Documentation for ***ProtModel***

*Selection of the best-fitting substitution model of protein evolution accounting for structural constrains for protein sequences alignments by approximate Bayesian computation*

Current version is 1.0

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# Disclaimer

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# Credits

This program was developed at,

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# Purpose

*ProtModel* is an evolutionary framework to estimate the best-fitting substitution model by approximate Bayesian computation (ABC) from protein sequence alignments. *ProtModel* is designed to be run either on Linux OS or Mac OSX and it is freely available from <https://github.com/DavidFerreiro/ProtModel>.

The user can specify a prior distribution of substitution rate as well as other evolutionary parameters that will be treated as nuisance parameters, like amino acid frequencies, proportion of invariable sites (+I) or heterogeneity change across sites according to a gamma distribution (+G). There are also available a graphical user interface (GUI) *ProtModel\_GUI* which allows for a user-friendly procedure to run *ProtModel* and a version to run it on clusters (*ProtModel\_Cluster*) to save computer time.

The computer simulations are performed via the coalescent program *ProteinEvolverProtABC*, an adapted version of the simulator *ProteinEvolver* (<https://github.com/MiguelArenas/proteinevolver>) (Arenas et al., 2013) to ABC. *ProtModel* implements a total of 7 summary statistics. Some of them explore the stability of the individual sequences of the alignment and the others are related with the physicochemical properties of the amino acids’ replacements. Conveniently, *ProtModel* can run the simulations on parallel, according to user specifications, to save computer time. It is highly recommended due to the simulations under substitution models which consider the stability of the protein and the stability calculations take a long time.

Three ABC methods are implemented in *ProtModel*, the “*rejection*” algorithm use the proportion of accepted simulations to estimate models’ posterior probabilities (Csilléry et al., 2012). With "*mnlogistic*" the posterior model probabilities are estimated using a multinomial logistic regression and using "*neuralnet*", neural networks are used to predict the probabilities of models based on the observed statistics (Csilléry et al., 2012). For all algorithms, the user must specify the number of simulations to use, the number of simulations to retain (tolerance of the rejection algorithm), the number of iterations and which summary statistics to use. *ProtModel* provides also several diagnostic plots to assess qualitatively how well the model fitted the data, namely: boxplots of simulations summary statistics for each model; histograms of the median distance between retained simulations summary statistics and the observed ones for each model; histograms of the summary statistics of the retained simulations; scatterplots of the summary statistics and each parameter; and a plot of the two principal components calculated from the summary statistics of the simulations. In all the plots the values for the target protein alignment data are superimposed in blue except for PCA plot, where the target protein alignment data value is represented with a black cross.

## Models

*ProtModel* is able to work with both empirical or site independent and with site-dependent substitution models that consider the stability of the protein, called structural constrain substitution (SCS) models (Arenas et al., 2015). In case of empirical substitution models, the user can select among Blosum62 (Henikoff & Henikoff, 1992), CpRev (Adachi et al., 2000), Dayhoff , DayhoffDCMUT (Kosiol & Goldman, 2005), HIVb (Nickle et al., 2007), HIVw (Nickle et al., 2007), JTT (Jones et al., 1992), JonesDCMUT (Kosiol & Goldman, 2005), LG (Le & Gascuel, 2008), Mtart (Abascal et al., 2006), Mtmam (Yang et al., 1998), Mtrev24 (Adachi & Hasegawa, s. f.), RtRev (Dimmic et al., 2002), VT (Müller & Vingron, 2000), WAG (Whelan & Goldman, 2001) and UserEAAM models (Arenas, 2015) (the latter is a user-defined model that must be provided as an additional input file with name UserEAAM, the format of this file is explained with details in the documentation of the simulator *ProteinEvolver* (Arenas et al., 2013)). Within SCS models, there are two available, mean-field Neutral (which does not consider population size) and mean-field Fitness (which needs user specified population size) (Arenas et al., 2015).

# Versions and Graphical User Interface

The *ProtModel* framework has two version, one to run on our computer and another to run in a cluster and they are placed in different folders, “*ProtModel*” and “*ProtModel\_Cluster*” respectively. The user can run the framework in their computer using the command lines or using a python graphical user interface (GUI), which allow the user to work with the program without using the command line. The GUI will display two consecutive windows. The first one which allow the user to choose between the mandatory parameters and to fill the optional (see section 4.1 in “*The Settings input file*”). Settings must be specified carefully, so that to have biological meaning. The GUI will check for incorrect methodological settings (e.g., it does not allow negative substitution rates), but it will not evaluate the settings in terms of biological meaning. Importantly, errors in *ProtModel* may be caused by incorrect user-specified settings or user-specified settings without biological meaning. The second windows will launch the simulations and the consecutive ABC analyses. While the program is running, it will inform about the progress and it will be show up a message when it ended.

This version of *ProtModel* can be also run on clusters because they are usually Linux OS environments. However, *ProtModel\_Cluster* version enables the user to work with many more processors and in parallel without sharing memory taking advance of MPI (with many more processors) interface. It is designed in the same way as the other version, having the same folders and executables names so the user must be careful not mixing the two version files.

# Executables and compilation

*ProtModel* requires that Python3 (<https://www.python.org/downloads/>) and R (https://www.r-project.org) are installed and there are some libraries required, which must be installed by the user (Table 1). Python libraries can be installed by the command: pip install ("*library\_name*") or, in the case of R, by the command install.packages("*library\_name*"). See further details in <https://docs.python.org/3/installing/index.html>, <http://cran.r-project.org/doc/manuals/R-admin.html#Installing-packages>. For example,

*pip install numpy*

*install.packages("abc")*

Our program also works using *ProteinEvolverProtABC* simulator (Arenas et al., 2013) and *DeltaGREM* software (Arenas et al., 2017), which are used to perform the protein sequences simulations and to calculate the protein free energy respectively. They are placed in the “*source*” folder with a csv file called “*Grantham*”, and they must not be modified by the user. In The folder “*GUI*” contains the GUI executable and two images needed to run it. *ProtModel* pipeline consists on 6 executable files needed to execute the framework but the user must only execute the main file called “*ProtModelGeneral.py*”. All these folders and files must not be modified to a properly execution of *ProtModel*.

*ProtModel* must be compiled for the OS through a Makefile file which is placed on the main directory. It will compile *ProteinEvolverProtABC* and *DeltaGREM* programs and will create the executables and place them in the folder “*bin*”.

Table 1. Libraries needed to run *ProtModel*

|  |  |  |
| --- | --- | --- |
| *Name* | *Language* | *Version* |
| abc | R | Command line and GUI |
| os | Python | All |
| sys | Python | All |
| Biopython | Python | All |
| random | Python | All |
| numpy | Python | All |
| warnings | Python | All |
| pandas | Python | All |
| csv | Python | All |
| multiprocessing | Python | Command line and GUI |
| re | Python | All |
| platform | Python | All |
| mpi4py | Python | Cluster |

# *ProtModel* usage

*ProtModel* have four mandatory input files:

* **Settings file**. This input file, called Settings.txt, must contain all the desired specifications for simulations and ABC estimation.
* **Multiple alignment of protein sequences**. The protein sequence alignment that will be analyzed must be provided by the user in sequential phylip format (.phy). Phylip requires that each sequence identifier is exactly 10 characters long, padded with spaces as necessary. We strongly recommend to use Phylogeny.fr (Dereeper et al., 2008) to convert into phylip format (<http://phylogeny.lirmm.fr/phylo_cgi/data_converter.cgi>) and check the number of characters of the sequences identifier.
* **Template structure**. This is not mandatory to run *ProtModel*, but it is needed to use two of the summary statistics and the SCS models. It has to be a .pdb file and it is recommended to select the structure with the highest homology with the multiple alignment of protein sequences. We advised to use SWISS-MODEL (Waterhouse et al., 2018) to find the best template (<https://swissmodel.expasy.org>).
* **Structures.in.** This file is needed to use two of the summary statistics too. It cannot be modified.

The recommend structure of *ProtModel* consists in create a folder in his/her desired location and copy there a Settings.txt file, the multiple alignment file, the template pdb, the structures.in file and all *ProtModel* material (including executable files and folders). Examples well prepared working directories for a standard *ProtModel* run are provided in the package (folder “*examples*”).

After concluding a full run of *ProtModel* two folders are created in the working directory, the “*ABCOutputs*” and the “*SimulationsOuputs*” folders (see details in section 4.3). Since the estimation phase is quite fast in comparison with the simulation phase, the user is advised to explore different settings for the ABC method (see section 4.4 in “*Re-analyzing data*”) if desired, without having to run again the simulation phases.

## The Settings input file

The Settings input file must contain all the information required to perform the analysis. It is highly recommended to be carefully checked by the user since mistakes may alter results (error messages will be displayed on the screen and may suggest stop the execution by typing CTRL+C) or use the GUI.

The file consists of two main blocks, the simulation phase and the estimation phase.

**Important notes when dealing with the Settings file**

- Parameter values must be introduced in the line after the parameter description, otherwise such parameter will be considered as “*not specified*” (see examples below).

- Do not modify the parameter description (it is used by the program to identify the parameter).

- Some parameters are mandatory and must be specified, these parameters contain an “*\**” (see below).

- Some parameters require a prior distribution (see section 4.1.1), this is a distribution within which the estimation should fall (the range of the prior distribution should include the estimation; for example, if the user believes that the recombination rate can be 0 then the prior distribution of the recombination rate should include 0).

### Available prior distributions

Several distributions are included in *ProtModel* to simulate protein data under different evolutionary scenarios (Table 2). In addition, most of them can be truncated at lowest and highest values. However, note that some parameters must be specified by an integer number (e.g., generation time) while others by a float number (e.g., amino acid frequencies). Therefore, not all distributions can be applied to any parameter. Details for each particular parameter are described in the following subsections.

Table 2. Available distributions in ProtModel.

|  |  |  |  |
| --- | --- | --- | --- |
| *Distribution* | *Description* | *Truncated* | *Examples* |
| fix | Fixed value (integer or non-integer) | n.a. | fix 4; fix 0.7 |
| Uniform (uniform) | Random between two values (integer or non-integer): lowest highest | n.a. | uniform 1.0e-8 1.0e-5; uniform 2e-9 5e-6; uniform 0 3 |
| Normal (norm) | Normal distribution (mean, sd) | t # # | norm 1.0e-8 1.0e-5; norm 1.0e-8 1.0e-5 t 1.0e-9 1.0e-6 |
| Exponential (exp) | Exponential distribution (rate) | t # # | exp 1.0e-7; exp 1.0e-7 t 1.0e-8 1.0e-6 |
| gamma | Gamma distribution (shape, rate “1/scale”) | t # # | gamma 1.0e-7 5.0e-7; gamma 1.0e-7 5.0e-7 t 2.0e-7 1.0e-6 |
| beta | Beta distribution (shape1, shape 2) | t # # | beta 1.0e-7 5.0e-7; beta 1.0e-7 5.0e-7 t 2.5e-7 1.0e-6 |
| dirichlet | Dirichlet distribution (alpha “vector”) | n.a. | dirichlet 1 1 1 1; dirichlet 1 1 1 1 1 1 |

### Simulation phase

***General settings for the simulation***

* **Name of the file with the target protein sequences alignment.** This specification is mandatory. Only specify the filename (i.e. no pathway) since the target alignment file needs to be placed in the same pathway as the Settings.txt file. For example,

*### Target alignment file ### # phylip format*

*\*NameOfPhylipFile=* *ProtSeq1.phy*

* **Number of simulations.** This parameter is mandatory. We recommend at least 1 000 computer simulations but with 10 000 simulations results should be accurate. However, the number of simulations required to obtain accurate estimates depends on many factors, especially the target multiple sequence alignment. More complex sequences (large molecular diversity) could require more simulations because the parametric landscape could be more irregular and should be more sampled. For example,

*### Total number of simulations ###*

*\*NumberOfSimulations=10000*

* **Consideration of indels.** This parameter is mandatory. The user specifies if indels (gaps) should be “*Ignored*” (by default and recommended) or considered as a “*NewState*”. This decision can affect the summary statistics if there are indels in the multiple sequence alignment. For example,

*# Consideration of indels. "Ignored" (indels are ignored), "NewState" (indels are considered as a new state)*

*\*Indels=Ignored*

* **Number of processors to run the simulations in parallel on a machine.** This parameter is mandatory (by default simulations run on all user computer processors). This parallelization works on machines using Linux OS with shared memory. Ideally, one should specify at the most the number of available processors of the machine. The number of processors must be specified in the settings file. For example,

*# Number of available processors to run the simulations in parallel. All by default*

*\*NumberOfProcessors=12*

* **Save simulated data.** This parameter is mandatory. It is recommended to not save the simulated because it requires a lot of space in the hard disk of user’s computer. If the user wants to save the data it will be placed in a compressed folder called “*Simulations.tar.gz*”. Note that even if choosing not to save simulated data, simulated data is created and saved temporally since this data is required for calculating the Summary Statistics in the following step. For example,

*#* *Save simulated data. “No”, “Yes” (but it requires space in the disk)*

*\** *SaveSimulations=No*

* **Show running information.** This parameter is mandatory. If the user chooses “*No*” the amount of information printed on the screen during ProtModel execution will be reduced. This option is recommended to save computing time (printing information on the screen requires more computer time). Regardless the option selected, the final results will not change, it only affects to the amount of information shown on the screen during the execution. For example,

*# Show running information (simulations and summary statistics) on the screen. “No”, “Yes” (but it slows down the running time)*

*\*ShowInformationScreen=No*

***Demographic settings***

* **Haploid/diploid simulated data.** This parameter is mandatory. Haploid is defined with a value of 1 and diploid with a value of 2. For example,

*# Haploid or Diploid data (haploid=1, diploid=2)*

*\*Haploid/Diploid=2*

* **Effective population size (*N*).** This parameter is mandatory. The parameter value must be an integer. For example,

*# Population size (i.e., 1000).*

*\*PopulationSize=1000*

***Longitudinal sampling***

* **Sampling at different times.** This parameter is optional. The user can specify the time at which the tip nodes of the tree (i.e. samples) were sampled in years. In the example, 4 sampling times are specified: sampled in 1995 - sequences 1 to 10; sampled in 2003 - sequences 11 to 16; sampled in 1997 - sequences 17 to 26; sampled in 2001: sequences 7 and 8. Note that this option does not work if a deme converges, backwards in time, before the last sampling time.For example,

*# Logitudinal sampling. Requires GenerationTime. See documentation for details*

*DatedTips=4 1995 1 10 2003 11 16 1997 17 26 2001 27 29*

* **Generation time.** This parameter is optional. The user can specify the time for each generation. The parameter value can be fixed (fix) or sampled from a uniform distribution (uniform). For example,

*# Generation time. fix, uniform; i.e., uniform 500 1000*

*GenerationTime=fix 1200*

* **Growth Rate.** This parameter is optional. The first number specifies the model, exponential growth rate (0) or demographic periods (1). These parameters are looking back in time, so it is not a good idea specify a negative growth rate for the last period, as the coalescent time could become infinite in the past. For an exponential growth per individual per generation, after “*0*” the growth rate must be specified. Example: 0 1e-5 (= 0 0.00001). For demographic periods, after “*1*” the user has to specify the number of periods (from the present to the past) and N during those periods. The first number here specifies the number of periods. For each period should be three consecutive numbers indicating the size N at the beginning and at the end of the period, and the duration of the period in generations. Example: 1 3 1000 1250 1000 1300 1550 2000 1560 1000 3000.

*# Generation time. fix, uniform; i.e., uniform 500 1000*

*GenerationTime=0 1e-5*

* **Migration Model.** This parameter is optional. The first number specifies the migration model (island model=1, stepping-stone model=2, continent-island model=3). The second number specifies the total number of demes or subpopulations sampled. The next n numbers specify the number of individuals (or sequences) per deme (note that the specified sample size must be equal to the sum of these). For the island-continent model, deme #1 will be the continent while the other demes will be islands. Example: 2 2 3 3 (a stepping-stone model, two demes with three samples each).

*# Migration model and population structure. See documentation for details*

*MigrationModel=2 2 3 3*

* **Migration Rate.** This parameter is optional. This parameter introduces the migration rate, which can be constant or variable with time according to temporal periods. The first number specifies the number of temporal periods, then: For only 1 period, the second number is the migration rate (constant). Example: 1 0.001 (only 1 period with migration rate = 0.001).

For more than 1 period, the second number/s are the time/s for the beginning of a new migration rate and the third/s numbers are the corresponding migration rate/s for each period. Example: 2 100 0.001 0.005 (2 periods, the first period occurs from t = 0 to t = 100 with a migration rate = 0.001, the second period occurs from t = 100 to the end of the simulation with a migration rate = 0.005). Example: 3 100 800 0.002 0.001 0.003 (3 periods: from t = 0 to t = 100 with migration rate = 0.002, from t = 100 to t = 800 with migration.

*# Migration rate (constant or variable with time according to temporal periods). See documentation for details*

*MigrationRate=3 100 800 0.002 0.001 0.003*

* **Convergence Demes.** This parameter is optional. The first number specifies the total number of convergent events. For each convergence event should be three consecutive numbers. The first number and the second number are the numbers of the demes to converge. The third number is the time to that convergence. With this option the user can build the demes evolutionary tree but it is only available when the migration model is activated (despite the migration rate could be zero). Examples: 1 1 2 2000 (for 2 initial demes (Migration Model = 2), convergence of deme 1with deme 2 at time 2000 to create a new deme 3). 3 1 2 400 3 4 1900 5 6 2000 (for 4 initial demes (Migration Model = 4) convergence of deme 1 with deme 2 at time 400 to create a new deme 5, convergence of deme 3 with deme 4 to create a new deme 6 at time 1900, convergence of deme 5 with deme 6 at time 2000 to create a final new deme 7).

*# Events of convergence of demes. See documentation for details*

*ConvergenceDemes= 1 1 2 2000*

***Protein evolution and substitution model***

* **Amino acid substitution rate per site.** This parameter is mandatory. Distributions allowed: fix, uniform, norm(t), exp(t), gamma(t), beta(t). For example,

*# Amino acid substitution rate. i.e., fix 7.0e-6.*

*\*SubstitutionRate=uniform 0 1.67e-4*

* **Model of empirical amino acid substitution.** This parameter is mandatory. The user have to specify all the desire empirical substitution model of protein evolution separated by a space among the following (Arenas, 2015): Blosum62, CpRev, Dayhoff, DayhoffDCMUT, HIVb, HIVw, JTT, JonesDCMUT, LG, Mtart, Mtmam, Mtrev24, RtRev, VT, WAG, UserEAAM (the latter is a user-defined model that must be provided as an additional input file with name UserEAAM, the format of this file is explained with details in the documentation of the simulator *ProteinEvolver* (Arenas et al., 2013)). For example,

*# Model of amino acid substitution (i.e., Blosum62, CpRev, Dayhoff, DayhoffDCMUT, HIVb, HIVw, JTT, JonesDCMUT, LG, Mtart, Mtmam, Mtrev24, RtRev, VT, WAG, UserEAAM)*

*\*SubstitutionModel=JTT*

* **Amino acid SCS models.** This parameter is mandatory. The user has to specify all the desire SCS models of protein evolution separated by a space among the following: Fitness, Neutral. For example,

*# Model of structural amino acid substitution (Fitness, Neutral)*

*\*StructuralSubstitutionModel=Fitness Neutral*

* **Amino acid frequencies.** This parameter is mandatory. Frequencies for each amino acid site along sequences. Distributions allowed: fix or dirichlet. If it is not specified the program will assume equally distributed frequencies (all fix with value 0.05). For example,

*# Amino acid frequencies. fix or dirichlet. By default, equally distributed frequencies. i.e., dirichlet 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1*

*\*AminoacidFrequencies=fix 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05*

* **Rate of heterogeneity across sites (+G).** This parameter is optional. Distributions allowed: fix, uniform, norm(t), exp(t), gamma(t), beta(t). For example,

*# Rate of heteregeneity across sites, +G. fix, uniform, gamma, beta, normal, exponential; i.e., fix 0.6*

*RateHetSites=uniform 0.76 0.90*

* **Proportion of invariable sites (+I).** This parameter is optional. Distributions allowed: fix, uniform, norm(t), exp(t), gamma(t), beta(t). For example,

*# Proportion of invariable sites, +I. fix, uniform, gamma, beta, normal, exponential; i.e., exponential 0.002 t 0 1.0*

*PropInvSites=uniform 0.3 0.5*

* **Template.** This parameter is mandatory. PDB protein structure used to structural substitution models and to calculate proteins free energy. For example,

*# PDB protein structure used to structural substitution models. See documentation for details*

*\*Template=3IXO.pdb*

* **Chain.** This parameter is mandatory. PDB protein chain used to structural substitution models and to calculate proteins free energy. For example,

*# Chain of the PDB protein structure used to structural substitution models. See documentation for details*

*\*Chain=A*

* **GMRCA.** This parameter is optional. By default, the GMRCA/MRCA sequence is simulated according to the amino acid frequencies. However, the user can optionally specify its own root sequence by a text file which must be located in the main directory. The file just contains in a single sequence. In case the user does not select any GMRCA/MRCA template sequence will be used, which is highly recommended.

*# GMRCA input file. See documentation for details*

*GMRCA=GMRCA.txt*

***Graphical settings***

* **Multiple pages.** This parameter is mandatory. PDF documents with multiple plost per page (No, Yes). For example,

*#* *Multiple pages. PDF documents with multiple plost per page (No, Yes)*

*\*MultiPage=Yes*

### Estimation phase

***ABC estimation***

* **ABC iterations.** This parameter is mandatory. Number of simulations to consider in the ABC analysis. Unless testing the methodology, the user is advised to use all the simulations performed. Note that the number of simulations to use has to be obviously equal or less to the number of simulations performed (specified in *NumberOfSimulations*).For example,

*#ABC iterations. Number of simulations to consider (Iterations <= NumberOfSimulations)*

*\*ABCIterations=100*

* **ABC tolerance.** This parameter is mandatory. Proportion of simulations closest to real data to retain in the ABC procedure. A tolerance of 0.01 can be enough but the best tolerance varies among multiple sequence alignments. It is recommended to explore different values of this parameter. For example,

*#ABC tolerance. % of simulations closest to real data to retain in the ABC procedure*

*\*ABCTolerance=0.01*

* **ABC method.** This parameter is mandatory. The ABC algorithm has to be specified. The user can choose between rejection, mnlogistic or neuralent.For example,

*#ABC method (rejection, mnlogistic, neuralnet).*

*\*ABCMethod=rejection*

* **Summary statistics to use.** This parameter is mandatory. The user needs to choose which summary statistics to use for the ABC estimation by specifying their numeric identifiers (see section 5 “*Models and Methods*” for further details).It is recommended to specify the 7 summary statistics implemented in the program but in some cases where the folding stability standard deviation is far from the real data value we recommend to not use it (summary statistic 2). For example,

*#Summary statistics to use. See documentation for details*

*\*SummaryStatistics= 1 2 3 4 5 6 7*

## Screen information with an example

The data shown below corresponds with the normal output information of a ProtModel run. To the first phase of the program, the user has to use computer terminal. The example corresponds with a rapid project, in which we tried to distinguish between the empirical HIV2and the SCS Neutral models. Before try this example, you have to have installed Python 3 and R.

### ProtModel compilation:

* First, we need to be in the main directory of ProtModel. The bold words bellow correspond with the mandatory input files, while the rest are the normal ProtModel directory available to download.

*Test\_ProtModel %* ***ls***

**3on9.pdb** LeerSettings.py Variables.py

ChangeVariablesPE.py Makefile source

Errores.py ProtModelGeneral.py **structures.in**

Functions.py **Settings.txt**

GUI **TNF.phy**

* Then, we only have to do a “*make all*” command to compile all the programs (ProteinEvolutionProtABC and DeltaGREM).

*Test\_ProtModel %* ***make all***

*Creating bin folder ...*

*Done!*

*Copying Grantham.csv file ...*

*Done!*

*Compiling DeltaGREM ...*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/DeltaGREM\_src clean*

*rm -fr main\_DeltaGREM.o REM.o random3.o Get\_pars\_DeltaGREM.o alignments.o mutations.o Codes.o Input.o gen\_code.o allocate.o output.o read\_pdb.o read.o Sec\_str\_all.o NeedlemanWunsch.o Profit\_aux.o DeltaGREM*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/DeltaGREM\_src all*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o main\_DeltaGREM.o main\_DeltaGREM.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o REM.o REM.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o random3.o random3.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o Get\_pars\_DeltaGREM.o Get\_pars\_DeltaGREM.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o alignments.o alignments.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o mutations.o mutations.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o Codes.o Codes.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o Input.o Input.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o gen\_code.o gen\_code.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o allocate.o allocate.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o output.o output.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o read\_pdb.o read\_pdb.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o read.o read.c*

*read.c:251:1: warning: control may reach end of non-void function*

*[-Wreturn-type]*

*}*

*^*

*1 warning generated.*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o Sec\_str\_all.o Sec\_str\_all.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o NeedlemanWunsch.o NeedlemanWunsch.c*

*gcc -O2 -Wall -std=c99 -pedantic -g -pg -D\_FILE\_OFFSET\_BITS=64 -D\_LARGE\_FILE\_SOURCE -c -o Profit\_aux.o Profit\_aux.c*

*gcc main\_DeltaGREM.o REM.o random3.o Get\_pars\_DeltaGREM.o alignments.o mutations.o Codes.o Input.o gen\_code.o allocate.o output.o read\_pdb.o read.o Sec\_str\_all.o NeedlemanWunsch.o Profit\_aux.o -o DeltaGREM -lm -L/usr/lib64/libg2c.so.0.0.0*

*ld: warning: directory not found for option '-L/usr/lib64/libg2c.so.0.0.0'*

*Done!*

*Compiling ProteinEvolverProtABC ...*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/ProteinEvolverProtABC clean*

*Removing object and executable files to save space*

*Finished cleanup.*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/ProteinEvolverProtABC all*

*Building ProteinEvolverProtABC version 1.2.0*

*gcc -c -O3 -Wall ProteinEvolverProtABC1.2.0.c*

*ProteinEvolverProtABC1.2.0.c:2035:16: warning: taking the absolute value of*

*unsigned type 'unsigned short' has no effect [-Wabsolute-value]*

*seed = seed + fabs(tmb.millitm);*

*^*

*ProteinEvolverProtABC1.2.0.c:2035:16: note: remove the call to 'fabs' since*

*unsigned values cannot be negative*

*seed = seed + fabs(tmb.millitm);*

*^~~~*

*ProteinEvolverProtABC1.2.0.c:16910:33: warning: using floating point absolute*

*value function 'fabs' when argument is of integer type [-Wabsolute-value]*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^*

*ProteinEvolverProtABC1.2.0.c:16910:33: note: use function 'abs' instead*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^~~~*

*abs*

*ProteinEvolverProtABC1.2.0.c:25094:33: warning: using floating point absolute*

*value function 'fabs' when argument is of integer type [-Wabsolute-value]*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^*

*ProteinEvolverProtABC1.2.0.c:25094:33: note: use function 'abs' instead*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^~~~*

*abs*

*ProteinEvolverProtABC1.2.0.c:25285:33: warning: using floating point absolute*

*value function 'fabs' when argument is of integer type [-Wabsolute-value]*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^*

*ProteinEvolverProtABC1.2.0.c:25285:33: note: use function 'abs' instead*

*arrayIndBreakpointsOrd\_C[i] = fabs(arrayIndBreakpointsOrd[i]/3);*

*^~~~*

*abs*

*ProteinEvolverProtABC1.2.0.c:27134:22: warning: format string is not a string*

*literal (potentially insecure) [-Wformat-security]*

*sprintf(FILE\_CODE, FILE\_CODE\_DEF);*

*^~~~~~~~~~~~~*

*/Library/Developer/CommandLineTools/SDKs/MacOSX.sdk/usr/include/secure/\_stdio.h:47:56: note:*

*expanded from macro 'sprintf'*

*\_\_builtin\_\_\_sprintf\_chk (str, 0, \_\_darwin\_obsz(str), \_\_VA\_ARGS\_\_)*

*^~~~~~~~~~~*

*ProteinEvolverProtABC1.2.0.c:27134:22: note: treat the string as an argument to*

*avoid this*

*sprintf(FILE\_CODE, FILE\_CODE\_DEF);*

*^*

*"%s",*

*/Library/Developer/CommandLineTools/SDKs/MacOSX.sdk/usr/include/secure/\_stdio.h:47:56: note:*

*expanded from macro 'sprintf'*

*\_\_builtin\_\_\_sprintf\_chk (str, 0, \_\_darwin\_obsz(str), \_\_VA\_ARGS\_\_)*

*^*

*ProteinEvolverProtABC1.2.0.c:27709:22: warning: format string is not a string*

*literal (potentially insecure) [-Wformat-security]*

*sprintf(FILE\_CODE, FILE\_CODE\_DEF);*

*^~~~~~~~~~~~~*

*/Library/Developer/CommandLineTools/SDKs/MacOSX.sdk/usr/include/secure/\_stdio.h:47:56: note:*

*expanded from macro 'sprintf'*

*\_\_builtin\_\_\_sprintf\_chk (str, 0, \_\_darwin\_obsz(str), \_\_VA\_ARGS\_\_)*

*^~~~~~~~~~~*

*ProteinEvolverProtABC1.2.0.c:27709:22: note: treat the string as an argument to*

*avoid this*

*sprintf(FILE\_CODE, FILE\_CODE\_DEF);*

*^*

*"%s",*

*/Library/Developer/CommandLineTools/SDKs/MacOSX.sdk/usr/include/secure/\_stdio.h:47:56: note:*

*expanded from macro 'sprintf'*

*\_\_builtin\_\_\_sprintf\_chk (str, 0, \_\_darwin\_obsz(str), \_\_VA\_ARGS\_\_)*

*^*

*ProteinEvolverProtABC1.2.0.c:33636:38: warning: for loop has empty body*

*[-Wempty-body]*

*{ int i; for (i=0; i<n; x[i]\*=a,i++) ; return(0); }*

*^*

*ProteinEvolverProtABC1.2.0.c:33636:38: note: put the semicolon on a separate*

*line to silence this warning*

*ProteinEvolverProtABC1.2.0.c:33638:40: warning: for loop has empty body*

*[-Wempty-body]*

*{ int i; for (i=0; i<n; y[i]=x[i],i++) ; return(0); }*

*^*

*ProteinEvolverProtABC1.2.0.c:33638:40: note: put the semicolon on a separate*

*line to silence this warning*

*ProteinEvolverProtABC1.2.0.c:52540:13: warning: unused function 'PrintTitle'*

*[-Wunused-function]*

*static void PrintTitle(FILE \*filep)*

*^*

*ProteinEvolverProtABC1.2.0.c:52709:13: warning: unused function 'PrintDate'*

*[-Wunused-function]*

*static void PrintDate (FILE \*filep)*

*^*

*10 warnings generated.*

*gcc -lm -O3 -Wall -o ProteinEvolverProtABC1.2.0 ProteinEvolverProtABC1.2.0.o*

*Finished compiling.*

*Done!*

*Note that R will require the following libraries: abc*

*These libraries can be installed from R by typing:*

*install.packages(abc)*

*And python will require the following libraries: os, sys, Biopython, rnamdom, numpy, warnings, pandas, csv, re, plataform and multiprocessing*

*These libraries can be installed from command line by typing:*

*pip install library\_name*

*See the documentation for additional details about ProtModel and for cluster version information*

*Compilation completed!*

*Test\_ProtModel %* ***make clean***

*Removing executables ...*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/DeltaGREM\_src clean*

*rm -fr main\_DeltaGREM.o REM.o random3.o Get\_pars\_DeltaGREM.o alignments.o mutations.o Codes.o Input.o gen\_code.o allocate.o output.o read\_pdb.o read.o Sec\_str\_all.o NeedlemanWunsch.o Profit\_aux.o DeltaGREM*

*/Library/Developer/CommandLineTools/usr/bin/make -C source/ProteinEvolverProtABC clean*

*Removing object and executable files to save space*

*Finished cleanup.*

*Done!*

* DeltaGREM executable file will be placed in the main directory and cannot be removed or moved. ProteinEvolverProtABC executable and the “*Grantham.csv*” file will be placed in a new folder called “*bin*” and it cannot be removed or change their directory. On the other hand, the ProtModel\_GUI should stay in its folder “*GUI*” but it can be moved as long as the images are kept in the same location.

*Test\_ProtModel %* ***ls***

*3on9.pdb* ***GUI*** *TNF.phy*

*ChangeVariablesPE.py LeerSettings.py Variables.py*

***DeltaGREM*** *Makefile* ***bin***

*Errores.py ProtModelGeneral.py source*

*Functions.py Settings.txt structures.in*

### 

### *ProtModel* execution:

A full execution may take time. In particular, simulations under SCS models and summary statistics calculation related with the protein stability may take a considerable amount of time especially when performing a large number of simulations or when the protein dataset includes a large number and long sequences. To reduce the execution time, simulations and summary statistics calculation phases can be run in parallel on a Linux environment if the user specifies this in the settings file and works with a machine presenting many processors or the user can run the analysis on cluster using many processors. If simulation settings are unexpected the analysis may fail (see section 4.6 about “*Message Errors*”).

To execute *ProtModel* all the python scripts (.py) must be placed within the mandatory input files (Settings.txt, the multiple alignment of protein sequence, template structure and structures.in). Then, once the user will be in the main folder and *ProtModel* was compiled, the user has to:

*python3 ProtMoldelGeneral.py*

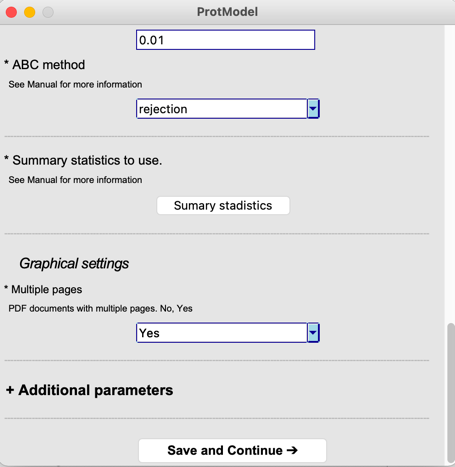
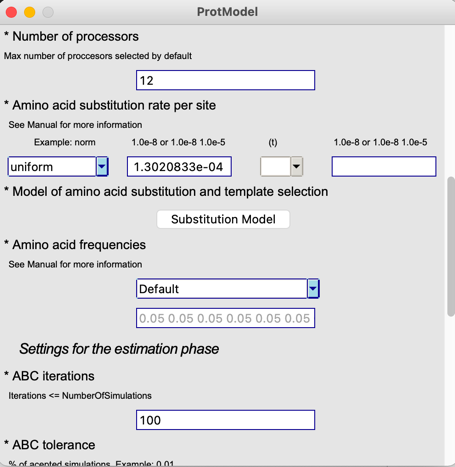
*ProtModel* was developed using python3.8 version and tested under most of python3 versions so is recommended to use python3.8 version but every version between python3 and python3.8 is expected to work.

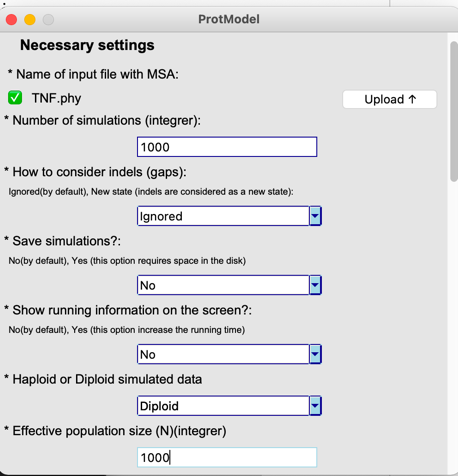
Note that this command on the cluster version won’t launch the framework, it will create the “*launch\_Simu.sh*” file ready to be send to the cluster queue system. This file is made to work on CESGA cluster so maybe the user has to modify the firsts lines (those which start with “*#*”) to adapt it to the user’s cluster. After these lines some cluster modules are loaded to be able to work with python and mpi4py module so maybe the user has to modify or delate it too.

*sbatch launch\_Simu.sh*

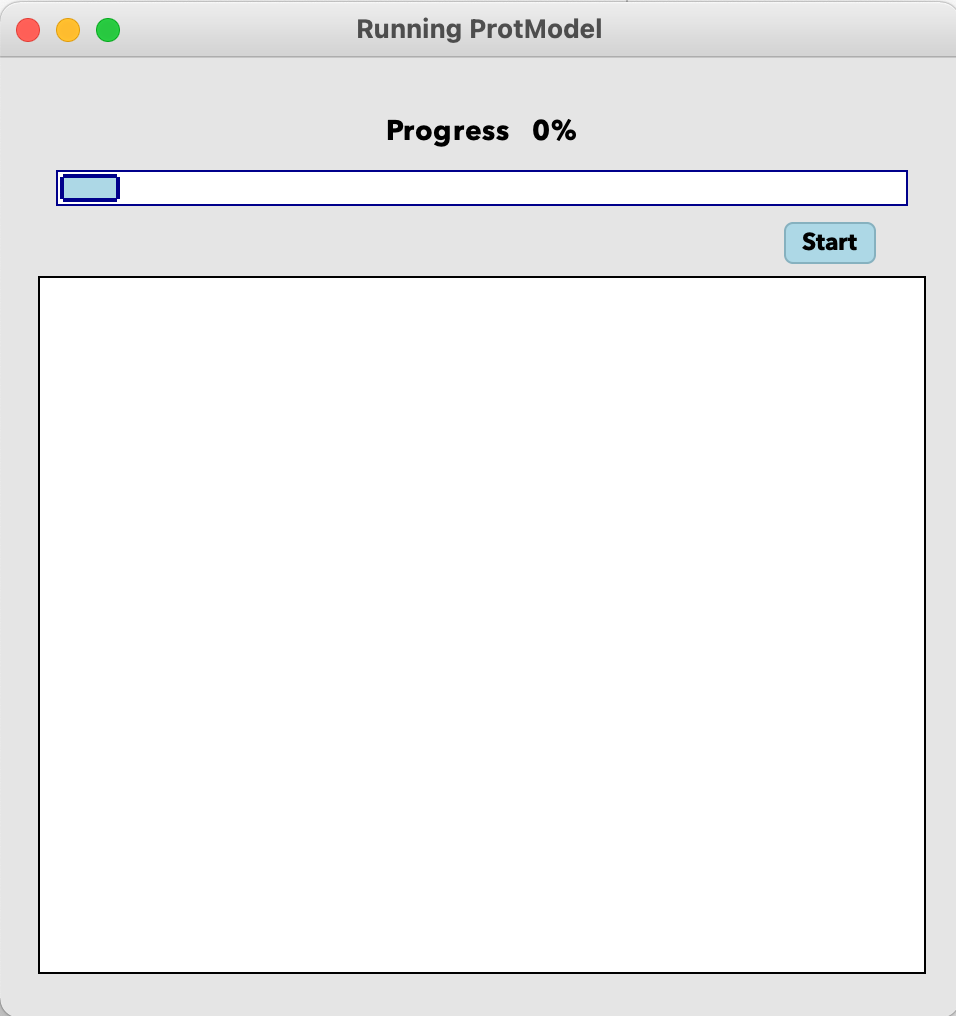
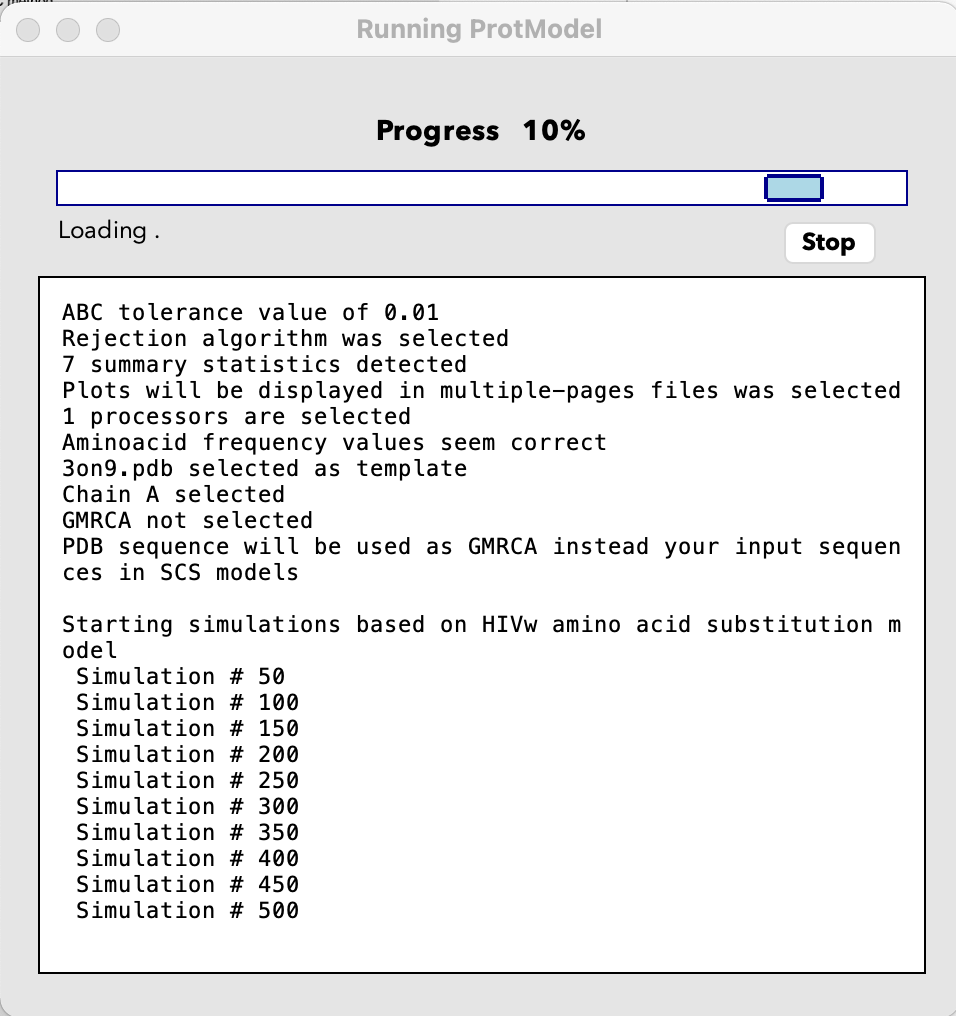
* **Execution with GUI:**

1. To open ProtModel GUI we need to go to the GUI directory and type “*python.3.8 ProtModel\_GUI.py*”. Remember that GUI must not be in the GUI folder, it can be moved but always with the corresponding images.
2. Now, the user must click or fill in all the mandatory entries. Once everything is filled, the user must click on the “*Save and* *Continue*” button to start the simulation and estimation phases. *ProtModel* will show up warnings if the user forgets to fill an entry or does it wrong, but user-specified settings without biological meaning won’t be detected, which would may produce incorrect estimations.

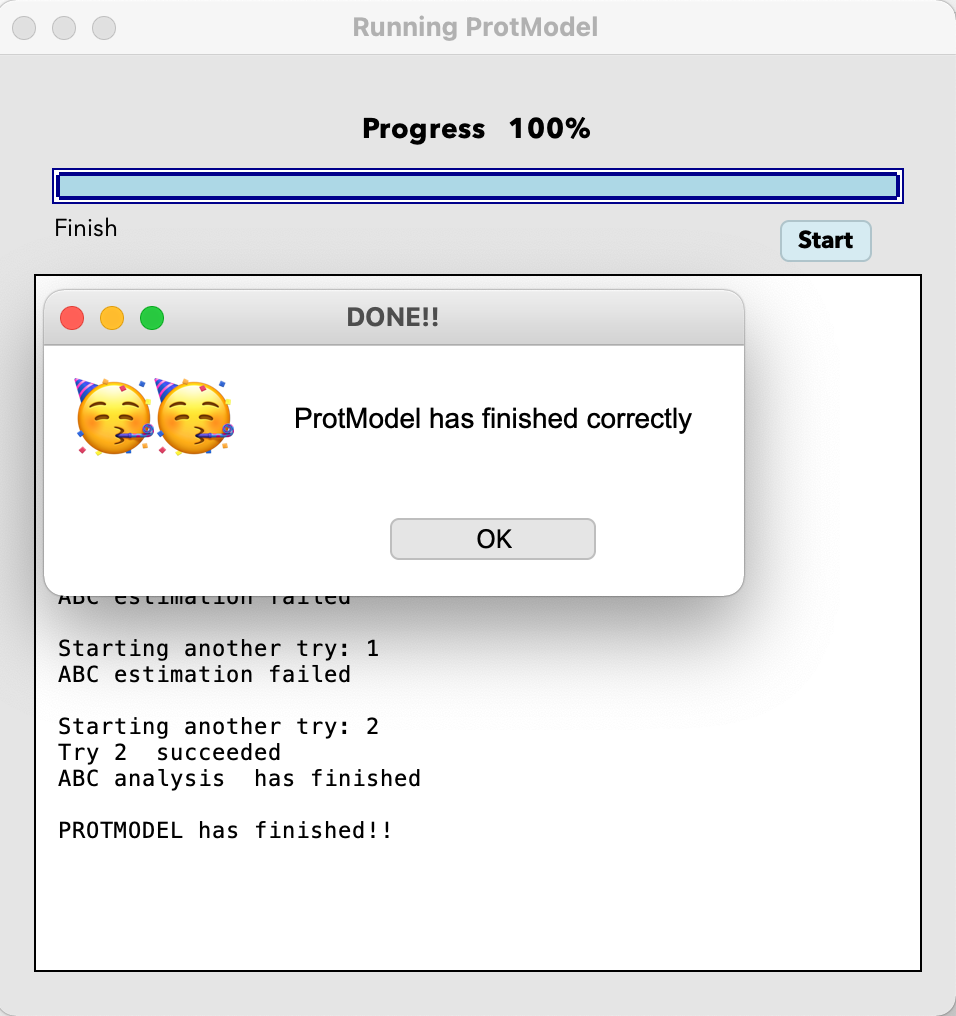
****

****

1. A new window will appear and to launch the simulation and the estimation phase the user only have to click in the “*Start*” button. Inside this window there are a box which will be display all the *ProtModel* execution information. It will be explained in the next section.

******

1. Once ProtModel is done, a new message will show up.

****

* **Execution with terminal:**

1. First, the user has to modify the Settings.txt file. Again, we recommend to not change anything more than the parameters values in the file.
2. Once we have our input files ready, we have to go to the ProtModel main directory and type “*python.3.8 ProtModelGeneral.py*”.

*Test\_ProtModel %* ***ls***

*3on9.pdb* ***GUI*** *TNF.phy*

*ChangeVariablesPE.py LeerSettings.py Variables.py*

***DeltaGREM*** *Makefile* ***bin***

*Errores.py ProtModelGeneral.py source*

*Functions.py Settings.txt structures.in*

*Test\_ProtModel %* **python3.8 ProtModelGeneral.py**

1. First, Settings.txt file is read and the main inputs are printed:

Alignment file exists

Alignment file exists

Correct number of simulations

Indels are ignored

Simulated data is saved

Running information will not be displayed on the screen

Diploid data are selected

PopulationSize selected

Parameter values sampled from a uniform distribution of 0 - 1.3020833e-04

Parameter values sampled from a uniform distribution of 0 - 1.3020833e-04 seem correct

HIVw substitution model selected

Fitness substitution model selected

Neutral substitution model selected

ABC iterations value of 100

ABC tolerance value of 0.01

Rejection algorithm was selected

7 summary statistics detected

Plots will be displayed in multiple-pages files was selected

12 processors are selected

Aminoacid frequency values seem correct

3on9.pdb selected as template

Chain A selected

GMRCA not selected

PDB sequence will be used as GMRCA instead your input sequences in SCS models

1. If any parameter is incorrect, ProtModel will stop the execution and print a message error (see section 4.6) as:

Number of processors introduce is higher than your computer number of processors

Please check your computer number of processors

ERROR!! Please check NumberOfProcessors value

1. Next, if the user has selected empirical substitution model, simulations will be launched:

Starting simulations based on HIVw amino acid substitution model

Simulation # 50

Simulation # 100

Simulation # 150

Simulation # 200

Simulation # 250

Simulation # 300

Simulation # 350

Simulation # 400

Simulation # 450

Simulation # 500

Simulation # 550

Simulation # 600

Simulation # 650

Simulation # 700

Simulation # 750

Simulation # 800

Simulation # 850

Simulation # 900

Simulation # 950

Simulation # 1000

Simulations based on HIVw amino acid substitution models ended

1. Later, if the user has selected structural substitution model, simulations will be launched:

Starting simulations based on Fitness amino acid substitution model

Simulation # 1050

Simulation # 1100

Simulation # 1150

Simulation # 1200

Simulation # 1250

Simulation # 1300

Simulation # 1350

Simulation # 1400

Simulation # 1450

Simulation # 1500

Simulation # 1550

Simulation # 1600

Simulation # 1650

Simulation # 1700

Simulation # 1750

Simulation # 1800

Simulation # 1850

Simulation # 1900

Simulation # 1950

Simulation # 2000

Simulations based on Fitness amino acid substitution models ended

Starting simulations based on Neutral amino acid substitution model

Simulation # 2050

Simulation # 2100

Simulation # 2150

Simulation # 2200

Simulation # 2250

Simulation # 2300

Simulation # 2350

Simulation # 2400

Simulation # 2450

Simulation # 2500

Simulation # 2550

Simulation # 2600

Simulation # 2650

Simulation # 2700

Simulation # 2750

Simulation # 2800

Simulation # 2850

Simulation # 2900

Simulation # 2950

Simulation # 3000

Simulations based on Neutral amino acid substitution models ended

1. In the next step ProtModel will calculate the summary statistics of the observed and of the simulated data:

Summary statistics calculation

Calculating TNF summary statistics

Calculating simulation # 50 summary statistics

Calculating simulation # 100 summary statistics

Calculating simulation # 150 summary statistics

Calculating simulation # 200 summary statistics

Calculating simulation # 250 summary statistics

Calculating simulation # 300 summary statistics

Calculating simulation # 350 summary statistics

Calculating simulation # 400 summary statistics

Calculating simulation # 450 summary statistics

Calculating simulation # 500 summary statistics

Calculating simulation # 550 summary statistics

Calculating simulation # 600 summary statistics

Calculating simulation # 650 summary statistics

Calculating simulation # 700 summary statistics

Calculating simulation # 750 summary statistics

Calculating simulation # 800 summary statistics

Calculating simulation # 850 summary statistics

Calculating simulation # 900 summary statistics

Calculating simulation # 950 summary statistics

Calculating simulation # 1000 summary statistics

Calculating simulation # 1050 summary statistics

Calculating simulation # 1100 summary statistics

Calculating simulation # 1150 summary statistics

Calculating simulation # 1200 summary statistics

Calculating simulation # 1250 summary statistics

Calculating simulation # 1300 summary statistics

Calculating simulation # 1350 summary statistics

Calculating simulation # 1400 summary statistics

Calculating simulation # 1450 summary statistics

Calculating simulation # 1500 summary statistics

Calculating simulation # 1550 summary statistics

Calculating simulation # 1600 summary statistics

Calculating simulation # 1650 summary statistics

Calculating simulation # 1700 summary statistics

Calculating simulation # 1750 summary statistics

Calculating simulation # 1800 summary statistics

Calculating simulation # 1850 summary statistics

Calculating simulation # 1900 summary statistics

Calculating simulation # 1950 summary statistics

Calculating simulation # 2000 summary statistics

Calculating simulation # 2050 summary statistics

Calculating simulation # 2100 summary statistics

Calculating simulation # 2150 summary statistics

Calculating simulation # 2200 summary statistics

Calculating simulation # 2250 summary statistics

Calculating simulation # 2300 summary statistics

Calculating simulation # 2350 summary statistics

Calculating simulation # 2400 summary statistics

Calculating simulation # 2450 summary statistics

Calculating simulation # 2500 summary statistics

Calculating simulation # 2550 summary statistics

Calculating simulation # 2600 summary statistics

Calculating simulation # 2650 summary statistics

Calculating simulation # 2700 summary statistics

Calculating simulation # 2750 summary statistics

Calculating simulation # 2800 summary statistics

Calculating simulation # 2850 summary statistics

Calculating simulation # 2900 summary statistics

Calculating simulation # 2950 summary statistics

Calculating simulation # 3000 summary statistics

1. Next, the ABC estimation will be launched. Sometimes, ABC can fail but if we repeat the estimation, it can success. So, ABC estimation will be performed up to 10 times.

Executing ABC analysis

ABC estimation failed

Starting another try: 1

ABC estimation failed

Starting another try: 2

Try 2 succeeded

ABC analysis has finished

1. Finally, a message will be printed to confirm that *ProtModel* has finished:

PROTMODEL has finished!!

* **Execution on Cluster:**

1. First, the user has to modify the Settings.txt file. Again, we recommend to not change anything more than the parameters values in the file.
2. Once we have our input files ready, we have to go to the ProtModel main directory and type “*python.3.8 ProtModel\_Clusterl.py*”.
3. Now, instead of launching the simulations and the summary statistics calculations as the other versions, some executables files are created. The user only has to focus on the main file, “*launch\_Simu.sh*”. It will be created following the CESGA cluster format so maybe it has to be adapted to the user’s cluster changing any of the first lines (those which start with “*#*”) or the next line, which load CESGA modules needed for the execution. However, change the other lines, which launch the simulations and the summary statistics calculations is not recommended. *ProtModel* running information will be the same as in the others versions.
4. In this version, the **ABC estimation will not be performed.** Our cluster didn´t have the R version needed to work with the “*abc library*” so the user must to download the folder and perform the estimation running the “*ABCAnalysis.r*” file. **The user must change the path to corresponding of the directory once downloaded.** See section 4.4.

## Output files

Several output files are generated by *ProtModel* during the different stages of the estimation. These files are saved in the output folders “*ABCOutputs*” and “*SimulationsOutputs*” that will be placed in the working directory.

### ABCOutputs

In this folder is saved the information of the ABC estimations and all the necessary files to run again the ABC estimation. A normal *ProtModel* run will produce:

* The file “*Histogram\_Priors.pdf*” show illustrative histogram of the prior distributions used for the simulations of substitution rates. In addition, a θ histogram is also provided.
* In case the user selects all summary statistics, the files “*Results\_SS\_Energy.pdf*” and “*Results\_SS\_AAReplacements.pdf*” will be created. “*Results\_SS\_Energy.pdf*” shows two boxplot graphs. The first one representing the stability mean of simulated proteins of every substitution model studied (ΔG kcal/mol) and the second one their corresponding standard deviation. “*Results\_SS\_AAReplacements.pdf*” shows five boxplot graphs. The first one with the number of segregating sites and the other four with the corresponding mean, standard deviation, skeaness and the kurtosis of the Grantham distances of the simulated proteins. If the user doesn’t select all de summary statistics, only one file called “*Results\_SS.pdf*” is created with the boxplots corresponding with the selected summary statistics. For more information about the summary statistics see section 5.2.
* The file “*Histogram\_GoodnessOfFit.pdf*” shows a histogram of every substitution model tested. It represents the median of the distance between accepted summary statistics and observed ones (represented by the blue line).
* The file “*PCA.pdf*” shows plot of the two first principal components of a principal component analysis (PCA) on the considered summary statistics. The black cross corresponds to a sample of the target sequences alignment and area inside the colours lines represent the retained simulations. Ideally the black cross will be inside only one model area. This plot is also used to assess if the model assumed for the simulations fits well the data.
* The file “*Results\_ConfusionMatrix\_100samp.pdf*” shows a plot of the confusion matrix, a specific table layout that allows visualization of the performance of the distinction between models.
* The file “*Results\_text.txt*” shows the results of the all the analysis performed. First, the confusion matrix followed by an ABC method matrix, second, the best-fitting substitution model estimation followed by a Bayes factors matrix and finally the pvalue of the goodness of fit analyses followed by simulations distances.
* One copy of the summary statistics computed from each simulated data are printed in “*SSSimulations.csv*” file.
* One copy of the summary statistics computed from the target multiple alignment of protein sequences are printed in the “*SSRealData.csv*” file.
* One copy of the file “*PSimulations.txt*” shows the values sampled from the substitution rate prior distribution and its corresponding θ value.
* The script “*ABCAnalysis.r*” contains all the instructions to the ABC estimation phase and to create all the plots.

### SimulationsOutputs

* The other copy of the summary statistics computed from each simulated data are printed in “*SSSimulations.csv*” file.
* The other copy of the summary statistics computed from the target multiple alignment of protein sequences are printed in the “*SSRealData.csv*” file.
* The other copy of the file “*PSimulations.txt*” shows the values sampled from the substitution rate prior distribution and its corresponding θ value.
* The simulated alignments are compressed into the file “*Simulations.tar.gz*” if the user specified in the settings file the option of save the simulations.

*ProtModel* executables files created during the framework execution will be places in this directory to allow user to modify code to adapt it to their corresponding clusters.

## Re-analysing data

*ProtModel* allows to choose a few different settings for the ABC estimation without having to repeat the high time-consuming simulation phase. These settings can be changed for a better tuning of the estimations. Among these, the most important to consider are the following:

- ABC iterations. This setting defines the number of simulations to consider in the ABC analysis. By considering different values of this setting, the user may get some insights on how well the entire space of the simulations is being characterized.

- ABC tolerance. This setting defines the number of simulations closest to the target sequences alignment that are retained. Choosing the tolerance is not trivial: it has to be large enough so that to provide a good characterization of the posterior distribution, but on the other hand, it has to be small enough so that the simulations retained are not very far from the target sequences alignment.

- Summary statistics to use. This setting defines which summary statistics to consider when performing ABC estimations. As the number of simulations and the tolerance interval, the choice on the summary statistics is not trivial in ABC. For the estimation of the best-fitting substitution model, we showed that the use of all the summary statistics provided in *ProtModel* works fairly well but, sometimes, the folding stability standard deviation of the real data is too high and far from the simulations value. In this case, we recommend to repeat the analysis without considering it. See section 5.2

To re-analyse the data, no matter the version used, the user must go to the ABCOutputs folder. Then, he or she must open the ABCAnalysis.r file and change the desired ABC parameters values in the first lines.

*#####################################################*

*################### ABC VARIABLES ###################*

*#####################################################*

*ABC\_Method <- "rejection"*

*ABC\_Tolerance <- 0.01*

*ABC\_N\_Iterations <- 100*

*#####################################################*

*#####################################################*

*#####################################################*

If the user needs to change the path, because of he or she used the Cluster version or due to other reasons, only one line has to be edited.

*#Path*

*address<-paste("/Users/****User route to the new directory****", sep="")*

*setwd(address)*

*############################*

## Examples

## Message errors and recommendations

Errors generated from incorrect settings are usually shown on the screen and the program will suggest abort the execution by typing CTRL+C in the command line. However, if the user uses the GUI, incorrect values will be automatically detected. If the user doesn’t want to work with GUI, I recommend copying a provided input file “Settings.txt” from the “examples” folder and edit it with the desired settings.

The input multiple sequence alignment must be presented in *standard phylip sequential* format. Importantly, the amino acid sequences of the input multiple sequence alignment should only include any of the 20 amino acids (one-letter code) or indels (as “-”). Other letters or symbols (i.e., X, $, ., etc) will produce an error displayed on the screen. Next, *ProtModel* check Settings.txt inputs. If any of them is incorrect (i.e., number when string is expected, parameters out of limits) *ProtModel* will stop the execution printing the parameter which is incorrect.

In general, note that the simulation phase can be computational costly with long running times. This is particularly found when performing a large number of simulations, or when the simulated data includes a large number and long sequences. Therefore, obviously, reducing the number of simulations and the size of the input alignment of protein sequences can dramatically reduce computer time. Sometimes reducing the input dataset is not be possible and then one can try to reduce the number of simulations. Indeed, running the simulations in parallel (if the machine has more than one processors) can reduce computer times. During sequences simulations some of them may failed due to intrinsic problems of simulation models, especially when Fitness SCS model is used. However, after last simulations was finished *ProtModel* will check if some of them failed and rerun each failed simulation.

During the ABC procedure the estimation may fail due to an incorrect value of the ABC tolerance or iterations or when mnlogistic or neuralnet methods are selected. If the ABC estimate cannot be carried out completely, no matter the reason, *ProtModel* will launch a big size error.

In this situation this the ABC estimation can be executed again (section 5.4) varying the ABC tolerance, iterations or method in the “*.r*” file. Concerning the ABC method, the rejection approach can be more robust (in terms of being able to work) than the others approaches to analyze real data. In addition to varying this values, one can increase the number of simulations. This could be enough to run successfully the ABC estimation but implies repeat the whole *ProtModel* execution with the new number of simulations.

If you find any unexpected error, or there is any doubt, do not hesitate to contact [ferreirogarciadavid@gmail.com](mailto:ferreirogarciadavid@gmail.com). Thanks for your contribution!

# Models and Methods

*ProtModel* is a framework to the best-fitting substitution model of protein sequence alignments using an ABC procedure. The simulator includes a variety of evolutionary models that may help to better mimic real scenarios. Then, informative summary statistics are calculated from the simulated data. Finally, the computation of the posterior distributions is performed using ABC methods.

## Evolutionary Models and Computer Simulations

*ProtModel* is able to work with both empirical or site independent and with site-dependent substitution models that consider the stability of the protein, called structural constrain substitution (SCS) models (Arenas et al., 2015). In case of empirical substitution models, the user can select among Blosum62 (Henikoff & Henikoff, 1992), CpRev (Adachi et al., 2000), Dayhoff , DayhoffDCMUT (Kosiol & Goldman, 2005), HIVb (Nickle et al., 2007), HIVw (Nickle et al., 2007), JTT (Jones et al., 1992), JonesDCMUT (Kosiol & Goldman, 2005), LG (Le & Gascuel, 2008), Mtart (Abascal et al., 2006), Mtmam (Yang et al., 1998), Mtrev24 (Adachi & Hasegawa, s. f.), RtRev (Dimmic et al., 2002), VT (Müller & Vingron, 2000), WAG (Whelan & Goldman, 2001) and UserEAAM models (Arenas, 2015) (the latter is a user-defined model that must be provided as an additional input file with name UserEAAM, the format of this file is explained with details in the documentation of the simulator *ProteinEvolver* (Arenas et al., 2013)). Within SCS models, there are two available, mean-field Neutral (which does not consider population size) and mean-field Fitness (which needs user specified population size) (Arenas et al., 2015).

The first step of *ProtModel* is based on the simulator *ProteinEvolverProtABC*, which is an adapted version of the program *ProteinEvolver* (<https://github.com/MiguelArenas/proteinevolver>) (Arenas et al., 2013) to perform ABC. *ProteinEvolverProtABC* implements a variety of evolutionary models and generates protein sequence alignments (see below) collected at same or different times (temporal longitudinal sampling or tip dates [see, Navascués et al., 2010]), from a population evolved using the coalescent approach (Hudson, 2002; Kingman, 1982). Protein sequences are evolved under a variety of empirical substitution models of protein evolution Blosum62 (Henikoff & Henikoff, 1992), CpRev (Adachi et al., 2000), Dayhoff , DayhoffDCMUT (Kosiol & Goldman, 2005), HIVb (Nickle et al., 2007), HIVw (Nickle et al., 2007), JTT (Jones et al., 1992), JonesDCMUT (Kosiol & Goldman, 2005), LG (Le & Gascuel, 2008), Mtart (Abascal et al., 2006), Mtmam (Yang et al., 1998), Mtrev24 (Adachi & Hasegawa, s. f.), RtRev (Dimmic et al., 2002), VT (Müller & Vingron, 2000), WAG (Whelan & Goldman, 2001) and UserEAAM models (Arenas, 2015). Of course, protein sequences can be evolved under SCS models of protein evolution too, suing mean-field Neutral (which does not consider population size) and mean-field Fitness (which needs user specified population size) (Arenas et al., 2015). Both haploid and diploid data can be simulated. Indeed, the program also implements heterogeneity across sites according to a gamma distribution (+G) and proportion of invariable sites (+I) (Yang, 1996).

## Summary Statistics

*ProtModel* includes a total of 7 summary statistics (Table 3). The first two summary statistics are related with the proteins folding stability. Folding stability was measured with another framework called *DeltaGREM* (Arenas et al., 2017) using Gibbs free energy difference between folded and unfolded states (ΔG kcal/mol). In addition, *ProtModel* uses the number of animo acids segregating sites and the mean, standard deviation, skeaness and kurtosis of Grantham distance between amino acids replacements per protein site. It assesses the difference between replaced amino acids (Grantham, 1974).

Table 3. Summary statistics implemented in *ProtModel* and their identifiers (ID).

|  |  |  |
| --- | --- | --- |
| *Name* | *Description* | *ID* |
| DGREM\_Mean | Mean of alignment folding stability | 1 |
| DGREM\_sd | Standard deviation of alignment folding stability | 2 |
| SegSites | Number of segregation sites | 3 |
| Grantham\_mean\_Position | Mean of the Grantham distance between aa replacements per protein site | 4 |
| Grantham\_sd\_Position | Standard deviation of the Grantham distance between aa replacements per protein site | 5 |
| Grantham\_sk\_Position | Skeaness of the Grantham distance between aa replacements per protein site | 6 |
| Grantham\_ku\_Position | Kurtosis of the Grantham distance between aa replacements per protein site | 7 |

## ABC Methods

Different ABC estimation methods are implemented in *ProtModel*. In the first one, “rejection”, the posterior probability of a given model is approximated by the proportion of accepted simulations given this model. This approximation holds when the different models are a priori equally likely, and the same number of simulations is performed for each model. On the other hand, “mnlogistic”, estimate the posterior probabilities using a multinomial logistic regression using neural networks and, finally, “neuralnet”, use neural networks too to predict the probabilities of models based on the observed statistics. This method can be useful if many summary statistics are used.

## *ProtModel* Performance

Substitution models of amino acids replacement are employed routinely to study evolutionary processes of protein evolution (Arenas 2015, Thorne 2000). However, there is a lack of evolutionary frameworks to select the best-fitting substitution model including SCS models, which successfully yielded accurate inferences of protein evolution overcoming empirical substitution models.

I performed an evaluation of *ProtModel* using computer simulations with *ProteinEvolverProtABC* considering other studies information (BIBLIO): (i) number of protein simulations (10 000, 50 000 and 100 000), (ii) ABC tolerance (0.005, 0.01, and 0.05) and (iii) ABC three different method (rejection, mnlogistic and neuralnet). We performed the analysis considering three substitution models Dayhoff (BIBLIO) (empirical model), Fitness and Neutral (Arenas et al., 2015) (both SCS models), using a dataset of 27 sequences and the 1TDE structure (BIBLIO) (Waksman et al., 1994) of the thioredoxin reductase protein family. For every combination of parameters (3 x 3 x 3 = 27 combinations), we tested if *ProtModel* can distinguish between the three substitution models with a cross-validation based on 100 pseudo-observed simulations (BIBLIO). We found that using the rejection ABC method, regardless the number of simulations we obtained similar results while the estimations accuracy decreases while increasing tolerance value. However, mnlogistic and neuralnet ABC methods failed when we consider 10 000, 50 000 and 100 000 simulations with any ABC tolerance value, using the rejection method instead of mnlogistic or neuralnet.

Next, I simulated 100 multiple sequence alignments under each substitution model considered (Dayhoff, Fitness and Neutral) (test datasets). Then, the framework was used to estimate the best-fitting substitution model in the test datasets under ABC based considering 10 000, 50 000 and 100 000 simulations parameterized under a tetha (θ) uniform (0, 500) prior distribution which encompass values that are commonly observed in real data (Lopes et al., 2014).As in previous studies (Arenas et al., 2020), we made a cross-validation of the ABC rejection method based on 100 pseudo-observed simulations with a tolerance of 0.005, 0.01 and 0.05 (Csilléry et al., 2012).

The results shows that the best-fitting substitution model estimation are generally accurate (Figure 2), being able to distinguish between the empirical and the SCS models but also between SCS models. The estimations effectiveness increases with decreasing the tolerance values but they are not almost influenced by the number of simulations, being 10 000 simulations per model enough to properly work.



## Assumptions and Limitations

ABC assumes that simulated data can mimic the real data. Simulations are based on models and are never fully representative of the real process. This is actually a limitation present in any estimation approach based on a model of evolution. The idea is to apply simulations as realistic as possible. The data-generating process assumed by *ProteinEvolverABC* consists of drawing a sample genealogy from the standard coalescent (Kingman, 1982), and then, given the genealogy, generating the sequences evolved under a substitution model of protein evolution (Arenas et al., 2013). This is a well-established methodology to simulate protein sequences upon evolutionary histories (Yang, 2006).

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