GE Healthcare
Life Sciences

Ready-To-Go RAPD Analysis Beads

Product Booklet

Codes: 27-9500-01 (100 rxns)
27-9502-01 (with primers)

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1. Legal

This product is designed for use in the Random Amplified Polymorphic DNA (RAPD) process covered by patents and pending applications including U.S. 5,126,239 owned by E. I. du Pont de Nemours and Company of Wilmington, DE. GE Healthcare has a limited license from E. I du Pont de Nemours and Company to produce and sell this product to the purchaser. The purchase of this product includes a fully paid-up, limited nonexclusive license under the RAPD patent to use this product to perform the RAPD process for research purposes, provided that the RAPD process is conducted using nucleic acid polymerase which has been licensed by Hoffmann-La Roche Inc. for use in the polymerase chain reaction ("PCR") process and provided that RAPD is conducted in conjunction with a thermocycler licensed by the Perkin-Elmer Corporation for use in the PCR process.

Purchase of this product conveys to the user the right to utilize the RAPD process for research purposes only. No right or license is conveyed to the purchaser to develop commercial product, to perform any diagnostic testing, or to offer any commercial service using the RAPD process.

Further information about acquiring rights under the RAPD process to perform commercial testing or for commercial product development can be obtained by contacting E. I. du Pont de Nemours and Company, New Business Development at facsimile number: 302-892-1581.

This product is sold under licensing arrangements with Roche Molecular Systems, F Hoffmann-La Roche Ltd and the Perkin-Elmer

Corporation. Purchase of this product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e. an authorized thermal cycler.

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only.* Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at ambient room temperature in the airtight foil pouch with the desiccant. Once opened, completely reseal the pouch, fold the sealed edge over several times and seal with a clip. In high humidity enviroments, store unopened and resealed pouches in a desiccator to maximize product lifetime.

Reconstituted control DNA and RAPD analysis primer should be stored at -20°C.

2.3. Quality control

Each batch of Ready-To-Go™ RAPD Analysis Beads is tested to ensure its ability to generate a differential banding pattern between the two control *E. coli* strains using the control primer.

3. Components of the kit

The following components are included in this product:

RAPD analysis beads	Room-temperature-stable bead containing buffer, dATP, dCTP, dGTP, dTTP, BSA, thermostable DNA polymerases.
Control *E. coli* BL21(DE3) DNA	1 µg of *E. coli* BL21(DE3) DNA; lyophilized.
Control *E. coli* C1a DNA	1 µg of *E. coli* C1a DNA; lyophilized.
RAPD analysis primer 2	2.5 nmol of lyophilized primer (5'-d[GTTTCGCTCC]-3').
RAPD primer set	Please refer to page 10 (supplied with 27-9502-01 only).

4. Materials not supplied

Reagents

- **10x TBE buffer**–Dissolve 108 g of Tris base and 55 g of boric acid in 900 ml of distilled water. Add 40 ml of 0.5 M EDTA (pH 8.0) and bring to a final volume of 1 liter with distilled water.
- **50x TAE buffer**–Dissolve 242 g of Tris base in 700 ml of distilled water. Add 57.1 ml of glacial acetic acid, 100 ml of 0.5 M EDTA (pH 8.0) and bring to a final volume of 1 liter with distilled water.
- **6x tracking buffer**–30% glycerol, 0.125% bromophenol blue, 20 mM Tris-HCl (pH 8.0).
- **Ethidium bromide**–10 mg/ml in distilled water.
- **Agarose**–GE Healthcare 17-0554-01, -02, -03.
- **Mineral oil**

5. Introduction

Random amplified polymorphic DNA (RAPD) analysis is a technique for rapidly detecting genomic polymorphisms (1), utilizing a single short oligonucleotide primer of arbitrary sequence in a polymerase chain reaction (PCR). The PCR is carried out under low stringency conditions to generate a reproducible array of strain-specific products that are subsequently analyzed by gel electrophoresis.

RAPD analysis has been used in numerous applications, including gene mapping, detection of strain diversity, population analysis, epidemiology, and the demonstration of phylogenetic and taxonomic relationships (reviewed in reference 2). RAPD analysis has become a widely used technique because it enables the quick detection of polymorphisms at a number of different loci using only nanogram quantities of genomic DNA. Furthermore, RAPD analysis can be carried out on organisms for which there is little or no information concerning genomic sequences or organization, thus making it possible to analyze polymorphisms for virtually any organism from which relatively pure genomic DNA can be isolated.

Ready-To-Go RAPD Analysis Beads provide the reagents for RAPD reactions in a convenient ambient-temperature-stable bead. The beads are manufactured using a proprietary technology licensed to GE Healthcare. Ready-To-Go RAPD Analysis Beads have been optimized for RAPD reactions and contain thermostable polymerases, dNTPs (0.4 mM each dNTP in a 25 µl reaction volume), BSA (2.5 µg) and buffer [3 mM $MgCl_2$, 30 mM KCl and 10 mM Tris, (pH 8.3)] in a 25 µl reaction volume. The two different thermostable polymerases, combined in a proprietary ratio, produces a more complex RAPD fingerprinting pattern than either of the polymerases alone. The only reagents that must be added to the reaction are an arbitrary primer and template DNA. The Ready-To-Go bead format significantly reduces the number of pipetting steps, thereby increasing the reproducibility of the RAPD technique and minimizing the risk of contamination.

A primer set consisting of six primers (10-mers) of arbitrary sequence is supplied with product 27-9502-01. This primer set has been successfully used in conjunction with Ready-To-Go RAPD Analysis Beads for the study of a variety of different organisms, including bacteria, fungi, insects, plants, algae and humans. This primer set is not sold separately.

Requirements for template DNA

RAPD analysis can be performed on genomic DNA from virtually any organism. The use of pure, high quality genomic DNA is critical for good RAPD analysis. Since the most frequent cause of poor quality RAPDs is poor quality DNA, it is important to purify genomic DNA using methods that will yield pure, undegraded DNA. To ensure high quality DNA, we recommend using genomicPrep DNA Isolation Kits from GE Healthcare (see page 23, "Related products"). To maximize the reproducibility of the RAPD technique, we recommend that all comparisons of RAPD reactions be made using DNA purified by the same procedure.

The amount of genomic DNA used in a RAPD reaction can vary from as little as 1 ng to as much as 100 ng. As a general rule, 5–50 ng is sufficient, but templates may differ in their optimal amounts. Sometimes a poor quality RAPD can be improved by decreasing or increasing the amount of template. Therefore, it is recommended that a range of template concentrations be tested to determine the amount needed to give the desired banding pattern.

E. coli cells can be added directly to a RAPD reaction without prior extraction of the DNA. However, the reproducibility of the RAPD technique is maximized if purified genomic DNA is used.

Requirements for RAPD Primers

Primers used for RAPD analysis should consist of a single oligonucleotide of arbitrary sequence. The size of the primer is typically 7–15 bases in length. Primers with different arbitrary sequences and different sizes will give different banding patterns with the same DNA. We have obtained the best results with a variety of different templates using primers that are 10 bases long.

RAPD primers should at a minimum be purified using a NAP™-10 column (17-0854-01, -02). Their GC content should be at least 60%, and they should contain no hairpin structures.

Different amounts of primer, relative to template, give different subsets of bands in a RAPD reaction. High concentrations of primer result in primarily low molecular weight bands, while low concentrations of primer result in a banding pattern comprised primarily of high molecular weight bands. We have found that for most templates, 25 pmol of primer and 10 ng of template is optimal for generating the most complex banding pattern.

RAPD Primers from GE Healthcare

GE Healthcare offers a set of six RAPD primers. Each primer is a 10-mer of arbitrary sequence that is specifically designed and tested for use in RAPD analysis. The Ready-To-Go RAPD Analysis Kit (27-9502-01) contains 2.5 nmol of each of the following primers:

RAPD analysis primer 1 - (5′-d[GGTGCGGGAA]-3′)
RAPD analysis primer 2 - (5′-d[GTTTCGCTCC]-3′)†
RAPD analysis primer 3 - (5′-d[GTAGACCCGT]-3′)
RAPD analysis primer 4 - (5′-d[AAGAGCCCGT]-3′)*
RAPD analysis primer 5 - (5′-d[AACGCGCAAC]-3′)*
RAPD analysis primer 6 - (5′-d[CCCGTCAGCA]-3′)*

† Primer 2 is also packaged as a component of Ready-To-Go RAPD Analysis Beads (27-9500-01).

* see reference 3.

When used with the control *E. coli* DNAs in a RAPD reaction, each of the RAPD primers will generate a unique banding pattern. Figure 1 (page 11) shows the approximate banding pattern that can be expected when the control DNA RAPD reactions are electrophoresed on a 2% agarose gel. Note, however, that slight variations in control RAPD patterns may result from differences in thermal cycling and/or electrophoresis conditions.

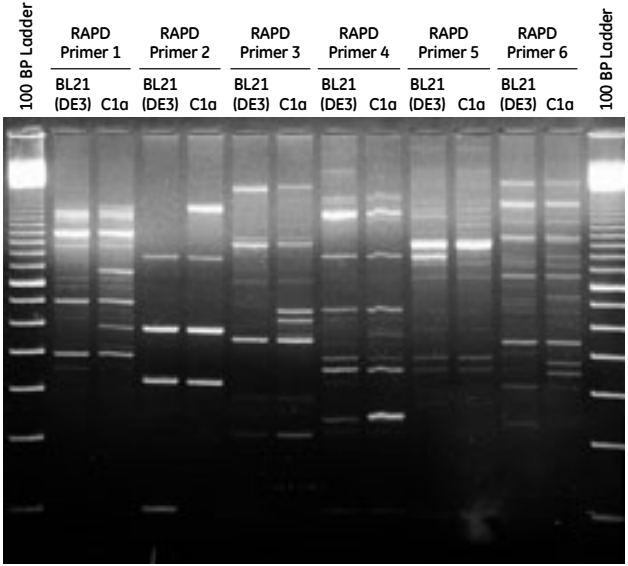

Figure 1. Analysis of control DNA RAPD reactions on a 2% agarose gel. Each reaction was performed with 10 ng of control DNA and 25 pmol of primer using the reaction conditions described in Protocol 1.

6. Protocols

Overview

The reaction conditions described in Protocol 1 have been optimized for genomic DNA from a wide variety of organisms. Any variations to the suggested conditions in relation to reaction volume, primer concentration, DNA template concentration or cycling profile can result in poor RAPD results (see the troubleshooting guide on page 16). It is strongly recommended that all RAPD reactions be performed initially using the conditions stated in Protocol 1. Subsequent reactions may then be further optimized to produce the desired banding pattern for a particular model system.

Control DNA and primer

The two *E. coli* strain DNAs and the RAPD analysis primer included in the kit are provided as controls to assay the ability of the RAPD beads to amplify DNA and identify polymorphisms. Each tube of *E. coli* DNA contains 1.0 µg of lyophilized genomic DNA which should be reconstituted with 200 µl of sterile water to give a final concentration of 5 ng/µl. Reconstituted control DNA should be stored at -20°C.

The tube of RAPD analysis primer 2 contains 2.5 nmol of primer. The primer should be reconstituted with 500 µl of sterile distilled water to give a final concentration of 5 pmol/µl. Reconstituted control primer should be stored at -20°C.

When 10 ng (2 µl) of *E. coli* DNA is used in a RAPD reaction with 25 pmol (5 µl) of RAPD analysis primer 2, a unique subset of PCR products is produced. When the two control DNAs are analyzed on a gel, they will display a differential banding pattern. Figure 2 (page 13) demonstrates the polymorphisms that are observed with the two control DNAs and RAPD primer 2 using the RAPD reaction conditions described in Protocol 1.

Additional reagents required for the procedures are listed in "Materials not supplied" (page 7).

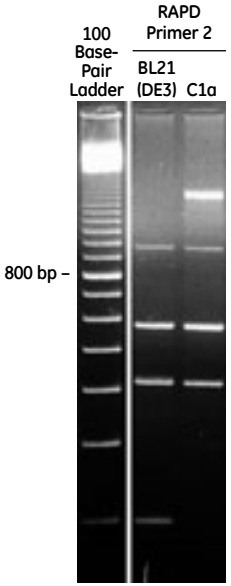

Figure 2. RAPD analysis of *E. coli* BL21 (DE3) and C1a DNA (10 ng each) using RAPD analysis primer 2 (25 pmol) and the reaction conditions described in Protocol 1.

6.1. RAPD reaction

When performing RAPD analysis, exercise extreme care to prevent DNA contamination. Always use sterile, filter pipette tips and avoid carry-over contamination of stock solutions.

1. Check that the bead is visible in the bottom of the tube of RAPD Analysis Beads. If necessary, tap the tube against a hard surface to force the bead to the bottom of the tube.

2. Add the following to a tube containing the RAPD Analysis Bead:

25 pmol of a single RAPD primer	X µl
5–50 ng template DNA	Y µl
Distilled water	to total of 25 µl

3. Mix the contents of the tube by gently vortexing, or by repeatedly pipetting the mixture up and down. Centrifuge briefly to collect the contents at the bottom of the tube.

4. Overlay the reaction with 50 µl of mineral oil if needed.

5. Place the samples in a thermal cycler and cycle using the following profile:

1 cycle: 95°C, 5 minutes
45 cycles: 95°C, 1 minute
36°C, 1 minute
72°C, 2 minutes

6. Continue with Protocol 2, "Gel Analysis".

6.2. Gel analysis

After amplification, the banding pattern of the randomly amplified DNA must be visualized and analyzed. RAPD analysis can be done on either agarose or polyacrylamide gels. For agarose gels:

1. Pour a long (20 cm) 2% agarose gel using 1x TAE or TBE buffer containing 0.5 µg/ml of ethidium bromide.

2. Add 1 µl of 6x tracking buffer to 5 µl of the amplified sample and load onto the gel.

3. Electrophorese the sample until good separation of RAPD bands is observed and the bromophenol blue from the tracking dye is 2.5 cm or less from the bottom of the gel (e.g. 150 volts for 3 hours).

For better resolution of low molecular weight bands, polyacrylamide gel systems may be used. The loading level for a polyacrylamide gel should be approximately 0.5 µl of the 25 µl RAPD reaction. For visualization on acrylamide gels, we recommend using the DNA Silver Staining Kit (17-6000-30) from GE Healthcare.

Note: Bands and/or smears in a "no template" control are normal in RAPD analysis. See page 21 for further information.

7. Troubleshooting

Problem	Possible causes/solutions
1. No bands are visible and/or excessive smearing appears on the gel.	**1.1.** Poor quality DNA is the most common reason for suboptimal RAPDs. Assay the template DNA on a gel to ensure that it is not degraded.
	1.2. Altering the recommended cycling conditions, especially the denaturation temperature, can affect the banding pattern (Figure 3). Use the thermal cycling conditions described in Protocol 1.
	1.3. Depending on the purification method used, the amount of DNA that produces a good banding pattern can vary (Figure 4). Titrate the genomic DNA in the reaction until the smearing is eliminated.

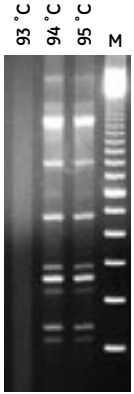

Figure 3. RAPD results of *E. coli* BL21(DE3) DNA (10 ng) using various denaturation temperatures during thermal cycling. All other reaction conditions were identical to those described in Protocol 1. M = 100 Base-Pair Ladder (27-4007-01).

Problem	Possible causes/solutions
	1.4. Improper cycling conditions can result in banding pattern variation and smearing. Check that your thermal cycler is functioning properly.

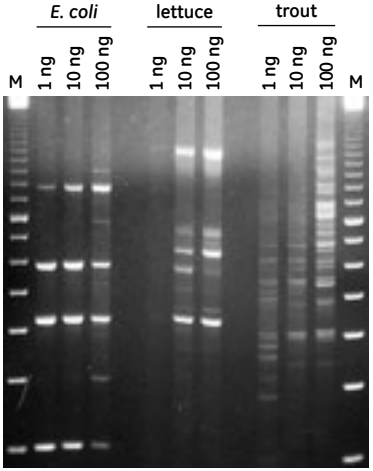

Figure 4. RAPD results using 25 pmol of primer with various amounts of template DNA purified as follows: *E. coli* DNA was isolated using RapidPrep Micro Genomic DNA Isolation Kit for Cells and Tissue (27-5225-01); lettuce DNA was isolated by the CTAB method (4); trout DNA was isolated by the proteinase K method of Grimberg *et al.* (5). All other reaction conditions were identical to those described in Protocol 1.
M = 100 Base-Pair Ladder (27-4001-01).

Problem	Possible causes/solutions
	1.5. The RAPD analysis beads should only be used in a final reaction volume of 25 µl (Figure 5).
	1.6. The RAPD Analysis Beads contain no primer. Therefore, primer must be added by the researcher.
	1.7. Too little primer can result in smearing while excessive primer:template ratios can cause an abundance of low molecular weight bands and smearing (Figure 6). Titrate the primer in the reaction until the smearing is eliminated.

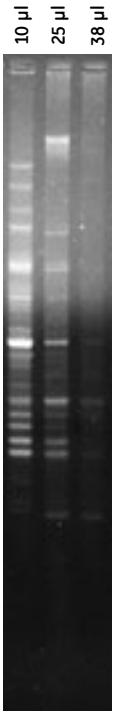

Figure 5. RAPD results using various reaction volumes with 10 ng of spinach DNA. All other reaction conditions were identical to those described in Protocol 1.

Problem	Possible causes/solutions
2. A reproducible banding pattern is not achieved when using the same template and primer.	**2.1.** Different primer to template ratios in a RAPD reaction can result in different banding patterns on a gel (Figure 4). It is critical that the exact same amount of template is used each time. Using "master mixes" (i.e. pre-mixed solutions of template and primer) will increase reproducibility.
	2.2. Different primer to template ratios in a RAPD reaction can result in different banding patterns on a gel (Figure 6). It is critical that the exact same amount of primer is used each time. Using "master mixes" (i.e. pre-mixed solutions of template and primer) will increase reproducibility.

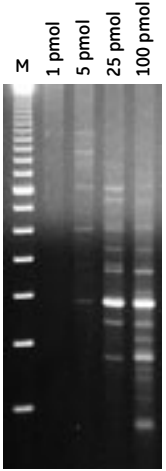

Fig 6. RAPD results using various amounts of primer with 10 ng of porcine DNA. All other reaction conditions were identical to those described in Protocol 1. M = 100 Base-Pair Ladder (27-4007-01).

Problem	Possible causes/solutions
	2.3. Degraded DNA generates a poor banding pattern. Analyze the template DNA on a gel to ensure that it is not degraded.
	2.4. Differences in thermal cycler design and ramping speeds can result in significant differences in banding patterns. Whenever possible, use the same instrument to avoid these variations. Improper cycling conditions can also result in banding variations. Check that your thermal cycler is functioning properly and switch to a different instrument if necessary. Variations within a single cycler can result from temperature variations across the block. This problem can be avoided by switching to another, less variable thermal cycler.

Problem	Possible causes/solutions
3. There are visible bands and/or smears in a "no template" control sample.	**3.** Bands and/or smears in a "no template" control are normal in RAPD analysis (1, 6, 7). The bands and/or smears probably arise from small amounts of DNA contamination in the polymerases. These bands are not observed when 10 ng of template is present in a reaction. At these levels, the template DNA will out-compete any contaminant DNA that might be present.

8. References

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2. Welsh, J. *et al., PCR 2: A Practical Approach,* McPherson, M. J., Hames, B. D., Taylor, G.R. eds., chapter 11, IRL Press (1995).

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4. Doyle, J. *et al., Phytochem. Bull.* **19**, 11 (1987).

5. Grimberg, J. *et al., Nucl. Acids Res.* **17**, 8390 (1989).

6. Klein-Lankhorst, R. M. *et al., Theor. Appl. Genet*. **83**, 108 (1991).

7. Hadrys, H. *et al., Molecular Ecology* **1**, 55 (1992).

9. Related products

Product	Pack size	Product number
genomicPrep Cells and Tissue DNA Isolation Kit (55 standard purifications)	1 kit	27-5237-01
genomicPrep Blood DNA Isolation Kit (100 purifications)	1 kit	27-5236-01
100 Base-Pair Ladder	100 µg	27-4007-01†
GNA 200 (cooling plate, tray, 22-well comb)	1 set	18-2300-02
DNA Silver Staining Kit	1 kit	17-6000-30

† Product must be shipped cold. There is an extra charge for insulated container and refrigerant.

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