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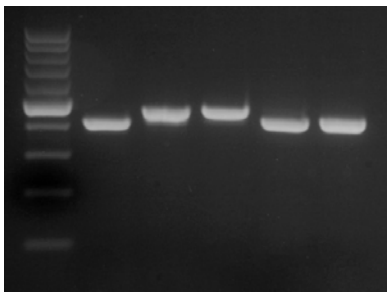
Direct Plant PCR Kit

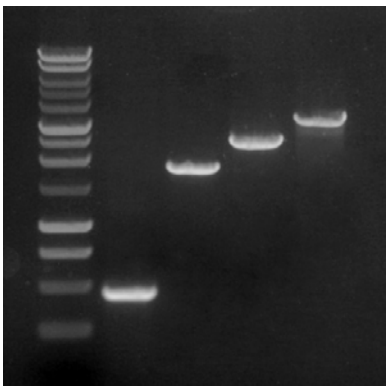
Direct Plant PCR Kit

Cat. No.	Size
E0960-01	100 reactions 50 µl each
E0960-02	500 reactions 50 µl each

Storage Conditions:
Store at -20˚C.



PCR amplification from different plant tissues and species using the Direct protocol of EURx Direct Plant PCR Kit.
Conserved PCR primers were used for amplification of chloroplast DNA (ca 400 bp fragment).
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder (E3131).
Lanes 1 to 5: PCR amplification reactions directly from (in order): grass leaf, parsley leaf, apple seed, rubber plant leaf, spruce needle.



PCR amplification from parsley leaf using the Extract protocol of EURx Direct Plant PCR Kit.
Conserved PCR primers were used for amplification of chloroplast DNA.
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder (E3131).
Lanes 1 to 4: PCR amplification reactions (fragments from 0.4 up to 3.5 kb) directly from parsley leaf using the Extract protocol of EURx Direct Plant PCR Kit.

Direct Plant PCR Kit enables to perform PCR reactions directly from plant samples without prior DNA extraction or purification.

Description:

- The Direct Plant PCR Kit allows to use samples such as: plant leaves, plant seeds and plant material stored on commercially available sampling cards.
- For use with fresh or frozen sample material.
- The Direct Plant PCR Kit employs a hot start and a genetically engineered thermostable DNA polymerase with high tolerance to plant inhibitors.
- "Hot Start" Plant DNA Polymerase activity is restored during a 10 min initial denaturation step.
- Direct Plant DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in 5'- 3' direction.
- The enzyme exhibits the 3' -5' proofreading activity, resulting in over 10-fold higher PCR fidelity as compared to Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to conduct short timed extension steps.
- Due to genetic modifications of the polymerase, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols.
- The Direct Plant PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols.
- The master mix contains a premixed gel loading reagent and dyes which allow direct sample loading on gels.
- Direct Plant PCR Kit allows to obtain a broad range of product size (exceeding 4 kb in length).

Plant DNA Polymerase Storage Buffer:

20 mM Tris-HCl (pH 9.0 at 22˚C), 100 mM KCl, 0.1 mM EDTA,
1 mM dithiothreitol, 50 % glycerol and stabilizers.

[2x] Plant PCR Master Mix:

The master mix contains 2 x concentrated optimized PCR buffer, 5 mM $MgCl_2$, dNTPs and two gel tracking dyes.

Direct Plant PCR Kit contains:
1. 2 x Plant PCR Master Mix
2. Plant DNA Polymerase
3. Extraction Solution
4. Dilution Solution
5. Water, nuclease free

Quality Control:

All preparations are assayed for contaminating 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95 % pure, as judged by SDS polyacrylamide gel electrophoresis.

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Direct Plant PCR Kit

PCR PROTOCOL (1)

Sample handling

To obtain small and uniform samples, it is recommended to use 0.35-0.5 mm diameter punchers or scalpel cut Plant samples. If the puncher or scalpel will be reused, it is mandatory to clean the cutting edges properly to prevent cross-contamination between samples. Use 2% NaClO solution for cleaning and cross-contamination prevention.

Choosing the protocol

The Direct Plant PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols. With a few exceptions, both Direct and Extract protocol are compatible with all sample types and application.

The Extract protocol is recommended:

- when working with new sample materials or a new primer pair,
- with difficult or long amplicons (exceeding 1 kb in length),
- when performing multiple reactions from the same sample.

For the Extract protocol use 20-50 µl reaction volume, for the Direct protocol use solely reaction volumes of 50 µl.

The samples in Extraction Buffer can be stored for up to 4-8 weeks at +4˚C or long-term at -20˚C before use in PCR reactions.

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- Take a sample of 0.35-0.5 mm in diameter from plant leaves, seeds or plant material stored on commercially available cards. For difficult samples (rich in polyphenols), a smaller 0.35 mm punch may give more robust results.
- It is recommended to use young leaves or dehulled seeds. For very small seeds, use 1-2 whole seeds.
- Place the sample directly into a 50 µl PCR reaction. Perform PCR (see the table on the next page).

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- Place the plant sample of 0.35-1 mm in diameter into 50 µl of Extraction Solution.
- Incubate the reaction for 5 minutes at room temperature.
- Add 50 µl of Dilution Solution.
- Mix the tube briefly and spin down the solution.
- Store the supernatant at +4˚C or -20˚C.
- The samples in mix of Extraction and Dilution Solutions can be stored for up to 8 weeks before using in PCR. For long term storage keep at -20˚C.

Use 1 µl of the supernatant as a template in a 20 µl PCR reaction.

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Direct Plant PCR Kit

PCR PROTOCOL (2)

Preparation of PCR Reaction from whole Plant:

Component	Volume / 20 µl Reaction	Volume / 50 µl Reaction	Final concentration
2x Plant PCR Master Mix, containing 6 mM $MgCl_2$.	10 µl	25 µl	1x 2.5 mM $MgCl_2$
Primer A	Variable	Variable	0.5 µM
Primer B	Variable	Variable	0.5 µM
Plant DNA Polymerase	0.4 µl	1.0 µl	
Sample Direct Protocol Extract Protoc.	- 1 µl	0.3-0.5 mm plant sample 2.5 µl liquid sample	
H_2O	Add to 20 µl	Add to 50 µl	
Premix Volume	**20 µl**	**50 µl**	

Notes:

1. **Thaw and Mix:** Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration.
2. **Reaction Assembly at Room Temperature:** Prepare reaction mixes at room temperature. Use of Plant DNA Polymerase allows room temperature reaction setup. Mix well.
3. **"Hot Start":** Reactions can be placed in a room temperature thermal cycler. Cycler preheating is not required.
4. **Direct Gel Loading:** The 2 x Plant PCR Master Mix allows PCR reactions to be loaded directly onto agarose gels without prior addition of gel loading buffer. The master mix contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
5. **PCR Optimization:** In most cases there is no need to add additives to PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO (provided) can be included to improve amplification. The recommended starting DMSO concentration (if needed) is 3 %.

Thermal Cycling Conditions:

Step	2-step protocol		3-step protocol		Number of Cycles
	Tempera-ture	Time	Tempera-ture	Time	
Initial Denat-uration	98˚C	10 min	98˚C	10 min	1
Denatura-tion	98˚C	5-10 s	98˚C	5-10 s	35-40
Annealing	-		X˚C	15-30 s	
Extension	72˚C	30 s / 1 kb	72˚C	30 s / 1 kb	
Final Exten-sion	72˚C	1 min	72˚C	1 min	1
Cooling	4˚C	Indefi-nite	4˚C	Indefi-nite	1

Notes:

1. **Plant Tissue Lysis:** A 10-minute initial denaturation at 98˚C enables lysis of cells and activates "Hot Start" Direct Plant DNA Polymerase.
2. **Annealing: Direct** Plant DNA Polymerase is capable of stabilizing primer-template hybridization. Melting temperatures (Tm) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. Tm's should be calculated with the base-stacking method (nearest-neighbor method). Use the calculator of the base-stacking method on Roboklons website (www.roboklon.de/eurx/blood-pcr). Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule, use an annealing temperature at T_m of the lower T_m primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. **Two-Step Protocol:** A 2-step protocol allows to perform combined annealing/extension step at 72˚C and is recommended for primers with T_m values of at least 72˚C. The 2 step protocol allows to save time of PCR reaction.
4. **Extension:** Extension time of 30 s/ 1 kb is recommended for most targets.

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