PathoSEEK® Total Yeast and Mold Count Assay with SenSATIVAx® TLP DNA Purification and Grim Reefer® Free DNA Removal

User Guide

Real-Time PCR (qPCR) Assay for the detection of Total Yeast and Mold in cannabis flower and MIP matrices

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Introduction

Yeasts and molds are known to cause deterioration and decomposition of cannabis. Certain species of yeast and mold, such as *Aspergillus fumigatus* can produce toxins and infect immuno-compromised patients with fatal Aspergillosis. The PathoSEEK® Total Yeast and Mold Count (TYM) Detection Assay combined with use of SenSATIVAx® Thaumatin-Like Protein (TLP) Extraction Enzyme purification protocol (MGC TYM Method) is a DNA Purification and qPCR method for the rapid enumeration of yeasts and molds in cannabis flower. The TLP is a beta glucanase that digests the glucan cell wall of some yeasts that are difficult to lyse. In 2020, *Candida albicans* became a popular TYM-cannabis reference standard. *C. albicans* is an excellent example of a yeast with a thick glucan cell wall that is difficult to lyse. While *C. albicans* has not been found on cannabis, it is reasonable to assume other yeast and molds may similarly produce thick glucan cell walls and robust lysis methods will be required for concordance with colony forming unit (CFU) based regulations.

Process Overview

PathoSEEK® Total Yeast and Mold (TYM) Detection Assay with Grim Reefer® Free DNA Removal and SenSATIVAx® TLP Enzyme Purification Protocol is a novel, real-time quantitative PCR assay that uses a multiplex system of primers to enumerate yeast and molds in cannabis matrices. Figure 1 shows a simplified depiction of the qPCR assay.

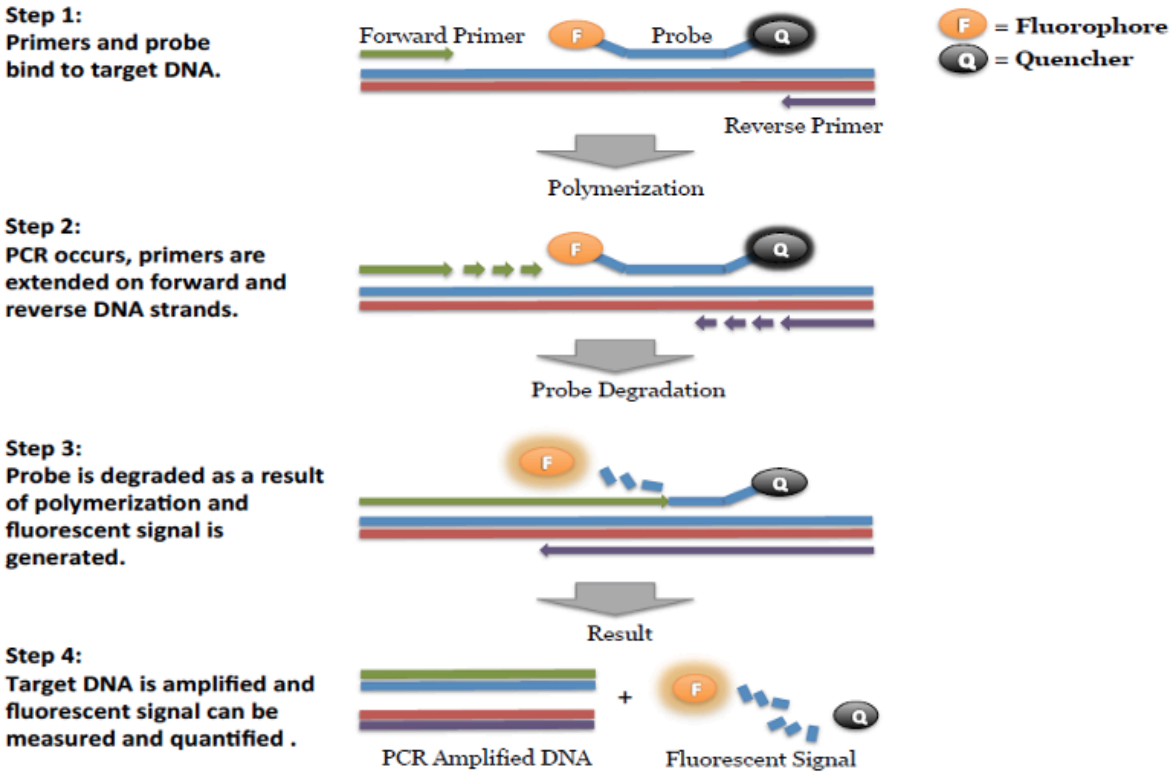
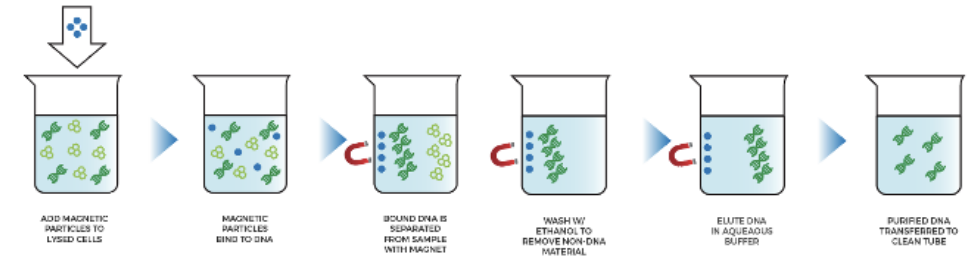


Figure 1: Overview of qPCR

SenSATIVAx® is a proprietary DNA isolation process that uses magnetic particles to isolate and purify both plant and microbial DNA from a raw, homogenized plant or MIP sample. The use of magnetic particles affords 8 or 96-tip automation, enabling high throughput applications. DNA can be isolated from a single test portion or a large batch in under 1 hour. Hands-on time is less than 45 minutes.

without Grim Reefer®



with Grim Reefer®

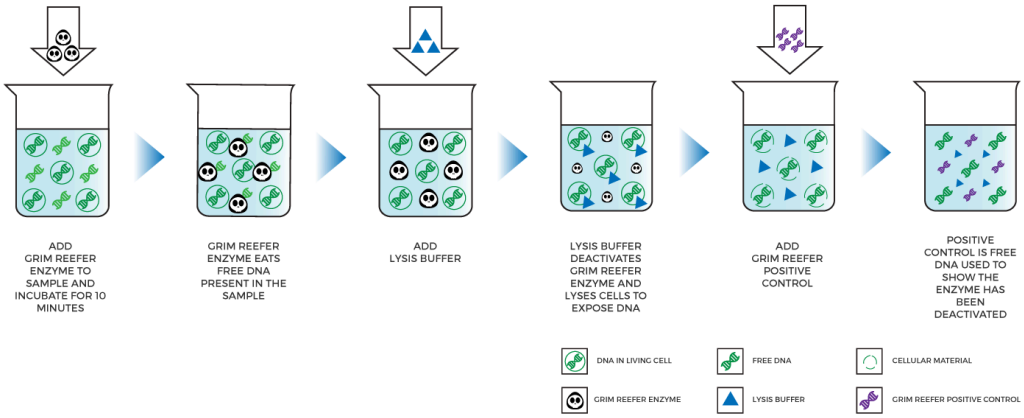


Figure 2: SenSATIVAx DNA Purification Protocols

Materials and Methods

Test Kit Name: PathoSEEK® Total Yeast and Mold Count Assay

Test Kit Information

1. SenSATIVAx® Flower/Leaf DNA Purification Kit - P/N 420001
2. SenSATIVAx® TLP Purification Enzyme - P/N 420206
3. SenSATIVAx® MIP/Extract DNA Purification Kit - P/N 420004/420005
4. PathoSEEK® qPCR Master Kit v3 - P/N 420201
5. PathoSEEK® Total Yeast and Mold Count Detection Assay - P/N 420103
6. PathoSEEK® Total Yeast and Mold Count Assay Positive Control - P/N 420303
7. Grim Reefer® Free DNA Removal Kit - P/N 420145
8. Grim Reefer® Free DNA Removal Control - P/N 420144
9. Grim Reefer® Free DNA Removal Assay - P/N 420143

Test Kit Components

1. SenSATIVAx® Flower/Leaf DNA Purification Kit - P/N 420001 (200 tests).
    a. MGC Lysis Buffer— 1 bottle (Store at 20-28°C). Expires 1 Year from Date of Manufacture.
    b. MGC Binding Buffer—1 bottle (Store at 2-8°C). Expires 1 Year from Date of Manufacture.
    c. MGC Elution Buffer—1 bottle (Store at 20-28°C). Expires 1 Year from Date of Manufacture.
2. SenSATIVAx® TLP Purification Enzyme - P/N 420206 (50 Purifications). Store at -15°C to -20°C. (for flower Purifications ONLY)
    a. 2.0 mL clear top - 1 vial
3. SenSATIVAx® MIP/Extract DNA Purification.— P/N 420004/420005 (200 tests).

a. MGC Binding Buffer — 1 bottle. Store at 2-8°C. Expires 1 Year from Date of Manufacture
b. MGC Elution Buffer — 1 bottle. Store at 20-28°C. Expires 1 Year from Date of Manufacture
c. Solution A — 1 bottle. Store at 20-28°C. Expires 1 Year from Date of Manufacture
d. Solution B — 1 bottle. Store at 20-28°C. Expires 1 Year from Date of Manufacture

4. PathoSEEK® qPCR Master Kit v3 - P/N 420201. Kit (Store at -15 to -20°C). Expires 2 Years from Date of Manufacture
    a. Reaction Buffer (10x) — 1 tube
    b. Nuclease Free Water —2 tubes
    c. qPCR Master Mix — 1 tube

5. PathoSEEK® Total Yeast and Mold Assay - P/N 420103 (200 tests)
    a. Assay — 1 tube (Store kit at -15 to -20°C). Expires 2 Years from Date of Manufacture.
6. PathoSEEK® Total Yeast and Mold Count Assay Positive Control - P/N 420303 (50 reactions)
    a. Control — 1 tube (Store at -15 to -20°C). Expires 2 Years from Date of Manufacture.
7. Grim Reefer® Reagents

a. Grim Reefer Free DNA Removal Kit— P/N 420145 - 1 kit (Store at -20ºC).

Expires 2 Years from Date of Manufacture.

i. MGC Grim Reefer Buffer.

ii. MGC Grim Reefer Enzyme.

b. Grim Reefer Free DNA Removal Control— P/N 420144 - 1 tube (Store at -20ºC).

Expires 2 Years from Date of Manufacture.

c. Grim Reefer Free DNA Removal Assay— P/N 420143 - 1 tube (Store at -20ºC).

Expires 2 Years from Date of Manufacture.

Supplies, Reagents, and Equipment

1. Agilent AriaMx Real-Time PCR System G8830A—Containing the following Optical Channels: FAM, HEX, and Cy5 (if using optional Grim Reefer).
   a. Agilent HP Notebook PC, or equivalent
   b. 96-Well Optical qPCR plate— Medicinal Genomics P/N 100164
   c. Adhesive optical seal for qPCR plates— Medicinal Genomics P/N 100177
   d. Optical Strip Caps (Optional) —Agilent #401425

   Note: If using adhesive seals instead of strip caps, use Applied Biosystems MicroAmp Optical Film Compression Pad, Fisher Scientific, #43-126-39 to prevent evaporation and cross-contamination between wells. Medicinal Genomics will provide at no charge if AriaMx is purchased through Medicinal Genomics.
2. Bio-Rad CFX96 Touch™ Real-Time System
   a. Bio-Rad Personal PC, or equivalent

b. 96-well Optical qPCR plate — Bio-Rad #HSP-96601 or Medicinal Genomics P/N 100164
c. Adhesive optical seal for qPCR plates — Bio-Rad #MSB-1001 or Medicinal Genomics P/N 100177

3. Adjustable, variable volume pipettes (single or multichannel)—P10, P20, P50, P200 or P300 and P1000
4. Adjustable, variable volume filter pipettes tips—For P10, P20, P50, P200, P300, and P1000
5. Crushed ice
6. 96-Well PCR Cryogenic Rack—VWR #89004-570
7. 1.5 mL Tube Benchtop Cryogenic Rack— VWR #89004-558, or equivalent
8. Freezer—Capable of maintaining -20°C
9. Tabletop Mini Plate Centrifuge—Fisher Scientific #14-100-143, or equivalent
10. Tabletop Mini Centrifuge—VWR #10067-588, #2631-0006, or equivalent
11. Vortex-Genie Pulse—Scientific Industries, SKU: SI-0236, or equivalent
12. High-Speed centrifuge— to accommodate 1.5mL tubes such as Eppendorf model 5414R or similar with ability to spin up to speeds of 14,000 – 19,300 RCF
13. Incubators capable of maintaining 37 ± 2ºC and 25 ± 1°C (VWR Personal Size Incubator # 97025-630, or similar)
14. MIPs only – Heat Block capable of maintaining 65ºC (VWR Advanced Mini Dry Block Heater #10153-318)
15. Filter Bags—Whirl-Pak #B01385WA or Medicinal Genomics P/N 100008
16. Beaker or Solo Cup (optional)

17. Molecular Biology Grade Nuclease Free Water (Medicinal Genomics P/N 420184)

18. Tryptic Soy Broth—Medicinal Genomics P/N 420205 (Store at 2-8°C)

19. 1.5 mL Eppendorf Tubes

20. 15 mL or 50 mL conical tubes (for MIP)

21. 96-well Plate Magnet— Medicinal Genomics P/N 420202

22. 96-well extraction plate— Medicinal Genomics P/N 100298

23. Eppendorf Tube Rack

24. Scientific Balance—Capable of measuring to milligrams

25. Refrigerator—Capable of maintaining 2–8°C

26. 25 mL Serological Pipette —VWR 89130-890 or 89130-900, or equivalent

27. 10% bleach

28. 70% Ethanol — Medicinal Genomics P/N 420030

29. Chloroform (Ethanol as preservative/ Certified ACS) – Fisher Scientific #C298

Safety Precautions and Recommendations for Best Results

Safety Precautions

The PathoSEEK® Total Yeast and Mold Count Assay is a qPCR detection assay for the rapid detection and enumeration of yeast and mold in cannabis matrices.

1. Assay users should observe standard microbiological practices and safety precautions when performing this assay. Wear protective gloves, lab coats, eye/face protection as indicated by your quality system.
2. It is the responsibility of each laboratory to handle waste and effluents processed according to their nature and degree of hazardousness. Waste and effluents processed must be treated and disposed of in accordance with all applicable local, state, and federal regulations.
3. Hazard Statement: 70% Ethanol
    a. Highly flammable liquid and vapor. May cause respiratory irritation.
    b. May cause drowsiness or dizziness. Causes damage to organs.
    c. May cause damage to organs through prolonged or repeated exposure.
    d. Please refer to the Safety Data Sheet (SDS) for more information and proper disposal.

4. Hazard Statement: Chloroform
    a. Harmful if inhaled or swallowed.

b. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Use only with adequate ventilation, which may require a chemical fume hood.

c. Keep the container tightly closed and sealed until ready for use. Wash thoroughly after handling.

d. Please refer to the Manufacturer Safety Data Sheet (SDS) for more information and proper disposal

Environment

1. The quality of results depends on the strict compliance with Good Laboratory Practices (for example, the EN ISO 7218 standard), especially concerning PCR.
    a. Never circulate lab equipment from one workstation to another.
    b. Always use a positive and negative control for each series of amplification reactions.
    c. Periodically verify the accuracy and precision of pipettes, as well as correct functioning of the instruments.
    d. Change gloves often, especially if you suspect contamination.
    e. Clean workspaces periodically with 10% bleach and other decontaminating agents.
    f. Use powder-free gloves.
    g. If using qPCR reaction strip tubes instead of plates, avoid fingerprints and writing on caps because both can interfere with data acquisition.

Intended User

The Total Yeast and Mold Count Detection Assay with SenSATIVAx® Purification and all components required to perform the method are intended for use by trained personnel familiar with laboratory techniques associated with pathogenic organism detection.

Sample Preparation

1. Aliquot Tryptic Soy Broth (TSB).

   Note: TSB is a very good growth medium for microbes. It is best to pour the approximate amount of TSB into another sterile tube or container to avoid contaminating the whole bottle. Inspect stock of TSB for flocculants or signs of growth prior to aliquoting. Return it to the 2-8°C refrigerator immediately after use.

2. Wipe down the workspace with a 10% bleach solution, including the benchtop and all equipment being used.
3. Remove the MGC Binding Buffer and TSB from the 2-8°C refrigerator (it should come to room temperature, 20-28°C, before use).
4. Prepare consumables. Label all the filter bags with "[sample name] [date]".
5. Prepare consumables. Label all the 1.5 mL centrifuge tubes needed with "[sample name]".
6. Label extraction plate with date, and if transferring eluted DNA to new plate label the destination plate also.

7. Remove the GR positive control from the -20°C freezer and allow to thaw at room temperature. Once thawed, keep on ice before using. Dilute GR Pos control (GRC) to 1:10:000.
    a. Label a new 1.5 mL Eppendorf tube (GRC 1:100), add 1 µl of GR positive control into 99 µl of sterile distilled water (dH2O). Vortex to mix thoroughly and quickly spin tube. Label another 1.5 mL Eppendorf tube (GRC 1:10,000), add 99 µl of dH2O, then add 1 µl of the GRC 1:100 dilution. Vortex to mix thoroughly and quickly spin the tube. This will result in a 1:10,000 dilution of the GR Positive Control.

       Note: It's easy to mis-pipette when trying to pipette only 1 µL of liquid. Visually check your pipette tip after aspirating 1 µL to ensure the liquid is in the tip before adding it to the tube for dilutions 1 and 2.
    b. Place GRC on ice until use.
8. Weigh flower or MIP test portions into Whirl-Pak bags or conical tubes:
    a. *Cannabis flower, **n** grams* —Weigh flower test portion material into one side of the mesh liner inside the Whirl-Pak bag. Add 19 x ***n*** mL of TSB to each test portion. This is a 1:20 initial dilution of the test portion. Close the Filter bag by folding the top over three times. Homogenize for 1 minute by hand.

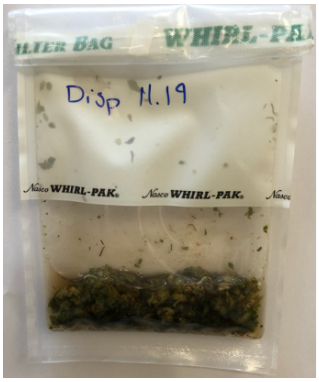


Figure 3: Homogenized cannabis flower and TSB.

b. *MIP and concentrates, **n** grams.* — Weigh the MIP matrix into a 15 mL conical tube, 50 mL conical tube, or Whirl-Pak bag depending on MIP volume. Add 2.4 x ***n*** mL of TSB to each test portion. Vortex or homogenize test portion with TSB and enrich for 16-24 hours at 37°C.

9. If processing multiple samples, be sure to change gloves between each sample to ensure that there is no cross contamination between test portions during the weighing process.
10. Allow a 1.5 mL tube rack to come to temperature in a 37 ± 2°C incubator.

SenSATIVAx® for Flower/Leaf DNA Purification

1. Aspirate 1 mL homogenate from the side of the filter bag free of plant debris and dispense into the 1.5 mL tube.
2. Spin tubes at 14,000 - 19,300 RCF for 5 minutes.

3. Using a pipette remove and discard 950 µL of the supernatant without disturbing the pellet.
4. Resuspend the pellet by adding 200 uL of Nuclease Free Water to each tube.
5. Pipette mix and then vortex tubes well to resuspend the pellet.
6. Add 28 µL of MGC Grim Reefer Buffer and 5 µL of MGC Grim Reefer Enzyme to the resuspended pellet in tubes. The buffer and enzyme are found in the Grim Reefer DNA Removal Kit.
    a. Vortex tubes for 10 seconds. Incubate tubes in tube rack in a 37 ± 2°C incubator for 10 minutes.
    b. Remove tubes from the incubator and add 12.5 µL of MGC Lysis Buffer into tubes. Vortex for 30 seconds.
    c. Allow tubes to incubate for 5 minutes at room temperature.

    Note: The addition of MGC Lysis Buffer deactivates the Grim Reefer Enzyme and should be done as quickly as possible.
    d. Add 2.5 µL of prepared 1:10,000 dilution of Grim Reefer Positive Control into tubes.
7. Add 12 µL of SenSATIVAx TLP Purification Enzyme into tubes. Vortex tubes for 30 seconds.
8. Incubate tubes in a 37 ± 2°C incubator for 30 minutes.
9. Remove tubes from the incubator and vortex tubes for 30 seconds.
10. Let tubes incubate on the bench for 2-5 minutes.

a. After incubation, spin tubes for a minimum 1-3 minutes in a benchtop mini centrifuge or high-speed centrifuge to pellet cellular debris.

Figure 4: Picture of clear lysate after centrifugation

Note: The supernatant should be translucent at this point. If the lysate is still opaque (cloudy) spin again for longer time. This is important for removing cellular debris.

11. Remove 200 µL of supernatant from the tubes containing the centrifuged lysate, being careful not to disturb the pellet at the bottom of the tube. Dispense lysate into the desired well of the previously labeled 96-well extraction plate.

    Note: Pellet size will vary depending on trichome density.

12. Vortex MGC Binding Buffer thoroughly before use for a minimum of 30 seconds. Be sure that the magnetic particles are completely re-suspended in buffer for at least 30 seconds.
13. Add 200 µL of MGC Binding Buffer to each supernatant, and gently pipette tip mix 15 times.

Note: Be careful to avoid adding too many bubbles by pipetting up and down gently when tip mixing to avoid contamination of other wells within the extraction plate.

14. Incubate the extraction plate on the bench for at least 5 minutes.
15. Place the extraction plate atop the 96-well Plate Magnet plate for at least 5 minutes.
16. After incubation, remove as much of the supernatant (400 µL) as possible. Be careful not to disturb or aspirate the beads.
17. Add 400 µL of 70% ethanol (EtOH) into each well with the extraction plate still on the Plate Magnet.
18. Wait at least 30 seconds and then remove all the EtOH from the wells.

    Note: Place the pipette tips at the bottom center of the well to remove all liquid.
19. Repeat addition of 400 µL of 70% EtOH wash with the extraction plate still on the Plate Magnet. Wait at least 30 seconds and remove all the EtOH.
20. After all the EtOH has been removed, let the beads dry at room temperature on the Plate Magnet plate for up to 15 minutes. Required drying time will vary based on complete removal of the second ethanol wash, as well as the laboratory environment. Visually inspect beads for residual ethanol before the elution step.

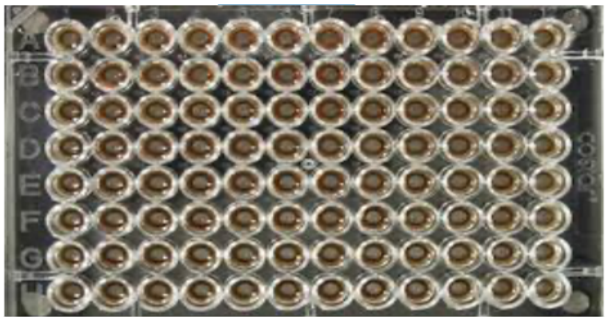


Figure 5: Extraction plate during wash step on magnetic plate.

Note: If EtOH still remains in the wells, go back in with a smaller pipette tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.

Note: It is important to NOT allow the beads to dry for an extended period of time. Over-drying can cause a reduction in DNA yield.

21. Remove the extraction plate from the Plate Magnet and add 50 µL of MGC Elution Buffer to each well.
    a. Mix beads in with buffer with tips approximately 15 times or until the beads are completely re-suspended. **Note: The re-suspensions may appear varied in their appearance, but the result will be the same. It may be helpful to allow the elution buffer to soak the beads prior to tip mixing.**

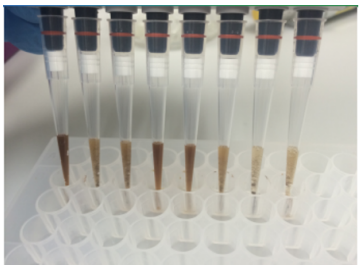


Figure 6: Multichannel pipette tips showing magnetic beads resuspended in elution buffer.

b. Incubate the extraction plate for at least 1 minute on the bench, then return the extraction plate to the Plate Magnet.

c. Let the extraction plate sit on the magnet for at least 1 minute. Use a pipette to transfer the eluant (50 µL) to a new extraction plate labeled with "Final Extract [date]".

d. Seal the extraction plate containing eluents with the adhesive seal, making sure to completely seal the plate wells using a pen or flat object to slide back and forth along the seal. Store plate at -20°C until ready to perform the qPCR protocol.

SenSATIVAx® for MIP/Extracts DNA Purification

NOTE: Non flower samples should be enriched and run as 'presence/absence'. If this test results in a Cq value, the sample should be plated on culture plates of your choice to determine CFU/g.

1. Prepare a SCCG positive control dilution of 1:5,000 (internal control)
    a. Label a new 1.5 mL Eppendorf tube (SCCG 1:50), add 1 µl of SCCG positive control into 49 µl of dH2O. Vortex to mix thoroughly and quick spin tube. Label another 1.5 mL Eppendorf tube (SCCG 1:5,000), add 99 µl of dH2O, then add 1 µl of the SCCG 1:50 dilution. Vortex to mix thoroughly and quick spin tube. This will result in a 1:5,000 dilution of SCCG.

    Note: It's easy to mis-pipette when trying to pipette only 1 µL of liquid. Visually check your pipette tip after aspirating 1 µL to ensure it is in the tip before adding it to the tube for dilutions 1 and 2.

    b. Place on ice until use.

    Note: The 100 µL dilution prepared above is enough diluted SCCG for approximately 10 Purifications. If more Purifications are going to be prepared at the same time, the initial 1:50 dilution can be used to make multiple 1:5,000 dilutions of SCCG.
2. Add initial test portion weight x 4.6 mL SenSATIVAx Solution A to conical tube with enriched test portion/TSB. Vortex the test portion vigorously until homogenized.
3. Transfer 1 mL of the homogenized test portion and Solution A into a 1.5 mL tube.
4. Add 10 µL of the SCCG internal control (1:5,000) to 1.5 mL tube and vortex to mix well.

5. Heat tube at 65 °C for 15 minutes in preheated heat block.
6. Vortex again and centrifuge for 10 minutes at 14,000 - 19,300 RCF using a high-speed benchtop centrifuge.
    a. If no benchtop centrifuge is available, centrifuge for 15 minutes using a mini centrifuge.

    Note: Some matrices will require the use of a high-speed centrifuge due to the presence of certain substances such as gelatin that hinder phase separation.
7. Transfer 600 µL of the solution to a new tube. Push pipette tip through the top solid layer (if one exists), without disturbing the pellet on the bottom to aspirate the sample.
8. Add 600 µL chloroform and vortex vigorously until the solution turns a milky white color throughout.

    Note: This may require longer vortexing for some matrices

Caution: ALWAYS WEAR GLOVES WHEN HANDLING CHLOROFORM

Fig 7. Example of a milky sample with chloroform in it.

9. Centrifuge for 5 minutes at 14,000 - 19,300 RCF using a benchtop centrifuge.
10. If no benchtop centrifuge is available, centrifuge for 15 minutes using a mini centrifuge.

Note: If there is still any color in your aqueous layer (top layer) after centrifugation another chloroform wash may help give you a strong internal control signal (HEX) for every assay. Transfer 300 µL of the top layer to a new 1.5 mL tube and add 300 µL chloroform, vortex and centrifuge again.

11. Transfer 100 µL of aqueous layer (TOP LAYER) from the centrifuged sample to a well of the labeled 96-well extraction plate. Be careful not to disturb the lower chloroform layer.
12. Add 100 µL of SenSATIVAx Solution B to the 100 µL sample in the 96-well extraction plate and mix by pipetting up and down 3 times.
13. Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in buffer for at least 30 seconds.
14. Add 200 µL of MGC Binding Buffer to each sample, and pipette tip mix 15 times.

    Note: Be careful to avoid adding too many bubbles by pipetting gently when tip mixing. This is extremely important so as to not contaminate the wells in proximity.

15. Incubate the plate on the bench for at least 5 minutes.
16. Place the extraction plate onto the 96-well plate magnet plate for at least 5 minutes.
17. After the 5 min incubation, remove as much of the 400 µL of the supernatant as possible. Be careful not to disturb or aspirate the beads.
    a. Add 400 µL of 70% ethanol (EtOH) with the extraction plate still on the magnet.
    b. Wait at least 30 seconds and remove all the EtOH.

    Note: Place the pipette tip at the bottom center of the well to remove all liquid.

18. Repeat 400 µL 70% EtOH wash with the extraction plate still on the magnet plate. Wait at least 30 seconds and remove all the EtOH.

    Note: If EtOH still remains in the wells, go back in with a smaller pipette tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.

19. After all the EtOH has been removed, let the beads dry at room temperature. Allow the beads to dry for up to 15 minutes. Necessary drying time will vary based on complete removal of the second ethanol wash, as well as lab environment. Visually inspect beads for residual ethanol before the elution step.

    Note: It is important to NOT allow the beads to dry for an extended period of time. Over-drying can cause a reduction in DNA yield.

20. Remove the extraction plate from the magnet plate and add 50 µL of MGC Elution Buffer.

    a. Tip mix approximately 15 times or until the beads are completely re-suspended.

       Note: The re-suspensions may appear varied in their appearance, but the result will be the same. It may be helpful to allow the elution buffer to soak the beads prior to tip mixing.

    b. Incubate the plate for at least 1 minute on the bench, before returning the plate to the magnetic plate.

    c. Let the plate sit on the magnet for at least 1 minute before transferring the eluant to a new extraction plate labeled with "Final Extract [date]".

21. Seal the plate with the adhesive seal, making sure to completely seal the plate wells using a pen or flat object to slide back and forth along the seal. Store at -20°C until ready to perform the qPCR protocol.

Real-Time Quantitative PCR (qPCR) Setup Protocol

1. Remove qPCR reagents including qPCR Master Mix, Nuclease Free Water, Reaction Buffer, PathoSEEK Total Yeast and Mold Count Detection Assay, PathoSEEK Total Yeast and Mold (TYM) Count Positive Control, and Grim Reefer Free DNA Removal Assay from the -20ºC freezer. Place qPCR Master Mix tube on ice or leave at -20°C until ready to use. Allow remaining tubes to thaw at room temperature. Once thawed, immediately place tubes on ice.
2. Before preparing the reaction master mix, mix reagent tubes.
    a. Total Yeast and Mold Count Detection Assay, Reaction Buffer, Total Yeast and Mold Count Positive Control, Grim Reefer Free DNA Removal Assay and Nuclease Free Water – Vortex tubes quickly followed by a pulse spin-down in a microcentrifuge.
    b. qPCR Master Mix – Invert the tube 5 times (do not vortex), followed by a pulse spin-down in a microcentrifuge.
    c. Return all reagents to the ice.

    Note: Do not vortex the qPCR Master Mix at any point during the protocol.
3. Make a separate master mix in a 1.5 mL tube sufficient for all test reactions being run. The Total Yeast and Mold Count Detection Assay contains the internal plant control (ICC) probe mix, and the probes for all microbial targets. Grim Reefer Free DNA Removal Assay contains the Grim Reefer Control detection probe mix. Label new master mix tube with TYM MM (Master Mix). Always prepare enough master mix for 1 or 2

additional reactions over the total number of tests to account for pipetting and dead volumes. An example of the TYM Master Mix can be found in Table 1.

Note: It is best to add the largest volume reagent first, in this case, water. Add qPCR Master mix last.

Note: It is important to avoid errors in pipet volumes, as these may affect PCR results.

Table 1: TYM Master Mix Reagent Volumes: Include Grim Reefer Free DNA Removal Assay

Reagents	1 Reaction	24 Reactions (Plus 1 excess rxn)	48 Reactions (Plus 2 excess rxn)
qPCR Master Mix	3.75 µL	93.75 µL	187.5 µL
Total Yeast and Mold Count Detection Assay	1 µL	25 µL	50 µL
Grim Reefer Free DNA Removal Assay	0.5 µL	12.5 µL	25 µL
Reaction Buffer	0.8 µL	20 µL	40 µL
Nuclease Free Water	7.7 µL	192.5 µL	385 µL
TYM Master Mix	13.75 µL	343.75 µL	687.5 µL

Note: The Grim Reefer Assay is detected in the Cy5 Channel of the qPCR instrument. Be sure to select the Cy5 channel when setting up the detection plate.

Table 2: TYM Mater Mix Reagent Volumes: No Grim Reefer Free DNA Removal Assay

Reagents	1 Reaction	24 Reactions (Plus 1 excess rxn)	48 Reactions (Plus 2 excess rxn)
qPCR Master Mix	3.75 µL	93.75 µL	187.5 µL
Assay Probe Mix	1 µL	25 µL	50 µL
Reaction Buffer	0.8 µL	20 µL	40 µL
Water	8.2 µL	205 µL	410 µL
Total Assay Probe MM	13.75 µL	343.75 µL	687.5 µL

4. Once combined, gently tip mix or invert the tube 5 times to combine the master mix together.
    a. Pulse spin-down tube in microcentrifuge.
    b. Place TYM Master Mix tubes on ice until used.
5. For the positive control, make a 1:10 dilution of TYM Count Positive Control.
    a. Vortex the stock TYM Positive Control tube and pulse spin-down. Add 1 µL of TYM Positive Control to 9 µL Nuclease Free Water (found in the kit) into a 1.5 mL tube and vortex to mix.

    Note: It is best to add the largest volume reagent first, in this case, the 9 µL water, then the 1 µL of TYM Positive Control. Pipette mix or vortex control dilution to ensure control DNA is in solution.
6. For the negative control, use Nuclease Free Water only.

7. Place the extraction plate on the Plate Magnet. This is to ensure that no magnetic beads are transferred into the qPCR reactions if there are some left over from the purification elution process.
8. Use a 96-Well Optical qPCR plate and label the plate "qPCR Plate_ [date]".
9. If frozen, let the DNA thaw completely. Spin the extraction plate in a mini plate centrifuge before removing the seal to avoid cross-contamination between samples. Carefully remove the seal from the extraction plate containing DNA samples.
10. Pipette mix the DNA in wells and place the extraction plate onto the Plate Magnet for one minute.

    Note: ALWAYS use a fresh tip for every liquid transfer into the qPCR plate.
11. Transfer 5 µL of each DNA sample into the corresponding well on the Optical qPCR plate, keeping the extraction plate on the Plate Magnet when aspirating the liquid (5 µL).
12. Add 5 µL of the diluted TYM Positive Control to the corresponding positive control well.
13. Add 5 µL of Nuclease Free Water to the corresponding negative control well.
14. Add 13.75 µL of the TYM Master Mix to each corresponding sample wells, positive control well, and negative control well in the Optical qPCR plate. Gently tip mix a few times after each addition of successive TYM master mix to plate wells. Be careful not to introduce bubbles during this mixing step.
15. Seal the Optical qPCR plate with strip caps or an adhesive seal.
16. Spin down Optical qPCR plate for at least 1 minute in mini plate centrifuge to bring well contents to the bottom of wells and help to rid of reaction bubbles.

Note: Check for bubbles at the bottom of the wells (minimal bubbles on the surface of the liquid is acceptable). If bubbles remain in the bottom of the wells, spin down for another minute.

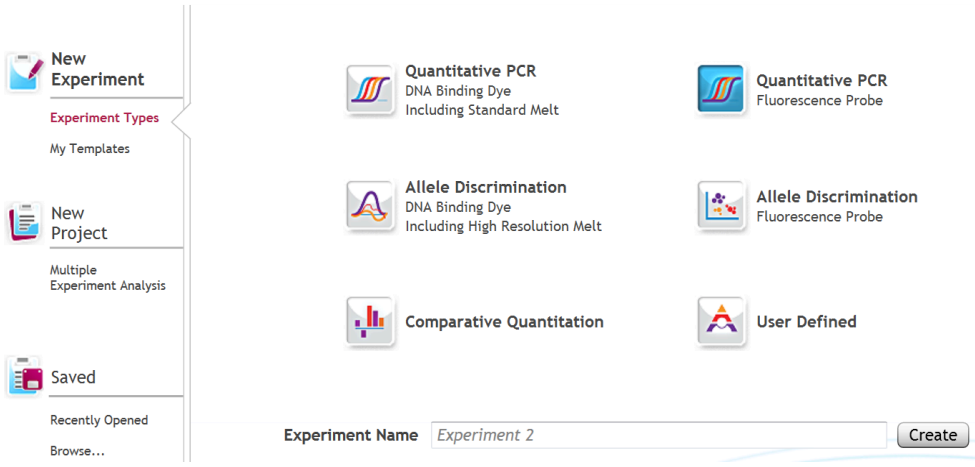
17. For the Agilent Aria: If using an adhesive seal on Optical qPCR plate, place the reusable MicroAmp Optical Film Compression Pad (gray side down) on the plate directly lining up the holes in the pad with the wells in the plate.
18. Place the sealed Optical qPCR plate onto the PCR instrument, positioning the A1 well in the top left corner of the system.
19. Follow the software-specific instructions to initiate the run.
20. Upon completion of the run save your results and proceed to confirmation if necessary.

Running the Agilent AriaMX

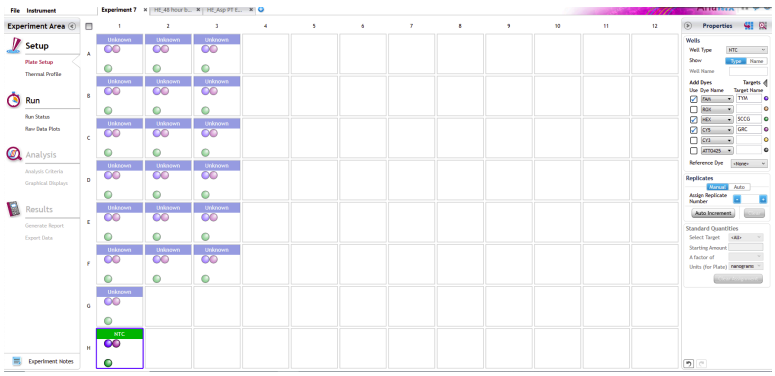
The following species will be detected on the following Fluorophores:

- Total Yeast and Mold: FAM
- Internal Plant Control: HEX
- Grim Reefer Control: Cy5

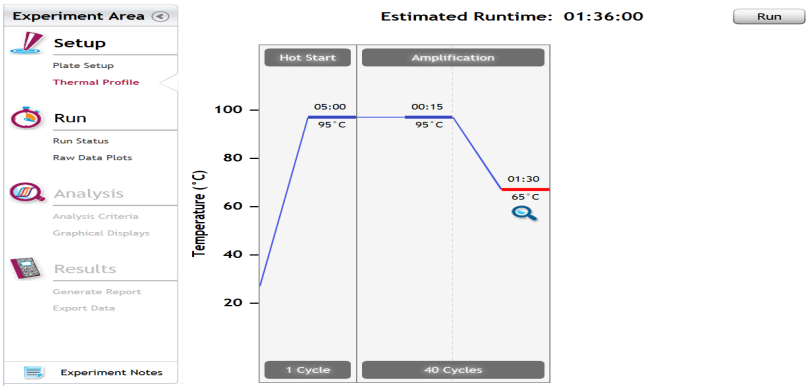
1. Create a New Experiment on the Agilent qPCR instrument.



2. Under Setup > Plate Setup, highlight **only** the wells that contain reactions and select FAM and HEX and Cy5 under **Add Dyes**. Having fluorescence detection turned on in empty wells can affect the calculations which the software makes to obtain the corrected data, ΔR log.



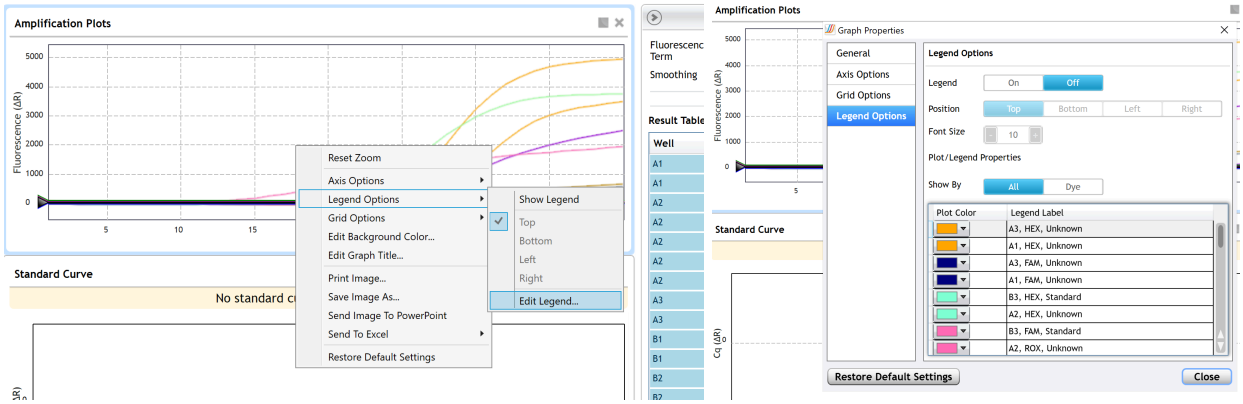
3. Change the well types to reflect your plate setup. Add Target names to include “pathogen name” for FAM and ICC (Internal Plant Control) for HEX and GRC (Grim Reefer Control) for Cy5.
4. Under Setup>Thermal Profile, create the following PCR thermal profile:
    a. Hot start at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 65°C for 90 seconds.



5. Close the lid and click “Start Run”.
6. Save the experiment with the [User] and [date].
7. When the run is complete, dispose of the plate. Do not open the plate seal after the run to avoid contamination in the lab.

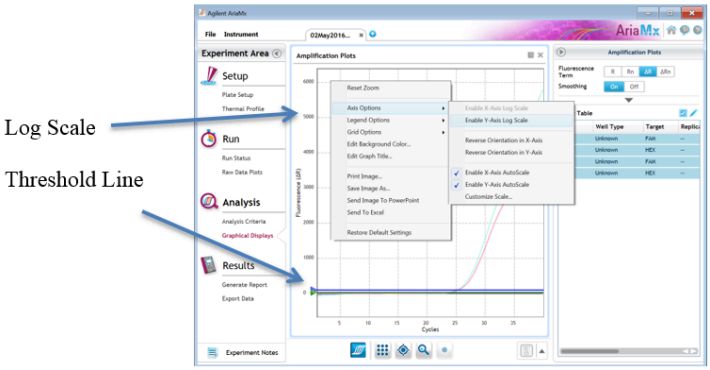
Data Analysis: Agilent AriaMX

1. Open the Data Analysis window when the run is complete.
2. Highlight the wells of interest in the Analysis Criteria under Analysis, then select Graphical Display.
    a. Amplification plots will be available for viewing
    b. The Cq values will appear to the right in the table
    c. Right-click inside the graph, select Edit Legend under Legend Options
    d. Change “All” to “Dye”
    e. All user settings for Plot/Legend Properties will be removed. Do you want to proceed? Select “Yes”.
    f. This will assign a single color to each fluorophore.

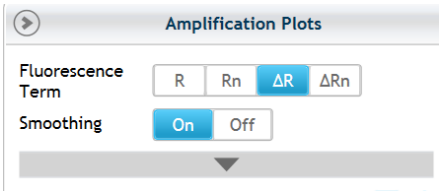


3. To analyze the results:

    a. Start by turning the graph to Log Scale by right-clicking on the chart and selecting Axis options. Enable y-axis log scale.



    b. Expand the amplification plots settings by clicking on the triangle (shown below).



    c. Manually adjust thresholds to 100 RFU for the FAM, HEX and Cy5 fluorophores.

Total Yeast and Mold Control Wells (POS and NEG):

1. Assay positive control (POS well) – Passing - on the FAM Fluorophore, has a Quantification Cycle (Cq) value ≤35.

    a. Visually confirm result with the curve on the graph. If FAM for positive well has a Cq value > 35 or a negative Cq result, then the qPCR run sample results are inconclusive and the qPCR must be re-run from the already extracted DNA.

2. Assay negative control (NEG well) – Passing - on the FAM Fluorophore, has no Cq value.

a. Visually confirm result with the curve on the graph. If FAM signal for the negative well is observed, then this indicates that the results are inconclusive, and the qPCR must be re-run from the already extracted DNA. Be sure to use all new qPCR reagents as a positive result for a negative well indicates a contaminant.

Internal Control:

3. Passing: Internal Control, on the HEX Fluorophore, has a Cq value ≤ 35 for flower samples and <40 for all other matrices.
    a. Visually confirm result with the curve on the graph.
    b. If no HEX signal or HEX signal has a Cq value >35 for flower sample, the DNA purification for sample should be repeated from beginning. This result is an indication that the purification process was not successful, or that the sample type contains very little cannabis DNA.

Grim Reefer FREE DNA:

4. Passing: GR Positive Control, on the Cy5 Fluorophore, has a Cq value between 22 and 30.
    a. If the Cq value is between 22–30 for Cy5, then the Grim Reefer Enzyme was properly deactivated.
    b. If the Cq value is less than 22:
        i. This may indicate a dilution error of the spiked GR Positive Control, or it may indicate that your flower sample is contaminated with russet mites.
        ii. If the Cq is less than 22, repeat the flower extraction from beginning, but now when repeat PCR analysis do not spike in the GR Positive Control.

iii. Once the samples have been re-extracted, analyze the newly extracted samples with the TYM assay including the Grim Reefer Assay probe. After repeat analysis, if there is still a Cy5 signal without the spiked GR Positive Control, this indicates the sample has russet mites.

iv. If the sample does have russet mites, the Grim Reefer treatment can be performed, but the GR Positive Control should not be spiked into the sample during the extraction, and no Grim Reefer components should be included in the qPCR setup (see Table 2, qPCR Reagent Volumes – No Grim Reefer Assay Probe Mix Included).

v. When not using the GR Positive Control, incorporate a TSB blank to ensure that the Grim Reefer Enzyme was properly deactivated by the addition of MGC Lysis Buffer. A TSB blank sample should be taken through the extraction process. The TSB blank should be spiked with the Grim Reefer Positive Control after the addition of lysis buffer. Your samples should NOT be spiked with the GR Positive Control. This sample should also be run with the addition of the Grim Reefer Free DNA Removal Assay to the qPCR master mix. If the Cy5 signal is between the Cq range of 22–30, the Grim Reefer Enzyme was properly deactivated

c. If the Cq value is greater than 30: this may indicate a dilution error of the spiked GR Positive Control, or it may indicate that the Grim Reefer Enzyme was not properly deactivated with the MGC Lysis Buffer. The entire DNA purification from beginning should be repeated.

Unknown Total Yeast and Mold targets:

5. A low CFU count or passing result (under threshold CFU for usage by vendor) for the unknown Total Yeast and Mold targets.
    a. Passing Sample Estimated CFU: Check Cq Value on the FAM Fluorophore. Use Cq to CFU conversion equation to determine approximate CFU/g. The equation employs an experimentally generated best fit line to correlate Cq to estimated CFU/g. See Table 3.

Table 3: Cq to CFU Conversion Equation for Flower

Matrix	Microbial Test	Cq to CFU Conversion Equation
Flower	Total Yeast and Mold	CFU/g = 10^[(-0.1267*Cq) + 6.6781] Multiply resulting CFU x 20 to account for upfront dilution factor

        i. Visually confirm with the curve on the graph.

    b. A high CFU count or failing result (over threshold CFU for disallowance by vendor) for the unknown Total Yeast and Mold targets.
        i. Failing Sample Estimated CFU: Check Cq value on the FAM Fluorophore. Use Cq to CFU conversion equation {10^[(-0.1267*Cq)+6.6781]}*20 to determine approximate CFU/g.
        ii. Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurs. Sometimes the background amplification will give a potential false-positive curve, especially when Cq is < 15. A potential false-positive

curve will appear to lack an exponential growth phase. Linear data view does not display a sigmoidal shaped curve. Raw data view shows a baseline that drifts upward throughout the run. These factors indicate that the results are inconclusive, and the qPCR must be re-run from the already extracted DNA.

Running the BioRad CFX96

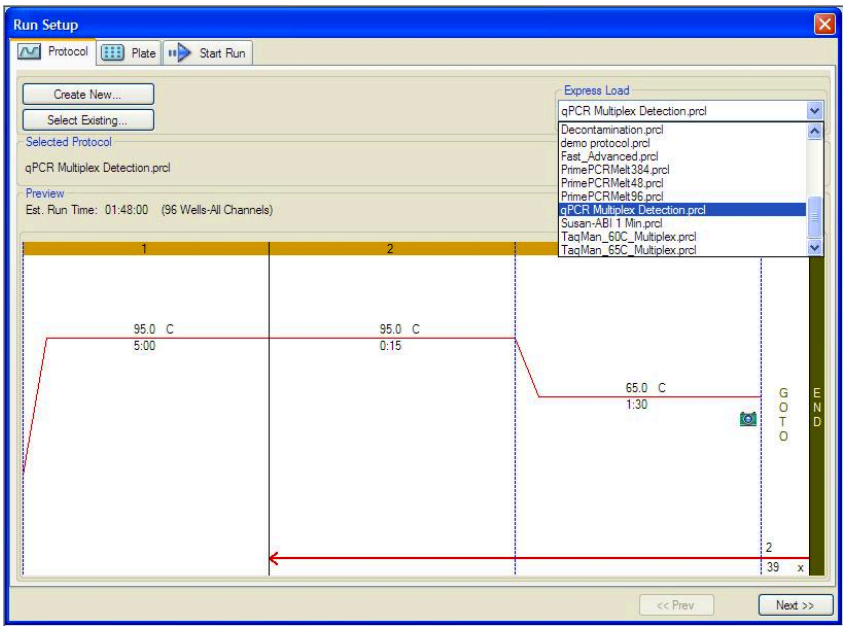
The following targets will be detected on the following fluorophores:

- Total Yeast and Mold: FAM
- Internal Plant Control: HEX
- Grim Reefer Control: Cy5

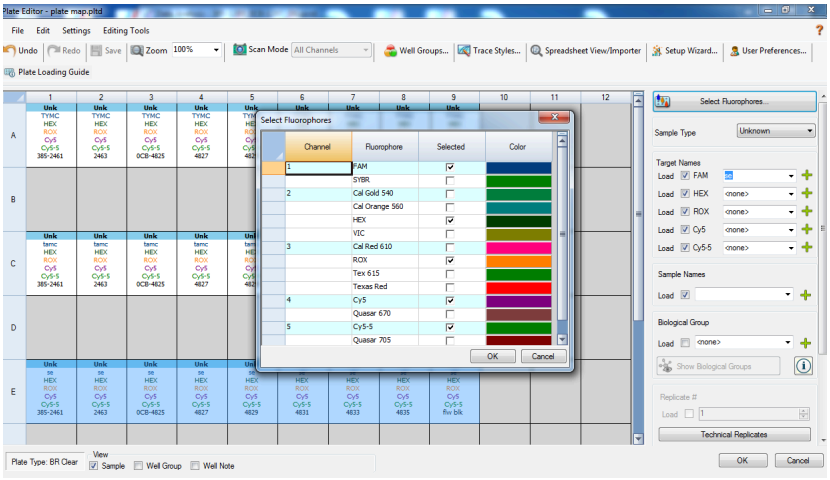
1. Start the qPCR Cycling program.
2. Select User-Defined in the Startup Wizard under Run setup.

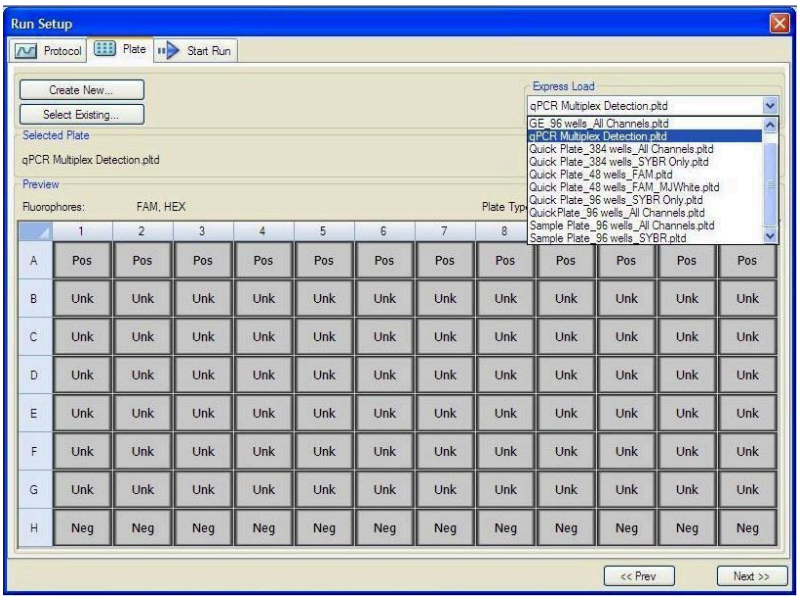
3. Use the Express Load dropdown menu to pick the qPCR Multiplex Detection Program and click “Next”.

4. If not already pre-programmed, create a cycling program with the following specifications and save as “qPCR Multiplex Detection”:
    a. Hot start at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 65°C for 1 minute, 30 seconds.



5. Design your plate under the plate tab in the Run Setup.
    a. Select the qPCR Multiplex Detection from the dropdown menu. If not already present, click “Create New”
    b. The plate editor window will appear. Choose FAM, HEX, and Cy5 fluorophores and click “OK”.





c. If plate layout previously saved, click “Edit Selected” to move to the Plate Editor Screen.

d. On the Plate Editor Screen, change the Sample Type to correlate with your specific plate setup.

Note: To select the Sample Type, highlight the wells you would like to define, then choose from the dropdown menu one of three types:

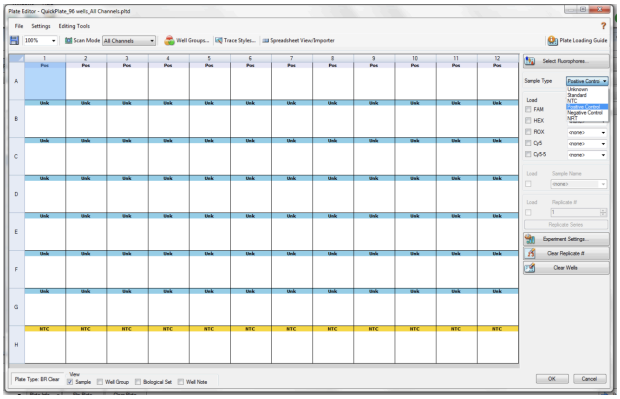
1. **Unknown**
2. **Positive Control**
3. **Negative Control**

e. Make sure “All Channels” is selected from the dropdown menu at the top.

f. Attach the fluorophores to the wells being used.

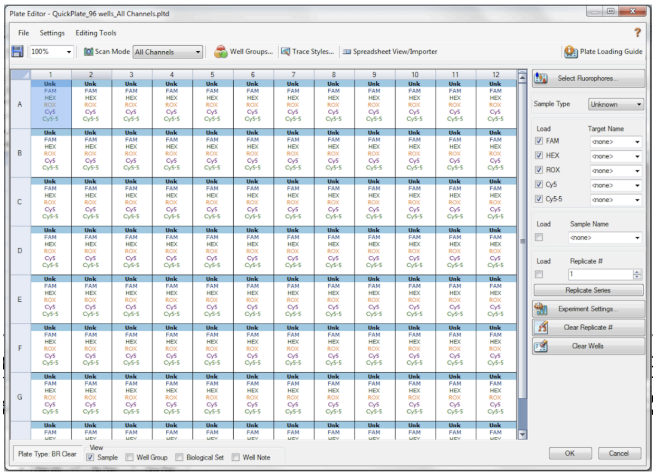
   i. Highlight all the wells being used.

   ii. For the Total Yeast and Mold Count Assay highlight the well locations and click on **FAM, HEX, and Cy5**.



g. When the plate is designed correctly, click OK.

h. Click “yes” to save your plate. If creating plate layout for the first time, save as “qPCR Multiplex Detection”. If you do not save the plate, it will return to the default plate.



Note: Saving will override the template but will not cause any issues.

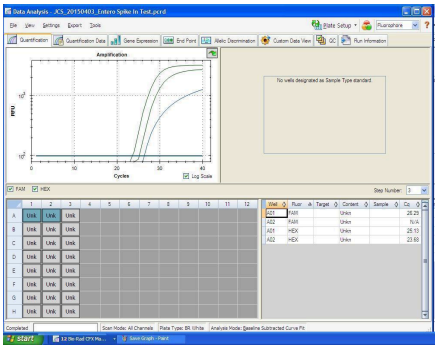
i. Close the lid and click Start Run.

j. Save the experiment with the [User] and [date].

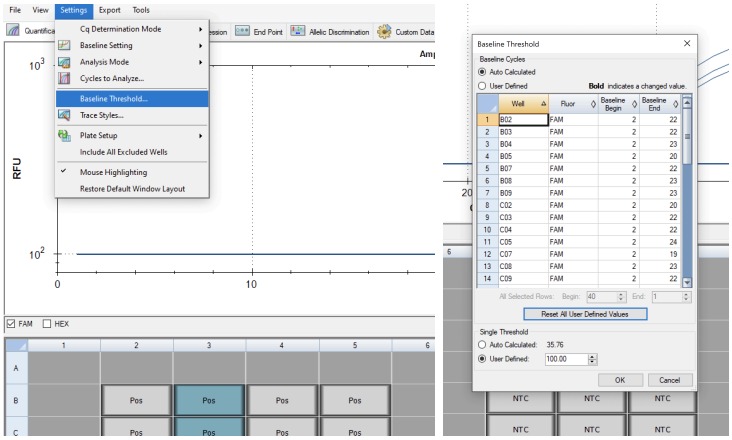
k. When the run is complete, immediately dispose of the plate after qPCR. Do not remove the plate seal after the run to avoid contamination in the lab.

Data Analysis: BioRad CFX96

1. The Data Analysis window will open automatically when the run is complete.
2. Highlight the well of interest.
3. The graph will appear above.
4. The Cq values will appear to the right.
5. To analyze the results:
    a. Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for all fluorophores.
        i. To turn the graph to Log Scale, click on the box at the bottom right of the graph.



        ii. To adjust the threshold, click on the horizontal lines, and move them to the specified value mentioned above on the y-axis. Alternatively, set an exact threshold value by selecting a single fluorophore at a time beneath the graph, then Settings > Baseline Threshold. In the next window select User Defined and enter 100.



Total Yeast and Mold Control Wells (POS and NEG):

1. Assay-specific positive control (well) – Passing - on the FAM Fluorophore, has a Cq value ≤35.
    a. Visually confirm result with the curve on the graph. If FAM for positive well has a Cq value >35 or negative Cq result, then the qPCR run sample results are inconclusive and the qPCR must be re-run from the already extracted DNA.
2. Assay-Specific negative control (well) – Passing - on the FAM Fluorophore, has no Cq value.
    a. Visually confirm result with the curve on the graph. If FAM signal for the negative control well is observed, then this indicates that the results are inconclusive, and the qPCR must be re-run from the already extracted DNA. Be sure to use all new qPCR reagents as a positive result for a negative well indicates a contaminant.
3. Passing: Internal Control, on the HEX Fluorophore, has a Cq value ≤35 for flower samples and <40 for all other matrices.
    a. Visually confirm result with the curve on the graph.
    b. If no HEX signal or HEX signal has a Cq value >35 for flower sample, the DNA purification for the sample should be repeated. This result is an indication that the

purification process was not successful or that the sample type contains very little cannabis DNA.

Grim Reefer FREE DNA:

4. Passing: GR Control, on the Cy5 Fluorophore, has a Cq value between 22 and 30.
    a. If the Cq value is between 22–30, then the Grim Reefer Enzyme was properly deactivated.
    b. If the Cq value is less than 22:
        i. This may indicate a dilution error of the spiked GR Positive Control, or it may indicate that your flower sample is contaminated with russet mites.
        ii. If the Cq value is less than 22, repeat the flower extraction, but now for repeat PCR analysis do not spike in the GR Positive Control.
        iii. Once the samples have been re-extracted, run the newly extracted samples with the TYM assay including the Grim Reefer Assay probe. After repeat analysis, if there is still a Cy5 signal without the spiked GR Positive Control, this indicates the sample has russet mites.
        iv. If the sample does have russet mites, the Grim Reefer treatment can be performed, but the Grim Reefer Positive Control should not be spiked into the sample during the extraction, and no Grim Reefer components should be included in the qPCR setup (see Table 2, qPCR Reagent Volumes – No Grim Reefer Assay Probe Mix Included).
        v. When not using the GR Positive Control, incorporate a TSB blank to ensure that the Grim Reefer Enzyme was properly deactivated by the addition of

MGC Lysis Buffer. A TSB blank sample should be taken through the extraction process. The TSB blank should be spiked with the GR Positive Control after the addition of lysis buffer. Your samples should NOT be spiked with the GR Positive Control. This sample should also be run with the addition of the Grim Reefer Free DNA Removal Assay to the qPCR master mix. If the Cy5 signal is between the Cq range of 22–30 the Grim Reefer Enzyme was properly deactivated.

c. If the Cq value is greater than 30, this may indicate a dilution error in the spiked GR Positive Control, or it may indicate that the Grim Reefer Enzyme was not properly deactivated with the MGC Lysis Buffer. The entire DNA purification from the beginning should be repeated.

Unknown Total Yeast and Mold targets:

5. A low CFU count or passing result (under threshold for CFU for usage by vendor) for the unknown Total Yeast and Mold targets.

    a. Passing Sample Estimated CFU: Check Cq Value on the FAM Fluorophore. Use Cq to CFU conversion equation to determine approximate CFU/g. The equation employs an experimentally generated best fit line to correlate Cq to estimated CFU/g. See Table 4.

Table 4. Cq to CFU Conversion Equation for Flower

Matrix	Microbial Test	Cq to CFU Conversion Equation
Flower	Total Yeast and Mold	CFU/g = 10^[(-0.1267*Cq) + 6.6781] Multiply resulting CFU x 20 to account for upfront dilution factor

i. Visually confirm with the curve on the graph.

b. A high CFU count or failing result (over threshold CFU for disallowance by vendor) for the unknown Total Yeast and Mold targets.

i. Failing Sample Estimated CFU: Check Cq value on the FAM Fluorophore. Use Cq to CFU conversion equation {10^[(-0.1267*Cq)+6.6781]}*20 to determine approximate CFU/g.

ii. Visually confirm with the curve on the graph. It is very important to confirm with the amplification curve when a high CFU count occurs. Sometimes the background amplification will give a potential false-positive curve, especially when Cq <15. A potential false-positive curve will appear to lack an exponential growth phase. Linear data view does not display a sigmoidal shaped curve. Raw data view shows a baseline that drifts upward throughout the run. These factors indicate that the results are inconclusive, and the qPCR must be re-run from the already extracted DNA.

Cq to CFU Conversion Equation

Table 5. Cq to Estimated CFU Conversion Equation for Flower

Matrix	Microbial Test	Cq to CFU Conversion Equation
Flower	Total Yeast and Mold Count	CFU/g = 10^[(-0.1267*Cq)+6.6781)]; Multiply resulting CFU x 20 to account for upfront dilution factor
Non-Flower	Total Yeast and Mold Count	IF Cq<40 after enrichment, plate confirm for enumeration

Non-Flower: If test results in a Cq value indicate the presence of the target organisms, an unenriched sample should be plated on culture plates of your choice to determine enumeration.

Conversion Equation Example:

A resulting Cq value for a flower sample should be plugged into the equation in Table 3 or 5.

See step by step instructions below. The following example assumes a resulting Cq value of 31.0

1. Multiply the Cq value by -0.1267
   = (31 x -0.1267) = -3.9277
2. Add 6.6781 to result in step 1
   = -3.9277 + 6.6781 = 2.7504
3. 10x the result from step 2
   = 102.7504 = 562.86
4. Multiply the result in step 3 by 20 = 562.86 x 20
   = Approximately 11, 257 CFU/gram cannabis flower
5. Determine if resulting CFU/g result is above or below the action limit set by the regulatory body

Confirmations

Test portions can be confirmed using dichloran rose bengal chloramphenicol (DRBC) agar or acidified potato dextrose agar (PDA) spread plates. Dilute portions as necessary to obtain 10–150 colonies/plate. Incubate plates at 25 ± 1°C. Do not stack plates higher than 3 and keep plates one or two inches apart. Do not invert plates. Enumerate colonies after 5 days. If no growth is present on the plate, return to the incubator for 2 additional days and enumerate (7 total days). Record results from plates with 10–150 colonies, multiply by the dilution factor, and report results in CFU/g.

AOAC *Performance Tested Method*℠ Certified Claim

The PathoSEEK® Total Yeast and Mold Detection Assay with SenSATIVAx® DNA Purification and Grim Reefer® Free DNA Removal has been validated according to the AOAC *Performance Tested Method*℠ Program. The validation study met the requirements as set forth in the AOAC *Standard Method Performance Requirements*℠ 2021.009 for viable yeast and mold count enumeration in cannabis and cannabis-infused products.

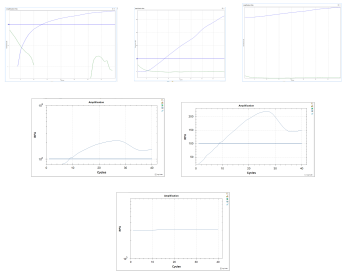
Matrix claims:

Dried cannabis flower (>0.3% THC), 10 g: detection and enumeration of total yeast and mold at contamination levels >10^3 CFU/g on the AriaMx and CFX-96 real-time PCR instruments. MIP and concentrates methods not included with AOAC claim.

In the inclusivity testing, *Botrytis cinerea* and *Scopulariopsis acremonium* were not detected by the method.

Troubleshooting Guide

Symptom	Reason	Solution
Internal Control Failure	Extraction Failure	Repeat SenSATIVAx™ and PathoSEEK™ by following the protocol.
	Residual ethanol in elution	Ethanol is an inhibitor to PCR. Return to the SenSATIVAxTM protocol and repeat all steps.
	Mix up in Reaction Setup	Repeat the qPCR by following the protocol.
	Missing Fluorophore on plate set up	In the Data Analysis window click on View/Edit Plate Setup from the Settings drop down. All wells should have both FAM and HEX. Once completed and the window is closed the analysis should automatically update.
	Matrix related (ex: age, remediation, ground)	Repeat SenSATIVAx and PathoSEEK following the protocol. If unacceptable internal control results are obtained a second time, Internal Cannabis Control may be spiked into samples during extraction for the third run. Please visit our Help Center for more information.
	qPCR inhibition	Dilute eluted samples 1:10 with nuclease free water and rerun qPCR. Multiply resulting CFU/g value by 10 to account for the additional dilution.
Internal Control Positive result on positive or negative control samples or samples that do not contain plant DNA No Cq or a Cq of more than 35 is acceptable. Any Cq lower than 35 constitutes a rerun	Plant DNA contamination in a reagent	Troubleshoot which reagent was contaminated; use new reagents, thoroughly clean all pipettes and bench areas with 10% bleach solution.
	qPCR bench too close to extraction area	Designate separate benches, pipettes etc. for extractions and qPCR setup
Positive Negative Control	Small Cq value <15	Visually confirm that there is an amplification curve. If not, this is a low level background and is to be expected.
	Contamination	Repeat the qPCR by following the protocol.
	Insufficient pre-setup bleaching	Wipe down the lab workspace and all equipment with 10% Bleach. Repeat qPCR.
Negative Positive Control	Mix up in Reaction Setup	Repeat the qPCR by following the protocol.
Potential false positive result	Small Cq value <15. Curve lacks an	Repeat the qPCR by following the

	exponential growth phase. Linear data view does not display a sigmoidal shaped curve. In the raw data the baseline appears to drift upwards for the entirety of the run. 	protocol.
Total run failure	Excessive vortex of the qPCR Master Mix	Repeat the qPCR by following the protocol.

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.

Polymerase Chain Reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

A **fluorophore** is a fluorescent chemical compound that can re-emit light upon light excitation.

The **Negative Controls** are the reactions where no Cq is expected. It helps to ensure that all Assay-specific reactions are clean of contaminants.

The assay-specific **Positive Controls** are the reactions where a Cq is expected. It helps ensure that all Assay-specific reactions are working correctly. The Assay specific Positive Control is targeting the pathogen using the FAM, ROX and Cy5 Fluorophores.

Amplification of the **Internal Control** is expected in every reaction containing cannabis DNA. It ensures the DNA isolation procedure was successful. The internal control targets plant DNA, or more specifically, a Single Copy Control Gene (SCCG), using the HEX Fluorophore.

MIP is short for Marijuana Infused Product. A MIP is cannabis plant material or concentrate mixed into a consumable.

Revision History

Version	Date	Description
v0	June 2021	1. New SOP - Full process in one document 2. Inclusion of v3 Master Kit for flower 3. Addition of TLP enzyme to SenSATIVAx protocol 4. Update Cq to CFU equation for flower
v1	June 2022	1. Update to User Guide Format 2. Inclusion of v3 Master Kit for MIPs 3. Changes to upfront dilution for flower 4. Addition of heating step in MIPs 5. Updates to Cq to CFU equations for flower and MIPs
v2	April 2023	1. Add Grim Reefer Free DNA Removal as an optional step in flower prep. 2. Update Cq to CFU equation for Flower 3. Removal of MIP enumeration option
v3	June 2024	1. AOAC PTM Certification 2. Update Grim Reefer Free DNA Removal as a required step as per AOAC
v4	February 2025	1. Add dilution of elution instructions when no HEX signal is observed in sample results

DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, for laboratory use. Any deviations from this protocol are not supported by MGC.

This test has not been validated on remediated (irradiated, ozone treated, acid treated, hydrogen peroxide treated, etc.) samples. Samples that have undergone remediation may cause discordant results between plating methods and PathoSEEK methods. When remediated samples produce a result above the action limit on qPCR, we recommend confirming viability with an approved plating method.

Results may vary based on laboratory conditions. Altitude and humidity are factors known to affect the growth of bacterial and fungal species.

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