Arcis Plant Nucleic Acid Extraction Protocol

Extraction of nucleic acids from plant material can be problematic due to challenges such as rigid cell walls, high polysaccharide content, and the frequent presence of compounds such as phenolics, tannins and other inhibitors. Classic protocols may be lengthy and involve combinations of physical disruption and enzymatic degradation of the plant material, in addition to lysis with hazardous agents such as alkaline solutions. The method presented here was designed to take advantage of the Arcis extraction technology to produce a rapid, inexpensive and reliable procedure for the extraction of total nucleic acids from plant cells. This simple method provides nucleic acids suitable for a range of downstream processes including qPCR and sequencing.

The following protocol describes the extraction of DNA from the leaves of the common bean *Phaseolus vulgaris*, and varies from the standard Arcis Sample Prep Kit method to maximize cell disruption; which may result in a variation in the number of reactions available in the kit. When taking samples from leaves it is advised to use younger leaves which have a higher cell density and have been potentially exposed to less inhibitory compounds. It is also advised that leaf punch samples should be taken in such a way as to avoid the mid rib of the leaf. The size of the leaf sample taken in the method below should be taken as an example protocol, with variation in sampling protocol dependant on species and availability- punches of as little as 1.5mm have also been successfully extracted.

Figure 1: *Common bean plant*

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 90 days.

Materials Provided in Arcis Sample Prep Kit:

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

Young leaves with a length of 2-3cm were removed from the plant and chosen for the extraction. 1cm diameter punches were taken from the *Phaseolus vulgaris* leaves.

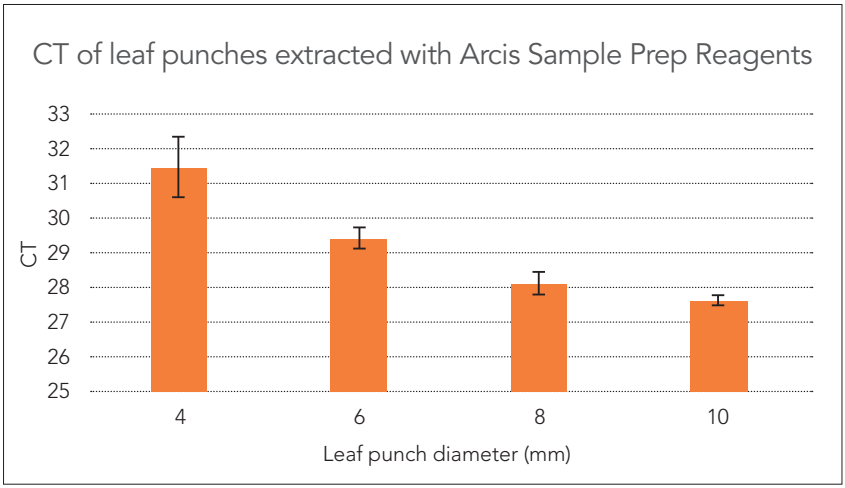
Method

- The leaf punch samples were directly added to 200µl of Arcis reagent 1 and homogenized using a micro tube homogenizer for 1 minute. At this point the samples turned the liquid green.This did not interfere with subsequent analysis.
- The samples were then centrifuged and the supernatant containing the released nucleic acids retained.
- The lysed mixture was then added to reagent 2 in a 1:4 ratio, with 5µl of the mixture being added to 20µl of reagent 2.
- 5µl was then added to the PCR Mastermix (IDT PrimeTime® Gene Expression Master Mix).
- Detection and identification of the plant DNA target through qPCR was performed using a Lightcycler and StepOnePlus.

Results

The qPCR results obtained using the Arcis extraction protocol are shown in Figure 2. The *Phaseolus vulgaris* actin assay (housekeeping gene) was developed in-house. Results show that this new extraction technology generates DNA of sufficient quality to be used directly in a qPCR mastermix without further purification.

Figure 2



Conclusion

The simple, rapid 2-step Arcis Sample Prep Kit can be used to extract nucleic acids from leaf material which can then be used directly in qPCR. The lysis and protection agent – reagent 1 is sufficiently powerful to rapidly break open cell walls without further digestion, and rapidly block degradation of the nucleic acids by released compounds within the cell. The protocol described here is a 3 minute extraction of nucleic acids from leaf material.