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ProductInformation

Automated Protocol for Extract-N-Amp™ Plant PCR Kits

Using the Tecan Freedom EVO® 150 Workstation

Extract-N-Amp Plant Product Codes **XNAR and XNAPR**

Automation Guide

I. Description

The Extract-N-Amp™ Plant PCR Kits (XNAR and XNAPR) have been developed for use as a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA from various plant leaves in a 96-well format. The Extract-N-Amp Plant PCR Kits provide a novel extraction system that eliminates the need for long enzymatic digestions and homogenization steps that are not amenable to automation. The XNAR Kit includes a specially formulated Extract-N-Amp PCR ReadyMix™ reagent that is a 2x reaction mixture of buffer, salts, dNTPs, and *Taq* polymerase. The ReadyMix reagent also contains Sigma's antibody mediated hot start mechanism, JumpStart™ Taq polymerase, for highly specific amplification of genomic DNA directly from the extract. The XNAPR Kit includes the REDExtract-N-Amp™ PCR ReadyMix reagent containing an inert tracking dye for convenient direct loading of the PCR reactions onto agarose gels for analysis.

The validated method created for use on the Freedom EVO 150 Liquid Handling Workstation from Tecan provides a high throughput protocol for all aspects of the Extract-N-Amp Plant PCR kit.

Extraction and amplification of genomic DNA from plant leaves is accomplished in 4 easy steps:

1. The Extraction Solution is added to a piece of leaf tissue.
2. Extracts are incubated for 10 minutes at 85 °C.
3. The Dilution Solution is added to the extract. Extracts are now stable for at least 6 months if stored at 2–8 °C.
4. PCR reactions are set up using 4 µl of the extracts.

In just 35 minutes, the Freedom EVO 150 can complete the extraction and PCR reaction setup for 96 plant tissue samples.

II. Product Components

Reagents Provided	Product Code	Extract-N-Amp Plant XNAR	REDExtract-N-Amp Plant XNAPR
	Package Size	1000 extractions 1000 amplifications	1000 extractions 1000 amplifications
Extraction Solution	E7526	120 ml	120 ml
Dilution Solution	D5688	120 ml	120 ml
Extract-N-Amp PCR Ready Mix	E3004 (for XNAR) R4775 (for XNAPR)	12 ml	12 ml

III. Storage

The Extract-N-Amp Plant PCR Kits can be stored at 2–8 °C for up to 3 weeks. For long-term storage, store at –20 °C. Do not use a frost-free freezer.

IV. Materials to Be Supplied by the User

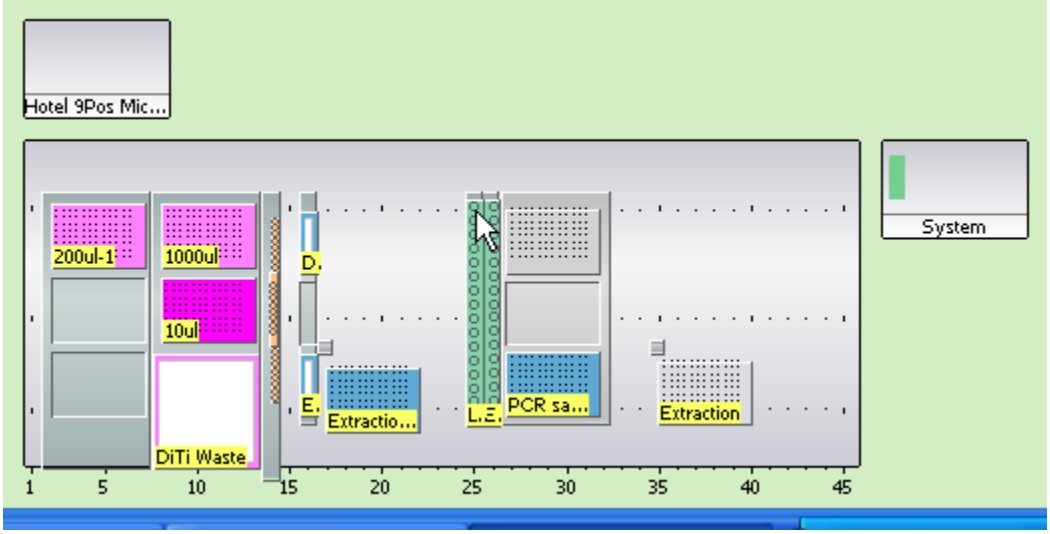
1. Plant leaf tissues
2. Paper punch (standard one-hole)
3. Forceps (small to medium in size)
4. Primers for genes of interest
5. (Optional) GenElute™ Plant Genomic DNA Miniprep Kit (Sigma, G2N10) for use as genomic DNA control.
6. Water, molecular biology reagent (Sigma, W4502)
7. 96 well PCR plates, with full skirt (Sigma, P4616)
8. Lid, universal (Fisher, 07200694)
9. 96-well PCR plates (Stratagene, 410088)
10. Cap strips (Stratagene, 410096)
11. PCR plate holder (Nunc, 251357)
12. 5 ml polypropylene round bottom tube (12 x 75 mm)
13. Microcentrifuge tubes (1.5 ml or 2 ml)
14. Aluminum sealing film (Sigma, A2350)
15. Heating device for 96-well plate (Inheco Industrial Heating & Cooling)
    - CPAC UltraFlat High Temperature (7000091)
    - TEC Control With RS 232 Interface (8900009)
    - 96-well PCR Plate Adapter (3200203)
16. Thermal cycler
17. Thermometer (Fisher, 15-077-26)

V. Instrument Requirements for the Freedom EVO 150 Workstation

(For detailed ordering information, contact Tecan sales representative)

Part Description	Qty
LiHa Arm (8 Channel) with Disposable Tip Option	1
RoMa Arm	1
1 ml syringes	8
DiTi 3 Position	1
DiTi 2 Position with Waste Slide and Cover	1
Wash Station	1
Te-Shake	1
Microplate Carrier (Landscape, 3-Position)	1
MP Hotel (9-Position)	1
16 Position Tube Carrier	1
16 Position Eppendorf Tube Carrier	1
100 ml Trough Carrier (3-Position)	1
100 ml Trough	2
25 ml Trough	2

VI. Worktable Setup



Grid Position	Equipment
2	DiTi 3 position Position 1: 200 µl tips
8	DiTi 2 position with waste slide and cover Position 1: 1000 µl tips Position 2: 10 µl tips Position 3: DiTi waste slide and cover
14	Wash Station Position 1: cleaner shallow Position 2: waste
16	Position 1: 100 ml trough with Dilution Solution Position 3: 100 ml trough with Extraction Solution
17	Te-Shake with 96-well PCR plate containing plant tissue samples
25	13 mm 16 position tube rack Position 16: PCR Master Mix
26	13 mm 16 position tube rack Positions 9–16: Control samples
27	3 position Microplate Carrier, Landscape Position 1: Lid Position 3: PCR amplification plate
35	Heating Device
37	MP Hotel for temporary storage of lid at position 1

VII. Temperature Control Device Setup

Set the temperature control device to the maximum setting of 110 °C with an offset of –4 °C (refer to the Watlow Temperature Control device User's Manual). Place a PCR plate containing 100 µl of water in each well on the device and measure the temperature inside the wells using thermometer probes. Verify that the temperature in the wells is at a minimum of 85 °C after 3 minutes. Approximately one hour prior to running the automated method, turn on the temperature control device and verify that the temperature display on the controller has reached the desired reading.

VIII. Plant Tissue Preparation

1. Rinse a paper punch and forceps in 70% ethanol prior to use and between different samples. Punch a 0.5–0.7 cm leaf tissue disk into a 96-well fully skirted PCR plate ensuring that each sample is centered down into the bottom of each well.
2. Chill the plate at 2–8 °C until needed or flash freeze the samples on dry ice/ethanol and keep at –70 °C.

IX. Reagent Preparation

1. *Extraction Solution*
   To process a single plate of 96 samples, add 10 ml of Extraction Solution to the 100 ml trough at grid location 16, position 3.

2. *Dilution Solution*
   To process a single plate of 96 samples, add 10 ml of Dilution Solution to the 100 ml trough at grid location 16, position 1.

3. *PCR Master Mix*
   The Extract-N-Amp Plant PCR ReadyMix is a 2X reaction mixture containing buffer, salts, dNTPs, and Taq polymerase. To prepare a Master mix, add water and primers (forward and reverse) to the Extract-N-Amp Plant PCR ReadyMix as described in table below.

	Water	PCR Mix	Forward Primer	Reverse Primer
Stock		E3004	100 µM	100 µM
Working (2 ml)	0.75 ml	1.25 ml	3.5 µl	3.5 µl

   To set up 20 µl PCR reactions in one 96-well plate, a total of 2 ml PCR master mix needs to be added to the 5 ml tube at grid location 25, position 16.

X. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated Extract-N-Amp Plant PCR method and can be customized to a variety of applications. To customize applications, see Section XII.

A. Getting Started

1. Turn on temperature control device.
2. Set up the worktable by placing the carriers and racks at the appropriate grid positions as described in section VI.
3. Add reagents to the appropriate troughs as described in section IX.
4. Run the method using Freedom EVOware® Software Version 1.0 SP1 or later.
5. At the completion of the method, place cap strips onto the PCR plate, vortex to mix the solution and briefly centrifuge. The PCR plate is now ready to be placed into a thermal cycler.
6. Seal the PCR plate containing plant tissue extracts with a sealing film. Plant tissue extracts can be stored for up to 6 months at 2–8 °C.

B. Method Overview

The *ExtractNAmpPlant* method performs all of the steps necessary to extract DNA from 96 plant tissue samples and set up PCR reactions using a master mix. For complete program details download the automation program at www.sigmaaldrich.com/automation.

1. Set DiTi positions for 1000 µl, 200 µl, and 10 µl disposable tips.
2. Extraction solution (50 µl) is dispensed to each well of the Extraction plate containing the plant tissue samples.
3. Mix the Extraction plate by shaking at 750 rpm for 30 seconds.
4. Transfer the Extraction plate to a heater for an incubation of 10 minutes at 85 °C.
5. Dilution solution (50 µl) is dispensed into each well of the Extraction plate.
6. PCR master mix (16 µl) is dispensed into all the wells of the PCR plate.
7. Plant tissue extracts (4 µl) are dispensed into each well of the PCR plate.
8. DNA controls (4 µl) are dispensed into wells of column 12 of the PCR plate.

XI. Recommended Parameters for PCR Amplification

Step	Temperature	Time	Cycles
Initial Denaturation	94–96 °C	3 minutes	1
Denaturation	94–96 °C	0.5–1 minutes	
Annealing	45–68 °C	0.5–1 minutes	30–40
Extension	72 °C	1–2 minutes (~1 kb/min)	
Final Extension	72 °C	10 minutes	1
Hold	4 °C	Indefinitely	

XII. Method Customization

A. Use of a different PCR plate

The automated method was created using the 96-well PCR amplification plates with half skirt from Stratagene. Other PCR plates including 384-well plates may be used in this method, but may require the creation of a new labware in the Freedom EVOware software.

B. PCR setup using multiple primer sets

To amplify genomic DNA from the 96 plant tissue extracts with different primer sets, primers can be added to microcentrifuge tubes and placed on the tube racks or added to the PCR ReadyMix and placed into the additional 100 ml or 25 ml troughs on the appropriate carriers. Additional steps will need to be added to the automated program.

XIII. Performance Characteristics

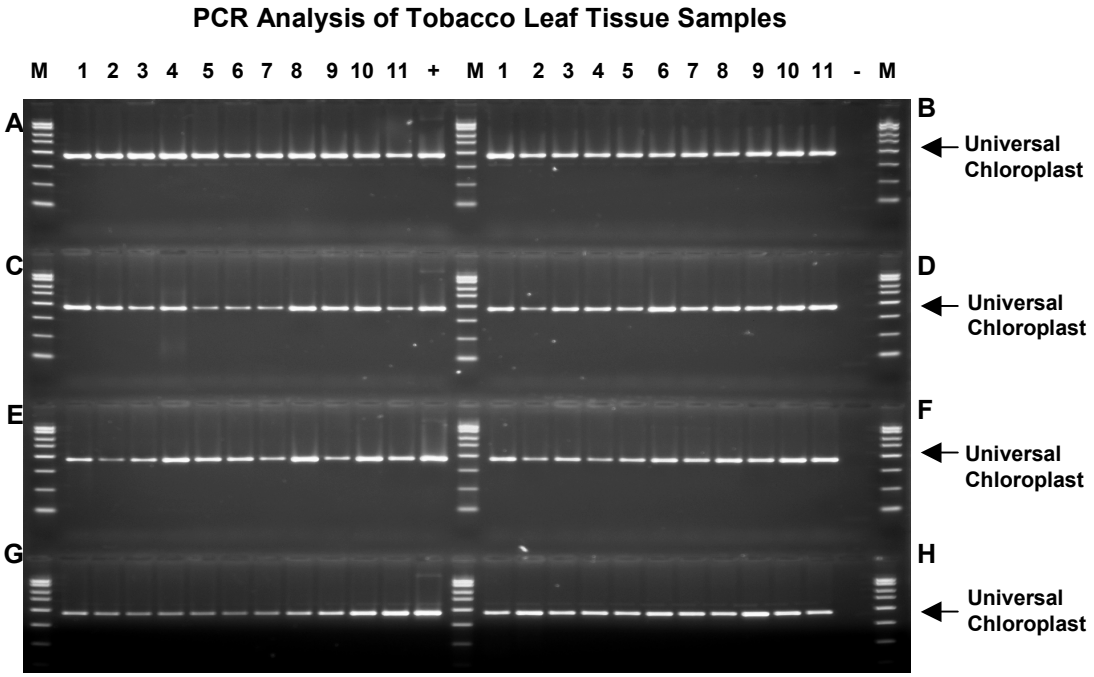


Figure 1. DNA was extracted from 88 Tobacco leaf samples. The 96-well plate was processed using the automated Extract-N-Amp Plant PCR procedure on the Tecan Freedom EVO workstation. Amplification of the 438 bp fragment of universal chloroplast genomic DNA is indicated by the arrow. M: PCR marker. (+): Maize genomic DNA control. (-): No DNA template control.

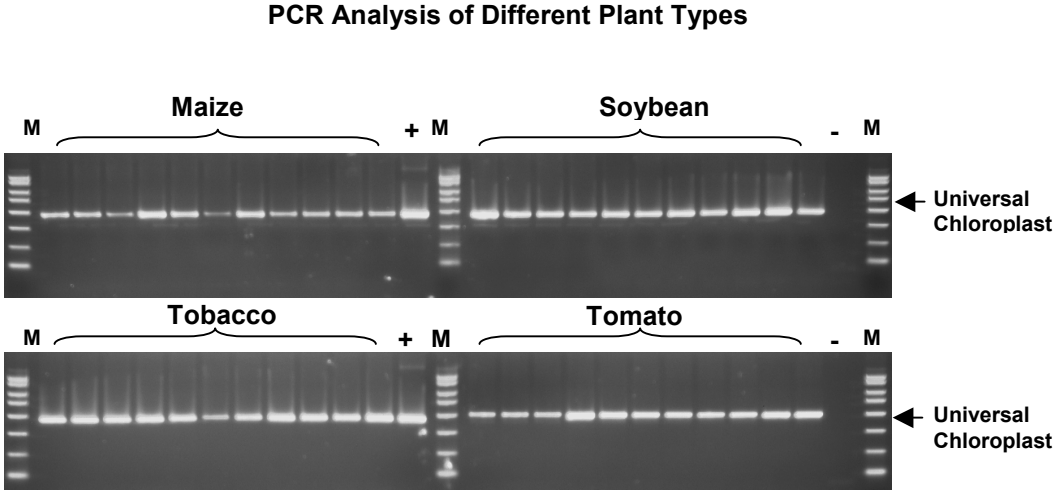


Figure 2. DNA was extracted from maize, soybean, tobacco, and tomato leaves using the automated Extract-N-Amp Plant PCR procedure on the Tecan Freedom EVO workstation. Amplification of the 438 bp fragment of universal chloroplast genomic DNA is indicated by the arrow. M: PCR marker. (+): Maize genomic DNA control. (-): No DNA template control.

Cross-Contamination Analysis

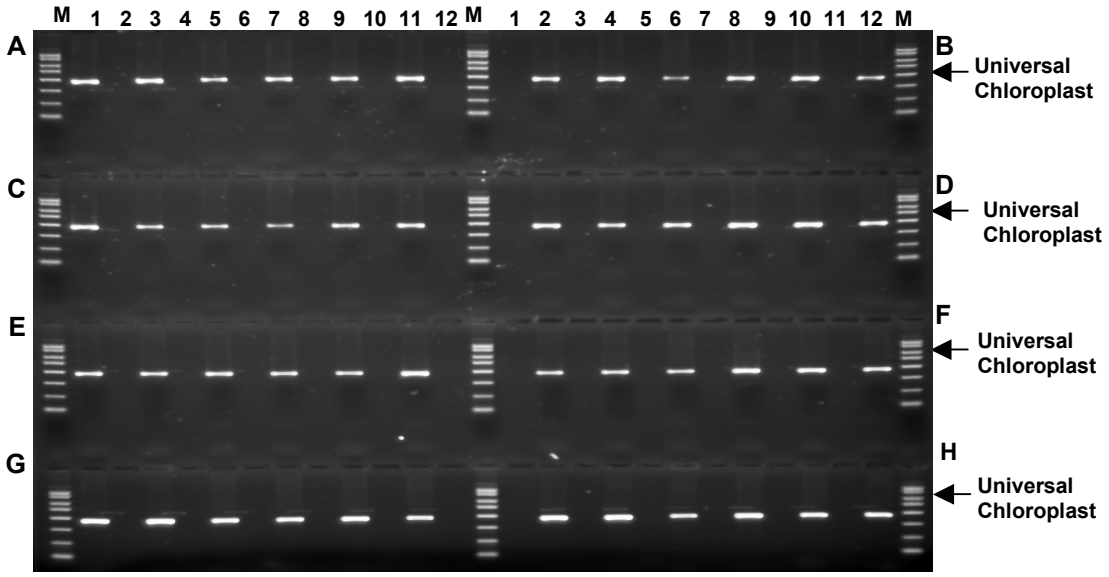


Figure 3. Tobacco leaf disks (0.5–0.7 cm) were placed in alternating wells of a 96-well plate. The plate was then processed using the automated Extract-N-Amp Plant PCR procedure on the Tecan Freedom EVO workstation. All samples were then subjected to amplification and 6 μl of the resultant products were electrophoresed on a 2% Agarose gel. PCR products were not detected in the wells without plant tissue samples.

XIV. Troubleshooting

Problem	Cause	Solution
Little or no PCR product is detected.	A PCR component is missing or degraded.	Run a positive control to ensure components are functioning.
	No leaf tissue extract is added to the PCR reactions.	Check the performance of liquid handler. Prime the system if needed. Adjust the aspiration position of the disposable tips in the extraction plate if the liquid detection function is inactivated.
	PCR reaction is inhibited due to contaminants in leaf tissue extract.	Use less extract or dilute the extract with 50:50 mix of Extraction and Dilution Solutions and repeat PCR.
	The mixing of Dilution Solution with leaf tissue DNA extract is not sufficient due to inefficient mixing by the Liquid Handler and/or the clogging of the pipette tip by the tissue samples.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps. Raise the aspiration position of the pipette tips in the mixing steps to avoid sucking up the tissue by the pipettors.
	Genomic DNA is sheared when the solution is mixed with the pipettor.	Reduce the aspiration and dispensing speed and/or cycle times in the mixing steps. It is critical for amplifying the large genomic DNA fragments.
	Too few amplification cycles are performed.	Increase the number of cycles (5−10 additional cycles at a time).
	Others	Refer to the Technical Bulletin of Extract-N-Amp Plant PCR Kits.
Negative control shows a PCR product or “false positive” results are obtained.	Reagents are contaminated.	Use new labware and new batch of reagents. Test a reagent blank without DNA template to determine if the reagents used in the extraction or PCR are contaminated.

XV. Contact Information

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