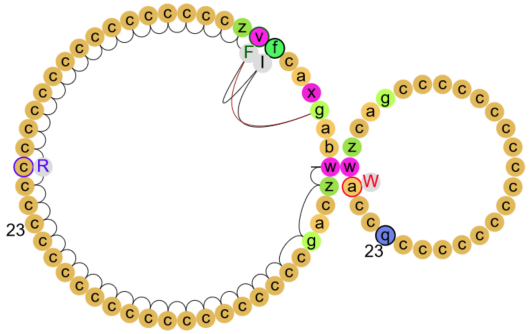
Exercise 1 - Exploring the Introduction of Genetic Variation by Random Mutation



During the process of reproduction, the parent Avidian's instruction c at position 23 mutated to instruction q in its offspring. Is this mutation more likely than another?

Student Learning Goals

- Students will be able to explain what it means to say that mutations occur at random.
- Students will be able to explain that mutations occurring during each individual's reproduction leads to genotypic variation in the population.

Questions to Consider While Doing Exercise #1

- How does genetic sequence variation originate in a population?
- Is there a pattern to how mutations occur?
- Could we predict which mutation(s) an organism's offspring will have?

Biological organisms can exhibit both genotypic (genetic information) and phenotypic (expressed trait) variation. Similarly, the organisms in Avida-ED – called Avidians – also have genotypes and phenotypes. An Avidian's genotype is the entire sequence of instructions in its genome, and its phenotype is its ability to reproduce and perform functions, for example NOT. Mutation is crucially important because it generates genotypic variation that might be expressed as phenotypic variation. In this exercise we will focus on inheritance and genotypic variation by investigating the fundamental source of variation – mutation.

Random Mutation and Genotypic Variation

Like bacteria, Avidians have single parent reproduction and a circular genome composed of simple genetic instructions that can undergo mutation. If mutations did not occur, an offspring would inherit the exact genetic sequence of its parent. Though an organism largely resembles its parent, it is generally never exactly identical because during the reproduction process, a few genetic sites may change due to mutations occurring at random in the sequence. Mutation creates genotypic variation in a population of organisms since different genetic sequence changes may occur during each individual's reproduction. For simplicity, Avida-ED allows only substitution mutation, which is a random change from one instruction to another in an organism's genome. Therefore, an Avidian genome in

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Avida-ED will always contain exactly 50 instructions.

Many genetics concepts do not apply to the simple genetic machinery of Avidians. For example, other types of mutation (insertions and deletions, chromosomal mutations), and the processes of transcription, translation, recombination, and horizontal transfer do not occur in Avidian genetics.

In this exercise, we will explore how mutations produce genotypic variation. In addition, we will ask whether each individual mutation event is a random event. Each student will guide an Avidian through its reproduction process and record all of the mutations that occur in the offspring individual. By carrying out this reproduction process for three independent replicates, each person in the class will be able to contribute the results of their three replicates to a class data set. With this much larger sample, we can investigate as a class whether or not there are trends in the occurrence of mutations. Where in the Avidian genome did the mutations occur? Did mutations occur such that certain mutant states were preferred? Finally, how many mutations occurred during Avidian reproduction?

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Before you begin collecting data. On the graph axes provided below, draw your expectations for the frequency distribution of the three features of mutation described in the previous paragraph. These distributions represent data you would expect to observe from very, very many (thousands of) experiments. Later, after we've examined the data collected by the entire class, we will return to this page to draw updated expected distributions. Because each graph will have two predictions for the distribution, make each distinct by using colored lines. *You must note this in the legend.*

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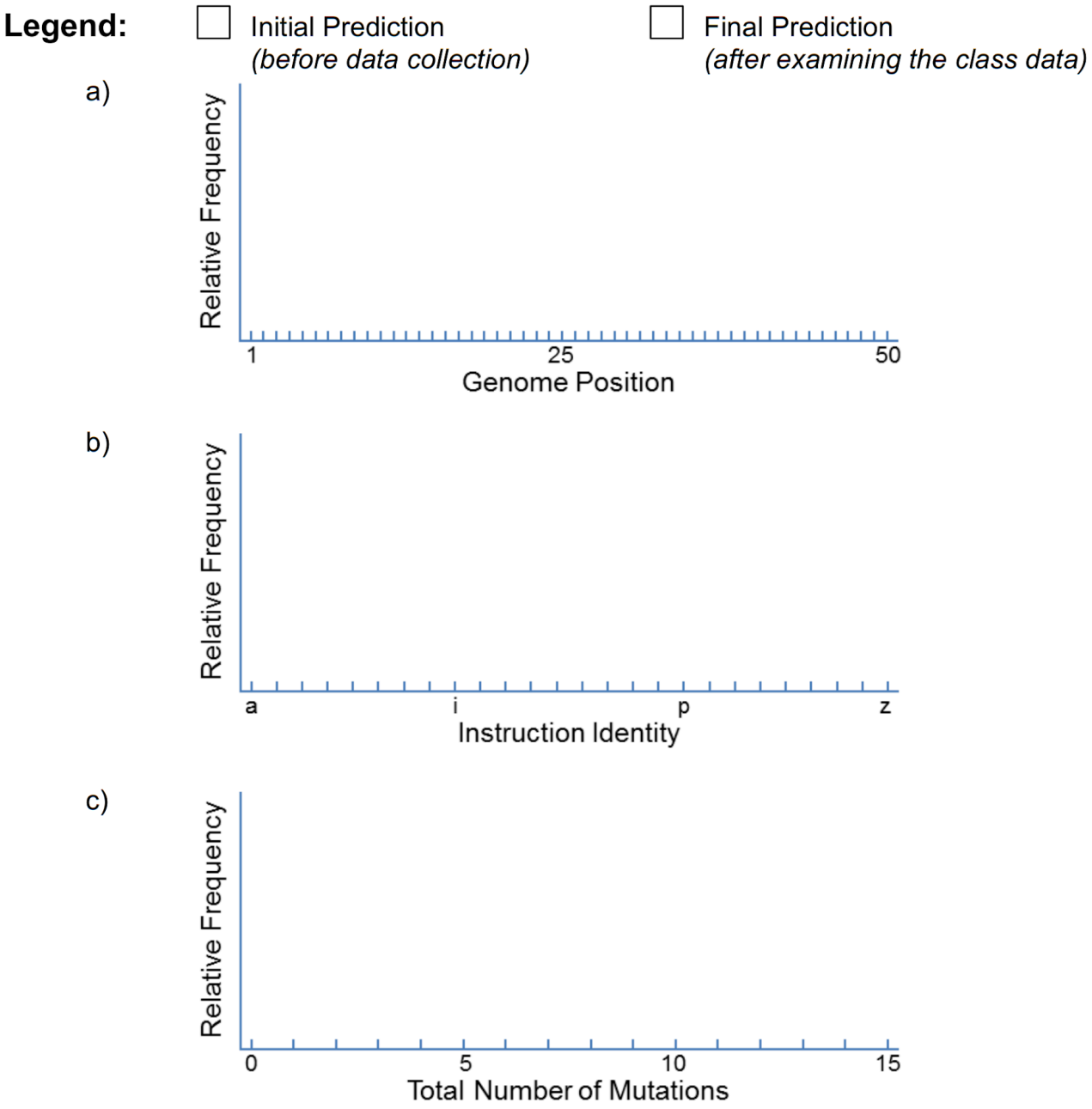


Figure 1. a) Predicted frequency of mutations by position across the Avidian genome.
b) Predicted frequency of mutations by identity of the final instruction state.
c) Predicted frequency of number of mutations per reproduction with 10% mutation rate

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AVIDA-ED

Recording Mutant Avidians

Observe how substitution mutations during reproduction change the genetic sequence from parent to offspring.

1. In the Organism viewer, select Settings.
2. Set the Per Site Mutation Rate to **10%**. Keep Repeatability Mode as Experimental.
3. Drag the "@ancestor" from the Freezer to the genetic code box.
4. Select Run to observe the Avidian executing its genomic instruction sequence, including the process of reproduction. Each mutation that occurs will be highlighted by a black outline around its instruction circle. You can display the genomic position (number) of any instruction by selecting it. Note that it is possible none may occur.
5. *Record your data as Replicate #1*. Use the provided ancestral genomic sequence template to record the mutations by crossing off the ancestral state and recording the new instruction directly beneath. *Be sure to mark both the location and the type of mutation that occurs.* Read the circular genome clockwise from the 3 o'clock position, which has the ancestral state W. As a guide, selecting an instruction circle will display its position in the genome.

Replicate #1: Mark differences from the ancestral genome.

```
Position    1       10        20        30        40        50
Ancestor    wzcagccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _____

Before performing additional replicates. Compare your Replicate 1 data with the data from a classmate. Note that in each case, you each began with the same parent (@ancestor) and allowed it to reproduce with a 10% mutation rate.

➔ In comparison to your classmate, did the same mutations and/or number of mutations occur during reproduction?

➔ What do you ***expect*** will happen if you repeat this experiment?

- Do you think the *specific* mutations will be the same? Explain your reasoning.
- Do you think the *number* of mutations will be the same? Explain your reasoning.

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Testing your prediction. After you have recorded your predictions, reproduce the ancestor twice more using the same settings. To begin Replicate #2, simply select Reset.

Replicate #2: Mark differences from the ancestral genome.

```
Position    1       10        20        30        40        50
Ancestor    wzcagccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _____

Replicate #3: Mark differences from the ancestral genome.

```
Position    1       10        20        30        40        50
Ancestor    wzcagccccccccccccccccccccccccccccccccczvfcaxgab
Mutations
```

Total number of mutations _____

Recording your data. Experiments often involve investigating processes or phenomena with lots of variation, we will therefore be examining the data generated by all students in the course. Enter your *mutation data* here:

INSTRUCTORS - PLEASE CREATE A NEW LINK FOR YOUR CLASS. THE ORIGINAL DATA COLLECTION SPREADSHEET CAN BE FOUND HERE AND SHOULD BE COPIED FOR YOUR COURSE USE.

Exercise 1 Data Collection Spreadsheet

Follow the “Example” in columns B-D of the data collection sheet. Find the first column on the right that does not contain data; enter your ***Name-Replicate#*** in row 4, and your observed mutations in rows 5-54 corresponding to genome positions 1-50 by entering the letter of the mutated final instruction state. The instructors will periodically collect this data, anonymize it, and add it to the course data set.

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Once you have entered your data, compared and discussed your group's data, and completed all responses and data entry except the Discussion Questions on the next page, you can view the course data and analysis.

INSTRUCTORS - IF YOU WISH TO USE OUR DATA ANALYSIS SPREADSHEET PLEASE CREATE A NEW LINK FOR YOUR CLASS. THE ORIGINAL FULL DATA ANALYSIS SPREADSHEET CAN BE FOUND HERE AND SHOULD BE COPIED FOR YOUR COURSE USE.

Exercise 1 Data Spreadsheet

Update your predicted distributions. After examining the course data, return to your prediction graphs and draw a new representation for each distribution – *not simply the exact course data*, but rather what you would expect if 200 or even 200,000 more students contributed to our investigation of random mutation in Avida-ED. Make sure your new distribution is distinct from your initial prediction by using colored lines. Note this distinction in the legend on that page.

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Discussion Questions and Wrap-up. After examining the course data, work with your lab team to respond to the following questions.

➔ How does this *experimental setup* demonstrate that mutations are random?

➔ Did each person in the course get the same mutations and number of mutations?

➔ How would you describe each of the three relative frequency distributions (genome position, instruction identity, total number of mutations) for the entire course data?

➔ How would you reconcile your responses to the above two questions – each person's individual experimental data versus data from the entire course?

➔ **Thought experiment –** How would the course's results be different if a 5% mutation rate was used instead? How would each relative frequency distribution appear?

➔ How is random mutation in Avida-ED *similar* to random mutation in biological systems?

➔ How is random mutation in Avida-ED *different* than random mutation in biological systems?

- We used Avida-ED and this experimental protocol to model what occurs when biological populations experience mutation. What are some limitations or constraints to our modeling in this exercise?

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Reflection and Metacognition
Think-Pair-Share: Work with your lab team to answer the following questions.

- What did you learn from this exercise?

- What are you still wondering about?

- What would you change in this exercise?

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