PlantDirect™ Multiplex PCR System

Technical Manual No. 0178 **Version 10112010**

I. DESCRIPTION

PlantDirect™ Multiplex PCR System is a powerful reagent kit for both easy and rapid genomic DNA preparation and multiplex PCR amplification. Genomic DNA is directly released from plant cells (leaves, roots, stems, and cell culture) using proprietary reagents in 12 minutes without DNA purification. The genomic DNA can be used immediately in PCR amplification of multiple gene targets (up to >1,000) or stored at +4 ^{o}C for future use (stable at least 6 months at +4 ^{o}C).

PlantDirect™ PCR System with Enzyme (L00201) contains TD-A Buffer, TD-B Buffer, TD-C Buffer, and PCR Premix. The fresh mixture of TD-A and TD-B at a 1:9 ratio is used to lyses cells and to release genomic DNA. TD-C is used to bring the conditions close to those for PCR. PCR Premix contains PCR buffer (TD-D), dNTP, Mg^{2+} and "HotStart" Script™ DNA polymerase for PCR amplification.

L00201 Components	100 Preps
TD-A Buffer	0.50 ml
TD-B Buffer	4.50 ml
TD-C Buffer	5.00 ml
2X PCR Premix	1.00 ml
PCR-grade Water	1.00 ml

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PlantDirect™ PCR System without Enzyme (L00200) is also available from GenScript Corporation. This kit allows our customers to use any other DNA polymerases that they prefer to use for PCR reaction. The kit contains TD-A Buffer, TD-B Buffer, TD-C Buffer, and TD-D Buffer. The fresh mixture of TD-A and TD-B at a 1:9 ratio is used to lyses cells and to release genomic DNA. TD-C is used to bring the conditions close to those for PCR. TD-D Buffer is an optimized 10X Script™ DNA polymerase buffer (without dNTP). Limited tests at GenScript show that this buffer is compatible with Taq DNA polymerases from other vendors and also increases PCR sensitivity.

L00200 Components	100 Preps
TD-A Buffer	0.50 ml
TD-B Buffer	4.50 ml
TD-C Buffer	5.00 ml
TD-D Buffer	0.20 ml
PCR-grade Water	1.00 ml

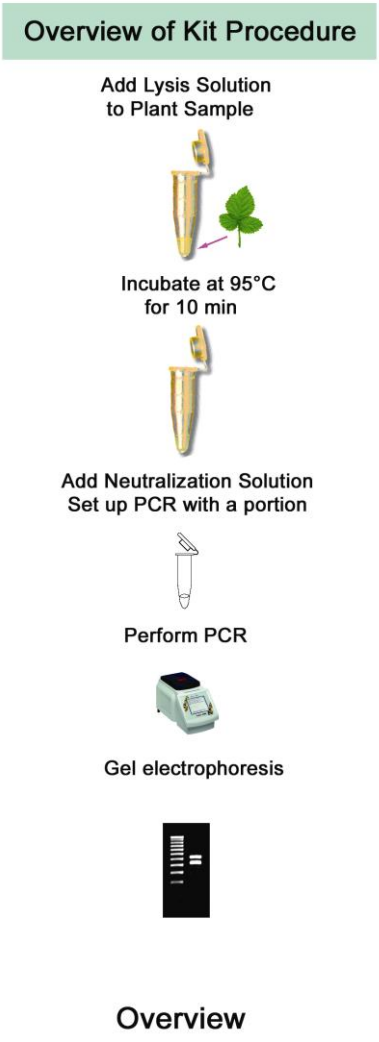
II. APPLICATIONS

For genomic DNA extraction from plant tissues or cells:

- Fresh plant tissue samples: leaf, stem, flower, seed, etc.
- Cultured plant cells
- Frozen grounded tissues
- Cryopreserved plant tissues or cells

And for application such as:

- SNP genotyping and mutation detection
- Target detection in transgenic plants
- DNA sequencing and cloning
- Quantitative PCR



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III. KEY FEATURES

- Easy to perform: very simple and rapid procedure to extract genomic DNA in 12 min.
- High specificity: highly specific amplification of genomic DNA using "HotStart" Script™ DNA polymerase (a GenScript proprietary DNA polymerase).
- Multiplex PCR: up to >1,000 DNA sequences can be amplified using multiplex PCR primers.
- Super sensitivity: genomic DNA from a single cell has been successfully used in multiplex PCR amplification of more than 1000 amplicons and subsequent DNA genotyping assays. The super sensitivity of this kit will dramatically cut down the tissue usage to save your precious tissue samples.

IV. SHIPPING AND STORAGE

This kit is shipped on blue ice. Store the kit at –20 °C after receiving.

V. SIMPLIFIED PROCEDURES

1. Thaw Buffer TD-A, TD-B and TD-C at room temperature and vortex the solutions. After thawing, keep TD-A buffer on ice, TD-B and TD-C can be kept on ice or at room temperature. Mix 5 μl TD-A and 45 μl TD-B (A to B ratio is 1:9) in a microcentrifuge tube to make the lysis solution. Add the lysis solution to tissue samples.
2. Mix and incubate the samples at 95 °C for 10 min.
3. Remove the tubes from incubation and add 50 μl of TD-C to each sample tube. Mix by tapping the tubes gently.
4. Spin down in a microcentrifuge at full-speed (~14,000 rpm) for 1 minute.
5. Set up and perform PCR reaction in a PCR cycler.
6. Analyze PCR reactions by agarose gel electrophoresis.

VI. DETAILED EXPERIMENTAL PROCEDURES

A. Genomic DNA Preparation

1. Thaw Buffer TD-A, TD-B and TD-C at room temperature and vortex the solutions. After thawing, keep TD-A buffer on ice, keep TD-B and TD-C on ice or at room temperature. Mix 5 μl TD-A and 45 μl TD-B (A to B ratio is 1:9) in a microcentrifuge tube to make the lysis solution.
2. Dispense the lysis solution from step 1 into the sample tube containing plant tissue or cells. Mix and incubate the samples at 95 °C for 10 min.
   a. Plant tissue: place small pieces of tissue (cut into small pieces with scissors or scalpel freshly rinsed in 70% ethanol) in the lysis solution. **Do not use more than 5 mg tissue.**
   b. Frozen ground tissue: add 50 μl of the lysis solution to frozen ground tissue **(less than 5 mg)** and mix by pipetting up and down.
   c. Cell culture: pipette 15 μl of cell culture into the lysis solution and mix by brief vortexing.

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3. Remove the tubes from incubation and add 50 μl of TD-C to each sample tube. Mix by tapping the tubes gently.

4. Spin down in a microcentrifuge at full-speed (~14,000 rpm) for 1 minute. Store the genomic DNA at 4 ^{o}C or use it immediately in PCR amplification.

Please note:

(1) The plant tissue is not expected to be completely digested after this procedure. Make sure to spin down the genomic DNA solution and take the supernatant for PCR reaction.

(2) Genomic DNA is fragile and high molecular weight DNA is sheared easily by mechanical forces. Do not vortex solutions containing genomic DNA.

B. PCR Amplification

One or multiple gene targets can be amplified using a pair of primers or multiple pairs of primers (multiplex PCR) from the genomic DNA prepared as described above. PCR reactions can be set up at room temperature since "HotStart" Script^{TM} DNA polymerase is used.

1. Set up 20 μl PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently. The table below is used only as a guide. For multiplex PCR, the primer concentrations and cycling parameters need to be optimized. Use only 1 μl of genomic DNA for 20 μl of PCR reaction.

Reagent	Volume	Final Concentration
Water, PCR grade	7 μl	
4 μM Forward primer	1 μl	200 nM
4 μM Reverse primer	1 μl	200 nM
Genomic DNA	1 μl	
PCR Premix	10 μl	
Total	20 μl	

If you are using PlantDirect^{TM} PCR System without Enzyme, set up 20 μl PCR reaction following your PCR kit instruction, and use 1 μl of genomic DNA prepared. As mentioned before, limited tests at GenScript show that TD-D buffer (without dNTP) is compatible with Taq DNA polymerases from different vendors.

2. The commonly used thermal profiles can be used for PCR amplification. The following two thermal profiles are recommended for the amplification of a single amplicon and multiple amplicons, respectively.

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a. Thermal profiles for amplification of a single amplicon with the primer concentration of 200 nM for each primer.

Activation of *Taq* DNA polymerase:		94 °C for 15 min
40 PCR cycles:	Denaturation:	94 °C for 40 sec
	Annealing:	55 °C – 60 °C for 1 min
	Extension:	72 °C for 30 sec to 2 min (~1 kb/min)
	Final extension:	72 °C for 3 min.

b. Thermal profiles for amplification of multiple amplicons with the each primer at concentration of 50 nM.

Activation of *Taq* DNA polymerase:		94 °C for 15 min.
40 PCR cycles:	Denaturation:	94 °C for 40 sec
	Annealing:	55 °C – 60 °C for 2 min
	Extension:	ramping from 55 °C to 72 °C for 5 min
	Final extension:	72°C for 3 min.

VII. EXAMPLES USING THE SYSTEM

Genomic DNA Preparation from Arabidopsis leaves and PCR Amplification.

PlantDirect™ PCR system with Enzyme was used to extract genomic DNA from Arabidopsis leaves. 12 pieces of Arabidopsis leaves (less than 5 mg) were cut from different Arabidopsis plants. Arabidopsis genomic DNA were extracted from all 12 pieces of Arabidopsis leaves and amplified using PlantDirect™ Multiplex PCR system following the kit instructions. A pair of primers was designed to amplify Arabidopsis *ubiquitin 10* gene with the sequences shown below:

Forward primer: 5'-GTCCTCAGGCTCCGTGGTG
Reverse primer: 5'-TGCCATCCTCCAACTGCTTTC

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The results were shown in Figure 1.

Figure 1 shows that the target PCR product of 180 bp fragment of Arabidopsis *ubiquitin 10* gene is seen from all 12 genomic DNA samples. There is little difference between genomic DNA samples extracted from different Arabidopsis leaves. This demonstrates the high quality and reproducibility of PlantDirect™ PCR System.

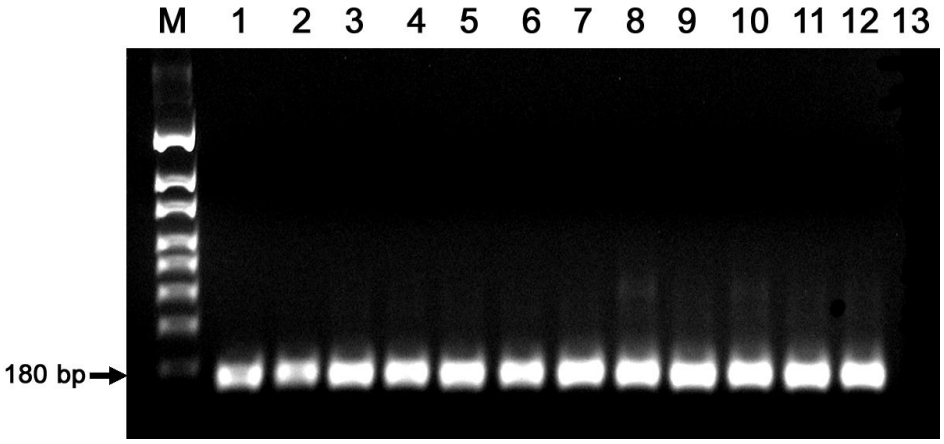

Figure 1. PCR analysis of genomic DNA extracted from Arabidopsis leaves. Genomic DNA was extracted from 12 pieces of Arabidopsis leaves using PlantDirect™ Multiplex PCR kit following the kit instructions. All genomic DNA samples were amplified using the PCR premix in the kit following the kit instructions. PCR products are 180 bp for Arabidopsis *ubiquitin 10* gene. Lane 13 is a negative control.

VIII. TROUBLESHOOTING

The table below is guideline for troubleshooting.

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Problem	Probable Cause	Solution
No PCR DNA	PCR may be inhibited by components in the tissue.	Dilute the genomic DNA 10 fold with a mixture of TD-A, TD-B and TD-C at the ratio of 1:9:10.
	One or more PCR components may be missing.	Always run a positive control side by side with PCR using genomic DNA prepared using the kit.
	PCR conditions are not optimized. The annealing temperature may be too high; More cycles may be needed; The denaturation time may be too short; The extension time may be too short.	Optimize the PCR conditions by decreasing annealing temperature in 2-4 ^{o}C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments, or increasing the extension time in 1minute increments. It is recommended to change one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step. Betaine, DMSO and formamide can help amplification of high GC-rich template.
	Genomic DNA is lost especially when a single cell is used.	Do not use pippet tips to mix. Tap the centrifuge tubes gently to mix.
Non-specific DNA products	The primers may not be designed optimally.	Primers may form dimers, or prime at non-specific target sequences. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4 ^{o}C increments, or decreasing the number of cycles.
High background (with your own Taq polymerase)	Too much Taq DNA polymerase may be used.	Optimize the PCR conditions by decreasing the amount of Taq DNA polymerase in 0.5 unit increments.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

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IX. ORDER INFORMATION

PlantDirect™ Multiplex PCR System without Enzyme Cat. No. L00200

PlantDirect™ Multiplex PCR System with Enzyme Cat. No. L00201

For Research Use Only.

- The PCR process is covered by US. Patent numbers 4683195 and 4683202 issued to Cetus and owned by Hoffman-La Roche Inc. GenScript does not encourage or support the unauthorized use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

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