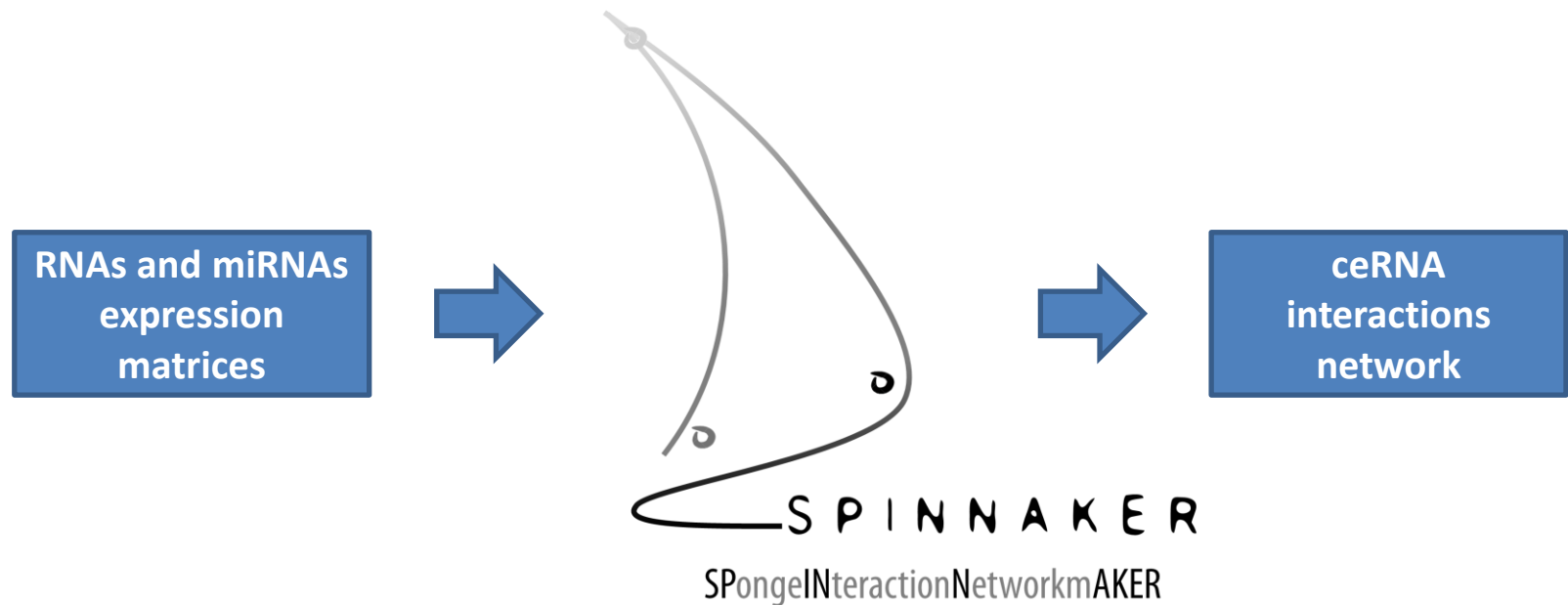
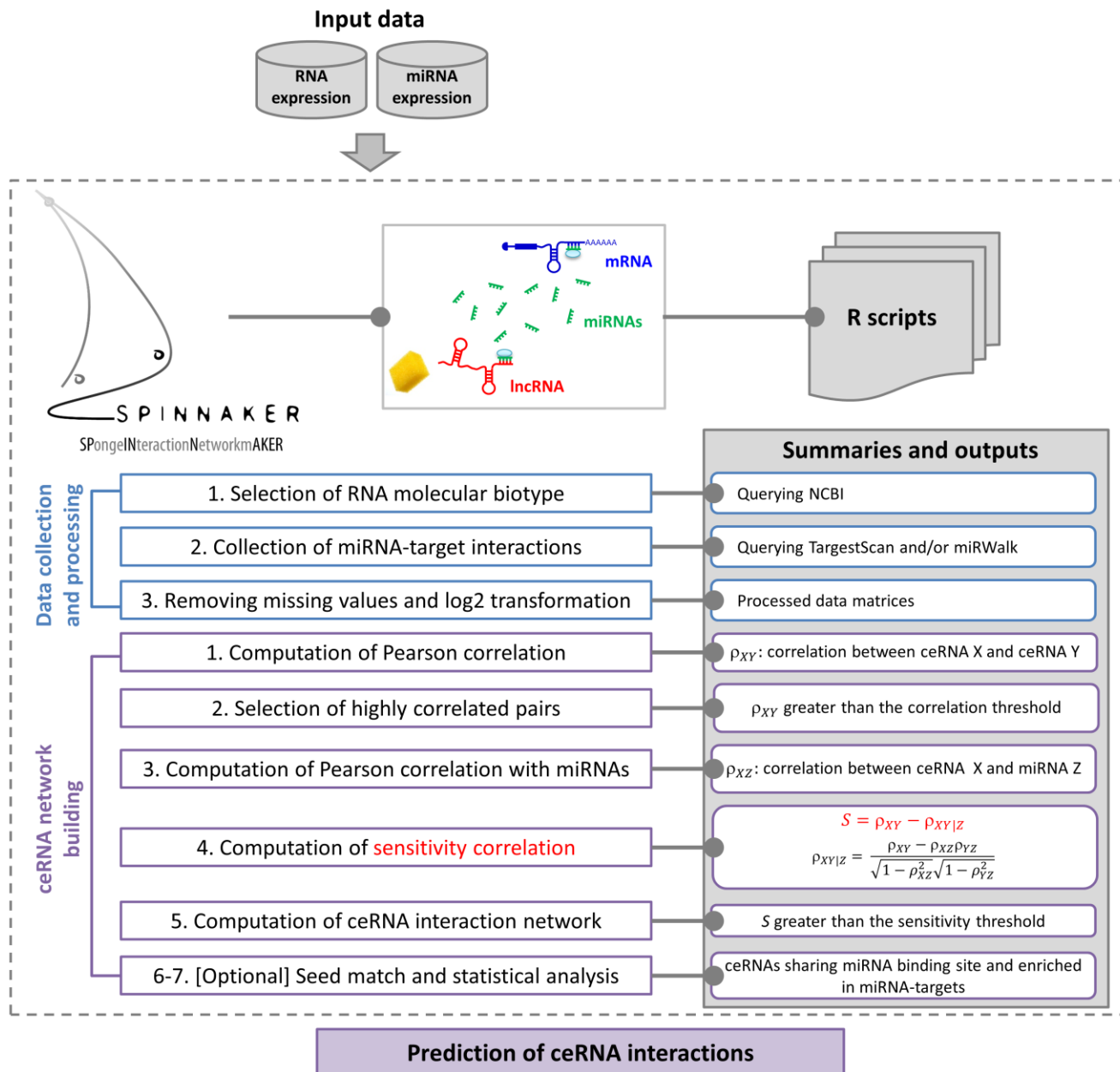



SPINNAKER (SPongeINteractionNetworkmAker) is the R-based implementation of a widely established mathematical model that we recently published for identifying putative competing endogenous RNA (ceRNA)*. Taking as input the expression levels of RNAs and miRNAs, SPINNAKER predicts the ceRNA interactions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







Module 0: Architecture



GETTING STARTED



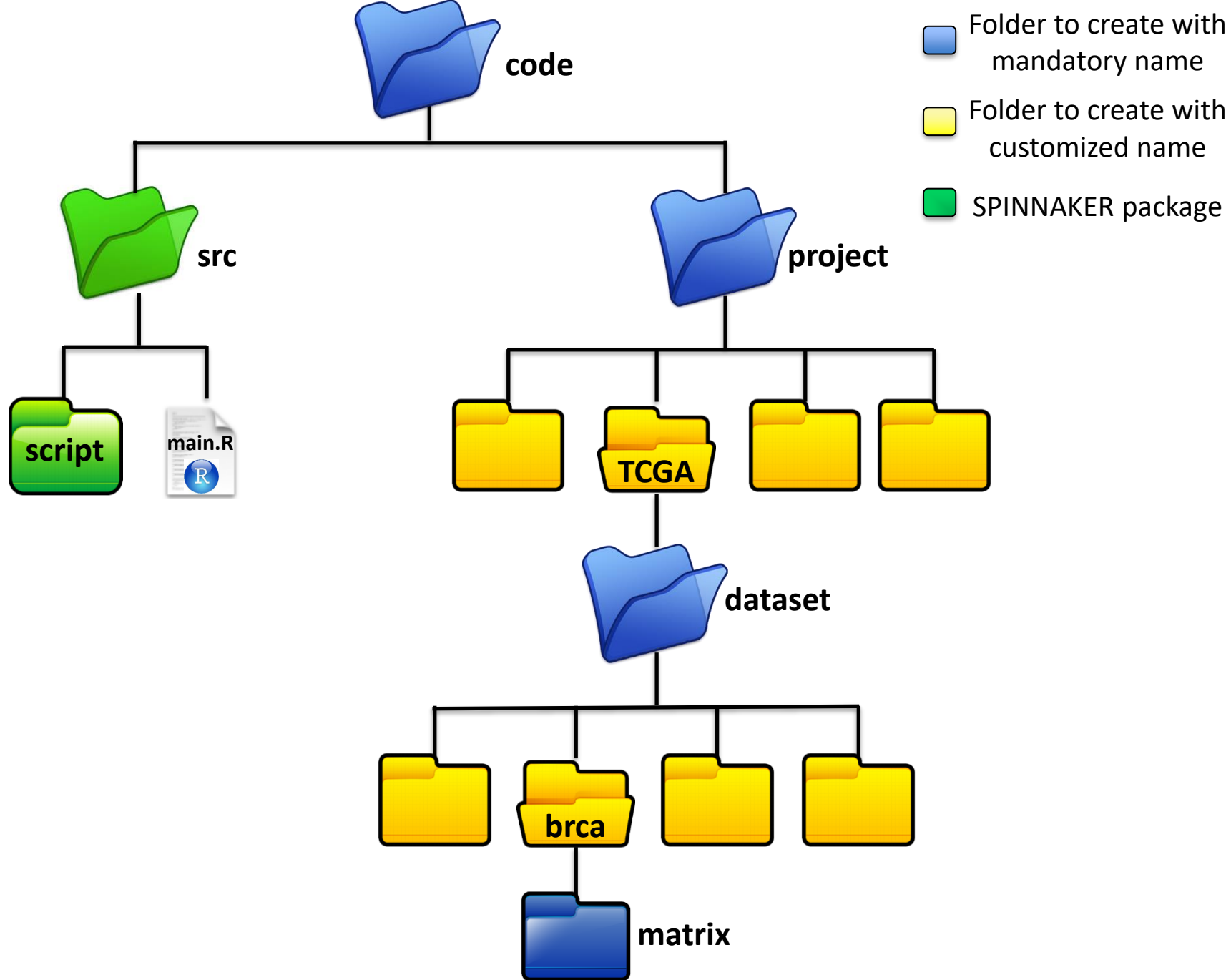
Software requirement

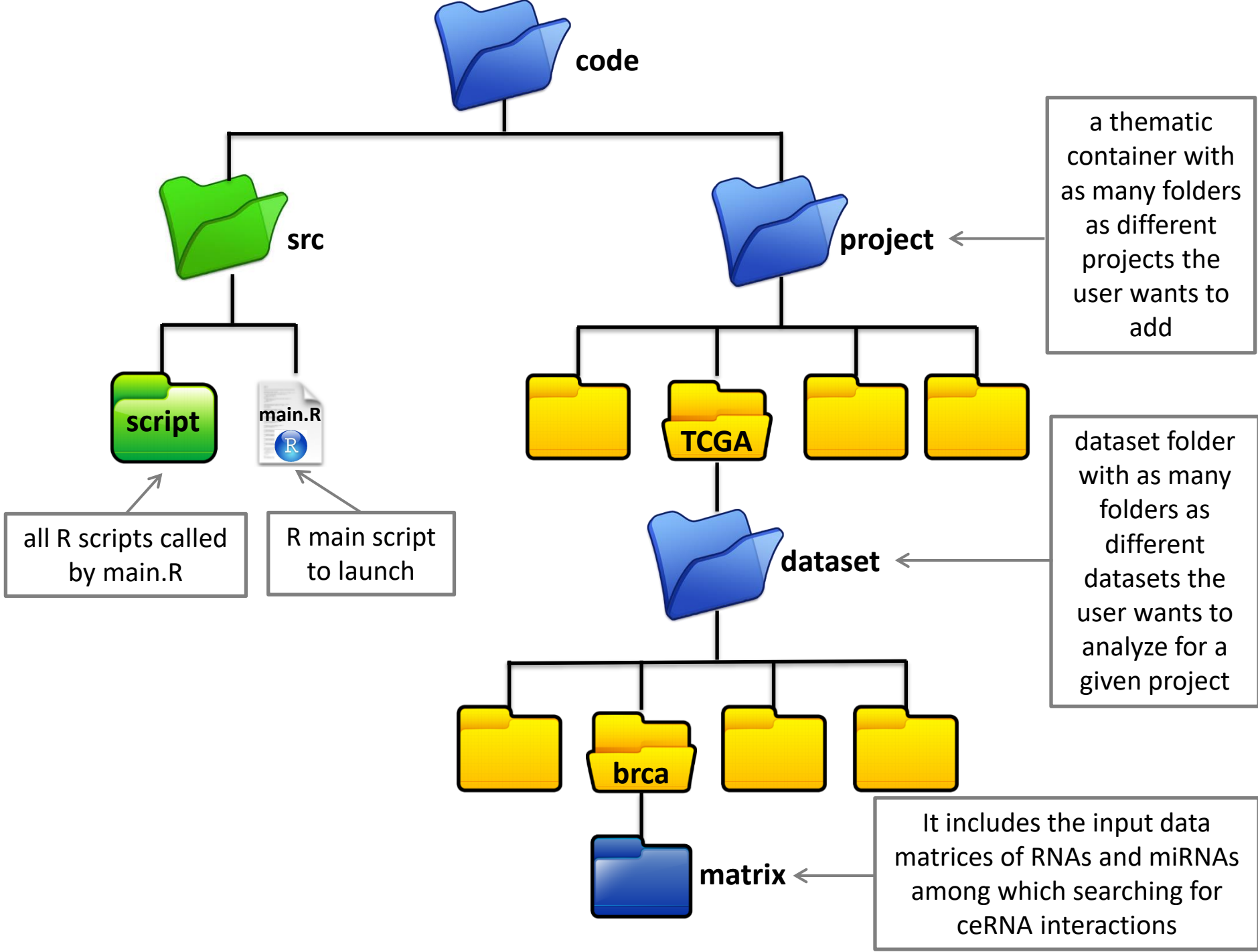
- SPINNAKER has been developed in R and tested on the following operative systems:
 - macOS High Sierra 10.13.6
 - Windows 10 Pro

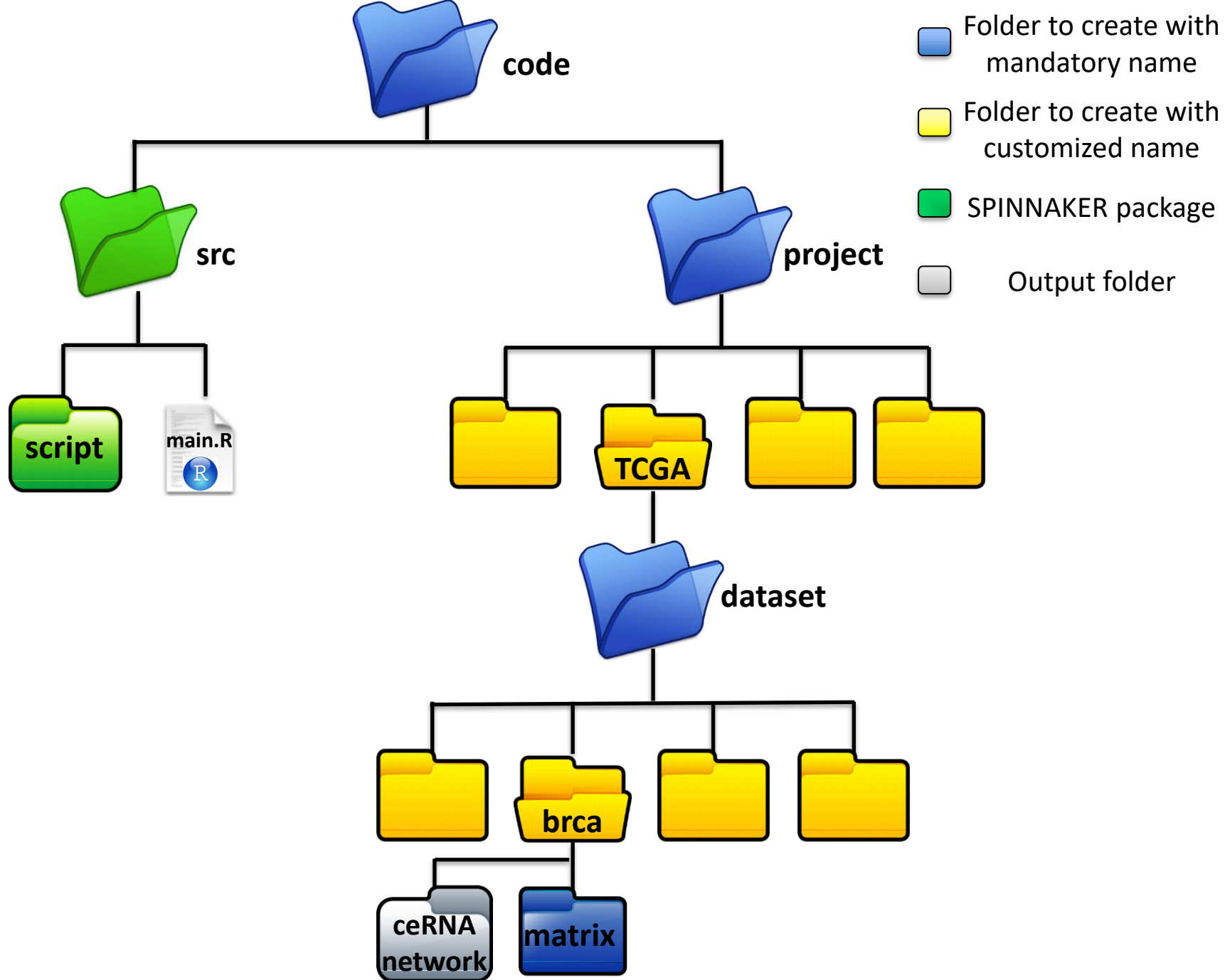
Setting up

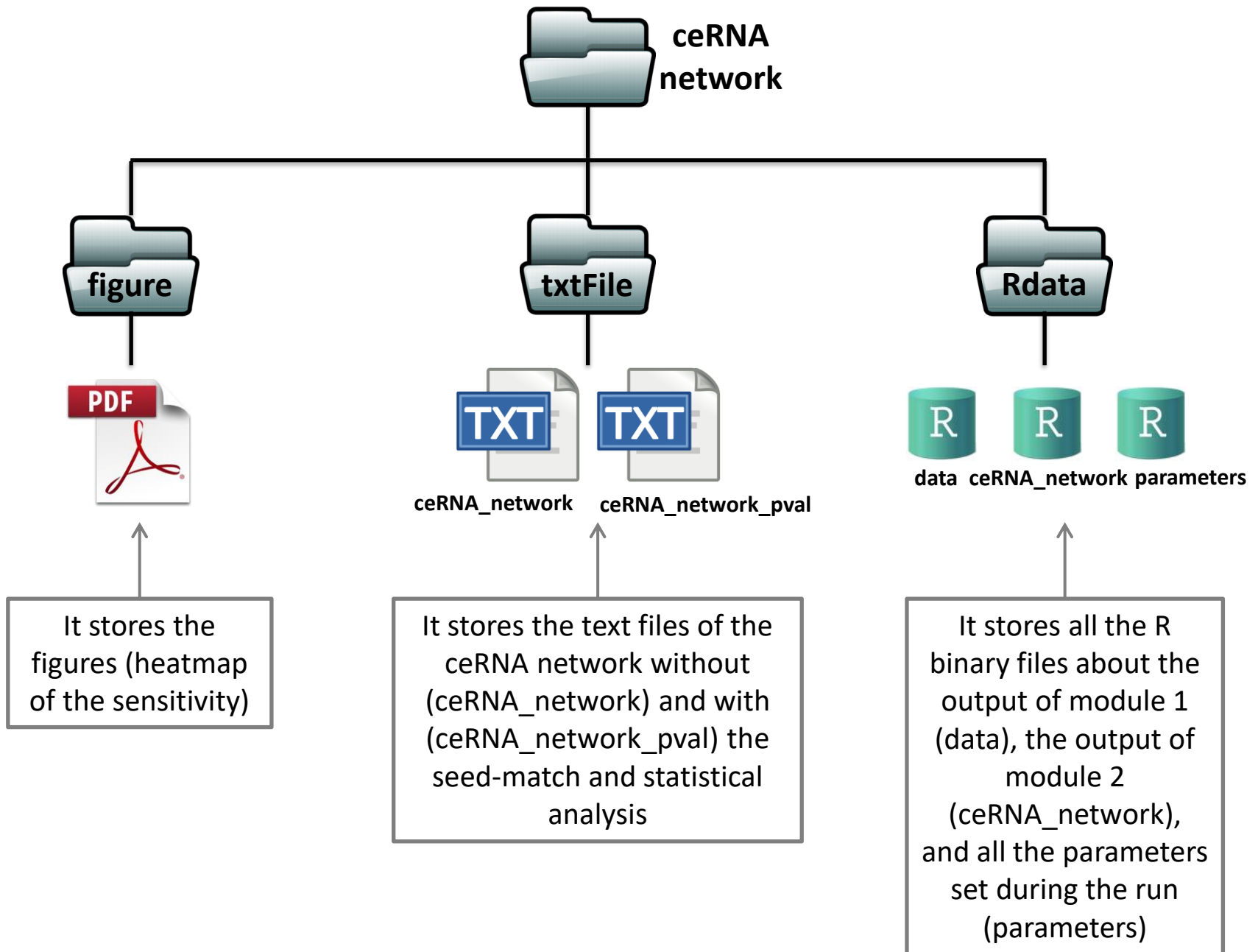
- Install R and R studio
- Download the SPINNAKER software package from

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



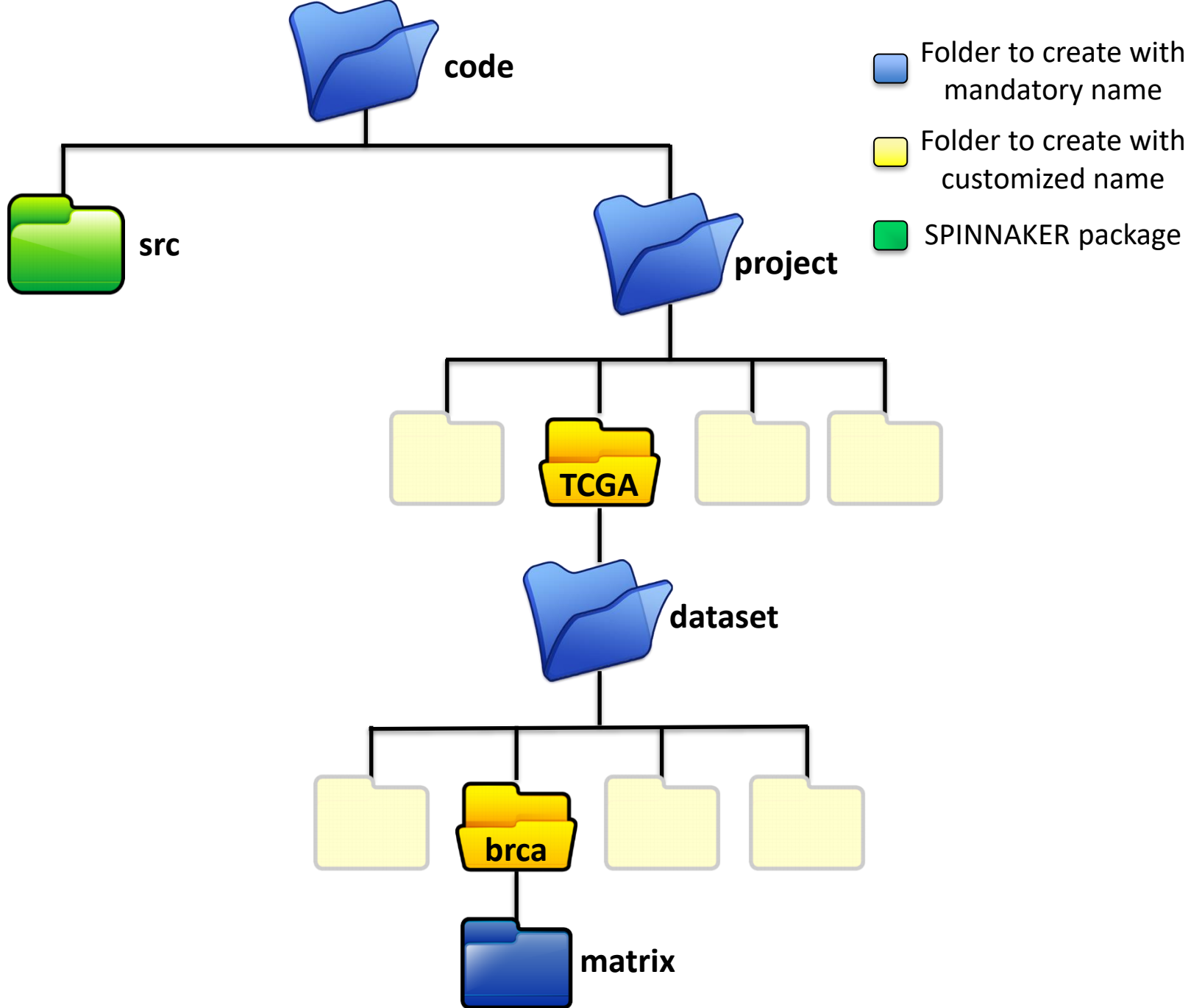


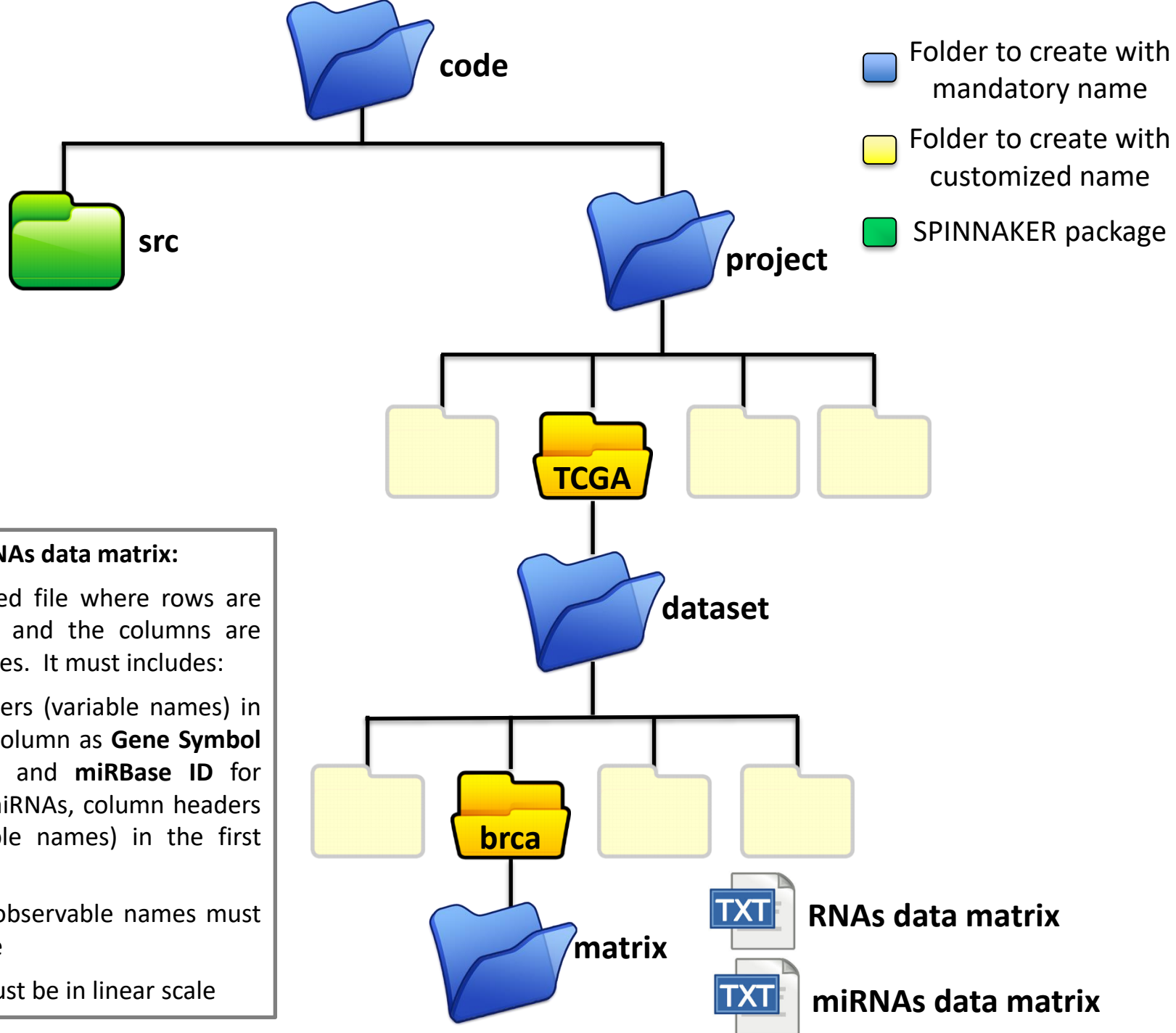




Let's get started







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																	
		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
Transcripts	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

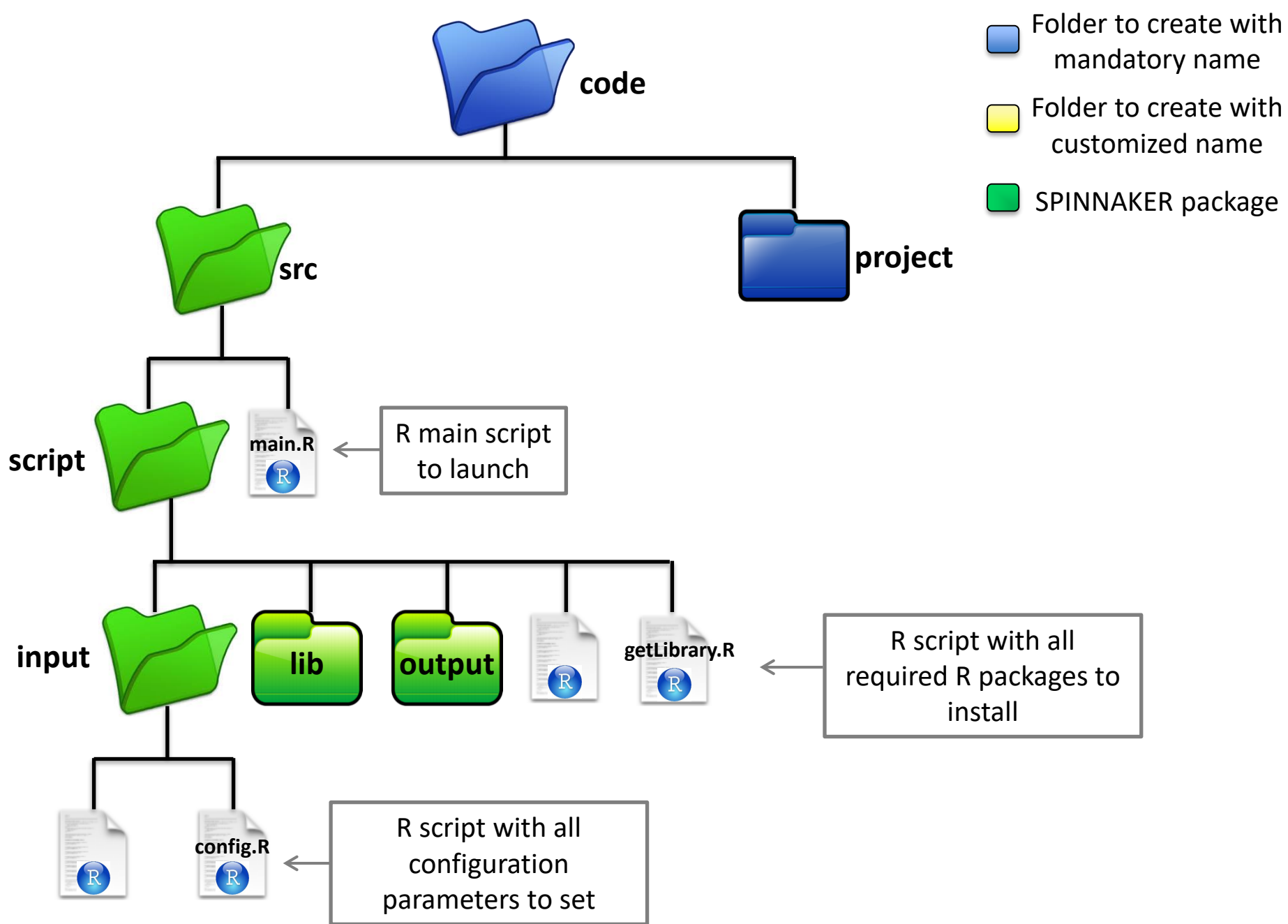
	TCGA-A7-A0CE-11A-21R-A089-07	TCGA-A7-A0D9-11A-53R-A089-07	TCGA-A7-A0D9-01A-31R-A056-07	TCGA-A7-A0DB-01A-11R-A00Z-07	TCGA-A7-A13E-11A-61R-A12P-07	TCGA-A7-A13E-01A-11R-A12P-07	TCGA-A7-A13G-11A-51R-A13Q-07	TCGA-AC-A23H-11A-12R-A157-07	TCGA-AC-A2FB-01A-11R-A17B-07	TCGA-AC-A2FM-11B-32R-A19W-07	...	TCGA-A7-A0CE-01A-11R-A00Z-07	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!





Main file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16
17 data <- DataCollectionProcessing()
18
19 ceRNA_network <- ceRNANetworkBuilding()
20
21 saveNetwork()
22
23 saveParameters()
24
```

main.R x

Source on Save Run Source

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, 12:21

Console ~/SPINNAKER/code/src/

>

Module 1

Module 2

Main file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16 data <- DataCollectionProcessing()
17 ceRNA_network <- ceRNANetworkBuilding()
18 saveNetwork()
19 saveParameters()
20
21
22
23
24
```

Run Source

Environment History Connections Tutorial

Import Dataset

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, 12:21

Console ~/SPINNAKER/code/src/

>

Module 1

Module 2

Main file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16
17 data <- DataCollectionProcessing()
18
19 ceRNA_network <- ceRNANetworkBuilding()
20
21 saveNetwork()
22
23 saveParameters()
24
```

Environment History Connections Tutorial

Import Dataset

R Global Environment

Environment is empty

Files Plots Packages Help Viewer

New Folder Delete Rename More

Home > SPINNAKER > code > src

	Name	Size	Modified
	..		
	script	498 B	Jun 11, 2021, 12:21
	main.R		

Module 1

Module 2

Console ~/SPINNAKER/code/src/

>

Main file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16 data <- DataCollectionProcessing()
17
18 ceRNA_network <- ceRNANetworkBuilding()
19
20 saveNetwork()
21
22 saveParameters()
23
24
```

Environment History Connections Tutorial

Import Dataset

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

Console ~/SPINNAKER/code/src/

>

Module 1

Module 2

Main file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16
17 data <- DataCollectionProcessing()
18
19 ceRNA_network <- ceRNANetworkBuilding()
20
21 saveNetwork()
22
23 saveParameters()
24
```

1:1 (Top Level) R Script

Console ~/SPINNAKER/code/src/

Environment History Connections Tutorial

Import Dataset

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 F

Configuration file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 config <- function(){
2
3   #####
4   project <- "TCGA"
5   dataset <- "brca"
6
7   path <- paste0("project/",project,"/dataset/",dataset)
8   #####
9   # input files
10
11   filename_data_RNA <- paste0(path,"/matrix/RNA_normal.txt")
12   filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal.txt")
13   #####
14   # input parameters
15
16   # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18   ceRNA1 <- "protein-coding"
19   ceRNA2 <- c("ncRNA","pseudo")
20
21   threshold_perc_missing_values <- 0.1
22   threshold_prc_corr <- 0.99
23   threshold_prc_sensitivity <- 0.99
24
25   searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
26   #####
27
28 }
```

Environment History Connections Tutorial

Import Dataset

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 F

Console ~/SPINNAKER/code/src/

> |

Configuration file



Initial settings

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 config <- function(){
2
3   #####
4   project <- "TCGA"
5   dataset <- "brca"
6
7   path <- paste0("project/",project,"/dataset/")
8   #####
9   # input files
10
11   filename_data_RNA <- paste0(path,"/matrix/RNA_normal1.txt")
12   filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal1.txt")
13   #####
14   # input parameters
15
16   # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18   ceRNA1 <- "protein-coding"
19   ceRNA2 <- c("ncRNA","pseudo")
20
21   threshold_perc_missing_values <- 0.1
22   threshold_prc_corr <- 0.99
23   threshold_prc_sensitivity <- 0.99
24
25   searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
26   #####
27
28 }
```

Insert *project* name

Insert *dataset* name

Project: (None)

Environment History Connections Tutorial

Import Dataset

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 F

Console ~/SPINNAKER/code/src/

> |

Configuration file



Input files

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

main.R x config.R x

```
1 config <- function(){
2
3 #####
4 project <- "TCGA"
5 dataset <- "brca"
6
7 path <- paste0("project/",project,"/dataset/",dataset)
8 #####
9 # input files
10
11 filename_data_RNA <- paste0(path,"/matrix/RNA_normal1.txt")
12 filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal1.txt")
13 #####
14 # input parameters
15
16 # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18 ceRNA1 <- "protein-coding"
19 ceRNA2 <- c("ncRNA","pseudo")
20
21 threshold_perc_missing_values <- 0.1
22 threshold_prc_corr <- 0.99
23 threshold_prc_sensitivity <- 0.99
24
25 searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
26 #####
27
28
29
30 }
```

Environment History Connections Tutorial

Import Dataset

R Global Environment

Insert filename for **RNA** data matrix

Insert filename for **miRNA** data matrix

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 F

Console ~/SPINNAKER/code/src/

> |

Configuration file



Input parameters

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function

Addins

main.R x config.R x

Source on Save

Run Source

Environment History Connections Tutorial

Import Dataset

R Global Environment

Environment is empty

```
1 config <- function(){
2
3 #####
4 project <- "TCGA"
5 dataset <- "brca"
6
7 path <- paste0("project/",project,"/dataset/",dataset)
8 #####
9 # input files
10
11 filename_data_RNA <- paste0(path,"/matrix/RNA-seq-matrix/")
12 filename_data_miRNA <- paste0(path,"/matrix/miRNA-seq-matrix/")
13 #####
14 # input parameters
15
16 # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18 ceRNA1 <- "protein-coding"
19 ceRNA2 <- c("ncRNA","pseudo")
20
21 threshold_perc_missing_values <- 0.1
22 threshold_prc_corr <- 0.99
23 threshold_prc_sensitivity <- 0.99
24
25 searchSeedMatch <- "YES" # if you don't
26 #####
27
28
29
30
31 }
```

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.

Viewer

Rename More

code > src > script > input

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

Console ~/SPINNAKER/code/src/

> |

Configuration file



Input parameters

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

```
1 config <- function(){
2
3 #####
4 project <- "TCGA"
5 dataset <- "brca"
6
7 path <- paste0("project/",project,"/dataset/",dataset)
8 #####
9 # input files
10
11 filename_data_RNA <- paste0(path,"/matrix/RNA-seq/matrix.RNA-seq")
12 filename_data_miRNA <- paste0(path,"/matrix/miRNA-seq/matrix.miRNA-seq")
13 #####
14 # input parameters
15
16 # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18 ceRNA1 <- "protein-coding"
19 ceRNA2 <- c("ncRNA","pseudo")
20
21 threshold_perc_missing_values <- 0.1
22 threshold_prc_corr <- 0.99
23 threshold_prc_sensitivity <- 0.99
24
25 searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
26 #####
27
28
29 }
```

Environment History Connections Tutorial

Import Dataset

R Global Environment

Environment is empty

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**

Console ~/SPINNAKER/code/src/

> |

inputFiles.R 878 B Jun 7, 2021, 9:50 A

config.R 1.4 KB Jun 8, 2021, 4:22 F

Configuration file



RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

main.R x config.R x

```
1 config <- function(){
2
3 #####
4 project <- "TCGA"
5 dataset <- "brca"
6
7 path <- paste0("project/",project,"/dataset/",dataset)
8 #####
9 # input files
10
11 filename_data_RNA <- paste0(path,"/matrix/RNA_normal1.txt")
12 filename_data_miRNA <- paste0(path,"/matrix/miRNA_normal1.txt")
13 #####
14 # input parameters
15
16 # NCBI biotype classes: "ncRNA","protein-coding","pseudo","rRNA","scrRNA","snoRNA","snRNA"
17
18 ceRNA1 <- "protein-coding"
19 ceRNA2 <- c("ncRNA","pseudo")
20
21 threshold_perc_missing_values <- 0.1
22 threshold_prc_corr <- 0.99
23 threshold_prc_sensitivity <- 0.99
24
25 searchSeedMatch <- "YES" # if you don't want to search for seed-match type "NO"
26 #####
27
28
29
30 }
```

Environment History Connections Tutorial

Import Dataset

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 F

Console ~/SPINNAKER/code/src/

```
> |
```

SPINNAKER launch!



RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Go to file/function Addins

main.R x config.R x

Source on Save Run Source

```
1 rm(list=ls())
2
3 options(stringsAsFactors = F)
4
5 setwd("~/SPINNAKER/code")
6 #####
7 source("src/script/getLibrary.R")
8 source("src/script/getSource.R")
9 #####
10 getLibrary()
11 getSource()
12 input_parameter <- config()
13 input_file <- inputFiles()
14 output_file <- outputFiles()
15 #####
16
17 data <- DataCollectionProcessing()
18
19 ceRNA_network <- ceRNANetworkBuilding()
20
21 saveNetwork()
22
23 saveParameters()
24
```

Environment History Connections Tutorial

Import Dataset

R Global Environment

Environment is empty

es Help Viewer

Delete Rename More

SPINNAKER > code > src > script > input

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

Console ~/SPINNAKER/code/src/

>

