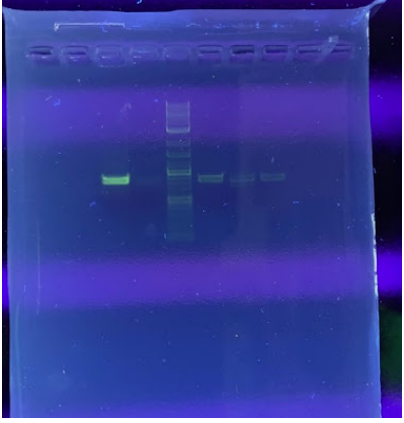
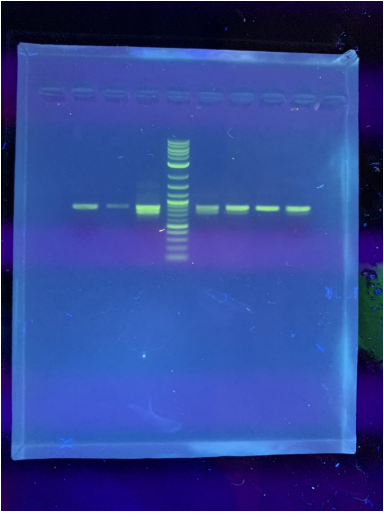
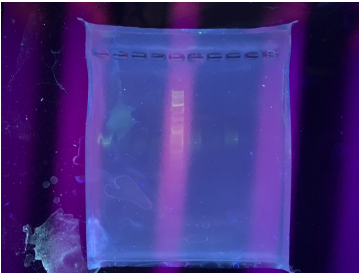
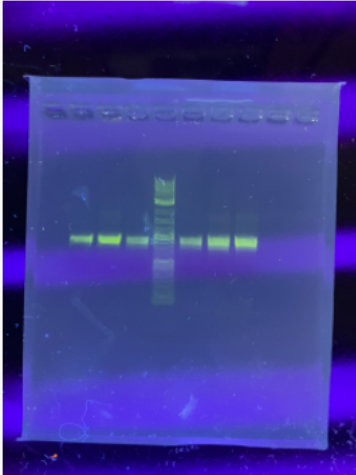
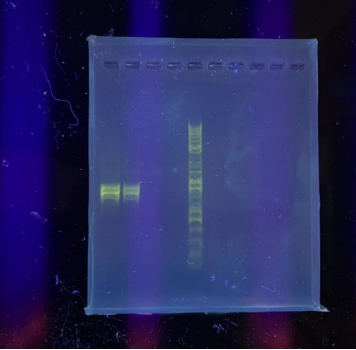
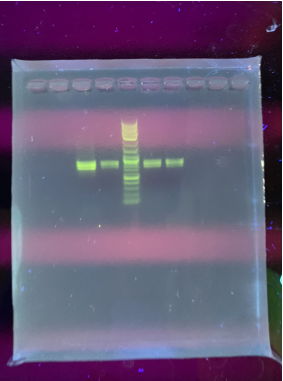
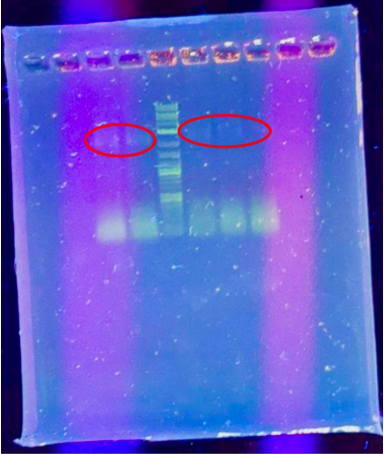
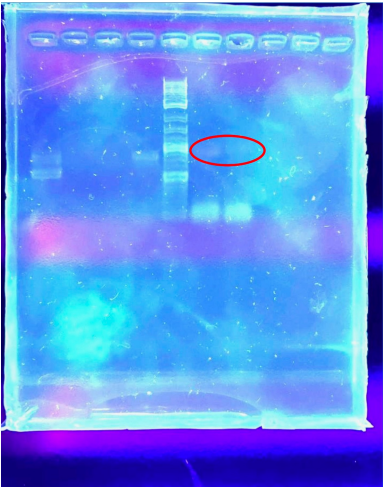


PPB Implementation Lab Notebook

| Date | Well Labeling: Note: Wells are labeled from left (1) to right (10) | Gel Picture | (Results) |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (8/16 - 8/20) | <ol style="list-style-type: none"> 1. Blank 2. Blank 3. Sink Strainer 4. Microneedle Patch 5. Ladder 6. Positive Control #1 7. Positive Control #2 8. Positive Control #3 9. Blank 10. Blank |  | <p>Results were successful, expression present for all samples, though some bands may be fainter than others.</p> <p>We decided to pursue with sink strainers based on the intensity of the sink strainer band combined with the messiness our Microneedle Stick had created, and poor nanodrop data during extraction.</p> |

| | | | |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>(8/23 - 8/27)</p> | <ol style="list-style-type: none"> 1. Blank 2. Control from first experiment* 3. Control from first experiment* 4. Without Nuclei Lysis 5. Ladder 6. Strainer 1 7. Strainer 2 8. New positive control 9. Strainer 3 10. Negative Control <p>* Old control refers to control DNA extracted in a prior week</p> |  | <p>Results were successful. Great expression (presence of bands) for all wells used in the experiment.</p> <p>Shows that nuclear lysis is not needed as a reagent in our experiment and can be replaced with water.</p> |
| <p>(8/30 - 9/3)</p> | <ol style="list-style-type: none"> 1. Positive Control 2. No TAE 3. 0.5X TAE 4. 1X TAE 5. Ladder 6. 10X TAE 7. 25X TAE 8. 50X 9. Negative Control 10. Blank |  | <p>Faint expressions for many concentrations including ladder. Could be due to usage of different types of RNase (low on reagents). Will make sure that we are more careful when conducting measurements and ensure that we have enough reagents.</p> |

| | | | |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>(9/7 -9/10)</p> | <ol style="list-style-type: none"> 1. Blank 2. Control 1 3. Control 2 4. 50X 5. Ladder 6. 25X 7. 10X 8. 1X 9. Negative Control 10. Blank |  | <p>Results were successful. Great expression (presence of bands) from all concentrations of TAE. We will now use 1X TAE for future extractions.</p> |
| <p>(9/13 - 9/17)</p> | <ol style="list-style-type: none"> 1. Original Process (PCR) 2. Original Process (PCR) Copy 3. RPA W/ RNASE & WASH 4. RPA W/o RNASE W/ WASH 5. Ladder 6. RPA W/ RNASE W/o WASH 7. RPA W/o RNASE W/o WASH 8. Positive Control 9. RPA Positive Control 10. Negative Control |  | <p>Unsuccessful results using RPA with no expression of bands</p> <p>Found that we used too small primers (20 base pairs) vs ideal RPA primers of 32-35 base pairs.</p> <p>We ordered elongated fungal primers which will arrive the week after fall break and retest with them.</p> |
| <p>(9/27 -10/1)</p> | <ol style="list-style-type: none"> 1. Blank 2. Blank 3. Original Process (PCR) 4. RPA W/ RNASE W/o WASH 5. Ladder 6. RPA W/o RNASE W/o WASH 7. Original Process (PCR) Copy 8. Negative Control 9. Blank 10. Blank |  | <p>Successful results, great expression amongst all bands, including those without RNase, and without a wash step.</p> <p>Will look to replicate this process, in the coming weeks.</p> |

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|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (10/4 -10/8) | <ol style="list-style-type: none"> 1. Blank 2. Blank 3. Yeast Positive Control (RPA) 4. Yeast Full Protocol (RPA) 5. Ladder 6. Yeast Shortened Protocol 1 (RPA) 7. Yeast Shortened Protocol 2 (RPA) 8. Yeast Shortened Protocol 3 (RPA) 9. Blank 10. Blank |  | <p>Displays the presence of very faint bands utilized for RPA. The brighter bands at the end of the gel represent extra nucleotides that weren't needed for our experimentation.</p> |
| (10/11-10/15) | <ol style="list-style-type: none"> 1. Shortened Protocol on Fusarium (PCR) 2. Shortened Protocol on Fusarium (PCR) 3. Shortened Protocol on Fusarium (PCR) 4. Fusarium Positive Control (PCR) 5. Ladder 6. Fusarium Positive Control (RPA) 7. Shortened Protocol on Fusarium (RPA) 8. Blank 9. Blank 10. Blank |  | <p>Shows that RPA was successful at amplifying genomic DNA of Fusarium, when a shortened extraction was done from infected root samples. The brighter bands at the end of the gel represent extra nucleotides that weren't needed for our experimentation.</p> |

Nanodrops:

| Name/Date | A260/280 | Concentration (ng/ul) |
|--------------------------|----------|-----------------------|
| 8/16 Sink Strainer | 2.00 | 285.5 |
| 8/16 Microneedle Patch | 0.76 | 16.9 |
| 8/16 Positive Control #1 | 1.70 | 227.1 |
| 8/16 Positive Control #2 | 2.13 | 299.0 |
| 8/16 Positive Control #3 | 1.62 | 156.9 |

| | | |
|---------------------------|------|-------|
| 8/23 Without Nuclei Lysis | 0.70 | 42.4 |
| 8/23 Strainer 1 | 1.16 | 10.2 |
| 8/23 Strainer 2 | 2.01 | 91.4 |
| 8/23 Strainer 3 | 1.63 | 30.0 |
| 8/23 New Positive Control | 1.71 | 39.2 |
| 8/30 Positive Control | 1.64 | 100.2 |
| 8/30 No TAE | 1.49 | 20.4 |
| 8/30 0.5X TAE | 1.36 | 198.7 |
| 8/30 1X TAE | 1.52 | 136.3 |
| 8/30 10X TAE | 1.49 | 23.1 |
| 8/30 25X TAE | 0.9 | 36.0 |
| 8/30 50X TAE | 0.97 | 54.7 |
| 9/7 Control 1 | 1.16 | 26.8 |
| 9/7 Control 2 | 1.64 | 471.8 |
| 9/7 50X | 1.22 | 36.4 |
| 9/7 25X | 1.97 | 25.1 |
| 9/7 10X | 1.57 | 95.2 |
| 9/7 1X | 1.62 | 47.9 |
| 9/14 1 | 1.52 | 17.1 |
| 9/14 2 | 1.69 | 39.6 |
| 9/14 3 | 1.41 | 69.0 |
| 9/14 4 | 1.60 | 145.3 |
| 9/14 5 | 1.83 | 39.7 |
| 9/14 6 | 1.83 | 94.6 |
| 9/29 + | 2.02 | 62.2 |
| 9/29 1 | 1.82 | 198.7 |
| 9/29 2 | 2.23 | 404.8 |

| | | |
|---------------------------------|------|-------|
| 9/29 3 | 0.53 | 2.8 |
| 9/29 6 | 1.92 | 119.0 |
| 9/29 7 | 2.07 | 337.4 |
| 9/29 10 | 2.02 | 497.4 |
| 10/4 Yeast Positive Control | 1.59 | 116.0 |
| 10/4 Yeast Shortened Protocol 1 | 1.60 | 189.2 |
| 10/4 Yeast Shortened Protocol 2 | 1.65 | 281.3 |
| 10/4 Yeast Shortened Protocol 3 | 1.68 | 357.4 |
| 10/14 SFUS 1 | 1.32 | 57.6 |
| 10/14 SFUS 2 | 1.37 | 69.6 |
| 10/14 SFUS RPA | 1.31 | 51.5 |