Submission ID #: 62559
Scriptwriter Name: Gaurav Vaidya
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=19080543

Title: Bead Loading Proteins and Nucleic Acids into Adherent Human Cells

Authors and Affiliations:

Charlotte Ayn Cialek[1], Gabriel Galindo[1], Amanda Lynn Koch[1], Matthew Neeley Saxton[1], Timothy John Stasevich[1,2]

[1]Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA
[2]World Research Hub Initiative, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8503, Japan

Corresponding Authors:
Timothy John Stasevich (tim.stasevich@colostate.edu)

Email Addresses for All Authors:

ccialek@colostate.edu; ccialek@gmail.com
Gabriel.Galindo@colostate.edu
amanda.koch@colostate.edu
Matthew.Saxton@colostate.edu
tim.stasevich@colostate.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? **no**

Current Protocol Length

Number of Steps: 14
Number of Shots: 36

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Charlotte Ayn Cialek:** Bead loading is an inexpensive assay for loading membrane-impermeable particles into live, adherent cells. This protocol has proven extremely useful for loading probes for single-cell or single-molecule fluorescence microscopy experiments.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.2.2.*

1.2. **Matthew Neeley Saxton:** Bead loading allows researchers to quickly load all sorts of molecules, including proteins, DNA, RNA, synthetic particles, or a combination of these, simultaneously into single cells.

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

OPTIONAL:

1.3. **Matthew Neeley Saxton:** When first performing bead loading, it is important to test out the tapping force. Cell lines respond differently to bead loading, and so the number and force of the taps can be modified for optimal cell loading while minimizing cell peeling.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.3.2.- 4.3.3.*

Introduction of Demonstrator on Camera

1.4. **Charlotte Ayn Cialek:** Demonstrating the procedure will be my colleagues Dr. Amanda Koch and Gabriel Galindo.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Clean, Sterilize, and Dry Glass Beads

2.1. Begin by measuring approximately 5 milliliters of glass beads in a 50-milliliter conical tube **[1]**. Add 25 milliliters of 2 molar sodium hydroxide to the tube **[2]** and mix gently for 2 hours using a shaker or a rotor **[3]**.

2.1.1. WIDE: Talent measuring the glass beads.

2.1.2. Talent adding sodium hydroxide to the glass beads.

2.1.3. Talent placing the conical tube on the shaker/rotor.

2.2. Spin down the tube of beads briefly in a centrifuge if the beads are in suspension **[1-TXT]**, then decant the sodium hydroxide while retaining as many beads as possible **[2]**.

2.2.1. Talent centrifuging the tube. **TEXT: 1 min at ~1000 × *g*, room temperature**

2.2.2. Talent decanting the sodium hydroxide.

2.3. Wash the beads thoroughly with cell culture-grade water until the pH is neutral **[1]** and decant the water each time **[2]**. Use a pH test strip on the eluent to confirm a neutral pH **[3]**.

2.3.1. Talent washing the beads with water.

2.3.2. Talent decanting the water.

2.3.3. Talent testing the pH of the eluent using a pH test strip.

2.4. Then, wash the beads thoroughly with 100 percent ethanol 2 to 3 times **[1]**, decanting the ethanol each time **[2]**.

2.4.1. Talent washing the beads with ethanol, with the labeled ethanol container in the shot.

2.4.2. Talent decanting the ethanol.

2.5. Dry the beads and sprinkle them to form a thin layer inside a sterile container **[1]**, then leave the container open inside a biosafety cabinet to air dry the beads overnight **[2]**. Gently tap or shake the container **[3]** and check that the beads have a sandy texture without clumping or flaking to ensure that the beads are completely dry **[4]**.

2.5.1. Talent sprinkling the beads inside the sterile container.

2.5.2. Talent placing the container in the biosafety cabinet and then opening it.

2.5.3. Talent shaking/tapping the container.

2.5.4. A shot of the dry beads.

3. Assemble the Bead Loader Apparatus

~~3.1. Cover the entire opening of the bead holding chamber using a polypropylene mesh or equivalent material with 105-micrometer openings to allow the beads to pass through **[1]**. Clamp the mesh between the male and female ends of a metal reusable imaging chamber **[2]**. *Videographer: This step is important!*~~

~~3.1.1. Talent covering the holding chamber with the mesh.~~

~~3.1.2. Talent fastening the mesh with the clamp.~~

NOTE: The VO and shots for step 3.1 are modified as below

3.1 Add the beads to the apparatus **[1A]** and cover the entire opening of the bead holding chamber using a polypropylene mesh or equivalent material with 105-micrometer openings to allow the beads to pass through **[1B]**. Clamp the mesh between the male and female ends of a metal reusable imaging chamber **[2]**. *Videographer: This step is important!*

3.1.1A. Added shot: Talent adding the beads to the apparatus.

3.1.1B. Talent covering the holding chamber with the mesh.

3.1.2. Talent fastening the mesh with the clamp.

~~3.2. Then, UV-sterilize the apparatus for 15 minutes **[1]**. Add the beads to the apparatus **[2]** and seal it tightly with the waxy film **[3]**.~~

~~3.2.1. Talent UV-sterilizing the apparatus.~~

~~3.2.2. Talent adding the beads to the apparatus.~~

~~3.2.3. Talent sealing the apparatus with a waxy film.~~

NOTE: The VO and shots for step 3.2 are modified as below

3.2. Then, seal it tightly with the waxy film **[1]** and UV-sterilize the apparatus for 15 minutes **[2]**.

3.2.1. Talent sealing the apparatus with a waxy film.

3.2.2. Talent UV-sterilizing the apparatus.

3.3. The bead loader apparatus can now be used to perform hundreds of loading assays. Store the apparatus in a dry container desiccated by silica gel or other desiccant medium **[1]** and seal it **[2]**. If the beads become damp, thoroughly dry, and sterilize the bead loader and replace it with fresh beads as demonstrated earlier **[3]**. *Videographer: This step is important!*

3.3.1. Talent storing the apparatus in the dry container.
3.3.2. Talent sealing the dry container.
3.3.3. A shot of the bead loader with dry beads.

4. Bead Loading Cells and Imaging the Bead-Loaded Cells

4.1. Remove the medium from the cells **[1]** and gently aspirate all medium from around the edges of the chamber **[2]**. Then, tilt the chamber at approximately a 45-degree angle and remove the remaining drop of media in the center microwell **[3-TXT]**.

4.1.1. Talent removing the medium from cells.
4.1.2. Talent aspirating the medium from the edges of the chamber.
4.1.3. Talent removing the media in the center microwell. **TEXT: Do not touch the aspirator tip to the microwell**

4.2. Pipette the bead loading solution gently onto the glass microwell present in the center of the chamber **[1]**. Use the bead loading apparatus to gently disperse a monolayer of glass beads on top of the cells and ensure that the beads cover the cells completely **[2]**. *Videographer: This step is important!*

4.2.1. Talent adding the bead loading solution to the glass microwell.
4.2.2. Talent dispersing a monolayer of beads on the cells.

4.3. Pinch the chamber with two fingers **[1]**. Lift it around 2 inches **[2]** and bring it down firmly using a force approximately equivalent to dropping the dish from that height. **[3-TXT].** *Videographer: This step is important!*

4.3.1. Talent pinching the chamber.
4.3.2. Talent lifting the chamber.
4.3.3. Talent bringing the chamber down firmly. **TEXT: Tap the chamber 10 times**

4.4. Gently add the medium back into the chamber by pipetting slowly onto the plastic side of the chamber **[1]**. Aspirate any floating beads without disturbing the cells **[2].** At this point, add more pre-warmed media if too much was removed **[3].** Then, incubate the cells for 0.5 to 2 hours in the incubator **[4]**. *Videographer: This step is important!*

4.4.1. Talent adding the medium back into the chamber.
4.4.2. Talent aspirating the floating beads.
4.4.3. Talent adding extra pre-warmed media to the chamber.
4.4.4. Talent incubating the cells in the incubator.

4.5. Before imaging, wash the cells three times with the medium to remove beads and excess loading components in the loading solution **[1-TXT]**.

4.5.1. Talent washing the cells three times. **TEXT: Do not pipette directly onto the cells**

4.6. Image the cells immediately or when required by the experiment using instructions in the text manuscript **[1]**.

4.6.1. Talent acquiring the images.

Results

5. Results: Proteins and Nucleic Acids Bead-loaded onto Adherent Human Cells

5.1. Cells that were successfully bead-loaded almost always had both Cy3- **[1]** and Alexa488-labeled proteins together **[2]**.

5.1.1. LAB MEDIA: Figure 2A. *Video Editor: Emphasize the second image from right.*
5.1.2. LAB MEDIA: Figure 2A. *Video Editor: Emphasize the second image from the left.*

5.2. One microgram of plasmid DNA encoding GFP **[1]** and 0.5 micrograms of Cy3-labeled protein was also introduced into the cells via bead loading, expressed, and visualized **[2]**. 40 percent of the cells were bead-loaded with Fab protein **[3]** and 21 percent of the bead-loaded cells expressed the co-loaded plasmid **[4]**.

5.2.1. LAB MEDIA: Figure 2B. *Video Editor: Emphasize the second image from the right.*
5.2.2. LAB MEDIA: Figure 2B. *Video Editor: Emphasize the second image from the left.*
5.2.3. LAB MEDIA: Figure 2B. *Video Editor: Emphasize the yellow text in the second image from the left.*
5.2.4. LAB MEDIA: Figure 2B. *Video Editor: Emphasize the yellow text in the second image from the right.*

5.3. The bead-loaded cells expressed varying levels of plasmids cell **[1]**. The results of the Fisher ratio test showed that although proteins 1 and 2 had similar intensity distributions **[2]**, each protein had a significantly smaller distribution than the plasmid **[3]**.

5.3.1. LAB MEDIA: Figure 2C.
5.3.2. LAB MEDIA: Figure 2D. *Video Editor: Emphasize the Protein 1 and 2 boxplots.*
5.3.3. LAB MEDIA: Figure 2D. *Video Editor: Emphasize the EGFP plasmid boxplot.*

5.4. The levels of bead-loaded proteins had little cell-to-cell variance **[1]**, and the levels of two simultaneously loaded proteins were highly correlated with each other **[2]**.

5.4.1. LAB MEDIA: Figure 2D. *Video Editor: Emphasize the Protein 1 and 2 boxplots.*
5.4.2. LAB MEDIA: Figure 2E. *Video Editor: Emphasize the first graph from the left.*

5.5. Proper bead loading had almost no noticeable effect on the number of human U2OS cells or their morphology when imaged directly before **[1]**, directly after **[2]**, and 24 hours after bead loading **[3]**.

5.5.1. LAB MEDIA: Figure 3A. *Video Editor: Emphasize the image labeled 'Before'.*
5.5.2. LAB MEDIA: Figure 3A. *Video Editor: Emphasize the image labeled 'After'.*
5.5.3. LAB MEDIA: Figure 3A. *Video Editor: Emphasize the images labeled '24 hours later'.*

5.6. Poor bead loading with excessive beads and tapping force caused cell loss, poor cell morphology, and clusters of beads remaining on the cover glass **[1]**.

5.6.1. LAB MEDIA: Figure 3B.

5.7. Even though cells are thought to undergo mechanical damage during bead loading, cells grew and proliferated in the properly bead-loaded chamber **[1]**. It was also observed that bead-loaded cells undergo cell division **[2]**.

5.7.1. LAB MEDIA: Figure 3A. *Video Editor: Emphasize the labeled 'Before' and '24 hours later'.*
5.7.2. LAB MEDIA: Figure 3C.

5.8. RPE1 and HeLa cell lines were bead-loaded with Fab to demonstrate the versatility of the bead-loading technique **[1].** U2OS cells were also bead-loaded with a Cy5-RNA 9-mer and Cy3-DNA 28-mer together **[2]**.

5.8.1. LAB MEDIA: Figure 4A and 4B.
5.8.2. LAB MEDIA: Figure 4C.

Conclusion

6. Conclusion Interview Statements

6.1. **Charlotte Ayn Cialek:** When attempting this protocol, ensure to add only a monolayer of beads to the cells, since too many beads cause cells to detach.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.2.2.*

6.2. **Matthew Neeley Saxton:** After a brief, 30-minute recovery post-bead loading, cells are ready for imaging immediately hours to days later. If the procedure requires further steps, such as staining, that can be done after the recovery period as well.

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