1. Purpose

1.1. The purpose of this procedure is to expand and harvest an adenoviral vector in a plaque.

1.2. The starting material may be an adenovirus plaque from a transfection.

1.3. 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 Practices

2.4. BSC Biological Safety Cabinet

2.5. CPE Cytopathic Effect

2.6. Antibiotics Penicillin/Streptomycin/Anti-mycotic

2.7. DMEM Dulbecco's Modified Eagle's Medium with 1% Antibiotics

2.8. FBS Fetal Bovine Serum

2.9. DMEM20 DMEM with 20% FBS and 2% Antibiotics

2.10. DMEM10 DMEM with 10% FBS

2.11. DMEM2 DMEM with 2% FBS

2.12. PBS Phosphate Buffered Saline

2.13. 70% Ethanol 70:30 (v/v) – Ethanol:Water

3. Equipment, Materials, 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 37°C/5% CO₂ incubator

3.1.2 BSC

3.1.3 Water bath
VDL103.3 PLAQUE EXPANSION AND HARVESTING ADENOVIRAL VECTOR

3.1.4 Pipet aide
3.1.5 Microscope
3.1.6 Tabletop low-speed centrifuge
3.1.7 Micro-centrifuge

3.2 Materials
3.2.1 Serological pipettes Corning
3.2.2 Cell culture dishes Corning
3.2.3 15 mL conical tubes Corning
3.2.4 1.7 mL tubes Axygen
3.2.5 Sterile pipet tips VWR
3.2.6 Pasteur pipets VWR

3.1 Reagents
3.1.1 Sterile Water Baxter
3.1.2 70% Ethanol VDL
3.1.3 DMEM Hyclone
3.1.4 DMEM10 VDL
3.1.5 DMEM20 VDL
3.1.6 DMEM2 VDL
3.1.7 Antibiotics Cellgro
3.1.8 FBS Atlas
3.1.9 PBS Invitrogen
3.1.10 Tris Sigma
3.1.11 50 mM Tris-HCl, pH 8.0 VDL

3.2 Starting Materials
3.2.1 1, 60 mm dish of 80% confluent 293 cells per plaque.
3.2.2 Adenoviral plaque in medium.
4. Procedure

   **Note:** Perform all steps in the BSC using aseptic techniques unless otherwise stated.

**Plaque Expansion**

4.1. Prior to infecting the cells with the adenoviral vector the solution containing the plaque must undergo three freeze/thaw cycles. If the plaque has been stored at -80°C, this can be counted as the first freeze.

   **Note:** It is important not to leave the thaws for longer than necessary, as the adenoviral vector will inactivate with time at 37°C.

4.2. Centrifuge the thawed virus for 2 minutes at 1000 x g.

4.3. Aspirate medium from the 60 mm dish.

4.4. Add 2 mL DMEM2 to each 60 mm dish.

4.5. Add 300 μL of each supernatant to one 60 mm dish and gently rock the dish to allow the virus to evenly distribute in the medium.

4.6. Repeat step 4.5 for each sample.

4.7. Place all 60 mm dishes in the 37°C/5% CO₂ incubator for overnight incubation.

4.8. Place the original plaque solution at -80°C.

4.9. After overnight incubation, add 2 mL of DMEM20 to bring the final concentration of FBS up to 10%.

4.10. Return the dishes to the 37°C/5% CO₂ incubator.

4.11. Check cultures daily under the microscope after the first 5 days for the onset of CPE.

**Harvest virus**

4.12. The virus is ready to harvest when 90-100% of the cells are floating.

4.13. Prepare 3, 1.5 mL tubes for each sample. Label each tube with the virus name, plaque number and date.

4.14. Prepare one 15 mL conical tube per sample. Label with virus name and plaque number.

4.15. Using a serological pipet, transfer floating cells and medium to the 15 mL conical tube. Wash dish with 4 mL sterile PBS. Transfer wash to the 15 mL conical tube containing the cells and medium.
4.16. Repeat steps 4.15 for all additional samples.

4.17. Pellet the cells in the 15 mL conical tube by centrifugation at 400 x g for 10 minutes.

4.18. Aspirate the media with a Pasteur pipet.

**Note: Use a new Pasteur pipet for each sample.**

4.19. Resuspend the cell pellet in 1 mL of sterile PBS and transfer to a new 1.5 mL tube.

4.20. Pellet the cells again by centrifugation at 1000 x g for 2 minutes.

4.21. Aspirate the PBS with a Pasteur pipet.

**Note: Use a new Pasteur pipet for each sample.**

4.22. Resuspend the cell pellet in 1.0 mL sterile 50 mM Tris-HCl, pH 8.0.

4.23. Divide each plaque solution between the three labeled tubes prepared in step 4.12.

   4.23.1. 400 μL for large-scale expansion
   4.23.2. 300 μL for VDL testing
   4.23.3. 300 μL for investigator testing

5. **Review and Revisions**

Written by: ______________________

Director, VDL

Reviewed by: ______________________

Director, Vector Production

Reviewed by: ______________________

Director, QA/QC

Date Issued: 12/1/06    Replaces VDL 103.1

**Annual Review:**

2011

Reviewed without changes    Changed and this version archived
VDL103.3 PLAQUE EXPANSION AND HARVESTING ADENOVIRAL VECTOR

Reviewed by: ___________________________ ___________________________

QA/QC by: ___________________________

Date Issued: 7/1/2011 Replaces VDL 103.2

2012

Reviewed without changes Changed and this version archived

Reviewed by: ___________________________ ___________________________

QA/QC by: ___________________________

Date: ___________________________

2013

Reviewed without changes Changed and this version archived

Reviewed by: ___________________________ ___________________________

QA/QC by: ___________________________

Date: ___________________________

Revisions