1. Purpose

1.1. The purpose of this protocol is to amplify an adenoviral vector from a crude virus stock for large-scale expansion.

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. CPE  Cytopathic Effect
2.6. Antibiotics  Penicillin/Streptomycin/anti-mycotic
2.7. FBS  Fetal Bovine Serum
2.8. DMEM  Dulbecco's Modified Eagle's Medium
2.9. DMEM10  DMEM with 10% FBS
2.10. EDTA  ethylenediaminetetraacetic acid
2.11. Trypsin  0.25% Trypsin-EDTA
2.12. PBS  Phosphate Buffer Saline
2.13. Tris  50mM Tris, pH 8.0

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.

3.1. Equipment

3.1.1. BSC
3.1.2. 37°C/5% CO₂ incubator
3.1.3. Pipette aid
3.1.4. Inverted microscope with 20X objective
3.1.5. Table top centrifuge
3.1.6. Water bath set at 37 °C
3.1.7. Microcentrifuge

3.2. Materials

3.2.1. 15 mL conical tubes  Corning
3.2.2. 150 mm cell culture dishes    Corning
3.2.3. Serological pipets    Corning
3.2.4. Sterile pipet tips    VWR
3.2.5. 250 mL centrifuge bottle    Sorvall
3.2.6. 100 mL glass bottle    Corning

3.3. Reagents
3.3.1. Trypsin    Cellgro
3.3.2. PBS    Invitrogen
3.3.3. DMEM    Invitrogen
3.3.4. FBS    Atlas
3.3.5. Antibiotics    Cellgro

3.4. Starting Materials
3.4.1. 400μL crude virus (from step 4.22.1 in SOP: Plaque Expansion and Harvesting Adenoviral Vector).
3.4.2. Five 150 mm dish of 80% confluent 293 cells.

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.
3.5.3. A barcoded label that also contains virus name will be applied to all tubes, dishes, and Cell Stack used in the production of the virus.

4. Procedure

Infection of 293 cells in five 150 mm dishes with adenoviral vector

4.1. Remove sample from -80°C.
4.2. Subject the cell suspension to three freeze/thaw cycles. This involves thawing the cell suspension by placing the tube it is contained in, in a 37°C water bath. When it is completely thawed the tube is transferred to a dry ice/ethanol bath to freeze the cell suspension. The initial placing of the cell suspension in the -80°C freezer for storage is considered the first freeze.

NOTE: As the total volume is 400 μL both the freeze and the thaw cycles should be short, less than 5min.

4.3. Pellet cellular debris by centrifugation at 400 x g for 5 minutes.
4.4. Transfer the cleared viral lysate to a sterile 100 mL bottle and Q.S. to 100 mL with DMEM2 and mix well by pipetting.

4.5. Remove media from the 5, 150 mm dishes containing 80-90% confluent 293 cells.

4.6. Add 20 mL DMEM2/adenoviral vector mixture to each of the dishes.

4.7. Place the 150 mm dishes in the 37°C/5% CO2 incubator.

4.8. On the next day, add 0.6 mL FBS to each plate.

**NOTE:** Microscopically inspect the cells every day in order to determine the degree of CPE. When 95-100% CPE is observed continue with the procedure. It will take 2-3 days before this stage of CPE is reached.

**Note:** If it takes longer than 4 days to reach 80-90% CPE, collect the viral lysate as described below in section 4.9-4.12, remove 1/5th of the volume (1mL) and repeat infection beginning with step 4.2.

### Harvesting of adenoviral vector from five 150mm dishes of infected 293 cells

4.9. When 95-100% CPE is observed (all cells should be detached), transfer cell suspension to a 250 mL centrifuge bottle. Wash each dish with 10 mL PBS to detach residual cells.

4.10. Transfer wash to centrifuge bottle.

4.11. Pellet cells by centrifugation at 1,500 x g for 20 minutes.

4.12. Remove supernatant and resuspend the cell pellet in 5 mL of Tris. Pipette to resuspend cells to obtain a single-cell suspension.

4.13. Transfer the cell suspension to a 15 mL conical tube and freeze at -80°C until use.

### 5. Review and Revisions

**Written by:** ____________________________
Director, VDL

**Reviewed by:** ____________________________
Director, Vector Production

**Reviewed by:** ____________________________
Director, QA/QC

**Date Issued:** 1/30/2006    **Replaces VDL 105.0**

**Annual Review:**

2011
VDL105.2 PREPARATION OF ADENOVIRAL VECTOR LYSATE

Reviewed without changes

Reviewed by: _____________________________

Date: _____________________________

QA/QC by: _____________________________

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2012

Reviewed without changes

Reviewed by: _____________________________

Date: _____________________________

QA/QC by: _____________________________

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2013

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Reviewed by: _____________________________

Date: _____________________________

QA/QC by: _____________________________

Date: _____________________________