cloning could be achieved by using this quick, alternative protocol.

Not only does this $Na_3VO_4$-inhibition procedure save time, but also note that in this protocol the adjustment of vector-to-insert ratio in the ligation reaction is not necessary. To ligate with the CIAP/$Na_3VO_4$-treated vector (ca. 50 ng), we recommend using as much excess insert as is practical. Eliminating the quantitation step for both vector and insert further simplifies the process and reduces the hands-on time of the cloning procedure.

In summary, the procedure we describe in this report should make the subcloning method using alkaline phosphatase not only a less costly but also a time-effective procedure. A high percentage of recombinants could be quickly achieved without using any special cloning vector or bacterial strain. We feel that this protocol is especially useful for cloning procedures having no appropriate screening method (e.g., blue/white colony selection) or no background reduction strategy [e.g., digesting the ligation product before transformation with a selection enzyme that cuts the vector between the two cloning sites (3,4)].

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## Photographic Recording of Fluorescent DNA Bands on Agarose Gels

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Electrophoretic separation of DNA fragments by size on agarose gels is one of the most important techniques in molecular biology, and Polaroid® (instant) photography is frequently used to record the fluorescent DNA banding patterns from this analysis. Polaroid photography allows a quick assessment of photographic quality and permits a repeat of the photography if needed. Small-format gels are often used to conserve agarose, to reduce run time and to conserve the DNA sample for other analyses. With less DNA per band, it becomes more important to have a sensitive and accurate method for recording the reduced fluorescence. With less distance between each band, lateral diffusion of DNA over time—longer than approximately 2 h between the end of electrophoresis and photography—reduces resolution, therefore further justifying the need for a quick and accurate record of low-intensity bands.

Here, we present a method to obtain a quick, sensitive and more accurate image of the fluorescent DNA banding patterns on agarose gels using Polaroid film (Polaroid, Cambridge, MA, USA). The principles and techniques presented are applicable to all photographic films for reduced light recordings (3) and have been applied to X-ray films used for autoradiographic records from weak β emitters (1,2).

Photographic exposure in normal light easily initiates silver grain formation on film and exhibits an exact reciprocal relationship for exposure time and aperture opening (*f*-stop). For example, the same silver grain density can be obtained with a 1/30-s exposure at $f = 8$ or a 1/15-s exposure at $f = 11$. Thus, in normal light, for the same amount of silver grain development, a doubling of exposure time is required with each 50% reduction in aperture opening.

Photography in low-light environments, such as ethidium bromide fluorescence (DNA staining), requires progressively greater exposure times to

obtain an image as the amount of light diminishes. In photography this is known as the reciprocity effect, a condition in which the 1:1 reciprocal relationship of exposure time and aperture opening for silver grain development is lost (3). The reciprocity effect results in recorded discrepancies between intensely fluorescent DNA bands and faintly fluorescent DNA bands.

The linear relationship between silver grain density and quantity of fluorescent light is restored by pre-activating the film with light to reach or exceed the threshold for reciprocity (1). Using illumination from copy stand spot lights or overhead lights for the initial exposure, we establish receiprocity of the film in the area of the agarose gel image by providing 1/2–2/3 of the exposure needed for a good photo of the ruler and channel labels on white paper (Figure 1A). The reflected light from the agarose gel on the UV transillumination filter is 3%–5% of the reflected light from the paper (ca. 5 *f*-stops). In practice, the first exposure should record the outside perimeter of the gel and sample wells, but should not be excessively bright in the body of the gel where the fluorescent DNA bands are to be recorded (Figure 1A).

The second exposure, to form the composite, is made with the Foto/Prep I™ UV Transilluminator (Fotodyne, Hartland, WI, USA) box turned on (with no other lights) to record the fluorescent DNA bands on the pre-activated film (Figure 1B). This exposure records both faint and intensely fluorescent

**Table 1. Fluorescence Recorded for DNA Bands of Different Intensities of the Film with Preexposure (Figure 1B) and without Preexposure (Figure 1C)**

| Channel Number | Photo B | Photo C | Reduction in Density |
|---|---|---|---|
| 3 | 16 327 | 15 398 | 5.70% |
| 4 | 10 433 | 7063 | 32.30% |
| 5 | 7255 | 4124 | 43.20% |
| 6 | 4466 | 2287 | 48.80% |

Photos B and C were scanned with an Arcus II Scanner (Agfa, Ridgefield Park, NJ, USA) set for linear gray scale (gamma 1.0) and using Adobe® Photoshop® 4 software (Mountain View, CA, USA). NIH Image Analysis Program (public domain) set for a gray-scale density of 0–256 and the accompanying gel-plotting macro were used to quantify the density of the DNA bands after field inversion (original background is dark) (4). The recorded density in lane 7 was subtracted as background for the recorded density in lanes 3–6 of the respective photograph.

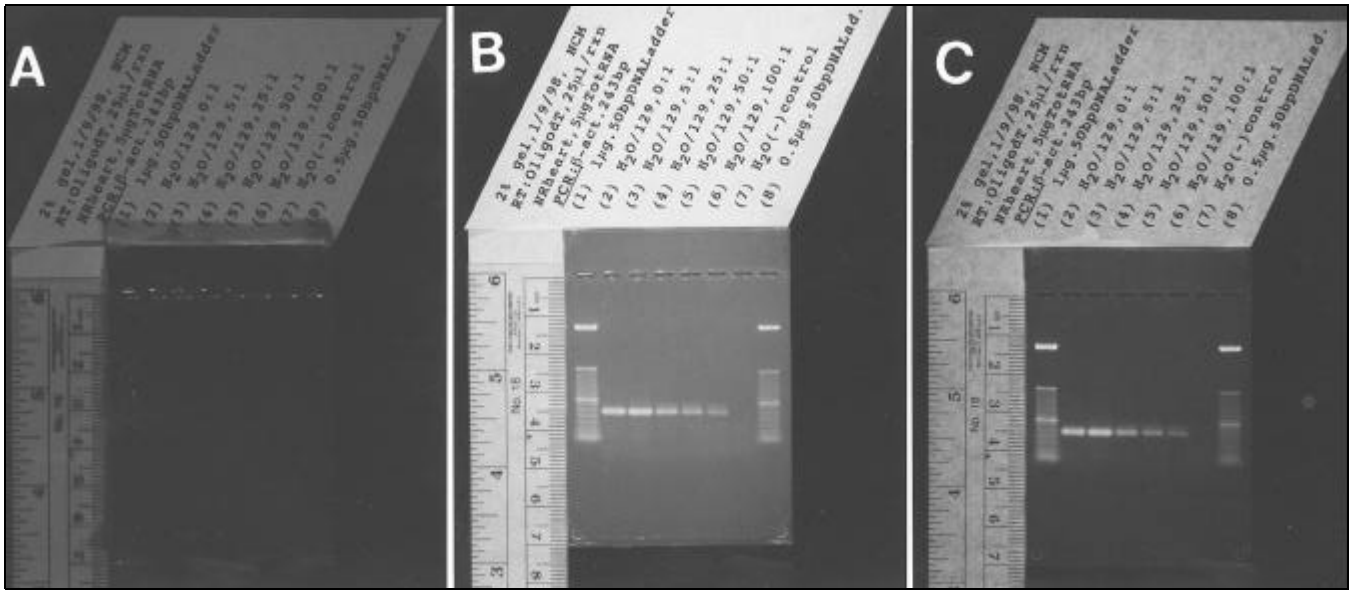


**Figure 1. Photographic results of the 2-exposure method that gives a more accurate and sensitive recording of ethidium bromide-stained DNA fragments on agarose gels.** The RT-PCR-generated, 243-bp β-actin fragment was from 5 µg of total rat heart RNA using limited (+) strand primer that was separated on a 2% agarose gel (6 × 8 × 0.5 cm). Lanes 2–7: (lane 2) 20 nM/mL (+) strand primer; (lanes 2–6) serial dilutions 1:5, 1:25, 1:50 and 1:100 of (+) strand primer, respectively, and (lane 7) no (+) strand primer. Lanes 1 and 8, respectively: 1.0 and 0.5 µg of a 50-bp DNA ladder (Bethesda Research Laboratories, Bethesda, MD, USA) ranging from 50–800 bp with an approximately 3× concentration in the 350-bp fragment. After electrophoresis (80 V for 50 min), the gel was stained with ethidium bromide (0.5 µg/mL in electrophoresis buffer [40.0 mM Tris-acetate, 1.0 mM EDTA, pH 8.0]) for 20 min and destained (water or electrophoresis buffer) 20–30 min (5). The agarose gel is placed on a Foto/Prep I UV transilluminator, located on the copy stand easel of an MP4+ Polaroid camera. The camera has a 135-mm, *f*-4.5 lens covered with a Tiffen, Series 6 (39.5 mm), No. 15 orange filter and uses Polaroid type 667 film (ISO 3000). Exact focus is obtained by opening the lens shutter (*f*-4.5) and using label information as a target. (Panel A) Photograph from pre-activation exposure only. The agarose gel was exposed for 1/125 s with an aperture of *f* = 22, using reflected illumination from 2 spot lights on the copy stand. (Panel B) The 2-exposure composite photograph was recorded; *(i)* with reciprocity established as in Panel A and *(ii)* with a second exposure of 8 s at *f* = 22 with only the UV transilluminator activated. We use 8 s and leave the aperture at *f* = 22 for simplicity; however, *f* = 5.6 for 1.0 s also works well because reciprocity has been restored. (Panel C) Photograph of fluorescence DNA bands without restoration of reciprocity. Film exposure was 8 s at *f* = 22.

DNA bands in proportion to the DNA amounts present within reasonable limits; however, bright fluorescent bands of DNA may overexpose (when the upper limit of the film is reached). In our experience, the time and *f*-stop combination for the second exposure is 8-fold–10-fold greater than the first exposure needed for pre-activation. However, all of the given exposure values are relative to the specific environment, the UV transilluminator used, the transformer settings for the UV box, if so equipped, and intensity of the other light sources used for the first exposure.

If film reciprocity is not established and only the second exposure is used to record the fluorescent DNA bands, a discrepancy between brightly and faintly fluorescing bands emerges (Figure 1C). Using the NIH Image Analysis Program (Reference 4; **http://rsb.info.nih.gov/nih-image/**) to compare the band in lanes 3–6 of Figure 1, B and C, the less intense the band the greater the difference in recorded intensity for the same DNA band (Table 1). To record a similar intensity of the faintly fluorescent DNA bands without first restoring reciprocity of the film requires 2–3 times greater exposure and results in film saturation for the brighter bands. Therefore, restoring reciprocity of film for consistency is an important consideration for photographically recording low-intensity fluorescent images if further analysis or quantitation is to be performed from the photograph.

Additional benefits of pre-activating film are that the first exposure can be used for recording labels for the lanes, identifying specific bands, referencing the date and the experiment and recording the distance of migration by placing a ruler next to the gel (Figure 1A). White photocopy paper has a bright blue-white fluorescence with UV light exposure, but little of the light produced is recorded on film due to the selective transmission of orange light through the orange filter. However, the added light recorded during the second exposure gives a sharp, crisp image of the labels and the ruler (Figure 1B). We rarely see overexposure of the label or ruler with the second exposure timed for the best resolution. However, if overexposure due to fluorescence is a problem, the paper, the ruler or both can be removed before the second exposure, or a heavier grade of paper, which has less fluorescence, can be used. Although any white or lightly colored ruler will work, we obtained the same intensity for the ruler and labels by placing a clear ruler on a strip of the same photocopy paper on which the labels are typed. The labels and ruler can still be read with only the UV exposure; however, the background has the mottled wood fiber appearance of paper (Figure 1C).

CLONTECH Laboratories (Palo Alto, CA, USA) and Diversified Biotech (Boston, MA, USA) provide fluorescent rulers for recording mobility of DNA bands when photographing agarose gels. The rulers' fluorescence does not necessarily match with the intensity of the DNA band(s) and can result in underexposure or overexposure of the ruler scale. Rulers of different fluorescent intensities have been produced to resolve this difficulty. Fluorescent rulers placed adjacent to gels can also refract generated light into the edge of the gel. Because the refracted light is also orange and not excluded by the orange filter, the light is recorded and can cause haze on one side of the gel image. This protocol dispenses the need to purchase expensive ruler sets of varying fluorescent intensities, which can cause recorded discrepancies on one side of the gel.

This procedure requires that you work out the settings for room light or copy stand exposure. However, after the first exposure (*f*-stop and timing) is determined for your particular light environment and written down, the extra expense for multiple exposures is minimized. More importantly, with reciprocity of the film restored, you should be able to standardize the second exposure for DNA gels and obtain an accurate and reproducible recording of DNA patterns. The necessity and expense of multiple exposures to record various intensity fluorescent bands because of the reciprocity effect are alleviated.

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