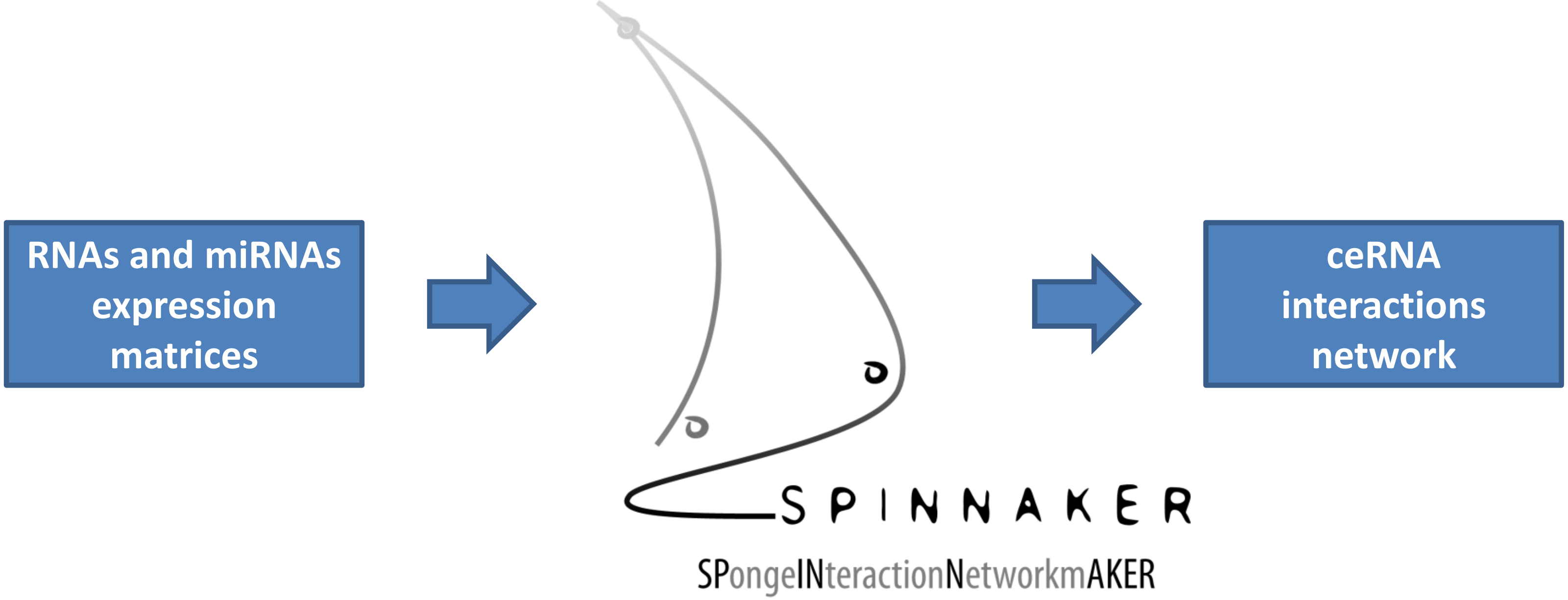
SPINNAKER (SPongeINteractionNetworkmAKER) is the R-based implementation of a widely established mathematical model that we recently published for identifying putative competing endogenous RNA (ceRNA). Taking as input the expression levels of RNAs and miRNAs, SPINNAKER predicts the ceRNA interactions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



RNAs and miRNAs expression matrices

SPINNAKER

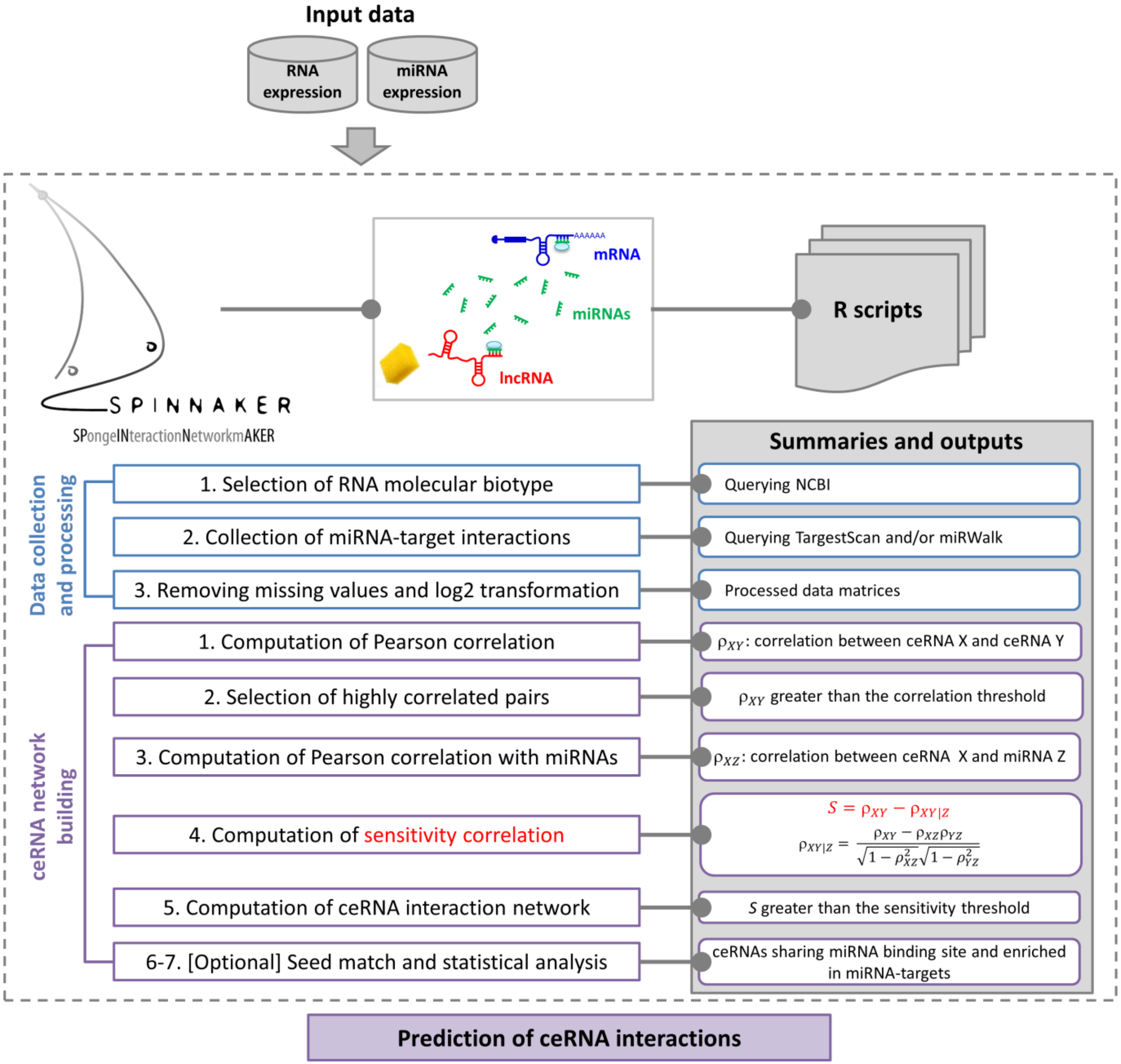
SPongeINteractionNetworkmAKER

ceRNA interactions network

BMC Systems Biology 2014, **8**(1):83

2021, SUBMITTED

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


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

Module 0: Architecture

GETTING STARTED

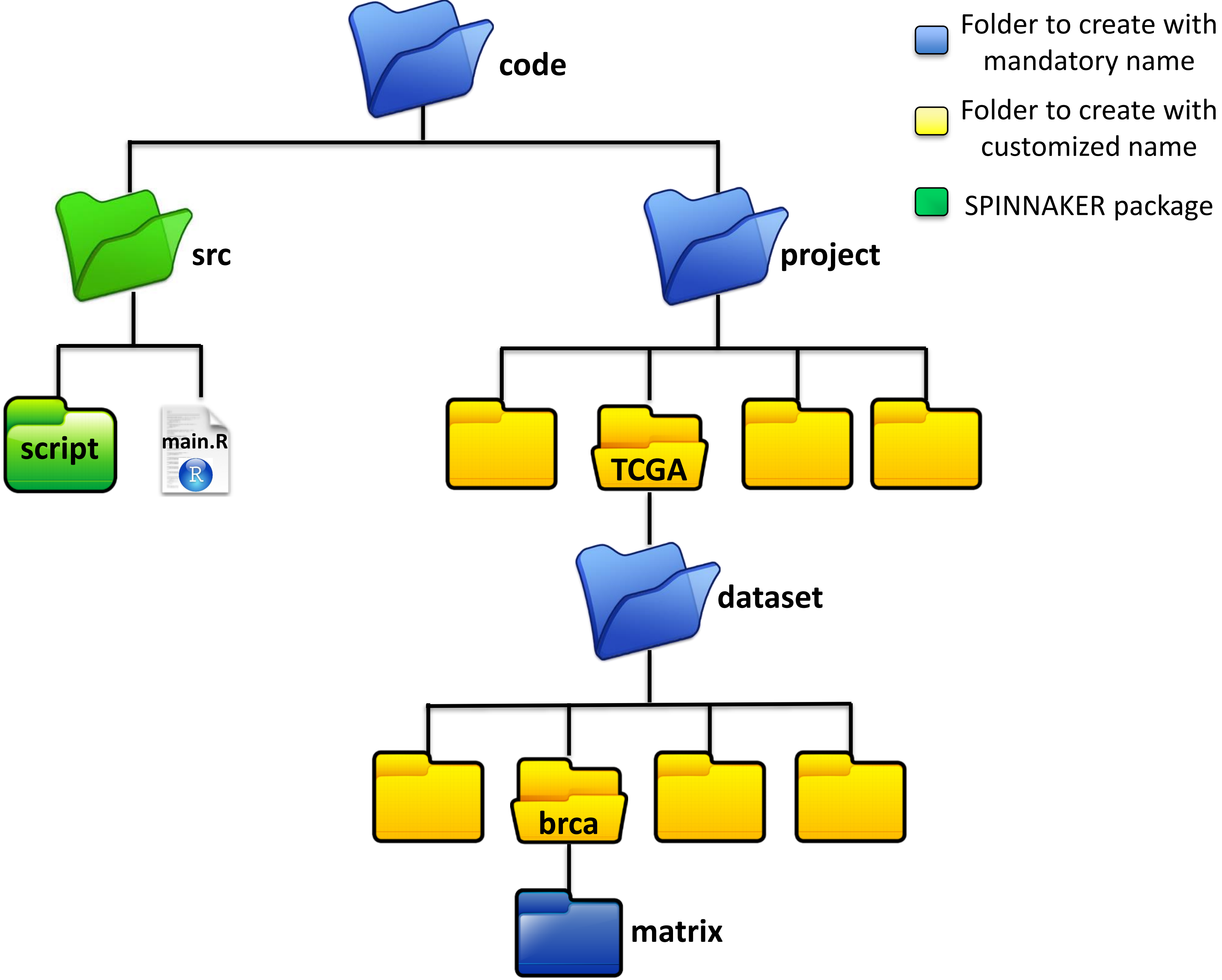
Software requirement

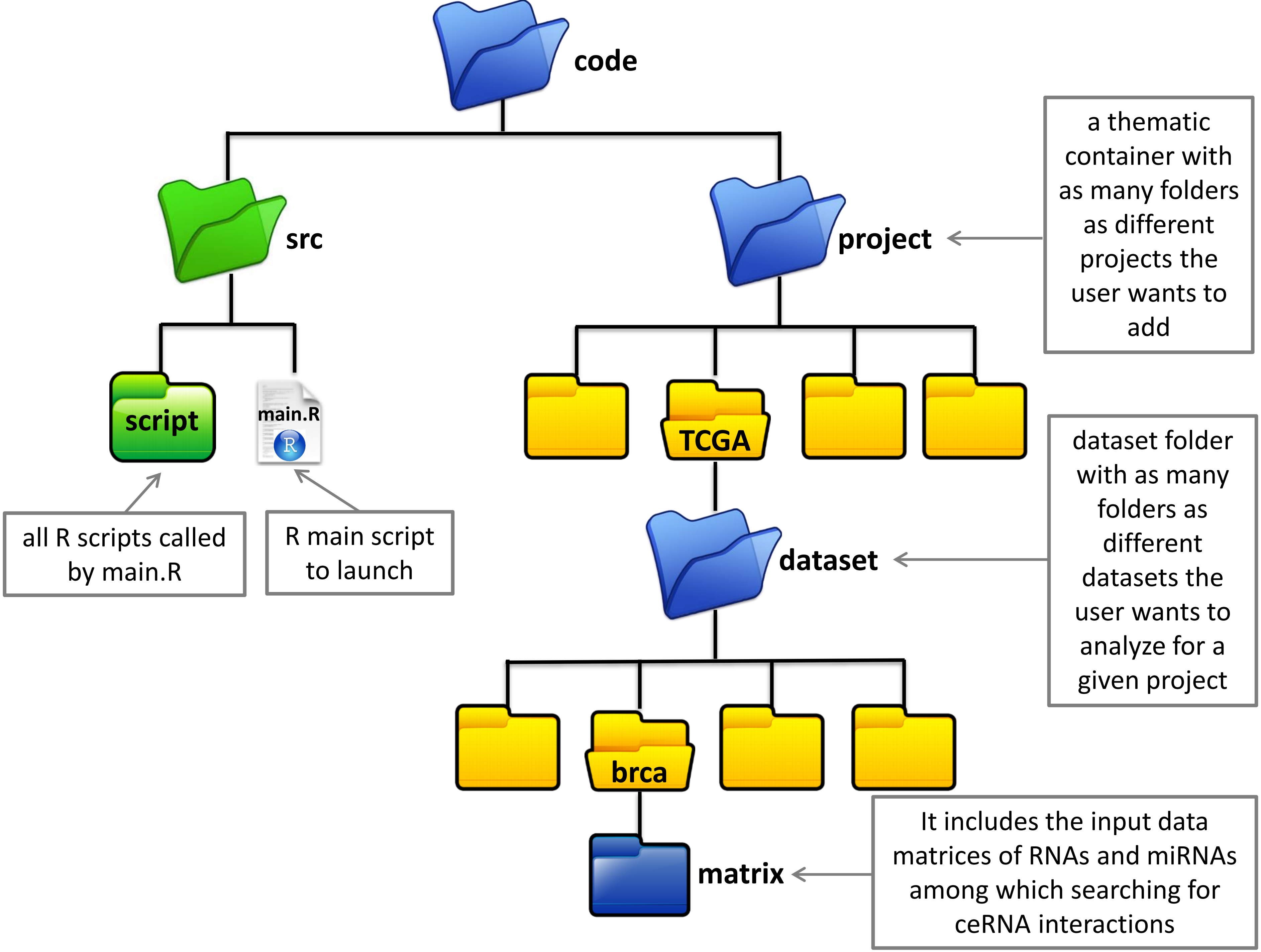
- SPINNAKER has been developed in R and tested on the following operative systems:
  - macOS High Sierra 10.13.6
  - Windows 10 Pro

Setting up

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

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


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


code
src
project
script
main.R
R
TCGA
dataset
brca
matrix

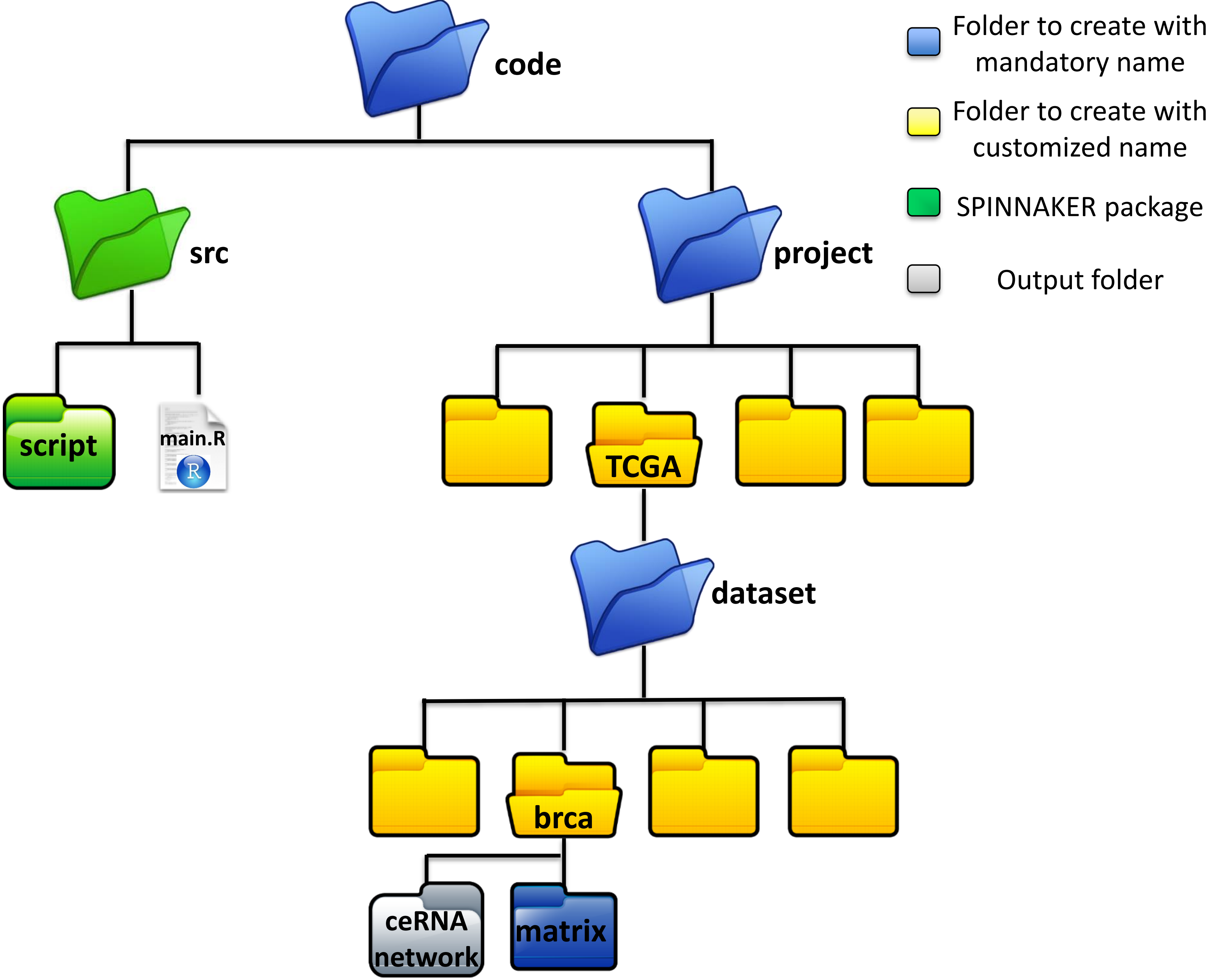
a thematic container with as many folders as different projects the user wants to add

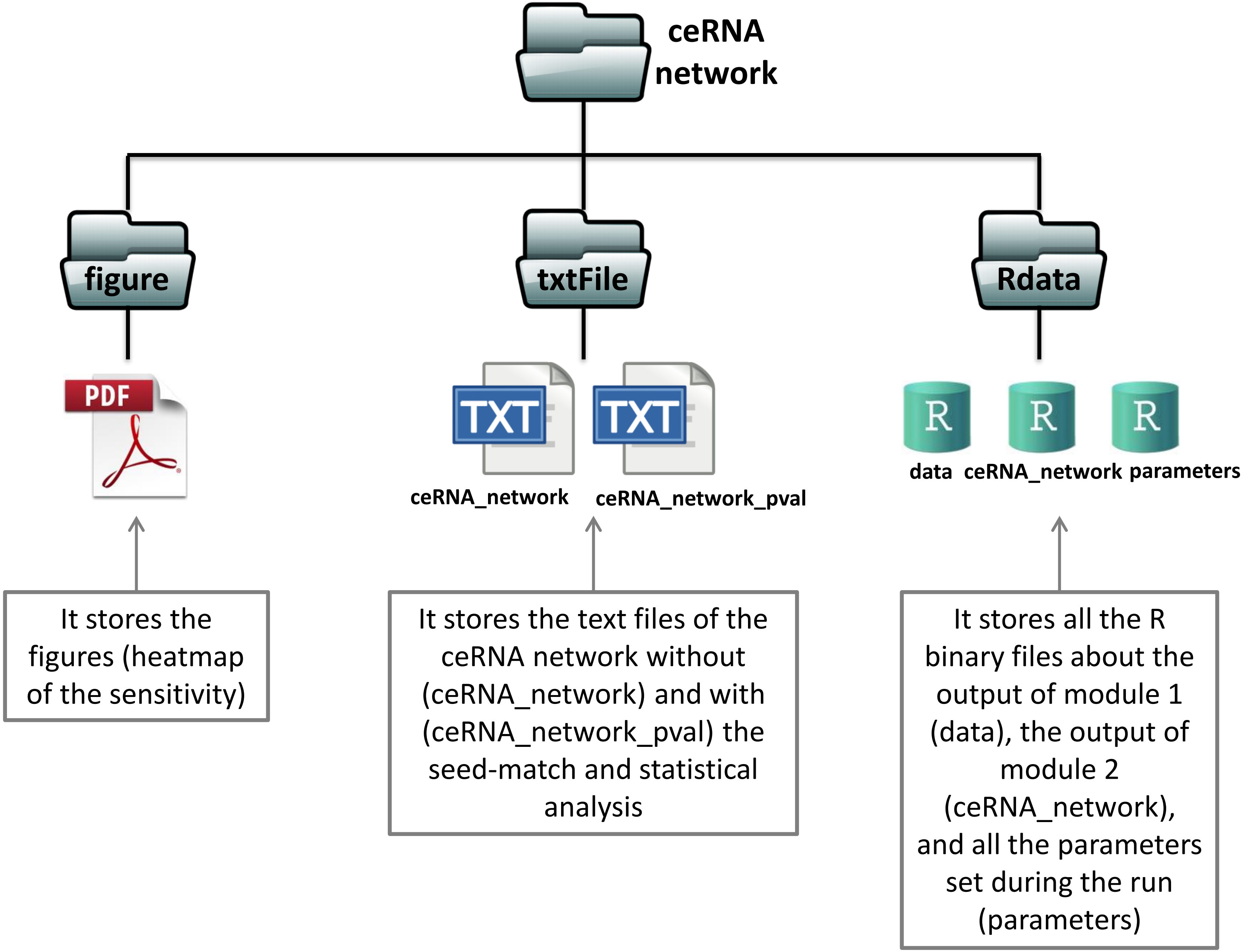
all R scripts called by main.R

R main script to launch

dataset folder with as many folders as different datasets the user wants to analyze for a given project

It includes the input data matrices of RNAs and miRNAs among which searching for ceRNA interactions


code
src
project
script
main.R
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

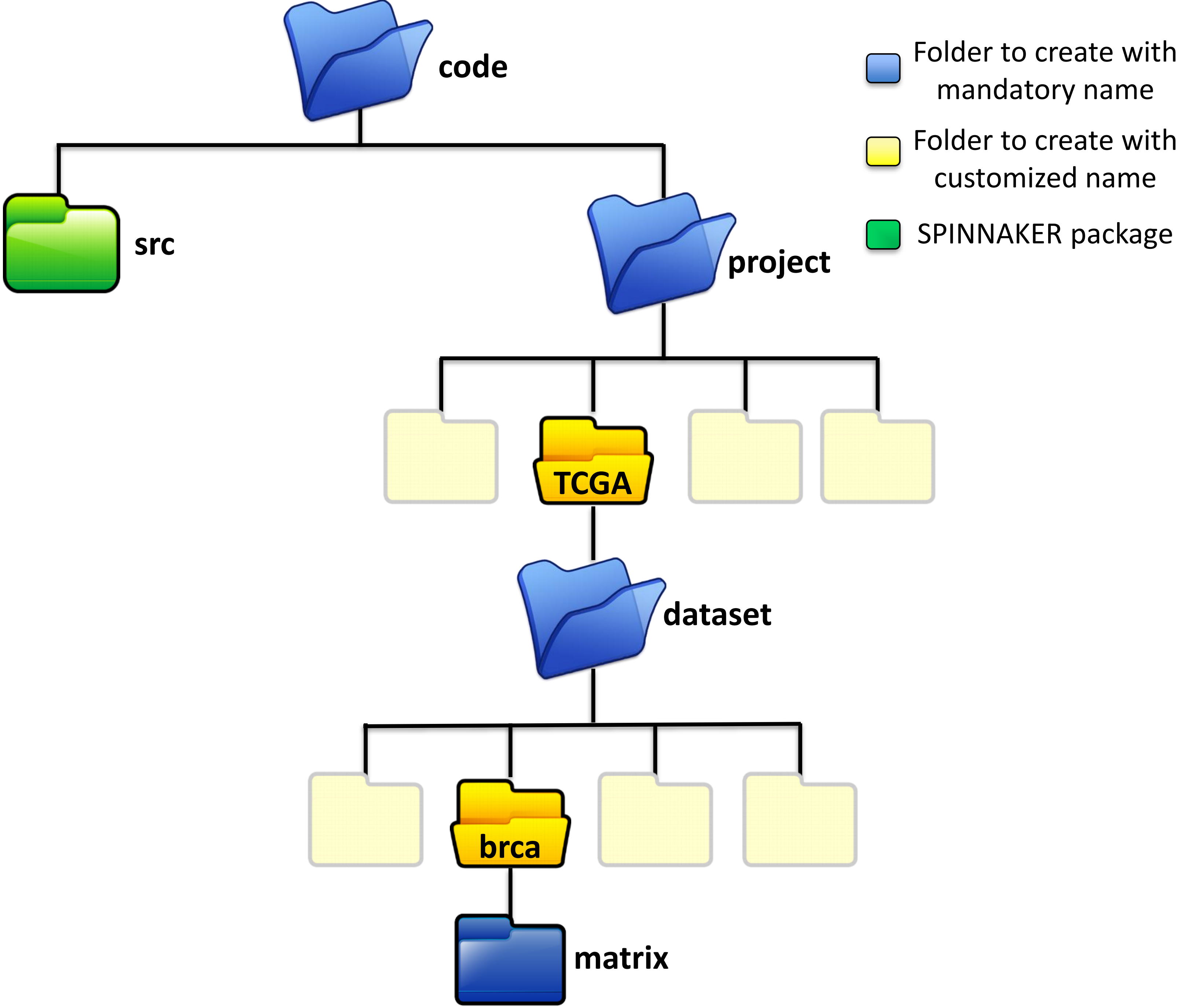
parameters

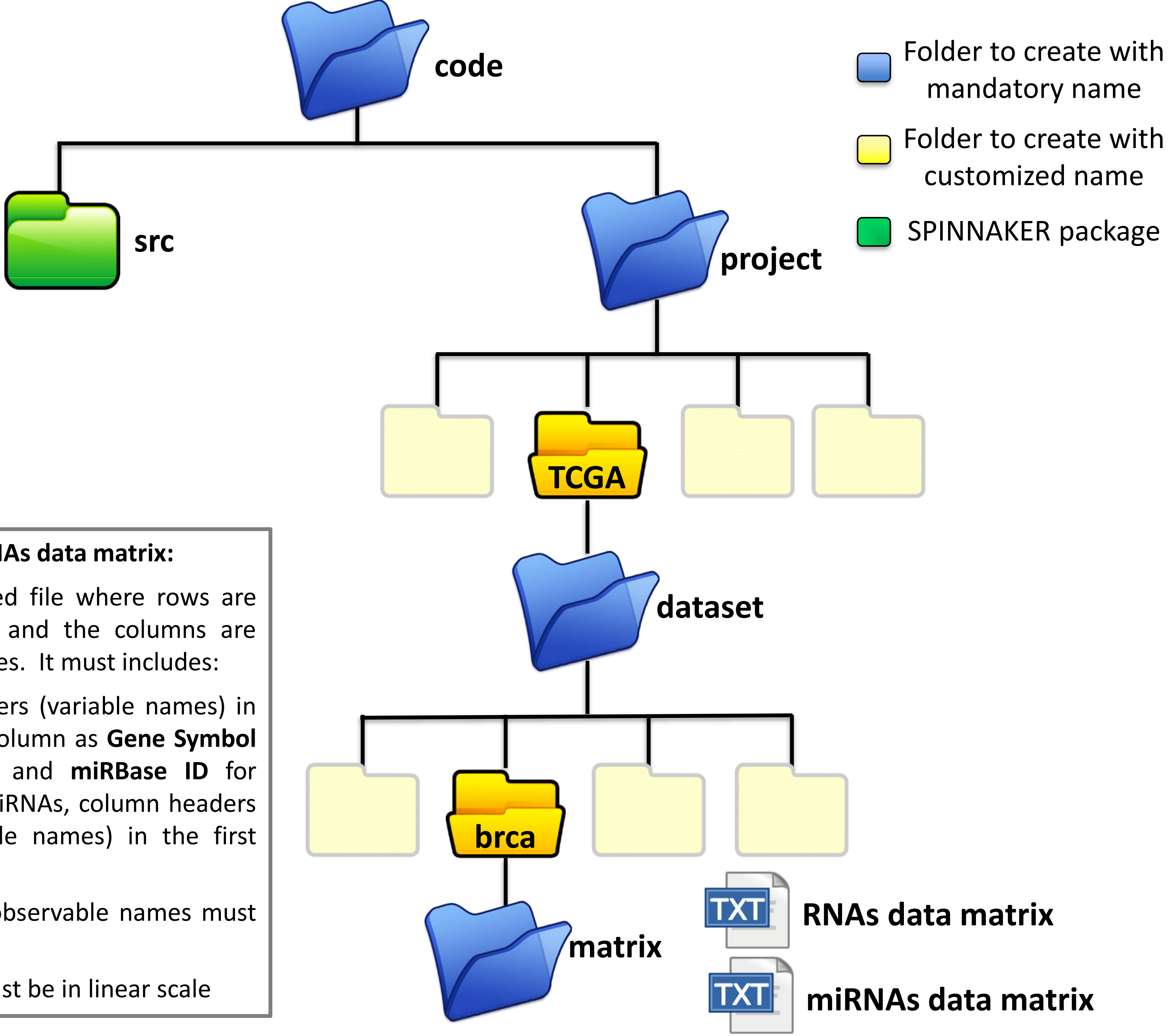
It stores the figures (heatmap of the sensitivity)

It stores the text files of the ceRNA network without (ceRNA_network) and with (ceRNA_network_pval) the seed-match and statistical analysis

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


code
src
project
TCGA
dataset
brca
matrix
TXT
RNAs data matrix
TXT
miRNAs data 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

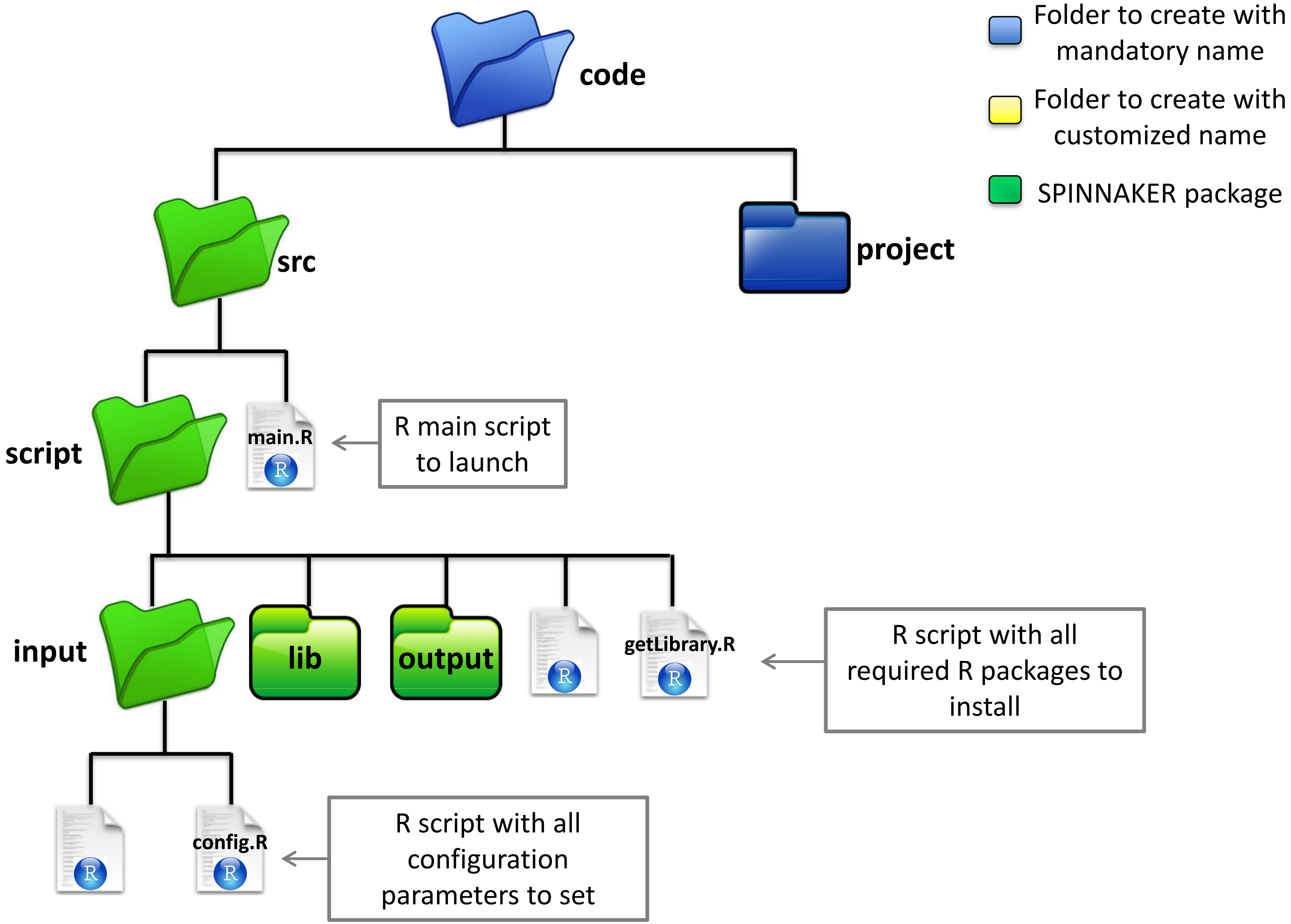
Samples

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
…											…							

⚠**veat:** 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. Samples **must be** the same as in RNA data matrix.

Ready to launch!


code
src
project
script
main.R
input
lib
output
getLibrary.R
config.R

🟦 Folder to create with mandatory name

🟨 Folder to create with customized name

🟩 SPINNAKER package

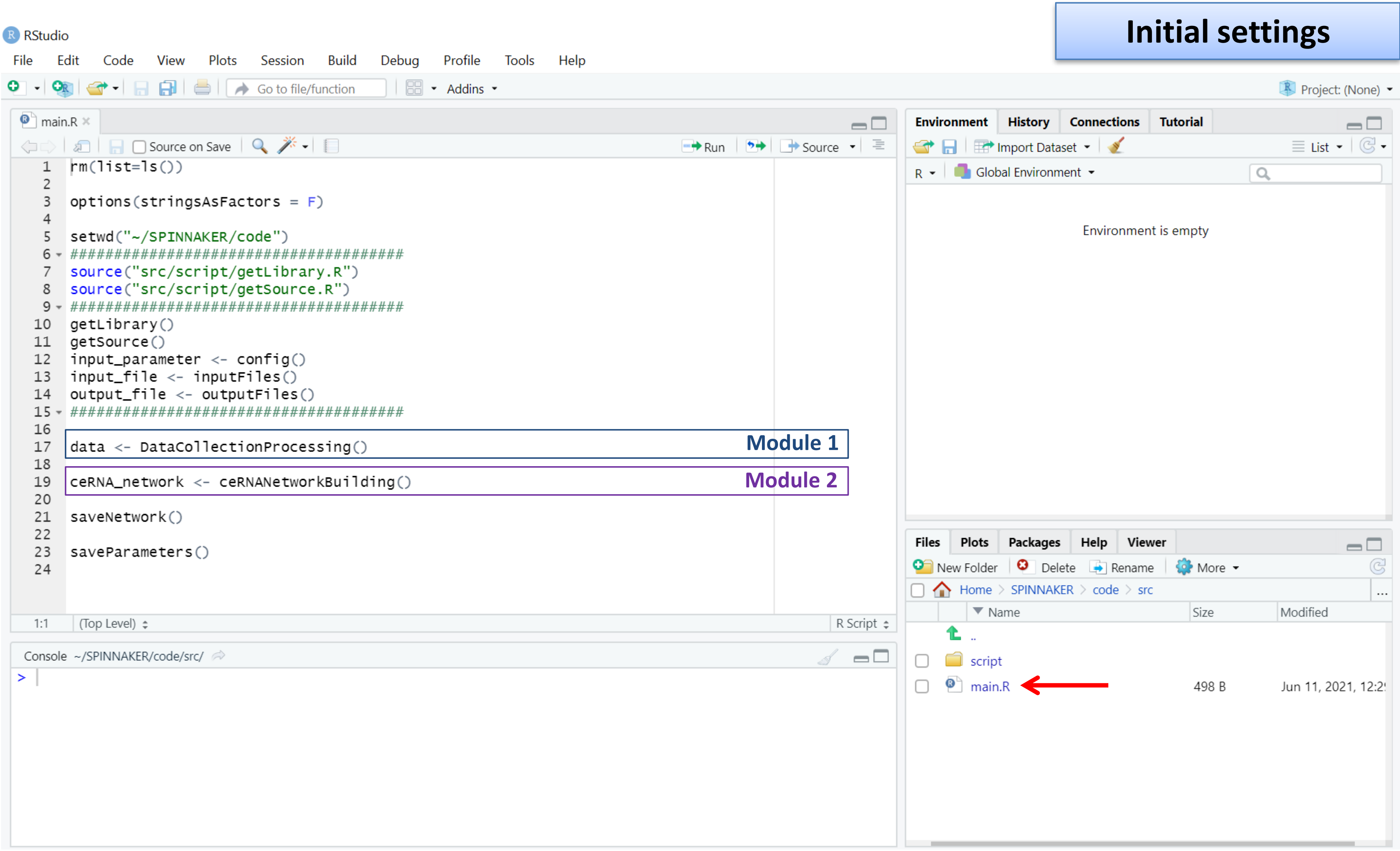
R main script to launch

R script with all required R packages to install

R script with all configuration parameters to set

Main file

Initial settings



```
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

Environment is empty

Console ~/SPINNAKER/code/src/

```
>
```

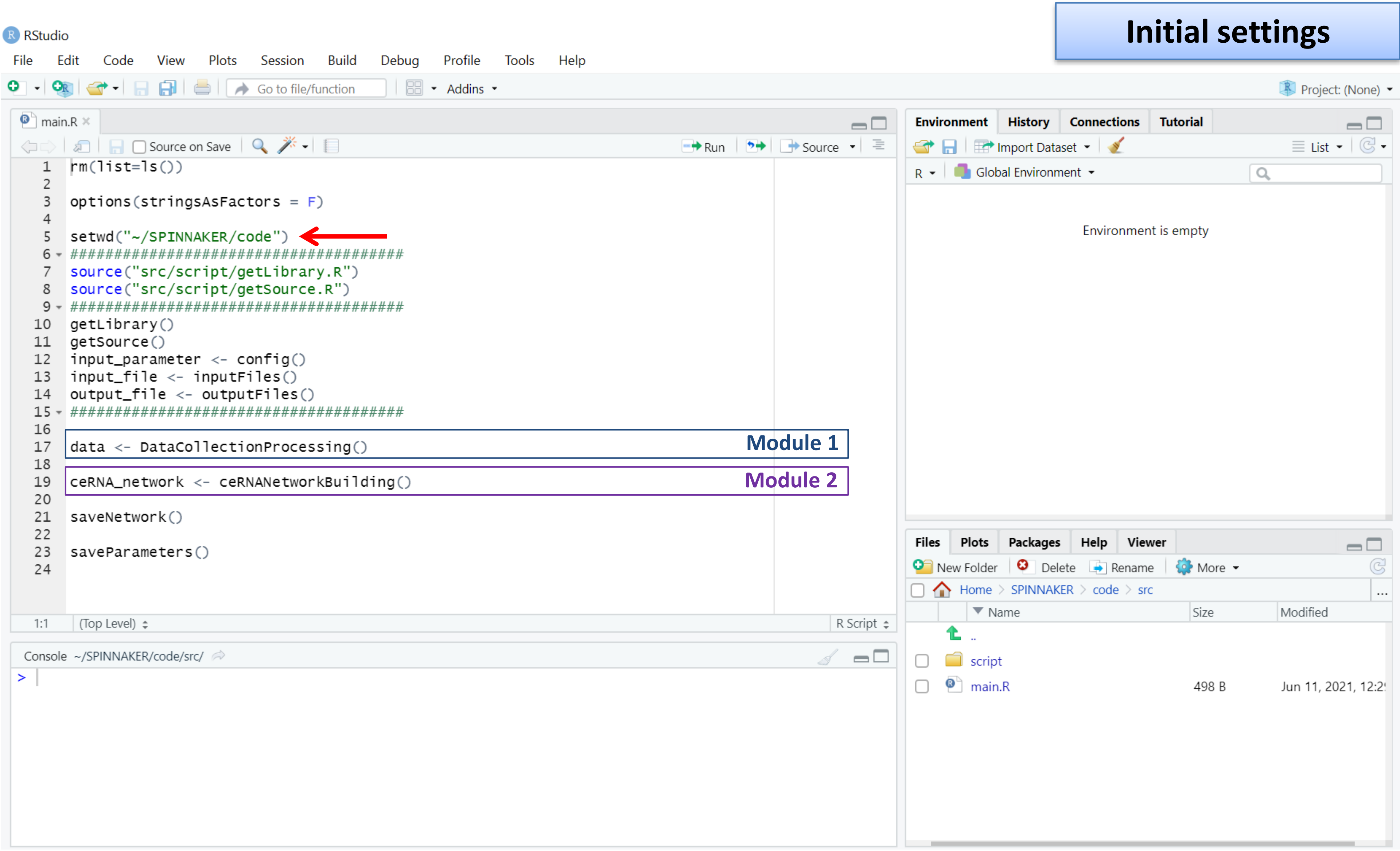
Home > SPINNAKER > code > src

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

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
1:1
(Top Level)
R Script
Environment
History
Connections
Tutorial
Import Dataset
List
R
Global Environment
Files
Plots
Packages
Help
Viewer
New Folder
Delete
Rename
More

Main file

Initial settings



```
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
######################################
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saveNetwork()

saveParameters()

```

Module 1

Module 2

Console ~/SPINNAKER/code/src/

Environment is empty

RStudio
File
Edit
Code
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Plots
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Tools
Help
Go to file/function
Addins
Project: (None)
main.R
Source on Save
Run
Source
1:1
(Top Level)
R Script
Environment
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Tutorial
Import Dataset
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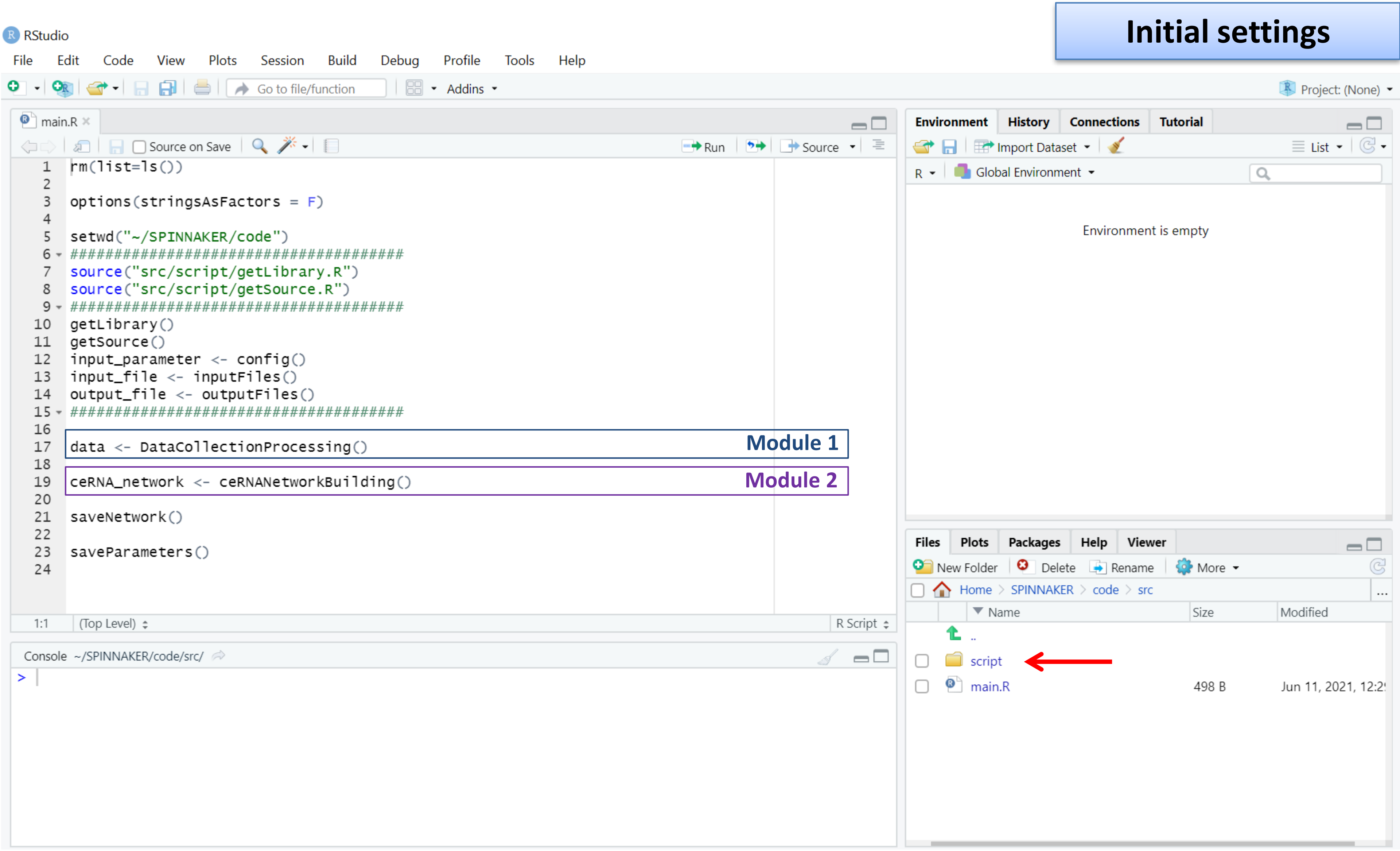
Global Environment
Files
Plots
Packages
Help
Viewer
New Folder
Delete
Rename
More
Home > SPINNAKER > code > src
Name
Size
Modified
..
script
main.R
498 B
Jun 11, 2021, 12:2

Main file

Initial settings



```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()

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saveNetwork()

saveParameters()

```

Module 1

Module 2

Environment is empty

```
Console ~/SPINNAKER/code/src/
>
```

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

RStudio
File
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Profile
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Help
Go to file/function
Addins
Project: (None)
main.R
Source on Save
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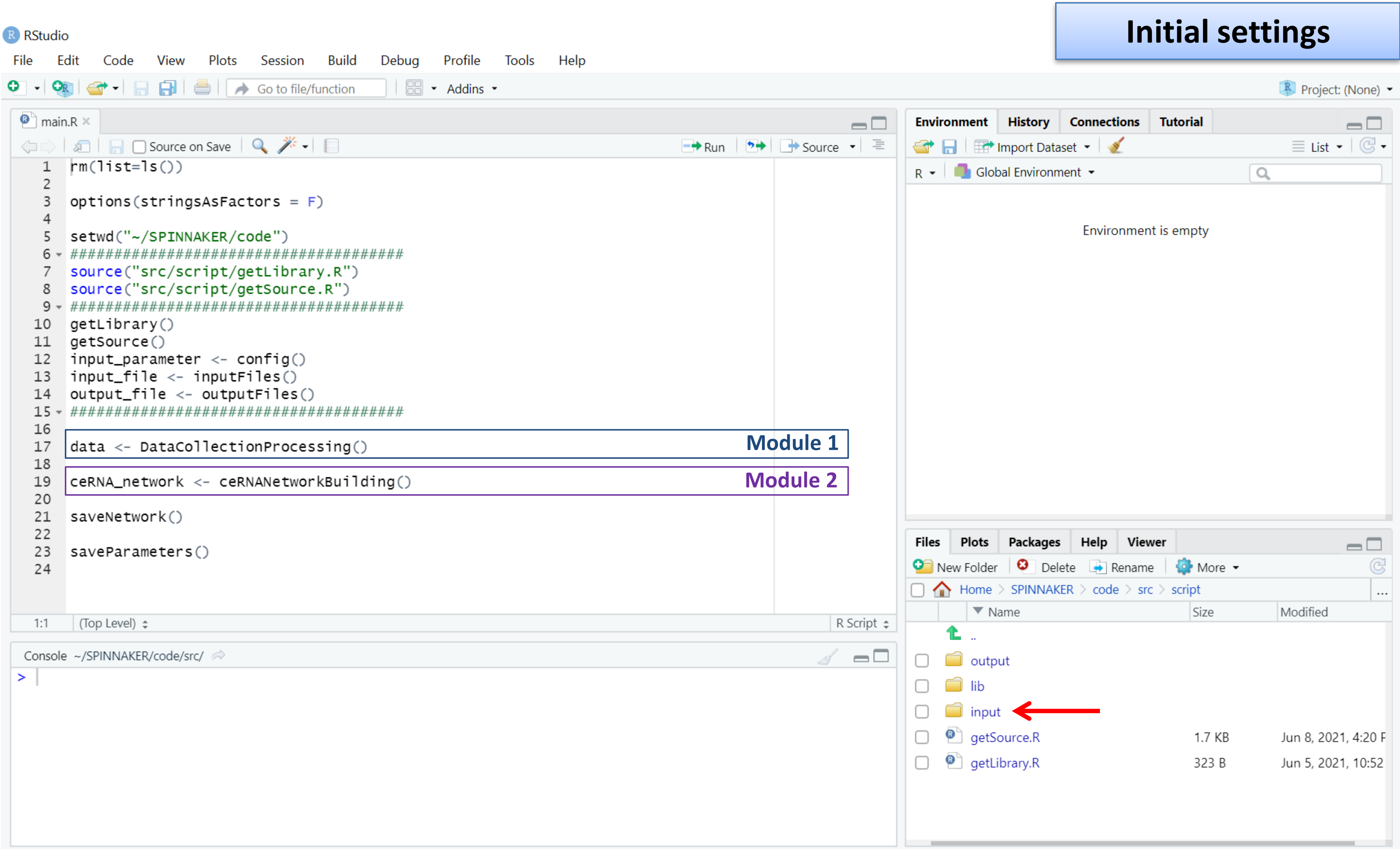
(Top Level)
R Script
Environment
History
Connections
Tutorial
Import Dataset
List
R
Global Environment
Files
Plots
Packages
Help
Viewer
New Folder
Delete
Rename
More
Home
SPINNAKER
code
src

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
Environment History Connections Tutorial
Import Dataset
List
R
Global Environment
Environment is empty
1:1
(Top Level)
R Script
Console ~/SPINNAKER/code/src/
Files Plots Packages Help Viewer
New Folder Delete Rename More
Home > SPINNAKER > code > src > script
Module 1
Module 2

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

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
######################################
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getSource()
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input_file <- inputFiles()
output_file <- outputFiles()
######################################

data <- DataCollectionProcessing()

ceRNA_network <- ceRNANetworkBuilding()

saveNetwork()

saveParameters()

```
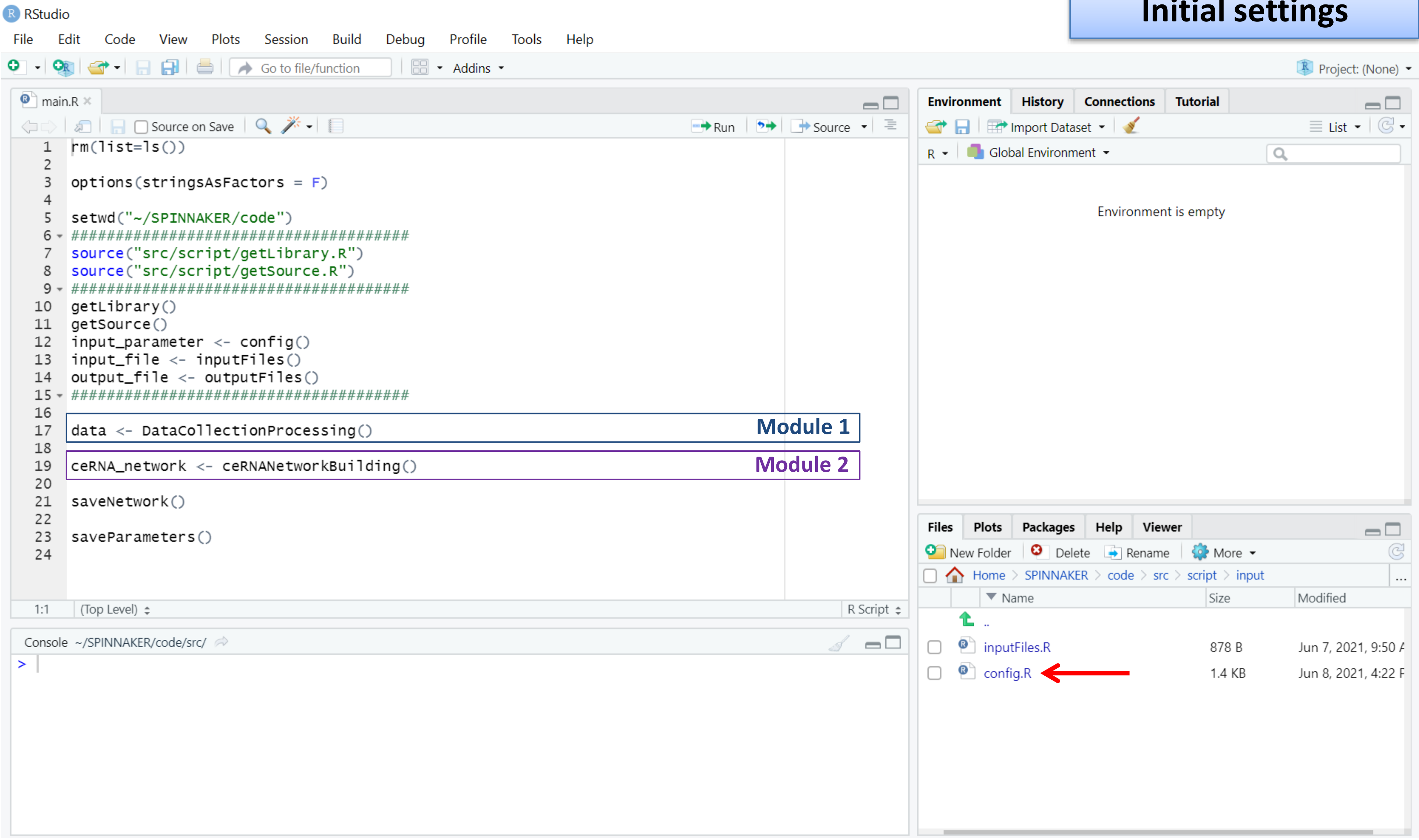

```
>
```

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

Main file

Initial settings



```
rm(list=ls())

options(stringsAsFactors = F)

setwd("~/SPINNAKER/code")
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######################################

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saveParameters()

```

Module 1

Module 2

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
1:1
(Top Level)
R Script
Console ~/SPINNAKER/code/src/
>
Environment
History
Connections
Tutorial
Import Dataset
List
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Global Environment
Environment is empty
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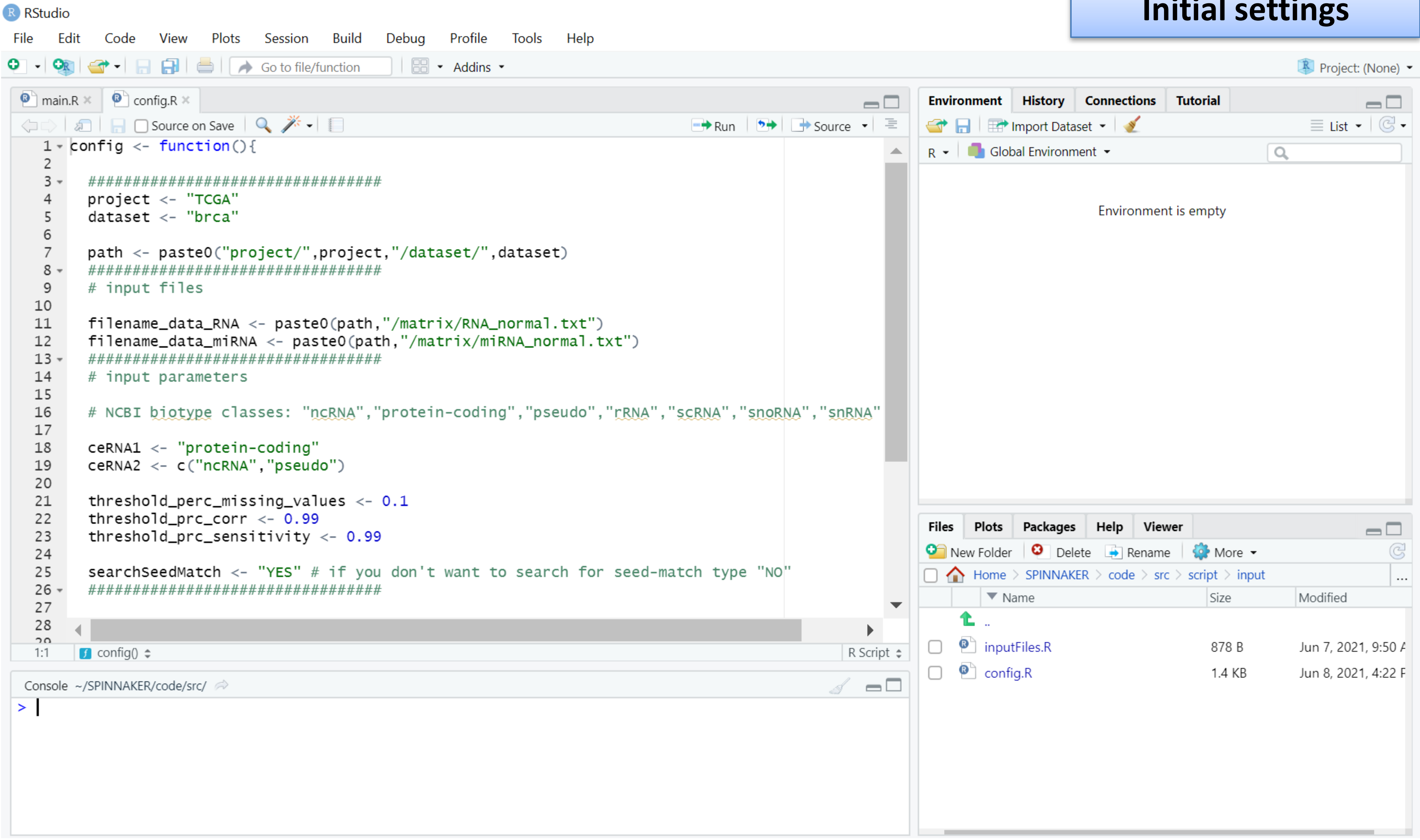
Packages
Help
Viewer
New Folder
Delete
Rename
More
Home > SPINNAKER > code > src > script > input
Name
Size
Modified
..
inputFiles.R 878 B Jun 7, 2021, 9:50
config.R 1.4 KB Jun 8, 2021, 4:22

Configuration file

Initial settings


RStudio
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Plots
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Debug
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Tools
Help
Go to file/function
Addins
Project: (None)
main.R
config.R
☐ Source on Save
Run
Source
1:1
config()
R Script
Console ~/SPINNAKER/code/src/
Environment
History
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Tutorial
Import Dataset
List
R
Global Environment
Files
Plots
Packages
Help
Viewer
New Folder
Delete
Rename
More
Home > SPINNAKER > code > src > script > input

```
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"
  #################################
```

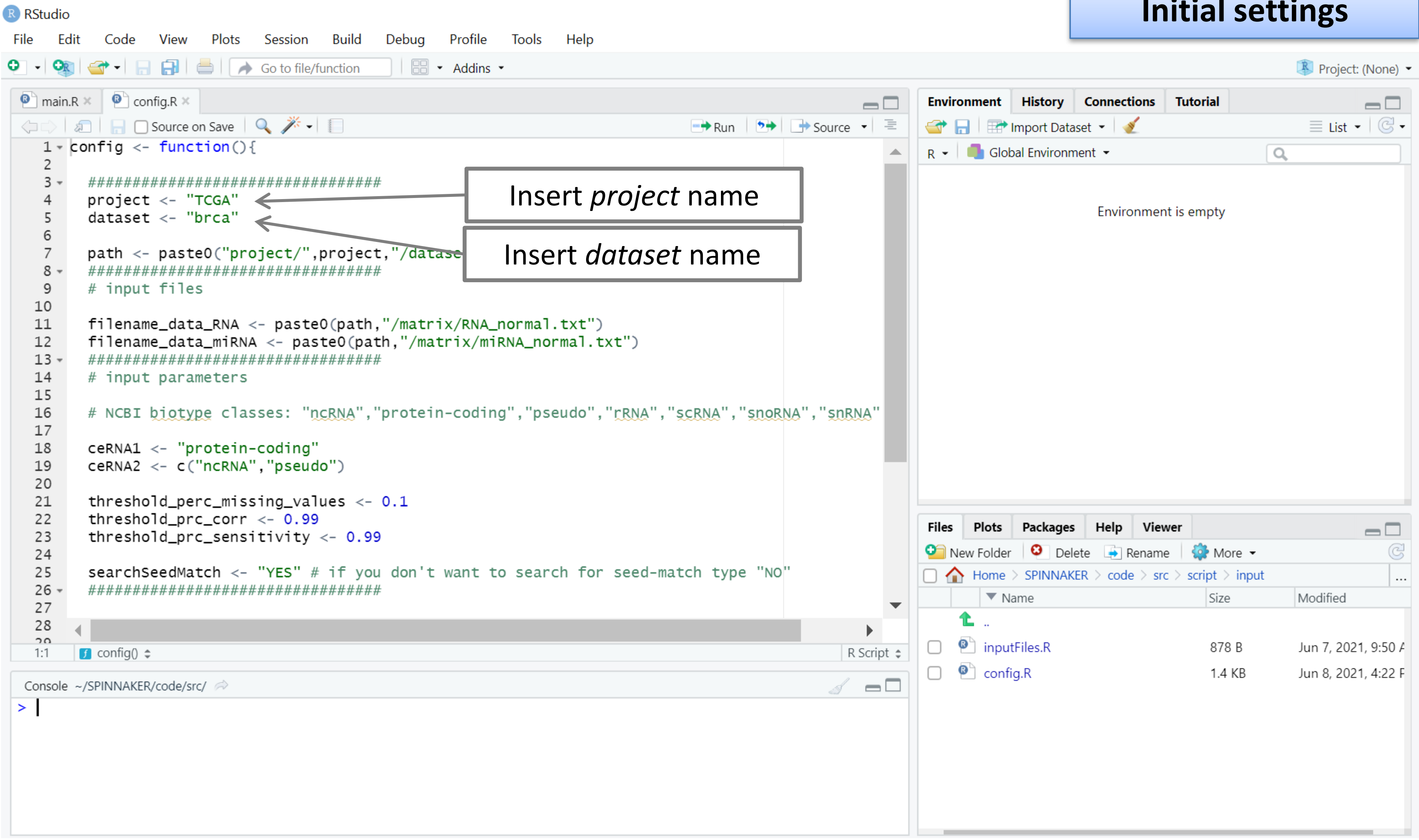
```
>
```

Environment is empty

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

Configuration file

Initial settings



RStudio
File
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Help
Go to file/function
Addins
Project: (None)
main.R
config.R
Source on Save
Run
Source
Environment
History
Connections
Tutorial
Import Dataset
List
R
Global Environment
Files
Plots
Packages
Help
Viewer
New Folder
Delete
Rename
More
Home
SPINNAKER
code
src
script
input
1:1
config()
R Script
Console ~/SPINNAKER/code/src/

```
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

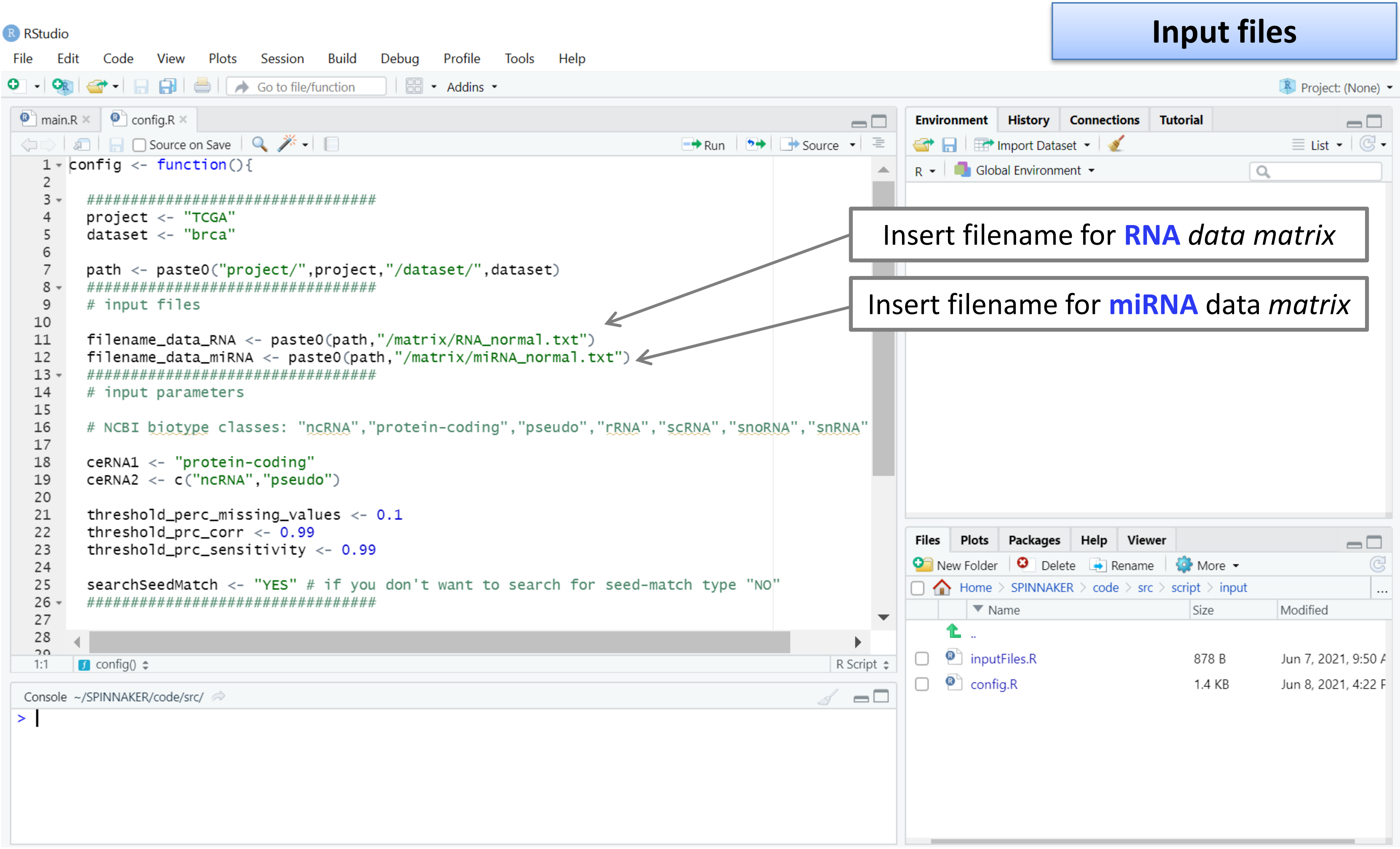
Environment is empty

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

```
>
```

Configuration file

Input files


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
Environment History Connections Tutorial
Import Dataset
List
R
Global Environment
1:1
config()
R Script
Console ~/SPINNAKER/code/src/
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
config.R 1.4 KB Jun 8, 2021, 4:22

```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"
  ##################################
```

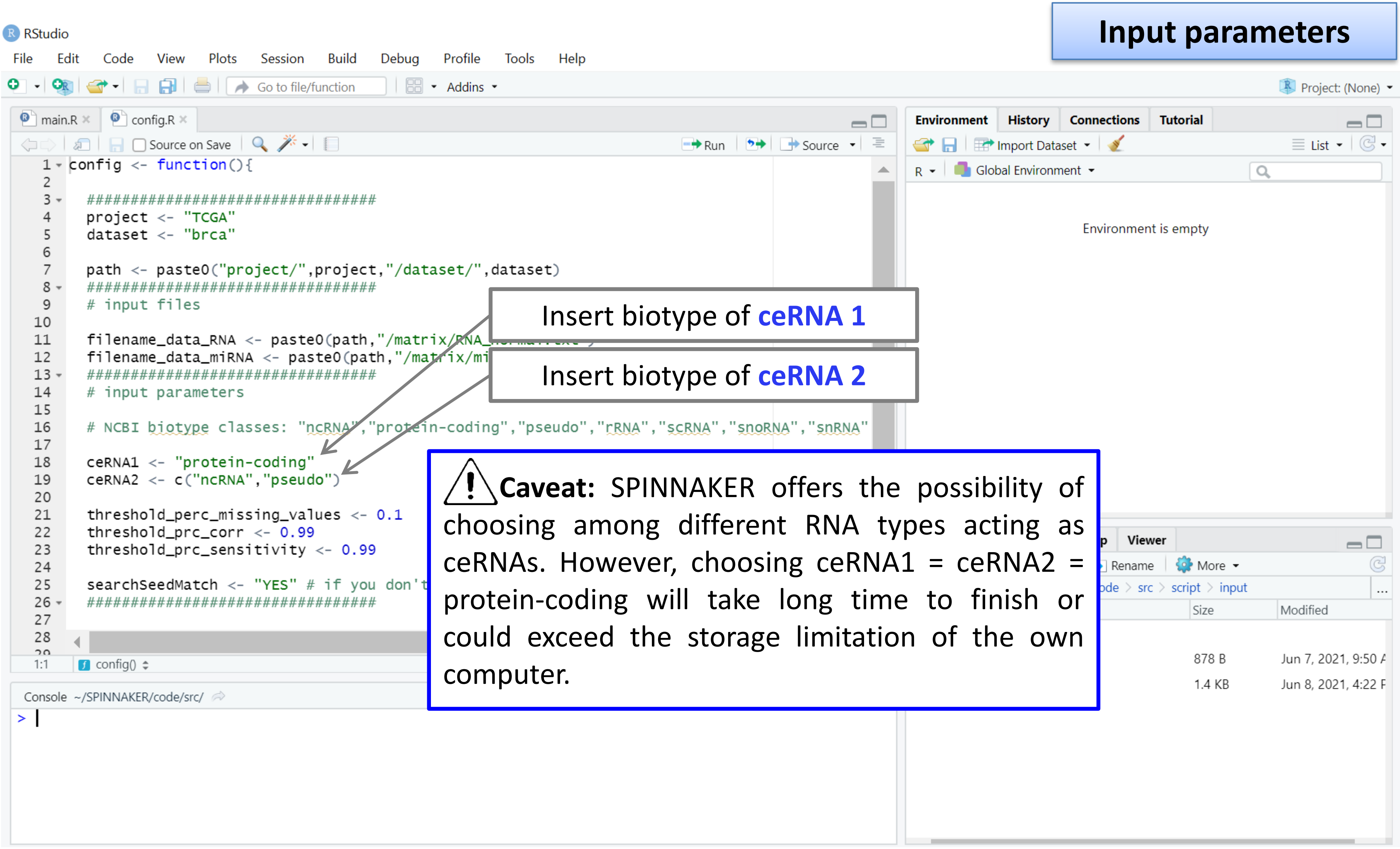
```
> 
```

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

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

Configuration file

Input parameters


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
Environment
History
Connections
Tutorial
Import Dataset
List
R
Global Environment
Environment is empty
Viewer
Rename
More
ode
src
script
input
Size
Modified
878 B
Jun 7, 2021, 9:50
1.4 KB
Jun 8, 2021, 4:22
1:1
config()
Console ~/SPINNAKER/code/src/

```r
config <- function(){

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

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

  filename_data_RNA <- paste0(path,"/matrix/RNA_
  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
  #################################
```

```
>
```

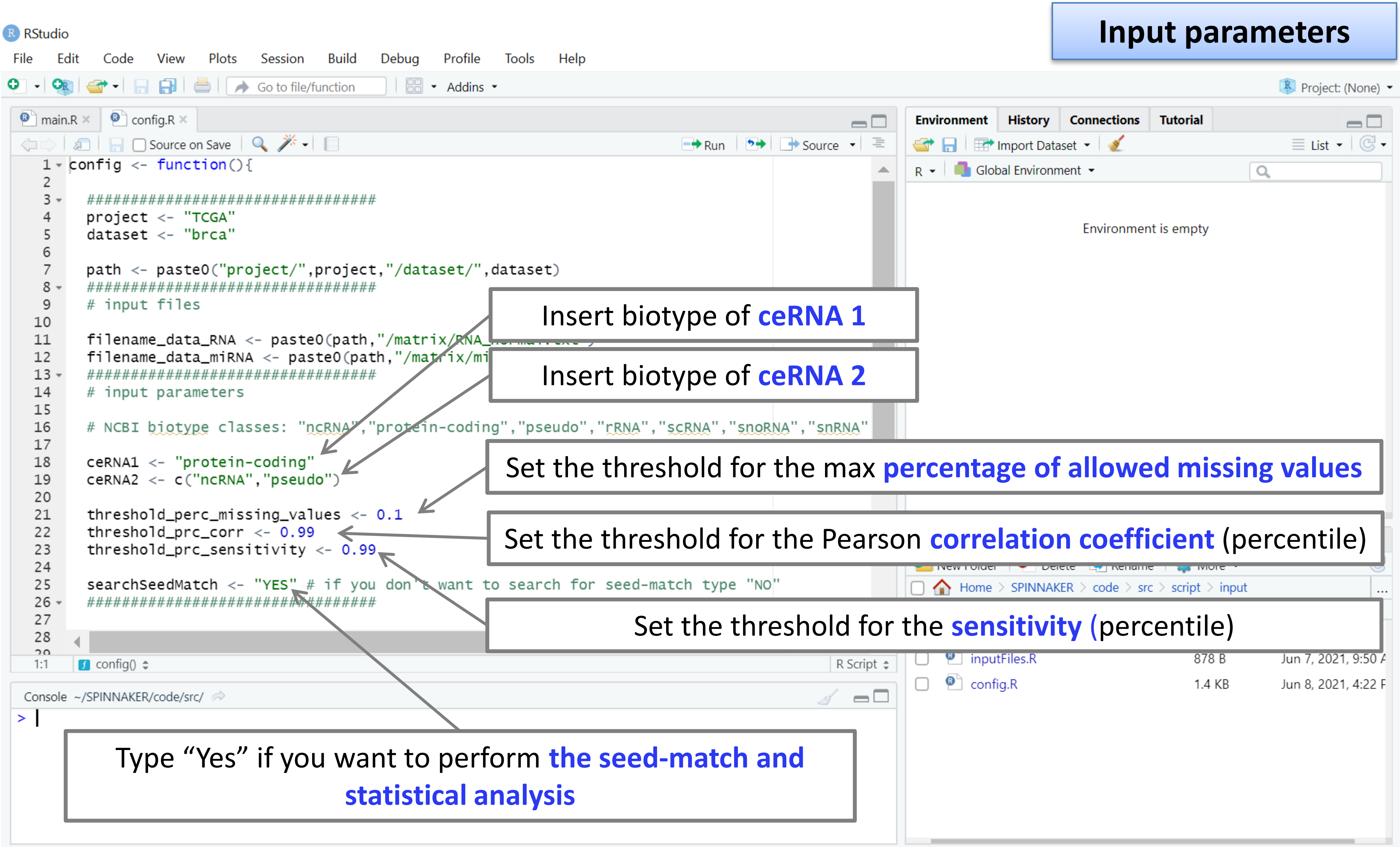
Insert biotype of **ceRNA 1**

Insert biotype of **ceRNA 2**

⚠ **Caveat:** SPINNAKER offers the possibility of choosing among different RNA types acting as ceRNAs. However, choosing ceRNA1 = ceRNA2 = protein-coding will take long time to finish or could exceed the storage limitation of the own computer.

Configuration file

Input parameters



```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/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"
  ##################################
```

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inputFiles.R 878 B Jun 7, 2021, 9:50
config.R 1.4 KB Jun 8, 2021, 4:22

Insert biotype of **ceRNA 1**

Insert biotype of **ceRNA 2**

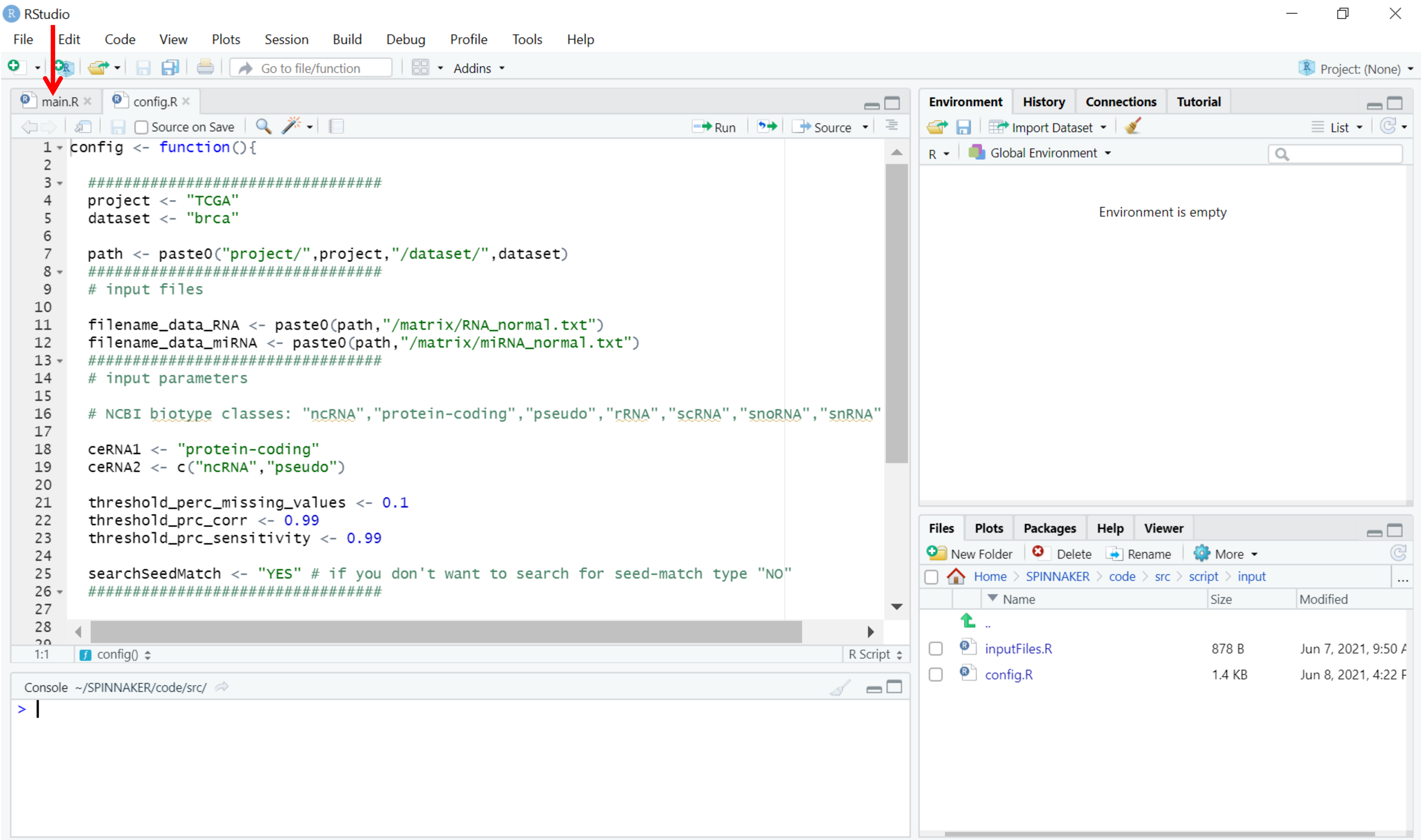
Set the threshold for the max **percentage of allowed missing values**

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

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

Type “Yes” if you want to perform **the seed-match and statistical analysis**

Configuration file



```
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 is empty

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

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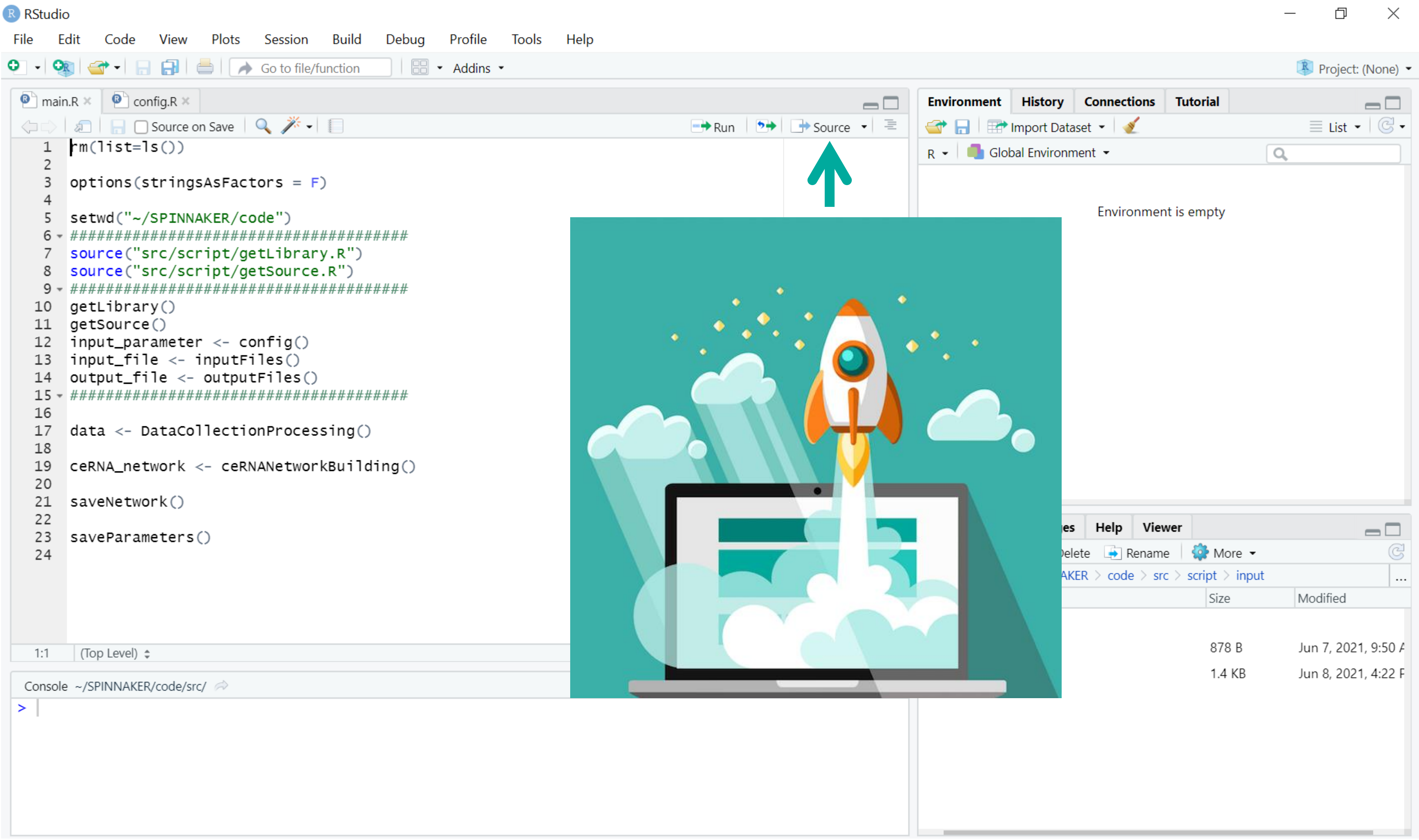
More

SPINNAKER launch!



```
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()
```

Environment is empty

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Project: (None)
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config.R
Source on Save
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