



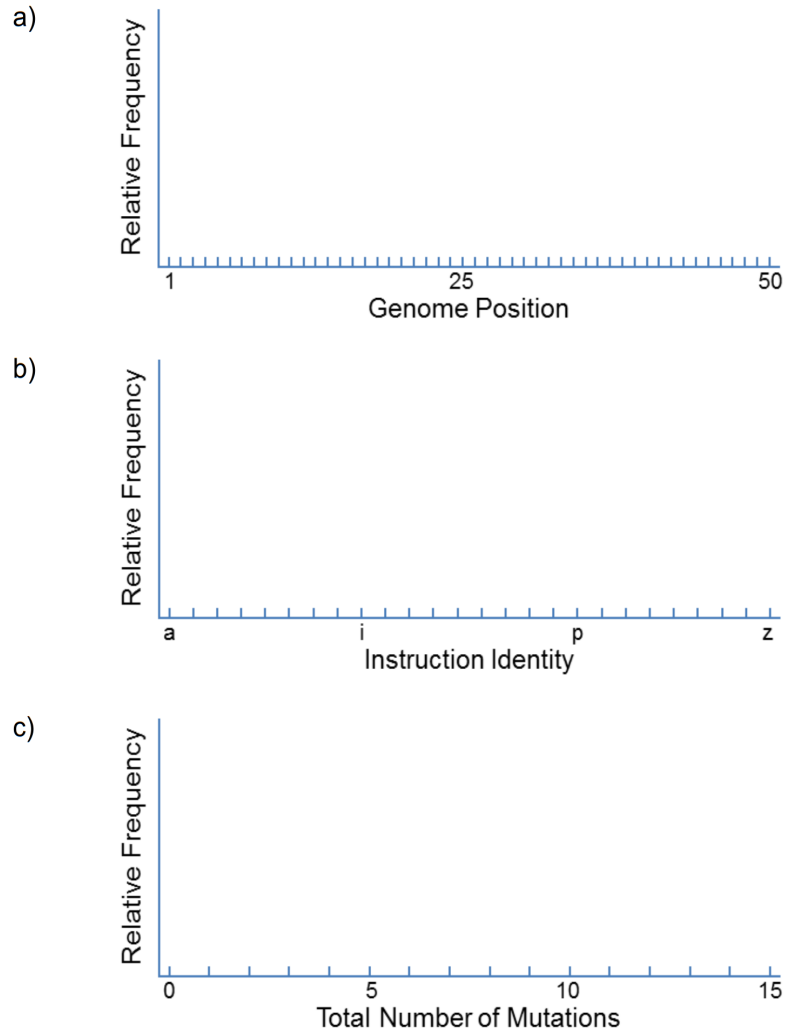


Many genetics concepts do not apply to the simple genetic machinery of Avidians. For example, other types of mutation (insertions and deletions, chromosomal mutations), and the processes of transcription, translation, recombination, and horizontal transfer do not occur in Avidian genetics.

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?



**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.



**Figure 1. Predictions about mutations, with respect to position, identity, and frequency.**  
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.

**Table 1. Differences from the Ancestor for three offspring Avidians.**

Position	ANC. ID	Rep #1	Rep #2	Rep #3		Position	ANC. ID	Rep. #1	Rep. #2	Rep. #3
1	w					26	c			
2	z					27	c			
3	c					28	c			
4	a					29	c			
5	g					30	c			
6	c					31	c			
7	c					32	c			
8	c					33	c			
9	c					34	c			
10	c					35	c			
11	c					36	c			
12	c					37	c			
13	c					38	c			
14	c					39	c			
15	c					40	c			
16	c					41	c			
17	c					42	z			
18	c					43	v			
19	c					44	f			
20	c					45	c			
21	c					46	a			
22	c					47	x			
23	c					48	g			
24	c					49	a			
25	c					50	b			



## 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 (Rep #1). Use the provided ancestral genomic sequence (ANC. ID) template to record the mutations by identifying the position and instructional change for any mutation. 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.

**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.



After you have compared your data, repeat the experiment two more times, dragging a new ancestor into the window for each replicate.

**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. Your instructor will provide you with a link where you can enter your data into a class spreadsheet.

#### [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.



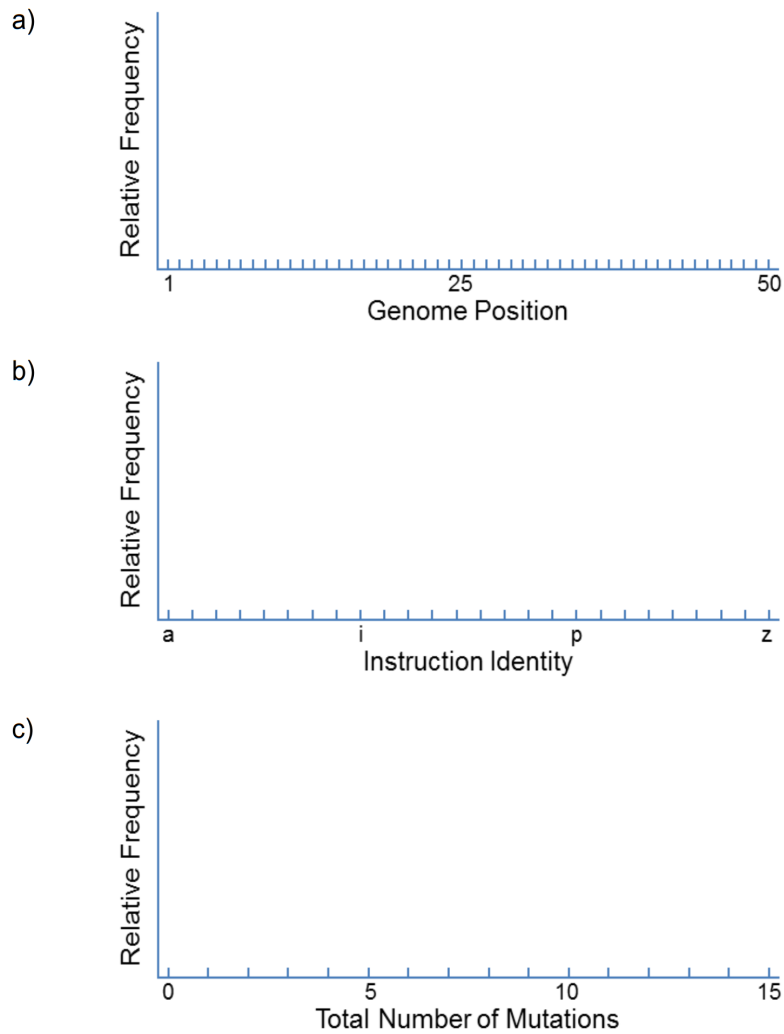
Once you have entered your data, compared and discussed your group's data, and Once you have entered your data, compared and discussed your group's data, and completed all responses and data entry except the Discussion Questions on the next page, you can view the course data and analysis.

### [Exercise 1 Data Analysis Spreadsheet](#)

**What did you find out?** Now that you have seen the course data, draw the observed distributions for each of the graphs you previously made a prediction about. Remember that these are experimental data, and therefore is unlikely to be perfectly in line with your expectations. It is more important to record what you actually observed.

In an experiment, researchers obtain data about a question. That data is specific to the individual experiment they are doing. This specificity is not only in terms of the question being asked and the method being used, but also to the individual replicate of the experiment they are conducting. If they were to do the exact same thing a second time, the exact numbers would likely be slightly different. That is because the data they obtain are being drawn from a larger true distribution of possible outcomes. When we perform many replicates of an experiment, part of what we are trying to do is to estimate that full distribution. In the same way, our class data is not necessarily a perfect representation of the true distribution. However, our data can inform us about what we think that underlying distribution is.

Go to Figure 2, and graph the data from the class as a whole. Next, add a line in a different color showing what you think the underlying distribution is. This is what you would expect if thousands or millions of additional replicates were performed.



**Figure 2. Observed data about mutations, with respect to position, identity, and frequency.**  
a) Observed frequency of mutations by position across the Avidian genome.  
b) Observed frequency of mutations by identity of the final instruction state.  
c) Observed frequency of number of mutations per reproduction with 10% mutation rate.

**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?

### **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?



