Cannabis Sex qPCR Detection Test

Version: 1.0
Release: April 2025
©2025 by miniPCR Dx™

CONTENTS

OVERVIEW

The *Cannabis* Sex qPCR Detection Test is a molecular test used to determine the sex of *Cannabis* plants. It is designed to simultaneously detect both a male-specific gene and an internal control gene (present in both female and male plants) from a DNA sample extracted from a leaf.

The reliability of the results is contingent upon meticulous sample collection and strict adherence to the provided protocol.

PROTOCOL AT A GLANCE

The test consists of the following steps:

Step		Time (approx.)
1	Sample collection	3 min per sample
2	Lysis setup	5 min
3	Sample preparation	3 min per sample
4	Lysis	100 min (hands-off)
5	qPCR setup	30 min
6	qPCR	100 min (hands off)
	*Optional stopping point: once you complete the qPCR run and save the file, you can proceed with data analysis at a later time*	
7	Result analysis	15 min

KIT COMPONENTS

Catalog number	Tests	Controls
DX-0422-02	42	6
DX-0422-04	84	12

SUPPLIED IN KIT

Reagents and Supplies	DX-0421-02	DX-0421-04	Storage
Enzyme A1	1 tube	2 tubes	Freezer
CannSex™ Probe Q1	1 tube	2 tubes	Freezer
HLVd Positive Control 1	1 tube	1 tube	Freezer
DEB™ Extraction Buffer	1 tube	2 tubes	Freezer
Punches	1 box	1 box	Room temp.
PCR tubes	1 bag	2 bags	Room temp.

SUPPLIED BY USER

Equipment

All items are available at https://dx.minipcr.com.
If you're starting your lab, we recommend the qPCR Bundle (QP-2510-50).

Item	Quantity	Recommended product	Cat. Number
Real time thermal cycler	1	q16 thermal cycler	QP-1100-01
Micropipettes	1 each	1-10 µl H-style	QP-1001-05
		2-20 µl H-style	QP-1001-01
		100-1000 µl H-style	QP-1002-02
		4 µl fixed volume minipette	QP-1003-01
		20 µl fixed volume minipette	QP-1003-03
Microcentrifuge tube rack	1	Microcentrifuge tube rack, 60-well	CM-1003-10
PCR tube rack	2	0.1 ml PCR tube rack - 96 wells	CM-1003-11
Microcentrifuge	1	Gyro™ Microcentrifuge, fixed speed	QP-1800-01

Consumables

All consumables are one-time use.

Item	Quantity	Recommended product	Cat. Number
Micropipette filter tips	2 boxes each (sufficient for processing up to 190 samples)	1-10 µl	4AA75
		2-20 µl	4AA76
		20-200 µl	4AA77
		100-1000 µl	4AA78
Microcentrifuge tubes	1 tube per run	Microcentrifuge tubes 1.5 ml	6AA02

Other common supplies

- Disposable laboratory gloves
- Protective eyewear
- Lab coat
- 2 Bottles or sprayers with bleach solution (prepare freshly by mixing 1 part household bleach +1 9 parts water)
- 1 Bottle or sprayer with distilled water
- 2 Fine point permanent markers
- 3 containers with lid to dispose of used tubes and tips
- Parchment paper or similar wax paper
- Tissue paper
- Labels to identify plants
- Zip-lock bags
- Disposable tweezers to help handle samples

BEFORE YOU START

LABORATORY GUIDELINES

qPCR is an extremely sensitive technique that can detect minute amounts of DNA. Always follow the practices outlined below to minimize the risk of contamination.

- Set up your lab in an area that is removed from possible sources of plant DNA (*e.g.*, away from the greenhouse area).
- Keep each step of the process in three separate areas and avoid unnecessary trafficking between them.

	Reagents area	**Sampling area**	**qPCR area**
Purpose	Preparing extraction buffer and master mix	Preparing samples. Transferring lysates and positive control to master mix	Running the lysis and the qPCR test Data analysis
Precautions	No plant material or completed PCR should be brought to this area.	Material exposed to the Sampling area should not be returned to the Reagents area. Carefully clean surfaces and clean or change gloves in between samples when collecting leaves.	Discard the tubes without opening them after the qPCR step.
Materials	*Reagents* DEB™ Extraction Buffer Enzyme A1 CannSex™ Probe Q1 *Equipment* 20-200 µl and 100-1000 µl micropipettes 20 µl fixed volume minipette Marker Tube rack Trash container Bottle with bleach solution *Consumables* Tissue paper Gloves 20 µl, 200 µl, and 1000 µl filter tips PCR and 1.5 ml tubes	*Reagents* CannSex™ Positive Control 1 *Equipment* Microcentrifuge 1-10 µl micropipette 4 µl fixed volume minipette Marker Tube rack Trash container Bottle with bleach solution Bottle with distilled water *Consumables* Tissue paper Gloves 10 µl and 20 µl filter tips Punches Parchment paper Disposable tweezers Tissue paper	*Reagents* No reagents *Equipment* q16 thermal cycler USB Drive USB extension cable Windows or Mac computer Trash container *Consumables* A few empty PCR tubes (they can be reused)

BEST PRACTICES

- Clean your hands and wear a clean lab coat before running a test, especially after contact with any plants or plant parts.
- Maintain clean work areas. Spray freshly prepared bleach solution (1 part bleach + 19 parts water) on work surfaces before and after every use.
- Always use filter tips. Do not use the tips included with the minipettes as those don't have filters.
- Change gloves between samples, or spray them with the bleach solution.
- Wear a clean lab coat when preparing the reagents.
- Discard materials such as tips, tubes, and gloves in a container that can be closed. Empty it often and clean it with a bleach solution.
- Keep all tubes closed except for the one that you are actively using.

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

CONTROLS

Positive and negative controls are mandatory.

To ensure accurate results and proper interpretation, each run **must include** both a negative and a positive control. Omitting these controls will hinder the software''s ability to automatically analyze and interpret the data.

Negative control (mandatory)

The negative control will always be assigned to position 1 in the q16 thermal cycler. Follow manufacturer recommendations for other qPCR thermal cyclers.
Amplification in the negative control indicates contamination and invalidates the results of the test batch.

Positive control (mandatory)

The positive control will always be assigned to position 2 in the q16 thermal cycler. Follow manufacturer recommendations for other qPCR thermal cyclers.

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.

Handle with care to avoid contaminating other tubes, equipment, and surfaces. Make sure the liquid is thawed and at the bottom of the tube before opening the tube. Contamination with the positive control will result in false positive tests.

Amplification in the positive control indicates the test is working as expected but does not preclude the possibility of contamination.

q16 THERMAL CYCLER

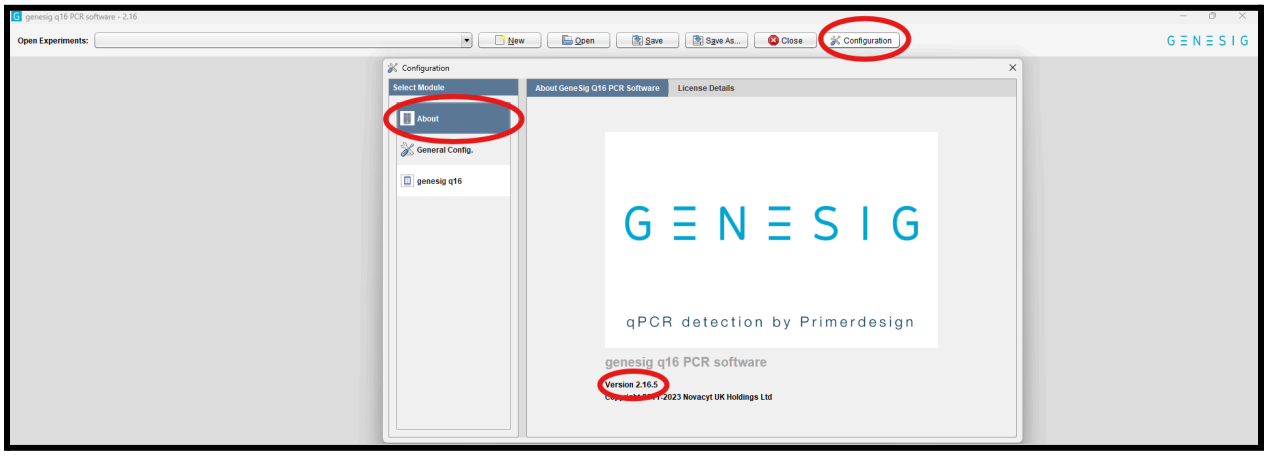
Read the q16 user's guide before proceeding. This is a quick guideline.

q16 software version 2.16.5 & q16 user's guide

Install the software version 2.16.5

- **The software version 2.16.5 is available on our website (dx.minipcr.com)**
- **The USB Drive provided with your q16 thermal cycler contains software for Windows and MacOS operating systems**. Please open the software file matching your chosen operating system.
    - *Windows:* Double-click on the Windows installer and follow the on-screen instructions to install your genesig® q16 software on Windows.
    - *MacOS:* Double-click on the disk image and follow the on-screen instructions to install your genesig®q16 software on your Mac computer.

IMPORTANT: make sure to install version 2.16.5 of the software. Previous versions are not compatible with this test. You can verify the installed version by clicking under Configuration/About.



PROCEDURE

Please read these instructions completely before continuing.

SETUP

Prepare sufficient reagents for screening all plants, plus two additional tests: one for the negative control and one for the positive control.

Keep track of the plant and samples ID during the entire process. Refer to the tables below for recommended sample labeling and the necessary number of tubes.

Suggested sample labeling

Plant ID	Lysis Tube ID	qPCR Tube ID	Default name on software
Not Applicable	1	1	Negative control
Not Applicable	No lysis tube needed	2	Positive control
Plant A	3	3	Sample 1
Plant B	4	4	Sample 2
.....	....	.....	.....
Plant N	16	16	Sample 14

Tubes needed for a full q16 run

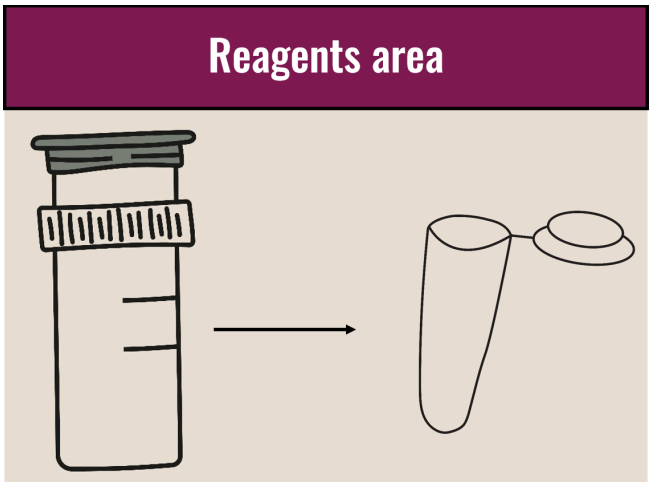
Reactions	Tubes Needed	
14 Tests 1 Negative control 1 Positive control	15 for lysis	16 for PCR

STEP 1: LEAVES COLLECTION

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.

1. Prepare the necessary material to collect the samples:
    a. Gloves
    b. Marker
    c. Plant labels
    d. Zip-lock bags
    e. Disposable tweezers (optional)
2. Label the plant with a unique ID.
3. Take a new zip-lock bag and label it with the same sample ID.
4. Collect the leaf sample and store it into a zip-lock bag.
    a. If you are going to execute the test immediately, store the sample in a zip-lock bag.
    b. Alternatively, wrap the leaf in a slightly moist paper towel inside a zip-loc bag and store it in a fridge. If desired, leaves can be collected up to a week in advance.
5. Change gloves or clean them with bleach between samples. Keep each leaf in a separate bag.
6. Repeat steps 1-5 for all your samples.

STEP 2. LYSIS SETUP



Once all samples are collected, proceed with the test as soon as possible.

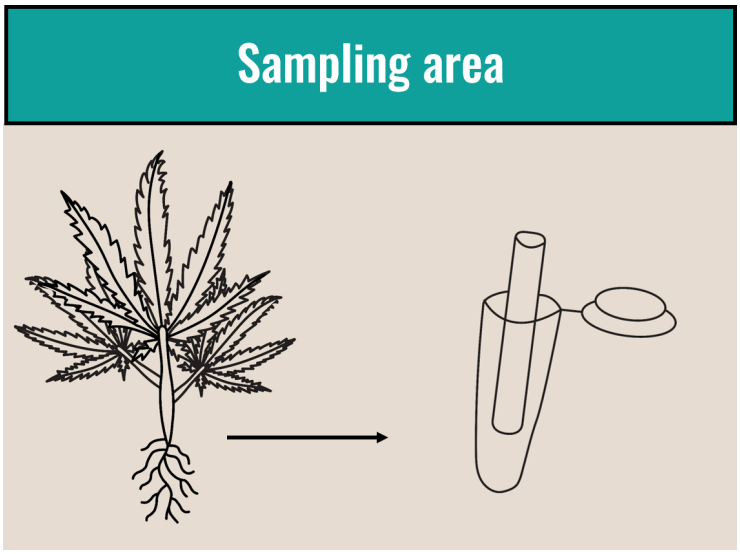
Prepare the DEB™ Extraction Buffer in the reagents area, away from where plant samples are processed and the qPCR is run to prevent contamination of the reagents with plant DNA or the positive control.

1. Wipe all surfaces with freshly prepared bleach solution.
2. Thaw the DEB™ Extraction Buffer at room temperature. Add **50 µl of DEB™ Extraction Buffer to each PCR tube.** You will need one PCR tube per plant sample and one tube for the negative control.
3. Label the tubes **on the top** with the assigned ID.

Sample	Lysis Tube ID
Negative control	1
Positive control	No lysis tube needed
Plant A	3
Plant B	4
.....	....
Plant N	16

4. Close all caps.
5. Any remaining DEB™ Extraction Buffer can be stored at room temperature.

STEP 3. SAMPLE PREPARATION



All sample preparation steps should be carried out in the dedicated sampling area.

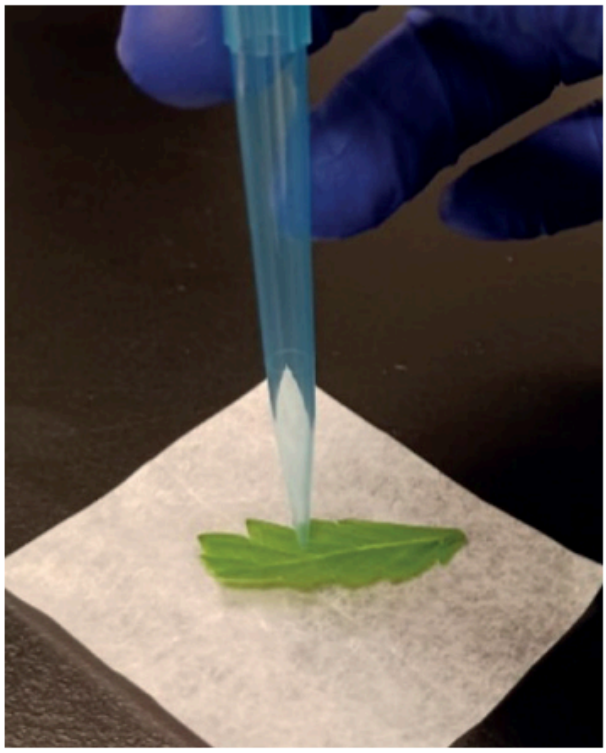
It is crucial to carefully clean the surfaces with the bleach solution before and after collecting the samples, and to clean or change gloves in between samples. This will prevent cross-contamination between samples.

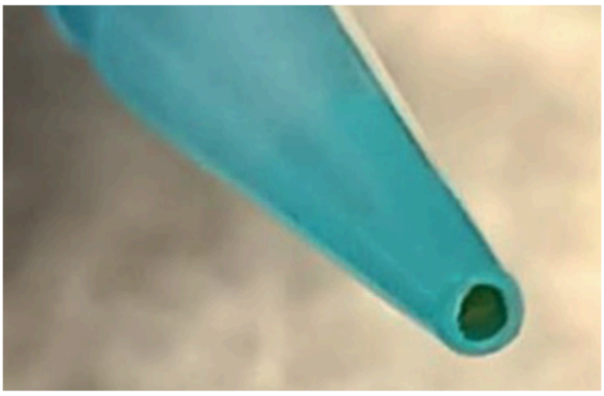
1. Prepare the materials to process the samples:
    a. Parchment paper
    b. Punches
    c. Tweezers (optional)

2. First, process the negative control tube.

The negative control tube should always be the first tube to be handled and should be closed before any other sample is processed.

3. Open tube labeled as “1” and insert a clean punch into the tube containing DEB buffer. 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. Discard the punch and close the tube. **Press strongly, the lid might be hard to close.** Make sure the cap is evenly seated on the rim of the tube.
5. Place the first leaf on a piece of parchment paper.
6. Use a clean punch to press firmly onto the leaf and rotate a quarter turn clock and counterclockwise three or four times.
7. Confirm that you collected a piece of leaf. You should see a hole in the leaf and a little green piece in the punch.



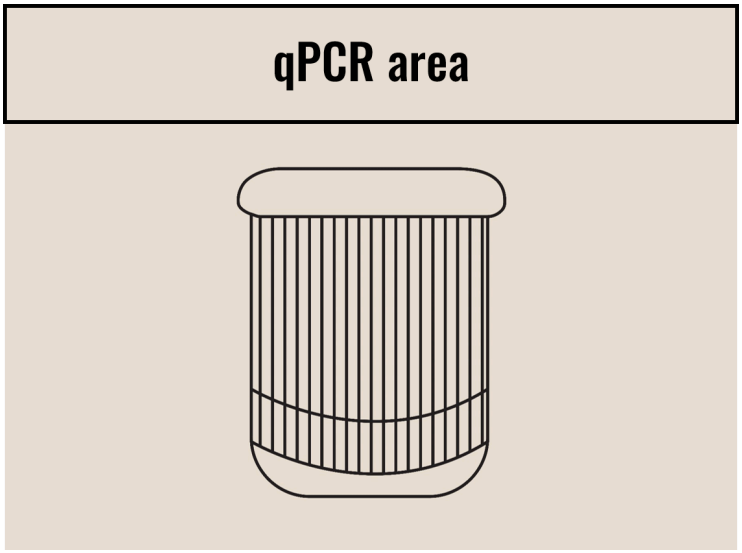


8. Open the corresponding tube.
9. Place the punch into the tube gently swirling in the liquid for about 10 seconds. Avoid tapping the punch.

The leaf disc should remain in the punch.

10. Discard the punch in a waste container and close the tube.
11. Remove the processed leaf and parchment paper from the working area.
12. Clean the surface and your gloves with 5% bleach.
13. Move on to the next samples by repeating steps 5-12.
14. Ensure all caps of the PCR tubes are fully closed. Inspect them individually.
15. Proceed to clean the roots and collect the samples.

STEP 4. LYSIS

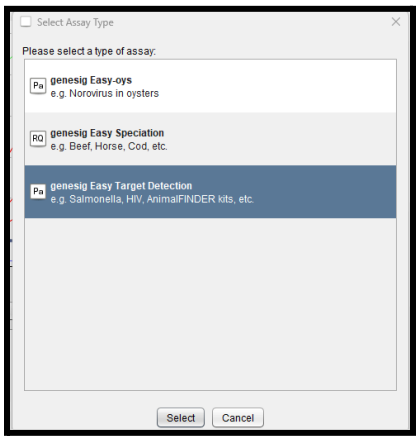


During this step you will extract DNA from the leaves using the q16 thermal cycler.

Please, read the genesig® q16 user's guide before operating the q16 thermal cycler.

1. Connect the q16 thermal cycler to the power supply, then plug the power supply into an electrical outlet. The machine will make an audible announcement and the LED will display a blue static light to signal that the machine is on. There is no on/off switch.
2. Connect also the USB adaptor cable to the bottom of the q16 thermal cycler.
3. Connect the USB Drive to your computer.
4. Open the q16 app on your computer.
5. Select "New".

6. Select "genesig Easy Target Detection".

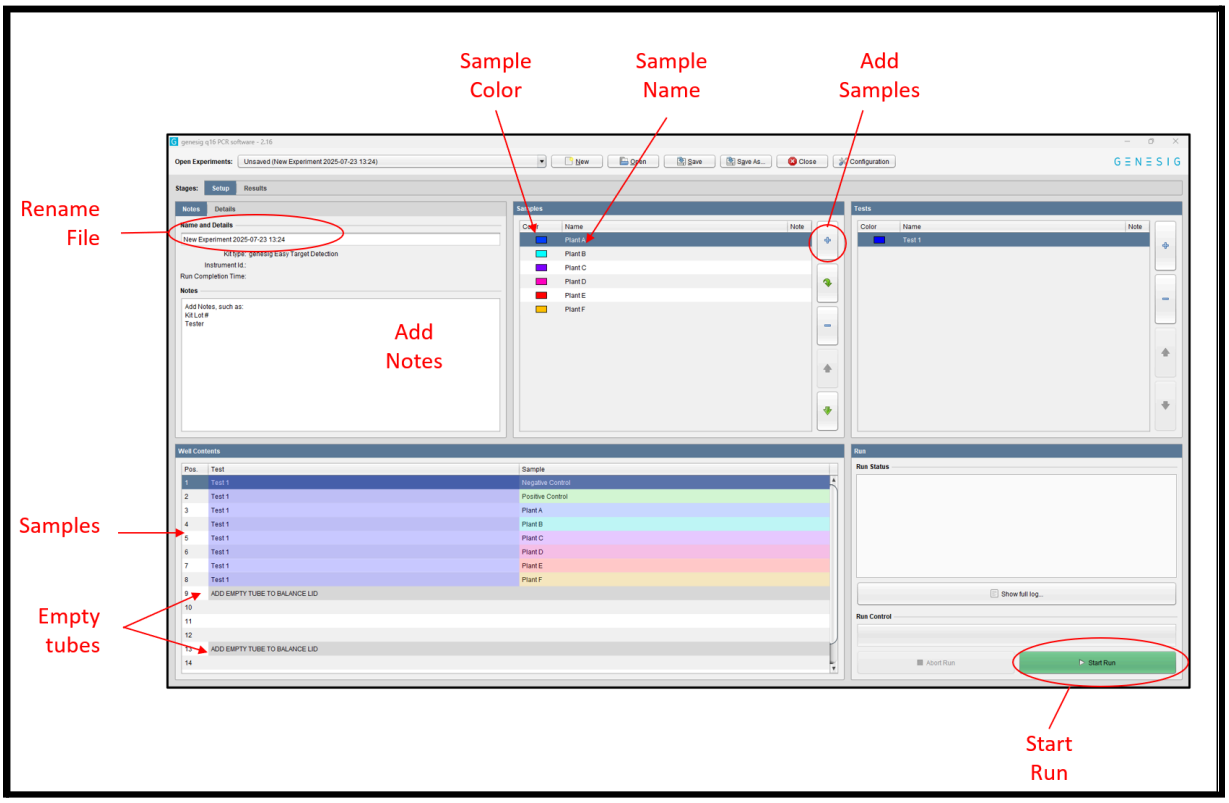


7. Rename the Experiment as you prefer.
8. Under “Samples” select “+” and add the number of samples based on your run.

   Notes:
   -The negative and positive control will be automatically assigned to position 1 and 2, respectively. **At this point, you will not run a positive control.**
   -The software will highlight where to add “empty” tubes, to make sure the lid will properly close.

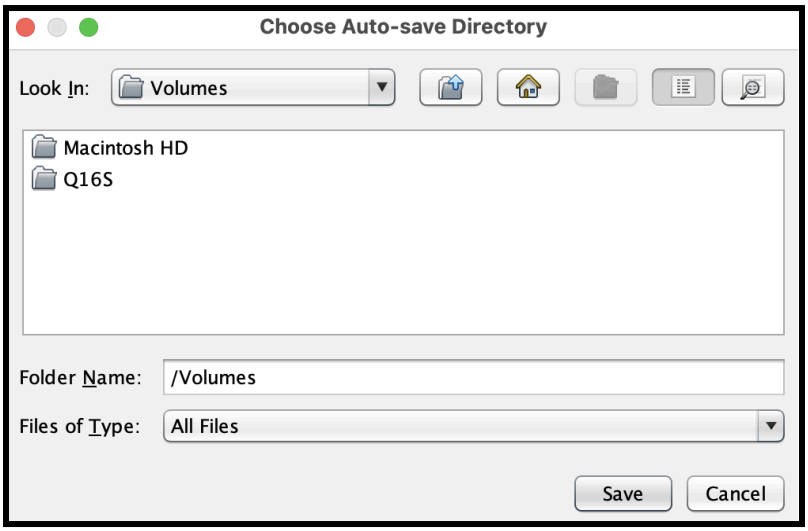
   Optional: rename the samples and change color by selecting the sample and typing in the box and/or selecting the relative box color.

9. Click on “Start Run”. Note that the q16 thermal cycler will not yet start the program.

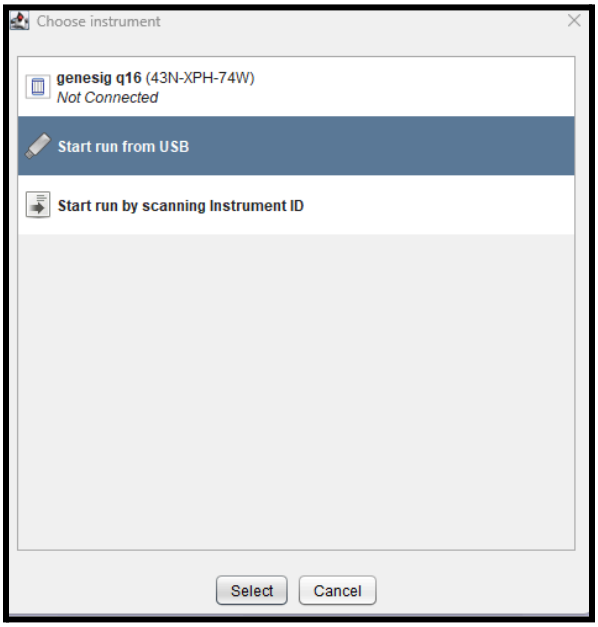


10. Select the *USB drive* as the directory for saving the data and click on “Ok”.

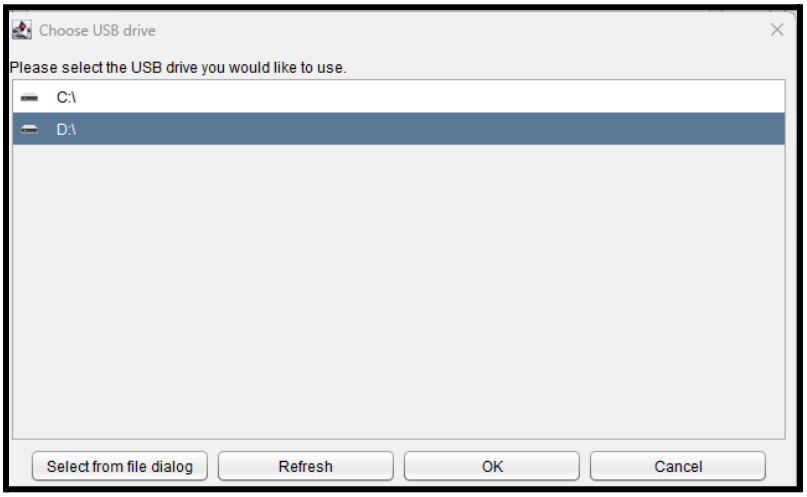
On a Mac, navigate to Volumes and select the name of the USB Drive.



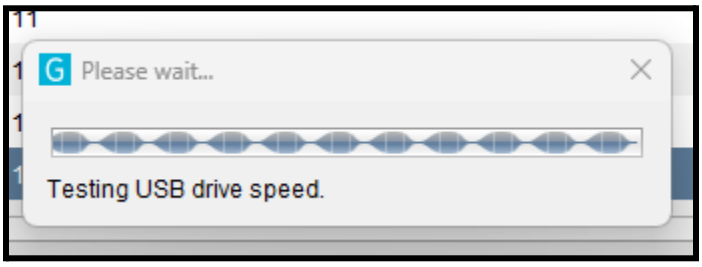
11. Select “Start run from USB” and click on “Select”.



12. Select the USB Drive and click “OK”.



13. The software will test the USB Drive. Once the test is correctly completed, remove the USB Drive from your computer.

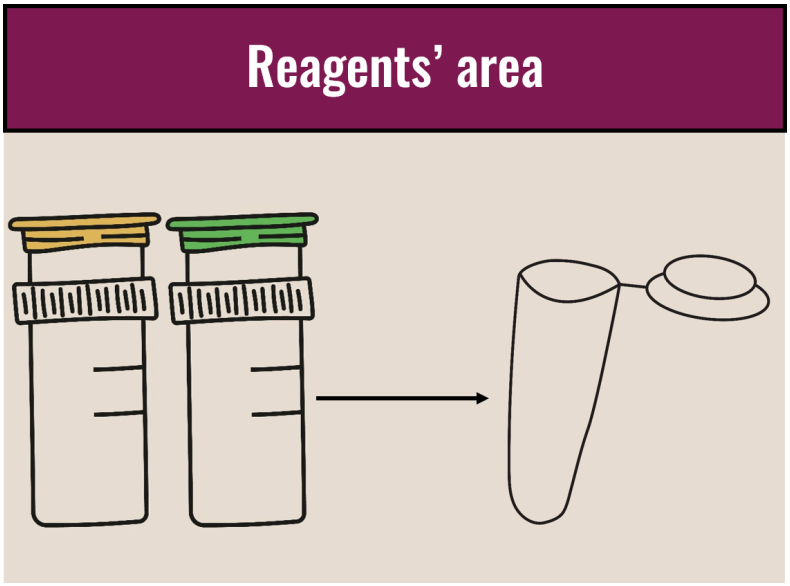


Note: If the software detected the USB Drive as too slow to load the program, try again repeating steps 10-13.

14. **To open the q16, push the lid down and twist a quarter turn anti-clockwise, then lift.**
15. **Load the samples in the assigned positions, making sure to add an empty tube in position 2 and to other positions assigned by the software, if needed. Close the lid.**
16. **Plug the USB Drive into the USB connector cable.**
17. When you start the run, the machine will make an audible announcement and the LED will turn green. The LED's color will change throughout the run, which takes approximately 1 hour and 40 minutes. A circling rainbow light pattern indicates that the program is completed. **We recommend preparing the qPCR reactions (STEP 5. qPCR SETUP) approximately 30 min before the run is completed.**

Important note: No data will be analyzed from this Lysis step.

STEP 5. qPCR SETUP



In this step, you will prepare the master mix in the reagents area. You will need the Enzyme A1, the CannSex Probe Q1, and the PCR tubes.

1. Wipe all surfaces with freshly prepared cleaning solution.
2. Thaw the provided reagents (Enzyme A1 and CannSex Probe Q1) at room temperature.
3. Prepare the master mix by following the instructions in the table below.
   -These calculations account for loss of volume during pipetting. You may have a small amount of liquid left in the tube.
   -The total number of reactions is the sum of the tests, the negative, and the positive controls.

Reagent	Volume for 1 reaction (µl)	Volume for 8 reactions (µl)	Volume for 16 reactions (µl)
Enzyme A1	12.5	100	200
CannSex Probe Q1	12.5	100	200
Total volume	**25**	**200**	**400**

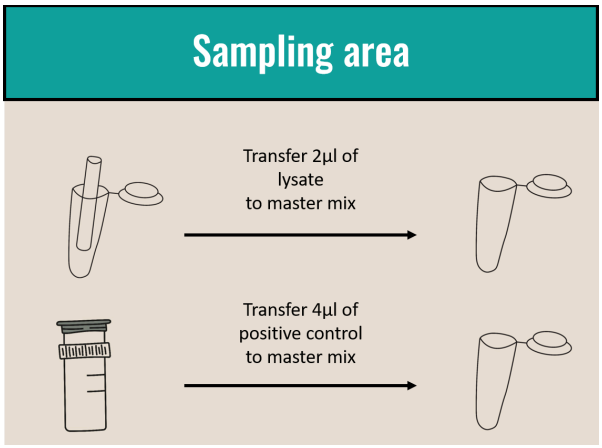
4. Add the reagents to a 1.5 ml microcentrifuge tube. Gently mix up and down until the solution is well mixed.
5. Put the Enzyme A1 and CannSex Probe Q1 back in the freezer.
6. Add **23 µl** of the qPCR mix to each PCR tube. There is no need to change tips at this time.
7. Close the tubes.

8. Label tubes with the proper ID on the top.

Test	PCR Tube ID
Negative control	1
Positive control	2
Sample 1	3
Sample 2	4
.....	....
Sample 14	16

9. Move the tubes to the sampling area.

STEP 6. qPCR



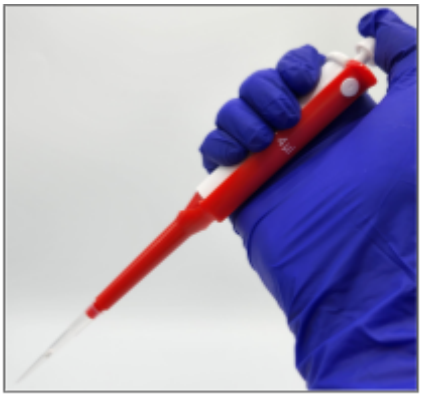
In this step, you will add the extracted DNA to the qPCR mix and run the qPCR in the q16 thermal cycler.

Transfer the lysates and the positive control

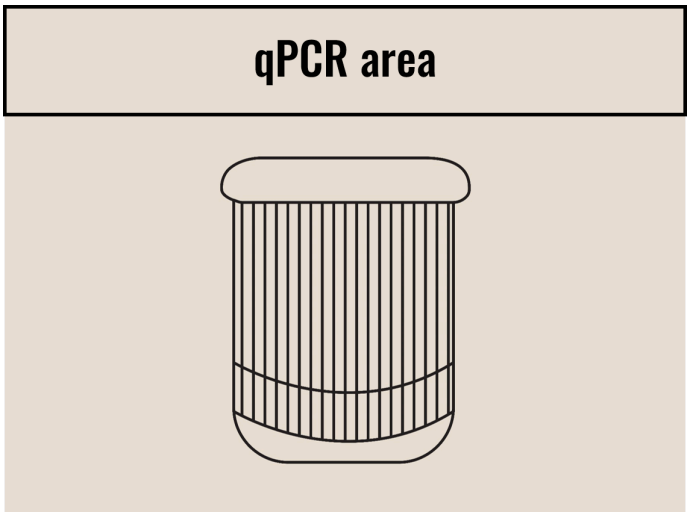
Open only one tube at a time to avoid contamination

1. Once the program in the genesig q16 thermal cycler is completed, gently open the lid and move the lysates to the **sampling area**.
2. First, process the negative control. Transfer 2 µl from the "lysate tube" to the PCR tube containing the master mix using the dedicated **H10 pipette** with a **1-10 µl filter tip**. Close both tubes tightly. Discard the tip.
3. Proceed with the root samples. Transfer 2 µl from the "lysate tube" to the PCR tube containing the complete mix. Close both tubes tightly. Discard the tip.
4. Process all samples one at a time by repeating step 3.
5. Thaw the positive control.
6. Using the dedicated **4 µl fixed volume minipette** and a **2-20 µl filter tip**, transfer 4 µl to the tube labeled "2". Close both tubes.
7. **Store the positive control in the freezer.**
8. **Check that all the PCR tubes containing the master mix are closed tightly.**
9. **Briefly spin down the microcentrifuge to remove bubbles** (please, refer to the Appendix on how to operate a microcentrifuge). Bubbles interfere with optical readings. Eliminating bubbles ensures accurate interpretation of the results.

Example of a 1-10 µl micropipette set to 2 µl

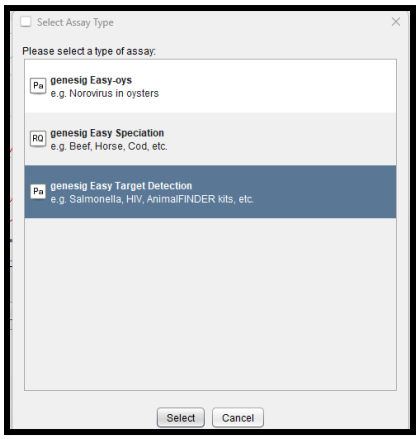

Example of 4 µl minipette with a 20 µl filter tip

Start the qPCR



1. Move to the qPCR area. Remove the USB Drive from the connector cable.
2. Connect the USB Drive to your computer.
3. Delete the lysis run file (PCR 1.USB) from the USB Drive.
4. Open the q16 app on your computer.
5. Select “New”.

6. Select “genesig Easy Target Detection”.



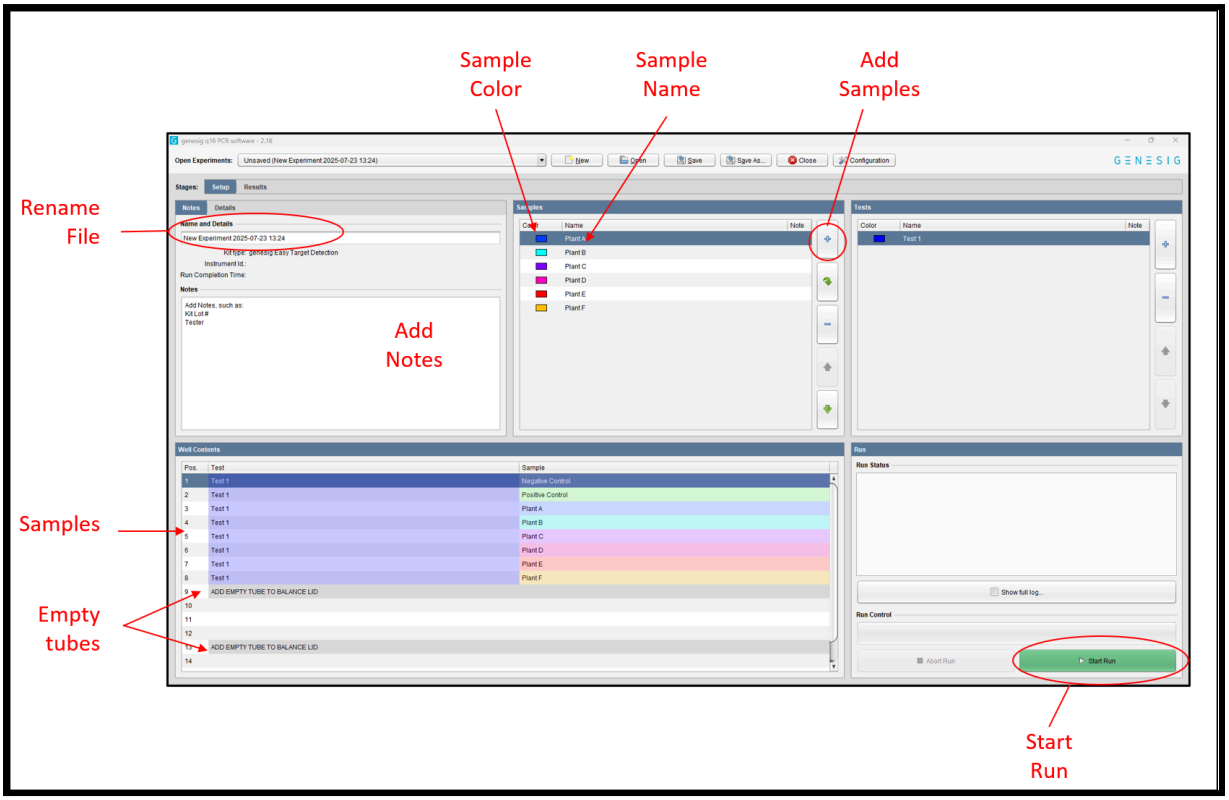
7. Rename the Experiment as you prefer.
8. Under “Samples” select “+” and add the number of samples based on your run.

Notes:
-The negative and positive control will be automatically assigned to position 1 and 2, respectively.

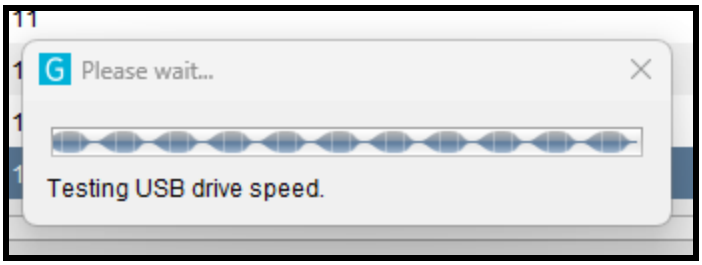
-The software will highlight where to add “empty” tubes, to make sure the lid will properly close.

Optional: rename the samples and change color by selecting the sample and typing in the box and/or selecting the relative box color.

9. Click on “Start Run”. Note that the q16 thermal cycler will not yet start the program.



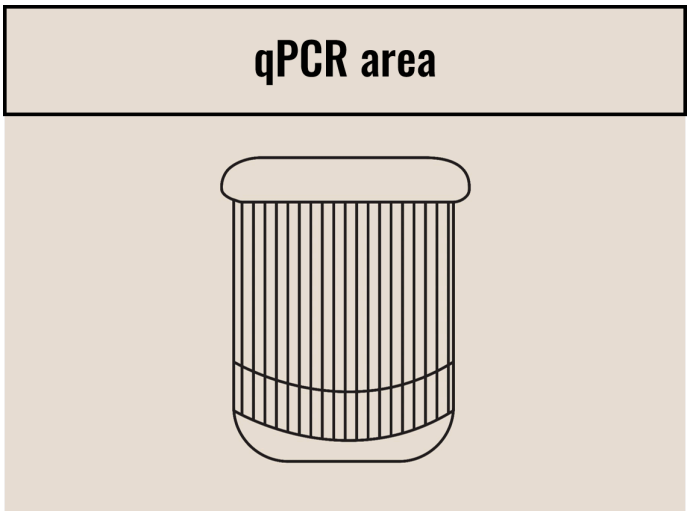
10. Select the USB Drive as the directory for saving the data and click on “Ok”.
11. Select “Start run from USB” and click on “Select”.
12. Select the USB Drive and click “OK”.
13. The software will test the USB Drive. Once the test is correctly completed, remove the USB Drive from your computer.



Note: If the software detected the USB as too slow to load the program, try again repeating steps 10-13.

14. **Load the samples in the assigned positions.**
    Reminder: the negative control should be always in position "1", while the positive control should be always in position "2".
15. Gently close the lid.
16. **Plug the USB Flash Drive into the USB connector cable.**
17. When you start the run, the machine will make an audible announcement and the LED will turn green. The LED's color will change throughout the run, which takes approximately 1 hour and 40 minutes. A circling rainbow light pattern indicates that the program is completed.
18. Once the run is completed, open the lid and discard the tubes. **Never open the PCR tubes once the qPCR step is completed!**
19. Gently close the lid, unplug the machine, and proceed with data analysis.

STEP 7. RESULT ANALYSIS



In this step you will analyze the results. Please, refer to the EXPECTED RESULTS section for more information.

The analysis requires checking both the automated result table as well as the amplification curves.

1. Remove the USB Drive from the USB connector cable and insert it in your PC. Transfer the run file (PCR 1. USB) into a directory where you store your results.
2. Open the q16 app and open the file you would like to analyze.
3. Click on the "Results" tab.
4. Click on "Details" close to the top "Summary" panel. Curves should appear.
5. Negative control is labeled as "PASS" in the result table.
6. Positive control is labeled as "PASS" in the result table.

Note: if either the Negative control or the Negative control failed, a warning message should appear in the Summary and the Notes panel.

7. **Manually check the amplification plot and Cq value for both the Internal Control and the Test channels for each sample**. **Refer to Result Interpretation, Expected Results, and Troubleshooting sections.**

    a. **Internal Control channel:** the sample shows a clear sigmoid curve and Cq < 36. Proceed with test channel analysis.

    Lack of a clear sigmoid curve and/or Cq > 36 indicates sub-optimal DNA extraction and the test should be considered inconclusive.

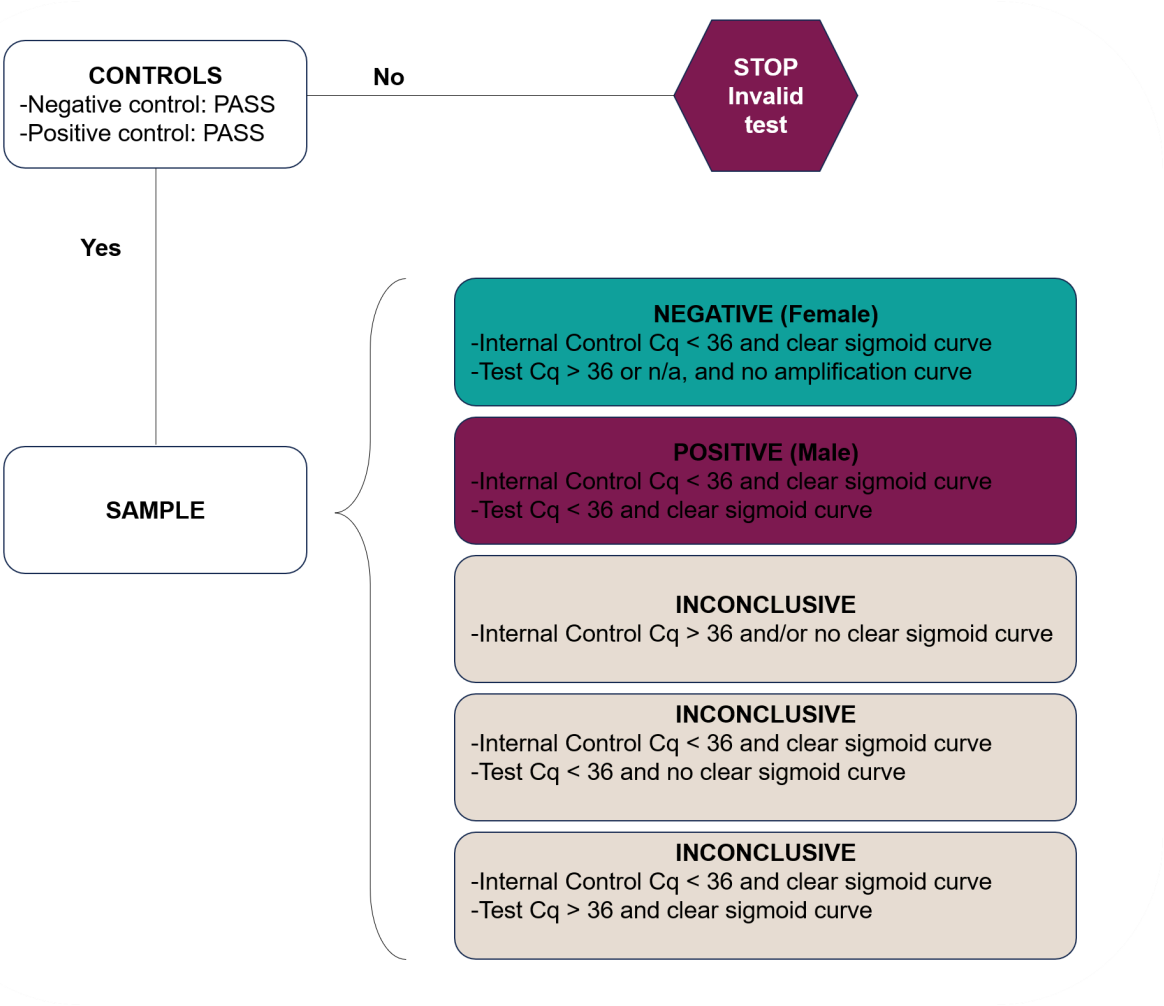
b. **Test channel:**
   i. **Negative (female)**: no sigmoid curve and no Cq detected (n/a). The software will label this as “NEGATIVE”.

   ii. **Inconclusive**: the sample does not show a clear sigmoid curve and/or Cq > 36. The software might indicate this sample as “POSITIVE”. However, conclusions from samples showing sub-optimal amplification should be taken consciously.

   Check the troubleshooting section and repeat the test.

   iii. **Positive (male)**: clear sigmoid curve and Cq < 36. The software will label this as “POSITIVE”.

8. Export the results for future record as .csv or .pdf (optional).
9. Delete the run file PCR 1. USB from the USB Drive.


CONTROLS
-Negative control: PASS
-Positive control: PASS
No
STOP
Invalid
test
Yes
SAMPLE
NEGATIVE (Female)
-Internal Control Cq < 36 and clear sigmoid curve
-Test Cq > 36 or n/a, and no amplification curve
POSITIVE (Male)
-Internal Control Cq < 36 and clear sigmoid curve
-Test Cq < 36 and clear sigmoid curve
INCONCLUSIVE
-Internal Control Cq > 36 and/or no clear sigmoid curve
INCONCLUSIVE
-Internal Control Cq < 36 and clear sigmoid curve
-Test Cq < 36 and no clear sigmoid curve
INCONCLUSIVE
-Internal Control Cq < 36 and clear sigmoid curve
-Test Cq > 36 and clear sigmoid curve

Sample	Software table	Curve Shape and Cq	Result Interpretation
Negative control	PASS	Both Internal control and Test show no clear sigmoid curve and/or Cq > 36.	Results as expected, the test is valid.
Negative control	FAILED	Internal Control and/or test show a clear sigmoid curve and/or Cq < 36.	Contamination. Tests should be considered invalid and they will be labeled as " TEST CONTAMINATED". Deeply clean the lab and repeat all the tests.
Positive control	PASS	Test: Clear sigmoid curve, 14 < Cq < 22.	Result as expected, the q16 thermal cycler correctly reads fluorescence.
Positive control	FAILED	Test: No clear sigmoid curve or Cq < 14 or Cq >22.	-Inaccurate pipetting when adding the positive control If the positive control is detected as FAILED, the tests will be labeled as "TEST FAILED". -If no signal is detected, this might indicate issues with the fluorescence reading. Contact support at dx@minipcr.com
Sample	NEGATIVE	Internal control: clear sigmoid curve and Ct < 36. Test: no clear sigmoid curve, Cq not detected (n/a).	**Female sample.**
Sample	NEGATIVE	Internal control: no clear sigmoid curve and/or Cq > 36.	**Inconclusive.** No clear sigmoid curve in the Internal Control indicates

			sub-optimal DNA extraction. Repeat the test.
Sample	POSITIVE	Internal control: clear sigmoid curve and Ct < 36. Test: clear sigmoid curve and Cq < 36.	**Male sample.**
Sample	POSITIVE	Internal control: clear sigmoid curve and Ct < 36. Test: not clear sigmoid curve and Cq < 36.	**Inconclusive.** Repeat the test.
Sample	SAMPLE PREPARATION FAILED	Internal control: no clear sigmoid curve and/or Cq > 36.	**Inconclusive.** No clear sigmoid curve in the Internal Control indicates sub-optimal DNA extraction. Repeat the test.
Sample	POSITIVE RESULT, POOR QUALITY SAMPLE	Internal control: no clear sigmoid curve and or Cq >36. Test: Cq < 36, clear sigmoid curve.	**Inconclusive.** No clear sigmoid curve in the Internal Control indicates sub-optimal DNA extraction. Repeat the test.

EXPECTED RESULTS

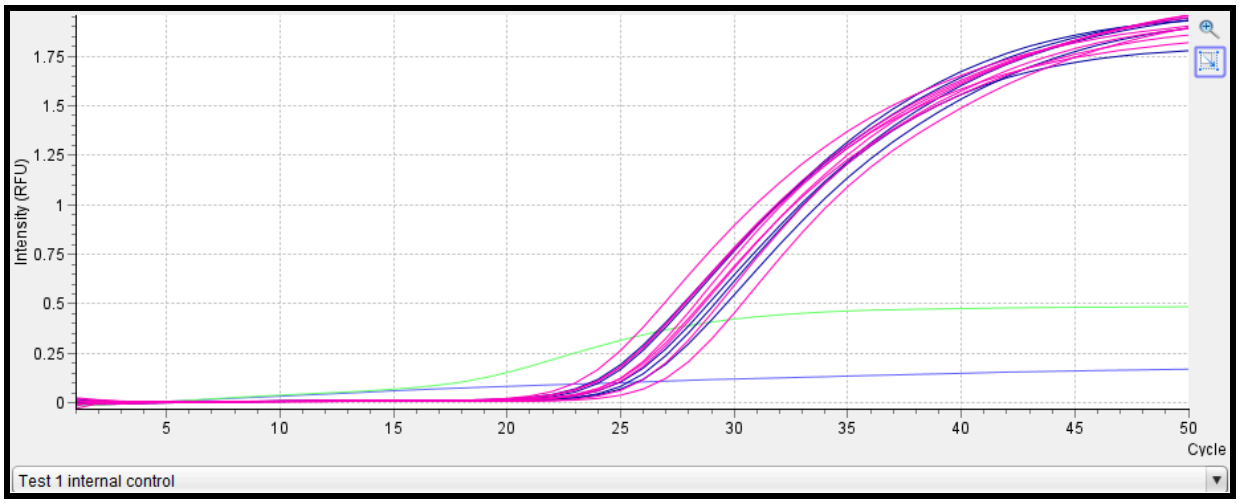
The test result must be interpreted integrating the analysis of the amplification curve shape and the Cq.

The Internal Control indicates whether DNA was extracted efficiently, and the Test is used to know if the sample is carrying the male-specific target gene.

Internal Control channel

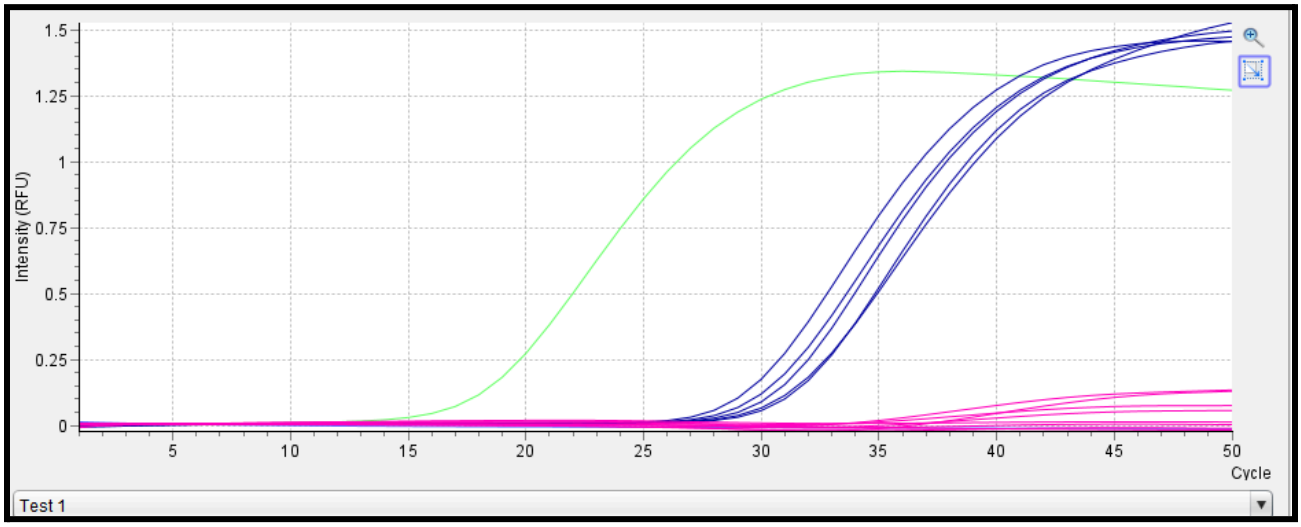
All samples except for the *negative control* (light blue, flat line) should show a clear sigmoid curve and Cq < 36.

- Female samples are in pink and male samples are in dark blue.



Test channel

- The positive control (green) and the male samples (dark blue) should show clear sigmoid curves and Cq < 36.
- The Negative control (light blue) and female plant samples (pink) show no amplification (flat lines).



Software table

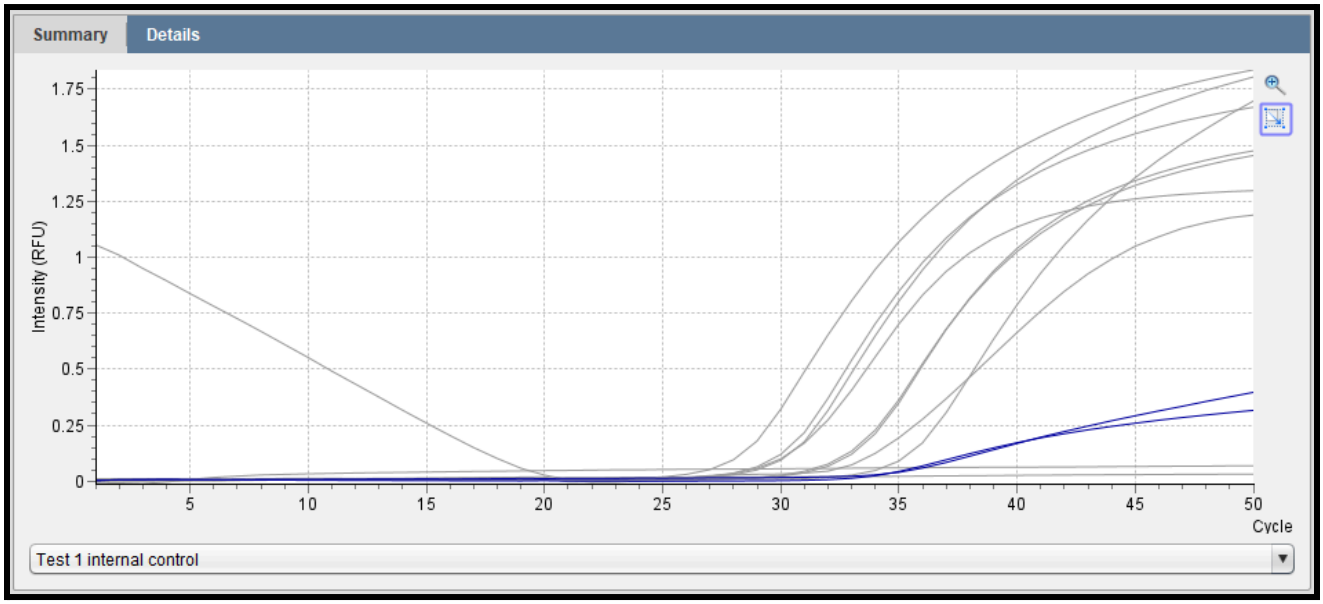
Sample Results | Details

#	Test	Sample	Status	Copy Number	Cq (Test)	Cq (I.C.)
1	Test 1	Negative Control	PASS	n/a	n/a	n/a
2	Test 1	Positive Control	PASS	n/a	17.45	17.40
3	Test 1	Male	POSITIVE	240	30.47	24.53
4	Test 1	Male 2	POSITIVE	539	29.30	23.62
5	Test 1	Male 3	POSITIVE	397	29.74	24.88
6	Test 1	Male 4	POSITIVE	922	28.53	23.33
7	Test 1	Male 5	POSITIVE	224	30.57	25.30
8	Test 1	Female	NEGATIVE	n/a	n/a	24.41
9	Test 1	Female 2	NEGATIVE	n/a	n/a	23.46
10	Test 1	Female 3	NEGATIVE	n/a	n/a	23.44
11	Test 1	Female 4	NEGATIVE	n/a	n/a	24.13
12	Test 1	Female 5	NEGATIVE	n/a	n/a	25.22
13	Test 1	Female 6	NEGATIVE	n/a	n/a	26.15
14	Test 1	Female 7	NEGATIVE	n/a	n/a	22.61

TROUBLESHOOTING

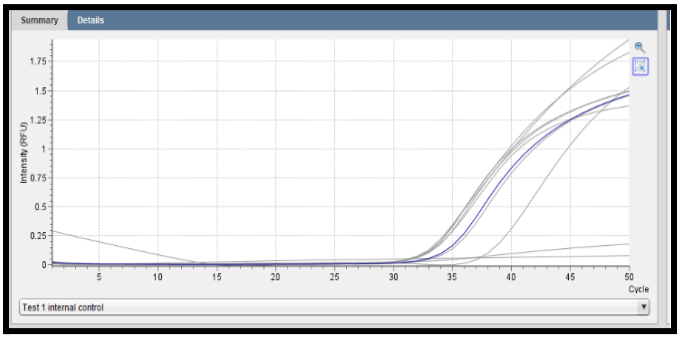
Inconclusive result, example I

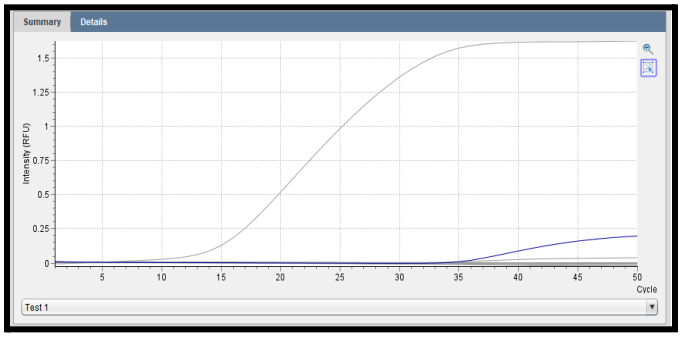
The samples in blue do not show the expected clear amplification curve (sigmoid shape) in the internal control channel. Indeed, the lines are diagonals. This indicates that the lysis was sub-optimal. We recommend repeating the test.



Inconclusive result, example II

Sample 1 (blue) shows a clear amplification curve and Cq < 36 for the Internal Control, this indicates that the lysis process was successful (left picture and table below).
The test channel shows a Cq < 36 (34.22). However, there is not a clear sigmoid curve for the test channel (right picture). Therefore, the test should be considered inconclusive.

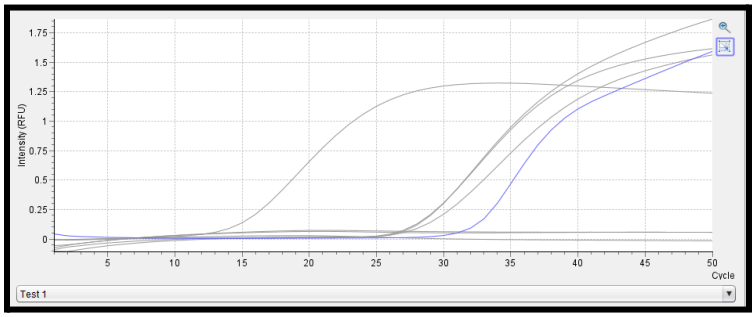




#	Test	Sample	Status	Copy Number	Cq (Test)	Cq (I.C.)
1	Test 1	Negative Control	PASS	n/a	n/a	n/a
2	Test 1	Positive Control	PASS	n/a	14.52	37.37
3	Test 1	Sample 1	POSITIVE	2	34.22	33.54

Invalid test, contamination

The negative control (light blue curve) shows a clear sigmoid curve and Cq < 36 for the Test channel. This indicates contamination.
We recommend discarding already opened tip boxes and current reagents aliquots.
Deeply clean the lab with bleach and clean the lab coats.
Start with new reagents and new tip boxes.



#	Test	Sample	Status	Copy Number	Cq(Test)	Cq(I.C.)
1	Test 1	Negative Control	FAIL	n/a	31.41	30.47

APPENDIX

TUBE TYPES

Tube type	Picture	Description
Screw-cap reagent tube		Reagents for DNA and RNA extraction and RT-qPCR.
1.5 ml microcentrifuge tubes		Used to prepare the master mix. Not included in the kit. Available at dx.minipcr.com
PCR tubes		Used for the lysis and PCR steps. Included in the kit.

MICROPIPETTING

Good micropipetting skills are essential to run the test successfully. Practice with water or colored liquids before using the reagents in this kit.

Micropipetting video tutorial

We recommend dedicated micropipettes and minipettes per area:
-Reagents area: one 100-1000 µl micropipette, one 20-200 µl micropipette, and one 20 µl fixed volume pipette.
-Sampling area: one 1-10 µl micropipette and one 4 µl fixed volume minipette.

To reduce the contamination risk:

- Dedicate specific micropipettes and filter tip boxes to each area
- Always use filter tips
- Use only the dedicated minipette for transferring the RT-PCR master mix and the positive control. In case of contamination, dispose of the minipette and use a new one.

Always select the right micropipette and tip based on the volume that you need to transfer according to the tables in the following page.

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

Volume	Minipette	Tips
4 µl		

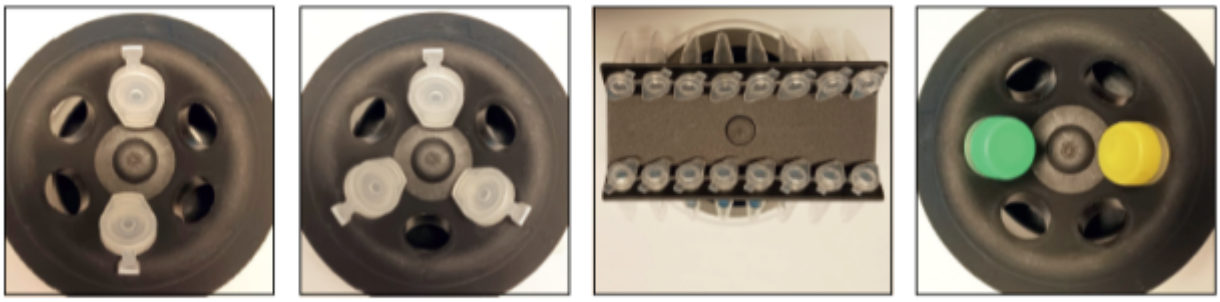
LIQUID HANDLING

Make sure that all liquid is collected at the bottom of the tubes before opening them.

Spin down the PCR tubes before the qPCR step. Bubbles can interfere with the qPCR process.

Follow these steps to use the microcentrifuge:

1. Ensure that the contents of the tubes are fully defrosted.
2. Place the tubes in the rotor, making sure they are balanced as shown in the pictures. The tubes should have approximately the same amount of liquid in them. If needed, prepare a tube with water to balance the rotor. Refer to the manufacturer's instructions for details.
3. Spin for 5 seconds at 10,000 RPM.



Examples of balanced microcentrifuges. Tubes match in size and contain the same volumes, making their weights comparable

SCIENTIFIC BASIS OF THE ASSAY

How to read and interpret qPCR data

A clear sigmoid curve in qPCR is crucial as it demonstrates successful and reliable amplification of the target DNA. This characteristic S-shaped curve signifies the exponential phase of PCR, where the amount of amplified product doubles with each cycle. The curve's plateau indicates the reaction has reached its endpoint, where amplification has ceased.

The cycle threshold (Ct or Cq) value is the cycle number at which the fluorescence signal crosses a defined threshold, indicating detectable amplification. It is inversely proportional to the initial amount of target DNA; a lower Cq indicates a higher starting concentration. Cq values exceeding 36 are generally considered unreliable because at such high Cq values, the signal is often very close to background noise, making it difficult to distinguish true amplification from random fluctuations. Additionally, potential inhibitors or degradation of reagents become more impactful in later cycles, further compromising the reliability of the results.