# A study into the detectability of Ionizing-Radiation Induced Mutations Using Single-Cell Whole-Genome Sequencing Simulations

Lexa Lowe[1], John Kildea[1]

1. McGill University, Montreal

## Introduction

Ionizing radiation is known to cause damage to DNA and, as a result, genomic mutations. The detection of these mutations is important for understanding the impact that ionizing radiation has on humans.

Recent research has shown that bulk-cell whole-genome DNA sequencing-based techniques can detect a mutational signature of ionizing radiation [1, 2]. However, this involves costly time and labour to clone sufficient cells, either as organisms, organoids or tumors.

Single-cell whole-genome DNA sequencing may offer an alternative method to detect radiation-induced changes in individual cells.

## Object

To estimate the sequencing parameters required for single-cell whole-genome sequencing of human cells that contain IR-induced mutations. The estimates will be verified and refined *in silico* using virtual sequencing simulations.

## References

1. S. Behjati, et al. Nat Commun. 7, (2016)
2. J. Youk, et al. Cell Genom. 4, (2024)
3. F. Matthew. github.com/kildealab/RadiSeq

## Contact

Lexa Lowe

hunter.lowe@mail.mcgill.ca

## Methods

The first task involves estimating appropriate simulation parameters based on known data related to IR-induced mutations and single-cell whole-genome DNA sequencing.

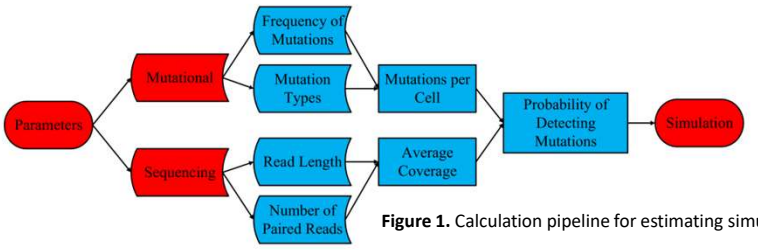


**Figure 1.** Calculation pipeline for estimating simulation parameters.

Sequencing simulations are carried out once the simulation parameters have been estimated. A virtual sequencer called RadiSeq [3] has been modified to carry out this work.

The simulations are important because single-cell whole-genome sequencing typically has poor coverage compared to bulk-cell sequencing.

Using simulated data, it's possible to refine the estimated sequencing parameters according to the simulated detectability of the mutations. This ensures that the bioinformatics tools can function correctly with poor coverage.

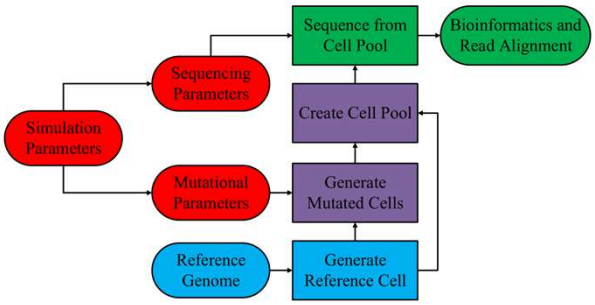


**Figure 2.** Simulation pipeline for measuring detectability of mutations.

The occurrence of long deletions, insertion-deletions, balanced inversions, and balanced translocations has been found to be at an increased rate in cells exposed to ionizing radiation [2].

These 4 general mutation types are inserted into cells *in silico* at rates corresponding to data from existing literature.

The mutations are Poisson distributed and should largely depend on the *detectable mean number of mutations* in the cells. Therefore, the estimations are based on setting a detectability threshold for depth of coverage and calculating detectability from a Poisson distribution depending on percent coverage and mutational frequency.

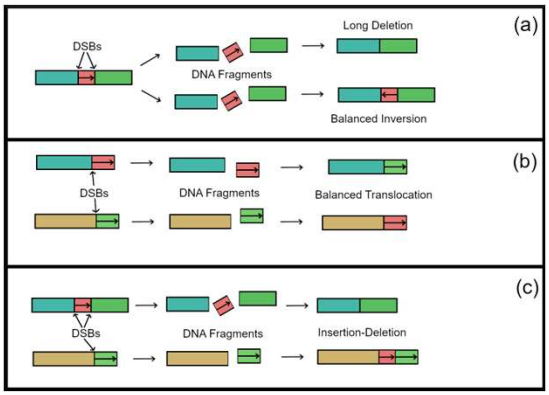


**Figure 3.** Somatic mutation types that are inserted into the simulation cell pool. Two DSBs in a single chromosome may lead to a long deletion or balanced inversion (a), two DSBs in two chromosomes may need to a balanced translocation (b) and three DSBs in 2 chromosomes may lead to an insertion-deletion.

## Preliminary Results & Discussion

Data from the literature has been used to predict cell sample sizes and parameters for the simulated single-cell whole-genome sequencing of cells exposed to ionizing radiation. A starting point for the simulations will be to simulate a dozen cells exposed to 2 Gy and sequence them with a mean coverage of 30x.

## Conclusions and Future Work

The detection of ionizing radiation induced somatic mutations is predicted to be reasonable with only few cells. Ongoing work largely involves conducting simulations to verify predictions and refine sequencing parameters.