



MONASH University

Fluent statistical computing interfaces for biological data analysis

Stuart Andrew Lee

B.MatComSci., University of Adelaide; MSc.Bioinformatics.

University of Melbourne

A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2020

Department of Econometrics and Business Statistics

Contents

Copyright notice	v
Abstract	vii
Declaration	ix
Acknowledgements	xi
Preface	xiii
1 Introduction	1
2 <i>plyranges</i>: a grammar of data transformation for genomics	3
2.1 Background	3
2.2 Results	5
2.3 Discussion	19
2.4 Conclusion	22
2.5 Acknowledgements	22
2.6 Availability of Data and Materials	23
3 Fluent genomics with <i>plyranges</i> and <i>tximeta</i>	25
3.1 Introduction	25
3.2 Import Data as a <i>SummarizedExperiment</i>	28
3.3 Model assays	34
3.4 Integrate ranges	41
3.5 Discussion	56
3.6 Software Availability	57
3.7 Acknowledgements	57
4 Tidy coverage analysis uncovers intron retention	59
5 Spurious embeddings from non-linear dimensionality reduction methods and how to find them	61
6 Conclusion and future plans	63
Bibliography	65

Copyright notice

© Stuart Lee (2020).

Abstract

Exploratory data analysis (EDA) is a vital element of the modern statistical workflow - it is an analyst's first pass at understanding their data; revealing all its messes and (possibly) uncovering hidden insights. It is an iterative process that can lead to new hypotheses that can be tested and formalised using modelling. However, as data grows in complexity and there is an increase in the number of observations, and variables EDA becomes increasingly difficult. This is certainly true in bioinformatic data analysis, where there are constraints computation time and memory, as well as a lack of guidelines or tooling for performing EDA.

This thesis presents a set of tools for performing exploratory data analysis in the context of genomics and transcriptomics. The first tool, "plyranges" provides a domain specific language for reasoning about range based genomics data that simplifies common operations such as overlaps and aggregations. Next I extend "plyranges" to develop "superintronic" which presents an approach for discovering interesting genomic regions within an experimental design. We apply this tool to explore intron signal in transcriptomic data. Finally, I turn my attention to focus on embedding methods currently used for visualisation of large data sets...

Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or equivalent institution, and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Stuart Lee

Acknowledgements

Preface

Chapter 1

Introduction

overview

describe main topic - research questions chapters 1, 2, 3, 4 (your contributions to the joint work) scope

Chapter 2

plyranges: a grammar of data transformation for genomics

2.1 Background

High-throughput genomics promises to unlock new disease therapies, and strengthen our knowledge of basic biology. To deliver on those promises, scientists must derive a stream of knowledge from a deluge of data. Genomic data is challenging in both scale and complexity. Innovations in sequencing technology often outstrip our capacity to process the output. Beyond their common association with genomic coordinates, genomic data are heterogeneous, consisting of raw sequence read alignments, genomic feature annotations like genes and exons, and summaries like coverage vectors, ChIP-seq peak calls, variant calls, and per-feature read counts. Genomic scientists need software tools to wrangle the different types of data, process the data at scale, test hypotheses, and generate new ones, all while focusing on the biology, not the computation. For the tool developer, the challenge is to define ways to model and operate on the data that align with the mental model of scientists, and to provide an implementation that scales with their ambition.

Several domain specific languages (DSLs) enable scientists to process and reason about heterogeneous genomics data by expressing common operations, such as range manipulation and overlap-based joins, using the vocabulary of genomics. Their implementations

either delegate computations to a database, or operate over collections of files in standard formats like BED. An example of the former is the Genome Query Language (GQL) and its distributed implementation GenAp which use a SQL-like syntax for fast retrieval of information of unprocessed sequencing data (Kozanitis, Christos et al., 2014; Kozanitis and Patterson, 2016). Similarly, the Genometric Query Language (GMQL) implements a DSL for combining genomic datasets (Kaitoua, A et al., 2017). The command line application BEDtools develops an extensive algebra for performing arithmetic between two or more sets of genomic regions (Quinlan and Hall, 2010). All of the aforementioned DSLs are designed to be evaluated either at the command line or embedded in scripts for batch processing. They exist in a sparse ecosystem, mostly consisting of UNIX and database tools that lack biological semantics and operate at the level of files and database tables.

The Bioconductor/R packages IRanges and GenomicRanges (R Core Team, 2018; Lawrence et al., 2013a; Huber et al., 2015a) define a DSL for analyzing genomics data with R, an interactive data analysis environment that encourages reproducibility and provides high-level abstractions for manipulating, modelling and plotting data, through state of the art methods in statistical computing. The packages define object-oriented (OO) abstractions for representing genomic data and enable interoperability by allowing users and developers to use these abstractions in their own code and packages. Other genomic DSLs that are embedded in programming languages include pybedtools and valr (Dale, Pedersen, and Quinlan, 2011; Riemondy et al., 2017), however these packages lack the interoperability provided by the aforementioned Bioconductor packages and are not easily extended.

The Bioconductor infrastructure models the genomic data and operations from the perspective of the power user, one who understands and wants to take advantage of the subtle differences in data types. This design has enabled the development of sophisticated tools, as evidenced by the hundreds of packages depending on the framework. Unfortunately, the myriad of data structures have overlapping purposes and important but obscure differences in behavior that often confuse the typical end user.

Recently, there has been a concerted, community effort to standardize R data structures and workflows around the notion of tidy data (Wickham, 2014). A tidy dataset is defined

as a tabular data structure that has observations as rows and columns as variables, and all measurements pertain to a single observational unit. The tidy data pattern is useful because it allows us to see how the data relate to the design of an experiment and the variables measured. The `dplyr` package (Wickham et al., 2017) defines an application programming interface (API) that maps notions from the general relational algebra to verbs that act on tidy data. These verbs can be composed together on one or more tidy datasets with the pipe operator from the `magrittr` package (Bache and Wickham, 2014). Taken together these features enable a user to write human readable analysis workflows.

We have created a genomic DSL called `plyranges` that reformulates notions from existing genomic algebras and embeds them in R as a genomic extension of `dplyr`. By analogy, `plyranges` is to the genomic algebra, as `dplyr` is to the relational algebra. The `plyranges` Bioconductor package implements the language on top of a key subset of Bioconductor data structures and thus fully integrates with the Bioconductor framework, gaining access to its scalable data representations and sophisticated statistical methods.

2.2 Results

2.2.1 Genomic Relational Algebra

Data Model

The `plyranges` DSL is built on the core Bioconductor data structure `GRanges`, which is a constrained table, with fixed columns for the chromosome, start and end coordinates, and the strand, along with an arbitrary set of additional columns, consisting of measurements or metadata specific to the data type or experiment (figure 2.1). `GRanges` balances flexibility with formal constraints, so that it is applicable to virtually any genomic workflow, while also being semantically rich enough to support high-level operations on genomic ranges. As a core data structure, `GRanges` enables interoperability between `plyranges` and the rest of Bioconductor. Adhering to a single data structure simplifies the API and makes it easier to learn and understand, in part because operations become endomorphic, i.e., they return the same type as their input.

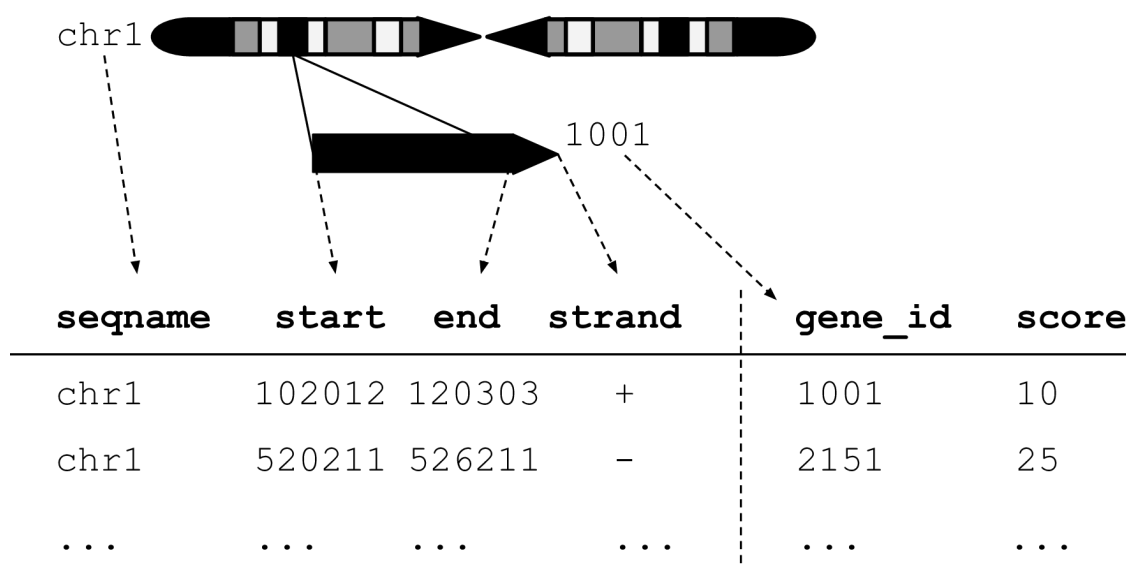


Figure 2.1: An illustration of the *GRanges* data model for a sample from an RNA-seq experiment. The core components of the data model include a *seqname* column (representing the chromosome), a *ranges* column which consists of *start* and *end* coordinates for a genomic region, and a *strand* identifier (either positive, negative, or unstranded). Metadata are included as columns to the right of the dotted line as annotations (*gene_id*) or range level covariates (*score*).

GRanges follow the intuitive tidy data pattern: it is a rectangular table corresponding to a single biological context. Each row contains a single observation and each column is a variable describing the observations. *GRanges* specializes the tidy pattern in that the observations always pertain to some genomic feature, but it largely remains compatible with the general relational operations defined by *dplyr*. Thus, we define our algebra as an extension of the *dplyr* algebra, and borrow its syntax conventions and design principles.

Algebraic operations

The *plyranges* DSL defines an expressive algebra for performing genomic operations with and between *GRanges* objects (see table 2.1). The grammar includes several classes of operation that cover most use cases in genomics data analysis. There are range arithmetic operators, such as for resizing ranges or finding their intersection, and operators for merging, filtering and aggregating by range-specific notions like overlap and proximity.

Arithmetic operations transform range coordinates, as defined by their *start*, *end* and *width*. The three dimensions are mutually dependent and partially redundant, so direct

	Verb	Description
Aggregate	summarize() <i>disjoin_ranges()</i> <i>reduce_ranges()</i>	aggregate over column(s) aggregate column(s) over the union of end coordinates aggregate column(s) by merging overlapping and neighboring ranges
Modify (Unary)	mutate() select() arrange() <i>stretch()</i> <i>shift_(direction)</i> <i>flank_(direction)</i> <i>%intersection%</i> <i>%union%</i>	modifies any column select columns sort by columns extend range by fixed amount shift coordinates generate flanking regions row-wise intersection row-wise union
Modify (Binary)	<i>compute_coverage</i> <i>%setdiff%</i> <i>between()</i> <i>span()</i>	coverage over all ranges row-wise set difference row-wise gap range row-wise spanning range
Merge	<i>join_overlap_*</i> () <i>join_nearest</i> <i>join_follow</i> <i>join_precedes</i> <i>union_ranges</i> <i>intersect_ranges</i> <i>setdiff_ranges</i> <i>complement_ranges</i>	merge by overlapping ranges merge by nearest neighbor ranges merge by following ranges merge by preceding ranges range-wise union range-wise intersect range-wise set difference range-wise set complement
Operate	<i>anchor_direction()</i> group_by() <i>group_by_overlaps()</i>	fix coordinates at direction partition by column(s) partition by overlaps
Restrict	filter() <i>filter_by_overlaps()</i> <i>filter_by_non_overlaps()</i>	subset rows subset by overlap subset by no overlap

Table 2.1: Overview of the *plyranges* grammar. The core verbs are briefly described and categorized into one of the following higher level categories: aggregate, modify, merge, operate, or restrict. A verb is given bold text if its origin is from the *dplyr* grammar.

manipulation of them is problematic. For example, changing the *width* column needs to change either the *start*, *end* or both to preserve integrity of the object. We introduce the *anchor* modifier to disambiguate these adjustments. Supported anchor points include the start, end and midpoint, as well as the 3' and 5' ends for strand-directed ranges. For example, if we anchor the start, then setting the width will adjust the end while leaving the start stationary.

The algebra also defines conveniences for relative coordinate adjustments: *shift* (unanchored adjustment to both start and end) and *stretch* (anchored adjustment of width). We can perform any relative adjustment by some combination of those two operations. The *stretch* operation requires an anchor and assumes the midpoint by default. Since *shift* is unanchored, the user specifies a suffix for indicating the direction: left/right or, for stranded features, upstream/downstream. For example, *shift_right* shifts a range to the right.

The *flank* operation generates new ranges that are adjacent to existing ones. This is useful, for example, when generating upstream promoter regions for genes. Analogous to *shift*, a suffix indicates the side of the input range to flank.

As with other genomic grammars, we define set operations that treat ranges as sets of integers, including *intersect*, *union*, *difference*, and *complement*. There are two sets of these: parallel and merging. For example, the parallel intersection (*x %intersect% y*) finds the intersecting range between *xi* and *yi* for *i* in $1 \dots n$, where *n* is the length of both *x* and *y*. In contrast, the merging intersection (*intersect_ranges(x, y)*) returns a new set of disjoint ranges representing wherever there was overlap between a range in *x* and a range in *y*. Finding the parallel union will fail when two ranges have a gap, so we introduce a *span* operator that takes the union while filling any gap. The *complement* operation is unique in that it is unary. It finds the regions not covered by any of the ranges in a single set. Closely related is the *between* parallel operation, which finds the gap separating *xi* and *yi*. The binary operations are callable from within arithmetic, restriction and aggregation expressions.

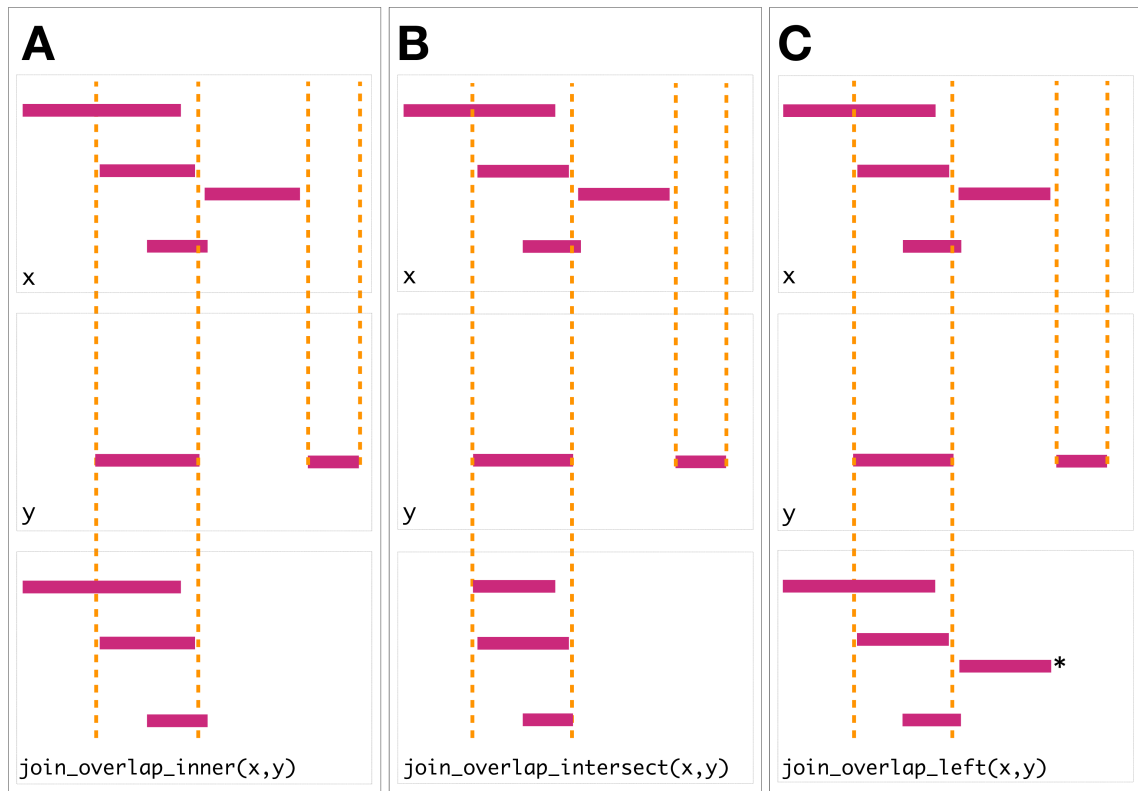


Figure 2.2: Illustration of the three overlap join operators. Each join takes two `GRanges` objects, `x` and `y` as input. A ‘Hits’ object for the join is computed which consists of two components. The first component contains the indices of the ranges in `x` that have been overlapped (the rectangles of `x` that cross the orange lines). The second component consists of the indices of the ranges in `y` that overlap the ranges in `x`. In this case a range in `y` overlaps the ranges in `x` three times, so the index is repeated three times. The resulting ‘Hits’ object is used to modify `x` by where it was ‘hit’ by `y` and merge all metadata columns from `x` and `y` based on the indices contained in the ‘Hits’ object. This procedure is applied generally in the `plyranges` DSL for both overlap and nearest neighbor operations. The join semantics alter what is returned: **A:** for an **inner** join the `x` ranges that are overlapped by `y` are returned. The returned ranges also include the metadata from the `y` range that overlapped the three `x` ranges. **B** An **intersect** join is identical to an inner join except that the intersection is taken between the overlapped `x` ranges and the `y` ranges. **C** For the **left** join all `x` ranges are returned regardless of whether they are overlapped by `y`. In this case the third range (rectangle with the asterisk next to it) of the join would have missing values on metadata columns that came from `y`.

To support merging, our algebra recasts finding overlaps or nearest neighbors between two genomic regions as variants of the relational join operator. A join acts on two `GRanges` objects: x and y . The join operator is relational in the sense that metadata from the x and y ranges are retained in the joined range. All join operators in the `plyranges` DSL generate a set of hits based on overlap or proximity of ranges and use those hits to merge the two datasets in different ways. There are four supported matching algorithms: *overlap*, *nearest*, *precede*, and *follow* (figure 2.2). We can further restrict the matching by whether the query is completely *within* the subject, and adding the *directed* suffix ensures that matching ranges have the same direction (strand).

For merging based on the hits, we have three modes: *inner*, *intersect* and *left*. The *inner* overlap join is similar to the conventional inner join in that there is a row in the result for every match. A major difference is that the matching is not by identity, so we have to choose one of the ranges from each pair. We always choose the left range. The *intersect* join uses the intersection instead of the left range. Finally, the overlap *left* join is akin to left outer join in Codd's relational algebra: it performs an overlap inner join but also returns all x ranges that are not hit by the y ranges.

Since the `GRanges` object is a tabular data structure, our grammar includes operators to filter, sort and aggregate by columns in a `GRanges`. These operations can be performed over partitions formed using the *group_by* modifier. Together with our algebra for arithmetic and merging, these operations conform to the semantics and syntax of the `dplyr` grammar. Consequently, `plyranges` code is generally more compact than the equivalent `GenomicRanges` code (figure 2.3).

2.2.2 Developing workflows with `plyranges`

Here we provide illustrative examples of using the `plyranges` DSL to show how our grammar could be integrated into genomic data workflows. As we construct the workflows we show the data output intermittently to assist the reader in understanding the pipeline steps. The workflows highlight how interoperability with existing Bioconductor infrastructure, enables easy access to public datasets and methods for analysis and visualization.


```

A library(plyranges)
    gwas <- read_bed('snps.bed')
    exons <- read_bed('exons.bed')
    res <- exons %>%
        join_overlap_inner(snps) %>%
        group_by(rsID) %>%
        summarise(n = n_distinct(exonID))

B library(GenomicRanges)
    library(rtracklayer)
    gwas <- import('snps.bed')
    exons <- import('exons.bed')
    hits <- findOverlaps(exons, gwas,
                        ignore.strand = FALSE)
    olap <- splitAsList(exons$name[queryHits(hits)],
                      gwas$name[subjectHits(hits)])
    n <- lengths(unique(olap))
    res <- DataFrame(rsID = names(n),
                    n = as.integer(n))

```

Figure 2.3: Idiomatic code examples for *plyranges* (A) and *GenomicRanges* (B) illustrating an overlap and aggregate operation that returns the same result. In each example, we have two BED files consisting of SNPs that are genome-wide association study (GWAS) hits and reference exons. Each code block counts for each SNP the number of distinct exons it overlaps. The *plyranges* code achieves this with an overlap join followed by partitioning and aggregation. Strand is ignored by default here. The *GenomicRanges* code achieves this using the ‘Hits’ and ‘List’ classes and their methods.

Peak Finding

In the workflow of ChIP-seq data analysis, we are interested in finding peaks from islands of coverage over chromosome. Here we will use *plyranges* to call peaks from islands of coverage above 8 then plot the region surrounding the tallest peak.

Using *plyranges* and the the Bioconductor package *AnnotationHub* (Morgan, 2017) we can download and read BigWig files from ChIP-Seq experiments from the Human Epigenome Roadmap project (Roadmap Epigenomics Consortium et al., 2015). Here we analyse a BigWig file corresponding to H3 lysine 27 trimethylation (H3K27Me3) of

primary T CD8+ memory cells from peripheral blood, focussing on coverage islands over chromosome 10.

First, we extract the genome information from the BigWig file and filter to get the range for chromosome 10. This range will be used as a filter when reading the file.

```
library(plyranges)
chr10_ranges <- bw_file %>%
  get_genome_info() %>%
  filter(seqnames == "chr10")
```

Then we read the BigWig file only extracting scores if they overlap chromosome 10. We also add the genome build information to the resulting ranges. This book-keeping is good practice as it ensures the integrity of any downstream operations such as finding overlaps.

```
chr10_scores <- bw_file %>%
  read_bigwig(overlap_ranges = chr10_ranges) %>%
  set_genome_info(genome = "hg19")
chr10_scores
```

```
#> GRanges object with 5789841 ranges and 1 metadata column:
```

```
#>           seqnames           ranges strand |           score
#>           <Rle>           <IRanges> <Rle> |           <numeric>
#> [1]    chr10           1-60602      * | 0.0422799997031689
#> [2]    chr10       60603-60781      * | 0.163240000605583
#> [3]    chr10       60782-60816      * | 0.372139990329742
#> [4]    chr10       60817-60995      * | 0.163240000605583
#> [5]    chr10       60996-61625      * | 0.0422799997031689
#> ...      ...      ...      ...      ...
#> [5789837] chr10 135524723-135524734      * | 0.144319996237755
#> [5789838] chr10 135524735-135524775      * | 0.250230014324188
#> [5789839] chr10 135524776-135524784      * | 0.427789986133575
#> [5789840] chr10 135524785-135524806      * | 0.730019986629486
```

```
#> [5789841] chr10 135524807-135524837 * | 1.03103005886078
#> -----
#> seqinfo: 25 sequences from hg19 genome
```

We then filter for regions with a coverage score greater than 8, and following this reduce individual runs to ranges representing the islands of coverage. This is achieved with the `reduce_ranges()` function, which allows a summary to be computed over each island: in this case we take the maximum of the scores to find the coverage peaks over chromosome 10.

```
all_peaks <- chr10_scores %>%
  filter(score > 8) %>%
  reduce_ranges(score = max(score))
all_peaks
```

```
#> GRanges object with 1085 ranges and 1 metadata column:
#>      seqnames      ranges strand |      score
#>      <Rle>        <IRanges> <Rle> |      <numeric>
#> [1] chr10      1299144-1299370      * | 13.2264003753662
#> [2] chr10      1778600-1778616      * |  8.20512008666992
#> [3] chr10      4613068-4613078      * |  8.76027011871338
#> [4] chr10      4613081-4613084      * |  8.43659973144531
#> [5] chr10           4613086      * |  8.11507987976074
#> ...      ...      ...      ...      ...
#> [1081] chr10 135344482-135344488      * |  9.23237991333008
#> [1082] chr10 135344558-135344661      * | 11.843409538269
#> [1083] chr10 135344663-135344665      * |  8.26965999603271
#> [1084] chr10 135344670-135344674      * |  8.26965999603271
#> [1085] chr10 135345440-135345441      * |  8.26965999603271
#> -----
#> seqinfo: 25 sequences from hg19 genome
```

Returning to the `GRanges` object containing normalized coverage scores, we filter to find the coordinates of the peak containing the maximum coverage score. We can then find a 5000 nt region centered around the maximum position by anchoring and modifying the width.

Finally, the overlap inner join is used to restrict the chromosome 10 coverage islands, to the islands that are contained in the 5000nt region that surrounds the max peak (figure 2.4).

```
#> GRanges object with 890 ranges and 2 metadata columns:
```

```
#>      seqnames      ranges strand |      score.x      score.y
#>      <Rle>      <IRanges> <Rle> |      <numeric>      <numeric>
#> [1] chr10 21805891-21805988      * | 0.0206599999219179 29.9573001861572
#> [2] chr10 21805989-21806000      * | 0.02112000006306171 29.9573001861572
#> [3] chr10 21806001-21806044      * | 0.0220699999948144 29.9573001861572
#> [4] chr10 21806045-21806049      * | 0.02159000000184774 29.9573001861572
#> [5] chr10 21806050-21806081      * | 0.02112000006306171 29.9573001861572
#> ...      ...      ...      ...      ...
#> [886] chr10      21810878      * | 5.24951982498169 29.9573001861572
#> [887] chr10      21810879      * | 5.83534002304077 29.9573001861572
#> [888] chr10 21810880-21810884      * | 6.44267988204956 29.9573001861572
#> [889] chr10 21810885-21810895      * | 7.07054996490479 29.9573001861572
#> [890] chr10 21810896-21810911      * | 6.44267988204956 29.9573001861572
#> -----
#> seqinfo: 25 sequences from hg19 genome
```

Computing Windowed Statistics

Another common operation in genomics data analysis is to compute data summaries over genomic windows. In `plyranges` this can be achieved via the `group_by_overlaps()` operator. We bin and count and find the average GC content of reads from a H3K27Me3 ChIP-seq experiment by the Human Epigenome Roadmap Consortium.

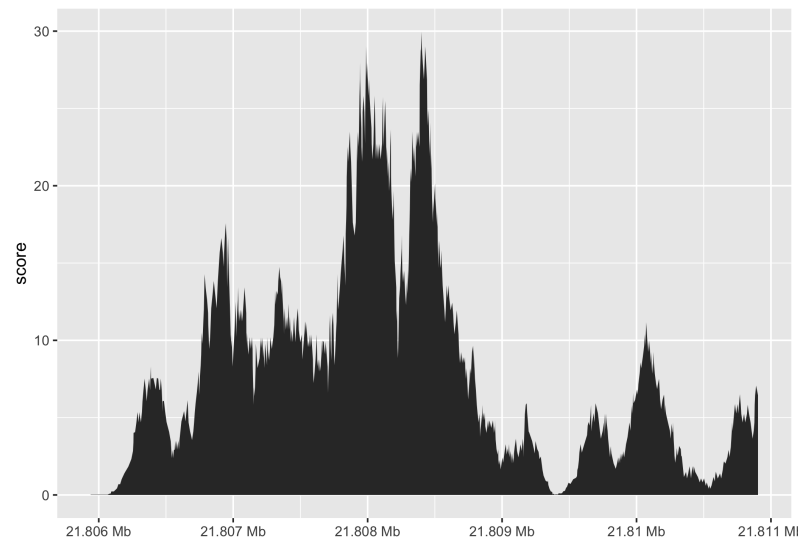


Figure 2.4: The final result of the *plyranges* operations to find a 5000nt region surrounding the peak of normalised coverage scores over chromosome 10, displayed as a density plot.

We can directly obtain the genome information from the header of the BAM file: in this case the reads were aligned to the hg19 genome build and there are no reads overlapping the mitochondrial genome.

```
bam <- read_bam(h1_bam_sorted, index = h1_bam_sorted_index)
locations <- bam %>%
  get_genome_info()
```

Next we only read in alignments that overlap the genomic locations we are interested in and select the query sequence. Note that the reading of the BAM file is deferred: only alignments that pass the filter are loaded into memory. We can add another column representing the GC proportion for each alignment using the `letterFrequency()` function from the `Biostrings` package (Pagès et al., 2018). After computing the GC proportion as the score column, we drop all other columns in the `GRanges` object.

```
alignments <- bam %>%
  filter_by_overlaps(locations) %>%
  select(seq) %>%
  mutate(
    score = as.numeric(letterFrequency(seq, "GC", as.prob = TRUE))
```

```

) %>%
  select(score)
alignments

#> GRanges object with 8275595 ranges and 1 metadata column:
#>
#>      seqnames      ranges strand |      score
#>      <Rle>        <IRanges> <Rle> |      <numeric>
#> [1] chr10      50044-50119      - | 0.276315789473684
#> [2] chr10      50050-50119      + |           0.25
#> [3] chr10      50141-50213      - | 0.447368421052632
#> [4] chr10      50203-50278      + | 0.263157894736842
#> [5] chr10      50616-50690      + | 0.276315789473684
#> ...      ...      ...      ...      ...
#> [8275591] chrY 57772745-57772805      - | 0.513157894736842
#> [8275592] chrY 57772751-57772800      + | 0.526315789473684
#> [8275593] chrY 57772767-57772820      + | 0.565789473684211
#> [8275594] chrY 57772812-57772845      + |           0.25
#> [8275595] chrY 57772858-57772912      + | 0.592105263157895
#> -----
#> seqinfo: 24 sequences from an unspecified genome

```

Finally, we create 10000nt tiles over the genome and compute the number of reads and average GC content over all reads that fall within each tile using an overlap join and merging endpoints.

```

bins <- locations %>%
  tile_ranges(width = 10000L)

alignments_summary <- bins %>%
  join_overlap_inner(alignments) %>%

```

```
disjoin_ranges(n = n(), avg_gc = mean(score))
alignments_summary
```

```
#> GRanges object with 286030 ranges and 2 metadata columns:
```

```
#>           seqnames           ranges strand |           n           avg_gc
#>           <Rle>           <IRanges> <Rle> | <integer>           <numeric>
#> [1]   chr10      49999-59997      * |      88 0.369019138755981
#> [2]   chr10      59998-69997      * |      65 0.434210526315789
#> [3]   chr10      69998-79996      * |      56 0.386513157894737
#> [4]   chr10      79997-89996      * |      71 0.51297257227576
#> [5]   chr10      89997-99996      * |      64 0.387746710526316
#> ...      ...      ...      ... .      ...      ...
#> [286026] chrY 57722961-57732958      * |      36 0.468201754385965
#> [286027] chrY 57732959-57742957      * |      38 0.469529085872576
#> [286028] chrY 57742958-57752956      * |      38 0.542936288088643
#> [286029] chrY 57752957-57762955      * |      42 0.510651629072682
#> [286030] chrY 57762956-57772954      * |     504 0.526942355889723
#> -----
#> seqinfo: 24 sequences from an unspecified genome; no seqlengths
```

Quality Control Metrics

We have created a GRanges object from genotyping performed on the H1 cell line, consisting of approximately two million single nucleotide polymorphisms (SNPs) and short insertion/deletions (indels). The GRanges object consists of 7 columns, relating to the alleles of a SNP or indel, the B-allele frequency, log relative intensity of the probes, GC content score over a probe, and the name of the probe. We can use this information to compute the transition-transversion ratio, a quality control metric, within each chromosome in GRanges object.

First we filter out the indels and mitochondrial variants. Then we create a logical vector corresponding to whether there is a transition event.

```
h1_snp_array <- h1_snp_array %>%  
  filter(!(ref %in% c("I", "D")), seqnames != "M") %>%  
  mutate(transition = (ref %in% c("A", "G") & alt %in% c("G", "A")) |  
          (ref %in% c("C", "T") & alt %in% c("T", "C")))
```

We then compute the transition-transversion ratio over each chromosome using `group_by()` in combination with `summarize()` (figure 2.5).

```
ti_tv_results <- h1_snp_array %>%  
  group_by(seqnames) %>%  
  summarize(n_snps = n(),  
            ti_tv = sum(transition) / sum(!transition))  
ti_tv_results
```

```
#> DataFrame with 24 rows and 3 columns  
#>   seqnames    n_snps      ti_tv  
#>   <factor> <integer>   <numeric>  
#> 1      Y      2226  1.4381161007667  
#> 2      6    154246  3.32013219807305  
#> 3     13     83736  3.40669403220714  
#> 4     10    120035  3.49400973418195  
#> 5      4    153243  3.29528828096533  
#> ...     ...      ...      ...  
#> 20     16     77538  3.19827819589583  
#> 21     12    113208  3.47851887016378  
#> 22     20     57073  3.7121036988111  
#> 23     21     32349  3.50480434479877  
#> 24      X     55495  3.58219800181653
```

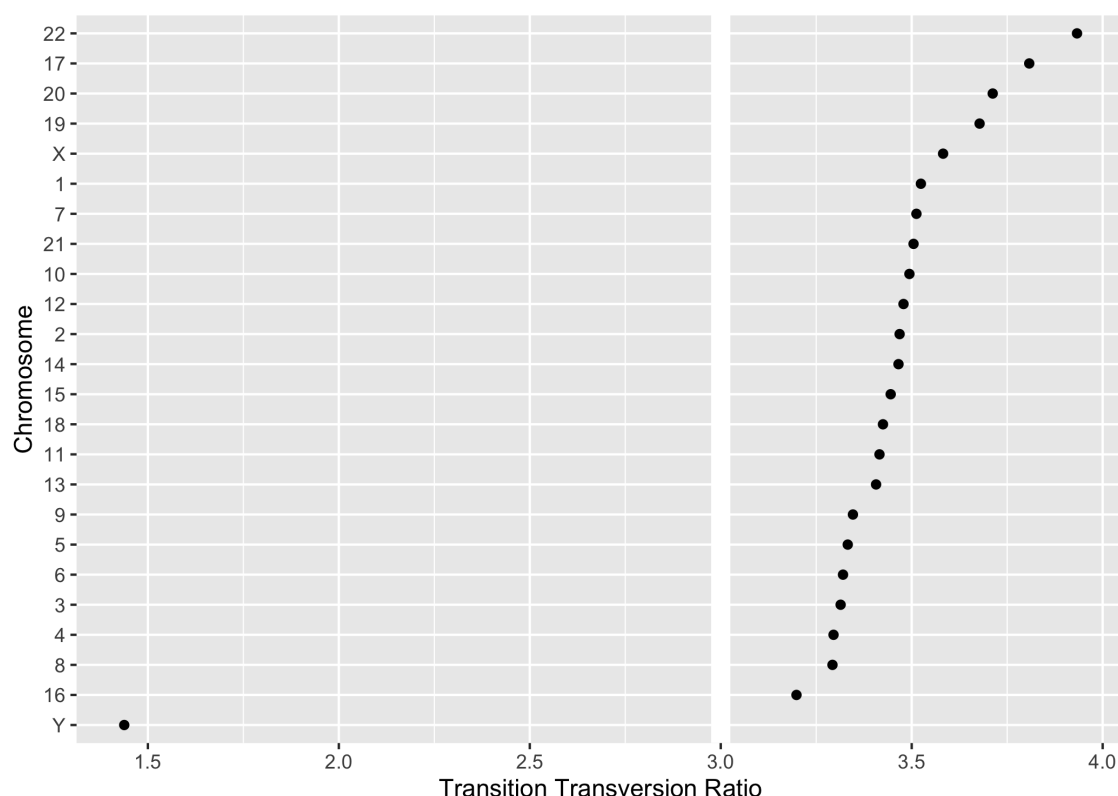



Figure 2.5: *The final result of computing quality control metrics over the SNP array data with `plyranges`, displayed as a dot plot. Chromosomes are ordered by their estimated transition-transversion ratio. A white reference line is drawn at the expected ratio for a human exome.”*

2.3 Discussion

The design of `plyranges` adheres to well understood principles of language and API design: cognitive consistency, cohesion, endomorphism and expressiveness (Green and Petre, 1996). To varying degrees, these principles also underlie the design of `dplyr` and the Bioconductor infrastructure.

We have aimed for `plyranges` to have a simple and direct mapping to the user’s cognitive model, i.e., how the user thinks about the data. This requires careful selection of the level of abstraction so that the user can express workflows in the language of genomics. This motivates the adoption of the tidy GRanges object as our central data structure. The basic `data.frame` and `dplyr` tibble lack any notion of genomic ranges and so could not easily support our genomic grammar, with its specific verbs for range-oriented data manipulation. Another example of cognitive consistency is how `plyranges` is insensitive

to direction/strand by default when, e.g., detecting overlaps. `GenomicRanges` has the opposite behavior. We believe that defaulting to purely spatial overlap is most intuitive to most users.

To further enable cognitive consistency, `plyranges` functions are cohesive. A function is defined to be cohesive if it performs a singular task without producing any side-effects. Singular tasks can always be broken down further at lower levels of abstraction. For example, to resize a range, the user needs to specify which position (start, end, midpoint) should be invariant over the transformation. The `resize()` function from the `GenomicRanges` package has a `fix` argument that sets the anchor, so calling `resize()` coalesces anchoring and width modification. The coupling at the function call level is justified since the effect of setting the width depends on the anchor. However, `plyranges` increases cohesion and decouples the anchoring into its own function call.

Increasing cohesion simplifies the interface to each operation, makes the meaning of arguments more intuitive, and relies on function names as the primary means of expression, instead of a more complex mixture of function and argument names. This results in the user being able to conceptualize the `plyranges` DSL as a flat catalog of functions, without having to descend further into documentation to understand a function's arguments. A flat function catalog also enhances API discoverability, particularly through auto-completion in integrated developer environments (IDEs). One downside of pushing cohesion to this extreme is that function calls become coupled, and care is necessary to treat them as a group when modifying code.

Like `dplyr`, `plyranges` verbs are functional: they are free of side effects and are generally endomorphic, meaning that when the input is a `GRanges` object they return a `GRanges` object. This enables chaining of verbs through syntax like the forward pipe operator from the `magrittr` package. This syntax has a direct cognitive mapping to natural language and the intuitive notion of pipelines. The low-level object-oriented APIs of Bioconductor tend to manipulate data via sub-replacement functions, like `start(gr) <- x`. These ultimately produce the side effect of replacing a symbol mapping in the current environment and thus are not amenable to so-called fluent syntax.

Expressiveness relates to the information content in code: the programmer should be able to clarify intent without unnecessary verbosity. For example, our overlap-based join operations are more concise than the multiple steps necessary to achieve the same effect in the original `GenomicRanges` API. In other cases, the `plyranges` API increases verbosity for the sake of clarity and cohesion. Explicitly calling `anchor()` can require more typing, but the code is easier to comprehend. Another example is the set of routines for importing genomic annotations, including `read_gff()`, `read_bed()`, and `read_bam()`. Compared to the generic `import()` in `rtracklayer`, the explicit format-based naming in `plyranges` clarifies intent and the type of data being returned. Similarly, every `plyranges` function that computes with strand information indicates its intentions by including suffixes such as *directed*, *upstream* or *downstream* in its name, otherwise strand is ignored. The `GenomicRanges` API does not make this distinction explicit in its function naming, instead relying on a parameter that defaults to strand sensitivity, an arguably confusing behavior.

The implementation of `plyranges` is built on top of Bioconductor infrastructure, meaning most functions are constructed by composing generic functions from core Bioconductor packages. As a result, any Bioconductor packages that uses data structures that inherit from `GRanges` will be able to use `plyranges` for free. Another consequence of building on top of Bioconductor generics is that the speed and memory usage of `plyranges` functions are similar to the highly optimized methods implemented in Bioconductor for `GRanges` objects.

A caveat to constructing a compatible interface with `dplyr` is that `plyranges` makes extensive use of non-standard evaluation in R via the `rlang` package (Henry and Wickham, 2017). Simply, this means that computations are evaluated in the context of the `GRanges` objects. Both `dplyr` and `plyranges` are based on the `rlang` language, because it allows for more expressive code that is free of repeated references to the container. Implicitly referencing the container is particularly convenient when programming interactively. Consequently, when programming with `plyranges`, a user needs to generally understand the `rlang` language and how to adapt their code accordingly. Users familiar with the tidyverse should already have such knowledge.

2.4 Conclusion

We have shown how to create expressive and reproducible genomic workflows using the `plyranges` DSL. By realising that the `GRanges` data model is tidy we have highlighted how to implement a grammar for performing genomic arithmetic, aggregation, restriction and merging. Our examples show that `plyranges` code is succinct, human readable and can take advantage of the interoperability provided by the Bioconductor ecosystem and the R language.

We also note that the grammar elements and design principles we have described are programming language agnostic and could be easily be implemented in another language where genomic information could be represented as a tabular data structure. We chose R because it is what we are familiar with and because the aforementioned Bioconductor packages have implemented the `GRanges` data structure.

We aim to continue developing the `plyranges` package and to extend it for use with more complex data structures, such as the `SummarizedExperiment` class, the core Bioconductor data structure for representing experimental results (e.g., counts) from multiple sample experiments in conjunction with feature and sample metadata. Although, the `SummarizedExperiment` is not strictly tidy, it does consist of three tidy data structures that are related by feature and sample identifiers. Therefore, the grammar and design of the `plyranges` DSL is naturally extensible to the `SummarizedExperiment`.

As the `plyranges` interface encourages tidy data practices, it integrates well with the grammar of graphics (Wickham, Hadley, 2016). To achieve responsive performance, interactive graphics rely on lazy data access and computing patterns, so the deferred mechanisms within `plyranges` should help support interactive genomics applications.

2.5 Acknowledgements

We would like to thank Dr Matthew Ritchie at the Walter and Eliza Hall Institute and Dr Paul Harrison for their feedback on earlier drafts of this work. We would also like to thank Lori Shepherd and H erve Pages for the code review they performed and users who have submitted feedback and pull requests.

2.6 Availability of Data and Materials

The BigWig file for the H3K27Me3 primary T CD8+ memory cells from peripheral blood ChIP-seq data from the Human Roadmap Epigenomics project was downloaded from the AnnotationHub package (2.13.1) under accession AH33458 (Morgan, 2017; Roadmap Epigenomics Consortium et al., 2015). The BAM file corresponding to the H1 cell line ChIP-seq data is available at NCBI GEO under accession [GSM433167](#) (Barrett et al., 2013; Roadmap Epigenomics Consortium et al., 2015). The SNP array data for the H1 cell line data is available at NCBI GEO under accession [GPL18952](#) (Roadmap Epigenomics Consortium et al., 2015).

The plyranges package is open source under an Artistic 2.0 license (Lee, Lawrence, and Cook, 2018). The software can be obtained via the Bioconductor project website <https://bioconductor.org> or accessed via Github <https://github.com/sa-lee/plyranges>.

Chapter 3

Fluent genomics with *plyranges* and *tximeta*

3.1 Introduction

In this workflow, we examine a subset of the RNA-seq and ATAC-seq data from Alasoo et al. (2018), a study that involved treatment of macrophage cell lines from a number of human donors with interferon gamma (IFN γ), *Salmonella* infection, or both treatments combined. Alasoo et al. (2018) examined gene expression and chromatin accessibility in a subset of 86 successfully differentiated induced pluripotent stem cells (iPSC) lines, and compared baseline and response with respect to chromatin accessibility and gene expression at specific quantitative trait loci (QTL). The authors found that many of the stimulus-specific expression QTL were already detectable as chromatin QTL in naive cells, and further hypothesize about the nature and role of transcription factors implicated in the response to stimulus.

We will perform a much simpler analysis than the one found in Alasoo et al. (2018), using their publicly available RNA-seq and ATAC-seq data (ignoring the genotypes). We will examine the effect of IFN γ stimulation on gene expression and chromatin accessibility, and look to see if there is an enrichment of differentially accessible (DA) ATAC-seq peaks in the vicinity of differentially expressed (DE) genes. This is plausible, as the transcriptomic

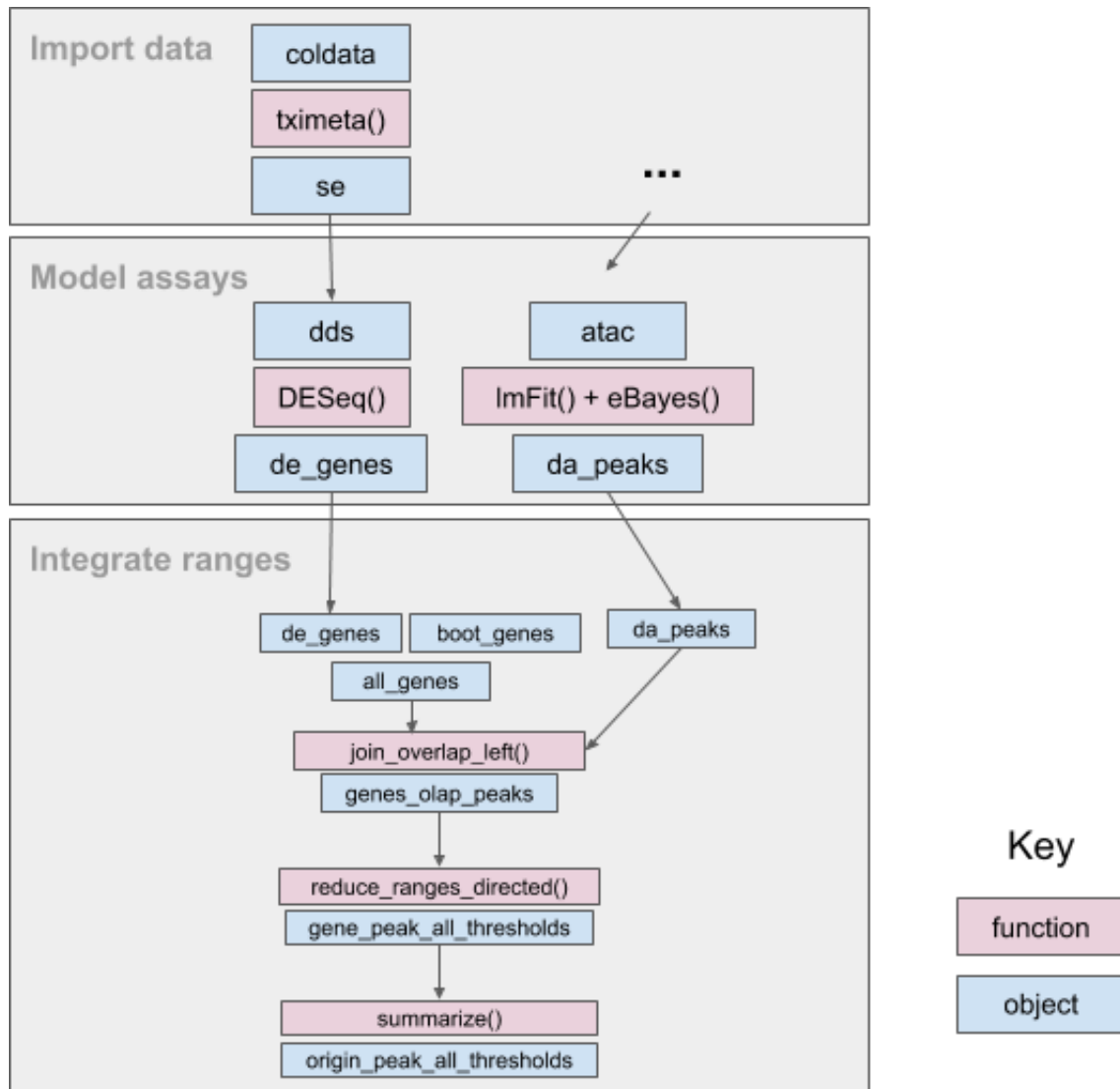


Figure 3.1: An overview of the fluent genomics workflow. First, we import data as a SummarizedExperiment object, which enables interoperability with downstream analysis packages. Then we model our assay data, using the existing Bioconductor packages *DESeq2* and *limma*. We take the results of our models for each assay with respect to their genomic coordinates, and integrate them. First, we compute the overlap between the results of each assay, then aggregate over the combined genomic regions, and finally summarize to compare enrichment for differentially expressed genes to non differentially expressed genes. The final output can be used for downstream visualization or further transformation.

response to IFN γ stimulation may be mediated through binding of regulatory proteins to accessible regions, and this binding may increase the accessibility of those regions such that it can be detected by ATAC-seq.

Throughout the workflow (Figure 3.1), we will use existing Bioconductor infrastructure to understand these datasets. In particular, we will emphasize the use of the Bioconductor

packages *plyranges* and *tximeta*. The *plyranges* package fluently transforms data tied to genomic ranges using operations like shifting, window construction, overlap detection, etc. It is described by Lee, Cook, and Lawrence (2019) and leverages underlying core Bioconductor infrastructure (Lawrence et al., 2013b; Huber et al., 2015b) and the tidyverse design principles Wickham et al. (2019).

The *tximeta* package described by Love et al. (2019) is used to read RNA-seq quantification data into R/Bioconductor, such that the transcript ranges and their provenance are automatically attached to the object containing expression values and differential expression results.

3.1.1 Experimental Data

The data used in this workflow is available from two packages: the macrophage Bioconductor *ExperimentData* package and from the workflow package *fluentGenomics*.

The macrophage package contains RNA-seq quantification from 24 RNA-seq samples, a subset of the RNA-seq samples generated and analyzed by Alasoo et al. (2018). The paired-end reads were quantified using *Salmon* (Patro et al., 2017), using the Gencode 29 human reference transcripts (Frankish, GENCODE-consortium, and Flicek, 2018). For more details on quantification, and the exact code used, consult the vignette of the *macrophage* package. The package also contains the Snakemake file that was used to distribute the *Salmon* quantification jobs on a cluster (Köster and Rahmann, 2012).

The *fluentGenomics* package contains functionality to download and generate a cached *SummarizedExperiment* object from the normalized ATAC-seq data provided by Alasoo and Gaffney (2017). This object contains all 145 ATAC-seq samples across all experimental conditions as analyzed by Alasoo et al. (2018). The data can be also be downloaded directly from the [Zenodo](#) deposition.

The following code loads the path to the cached data file, or if it is not present, will create the cache and generate a *SummarizedExperiment* using the *BiocFileCache* package (Shepherd and Morgan, 2019).

```
library(fluentGenomics)
path_to_se <- cache_atac_se()
```

We can then read the cached file and assign it to an object called `atac`.

```
atac <- readRDS(path_to_se)
```

A precise description of how we obtained this *SummarizedExperiment* object can be found in section [3.2.2](#).

3.2 Import Data as a *SummarizedExperiment*

3.2.1 Using *tximeta* to import RNA-seq quantification data

First, we specify a directory `dir`, where the quantification files are stored. You could simply specify this directory with:

```
dir <- "/path/to/quant/files"
```

where the path is relative to your current R session. However, in this case we have distributed the files in the *macrophage* package. The relevant directory and associated files can be located using `system.file`.

```
dir <- system.file("extdata", package="macrophage")
```

Information about the experiment is contained in the `coldata.csv` file. We leverage the *dplyr* and *readr* packages (as part of the *tidyverse*) to read this file into R (Wickham et al., 2019). We will see later that *plyranges* extends these packages to accommodate genomic ranges.

```
library(readr)
library(dplyr)
colfile <- file.path(dir, "coldata.csv")
```

```
coldata <- read_csv(colfile) %>%
  dplyr::select(
    names,
    id = sample_id,
    line = line_id,
    condition = condition_name
  ) %>%
  dplyr::mutate(
    files = file.path(dir, "quants", names, "quant.sf.gz"),
    line = factor(line),
    condition = relevel(factor(condition), "naive")
  )
coldata
```

```
#> # A tibble: 24 x 5
#>   names      id    line condition  files
#>   <chr>     <chr> <fct> <fct>     <chr>
#> 1 SAMEA1038~ diku_A diku~ naive      /Library/Frameworks/R.framework/Versions/~
#> 2 SAMEA1038~ diku_B diku~ IFNg      /Library/Frameworks/R.framework/Versions/~
#> 3 SAMEA1038~ diku_C diku~ SL1344    /Library/Frameworks/R.framework/Versions/~
#> 4 SAMEA1038~ diku_D diku~ IFNg_SL13~ /Library/Frameworks/R.framework/Versions/~
#> 5 SAMEA1038~ eiwy_A eiwy~ naive      /Library/Frameworks/R.framework/Versions/~
#> 6 SAMEA1038~ eiwy_B eiwy~ IFNg      /Library/Frameworks/R.framework/Versions/~
#> 7 SAMEA1038~ eiwy_C eiwy~ SL1344    /Library/Frameworks/R.framework/Versions/~
#> 8 SAMEA1038~ eiwy_D eiwy~ IFNg_SL13~ /Library/Frameworks/R.framework/Versions/~
#> 9 SAMEA1038~ fikt_A fikt~ naive      /Library/Frameworks/R.framework/Versions/~
#> 10 SAMEA1038~ fikt_B fikt~ IFNg      /Library/Frameworks/R.framework/Versions/~
#> # ... with 14 more rows
```

After we have read the `coldata.csv` file, we select relevant columns from this table, create a new column called `files`, and transform the existing `line` and `condition` columns into

factors. In the case of condition, we specify the “naive” cell line as the reference level. The `files` column points to the quantifications for each observation – these files have been gzipped, but would typically not have the ‘gz’ ending if used from *Salmon* directly. One other thing to note is the use of the pipe operator, `%>%`, which can be read as “then”, i.e. first read the data, *then* select columns, *then* mutate them.

Now we have a table summarizing the experimental design and the locations of the quantifications. The following lines of code do a lot of work for the analyst: importing the RNA-seq quantification (dropping *inferential replicates* in this case), locating the relevant reference transcriptome, attaching the transcript ranges to the data, and fetching genome information. Inferential replicates are especially useful for performing transcript-level analysis, but here we will use a point estimate for the per-gene counts and perform gene-level analysis.

The result is a *SummarizedExperiment* object.

```
suppressPackageStartupMessages(library(SummarizedExperiment))
library(tximeta)
se <- tximeta(coldata, dropInfReps=TRUE)
se
```

```
#> class: RangedSummarizedExperiment
#> dim: 205870 24
#> metadata(6): tximetaInfo quantInfo ... txomeInfo txdbInfo
#> assays(3): counts abundance length
#> rownames(205870): ENST00000456328.2 ENST00000450305.2 ...
#>   ENST00000387460.2 ENST00000387461.2
#> rowData names(3): tx_id gene_id tx_name
#> colnames(24): SAMEA103885102 SAMEA103885347 ... SAMEA103885308
#>   SAMEA103884949
#> colData names(4): names id line condition
```

On a machine with a working internet connection, the above command works without any extra steps, as the `tximeta` function obtains any necessary metadata via FTP, unless it is already cached locally. The `tximeta` package can also be used without an internet connection, in this case the linked transcriptome can be created directly from a *Salmon* index and `gtf`.

```
makeLinkedTxome(  
  indexDir=file.path(dir, "gencode.v29_salmon_0.12.0"),  
  source="Gencode",  
  organism="Homo sapiens",  
  release="29",  
  genome="GRCh38",  
  fasta="gencode.v29.transcripts.fa.gz", # ftp link to fasta file  
  gtf=file.path(dir, "gencode.v29.annotation.gtf.gz"), # local version  
  write=FALSE  
)
```

Because `tximeta` knows the correct reference transcriptome, we can ask `tximeta` to summarize the transcript-level data to the gene level using the methods of Soneson, Love, and Robinson (2015).

```
gse <- summarizeToGene(se)
```

One final note is that the start of positive strand genes and the end of negative strand genes is now dictated by the genomic extent of the isoforms of the gene (so the start and end of the reduced *GRanges*). Another alternative would be to either operate on transcript abundance, and perform differential analysis on transcript (and so avoid defining the TSS of a set of isoforms), or to use gene-level summarized expression but to pick the most representative TSS based on isoform expression.

3.2.2 Importing ATAC-seq data as a *SummarizedExperiment* object

The *SummarizedExperiment* object containing ATAC-seq peaks can be created from the following tab-delimited files from Alasoo and Gaffney (2017):

- The sample metadata: `ATAC_sample_metadata.txt.gz` (<1M)
- The matrix of normalized read counts: `ATAC_cqn_matrix.txt.gz` (109M)
- The annotated peaks: `ATAC_peak_metadata.txt.gz` (5.6M)

To begin, we read in the sample metadata, following similar steps to those we used to generate the `coldata` table for the RNA-seq experiment:

```
atac_coldata <- read_tsv("ATAC_sample_metadata.txt.gz") %>%  
  select(  
    sample_id,  
    donor,  
    condition = condition_name  
  ) %>%  
  mutate(condition = relevel(factor(condition), "naive"))
```

The ATAC-seq counts have already been normalized with *cqn* (Hansen, Irizarry, and Wu, 2012) and log2 transformed. Loading the *cqn*-normalized matrix of log2 transformed read counts takes ~30 seconds and loads an object of ~370 Mb. We set the column names so that the first column contains the rownames of the matrix, and the remaining columns are the sample identities from the `atac_coldata` object.

```
atac_mat <- read_tsv(  
  "ATAC_cqn_matrix.txt.gz",  
  skip = 1,  
  col_names = c("rownames", atac_coldata[["sample_id"]])  
)  
rownames <- atac_mat[["rownames"]]
```

```
atac_mat <- as.matrix(atac_mat[, -1])  
rownames(atac_mat) <- rownames
```

We read in the peak metadata (locations in the genome), and convert it to a *GRanges* object. The `as_granges()` function automatically converts the *data.frame* into a *GRanges* object. From that result, we extract the `peak_id` column and set the genome information to the build “GRCh38”. We know this from the [Zenodo entry](#).

```
library(plyranges)  
peaks_df <- read_tsv(  
  "ATAC_peak_metadata.txt.gz",  
  col_types = c("cidciicdc")  
)  
  
peaks_gr <- peaks_df %>%  
  as_granges(seqnames = chr) %>%  
  select(peak_id=gene_id) %>%  
  set_genome_info(genome = "GRCh38")
```

Finally, we construct a *SummarizedExperiment* object. We place the matrix into the `assays` slot as a named list, the annotated peaks into the `rowRanges` slot, and the sample metadata into the `colData` slot:

```
atac <- SummarizedExperiment(  
  assays = list(cqndata=atac_mat),  
  rowRanges = peaks_gr,  
  colData = atac_coldata  
)
```

3.3 Model assays

3.3.1 RNA-seq differential gene expression analysis

We can easily run a differential expression analysis with DESeq2 using the following code chunks (Love, Huber, and Anders, 2014). The design formula indicates that we want to control for the donor baselines (line) and test for differences in gene expression on the condition. For a more comprehensive discussion of DE workflows in Bioconductor see Love et al. (2016) and Law et al. (2018).

```
library(DESeq2)
dds <- DESeqDataSet(gse, ~line + condition)
# filter out lowly expressed genes
# at least 10 counts in at least 6 samples
keep <- rowSums(counts(dds) >= 10) >= 6
dds <- dds[keep,]
```

The model is fit with the following line of code:

```
dds <- DESeq(dds)
```

Below we set the contrast on the condition variable, indicating we are estimating the \log_2 fold change (LFC) of IFNg stimulated cell lines against naive cell lines. We are interested in LFC greater than 1 at a nominal false discovery rate (FDR) of 1%.

```
res <- results(dds,
               contrast=c("condition", "IFNg", "naive"),
               lfcThreshold=1, alpha=0.01)
```

To see the results of the expression analysis, we can generate a summary table and an MA plot:

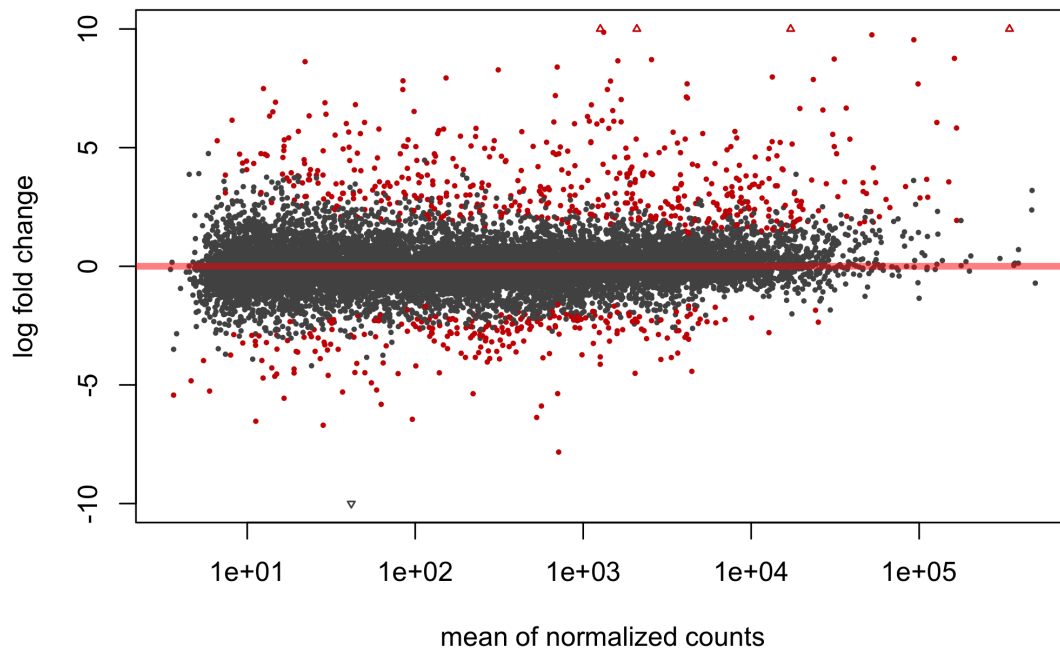


Figure 3.2: Visualization of *DESeq2* results as an “MA plot”. Genes that have an adjusted p-value below 0.01 are colored red.

```
summary(res)
```

```
#>
#> out of 17806 with nonzero total read count
#> adjusted p-value < 0.01
#> LFC > 1.00 (up)      : 502, 2.8%
#> LFC < -1.00 (down)  : 247, 1.4%
#> outliers [1]        : 0, 0%
#> low counts [2]       : 0, 0%
#> (mean count < 3)
#> [1] see 'cooksCutoff' argument of ?results
#> [2] see 'independentFiltering' argument of ?results
```

```
DESeq2::plotMA(res, ylim=c(-10,10))
```

We now output the results as a *GRanges* object, and due to the conventions of *plyranges*, we construct a new column called `gene_id` from the row names of the results. Each row now contains the genomic region (`seqnames`, `start`, `end`, `strand`) along with corresponding metadata columns (the `gene_id` and the results of the test). Note that *tximeta* has correctly identified the reference genome as “hg38”, and this has also been added to the *GRanges* along the results columns. This kind of book-keeping is vital once overlap operations are performed to ensure that *plyranges* is not comparing across incompatible genomes.

```
suppressPackageStartupMessages(library(plyranges))
de_genes <- results(dds,
                    contrast=c("condition", "IFNg", "naive"),
                    lfcThreshold=1,
                    format="GRanges") %>%
  names_to_column("gene_id")
de_genes
```

#> GRanges object with 17806 ranges and 7 metadata columns:

```
#>      seqnames      ranges strand |      gene_id
#>      <Rle>      <IRanges> <Rle> |      <character>
#> [1]   chrX 100627109-100639991   - | ENSG000000000003.14
#> [2]  chr20  50934867-50958555   - | ENSG000000000419.12
#> [3]   chr1 169849631-169894267   - | ENSG000000000457.13
#> [4]   chr1 169662007-169854080   + | ENSG000000000460.16
#> [5]   chr1  27612064-27635277   - | ENSG000000000938.12
#> ...      ...      ...      ... .      ...
#> [17802] chr10  84167228-84172093   - | ENSG00000285972.1
#> [17803]  chr6  63572012-63583587   + | ENSG00000285976.1
#> [17804] chr16  57177349-57181390   + | ENSG00000285979.1
#> [17805]  chr8 103398658-103501895   - | ENSG00000285982.1
#> [17806] chr10 12563151-12567351   + | ENSG00000285994.1
```

```
#>           baseMean      log2FoldChange      lfcSE
#>           <numeric>           <numeric>           <numeric>
#> [1] 171.570646163445 -0.282245015065582 0.300571026277417
#> [2] 967.751278980391 0.0391222756936352 0.0859707605047955
#> [3] 682.432885098654 1.2846178585311 0.196906721741941
#> [4] 262.963397841117 -1.47187616421189 0.218691645887265
#> [5] 2660.10225731917 0.675478091290521 0.236053041372838
#> ...           ...           ...           ...
#> [17802] 10.0474624496157 0.548451844773876 0.444318686394084
#> [17803] 4586.34616821518 -0.033929582570062 0.188004977365846
#> [17804] 14.2965310090402 0.312347650582085 0.522699844356108
#> [17805] 27.7629588245413 0.994518742790125 1.58237312176743
#> [17806] 6.60408582708505 0.25399752352481 0.5957511892896
#>           stat           pvalue           padj
#>           <numeric>           <numeric>           <numeric>
#> [1]           0           1           1
#> [2]           0           1           1
#> [3] 1.44544511235177 0.148332899695748 1
#> [4] -2.15772377722715 0.0309493141635637 0.409727500369082
#> [5]           0           1           1
#> ...           ...           ...           ...
#> [17802]           0           1           1
#> [17803]           0           1           1
#> [17804]           0           1           1
#> [17805]           0           1           1
#> [17806]           0           1           1
#> -----
#> seqinfo: 25 sequences (1 circular) from hg38 genome
```

From this, we can restrict the results to those that meet our FDR threshold and select (and rename) the metadata columns we're interested in:

```
de_genes <- de_genes %>%  
  filter(padj < 0.01) %>%  
  dplyr::select(  
    gene_id,  
    de_log2FC = log2FoldChange,  
    de_padj = padj  
  )
```

We now wish to extract genes for which there is evidence that the LFC is *not* large. We perform this test by specifying an LFC threshold and an alternative hypothesis (`altHypothesis`) that the LFC is less than the threshold in absolute value. To visualize the result of this test, you can run `results` without `format="GRanges"`, and pass this object to `plotMA` as before. We label these genes as `other_genes` and later as “non-DE genes”, for comparison with our `de_genes` set.

```
other_genes <- results(dds,  
  contrast=c("condition", "IFNg", "naive"),  
  lfcThreshold=1,  
  altHypothesis="lessAbs",  
  format="GRanges") %>%  
  filter(padj < 0.01) %>%  
  names_to_column("gene_id") %>%  
  dplyr::select(  
    gene_id,  
    de_log2FC = log2FoldChange,  
    de_padj = padj  
  )
```

3.3.2 ATAC-seq peak differential abundance analysis

The following section describes the process we have used for generating a *GRanges* object of differential peaks from the ATAC-seq data in Alasoo et al. (2018).

The code chunks for the remainder of this section are not run.

For assessing differential accessibility, we run *limma* (Smyth, 2004), and generate the a summary of LFCs and adjusted p-values for the peaks:

```
library(limma)
design <- model.matrix(~donor + condition, colData(atac))
fit <- lmFit(assay(atac), design)
fit <- eBayes(fit)
idx <- which(colnames(fit$coefficients) == "conditionIFNg")
tt <- topTable(fit, coef=idx, sort.by="none", n=nrow(atac))
```

We now take the *rowRanges* of the *SummarizedExperiment* and attach the LFCs and adjusted p-values from *limma*, so that we can consider the overlap with differential expression. Note that we set the genome build to “hg38” and restyle the chromosome information to use the “UCSC” style (e.g. “chr1”, “chr2”, etc.). Again, we know the genome build from the Zenodo entry for the ATAC-seq data.

```
atac_peaks <- rowRanges(atac) %>%
  remove_names() %>%
  mutate(
    da_log2FC = tt$logFC,
    da_padj = tt$adj.P.Val
  ) %>%
  set_genome_info(genome = "hg38")

seqlevelsStyle(atac_peaks) <- "UCSC"
```

The final *GRanges* object containing the DA peaks is included in the workflow package and can be loaded as follows:

```
library(fluentGenomics)
```

```
peaks
```

```
#> GRanges object with 296220 ranges and 3 metadata columns:
```

```
#>           seqnames           ranges strand |           peak_id
#>           <Rle>           <IRanges> <Rle> |           <character>
#> [1]      chr1      9979-10668      * | ATAC_peak_1
#> [2]      chr1     10939-11473      * | ATAC_peak_2
#> [3]      chr1     15505-15729      * | ATAC_peak_3
#> [4]      chr1     21148-21481      * | ATAC_peak_4
#> [5]      chr1     21864-22067      * | ATAC_peak_5
#> ...      ...      ...      ...      ...
#> [296216] chrX 155896572-155896835      * | ATAC_peak_296216
#> [296217] chrX 155958507-155958646      * | ATAC_peak_296217
#> [296218] chrX 156016760-156016975      * | ATAC_peak_296218
#> [296219] chrX 156028551-156029422      * | ATAC_peak_296219
#> [296220] chrX 156030135-156030785      * | ATAC_peak_296220
#>           da_log2FC           da_padj
#>           <numeric>           <numeric>
#> [1] 0.266185396736073 9.10672732956434e-05
#> [2] 0.32217712436691 2.03434717570469e-05
#> [3] -0.574159538548115 3.41707743345703e-08
#> [4] -1.14706617895329 8.22298606986521e-26
#> [5] -0.896143162633654 4.79452571676397e-11
#> ...      ...      ...
#> [296216] -0.834628897017445 1.3354605397165e-11
#> [296217] -0.147537281935847 0.313014754316915
#> [296218] -0.609732301631964 3.62338775135558e-09
#> [296219] -0.347678474957794 6.94823191242968e-06
#> [296220] 0.492442459200901 7.07663984067763e-13
```

```
#> -----  
#> seqinfo: 23 sequences from hg38 genome; no seqlengths
```

3.4 Integrate ranges

3.4.1 Finding overlaps with *plyranges*

We have already used *plyranges* a number of times above, to filter, mutate, and select on *GRanges* objects, as well as ensuring the correct genome annotation and style has been used. The *plyranges* package provides a grammar for performing transformations of genomic data (Lee, Cook, and Lawrence, 2019). Computations resulting from compositions of *plyranges* “verbs” are performed using underlying, highly optimized range operations in the *GenomicRanges* package (Lawrence et al., 2013b).

For the overlap analysis, we filter the annotated peaks to have a nominal FDR bound of 1%.

```
da_peaks <- peaks %>%  
  filter(da_padj < 0.01)
```

We now have *GRanges* objects that contain DE genes, genes without strong signal of DE, and DA peaks. We are ready to answer the question: is there an enrichment of DA ATAC-seq peaks in the vicinity of DE genes compared to genes without sufficient DE signal?

3.4.2 Down sampling non-differentially expressed genes

As *plyranges* is built on top of *dplyr*, it implements methods for many of its verbs for *GRanges* objects. Here we can use `slice` to randomly sample the rows of the `other_genes`. The `sample.int` function will generate random samples of size equal to the number of DE-genes from the number of rows in `other_genes`:

```
size <- length(de_genes)
slice(other_genes, sample.int(plyranges::n(), size))
```

```
#> GRanges object with 749 ranges and 3 metadata columns:
```

```
#>      seqnames      ranges strand |      gene_id
#>      <Rle>        <IRanges> <Rle> |      <character>
#> [1]   chr17  38869859-38921770      + | ENSG00000002834.17
#> [2]   chr3 124730366-124749273      + | ENSG000000114491.13
#> [3]   chr17  58205437-58219605      - | ENSG000000011143.16
#> [4]   chr13  79941531-80074640      - | ENSG000000284196.2
#> [5]   chr14  30893799-31026401      - | ENSG000000196792.11
#> ...      ...      ...      ...      ...
#> [745] chr19 19516227-19536076      + | ENSG000000258674.5
#> [746] chr19  3572777-3579088      + | ENSG000000064961.18
#> [747] chr7  43875894-43926411      - | ENSG000000106608.16
#> [748] chr4   6575175-6623362      + | ENSG000000013288.8
#> [749] chr9 100099256-100301000      + | ENSG000000119509.12
#>      de_log2FC      de_padj
#>      <numeric>      <numeric>
#> [1] 0.0536201277817726 1.77259338140751e-06
#> [2] -0.29917199446035 9.77614496406817e-05
#> [3] 0.177304933924909 0.00577260932377875
#> [4] 0.0921387699702874 0.0049759626704761
#> [5] 0.21195109721739 5.51488326580291e-07
#> ...      ...      ...
#> [745] 0.10148592994 0.00106456634166927
#> [746] -0.335966379675693 1.48705435662573e-05
#> [747] -0.0675449918352472 8.1159946932927e-16
#> [748] 0.508158753677247 0.00018268760369752
#> [749] 0.113534202189026 4.76119242689745e-17
```



```
#> -----
```

```
#> seqinfo: 25 sequences (1 circular) from hg38 genome
```

We can repeat this many times to create many samples via `replicate`. By replicating the sub-sampling multiple times, we minimize the variance on the enrichment statistics induced by the sampling process.

```
# set a seed for the results
set.seed(2019-08-02)
boot_genes <- replicate(10,
                        slice(other_genes, sample.int(plyranges::n(), size)),
                        simplify = FALSE)
```

This creates a list of *GRanges* objects as a list, and we can bind these together using the `bind_ranges` function. This function creates a new column called “resample” on the result that identifies each of the input *GRanges* objects:

```
boot_genes <- bind_ranges(boot_genes, .id = "resample")
```

Similarly, we can then combine the `boot_genes` *GRanges*, with the DE *GRanges* object. As the `resample` column was not present on the DE *GRanges* object, this is given a missing value which we recode to a 0 using `mutate()`

```
all_genes <- bind_ranges(
  de=de_genes,
  not_de = boot_genes,
  .id="origin"
) %>%
  mutate(
    origin = factor(origin, c("not_de", "de")),
    resample = ifelse(is.na(resample), 0L, as.integer(resample))
  )
all_genes
```

```
#> GRanges object with 8239 ranges and 5 metadata columns:
#>           seqnames           ranges strand |           gene_id
#>           <Rle>           <IRanges> <Rle> |           <character>
#> [1]      chr1 196651878-196747504      + | ENSG00000000971.15
#> [2]      chr6  46129993-46146699      + | ENSG00000001561.6
#> [3]      chr4  17577192-17607972      + | ENSG00000002549.12
#> [4]      chr7 150800403-150805120      + | ENSG00000002933.8
#> [5]      chr4  15778275-15853230      + | ENSG00000004468.12
#> ...      ...      ...      ...      ...
#> [8235] chr17  43527844-43579620      - | ENSG00000175832.12
#> [8236] chr17  18260534-18266552      + | ENSG00000177427.12
#> [8237] chr20  63895182-63936031      + | ENSG00000101152.10
#> [8238] chr1   39081316-39487177      + | ENSG00000127603.25
#> [8239] chr8   41577187-41625001      + | ENSG00000158669.11
#>           de_log2FC           de_padj resample  origin
#>           <numeric>           <numeric> <integer> <factor>
#> [1]  4.98711071930695 1.37057050625117e-13      0      de
#> [2]  1.92721595378787 3.1747750217733e-05      0      de
#> [3]  2.93372501059128 2.0131038573066e-11      0      de
#> [4]  3.16721751137972 1.07359906028984e-08      0      de
#> [5]  5.40894352968188 4.82904694023763e-18      0      de
#> ...      ...      ...      ...      ...
#> [8235] -0.240918426099239 0.00991611085813261      10  not_de
#> [8236] -0.166059030395757 9.1205141062356e-05      10  not_de
#> [8237] 0.250538999517482 1.74084544559733e-09      10  not_de
#> [8238] -0.385053503003028 0.00265539384929076      10  not_de
#> [8239] 0.155922038318879 2.9637514745875e-17      10  not_de
#> -----
#> seqinfo: 25 sequences (1 circular) from hg38 genome
```

3.4.3 Expanding genomic coordinates around the transcription start site

Now we would like to modify our gene ranges so they contain the 10 kilobases on either side of their transcription start site (TSS). There are many ways one could do this, but we prefer an approach via the anchoring methods in *plyranges*. Because there is a mutual dependence between the start, end, width, and strand of a *GRanges* object, we define anchors to fix one of start and end, while modifying the width. As an example, to extract just the TSS, we can anchor by the 5' end of the range and modify the width of the range to equal 1.

```
all_genes <- all_genes %>%  
  anchor_5p() %>%  
  mutate(width = 1)
```

Anchoring by the 5' end of a range will fix the end of negatively stranded ranges, and fix the start of positively stranded ranges.

We can then repeat the same pattern but this time using `anchor_center()` to tell *plyranges* that we are making the TSS the midpoint of a range that has total width of 20kb, or 10kb both upstream and downstream of the TSS.

```
all_genes <- all_genes %>%  
  anchor_center() %>%  
  mutate(width=2*1e4)
```

3.4.4 Use overlap joins to find relative enrichment

We are now ready to compute overlaps between RNA-seq genes (our DE set and bootstrap sets) and the ATAC-seq peaks. In *plyranges*, overlaps are defined as joins between two *GRanges* objects: a *left* and a *right* *GRanges* object. In an overlap join, a match is any range on the *left* *GRanges* that is overlapped by the *right* *GRanges*. One powerful aspect of the overlap joins is that the result maintains all (metadata) columns from each of the *left* and *right* ranges which makes downstream summaries easy to compute.

To combine the DE genes with the DA peaks, we perform a left overlap join. This returns to us the `all_genes` ranges (potentially with duplication), but with the metadata columns from those overlapping DA peaks. For any gene that has no overlaps, the DA peak columns will have NA's.

```
#> GRanges object with 27766 ranges and 8 metadata columns:
```

```
#>           seqnames           ranges strand |           gene_id
#>           <Rle>           <IRanges> <Rle> |           <character>
#> [1]      chr1 196641878-196661877      + | ENSG00000000971.15
#> [2]      chr6  46119993-46139992      + | ENSG00000001561.6
#> [3]      chr4  17567192-17587191      + | ENSG00000002549.12
#> [4]      chr4  17567192-17587191      + | ENSG00000002549.12
#> [5]      chr4  17567192-17587191      + | ENSG00000002549.12
#> ...      ...      ...      ...      ...
#> [27762] chr1  39071316-39091315      + | ENSG00000127603.25
#> [27763] chr1  39071316-39091315      + | ENSG00000127603.25
#> [27764] chr8  41567187-41587186      + | ENSG00000158669.11
#> [27765] chr8  41567187-41587186      + | ENSG00000158669.11
#> [27766] chr8  41567187-41587186      + | ENSG00000158669.11
#>           de_log2FC           de_padj resample  origin
#>           <numeric>           <numeric> <integer> <factor>
#> [1] 4.98711071930695 1.37057050625117e-13      0      de
#> [2] 1.92721595378787 3.1747750217733e-05      0      de
#> [3] 2.93372501059128 2.0131038573066e-11      0      de
#> [4] 2.93372501059128 2.0131038573066e-11      0      de
#> [5] 2.93372501059128 2.0131038573066e-11      0      de
#> ...      ...      ...      ...
#> [27762] -0.385053503003028 0.00265539384929076     10 not_de
#> [27763] -0.385053503003028 0.00265539384929076     10 not_de
#> [27764] 0.155922038318879 2.9637514745875e-17     10 not_de
#> [27765] 0.155922038318879 2.9637514745875e-17     10 not_de
```

```
#> [27766] 0.155922038318879 2.9637514745875e-17 10 not_de
#>          peak_id          da_log2FC          da_adj
#>          <character>          <numeric>          <numeric>
#> [1] ATAC_peak_21236 -0.546582189082724 0.000115273676444232
#> [2] ATAC_peak_231183 1.45329684862127 9.7322474682763e-17
#> [3] ATAC_peak_193578 0.222371496904895 3.00939005719989e-11
#> [4] ATAC_peak_193579 -0.281615137872819 7.99888515457195e-05
#> [5] ATAC_peak_193580 0.673705317951604 7.60042918890061e-15
#> ...
#> [27762] ATAC_peak_5357 -1.05823584693303 3.69051674661467e-16
#> [27763] ATAC_peak_5358 -1.31411238041643 6.44280493172654e-26
#> [27764] ATAC_peak_263396 -0.904080135059089 8.19576651692093e-13
#> [27765] ATAC_peak_263397 0.364737985368599 2.08834835864614e-08
#> [27766] ATAC_peak_263399 0.317386691052334 1.20088116314111e-08
#> -----
#> seqinfo: 25 sequences (1 circular) from hg38 genome
```

Now we can ask, how many DA peaks are near DE genes relative to “other” non-DE genes? A gene may appear more than once in `genes_overlap_peaks`, because multiple peaks may overlap a single gene, or because we have re-sampled the same gene more than once, or a combination of these two cases.

For each gene (that is the combination of chromosome, the start, end, and strand), and the “origin” (DE vs not-DE) we can compute the distinct number of peaks for each gene and the maximum peak based on LFC. This is achieved via `reduce_ranges_directed`, which allows an aggregation to result in a *GRanges* object via merging neighboring genomic regions. The use of the directed suffix indicates we’re maintaining strand information. In this case, we are simply merging ranges (genes) via the groups we mentioned above. We also have to account for the number of resamples we have performed when counting if there are any peaks, to ensure we do not double count the same peak:

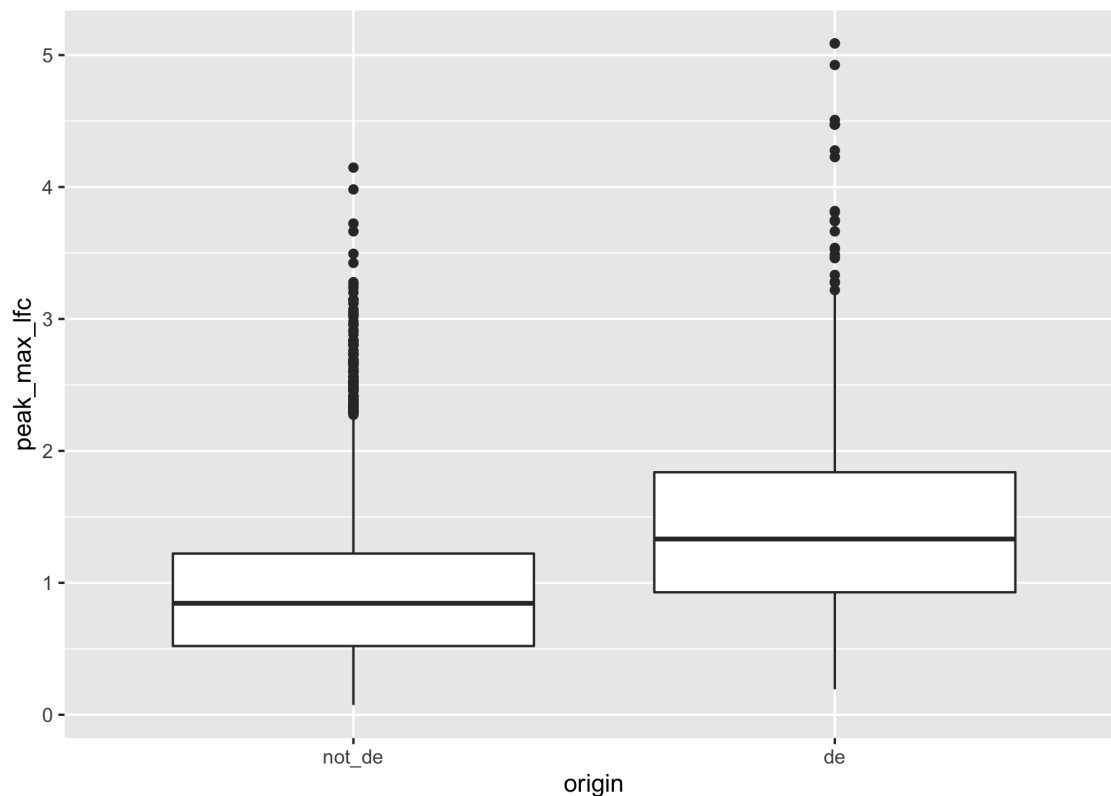


Figure 3.3: A boxplot of maximum LFCs for DA peaks for DE genes compared to non-DE genes where genes have at least one DA peak.

We can then filter genes if they have any peaks and compare the peak fold changes between non-DE and DE genes using a boxplot:

```
library(ggplot2)
gene_peak_max_lfc %>%
  filter(peak_count > 0) %>%
  as.data.frame() %>%
  ggplot(aes(origin, peak_max_lfc)) +
  geom_boxplot()
```

In general, the DE genes have larger maximum DA fold changes relative to the non-DE genes.

Next we examine how thresholds on the DA LFC modify the enrichment we observe of DA peaks near DE or non-DE genes. First, we want to know how the number of peaks within DE genes and non-DE genes change as we change threshold values on the peak

LFC. As an example, we could compute this by arbitrarily chosen LFC thresholds of 1 or 2 as follows:

```
origin_peak_lfc <- genes_olap_peaks %>%
  group_by(origin) %>%
  summarize(
    peak_count = sum(!is.na(da_padj)) / plyranges::n_distinct(resample),
    lfc1_peak_count = sum(abs(da_log2FC) > 1, na.rm=TRUE) / plyranges::n_distinct(resa
    lfc2_peak_count = sum(abs(da_log2FC) > 2, na.rm=TRUE) / plyranges::n_distinct(res
  )
origin_peak_lfc
```

```
#> DataFrame with 2 rows and 4 columns
#>   origin peak_count lfc1_peak_count lfc2_peak_count
#>   <factor> <numeric>      <numeric>      <numeric>
#> 1 not_de    2391.8        369.5          32.5
#> 2 de        3416         1097           234
```

Here we see that DE genes tend to have more DA peaks near them, and that the number of DA peaks decreases as we increase the DA LFC threshold (as expected). We now show how to compute the ratio of peak counts from DE compared to non-DE genes, so we can see how this ratio changes for various DA LFC thresholds.

For all variables except for the `origin` column we divide the first row's values by the second row, which will be the enrichment of peaks in DE genes compared to other genes. This requires us to reshape the summary table from long form back to wide form using the *tidyr* package. First we pivot the results of the `peak_count` columns into name-value pairs, then pivot again to place values into the `origin` column. Then we create a new column with the relative enrichment:

```
origin_peak_lfc %>%
  as.data.frame() %>%
  tidyr::pivot_longer(cols = -origin) %>%
```

```
tidyr::pivot_wider(names_from = origin, values_from = value) %>%  
mutate(enrichment = de / not_de)
```

```
#> # A tibble: 3 x 4  
#>   name          not_de    de enrichment  
#>   <chr>        <dbl> <dbl>      <dbl>  
#> 1 peak_count    2392.   3416        1.43  
#> 2 lfc1_peak_count 370.   1097        2.97  
#> 3 lfc2_peak_count  32.5    234        7.2
```

The above table shows that relative enrichment increases for a larger LFC threshold.

Due to the one-to-many mappings of genes to peaks, it is unknown if we have the same number of DE genes participating or less, as we increase the threshold on the DA LFC. We can examine the number of genes with overlapping DA peaks at various thresholds by grouping and aggregating twice. First, the number of peaks that meet the thresholds are computed within each gene, origin, and resample group. Second, within the origin column, we compute the total number of peaks that meet the DA LFC threshold and the number of genes that have more than zero peaks (again averaging over the number of resamples).

```
genes_olap_peaks %>%  
  group_by(gene_id, origin, resample) %>%  
  reduce_ranges_directed(  
    lfc1 = sum(abs(da_log2FC) > 1, na.rm=TRUE),  
    lfc2 = sum(abs(da_log2FC) > 2, na.rm=TRUE)  
  ) %>%  
  group_by(origin) %>%  
  summarize(  
    lfc1_gene_count = sum(lfc1 > 0) / plyranges::n_distinct(resample),  
    lfc1_peak_count = sum(lfc1) / plyranges::n_distinct(resample),  
    lfc2_gene_count = sum(lfc2 > 0) / plyranges::n_distinct(resample),
```



```
lfc2_peak_count = sum(lfc2) / plyranges::n_distinct(resample)
)
```

```
#> DataFrame with 2 rows and 5 columns
```

```
#>   origin lfc1_gene_count lfc1_peak_count lfc2_gene_count lfc2_peak_count
#>   <factor>      <numeric>      <numeric>      <numeric>      <numeric>
#> 1  not_de          271.2          369.5          30.3          32.5
#> 2    de           515          1097          151          234
```

To do this for many thresholds is cumbersome and would create a lot of duplicate code. Instead we create a single function called `count_above_threshold` that accepts a variable and a vector of thresholds, and computes the sum of the absolute value of the variable for each element in the thresholds vector.

```
count_if_above_threshold <- function(var, thresholds) {
  lapply(thresholds, function(.) sum(abs(var) > ., na.rm = TRUE))
}
```

The above function will compute the counts for any arbitrary threshold, so we can apply it over possible LFC thresholds of interest. We choose a grid of one hundred thresholds based on the range of absolute LFC values in the `da_peaks` *GRanges* object:

```
thresholds <- da_peaks %>%
  mutate(abs_lfc = abs(da_log2FC)) %>%
  with(
    seq(min(abs_lfc), max(abs_lfc), length.out = 100)
  )
```

The peak counts for each threshold are computed as a new list-column called `value`. First, the *GRanges* object has been grouped by the gene, origin, and the number of resamples columns. Then we aggregate over those columns, so each row will contain the peak counts for all of the thresholds for a gene, origin, and resample. We also maintain another list-column that contains the threshold values.

```

genes_peak_all_thresholds <- genes_olap_peaks %>%
  group_by(gene_id, origin, resample) %>%
  reduce_ranges_directed(
    value = count_if_above_threshold(da_log2FC, thresholds),
    threshold = list(thresholds)
  )
genes_peak_all_thresholds

```

```
#> GRanges object with 8239 ranges and 5 metadata columns:
```

```

#>           seqnames           ranges strand |           gene_id   origin
#>           <Rle>           <IRanges> <Rle> |           <character> <factor>
#> [1]      chr1 196641878-196661877      + | ENSG00000000971.15      de
#> [2]      chr6  46119993-46139992      + | ENSG00000001561.6      de
#> [3]      chr4  17567192-17587191      + | ENSG00000002549.12      de
#> [4]      chr7 150790403-150810402      + | ENSG00000002933.8      de
#> [5]      chr4  15768275-15788274      + | ENSG00000004468.12      de
#> ...      ...      ...      ...      ...      ...
#> [8235] chr17 43569620-43589619      - | ENSG00000175832.12 not_de
#> [8236] chr17 18250534-18270533      + | ENSG00000177427.12 not_de
#> [8237] chr20 63885182-63905181      + | ENSG00000101152.10 not_de
#> [8238] chr1 39071316-39091315      + | ENSG00000127603.25 not_de
#> [8239] chr8 41567187-41587186      + | ENSG00000158669.11 not_de
#>           resample           value
#>           <integer> <IntegerList>
#> [1]           0      1,1,1,...
#> [2]           0      1,1,1,...
#> [3]           0      6,6,6,...
#> [4]           0      4,4,4,...
#> [5]           0     11,11,11,...
#> ...      ...      ...
#> [8235]        10      1,1,1,...

```

```
#> [8236]      10      3,3,2,...
#> [8237]      10      5,5,5,...
#> [8238]      10      3,3,3,...
#> [8239]      10      3,3,3,...
#>                                     threshold
#>                                     <NumericList>
#> [1] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [2] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [3] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [4] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [5] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> ...                                     ...
#> [8235] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [8236] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [8237] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [8238] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> [8239] 0.0658243106359027,0.118483961449043,0.171143612262182,...
#> -----
#> seqinfo: 25 sequences (1 circular) from hg38 genome
```

Now we can expand these list-columns into a long *GRanges* object using the `expand_ranges()` function. This function will unlist the value and threshold columns and lengthen the resulting *GRanges* object. To compute the peak and gene counts for each threshold, we apply the same summarization as before:

```
origin_peak_all_thresholds <- genes_peak_all_thresholds %>%
  expand_ranges() %>%
  group_by(origin, threshold) %>%
  summarize(
    gene_count = sum(value > 0) / plyranges::n_distinct(resample),
    peak_count = sum(value) / plyranges::n_distinct(resample)
```

```
)  
origin_peak_all_thresholds  
  
#> DataFrame with 200 rows and 4 columns  
#>      origin      threshold gene_count peak_count  
#>   <factor>    <numeric> <numeric>  <numeric>  
#> 1    not_de 0.0658243106359027      708    2391.4  
#> 2    not_de 0.118483961449043      698.8    2320.6  
#> 3    not_de 0.171143612262182      686.2    2178.6  
#> 4    not_de 0.223803263075322      672.4    1989.4  
#> 5    not_de 0.276462913888462      650.4    1785.8  
#> ...      ...      ...      ...      ...  
#> 196      de 5.06849113788419        2        2  
#> 197      de 5.12115078869733        0        0  
#> 198      de 5.17381043951047        0        0  
#> 199      de 5.22647009032361        0        0  
#> 200      de 5.27912974113675        0        0
```

Again we can compute the relative enrichment in LFCs in the same manner as before, by pivoting the results to long form then back to wide form to compute the enrichment. We visualize the peak enrichment changes of DE genes relative to other genes as a line chart:

```
origin_threshold_counts <- origin_peak_all_thresholds %>%  
  as.data.frame() %>%  
  tidyr::pivot_longer(cols = -c(origin, threshold),  
    names_to = c("type", "var"),  
    names_sep = "_",  
    values_to = "count") %>%  
  dplyr::select(-var)  
  
origin_threshold_counts %>%
```

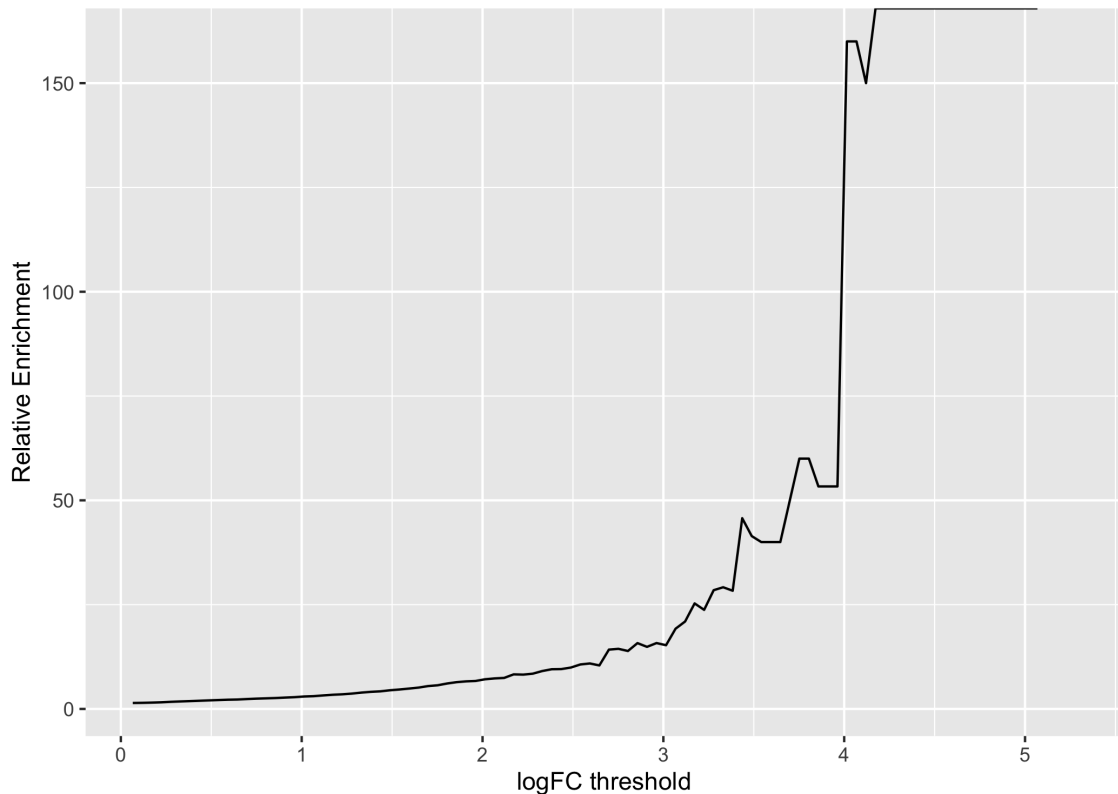


Figure 3.4: A line chart displaying how relative enrichment of DA peaks change between DE genes compared to non-DE genes as the absolute DA LFC threshold increases.

```
filter(type == "peak") %>%
tidyr::pivot_wider(names_from = origin, values_from = count) %>%
mutate(enrichment = de / not_de) %>%
ggplot(aes(x = threshold, y = enrichment)) +
geom_line() +
labs(x = "logFC threshold", y = "Relative Enrichment")
```

We computed the sum of DA peaks near the DE genes, for increasing LFC thresholds on the accessibility change. As we increased the threshold, the number of total peaks went down (likewise the mean number of DA peaks per gene). It is also likely the number of DE genes with a DA peak nearby with such a large change went down. We can investigate this with a plot that summarizes many of the aspects underlying the enrichment plot above.

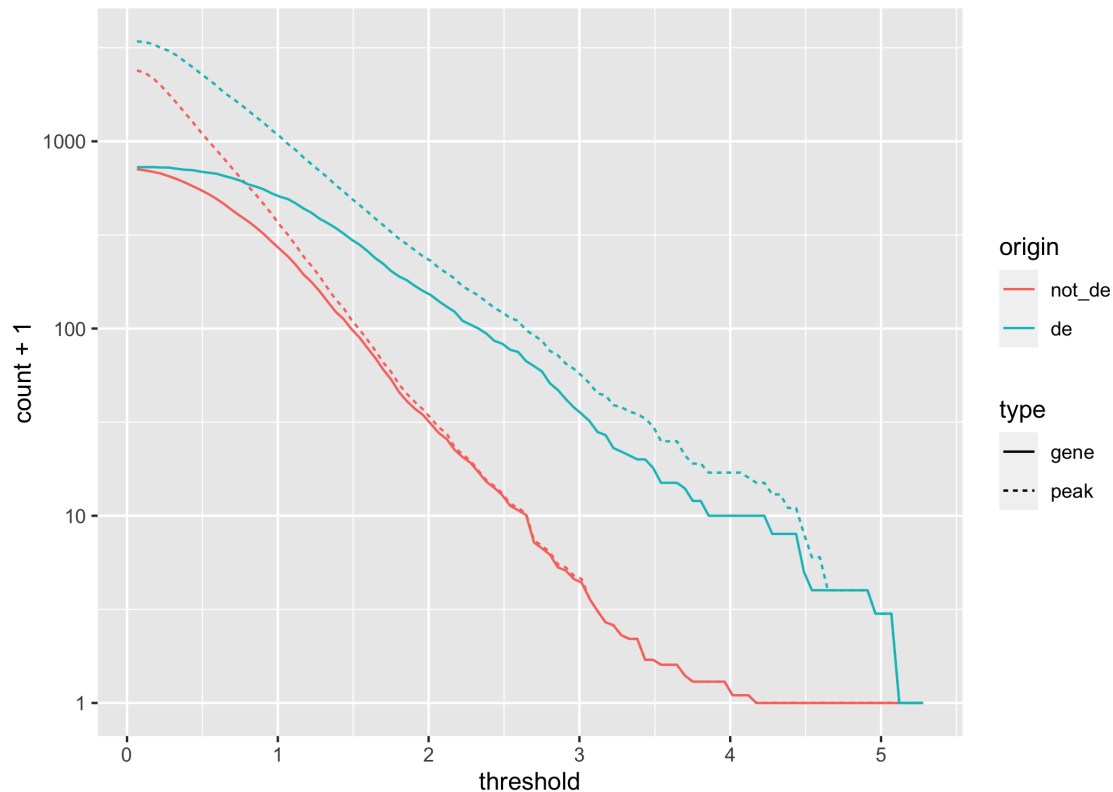


Figure 3.5: A line chart displaying how gene and peak counts change as the absolute DA LFC threshold increases. Lines are colored according to whether they represent a gene that is DE or not. Note the x-axis is on a \log_{10} scale.

```
origin_threshold_counts %>%
  ggplot(aes(x = threshold,
             y = count + 1,
             color = origin,
             linetype = type)) +
  geom_line() +
  scale_y_log10()
```

3.5 Discussion

We have shown that by using *plyranges* and *tximeta* (with support of Bioconductor and tidyverse ecosystems) we can fluently iterate through the biological data science workflow: from import, through to modeling, and data integration.

There are several further steps that would be interesting to perform in this analysis; for example, we could modify window size around the TSS to see how it affects enrichment, and vary the FDR cut-offs for both the DE gene and DA peak sets. We could also have computed variance in addition to the mean of the bootstrap set, and so drawn an interval around the enrichment line.

Finally, our workflow illustrates the benefits of using appropriate data abstractions provided by Bioconductor such as the *SummarizedExperiment* and *GRanges*. These abstractions provide users with a mental model of their experimental data and are the building blocks for constructing the modular and iterative analyses we have shown here. Consequently, we have been able to interoperate many decoupled R packages (from both Bioconductor and the tidyverse) to construct a seamless end-to-end workflow that is far too specialized for a single monolithic tool.

3.6 Software Availability

The workflow materials can be fully reproduced following the instructions found at the Github repository [sa-lee/fluentlyGenomics](https://github.com/sa-lee/fluentlyGenomics). Moreover, the development version of the workflow and all downstream dependencies can be installed using the BiocManager package by running:

```
# development version from Github
BiocManager::install("sa-lee/fluentlyGenomics")
# version available from Bioconductor
BiocManager::install("fluentlyGenomics")
```

3.7 Acknowledgements

We would like to thank all participants of the Bioconductor 2019 and BiocAsia 2019 conferences who attended and provided feedback on early versions of this workflow paper.

Chapter 4

Tidy coverage analysis uncovers intron retention

Chapter 5

Spurious embeddings from non-linear dimensionality reduction methods and how to find them

Chapter 6

Conclusion and future plans

Bibliography

- Alasoo, K, J Rodrigues, S Mukhopadhyay, A Knights, A Mann, K Kundu, HIPSCI-Consortium, C Hale, D G, and D Gaffney (2018). Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response. *Nature Genetics* **50**, 424–431.
- Alasoo, K and D Gaffney (2017). *Processed read counts from macrophage RNA-seq and ATAC-seq experiments*. <https://doi.org/10.5281/zenodo.1188300>.
- Bache, SM and H Wickham (2014). *magrittr: A Forward-Pipe Operator for R*. R package version 1.5. <https://CRAN.R-project.org/package=magrittr>.
- Barrett, T, SE Wilhite, P Ledoux, C Evangelista, IF Kim, M Tomashevsky, KA Marshall, KH Phillippy, PM Sherman, M Holko, A Yefanov, H Lee, N Zhang, CL Robertson, N Serova, S Davis, and A Soboleva (2013). NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research* **41**(D1), D991–D995. eprint: [/oup/backfile/content_public/journal/nar/41/d1/10.1093/nar/gks1193/2/gks1193.pdf](http://oup/backfile/content_public/journal/nar/41/d1/10.1093/nar/gks1193/2/gks1193.pdf).
- Dale, RK, BS Pedersen, and AR Quinlan (2011). Pybedtools: a flexible Python library for manipulating genomic datasets and annotations. *en. Bioinformatics* **27**(24), 3423–3424.
- Frankish, A, GENCODE-consortium, and P Flicek (2018). GENCODE reference annotation for the human and mouse genomes. *Nucleic Acids Research*.
- Green, TRG and M Petre (1996). Usability Analysis of Visual Programming Environments: A ‘Cognitive Dimensions’ Framework. *Journal of Visual Languages & Computing* **7**(2), 131–174.
- Hansen, KD, RA Irizarry, and Z Wu (2012). Removing technical variability in RNA-seq data using condition quantile normalization. *Biostatistics* **13** (2), 204–16.

- Henry, L and H Wickham (2017). *rlang: Functions for Base Types and Core R and 'Tidyverse' Features*. <http://rlang.tidyverse.org>, <https://github.com/r-lib/rlang>. <http://rlang.tidyverse.org>.
- Huber, W, VJ Carey, R Gentleman, S Anders, M Carlson, BS Carvalho, HC Bravo, S Davis, L Gatto, T Girke, R Gottardo, F Hahne, KD Hansen, RA Irizarry, M Lawrence, MI Love, J MacDonald, V Obenchain, AK Oleś, H Pagès, A Reyes, P Shannon, GK Smyth, D Tenenbaum, L Waldron, and M Morgan (2015a). Orchestrating high-throughput genomic analysis with Bioconductor. en. *Nat. Methods* **12**(2), 115–121.
- Huber, W, VJ Carey, R Gentleman, S Anders, M Carlson, BS Carvalho, HC Bravo, S Davis, L Gatto, T Girke, R Gottardo, F Hahne, KD Hansen, RA Irizarry, M Lawrence, MI Love, J MacDonald, V Obenchain, AK Oleś, H Pagès, A Reyes, P Shannon, GK Smyth, D Tenenbaum, L Waldron, and M Morgan (2015b). Orchestrating high-throughput genomic analysis with Bioconductor. en. *Nature Methods* **12**(2), 115–121.
- Kaitoua, A, Pinoli, P, Bertoni, M, and Ceri, S (2017). Framework for Supporting Genomic Operations. *IEEE Trans. Comput.* **66**(3), 443–457.
- Köster, J and S Rahmann (2012). Snakemake—a scalable bioinformatics workflow engine. *Bioinformatics* **28**(19), 2520–2522.
- Kozanitis, Christos, Heiberg, Andrew, Varghese, George, and Bafna, Vineet (2014). Using Genome Query Language to uncover genetic variation. en. *Bioinformatics* **30**(1), 1–8.
- Kozanitis, C and DA Patterson (2016). GenAp: a distributed SQL interface for genomic data. en. *BMC Bioinformatics* **17**, 63.
- Law, CW, M Alhamdoosh, S Su, X Dong, L Tian, GK Smyth, and ME Ritchie (2018). RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. en. *F1000 Research* **5**(1408), 1408.
- Lawrence, M, W Huber, H Pagès, P Aboyoun, M Carlson, R Gentleman, M Morgan, and V Carey (2013a). Software for Computing and Annotating Genomic Ranges. *PLoS Comput. Biol.* **9**.
- Lawrence, M, W Huber, H Pagès, P Aboyoun, M Carlson, R Gentleman, M Morgan, and V Carey (2013b). Software for Computing and Annotating Genomic Ranges. *PLoS Comput. Biol.* **9**.

- Lee, S, D Cook, and M Lawrence (2019). plyranges: a grammar of genomic data transformation. *Genome Biology* **20**(1), 4.
- Lee, S, M Lawrence, and D Cook (2018). *plyranges: a grammar of genomic data transformation*. <https://doi.org/10.5281/zenodo.1469841>.
- Love, MI, S Anders, V Kim, and W Huber (2016). RNA-Seq workflow: gene-level exploratory analysis and differential expression. en. *F1000 Research* **4**(1070), 1070.
- Love, MI, W Huber, and S Anders (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15** (12), 550.
- Love, MI, C Soneson, PF Hickey, LK Johnson, N Tessa Pierce, L Shepherd, M Morgan, and R Patro (2019). Tximeta: reference sequence checksums for provenance identification in RNA-seq. en. *bioRxiv*, 777888.
- Morgan, M (2017). *AnnotationHub: Client to access AnnotationHub resources*. R package version 2.13.1.
- Pagès, H, P Aboyoun, R Gentleman, and S DebRoy (2018). *Biostrings: Efficient manipulation of biological strings*. R package version 2.49.0.
- Patro, R, G Duggal, M Love, R Irizarry, and C Kingsford (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* **14**, 417–419.
- Quinlan, AR and IM Hall (2010). BEDTools: a flexible suite of utilities for comparing genomic features. en. *Bioinformatics* **26**(6), 841–842.
- R Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. <https://www.R-project.org/>.
- Rielly, KA, RM Sheridan, A Gillen, Y Yu, CG Bennett, and JR Hesselberth (2017). valr: Reproducible Genome Interval Arithmetic in R. *F1000Research*.
- Roadmap Epigenomics Consortium, A Kundaje, W Meuleman, J Ernst, M Bilenky, A Yen, A Heravi-Moussavi, P Kheradpour, Z Zhang, J Wang, MJ Ziller, V Amin, JW Whitaker, MD Schultz, LD Ward, A Sarkar, G Quon, RS Sandstrom, ML Eaton, YC Wu, AR Pfenning, X Wang, M Claussnitzer, Y Liu, C Coarfa, RA Harris, N Shores, CB Epstein, E Gjoneska, D Leung, W Xie, RD Hawkins, R Lister, C Hong, P Gascard, AJ Mungall, R Moore, E Chuah, A Tam, TK Canfield, RS Hansen, R Kaul, PJ Sabo, MS Bansal, A Carles, JR Dixon, KH Farh, S Feizi, R Karlic, AR Kim, A Kulkarni, D Li, R Lowdon, G Elliott, TR Mercer, SJ Neph, V Onuchic, P Polak, N Rajagopal, P Ray,

- RC Sallari, KT Siebenthall, NA Sinnott-Armstrong, M Stevens, RE Thurman, J Wu, B Zhang, X Zhou, AE Beaudet, LA Boyer, PL De Jager, PJ Farnham, SJ Fisher, D Haussler, SJM Jones, W Li, MA Marra, MT McManus, S Sunyaev, JA Thomson, TD Tlsty, LH Tsai, W Wang, RA Waterland, MQ Zhang, LH Chadwick, BE Bernstein, JF Costello, JR Ecker, M Hirst, A Meissner, A Milosavljevic, B Ren, JA Stamatoyannopoulos, T Wang, and M Kellis (2015). Integrative analysis of 111 reference human epigenomes. *Nature* **518**(7539). NCBI GEO accession numbers GSM433167 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM433167> and GPL18952 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL18952>.
- Shepherd, L and M Morgan (2019). *BiocFileCache: Manage Files Across Sessions*. R package version 1.10.2.
- Smyth, GK (2004). Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. *Statistical Applications in Genetics and Molecular Biology* **3**(1).
- Soneson, C, MI Love, and M Robinson (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research* **4** (1521).
- Wickham, Hadley (2016). *ggplot2: Elegant Graphics for Data Analysis*. en. Use R! Springer International Publishing.
- Wickham, H (2014). Tidy Data. *Journal of Statistical Software, Articles* **59**(10), 1–23.
- Wickham, H, M Averick, J Bryan, W Chang, LD McGowan, R François, G Grolemund, A Hayes, L Henry, J Hester, M Kuhn, TL Pedersen, E Miller, SM Bache, K Müller, J Ooms, D Robinson, DP Seidel, V Spinu, K Takahashi, D Vaughan, C Wilke, K Woo, and H Yutani (2019). Welcome to the tidyverse. *Journal of Open Source Software* **4**(43), 1686.
- Wickham, H, R Francois, L Henry, and K Müller (2017). *dplyr: A Grammar of Data Manipulation*. R package version 0.7.4. <https://CRAN.R-project.org/package=dplyr>.