Tree and plant root samples for RNA-Seq

How to dig, clean and preserve root tissue (liquid nitrogen or RNAlater) so the RNA stays intact for RNA-Seq, with quality targets for difficult, polyphenol-rich roots.

Roots contain polysaccharides, polyphenols, soil inhibitors and active RNases, so careful sampling and fast preservation are critical.

1. Plan the experiment

- Choose the root type for your question: fine roots (under 2 mm) for nutrient uptake and stress; lateral roots for branching; tap roots for growth; root tips (1–2 cm) for cell division and gene expression.
- At least **3 biological replicates** (independent trees or plants) per treatment, at the same developmental stage and the same time of day.
- Avoid decayed, dark brown or black, or damaged roots.

2. Prepare before going to the field

- Liquid nitrogen and cryovials (or RNAlater and RNase-free 2 mL tubes), RNase-free forceps and scissors, gloves, cool box with ice packs, 70% ethanol and an RNase decontaminant (for example RNaseZAP), labels and waterproof marker.
- Label tubes in advance: sample ID, species, location, root type, treatment, date and time, replicate.
- Clean tools between replicates; new gloves for each sample.

3. Dig and collect

- Remove soil gently with sterile brushes or forceps; do not pull or crush roots.
- Select healthy roots and cut 2–5 cm segments, or 1–2 cm white, actively growing tips.
- Collect **100–300 mg** fresh root tissue per sample (more for low-yield species).

4. Remove soil quickly

Shake off loose soil, rinse briefly in RNase-free water if needed and blot dry. Do not wash for long, as this changes stress-gene expression. Aim for **less than 2–3 minutes** from digging to preservation.

5. Preserve immediately

Option 1: Liquid nitrogen (best)

Put roots straight into cryovials and snap-freeze. Keep in liquid nitrogen or on dry ice during fieldwork, then store at −80 °C.

Option 2: RNAlater (when liquid nitrogen is not available)

1. Cut roots into 0.5–1 cm pieces and put them into RNAlater within about 1 minute.
2. Use 5–10 volumes of RNAlater: about 1 mL per 100 mg tissue. Every piece must be fully covered.
3. Close tightly and invert a few times.
4. Keep at 4 °C for 12–24 hours so RNAlater soaks in, then move to −80 °C.

Storage in RNAlater	Up to
25 °C	24 hours
4 °C	1 month
−20 °C	Several months
−80 °C	More than a year (recommended)

6. Transport

Ship in an insulated box with ice packs (RNAlater samples) or dry ice (frozen samples). Keep below 30 °C and out of the sun. Avoid freeze–thaw.

7. Root-specific challenges

Challenge	Effect	What to do
Polysaccharides	Low yield	CTAB/PVP-based extraction
Polyphenols	RNA oxidation	β-mercaptoethanol and PVP during extraction
Soil	Inhibitors, microbial RNA	Clean roots well before preserving
RNases, slow preservation	Degradation, biased profiles	Preserve within 1 minute
Rough handling	Wound-response genes switched on	Handle gently

8. Quality targets for extracted RNA

Parameter	Target
Concentration / total	At least 100 ng/µL and 1 µg
A260/280	1.9–2.1
A260/230	Above 2.0
RIN	7 or higher (preferably above 8)
DNA, protein, phenol	Not detectable

> Before extraction, pour off excess RNAlater and blot the tissue; carried-over RNAlater can interfere with some kits.

Questions before sending samples? WhatsApp / phone +880 1308-222824 · bdgenome@yahoo.com · bdgenomes.com/guides/plant-root-rna/