

Package ‘MSstatsShiny’

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Type Package

Title MSstats GUI for Statistical Analysis of Proteomics Experiments

Version 1.3.2

Description MSstatsShiny is an R-Shiny graphical user interface (GUI) integrated with the R packages MSstats, MSstatsTMT, and MSstatsPTM. It provides a point and click end-to-end analysis pipeline applicable to a wide variety of experimental designs. These include data-dependent acquisitions (DDA) which are label-free or tandem mass tag (TMT)-based, as well as DIA, SRM, and PRM acquisitions and those targeting post-translational modifications (PTMs). The application automatically saves users selections and builds an R script that recreates their analysis, supporting reproducible data analysis.

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Depends R (>= 4.2)

Imports shiny, shinyBS, shinyjs, shinybusy, dplyr, ggplot2, data.table, Hmisc, MSstats, MSstatsTMT, MSstatsPTM, MSstatsConvert, gplots, marray, DT, readxl, ggrepel, uuid, utils, stats, htmltools, methods, tidyr, grDevices, graphics

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annotation.mine	<i>Example annotation file for Spectromine</i>
-----------------	--

Description

data.frame mapping Spectromine run names to the corresponding bioreplicates and conditions. Used as input to preprocessing function, converting data into MSstats format.

Format

data.frame

Examples

```
data(annotation.mine)
head(annotation.mine)
```

annotation.mq	<i>Example annotation file for MaxQuant</i>
---------------	---

Description

data.frame mapping MaxQuant run names to the corresponding bioreplicates and conditions. Used as input to preprocessing function, converting data into MSstats format.

Format

data.frame

Examples

```
data(annotation.mq)
head(annotation.mq)
```

annotation.pd

Example annotation file for PD

Description

data.frame mapping PD run names to the corresponding bioreplicates and conditions. Used as input to preprocessing function, converting data into MSstats format.

Format

data.frame

Examples

```
data(annotation.pd)
head(annotation.pd)
```

apply_adj

Main PTM adjustment function

Description

Main PTM function to model MSstatsShiny data.

Usage

```
apply_adj(ptm_model, protein_model)
```

Arguments

ptm_model output of MSstats modeling function modeling PTMs
protein_model output of MSstats modeling function modeling unmodified proteins

Value

list of PTM modeling results

Examples

```
model = MSstatsPTM::groupComparisonPTM(MSstatsPTM::summary.data,
                                         data.type = "LabelFree")
apply_adj(model$PTM.Model, model$PROTEIN.Model)
```

dia_skyline_model	<i>Example of Skyline DDA dataset modeled using MSstats groupComparison function.</i>
-------------------	---

Description

Data includes one list with two data.tables named ComparisonResult and ModelQC and another list of model details named FittedModel. ComparisonResult shows an overview of all proteins modeled in the system. ModelQC provides a report on the quality control checks of each protein in the dataset.

Format

list

Examples

```
data(dia_skyline_model)
head(dia_skyline_model)
```

dia_skyline_summarized	<i>Example of Skyline DDA dataset processed using MSstats summarization function.</i>
------------------------	---

Description

Data includes one list with two data.tables named FeatureLevelData and ProteinLevelData and a string value SummaryMethod. FeatureLevelData shows the unsummarized feature level data. ProteinLevelData shows the data summarized up to the protein level and is used for modeling the data.

Format

list

Examples

```
data(dia_skyline_summarized)
head(dia_skyline_summarized)
```

evidence	<i>Example evidence file for MaxQuant</i>
----------	---

Description

data.frame containing output of MaxQuant. Used in examples.

Format

data.frame

Examples

```
data(evidence)
head(evidence)
```

example_dia_skyline	<i>Example of input Skyline DDA dataset.</i>
---------------------	--

Description

Used as input data to MSstats workflow. Data includes one data.table which is the output of Skyline.

Format

data.frame

Details

The raw data (input data for MSstats) is required to contain variable of ProteinName, PeptideSequence, PrecursorCharge, FragmentIon, ProductCharge, IsotopeLabelType, Condition, BioReplicate, Run, Intensity. The variable names should be fixed. If the information of one or more columns is not available for the original raw data, please retain the column variables and type in fixed value. For example, the original raw data does not contain the information of PrecursorCharge and ProductCharge, we retain the column PrecursorCharge and ProductCharge and then type in NA for all transitions in RawData. Variable Intensity is required to be original signal without any log transformation and can be specified as the peak of height or the peak of area under curve.

Examples

```
data(example_dia_skyline)
head(example_dia_skyline)
```

example_skyline_annotation
<i>Example annotation file</i>

Description

data.frame mapping Skyline run names to the corresponding bioreplicates and conditions. Used as input to preprocessing function, converting data into MSstats format.

Format

data.frame

Examples

```
data(example_skyline_annotation)
head(example_skyline_annotation)
```

expdesServer	<i>Expdes Server module for future experiments</i>
--------------	--

Description

This function sets up the Expdes server to process data based on user selected inputs

Usage

```
expdesServer(
  input,
  output,
  session,
  parent_session,
  loadpage_input,
  qc_input,
  statmodel_input,
  data_comparison
)
```

Arguments

input	input object to capture different ui element values
output	to render and create elements
session	session current module
parent_session	session of the main calling module

loadpage_input input object from loadpage UI
qc_input input object from QC UI
statmodel_input input object from Statmodel UI
data_comparison function for group comparisons

Value

list object with user selected options and matrix build

expdesUI	<i>Expdes UI module for future experiments UI.</i>
----------	--

Description

This function sets up the Expdes UI where it consists of several, options for users to select and generate plots.

Usage

expdesUI(id)

Arguments

id namespace prefix for the module

Value

This function returns nothing, as it sets up the Expdes UI

groupComparisonPlots2	<i>Plotting funcitonality for QC plots</i>
-----------------------	--

Description

General plotting code to produce all QC plots in the application

Usage

```

groupComparisonPlots2(
  data = data,
  type = type,
  sig = 0.05,
  FCcutoff = FALSE,
  logBase.pvalue = 10,
  ylimUp = FALSE,
  ylimDown = FALSE,
  xlimUp = FALSE,
  x.axis.size = 10,
  y.axis.size = 10,
  dot.size = 3,
  text.size = 4,
  legend.size = 13,
  ProteinName = TRUE,
  colorkey = TRUE,
  numProtein = 100,
  clustering = "both",
  width = 10,
  height = 10,
  which.Comparison = "all",
  which.Protein = "all",
  address = "",
  savePDF = FALSE
)

```

Arguments

data	'ComparisonResult' in testing output from function groupComparison.
type	choice of visualization. "VolcanoPlot" represents volcano plot of log fold changes and adjusted p-values for each comparison separately. "Heatmap" represents heatmap of adjusted p-values for multiple comparisons. "ComparisonPlot" represents comparison plot of log fold changes for multiple comparisons per protein.
sig	FDR cutoff for the adjusted p-values in heatmap and volcano plot. level of significance for comparison plot. 100(1-sig)% confidence interval will be drawn. sig=0.05 is default.
FCcutoff	for volcano plot or heatmap, whether involve fold change cutoff or not. FALSE (default) means no fold change cutoff is applied for significance analysis. FC-cutoff = specific value means specific fold change cutoff is applied.
logBase.pvalue	for volcano plot or heatmap, (-) logarithm transformation of adjusted p-value with base 2 or 10(default).
ylimUp	for all three plots, upper limit for y-axis. FALSE (default) for volcano plot/heatmap use maximum of -log2 (adjusted p-value) or -log10 (adjusted p-value). FALSE (default) for comparison plot uses maximum of log-fold change + CI.

<code>ylimDown</code>	for all three plots, lower limit for y-axis. FALSE (default) for volcano plot/heatmap use minimum of $-\log_2$ (adjusted p-value) or $-\log_{10}$ (adjusted p-value). FALSE (default) for comparison plot uses minimum of log-fold change - CI.
<code>xlimUp</code>	for Volcano plot, the limit for x-axis. FALSE (default) for use maximum for absolute value of log-fold change or 3 as default if maximum for absolute value of log-fold change is less than 3.
<code>x.axis.size</code>	size of axes labels, e.g. name of the comparisons in heatmap, and in comparison plot. Default is 10.
<code>y.axis.size</code>	size of axes labels, e.g. name of targeted proteins in heatmap. Default is 10.
<code>dot.size</code>	size of dots in volcano plot and comparison plot. Default is 3.
<code>text.size</code>	size of ProteinName label in the graph for Volcano Plot. Default is 4.
<code>legend.size</code>	size of legend for color at the bottom of volcano plot. Default is 7.
<code>ProteinName</code>	for volcano plot only, whether display protein names or not. TRUE (default) means protein names, which are significant, are displayed next to the points. FALSE means no protein names are displayed.
<code>colorkey</code>	TRUE(default) shows colorkey.
<code>numProtein</code>	The number of proteins which will be presented in each heatmap. Default is 100. Maximum possible number of protein for one heatmap is 180.
<code>clustering</code>	Determines how to order proteins and comparisons. Hierarchical cluster analysis with Ward method(minimum variance) is performed. 'protein' means that protein dendrogram is computed and reordered based on protein means (the order of row is changed). 'comparison' means comparison dendrogram is computed and reordered based on comparison means (the order of comparison is changed). 'both' means to reorder both protein and comparison. Default is 'protein'.
<code>width</code>	width of the saved file. Default is 10.
<code>height</code>	height of the saved file. Default is 10.
<code>which.Comparison</code>	list of comparisons to draw plots. List can be labels of comparisons or order numbers of comparisons from levels(data\$Label), such as levels(testResultMultiComparisons\$Comparison). Default is "all", which generates all plots for each protein.
<code>which.Protein</code>	Protein list to draw comparison plots. List can be names of Proteins or order numbers of Proteins from levels(testResultMultiComparisons\$ComparisonResult\$Protein). Default is "all", which generates all comparison plots for each protein.
<code>address</code>	the name of folder that will store the results. Default folder is the current working directory. The other assigned folder has to be existed under the current working directory. An output pdf file is automatically created with the default name of "VolcanoPlot.pdf" or "Heatmap.pdf" or "ComparisonPlot.pdf". The command address can help to specify where to store the file as well as how to modify the beginning of the file name. If address=FALSE, plot will be not saved as pdf file but showed in window.
<code>savePDF</code>	Boolean input passed from user on whether or not to save the plot to a PDF.

Value

PDF or console plot

Examples

```
data("dia_skyline_model")
groupComparisonPlots2(dia_skyline_model$ComparisonResult, type="VolcanoPlot",
                      address=FALSE)
```

helpUI

Help UI module for help page.

Description

This module shows the help page for general documentation

Usage

```
helpUI(id)
```

Arguments

id namespace prefix for the module

Value

This function returns nothing, as it sets up the Help UI

homeUI

Home UI module for home page.

Description

This function generates the home user interface for MSstatsShiny, a web tool for the statistical analysis of quantitative proteomic data built around the R packages MSstats, MSstatsTMT, and MSstatsPTM.

Usage

```
homeUI(id)
```

Arguments

id namespace prefix for the module

Value

This function returns nothing, as it sets up the Home UI

launch_MSstatsShiny	<i>Run MSstatsShiny Application</i>
---------------------	-------------------------------------

Description

Main function to run MSstatsShiny. All other functions in this package are run automatically.

Usage

```
launch_MSstatsShiny(  
  launch_app = TRUE,  
  port = getOption("shiny.port"),  
  host = getOption("shiny.host", "127.0.0.1"),  
  testMode = FALSE  
)
```

Arguments

launch_app	One of TRUE or FALSE indicating whether or not to run application. Default is TRUE.
port	(optional) Specify port the application should list to.
host	(optional) The IPv4 address that the application should listen on.
testMode	One of TRUE or FALSE indicating whether or not to run the application in test mode. Default is FALSE.

Value

Running Shiny Application

Examples

```
## Not run:  
## To run app set launch_app=TRUE  
launch_MSstatsShiny(launch_app=FALSE, testMode=FALSE)  
  
## End(Not run)
```

lf_model	<i>Main LF modeling function for MSstatsShiny application</i>
----------	---

Description

Main LF function to model MSstatsShiny data.

Usage

```
lf_model(data, contrast.matrix, busy_indicator = TRUE)
```

Arguments

`data` summarized data from output of MSstats summarization function.
`contrast.matrix` contrast matrix specifying which conditions should be compared
`busy_indicator` Boolean indicator indicating whether or not to display shiny waiting indicator.

Value

list of LF modeling results

Examples

```
data("dia_skyline_summarized")
comparison <- matrix(c(1, -1, 0, 0, 0, 0, 0, 0, 0, 0), nrow=1)
row.names(comparison) = "1 vs 128"
colnames(comparison) = c("1", "128", "16", "2", "256",
                        "32", "4", "512", "64", "8")
model_lf_test = lf_model(dia_skyline_summarized, comparison,
                        busy_indicator = FALSE)
```

lf_summarization_loop	<i>Main LF calculation summarization function for MSstatsShiny application</i>
-----------------------	--

Description

Main LF function to calculate MSstatsShiny results.

Usage

```
lf_summarization_loop(data, qc_input, loadpage_input, busy_indicator = TRUE)
```

Arguments

data Data converted into MSstats format.
qc_input options for data processing input by the user from data processing page.
loadpage_input options for data processing input by the user from data upload page.
busy_indicator Boolean indicator indicating whether or not to display shiny waiting indicator.

Value

list of LF Summarization results

Examples

```

data("example_dia_skyline")
data("example_skyline_annotation")
testdata = MSstats::SkylinetoMSstatsFormat(example_dia_skyline,
                                           annotation = example_skyline_annotation,
                                           filter_with_Qvalue = TRUE,
                                           qvalue_cutoff = 0.01,
                                           fewMeasurements="remove",
                                           removeProtein_with1Feature = TRUE,
                                           use_log_file = FALSE)

## Source app functionality
qc_input = list()
loadpage_input = list()
qc_input$norm = "equalizeMedians"
qc_input$log = 2
qc_input$names = NULL
qc_input$features_used = "all"
code_n_feat=3
qc_input$censInt = "NA"
qc_input$MBi = TRUE
qc_input$remove50 = FALSE
qc_input$maxQC = 0.999
qc_input$null = FALSE
qc_input$null1 = FALSE
loadpage_input$DDA_DIA = "LF"
lf_summarization_loop(testdata, qc_input,loadpage_input, busy_indicator=FALSE)

```

loadpageServer

Loadpage Server module for data selection and upload server.

Description

This function sets up the loadpage server where it consists of several, options for users to select and upload files.

Usage

```
loadpageServer(id, parent_session)
```

Arguments

id namespace prefix for the module
parent_session session of the main calling module

Value

input object with user selected options

loadpageUI	<i>Loadpage UI module for data selection and upload UI.</i>
------------	---

Description

This function sets up the loadpage UI where it consists of several, options for users to select and upload files.

Usage

```
loadpageUI(id)
```

Arguments

id namespace prefix for the module

Value

This function returns nothing, as it sets up the loadpage UI

msstatsHelpUI	<i>Help MSStats UI module for mssatst help page.</i>
---------------	--

Description

This module shows the msstats help page for general documentation

Usage

```
msstatsHelpUI(id)
```

Arguments

id namespace prefix for the module

Value

This function returns nothing, as it sets up the MSstats Help UI

MSstatsShiny	<i>MSstatsShiny: An R-shiny based package for detecting differentially abundant proteins, integrated with the MSstats family of packages.</i>
--------------	---

Description

A set of tools for detecting differentially abundant proteins in shotgun mass spectrometry-based proteomic experiments. The package can handle a variety of acquisition types, including label free, DDA, DIA, and TMT. The package includes tools to convert raw data from different spectral processing tools, summarize feature intensities, and fit a linear mixed effects model. The GUI supports different biological queries including those targeting the global proteome and post translational modifications. Additionally the package includes functionality to plot a variety of data visualizations.

functions

- `launch_MSstatsShiny` : Main function to launch the application.
- `groupComparisonPlots2` : Generates MSstatsShiny plots.
- `lf_summarization_loop` : Summarization for LF experiments.
- `tmt_summarization_loop` : Summarization for TMT experiments.
- `lf_model` : Modeling for LF experiments.
- `tmt_model` : Modeling for TMT experiments.

proteinGroups	<i>Example ProteinGroups file for MaxQuant</i>
---------------	--

Description

data.frame containing output of ProteinGroups MaxQuant file. Used in examples.

Format

data.frame

Examples

```
data(proteinGroups)
head(proteinGroups)
```

qcServer	<i>QC Server module for data processing</i>
----------	---

Description

This function sets up the QC server to process data based on user selected inputs

Usage

```
qcServer(input, output, session, parent_session, loadpage_input, get_data)
```

Arguments

input	input object to capture different ui element values
output	to render and create elements
session	session current module
parent_session	session of the main calling module
loadpage_input	input object from loadpage UI
get_data	stored function that returns the data from loadpage

Value

input object with user selected options

qcUI	<i>QC UI module for data processing UI.</i>
------	---

Description

This function sets up the QC UI where it consists of several, options for users to process data based on previously selected fragments.

Usage

```
qcUI(id)
```

Arguments

id	namespace prefix for the module
----	---------------------------------

Value

This function returns nothing, as it sets up the QC UI

QC_check	<i>Quick QC value check</i>
----------	-----------------------------

Description

Quick QC value check for LF vs TMT

Usage

```
QC_check(qc_input, loadpage_input)
```

Arguments

qc_input options for data processing input by the user from data processing page.
 loadpage_input options for data processing input by the user from data upload page.

Value

string

Examples

```
qc_input = list(null=TRUE)
loadpage_input = list(null=TRUE)
QC_check(qc_input,loadpage_input)
```

radioTooltip	<i>Custom function to create radio tool tips</i>
--------------	--

Description

Used in UI files to create HTML vizualizations

Usage

```
radioTooltip(
  id,
  choice,
  title,
  placement = "bottom",
  trigger = "hover",
  options = NULL
)
```

Arguments

id	input id
choice	user selection
title	title of object
placement	where should tooltip be shown
trigger	how should prompt be shown
options	additional options to pass to function

Value

HTML object

Examples

```
radioTooltip("testid", "test_choice", "test_title")
```

raw.mine

Example output file Spectromine

Description

data.frame containing output of Spectromine. Used in examples.

Format

data.frame

Examples

```
data(raw.mine)
head(raw.mine)
```

`raw.om`*Example output file Spectromine*

Description

data.frame containing output of Spectromine. Used in examples.

Format

data.frame

Examples

```
data(raw.om)
head(raw.om)
```

`raw.pd`*Example output file PD*

Description

data.frame containing output of PD. Used in examples.

Format

data.frame

Examples

```
data(raw.pd)
head(raw.pd)
```

statmodelServer	<i>Statmodel Server module for stat inference</i>
-----------------	---

Description

This function sets up the Statmodel server to process data based on user selected inputs

Usage

```
statmodelServer(  
  input,  
  output,  
  session,  
  parent_session,  
  loadpage_input,  
  qc_input,  
  get_data,  
  preprocess_data  
)
```

Arguments

input	input object to capture different ui element values
output	to render and create elements
session	session current module
parent_session	session of the main calling module
loadpage_input	input object from loadpage UI
qc_input	input object from QC UI
get_data	stored function that returns the data from loadpage
preprocess_data	stored function that returns preprocessed data

Value

list object with user selected options and matrix build

statmodelUI	<i>Statmodel UI module for statistical inference UI.</i>
-------------	--

Description

This function sets up the Statmodel UI where it consists of several, options for users to select and upload files.

Usage

```
statmodelUI(id)
```

Arguments

id	namespace prefix for the module
----	---------------------------------

Value

This function returns nothing, as it sets up the Statmodel UI

tmt_model	<i>Main TMT modeling function for MSstatsShiny application</i>
-----------	--

Description

Main TMT function to model MSstatsShiny data.

Usage

```
tmt_model(data, input, contrast.matrix, busy_indicator = TRUE)
```

Arguments

data	summarized data from output of MSstats summarization function.
input	options for data processing input by the user
contrast.matrix	contrast matrix specifying which conditions should be compared
busy_indicator	Boolean indicator indicating whether or not to display shiny waiting indicator.

Value

list of TMT modeling results

Examples

```
data(raw.pd, package = "MSstatsTMT")
data(annotation.pd, package = "MSstatsTMT")

testdata <- MSstatsTMT::PDtoMSstatsTMTFormat(raw.pd,
                                              annotation.pd,
                                              use_log_file = FALSE
                                              )#'

qc_input = list()
loadpage_input = list()
qc_input$summarization = "msstats"
qc_input$norm = "equalizeMedians"
qc_input$log = 2
qc_input$names = NULL
qc_input$features_used = "all"
code_n_feat=3
qc_input$censInt = "NA"
qc_input$MBi = TRUE
qc_input$remove50 = FALSE
qc_input$maxQC = 0.999
qc_input$null = FALSE
qc_input$null1 = FALSE
loadpage_input$DDA_DIA = "LF"
qc_input$global_norm = TRUE
qc_input$reference_norm = TRUE
qc_input$remove_norm_channel = TRUE
qc_input$maxQC1 = NULL
qc_input$moderated = FALSE

summarization_tmt_test = tmt_summarization_loop(testdata, qc_input, loadpage_input,
                                              busy_indicator = FALSE)

comparison=matrix(c(-1,0,0,1),nrow=1)
row.names(comparison) = "1-0.125"
colnames(comparison) = c("0.125", "0.5", "0.667", "1")

model_tmt_test = tmt_model(summarization_tmt_test, qc_input, comparison,
                          busy_indicator = FALSE)
```

tmt_pd_model	<i>Example of TMT dataset modeled using MSstatsTMT groupComparisonTMT function.</i>
--------------	---

Description

Data includes one list with two data.tables named ComparisonResult and ModelQC and another list of model details named FittedModel. ComparisonResult shows an overview of all proteins modeled in the system. ModelQC provides a report on the quality control checks of each protein in the dataset.

Format

list

Examples

```
data(tmt_pd_model)
head(tmt_pd_model)
```

tmt_pd_summarized	<i>Example of TMT dataset processed using MSstatsTMT summarization function.</i>
-------------------	--

Description

Data includes one list with two data.tables named FeatureLevelData and ProteinLevelData. FeatureLevelData shows the unsummarized feature level data. ProteinLevelData shows the data summarized up to the protein level and is used for modeling the data.

Format

list

Examples

```
data(tmt_pd_summarized)
head(tmt_pd_summarized)
```

tmt_summarization_loop	<i>Main TMT summarization calculation function for MSstatsShiny application</i>
------------------------	---

Description

Main TMT function to calculate MSstatsShiny results.

Usage

```
tmt_summarization_loop(data, qc_input, loadpage_input, busy_indicator = TRUE)
```


Arguments

`data` Data converted into MSstats format.

`qc_input` options for data processing input by the user from data processing page.

`loadpage_input` options for data processing input by the user from data upload page.

`busy_indicator` Boolean indicator indicating whether or not to display shiny waiting indicator.

Value

list of TMT summarization results

Examples

```
data(raw.pd, package = "MSstatsTMT")
data(annotation.pd, package = "MSstatsTMT")

testdata <- MSstatsTMT::PDtoMSstatsTMTFormat(raw.pd,
                                              annotation.pd,
                                              use_log_file = FALSE
                                              )

qc_input = list()
loadpage_input = list()
qc_input$summarization = "msstats"
qc_input$norm = "equalizeMedians"
qc_input$log = 2
qc_input$names = NULL
qc_input$features_used = "all"
code_n_feat=3
qc_input$scensInt = "NA"
qc_input$MBi = TRUE
qc_input$remove50 = FALSE
qc_input$maxQC = 0.999
qc_input$null = FALSE
qc_input$null1 = FALSE
loadpage_input$DDA_DIA = "LF"
qc_input$global_norm = TRUE
qc_input$reference_norm = TRUE
qc_input$remove_norm_channel = TRUE
qc_input$maxQC1 = NULL
summarization_tmt_test = tmt_summarization_loop(testdata, qc_input,loadpage_input,
                                              busy_indicator = FALSE)
```

Description

This functions generates the UI object for MSstatsShiny app. Responsible for generating 5 nav pages homepage, data upload page, data processing page, statistical inference and future experiments.

Usage

```
uiObject()
```

Value

UI object for shinyUI

Examples

```
## Not run:  
library(shiny)  
shinyUI(uiObject())  
  
## End(Not run)
```

xy_str

Simple function to return coordinates

Description

Used in experimental design to create vizualization

Usage

```
xy_str(e)
```

Arguments

e input function provided by user

Value

Character with x and y coordinates

Examples

```
xy_str(list(x=5.0,y=2.0))
```

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