1. Purpose
1.1. The purpose of this protocol is to produce retrovirus from transfected plasmid DNA.
1.2. This procedure is routinely performed in the Vector Development Laboratory (VDL) following Good Laboratory Practices (GLP).

2. Abbreviations and Definitions
2.1. SOP  Standard Operating Procedure
2.2. VDL  Vector Development Laboratory
2.3. GLP  Good Laboratory Practice
2.4. BSC  Biological Safety Cabinet
2.5. DMEM  Delbecco’s Modified Eagle Medium
2.6. FBS  Fetal Bovine Serum
2.7. Antibiotics  Penicillin/Streptomycin/Anti-mycotic Solution
2.8. DMEM10  DMEM with 10% FBS, 1% Antibiotics
2.9. DMEM2  DMEM with 2% FBS, 1% Antibiotics
2.10. HBS  HEPES Buffered Saline
2.11. PBS  Phosphate Buffered Saline without Ca\(^2\+) and Mg\(^2\+)

3. Equipment, Materials, and Reagents

**NOTE:** All materials in contact with cells must be sterile, pyrogen-free and used according to the manufacturer's directions unless stated otherwise. Equivalent materials and equipment may be used but all changes must be recorded in the laboratory notebook.

3.1. Equipment
3.1.1. BSC
3.1.2. 37°C/5% CO\(_2\) incubator
3.1.3. Pipette aid
3.1.4. Water bath set at 37 °C
3.1.5. Inverted microscope with 20X objective
3.1.6. Microcentrifuge
3.1.7. Vortex

3.2. Materials
3.2.1. 60 mm dishes  Corning
3.2.2. Serological pipets  Corning
3.2.3. Sterile pipet tips  VWR
3.2.4. 1.7 ml tubes     Axygen
3.2.5. 0.45-µm filter     Millipore
3.2.6. Cryotubes     Sorenson

3.3. **Reagents**

3.3.1. DMEM      Hyclone
3.3.2. DMEM10    VDL
3.3.3. DMEM2     VDL
3.3.4. PBS       Invitrogen
3.3.5. FBS       Atlas
3.3.6. Antibiotics     Cellgro
3.3.7. Deionized Water    Baxter
3.3.8. Profection ® Mammalian Transfection Kit     Promega

3.4. **Starting Materials**

3.4.1. 2 x 60 mm dish of confluent GP2-293
3.4.2. Expression vector, 10 µg
3.4.3. pVSV-G vector, 10 µg

3.5. **Test Sample Identification**

3.5.1. The bar code on the plasmid will be scanned and compared to the computer database to ensure the correct sample is being processed.
3.5.2. One copy of the barcode will be printed and applied to a copy of this SOP for the final record.

4. **Procedure**

4.1. **Preparation of Cells**

4.1. Plate GP2-293 retrovirus packaging cells at 5x10^5 cells per 60mm dish in DMEM10.

**Note:** Cell should be plated so that 50% confluency is achieved after overnight incubation.

4.2. Transfer cells to the 37°C/5% CO₂ incubator for overnight incubation.

4.3. Check the cells to ensure they are at the proper density. If not contact laboratory director.
4.4. Three hours prior to transfection, aspirate the media from the cells and gently replace with 5 mL DMEM2.

4.5. Return cells to 37°C/5% CO₂ incubator until use.

**Preparation of Kit Reagents**

4.6. Remove Profection ® Mammalian Transfection Kit from the -20°C freezer and allow reagents (sterile water, CaCl₂, 2X HBS) to warm to room temperature.

**Transfection**

**NOTE:** Perform all steps in a certified BSC, using aseptic technique.

4.7. Mix kit reagents thoroughly by swirling the container or vortexing.

4.8. For each transfection, prepare the DNA and 2X HBS solutions in separate tubes.

4.9. Set up the reactions as follows:

<table>
<thead>
<tr>
<th>Tube 1</th>
<th>(µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression vector</td>
<td>5 µg</td>
</tr>
<tr>
<td>pVSV-G</td>
<td>5 µg</td>
</tr>
<tr>
<td>2 M CaCl₂</td>
<td>37 µL</td>
</tr>
<tr>
<td>Sterile water</td>
<td>Q.S to 300 µL</td>
</tr>
<tr>
<td>Tube 2</td>
<td></td>
</tr>
<tr>
<td>2X HBS</td>
<td>300 µL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>600 µL</td>
</tr>
</tbody>
</table>

4.10. Gently vortex the tube containing the 2X HBS.

**Note:** The speed should be adjusted such that the tube can be vortexed safely with the cap off and can accommodate the addition of the prepared DNA solution.

4.11. Continue to vortex while adding the prepared DNA solution in tube 1 drop-wise.

4.12. Incubate the solution at room temperature for 30 minutes.

4.13. Vortex the transfection solution again just prior to adding it to the cells.


4.15. Gently swirl the dishes to distribute the precipitate evenly over the cells.

4.16. Return the plates to the 37°C/5% CO₂ incubator for 12-16 hours.

4.17. Return cells to BSC and aspirate the medium. Add 5 mL of DMEM10.

4.18. Harvest retroviral supernatant at 48 hr following transfection by gently removing the supernatant from the cells. Filter the supernatant through a 0.45-µm filter.
4.19. If the retroviral supernatant is to be used within 2 hours, keep it on ice. For longer intervals, freeze the supernatant on dry ice and transfer it to –80°C.

**Note:** Retroviral half-life is 3 to 6 hr at 37°C (Sanes et al., 1986). To maintain the high titer, supernatants should be left on ice or frozen following harvest. The supernatant is aliquoted in 100 μL volumes so that subsequent infections can be performed with multiple freeze/thaw cycles. To thaw frozen supernatants, warm for a minimal period at 37°C and use immediately.

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5. **Data Collection and Management**

5.1.1. All data will be collected in a laboratory notebook.

5.1.2. Deviations of the protocol will be recorded in the laboratory notebook and on the Lab Meeting Sheet.

6. **Review and Revisions**

Written by: __________________________

Director, VDL

Reviewed by: __________________________

Director, Vector Production

Reviewed by: __________________________

Director, QA/QC

Date Issued: 12/1/06    Replaces VDL 300.1

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**Annual Review:**

2011

Reviewed without changed

Reviewed by: __________________________

QA/QC by: __________________________

Date Issued: 6/1/2011    Replaces VDL 300.2