

Submission ID #: 70016

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21354928>

Title: Profiling Epidermal Growth Factor Receptor DNA Mutations in Circulating Rare Cells Using a Cell Isolation System and Droplet Digital PCR

Authors and Affiliations:

Yoon-Tae Kang, Srikanth Perike, Adam Corner, Elizabeth Dreskin, Nathan Knapp

Bio-Rad Laboratories

Corresponding Authors:

Yoon-Tae Kang	Yoontae_kang@bio-rad.com
Nathan Knapp	Nathan_knapp@bio-rad.com

Email Addresses for All Authors:

Srikanth Perike	Srikanth_perike@bio-rad.com
Adam Corner	Adam_corner@bio-rad.com
Elizabeth Dreskin	liz_jordan@bio-rad.com
Yoon-Tae Kang	Yoontae_kang@bio-rad.com
Nathan Knapp	Nathan_knapp@bio-rad.com

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!**
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. **Yes**

Current Protocol Length

Number of Steps: 24

Number of Shots: 57 (13 SC)

Introduction

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

Videographer's Note: Interview lav audio was wired directly to the camera.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Nathan Knapp:** We isolate circulating tumor cells from blood and perform sensitive genetic mutation analysis using microfluidics and droplet digital PCR.

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

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Nathan Knapp:** This protocol supports liquid biopsy research, oncology studies, and monitoring tumor mutations from blood samples.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Yoontae Kang:** This protocol enables sensitive detection and quantification of genetic mutations in circulating tumor cells, with EGFR as one example.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Yoontae Kang:** Maintaining consistent recovery efficiency and preserving DNA quality are critical for accurate downstream mutation analysis.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Nathan Knapp:** Future work can expand mutation panels, enable longitudinal monitoring, and integrate circulating tumor cell and circulating tumor DNA analyses.

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

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.8. **Yoontae Kang, Staff Application Scientist, Bio-Rad Laboratories** : (authors will present their testimonial statements live)

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

What value do you expect this video article to have for other researchers working in your field?

- 1.9. **Yoontae Kang, Staff Application Scientist, Bio-Rad Laboratories** : (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at Discovery Life Sciences

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

Videographer's Note: For combined shots, the individual shots were verbally called out throughout the take.

2. Circulating Tumor Cells Isolation from Human Blood Sample

Demonstrator: Nathan Knapp

***Videographer:** Please capture the instrument's touch screen interface for shots designated as "SCREEN."*

Authors: Based on your response that all the "SCREEN" shots in this section will be performed on the instrument's interactive touchscreen, we have removed the yellow highlighting for the word "SCREEN". These shots will now be filmed by the videographer.

- 2.1. To begin, power on the Genesis Cell Isolation System and wait for the system to initialize for up to 5 minutes [1].
 - 2.1.1. WIDE: Talent powering on the Genesis Cell Isolation System and waiting for the initialization screen to appear. ***Videographer:** Please obtain CU shot as well for this shot.*
- 2.2. Place the waste jar in position at the base of the station bays [1]. Attach the waste jar tubing to the waste jar [2]. Attach the semi-automated cell isolation system tubing to the waste jar outlet [3].
 - 2.2.1. Talent placing the waste jar at the base of the station bays.
 - 2.2.2. Talent attaching the waste jar tubing to the waste jar. **Note:** Shots 2.2.2 and 2.3.2 were combined, and the narration is updated for the combined shot from the other step.
- 2.3. Press the tubing into the pinch valve [1]. ~~Attach the semi-automated cell isolation system tubing to the waste jar outlet [2].~~
 - 2.3.1. Close-up shot of the talent pressing the tubing into the pinch valve.
 - 2.3.2. Talent attaching the semi-automated cell isolation system tubing to the waste jar outlet. **Note:** This shot was combined with 2.2.2, and the corresponding narration is moved.
- 2.4. Run a self-test by pressing **Start** from the home screen [1]. Touch **Self-Test** on the screen to begin the procedure [2].
 - 2.4.1. SCREEN: Show the home screen of the Genesis Cell Isolation System and click **Start**.

- 2.4.2. SCREEN: Show the selection of **Self-Test** on the touchscreen interface.
- 2.5. When the self-test is complete, select **2.0 Kit Enrichment** from the protocol options [1].
- 2.5.1. SCREEN: Show the protocol selection menu and highlight **2.0 Kit Enrichment** being selected.
- 2.6. Follow the instructions on the screen and populate the required data entry fields [1]. Complete the priming and assembly procedure using 70 percent ethanol and 1X phosphate-buffered saline [2]. Allow the priming procedure to run for approximately 20 minutes [3].
- 2.6.1. SCREEN: Show the protocol instructions and required data entry fields being completed. **Author's Note: A: what 2.6.1 should have been**
- 2.6.2. Talent loading 70% ethanol and 1X phosphate-buffered saline for the priming and assembly procedure. **Note: 2.6.2 and 2.6.3 are combined.**
- 2.6.3. Shot of the instrument showing the running of the priming procedure.
- 2.7. ~~Ensure that the correct enrichment program, **2.0 Kit Enrichment**, is selected [1].~~ After verifying the tubing and slide placements, gently dispense the model circulating tumor cells, or CTC (*C-T-C*) sample containing whole human blood spiked with A549 (*A-5-4-9*) and H1975 (*H - nineteen-seventy-five*) lung cancer cells into the inlet funnel, avoiding air bubbles to ensure consistent flow and efficient cell capture [2]. Press **Resume** on the semi-automated cell isolation system screen to continue the enrichment protocol [3].
- ~~2.7.1. SCREEN: Show the selected protocol as **2.0 Kit Enrichment** on the display. **Note: This shot and its corresponding narration are removed.**~~
- 2.7.2. Talent gently dispenses the prepared sample into the inlet funnel.
- 2.7.3. SCREEN: Show the operator pressing **Resume** on the instrument screen.
- 2.8. When the CTC capture is complete, remove the waste jar [1] and reattach the priming reservoir as prompted by the system [2].
- 2.8.1. SCREEN: Show the completion prompt instructing the user to ~~remove the waste jar.~~ attach the priming reservoir.
- 2.8.2. Talent reattaching the priming reservoir to the system.
- 2.9. Detach the semi-automated cell isolation system tubing from the waste jar [1]. Pipette 4 milliliters of dilution buffer into the priming reservoir [2].
- 2.9.1. Talent detaching the tubing from the waste jar.
- 2.9.2. Talent pipetting 4 milliliters of dilution buffer into the priming reservoir.
- 2.10. Attach the backflow adaptor to the priming reservoir [1]. Reattach the priming reservoir to the semi-automated cell isolation system tubing [2]. Press **Resume** on the screen to continue the enrichment protocol [3]. Release the captured cells in the microfluidic slide into the inlet funnel by applying backflow [4].

- 2.10.1. Talent attaching the backflow adaptor to the priming reservoir.
- 2.10.2. Talent reconnecting the priming reservoir to the tubing system.
- 2.10.3. SCREEN: Show the operator pressing **Resume** on the system screen.
- 2.10.4. Shot of fluid backflow releasing captured cells into the inlet funnel.
- 2.11. Pipette the aqueous sample completely from the inlet funnel into a 5 milliliter Eppendorf tube to collect the recovered cells [1]. Centrifuge the recovered sample at 600 g for 5 minutes to pellet the captured cells before cell lysis [2]. Carefully remove the supernatant from the pelleted sample, then proceed to DNA extraction [3].
 - 2.11.1. Talent pipetting recovered cells from the inlet funnel into a 5 milliliter Eppendorf tube.
 - 2.11.2. Talent placing the recovered sample into the centrifuge.
 - 2.11.3. Talent removing the supernatant from the sample tube.

3. EGFR Mutation Analysis using ddPCR

Demonstrator: Yoontae Kang

- 3.1. Thaw the digital droplet polymerase chain reaction, or ddPCR (*D-D-P-C-R*) Supermix and Mutation Detection Assays on ice [1]. Vortex each tube to ensure the contents are homogeneous [2].
 - 3.1.1. Talent placing the ddPCR supermix and mutation detection assay tubes on ice.
 - 3.1.2. Talent vortexing individual reagent tubes.
- 3.2. Centrifuge the tubes briefly to sediment the contents at the bottom of each tube [1]. Place the tubes back on ice [2].
 - 3.2.1. Talent placing the reagent tubes into the centrifuge.
 - 3.2.2. Talent placing the centrifuged tubes on ice.
- 3.3. Prepare the extracted DNA samples in the final DNA concentration range between 50 femtograms and 100 nanograms [1]. Then, prepare the reaction mix by adding the sample DNA individually as the final reaction component [2]
 - 3.3.1. Talent preparing DNA samples for reaction mix preparation.
 - 3.3.2. LAB MEDIA: Table 1
- 3.4. Load 20 microliters of each reaction mix into the sample wells of a DG8 cartridge [1]. Load 75 microliters of Droplet Generation Oil for Probes into the oil wells [2].
 - 3.4.1. Talent pipetting reaction mix into the DG8 cartridge sample wells.
 - 3.4.2. Talent loading droplet generation oil into the oil wells.

- 3.5. Cover the cartridge with a gasket [1]. Place the cartridge into the QX200 (Q-X-200) Droplet Generator [2].
 - 3.5.1. Talent placing a gasket over the cartridge.
 - 3.5.2. Talent inserting the cartridge into the QX200 Droplet Generator.
- 3.6. Press the button on the droplet generator to close the lid and allow droplet generation to begin automatically [1]. Program the thermal cycling conditions on the thermal cycler [2].
 - 3.6.1. Talent pressing the lid button on the droplet generator.
 - 3.6.2. LAB MEDIA: Table 2
- 3.7. When droplet generation is complete and all indicator lights are solid green, press the button to open the droplet generator [1]. Remove the cartridge holder and cartridge from the instrument [2].
 - 3.7.1. Talent pressing the button to open the droplet generator. *Videographer: Please capture the shot of the droplet generator displaying solid green lights.*
 - 3.7.2. Talent removing the cartridge holder and cartridge from the instrument.
- 3.8. After removing the disposable gasket from the cartridge, aspirate 45 microliters of droplets from the cartridge while holding the pipette tip at a slight angle to prevent droplet shredding [1]. Dispense the droplets into the ddPCR 96-well plate [2]. Use the PX1 (P-X-one) polymerase chain reaction or PCR (P-C-R) plate sealer to seal the plate with a Plate Heat Seal [3]. Start the thermal cycling procedure after transferring the sealed 96-well plate containing droplets to the prepared thermal cycler [4].
 - 3.8.1. Close-up shot of droplets being aspirated carefully at an angle.
 - 3.8.2. Talent dispensing droplets into the 96-well plate.
 - 3.8.3. Talent sealing the plate using the PX1 PCR plate sealer.
 - 3.8.4. SCREEN: Show the thermal cycler run being started.
- 3.9. Initiate the ddPCR droplet reader instrument and the connected computer with QX (Q-X) Manager Software installed and configured [1].
 - 3.9.1. WIDE: Talent powering on the droplet reader instrument and connected computer.
- 3.10. After thermal cycling is complete, transfer the sealed 96-well plate to the droplet reader instrument [1]. In the QX Manager Software, click the **Add Plate** icon to enable the **Configure Plate** button, and configure a new plate layout based on the experimental design [2].
 - 3.10.1. Talent moving the sealed 96-well plate into the droplet reader instrument.
 - 3.10.2. SCREEN: Take-3_3p10p2_3p11p5.mp4. 00:00–00:09

3.11. To fill in the **plate information**, under the **Create New Plate** menu, enter the plate name, supermix, and save fields, then select **Acquire Wells By Columns [1]**. Next, under the **Well Selection** menu, select the required wells, then click **Include selected wells [2]**. Under the **Well Information** menu, click to automatically input the experiment type and assay type fields, then click on **Apply** to assign the details to the selected wells **[3]**. Select the individual wells, enter the sample and target information descriptions, then click **Apply** to designate the input target conditions for the selected wells before starting the Run **[4]**.

3.11.1. SCREEN: Take-3_3p10p2_3p11p5.mp4. 00:12–00:18; 00:20–00:25

3.11.2. SCREEN: Take-3_3p10p2_3p11p5.mp4. 00:26–00:32

3.11.3. SCREEN: Take-3_3p10p2_3p11p5.mp4. 00:41–00:47

3.11.4. SCREEN: Take-3_3p10p2_3p11p5.mp4. 00:50–00:53; 1:01–1:04; 1:10–1:13; 1:19–1:22; 1:26–1:29

3.12. After completing the droplet reading process, click on Browse under the data analysis section to open the data file **[1]**. Once the data is launched, click on Plate Editor to review the well information **[2]**. Revert to the 2D Amplitude View, select the desired well from the plate layout, click on the settings gear icon to choose the scale, and enter the axes thresholds values to distinguish the wild-type and mutant signals **[3]**. Check the reported concentration values of the mutant and wildtype signals, displayed as copy numbers per microliter of the final 1X dddPCR reaction **[4]**. Review the copy numbers per microliter values across wells within the plate to analyze EGFR mutation levels in different cell lines **[5]**.

3.12.1. SCREEN: Take2_3p12p1_3p13p2.mp4. 00:00–00:05; 00:12–00:15

3.12.2. SCREEN: Take2_3p12p1_3p13p2.mp4. 00:16–00:20

3.12.3. SCREEN: Take2_3p12p1_3p13p2.mp4. 00:21–00:35

3.12.4. SCREEN: Take2_3p12p1_3p13p2.mp4. 00:38–00:44

3.12.5. SCREEN: Take2_3p12p1_3p13p2.mp4. 00:46–00:49; 00:52–00:55; 00:58–01:06

Results

4. Results

- 4.1. The Genesis cell isolation system with microfluidic cell capture slides isolated an average of 78.1 percent for spiked A549 cells [1] and 90 percent for H1975 cells in blood samples at 25 to 250 cells per milliliter [2].
 - 4.1.1. LAB MEDIA: Figure 3A. *Video editor: Please highlight the bar showing 78.1% value.*
 - 4.1.2. LAB MEDIA: Figure 3A. *Video editor: Please highlight the bar showing 90.0% value.*
- 4.2. ddPCR plots showed positive epidermal growth factor receptor, or EGFR (E-G-F-R) mutation signals in H1975 cells [1], whereas no mutation signals were detected in the negative control, A549 cells [2].
 - 4.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the red-circled positive signal area shown for H1975 cells in both L858R and T790M rows.*
 - 4.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight the corresponding area without red circle for A549 cells in both L858R and T790M rows.*

Pronunciation Guide:

1. Circulating
Pronunciation link: <https://www.merriam-webster.com/dictionary/circulating>
IPA: /'sə:kjə.leɪtɪŋ/
Phonetic Spelling: sur·kyuh·lay·ting
2. Tumor
Pronunciation link: <https://www.merriam-webster.com/dictionary/tumor>
IPA: /'tu:mər/
Phonetic Spelling: too·mer
3. Isolation
Pronunciation link: <https://www.merriam-webster.com/dictionary/isolation>
IPA: /,aɪsə'leɪʃən/
Phonetic Spelling: eye·suh·lay·shuhn
4. Genesis
Pronunciation link: <https://www.merriam-webster.com/dictionary/genesis>
IPA: /'dʒenəsəs/
Phonetic Spelling: jen·uh·suhs
5. Semi-automated
Pronunciation link: <https://www.merriam-webster.com/dictionary/semi-automated>

IPA: /,semi'ʊtə,meɪtɪd/, /,semaɪ'ʊtə,meɪtɪd/

Phonetic Spelling: sem·ee·aw·tuh·may·tid

6. Pinch valve

Pronunciation link: <https://www.merriam-webster.com/dictionary/valve>

IPA: /vælʌv/

Phonetic Spelling: valv

7. Ethanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>

IPA: /'eθə,nɔ:l/

Phonetic Spelling: eth·uh·nawl

8. Phosphate-buffered saline

Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>

IPA: /'fɑ:s,feɪt/

Phonetic Spelling: fos·fayt

9. Priming

Pronunciation link: <https://www.merriam-webster.com/dictionary/priming>

IPA: /'praɪmɪŋ/

Phonetic Spelling: pry·ming

10. Enrichment

Pronunciation link: <https://www.merriam-webster.com/dictionary/enrichment>

IPA: /ɪn'rɪtʃmənt/

Phonetic Spelling: in·rich·muhnt

11. Circulating tumor cells

Pronunciation link: <https://www.merriam-webster.com/dictionary/circulating>

IPA: /'sɜ:kjə,leɪtɪŋ/, /'tu:mər/, /selz/

Phonetic Spelling: sur·kyuh·lay·ting too·mer selz

12. Microfluidic

Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidics>

IPA: /,maɪkroʊflu'ɪdɪk/

Phonetic Spelling: my·kroh·floo·id·ik

13. Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /'sentərə,fju:dʒ/

Phonetic Spelling: sen·truh·fyooj

14. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /,su:pər'nextənt/

Phonetic Spelling: soo·per·nay·tuhnt

15. Pelleted
Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /'pelət/
Phonetic Spelling: pel·it
16. Lysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>
IPA: /'laɪsɪs/
Phonetic Spelling: ly·suhs
17. EGFR
Pronunciation link: No confirmed link found
IPA: /,iː dʒiː ɛf 'ɑːr/
Phonetic Spelling: ee·jee·ef·ar
18. Mutation
Pronunciation link: <https://www.merriam-webster.com/dictionary/mutation>
IPA: /mjuː'teɪʃən/
Phonetic Spelling: myoo·tay·shuhn
19. ddPCR
Pronunciation link: No confirmed link found
IPA: /,diː ,diː ,piː ,siː 'ɑːr/
Phonetic Spelling: dee·dee·pee·see·ar
20. Polymerase
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
IPA: /pə'limərəˌseɪs/
Phonetic Spelling: puh·lim·uh·ruh·says
21. Homogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /,həʊmə'dʒiːniəs/
Phonetic Spelling: hoh·muh·jee·nee·uhs
22. Femtograms
Pronunciation link: <https://www.merriam-webster.com/dictionary/femtogram>
IPA: /'femtoʊˌgræm/
Phonetic Spelling: fem·toh·gram
23. Nanograms
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanogram>
IPA: /'nænəˌgræm/
Phonetic Spelling: nan·uh·gram
24. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

IPA: /'maɪkroʊˌliːtər/

Phonetic Spelling: my·kroh·lee·ter

25. Cartridge

Pronunciation link: <https://www.merriam-webster.com/dictionary/cartridge>

IPA: /'kɑːrtɹɪdʒ/

Phonetic Spelling: kar·trij

26. Droplet

Pronunciation link: <https://www.merriam-webster.com/dictionary/droplet>

IPA: /'drɑːplət/

Phonetic Spelling: drop·lit

27. Gasket

Pronunciation link: <https://www.merriam-webster.com/dictionary/gasket>

IPA: /'gæskət/

Phonetic Spelling: gas·kit

28. Aspirate

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>

IPA: /'æspəˌreɪt/

Phonetic Spelling: ass·puh·rayt

29. Thermal cyclor

Pronunciation link: <https://www.merriam-webster.com/dictionary/thermal>

IPA: /'θɜːməl/, /'saɪklər/

Phonetic Spelling: thur·muhl sy·kler

30. Amplitude

Pronunciation link: <https://www.merriam-webster.com/dictionary/amplitude>

IPA: /'æmpləˌtuːd/, /'æmpləˌtjuːd/

Phonetic Spelling: am·pluh·tood

31. Wild-type

Pronunciation link: <https://www.merriam-webster.com/dictionary/wild-type>

IPA: /'waɪldˌtaɪp/

Phonetic Spelling: wyld·type

32. Mutant

Pronunciation link: <https://www.merriam-webster.com/dictionary/mutant>

IPA: /'mjuːtənt/

Phonetic Spelling: myoo·tuhnt

33. Aqueous

Pronunciation link: <https://www.merriam-webster.com/dictionary/aqueous>

IPA: /'eɪkwɪəs/

Phonetic Spelling: ay·kwee·uhs

34. Eppendorf

Pronunciation link: <https://www.howtopronounce.com/eppendorf>

IPA: /'ɛpəndɔːrf/

Phonetic Spelling: ep·uhn·dorf

35. A549

Pronunciation link: No confirmed link found

IPA: /,eɪ faɪv fɔːr 'naɪn/

Phonetic Spelling: ay·five·for·nine

36. H1975

Pronunciation link: No confirmed link found

IPA: /,eɪtʃ ,naɪn'tiːn ,sevənti 'faɪv/

Phonetic Spelling: aych·nine·teen·sev·uhn·tee·five