MGC
2024
v1

SenSATIVAx® DNA Purification

QUICK GUIDE

Process Overview

SenSATIVAx is a proprietary DNA purification 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 sample or a large batch in less than one hour. Hands-on time is less than 45 minutes. Upon completion of this process, samples can be tested for Salmonella, STEC and Aspergillus contamination via PathoSEEK qPCR on the Bio-Rad CFX96, Agilent AriaMX or Biomolecular Systems MIC.

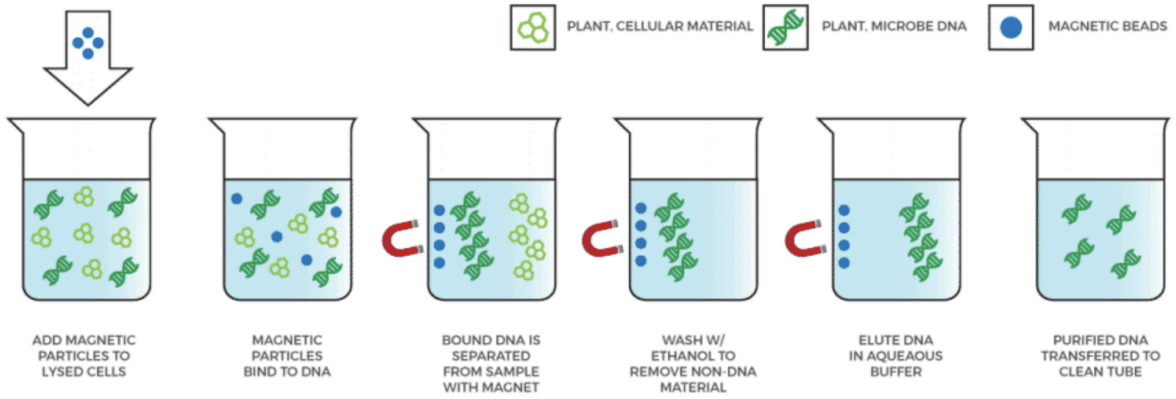

Figure 1: SenSATIVAx Purification Protocol

Test Kit Information

Assay Components:

1. SenSATIVAx Flower & Leaf DNA Purification Kit — P/N 420001 (200 extractions).
    a. MGC Lysis Buffer.— 1 bottle. Store at 20-28°C. Expires 1 Year from DOM
    b. MGC Binding Buffer.—1 bottle. Store at 2-8°C. Expires 1 Year from DOM
    c. MGC Elution Buffer.—1 bottle. Store at 20-28°C. Expires 1 Year from DOM
2. SenSATIVAx MIP DNA Purification Kit — P/N 420004 (200 extractions).
    a. Binding Buffer — 1 bottle. Store at 2-8°C. Expires 1 Year from DOM
    b. Elution Buffer — 1 bottle. Store at 20-28°C. Expires 1 Year from DOM
    c. Solution A — 1 bottle. Store at 20-28°C. Expires 1 Year from DOM
    d. Solution B — 1 bottle. Store at 20-28°C. Expires 1 Year from DOM
3. SCCG Control (referred to as ICC in this user guide) - P/N 420326. (60 ul)
    a. Control - 1 tube. Store at -15 to -20°C. Expires 2 Years from DOM

Consumables:

1. 96-well Extraction Plate—Medicinal Genomics P/N 100298 (Perkin Elmer P/N 6008290)
2. Adhesive optical seal for qPCR plates - Medicinal Genomics P/N 100177
3. Adjustable, variable volume pipettes (single or multichannel).—P10, P20, P50, P200, 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 (optional) —VWR #89004-570
7. 1.5 µL 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 5425 or similar with ability to spin up to speeds of 14,000-19,300 RCF
13. Incubator—Capable of maintaining 37 ± 2°C, VWR #97025-630 or equivalent
14. Filter Bags—Medicinal Genomics P/N 100008 (Whirl-Pak #B01385WA)
15. Beaker or Solo Cup (optional)
16. Tryptic Soy Broth— Medicinal Genomics P/N 420205 (Store at 2-8°C)
17. 1.5 mL Eppendorf Tubes
18. 15 mL or 50 mL conical tubes
19. 96-well Magnet Plate— Medicinal Genomics P/N 420202
20. Eppendorf Tube Rack
21. Scientific Scale—Capable of measuring to milligrams
22. Refrigerator—Capable of maintaining 2–8°C
23. 25mL Serological Pipette—VWR 89130-890 or 89130-900 or equivalent
24. 10% bleach
25. 70% Ethanol— Medicinal Genomics P/N 420030
26. Chloroform (Ethanol as preservative/ Certified ACS) - Fisher Scientific, C298

Sample Preparation

1. Aliquot Tryptic Soy Broth (TSB).
   Note: TSB is a very good growth medium for microbes. Due to this, it is best to pour the approximate amount of TSB into another sterile tube or container so as to not contaminate the whole bottle. Return to 2-8°C refrigerator immediately after use.
2. Wipe down the workspace with a 10% bleach solution, including the bench top 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.
7. Before weighing out the sample to be tested, make sure that the entire sample is broken up and thoroughly homogenized. A well-homogenized sample will ensure more accurate testing.
8. *Cannabis flower, **n** grams* —Weigh flower sample material into one side of the mesh liner inside the Whirl-Pak bag. Add 9 x *n* mL of TSB to each test portion. Close the Filter bag by folding the top over three times. Mix for 1 minute by hand.
9. *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 sample and TSB.
10. Incubate Samples
    a. Salmonella and STEC: 16-24h at 37 ± 1°C.
    b. Aspergillus: 24-48h at 37 ± 1°C.
11. If processing multiple samples, be sure to change gloves between each, to ensure there is no cross-contamination between samples during the weighing process.

DNA Purification

SenSATIVAx for Flower DNA Purification

1. Remove Whirl-Pak from the incubator and hand homogenize for 1 minute.
2. Aspirate 1 mL from the side of the filter bag, free of plant debris, and dispense into the 1.5 mL tube.
3. Add 50 µL of MGC Lysis buffer and vortex for 10 seconds then let incubate on the bench for 2 minutes.
4. Pellet cellular debris by spinning in a high-speed centrifuge for 1-3 minutes.
   Note: The supernatant should be translucent at this point. If the sample is still opaque (cloudy) spin for longer. This is important for removing cellular debris.
5. Transfer 200 µL of supernatant to a labeled 96-well extraction plate. Label the extraction plate with the [date].
   Note: Do not disturb the pellet in the 1.5ml tube, the pellet size will vary depending on trichome density.
6. Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in the buffer for at least 30 seconds.
7. 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 as to not contaminate the wells in proximity.
8. Incubate the plate on the bench for at least 5 minutes.
9. Place the extraction plate onto the 96-well plate magnet plate for at least 5 minutes.
10. After the 5 minute 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 plate.
    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.
11. 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.

12. After all the EtOH has been removed, allow the beads to dry for up to 15 minutes. Necessary drying time will vary based on the complete removal of the second ethanol wash, as well as the 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.
13. Remove the extraction plate from the magnet plate and add 25 µL or 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.
    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 eluent to a new extraction plate labeled with "Final Extract [date]".
14. Seal the plate with the adhesive seal, making sure to completely seal the plate wells. Slide a pen or flat object back and forth along the seal. Store at -20°C until ready to perform the qPCR protocol.

SenSATIVAx® for MIP/Extracts DNA Purification

1. Prepare the Internal Cannabis Control (ICC) control dilution of 1:5,000
    a. Label a new 1.5 mL Eppendorf tube (ICC 1:50), add 1µl of ICC positive control into 49 µl of dH2O. Vortex to mix thoroughly and quick spin tube. Label another 1.5 mL Eppendorf tube (ICC 1:5,000), add 99 µl of dH2O, then add 1µl of the ICC 1:50 dilution. Vortex to mix thoroughly and quick spin tube. This will result in a 1:5,000 dilution of ICC.
    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 ICC for approximately 10 extractions. If more extractions 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 ICC.

2. Add initial sample weight x 4.6 mL SenSATIVAx Solution A to conical tube with enriched sample/TSB. Vortex the sample vigorously until homogenized.
3. Transfer 1 mL of the homogenized sample into a 1.5 mL tube.
4. Add 10µL of the internal cannabis control (1:5,000) to 1.5 mL tube and vortex to mix well.
5. 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.
6. 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.
7. Add 600 µL chloroform and vortex vigorously until solution turns a milky white color.

    Note: This may require longer vortexing for some matrices

Caution: ALWAYS WEAR GLOVES WHEN HANDLING CHLOROFORM

8. Centrifuge for 5 minutes at 14,000 - 19,300 RCF using a benchtop centrifuge.
    a. 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 cannabis 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.
9. Transfer 100 µL of aqueous layer (TOP LAYER) from Step 8 to a well of the labeled 96-well extraction plate. Be careful not to disturb the lower chloroform layer.
10. 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.
11. Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in buffer for at least 30 seconds.
12. 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 as to not contaminate the wells in proximity.
13. Incubate the plate on the bench for at least 5 minutes.
14. Place the extraction plate onto the 96-well plate magnet plate for at least 5 minutes.
15. 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 plate.

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.

16. 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.

17. After all the EtOH has been removed, 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.

18. Remove the extraction plate from the magnet plate and add 25 µL or 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.

    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 eluent to a new extraction plate labeled with "Final Extract [date]".

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

REVISION HISTORY

Version	Date	Description
v1	February 2024	Stand Alone SenSATIVAx in Quick Guide Format

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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