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Ready-To-Go RAPD Analysis Beads

Product Booklet

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27950001PL AE

Table of Contents

1 Introduction

Product codes

27950001 (100 rxns)

27950201 (with primers)

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

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.

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.

2 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 *RAPD Primers from Cytiva, on page 8* (supplied with 27950201 only).

3 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**–Cytiva 17055401, -02, -03.
- **Mineral oil**

4 Background

Random amplified polymorphic DNA (RAPD) analysis is a technique for rapidly detecting genomic polymorphisms (see reference 1 in *Chapter 8 References, on page 20*), 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 in *Chapter 8 References, on page 20*). 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 Cytiva. 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 27950201. 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 Cytiva, see *Chapter 7 Related products, on page 19*. 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 (17085401, -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 Cytiva

Cytiva 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 (27950201) 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′)[1]

RAPD analysis primer 3 - (5′-d[GTAGACCCGT]-3′)

RAPD analysis primer 4 - (5′-d[AAGAGCCCGT]-3′)[2]

RAPD analysis primer 5 - (5′-d[AACGCGCAAC]-3′)[2]

RAPD analysis primer 6 - (5′-d[CCCGTCAGCA]-3′)[2]

[1] Primer 2 is also packaged as a component of Ready-To-Go RAPD Analysis Beads (27950001).

[2] See reference 3 in *Chapter 8 References, on page 20*

When used with the control *E. coli* DNAs in a RAPD reaction, each of the RAPD primers will generate a unique banding pattern. *Fig. 1, on page 9* 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.

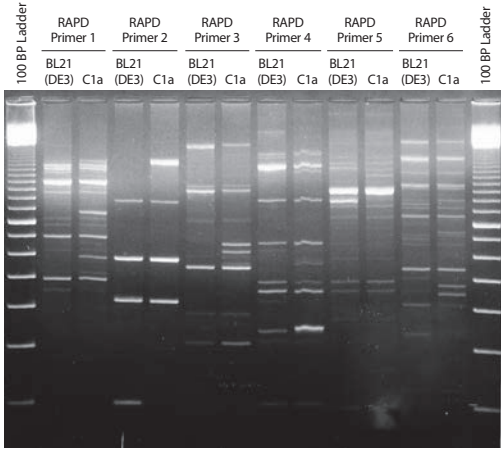

Fig 1. Analysis of control DNA RAPD reactions on a 2% agarose gel. Each reaction was performed with 10 ng of control DNA and 25 pmol of primer using the reaction conditions described in ***RAPD reaction, on page 12***.

5 Protocols

Overview

The reaction conditions described in *RAPD reaction, on page 12* 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 *Troubleshooting, on page 14*. It is strongly recommended that all RAPD reactions be performed initially using the conditions stated in *RAPD reaction, on page 12*. 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. *Fig. 2, on page 11* demonstrates the polymorphisms that are observed with the two control DNAs and RAPD primer 2 using the RAPD reaction conditions described in *RAPD reaction, on page 12*.

Additional reagents required for the procedures are listed in *Chapter 3 Materials not supplied, on page 4*.

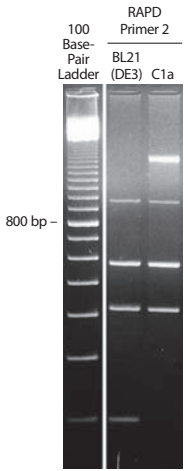

Fig 2. RAPD analysis of E. coli BL21 (DE3) and C1a DNA (10 ng each) using RAPD analysis primer 2 (25 pmol) and the reaction conditions described in ***RAPD reaction, on page 12***.

RAPD reaction

Note: *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.*

Step	Action
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

Step	Action	
	45 cycles: 95°C	1 minute
	36°C	1 minute
	72°C	2 minutes
6	Proceed to the next part of the protocol.	

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:

Step	Action
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 (17600030) from Cytiva.

Note: *Bands and/or smears in a "no template" control are normal in RAPD analysis. See the Troubleshooting section below for further information.*

6 Appendix

Troubleshooting

No bands are visible and/or excessive smearing appears on the gel.

- 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.
- Altering the recommended cycling conditions, especially the denaturation temperature, can affect the banding pattern (see the figure below). Use the thermal cycling conditions described in *RAPD reaction, on page 12*.

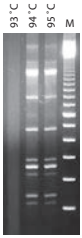

Fig 3. RAPD results of E. coli BL21(DE3) DNA (10 ng) using various denaturation temperatures during thermal cycling. All other reaction conditions were identical to those described in ***RAPD reaction, on page 12***. M = 100 Base-Pair Ladder (27400701).

- Depending on the purification method used, the amount of DNA that produces a good banding pattern can vary (see the figure below). Titrate the genomic DNA in the reaction until the smearing is eliminated.

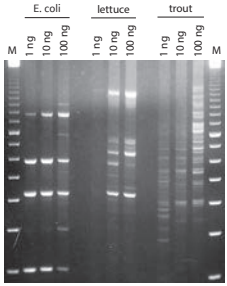

Fig 4. RAPD results using 25 pmol of primer with various amounts of template DNA purified as follows: E. coli DNA was isolated using RapidPrep Micro Genomic DNA Isolation Kit for Cells and Tissue (27522501); lettuce DNA was isolated by the CTAB method (see reference 4 in ***Chapter 8 References, on page 20***); trout DNA was isolated by the proteinase K method of Grimberg et al. (see reference 5 in ***Chapter 8 References, on page 20***). All other reaction conditions were identical to those described in ***RAPD reaction, on page 12***. M = 100 Base-Pair Ladder (27400101).

- Improper cycling conditions can result in banding pattern variation and smearing. Check that your thermal cycler is functioning properly.
- The RAPD analysis beads should only be used in a final reaction volume of 25 µL (*Fig. 5, on page 17*).

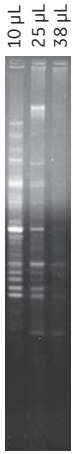

Fig 5. RAPD results using various reaction volumes with 10 ng of spinach DNA. All other reaction conditions were identical to those described in ***RAPD reaction, on page 12***.

- The RAPD Analysis Beads contain no primer. Therefore, primer must be added by the researcher.
- Too little primer can result in smearing while excessive primer:template ratios can cause an abundance of low molecular weight bands and smearing (*Fig. 6, on page 18*). Titrate the primer in the reaction until the smearing is eliminated.

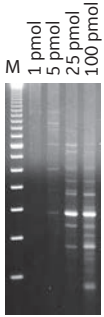

Fig 6. RAPD results using various amounts of primer with 10 ng of porcine DNA. All other reaction conditions were identical to those described in ***RAPD reaction, on page 12***. M = 100 Base-Pair Ladder (27400701).

A reproducible banding pattern is not achieved when using the same template and primer.

- Different primer to template ratios in a RAPD reaction can result in different banding patterns on a gel (*Fig. 4, on page 16*). 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.
- Different primer to template ratios in a RAPD reaction can result in different banding patterns on a gel (*Fig. 6, on page 18*). It is critical that the exact same amount of primer is used each time. Using "master mixes" (i.e. premixed solutions of template and primer) will increase reproducibility.
- Degraded DNA generates a poor banding pattern. Analyze the template DNA on a gel to ensure that it is not degraded.

- 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.

There are visible bands and/or smears in a "no template" control sample.

- Bands and/or smears in a "no template" control are normal in RAPD analysis, see reference 1, 6 and 7 in *Chapter 8 References, on page 20*. 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 outcompete any contaminant DNA that might be present.

7 Related products

Product	Pack size	Product number
genomicPrep Cells and Tissue DNA Isolation Kit (55 standard purifications)	1 kit	27523701
genomicPrep Blood DNA Isolation Kit (100 purifications)	1 kit	27523601
100 Base-Pair Ladder	100 µg	27400701[1]

Product	Pack size	Product number
GNA 200 (cooling plate, tray, 22-well comb)	1 set	18230002
DNA Silver Staining Kit	1 kit	17600030

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

8 References

1. Williams, J. G. *et al., Nucl. Acids Res.* **18**, 6531 (1990).

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).

3. Akopyanz, N. *et al., Nucl. Acids Res.* **20**, 5137 (1992).

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

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

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

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