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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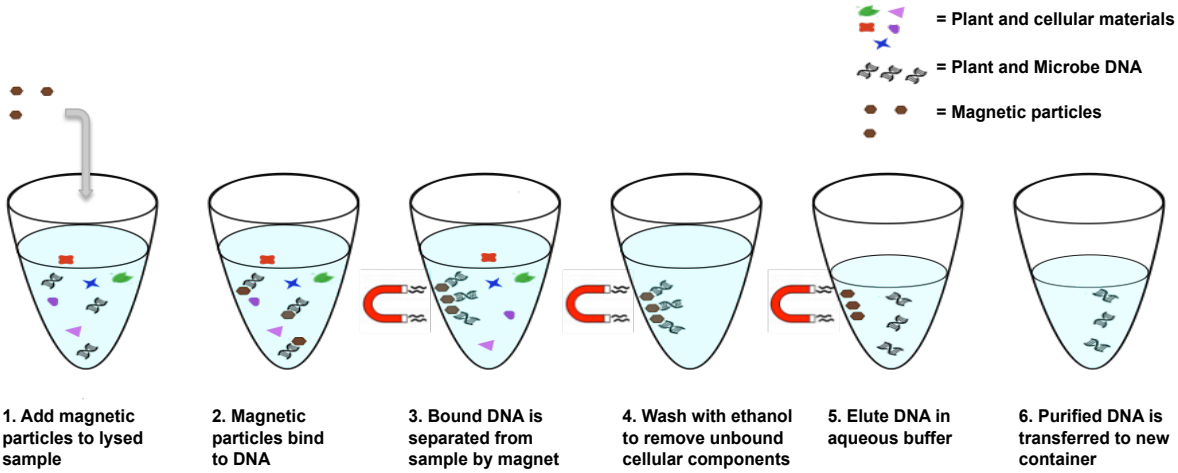
Introduction

SenSATIVAx™ for MIP/Extract is a proprietary DNA isolation process that uses magnetic particles to isolate and purify both plant and microbial DNA from a Marijuana infused product or a flower extract. 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 minimal entry costs and high throughput applications. DNA can be isolated in less than 1 hour. Hands-on time is less than 45 minutes. Magnet plates are available for purchase from Medicinal Genomics (part #420202).

Process Overview



Kit Specifications

The SenSATIVAx™ MIP/Extract DNA Purification Kit contains 200 reactions (Medicinal Genomics #420004) 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)

Additional Materials to Purchase from Medicinal Genomics

- SCCG Positive Control (Store at -20°C) (Medicinal Genomics #420326)
- MGC Enrichment Broth (store at 2º-8ºC) (Medicinal Genomics #420205)

Materials Supplied by the User:

Consumables & Hardware:

- 15mL Conical Tubes (USA scientific, PN 1475-0511)
- Solo Cups or Beaker (optional)
- 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, +4C (for storage of MGC Binding Buffer)
- Incubator, that can reach 37ºC (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 (USA Scientific, # 2631-0006, or similar)

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

Reagents:

- 10% Bleach
- 70% Ethanol (EtOH) (American Bioanalytical product # AB00844-01000)
- Chloroform (Fisher Scientific, C298-1)

Extraction #1 Protocol (CFU Threshold Assays):

1. Begin with a 10% bleach wipe down of the workspace, including the bench top and all equipment being used.
2. Remove the MGC Binding Buffer and the MGC Enrichment Broth from the 2-8°C refrigerator (let come to room temperature before use).
3. Label a new 15mL conical tube with the "[sample name] [date]". Weigh out **0.22-0.28g MIP or Extract sample** and put into the labeled conical tube. If processing multiple MIP/extracts, be sure to change gloves between each sample to ensure there is no cross contamination of MIP/Extract during the weighing process.
4. If performing threshold test, proceed to step 5. If performing a presence absence test (*Salmonella*, *E. coli*, *Aspergillus*) skip to 'Extraction # 2" part of protocol on page 6.
5. Add **1.75 mL** of SenSATIVAx Solution A to conical tube. Vortex the MIP/extract vigorously until thoroughly homogenized.
6. Prepare a SCCG positive control dilution of 1:5,000
   a. Label a new 1.5mL Eppendorf tube (SCCG 1:50), add 1ul of SCCG positive control into 49ul of dH2O. Vortex well to mix thoroughly and quick spin tube. Label another 1.5mL Eppendorf tube (SCCG 1:5,000), add 99ul of dH2O, then add 1ul of the SCCG 1:50 dilution. Vortex well 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 uL of liquid. Visually check your pipette tip after aspirating 1 uL 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 uL dilution prepared in step 6a is enough diluted SCCG for approximately 20 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.
7. Label a new 1.5mL tube with the "[sample name] [date]". Transfer **500ul** of sample from step 4 and **5ul** of diluted SCCG positive control (1: 5,000) to tube. Vortex briefly to mix.
8. Centrifuge for **10 minutes** at 14,000 rpm using a high-speed bench top centrifuge
   a. 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.
9. Label a new 1.5mL tube with the "[sample name] [date]". Transfer **300ul** of the solution from Step 7 into the tube. Place pipet tip through the top solid layer (if one exists), without disturbing the pellet on the bottom to aspirate the sample.
   a. Add 300uL of Chloroform to the tube.
   ALWAYS WEAR GLOVES WHEN HANDLING CHLOROFORM
   b. Vortex vigorously until solution turns a milky white color throughout, this may require longer vortexing for some matrices.

10. Centrifuge for **10 minutes** at 14,000 rpm using a high-speed bench top centrifuge
    a. 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.

11. Label a 96 well extraction plate with 'Extraction Plate Day 1 [date]" or "Extraction Plate Day 2 [date]". Transfer **100**µL of supernatant (TOP LAYER) from Step 9 to a well of the labeled 96 well extraction plate. Be careful not to disturb the lower chloroform layer.
    a. If it is difficult to remove 100 uL of supernatant without aspirating some of the interphase or organic (lower) phase, see Appendix I: Larger Volume Protocol.

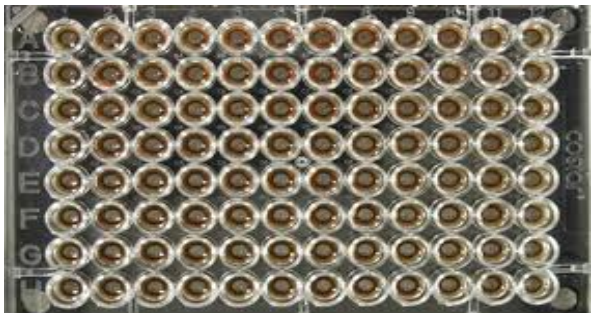
12. Add **100ul** of SenSATIVAx Solution B to the 100ul sample in the 96 well extraction plate and mix.

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

14. Add **200µL** of MGC Binding Buffer (this liquid is very viscous) to the 200µL sample, and pipette tip mix 15 times.
    a. Incubate the plate on the bench for at least **5 minutes**.
    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.

15. Place the extraction plate onto the 96 well plate magnet plate for at least **5 minutes**.

16. After 5 min incubation, remove as much of the 400ul of the supernatant as possible. Be careful not to disturb or aspirate the beads.
    a. Add **400µL** 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: Take the pipet tip to the bottom center of the well to remove liquid.



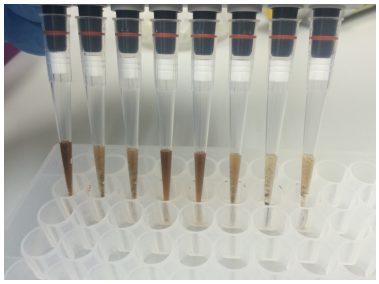
17. Again, add **400µ**L of 70% EtOH with the extraction plate still on the magnet plate. Wait at least **30 seconds** and remove all the EtOH.

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.

18. 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.

19. Remove the extraction plate from the magnet plate and add **50µL** 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 magnet 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 Day 1 [date]” or ”Final Extract Day 2 [date]”.
Note: To save space and consumables, both day 1 and day 2 extracts can be stored in separate wells on the same extraction plate if space allows.

20. Seal the plate with an adhesive seal. Make 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 qPCR protocol.

Extraction #2 Protocol (Presence/Absence)

1. If performing a presence/absence test (E. coli, Salmonella or Aspergillus), add 600ul TSB to conical tube with weighed sample. Incubate the conical tube at 37ºC for **16-24 hours**. Incubate a full 24 hours for Aspergillus testing.
2. After **16-24 hour** incubation at 37ºC, add **1.15mL** SenSATIVAx solution A to conical tube. Vortex the sample vigorously until homogenized.
   Note: If using the Aspergillus Specific Detection Assays, you must incubate for the full 24 hours to insure proper growth.
3. Repeat steps 6-20 from Extraction #1 Protocol.

Appendix I: Larger Volume Protocol

NOTE: These steps should be followed in place of steps 7-10 above when processing samples where it is very challenging to remove 100 uL post chloroform spin

1. Label a new 1.5mL tube with the "[sample name] [date]". Transfer **1000 ul (1mL)** of sample from step 4 and **10ul** of diluted SCCG positive control (1: 5,000) to tube. Vortex well to mix.
2. Centrifuge for **10 minutes** at 14,000 rpm using a high-speed bench top centrifuge
   a. 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.
3. Label a new 1.5mL tube with the "[sample name] [date]". Transfer **600ul** of the solution from Step 7 into the tube. Place pipet tip through the top solid layer (if one exists), without disturbing the pellet on the bottom to aspirate the sample.
   a. Add 600uL of Chloroform to the tube.
   ALWAYS WEAR GLOVES WHEN HANDLING CHLOROFORM

   b. Vortex vigorously until solution turns a milky white color throughout, this may require longer vortexing for some matrices.

4. Centrifuge for **10 minutes** at 14,000 rpm using a high-speed bench top centrifuge
5. 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.

Continue to step 11, page 6

Troubleshooting Guide:

Symptom	Reason	Solution
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 residual 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 **eluent** is a solution containing the DNA released from the MCG 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 positive control for extractions