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Rapid screening of PCR products using a novel agarose gel electrophoresis system

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NUCLEIC ACID AMPLIFICATION

Ready-To-Run™ Electrophoresis System is designed for the rapid screening of large numbers of PCR products. The modular system features precast gel cassettes; a separation unit with built-in power supply, timer and electrodes; sample loading guide plate; and satellite separation units, each with built-in timer and electrodes. Gel cassettes contain integral buffer strips and are directly compatible with standard 96-well microplates, maintaining the 8 × 12 grid for easy sample identification. Using the system, 96 samples can be processed in about 10 min. This compares with over 2 h using traditional agarose gel electrophoresis.

Introduction

Many high-throughput DNA analysis methods, including sequencing, array production, library screening, DNA mapping, and clone analysis, use PCR products as starting material. To produce the large number of samples required, PCR is typically performed in standard 96-well or 384-well microplates. The amplified products must be screened prior to use to verify their quality.

Screening is usually performed by agarose gel electrophoresis. Gel preparation and electrophoresis time for a 200-sample gel can take as long as 3.5 h. Large volumes of buffer are required that may need special handling for disposal if ethidium bromide or other dyes were used. Commercially available precast gels can reduce preparation time, but are expensive for large numbers of samples.

Ready-To-Run Electrophoresis System was developed to make agarose gel electrophoresis convenient for the rapid analysis of large numbers of PCR products (Fig 1). The system consists of precast Ready-To-Run agarose gels in UV-transparent acrylic cassettes, the Ready-To-Run Separation Unit with a built-in power supply, and Sample Loading Guide Plates to aid application of large numbers of samples to the gel. In addition, where greater sample throughput is required, up to three, optional Ready-To-Run Satellite Separation Units can be added to the system to separate 384 samples at once.



Fig 1. Ready-To-Run Electrophoresis System features a Separation Unit with a built-in power supply, pre-cast agarose gels that match the standard 96-well microplate format, and Sample Loading Guide Plate for easy gel loading. Sample well positions are compatible with standard 8- and 12-channel pipettes.

Novel gel system

The heart of the system is the precast agarose gel (1.2% agarose in TBE) that, in overall dimensions and sample placement, conforms to the industry standard 96-well microplate. The patent-pending cassette design includes buffer strips as a part of the agarose gel, which means that no liquid buffer and no manual placing of buffer strips are required.

In addition, ethidium bromide is included in the gels (0.5 µg/ml) to eliminate the need to stain, destain, and dispose of dye-containing buffer. The gel cassettes are UV transparent. Any standard method of visualizing ethidium bromide-stained DNA can be used with Ready-To-Run gels.

Two formats of gels are available: one with 96 sample wells and 16 reference wells, and one with 48 sample wells and 8 reference wells. The gels are suitable for separating DNA samples 100–5000 bp in size. Marker fragments of 800, 1200, and 2000 bp can be clearly resolved in a 6-minute separation.

◇ See licensing information on page 3.

Simplified sample application

The Sample Loading Guide Plate facilitates loading large numbers of samples quickly and accurately. A guide plate has 112 holes corresponding precisely to the positions of the 96 sample wells and 16 reference wells in the Ready-To-Run 96-well gel. The wide openings in the top of the plate guide the tips to the well positions, but the narrow bottom diameter of the holes prevents the tips from penetrating deeply enough to gouge the gel. A guide plate can be used on either a 96- or a 48-well Ready-To-Run gel. Two versions of Sample Loading Guide Plate are available to accommodate a variety of tips from different manufacturers.

The actual time required for electrophoresis in the Ready-To-Run 96-well gel is between 5 and 6 min at the highest voltage setting. Including loading, running, and documentation, 96 samples can be processed in about 10 min. With three Satellite Separation Units connected to a single Separation Unit, 384 samples can be processed in less than 20 min. For the Ready-To-Run 48-well gel, the sample separation is complete in about 12 min.

Rapid performance

To test the utility of the Ready-To-Run System, we compared the new system performance with the performance of a “standard” agarose gel apparatus for checking 96 PCR products prior to spotting onto microarray slides (1). The PCR products (500 bp) were amplified from human genomic DNA and purified prior to evaluation. Samples were evaluated for the presence or absence of single or multiple bands and smears. Results are shown in Figure 2.

Both electrophoresis systems were able to detect smears and multiple bands. The advantage of the Ready-To-Run system was in processing time (6 min for Ready-To-Run vs. 2 h plus additional time to pour and cool the gel for the standard method), and waste stream reduction (standard method required disposal of 200 ml of SYBR™ Green containing buffer).

Conclusion

Many high-throughput DNA applications require the screening of several thousand PCR products. Ready-To-Run Electrophoresis System provides a cost-effective method for rapidly processing large numbers of samples, minimizing handling time and reagent consumption.

Reference

1. Worley, J. *et al.*, in *Microarray Biochip Technology*, (Scheda, M., ed.), Biotechniques Book, Natick, MA, pp. 65–86 (2000).

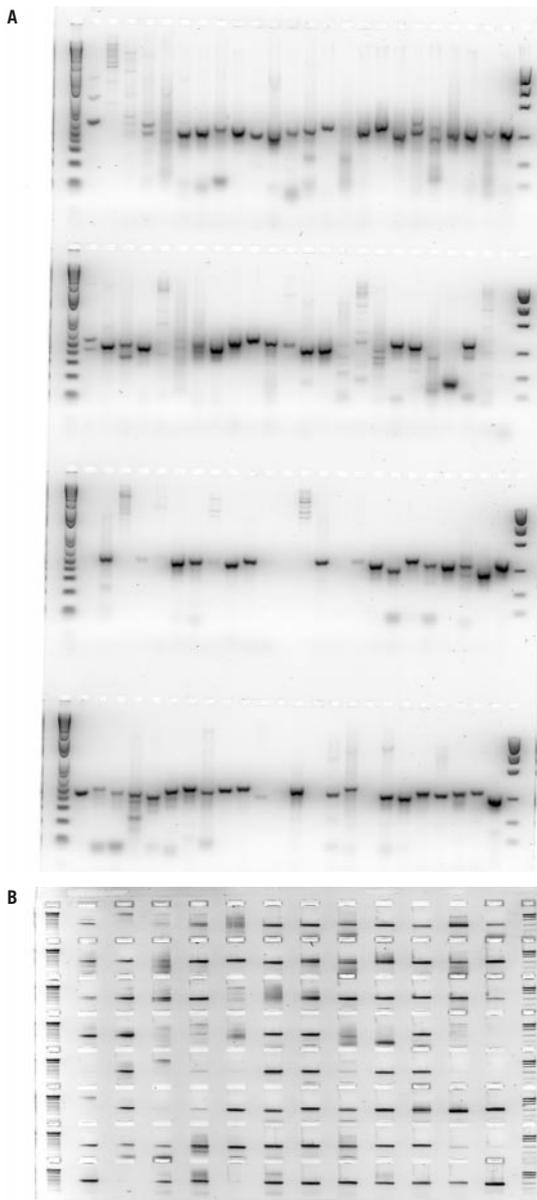


Fig 2. Comparison of separations on a large-format agarose gel vs. a Ready-To-Run gel. (A) 1 µl of a 1:10 dilution of PCR product separated in a 2% agarose gel containing SYBR Green I at 83 V for approximately 2 h. (B) 1 µl of a 1:5 dilution of PCR product separated in a 1.5% agarose Ready-To-Run gel at 120 V for approximately 6 min. Both gels were imaged using FluorImager laser scanner. Markers for both gels were a 1 kb ladder on the right side and a 100 bp ladder on the left side.

ORDERING INFORMATION		
Ready-To-Run Separation Unit	1	80-6460-95
Ready-To-Run Satellite Unit	1	80-6461-14
Ready-To-Run 96 well, 1.2% agarose gel	10 gels	80-6461-33
Ready-To-Run 48 well, 1.2% agarose gel	10 gels	80-6461-71
Ready-To-Run Loading Guide Plates	2	80-6462-09



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