

An improved method for sequencing of RNA templates

O.Fichot and M.Girard
Unité de Virologie Moléculaire, URA CNRS 545, Institut Pasteur, 25, rue du Dr Roux, 75724 Paris Cedex 15, France

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RESULTS

Application of the dideoxy chain termination procedure[1] to sequence RNA templates using Avian Myeloblastosis Virus (AMV) or Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase[2], is subject to difficulties related to the low concentration of the radioactively labelled dNTP which results in non-specific chain termination. To avoid this inconvenience, we have adapted to RNA sequencing the method described by Tabor and Richardson[3] for DNA sequencing, which separates the sequencing reaction into a labelling step and an extension/termination step.

Purified poliovirus RNA (1 μg), or total cytoplasmic RNA from poliovirus-infected HeLa cells[4] (10 μg) was mixed with 50 ng (10 pmol) of a 15–20 bases long oligonucleotide primer in 10 μl of 60 mM Tris, pH 8.3, 75 mM NaCl, 7.5 mM $MgCl_2$, 5 mM DTT. After incubation for 10 min at room temperature, 2 μl of labelling mix (2 μM dGTP, dTTP, dCTP; 20 μCi [^{35}S]-dATP (600 Ci/mmol; Amersham) and 1 μl (15 U) of M-MuLV or AMV reverse transcriptase were added. After 5 min. incubation at room temperature, 3.5 μl of the labelling reaction mixture were pipetted into 4 separate tubes, each containing 2 μl of specific elongation mix containing 0.5 mM dATP, dCTP, dTTP, 1 mM dGTP, and 0.25 mM of either ddATP (Tube A), ddTTP (Tube T), ddCTP (Tube C) or 0.5 mM of ddGTP (Tube G). The elongation/termination step was for 15 min at 37°C with M-MuLV reverse transcriptase or at 42°C with AMV reverse transcriptase. Reactions were terminated by addition of 3 μl of stop solution (0.1% xylene cyanol, 0.1% bromophenol blue, 1 mM EDTA, 80% formamide) and concentrated for 10 min in a speed vac evaporator. DNA was denatured for 2 min at 95°C immediately prior to loading onto a 7% denaturing polyacrylamide gel.

The method described here allows one to read the sequence up to approximately 250–300 nucleotides from the primer after exposure times of 48–60 hrs (Fig. 1).

We have applied this procedure to the determination of genomic sequences of different viruses, using as template, either purified viral RNA or total cytoplasmic RNA from infected cells.

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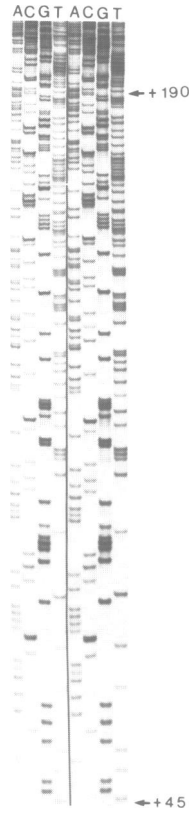

Figure 1. Sequence of poliovirus type 1 RNA with AMV reverse transcriptase using total cytoplasmic RNA from infected HeLa cells as a template and a 15 bases oligonucleotide complementary to nt 849–864 of the viral genome. Numbers refer to the number of nucleotides from the primer.