

Product Information

REExtract-N-Amp™ Plant PCR Kits Product Codes XNAPS, XNAP, XNAPE, and XNAPR

TECHNICAL BULLETIN

Product Description

The REExtract-N-Amp™ Plant PCR Kits contain all 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 REExtract-N-Amp PCR ReadyMix™ and user-provided PCR primers to amplify target DNA. The REExtract-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 REDTaq™ dye to allow direct loading of the PCR product onto an agarose gel.

Reagents Provided (100 preparations)	Product Code	XNAPS 10 extractions, 10 amplifications	XNAP 100 extractions, 100 amplifications	XNAPE 100 extractions, 500 amplifications	XNAPR 1,000 extractions, 1,000 amplifications
Extraction Solution	E 7526	1.2 ml	12 ml	12 ml	120 ml
Dilution Solution	D 5688	1.2 ml	12 ml	12 ml	120 ml
REExtract-N-Amp PCR ReadyMix. This is a 2x PCR reaction mix containing buffer, salts, dNTPs, Taq polymerase and JumpStart Taq antibody.	R 4775	0.15 ml	1.2 ml	5 x 1.2 ml	12 ml
Collection Tubes, 2 ml	T 7813	Not included	2 x 50 each	2 x 50 each	Not included

Reagents and Equipment Required But Not Provided

- Paper punch
- Forceps (small to medium in size)
- Heat block or water bath at 95 °C
- PCR primers
- Water, PCR reagent, Product Code W 1754

Precautions and Disclaimer

The REExtract-N-Amp Plant PCR Kits are for laboratory use only. Not for drug, household or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Storage

The Extraction Solution, Dilution Solution and REExtract-N-Amp PCR ReadyMix can be stored at 2-8 °C for short-term; -20 °C for long-term. Do not store in a "frost-free" freezer.

Procedure

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

A. DNA extraction

1. Rinse the paper punch and forceps in 70% ethanol prior to use and between handling different samples.

- 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.
- Add 100 μ l of Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the disk is covered by the Extraction Solution.
- Incubate at 95 $^{\circ}$ C for 10 minutes. Note that leaf tissues usually do not appear to be degraded after this treatment.
- Add 100 μ l of Dilution Solution and vortex to mix.
- Store the diluted leaf extract at 2-8 $^{\circ}$ C. It is not necessary to remove the leaf disk before storage.

B. PCR amplification

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

Typical final primer concentrations are \sim 0.4 μ M each. The optimal primer concentration and cycling parameters will depend on the system being used.

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

Reagent	Volume
Water, PCR reagent	x μ l
REExtract-N-Amp PCR ReadyMix	10 μ l
Forward primer	y μ l
Reverse primer	y μ l
Leaf disk extract	4 μ l*
Total volume	20 μ l

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

- Mix gently and briefly centrifuge to collect all components at the bottom of the tube.
- For thermal cyclers without a heated lid, add 20 μ l of mineral oil to the top of each tube to prevent evaporation.

- The amplification parameters should be optimized for individual primers, template, and thermal cycler.

Common cycling parameters:

Step	Temperature	Time	Cycles
Initial Denaturation	94 $^{\circ}$ C	3 minutes	1
Denaturation	94 $^{\circ}$ C	0.5-1 minutes	30-35
Annealing	45 to 68 $^{\circ}$ C	0.5-1 minutes	
Extension	72 $^{\circ}$ C	1-2 minutes (~1 kb/min)	
Final Extension	72 $^{\circ}$ C	10 minutes	1
Hold	4 $^{\circ}$ C	Indefinitely	

- 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.

References

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- 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 reaction 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 insure 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.
Multiple products	JumpStart Taq antibody is not working correctly.	Do not use DMSO or formamide with REExtract-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 T_m of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer T_m for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.
Contamination	Reagents are contaminated.	Sigma recommends that 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.

Related Products Product Code

- Tubes for PCR Z37,487-3, Z37,496-2, and Z37,488-1.
- PCR Marker P 9577.
- Precast Agarose Gels P 5597, P 5847, and P 6097.
- TBE Buffer T 4415, T 6400, and T 9525.
- Ethanol, 200 proof 45,983-6.

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