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A simplified method for single-cell RT-PCR that can detect and distinguish genomic DNA and mRNA transcripts

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It is now possible to analyze mRNA transcribed in single cells by the reverse transcriptase-polymerase chain reaction (RT-PCR) technique (1,2,3). Isolation of mRNA from single cell suspensions may lead to significant loss of material due to the required extraction step (1) or repeated column passages (2). Another approach, analyzing mRNA from cellular contents withdrawn via a glass micropipette electrode, requires high cycle numbers in two stages of amplification. The limits of sensitivity of this method have not been established, nor does it permit distinction between genomic DNA and mRNA products (3). Here we introduce a simplified single cell RT-PCR method, which results in sensitive detection and can distinguish between genomic and mRNA derived RT-products. The ability to remove genomic products is important for evaluation of transcription of intronless gene.

Experiments described here were performed on single human embryonic kidney (HEK 293) cells, using primers for β-actin. Under the microscope, the cell's cytoplasm was harvested into a negative pressure glass patch clamp micro-electrode pipette (4): in most cases the nucleus (and genomic DNA) appear to have been harvested as well. The tip of the pipette was then broken into a test tube and the pipette's contents expelled into 16 μl of solution A [1.25×PCR buffer (200 mM Tris−HCl, 500 mM KCl), 6.25 mM $MgCl_2$, 5 units RNasin (Promega), 2 mM DTT]. In the illustrated experiments (Figure 1), each lane corresponds to the contents of a single cell, and conditions were employed to amplify both genomic and mRNA productions (Lanes 1), mRNA products alone (lane 2) and to demonstrate complete elimination of genomic DNA products (lane 3). To samples in lane 2 and 3 was added 1 unit DNase I (Ambion) to remove genomic contaminants, followed by incubation at 37°C for 1 hour, and then heating to 95°C for 15 minutes to inactivate DNase I. No DNase was added to the sample in lane 1, but other steps were identical. After cooling to room temperature, to the samples in lane 1 and 2 was added 4 μl of RT mixture [5 mM dNTPs, 2.5 μM oligo-dT primers, 5 units RNasin, 100 units M-MULV reverse transcriptase (BioLabs)] for the RT-PCR reaction. To the sample in lane 3 was added the same RT reaction mixture, but without M-MULV: all samples were thereafter treated the same. The reverse transcription reaction was performed at 45°C for 30 minutes, then 99°C for 5 minutes. Finally, the PCR reaction was begun by adding 30 μl PCR mixture [1×PCR buffer, 0.33 μM β-actin primers (Stratagene) and 2.5 units Taq DNA polymerase] and mineral oil. Products were analyzed by agarose gel electrophoresis and u.v. visualization or by Southern blot.

The specificity of RT-PCR products was confirmed by their sizes on agarose gels and by Southern blot hybridization using a Digoxigenin-labeled β-actin probe. On agarose gels, only one band of ~660 bp was seen in the RT-PCR reaction samples (Lanes 1, 2) [the predicted RT-PCR β-actin product = 661 bp (5)]. With u.v. detection we consistently observed a single product band in the presence (Lane 1) or absence (Lane 2) of DNase treatment. However, on Southern blots we always detected an additional faint band of ~870 bp when DNase treatment was omitted (Lane 4). [The PCR product from the β-actin functional genomic DNA is 868 bp (6), and we confirmed the amplification of this sized product from both HEK cell DNA and from samples of human genomic DNA]. There were no detectable PCR products from single cell preparations after DNase I treatment (Lanes 3, 6). Thus, inclusion of the DNase step appears to prevent amplification of contaminating genomic sequences. These results therefore indicate that the RT-PCR products in Lane 2 came from β-actin mRNA.

Compared to other RT-PCR methods, the advantages of this method are simplicity and increased sensitivity. All reactions (DNase treatment, inactivation, RT-PCR) were performed in the same tube, reducing the loss of mRNA from single cells. We detect the PCR product after 40 thermal cycles by u.v. When combined with more sensitive Southern blot detection, it appears

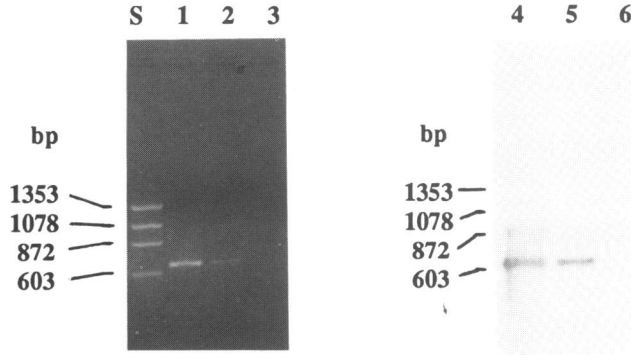

Figure 1.

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possible to observe the single cell genomic sequence (e.g. 1–2 gene copies, depending on harvesting efficiency) (Lane 4). Other reported methods have required ~60 cycles (1) or even 105 cycles (2), requiring Southern blots for detection (2). In experiments not illustrated, we have reproducibly been able to divide contents of a single cell into equal aliquots for the separate detection of voltage- sensitive Na channel α (7) and β_1 (8) gene products.

The method described here is useful for detecting mRNA of rare samples (9), for use in combination with electrophysiological measurements (3,10), tracing mRNA from histological sections (11) and for constructing PCR based cDNA libraries from single or countable numbers of cells (12).

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