Agri-Services

CGIAR HTPG PROJECT

Sampling instructions for SNP verification and routine SNP analysis

This document describes the general instructions for sampling leaf tissue for SNP analysis, part of the CGIAR HTPG Project.

It includes sampling for

1) Both verification and routine sampling
2) All crops
3) Sampling in either greenhouse or field
4) Leaf tissue only

If other kinds of tissues or sampling would be desired, then this should be discussed in advance and additional instructions should apply.

SNP verification versus routine SNP analysis

It is essential that the type, quality and quantity of the sample sent for SNP verification is comparable to the samples sent for routine SNP analysis.

A 'SNP verification test' is carried out by the Intertek lab on a panel of Reference material, such as Trait specific control plants, Parental lines and/or F1 Hybrids, and has various reasons:

1) Confirmation (= Verification) that the SNP is trait specific and/or differentiating Parental lines
2) Confirmation that the KASP assay obtained from LGC is technically working
3) Confirmation that the DNA extraction and SNPline are technically working in an optimal way
4) Confirmation that the DNA in leaf tissues is adequate and uniform in concentration for routine genotyping.

SNP verification is highly recommended for the above reasons to ensure the highest quality of data return on routine genotyping jobs. (Note: Even KASP markers previously used on a LGC system may potentially provide slightly different results due to hardware differences.)

Sampling plates and seals

Use Intertek approved plastic ware and seals/mats only. The sampling plates should be 96-well format, and the wells/tubes should be of the round bottom style (not conical) in order to sustain thorough grinding of the tissue. If tube strips or single tubes are used, then they should be properly secured to the plate before shipment. Some recommended supplies: 1,2 ml AbGene Storage Plate (AB056) and Sealing Mats (AB0674).

Sampling device

Various sampling devices can be used as long as the samples can be easily identified and uniform sampling can be maintained for all samples. The devices can be scissors, single-hole punchers or (semi-) automated samplers such as the PlantTrak Hx system or AK-EP100.

Leaf tissue

The leaf tissue should be of 'good' and even quality to be able to extract good quality DNA. This means that healthy and 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, newly stretched and opened up, would provide the DNA quality and quantity needed for genotyping.

Sampling instructions

1) Select the plants to be sampled.
2) Make sure that your logistics are in order before you start sampling.
3) Especially in case you use a fixed well format sample plate, decide on your plate layout before you start sampling. Documentation is essential to link the sample and the plant.
4) Keep the last two wells H11 and H12 of the sampling plate empty for lab controls.
5) Label the sample plate, both with human readable text and a barcode.
6) Select the kinds of leaves to be sampled (developmental stage and quality).
7) Sample the leaves. In general, 2 leaf disks of 5 to 6 mm diameter punches give good quality and quantity DNA.
8) Do NOT sample more than agreed upon. A general rule is that one should never sample more than the size of half the size of a thumb nail. (Too much tissue will prohibit thorough drying and grinding; it will increase the amount of PCR inhibitors and may negatively affect the quality of the SNP results.)
9) Avoid sampling the main nerves/mid rib in the leaves. Best to draw a sample from distal end of the leaf.
10) Avoid sampling leaves with soil and/or dirt.
11) Avoid contamination at all steps.
12) Dry the samples. Preferably by lyophilizing, because this results in the best quality DNA, but drying in an oven at 40-50 ^{o}C for 12-24 hours is sufficient for most crops.
13) Seal the plates or tubes with Intertek approved silicone mats, caps or seals.
14) Apply the lid of the box when applicable and fix this with the clips and/or rubber bands. Alternatively, wrap the sample plates in (a) plastic bag(s) and seal this with tape or rubber bands. If available, add silica gel to the plastic bags to avoid moister.
15) Add the sealed plates, together with a printed Order form to the outer package (mostly a carton box). Send it to desired lab, Intertek Sweden or India according to the instructions. In parallel, send the same Intertek Order form electronically to the desired lab.
16) The order should be announced by mail at least 15 days prior to sending leaf samples to your contact person of respective INTERTEK labs in order to ensure the 14 days turn-around time. You can use the draft order form to announce the shipment. For larger sample volume (>10,000 samples), please submit the order at least 25 days prior to sending the leaf samples.

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How to send samples

It is **REQUIRED** that samples from plant materials are labelled with the following text: ***"For analytical purposes only, not for breeding, will be destroyed"***

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.

All customers will receive an order confirmation as soon as the samples have arrived.

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General comments

When sampling occurs in nurseries, very young leaf tissue or even cotyledons could be considered, but it has to be taken into account that this might have an effect on the quality and/or quality of the DNA and that by such some SNP assays might fail.

As a rough guide, Intertek typically extracts 1 µg of DNA per 2 leaf disk punches, although this obviously varies considerably according to species, age of tissue, etc.

It is of importance to take the sampling seriously. Bad quality samples only seldom lead to good quality results. SNP results lose their meaning when the tracking of the samples fails or the link to the plants is lost.

For more information and shipping solutions, contact respective laboratory.