Rapid and convenient method of autoradiography for DNA cloning using digital imaging analysis

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Abstract : Digital image analysis has been used for various biochemical and molecular biological analyses instead of autoradiography with X-ray film. However, in such cases the data manipulated by an imaging analyzer was generally printed out on normal printing paper. With normal paper, it is difficult to align the signal or its position relative to the original sample. Here in, we demonstrate it to be convenient and accurate to align signal obtained by imaging analyzer with OHP film. J. Med. Invest. 45 : 111-113, 1998

Key words **:** *screening, digital image analysis, cloning, autoradiography*

INTRODUCTION

Use of digital imaging analysis in Northern blotting, Southern blotting and thin layer chromatography markedly decreases exposure time and enhances sensitivity compared to conventional autoradiography. We have used the Fujix Bio-imaging-analyzer BAS 2000 (Fuji Photo Film Co., Ltd., Kanagawa, Japan), instead of routine autoradiography, because of its high sensitivity (100-fold or more that of X-ray film) and resolution (100 or 200 μm). However, this system is not suited for analyzing plaque and colony hybridization. Since the results are printed on normal paper with a laser printer, it is difficult to ensure that orientation markers in the paper are aligned with the original plate especially on screening for cDNA or genomic DNA cloning. On the other hand, hybridization signals on X-ray film are easily aligned with the plate. To circumvent this problem, we used OHP film instead of normal laser printer paper.

MATERIALS AND METHODS

DNA cloning was performed as previously described (1-3). A human genomic DNA library in λEMBL 3 (Clonetech, CA, USA) was plated on LB/agar plates at $2x10^4$ plaques/100mm dish. Plaques were transferred to Hybond C Extra nitrocellulose membranes (Amersham, UK), denatured by alkaline treatment, and fixed by baking at 80℃ for 2 hours. Prehybridization was carried out at 65℃ for 4 hours in hybridization buffer (50% formamide, 5 X SSPE, 5 X Denhardt's, 0.1% SDS, 0.1mg/ml salmon sperm DNA). Hybridization was carried out at 42℃ for 12 hours in hybridization buffer containing radiolabeled probe. The cDNA probe for the human sodium-phosphate cotransporter gene (NPT-1) (4) was prepared using the Megaprime DNA labeling kit (Amersham, UK) with [α-^{32}P] dCTP (110TBq/mmol:Amersham,UK) according to the manufacture's instructions. Washing was carried out as follows : 2X SSPE, 0.1% SDS, room temperature, 5 minutes, three times ; 0.5 X SSPE, 0.1% SDS, 55℃, 10 minutes, three times. After washing off excess of radiolabeled probe, membranes were wrapped in plastic wrap and exposed to a digital analysis imaging plate (IP), instead of X-ray film, for 2 hours. Following exposure, the imaging plate was set in an IP magazine and scanned with the BAS2000. Scanning parameters were as follows:gradation, 1024;resolution, 200 μm ; sensitivity, 10,000 ; latitude, 4. The scanned data were analyzed and printed out on OHP film (210 x 297 mm, polyester). Then the same membrane was reexposed to conventional X-ray film for 3 days at -80℃ for comparison with the data from BAS2000.

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RESULTS AND DISCUSSION

Figure 1 A shows the results of autoradiography with the Imaging-analyzer for the first plaque hybridization screen. A positive signal was seen clearly on OHP film following 2hr exposure. In contrast, exposure for 3 days was required to detect the same signal on X-ray film at a similar intensity (Figure 1 B). As shown in Figure 1, the signal obtained with OHP film was clearer with lower background than that seen with conventional X-ray film. These results indicate that this method markedly reduces exposure time and minimizes background compared to conventional autoradiography while keeping several of the advantages of X-ray film.

The use of X-ray film to visualize and retain a permanent record of data is essential in many research applications. However, the quality of an autoradiograph and the signal strength are often not known until the film has been developed. A high background on the film reduces the signal/noise ratio and makes data interpretation difficult. Such a situation may lead to repetition of experiments solely to obtain publication quality data. Here we describe and recommend a rapid, convenient, and accurate method for autoradiography using digital image analysis with OHP film. With this method the signal is more visible and the data interpreted more easily than with conventional X-ray film. The method is particularly applicable to initial low-stringency screening, which frequently gives rise to low intensity positive signals. The method also is applicable to Northern and Southern blotting and is extremely useful in minimizing unnecessary repetition of experiments.

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REFERENCES

1. Sambrook J, Fritsch EF, Maniatis T : Molecular Cloning : Laboratory Manual 2 nd. Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989, pp.9.14-9.23
2. Taketani Y, Miyamoto K, Tanaka K, Katai K, Chikamori M, Tatsumi S, Segawa H, Yamamoto H, Morita K, Takeda E : Gene structure and functional analysis of the human Na^+/phosphate co-transporter. Biochem J 324 : 927-934, 1997
3. Taketani Y, Miyamoto K, Chikamori M, Tanaka K, Yamamoto H, Tatsumi S, Morita K, Takeda

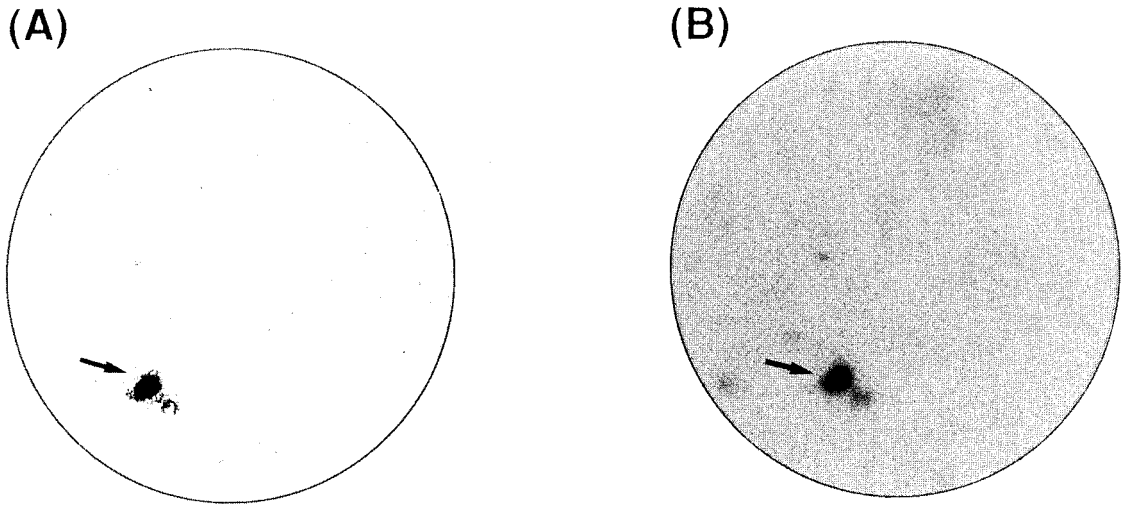

Fig.1. Result of autoradiography using the Imaging-analyzer with OHP film (A) and using conventional X-ray film (B). Arrows indicate positive signals corresponding to plaques containing the human sodium-phosphate cotransporter (NPT-1) gene at the same position in both panels A and B.
The exposure time of the membrane onto the imaging plate was 2 hours. The data were analyzed on a BAS 2000 image analyzer and printed onto OHP film with a laser printer. The same membrane was reexposed to X-ray film for 3 days.

E : Characterization of the 5' flanking region of the human NPT-1 Na^+/phosphate cotransporter gene. Biochem Biophys Acta 1396:267-272, 1998

4. Miyamoto K, Tatsumi S, Sonoda T, Yamamoto H, Minami H, Taketani Y, Takeda E : Cloning and functional expression of a Na^+-dependent phosphate co-transporter from human kidney : cDNA cloning and functional expression. Biochem J 305 : 81-85, 1995