Package: Azimuth (via r-universe)

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Type Package

Title A Shiny App Demonstrating a Query-Reference Mapping Algorithm for Single-Cell Data

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Description Azimuth uses an annotated reference dataset to automate the processing, analysis, and interpretation of a new single-cell RNA-seq or ATAC-seq experiment. Azimuth leverages a 'reference-based mapping' pipeline that inputs a counts matrix and performs normalization, visualization, cell annotation, and differential expression (biomarker discovery).

URL https://github.com/satijalab/azimuth

BugReports https://github.com/satijalab/azimuth/issues

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Collate 'RcppExports.R' 'zzz.R' 'ui.R' 'helpers.R' 'azimuth.R'
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Contents

Azimuth-package   *Azimuth: A Shiny App Demonstrating a Query-Reference Mapping
                          Algorithm for Single-Cell Data*

Description

Azimuth uses an annotated reference dataset to automate the processing, analysis, and interpretation
of a new single-cell RNA-seq or ATAC-seq experiment. Azimuth leverages a 'reference-based map-
ping' pipeline that inputs a counts matrix and performs normalization, visualization, cell annotation,
and differential expression (biomarker discovery).

Package options

> **Azimuth** uses the following options to control the behavior of the app. Users can provide these as named arguments to `AzimuthApp` through dots (...), specify these in the config file, or configure these with `options`.

> **App options:** The following options control app behavior

> `Azimuth.app.default_adt` ADT to select by default in feature/violin plot

> `Azimuth.app.default_gene` Gene to select by default in feature/violin plot

> `Azimuth.app.default_metadata` Default metadata transferred from reference.

> `Azimuth.app.demodataset` Path to data file (in any Azimuth-supported format) to automatically load when the user clicks a button. The button is only available in the UI if this option is set

> `Azimuth.app.googlesheet` Google Sheet identifier (appropriate for use with `googlesheets4:gs4_get`) to write log records. Logging is only enabled if this and other google* options are set

> `Azimuth.app.googletoken` Path to directory containing Google Authentication token file. Logging is only enabled if this and other google* options are set

> `Azimuth.app.googletokenemail` Email address corresponding to the Google Authentication token file. Logging is only enabled if this and other google* options are set

> `Azimuth.app.max_cells` Maximum number of cells allowed to upload

> `Azimuth.app.metadata_notransfer` Metadata to annotate in reference but not transfer to query

> `Azimuth.app.mito` Regular expression pattern indicating mitochondrial features in query object

> `Azimuth.app.plotseed` Seed to shuffle colors for cell types

> `Azimuth.app.reference` URL or directory path to reference dataset; see `LoadReference` for more details

> `Azimuth.app.refuri` URL for publicly available reference dataset, used in the downloadable analysis script in case `Azimuth.app.reference` points to a directory

> `Azimuth.app.refdescriptor` Provide (as a string) the html to render the reference description on the welcome page

> `Azimuth.app.welcomebox` Provide (as a string) the code to render the box on the welcome page (quotes escaped). Example:
> ```
> box(
>   h3(\"Header\"),
>   \"body text\",
>   a(\"link\", href=\"www.satijalab.org\", target=\"_blank\"),
>   width = 12
> )
> ```

> `Azimuth.app.homologs` URL or path to file containing the human/mouse homolog table.

> `Azimuth.app.metatableheatmap` Display the meta.data table as a heatmap rather than in tabular form. defaults to FALSE.

> `Azimuth.app.overlayedreference` Display the mapped query on top of greyed out reference in the 'Cell Plots' tab. defaults to FALSE

> **Control options:** These options control mapping and analysis behavior

> `Azimuth.map.ncells` Minimum number of cells required to accept uploaded file defaults to 100

> `Azimuth.map.ngenes` Minimum number of genes in common with reference to accept uploaded file; defaults to 250

`Azimuth.map.nanchors` Minimum number of anchors that must be found to complete mapping. Defaults to 50

`Azimuth.map.panchorscolors` Configure the valuebox on the main page corresponding to the values for failure, warning, success for fraction of unique query cells that participate in anchor pairs. Failure corresponds to [0:Azimuth.map.fracanchorscolors[1]), warning to [Azimuth.map.fracanchorscolors[1]:Azimuth.map.fracanchorscolors[2]), and success is >= Azimuth.map.fracanchorscolors[2]. Defaults to c(5, 15)

`Azimuth.map.postmapqccolors` Configure the valuebox on the main page corresponding to the values for failure, warning, success for the post mapping cluster based QC metric. Failure corresponds to [0:Azimuth.map.postmapqc[1]), warning to [Azimuth.map.postmapqc[1]:Azimuth.map.postmapq and success is >= Azimuth.map.postmapqc[2]. Defaults to c(0.15, 0.25)

`Azimuth.map.postmapqcds` Set the amount of query random downsampling to perform before computing the mapping QC metric. Defaults to 5000

`Azimuth.map.ntrees` Annoy (approximate nearest neighbor) n.trees parameter Defaults to 20

`Azimuth.map.ndims` Number of dimensions to use in FindTransferAnchors and TransferData Defaults to 50

`Azimuth.de.mincells` Minimum number of cells per cluster for differential expression; defaults to 15

`Azimuth.de.digits` Number of digits to round differential expression table to; defaults to 3

`Azimuth.sct.ncells`, `Azimuth.sct.nfeats` Number of cells and features to use for [SCTransform](), respectively. Defaults to 2000 for each

External options: The following options are used by external dependencies that have an effect on **Azimuth**'s behavior. Refer to original package documentation for more details

[shiny.maxRequestSize]() User-configurable; used for controlling the maximum file size of uploaded datasets. Defaults to 500 Mb

[DT.options]() User-configurable; used for controlling biomarker table outputs. Defaults to setting `pageLength` to 10

[future.globals.maxSize]() **Non-configurable**; used for parallelization. Defaults to `Azimuth.app.max_cells * 320000`

Author(s)

Maintainer: Paul Hoffman <phoffman@nygenome.org> ([ORCID]())

Authors:

- Andrew Butler <abutler@nygenome.org> ([ORCID]())
- Charlotte Darby <cdarby@nygenome.org> ([ORCID]())
- Yuhan Hao <yhao@nygenome.org> ([ORCID]())
- Austin Hartman <ahartman@nygenome.org> ([ORCID]())
- Gesmira Molla <gmolla@nygenome.org> ([ORCID]())
- Rahul Satija <rsatija@nygenome.org> ([ORCID]())

Other contributors:

- Jaison Jain <jjain@nygenome.org> ([ORCID]()) [contributor]
- Satija Lab and Collaborators [funder]

See Also

Useful links:

- <https://github.com/satijalab/azimuth>
- Report bugs at <https://github.com/satijalab/azimuth/issues>

| AzimuthApp | *Launch the mapping app* |

Description

Launch the mapping app

Usage

```
AzimuthApp(config = NULL, ...)
```

Arguments

config	Path to JSON-formatted configuration file specifying options; for an example config file, see system.file("resources", "config.json", package = "Azimuth")
...	Options to set, see ?`Azimuth-package` for details on **Azimuth**-provided options

Value

None, launches the mapping Shiny app

Specifying options

R options can be provided as named arguments to AzimuthApp through dots (...), set in a config file, or set globally. Arguments provided to AzimuthApp through dots take precedence if the same option is provided in a config file. Options provided through dots or a config file take precedence if the same option was set globally.

Options in the Azimuth.app namespace can be specified using a shorthand notation in both the config file and as arguments to AzimuthApp. For example, the option Azimuth.app.reference can be shortened to reference in the config file or as an argument to AzimuthApp

See Also

Azimuth-package

Examples

```
if (interactive()) {
  AzimuthApp(system.file("resources", "config.json", package = "Azimuth"))
}
```

AzimuthBridgeReference

Create a Seurat object compatible with Azimuth.

Description

Create a Seurat object compatible with Azimuth.

Usage

```
AzimuthBridgeReference(
  object,
  reference.reduction = "spca",
  bridge.ref.reduction = "ref.spca",
  bridge.query.reduction = "slsi",
  laplacian.reduction = "lap",
  refUMAP = "wnn.umap",
  refAssay = "SCT",
  dims = 1:50,
  plotref = "wnn.umap",
  plot.metadata = NULL,
  ori.index = NULL,
  colormap = NULL,
  assays = c("Bridge", "RNA"),
  metadata = NULL,
  reference.version = "0.0.0",
  verbose = FALSE
)
```

Arguments

object	Seurat object
refUMAP	Name of UMAP in reference to use for mapping
refAssay	Name of SCTAssay to use in reference
dims	Dimensions to use in reference neighbor finding
plotref	Either the name of the DimReduc in the provided Seurat object to use for the plotting reference or the DimReduc object itself.
plot.metadata	A data.frame of discrete metadata fields for the cells in the plotref.
ori.index	Index of the cells used in mapping in the original object on which UMAP was run. Only need to provide if UMAP was run on different set of cells.
colormap	A list of named and ordered vectors specifying the colors and levels for the metadata. See `CreateColorMap` for help generating your own.
assays	Assays to retain for transfer
metadata	Metadata to retain for transfer

reference.version	
	Version of the Azimuth reference
verbose	Display progress/messages
refDR	Name of DimReduc in reference to use for mapping
k.param	Defines k for the k-nearest neighbor algorithm

Value

Returns a Seurat object with AzimuthData stored in the tools slot for use with Azimuth.

AzimuthData-class *AzimuthData*

Description

The AzimuthData class is used to store reference info needed for Azimuth

Slots

plotref DimReduc object containing UMAP for plotting and projection. This should also contain
the cell IDs in the misc slot

colormap Vector of id-color mapping for specifying the plots.

seurat.version Version of Seurat used in reference construction

azimuth.version Version of Azimuth used in reference construction

reference.version Version of the Azimuth reference

AzimuthReference *Create a Seurat object compatible with Azimuth.*

Description

Create a Seurat object compatible with Azimuth.

Usage

```
AzimuthReference(
  object,
  refUMAP = "umap",
  refDR = "spca",
  refAssay = "SCT",
  dims = 1:50,
  k.param = 31,
  plotref = "umap",
  plot.metadata = NULL,
```

```
        ori.index = NULL,
        colormap = NULL,
        assays = NULL,
        metadata = NULL,
        reference.version = "0.0.0",
        verbose = FALSE
    )
```

Arguments

object	Seurat object
refUMAP	Name of UMAP in reference to use for mapping
refDR	Name of DimReduc in reference to use for mapping
refAssay	Name of SCTAssay to use in reference
dims	Dimensions to use in reference neighbor finding
k.param	Defines k for the k-nearest neighbor algorithm
plotref	Either the name of the DimReduc in the provided Seurat object to use for the plotting reference or the DimReduc object itself.
plot.metadata	A data.frame of discrete metadata fields for the cells in the plotref.
ori.index	Index of the cells used in mapping in the original object on which UMAP was run. Only need to provide if UMAP was run on different set of cells.
colormap	A list of named and ordered vectors specifying the colors and levels for the metadata. See `CreateColorMap` for help generating your own.
assays	Assays to retain for transfer
metadata	Metadata to retain for transfer
reference.version	
	Version of the Azimuth reference
verbose	Display progress/messages

Value

Returns a Seurat object with AzimuthData stored in the tools slot for use with Azimuth.

ConvertGeneNames	*Converts gene names of query to match type/species of reference names (human or mouse).*

Description

Converts gene names of query to match type/species of reference names (human or mouse).

Usage

```
ConvertGeneNames(object, reference.names, homolog.table)
```

Arguments

`object`	Object to convert, must contain only RNA counts matrix
`reference.names`	
	Gene names of reference
`homolog.table`	Location of file (or URL) containing table with human/mouse homologies

Value

query object with converted feature names, likely subsetted

`CreateAzimuthData`   *Create an* [`AzimuthData`] *object*

Description

Create an auxiliary [`AzimuthData`] object for storing necessary info when generating an Azimuth reference.

Usage

```
CreateAzimuthData(
  object,
  plotref = "umap",
  plot.metadata = NULL,
  colormap = NULL,
  reference.version = "0.0.0"
)
```

Arguments

`object`	Seurat object
`plotref`	Either the name of the DimReduc in the provided Seurat object to use for the plotting reference or the DimReduc object itself.
`plot.metadata`	A data.frame of discrete metadata fields for the cells in the plotref.
`colormap`	A list of named and ordered vectors specifying the colors and levels for the metadata. See [`CreateColorMap`] for help generating your own.
`reference.version`	
	Version of the Azimuth reference

Value

Returns an [`AzimuthData`] object

CreateColorMap   *Create A Color Map*

Description

Create mapping between IDs and colors to use with reference plotting in Azimuth

Usage

```
CreateColorMap(object, ids = NULL, colors = NULL, seed = NULL)
```

Arguments

object   Seurat object

ids   Vector of IDs to link to colors

colors   Vector of colors to use

seed   Set to randomly shuffle color assignments

Value

A named vector of colors

GetColorMap   *Get Azimuth color mapping*

Description

Pull ID-color mapping for Azimuth plotting

Usage

```
GetColorMap(object, ...)

## S3 method for class 'AzimuthData'
GetColorMap(object, ...)

## S3 method for class 'Seurat'
GetColorMap(object, slot = "AzimuthReference", ...)
```

Arguments

object   An object

...   Arguments passed to other methods

slot   Name of tool

Value

A named vector specifying the colors for all reference IDs

GetPlotRef   *Get Azimuth plotref*

Description

Pull DimReduc used in Azimuth plotting/projection

Usage

```
GetPlotRef(object, ...)

## S3 method for class 'AzimuthData'
GetPlotRef(object, ...)

## S3 method for class 'Seurat'
GetPlotRef(object, slot = "AzimuthReference", ...)
```

Arguments

object	An object
...	Arguments passed to other methods
slot	Name of tool

Value

A DimReduc object

GetTranscripts   *Get transcripts modified from Signac::GeneActivity*

Description

Get transcripts modified from Signac::GeneActivity

Usage

```
GetTranscripts(
  object,
  assay = NULL,
  features = NULL,
  extend.upstream = 2000,
  extend.downstream = 0,
  biotypes = "protein_coding",
  max.width = 5e+05,
  process_n = 2000,
  gene.id = FALSE,
  verbose = TRUE
)
```

Arguments

`object`	A Seurat object
`assay`	Name of assay to use. If NULL, use the default assay
`features`	Genes to include. If NULL, use all protein-coding genes in the annotations stored in the object
`extend.upstream`	
	Number of bases to extend upstream of the TSS
`extend.downstream`	
	Number of bases to extend downstream of the TTS
`biotypes`	Gene biotypes to include. If NULL, use all biotypes in the gene annotation.
`max.width`	Maximum allowed gene width for a gene to be quantified. Setting this parameter can avoid quantifying extremely long transcripts that can add a relatively long amount of time. If NULL, do not filter genes based on width.
`process_n`	Number of regions to load into memory at a time, per thread. Processing more regions at once can be faster but uses more memory.
`gene.id`	Record gene IDs in output matrix rather than gene name.
`verbose`	

Value

Transcripts

LoadBridgeReference   *Load the extended reference RDS file for bridge integration*

Description

Read in a precomputed extended reference. This function can read either from URLs or a file path. The function looks for a file called ext.Rds for the extended reference `Seurat` object

Usage

```
LoadBridgeReference(path, seconds = 10L)
```

Arguments

path	Path or URL to the RDS file
seconds	Timeout to check for URLs in seconds

Value

A list with two entries:

map The extended reference [Seurat](#) object

plot The reference Seurat object (for plotting)

Examples

```
## Not run:
# Load from a URL
ref <- LoadBridgeReference("https://seurat.nygenome.org/references/pbmc")
# Load a file from the path to a directory
ref2 <- LoadBridgeReference("path/")
# Load a file directly
ref3 <- LoadBridgeReference("ext.Rds")

## End(Not run)
```

LoadFileInput   *Load file input into a* Seurat *object*

Description

Take a file and load it into a [Seurat](#) object. Supports a variety of file types and always returns a
Seurat object

Usage

```
LoadFileInput(path, bridge = FALSE)
```

Arguments

path	Path to input data

Details

LoadFileInput supports several file types to be read in as Seurat objects. File type is determined
by extension, matched in a case-insensitive manner See sections below for details about supported
filtypes, required extension, and specifics for how data is loaded

Value

A [Seurat](#) object

10X H5 File (extension h5**)**

10X HDF5 files are supported for all versions of Cell Ranger; data is read in using [Read10X_h5](#).
Note: for multi-modal 10X HDF5 files, only the *first* matrix is read in

Rds File (extension rds**)**

Rds files are supported as long as they contain one of the following data types:

- A [Seurat](#) V3 object
- An S4 [Matrix](#) object
- An S3 [matrix](#) object
- A [data.frame](#) object

For S4 Matrix, S3 matrix, and data.frame objects, a Seurat object will be made with [CreateSeuratObject](#) using the default arguments

h5Seurat File (extension h5seurat**)**

h5Seurat files and all of their features are fully supported. They are read in via [LoadH5Seurat](#).
Note: only the "counts" matrices are read in and only the default assay is kept

AnnData H5AD File (extension h5ad**)**

Only H5AD files from AnnData v0.7 or higher are supported. Data is read from the H5AD file in
the following manner

- The counts matrix is read from "/raw/X"; if "/raw/X" is not present, the matrix is read from
  "/X"
- Feature names are read from feature-level metadata. Feature level metadata must be an HDF5
  group, HDF5 compound datasets are **not** supported. If counts are read from /raw/X, features
  names are looked for in "/raw/var"; if counts are read from "/X", features names are looked
  for in "/var". In both cases, feature names are read from the dataset specified by the "_index"
  attribute, "_index" dataset, or "index" dataset, in that order
- Cell names are read from cell-level metadata. Cell-level metadata must be an HDF5 group,
  HDF5 compound datasets are **not** supported. Cell-level metadata is read from "/obs". Cell
  names are read from the dataset specified by the "_index" attribute, "_index" dataset, or "in-
  dex" dataset, in that order
- Cell-level metadata is read from the "/obs" dataset. Columns will be returned in the same order
  as in the "column-order", if present, or in alphabetical order. If a dataset named "__categories"
  is present, then all datasets in "__categories" will serve as factor levels for datasets present in
  "/obs" with the same name (eg. a dataset named "/obs/__categories/leiden" will serve as the
  levels for "/obs/leiden"). Row names will be set as cell names as described above. All datasets
  in "/obs" will be loaded except for "__categories" and the cell names dataset

---

LoadH5ADobs *Load obs from a H5AD file*

Description

Read in only the metadata of an H5AD file and return a data.frame object

Usage

```
LoadH5ADobs(path, cell.groups = NULL)
```

LoadReference *Load the reference RDS files*

Description

Read in a reference [Seurat](#) object and annoy index. This function can read either from URLs or a file path. In order to read properly, there must be the following files:

- "ref.Rds" for the downsampled reference Seurat object (for mapping)
- "idx.annoy" for the nearest-neighbor index object

Usage

```
LoadReference(path, seconds = 10L)
```

Arguments

path	Path or URL to the two RDS files
seconds	Timeout to check for URLs in seconds

Value

A list with two entries:

map The downsampled reference [Seurat](#) object (for mapping)

plot The reference Seurat object (for plotting)

Examples

```
## Not run:
# Load from a URL
ref <- LoadReference("https://seurat.nygenome.org/references/pbmc")
# Load from a directory
ref2 <- LoadReference("/var/www/html")

## End(Not run)
```

ReferenceVersion   *Get Azimuth reference version number*

Description

Pull the reference version information

Usage

```
ReferenceVersion(object, ...)

## S3 method for class 'AzimuthData'
ReferenceVersion(object, ...)

## S3 method for class 'Seurat'
ReferenceVersion(object, slot = "AzimuthReference", ...)
```

Arguments

object	Seurat or AzimuthData object
...	Not used
slot	Name of the version to pull. Can be "seurat.version", "azimuth.version", or "reference.version".

Value

A character string specifying the reference version

RunAzimuth.Seurat   *Run Azimuth annotation*

Description

Run Azimuth annotation

Usage

```
## S3 method for class 'Seurat'
RunAzimuth(
  query,
  reference,
  query.modality = "RNA",
  annotation.levels = NULL,
  umap.name = "ref.umap",
  do.adt = FALSE,
```

```
    verbose = TRUE,
    assay = NULL,
    k.weight = 50,
    n.trees = 20,
    mapping.score.k = 100,
    ...
)

## S3 method for class 'character'
RunAzimuth(query, ...)

RunAzimuth(query, ...)
```

Arguments

query
: Seurat object or following type of path:

    - A .h5 matrix
    - A .rds file containing a Seurat object
    - A .h5ad anndata object
    - A .h5seurat object

reference
: Name of reference to map to or a path to a directory containing ref.Rds and idx.annoy

annotation.levels
: list of annotation levels to map. If not specified, all will be mapped.

umap.name
: name of umap reduction in the returned object

do.adt
: transfer ADT assay

assay
: query assay name

Value

Seurat object with reference reductions and annotations

Returns a Seurat object containing celltype annotations

RunAzimuthATAC.Seurat *Run Azimuth annotation for ATAC query*

Description

Run Azimuth annotation for ATAC query

Usage

```
## S3 method for class 'Seurat'
RunAzimuthATAC(
  query,
  reference,
  fragment.path = NULL,
  annotation.levels = NULL,
  umap.name = "ref.umap",
  verbose = TRUE,
  assay = NULL,
  k.weight = 50,
  n.trees = 20,
  mapping.score.k = 100,
  dims.atac = 2:50,
  dims.rna = 1:50
)

## S3 method for class 'character'
RunAzimuthATAC(query, ...)

RunAzimuthATAC(query, ...)
```

Arguments

query   Seurat object or following type of path:

- A .h5 matrix
- A .rds file containing a Seurat object
- A .h5ad anndata object
- A .h5seurat object

reference   Name of reference to map to or a path to a directory containing ext.Rds

annotation.levels

                    list of annotation levels to map. If not specified, all will be mapped.

umap.name   name of umap reduction in the returned object

assay   query assay name

dims.atac   dimensions

dims.rna   dimensions

do.adt   transfer ADT assay

Value

Seurat object with reference reductions and annotations

Returns a Seurat object containing celltype annotations

SaveAzimuthReference   *Save* Azimuth *references and neighbors index to same folder*

Description

Save Azimuth references and neighbors index to same folder

Usage

```
SaveAzimuthReference(object = NULL, folder = NULL)
```

Arguments

object	An [Azimuth](#) reference
file	Path to save Azimuth reference to; defaults to file.path(getwd(), "azimuth_reference/"))
...	Arguments passed on to [base::saveRDS](#)
	ascii a logical. If TRUE or NA, an ASCII representation is written; otherwise (default), a binary one is used. See the comments in the help for [save](#).
	version the workspace format version to use. NULL specifies the current default version (3). The only other supported value is 2, the default from R 1.4.0 to R 3.5.0.
	compress a logical specifying whether saving to a named file is to use "gzip" compression, or one of "gzip", "bzip2" or "xz" to indicate the type of compression to be used. Ignored if file is a connection.
	refhook a hook function for handling reference objects.

Value

Invisibly returns file

See Also

[saveRDS()](#) [readRDS()](#)

Examples

```
# Make Azimuth Reference object
obj.azimuth <- AzimuthReference(object)

# Save
SaveAzimuthReference(object = obj.azimuth, folder = "azimuth_reference")

# Run Azimuth

query <- RunAzimuth(query = query,
                    reference = "azimuth_reference",
                    ...)
```

SetColorMap   *Set Azimuth color mapping*

Description

Set ID-color mapping for Azimuth plotting

Usage

```
SetColorMap(object, ...)

## S3 method for class 'AzimuthData'
SetColorMap(object, value, ...)

## S3 method for class 'Seurat'
SetColorMap(object, slot = "AzimuthReference", value, ...)
```

Arguments

object	An object
...	Arguments passed to other methods
value	New colormap to assign
slot	Name of tool

Value

An object with the colormap slot set

ValidateAzimuthReference
                       Validate References for Azimuth

Description

Validate aspects of a Seurat object to be used as an Azimuth reference

Usage

```
ValidateAzimuthReference(object, ad.name = "AzimuthReference")
```

Arguments

`object`	Seurat object
`ad.name`	Name in the tools slot containing the AzimuthData object.

Value

No return value

Index