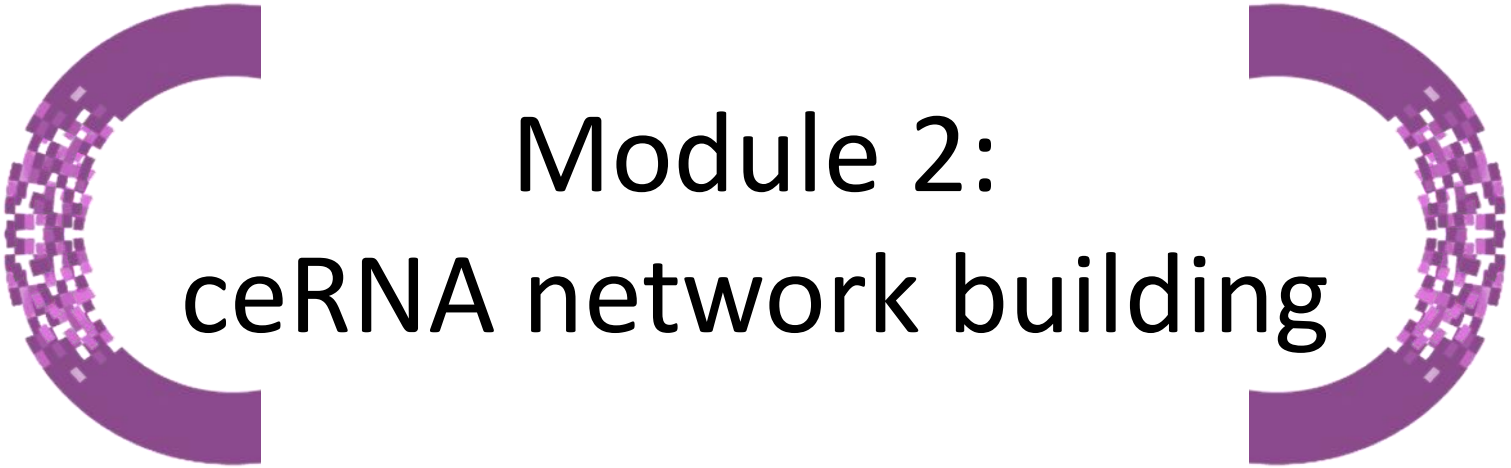
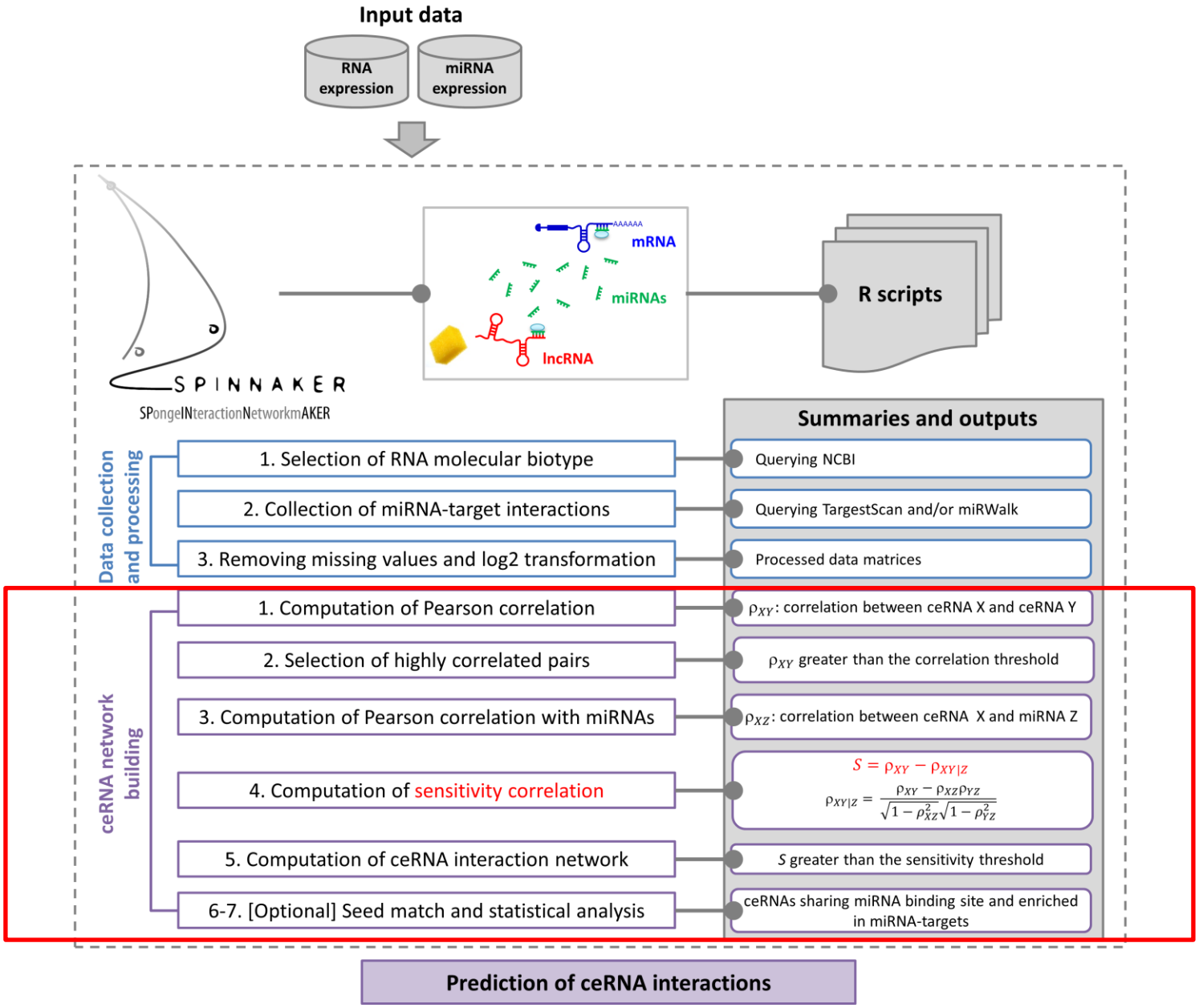


Module 2:
ceRNA network building


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

ceRNA network building

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

...

- The goal of this module is to build the **ceRNA interactions network**
- This module is composed of seven steps:
  i. Computation of Pearson correlation
  ii. Selection of highly correlated pairs
  iii. Computation of Pearson correlation with miRNAs
  iv. Computation of sensitivity correlation
  v. Computation of ceRNA interaction network
  vi. [Optional] Search seed-match for all triplets
  vii. [Optional] Compute statistical analysis

ceRNA network building

...

```
  ######################################
  # STEP 4

  print("STEP 4: compute sensitivity correlation")

  triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
  ######################################
  # STEP 5

  print("STEP 5: compute ceRNA interaction network")

  threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

  ceRNA <- selectTriplets(triplets,threshold_sensitivity)

  res <- list(ceRNA = ceRNA,
              threshold_corr = threshold_corr,
              threshold_sensitivity = threshold_sensitivity)
  ######################################

  if(searchSeedMatch == "YES"){

    ######################################
    # STEP 6

    print("STEP 6: search seed-match for all triplets")

    triplets <- searchSeedMatch(triplets,miRNAtarget)
    ######################################
    # STEP 7

    print("STEP 7: compute statistical analysis")

    ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

    res_stat <- list(ceRNA_pval = ceRNA_pval)
    ######################################

    res <- c(res,res_stat)

  }

  return(res)
}
```

- The goal of this module is to build the **ceRNA interactions network**
- This module is composed of seven steps:
  i. Computation of Pearson correlation
  ii. Selection of highly correlated pairs
  iii. Computation of Pearson correlation with miRNAs
  iv. Computation of sensitivity correlation
  v. Computation of ceRNA interaction network
  vi. [Optional] Search seed-match for all triplets
  vii. [Optional] Compute statistical analysis

Pearson correlation bewteen RNA pairs

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

Step i: the Pearson correlation coefficients are computed

- The existence of a linear relationship between two normally distributed continuous variables (e.g., gene expression values of RNA X and RNA Y) can be expressed by the **Pearson correlation coefficient ρ**

$$\rho_{xy} = \frac{\sum_{i=1}^{col}\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\sqrt{\sum_{i=1}^{col}\left(x_i - \bar{x}\right)^2 \sum_{i=1}^{col}\left(y_i - \bar{y}\right)^2}}$$

- ρ coefficient varies between -1 and 1 and shows strength (value) and direction (sign) of correlation

Pearson correlation bewteen RNA pairs

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

Step i: the Pearson correlation coefficients are computed

- SPINNAKER computes the Pearson correlation between the expression profiles of RNA pairs (ρ_{XY})

```
computeCorrelation <- function(data1,data2){

  dim_min <- min(nrow(data1),nrow(data2),2000)

  size <- round_any(dim_min, 100, f = floor)

  rho <- bigcor(t(data1), t(data2), size = size, fun = "cor",
                verbose = F, use = "pairwise.complete.obs")

  rho <- rho[1:nrow(data1), 1:nrow(data2)]
  rownames(rho) <- rownames(data1)
  colnames(rho) <- rownames(data2)

  return(rho)
}
```

Select Pairs

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

Step ii: the highly correlated pairs are selected

- SPINNAKER selects the pairs with ρ_{XY} greater than a defined threshold (by default equal to the 99th percentile)

```
selectPairs <- function(rho,thr){

  ind <- which(rho >= thr, arr.ind = T)

  if( nrow(ind) > 0 ){

    pairs <- data.frame(ceRNA1 = rownames(rho)[ind[,1]],
                        ceRNA2 = colnames(rho)[ind[,2]],
                        correlation = rho[ind])

  }else{

    stop("No pairs with the selected correlation threshold")

  }

  return(pairs)
}
```

Select Pairs

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

Step ii: the highly correlated pairs are selected

- SPINNAKER selects the pairs with ρ_{XY} greater than a defined threshold (by default equal to the 99th percentile)

```
selectPairs <- function(rho,thr){

  ind <- which(rho >= thr, arr.ind = T)

  if( nrow(ind) > 0 ){

    pairs <- data.frame(ceRNA1 = rownames(rho)[ind[,1]],
                        ceRNA2 = colnames(rho)[ind[,2]],
                        correlation = rho[ind])

  }else{

    stop("No pairs with the selected correlation threshold")

  }

  return(pairs)
```

⚠ **Caveat:** if the chosen threshold does not allow to select any pairs an error message appears, SPINNAKER stops running, and you have to change the input parameter.

Pearson correlation with miRNAs

```
ceRNANetworkBuilding <- function(){

  ######################################
  # input parameters

  ceRNA1 <- input_parameter$ceRNA1
  ceRNA2 <- input_parameter$ceRNA2

  data_ceRNA1 <- data$data_ceRNA1
  data_ceRNA2 <- data$data_ceRNA2
  data_miRNA <- data$data_miRNA

  threshold_prc_corr <- input_parameter$threshold_prc_corr
  threshold_prc_sensitivity <- input_parameter$threshold_prc_sensitivity

  searchSeedMatch <- input_parameter$searchSeedMatch

  if(searchSeedMatch == "YES") {
    miRNAtarget <- data$miRNAtarget
  }

  filename_heatmap <- output_file$filename_heatmap
  ######################################
  # STEP 1

  print("STEP 1: compute Pearson correlation")

  rho_ceRNA1_ceRNA2 <- computeCorrelation(data_ceRNA1,data_ceRNA2)

  if(all(ceRNA1 == ceRNA2)){
    rho_ceRNA1_ceRNA2[upper.tri(rho_ceRNA1_ceRNA2,diag = T)] <- NA
  }
  ######################################
  # STEP 2

  print("STEP 2: select highly correlated pairs")

  threshold_corr <- as.numeric(quantile(c(rho_ceRNA1_ceRNA2),threshold_prc_corr,na.rm = T))

  pairs <- selectPairs(rho_ceRNA1_ceRNA2,threshold_corr)

  data_ceRNA1 <- data_ceRNA1[unique(pairs$ceRNA1),]
  data_ceRNA2 <- data_ceRNA2[unique(pairs$ceRNA2),]
  ######################################
  # STEP 3

  print("STEP 3: compute Pearson correlation with miRNAs")

  rho_ceRNA1_miRNA <- computeCorrelation(data_ceRNA1,data_miRNA)
  rho_ceRNA2_miRNA <- computeCorrelation(data_ceRNA2,data_miRNA)
  ######################################
```

Step iii: the Pearson correlation coefficients are computed

- SPINNAKER computes the Pearson correlation between the expression profiles of the RNA X and miRNA Z (ρ_{XZ}) and of the RNA Y and miRNA Z (ρ_{YZ})

```
computeCorrelation <- function(data1,data2){

  dim_min <- min(nrow(data1),nrow(data2),2000)

  size <- round_any(dim_min, 100, f = floor)

  rho <- bigcor(t(data1), t(data2), size = size, fun = "cor",
                verbose = F, use = "pairwise.complete.obs")

  rho <- rho[1:nrow(data1), 1:nrow(data2)]
  rownames(rho) <- rownames(data1)
  colnames(rho) <- rownames(data2)

  return(rho)
}
```

Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed

- SPINNAKER computes the **sensitivity correlation** as:

$$S = \rho_{XY} - \rho_{XY|Z}$$

where ρ_{XY} is the Pearson and $\rho_{XY|Z}$ the **partial correlation** between RNA X and RNA Y controlling for the miRNA Z defined as:

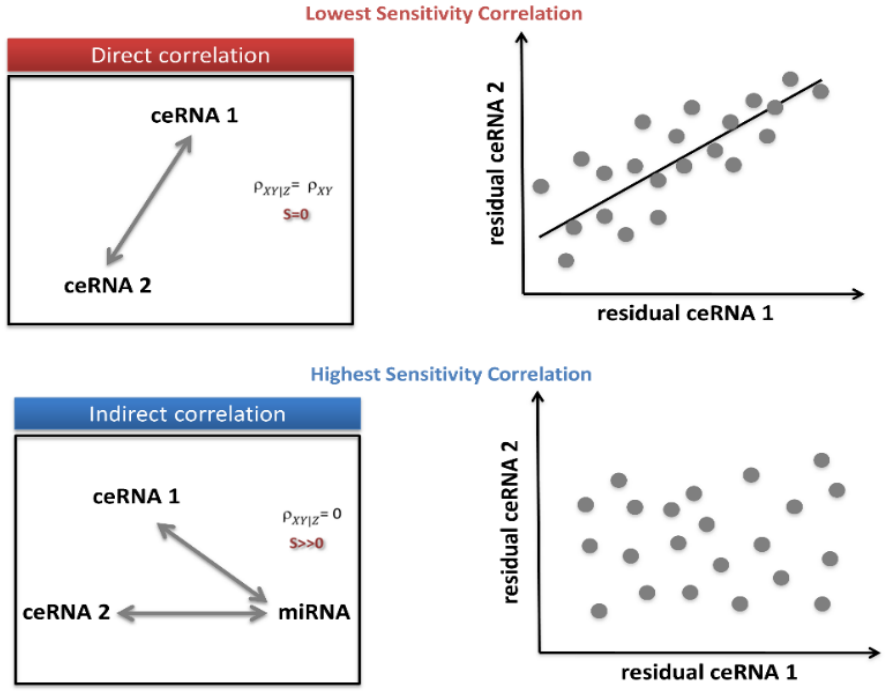
$$\rho_{XY|Z} = \frac{\rho_{XY} - \rho_{XZ}\rho_{YZ}}{\sqrt{1-\rho_{XZ}^2}\sqrt{1-\rho_{YZ}^2}}$$

Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed



Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed

```
computeSensitivity <- function(pairs_xy,rho_xz,rho_yz,filename_heatmap){

  z <- colnames(rho_xz)

  list <- apply(pairs_xy,1,function(pair){

    x <- pair[1]
    y <- pair[2]
    rxy <- as.numeric(pair[3])

    rxz <- rho_xz[x,]
    ryz <- rho_yz[y,]

    pc <- ( rxy - (rxz * ryz) ) / ( sqrt(1 - rxz^2) * sqrt(1 - ryz^2) )

    s <- rxy - pc

    df <- data.frame(ceRNA1 = x, ceRNA2 = y, miRNA = z,
                     correlation = rxy, partial_correlation = pc,
                     sensitivity = s, row.names = NULL)

  })

  triplets <- rbindlist(list)

  makeHeatmap(z,list,filename_heatmap)

  return(triplets)
}
```

Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed

```
computeSensitivity <- function(pairs_xy,rho_xz,rho_yz,filename_heatmap){

  z <- colnames(rho_xz)

  list <- apply(pairs_xy,1,function(pair){

    x <- pair[1]
    y <- pair[2]
    rxy <- as.numeric(pair[3])

    rxz <- rho_xz[x,]
    ryz <- rho_yz[y,]

    pc <- ( rxy - (rxz * ryz) ) / ( sqrt(1 - rxz^2) * sqrt(1 - ryz^2) )

    s <- rxy - pc

    df <- data.frame(ceRNA1 = x, ceRNA2 = y, miRNA = z,
                     correlation = rxy, partial_correlation = pc,
                     sensitivity = s, row.names = NULL)

  })

  triplets <- rbindlist(list)

  makeHeatmap(z,list,filename_heatmap)

  return(triplets)
}
```

Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed

- SPINNAKER computes the **heatmap** representing the sensitivity correlation S, computed for the top-correlated ceRNA pairs

```
makeHeatmap <- function(mir,pairs,filename_heatmap){

  size <- 5000

  n_pairs <- length(pairs)

  n_group <- floor(n_pairs/size)

  for(i in 1:n_group){

    start <- 1 + size * (i-1)
    end <- size * i

    mat <- sapply(pairs[start:end], function(x){ x$sensitivity })

    mat[mat<0] <- 0
    mat <- t(mat)
    colnames(mat) <- mir
    mat <-mat[,sort(colnames(mat))]

    filename_heatmap_i <- paste0(filename_heatmap,"_",i,".pdf")

    plotHeatmap(mat,filename_heatmap_i)

  }
}
```

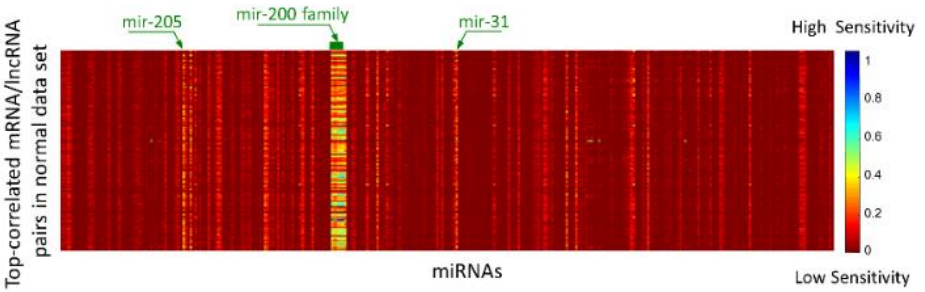
Sensitivity correlation

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step iv: the sensitivity correlation is computed

- SPINNAKER computes the **heatmap** representing the sensitivity correlation S, computed for the top-correlated ceRNA pairs



Select Triplets

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step v: the highly S triplets are selected

- SPINNAKER selects the XYZ triplets with S greater than a defined threshold (by default equal to the 99th percentile)

```
selectTriplets <- function(df,thr){

  s <- df$sensitivity

  ind <- which(s >= thr)

  if( length(ind) > 0 ){

    ceRNA <- df[ind,]

  }else{

    stop("No triplets with the selected sensitivity threshold")

  }

  return(ceRNA)
}
```

⚠ **Caveat:** if the chosen threshold does not allow to select any triplets an error message appears, SPINNAKER stops running, and you have to change the input parameter.

Select Triplets

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step v: the highly S triplets are selected

- The output of this step is the **ceRNA interactions network** where nodes represent ceRNAs with highly correlated expression profiles, while edges represent miRNAs mediating their interactions
- A link between two nodes (ceRNAs) occurs if they fulfilled the following conditions:
  1. matching **high** values of the **Pearson correlation** between their expression profiles
  2. matching **high** values of the **sensitivity correlation**

ceRNA interaction network

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step v: the highly S triplets are selected

source **target** **interaction**

	A	B	C	D	E	F
1	ceRNA1	ceRNA2	miRNA	correlation	partial_correlation	sensitivity
2	ABCC9	AGAP11	hsa-miR-452-5p	0.7251236	0.46708507	0.258039
3	ABHD15	AGAP11	hsa-miR-224-5p	0.7250564	0.477516646	0.24754
4	ABHD15	AGAP11	hsa-miR-452-5p	0.7250564	0.448402624	0.276654
5	ACACB	AGAP11	hsa-miR-452-5p	0.7299151	0.460145715	0.269769
6	ACSL4	AGAP11	hsa-miR-452-5p	0.7003977	0.443672326	0.256725
7	ACSM5	AGAP11	hsa-miR-224-5p	0.7034438	0.457025091	0.246419
8	ACSM5	AGAP11	hsa-miR-452-5p	0.7034438	0.39869897	0.304745
9	ADIPOQ	AGAP11	hsa-miR-452-5p	0.7204383	0.438231989	0.282206
10	ADRB1	AGAP11	hsa-miR-224-5p	0.7099421	0.462706234	0.247236
11	ADRB1	AGAP11	hsa-miR-452-5p	0.7099421	0.426378342	0.283564
12	ANO3	AGAP11	hsa-miR-224-5p	0.6939186	0.425771987	0.268147
13	ANO3	AGAP11	hsa-miR-452-5p	0.6939186	0.401611936	0.292307
14	ANTXR2	AGAP11	hsa-miR-452-5p	0.748311	0.502458952	0.245852
15	AOC3	AGAP11	hsa-miR-224-5p	0.7265339	0.468498677	0.258035
16	AOC3	AGAP11	hsa-miR-452-5p	0.7265339	0.425578625	0.300955

Seed-match analysis

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vi: the seed-match analysis is performed

- SPINNAKER searches for the **seed-match** of all the highly correlated pairs with the miRNA mediating their interactions, in order to restrict the selected triplets to those including only ceRNAs that are targets of the shared miRNA

⚠ **Caveat:** this step is performed if searchSeedMatch = “YES” in config.R

⚠ **Caveat:** this step could take some time

Seed-match analysis

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vi: the seed-match analysis is performed

```
searchSeedMatch <- function(triplets,miRNAtarget){

  mir_common <- intersect(unique(triplets$miRNA),names(miRNAtarget))

  list <- lapply(mir_common,function(x){

    target <- miRNAtarget[[x]]

    ind <- which(triplets$miRNA == x)
    pairs <- triplets[ind,c("ceRNA1","ceRNA2")]

    seed_match <- apply(pairs,1,function(p){

      condition <- length(intersect(p,target)) == 2

      ifelse(condition,"yes","no")

    })

    df <- data.frame(pairs,miRNA=x,seed_match)

  })

  df_tmp <- rbindlist(list)

  triplets <- merge(triplets, df_tmp, by = c("ceRNA1","ceRNA2","miRNA"), all.x = T)

  return(triplets)
}
```

Statistical analysis

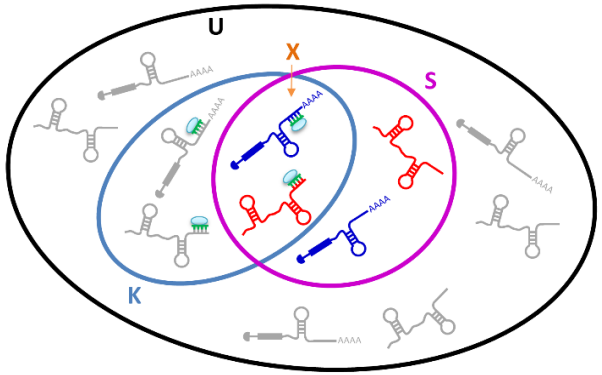
...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed

- SPINNAKER performs a seed-match enrichment analysis by calculating the following p-value resulting from the hypergeometric test:

$$p = 1 - \sum_{i=0}^{X-1} \frac{\binom{K}{i}\binom{U-K}{S-i}}{\binom{U}{S}} = \sum_{i=X}^{S} \frac{\binom{K}{i}\binom{U-K}{S-i}}{\binom{U}{S}}$$



- **U (universe) :** number of the top correlated RNA pairs
- **K (property):** number of RNA pairs sharing the binding site for the miRNA under test
- **S (selection):** number of RNA pairs with high sensitivity correlation for the miRNA under test
- **X (intersection):** number of RNA pairs with high sensitivity correlation sharing the binding site for the miRNA under test

Statistical analysis

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed

- SPINNAKER performs a seed-match enrichment analysis by calculating the following p-value resulting from the hypergeometric test:

$$p = 1 - \sum_{i=0}^{X-1} \frac{\binom{K}{i}\binom{U-K}{S-i}}{\binom{U}{S}} = \sum_{i=X}^{S} \frac{\binom{K}{i}\binom{U-K}{S-i}}{\binom{U}{S}}$$

⚠ **Caveat:** this step is performed if searchSeedMatch = “YES” in config.R

Statistical analysis

...

```
#######################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#######################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#######################################

if(searchSeedMatch == "YES"){

  #######################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #######################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #######################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed

```
statisticalAnalysis <- function(pairs,triplets,ceRNA,miRNAtarget){

  ceRNA <- removeNoTarget(ceRNA,miRNAtarget)

  # universe: highly correlated pairs (rho > thr)
  u <- nrow(pairs)

  mir_common <- intersect(unique(ceRNA$miRNA),names(miRNAtarget))

  list <- lapply(mir_common, function(m){

    ind <- which(triplets$miRNA == m)
    triplets_mir <- triplets[ind,]

    found_yes <- which(triplets_mir$seed_match == "yes")

    df <- NULL

    if( length(found_yes) > 0 ){

      # property: highly correlated pairs with the binding site for that mir
      pairs_property <- triplets_mir[found_yes,c("ceRNA1","ceRNA2","miRNA")]
      k <- nrow(pairs_property)

      # selection: highly correlated pairs with sensitivity > thr for that mir
      pairs_selection <- ceRNA[ceRNA$miRNA == m,c("ceRNA1","ceRNA2","miRNA")]
      s <- nrow(pairs_selection)

      pairs_intersection <- merge(pairs_property, pairs_selection,
                                  by = c("ceRNA1","ceRNA2","miRNA"), all = F)
      x <- nrow(pairs_intersection)

      n <- u - k

      p_value <- sum(dhyper(x:s,k,n,s))

      if (nrow(pairs_intersection) > 0){
        df <- data.frame(pairs_intersection, p_value = p_value,
                         check.names = F, row.names = NULL)
      }else{
        df <- NULL
      }
    }

    return(df)
  })

  df <- rbindlist(list)

  if(nrow(df) > 0){

    ceRNA_pval <- merge(ceRNA, df, by = c("ceRNA1","ceRNA2","miRNA"), all = F)

  }else{

    ceRNA_pval <- NULL

  }

  return(ceRNA_pval)
}
```

Statistical analysis

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed

- The output of this step is the **ceRNA interaction network** with the additional information of p-values
- A link between two nodes (ceRNAs) occurs if they fulfilled the following conditions:
  1. matching **high** values of the **Pearson correlation** between their expression profiles
  2. matching **high** values of the **sensitivity correlation**
  3. sharing the **binding sites** for miRNAs

ceRNA interaction network with p-value

…

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed

source **target** **interaction** **p-value**

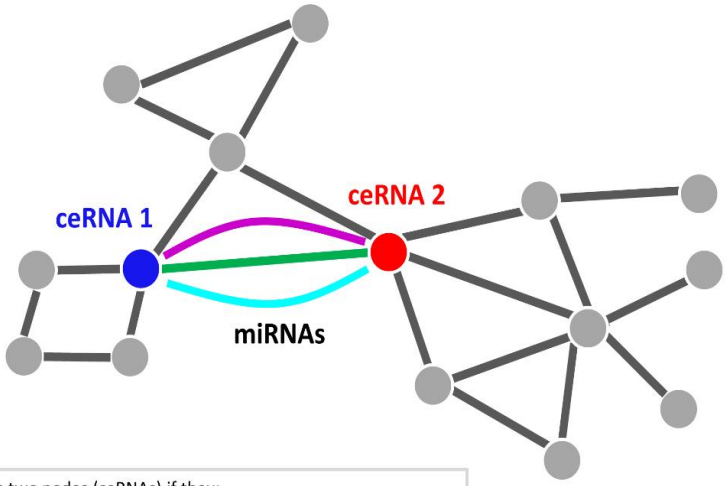
	A	B	C	D	E	F	G
1	ceRNA1	ceRNA2	miRNA	correlatio	partial_co	sensitivity	p_value
2	ABAT	EGOT	hsa-miR-200b-3p	0.723733	0.378208	0.345525418	9.55E-89
3	ABAT	EGOT	hsa-miR-200c-3p	0.723733	0.3842	0.339532842	4.59E-81
4	ABCA1	EMX2OS	hsa-miR-200b-3p	0.750324	0.493991	0.256333696	9.55E-89
5	ABCC9	H19	hsa-miR-200b-3p	0.698159	0.395393	0.302765546	9.55E-89
6	ABCC9	H19	hsa-miR-200c-3p	0.698159	0.431533	0.266625932	4.59E-81
7	ABI2	EGOT	hsa-miR-200b-3p	0.81511	0.401184	0.4139269	9.55E-89
8	ABI2	EGOT	hsa-miR-200c-3p	0.81511	0.415847	0.399263465	4.59E-81
9	ABI2	EGOT	hsa-miR-205-5p	0.81511	0.530219	0.284891767	4.24E-50
10	ABI2	FAM66C	hsa-miR-200b-3p	0.819644	0.544121	0.275523135	9.55E-89
11	ABI2	FAM66C	hsa-miR-200c-3p	0.819644	0.546277	0.273367104	4.59E-81
12	ABI2	PART1	hsa-miR-200b-3p	0.774807	0.127118	0.647689599	9.55E-89
13	ABI2	PART1	hsa-miR-200c-3p	0.774807	0.188392	0.586415039	4.59E-81
14	ABI2	PART1	hsa-miR-205-5p	0.774807	0.491132	0.283675212	4.24E-50
15	ABI2	PART1	hsa-miR-429	0.774807	0.437099	0.337707791	4.59E-17
16	ABI2	PVT1	hsa-miR-200b-3p	0.811366	0.271848	0.539518214	9.55E-89

ceRNA interaction network with p-value

...

```
#####################################
# STEP 4

print("STEP 4: compute sensitivity correlation")

triplets <- computeSensitivity(pairs,rho_ceRNA1_miRNA,rho_ceRNA2_miRNA,filename_heatmap)
#####################################
# STEP 5

print("STEP 5: compute ceRNA interaction network")

threshold_sensitivity <- as.numeric(quantile(triplets$sensitivity,threshold_prc_sensitivity))

ceRNA <- selectTriplets(triplets,threshold_sensitivity)

res <- list(ceRNA = ceRNA,
            threshold_corr = threshold_corr,
            threshold_sensitivity = threshold_sensitivity)
#####################################

if(searchSeedMatch == "YES"){

  #####################################
  # STEP 6

  print("STEP 6: search seed-match for all triplets")

  triplets <- searchSeedMatch(triplets,miRNAtarget)
  #####################################
  # STEP 7

  print("STEP 7: compute statistical analysis")

  ceRNA_pval <- statisticalAnalysis(pairs,triplets,ceRNA,miRNAtarget)

  res_stat <- list(ceRNA_pval = ceRNA_pval)
  #####################################

  res <- c(res,res_stat)

}

return(res)
}
```

Step vii: the statistical analysis is performed



A link occurs between two nodes (ceRNAs) if they:
1) show high values of the Pearson correlation between their expression profiles
2) show high values of the sensitivity correlation
3) [optional] share the binding site for the miRNA mediating their interaction

At the end of module 2, you will obtain the ceRNA interactions network