

Package ‘AMARETTO’

November 18, 2025

Type Package

Title Regulatory Network Inference and Driver Gene Evaluation using
Integrative Multi-Omics Analysis and Penalized Regression

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Depends R (>= 3.6), impute, doParallel, grDevices, dplyr, methods,
ComplexHeatmap

Description Integrating an increasing number of available multi-omics cancer data remains one of the main challenges to improve our understanding of cancer. One of the main challenges is using multi-omics data for identifying novel cancer driver genes. We have developed an algorithm, called AMARETTO, that integrates copy number, DNA methylation and gene expression data to identify a set of driver genes by analyzing cancer samples and connects them to clusters of co-expressed genes, which we define as modules. We applied AMARETTO in a pancancer setting to identify cancer driver genes and their modules on multiple cancer sites. AMARETTO captures modules enriched in angiogenesis, cell cycle and EMT, and modules that accurately predict survival and molecular subtypes. This allows AMARETTO to identify novel cancer driver genes directing canonical cancer pathways.

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LazyLoad yes

LazyData true

Encoding UTF-8

biocViews

StatisticalMethod,DifferentialMethylation,GeneRegulation,GeneExpression,MethylationArray,Transcription,Preprocessing

Suggests testthat, MASS, knitr, BiocStyle

NeedsCompilation no

Imports callr (>= 3.0.0.9001), Matrix, Rcpp, BiocFileCache, DT,
MultiAssayExperiment, circlize, curatedTCGAData, foreach,
glmnet, httr, limma, matrixStats, readr, reshape2, tibble,
rmarkdown, graphics, grid, parallel, stats, knitr, ggplot2,
gridExtra, utils

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AMARETTO_CreateModuleData
<i>AMARETTO_CreateModuleData</i>

Description

AMARETTO_CreateModuleData

Usage

AMARETTO_CreateModuleData(AMARETTOinit, AMARETTOresults)

Arguments

- AMARETTOinit List output from AMARETTO_Initialize().
- AMARETTOresults List output from AMARETTO_Run()

Value

result

Examples

```
data('ProcessedDataLIHC')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   NrModules = 2, VarPercentage = 50)
AMARETTOresults <- AMARETTO_Run(AMARETTOinit)
AMARETTO_MD <- AMARETTO_CreateModuleData(AMARETTOinit, AMARETTOresults)
```

AMARETTO_CreateRegulatorPrograms

AMARETTO_CreateRegulatorPrograms

Description

AMARETTO_CreateRegulatorPrograms

Usage

```
AMARETTO_CreateRegulatorPrograms(AMARETTOinit, AMARETTOresults)
```

Arguments

AMARETTOinit List output from AMARETTO_Initialize().

AMARETTOresults
 List output from AMARETTO_Run()

Value

result

Examples

```
data('ProcessedDataLIHC')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   NrModules = 2, VarPercentage = 50)
AMARETTOresults <- AMARETTO_Run(AMARETTOinit)
AMARETTO_RP <- AMARETTO_CreateRegulatorPrograms(AMARETTOinit,AMARETTOresults)
```

AMARETTO_Download	<i>AMARETTO_Download</i>
-------------------	--------------------------

Description

Downloading TCGA dataset for AMARETTO analysis

Usage

```
AMARETTO_Download(CancerSite = "CHOL",
  TargetDirectory = TargetDirectory)
```

Arguments

CancerSite	TCGA cancer code for data download
TargetDirectory	Directory path to download data

Value

result

Examples

```
TargetDirectory <- file.path(getwd(),"Downloads/");dir.create(TargetDirectory)
CancerSite <- 'CHOL'
DataSetDirectories <- AMARETTO_Download(CancerSite,TargetDirectory = TargetDirectory)
```

AMARETTO_EvaluateTestSet	<i>AMARETTO_EvaluateTestSet</i>
--------------------------	---------------------------------

Description

Code to evaluate AMARETTO on a new gene expression test set. Uses output from AMARETTO_Run() and CreateRegulatorData().

Usage

```
AMARETTO_EvaluateTestSet(AMARETTOresults = AMARETTOresults,
  MA_Data_TestSet = MA_Data_TestSet,
  RegulatorData_TestSet = RegulatorData_TestSet)
```

Arguments

AMARETTOresults
 AMARETTO output from AMARETTO_Run().

MA_Data_TestSet
 Gene expression matrix from a test set (that was not used in AMARETTO_Run()).

RegulatorData_TestSet
 Test regulator data from CreateRegulatorData().

Value

result

Examples

```
data('ProcessedDataLIHC')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                     NrModules = 2, VarPercentage = 50)

AMARETTOresults <- AMARETTO_Run(AMARETTOinit)
AMARETTOtestReport <- AMARETTO_EvaluateTestSet(AMARETTOresults = AMARETTOresults,
                                                MA_Data_TestSet = AMARETTOinit$MA_matrix_Var,
                                                RegulatorData_TestSet = AMARETTOinit$RegulatorData)
```

AMARETTO_ExportResults

AMARETTO_ExportResults

Description

Retrieve a download of all the data linked with the run (including heatmaps)

Usage

```
AMARETTO_ExportResults(AMARETTOinit, AMARETTOresults, data_address,
                        Heatmaps = TRUE, CNV_matrix = NULL, MET_matrix = NULL)
```

Arguments

AMARETTOinit AMARETTO initialize output

AMARETTOresults
 AMARETTO results output

data_address Directory to save data folder

Heatmaps Output heatmaps as pdf

CNV_matrix CNV_matrix

MET_matrix MET_matrix

Value

result

Examples

```
data('ProcessedDataLIHC')
TargetDirectory <- file.path(getwd(),"Downloads/");dir.create(TargetDirectory)
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   NrModules = 2, VarPercentage = 50)

AMARETTOresults <- AMARETTO_Run(AMARETTOinit)
AMARETTO_ExportResults(AMARETTOinit,AMARETTOresults,TargetDirectory,Heatmaps = FALSE)
```

AMARETTO_HTMLreport	<i>AMARETTO_HTMLreport</i>
---------------------	----------------------------

Description

Retrieve an interactive html report, including gene set enrichment analysis if asked for.

Usage

```
AMARETTO_HTMLreport(AMARETTOinit, AMARETTOresults, ProcessedData,
  show_row_names = FALSE, SAMPLE_annotation = NULL, ID = NULL,
  hyper_geo_test_bool = FALSE, hyper_geo_reference = NULL,
  output_address = "./", MSIGDB = TRUE, driverGSEA = TRUE,
  phenotype_association_table = NULL)
```

Arguments

AMARETTOinit	AMARETTO initialize output
AMARETTOresults	AMARETTO results output
ProcessedData	List of processed input data
show_row_names	if True, sample names will appear in the heatmap
SAMPLE_annotation	SAMPLE annotation will be added to heatmap
ID	ID column of the SAMPLE annotation data frame
hyper_geo_test_bool	Boolean if a hyper geometric test needs to be performed. If TRUE provide a GMT file in the hyper_geo_reference parameter.
hyper_geo_reference	GMT file with gene sets to compare with.
output_address	Output directory for the html files.
MSIGDB	TRUE if gene sets were retrieved from MSIGDB. Links will be created in the report.

driverGSEA if TRUE, module drivers will also be included in the hypergeometric test.
 phenotype_association_table
 a Data Frame, containing all modules phenotype association data. Optional.

Value

result

Examples

```
## Not run:
data('ProcessedDataLIHC')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   NrModules = 2, VarPercentage = 50)

AMARETTOresults <- AMARETTO_Run(AMARETTOinit)

AMARETTO_HTMLreport(AMARETTOinit= AMARETTOinit,AMARETTOresults= AMARETTOresults,
                    ProcessedData = ProcessedDataLIHC,
                    hyper_geo_test_bool=FALSE,
                    output_address='./')

## End(Not run)
```

AMARETTO_Initialize *AMARETTO_Initialize (version: reorder and filter MA_Matrix)*

Description

Code used to initialize the seed clusters for an AMARETTO run. Requires processed gene expressions (rna-seq or microarray), CNV (usually from a GISTIC run), and methylation (from MethylMix, provided in this package) data. Uses the function CreateRegulatorData() and results are fed into the function AMARETTO_Run().

Usage

```
AMARETTO_Initialize(ProcessedData = ProcessedData, Driver_list = NULL,
                    NrModules, VarPercentage, PvalueThreshold = 0.001,
                    RsquareThreshold = 0.1, pmax = 10, NrCores = 1, OneRunStop = 0,
                    method = "union", random_seeds = NULL, convergence_cutoff = 0.01)
```

Arguments

ProcessedData List of Expression, CNV and MethylMix data matrices, with genes in rows and samples in columns.

Driver_list Custom list of driver genes to be considered in analysis

NrModules	How many gene co-expression modules should AMARETTO search for? Usually around 100 is acceptable, given the large number of possible driver-passenger gene combinations.
VarPercentage	Minimum percentage by variance for filtering of genes; for example, 75% would indicate that the CreateRegulatorData() function only analyses genes that have a variance above the 75th percentile across all samples.
PvalueThreshold	Threshold used to find relevant driver genes with CNV alterations: maximal p-value.
RsquareThreshold	Threshold used to find relevant driver genes with CNV alterations: minimal R-square value between CNV and gene expression data.
pmax	'pmax' variable for glmnet function from glmnet package; the maximum number of variables ever to be nonzero. Should not be changed by user unless she/he fully understands the AMARETTO algorithm and how its parameters choices affect model output.
NrCores	A numeric variable indicating the number of computer/server cores to use for parallelization. Default is 1, i.e. no parallelization. Please check your computer or server's computing capacities before increasing this number. Parallelization is done via the RParallel package. Mac vs. Windows environments may behave differently when using parallelization.
OneRunStop method	OneRunStop Perform union or intersection of the driver genes evaluated from the input data matrices and custom driver gene list provided.
random_seeds	A numeric vector of length 2, containing two seed numbers for randomization : 1st for kmeans and 2nd for glmnet
convergence_cutoff	A numeric value (E.g. 0.01) representing the fraction of the total number of genes, in which, The algorithm is considered reaching convergence and will stop, if Nr of Gene-replacements in an iteration falls below this threshold * total number of genes.

Value

result

Examples

```

data('ProcessedDataLIHC')
data('Driver_Genes')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   NrModules = 2, VarPercentage = 50)

## Not run:
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                   Driver_list = Driver_Genes[['MSigDB']],
                                   NrModules = 2, VarPercentage = 50)

## End(Not run)

```

AMARETTO_LarsenBased	<i>AMARETTO_LarsenBased</i>
----------------------	-----------------------------

Description

AMARETTO_LarsenBased

Usage

AMARETTO_LarsenBased(Data, Clusters, RegulatorData, Parameters, NrCores, random_seeds, convergence_cutoff)

Arguments

- Data
- Clusters
- RegulatorData
- Parameters
- NrCores
- random_seeds
- convergence_cutoff

Value

result

AMARETTO_LearnRegulatoryProgramsLarsen	<i>AMARETTO_LearnRegulatoryProgramsLarsen</i>
--	---

Description

AMARETTO_LearnRegulatoryProgramsLarsen

Usage

AMARETTO_LearnRegulatoryProgramsLarsen(Data, Clusters, RegulatorData, RegulatorSign, Lambda, AutoRegulation, alpha, pmax, random_seeds)

Value

result

AMARETTO_Preprocess	<i>AMARETTO_Preprocess</i>
---------------------	----------------------------

Description

Wrapper code that analyzes process TCGA GISTIC (CNV) and gene expression (rna-seq or microarray) data via one call

Usage

```
AMARETTO_Preprocess(DataSetDirectories = DataSetDirectories,
  BatchData = BatchData)
```

Arguments

DataSetDirectories	
	DataSetDirectories
BatchData	BatchData

Value

result

Examples

```
## Not run:
TargetDirectory <- "Downloads" # path to data download directory
CancerSite <- 'CHOL'
DataSetDirectories <- AMARETTO_Download(CancerSite,TargetDirectory)
ProcessedData <- AMARETTO_Preprocess(DataSetDirectories,BatchData)

## End(Not run)
```

AMARETTO_ReassignGenesToClusters	
	<i>AMARETTO_ReassignGenesToClusters</i>

Description

AMARETTO_ReassignGenesToClusters

Usage

```
AMARETTO_ReassignGenesToClusters(Data, RegulatorData, Beta, Clusters,
  AutoRegulation)
```


Arguments

AMARETTOinit List output from AMARETTO_Initialize().
 AMARETTOresults List output from AMARETTO_Run().
 ProcessedData List of processed input data
 ModuleNr Module number to visualize
 show_row_names If TRUE, row names will be shown on the plot.
 SAMPLE_annotation Matrix or Dataframe with sample annotation
 ID Column used as sample name
 order_samples Order samples in heatmap by mean or by clustering

Value

result

Examples

```

data('ProcessedDataLIHC')
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,
                                     NrModules = 2, VarPercentage = 50)

AMARETTOresults <- AMARETTO_Run(AMARETTOinit)

AMARETTO_VisualizeModule(AMARETTOinit = AMARETTOinit, AMARETTOresults = AMARETTOresults,
                          ProcessedData = ProcessedDataLIHC, ModuleNr = 1)

```

aprior

aprior

Description

Following four find empirical hyper-prior values

Usage

```
aprior(gamma.hat)
```

Value

result

BatchData	<i>BatchData</i>
-----------	------------------

Description

A dataset for conducting batch corerction in TCGA samples

Usage

BatchData

Format

A data frame with 23263 observations and 3 variables:

Source

AMARETTO

Beta.NA	<i>Beta.NA</i>
---------	----------------

Description

Beta.NA

Usage

Beta.NA(y, X)

Value

result

bprior	<i>bprior</i>
--------	---------------

Description

bprior

Usage

bprior(gamma.hat)

Value

result

build.design	<i>build.design</i>
--------------	---------------------

Description

Next two functions make the design matrix (X) from the sample info file

Usage

```
build.design(vec, des = NULL, start = 2)
```

Value

result

cacheResource	<i>cacheResource</i>
---------------	----------------------

Description

cacheResource

Usage

```
cacheResource(TargetDirectory = TargetDirectory, resource = resource)
```

Value

result

ComBat_NoFiles	<i>ComBat_NoFiles</i>
----------------	-----------------------

Description

ComBat_NoFiles

Usage

```
ComBat_NoFiles(dat, saminfo, type = "txt", write = FALSE,  
  covariates = "all", par.prior = TRUE, filter = FALSE, skip = 0,  
  prior.plots = FALSE)
```

Value

result

computeGisticURL	<i>computeGisticURL</i>
------------------	-------------------------

Description

computeGisticURL

Usage

```
computeGisticURL(url = NULL, acronym = "CHOL")
```

Value

result

CreateRegulatorData	<i>CreateRegulatorData</i>
---------------------	----------------------------

Description

Determine potential regulator genes.

Usage

```
CreateRegulatorData(MA_matrix = MA_matrix, CNV_matrix = NULL,  
  MET_matrix = NULL, Driver_list = NULL, PvalueThreshold = 0.001,  
  RsquareThreshold = 0.1, method = "union")
```

Value

result

design.mat	<i>design.mat</i>
------------	-------------------

Description

design.mat

Usage

```
design.mat(saminfo)
```

Value

result

Driver_Genes	<i>Driver_Genes</i>
--------------	---------------------

Description

A list of cancer driver genes described in literature.

Usage

Driver_Genes

Format

List

Source

AMARETTO

filter.absent	<i>filter.absent</i>
---------------	----------------------

Description

filters data based on presence/absence call

Usage

```
## S3 method for class 'absent'
filter(x, pct)
```

Value

result

FindTranscriptionallyPredictive_CNV
<i>FindTranscriptionallyPredictive_CNV</i>

Description

Function to identify which genes CNV significantly predict expression of that gene.

Usage

```
FindTranscriptionallyPredictive_CNV(MA_matrix, CNV_matrix,  
  PvalueThreshold = 0.001, RsquareThreshold = 0.1)
```

Value

result

geneFiltering	<i>geneFiltering</i>
---------------	----------------------

Description

Function to filter gene expression matrix

Usage

```
geneFiltering(Type, MAdat, Percentage)
```

Value

result

GeneSetDescription	<i>GeneSetDescription</i>
--------------------	---------------------------

Description

GeneSetDescription

Usage

GeneSetDescription(filename, MSIGDB)

Arguments

- filename The name of the gmt file.
- MSIGDB If True, the gene set description column will be provided from MSIGDB.

Value

result

get_firehoseData	<i>get_firehoseData</i>
------------------	-------------------------

Description

Downloading TCGA dataset via firehose

Usage

```
get_firehoseData(TargetDirectory = "./",
  TCGA_acronym_uppercase = "LUAD", dataType = "stddata",
  dataFileTag = "mRNAseq_Preprocess.Level_3", FFPE = FALSE,
  fileType = "tar.gz",
  gdacURL = "http://gdac.broadinstitute.org/runs/", untarUngzip = TRUE,
  printDisease_abbr = FALSE)
```

Value

result

GmtFromModules	<i>GmtFromModules</i>
----------------	-----------------------

Description
GmtFromModules

Usage
GmtFromModules(AMARETTOinit, AMARETTOresults, driverGSEA)

Arguments
AMARETTOinit List output from AMARETTO_Initialize().
AMARETTOresults List output from AMARETTO_Run().
driverGSEA if TRUE , module driver genes will also be added to module target genes for GSEA.

Value
result

HyperGTestGeneEnrichment	<i>Hyper Geometric Geneset Enrichment Test</i>
--------------------------	--

Description
Calculates the p-values for unranked gene set enrichment based on two gmt files as input and the hyper geometric test.

Usage
HyperGTestGeneEnrichment(gmtfile, testgmtfile, NrCores, ref.numb.genes = 45956)

Arguments
gmtfile The gmt file with reference gene set.
testgmtfile The gmt file with gene sets to test. In our case, the gmt file of the modules.
NrCores Number of cores used for parallelization.
ref.numb.genes The total number of genes teste, standard equal to 45 956 (MSIGDB standard).

Value
result

int.eprior	<i>int.eprior</i>
------------	-------------------

Description

Monte Carlo integration function to find the nonparametric adjustments

Usage

int.eprior(sdat, g.hat, d.hat)

Value

result

it.sol	<i>it.sol</i>
--------	---------------

Description

Pass in entire data set, the design matrix for the entire data, the batch means, the batch variances, priors (m, t2, a, b), columns of the data matrix for the batch. Uses the EM to find the parametric batch adjustments

Usage

it.sol(sdat, g.hat, d.hat, g.bar, t2, a, b, conv = 1e-04)

Value

result

L	<i>L</i>
---	----------

Description

likelihood function

Usage

L(x, g.hat, d.hat)

Value

result

Lambda_Sequence	<i>Lambda_Sequence</i>
-----------------	------------------------

Description

Lambda_Sequence

Usage

Lambda_Sequence(sx, sy)

Value

result

list.batch	<i>list.batch</i>
------------	-------------------

Description

Makes a list with elements pointing to which array belongs to which batch

Usage

list.batch(saminfo)

Value

result

MsigdbMapping	<i>MsigdbMapping</i>
---------------	----------------------

Description

A dataset containing all MSIGDB pathways and their descriptions. .

Usage

MsigdbMapping

Format

List

Source

AMARETTO

plot_run_history	<i>Title plot_run_history</i>
------------------	-------------------------------

Description

Title plot_run_history

Usage

```
plot_run_history(AMARETTOinit, AMARETTOresults)
```

Arguments

AMARETTOinit AMARETTO initialize output
AMARETTOresults AMARETTO results output

Value

plot

Examples

```
data('ProcessedDataLIHC')  
AMARETTOinit <- AMARETTO_Initialize(ProcessedData = ProcessedDataLIHC,  
                                     NrModules = 2, VarPercentage = 50)  
  
AMARETTOresults <- AMARETTO_Run(AMARETTOinit)  
  
plot_run_history(AMARETTOinit,AMARETTOresults)
```

postmean	<i>postmean</i>
----------	-----------------

Description

postmean

Usage

```
postmean(g.hat, g.bar, n, d.star, t2)
```

Value

result

postvar	<i>postvar</i>
---------	----------------

Description

postvar

Usage

postvar(sum2, n, a, b)

Value

result

Preprocess_MAdata	<i>Preprocess_MAdata</i>
-------------------	--------------------------

Description

Preprocess_MAdata

Usage

Preprocess_MAdata(CancerSite = CancerSite, MAEO_ge = MAEO_ge,
BatchData = BatchData)

Value

result

printf	<i>printf</i>
--------	---------------

Description

Wrapper function for C-style formatted output.

Usage

printf(...)

Value

result

ProcessedDataLIHC	<i>ProcessedDataLIHC</i>
-------------------	--------------------------

Description

A list of dataframes of processed toy example dataset from TCGA-LIHC.

Usage

ProcessedDataLIHC

Format

List

Source

AMARETTO

readGMT	<i>readGMT</i>
---------	----------------

Description

readGMT

Usage

readGMT(filename)

Arguments

filename

Value

result

read_gct	<i>read_gct</i>
----------	-----------------

Description

Function to turn a .gct data files into a matrix format

Usage

```
read_gct(file_address)
```

Arguments

file_address Address of the input gct file.

Value

result

Examples

```
data_matrix<-read_gct(file_address="")
```

Save_CancerSite	<i>Save_CancerSite</i>
-----------------	------------------------

Description

Save_CancerSite

Usage

```
Save_CancerSite(CancerSite, TargetDirectory, DataSetDirectories,  
ProcessedData)
```

Value

result

TCGA_BatchCorrection_MolecularData
<i>TCGA_BatchCorrection_MolecularData</i>

Description

TCGA_BatchCorrection_MolecularData

Usage

```
TCGA_BatchCorrection_MolecularData(GEN_Data = GEN_Data,  
  BatchData = BatchData, MinInBatch = MinInBatch)
```

Value

result

TCGA_GENERIC_BatchCorrection
<i>TCGA_GENERIC_BatchCorrection</i>

Description

TCGA_GENERIC_BatchCorrection

Usage

```
TCGA_GENERIC_BatchCorrection(GEN_Data = GEN_Data,  
  BatchData = BatchData)
```

Value

result

TCGA_GENERIC_CheckBatchEffect
<i>TCGA_GENERIC_CheckBatchEffect</i>
Description
TCGA_GENERIC_CheckBatchEffect
Usage
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result
TCGA_GENERIC_CleanUpSampleNames
<i>TCGA_GENERIC_CleanUpSampleNames</i>
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TCGA_GENERIC_CleanUpSampleNames
Usage
TCGA_GENERIC_CleanUpSampleNames(GEN_Data = GEN_Data, IDlength = 12)
Value
result
TCGA_GENERIC_GetSampleGroups
<i>TCGA_GENERIC_GetSampleGroups</i>
Description
TCGA_GENERIC_GetSampleGroups
Usage
TCGA_GENERIC_GetSampleGroups(SampleNames)
Value
result

TCGA_GENERIC_MergeData
<i>TCGA_GENERIC_MergeData</i>

Description

TCGA_GENERIC_MergeData

Usage

TCGA_GENERIC_MergeData(NewIDListUnique, DataMatrix, MergeMethod)

Value

result

TCGA_Load_GISTICdata	<i>TCGA_Load_GISTICdata</i>
----------------------	-----------------------------

Description

TCGA_Load_GISTICdata

Usage

TCGA_Load_GISTICdata(GisticDirectory)

Value

result

TCGA_Load_MolecularData
<i>TCGA_Load_MolecularData</i>

Description

TCGA_Load_MolecularData

Usage

TCGA_Load_MolecularData(MAEO_ge)

Value

result

trim.dat	<i>trim.dat</i>
----------	-----------------

Description

Trims the data of extra columns, note your array names cannot be named 'X' or start with 'X.'

Usage

```
trim.dat(dat)
```

Value

result

write_gct	<i>write_gct</i>
-----------	------------------

Description

write_gct

Usage

```
write_gct(data_in, file_address)
```

Value

result

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