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   0.795
## 2 1     a            2 FALSE   0.687
## 3 1     a            3 FALSE   0.963
## 4 2     a            1 FALSE   0.991
## 5 2     a            2 FALSE   0.209
## 6 2     a            3 FALSE   0.629
```

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.
- `group_by()` perform any operation by group.

https://dplyr.tidyverse.org/

Summarize after grouping

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

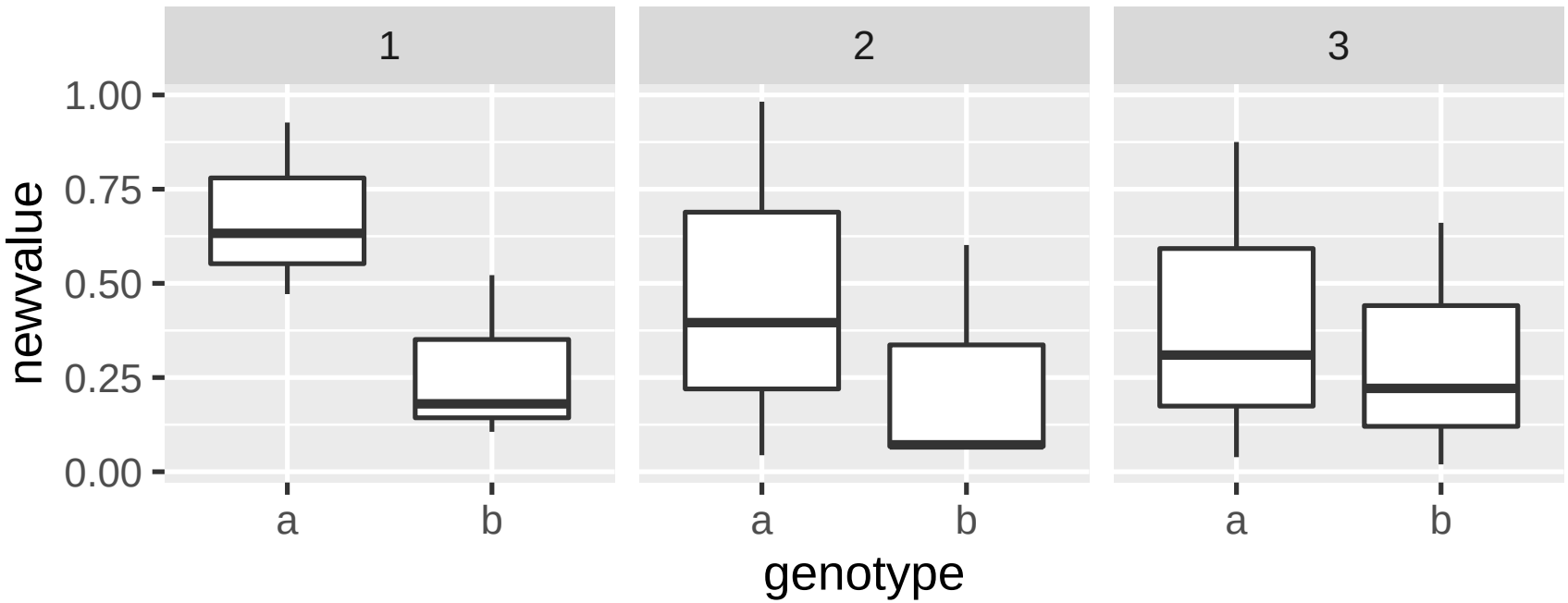
```
dat %>%
  filter(!outlier) %>%
  group_by(drug, genotype) %>%
  summarize(mu_hat = mean(value))
```

Summarized output

```
## # A tibble: 6 x 3
## # Groups:   drug [3]
##   drug  genotype mu_est
##   <fct> <chr>     <dbl>
## 1 1     a         0.815
## 2 1     b         0.573
## 3 2     a         0.610
## 4 2     b         0.431
## 5 3     a         0.566
## 6 3     b         0.474
```

Piping directly 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

Tidy advantages

- Many already familiar with dplyr and ggplot2
- Avoid intermedite variables, e.g.:

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

- Aim is for *readable* code

Why "tidy analysis" for genomics?

- Encourages exploration
- Encourages efficiency: fewer calls out of R
- Generalizes from simple to complex cases
- Developer side: modularity is easier to maintain

Bringing range data into R

ENCODE mouse embryonic fibroblast, H3k4me1:

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

Alternatively:

```
pks <- read.csv("file.csv") %>%
  rename(seqnames = chr) %>%
  as_granges()
```

Bringing range data into R

```
pks %>% slice(1:3) %>% select(signalValue)
```

```
## GRanges object with 3 ranges and 1 metadata column:
##       seqnames              ranges strand | signalValue
##          <Rle>           <IRanges>  <Rle> |   <numeric>
##   [1]     chr1 100122272-100122495      * |     4.24709
##   [2]     chr1 100148963-100149149      * |     5.42118
##   [3]     chr1   10035626-10035783      * |     4.24908
##   -------
##   seqinfo: 22 sequences (1 circular) from mm10 genome
```

Example plyranges

- For a set of query ranges, `tiles` (here three 1 Mb ranges)
- Find overlaps between `pks` and `tiles`
- Optional specification of `maxgap` and/or `minoverlap`

```
tiles
```

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

Overlaps with `join`

- Join-by-overlaps is a flexible paradigm for performing overlaps
- "Left" vs. "inner" joins: 0-matches are / aren't included

```
pks %>%
  select(score) %>% # just `score` column
  join_overlap_inner(tiles) %>% # overlap -> add cols from tiles
  group_by(tile_id) %>% # group matches by which tile`
  slice(which.max(score)) # take the top scoring peak
```

```
## GRanges object with 3 ranges and 2 metadata columns:
## Groups: tile_id [3]
##       seqnames              ranges strand |     score   tile_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
```

Counting overlaps

- Use `.` to specify self within a command
- Add number of overlaps to each entry in `tiles`:

```
tiles %>% mutate(n_overlaps = count_overlaps(., pks))
```

```
## GRanges object with 3 ranges and 2 metadata columns:
##       seqnames              ranges strand |   tile_id n_overlaps
##          <Rle>           <IRanges>  <Rle> | <integer>  <integer>
##   [1]     chr1 51000001-52000000      * |         1         73
##   [2]     chr1 52000001-53000000      * |         2         36
##   [3]     chr1 53000001-54000000      * |         3         22
##   -------
##   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 (Euphy Wu, Lexi Bounds, Pat Sullivan)

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)
pks %>%
  join_overlap_inner(tiles) %>%
  as_tibble() %>%
  select(tile_id, score, qValue) %>%
  nest(data = -tile_id) %>%
  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
##    tile_id 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
```

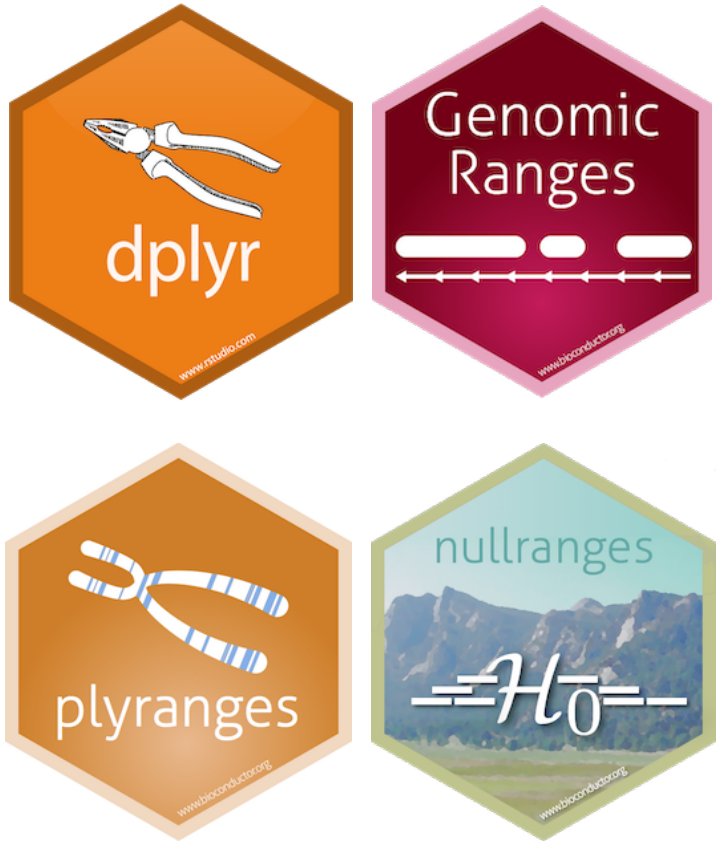
Some pointers

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

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
- See `#tidiness_in_bioc` and `#nullranges` Bioc Slack channels

Summary: tidy analysis for genomic data



nullranges development sponsored by CZI EOSS

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 sa-lee.github.io/plyranges
- Tidy Ranges Tutorial nullranges.github.io/tidy-ranges-tutorial
- *nullranges*: bootRanges, matchRanges nullranges.github.io/nullranges
- excluderanges dozmorovlab.github.io/excluderanges

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
- tidySE, tidySCE, tidyseurat stemangiola.github.io/tidytranscriptomics