ELSEVIER

A simple method for isolation of PCR fragments from silver-stained polyacrylamide gels by scratching with a fine needle

Gabriele B. Stumm[a], Helmut Vedder[b] and Jürgen Schlegel[a]

[a]Department of Neuropathology, University of Marburg, D 35033, Marburg, Germany
[b]Department of Psychiatry, University of Marburg, D 35033, Marburg, Germany

▼PCR-based DNA and RNA fingerprinting techniques, including arbitrarily primed PCR (AP-PCR) and differential display reverse transcriptase PCR (ddRT-PCR), represent comprehensive genetic screening methods (Ref. 1). Recently, sensitive nonradioactive detection techniques have been introduced for visualization of fingerprints using silver staining of the PCR products after polyacrylamide gel electrophoresis (Ref. 2, 3). Characterization of polymorphic PCR fragments depends on prior isolation of the corresponding bands from stained and dried gels.

In most protocols, PCR products of interest have been cut out of the dried gel complete with the paper support, and DNA eluted by boiling in elution buffer (Ref. 4). Reamplification of the eluted PCR fragments has been successful in up to 75% of the experiments. However, additional bands neighbouring the fragment of interest were frequently coamplified. To avoid coamplification of contaminating bands, the whole procedure of cutting, boiling and reamplification was repeated up to five times by some protocols (Ref. 5).

Here we demonstrate simple and rapid reamplification of PCR products after isolation by gently scratching over the silver-stained band using a sterile disposable needle [Microlance 3 (Becton-Dickinson; 0.7 mm)] prewetted with PCR mastermix, without destroying the gel. After scratching the band from the gel, the needle is placed into 20 μl standard PCR reaction mix for 1 min and then discarded. The scratched PCR products are reamplified by 30 PCR cycles (Fig. 1). Using this scratching technique, we are able to reamplify PCR products of interest in more than 90% of the experiments. In addition to the simplicity and speed of the procedure, coamplification of neighbouring PCR fragments is infrequent and the original fingerprint gel is not destroyed. This technique is applicable to all nonradioactive PCR procedures based on silver staining including AP-PCR or differential display.

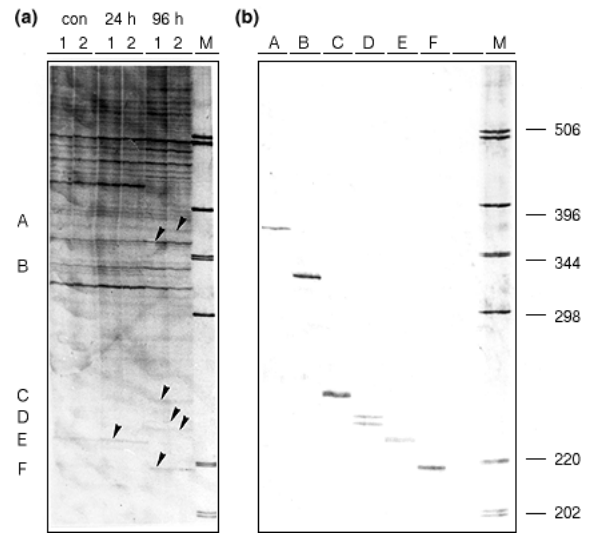

FIGURE 1. (a) Original RNA fingerprint generated by RNA arbitrarily primed PCR (RAP-PCR) of embryonal neurons from rat cortex after 24 h and 96 h of metamphetamine treatment using two different RNA concentrations (1, 150 ng RNA; 2, 200 ng RNA; con, control; M, 1 kb DNA ladder) and an arbitrary 11-mer primer (5′-ACCAGGGGCA-3′). (b) Reamplification of fragments A–F isolated by the scratching technique revealed distinct bands of the expected size. The source of re-amplified DNA fragments A–F is indicated by arrows on the original fingerprint.

Corresponding author: stumm@mailer.uni-marburg.de

Protocol

1. Prepare PCR reaction mix as used for standard specific PCR amplifications in a reaction volume of 20 μl.
2. Prewet a sterile needle [e.g. Microlance 3 (Becton-Dickinson; 0.7 mm)].
3. Scratch gently along the silver-stained PCR product of interest.
4. Place the needle into the 20 μl PCR reaction mix in a thin-walled PCR tube for 1 min and then discard it.
5. Reamplify the PCR product in 30 PCR cycles of 1 min at 95°C, 30 s at an annealing temperature specific for the primer used, 2 min at 72°C, with an initial 5 min denaturation at 95°C and a final 5 min elongation at 72°C.

References

1 Welsh, J. and McClelland, M. (1990) *Nucleic Acids Res.* 18, 7213–7218.
2 Budowle, B. *et al.* (1991) *Am. J. Hum. Genet.* 48, 137–144.
3 Schlegel, J. *et al.* (1996) *BioTechniques* 20, 178–180.
4 Sanguinetti, C.J., Neto, E. and Simpson, A.J.G. (1994) *BioTechniques* 17, 914–921.
5 Weaver, K.R. *et al.* (1994) *BioTechniques* 16, 226–227.

Products Used

Microlance 3: Microlance 3 from Becton Dickinson

Microlance 3: Microlance 3 from Becton Dickinson