# e3SIM
# Epidemiological-ecological-evolutionary simulation framework for genomic epidemiology

immediate

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# Contents

# Glossary

drug resistance
: The trait value affecting the probability of pathogen survival. A higher value of drug resistance is associated with a higher probability of survival at each tick. 28, 40

effective contact
: An effective contact involves a pair of hosts connected by an edge, where one host is infected (the infector) and the other is capable of being infected (the infectee). When super-infection is not activated, only "susceptible" state hosts are capable of being infected, while connect hosts in susceptible, exposed and infected states are capable of being infected when super-infection is activated. 39

epoch
: A range of consecutive ticks. Within each epoch, the base rates for compartmental transitions and the genetic architecture for each trait remain constant. 39, 43

host
: The larger organism that harbours one or more pathogens. 7, 38

initial sequences
: The pathogen genome(s) that infect a fully susceptible host population as outbreak simulation starts. 17

pathogen
: The organism whose transmission between hosts and genomes are being explicitly modeled. 7

tick
: Analogous to the definition of **tick** defined in population genetics simulation framework SLiM. It is the time unit of the epidemiological events, the time unit of mutation happening, and the unit of branch length of the genealogy output. 18, 36, 39, 43

transmissibility
: The trait affecting the probability of pathogen transmission per contact. A higher value of transmissibility is associated with a higher probability of transmitting the pathogen to a connected host at each tick. 28, 39

Glossary

# Preface

**Contents**



## 0.1 An Overview of e3SIM

e3SIM (**E**pidemiological-**e**cological-**e**volutionary **sim**ulation framework for genetic epidemiology) is an agent-based, discrete, forward-in-time outbreak simulator that models pathogen evolution and transmission dynamics featuring host population contact structures. The tool consists of 3 components: pre-simulation, main simulation, and post-simulation, all accessible through the command line. Additionally, we offer a graphic user interface (GUI) to let users interactively conduct all the pre-simulation modules and setting a configuration for the main module. The GUI is structured to guide users step-by-step through the process of configuring and initiating simulations, providing clear and intuitive interaction with the software's features.

### 0.1.1 Introduction

e3SIM implements an agent-based, discrete and forward-in-time approach to simultaneously simulate the transmission dynamics and molecular evolution of a haploid transmissible pathogen population within a static host population structure. It highlights the coupling of evolutionary and epidemiological processes and explicitly models transmission on top of a host contact network. Utilizing a SEIRS compartmental model, e3SIM adeptly balances the simplification of real-world processes with the theoretical constructs inherent in both epidemiological and quantitative genetic models. For a thorough introduction of the background and principle which e3SIM is built upon, please refer to our manuscript.

The package integrates Python, R, and SLiM scripts. Its first component, the pre-simulation modules generates essential input files for the main module to run. The second part executes the main simulation using SLiM — a script-based simulator designed for population genetics — as the back-end engine. We allow various permissible transitions between compartments, where transmissions and treatments are affected by genetic-based trait values. The third component, executed together with the main module, includes analysis and visualizations for the simulation output, including raw whole genome sequence, summary statistics, and graphic representations of results, such as the phylogeny of the transmission tree and the compartments' trajectories averaged over all simulation replicates.

### 0.1.2 A quick summary of usage

e3SIM contains 5 modules. 4 modules belong to the pre-simulation component: *NetworkGenerator* (Chapter 7), *SeedGenerator* (Section 2.2), *GeneticEffectGenerator* (Section 2.3), *HostSeedMatcher* (Section 2.4), and 1 module

belongs to the main component: *OutbreakSimulator* (Chapter 3), which also executes the post-simulation modules. The software can be run in one of the following ways.

- **Sequential**: Run each module individually and sequentially on the command line.
- **Streamlined**: Run programs streamlined on the command line with a complete configuration file. In this option, users can run all pre-simulation, main, and post-processing modules together with a single command by providing a comprehensive configuration file. However, this is not recommended when users are still adjusting their parameters, as e3SIM doesn't provide an option to cache the program and start straight from the stopped timepoint next execution.
- **GUI**: Run pre-simulation modules sequentially in GUI and then execute *OutbreakSimulator* on the command line, using the configuration file generated by the GUI.

The pre-simulation modules generate several specific input files necessary for the main simulation. Users can also create these input files themselves, but the files should follow specific format. The required files for the main simulation include:

1. **Host contact network**: a tab-delimited adjacency list file defining the host contact network, where each row represents a host in order and lists its direct connections. This file is generated by the `NetworkGenerator` module (Chapter 7).
2. **Seeding pathogen sequences**: One file for each seed in the Variant Call Format (`VCF`) format containing the mutation profiles of the seeding pathogen sequences, which are introduced to the host population at the start of the simulation. These files are generated by the `SeedGenerator` module (Section 2.2) through stochastic simulation from the reference genome, unless users choose to seed the host population with the reference genome. The phylogeny of the seeding sequences can also be provided or generated in the Newick (`NWK`) format.
3. **Genetic architecture specification**: A `CSV` file detailing the genetic architecture of pathogen genomes, specifying the effect sizes for traits such as transmissibility and drug resistance associated with mutations in designated genetic elements. This file is generated by the `GeneticEffectGenerator` module (Section 2.3).
4. **Host-seed assignment**: A `csv` file that maps each seeding pathogen sequence to a specific host within the contact network. This file is generated by the `HostSeedMatcher` module (Section 2.4).
5. **Main module configuration**: A `JSON` file that provides the epi-eco-evo parameters used in the main module. If the GUI is used to run the pre-simulation modules, this file will be generated by the GUI. For users who prefer directly interacting with the configuration file, a template of the configuration is provided in our github repository for reference.

Templates for all these necessary input files are available in our Github repository. If the user chooses to create these input files not via the e3SIM pre-simulation modules but by themselves, we still recommend running the relevant pre-simulation modules in the `user_input` mode (Adding `-method user_input` flag to each pre-simulation module). This will validate the user-provided input files and make a copy of the files with fixed file names in the working directory specified.

## 0.2 Installation

Please follow the `README.md`.

# Part I

# Pre-simulation modules

# Chapter 1

# Overview

Before diving into how to navigate the software, let us take a look at the prerequisites for running the pre-simulation modules:

- A **reference genome** in `FASTA` format
- Path to a **working directory** (All e3SIM pre-simulation modules, when running on the command line, require a consistent path to the working directory (provided by `-wkdir` option), so that all input files will be generated in the same directory.

Other inputs may be required depending on specific user needs. For example, in order to randomly generate a genetic architecture for the genome, a `GFF` (general feature format) file is required when running ***GeneticEffectGenerator*** program in random generation mode. We will introduce all required and optional inputs below and include more details in the respective chapters.

Each pre-simulation module should be run in order to ensure proper functioning of dependent programs. Below is a typical workflow of running pre-simulation modules:

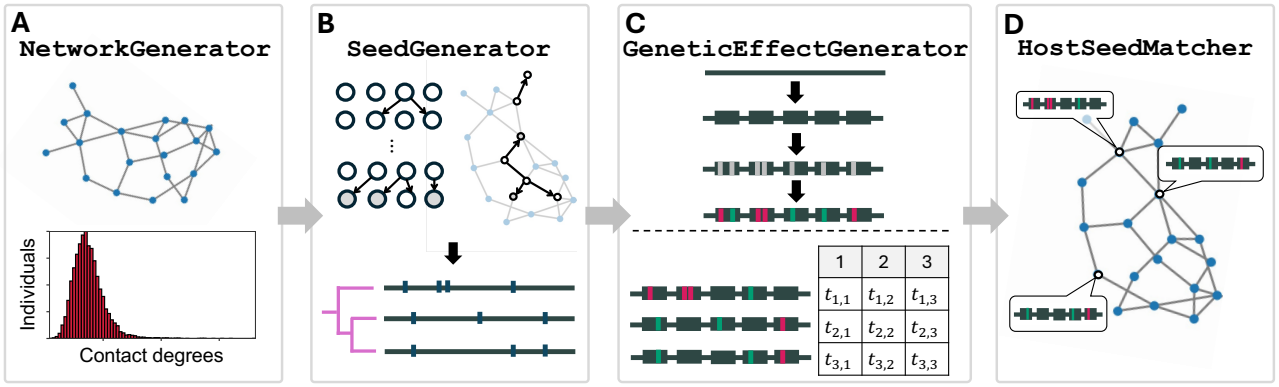



Figure 1.1: **Pre-simulaton modules.**

1. Run ***NetworkGenerator*** to create the host population's contact network. The network is required by ***OutbreakSimulator***, but also needed for ***SeedGenerator*** if you want to generate seed sequences using a burn-in by an epidemiological model, for example.
2. Run ***SeedGenerator*** if you want your seeded sequences to be different from the provided reference genome. If you want to use the unchanged reference genome as the initial sequence for all your seeds, this step could be skipped.

3. Run ***GeneticEffectGenerator*** if you want to enable non-neutral mutations in your simulation. When this step is skipped, mutations are still modeled but do not affect the trait values of the pathogens.

4. Run ***SeedHostMatcher*** after ***NetworkGenerator*** and ***SeedGenerator*** to match each initial sequence to a specific host.

In the following chapters, different options to interact with the pre-simulation modules are introduced.

# Chapter 2

# Run each pre-simulation module

**Contents**



## 2.1 NetworkGenerator

e3SIM models the host population and their contact network explicitly. Hosts are modeled as "subpopulations" in SLiM. The host contact network is modeled as an undirected, unweighted graph, with nodes representing hosts and edges indicating contacts between them. The network structure remains static throughout the main simulation. In the main module, only connected hosts can transmit pathogens between each other.

*NetworkGenerator* is used to generate an adjacency list in the working directory that stores the host contact network. To run *NetworkGenerator*, the required inputs are path of the working directory (`-wkdir`), host population size (`-popsize`), and method of specifying the contact network (`-method`), which could either be random generation (`-method randomly_generate`) or user-defined contact network (`-method user_input`). In the user-input mode, users have to specify the path to a cumtomized adjacency list, rather than use *NetworkGenerator* to randomly generate one, but it is still recommended to execute this program to perform a validation check on your network file. Here is how you can run *NetworkGenerator*:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize ${HOST_SIZE} \
    -method ${METHOD} \
    [Other params]
```

Listing 2.1: NetworkGenerator Usage

We introduce all the options:

- `wkdir`: [*REQUIRED] (`string`)
  Absolute path to the working directory, which should be consistent in one workflow for running each module.

- `popsize`: [*REQUIRED] (`integer`)
  Size of the host population, i.e. Number of nodes in the network graph.

- `method`: [*REQUIRED] (`string`) `user_input` or `randomly_generate`
  The method of generating the network, which can only be one of the two methods above. By specifying `user_input`, users are required to provide their own contact network file by `-path_network`. By specifying `randomly_generate`, users are required to provide a random network model by `-model` and corresponding parameters.

- `path_network`: [*OPTIONAL] (`string`)
  Required if `method` is specified as `user_input`. Absolute path to the customized contact network file. The file has to be in an adjacency list format.

- `model`: [*OPTIONAL] (`string`) `ER` or `BA` or `RP`
  Required if `method` is specified as `randomly_generate`. The random graph model you want to apply for generating the contact network. The choices are `ER` (Erdős–Rényi network), `BA` (Barabási–Albert network) or `RP` (Random partition network that has two partitions).

- `p_ER`: [*OPTIONAL] (`float`)
  Required if `model` is specifed as `ER`. It is the parameter for generating an Erdős–Rényi network, specifying the probability of presence of an edge between two host individuals.

- `rp_size`: [*OPTIONAL] (`list of ints`)
  Required if `model` is specifed as `RP`. It is the parameter for generating a random partition network, specifying the size of each partition. As of now, we only support a two-partition model, thus this option has to be in the format of an integer list of size 2, which sums up to be the size of the host population provided by `-popsize`.

- `p_within`: [*OPTIONAL] (`list of floats`)
  Required if `model` is specifed as `RP`. It is the parameter for generating a random partition network, specifying the probability of edge creation between host individuals within the same partition. As of now, we only support a two-partition model, thus this option has to be a float list of size 2, each representing a contact probability between individuals within each partition, corresponding to the order of population specified in `rp_size`.

- `p_between`: [*OPTIONAL] (`float`)
  Required if `model` is specifed as `RP`. It is the parameter for generating a random partition network, specifying the probability of edge creation between individuals from different partitions.

- `m`: [*OPTIONAL] (`int`)
  Required if `model` is specifed as `BA`. It is the parameter for generating a Barabási–Albert network (Number of contacts to attach from a new individual to existing individuals when creating this network).

- `random_seed`: [*OPTIONAL] (`int`)
  The value that help generate random numbers in the module. Using the same `random_seed` ensures identical output when the module is rerun with the same parameters.

In the upcoming sections, we introduce logistics and examples about the generation of various host contact networks.

### 2.1.1 User input

If the user opts to supply their own contact network, they need to make sure the contact network file is in an adjacency list format, which is a space-delimited file. Lines starting with `#` will be marked as comments. A typical command to run this mode is:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize ${HOST_SIZE} \
    -method user_input \
    -path_network ${NETWORK_PATH}
```

Listing 2.2: NetworkGenerator user_input mode

*NetworkGenerator* will read in the network from the file path user provided and check its format. If the file satisfies the format requirements, the network will be rewritten into a file named `contact_network.adjlist` in the working directory. Note that *OutbreakSimulator* cannot be run without a properly formatted and named `contact_network.adjlist` in the working directory. The step is recommended even if you're providing your own contact network. However, you can modify `contact_network.adjlist` by adding/deleting new contact edges manually as well, but we recommend rerunning *NetworkGenerator* by taking the absolute path of `${WKDIR}/contact_network.adjlist` as input for `-path_network` after your manual modification to make sure it is in a proper format except for non-canonical usage.

Non-canonical usage includes using weighted graph; by default the contact network is unweighted. Any connection between two hosts will only appear once in the file `contact_network.adjlist`, since the network is undirected and unweighted by default. In the main module of e3SIM, each connection will only be evaluated once for transmission per tick. Users can add weight to an edge by duplicating the specific node ids in the relevant lines of `contact_network.adjlist`, so that the same contact will be evaluated multiple times per tick, imitating adding weight to an edge. Do not rerun the program or the weight of edges will not be retained, but users should pay attention to keep proper formatting when making this edit.

### 2.1.2 Well-mixed Host population

To generate a contact network for a specified host population size and a well-mixed host population, where all hosts have the same number of expected contacts, users can utilize the Erdős–Rényi (ER) random graph model. The ER model is parameterized by `p_ER`, which represents the probability of any edge being present in the network. The degrees of any node of the generated network will follow a binomial distribution, with the expected number of edges per individual calculated as `p_ER`×`popsize`.

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize ${HOST_SIZE} \
    -method randomly_generate \
    -model ER \
    -p_ER ${PROB_ER}
```

Listing 2.3: NetworkGenerator ER network

*NetworkGenerator* will use the parameters provided to generate an Erdős–Rényi graph and write down the network into a file named `contact_network.adjlist` in the working directory. For example, to generate a uniform host population of $10,000$ hosts where each host has an expected degree of contact being $10$ $(10 = 10000 * 0.001)$, we run:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize 10000 \
    -method randomly_generate \
    -model ER \
    -p_ER 0.001
```

Listing 2.4: NetworkGenerator ER network example

### 2.1.3 Superspreaders

Using this option will generate a host contact network using the Barabási–Albert (BA) random network model that features unbalanced connectivity, where a few potential "superspreaders" have high connectivity and the rest of the population have low connectivity, representative of a real-world social network for sexually transmitted disease, like HIV. The BA model has a single parameter, `-m`, specifying number of edges to attach from a new node to existing nodes in a BA graph.

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize ${HOST_SIZE} \
    -method randomly_generate \
    -model BA \
    -m ${m}
```

Listing 2.5: NetworkGenerator BA network

*NetworkGenerator* will use the parameters provided to generate an Barabási–Albert random network model and write down the network into a file named `contact_network.adjlist` in the working directory. For examples, to generate a host population of $10,000$ hosts and has $m = 2$, we run:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize 10000 \
    -method randomly_generate \
    -model BA \
    -m 2
```

Listing 2.6: NetworkGenerator BA network example

### 2.1.4 Two Sub-populations

This option is designed for a host contact network with sub-population structures, where there are two partitions in the whole graph. Each partition has their own intra-partition contact probability and there is an inter-partition contact probability for nodes between these two partitions. This sub-population configuration can represent separate rural and urban areas, two countries, an ecological barrier in natural world, and so on. A typical command for this option is:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize ${HOST_SIZE} \
    -method randomly_generate \
    -model RP \
    -rp_size ${POP1_SIZE} ${POP2_SIZE} \
    -p_within ${POP1_PROB} ${POP2_PROB} \
    -p_between ${PROB_BETWEEN_POP1_POP2}
```

Listing 2.7: NetworkGenerator RP network

*NetworkGenerator* will use the parameters provided to generate a random partition graph and write down the network into a file named `contact_network.adjlist` in the working directory. For example, the following command generates a host population of $10,000$ individuals, of which $6000$ hosts come from an urban area where expected connectivity for each host is high as $12 (= 6000 * 0.002)$, and the rest $4000$ come from a rural area where expected connectivity for each host is as low as $4 (= 4000 * 0.001)$. Meanwhile, there are a few connections between the two area so that hosts from one partition are expected to be connected with $3 (= 6000 * 0.0005)$ hosts from the other partition:

```
python network_generator.py \
    -wkdir ${WKDIR} \
    -popsize 10000 \
    -method randomly_generate \
    -rp_size 4000 6000 \
    -p_within 0.001 0.002 \
    -p_between 0.0005
```

Listing 2.8: NetworkGenerator RP network example

## 2.2 SeedGenerator

Initial sequences (a.k.a. seeds) are pathogen genomes that are planted in the host population at the start of the simulation of the main module. They can have distinct genomic sequences and different trait values such as transmissibility and drug-resistance. These trait values are directly affected by their genomic sequences (2.3). If the user wants simulations to seed the main module with the reference genome(s) instead of mutated genome(s) for each initial sequences compared to the reference genome, this module can be skipped. Note that using the reference genome as the initial sequence has to be specified when running *OutbreakSimulator* (Chapter 3). Otherwise, user can run *SeedGenerator* in random generation mode to generate mutated pathogen genomes by forward-in-time simulation, which serve as initial sequences in main simulator. If users choose to provide their own initial sequences, it is recommended to run *SeedGenerator* in user input mode to check the format of the user-provided genomic sequences (in `VCF` format).

*SeedGenerator* will create a new folder named `originalvcfs` under the provided working directory and store one VCF file for each initial sequence, named `seed.X.vcf` in the `originalvcfs` folder (`X` ranges from 0 to `${NUM_INIT_SEQ}` − 1). Please note that any existing `originalvcfs` folder in the working directory will be overwritten when running *SeedGenerator*.

*SeedGenerator* also stores the genealogy of the initial sequences in the working directory if applicable. If random generation mode is being used, it is not guaranteed that the generated seeds have a single common ancestor. If there is a single common ancestor, the genealogy will be stored in a `NWK` format file named `seeds.nwk` in the working directory; Otherwise, *SeedGenerator* will create a folder named `seeds_phylogeny_uncoalesced` in the working directory and write a `NWK` file for each common ancestor for seeds. In the `NWK` file(s), tip labels of the tree are integer numbers from 0 to `${NUM_INIT_SEQ}` − 1, corresponding to the initial sequences' IDs in the `originalvcfs` folder. The `seeds.nwk` file, if present in the working directory, will be used by the post-simulation modules to generate a complete genealogy of the sampled pathogens during the main simulation module. Please see more details in Chapter 3.

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq ${NUM_INIT_SEQ} \
    -method ${METHOD} \
    [Other params]
```

Listing 2.9: SeedGenerator Usage

We now introduce all the options:

- `wkdir`: [*REQUIRED] (`string`)
  Absolute path to the working directory, which should be consistent for each module in one workflow.

- `num_init_seq`: [*REQUIRED] (`integer`)
  Number of initial sequences for the main simulator, i.e. number of infected hosts at the start of the main simulation. Note that this quantity is for the main simulator, rather than the number of starting sequences for running the current module.

- `method`: [*REQUIRED] (`string`) `user_input` or `SLiM_burnin_WF` or `SLiM_burnin_epi`
  Method used for generating the initial sequences, which can only be one of the three choices: `user_input` or `SLiM_burnin_WF` or `SLiM_burnin_epi`. By specifying `user_input`, users are required to provide their own `VCF` file for the initial sequences by `-seed_vcf`. By specifying one of the SLiM-burnin methods, you are required to provide a reference genome by `-ref_path` and other corresponding parameters for the method you choose.

- `init_seq_vcf`: [*OPTIONAL] (`string`)
  Required if `method` is specified as `user_input`. Absolute path to a `VCF` file containing the mutation profiles of the initial sequences.

- `path_init_seq_phylogeny`: [*OPTIONAL] (`string`)
  Optional if `method` is specified as `user_input`, not required otherwise. Absolute path to the initial sequences' phylogeny in `NWK` format.

- `ref_path`: [*OPTIONAL] (`string`)
  Required if `method` is specified as `SLiM_burnin_WF` or `SLiM_burnin_epi`. Absolute path to the reference genome of the pathogen you want to simulate with.

- `use_subst_matrix`: [*OPTIONAL] (`bool`)
  Optional if `method` is specified as `SLiM_burnin_WF` or `SLiM_burnin_epi`. Whether to use a substitution probability matrix to parametrize mutation probability, default is `False`. When False, the substitution model will be default to a Jukes–Cantor model.

- `mu`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_WF` or `SLiM_burnin_epi`, and that `-use_subst_matrix` is specified to be False. Mutation probability of the genomes during burn-in, which is the expected number of mutations per site for each genome in one tick, which is the parameter $\alpha$ for a Jukes–Cantor model (3.2.2). `mu` need not have the same value when running *OutbreakSimulator*, but note that this will cause the branch lengths of the generated phylogenies to be measured in different units, which may be misleading if directly compared. Manual re-scaling the branch length of the initial sequences' phylogeny might be of interest after running *SeedGenerator* in this case.

- `mu_matrix`: [*OPTIONAL] (`str`)
  `'{"A": [0, p_ac, p_ag, p_at], "C": [p_ca, 0, p_cg, p_ct], "G": [p_ga, p_gc, 0, p_gt], "T": [p_ta, p_tc, p_tg, 0]}'`
  Required if `method` is specified as `SLiM_burnin_WF` or `SLiM_burnin_epi`, and that `-use_subst_matrix` is specified to be True. A `JSON` string having the format shown above, where `p_ij` gives the probability of one site in allele `i` mutating to allele `j` in one tick, thus they have to be within the range of $[0, 1]$. Following the tradition of SLiM, `p_ii=0` for $i \in \{A,C,G,T\}$, but in practice the probability for a site in allele `i` staying in allele `i` during one tick will be calculated as $1 - \sum_{j \neq i} \texttt{p\_ij}$. For details of the evolution model, please refer to Section 3.2.2.

- `n_gen`: [*OPTIONAL] (`int`)
  Required if `method` is specified as `SLiM_burnin_WF` or `SLiM_burnin_epi`. Number of ticks/generations that will be run during the seed generation.

- `Ne`: [*OPTIONAL] (`int`)
  Required if `method` is specified as `SLiM_burnin_WF`. The effective population size of a Wright–Fisher model.

- `host_size`: [*OPTIONAL] (`int`)
  Required if `method` is specified as `SLiM_burnin_epi`. Host population size, which needs to be consistent with the size of the network in the `contact_network.adjlist` file in the working directory provided by `-wkdir`.

- `seeded_host_id`: [*OPTIONAL] (`list of ints`)
  Required if `method` is specified as `SLiM_burnin_epi`. ID(s) of the host(s) that will be infected with a pathogen whose genome is identical to the reference genome at first tick of the seed generation process. The host IDs have to be chosen from 0 to host_size - 1.

- `S_IE_prob`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_epi`. The probability of a successful transmission for one effective contact (an infected host and a susceptible host that are connected to each other) per tick. The default value is 0.

- `E_I_prob`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_epi` and `-latency_prob` is also greater than 0. The probability of an exposed host becoming infected per tick. The default value is 0.

- `E_R_prob`: [*OPTIONAL] (`float`)
  Could be supplied if `method` is specified as `SLiM_burnin_epi` and `-latency_prob` is greater than 0. The probability of an exposed host recovering per tick. The default value is 0.

- `latency_prob`: [*OPTIONAL] (`float`)
  Could be supplied if `method` is specified as `SLiM_burnin_epi`. The probability of a susceptible host becoming exposed upon transmission per tick. The default value is 0, meaning that all susceptible hosts become infected upon a relevant transmission.

- `I_R_prob`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_epi`. The probability of an infected host recovering per tick. The default value is 0.

- `I_E_prob`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_epi`. The probability of an infected host deactivating (transitioning to exposed state) per tick. The default value is 0.

- `R_S_prob`: [*OPTIONAL] (`float`)
  Required if `method` is specified as `SLiM_burnin_epi`. The probability of a recovered host losing immunity (going back to susceptible) per tick. The default value is 0, meaning that infected hosts will not go back to the susceptible state. Note that if this variable is zero, the outbreak will eventually diminish, which might cause insufficient number of seeding sequences to sample from.

- `random_seed`: [*OPTIONAL] (`int`)
  The value that help generate random numbers in the module. Using the same `random_seed` ensures identical output when the module is rerun with the same parameters.

### 2.2.1 User input

If the user wants to provide their own initial sequences' mutation information, they need to make sure the provided file is in VCF format. The `.vcf` file should contain the exact number of samples as specified by `-num_seq_init`, and sample names do not matter, as initial sequences will be re-named based on the order in the provided `.vcf` file from 0 to `NUM_INIT_SEQ` − 1. If the provided `.vcf` file has more individuals than

what is specified by `-num_seq_init`, *SeedGenerator* will take the first `NUM_INIT_SEQ` columns for the initial sequences' genetic information, and the subsequent columns will be ignored. Please note that since e3SIM only supports haploid pathogens for now, the phasing won't be informative in the user-provided `.vcf` file. i.e. `0/1` and `1/0` or `1/1` in the sample columns will be all rewritten as `1` during validation. To run this mode:

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq ${NUM_INIT_SEQ} \
    -method user_input \
    -init_seq_vcf ${PATH_TO_INIT_SEQ_VCF}
```

Listing 2.10: SeedGenerator Usage user_input

### 2.2.2 burn-in by Wright-Fisher model

The user can generate initial pathogen genomes with desired genetic diversity using the option to run a forward-in-time simulation using a Wright–Fisher model with SLiM. We run the WF model with `-Ne` constant population size for `-n_gen` generations with a Jukes–Cantor model for substitution model. Here is a template for how to initiate the seed generation process:

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq ${NUM_INIT_SEQ} \
    -method SLiM_burnin_WF \
    -ref_path ${REF_PATH} \
    -use_subst_matrix F \
    -mu ${MU} \
    -Ne ${Ne} \
    -n_gen ${NUM_GENS_BURNIN}
```

Listing 2.11: SeedGenerator Usage SLiM_burnin_WF

At the last generation/tick in *SeedGenerator*, initial sequences will be sampled from the current population. *SeedGenerator* will create a new folder named `originalvcfs` in the provided working directory and write one `.vcf` file for each initial sequence in the `$WKDIR/originalvcfs` directory named `seed.X.vcf` where $X \in \{0, \cdots, \$\texttt{NUM\_INIT\_SEQ} - 1\}$. *SeedGenerator* records the phylogeny of the sequences sampled, but sometimes not all sampled seeding sequences can be traced back to a single common ancestor. If one single common ancestor exists, the initial sequences' phylogeny will be written to the working directory as `seeds.nwk` in NWK format. If multiple ancestors exist, *SeedGenerator* will create a folder named `seeds_phylogeny_uncoalesced` in the working directory and write a `.nwk` file for each separate progeny.

For example, if we want to run a burn-in process for COVID-19 genomes using the WF model, we download COVID-19 genome from the NCBI website into the current directory, retrieving a file named `EPI_ISL_402124.fasta`. We specify the mutation rate as $1 \times 10^{-6}$, which corresponds to the mutation rate of COVID-19 per day in real-time scale. We can run the burn-in for 3650 ticks to simulate 10 years' burn-in period in real-time scale. Let us use an effective population size of 1000 and sample 5 initial sequences:

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq 5 \
    -method SLiM_burnin_WF \
    -ref_path EPI_ISL_402124.fasta \
    -mu 1e-6 \
    -Ne 1000 \
    -n_gen 3650
```

Listing 2.12: SeedGenerator Usage SLiM_burnin_WF example

### 2.2.3 burn-in by epidemiological model

Sometimes a burn-in process using the epidemiological model (which can be the same model and parameters as the real simulation) is desirable. in this case, the user must run *NetworkGenerator* program before they proceed to this step. This mode can be viewed as a minimal version of the *OutbreakSimulator* with sampling only in the last generation/tick with no genetic effects being simulated. In this example, we use a customized substitution probability matrix for the evolution model by specifying `-use_subst_matrix T`.

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq ${NUM_INIT_SEQ} \
    -method SLiM_burnin_epi \
    -ref_path ${REF_PATH} \
    -use_subst_matrix T \
    -mu_matrix '{"A":[0,,,], "C":[,0,,], "G":[,,0,], "T":[,,,0] }' \
    -n_gen ${NUM_GENS_BURNIN} \
    -host_size ${HOST_SIZE}  \
    -seeded_host_id ${SEEDED_HOST_ID} \
    -S_IE_prob ${S_IE_prob} \
    -E_I_prob ${E_I_prob} \
    -E_R_prob ${E_R_prob} \
    -latency_prob ${latency_prob} \
    -I_R_prob ${I_R_prob} \
    -I_E_prob ${I_E_prob} \
    -R_S_prob ${R_S_prob}
```

Listing 2.13: SeedGenerator Usage SLiM_burnin_epi

Note that the transition probabilities all have a default value of 0 and are thus not strictly required to be otherwise specified. However, not providing non-zero probabilities for some transitions can lead to undesired effects. You often want to avoid making any state an absorbing dead end since that can lead to an epidemic dying out before any sequence get sampled. If the number of existing pathogens is less than the desired quantity of initial sequences, the program will instruct you to rerun the burn-in process or modify the parameters. The SEIR trajectory during the burn-in process is recorded and written as a compressed `.csv` file in the working directory provided by `-wkdir`, so users are welcome to inspect the file (named `burn_in_SEIR_trajectory.csv.gz`) to get an impression of the dynamics produced by your current rate parameter settings. If sufficient pathogens are present, *SeedGenerator* will create a new folder named `originalvcfs` under the provided working directory and write one `seed.X.vcf` file for each initial sequence.

As with the `SLiM_burnin_WF` option, *SeedGenerator* records the phylogeny of the sequences sampled, but sometimes not all sampled seeding sequences can be traced back to a single common ancestor. If one single common ancestor exists, the initial sequences' phylogeny will be written to the working directory as `seeds.nwk` in NWK format.

For example, if we want to run a burn-in process for COVID-19 genomes using an epidemiological model, we download COVID-19 genome from the NCBI website into the current directory, retrieving a file named `EPI_ISL_402124.fasta`. We want to specify a mutation probability matrix as such:

| | A | C | G | T |
|---|---|---|---|---|
| A | – | $7.16 \times 10^{-8}$ | $2.84 \times 10^{-7}$ | $5.45 \times 10^{-8}$ |
| C | $1.17 \times 10^{-7}$ | – | $3.51 \times 10^{-8}$ | $1.5 \times 10^{-6}$ |
| G | $4.32 \times 10^{-7}$ | $3.27 \times 10^{-8}$ | – | $2.32 \times 10^{-7}$ |
| T | $5.05 \times 10^{-8}$ | $8.54 \times 10^{-7}$ | $1.42 \times 10^{-7}$ | – |

We can run the burn-in for 3650 ticks to simulate 10 years' burn-in period in real-time scale. Say that we used *NetworkGenerator* to generate a contact network with 10000 hosts and decide that we want to start seed

simulation at individual 256. We want to specify an epidemiological model where each infected host infects each of its connections by a probability of 0.03, and all infected hosts will go through a latent stage upon infection. Since the latency period for COVID-19 is usually 6 days after infection, we choose an activation probability $0.16(\approx \frac{1}{6})$. Since the recovery period for COVID-19 is usually 28 days after infection, we choose a recovery probability of $0.035(\approx \frac{1}{28})$. Post-recovery immunity to COVID-19 lasts for up to 6 months but is highly protective only for the first 2 months, so we set 2 months as the expected time for losing immunity by choosing the probability of immunity loss to be $0.018(\approx \frac{1}{56})$. We do not want to allow infected hosts to go back to latency, or let latent hosts recover. Finally, we want to sample 5 initial sequences. To realize the specification, we run the following:

```
python seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq 5 \
    -method SLiM_burnin_epi \
    -ref_path EPI_ISL_402124.fasta \
    -use_subst_matrix T \
    -mu_matrix '{"A": [0,7.16e-08,2.848e-07,5.45e-08], "C": [1.17e-07,0,3.51e-08,1.5e
    -06], "G": [4.32e-07,3.27e-08,0,2.32e-07], "T": [5.05e-08,8.54e-07,1.42e-07,0]}'
    \
    -n_gen 3650 \
    -host_size 10000 \
    -seeded_host_id 256 \
    -S_IE_prob 0.003 \
    -E_I_prob 0.16 \
    -E_R_prob 0  \
    -latency_prob 1 \
    -I_R_prob 0.035 \
    -I_E_prob 0  \
    -R_S_prob 0.018
```

Listing 2.14: SeedGenerator Usage SLiM_burnin_epi example

For `-seeded_host_id`, you can manually check your contact network file to find a host that you think is good, or you can utilize *HostSeedMatch* program (Section 2.4) to find a specific host that satisfy your need.

## 2.3 GeneticEffectGenerator

*GeneticEffectGenerator* module in e3SIM is designed to configure the causal genomic elements for the main simulator module. *GeneticEffectGenerator* will create `causal_gene_info.csv` in the working directory, where each row represents one causal site. The first columns gives the position on the genome, and the other columns represent the effect sizes for mutations located in each causal genomic element for each trait. Effect size columns for transmissibility and drug resistance are named as `transmissibility_X` or `drug_resistance_X`, where `X` is the ID of the trait in each trait type, 1-indexed.

Code block 2.15 shows an example of a `causal_gene_info.csv` generated by *GeneticEffectGenerator*, where three causal sites conferring additive effects to three different traits are specified.

```
Sites,transmissibility_1,drug_resistance_1,drug_resistance_2
450,0.0,0.3,0.0
651,-0.2,0.0,0.0
4608,0.0,0.0,1.3
```

Listing 2.15: Example of an output file of GeneticEffectGenerator

For how the causal sites affect relevant event probabilities, please refer to Section 4.3. As shown in the example above, *GeneticEffectGenerator* supports more than one trait for transmissibility and/or drug resistance, like `drug_resistance_1` and `drug_resistance_2`. e3SIM operates in an epoch manner (Section 4.1), so

that within one epoch, only one of the traits for the same trait type could be utilized. In this example, during one epoch, users could activate drug treatment by using either `drug_resistance_1` or `drug_resistance_2`, which could differ among epochs. For details about how to specify this in the main module, please refer to Section 4.1.

The causal genomic element configuration file generated by *GeneticEffectGenerator* could come from two modes, user-defined or randomly generated. For a user-defined architecture, users are recommended to run the user-defined mode to check the format of the file they provided, which should be in the same format as Code block 2.15. To randomly generate a genetic architecture, a `CSV` file specifying genomic regions that contain candidate causal sites for each trait should be provided through the `-csv` option. In the candidate causal regions file, each row represent a genomic region. The first two columns specify the starting and ending position of the region on the genome, and the other columns specify the causal status of this region to each trait. For example, Code block 2.16 specifies three genomic regions, with the first region (position 1–50000) causal to the transmissibility trait, the second region (position 50001–100000) causal to the drug resistance trait, and the third region (position 200000–300000) causal to both traits. The causal sites for each trait will be drawn from these causal regions. Please note that though one genomic element can be causal to multiple traits, since e3SIM does not provide random generation of pleiotropic effect size structures, the drawn causal sites won't overlap across traits i.e. one site will only be causal to at most 1 trait.

```
Start,End,transmissibility_1,drug_resistance_1
1,50000,1,0
50001,100000,0,1
200000,300000,1,1
```

Listing 2.16: Example of an input file of GeneticEffectGenerator random generation mode

In this random generation mode, *GeneticEffectGenerator* generate the genetic architecture in a 4-step process:

**Step 1. Choose causal sites from the specified causal regions for each trait**

For trait $i$, let $S_0^{(i)}$ be the trait-specific candidate set of genomic sites (the union of causal regions for trait $i$ specified in the provided genomic region file), and $n_i = \left|S_0^{(i)}\right|$ be the cardinality of $S_0^{(i)}$. Let $c_{ij} \in \{0, 1\}$ for the causal indicator that site $j \in S_0^{(i)}$ is causal for trait $i$, and define the number of causal site for trait $i$ as $K_i = \sum_{j \in S_0^{(i)}} c_{ij}$.

We model heterogeneity in polygenicity across traits by placing a Beta–Binomial prior on $K_i$:

$$\pi_i \sim \text{Beta}(a_i, b_i), \quad K_i \mid \pi_i \sim \text{Binomial}(n_i, \pi_i).$$

To make hyperparameters interpretable, we re-parameterize the Beta prior for per-trait polygenicity by its mean $\mu_i = \mathbb{E}[\pi_i]$ and concentration $\xi$, such that $a_i = \mu_i \xi$ and $b_i = (1 - \mu_i)\xi$. Under this parameterization,

$$\mathbb{E}[K_i] = n_i \mu_i, \quad \text{Var}(K_i) = n_i \mu_i (1 - \mu_i) \frac{\xi + n_i}{\xi + 1}.$$

Users specify an expected fraction of causal sites $\mu_i$ with default at 0.003 through option `-site_frac`, and an optional prior strength $\xi$ controlling dispersion around $\mu_i$ through option `-site_disp`. We default to a moderately informative choice $\xi = 100$.

Given $K_i$, causal sites are sampled uniformly without replacement from the available candidate set for that trait. When more than one trait is simulated, to avoid spurious pleiotropy, we enforce trait exclusivity so that each site can be causal for at most one trait by requiring $\sum_i c_{ij} \leq 1$ for all genomic sites $j$. We process traits in a random permutation, and then iterate over the traits in that order to minimize order effects. For trait $i$, define the available candidate site set $A^{(i)} = S_0^{(i)} \setminus \bigcup_{\ell<i} S^{(\ell)}$ and draw $S^{(i)} \subseteq A^{(i)}$ uniformly without replacement with $\left|S^{(i)}\right| = K_i$ and set $c_{ij} = 1$ for $j \in S^{(i)}$ and 0 otherwise. If $\left|A^{(i)}\right| < K_i$, we set $S^{(i)} = A^{(i)}$ (thus effectively capping $K_i^* = \left|A^{(i)}\right|$) and throw a warning.

**Step 2. Draw non-zero effect sizes for each causal site**

Conditional on $c_{ij} = 1$, we draw $q_{ij}$ on the link scale from one of the following parametric families based on user's input:

1. Normal (point-normal): $q_{ij} \sim \mathcal{N}(0, \tau_i^2)$ with sparsity handled in Step 1. Users can specify the variance of the point normal distribution $\tau_i^2$ for each trait via option `-taus`, default to 1.0.

2. Laplace (Bayesian lasso): $q_{ij} \sim \text{Laplace}(0, \eta_i)$ (heavy-tailed with shrinkage near zero). Users can specify the scale of the Laplace distribution $\eta_i$ for each trait via option `-bs`, default to 1.0.

3. Student-t: $q_{ij} \sim t_\nu(0, s_i)$ (heavy-tailed to allow a few large effects). Users can specify the scale $s_i$ for each trait (default = 1.0) via option `-s` and a degree of freedom value $\nu$ across all traits (default = 3) via option `-nv`.

We require $\eta_i > 0$, $s_i > 0$, and recommend $\nu > 2$ for a finite slab variance $\text{Var}(q_{ij}) = s_i^2 \nu/(\nu - 2)$.

**Step 3. Standardize genetic values and effect sizes**

If the calibration option is activated (`-calibration T`), `GeneticEffectGenerator` standardizes the genetic architecture effect sizes based on the genetic values of the seeding sequences. This option is default to be True when using the random generation mode, and default to be False when using the user-input mode. Given additive link-scale genetic values $G_i = \sum_{j \in S^{(i)}} q_{ij} x_j$ where $x_j \in \{0, 1\}$ indicates the presence of the effect allele (any alternative allele different from the reference allele are defined as effect allele in e3SIM) at site $j$ in the haploid pathogen genome for seeds and trait $i$, we standardize $G_i$ to mean zero and a user-specified variance (specified via option `var_target`, default to 1.0) on the link scale.

We adopt allele-frequency centering, which is standard in quantitative genetics. Let $m$ be the number of seed genomes, and let $G_{i,k}$ be the raw link-scale genetic value for trait $i$ in seed genome $k = 1, \ldots, m$. Let $x_{j,k} \in \{0, 1\}$ denote the derived allele indicator at site $j$ in seed $k$. Define the allele frequency of mutation $j$ in the seeding population and the centered genotype as:

$$\hat{p}_j = \frac{1}{m} \sum_{k=1}^{m} x_{j,k}, \quad z_{j,k} = x_{j,k} - \hat{p}_j.$$

The centered genetic value is then

$$G'_{i,k} = \sum_{j \in S^{(i)}} q_{ij} z_{j,k}.$$

Because $\mathbb{E}[z_{j,k}] = 0$ by construction, $\frac{1}{m} \sum_{k=1}^{m} G'_{i,k} = 0$ for any fixed effect vector $(q_{ij})$.

The across-seed dispersion of genetic values is then fixed by moment matching. We first compute the empirical mean and variance of $G_{i,k}$ across seeds:

$$\hat{\mu}_i = \frac{1}{m} \sum_{k=1}^{m} G'_{i,k} = 0, \quad \hat{V}_i = \frac{1}{m-1} \sum_{k=1}^{m} (G'_{i,k} - \hat{\mu}_i)^2.$$

Given a user-chosen target variance $V_{i,\text{target}} > 0$, define the standardized trait value and scaled effect sizes as:

$$\widetilde{G}_{i,k} = r_i (G'_{i,k} - \hat{\mu}_i), \quad \widetilde{q}_{ij} = r_i q_{ij}, \quad \text{where } r_i = \sqrt{\frac{V_{i,\text{target}}}{\hat{V}_i}},$$

By construction, $\frac{1}{m} \sum_{k=1}^{m} \widetilde{G}_{i,k} = 0$ and $\frac{1}{m-1} \sum_{k=1}^{m} \widetilde{G}_{i,k}^2 = V_{i,\text{target}}$. If seeds are monomorphic at all causal sites for trait $i$, $\hat{V}_i = 0$, so empirical per-SD (standard deviation) calibration is undefined. We therefore

standardize using the expectation of additive genetic variance under a Beta prior on allele frequencies $p_j$ rather than the empirical seed variance:

$$V_i^* = \sum_{j \in S^{(i)}} q_{ij}^2 \mathbb{E}\left[p_j(1-p_j)\right].$$

By default, we assume loci are in linkage equilibrium and use a uniform Beta prior to set a reference scale, for which $\mathbb{E}\left[p_j(1-p_j)\right] = 1/6$. We then rescale effects by $\widetilde{q}_{ij} = r_i q_{ij}$ with:

$$r_i = \sqrt{\frac{V_{i,\text{target}}}{V_i^*}},$$

giving a fixed reference unit $\text{SD}_i = \sqrt{V_{i,\text{target}}}$.

The standardized (or unstandardized if `-calibration F`) effect sizes of causal sites will be stored in the working directory as the genetic architecture file used in the main simulation, named `causal_gene_info.csv`, with the format shown in Code block 2.15. Using this final genetic architecture, the trait values for all seeding sequences, based on their mutation profiles will be calculated and stored as a file named `seeds_trait_values.csv` in the working directory, which could be inspected by the user manually before running the next module. If using the GUI, this trait information will be shown in the `HostSeedMacher` tab for users to inspect. The format is as follows:

```
Seed_ID,transmissibility_1,drug_resistance_1
0,0.72,0.0
1,0.2,-0.34
2,0.72,-0.8
```

Listing 2.17: Example of seeds_trait_values.csv format

**Step 4. Calibrate the link scale slope**

If the calibration option for link scale slope is activated (`calibration_link T`), `GeneticEffectGenerator` calibrate the link scale slope $\alpha$ by specifying the per-SD effect multipliers via option (`-Rs`).

- Logit link: user supplies per-SD odds ratio $R_{i,\text{OR}} > 0$ for trait $i$; set:

$$\alpha_i = \frac{\log R_{i,\text{OR}}}{\text{SD}_i}.$$

- Cloglog link transmissibility: user supplies per-SD transmission hazard ratio $R_{\text{trans,HR}} > 0$; set:

$$\alpha_{\text{trans}} = \frac{\log R_{\text{trans,HR}}}{\text{SD}_{\text{trans}}}.$$

- Cloglog link drug resistance: user supplies per-SD clearance hazard ratio $R_{\text{drug,clr}} > 0$; set:

$$\alpha_{\text{drug}} = -\frac{\log R_{\text{drug,clr}}}{\text{SD}_{\text{drug}}}.$$

A typical command to run *GeneticEffectGenerator* is:

```
python genetic_effect_generator.py \
    -wkdir ${WKDIR} \
    -method ${METHOD} \
    [Other params]
```

Listing 2.18: GeneticEffectGenerator Usage

We now introduce all the options:

- `wkdir`: [*REQUIRED] (`string`)
  Absolute path to the working directory, which should be the same for all modules.

- `method`: [*REQUIRED] (`string`) `user_input` or `randomly_generate`
  The method of setting the genetic architecture, which can only be `user_input` or `randomly_generate`. By specifying `user_input`, users are required to provide their own effect size file via the option `-csv`, that has the format shown in code block 2.15. In this mode, *GeneticEffectGenerator* will not perform the random generation of causal sites and effect sizes, but can do effect size calibration if required. It will mandatorily check the format of provided files and calculate the trait values of the seeding sequences. By specifying `randomly_generate`, you are required to provide the relevant parameters and options to randomly generate causal sites and associated effect sizes from a candidate genomic region file (See the format from Code block 2.16) through the option `-csv`.

- `trait_n`: [*REQUIRED] (`JSON string`) `'{"transmissibility": x, "drug-resistance": y}'`
  A `JSON` string specifying the number of transmissibility and drug resistance traits. `x` and `y` are integer numbers specifying the number of traits for each trait type.

- `num_init_seq`: [*REQUIRED] (`integer`)
  Number of initial sequences for the main simulator, i.e. number of infected hosts at the start of the main simulation. This quantity will be used when calculating the trait values for each initial seeding sequences, and should be the same as specified when running the previous pre-simulation programs. If more sequences than specified are detected in the working directory, only the first `num_init_seq` sequences will be used. The program will throw an error and terminate if fewer sequences than the specified number are detected.

- `csv`: [*REQUIRED] (`string`)
  Absolute path to the user-provided effect size file when `method` is specified as `user_input` (See the format from Code block 2.15); Absolute path to the user-provided candidate causal regions file when `method` is specified as `randomly_generate` (See the format from Code block 2.16).

- `site_frac`: [*OPTIONAL] (`list of floats`)
  Could be specified if `method` is specified as `randomly_generate`. The expected fraction of causal sites to be sampled for each trait from the candidate causal regions. Should be a float list with the same length of the number of traits in total. Each element of the list should be a float within the range (0,1]. Default to 0.003. See the details in the Step 1 of `GeneticEffectGenerator`, $\mu_i$ in the section.

- `site_disp`: [*OPTIONAL] (`float`)
  Could be specified if `method` is specified as `randomly_generate`. Modeling the dispersion of the expected fraction of causal sites in the candidate causal regions. Default to 100. See the details in the Step 1 of `GeneticEffectGenerator`, $\xi$ in the section.

- `func`: [*OPTIONAL] (`string`) `n` or `l` or `st`
  Required if `method` is specified as `randomly_generate`. The distribution to sample effect sizes from given selected causal sites. By specifying `n`, the distribution will be a normal distribution, where users have to provide the variance of each trait's causal effect sizes via `-taus` option. By specifying `l`, the distribution will be a laplace distribution, where users have to provide the scales of each trait's causal effect sizes via `-bs` option. By specifying `st`, the distribution will be a Student's t distribution, where users have to provide the scales of each trait's causal effect sizes via `-s` option, and optionally the degree of freedom via `-nv` option.

- `taus`: [*OPTIONAL] (`list of floats`)
  Could be specified if `method` is specified as `randomly_generate` and `func` is specified as `n`. The variance parameter of the normal distribution for the effect sizes. Should be a float list with the same length of the number of traits in total. Each element of the list should be a number within the range $(0, \infty)$. Default is 1.

- `bs`: [*OPTIONAL] (`list of floats`)
  Could be specified if `method` is specified as `randomly_generate` and `func` is specified as `l`. The scale parameter of the Laplace distribution for the effect sizes. Should be a float list with the same length of the number of traits in total. Each element of the list should be a number within the range $(0, \infty)$. Default is 1.

- `s`: [*OPTIONAL] (`list of floats`)
  Could be specified if `method` is specified as `randomly_generate` and `func` is specified as `st`. The scale parameter of the Student's t's distribution for the effect sizes. Should be a float list with the same length of the number of traits in total. Each element of the list should be a number within the range $(0, \infty)$. Default is 1.

- `nv`: [*OPTIONAL] (`float`)
  Could be specified if `method` is specified as `randomly_generate` and `func` is specified as `st`. The degree of freedom parameter of the Student's t's distribution for the effect sizes across all traits. Should be a positive float. Default is 3. It's recommended to have the value bigger than 2.

- `calibration`: [*OPTIONAL] (`bool`)
  Optional. Whether to calibrate the sampled effect sizes in order to match the initial seeding population's trait values to the variance specified by the users. Default is `False` when `method` is specified as `csv`, and default is `True` when `method` is specified as `gff`. If specified as `True`, the users have to specify the target trait variance via `var_target` option.

- `var_target`: [*OPTIONAL] (`list of floats`)
  Could be specified if `calibration` is specified as `True`. The target variance of the trait values in the seeding populations, will be used for calibrating the effect sizes. Default is 1.

- `calibration_link`: [*OPTIONAL] (`bool`)
  Optional. Whether to calibrate and provide a link slope $\alpha$ for each trait, according to the seeding population trait variances. Default is `False`. If specified as `True`, the users have to specify the link type of the link function via `link` option, and the odds ratio for the transmission/survival per SD of trait values under logit, or the hazard ratio per SD under cloglog via `Rs` option.

- `link`: [*OPTIONAL] (`string`) `logit` or `cloglog`
  Required if `calibration_link` is specified as `True`. The link function to map the trait values to event type. If sepecified as `logit`, the option `Rs` will represent the odds ratio for the transmission/survival per SD of trait values. If sepecified as `cloglog`, the option `Rs` will represent hazard ratio per SD of the trait values.

- `Rs`: [*OPTIONAL] (`list of floats`)
  Required if `calibration` is specified as `True`. The odds ratio for the transmission/survival per SD of trait values or the hazard ratio per SD of the trait values. Should be a positive float list with the same length of the number of traits in total. Default is 1.5 for all traits with logit link or transmissibility trait with cloglog link; default is 0.67 (1/1.5) for drug resistance trait with cloglog link.

- `random_seed`: [*OPTIONAL] (`float`)
  The value that help generate random numbers in the module. Using the same `random_seed` ensures identical output when the module is rerun with the same parameters.

### 2.3.1 User input

By specifying `-method csv`, user could provide a customized genetic architecture file. *GeneticEffectGenerator* will read the user-provided effect size file, check the format and entry of the file, and then rewrite it to `$WKDIR/causal_gene_info.csv`. *GeneticEffectGenerator* also calculates the trait values for each seed based on the genetic architecture file provided, and stores the trait values in `$WKDIR/seeds_trait_values.csv`. The provided genetic architecture file has to be in the format shown in Code block 2.15 with correct format of column.

```
python genetic_effect_generator.py \
    -wkdir ${WKDIR} \
    -method csv \
    -trait_n
    '{"transmissibility": ${NUM_SET_TRANSMISSIBILITY}, "drug_resistance": ${
    NUM_SET_DRUGRESIST}}' \
    -num_init_seq ${NUM_SEED}$
    -csv ${EFF_SIZE_CSVPATH}
```

Listing 2.19: GeneticEffectGenerator usage in the user input mode

### 2.3.2 Generate from candidate regions

The user can also randomly generate an effect size file using a candidate regions as input. A candidate genomic region file has the format like Code block 2.16.

The genetic architecture for one trait is a set of causal sites along with their effect sizes selected for the trait. Several traits for one trait type could be specified. When executing *GeneticEffectGenerator* in the random generation mode, users are required to specify numbers of traits for the two different trait types: transmissibility and drug resistance according to the needs in the main simulator. For example, two traits for drug resistance with different causal genomic regions and effect sizes could be generated, representing resistance for two different types of treatments. In *OutbreakSimulator*, the user could specify two epochs with those two different treatments by activating different drug resistance traits for different epochs.

`GeneticEffectGenerator` contains two main steps to randomly generate the genetic architecture, as discussed earlier in the section. First, the causal sites are drawn from the candidate regions in the `csv` file by independent draws. Given the causal sites, effect sizes for each site will be drawn from a normal ditribution (`-func n`), a Laplace distribution (`-func l`) or a Student's t distribution (`-func st`). All the distributions assume the mean value being 0, and the users specify the dispersion. For normal distribution, users specify the variance. For Laplace or Student's t's distribution, users specify the scale.

Moreover, if `-calibration T` is specified, the effect sizes will be re-scaled according to the existing mutations of the seeding populations. The trait values of seeding sequences will be calculated and the variance of the seeding sequences population will be anchored to rescale the effect sizes such that rescaled trait variance match a user-specified target variance (`-var_target`). To run genetic effect generators without effect size calibration:

```
python genetic_effect_generator.py \
    -wkdir ${WKDIR} \
    -method randomly_generate \
    -num_init_seq ${NUM_SEED}$
    -csv ${CSV_PATH} \
    -trait_n
```

```
    '{"transmissibility": ${NUM_SET_TRANSMISSIBILITY}, "drug_resistance": ${
    NUM_SET_DRUGRESIST}}' \
    -causal_size_each ${NUM_GENE_SET_1} ... ${NUM_GENE_SET_N} \
    # N = ${NUM_SET_TRANSMISSIBILITY}+${NUM_SET_DRUGRESIST}
    -site_disp ${DISPERSION} \
    -site_frac ${FRAC_CAUSALTRAIT_1} ... ${FRAC_CAUSALTRAIT_N} \
    -func n -taus ${VAR_EFFSIZE_TRAIT_1} ... ${VAR_EFFSIZE_TRAIT_N}
```

Listing 2.20: GeneticEffectGenerator Usage randomly generate

If the user would like to add calibration of the effect sizes they will have to specify a few more options:

```
...
-calibration T \
-var_target ${VAR_TARGET_TRAIT_1} ... ${VAR_TARGET_TRAIT_N}
```

Listing 2.21: Calibration of effect sizes

Except for the generation of genetic architecture, `GeneticEffectGenerator` can also be used to calibrate the link scale slope according to the seeding population trait values. This action can be activated by specifying the following options:

```
...
-calibration_link T \
-link logit \
-Rs ${R_TRAIT_1} ... ${R_TRAIT_N}
```

Listing 2.22: Calibration of the link scale slope

For example, now we want to generate the genetic architecture for simulating a *Mtb* outbreak. *Mtb* has a 4.4 megabase genome, among which we would like to select from the candidate regions specified in Code block 2.16, and the file is named as `candidate_regions.csv`. We want to have two sets of drug-resistance traits, so that we can simulate two different treatment stages later. We want to generate the effect sizes for these traits from normal distributions. For the drug resistance traits, we want to generate effect sizes from normal distributions $\mathcal{N}(0, 2)$ and $\mathcal{N}(0, 3)$, respectively. For the first drug resistance trait, we want to select an expected number of 0.1% sites from the candidate regions, while for the second drug resistance trait, we would like the expected fraction being 0.2%. We would like a dispersion slightly bigger than the default value, so we choose $\xi = 50$ In this case, we have already generated 10 seeding sequences from `SeedGenerator`. We want to calibrate our effect sizes such that the variance of drug-resistance traits are 2 and 3, respectively, among the initial sequences. We then execute:

```
python genetic_effect_generator.py \
    -wkdir ${WKDIR} \
    -init_num_seq 10 \
    -method randomly_generate \
    -csv candidate_regions.csv \
    -trait_n  '{"transmissibility": 0, "drug_resistance": 2}' \
    -site_frac 0.001 0.002 \
    -site_disp 50 \
    -func n \
    -taus 3 2 1 \
    -calibration_link T \
    -Rs 2 3
```

Listing 2.23: GeneticEffectGenerator Usage randomly generate example

The generated causal gene information file can be inspected and manually modified by users as well. It is recommended to rerun by `user_input` mode after modifications, so that trait values for each seed can be recalculated and centered. In the random generation mode of `GeneticEffectGenerator`, it is prevented that any causal site has non-zero effect sizes for more than one trait, essentially preventing the program from

generating **pleiotropic** genetic architecture. However, pleiotropy can be supported in the user-input mode. For example, the user can define a site where alternative alleles induce lower transmissibility and higher drug-resistance at the same time.

Please note that e3SIM is not intended to be a comprehensive quantitative trait simulator. Users who require biologically calibrated genetic architectures should either derive them from empirical studies or generate them with dedicated architecture/phenotype simulator.

## 2.4 HostSeedMatcher

After configuring the genetic architectures of the pathogen genomes, the last step before running the main simulator module is to determine the start of the simulation, which is, determining which hosts are patient zero(s) of the simulated outbreak, and which seeding sequence each patient zero is infected by. *HostSeedMatcher* provides different methods to match the seeding sequences to hosts. A typical command to run *HostSeedMatcher* is:

```
python seed_host_match_func.py \
    -wkdir ${WKDIR} \
    -method ${METHOD} \
    -num_init_seq ${NUMBER_OF_INITIAL_SEQUENCES} \
    [Other params]
```

Listing 2.24: HostSeedMatch Usage

Upon successful execution of this module, one `csv` file named `seed_host_match.csv` is produced in the working directory, which has the format of: 1) two columns named `host_id` and `seed`; 2) each row representing one pair of seed-host match; 3) all seeds must be matched to one host; 4) The rows are ordered in the order of the ID of hosts. One example of the output file is:

```
seed,host_id
4,1188
3,1290
1,4059
0,4446
2,7100
```

Listing 2.25: Example of an output file of HostSeedMatcher

Five seeding sequences are provided in this file, each is matched to one of the hosts. The seeds are ordered as $4, 3, 1, 0, 2$ due to the ID of the hosts they are matched to. The host IDs that are smaller appear earlier, which is required for the correct execution of the main simulator module.

The available options for running *HostSeedMatcher* are as follows:

- `wkdir`: [*REQUIRED] (`string`)
  Absolute path to the working directory, which should consistent with the directory used for running previous modules. It is assumed that *NetworkGenerator* has been run, so that `contact_network.adjlist` file is in this directory.

- `method`: [*REQUIRED] (`string`) `user_input` or `randomly_generate`
  The method used matching, which can only be `user_input` or `randomly_generate`. When specifying `user_input`, users are required to provide their own matching file using the `-path_matching` option. On the other hand, specifying `randomly_generate` necessitates providing a match scheme and matching parameters. (`-match_scheme` and `-match_scheme_param`).

- `num_seq_init`: [*REQUIRED] (`int`)
  Total number of seeding sequences to be matched to the hosts.

- `path_matching`: [*OPTIONAL] (`string`)
  Required when `method` is set to `user_input`. Absolute path to the user-provided matching file, which should have the format shown in Code block 2.25.

- `match_scheme`: [*OPTIONAL] (`JSON string`) `'{"0": M0, "1": M1 ...}'`
  Required when `method` is set to `randomly_generate`. A dictionary specifying the matching scheme for each initial sequence, having the format shown above. The keys are the IDs of the seeds, and the values (M0, M1...) are the matching scheme for each seeding sequence, respectively. The values (M0, M1...) should be one of `["ranking", "percentile", "random"]`. If all seeding sequences are to be matched randomly, the dictionary can be replaced by just a single "`random`". For seeding sequences that were not specified with a matching scheme, "`random`" will be the default.

- `match_scheme_param`: [*OPTIONAL] (`JSON string`) `'{"0": P0, "1": P1 ...}'`
  Required when `method` is set to `randomly_generate`. A dictionary containing parameters for the matching scheme of each seeding sequences. For example, if for seeding sequence 0, "`ranking`" is specified in `-match_scheme`, then `P0` here should be an integer number specifying the rank of the host. For detailed introduction, please refer to the sections below for the format of parameters corresponding to each matching method.

- `random_seed`: [*OPTIONAL] (`int`)
  The value that help generate random numbers in the module. Using the same `random_seed` ensures identical output when the module is rerun with the same parameters.

### 2.4.1 User-provided matching file input

If users would like to match host(s) with exact ID(s) to the seeding sequences, one customized seed-host matching file could be provided and checked by *HostSeedMatch* module in the `user_input` mode. The provided matching file has to be in specific format as described by Code block 2.25. An example for running *HostSeedMatch* in this mode is:

```
python seed_host_matcher.py \
    -wkdir ${WKDIR} \
    -method user_input \
    -num_init_seq 10 \
    -path_matching ${PATH_TO_MATCHING_CSV_FILE}
```

Listing 2.26: HostSeedMatch user input example

### 2.4.2 Generate matching based on user-specified methods & parameters

e3SIM provides three different matching schemes: `ranking`, `percentile` and `random`. Different matching scheme could be chosen for each seeding sequences, specified by the `-match_scheme` option. Separate matching scheme parameters have to be provided for each seeding sequences. All three schemes are explained below with an example:

#### Ranking Method (`'ranking'`)

- This scheme assigns seeds to specific hosts based on the host's contact degree ranking. The required parameter is one integer number denoting the host ranking. For example, the host with the highest number of contacts receives the first seed, the second-highest receives the second seed, and so on. Each seed can be assigned to a host with any rank.

- Lower ranks (smaller numbers) correspond to hosts with higher connectivity. For example, rank 1 corresponds to the host with the highest connectivity in the contact network.
- EXAMPLE: To assign seeding sequnece 1 to a host that ranks 100 in connectivity: `-match_scheme '{..., "1": "ranking", ...}'`, `-match_scheme_param '{..., "1": 100, ...}'`

**Percentile Method (`'percentile'`)**

- This scheme assigns seeds to hosts within a connectivity range, specified by percentiles of the host contact degree distribution. The required parameter is an integer list of length two, specifying one percentile range to choose from. Thus the integer numbers in the list has to be within the closed interval [0, 100], with the first number smaller than the second number.
- All hosts are assigned to different percentiles based on the contact degree distribution. By specifying a percentile range, one host within that range is uniformly drawn to be the matched host.
- EXAMPLE: To assign seeding sequence 1 to a host whose connectivity is in the top 25%: `-match_scheme '{..., "1": "percentile", ...}'`, `-match_scheme_param '{..., "1": [0, 25], ...}'`

**Random Method (`'random'`)**

- This scheme randomly select one host from the available host lists. No parameter is required for this scheme. It is also the default method for all seeding sequences. For each seeding sequence, if no specific matching scheme is provided, it is matched by this scheme.
- EXAMPLE: If users want to match all seeding sequences randomly, they don't need to provide `-match_scheme` and `-match_scheme_param` option.

We hereby show a practical example for executing `HostSeedMatcher`: Consider the following scenario that matches 3 seeding sequences by three different schemes. We want to match the first sequence to a host completely at random. We want to match the second sequence to the host possessing the highest number of contacts within the network. Finally, we want to match the third sequence with a host from that is less connected than the medium value. To achieve this customized instance of matching, the following command would be executed:

```
python seed_host_matcher.py \
    -wkdir ${WKDIR} \
    -num_init_seq 3 \
    -method randomly_generate \
    -match_scheme '{"0": "random", "1": "ranking", "2": "percentile"}'
    -match_scheme_param '{"1": 1, "2": [50, 100]}'
```

Listing 2.27: HostSeedMatch user specified matching method(s) example

# Part II

# Simulator

# Chapter 3

# Overview of the usage

**Contents**



## 3.1 Run OutbreakSimulator

In this chapter, we will introduce the main simulator module of e3SIM: *OutbreakSimulator*. To run this module, users should make sure that they have already run necessary pre-simulation modules. *OutbreakSimulator* requires the existence of several files with specific format **in the specified working directory** (e.g., `${WKDIR}`), which are generated by the pre-simulation modules. These files are listed below:

- `contact_network.adjlist`: Required. Generated by *NetworkGeneratror*, an adjacency list specifying the contact network of the host population (Chapter 7).
- `originalvcfs`: Required if the user would like to use seeding sequences different from the reference genome. Generated by *SeedGenerator*, a folder where the `VCF` files for all seeding sequences locates (The `VCF` files are named as `seed.X.vcf` where `X` is the ID of the seeding sequences starting from 0) (Section 2.2)
- `seed_host_match.csv`: Required. Generated by *HostSeedMatch*, a `CSV` file specifying the matching of seeding sequences to the hosts (Section 2.4).
- `seeds.nwk`: Optional. Generated by *SeedGenerator* (Section 2.2), a `NWK` file specifying the genealogy of the seeding sequences. If the file exists, in the post-simulation module, *OutbreakSimulator* could generate the full pathogen genealogy by binding the genealogy of the progeny of each seeding sequence to the the genealogy of the seeding sequences.
- `causal_gene_info.csv`: Required if the user would like to incorporate the coupling of evolutionary process and epidemiological processes in the simulation. Generated by *GeneticEffectGenerator*, a `CSV` file specifying the positions of causal sites and their effect sizes for modeled traits (Section 2.3).
- A configuration file in `JSON` format: Required. If the user used the GUI to perform the pre-simulation modules, the configuration file will be generated in the working directory. Otherwise, users are required to provide a customized configuration file to *OutbreakSimulator*, and the template to the configuration file is in our repository.

To execute `OutbreakSimulator`, only one option is required, which is the absolute path to the configuration file that specifies all the parameters of the simulation. An example to run `OutbreakSimulator` is:

```
python outbreak_simulator.py \
    -config ${YOUR_SIM_CONFIG_PATH}
```

Listing 3.1: OutbreakSimulator usage

- `config` [*REQUIRED] (`string`)
  Absolute path to the configuration file of running *OutbreakSimulator* in a specific format.

We will explain the structure and contents of the configuration file in this chapter. However, it is recommended to go through Chapter 4, that breaks down the components of `OutbreakSimulator` and explains how the simulation works in detail in order to fully understand the meaning of the parameters. In Chapter 5, we will walk through some realistic examples on how to specify and simulate your own model.

## 3.2 Configuration file

There are several sections in the configuration file: `"BasicRunConfiguration"`, `"EvolutionModel"`, `"SeedsConfiguration"`, `"GenomeElement"`, `"NetworkModelParameters"`, `"EpidemiologyModel"` and `"Postprocessing_options"`. In the following paragraphs, we introduce the format and meaning for all options within the configuration file in detail.

### 3.2.1 BasicRunConfiguration

This section specifies basic configurations to execute the simulator.

- `"cwdir"`: (`string`)
  Absolute path to the working directory, which should be the same path provided to run all the pre-simulation programs, so it is expected that necessary files are present in this directory.
- `"n_replicates"`: (`int`)
  The number of replicates to run with the configuration specified in this file.
  (`OutbreakSimulator` will create sub-directories for the output of each replication in the working directory specified by `"cwdir"`).

### 3.2.2 EvolutionModel

This section specifies the evolution model used in the simulation.

- `"n_generation"`: (`int`)
  The number of ticks the user wants the simulation to run.
- `"subst_model_parameterization"`: (`string`) `mut_rate_matrix` or `mut_rate`
  How to parameterize the molecular evolution model. If `mut_rate` is specified, `"mut_rate"` needs to be specified in the configuration file. If `mut_rate_matrix` is specified, `"mut_rate_matrix"` option needs to be specified in the configuration file.
- `"mut_rate"`: (`float`)
  Needed to be specified when `"subst_model_parameterization"`is specified to be `mut_rate`. A single mutation probability each tick for any site in the pathogen genome. By using the `"mut_rate"` mode

for evolution model, `OutbreakSimulator` uses the Jukes–Cantor model in SLiM, thus, the probability of one site mutating into each of the alternative allele (for example, A to C) per tick will be `mut_rate`/3, and the overall mutation probability will be `mut_rate`.

- `"mut_rate_matrix"`: (`list`)
  `[[0,p_AC,p_AG,p_AT],[p_CA,0,p_CG,p_CT],[p_GA,p_GC,0,p_GT],[p_TA,p_TC,p_TG,0]]`
  Needed to be specified when `"subst_model_parameterization"`is specified to be `mut_rate_matrix`. A numerical list of length 4 showing the mutation probability matrix to be used in the simulation. The 4 sub-lists in the list represents the probability of mutation per tick for a site that has allele A,C,G,T. `p_uv` represents the probability of allele $u$ mutating into allele $v$ per tick. All the probabilities should be a numerical value within the closed interval [0,1], and the sum of the probabilities for each sub-list should not exceed 1.

- `"within_host_reproduction"`: (`bool`) `true` or `false`
  Whether to activate within-host reproduction. If activated, pathogens are permitted to reproduce within the same host in a probability specified by `"within_host_reproduction_rate"`.

- `"within_host_reproduction_rate"`: (`float`)
  Probability of reproducing within-host for each existing pathogen genome during each tick. This parameter will not be applied unless `"within_host_reproduction"` is set to `true`.

- `"cap_withinhost"`: (`int`)
  The maximum number of pathogens that is allowed to exist in one host. i.e., if the cap is reached for one host, then no within-host reproduction will happen for the host, and the host cannot be an infectee in a transmission event. The parameter is default to 1. However, if within-host reproduction and/or superinfection is specified to be true, it is recommended to set a different value for `"cap_withinhost"`.

### 3.2.3 SeedsConfiguration

This section specifies the initial sequences.

- `"seed_size"`: (`int`)
  Number of seeding pathogen sequences. Should be the same as what is used for *SeedGenerator* and *HostSeedMatcher*. For example, it is expected that the `originalvcfs` folder in the working directory contains the same number of `VCF` files if applicable.

- `"use_reference"`: (`bool`) `true` or `false`
  Whether to use the reference genome as the seeding sequence(s). If `true`, then it is not required to run *SeedGenerator* in the pre-simulation modules.

### 3.2.4 GenomeElement

This section specifies the causal genomic elements settings that is going to be used in the simulation.

- `"use_genetic_model"`: (`bool`) `true` or `false`
  Whether to let mutation profiles to affect trait values such as transmissibility and drug-resistance. If `true`, then it is required to run *GeneticEffectGenerator* in the pre-simulation modules.

- `"ref_path"`: (`string`)
  Absolute path to the reference genome in `FASTA` format. It should be the same as the one used for *SeedGenerator* if applicable. The `FASTA` file should contain only one sequence, and only composed by the four alleles A,C,G and T.

- `"traits_num"`: (`JSON string`) `{"transmissibility": x, "drug-resistance": y}`
  A `JSON` string specifying the number of traits (the genetic architectures) for to `'transmissibility'` and `'drug_resistance'`, which should be the same as what is used in *GeneticEffectGenerator*'s `-trait_n` option. e.g., `"traits_num": {"transmissibility": 1, "drug_resistance": 2}` represents 1 set for transmissibility and 2 sets for drug-resistance.

- `"trait_prob_link"`:
  This subsection specifies the link function of the genetic architecture i.e. How to link genetic values to probability of events. Please see more details about the genetic architecture and link function in Section 4.3.

  - `"link"`: (`string`) `logit` or `cloglog`
    The link function that will be used to map trait values to the probability scale. If `"logit"` is specified here, then `"alpha_trans"` and `"alpha_drug"` under the `"logit"` section has to be specified, and `OutbreakSimulator` will use the logit link function (4.2). If `"cloglog"` is specified here, then `"alpha_trans"` and `"alpha_drug"` under the `"cloglog"` section has to be specified, and `OutbreakSimulator` will use the complementary log-log link function (4.3).
  - `"logit"` or `"cloglog"`: This subsection specifies the link scale slope for each trait. Only one of the sections need to be filled in depending on which link function type is used. The link function type stays the same across trait types and simulation epochs.
    * `"alpha_trans"`: (`list of floats`)
      The link scale slope $\alpha_{\text{trans}}$ for each transmissibility trait. Has to be a list with the same length as the number of transmissibility traits specified in `traits_num`.
    * `"alpha_drug"`: (`list of floats`)
      The link scale slope $\alpha_{\text{drug}}$ for each drug-resistance trait. Has to be a list with the same length as the number of drug-resistance traits specified in `traits_num`.

### 3.2.5 NetworkModelParameters

This section specifies information about the host population.

- `"host_size"`: (`int`)
  host population size, which should be the same as what was specified in *NetworkGenerator*.

### 3.2.6 EpidemiologyModel

This section specifies the epidemiological model, which includes the compartmental model and the transition probabilities between compartments.

- `"slim_replicate_seed_file_path"`: (`string`)
  Absolute path to a `.csv` file with a single column named `random_number_seed` that stores the random number generator ("seeds" in computational science generally, different from the seeding sequences in e3SIM) for each replicate. If not specified, the random number generator will be chosen by SLiM, and could be checked afterwards in the logging file of SLiM.

- `"model"`: (`string`) `SIR` or `SEIR`
  The compartmental model that will be applied. The user can choose from `"SIR"` or `"SEIR"`. See Section 4.4 for the permitted compartments and transitions. The transitions involving the exposed state will be disabled if `"SIR"` is chosen.

- "epoch_changing":
  This subsection specifies the epoch setting of the simulation. Ticks, the time units in e3SIM, are organized into *epochs*. Each epoch comprises a sequence of consecutive ticks. Within each epoch, one set of epidemiological parameters and genetic architecture is used. Please see more details about epochs in Section 4.1.

  - "n_epoch": (int)
    Number of epochs used in the simulation. By specifying this parameter, all configurations in "genetic_architecture" and "transition_prob" section will be lists with the length of n_epoch, as one set of parameters needs to be specified for each epoch.
  - "epoch_changing_generation": (list of ints)
    The tick(s) at which the simulation switches to the next epoch and its corresponding set of parameters. It should be an integer list with the length of n_epoch - 1. For example, if "n_epoch": 3 is specified, then you should specify "epoch_changing_generation":[t1, t2], where $t1, t2 \in \{1, \cdots, \text{n\_generation}\}$ ("n_generation" was specified in Section 3.2.2).

- "genetic_architecture":
  This subsection specifies whether to invoke genetic architecture, and which trait to use in each epoch. Each parameter listed in this subsection should take the format of a numerical list whose length is the same to n_epoch.

  - "transmissibility": (list of ints)
    An integer list that specifies which transmissibility trait should be used for each epoch. Should be a list of length n_epoch, and each element is an integer representing the trait ID. For example, if "traits_num" is specified to be '{"transmissibility": 2, "drug_resistance": 3}', meaning that there are 2 traits for transmissibility, then here each element in "transmissibility" should be one of $\{0, 1, 2\}$, where 0 represents not using genetic effect on transmissibility in this epoch, and 1 or 2 denotes that the first or second transmissibility genetic architecture will be used in an epoch.
  - "drug_resistance": (list of int)
    An integer list that specifies which drug resistance trait should be used for each epoch. Should be a list of length n_epoch, where each element is an integer representing the trait id. For example, if "traits_num" is specified to be '"transmissibility": 2, "drug_resistance": 3', meaning that you have 3 effect size sets for drug-resistance, then here each element in "drug_resistance" should be one of $\{0, 1, 2, 3\}$, where 0 represents not using genetic effect on drug-resistance in an epoch, and 1 or 2 or 3 denotes that the first, second or third drug resistance genetic architecture will be used in an epoch.

- "transition_prob":
  This subsection specifies the (base) transition probability between compartments in each epoch. Each parameter listed in this subsection should take the format of a numerical list whose length is the same to n_epoch.

  - "S_IE_prob": (list of floats)
    The base probability of a successful transmission for an effective contact in one tick. The actual probability of transmission for one contact could be modified by transmissibility trait of the pathogen from the infector if "transmissibility" isn't 0 in this epoch.
  - "I_R_prob": (list of float)
    The base probability of an infected host recovering for each epoch. In epochs where "drug_resistance"

isn't 0, it represents the probability of an infected host being evaluated for recovery per tick, and the actual recovery probability is modified by drug resistance trait of all the pathogens in the host.

- `"R_S_prob"`: (`list of float`)
  The probability of a recovered host losing immunity per tick (going back to susceptible state) during each epoch.
- `"latency_prob"`: (`list of float`)
  The probability of a susceptible host becoming exposed upon undergoing a successfull transmission for each epoch.
- `"E_I_prob"`: (`list of float`)
  The probability of an exposed host activating (going to the infected state) per tick for each epoch.
- `"I_E_prob"`: (`list of float`)
  The probability of an infected host deactivating (going to the exposed state) for each epoch.
- `"E_R_prob"`: (`list of float`)
  The probability of an exposed host recovering per tick for each epoch.
- `"sample_prob"`: (`list of float`)
  The sequential sampling probability of an infected host per tick for each epoch.
- `"recovery_prob_after_sampling"`: (`list of float`)
  The probability of a host recovering upon being sequentially sampled for each epoch.

- `"massive_sampling"`: A subsection that specifies concerted sampling events (if any). Please refer to 4.4 for how these events work.
  - `"event_num"`: (`int`)
    Number of concerted sampling events to happen.
  - `"generation"`: (`list of int`)
    Ticks when each concerted sampling event happens. Should be a list of length `event_num`.
  - `"sampling_prob"`: (`list of float`)
    Probability of infected hosts being sampled in each concerted sampling event. Should be a list of length `event_num`.
  - `"recovery_prob_after_sampling"`: (`list of float`)
    Probability of infected hosts recovering upon being sampled in each concerted sampling event. Should be a list of length `event_num`.

- `"super_infection"`: (`bool`) `true` or `false`
  Whether to permit super-infections. If this option is set to `true`, the host can be an infectee regardless of existing infection, as long as the number of pathogens within the host does not exceed the specified maximum capacity (`"cap_withinhost"`), and all successful transmissions to this host within a single tick are retained.

### 3.2.7 Postprocessing_options

This subsection specifies whether to do post-simulation data processing and how to visualize the simulated data. Please refer to Part III for all post-processing details.

- `"do_postprocess"`: (`bool`) `true` or `false`
  Whether to do post-simulation processing. If `true`, then some plots and metadata files will be generated. If `false`, then for each replicate, only logging files for epidemiological events, treesequence files of the sampled pathogen genomes will be generated.

- `"tree_plotting"`:
  This sub-subsection specifies how to produce the plotted genealogy of sampled pathogen genomes.
  - `"branch_color_trait"`: (`int`)
    How to color the branches in the generated tree plot, given that `"do_postprocess": true`. If specified as 0, branches will be colored by seed ID. If specified as IDs of other traits (both transmissibility and drug resistance traits are ordered together), branches will be colored by relative trait value (lowest being blue, highest being red). For example, if `"traits_num"` is specified to be `'{"transmissibility": 2, "drug_resistance": 3}'`, then specifying `"branch_color_trait": 2` will generate a tree plot with branches colored by the second transmissibility trait, while specifying `"branch_color_trait": 4` will generate a tree plot with branches colored by the second drug resistance trait.
  - `"heatmap"`: (`string`) `none` or `transmissibility` or `drug_resistance`
    Whether and which trait to plot as a heatmap for each sample in the transmission tree plot, given that `"do_postprocess": true` is specified.
- `"sequence_output"`:
  This sub-subsection specifies how to export the sequences of the sampled pathogen genomes.
  - `"vcf"`: (`bool`) `true` or `false`
    Whether to output `vcf` format of the sampled sequences.
  - `"fasta"`: (`bool`) `true` or `false`
    Whether to output `fasta` format of the sampled sequences.

# Chapter 4

# Modules of the simulator

**Contents**



## 4.1 Epochs and ticks

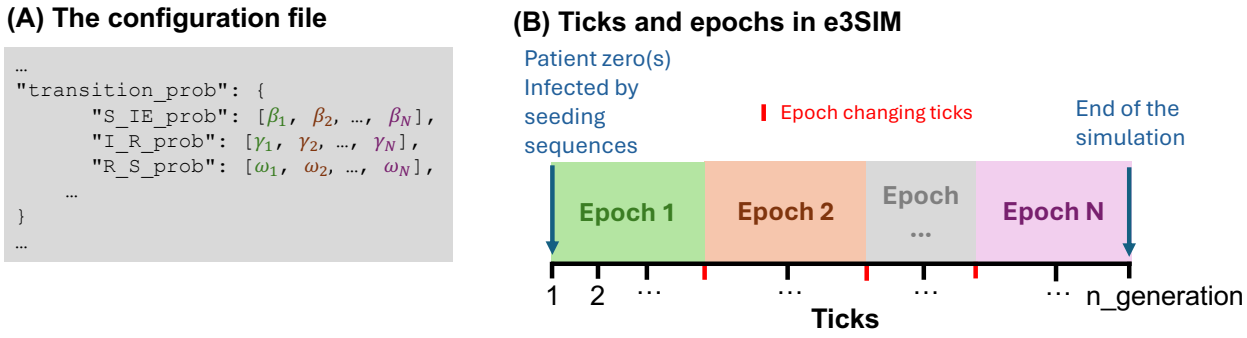



Figure 4.1: **Ticks and epochs in e3SIM.** (A) The configuration file for this simulation. (B) All the ticks are assigned into $N$ epochs, and each epoch uses one set of epidemiological parameters, as colored in (A).

`OutbreakSimulator` operates in discrete time, where each step, or tick, represents the unit of time during which actions occur for each pathogen and host. The simulation concludes when a predefined number of ticks is reached. In every tick, decisions are made for all possible reproduction and compartmental transitions. Ticks are organized into epochs, each comprising a sequence of consecutive ticks. Within each epoch, the base rates for compartmental transitions and the genetic architecture for each trait remain constant. These parameters can change to new values when the simulation transitions to a new epoch. The epoch structure allows the simulation to incorporate time-dependent environmental changes, such as implementing new intervention strategies at specific time points after the outbreak starts. For example, in the following configuration file (only part of it is shown), two epochs are specified in the configuration file:

```
...
"epoch_changing": {
```

```
  "n_epoch": 2,
  "epoch_changing_generation": [100]
},
"genetic_architecture": {
  "transmissibility": [0, 1],
  "drug_resistance": [1, 2],
  ...
},
"transition_prob": {
  "S_IE_prob": [0.1, 0.2],
  "I_R_prob": [0.3, 0.3],
  ...
}
...
```

Listing 4.1: Config file for multiple epochs

In the first epoch (tick 1 through tick 99), no genetic architecture is specified for transmissibility trait, and the first drug resistance trait is used. The base transmission probability and base recovery probability are 0.1 and 0.3, respectively. In the second epoch (tick 100 through the end of the simulation), the first transmissibility trait is used, and the second drug resistance trait is used. The base transmission probability and base recovery probability are 0.2 and 0.3, respectively.

## 4.2 Evolutionary model

In e3SIM, mutations accumulate in all existing pathogen genomes at each time tick according to a user-specified substitution model. The transition probability matrix $\mathbf{P}$ for nucleotides is a $4 \times 4$ matrix where $P_{ij}$ $(i \neq j)$ represents the probability of a nucleotide in state $i$ transitioning to state $j$ in one time tick $(i, j \in \{\text{A, C, G, T}\})$. The overall probability of a site in state $i$ acquiring a mutation in each tick is given by $\sum_{j \neq i} P_{ij}$. If no transition probability matrix is provided, users must specify a mutation rate, defaulting the molecular evolution model to the Jukes–Cantor model (`"subst_model_parameterization": "mut_rate"`).

Otherwise, a mutation probability matrix should be provided (`"subst_model_parameterization": "mut_rate_matrix"`), specifying $P_{ij}$ $(i \neq j)$. Following the format of mutation probability matrix in SLiM, the diagonal values of the mutation probability matrix should be zero (while in practice, the probability of not mutating for allele $i$ is $1 - \sum_{j \neq i} P_{ij}$). To be noted, the provided mutation probability matrix is essentially a transition **probability** matrix in a discrete-time Markov chain, where each time unit is one tick. To simulate the evolution of some specific pathogen, the mutation probability matrix should be scaled to the real time $t_0$ that each tick represents (unit: real time/tick). For example, a transition rate matrix $\mathbf{Q}$ estimated by popular maximum likelihood inference methods such as RAxML or iqtree, represents instantaneous rate of transition, in the time unit of the time needed for 1 mutation to happen per site. To specify an appropriate transition probability matrix for e3SIM, the molecular clock $\mu_0$ (unit: mutation/site/real time) of this pathogen should be used to calculate the transition probability matrix. The calculated transition probability matrix should be

$$\mathbf{P} = e^{\mathbf{Q}t}$$

where $t = t_0 \times \mu_0$. After changing all the diagonal values to 0s, this matrix could be supplied to e3SIM.

Mutations occur stochastically at a constant probability each tick, as defined by the mutation probability matrix, regardless of whether the pathogen reproduces. Trait values, influenced by the mutation profiles of the pathogens, are recalculated before transmission events. These recalculated trait values subsequently alter the probabilities of relevant transitions, such as transmission and recovery. This mechanism captures the crucial interaction between epi-eco-evo processes, which is often overlooked in classical epidemiological simulators

## 4.3 Genetic effects to trait values

We model traits via standard quantitative-genetic additive effects. For trait $i$, let $S^{(i)}$ denote the set of causal sites, set up by `GeneticEffectGenerator` (For details, see Section 2.3). We define the additive genetic value of trait $i$ for a pathogen genome as:

$$G_i = \sum_{j \in S^{(i)}} q_{ij} x_j, \tag{4.1}$$

where $x_j \in \{0, 1\}$ indicates the presence of the effect allele at site $j$ in the haploid pathogen genome, and $q_{ij}$ is the additive effect on the link scale of site $j$ on trait $i$.

We map the genetic value $G_i$ of trait $i$ to the corresponding event probability for each pathogen using standard GLM links: the logit link or the complementary log-log (cloglog) link. Here, we illustrate two traits, transmissibility and drug resistance, but the e3SIM supports any user-defined pathogen genetic traits.

**Logit link (log-odds scale).**
Let $\beta \in (0, 1)$ be the baseline per-contact transmission probability at $G_{\text{trans}} = 0$, and $s \in (0, 1)$ the baseline per-tick pathogen survival probability under treatment at $G_{\text{drug}} = 0$. We map the additive genetic values to probabilities via:

$$\begin{aligned} P(\text{transmission}) &= \sigma\left(\text{logit}(\beta) + \alpha_{\text{trans}} G_{\text{trans}}\right), \\ P(\text{survival} \mid \text{treatment}) &= \sigma\left(\text{logit}(s) + \alpha_{\text{drug}} G_{\text{drug}}\right), \end{aligned} \tag{4.2}$$

where $\text{logit}(p) = \log\left(p/(1-p)\right)$, $\sigma(z) = \text{logit}^{-1}(z) = 1/(1+e^{-z})$, and $\alpha_{\text{trans}}, \alpha_{\text{drug}} > 0$ are slope parameters. Thus, under this link, a unit increase in $G_{\text{trans}}$ multiplies the odds of transmission by $e^{\alpha_{\text{trans}}}$, and a unit increase in $G_{\text{drug}}$ multiplies the odds of survival by $e^{\alpha_{\text{drug}}}$.

**Complementary log–log (cloglog) link (discrete-time proportional hazards).**
Let $\lambda = -\log(1-\beta) > 0$ be the baseline transmission hazard per contact and $\kappa = -\log(s) > 0$ the baseline pathogen clearance hazard per tick under treatment (so baseline pathogen survival equals $e^{-\kappa}$). Then,

$$\begin{aligned} P(\text{transmission}) &= 1 - \exp\left(-\lambda e^{\alpha_{\text{trans}} G_{\text{trans}}}\right), \\ P(\text{survival} \mid \text{treatment}) &= \exp\left(-\kappa e^{-\alpha_{\text{drug}} G_{\text{drug}}}\right). \end{aligned} \tag{4.3}$$

Thus, $e^{\alpha_{\text{trans}}}$ is the transmission hazard ratio per $+1$ unit of $G_{\text{trans}}$, and $e^{-\alpha_{\text{drug}}}$ is the clearance hazard ratio per $+1$ unit of $G_{\text{drug}}$ (so larger $G_{\text{drug}}$ increases survival by reducing clearance hazard). These expressions correspond to standard discrete-time proportional hazards models.

## 4.4 Compartmental model

e3SIM employs a classical compartmental epidemiological model by dividing all hosts into four compartments: susceptible (S), exposed (E), infected (I), and recovered (R). Transitions between compartments have base rates that are specified in the configuration file. e3SIM supports a wide range of transitions:

In every tick, **reproduction** happens first. There are two types of reproduction events in e3SIM: transmission and within-host replication.

Transmission occurs when a pathogen infects a new host by producing offspring within the new host, which can only happen when the hosts are in direct contact. During each tick, all effective contacts are evaluated for potential transmissions. An effective contact involves a pair of hosts connected by an edge, where one host is infected (the infector) and the other is capable of being infected (the infectee). For each effective contact, a single pathogen is randomly selected from the infector's pathogen population. The transmission success of the chosen pathogen is determined by a Bernoulli trial with the success probability calculated from both the base transmission probability $\beta$ and the transmissibility trait (Equation 4.2, Equation 4.3)

Successful trials result in pathogen transmission to the infectee. e3SIM assumes a transmission bottleneck, where only one pathogen genome from the infector is transmitted to the infectee per successful transmission.

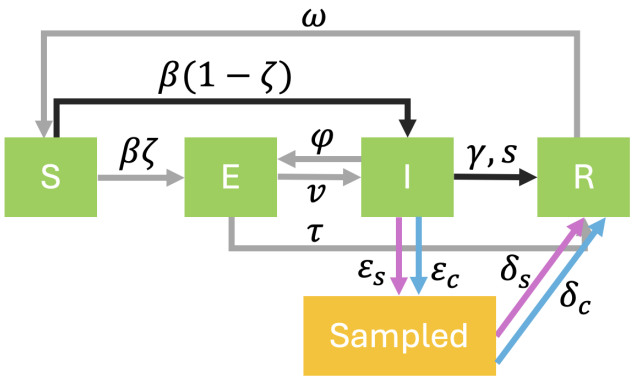



Figure 4.2: **The compartmental model in e3SIM.** Green blocks are the compartments of hosts. The grey arrows show the optional transitions whose probabilities could be specified as a constant, the red arrows are the transition whose probabilities are affected by the relevant trait values of pathogens. The greek letters are the corresponding base transition probabilities, which are fixed within one tick.

Table 4.1: List of symbols for epidemiological parameters. All the probabilities on a per-tick basis.

| Symbol | Description | Keys |
|---|---|---|
| $\omega$ | Probability of immunity loss for a recovered host | `R_S_prob` |
| $\beta$ | Base probability of successful infection event for one effective contact | `S_IE_prob` |
| $\zeta$ | Probability of latent infection for each successful infection event | `latency_prob` |
| $\phi$ | Probability of deactivation for an infected host | `I_E_prob` |
| $\nu$ | Probability of activation for an exposed host | `E_I_prob` |
| $\tau$ | Probability of transitioning to recovered state for an exposed host | `E_R_prob` |
| $\gamma$ | In treatment-free epochs: Base probability of recovery for an infected host; In treatment epochs: Probability of recovery evaluation for an infected host. | `I_R_prob` |
| $s$ | Base probability of pathogen survival under treatment per tick. | `surviv_prob` |
| $\epsilon_s$ | Probability of being sampled in a sequential sampling event for an infected host | `sample_prob` |
| $\delta_s$ | Probability of recovery for an infected host following a sequential sampling event | `recovery_prob_after_sampling` |
| $\epsilon_c$ | Probability of being sampled in a concerted sampling event for an infected host | `sampling_prob` |
| $\delta_c$ | Probability of recovery for an infected host following a concerted sampling event | `recovery_prob_after_sampling` |

"Super-infection" refers to a scenario in which a host can be infected by multiple sources, either within the same tick or across different ticks. If disabled, a host can only be infected if it is in the susceptible state. In this case, only one successful transmission per tick is retained, with the infecting source randomly chosen from the pool of successful transmissions. If super-infection is enabled, the host can be an infectee regardless of existing infection, as long as the number of pathogens within the host does not exceed the specified maximum capacity. All successful transmissions to this host within a single tick are retained.

Within-host replication occurs when a pathogen reproduces within a single host. This process is enabled only if the within-host replication mechanism is active and the total number of pathogens within the host

does not exceed the user-defined maximum capacity. Under these conditions, each pathogen has a probability of producing exactly one offspring within the same host during each simulation tick. Mutations accumulate over time independently of the Section 3.2.2. Therefore, all pathogens, regardless of their reproduction status, have the same probability of mutation per tick.

State changes occur after reproduction events. The transitions for hosts in different states are listed below:

- A newly infected host that is in **susceptible** state before being infected enters the exposed state with a probability $\zeta$ and the infected state with a probability $1-\zeta$. If `superinfection=false`, for hosts that are infected more than once in this tick, only one of the infection events, selected at random, will be kept and all other infection events are omitted. Susceptible hosts that are not involved in transmission events in the current tick remain in the susceptible state.

- For **exposed** hosts, new states are decided by a random draw from a multinoulli distribution with event probabilities $\upsilon$, $\tau$ and $1-\tau-\upsilon$, which represent the probability of activation (`E_I_prob`), recovery (`E_R_prob`), and staying exposed.

- For **infected** hosts, in non-treatment epochs (when `"drug_resistance"` in the `"genetic_architecture"` section is specified as 0 for the current epoch) new states are decided by a random draw from a multinoulli distribution with event probabilities $\gamma$, $\epsilon$, $\phi$ and $1-\gamma-\epsilon-\phi$, which represent the probability of recovery evaluation (`I_R_prob`), being sequentially sampled (`sample_prob`), deactivation (`I_E_prob`), and staying infected. For those chosen to be sequentially sampled, an additional Bernoulli trial with parameter $\delta$ (`recovery_prob_after_sampling`) determines whether the host ends in the recovered or infected state. If drug resistance is inactive in the current tick, all recovery evaluation yields a successful result so that the hosts transit into the recovered state. However, in treatment epochs (when `"drug_resistance"` in the `"genetic_architecture"` section is specified as a drug-resistance trait set different than 0 for the current epoch), then for hosts that are first evaluated for recovery before undergoing other events. We first evaluate the innate recovery event with probability $\gamma$; on success, the host is set to recovered, and all within-host pathogens are cleared. If the immune trial fails, we then evaluate drug-induced clearance at the pathogen level: each pathogen $k$ present at the start of the tick survives treatment independently with probability $p_k = P(\text{survival} \mid \text{treatment})$, mapped from its drug-resistance genetic value (Equation 4.1) via the specified link (Equation 4.2 and 4.3) and baseline survival $s$ at $G_{\text{drug}} = 0$. The host recovers if and only if all within-host pathogens fail their survival trials in that tick; otherwise, the host remains in its current infected state with the surviving pathogens. After the recovery evaluation, the remaining hosts in the infected state undergo sequential sampling event with probability $\epsilon_s$, deactivation event with probability $\phi$, and stay unchanged with probability $1-(\phi+\epsilon_s)$, modeled by random draws from a multinomial distribution.

- For **recovered** hosts, new states are decided by a Bernoulli trial with probability $\omega$ (`R_S_rate`) to see whether they lose their immunity and become susceptible again.

After these sequential state changing events, concerted sampling events happens if specified (specified in the `massive_sampling` section of the configuration file). Concerted sampling only happens in specific ticks, and each event has to be specified by its time-of-happening, sampling probability ($\epsilon_c$) and recovery probability after sampling ($\delta_c$).

# Chapter 5

# Specifying your own configuration

**Contents**



In this chapter, examples of different scenarios are provided to help understand how the configuration file and *OutbreakSimulator* works.

## 5.1 A minimal model for transmissibility

In this section, we will simulate a Tuberculosis (*M.tb*) outbreak in a host population of size $10,000$ for $10$ years. The time scale is that we want each tick to represent $1$ day, so we want to simulate $3650$ ticks. The mutation rate for tuberculosis genome is $0.5$ SNPs per genome per year, which scale to be $3.12 \times 10^{-10}$ SNPs/bp/day. You can set a random seed for this pipeline for reproducibility by setting `RANDOMSEED=1100` before running the pipeline, that should reproduce the results we provide in the repository.

1. The first step is to run *NetworkGenerator*. We want to simulate a well-mixed population with every individual having $10$ contacts. We thus run:

```
python ${e3SIM}/network_generator.py \
    -popsize 10000 \
    -wkdir ${WKDIR} \
    -method randomly_generate \
    -model ER \
    -p_ER 0.001 \
    -random_seed ${RANDOMSEED}
```

Listing 5.1: NetworkGenerator model 1

2. We then run *SeedGenerator* to generate the seeds. We want to run a Wright-Fisher model of $N_e = 1000$ and run for $4000$ generations. During the burn-in, we want to let one generation scale to one year, and thus use mutation rate $1.1 \times 10^{-7}$. We want to generate $5$ seeds. The reference genome we use here is Tuberculosis genome, which we put in our repository as well.

```
python -u ${e3SIM}/seed_generator.py \
    -wkdir ${WKDIR} \
    -num_init_seq 5 \
    -method SLiM_burnin_WF \
    -Ne 1000 \
```

```
        -ref_path GCF_000195955.2_ASM19595v2_genomic.fna \
        -mu 1.1e-7 \
        -n_gen 4000 \
        -random_seed ${RANDOMSEED}
```

Listing 5.2: SeedGenerator model 1

If all the seeds have a single common ancestor, `seeds.nwk` will appear in the working directory, and we can manually scale the branch lengths by a factor of 365 to scale the tree to based on daily mutation rate. For now, we leave `seeds.nwk` unchanged. It's fine if there isn't a `seeds.nwk` file but we won't be able to generate a full phylogeny tree post *OutbreakSimulator*; rerun this program until all seeds coalesce if desired.

3. Next, we would like to run *GeneticEffectGenerator* to generate causal sites from candidate regions. A `CSV` file named `candidate_regions.csv` that contains 100 genes from the TB genome annotation is provided in our repository. We would like to use the default parameters to select the causal sites, and use a normal distribution with variance 1.5 to generate effect sizes. By default, the effect sizes will be calibrated by seeding sequences' mutatoin profiles. We also would like to get the calibrated link scale slope $\alpha$, by setting the transmission odds ratio as 2. We thus run:

```
    python ${e3SIM}/genetic_effect_generator.py \
        -wkdir ${WKDIR} \
        -method randomly_generate \
        -num_init_seq 5 \
        -trait '{"transmissibility": 1, "drug_resistance": 0}' \
        -csv ${WKDIR}/candidate_regions.csv \
        -func n \
        -taus 1.5 \
        -calibration_link T \
        -Rs 2 \
        -random_seed ${RANDOMSEED}
```

Listing 5.3: GeneticEffectGenerator model 1

Running *GeneticEffectGenerator* should output `causal_gene_info.csv` and `seeds_trait_values.csv`. `seeds_trait_values.csv` shows the transmissibility value for each seed based on the genetic architecture that was generated and stored in `causal_gene_info.csv`.

Using the provided random seed, all seeding sequences resulted in zero trait values because no mutations occurred at the designated causal sites. To generate polymorphic trait values, you can rerun the program with a different random seed or manually modify the file `causal_gene_info.csv` and rerun the program using the `-method user_input` option to adjust the genetic effects.

You will also see the calibrated link scale slope in the terminal. Using the random seed we provided, you should see the following information in terminal:

```
The calibrated link-scale slopes under the logit link are as follows.
  transmissibility_1: 0.6648
Please write the following part to your configuration file under the "
    trait_prob_link" key:
{
  "link": "logit",
  "logit": {
    "alpha_trans": [
      0.6648
    ],
```

```
    "alpha_drug": []
  }
}
```

Listing 5.4: Printout message for GeneticEffectGenerator's link scale slope calibration

This gives the calibrated $\alpha$ for all the traits, in this case only one $\alpha_{\text{trans}} = 0.6648$ since this is the only trait generated. The value will not be automatically written to the configuration file, so you will have to manually write that in the configuration file, as shown in Configuration file 5.6.

4. The last step before running *OutbreakSimulator* is matching the seeds to hosts using *HostSeedMatcher*. This time, we want all the seeds to be matched randomly. We can run:

```
python ${e3SIM}/seed_host_matcher.py \
-wkdir ${WKDIR} \
-num_init_seq 5 \
-method randomly_generate \
-random_seed ${RANDOMSEED}
```

Listing 5.5: HostSeedMatcher model 1

`seed_host_match.csv` should appear in the working directory.

5. Now, we can run *OutbreakSimulator*. We first download the template configuration file from the repository, then make the desired modifications. According to the CDC, latency period for TB can be around 3 months, and recovery time can be 4 months for a rifapentine-moxifloxacin treatment regimen. We want to model all new infections initially leading to latency. Though it's controversial, several studies indicate that immunity doesn't exist in TB. We thus assume that immunity loss occurs at around 20 days. For base transmission probability, we assume an arbitrary probability of 0.004, meaning that for each contact pair, the per-day infection probability is 0.4%. We don't want to model other transitions between compartments, and we don't want to model massive sampling events. This is the configuration file we specify using these parameters:

```
{
  "BasicRunConfiguration": {
    "cwdir": "5.1_test_minimal_model",
    "n_replicates": 3
  },
  "EvolutionModel": {
    "subst_model_parameterization": "mut_rate",
    "n_generation": 3650,
    "mut_rate": 3.12e-10,
    "within_host_reproduction": false,
    "within_host_reproduction_rate": 0,
    "cap_withinhost": 1
  },
  "SeedsConfiguration": {
    "seed_size": 5,
    "use_reference": false
  },
  "GenomeElement": {
    "use_genetic_model": true,
    "ref_path": "GCF_000195955.2_ASM19595v2_genomic.fna",
    "traits_num": {
      "transmissibility": 1,
      "drug_resistance": 0
    },
```

```
    "trait_prob_link": {
      "link": "logit",
      "logit": {
        "alpha_trans": [
          0.6648
        ],
        "alpha_drug": []
      }
    }
  },
  "NetworkModelParameters": {
    "host_size": 10000
  },
  "EpidemiologyModel": {
    "model": "SEIR",
    "slim_replicate_seed_file_path": "slimseeds.csv",
    "epoch_changing": {
      "n_epoch": 1,
      "epoch_changing_generation": []
    },
    "genetic_architecture": {
      "transmissibility": [
        1
      ],
      "drug_resistance": [
        0
      ]
    },
    "transition_prob": {
      "S_IE_prob": [
        0.004
      ],
      "I_R_prob": [
        0.008
      ],
      "R_S_prob": [
        0.05
      ],
      "latency_prob": [
        1
      ],
      "E_I_prob": [
        0.01
      ],
      "I_E_prob": [
        0
      ],
      "E_R_prob": [
        0
      ],
      "sample_prob": [
        1e-05
      ],
      "recovery_prob_after_sampling": [
        0
      ]
    },
    "massive_sampling": {
      "event_num": 0,
      "generation": [],
```

```
      "sampling_prob": [],
      "recovery_prob_after_sampling": []
    },
    "super_infection": false
  },
  "Postprocessing_options": {
    "do_postprocess": true,
    "tree_plotting": {
      "branch_color_trait": 1,
      "heatmap": "drug_resistance"
    },
    "sequence_output": {
      "vcf": true,
      "fasta": false
    }
  }
}
```

Listing 5.6: config model 1

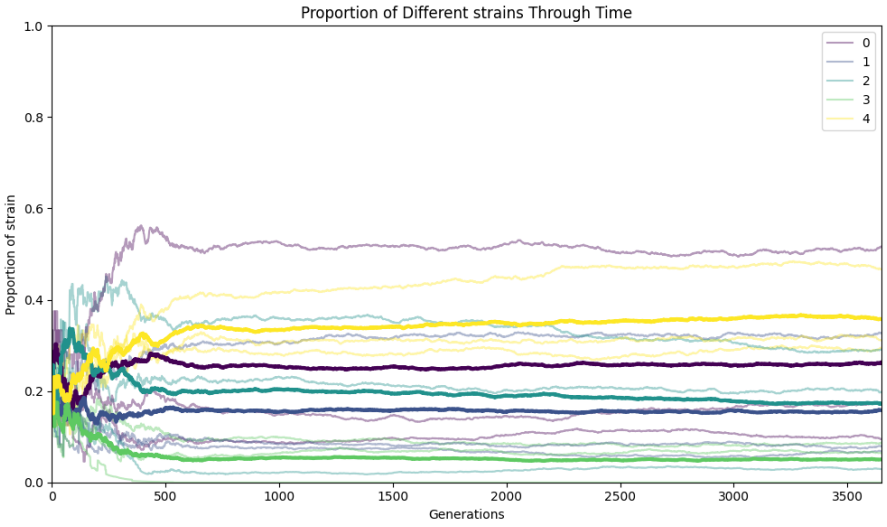
We thus use the absolute path to this configuration file to run *OutbreakSimulator* by:

```
python -u outbreak_simulator.py \
        -config ${PATH_TO_THIS_CONFIG}
```

Listing 5.7: OutbreakSimulator model 1

*OutbreakSimulator* will print logging information to standard output as it progresses through the simulation. When the program finishes, you can look in the working directory and each replicate's subdirectory for the results. Please note that the file paths in the configurations has to be changed to full path of your own directories before running with the configuration in the above directory.

6. For detailed instructions on how to analyze the output files, please refer to Chapter 6. Using the current setup and random seed, you will encounter a terminal warning message: "WARNING: There's no mutations related to any trait in samples from this replication." This occurs because Mycobacterium tuberculosis (Mtb) has an extremely low mutation rate, and none of the sampled genomes carry mutations at the causal sites defined in Step 3. Consequently, the resulting plotted phylogenetic trees will appear entirely gray, as they are colored by the transmissibility trait, which remains identical across all sampled genomes. Similarly, the strain-frequency plot over time will appear largely homogeneous, since no mutation confers a selective advantage—most strains spread at approximately equal probabilities.




## 5.2 Multi-stage drug treatment

In this section, we will simulate a COVID-19 outbreak in a host population of size 10,000 for 2 years. We want each tick to represent 1 day, so we want to simulate 730 ticks. The mutation rate of coronavirus is $6.67 \times 10^{-3}$ SNPs/bp/year, which is $1.8 \times 10^{-5}$ SNPs/bp/day. In this simulation, we model realistic host population structure and different treatment stages. You can set a random seed for this pipeline for reproducibility by setting `RANDOMSEED=1100` before running the pipeline, that should reproduce the results we provide in the repository.

1. The first step is to run *NetworkGenerator*. We want to simulate a realistic host population structure using a scale-free graph. We thus run:

```
python ${e3SIM}/network_generator.py \
    -wkdir ${WKDIR} \
    -popsize 10000 \
    -method randomly_generate \
    -model BA \
    -m 2 \
    -random_seed ${RANDOMSEED}
```

Listing 5.8: NetworkGenerator model 2

2. In this example, we are starting from the reference genome, thus we dont need to run *SeedGenerator*.

3. We then run *GeneticEffectGenerator*. In this example, we are using a pre-defined effect size file `causal_gene_info.csv` in the working directory. We do not want to do calibration, since we are using the reference genome as the seeding sequences.

```
python ${e3SIM}/genetic_effect_generator.py \
    -wkdir ${WKDIR} \
    -method user_input \
    -num_init_seq 1 \
    -csv ${WKDIR}/causal_gene_info.csv \
    -trait_n '{"transmissibility": 1, "drug_resistance": 2}' \
    -calibration F \
    -calibration_link T \
    -random_seed ${RANDOMSEED}
```

Listing 5.9: GeneticEffectGenerator model 2

Here `-trait_n` tells e3SIM the provided effect size file contains one transmissibility and two drug resistance trait sets. You should see `causal_gene_info.csv` appearing in your working directory, along with a `seeds_trait_values.csv` file where all the traits of the single seeding sequence are shown as 0s. We calibrated the link scale slope here as well, but here we decided to use different link scale slope in the simulation, as shown in the configuration file 5.11.

4. We then run *HostSeedMatcher*. In this example, we are using only one seed, and we want to match it to a host that has median contact degree. We thus utilize the ranking method to match seed and host.

```
python ${e3SIM}/seed_host_matcher.py \
   -wkdir ${WKDIR} \
   -method randomly_generate \
    -num_init_seq 1 \
    -match_scheme '{"0": "ranking"}' \
    -match_scheme_param '{"0": 5000}' \
    -random_seed ${RANDOMSEED}
```

Listing 5.10: SeedHostMatcher model 2

You should see `seed_host_match.csv` in your working directory.

5. Now, we can run *OutbreakSimulator*. We first download the template configuration file from our Github repository, then make the desired modifications. According to the CDC, latency period for COVID is around 3 days, and recovery time is around 2 weeks. We want to model all new infections initially leading to latency. Though it's controversial, but immunity can lose in 30 days. For basic transmission probability, we assume an arbitrary probability of $0.03$, meaning that for each contact pair, an everyday infection probability is $3\%$. We don't want to model other transitions between compartments, and we don't want to model massive sampling events. We conduct post-processing, asking *OutbreakSimulator* to plot genealogies of the sampled pathogens and include a drug resistance heatmap. This is the configuration file we specify using these parameters:

```
{
  "BasicRunConfiguration": {
    "cwdir": "5.2_test_drugresist",
    "n_replicates": 3
  },
  "EvolutionModel": {
    "subst_model_parameterization": "mut_rate",
    "n_generation": 730,
    "mut_rate": 1.8e-06,
    "within_host_reproduction": false,
    "within_host_reproduction_rate": 0,
    "cap_withinhost": 1
  },
  "SeedsConfiguration": {
    "seed_size": 1,
    "use_reference": true
  },
  "GenomeElement": {
    "use_genetic_model": true,
    "ref_path": "COVID/EPI_ISL_402124.fasta",
    "traits_num": {
      "transmissibility": 1,
```

```
      "drug_resistance": 2
    },
    "trait_prob_link": {
      "link": "logit",
      "logit": {
        "alpha_trans": [
          0.405
        ],
        "alpha_drug": [
          0.0116,
          0.0273
        ]
      }
    }
  },
  "NetworkModelParameters": {
    "host_size": 10000
  },
  "EpidemiologyModel": {
    "model": "SEIR",
    "slim_replicate_seed_file_path": "slimseeds.csv",
    "epoch_changing": {
      "n_epoch": 3,
      "epoch_changing_generation": [
        250,
        500
      ]
    },
    "genetic_architecture": {
      "transmissibility": [
        1,
        1,
        1
      ],
      "drug_resistance": [
        0,
        1,
        2
      ]
    },
    "transition_prob": {
      "S_IE_prob": [
        0.04,
        0.02,
        0.02
      ],
      "I_R_prob": [
        0.03,
        0.06,
        0.05
      ],
      "R_S_prob": [
        0.05,
        0.1,
        0.1
      ],
      "latency_prob": [
        1,
        1,
        1
```

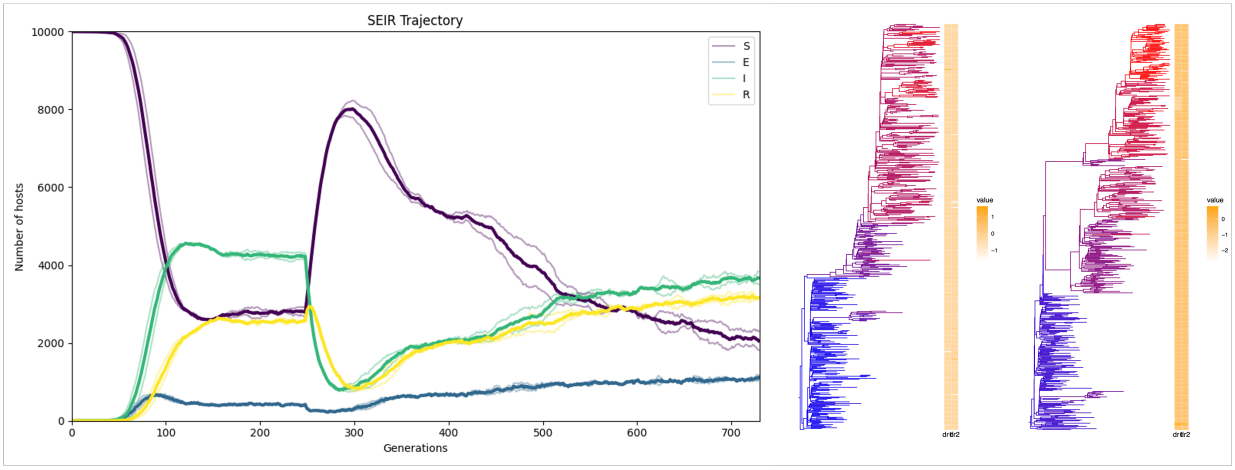
```
      ],
      "E_I_prob": [
        0.3,
        0.3,
        0.3
      ],
      "I_E_prob": [
        0,
        0,
        0
      ],
      "E_R_prob": [
        0,
        0,
        0
      ],
      "sample_prob": [
        0.0002,
        0.0003,
        0.0003
      ],
      "recovery_prob_after_sampling": [
        0,
        0,
        0
      ],
      "surviv_prob": [
        0.01,
        0.96,
        0.96
      ]
    },
    "massive_sampling": {
      "event_num": 0,
      "generation": [],
      "sampling_prob": [],
      "recovery_prob_after_sampling": []
    },
    "super_infection": false
  },
  "Postprocessing_options": {
    "do_postprocess": true,
    "tree_plotting": {
      "branch_color_trait": 1,
      "heatmap": "drug_resistance"
    },
    "sequence_output": {
      "vcf": true,
      "fasta": false
    }
  }
}
```

Listing 5.11: config model 2

*OutbreakSimulator* will print logging information to standard output as it progresses through the simulation. Since this is a stochastic simulation, it isn't guaranteed that the outbreak will reach an endemic stage, i.e. the outbreak might end quickly enough that there aren't many samples retrieved. If no samples are retrieved at all, *OutbreakSimulator* will give a warning and there will be no post-simulation

processing steps. Using the current setup and the random seed we provided, you will see that the second replicate has the epidemic die out early in the simulation, with a warning message in the terminal: "WARNING: Replicate 2 failed or produced no output".

6. When the program finishes, you can look in the working directory and each replicate's subdirectory for the results. For detailed instructions on how to analyze the output files, please refer to Chapter 6. The results and configurations of one test run are in our repository for comparison. Below, we show the aggregated SEIR trajectory for two replicates (since one of the replicates fail to produce samples) from one simulation run. The different behavior of the outbreak in different epochs can be clearly seen. We also show examples of the transmission trees for two replicates; in both replicates, the population accumulate higer transmissibility through mutations over time.



# Part III

# Post-simulation processing

# Chapter 6

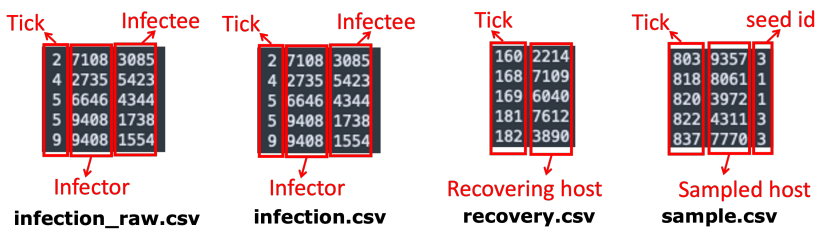
# Output

In this chapter, we describe the structure of the output of e3SIM and how to read the outputs.

## 6.1 Output structure

For every replicate, e3SIM creates a folder named the replicate id (starting from 0) in the working directory. In each replicate folder, there will be the following output files:

- `infection_raw.csv.gz`: Compressed csv file that stores all the raw transmission events (including those events that were omitted when super-infection is deactivated, which do not exist in `infection.csv.gz`). Every row in the file describes one infection event. The first column gives the tick number, and the second and third columns gives the infector and infectee's host ids respectively.
- `infection.csv.gz`: Compressed csv file that stores all the preserved transmission events. For example, when super-infection is deactivated, only one of the transmission events for an infectee is preserved per tick, all the other transmissions were unsuccessful, thus not stored in `infection.csv.gz`. Every row in the file describes one infection event. The first column gives the tick number, and the second and third columns gives the infector and infectee's host ids respectively.
- `recovery.csv.gz`: Compressed csv file that stores all the recovery events that happened. Every row in the file describes one recovery event. The first column gives the tick number and the second column gives the recovering host id.
- `sample.csv.gz`: Compressed csv file that stores all the sampling events that happened. Every row in the file describes one recovery event. The first column gives the tick number and the second column gives the sampled host id. The third column

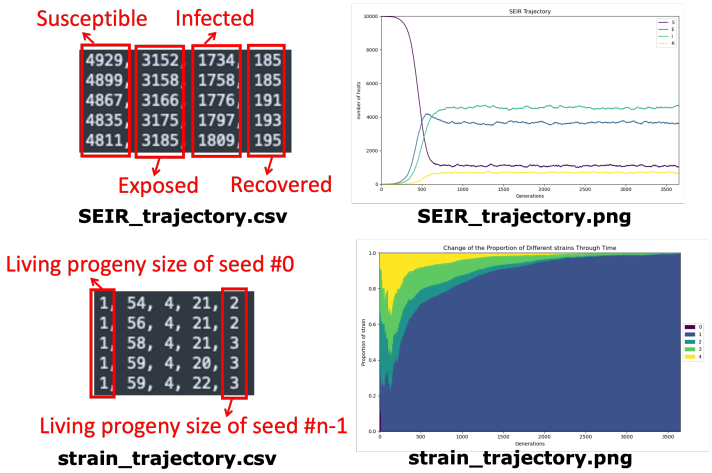



- `SEIR_trajectory.csv.gz`: Compressed csv file that stores the SEIR compartmental size for each tick. It has the row number equals to the total number of ticks. The four columns represents the size of susceptible, exposed, infected and recovered respectively.

- `strain_trajectory.csv.gz`: Compressed csv file that stores the size of the progeny of each strain for each tick. It has the row number equals to the total number of ticks. Each column represents size of the progeny of one seed (strain), in the order of seeds' ids.

- `SEIR_trajectory.png`: Plotted `SEIR_trajectory.csv.gz`.

- `strain_trajectory.png`: Plotted `strain_trajectory.csv.gz` in proportion.

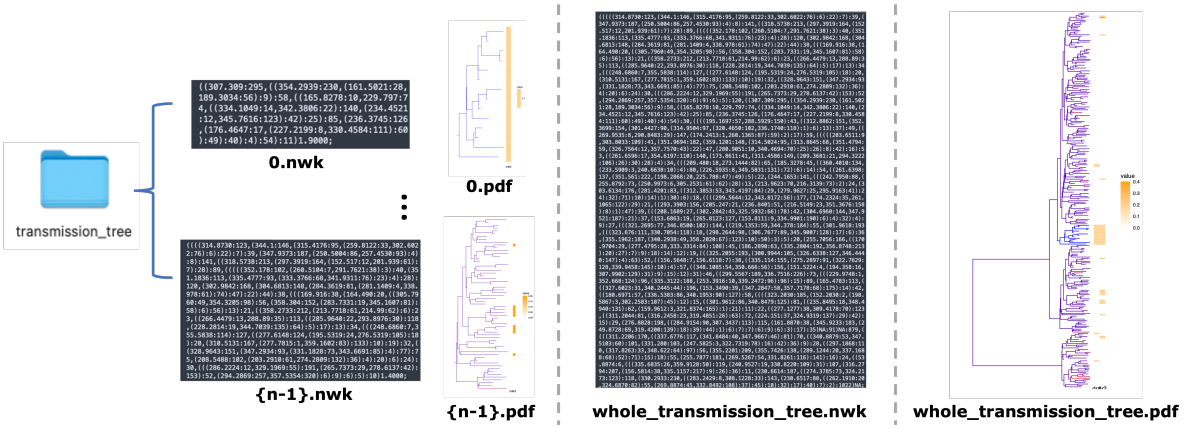



- `transmission_tree`: A folder that stores transmission tree for each seeds' progeny, within which `x.nwk` for each seed id x will present. For seeds that has more than one sampled offspring, a `tree.x.pdf` that is a plotted tree will be present.

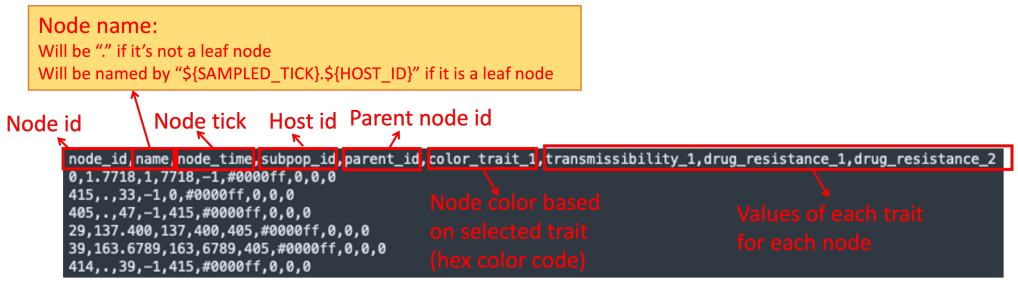
  The leaf labels in the `x.nwk` file is in the format of `${SAMPLED_TICK}.${SAMPLED_HOST_ID}`, which corresponds to the sample names in the `.vcf file`

- `whole_transmission_tree.pdf`: Plotted transmission tree, concatenating the seeds' phylogeny and each seed's transmission tree. Presents only one seed phylogeny presents in the working directory.

- `whole_transmission_tree.nwk`: Transmission tree, concatenating the seeds' phylogeny and each seed's transmission tree. Presents only one seed phylogeny presents in the working directory.




- `sampled_genomes.trees`: Treesequence of the sampled genomes, output by SLiM in `OutbreakSimulator`.

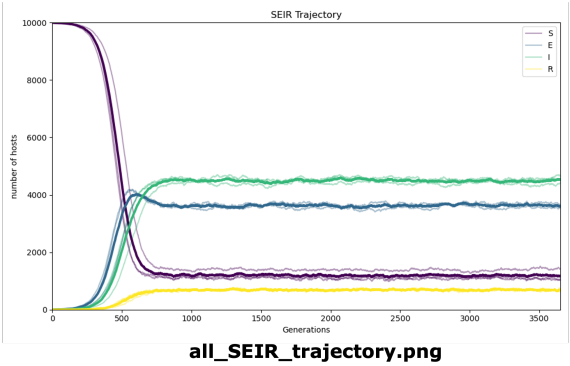



**transmission_tree_metadata.nwk**

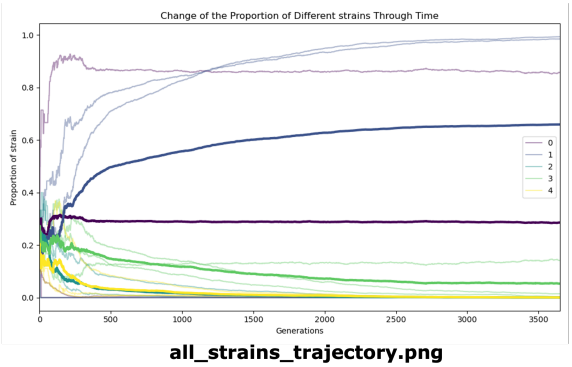
- `transmission_tree_metadata.pdf`: Metadata of the transmission tree, storing the trait values for each node in the transmission tree and the coloring of each node.
- `sampled_pathogen_sequences.vcf`: The mutation profiles of the sampled pathogen sequences in VCF format, where sample names correspond to the naming criteria in `whole_transmission_tree.nwk`.
- `sample.SNPs_only.fasta`: A FASTA file showing the concatenated SNPs for each sampled pathogen, where sample names correspond to the naming criteria in `whole_transmission_tree.nwk`.
- `final_samples_snp_pos.csv`: A CSV file showing the positions of each site corresponding to the reference genome in the concatenated SNPs for each sampled pathogen.
- `sample.wholegenome.fasta`: A FASTA file showing the sampled pathogens whole genome sequences, where sample names correspond to the naming criteria in `whole_transmission_tree.nwk`.

In the working directory, there will be a folder called `output_trajectories`, with the contents being:

- `all_SEIR_trajectory.png`: Plot the aggregated results from `SEIR_trajectory.csv.gz` for each replicate, with the average trajectory plotted out.
- `all_strain_trajectory.png`: Plot the aggregated results from `strain_trajectory.csv.gz` for each replicate, with the average trajectory plotted out.




**all_SEIR_trajectory.png**




**all_strains_trajectory.png**

# Part IV

# GUI

# Chapter 7

# Introduction to the GUI

e3SIM provides a graphical user interface for users who prefer visual interactions over command line tools. The GUI could be used for executing all the pre-simulation modules and generating the configuration file for the main simulator module.

To launch the GUI, first locate the gui subdirectory within the `e3SIM_codes` folder of your e3SIM installation. Assuming the path to the `e3SIM_codes` folder is defined as $e3SIM, the GUI can be started with the following command:

```
python ${e3SIM}/gui
```

Listing 7.1: Launch the GUI

It is recommended to launch the program from the working directory where you intend to run your simulation, so that the GUI will automatically use the current directory as the default for file and path selections.

Please note that when pre-simulation modules are run in the backend, the GUI will freeze until the step gets finished. In this section, all the screenshots demonstrate values we set for a same simulation as what was done in Section 5.2, i.e. it should generate a configuration file that could be used to run the same simulation as shown in Configuration file 5.11.

A GUI window pops up with several tabs:

In the "basic configuration" tab (Figure 7.1), some basic parameters are set, including working directory and the random number generator, which are used in subsequent tabs where pre-simulation modules are executed. By clicking on the "choose directory" button after the "working directory" item, you can navigate to the path you'd like to choose as your working directory, and all subsequent pre-simulation modules will generate files in this path you chose. To be noted, when clicking on the button, the default path popping out will be the current path where you launched the gui. Then, you can navigate to choose the reference genome in fasta format by clicking on the "Choose File" button. If the reference genome has a wrong format, an error mesage will pop out. The "Number of simulation replicates" item let you choose how many simulations with the same parameter sets but different random seeds do you want to choose, the integer you entered here will be logged in the "n_replicates" key in the "BasicRunConfiguration" section of the configuration file (Section 3.2.1). Lastly, you could optionally specify a random seed, that will be used for all the randomization pre-simulation modules in the GUI, and will be logged in the "random_number_seed" key in the "BasicRunConfiguration" section of the configuration file (Section 3.2.1). After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file.

In this tab (Figure 7.2), configurations for the evolutionary model and the within-host reproduction mode are set. This evolutionary model will be used in the main simulator module, and can also be directly loaded in the *SeedGenerator* module. To be noted, all the settings in this tab configures the *OutbreakSimulator* model, rather than the seeding sequences generation process, which will be specified separately and executed in the fourth tab. The "Number of Generations" item let you specify the number of ticks you will run for the simulation (See Section 4.1 for the definition of ticks). You can use the drop-down menu to choose a substitution

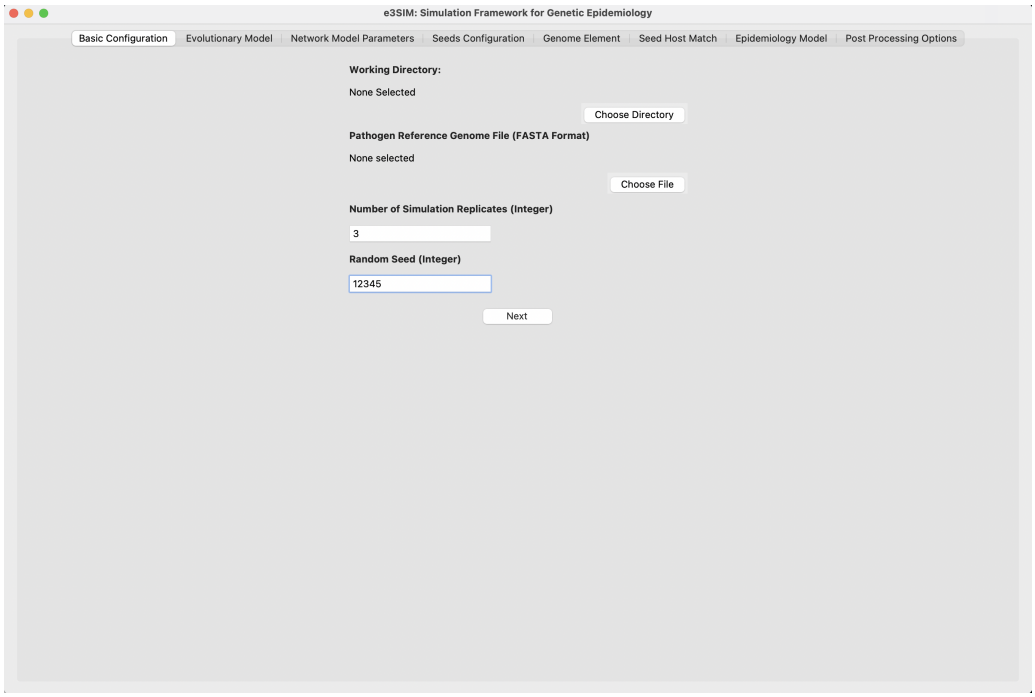



Figure 7.1: **The first tab of the GUI: Basic configuration.**

model: If you choose "mutation rate (single)", an item will pop up to let you define a mutation rate per site per generation (tick) for the pathogen genome, and the substitution model will be default to Jukes-Cantor (See Section 4.1 for details); if you choose "mutation rate matrix" in the drop down menu, a substitution probability matrix will pop up, as shown in Figure 7.2. The rows show the current allele in the order of ACGT, and the columns show the target mutated allele in the order of ACGT. For example, the number in the first row and third column specifies the probability of transition from an A allele to a G allele per tick. These settings will be logged in the "EvolutionModel" section of the configuration file. You can choose to enable within-host reproduction or not, and only when you choose "Yes" (In the "within_host_reproduction" key of the configuration file), the "within-host reproduction rate" will be available for you (In the "within_host_reproduction_rate" key of the configuration file). The maximum number of pathogens within host can also be specified (In the "cap_withinhost" key of the configuration file). After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file.

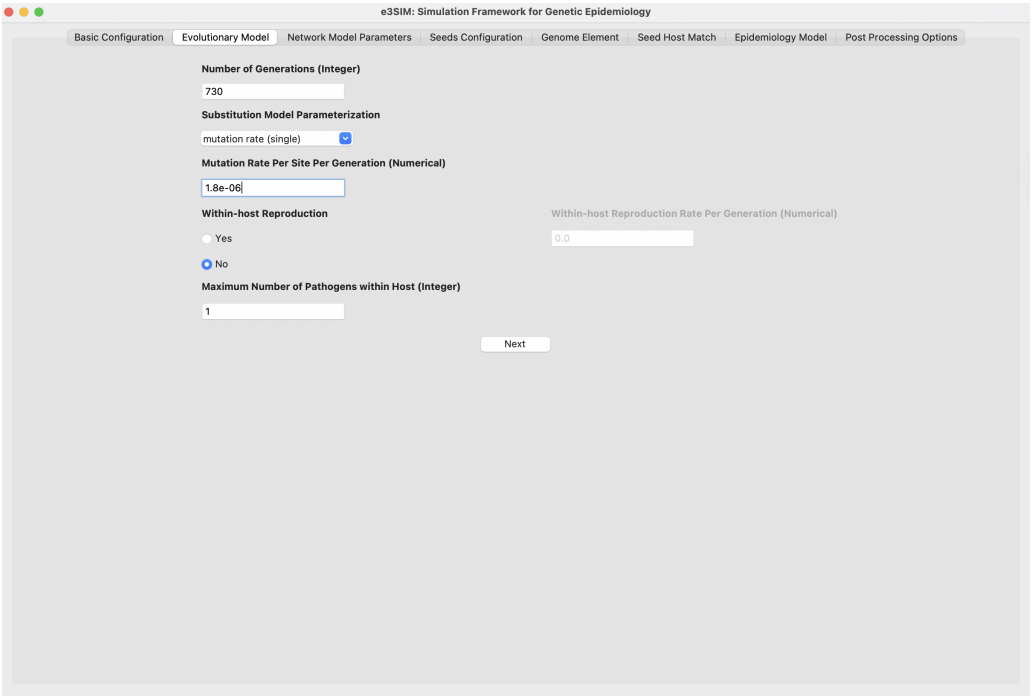



Figure 7.2: **The second tab of the GUI: Evolutionary model.**

In this tab (Figure 7.3), configurations for the host population are set, and *NetworkGenerator* is run. The generated contact network will be used in the main simulator and the *HostSeedMatcher* module. After setting all the parameters, clicking on the "Run Network Generation" button will generate a random network and visualize the network in the left panel. Please see Section  for all the modes of the host contact network generation. Once a network model method is selected, corresponding variables required for the selected method will pop up. For example, in Figure 7.3, users specifies a random generation method for the network and specifies an Barabasi–Albert graph, so that the "BA probability parameter" is enabled. After running the network generation and viewing the degree distribution to make sure this is the contact network desired, click on the "Next" button to proceed and store all your choices in the "NetworkModelParameters" section of the configuration file. After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file.

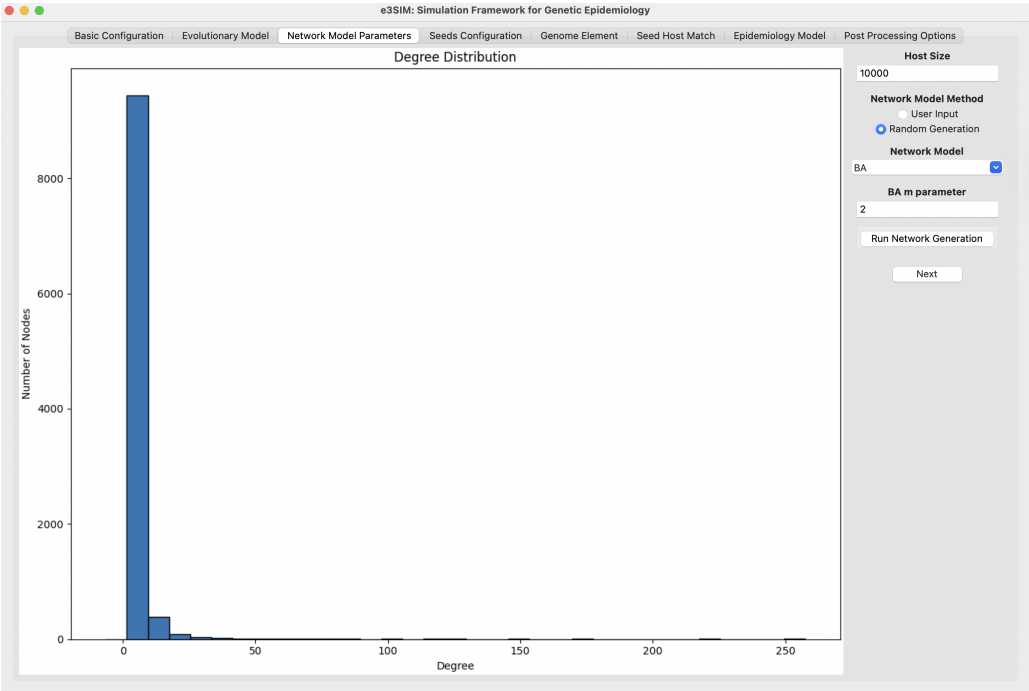



Figure 7.3: **The third tab of the GUI: Network Model Parameters.**

In this tab (Figure 7.4), configurations for the seeding sequences are set, and *SeedGenerator* is run. The generated seeding sequences will be used in the main simulator, the the *GeneticEffectGenerator* module and the *HostSeedMatcher* module. After setting all the parameters, clicking on the "Run Seed Generation" button will generate seeding sequences and their genealogy to store in the working directory. Please refer to Section 2.2 for how and why these parameters should be specified. The mutation specifications could be different from the main simulator specifications specified in tab2, but can also be directly imported from tab2 by clicking on "Load mut.rate(s) from Evolutionary Model". Since this step including running a SLiM simulation, it might take some time to finish, during which the gui will be frozen. A pop-up window indicating the finishing of the seed generation will appear once finished. After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file. Settings here will be stored in the "SeedsConfiguration" section of the configuration file, and only the "Number of seeding pathogens" will be used to run "OutbreakSimulator", while all other settings are just for generating the seeding sequences, irrelevant to the actual simulation.

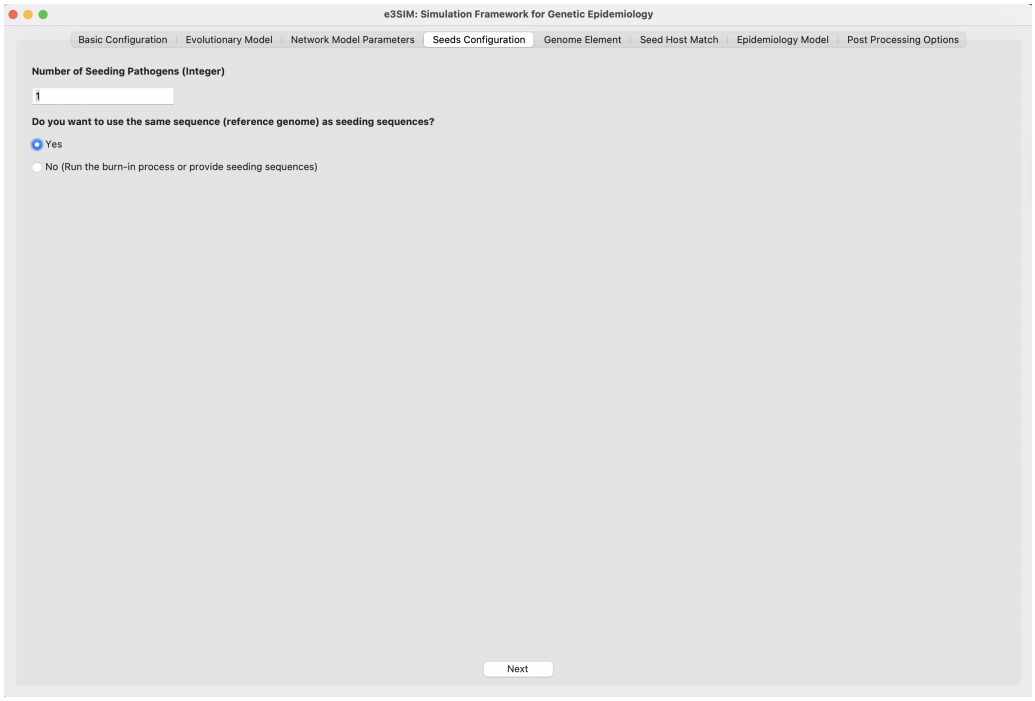



Figure 7.4: **The fourth tab of the GUI: Seeds Configuration.**

In this tab (Figure 7.5), the genetic architecture are set, and *GeneticEffectGenerator* is run to calculate and store the trait values of the seeding sequences. The genetic architecture is stored in the working directory. Please refer to Section 2.3 for how and why these parameters should be specified. If you choose "No" for the question "Do you want to use genetic architecture", all the subsequent options will disappear from the GUI, and your simulation will be an neutral simulation where mutations don't affect epidemiological process; if you choose "Yes", you will be prompted to set up the genetic architecture for transmissibility and drug-resistance. Users can choose the method to set up the effect sizes, and provide either the candidate regions or a provided genetic architecture file. For the random generation mode, expected fractions and dispersion of the fraction need to be provided. If users want to calibrate the effect sizes, they should select "Yes" for the "Whether to calibrate effect sizes by the provided seeding populations", and click on "Run effect size generation" again to calibrate the effect sizes. The link scale slope has to be set for all traits. Users can click on the "Run slope calibration" button at the bottom of the page, and the calibrated slope alpha will be shown in the terminal. Users will have to enter the alpha to the GUI manually.

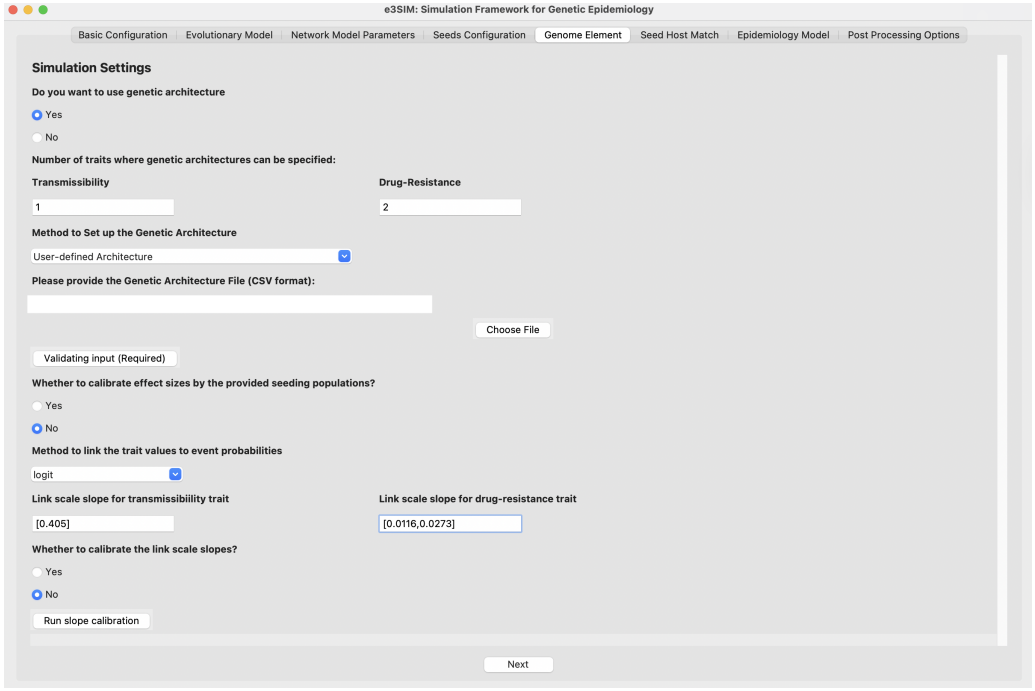



Figure 7.5: **The fifth tab of the GUI: Genome Element.**

In this tab (Figure 7.6), the generated or input network is loaded, and *SeedHostMatcher* is run to match the seeding sequences to the patient 0s' IDs. The matching file is stored in the working directory. The table in the panel below the degree distribution plot shows the trait values of each seeding sequence, using the sequence generated in the "Seeds Configuration" tab and the genetic architecture defined in the "Genome Element" tab. For each seed, one matching method can be chosen. All the seeds are default to random matching, which matches seeds to a random host from the host contact network. However, you can change the method to "ranking" or "percentile" by clicking on the drop-down menu for each seed in the "match method" column. By choosing methods different from random, parameters are required per each method. Please refer to section 2.4 for details on how to set these parameters. They should be typed in the "method parameter" columns. After clicking on the "Match Hosts" button, the "Host id" column will be filled with the matched hosts' ID according to the method you chose, and the contact degree of the matched hosts will be plotted on the above panel as vertical lines in the degree distribution plot. You can adjust the methods and parameters to re-match until you are satisfied with the matching. After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file.

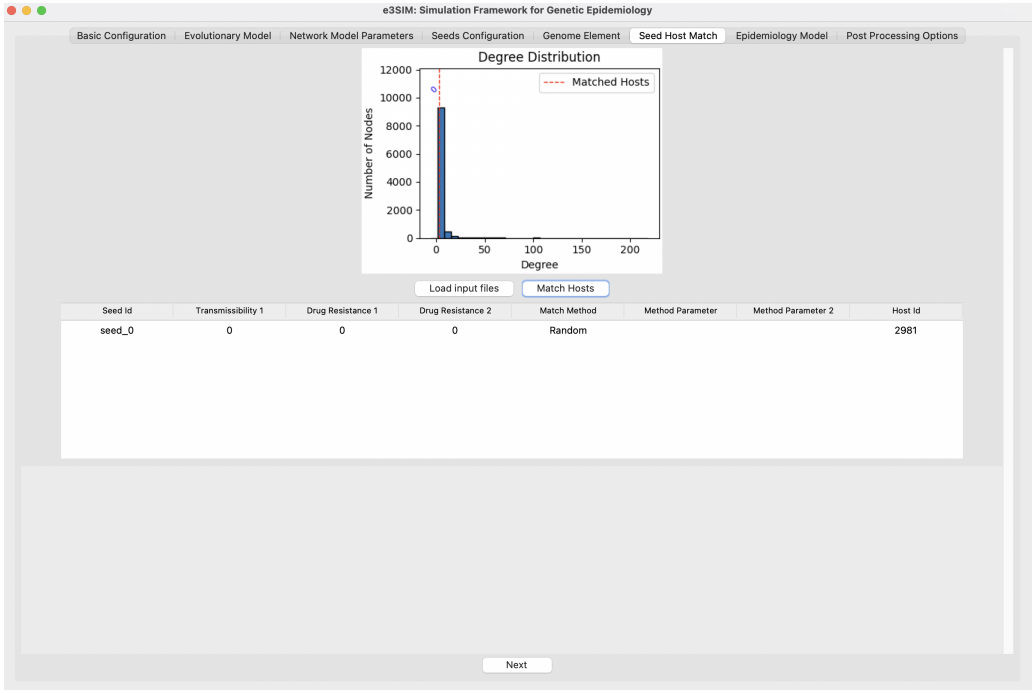



Figure 7.6: **The sixth tab of the GUI: Seed Host Match.**

In this tab (Figure 7.7), the configuration of the epidemiological model of the simulation is set. The epoch structure is also specified. Please refer to Section 4.4 for the correct format of these parameters. After configuring everything, make sure to click on the "Next" button to store all your choices in the configuration file.

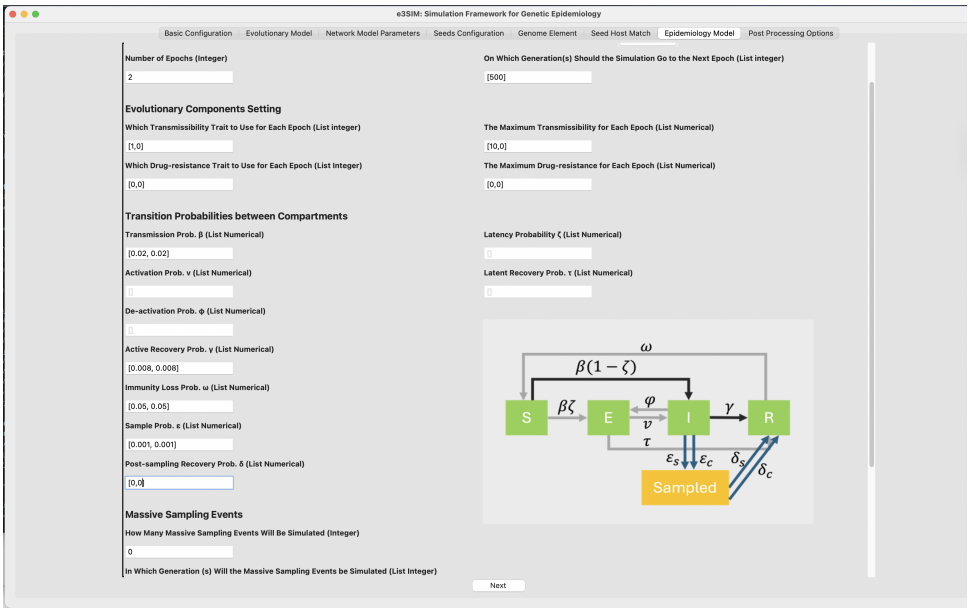



Figure 7.7: **The seventh tab of the GUI: Epidemiology Model, with valid example of values entered.**

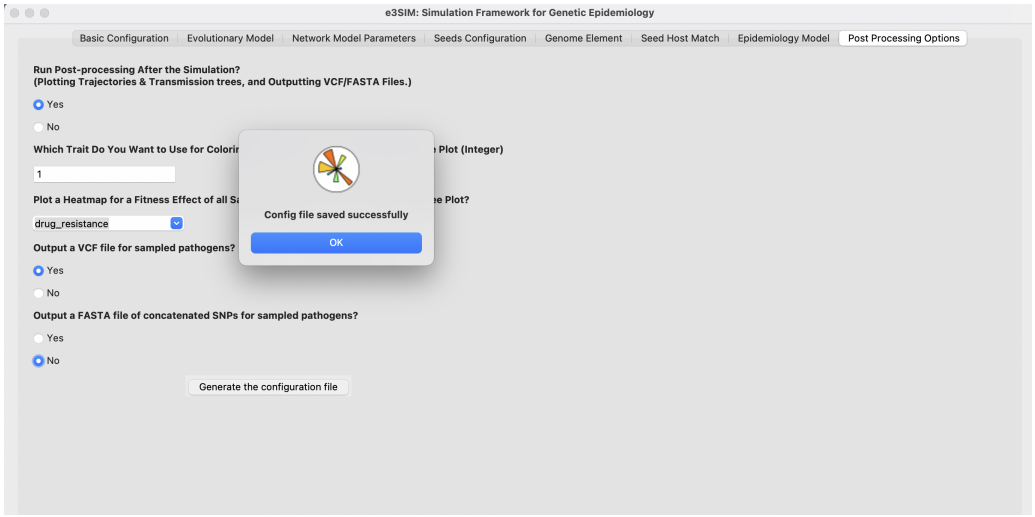



Figure 7.8: **The eighth tab of the GUI: Post Processing Options.**

In this tab (Figure 7.8), the post-processing steps after the simulation are configured. For the question "Which trait do you want to use for coloring the branches in the transmission tree plot", if "0" is specified, they will be colored by seed ID. If "1" is specified, the first trait value will be used. (If 3 traits are specified, where 2 are transmissibility and 1 is drug resistance, then "1" refers to the first transmissibility trait, and "3" refers to the drug-resistance trait). Please see Section III and Section 3.2.7 for more details of the post-processing options. Please make sure to click on the button to generate the full configuration file in the working directory before you exit the GUI.

It is recommended not to close the GUI before making sure every entry is correct for first-time users since the GUI doesn't perform validity check for all the entries. The complete validity check will be performed when you run `OutbreakSimulator.py`. You can go back to previous tabs to make edits and regenerate the configuration file as many times as you wish.

# Part V

# Advanced usage

# Chapter 8

# SLiM codes of `outbreakSimulator`

The main simulator module of e3SIM uses SLiM 4 in the back-end. In `outbreakSimulator`, e3SIM assembled Eidos code blocks to generate a customized script for the simulation configuration user specified and executes it. Advanced user could modify these code blocks to implement their own model. All the Eidos code blocks are located in the `slim_scripts` directory. For how to write and modify Eidos code and the logic of SLiM, please refer to SLiM's tutorial. In this chapter, we provide the logic of how e3SIM assembles the code blocks, so that experienced users could modify the codes accordingly.

To be noted, the random number generator for the SLiM simulations can be found in the logging files of SLiM, which is the `slim.stdout` file in each replicate's directory after finishing the simulation.

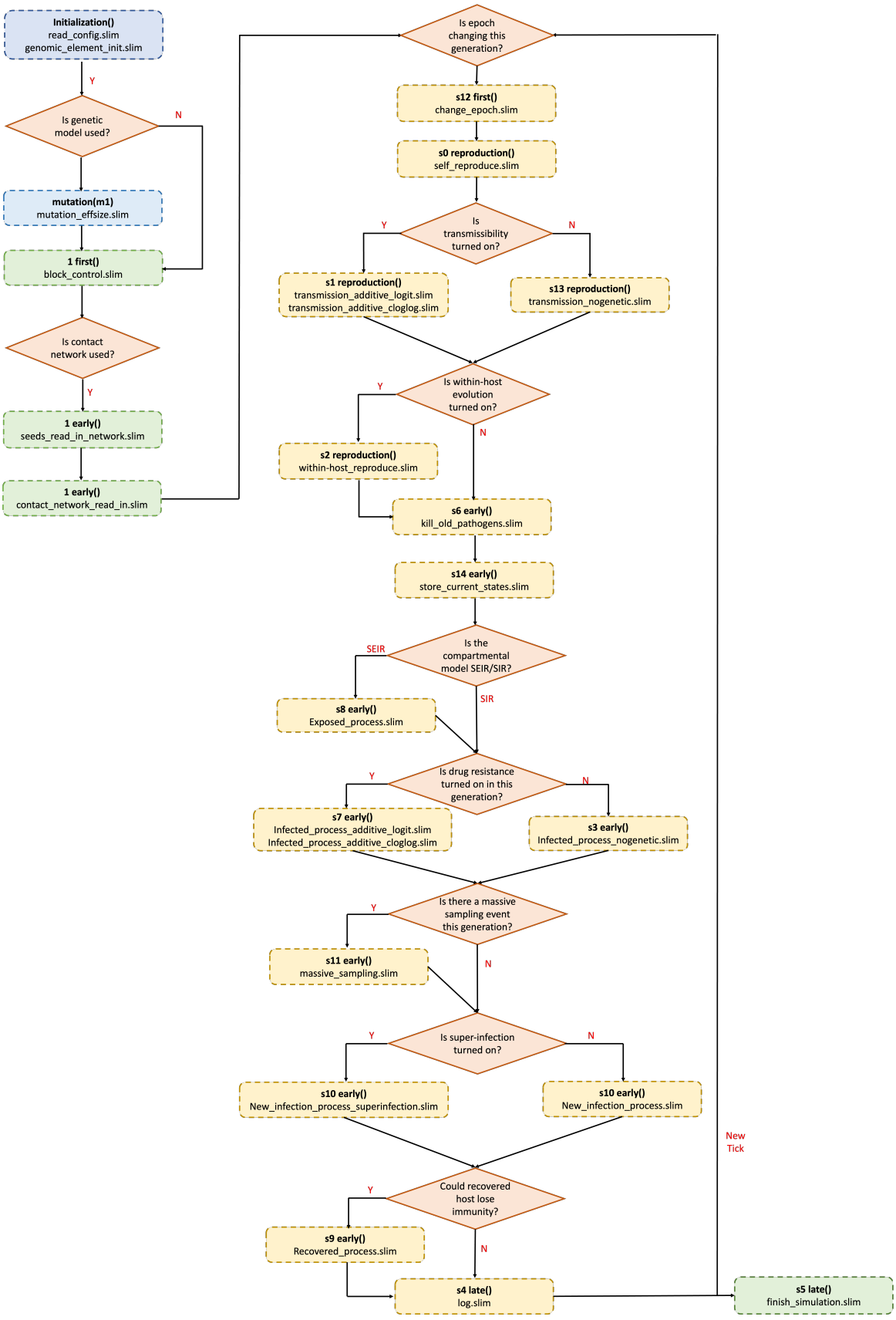



Figure 8.1: Flow chart showing the logic of code block assembling for **OutbreakSimulator**