1. Sample collection by direct leaf press:

WHAT YOU NEED
FTA PlantSaver card
Parafilm
Pestle & Mortar (or minipestle and tube)
Plastic clip top bag, desiccator and silica gel desiccant

Always wear gloves when handling FTA PlantSaver Cards to avoid contamination of the cards.

Store unused FTA PlantSaver Cards in a desiccator. (Avoid light and excessive humidity).

1. Label the FTA PlantSaver Card with the appropriate sample identification. Divide each of the four sample areas on the card into 2 or 4 sections so that the card can accommodate 8-16 samples (see page 18 Use of FTA PlantSaver Cards).
2. Place the leaf over the marked area (the underside of the leaf facing down) on top of the FTA matrix card.
3. Overlay the leaf, either with Parafilm or replace the cover sheet.

1. Sample collection by direct leaf press (cont'd):

4. Using a heavy blunt object (such as a small porcelain pestle, tack hammer or screwdriver handle) pound each sample area with moderate force for 15 seconds[1]. This will burst the cell walls of the plant tissue.
5. It is important that the samples be pressed by a smooth surface so that the filter matrix is not damaged.
6. Verify that sufficient plant material has been transferred to the paper by checking the back of the FTA card: plant tissue should be visible on the other side of the matrix.
7. **Use great care not to damage the matrix.**
8. Ensure that no large pieces of plant tissue remain adhered to the FTA card as this may interfere with later processing.
9. When samples have been transferred to the paper allow the FTA card to air dry for a minimum of one hour at room temperature.
10. If sample is to be archived, place in a clip top bag and store in the desiccator with active silica gel (all items supplied by KT).

Notes

[1] A quality leaf press is the most important factor for obtaining good quality plant DNA sample on the FTA card. A leaf press made with too little force will not transfer enough DNA to the card. For the best results it is important to apply quick, strong pressure to the Parafilm/leaf/card sandwich. This will provide enough momentum to break the cell walls. Applying too much or too little pressure or pressure that is uneven is not likely to work well. Also, hitting the card too strongly will damage the matrix making it too fragile for processing. When applying force, do not use a rubbing motion.

2. Sample collection of plant homogenate:

WHAT YOU NEED
FTA PlantSaver card
Parafilm
Pestle & Mortar (or minipestle and tube)
Phosphate-buffered Saline (PBS) buffer
Plastic clip top bag, desiccator, and silica gel desiccant

1. Label the FTA PlantSaver card with the appropriate sample identification.
2. Use a minimum of 10 mg of young plant tissue. Add 1 part plant tissue to 5 parts PBS and using a mortar and pestle grind leaf material to a smooth homogenate (if preferred use a micropestle and microfuge tube). The ratio of 1 part plant material and 5 parts PBS is critical for good results. [For soybean and some species of cereal it may be necessary to add dithiothreitol (DTT) to improve the amount of DNA that binds to the FTA].
3. Apply the homogenate to the FTA PlantSaver card matrix inside the marked circle using a wide mouth pipette or a pipette tip that has been cut to give a 1.5 to 2.0 mm opening (the sample will likely be too viscous to use pipette tips with narrow openings). Allow the sample to air dry on the FTA card for a minimum of 2 hours at room temperature.
4. If all of the plant tissue cannot be homogenised completely, the semi -homogenised tissue can be pressed against the card and then discarded.
5. If the sample is to be archived, place in a clip-top bag or store in a desiccator with active silica gel (all items supplied by KT).

3. Removing a sample disc from an FTA Card for analysis:

WHAT YOU NEED
FTA PlantSaver card
Harris 2 mm punch or similar
Cutting mat

1. To prevent carry-over between samples, always ensure that the sample applied is dry before taking a punch.
2. Place the FTA PlantSaver card on a cutting mat. For cards with outer paper layers, ensure that the mat is directly beneath the FTA card with no paper layer in between.
3. Place the tip of the coring punch, e.g. a Harris 2 mm micropunch, over the area to be sampled. Do NOT depress the ejection plunger at this time.
4. Press down firmly on barrel of the coring device and twist one quarter turn to cut the disc out of the card.
5. Once the disc is in the corer, transfer the disc to the desired PCR tube or tray by depressing the ejection plunger and ejecting the disc.
6. Care should be taken when handling the dry FTA discs because the static charge that can develop on some plastic labware can cause the disc to be ejected from the tubes and adhere to other surfaces.
7. In order to ensure there is no cross-contamination between samples, the coring device can be cleaned using one of the two methods described below. Use the method which fits best your laboratory workflow.

4. Cleaning the corer tip:

1. Rinse the tip with ethanol between samples and dry with a sterile wipe.

   OR

2. Take one punch from blank filter paper or an unspotted area of the FTA PlantSaver Card between samples.

5. Preparing the FTA for DNA analysis:

WHAT YOU NEED
FTA Purification Reagent - TE buffer pH 8 1X with 1% Triton X
Microfuge tubes
TE^{-1} pH 8.0 buffer

1. Take a sample disc from the dried spot (follow the instructions in Protocol 3, Page 20). For plant samples, a 2 mm disc is recommended.
2. Place disc in a 0.5 mL or 1.5 mL micro-centrifuge tube.
3. Add 200 µL of FTA Purification Reagent to the tube.
4. Incubate for 5 minutes at room temperature with moderate manual mixing.

5. Preparing the FTA for DNA analysis (cont'd):

5. Remove and discard the used FTA Purification Reagent with a pipette.
6. Repeat steps 3-5 once, for a total of 2 washes with FTA Purification Reagent.
7. At this stage the FTA disc should be white (i.e. no chlorophyll). If the disc is still very green, repeat steps 3-5 with an additional FTA Reagent wash.
8. Add 200 µL of TE^{-1} Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).
9. Incubate for 5 minutes at room temperature.
10. Remove and discard all used TE^{-1} Buffer with a pipette.
11. Repeat steps 8-10 once for a total of 2 washes with TE^{-1} Buffer.
12. Ensure that all the liquid has been removed before performing analysis. The disc maybe allowed to dry[1].

Notes:
[1] It is recommended that analysis be conducted within 3 hours of the disc washing. If this is not possible, the punch can be stored at 4 °C or -20 °C in a dark environment for up to 1 week.

6. Modified protocol for enhanced sample washing:

With some plant species removal of chlorophyll from the punch becomes difficult. This may interfere with downstream analysis. The following protocol is an enhanced washing method developed to remove the chlorophyll from such samples:

1. After washing with the FTA Purification Reagent, wash the punches with 200 µL iso-propanol.
2. Incubate for 2 minutes, pipette up and down a couple of times and discard. Repeat for a total of 2 washes with isopropanol.
3. Dry the punches at room temperature to ensure that the isopropanol has been completely removed.

7. PCR of washed punch:

1. The washed and air-dried disc is now ready for analysis by PCR using standard protocols.
2. The disc is included in the PCR reaction.
3. There is no need to change reaction volume or PCR conditions due to the presence of the disc.
4. For the PCR it can be safely assumed that the punch + DNA constitutes zero added volume.
5. Recommended reaction volume of the PCR analysis of plant DNA is between 25-50 µL.
6. For some species of plant a 2 mm punch may contain too much DNA causing the PCR reaction to be inhibited. In this situation a smaller 1.2 mm punch or similar can be used in as much as 50 µL of PCR reaction mix.