Tidy Analysis of Genomic Data

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Data organization depends on purpose

Table 1

	Genotype A			Genotype B		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Drug 1	0.084	0.853	0.096	0.067	**0.367**	0.392
Drug 2	0.696	0.998	0.182	0.085	0.698	0.791
Drug 3	0.409	0.093	**0.495**	0.003	0.768	0.689
	Key:	**Potential outlier**				

"Tidy data" is organized for programming

One row per observation, one column per variable

```
head(dat)
```

```
## # A tibble: 6 x 5
##   drug  genotype   rep outlier  value
##   <fct> <chr>    <dbl> <lgl>    <dbl>
## 1 1     a            1 FALSE   0.750
## 2 1     a            2 FALSE   0.885
## 3 1     a            3 FALSE   0.982
## 4 2     a            1 FALSE   0.0243
## 5 2     a            2 FALSE   0.417
## 6 2     a            3 FALSE   0.701
```

The pipe

```
command | command | command > output.txt
```

> *"Pipes rank alongside the hierarchical file system and regular expressions as one of the most powerful yet elegant features of Unix-like operating systems."*

http://www.linfo.org/pipe.html

In R we use `'%>%'` instead of `'|'` to chain operations.

Verb-based operations

In the R package *dplyr*:

- `mutate()` adds new variables that are functions of existing variables.
- `select()` picks variables based on their names.
- `filter()` picks cases based on their values.
- `slice()` picks cases based on their position.
- `summarize()` reduces multiple values down to a single summary.
- `arrange()` changes the ordering of the rows.

https://dplyr.tidyverse.org/

Summarize after grouping

A useful paradigm is to *group* data and then *summarize*:

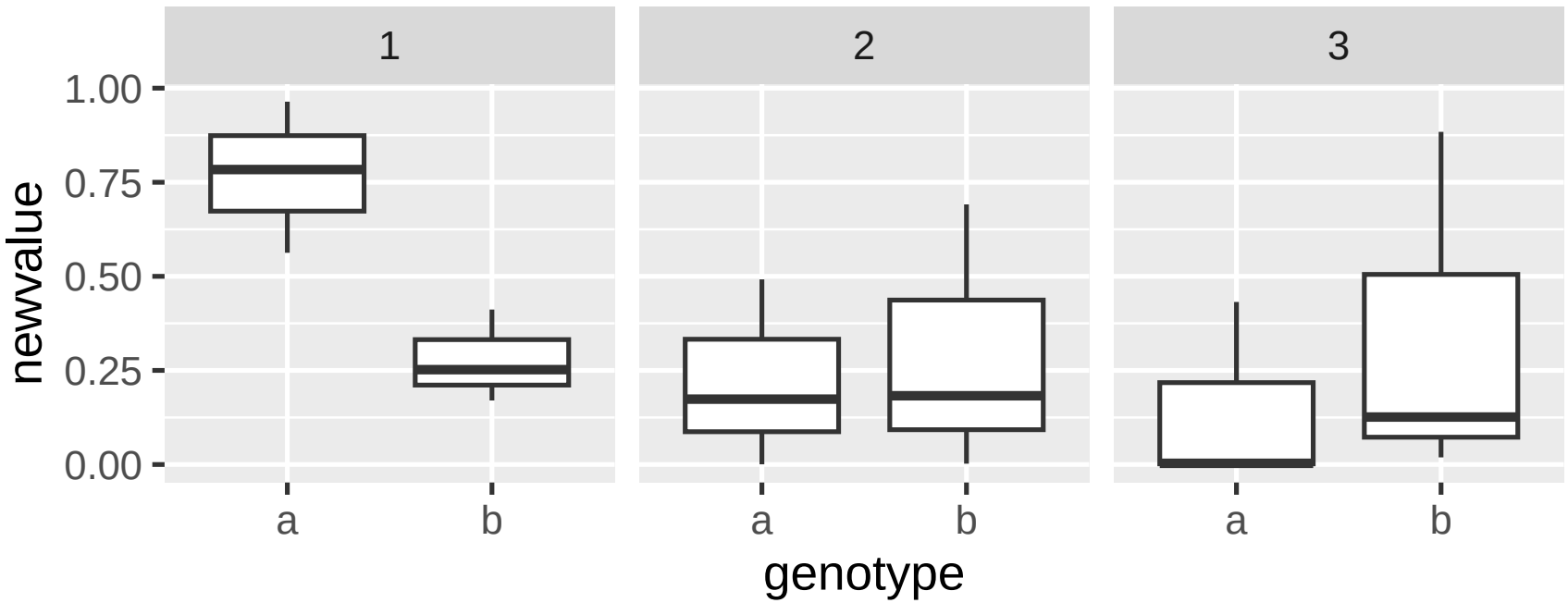
```
dat %>%
  filter(!outlier) %>%
  group_by(drug, genotype) %>%
  summarize(mu_hat = mean(value))
```

Summarized output

```
## # A tibble: 6 x 3
## # Groups:   drug [3]
##   drug  genotype mu_est
##   <fct> <chr>     <dbl>
## 1 1     a         0.872
## 2 1     b         0.457
## 3 2     a         0.381
## 4 2     b         0.435
## 5 3     a         0.0383
## 6 3     b         0.478
```

Piping into plots

```
dat %>%
  mutate(newvalue = value^2) %>%
  ggplot(aes(genotype, newvalue)) +
  geom_boxplot() +
  facet_wrap(~drug)
```



Genomic range data is often already tidy

chr1	100122271	100122495	Peak_75319	65	.	4.24709	6.53
chr1	100148962	100149149	Peak_47035	78	.	5.42118	7.87
chr1	10035625	10035783	Peak_83599	60	.	4.24908	6.01
chr1	10113652	10114012	Peak_22696	102	.	5.88792	10.2
chr1	10165234	10165473	Peak_61426	70	.	4.89948	7.04
chr1	10166426	10166654	Peak_52303	75	.	4.05875	7.56
chr1	10166709	10167142	Peak_101485	56	.	4.29447	5.62
chr1	10228978	10229286	Peak_56552	73	.	4.40606	7.37
chr1	10233774	10233984	Peak_54437	74	.	4.78393	7.43
chr1	10257595	10257832	Peak_144324	43	.	3.23111	4.35
chr1	10300983	10301435	Peak_55477	74	.	4.26907	7.41
chr1	10485619	10485897	Peak_128866	48	.	3.79116	4.85
chr1	10486926	10487197	Peak_64148	68	.	4.92835	6.83
chr1	105184501	105185026	Peak_98454	56	.	4.04794	5.69
chr1	105199317	105199602	Peak_117608	49	.	3.59369	4.96
chr1	105310436	105310779	Peak_23716	100	.	5.55389	10.0
chr1	105312808	105313002	Peak_104599	54	.	3.38229	5.46
chr1	105367824	105367998	Peak_12375	123	.	7.39252	12.3

Practical considerations

- Many comp students are already familiar with dplyr/ggplot2, so helps with onboarding
- Piping can help to avoid hard-to-read variable names, e.g.:

```
dat3 <- dat2[dat2$signal > x]
```

- It's not necessarily *less code*, but aims for readable code

Why consider "tidy analysis" paradigm for genomics?

- Encourages exploratory analysis, vs. "all-in-one" functions for performing summarization or enrichment analysis
- Encourages efficiency: fewer calls to C code
- Generalizes from simple to complex cases

Bringing range data into R

ENCODE mouse embryonic fibroblast, H3k4me1:

```
library(plyranges)
pks <- read_narrowpeaks("ENCFF231UNV.bed.gz")
```

Bringing range data into R

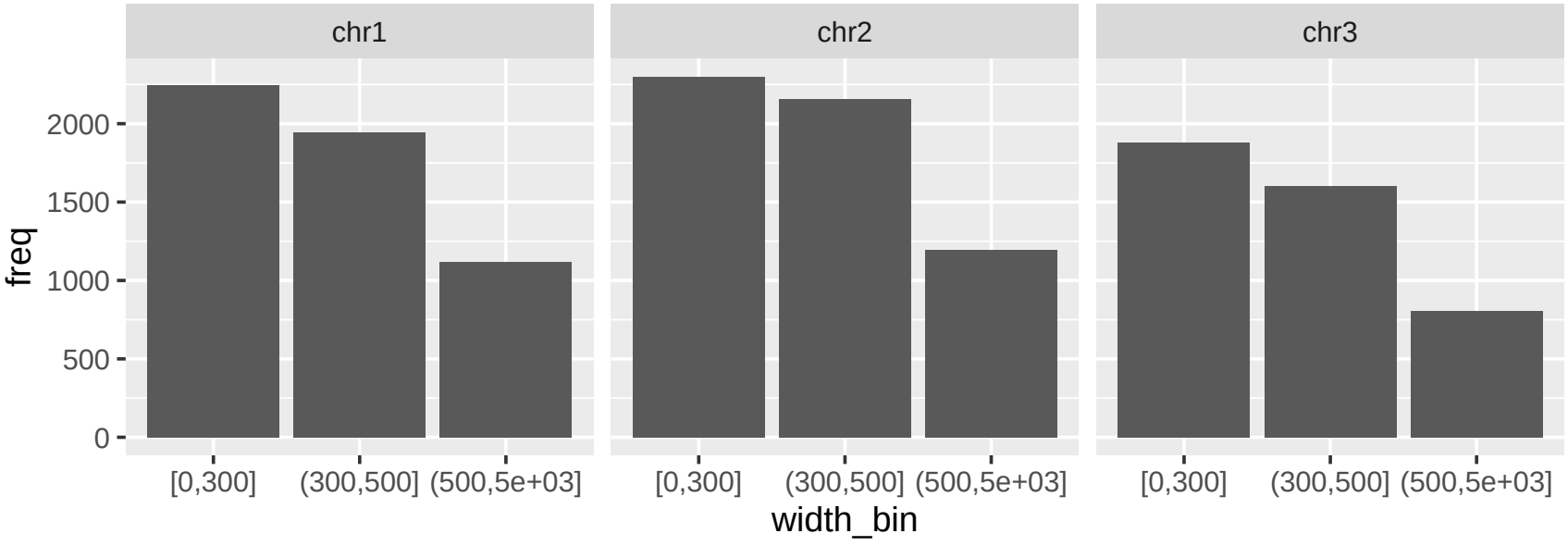
```
pks
```

```
## GRanges object with 74284 ranges and 6 metadata columns:
##           seqnames              ranges strand |        name     score signalValue    pValue    qValue
##              <Rle>           <IRanges>  <Rle> | <character> <numeric>   <numeric> <numeric> <numeric>
##       [1]     chr1 100122272-100122495      * |  Peak_75319        65     4.24709   6.53821   4.00852
##       [2]     chr1 100148963-100149149      * |  Peak_47035        78     5.42118   7.87250   5.08650
##       [3]     chr1   10035626-10035783      * |  Peak_83599        60     4.24908   6.01848   3.55308
##       [4]     chr1   10113653-10114012      * |  Peak_22696       102     5.88792  10.26247   7.07929
##       [5]     chr1   10165235-10165473      * |  Peak_61426        70     4.89948   7.04738   4.40822
##       ...      ...                 ...    ... .         ...       ...         ...       ...       ...
##   [74280]     chrX   99530000-99530373      * |  Peak_24840       100     6.40685  10.04640   6.89683
##   [74281]     chrX   99530681-99531004      * |  Peak_84432        60     3.66625   6.01494   3.54969
##   [74282]     chrX   99550895-99551287      * |  Peak_56421        74     5.12695   7.40639   4.71603
##   [74283]     chrX   99567509-99567986      * |  Peak_91747        58     4.43250   5.84328   3.41019
##   [74284]     chrY     1116052-1116527      * |  Peak_30698        92     5.51992   9.29023   6.26238
##               peak
##           <integer>
##       [1]       126
##       [2]        90
##       [3]       134
##       [4]       138
##       [5]       107
##       ...       ...
##   [74280]       242
##   [74281]       231
##   [74282]       272
##   [74283]       399
##   [74284]       118
##   -------
##   seqinfo: 22 sequences (1 circular) from mm10 genome
```

Example operations with plyranges

```
pks %>%
  filter(seqnames %in% paste0("chr",1:3),
         qValue > 2) %>%
  mutate(width_bin = cut(width,
                         breaks=c(0,300,500,5000),
                         include.lowest=TRUE)) %>%
  group_by(width_bin, seqnames) %>%
  summarize(freq = n()) %>%
  as_tibble() %>%
  ggplot(aes(width_bin, freq)) +
  geom_col() +
  facet_wrap(~seqnames)
```

Example operations with plyranges



Making use of range information

- Suppose a query set of ranges, `gr` (here three ranges).
- The following chunk groups `pks` by the query ranges.
- Optional specification of `maxgap` and/or `minoverlap`.
- Range information for the `pks` is lost though.

```
gr %>%
  group_by_overlaps(pks) %>%
  summarize(mu=mean(score), sd=sd(score), n=n())
```

```
## DataFrame with 3 rows and 4 columns
##       query         mu         sd         n
##   <integer> <numeric> <numeric> <integer>
## 1         1  101.4521   42.9574        73
## 2         2   78.2500   27.9616        36
## 3         3   95.6818   49.8114        22
```

Picking out a representative

```
gr %>%
  group_by_overlaps(pks) %>%
  slice(which.max(score)) %>%
  ungroup() %>%
  select(score)
```

```
## GRanges object with 3 ranges and 1 metadata column:
##       seqnames              ranges strand |     score
##          <Rle>           <IRanges>  <Rle> | <numeric>
##   [1]     chr1 51000001-52000000      * |       283
##   [2]     chr1 52000001-53000000      * |       177
##   [3]     chr1 53000001-54000000      * |       265
##   -------
##   seqinfo: 22 sequences (1 circular) from mm10 genome
```

Keeping the ranges of the representative

- Join-by-overlaps is a more flexible paradigm.
- "Left" and "inner" joins differ by how missing IDs in the first table are handled.

```
pks %>%
  select(score) %>%
  join_overlap_inner(gr) %>%
  group_by(query_id) %>%
  slice(which.max(score))
```

```
## GRanges object with 3 ranges and 2 metadata columns:
## Groups: query_id [3]
##       seqnames              ranges strand |     score  query_id
##          <Rle>           <IRanges>  <Rle> | <numeric> <integer>
##   [1]     chr1 51507255-51507557      * |       283         1
##   [2]     chr1 52253831-52254329      * |       177         2
##   [3]     chr1 53757564-53757891      * |       265         3
##   -------
##   seqinfo: 22 sequences (1 circular) from mm10 genome
```

More complex cases

- The most common cases are computing summaries, overlaps
- More complex computations are possible, e.g.:
  - For peaks near genes, compute correlation of cell-type-specific accessibility and expression (Wancen Mu)
  - For regulatory variants falling in ATAC peaks, visualize their distribution stratified by SNP and peak categories (Jon Rosen)
  - For looped and unlooped enhancer-promoter pairs, compare average ATAC and RNA time series, while controlling for genomic distance and contact frequency (Eric Davis)
  - For DHS in a region of interest with particular genomic characteristics, compare overlap with functional annotation within and in comparison to matched regions from elsewhere in genome (Lexi Bounds, Pat Sullivan, *et al.*)

Going further: extracting info from fitted models

- Nest $\rightarrow$ map $\rightarrow$ unnest
- Allows model fitting within data groups, see also `glance` and `augment`

```
library(broom)
gr %>%
  group_by_overlaps(pks) %>%
  as_tibble() %>%
  select(query, score, qValue) %>%
  nest(data = -query) %>%
  mutate(fit = map(data, ~lm(score ~ qValue, data=.)),
         fitted = map(fit, ~.x$fitted)) %>%
  unnest(c(data, fitted))
```

Going further: extracting info from fitted models

```
## # A tibble: 131 x 5
##    query score qValue fit    fitted
##    <int> <dbl>  <dbl> <list>  <dbl>
##  1     1    92   6.25 <lm>     91.9
##  2     1   135   9.85 <lm>    134.
##  3     1    68   4.22 <lm>     67.9
##  4     1    75   4.84 <lm>     75.2
##  5     1    43   2.23 <lm>     44.4
##  6     1    68   4.22 <lm>     67.9
##  7     1    98   6.77 <lm>     98.0
##  8     1   100   6.90 <lm>     99.5
##  9     1    36   1.70 <lm>     38.1
## 10     1    68   4.22 <lm>     67.9
## # ... with 121 more rows
```

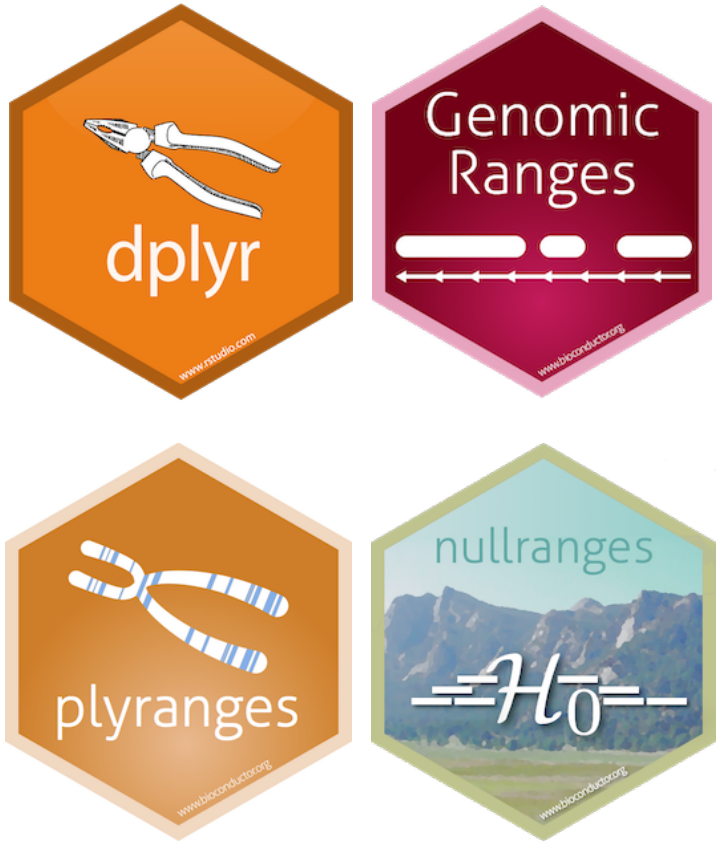
Some pointers

- TSS: `anchor_5p() %>% mutate(width=1)`
- Overlaps can specify `*_directed` or `*_within`
- Flatten/break up ranges: `reduce_ranges`, `disjoin_ranges`
- Concatenating ranges: `bind_ranges`
- Overlaps are handled often with "joins": `join_overlap_*`, `join_nearest`, `join_nearest_downstream`, etc.
- Load *plyranges* last to avoid name masking

More tutorials online

- *plyranges* vignettes (on Bioc and GitHub)
- Enrichment of peaks and genes: "Fluent Genomics" workflow
- *nullranges* vignettes (on Bioc and GitHub), which provides block bootstrap and matching functionality that pairs easily with *plyranges*
- Other examples, incl. bootstrap: "Tidy Ranges Tutorial"
- BioC2022: Wancen Mu & Eric Davis *nullranges* workshop

Summary: tidy analysis for genomic data



- Encourages exploratory analysis, vs. "all-in-one" functions
- Encourages efficiency: fewer calls to C code
- Generalizes from simple to complex cases

Reading

- Lee, S., Cook, D. & Lawrence, M. plyranges: a grammar of genomic data transformation. Genome Biology 20, 4 (2019). https://doi.org/10.1186/s13059-018-1597-8
- Lee S, Lawrence M and Love MI. Fluent genomics with plyranges and tximeta. F1000Research 2020, 9:109 https://doi.org/10.12688/f1000research.22259.1
- plyranges vignettes https://sa-lee.github.io/plyranges
- Tidy Ranges Tutorial https://nullranges.github.io/tidy-ranges-tutorial
- bootRanges, matchRanges, see: https://nullranges.github.io/nullranges

Tidy analysis for matrix data:

- Mangiola, S., Molania, R., Dong, R. et al. tidybulk: an R tidy framework for modular transcriptomic data analysis. Genome Biology 22, 42 (2021). https://doi.org/10.1186/s13059-020-02233-7
- tidySummarizedExperiment, see: https://stemangiola.github.io/tidySummarizedExperiment