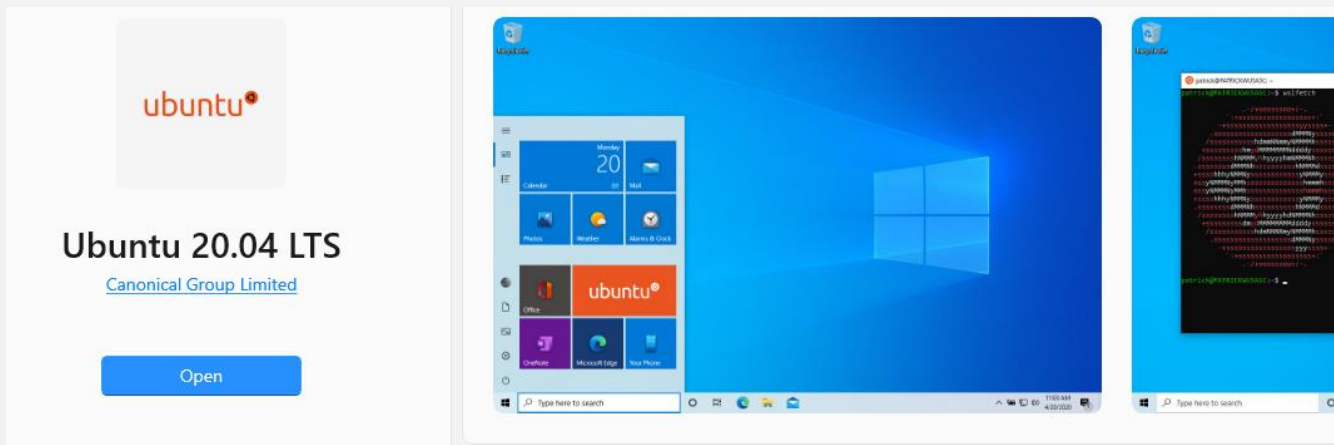


COMPUTER RESOURCES

- Computer
- Some kind of Linux
 - You use Linux? → you're fine (but need Win/Mac to run Loupe browser)
 - You use Windows? → use Windows Subsystem for Linux → you're fine



- You use Mac OS? → you're mostly fine, but I can't help you
 - My Mac experience dates back to Mac OS X Leopard!

GIT REPOSITORY

The screenshot shows the GitHub interface for the repository 'mjoppich / game_cibog_2022'. The repository is private and has 1 branch (main) and 0 tags. The file list shows a folder structure with files like 'alignments/bulk', 'bulk', 'bulk_own', 'code', 'images', 'reads', 'ready_to_use_data', 'references', 'sc', 'tools', and 'README.md'. A green arrow points to the 'ready_to_use_data' folder with the text 'Data for direct use'. The README.md file is also visible, containing the text 'game_cibog_2022' and 'Workshop material for GAME-CIBoG workshop'.

File/Folder	Type	Commit
alignments/bulk	folder structure	2 minutes ago
bulk	folder structure	2 minutes ago
bulk_own	folder structure	2 minutes ago
code	folder structure	2 minutes ago
images	folder structure	2 minutes ago
reads	folder structure	2 minutes ago
ready_to_use_data	folder structure	2 minutes ago
references	folder structure	2 minutes ago
sc	preprocessed data	2 minutes ago
tools	folder structure	2 minutes ago
README.md	Initial commit	10 minutes ago

- git clone https://github.com/mjoppich/game_cibog_2022.git

- Folder structure useful (not required though!) for workshop

- Send eMail to joppich@bio.ifl.lmu.de
with Github username to get access


SOFTWARE RESOURCES

- You need some R version

```
mjoppich@spectre3:/mnt/c/Users/mjopp$ R --version
R version 4.0.3 (2020-10-10) -- "Bunny-Wunnies Freak Out"
Copyright (C) 2020 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)
```

- You may want to use jupyter+R kernel
 - `install.packages("devtools")`
 - `devtools::install_github("IRkernel/IRkernel")`
 - `IRkernel::installspec()`

- Some kind of IDE or jupyter-lab in the browser

- Big fan of Visual Code 
 - View PDF, PNG, JPEG
 - Supports jupyter notebooks and plots

```
options(jupyter.plot_mimetypes = 'image/png')
options(repr.plot.width = 1, repr.plot.height = 0.75, repr.plot.res = 100)
```

REQUIRED TOOLS FOR DOWNLOADING DATA

Getting the read data

- sratoolkit for downloading files
 - `wget --output-document tools/sratoolkit.tar.gz http://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/current/sratoolkit.current-ubuntu64.tar.gz`
 - `cd tools && tar -vxzf sratoolkit.tar.gz`
 - `vdb-config -interactive`
 - Make path to sratoolkit visible:
 - Add
 - `export PATH=$PATH:/mnt/t/workshop/tools/sratoolkit.2.11.2-ubuntu64/bin`
 - to `~/.bashrc` and source `~/.bashrc`
- Then we can call *fastq-dump* as
 - `fastq-dump --gzip SRR9048093`
 - for downloading specific SRA-files

REQUIRED FILES AND REFERENCES

Doing the alignment

- Hisat2
 - `sudo apt install hisat2`
 - <http://daehwankimlab.github.io/hisat2/>
 - Index/Reference genome: H. sapiens, GRCh38, https://genome-idc.s3.amazonaws.com/hisat/grch38_genome.tar.gz
 - `tar xzf grch38_genome.tar.gz`
- featureCounts / subread
 - `sudo apt install subread`
 - <https://sourceforge.net/projects/subread/files/subread-2.0.3/>
- Genome Annotation File:
 - http://ftp.ensembl.org/pub/release-105/gtf/homo_sapiens/Homo_sapiens.GRCh38.105.gtf.gz
 - `gunzip Homo_sapiens.GRCh38.105.gtf.gz`

Binaries


Version: HISAT2 2.2.1


Release Date: 7/24/2020


Source	https://cloud.biohpc.swmed.edu/index.php/s/fe9QCsX3NH4QwBi/download
OSX_x86_64	https://cloud.biohpc.swmed.edu/index.php/s/zMgEtnF6LjnJFrr/download
Linux_x86_64	https://cloud.biohpc.swmed.edu/index.php/s/oTtGWbWjaxsQ2Ho/download

Gene annotation

What can I find? Protein-coding and non-coding genes, splice variants, cDNA and protein sequences, non-coding RNAs.

 [More about this genebuild](#)

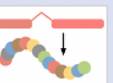
 [Download FASTA files for genes, cDNAs, ncRNA, proteins](#)

 [Download GTF or GFF3 files for genes, cDNAs, ncRNA, proteins](#)

 [Update your old Ensembl IDs](#)



Example gene



Example transcript

R PACKAGES

```
# ensures we can install everything
```

```
library(devtools) # => install.packages("devtools")
```

```
# accessing files, plotting
```

```
library(readr) # => install.packages("readr")  
library(ggplot2) # => install.packages("ggplot2")  
library(cowplot) # => install.packages("cowplot")  
library(ggrepel) # => install.packages("ggrepel")  
library(pheatmap) # => install.packages("pheatmap")  
library(RColorBrewer) # => install.packages(" RColorBrewer ")
```

```
# Bioconductor package manager => required for basically all bioinformatics tasks
```

```
if (!requireNamespace('BiocManager', quietly = TRUE))  
  install.packages('BiocManager')
```

```
library(EnhancedVolcano) # => BiocManager::install('EnhancedVolcano') or devtools::install_github('kevinblighe/EnhancedVolcano')  
library(DESeq2) # => BiocManager::install('DESeq2')  
library(clusterProfiler) # => BiocManager::install('clusterProfiler')  
library(DOSE) # => BiocManager::install('DOSE')  
library(enrichplot) # => BiocManager::install('enrichplot')  
library(ReactomePA) # => BiocManager::install('ReactomePA')  
library(org.Hs.eg.db) # => BiocManager::install('org.Hs.eg.db')
```

```
library(disgenet2r) # => devtools::install_bitbucket("ibi_group/disgenet2r")
```

DOWNLOADING CELLRANGER+LOUPE

Cell Ranger - 6.1.2 (October 25, 2021)

- Self-contained, relocatable tar file. Does not require centralized installation.
- Contains binaries pre-compiled for CentOS/RedHat 6.0+ and Ubuntu 12.04+.
- [Download - Linux 64-bit - 768 MB](#) - md5sum: 310d4453acac0e0ec52e76aded14024c

curl	wget
------	------

```
wget -O cellranger-6.1.2.tar.gz "https://cf.10xgenomics.com/releases/cell-exp/cellranger-6.1.2.tar.gz?Expires=1642730466&Policy=eyJ0IGZlbnQ1bm90dGl0IiwiaWF0IjE1LjIwMjUyOTYzNjUyMDk1MTIwIiwiaHR0cHM1YjIzIiwiaHhndWVsbWJlc3Vjb2N0cmVzfXZmZXMyV2VsY2VsbC1leAAYyY2VsbHJhbmdldi02LjEuMiS0YXIUXz0iLCJDID25kaXRpb240nsIRGF0ZUxlc3NUAGFuIjp7IkFkFUzPfcG9jaFRpbWUiOjE2NDIUMzMA0NJ29FXldfQeSignature=USFWTdaZIygJwvTt1CS0xZdwZmDq2CH-w-0t1Wg1CUdTUVN-LKbcgjYXLN8IAIEaSignackCckQ4AJRw-bbNRGWf70MS9oB5WheoRAopYzKXuNaCT97tkS9NN1UpwAbRDgdDJfZ1QdKMCT3cg3-AXRL0xFnxhNVvfjcpcjrN1dxv7qcS862tVezERtC9OKrwCR1syjfFnCKKhmiO4xPARAdQs8UhoFloLxLb-WslM7f-CSNJ4-dv-RFRGS-0ePVUu-s2mOGddtOF66je12kZo433Z7SlEx11GWK6ktwpJBESqksNoJFYCDTwPHCXU-F4X8nTh6rpGNEvS48QEha__&Key-Pair-Id=APWKAT156ASRYOXBWPRDA"
```

- Human reference (GRCh38) dataset required for Cell Ranger.
- [Download – 11 GB](#) – md5sum: dfd654de39bff23917471e7fcc7a00cd
- [Build steps](#)

curl	wget
------	------

```
wget https://cf.10xgenomics.com/supp/cell-exp/refdata-gex-GRCh38-2020-A.tar.gz
```

- <https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest>
- Might require brief registration



Cell Ranger 6.1.2

Single Cell Analysis Pipelines




Loupe Browser 6.0.0

Interactive Analysis

Loupe Browser 6.0.0 (October 7, 2021)

Please follow the [install instructions](#) after downloading one of the installers below.

[Read about what's new in Loupe Browser 6.0](#)



```
filesize: 694 MiB
```

```
md5sum: 8fce42ab47c3d040d7a1538be8d33900
```

 [Download for macOS](#)

```
filesize: 784 MiB
```

```
md5sum: aee779daa98bc101092169666fd1c2aa
```

DOWNLOAD RSTUDIO

Deliver open source data science at *scale*

Learn how RStudio's enterprise-ready pro products help data science teams securely deploy their work, collaborate, and communicate with decision makers.

SEE OUR PRO PRODUCTS IN ACTION



RStudio Desktop 2021.09.2+382 - [Release Notes](#)

1. Install R. RStudio requires [R 3.0.1+](#).
2. Download RStudio Desktop. Recommended for your system:

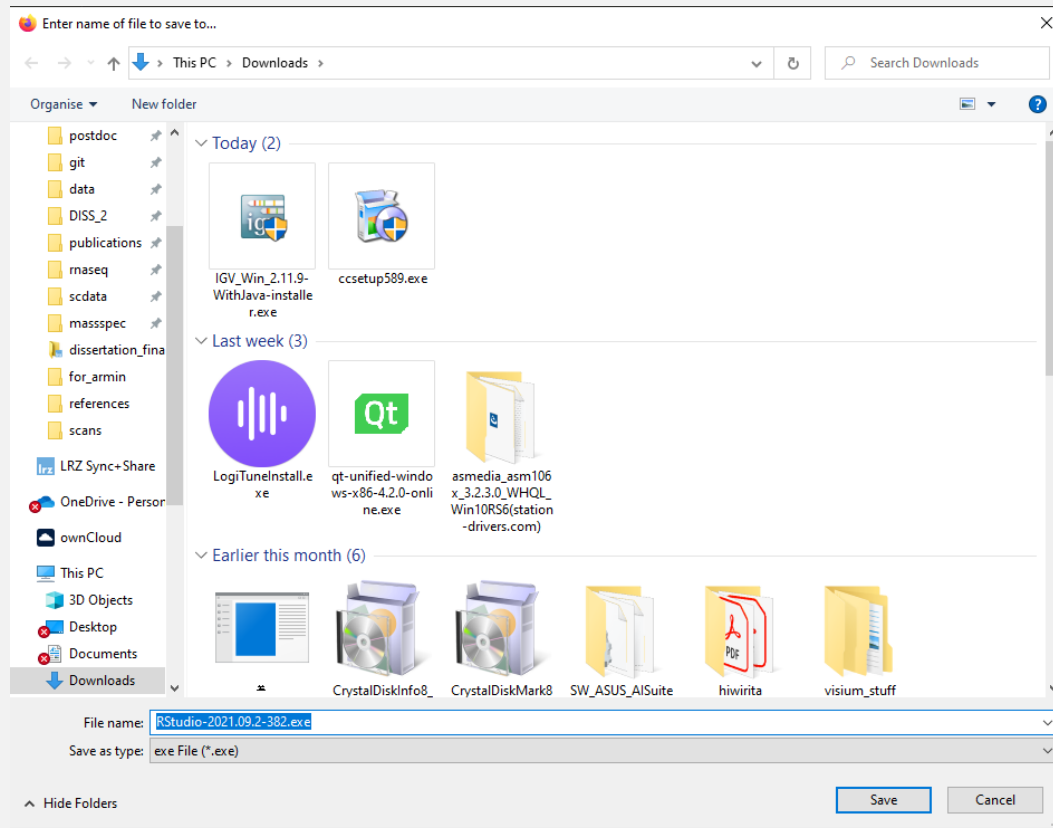


Requires Windows 10 (64-bit)



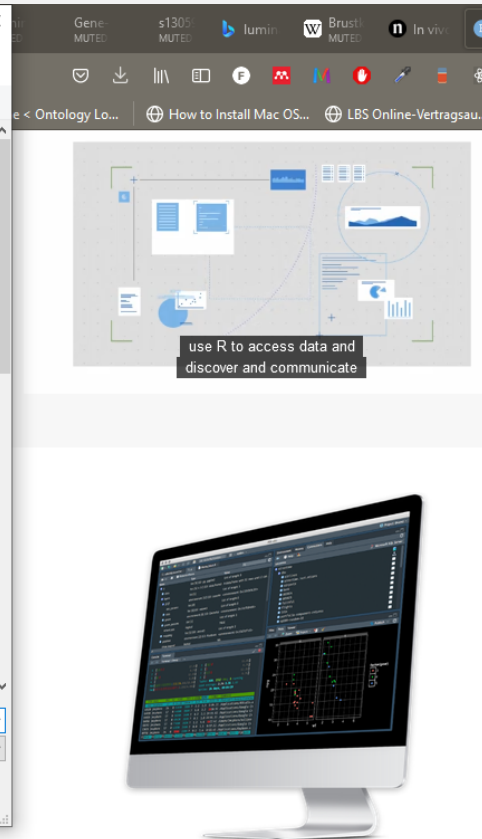
<https://www.rstudio.com/products/rstudio/download/#download>

DOWNLOAD RSTUDIO

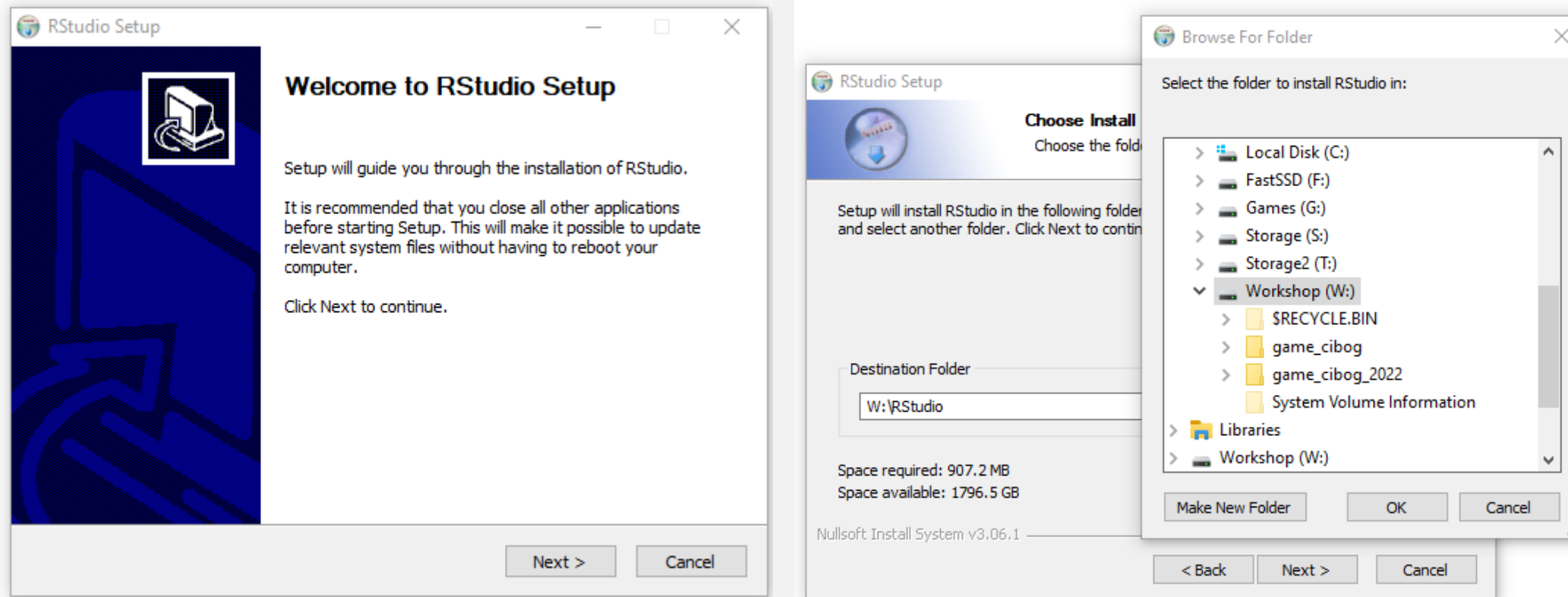


Requires Windows 10 (64-bit)

All Installers

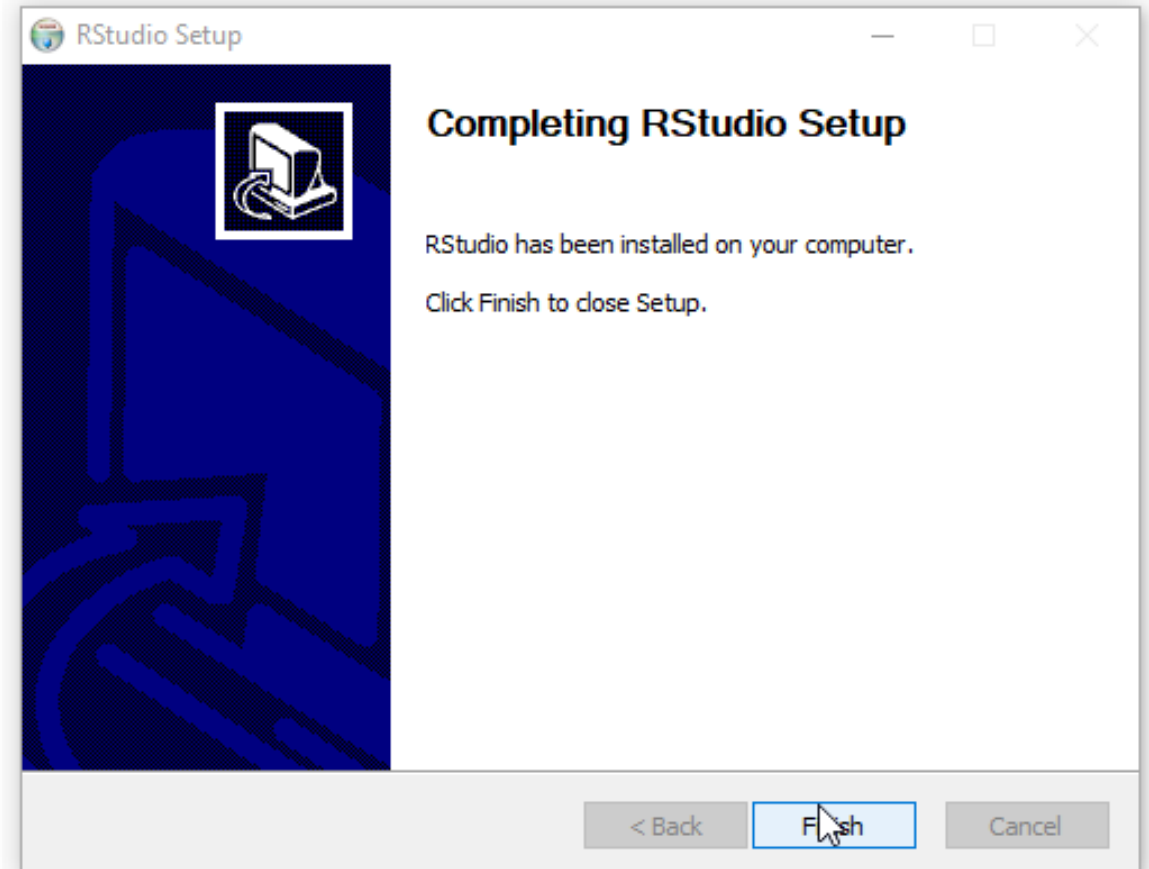
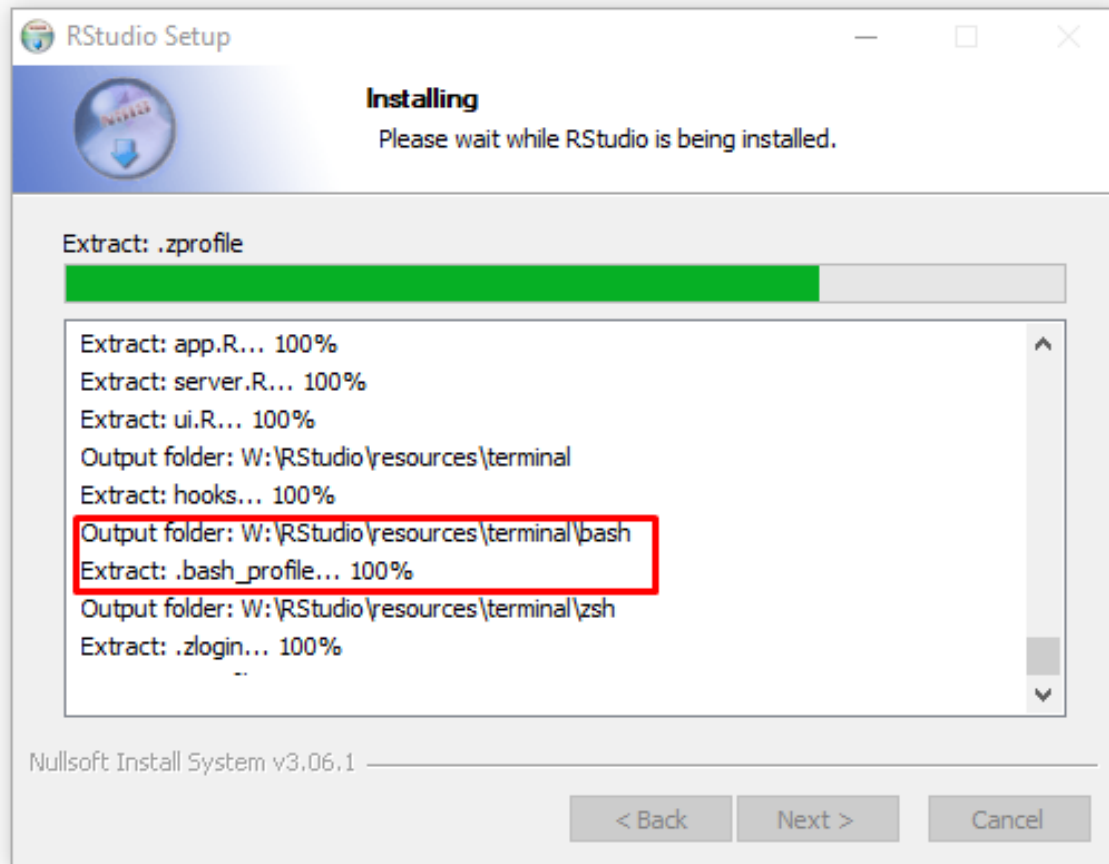


DOWNLOAD RSTUDIO

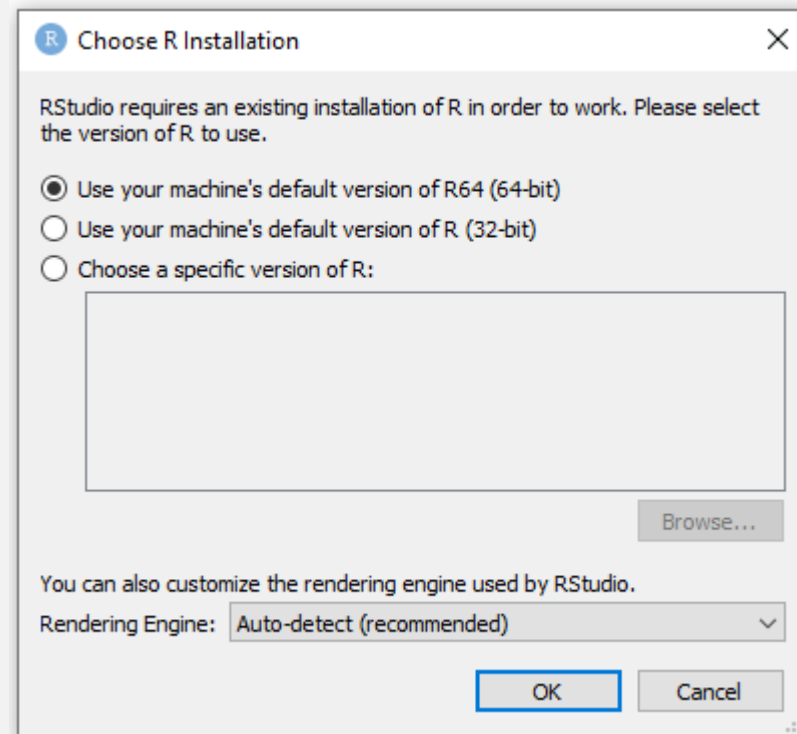
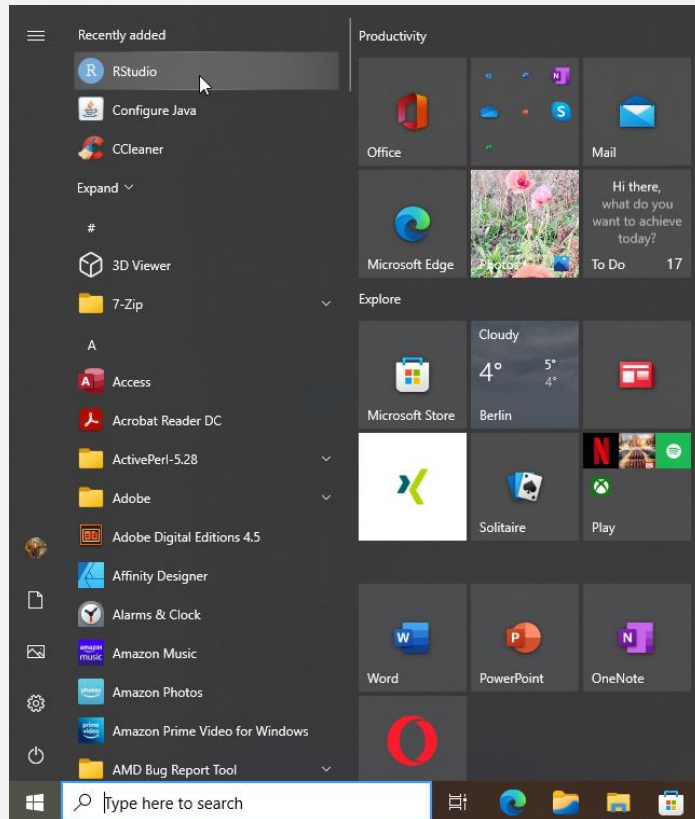


- Select a volume/harddisk/folder with enough space!

DOWNLOAD RSTUDIO

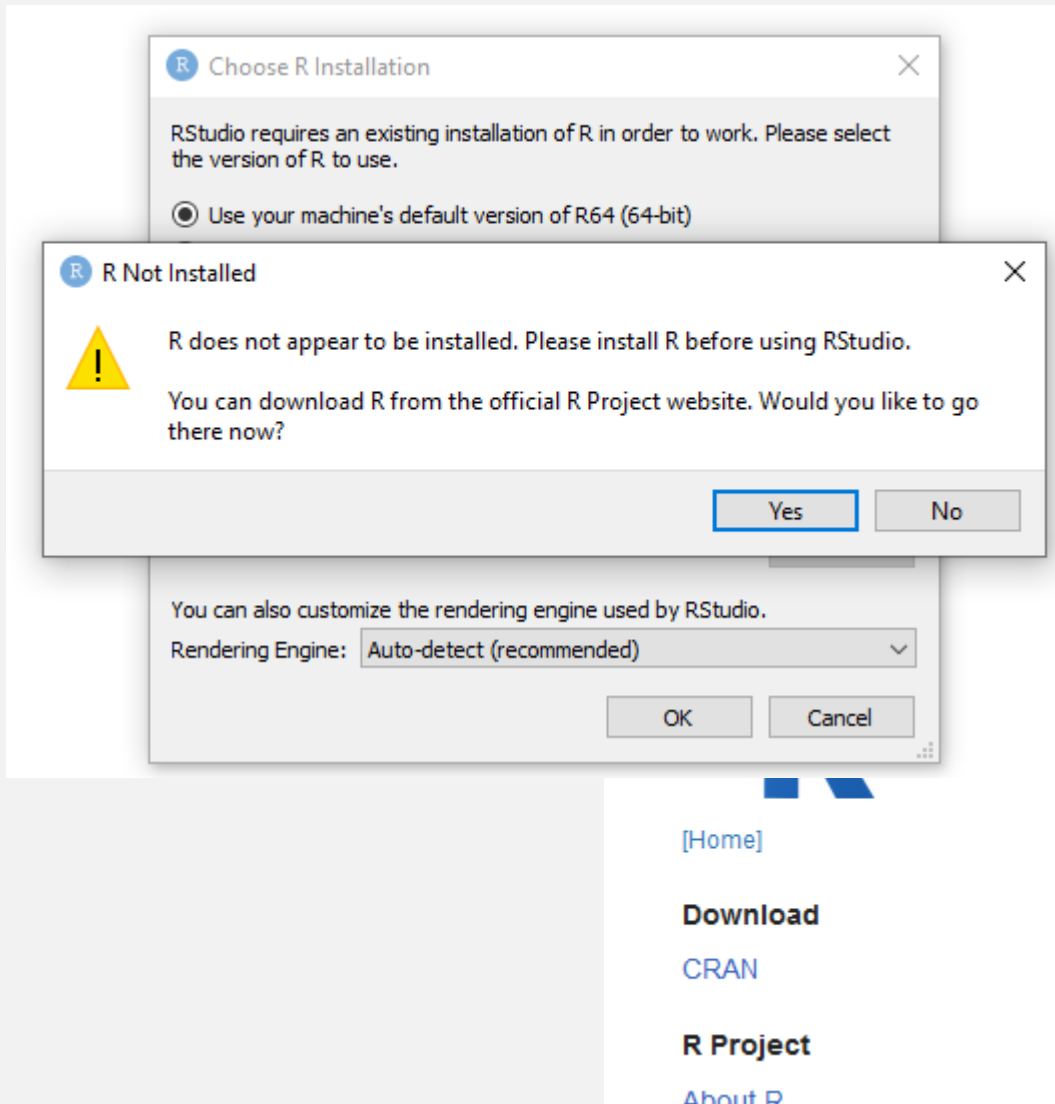


STARTING RSTUDIO



- When you start Rstudio the first time, select R version
 - If you have no R installed yet, select “Use your machine’s default version of R64”

INSTALLING R



The R Project for Statistical Computing

Getting Started

R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. To [download R](#), please choose your preferred [CRAN mirror](#).

If you have questions about R like how to download and install the software, or what the license terms are, please read our [answers to frequently asked questions](#) before you send an email.

DOWNLOADING R

CRAN Mirrors

The Comprehensive R Archive Network is available at the following URLs, please choose a location close to you. Some statistics on the status of the mirrors can be found here: [main page](#), [windows release](#), [windows old release](#).

If you want to host a new mirror at your institution, please have a look at the [CRAN Mirror HOWTO](#).

0-Cloud

<https://cloud.r-project.org/>

Automatic redirection to servers worldwide, currently sponsored by Rstudio

Algeria

<https://cran.usthb.dz/>

University of Science and Technology Houari Boumediene

Argentina

<http://mirror.fcaglp.unlp.edu.ar/CRAN/>

Universidad Nacional de La Plata

Australia

<https://cran.csiro.au/>

CSIRO

<https://mirror.aarnet.edu.au/pub/CRAN/>

AARNET



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[About R](#)

[R Homepage](#)

[The R Journal](#)

[Software](#)

[R Sources](#)

The Comprehensive R Archive Network

Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- [Download R for Linux](#) ([Debian](#), [Fedora/Redhat](#), [Ubuntu](#))
- [Download R for macOS](#)
- [Download R for Windows](#)

R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.

Source Code for all Platforms

Windows and Mac users most likely want to download the precompiled binaries listed in the upper box, not the source code. The sources have to be compiled before you can use them. If you do not know what this means, you probably do not want to do it!

- The latest release (2021-11-01, Bird Hippie) [R-4.1.2.tar.gz](#), read [what's new](#) in the latest version.

DOWNLOADING R



CRAN
[Mirrors](#)
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[Search](#)

About R
[R Homepage](#)
[The R Journal](#)

Software

R for Windows

Subdirectories:

[base](#)
[contrib](#)

[old contrib](#)
[Rtools](#)

Binaries for base distribution. This is what you want to [install R for the first time](#).

Binaries of contributed CRAN packages (for R \geq 2.13.x; managed by Uwe Ligges). There is also information on [third party software](#) available for CRAN Windows services and corresponding environment and make variables.

Binaries of contributed CRAN packages for outdated versions of R (for R $<$ 2.13.x; managed by Uwe Ligges).

Tools to build R and R packages. This is what you want to build your own packages on Windows, or to build R itself.

Please do not submit binaries to CRAN. Package developers might want to contact Uwe Ligges directly in case of questions / suggestions related to Windows binaries.

You may also want to read the [R FAQ](#) and [R for Windows FAQ](#).

Note: CRAN does some checks on these binaries for viruses, but cannot give guarantees. Use the normal precautions with downloaded executables.



CRAN
[Mirrors](#)
[What's new?](#)
[Task Views](#)
[Search](#)

About R
[R Homepage](#)
[The R Journal](#)

Software
[R Sources](#)

R-4.1.2 for Windows (32/64 bit)

[Download R 4.1.2 for Windows](#) (86 megabytes, 32/64 bit)
[Installation and other instructions](#)
[New features in this version](#)

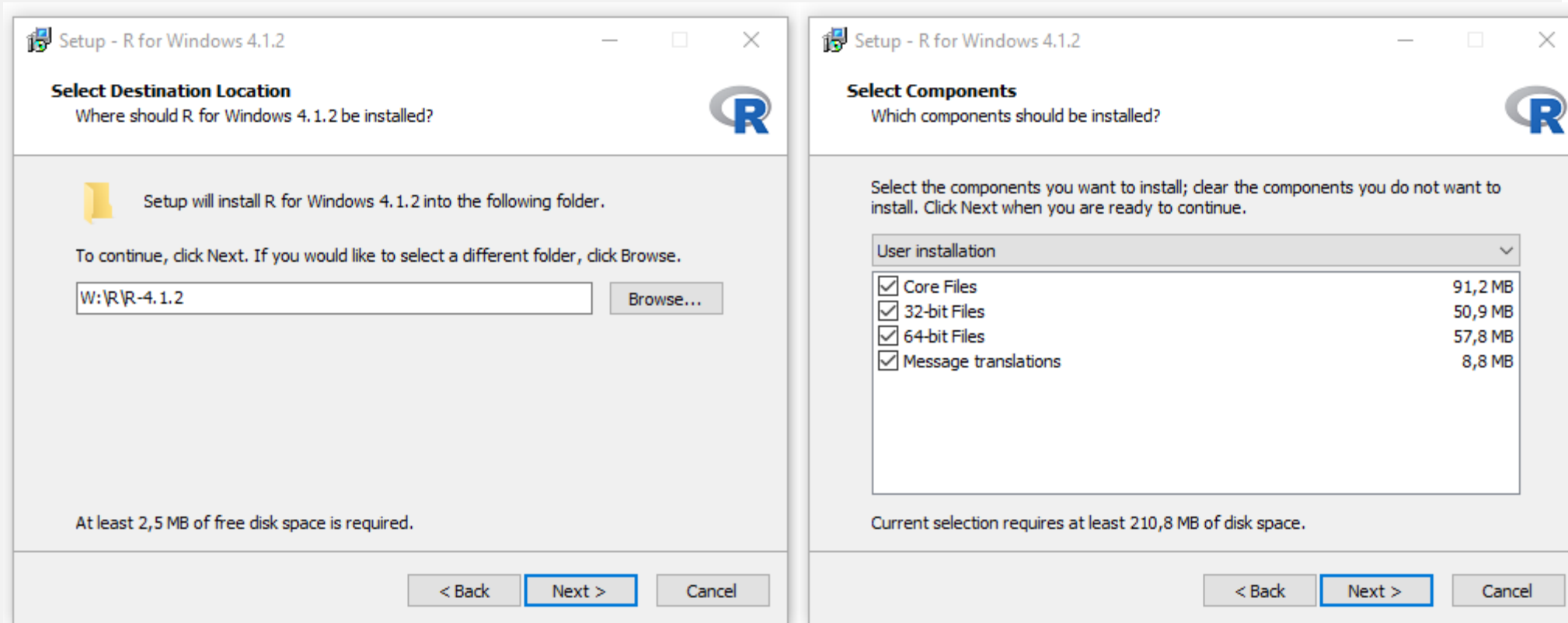
If you want to double-check that the package you have downloaded matches the package distributed by CRAN, you can compare the [md5sum](#) of the .exe to the [fingerprint](#) on the master server. You will need a version of md5sum for windows: both [graphical](#) and [command line versions](#) are available.

Frequently asked questions

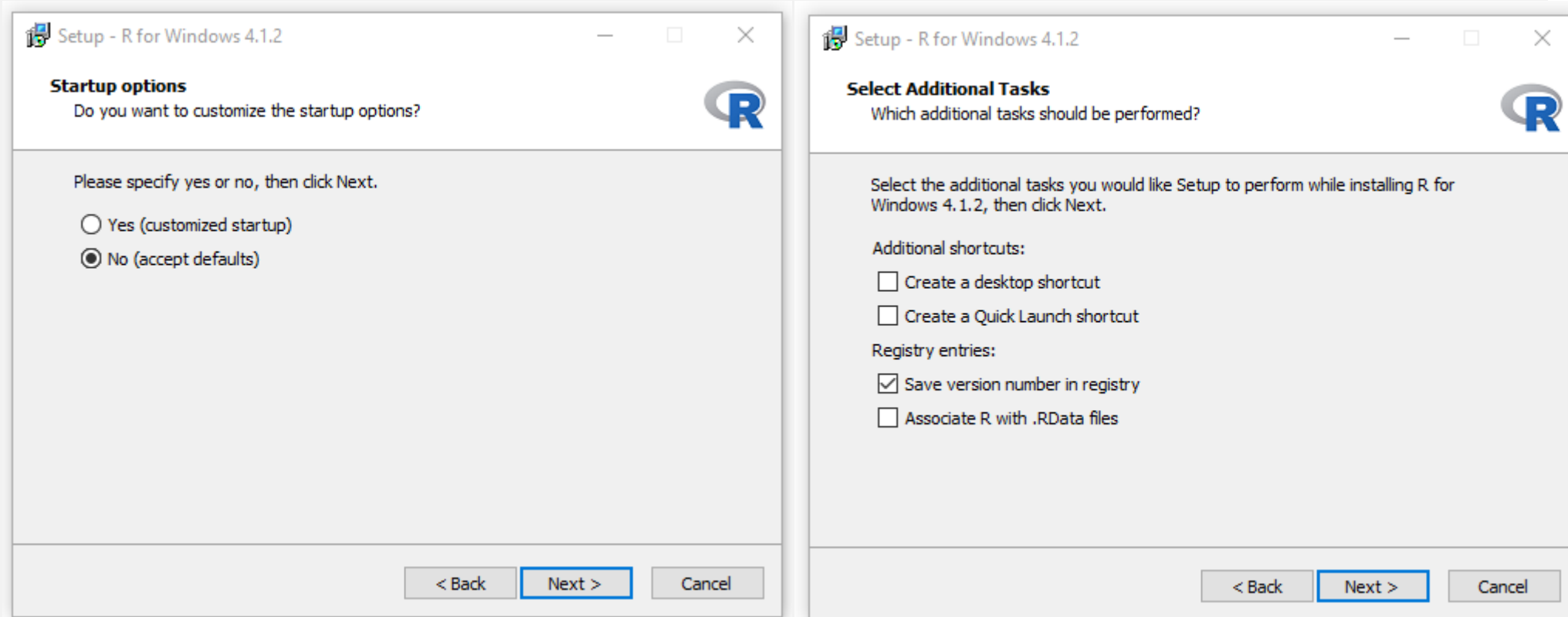
- [Does R run under my version of Windows?](#)
- [How do I update packages in my previous version of R?](#)
- [Should I run 32-bit or 64-bit R?](#)

Please see the [R FAQ](#) for general information about R and the [R Windows FAQ](#) for Windows-specific information.

INSTALLING R



INSTALLING R



The image displays two side-by-side screenshots of the 'Setup - R for Windows 4.1.2' installer windows. The left window is titled 'Startup options' and asks 'Do you want to customize the startup options?'. It contains two radio button options: 'Yes (customized startup)' and 'No (accept defaults)', with the latter being selected. The right window is titled 'Select Additional Tasks' and asks 'Which additional tasks should be performed?'. It contains two sections: 'Additional shortcuts' with two unchecked checkboxes for 'Create a desktop shortcut' and 'Create a Quick Launch shortcut', and 'Registry entries' with two checkboxes: 'Save version number in registry' (checked) and 'Associate R with .RData files' (unchecked). Both windows feature a blue R logo in the top right corner and navigation buttons ('< Back', 'Next >', and 'Cancel') at the bottom.

Startup options
Do you want to customize the startup options?

Please specify yes or no, then click Next.

☐ Yes (customized startup)
☒ No (accept defaults)

< Back Next > Cancel

Select Additional Tasks
Which additional tasks should be performed?

Select the additional tasks you would like Setup to perform while installing R for Windows 4.1.2, then click Next.

Additional shortcuts:

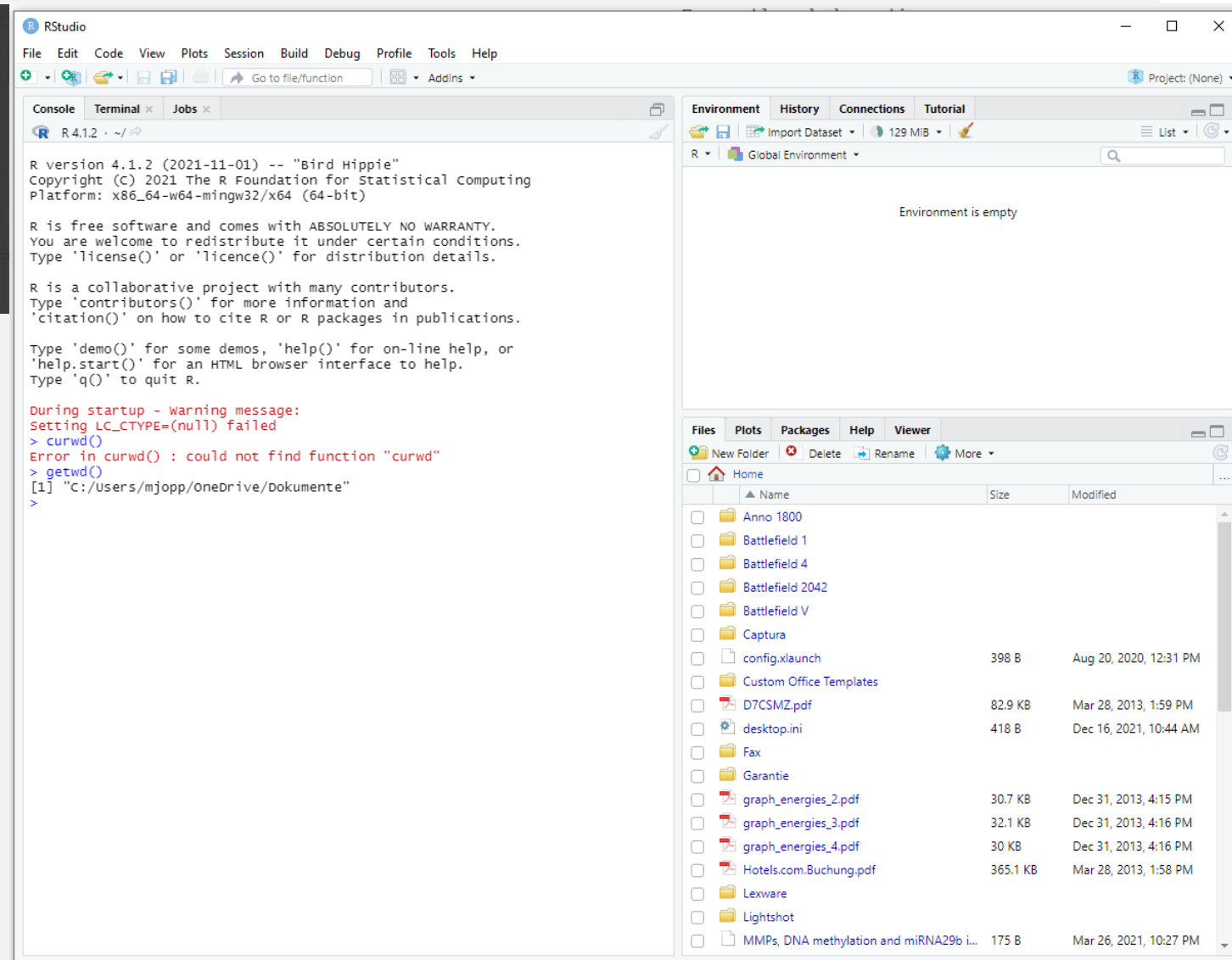
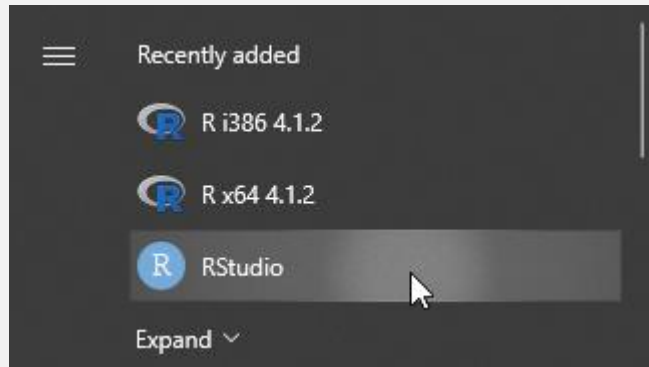
☐ Create a desktop shortcut
☐ Create a Quick Launch shortcut

Registry entries:

☒ Save version number in registry
☐ Associate R with .RData files

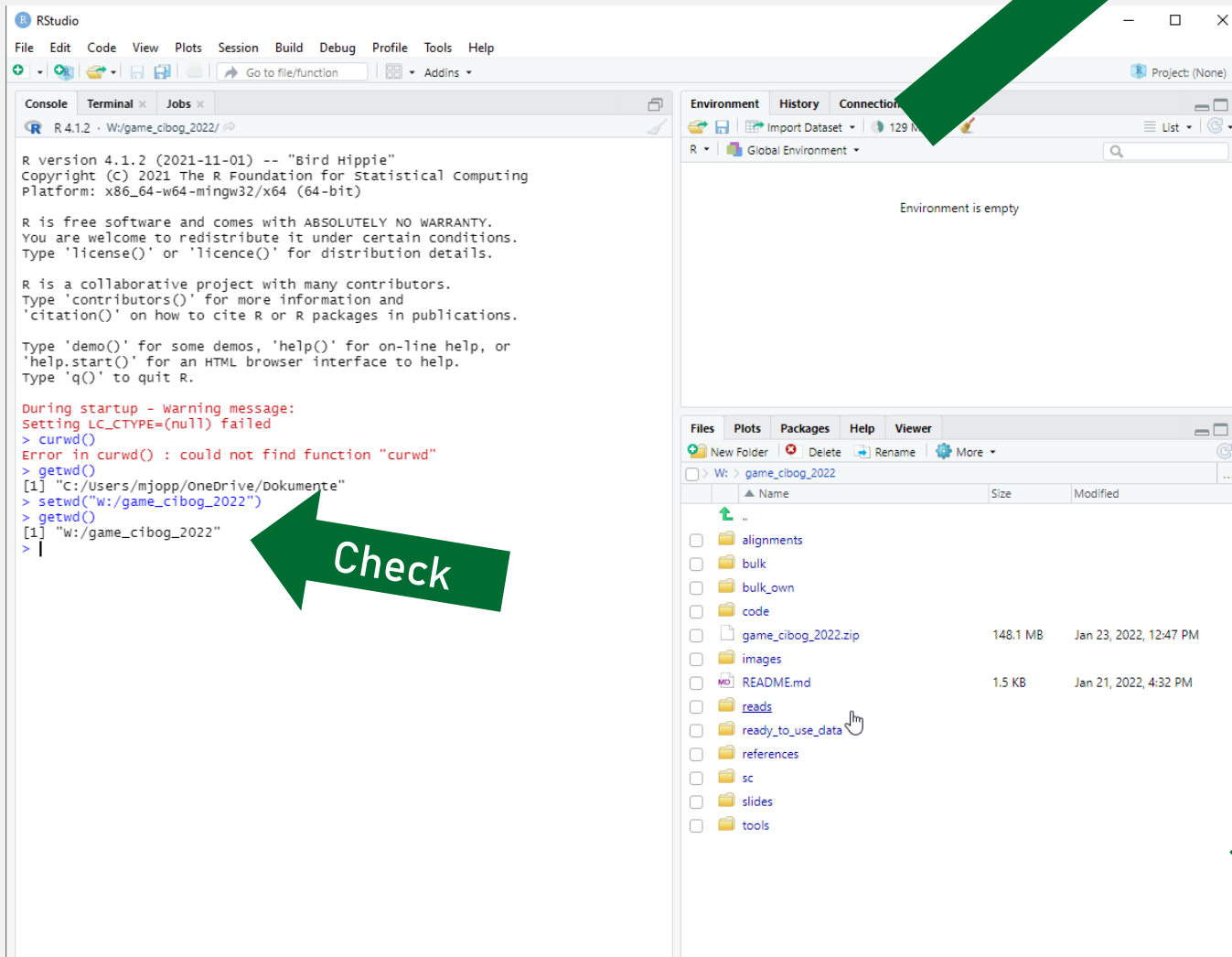
< Back Next > Cancel

STARTING RSTUDIO

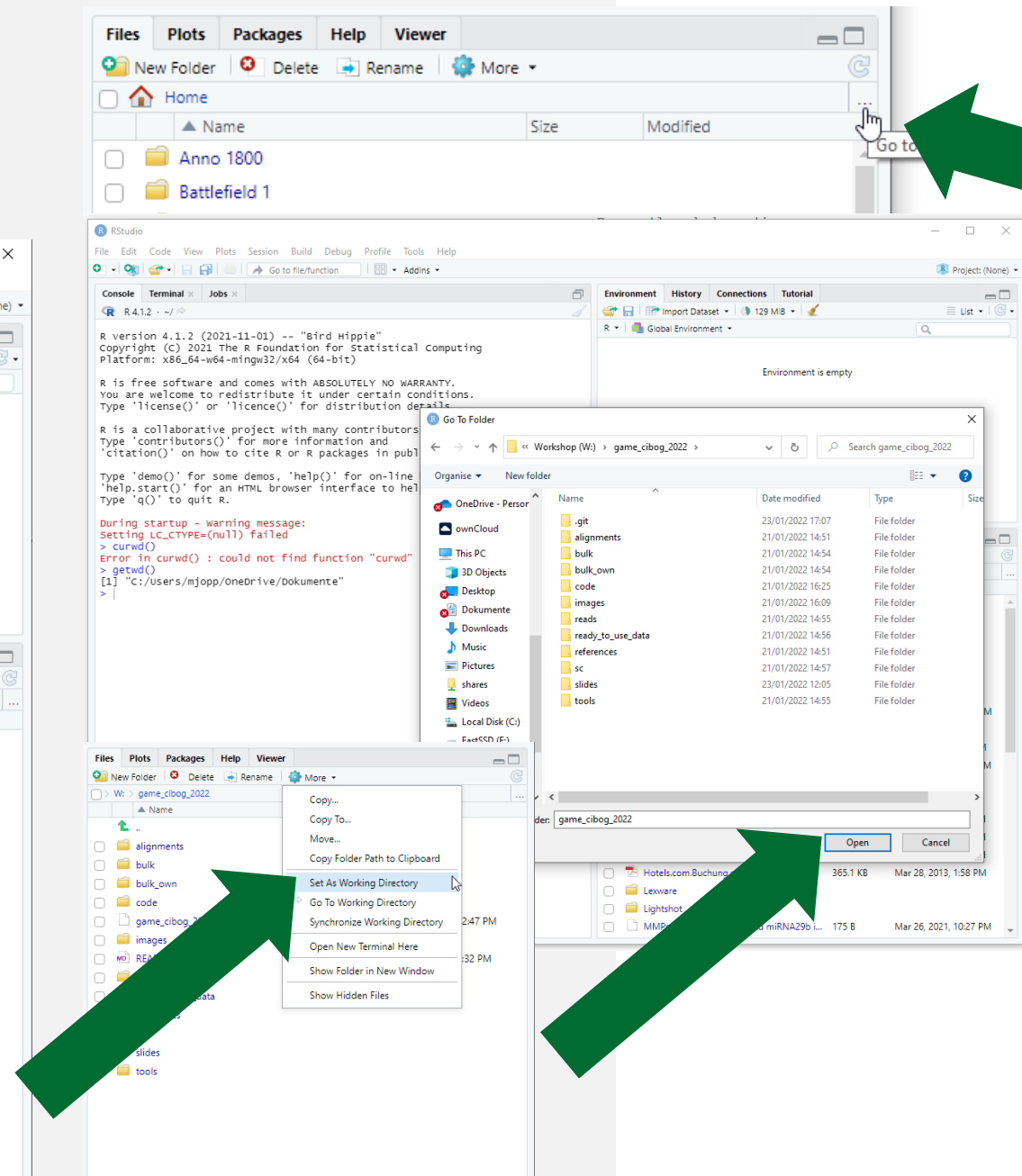


STARTING RSTUDIO

Changing the current working directory

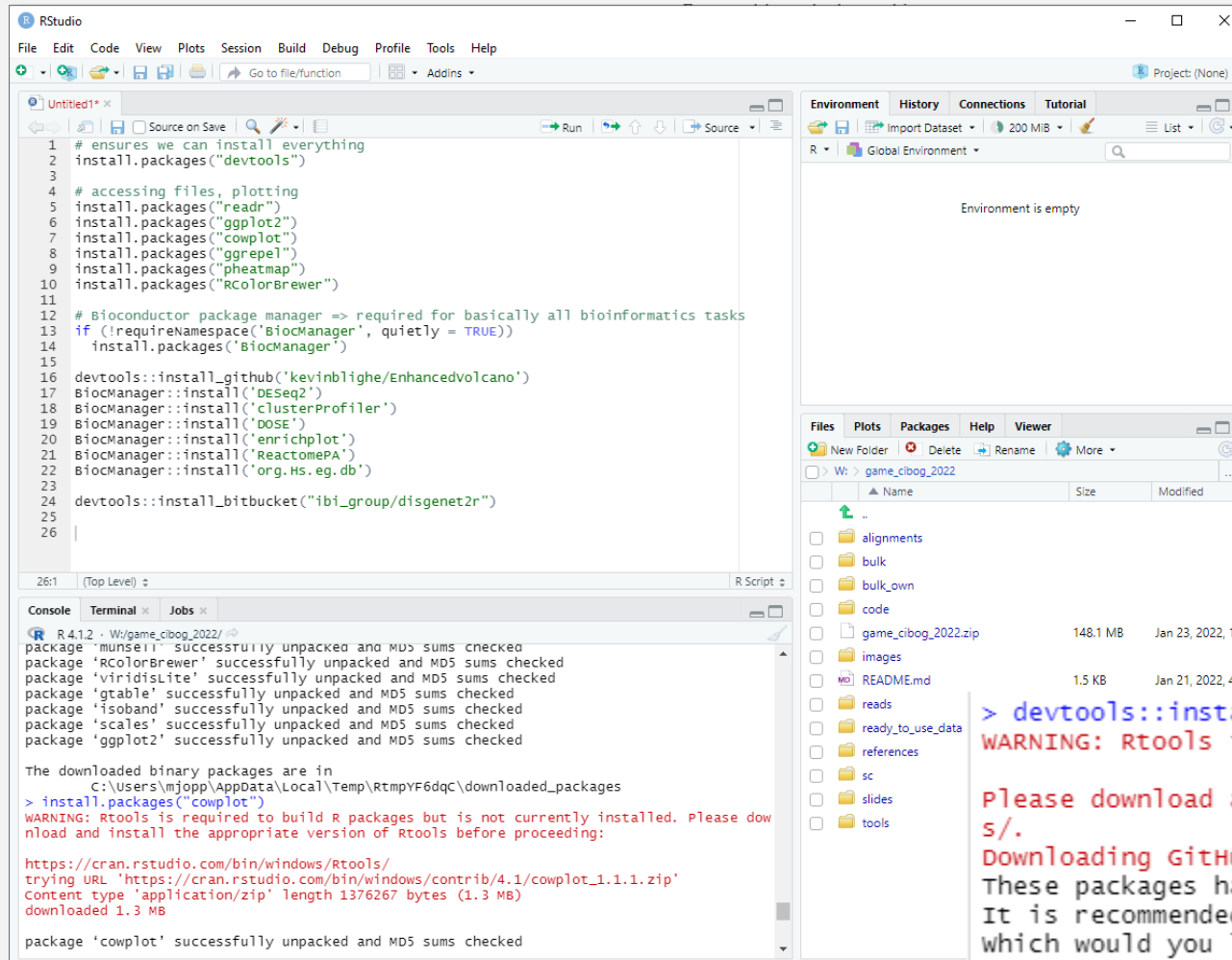


Check



RTOOLS REQUIRED

Some libraries require compilation from source. On Windows: Rtools required



The screenshot shows the RStudio interface. The script editor contains R code for installing various packages. The console shows the output of the script execution, including a warning about Rtools not being installed.

```
# ensures we can install everything
install.packages("devtools")

# accessing files, plotting
install.packages("readr")
install.packages("ggplot2")
install.packages("cowplot")
install.packages("ggrepel")
install.packages("pheatmap")
install.packages("RColorBrewer")

# Bioconductor package manager => required for basically all bioinformatics tasks
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")

devtools::install_github("kevinblighe/EnhancedVolcano")
BiocManager::install("DESeq2")
BiocManager::install("clusterProfiler")
BiocManager::install("DOSE")
BiocManager::install("enrichplot")
BiocManager::install("ReactomePA")
BiocManager::install("org.Hs.eg.db")

devtools::install_bitbucket("ibi_group/disgenet2r")
```

Console output:

```
R 4.1.2 - W:\game_cibog_2022\
package 'munseil' successfully unpacked and MD5 sums checked
package 'RColorBrewer' successfully unpacked and MD5 sums checked
package 'viridisLite' successfully unpacked and MD5 sums checked
package 'gttable' successfully unpacked and MD5 sums checked
package 'isoband' successfully unpacked and MD5 sums checked
package 'scales' successfully unpacked and MD5 sums checked
package 'ggplot2' successfully unpacked and MD5 sums checked

The downloaded binary packages are in
C:\Users\mjopp\AppData\Local\Temp\RtmpYF6dqC\downloaded_packages
> install.packages("cowplot")
WARNING: Rtools is required to build R packages but is not currently installed. Please download and install the appropriate version of Rtools before proceeding:
https://cran.rstudio.com/bin/windows/Rtools/
trying URL 'https://cran.rstudio.com/bin/windows/contrib/4.1/cowplot_1.1.1.zip'
Content type 'application/zip' length 1376267 bytes (1.3 MB)
downloaded 1.3 MB

package 'cowplot' successfully unpacked and MD5 sums checked
```

```
> devtools::install_github('kevinblighe/EnhancedVolcano')
WARNING: Rtools is required to build R packages, but is not currently installed.

Please download and install Rtools 4.0 from https://cran.r-project.org/bin/windows/Rtools/.
Downloading Github repo kevinblighe/EnhancedVolcano@HEAD
These packages have more recent versions available.
It is recommended to update all of them.
which would you like to update?
```

INSTALL RTOOLS

Using Rtools4 on Windows

Starting with R 4.0.0 (released April 2020), R for Windows uses a toolchain bundle called **rtools4**. This version of Rtools4 contains 3 toolchains:

- C:\rtools40\mingw32: the 32-bit gcc-8-3.0 toolchain for R 4.0 - 4.1
- C:\rtools40\mingw64: the 64-bit gcc-8-3.0 toolchain for R 4.0 - 4.1
- C:\rtools40\ucrt64: a 64-bit gcc-10.3.0 ucrt toolchain for R 4.2 ¹

The [msys2 documentation](#) gives an overview of the supported environments in msys2 and a comparison of dynamic linking, whereas upstream msys2 prefers dynamic linking. The references at the bottom of this document correspond to the current version of Rtools.

The current version of Rtools is maintained by Jeroen Ooms. [Older editions](#) were put together by Prof. Brian Ripley.

Installing Rtools

Note that Rtools is only needed to build R packages with C/C++/Fortran code from source. By default, R for Windows does not install Rtools.

To use rtools, download the installer from CRAN:

- On Windows 64-bit: [rtools40-x86_64.exe](#) (includes both i386 and x64 compilers). Permanent url: [rtools40-x86_64.exe](#)
- On Windows 32-bit: [rtools40-i386.exe](#) (i386 compilers only). Permanent url: [rtools40-i386.exe](#)

Note for RStudio users: you need at least RStudio version 1.2.5042 to work with rtools4.

Setup - Rtools version 4.0

Select Additional Tasks

Which additional tasks should be performed?

Select the additional tasks you would like Setup to perform while installing Rtools, then click Next.

- ☒ Save version information to registry
- ☒ Create start-menu icons to msys2 shells

Folder: : folder, click Browse.

< Back **Next >** Cancel **Next >** Cancel

Setup - Rtools version 4.0

Installing

Please wait while Setup installs Rtools on your computer.

Extracting files...

C:\rtools40\mingw64\x86_64-w64-mingw32\lib\brshx32.a

- <https://cran.rstudio.com/bin/windows/Rtools/rtools40.html>

MAKING RTOOLS USABLE

The image displays two RStudio windows side-by-side, illustrating the process of installing Rtools.

Left Window: The R script in the editor contains the following code:

```
1 # ensures we can install everything
2 install.packages("devtools")
3
4 # accessing files, plotting
5 install.packages("readr")
6 install.packages("ggplot2")
7 install.packages("cowplot")
8 install.packages("ggrepel")
9 install.packages("pheatmap")
10 install.packages("RcolorBrewer")
11
12 # Bioconductor package manager => required for basically all bioinformatics tasks
13 if (!requireNamespace("BiocManager", quietly = TRUE))
14   install.packages("BiocManager")
15
16 devtools::install_github("kevinblighe/EnhancedVolcano")
17 BiocManager::install("DESeq2")
18 BiocManager::install("clusterProfiler")
19 BiocManager::install("DOSE")
20 BiocManager::install("enrichplot")
21 BiocManager::install("ReactomePA")
22 BiocManager::install("org.Hs.eg.db")
23
24 devtools::install_bitbucket("ibi_group/disgenet2r")
25
26
```

The file explorer on the right shows the directory structure, with the 'tools' folder highlighted. The console shows the R script being executed.

Right Window: The R script is the same as the left window. The terminal output shows the installation process:

```
R 4.1.2 - ~/
Platform: x86_64-w64-mingw32/x64 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

During startup - warning message:
Setting LC_CTYPE=(null) failed
> Sys.which("make")
[1] "C:\\Program Files\\Microsoft Visual Studio\\2019\\BuildTools\\bin\\x64\\make.exe"
> |
```

A green arrow points to the terminal output with the word "Check".

R PACKAGES

```
# ensures we can install everything
```

```
library(devtools) # => install.packages("devtools")
```

```
# accessing files, plotting
```

```
library(readr) # => install.packages("readr")  
library(ggplot2) # => install.packages("ggplot2")  
library(cowplot) # => install.packages("cowplot")  
library(ggrepel) # => install.packages("ggrepel")  
library(pheatmap) # => install.packages("pheatmap")  
library(RColorBrewer) # => install.packages(" RColorBrewer ")
```

```
# Bioconductor package manager => required for basically all bioinformatics tasks
```

```
if (!requireNamespace('BiocManager', quietly = TRUE))  
  install.packages('BiocManager')
```

```
library(EnhancedVolcano) # => BiocManager::install('EnhancedVolcano') or devtools::install_github('kevinblighe/EnhancedVolcano')  
library(DESeq2) # => BiocManager::install('DESeq2')  
library(clusterProfiler) # => BiocManager::install('clusterProfiler')  
library(DOSE) # => BiocManager::install('DOSE')  
library(enrichplot) # => BiocManager::install('enrichplot')  
library(ReactomePA) # => BiocManager::install('ReactomePA')  
library(org.Hs.eg.db) # => BiocManager::install('org.Hs.eg.db')
```

```
library(disgenet2r) # => devtools::install_bitbucket("ibi_group/disgenet2r")
```

INSTALL ALL THE LIBRARIES!

The image displays two side-by-side screenshots of the RStudio interface, illustrating the process of installing the 'pillar' package and its dependencies.

Left Screenshot: The R console shows the R version (4.1.2) and the installation of the 'pillar' package. The output indicates that the package is being installed from source, and the dependencies are being installed. The console output includes:

```
R version 4.1.2 (2021-11-01) -- "Bird Hippie"
Copyright (C) 2021 The R Foundation for Statistical Computing
Platform: x86_64-w64-mingw32/x64 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

During startup - warning message:
Setting LC_CTYPE=(null) failed
> curwd()
Error in curwd() : could not find function "curwd"
> getwd()
[1] "C:/Users/mjopp/OneDrive/Dokumente"
> setwd("w:/game_cibog_2022")
> getwd()
[1] "w:/game_cibog_2022"
> install.packages("devtools")
WARNING: Rtools is required to build R packages but is not currently install
ed. Please download and install the appropriate version of Rtools before pro
ceeding:

https://cran.rstudio.com/bin/windows/Rtools/
also installing the dependencies 'utf8', 'pillar', 'pkgconfig', 'vctrs', 'as
kpass', 'credentials', 'sys', 'zip', 'gitcreds', 'ini', 'fastmap', 'highr',
'xfun', 'diffobj', 'fansl', 'rematch2', 'tibble', 'clipr', 'crayon', 'cur
1', 'gert', 'gh', 'glue', 'jsonlite', 'purrr', 'rappdirs', 'rprojroot', 'whi
sker', 'yaml', 'processx', 'R6', 'mime', 'openssl', 'cachem', 'prettyunits',
'digest', 'xopen', 'brew', 'commonmark', 'knitr', 'stringi', 'stringr', 'xm
l2', 'cpp11', 'brio', 'evaluate', 'magrittr', 'praise', 'ps', 'waldo', 'uset
his', 'callr', 'cli', 'desc', 'ellipsi', 'fs', 'http', 'lifecycle', 'memois
e', 'pkgbuild', 'pkgload', 'rcmdcheck', 'remotes', 'rlang', 'roxygen2', 'rst
udioapi', 'rversions', 'sessioninfo', 'testthat', 'withr'

There are binary versions available but the source versions
are later:
  binary source needs_compilation
pillar 1.6.4 1.6.5 FALSE
glue 1.6.0 1.6.1 TRUE
yaml 2.2.1 2.2.2 TRUE

Binaries will be installed
trying URL 'https://cran.rstudio.com/bin/windows/contrib/4.1/utf8_1.2.2.zip'
```

Right Screenshot: The R console shows the output of the 'pillar' package installation. The output indicates that the package is being installed from source, and the dependencies are being installed. The console output includes:

```
finding HTML links ... done
align      html
char       html
colonnade  html
ctl_new_pillar html
deprecated html
dim_desc   html
expect_known_display html
extra_cols html
format_glimpse html
format_tbl html
format_type_sum html
get_extent html
glimpse     html
new_ornament html
new_pillar  html
new_pillar_component html
new_pillar_shaft html
new_pillar_title html
new_pillar_type html
new_tbl_format_setup html
num         html
pillar-package html
pillar      html
pillar_options html
pillar_shaft html
scale_x_num html
squeeze     html
style_subtle html
tbl_format_body html
tbl_format_footer html
tbl_format_header html
tbl_format_setup html
tbl_sum     html
type_sum    html
*** copying figures
** building package indices
** installing vignettes
** testing if installed package can be loaded from temporary location
*** arch - i386
*** arch - x64
** testing if installed package can be loaded from final location
*** arch - i386
*** arch - x64
** testing if installed package keeps a record of temporary installation pat
h
* DONE (pillar)
Making 'packages.html' ... done

The downloaded source packages are in
'C:/Users/mjopp/AppData/Local/Temp/RtmpYF6dqc/downloaded_packages'
```

The right screenshot also shows the Environment pane, which is empty, and the Files pane, which displays the contents of the 'game_cibog_2022' directory, including files like 'alignments', 'bulk', 'bulk_own', 'code', 'game_cibog_2022.zip', 'images', 'README.md', 'reads', 'ready_to_use_data', 'references', 'sc', 'slides', and 'tools'.

WHEN SOMETHING GOES WRONG

```
* installing *source* package 'glue' ...
** package 'glue' successfully unpacked and MD5 sums checked
ERROR: cannot remove earlier installation, is it in use?
* removing 'w:/R/R-4.1.2/library/glue'
* restoring previous 'w:/R/R-4.1.2/library/glue'
Warning in file.copy(lp, dirname(pkgdir), recursive = TRUE, copy.date = TRUE) :
  problem copying w:\R\R-4.1.2\library\00LOCK-glue\glue\libs\x64\glue.dll to w:\R\R-4.1.2
\library\glue\libs\x64\glue.dll: Permission denied
```

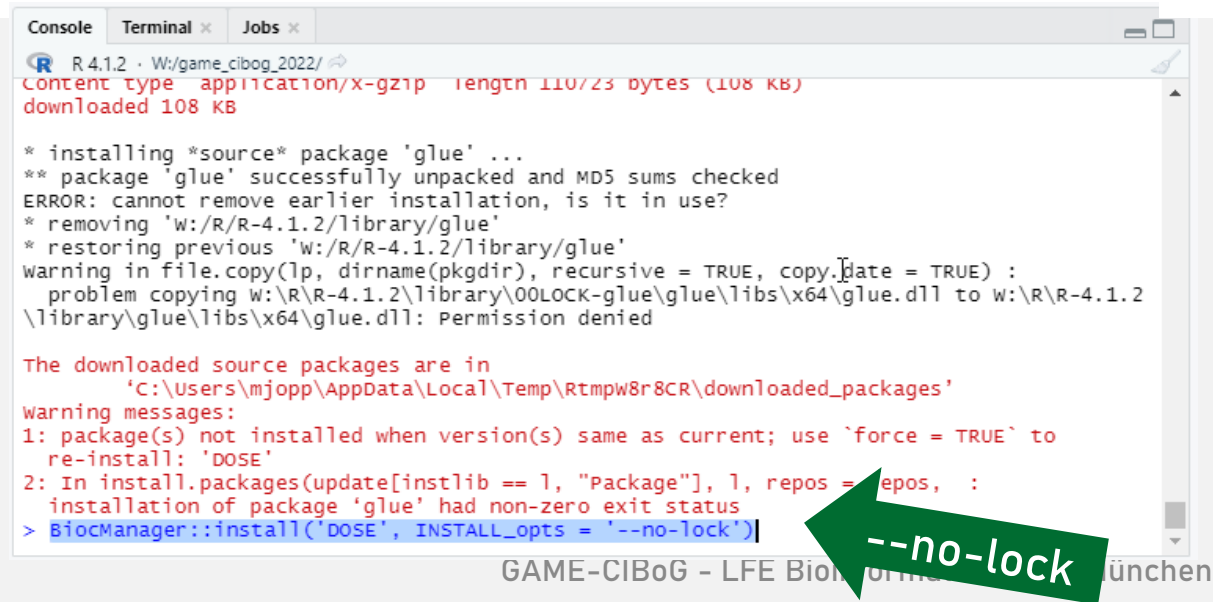
The downloaded source packages are in
'C:\Users\mjopp\AppData\Local\Temp\Rtmpw8r8CR\downloaded_packages'

Warning messages:

- 1: package(s) not installed when version(s) same as current; use `force = TRUE` to re-install: 'DOSE'
- 2: In install.packages(update[instlib == 1, "Package"], 1, repos = repos, : installation of package 'glue' had non-zero exit status

> |

```
* installing *source* package 'glue' ...
** package 'glue' successfully unpacked and MD5 sums checked
ERROR: cannot remove earlier installation, is it in use?
* removing 'w:/R/R-4.1.2/library/glue'
```



```
R 4.1.2 · W:/game_cibog_2022/
content type application/x-gzip length 110723 bytes (108 KB)
downloaded 108 KB

* installing *source* package 'glue' ...
** package 'glue' successfully unpacked and MD5 sums checked
ERROR: cannot remove earlier installation, is it in use?
* removing 'w:/R/R-4.1.2/library/glue'
* restoring previous 'w:/R/R-4.1.2/library/glue'
warning in file.copy(lp, dirname(pkgdir), recursive = TRUE, copy.date = TRUE) :
  problem copying w:\R\R-4.1.2\library\00LOCK-glue\glue\libs\x64\glue.dll to w:\R\R-4.1.2
\library\glue\libs\x64\glue.dll: Permission denied

The downloaded source packages are in
  'C:\Users\mjopp\AppData\Local\Temp\Rtmpw8r8CR\downloaded_packages'
warning messages:
1: package(s) not installed when version(s) same as current; use `force = TRUE` to
  re-install: 'DOSE'
2: In install.packages(update[instlib == 1, "Package"], 1, repos = repos, :
  installation of package 'glue' had non-zero exit status
> BiocManager::install('DOSE', INSTALL_opts = '--no-lock')
```

CONVERT IPYNB TO RMD

All notebooks expect working directory to be the 'code' directory!

The screenshot displays the RStudio interface with three main panes illustrating the conversion process from an IPYNB notebook to an RMD file.

Left Pane (Original IPYNB Notebook): Shows the R script code for converting an IPYNB notebook to an RMD file. The code includes loading the 'rmarkdown' package, setting the input notebook path, and using the `convert_ipynb` function.

```
1 library("rmarkdown")
2
3 input_notebook = "code/00_get_data.ipynb"
4
5 convert_ipynb(input=input_notebook, output = xfun::with_ext(input_notebook, "Rmd"))
```

Middle Pane (Converted RMD File): Shows the converted RMD file content, which includes a title, output format, options, and R code for reading data files.

```
1 ---
2 title: An R Markdown document converted from "code/02_bulk_preprocessed.ipynb"
3 output: html_document
4 ---
5
6 {r}
7 options(jupyter.plot_mimetypes = 'image/png')
8 options(repr.plot.width = 1, repr.plot.height = 0.75, repr.plot.res = 100)
9
10 {r}
11
12 library(readr)
13 library(DESeq2)
14 library(ggplot2)
15 library(cowplot)
16
17
18 Let's start with reading in the 24h samples
19
20 {r}
21 SUM159_24h_r1 = read_tsv("../bulk/GSM3763467_SUM159_DMSO_24h.R1.counts.txt.gz",
22 col_names=F)
23 SUM159_24h_r2 = read_tsv("../bulk/GSM3763468_SUM159_DMSO_24h.R2.counts.txt.gz",
24 col_names=F)
25 SUM159jq1r_24h_r1 = read_tsv("../bulk/GSM3763477_SUM159_JQ1R_DMSO_24h.R1.counts.txt
26 .gz", col_names=F)
```

Right Pane (File Explorer): Shows the file explorer with the 'code' directory highlighted. A context menu is open, showing options like 'Set As Working Directory' and 'Go To Working Directory'.

Console: Shows the R console output, indicating errors related to the 'input' and 'input_notebook' objects not being found. The errors are:

```
Error in xfun::with_ext(input, "Rmd") : object 'input' not found
Error in xfun::with_ext(input_notebook, "Rmd") : object 'input_notebook' not found
Error in xfun::with_ext(input, "Rmd") : object 'input' not found
Error in xfun::with_ext(input_notebook, "Rmd") : object 'input_notebook' not found
```

RUNNING THE RMD

- Execute cells
- Check output
 - It will tell you if something did not work!

The screenshot displays the RStudio interface with the following components:

- Source Editor:** Contains R code for loading libraries and reading data files. The code includes comments and uses `read_tsv()` to load data from gzipped files.
- Environment:** Shows the current environment with variables like `SUM159_24h...` and `SUM159jq1r...`.
- Files:** A file explorer showing the directory structure, including files like `GSM3763477_SUM159_JQ1R_DMSO...`.
- Console:** Displays the output of the R code, including column specifications and data loading progress.

```
8 options(repr.plot.width = 1, repr.plot.height = 0.75, repr.plot.res = 100)
9
10
11 ```{r}
12 library(readr)
13 library(DESeq2)
14 library(ggplot2)
15 library(cowplot)
16
17
18 Let's start with reading in the 24h samples
19
20 ```{r}
21 SUM159_24h_r1 = read_tsv("../bulk/GSM3763467_SUM159_DMSO_24h_R1.counts.txt.gz",
22 col_names=F)
23 SUM159_24h_r2 = read_tsv("../bulk/GSM3763468_SUM159_DMSO_24h_R2.counts.txt.gz",
24 col_names=F)
25 SUM159jq1r_24h_r1 = read_tsv("../bulk/GSM3763477_SUM159_JQ1R_DMSO_24h_R1.counts.txt
26 .gz", col_names=F)
27 SUM159jq1r_24h_r2 = read_tsv("../bulk/GSM3763478_SUM159_JQ1R_DMSO_24h_R2.counts.txt
28 .gz", col_names=F)
```

Output from the console:

```
Rows: 23841 Columns: 2
-- Column specification -----
Delimiter: "\t"
chr (1): X1
dbl (1): X2

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
Rows: 23841 Columns: 2
-- Column specification -----
Delimiter: "\t"
chr (1): X1
dbl (1): X2

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```