

Quantifying the Dynamics of Kaposi's Sarcoma-Associated Herpesvirus Persistence

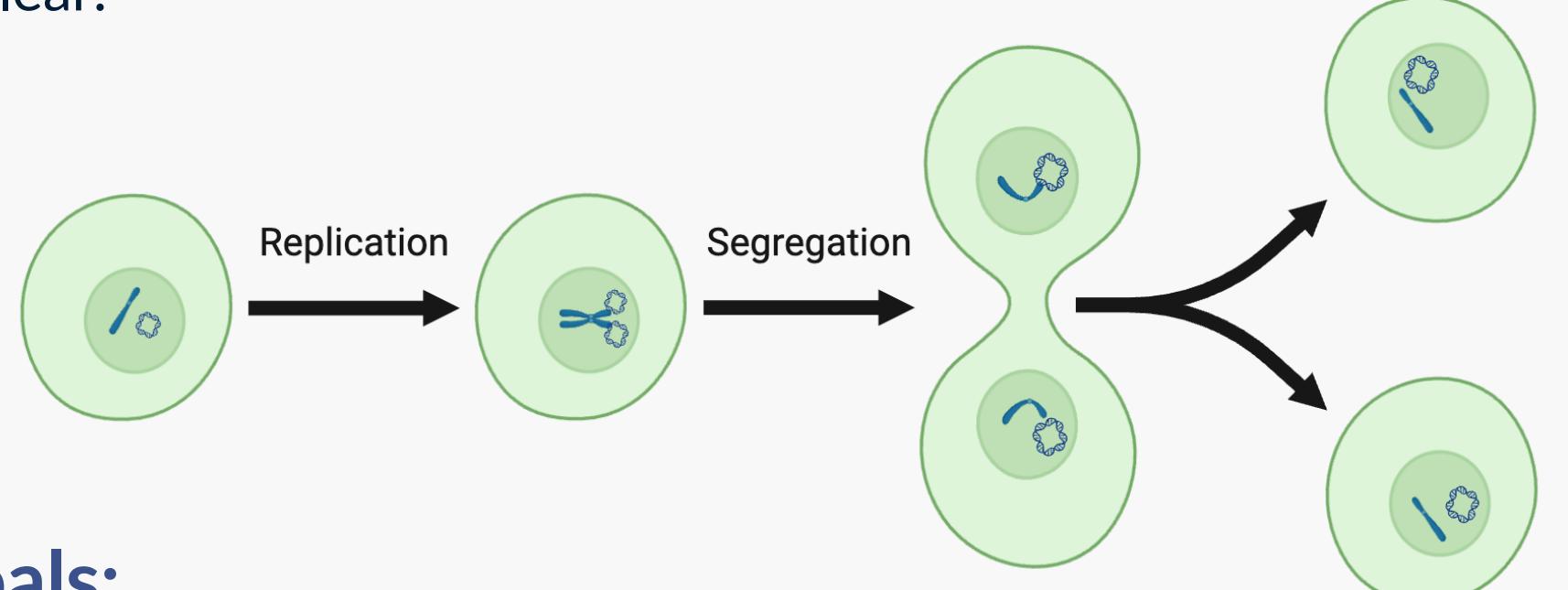
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Introduction

Motivating question: What are the mechanisms of lifelong viral persistence for KSHV?

Kaposi's sarcoma-associated herpesvirus (KSHV) is a causative agent of several lymphoproliferative diseases, particularly in immunocompromised individuals. These malignancies often originate from latently infected B cells, where KSHV persists as extrachromosomal, circularized episomes. Although the viral protein LANA is essential for viral maintenance during latency¹⁻³, the precise mechanisms by which latent KSHV is replicated and passed to daughter cells to enable lifelong viral persistence remain unclear.

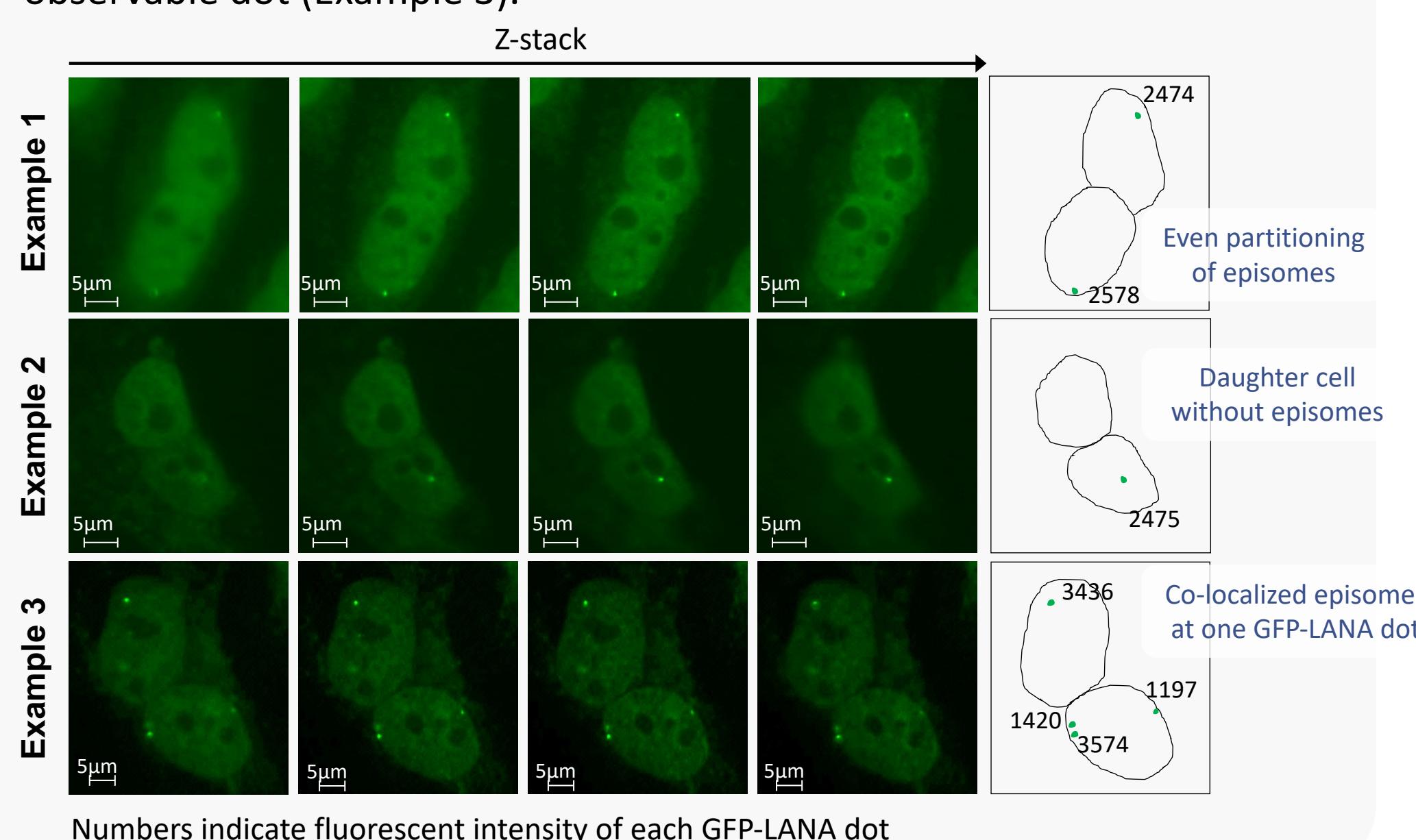


Goals:

- Determine if there is a non-random partitioning mechanism for latent KSHV episomes by estimating the efficiency of replication and segregation
- Understand the role of replication and segregation in maintaining latent virus in a dividing cell population for decades
- Estimate the required level of disruption to replication and/or segregation to reduce tumor burden in KSHV-dependent malignancies

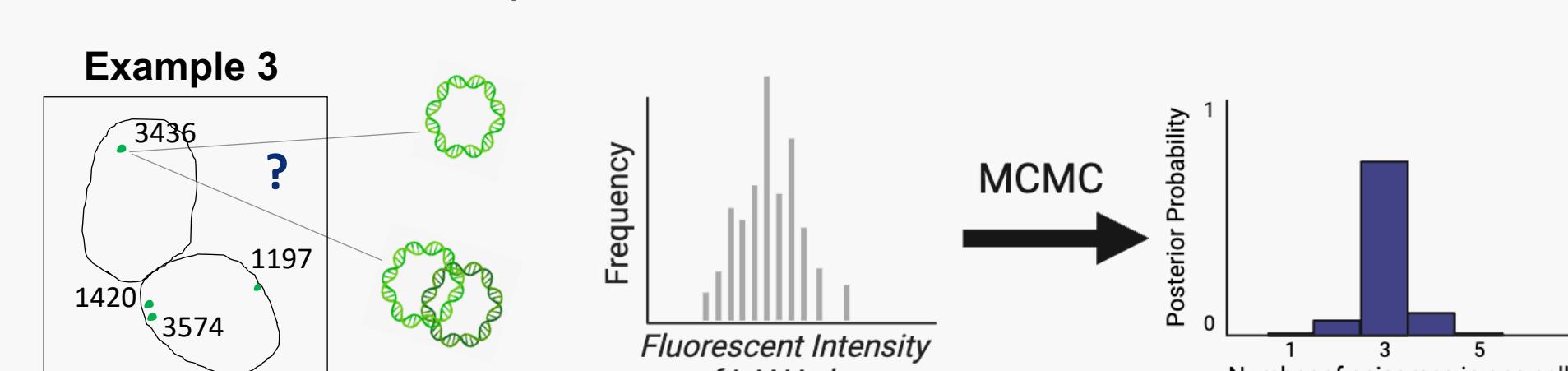
Experimental Data

Fluorescence microscopy images of GFP-tagged LANA bound to episomes post cell division suggest that replication and/or segregation may be imperfect (Example 2). Furthermore, variation in intensity of GFP-LANA dots indicate that multiple episomes may be co-localized at each observable dot (Example 3).



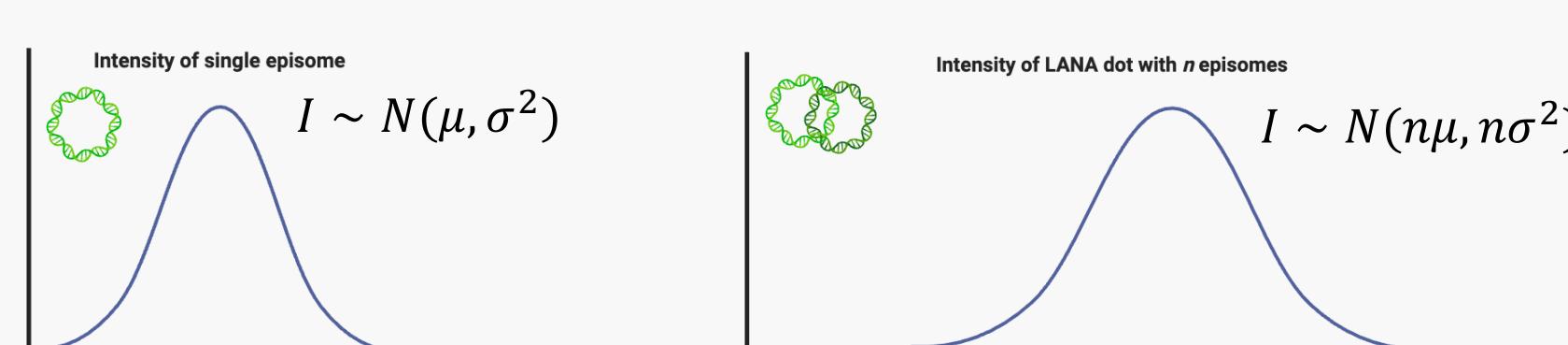
Enumerating Episomes from Images with Gibbs Sampling

The number of episomes in a cell cannot be precisely determined from the images alone, as multiple episomes may spatially cluster or overlap, thus appearing as one GFP-tagged dot. We built a Gibbs sampler to sample the posterior distribution of episome counts per cell from the intensity of GFP-LANA dots measured in each cell.

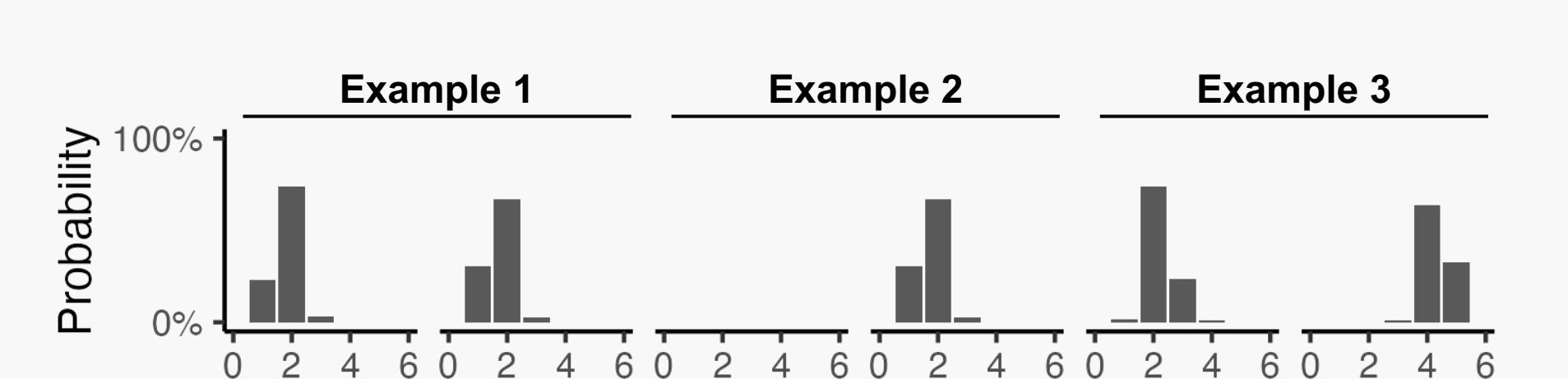


The sampler is based on two foundational assumptions:

- 1) The intensity of a single episome follows a normal distribution
- 2) The intensity of a cluster is proportional to the number of episomes in the cluster

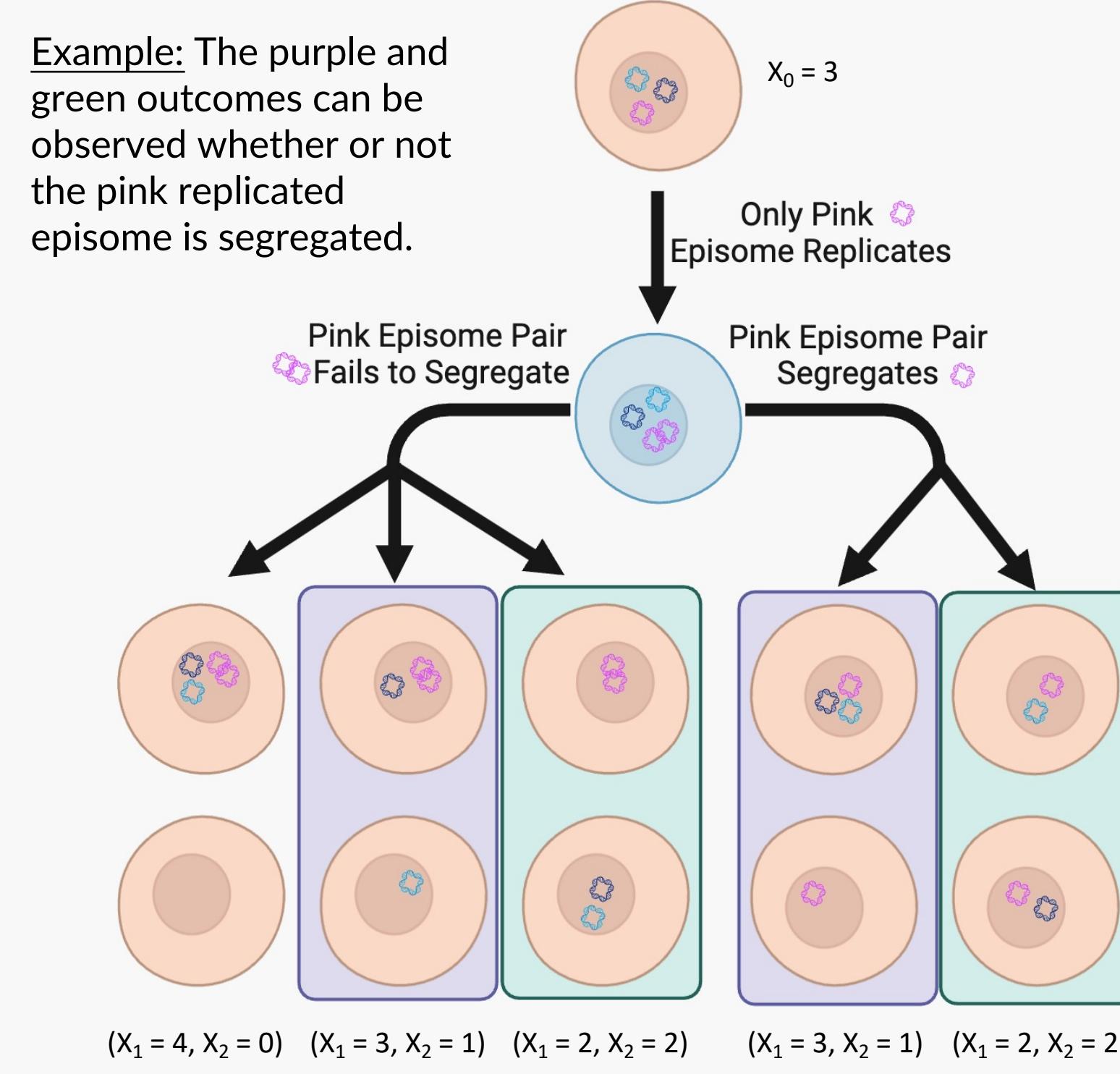


Uncertainty in the number of episomes per cell is propagated to the estimates of replication and segregation efficiency (see middle panel). Posterior distributions for the number of episomes per cell in examples 1-3 (above) are shown below.



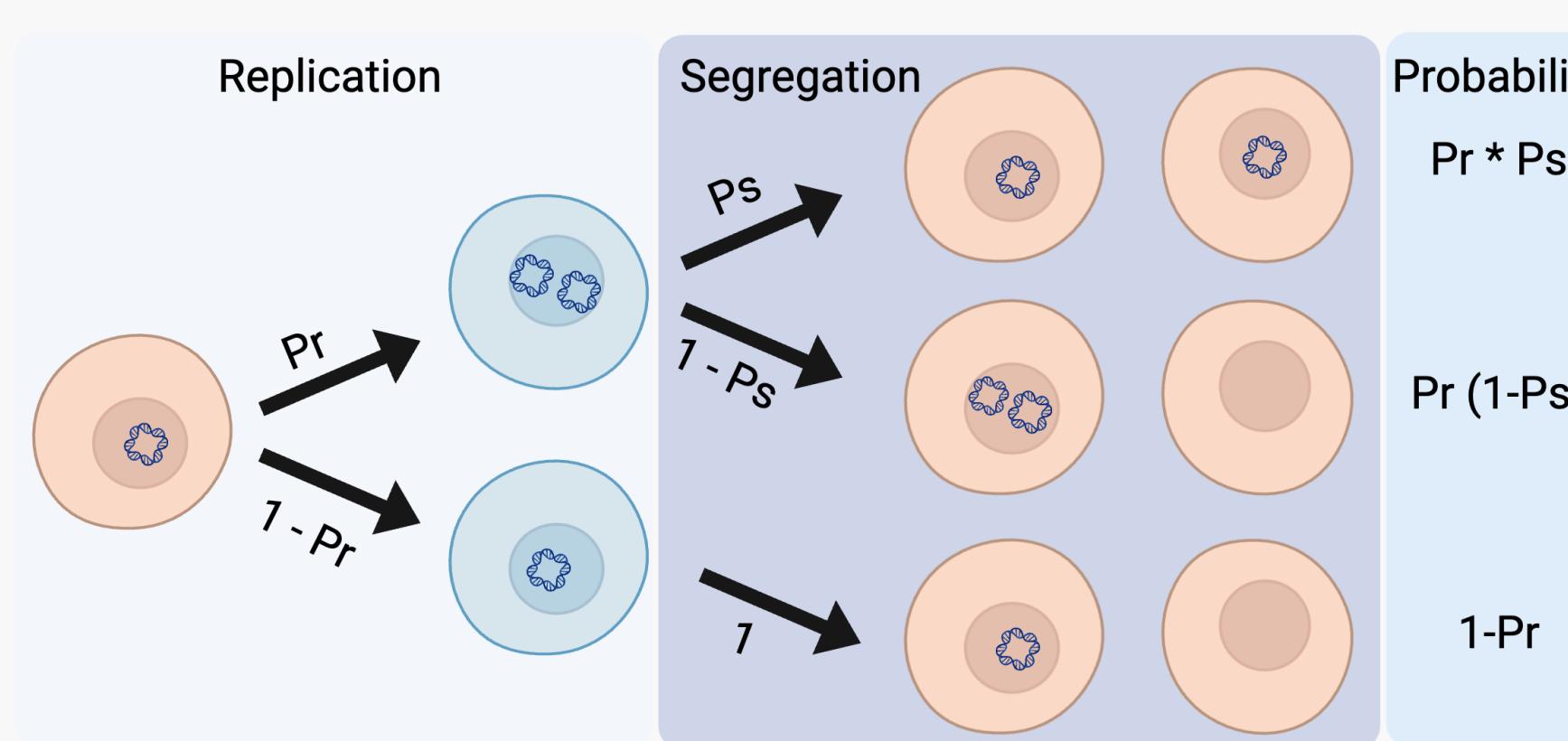
Mathematical Model

Why do we need a mathematical model? Some combinations of replication and segregation are indistinguishable just by examining the number of episomes in each cell post-division. A model allows us to leverage information from a collection of division events to jointly estimate replication and segregation efficiency and quantify the uncertainty in our estimates.



The mathematical model has two parameters:

- Replication Efficiency (Pr):** The probability that an episome will be replicated
- Segregation Efficiency (Ps):** The probability that a replicated episome pair will segregate to separate daughter cells



We generalize this simple example to account for mother cells with more multiple episomes (X_0) to define the likelihood of observing a daughter cell pair with (X_1, X_2) episomes as:

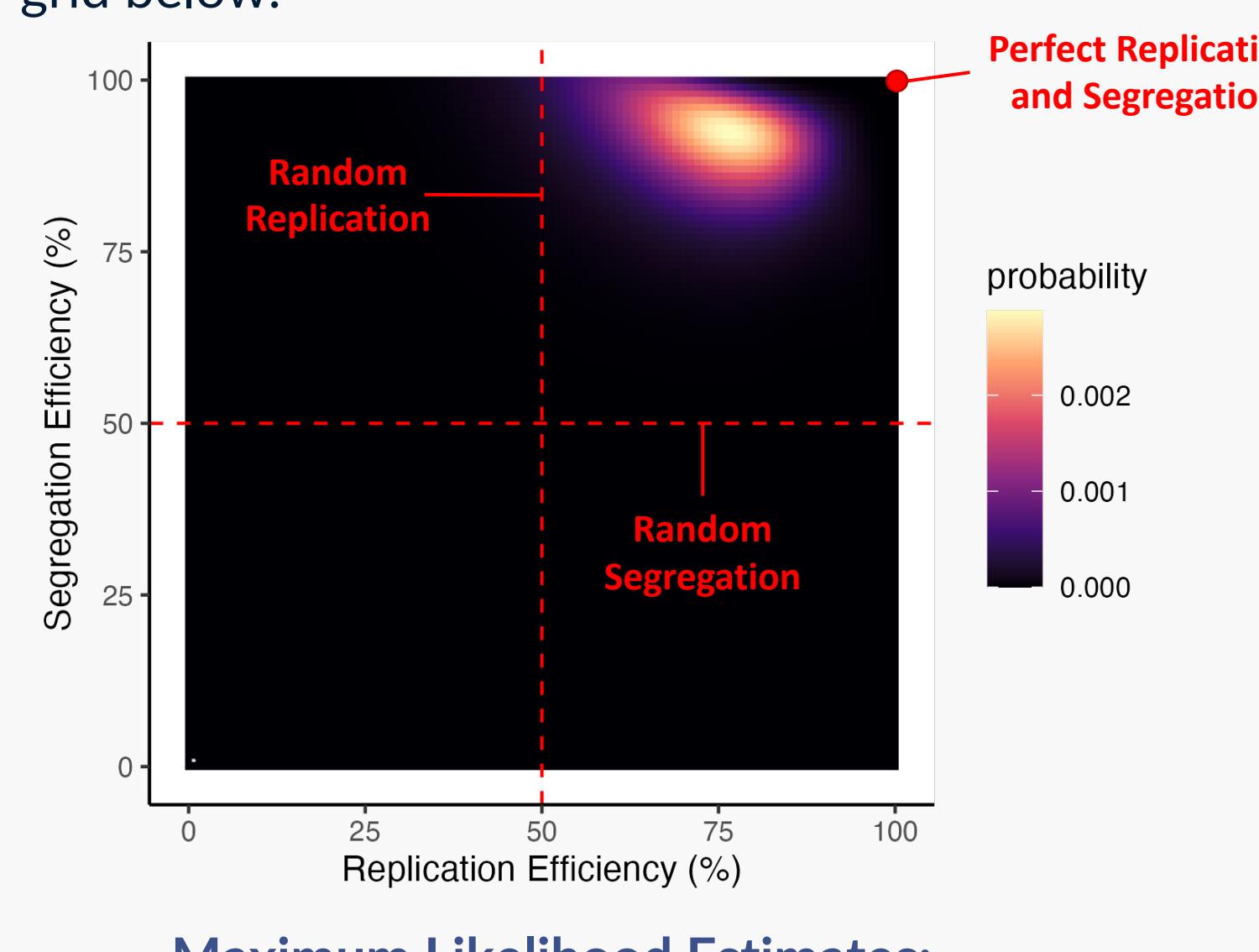
$$L(X_1, X_2 | X_0, P_r, P_s) = \frac{2^{ABS(SIGN(X_1-X_2))} \binom{X_0}{R} P_r^R (1-P_r)^{X_0-R}}{\sum_{S=0}^R \binom{R}{S} P_s^S (1-P_s)^{R-S}} \sum_{k=0}^{X_0-S} \frac{1}{2} \binom{X_0-S}{2k} \binom{X_0-R}{k} \binom{X_1-S}{2k}$$

Probability of $R = X_1 + X_2 - X_0$ episomes replicating Sum over possible segregation events Sum over possible random groupings of episomes that do not segregate

Using synthetic data, we confirmed that the model could provide unbiased estimates of replication and segregation efficiency.

Replication and Segregation are Imperfect and Non-random

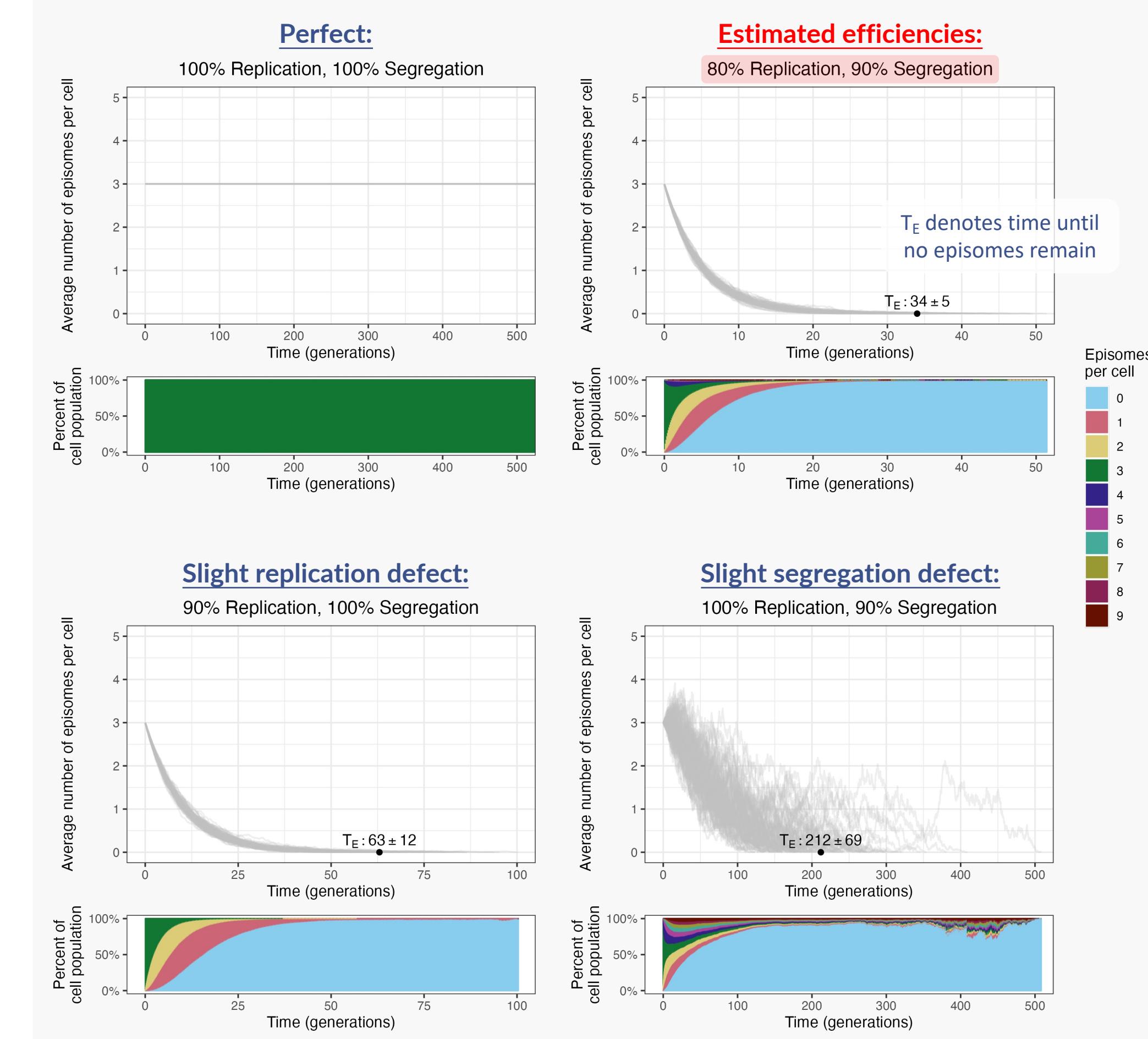
We used the model to evaluate every combination of replication and segregation efficiency and identify the maximum likelihood estimates. Uncertainty in the number of episomes per cell was propagated by taking 100 bootstrapped samples from the posterior distributions of the number of episomes per cell and calculating the probability of each parameter combination. Probabilities were summed from all samples and normalized to create the final probability grid below.



References: [1] Ballestas, M. E., Chatis, P. A. & Kaye, K. M., *Science* (1999). [2] Ballestas, M. E. & Kaye, K. M., *J Virol* (2001). [3] Hu, J. & Renne, R., *J Virol* (2005). Figures created with Biorender.com. Experimental design and data collection by FJ, AS, and KK. Modeling and simulation by MG*, JG, and AH. *email: mgaston1@jh.edu

Projecting Dynamics in a Dividing Cell Population

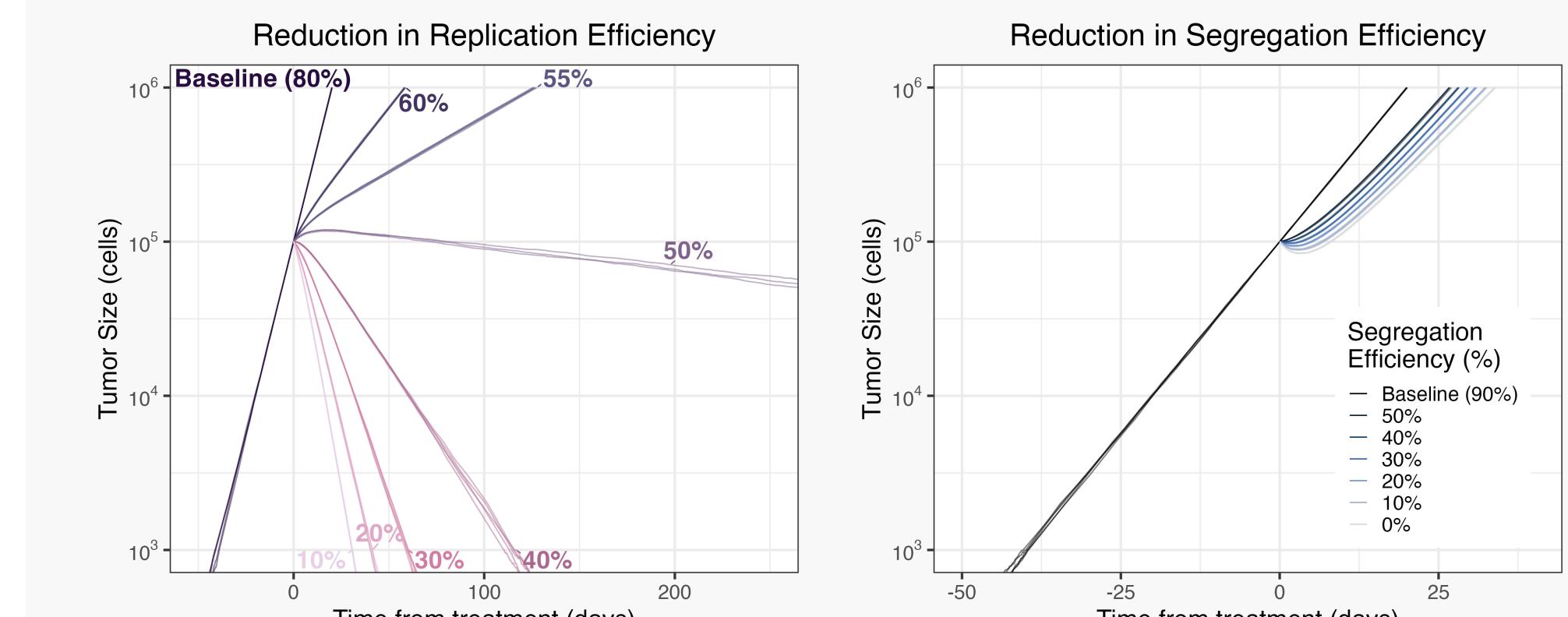
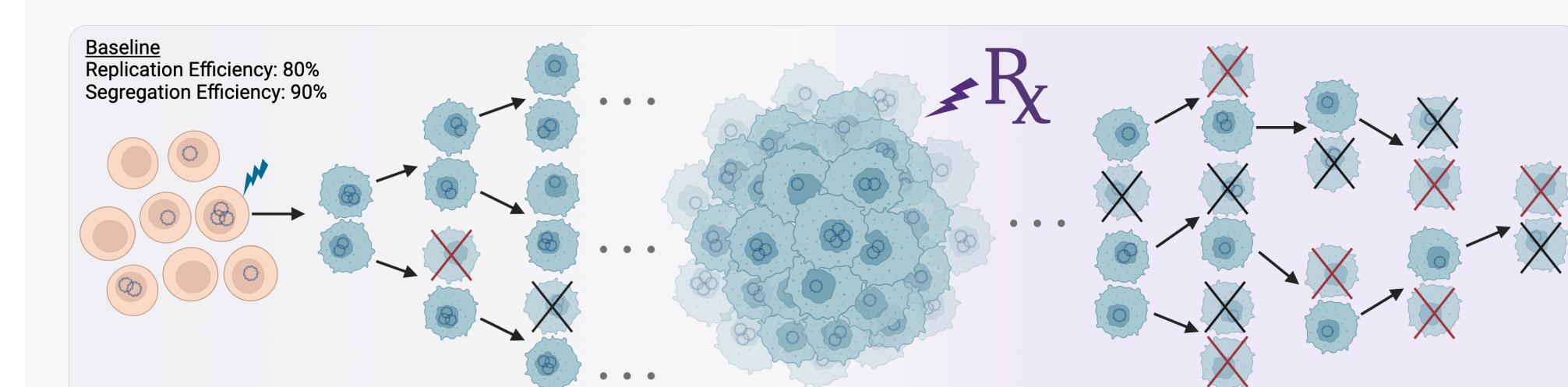
To represent KSHV persistence in a population of B-cells, we simulate a dividing cell population with a balanced birth and death rate. Stochastic simulations of 100 dividing cell populations fluctuating around a size of 1000 cells reveal that imperfect replication or segregation leads to eventual loss of episomes.



Replication efficiency is more influential than segregation efficiency for duration of latent episome maintenance. Segregation defects lead to increased variation in episomes per cell.

Reducing Tumor Burden via Disruption of Episome Replication

In KSHV-associated malignancies that are dependent on episomes for survival, increasing the rate of episome loss via therapeutic agents targeting KSHV replication or segregation efficiency may be effective in reducing tumor burden. As a proof of principle, we simulated an exponentially growing tumor starting from a single infected cell with 80% replication efficiency and 90% segregation efficiency, where tumor cells born without any episomes die immediately. Once the tumor grew to a defined size, we simulated a theoretical therapeutic intervention that reduced either replication or segregation efficiency. We assume the tumor doubles every 6 days and tumor cells live an average of 5 days.



There is no level of reduction in segregation efficiency that will stop tumor growth, but lowering replication efficiency below a threshold can effectively reduce tumor burden. The required reduction in replication depends on how quickly tumor cells divide; slower dividing tumors require a larger disruption to replication.

Insights

- KSHV persists by employing an active partitioning mechanism, as opposed to random segregation
- Decades-long persistence in dividing cell populations is not possible without the assistance of additional mechanisms such as cell-survival benefits to infection or occasional lytic replication
- Replication defects limit duration of latent episome maintenance
- Segregation defects allow episomes to accumulate in cells
- Sufficient reduction of episome replication, but not segregation, can reduce tumor burden in KSHV-dependent malignancies
- The degree of replication reduction required for success depends on the growth characteristics of the tumor
- This inference framework can be applied to other circularized viruses