One-step amplification of transcripts in total RNA using the polymerase chain reaction

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Submitted January 3, 1989

We describe here a simplified protocol for detection of RNA transcripts using the polymerase chain reaction (PCR) method. This protocol differs from others (1-7) in using the same buffer for both the reverse transcriptase (RT) and the Taq DNA polymerase enzymes.

Total RNA from mouse skeletal muscle was prepared (8) and a range of quantities was mixed with 100 ng of each of two myosin heavy chain (MHC) primers (Panel A). RNA and primers in 30 μ l water were heated at 65°C for 15 minutes and then cooled on ice. A mix of 20 μ l 2.5X PCR buffer (168mM Tris-HCl, pH 8.8 at 25°C, 41.5mM $(\text{NH}_4)_2\text{SO}_4$, 16.8mM MgCl_2 , 25mM β ME, 0.42mg/ml BSA) purchased from Bioexcellence, AMV RT (see legend), 1 U Taq DNA polymerase (Bioexcellence) and 500 μ M dNTPs (Boehringer) was added to each tube and overlaid with 100 μ l paraffin. After incubation at 42°C for 15 minutes, 40 cycles of PCR were performed in a Hybaid apparatus. A single cycle consisted of: 1 s at 92°C for denaturation, 2 min at 55°C for annealing, and 2 min at 72°C for elongation; the 40th cycle had a 5 min elongation step to fully extend incomplete DNA fragments. Ten μ l were electrophoresed in an agarose gel.

In panel B, lanes a-d show that 10 ng of total RNA is sufficient to produce a band from this relatively abundant mRNA which is detectable by staining with ethidium bromide; this band hybridizes with a cloned genomic probe overlapping the 220 bp region (not shown). Using 100 ng of total RNA the amount of AMV RT can be reduced to 0.04 U (lane i). Using only 20 cycles still allows detection of the transcript (lane l).

Panel A. Schematic localization of MHC primers used for DNA or RNA amplification.

Panel B. Electrophoresis of PCR-amplified fragments through a 1.4% NuSieve (FMC), 0.6% Type II agarose (Sigma) gel. Lanes a-d: 40 cycles using RNA and AMV RT respectively at (a) 10 μ g, 2.5 U (b) 1 μ g, 1.25 U (c) 100 ng, 1 U (d) 10 ng, 0.02 U. Lane e: 1 ng cloned MHC genomic DNA (40 cycles). Lane f: Hae III digest of Φ X174. Lanes g-i: 40 cycles using 100 ng RNA and AMV RT at (g) 1.25 U, (h) 0.2 U, (i) 0.04 U. Lane j: 1 ng cloned MHC genomic DNA (20 cycles). Lanes k-l: 100 ng of RNA, 0.2 U AMV RT (k) 40 cycles and (l) 20 cycles.

Acknowledgements. We are grateful to L. Legault for help with RNA preparation and to S. Takeda for design of the primers. This work has been supported by a grant from the NIH, Muscular Dystrophy Association, INSERM and the Association Française contre les Myopathies.

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