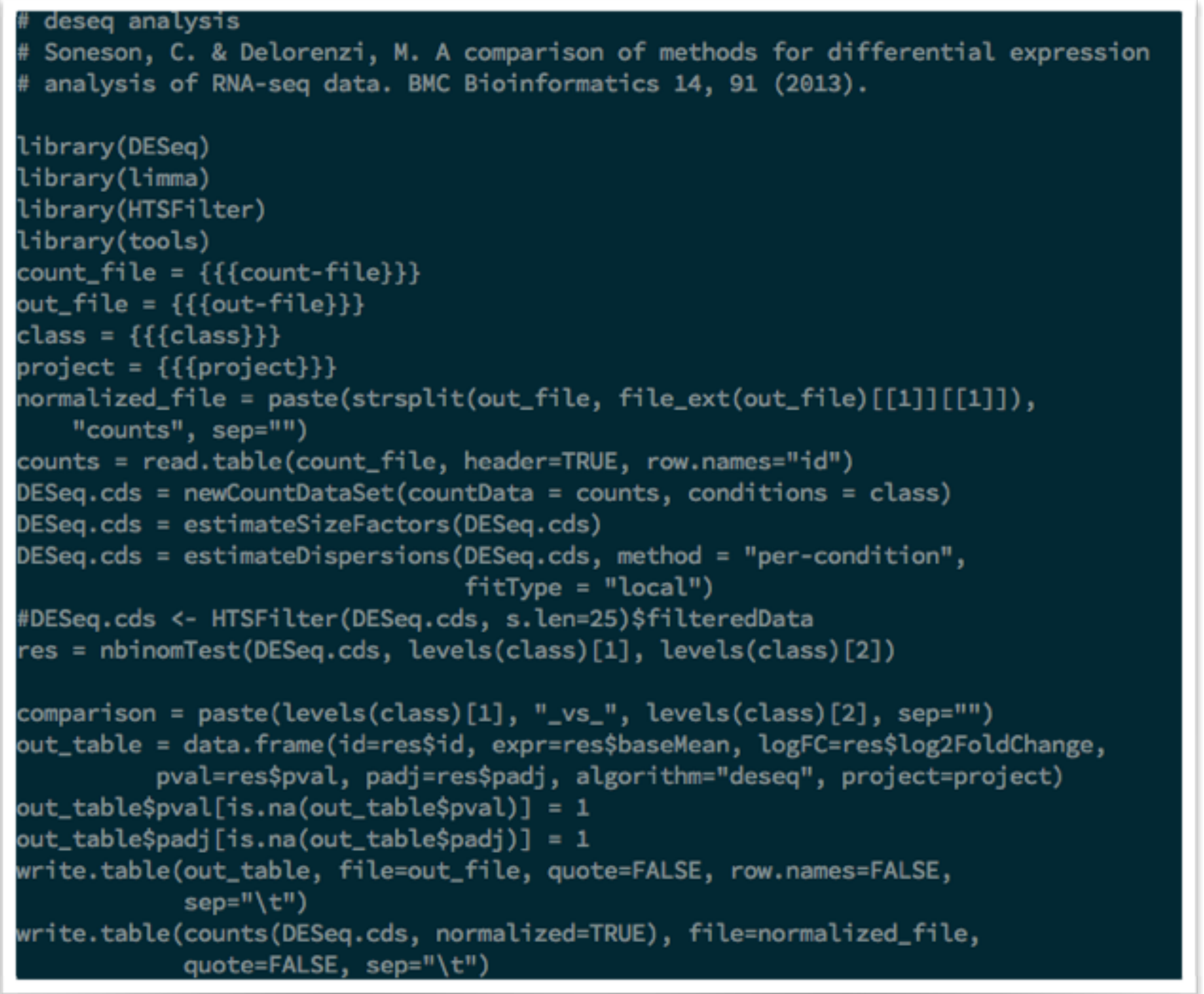
BaySeq

voom + limma

Cuffdiff

NOISeq*

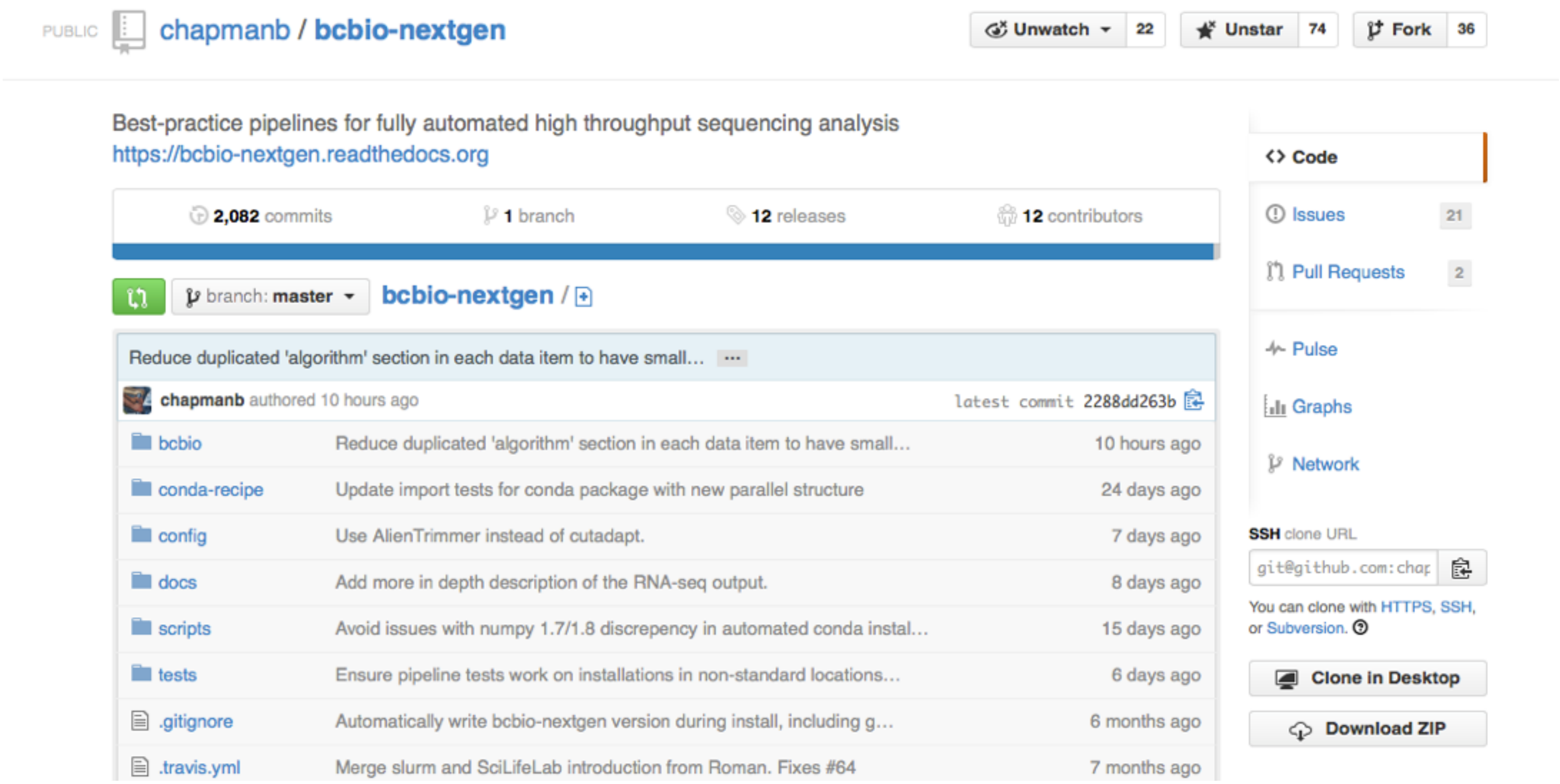
DERFinder*



```
# deseq analysis
# Soneson, C. & Delorenzi, M. A comparison of methods for differential expression
# analysis of RNA-seq data. BMC Bioinformatics 14, 91 (2013).

library(DESeq)
library(limma)
library(HTSFilter)
library(tools)
count_file = {{{count-file}}}
out_file = {{{out-file}}}
class = {{{class}}}
project = {{{project}}}
normalized_file = paste(strsplit(out_file, file_ext(out_file)[[1]][[1]]),
    "counts", sep="")
counts = read.table(count_file, header=TRUE, row.names="id")
DESeq.cds = newCountDataSet(countData = counts, conditions = class)
DESeq.cds = estimateSizeFactors(DESeq.cds)
DESeq.cds = estimateDispersions(DESeq.cds, method = "per-condition",
                                fitType = "local")
#DESeq.cds <- HTSFilter(DESeq.cds, s.len=25)$filteredData
res = nbinomTest(DESeq.cds, levels(class)[1], levels(class)[2])

comparison = paste(levels(class)[1], "_vs_", levels(class)[2], sep="")
out_table = data.frame(id=res$id, expr=res$baseMean, logFC=res$log2FoldChange,
          pval=res$pval, padj=res$padj, algorithm="deseq", project=project)
out_table$pval[is.na(out_table$pval)] = 1
out_table$padj[is.na(out_table$padj)] = 1
write.table(out_table, file=out_file, quote=FALSE, row.names=FALSE,
            sep="\t")
write.table(counts(DESeq.cds, normalized=TRUE), file=normalized_file,
            quote=FALSE, sep="\t")
```



PUBLIC chapmanb / bcbio-nextgen

Best-practice pipelines for fully automated high throughput sequencing analysis
https://bcbio-nextgen.readthedocs.org

2,082 commits · 1 branch · 12 releases · 12 contributors

Reduce duplicated 'algorithm' section in each data item to have small… ···

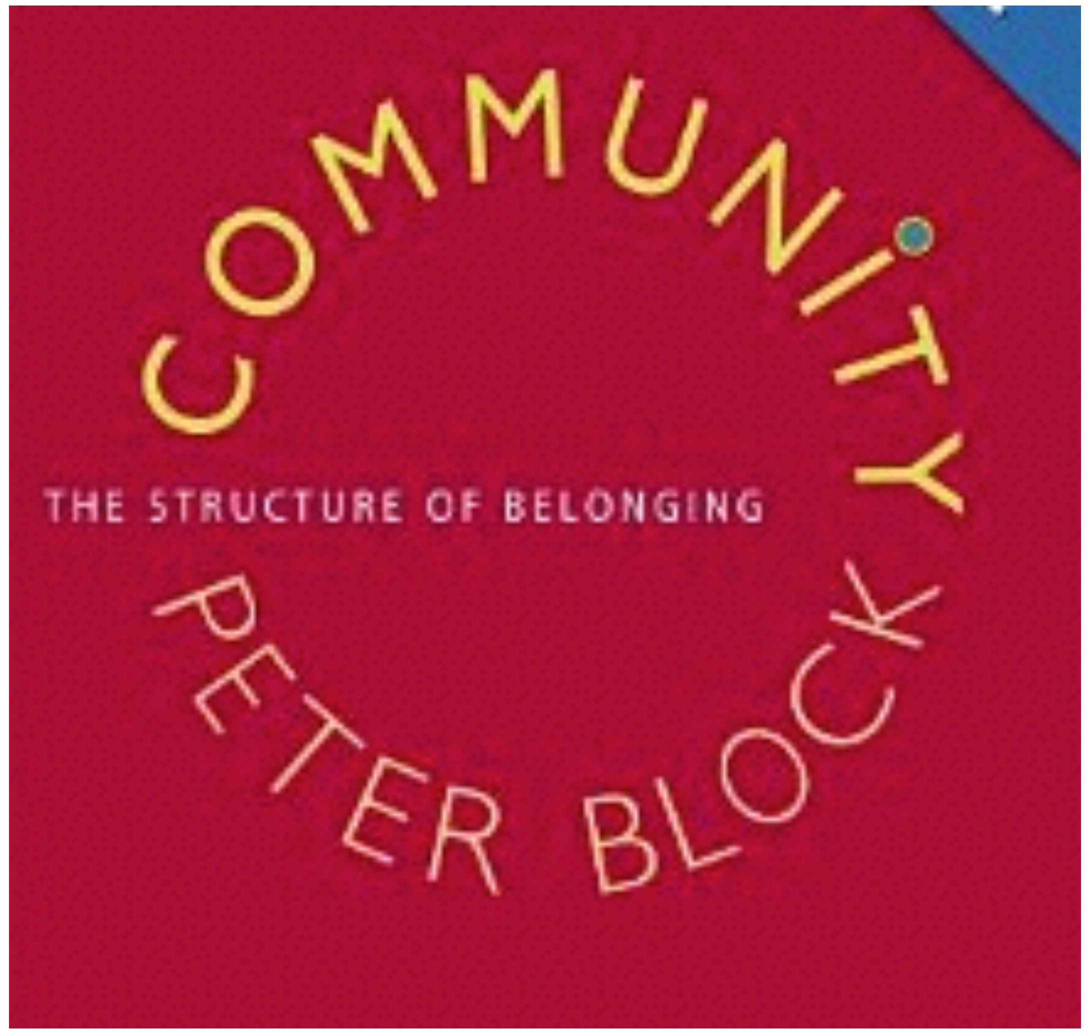
chapmanb authored 10 hours ago — latest commit 2288dd263b

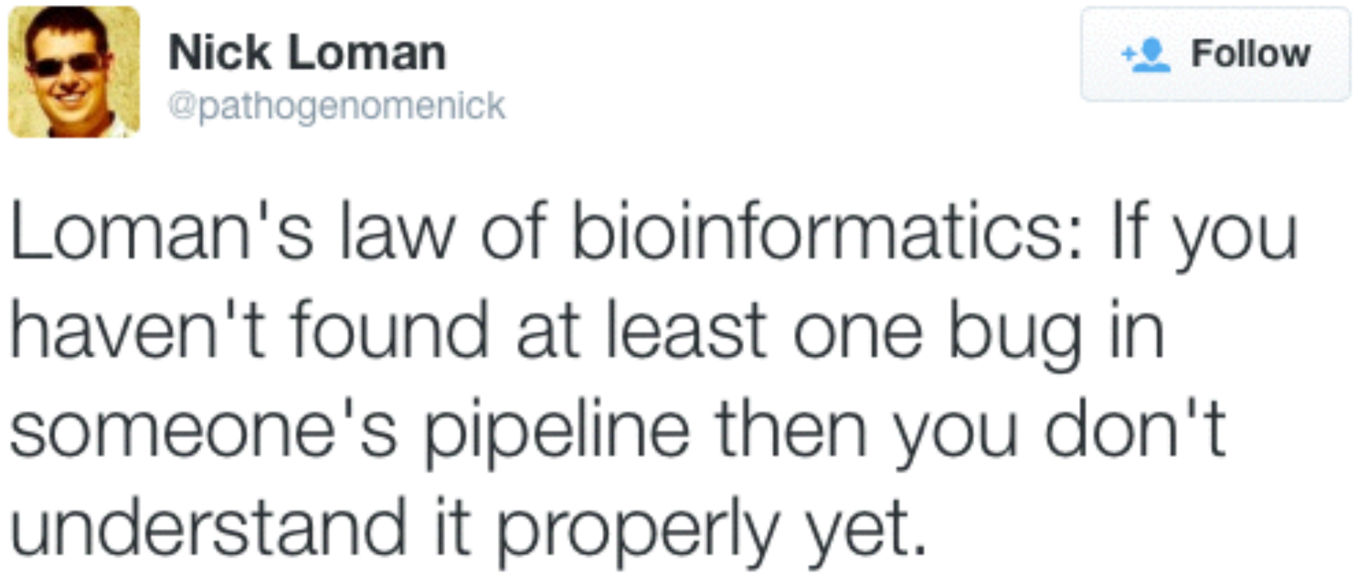
bcbio	Reduce duplicated 'algorithm' section in each data item to have small…	10 hours ago
conda-recipe	Update import tests for conda package with new parallel structure	24 days ago
config	Use AlienTrimmer instead of cutadapt.	7 days ago
docs	Add more in depth description of the RNA-seq output.	8 days ago
scripts	Avoid issues with numpy 1.7/1.8 discrepency in automated conda instal…	15 days ago
tests	Ensure pipeline tests work on installations in non-standard locations…	6 days ago
.gitignore	Automatically write bcbio-nextgen version during install, including g…	6 months ago
.travis.yml	Merge slurm and SciLifeLab introduction from Roman. Fixes #64	7 months ago

SSH clone URL
git@github.com:chap

You can clone with HTTPS, SSH, or Subversion.

Community





Nick Loman
@pathogenomenick

Loman's law of bioinformatics: If you haven't found at least one bug in someone's pipeline then you don't understand it properly yet.

Community