PK-7 and RD2-MolPack-Chim3.14, two packaging cell clones for semi-stable and stable production of HIV-based lentiviral vectors

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Abstract
We conceived a novel strategy based on the sequential insertion of the viral genes into HEK-293T cells by integrating vectors: 1. baculo-AAV hybrid vector expressing gag-pol-rev genes 2. self-inactivating lentiviral vector (SIN-LV) expressing the RD114-TR env gene 3. SIN-LV expressing the tat gene
We obtained the PK-7 intermediate clone for semi-stable production of LV and the RD2-MolPack-Chim3.14 producer clone expressing the anti-HIV transgene Chim3.14 as an example of a prototype stable packaging cell line for the production of 2nd generation LV.

Objective
Generate two packaging cell lines for semi-stable: (PK-7 clone - 2 and 3 generation) and stable: (RD2-MolPack-Chim3.14 clone - 2 generation) production of HIV-based LV

Materials and Methods
To obtain the PK-7 clone, HEK-293T cells were transfected with the AAV-Rep78 plasmid and then infected with the hybrid baculo-AAV-gag-pol-rev vector. From PK-7 we generated the RD2-MolPack-Chim3.14 clone via the sequential integration of the SIN-LV-Tat and SIN-RD114-TR vectors and the Tat-dependent LV vector expressing the anti-HIV Vif dominant negative transgene Chim3.14.

Results: PK-7 semi-stable

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Table 1. Reduction of total amount of plasmid DNA (small-scale)

<table>
<thead>
<tr>
<th>MOI</th>
<th>0.3 mg</th>
<th>0.6 mg</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>1.2 × 10^7</td>
<td>4.9 × 10^6</td>
<td>1.1 × 10^7</td>
</tr>
<tr>
<td>D2</td>
<td>3.5 × 10^6</td>
<td>1.0 × 10^7</td>
<td>4.1 × 10^7</td>
</tr>
</tbody>
</table>

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Table 2. Transduction ability of RD114-TR-LV from RD2-MolPack-Chim3.14

<table>
<thead>
<tr>
<th>MOI</th>
<th>0.1</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>10</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>D2</td>
<td>40</td>
<td>10</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Conclusions

PK-7 cells: 
- 2 copies, head-to-tail oriented, of the ITR-flanked cassette
- transduced as efficiently as the parental cell line (>95%) also in medium containing 5% FBS
- produce VSV-G-LV with a titer of 1 × 10^6 to 1 × 10^7 TU/ml, after 1 year of continuous culture (ca 420 doublings)
- Low amount of plasmid DNA is sufficient

RD2-MolPack-3.14 cells:
- grow in suspension for longer than 2 months of continuous culture
- produce RD114-TR-LV with a titer of 1 × 10^7 TU/ml
- transduction of human CD34+ cells by RD114-TR-LVs is very efficient as previously described.

References