SenSATIVAx® Kit de Purificación ADN Hoja y Flor

MEDICINAL GENOMICS

Introduction

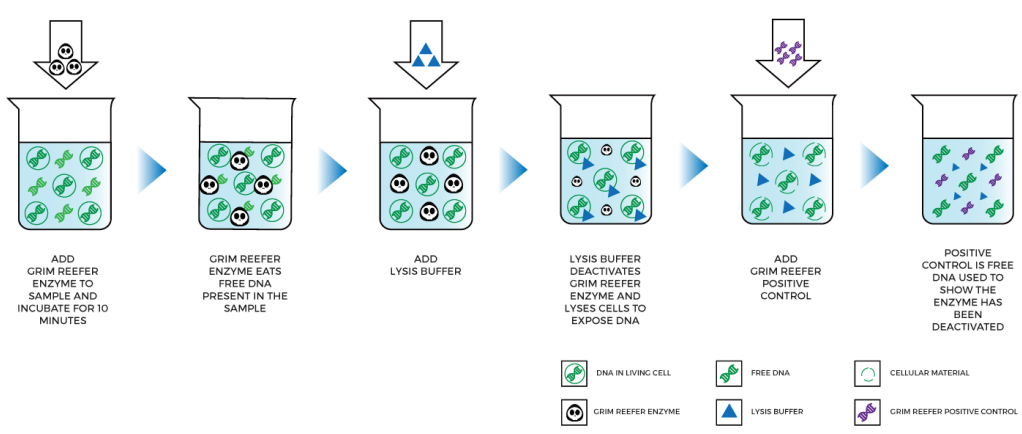
SenSATIVA x™ is a proprietary DNA isolation process that uses magnetic particles to isolate and purify both plant and microbial DNA from a raw, homogenized plant sample. This approach is designed for ease of use and minimal requirement of laboratory equipment. Large centrifuges have been replaced with 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



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Kit Specifications

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

Materials Supplied in the SenSATIVAx 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)
- 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

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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 MCG Binding Buffer and the MGC Enrichment Broth from the 2-8°C refrigerator (it should come to room temperature before use).

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

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

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, weigh out **0.22-0.28g,** and put 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. 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 **3.55mL** of MGC Enrichment Broth to Whirl-Pak bag.
   Note: MGC 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 2-8°C refrigerator immediately after use.
      i. Close the Whirl-Pak bag by folding the top over three times.
   b. Mix the homogenized plant material in MGC Enrichment 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] [date]". Aspirate **500 µL** from the side of the filter bag, free of plant debris, and dispense into the 1.5mL tube.

   NOTE: If using a presence/absence test (*E.coli*, STEC, Salmonella or Aspergillus), save and incubate the Whirl-Pak bag at 37°C for **16-24 hours**, and proceed to the Extraction #2 set up**. Incubate a full 24 hours for Aspergillus testing.**

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If Performing the Grim Reefer extracellular DNA removal step perform the following steps. Otherwise proceed directly to step 6

- Add 56uL of 10X GR Buffer and mix well by vortexing (briefly spin to remove liquid from cap).
- After vortexing add 10uL of GR Enzyme and mix well by vortexing (briefly spin to remove liquid from cap).
- After addition of the GR Enzyme immediately incubate at 37C for 10 minutes. At the end of 10-minute incubation immediately proceed to step 6, add lysis buffer and vortex to inactivate the GR enzyme

6. Add 25 µL of MGC Lysis buffer to the sample.
    a. Vortex for 5 seconds and incubate on the bench for 2 minutes.
    b. **If processing with Grim Reefer Free DNA Removal** add 5uL 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.

7. After 2-minute incubation, spin for at least **30 seconds** in a bench top mini centrifuge.

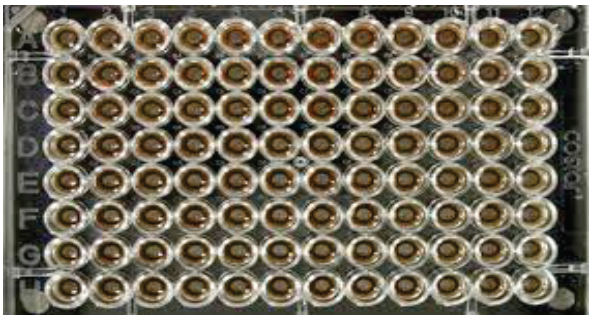
8. Remove the **200**µL of supernatant from the 1.5ml tube containing the centrifuged sample, being careful not to disturb the pellet at the bottom of the tube. Place the 200µL in a labeled 96 well extraction plate labeled with "Extraction Plate Day1 [date]" or "Extraction Plate Day2 [date]".
    Note: Pellet size will vary depending on trichome density.

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

10. 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.

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

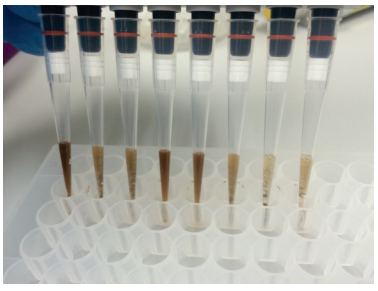
12. After 5 min incubation, remove as much of the 400ul of the supernatant as possible. Be careful not to disturb or aspirate the beads.
    a. Add **400µL** of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
    b. Wait at least **30 seconds and** remove all the EtOH.
    Note: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



13. Again, add **400µL** 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.

14. 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.

15. Remove the extraction plate from the magnet plate and add **50µL** (**25 uL if using eluted DNA for the 5-Color Aspergillus Assay**) 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 eluent 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.

16. 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 -20°C until ready to perform qPCR protocol.

Extraction #2 Protocol (Presence/Absence)

After an **16-24-hour** incubation at 37°C has occurred remove **500µL** from the side of the filter bag free of plant debris, and put into a 1.5mL tube and repeat steps 5-16.
Note: If using the Aspergillus Specific Detection Assays, you must incubate for the full 24 hours to ensure proper growth.

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.

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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.