R: A Hitchhikers Guide to Reproducible Research

- Everything in it's right place

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CRF-C
HRB Clinical Research Facility Cork

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University College Cork, Ireland
Coláiste na hOllscoile Corcaigh
School of Public Health

How is research presented?

Papers

Journal of Virology

Network Analysis of the Chronic Hepatitis C Virome Defines Hypervariable Region 1 Evolutionary Phenotypes in the Context of Humoral Immune Responses

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ABSTRACT

Hypervariable region 1 (HVR1) of hepatitis C virus (HCV) comprises the first 27 N-terminal amino acid residues of E2. It is classically seen as the most heterogeneous region of the HCV genome. In this study, we assessed HVR1 evolution by using ultradeep pyrosequencing for a cohort of treatment-naive, chronically infected patients over a short, 16-week period. Organization of the sequence set into connected components that represented single nucleotide substitution events revealed a network dominated by highly connected, centrally positioned master sequences. HVR1 phenotypes were observed to be under strong purifying (stationary) and strong positive (antigenic drift) selection pressures, which were coincident with advancing patient age and cirrhosis of the liver. It followed that stationary viromes were dominated by a single HVR1 variant surrounded by minor variants comprised from conservative single amino acid substitution events. We present evidence to suggest that neutralization antibody efficacy was diminished for stationary-virome HVR1 variants. Our results identify the HVR1 network structure during chronic infection as the preferential dominance of a single variant within a narrow sequence space.

IMPORTANCE

HCV infection is often asymptomatic, and chronic infection is generally well established in advance of initial diagnosis and subsequent treatment. HVR1 can undergo rapid sequence evolution during acute infection, and the variant pool is typically seen to diverge away from ancestral sequences as infection progresses from the acute to the chronic phase. In this report, we describe HVR1 viromes in chronically infected patients that are defined by a dominant epitope located centrally within a narrow variant pool. Our findings suggest that weakened humoral immune activity, as a consequence of persistent chronic infection, allows for the acquisition and maintenance of host-specific adaptive mutations at HVR1 that reflect virus fitness.

Hepatitis C virus (HCV) infection is a global health issue and is recognized as a major etiological agent of liver-related diseases (1). It has been estimated that the current prevalence of HCV represents approximately 2% of the global adult (15 years of age and older) population (2). Following transmission, HCV infection may remain asymptomatic for decades, resulting in the majority of infections initially passing undetected (3). It is estimated that up to 4 million Americans are living with the virus, the majority of whom became infected prior to the isolation and identification of the virus (4, 5). Consequently, the U.S. Centers for Disease Control and Prevention now recommend that Americans born from 1945 to 1965 be screened for the presence of the virus notwithstanding the presence of clinical symptoms (3, 5).

HCV is a single-stranded positive-sense RNA virus of considerable genomic heterogeneity. A recent reclassification defined the HCV global distribution into 7 genotypes and 67 subtypes, with genotypes 1 and 3 accounting for the majority of infections worldwide (6, 7). An error-prone RNA-dependent RNA polymerase, together with an inherent tolerance of defined hypervariable regions (HVR), accounts for much of this variability. Three HVRs are located within the envelope glycoprotein E2. The greatest heterogeneity has been identified at the 27-amino-acid HVR1 (residues 384 to 410 of the H77 reference strain), located at the amino-terminal end of the E2 glycoprotein (8). Recent studies indicated that the central region of E2 (residues 456 to 656) is globular and surprisingly compact, whereas the first 80 amino acids (including HVR1) lack this structural rigidity (9). This observation is consistent with a region that is proposed to shield conserved neutralizing epitopes and to participate in high-density lipoprotein enhancement of infection via scavenger receptor class B type I (SRBI) interactions and is itself targeted by neutralizing antibodies (nAb) (10–16).

Mutational flexibility at HVR1 was characterized soon after the initial identification of HCV (8, 17). Rapid mutational change of HVR1 has been documented over weeks during the acute phase of infection, where HVR1 evolution is governed predominantly by strong selective pressures, with fixation of beneficial mutations (11, 18, 19). Reports examining samples collected over years to decades have documented the emergence of convergent HVR1

Received 25 November 2015 Accepted 22 December 2015
Accepted manuscript posted online 30 December 2015
Citation Palmer BA, Schmidt-Martin D, Dimitrova Z, Skums P, Crosbie O, Kenny-Walsh E, Fanning LJ. 2016. Network analysis of the chronic hepatitis C virome defines hypervariable region 1 evolutionary phenotypes in the context of humoral immune responses. J Virol 90:3318–3329. doi:10.1128/JVI.02995-15.
Editor M. S. Diamond
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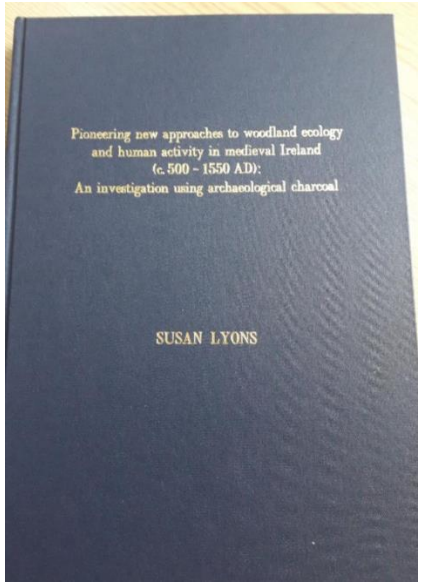
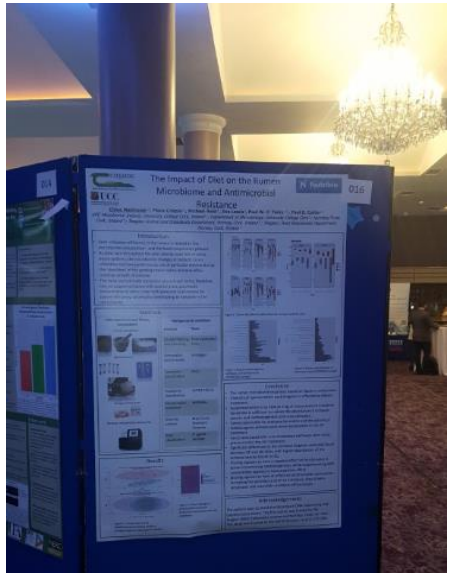
Books

Theses



Talks

Posters

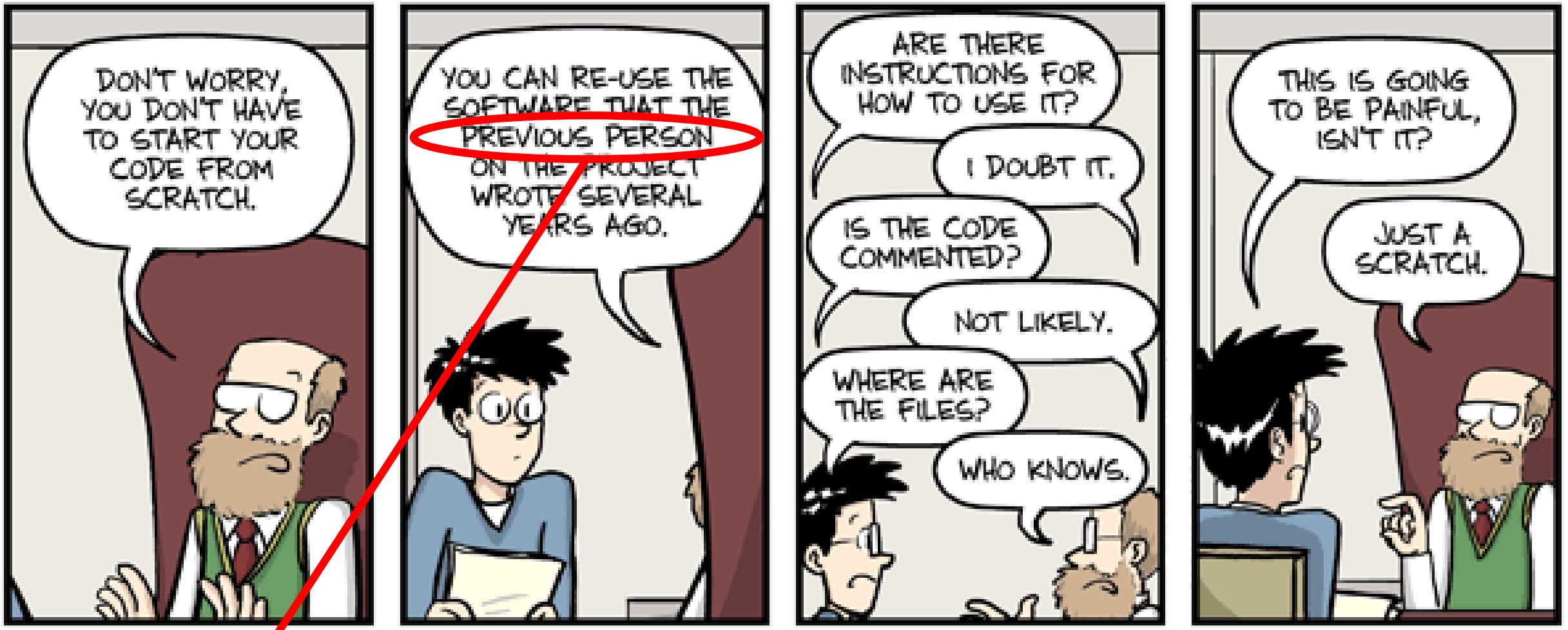


But what does it really look like?

Zheng Lab - Bad Project (Lady Gaga parody)

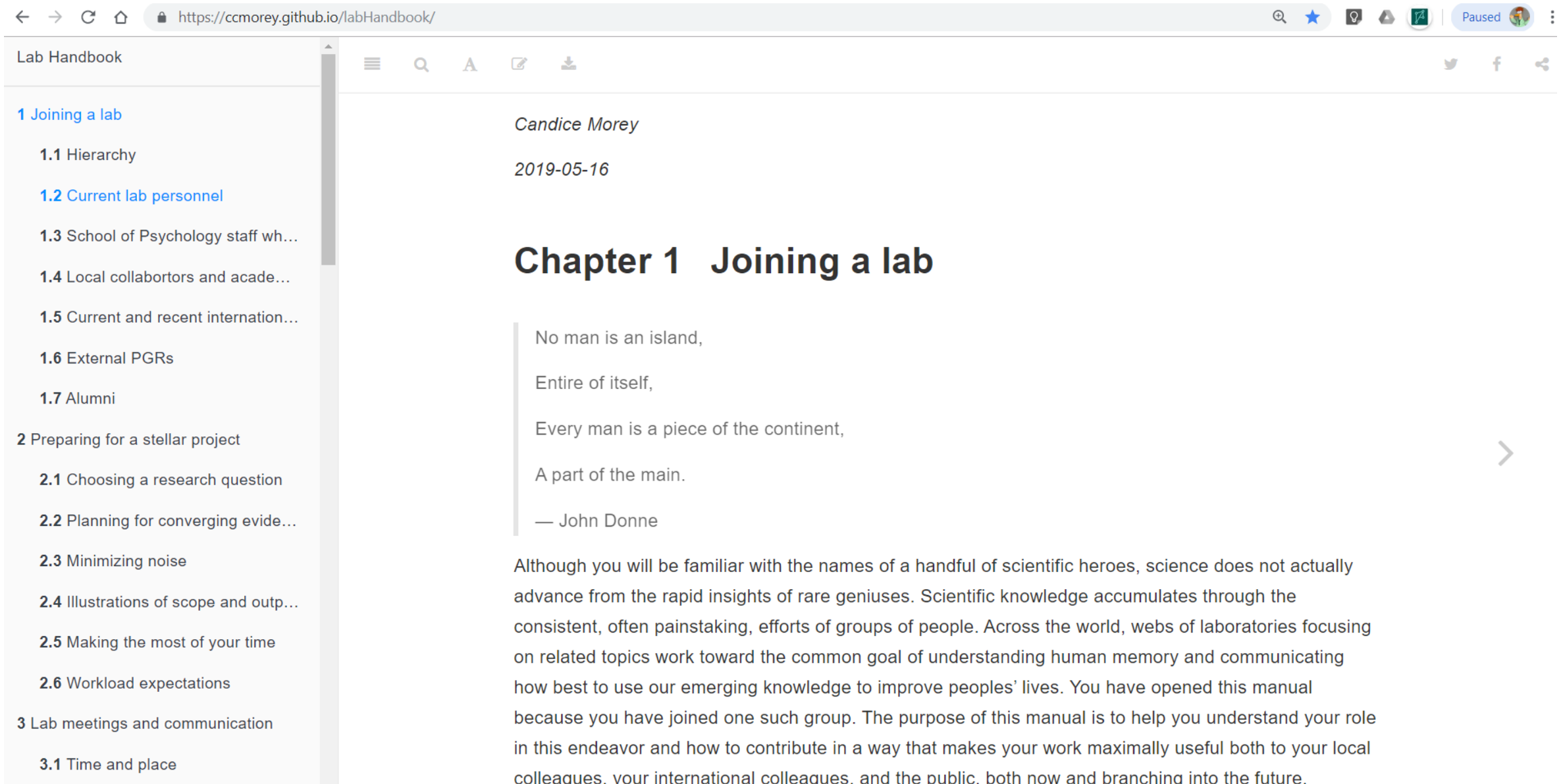
Zheng Lab - Bad Project (Lady Gaga parody)

Zheng Lab - Bad Project (Lady Gaga parody)



THIS PERSON IS likely to be YOU BTW!!

Reproducibility comes in many forms



Work from the raw data ALWAYS!!

Tom Webb @tomjwebb · 16 Jan 2015

If you could tell a new PhD student one thing to help make their data more useful/shareable, what would it be?

27 11 7

Dr Gavin Simpson

@ucfagls

Follow

Replying to @tomjwebb

@tomjwebb don't, not even with a barge pole, not for one second, touch or otherwise edit the raw data files. Do any manipulations in script

7:15 AM - 16 Jan 2015

Still haven't found what I'm looking for

- Help your future-self

B_Palmer_Medicine_Files › 4a Project › Pyrosequencing_analysis › Pyrosequencing_Paper › Draft_Paper_incl_Figs › Submission › JVI_Resubmission › JVI_resubmission_files › Final Final version

Name	Date modified
Cover_letter_B_A_Palmer_Sept_2014	10/09/2014 17:05
Fig_1_Sept_14	11/09/2014 10:31
Fig_1_Sept_14	10/09/2014 23:07
Fig_2_Sept_14	11/09/2014 10:31
Fig_2_Sept_14	10/09/2014 23:07
Fig_3_Sept_14	11/09/2014 10:31
Fig_3_Sept_14	10/09/2014 23:07
Fig_4_Sept_14	11/09/2014 10:31
Fig_4_Sept_14	10/09/2014 23:07
Fig_5_Sept_14	11/09/2014 10:33
Fig_5_Sept_14	10/09/2014 23:07
HCV_UDPS_B_A_Palmer_Sept_14	17/09/2014 12:21
Response_to_Reviewer_Sept_14	10/09/2014 22:42
Supplementry_Figure_B_A_Palmer_Sept_14	29/08/2014 13:21
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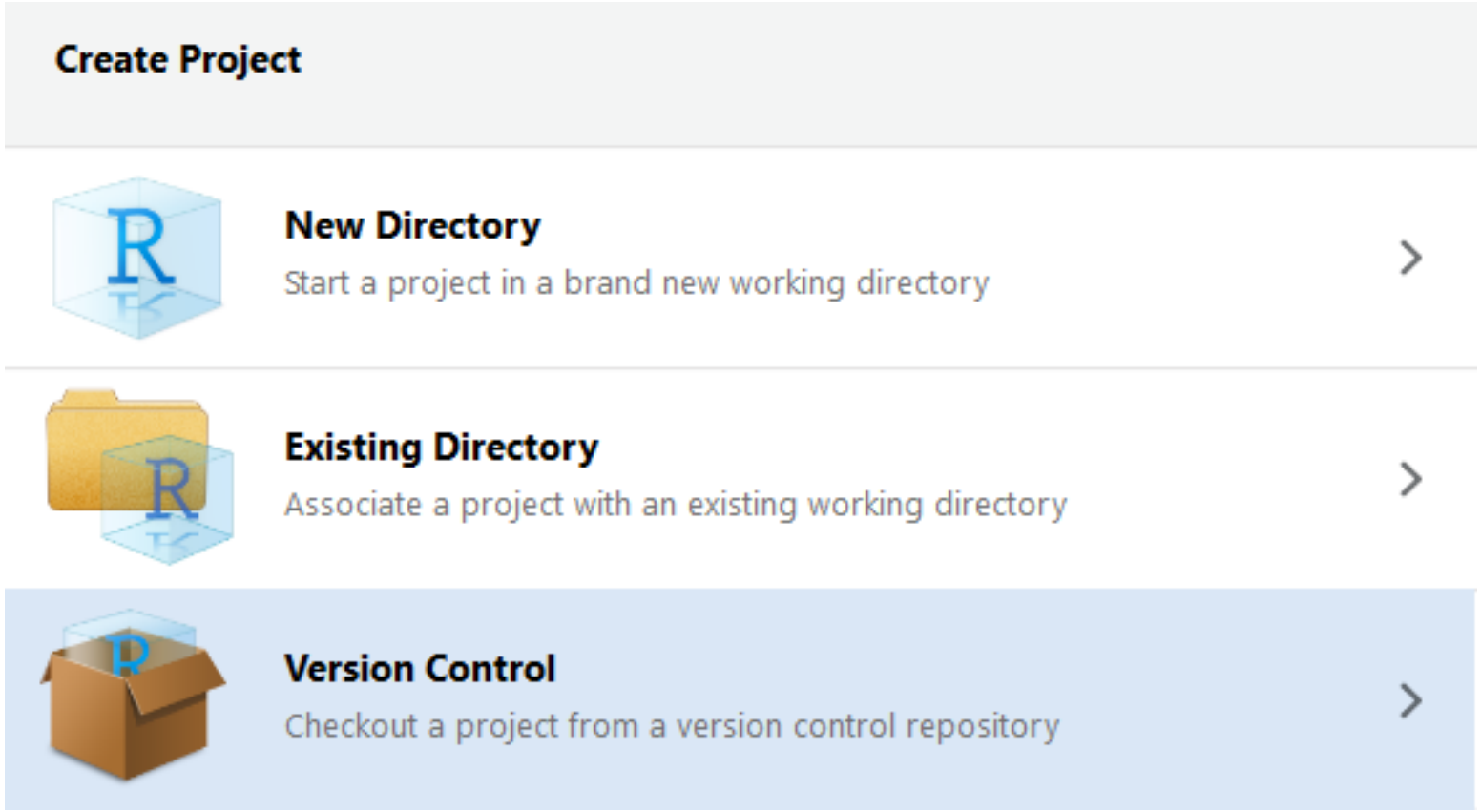
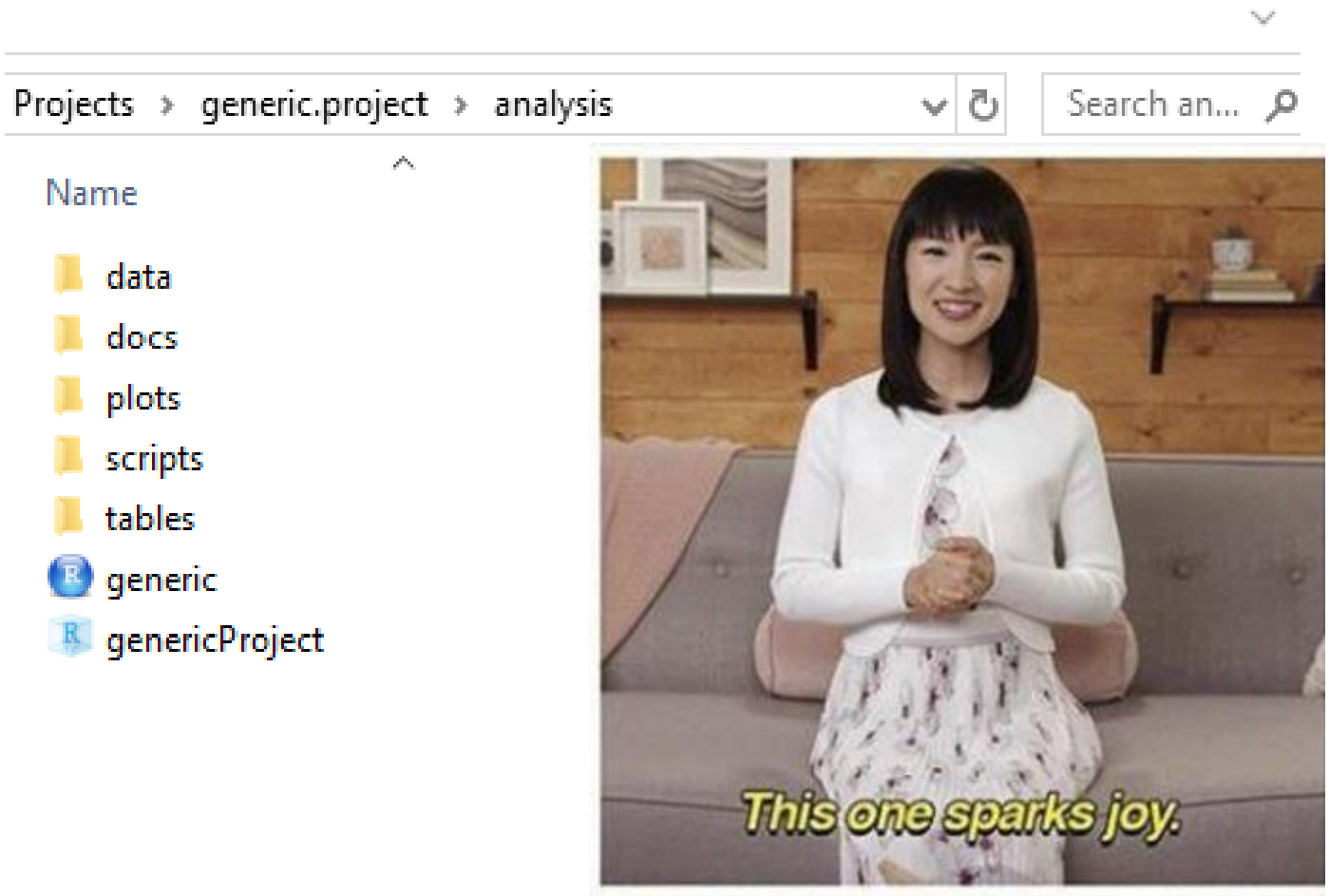
Tables_B_A_Palmer_Sept_2014	10/09/2014 22:09

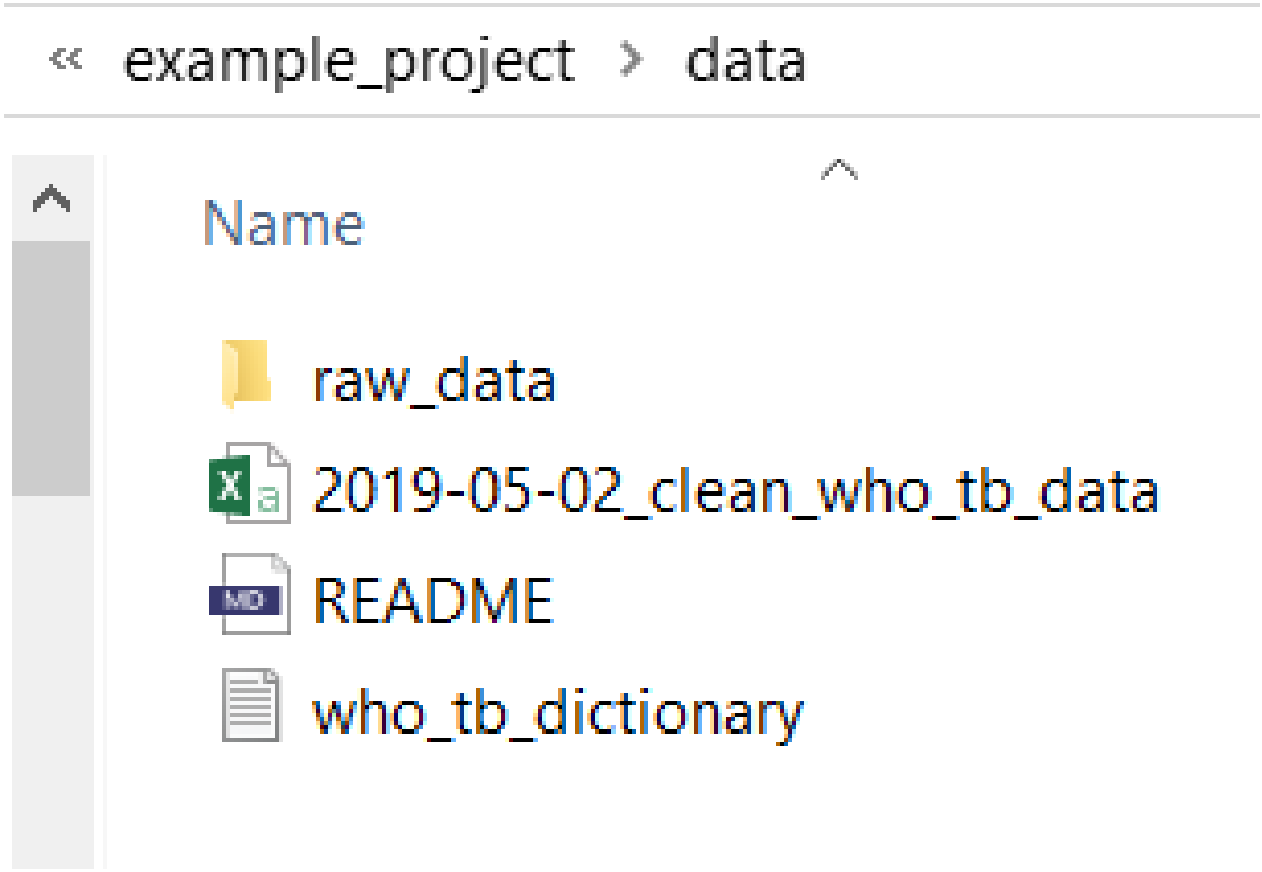
R-projects


Create Project
New Directory
Start a project in a brand new working directory
Existing Directory
Associate a project with an existing working directory
Version Control
Checkout a project from a version control repository

Step 1: Define a generic project structure

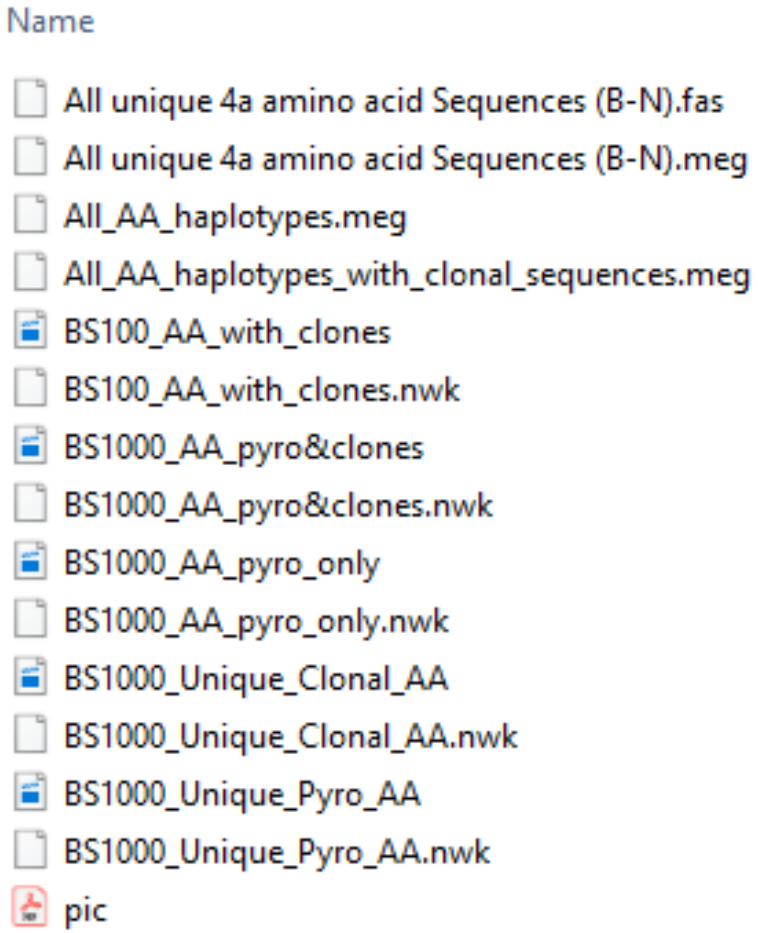


Step 2: Give your files informative names

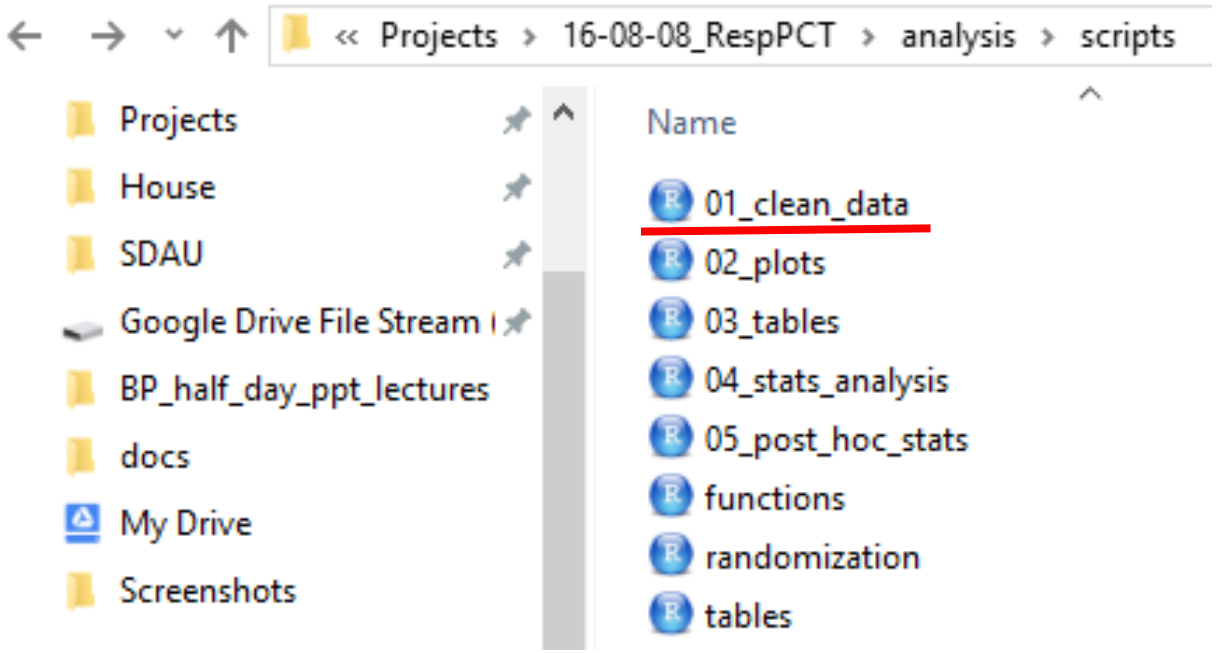


Step 3: Make you file names machine readable, human readable and work with default ordering

NO



Yes



Step 4: Outline a file naming convention

Machine readable:

- Inherent order
- Avoid spaces
- Avoid punctuation
- Remove case-sensitivity

Human readable:

- Contains info on content
- Avoid spaces
- Avoid punctuation
- Remove case sensitivity

Metadata:

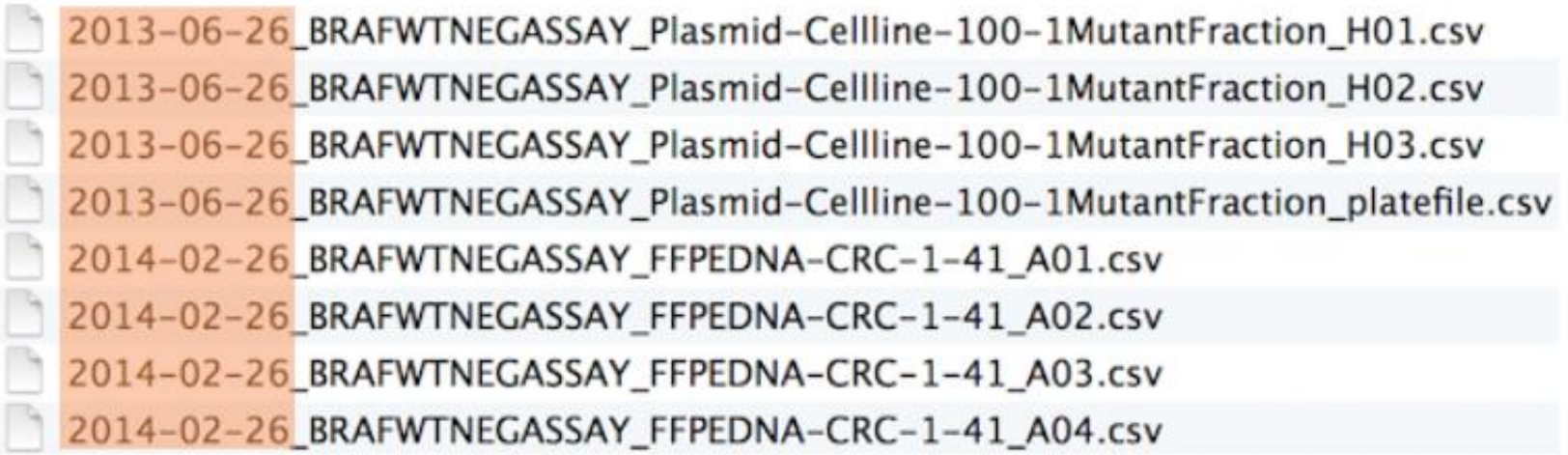
Separate with underscores ("_")

- Avoid punctuation
- Remove case-sensitivity

```
01_marshal-data.r

02_pre-dea-filtering.r

03_dea-with-limma-voom.r

04_explore-dea-results.r

90_limma-model-term-name-fiasco.r

helper01_load-counts.r

helper02_load-exp-des.r

helper03_load-focus-statinf.r

helper04_extract-and-tidy.r
```

Step 4: Outline a file naming convention

Chronological order:



Logical order:

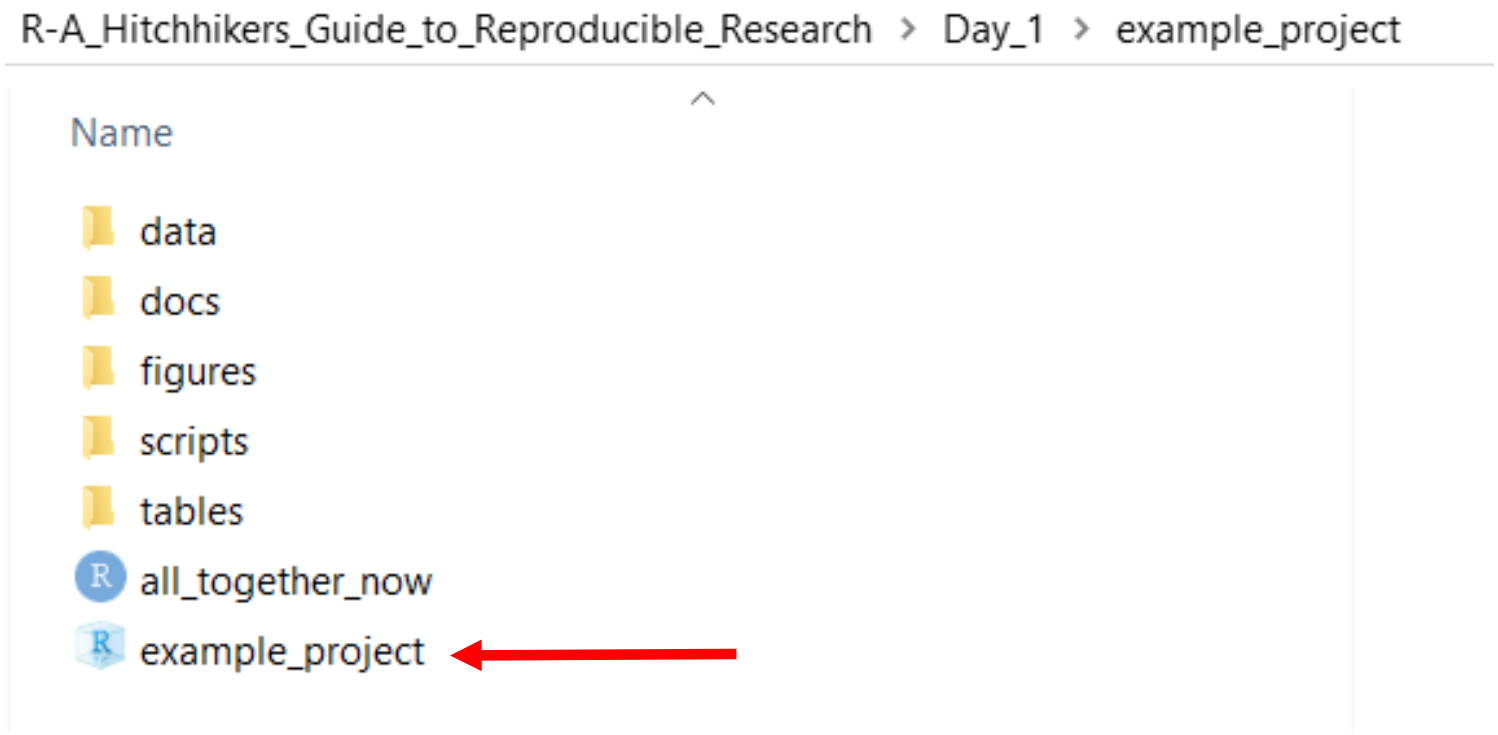
```
01_marshal-data.r
02_pre-dea-filtering.r
03_dea-with-limma-voom.r
04_explore-dea-results.r
90_limma-model-term-name-fiasco.r
helper01_load-counts.r
helper02_load-exp-des.r
helper03_load-focus-statinf.r
helper04_extract-and-tidy.r
```

Step 5: Joined up thinking

- The R scripts you generate should be human readable
    - Annotate the code
    - Break up the scripts into dedicated tasks
    - Interlink with other within project scripts

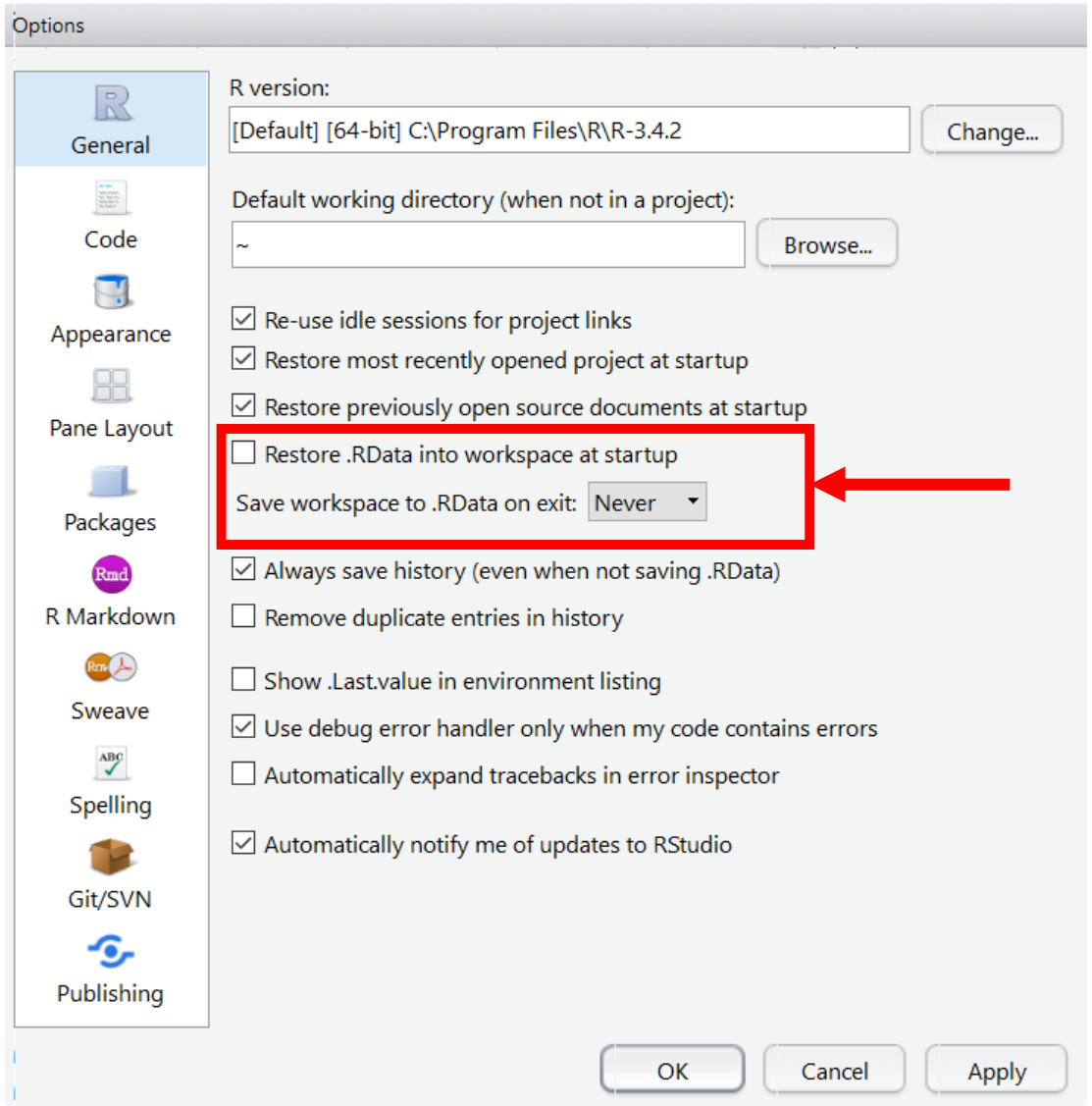
```r
# Script: 04_stats_analysis.R

# Data ----
# Four tibbles will be returned from scripts/01_clean_data.R
# 1. abx => details of the antibiotic consumption by type
# 2. monitoring => patient condition over time. Also WCC, CRP
# 3. pct => PCT values from the PCT arm of the trial
# 4. pt_info => general patient information

# Load the cleaned data sets
source("scripts/01_clean_data.R")

#Load the necessary add-on packages
library(knitr)
library(broom)
library(survminer)
```

Step 6: R-projects



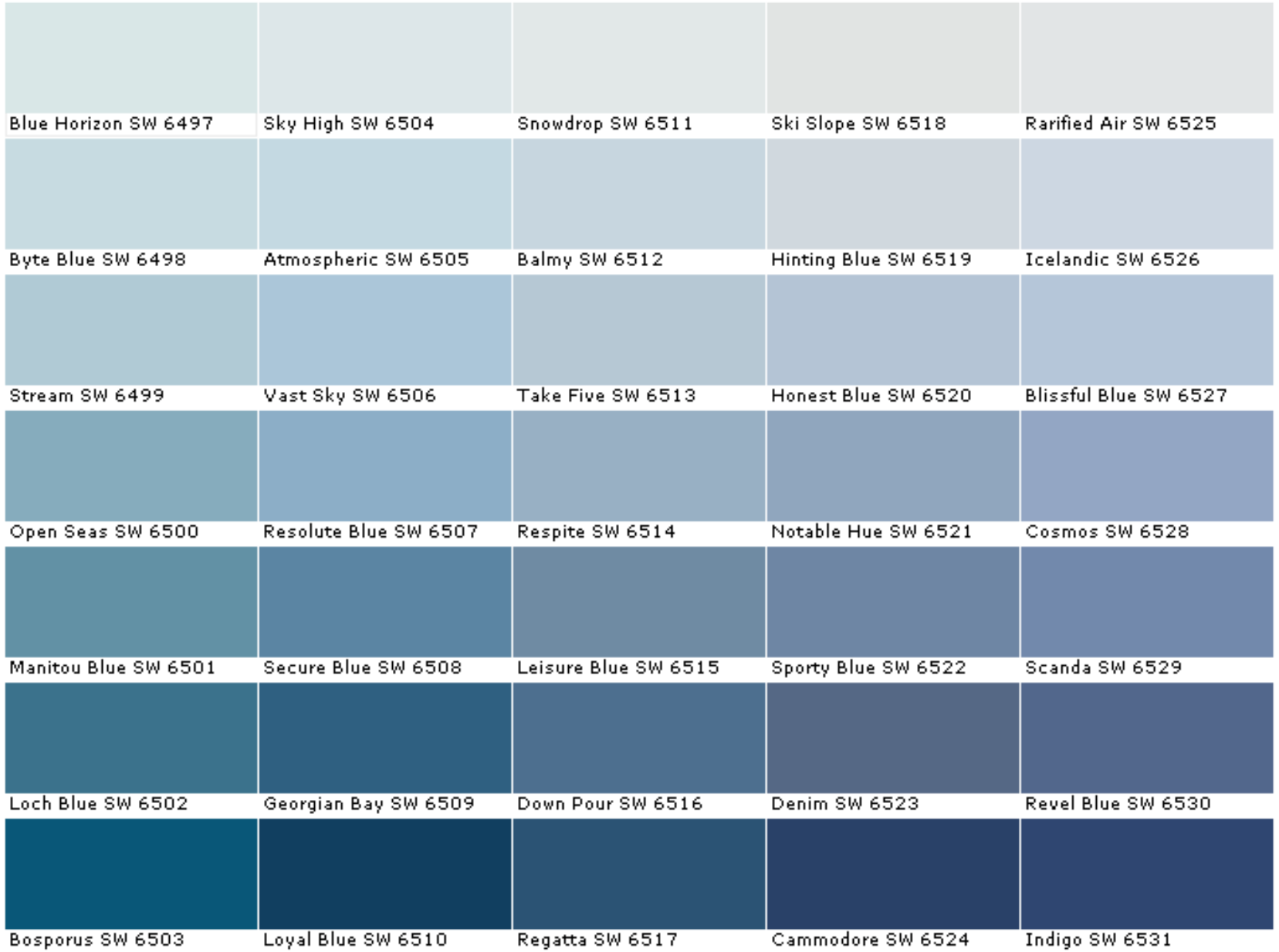
- Switch to the R-project file… Day_1/example_project/example_project.Rproj
- Open the scripts 01_eg_clean_data.R, 02_eg_figures.R and 03_eg_analysis.R

Other points to note



- You might consider your environment as "real"
- If you continue to use R, it is better for you to consider your R scripts as "real", as these should recreate the environment
- You may suffer short term pain
- This will prevent long term agony

Is too much choice good or bad?



Inconsistent function names, inconsistent syntax

- R is a very versatile language
  - Sometimes it can be too versatile
  - Do you want to use…

```
row.names or rownames
rowSums or rowsum
Sys.time, system.time
```

  - Should it be written as…

```
newobject or new.Object
x = 5 or x <- 5
mapping=aes(x,y) or mapping = aes(x, y)
```

Variable selection

```
summary(starwars$name)

summary(starwars$"name")

summary(starwars["name"])

summary(starwars[ , "name"])

summary(starwars[1])

summary(starwars[ , 1])

summary(starwars[[1]])
```

- Open the script 04_too_much.choice.R

Motivation to move on from poorly written code

am_bad_habits.R

Source on Save | Run | Source

```
21 sites1<-as.list(unique(RL6.7$Var1))
22 sites2<-as.list(unique(RL6.7$Var2))
23
24 sites<-as.data.frame(t(merge(sites1,sites2)))
25 colnames(sites)[1]<-"Position"
26
27 for(i in 1:nrow(sites)){
28 ans<-(sites$Position[i]<=65)
29 sites$E1[i]<-ans
30 }
31
32 # Start building network
33 RL6.7_topology<-subset(RL6.7[2:3])
34 g2<-graph.data.frame(RL6.7_topology,vertices=sites,directed=FALSE)
35 V(g2)$color<-ifelse(V(g2)$E1==TRUE,"white","grey")
36 V(g2)$color<-ifelse(V(g2)$E1==TRUE,"white","grey")
37 plot(g2,vertex.label.color="black",vertex.size=20,edge.color="black",edge.width=1.5)
```

Environment | History | Connections

Import Dataset | Grid

Global Environment

Name	Type	Length	Size	Value
ans	logical	1	56 B	TRUE
df	spec_tbl…	4	5.9 KB	39 obs. of 4 variables
g2	igraph	10	2.4 KB	List of 10
i	integer	1	56 B	4L
RL1.2	tbl_df	4	1.4 KB	3 obs. of 4 variables
RL2.3	tbl_df	4	1.4 KB	3 obs. of 4 variables
RL3.4	tbl_df	4	1.3 KB	1 obs. of 4 variables
RL4.5	tbl_df	4	1.8 KB	9 obs. of 4 variables
RL5.6	tbl_df	4	1.9 KB	20 obs. of 4 variables
RL6.7	tbl_df	4	1.3 KB	2 obs. of 4 variables
RL6.7_topolo…	tbl_df	2	1016 B	2 obs. of 2 variables
sites	data.fra…	2	1.1 KB	4 obs. of 2 variables
sites1	list	2	176 B	List of 2
sites2	list	2	176 B	List of 2

Lack of annotation
Poor naming conventions
Poor readability
Spacing absent

Cluttered environment
Intermediate objects

- Open the script 05_bad_habits.R

Writing clearer code

- Annotation

- Object names
  - should use only lowercase letters, numbers, and "_"

- Spacing
  - Put a space before and after =
  - Put a space after a ,
  - Operators should be surrounded by spaces e.g. ==, <-, +

- For a more complete list visit
  - http://style.tidyverse.org/syntax.html

- Open the script 06_good_habits.R