

## Computational Modeling of Yeast Telomere Dynamics: Overview

We developed a highly-efficient computational model of telomere dynamics that simulates telomerase-negative populations of cells, with telomere shortening (erosion), senescence, and potential recovery (formation of ALT survivors from post-senescence cells) based fully stochastic population genetics principles in finite populations. As in Kockler et al. (2021), our model mimics a case where each telomere of every dividing cell erodes, and replicative senescence is activated when the shortest telomere in a cell reaches a critically short threshold length. Senescent cells, on the other hand, are allowed to recombine through multiple modalities (see below), which can potentially lead to cells in which all telomeres surpass the critical length threshold, leading to resumption of cell cycling. Additional to telomere repeats, our model considers pre-telomeric regions, which play important roles in cell dynamics and formation of ALT survivors when senescent cells are allowed to recombine. Notably, our computational model allows the following of very large populations—approximating realistic experimental cell populations—which enables the identification of critical dynamics caused by very low frequency events that would be missed when investigating smaller populations. Also important, our simulations track all per-chromosome-end features for each cell in the population, thus allowing capturing the full heterogeneity and dynamics over time of chromosome-end structures within and between cells. Because the modeling is based on directly following every cell and every chromosome end structure within each cell in a population over time, it naturally restricts properties of cell division and chromosome-ends to biologically relevant ranges and allows the inclusion of complex modes of recombination.

**General chromosome-end properties and population processes.** Our simulations start with a cell with 32 chromosome ends (16 chromosomes), each characterized by a telomere repeat, possible Y' elements and an X element. The average telomere length as well as the number and distribution of Y' elements across chromosome ends can be based on a user-defined averages or to follow specific values from the sequenced strain under study. As in Kockler et al. (2021), the model follows exponential growth of this ancestral cell, with telomeres randomly increasing or decreasing in length every cell division to generate a large and heterogeneous population prior to telomerase inactivation.

When modeling of telomerase-negative cell populations begins, one randomly chosen cell (or a number defined by the user) from the pre-evolved population is used as starting point per replicate, and the study of multiple replicates allows capturing variation in dynamics due to differences in initial telomere length composition. Populations expand exponentially and cell properties are analyzed every population doubling (PD). Simulations can also mimic the experimental design with passages (sub-samples of the cell pool) at specific PDs. For every PD, population expansion is based on randomly choosing cells one by one from the whole pool of  $N$  cells, either non-senescent or senescent, until  $2N$  cells are achieved. This approach adds random genetic drift as well as heterogeneity to division rates among non-senescent cells, which simulates observations from microfluidics experiments of telomerase-negative single cell lineages (Xu et al. 2015). If the chosen cell is non-senescent, it divides creating a daughter cell, with telomere shortening occurring on both the parent and the daughter cells independently on all chromosomes. Shortening follows an average of  $n$  nucleotides per chromosome end per

division, with each chromosome eroding one of the two telomeres (chosen at random) with Poisson-distributed average  $2n$  nucleotides to follow end-replication properties. If a chosen cell is senescent, with one or more chromosome ends shorter than a threshold length ( $L_s$ ), we apply either death (removed from the pool of cells with probability  $P_{death}$ ) or recombination (1-  $P_{death}$ ).

**Recombination in senescent cells.** During the replication process, if a senescent cell is chosen, it cannot divide, but it can attempt recombination. The model assumes that chromosome ends with telomeres shorter than  $L_s$  go through long-distance 5'-3' end resection (Tsai et al. 2026), generating ssDNA at these chromosome ends that can only find homology with dsDNA of non-resected chromosome ends (with telomeres longer than  $L_s$ ). It also assumes that end resection will regularly extend beyond the telomere repeat region (Churikov et al. 2014; Kockler et al. 2021). Therefore, the recombination scheme allows resected chromosome ends to recombine through homologous recombination (HR) at either telomeric repeats or at sub-telomeric regions, including Y' elements (when present) and the X element.

For each resected chromosome (with telomeres  $< L_s$ ) in senescent cells, the model first identifies the element that will attempt HR. As a simplifying assumption, we assign equal probability of HR along the resected segment to the telomere repeat, each individual Y' element (if any) and the X element. When an X element is assigned for recombination, it randomly chooses among the X elements of non-resected chromosome ends for HR, capturing the X element as well as terminal regions (Y' elements and telomere repeats) of the donor chromosome end. When a Y' element is chosen for recombination, HR occurs with a Y' element chosen randomly chosen among all the Y' elements present in non-resected chromosome ends of the cell, capturing all terminal regions of the donor chromosome end (terminal Y' elements, if any, and the telomere repeat).

When recombination is assigned to a resected telomere repeat, the model allows for HR between telomere repeats or between the repeats and t-circles, if present, the latter with probability  $P_{t-circle}$  for each recombination attempt. When a resected telomere repeat recombines with a donor telomere repeat, the model also incorporates the possibility that the recombining resected telomeres can engage in subsequent recombination events through a model equivalent to template switching by microhomology with other non-resected donor telomere ends based on probability  $P_{ts}$ . When template switching occurs, the terminal end of the ssDNA telomere repeat randomly identifies a location along the donor telomere, extending the length of repeats. If template switching occurs, the model further allows for additional template switches (each with probability  $P_{ts}$ ), with a maximum of 5 consecutive switches.

Finally, our model investigates the potential impact of t-circles emerging from the dynamics of template switching at microhomologies following initial HR, where ssDNA telomeric repeat segments could be long enough to generate a t-circle (Vafabakhsh and Ha 2012). This possibility allows the probability of recombination between telomere repeats and t-circles to be dynamic over time for a given cell lineage, starting with no circles and increasing as senescent cells go through recombination events at telomeres, with each new generated t-circle adding  $P_{dyn-t-circle}$  to  $P_{t-circle}$ . Each cell in the population, therefore, can have a different  $P_{t-circle}$  that, on division, will be equally split between parent and daughter cells. In this model, therefore,

survivor cells that successfully elongated telomeres with t-circles, are predicted to reduce the number of t-circles over time.

**Parameters for yeast telomere dynamics.** To speed up the simulation process and the identification of adequate range of parameters, we recommend to initially limit the number of cells to  $1 \times 10^5$  —  $10^6$ , at which point a moderate subsampling (e.g., 10%) can be applied before resuming exponential growth. This approach still allows capturing consequences of low frequency events. Follow up simulations, however, can take the population size to even larger values (eg,  $1 \times 10^7$  —  $10^8$ ), without challenging too much memory limits and run time. To also simulate experimental conditions with passages, the model allows sub-sampling a specific number of cells at specific PDs (e.g., 10,000 cells at 10 PD intervals). Based on Kockler et al. (2021), an erosion rate per telomere per division of  $n = 6$  nt is used as default. For  $L_s$ , it is recommended to consider a wide range (e.g., between 40 and 70 bp). When t-circles are considered, telomeres recombining with t-circles add a certain length to telomere repeats (e.g. 2,000 bp).

Finally, we recommend a minimum of 100 independent population replicates, except for non-recombination conditions to study dynamics of senescence, where  $>10,000$  replicates from pre-evolved populations may be needed due to much larger variances. These non-recombinant simulations are, however, quite fast given that the maximum number of PDs before population senescence is limited (often  $< 30$ ).

**Output:** At every PD, number of cells, fraction of senescent cells, telomere length parameters (mean, 5%, 10%, 50%, 90% and 95%), and Y' number parameters (mean, 5%, 10%, 50%, 90% and 95%) are estimated. Two files are generated, showing parameters for each individual replicate and mean parameters across replicates.

**Citation:** If you use this simulator in a publication, please cite the original article (Tsai et al. 2026) and this github repository.

## References:

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