APPLICATION NOTE

Direct PCR from Crude Plant Extracts Using DeCodi-Fi High-Fidelity Polymerase

Introduction

High-fidelity polymerases capable of amplifying plant DNA directly from crude extracts are important for sequencing-based studies in agriculture, helping to reduce processing time and simplify workflows without compromising accuracy. However, most high-fidelity polymerases struggle with natural inhibitors in plant tissues, making amplification challenging without extensive purification. **DeCodi-Fi High Fidelity Polymerase** can overcome these limitations, improve plant genomics workflows, and **enable more efficient sample preparation for sequencing**. This application note evaluates the performance of **DeCodi-Fi High-Fidelity Polymerase for direct PCR from crude plant extracts**, using tomato, strawberry, and maize as representative samples following HotShot DNA extraction.

Materials and Methods

- **Sample type:** Maize leaves and seeds, strawberry leaves, and tomato peel.
- **HiFi Polymerase Used:** DeCodi-Fi High-Fidelity PCR Kit was used for amplification, with Q5 High-Fidelity Polymerase included for comparison.
- **Primers:** Primers targeting a conserved chloroplast region (~300 bp amplicon) were used. All primers featured 3' phosphorothioate bonds to enhance stability and resistance to exonuclease degradation.

Sample Preparation: DNA Extraction Using HotShot Protocol

DNA extraction was performed using the HotShot method prior to PCR. A 1 to 4 mm^2 sample was excised and placed in 90 µL of 25 mM NaOH + 0.2 mM EDTA. The mixture was incubated at 95°C for 1 hour and then subsequently cooled to room temperature. Neutralization was carried out by adding 90 µL of 40 mM Tris-HCl, and the extract was stored at 4 °C until use.

Annealing Temperature Optimization

A qPCR-based annealing temperature gradient (from 55 °C to 72°C) was conducted to determine the optimal temperature for primers, ensuring specific and efficient amplification. A strawberry leaf sample served as a template to determine the annealing temperature, which was then applied to all samples. The optimal annealing temperature was established at 59°C.

PCR Reaction Setup

A 20% (v/v) crude extract was incorporated into a 25 µL PCR reaction, corresponding to 5 µL of sample per reaction. Likewise, 5 µL of purified DNA was used for the same reaction volume. Up to 20% (v/v) of the crude extract can be used without compromising DeCodi-Fi performance. The same conditions, including primers and dNTPs, were applied to both DeCodi-Fi and Q5.

PCR Cycling conditions:

The same cycling conditions were applied to both purified and crude extract samples, as well as for both Q5 and DeCodi-Fi High-Fidelity Polymerase to ensure a consistent comparison of amplification performance.

Table 1: PCR Cycling conditions

Step	Temperature	Time	Cycle
Initial Denaturation	95 °C	2 min	1
Denaturation	95 °C	20 sec	35 cycles
Annealing	59 °C	30 sec	
Extension	72 °C	30 sec	

Results:

A 300 bp fragment from a conserved chloroplast region was successfully amplified from maize leaf and seed, strawberry leaf, and tomato peel following HotShot DNA extraction. DeCodi-Fi High-Fidelity Polymerase generated comparable yields in crude and purified samples, producing more intense bands than Q5.

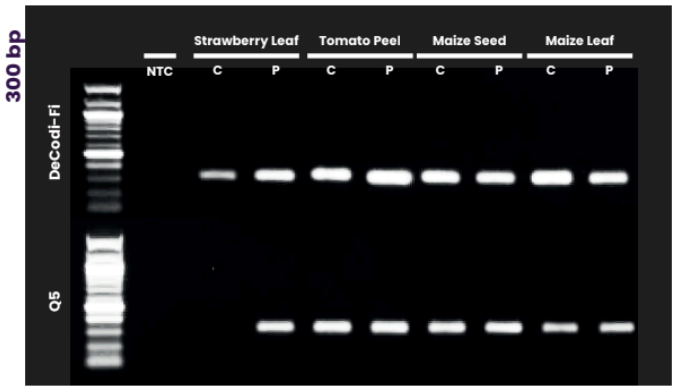

Figure 1. Amplification of a 300 bp conserved chloroplast region from plant samples following HotShot DNA extraction. PCR was performed using DeCodi-Fi High-Fidelity Polymerase and a Q5 (for comparison). Samples included strawberry and maize leaves, maize seed, and tomato peel. A 100 bp DNA ladder was used as the size standard. C: crude sample (post-HotShot extraction), P: purified DNA sample, NTC: no template control.

Conclusions:

DeCodi-Fi High-Fidelity Polymerase successfully amplified DNA from crude plant extracts, including strawberry samples, which contain high levels of inhibitors such as polysaccharides and polyphenols. **DeCodi-Fi** also demonstrated **efficient amplification across different plant tissue types**, including leaves, seeds, and peel. Its **robust inhibitor tolerance** enables direct PCR without extensive purification, **streamlining workflows for plant research**.

March 20, 2025, v1.0

1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448