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

```
## # A tibble: 6 x 5
##   drug  genotype   rep outlier value
##   <fct> <chr>    <dbl> <lgl>   <dbl>
## 1 1     a            1 FALSE   0.267
## 2 1     a            2 FALSE   0.524
## 3 1     a            3 FALSE   0.974
## 4 2     a            1 FALSE   0.786
## 5 2     a            2 FALSE   0.283
## 6 2     a            3 FALSE   0.527
```

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 %>% or |> 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*:

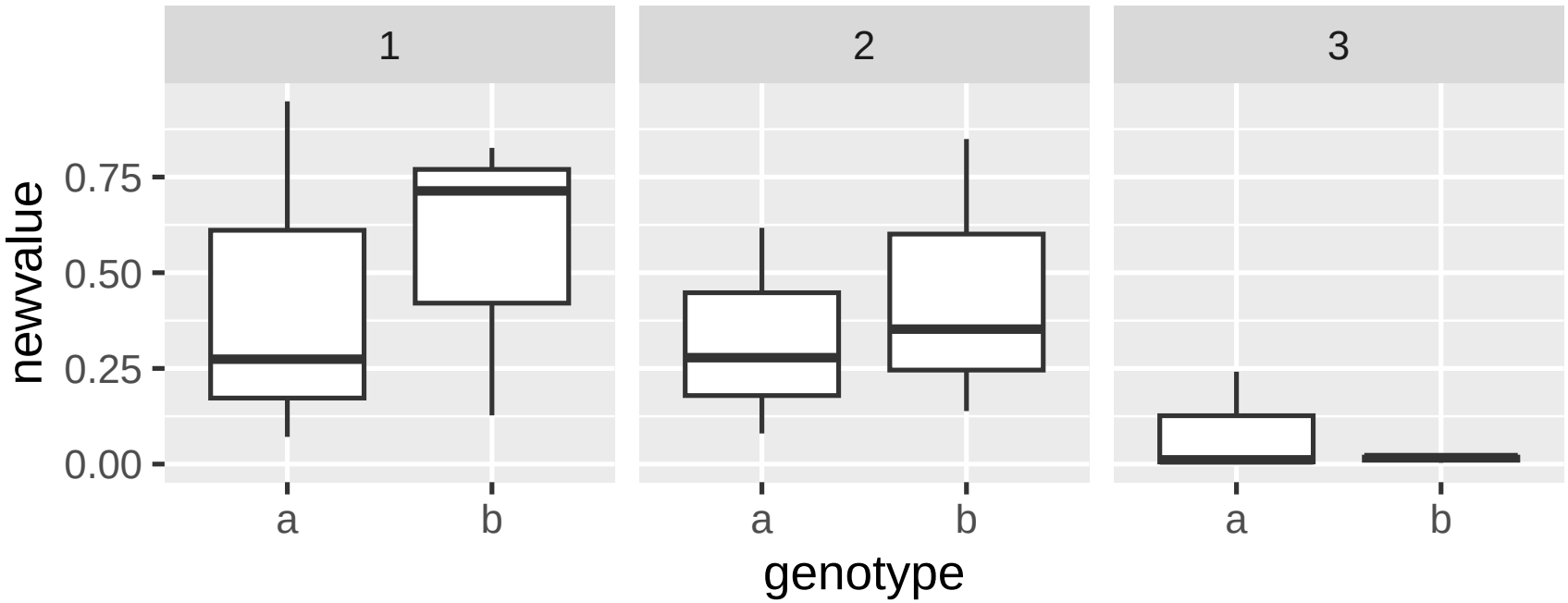
```
dat %>%
  filter(!outlier) %>%
  group_by(drug, genotype) %>%
  summarize(mu_hat = mean(value))
```

Summarized output

```
## # A tibble: 6 x 3
## # Groups:   drug [3]
##   drug  genotype mu_est
##   <fct> <chr>     <dbl>
## 1 1     a         0.588
## 2 1     b         0.877
## 3 2     a         0.532
## 4 2     b         0.629
## 5 3     a         0.252
## 6 3     b         0.110
```

Piping directly into plots facilitates data exploration

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



Summary I

- I teach both base R and "tidy"
- Both are wrappers, choose based on 1) efficiency 2) flow
- I use the former for writing software, latter for scripting
- Students know dplyr/ggplot2 already
- Next:
  - tidy for genomic ranges
  - tidy for matrix data (scRNA-seq)

Genomic range data is 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

Great packages in Bioconductor to work with ranges

- LOLA - facilitates testing overlaps, fast, useful databases
- COCOA - explore sample variation along genome
- GenomicDistributions - annotate, visualize distribution with respect to other features (genes)
- regioneR - permutation testing
- ChIPpeakAnno - facilitates downstream analysis

Going to talk now about data exploration

Exploring data with tidy syntax

Helps avoid intermediate variables, and tucks away control code

```
dat3 <- dat2[dat2$signal > 5]

# vs.

dat %>%
  filter(signal > 5)
```

This is *plyranges* from Stuart Lee, Michael Lawrence and Di Cook

Bringing range data into R

ENCODE mouse embryonic fibroblast, H3K4me1:

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

or equivalently:

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

Another common paradigm, separating single column

```
pks <- read.delim("file.tsv") %>%
  tidyr::separate_wider_delim(
    location,
    delim=":|-", # e.g. chr1:123-456
    into=c("seqnames","start","end")
  ) %>%
  as_granges()
```

Ranges are rows, metadata are columns

```
pks %>%
  slice(1:3) %>% # first 3 ranges
  select(signalValue) # just one metadata column
```

```
## 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 use of *plyranges*

- Suppose query ranges, `tiles` (e.g. ~1 Mb)
- Find all overlaps between `pks` and `tiles`
- Perform computation on the overlaps
- Many other choices in Bioc for enrichment (e.g. LOLA)

Example use of *plyranges*

Created with `tile_ranges` (see also `tileGenome`):

```
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
```

Consider genomic overlaps as a `join`

- We are joining two sources of information by match
- How would you then pick top scoring peak (`pks`) per `tile`?
- What verbs would be involved?

Consider overlaps as a `join`

```
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`:
- Can specify `maxgap` and/or `minoverlap`

```
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

- For peaks near genes, compute correlation of cell-type-specific accessibility and expression (Wancen Mu) $\rightarrow$ similar to COCOA
- For regulatory variants falling in open chromatin peaks, visualize their distribution stratified by SNP and peak categories (Jon Rosen)
- For looped and un-looped enhancer-promoter pairs, compare average ATAC and RNA time series, while controlling for genomic distance and contact frequency (Eric Davis)

Nest → map → unnest

```
library(purrr)
library(broom)
pks %>%
  join_overlap_inner(tiles) %>%
  as_tibble() %>%
  select(tile_id, signalValue, qValue) %>%
  nest(data = -tile_id) %>%
  mutate(fit = map(data,
                   ~lm(signalValue ~ qValue, data=.)
                   ),
         stats = map(fit, glance)) %>%
  unnest(stats)
```

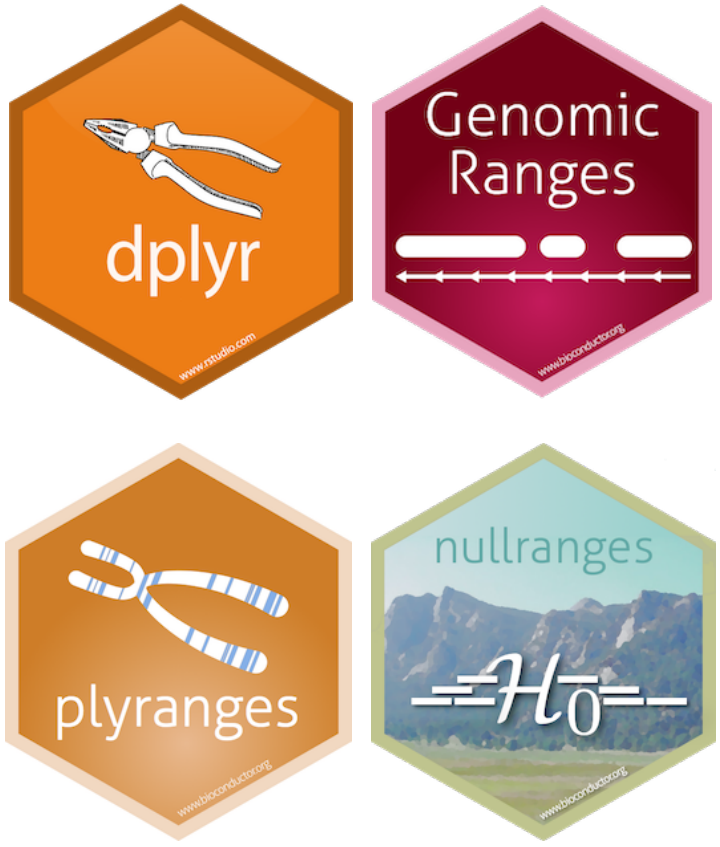
Nest $\rightarrow$ map $\rightarrow$ unnest

```
## # A tibble: 3 x 4
##   tile_id data                fit    r.squared
##     <int> <list>              <list>     <dbl>
## 1       1 <tibble [73 x 2]> <lm>       0.864
## 2       2 <tibble [36 x 2]> <lm>       0.797
## 3       3 <tibble [22 x 2]> <lm>       0.954
```

More *plyranges*-based tutorials online

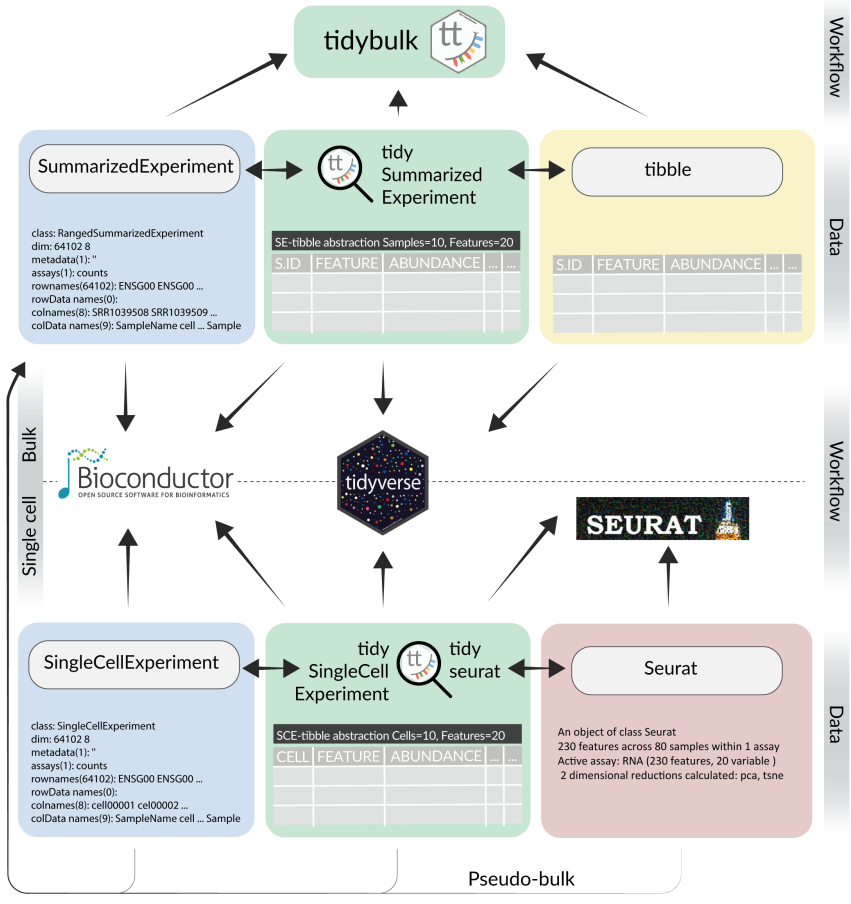
- *plyranges* vignettes (on Bioc and GitHub)
- Enrichment of peaks and genes: "Fluent Genomics" workflow
- Null regions: *nullranges* vignettes (on Bioc and GitHub)
- Other examples, incl. bootstrap: "Tidy Ranges Tutorial"
- `#tidiness_in_bioc` and `#nullranges` Slack channels

Summary: tidy analysis for genomic range data



nullranges development sponsored by CZI EOSS

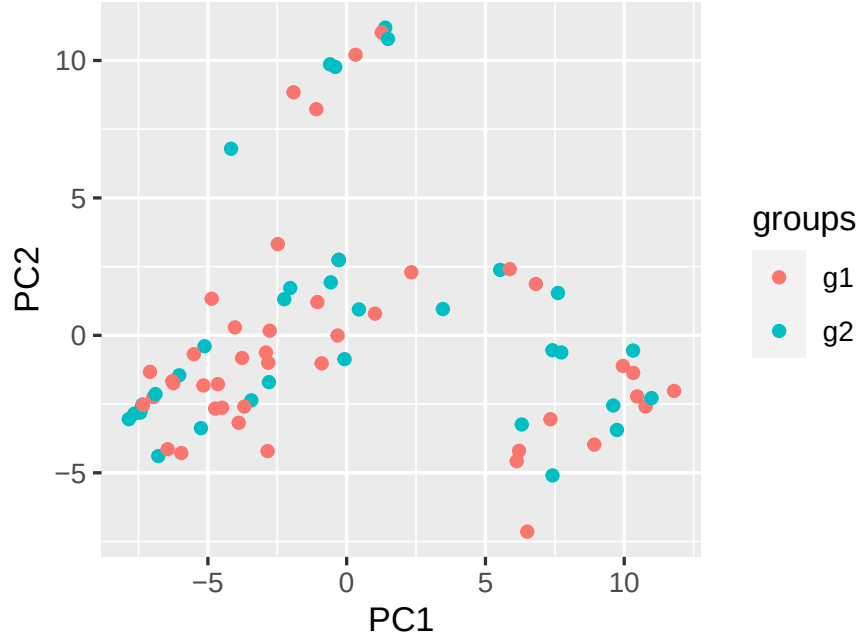
Tidy analysis of matrix data



tidy-* from Stefano Mangiola (WEHI) *et al.*

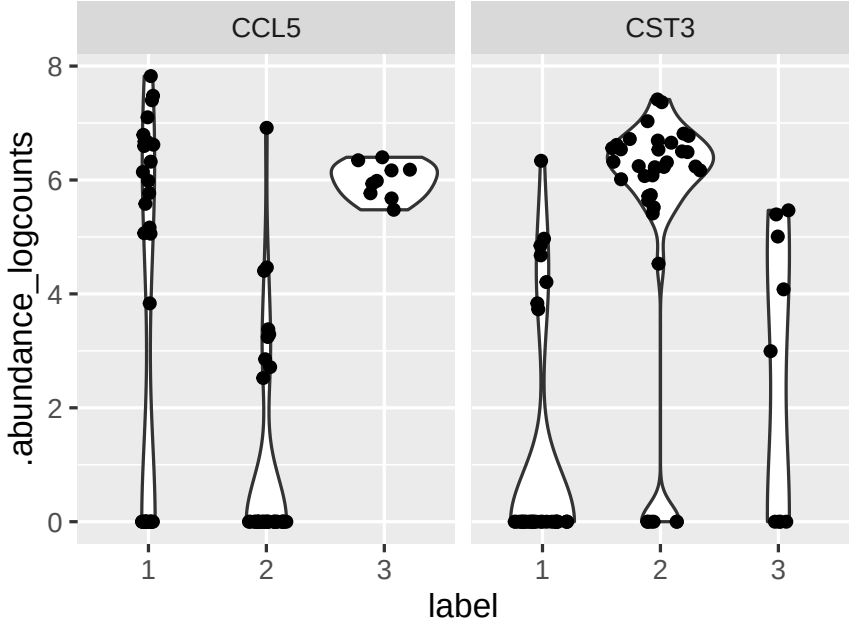
Example use of tidySingleCellExperiment

```
sce %>%
  scater::runPCA(ncomp=2, subset_row=var_genes) %>%
  ggplot(aes(PC1, PC2, color=groups)) +
  geom_point()
```



Example use of tidySingleCellExperiment

```
sce %>%
  join_features(c("CCL5","CST3")) %>%
  ggplot(aes(label, .abundance_logcounts)) +
  geom_violin() +
  geom_sina() +
  facet_wrap(~.feature)
```



More complex cases

- Join extra cell-level data
- Perform nested analyses per cell population
- Create a custom expression signature from subset of genes
- Find genes near ChIP-seq peaks, convert to pseudobulk, plot

See our Bioc2023 workshop and tidyseurat / tidySCE

Altogether, “tidyomics”

https://github.com/tidyomics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Stefano Mangiola stemangiola


Stuart Lee sa-lee

Reading

- Hutchison, WJ, Keyes, TJ, *et al.* The tidyomics ecosystem: Enhancing omic data analyses *bioRxiv* (2023) 10.1101/2023.09.10.557072
- Lee, S, Cook, D, Lawrence, M. plyranges: a grammar of genomic data transformation. *Genome Biology* (2019) 10.1186/s13059-018-1597-8
- Lee S, Lawrence M, Love MI. Fluent genomics with plyranges and tximeta. *F1000Research* (2020) 10.12688/f1000research.22259.1

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* (2021) 10.1186/s13059-020-02233-7
- tidySE, tidySCE, tidyseurat stemangiola.github.io/tidytranscriptomics

Extra slides

plyranges 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*