HINTS AND TIPS

Recovering cDNA Bands from Differential Display RT-PCR Gels Using a Transparency Film Mask

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Abstract

We describe here the use of a transparency film as a mask for recovering bands from differential display reverse transcription-polymerase chain reaction (DDRT-PCR) gels. This method represents a simple and rapid way to isolate the differentially expressed bands from dried and nondried polyacrylamide, radioactive and nonradioactive, denaturing and native DDRT gels. A transparency film is overlaid on the DDRT autoradiogram, and the marks and bands of interest are drawn using a permanent marker. The reproduced band marks are cut out of the transparency sheet with a scalpel to facilitate the recovery of the desired bands. The transparency film mask is then overlaid on the top of the gel and the bands are recovered from the gel. The use of the transparency film mask avoids damage to the autoradiogram and is also extremely useful in DDRT-PCR experiments involving different RNA samples that produce band patterns of different intensities that require many X-ray exposures for different periods of time.

Index Entries: mRNA differential display PCR; cDNA; band recovery; polyacrylamide gel; DDRT-PCR.

1. Introduction

The differential display reverse transcription-polymerase chain reaction (DDRT-PCR) technique for cDNA analysis has been proven to be an efficient method for preliminary identification and characterization of genes that are expressed in different cell types or tissues, or that are expressed upon different stimuli (1-4). In order to recover and clone the putative differentially expressed amplified fragments from the gels, published procedures usually describe the use of the DDRT autoradiogram itself as a mask to overlay the gel and cutting both the autoradiogram and the gel (1,2). At the end, the autoradiogram cannot be used for documentation. Here we suggest a simple, safe, economical, easy and reliable method for recovering cDNA fragments from DDRT-PCR gels with no damage to the autoradiogram and no need to overlay two pieces of X-ray film onto the gel simultaneously. We have used the DDRT-PCR strategy to compare the mRNA population from primary cell cultures obtained from skin biopsies of systemic sclerosis (SSc) patients and normal individuals. Normal and SSc fibroblasts were grown from explants of the skin biopsies and propagated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) containing glutamine and antibiotics. Total RNA was extracted from fibroblast monolayers as described previously (5). DDRT-PCR was performed as described elsewhere (1,2) in the presence of α^{32}P- dCTP. Four microliters of each reaction were loaded onto a 6% sequencing gel and electrophoresed at 80W for approximately 2-3 h, until xylene cyanol reached about 3/4 of the gel.

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The gel was transferred to a used X-ray film, and nonsymmetrical marks were placed on the corners and sides of the gel using a mix containing black Indian ink plus traces of a radioactive isotope (Fig. 1A). Native polyacrylamide gels (without urea) are also used to resolve DDRT-PCR fragments; in that case, gel drying is required. The gel was then covered with a PVC film (Saran-Wrap®, Dow Chemical Company, USA) and exposed to X-ray film at -70°C for different periods of time and developed. In different experiments the X-ray exposure times varied from 4–18 h to 5 d.

To prepare the mask for cutting the bands, a transparency film (or any acetate sheet) was overlaid on top of the DDRT-PCR autoradiogram. The nonsymmetrical marks of the autoradiogram and the differentially expressed bands of interest were reproduced using a permanent marker. The reproduced band marks were cut out of the transparency film with a scalpel to facilitate the recovery of the desired bands (Fig. 1B and 1C). When excising the bands, the Saran-Wrap® film was removed and the transparency film mask was placed on the top of the sequencing gel, making sure that the markers were coincident, and the desired bands were

Fig. 1. Diagram of differentially expressed band recovery from DDRT-PCR gels. (A) Non symmetrical marks are made on corners and sides of the gel using a mix containing traces of radioactive nucleotide plus Indian ink. (B) After developing the film, marks and bands are seen on the autoradiogram. (C) A transparency film is overlaid on the autoradiogram, and the marks and bands of interest are drawn using a permanent marker. The reproduced band marks are cut out of the transparency sheet with a scalpel to facilitate the recovery of the desired bands. The transparency film is then overlaid on the top of the gel and the bands are recovered from the gel. See text for additional details.