**Identifying a Potato Killer: Using DNA Extraction and PCR Analysis** **Day ONE**

**Problem:** Lesions caused by different plant pathogens can look alike. Identifying which pathogen that has infected a particular agricultural crop is important in determining which type of intervention should be used to prevent the entire crop from being destroyed. In your laboratory exercise today, you will isolate DNA from dried healthy potato leaves and from potato leaves infected with *P. infestans*. Thus, you will use the DNA you have isolated to perform PCR analysis to verify that the pathogen in the infected leaf was indeed *P. infestans*, the pathogen responsible for the Irish Potato Famine.

**Objectives:** By the end of this activity, you will be able to…

- Use DNA extraction techniques to isolate pathogen DNA from leaf samples.
- Amplify DNA using Polymerase Chain Reaction (PCR).
- Identify the plant pathogen (*P. infestans*) that is responsible for the Irish Potato Famine.

**Introduction**:

More than 150 years ago, the late blight pathogen *Phytophthora infestans* struck the Irish potato crop, leading to famine. Epidemics first began in North America in the 1840s. In the late summer of 1845, an epidemic swept across Europe to Ireland destroying potato crops. The Irish people were dependent on potatoes as a sole food source, and when the crop failed, millions suffered the consequences. In the aftermath of the potato crop failure over one million people perished and another two million emigrated from Ireland, with many of these "agricultural refugees" coming to the United States.

Late blight is a reemerging disease of both potato and tomato crops in the United States and abroad due to the widespread occurrences of new genotypes of the pathogen that are highly resistant to the commonly applied fungicide **metalaxyl**. Most isolates of the pathogen found today are now resistant to this fungicide. Symptoms on plants include black lesions on the stems and leaves with rapid wilting. Within three weeks an entire field can be destroyed if proper interventions are not used to prevent the spread of the pathogen. Understanding past epidemics may help us prevent destruction of crops today. In the meantime, it is important to develop assays that can specifically detect the presence of *P. infestans* in crops prior to the pathogen causing major damage. Such assays are needed to prevent movement of P. infestans in modern day potatoes that are shipped nationally and internationally and that are used as seed potatoes for new crops each year. This laboratory demonstrates one such assay.

**Student Materials: (6 Lab Setups, 1 per table group)**

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| 1 tube of 20 µl of primer (on ice) | 1 tube of 20 µl control DNA from *P. infestans* (on ice) | 1 tray of ice |
| 2 sterilized pellet pestles | 1 0.4 ml tube of 0.5 M Sodium Hydroxide (NaOH) | Latex gloves |
| 4 Ready-To-Go™ PCR beads | 1 5 ml bottle of 100 mM Tris, pH 8.0 | 1 ml of $H_20$ |
| 1 Permanent Marker & Lighter | Student Lab sheet (1 per person for portfolio project) | 1 Trash Cup |
| 1 Dried healthy potato leaf | 1 Dried infected potato leaf | 1 Forceps |
| Pipette Tips & 2 1.5µl Tubes | 2 Pipettes (1 being 1-20 µl, 1 being 20-200 µl) | 4 PCR tubes |

**Safety Considerations**: The drying process for the leaves in your lab today kills the pathogen that is present in these leaves, so it is no longer infectious. However, you will be working with 0.5 M NaOH, a very powerful base. Please wear your **safety glasses and gloves** at all times!

**Special Instructions:** It only takes a miniscule amount of DNA to contaminate PCR reactions. Therefore, avoid handling the positive control DNA until you are using it to set up the PCR reaction. In particular, make sure to extract DNA from the healthy leaf **before** you extract DNA from the infected leaf. Also, use **new tips** for your pipette any time you move between samples. This will help prevent any cross contamination of your samples.

**Instructions**: ***(Part I: DNA Extraction)***

1. Using your pair of forceps, sterilize them with your lighter. Then, remove a 2 mm in diameter piece of leaf tissue from the **healthy potato leaf** and place it in a clean 1.5 ml tube labeled **H1**.
2. Add 90 µl of 0.5 M NaOH to the tube. (Make sure you are wearing gloves and eye protection!)
3. Grind the tissue using a clean pellet pestle until it is liquefied *(approximately 1-2 minutes.)* You may see very small particulate matter still floating in the liquid. Place the used pestle into your Trash cup.
4. To a new 1.5µl tube labeled **H2**, add 300 µl of 100 mM Tris buffer, pH 8.0 (1:100 dilution).
5. Transfer 3 µl of the solution of the ground leaf (**H1**) to the second tube **H2**.

6. Cap the tube tightly and mix or shake vigorously until the tube contents are well mixed. Then place it in ice tray.
7. Repeat steps 1 through 5 using the **infected leaf sample**. (This time using the 1.5 µl tubes labeled **I1** and **I2**.) Make sure to sample from a visible area of lesion on the leaf. Also, ensure that you use a **clean pellet pestle to grind the sample and a clean pipet tip each time!** Place all discarded items into your trash cup to prevent any cross contamination.

**Instructions**: ***(Part II: PCR Analysis)***

8. Locate your 4 smaller PCR tubes on your lab bench. Add **1 Ready-To-Go™ PCR bead** to each tube.
9. Label your tubes 1, 2, 3, and 4. Then add 13 µl of sterile $H_2O$ and 4µl of primers to each tube (1, 2, 3, and 4.)
10. After adding your two reagents, transfer all tubes (1, 2, 3, and 4) to your **ice cup**. DO NOT USE the larger tubes!

**VERY IMPORTANT: Make sure to follow each of the steps below exactly!**
Add the following reagents as described below. Gently mix each by pipeting the mixture up and down with micropipette.

11. Take out **Tube 1** from your ice cup and add 8µl of sterile H2O. (This is your negative control.) Return to ice.
12. Take out **Tube 2** from your ice cup and add 8µl of diluted DNA from **healthy** potato leaf. Return to ice.
13. Take out **Tube 3** from your ice cup and add 8µl of diluted DNA from **infected** potato leaf. Return to ice.
14. Take out **Tube 4** from your ice cup and add 8µl of positive control DNA. Return to ice.

15. Once you are finished, label your cup with your group name and give to your teacher to begin PCR analysis.
16. Cleanup and reset your lab station as per your teacher's instructions.

**Analysis & Conclusions:** Using your data, answer the following questions on a **separate sheet of paper and typed**!

***You should make notes below now during lab time to refer to later when typing up your formal response.***

1. Based upon the introduction and in your own words, explain why this assay you performed is important for understanding historical plant epidemics as well as identifying current plant epidemics today.

2. Recall from the web quest, you ran a **negative** control reaction (**Tube #1**) in which no DNA was added to the reaction. What was the purpose of running this negative control reaction?

3. Recall from the web quest, you ran a **positive** control reaction (**Tube #4**) using DNA that had already been isolated from a known sample of *P. infestans*. What was the purpose of running this positive control reaction?

In order for you to answer the last two questions, please refer to the following web page and your Virtual Lab B on PCR: http://www.dnalc.org/resources/animations/pcr.html

4. In your own words, describe each of the following PCR reaction steps:

    1. The denaturing step. 2. The annealing step. 3. The extension step.

5. You have **two single stranded copies** of target sequence DNA (*the sequence you want to amplify)* at the start of a PCR reaction. Figure out how many single stranded copies of this same target sequence would have in the reaction at the end of 10 cycles in the PCR machine. *Assume that the PCR works with 100% efficiency.*

    You may wish to draw a diagram to help you, but it is not necessary.