Please refer to http://www.medicinalgenomics.com/product-literature/ for updated protocols and Material Safety Data Sheets (MSDS). Consult MSDS before using any new product.

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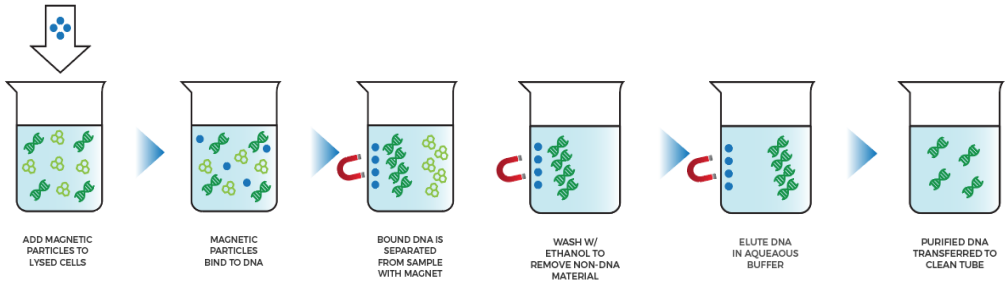
Introduction

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 sample. This approach is designed for ease of use and minimal requirement of laboratory equipment. Large centrifuges have been replaced with lightweight mini-fuges, magnetic particles, and magnets. The use of magnetic particles affords 8 tip or 96 tip automation, enabling both minimal entry costs and high throughput applications. DNA can be isolated from a single sample or a large batch in under 1 hour. Hands-on time is less than 45 minutes.

To enable minimal laboratory overhead, all organic solvents have been replaced with non-caustic reagents and 70% EtOH. Magnet plates are available for purchase from Medicinal Genomics (part #420202).

Process Overview



Kit Specifications

The SenSATIVAx™ MIP/Extract DNA Purification Kit (1g) contains 200 reactions (Medicinal Genomics #420005) worth of reagents.

Materials Supplied in the Kit

- MGC Binding Buffer (Store at 2-8ºC)
- SenSATIVAx Solution A (Store at Room Temperature, 20ºC to 28ºC)
- SenSATIVAx Solution B (Store at Room Temperature, 20ºC to 28ºC)
- MGC Elution Buffer (Store at Room Temperature, 20ºC to 28ºC)

Materials Supplied by the User

Consumables & Hardware:

- 15mL or 50mL Conical Tubes (USA scientific, PN 1475-0511)
- Solo Cups or Beaker (optional)
- MGC Enrichment Broth, store at 2 ºC-8 ºC (Medicinal Genomics #420205)
- 1.5 mL Eppendorf tubes (Multiple Suppliers)
- 96 well plate magnet (Medicinal Genomics #420202)
- 96 well extraction plate (Perkin Elmer #6008290)
- Adhesive optical seal for qPCR plates (Bio-Rad Microseal® # MSB-1001 or USA Scientific TempPlate® RT Optical Film # 2978-2100)
- Multi-channel pipettes P20 and P300, or P50 and P1000 (optional)
- Single channel pipettes P20, P200, & P1000
- Filtered pipette tips for P20, P50, P200, & P1000
- Eppendorf tube rack
- Scientific scale (milligram)
- Refrigerator, +4 ºC (for storage of MGC Binding Buffer)
- 25 mL Sterile Serological Pipettes (VWR #89130-890 or #89130-900, or similar

- Incubator, that can reach 37 ºC (VWR® Personal Size Incubator # 97025-630, or similar)

- High Speed centrifuge to accommodate 1.5 mL tubes such as Eppendorf model 5414 R or similar with the ability to spin up to speeds of 14,000 rpm.

- Table top mini tube centrifuge (VWR® Mini Centrifuge #10067-588 or 6-place personal microcentrifuge for 1.5/2.0 mL tubes # 2631-0006, or similar)

- Table top Vortex Genie (Scientific Industries #SI-0236 or Similar)

Reagents:

- 10% Bleach
- 70% Ethanol (EtOH) (Medicinal Genomics, #420030)
- Chloroform (Fisher Scientific, C298-1)

Hazard Statement: 70% Ethanol
Highly flammable liquid and vapor May cause respiratory irritation
May cause drowsiness or dizziness Causes damage to organs
May cause damage to organs through prolonged or repeated exposure
Please refer to the Material Safety Data Sheet (MSDS) for more information and proper disposal

Quantification Tests (Total Count Tests)

To quantify the total microbes present in a sample no enrichment is necessary. For this protocol MIPs/Extract samples go directly into Solution A.

Enrichment (Presence/Absence Tests)

Enrichment of microbes from cannabis products is required for accurate detecting the presence of microbes down to **1 CFU**. Detection of ***Aspergillus*** requires a **24 hours** enrichment at **37 ºC**. ***Salmonella*** and ***E. coli*** also require **16-24 hours** of enrichment also at **37 ºC**.
For enrichment add the sample to **600µL TSB** and place the sample in an incubator for to enrich.

Before Weighing and Preparing Samples

1. Aliquot out your TSB enrichment broth. *Note: TSB Enrichment Broth is a growth medium and the perfect condition for microbes to grow. Due to this, it is best to pour the approximate amount of MGC Enrichment Broth into another sterile tube or container as to not contaminate the whole bottle. Nothing should go into this bottle. Return it to the 2-8ºC refrigerator immediately after use.*
2. 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.
3. Prepare your consumables. Label all the conical tubes you'll need with "[sample name] [date]".

Weigh and Prepare Samples for Quantification Tests (Total Count Tests)

1) Weigh out **0.22-0.28g** of sample into the labeled 15mL (or 50mL) conical tube.
   Note: Extracts and concentrates may not go completely into solution.

2) Add **1.75mL** of **SenSATIVAx Solution A** the conical tube. Vortex the MIP/extract vigorously until thoroughly homogenized.

Fig. 1 Conical tube containing sample and TSB.

Weigh and Prepare Samples for Enrichment (Presence/Absence Tests)

1) Weigh out **0.22-0.28g** of sample into the labeled 15mL conical tube.
   Note: Extracts and concentrates may not go completely into solution.

2) Add **600µL** of **TSB Broth** to the conical tube. Vortex the MIP/extract vigorously until thoroughly homogenized. Place in incubator to enrich.

Before Beginning DNA Extraction

1. Wipe down the workspace with a 10% bleach solution, including the bench top and all equipment being used.
2. Remove the MGC Binding Buffer from the 2-8°C refrigerator (it should come to room temperature before use).
3. Prepare your consumables. Label all the 1.5mL centrifuge tubes needed 2 per sample "[sample name.1], [Sample name.2]"
4. Label extraction plate with date, and if transferring eluted DNA to new plate
5. Prepare a SCCG positive control dilution of 1:5,000 (internal control)

    a. Label a new 1.5mL Eppendorf tube (SCCG 1:50), add **1µl** of SCCG positive control into **49µl** of dH2O. Vortex to mix thoroughly and quick spin tube. Label another 1.5mL Eppendorf tube (SCCG 1:5,000), add **99µl** of dH2O, then add **1µl** of the SCCG 1:50 dilution. Vortex to mix thoroughly and quick spin tube. This will result in a 1:5,000 dilution if 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 in step 6a is enough diluted SCCG 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 SCCG.

DNA Extraction Protocol

1. Lysing the sample.
   - **For total count tests:** Move to Step 2. The sample should already be in **Solution A**.
   - **For absence/presence tests:** After **24 hour** incubation at 37°C, add **1.15mL** SenSATIVAx **Solution A** to conical tube. Vortex the sample vigorously until homogenized.

2. Transfer 1000µL to the first 1.5mL centrifuge tube

3. Add 10µL of the SCCG internal control (1:5,000) and vortex to mix well

4. Centrifuge for **10 minutes** at 14,000 rpm using a high-speed bench top centrifuge
   - If no bench top 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.
5. Transfer **600µL** of the solution to a new tube. Push pipet tip through the top solid layer (if one exists), without disturbing the pellet on the bottom to aspirate the sample.

6. Add **600µL** chloroform and vortex vigorously until solution turns a milky white color throughout
   Note: This may require longer vortexing for some matrices
   Caution: ALWAYS WEAR GLOVES WHEN HANDLING CHLOROFORM

Fig 2. Example of milky sample with chloroform in it.

7. Centrifuge for **5 minutes** at 14000 rpm using a bench top centrifuge
   - If no bench top 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 **300µl** of the top layer to a new 1.5mL tube and add **300µL** chloroform, vortex and centrifuge again.

8. Transfer **100µL** of aqueous layer (TOP LAYER) from Step 7 to a well of the labeled 96 well extraction plate. Be careful not to disturb the lower chloroform layer.

9. Add **100µL**of SenSATIVAx Solution B to the 100ul sample in the 96 well extraction plate.

Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely resuspended in buffer at least 30 seconds.

10. Add **200µL** 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.

11. Incubate the plate on the bench for at least **5 minutes**.
12. Place the extraction plate onto the 96 well plate magnet plate for at least **5 minutes**.
13. Remove as much of the 400µL of the supernatant as possible.
    Be careful not to disturb or aspirate the beads.

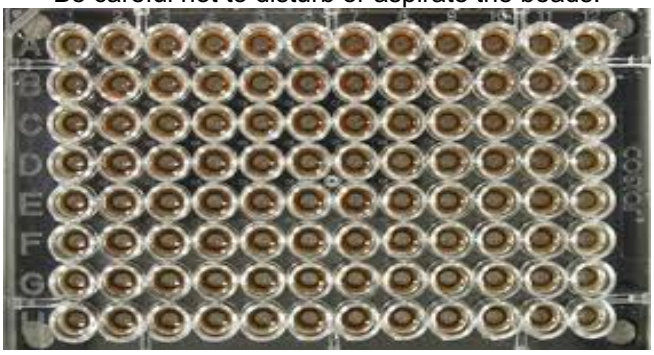


Fig. 3 Extraction plate during wash step on magnetic plate.

14. Add **400µL** of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.

15. Wait at least **30 seconds** and remove all the EtOH.
    Note: Place the pipet tip at the bottom center of the well to remove all liquid.

16. Repeat **400µL** 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 pipet tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.

17. After all the EtOH has been removed let the beads dry at room temperature on the magnet plate for **15 minutes**.
    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 **50µL** of MGC Elution Buffer. (**25µL if using eluted DNA for the 5-Color Aspergillus Assay**)
    - 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.

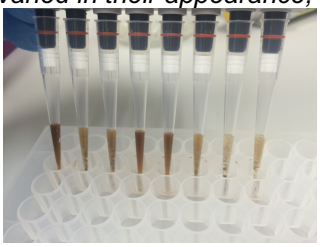


Fig. 4 Multichannel pipette tips showing magnetic beads resuspended in Elution Buffer.

    - Incubate the plate for at least **1 minute** on the bench, then return to the magnetic rack for at least **1 minute.**
19. Transfer as much as possible of the **50µL** of elution buffer with DNA to a newly labeled plate.
    NOTE: Elute with 25µL if using sample DNA with the 5-Color Aspergillus Assay

20. 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 4 ºC overnight until ready to perform qPCR protocol (if storing longer freeze at -20 ºC.)

Troubleshooting Guide

Symptom	Reason	Solution
Clumpy/Grainy Beads	Over-manipulation of plant with MGC Enrichment Broth	Over manipulation of the plant can cause the release of extra cellular debris therefore clogging the beads with extra material. To ensure this does not occur, only manipulate the plant material for 1 minute.
	Too many tricomes and/or insufficient spinning	Some plants produce more tricomes than others resulting in carry-over into extraction plate. To ensure this doesn't happen, it may be necessary to spin the tube for longer than the recommended 30 seconds. Also, be sure not to disturb the pellet. If the pellet is disturbed or tricomes are still visible, re-centrifuge the tube and try again.
Bead Loss	Insufficient time on the magnet	Make sure the supernatant has fully cleared before removing. Failure to do so will result in bead loss, which will result in DNA loss.
	Insufficient pipetting	Make sure no beads are aspirated during supernatant removal; dispense back supernatant, and attempt again with a smaller volume after beads have re-settled.
Extra elution volume	Insufficient removal of Ethanol	Make sure ALL ethanol is removed before drying. This may require a second or third aspiration. Carry-over ethanol can cause inhibition in qPCR.

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.

A **supernatant** is the liquid lying above the solid residue after centrifugation.

An **eluant** is a solution containing the DNA released from the MGC Binding Buffer.

Homogenize is to make uniform or similar.

MIP stands for Marijuana Infused Product

SCCG stands for 'Single Copy Cannabis Gene' and is DNA used as a Internal control for DNA extractions from samples without cannabis plant material.