Fastract Direct PCR Kit ® for Plant

#G45311B **500 Reactions**

Store at 4^{O}C and Dark

Introduction

Plant DNA isolation is a time consuming, laborious procedure specially when working with a huge number of samples. FasTract Direct PCR Kit® provides ease for direct PCR from plant leaves without isolation of total genomic DNA. Fastract Direct PCR Kit is designed to perform PCR directly from plant leaves without prior DNA isolation and purification. Fresh plants, plant material stored at +4°C or frozen are all suitable templates for this kit. Plant leaves could also be collected directly in the FasTract Lysis Buffer and stored at room temperature for at least 48hr, at 4^{0}C for 5 days and at -20^{0}C for at least one month. Caotrophs and detergents present in FasTract Lysis Buffer ensure better and efficient lysis even with tough samples like palm and coconut. FasTract PCR Mix contains a genetically modified Hi Fidelity DNA polymerase which can amplify even in presence of PCR inhibitors in plant lysate. The kit ensures high yield in PCR amplification and is time saving as DNA isolation from samples can be avoided prior to PCR. The control primers provided can amplify a highly conserved region from plant DNA. Purified plant gDNA is also provided as a positive control for the PCR reactions. The kit is recommended for end-point PCR.

Kit Components

Components	Amount
Fastract Lysis Buffer	25ml
Fastract 1X PCR Mix FasTract DNA Polymerase, Reaction Buffer, dNTP (10mM), enhancer solution.	12.5ml
Control Primer (Forward),10uM	10ul
Control Primer (Reverse),10uM	10ul

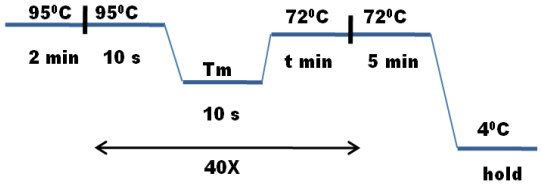
Guidelines for sample handling

- Single hole paper puncher must be used to obtain small and uniform leaf discs (2mm diameter)
- It is very important to clean the cutting edge every time before sampling to prevent cross-contamination between samples
- 2 % sodium hypochlorite solution or 70% Ethanol should be used for cleaning.
- Punch out a disc from the plant leaf using the sampling tool and place disc directly into the PCR tubes.
- Add 50µl FastTrack Lysis Buffer into each tube, vortex well and incubate them at 95^{0}C for 15mins.
- Use2. 5µl of this lysate as template for the PCR reaction
- ***The FasTract Lysis buffer can be used for collection and storage of leaf disc samples.***

Guidelines for PCR setup

- Take a single leaf disk in a PCR tube and add 50µl of FasTract Lysis Buffer in it.
- For samples collected directly in FasTract Lysis buffer, next step is not required for soft plant leaves. Still, heating the samples will ensure better amplification.
- Vortex the sample vigorously and incubate the FasTract Lysis Buffer added sample at 95^{0}C for 15 min in a PCR machine lead heating condition.
- Add 22µl 1X FasTract PCR mix in a Flat Capped PCR tube.
- Add 0.25µl of each Primers (from 10µM stock) to the master mix.
- Use 2.5µl of FasTract lysate as template for the PCR reaction.
- Set up PCR as bellow.

PCR Cycle:



t min= extension time: 1min/kb

Guidelines for Control reactions

We recommend setting up a separate control PCR reaction (use provided primer as working stock) with the FasTract leaf disc lysate used in the actual experiment to ensure that the PCR conditions are optimal. Tm for control primer is 50^{0}C and it amplifies a 550bp fragment from a highly conserved region of plant genomic DNA. It is recommended to add a no-template control to all PCR assays

Important Notes

- Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature.
- **Always add the plant sample last to the reaction.**
- It is recommended to eject the leaf disc into the empty tube and add the lysis buffer to ensure that the entire disc is dipped into buffer. Make sure that you see the sample disc in the solution.
- We recommend using fresh plant material for best results, even though plant material stored at +4°C or -20^{0}C can also be used.
- For extension, use 1min for 1Kb amplicon size .

The FasTract Lysate must be added such that it is 10% of the total reaction volume i.e if the total reaction volume is 25µl then 2.5µl of leaf disc lysate must be added to the reaction.

Troubleshooting

For tough and dry leaf samples increase the incubation time at 95^{0}C
If PCR fails due to high amount of PCR inhibitors in the leaf disc lysate, then the amount of lysis buffer can be increased to 75-100ml
If PCR fails even after increasing the volume of lysis buffer, incubate half of the leaf disc in lysis buffer instead of the entire disc.