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Title: Production of A SARS-CoV-2 Virus-Like Particle System to Investigate Viral Life Cycles In Vitro

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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. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 26
Number of Shots: 45

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

REQUIRED:

1.1. **Wenxin Dai:** This research focuses on understanding the biology of the SARS-CoV-2 virus and aims to identify potential drugs, particularly from traditional Chinese medicine, to combat SARS-CoV-2.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.1., 2.7.2., 2.7.3.*

What are the current experimental challenges?

1.2. **Wenxin Dai:** All studies involving live SARS-CoV-2 must be conducted in biosafety level 3 laboratories. This requirement poses a significant experimental limitation, making such research feasible only in a handful of laboratories.

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

What technologies are currently used to advance research in your field?

1.3. **Wenxin Dai:** The SARS-CoV-2 virus-like particle method enables the study of the virus without the constraints of a biosafety level 3 lab, making it a highly valuable approach for advancing research in this area.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.2.*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Procedure for Generating SC2-VLPs

Demonstrator: Wenxin Dai

2.1. To begin, seed approximately three million HEK-293T *(Heck two ninety-three tee)* cells in a 10-centimeter diameter tissue culture plate with DMEM *(D-M-E-M)* complete medium supplemented with 10% FBS and 1% penicillin-streptomycin **[1]**. Culture the cells at 37 degrees Celsius and 5% carbon dioxide for approximately 24 hours **[2]**. Check the cell confluency under the microscope **[3-TXT]**.

2.1.1. WIDE: Talent pipetting HEK-293T cells into a 10-centimeter culture plate containing DMEM complete medium + 10% FBS + 1% penicillin-streptomycin.

2.1.2. Talent placing the culture plate in an incubator.

2.1.3. Talent checking cell confluency using a microscope. **TXT: Proceed if the cells are ~70% confluent**

2.2. Dilute 60 microliters of PEI *(P-E-I)* from a 1 milligram per milliliter stock solution with serum-free medium to reach a final volume of 200 microliters **[1]**.

2.2.1. Talent pipetting 60 microliters of PEI stock into a microcentrifuge tube and adding serum-free medium to the tube.

2.3. Now, take 200 microliters of serum-free medium and add 6.7 micrograms of N plasmid *(N-Plasmid)*, 10 micrograms of Luc-T20 plasmid *(Luke Tee Twenty plasmid)*, 0.016 micrograms of S plasmid, and 3.3 micrograms of M-IRES-E plasmid *(M eye-rez E plasmid)* into it **[1]**.

2.3.1. Talent sequentially adding the specified plasmids into a microcentrifuge tube containing serum-free medium.

2.4. Gently add the diluted PEI solution into the solution containing plasmids coding for viral structure proteins, and incubate the mixture at room temperature for 10 minutes. This is the transfection solution **[1]**.

2.4.1. Talent gently pipetting the diluted PEI into the plasmid solution and setting the tube aside.

2.5. Carefully drop the transfection solution onto the HEK-293T cells **[1]** and gently swirl the tissue culture plate to ensure thorough mixing **[2]**.

2.5.1. Talent adding transfection solution dropwise to the culture plate containing HEK-293T cells.

2.5.2. Talent swirling the plate for mixing.

2.6. Exchange the cell culture medium with DMEM complete medium 6 hours post-infection **[1]**, and incubate the transfected HEK-293T cells at 37 degrees Celsius with 5 percent carbon dioxide for 48 hours **[2]**.

2.6.1. Talent aspirating the old medium from the plate and replacing it with fresh DMEM complete medium.

2.6.2. Talent placing the plate into the incubator.

2.7. Collect the supernatant of the infected HEK-293T cells, which contains the SARS-CoV-2 *(Sarz-Koh-Vee Two)* virus-like particles **[1]**. Filter the collected supernatant through a 0.45-micrometer syringe filter to remove cellular debris **[2]**. This is the SC2-VLP *(S-C-two V-L-P)* medium **[3]**.

2.7.1. Talent collecting the supernatant from the culture plate using a pipette.

2.7.2. Talent filtering the supernatant through a 0.45 micrometer syringe filter.

2.7.3. A shot of the collected SC2-VLP medium.

3. Procedure for Assessing SC2-VLP Effectiveness and Composition

3.1. Seed forty thousand HEK-293T cells with stable expression of Angiotensin-Converting Enzyme 2 or ACE2 *(A-C-E-two)* and TMPRSS2 *(T-M-P-R-S-S-two)* in a 96-well plate **[1]** and add 50 microliters of SC2-VLP medium **[2]**.

3.1.1. Talent pipetting HEK-293T ACE2/TMPRSS2 cells in a 96-well plate.

3.1.2. Talent adding SC2-VLP medium into each well.

3.2. Incubate the 96-well tissue culture plate at 37 degrees Celsius with 5 percent carbon dioxide for 24 hours **[1]**.

3.2.1. Talent placing the plate into a CO_2 incubator.

3.3. After incubation, remove the medium from each well of the 96-well plate **[1]**, and wash once with 100 microliters of PBS pre-warmed to 37 degrees Celsius **[2]**.

3.3.1. Talent aspirating medium from each well using a multichannel pipette.

3.3.2. Talent pipetting PBS into the wells for washing.

3.4. Lyse the HEK-293T cells seeded with ACE2/TMPRSS2 *(A-C-E-two T-M-P-R-S-S-two)* cells with 20 microliters of passive lysis buffer **[1]**, and gently rock the sample on an orbital shaker for 15 minutes at room temperature **[2]**.

3.4.1. Talent pipetting lysis buffer into each well containing HEK-293T ACE2/TMPRSS2 cells.

3.4.2. Talent placing the plate on an orbital shaker.

3.5. Spin the 96-well plate at 4,000 *g* for 15 minutes at 4 degrees Celsius using a refrigerated microplate centrifuge **[1]**, and then immediately transfer the plate to an ice bath **[2]**.

3.5.1. Talent placing the 96-well plate into the refrigerated microplate centrifuge.

3.5.2. Talent transferring the plate into an ice bath.

3.6. Take 100 microliters of reconstituted luciferase assay buffer into a new opaque white 96-well plate **[1]**, and add 20 microliters of lysate into each well **[2]**. Mix briefly by pipetting up and down 2 to 3 times **[3]**.

3.6.1. Talent pipetting luciferase assay buffer into a new opaque white plate.

3.6.2. Talent adding lysate into the white plate containing the luciferase buffer.

3.6.3. Talent pipetting up and down to mix the contents gently.

3.7. Measure the luminescence signal using a plate reader **[1]**.

3.7.1. Talent placing the opaque white plate into the plate reader and initiating the measurement.

3.8. Next, to assess the composition of the SC2-VLP medium, add 1.36 milliliters of PEG 8000 *(Peg-eight-thousand)* solution to 10 milliliters of SC2-VLP medium **[1]**.

3.8.1. Talent pipetting PEG 8000 solution into a tube containing 10 milliliters of SC2-VLP medium.

3.9. Place the mixture on an orbital shaker and slowly mix at 4 degrees Celsius overnight **[1]**.

3.9.1. Talent placing the tube on an orbital shaker and setting the temperature.

3.10. Centrifuge the solution at 4 degrees Celsius and 2,000 *g* for 30 minutes **[1]** and collect the SC2-VLP pellet for western blotting analysis **[2]**.

3.10.1. Talent loading the tube into a centrifuge.

3.10.2. Talent collecting the pellet.

4. Subcellular Localization Analysis of S and Its Mutants in SC2-VLP-Producing Cells

4.1. Seed approximately three million HEK-293T cells evenly in a glass-bottom culture dish with a 15-millimeter diameter, and allow the cells to adhere and grow **[1]** until they reach approximately 70 percent confluency **[2]**.

4.1.1. Talent pipetting cells into a glass-bottom culture dish with a 15-millimeter diameter.

4.1.2. A shot of the 70 percent confluent cells.

4.2. After transfecting the cells as demonstrated earlier with the modified plasmid quantities, gently wash the culture dish twice with 1 milliliter of ice-cold PBS **[1-TXT]**. Add 1 milliliter of 4 percent paraformaldehyde fixation solution at room temperature and incubate for 15 minutes **[2]**.

4.2.1. Talent washing the culture dish containing transfected cells with ice-cold PBS. **TXT: N plasmid: 1.3 µg; Luc-T20 plasmid: 2 µg; S plasmid: 0.0032 µg; M-IRES-E plasmid: 0.66 µg**

4.2.2. Talent pipetting paraformaldehyde into the dish.

4.3. Wash the cells twice for 5 minutes each with 1 milliliter of PBS at room temperature **[1]**, and permeabilize the cells by adding 1 milliliter of 0.25 percent Triton X-100 *(Ex-hundred)* for 10 minutes **[2]**.

4.3.1. Talent washing the cells with PBS.

4.3.2. Talent adding Triton X-100 to the dish.

4.4. Again, wash the cells twice for 5 minutes each with 1 milliliter of PBS at room temperature **[1]**, then add 1 milliliter of 5 percent bovine serum albumin for 1 hour to block nonspecific antibody interactions **[2]**.

4.4.1. Talent washing the cells with PBS.

4.4.2. Talent adding bovine serum albumin to the dish.

4.5. Add approximately 200 microliters of primary antibody solution to cover the glass bottom **[1]**, and incubate at 4 degrees Celsius overnight **[2]**.

4.5.1. Talent pipetting the primary antibody solution to cover the glass bottom.

4.5.2. Talent placing the dish in a refrigerator.

4.6. Then, remove the primary antibody solution **[1]**, and wash the cells three times for 5 minutes each with 1 milliliter of PBS at room temperature **[2]**.

4.6.1. Talent aspirating the primary antibody solution.

4.6.2. Talent washing the cells with PBS.

4.7. Now, add a fluorescence-conjugated secondary antibody solution and incubate at room temperature for 1 hour **[1]**.

4.7.1. Talent adding secondary antibody and covering the dish.

4.8. After washing the cells three times with PBS, stain the nuclei with 2.5 micrograms per milliliter of Hoechst solution at room temperature for 5 minutes **[1]**.

4.8.1. Talent adding Hoechst solution to the dish.

4.9. Finally, after washing the cells with PBS, observe the S protein or organelle staining before acquiring images using a confocal microscope **[1]**.

4.9.1. A shot of the observed S protein or organelle staining.

Results

5. Results

5.1. This figure illustrates the sensitivity of SC2-VLP production to varying transfection amounts of the plasmid encoding the spike protein **[1]**. SC2-VLP titer was highest when 0.016 micrograms of S plasmid were transfected **[2]**, and decreased significantly with 0.16 and 1.6 micrograms of S plasmid **[3]**.

5.1.1. LAB MEDIA: Figure 2.

5.1.2. LAB MEDIA: Figure 2. *Video Editor: Highlight the tallest blue bar in B.*

5.1.3. LAB MEDIA: Figure 2. *Video Editor: Highlight the two smaller blue bars in B.*

5.2. The H1271E *(H one thousand two hundred seventy one to E)* mutation in the spike protein significantly reduced SC2-VLP titer compared to wild type **[1]**.

5.2.1. LAB MEDIA: Figure 3A. *Video Editor: Highlight the blue bar labeled H1271E.*

5.3. The E1262H *(E one thousand two hundred sixty two to H)* mutation led to a moderate reduction in SC2-VLP titer **[1]**, while the E1262H/H1271E *(E one thousand two hundred sixty two to H H one thousand two hundred seventy one to E)* double mutation abolished production entirely **[2]**.

5.3.1. LAB MEDIA: Figure 3A. *Video Editor: Highlight the blue bars at E1262H.*

5.3.2. LAB MEDIA: Figure 3A. *Video Editor: Highlight the small blue bar at Double mut.*

5.4. Full-length S and S2 protein bands were decreased in the E1262H and double mutant lanes compared to wild type **[1]**.

5.4.1. LAB MEDIA: Figure 3B.

5.5. S packaging efficiency was also reduced in E1262H and H1271E mutants **[1]** and nearly abolished in the double mutant **[2]**.

5.5.1. LAB MEDIA: Figure 3C. *Video Editor: Highlight the red bars at E1262H and H1271E.*

5.5.2. LAB MEDIA: Figure 3C. *Video Editor: Highlight the small red bar at Double mut.*

5.6. VLP abundance remained largely unchanged across wild type and all S mutants **[1]**.

5.6.1. LAB MEDIA: Figure 3D.

5.7. Wild-type S protein colocalized with the cis-Golgi *(cis Golgi)* marker GM130 *(G-M-one hundred thirty)* **[1]**, but not with the ER *(E-R)* marker Sec61β *(seek sixty one beta)* **[2]** or ERGIC marker ERGIC-53 *(R-jik fifty-three)* **[3]**.

5.7.1. LAB MEDIA: Figure 4. *Video Editor: Highlight I.*

5.7.2. LAB MEDIA: Figure 4. *Video Editor: Highlight F.*

5.7.3. LAB MEDIA: Figure 4. *Video Editor: Highlight C.*

5.8. The H1271E mutant S protein displayed diffuse cytoplasmic distribution and lacked colocalization with GM130 **[1]**.

5.8.1. LAB MEDIA: Figure 4. *Video Editor: Highlight L.*

Pronunciation Guides:

1. Penicillin-Streptomycin
Pronunciation link:
- Penicillin: https://www.merriam-webster.com/dictionary/penicillin
- Streptomycin: https://www.merriam-webster.com/dictionary/streptomycin
  IPA:
- Penicillin: /ˌpɛnɪˈsɪlɪn/
- Streptomycin: /ˌstrɛptəˈmaɪsɪn/
  Phonetic Spelling:
- Penicillin: pen-ih-sil-in
- Streptomycin: strep-tuh-my-sin

2. Plasmid
Pronunciation link: https://www.merriam-webster.com/dictionary/plasmid
IPA: /ˈplæzmɪd/
Phonetic Spelling: plaz-mid

3. Transfection
Pronunciation link: https://www.merriam-webster.com/dictionary/transfection
IPA: /trænˈsfɛkʃən/
Phonetic Spelling: tran-sfek-shun

4. Supernatant
Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˈsuːpərˌneɪtənt/
Phonetic Spelling: soo-per-nay-tuhnt

5. SARS-CoV-2
Pronunciation link: https://www.merriam-webster.com/dictionary/SARS-CoV-2
IPA: /ˌsɑːrz koʊ ˈviː tuː/
Phonetic Spelling: sarz-koh-vee too

6. Lysis
Pronunciation link: https://www.merriam-webster.com/dictionary/lysis

IPA: /ˈlaɪsɪs/
Phonetic Spelling: lie-sis
7. Luciferase
Pronunciation link: https://www.merriam-webster.com/dictionary/luciferase
IPA: /ˈluːsɪfəˌreɪs/
Phonetic Spelling: loo-sih-fuh-race
8. Western Blotting
Pronunciation link: No confirmed link found
IPA: /ˈwɛstərn ˈblɒtɪŋ/
Phonetic Spelling: west-ern blot-ing
9. Paraformaldehyde
Pronunciation link: No confirmed link found
IPA: /ˌpærəˈfɔːrməlˌdeɪhaɪd/
Phonetic Spelling: par-uh-form-al-de-hide
10. Triton X-100
Pronunciation link: No confirmed link found
IPA: /ˈtraɪtɒn ɛks ˈhʌndrəd/
Phonetic Spelling: try-ton ex hundred
11. Bovine Serum Albumin
Pronunciation link: No confirmed link found
IPA: /ˈboʊvaɪn ˈsɪərəm ˈæl.bjuː.mɪn/
Phonetic Spelling: boh-vine seer-um al-byoo-min
12. Hoechst
Pronunciation link: No confirmed link found
IPA: /ˈhɔɪst/
Phonetic Spelling: hoyst
13. Confocal Microscope
Pronunciation link: No confirmed link found
IPA: /kɒnˈfoʊkəl ˈmaɪ.krəˌskoʊp/
Phonetic Spelling: kon-foh-kul my-kro-scope