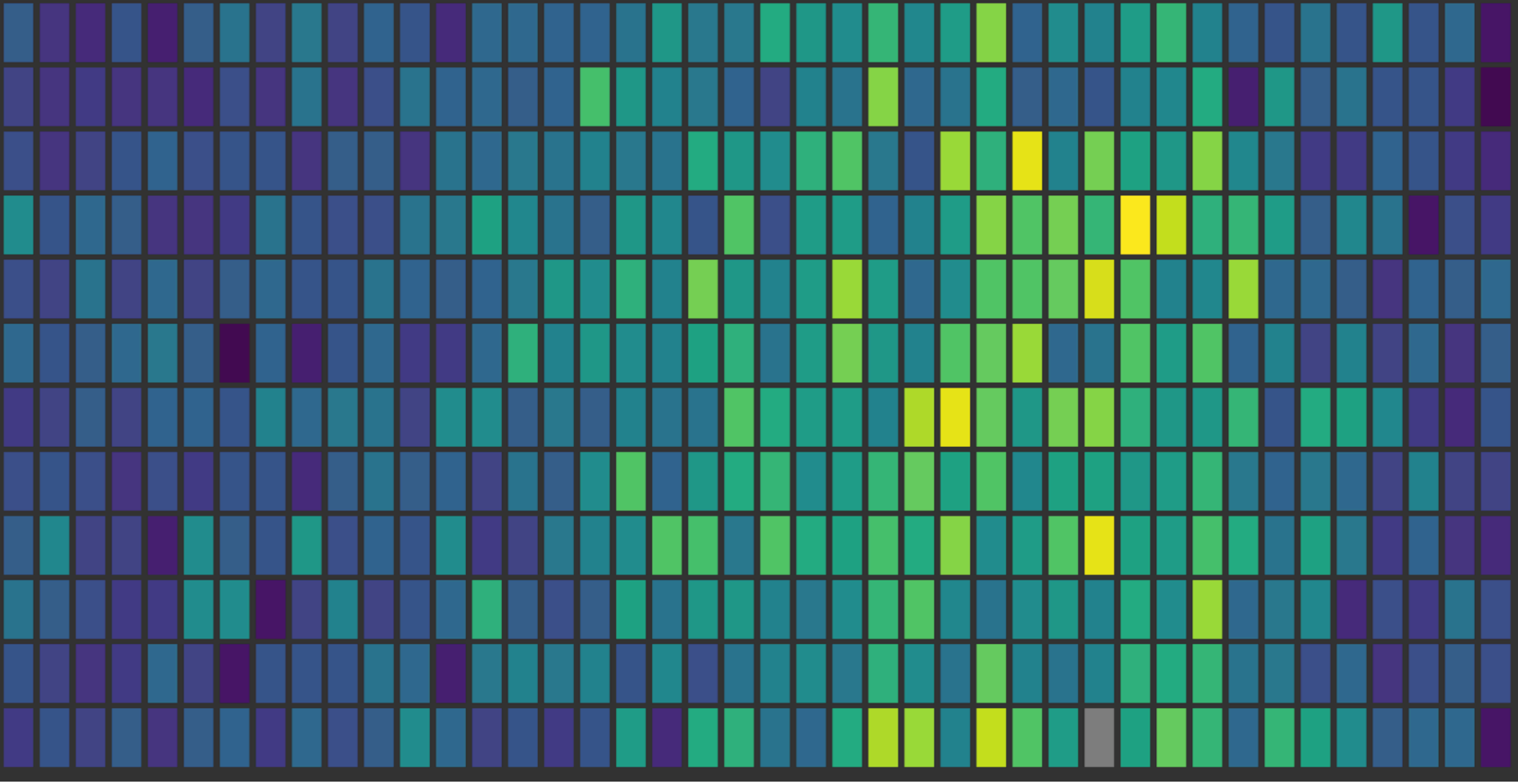


# Graphics and Visualisation

W.Huber;  some slides adapted from Laura Symul & Susan Holmes

# Why graphics?

- To explore data
- To communicate data patterns & preliminary insights with collaborators
- To display results and convey findings in a publication

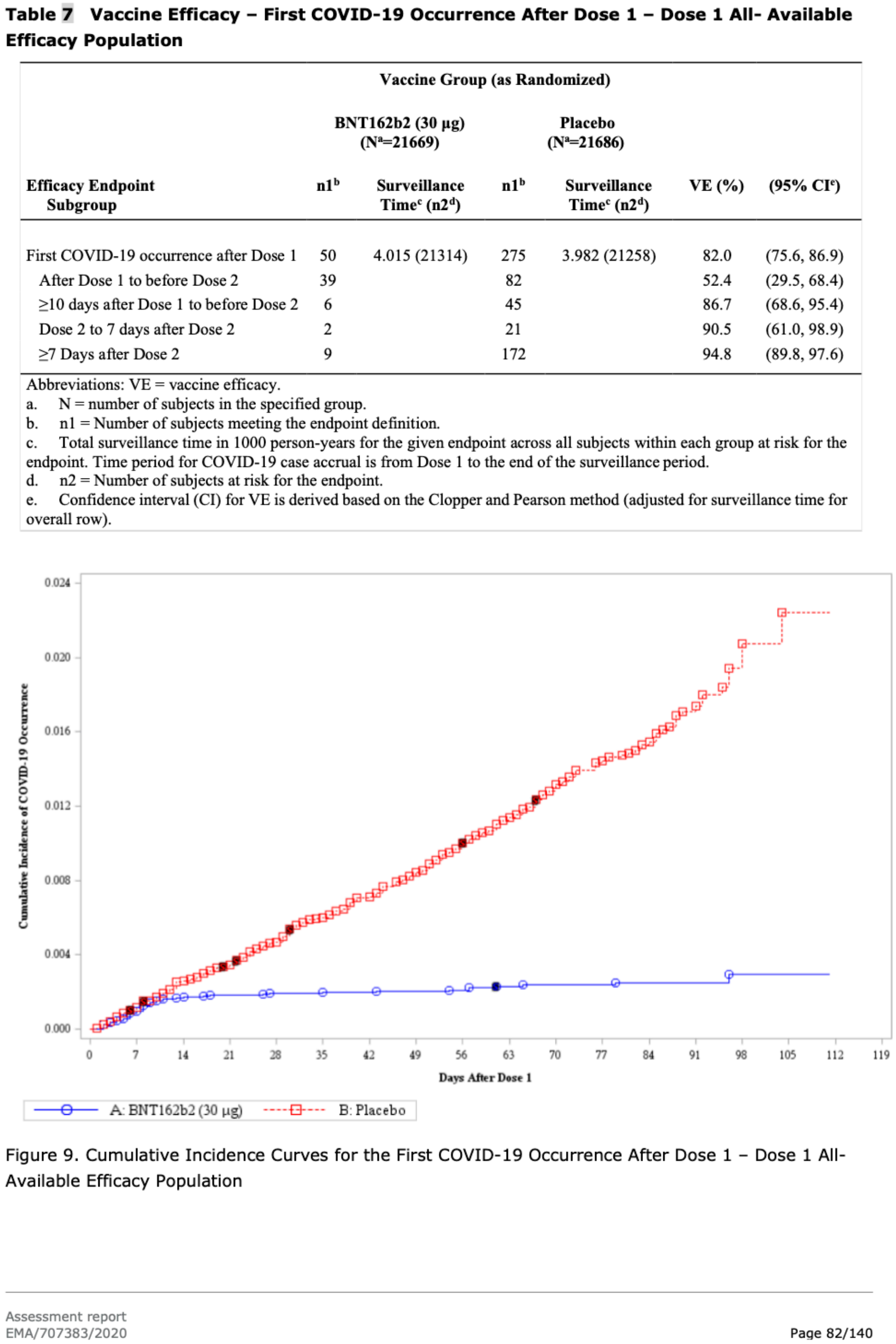


**Table 7 Vaccine Efficacy – First COVID-19 Occurrence After Dose 1 – Dose 1 All- Available Efficacy Population**

| | Vaccine Group (as Randomized) | | | | | |
|---|---|---|---|---|---|---|
| | BNT162b2 (30 µg)<br>(N[a]=21669) | | Placebo<br>(N[a]=21686) | | | |
| Efficacy Endpoint<br>Subgroup | n1[b] | Surveillance Time[c] (n2[d]) | n1[b] | Surveillance Time[c] (n2[d]) | VE (%) | (95% CI[e]) |
| First COVID-19 occurrence after Dose 1 | 50 | 4.015 (21314) | 275 | 3.982 (21258) | 82.0 | (75.6, 86.9) |
| After Dose 1 to before Dose 2 | 39 | | 82 | | 52.4 | (29.5, 68.4) |
| ≥10 days after Dose 1 to before Dose 2 | 6 | | 45 | | 86.7 | (68.6, 95.4) |
| Dose 2 to 7 days after Dose 2 | 2 | | 21 | | 90.5 | (61.0, 98.9) |
| ≥7 Days after Dose 2 | 9 | | 172 | | 94.8 | (89.8, 97.6) |

Abbreviations: VE = vaccine efficacy.

a. N = number of subjects in the specified group.

b. n1 = Number of subjects meeting the endpoint definition.

c. Total surveillance time in 1000 person-years for the given endpoint across all subjects within each group at risk for the endpoint. Time period for COVID-19 case accrual is from Dose 1 to the end of the surveillance period.

d. n2 = Number of subjects at risk for the endpoint.

e. Confidence interval (CI) for VE is derived based on the Clopper and Pearson method (adjusted for surveillance time for overall row).

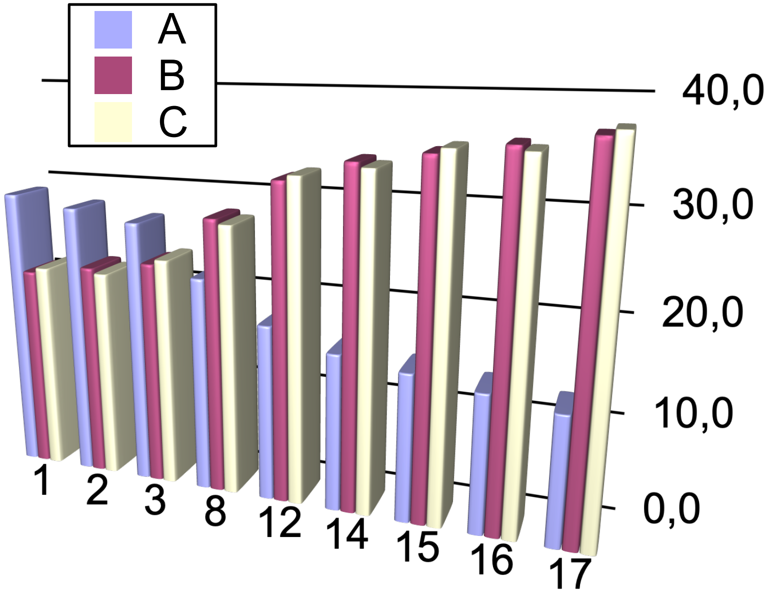
Figure 9. Cumulative Incidence Curves for the First COVID-19 Occurrence After Dose 1 – Dose 1 All-Available Efficacy Population

# A picture says more than a thousand words

**Source:**
Assessment report
EMA/707383/2020
21 December 2020
Committee for Medicinal Products for Human Use (CHMP)

Comirnaty
Common name: COVID-19 mRNA vaccine (nucleoside-modified)
Procedure No.: EMEA/H/C/005735/0000
Page 82 / 140

# Horror Picture Show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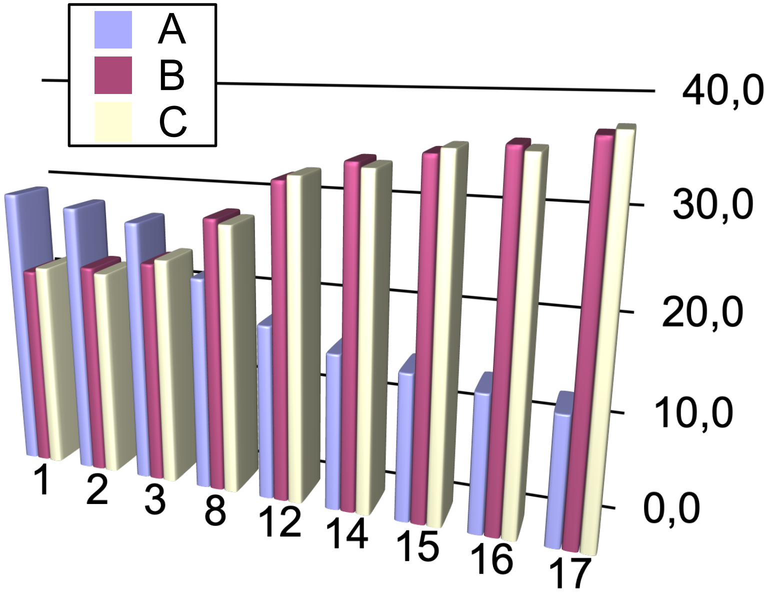
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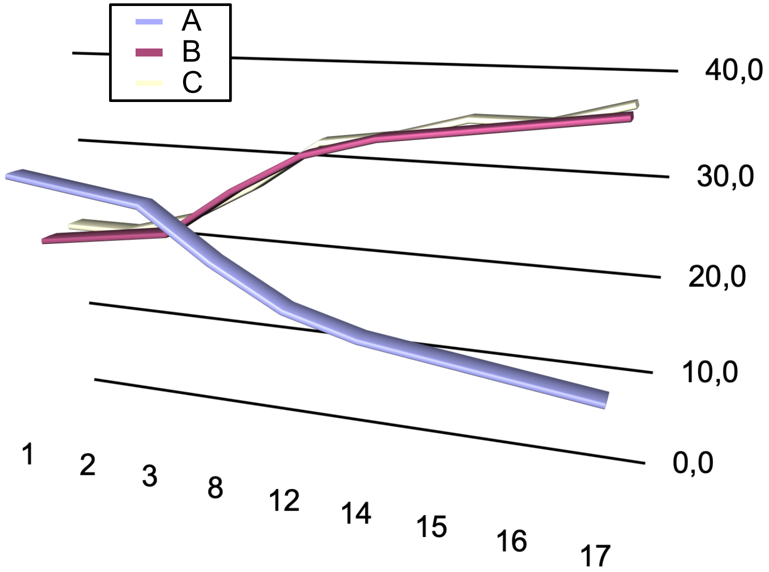
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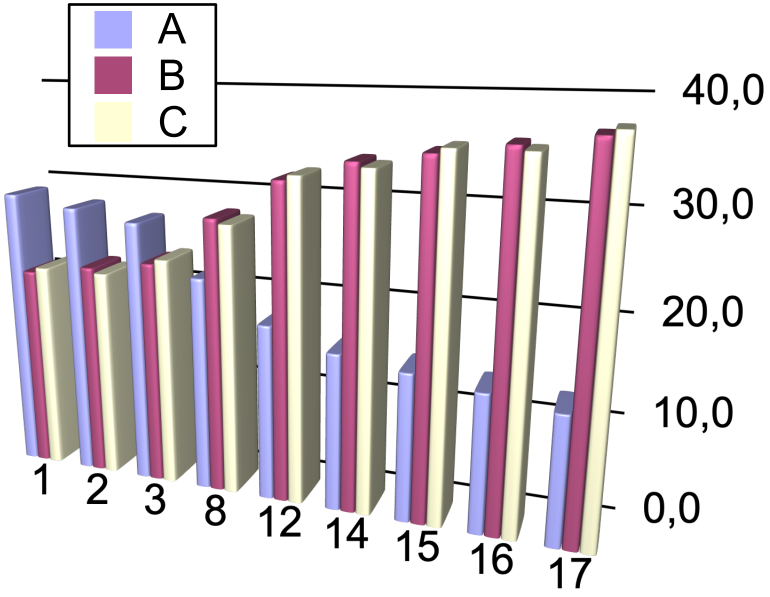
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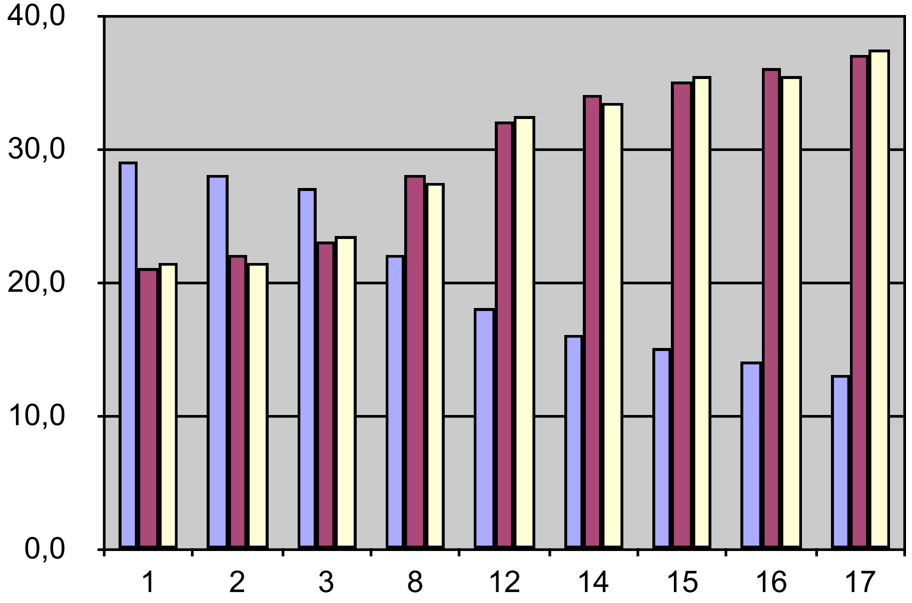
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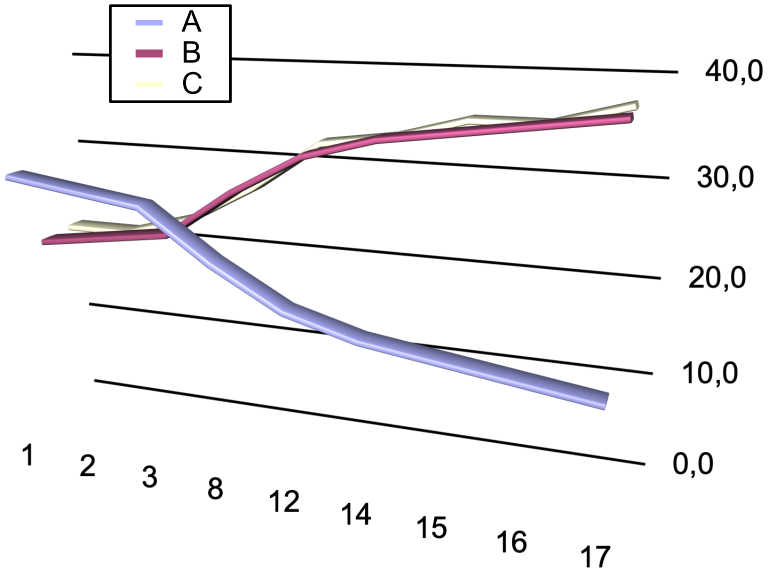
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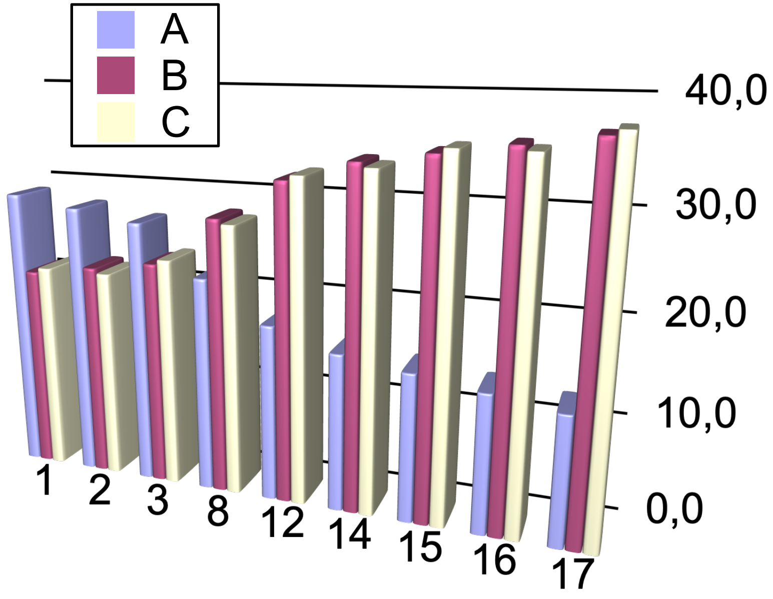

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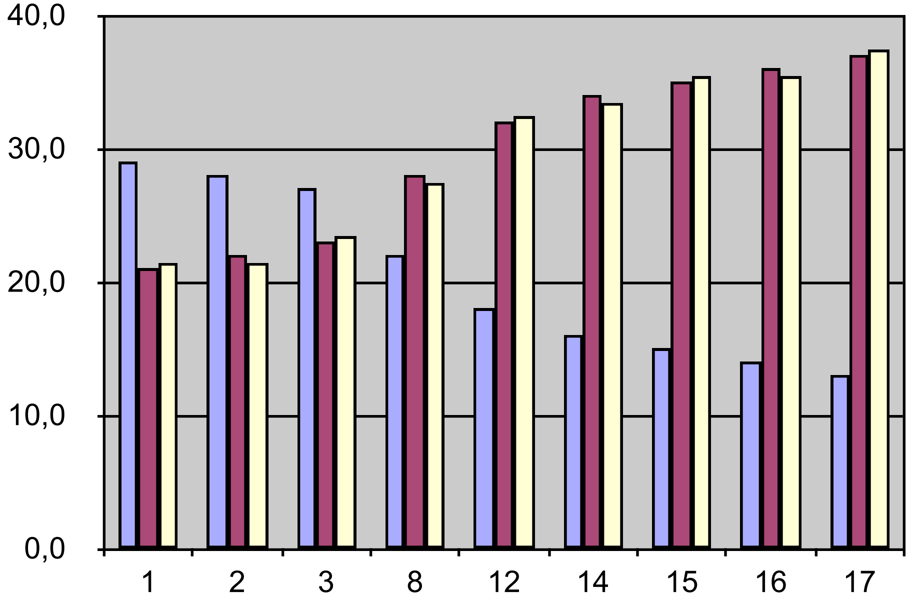

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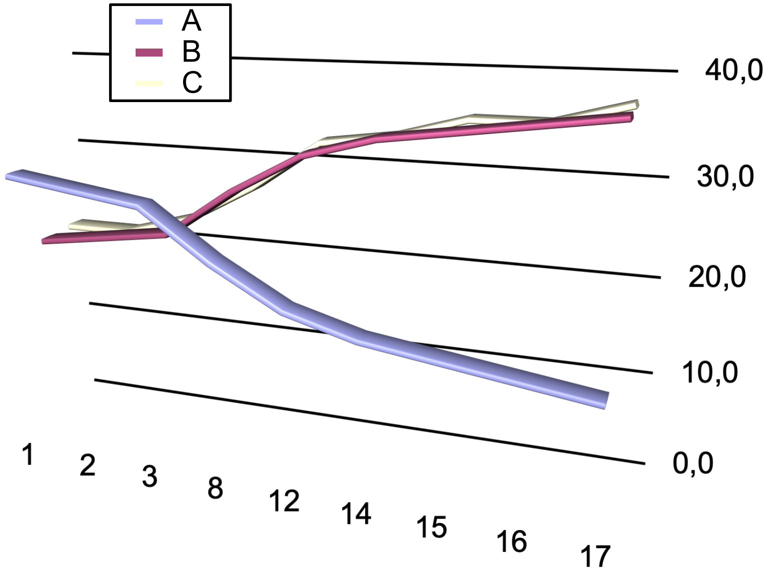

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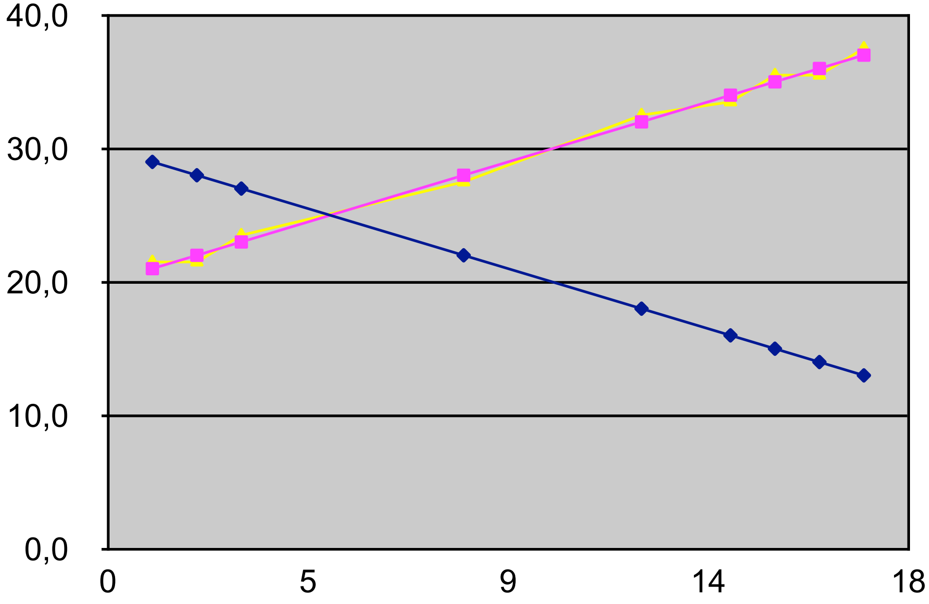

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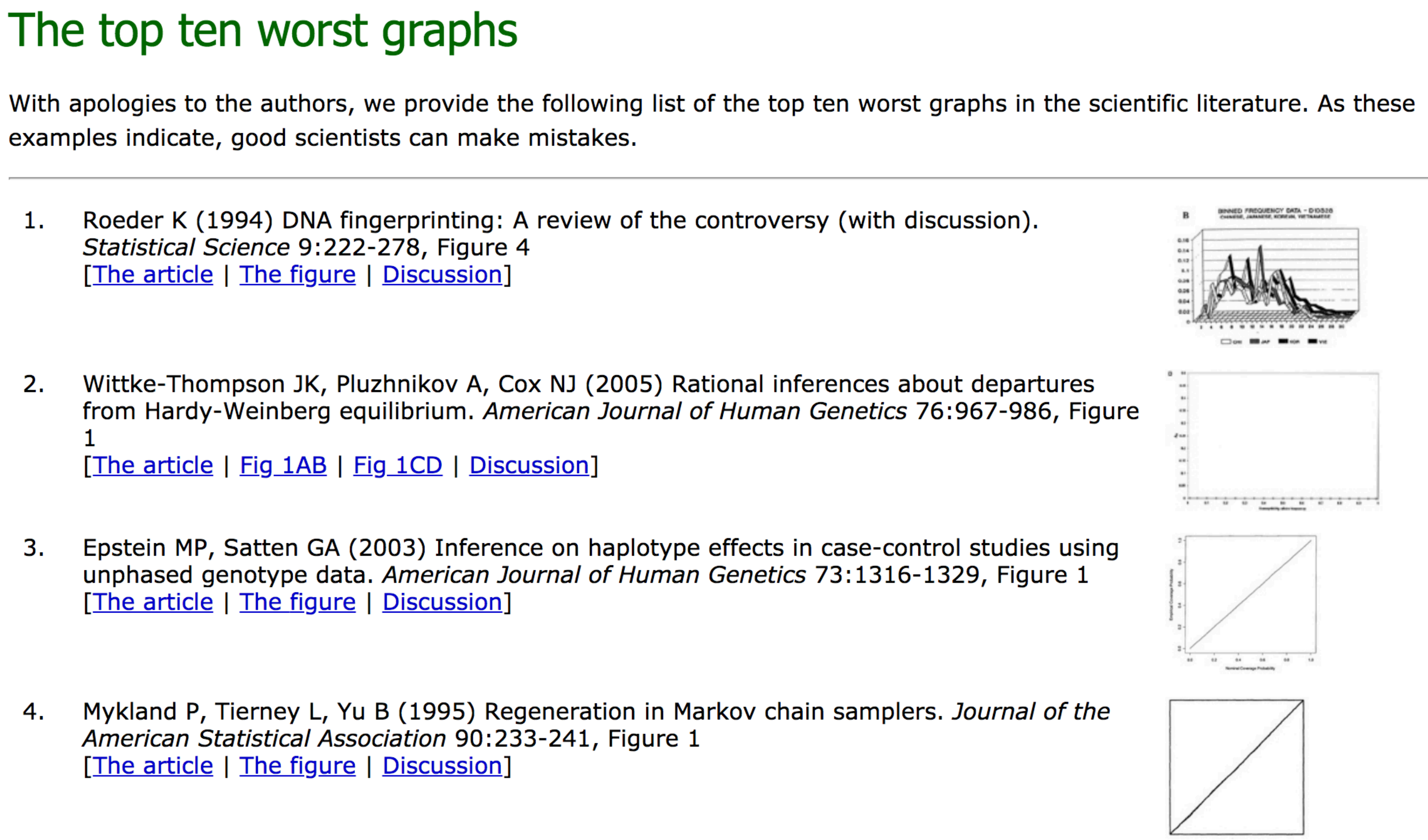


# The top ten worst graphs

With apologies to the authors, we provide the following list of the top ten worst graphs in the scientific literature. As these examples indicate, good scientists can make mistakes.

1. Roeder K (1994) DNA fingerprinting: A review of the controversy (with discussion). *Statistical Science* 9:222-278, Figure 4
[The article | The figure | Discussion]

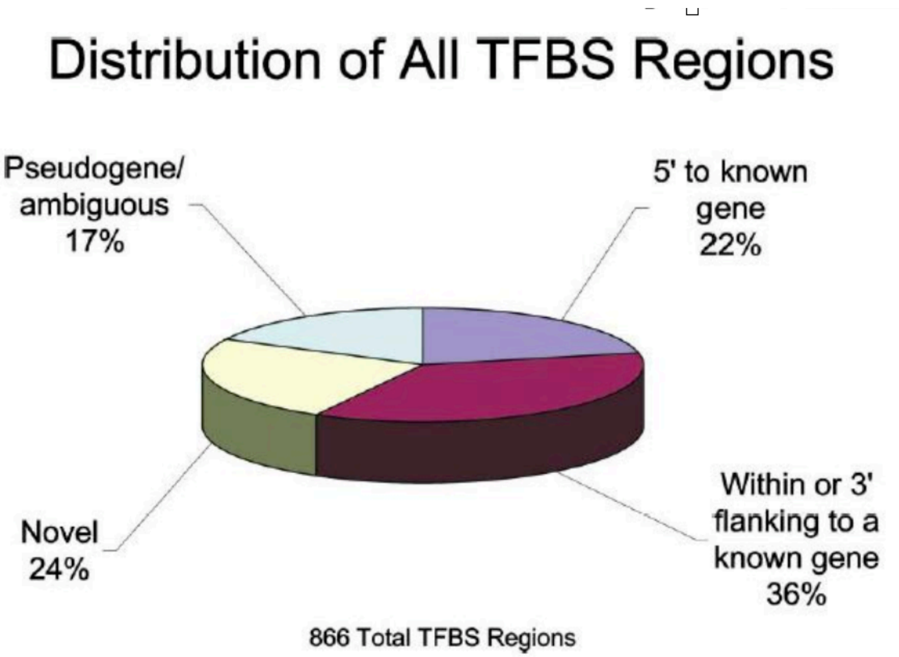
B
BINNED FREQUENCY DATA - D10S28
CHINESE, JAPANESE, KOREAN, VIETNAMESE
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
CHI
JAP
KOR
VIE

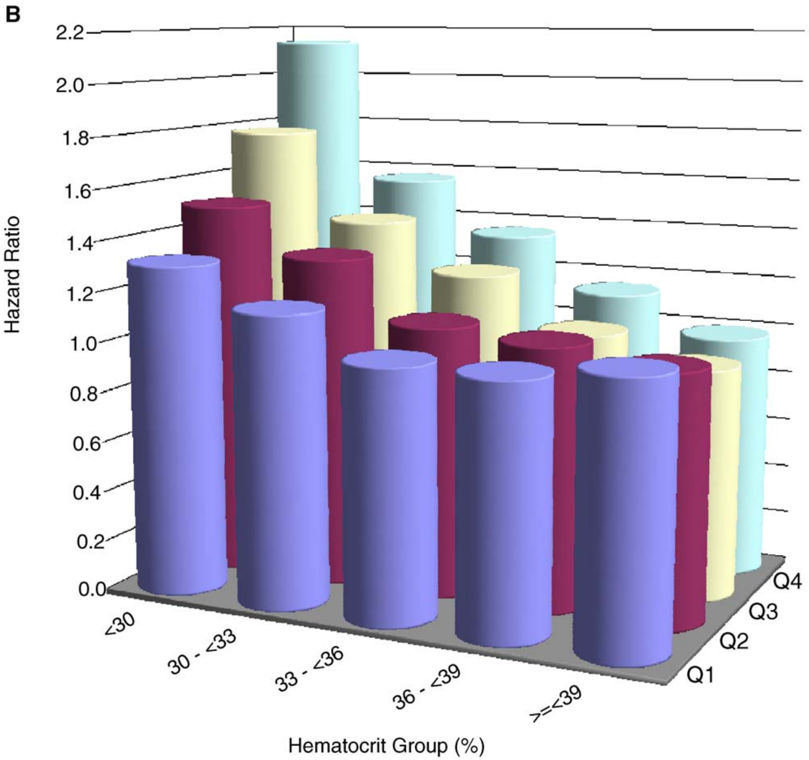
2. Wittke-Thompson JK, Pluzhnikov A, Cox NJ (2005) Rational inferences about departures from Hardy-Weinberg equilibrium. *American Journal of Human Genetics* 76:967-986, Figure 1
[The article | Fig 1AB | Fig 1CD | Discussion]

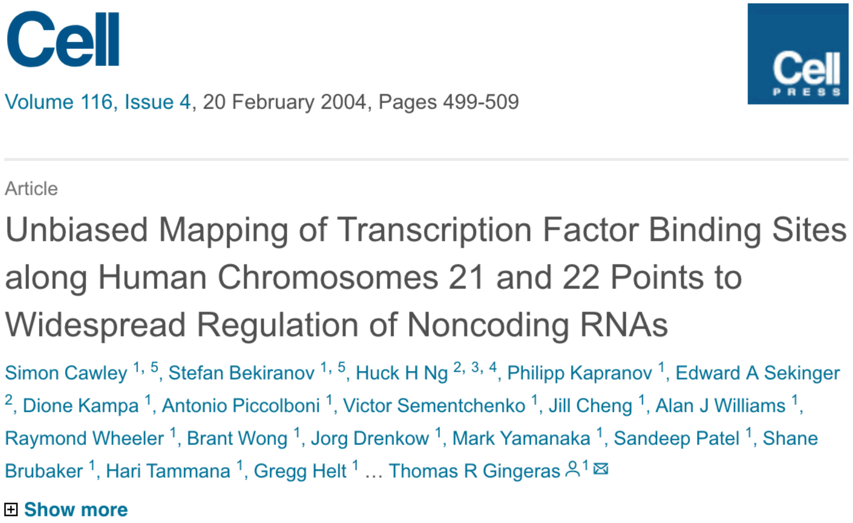
3. Epstein MP, Satten GA (2003) Inference on haplotype effects in case-control studies using unphased genotype data. *American Journal of Human Genetics* 73:1316-1329, Figure 1
[The article | The figure | Discussion]

4. Mykland P, Tierney L, Yu B (1995) Regeneration in Markov chain samplers. *Journal of the American Statistical Association* 90:233-241, Figure 1
[The article | The figure | Discussion]

Source: Karl Broman https://www.biostat.wisc.edu/~kbroman/topten_worstgraphs/






Cell

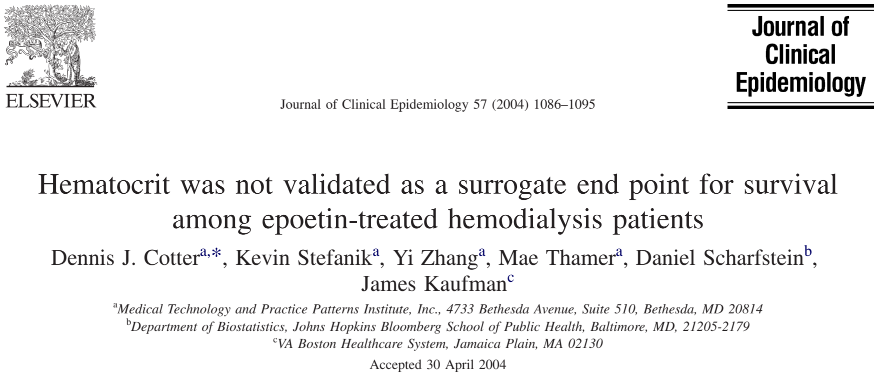
Volume 116, Issue 4, 20 February 2004, Pages 499-509

Article

## Unbiased Mapping of Transcription Factor Binding Sites along Human Chromosomes 21 and 22 Points to Widespread Regulation of Noncoding RNAs

Simon Cawley [1, 5], Stefan Bekiranov [1, 5], Huck H Ng [2, 3, 4], Philipp Kapranov [1], Edward A Sekinger [2], Dione Kampa [1], Antonio Piccolboni [1], Victor Sementchenko [1], Jill Cheng [1], Alan J Williams [1], Raymond Wheeler [1], Brant Wong [1], Jorg Drenkow [1], Mark Yamanaka [1], Sandeep Patel [1], Shane Brubaker [1], Hari Tammana [1], Gregg Helt [1] … Thomas R Gingeras [1]

⊞ Show more


ELSEVIER

Journal of Clinical Epidemiology 57 (2004) 1086–1095

Journal of Clinical Epidemiology

## Hematocrit was not validated as a surrogate end point for survival among epoetin-treated hemodialysis patients

Dennis J. Cotter[a,*], Kevin Stefanik[a], Yi Zhang[a], Mae Thamer[a], Daniel Scharfstein[b], James Kaufman[c]

[a]Medical Technology and Practice Patterns Institute, Inc., 4733 Bethesda Avenue, Suite 510, Bethesda, MD 20814
[b]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, 21205-2179
[c]VA Boston Healthcare System, Jamaica Plain, MA 02130

Accepted 30 April 2004

[1] https://doi.org/10.1016/S0092-8674(04)00127-8
[2] https://doi.org/10.1016/j.jclinepi.2004.05.002

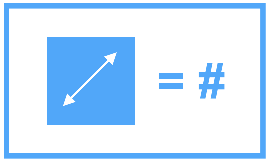
# Goals for this lecture

1. Discuss the principles of **good *vs* bad** data viz
2. Review base R plotting
3. Understand the **grammar of graphics** concept
4. Introduce, explain and use the `ggplot()` function
5. Discuss how to plot 1D, 2D, 3-5D data and select the most appropriate plot type. Use faceting
6. Use visualization for the inspection of large datasets and discovery of global trends (e.g. batch effects)
7. Implement interactive (3D) visualization

# Respect **Graphical Integrity** principles

## Representation of numbers should match the true proportions

*Visual Display of Quantitative Information*
*E. Tufte*

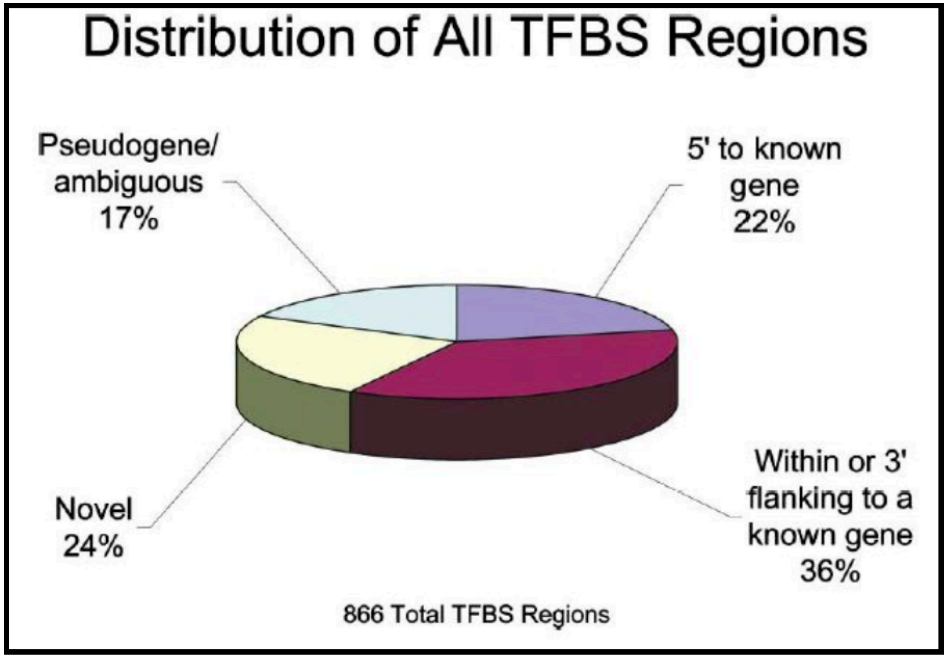


# Respect **Graphical Integrity** principles

## Representation of numbers should match the true proportions

*Visual Display of Quantitative Information*
*E. Tufte*

⤢ = #

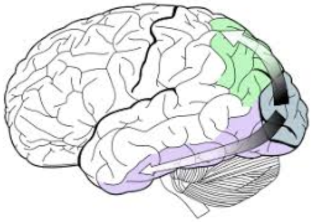


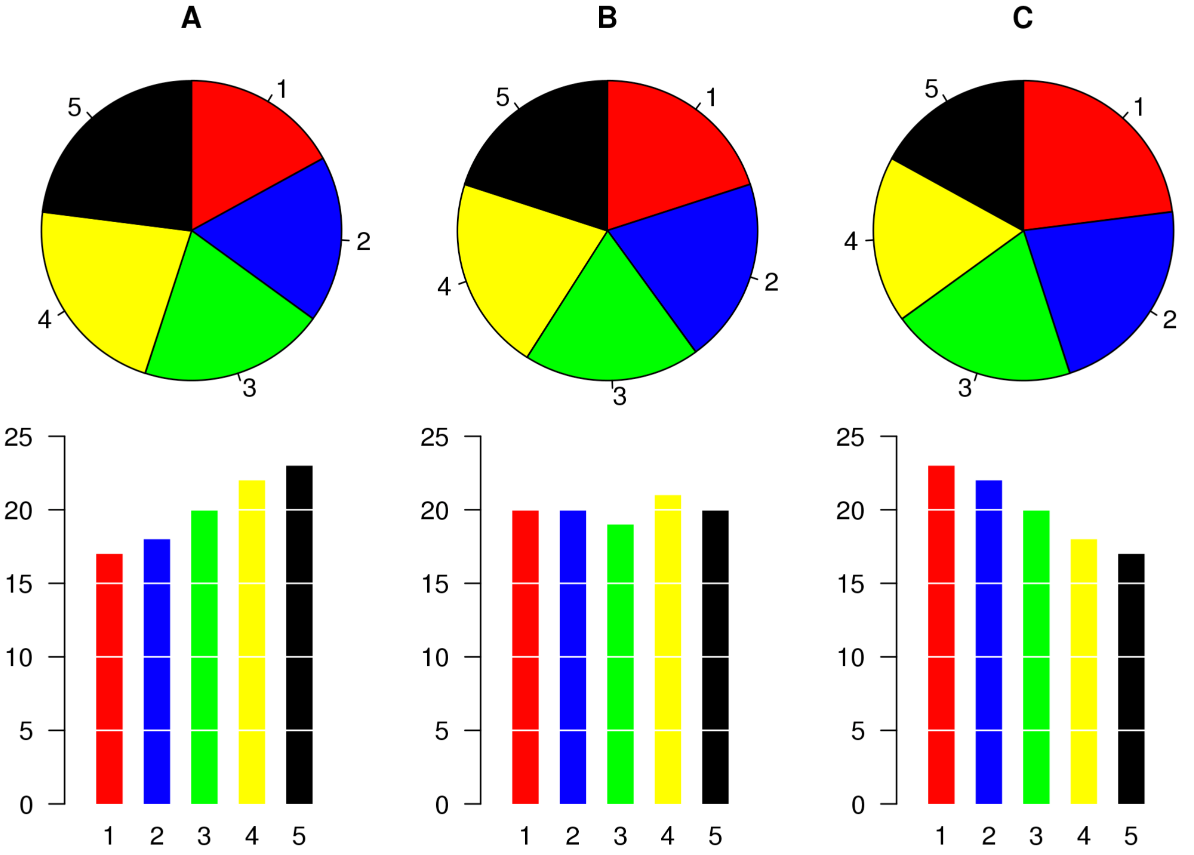
The problem with the “3D perspective”:

The area (or indeed the actual angles) occupied by each category on the plot is not proportional to the actual numbers

This principle also applies to inclusion of the baseline (e.g., 0) in bar charts, scatterplots…

# Respect humans' visual abilities





(but the colours…)

Pie charts are bad because the human brain is not good at differentiating angles. (Especially angles that do not have a horizontal or vertical edge)

https://medium.com/@kennelliott/39-studies-about-human-perception-in-30-minutes-4728f9e31a73

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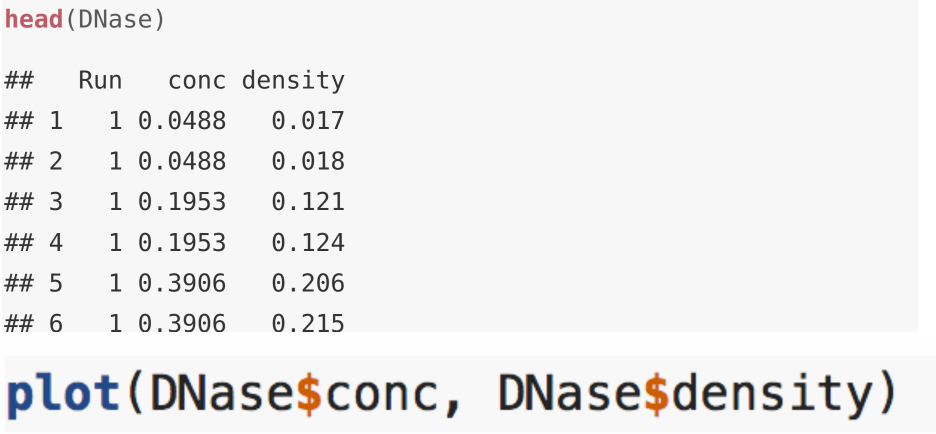
# base R plotting

**canvas model:**
a series of instructions that **sequentially** fill the plotting canvas



```
head(DNase)

##    Run   conc density
## 1    1 0.0488   0.017
## 2    1 0.0488   0.018
## 3    1 0.1953   0.121
## 4    1 0.1953   0.124
## 5    1 0.3906   0.206
## 6    1 0.3906   0.215
```

```
plot(DNase$conc, DNase$density)
```

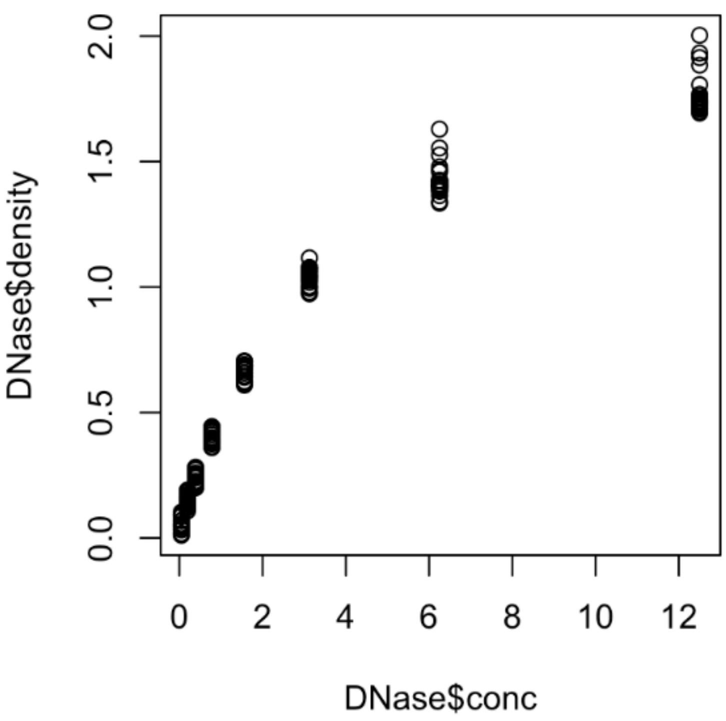
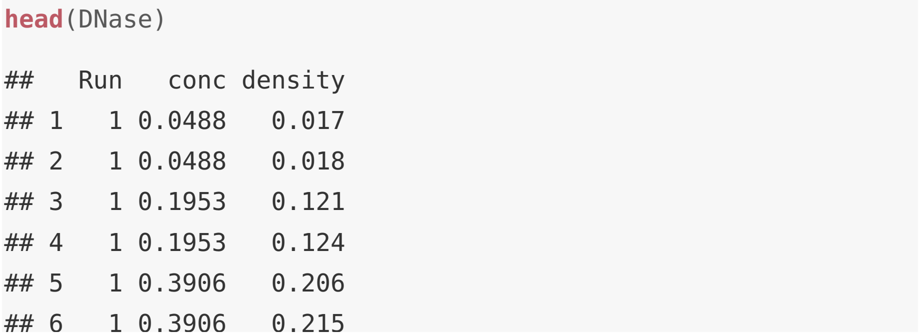


Figure 3.2: Plot of concentration vs. density for an ELISA assay of DNase.

# base R plotting

**canvas model:**
a series of instructions that **sequentially** fill the plotting canvas

**Great for quick data exploration!**



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head(DNase)

##   Run   conc density
## 1   1 0.0488   0.017
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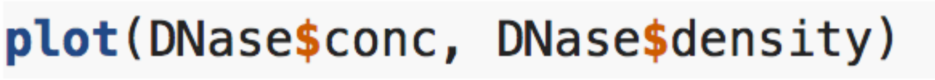
## 6   1 0.3906   0.215
```



```
plot(DNase$conc, DNase$density)
```

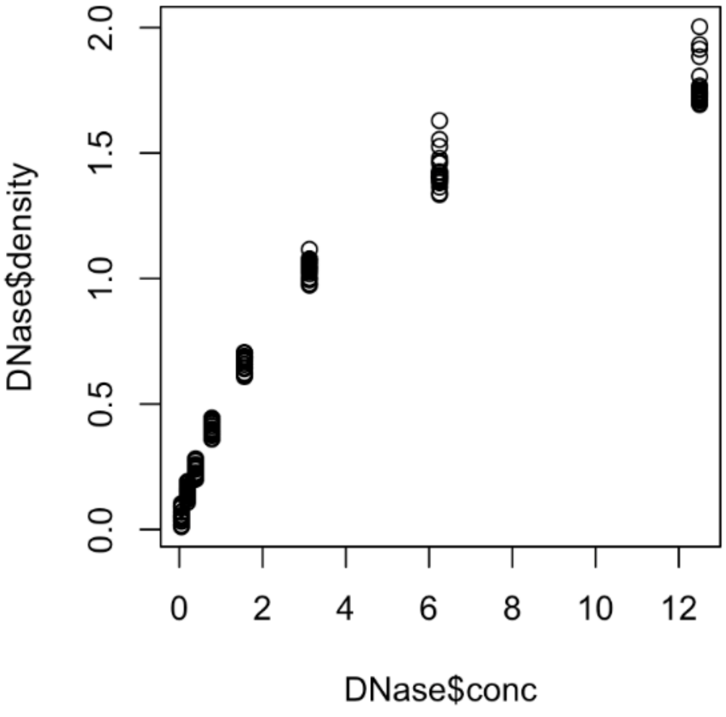
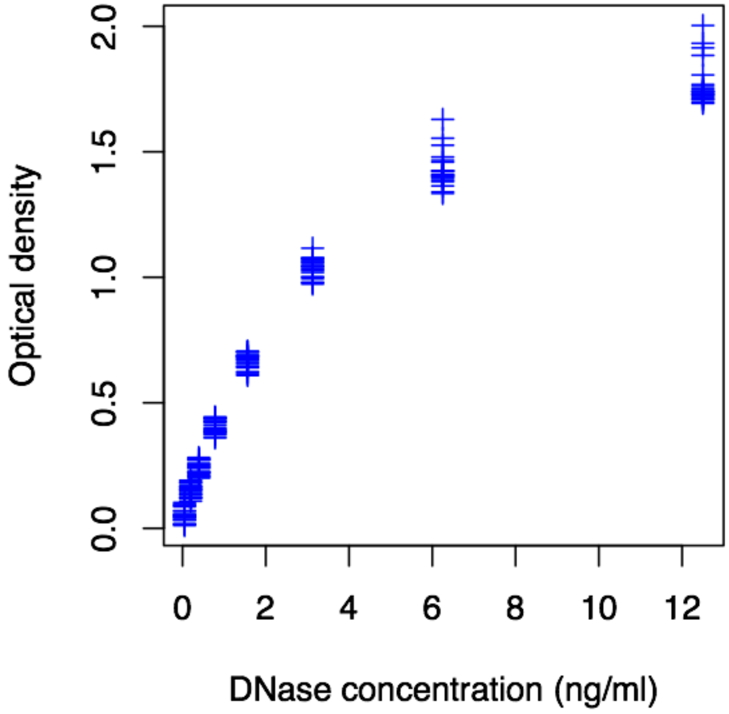


Figure 3.2: Plot of concentration vs. density for an ELISA assay of DNase.

# base R plotting

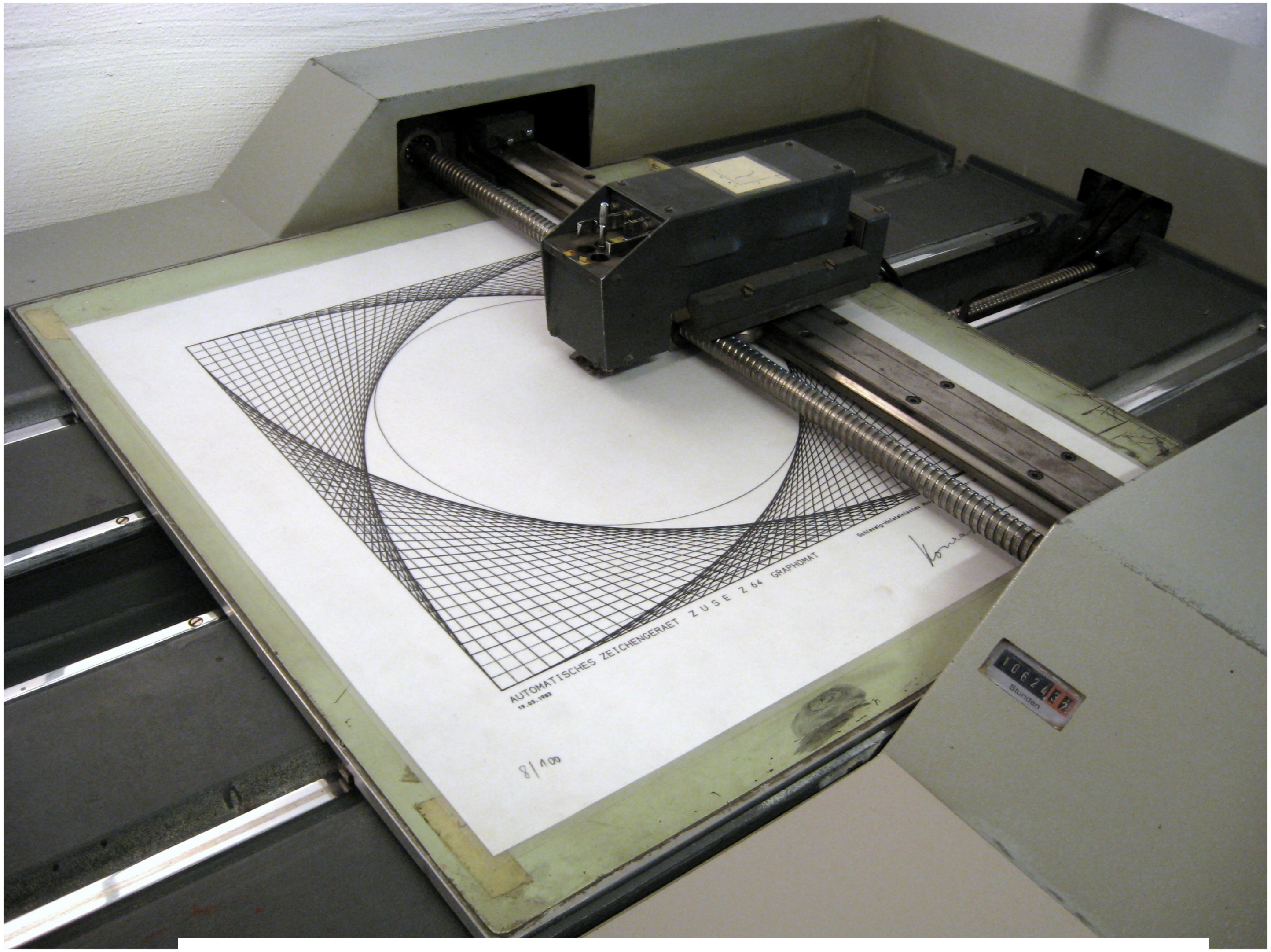
**canvas model:**
a series of instructions that **sequentially** fill the plotting canvas

**Inefficient for customization and generating complex plots.**



```r
plot(DNase$conc, DNase$density,
ylab = attr(DNase, "labels")$y,
xlab = paste(attr(DNase, "labels")$x, attr(DNase, "units")$x),
pch = 3, col = "blue")
```

# base R plotting



ZUSE Plotter Z64 (presented in 1961).

# base R plotting

**Drawbacks:**

- **Layout choices have to be made at the beginning** with no overview over what may still be coming
- **Different functions for different plot types**, with different interfaces
- Routine tasks can require lots of **boilerplate code**
- **No concept of facets / lattices**
- Only a **single global coordinate** system allowed per plot
- **Poor default colours**
- **Resizing** often leads to unsatisfactory results

# Goals for this lecture

1. Discuss the principles of **good *vs* bad** data viz
2. Review base R plotting
3. Understand the **grammar of graphics** concept
4. Introduce, explain and use the `ggplot()` function
5. Discuss how to plot 1D, 2D, 3-5D data and select the most appropriate plot type. Use faceting
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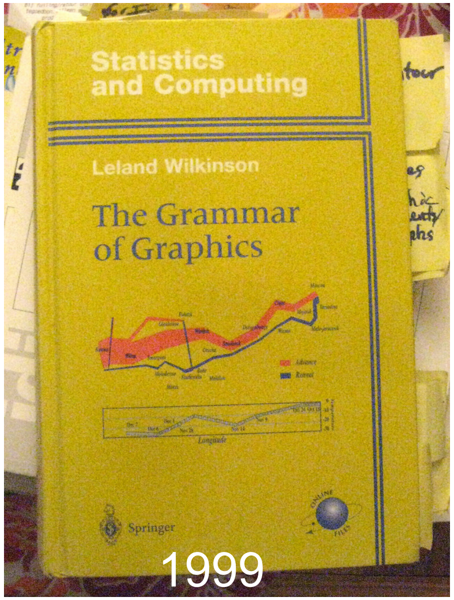
# The Grammar of Graphics

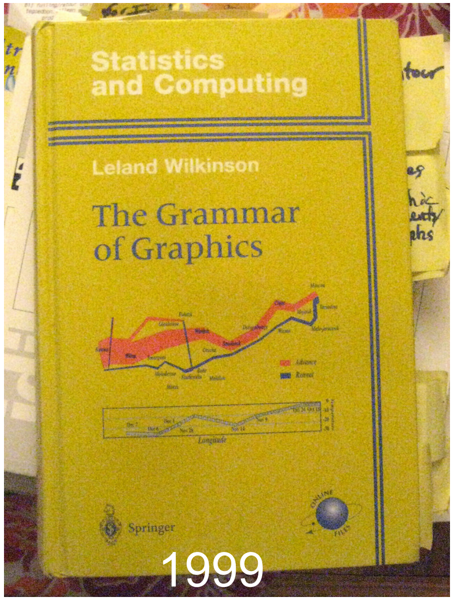


1999

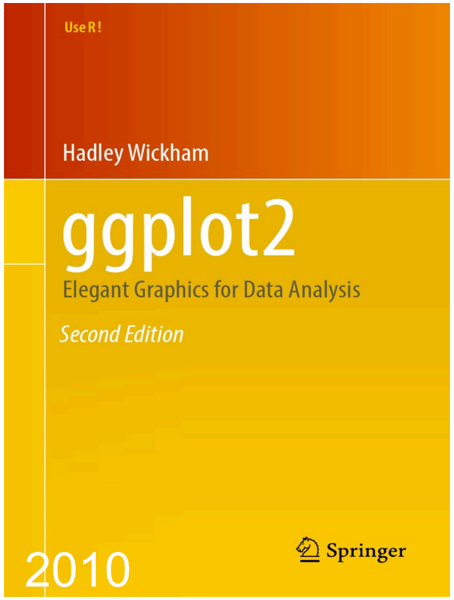
Concept **coined by Leland Wilkinson in 1999**.

An **abstraction** which facilitates reasoning and communicating graphics.

# The Grammar of Graphics



1999



2010

Concept **coined by Leland Wilkinson in 1999**.
An **abstraction** which facilitates reasoning and communicating graphics.

ggplot2 is an implementation of **a layered grammar of graphics** that enables users to independently specify the building blocks of a plot and combine them to create just about any kind of graphical display.

# `ggplot2` grammar of graphics

The components of ggplot2's grammar of graphics are

- **datasets** (*nouns*)
- **geometric objects** (*verbs*), visual representations of the data, e.g. points, lines, rectangles, contours,
- **aesthetics** (*adverbs*), instructions on how to map variables to geometric objects,
- **statistical transformation/summaries** e.g. line fitting, binning,
- **coordinate systems** and associated **scales** e.g. linear, log, rank,
- **facets** separating subsets of data into multiple subplots,
- optional parameter settings e.g. text size, font, alignment, legend positions

# ggplot2 grammar of graphics

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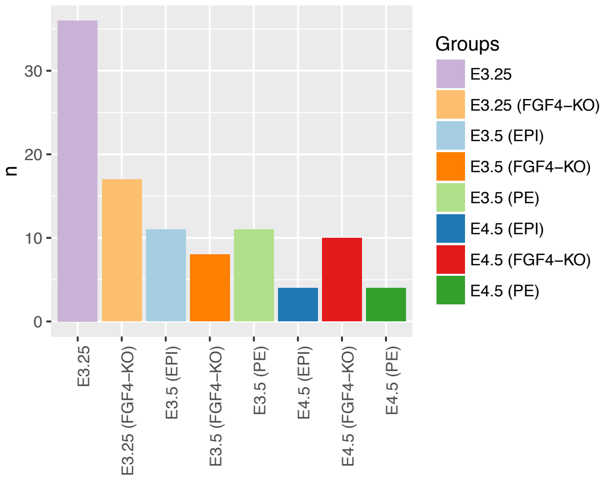
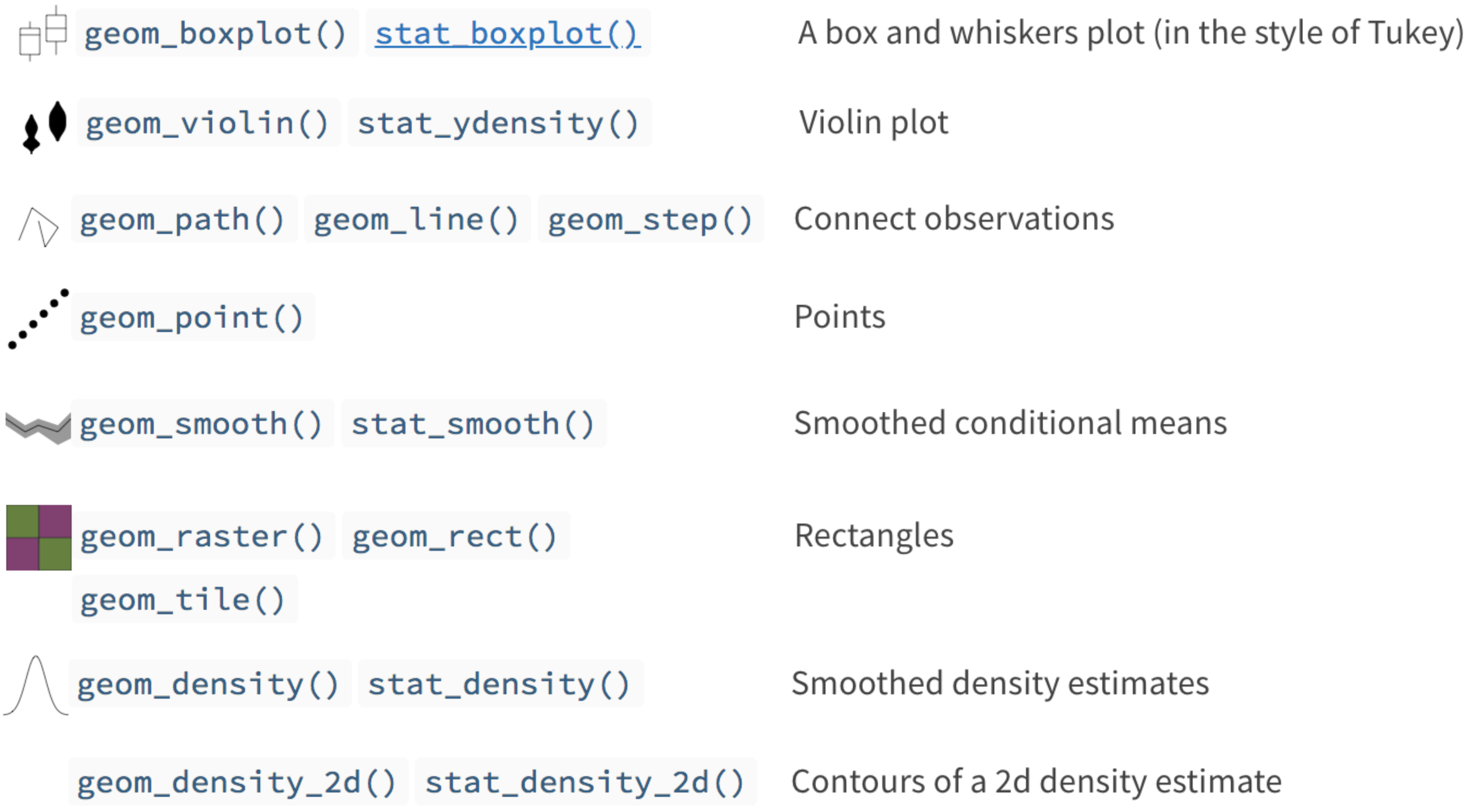
- **coordinate systems** and associated **scales** e.g. linear, log, rank,
- **facets** separating subsets of data into multiple subplots,
- optional parameter settings e.g. text size, font, alignment, legend positions

```r
ggplot(groups, aes(x = sampleGroup, y = n, fill = sampleGroup)) +
  geom_bar(stat = "identity") +
  scale_fill_manual(values = groupColour, name = "Groups") +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```



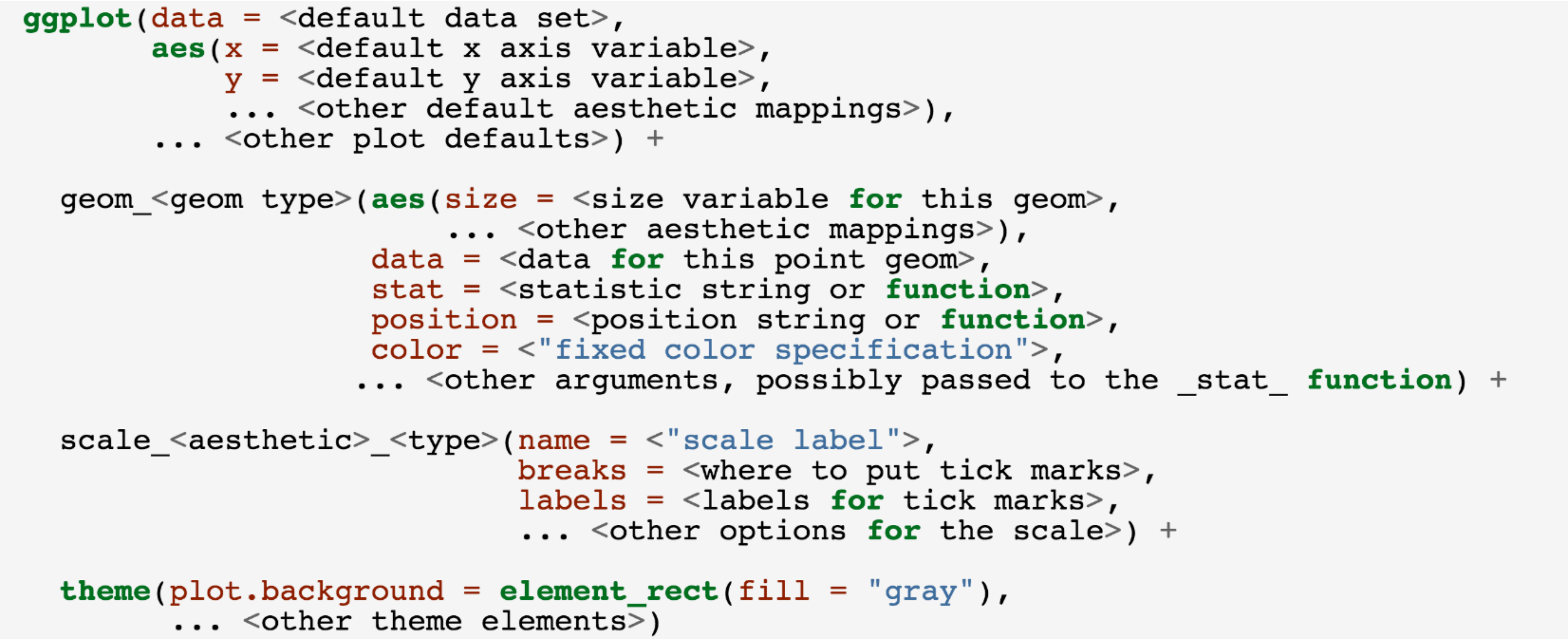
# geometric objects



| | |
|---|---|
| `geom_boxplot()` `stat_boxplot()` | A box and whiskers plot (in the style of Tukey) |
| `geom_violin()` `stat_ydensity()` | Violin plot |
| `geom_path()` `geom_line()` `geom_step()` | Connect observations |
| `geom_point()` | Points |
| `geom_smooth()` `stat_smooth()` | Smoothed conditional means |
| `geom_raster()` `geom_rect()` `geom_tile()` | Rectangles |
| `geom_density()` `stat_density()` | Smoothed density estimates |
| `geom_density_2d()` `stat_density_2d()` | Contours of a 2d density estimate |

Cheat sheet: https://www.rstudio.com/wp-content/uploads/2015/03/ggplot2-cheatsheet.pdf

# ggplot() template



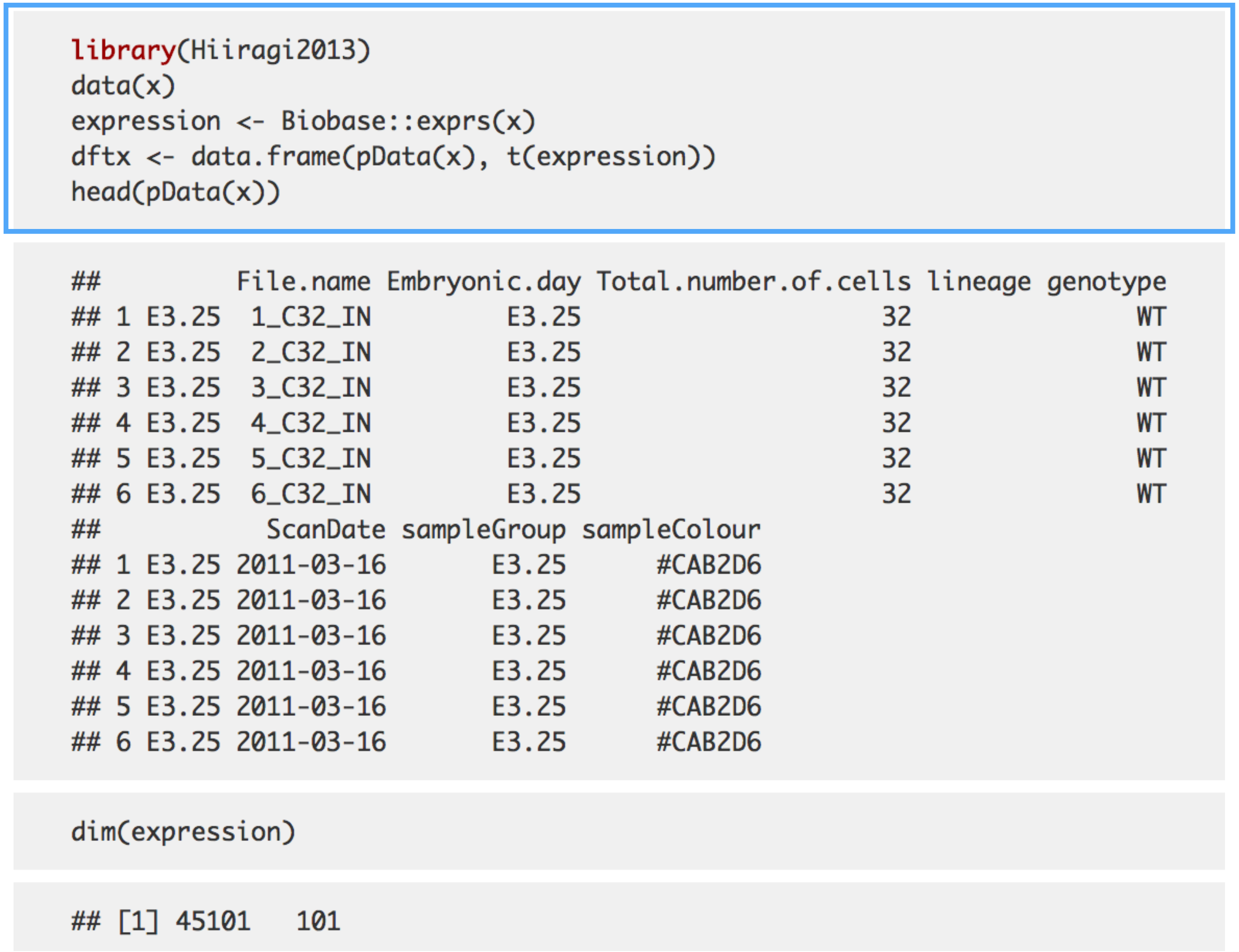
```
ggplot(data = <default data set>,
       aes(x = <default x axis variable>,
           y = <default y axis variable>,
           ... <other default aesthetic mappings>),
       ... <other plot defaults>) +

  geom_<geom type>(aes(size = <size variable for this geom>,
                       ... <other aesthetic mappings>),
                   data = <data for this point geom>,
                   stat = <statistic string or function>,
                   position = <position string or function>,
                   color = <"fixed color specification">,
                  ... <other arguments, possibly passed to the _stat_ function) +

  scale_<aesthetic>_<type>(name = <"scale label">,
                           breaks = <where to put tick marks>,
                           labels = <labels for tick marks>,
                           ... <other options for the scale>) +

  theme(plot.background = element_rect(fill = "gray"),
        ... <other theme elements>)
```

# Data must be in *dataframe* format



```
library(Hiiragi2013)
data(x)
expression <- Biobase::exprs(x)
dftx <- data.frame(pData(x), t(expression))
head(pData(x))
```

```
##         File.name Embryonic.day Total.number.of.cells lineage genotype
## 1 E3.25  1_C32_IN         E3.25                    32               WT
## 2 E3.25  2_C32_IN         E3.25                    32               WT
## 3 E3.25  3_C32_IN         E3.25                    32               WT
## 4 E3.25  4_C32_IN         E3.25                    32               WT
## 5 E3.25  5_C32_IN         E3.25                    32               WT
## 6 E3.25  6_C32_IN         E3.25                    32               WT
##           ScanDate sampleGroup sampleColour
## 1 E3.25 2011-03-16       E3.25      #CAB2D6
## 2 E3.25 2011-03-16       E3.25      #CAB2D6
## 3 E3.25 2011-03-16       E3.25      #CAB2D6
## 4 E3.25 2011-03-16       E3.25      #CAB2D6
## 5 E3.25 2011-03-16       E3.25      #CAB2D6
## 6 E3.25 2011-03-16       E3.25      #CAB2D6
```

```
dim(expression)
```

```
## [1] 45101   101
```

`ggplot()` requires input data in form of a dataframe

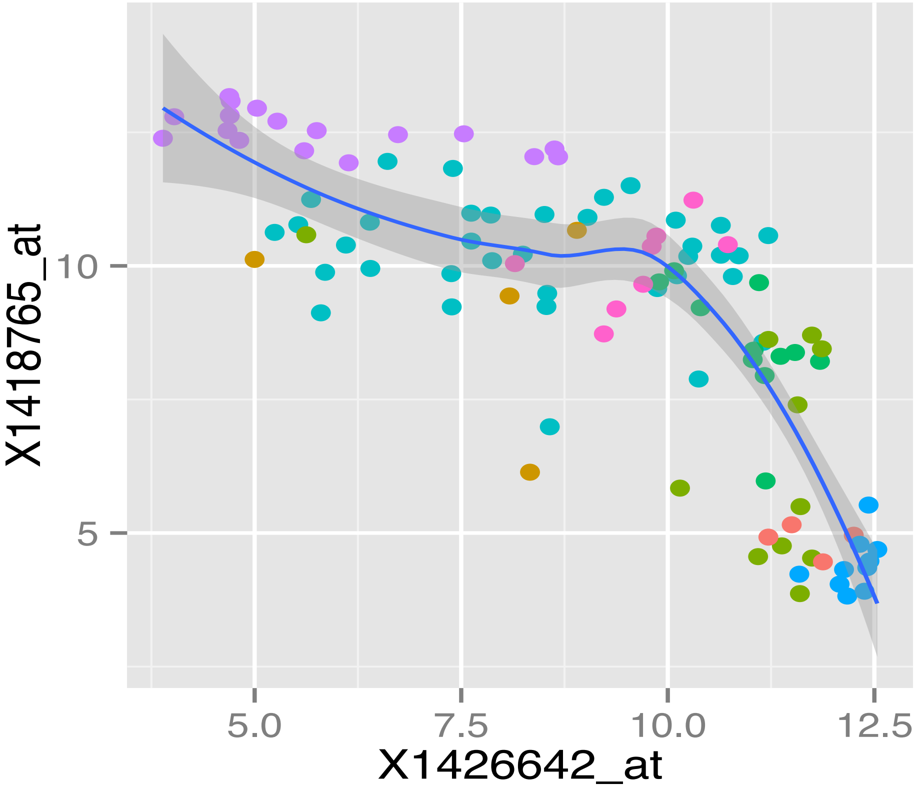
Gene expression **microarray dataset on early development of mouse embryos**

transcriptomes of ~100 individual cells at different time points in. [1]

[1] Cell-to-cell expression variability followed by signal reinforcement progressively segregates early mouse lineages by Ohnishi et al., Nature Cell Biology (2014) 16(1): 27-37. doi: 10.1038/ncb2881.

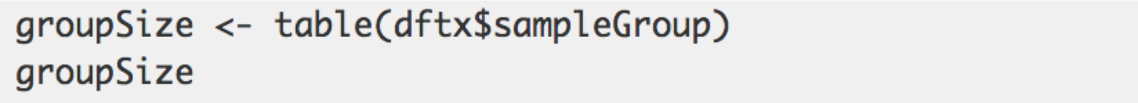
# Multiple layers can be superposed

```
ggplot( dftx, aes( x = X1426642_at, y = X1418765_at ))  +
  geom_point( aes( colour = sampleColour), shape = 19 ) +
  geom_smooth( method = "loess" ) +
  scale_colour_discrete( guide = FALSE )
```

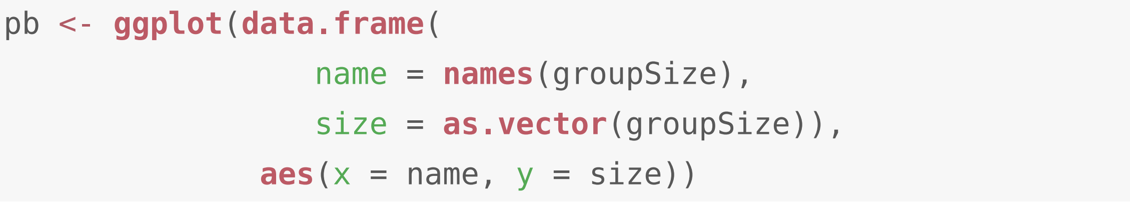


Here, the first layers holds the points, the second holds the smoothed average.

# Using the same plot, we can easily change the coordinates

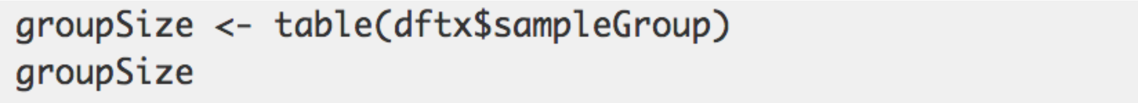


```
groupSize <- table(dftx$sampleGroup)
groupSize
```



```
pb <- ggplot(data.frame(
                  name = names(groupSize),
                  size = as.vector(groupSize)),
               aes(x = name, y = size))
```

# Using the same plot, we can easily change the coordinates



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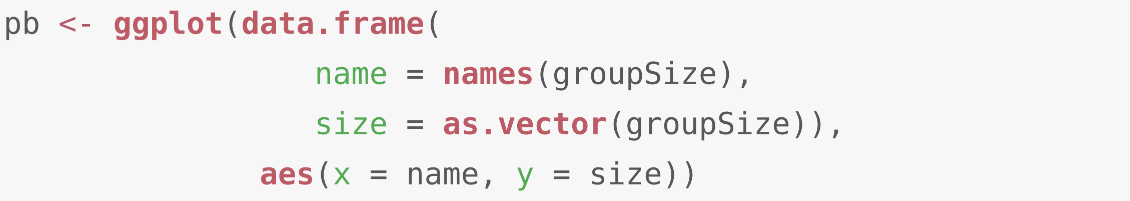
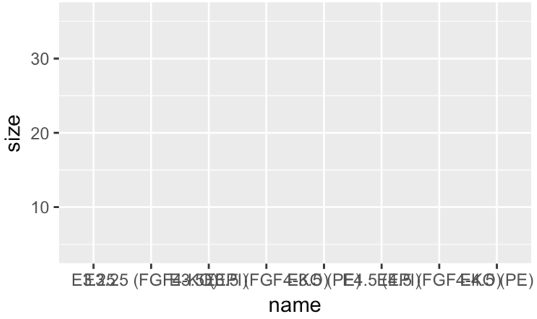
groupSize
```



```
pb <- ggplot(data.frame(
                 name = names(groupSize),
                 size = as.vector(groupSize)),
               aes(x = name, y = size))
```

No geom defined yet!



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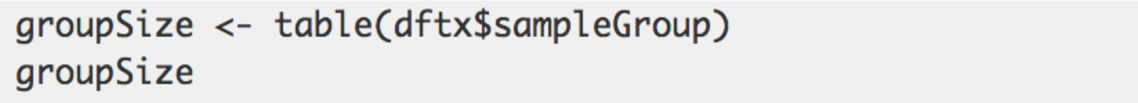

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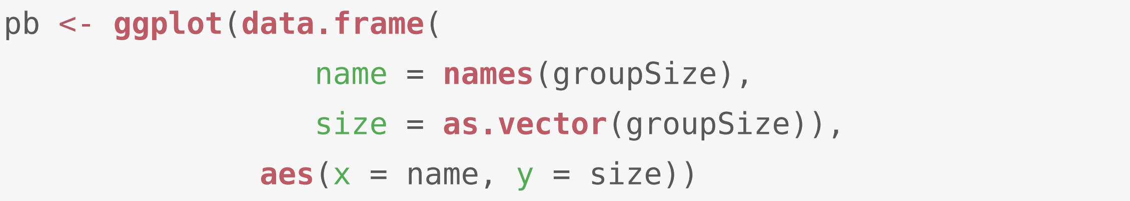

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                  size = as.vector(groupSize)),
                aes(x = name, y = size))
```

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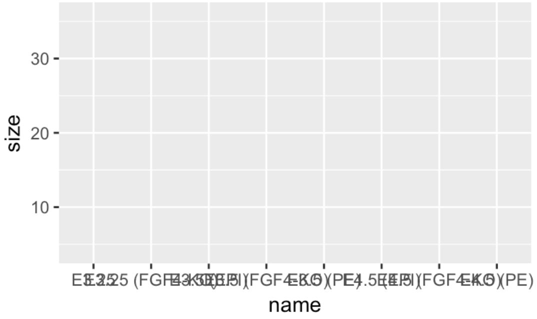
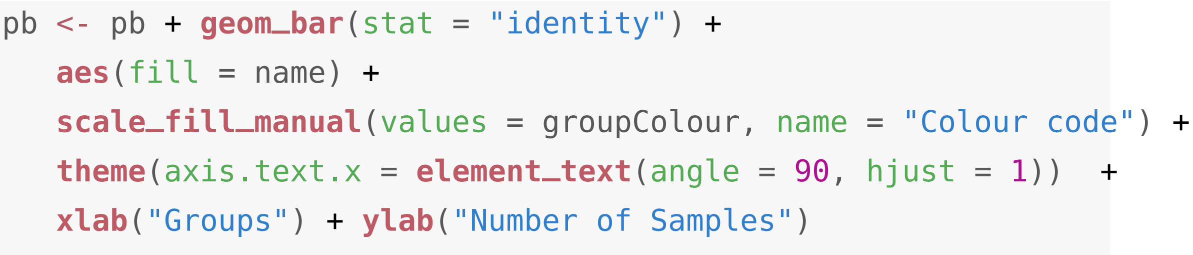




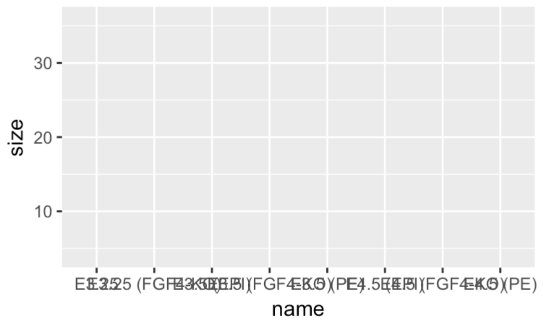
```
pb <- pb + geom_bar(stat = "identity") +
   aes(fill = name) +
   scale_fill_manual(values = groupColour, name = "Colour code") +
   theme(axis.text.x = element_text(angle = 90, hjust = 1))  +
   xlab("Groups") + ylab("Number of Samples")
```

# Using the same plot, we can easily change the coordinates

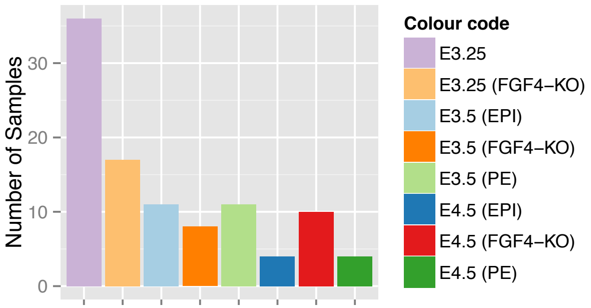
```
groupSize <- table(dftx$sampleGroup)
groupSize
```

```
pb <- ggplot(data.frame(
                name = names(groupSize),
                size = as.vector(groupSize)),
             aes(x = name, y = size))
```

No geom defined yet!



```
pb <- pb + geom_bar(stat = "identity") +
   aes(fill = name) +
   scale_fill_manual(values = groupColour, name = "Colour code") +
   theme(axis.text.x = element_text(angle = 90, hjust = 1))  +
   xlab("Groups") + ylab("Number of Samples")
```



# Using the same plot, we can easily change the coordinates

```
groupSize <- table(dftx$sampleGroup)
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pb <- ggplot(data.frame(
                  name = names(groupSize),
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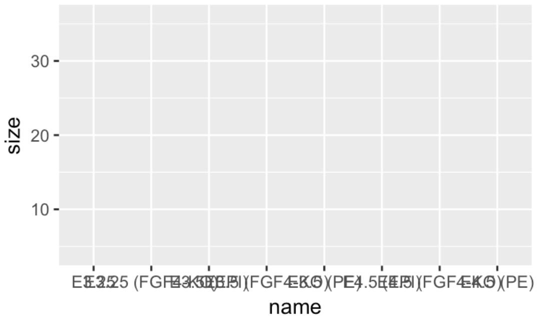

```
pb <- pb + geom_bar(stat = "identity") +
   aes(fill = name) +
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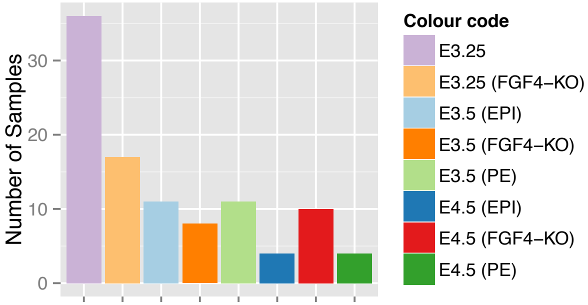
```



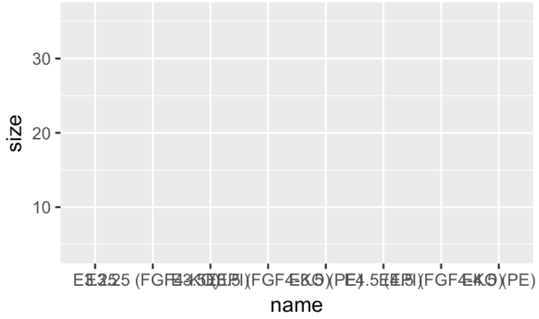
```
pb.polar <- pb + coord_polar() +
  theme(axis.text.x = element_text(angle = 0, hjust = 1),
        axis.text.y = element_blank(),
        axis.ticks = element_blank()) +
  xlab("") + ylab("")
pb.polar
```

# Using the same plot, we can easily change the coordinates

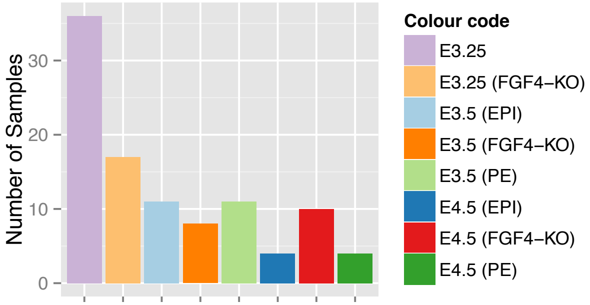
```
groupSize <- table(dftx$sampleGroup)
groupSize
```

```
pb <- ggplot(data.frame(
                  name = names(groupSize),
                  size = as.vector(groupSize)),
                aes(x = name, y = size))
```

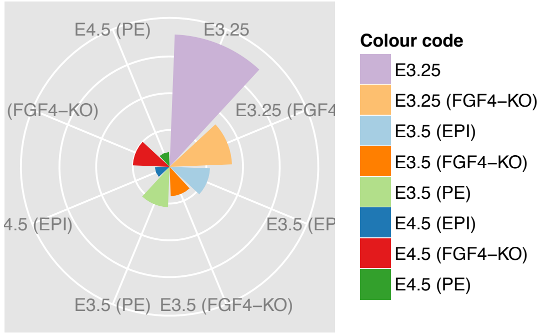
No geom defined yet!



```
pb <- pb + geom_bar(stat = "identity") +
   aes(fill = name) +
   scale_fill_manual(values = groupColour, name = "Colour code") +
   theme(axis.text.x = element_text(angle = 90, hjust = 1))  +
   xlab("Groups") + ylab("Number of Samples")
```



```
pb.polar <- pb + coord_polar() +
  theme(axis.text.x = element_text(angle = 0, hjust = 1),
        axis.text.y = element_blank(),
        axis.ticks = element_blank()) +
  xlab("") + ylab("")
pb.polar
```



# Themes can change the look

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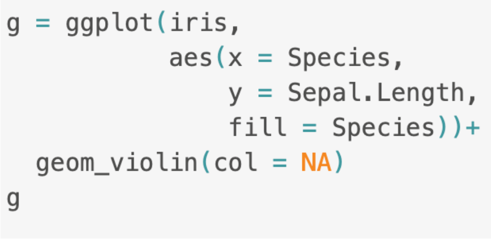
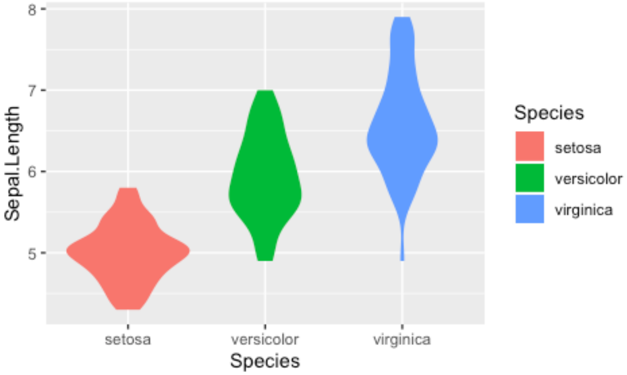
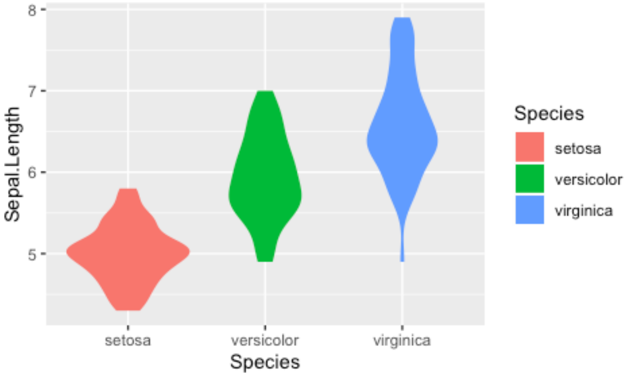


```r
g = ggplot(iris,
           aes(x = Species,
               y = Sepal.Length,
               fill = Species))+
  geom_violin(col = NA)
g
```

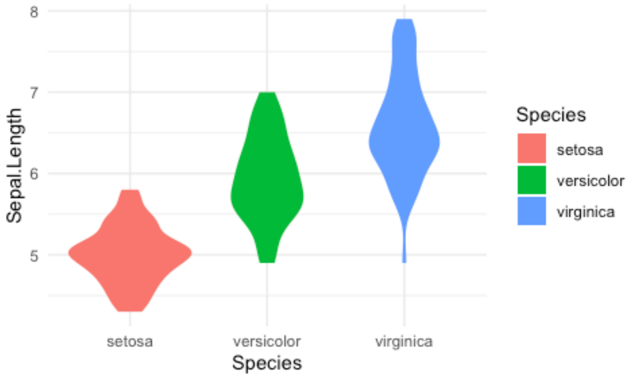


# Themes can change the look

```
g = ggplot(iris,
           aes(x = Species,
               y = Sepal.Length,
               fill = Species))+
  geom_violin(col = NA)
g
```



```
g + theme_minimal()
```



# Themes can change the look

```r
g = ggplot(iris,
           aes(x = Species,
               y = Sepal.Length,
               fill = Species))+
  geom_violin(col = NA)
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```
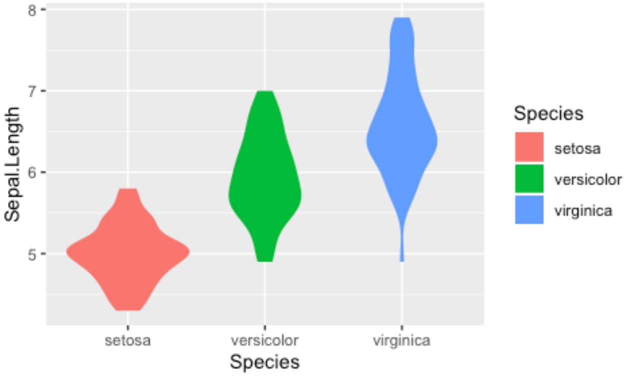
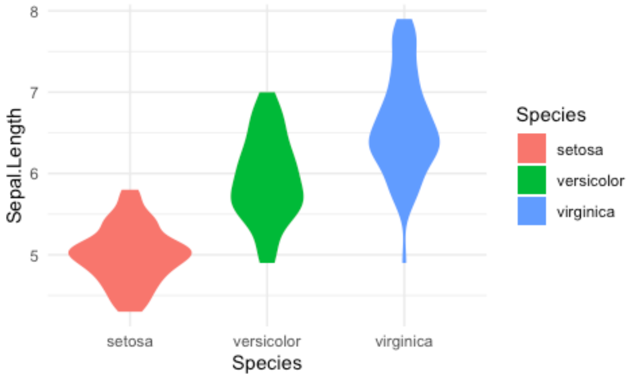
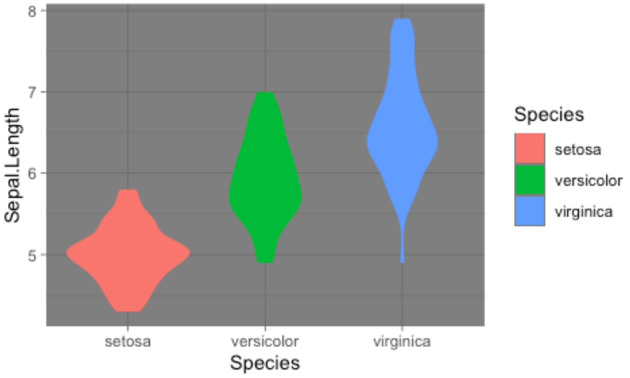



```r
g + theme_minimal()
```

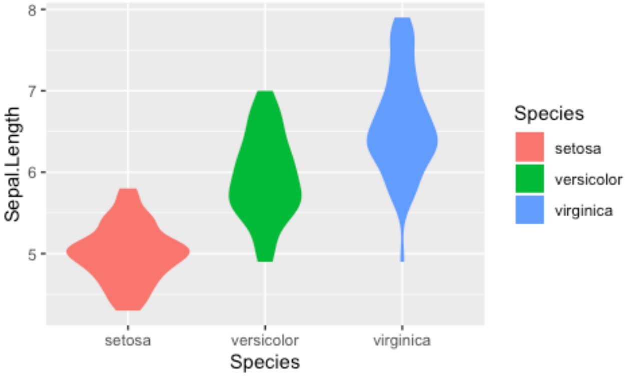


```r
g + theme_dark()
```



# Themes can change the look

```r
g = ggplot(iris,
           aes(x = Species,
               y = Sepal.Length,
               fill = Species))+
  geom_violin(col = NA)
g
```



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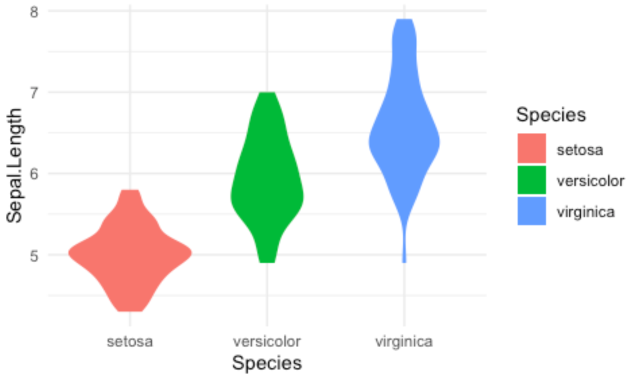
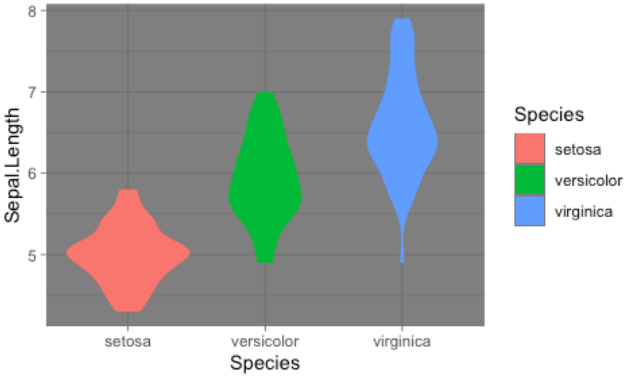
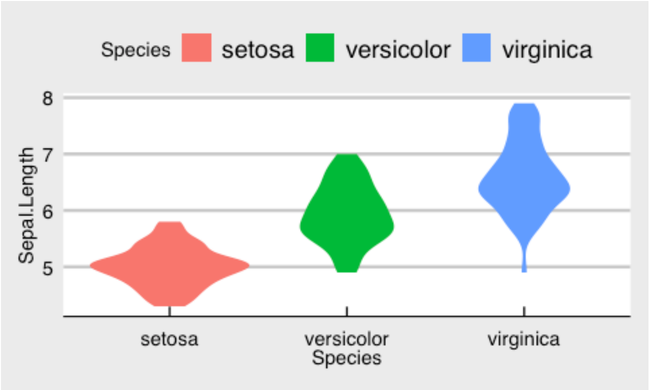
```



```r
g + theme_dark()
```



```r
library(ggthemes)
g + theme_economist_white()
```



# Goals for this lecture

1. Discuss the principles of **good *vs* bad** data viz
2. Review base R plotting
3. Understand the **grammar of graphics** concept
4. Introduce, explain and use the `ggplot()` function
5. Discuss how to plot 1D, 2D, 3-5D data and select the most appropriate plot type. Use faceting
6. Use visualization for the inspection of large datasets and discovery of global trends (e.g. batch effects)
7. Implement interactive (3D) visualization

# 1D plot types

What do you use to show or compare 1D distributions?

# 1D plot types

## What do you use to show or compare 1D distributions?

Boxplot makes sense for **unimodal** distributions

Histogram requires definition of bins/binwidths/break positions. It can create visual artifacts esp. if the number of data points is not large

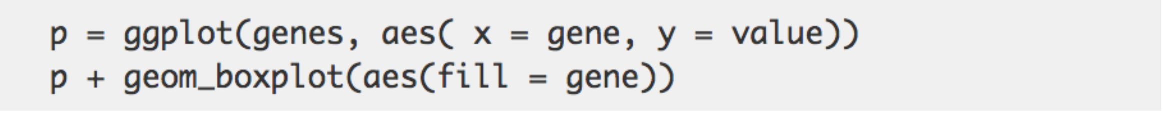
Density requires setting of **bandwidth parameter**; obscures the sample size (i.e. the uncertainty of the estimate)

ECDF (Empirical Cumulative Density Function) does not have these problems, but is more abstract and its interpretation requires more training.

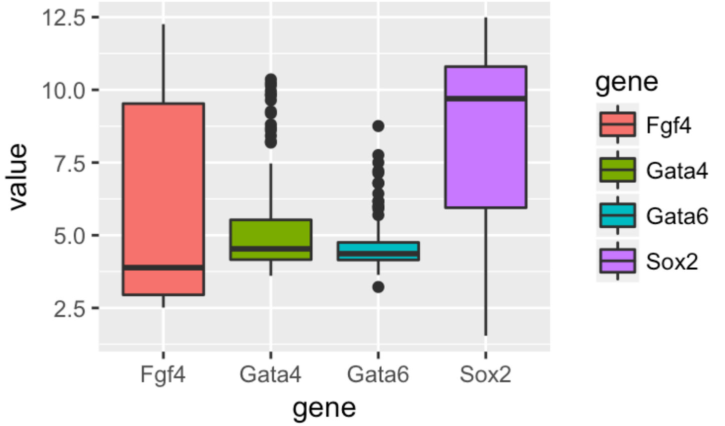
If you have only up to a **few dozens of points** just **show the raw data**! (e.g. with beeswarm)

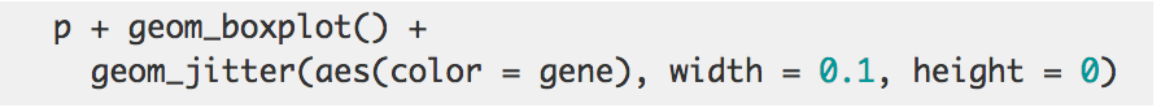
# Boxplot

Boxplots are good for plotting summary of 1D continuous data; they allow you to **compare quantiles of data distributions.**

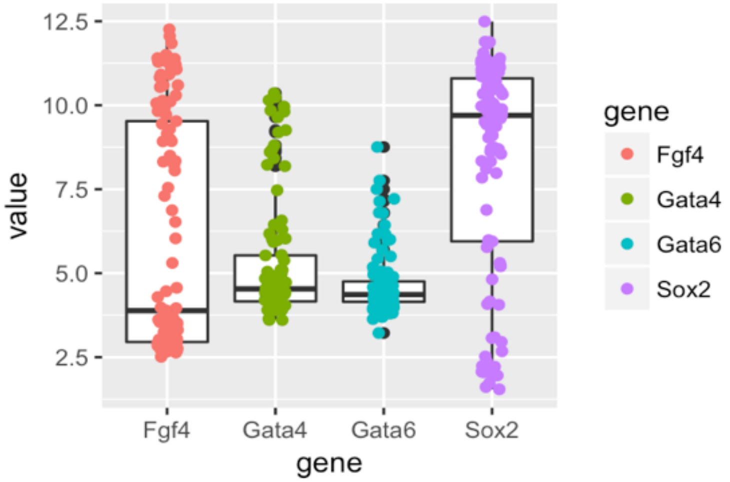


```
p = ggplot(genes, aes( x = gene, y = value))
p + geom_boxplot(aes(fill = gene))
```





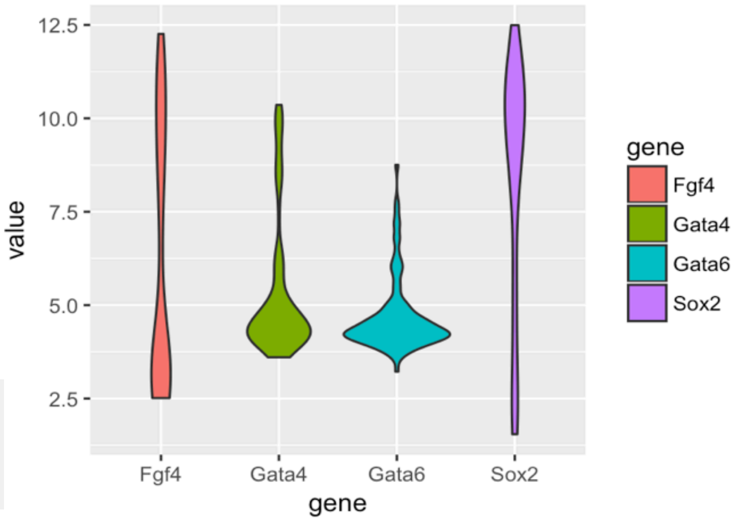
```
p + geom_boxplot() +
  geom_jitter(aes(color = gene), width = 0.1, height = 0)
```



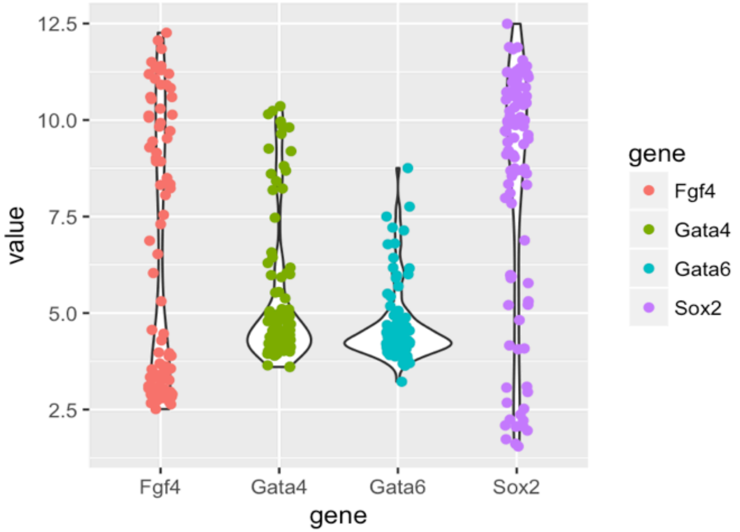
# Violin Plot

If there are many observations in the dataset, we can **show the estimated distribution with violin plots.**

```
p = ggplot(genes, aes( x = gene, y = value))
p + geom_violin(aes(fill =  gene))
```

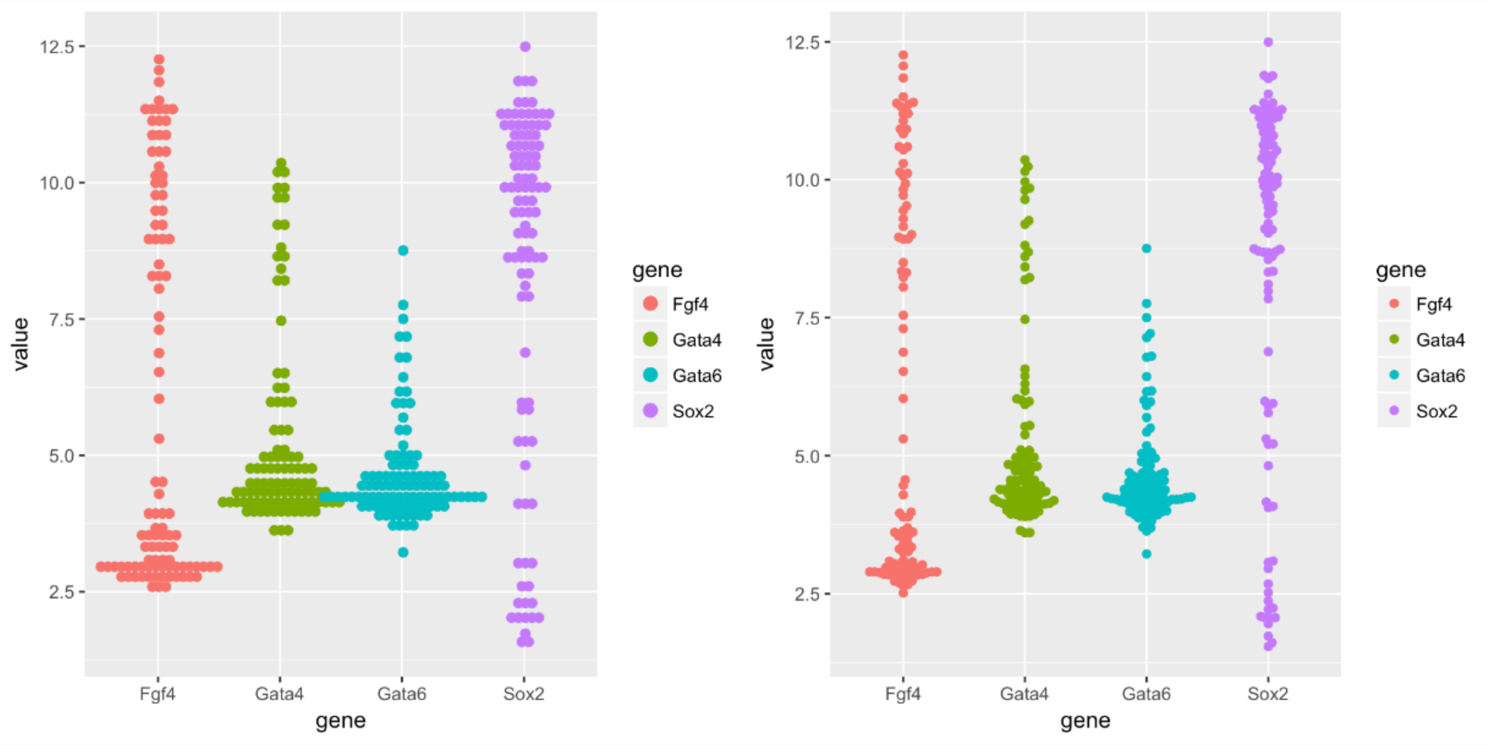


```
p + geom_boxplot() +
  geom_jitter(aes(color = gene), width = 0.1, height = 0)
```



# Dot & Beeswarm Plot

```
p + geom_dotplot(binaxis = "y", binwidth = 1/6,
        stackdir = "center", stackratio = 0.75,
        aes(color = gene))
library("ggbeeswarm")
p + geom_beeswarm(aes(color = gene))
```



# Bar charts with error bars

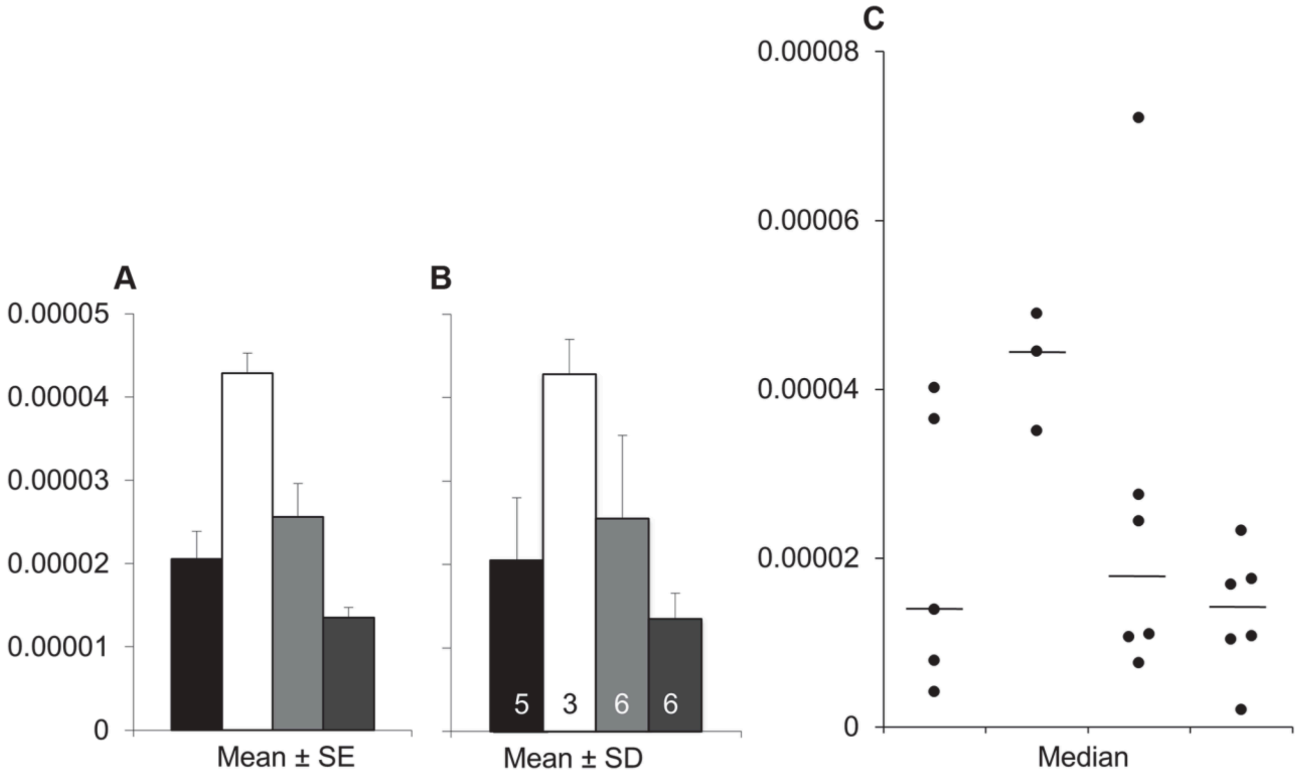


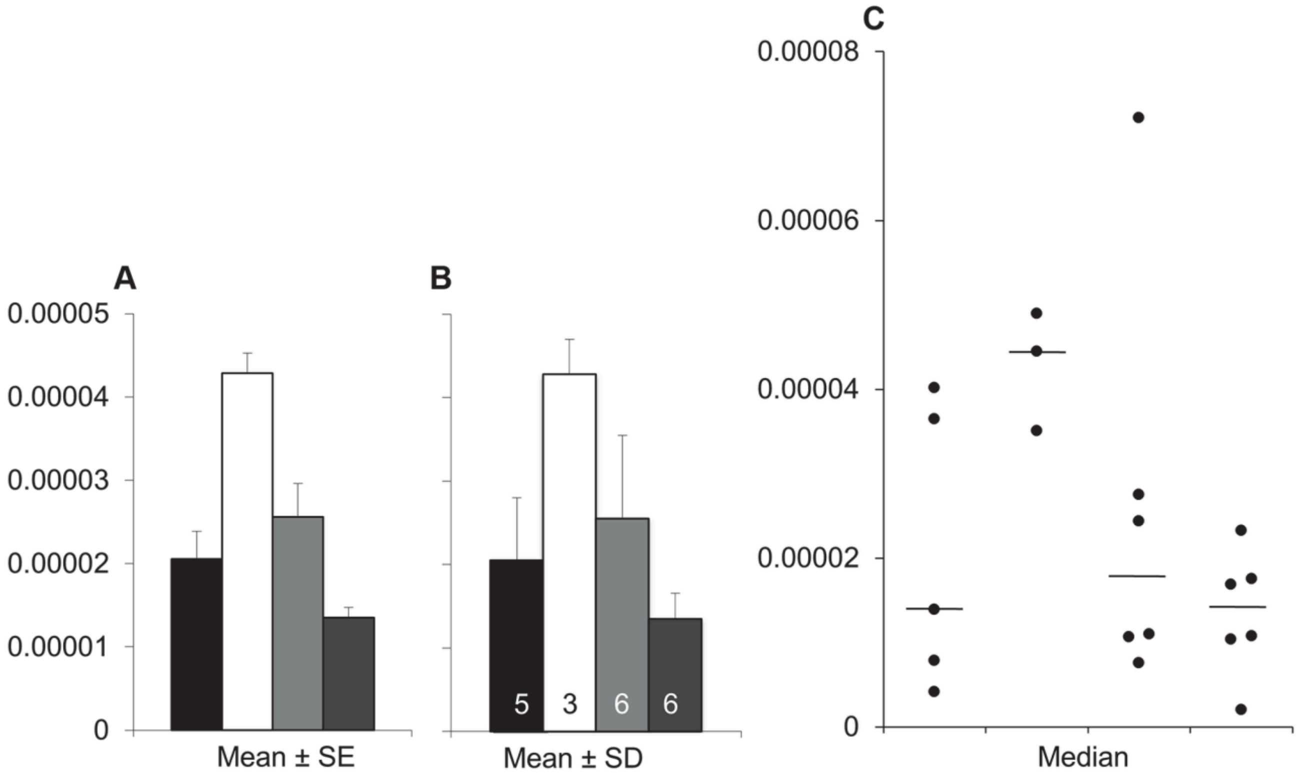
**Fig 3. Bar graphs and scatterplots convey very different information.** While scatterplots prompt the reader to critically evaluate the statistical tests and the authors' interpretation of the data, bar graphs discourage the reader from thinking about these issues. Placental endothelin 1 (*EDN1*) mRNA data for four different groups of participants is presented in bar graphs showing mean ± SE (Panel A), or mean ± SD (Panel B), and in a univariate scatterplot (Panel C). Panel A (mean ± SE) suggests that the second group has higher values than the remaining groups; however, Panel B (mean ± SD) reveals that there is considerable overlap between groups. Showing SE rather than SD magnifies the apparent visual differences between groups, and this is exacerbated by the fact that SE obscures any effect of unequal sample size. The scatterplot (Panel C) clearly shows that the sample sizes are small, group one has a much larger variance than the other groups, and there is an outlier in group three. These problems are not apparent in the bar graphs shown in Panels A and B.

doi:10.1371/journal.pbio.1002128.g003

Weissgerber TL, et al. (2015) Beyond Bar and Line Graphs: Time for a New Data Presentation Paradigm. PLOS Biology 13(4): e1002128.

# Bar charts with error bars

## What is wrong with {bar charts + error bars} ?



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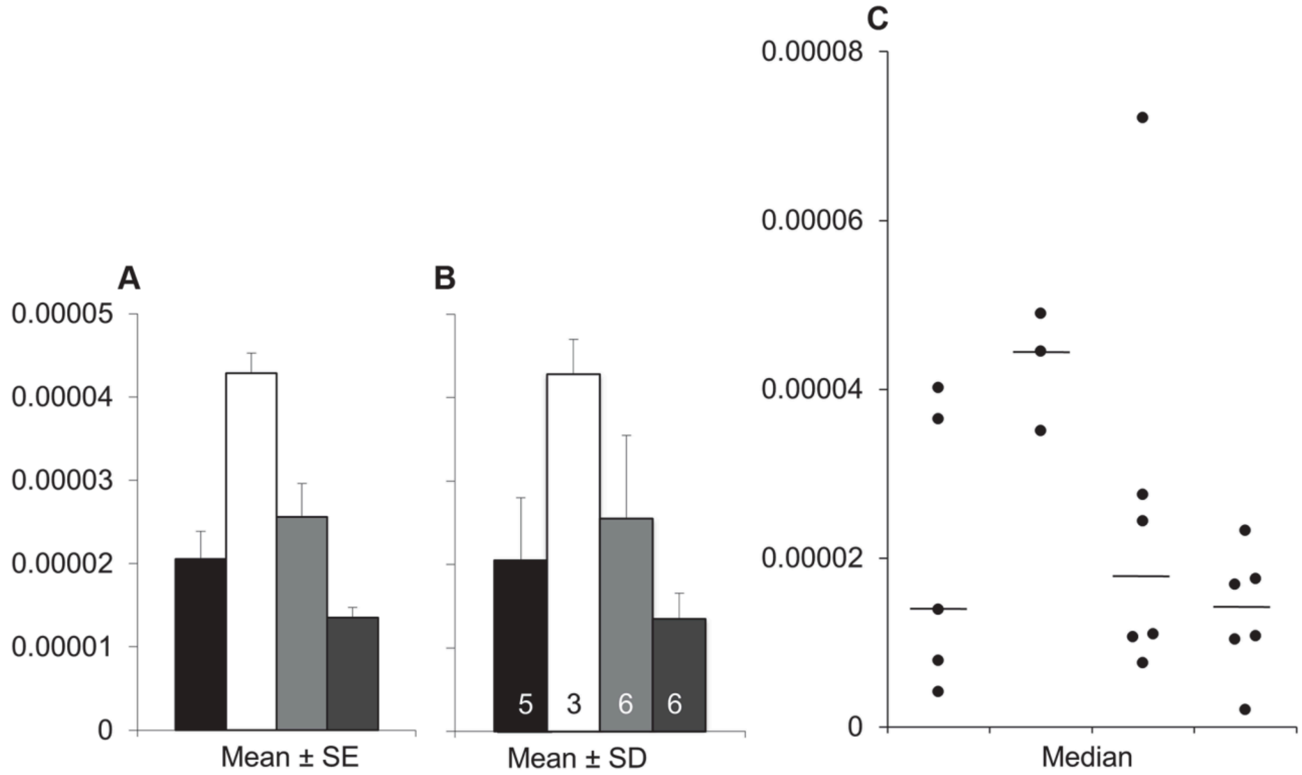

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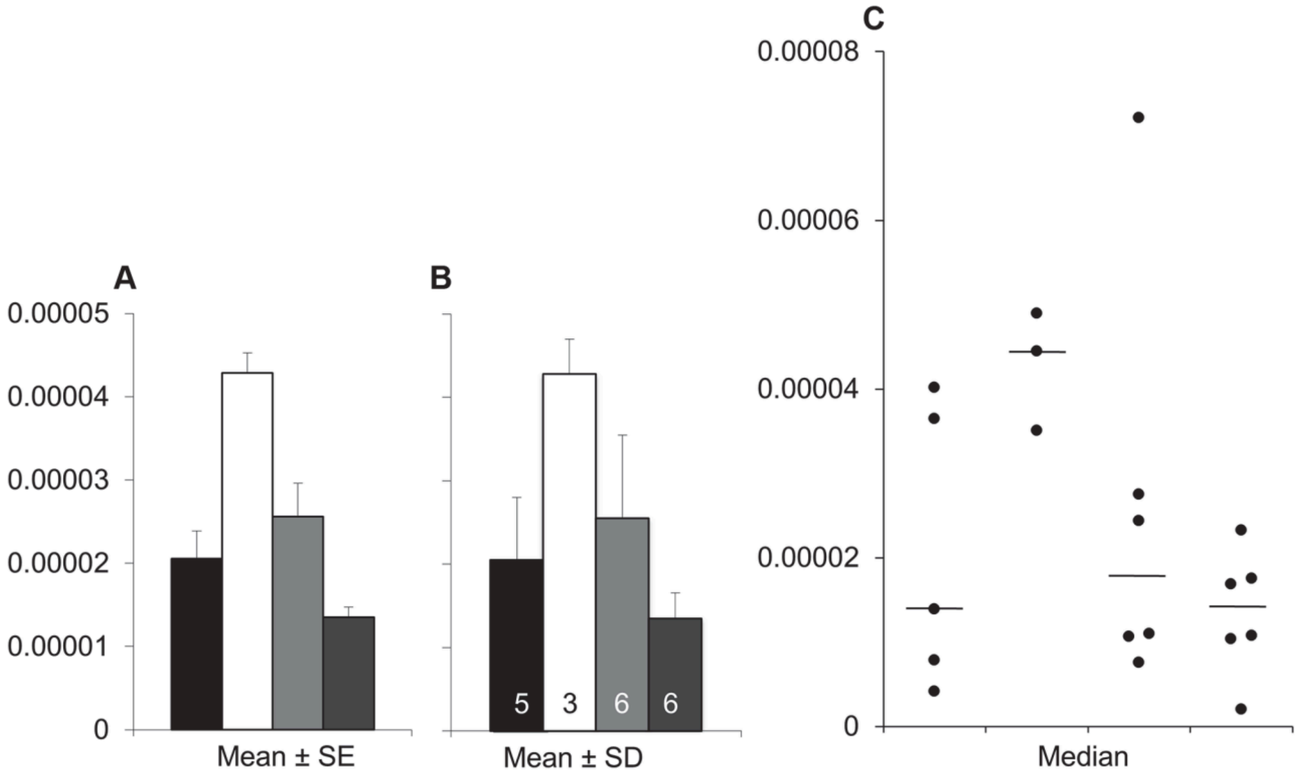
Bar charts (with error bars) **not good for showing distributions**

Use bar charts **only to show class counts.**

# ~~Bar charts with error bars~~

## What is wrong with {bar charts + error bars} ?



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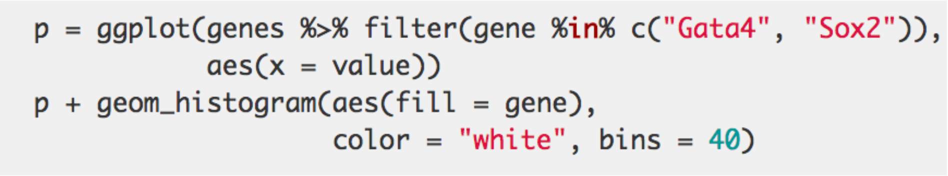
Bar charts (with error bars) **not good for showing distributions**

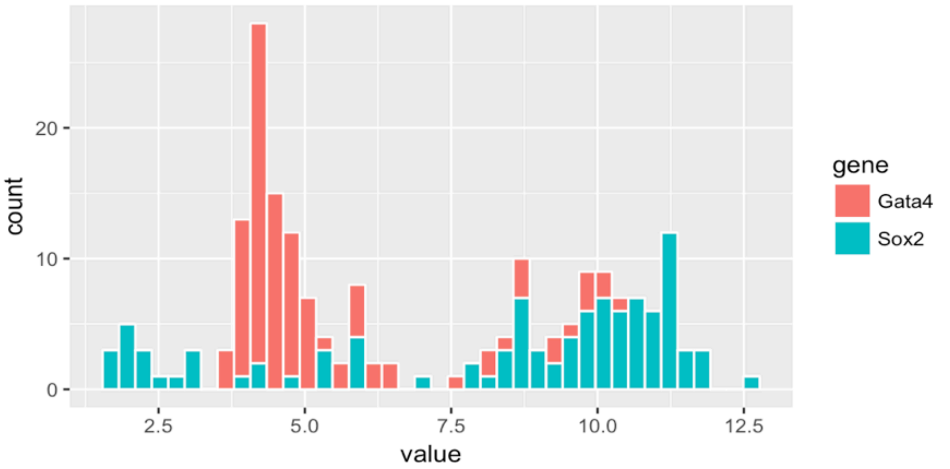
Use bar charts **only to show class counts.**

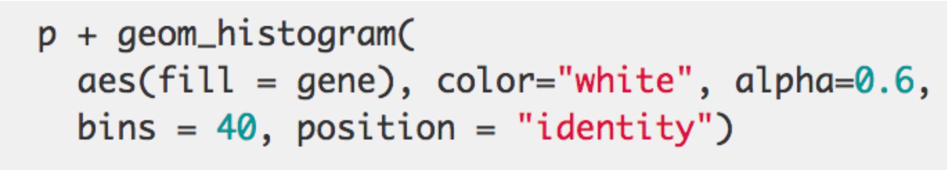
# Histograms



```
p = ggplot(genes %>% filter(gene %in% c("Gata4", "Sox2")),
           aes(x = value))
p + geom_histogram(aes(fill = gene),
                   color = "white", bins = 40)
```

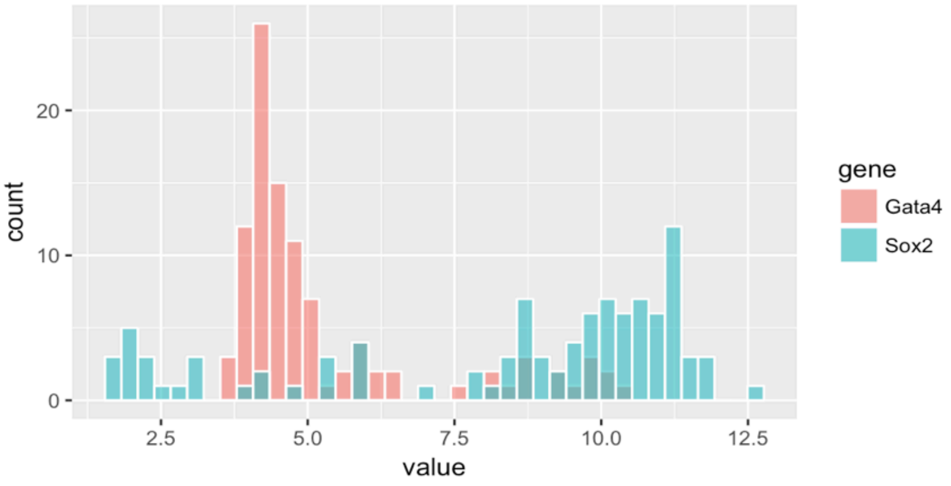
Stacked





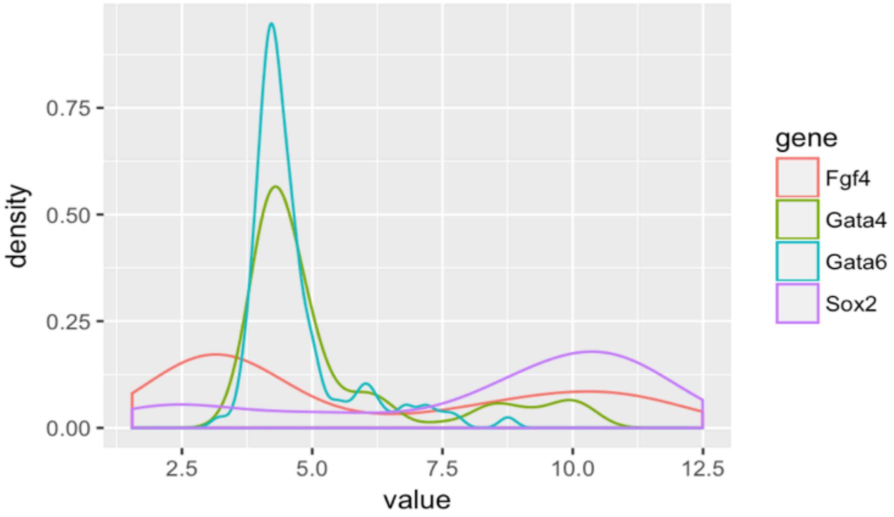
```
p + geom_histogram(
  aes(fill = gene), color="white", alpha=0.6,
  bins = 40, position = "identity")
```

Overlapping

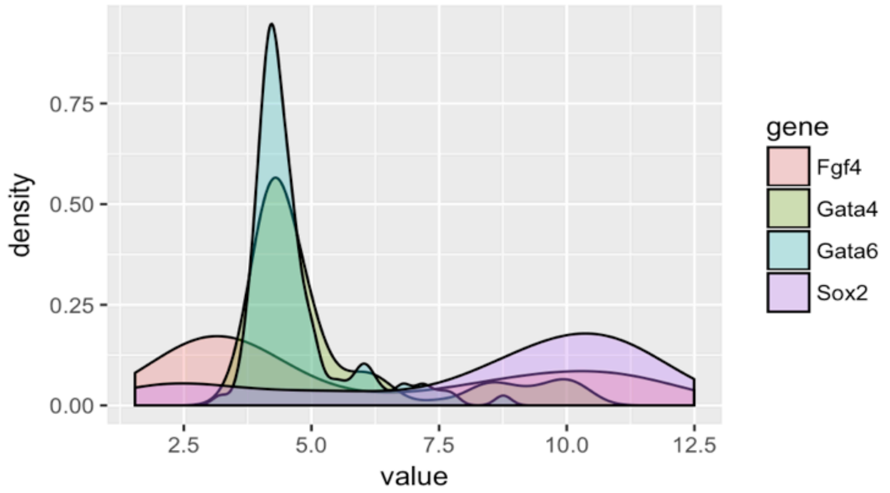


# Density plots

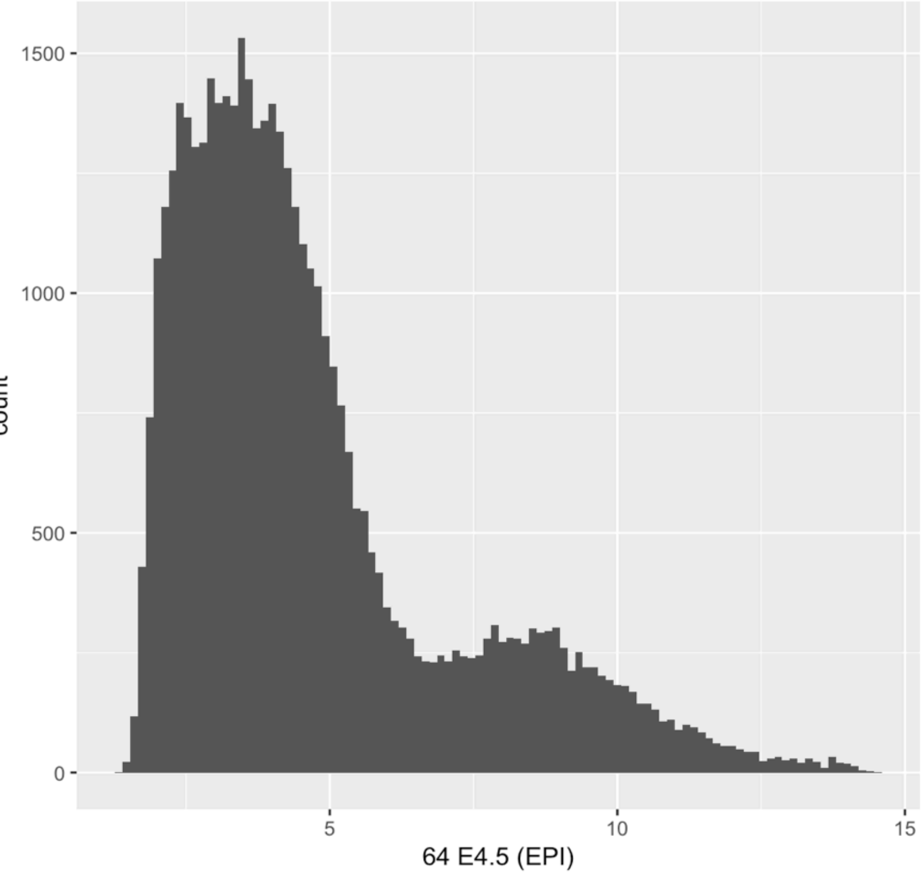
```
p = ggplot(genes, aes( x = value, color = gene))
p + geom_density()
```

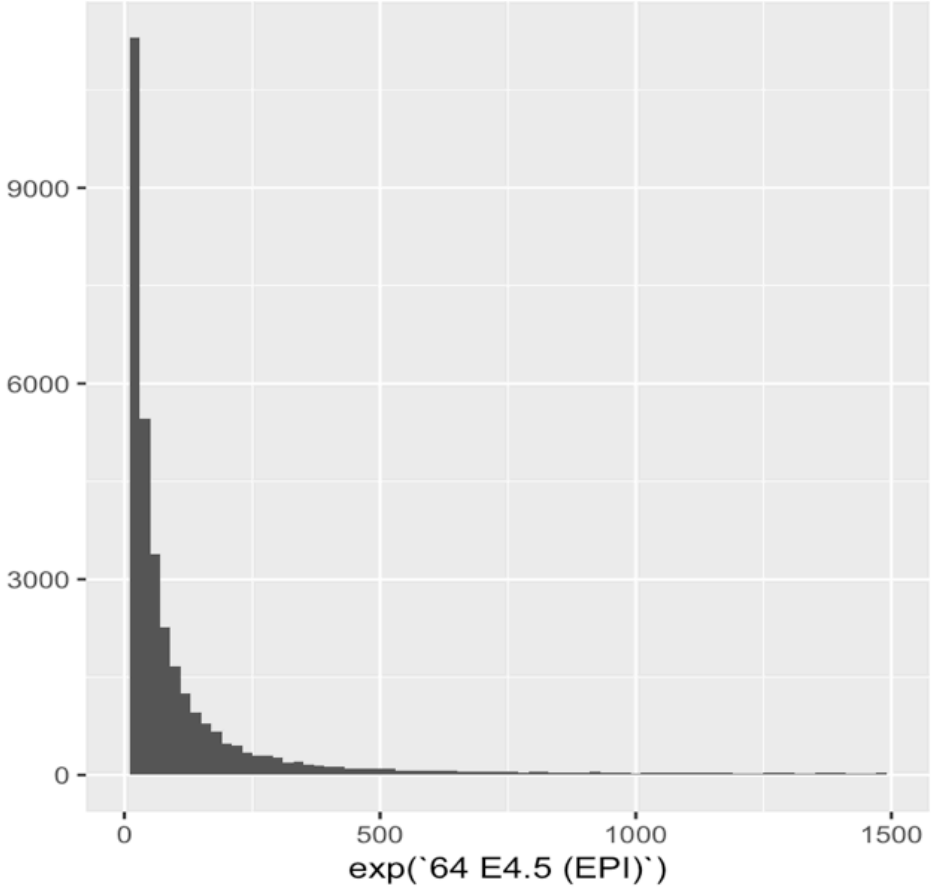


```
p = ggplot(genes, aes( x = value, fill = gene))
p + geom_density(alpha = 0.3)
```



# **Non-linear transformations** change the shape of a density

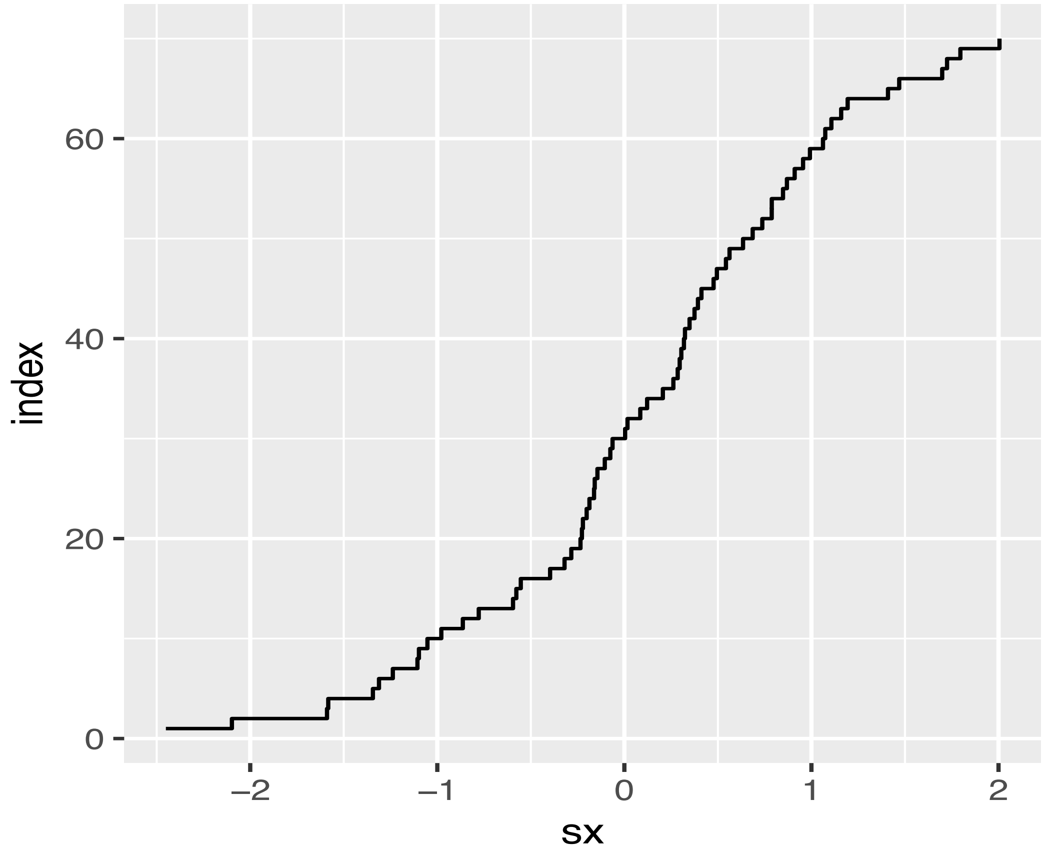




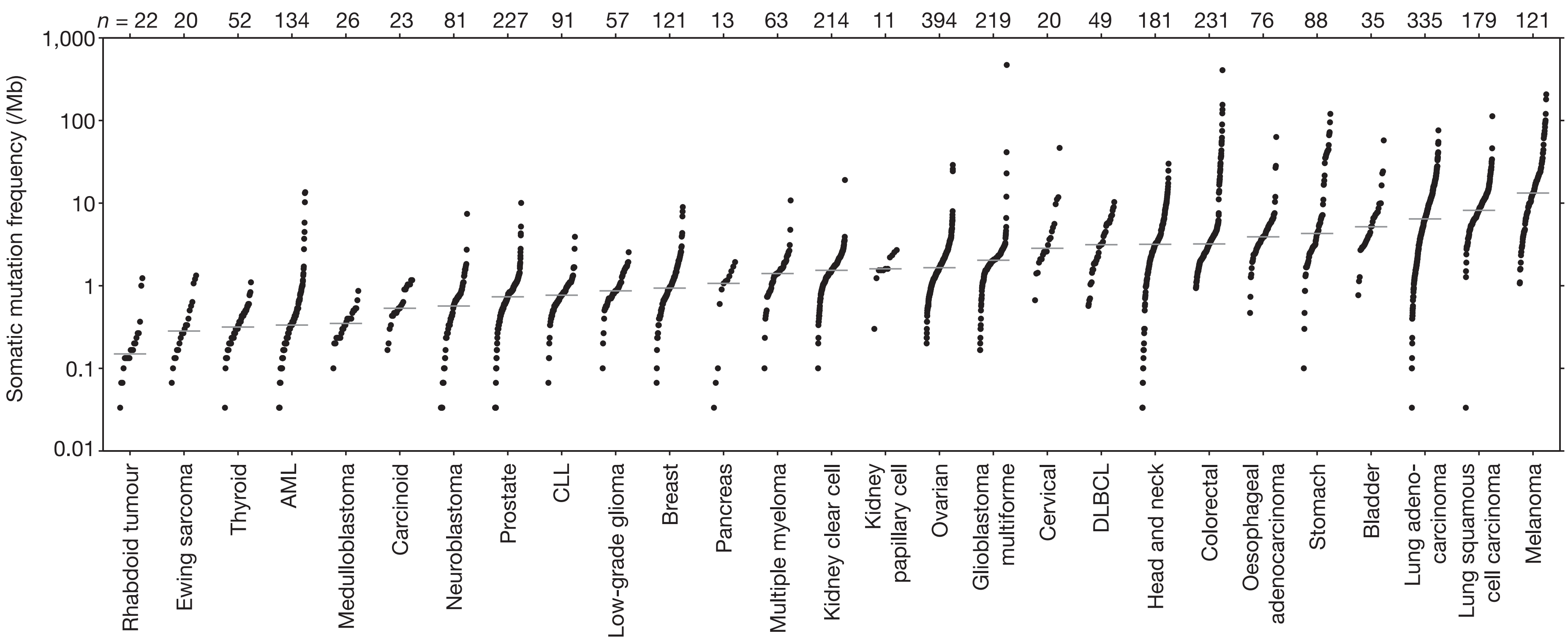
- The **mode** of a distribution is an **infinitesimal concept**.
- Need either an infinite amount of data or choose smoothing / binning bandwidth
- **Number of modes (let alone their positions) can change under non-linear data transformations**

# The empirical cumulative distribution

$$F_n(x) = \frac{\text{number of } i \text{ for which } x_i \leq x}{n} = \frac{1}{n}\sum_{i=1}^{n} \mathbb{1}(x \leq x_i)$$



```
simdata = rnorm(70)
simdf <- data.frame(index = seq(along = simdata), sx = sort(simdata))
ggplot(simdf, aes(x = sx, y = index)) +  geom_step()
```



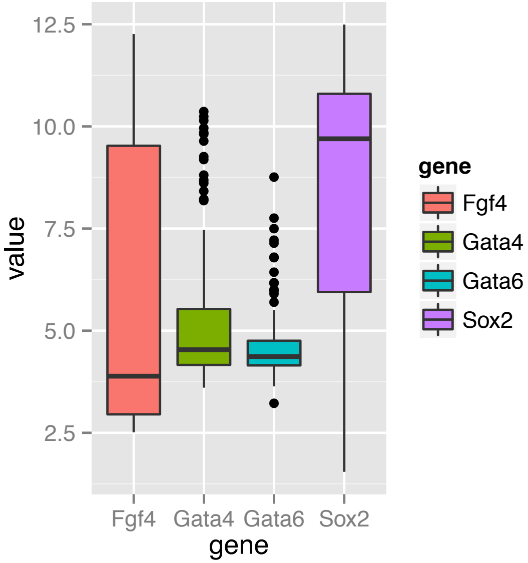
# LETTER

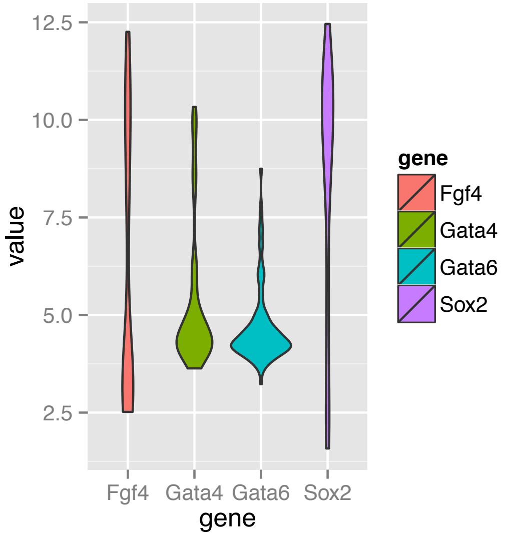
doi:10.1038/nature12213

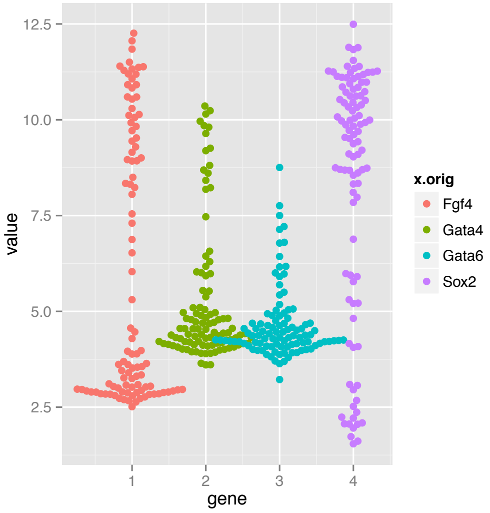
# Mutational heterogeneity in cancer and the search for new cancer-associated genes

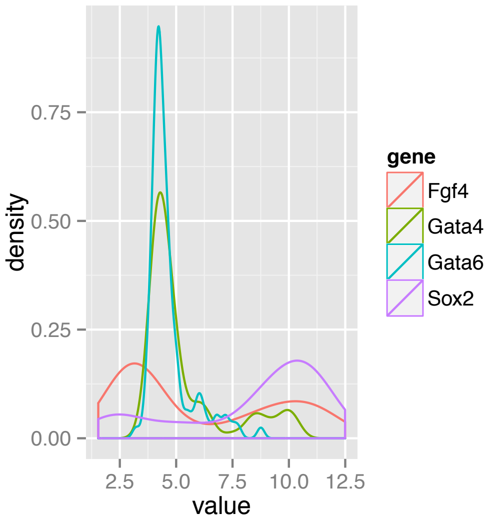
Michael S. Lawrence[1]*, Petar Stojanov[1,2]*, Paz Polak[1,3,4]*, Gregory V. Kryukov[1,3,4], Kristian Cibulskis[1], Andrey Sivachenko[1], Scott L. Carter[1], Chip Stewart[1], Craig H. Mermel[1,5], Steven A. Roberts[6], Adam Kiezun[1], Peter S. Hammerman[1,2], Aaron McKenna[1,7], Yotam Drier[1,3,5,8], Lihua Zou[1], Alex H. Ramos[1], Trevor J. Pugh[1,2,3], Nicolas Stransky[1,9], Elena Helman[1,10], Jaegil Kim[1], Carrie Sougnez[1], Lauren Ambrogio[1], Elizabeth Nickerson[1], Erica Shefler[1], Maria L. Cortés[1], Daniel Auclair[1], Gordon Saksena[1], Douglas Voet[1], Michael Noble[1], Daniel DiCara[1], Pei Lin[1], Lee Lichtenstein[1], David I. Heiman[1], Timothy Fennell[1], Marcin Imielinski[1,5], Bryan Hernandez[1], Eran Hodis[1,2], Sylvan Baca[1,2], Austin M. Dulak[1,2], Jens Lohr[1,2], Dan-Avi Landau[1,2,11], Catherine J. Wu[2,3], Jorge Melendez-Zajgla[12], Alfredo Hidalgo-Miranda[12], Amnon Koren[1,3], Steven A. McCarroll[1,3], Jaume Mora[13], Ryan S. Lee[2,3,14], Brian Crompton[2,14], Robert Onofrio[1], Melissa Parkin[1], Wendy Winckler[1], Kristin Ardlie[1], Stacey B. Gabriel[1], Charles W. M. Roberts[2,3,14], Jaclyn A. Biegel[15], Kimberly Stegmaier[1,2,14], Adam J. Bass[1,2,3], Levi A. Garraway[1,2,3], Matthew Meyerson[1,2,3], Todd R. Golub[1,2,3,8], Dmitry A. Gordenin[6], Shamil Sunyaev[1,3,4], Eric S. Lander[1,3,10] & Gad Getz[1,5]

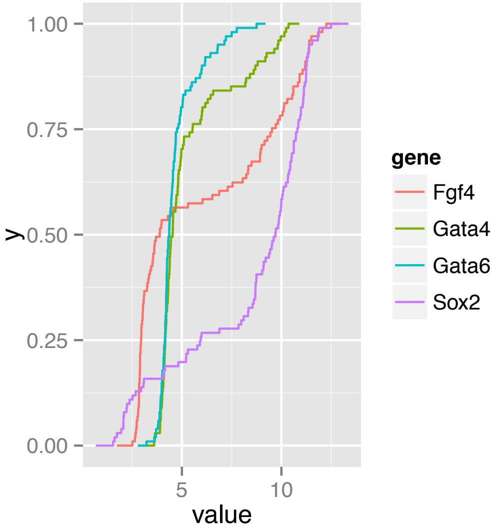
# Summary: Visualizing distributions in 1D











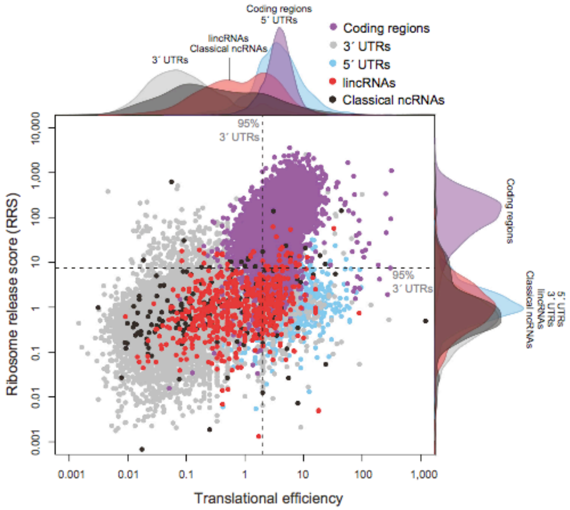
# 2D data plots

# 2D data plots

Scatterplots (x,y)-point plots

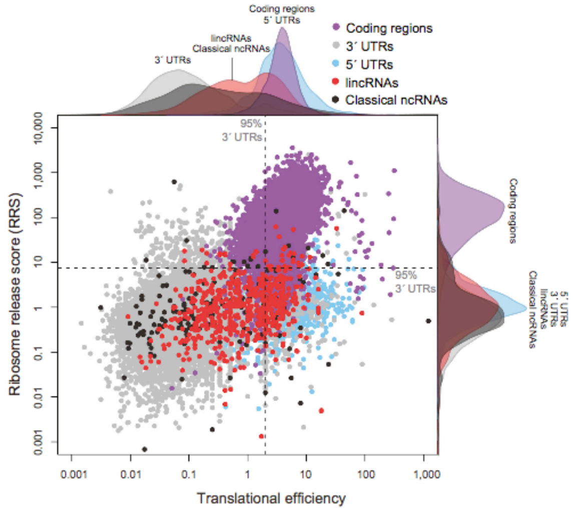
# 2D data plots

Scatterplots (x,y)-point plots

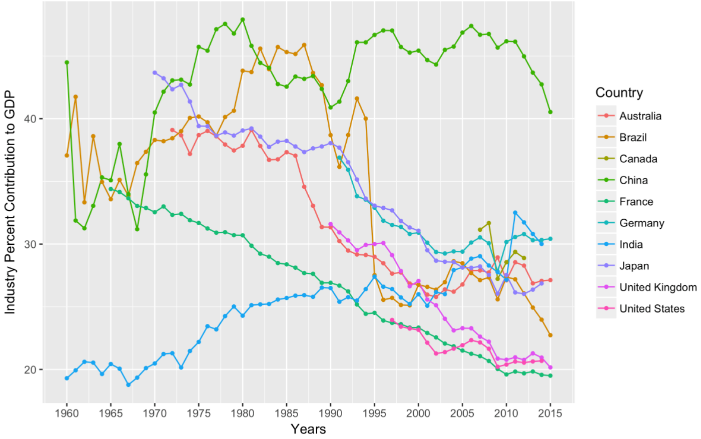


# 2D data plots

Scatterplots (x,y)-point plots

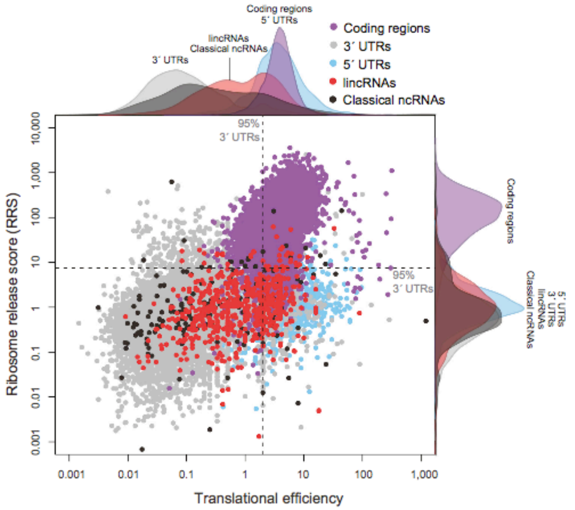


Line plots (x,y)-line plots

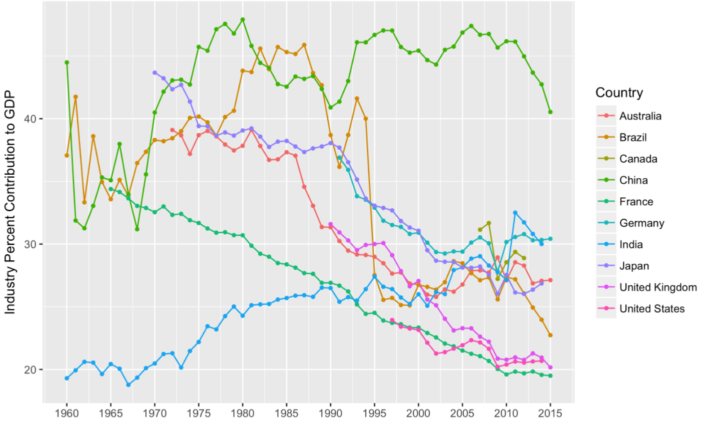


# 2D data plots

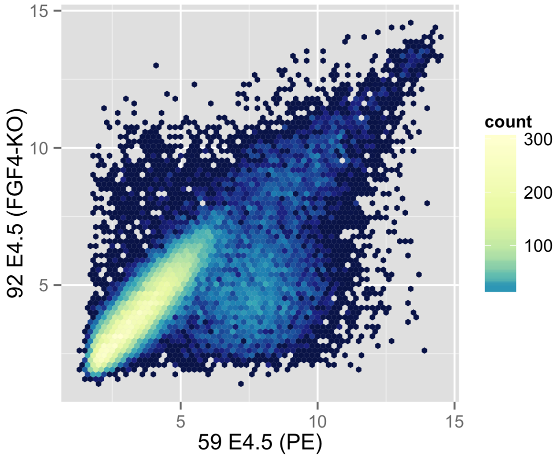
Scatterplots (x,y)-point plots



Line plots (x,y)-line plots

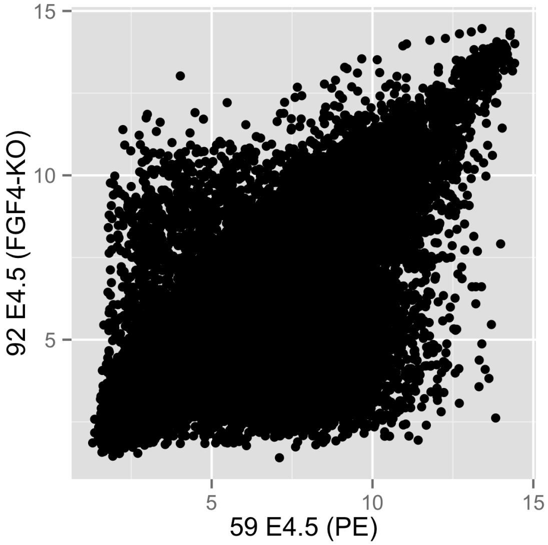


2D density requires the choice of bandwidth; obscures the sample size (i.e. the uncertainty of the estimate)



# Showing distributions in 2D

```
scp <- ggplot(dfx, aes( x = ‘59 E4.5 (PE)‘ ,
                        y = ‘92 E4.5 (FGF4-KO)‘))
scp + geom_point()
```



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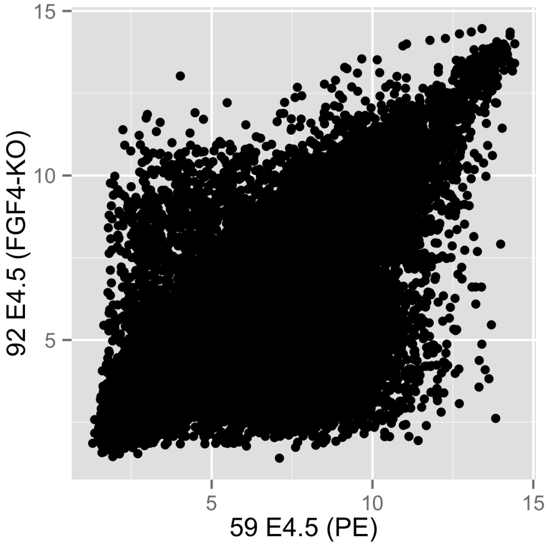
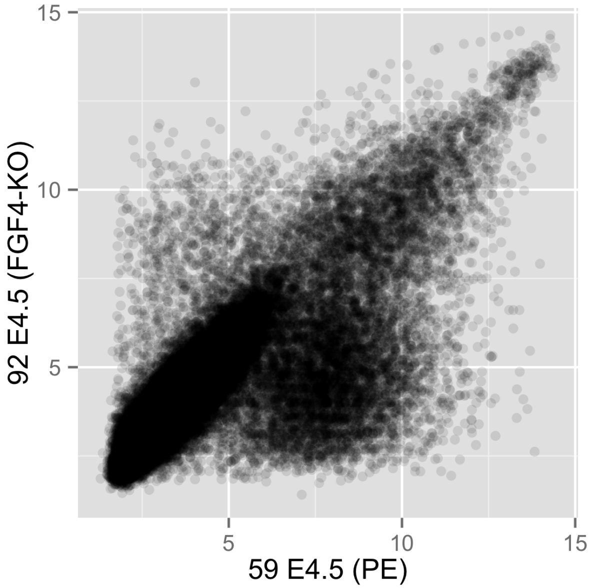
scp + geom_point()
```



```
scp  + geom_point(alpha = 0.1)
```



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```
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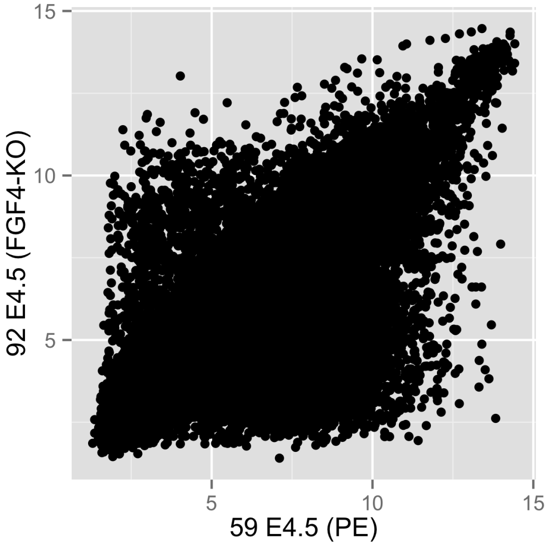
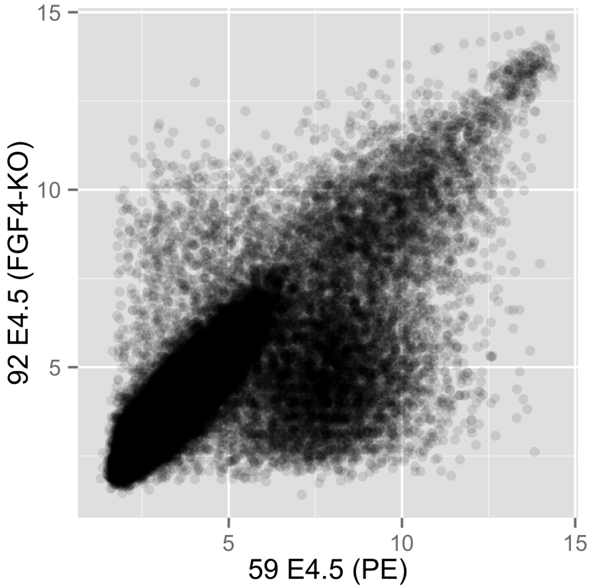
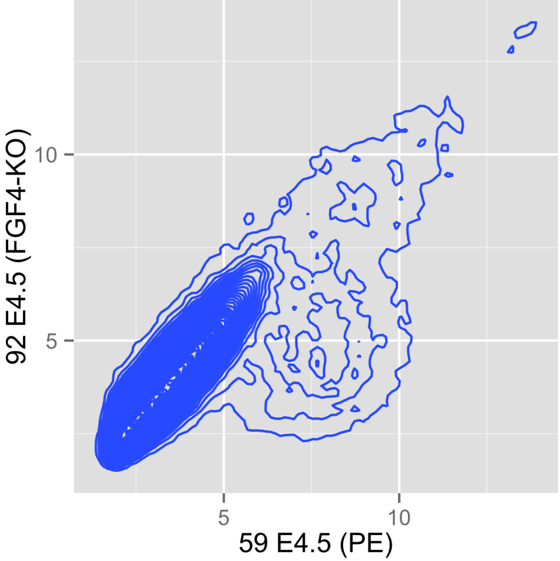
scp + geom_point()
```



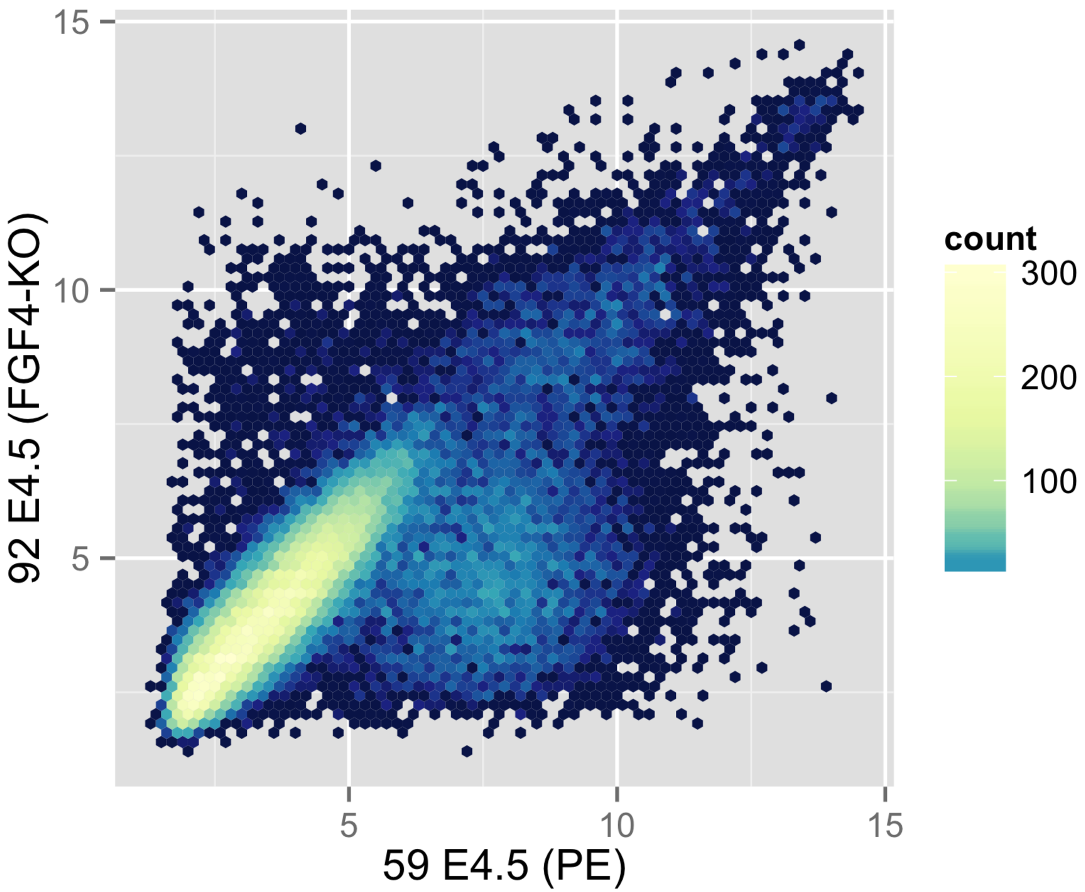


```
scp  + geom_point(alpha = 0.1)
```



```
scp + geom_density2d(h = 0.5, bins = 60)
```

## **binhex** is a good, easy to read, option to show 2D density



```
scp + stat_binhex(binwidth = c(0.2, 0.2)) + colourscale +
  coord_fixed()
```

# How to show more than 2D?

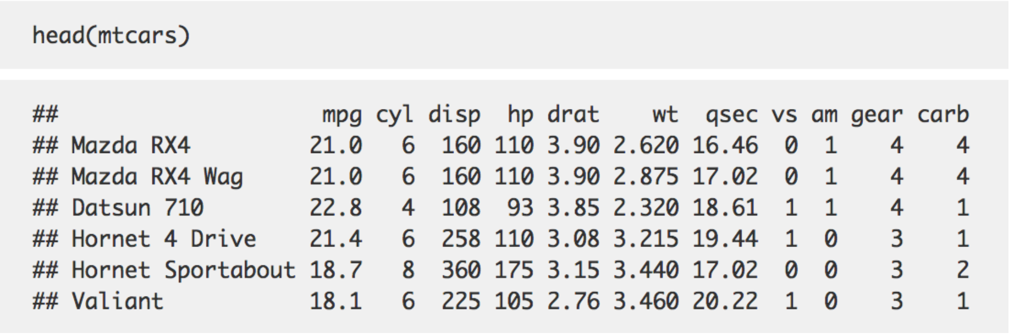
# 3-5D: **aesthetics** allow to show more than 2D

**geom_point's**

aesthetics

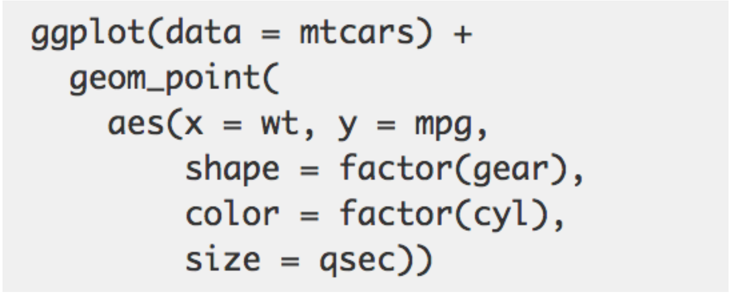
(apart from x and y):

- fill / color
- shape
- size
- alpha

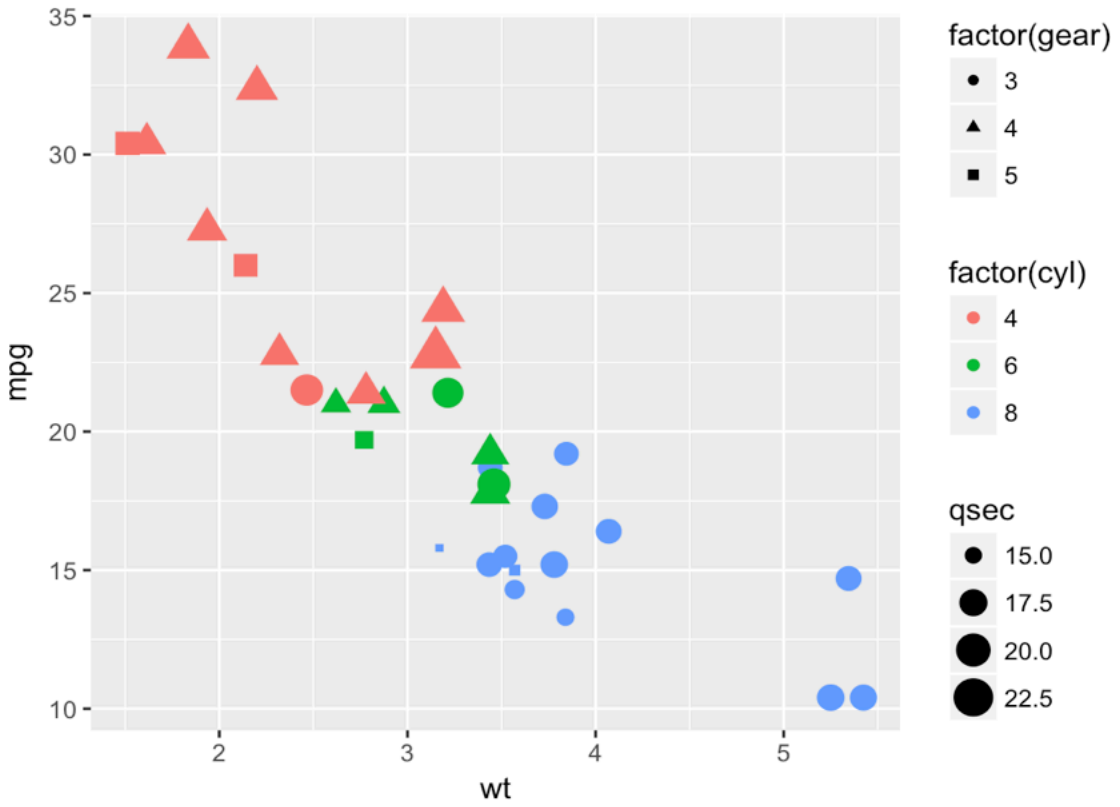


```
head(mtcars)
```

```
##                    mpg cyl disp  hp drat    wt  qsec vs am gear carb
## Mazda RX4         21.0   6  160 110 3.90 2.620 16.46  0  1    4    4
## Mazda RX4 Wag     21.0   6  160 110 3.90 2.875 17.02  0  1    4    4
## Datsun 710        22.8   4  108  93 3.85 2.320 18.61  1  1    4    1
## Hornet 4 Drive    21.4   6  258 110 3.08 3.215 19.44  1  0    3    1
## Hornet Sportabout 18.7   8  360 175 3.15 3.440 17.02  0  0    3    2
## Valiant           18.1   6  225 105 2.76 3.460 20.22  1  0    3    1
```



```
ggplot(data = mtcars) +
  geom_point(
    aes(x = wt, y = mpg,
        shape = factor(gear),
        color = factor(cyl),
        size = qsec))
```



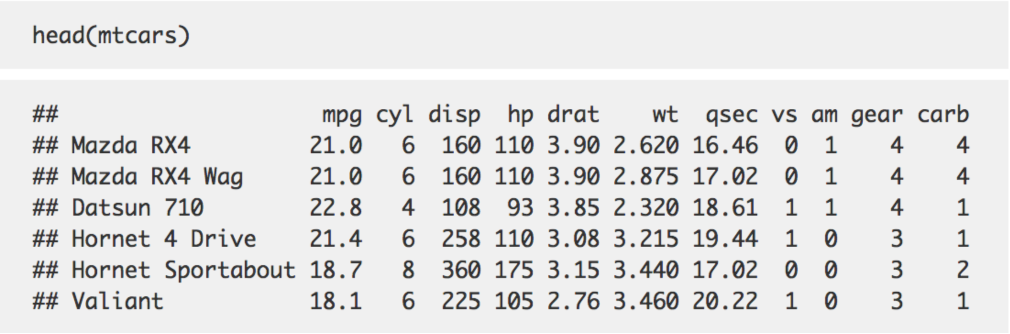
# 3-5D: **aesthetics** allow to show more than 2D

**geom_point's**

aesthetics

(apart from x and y):

- fill / color
- shape
- size
- alpha



```
head(mtcars)
```

```
##                    mpg cyl disp  hp drat    wt  qsec vs am gear carb
## Mazda RX4         21.0   6  160 110 3.90 2.620 16.46  0  1    4    4
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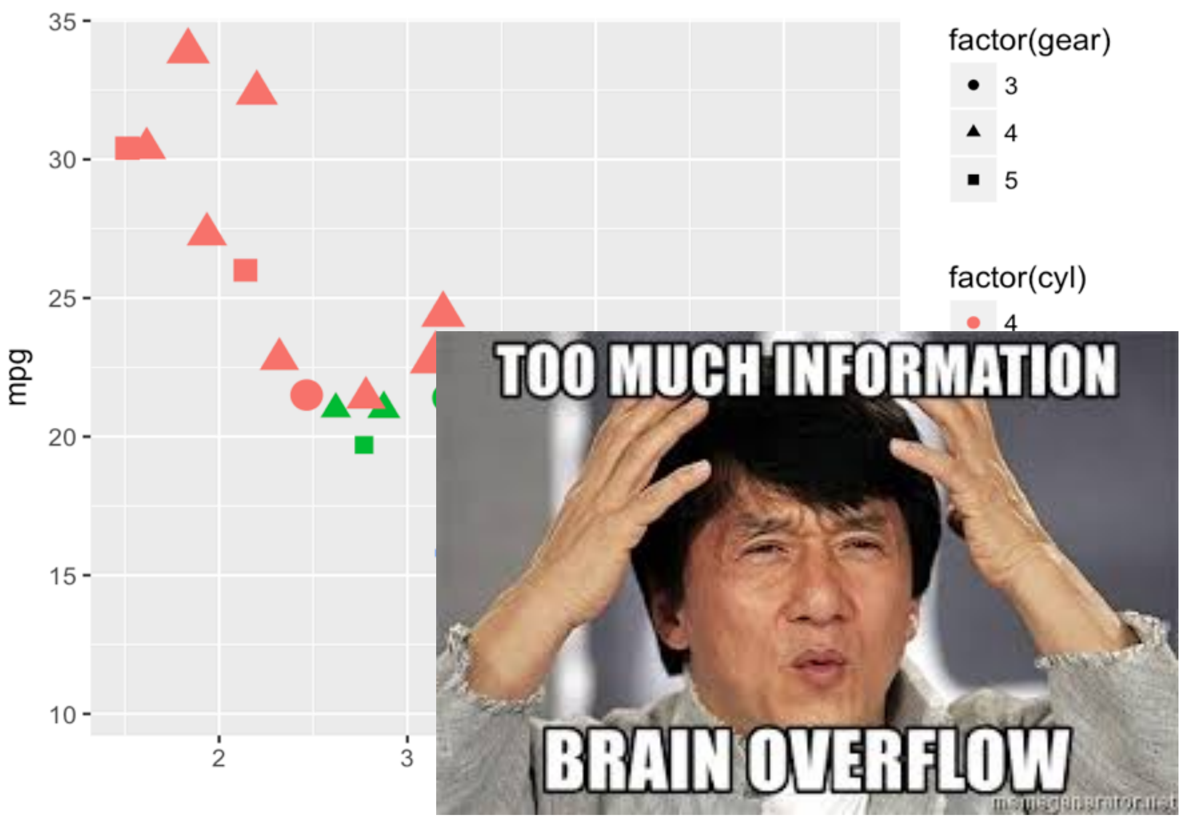
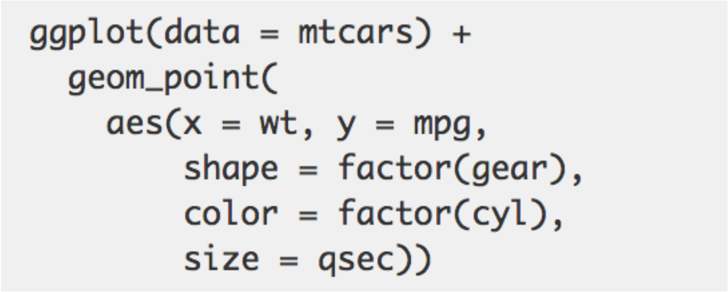
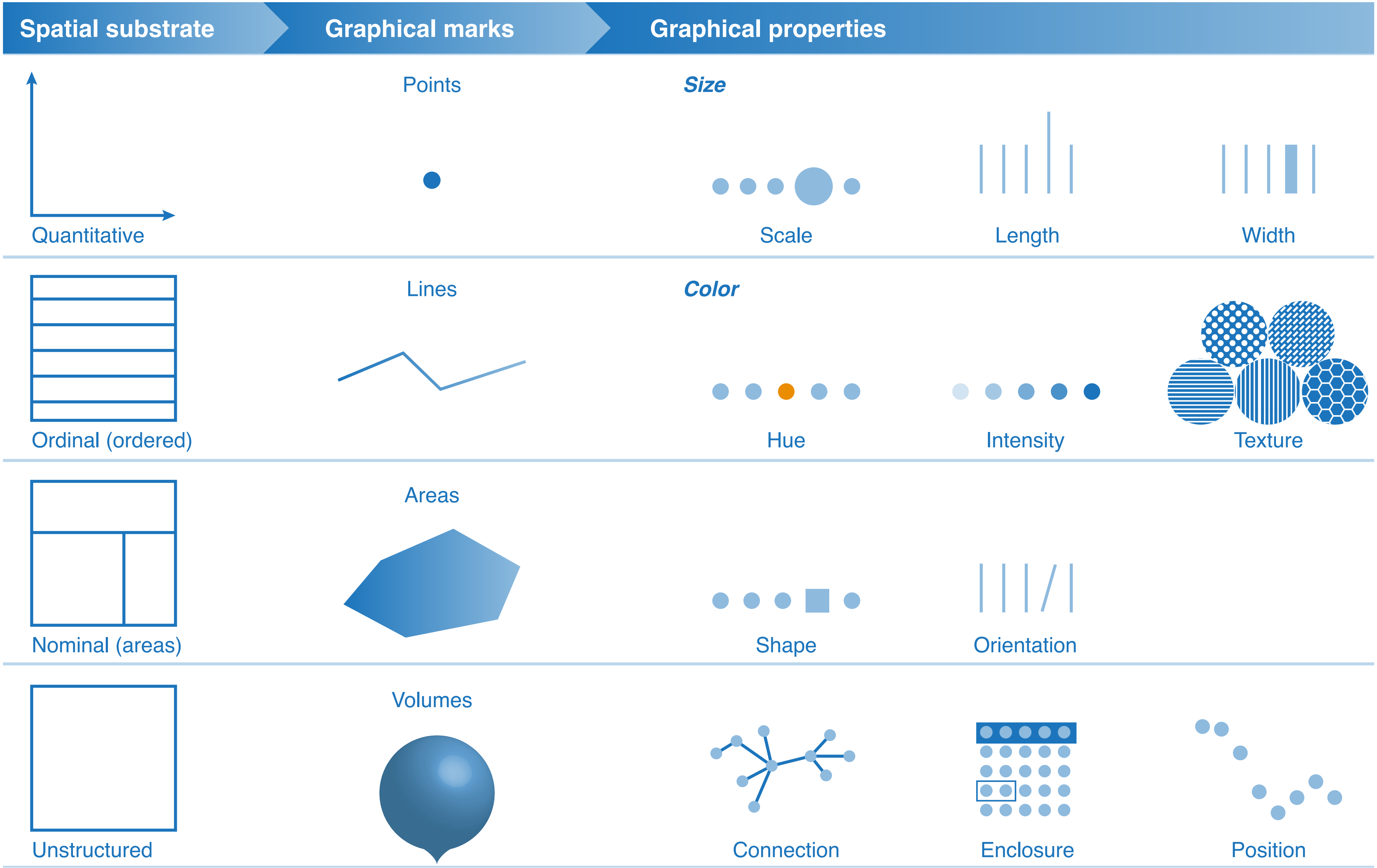
## Datsun 710        22.8   4  108  93 3.85 2.320 18.61  1  1    4    1
## Hornet 4 Drive    21.4   6  258 110 3.08 3.215 19.44  1  0    3    1
## Hornet Sportabout 18.7   8  360 175 3.15 3.440 17.02  0  0    3    2
## Valiant           18.1   6  225 105 2.76 3.460 20.22  1  0    3    1
```





```
ggplot(data = mtcars) +
  geom_point(
    aes(x = wt, y = mpg,
        shape = factor(gear),
        color = factor(cyl),
        size = qsec))
```

# A diversity of **graphical properties (aesthetics)** are available to show dimensions



**Spatial substrate**

**Graphical marks**

**Graphical properties**

Points

***Size***

Quantitative

Scale

Length

Width

Lines

***Color***

Ordinal (ordered)

Hue

Intensity

Texture

Areas

Nominal (areas)

Shape

Orientation

Volumes

Unstructured

Connection

Enclosure

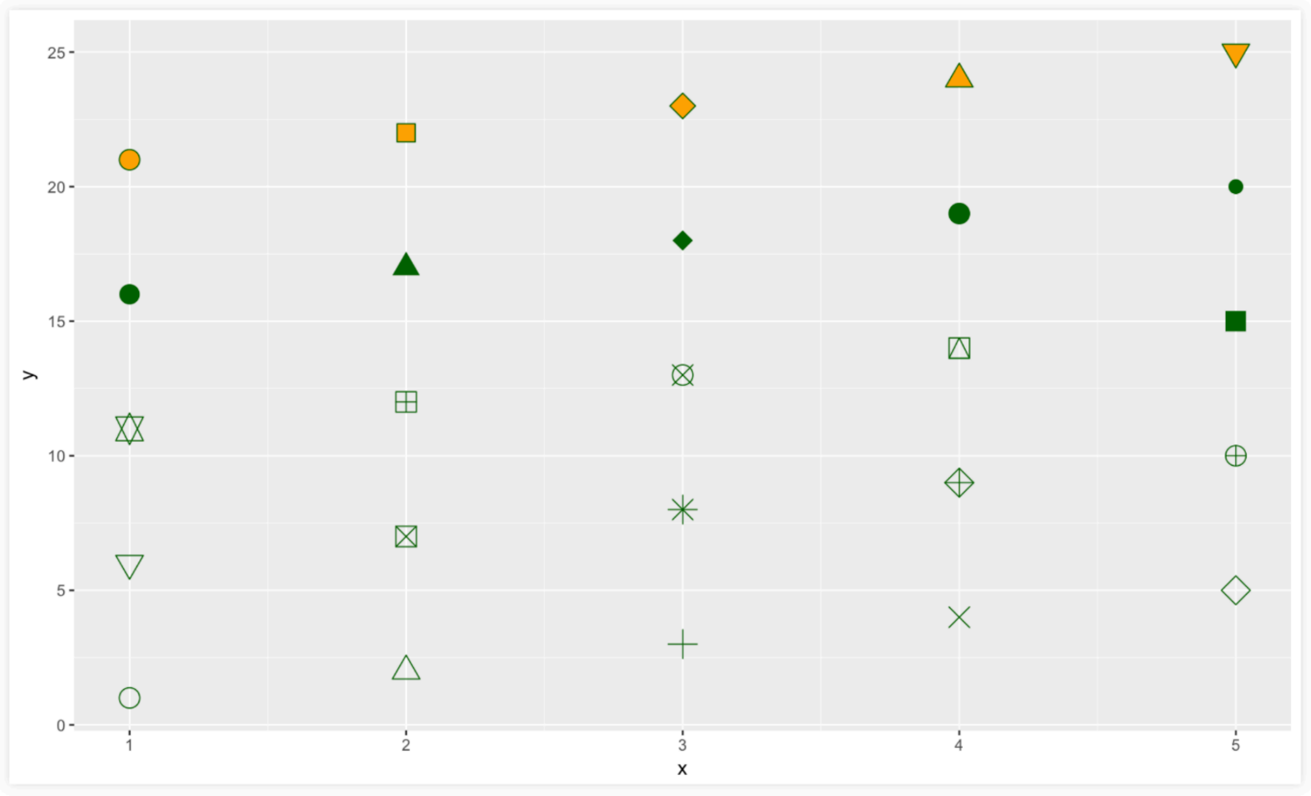
Position

# Marker shapes and colors in R

**geom_point's**

aesthetics
(beyond x and y):

- **fill / color**
- **shape**
- size
- alpha

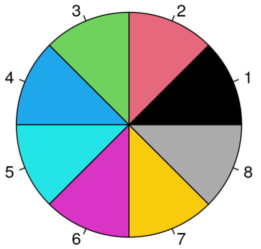


```
ggplot(data.frame(x = 1:5 , y = 1:25, z = 1:25), aes(x = x, y = y)) +
  geom_point(aes(shape = z), size = 5, colour = "darkgreen", fill = "orange") +
  scale_shape_identity()
```

# Color Usage

**Default** color scheme in base R plot:

```
pie(rep(1, 8), col=1:8)
```



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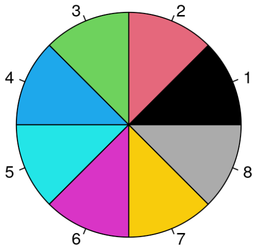
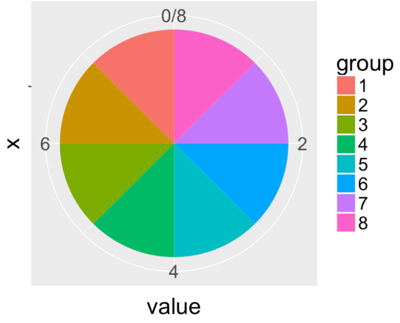


**Default** color scheme in ggplot:

```
ggplot(data.frame(group = factor(seq_len(8)), value = rep(1, 8)),
       aes(x="", y=value, fill=group)) +
  geom_bar(width = 1, stat = "identity") +
  coord_polar("y", start=0) +
  theme(text = element_text(size = 20))
```



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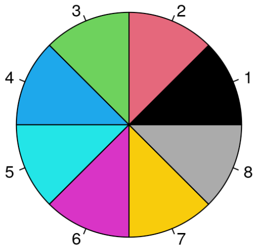

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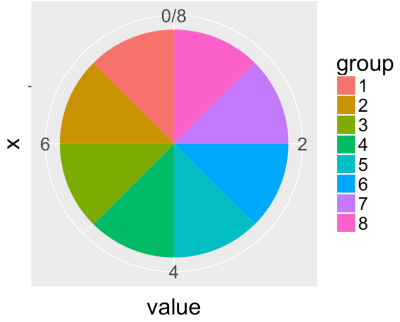
ggplot(data.frame(group = factor(seq_len(8)), value = rep(1, 8)),
       aes(x="", y=value, fill=group)) +
  geom_bar(width = 1, stat = "identity") +
  coord_polar("y", start=0) +
  theme(text = element_text(size = 20))
```



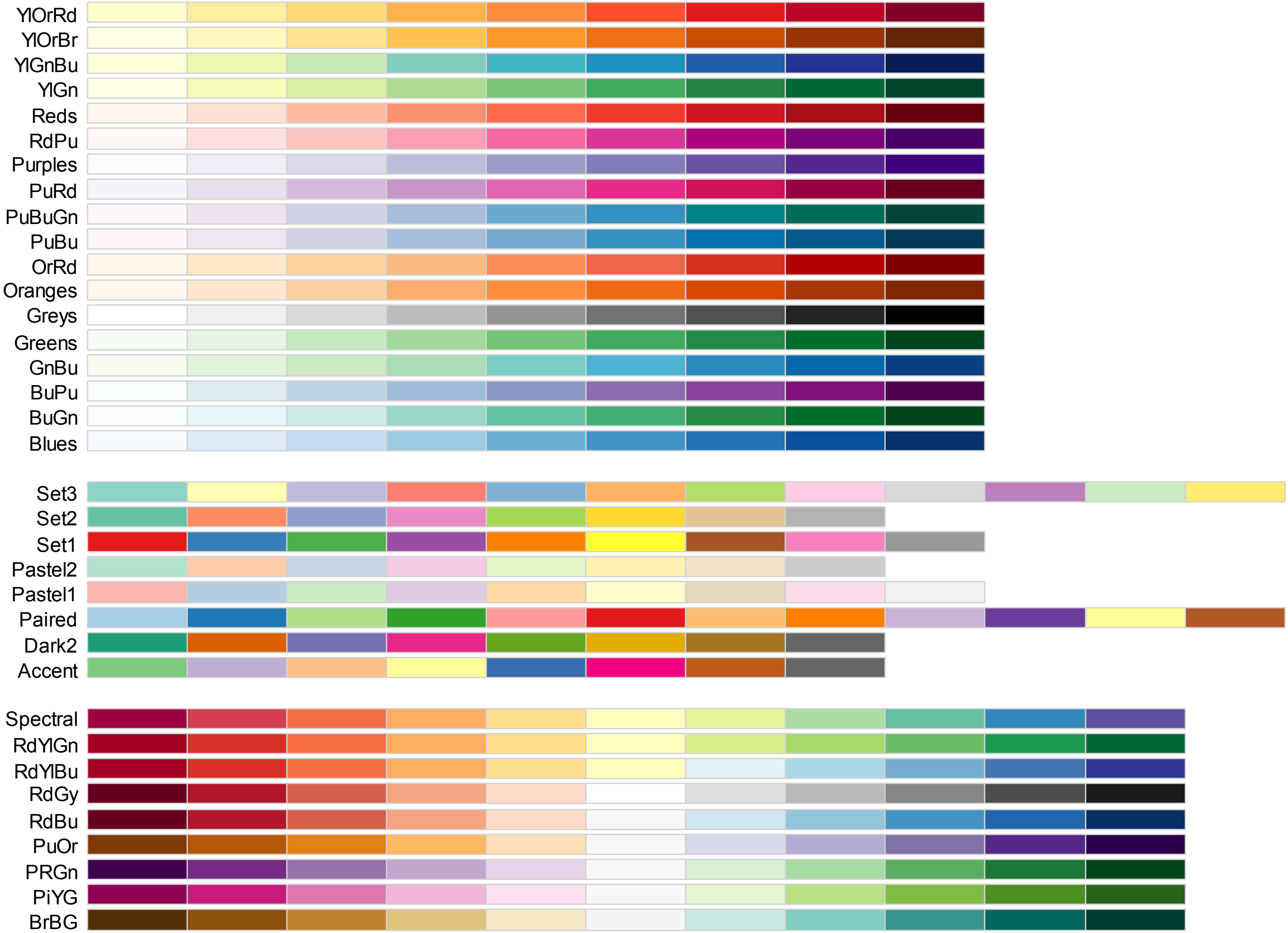
When choosing a coloring scheme, consider these:

- Different requirements for line & area colors
- Many people are **red-green color-blind**
- Lighter colors tend to make areas look larger than darker colors

  → **use colors of equal luminance for filled areas**.

# RColorBrewer

```
display.brewer.all()
```



**sequential**

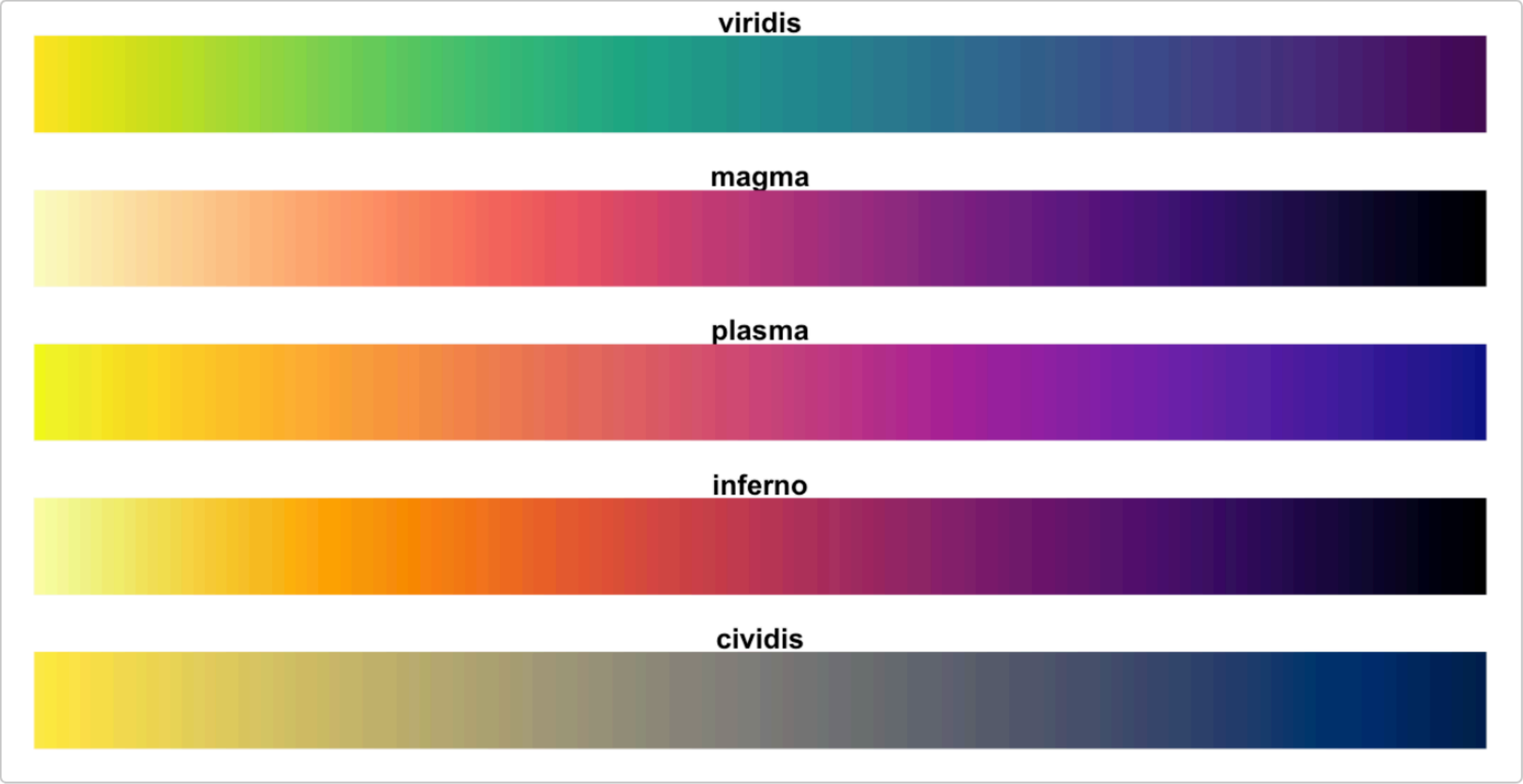
**qualitative**

**diverging**

# Viridis Palettes

```
install.packages("viridis")
library(viridis)
```

Simply add: `scale_color_viridis()` `scale_fill_viridis()`. to your plot

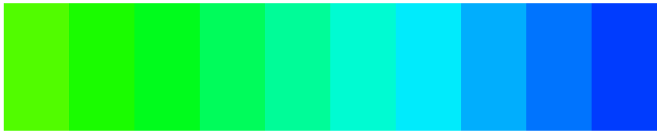


# Viridis Palettes

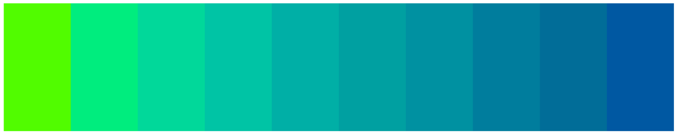
Color scales are designed to be:

- **Colorful and Pretty**, spanning as wide a palette as possible so as to make differences easy to see,
- **Perceptually uniform**, the perceived difference between two colors is proportional to the Euclidian distance within the color space
- **Robust to colorblindness**, looks good in grey scale and to people with common forms of colorblindness

You can hear more about the science behind creating these color scales, on Walt and Smith's talk at SciPy 2015.

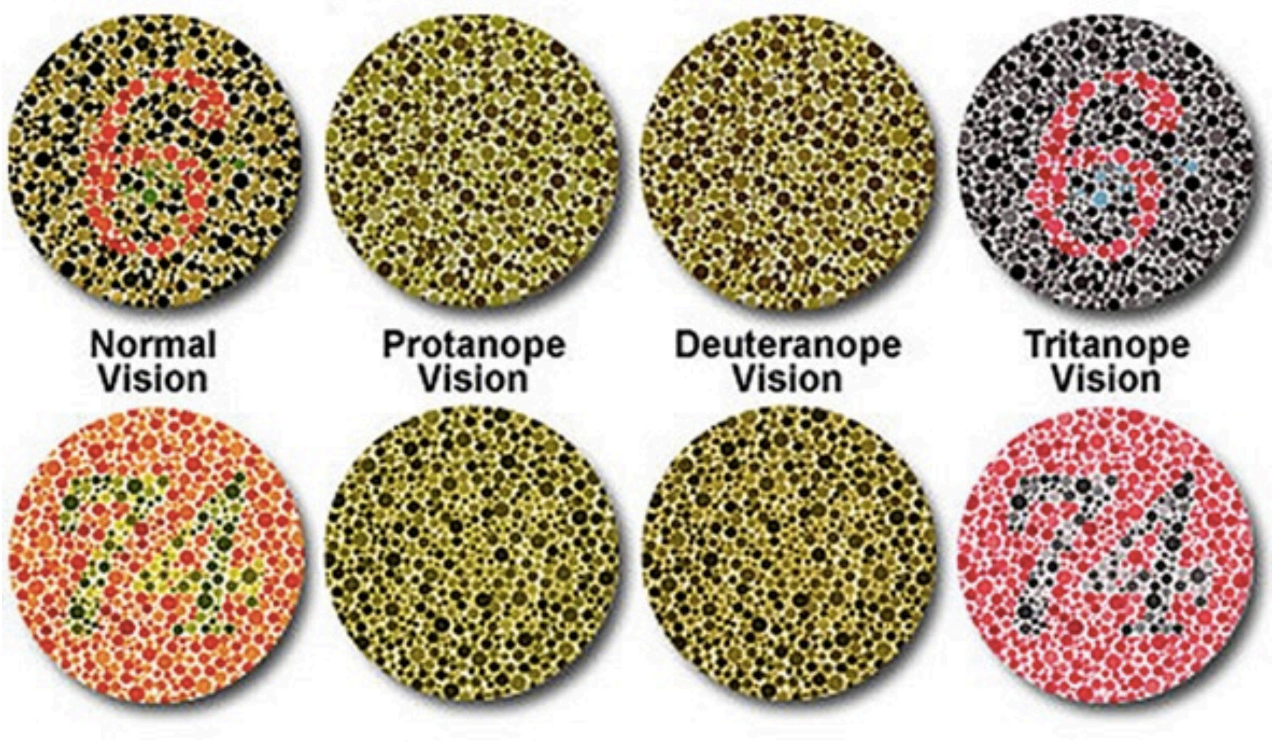


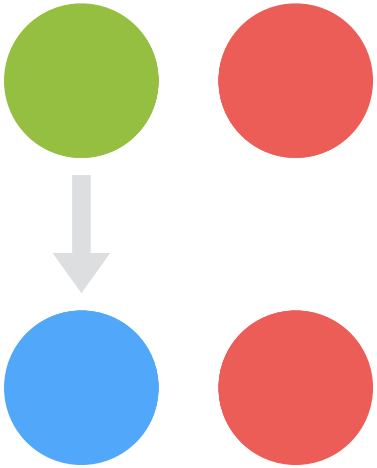
**not perceptually uniform**



**perceptually uniform**

# Be kind to colorblind people

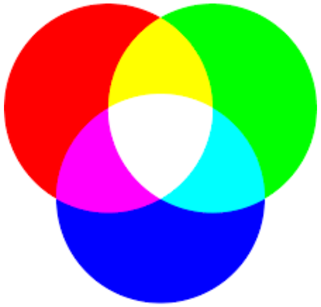




Simple solution: replace greens by blues.
Blues also display better on most monitors than greens.

# Colour models

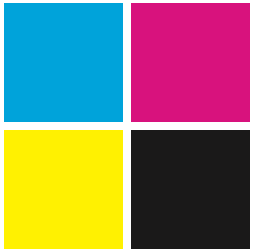
## How are colours defined?



**RGB**

Light emitting screens

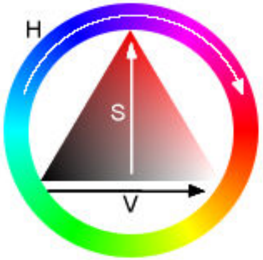
**R**ed **G**reen **B**lue
additive



**CMYK**

Printing, ink

**C**yan **M**agenta **Y**ellow Blac**k**
subtractive



**HSV**
**HSB**

coordinates in
human perception
space

**Faceting** is useful to show more dimensions without overcrowding the graph

# **Faceting** is useful to show more dimensions without overcrowding the graph

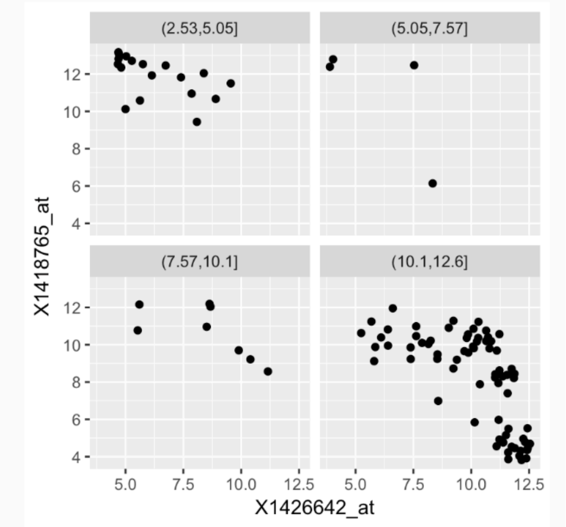
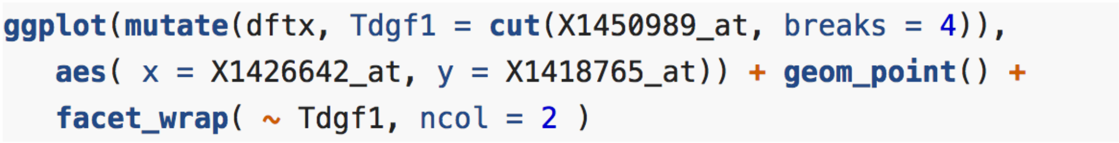


Figure 3.33: **Faceting**: the same data as in Figure 3.9, split by the continuous variable `X1450989_at` and arranged by `facet_wrap`.

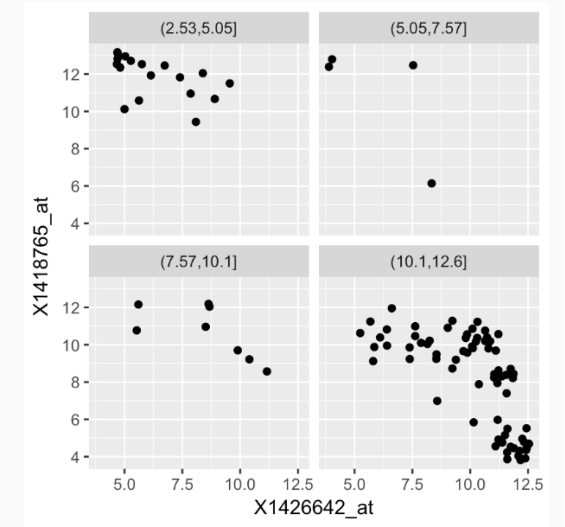
**Trellis** — chart that uses multiple instances of the same chart

`facet_wrap`



```
ggplot(mutate(dftx, Tdgf1 = cut(X1450989_at, breaks = 4)),
   aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_wrap( ~ Tdgf1, ncol = 2 )
```

# **Faceting** is useful to show more dimensions without overcrowding the graph



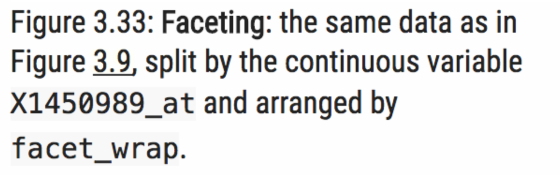
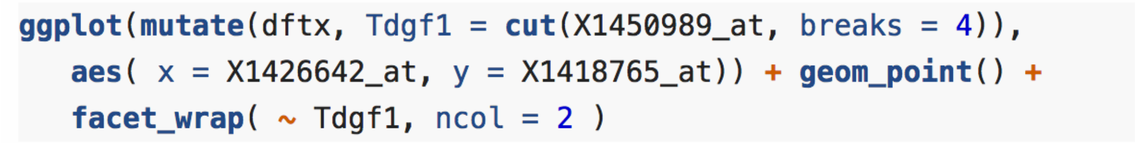


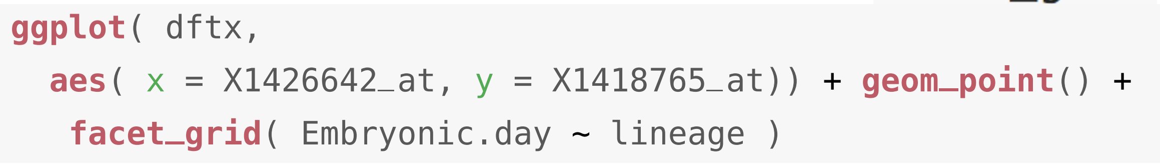
Figure 3.33: Faceting: the same data as in Figure 3.9, split by the continuous variable X1450989_at and arranged by facet_wrap.

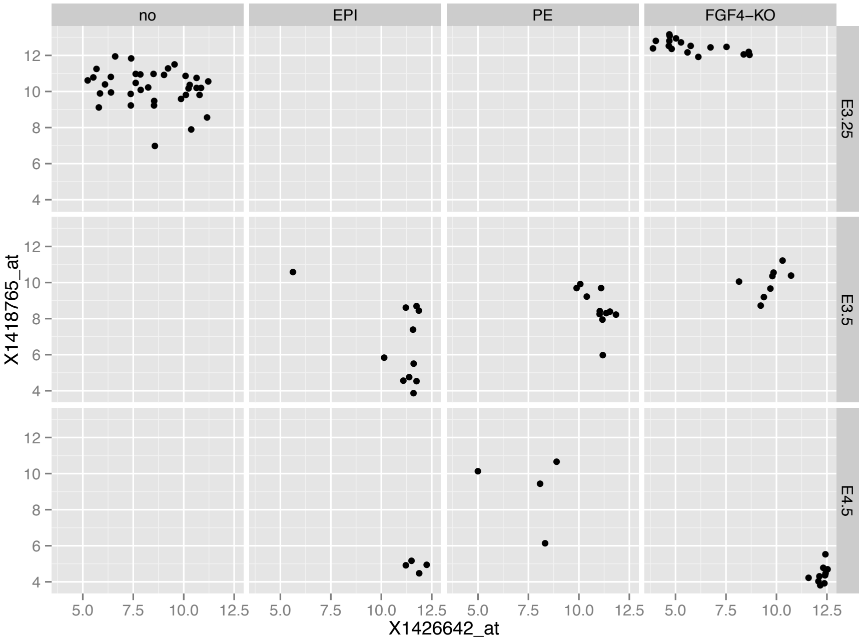
**Trellis** — chart that uses multiple instances of the same chart

facet_wrap


```
ggplot(mutate(dftx, Tdgf1 = cut(X1450989_at, breaks = 4)),
   aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_wrap( ~ Tdgf1, ncol = 2 )
```

facet_grid


```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```



# **Faceting** is useful to show more dimensions without overcrowding the graph

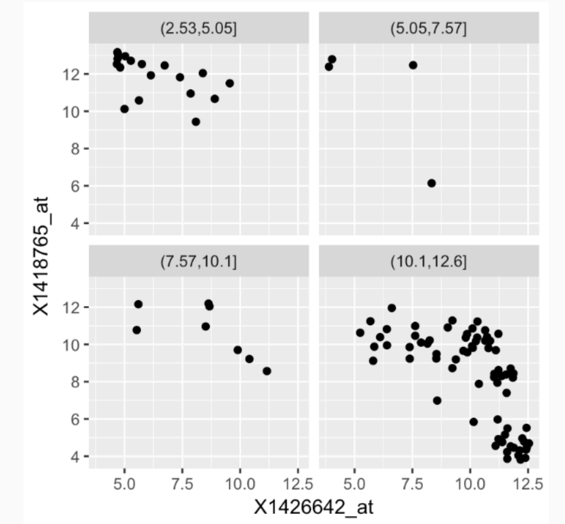
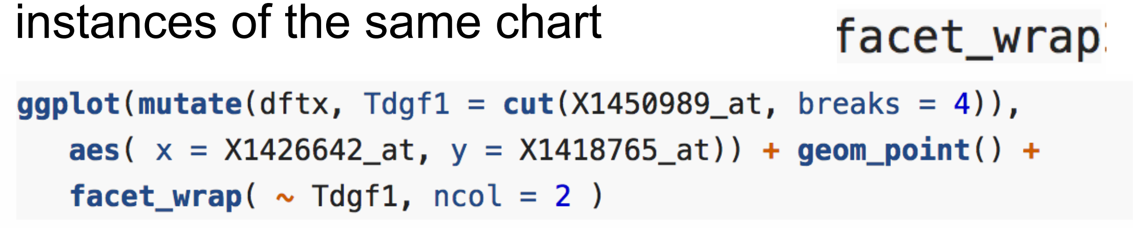


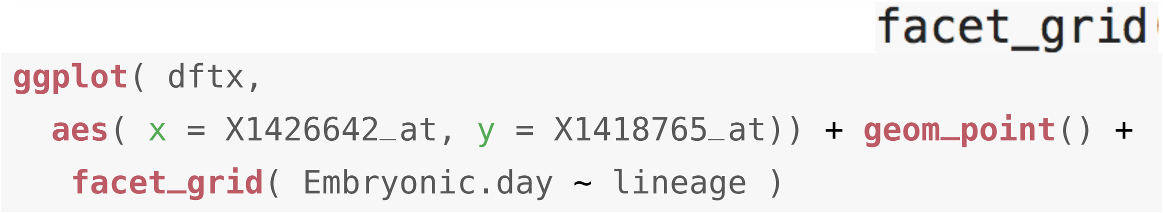
Figure 3.33: Faceting: the same data as in Figure 3.9, split by the continuous variable X1450989_at and arranged by facet_wrap.

**Trellis** — chart that uses multiple instances of the same chart



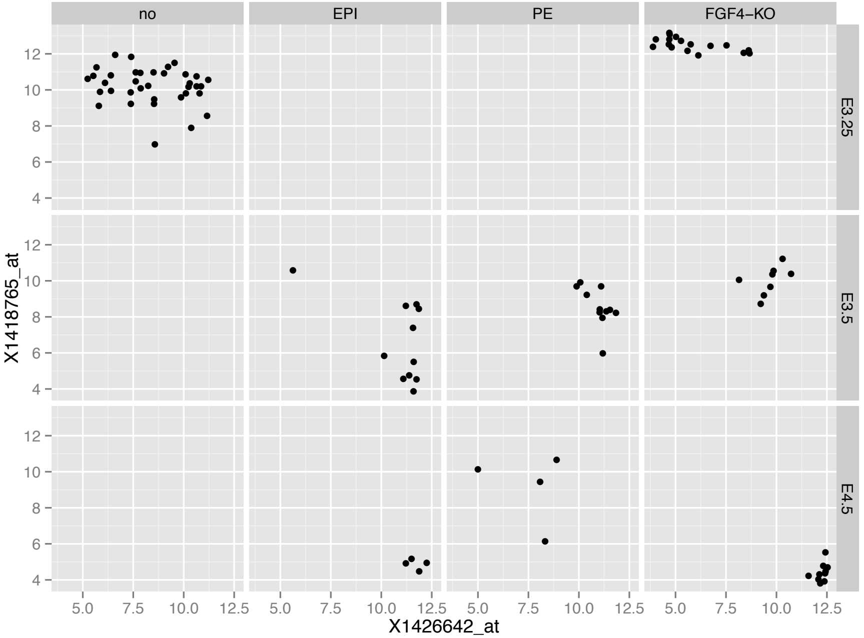
facet_wrap

```
ggplot(mutate(dftx, Tdgf1 = cut(X1450989_at, breaks = 4)),
   aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_wrap( ~ Tdgf1, ncol = 2 )
```

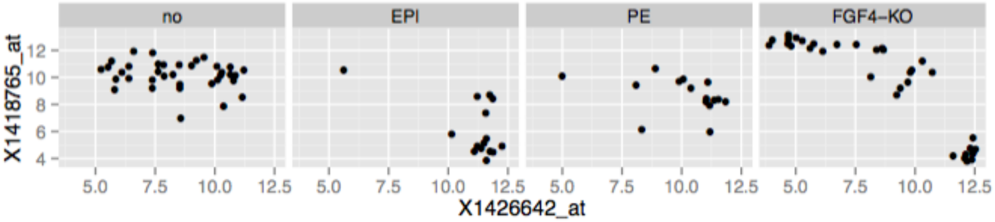


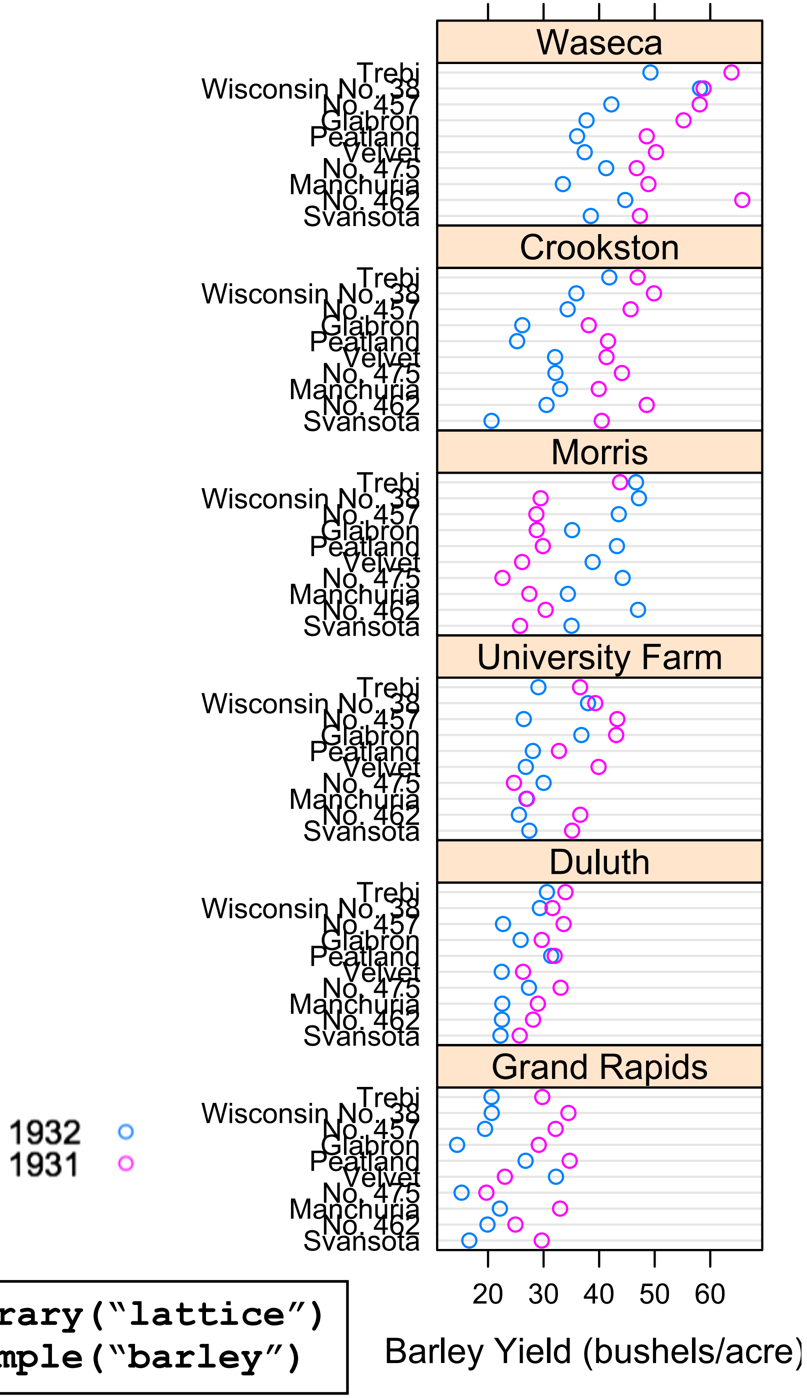
facet_grid

```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```



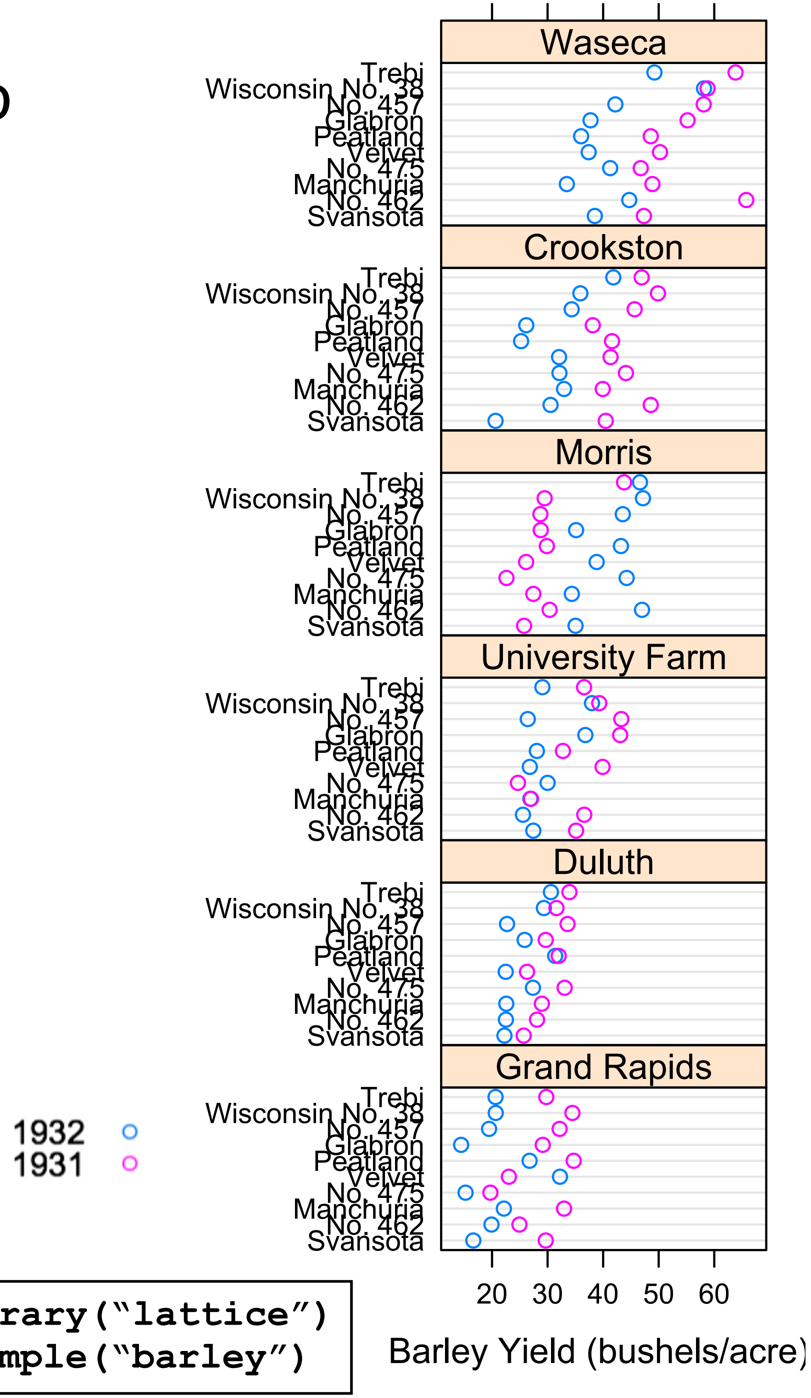
```
ggplot(dftx, aes( x = X1426642_at, y = X1418765_at)) +
  geom_point() + facet_grid( . ~ lineage )
```





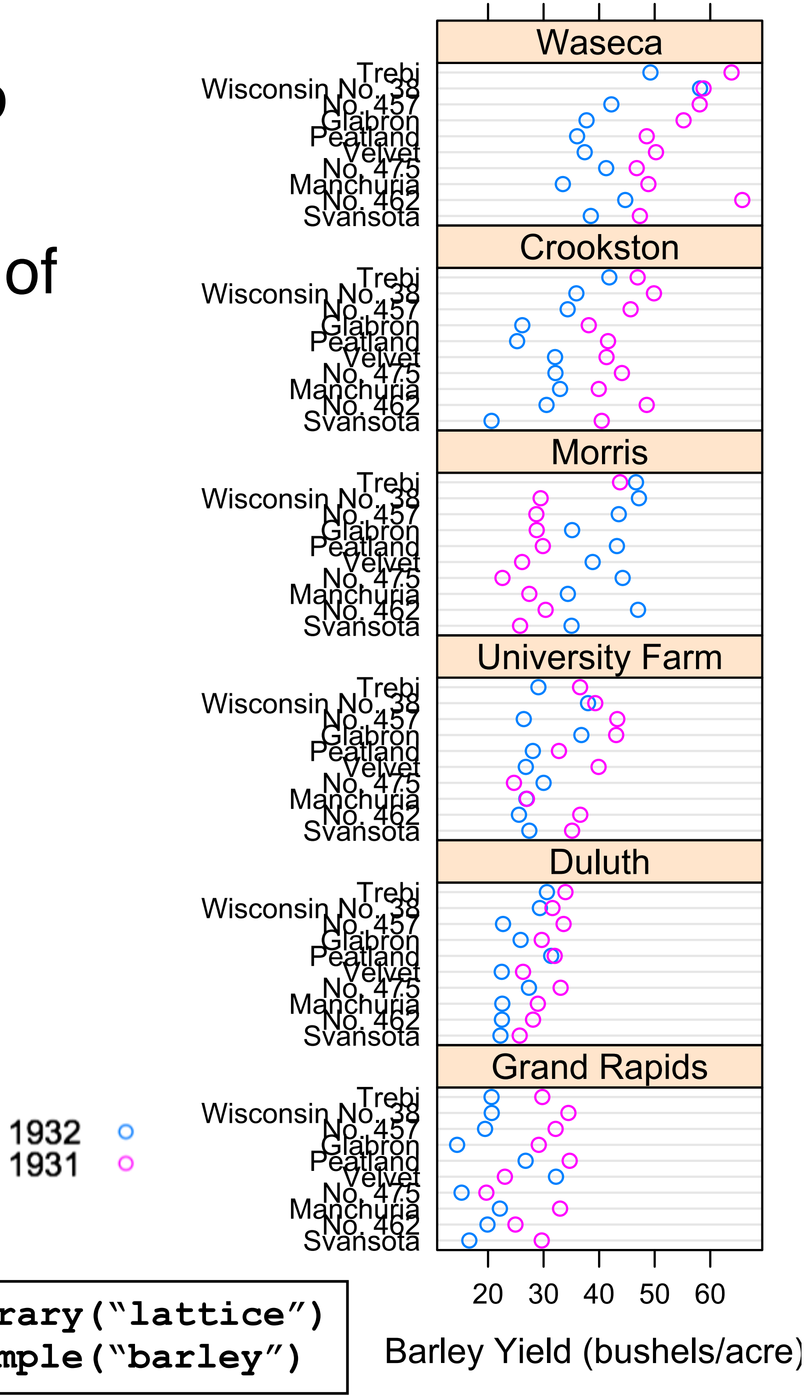
```
library("lattice")
example("barley")
```

Data from an agricultural field trial to study the crop barley.



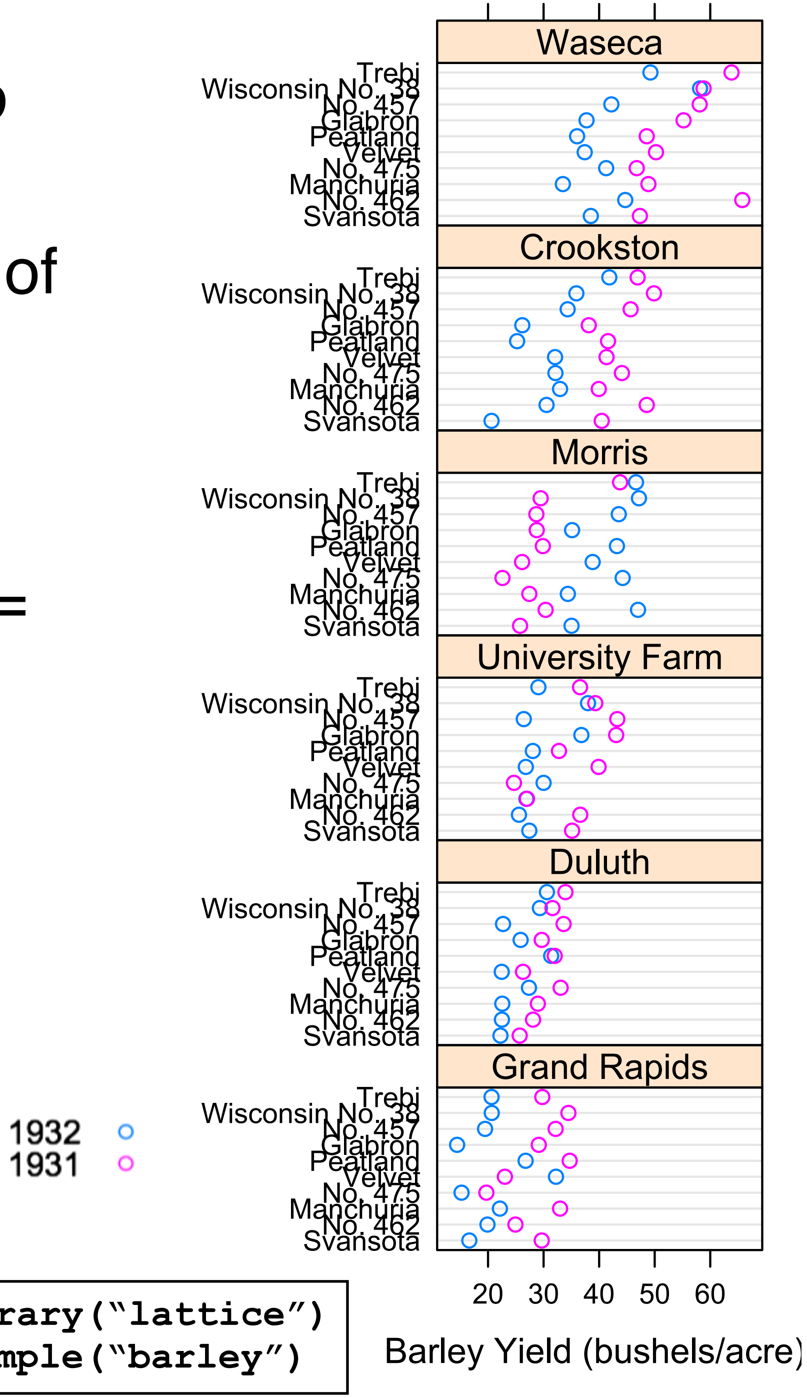
```
library("lattice")
example("barley")
```

- Data from an agricultural field trial to study the crop barley.
- At 6 sites in Minnesota, 10 varieties of barley were grown in each of two years.



```
library("lattice")
example("barley")
```

- Data from an agricultural field trial to study the crop barley.
- At 6 sites in Minnesota, 10 varieties of barley were grown in each of two years.
- Data: yield, for all combinations of site, variety, and year (6 x 10 x 2 = 120 observations)



```
library("lattice")
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```

- Data from an agricultural field trial to study the crop barley.
- At 6 sites in Minnesota, 10 varieties of barley were grown in each of two years.
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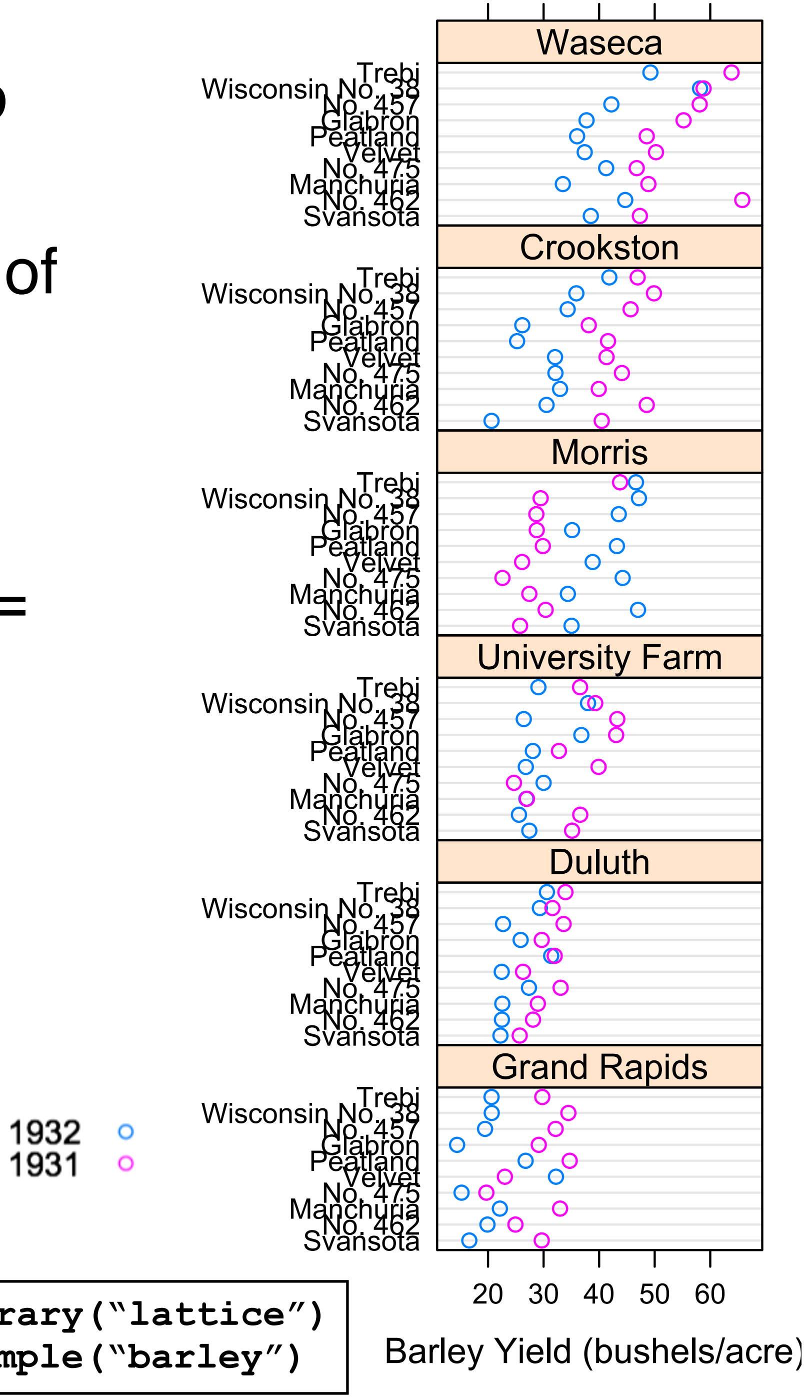


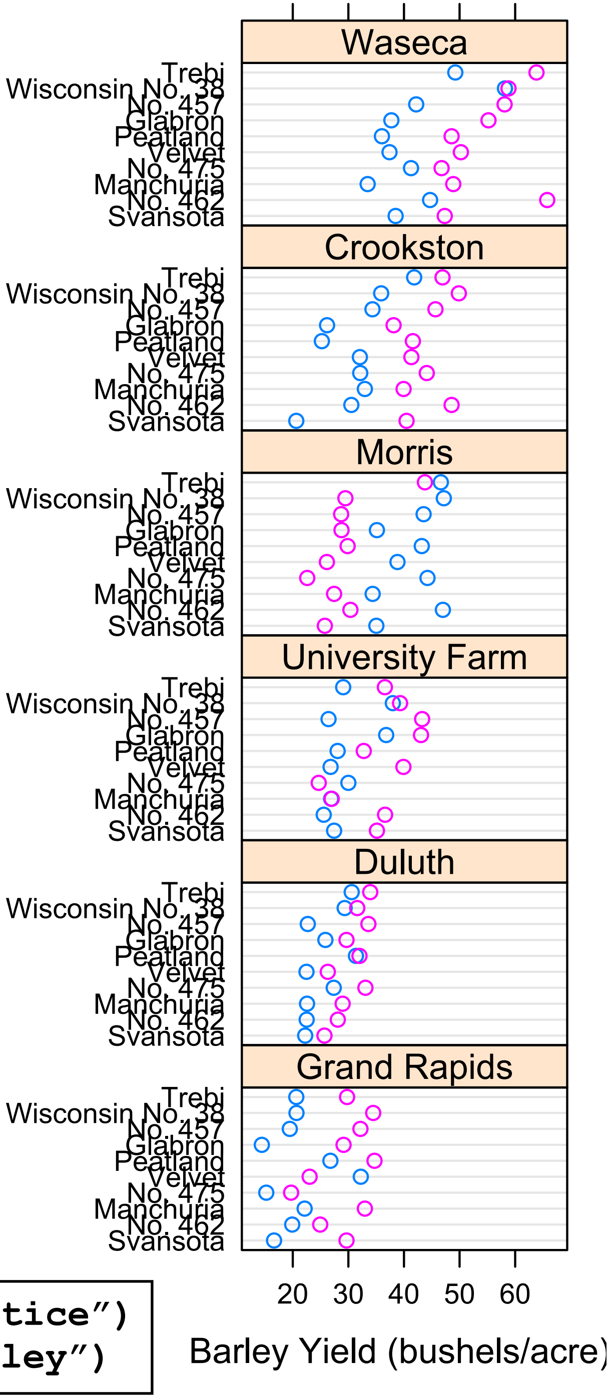
```
library("lattice")
example("barley")
```

Data from an agricultural field trial to study the crop barley.

At 6 sites in Minnesota, 10 varieties of barley were grown in each of two years.

Data: yield, for all combinations of site, variety, and year (6 x 10 x 2 = 120 observations)

Note the data for **Morris** - reanalysis in the 1990s using Trellis revealed that the years had been flipped!



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```

Data from an agricultural field trial to study the crop barley.

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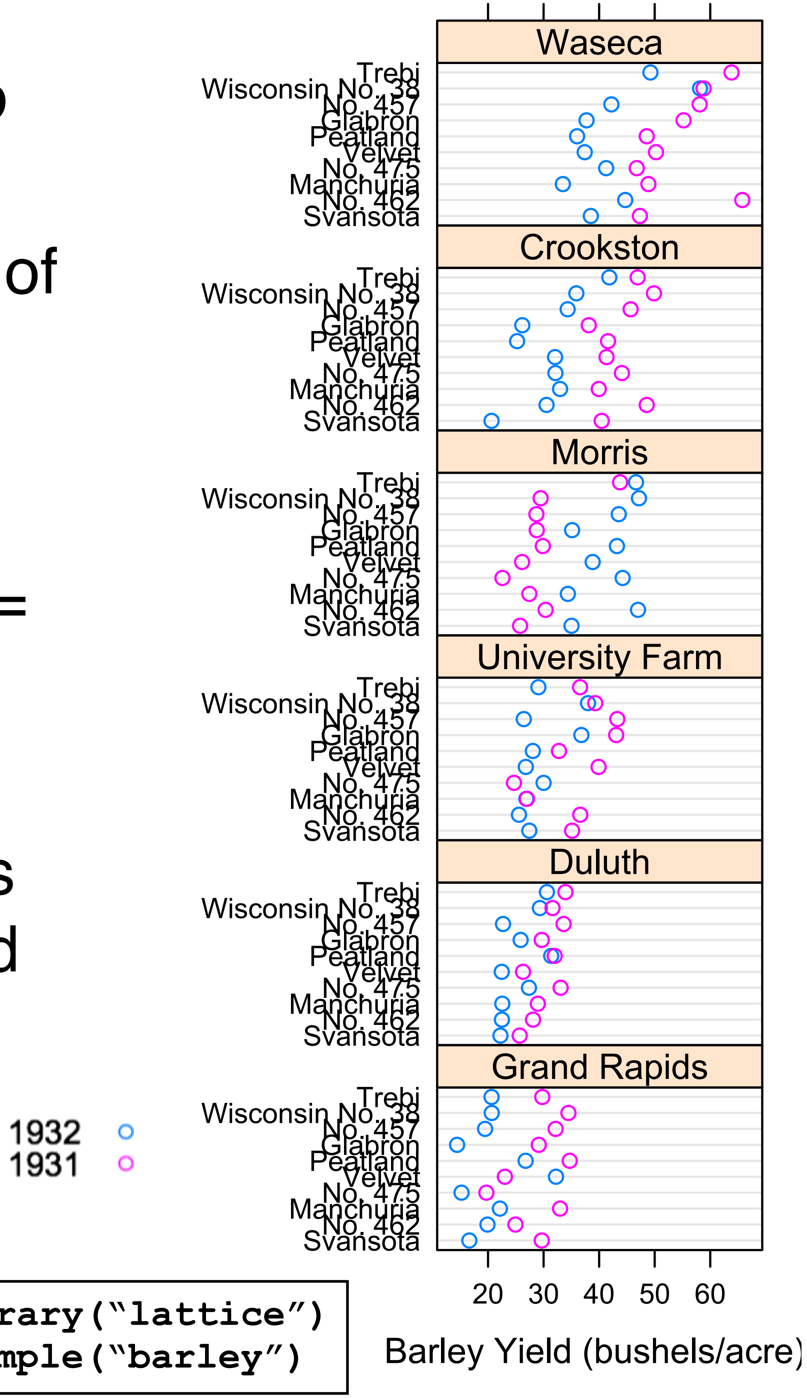
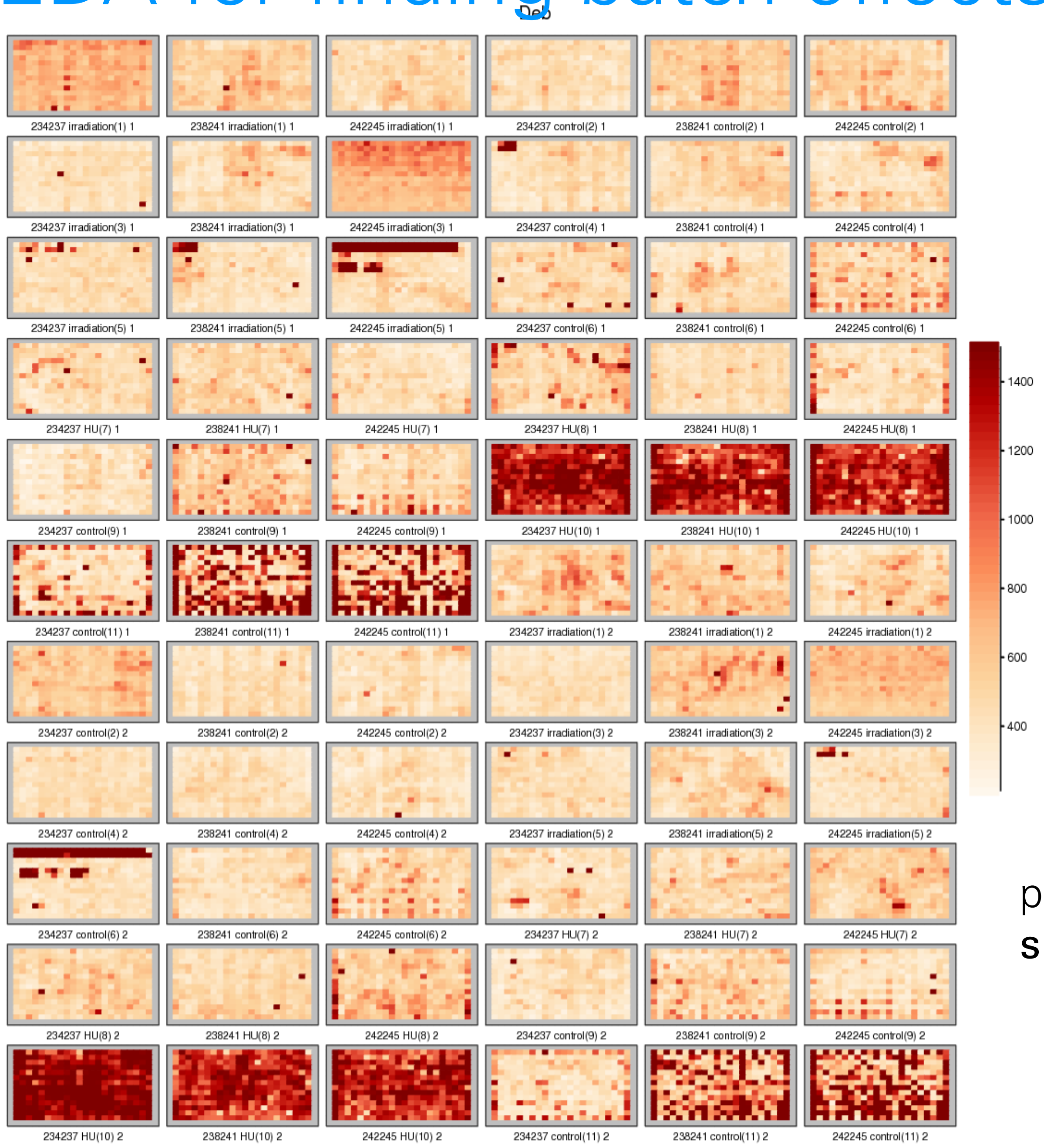


How could you quickly check for potential batch effects?

```
library("lattice")
example("barley")
```

# EDA for finding batch effects



package
**splots**

# **Tidying data** to use columns as aesthetics

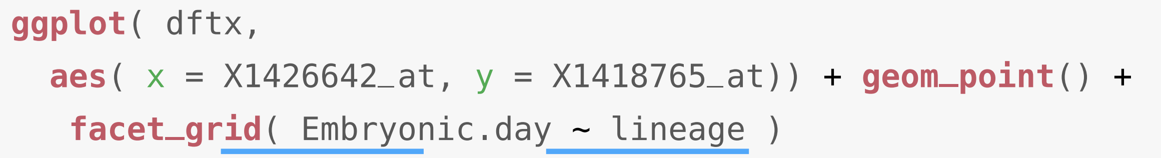
```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```

# **Tidying data** to use columns as aesthetics

```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```

Data.frame in R can be in:

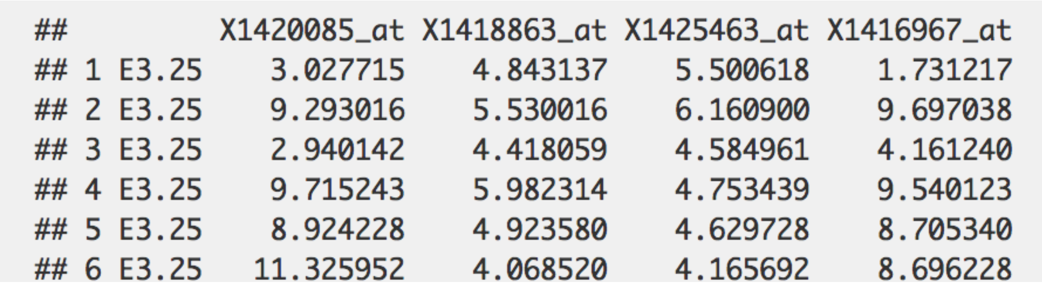
# **Tidying data** to use columns as aesthetics



```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```

Data.frame in R can be in:

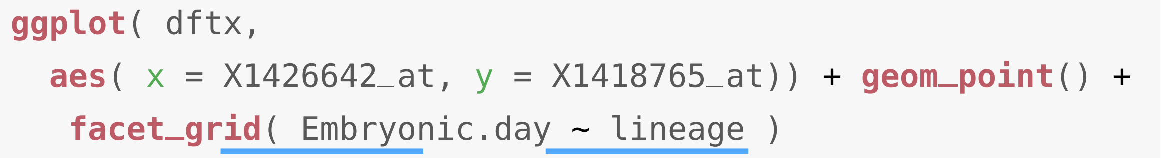
**wide format**



```
##          X1420085_at X1418863_at X1425463_at X1416967_at
## 1 E3.25     3.027715    4.843137    5.500618    1.731217
## 2 E3.25     9.293016    5.530016    6.160900    9.697038
## 3 E3.25     2.940142    4.418059    4.584961    4.161240
## 4 E3.25     9.715243    5.982314    4.753439    9.540123
## 5 E3.25     8.924228    4.923580    4.629728    8.705340
## 6 E3.25    11.325952    4.068520    4.165692    8.696228
```

e.g. a expression matrix with each raw containing a gene expression for all samples

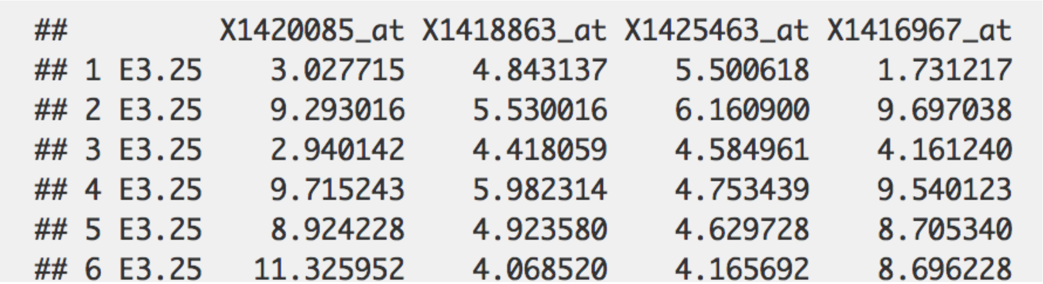
# **Tidying data** to use columns as aesthetics



```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```

Data.frame in R can be in:

**wide format**



```
##          X1420085_at X1418863_at X1425463_at X1416967_at
## 1 E3.25     3.027715    4.843137    5.500618    1.731217
## 2 E3.25     9.293016    5.530016    6.160900    9.697038
## 3 E3.25     2.940142    4.418059    4.584961    4.161240
## 4 E3.25     9.715243    5.982314    4.753439    9.540123
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## 6 E3.25    11.325952    4.068520    4.165692    8.696228
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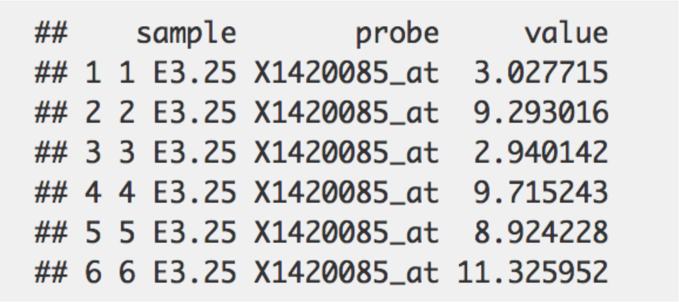
e.g. a expression matrix with each raw containing a gene expression for all samples

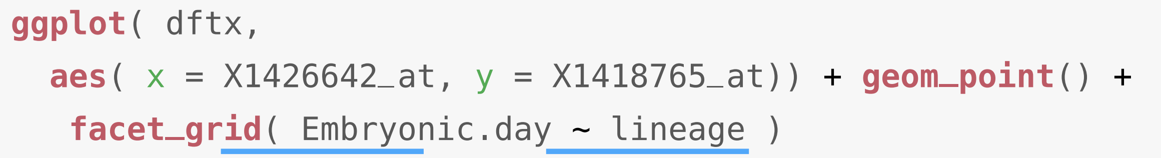
**long format**



```
##      sample       probe     value
## 1 1 E3.25 X1420085_at  3.027715
## 2 2 E3.25 X1420085_at  9.293016
## 3 3 E3.25 X1420085_at  2.940142
## 4 4 E3.25 X1420085_at  9.715243
## 5 5 E3.25 X1420085_at  8.924228
## 6 6 E3.25 X1420085_at 11.325952
```

e.g. a collapsed expression data with each row corresponding to a gene-sample pair

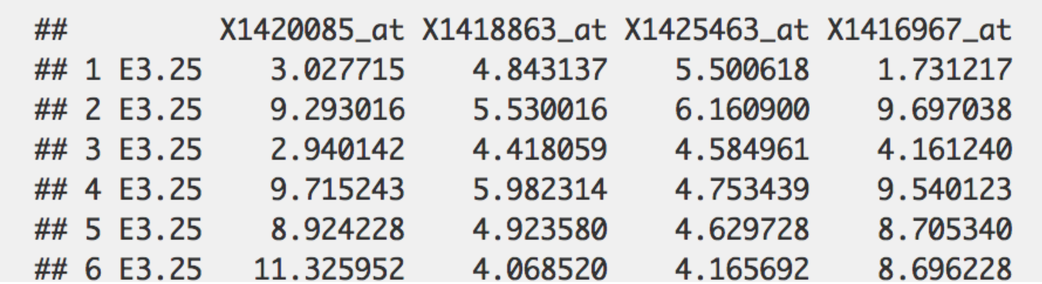
# **Tidying data** to use columns as aesthetics



```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
   facet_grid( Embryonic.day ~ lineage )
```

Data.frame in R can be in:

**wide format**



```
##         X1420085_at X1418863_at X1425463_at X1416967_at
## 1 E3.25    3.027715    4.843137    5.500618    1.731217
## 2 E3.25    9.293016    5.530016    6.160900    9.697038
## 3 E3.25    2.940142    4.418059    4.584961    4.161240
## 4 E3.25    9.715243    5.982314    4.753439    9.540123
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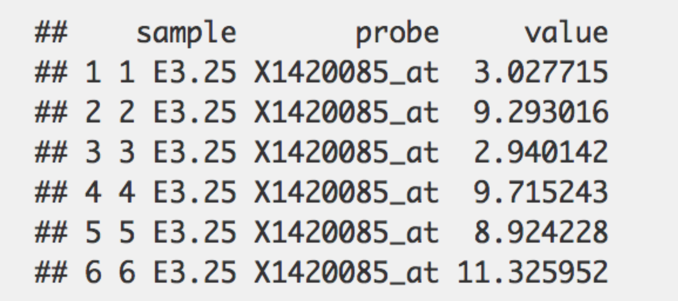
```

e.g. a expression matrix with each raw containing a gene expression for all samples

Each **row** corresponds to a **sample** and each **column** to a **feature** (or vice versa).

**long format**



```
##     sample       probe     value
## 1 1 E3.25 X1420085_at  3.027715
## 2 2 E3.25 X1420085_at  9.293016
## 3 3 E3.25 X1420085_at  2.940142
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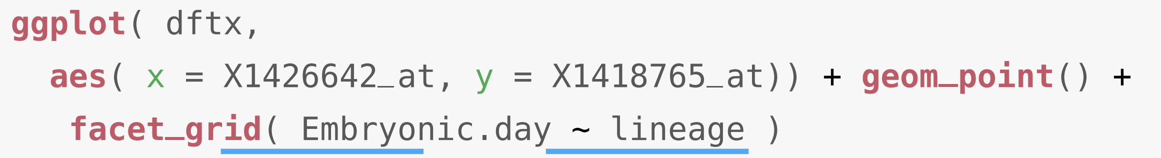

```
ggplot( dftx,
  aes( x = X1426642_at, y = X1418765_at)) + geom_point() +
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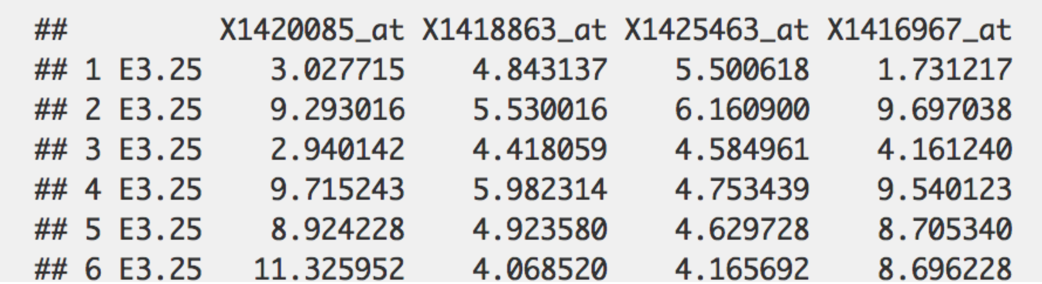
```

Data.frame in R can be in:

## wide format



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##          X1420085_at X1418863_at X1425463_at X1416967_at
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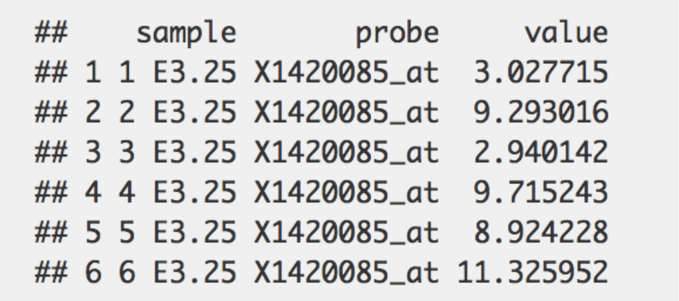
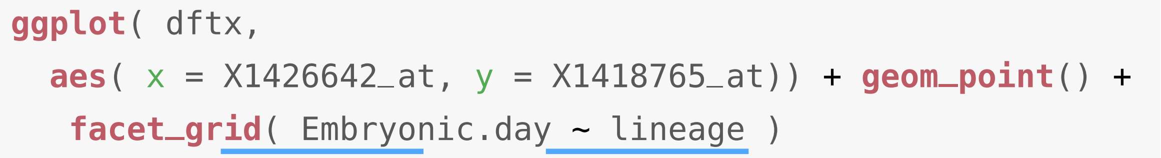


```
##     sample       probe     value
## 1 1 E3.25 X1420085_at  3.027715
## 2 2 E3.25 X1420085_at  9.293016
## 3 3 E3.25 X1420085_at  2.940142
## 4 4 E3.25 X1420085_at  9.715243
## 5 5 E3.25 X1420085_at  8.924228
## 6 6 E3.25 X1420085_at 11.325952
```

e.g. a collapsed expression data with each row corresponding to a gene-sample pair

**Feature** and **sample** information is stored separately for each measurement in data columns.

# **Tidying data** to use columns as aesthetics



```
ggplot( dftx,
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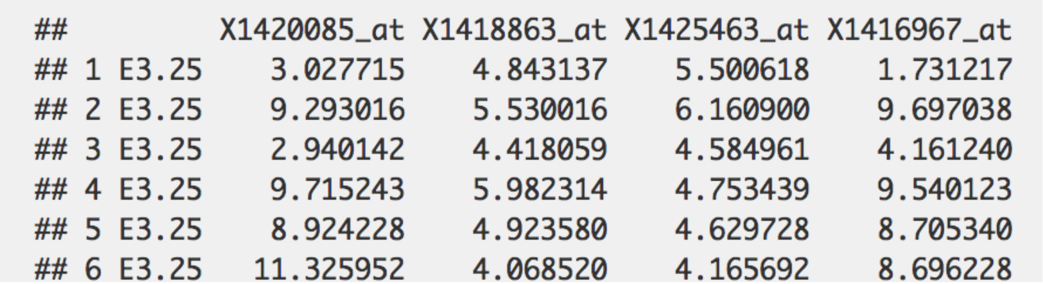
   facet_grid( Embryonic.day ~ lineage )
```

Data.frame in R can be in:

### wide format



```
##          X1420085_at X1418863_at X1425463_at X1416967_at
## 1 E3.25     3.027715    4.843137    5.500618    1.731217
## 2 E3.25     9.293016    5.530016    6.160900    9.697038
## 3 E3.25     2.940142    4.418059    4.584961    4.161240
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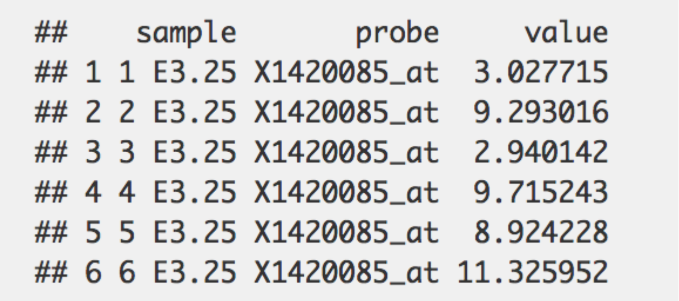

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##     sample       probe     value
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## 2 2 E3.25 X1420085_at  9.293016
## 3 3 E3.25 X1420085_at  2.940142
## 4 4 E3.25 X1420085_at  9.715243
## 5 5 E3.25 X1420085_at  8.924228
## 6 6 E3.25 X1420085_at 11.325952
```

e.g. a collapsed expression data with each row corresponding to a gene-sample pair

**Feature** and **sample** information is stored separately for each measurement in data columns.

To switch wide ↔ long:     pivot_longer, pivot_wider

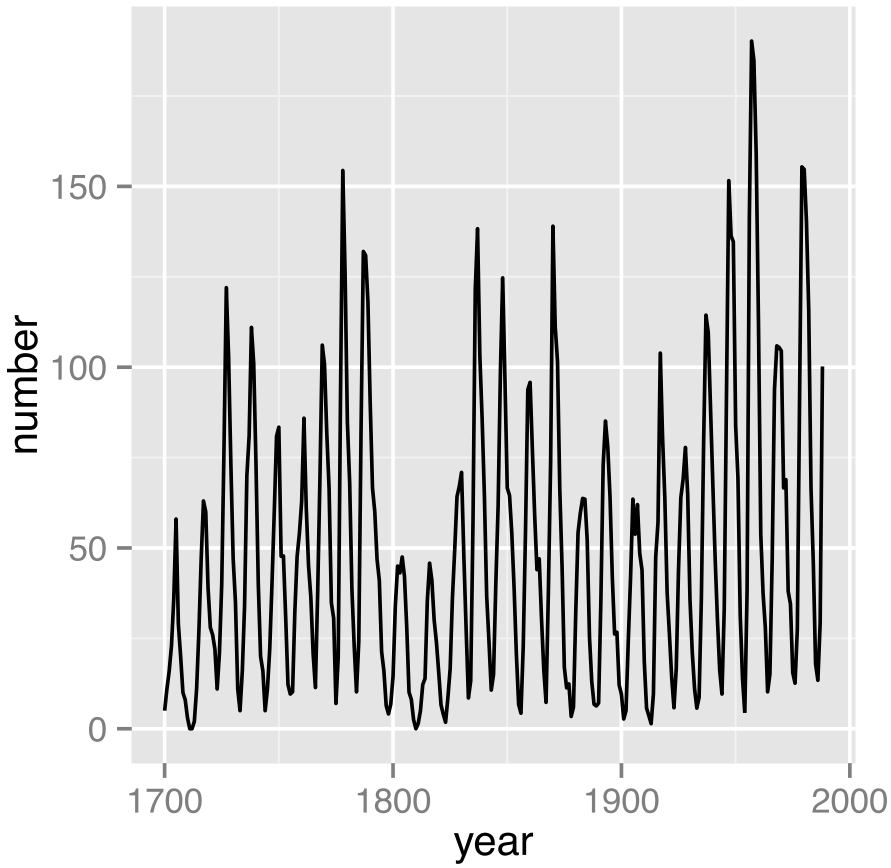
# Choose **aspect ratio** so that banking = 45%

Yearly sunspot numbers 1849-1924 - changes in amplitude

**Banking to 45 degrees:**

Choose aspect ratio so that the median absolute slope is 1, i.e. at 45 degrees angle.

**Sawtooth**: Sunspot cycles typically rise more rapidly than they fall — steep rise and slow decline.



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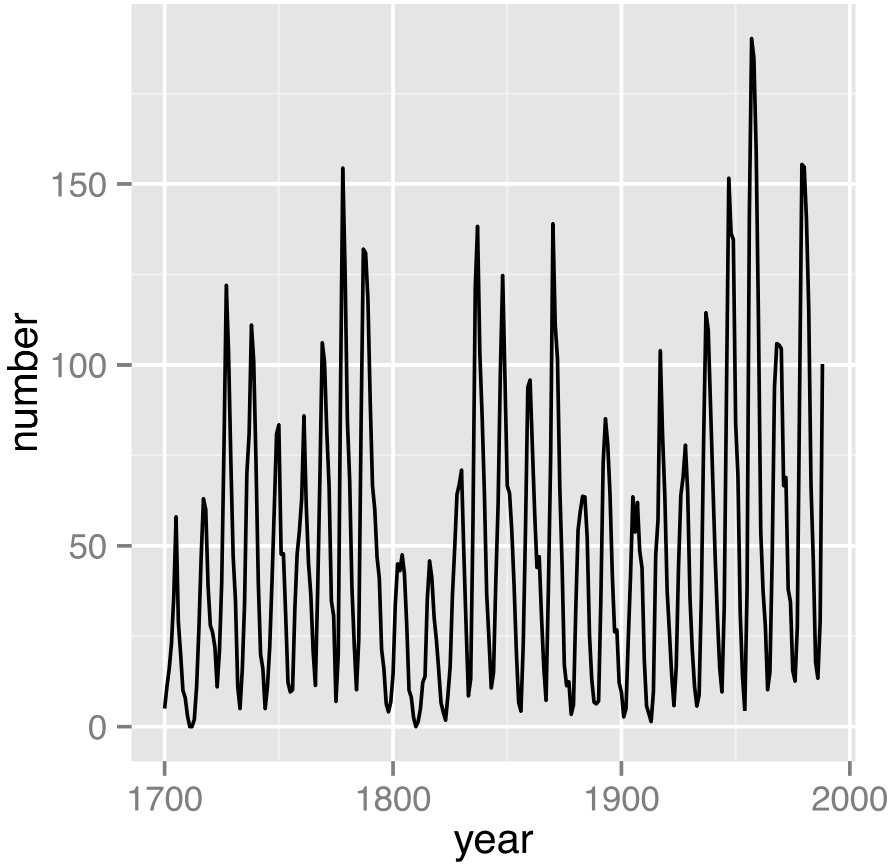

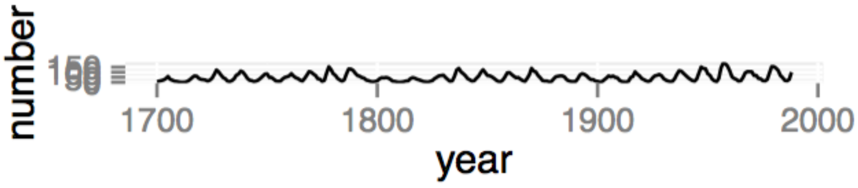

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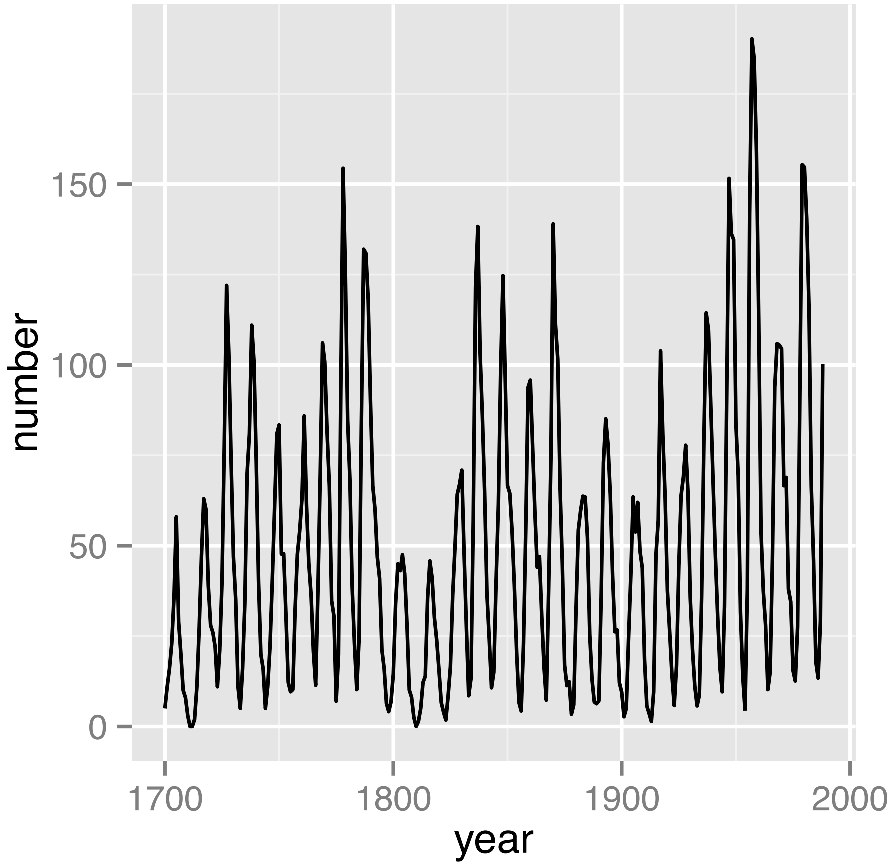
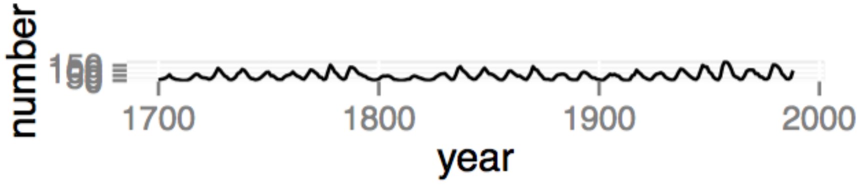
Choose aspect ratio so that the median absolute slope is 1, i.e. at 45 degrees angle.

**Sawtooth**: Sunspot cycles typically rise more rapidly than they fall — steep rise and slow decline.





**For plots where x- and y-axis have same units: use 1:1 aspect ratio**

# Heatmaps for visualizing large matrices

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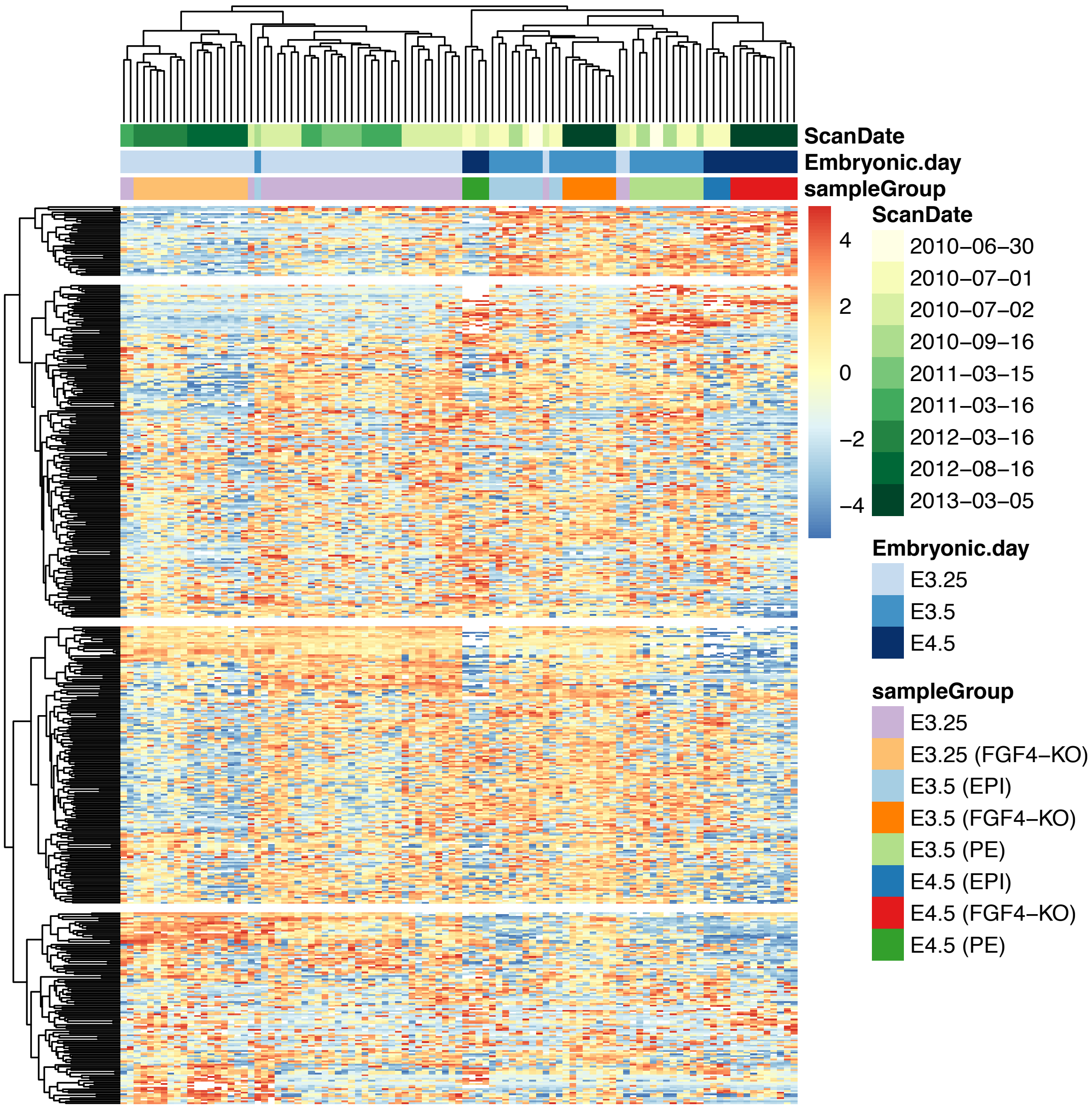

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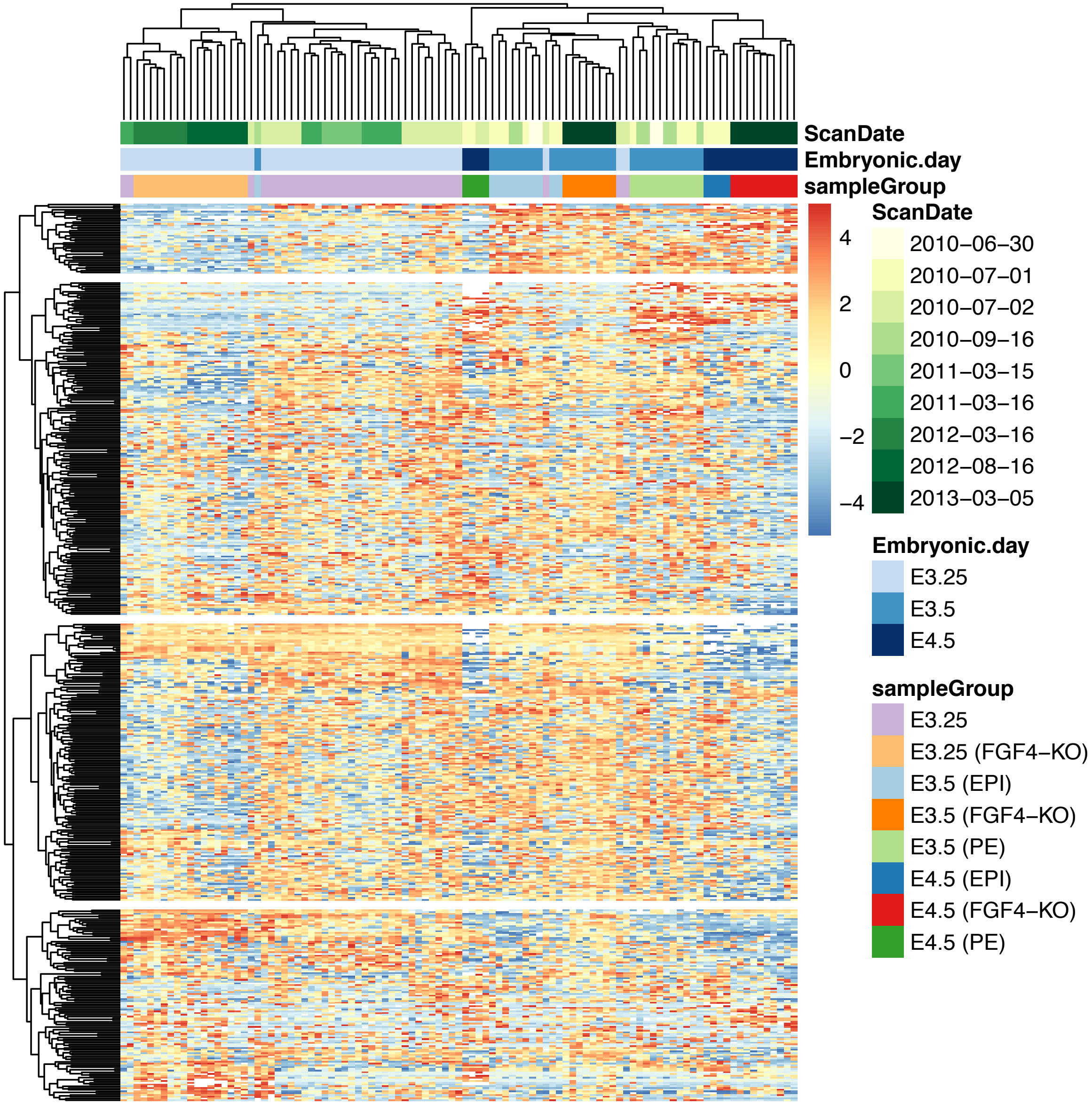
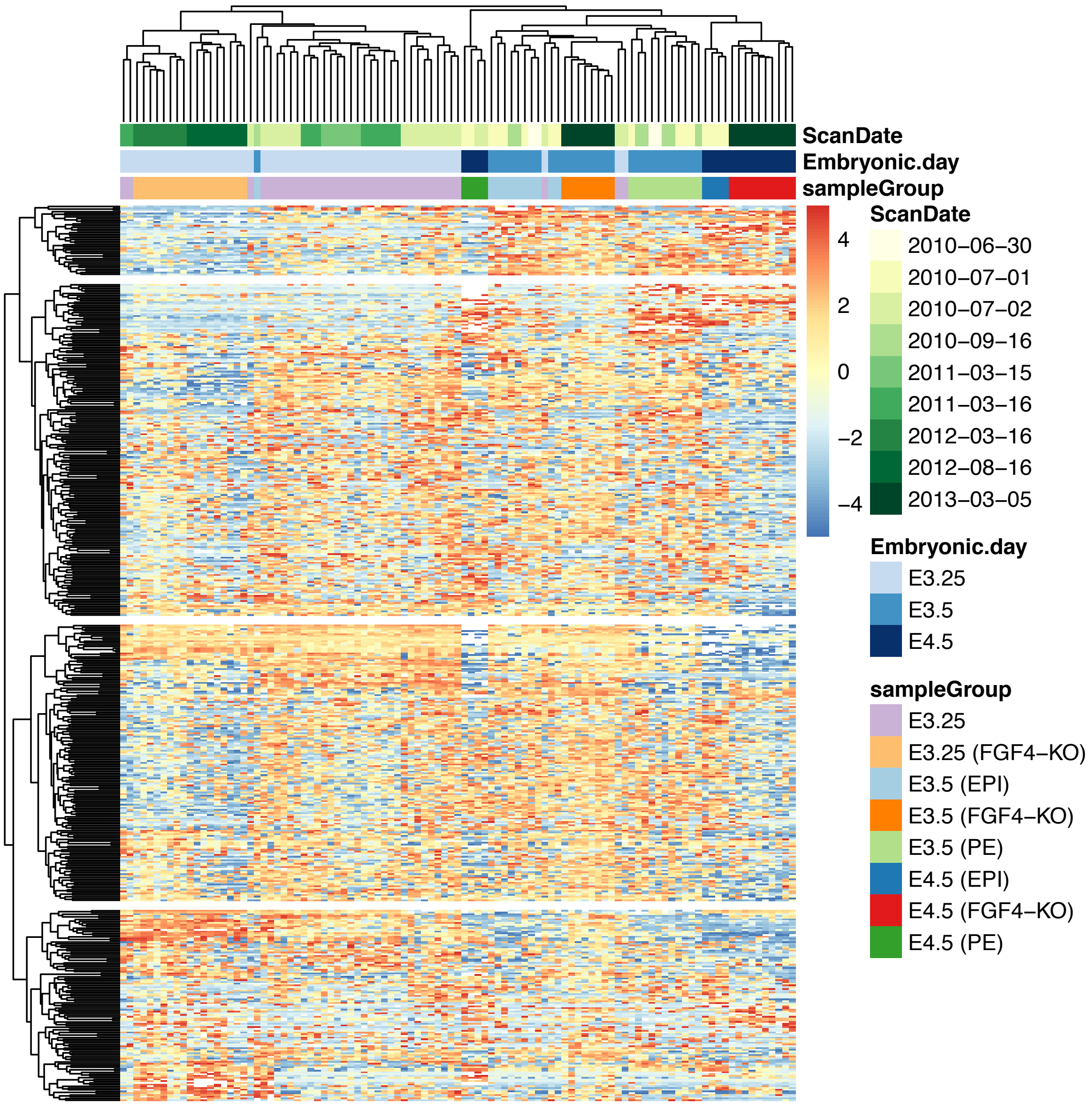


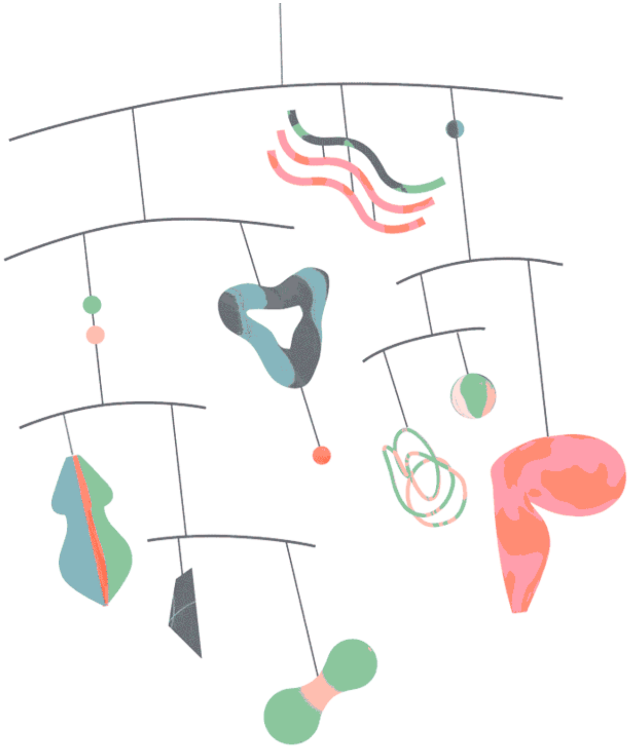
*pheatmap*

- many “reasonable” defaults
- easy to add column and row ‘metadata’ at the sides

See also *ComplexHeatmap* package

# The **order** of dendrogram **branches** is not unique





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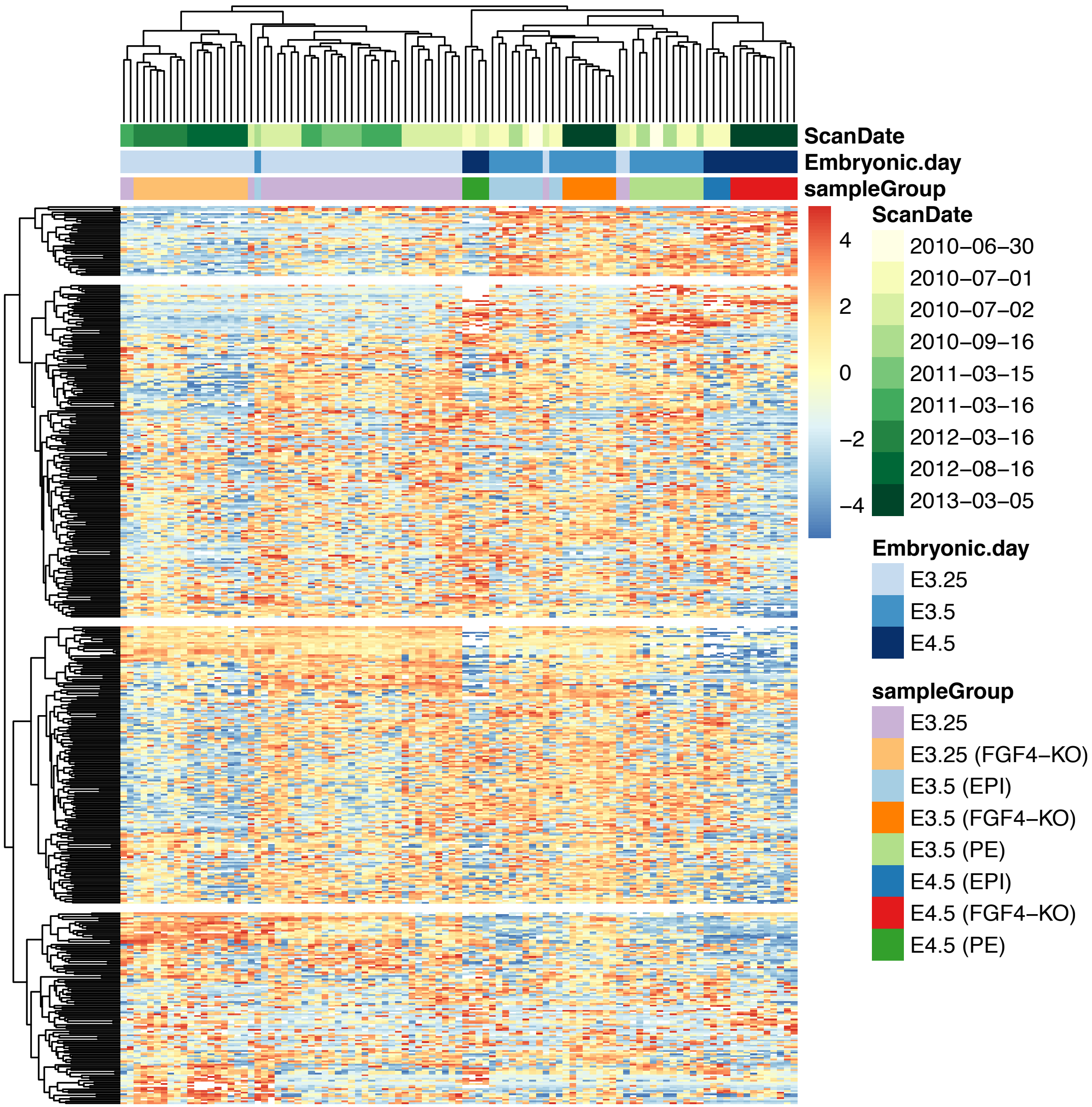

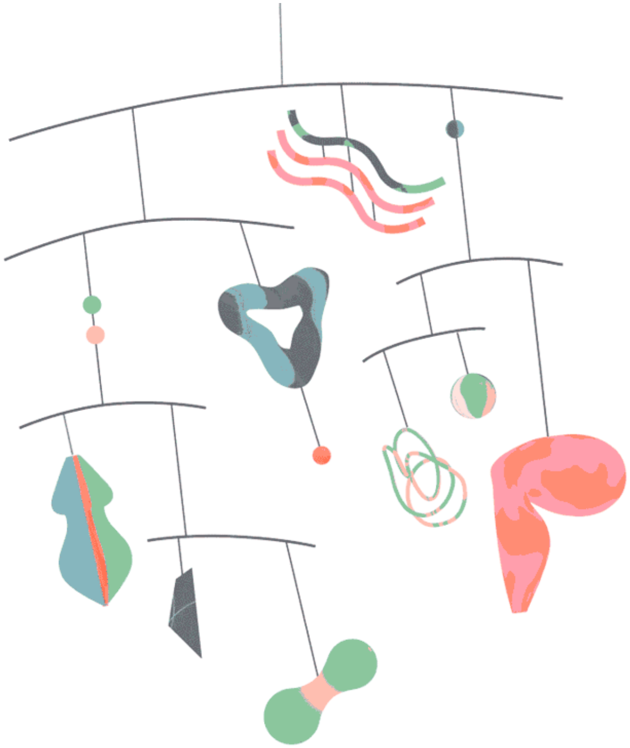

# Goals for this lecture

1. Discuss the principles of **good *vs* bad** data viz
2. Review base R plotting
3. Understand the **grammar of graphics** concept
4. Introduce, explain and use the `ggplot()` function
5. Discuss how to plot 1D, 2D, 3-5D data and select the most appropriate plot type. Use faceting
6. Use visualization for the inspection of large datasets and discovery of global trends (e.g. batch effects)
7. Implement interactive (3D) visualization

# Interactivity

Use shiny or plotly

https://shiny.rstudio.com/gallery/genome-browser.html

Animations (time-dependent plots):
https://gganimate.com

Linked Charts
https://anders-biostat.github.io/linked-charts/

NB: ggvis is senescent

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More on `plotly` can be found at https://plotly-book.cpsievert.me/

# `plotly` interactive graphics demo

see https://www.huber.embl.de/users/whuber/2021-M5Bioinfo/graphics

# Acknowledgements

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