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Introduction:

youPCR™ is a mobile DNA amplification platform that can detect various genetic markers in the cannabis plant. youPCR requires a simple 55-minute hands off lysis of a hole punch from a leaf and a subsequent colorimetric reaction. After this process the sample visually changes color and can be captured with a mobile phone camera. This can be used for Y chromosome detection, Bd allele (CBD) detection, THC Synthase detection, and pathogen detection. This protocol describes how to run youPCR using a **96-well 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 #420225) (Store in freezer, -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)
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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)

Supplied by the User (page 11 organizes materials by vendor for easier purchasing):

- Thermal Cycler, 96-well format with a heated lid, fits skirted plates
- PlateFuge MicroPlate MicroCentrifuge (Emerald Scientific, #C2000)
- Dual rotor personal microcentrifuge, (USA Scientific #2641-0016)
- Refrigerator, 4°C (we recommend a different refrigerator than where food and drink are kept)
- Freezer, -20°C (we recommend a different freezer than where food and drink are kept)
- 96 well fully skirted plates (Fisher Scientific #AB2396)
- 96 well plate seals (USA Scientific #2978-2100)
- 1.5mL tubes (USA Scientific #1415-2600)
- 15mL Conical Tubes (USA scientific, # 1475-0511)
- Reagent Reservoirs (USA Scientific, #1930-2535)
- P2-20uL micropipette (USA Scientific, 7100-0220)
- P20-200 micropipette (USA Scientific, 7100-2200)
- P100-1000 (USA Scientific, 7110-1000)
- Multi-channel pipettes P 0.5-10µL & P 10-100µL (USA Scientific # 7031-2219 & 7031-2235)
- Filtered pipette tips, 0.5-10uL (USA Scientific, 10 uL TipOne filter tip,#1120-3810) – For use with P0.5-10 uL multichannel pipette
- Filtered pipette tips, 1-200µl (USA Scientific, 200 µL TipOne filter tip, 1120-8810)
- Filtered pipette tips, 100-1000µl (USA Scientific 1000uL TipOne filter tip - 1126-7810)
- Laboratory Gloves, (USA Scientific, # 4904-3300 or similar)
- Eppendorf tube rack (USA Scientific, #2380-1008 or similar)
- Permanent Marker (Sharpie)
- Plant Stakes
- Toothpicks for punching out leaf punches (can use pipette tips as well)
- Waste Container
- 10% Bleach Solution

General Instructions & Experimental Setup:

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1. **READ THROUGH AND REVIEW THE ENTIRE PROTOCOL/INSTRUCTION MANUAL BEFORE BEGINNING.**
2. Familiarize yourself with how to use a pipette.
 - a. Pipettes 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
3. Watch our youPCR set up video 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.



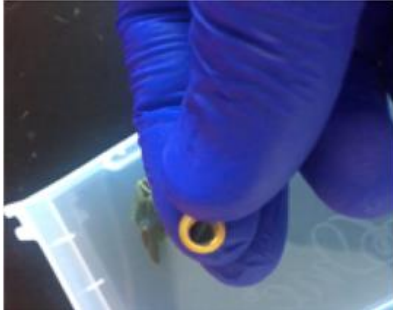
Figure 1: Example of Bench Setup

Leaf Sampling:

1. **READ THIS SECTION COMPLETELY BEFORE BEGINNING LEAF SAMPLING.**

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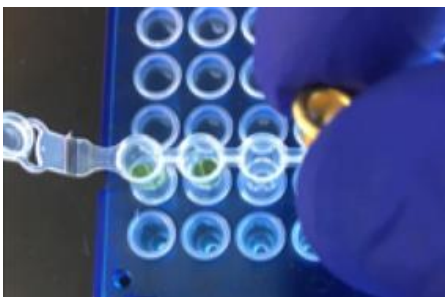
2. Make sure your table or lab bench and equipment are setup and ready to begin by following the instructions on the General Instructions & Experimental Setup section of this Protocol/Instruction Manual
3. Put on a new pair of sterile gloves.
4. Place a strip tube containing pre-aliquoted youPCR Solution 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 Solution A strip tubes being used and be sure to label one “-” for negative control.
 - a. The (-) control well 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.



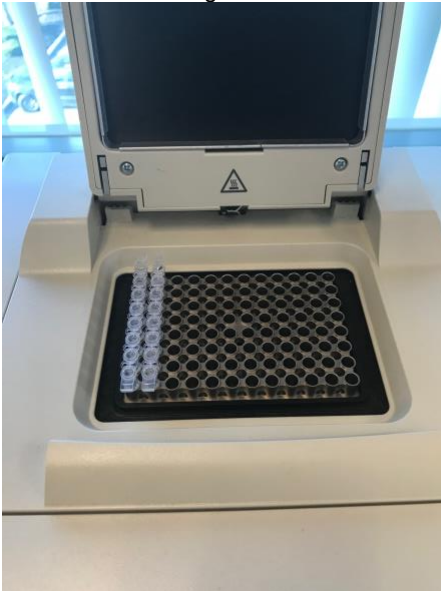
8. Close cap of strip tube and discard leaf punch.



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, run youPCR lysis on thermal cycler.

youPCR Lysis Reaction Setup:

1. **READ THIS SECTION COMPLETELY BEFORE BEGINNING THE youPCR LYSIS REACTION SETUP.**
2. Make sure all of the strip tube caps are firmly snapped into place on each tube.
3. Briefly spin all strip tubes using mini-centrifuge with strip tube attachment to ensure that the youPCR Solution A and leaf punch are in the bottom of the wells of the strip tubes. In some cases, the leaf punch may float onto top of youPCR Solution A. This is OK as long as it is in contact with the liquid.
4. Place strip tubes onto the thermal cycler.
 - a. You should have one tube containing solution A, labeled "-". This will be used for your negative control. Close the lid and tighten the knob.



5. Program the thermal cycler to do the following:
 - a. 45 minutes at 60°C
 - b. 10 minutes at 95°C*Set Heated lid to 105°C
6. After approximately 55 minutes when the program is complete, remove the strip tubes from the thermal cycler and briefly spin in mini-centrifuge with strip tube attachment to bring contents of each well to bottom.
7. **Allow the samples to come to room temperature for at least 5 minutes before continuing to youPCR detection assay setup.** The Strip tubes now contain 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, youPCR Solution B and youPCR Positive Control from the freezer and allow to thaw. Make sure you remove enough of each to process the number of samples you're running.
 - i. **Once youPCR Solution C is thawed use immediately or place in the refrigerator or on ice until ready to use, no longer than 30 minutes.**
 - ii. Thawing should take approximately 15-20 minutes and can be started 25 minutes into the youPCR Lysis program on the thermal cycler.
4. Properly mix all solutions by vortexing or inverting the tube making sure the solution moves from the bottom to the top of the tube and back again. If there is a white precipitate on the bottom of the Solution C to make sure it is fully mixed and resuspended.
 - a. Quick spin in mini-tube centrifuge to bring all liquid back to the bottom of the tube and away from the caps.
5. Label two 1.5ml tubes and a 96 well plate 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.
6. Make the diluted positive control using the P2-20µL micropipette and the 200µl-filtered pipette tips.
 - a. To make the diluted positive control you will make a 1:10 dilution.
 - i. Add **18µl** of Nuclease Free Water to the bottom of the pre-labeled "PC" 1.5ml tube.
 - ii. Add **2µl** of the youPCR Positive Control to the water already in the bottom of the tube.
 - b. Once the solutions have been added mix well by vortexing or inverting the tube 5 times
 - c. Quick spin in mini-tube centrifuge to bring all liquid back to the bottom of the tube.
7. Make the master mix using the P20-200 or P100-1000µl micropipette and the 200µl or 1000µl-filtered pipette tips depending on volumes of reagents being used.

- a. Follow the table below adding the solutions to the pre-labeled "MM" 1.5mL tube. If the total volume exceeds 1,500µL (1.5 mL) make the master mix in a 15mL Conical Tube.
- b. Include a positive and negative control in your # of reactions and enough overage to account for dead volumes in the tube or reservoir.

Solution	1 Reaction	50 Reactions (plus 10 reaction excess)	96 Reactions (plus 10 reaction excess)
Nuclease Free Water	6µL	360µL	636µL
youPCR Solution B	2µL	120µL	212µL
youPCR Solution C	10µL	600µL	1,060µL
Total	18µL	1,080µL	1,908µL

- c. Once the solutions have been added mix well by vortexing or inverting the tube 5 times making sure the solution moves from the bottom to the top of the tube and back again.
 - d. Quick spin in mini-tube centrifuge to bring all liquid back to the bottom of the tube.
8. Using a P2-20 micropipette (or P0.5-10µL multichannel) and the 200µl-filtered pipette tips (or P10µL filtered pipette tips) transfer **2µl** of the plant DNA to the bottom of each well of your 96 well plate.
 - a. When aspirating plant DNA, try not to disturb the solution A beads at the bottom of each well.
IMPORTANT: Make sure you change pipette tips from sample to sample.
 9. Transfer 2µL of your prepared positive control to the bottom of the designated positive control well in your 96 well plate.
 10. Transfer 2µL of the blank solution A "-" to the bottom of the designated negative control well to serve as the negative control in your 96 well plate.
 11. Using a P2-20µL micropipette and the P200µL-filtered pipette tips add **18µl** of the Master Mix from step 7 to the sample in each sample containing well of the pre-labeled Detection Assay 96-well plate and pipette tip mix well. Use new tips for every transfer to avoid cross contamination of samples and bubbling that can occur in the tips and cause inaccurate volume transfers.
 - a. If using multiple wells or the entire 96-well plate transfer the Master Mix to a reservoir. Using the P10-100µL multichannel pipette and the P200µL-filtered pipette tips, add **18µl** of the Master Mix from step 7 to the samples each well of the pre-labeled Detection Assay 96-well plate.

12. After transferring all lysed samples as well as the negative control and positive control DNA, store the Lysis Setup strip tubes, which contain the plant DNA at 4°C, for potential future use with another youPCR detection assay.
13. Seal the 96-well plate with the adhesive seals and briefly use the table-top mini plate centrifuge to spin down the samples in your plate.

IMPORTANT: *Be sure to seal the plate well to avoid evaporation during thermal cycling*

- a. At this point all the wells will be pink.



14. Place the 96-well plate on the thermal cycler, close the lid, and tighten the knob.
15. If using the **youPCR Gender**, Program your thermal cycler using the following conditions.
 - a. 35 minutes at 65°C
 - b. 5 minutes at 80°C
 - c. 30 minutes at 4°C or set to 4°C Forever
*Set Heated lid to 90°C
16. If using the **CBD, or Fusarium Assay**, Program your thermal cycler using the following conditions.
 - a. 40 minutes at 65°C
 - b. 5 minutes at 80°C
 - c. 30 minutes at 4°C or set to 4°C Forever
*Set Heated lid to 90°C
17. If using the **youPCR Powdery Mildew, THC, Russet Mite or Botrytis Assay** Program your thermal cycler using the following conditions.
 - a. 50 minutes at 65°C
 - b. 5 minutes at 80°C
 - c. 30 minutes at 4°C or set to 4°C Forever
*Set Heated lid to 90°C
18. When the program is complete, remove the 96-well plate from the thermal cycler.
19. Make sure the plate has fully cooled before reading the color change.
20. Reading the results
 - a. Samples that remain the same pink color from before incubation are negative.
 - b. Samples that change from pink to yellow are positive.
 - c. The negative control should remain pink while the positive control should change to a yellow color.
Note: *If the negative control changes to yellow or the positive control stays pink, assay must be repeated, as the results cannot be trusted.*



youPCR Troubleshooting:

Symptom	Reason	Solution
If the Negative Control turns yellow or positive	The NTC is contaminated Thermal cycler was not run properly or is defective.	Re-run the samples using fresh youPCR solution A. Solution A was not taken through the lysis step.
If the Positive Control does not turn yellow	Positive Control may not have been added. The thermal cycler was not run properly. Thermal cycler program set up incorrectly	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. Thermal cycler program set up incorrectly	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:

Emerald Scientific

- PlateFuge MicroPlate MicroCentrifuge (Emerald Scientific, #C2000)

Fisher Scientific

- 96 well fully skirted plates (Fisher Scientific #AB2396)

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 Solution C (Medicinal Genomics #420225) (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 #420225) (Store in freezer, -20°C)
- Nuclease Free Water (Medicinal Genomics # 420184)

USA Scientific

- Reagent Reservoirs (USA Scientific, #1930-2535)
- P2-20uL micropipette (USA Scientific, 7100-0220)
- P20-200 micropipette (USA Scientific, 7100-2200)
- P100-1000 (USA Scientific, 7110-1000)
- 1.5mL tubes (USA Scientific #1415-2600)
- 15mL Conical Tubes (USA scientific, PN 1475-0511)
- 96 well plate seals (USA Scientific #2978-2100)
- Compact PCR Tube Rack (USA Scientific, #2300-9602 or similar)
- Eppendorf tube rack (USA Scientific, #2380-1008 or similar)
- Filtered pipette tips, 2-20 ul (USA Scientific, 20 uL TipOne filter tip, 1123-1180)
- Filtered pipette tips, 20-200 ul (USA Scientific, 200 uL TipOne filter tip, 1120-8810)
- Filtered pipette tips, 100-1000ul (USA Scientific 1000uL TipOne filter tip - 1126-7810)
- Laboratory Gloves, (USA Scientific, # 4904-3300 or similar)
- Multi-channel pipettes 0.5-10uL & 10-100uL (USA Scientific# 7031-2219 & 7031-2235)
- Dual rotor personal microcentrifuge (USA Scientific #2641-0016)

Other

- Thermal Cycler, 96-well format with a heated lid
- 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
- Toothpicks (pipette tips can be used instead)
- Waste Container
- Bleach for 10% bleach solution

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