Nucleic Acid Amplification

Quantitative PCR

SYBR® Green JumpStart™ Taq ReadyMix™ for Quantitative PCR

S 4438 SYBR® Green JumpStart Taq ReadyMix for Quantitative PCR combines the advantages of a hot start enzyme with a ready-to-use mix for high throughput, quantitative PCR. The ReadyMix includes SYBR Green I fluorescent dye, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in an optimized 2× concentrate. SYBR Green JumpStart Taq ReadyMix is recommended for single product real-time amplification experiments. It can also be used for PCR optimization prior to manufacture of fluorescent-labeled probes. Fluorescent labeled probes are not recommended for use with SYBR Green I dye.

100 reactions
500 reactions

–20°C
WET ICE

SYBR Green I fluorescent dye binds selectively to double-stranded DNA. Detection of the DNA can then be monitored by measuring the increased fluorescence throughout the PCR cycles. SYBR Green I dye is the most common nonspecific detection method used for quantitative PCR/RT-PCR.

At room temperature, the Taq DNA polymerase remains inactive due to JumpStart Taq antibody binding. The elevated temperature of the first denaturation cycle is sufficient to disrupt the complex, restoring full enzyme activity. No special preparations or protocol changes are required. By preventing nonspecific product formation, JumpStart Taq DNA Polymerase results in more accurate C_T values and an improved standard curve for sample quantitation.

To prepare a reaction, 25 µl of ReadyMix is added to primers, template and water for a final reaction volume of 50 µl. Sigma's Reference Dye for Quantitative PCR is included separately with this ReadyMix for normalization of the reaction data. The dye has a maximum excitation of 586 nm, and a maximum emission of 605 nm. The instrument settings for ROX reference dye are satisfactory for the measurement of the Reference Dye for Quantitative PCR.

Features and Benefits

- Double-stranded DNA detection by SYBR Green I dye
- Hot start PCR for more accurate C_T values and improved quantitation
- High throughput ReadyMix contains all necessary components for quantitative PCR except for primers and template.

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C.

Concentration. . . . . 1.25 units/reaction Taq DNA polymerase (50 µl reaction volume)

R: 36/37/38 S: 26-36

Reference Dye for Quantitative PCR

R 4526 **100 ×, solution** 0.3 mL

2–8°C

For use with quantitative (real-time) PCR.

The dye is used for normalization of reaction data when using SYBR Green I dye, Molecular Beacons, or dual-labeled probe chemistries for real-time detection in a non-capillary format. The dye comes as a 100× solution with a maximum excitation of 586 nm, and a maximum emission of 605 nm. Instrument settings for ROX reference dye are satisfactory for the measurement of the Reference Dye for Quantitative PCR.

sufficient for minimum600 reactions

Genome Mapping and Genotyping

Kits for Genotyping

Extract-N-Amp™ Plant PCR Kits

–20-0°C
WET ICE
NEW

The Extract-N-Amp Plant PCR Kits contain all the reagents necessary to rapidly extract genomic DNA from plant leaves and amplify targets of interest by PCR (Fig. 1). A novel Extraction Solution eliminates the need for conventional freezing of plant tissues with liquid nitrogen, mechanical disruption, organic extraction, column purification, or precipitation of DNA. The kit also includes a PCR ReadyMix™, specially formulated for amplification directly from extract. This formulation uses an antibody based hot start for specific amplification. The mix comes in two formulations: Extract-N-Amp PCR ReadyMix and REDExtract-N-Amp™ Plant PCR ReadyMix. The REDExtract-N-Amp PCR ReadyMix contains a dye that acts as a tracking dye and allows for convenient direct loading of PCR reactions onto agarose gels for analysis.

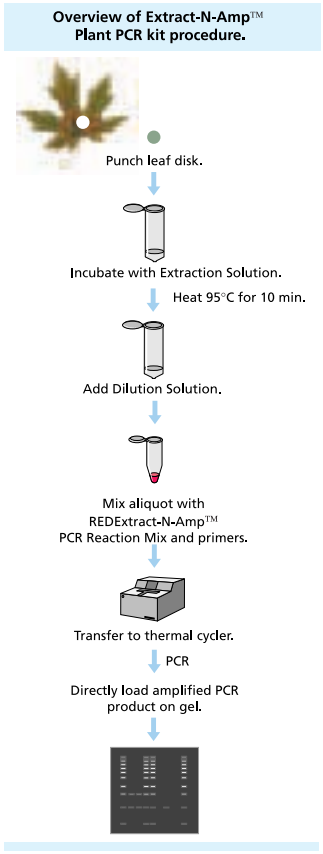
Genomic DNA is extracted from 0.5 to 0.7 cm plant leaf disks that have been cut with a standard paper punch and simply incubated in Extraction Solution at 95 °C for 10 minutes. An equal volume of Dilution Solution is added to the extract to neutralize inhibitory substances prior to PCR. A portion of the DNA extract is then added to a PCR reaction containing primers and either the REDExtract-N-Amp or Extract-N-Amp PCR ReadyMix.

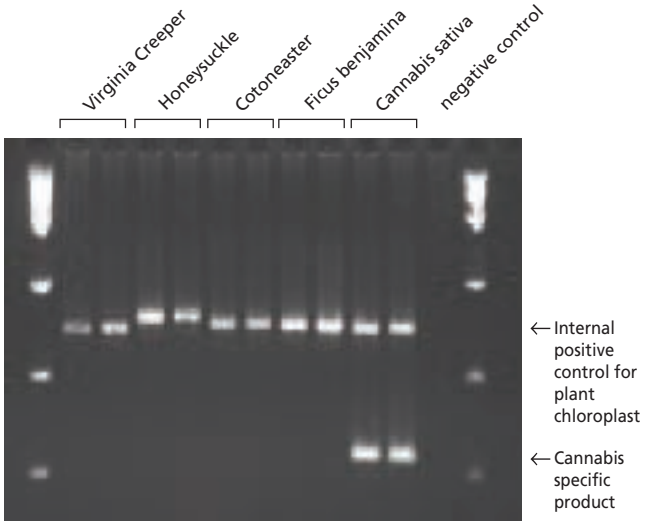
Features and Benefits

- Single-step extraction of plant genomic DNA for PCR in less than 15 minutes
- No freezing, mechanical disruption, organic extraction, column purification or precipitation required
- Specially formulated PCR ReadyMix for use with extract
- Hot Start antibody for highly specific PCR amplification of genomic DNA
- REDExtract-N-Amp requires no loading buffers or tracking dyes required for gel analysis
- Compatible with high-throughput requirements for genetic analysis of plants
- Extract stable at 4 °C for at least 6 months (Fig. 3)

Genome Mapping and Genotyping

Kits for Genotyping





PCR analyses of genomic DNA extracted from 5 different species using Sigma's Extract-N-Amp™ Plant Kit.

Figure 1. Extract-N-Amp™ Plant PCR Kit used to isolate and amplify genomic DNA from various plant sources. Genomic DNA was extracted from 0.5 cm leaf disks that were cut using a standard paper punch. DNA was extracted using the Extract-N-Amp™ Plant PCR Kit in less than 15 minutes. All samples were then amplified using the specially formulated hot start PCR ReadyMix™. The products were generated from a 30-cycle duplex reaction containing primers specific to plant chloroplast (upper band) and primers specific to *Cannabis sativa* DNA (lower band). MW ladder is 100, 200, 400 and 800 bp.
Data provided by Andy Hopwood, Forensic Science Service, Birmingham, England.

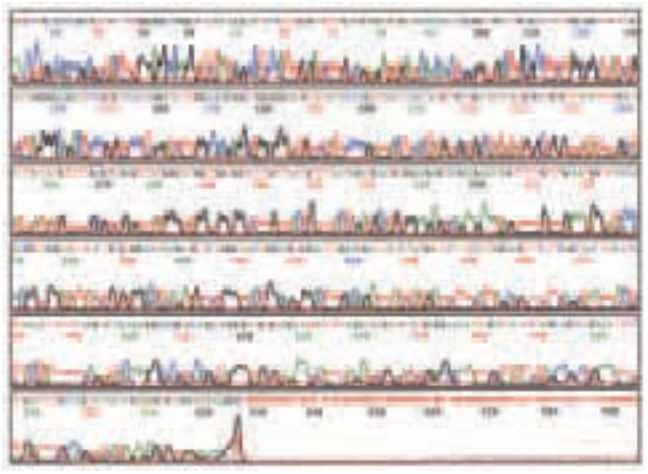


Figure 2. Sequence was resolved on a ABI 310 from a purified, 645 bp corn leaf PCR product. The PCR product was prepared with the GenElute™ PCR Clean-Up Kit (Product Code NA1020). The DNA extraction and PCR were performed using Sigma's Extract-N-Amp™ Plant PCR Kit. The sequence was obtained by using ABI BigDye™ terminator chemistry and the same primers as the original PCR.

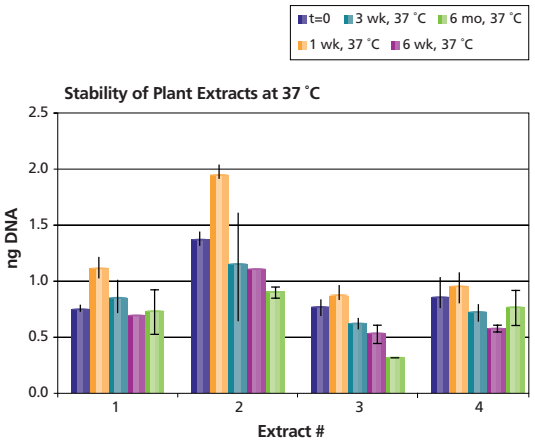


Figure 3. Eight disks were punched from a corn leaf, and DNA was extracted according to the procedure in the Technical Bulletin for the Extract-N-Amp Plant PCR Kit. Two 4-µl aliquots from each were analyzed immediately by quantitative PCR with SYBR® Green detection on an ABI Prism® 7700. DNA standards for quantitative PCR were purified DNA prepared from corn leaf tissue with the GenElute Plant Genomic DNA kit (Product Code G2N70). Half of the leaf extracts were stored at 4 °C (recommended storage conditions) and the other half at 37 °C (accelerated storage). Quantitative PCR was repeated after 1, 3, and 6 months from extracts at 4 °C, and after 1 week, 3 weeks, 6 weeks, and 6 months from extracts at 37 °C. Results for storage at 37 °C are shown. The average of 2 replicate PCR assays from each extract is plotted. Error bars represent one standard deviation. Results for storage at 4 °C are essentially the same as those shown for 37 °C.

XNAPS -20-0°C **REDExtract-N-Amp™ Plant PCR Kit sufficient for 10 extractions, sufficient for 10 amplifications** 1 kit
R: 36/37/38 S: 26-36

XNAP -20-0°C **REDExtract-N-Amp™ Plant PCR Kit sufficient for 100 extractions, sufficient for 100 amplifications** 1 kit
R: 36/37/38 S: 26-36

XNAPE -20-0°C **REDExtract-N-Amp™ Plant PCR Kit sufficient for 100 extractions, sufficient for 500 amplifications** 1 kit
R: 36/37/38 S: 26-36

XNAPR -20-0°C **REDExtract-N-Amp™ Plant PCR Kit sufficient for 1000 extractions, sufficient for 1000 amplifications** 1 kit
R: 36/37/38 S: 26-36

Genome Mapping and Genotyping

Kits for Genotyping

(Continuation of)

Extract-N-Amp™ Plant PCR Kits

XNAPRE –20-0°C — **REDExtract-N-Amp™ Plant PCR Kit** 1 kit
sufficient for 1000 extractions, sufficient for 5000 amplifications
R: 36/37/38 S: 26-36

XNAP2 –20-0°C — **Extract-N-Amp™ Plant PCR Kit** 1 kit
sufficient for 100 extractions, sufficient for 100 amplifications

XNAP2E –20-0°C — **Extract-N-Amp™ Plant PCR Kit** 1 kit
sufficient for 100 extractions, sufficient for 500 amplifications
R: 36/37/38 S: 26-36

XNAR –20-0°C — **Extract-N-Amp™ Plant PCR Kit** 1 kit
sufficient for 1000 extractions, sufficient for 1000 amplifications

XNAP2RE –20-0°C — **Extract-N-Amp™ Plant PCR Kit** 1 kit
sufficient for 1000 extractions, sufficient for 5000 amplifications
R: 36/37/38 S: 26-36

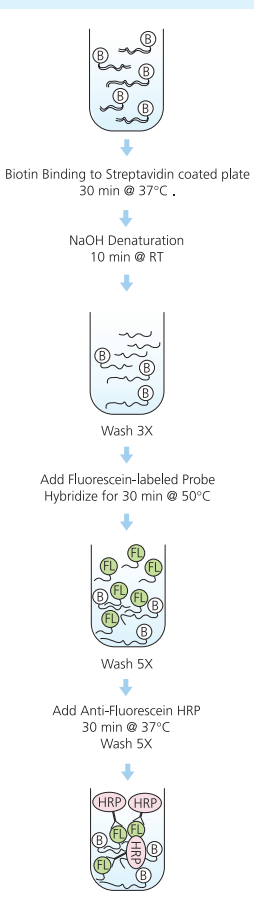
PCR Plate Detection Kit

PPD-1 2-8°C — 1 kit

Perform solid-phase capture and sequence-specific detection of PCR products in an easy-to-use, automatable format and allele-specific hybridization for the detection and genotyping of point mutations. PCR amplification is carried out with one 5′-biotinylated primer and one unlabeled primer. The amplified products are immobilized in streptavidin-coated strip-well plates. The non-biotinylated strand is removed by sodium hydroxide denaturation, and the biotinylated strand is hybridized to a sequence-specific fluorescein-labeled probe. After a wash step, the probe is detected with a peroxidase-conjugated anti-fluorescein antibody and the chromogenic peroxidase substrate TMB. The assay can be completed in 2.5 hours.
sufficient for 480 detection reactions

Components:
Anti-fluorescein-peroxidase conjugate, 500X stock, 300 µL
Denaturation solution, 100 mL
DNA dilution buffer, 250 mL
PlateHyb hybridization buffer, 200 mL
Stop solution, 100 mL
Streptavidin-coated 8 × 12 strip-well plates, 5 each
Thermoplate covers, 15 each
TMB Liquid Substrate System, 2×100 mL
Wash buffer dry packs (phosphate buffered saline with Tween 20, pH 7.4), 10 each

Protocol for Microplate Detection of Amplicons



Genome Walking

Universal Vectorette System

UVS-1 –20°C ◆ WET ICE — 1 kit

The Vectorette™ system is a PCR-based method for DNA walking and mapping that uses a form of unidirectional PCR for amplifying and sequencing unknown genomic or large construct DNA. The system eliminates the time-consuming need to make and screen libraries to obtain overlapping clones that use conventional nucleic acid purification and screening procedures. A Vectorette unit is employed, which consists of a double stranded linker with an internal mismatched region and a sticky end.
The Universal Vectorette system uses three simple steps to obtain DNA sequence information:

Step 1: Genomic or large construct DNA containing target sequence is digested with a restriction enzyme and ligated to a Vectorette unit to create a Vectorette library. The Vectorette library consists of DNA fragments that have a Vectorette unit on each end.
Step 2: PCR is performed on the Vectorette library using a primer complementary to the mismatched region of the Vectorette unit (Vectorette primer provided) and a specific primer to known DNA sequence. In the first PCR cycle, primer extension occurs only from the specific PCR primer that

Genome Mapping and Genotyping

Genome Walking

hybridizes to the known sequence in the DNA fragment within the Vectorette library. Extension from this primer generates a unique sequence as the polymerase reads through the mismatched portion of the Vectorette. Subsequent PCR cycles generate a DNA fragment between the known sequence and the Vectorette unit on the end of the fragment. Any Vectorette fragment that does not contain a sequence that is complementary to the specific primer will not generate a PCR product.

Step 3: A separate sequencing primer is included (slightly nested) that can be used to perform a sequencing reaction from the Vectorette end. PCR products are typically obtained from a single PCR run, however, nested primers are included to increase specificity when amplifying more complex templates. The PCR products generated by the Vectorette system can be used directly for cycle sequencing or cloned into commercially available vectors for further characterization.

Features and Benefits

- Cell-free gene manipulation replaces cloning and subcloning in many molecular genetics projects
- Two and three-step procedures can be performed in a single day
- High fidelity, highly specific amplifications up to 6 kb from genomic DNA
- Eliminates the need for nested PCR in most applications

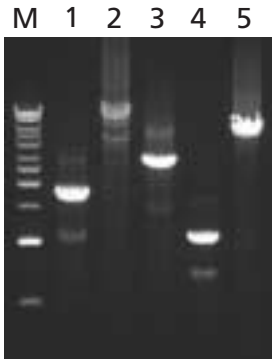
The Vectorette Genomic Library is a preconstructed set of genomic human DNA digested with a specific restriction enzyme and ligated to a Vectorette unit on each end of the digest to create a Vectorette library. This provides added convenience by completing the first step of the Universal Vectorette system. The Vectorette Genomic Library has been constructed using four restriction enzymes: *Bgl* II, *EcoR* I, *Hind* III, and *Pvu* II (blunt end).

Ideal for:

Genome walking
Sequencing of yeast artificial chromosome (YAC) termini
Sequencing of cosmid insert termini
Mapping of promoters, introns, microsatellites, SSR's and STR's
Sequencing of large clones without sub-cloning
Mapping of regions containing deletions, insertions and translocations
Gap-filling in genome mapping projects
Identification of flanking genomic sequences of transgenes in transgenic organisms
1 kit sufficient for 20 PCR reactions
1 kit sufficient for 25 ligation reactions

Components:

JumpStart REDAccuTaq LA DNA Polymerase Mix,
10× AccuTaq LA Buffer,
10mM dNTP mix,
Lyophilized Vectorette *Eco* RI,
Lyophilized Vectorette *Hind* III,
Lyophilized Vectorette *Bam* HI,
Lyophilized Vectorette *Cla* I,
Lyophilized Vectorette Blunt End,
Vectorette primer,
Nested Vectorette primer,
Sequencing primer,
T4 DNA Ligase,
Control lambda DNA,
Lambda PCR control primer,
Lambda nested PCR control primer,
100 mM ATP,
100 mM DTT,



Positive control PCR results for 5 different vectorette libraries. This gel illustrates a common primer to a known sequence generating different amplicon size fragments on five different vectorette libraries.
Lane M. 1 kb DNA Ladder; Lane 1. *Bam* HI vectorette amplicon, 1.9 kb
Lane 2. *Cla I* vectorette amplicon, 8.1 kb
Lane 3. *Eco*RI vectorette amplicon, 3 kb
Lane 4. *Hind* III vectorette amplicon, 1.1 kb
Lane 5. *Sma* I vectorette amplicon, 4.8 kb