

PowerExplorer Manual

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Abstract

This vignette demonstrates R package **PowerExplorer** as a power and sample size estimation tool for RNA-Seq and quantitative proteomics data.

PowerExplorer contains the following main features:

- Estimation of power based on the current data
- Prediction of power corresponding to the increased sample sizes
- Result visualizations

Introduction

Power and sample size estimation is one of the important principles in designing next-generation sequencing experiments to discover differential expressions. **PowerExplorer** is a power estimation and prediction tool currently applicable to RNA-Seq and quantitative proteomics experiments.

The calculation procedure starts with estimating the distribution parameters of each gene or protein. With the obtained prior distribution of each feature, a specified amount of simulations are executed to generate data (read counts for RNA-Seq and protein abundance for proteomics) repetitively for each entry based on null and alternative hypotheses. Furthermore, the corresponding statistical tests (t-test or Wald-test) are performed and the test statistics are collected. Eventually the statistics will be summarized to calculate the statistical power.

Input Data Preparation

For both RNA-Seq (gene expression levels) and quantitative proteomics (protein abundance levels) datasets, the data matrix should be arranged as genes/proteins in rows and samples in columns. Here we show a RNA dataset as an example:

```
library(PowerExplorer)
data("exampleProteomicsData")
head(exampleProteomicsData$dataMatrix)
#>      Sample_A_1 Sample_A_2 Sample_A_3 Sample_A_4 Sample_A_5
#> Protein_1      888390      939871      1040976      668450      1008080
#> Protein_2      1159451      1171040      806536      808084      775754
#> Protein_3      996873      1041867      851849      872192      889652
#> Protein_4      730004      976224      1102569      1471498      1202674
#> Protein_5      1075502      832203      1006920      1412591      1282106
#> Protein_6      1274303      1136278      1146456      1028072      915348
#>      Sample_B_1 Sample_B_2 Sample_B_3 Sample_B_4 Sample_B_5
#> Protein_1      3093632      1837451      2760696      2012186      4359111
#> Protein_2      3052050      1079945      5479397      714079      1517852
#> Protein_3      3180328      3265779      2329892      4604491      4050628
#> Protein_4      2123667      1797917      1768735      1652113      2300376
#> Protein_5      1345427      2115544      776261      2704702      2035877
#> Protein_6      3957101      2438872      2844246      1134957      1366432
#>      Sample_C_1 Sample_C_2 Sample_C_3 Sample_C_4 Sample_C_5
#> Protein_1      3303614      4113386      3974813      3723468      2570479
#> Protein_2      4213595      9508269      4342755      5278913      4058501
#> Protein_3      5350451      5616083      5167265      5666823      3950354
#> Protein_4      4557161      1607928      2938020      3850669      3699732
#> Protein_5      3708197      2707825      2818278      3078232      3234728
#> Protein_6      1837780      3608012      4149520      2328815      8972975
```

A grouping vector indicating the sample groups to which all the samples belong should also be created, for example:

```
show(exampleProteomicsData$groupVec)
#> [1] "A" "A" "A" "A" "A" "B" "B" "B" "B" "B" "C" "C" "C" "C" "C"
```

The sample groups corresponding to the data:

```
colnames(exampleProteomicsData$dataMatrix)
#> [1] "Sample_A_1" "Sample_A_2" "Sample_A_3" "Sample_A_4" "Sample_A_5"
#> [6] "Sample_B_1" "Sample_B_2" "Sample_B_3" "Sample_B_4" "Sample_B_5"
#> [11] "Sample_C_1" "Sample_C_2" "Sample_C_3" "Sample_C_4" "Sample_C_5"
```

Note that the grouping vector length should be equal to the column number of the data matrix.

Power Estimation

Here we use a randomly generated Proteomics dataset `exampleProteomicsData` as an example to estimate the current power of the dataset. The input dataset is named as `dataMatrix` and the grouping vector as `groupVec`.

To run the estimation, apart from the input, we still need to specify the following parameters:

- `isLogTransformed`: FALSE; the input data is not log-transformed.
- `dataType`: "Proteomics"; the datatype can be declared as "Proteomics" or "RNA-Seq".
- `minLFC`: 0.5; the threshold of Log2 Fold Change, proteins with lower LFC will be discarded.
- `enableROTS`: TRUE; Using Reproducibility-Optimized Test Statistic (ROTS) as the statistical model.
- `paraROTS`: the parameters to be passed to ROTS (if enabled). Check ROTS documentation for further details on the parameters.
- `alpha`: 0.05; the controlled false positive (Type I Error) rate.
- `ST`: 50; the simulation of each gene will be run 50 times (`ST > 50` is recommended).
- `seed`: 345; optional, a seed value for the random number generator to maintain the reproducibility.
- `showProcess`: FALSE; no detailed processes will be shown, set to TRUE if debug is needed.
- `saveSimulatedData`: FALSE; if TRUE, save the simulated data in `./savedData` directory.

The results will be summarized in barplot, boxplot and summary table.

```
library(PowerExplorer)
data("exampleProteomicsData")
res <- estimatePower(inputObject = exampleProteomicsData$dataMatrix,
                    groupVec = exampleProteomicsData$groupVec,
                    isLogTransformed = FALSE,
                    dataType = "Proteomics",
                    minLFC = 0.5,
                    enableROTS = TRUE,
                    paraROTS = list(B = 1000, K = NULL),
                    alpha = 0.05,
                    ST = 50,
                    seed = 345,
                    showProcess = FALSE,
                    saveResultData = FALSE
                    )
#> ##----- Tue Oct 29 23:02:28 2019 -----##
#> Sample groups:           A, B, C
#> Num. of replicates:      5, 5, 5
#> Num. of simulations:     50
#> Min. Log Fold Change:    0.5
#> False Postive Rate:     0.05
#> Log-transformed:        FALSE
#> ROTS enabled:           TRUE
#> Parallel:               FALSE
#>
#> 0 of 110 entries are filtered due to excessive zero counts
#> [vsn] Transforming data...
#>
#> ----- <A.vs.B> -----
#>
#> Log2 Fold Change Quantiles:
#>  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.01 0.06 0.10 0.14 0.18 0.24 0.31 0.40 0.50 1.82
```

```

#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 0.12, a2 = 1
#>
#> 11 of 110 proteins are over minLFC threshold 0.5.
#>
#> ----- <A.vs.C> -----
#>
#> Log2 Fold Change Quantiles:
#>  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.03 0.07 0.11 0.15 0.20 0.27 0.34 0.43 0.64 2.39
#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 0.82, a2 = 1
#>
#> 15 of 110 proteins are over minLFC threshold 0.5.
#>
#> ----- <B.vs.C> -----
#>
#> Log2 Fold Change Quantiles:
#>  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.03 0.07 0.13 0.15 0.18 0.23 0.29 0.42 0.60 1.59
#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 4.6, a2 = 1
#>
#> 15 of 110 proteins are over minLFC threshold 0.5.
#>
#> Simulation in process, it may take a few minutes...
#>
#> Power Estimation between groups A.vs.B:
#> Completed.
#> Simulation in process, it may take a few minutes...
#>
#> Power Estimation between groups A.vs.C:
#> Completed.
#> Simulation in process, it may take a few minutes...
#>
#> Power Estimation between groups B.vs.C:
#> Completed.

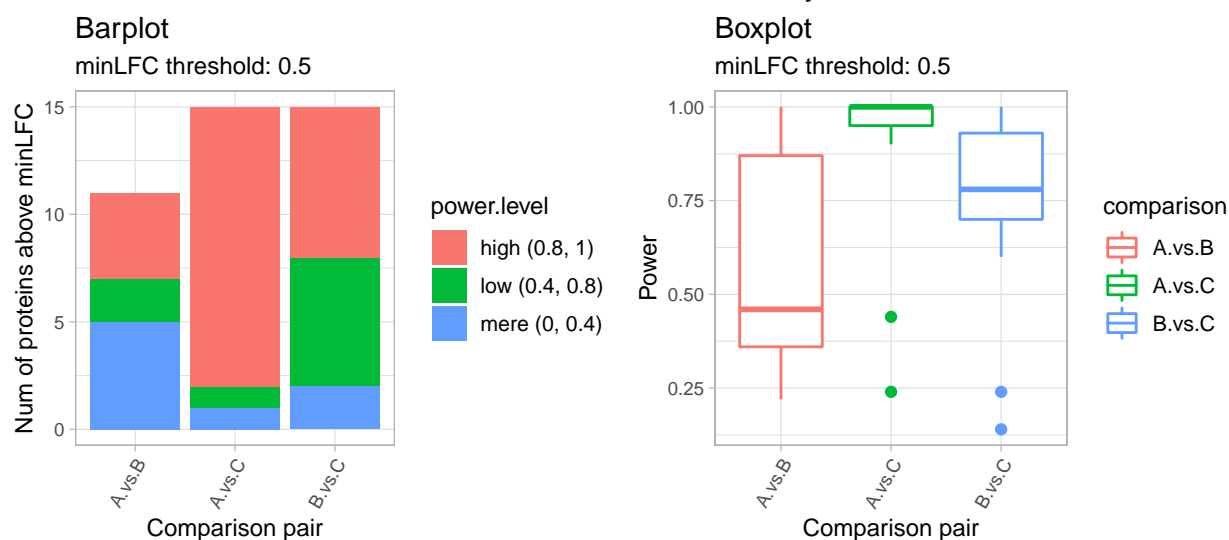
```

Visualization

The estimated results can be summarized using `plotEstPwr`, the only input needed is the `estimatedPower`, which should be the estimated power object returned from `estimatePower`.

```
plotEstPwr(res)
#> Warning in melt(as.matrix(estimatedPower)): The melt generic in data.table
#> has been passed a matrix and will attempt to redirect to the relevant
#> reshape2 method; please note that reshape2 is deprecated, and this
#> redirection is now deprecated as well. To continue using melt methods from
#> reshape2 while both libraries are attached, e.g. melt.list, you can prepend
#> the namespace like reshape2::melt(as.matrix(estimatedPower)). In the next
#> version, this warning will become an error.
#> Warning in melt(numSum): The melt generic in data.table has been passed
#> a matrix and will attempt to redirect to the relevant reshape2 method;
#> please note that reshape2 is deprecated, and this redirection is now
#> deprecated as well. To continue using melt methods from reshape2 while both
#> libraries are attached, e.g. melt.list, you can prepend the namespace like
#> reshape2::melt(numSum). In the next version, this warning will become an
#> error.
```

Estimated Power Summary



| Comp. | Protein Num. | Avg. Power | H (0.8, 1) | L (0.4, 0.8) | M (0, 0.4) |
|--------|--------------|------------|------------|--------------|------------|
| A vs B | 11 | 0.60 | 4 (36%) | 2 (18%) | 5 (45%) |
| A vs C | 15 | 0.90 | 13 (87%) | 1 (7%) | 1 (7%) |
| B vs C | 15 | 0.75 | 7 (47%) | 6 (40%) | 2 (13%) |

The graph contains 3 plots, the **barplot** vertically shows the number of genes/proteins above the minLFC threshold, columns indicates the comparison pairs, each column presents the proportions of three power levels in three colours as indicated in the legend `power.level`; The **boxplot** shows the overall power distribution of each comparison; And the **summary table** summarize the power in a numerical way with the same information shown in the previous two plots.

Result Summary

With the result `PowerExplorerStorage` object, summarized information can be shown by `show` method.

```
res
#> ##--Parameters--##
#> -dataType: Proteomics
#> -original repNum: 5, 5, 5
#> -Comparison groups: A, B, C
#> -False positive rate: 0.05
#> -LFC threshold: 0.5
#> -Simulations: 50
#>
#> ##--Log2 Fold Change Range--##
#>           A.us.B A.us.C B.us.C
#> minLFC    0.00  0.00  0.00
#> maxLFC    1.82  2.39  1.59
#>
#> ##--Average Estimated Power--##
#>           A.us.B   A.us.C   B.us.C
#> 0.5963636 0.8986667 0.7480000
#>
#> ##--Average Predicted Power--##
#> NA
```

If interested in specific genes/proteins or a ranking list, one can use `listEstPwr` with the following parameters:

- `inputObject`: A `PowerExplorerStorage` returned from `PowerExplorer` as input
- `decreasing`: logical; TRUE, decreasing order; FALSE, increasing order.
- `top`: numeric; the number of genes/proteins in the top list
- `selected`: default as NA; specify as a list of geneID or protein ID to show power of a list of interested genes/proteins.

To show the top 10 genes in an example result object `exampleObject` in decreasing order:

```
data(exampleObject)
listEstPwr(exampleObject, decreasing = TRUE, top = 10)
#>           A.us.B
#> ENSMUSG00000000402      1
#> ENSMUSG00000000958      1
#> ENSMUSG00000001473      1
#> ENSMUSG00000003477      1
#> ENSMUSG00000004341      1
#> ENSMUSG00000006154      1
#> ENSMUSG00000006403      1
#> ENSMUSG00000007035      1
#> ENSMUSG00000015484      1
#> ENSMUSG00000015852      1
```

To show the results of specific genes:

```
listEstPwr(exampleObject,
            selected = c("ENSMUSG00000000303",
                         "ENSMUSG000000087272",
                         "ENSMUSG000000089921"))
#>           A.us.B
#> ENSMUSG00000000303  0.34
```

```
#> ENSMUSG00000087272 0.25  
#> ENSMUSG00000089921 0.04
```


Power Predictions

With the same dataset, to run a prediction, a different parameter is needed:

- `rangeSimNumRep`: the power of replicate number 5 to 20 will be predicted.

Similar to the estimation process, however, the simulations will be executed with each sample size specified in `rangeSimNumRep`. (Note: the term sample size in this vignette refers to the replicate number of each group/case)

It is possible to append the prediction results within the same object by using the same result object as an input.

```
data("exampleProteomicsData")
res <- predictPower(inputObject = res,
                    groupVec = exampleProteomicsData$groupVec,
                    isLogTransformed = FALSE,
                    dataType = "Proteomics",
                    minLFC = 0.5,
                    rangeSimNumRep = c(5, 10, 15, 20),
                    enableROTS = TRUE,
                    paraROTS = list(B = 1000, K = NULL),
                    alpha = 0.05,
                    ST = 50,
                    seed = 345)
#> ##----- Tue Oct 29 23:02:55 2019 -----##
#> Sample groups:      A, B, C
#> Replicates of prediction:      5, 10, 15, 20
#> Num. of simulations: 50
#> Min. Log Fold Change:      0.5
#> False Postive Rate:      0.05
#> Transformed: FALSE
#> ROTS enabled:          TRUE
#> Parallel:            FALSE
#>
#> 0 of 110 entries are filtered due to excessive zero counts
#> [vsn] Transforming data...
#>
#> ----- <A.vs.B> -----
#>
#> Log2 Fold Change Quantiles:
#>   0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.01 0.06 0.10 0.14 0.18 0.24 0.31 0.40 0.50 1.82
#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 0.12, a2 = 1
#>
#> 11 of 110 proteins are over minLFC threshold 0.5.
#>
#> ----- <A.vs.C> -----
#>
#> Log2 Fold Change Quantiles:
#>   0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.03 0.07 0.11 0.15 0.20 0.27 0.34 0.43 0.64 2.39
```

```

#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 0.82, a2 = 1
#>
#> 15 of 110 proteins are over minLFC threshold 0.5.
#>
#> ----- <B.us.C> -----
#>
#> Log2 Fold Change Quantiles:
#>  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
#> 0.00 0.03 0.07 0.13 0.15 0.18 0.23 0.29 0.42 0.60 1.59
#>
#> [ROTS] Estimating statistics optimizing parameters...
#> [ROTS] optimization parameters:
#> a1 = 4.6, a2 = 1
#>
#> 15 of 110 proteins are over minLFC threshold 0.5.
#>
#> ##--Simulation with 5 replicates per group--##
#>
#> [repNum:5] Simulation in process, it may take a few minutes...
#>
#> [repNum:5] Power Estimation between groups A.us.B:
#> Completed.
#> [repNum:5] Simulation in process, it may take a few minutes...
#>
#> [repNum:5] Power Estimation between groups A.us.C:
#> Completed.
#> [repNum:5] Simulation in process, it may take a few minutes...
#>
#> [repNum:5] Power Estimation between groups B.us.C:
#> Completed.
#> ##--Simulation with 10 replicates per group--##
#>
#> [repNum:10] Simulation in process, it may take a few minutes...
#>
#> [repNum:10] Power Estimation between groups A.us.B:
#> Completed.
#> [repNum:10] Simulation in process, it may take a few minutes...
#>
#> [repNum:10] Power Estimation between groups A.us.C:
#> Completed.
#> [repNum:10] Simulation in process, it may take a few minutes...
#>
#> [repNum:10] Power Estimation between groups B.us.C:
#> Completed.
#> ##--Simulation with 15 replicates per group--##
#>
#> [repNum:15] Simulation in process, it may take a few minutes...
#>
#> [repNum:15] Power Estimation between groups A.us.B:
#> Completed.

```

```
#> [repNum:15] Simulation in process, it may take a few minutes...
#>
#> [repNum:15] Power Estimation between groups A.vs.C:
#> Completed.
#> [repNum:15] Simulation in process, it may take a few minutes...
#>
#> [repNum:15] Power Estimation between groups B.vs.C:
#> Completed.
#> ##--Simulation with 20 replicates per group--##
#>
#> [repNum:20] Simulation in process, it may take a few minutes...
#>
#> [repNum:20] Power Estimation between groups A.vs.B:
#> Completed.
#> [repNum:20] Simulation in process, it may take a few minutes...
#>
#> [repNum:20] Power Estimation between groups A.vs.C:
#> Completed.
#> [repNum:20] Simulation in process, it may take a few minutes...
#>
#> [repNum:20] Power Estimation between groups B.vs.C:
#> Completed.
```

Visualization

The predicted results can be summarized using `plotPredPwr`. The input should be the predicted power object returned from `predictPower`, the summary can be optionally visualized by setting the following parameters:

- `inputObject`: A `PowerExplorerStorage` returned from `PowerExplorer` as input
- `minLFC` and `maxLFC`: to observe power in a specific range of LFC
- `LFCscale`: to determine the LFC scale of the observation

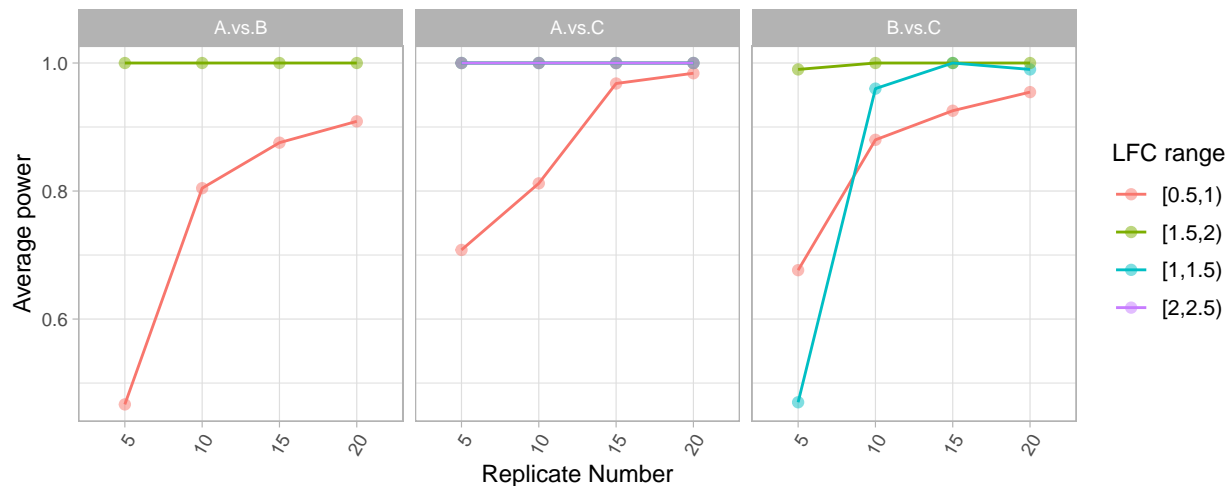
Lineplot (`LFCscale = 0.5`):

```
plotPredPwr(res, LFCscale = 0.5)
```

```
#> Warning in melt(predPwr): The melt generic in data.table has been passed
#> a list and will attempt to redirect to the relevant reshape2 method;
#> please note that reshape2 is deprecated, and this redirection is now
#> deprecated as well. To continue using melt methods from reshape2 while both
#> libraries are attached, e.g. melt.list, you can prepend the namespace like
#> reshape2::melt(predPwr). In the next version, this warning will become an
#> error.
#> Warning in melt(lfc): The melt generic in data.table has been passed
#> a matrix and will attempt to redirect to the relevant reshape2 method;
#> please note that reshape2 is deprecated, and this redirection is now
#> deprecated as well. To continue using melt methods from reshape2 while
#> both libraries are attached, e.g. melt.list, you can prepend the namespace
#> like reshape2::melt(lfc). In the next version, this warning will become an
#> error.
```

Average Predicted Power within LFC ranges

segmented by every 0.5 Log2FoldChange (minLFC: 0.5, maxLFC: 2.39)



| Comp. | repNum:5 | repNum:10 | repNum:15 | repNum:20 |
|--------|----------|-----------|-----------|-----------|
| A vs.B | 0.56 | 0.84 | 0.9 | 0.93 |
| A vs.C | 0.9 | 0.94 | 0.99 | 0.99 |
| B vs.C | 0.69 | 0.91 | 0.95 | 0.97 |

The output figure contains a lineplot and a summary table. For each comparison, the lineplot shows the power tendency across every Log2 Fold Change segment resulted from a complete LFC list divided by a specified `LFCscale`. Each dot on the lines represents the average power (y-axis) of the genes/proteins at a

certain sample size (x-axis) within different LFC ranges. In addition, a summary table below displays the average power of each comparison across the sample sizes.

For instance, the line plot here shows the average power at four different sample sizes (5 to 30, with increment of 5) in LFCscale of 0.5. The LFC ranges from 0 to 5, and within each LFC segment, the graph shows the average power of the genes/proteins. Here, the higher LFC shows higher power, the average power of each LFC range increases with the larger sample sizes, as expected.

Result Summary

With the result `PowerExplorerStorage` object, summarized information can be shown by `show` method. Both estimated and predicted results can be summarized.

```
res
#> ##--Parameters--##
#> -dataType: Proteomics
#> -original repNum: 5, 5, 5
#> -Comparison groups: A, B, C
#> -False positive rate: 0.05
#> -LFC threshold: 0.5
#> -Simulations: 50
#>
#> ##--Log2 Fold Change Range--##
#>           A.vs.B A.vs.C B.vs.C
#> minLFC    0.00  0.00  0.00
#> maxLFC    1.82  2.39  1.59
#>
#> ##--Average Estimated Power--##
#>           A.vs.B  A.vs.C  B.vs.C
#> 0.5963636 0.8986667 0.7480000
#>
#> ##--Average Predicted Power--##
#> $`repNum: 5`
#>           A.vs.B  A.vs.C  B.vs.C
#> 0.5636364 0.9026667 0.6906667
#>
#> $`repNum: 10`
#>           A.vs.B  A.vs.C  B.vs.C
#> 0.8400000 0.9373333 0.9066667
#>
#> $`repNum: 15`
#>           A.vs.B  A.vs.C  B.vs.C
#> 0.8981818 0.9893333 0.9453333
#>
#> $`repNum: 20`
#>           A.vs.B  A.vs.C  B.vs.C
#> 0.9254545 0.9946667 0.9653333
```

If interested in specific genes/proteins or a ranking list of predicted power at each sample size, one can use `listPrePwr` with the following parameters:

- `inputObject`: A `PowerExplorerStorage` returned from `PowerExplorer` as input
- `decreasing`: logical; TRUE, decreasing order; FALSE, increasing order.
- `top`: numeric; the number of genes/proteins in the top list
- `selected`: default as NA; specify as a list of geneID or protein ID to show power of a list of interested genes/proteins.

To show the top 10 genes in an example result object `exampleObject` in decreasing order at each sample size:

```
listPredPwr(exampleObject, decreasing = TRUE, top = 10)
#> $`repNum: 10`
#>           A.vs.B
#> ENSMUSG00000000402      1
#> ENSMUSG00000000958      1
```

```

#> ENSMUSG00000001473      1
#> ENSMUSG00000003477      1
#> ENSMUSG00000004341      1
#> ENSMUSG00000005553      1
#> ENSMUSG00000006154      1
#> ENSMUSG00000006403      1
#> ENSMUSG00000007035      1
#> ENSMUSG00000011263      1
#>
#> `$repNum: 15`
#>                               A.vs.B
#> ENSMUSG00000000402      1
#> ENSMUSG00000000958      1
#> ENSMUSG00000001473      1
#> ENSMUSG00000001493      1
#> ENSMUSG00000003477      1
#> ENSMUSG00000004341      1
#> ENSMUSG00000005553      1
#> ENSMUSG00000005681      1
#> ENSMUSG00000006154      1
#> ENSMUSG00000006403      1
#>
#> `$repNum: 20`
#>                               A.vs.B
#> ENSMUSG00000000402      1
#> ENSMUSG00000000958      1
#> ENSMUSG00000001473      1
#> ENSMUSG00000001493      1
#> ENSMUSG00000003477      1
#> ENSMUSG00000004341      1
#> ENSMUSG00000004709      1
#> ENSMUSG00000005553      1
#> ENSMUSG00000005681      1
#> ENSMUSG00000006154      1
#>
#> `$repNum: 30`
#>                               A.vs.B
#> ENSMUSG00000000402      1
#> ENSMUSG00000000958      1
#> ENSMUSG00000001473      1
#> ENSMUSG00000001493      1
#> ENSMUSG00000003355      1
#> ENSMUSG00000003477      1
#> ENSMUSG00000004341      1
#> ENSMUSG00000004709      1
#> ENSMUSG00000005553      1
#> ENSMUSG00000005677      1

```

To show the results of specific genes at each sample size:

```

listPredPwr(exampleObject,
             selected = c("ENSMUSG00000000303",
                          "ENSMUSG000000087272",
                          "ENSMUSG000000089921"))

```

```

#> `$repNum: 10`
#>           A.vs.B
#> ENSMUSG0000000303 0.28
#> ENSMUSG00000087272 0.16
#> ENSMUSG00000089921 0.01
#>
#> `$repNum: 15`
#>           A.vs.B
#> ENSMUSG0000000303 0.39
#> ENSMUSG00000087272 0.17
#> ENSMUSG00000089921 0.00
#>
#> `$repNum: 20`
#>           A.vs.B
#> ENSMUSG0000000303 0.37
#> ENSMUSG00000087272 0.35
#> ENSMUSG00000089921 0.02
#>
#> `$repNum: 30`
#>           A.vs.B
#> ENSMUSG0000000303 0.64
#> ENSMUSG00000087272 0.56
#> ENSMUSG00000089921 0.01

```

Parallel computation

The calculation may take much longer time when an input dataset contains more than thousands of features, especially for the power prediction process. The computational time can be significantly shortened by using parallelised computation, and the simulations will be distributed to multiple cores. This can be done by loading Bioconductor package `BiocParallel` and then set the following arguments of `estimatePower` and `predictPower`: `parallel=TRUE` and `BPPARAM=bpparam()`. This will distribute the jobs to all the available cores. One can register the number of cores to be used by setting `BPPARAM=MulticoreParam(4)`, for instance, distributing simulations (jobs) to 4 cores. However, `MulticoreParam()` only supports non-Windows platforms. For Windows platforms, one can use `SnowParam()` instead. For further details, please check the `BiocParallel` documentation.