Y-SPOTTER™ *Cannabis* DNA SEX TEST

Y-spotter™
Cannabis DNA Sex Test

Version: 3.0
Release: Feb 2026

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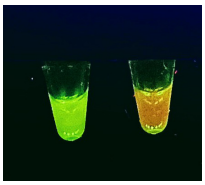
OVERVIEW

The Y-spotter™ *Cannabis* DNA Sex Test is a molecular diagnostic test intended for the detection of male *Cannabis sativa* plants. Using the Polymerase Chain Reaction (PCR), small amounts of male plant DNA can be amplified. Green fluorescence indicates presence of male DNA.

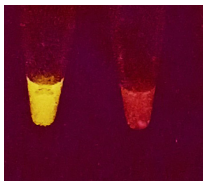
RESULT SUMMARY

Visualization device:
P51™ Molecular Fluorescence Viewer



Male Female

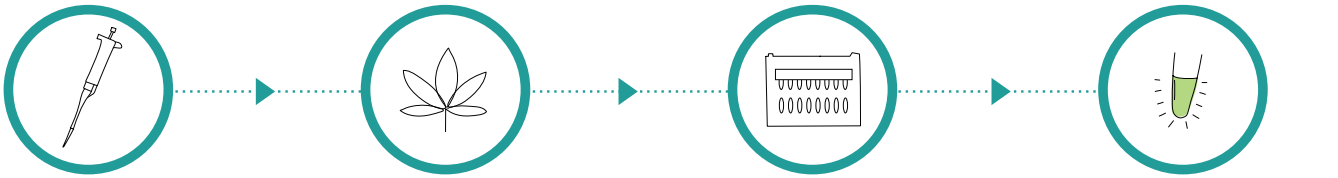
Visualization device:
blueBox™ transilluminator



Male Female

PROCEDURE SUMMARY

The test consists of four steps: reagent set up, sample collection, PCR, and fluorescence readout. The total time to process 16 samples is approximately 2 hours, with only 30 min of hands-on work.



SETUP
10 MINS

SAMPLE COLLECTION
10 MINS

PCR
90 MINS

VISUALIZATION
20 MINS

KIT COMPONENTS

The Y-Spotter™ *Cannabis* DNA Sex Test - 60 tests kit provides enough reagents for testing 60 leaves and processing 4 controls.

The Y-Spotter™ *Cannabis* DNA Sex Test - 480 tests kit provides enough reagents for testing 480 leaves and processing 32 controls.

The Y-Spotter™ *Cannabis* DNA Sex Test - 960 tests kit provides enough reagents for testing 960 leaves and processing 64 controls.

SUPPLIED IN KIT

Reagents and supplies	60 tests kit	480 tests kit	960 tests kit	Storage
2X AMPD™ Enzyme	**1 tube**	**8 tubes**	**16 tubes**	Freezer
2X CannSex™ Probe	**1 tube**	**8 tubes**	**16 tubes**	Freezer
Y-Spotter™ Positive Control	**1 tube**	**1 tube**	**2 tubes**	Freezer
PCR tubes	**8 strips**	**64 strips**	**128 strips**	Room temp.
Disposable blue punches	**1 box**	**6 boxes**	**11 boxes**	Room temp.

SUPPLIED BY USER

	Reagents and supplies	Amount needed
Available at dx.minipcr.com	**Thermal cycler** e.g., miniPCR® mini8 or mini16 thermal cycler	**1**
	Viewer P51™ Molecular Fluorescence Viewer or blueBox™ S Transilluminator with Imaging Hood	**1**
	1.5ml microcentrifuge tubes	**various**
	PCR and microcentrifuge tube racks (optional)	**1 each**
	Micropipettes · 1-10 µl (H10) · 2-20 µl (H20) · 20-200 µl (H200) · 100-1000 µl (H1000)[1]	**1 each**
	Micropipette filter tips · 1-10 µl · 2-20 µl · 20-200 µl · 100-1000 µl[1]	**2 boxes of each recommended**
	Microcentrifuge (optional)	**1**

[1] Recommended when processing more than 8 samples at a time.

Other laboratory supplies

- Disposable laboratory gloves
- Protective eyewear
- Lab coat
- Bottle or sprayer with 5% bleach (1 part bleach + 19 parts water)
- Permanent marker
- Container with lid to dispose of tubes and tips
- Parchment paper or similar to place leaves when punching

BEFORE YOU START

LABORATORY GUIDELINES

PCR is an extremely sensitive technology that can detect minute amounts of DNA. Always follow best laboratory practices to prevent contamination. This guide is a good start.

Follow these practices closely to prevent the spread of male DNA that can result in female plants being detected as male.

- Keep all tubes closed except for the one that you are actively using.
- Never open tubes after the PCR step.
- Change gloves often. If you have touched any tissue or material that contains DNA, change gloves or spray 5% bleach on them.
- Maintain a clean work area. Spray 5% bleach on work surfaces before and after every use.
- Use filter tips when preparing the reagents.
- Discard materials such as tips, tubes, and gloves in a closed container. Empty it and clean it often.
- Set up your lab in an area that is removed from possible sources of plant DNA (e.g., away from the greenhouse).
- Clean your hands and change clothes or wear a lab coat before running a test, especially if you have been in contact with plant DNA sources (e.g., pollen, leaves, and flowers).
- Keep each step of the process in separate areas:
  - Reagent preparation area
  - Leaf processing area
  - PCR area
- Avoid unnecessary traffic between areas.
- Wear different lab coats for each of the working areas.
- Label the plant, the leaf, and the tube corresponding to a given test, so you can easily identify plants after the test.
- Do not discard the leaves in case you need to repeat the test. Wrap the leaf in a slightly moist paper towel inside a zip-loc bag and store it in a fridge for up to a week. Keep each leaf in a separate bag.

Failure to follow good laboratory practices can result in contamination that will invalidate results. A contaminated laboratory is difficult to clean up.

CONTROLS

We recommend running batches of 30 leaves with one negative and one positive control.

Negative control

We strongly encourage you to run a test without a leaf sample. Fluorescence in the negative control indicates contamination with male DNA and invalidates the results of all samples tested with the same PCR mix. We recommend running one negative control tube every time you prepare reagents.

Positive control

We also encourage you to run a positive control reaction every time you prepare a PCR mix. A positive result indicates that the test was set up properly and that the PCR worked as expected.
As positive control you can use either a leaf from a known male plant, or the positive control provided with the kit.

The positive control provided in the kit contains purified male DNA and should be used carefully to prevent contamination of other tubes, equipment, and surfaces.

MICROPIPETTING

Good micropipetting skills are essential to run the test successfully. This video explains the basics.
Practice with water or colored liquids before using the reagents in this kit.
Ensure that you chose the right micropipette and tip based on the volume that you need to transfer.

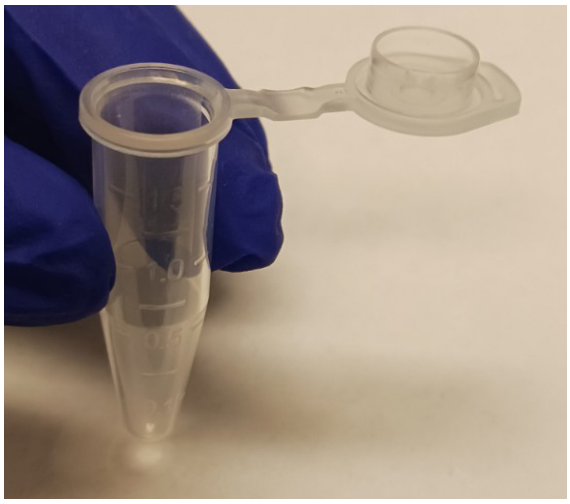
Volume Range	Micropipette		Tips
1-10 µl		**H10**	
2-20 µl		**H20**	
2-200 µl		**H200**	
100-1000 µl		**H1000**	

TUBES HANDLING AND CENTRIFUGING

You will use three types of tubes:

1. Liquid component tubes: these tubes contain the liquid components and have different labels and caps.

2. 1.5 ml microcentrifuge tubes: in this type of tube, you will prepare the PCR Mix.

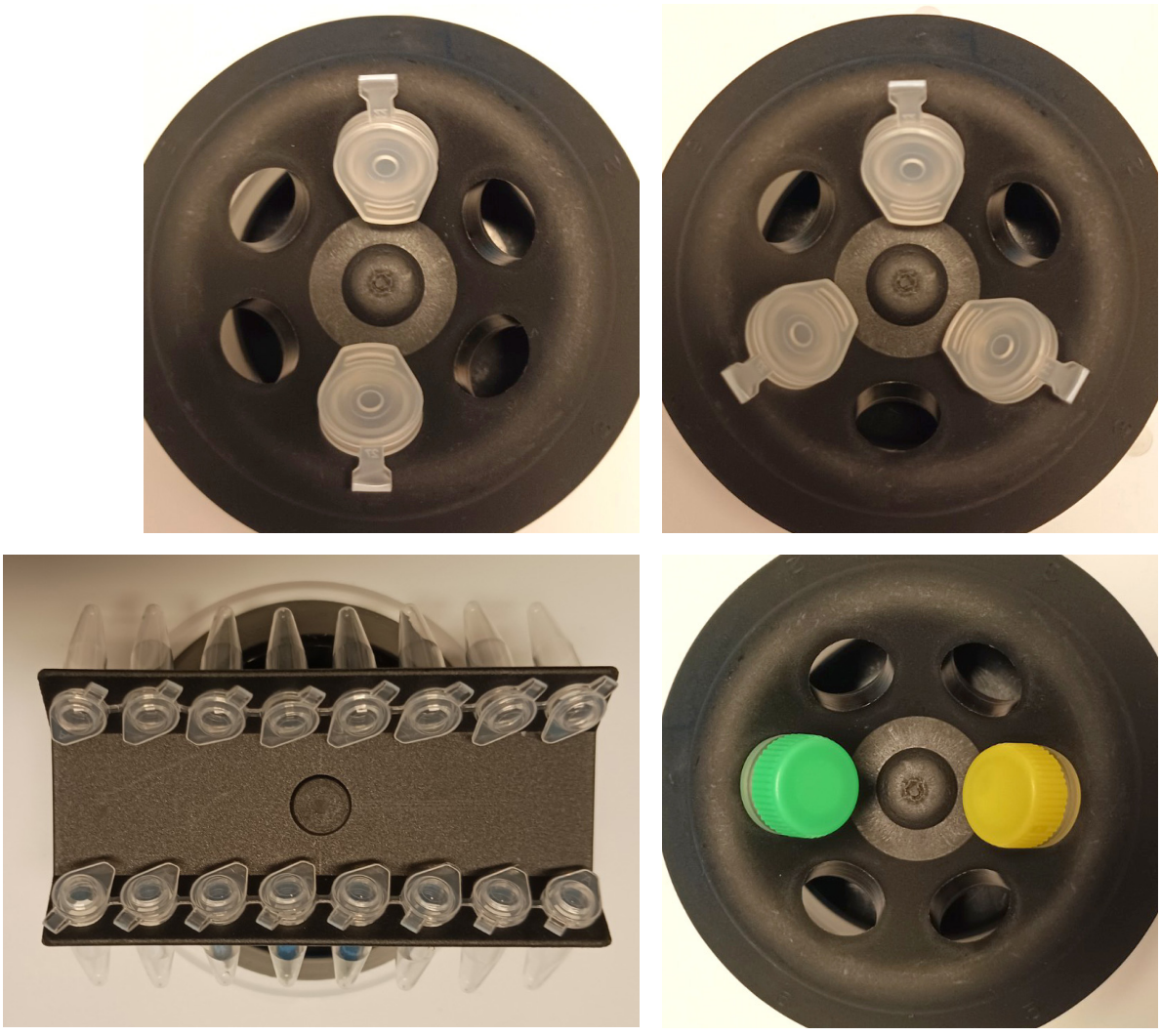


3. PCR-tubes: in these tubes you will add the PCR mix and the sample, run the PCR, and visualize the result. These tubes come as trips of 8 tubes.

For all type of tubes, to avoid contamination, always make sure that the liquid is at the bottom of the tube before opening them.

For making sure that the liquid is at the bottom of the tube, we strongly recommend to spin the tubes into the microcentrifuge. Alternatively, bring the volume down with one strong and quick flick of the wrist.

Always balance the microcentrifuge by making sure that the weight is equally distributed between the sides of the rotor. Make sure to use tubes that are containing similar volumes and have similar weight.

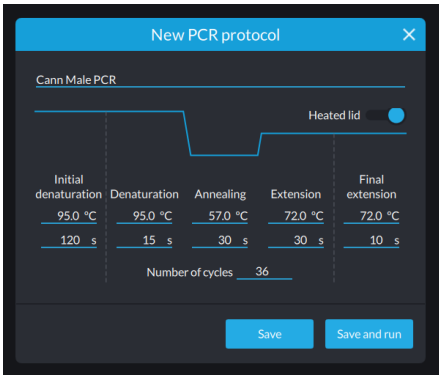


Examples of balanced microcentrifuges. Tubes match in style and contain the same volume, making their weight comparable.

PROGRAM THE MINIPCR® OR OTHER THERMAL CYCLER

This is a one-time setup. The program will be saved in the software library and can be reused. Please refer to the miniPCR® User's guide or video for details.

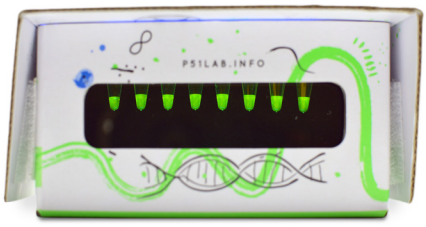
4. Click or tap on the (+) symbol on the top right corner of the app
5. Select "PCR"
6. Enter
   a. Name: "Cann Male PCR" or name of your choice
   b. Initial denaturation 95 °C, 120 sec
   c. Denaturation 95 °C, 15 sec
   d. Annealing 57 °C, 30 sec
   e. Extension 72 °C, 30 sec
   f. Number of cycles 36
   g. Final extension 72 °C, 10 sec
7. Click or tap "Save"



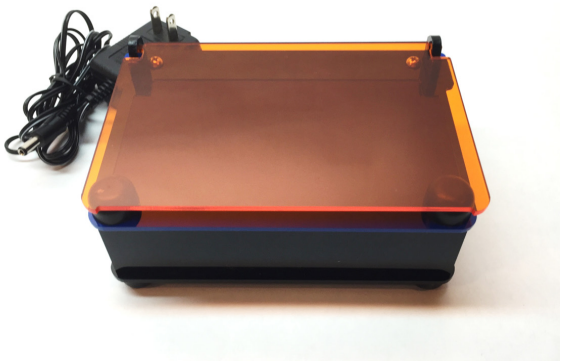
SET UP THE FLUORESCENCE VIEWER

You can use either the P51TM Molecular Fluorescence Viewer or blueBoxTM transilluminator. If using P51TM Molecular Fluorescence Viewer, please assemble the device as explained in this guide, using the orange filter.

P51™ Molecular Fluorescence Viewer



blueBox™ S Transilluminator with Imaging Hood



PROCEDURE

If desired, leaves can be collected up to a week in advance. Wrap leaves in a slightly moist paper towel inside a zip-loc bag and store it in a fridge. Keep each leaf in a separate bag.

Read the entirety of the protocol before proceeding.

STEP 1. SET UP (10 MINUTES)

"PCR mix" refers to the combination of 2X AMPD™ Enzyme (green cap) and 2X CannSex™ Probe (yellow cap).

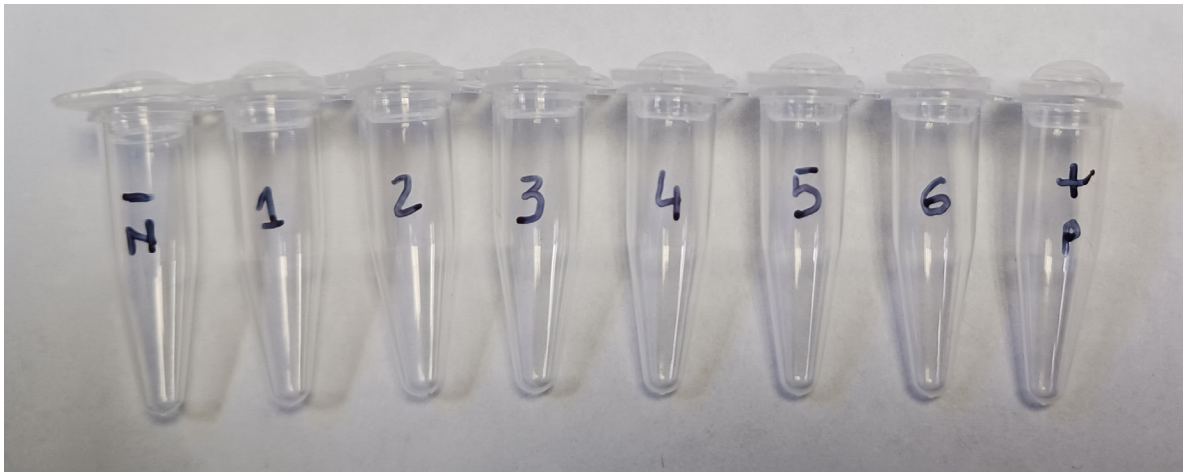
Prepare the PCR mix away from where plants are processed and PCR is performed to prevent contamination of the PCR mix with plant DNA.

When calculating the amount of reagents to prepare, take into account the number of leaves that you are testing, the negative control, and the positive control.

Leaf samples	Negative Control	Positive Control	Total tests per batch
6	1	1	8
14	1	1	16
30	1	1	32

1. Thaw the 2X AMPD™ Enzyme and the 2X CannSex™ Probe at room temperature.

2. Prepare tubes:
   a. You will need one tube per test and controls.
   b. Label tubes with a sample ID on the upper side wall (not on the lid).
   c. Place tubes for the negative and positive controls as far apart from each other as possible. For example, use position 1 for the negative control and position 8 for the positive control of a tube strip.



Example of labelled PCR tubes.
The negative control tube (labelled as " – N") is in position 1, while the positive control tube (labelled as " + P") is in position 8.

3. Calculate the amount of the 2X AMPD™ Enzyme (green cap) and of the 2X CannSex™ Probe (yellow cap) needed:
   a. Each test requires 25 µl of the PCR mix.
   b. Some liquid may be lost when pipetting multiple times. Use the tables below to prepare the appropriate reagent volumes. The calculations account for a 5% loss.

Table 1. Reagent volumes for commonly used test quantities.

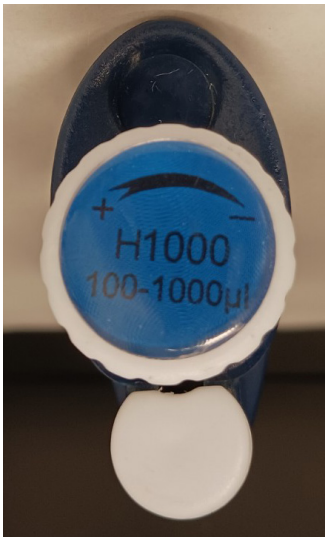
Reagent	Total tests and controls			
	8	**16**	**32**	**64**
2X AMPD™ Enzyme (µl)	105	210	420	840
2X CannSex™ Probe (µl)	105	210	420	840
Total PCR mix (µl)	210	420	840	1680

Table 2. Calculate the volume of PCR mix for any number of samples.

Reagent	Volume for 1 test		Total tests and controls		Total volume
2X AMPD™ Enzyme	13.125 µl	x		=	..... µl
2X CannSex™ Probe	13.125 µl	x		=	..... µl
				Total	..... µl

Note

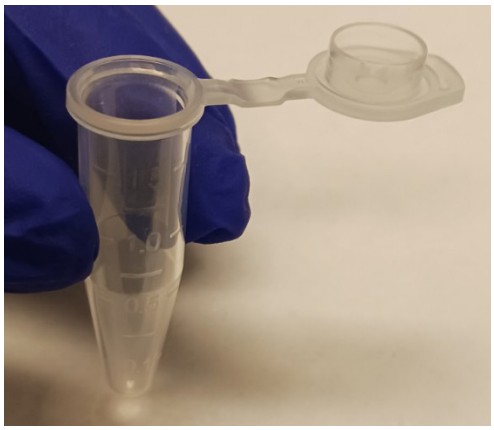
Use a H1000 pipette when preparing PCR mix for 16 or more samples. If not available, pipette multiple times with an H200 pipette. Do not fill 1.5 ml microcentrifuge tubes with more than 1.2 ml of liquid to prevent spilling when mixing the reagents. 64 or more tests will need more than one microcentrifuge tube.



Example of a H1000 pipette set to 420 µl.

Example of a H200 pipette set to 105 µl.

4. Add the calculated volumes to a 1.5 ml microcentrifuge tube and mix well by pipetting gently up and down four or five times. Be careful not to introduce air bubbles.



Example of 1.5 ml tube, where the components of the PCR mix will be mixed.

Optional: Briefly spin the tubes in a microcentrifuge to bring all liquid to the bottom and eliminate bubbles. Remember to balance the microcentrifuge.

5. Open all PCR tubes.
6. Add 25 µl of the PCR mix to each tube using the H200 pipette and the appropriate filter tip.

Example of a H200 pipette set to 25 µl.

7. Close all tubes. Make sure that all the liquid is at the bottom of the tubes.

Optional: Briefly spin the tubes in a microcentrifuge to bring all liquid to the bottom and eliminate bubbles. Remember to balance the microcentrifuge.

Reminder: bring the 2X AMPD™ Enzyme (green cap) and the 2X CannSex™ Probe (yellow cap) back to the freezer.

STEP 2. SAMPLE COLLECTION (APPROX. 1 MINUTE PER SAMPLE)

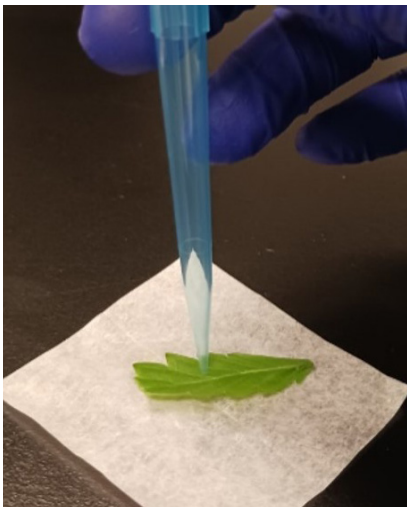
In this step, you will add a small leaf piece to tubes containing the PCR mix.

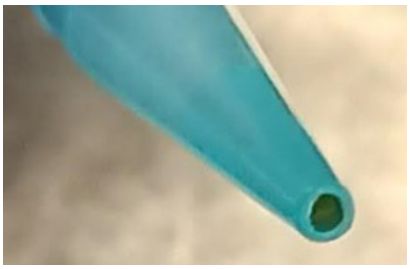
Open only one tube at a time to avoid contamination.

1. Place the number of blue punches that you will need (one per sample) on a clean paper towel or surface. Close and store the punch box away from the working area.
2. Open the negative control tube.

The tube containing the negative control should always be the first tube to be handled.

3. Place a clean punch into the tube and mix by gently swirling it for about 10 seconds. Make sure that the punch is in contact with the liquid. Hold the punch with your hand.
4. Close the tube.
5. Discard the punch in a waste container.
6. Place the first leaf on a piece of parchment paper.
7. Use a clean punch to press firmly onto the leaf and rotate a quarter turn clock and counter-clockwise three or four times.
8. Confirm that you collected a piece of leaf. You should see a hole in the leaf and a little green piece in the punch.





9. Open the corresponding tube.
10. Place the punch into the tube gently swirling in the liquid for about 10 seconds. Avoid tapping the punch.

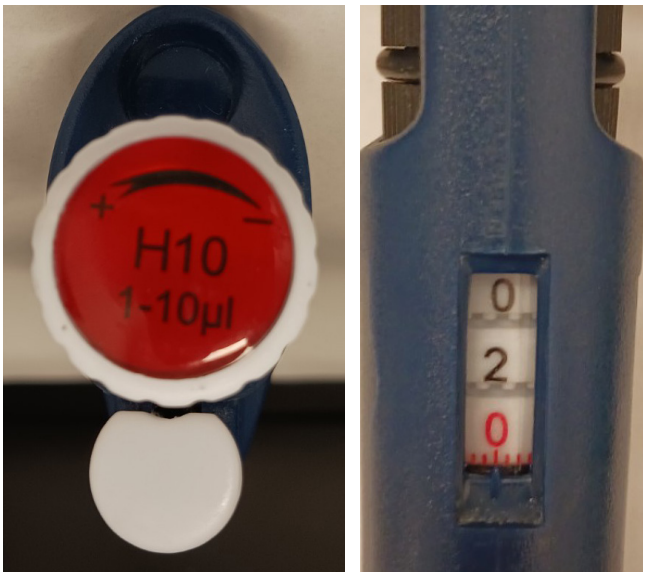
The leaf disc should remain in the punch. If the disc falls into the PCR mix, the result might be difficult to interpret.

11. Close the tube.
12. Discard the punch in a waste container.
13. Remove the processed leaf and parchment paper from the working area.
14. Clean the surface and your gloves with 5% bleach.
15. Move on to the next samples by repeating steps 7-14.
16. Ensure all caps of the PCR strips are fully closed. Inspect them individually.
17. If using the Y-Spotter™ Positive Control (clear cap) instead of a known male leaf, thaw the tube at room temperature. The Y-Spotter™ Positive Control tube should always be the last one to be processed.

Y-spotter™ Positive Control tube.

18. Tap the tube gently on the bench or briefly spin it in a micro centrifuge to ensure that the liquid is at the bottom.

19. Open the tube and the PCR tube assigned as positive control. Using a H10 or a H20 micropipette with a filter tip, transfer 2 µl of Y-spotter™ Positive Control into the assigned tube.



Example of a H10 pipette set to 2 µl.

Example of a H20 pipette set to 2 µl.

20. Discard the tip.
21. Close the Y-spotter™ Positive Control and PCR tubes.
22. Make sure that all the liquid in the PCR tubes is at the bottom.

Example of PCR tubes ready for the PCR step.
Tubes 1 to 7 contain 25 µl of PCR mix.
Tube 8 (positive control, "+ P") contains 27 µl (25 µl of PCR mix plus 2 µl of Y-spotter™ positive control).

Bring the Y-spotter™ Positive Control tube (clear cap) back to the freezer.

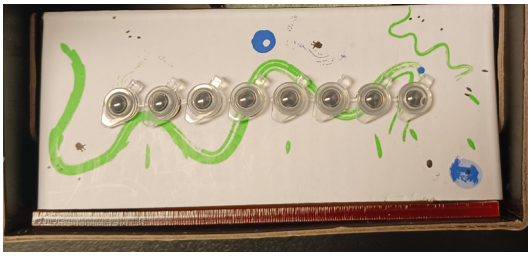
STEP 3. PCR (90 MINUTES)

1. Place the tubes or strips in the miniPCR® thermal cycler. Close the lid and tighten the knob (if available).
2. Start the Cann Male PCR program from the miniPCR® app and let it run to completion.

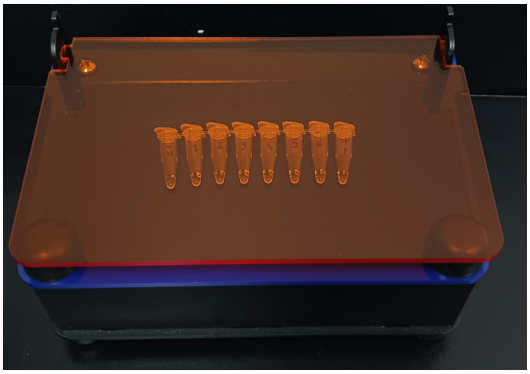
STEP 4. VISUALIZATION AND DOCUMENTATION (20 MINUTES)

1. Remove the tubes from the miniPCR® thermal cycler and place them inside the P51™ Molecular Fluorescence Viewer with the orange filter or in a blueBox™ S Transilluminator with Imaging Hood (place the samples on the blue surface and cover them with the orange filter).
2. Turn the illumination on (blue light will appear).
3. Leave the samples inside the device with the blue light turned on for 15 minutes.
4. If using P51™ Molecular Fluorescence Viewer, dim the ambient light and observe the samples. If using or blueBox™ S Transilluminator, cover it with the Imaging Hood and observe the samples.

   Optional: take a picture to keep as reference.



If using the P51™ Molecular Fluorescence Viewer, place the samples inside the holder and close the cover flaps. Switch on the blue light and leave the samples in the device for 15 minutes. Finally, dim the room lights, observe the samples, and take a picture with your phone.



If using blueBox S Transilluminator with Imaging Hood, place the samples on the blue surface, under the orange filter. Switch on the blue light and leave the samples in the device for 15 minutes. Then, cover the system with the Imaging Hood, observe the samples, and take a picture with your phone.

Note:

Do not rely solely on pictures to interpret results. Phone cameras may enhance or diminish fluorescence signals and can make it difficult to interpret results. We recommend manually adjusting the phone camera's brightness and contrast to maximize the difference between positive and negative controls while taking the picture.

EXPECTED RESULTS

Positive control: bright green fluorescence.
Negative control: no fluorescence.
Male plants: bright green/yellow fluorescence.
Female plants: no color, very dim opaque green, or red-pink fluorescence. Red or pink fluorescence does not indicate a positive result.

All results are invalid if the positive control does not fluoresce and/or the negative control fluoresces.

If the leaf disc fell into the tube, the result of only that specific reaction might be difficult to interpret, as the chlorophyll contained in the leaf disc can mask the test fluorescence.

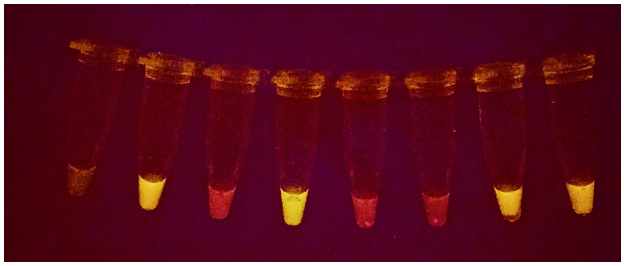
RESULT EXAMPLE

Tube 1 Negative control (N)
Tube 2, 4, 7 male plants (M)
Tubes 3, 5, 6 female plants (F)
Tubes 8 positive control (P)

P51™ Molecular Fluorescence Viewer

N M F M F F M P

blueBox™ S Transilluminator with Imaging Hood



N M F M F F M P

Please contact dx@minipcr.com or call +1-781-990-8727 if you have any questions. We are happy to help.