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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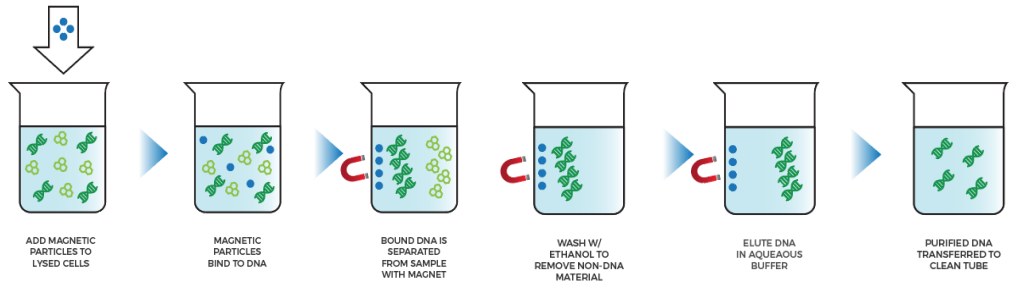
Introduction

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.

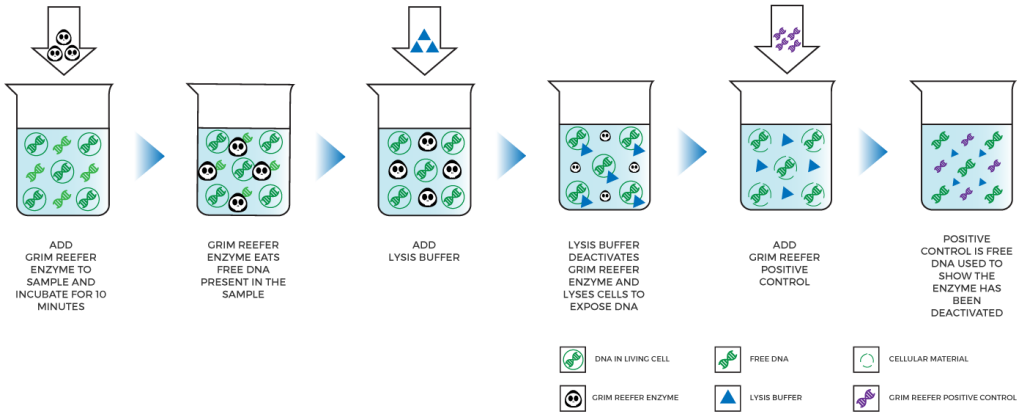
To enable minimal laboratory overhead, all organic solvents have been replaced with non-caustic reagents and 70% EtOH. Magnet plates are available for purchase from Medicinal Genomics (part #420202).

Process Overview

NO Grim Reefer



+ Grim Reefer



Kit Specifications

The SenSATIVAx™ Plant/Microbial DNA Purification Kit contains 200 reactions.

Materials Supplied in the Kit

- MGC Lysis Buffer (Store at Room Temperature, 20°C to 28°C)
- MGC Binding Buffer (Store at 2-8°C)
- MGC Elution Buffer (Store at Room Temperature, 20°C to 28°C)

If performing Optional Grim Reefer Free DNA Removal Step the following reagents are also required:

- Grim Reefer Free DNA Removal Kit, Medicinal Genomics #420145 (Store at -20°C) (used in this SOP)
- Grim Reefer Free DNA Removal Control, Medicinal Genomics #420144 (Store at -20°C) (used in this SOP)
- Grim Reefer Free DNA Removal Assay, Medicinal Genomics #420143 (Store at -20°C) (used in qPCR Setup SOP)

Materials Supplied by the User

Consumables & Hardware:

- Whirl-Pak bags (Nasco #B01385WA)
- Solo Cups or Beaker (optional)
- MGC Enrichment Broth, store at 2°C-8°C (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, +4C (for storage of MGC Binding Buffer)
- 25 mL Sterile Serological Pipettes (VWR #89130-890 or #89130-900, or similar

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

- 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)
  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 there should be no enrichment. Once your sample is homogenized in TSB broth start the DNA extraction process.

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**. If you are running total counts along with presence/absence, pull your 1mL to extract and then place the sample in the incubator.

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 whirlpak bags you'll need with "[sample name] [date]".

Weigh and Prepare Samples

1. Weigh out **1.00-1.03 g** of sample into the labeled Whirl-Pak bag.
   Make sure to add all of the sample material to one side of the mesh layer inside the Whirl-Pak bag
2. Add **14.2 mL** of **TSB Broth** to Whirl-Pak bag. Mix the homogenized plant material in TSB Enrichment Broth for at least **1 minute** with your fingers, one sample at a time.

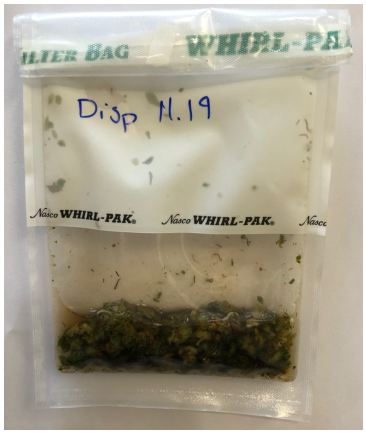


Fig. 1 Whirlpak bag containing homogenized plant sample and TSB.

- **If performing a total count test, proceed directly to section "Before Beginning DNA Extraction"**
- **If performing presence/absence tests, incubate for 24 hours to enrich the sample.**

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 2-8ºC refrigerator (it should come to room temperature before use).
3. Prepare your consumables. Label all the 1.5mL centrifuge tubes needed "[sample name]
4. Label extraction plate with date, and if transferring eluted DNA to new plate

If performing the Grim Reefer extracellular DNA removal step do the following to prepare:

1. Allow a 1.5mL tube rack to come to temperature in a 37 ºC incubator
2. Thaw the 10X GR Buffer
3. Thaw Stock GR Positive Control and make a 1:10,000 dilution:
   (i) Make a 1:100 dilution (1µL positive control + 99µL water mix well)
   (ii) Make a second 1:100 dilution (1µL of 1st 1:100 dilution + 99µL water mix well). This is your 1:10,000 dilution.

Grim Reefer can be helpful if you are concerned about free/dead DNA causing a false presence result in your samples. Learn more about Grim Reefer here: https://www.medicinalgenomics.com/free-dna-removal-kit/

DNA Extraction Protocol

1. Aspirate **1 mL** from the side of the filter bag, free of plant debris, and dispense into the 1.5mL tube.

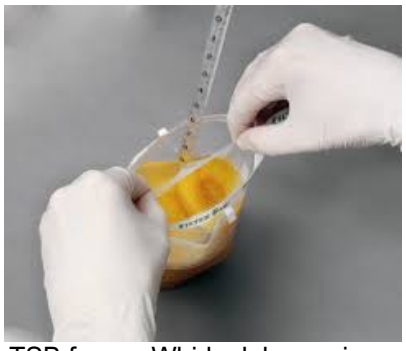


Fig 2. Aspirating TSB from a Whirlpak bag using serological pipette

If Performing the Grim Reefer extracellular DNA removal step perform the following steps. Otherwise proceed directly to **Step 2**

a. Add 112µL of 10X GR Buffer and mix well by vortexing
b. After vortexing add 20µL of GR Enzyme and mix well by vortexing.
c. After addition of the GR Enzyme immediately incubate at 37C for 10 minutes.

2. Add **50µL** of MGC Lysis buffer and vortex for 10 seconds then let incubate on the bench for **2 minutes.**
   - **If processing with Grim Reefer Free DNA Removal** add 10µL of the diluted GR Positive control (1:10,000) and mix by vortexing.
     Note: The GR Positive Control is used to show the GR enzyme was completely deactivated during the lysis step.

3. After 2-minute incubation, spin for at least **1-3 minutes** in a bench top mini centrifuge.
   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.

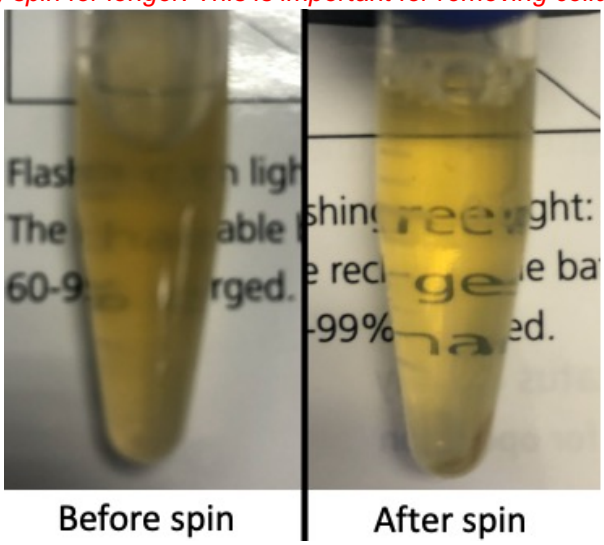


Fig. 3 Example of translucent lysate after spinning, notice pellet of cellular debris

4. Remove the **200**µL of supernatant from the 1.5ml tube in a 96 well extraction plate
   Be careful not to disturb the pellet at the bottom of the tube.

   Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in buffer at least 30 seconds.
5. 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.
6. Incubate the plate on the bench for at least **5 minutes**.
7. Place the extraction plate onto the 96 well plate magnet plate for at least **5 minutes**.
8. Remove as much of the 400µL of the supernatant as possible.
   Note: Be careful not to disturb or aspirate the beads.

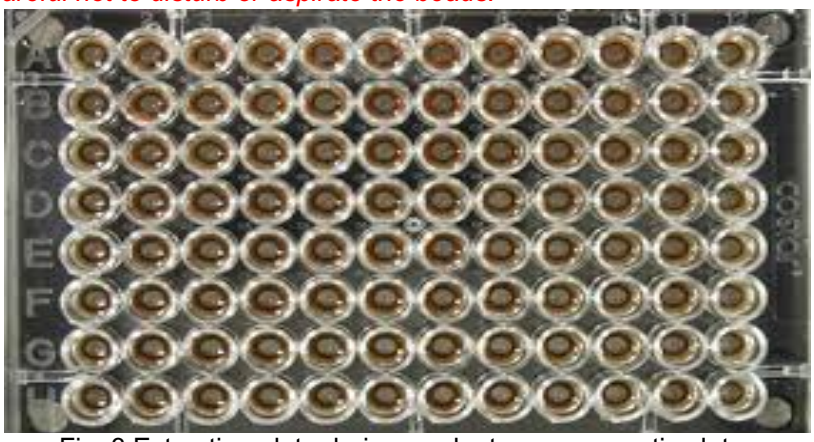


Fig. 3 Extraction plate during wash step on magnetic plate.

9. Add **400µL** of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
10. 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.
11. 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.
12. 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.
13. 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**)
    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.

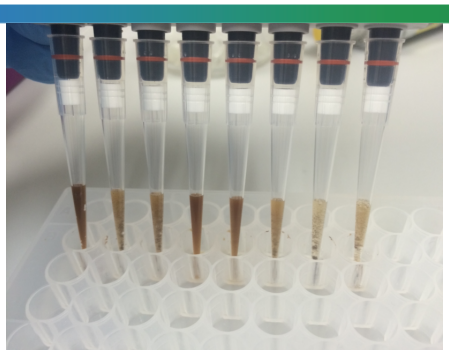


Fig. 4 Multichannel pipette tips showing magnetic beads resuspended in Elution Buffer.

b. Incubate the plate for at least **1 minute** on the bench, then return to the magnetic rack for at least **1 minute.**

14. Transfer as much as possible of the **50µl** of elution buffer with DNA to a newly labeled plate.

15. 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.