Direct PCR from plant material is a challenging application as a result of the diversity of plant tissue types and the potent PCR inhibitors contained within the tissue.

Success rates with direct PCR are typically low due to the high concentrations of inhibitors present in even small amounts of plant material, and the precise control of sample size required. In this Application Note, we present a systematic approach for reaction optimization with the KAPA3G Plant PCR Kit, as well as a robust method for successful crude sample PCR.

Introduction

Amplification of DNA directly from plant material is a challenging application due to the diversity of plant tissue types and the potent PCR inhibitors contained within the tissue. The KAPA3G Plant PCR Kit is optimized for the successful amplification of DNA from plant samples added directly to the PCR (direct PCR), DNA obtained with crude extraction methods (crude sample PCR), as well as purified DNA.

The KAPA3G Plant PCR Kit contains a novel DNA polymerase, engineered via a process of directed evolution, for improved tolerance to common plant-derived PCR inhibitors such as polyphenolics and polysaccharides. The unique characteristics of the enzyme result in robust amplification across a wide range of plant, template and amplicon types.

Despite the improved inhibitor tolerance of the KAPA3G Plant DNA Polymerase, success rates with direct PCR are typically low due to the high concentration of inhibitors present, even in very small amounts of most plant types. This problem can be addressed by either controlling the size of sample used in the PCR with a sampling tool, or by performing crude sample PCR using a crude extraction method to release DNA into solution and dilute inhibitory compounds to levels that do not affect PCR. In this Application Note, we describe a detailed strategy for optimizing Plant PCR with the KAPA3G Plant PCR Kit, and a quick, easy method for crude sample PCR.

This strategy involves first optimizing reaction conditions (particularly the annealing temperature) using purified DNA as template. Once this has been achieved, direct PCR may be evaluated to determine whether a particular assay can be converted to direct PCR. Should direct PCR not be possible or desirable, a detailed method for crude sample PCR is described. This method has many advantages over direct PCR, including higher success rates across a wide range of plant species and sample types, the ability to perform multiple PCRs from the same sample, and less need for precise control over sample size.

PCR from purified DNA

Annealing temperature optimization was performed with two universal primer sets, Tab a/b and Tab c/d, targeting the trnL intron.[1] Genomic DNA purified from Erica cerinthoides and Cinnamonium camphora was used as template. Results (Figure 1) showed that the Tab a/b primer set worked best at an annealing temperature of 56°C, while the Tab c/d primer set worked well across the entire annealing temperature range tested. The Tab c/d primer set was selected for this study, using an annealing temperature of 55°C. In the next experiment (Figure 2), the Tab c/d primer set was used with genomic DNA purified from 18 different plant sample types from various species. Amplification was successful for all 18 plants tested.

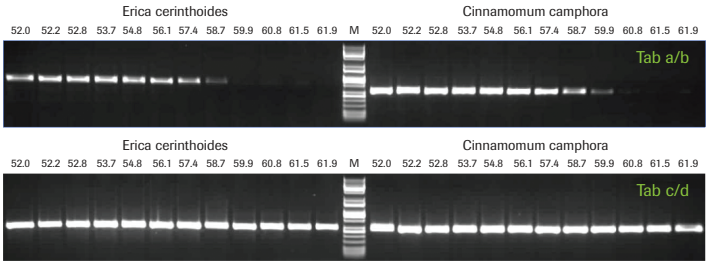

Figure 1. Annealing temperature optimization with purified DNA. Annealing temperature gradient PCR (52º to 62°C) was performed to determine the optimal annealing temperature for two primer sets targeting the trnL intron, Tab a/b and Tab c/d.[1] Reactions (50 µL) contained 1X KAPA Plant PCR Buffer (providing 1.5 mM $MgCl^2$ and 0.2 mM of each dNTP), 0.3 µM of each primer, 1 U of KAPA3G Plant DNA Polymerase and 1 µL of purified DNA extracted from Erica cerinthoides and Cinnamomum camphora using the QIAGEN® DNeasy Plant Mini Kit (10 ng per reaction).

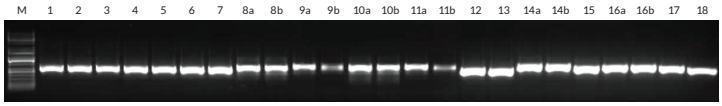

Figure 2. Plant PCR with purified DNA from 18 sample types. Reactions (50 µL) contained 1X KAPA Plant PCR Buffer (providing 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP), 0.3 µM of each primer for the Tab c/d primer set, targeting the rnL intron,[1] 1 U of KAPA3G Plant DNA Polymerase and 1 µL of purified DNA extracted using the QIAGEN DNeasy Plant Mini Kit (1 – 10 ng per reaction). Plants used were 1–7: Erica spp. (leaves); 8–12: Euphorbia spp. (leaves [a] and thorns [b]); 13: Euryops tysonii (leaf); 14: Schotia brachypetala (leaf [a] and seedpod [b]); 15: Hyaenanche globosa (leaf); 16: Acacia sieberiana (leaf [a] and seedpod [b]); 17: Cinnamomum camphora (leaf); 18: Fragaria ananassa (leaf). A standard 35-cycle Plant PCR was performed, with an annealing temperature of 55°C, and 15 seconds extension at 72°C.

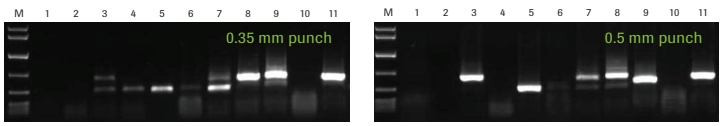

Figure 3. Results obtained with direct PCR. Direct PCR with the Tab c/d primer pair was performed on leaves and/or seeds from 8 plants, sampled using either a 0.35 mm punch (left) or 0.5 mm punch (right). Reactions were set up as described in the KAPA3G Plant PCR Kit TDS, and 45 cycles of PCR performed with annealing at 55°C, and 20 sec extension at 72°C per cycle.

1: Eucalyptus; 2: Grapevine; 3: Wheat leaf; 4: Wheat seed; 5: Canola leaf; 6: Canola seed; 7: Rice seed; 8: Barley seed; 9: Corn kernel; 10: Cotton seed; 11: Cotton leaf.

Direct PCR

The success rate of direct PCR with 8 plant species, including several crops, was evaluated using either a 0.35 mm of 0.5 mm piece of leaf/seed. Results are shown in Figure 3. Generally, leaf samples amplified well, with the exception of grapevine and Eucalyptus spp. Seeds had a lower success rate, and tended to result in nonspecific amplification. Crude sample extraction and PCR Crude extracts were prepared from leaves/seeds of the same 8 plant species used for direct PCR. Crude extracts were prepared as described in the KAPA3G Plant PCR Kit TDS, both with and without heat treatment (5 min at 95°C). Of this crude extract, 1 µL was used as template in PCR. Results (Figure 4) show that all samples amplified successfully. The heat treatment increased yields slightly for all of the plants with the exception of Eucalyptus.

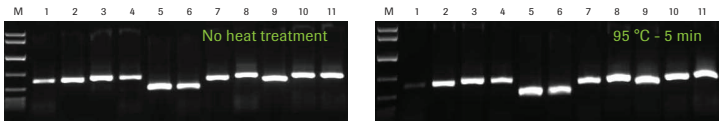

Figure 4. Results obtained with crude sample PCR. Crude sample PCR with the Tab c/d primer pair was performed on leaves and/or seeds from 8 plants, with 1 µL of crude extract prepared without heat treatment (left) or 1 µL of crude extract prepared with heat treatment (right). Reactions were set up as described in the KAPA3G Plant PCR Kit TDS, and 45 cycles of PCR performed with annealing at 55°C, and 20 sec extension at 72°C per cycle.

1: Eucalyptus; 2: Grapevine; 3: Wheat leaf; 4: Wheat seed; 5: Canola leaf; 6: Canola seed; 7: Rice seed; 8: Barley seed; 9: Corn kernel; 10: Cotton seed; 11: Cotton leaf.

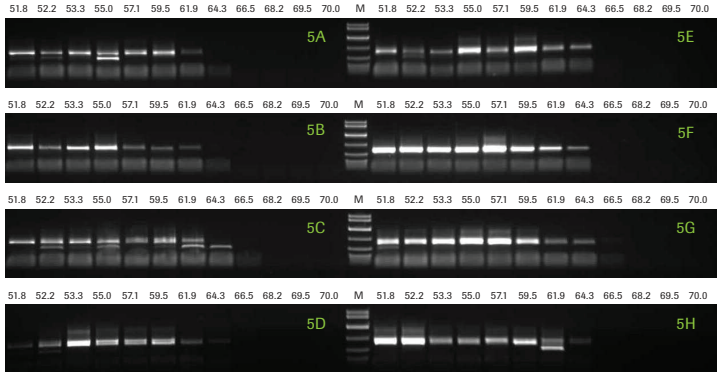

Figure 5. Results obtained with optimization of crude sample from linseed. Crude sample PCR from linseed was optimized by performing 8 sets of annealing temperature gradient PCRs (52º to 70°C), all containing 1 µL of crude extract (prepared without heat treatment). Reactions were performed with various combinations of enzyme, MgCl2 and KAPA Plant PCR Enhancer, as shown in the table above. Conditions 5F and 5G produced the best results.

Optimization of crude sample plant PCR

While success rates with crude sample PCR are typically high, there are exceptions where optimization is required in addition to the initial optimization with purified DNA. Reaction parameters to be optimized include the enzyme and $MgCl_2$ concentrations, and the use of KAPA Plant PCR Enhancer. Cycling parameters generally do not require much optimization, but in certain cases, the cycle number and extension time may have to be increased. Reoptimization f the annealing temperature may also be necessary. To demonstrate the optimization process, crude extracts prepared from linseed were amplified with 8 combinations of enzyme (1 or 2 U), $MgCl_2$ (2 or 3 mM) and KAPA Plant PCR Enhancer (0 or 0.2X). Refer to Table 1 for full details. This was combined with annealing temperature gradient PCR at each condition, to determine whether the temperature optimized initially remained optimal. Two of the reaction conditions (5F and 5G, highlighted in green in Table 1) were shown to improve the yield of product obtained, while an annealing temperature of 55°C remained suitable for this assay. Improved results were obtained with 2 U of enzyme and 3 mM $MgCl_2$ (panel 5G), or with 2 U of enzyme, 2 mM $MgCl_2$, and 0.2X KAPA Plant PCR Enhancer (panel 5F). Should results not have improved significantly with the combinations tested, the cycle number and extension time would have been increased in future rounds of optimization.

Conclusion

Direct PCR with the KAPA3G Plant PCR Kit is a quick and easy method for some plant species, but even with precise control over sample size, some sample types cannot be amplified successfully. Crude sample PCR is a highly robust, quick, and easy method of Plant PCR with the KAPA3G Plant PCR Kit, with even the most challenging plant species. Much higher success rates across a wide range of plant species and sample types can be achieved with crude sample PCR, also enabling multiple PCRs from the same PCR and eliminating the need for precise control of sample size.

Generally, very little optimization is required, provided that reaction conditions were optimized with purified DNA before attempting crude sample PCR. For those sample types that do require additional optimization, combinations of increased enzyme and $MgCl_2$, as well as the use of the KAPA Plant PCR Enhancer, can be evaluated to determine optimal reaction conditions. In exceptional cases, further optimization of cycling parameters may be necessary.

For more information on reaction setup, cycling parameters and optimization, and plants that these methods have been validated with, please consult the **KAPA3G Plant PCR Kit Technical Data Sheet**.

References

1. Taberlet, et al. *Plant Molecular Biology.* 1991; 17: 1105 – 1109.

Table 1: Parameters optimized for crude sample PCR from linseed

Panel	5A	5B	5C	5D	5E	5F	5G	5H
Enzyme	1 U	1 U	1 U	1 U	2 U	2 U	2 U	2 U
$MgCl_2$	2 mM	2 mM	3 mM	3 mM	2 mM	2 mM	3 mM	3 mM
Enhancer	0	0.2X	0	0.2X	0	0.2X	0	0.2X

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