Subclonal variant calling with multiple samples and prior knowledge using `shearwater`

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Contents

1 Introduction

The `shearwater` algorithm was designed for calling subclonal variants in large ($N = 10 \dots 1,000$) cohorts of deeply (∼100x) sequenced unmatched samples. The large cohort allows for estimating a base-specific error profile on each position, which is modelled by a beta-binomial. A prior can be useded to selectively increase the power of calling variants on known mutational hotspots. The algorithm is similar to deepSNV, but uses a slightly different parametrization and a Bayes factors instead of a likelihood ratio test.
If you are using `shearwater`, please cite

- Gerstung M, Papaemmanuil E, Campbell PJ (2014). "Subclonal variant calling with multiple samples and prior knowledge." *Bioinformatics*, **30**, 1198-1204.

2 The statistical model

2.1 Definition

Suppose you have an experimental setup with multiple unrelated samples. Let the index i denote the sample, j the genomic position and k a particular nucleotide. Let X_{ijk} and X'_{ijk} denote the counts of nucleotide k in sample i on position j in forward and reverse read orientation, respectively. We assume that

$$\begin{aligned} X &\sim \mathrm{BetaBin}(n, \mu, \rho) \\ X' &\sim \mathrm{BetaBin}(n', \mu', \rho). \end{aligned} \tag{1}$$

are beta-binomially distributed. To test if there is a variant k in sample i, we compare the counts to a compound reference $X_{ijk} = \sum_{h \in H} X_{hjk}$ and $X'_{ijk} = \sum_{h \in H} X'_{hjk}$. The subset of indeces H is usually chosen such that $H = \{h : h \neq j\}$, that is the row sums X_{ijk} and X'_{ijk}. To reduce the effect of true

variants in other samples entering the compound reference, one may also choose H such that it only includes sample h with variant allele frequencies below a user defined threshold, typically 10%.
We model the compound reference again as a beta-binomial,

$$\begin{aligned} \mathbf{X} &\sim \text{BetaBin}(\mathbf{n}, \nu, \rho) \\ \mathbf{X}' &\sim \text{BetaBin}(\mathbf{n}', \nu', \rho). \end{aligned} \tag{2}$$

2.2 Testing for variants

Testing for the presence of a variant can now be formulated as a model selection problem in which we specify a null model and an alternative. Here we consider two options, "OR" and "AND".

2.2.1 The OR model

The OR model is defined in the following way:

$$\begin{aligned} M_0: &\quad \mu = \nu \quad \vee \quad \mu' = \nu' \\ M_1: &\quad \mu = \mu' > \nu, \nu'. \end{aligned} \tag{3}$$

Under the null model M_0, the mean rates of the beta-binomials are identical in sample i and the compound reference on at least one strand. Under the alternative model M_1, the mean rates μ, μ' are identical on both strands and greater than the mean in the compound reference on both strands.
Here we use the following point estimates for the parameters:

$$\begin{aligned} \hat{\mu} &= (X + X')/(n + n') \\ \hat{\nu} &= \mathbf{X}/\mathbf{n} \\ \hat{\nu}' &= \mathbf{X}'/\mathbf{n}' \\ \hat{\nu}_0 &= (X + \mathbf{X})/(n + \mathbf{n}) \\ \hat{\nu}_0' &= (X' + \mathbf{X}')/(n' + \mathbf{n}') \\ \hat{\mu}_0 &= X/n \\ \hat{\mu}_0' &= X'/n'. \end{aligned} \tag{4}$$

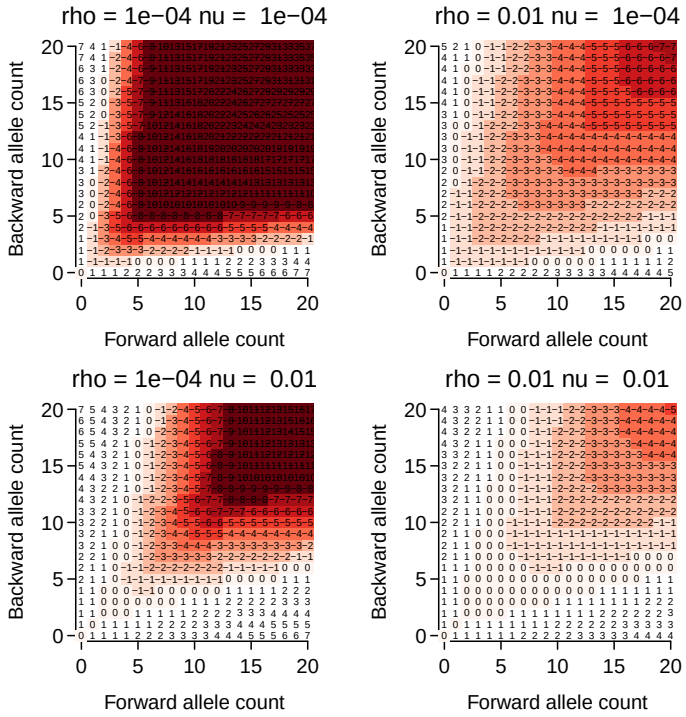
Using these values, the Bayes factor is approximated by

$$\begin{aligned} \frac{\Pr(D \mid M_0)}{\Pr(D \mid M_1)} = &\frac{\Pr(X|\hat{\nu}_0)\Pr(X'|\hat{\mu}_0')\Pr(\mathbf{X}|\hat{\nu}_0)}{\Pr(X|\hat{\mu})\Pr(X'|\hat{\mu})\Pr(\mathbf{X}|\hat{\nu})} \\ &+ \frac{\Pr(X|\hat{\mu}_0)\Pr(X'|\hat{\nu}_0')\Pr(\mathbf{X}'|\hat{\nu}_0')}{\Pr(X|\hat{\mu})\Pr(X'|\hat{\mu})\Pr(\mathbf{X}'|\hat{\nu}')} \\ &- \frac{\Pr(X|\hat{\nu}_0)\Pr(\mathbf{X}|\hat{\nu}_0)\Pr(X'|\hat{\nu}_0')\Pr(\mathbf{X}'|\hat{\nu}_0')}{\Pr(X|\hat{\mu})\Pr(\mathbf{X}|\hat{\nu})\Pr(X'|\hat{\mu})\Pr(\mathbf{X}'|\hat{\nu}')} \end{aligned} \tag{5}$$

Example The Bayes factors can be computed using the `bbb` command:

```
library(deepSNV)
library(RColorBrewer)
n <-  100 ## Coverage
n_samples <- 1000 ## Assume 1000 samples
x <-  0:20 ## Nucleotide counts
X <-  cbind(rep(x, each = length(x)), rep(x, length(x))) ## All combinations forward and reverse
par(bty="n", mgp = c(2,.5,0), mar=c(3,3,2,2)+.1, las=1, tcl=-.33, mfrow=c(2,2))
for(nu in 10^c(-4,-2)){ ## Loop over error rates
        ## Create counts array with errors
        counts = aperm(array(c(rep(round(n_samples*n* c(nu,1-nu,nu,1-nu)), each=nrow(X)), cbind(n - X
                                        dim=c(nrow(X) ,4,2)), c(3,1,2))
        for(rho in c(1e-4, 1e-2)){ ## Loop over dispersion factors
                ## Compute Bayes factors
                BF = bbb(counts, rho=rho, model="OR", return="BF")
```

```
                ## Plot
                image(z=log10(matrix(BF[2,,1], nrow=length(x))),
                                x=x,
                                y=x,
                                breaks=c(-100,-8:0),
                                col=rev(brewer.pal(9,"Reds")),
                                xlab = "Forward allele count",
                                ylab="Backward allele count",
                                main = paste("rho =", format(rho, digits=2), "nu = ", format(nu, dig
                                font.main=1)
                text(X[,1],X[,2],ceiling(log10(matrix(BF[2,,1], nrow=length(x)))), cex=0.5)
        }
}
```



Here we have used a coverage of $n = 100$ on both strands and computed the Bayes factors assuming 1,000 samples to estimate the error rate $\nu = \nu'$ from. Shown are results for fixed values of $rho = \{10^{-4}, 10^{-2}\}$.

2.2.2 The AND model

The AND model is defined in the following way:

$$\begin{aligned} M_0: &\quad \mu = \nu \quad \wedge \quad \mu' = \nu' \\ M_1: &\quad \mu = \mu' > \nu, \nu'. \end{aligned} \tag{6}$$

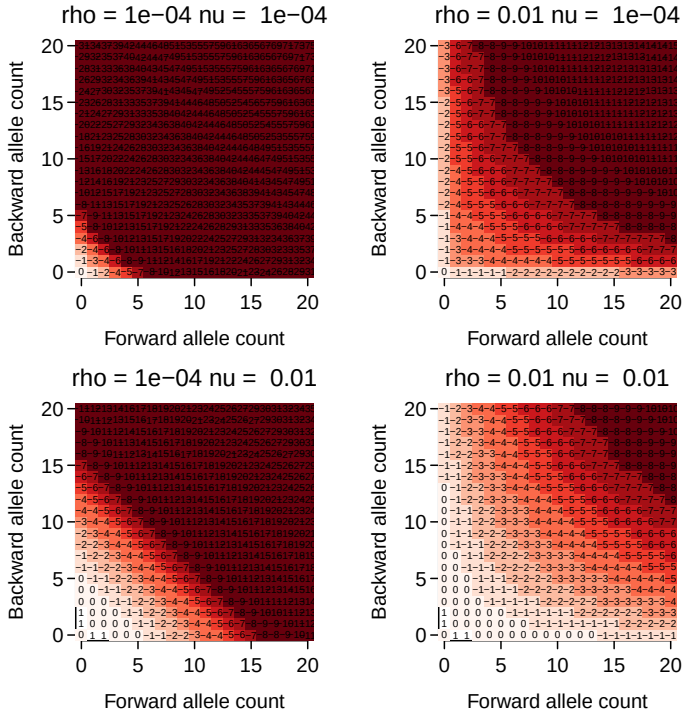
Here the null model states that the error rates $\nu = \mu$ and $\nu' = \mu'$ are identical on both strands, which is more restrictive and hence in favour of the alternative.
In this case the Bayes factor is approximately

$$\frac{\Pr(D \mid M_0)}{\Pr(D \mid M_1)} = \frac{\Pr(X|\hat{\nu}_0)\Pr(\mathbf{X}|\hat{\nu}_0)\Pr(X'|\hat{\nu}'_0)\Pr(\mathbf{X}'|\hat{\nu}'_0)}{\Pr(X|\hat{\mu})\Pr(\mathbf{X}|\hat{\nu})\Pr(X'|\hat{\mu})\Pr(\mathbf{X}'|\hat{\nu}')} \tag{7}$$

Example The behaviour of the AND model can be inspected by the following commands

```
par(bty="n", mgp = c(2,.5,0), mar=c(3,3,2,2)+.1, las=1, tcl=-.33, mfrow=c(2,2))
for(nu in 10^c(-4,-2)){ ## Loop over error rates
        ## Create counts array with errors
```

```
        counts = aperm(array(c(rep(round(n_samples*n* c(nu,1-nu,nu,1-nu)), each=nrow(X)), cbind(n - 
                                    dim=c(nrow(X) ,4,2)), c(3,1,2))
        for(rho in c(1e-4, 1e-2)){ ## Loop over dispersion factors
                ## Compute Bayes factors, mode = "AND"
                BF = bbb(counts, rho=rho, model="AND", return="BF")
                ## Plot
                image(z=log10(matrix(BF[2,,1], nrow=length(x))),
                                x=x,
                                y=x,
                                breaks=c(-100,-8:0),
                                col=rev(brewer.pal(9,"Reds")),
                                xlab = "Forward allele count",
                                ylab="Backward allele count",
                                main = paste("rho =", format(rho, digits=2), "nu = ", format(nu, digi
                                font.main=1)
                text(X[,1],X[,2],ceiling(log10(matrix(BF[2,,1], nrow=length(x)))), cex=0.5)
        }
}
```



One realises that for small dispersion the Bayes factor depends mostly on the sum of the forward and reverse strands in the AND model.

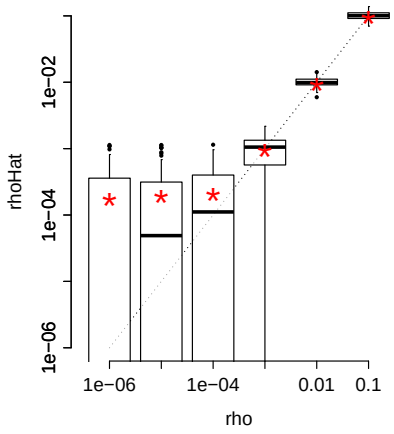
2.3 Estimating ρ

If the dispersion parameter ρ is not specified, it is estiated at each locus using the following method-of-moment estimator:

$$\begin{aligned}\hat{\rho} &= \frac{Ns^2/(1-\hat{\nu})/\hat{\nu} - \sum_{i=1}^{N} 1/n_i}{N - \sum_{i=1}^{N} 1/n_i} \\ s^2 &= \frac{N\sum_{i=1}^{N} n_i(\hat{\nu} - \hat{\mu}_i)^2}{(N-1)\sum_{i=1}^{N} n_i}.\end{aligned} \tag{8}$$

This yields consistent estimates over a range of true values:

```
rho = 10^seq(-6,-1)
rhoHat <- sapply(rho, function(r){
                        sapply(1:100, function(i){
                                                n = 100
                                                X = rbetabinom(1000, n, 0.01, rho=r)
                                                X = cbind(X, n-X)
                                                Y = array(X, dim=c(1000,1,2))
                                                deepSNV:::estimateRho(Y, Y/n, Y < 1000)[1,1]})
                })
par(bty="n", mgp = c(2,.5,0), mar=c(3,4,1,1)+.1,  tcl=-.33)
plot(rho, type="l", log="y", xaxt="n", xlab="rho", ylab="rhoHat", xlim=c(0.5,6.5), lty=3)
boxplot(t(rhoHat+ 1e-7) ~ rho, add=TRUE, col="#FFFFFFAA", pch=16, cex=.5, lty=1, staplewex=0)
points(colMeans(rhoHat), pch="*", col="red", cex=2)
```



2.4 Using a prior

`shearwater` calls variants if the posterior probability that the null model M_0 is true falls below a certain threshold. Generally, the posterior odds is given by

$$\frac{\Pr(M_0 \mid D)}{\Pr(M_1 \mid D)} = \frac{1-\pi(M_1))}{\pi(M_1)} \frac{\Pr(D \mid M_0)}{\Pr(D \mid M_1)} \tag{9}$$

where $\pi = \pi(M_1)$ is the prior probability of that a variant exists. These probabilities are not uniform and may be calculated from the distribution of observed somatic mutations. Such data can be found in the COSMIC data base `http://www.sanger.ac.uk/cosmic`.
As of now, the amount of systematic, genome-wide screening data is still sparse, which makes it difficult to get good estimates of the mutation frequencies in each cancer type. However, a wealth of data exists for somatic mutations within a given gene. Assume we know how likely it is that a gene is mutated. We then model

$$\pi = \begin{cases} \pi_{\text{gene}} \times \frac{\text{\# Mutations at given position}}{\text{\# Mutations in gene}} & \text{if variant in COSMIC} \\ \pi_{\text{background}} & \text{else.} \end{cases} \tag{10}$$

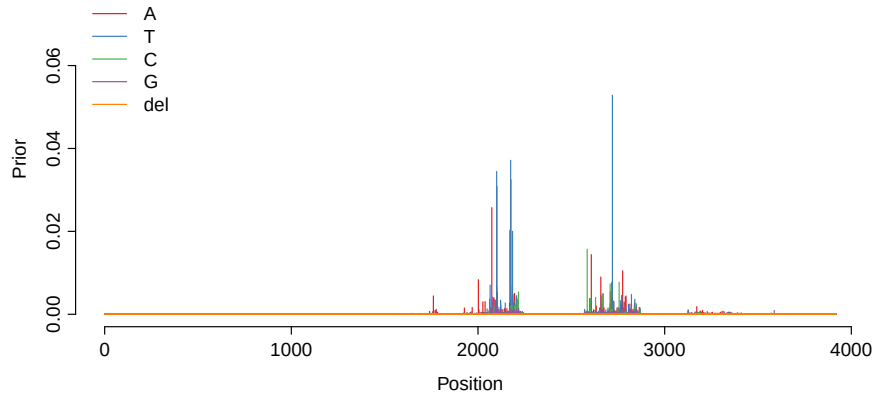
Suppose you have downloaded the COSMIC vcf `"CosmicCodingMuts_v63_300113.vcf.gz"` from `ftp://ngs.sanger.ac.uk/production/cosmic`.

```
## Not run..
## Load TxDb
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
seqlevels(txdb) <- sub("chr","",seqlevels(txdb))

## Make prior
```

```
regions <- reduce(exons(txdb, filter=list(gene_id='7157'))) ## TP53 exons
cosmic <- readVcf("CosmicCodingMuts_v63_300113.vcf.gz", "hg19", param=ScanVcfParam(which=regions))
pi <- makePrior(cosmic, regions, pi.gene = 1)
```

The resulting prior can be visualised:

```
## Load pi
data(pi, package="deepSNV")

## Plot
par(bty="n", mgp = c(2,.5,0), mar=c(3,3,2,2)+.1, tcl=-.33)
plot(pi[,1], type="h", xlab="Position", ylab="Prior", col=brewer.pal(5,"Set1")[1], ylim=c(0,0.075))
for(j in 2:5)
        lines(pi[,j], type="h", col=brewer.pal(5,"Set1")[j])
legend("topleft", col=brewer.pal(5,"Set1"), lty=1, bty="n", c("A","T","C","G","del"))
```



The data shows that the distribution of somatic variants is highly non-uniform, with multiple mutation hotspots.

3 Using shearwater

To run shearwater you need a collection of .bam files and the set of regions you want to analyse as a GRanges() object. Additionally, you may calculate a prior from a VCF file that you can download from `ftp://ngs.sanger.ac.uk/production/cosmic`.

3.1 Minimal example

Here is a minimal example that uses two .bam files from the deepSNV package. The data is loaded into a large array using the `loadAllData()` function:

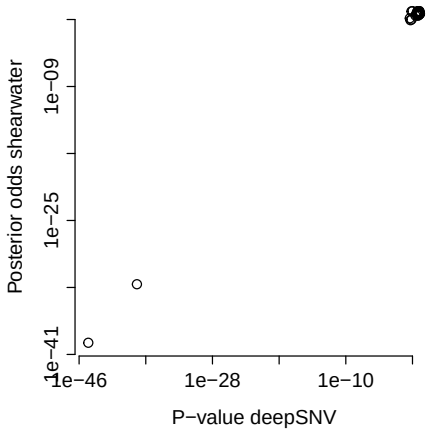
```
## Load data from deepSNV example
regions <- GRanges("B.FR.83.HXB2_LAI_IIIB_BRU_K034", IRanges(start = 3120, end=3140))
files <- c(system.file("extdata", "test.bam", package="deepSNV"), system.file("extdata", "control.ba
counts <- loadAllData(files, regions, q=10)
dim(counts)

## [1]  2 21 10
```

The dimension of `counts` for N samples, a total of L positions is $N \times L \times 2|B|$, where $|B| = 5$ is the size of the alphabet $B = \{A, T, C, G, -\}$ and the factor of 2 for the two strand orientations.
The Bayes factors can be computed with the `bbb` function:

```
## Run (bbb) computes the Bayes factor
bf <- bbb(counts, model = "OR", rho=1e-4)
dim(bf)

## [1]  2 21  5

vcf <- bf2Vcf(bf, counts, regions, cutoff = 0.5, samples = files, prior = 0.5, mvcf = TRUE)
show(vcf)

## class: CollapsedVCF
## dim: 8 2
## rowRanges(vcf):
##   GRanges with 4 metadata columns: REF, ALT, QUAL, FILTER
## info(vcf):
##   DataFrame with 4 columns: ER, PI, AF, LEN
## info(header(vcf)):
##        Number Type  Description
##    ER  1      Float Error rate
##    PI  1      Float Prior
##    AF  1      Float Allele frequency in cohort
##    LEN 1      Float Length of the alt allele
## geno(vcf):
##   List of length 8: GT, GQ, BF, VF, FW, BW, FD, BD
## geno(header(vcf)):
##       Number Type    Description
##    GT 1      String  Genotype
##    GQ 1      Integer Genotype Quality
##    BF 1      Float   Bayes factor
##    VF 1      Float   Variant frequency in sample
##    FW 1      Integer Forward variant read count
##    BW 1      Integer Backward variant read count
##    FD 1      Integer Read Depth forward
##    BD 1      Integer Read Depth backward
```

The resulting Bayes factors were thresholded by a posterior `cutoff` for variant calling and converted into a `VCF` object by `bf2Vcf`.
For two samples the Bayes factors are very similar to the p-values obtained by `deepSNV`:

```
## Shearwater Bayes factor under AND model
bf <- bbb(counts, model = "AND", rho=1e-4)
## deepSNV P-value with combine.method="fisher" (product)
dpSNV <- deepSNV(test = files[1], control = files[2], regions=regions, q=10, combine.method="fisher")
## Plot
par(bty="n", mgp = c(2,.5,0), mar=c(3,3,2,2)+.1, tcl=-.33)
plot(p.val(dpSNV), bf[1,,]/(1+bf[1,,]), log="xy",
                xlab = "P-value deepSNV",
                ylab = "Posterior odds shearwater"
                )
```



3.2 More realistic example

Suppose the bam files are in folder `./bam` and the regions of interest are stored in a `GRanges()` object with metadata column `Gene`, indicating which region (typically exons for a pulldown experiment) belongs to which gene. Also assume that we have a tabix indexed vcf file `CosmicCodingMuts_v63_300113.vcf.gz`. The analysis can be parallelized by separately analysing each gene, which is the unit needed to compute the prior using `makePrior`.

```
## Not run
files <- dir("bam", pattern="*.bam$", full.names=TRUE)
MC_CORES <- getOption("mc.cores", 2L)
vcfList <- list()
for(gene in levels(mcols(regions)$Gene)){
        rgn <-  regions[mcols(regions)$Gene==gene]
        counts <-  loadAllData(files, rgn, mc.cores=MC_CORES)
        ## Split into
        BF <-  mcChunk("bbb", split = 200, counts, mc.cores=MC_CORES)
        COSMIC <-  readVcf("CosmicCodingMuts_v63_300113.vcf.gz", "GRCh37", param=ScanVcfParam(which=r
        prior <- makePrior(COSMIC, rgn, pi.mut = 0.5)
        vcfList[[gene]] <- bf2Vcf(BF = BF, counts=counts, regions=rgn, samples = files, cutoff = 0.5
}
## Collapse vcfList
vcf <- do.call(rbind, vcfList)
```

The `mcChunk` function splits the `counts` objects into chunks of size split and processes these in parallel using `mclapply`.
Instead of using a for loop one can also use a different mechanism, e.g. submitting this code to a computing cluster, etc.

sessionInfo()

- R version 4.3.1 (2023-06-16), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_GB`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Time zone: `America/New_York`
- TZcode source: `system (glibc)`
- Running under: `Ubuntu 22.04.3 LTS`
- Matrix products: default

- BLAS: `/home/biocbuild/bbs-3.18-bioc/R/lib/libRblas.so`
- LAPACK: `/usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, splines, stats, stats4, utils
- Other packages: Biobase 2.62.0, BiocGenerics 0.48.0, Biostrings 2.70.0, GenomeInfoDb 1.38.0, GenomicRanges 1.54.0, IRanges 2.36.0, MatrixGenerics 1.14.0, RColorBrewer 1.1-3, Rsamtools 2.18.0, S4Vectors 0.40.0, SummarizedExperiment 1.32.0, VGAM 1.1-9, VariantAnnotation 1.48.0, XVector 0.42.0, deepSNV 1.48.0, knitr 1.44, matrixStats 1.0.0
- Loaded via a namespace (and not attached): AnnotationDbi 1.64.0, BSgenome 1.70.0, BiocFileCache 2.10.0, BiocIO 1.12.0, BiocParallel 1.36.0, DBI 1.1.3, DelayedArray 0.28.0, GenomeInfoDbData 1.2.11, GenomicAlignments 1.38.0, GenomicFeatures 1.54.0, KEGGREST 1.42.0, Matrix 1.6-1.1, R6 2.5.1, RCurl 1.98-1.12, RSQLite 2.3.1, Rhtslib 2.4.0, S4Arrays 1.2.0, SparseArray 1.2.0, XML 3.99-0.14, abind 1.4-5, biomaRt 2.58.0, bit 4.0.5, bit64 4.0.5, bitops 1.0-7, blob 1.2.4, bslib 0.5.1, cachem 1.0.8, cli 3.6.1, codetools 0.2-19, compiler 4.3.1, crayon 1.5.2, curl 5.1.0, dbplyr 2.3.4, digest 0.6.33, dplyr 1.1.3, evaluate 0.22, fansi 1.0.5, fastmap 1.1.1, filelock 1.0.2, generics 0.1.3, glue 1.6.2, grid 4.3.1, highr 0.10, hms 1.1.3, htmltools 0.5.6.1, httr 1.4.7, jquerylib 0.1.4, jsonlite 1.8.7, lattice 0.22-5, lifecycle 1.0.3, magrittr 2.0.3, memoise 2.0.1, pillar 1.9.0, pkgconfig 2.0.3, png 0.1-8, prettyunits 1.2.0, progress 1.2.2, rappdirs 0.3.3, restfulr 0.0.15, rjson 0.2.21, rlang 1.1.1, rmarkdown 2.25, rtracklayer 1.62.0, sass 0.4.7, stringi 1.7.12, stringr 1.5.0, tibble 3.2.1, tidyselect 1.2.0, tools 4.3.1, utf8 1.2.4, vctrs 0.6.4, xfun 0.40, xml2 1.3.5, yaml 2.3.7, zlibbioc 1.48.0