Reproducible data analysis using R and RStudio

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Data Science for Developing Scholars in Down Syndrome Research (DS3) 2025







Useful links for this session

https://github.com/DS3-2025/installing_updating_R-RStudio

https://docs.posit.co/ide/user/ide/get-started/
https://rstudio.github.io/cheatsheets/base-r.pdf
https://posit.co/download/rstudio-desktop/
https://rstudio.github.io/cheatsheets/html/rstudio-ide.html

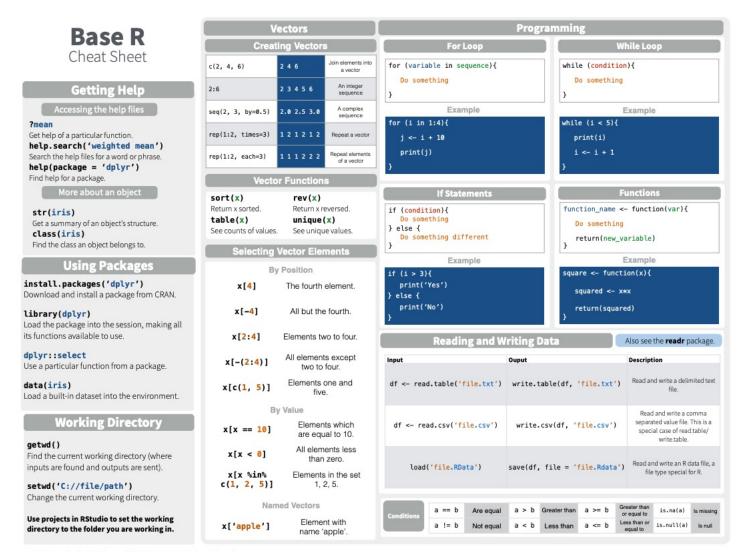
https://github.com/DS3-2025/Rproject_template

https://support.rstudio.com/hc/en-us/articles/200526207-Using-RStudio-Projects
https://r4ds.had.co.nz/workflow-projects.html

https://tidyverse.tidyverse.org/

https://ggplot2.tidyverse.org/ https://ggplot2-book.org/





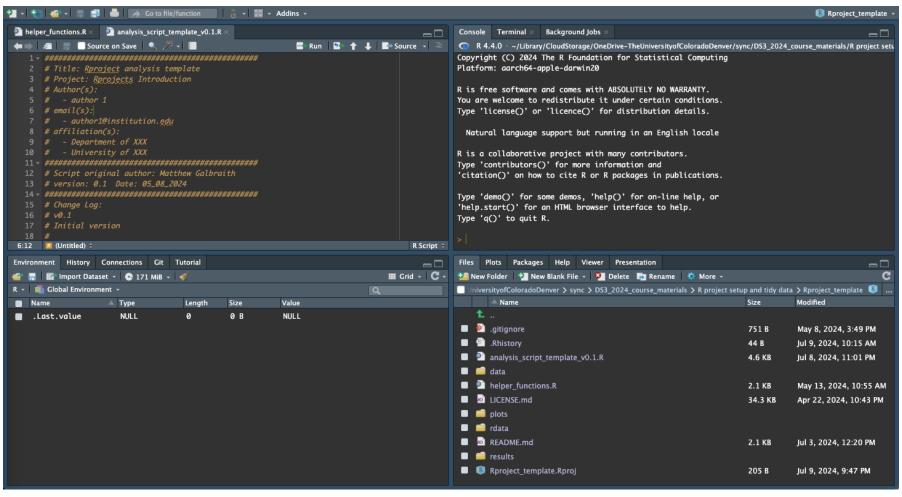
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Learn more at web page or vignette + package version + Updated: 3/15



Integrated Development Environment (IDE) for R

- Edit + execute R code
- Syntax highlighting, code completion, debugging
- View output, plots, help, environment

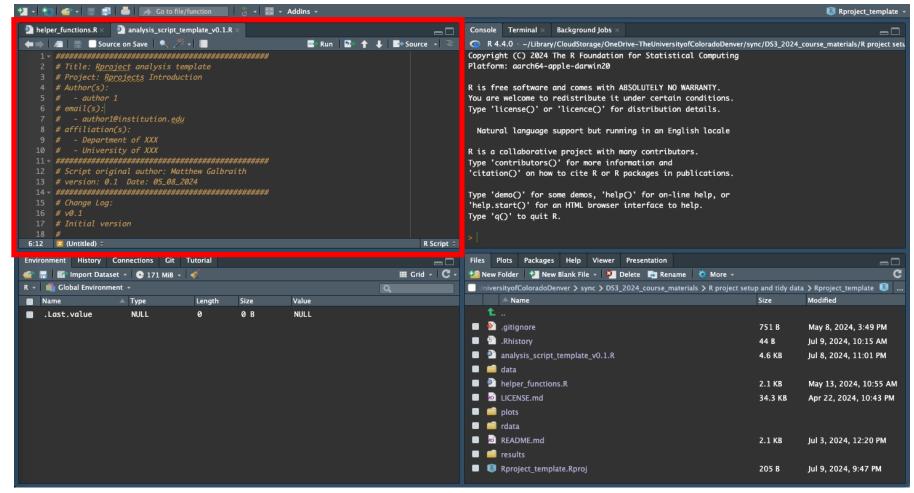




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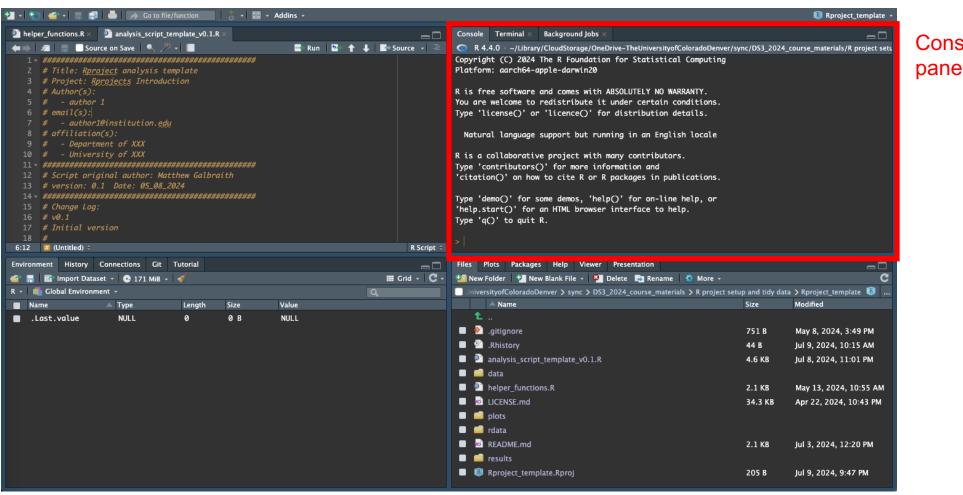
Source pane





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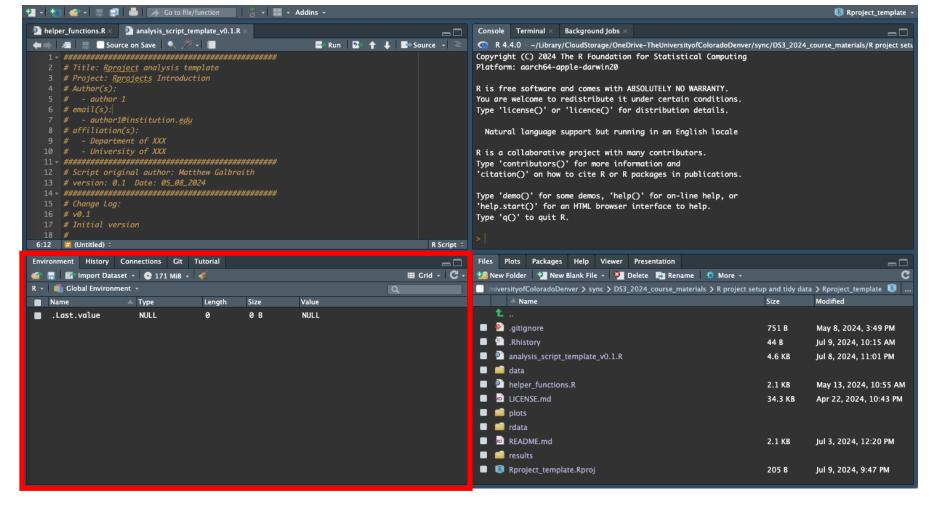


Console



Integrated Development Environment (IDE) for R

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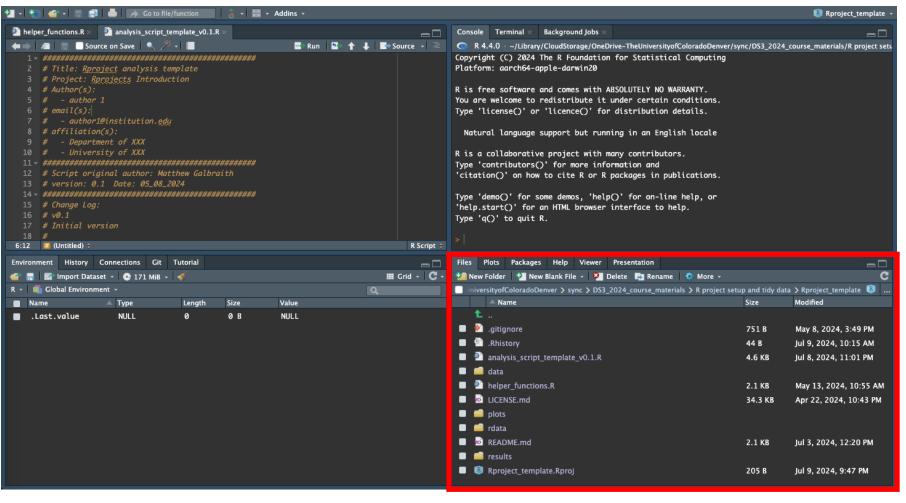


Environment etc pane



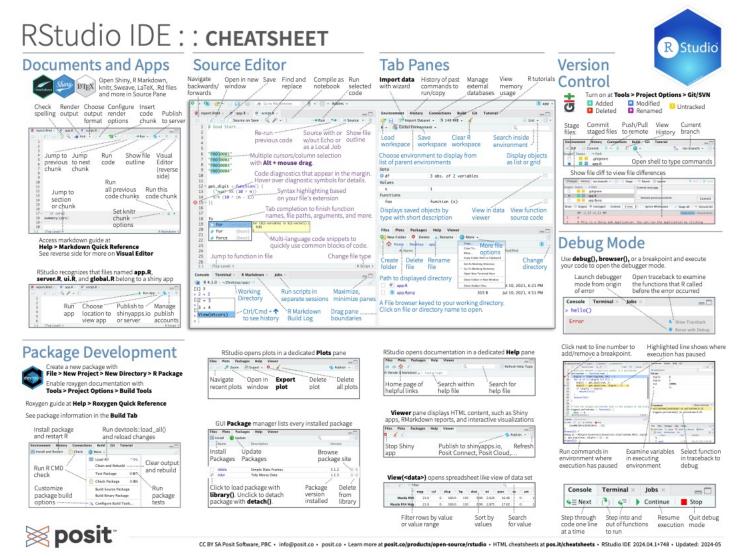
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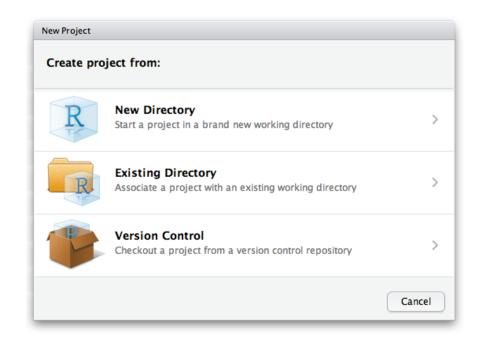
Files, Plots, Help etc pane

RStudio cheatsheet



https://rstudio.github.io/cheatsheets/html/rstudio-ide.html

Reproducible data analysis: RStudio Projects



Project_directory

- /data
- /results
- -/plots
- /rdata
- analysis_script.R
- helper_functions.R
- project.Rproj

- Usually open project via .Rproj file
- Automatically sets your working directory
- Self-contained set of directories, scripts, and data files (very important for multiple projects)

Organizing your Rstudio Projects

- Only /data and R scripts are required everything else can be recreated (incl. earlier versions)
- Treat /data directory as read-only
- Analysis outputs go to /results or /plots (with version info)
- R workspace and large intermediate files stored in /rdata
- Additional directories added as needed, eg /Archive
- Compatible with manual or other version control

Reproducible data analysis: Package management using renv



- install.packages("renv")
- renv::init() to initialize a new project-local environment with a private R library
- renv::install() to install packages after initialization
- renv::snapshot() to save the state of your project to renv.lock
- renv::restore() to restore the state of your project from renv.lock

Project_directory - /data - /results - /plots - /rdata - /renv - renv.lock

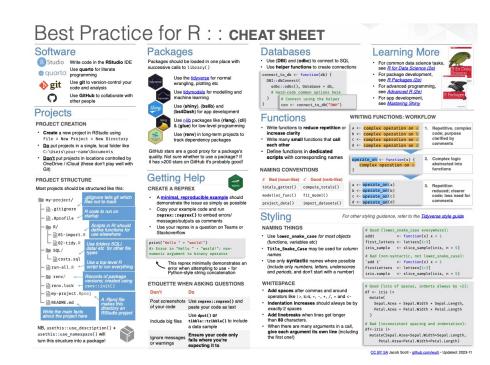
helper functions.R

analysis script.R

Allows for fully self-contained R projects (Usually) takes care of installing packages

Reproducible data analysis: Best Practice

- Main analysis R script with standardized workflow (see next slides)
- Write functions to reduce repetition or increase clarity
- Define functions in dedicated script(s) (e.g. helper_functions.R)
 keeps main script workflow clean + makes editing easier
- Comment your code!! (but don't just write what the code is doing)
- Adopt a coding style for consistency (e.g. <u>Tidyverse style guide</u>)
- Track versions and log changes + filenames with versions
- Inspect the data!!!!
- Check each step!!
- Visualize your data!!!!
- Keep track of any random seeds used
- Optional: use R notebooks/R markdown/Quarto/Roxygen to allow generation of reports with code (html, pdf, etc)



Title, Project, Author(s)

```
# Title: Rproject analysis template
# Project: Rprojects Introduction
 Author(s):
  - author 1
# email(s):
  author1@institution.edu
# affiliation(s):

    Department of XXX

  - University of XXX
# Script original author: Matthew Galbraith
# version: 0.1 Date: 05_08_2024
# Change Log:
# v0.1
# Initial version
```

Keep a change log

- What was changed and why?
- When?

Explain the purpose of the script + data types used

```
### Summary:
# Description of data wrangling and/or analysis being performed.
### Data type(s):
    A. Meta data
       Where/who did this data come from?
       What is the source of the original data and where is it stored?
    B. Data type 1
       Where/who did this data come from?
       What is the source of the original data and where is it stored?
    C. Data type 2
       Where/who did this data come from?
       What is the source of the original data and where is it stored?
### Workflow:
   1. Step 1 description
   2. Step 2 description
   3. Step 3 description
    4. Step 4 description
## Comments:
   Any further relevant details?
```

Outline the workflow steps (especially for longer scripts)

Load package libraries and custom functions

Packages should be loaded in one place with successive calls to library()

```
# 0 General Setup ----
# Initialize and install packages with renv
# renv::init()
## 0.1 Load required libraries ----
library("tidyverse")
library("readxl") # read .xlsx files
library("openxlsx") # data export to Excel workbooks
library("skimr") # data summary and validation
library("janitor") # data cleaning etc
library("ggrepel") # labelling points in plots
library("ggforce") # sina plots etc
library("patchwork") # arranging plots
library("tidyHeatmap") # tidy interface to ComplexHeatmap
library("plotly") # generating interactive plots
library("tictoc") # timer
library("conflicted") # force all conflicts to become errors
conflict_prefer("filter", "dplyr")
conflict_prefer("select", "dplyr")
conflict_prefer("count", "dplyr")
library("here") # generate path to current project directory
source(here("helper_functions.R")) # load helper functions
```

Use comments to explain what packages are being used for Note use of the *here* package which defines and stores project directory path Note use of the *conflicted* package which helps manage function conflicts

Define file locations and other global variables (all input files should be in /data)

```
## 0.2 Set required parameters ----
# Input data files
meta_data_file <- here("data", "meta_data_file.txt") # comments/notes on this file?
data_type1_file <- here("data", "data_type1_file.txt") # comments/notes on this file?
data_type2__file <- here("data", "data_type1_file.txt") # comments/notes on this file?
# Other parameters
standard_colors <- c("Group1" = "#F8766D", "Group2" = "#00BFC4")
out_file_prefix <- "analysis_script_template_v0.1_"
# End required parameters ###</pre>
```

All plots and results exports should use *out_file_prefix* (and go to /plots or /results) Include script version number in *out_file_prefix*

Read in and inspect meta data (sample and/or experiment information)

```
# 1 Read in and inspect data ----
## 1.1 Read in meta data ----
meta_data <- meta_data_file |>
    read_tsv() |>
    janitor::clean_names(case = "none")
# inspect
meta_data
meta_data |> skimr::skim()
#
```

How many samples do you expect? How many measurements do you expect? Which identifiers are unique?

Read in and inspect your main data

```
## 1.2 Read in data type 1 ----
data_type1 <- data_type1_file |>
    read_tsv() |>
    janitor::clean_names(case = "none")
# inspect
data_type1
data_type1 |> skimr::skim()
#

## 1.3 Read in data type 2 ----
data_type2 <- data_type2_file |>
    read_tsv() |>
    janitor::clean_names(case = "none")
# inspect
data_type2
data_type2 |> skimr::skim()
#
```

How many samples do you expect? How many measurements do you expect? Which identifiers are unique?

Join meta data with main data

```
## 1.3 Join meta data with data type 1 and data type 2 ----
combined <- meta_data |>
  inner_join(data_type1) |>
  inner_join(data_type2)
# check number of rows returned
```

Do you have all sample rows after join? CHECK

Analysis specific sections:

- Data exploration
- Statistical testing
- Plot results
- Export results
- Plot individual features

```
# 2 Data exploration / summary ----
# check data distribution(s), outliers etc

# 3 Analysis ----
# statistical testing
# assemble and export results

# 4 Plot results ----
# plot results summaries

# 5 Plot individual features ----
# plot interesting/significant features
```

Finally: save and/or reload workspace

This can save time when resuming, but make sure to resave if something is changed or errors fixed!!

Into the Tidyverse



https://tidyverse.tidyverse.org/

Base R: install.packages("tidyverse")

renv: renv::install("tidyverse")

Core tidyverse packages:

ggplot2, for data visualisation.

dplyr, for data manipulation.

tidyr, for data tidying.

readr, for data import.

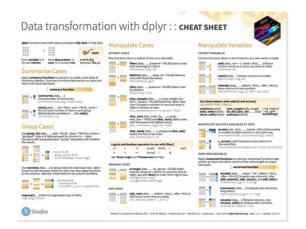
purrr, for functional programming.

tibble, for tibbles, a modern re-imagining of data frames.

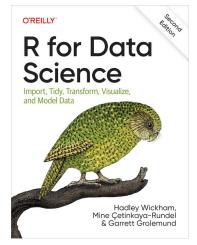
stringr, for strings.

forcats, for factors.

<u>lubridate</u>, for date/times.







Into the Tidyverse: Pipes



Pipe operator %>%

- Avoids nesting
- Minimizes need to create objects and functions
- Structure sequences of operations left-to-right or top-to-bottom
- Easy to inspect and add steps anywhere
- x %>% f is equivalent to f(x)
- x %>% f(y) is equivalent to f(x, y)
- x %>% f %>% g %>% h is equivalent to h (g(f(x)))
- Keyboard shortcut in RStudio: cmd/ctrl+shift+m
- Note: R 4.1.0 introduced a native pipe operator |>
 with some minor differences
 https://www.tidyverse.org/blog/2023/04/base-vs-magrittr-pipe/

Into the Tidyverse: syntax

Base R "dollar sign" syntax

Example - summary statistics:

one continuous variable:

mean (mtcars\$mpg)

one categorical variable:

table (mtcars\$cyl)

two categorical variables:

table(mtcars\$cyl, mtcars\$am)

one continuous, one categorical:

```
mean (mtcars$mpg[mtcars$cyl==4])
mean (mtcars$mpg[mtcars$cyl==6])
mean (mtcars$mpg[mtcars$cyl==8])
```

Tidyverse syntax

Example - summary statistics:

one continuous variable:

```
mtcars %>% dplyr::summarize(mean(mpg))
one categorical variable:
```

```
mtcars %>%
    dplyr::group_by(cyl)%>%
    dplyr::summarize(n())
```

two categorical variables:

```
mtcars %>%
    dplyr::group_by(cyl, am) %>%
    dplyr::summarize(n())
```

one continuous, one categorical:

```
mtcars %>%
    dplyr::group_by(cyl)%>%
    dplyr::summarize(mean(mpg))
```

Into the Tidyverse

Z-score calculation with base R:

```
x <- sweep(sweep(t(dat), 1,
apply(t(dat),1, mean, na.rm=T), FUN = "-"),
1, apply(t(dat),1,sd,na.rm=T), FUN = "/")
```

- hard to decipher (learning barrier)
- have to enter target object name in several places

Z-score calc with tidyverse + scale():

```
zscores <- dat |>
      select(LabID, Analyte, Value) |>
      pivot wider() |>
      scale()
```

 Somewhat easier to decipher, but not obvious that this calculates Z-scores, even looking at ?scale defaults (center = TRUE, scale = TRUE)

Manual Z-score calc with tidyverse:

```
zscores <- dat |>
      select(LabID, Analyte, Value) |>
      group by(Analyte) |>
      mutate(
       zscore = (Value - mean(Value, na.rm = TRUE)) / sd(Value, na.rm = TRUE)
      ) |>
      ungroup()
```

- Naming of new variable
- Easier to see how calculation was performed
- Easy to keep both original and transformed values for comparison

Into the Tidyverse

Z-score calculation with base R:

```
x <- sweep(sweep(t(dat), 1,
apply(t(dat),1,mean,na.rm=T), FUN = "-"),
1, apply(t(dat),1,sd,na.rm=T), FUN = "/")</pre>
```

Even more verbose tidyverse version:

```
zscores <- dat |>
    select(LabID, Analyte, Value) |>
    group_by(Analyte) |>
    mutate(
        mean = mean(Value, na.rm = TRUE),
        sd = sd(Value, na.rm = TRUE),
        zscore = (Value - mean) / sd
    ) |>
        ungroup()
```

- hard to decipher (learning barrier)
- have to enter target object name in several places

- Naming of new variable
- Easier to see how calculation was performed
- Easy to keep both original and transformed values for comparison







Tibbles = enhanced data frames

- Easier preview of data
- Concise summary information including data types

Importing delimited data

- Easy reading in of data from .txt, .csv, .tsv, .xlsx
- Guessing of column types
- Will not convert strings
- Imported as tibble

_															
>	> mpg %>% as.data.frame()														
	manufact	turer		mo	odel	displ	year	cyl	trans	drv	cty	hwy	fl	class	
1	L	audi			a4	1.8	1999	4	auto(15)	f	18	29	р	compact	
2	2	audi			a4	1.8	1999	4	manual(m5)	f	21	29	р	compact	
3	3	audi			a4	2.0	2008	4	manual(m6)	f	20	31	р	compact	
4	1	audi			a4	2.0	2008	4	auto(av)	f	21	30	р	compact	
5	5	audi			a4	2.8	1999	6	auto(15)	f	16	26	р	compact	
6	5	audi			a4	2.8	1999	6	manual(m5)	f	18	26	р	compact	
7	7	audi			a4	3.1	2008	6	auto(av)	f	18	27	р	compact	
5	33	ford	Α\	xplorer	4wd	5.0	1999	8	auto(l4)	4	13	17	r	suv	
	34	ford		pickup			1999	6	auto(14)	4	14	17	r	pickup	
_	35	ford		pickup			1999		manual(m5)	4	14	17	r	pickup	
_															
	36	ford		pickup			1999	8		4	13	16	r	pickup	
_	37	ford		pickup			1999	8	auto(14)	4	13	16	r	pickup	
8	38	ford		pickup			2008	8	auto(l4)	4	13	17	r	pickup	
8	39	ford	f150	pickup	4wd	5.4	1999	8	auto(l4)	4	11	15	r	pickup	
9	90	ford	f150	pickup	4wd	5.4	2008	8	auto(l4)	4	13	17	r	pickup	
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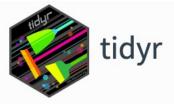
VS.

manufacturer	model	displ	year	cvl	trans	drv	cty	hwy	fl	class
<chr></chr>	<chr></chr>		<int></int>			<chr></chr>	<int></int>			<chr></chr>
1 audi	a4	1.8	<u>1</u> 999	4	auto(15)	f	18	29	р	compac
2 audi	a4	1.8	<u>1</u> 999	4	manual(m5)	f	21	29	р	compac
3 audi	a4	2	<u>2</u> 008	4	manual(m6)	f	20	31	р	compac
4 audi	a4	2	<u>2</u> 008	4	auto(av)	f	21	30	р	compac
5 audi	a4	2.8	<u>1</u> 999	6	auto(15)	f	16	26	р	compac
6 audi	a4	2.8	<u>1</u> 999	6	manual(m5)	f	18	26	p	compac
7 audi	a4	3.1	<u>2</u> 008	6	auto(av)	f	18	27	р	compac
8 audi	a4 quattro	1.8	<u>1</u> 999	4	manual(m5)	4	18	26	р	compac
9 audi	a4 quattro	1.8	<u>1</u> 999	4	auto(15)	4	16	25	р	compac
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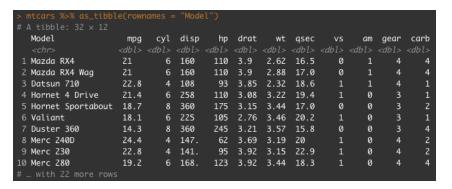
Data manipulation

- mutate() adds new variables that are functions of existing variables.
- select() picks variables based on their names.
- filter() picks rows based on their values.
- summarize () reduces multiple values down to a single summary.
- arrange () changes the ordering of the rows.
- group by () perform group-wise operations.



Reshaping data

- Conversion to/from Tidy data where each column is a variable and each row is an observation.
- pivot_longer() converts to Tidy/long format.
- pivot wider() converts to wide format.
- tibble::column_to_rownames(var = "id_col") converts to data frame (required for some functions).



VS.

+ add additional variables



Character string manipulations

- str_detect(x, pattern) looks for match to the pattern; commonly used with dplyr::filter()
- str_extract(x, pattern) extracts the text of the match; commonly used with dplyr::mutate()
- str_replace(x, pattern, replacement) replaces the matches with new text; commonly used with dplyr::mutate()



Managing factors

- R uses factors to handle categorical variables
- Often important to control the ordering of factors eg for plotting or modelling
- fct_relevel() changes the order of a factor as specified by
 a character vector
- fct_inorder() changes the order of a factor as specified by current order; commonly used with dplyr::arrange()



Functional programming tools for iterating with functions and vectors

- map() family of functions to replace for loops
- see the <u>Iteration</u> chapter of R for Data Science to learn more <u>https://github.com/rstudio/cheatsheets/blob/master/purrr.pdf</u>





Summarizes key statistical model information in tidy format

- tidy() summarizes information about model components.
- glance() reports information about the entire model.
- augment () adds information about observations to a dataset (eg residuals).
- Works with 100+ model objects.
- Plays well with the nest/unnest functions in tidyr and the map functions in purr https://broom.tidymodels.org/articles/broom_and_dplyr.html



Publication-quality data visualization

- Implements a "grammar of graphics"
- Start by defining the data to be plotted ("aesthetics"):

 ggplot(aes(x, y, color, fill, shape, alpha, linetype))
- Then add layers ("geoms") to specify how data is plotted, eg:
 + geom point()
- Add additional geom layers, eg:
 + geom boxplot()
- Can split into separate plots, eg male vs. female, by "faceting":
 + facet_wrap (~ Sex)
- Finally add title and modify theme:

```
+ labs(title = "Plot title", subtitle = "plot details"
+ theme(aspect.ratio = 1)
```

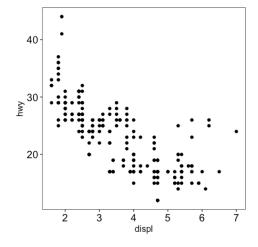


Backs

With a graph of the property of graphs and the property of the property of the property of graphs and the property of the property of graphs and the property of the pr

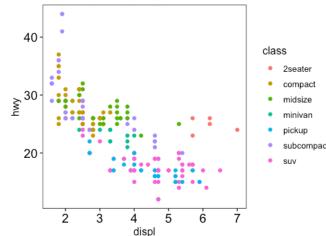


```
mpg %>%
   ggplot(aes(displ, hwy)) +
   geom_point()
```



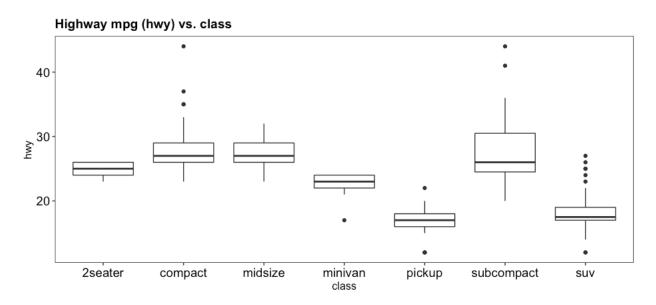
```
mpg %>%
    ggplot(aes(displ, hwy, color = class)) +
    geom_point() +
    theme(aspect.ratio = 1) +
    labs(title = "Highway mpg (hwy) vs. displacement")
```

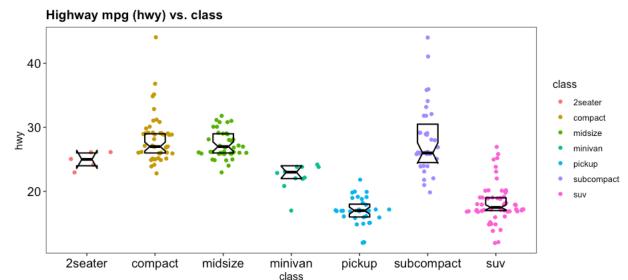
Highway mpg (hwy) vs. displacement





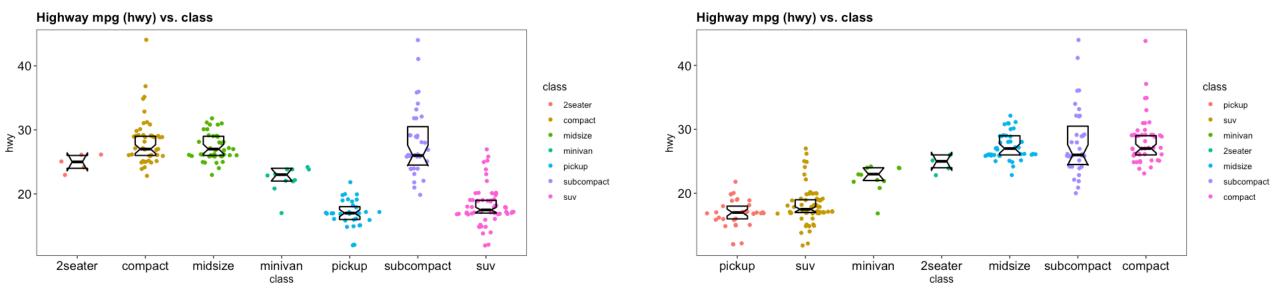
```
mpg %>%
    ggplot(aes(class, hwy)) +
    geom_boxplot() +
    labs(title = "Highway mpg (hwy) vs. class")
```



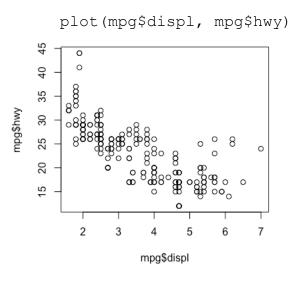


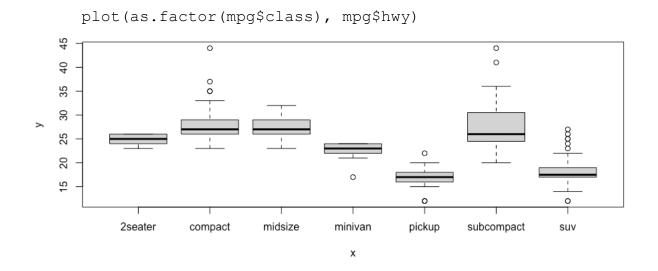


```
mpg %>%
    group_by(class) %>%
    mutate(mean = mean(hwy)) %>%
    ungroup() %>%
    arrange(mean) %>%
    mutate(class = fct_inorder(class)) %>%
    ggplot(aes(class, hwy, color = class)) +
    ggforce::geom_sina() +
    geom_boxplot(
        notch=TRUE, varwidth=FALSE, outlier.shape=NA, coef=FALSE, width=0.3, color="black", fill="transparent", size=0.75") +
    labs(title = "Highway mpg (hwy) vs. class")
```



Equivalent plots using base R





Other commonly used packages

ggforce

Additional plot geoms, including geom_sina() https://ggforce.data-imaginist.com/

ggrepel

Repel overlapping text labels https://ggrepel.slowkow.com/index.html

ggrastr

Rasterize ggplot objects or layers
https://cran.r-
project.org/web/packages/ggrastr/vignettes/Raster geoms.html

ggsignif

Test and/or add significance brackets to plots https://cran.r-project.org/web/packages/ggsignif/vignettes/intro.html

tidyheatmap

Tidy interface to ComplexHeatmap https://cran.r-

project.org/web/packages/tidyHeatmap/vignettes/introduction.html

janitor

cleaning variable names
https://cran.r-project.org/web/packages/janitor/vignettes/janitor.html

rstatix

Tidy and pipe-friendly statistics, e.g. t-test, Wilcoxon/Mann-Whitney https://cran.r-project.org/web/packages/rstatix/index.html

patchwork

Assemble multiple ggplot objects https://patchwork.data-imaginist.com/index.html



The Bioconductor Project

https://bioconductor.org/