Submission ID #: 61449
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18741993

Title: Detection of Cell-Free DNA in Blood Plasma Samples of Cancer Patients

Authors and Affiliations: Vikrant Palande[1], Dorith Raviv Shay[1], and Milana Frenkel-Morgenstern[1,2,3]

[1]Cancer Genomics and BioComputing of Complex Diseases laboratory, The Azrieli Faculty of Medicine, Bar-Ilan University
[2]The Data Science Institute, Bar-Ilan University
[3]The Dangoor Centre For Personalized Medicine, Bar-Ilan University

Corresponding Author:
Milana Frenkel-Morgenstern
milana.morgenstern@biu.ac.il

Co-Authors:
vikrant.palande@biu.ac.il
dorith.raviv-shay@biu.ac.il

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

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 the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, 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 (greater than walking distance)? **N**

Protocol Length
Number of Shots: **51**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Milana Frenkel-Morgenstern**: Liquid biopsy using cell-free DNA may identify tumor mutations and fusions to potentially diagnose tumor type and its drug response **[1]**.

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

REQUIRED:

1.2. **Vikrant Palande**: In contrast to tissue biopsy, cell-free DNA-based liquid biopsy offers a non-invasive approach that can be performed multiple times to monitor cancer disease progression **[1]**.

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

Protocol

2. Circulating Cell-Free DNA (cfDNA) Purification

2.1. To isolate circulating cell-free DNA from a patient plasma sample, add 100 microliters of Proteinase K and 800 microliters of lysis buffer supplemented with 1 microgram of carrier RNA to 1 milliliter of patient plasma **[1-TXT]**.

2.1.1. WIDE: Talent adding Proteinase K and/or plasma and/or buffer to tube, with Proteinase K, plasma, and lysis buffer containers visible in frame **TEXT: See text for all buffer and solution preparation details**

2.2. Thoroughly pulse-vortex the solution for 30 seconds **[1]** before incubating for 30 minutes at 60 degrees Celsius **[2]**.

2.2.1. Solution being vortexed

2.2.2. Talent placing tubes at 60 °C

2.3. At the end of the incubation, add 1.8 milliliters of binding buffer to the tube **[1]** and thoroughly mix with 15-30 seconds of pulse vortexing **[2]**.

2.3.1. Talent adding buffer to tube, with buffer container visible in frame

2.3.2. Solution being vortexed

2.4. After a 5-minute incubation on ice, insert a silica membrane column into a vacuum apparatus connected to a vacuum pump **[1]** and firmly insert a 20-milliliter tube extender into the open column to prevent sample leakage **[2]**.

2.4.1. Talent inserting column into apparatus *Videographer: Important step*

2.4.2. Extender being inserted into column *Videographer: Important step*

2.5. At the end of the incubation, carefully pour the mixture into the tube extender **[1]** and switch on the vacuum pump **[2]**.

2.5.1. Talent pouring mixture into extender *Videographer: Important step*

2.5.2. Talent turning on pump *Videographer: Important step*

2.6. When all of the lysate has completely run through the columns, switch off the vacuum pump **[1]**, release the pressure to 0 millibars **[2]**, and carefully discard the tube extender without contaminating the adjacent columns **[3]**.

2.6.1. Talent switching off pump

~~2.6.3. Talent removing/discarding extender~~

2.7. Transfer the column into a collection tube for centrifugation to remove any residual lysate **[1-TXT]**.

2.7.1. Talent placing column into collection tube **TEXT: 30 s, 11,000 x g**

2.8. After discarding the flow through, add 600 microliters of wash buffer 1 to the column for a second centrifugation **[1-TXT]**.

2.8.1. Talent adding wash buffer to column, with buffer container visible in frame **TEXT: 1 min, 11,000 x g**

2.9. After discarding the flow through, centrifuge the column again with 750 microliters of wash buffer 2 **[1]**.

2.9.1. Talent placing tube into centrifuge

2.10. After discarding the flow through, add 750 microliters of 96-100% ethanol to the column for an additional centrifugation **[1]**.

2.10.1. Talent adding ethanol to tube, with ethanol container visible in frame

2.11. Transfer the column into a new 2-milliliter collection tube for another centrifugation **[1-TXT]** before placing the membrane column assembly into a new 2-milliliter collection tube at 56 degrees Celsius for 10 minutes **[2]**.

2.11.1. Column being placed into new tube **TEXT: 3 min, 20,000 x g**

2.11.2. Talent placing tube at 56 °C

2.12. At the end of the incubation, transfer the column into a new 1.5-milliliter elution tube ~~**[1]**~~ and add 50 microliters of elution buffer to the column for a 3-minute incubation at room temperature **[2]**.

~~2.12.1.~~

2.12.2. Talent adding buffer to column, with buffer container visible in frame

2.13. Then centrifuge the recovered solution for 1 minute at 20,000 x g to elute the nucleic acids **[1-TXT]**

2.13.1. Talent placing tube into centrifuge **TEXT: Optional: Store sample at -20 °C for later analysis**

3. DNA Fragment Size Analysis

3.1. To analyze the size of the DNA fragments, first secure the base plate to the chip priming station ~~**[1]**~~ and adjust the clip at the lowest position **[2]**.

3.1.1. WIDE: Talent attaching plate to station NOTE: This shot to be used after 3.1.2, instead of 3.2.1 that was not taken.
3.1.2. Talent adjusting clip position

3.2. Place a new high sensitivity DNA chip onto the chip priming station **[1]** and add 9 microliters of gel-dye mix to the bottom of the G chip well **[2]**.

3.2.1. ~~Talent placing chip onto priming station~~ *Videographer: Important step* NOTE: Use shot 3.1.1 for this step
3.2.2. Dye being added to chip well *Videographer: Important step*

3.3. Position the plunger at 1 milliliter **[1]** and close the chip priming station **[2]**.

3.3.1. Talent positioning plunger *Videographer: Difficult step*
3.3.2. Talent closing station *Videographer: Difficult step*

3.4. Close the lock of the latch until it clicks **[1]**, set the timer to 60 seconds **[2]**, and press down the plunger until it is held by the clip **[3]**.

~~3.4.1.~~
3.4.2. Talent setting timer *Videographer: Difficult step* NOTE: This step was combined into step 3.4.3
3.4.3. Talent pressing plunger and setting timer *Videographer: Difficult step*

3.5. After exactly 60 seconds, use the clip-release mechanism to release the plunger **[1]**.

3.5.1. Plunger being released

3.6. When the plunger retreats to at least the 300-microliter mark **[1]**, wait for 5 seconds before slowly retracting the plunger to the 1-milliliter position **[2]**.

~~3.6.1.~~
3.6.2. Shot of retreated plunger, then plunger being pulled to 1 mL

3.7. Open the chip priming station to remove 9 microliters of the gel-dye mix **[1]** and transfer the gel to the bottom of the high sensitivity DNA chip G well **[2]**.

~~3.7.1.~~
3.7.2. Dye being added to chip well

3.8. To load the DNA marker, dispense 5 microliters of DNA marker into the ladder well and to the 11 sample wells **[1]**.

3.8.1. Talent adding DNA marker to ladder well, with DNA marker container visible in frame
~~3.8.2.~~

3.9. To load the ladder and samples, add 1 microliter of the DNA ladder to the DNA ladder well **[1]**, 1 microliter of sample to the used sample wells, and 1 microliter of marker to the unused sample wells **[2]**.

3.9.1. DNA ladder being added to ladder well, with ladder container visible in frame
3.9.2. Sample being added to sample well(s), with sample and marker containers visible in frame

3.10. Place the high sensitivity DNA chip horizontally in the adapter **[1]** for 60 seconds of vortexing at 2400 revolutions per minute, taking care that the bulge that fixes the high sensitivity DNA chip is not damaged **[2]**.

3.10.1. Talent placing chip into adapter *Videographer: Important step*
3.10.2. Chip being vortexed *Videographer: Important step*

3.11. After vortexing, confirm that the electrode cartridge is properly inserted ~~**[1]**~~ and that the chip selector is positioned to “double stranded high sensitivity DNA” **[2]**.

~~3.11.1.~~

3.11.2. Shot of chip selector in dsHS DNA position

3.12. Carefully mount the high sensitivity DNA chip into the receptacle **[1-TXT]** and confirm that the electrode cartridge is fit exactly into the wells of the chip before closing the lid **[2]**.

3.12.1. Chip being mounted **TEXT: Chip fits only one way** *Videographer: Important step*
3.12.2. Shot of cartridge fitting in chip wells *Videographer: Important step*

3.13. The fragment analyzer software screen will display a chip icon to indicate that the chip has been inserted and the lid has been closed **[1]**.

3.13.1. Shot of screen NOTE: 3.13.1 – 3.15.2 not filmed. Authors provided screen capture videos for 3.13.1 – 3.15.2: 3.13.1 to 3.15.2.MP4 *Video Editor: please emphasize chip icon*

3.14. To initiate the analysis, open the **Assay** menu and select the double stranded **DNA High Sensitivity Assay [1]**.

3.14.1. Menu being opened, then assay being selected

3.15. Enter the sample names into the Table **[1]** and click **Start** to initiate the chip run **[2]**.

3.15.1. Sample name(s) being entered
3.15.2. Start being clicked

3.16. At the end of the run, immediately remove and discard the chip according to good laboratory practice **[1-TXT]**.

3.16.1. Talent discarding chip into biohazardous waste container **TEXT: See text for instrument cleaning details**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.4., 2.5., 3.2., 3.10., 3.12.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.3., 3.4.

Results

4. Results: Representative Glioma Patient cfDNA Bioanalyses

4.1. Here the expected cell free DNA bioanalyzer graph from a representative glioma patient **[1]**, in which cell free DNA fragments are enriched at 166 base pairs **[2]** and there is no genomic DNA contamination in the sample, can be observed **[3]**.

4.1.1. LAB MEDIA: Figure 1
4.1.2. LAB MEDIA: Figure 1 *Video Editor: please emphasize 166 bp peak*
4.1.3. LAB MEDIA: Figure 1

4.2. Here the same fragment enrichment graph can be observed after next generation sequencing library preparation of the cell free DNA **[1]**. Note the fragment enrichment shift from 166 to 291 base pairs due to the attachment of 125 base pair indexes and adapters **[2]**.

4.2.1. LAB MEDIA: Figure 2
4.2.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize 291 bp peak*

4.3. For this second representative patient, a very slight enrichment of the cell free DNA fragments can be observed **[1]**.

4.3.1. LAB MEDIA: Figure 3

4.4. Despite the low cell free DNA concentration, the addition of next generation sequencing adapters to the cell free DNA and PCR amplification **[1]** leads to a visible cell free DNA library peak on the fragment enrichment graph **[2]**, indicating that the library was prepared successfully from this low concentration cell free DNA sample **[3]**.

4.4.1. LAB MEDIA: Figure 4
4.4.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize 291-295 bp peak*
4.4.3. LAB MEDIA: Figure 4

4.5. For this third representative patient **[1]**, the cell free DNA fragments peaked near 166 base pairs **[2]** but genomic DNA contamination was also apparent near the 10,380 base pair reference ladder peak **[3]**.

4.5.1. LAB MEDIA: Figure 5
4.5.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize 166 bp peak*
4.5.3. LAB MEDIA: Figure 5 *Video Editor: please emphasize 10380 peak*

Conclusion

5. Conclusion Interview Statements

5.1. **Dorith Raviv Shay**: Plasma sample lysis is a crucial step in this protocol, as incomplete lysis will affect the cfDNA yield and the entire downstream process **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.1., 2.2.)