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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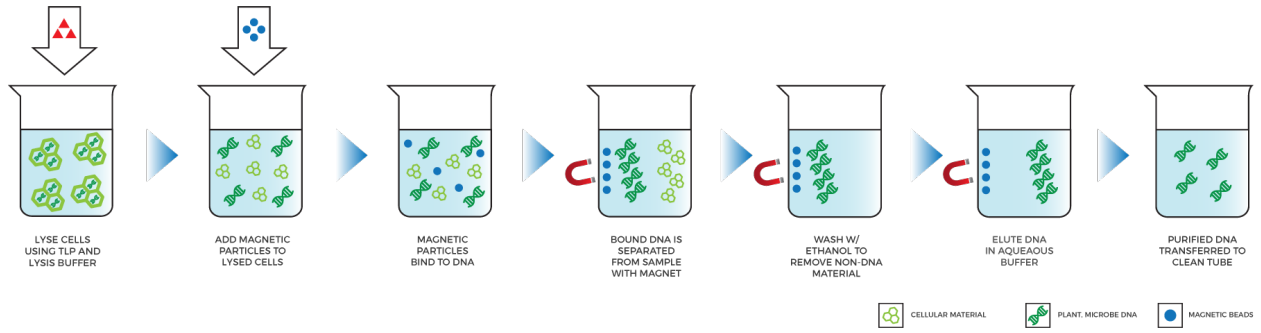
Table of Contents:

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® Plant/Microbial DNA Purification Kit contains reagents for 200 extractions. (Medicinal Genomics #420001)

Materials Supplied in the Kit

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

Extraction enzyme ordered separately:

- SenSATIVAx® TLP Extraction Enzyme, store at -20 ºC (50 reactions) (Medicinal Genomics #420206)

NOTE: Grim Reefer Extracellular DNA removal kit can NOT be used with this assay.

Materials Supplied by the User:

Consumables & Hardware:

- Whirl-Pak bags (Nasco #B01385WA)
- Solo Cups or Beaker (optional)
- Tryptic Soy Broth, store at 2ºC-8ºC (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, 2ºC-8ºC (for storage of Tryptic Soy Broth and MGC Binding Buffer)
- 25 mL Sterile Serological Pipettes (VWR #89130-890 or #89130-900, 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.

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

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

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

Reagents:

- PCR Grade Nuclease Free Water (Medicinal Genomics, #420184)
- 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

Extraction Protocol:

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 Tryptic Soy Broth from the 2-8ºC refrigerator (it should come to room temperature before use).

3. 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.

4. Label a new Whirl-Pak bag with the "[sample name] [date]". After homogenization, transfer desired weight of sample to be tested into the labeled Whirl-Pak bag. Make sure to add the sample material to one side of the mesh lining inside the Whirl-Pak bag. If processing multiple plant samples, be sure to change gloves between each, to ensure there is no cross contamination of flowers during the weighing process.

    a. Add Tryptic Soy Broth to sample in Whirl-Pak bag. Volume of TSB is determined by multiplying grams of sample weight by 9mL. For example, a 1g sample would require 9 mLs of TSB. Close the Whirl-Pak bag by folding the top over three times.
    Note: Tryptic Soy 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 Tryptic Soy 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.

    b. Mix the homogenized plant material in Tryptic Soy Broth for at least **1 minute** with your fingers, one sample at a time.

5. Prepare and label a 1.5mL tube with the "[sample name]". Aspirate **1 mL** from the side of the filter bag, free of plant debris, and dispense into the 1.5mL tube.
6. Spin tubes at 12,000 – 14,000 rpm for 5 minutes
7. Carefully and slowly remove and discard **950 uL** of the supernatant by aspirating liquid with 1mL pipette without disturbing the pellet.
8. Resuspend the pellet in **200 µL** of nuclease free water
9. Add **12µL** of TLP Extraction Enzyme to Cell/Water mixture and vortex 30 seconds
10. Incubate tubes in 37 ºC incubator for 30 minutes
11. Add **12.5µL** of MGC cell lysis buffer and vortex 30 seconds
12. Let incubate on the bench for 5 minutes
13. After 5-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.

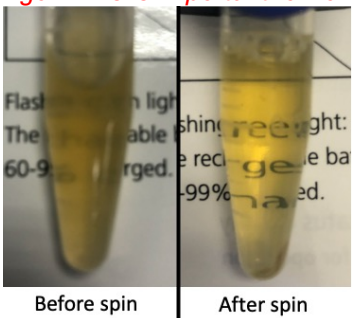


Fig. 3 Example of translucent lysate after spinning notice pellet of cellular debris

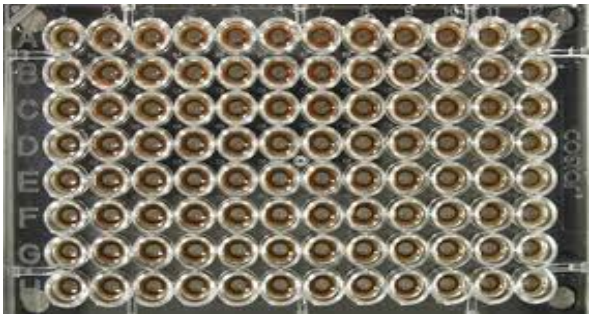
14. Remove the **200µL** of supernatant from the 1.5ml tube containing the centrifuged sample, being careful not to disturb the pellet at the bottom of the tube. Place the 200µL in a labeled 96 well extraction plate labeled with "Extraction Plate [date]".
    Note: Pellet size will vary depending on trichome density.

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

16. Add **200µL** of MGC Binding Buffer (this liquid is very viscous) to the 200µL sample, and pipette tip mix thoroughly.
    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.

17. Place the extraction plate onto the 96 well plate magnet plate for at least **5 minutes** to allow magnetic beads to form rings at the bottom of each well.

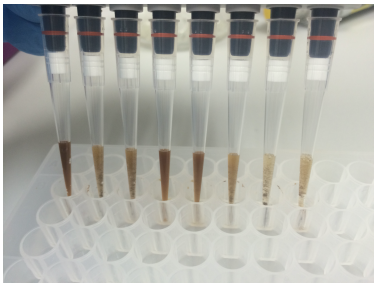
18. After 5 min incubation, remove as much of the 400ul of the supernatant as possible. Be careful not to disturb or aspirate the beads.
    a. Add **400µL** of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
    b. Wait at least **30 seconds** and remove all the EtOH.
    Note: Take the pipet tip to the bottom center of the well to remove liquid.



19. 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.

20. 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.

21. Remove the extraction plate from the magnet plate and add **50µL** MGC Elution Buffer
    a. Tip mix approximately 15 times or until the bead rings are completely re-suspended in the elution buffer.
    Note: The re-suspensions may appear varied in their appearance.



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 clean part of the same place or a new extraction plate labeled with" Final Extract [date]".

22. 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. If setting up qPCR reactions right away, the plate can stay at room temp or **4ºC**. If the qPCR reactions will not be prepared right away, store at **-20ºC** until ready to perform qPCR protocol.

Troubleshooting Guide:

Symptom	Reason	Solution
Clumpy/Grainy Beads	Homogenizing flower material too fine or over-manipulation of plant with Tryptic Soy 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.

Safety Precautions

Assay users should observe standard microbiological practices and safety precautions when performing this extraction. Wear protective gloves, lab coats, eye/face protection as indicated by your quality system.
It is the responsibility of each laboratory to handle waste and effluents processed according to their nature and degree of hazardousness and to treat and dispose of them in accordance with applicable local, state, and federal regulations.

DISLCAIMER

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.

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