Optimizing HIV-1 Viral Production via a Single Plasmid Transfection System

Shannon Zheng1,2, Yvetane Moreau2, Manish Sagar, MD2
Dougherty Valley High School, 10550 Albion Road, San Ramon, CA 945821, Department of Medicine, Boston University Medical School, 650 Albany Street, Boston, MA 021182

Objective
High genetic variability of HIV-1 envelope proteins pose challenges to generating an effective vaccine. By optimizing transfection using a single plasmid, the lab will be able to generate diverse virus stocks more efficiently, accelerating HIV-1 antibody studies and spurring vaccine development.

Background
- The HIV-1 envelope mediates infection by binding to the CD4 receptor and coreceptors (primarily CCR5 or CXCR4) of susceptible cells.
- A panel of CCR5 envelope variants (termed Global Panel or GP) effectively represents the global diversity of HIV-1.
- The single hybrid plasmid would contain the proviral backbone of HIV-1 called NL4-3 (X4) and the envelope of a global panel virus (R5).
- A coreceptor assay phenotypically confirms if the recombinant virus successfully acquired the global panel envelope that would make it an R5 virus.
- Genetic sequencing further indicates the integration of the global panel envelope gene into the hybrid virus genome.

Methods
Restriction digest with enzymes Xho1 and P1Ml/Van911

Gel electrophoresis to isolate NL4-3 HIV-1 backbone (12kb) and global panel envelope (3kb)

DNA extraction and purification

Ligation of hybrid plasmid

Transform E. coli

Pick colonies and perform miniprep

PCR to amplify the envelope

Transfection of HEK-293T cells

Sequence comparison

ATCGGTGTC
TACGCACG

Results
Gel of PCR products confirmed the presence of the envelope in hybrid plasmid

Amplification of the envelope region by PCR in Figure 1 shows that the envelope sequences of the hybrid plasmids (lanes 4-13) are the same length (3kb) as the envelope sequence of the positive control, NL4-3 (lane 3).

Restriction digest of miniprep products verified that the hybrid plasmid contained the complete HIV-1 genome

Figure 2 shows that the hybrid plasmid in lane 8 had both the HIV-1 backbone of NL4-3 and the envelope of a global panel virus. The lengths of the hybrid plasmid’s proviral backbone and envelope are comparable to those of the positive control, wild-type global panel virus 398F1 (lane 4).

Coreceptor assay displayed coreceptor tropism of control and hybrid samples

Figure 3: Hybrid virus infection in DMEM

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Given Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL4-3</td>
<td>Global Panel 1</td>
</tr>
<tr>
<td>CE1176</td>
<td>Global Panel 2</td>
</tr>
<tr>
<td>246F3</td>
<td>Global Panel 3</td>
</tr>
<tr>
<td>BOK9000</td>
<td>Global Panel 4</td>
</tr>
<tr>
<td>U1176</td>
<td>Global Panel 5</td>
</tr>
<tr>
<td>25710</td>
<td>Global Panel 6</td>
</tr>
<tr>
<td>398F1</td>
<td>Global Panel 12</td>
</tr>
<tr>
<td>CBE</td>
<td>Global Panel 11</td>
</tr>
</tbody>
</table>

Low RLU (relative light unit) values in Figure 3 indicate that the overall infection rate was low for all hybrid viruses other than Global Panel 12. X4-tropic (NL4-3), R5-tropic (YU2, 246F3), and dual tropic (89.6) viruses were used as positive controls to reflect the expected phenotypes. Wells without an active inhibitor had higher RLU, as highlighted in Figure 4.

Discussion and Conclusions
In this experiment, NL4-3 and global panel HIV-1 strains were cut correctly using restriction enzymes Xho1 and P1Ml/Van911 (see Methods). Successful transformation of E. coli with the hybrid plasmids demonstrated that the HIV-1 backbones of NL4-3 properly ligated to the global panel envelopes. The coreceptor assay revealed that a functioning hybrid virus containing the Global Panel 12 envelope was created (Figure 4). The remaining hybrid viruses were weak and failed to induce infection (Figure 3). This was most likely due to poor selection of colonies and lowered DNA concentrations following miniprep. Sequencing results were generally inconclusive. Various experimental strains came back >90% positive for the Global Panel 1 virus genome. Cross contamination most likely occurred during PCR. To optimize future production of single plasmid transfection systems, try increasing the strength of ampicillin on LB plates and maintaining higher systems, try increasing the strength of ampicillin on LB plates and maintaining higher

Acknowledgements
Special thanks to Dr. Manish Sagar for allowing me to work in his lab, to Yvetane Moreau for being the most patient and caring mentor, and to Alex Olson & John Isaac for their helpful guidance. Additionally, I would like to thank the RISe program for this opportunity.