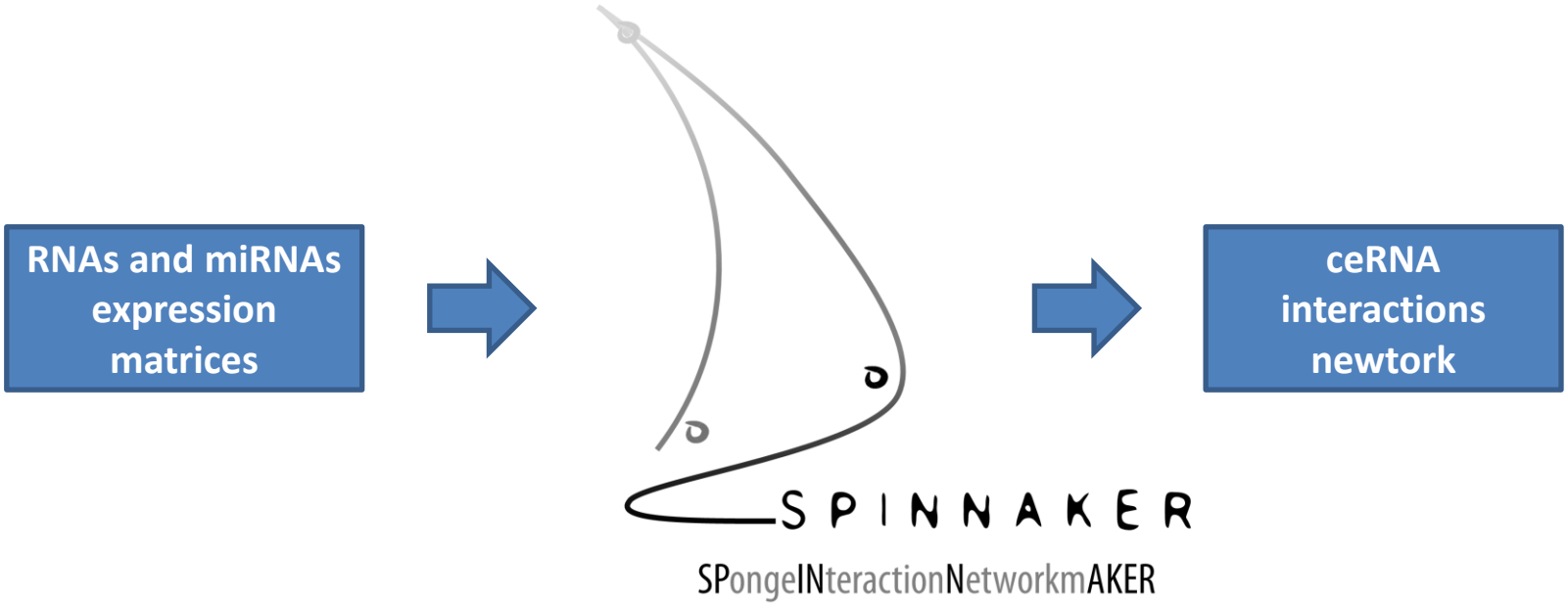
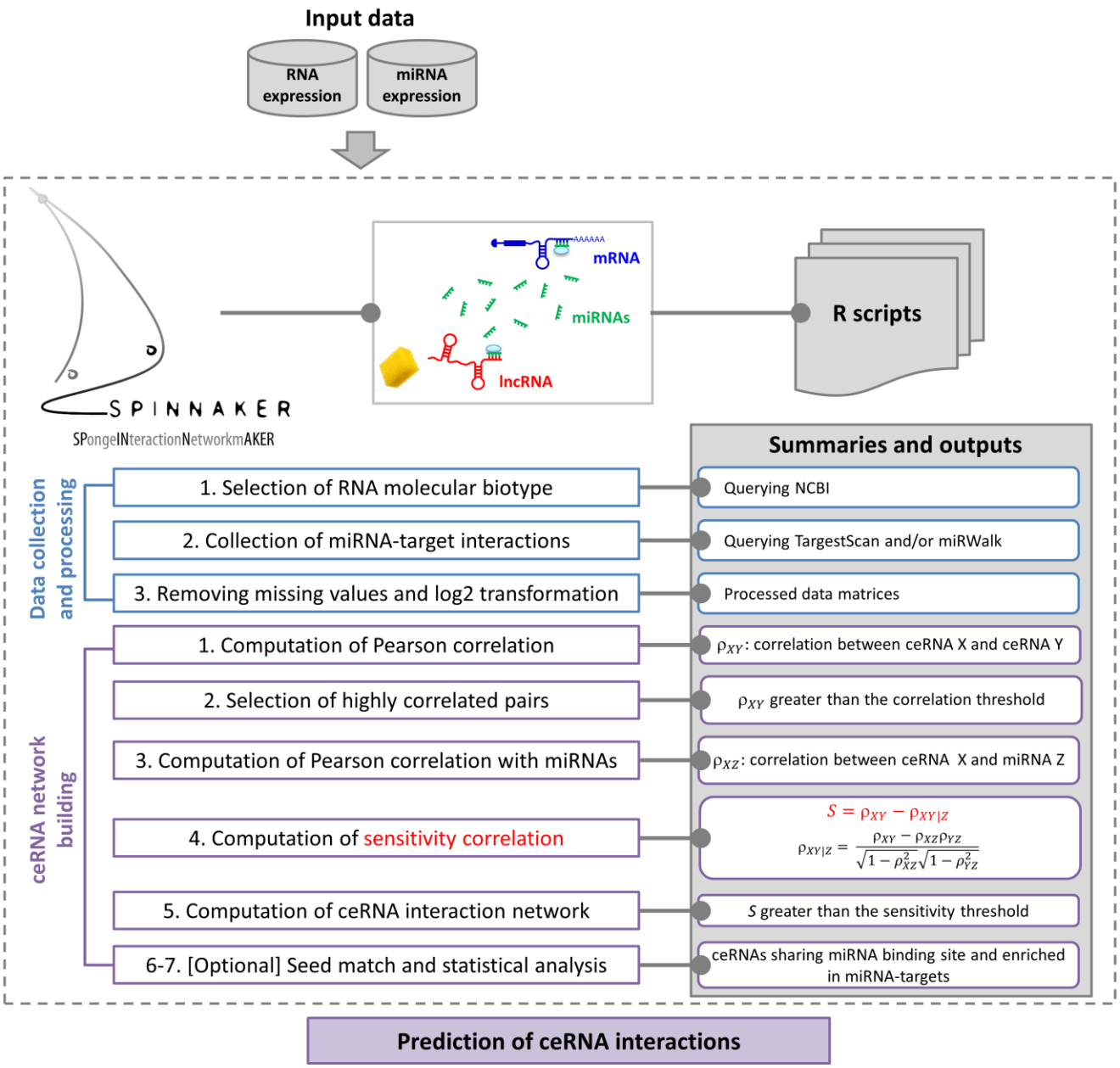
SPINNAKER (SPongeINteractionNetworkmAKER) is the R-based implementation of a widely established mathematical model that we recently published for identifying putative competing endogenous RNA (ceRNA)*. Taking as input the expression levels of RNAs and miRNAs, SPINNAKER predicts the ceRNA interactions network by implementing two modules: 1) data collection and processing, 2) ceRNA network building.



*BMC Syst Biol 2014 Jul 17;8:83. doi: 10.1186/1752-0509-8-83


Input data
RNA expression
miRNA expression
SPINNAKER
SPongeINteractionNetworkmAKER
mRNA
miRNAs
lncRNA
R scripts
Summaries and outputs
Data collection and processing
1. Selection of RNA molecular biotype
Querying NCBI
2. Collection of miRNA-target interactions
Querying TargestScan and/or miRWalk
3. Removing missing values and log2 transformation
Processed data matrices
ceRNA network building
1. Computation of Pearson correlation
ρ_{XY}: correlation between ceRNA X and ceRNA Y
2. Selection of highly correlated pairs
ρ_{XY} greater than the correlation threshold
3. Computation of Pearson correlation with miRNAs
ρ_{XZ}: correlation between ceRNA X and miRNA Z
4. Computation of sensitivity correlation
$S = \rho_{XY} - \rho_{XY|Z}$
$\rho_{XY|Z} = \frac{\rho_{XY} - \rho_{XZ}\rho_{YZ}}{\sqrt{1-\rho_{XZ}^2}\sqrt{1-\rho_{YZ}^2}}$
5. Computation of ceRNA interaction network
S greater than the sensitivity threshold
6-7. [Optional] Seed match and statistical analysis
ceRNAs sharing miRNA binding site and enriched in miRNA-targets
Prediction of ceRNA interactions

Module 0: Architecture

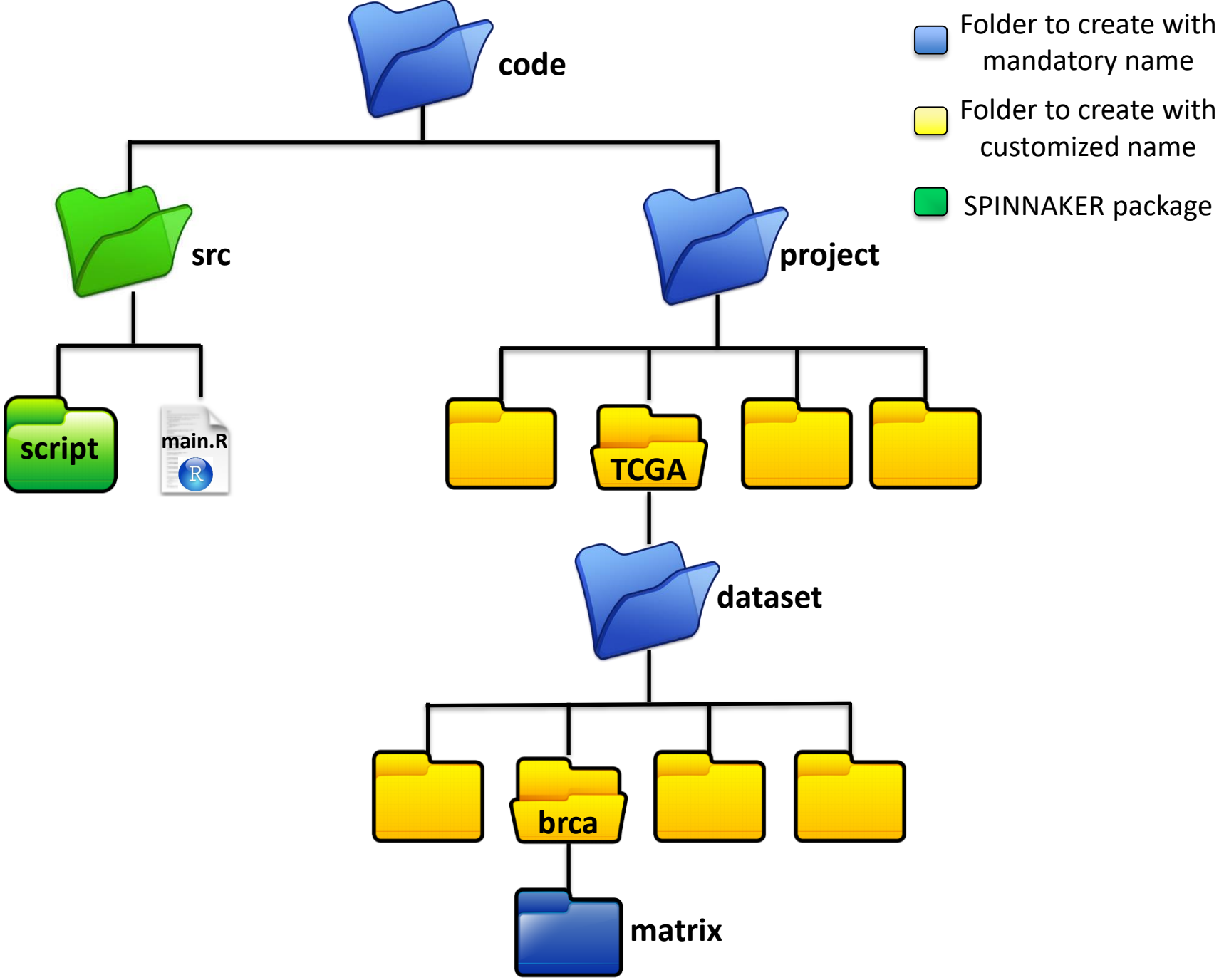
Software requirement

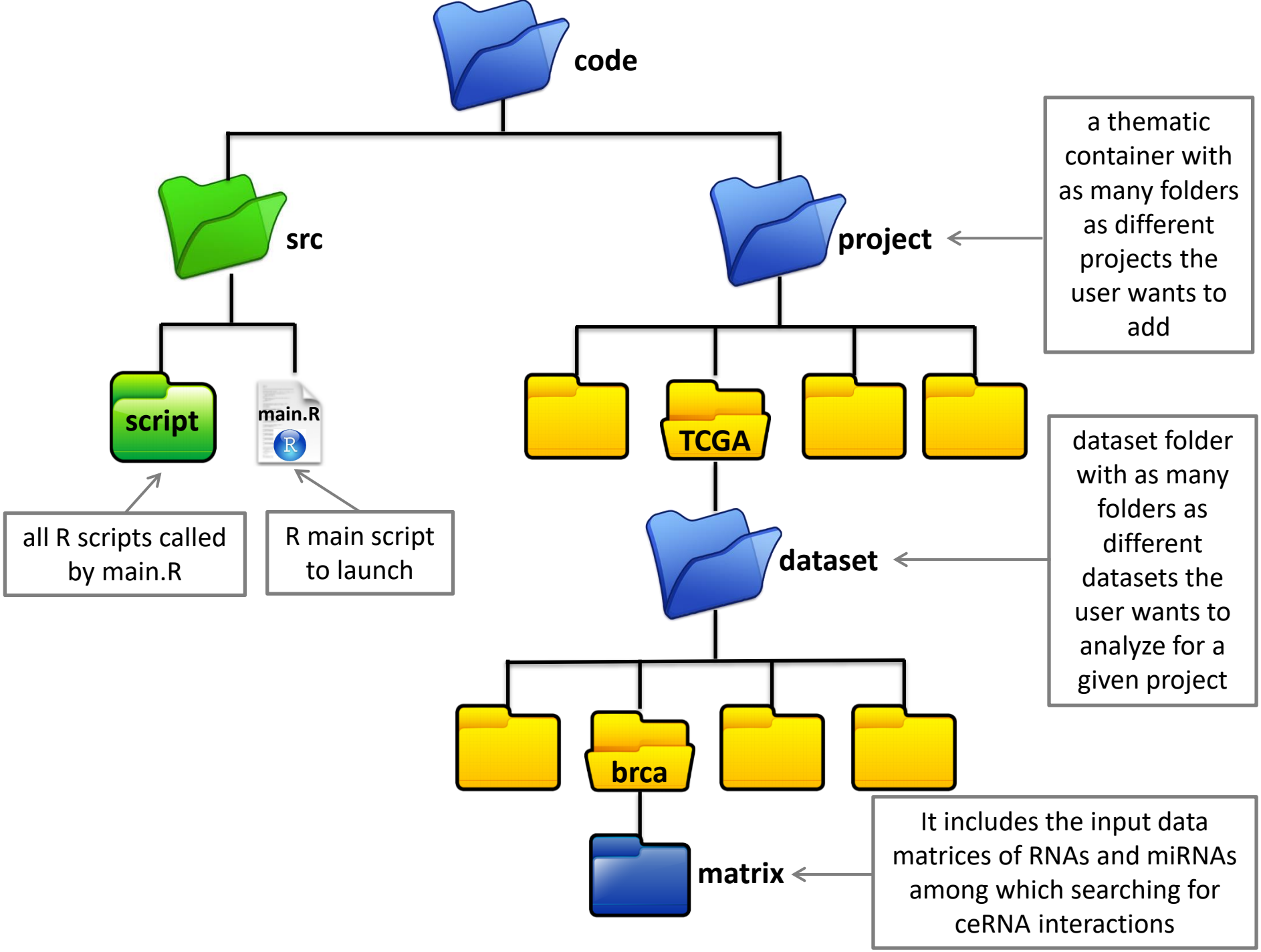
- SPINNAKER has been developed in R and tested on the following operative systems:
  - macOS High Sierra 10.13.6
  - Windows 10 Pro

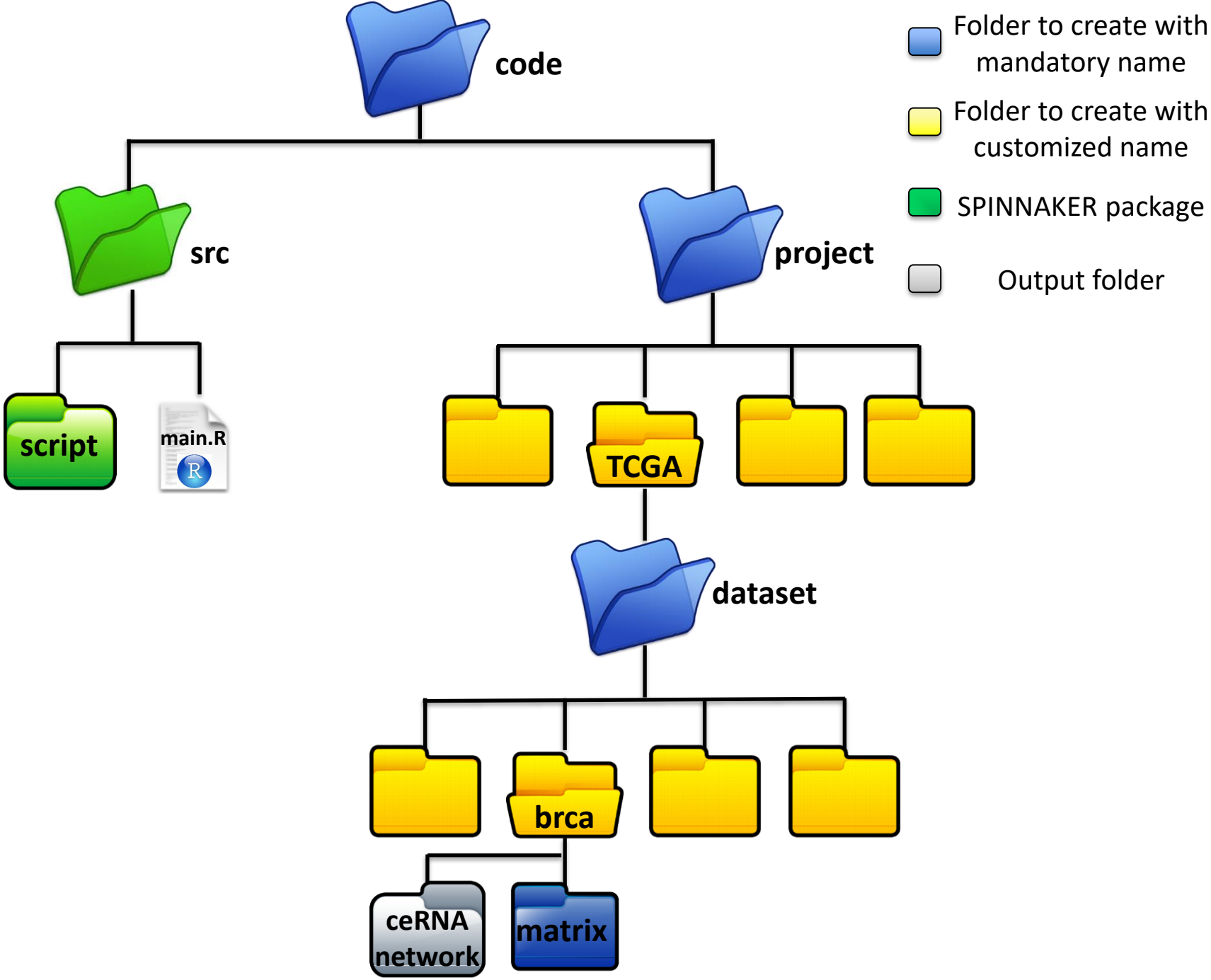
Setting up

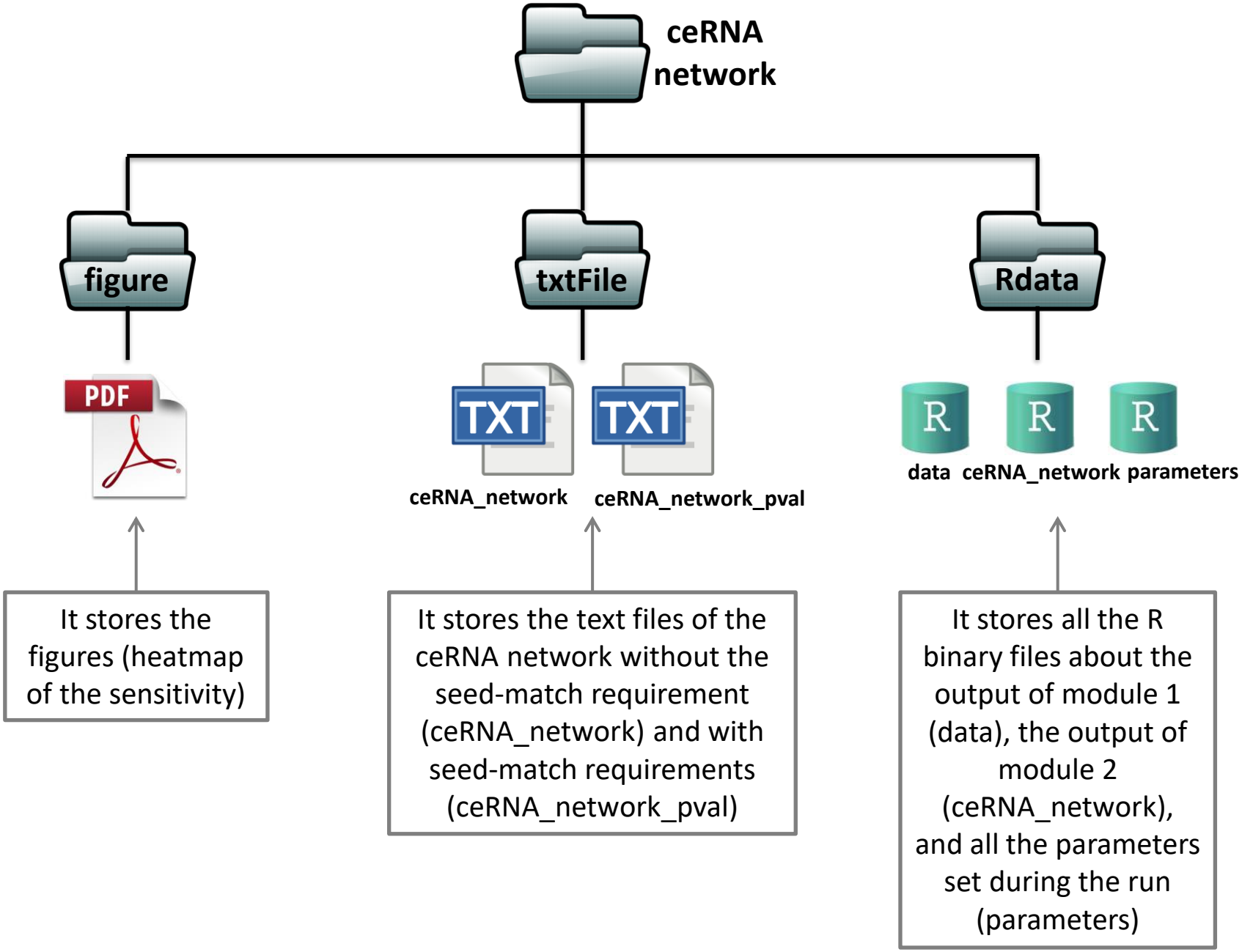
- Install R and R studio
- Download the SPINNAKER software package from

https://github.com/sportingCode/SPINNAKER.git

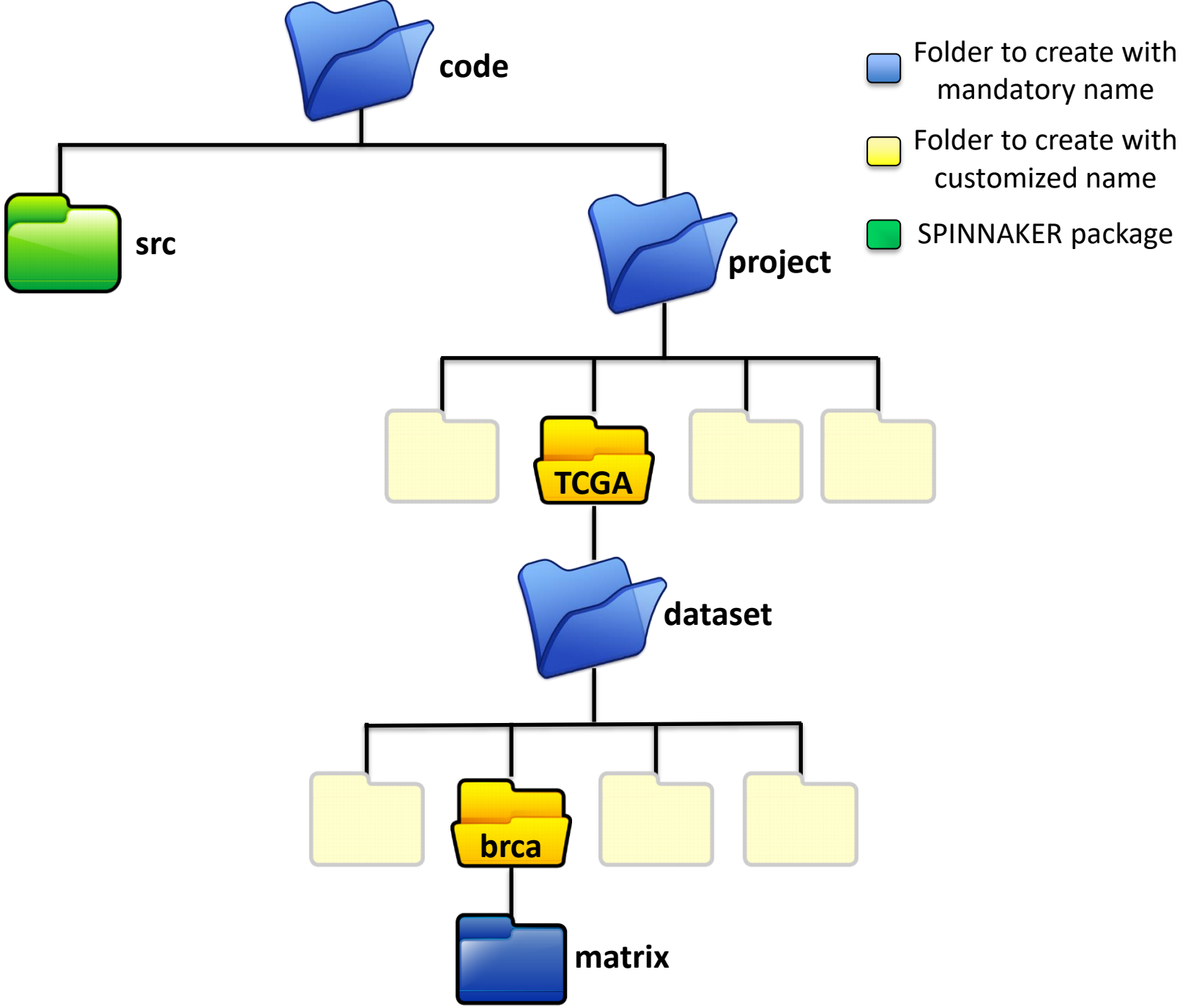

code
Folder to create with mandatory name
Folder to create with customized name
SPINNAKER package
src
project
script
main.R
TCGA
dataset
brca
matrix

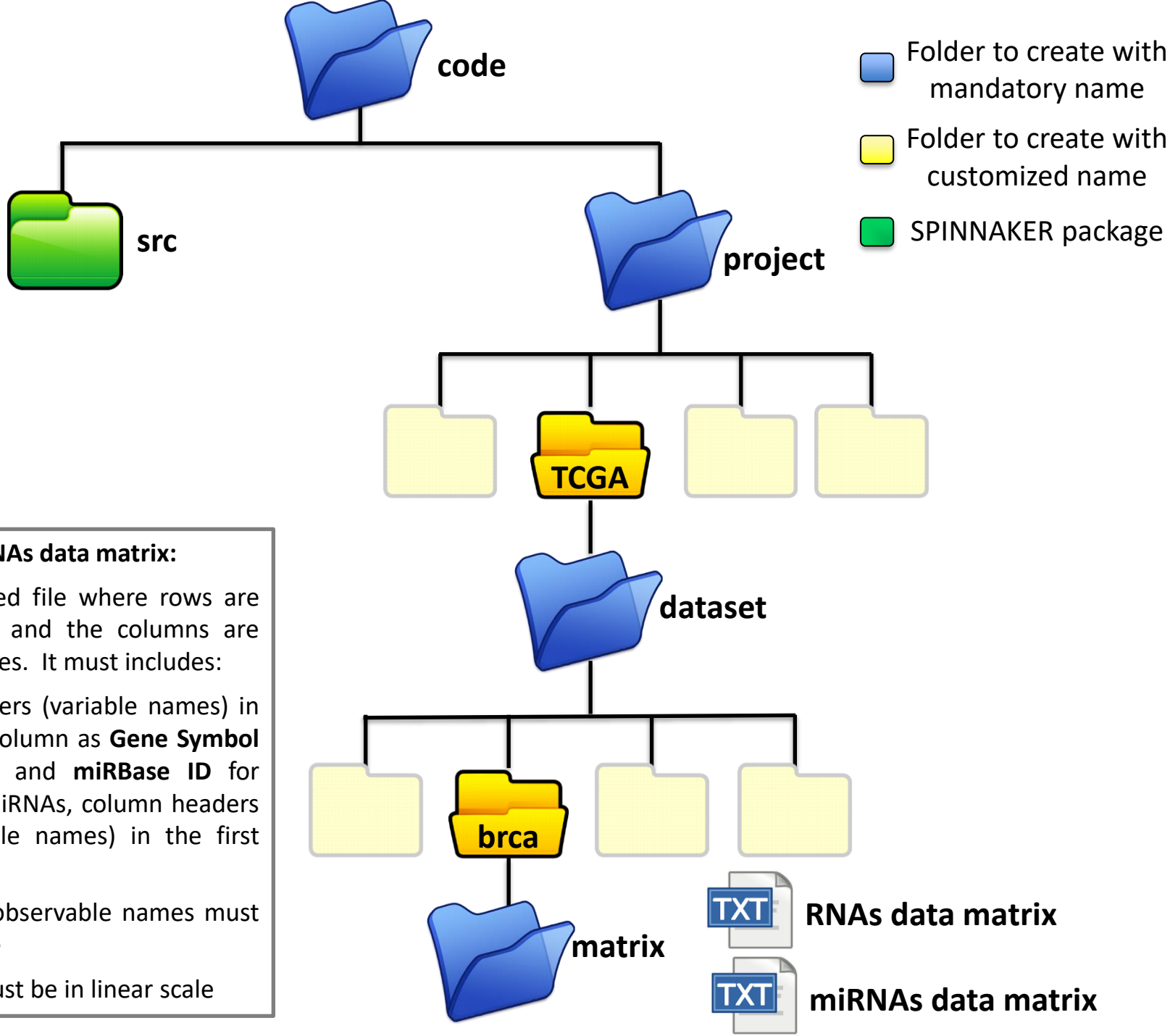

code
src
project
a thematic container with as many folders as different projects the user wants to add
script
main.R
all R scripts called by main.R
R main script to launch
TCGA
dataset
dataset folder with as many folders as different datasets the user wants to analyze for a given project
brca
matrix
It includes the input data matrices of RNAs and miRNAs among which searching for ceRNA interactions


code
src
project
script
main.R
TCGA
dataset
brca
ceRNA network
matrix
Folder to create with mandatory name
Folder to create with customized name
SPINNAKER package
Output folder


ceRNA network
figure
txtFile
Rdata
PDF
TXT
TXT
R
R
R
ceRNA_network
ceRNA_network_pval
data
ceRNA_network
parameters
It stores the figures (heatmap of the sensitivity)
It stores the text files of the ceRNA network without the seed-match requirement (ceRNA_network) and with seed-match requirements (ceRNA_network_pval)
It stores all the R binary files about the output of module 1 (data), the output of module 2 (ceRNA_network), and all the parameters set during the run (parameters)

Let’s get started


code
src
project
TCGA
dataset
brca
matrix
Folder to create with mandatory name
Folder to create with customized name
SPINNAKER package



RNAs or miRNAs data matrix:

a tab-delimited file where rows are the variables and the columns are the observables. It must includes:

- row headers (variable names) in the first column as **Gene Symbol** for RNAs and **miRBase ID** for mature miRNAs, column headers (observable names) in the first row
- Variable/observable names must be unique
- Values must be in linear scale

RNA data matrix - example

Data matrix
N x M

- N rows → Transcripts
- M columns → Samples

Samples →

Transcripts ↑

	TCGA-A7-A0CE-11A-21R-A089-07	TCGA-A7-A0D9-11A-53R-A089-07	TCGA-A7-A0D9-01A-31R-A056-07	TCGA-A7-A0DB-01A-11R-A00Z-07	TCGA-A7-A13E-11A-61R-A12P-07	TCGA-A7-A13E-01A-11R-A12P-07	TCGA-A7-A13G-11A-51R-A13Q-07	TCGA-AC-A23H-11A-12R-A157-07	TCGA-AC-A2FB-01A-11R-A17B-07	TCGA-AC-A2FM-11B-32R-A19W-07	...	TCGA-A7-A0CE-01A-11R-A00Z-07	TCGA-A7-A0D9-11A-53R-A089-07	TCGA-A7-A0DB-11A-33R-A089-07	TCGA-A7-A0DC-01A-11R-A00Z-07	TCGA-A7-A13G-11A-51R-A13Q-07	TCGA-A7-A13F-01A-11R-A12P-07	TCGA-A7-A13G-01A-11R-A13Q-07
AB1G	4.3	3.1	3.3	5.6	2.1	1.6	6.3	5.9	11.2	0.4	...	11.1	26.8	0.9	12.8	4.3	0.0	13.1
ADAM10	3.9	0.0	0.8	13.5	3.3	4.7	4.7	8.5	3.9	1.2	....	3.5	13.5	5.2	11.6	4.3	1.2	6.2
...											...							

⚠ **Caveat:** Transcript and sample names **must be** unique. Matrix values **must be** linear. Transcript names **must be** in Gene Symbol.

miRNA data matrix - example

Data matrix
N x M

- N rows → mature miRNAs
- M columns → Samples

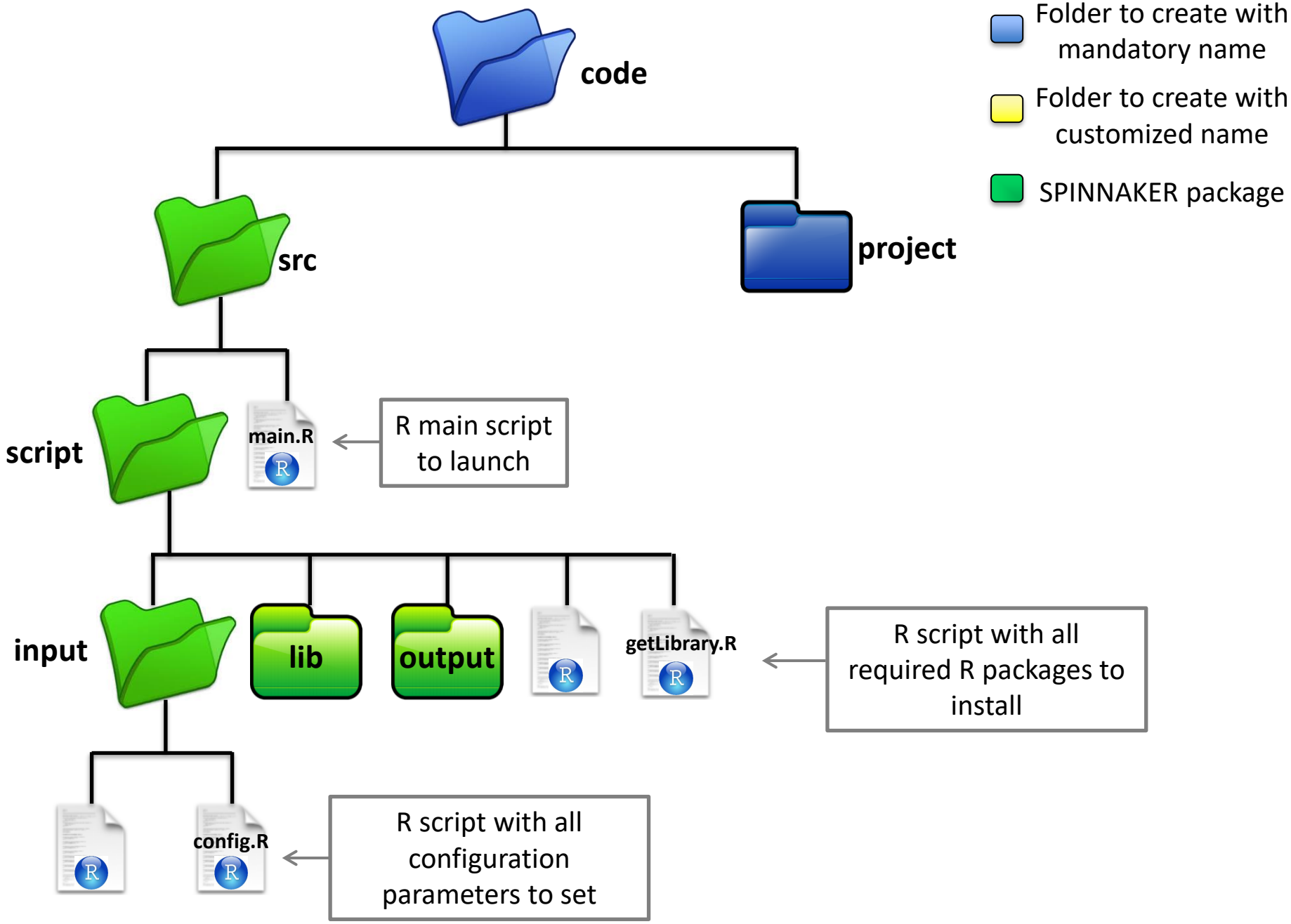
Samples →

Mature miRNAs ↑

	TCGA-A7-A0CE-11A-21R-A089-07	TCGA-A7-A0D9-11A-53R-A089-07	TCGA-A7-A0D9-01A-31R-A056-07	TCGA-A7-A0DB-01A-11R-A00Z-07	TCGA-A7-A13E-11A-61R-A12P-07	TCGA-A7-A13E-01A-11R-A12P-07	TCGA-A7-A13G-11A-51R-A13Q-07	TCGA-AC-A23H-11A-12R-A157-07	TCGA-AC-A2FB-01A-11R-A17B-07	TCGA-AC-A2FM-11B-32R-A19W-07	...	TCGA-A7-A0CE-01A-11R-A00Z-07	TCGA-A7-A0D9-11A-53R-A089-07	TCGA-A7-A0DB-11A-33R-A089-07	TCGA-A7-A0DC-01A-11R-A00Z-07	TCGA-A7-A13G-11A-51R-A13Q-07	TCGA-A7-A13F-01A-11R-A12P-07	TCGA-A7-A13G-01A-11R-A13Q-07
hsa-miR-200a-3p	13.5	3.3	4.7	4.7	8.5	3.9	3.9	0.0	0.8	13.5	...	11.1	4.7	8.5	3.9	4.3	0.0	13.1
hsa-miR-205	11.1	26.8	0.9	12.8	11.1	3.5	13.5	5.2	11.6	3.5	....	3.5	12.9	1171	3.5	4.3	1.2	6.2
...											...							

⚠ **Caveat:** miRNA and sample names **must be** unique. Matrix values **must be** linear. miRNA names **must be** in miRBase ID (i.e., hsa-miR-200a-3p) and as mature forms.

Ready to launch!


Folder to create with mandatory name
Folder to create with customized name
SPINNAKER package
code
src
project
script
main.R
R main script to launch
input
lib
output
getLibrary.R
R script with all required R packages to install
config.R
R script with all configuration parameters to set

Main file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R

Source on Save | Run | Source

```
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
getLibrary()
getSource()
input_parameter <- config()
input_file <- inputFiles()
output_file <- outputFiles()
#####################################

data <- DataCollectionProcessing()

ceRNA_network <- ceRNANetworkBuilding()

saveNetwork()

saveParameters()
```

Module 1

Module 2

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

>

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src

Name	Size	Modified
..		
script		
main.R	498 B	Jun 11, 2021, 12:2

Main file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R

Source on Save | Run | Source

```r
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
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```

Module 1

Module 2

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

>

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src

Name	Size	Modified
..		
script		
main.R	498 B	Jun 11, 2021, 12:2

Main file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R

Source on Save | Run | Source

```
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
getLibrary()
getSource()
input_parameter <- config()
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output_file <- outputFiles()
#####################################

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ceRNA_network <- ceRNANetworkBuilding()

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saveParameters()
```

Module 1

Module 2

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

>

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src

Name	Size	Modified
..		
script		
main.R	498 B	Jun 11, 2021, 12:2

Main file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R

Source on Save | Run | Source

```r
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
getLibrary()
getSource()
input_parameter <- config()
input_file <- inputFiles()
output_file <- outputFiles()
#####################################

data <- DataCollectionProcessing()

ceRNA_network <- ceRNANetworkBuilding()

saveNetwork()

saveParameters()
```

Module 1

Module 2

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

>

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src > script

Name	Size	Modified
..		
output		
lib		
input		
getSource.R	1.7 KB	Jun 8, 2021, 4:20 P
getLibrary.R	323 B	Jun 5, 2021, 10:52

Main file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R

Source on Save | Run | Source

```r
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
getLibrary()
getSource()
input_parameter <- config()
input_file <- inputFiles()
output_file <- outputFiles()
#####################################

data <- DataCollectionProcessing()

ceRNA_network <- ceRNANetworkBuilding()

saveNetwork()

saveParameters()
```

Module 1

Module 2

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

>

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src > script > input

Name	Size	Modified
..		
inputFiles.R	878 B	Jun 7, 2021, 9:50 A
config.R	1.4 KB	Jun 8, 2021, 4:22 P

Configuration file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R | config.R

Source on Save | Run | Source

```r
config <- function(){

  ################################
  project <- "TCGA"
  dataset <- "brca"

  path <- paste0("project/",project,"/dataset/",dataset)
  ################################
  # input files

  filename_data_RNA <- paste0(path,"/matrix/RNA_normal.txt")
  filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal.txt")
  ################################
  # input parameters

  # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scRNA","snoRNA","snRNA"

  ceRNA1 <- "protein-coding"
  ceRNA2 <- c("ncRNA","pseudo")

  threshold_perc_missing_values <- 0.1
  threshold_prc_corr <- 0.99
  threshold_prc_sensitivity <- 0.99

  searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
  ################################
```

1:1 | config() | R Script

Console ~/SPINNAKER/code/src/

```
>
```

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src > script > input

Name	Size	Modified
..		
inputFiles.R	878 B	Jun 7, 2021, 9:50 A
config.R	1.4 KB	Jun 8, 2021, 4:22 P

Configuration file

Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R | config.R

Source on Save | Run | Source

```
config <- function(){

  ################################
  project <- "TCGA"
  dataset <- "brca"

  path <- paste0("project/",project,"/datase
  ################################
  # input files

  filename_data_RNA <- paste0(path,"/matrix/RNA_normal.txt")
  filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal.txt")
  ################################
  # input parameters

  # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scRNA","snoRNA","snRNA"

  ceRNA1 <- "protein-coding"
  ceRNA2 <- c("ncRNA","pseudo")

  threshold_perc_missing_values <- 0.1
  threshold_prc_corr <- 0.99
  threshold_prc_sensitivity <- 0.99

  searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
  ################################
```

Insert *project* name

Insert *dataset* name

1:1 | config() | R Script

Console ~/SPINNAKER/code/src/

```
>
```

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Files | Plots | Packages | Help | Viewer

New Folder | Delete | Rename | More

Home > SPINNAKER > code > src > script > input

Name	Size	Modified
..		
inputFiles.R	878 B	Jun 7, 2021, 9:50 A
config.R	1.4 KB	Jun 8, 2021, 4:22 P

Configuration file

Input files

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

main.R | config.R

```r
config <- function(){

  ################################
  project <- "TCGA"
  dataset <- "brca"

  path <- paste0("project/",project,"/dataset/",dataset)
  ################################
  # input files

  filename_data_RNA <- paste0(path,"/matrix/RNA_normal.txt")
  filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal.txt")
  ################################
  # input parameters

  # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scRNA","snoRNA","snRNA"

  ceRNA1 <- "protein-coding"
  ceRNA2 <- c("ncRNA","pseudo")

  threshold_perc_missing_values <- 0.1
  threshold_prc_corr <- 0.99
  threshold_prc_sensitivity <- 0.99

  searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
  ################################
```

Insert filename for **RNA** *data matrix*

Insert filename for **miRNA** data *matrix*

Console ~/SPINNAKER/code/src/

Environment | History | Connections | Tutorial

Files | Plots | Packages | Help | Viewer

Home > SPINNAKER > code > src > script > input

Name	Size	Modified
..		
inputFiles.R	878 B	Jun 7, 2021, 9:50 A
config.R	1.4 KB	Jun 8, 2021, 4:22 P

Configuration file

Input parameters

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

main.R | config.R

```r
config <- function(){

  ################################
  project <- "TCGA"
  dataset <- "brca"

  path <- paste0("project/",project,"/dataset/",dataset)
  ################################
  # input files

  filename_data_RNA <- paste0(path,"/matrix/RNA_normalized")
  filename_data_miRNA <- paste0(path,"/matrix/mi
  ################################
  # input parameters

  # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scRNA","snoRNA","snRNA"

  ceRNA1 <- "protein-coding"
  ceRNA2 <- c("ncRNA","pseudo")

  threshold_perc_missing_values <- 0.1
  threshold_prc_corr <- 0.99
  threshold_prc_sensitivity <- 0.99

  searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
  ################################
```

Insert biotype of **ceRNA 1**

Insert biotype of **ceRNA 2**

Set the threshold for the max **percentage of allowed missing values**

Set the threshold for the Pearson **correlation coefficient** (percentile)

Set the threshold for the **sensitivity** (percentile)

Set the threshold for the **sensitivity** (percentile)

Type "Yes" if you want to perform **the seed-match analysis**

Configuration file

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

main.R | config.R

```r
config <- function(){

  ################################
  project <- "TCGA"
  dataset <- "brca"

  path <- paste0("project/",project,"/dataset/",dataset)
  ################################
  # input files

  filename_data_RNA <- paste0(path,"/matrix/RNA_normal.txt")
  filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal.txt")
  ################################
  # input parameters

  # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scRNA","snoRNA","snRNA"

  ceRNA1 <- "protein-coding"
  ceRNA2 <- c("ncRNA","pseudo")

  threshold_perc_missing_values <- 0.1
  threshold_prc_corr <- 0.99
  threshold_prc_sensitivity <- 0.99

  searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
  ################################
```

Console ~/SPINNAKER/code/src/

Environment | History | Connections | Tutorial

Environment is empty

Files | Plots | Packages | Help | Viewer

Home > SPINNAKER > code > src > script > input

Name	Size	Modified
..		
inputFiles.R	878 B	Jun 7, 2021, 9:50 A
config.R	1.4 KB	Jun 8, 2021, 4:22 P

SPINNAKER launch!

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function | Addins | Project: (None)

main.R | config.R

Source on Save | Run | Source

```r
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
#####################################
source("src/script/getLibrary.R")
source("src/script/getSource.R")
#####################################
getLibrary()
getSource()
input_parameter <- config()
input_file <- inputFiles()
output_file <- outputFiles()
#####################################

data <- DataCollectionProcessing()

ceRNA_network <- ceRNANetworkBuilding()

saveNetwork()

saveParameters()
```

1:1 (Top Level)

Console ~/SPINNAKER/code/src/

```
>
```

Environment | History | Connections | Tutorial

Import Dataset | List

R | Global Environment

Environment is empty

Help | Viewer

Delete | Rename | More

AKER > code > src > script > input

	Size	Modified
	878 B	Jun 7, 2021, 9:50 A
	1.4 KB	Jun 8, 2021, 4:22 P