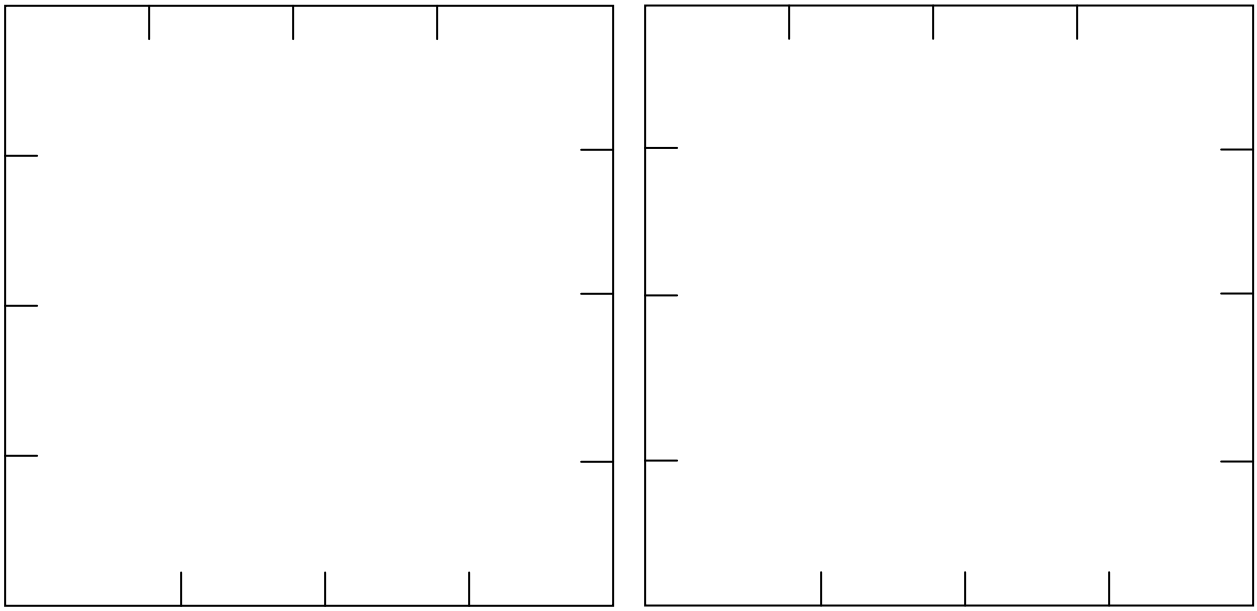
Name: _____ Date: _____ Period: _____

Student Sheet 1: Observing Worms - Draw what you see inside the square on both worm plates.

Wild Type **Mutant**



Scale of drawing is 20x:
4 mm in microscope field = 8 cm (80 mm) on diagram.

Discussion questions:

1. Where did you find most of the worms? Why do you think they are there?

2. Why do you think nematodes make a good model organism for understanding humans?

3. How are the wild type and mutant worms similar?

4. How are the wild type and mutant worms different?

Student Sheet 2: How Does High Salt Affect the Activity and Growth of *C. elegans*?

Overview
Normally, nematodes live, move, eat, and reproduce in moist soil. Sometimes they encounter conditions that are not good for their growth. In this experiment, you will study what happens when wild type and mutant are put into a high salt environment. You will observe both types of worms over several days and record what you see. At the end of the experiment, you will describe what you saw and propose a model for what was going on.

In the table below, **predict** what you think will happen to wild type worms on high salt.

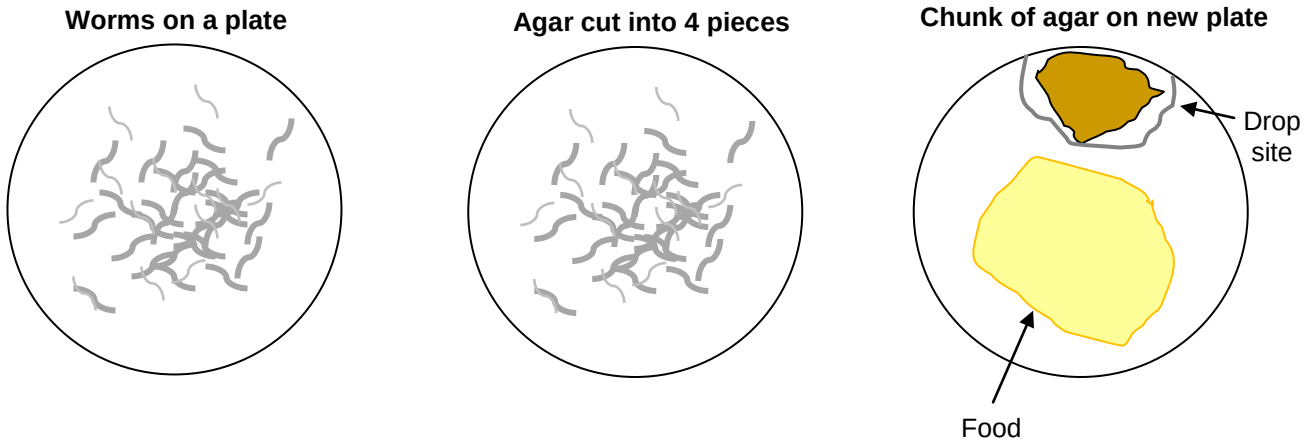
	Low Salt	**High Salt**
Wild type		
Mutant		

Materials
Dissecting microscope
Small NGM plate of **wild type** or **mutant** worms
NGM plates containing **0.05 (low)** and **0.45 M (high)** NaCl
Bunsen burner and lighter
Square-ended spatula

Procedure: Transferring worms from the parent plate to **0.05 M** (low) and **0.45 M** (high) NaCl plates

1. Remove the lid and **look at your plate of worms under the microscope at low power**, and notice where most of the worms are.
2. **Heat the flat end of the spatula** in the Bunsen burner for a few seconds. Let the spatula cool for a few seconds, and touch it lightly to part of the agar that does not have worms on it to make sure it's cool.
3. Use the flat end of the spatula to **cut around the part of the worm plate that contains most of the worms. Then cut this piece into four (4) pieces** with the same number of worms on each.
4. Heat and cool the end of the spatula again. Slide spatula under one of the three chunks of agar and **place the chunk, worm side down, onto the fresh plate containing 0.05 M NaCl** within the circle drawn near the edge of the plate (the "drop site"). Record the time that you do this on the data table, and drop the chunk of agar into the waste container.
6. **Look at the worms under the microscope** to make sure that you have transferred some.
7. **Repeat steps 1-6** with another chunk of worms onto the **0.45 M** NaCl plate.

8. **<u>Look at the worms and record</u> what you see *15 minutes*** after you transfer to each plate. Here are some things for you to observe and record:
   - **Who:** How many adults, larvae, and eggs were transferred?
   - **What:** What are the worms doing? Are they moving or still? Have they moved from the drop spot or are they still in the same place?
   - **Where:** Are the worms at the drop site or are they somewhere else? Are they on the food or the agar?



<u>Additional worm observations after 24 and 48 hours</u>:

1. Observe your worms on your plates, and record your observations in Data Table (*next page*).

   <u>Here are some things for you to look for and record</u>:
   - **Evidence that worms have moved:** Worms are no longer at the drop site; worms are clearly moving; there are worm tracks in the food
   - **Evidence that worms are eating:** They are in the food; the amount of food is less since the previous day; food is all gone
   - **Evidence that worms are growing:** There are more large larvae or adults than on previous day; eggs at the drop site are no longer there because they have hatched
   - **Evidence that worms are reproducing:** There are eggs in places other than the drop site

2. Discuss your results for the two worm strains with your team members.

Discussion Question:

After 15 minutes what are the effects of low and high salt on the wild type and mutant worms? Do wild type and mutant act the same or different?

Data Table #1 - Effect of Salt Concentration on **Wild type** *C.elegans*

Worm Observations: Wild Type (N2)

[NaCl] M	Time	*Who? Eggs, Larvae or Adults?*	What? Moving not moving?	Where? Location of worms?
Low (0.05)	**15 minutes** ***Actual time:*** _____			
Low (0.05)	**24 hours** ***Actual time:*** _____			
Low (0.05)	**48 hours** ***Actual time:*** _____			
High (0.45)	**15 minutes** ***Actual time:*** _____			
High (0.45)	**24 hours** ***Actual time:*** _____			
High (0.45)	**48 hours** ***Actual time:*** _____			

Data Table #2 - Effect of Salt Concentration on **Mutant** *C.elegans*

Worm Observations (*circle your strain*):

Mutant #1 (MT3643) **Mutant #2 (JT89)** **Mutant #3 (MT3564)**

[NaCl] M	Time	*Who? Eggs, Larvae or Adults?*	What? Moving not moving?	Where? Location of worms?
Low (0.05)	15 minutes *Actual time:* _____			
Low (0.05)	24 hours *Actual time:* _____			
Low (0.05)	48 hours *Actual time:* _____			
High (0.45)	15 minutes *Actual time:* _____			
High (0.45)	24 hours *Actual time:* _____			
High (0.45)	48 hours *Actual time:* _____			

Student Sheet 3: Effect of Glycerol on Worms in Salt

A. Entry Task: Pre-lab Questions

1. How does glycerol content of wild type and mutant worms compare? **(*see* Figure 1A)**

2. Since glycerol has the ability to bind to water, how do you think glycerol content (amount of glycerol in worm) affects a worm? Is glycerol good or bad?

3. Based on your answers above, is it better to be a wild type or a mutant worm? Why?

B. Dialysis Demonstration: Modeling the Effect of Glycerol on Worms in Salt

1. In a group of four, setup your dialysis tube experiment according to **Figure 2**.
2. Make sure to record, in the table below, the initial mass (in grams) of dialysis tubes, containing solutions, before putting in tray with salt.

	Initial Mass (grams)	**Final Mass (grams)**	**% Change = (Initial – Final) / Initial * 100**
Low glycerol *"Wild type"*			
High glycerol *"Mutant"*			

3. After your group has setup both a "mutant" and "wild type" worm, draw what you predict will happen to the two tubes after sitting in salt for 40-60 minutes, and explain why.

4.

Tube with LOW glycerol	**Tube with HIGH glycerol**
Drawing:	***Drawing:***
Explanation:	***Explanation:***

C. Dialysis Demonstration: Results of Dialysis Tube Demonstration

1. Weigh each of the two dialysis tubes, after briefly dipping them in water to remove salt sticking to the outside of the tube and drying with a paper towel. Then record their weights in the table on the previous page.
2. In the table below, draw and explain what happened to your dialysis tubes after sitting in salt for 40-60 minutes (*can leave overnight*).

Tube with LOW glycerol	**Tube with HIGH glycerol**
Drawing:	***Drawing:***
Explanation:	***Explanation:***

Do your observations agree with the prediction you made before setting up the dialysis? ***Explain.***

Discussion Questions

1. After 24 hours on low and high salt, how does the movement of the wild type worms compare to the mutant worms? Explain.

2. Were you surprised by what you observed on the high salt plates after 24 hours? What do you think is happening to the worms to account for what you saw?

Complete after observing worms at start of Day 4:

3. After 48 hours on low and high salt, is there a difference in the population sizes of the wild type and mutant worms? Explain.

D. Discussion Questions: Developing an explanation for worm observations

1. Looking at **Figure 1A** again, what is different about the mutant compared to the wild type worms?

2. Looking at **Figure 1B**, how does growing on different salt concentrations affect glycerol levels inside wild type worms?

2. Look at **Figure 1C**, and think about what you observed with your wild type worms when you grew them on **0.45 M NaCl (high salt)**. How might glycerol production inside the worms affect their level of activity right after being transferred to high salt? 24 hours after transfer?

4. GPD is the enzyme that carries out the final step in making glycerol inside worm cells. **Figure 1D** shows the amount of GPD in wild type and mutant worms at two salt concentrations. What do you notice about the level of this enzyme in the two worm strains at normal and high salt? How would this affect how much glycerol they produced?

5. Based on your observations and the results of your experiment(s), is there an advantage or disadvantage to making glycerol **only at high salt** (***not all the time***). Is there an advantage or disadvantage to making glycerol all the time, as in the **mutants**? *Please be as thorough as possible in your answer.*

Student Sheet 4: Effect of Single Nucleotide Change (Mutation) in Gene on Worm Response to Salt

The scientific name for Mutation 1 is JT89. It has a mutation in the *osm-7* gene, which codes for a protein called T05D4.4 (not a very informative name!). Here are some facts about this strain compared to the wild type:

- The *osm-7* gene of the mutant differs by one nucleotides compared to the wild type gene.
- The *osm-7* mRNA is 1700 nucleotides long, and the wild type and mutant differ by one nucleotide. The mutation is
- The protein made from the wild type mRNA is 562 amino acids long.

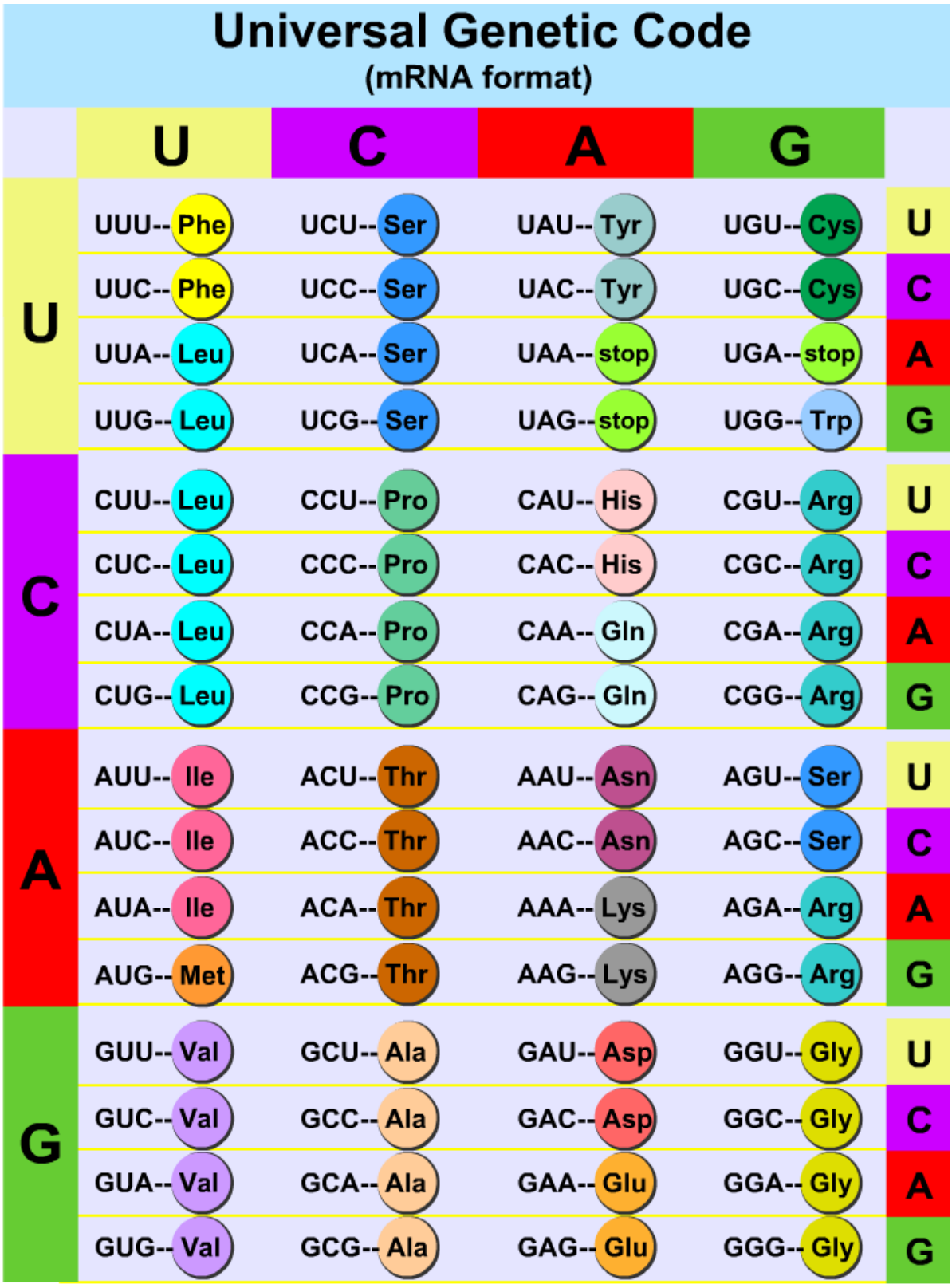
Complete the following activity to learn how a single base change can affect the protein made from that gene.

1. Use the Universal Genetic Code to decide which amino acid is coded for by each nucleotide triplet in the mRNA for the wild type mRNA. Write the name of each amino acid using the three letter abbreviation in the circle below the mRNA sequence.
2. Now use the Universal Genetic Code to translate the mutant mRNA gene.

Discussion Questions

1. How are the two resulting proteins different?

2. How would this difference affect the protein made? Do you think the mutant protein would still function properly?



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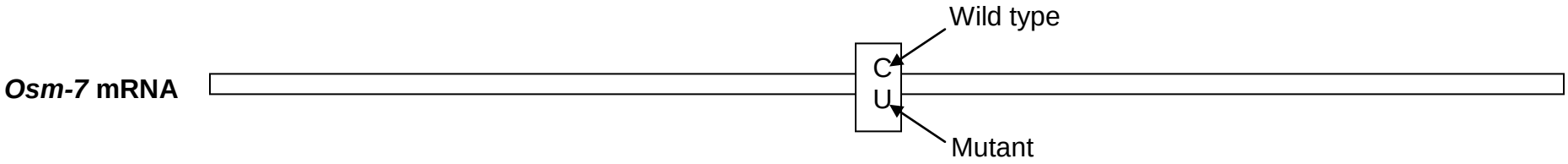
How does changing one nucleotide in the *osm-7* gene affect the protein it codes for?



The two figures below show 15 nucleotides around the mutation. Use the genetic code chart to translate the mRNA into amino acids for the wild type and mutant.