INSTRUCTIONS FOR THE COLLECTION OF RSD SAMPLES

QPCR ASSAY XYLEM EXTRACTS

Sugar Research Australia

STALKS PER CROP: 16

Extract from 16 stalks to be placed into one 5ml tube.

EXTRACT SAMPLE TUBES

- ***Bulking extracts:*** extracts from a crop should be placed into one (5mL) tube; this will require just a few drops from each stalk. 0.5ml is the minimum needed to undertake a qPCR assay.
- ***Barcode:*** each 5ml tube must be labelled with a small bar code (Provided)

  Please place the barcode down the 5mL tube, not around the tube.
  As shown in picture (below right).
- ***Barcode records:*** should be recorded on the registration sheet. (Provided)
  - The sample registration form can be downloaded from the SRA website.
- ***Sample preservation:*** samples should be frozen as soon after collection as possible and sent by Express post to the RSD assay lab
  - sample tubes need to be kept cool during transportation by placing them in a cooler bag or cooler box along with 2-3 frozen gel packs.
- ***Sample details:*** please send your completed form by email to RSDLab@sugarresearch.com.au

DECONTAMINATION OF RSD SAMPLE COLLECTION TOOLS

Introduction: with the use of the more sensitive qPCR assay, an associated change is needed in the decontamination procedure – to ensure that RSD DNA is removed from all cutting surfaces. Although 70% methylated spirits will kill the bacterium, it does not remove all the DNA. There are two proposed methods for decontaminating cutting surfaces, one involves sodium hypochlorite (household bleach), the other ethanol in association with a clean water rinse of the equipment.

METHODS

Ethanol:

1. Ethanol 70% ethanol (70% ethanol / 30% clean water).
   - This ratio is needed as 100% ethanol is ineffective.
   - Treatment time: a 10min soak in the solution is needed to be effective
   - Duplicate cutting equipment: ensure you have at least 2 sets of cutting equipment
     - leave one set to soak in a bucket of 70% ethanol,
     - the other cutting equipment can be used to take the required samples
       > Then swap the equipment over.
2. ***Water flush:*** It is important to flush the DNA from the cutting equipment. Take a water container on the back of the vehicle and use this for rinsing off the disinfectant, once it has been soaked for a minimum of 10mins. It is unlikely that running water (from a hose) will be available to be accessed when sampling in the field.
3. ***Overnight:*** at the end of the day sampling tools should be left to soak in the 70% ethanol overnight in a bucket or similar and washed down the next day with water.
   - After sampling has finished for the day, the rubber connector should be removed from the air hose and placed into the 70% ethanol solution
   - the tubing should also be soaked overnight and rinsed in the morning with water.
   - If your rubber connector is cracked or worn, replace it with a new one.
4. ***Secateurs:*** if these are attached to a wooden block, the wooden/metal base plus the secateurs should be sprayed with the ethanol mixture until wet and also left for ten minutes.

Bleach: – Method can be downloaded from the SRA website.

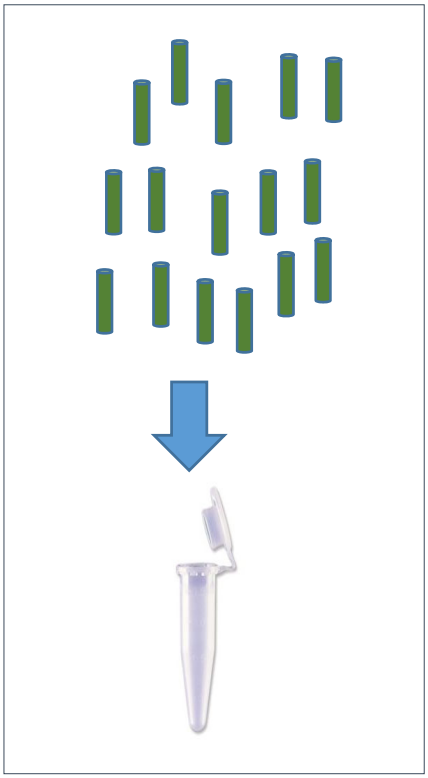

*Diagram showing the 16 stalks being placed in a 5ml tube. **(Above)** 5ml tube with barcode.*

5ml tube with barcode.

5ml tubes can be purchased from LABGEAR Australia for $48 (200/bag)

Product details: [SSIB1410-00S] SSI Centrifuge Tube - 5.0 mL, Graduated, Clear (200/bag)

LabGear
T 1800 522 432
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