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Image Enhancement of RFLP Autoradiograms Through the Use of Neutral Density Filters

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ABSTRACT: Autorads that were difficult to size due to unequal band intensities were evaluated and a simple inexpensive procedure developed to allow accurate sizing of these unbalanced films. The procedure uses neutral density filters to increase the working range of video systems employed in autorad assessment.

KEYWORDS: forensic science, DNA, autoradiographs, DNA typing, restriction fragment length polymorphism, image analysis, neutral density filters, photography

Restriction fragment length polymorphism analysis (RFLP) is predominately a comparative technique. DNA fragments can be visualized with this procedure and their migration distance compared to that of other DNA fragments. Often much can be inferred from this comparison. These fragments that have been visualized through the use of specific probes are often described as "bands." These bands appear as dark rod shaped markings on photographic film. This film has been termed an autoradiograph if the image was produced with a radioactive isotope or a lumigraph if it was produced by a chemiluminescence procedure. In an attempt to quantitate the migration of these fragments, molecular size makers are used during the RFLP procedure to bracket the samples in question. The resulting films are visually reviewed and can be further subjected to a computer image analysis. With the assistance of this computer imaging, the band centers for both the molecular weight markers and the questioned samples can be determined. Through appropriate software the molecular weight of the questioned samples can then be estimated. Due to a number of variables the films may not be balanced in band intensity, with the images produced by the molecular weight maker or the samples displaying an unequal intensity across the film. These films with large signal variations can often be easily assessed by eye. Some imaging systems, however, can not accommodate such variations making interpretation of these films difficult. We describe in this report a technique that facilitates the computer image analysis of films with unequal intensity patterns, using nothing more than simple neutral density filters.

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Materials and Methods

Autoradiograms (autorads) were prepared using procedures modeled after the FBI RFLP procedure (1,2). The molecular weight marker was a 30 band ladder produced by Gibco/BRL and spanned the weight range of 0.5 to 22 Kb. The film used to produce the autorads was Kodak XAR-5. The imaging system developed by the FBI was used to evaluate the autorads (3,4). Briefly, the imaging system is composed of a light table used to illuminate an autorad, with a video camera placed above to capture the image and transfer it to a computer. The captured image is displayed by an accessory monitor. On-board software is then used to assist the analyst in locating and marking the center of the appropriate ladder and sample bands. Once the centers are marked, fragment sizes are then determined based upon the marked position of the band. Development times for autoradiograms varied from a few days to over a week.

Neutral density filters were prepared by developing unexposed Kodak XAR-5 film. The film was then cut into strips of varying width to be placed over or under lanes of an autorad allowing for individual lanes to be adjusted for imaging.

Results

Autorads with large intensity variations were evaluated with the imaging system described. Figure 1 shows an autorad where all

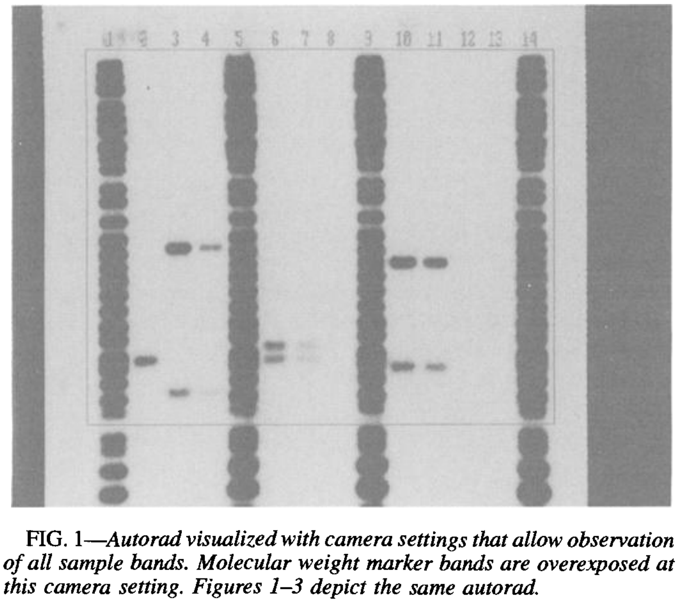

FIG. 1—*Autorad visualized with camera settings that allow observation of all sample bands. Molecular weight marker bands are overexposed at this camera setting. Figures 1–3 depict the same autorad.*

the sample bands are visible. Under these camera settings and light conditions, the molecular weight marker lanes are overexposed making the placement of the band centers subjective. Attempts to make these overly exposed bands clearly visible by increasing the light passing through the autorad or by varying the f-stop of the camera often simply allows one set of bands to become distinct while washing out those less exposed. An example of this is shown in Fig. 2. In this figure the camera lens was opened, allowing the imaging system to obtain a distinct image of the molecular weight marker bands. Many sample bands under these lighting conditions are "washed out" and are no longer visible. Figure 3 shows the same autorad at the identical light and camera settings as Figure 2 but with neutral density filters placed on top of the autorad at appropriate lane locations. The images in lanes 6 and 7 were visualized through the use of two layers of the neutral density filter. Lane 4 used one layer for the top band and two layers for the bottom. Lane 2 employed one layer while one layer was used for the bottom band only in lane 3. No filters were employed in lanes 10 and 11 and hence the bottom bands are not visible.

Another situation in which we have found this technique useful, is the location of the centers of closely spaced doublets. These bands that are spaced very closely together on the autorad, may appear as a single large band when imaged. (Data not shown.) The doublets can often be clearly seen when the lighting and camera f-stop are appropriately adjusted. When these adjustments are made other bands may be washed out but can be brought back into view with neutral density filters.

Discussion

The RFLP process is a labor intensive and time consuming procedure that may require the consumption of the majority of a sample for analysis. The appropriate evaluation of an autorad under these circumstances can be of paramount importance. The method described here facilitates the evaluation of imbalanced autorads through the application of a very simple technique. This process does not alter or change the autorad in any way, permitting independent analysis of the same autorad at a later date. The filters do nothing more than allow selective f-stops to be employed within the autorad. These filters can be customized to fit particular lanes of an autorad with the number of the filter layers required per section dependent upon the extent of the intensity differences existing within the autorad. As demonstrated, the use of these neutral density filters can achieve an image balance which allows for the accurate determination of band centers. Through the use of these filters, a more accurate representation of the center of a band can be obtained, yielding a better estimation of all bands evaluated on a particular autorad.

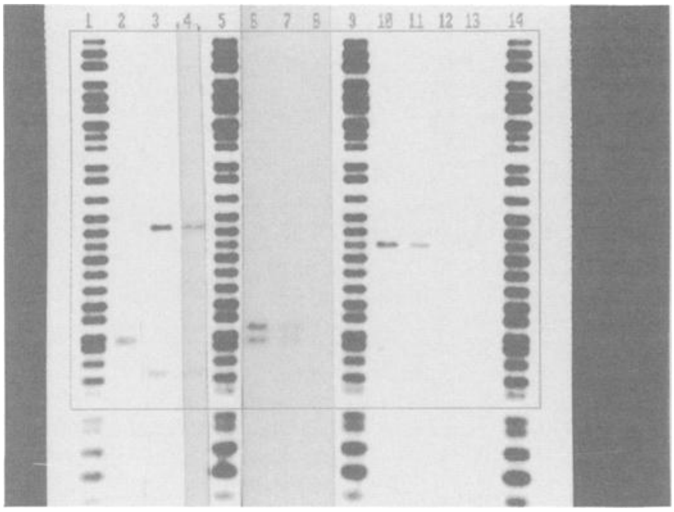

FIG. 3—*Depiction of how bands can be visualized with neutral density filters. No change in camera setting from that used in Fig. 2 but filters were added to lanes 2,3,4,6 and 7 to retrieve the "washed out" images.*

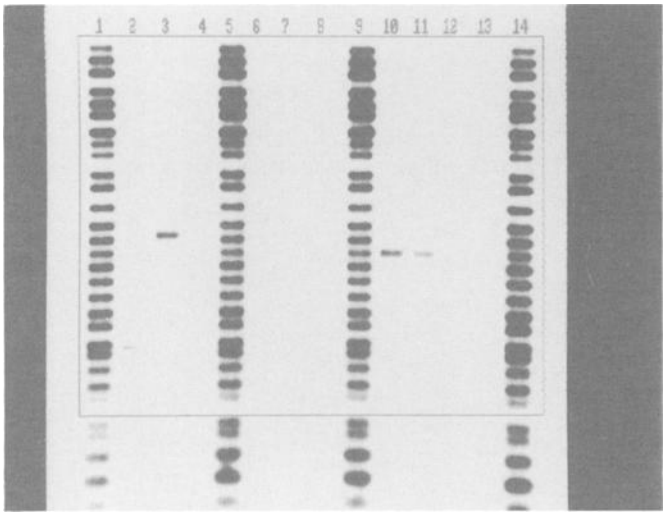

FIG. 2—*Camera f-stop opened to optimize the evaluation of the molecular weight marker bands. Some sample bands are "washed out" at these settings.*

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