SCALABLE LENTIVIRAL VECTOR PRODUCTION USING STABLE PRODUCER CELL LINES IN PERFUSION MODE

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Lentiviral vectors (LVs) are becoming an important tool in gene and cell therapy and are being utilized in several clinical studies against genetic and acquired diseases, as well as in cancer therapies. To address the challenges linked to the generation of preclinical and clinical supply, the National Research Council Canada has developed packaging cell lines and stable producer cell lines for the production of LVs which can grow in suspension in serum-free media and produce LV in the $10^6$ TU/ml range without optimization. We focus on the development of perfusion processes to both intensify the process and harvest produced LV rapidly.

To facilitate titration, a producer cell line for LV expressing GFP regulated by the strong constitutive CMV promoter was generated (HEK293SF-LVP-CMVGFp92). Transcription of Rev and the envelope protein (VSVG) is under the control of the tetracycline and cumate switches, which means that addition of doxycycline and cumate is required to induce the production of LV. Results obtained demonstrate that the system is scalable and up to 15 fold increase in total yield was obtained in perfusion mode when compared to batch mode (Fig. 1), using perfusion rates of up to 1 vessel volume exchange per day (vvd) after induction.

Figure 1 – Comparison of the total amount of LV produced in 3L bioreactor in batch mode (n=4) and during two runs operated in perfusion mode using an acoustic filter. Cells were induced at $10^5$E06 cells/ml for perfusion 1, and $5E06$ cells/ml for perfusion 2. Titers were obtained using a gene transfer assay.

<table>
<thead>
<tr>
<th>Cell density at induction (E06 cells/ml)</th>
<th>Perfusion rate after induction (vvd)</th>
<th>Cumulative titer (E10 TU)</th>
<th>Specific production (TU/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>21.1</td>
<td>12.4</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>18.9</td>
<td>7.2</td>
</tr>
<tr>
<td>10</td>
<td>0.65</td>
<td>8.07</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Our data indicate that the specific productivity decreases as the cell density at induction increases (Table 1). Additional improvements are expected by optimizing the perfusion rate, selecting a different media and adding feeds and supplements. In conclusion, through a combination of induction at high cell density and operation in perfusion mode, cumulative functional LV titers were increased by >15-fold, reaching up to $1E11$/L of bioreactor culture. Our scalable approach is directly transferable to GMP facilities for cell and gene therapy manufacturing.