tugHall version 3.0: USER-GUIDE

Requirements for tugHall simulation:

R version **3.6.0** or later

libraries: **stringr, actuar, tidyr**

tugHall is a tool based on the model described in the paper Iurii S Nagornov, Mamoru Kato. tugHall: a simulator of cancer-cell evolution based on the hallmarks of cancer and tumor-related genes. Bioinformatics, V.36, N11, June 2020, pp. 3597–3599. The parameters of the model are described in the Supplementary materials of the paper.

Note that the program has two different procedures in general: the first is the simulation and the second is the analysis of the simulation results. Please, pay attention that the requirements for these procedures are **different**. This User-Guide pertains to the **simulation procedure** alone. Please, also note that plots and tables of this document are related to the data files from **/Documentation/Example/** folder.

Table of Contents

1. Quick start guide

The simplest way to run tugHall:

- Save the **/CNA/** directory to the working folder;
- Run **tugHall_3.0.R**.

The code has its initial input parameters and input files in the **/Input/** folder. After the simulation the user can see results of the simulation (please, see **User-Guide-Analysis_3** for details), which will save to the **/Output/** and **/Figures/** folders. Note that the analysis procedure requires additional libraries and a higher version of R - 3.6.0.

To test code, please, be kind to run tests in the **/Tests/** folder, for details see **/Tests/README.md** file.

2. Structure of directories

The root directory /CNA/:

dir **/Code/** - the folder with a code and a function library.

dir **/Input/** - the folder with the input files.

dir **/Output/** - the folder with the output files.

dir **/Figures/** - the folder with the plot figures.

dir **/Documentation/** - the folder with documentation and example of a simulation.

dir **/Tests/** - the folder with tests to check functions of tugHall.

file **tugHall_3.0.R** - R script to run a simulation and to define the parameters.

file **LICENSE** - the license of the usage of the software based on GNU GENERAL PUBLIC LICENSE version 3.

file **README** - the ReadMe file in markdown format.

file **Sim_monitoring.txt** - the file which is generated during simulation to show the running process.

/Documentation/ directory

file **User-Guide-tugHall_v_3.0** - user guide for a simulation in Rmd or pdf formats.

file **User-Guide-Analysis_v3.0** - user guide for the generation of an analysis and a report in Rmd or pdf formats.

dir **/Example/** - there are files of a simulation as the data resource for tables of the documentation.

/Code/ directory:

tugHall_3.0_functions.R - the file that contains the functions for the simulation / core of program.

read_maps.R - the file to read chromosomal locations got genes of interest from CCDS.current.txt file in the *Input/* folder.

Functions_clones.R - the file with the functions for the analysis of results.

my_plots.R - the file with the functions to plot results of a simuation.

/Input/ directory:

cloneinit.txt - the file with a list of initial cells with/without destroyed genes.

gene__hallmarks.txt - the file with hallmark variables and weights.

CCDS.current.txt - the file with information about chromosomal locations that was getting from CCDS database.

CF.txt - the file with the coefficients of Compaction Factors for each hallmark.

gene__map.txt - the file with information about chromosomal locations for *genes of interest* only.

parameters.txt - the file to read all the parameters which are used in a simulation.

/Output/ directory:

cloneout.txt - the file with simulation output.

geneout.txt - the file with information about hallmark variables and the weights.

log.txt - the file with information about all the parameters.

Weights.txt - the file with information about weights between hallmarks and genes.

point__mutations.txt - the file contains information about point mutations in genome of clones.

CNA__mutations.txt - the file contains information about copy number alterations in genome of clones.

gene__map.txt - the file with information about chromosomal locations for *genes of interest* only.

order__genes__dysfunction.txt - the file with data of order of genes' dysfunction.

VAF.txt and **VAF__data.txt** - the files with data related to variant allele frequencies for each mutated site of genes.

/Tests/ directory:

README.md - the file with short description how to run tests.

tests__clones.R - the file with tests for the functions related to operations with clones.

tests__cna.R - the file with tests for the functions related to copy number alterations.

tests__Model.R - the file with tests for the main function Model and a simulation.

/GENE__MAP/, **/Clones/**, **/CNA/**, **/Model/**, **/Input/** are directories with correct data to compare with the data from running tests.

/Figures/ directory

In the **/Figures/** directory there are figures in *.pdf format which will appear after the analysis of the simulation results. See **USER-GUIDE-Analysis__3**.

3. Inputs

Input of hallmark variables and gene weights

The file **CNA/Input/gene__hallmarks.txt** defines the hallmark variables and weights:

Table 1: **Table 1. Input file for genes.** Example of input file for hallmarks and weights in the file **CNA/Tests/Input/gene__hallmarks.txt**.

Genes	Suppressor or Oncogene	Hallmark	Weights
APC	s	apoptosis	0.2616483
APC	s	growth	0.3285351
APC	s	invasion	0.3746081
KRAS	o	apoptosis	0.2099736
KRAS	o	growth	0.2881968
KRAS	o	immortalization	0.4735684
KRAS	o	angiogenesis	0.3525394
KRAS	o	invasion	0.0446472
TP53	s	apoptosis	0.2543523
TP53	s	growth	0.3076387
TP53	s	angiogenesis	0.4012288
TP53	s	immortalization	0.5264316
TP53	s	invasion	0.0645107
PIK3CA	o	invasion	0.3588945
PIK3CA	o	growth	0.2879753
PIK3CA	o	angiogenesis	0.3261495
PIK3CA	o	apoptosis	0.2938981

1. **Genes -** name of gene, e.g., TP53, KRAS. The names must be typed carefully. The program detects all the unique gene names.
2. **Suppressor or oncogene. -** Distinction of oncogene/suppressor:

- o: oncogene

- s: suppressor
- ?: unknown (will be randomly assigned) Note that gene malfunction probabilities shown below for “Suppressor” and “Oncogene” are defined separately.

3. **Hallmark** - hallmark name, e.g., “apoptosis”. Available names:

- apoptosis
- immortalization
- growth
- anti-growth
- angiogenesis
- invasion

Note that “growth” and “anti-growth” are related to the single hallmark “growth/anti-growth”. Note that “invasion” is related to “invasion/metastasis” hallmark.

4. **Weights** - Hallmark weights for genes, e.g., 0.333 and 0.5. For each hallmark, the program checks the summation of all the weights. If it is not equal to 1, then the program normalizes it to reach unity. Note that, if the gene belongs to more than one hallmark type, it must be separated into separate lines.

After that, the program defines all the weights. **Unspecified weights** are set to 0. Program performs normalization so that the sum of all weights should be equal to 1 for each column (see next table). The **CNA/Output/Weights.txt** file saves these final input weights for the simulation.

Table 2: **Table 2. Weights for hallmarks.** Example of weights for hallmarks and genes from ***CNA/Documentation/Example/Weights.txt*** file. Unspecified values equal 0.

Genes	Apoptosis, H_a	Angiogenesis, H_b	Growth / Anti-growth, H_d	Immortalization, H_i	Invasion / Metastasis, H_{im}
APC	0.2565501	0.0000000	0.2709912	0.0000000	0.4445540
KRAS	0.2058822	0.3264502	0.2377183	0.4735684	0.0529836
TP53	0.2493962	0.3715365	0.2537549	0.5264316	0.0765560
PIK3CA	0.2881715	0.3020133	0.2375356	0.0000000	0.4259064

1. **Genes** - name of genes.
2. **Apoptosis,** H_a - weights of hallmark “Apoptosis”.
3. **Angiogenesis,** H_b - weights of hallmark “Angiogenesis”.
4. **Growth / Anti-growth,** H_d - weights of hallmark “Growth / Anti-growth”.

5. **Immortalization,** H_i - weights of hallmark "Immortalization".
6. **Invasion / Metastasis,** H_{im} - weights of hallmark "Invasion / Metastasis".

Input the probabilities

The input of the probabilities used in the model is possible in the code for parameter value settings, see function **define_paramaters()** in the file **"tugHall_3.0.R"**:

Probability variable and value	Description	Units
E0 = 2E-4	Parameter E_0 related to environmental resource limitation	*
F0 = 1E0	Parameter F_0 related angiogenesis	*
m = 1E-6	Point mutation probability m'	per cell's division per base pair
uo = 0.5	Gene malfunction probability by point mutation for oncogene u_o	per mutation
us = 0.5	Gene malfunction probability by point mutation for suppressor u_s	per mutation
s = 10	Parameter in the sigmoid function s	*
k = 0.1	Environmental death probability k'	per time-step
m_dup = 0.01	CNA duplication probability m_{dup}	per cell's division
m_del = 0.01	CNA deletion probability m_{del}	per cell's division
lambda_dup = 7000	CNA duplication average length λ_{dup}	the geometrical distribution for the length
lambda_del = 5000	CNA deletion average length λ_{del}	the geometrical distribution for the length
uo,dup = 0.8	Gene malfunction probability by CNA duplication for oncogene $u_{o,dup}$	per mutation
us,dup = 0	Gene malfunction probability by CNA duplication for suppressor, $u_{s,dup}$. Currently, 0 is assumed.	per mutation
uo,del = 0	Gene malfunction probability by CNA deletion for oncogene $u_{o,del}$. Currently, 0 is assumed.	per mutation
us,del = 0.8	Gene malfunction probability by CNA deletion for suppressor, $u_{s,del}$.	per mutation
d0 = 0.35	Initial division rate	per time-step
censore_n = 30000	Max cell number where the program forcibly stops	number of cells
censore_t = 200	Max time where the program forcibly stops	in time-steps
Compaction_factor = TRUE	Indicator about an usage of compaction factor	Logical

Probability variable and value	Description	Units
model_name = 'proportional_metastatic'	Model definition, it can be 'proportional_metastatic' or 'threshold_metastatic' or 'simplified'	string/character variable
time_stop = 120	Max time of running after that the program forcibly stops	in seconds
n_repeat = 1	Max number of repetition of the program until the NON-ZERO output will be getting	must be integer
monitor = TRUE	The indicator to safe or not to the monitoring file *Sim_monitoring.txt* during a simulation	logical

* see Suplementary materials in Bioinformatics,V.36,N11,2020,p.3597

User can also define input parameters from the file **parameters.txt** and print all the parameters like:

```
define_paramaters( read_fl = TRUE , file_name = './Input/parameters.txt' )
print_parameters()
```

Compaction factor

If the model 'proportional_metastatic' is used then an user should to define compaction factors which reduce hallmark values like:

```
define_compaction_factor( read_fl = TRUE , file_name = './Input/CF.txt' )
```

where file **'CNA/Input/CF.txt'** contents data of compaction factors:

Hallmark's name	Factor
apoptosis	0.9
growth	0.85
immortalization	0.79
angiogenesis	0.82
invasion	0.97

Filename input

Also in the code **"tugHall_3.3.R"** user should define names of input and output files using function **define_files_names()** before a simulation:

Variables and file names	Description
genefile = 'gene_hallmarks.txt'	File with information about gene-hallmarks weights
mapfile = 'gene_map.txt'	File with information about genes' map
clonefile = 'cloneinit.txt'	Initial Cells
geneoutfile = 'geneout.txt'	Gene Out file with hallmarks
cloneoutfile = 'cloneout.txt'	Output information of simulation
logoutfile = 'log.txt'	Log file to save the input information of simulation

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Input of the initial clones

Please, pay attention, it works for driver point mutation only.

The initial states of cells are defined in **"CNA/Input/cloneinit.txt"** file:

Clone ID	List of malfunctioned genes	Number of cells
1	""	1000
2	"APC"	10
3	"APC, KRAS"	100
4	"KRAS"	1
5	"TP53, KRAS"	1
...	...	100
1000	""	10

1. **Clone ID -** ID of clone, e.g., 1, 324.
2. **List of malfunctioned genes -** list of malfunctioned genes for each clone, e.g. "",”KRAS, APC“. The values are comma separated. The double quotes (”") without gene names indicate a clone without malfunctioned genes.
3. **Number of cells -** number of cells in each clone, e.g., 1, 1000.

Input of the genes' maps

This new version of **tugHall** allows to calculate CNAs in the genome. The breakpoints of CNAs may fall on genic regions consisting of exons and introns. That's why it's needed to enter information about gene's map. In the **/Input/** directory you can find **CCDS.current.txt**, which was getting from CCDS database at the National Center for Biotechnology Information and has information about genes. At the beginning of simulation, the program reads this file and extracts genes' map using function **define_gene_location()**, which is put into **"CNA/Input/gene_map.txt"**. For example, the map is shown as follow:

Chr	CCDS_ID	Gene	Start	End	Len
5	CCDS4107.1	APC	112754890	112755024	135
5	CCDS4107.1	APC	112766325	112766409	85
5	CCDS4107.1	APC	112767188	112767389	202
5	CCDS4107.1	APC	112775628	112775736	109
5	CCDS4107.1	APC	112780789	112780902	114
5	CCDS4107.1	APC	112792445	112792528	84
5	CCDS4107.1	APC	112801278	112801382	105
5	CCDS4107.1	APC	112815494	112815592	99
5	CCDS4107.1	APC	112818965	112819343	379
5	CCDS4107.1	APC	112821895	112821990	96

1. **Chr** - Name of the chromosome, e.g., 1, 12, X, Y.
2. **CCDS_ID** - ID of the gene in the CCDS database.
3. **Gene** - the name of the gene.
4. **Start** - the start position of each exon of the gene.
5. **End** - the final position of each exon of the gene.
6. **Len** - the length of gene's location *Len = End - Start + 1*

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4. Outputs

The output data consists of several files after the simulation.

"log.txt" file

The file **"log.txt"** contains information about probabilities and file names. These variables are explained in the "Inputs".

Table 8: **The log.txt file.** Example of log.txt file.

Variable	Value
genefile	Input/gene_hallmarks.txt
clonefile	Input/cloneinit.txt
geneoutfile	Output/geneout.txt
cloneoutfile	Output/cloneout.txt
logoutfile	Output/log.txt
E0	1e-04
F0	10
m0	1e-07
uo	0.9
us	0.9
s	10
k	0.285714285714286
m_dup	1e-08
m_del	1e-09
lambda_dup	5000
lambda_del	7000
uo_dup	0.8
us_dup	0.8
uo_del	0
us_del	0.8
censore_n	1e+05
censore_t	100
d0	0.5
Compaction_factor	TRUE
model_name	proportional_metastatic
time_stop	120

"geneout.txt" file

The file **"geneout.txt"** contains input information about the weights that connect the hallmarks and genes, which are defined by the user. These variables also are explained in the "Inputs".

Table 9: **The geneout.txt file.** Given below is an example of the geneout.txt file.

Gene_name	Hallmark_name	Weight	Suppressor_or_oncogene
APC	apoptosis	0.2565501	s
KRAS	apoptosis	0.2058822	o
TP53	apoptosis	0.2493962	s
PIK3CA	apoptosis	0.2881715	o
KRAS	immortalization	0.4735684	o
TP53	immortalization	0.5264316	s
APC	growth|anti-growth	0.2709912	s
KRAS	growth|anti-growth	0.2377183	o
TP53	growth|anti-growth	0.2537549	s
PIK3CA	growth|anti-growth	0.2375356	o
KRAS	angiogenesis	0.3264502	o
TP53	angiogenesis	0.3715365	s
PIK3CA	angiogenesis	0.3020133	o
APC	invasion	0.4445540	s
KRAS	invasion	0.0529836	o
TP53	invasion	0.0765560	s
PIK3CA	invasion	0.4259064	o

“cloneout.txt” file

The file **“cloneout.txt”** contains the results of the simulation and includes the evolution data: all the output data for each clone at each time step (only the first 10 lines are presented):

Table 10: **The Output data from cloneout.txt file.** Example of output data for all clones. The names of columns are related to the description in the Tables 1,2 and *USER-GUIDE-Analysis_3*'s figures. Columns are from 1 to 13.

Time	AvgOrIndx	ID	N_cells	ParentID	Birth_time	c	d	i	im	a	k	E
0	avg	-	-	-	-	0	0.5692	0.8129	0	0.0033	0.2857	6.3598
0	1	1	500	0	0	0	0.45	1	0	0.0066	0.2857	1e-04
0	2	2	500	0	0	0	0.6884	0.6258	0	0	0.2857	2.7196
1	avg	-	-	-	-	0.5746	0.5744	0.7969	0.0007	0.0031	0.2857	6.0468
1	1	1	494	0	0	0.4460	0.441	1	0	0.0066	0.2857	1e-04
1	2	2	588	0	0	0.6828	0.6860	0.6258	0	0	0.2857	2.7196
1	3	3	1	1	0	0.4460	0.6713	1	0	0	0.2857	1e-04
1	4	4	1	1	0	0.4460	0.441	1	0	0.0066	0.2857	1e-04
1	5	5	1	2	0	0.6828	0.8943	0.6258	0.8222	0.0553	0.2857	1.6251
2	avg	-	-	-	-	1.1665	0.5761	0.7827	0	0.0030	0.2857	5.7729

1. **Time -** the time step, e.g., 1, 50.
2. **N__cells -** the number of cells in this clone, e.g. 1000, 2.
3. **AvgOrIndx -** “avg” or “index”: “avg” is for a line with averaged values across different (index) lines at the same time step; “index” shows the cell's index at the current time step, e.g., avg, 4,7.
4. **ID -** the unique ID of clone, e.g., 1, 50.
5. **Parent__ID -** the parent index, e.g., 0, 45.
6. **Birth__time -** the time step of the clone's birth, e.g., 0, 5.
7. **c -** the counter of cell divisions for the clone, it equals average counter across all the cells in the clone.
8. **d -** the probability of division for the cell, e.g., 0.1, 0.8 [per time-step].
9. **i -** the probability of immortalization for the cell, e.g., 0.1, 0.8 [per time-step].
10. **im -** the probability of invasion/metastasis for the cell, e.g., 0.1, 0.8 [per time-step].
11. **a -** the probability of apoptosis for the cell, e.g., 0.1, 0.8 [per time-step].
12. **k -** the probability of death due to the environment, e.g., 0.1, 0.8 [per time-step].
13. **E -** the E coefficient for the function of the division probability, e.g., 10ˆ4, 10ˆ5.

Table 11: **cloneout.txt** Columns are from 14 to 24.

Time	ID	N_normal	Nmax	N_primary	N_metastatic	Ha	Him	Hi	Hd	Hb	type	mut_den
0	-	500	23384.4	500	0	0.09264	0	0.18705	0.10103	0.13384	-	0.125
0	1	500	10000	500	0	0	0	0	0	0	normal	0
0	2	500	36768.9	500	0	0.18529	0	0.37411	0.20206	0.26768	primary	0.25
1	-	495	24554.5	590	0	0.10104	0.00075	0.20309	0.11008	0.14554	-	0.13617
1	1	495	10000	590	0	0	0	0	0	0	normal	0
1	2	495	36768.9	590	0	0.18529	0	0.37411	0.20206	0.26768	primary	0.25
1	3	495	10000	590	0	0.23089	0	0	0.23034	0	primary	0.25
1	4	495	10000	590	0	0	0	0	0	0	normal	0
1	5	495	61534.0	590	0	0.44464	0.82220	0.37411	0.40396	0.51534	primary	0.5
2	-	526	25542.5	733	0	0.10868	0	0.21722	0.11841	0.15542	-	0.14634
2	1	526	10000	733	0	0	0	0	0	0	normal	0
2	2	526	36768.9	733	0	0.18529	0	0.37411	0.20206	0.26768	primary	0.25
2	6	526	10000	733	0	0.23089	0	0	0.23034	0	primary	0.25
2	7	526	10000	733	0	0	0	0	0	0	normal	0
2	8	526	10000	733	0	0.23089	0	0	0.23034	0	primary	0.25
2	9	526	36768.9	733	0	0.41618	0	0.37411	0.43240	0.26768	primary	0.5
2	10	526	36768.9	733	0	0.41618	0	0.37411	0.43240	0.26768	primary	0.5
2	11	526	36768.9	733	0	0.41618	0	0.37411	0.43240	0.26768	primary	0.5

14. **N_normal -** the number of normal cells at this time step, e.g., 134, 5432.
15. **Nmax -** the theoretically maximal number of primary tumor cells, e.g., 10000, 5000.
16. **N_primary -** the number of primary tumor cells at this time step, e.g., 134, 5432.
17. **N_metastatic -** the number of metastatic cells at this time step, e.g., 16, 15439.
18. **Ha -** the value of the hallmark “Apoptosis” for the cell, e.g., 0.1, 0.4444.
19. **Him -** the value of the hallmark “Invasion / Metastasis” for the cell, e.g., 0.1, 0.4444.
20. **Hi -** the value of the hallmark “Immortalization” for the cell, e.g., 0.1, 0.4444.
21. **Hd -** the value of the hallmark “Growth / Anti-growth” for the cell, e.g., 0.1, 0.4444 .
22. **Hb -** the value of the hallmark “Angiogenesis” for the cell, e.g., 0.1, 0.4444 .
23. **type -** the type of the cell: ‘normal’ or ‘primary’ or ‘metastatic’.
24. **mut_den -** the density of mutations for the cell, it equals to ratio a number of mutated driver genes to a number of all the genes, e.g., 0, 0.32.

Table 12: **cloneout.txt** Columns are from 25 to 28.

Time	ID	driver_genes	passenger_genes	PointMut_ID	CNA_ID
0	-	-	-	-	-
0	1	0 0 0 0	0 0 0 0	0	0
0	2	0 1 0 0	0 0 0 0	1	0
1	-	-	-	-	-
1	1	0 0 0 0	0 0 0 0	0	0
1	2	0 1 0 0	0 0 0 0	1	0
1	3	1 0 0 0	0 0 0 0	3	0
1	4	0 0 0 0	1 0 0 0	0	1
1	5	0 1 0 1	0 0 0 0	1	2
2	-	-	-	-	-
2	1	0 0 0 0	0 0 0 0	0	0
2	2	0 1 0 0	0 0 0 0	1	0
2	6	1 0 0 0	0 0 0 0	5	0
2	7	0 0 0 0	1 0 0 0	0	3
2	8	1 0 0 0	0 0 0 0	7	0
2	9	1 1 0 0	0 0 0 0	1	4
2	10	1 1 0 0	0 0 0 0	1	5
2	11	1 1 0 0	0 0 0 0	1	6

25. **driver_genes -** the binary numbers indicate the driver mutation at the gene related to order of genes in onco as well as order of the next columns with genes' names, e.g., '1 0 0 0' means that the first gene has a driver mutation and other genes have no.
26. **passenger_genes -** the binary numbers indicate the passenger mutation at the gene related to order of genes in onco as well as order of the next columns with genes' names, e.g., '0 0 1 0' means that the third gene has a passanger mutation and other genes have no.
27. **PointMut_ID -** the index of data row for point mutation data frame saved at the end of simulation in the file **Point_mutations.txt**, e.g., 23, 32.
28. **CNA_ID -** the index of data row for CNA data frame saved at the end of simulation in the file **CNA.txt**, e.g., 44, 21.

There are two columns (25th and 26th) with the indexes of point mutations and CNAs. Each index corresponds to index in the related data frames for point mutations and for CNAs represented in additional tables below.

Table 13: **cloneout.txt** Columns are from 29 to 32.

Time	ID	Chr1_CDS_APC	Chr1_CDS_KRAS	Chr1_CDS_TP53	Chr1_CDS_PIK3CA
0	-	-	-	-	-
0	1	8532	567	1182	3207
0	2	8532	567	1182	3207
1	-	-	-	-	-
1	1	8532	567	1182	3207
1	2	8532	567	1182	3207
1	3	8532	567	1182	3207
1	4	8532	567	1182	3207
1	5	8532	567	1182	3207
2	-	-	-	-	-
2	1	8532	567	1182	3207
2	2	8532	567	1182	3207
2	6	8532	567	1182	3207
2	7	8532	567	1182	3207
2	8	8532	567	1182	3207
2	9	8532	567	1182	3207
2	10	8563	567	1182	3207
2	11	5560	567	1182	3207

29-32. **Chr1_CDS_(gene's name)**, for example **Chr1_CDS_APC -** the length of CDS for each gene in the order of names of genes for FIRST parental chromosome of a clone. The CDS length of genes for second parental chromosome can be different in principle. The point mutation is proportional to **CDS_(gene's name)** od each chromosome.

Table 14: **cloneout.txt** Columns are from 33 to 36.

Time	ID	Chr1_Len_APC	Chr1_Len_KRAS	Chr1_Len_TP53	Chr1_Len_PIK3CA
0	-	-	-	-	-
0	1	89236	35590	6986	35539
0	2	89236	35590	6986	35539
1	-	-	-	-	-
1	1	89236	35590	6986	35539
1	2	89236	35590	6986	35539
1	3	89236	35590	6986	35539
1	4	89236	35590	6986	35539
1	5	89236	35590	6986	35539
2	-	-	-	-	-
2	1	89236	35590	6986	35539
2	2	89236	35590	6986	35539
2	6	89236	35590	6986	35539
2	7	89236	35590	6986	35539
2	8	89236	35590	6986	35539
2	9	89236	35590	6986	35539
2	10	89267	35590	6986	35539
2	11	86264	35590	6986	35539

33-36. **Chr1_Len_(gene's name)**, for example **Chr1_Len_APC -** the length of gene in the order of names of genes for ONLY FIRST chromosome of a clone. The length of genes for second chromosome can be different in principle. The CNA mutation is proportional to **Len_(gene's name)**.

Table 15: **cloneout.txt** Columns are from 37 to 40.

Time	ID	Chr1__p0	Chr1__prob__point__mut	Chr1__prob__del	Chr1__prob__dup
0	-	-	-	-	-
0	1	0.996815	0.422866	0.052466	0.524667
0	2	0.996815	0.422866	0.052466	0.524667
1	-	-	-	-	-
1	1	0.996815	0.422866	0.052466	0.524667
1	2	0.996815	0.422866	0.052466	0.524667
1	3	0.996815	0.422866	0.052466	0.524667
1	4	0.996815	0.422866	0.052466	0.524667
1	5	0.996815	0.422866	0.052466	0.524667
2	-	-	-	-	-
2	1	0.996815	0.422866	0.052466	0.524667
2	2	0.996815	0.422866	0.052466	0.524667
2	6	0.996815	0.422866	0.052466	0.524667
2	7	0.996815	0.422866	0.052466	0.524667
2	8	0.996815	0.422866	0.052466	0.524667
2	9	0.996815	0.422866	0.052466	0.524667
2	10	0.996811	0.423381	0.052419	0.524198
2	11	0.997144	0.367722	0.057479	0.574798

37. **Chr1__p0 -** the probability that during a trial, a cell of the clone has **NO** mutation [per time-step]. Applied to all cells in the clone.
38. **Chr1__prob__point__mut -** the **conditional** probability that if cell will have a mutation it should be a **point mutation**.
39. **Chr1__prob__del -** the **conditional** probability that if cell will have a mutation it should be a **deletion**.
40. **Chr1__prob__dup -** the **conditional** probability that if cell will have a mutation it should be a **duplication**.

Last columns (from 41 to the end) show the data related to the **SECOND** parental chromosome with the same set of data as for the first parental chromosome.

Table 16: Point mutation data frame which will be saved to the file **Point_mutations.txt** at the end of simulation.

PointMut_ID	Parental_1or2	Chr	Ref_pos	Phys_pos	Delta	Copy_number	Gene_name	MalfunctionedByPointMut	mut_order
1	2	5	112766358	[112766358]	[0]	1	APC	TRUE	1
1	1	5	112766358	[NA]	[NA]	1	APC	NA	1
3	2	12	25227294	[25227294]	[0]	1	KRAS	TRUE	2
3	1	12	25227294	[NA]	[NA]	1	KRAS	NA	2
5	1	5	112834962	[112834962]	[0]	1	APC	TRUE	3
5	2	5	112834962	[NA]	[NA]	1	APC	NA	3
7	1	5	112838048	[112838048]	[0]	1	APC	TRUE	4
7	2	5	112838048	[NA]	[NA]	1	APC	NA	4
9	2	5	112819291	[112819291]	[0]	1	APC	TRUE	5
9	1	5	112819291	[NA]	[NA]	1	APC	NA	5
11	2	5	112828919	[112828919]	[0]	1	APC	TRUE	6
11	1	5	112828919	[NA]	[NA]	1	APC	NA	6
13	2	3	179221099	[179221099]	[0]	1	PIK3CA	TRUE	7
13	1	3	179221099	[NA]	[NA]	1	PIK3CA	NA	7
15	1	5	112840163	[112840163]	[0]	1	APC	TRUE	8
15	2	5	112840163	[NA]	[NA]	1	APC	NA	8
17	1	5	112838486	[112838486]	[0]	1	APC	TRUE	9
17	2	5	112838486	[NA]	[NA]	1	APC	NA	9
19	2	17	7670695	[7670695]	[0]	1	TP53	TRUE	10
19	1	17	7670695	[NA]	[NA]	1	TP53	NA	10

1. **PointMut_ID -** ID of point mutation, first ID is related to variant allele 'B' and same *second* ID - to the original allele A.
2. **Parental_1or2 -** indicates either of the two parental chromosomes.
3. **Chr -** name of a chromosome.
4. **Ref_pos -** the reference position of an allele. The reference position is on the coordinate system of the human reference genome.
5. **Phys_pos -** the physical position of an allele. The physical length of a (parental) chromosome is extended or shrunk by CNA duplications or deletions, respectively. When a duplication happens, the reference position is divided into two or more physical positions, which are represented by multiple elements in a vector. When a deletion happens and the allele is lost, the lost is represented by "-" on the coordinate system of physical positions.
6. **Delta -** difference between the reference and physical positions.
7. **Copy_number -** the copy number of an allele.
8. **Gene_name -** the name of a gene.
9. **MalfunctionedByPointMut -** logical indicator of whether or not the gene is malfunctioned by the point mutation.
10. **mut_order -** indicator of mutation order in the simulation, it's used to detect order of mutations in the clone at each chromosome.

Table 17: **CNA mutation data frame which will be saved to the file** CNA.txt** at the end of simulation.

CNA_ID	Parental_1or2	dupOrdel	Chr	Ref_start	Ref_end	Gene_names	MalfunctionedByCNA	mut_order
1	1	dup	17	7673762	7676402	TP53	TRUE	4
2	1	del	17	7673606	7676593	TP53	FALSE	9
3	1	dup	5	112843870	112844125	APC	FALSE	13
4	2	dup	12	25245297	25245383	KRAS	TRUE	14
5	2	dup	5	112827201	112828201	APC	TRUE	17
6	2	dup	5	112819264	112828971	APC	FALSE	19
7	2	dup	17	7676251	7676593	TP53	TRUE	22
8	1	dup	5	112838988	112840084	APC	TRUE	31
9	2	dup	3	179234103	179234363	PIK3CA	FALSE	38
10	1	dup	5	112839427	112844125	APC	TRUE	56
11	2	dup	5	112841115	112844125	APC	TRUE	64
12	2	dup	5	112842377	112844125	APC	TRUE	73
13	2	dup	3	179229358	179230708	PIK3CA	TRUE	78
14	2	dup	3	179225964	179227090	PIK3CA	FALSE	82
15	1	dup	5	112840818	112844125	APC	TRUE	84
16	1	dup	12	25209872	25209910	KRAS	TRUE	91
17	1	dup	5	112838547	112838607	APC	TRUE	102
18	1	dup	5	112842702	112844125	APC	TRUE	109
19	1	dup	3	179198925	179200903	PIK3CA	TRUE	110
20	2	dup	3	179219615	179224820	PIK3CA	TRUE	111

1. **CNA_ID -** ID of CNA.
2. **Parental_1or2 -** indicates either of the two parental chromosomes.
3. **DuplicationOrDeletion -** indicator of duplication or deletion for CNA.
4. **Chr -** name of a chromosome.
5. **Reference_start -** the reference position of the CNA start.
6. **Reference_end -** the reference position of the CNA end.
7. **Gene_name -** the name(s) of a gene(s).
8. **MalfunctionedByCNA -** logical indicator of whether or not the gene(s) is malfunctioned by the CNA.
9. **mut_order -** indicator of mutation order in the simulation, it's used to detect order of mutations in the clone at each chromosome.

Table 18: **Sim_monitoring.txt** file with data getting during a simulation.

Time	N_clones	N_normal	N_primary	N_metastatic	N_point_mutations	N_duplications	N_deletions
1	12	524	616	0	9	1	1
2	13	513	763	2	9	3	0
3	16	514	936	6	11	4	0
4	19	502	1137	6	16	2	0
5	30	491	1359	9	25	4	0
6	37	418	1604	12	32	4	0
7	45	413	1919	15	38	6	0
8	59	369	2264	20	50	8	0
9	63	341	2656	32	52	10	0
10	71	287	3090	39	58	12	0
11	81	252	3591	54	66	14	0
12	90	198	4145	66	70	19	1
13	108	150	4742	76	87	22	1
14	117	111	5373	81	96	23	1
15	135	85	5985	112	103	33	1
16	139	63	6513	164	106	33	2
17	155	36	7079	212	119	37	2
18	171	25	7472	255	135	36	2
19	197	20	8084	336	155	38	5
20	211	14	8511	399	164	44	5

1. **Time** - time step.
2. **N_clones** - total number of clone at a time step.
3. **N_normal** - total number of normal cells at a time step.
4. **N_primary** - total number of primary tumor cells at a time step.
5. **N_metastatic** - total number of metastatic cells at a time step.
6. **N_point_mutations** - total number of unique point mutations at a time step.
7. **N_duplications** - total number of unique duplications at a time step.
8. **N_deletions** - total number of unique deletions at a time step.

5. How to run

In order to make the simulation, please follow the procedure:

1. Copy **/CNA/** directory into the working directory.
2. CD to the **/CNA/** directory.
3. Run the **tugHall_3.0.R** file, using the command line like

```
R --vanilla < tugHall_3_0.R
```

or using the line by line procedure in **R Studio** in the **tugHall_3.0.R** file.

4. To obtain analysis reports of the simulation, please refer to **User-Guide-Analysis_v3.0.RMD**. In **User-Guide-Analysis_v3.0.RMD** and **User-Guide-tugHall_v3.0.RMD**, commands are embedded to include files under **/Documentation/Example/**.

6. Differences with cell-based code and version 2.0

6.1. Reason to develop clone-based code

- Clone-based code was designed to accelerate calculation and increase number of tumor cell. Advantage of clone-based algorithm is making trial for all cells at 1 clone with one application of **trial()** function. In cell-based algorithm **trial()** applies to each cell. But if number of cells equal number of clones, then speed up is 1. That's why clone-based code works faster for any cases.
- Another reason is a case, when we need to simulate huge number of cells like 10^7 or 10^9, but mutation rate is very low. Cell-based algorithm takes a huge computational cost, and vice versa clone-based algorithm will work very fast, if mutated cells will appear slowly.

6.2. Usage of *trial()* function

- In **trial()** function program applies several trials like environmental death, apoptosis death, division process, etc. We changed the trials with probability p (for some death process) for each cell in the clone with for 1 trial with procedure:

$N_{cells} = N_{cells} - Binom(p, N_{cells})$,

where $Binom(p, N_{cells})$ is random number from the binomial distribution with probability p, N_{cells} is a number of cells in a clone. Probability p is one of probabilities of death processes, for example, for apoptosis death $p = a'$ or for environment death $p = k$ etc.

- For cell division with probability d' the new number of cells will be:

$N_{cells} = N_{cells} + Binom(d', N_{cells})$

- Check at the end of **trial()** function: if $N_{cells} = 0$, then the clone has died.

6.3. Usage of mutation function

- In mutation function we have changed the mutation to birth of a new clone (one mutation is a birth of one clone):

$N_{new\_clones} = Binom(m, N_{new\_cells})$,

$N_{new\_cells} = Binom(d', N_{cells})$.

- Passenger or Driver mutations do not matter for new clone's generation. Only during analysis, we will distinguish Passengers or Drivers clones.

6.4. Average function

- The average values $\overline{x}$ of probabilities or hallmarks are found by summation on the x_i with multiplication by cells number $N_{cells,i}$ of this clone:

$\overline{x} = \sum_i x_i \times w_i$,

where $w_i = \frac{N_{cells,i}}{N_{cells,tot}}$ is ith clone's occupancy in whole cell population $N_{cells,tot} = \sum_i N_{cells,i}$, x_i is the value for ith clone, summation applies for all clones $i = 1..N_{clones}$.

- For this purpose, we added the calculation of cells number (primary and metastasis) before average and hallmarks update.

6.5. Difference with version 2.0

In the current version we use library *actuar* to make non-zero-binom calculation faster, and we use the approximation for big numbers of cells in **trial()** function, because **rbinom()** function in R has restriction for big numbers like $n \times p > 10^{12}$.

7. Differences with clone-based code and version 2.1

7.1. Reason to develop CNA-based code

New version of tugHall with copy number alteration (CNA) was designed for correct calculation of VAF influenced by CNA and tumor purity. It's expected that this design should improve comparison between observation VAF $\in [0; 1]$ and calculated VAF. The previous versions of tugHall have VAF in the range $[0; 0.5]$ because of the neglect of CNA and tumor purity.

7.2. Changing the formula of the cell division coefficient

In the cell division process, the logistic growth applies to primary-tumor cells and normal cells, where normal cells are cells without any driver mutations. Meanwhile, N_p of the friction term in the logistic equation is the number of primary-tumor cells. N_p, N_m, and N_n are the numbers of primary-tumor, metastatic-tumor, and normal cells. So, the division coefficient now is calculated by next formula:

$$d' = \begin{cases} (d_0 + H_d)(1 - E' \times N_p), & \text{when logistic growth,} \\ d_0 + H_d, & \text{when exponential growth} \end{cases}$$

where H_d is division hallmark, d_0 is initial division coefficient, $E' = \frac{E_0}{(1+F_0 \times H_b)}$, H_b is angiogenesis hallmark, F_0 is a friction coefficient.

7.3. Calculation of point and CNA mutations

Probabilities of CNA mutations are calculated in the same way as point mutations:

- $m_{point} = m_0 \times l_{CDS}$ - for point mutation of a gene, where l_{CDS} is the length of all exons of a gene (*CDS_(gene's name)* is denoted in the table above) and m_0 is a constant per base pairs per cell's division defined by users;
- $m_{0,dup}$ and $m_{0,del}$, or we collectively call $m_{0,CNA}$, indicate the first breakpoint event of a CNA and it is a constant per base pairs per cell's division defined by users. $m_{CNA} = m_{0,CNA} \times l_{genes}$, where l_{genes} is the total region size of all genes of interest which consists of exons as well as introns (*Len_(gene's name)* is denoted in the table above).
- a length of CNA is calculated using geometrical distribution: $l_{CNA} = rgeom(1, 1/\lambda_{CNA})+1$, where λ_{CNA} is average base-pair length defined by users (λ_{CNA} is either λ_{dup} or λ_{del}).
- probability of malfunctioning a gene $u = u_{s,CNA}$ for suppressor and $u = u_{o,CNA}$ for oncogene.

So, the algorithm of CNA is as follow:

```
if ( runif(1) < m_dup + m_del ) then 'Generate CNA':
  - define which event should occur - duplication or deletion using ratio m_dup/m_del like:
      event  <-  sample(c('dup', 'del'), 1, prob = c( m_dup, m_del )/sum(m_dup, m_del) )

  - find randomly first position within the regions of genes of interest;
  - find the length of CNA from geometrical distribution
  - define with probability 0.5 is it the parental chromosome 1 or 2;
  - define the list of genes in CNA;
  - define with probabilty u = {u_o or u_s} is it malfunction for each gene;
  - check overlap of position for other mutations (and if it's necessesary change their positions).
```

The calculation of probabilities and hallmarks variables is not changed.

At the end of a simulation the VAF frequencies are calculated in accordance with formulation:

$$VAF^i = \frac{(1-\rho)n^i_{B,N} + \sum_{s=1}^{\#sp} \tau_s n^i_{B,S}}{(1-\rho)(n^i_{A,N} + n^i_{B,N}) + \sum_{s=1}^{\#sp} \tau_s (n^i_{A,S} + n^i_{B,S})},$$

where:

i is position (site) index,

s is subpopulation (clone's) index,

τ is subpopulation (clone's) fraction,

ρ is tumor purity: $\rho = \sum_{s=1}^{\#sp} \tau_s$,

n is copy number,

A denotes an original allele A, B - variant B, N - normal, S - tumor.

In usual application we used for normal cells $n^i_{A,N} = 2$ and $n^i_{B,N} = 0$, so VAF can be calculated as follow:

$$VAF^i = \frac{\sum_{s=1}^{\#sp} \tau_s n^i_{B,S}}{2(1-\rho) + \sum_{s=1}^{\#sp} \tau_s (n^i_{A,S} + n^i_{B,S})}$$

In tugHall, under the given admixture rate of intact normal cells ρ_N, values of VAF are calculated using next formulation:

$$VAF^i = \frac{(1-\rho_N) \sum_{s=1}^{\#sp} \lambda_s n^i_{B,S}}{2\rho_N + (1-\rho_N) \sum_{s=1}^{\#sp} \lambda_s (n^i_{A,S} + n^i_{B,S})},$$

where $\lambda_s = (N^s_T + N^s_{SN})/(N_T + N_{SN})$, N_T and N_{SN} are total number of primary tumor and speckled normal cells respectively at the last time step of a simulation, index s related to cells with mutated site s. Observation subpopulation (clone's) fraction τ_s and simulation one λ_s are proportional with $\tau_s = (1-\rho_N)\lambda_s$. Please, pay attention that for metastatic cells, we put $\rho_N = 0$.