Product Information

REDExtract-N-Amp™ Plant PCR Kits

XNAPS, XNAP, XNAPE, XNAPR

Product Description

The REDExtract-N-Amp™ Plant PCR Kits for direct PCR contain the reagents needed to rapidly extract and amplify genomic DNA from plant leaves. Briefly, the DNA is extracted from a piece of leaf tissue, a 0.5 to 0.7 cm disk cut with a standard paper punch, by incubation in Extraction Solution at 95 °C for 10 minutes. There is no need for freezing plant tissue in liquid nitrogen, mechanical disruption, organic extraction, column purification or precipitation of the DNA. After an equal volume of Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR.

An aliquot of the diluted extract is then combined with the REDExtract-N-Amp™ PCR ReadyMix and user-provided PCR primers to amplify target DNA. The REDExtract-N-Amp™ PCR ReadyMix is a 2X reaction mix containing buffer, salts, dNTPs, and *Taq* polymerase. It is optimized specifically for use with the extraction reagents. It also contains JumpStart™ *Taq* antibody for hot start PCR to enhance specificity and RED*Taq*® dye to allow direct loading of the PCR product onto an agarose gel.

Reagents Provided	**Cat. No.**	**XNAPS** 10 extractions, 10 amplifications	**XNAP** 100 extractions, 100 amplifications	**XNAPE** 100 extractions, 500 amplifications	**XNAPR** 1,000 extractions, 1,000 amplifications
Extraction Solution	E7526	1.2 mL	12 mL	12 mL	120 mL
Dilution Solution	D5688	1.2 mL	12 mL	12 mL	120 mL
REDExtract-N-Amp™ PCR ReadyMix is a 2X PCR mix containing buffer, salts, dNTPs, *Taq* polymerase and JumpStart™ *Taq* antibody.	R4775	0.15 mL	1.2 mL	5 x 1.2 mL	12 mL
Collection Tubes, 2 mL	T5449 or T7813	Not included	2 x 50 each	2 x 50 each	Not included

Reagents and Equipment Required

(Not Provided)

- Paper punch
- Forceps (small to medium in size)
- Heat block or water bath at 95 °C
- PCR primers, Cat. No. OLIGO
- Water, PCR reagent, Cat. No. W1754

Precautions and Disclaimer

The REDExtract-N-Amp™ Plant PCR Kits are for R&D use only. Not for drug, household or other uses. Consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

The Extraction Solution, Dilution Solution and REDExtract-N-Amp™ PCR ReadyMix can be stored at 2-8 °C for up to 3 weeks. For longer storage, store at -20 °C. Do not store in a "frost-free" freezer.

Procedure

All steps are carried out at room temperature unless otherwise noted.

DNA extraction

1. Rinse the paper punch and forceps in 70% ethanol prior to use and between handling different samples.
2. Punch a 0.5 to 0.7 cm disk of leaf tissue into a 2 mL collection tube using a standard one-hole paper punch. If frozen plant tissue is used, keep the leaves on ice while punching disks.
3. Add 100 µL of Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the disk is covered by the Extraction Solution.
4. Incubate at 95 °C for 10 minutes. Note that leaf tissues usually do not appear to be degraded after this treatment.
5. Add 100 µL of Dilution Solution and vortex to mix.
6. Store the diluted leaf extract at 2-8 °C. It is not necessary to remove the leaf disk before storage.

PCR Amplification

The REDExtract-N-Amp™ PCR ReadyMix contains JumpStart™ *Taq* antibody for specific hot start amplification. Therefore, PCR mixtures can be assembled at room temperature without premature *Taq* DNA polymerase activity.

Typical final primer concentrations are ~0.4 µM each. The optimal primer concentration and cycling parameters will depend on the system being used.

1. Add the following reagents to a thin-walled PCR microcentrifuge tube:

Reagent	Volume
Water, PCR reagent	Variable
REDExtract-N-Amp™ PCR ReadyMix	10 µL
Forward primer	Variable
Reverse primer	Variable
Leaf disk extract	4 µL *
Total volume	20 µL

*The REDExtract-N-Amp™ PCR ReadyMix is formulated to compensate for components in the Extraction and Dilution Solutions. If less than 4 µL of leaf disk extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction and Dilution Solutions to bring the volume of leaf disk extract up to 4 µL.

2. Mix gently and briefly centrifuge to collect all components at the bottom of the tube.
3. For thermal cyclers without a heated lid, add 20 µL of mineral oil to the top of each tube to prevent evaporation.
4. The amplification parameters should be optimized for individual primers, template, and thermal cycler.

Common cycling parameters

Step	Temperature	Time	Cycles
Initial Denaturation	94 °C	3 minutes	1
Denaturation	94 °C	30 seconds	30-35
Annealing	45 to 68 °C	30 seconds	
Extension	72 °C	1-2 minutes (1 min/kb)	
Final Extension	72 °C	10 min	1
Hold	4 °C	Indefinitely	

5. The amplified DNA can be loaded directly onto an agarose gel after the PCR is completed. It is not necessary to add a separate loading buffer/tracking dye.

Note: PCR products can be purified, if desired, for downstream applications, such as sequencing, with the GenElute™ PCR Clean-Up Kit, Cat. No. NA1020.

References

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5. Innis, M.A., et al. (Eds.) *PCR Strategies*, Academic Press, New York (1995).
6. Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990).
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9. Roux, K.H. Optimization and troubleshooting in PCR. *PCR Methods Appl.*, 4, 5185-5194 (1995).
10. Saiki, R., PCR Technology: Principles and Applications for DNA Amplification, Stockton, New York (1989).

Troubleshooting Guide

Problem	Cause	Solution
Little or no PCR product is detected	PCR may be inhibited due to contaminants in the plant extract.	Dilute the extract with a 50:50 mix of Extraction and Dilution solutions. To test for inhibition, include a DNA control and/or spike a known amount of template (100-500 copies) into the PCR mixture along with the plant extract.
	A PCR component may be missing or degraded.	Run a positive control to ensure components are functioning. A checklist is also recommended when assembling reactions.
	There may be too few cycles performed.	Increase the number of cycles (5-10 additional cycles at a time).
	The annealing temperature may be too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers may not be designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 22 nucleotides long, try to lengthen the primer to 25-30 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%.
	The denaturation temperature may be too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time may be too long or too short.	Optimize the denaturation time by increasing or decreasing the time in 10 second increments.
	The extension time may be too short.	Increase the extension time in 1-minute increments, especially for long templates.
	Target template is difficult.	In most cases, inherently difficult targets are due to unusually high GC content and/or secondary structure. Betaine has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.

Problem	Cause	Solution
Multiple products	JumpStart™ *Taq* antibody is not working correctly.	Do not use DMSO or formamide with REDExtract-N-Amp™ PCR ReadyMix. It can interfere with the enzyme-antibody complex. Other cosolvents, solutes (e.g., salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart™ *Taq* antibody for *Taq* polymerase and thereby compromise its effectiveness.
	Touchdown PCR may be needed.	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications. Touchdown PCR uses an annealing/extension temperature that is higher than the Tm of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer Tm for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.
Contamination	Reagents are contaminated.	Include a reagent blank without DNA template be included as a control in every PCR run to determine if the reagents used in extraction or PCR are contaminated with a template from a previous reaction.

Product Ordering

Order products online at SigmaAldrich.com.

Related Products	Cat. Nos.
Tubes for PCR	Z374873, Z374962, and Z374881
PCR Marker	P9577
Precast Agarose Gels	P6097
TBE Buffer	T4415, T6400 and T9525
Ethanol	E7148, E7023 and 459836
Mineral oil	M8662

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