plyranges: a grammar for transforming genomics data

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Abstract

The Bioconductor project has created many useful data abstractions for analysing high-throughput genomics experiments. However, there is a cognitive load placed on a user in learning a data abstraction and understanding its appropriate use. Throughout a standard workflow, a user must navigate and know many of these abstractions to perform an genomic analysis task, when a single data abstraction, a GRanges object will suffice. The GRanges class naturally represent genomic intervals and their associated measurements. By recognising that the GRanges class follows 'tidy' data principles we have created a grammar of genomic data transformation. The grammar defines verbs for performing actions on and between genomic interval data. It provides a principled way of performing common genomic data analysis tasks through a coherent interface to existing Bioconductor infrastructure, resulting in human readable analysis workflows. We have implemented this grammar as a Bioconductor/R package called plyranges.

Introduction

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 an SQL-like syntax for fast retrieval of information of unprocessed sequencing data [1]; [2]. Similarly, the Genometric Query Language (GMQL) implements a relational algebra for combining genomic datasets [3]. The command line application BEDtools develops an

extensive algebra for performing arithmetic between two or more sets of genomic regions [4]. 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` [5–7] define a DSL for analysing 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 [8,9], 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 [10]. 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 [11] defines an API that maps notions from the general relational algebra to operations on tidy data. It expresses each operation as a cohesive, endomorphic verb. 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.

Genomic relational algebra

Data model

The `plyranges` DSL is built on the core Bioconductor data structure GRanges, which is capable of representing all types of genomic data at a semantic level that roughly matches the intuition of most users. A GRanges is essentially a table, with 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 1).

By definition GRanges follows the 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 about that observation. Hence, we have designed the `plyranges` DSL to extend the grammar and design principles of `dplyr`: cohesion,

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

Table 1. Overview of the `plyranges` grammar. The core verbs are briefly described and categorised into one of: aggregation, unary or binary arithmetic, merging, modifier, or restriction. A verb is given bold text if its origin is from the `dplyr` grammar.

Fig 1. An illustration of the GRanges data model for a sample from an RNA-seq experiment. The core components of the data model inlcude a seqnames 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 (GC score and count).

consistency, endomorphism, and fluency. All of these principles are defined and discussed below in the context of the GRanges class and an overview of the grammar is provided in table 1. Where applicable we contrast our design to the existing Bioconductor infrastructure.

Algebraic operations

The `plyranges` DSL defines an expressive algebra for performing genomic operations with and between GRanges objects (see table 1). The grammar includes several classes of operation that cover most use cases in genomics data analysis. There are operators for performing arithmetic such as modifying width or performing set operations, merging such as finding overlaps between GRanges, subsetting by genomic regions, and aggregating over columns within in a GRanges object.

GRanges describes the within-sequence coordinates of a range by its *start*, *end* and *width*. Those three variables are mutually dependent and partially redundant, so direct 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. The parallel operations map to infix operators, which we surround with % symbols in accordance with R syntax rules. For example, the parallel

intersection (*x %intersect% y*) finds the intersecting range between *xi* and *yi* for *i* in *1...n*, where *n* is the length of both *x* and *y*. In constrast, the merging intersection (*intersect_ranges(x, y)*) returns a new set of disjoint ranges representing whereever there was overlap between a range in *x* and a range in *y*. We use the infix syntax for the parallel operations, since it is the conventional syntax for parallel, binary operations in R. 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 the arithmetic, restriction and aggregation expressions.

Our algebra recasts finding overlaps or nearest neighbours between two genomic regions as variants of the relational join operator. A join acts on two GRanges objects, a query and a subject. The join operator is relational in the sense that metadata from the query and subject ranges is 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*. 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 Cobb's relational algebra: it performs an overlap inner join but also returns all query ranges that are not hit by the subject.

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 within GRanges object using the *group_by* modifier. Together with our algebra for arithmetic and merging, these operations enable flexible and reproducible analysis using the desgin principles of the `dplyr` grammar.

Cohesion

A function is cohesive if it performs a singular task and does not produce any side-effects. In the `plyranges` DSL our algebra for performing genomic arithmetic is a key example of cohesion. We define anchoring operators that decorate a GRanges object with an 'anchor' and modify the semantics of performing genomic arithmetic. The anchoring operators amount to fixing a GRanges object by its start, center, or end coordinates (or fixing these coordinates by strand). This enables any arithmetic function that performs a coordinate transformation to remain cohesive: that is they always perform the same operation regardless of whether a GRanges object is anchored or not. The only difference is the result of performing the arithmetic changes when contextual information is added to the GRanges object.

Another example of our algebra altering object semantics, while maintaining cohesion is through the 'group_by' operator. Like anchoring, this operator decorates a GRanges object with a column name (or names) that defines a partitioning of the GRanges by the unique values in the column(s). Functions defined in the `plyranges` DSL that perform restriction, aggregation, or column modification still perform those singular tasks, however grouping changes how those tasks are performed.

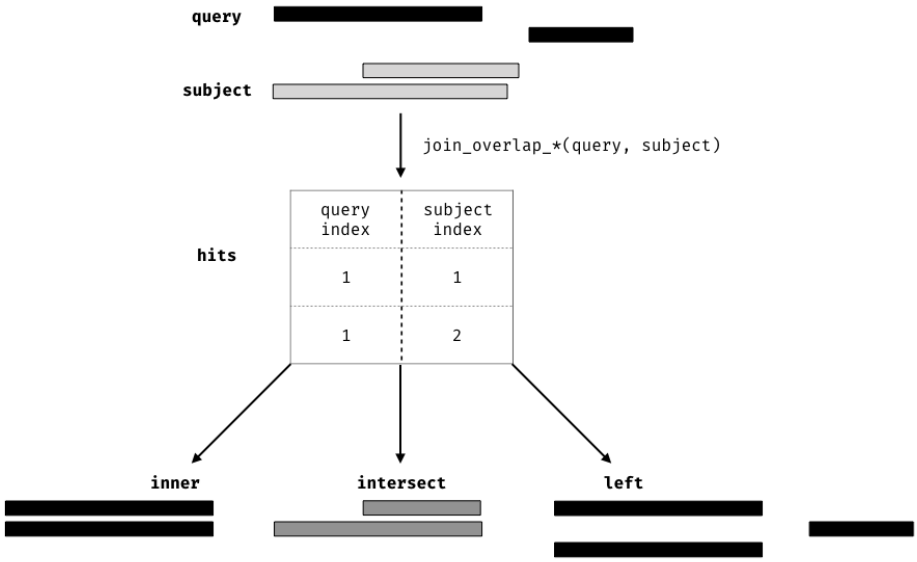

Fig 2. Illustration of the three overlap join operators. Each join takes query and subject range as input (black and light gray rectangles, respectively). An index for the join is computed, returning a Hits object, which contains the indices of where the subject overlaps the query range. This index is used to expand the query ranges by where it was 'hit' by the subject ranges. The join semantics alter what is returned: for an *inner* join the query range is returned for each match, for an intersect *join* the intersection is taken between overlapping ranges, and for a *left* join all query ranges are returned even if the subject range does not overlap them. This principle is gnerally applied through the 'plyranges' DSL for both overlaps and nearest neighbour operations.

Consistency

A core design principle of the `plyranges` DSL is interface consistency: a user should not be surprised by the input or output of `plyranges` code. A key example of consistency is how `plyranges` handles strand information. Every 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. This strongly differs from the Bioconductor packages, which produces surprising output by assuming the user is always interested in using strand in the majority of circumstances (unless a user is computing coverage or finding flanking ranges).

Our use of suffixes in function names, also highlights are core tenet of the `plyranges` design: avoid complex generic functions with many arguments and instead use cohesive functions with a minimal number of arguments. As an example, we have written a consistent framework for reading and writing files from and to common genomic data formats as GRanges, using the `rtracklayer` package as a back-end [12]. We have replaced that packages generic functions for importing files with a family of reader functions that all take the exact same arguments.

Endomorphism

Most function calls in `plyranges` are endomorphisms: when the input is GRanges object the output will also be a GRanges object. The use of endomorphism in the `plyranges` DSL enables a user to predict the structure of the output of their computations and does not require them to learn any additional classes beyond GRanges and DataFrames.

As an example, in `plyranges` when we compute coverage over a GRanges object, the result of the operation is an expanded GRanges object with a score column, corresponding the estimated coverage over a genomic region. Hence, the resulting coverage score can easily be composed with other expressions in our algebra. This pattern strongly deviates from the design of the OO interface in `GenomicRanges`, which in the case of computing coverage would return an RleList, an object that has many new methods and that is potentially unfamiliar to a new user. While, these low-level classes enable efficient computing, the increase complexity, and as a consequence are abstracted away in the `plyranges` DSL.

Fluency

As a consequence of the design principles defined above every function in `plyranges` performs a single action on GRanges objects. The `plyranges` DSL implements the core verbs from the `dplyr` package and implements a genomic relational algebra for transforming GRanges objects (table x, y). Each verb preserves the semantics of GRanges object and works with derivatives of the GRanges class. Both of these aspects reduce the cognitive load on a new user since most manipulations can be performed with a vocabulary of several verbs, rather than having to memorise function names that are nouns.

This approach strongly contrasts the `GenomicRanges/IRanges` OO interface, which emphasises the use of setter and getter methods. In that interface, core components and metadata are updated via replacement methods (requiring knowledge of the class components), while our interface requires only a single call to `mutate()` to perform the modification.

Workflows can be composed by chaining verbs together into 'sentences' via the forward pipe operator,`%>%` (exported from the R package `magrittr` [13]), which can be

read as the word 'then'. Overall, this allows users to write human readable code because workflows describe what the code is doing rather than how its doing it.

Opportunities

A caveat to constructing a compatible interface with `dplyr` is that `plyranges` makes extensive use of non-standard evaluation in R (achieved via the `rlang` package [14]). Simply, this means that computations are performed and evaluated in the context of the GRanges objects; emphasising the interactive nature of our API. Consequently, when programming with `plyranges` a user needs to be aware of how non-standard evaluation in R works and how to adapt their code accordingly. However, with the rise of R packages like `rlang` this process is becoming less difficult.

While GRanges are an intuitive representation for data measured on genomic regions, more flexible data structures are required to represent data from multiple sample experiments. The Bionconductor class SummarizedExperiment is the canonical data structure for representing data for combining multiomic measurements from multiple samples. The grammar and design of the `plryanges` DSL can be naturally extended to the SummarizedExperiment.

Results

Here we provide illustrative examples by using the `plyranges` DSL to show how our grammar could be integrated into genomic data workflows. We also highlight how interoperability with existing Bioconductor infrastructure, enables easy access to public datasets and methods for analysis and visualisation.

Peak Finding

The Bioconductor package `AnnotationHub` [15] can be used to search for BigWig files from ChIP-Seq experiments from the Human Epigenome Roadmap project [16]. Here we focus on assays for primary T CD8+ memory cells from peripheral blood. Using `plyranges` we will read the BigWig file corresponding to the H3 lysine 27 trimethylation (H3K27Me3) methylation mark over chromosome 10.

First, we gather the BigWig file and extract its annotation information and filter it to chromosome 10.

```
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. The annotation information from the file is automatically included (in this case the hg19 genome build).

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

The `reduce_ranges()` operation is used to find coverage peaks across chromosome 10. We can manually set a threshold to restrict genomic regions to have a coverage score of greater than 8, and then merge nearby regions. The maximum coverage is computed over all the coverage scores in the regions that were reduced.

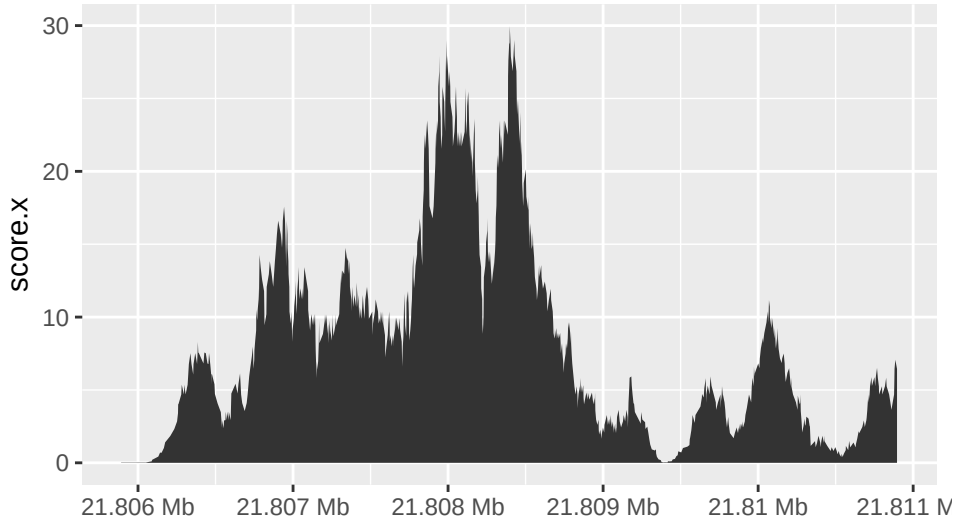

Fig 3. Visualisation of normalised coverage scores accross a 5000nt region of chromosome 10 from H3K27Me3 ChIP-Seq assay from the Human Epigenome Roadmap project.

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

Returning to the GRanges object containing normalised 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 the width.

```
chr10_max_score_region <- chr10_scores %>%
  filter(score == max(score)) %>%
  anchor_center() %>%
  mutate(width = 5000)
```

Finally, the overlap inner join is used to restrict the chromosome 10 normalised coverage scores that are within the 5000nt region that contain the max peak on chromosome 10 (figure 3).

```
peak_region <- chr10_scores %>%
  join_overlap_inner(chr10_max_score_region)
```

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. Continuing with the data from the Human Epigenome Roadmap Consortium data, we can count the number of reads that fall into a fixed bins of size 10000bp over a BAM file of H3K27Me3 methylation marks from the H1 cell line. We extract reads that have a mapping quality score greater than 20:

```
alignments <- read_bam(h1_bam) %>%
  filter(mapq > 20)
```

Note that the BAM file is only read into memory once we perform an operation on it. Because of interoperability over Bioconductor, we can generate genomic bins using the `tile` function from `GenomicRanges`:

```
bins <- tile(h1_bam, width = 10000)
```

Finally, we can use `group_by_overlaps` with `summarise` to compute the total number of reads within each window.

```
h1_bam_read_summary <- h1_bam %>%
  group_by_overlaps(bins) %>%
  summarise(n_reads = n())
```

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 (SNP) and short insertion/deletions (indel). 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 any insertion or deletion alleles and the SNPs present on the mitochondria then 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 can then compute the transition-transversion ratio using the `group_by` and `summarise` pattern, it is computed as the total number of transition SNPs divided by the total number of transversion SNPs within each chromosome (figure 4).

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

Availablilty and Future Work

The `plyranges` package is available on the Bioconductor project website `https://bioconductor.org` or can be accessed via Github `https://github.com/sa-lee/plyranges`. We aim to continue developing the `plyranges` package and extend it for use with more complex data structures such as the SummarizedExperiment class, which can be used for analysing transcriptomic and variant data. As the `plyranges` interface encourages tidy data practices it integrates well with the principles of the grammar of graphics, we aim to use it to prepare data for the visualisation of multimodal biological datasets.

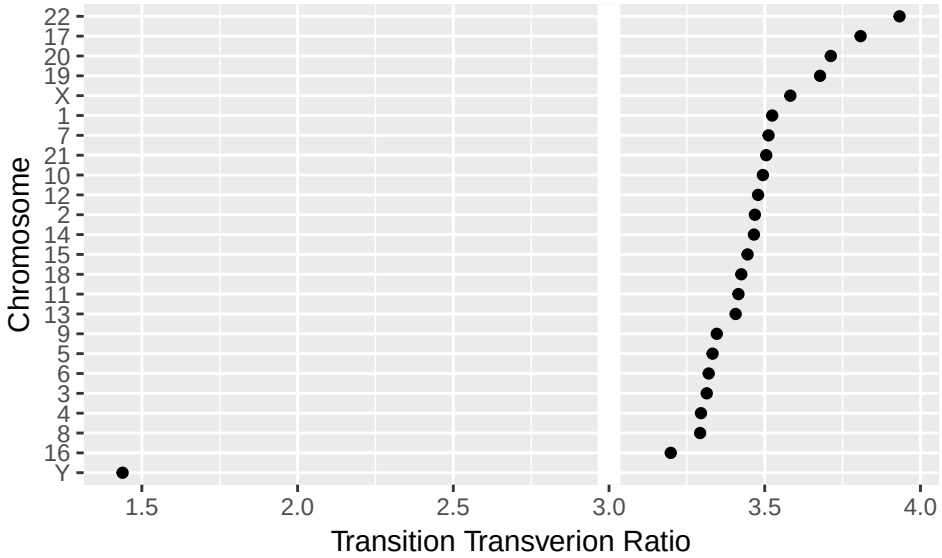

Fig 4. Dot plot of chromosomes ordered by estimated transition-transversion ratio. A white reference line is drawn at the expected ratio for a human exome.

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