ARMT

Description

ARMT is auto RNA-seq data minning tool.

The aim of ARMT is:

- i) integrate and facilitate the downstream analysis of RNA-seq data,
- ii) provide the way to analyze genes set according to GSVA,
- iii) explore the correlation of gene expression and mutation.

Author:

Guanda Huang —— 202010108432@mail.scut.edu.cn

Hongli Du — hldu@scut.edu.cn

Year: 2021

License: GPL(>=3)

Function

Provide TCGA clinical data
Create .gmt file for arbitrary gene sets
Normalization of counts matrix (TPM)
Gene set variant analysis
Survival analysis
Differential analysis
Correlation analysis
Analysis for multiple sets of data
Plot the mutation information

Feature

Easy to use

Automation

Visualization

Comprehensiveness

Integrating GSVA score analysis

Catalog

ARN	ЛТ			
	Des	scription		
	Fun	nction	1	
	Fea	Feature		
	1.	The structure of ARMT	1	
	2.	Dependencies	1	
	3.	3. Installation		
	4.	Quick Star	2	
	5.	Graphical User Interface (GUI) of ARMT	2	
	6.	The page of ARMT		
		.6.1. Data	3	
		6.2. Normalization&GSVA	4	
		6.3. Integration&Analysis	4	
		6.3.1. Data Integration	4	
		6.3.2. Survival analysis&Cox proportional hazards regression analysis	5	
		6.3.3 Differential analysis	6	
		6.3.4. Enrichment analysis	8	
		6.3.5. Correlation analysis	9	
		6.4. Mutant mapping		
	7.	Format of Input File	13	
		7.1. Gene sets	13	
		7.2. Gene expression matrix	13	
		7.3. Gene mutation	14	
		7.4. GSVA score matrix		
		7.5. Clinical information		

1. The structure of ARMT

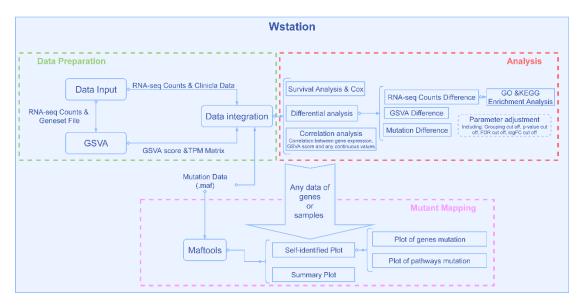


Figure 1. The workflow and structure of ARMT

2. Dependencies

R >=4.0.2, Rstudio, R packages("devtools" or "remotes")

The other dependent R packages are automatically installed, including:

maftools

GSVA

GSVAdata

limma

GSEAbase

org.Hs.eg.db

edgeR

survival

clusterProfiler

DOSE

. . .

By default, outdated dependencies are automatically upgraded. In interactive sessions you can select a subset of the dependencies to upgrade.

3. Installation

To install this package from Github, please, use the code below.

```
if (!requireNamespace("devtools", quietly = TRUE))
install.packages("devtools")
devtools::install_github("Dulab2020/ARMT")
```

4. Quick Star

The following commands should be used to start the graphical user interface (GUI).

ARMT::run_app()

5. Graphical User Interface (GUI) of ARMT

The GUI is developed based on 'shiny' package, and has four pages including: **Data, Normalization&GSVA, Integration&Analysis, Mutant mapping**

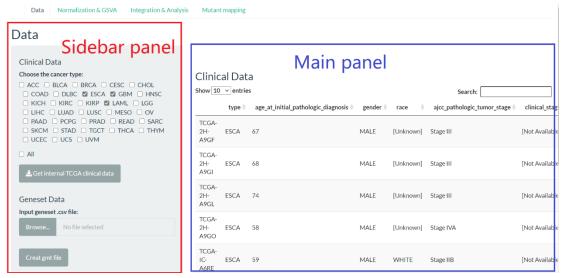


Figure 2. GUI of ARMT: The GUI consists of two parts, sidebar panel at left and main panel at right.

The sidebar panel is used to input data and adjust parameters; the main panel is used to demonstrate and save the results.

6. The page of ARMT

.6.1. Data

This page of ARMT provides TCGA clinical data and builds .gmt file of arbitrary gene sets. Through the internal datasets of ARMT, you can get TCGA clinical data of 33 cancer types. (*Figure2*)

To build a .gmt file, a .csv file should be provided, and it must contain two column as shown in *Figure3*.

geneset	genes
Glycolysis	ALDOA
Glycolysis	ALDOB
Glycolysis	ALDOC
Glycolysis	ENO1
Glycolysis	ENO2
Glycolysis	ENO3
Glycolysis	GAPDH
Glycolysis	GPI
Glycolysis	HK1
Glycolysis	HK2
Glycolysis	НК3
Glycolysis	LDHA
Glycolysis	PFKL
Glycolysis	PFKM
Glycolysis	PFKP
Glycolysis	PGAM1
Glycolysis	PGAM4
Glycolysis	PGK1
Glycolysis	PKLR
Glycolysis	PKM
Glycolysis	SLC2A1
Glycolysis	TPI1
Hypoxia	ACOT7
Hypoxia	ADM
Hypoxia	ALDOA
Hypoxia	CDKN3
Hypoxia	ENO1
Gure 3 The csv file	IDHA

Figure 3. The .csv file to create .gmt file

This file in *Figure3* has two columns. The first column declares gene sets. The second one contains genes in corresponding gene set.

6.2. Normalization&GSVA

In this page, ARMT automatically normalize the expression counts matrix to TPM matrix, and use log2(TPM) to process gene set variant analysis (GSVA). The input data must be gene expression matrix with **Ensembl** ID, and the ID can be transformed to **Symbol** through normalization.

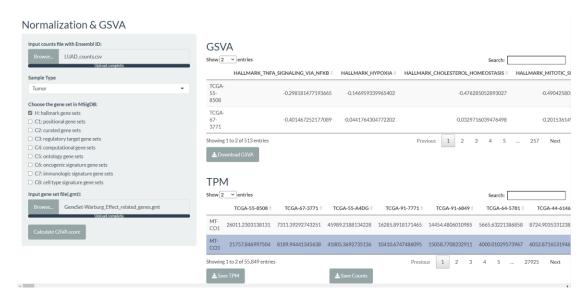


Figure 4. Normalization&GSVA

There are 9 gene sets from MSigDb in ARMT internal datasets available for direct selection to process GSVA, and it is also supported to input a .gmt file of arbitrary gene set from 'Data' page. If the input data is from TCGA, you can select normal, tumor or all samples to process normalize and GSVA (default: tumor).

6.3. Integration&Analysis

ARMT can integrate clinical, GSVA, gene expression (TPM), gene mutation data together, and use these data to carry out analysis including: survival analysis&cox proportional hazards regression analysis, differential analysis, enrichment analysis and correlation analysis.

6.3.1. Data Integration

The integrated data should contain the common samples. You should input the clinical, GSVA, TPM data by .csv file which can be produced by 'Data' and 'Integration&Analysis' page, and the mutation data should be entered by .maf file. The next analysis is carried out according to these integrated data. You can choose a column in integrated data table as the 'Group Column' to separate the data into multiple groups to analyze respectively. Also, you can input your own data in the same format.

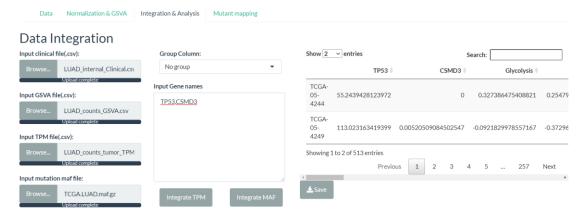


Figure 5. Data Integration

In this page, clinical data and GSVA data will be merged automatically, and you can enter your interested gene name in your TPM and mutation data to integrate their expression and mutation information by clicking 'Integrate TPM' and 'Integrate Maf' button. The integrated data is showed in main panel.

6.3.2. Survival analysis&Cox proportional hazards regression analysis

In this page, you should choose two columns in integrated data table as the survival time and status of samples. Each column of integrated data table can be seen as a factor of samples to carried out survival analysis and cox proportional hazards regression analysis.

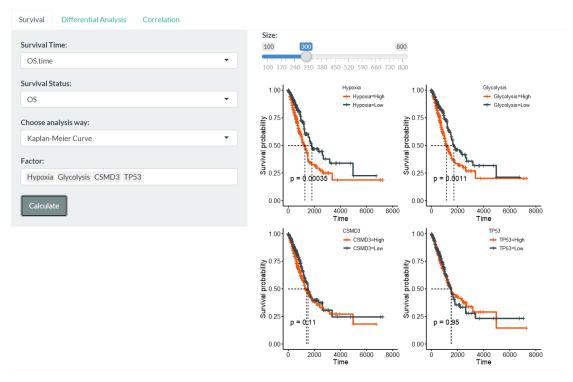


Figure 6. Survival Analysis

If the survival factor is a continuous variable, the samples will be grouped into two parts (high&low). You can choose multiple factors to obtain multiple analysis results (K-M curve).

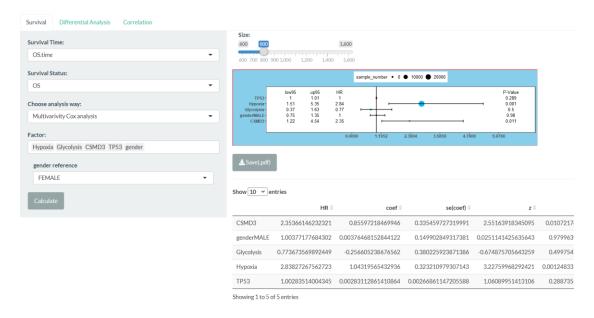


Figure 7. Cox Proportional Hazards Regression Analysis

The cox analysis can focus on single-variable or multiple-variable. If the factor in cox analysis is not numeric variable, one value of this variable should be set as a reference.

6.3.3 Differential analysis

ARMT can carry out differential analysis to GSVA score, gene mutation and expression. The difference factor should be selected in the integrated data, and the samples will be grouped by it. If the difference factor is a numeric variable, the samples will be grouped into high and low, and the low part will be set as control group. The group cut off value 't' is an adjustable parameter in ARMT(upper and lower t*100%). If the difference factor is not a numeric variable, you should select two values used to group the samples into experiment group and control group. You can filter the result by p-value, logFC and FDR (adj.p) after analysis and visualize it in main panel.

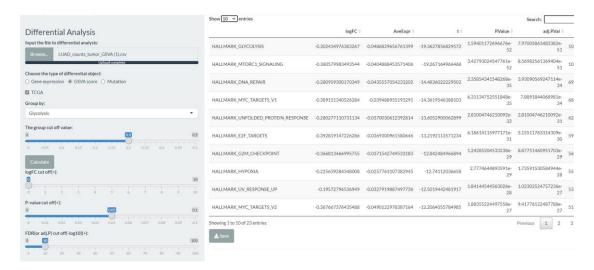


Figure 8. The differential analysis of GSVA score

To analyze difference of GSVA score, the GSVA result of samples in integrated data should be entered through .csv file, and you can get this file in 'Normalization&GSVA' page.

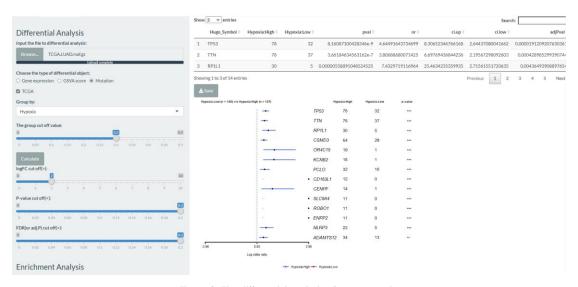


Figure 9. The differential analysis of gene mutation

To analyze difference of gene mutation, the mutation information of samples in integrated data should be entered through .maf file. The result is demonstrated with forest plot in main panel.

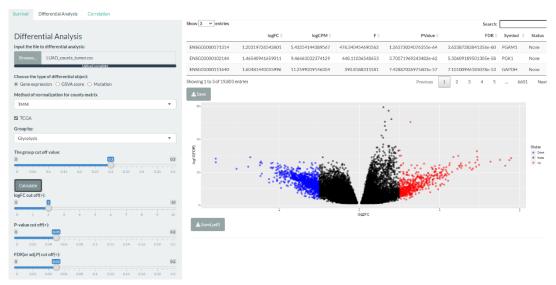


Figure 10. The differential analysis of gene expression

To analyze difference of gene expression (DEG), the counts matrix of samples in integraated data should be entered through .csv file. ARMT provides four methods to normalize counts matrix: TMM, TMMwsp, RLE, upperquartile. The result is demonstrated with volcano plot in main panel.

6.3.4. Enrichment analysis

The enrichment analysis ways in ARMT include GO and KEGG. It can be processed for differential expression genes (DEG) from above differential analysis or for arbitrary gene list input by user.

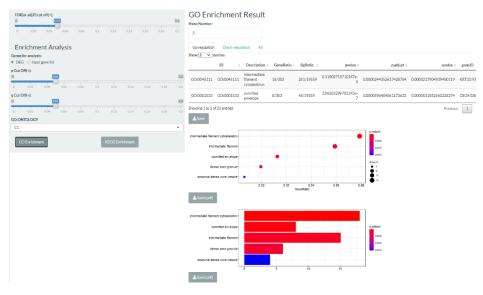


Figure 11. Enrichment Analysis

You should set up the p-value and q-value cut off before enrichment analysis, and you can select interested ontology (MF, CC, BP) of GO enrichment. The enriched genes are up

regulation and down regulation in DEG, and their enrichment results (up regulation, down regulation and all DEG) are showed in different pages of main panel. These results are demonstrated with bar plot and dot plot.

6.3.5. Correlation analysis

ARMT can calculate the correlation between any continuous variable factors of integration data, such as TPM and GSVA score. The result is demonstrated in main panel with correlation coefficient matrix and heat map, and it can be filtered by p-value and correlation coefficient (r, Spearman or Pearson).

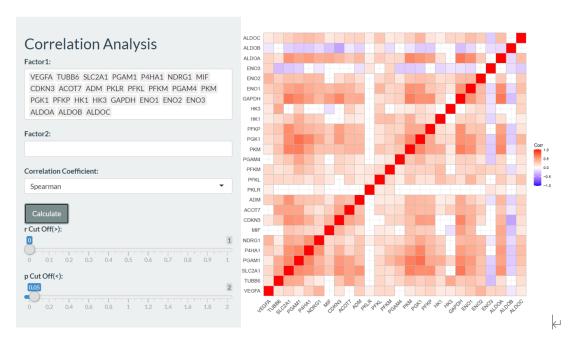


Figure 12. ARMT can calculate correlation coefficient of each pair-factors.

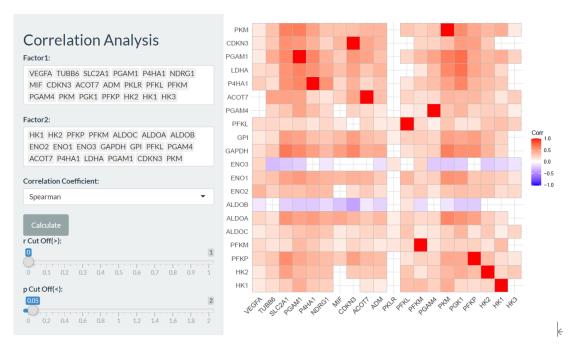


Figure 13. ARMT can calculate correlation coefficient between two list of factors.

If the integrated data is grouped by 'Group Column', ARMT can calculate correlation in each group of data by using single factor or all factors in the list 'Factor1'

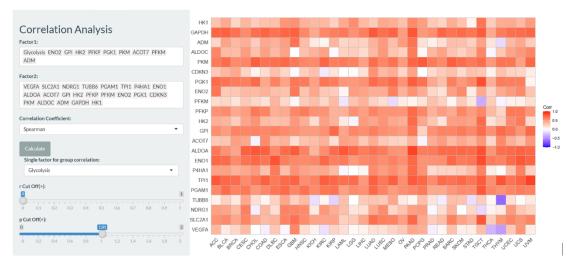


Figure 14. For each group of data, the correlation coefficient between single factor and 'Factor2' list is put together in one result, and the single factor name is replaced by group name in correlation matrix. It is a case of pan-cancer correlation analysis.

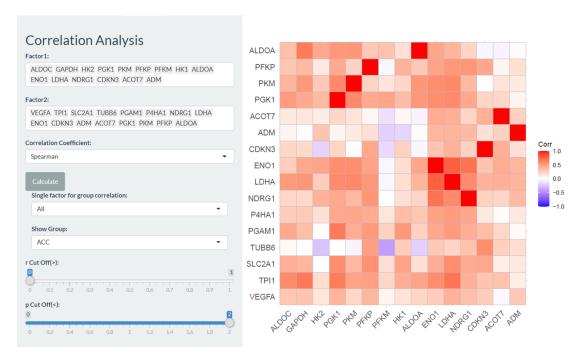


Figure 15. When all factors in 'Factor1' is demonstrated, you can choose one result of multiple groups in sidebar panel to show in main panel.

6.4. Mutant mapping

In corporation with 'maftools', ARMT can visualize the gene mutation in .maf file. There are two plot modes: Maftools Summary and Self-defined. The number of top mutant genes plot out can be set in sidebar panel.

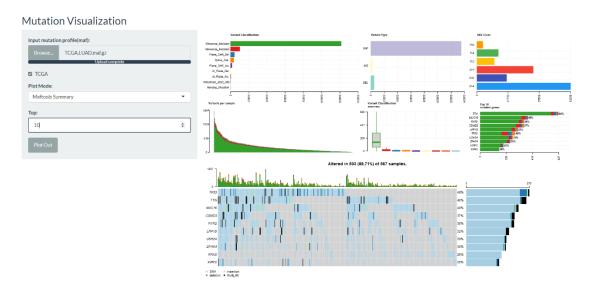




Figure 16. Maftools Summary

ARMT can plot the maf summary and illustrate the enrichment of known Oncogenic Signaling Pathways in TCGA cohorts. It is also supported to draw the oncoplot of interested pathway completely.

In Self-defined mode, ARMT can plot the map for any genes or gene sets, and the mutation types (deletion, insert, SNV) can be specified.

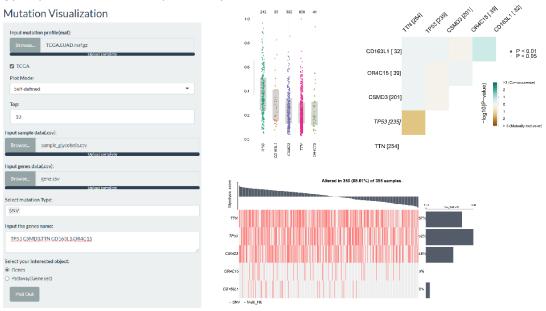


Figure 17. Mutant map plot for genes

About the specific genes, ARMT can plot out the VAF (Variant Allele Frequencies) as a boxplot, detect genes mutually exclusive or co-occurring, and produce oncoplot with any gene

information and sample data.

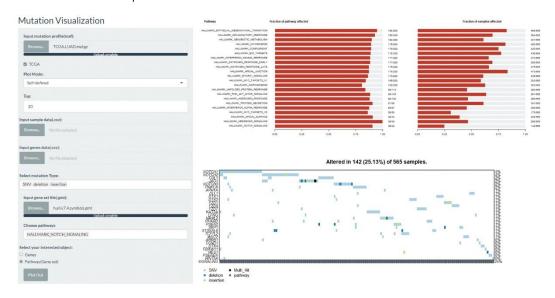


Figure 18. Mutant map plot for gene sets

Input the gmt file, ARMT shows the enrichment of mutation, and can plot a oncoplot of specific gene sets.

7. Format of Input File

The input data of ARMT include gene sets, gene expression matrix, gene mutation, GSVA score matrix and clinical information.

7.1. Gene sets

In 'Data' page, the gene set file (.csv, Figure3) is used to create .gmt file, and the .gmt file is the main format of gene sets input.,

7.2. Gene expression matrix

For gene expression, the counts matrix can be normalized to TPM matrix in ARMT.

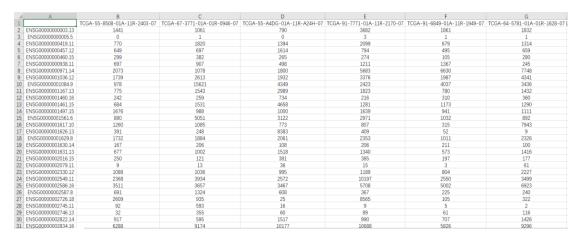


Figure 19. Counts matrix must be in a .csv file. The row name is gene Ensembl ID and the column name is the sample ID. It is used to normalization and differential analysis.



Figure 20. TPM matrix must be in a .csv file. The row name is gene symbol ID and the column name is the sample ID. It can be integrated with other data.

7.3. Gene mutation

To get the mutation information, ARMT requires standard .maf file like the mutation annotation format file in TCGA.

7.4. GSVA score matrix

The GSVA score matrix can be obtained in 'Normalization&GSVA' page.

4	A	В	С	D	E	F	G
1		HALLMARK_TNFA_SIGNALING_VIA_NFKB	HALLMARK_HYPOXIA	HALLMARK_CHOLESTEROL_HOMEOSTASIS	HALLMARK_MITOTIC_SPINDLE	HALLMARK_WNT_BETA_CATENIN_SIGNALING	IALLMARK_TGF_BETA_SIGNALIN
2	TCGA-55-8508	-0.298181477	-0.14695934	-0.476285053	-0.490425805	-0.025793981	-0.301616096
3	TCGA-67-3771	-0.401467252	-0.04417643	-0.032971604	-0.20153615	-0.254657751	-0.294513222
4	TCGA-55-A4DG	-0.400635586	-0.438587803	-0.337369344	-0.373089689	0.032845079	-0.461494455
5	TCGA-91-7771	0.238494726	-0.008675578	-0.216460863	-0.166274071	-0.154668647	0.098186722
6	TCGA-91-6849	0.37211698	0.094365426	0.470551398	-0.467763984	-0.028943508	0.057891694
7	TCGA-64-5781	0.50964787	0.269743125	0.099995907	-0.334381022	-0.320828151	0.02112543
8	TCGA-44-6146	-0.518076031	-0.568135784	-0.632400539	-0.410801656	-0.555839778	-0.101603954
9	TCGA-97-7552	0.088901744	-0.117089144	-0.153580111	0.147219515	0.249062945	0.219725526
10	TCGA-80-5608	-0.145975529	0.149789493	0.406927406	-0.002592738	-0.192207006	-0.17313599
11	TCGA-91-6829	-0.328284401	0.043129857	0.184359399	0.453457124	0.224574779	0.462671651
12	TCGA-49-AARE	-0.501489346	-0.331561028	-0.009124415	-0.408122195	-0.484724662	-0.530880984
13	TCGA-97-A4M1	-0.460638311	-0.436513882	-0.103411023	-0.416215884	-0.115566818	-0.349014866
14	TCGA-83-5908	0.466123875	-0.055500078	-0.204240118	0.563310702	0.040202105	0.262440806
15	TCGA-97-8179	-0.474609407	-0.364264083	-0.031474989	0.130473825	0.18795017	-0.067496851
16	TCGA-64-1680	-0.162801436	-0.144346494	0.011950065	-0.493143217	-0.461486224	-0.362574873
17	TCGA-73-4670	0.223277099	0.385237274	-0.011874063	-0.073657692	-0.241740732	-0.030996154
18	TCGA-44-3396	0.553139396	0.177955331	-0.283144059	-0.215901659	-0.252598055	-0.190981141
19	TCGA-80-5611	0.101100844	0.175335045	0.306364832	0.380942134	-0.090657648	-0.205206822
20	TCGA-53-7624	-0.062322707	0.109092354	0.129833039	0.439014629	-0.222268913	0.128293734
21	TCGA-91-6835	0.304470845	-0.100971734	0.098145495	0.504456739	0.365815096	0.29080828
22	TCGA-L9-A50W	-0.347452806	-0.177680679	0.014832847	-0.399896372	-0.073866756	-0.016053632
23	TCGA-86-7713	-0.295351714	-0.030412753	0.060566714	0.483035053	0.277048485	0.167823015
24	TCGA-50-5044	0.029302267	0.200827915	-0.303176959	-0.151492472	-0.085790154	0.137580237
25	TCGA-97-7941	-0.336901471	-0.180450069	-0.334354036	-0.310156932	0.105575113	-0.135809056
26	TCGA-86-7714	-0.217766115	-0.145155879	-0.044227871	0.173808164	0.066300437	0.309098661
27	TCGA-62-8402	-0.064235448	-0.2361396	0.190006499	0.274204358	0.28209578	-0.020856682
28	TCGA-78-7162	-0.024789208	0.047477261	0.30327317	-0.006378353	0.388722782	0.380009472
29	TCGA-49-AAR0	-0.286506465	-0.26192259	-0.146640322	-0.473166295	-0.188097513	-0.52066299
30	TCGA-35-4122	0.337650847	0.16015702	0.155597354	-0.532696205	-0.578788052	-0.236053936
31	TCGA-55-7726	0.365429118	0.241024252	-0.263701483	0.330382713	0.18083916	0.484278312

Figure 21. The GSVA score is saved in a matrix of .csv file. The row name is the sample ID, and the column name is gene set name.

7.5. Clinical information

The TCGA clinical information can be obtained in 'Data' page.

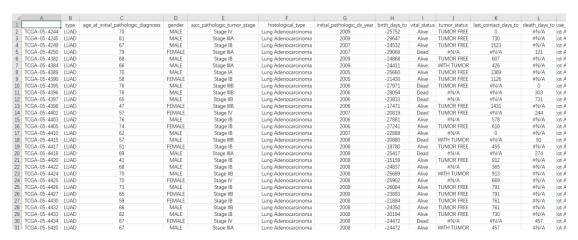


Figure 22. The clinical data must be in a .csv file, and the row name is sample ID and column name is sample characteristic.

Any information about samples in this format file can be input as clinical data.

The example data has been uploaded to https://github.com/Dulab2020/ARMT.