

# Intertek DNA Extraction

## Sampling instructions for DNA extraction of plant leaf samples in 96-format plates

### Key to font color annotations used in this document:

Recommendation, advice, or tips

Cautionary note

Requirement

### Introduction

- This document describes the general instructions for sampling plant leaf tissue for DNA Extraction Services at Intertek.
- If other types of tissue or samples are desired, then this should be discussed in advance and additional instructions should apply.

### Communication

- All email communications with Intertek should be addressed to Agritech Sweeden - <mailto:agritech.sweden@intertek.com>.
- Please keep Petra Van Roggen - <mailto:petra.vanroggen@intertek.com>, and Breeding Insight coordinators copied in all communications.

### Sampling plates and seals

- Use Intertek approved plastic ware and seals/mats only.
- The sampling plates should be in 96-well format and the wells/tubes should be of the round bottom style (not conical).
- If tube strips or single tubes are used, they should be properly secured to the plate before shipment to avoid sample mix-up and to withstand thorough grinding of the tissue.
- Recommended supplies: 1.2 ml AbGene Storage Plate (AB0564) and Sealing Mats (AB0674).

### Sampling devices

- Various sampling devices can be used as long as the samples are easily identified, and uniform sampling is maintained for all samples.
  - Scissors (grass-like leaves), single-hole punchers (broad-leaves) or (semi-) automated samplers such as the PlantTrak Hx system or AK-EP100 can be used.
  - A puncher should be dried and cleaned between samples by punching two or three punches of clean thick tissue paper. Punchers can also be sprayed or wiped with a paper towel dampened with 80% ethanol.

### Leaf tissue quality

- The sampled leaf tissue should be of 'good' and even quality to be able to extract good quality DNA. Only healthy, tender, young green tissue should be sampled.

- In order to create sets of samples with equal quality, it is important to always sample leaves of similar developmental stage.
- The stage is crop dependent, but in general a newly developed leaf, which is newly stretched and opened up, will provide the DNA quality and quantity needed for genotyping.

### Sampling instructions

- 1) Select the plants to be sampled. Label (tag) the plants before you sample. **Caution: Bad sample quality leads to low quality results.**
- 2) Make sure that your logistics are in order before you start sampling. **Advice: keep the samples and plates cool (on wet ice) while sampling.**
- 3) If you use a fixed-well format sample plate, decide on your plate layout before you start sampling. **Tip: Documentation is essential to link the sample ID and the plant ID.**
- 4) **Keep the two wells of the sampling plate G12 and H12 empty for lab controls.**
- 5) Label the sample plate, preferably both with human readable text and a barcode.
- 6) Select the kind of leaf to be sampled (developmental stage and quality):
  - a. Avoid sampling the main nerves/mid rib in the leaves. It's best to draw a sample from distal end of the leaf.
  - b. Avoid sampling leaves with soil and/or dirt.
  - c. Avoid cross-contamination at all steps. Clean the sampling tool before you start sampling and thereafter every sample.
  - d. If a plant is missing, or can't be sampled, then mark the well on the plate and make a note in the sampling sheet as this helps avoiding mis-sampling. **Tip: Wells for a missing sample can be marked by coloring the edge of the well with a marker pen or by placing a temporary cocktail stick or toothpick that is removed when sampling is completed.**
- 7) Sample size:
  - a. 4 leaf discs/punches of 4 to 6 mm diameter (no more than the area of a thumb nail) will give good quality and quantity DNA. **Do NOT sample more than agreed upon as too much tissue will hinder thorough drying and grinding and it will increase the amount of PCR and/ or sequencing inhibitors.**
  - b. **Note: For specific projects, other amounts of tissue outside of the recommendation can be agreed upon prior to sampling.**
- 8) Drying of samples:
  - a. Preferred: Lyophilization
  - b. Acceptable: Drying in an oven at 40-50 °C for 12-24 hours
  - c. **Caution: Do not use silica gel in tissue sample wells.**
- 9) Seal the plates or tubes with Intertek approved silicone mats, caps or seals. **Be sure to press down on the seal to secure it fully and completely on the plate.**
  - a. If tubes are used, apply a lid on the box and affix this with the clips and/or rubber bands. All plates can then be wrapped in (a) plastic bag(s) and sealed with tape or rubber bands.
  - b. If possible, add silica gel to the plastic bags to remove moisture during shipping.
- 10) Add the sealed plates, together with a printed Order form to the outer package (typically a cardboard box). Send it to the Intertek lab according to the instructions (see **How to send samples** below).
- 11) In parallel, send the same Order form electronically to the desired Intertek-Agritech lab.

- a. **The order should be announced by mail at least 15 days prior to sending leaf samples through the Intertek lab email, in order to ensure the fast turn-around time.** You can use a draft order form and email to announce the shipment.
- b. **For larger sample volume (>10,000 samples), please submit the order at least 25 days prior to sending the leaf samples.**

#### How to send samples

- **It is REQUIRED that the paperwork on the outside of the parcel with samples from plant materials is labelled: “For analytical purposes only, not for breeding, will be destroyed”**
- When filling in the **Commercial Invoice**, you can use the **Commodity Code 140490** for dried leaf samples of all crops ('Vegetable products, not elsewhere specified or included')
- Intertek recommend the courier DHL, for faster logistics, but other couriers could also be used.
- Send a printed copy of the **Order form** together with the samples AND in parallel, electronically to the desired lab e-mail address. Make sure to provide courier tracking information in the email.
- All customers will receive an order confirmation as soon as the samples have arrived.

#### INTERTEK SWEDEN LAB:

Elevenborgsvägen 2,  
230 53 Alnarp Sweden  
Phone: +46 40 69 28 001  
[agritech.sweden@intertek.com](mailto:agritech.sweden@intertek.com)

#### Additional comments

- When sampling in nurseries, very young leaf tissue or even cotyledons can be samples, however, please note that this may affect quantity and/or quality of the DNA hence some PCR/SNP assays and/or sequencing might fail.
- Typically, 1 µg of DNA is extracted from 2 leaf disk punches, although this varies considerably according to species, age of tissue, and other factors.
- For more information and shipping solutions, please contact respective laboratory.