Submission ID #: 62677
Scriptwriter Name: Mithila Boche
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=19116533

Title: Growth, Purification, and Titration of Oncolytic Herpes Simplex Virus

Authors and Affiliations:
Hong-My Nguyen[1,*], Naresh Sah[1,*], Melissa R. M. Humphrey[2], Samuel D. Rabkin[2], Dipongkor Saha[1]

* Authors contributed equally

[1]Department of Immunotherapeutics and Biotechnology, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center
[2]Molecular Neurosurgery Laboratory and the Brain Tumor Research Center, Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School

Corresponding Authors:
Dipongkor Saha dipongkor.saha@ttuhsc.edu

Email Addresses for All Authors:
My.Nguyen@ttuhsc.edu
Naresh.Sah@ttuhsc.edu
mmarinelli@mgh.harvard.edu
rabkin@mgh.harvard.edu
dipongkor.saha@ttuhsc.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? ~20 ft

Current Protocol Length

Number of Steps: 16
Number of Shots: 39

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Hong-My Nguyen:** Oncolytic herpes simplex virus is an emerging form of cancer immunotherapy. An efficient system of virus propagation, purification, and titer determination is critical for their use in experimental studies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.3 for 'virus propagation' and 3.2.1 for 'purification'*

1.2. **Naresh Sah:** This method is very simple and can be easily adopted in any Biosafety Level 2 laboratory setting to achieve a high-quality viral stock for preclinical studies.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.2 'viral stock for preclinical studies'*

OPTIONAL:

1.3. **Dipongkor Saha:** Production of a high-quality/pure viral stock is crucial since it is used to study anti-cancer immune response and to develop a novel form of virus-based cancer immunotherapy.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.2 for 'viral stock'*

1.4. **Hong-My Nguyen:** This protocol can be used to purify any wild-type or genetically engineered herpes simplex virus.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.5. **Naresh Sah:** This protocol should be easy-to-read and easy-to-follow by an individual who has never performed this technique before. The listed materials and reagents must be in place before trying this technique for the first time.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Oncolytic Herpes Simplex Virus (oHSV) Growth

2.1. Begin by washing the T-150 square centimeter flasks with 2x high-glucose DPBS supplemented with 1% IFCS **[1-TXT]**. Aspirate the DPBS **[2]** and add 7 milliliters of virus inoculum per flask **[3]**.

2.1.1. WIDE: Establishing shot of talent washing the flasks. **TEXT: IFCS: heat-inactivated fetal calf serum**

2.1.2. Talent aspirating DPBS

2.1.3. Talent adding virus inoculum in the flasks.

2.2. Gently rock the flasks for 5 minutes **[1]** and incubate at 37 degrees Celsius for 1.5 to 2 hours **[2]**.

2.2.1. The flasks shaking on the rocker. *Videographer: This step is important!*

2.2.2. Talent placing flasks in an incubator.

2.3. Then, remove the inoculum **[1]** and add 25 milliliters of DMEM supplemented with 1% IFCS to each flask **[2]**.

2.3.1. Talent removing inoculum.

2.3.2. Talent adding DMEM in a flask.

2.4. After incubation at 37 degrees Celsius and 5% carbon dioxide for 2 to 4 days, collect 20 milliliters of the culture supernatant from each flask into 50-milliliter centrifuge tubes **[1-TXT]**. Using a cell scraper, scrape the cells from the bottom of the flasks **[2]**.

2.4.1. Talent removing flasks from an incubator and collecting 20mL of the supernatant in labeled tubes. **TEXT: Leave 5 mL of supernatant in each flask**

2.4.2. Talent scraping off the cells. *Videographer: This step is important!*

2.5. Add approximately 15 milliliters of the previously collected culture supernatant to each flask to bring the volume up to 20 milliliters **[1]**, then use a 10-milliliter sterile serological pipette to gently wash the bottom of the flasks a few times **[2]**.

2.5.1. Talent adding supernatant from labeled tube to the flask.

2.5.2. Talent washing the bottom of the flasks. *Videographer: This step is important!*

2.6. Collect the cells with medium into 50-milliliter conical centrifuge tubes placed on ice **[1]**. Centrifuge the tubes at 300 × *g* for 10 minutes at 4 degrees Celsius **[2]**.

2.6.1. Talent collecting the cells in tubes.

2.6.2. Talent placing tubes in the centrifuge and closing the lid.

2.7. Resuspend each pellet thoroughly in 1.25 milliliters of Virus Buffer, or VB, and 1.25 milliliters of previously collected culture supernatant **[1]**. Mix all re-suspended pellets into one 50-milliliter conical centrifuge tube **[2]**. Snap-freeze the resuspended cells using dry ice and 100% ethanol **[3]** and store at minus 80 degrees Celsius **[4]**.

2.7.1. Talent adding virus buffer and culture supernatant to the tubes and resuspending the cell pellet.

2.7.2. Talent mixing all cells in one tube.

2.7.3. Talent snap-freezing the cells on dry ice and 100 % ethanol.

2.7.4. Talent keeping tube in a deep freezer.

3. Oncolytic Herpes Simplex Virus (oHSV) Purification

3.1. Thaw the previously snap-frozen cells in a warm water bath at 37 degrees Celsius **[1]** and sonicate for 1 minute for three cycles in a water bath **[2]**.

3.1.1. WIDE: Talent placing tube in a water bath.

3.1.2. Tube being sonicated in the water bath.

3.2. Add 175 units of Benzonase Nuclease and 2 microliters of 2 millimolar magnesium chloride per milliliter of the cell lysate **[1-TXT]** and vortex **[2]**. After an incubation of 30 minutes in warm water bath at 37 degrees Celsius **[3]**, place the tube on ice **[4]**.

3.2.1. Talent adding Benzonase Nuclease in the tube. **TEXT: 2 µL/mL from 1M stock of $MgCl_2$**

3.2.2. Talent vortexing the tube.

3.2.3. Talent placing the tube in a warm water bath.

3.2.4. Talent removing the tube from warm water bath and placing it on ice.

3.3. Spin the cell lysate at 300 × *g* for 10 minutes **[1]**. Collect the supernatant in a new 50-milliliter conical centrifuge tube **[2]** and resuspend the cell pellet in 500 microliters of VB. Designate it as pellet 1 **[3]**.

3.3.1. Talent placing tube in a centrifuge and closing the lid.

3.3.2. Talent withdrawing supernatant in a labeled tube.

3.3.3. Talent resuspending pellet in VB and labeling the tube.

3.4. Spin the collected supernatant again at 500 × *g* for 10 minutes **[1]** and store the supernatant separately in a fresh 50-milliliter conical centrifuge tube to use later **[2]**. Resuspend the cell pellet in 500 microliters of VB, designating it as pellet 2 **[3]**.

3.4.1. Talent placing labeled tube containing supernatant in a centrifuge and closing the lid.

3.4.2. Talent collecting supernatant in a labeled fresh tube.

3.4.3. Talent resuspending pellet in VB and labeling the tube.

3.5. Combine the resuspended pellet 1 and pellet 2 in a new 1.7-milliliter centrifuge tube **[1]** and vortex and sonicate twice in a water bath before spinning the tubes at 400 × *g* for 10 minutes **[2]**. Collect the supernatant from 1.7-milliliter centrifuge tube and combine it with the supernatant previously collected 50-milliliter conical centrifuge tube **[3]**.

3.5.1. Talent combining cell suspensions from two labeled tubes. NOTE: Maybe slated as 3.5.1a

3.5.2. Talent vortexing/sonicating tubes and placing them in a centrifuge.

NOTE: This shot was broken up into 3 shots: 3.5.2a, 3.5.2b, 3.5.2c

3.5.2a. Added shot: Talent vortexing the tube. NOTE: Discrepancy between author and videographer notes. This could be slated as 3.5.2a or 3.5.2b.

3.5.2b. Added shot: Talent sonicating the tube. NOTE: Discrepancy between author and videographer notes. This could be slated as 3.5.2b or 3.5.2c

3.5.2c. Added shot: Talent placing tube in a centrifuge and closing the lid.

3.5.3. Talent collecting supernatant from 1.7-milliliter centrifuge tube and combining it with the supernatant stored in a 50-milliliter conical centrifuge tube.

3.6. Filter the supernatant through a sterile 5-micrometer PVDF membrane filter into a new 50-milliliter centrifuge tube called TUBE 1 **[1-TXT]**.

3.6.1. Talent drawing the supernatant by a 10-milliliter syringe and filtering supernatant into a labeled tube 1. **TEXT: polyvinylidene difluoride** *Videographer: This step is important!*

3.7. Add 1 milliliter of VB to the 50-milliliter centrifuge tube emptied after filtering the supernatant from the previous step, vortex **[1]**, and pass it through the same PVDF membrane filter placed on TUBE 1 **[2]**.

3.7.1. Talent adding VB in emptied labeled tube, vortexing, and placing the tube on ice. *Videographer: This step is important!*

3.7.2. Talent drawing VB suspension by a 10-milliliter syringe and filtering the suspension/solution into labeled tube 1.

3.8. Pass the filtrate from TUBE 1 through a sterile 0.8-micrometer MCE membrane filter placed on a new 50-milliliter centrifuge tube called TUBE 2 **[1-TXT]**. Add 1 milliliter of VB to the emptied TUBE 1, vortex **[2]**, and pass it through the same MCE filter placed on TUBE 2 **[3].**

3.8.1. Talent drawing tube 1 content by a 10-milliliter syringe and filtering it into labeled tube 2. **TEXT: MCE: mixed cellulose ester**

3.8.2. Talent adding VB in emptied tube 1, vortexing, and placing the tube on ice.

3.8.3. Talent drawing tube 1 content by a 10-milliliter syringe and filtering it into tube 2.

3.9. Pass the filtrate from TUBE 2 through a sterile 0.45-micrometer PVDF filter placed on a new 50-milliliter centrifuge tube labeled TUBE 3 **[1]**. As described before, repeat the washing of TUBE 2 with 1 milliliter of VB to add filtrate to TUBE 3 **[2]**.

3.9.1. Talent filtering content of tube 2 into tube 3.

3.9.2. Talent adding VB in emptied tube 2, vortexing, and filtering tube 2 content into tube 3.

Results

4. Results: Microscopic Analysis of Vero Cells Infected with oHSV

4.1. In the representative analysis, the cytopathic effect could be observed in Vero cells at 36, 48, and 72 hours post-oHSV infection **[1]** with the rounding of the affected cells **[2]**.

4.1.1. LAB MEDIA: Figure 2.

4.1.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize black arrows from the left panel.*

4.2. When stained with mCherry, the increasing level of the CPE over time was evident **[1]**.

4.2.1. LAB MEDIA: Figure 2. *Video Editor: Emphasize the right panel.*

4.3. At 72 hours post-infection, viral plaques were stained with X-gal to visualize oHSVs with *lacZ* expression **[1]**.

4.3.1. LAB MEDIA: Figure 4.

Conclusion

5. Conclusion Interview Statements

5.1. **Hong-My Nguyen:** Gentle scraping off virus-infected cells and gentle washing the bottom of flasks is critical to keep oHSV infected cells intact so that a high titer viral stock can be obtained.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.2 for 'gentle scraping' and 2.5.3 for 'washing the bottom of flasks'*