

LEAF SHEATH BIOPSY (LSB) COLLECTION PROTOCOL FOR RSD LAB - 2021

OVERVIEW

For each block/field, collect 50 discs from lowest green leaf sheaths, add 4 ml water to submerge discs, incubate overnight, transfer liquid to sample tube.

EQUIPMENT LIST

Item	Specifications	Supplier	Product Name/Notes
Biopsy punch tools	8 mm diameter	SIGNET	Meister - Sterile Dermal Biopsy Punch
Wire 'pokers'	~2 mm diameter	Homemade	Size to fit hole in biopsy punch
Ziplock bags	75mm x 100 mm	SIGNET	Signet's Own - Selfseal bags
Disposable pipettes	3 ml	LabTek	650.050.503 Pipette Transfer 3mL Graduated
Sample tubes	5 ml	LabGear	SSI Centrifuge Tube - 5.0 mL Sterile, Clear (200/bag)
Household bleach	4-5% hypochlorite	Supermarket	White King Bleach Standard 2.5L
Deionised water	-	Supermarket	Demineralised / deionised water
Falcon tubes	50 ml (or similar)	-	Or any clean disposable container for dispensing water
Tray/container for bleach	-	-	e.g Round screw top watertight canister 0.5 - 1L
Tray/container for water	-	-	e.g Round screw top watertight canister 0.5 - 1L
Disposable gloves/ wet-wipes	-	-	-
Esky and ice bricks	-	-	-

COLLECTION METHOD

IN FIELD

1. Use a clean biopsy punch to collect discs from around the **base** and **centre** of the lowest green leaf sheath:
2. Use a slight twisting motion to push the tool into one layer only (try not to cut into the underlying stalk)
3. Remove the tool and ensure the disc has been collected
4. Place the head of the biopsy punch tool into the sample bag
5. Use the wire to push out the leaf sheath disc, then pull the wire out to continue sampling
6. Collect 50 discs into one ziplock bag (one sample). Approx ~5 discs can be collected before pushing them out with wire, more than this may clog up the tool
7. Close and label the ziplock bag
8. Keep samples cool while continuing sampling (e.g. in esky with ice bricks)
9. Use a clean biopsy punch and wire for the next sample (do not reuse punch and wire for sampling until cleaned - see below)
10. Clean hands between samples/fields with soap and water or a wet-wipe, or use disposable gloves

IN OFFICE/LAB

1. After collecting samples for the day, add water to the LSB sample bags:
2. In a clean working area, pour deionised water into a clean falcon tube or clean beaker/container (you will need 4 ml per sample e.g. for 10 samples, add a little over 40 ml)
3. Carefully open sample bag
4. Using a disposable pipette, carefully transfer 4 ml deionised water into the sample bag over the leaf discs, ensuring the pipette tip does NOT touch the sample bag (dispose of the pipette if it touches the bag to prevent cross-contamination into the next sample)
5. Ensure all discs are submerged in water at the bottom of the bag
6. Keeping the sample bags upright rather than laying flat will help keep the discs submerged in water (using a bulldog clip, or by placing the bags in a small container)
7. Incubate sample bags overnight (16 - 24 hrs) in the fridge (4 degrees) - no longer than 24 hrs
8. The next day, the liquid suspension will need to be transferred to 5 ml tubes for sending to the RSD lab:
9. Set up a tube rack with one new 5 ml tube for each sample
10. Using a pipette, transfer the maximum volume of liquid from each sample bag into the labelled tube (approx. 3 ml, as the discs will absorb some water) and close lids
11. Use a new pipette for each sample
12. Freeze samples upright to prevent lids popping open, deliver/post to RSD lab for testing

METHOD FOR CLEANING EQUIPMENT

OVERVIEW

1. Soak in bleach for 5 - 10 mins (10% solution of household bleach = approx. 0.4-0.6% hypochlorite)
2. Rinse thoroughly with water

METHOD DETAILS

1. Make up a fresh 10% bleach solution by diluting 1 part household bleach (usually 4 % - 6% hypochlorite) to 9 parts tap water
2. E.g. Add 100 ml bleach to 900 ml water to make up 1 L of diluted bleach solution
3. If necessary, first rinse sampling equipment (biopsy punches, wire) to remove any leaf material
4. Place equipment in a tray or bucket of 10% bleach solution to submerge (round canisters with watertight screw-top lids are handy for in the field/utes)
5. Keep in bleach for 5 - 10 minutes (do not leave for too long in bleach as the metal will corrode)
6. Transfer to a second tray/bucket of clean water to rinse off the bleach
7. Transfer to a clean rack or paper towel to dry
9. The biopsy punches and wire are now ready to reuse (keep in a clean bag or container)

Note: A fresh bleach solution needs to be made up each day, as the chlorine will evaporate over time and will become less effective.

SUMMARY OF LSB RESEARCH FINDINGS (TIPS FOR MAXIMISING CHANCE OF DETECTION):

Timing

For PLANT crops, wait until plants are ~7 months to take LSBs. For RATOON crops, you can take LSB samples as soon as the leaf sheath is formed enough to take samples.

Field strategy

Take discs from as many different parts of block as possible to maximise chance of detection (i.e. not just one or two corners).

Biopsy punch tool/diameter

Exact disc diameter is not so important, we use 8mm diameter discs as they are easy to take samples. Biopsy punches can be re-used until blunt.

Number of discs

50 discs per sample (per ziplock bag) is currently recommended to give an appropriate sample size of block.

Leaf sheath selection

Lowest green leaf sheath, avoid red/brown patches. If lowest green leaf sheath has red/brown patches, use second lowest if it is greener.

Position on leaf sheath

Middle base of leaf sheath rather than edges. Target green, fleshy material and avoid red/brown/dried out areas.

Ziplock bag

Small ziplock bag (e.g. 75mm x 100 mm) so all discs are covered once water is added.

Water volume

Add 4 ml deionised/demineralised water (available from supermarket) for 50 x 8 mm discs. If using a different size punch, adjust volume to make sure all discs are covered with water.

Incubation time

Overnight (~16hrs) or up to 24 hrs at 4 degrees (refrigerated). Keep ziplock bags upright to keep discs submerged e.g. use a bulldog clip and/or or place in a small container.

Suspension transfer

Using a clean disposable pipette, transfer as much liquid as possible from each bag into a sample tube (5ml plastic tube with RSD lab barcode attached).

Storage and postage to RSD Lab

Freeze samples upright in 5 ml tubes, deliver/post to RSD lab with ice bricks to keep as cold as possible.

Decontamination of equipment

Soak biopsy punch and wire in 10% bleach for 5-10 mins followed by water rinse. Other cleaning methods are not as effective: we found approx. **30%** chance of carry-over contamination to subsequent sample after a positive sample if tools were cleaned with 70% metho/ethanol and water rinse, and approx. **50%** chance with no wash, compared to **0%** when cleaned with bleach.



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