Table 1. Composition of the Different Sets of RNA Size Markers

<table>
<thead>
<tr>
<th>Size (nt)</th>
<th>Enzyme</th>
<th>Proportion in Template Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>148</td>
<td>Rsal</td>
<td>6</td>
</tr>
<tr>
<td>244</td>
<td>Ndel</td>
<td>4</td>
</tr>
<tr>
<td>255</td>
<td>Dral</td>
<td>4</td>
</tr>
<tr>
<td>390</td>
<td>BstNI</td>
<td>2</td>
</tr>
<tr>
<td>497</td>
<td>BglII</td>
<td>2</td>
</tr>
<tr>
<td>688</td>
<td>Accl</td>
<td>1.5</td>
</tr>
<tr>
<td>767</td>
<td>Sspl</td>
<td>1.5</td>
</tr>
<tr>
<td>977</td>
<td>Xhol</td>
<td>1</td>
</tr>
<tr>
<td>1106</td>
<td>BsmAI</td>
<td></td>
</tr>
<tr>
<td>1569</td>
<td>PvuII</td>
<td>1</td>
</tr>
<tr>
<td>1816</td>
<td>HindIII</td>
<td>2</td>
</tr>
<tr>
<td>2020</td>
<td>Asel</td>
<td>2</td>
</tr>
<tr>
<td>3621</td>
<td>Scal</td>
<td>1</td>
</tr>
</tbody>
</table>

The table indicates the size of the RNA synthesized (first column) when the pBluescript-based pXLPP2Ac plasmid was linearized with the corresponding restriction enzyme (second column). The proportions (weight) of the different templates in the ready-to-use mixtures for the low-, medium- and high-range markers are indicated in the third, fourth and fifth columns.

size markers, we calculated that the length of the ODC mRNA was 2100 ± 25 nt in the unfertilized egg RNA sample (lane 1) and 2225 ± 25 nt in the sample from four-hour embryos (lane 2). The length of this mRNA was previously estimated, relative to the rRNAs, to be 2400 nt (2).

In conclusion, we have described a series of in vitro transcription templates that allow three series of radiolabeled RNA size markers to be produced using well-proven methods and commercially available in vitro transcription kits. The size of these markers ranges from 148 to 3621 nt. Therefore, for a laboratory of 5 to 10 research workers, a single large-scale preparation can produce a stock of templates sufficient to reproducibly make these RNA size markers for several years.

REFERENCES


The authors thank R. Hartley for her comments on the manuscript. This work was supported by grants from the European Economic Community, DG12—Biotechnology Program (No. B164-CT95-0045), the Ministère Chargé de la Recherche (No. ACC-SV4), the Association pour la Recherche sur le Cancer (Contract No. 6788) and the Ligue National de Lutte Contre le Cancer. Address correspondence to H.B. Osborne, UPR 41 CNRS, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France. Internet: beverley.osborne@univ-rennes1.fr

Received 28 January 1997; accepted 18 April 1997.

Yann Audic, Francis Omilli, H. Beverley Osborne and Laëtitia Landais¹

Université de Rennes 1
Reze, France

RT-PCR Detection of RNA Viruses in Stool Specimens

BioTechniques 23:616-618 (October 1997)

The use of reverse transcription polymerase chain reaction (RT-PCR) to detect viral nucleic acid in stool specimens has been described for both enteroviruses (9) and small round structured viruses (SRSV) (1,3,7). One of the main problems associated with stool RT-PCR is the presence of amplification inhibitors in fecal specimens (5). To overcome this problem, several procedures have been designed that use antigen capture of viral material in stools (6) or specific oligonucleotides attached to magnetic beads that concentrate virus RNA away from the rest of the fecal specimen (8). Other proce-
dures have combined phenol/chloroform extractions with other extraction steps using freon and cetylhexadecyltrimethylammonium bromide (CTAB) in combination with polyethylene glycol (PEG) precipitations (7) or applying Sephadex G200 gel chromatography (Pharmacia Biotech, Piscataway, NJ, USA) for the purification of virus material away from inhibitors (3,9). Other researchers have also used organic extraction procedures with viral nucleic acid adsorption to size-fractioned silicon dioxide particles in attempting to remove inhibitors (1).

Although all these procedures appear to do an adequate job of removing inhibitors, they are relatively complex to set up and involve a significant number of steps before completion of RT-PCR. We describe a reasonably rapid and sensitive procedure for RT-PCR of numerous stool specimens that uses a commercially available mixture of guanidine salts, urea, detergent and phenol (Ultraspec™-3 reagent; Biotecx Laboratories, Houston, TX, USA) in combination with a hot-start PCR. The inclusion of guanidine salts with the other denaturants present in the Ultraspec-3 should both denature protein and inhibit RNase activity, thus decreasing the degradation of RNA (2).

During the extraction procedure, there is a partitioning of RNA into the aqueous phase while DNA and protein remain in the organic phase.

Fecal suspensions (500 µL of a 10% suspension in sterile water) not containing any enterovirus were placed in 1.5 mL microcentrifuge tubes and spiked with 1-µL dilutions of poliovirus 3 (Sabin strain) culture fluid. Microcentrifuge tubes containing 500-µL volumes of 10% suspensions harboring SRSV strains from independent outbreaks of gastroenteritis were also prepared. The tubes of polio and SRSV suspensions were then spun at 3000× g for 3–4 min. The clarified supernatant was transferred to a fresh sterile tube and gently vortex-mixed for 30 s to further disrupt any fine particulate stool materials and virus that could hinder subsequent extraction of viral RNA. The tubes were spun at 16,000× g for 5 min and the supernatant transferred to another fresh sterile tube. A 100-µL aliquot of the clarified stool supernatants was then extracted with Ultraspec-3 RNA reagent following the manufacturer’s instructions. RNA pellets were resuspended in 25 µL of diethyl pyrocarbonate (DEPC)-treated water.

Using a 4-µL aliquot of the resuspended RNA, an RT-PCR was performed as previously described by Drebot et al. (4) with the following modifications. Upon synthesis of cDNA, a wax bead (AmpliWax® PCR Gem 100; Perkin-Elmer, Norwalk, CT, USA) was added and the mixture
heated at 96°C for 10 min and then placed on ice. A PCR mixture (4) containing 50 pmol of the SR33 and SR46 primers (1) or enterovirus-specific primers (4) was then added and a hot-start PCR performed. Samples were preheated for 4 min at 94°C and PCR amplification carried out in 40 cycles at 94°C for 1 min, 50°C for 1.5 min and 72°C for 2 min. At the end of the 40 cycles, the reaction mixtures were left at 72°C for 15 min. Amplifications were carried out in a GeneAmp® PCR System 9600 Thermal Cycler (Perkin-Elmer).

Figure 1 shows the amplification products generated using the stool extraction procedure and hot-start RT-PCR described above. Lanes 1 and 2 show that the predicted SRSV PCR products (123 bp) were generated from representative stool specimens obtained from two independent outbreak sites. Since SRSV viruses cannot be cultured for stool seeding experiments, we determined the sensitivity of the extraction procedure by making serial dilutions of the RNA suspensions obtained after extraction. Lanes 3–7 show that the RNA suspension could be diluted 10,000× and still produce a fragment observable on an ethidium bromide-stained gel from stools spiked with only 10 plaque-forming units (pfu) of poliovirus. Although one tenth less sensitive than RT-PCR procedures involving non-stool specimens (4,10), the sensitivity should be efficient in detecting the presence of enteroviruses in stools during infection. As mentioned above, increased sensitivity could also be added by combining our RT-PCR procedure with Southern hybridization.

One notable feature of the procedure is the inclusion of a hot-start PCR step. We found that the efficiency of stool RT-PCR was very much reduced without performance of the hot-start step as described. It is possible with all the components present in stool including nucleic acid from bacterial and human cells in the feces that only under specific hot-start conditions will viral RNA be amplified. Without the specificity contributed by the hot-start step, primer annealing may occur to a number of templates in the stool preparation. Since introducing this procedure in the laboratory, we have observed consistently similar enhanced sensitivity in experiments where enteroviruses in cerebrospinal fluid specimens were processed using a hot-start RT-PCR procedure.

The extraction procedure and hot-start RT-PCR also worked well with the stool suspensions spiked with the poliovirus 3 particles (Figure 1, lanes 8–11). In this case, the RT-PCR was able to amplify products observable on an ethidium bromide-stained gel from stools spiked with only 10 plaque-forming units (pfu) of poliovirus. Although one tenth less sensitive than RT-PCR procedures involving non-stool specimens (4,10), the sensitivity should be efficient in detecting the presence of enteroviruses in stools during infection. As mentioned above, increased sensitivity could also be added by combining our RT-PCR procedure with Southern hybridization.

REFERENCES


Address correspondence to Spencer H.S. Lee, National Centre for Enteroviruses, Division of Microbiology, Queen Elizabeth II Health Sciences Centre, Victoria General Hospital Site, 5788 University Avenue, Halifax, NS, Canada B3H 1V8. Internet: slee2@is.dal.ca

Received 11 February 1997; accepted 5 May 1997.

Michael A. Drebot and Spencer H.S. Lee
Queen Elizabeth II Health Sciences Centre
Halifax, NS, Canada