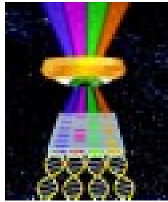

Focal Points

Application Note FP-119

UVP

BioImaging Systems

Using the UVP BioDoc-It Imaging System for Analysis of Differential Display RT-PCR Gels

Cathey Serrano and Todd P. Primm, Ph.D.
Department of Biological Sciences
Border BioMedical Research Center
The University of Texas at El Paso
El Paso, Texas

Differential display reverse transcriptase polymerase chain reaction (DD RT-PCR) is a cutting-edge technique in molecular biology, which allows the detection of genes, which are positively or negatively regulated transcriptionally under conditions of interest, without knowing the gene sequences in advance. This technique has been used successfully in eukaryotes and prokaryotes. Basically, the bacterial protocol involves exposing the organism of interest to two or more conditions, then extracting total RNA. Following reverse transcription, the cDNA generated serves as a template for arbitrarily primed PCR. To reveal RNAs, which were differentially expressed, the PCR products from different conditions are run side-by-side in an agarose or polyacrylamide gel. Imaging of bands for DD RT-PCR can be quite challenging. One must have the capacity to detect not only faint bands, but also in the presence of strong bands (demanding a wide dynamic range of detection in one image). In addition to sensitivity and broad range, the capacity to clone and sequence bands of interest following detection is critical. Radiolabeling during the PCR step with ^{32}P or ^{33}P is typical. In efforts to examine non-radioactive protocols, we have compared the sensitivity of imaging agarose gels using the UVP BioDoc-It system following ethidium bromide staining to silver staining (BioRad Silver Stain Plus kit). We were surprised to discover that imaging was superior in sensitivity to silver staining. Further, the dynamic range of imaging was more broad and the ability to sequence bands would be expected to be much greater.

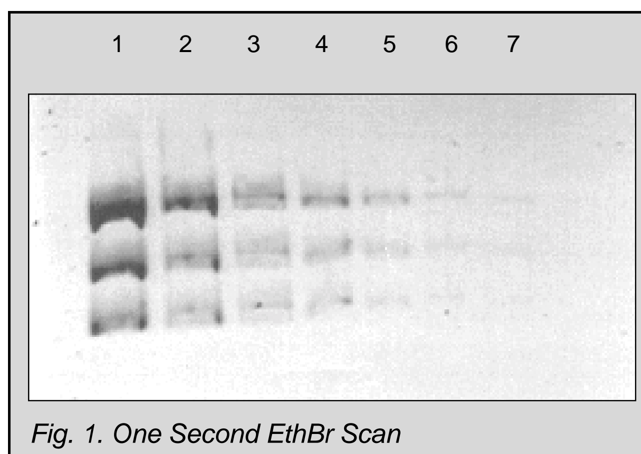

Fig. 1. One Second EthBr Scan

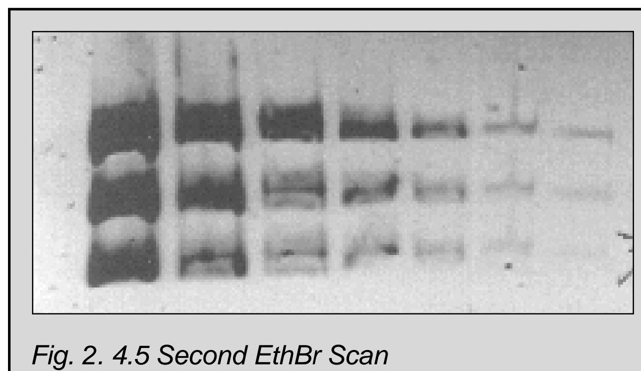

Fig. 2. 4.5 Second EthBr Scan

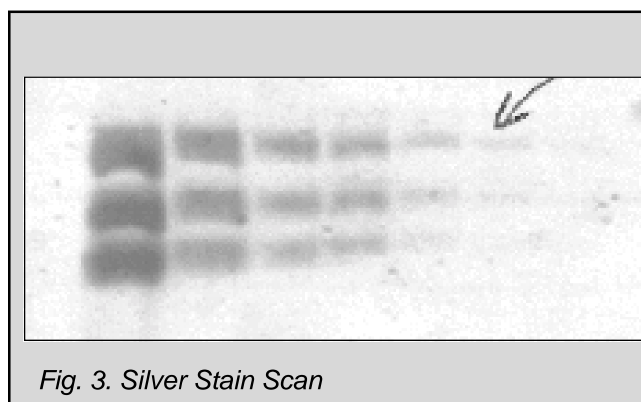

Fig. 3. Silver Stain Scan

The one second EthBr scan (Fig. 1) is an image derived from integrating for one second using the UV lightbox in the BioDoc-It system, and likewise for the 4.5 second scan (Fig. 2). The 20 mL TAE 0.7% agarose gel contained 0.5 µg/mL of ethidium bromide when poured. No other staining was performed before imaging. Following imaging,

the gel (Fig. 3) was dried and subjected to the BioRad silver stain protocol, then imaged again using the visible lightbox on the UVP system. The three bands in the leftmost lane contain 100 ng (1000 bp in size), 70 ng (700 bp), and 50 ng (500 bp) respectively, from top to bottom (ng = nanograms of double-stranded DNA). These sizes are typical of DD RT-PCR products. The bands were generated using the BioRad EZ Load Mass Ladder. The third lane from the left (numbers above the one second scan for reference) contains bands of 20, 14, and 10 ng each. The fifth lane contains bands of 4, 2.8, and 2ng, while the sixth lane contains bands of 2, 1.4 and 1 ng. The seventh and last lane contains bands of 1, 0.7, and 0.5 ng. The maximum sensitivity of the silver stain was the 2 ng band in the sixth lane (indicated with an arrow). Bands less than 2 ng were too faint to be discerned significantly. No bands in the seventh lane are visible. Enhancing contrast of the image was of little help. Additionally, the bands in the first lane all appear with equivalent intensity. With only one development of the silver stain possible, the dynamic range is limited. In contrast, the one second ethidium bromide scan reveals the differences in intensities in the bands of the first lane.

Four and a half seconds was chosen because this was empirically determined to be the setting for maximum sensitivity. At that setting, the 0.7 ng and 0.5 ng bands can be detected in the image. Thus, the UVP imager gives ethidium bromide staining 2-4 times the sensitivity of silver staining, with a much greater dynamic range. Integration of a fluorescent image is obviously far more effective than of a simple visible image. Basic quantitation of the fluorescence was also possible using UVP's LabWorks software, but we did not extensively investigate this possibility. EthBr can be easily removed from the DNA, and will not interfere with downstream cloning, sequencing, or secondary PCR procedures.

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UVP, Inc. • 2066 W.11th Street, Upland, CA 91786 • (800) 452-6788
(909) 946-3197 • Fax: (909) 946-3597 • E-Mail: uvp@uvp.com

Ultra-Violet Products Ltd. • Unit 1, Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG UK
+44(0)1223-420022 • Fax: +44(0)1223-420561 • E-Mail: uvp@uvp.co.uk