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# Hydroponic Seedling Culture for Low-Contaminant Wheat Tissue Collection

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**We use this protocol and it's working**

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## Abstract

De-novo gene annotation of wheat genome assemblies and other RNA-seq studies require tissue collection from various tissues, including roots, for RNA extraction and sequencing. Obtaining clean tissue samples from roots can be difficult and tedious when seedlings are grown in soil or potting mix. Here, wheat plant growth for tissue collection is adapted to a hydroponic method suitable for collection from young seedling plants. The steps explain set up a small-scale hydroponic system within a growth chamber using a shallow water tank and air pump in addition to germination and maintenance of wheat plants. Finally, steps for collecting flash-frozen tissue samples are detailed. This protocol establishes a hydroponic method for wheat seedling culture that allows for easy, low-contaminant root and shoot tissue collection. It is applicable to high molecular weight DNA and RNA extractions from young wheat seedlings for various omics studies.

## Guidelines

METADATA:

The following metadata must be recorded digitally for each sample; write the bolded information on the sample tube as well (Ex. "AGS2000.root.1 2023-01-31"):

- **Genotype (Ex. "AGS2000")**
- **Tissue type (Ex. "root", "shoot")**
- **Replicate (Ex. "1")**
- **Collection date (Ex. "2023-01-31")**
- Growth stage (using **Zadoks** single digit scale)
- PI Accession # (if available)
- NCBI BioProject # (if available)

SEED PURITY:

For data integrity, it is imperative that tissue is collected from the intended genotype. In bread wheat, these are typically highly inbred 'pure lines' that have been selfed for 8+ generations. Use only these 'pure lines' and ensure that they have been meticulously managed.

Best practice: To safeguard against any residual outcrossing, isolate plants of or bag heads during flowering for at least two generations prior to use.

## Materials

**Seed Germination**

- Small clear plastic box with lid, such as petri dish
- Paper towels
- Sterile water

**Growth Chamber  Preparation**

- 32 L tank (10-12" deep)
- Air pump and tubing
- Opaque plastic tray cut to cover tank, with 20 holes each 3" in diameter
- 3" solid foam tubing, cut into sections (approx. 4")

**Flash-Frozen Tissue Collection**

- Liquid nitrogen (LN2)
- LN2 cannister
- Tongs
- Digital scale
- 80% ethanol
- Sterile 15 ml collection tubes
- Gloves

## Safety warnings

Flash-frozen tissue collection requires the use of liquid nitrogen (LN2). Safety training is required.

## Before start

Obtain all required equipment prior to starting.

Note: This protocol was developed for subsequent sequencing library preparation facilitated by the Genomic Sciences Laboratory (**GSL**) at NC State. If you are planning to use a sequencing facility other than the GSL, check in regarding their input and transfer requirements and adjust this protocol as needed.

## Seed Germination

1w

1 Prepare one germination box per genotype.

1.1 Line the bottom of a clear plastic germination box with a moistened, but not wet, paper towel.

1.2 Place 10-20 seeds evenly across the paper towel. To avoid cross-contamination, never germinate different genotypes concurrently in the same box.

1.3 Return the clear lid back to the box and place it near a window to germinate.

2 Monitor germination.

1w

2.1 Check routinely. Every few days, replace the moistened paper towel with fresh materials to avoid mold growth.

2.2 Once the seedlings have 3-5 roots and the shoot is exiting the coleoptile, they are ready to be transplanted.

## Growth Chamber Preparation

1h

3 Prepare a cover tray for the tank.

3.1 Using aluminum foil, wrap the cover tray entirely. Gently create holes in the positions you plan to grow seedlings in and fold foil around the edges.

3.2 Cut styrofoam tubes in thirds and cut a slit halfway into one side of each slice. Place a styrofoam slice in each opened position in the cover tray.

4 Prepare 1:1 dilution of nutrient solution.

4.1 Prepare a nutrient solution, such as the NCSU Phytotron Nutrient Solution given in the References section. If using the growth chambers at the Phytotron, this can be obtained

directly from the watering hose.

4.2 Add 16 L of nutrient solution to the tank.

4.3 Add 16 L of distilled water to the tank and mix.

5 Set up the air pump by attaching tubing to the pump and placing at the bottom of the water tank. Plug in the pump and run at a low setting. Situate the pump and tubing in the tank such that the water is evenly oxygenated.

6 Return the cover tray to the top of the tank.

## Transplanting Seedlings

30m

7 Gently transfer a single germinated seedling into a 3" foam slice, taking care to ensure the roots are beyond the bottom of the foam and the shoot is pointed upwards.

8 Place the seedling-filled foam plug into the cover tray, taking care not to damage roots. The foam plugs should fit snugly in the cover tray.

9 Repeat these steps for the desired number of seedlings.

10 Place the cover tray, now filled with seedlings, into the top of the hydroponic tank. To avoid microbial growth, ensure that any remaining holes in the cover tray are covered with foil or foam.

## Plant Maintenance

2w

11 Monitor growth of the seedlings.

2w

11.1 Routinely change the 1:1 nutrient solution used in the tank, every 3-4 days.

11.2 Seedlings are ready for tissue collection when they have reached the 5-leaf stage.

## Flash-Frozen Tissue Collection

1h

12 Following safety precautions, fill a small cannister approximately halfway with liquid nitrogen (LN2). This will be used to flash-freeze the tissue.

13 Prepare a workspace.

13.1 Get the prepared LN2 cannister, a scale, 80% ethanol, gloves, and sterile 15 ml tubes.

13.2 Carefully remove each plant to be sampled with its foam plug, taking care to detangle and keep the roots. Gently shake excess water from roots. Place plants on clean paper towels and allow them to drip dry while transferring them to the prepared workplace.

14 Collect **shoot tissue** from a single plant/experimental unit only. Try to move quickly when handling cut tissue so that the RNA does not degrade.

14.1 Tare an empty, labeled, 15 ml tube on the scale. Follow the labelling instructions listed under "METADATA" in the Guidelines & Warnings section.

14.2 Wearing gloves and spraying with 80% ethanol between samples, remove the main tiller shoot from the plant by gently tearing at the base of the stem. Take care to collect only young, healthy tissue. Remove and discard chlorotic and/or necrotic areas.

14.3 Return the tube cap and weigh the sample.

14.4 If <30 mg, add additional leaf tissue from the youngest leaves to the sample tube until 30-50 mg is obtained.

14.5 Securely tighten the tube cap and, using tongs, carefully immerse the sample tube fully in LN2 to flash-freeze.

15 Collect 30-50 mg of **root tissue** from the same experimental unit. Try to move quickly when handling cut tissue so that the RNA does not degrade.

15.1 Tare an empty, labeled, 15 ml tube on the scale. Follow the labelling instructions listed under "METADATA" in the Guidelines & Warnings section.

15.2 Wearing gloves and spray cleaning them with 80% ethanol between samples, gently remove a small section of root tissue. Take care to collect only healthy tissue.

15.3 Gently shake off excess water on the root sample, then add them to the sample tube.

15.4 Return the tube cap and weigh the sample.

15.5 If <30 mg, add additional healthy, air-dry root tissue to the sample tube until 30-50 mg is obtained.

15.6 Securely return the lid to the sample tube and, using tongs, carefully immerse the tube fully in LN2 to flash-freeze.

16 Transfer flash-frozen tissue to long-term storage.

16.1 Allow flash-frozen samples to rest in LN2 for at least 20 minutes to fully freeze before transfer.

16.2 Using tongs, quickly transfer samples to a -80°C freezer for long-term storage. Take care not to allow samples to thaw during transfer.

## Protocol references

NCSU Phytotron Nutrient Solution: https://phytotron.ncsu.edu/wp-content/uploads/2016/10/Nutrient-info.pdf

Zadoks Growth Scale: https://www.agric.wa.gov.au/grains/zadoks-growth-scale

*NCSU Collaborator Links*

Phytotron: **https://phytotron.ncsu.edu/**

Genomic Sciences Laboratory: **https://research.ncsu.edu/gsl/**

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