

Genomic DNA Amplification

Genomic DNA Amplification Product Listing

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D8312	REDTaq Genomic DNA Polymerase with 10× Reaction Buffer containing MgCl ₂	34
D2812	REDTaq Genomic DNA Polymerase with 10× Reaction Buffer without MgCl ₂	34
UVS1	Universal Vectorette System	36

REDTaq® Genomic DNA Polymerase

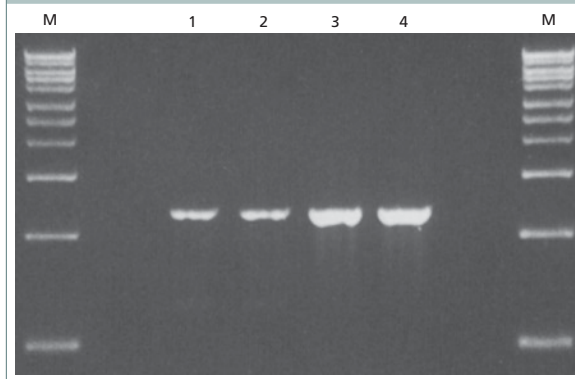
REDTaq Genomic DNA Polymerase is a special formulation of REDTaq DNA Polymerase designed to provide enhanced amplification of more complex genomic templates. REDTaq Genomic DNA Polymerase is more sensitive, produces higher yields and is more capable of generating longer product lengths. It has all the advantages of REDTaq DNA Polymerase, such as easy visualization of enzyme addition and complete reaction mixing, and direct loading to an agarose gel. The dye migrates slightly faster than bromophenol blue at the same rate as a 125 base pair fragment.

The inert red dye has no effect on automated or manual sequencing, restriction digestions, ligation or other downstream applications. However, if dye removal is desired, this can easily be accomplished using any standard purification method.

Features and Benefits

- Enhanced amplification on genomic and difficult DNA templates
- No loading buffers or tracking dyes required. The PCR product is loaded directly onto an agarose gel after amplification
- Quick recognition when the REDTaq has been added to the reaction tube
- Confirms proper mixing at a glance for greater consistency across reactions
- PCR samples can be easily re-amplified as in nested PCR
- The unique red dye migrates slightly faster than bromophenol blue

Higher Yields from Genomic Templates with REDTaq Genomic DNA Polymerase



Higher Yields from Genomic Templates with REDTaq Genomic DNA Polymerase. PCR reactions were set up using 1 µl of mouse genomic DNA and 1 unit of polymerase. The resulting amplicon is a specific 1181 bp fragment. Each sample was prepared in duplicate, and conditions for both sets were identical with the exception of the enzyme used.

Lanes M: 1 kb DNA Ladder ([D3937](#))

Lanes 1, 2: REDTaq DNA Polymerase

Lanes 3, 4: REDTaq Genomic DNA Polymerase

Components: REDTaq Genomic DNA Polymerase
10× PCR Buffer or 10× PCR Buffer without MgCl₂
Separate vial of 25 mM MgCl₂ included with D2812

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

Concentration: 1 unit per µl

Storage: -20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
D8312	REDTaq Genomic DNA Polymerase with 10×	250 units
	Reaction Buffer	1,000 units
	2,500 (10 × 250) units containing MgCl ₂	
D2812	REDTaq Genomic DNA Polymerase with 10×	250 units
	Reaction Buffer	1,000 units
	2,500 (10 × 250) units without MgCl ₂ .	
	Includes a separate tube of 25 mM MgCl ₂	

The Universal Vectorette™ System A PCR-based method for DNA walking and mapping

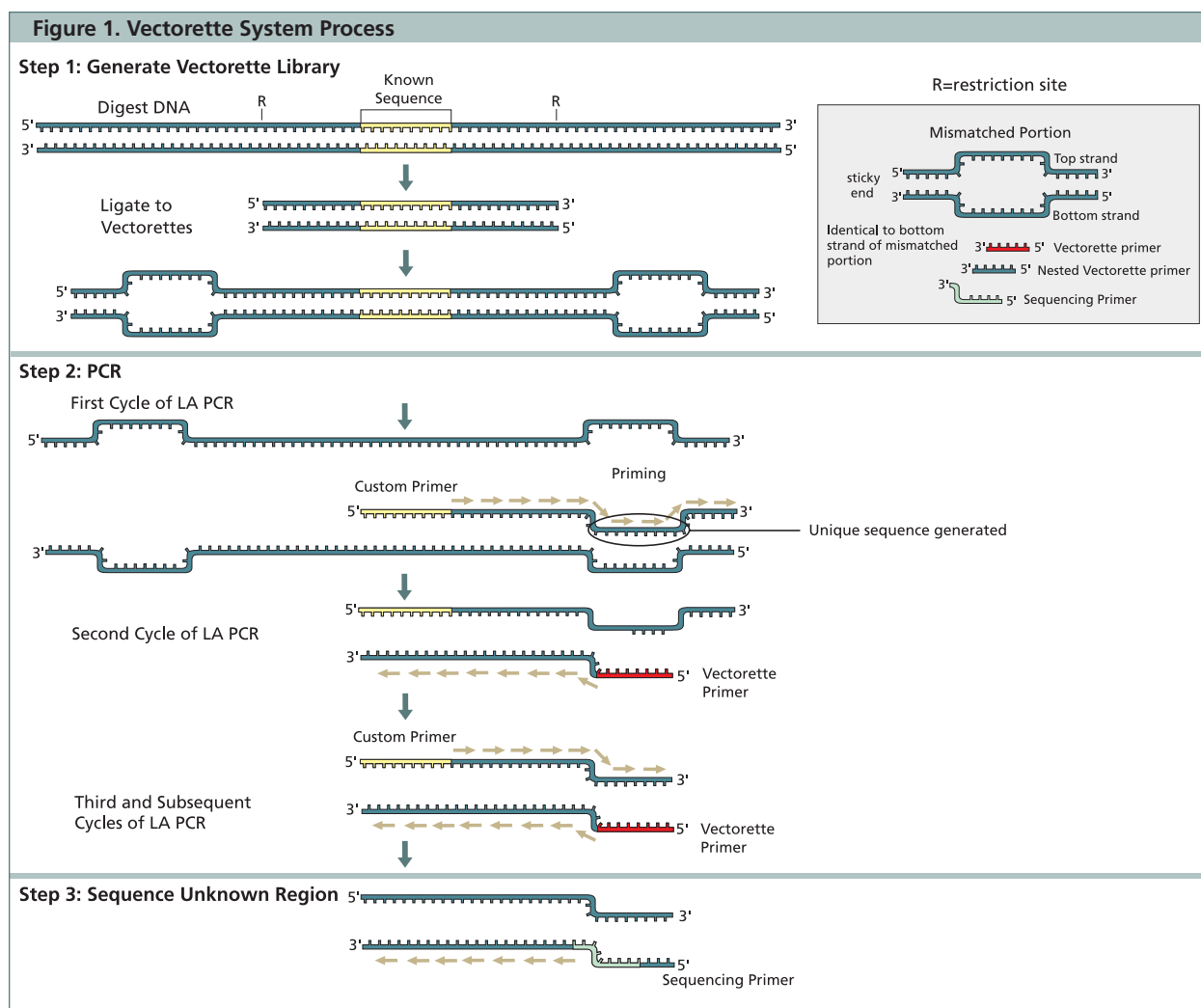
The Vectorette system is a PCR-based method for DNA walking and mapping that uses a form of unidirectional PCR for amplifying and sequencing unknown genomic or large construct DNA. The system eliminates the time-consuming process of making and screening libraries to obtain overlapping clones and using 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 (Fig. 1):

Step 1: Genomic or large construct DNA containing the target sequence is digested with a restriction enzyme and Vectorette units are ligated to the 5' and 3' ends to create a Vectorette library.

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 primer specific to the known DNA sequence. In the first PCR cycle, primer extension occurs only from the specific PCR primer that 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.



Genomic DNA Amplification

The Universal Vectorsystem offers the flexibility to generate Vectorsystem libraries from purified genomic DNA by *Bam* H I, *Cla* I, *Eco* R I, *Hind* III or blunt restriction enzyme digests. This system can be used with 1 µg template genomic DNA or less, and provides a time saving alternative to traditional library construction and screening. The protocol can also be modified for high throughput applications.

Ideal for:

Genome walking
Sequencing of yeast artificial chromosome (YAC) termini
Sequencing of cosmid insert termini
Mapping of promoters, introns, microsatellites, SSRs and STRs
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

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 20 kb from genomic DNA
- Eliminates the need for nested PCR in most applications

Storage: -20 °C

Shipped in wet ice

Figure 2.

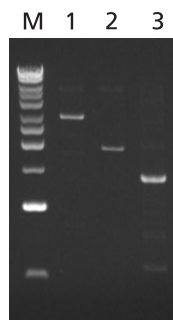


Figure 2. Three different primers were used on a *Cla* I human genomic DNA Vectorsystem library to generate three different sizes of amplicons. The fragments generated are from different regions of the human globin gene.

Lane M: 1 kb DNA Ladder (D3937)

Lane 1: 3 kb amplicon

Lane 2: 1.9 kb amplicon

Lane 3: 1.3 kb amplicon

Figure 3.

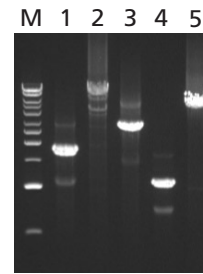


Figure 3. Positive control PCR results for 5 different Vectorsystem libraries. This gel illustrates a common primer to a known sequence generating different amplicon size fragments on five different Vectorsystem libraries.

Lane M: 1 kb DNA Ladder (D3937)

Lane 1: *Bam* H I Vectorsystem amplicon, 1.9 kb

Lane 2: *Cla* I Vectorsystem amplicon, 8.1 kb

Lane 3: *Eco* R I Vectorsystem amplicon, 3 kb

Lane 4: *Hind* III Vectorsystem amplicon, 1.1 kb

Lane 5: *Sma* I Vectorsystem amplicon, 4.8 kb

Ordering Information

Cat. No.	Product Description	Quantity
UVS1	Universal Vectorsystem 1 kit sufficient for 25 ligation reactions and 20 PCR reactions (50 µl reaction volume)	1 kit

Human Genomic DNA

Human Random Control DNA Panels for use as reference standards

Sigma-Aldrich and ECACC have teamed together to provide researchers with control populations of human genomic DNA for gene regulation and quantitative PCR research. The range of Human Random Control (HRC) DNA samples represents a control population of 480 UK Caucasian blood donors. The HRC DNA is extracted from lymphoblastoid cell lines derived by Epstein Barr Virus (EBV) that can be continuously propagated in culture. This ensures an infinite supply of the unvarying DNA panels. The composition of each panel is completely defined and standardized so that each lot will be identical. Therefore, the HRC DNA Panels can be used as reference standards for routine quality control in the laboratory.

Features and Benefits

- Consistent control samples – the DNA is extracted from immortalized cell lines held as cryopreserved banks, so there is no batch-to-batch variation
- Convenient and ready to use – ideal for most standard genetic research applications and avoids errors in preparing controls
- Cost effective – the 96 × 2 µg format provides enough purified HRC DNA for thousands of PCR assays

The purified HRC DNA is available in 5 different panels (HRC1 through 5) consisting of 96 individuals and containing 2 µg DNA each at a concentration of 100 ng/µl. For convenience, all of the panels are available in a PCR compatible 96-well format.

Human Genetic Disease DNA Panel for Breast Cancer

Also available is a Human Genetic Disease DNA Panel for Breast Cancer (HGDBC1). The samples are taken from female patients that have all been diagnosed as having breast cancer. The HGDBC1 panel is provided in a 96-well plate and can be used directly in automated gene analysis systems.



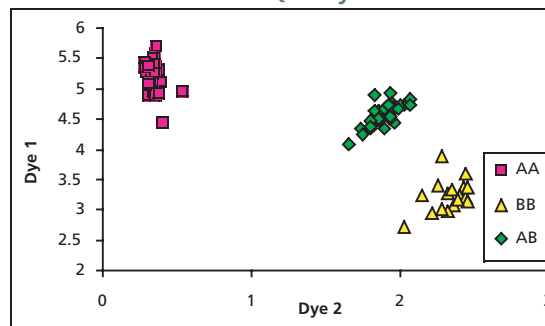
ecacc

SIGMA[®]

Working in Partnership
ecacc.org.uk sigma-aldrich.com

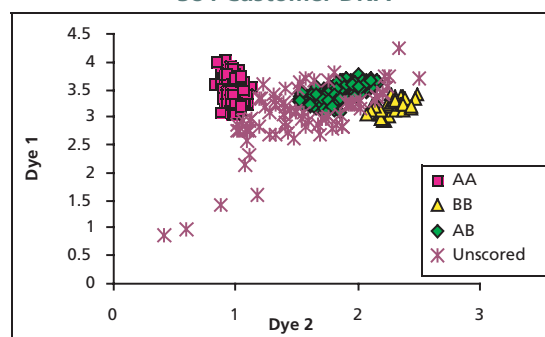
Increased Effectiveness and Reliability of HRC DNA Panels

ECACC DNA Quality Control



(A)

384 Customer DNA



(B)

Increased Effectiveness and Reliability of HRC DNA Panels.

Demonstration of how DNA quality can affect assay performance. The results of the 5' nuclease assay using 96 ECACC Human Random Control (HRC) DNA samples (plot A) and 384 Customer DNA samples (plot B). Homozygotes for allele 1 (AA, top left), heterozygotes for alleles 1 and 2 (AB, middle), homozygotes for allele 2 (BB, lower right). In plot A, using the ECACC HRC1 DNA, the lack of scatter and tight clustering of data points makes scoring the two alleles quite clear. Whereas in plot B, the wide scatter and lack of clustering, make scoring the different alleles very difficult, showing how DNA quality can affect assay performance. Data provided courtesy of MRC Geneservice, Cambridge.

Ordering Information

Cat. No.	Product Description	Quantity
HRC1	Human Random Control Panel 1 Concentration: 100 ng/µl	2 µg
HRC2	Human Random Control Panel 2 Concentration: 100 ng/µl	2 µg
HRC3	Human Random Control Panel 3 Concentration: 100 ng/µl	2 µg
HRC4	Human Random Control Panel 4 Concentration: 100 ng/µl	2 µg
HRC5	Human Random Control Panel 5 Concentration: 100 ng/µl	2 µg
HGDBC1	Human Genetic Disease – Breast Cancer Concentration: 100 ng/µl 96-well plate (20 µl/well)	1 each