Serum-Free Production, Concentration and Purification of Recombinant Retroviruses


Concentrated and purified retroviral preparations are useful in gene therapy protocols and in the study of retroviral function. For the transduction of refractory cell types, such as hematopoietic stem cells and for in vivo applications such as liver-directed gene therapy after induction of liver regeneration, gene transfer efficiencies can be increased by concentrating packaging cell-line supernatants (3,4). The study of retroviral fusion with cellular or model membranes has been hampered by the difficulty in obtaining high titer-purified viral preparations (8). A major problem in retroviral concentration is the instability of the viral particles. During purification procedures, shear forces dissociate the envelope proteins from the gag core of the retroviruses, leading to a noninfectious particle (2,7,12,16).

Several methods for the concentration of retroviruses have been described. Retroviruses can be concentrated by passing producer-line supernatant through filtration devices (6,9,13,14). The main disadvantage of these methods is that most media components are co-concentrated as well. In case of an in vivo application, this may generate a strong immune response in the treated animal. The presence of animal products, such as bovine serum, in the cell culture media is a concern because of the possible presence of viruses or prion proteins. Large amounts of nonviral proteins will disturb assays to monitor membrane fusion. The concentrate will also contain cytokines and growth factors from bovine serum or produced by the packaging cells. These cytokines and growth factors might subsequently interfere with the target cells. Furthermore, the concentrated supernatant will also contain inhibitors of viral function, such as free envelope proteins and proteoglycans (1,10). More elaborate procedures to concentrate and purify retroviruses have been described. These procedures involve multiple steps including density gradient centrifugation, or gel filtration, and are consequently time-consuming and have a low yield (3,4).

By combining the production of retroviruses in serum-free media and a simple low-speed centrifugation procedure, we were able to concentrate and purify retroviruses in one step.

A retroviral producer line, based on PA317, producing the LNFZ vector (15) capable of transferring the E. coli β-galactosidase cDNA was used for all experiments described in this paper. Producer lines were cultured in Dulbecco’s modified Eagle medium (D-MEM; Life Technologies, Gaithersburg, MD, USA), supplemented with 2 mM l-glutamine and 100 U/mL penicillin-G/streptomycin plus 10% fetal calf serum (FCS; Summit Biotechnology, Fort Collins, CO, USA) until confluent. Viral supernatants were collected from 13.5-cm-diameter plates in 15 mL of medium. The cells were washed with D-MEM without serum, and serum-free medium was added. After 24 h, the supernatant was collected, filtered through a 0.22-µm filter and used for concentration or titration. Retrovirus titers were determined by diluting the different retroviral preparations 10^4–10^5 and infecting 5 × 10^5 NIH3T3 TK- cells in 6-cm-diameter plates in the presence of 4 µg/mL polybrene overnight. The day after infection, the cells were split 1/10 and plated in D-MEM, 10% FCS and 1 mg/mL G-418 (Life Technologies). After one week, the colonies were fixed and stained in 50% methanol/10% glacial acetic acid/H2O containing 0.1% Coomassie Brilliant Blue® G250 and counted. In initial experiments, it was found that culturing of retroviral producer lines in D-MEM without FCS led to a significant decrease in viral titers, as previously published (13). Several serum-free formulations were tested. Opti-MEM®, D-MEM/F-12 and viral production (VP) media were obtained from Life Technologies. HL-1 supplement was obtained from BioWhittaker (Walkersville, MD, USA). Opti-MEM and VP yielded virus at comparable titers to D-MEM plus FCS. Titers in D-MEM/F-12 + HL-1 were significantly lower than titers in D-MEM + 10% FCS; P < 0.0002 and P < 0.0025, respectively. Titers in Opti-MEM and VP were not significantly different from titers in D-MEM + 10% FCS.

### Table 1. Influence of Serum-Free Media on Viral Titers

<table>
<thead>
<tr>
<th>Media</th>
<th>Titer (cfu/mL)</th>
<th>N</th>
<th>± SD</th>
<th>N</th>
<th>PTI-MEM</th>
<th>N</th>
<th>PTI-MEM/F-12</th>
<th>HL-1</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-MEM + 10% FCS</td>
<td>4.5 × 10^6</td>
<td>8</td>
<td>± 1.6 × 10^6</td>
<td>6</td>
<td>0.9 × 10^6</td>
<td>6</td>
<td>1.6 × 10^6</td>
<td>4</td>
<td>3.5 × 10^6</td>
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<tr>
<td>D-MEM</td>
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<tr>
<td>VP</td>
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<td>Opti-MEM</td>
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<tr>
<td>D-MEM/F-12 + HL-1</td>
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Different media were added to plates containing monolayers of PA317/LNFZ retroviral producer cells. Supernatant was collected for 24 h, and the viral titer was determined. Virus was collected in D-MEM + 10% FCS, D-MEM only, viral production medium, Opti-MEM and D-MEM/nutrient mixture F-12 1/1 + HL-1 supplement. The number of independent experiments and the standard deviations (SD) are indicated. As determined by Student’s t-test, titers in D-MEM without FCS and D-MEM/F-12 + HL-1 were significantly lower than titers in D-MEM + 10% FCS; P < 0.0002 and P < 0.0025, respectively. Titers in Opti-MEM and VP were not significantly different from titers in D-MEM + 10% FCS.
Table 2. Concentrations

<table>
<thead>
<tr>
<th>Starting Titer (cfu/mL)</th>
<th>Purified Titer (cfu/mL)</th>
<th>Concentration Factor</th>
<th>Recovery in Pellet</th>
<th>Total Recovery (pellet + supernatant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7 × 10^6</td>
<td>2.7 × 10^7</td>
<td>12</td>
<td>47%</td>
<td>87%</td>
</tr>
<tr>
<td>± 2.1 × 10^6</td>
<td>± 1.6 × 10^7</td>
<td>± 4</td>
<td>± 17%</td>
<td>± 14%</td>
</tr>
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</table>

N = 5

Viral supernatants were collected and concentrated by centrifugation. The average and standard deviations of five independent purification procedures are depicted.

To ascertain the purity of the retroviral preparations, 1 mL of VP, 1 mL of supernatant from NIH3T3, 1 mL of supernatant from PA317, 1 mL of supernatant from PA317 producing LNFZ, corresponding to 10^6 colony-forming units (cfu) and 100 µL of concentrated virus, also corresponding to 10^6 cfu, were precipitated with trichloroacetic acid (TCA) and separated using a 15% sodium dodecyl sulfate (SDS) polyacrylamide gel. Proteins were stained with Coomassie Brilliant Blue (Figure 1). The PA317 packaging cell line is derived from NIH3T3 TK- (11). The PA317 packaging cell line is a useful method to obtain relatively pure retroviruses. These preparations may be useful in gene therapy and virological studies.

REFERENCES


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