mRNA phenotyping by enzymatic amplification of randomly primed cDNA

Kevin E.Noonan and Igor B.Roninson

Department of Genetics, University of Illinois College of Medicine, Chicago, IL 60612, USA

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A highly sensitive technique for the analysis of gene expression employs the polymerase chain reaction (PCR) (1) to amplify *in vitro* cDNA sequences corresponding to different mRNAs. This approach has been termed mRNA phenotyping (2). Prior to PCR amplification, cellular mRNA is converted to cDNA by reverse transcription using either an oligo(dT) primer (2) or primers specific for particular genes (3). We here report a novel modification of this technique which uses a random hexadeoxynucleotide primer for reverse transcription. Products of a single reverse transcription reaction may then be used for amplification of multiple mRNA sequences, either in the same PCR assay or separately, facilitating the use of internal standards for quantitation. The use of a random primer rather than oligo(dT) minimizes the effects of sequence complexity, mRNA secondary structure and distance of the primer sequences from the poly(A) tail. The latter is particularly important for analysis of partially degraded samples of cellular RNA, as are commonly isolated from tumors and other clinical tissue specimens.

Procedure: Total cellular RNA was isolated from human KB-8-5-11 cells (4) by standard procedures. cDNA was prepared by reverse transcription of 1 ug of total RNA using 100 ng of random primer (P-L Biochemicals) and 100 units of MoMuLV reverse transcriptase (Bethesda Research Labs) under the conditions recommended by the supplier. cDNA aliquots, corresponding to 100 ng of RNA, were used for enzymatic amplification by PCR using 1 unit of *Taq* DNA polymerase (Perkin Elmer Cetus) and 1.5 μM of gene-specific primers (amplimers). Amplimer pairs were selected from different exons separated by a long intron, to prevent amplification of genomic DNA. 30 cycles of PCR were carried out in 50 μl volume using a thermal cycler (Perkin Elmer Cetus; each cycle includes 1 minute at 94°C and 5 minutes at 65°C).

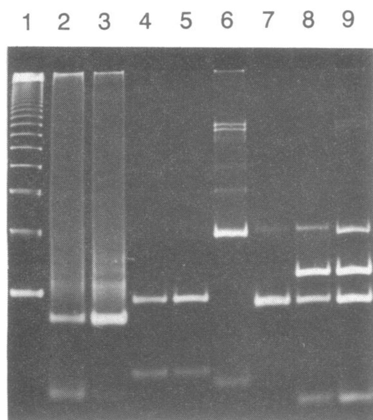

Figure 1: PCR products separated in a 12% polyacrylamide gel. cDNA in lanes 2 and 4 was prepared using an oligo(dT) primer, in all the other lanes cDNA was made with a random primer. Lane 1: 123 bp ladder (size markers); lanes 2 and 3: amplimers from the 5' end of the *mdr*1 gene (positions 302-321 and 389-408) (5); lanes 4 and 5: amplimers from the 3' end of the *mdr*1 gene (2633-2652 and 2730-2749) (5); lane 6: amplimers for dihydrofolate reductase (DHFR) gene (13-32, exon 5 and 99-118, exon 6) (6); lane 7: amplimers for β_2-microglobulin (B2M) gene (1552-1572 and 3218-3237) (7); lane 8: simultaneous amplification using amplimers for B2M and *mdr*1 (2596-2615 and 2744-2763) (5); lane 9: simultaneous amplification using B2M, DHFR and *mdr*1 amplimers. By ethidium bromide staining of gel-separated PCR products, expression of the DHFR gene is detectable in less than 20 pg of total RNA (data not shown).

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