

Tidy Analysis of Genomic Data

Michael Love
Dept of Genetics &
Dept of Biostatistics
UNC-Chapel Hill

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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*:

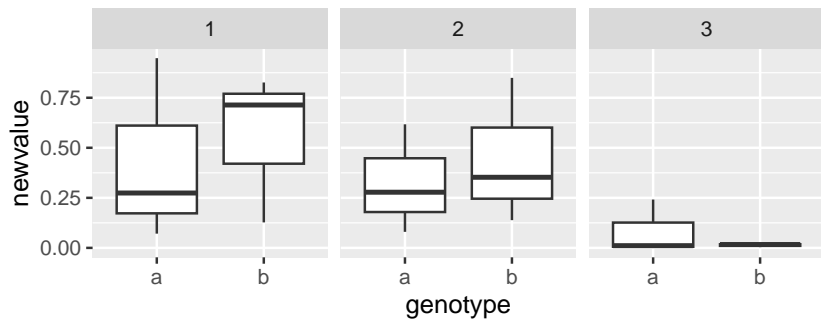
```
dat %>%  
  filter(!outlier) %>%  
  group_by(drug, genotype) %>%  
  summarize(mu_hat = mean(value))
```

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
- ▶ [COCO](#) - explore sample variation along genome
- ▶ [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 510000001-520000000      * |         1
## [2]      chr1 520000001-530000000      * |         2
## [3]      chr1 530000001-540000000      * |         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) → 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 → map → 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

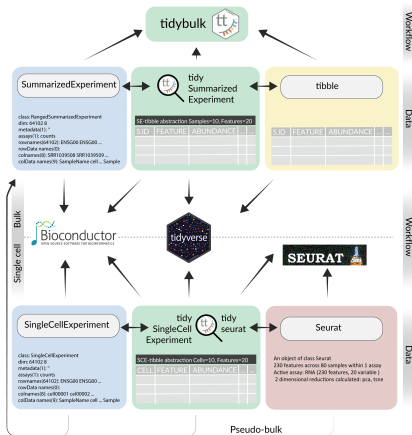
Summary: tidy analysis for genomic range data



nullranges development sponsored by CZI EOSS



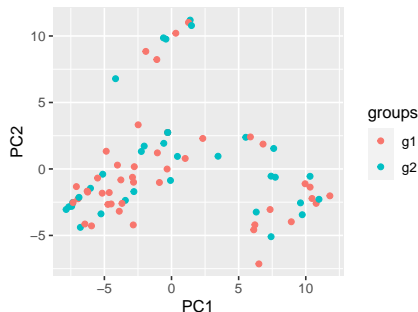
Tidy analysis of matrix data



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

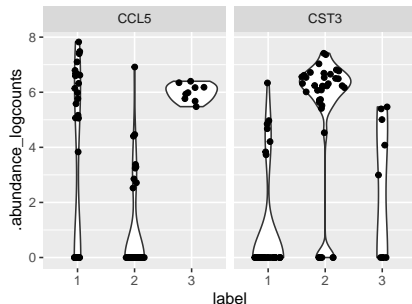
Example use of tidySingleCellExperiment

```
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>



Timothy Keyes keyes-timothy



Helena L. Crowell HelenaLC



Jacques Serizay js2264



Ji-Eun Park jennprk



Eric Davis EricSDavis



ppaxisa



Wancen Mu Wancen



Abdullah Al Nahid nahid18



william-hutchison



Mike Love mikelove



Laurent Gatto lgatto



Stephanie Hicks stephaniehicks



Ming Tang crazyhottommy



Maria Doyle mblue9



Charlotte Soneson csoneson



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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/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_nearest_distance`
- ▶ Load *plyranges* last to avoid name masking with *AnnotationDbi* and *dplyr*