Student Guide

Cyanogenesis Assay Procedure
Activity B

Materials:

- 4 clover plants
- 48 well tissue culture plate with lid
- 2- 20 µl pipette tips or eye dropper
- 24 toothpicks
- linamarin solution
- linamarase solution
- 1 sheet of Feigl-Anger test paper (Keep test paper in a cool dry location, wrapped in foil at all times)
- 6 large binder clips
- 20 µl pipettor or eye dropper
- Distilled water

Procedure:

1. Label the clover plants #1-4.

2. Inspect the 48 well culture plate. Find the letters and numbers that identify the rows and columns. Label the 48 well culture plate and lid with your lab group number. Label each Row as follows:

A and B= "TO" for tissue only
C and D= "E" for positive for Enzyme
E and F= "C" for positive for Cyanide Compound

3. Label the well plate diagram across top with the identity of each of your 4 clover plants

1 leaf= 3 leaflets

4. For plant 1, place 3 fresh, young, green leaves into each of the wells in column #1, a total of 6 wells. These will be wells A1-F1. Skip a Column of wells and Place 3 leaves from plant #2 in wells A3-F3 and so on, for all four plants. Label your diagram carefully as to which plant is in each well.

5. Place the lid on top of the plate and place it in a -20^{o}C freezer (normal freezer) for 45 minutes.

*Optional stopping point. Plates can be left in freezer until next class, or even longer if necessary.

6. Remove the plate from the freezer and thaw the samples by placing the plate in a 37°C incubator for 10 minutes. If an incubator is not available, plants can be thawed in a warm, dry area of the room for about 20 minutes, or teacher can remove them prior to the class period.

7. Remove plate from incubator and add the reagents as follows:

- In wells A and B: Marked "TO" leaf tissue only + 20 μl or 1 drop of distilled water (no reagents added)
- In wells C and D: Marked "E" leaf tissue + 20 μl or 1 drop of **linamarin** solution
- In wells E and F: Marked "C" leaf tissue + 20 μl or 1 drop of **linamarase** solution
- Repeat for all plants and all wells.

8. Use well F8 as a positive control by placing 20 μl or 1 drop linamarin solution + 20 μl or 1 drop linamarase, no plant tissue. Be sure to mix the contents of the control well

9. Using a new toothpick for each well, mash the tissue and reagents in each well to mix compounds. Mash until a small amount of liquid is visible.
*Be sure to use a new toothpick for each well.

2. Wipe off the top of the plate to remove any moisture. Label your Feigl-Anger paper with your initials, and Place the test paper on top of the well-plate. Place the lid over the paper on the plate and then clamp together using 6 large binder clips (prevents bleed-over of HCN between wells).

3. Incubate the plate in a 37°C incubator for 30 minutes to 2 hours (or longer, if needed, but not longer than 6 hours) then photograph or record results. If an incubator is not available, cover plate with aluminum foil or place in a thick envelope, and place in a warm dry area for approximately 90 minutes. Check plate after 30 minutes and remove any tissue from already positive wells.

For reliable scoring of positive results in weakly cyanogenic plants, a minimum of 90 minutes in an incubator and 3 hours at room temperature. Scoring plants after shorter durations may yield false negative results for plants that produce low levels of cyanogenic compounds.

Ask your teacher what to do with your plants once you have finished.

Well-Plate Diagram
Individual Group Data

	1	2	3	4	5	6	7	8
→	Plant 1		Plant 2		Plant 3		Plant 4	
A (TO)								Distilled water only
B (TO)								Distilled water only
C (E)								Add linamarin to activate enzyme presence
D (E)								Add linamarin to activate enzyme presence
E (C)								Add linamarase to test for cyanide sugar
F (C)								Add linamarase to test for cyanide sugar **Control Goes Here**
Genotype								

RESULTS

1. If cyanide is produced, a blue color change will appear on the paper. Carefully inspect your paper. Record on your well-plate diagram which wells turned blue and which did not. Inspect the paper from the underside, if results are questionable.

   a. If the clover has the ability to produce cyanide, which wells will be blue?

   b. If the clover produces the sugar-cyanide compound, but not the enzyme, which wells will be blue?

   c. If the clover plant produces the enzyme, but not the sugar-cyanide compound, which wells will be blue?

   d. If the clover plant produces neither sugar-cyanide compound nor the enzyme, which wells will be blue?

   e. What is the purpose of well F8?

   2. Record the phenotype and the genotype of your plants:

Plant	1	2	3	4
Cyanide producer(y/n)				
Produces Linamarase only (y/n)				
Produces Linamarin only (y/n)				
Genotype				

   3. Collect class data.

   4. Calculate the percentage of plants that are cyanogenic.

   5. Compare the percentage of plants that are cyanogenic to your hypothesis and to the percentages from other climates.

   6. Based on climate, do the results for your local area make sense? Explain

7. Calculate the percentage of the plants that contain the gene for the linamarase ONLY:

8. Calculate the percentage of the plants that contain the gene for cyanogenic glucosides ONLY:

9. Calculate the percentage of the plants that contain NEITHER gene for cyanogenic glucosides or linamarase.

10. Explain why natural selection would favor plants that produce cyanide in warm climates, but not in cold climates.

11. Explain why natural selection would favor plants that produce neither compound in cold climates versus a plant that produces only one of the compounds.

12. Assume more plants express the gene for the production of the sugar-cyanide compound than express the gene for the production of the enzyme. What can you infer about the relative metabolic "cost" of producing the enzyme?

13. Some plants in the class may have appeared stronger in the levels of cyanogenic compounds produced in the class plants as a whole. What are some possibilities as to why there would be varying levels of cyanogenic compounds in clover plants growing in the same general area?