FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69090
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21072498

Title: Standardized SDS-PAGE Workflow for Personalized Protein Corona Profiling in Early Cancer Detection

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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? **Yes, all done**

   If **Yes**, we will need you to record using screen recording software.

   We recommend using the screen capture program OBS. JoVE's tutorial for using OBS Studio is provided at this link: https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k

   As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible.

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

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 24
Number of Shots: 50

Introduction

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

AUTHORS : Please note that only 2 introductory and 3 conclusion statements may be presented. Some statements have been edited to meet the word limit of 20 words

INTRODUCTION:

1.1. **Erica Quagliarini:** We study how nanoparticles interact with blood to reveal early cancer signs, aiming for simple, reliable diagnostic tests.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

1.2. **Erica Quagliarini:** Recent advances focus on automated, standardized nanoparticle-protein corona profiling, improving cancer diagnostics through reproducibility, sensitivity, and clinical translation.

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

CONCLUSION:

1.3. **Erica Quagliarini:** We demonstrated that standardized nanoparticle-protein corona profiling enhances reproducibility and reveals disease-specific fingerprints, supporting early cancer detection strategies.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.4*

1.4. **Erica Quagliarini:** We address the lack of standardized, reproducible methods for nanoparticle-protein corona analysis, enabling reliable comparisons across laboratories and studies.

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

1.5. **Erica Quagliarini:** Our protocol is rapid, user-friendly, WHO-compliant, ensuring standardized, reproducible protein corona fingerprints with strong diagnostic relevance.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.6*

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

Protocol

2. Isolation and Characterization of the Protein Corona Formed on Graphene Oxide Nanosheets via SDS-PAGE

Demonstrator: Manuela Iacobini

2.1. To begin, dilute the 4 milligrams per milliliter stock solution of graphene oxide nanosheets in ultrapure water to the desired concentration **[1]**. Sonicate the diluted solution for 2 minutes at 28 percent amplitude with a pulse setting of 0.8 seconds on and 0.6 seconds off to ensure uniform dispersion **[2]**.

2.1.1. WIDE: Talent pipetting graphene oxide nanosheet stock solution into a tube containing ultrapure water.

2.1.2. Talent placing the tube in the sonicator and setting the amplitude and pulse settings before starting sonication.

2.2. Quantify the concentration of the dispersed solution using ultraviolet-visible spectroscopy **[1]**.

2.2.1. Talent placing a cuvette containing the sample into the ultraviolet-visible spectrophotometer and starting the measurement.

2.3. Then measure the hydrodynamic size and zeta potential of the solution using dynamic light scattering with a 633-nanometer helium-neon laser **[1]**. Dilute the sample in ultrapure water according to the cuvette detection limits **[2]**.

2.3.1. Talent operating the dynamic light scattering instrument and selecting the appropriate laser wavelength.

2.3.2. Talent adding ultrapure water to the sample and placing it into the instrument.

2.4. Next, dilute the commercial lyophilized human plasma with ultrapure water as required **[1]**. Centrifuge the reconstituted plasma at 18,620 *g* for 15 minutes at 4 degrees Celsius **[2]**. Collect the supernatant **[3]** and store it at minus 80 degrees Celsius until further use **[4]**.

2.4.1. Talent reconstituting lyophilized plasma by adding ultrapure water and gently mixing.

2.4.2. Talent placing the reconstituted plasma into the centrifuge and setting the temperature and speed.

2.4.3. Talent pipetting out the clear supernatant into a labelled tube.

2.4.4. Talent transferring the tubes for storage in the ultra-low freezer.

2.5. Now, quantify the protein concentration using the bicinchoninic acid assay to adjust plasma dilution and determine the optimal nanoparticle to plasma ratio **[1]**. Add 100 microliters of nanoparticle suspension to 100 microliters of plasma to achieve the target plasma concentrations **[2]**. Incubate the mixture at 37 degrees Celsius for 1 hour **[3]**.

2.5.1. Talent preparing BCA reagents and loading samples and standards into a microplate to begin the assay.

2.5.2. Talent pipetting equal volumes of nanoparticle suspension and plasma into a microcentrifuge tube.

2.5.3. Talent placing the tubes in a temperature-controlled incubator and setting the timer.

2.6. To isolate the protein corona, centrifuge the nanoparticle-plasma samples at 18,620 *g* at 4 degrees Celsius for 15 minutes **[1]**. Wash the resulting pellet with 200 microliters of ultrapure water **[2]** then resuspend it and centrifuge again **[3]**.

2.6.1. Talent placing the tubes in the centrifuge and starting the run with the specified settings.

2.6.2. Talent adding ultrapure water to the pellet.

2.6.3. Shot of the pellet being mixing gently, and the tube being placed in a centrifuge. **TXT: Perform wash 2 more times**

2.7. After the final wash, confirm the presence of a compact pellet as evidence of nanoparticle-protein complex isolation **[1]**.

2.7.1. Close-up of the compact pellet remaining in the bottom of the tube.

2.8. Next, mix the reducing agent, SDS sample buffer and water to reach the calculated loading buffer volume **[1-TXT]**. Resuspend the pellet in the prepared loading buffer **[2].** Then boil the suspension at 100 degrees Celsius for 10 minutes **[3]**.

2.8.1. Talent pipetting appropriate volumes of reducing agent, SDS buffer, and water into a labeled tube and mixing well. **TXT: Reducing agent (1:10), SDS buffer (1:2)**

2.8.2. Talent adding 1x loading buffer to the pellet.

2.8.3. Talent placing the tube in a heat block set to 100 degrees Celsius.

2.9. Centrifuge the boiled sample at 18,620 *g* at 4 degrees Celsius for 15 minutes **[1].** Collect the supernatant into a new tube **[2]**.

2.9.1. Talent placing the tube with the boiled sample in a centrifuge.

2.9.2. Talent pipetting out the clear supernatant into a new tube.

2.10. Now prepare the protein ladder by diluting the molecular weight standard with the

loading buffer **[1]**. Dilute 10x Tris-Glycine-SDS running buffer to 1x concentration **[2]**.

2.10.1. Talent pipetting loading buffer into the molecular weight standard tube and mixing gently.

2.10.2. Talent measuring and mixing the running buffer with water to achieve a 1x dilution.

2.11. Assemble the electrophoresis chamber using a 4 to 20 percent stain-free gradient gel **[1].** Then pour in the running buffer until it reaches the recommended level indicated by the manufacturer **[2]**.

2.11.1. Talent inserting the gradient gel into the electrophoresis chamber. **NOTE**: 2.11.1 AND 2.11.1 were shot together

2.11.2. Talent pouring in running buffer until the correct level is reached.

2.12. Load 10 microliters of sample into each well **[1].** Then pipette 7 microliters of ladder into each well **[2].** Run the gel at 150 volts for approximately 90 minutes at room temperature **[3]**.

2.12.1. Talent pipetting 10 µl of the sample into each well.

2.12.2. Talent pipetting 7 µL of the ladder into the gel wells.

2.12.3. Talent initiating the electrophoresis run.

3. Automated Quantitative Analysis of Gel Electrophoresis Profiles Using MATLAB-Based Scripting

Demonstrator: Manuela Iacobini

3.1. Store the gel image as a .tif *(dot-tiff)* file in the same folder as the scripts, then open "gel_processing.m" *(gel-processing-dot-m)* in MATLAB *(Mat-lab)* **[1]**. Enter the name of the gel image as "image_name" *(image-name)* **[2]**. Then enter the index of one ladder lane as "index_marker" *(index-marker)* **[3]**.

3.1.1. FILE: 69090_screenshot_1.mp4 00:00–00:14

3.1.2. FILE: 69090_screenshot_1.mp4 00:15–00:17

3.1.3. FILE: 69090_screenshot_1.mp4 00:18–00:34

3.2. Run the script. The completion of the steps will be indicated in the Command Window , and four windows will pop up on the screen **[1]**.

3.2.1. FILE: 69090_screenshot_1.mp4 00:36–00:55

3.3. Now open **Figure 1** with the original gel image on top and its correction for the oblique-lane effect at the bottom **[2]**.

3.3.1. FILE: 69090_screenshot_2.mp4 00:00–00:05

3.4. Modify the values of **left_corr** *(left-correction)* and right_corr *(right-correction)* to tune the correction parameters **[1]**.

3.4.1. FILE: 69090_screenshot_2.mp4 00:06–00:20

3.5. Open **Figure 2**. It contains the image corrected for the oblique lane effect on top and the background-removed image at the bottom **[1]**. Adjust the value of bkg_par_x *(B-K-G-par-x)* in the script to tune the background removal **[2]**. Observe that all identified lanes are indicated as vertical white lines in "**Figure 2**" **[3]**.

3.5.1. FILE: 69090_screenshot_3.mp4 00:00–00:10

3.5.2. FILE: 69090_screenshot_3.mp4 00:10–00:17

3.5.3. FILE: 69090_screenshot_3.mp4 00:18–00:25

3.6. Now, open "**Figure 3**" containing the intensity profile of the marker lane, with the detected peaks on the left and a nonlinear fit with R^2 *(R-Square)* value on the right **[1]**. Double-check that the number of expected bands in the ladder lane matches the number of detected peaks used for the fitting procedure **[2]**.

3.6.1. FILE: 69090_screenshot_4.mp4 00:00–00:13

3.6.2. FILE: 69090_screenshot_4.mp4 00:14–00:22

3.7. Then open "**Figure 4**" containing the normalized intensity profiles for all detected lanes in the gel **[1]**. Set the export parameter to 1 to export the intensity profiles as functions of molecular weights in an Excel file **[2]**.

3.7.1. FILE: 69090_screenshot_4.mp4 00:23–00:29

3.7.2. FILE: 69090_screenshot_5.mp4 00:00–00:07

3.8. Run the script. After completion, an excel sheet will be generated in the working folder, containing the absolute and normalized profiles as functions of molecular weight in the first column **[1]**.

3.8.1. FILE: 69090_screenshot_5.mp4 00:08–00:35

3.9. Then open "gel_profiles_pro.m" *(gel-profiles-pro-dot-m)* file and run the script **[1]**. Four windows will pop up on the screen **[2]**.

3.9.1. FILE: 69090_screenshot_5.mp4 00:36–00:58

3.9.2. FILE: 69090_screenshot_5.mp4 00:46–00:58

3.10. Open **Figure 5** containing the absolute intensity profiles at the top and the corresponding total intensities at the bottom for each lane **[1]** and **Figure 6** containing the normalized intensity profiles for each lane, with the molecular weight axis subdivided according to the ranges specified as "**reg**" *(reg)* in the script **[2].**

3.10.1. FILE: 69090_screenshot_6.mp4 00:00–00:12

3.10.2. FILE: 69090_screenshot_6.mp4 00:13–00:29

3.11. Set the export data to 1 to export the absolute and normalized integral areas **[1]**. Run the script . After completion, a spreadsheet will be generated in the working folder, containing the exported absolute and normalized areas within the specified molecular weight ranges **[2]**.

3.11.1. FILE: 69090_screenshot_6.mp4 00:30–00:32

3.11.2. FILE: 69090_screenshot_6.mp4 00:33–00:59

3.12. Lastly, open figure 7 and 8. They contain the histogram of absolute and normalized areas for each lane, grouped by lane and by area respectively **[1]**.

3.12.1. FILE: 69090_screenshot_6.mp4 01:00–01:25

Results

4. Results

4.1. The SDS-PAGE profiles obtained across all conditions were highly reproducible **[1]**. Increased plasma concentrations from 5% to 50% resulted in stronger signal intensities in the 30 to 80 kilodalton region of the SDS-PAGE profiles **[2]**. The automated pipetting workflow yielded nearly identical electrophoretic profiles **[3]**.

4.1.1. LAB MEDIA: Figure 3.

4.1.2. LAB MEDIA: Figure 3. *Video editor: Sequentially highlight the rows (5% to 50%)*

4.1.3. LAB MEDIA: Figure 3. *Video editor: Highlight the side-by-side traces labeled "mO" and "aO" for all panels*

4.2. Graphene oxide consistently produced well-resolved, stable corona profiles, attributed to its high surface area and charge properties **[1]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the leftmost column of electrophoretic profiles corresponding to graphene oxide (GO).*

4.3. Size distributions of small, medium, and large graphene oxide samples were narrow, non-overlapping, and centered around 100, 300, and 750 nanometers, respectively **[1]**.

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Emphasize the three separate colored peaks labeled sGO, mGO, and lGO*

4.4. SDS-PAGE profiles of protein coronas formed on graphene oxide were similar across sizes, while distinct differences were observed between different dilutions **[1]**. Lower plasma concentrations produced more intense bands in specific molecular weight ranges, indicating selective protein enrichment **[2]**.

4.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the 3 curves in one row (A, B, or C) then highlight each row sequentially*

4.4.2. LAB MEDIA: Figure 4B. *Video editor: Highlight c*

4.5. At intermediate plasma dilutions, the densitometric profiles revealed disease-specific differences, especially in the 20–30 kilodalton region, where PDAC samples showed reduced signal intensity **[1]**.

4.5.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the 20–30 kilodalton region across rows C to J*

4.6. The combined diagnostic model incorporating SDS-PAGE corona profiles and CA 19-9 levels achieved the highest accuracy, sensitivity, and specificity in classifying PDAC patients **[1]**. The SDS-PAGE-based profiling model alone correctly identified both PDAC and control samples with 83.0% accuracy and balanced sensitivity and specificity **[2]**.

4.6.1. LAB MEDIA: Figure 5 Panels I–L. *Video editor: Emphasize the statistics in panel L, then highlight the class separation plot in panel K and confusion matrix in panel I.*

4.6.2. LAB MEDIA: Figure 5 Panels A–D. *Video editor: Highlight the confusion matrix in panel A and the metrics summary in panel D.*

- **graphene oxide**

Pronunciation link: No confirmed link found
IPA: /ˌgræfˈiːn ˈɑːksaɪd/
Phonetic Spelling: graf-EEN OX-ide

- **nanosheets**

Pronunciation link: https://www.merriam-webster.com/dictionary/nanosheet
IPA: /ˈnænoʊˌʃiːts/
Phonetic Spelling: NAN-oh-sheets

- **ultrapure**

Pronunciation link: https://www.merriam-webster.com/dictionary/ultrapure
IPA: /ˌʌltrəˈpjʊr/
Phonetic Spelling: UL-truh-pure

- **sonicate**

Pronunciation link: https://www.merriam-webster.com/dictionary/sonicate
IPA: /ˈsɒnɪˌkeɪt/ (American: /ˈsɑːnɪˌkeɪt/)
Phonetic Spelling: SON-ih-kate

- **hydrodynamic**

Pronunciation link: https://www.merriam-webster.com/dictionary/hydrodynamic
IPA: /ˌhaɪdroʊdaɪˈnæmɪk/
Phonetic Spelling: HY-droh-dy-NAM-ik

- **zeta potential**

Pronunciation link: https://www.merriam-webster.com/dictionary/zeta
IPA: /ˈzeɪtə pəˈtɛnʃəl/
Phonetic Spelling: ZAY-tuh puh-TEN-shuhl

- **lyophilized**

Pronunciation link: https://www.merriam-webster.com/dictionary/lyophilization

IPA: /ˌlaɪəˈfaɪlaɪzd/
Phonetic Spelling: LYE-oh-fye-lyzd

- **bicinchoninic acid**

Pronunciation link: https://www.howtopronounce.com/bicinchoninic-acid
IPA: /ˌbaɪsɪnʧəˈnɪnɪk ˈæsɪd/
Phonetic Spelling: bye-sin-chuh-NIN-ik AS-id

- **electrophoresis**

Pronunciation link: https://www.merriam-webster.com/dictionary/electrophoresis
IPA: /ɪˌlɛktroʊfəˈriːsɪs/
Phonetic Spelling: ih-LEK-troh-foh-REE-sis

- **microcentrifuge**

Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge
IPA: /ˌmaɪkroʊˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: MY-kroh-sen-truh-FYOODJ