# Extract-N-Amp™ Seed PCR Kit: Rapid Genomic DNA Extraction from Seeds Coupled with PCR

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Application Notes

- Rapid 15-minute extraction of genomic DNA for PCR
- Compatible with a wide variety of seed sources
- Genomic DNA extracts are stable at 4 °C for at least 6 months
- Hot Start antibody for highly specific PCR amplification of genomic DNA
- No phenol/chloroform extraction or alcohol precipitation required
- No column purification or centrifugation required

## Introduction

The genotyping of plants can be a time-consuming process. This screening time increases if seeds must be germinated and plants grown. Furthermore, for some screening methods plants must be grown until root and adult leaf tissues are differentiated. In addition to the time that this methodology takes, valuable resources can be tied up as large amounts of greenhouse space are needed to screen large numbers of variants. Extraction of DNA directly from seeds for transgenic screening is an advantageous way to sidestep the requirement of growing plants. Extracting DNA directly from seeds can save a great deal of time and money.

Standard methods for extracting DNA from seeds, such as phenol-chloroform extraction and bind and elute methods, can be laborious and time consuming. The Extract-N-Amp Seed PCR Kit (Product Code XNAS2) was developed to simplify the extraction of genomic DNA for PCR by providing a method to rapidly extract and amplify genomic DNA from seed tissues. This system eliminates the need for organic extraction, centrifugation, column purification, filtration, or alcohol precipitation. The antibody-based hot start PCR ReadyMix™ that is supplied with the kit is specially formulated to work with the extraction reagents, allowing the DNA extract to be added directly to the PCR reaction. PCR is then initiated without any further manipulation or purification.

## Complete kit ensures fast extraction and stable DNA

The Extract-N-Amp Seed PCR Kit was used to extract and successfully amplify target DNA from various seed sources including soybean, corn, cotton, sorghum, canola, *Arabidopsis,* and wheat according to the procedure shown in Figure 1. The various seed extracts were used in PCR to amplify products from 500 bp-2.2 kb (data not shown). The extracts were also used in multiplex PCR, as is shown in Figure 2. The gel image shows the products produced by amplifying the various seed extracts in a reaction containing the 2x Extract-N-Amp PCR Mix and 2 sets of primers, one for the acetylcoenzyme A carboxylase gene in wheat* and the other for a universal chloroplast gene+. The 964 bp band amplified from the wheat acetylcoenzyme A carboxylase gene is only seen after PCR on the wheat extract, whereas the band for the universal chloroplast gene is seen with all of the various seed extracts.

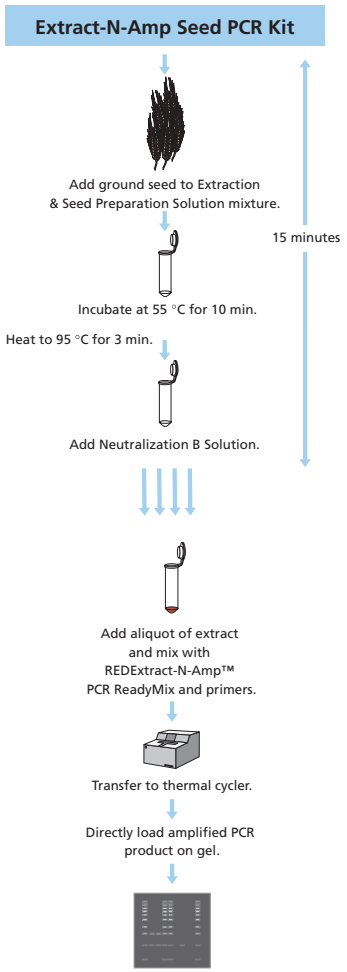


*Figure 1. Overview of Extract-N-Amp seed procedure*

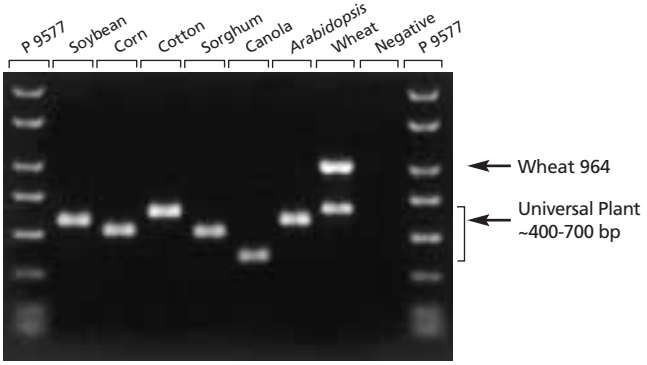


***Figure 2. Multiplex PCR Analysis of Extracts from Various Seed Types.*** *Genomic DNA was extracted from seeds using the protocol as described in the Extract-N-Amp Seed Technical Bulletin. All extracts were then amplified using the specially formulated JumpStart™ PCR ReadyMix and PCR primers multiplexed for both a universal chloroplast gene (~400-700 bp) and the acetylcoenzyme A carboxylase gene specific to wheat (964 bp).*

PCR products produced with the Extract-N-Amp Seed PCR Kit can be sequenced directly, however higher quality sequence is often obtained after purifying the product by a routine clean-up procedure. Clean sequencing traces have been obtained from wheat seed extracts by sequencing with the original PCR primers for acetylcoenzyme A carboxylase (data not shown). Prior to sequencing, the PCR product was purified using the GenElute™ PCR Clean-Up Kit (Product Code NA1020).

To test the stability of the DNA extracts prepared with the Extract-N-Amp Seed PCR Kit, stability tests were set up with soybean seed extracts using quantitative real time PCR. Two soybean seeds were extracted according to the technical bulletin (XNAS2) and 4-µl aliquots were analyzed immediately by quantitative PCR with SYBR® Green dye detection on an ABI PRISM® 7700 (Applied Biosystems, Foster City, CA). The Extract-N-Amp Seed PCR Kit extracts were amplified without significant quenching of the SYBR Green signal. The remaining extracts were stored at the recommended storage temperature of 4 °C. Quantitative PCR was performed after 2.5 weeks, 5 weeks, 2 months, and 6 months for all extracts and on separate aliquots of the same standards used at time zero. As shown in Figure 3, the target DNA in the soybean seed extracts is stable at 4 °C for all time points out to 6 months. Results from stability experiments performed on seed extracts incubated at 37 °C indicate that DNA will be detectable much longer than 6 months when stored at the recommended temperature of 4 °C.

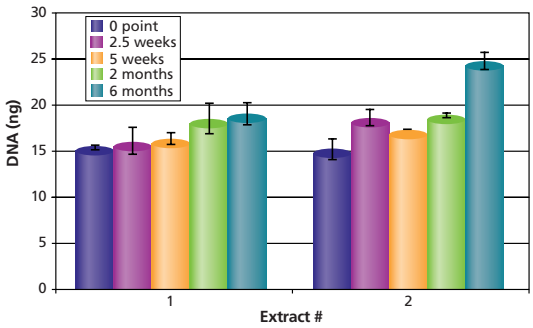


***Figure 3. Stability of Soybean Seed Extracts at 4 °C.*** *Genomic DNA was extracted from soybean seeds with the Extract-N-Amp Seed PCR Kit. 4-µl aliquots were analyzed immediately by quantitative PCR with SYBR® Green detection on an ABI PRISM® 7700. DNA standards for quantitative PCR were purified DNA prepared from soybean seeds using the GenElute™ Plant Genomic DNA MiniPrep Kit (Product Code G2N70) and stored as single use aliquots at –20 °C. The soybean seed extracts were stored at 4 °C (recommended storage). Quantitative PCR was repeated after 2.5 and 5 weeks and then 2 and 6 months for the 4 °C samples. These results show that the extracts will be stable for at least 6 months at the recommended storage temperature of 4 °C.*

## Summary

The Extract-N-Amp Seed PCR Kit can be used to extract genomic DNA that is suitable for PCR in just 15 minutes, using a straightforward extraction protocol. The PCR product that is amplified using the Extract-N-Amp Seed PCR Kit is suitable for direct sequencing. Finally, the DNA extracted by the Extract-N-Amp Seed PCR Kit is stable for at least 6 months at 4 °C, allowing for multiple reassays.

## Primers

*acetylcoenzyme A carboxylase gene

5′-CGTGCTTGCCCTACCT-3′

5′-GTGCCGTTTCTGTTGTTG-3′

+ universal chloroplast

C 5′-CGAAATCGGTAGACGCTACG-3′

D 5′-GGGGATAGAGGGACTTGAAC-3′

## Ordering Information

| Product | Description | Extractions/ Amplifications |
|---|---|---|
| XNASS | REDExtract-N-Amp™ Seed PCR Kit | 10 |
| XNAS | REDExtract-N-Amp™ Seed PCR Kit | 100 |
| XNAS2 | Extract-N-Amp Seed PCR Kit | 100 |