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## **Introduction:**

youPCR is a mobile DNA amplification platform that can detect various genetic markers or plant pathogens in the cannabis plant. youPCR requires a simple 55-minute hands off boil of a hole punch from a leaf and a subsequent colorimetric reaction incubation. After this process the sample visually changes color and can be captured with a mobile phone camera. This can be helpful for Y chromosome detection, Bd allele (CBDA) detection, THCA Synthase detection and pathogen detection. This protocol is to instruct how to run youPCR using the miniPCR™ mini8 or mini16 thermal cycler.

## **Materials:**

### **Supplied by Medicinal Genomics:**

Used with all assays:

- youPCR Solution A (Medicinal Genomics #420210) (Store Refrigerated, 4°C or at Room Temp)
- Leaf hole punches (included with Solution A)
- youPCR Solution C (Medicinal Genomics #420205) (Store at -20° C)
- Nuclease Free Water (Medicinal Genomics # 420184)

If running Gender Detection:

- youPCR Solution B - Gender, Medicinal Genomics #420211 (Store in freezer, -20°C)
- youPCR Gender Positive Control (Medicinal Genomics #420213) (Store in freezer, -20°C)

If running CBD Detection

- youPCR Solution B – CBD (Medicinal Genomics #420212) (Store in freezer, -20°C)
- youPCR CBD Positive Control (Medicinal Genomics #420214) (Store in freezer, -20°C)

If running Powdery Mildew Detection

- youPCR Solution B – Powdery Mildew (Medicinal Genomics #420215) (Store in freezer, -20°C)
- youPCR Powdery Mildew Positive Control (Medicinal Genomics #420216) (Store in freezer, -20°C)

If running THC Detection

- youPCR Solution B – THC (Medicinal Genomics #420217) (Store in freezer, -20°C)
- youPCR THC Positive Control (Medicinal Genomics #420218) (Store in freezer, -20°C)

If running Fusarium Detection

- youPCR Solution B – Fusarium (Medicinal Genomics #420219) (Store in freezer, -20°C)
- youPCR Fusarium Positive Control (Medicinal Genomics #420220) (Store in freezer, -20°C)

If running Russet Mite Detection

- youPCR Solution B – Russet Mite (Medicinal Genomics #420223) (Store in freezer, -20°C)
- youPCR Russet Mite Positive Control (Medicinal Genomics #420224) (Store in freezer, -20°C)

If running Botrytis Detection

- youPCR Solution B – Botrytis (Medicinal Genomics #420221) (Store in freezer, -20°C)
- youPCR Botrytis Positive Control (Medicinal Genomics #420222) (Store in freezer, -20°C)

**Materials supplied by the User:**

**(page 13 organizes materials by vendor for easier purchasing):**

- miniPCR™ mini8 thermal cycler (miniPCR™ QP-1000-01) or miniPCR™ mini16 thermal cycler (miniPCR™ QP-1016-01)
- Refrigerator, 4°C or 39°F (we recommend a different refrigerator than where food and drink are kept)
- Freezer, -20°C or 0°F (we recommend a different freezer than where food and drink are kept)
- Eppendorf tube rack (USA Scientific, #2380-1008 or similar)
- TempAssure 0.1 mL PCR 8–Tube Strips, Att. Optical Caps (USA Scientific, #1402-2300) or 0.2 ml PCR Amplitube strip tubes (VWR, #89133-910)
- 1.5mL tubes (USA Scientific #1415-2600)
- Dual rotor personal microcentrifuge, (USA Scientific #2641-0016)
- P2-20uL micropipette (USA Scientific, 7100-0220)
- P20-200 micropipette (USA Scientific, 7100-2200)
- Filtered pipette tips, 1-200 ul (USA Scientific, 200 uL TipOne filter tip, 1120-8810)
- 10% Bleach Solution
- Laboratory Gloves, (USA Scientific, # 4904-3300 or similar)
- Permanent Marker (Sharpie)
- Plant Stakes
- Toothpicks for punching out leaf punches (can use pipette tips as well)
- Waste Container

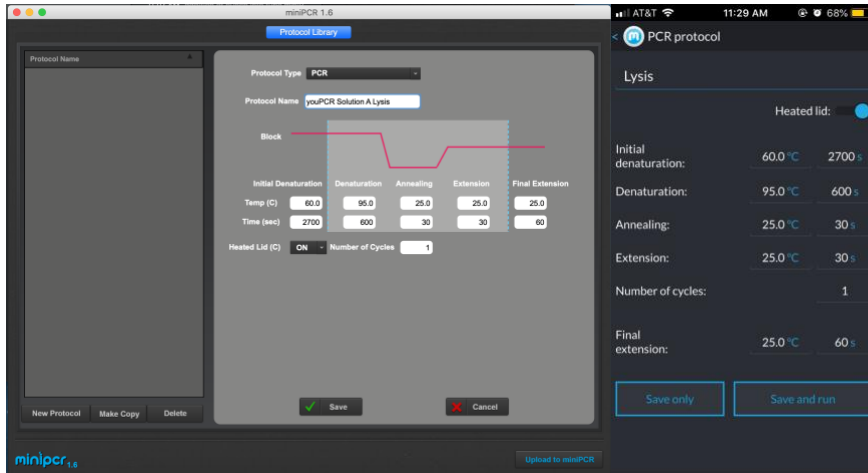
## General Instructions & Experimental Setup:

1. **READ THROUGH AND REVIEW THE ENTIRE PROCTOL/INSTRUCTION MANUAL BEFORE BEGINNING.**
2. Familiarize yourself with how to use a micropipette.
  - a. Micropipettes may be difficult to use for the first time. It will be helpful to watch the following video covering general information and instruction.
    - i. **Video Link:** [https://www.youtube.com/watch?v=uEy\\_NGDfo\\_8](https://www.youtube.com/watch?v=uEy_NGDfo_8)
3. Watch our youPCR set up video on to familiarize yourself with the process before starting: <https://youtu.be/X9SdrBXYo1g>
4. Prepare your workspace.
  - a. Before beginning any type of “laboratory” process, it is good practice to prepare your workspace and print out a copy of the Protocol/Instruction Manual.
    - i. **Start with cleaning your tabletop, centrifuge, and micropipettes with a 10% bleach solution. Allow solution to evaporate before setting up assays.**
    - ii. Place a tabletop/lab bench covering on the workspace you are planning to use.
    - iii. Arrange everything needed for use in this Protocol/Instruction Manual within reach of where you are seated.

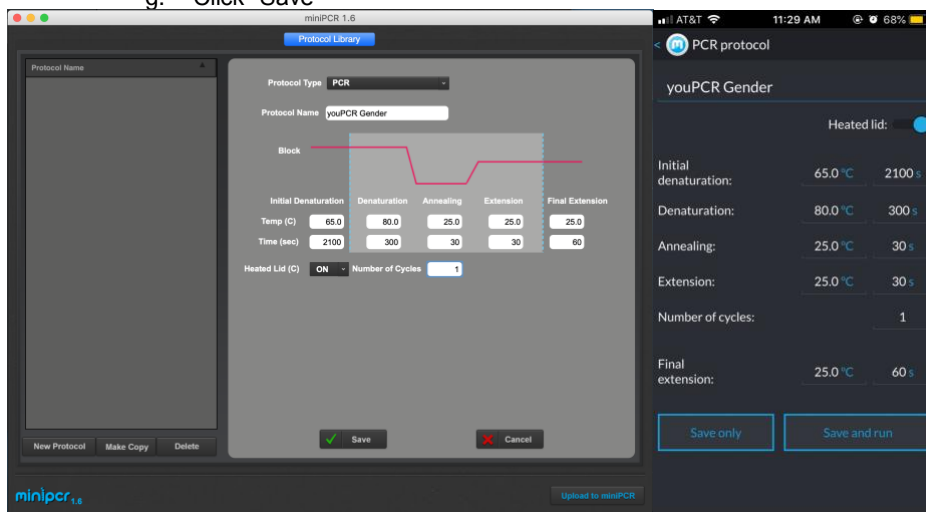


**Figure 1: Example of Bench Setup**

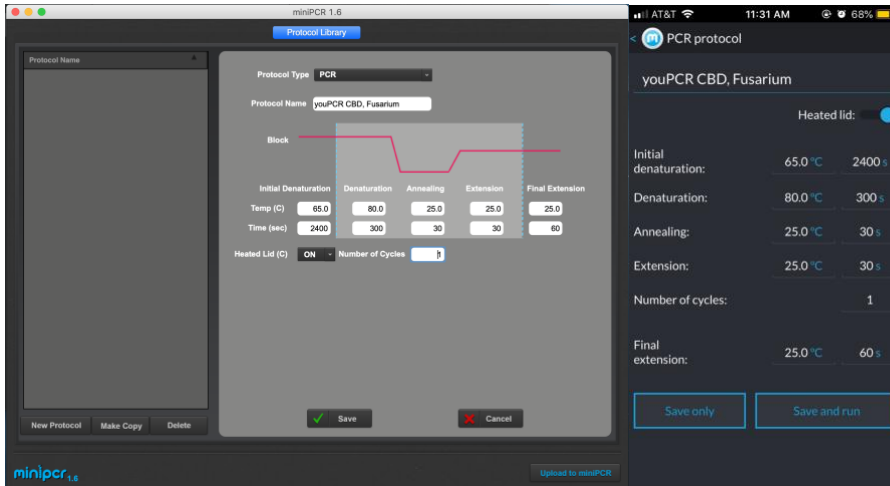
5. Prepare the miniPCR™ mini8 or mini16 thermal cycler for use.
  - a. Download Software
    - i. Download the recommended software for your computer or phone at the following web address
      1. <http://www.minipcr.com/downloads/#toggle-id-1>
    - ii. Download [the miniPCR user guide](#)
      1. [http://www.minipcr.com/wp-content/uploads/MiniPCR\\_Instruction\\_Manual\\_Revised\\_7.pdf](http://www.minipcr.com/wp-content/uploads/MiniPCR_Instruction_Manual_Revised_7.pdf)
  - b. Be certain the instrument is turned on. The software will run even if the instrument is turned off. There are LED indicators on the control board that indicate power status.
6. Program the Lysis miniPCR Protocol
  - a. Click on the New Protocol button on the lower left window. (60°C for 45 minutes, 95°C for 10 minutes)
    - i. If using the iOS or Android app tap the + icon to create a new protocol
  - b. Select on the Protocol Type as (PCR).
  - c. Enter “Lysis” in the Protocol Name field
  - d. Set the time and temperatures to match the screenshot below.
  - e. Select “ON” from the Heated Lid (C) dropdown
  - f. Enter “1” for Number of Cycles
  - g. Click “Save”



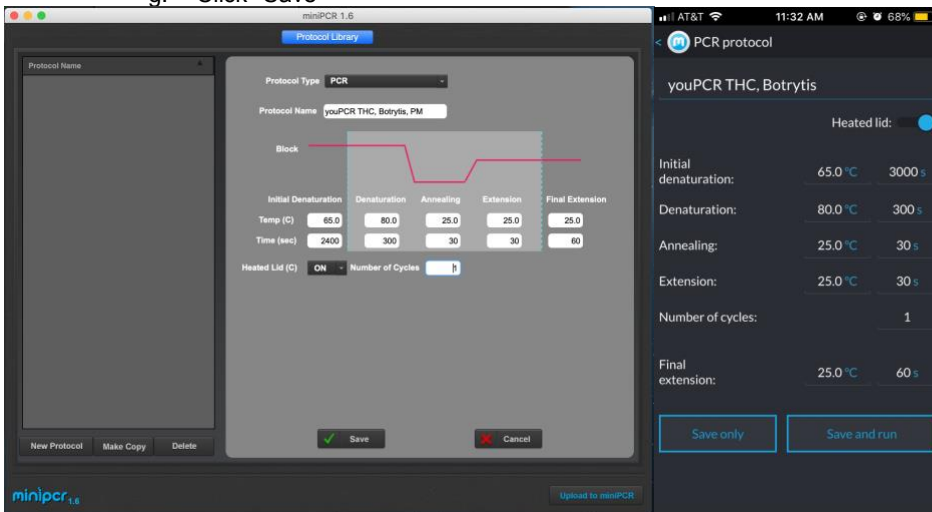
7. Program the youPCR Detection Assay Protocol for the Gender Detection test.
  - a. Click on the New Protocol button on the lower left window.
    - i. If using the iOS or Android App tap the + icon to create a new protocol.
  - b. Select on the Protocol Type as (PCR).
  - c. Enter “youPCR Gender” in the Protocol Name field
  - d. Set the time and temperatures to match the screenshot below.
  - e. Select “ON” from the Heated Lid (C) dropdown
  - f. Enter “1” for Number of Cycles
  - g. Click “Save”



8. Program the youPCR Detection Assay Protocol for the CBD and Fusarium tests.
  - a. Click on the New Protocol button on the lower left window.
  - b. Select on the Protocol Type as (PCR).
  - c. Enter “youPCR CBD\_Fusarium” in the Protocol Name field
  - d. Set the time and temperatures to match the screenshot below.
  - e. Select “ON” from the Heated Lid (C) dropdown
    - i. If using the iOS or Android app slide the Heated Lid setting to “ON”
  - f. Enter “1” for Number of Cycles
  - g. Click “Save”

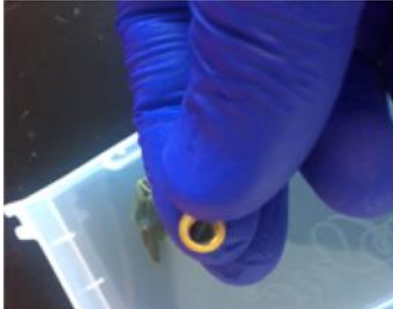


9. Program the youPCR Detection Assay Protocol for the Powdery Mildew, THC, Russet Mite and Botrytis Detection tests.
  - a. Click on the New Protocol button on the lower left window.
  - b. Select on the Protocol Type as (PCR).
  - c. Enter "youPCR Powdery Mildew\_THC\_Russet Mite\_Botrytis" in the Protocol Name field
  - d. Set the time and temperatures to match the screenshot below.
  - e. Select "ON" from the Heated Lid (C) dropdown
    - i. If using the iOS or Android app slide the Heated Lid setting to "ON"
  - f. Enter "1" for Number of Cycles
  - g. Click "Save"

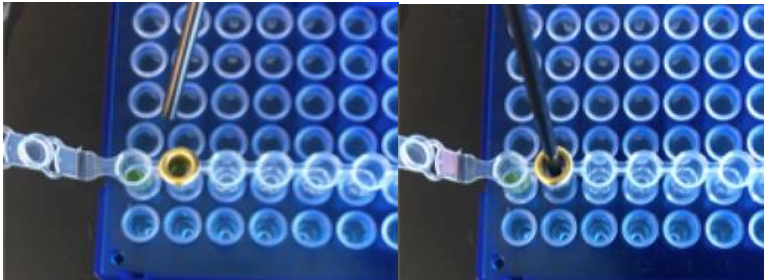


## Leaf Sampling:

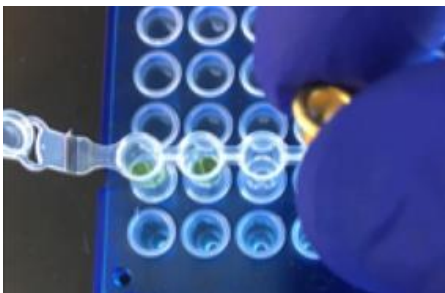
1. **READ THIS SECTION COMPLETELY BEFORE BEGINNING LEAF SAMPLING.**
2. Make sure your table or lab bench and equipment are setup and ready to begin by following the instructions on the General Instructions & Experimental Setup section of this Protocol/Instruction Manual
3. Put on a new pair of sterile gloves.
4. Place a strip tube containing pre-aliquoted youPCR Solution A onto the PCR tube rack. Briefly spin strip tube using mini-centrifuge with strip tube attachment to ensure that the youPCR Solution A is in the bottom of the wells of the strip tube.
5. Label the wells of the strip tube(s) in order to distinguish between samples, label one tube with a "-" to be used for the negative control.
  - a. The well marked (-) will **not** receive a plant sample.
6. Obtain a disposable leaf punch. Press leaf punch down into leaf on sterile surface and rotate to remove a small section.



7. Remove cap from one strip tube and place leaf punch into the top of the strip tube. Discharge leaf punch with toothpick or pipette tip. Use a fresh toothpick or pipette tip for every leaf punch.



8. Close cap of strip tube and discard leaf punch.



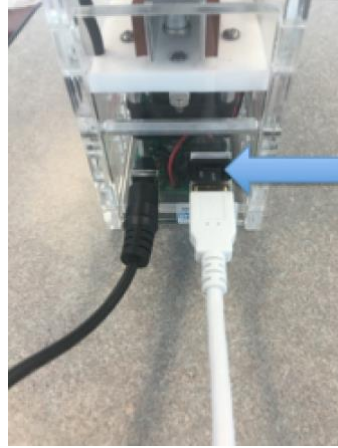
9. Repeat steps 5-8 for each sample being tested.
10. If gloves come in direct contact with leaves, change gloves between sampling.
11. Once finished with leaf sampling, spin down tubes and start youPCR lysis program on miniPCR.

## youPCR Lysis Reaction Setup:

1. **READ THIS SECTION COMPLETELY BEFORE BEGINNING THE youPCR LYSIS REACTION SETUP.**
2. Put on a new pair of sterile gloves.
3. Using the centrifuge briefly spin to ensure that the youPCR Solution A and leaf punch are in the bottom of the wells of the strip tubes.
4. Place the strip tube on the miniPCR™ mini8 thermal cycler or miniPCR™ mini16 thermal cycler, close the lid, and tighten the knob.

**Note:** Remember not to over tighten the lid by turning the knob when you feel resistance.

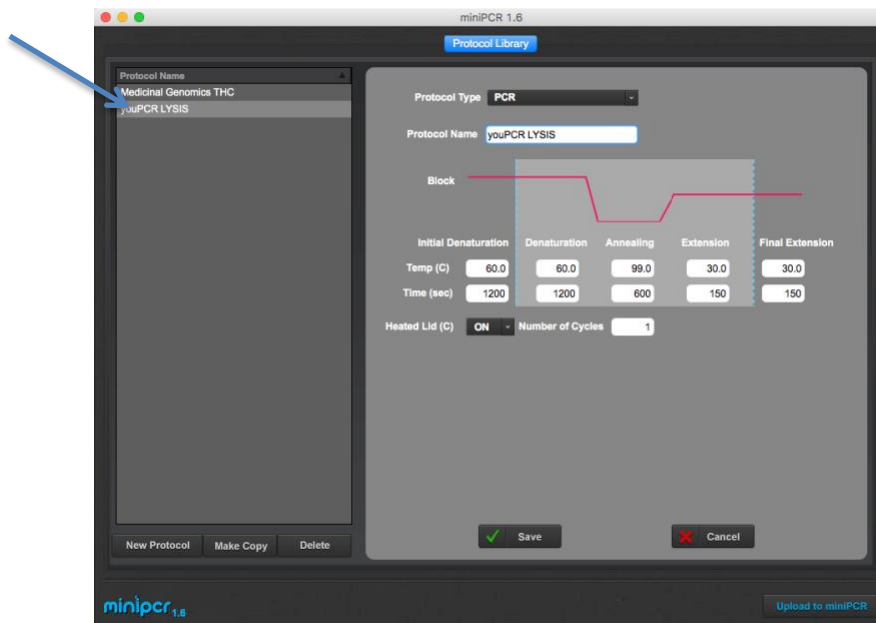
- a. Make sure that the On/Off switch is turned On. The software will run even if the instrument is turned off. There are LED indicators on the control board that indicate power status.



5. Run the pre-programmed “Lysis” Protocol.

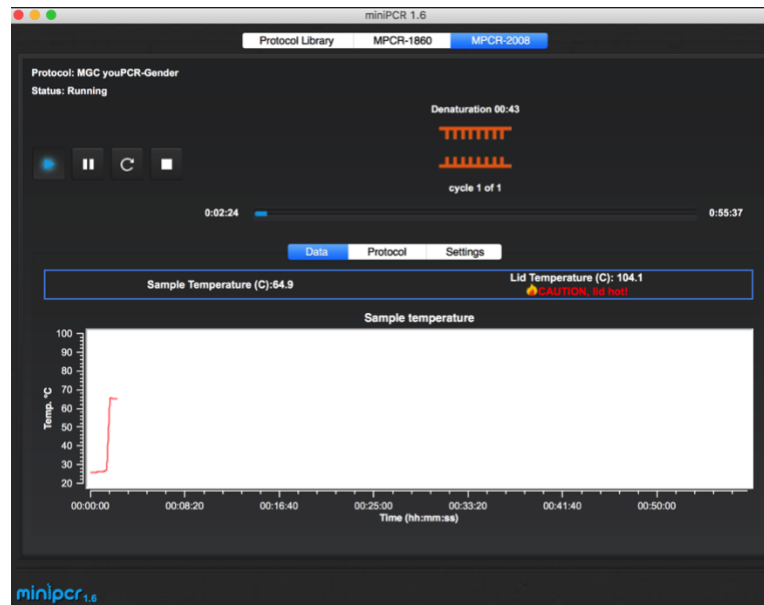
**Note:** Programing and setup instructions for the miniPCR™ mini8 or mini16 thermal cycler can be found in the General Instructions & Experimental Setup section of this Protocol/ Instruction Manual.

- a. Double click on ‘youPCR Lysis’ under the Protocol Name list to the left of the software window to start the run.

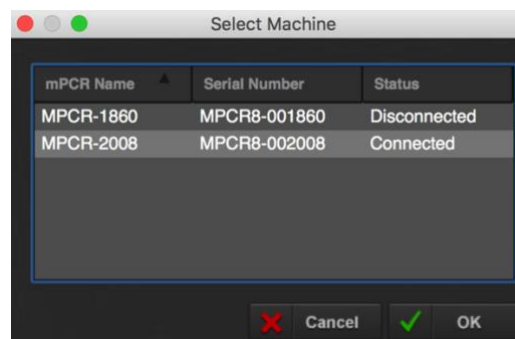


- b. If your instrument is running properly you should see a temperature chart pop open like the one below. If you need to stop the run for any reason, the Play, Pause, Replay and Stop icons are on the upper left of the screen.





- c. If you have more than one instrument plugged in, you will be shown a window like the one below to select the instrument you want to run. The number that appears should match the Serial number near the power jack on the miniPCR instrument.



6. After approximately 55 minutes when the program is complete, remove the strip tube and briefly spin using centrifuge.
7. Allow the samples to come to room temperature for 5 minutes before continuing to youPCR detection assay setup.
  - a. This strip tube now contains the plant DNA to be tested in all youPCR detection assays.

## youPCR Detection Assay Setup:

1. **READ THIS SECTION COMPLETELY BEFORE BEGINNING THE youPCR DETECTION ASSAY SETUP.**
2. Put on a new pair of sterile gloves.
3. Remove the youPCR detection assay solutions
  - a. Remove the youPCR Solution C from the freezer and allow to thaw.
    - i. **Once thawed, use immediately or place in the refrigerator until use, no longer than 30 minutes.**
    - ii. Thawing should take approximately 15-20mins and can be started 25 minutes into the youPCR Lysis program on the miniPCR™ mini8 or mini16 thermal cycler.
  - b. Remove youPCR Solution B and youPCR Positive Control from the refrigerator immediately before starting step 5.
4. Properly mix all solutions by both flicking with your finger and inverting the tube 5 times making sure the solution moves from the bottom to the top of the tube and back again.
  - a. Tap the bottom of the 1.5ml tube on the table until all liquid is at the bottom.
5. Label two empty 1.5ml tubes and one strip tube with a permanent marker.
  - a. Label one 1.5ml tube with “MM” for Master Mix.
  - b. Label one 1.5ml tube with “PC” for Positive Control.
  - c. Label the wells of the strip tube 1-6 in order, well 7 label with a “-”, and well 8 with a “+”.
    - i. Well 8(+) will get be filled in this setup.
6. Make the diluted positive control using the P20uL micropipette and the p200ul-filtered pipette tips.
  - i. Add 18ul of Nuclease Free Water to the bottom of the pre-labeled “PC” 1.5ml tube.
  - ii. Add 2ul of the youPCR Positive Control to the water in the bottom of the “PC” tube.

**Note:** If only a P20 pipette is available simply double the volumes in I and ii.

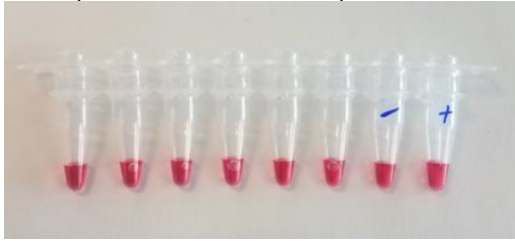
- b. Once the solutions have been added mix well by both flicking with your finger or vortexing to mix.
  - c. Tap the bottom of the 1.5ml tube on the table until all liquid is at the bottom.
7. Make the master mix using the P20-200uL micropipette and the 200ul-filtered pipette tips.
    - a. Follow the table below adding the solutions to the pre-labeled “MM” 1.5ml tube.

Reagent	1 Reaction	8 Reactions (plus excess)
Nuclease Free water	6µL	54µL
youPCR Solution B	2uL	18 µL
youPCR Solution C	10µL	90µL
Total	18µL	162µL

- b. Once the solutions have been added mix well by both flicking with your finger and inverting the tube 5 times making sure the solution moves from the bottom to the top of the tube and back again.
  - c. Tap the bottom of the 1.5ml tube on the table until all liquid is at the bottom.
8. Using a P2-20uL micropipette and the 200ul-filtered pipette tips transfer 2uL of the plant DNA from the lysis step to the “MM” in the bottom of each well. For the negative control transfer 2uL from the “-“ well to the MM in the bottom of designated negative control well of your detection assay strip tube.
    - a. Remember to match wells from the Lysis Setup strip tube to Detection Assay Setup strip tube.
    - Note:** Make sure you change pipette tips from sample to sample.
    - b. Close the plant DNA strip tube for use with another youPCR detection assay.
  9. Using a P2-20uL micropipette and the 200ul-filtered pipette tips transfer 2uL of the “PC” (+) to the designated positive control well of your Detection assay strip tube.
  10. Using a P2-20uL micropipette and the 200ul-filtered pipette tips transfer 2uL of the from well 7 of the solution A strip tube to the designated “-“ control well of your Detection assay strip tube.
  11. Use a P2-20uL micropipette and the 200ul-filtered pipette tips to add 18ul of the “MM” from step 7 to the bottom of each well of the pre-labeled Detection Assay strip tube. Pipette up and down 5-10 times to properly mix the solutions.
 

**Note:** Make sure you change pipette tips from sample to sample
  12. Close the tubes with the strip tube caps and briefly use the table-top mini centrifuge to spin down the samples.

13. At this point all the wells will be pink.



14. Place the strip tube on the miniPCR™ mini8 or mini16 thermal cycler, close the lid, and tighten the knob. Make sure that the On/Off switch is turned On.

**Note:** Remember not to over tighten the lid by turning the knob when you feel resistance.

15. Run the pre-programmed MGC youPCR Detection Assay Protocol that is appropriate for the test you are running.

**Note:** Programming and setup instructions for the miniPCR™ mini8 or mini16 thermal cycler can be found in the General Instructions & Experimental Setup section of this Protocol/ Instruction Manual.

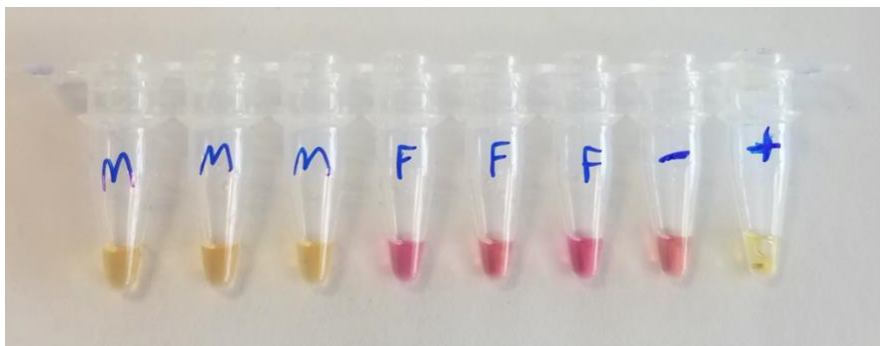
16. After the program is complete, remove the strip tube from the miniPCR™ mini8 or mini16 thermal cycler.

- a. Make sure the plate has fully cooled before reading the color change. It may be necessary to place on ice or in the refrigerator for 5-10 minutes to allow the samples to fully cool.

17. Reading the results

- a. Samples that remain the same pink color from before incubation are negative.  
b. Samples that change from pink to yellow/orange/peach are positive.  
c. The negative control should remain pink while the positive control should change to a yellow color.

**Note:** If either the negative control changes to yellow or the positive control stays pink; Results cannot be trusted. Repeat the process from the beginning.



**youPCR Troubleshooting:**

Symptom	Reason	Solution
If the Negative Control turns yellow or positive	The NTC is contaminated	Re-run the samples using fresh youPCR solution A
If the Positive Control does not turn yellow	Positive Control may not have been added. The miniPCR was not run properly. The temperature profile was not correct.	Re-run the samples making sure the positive control was properly diluted and added. Make sure the instrument is turned on and the temperature profile is correct.
Unsure if a sample is negative or positive	Pipetting error.	Re-run the samples using fresh reagents
Color change was not complete, samples have a peachy orange color	It's possible there is contaminating detergents or pH altering substances coming off the leaves	Dilute lysed samples 1:10 and run colorimetric assay again with 2 uL of the 1:10.

**Glossary and Definitions:**

**Deoxyribonucleic acid (DNA)** is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.

The **Negative Controls** are the reactions where no color change is expected. It helps to ensure that all reactions are clean of contaminants.

The assay specific **Positive Controls** are the reactions where a color change to yellow is expected. It helps ensure that all reactions are working correctly.

Cell **lysis** refers to the breaking down of the membrane of the cell, releasing all the contents of the cell. The contents can then be purified to recover only the molecules needed for future tests, in this case the DNA.

**Reagent** is a substance or mixture for use in a chemical reaction

## **Materials List by Vendor:**

### **Medicinal Genomics**

- youPCR Solution A (Medicinal Genomics #420210) (Store Refrigerated, 4°C or Room Temperature)
- youPCR Solution B - Gender, Medicinal Genomics #420211 (Store in freezer, -20°C)
- youPCR Gender Positive Control (Medicinal Genomics #420213) (Store in freezer, -20°C)
- youPCR Solution B – CBD (Medicinal Genomics #420212) (Store in freezer, -20°C)
- youPCR CBD Positive Control (Medicinal Genomics #420214) (Store in freezer, -20°C)
- youPCR Solution B – Powdery Mildew (Medicinal Genomics #420215) (Store in freezer, -20°C)
- youPCR Powdery Mildew Positive Control (Medicinal Genomics #420216) (Store in freezer, -20°C)
- youPCR Solution B – THC (Medicinal Genomics #420217) (Store in freezer, -20°C)
- youPCR THC Positive Control (Medicinal Genomics #420218) (Store in freezer, -20°C)
- youPCR Solution B – Fusarium (Medicinal Genomics #420219) (Store in freezer, -20°C)
- youPCR Fusarium Positive Control (Medicinal Genomics #420220) (Store in freezer, -20°C)
- youPCR Solution B – Russet Mite (Medicinal Genomics #420223) (Store in freezer, -20°C)
- youPCR Russet Mite Positive Control (Medicinal Genomics #420224) (Store in freezer, -20°C)
- youPCR Solution B – Botrytis (Medicinal Genomics #420221) (Store in freezer, -20°C)
- youPCR Botrytis Positive Control (Medicinal Genomics #420222) (Store in freezer, -20°C)
- youPCR Solution C (Medicinal Genomics #420205) (Store at -20° C)
- Nuclease Free Water (Medicinal Genomics # 420184)

### **miniPCR**

- mini8 thermal cycler (miniPCR™ QP-1000-01)
- mini8 thermal cycler (miniPCR™ QP-1016-01)

### **USA Scientific**

- TempAssure 0.1 mL PCR 8–Tube Strips, Att. Optical Caps (USA Scientific, #1402-2300)
- 1.5mL tubes (USA Scientific #1415-2600)
- Eppendorf tube rack (USA Scientific, #2380-1008 or similar)
- Filtered pipette tips, 1-200 ul (USA Scientific, 200 uL TipOne filter tip, 1120-8810)
- Laboratory Gloves, (USA Scientific, # 4904-3300 or similar)
- P2-20uL micropipette (USA Scientific, 7100-0220)
- P20-200 micropipette (USA Scientific, 7100-2200)

### **VWR**

- Dual rotor personal microcentrifuge, (USA Scientific #2641-0016)
- 0.2 ml PCR Amplitube strip tubes (VWR, #89133-910)

### **Other**

- Refrigerator, 4°C or 39°F (we recommend a different refrigerator than where food and drink are kept)
- Freezer, -20°C or 0°F (we recommend a different freezer than where food and drink are kept)
- Permanent Marker (Sharpie)
- Plant Stakes
- Waste Container
- Toothpicks (pipette tips can be used instead)
- Bleach to prepare 10% bleach solution

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