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Title: Detection of Targetable Alterations in Non-Small Cell Lung Cancer Using Next-Generation Sequencing

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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**

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: 17
Number of Shots: 29

Introduction

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

INTRODUCTION:

1.1. **Weihua Li:** My research discovers novel and rare genetic mutations in non-small cell lung cancer to improve diagnosis and overcome drug resistance.
   1.1.1. **INTERVIEW:** Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

What advantage does your protocol offer compared to other techniques?

1.2. **Weihua Li:** NGS provides a comprehensive genetic profile for non-small cell lung cancer by detecting common, rare and novel genes simultaneously.
   1.2.1. **INTERVIEW:** Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 3.5*

What questions will future research focus on?

1.3. **Weihua Li:** Combining molecular pathology with artificial intelligence to explore new biomarkers, disease subtype classification, and prognostic models.
   1.3.1. **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.

Protocol

2. Pre-Testing Steps and QC of Non-Small Cell Lung Cancer FFPE Sample

Demonstrator: Mengli Wang

2.1. To begin, register the sample information in the laboratory information management system **[1]** and fill out the informed consent form for gene mutation testing **[2]**.

2.1.1. WIDE: Talent typing sample details into the laboratory information management system.

2.1.2. Talent completing the informed consent form for gene mutation testing.

2.2. Using disposable microtome blades, cut sections from the non-small cell lung cancer formalin-fixed paraffin-embedded sample **[1-TXT]**.

2.2.1. Talent cutting tissue sections using a disposable microtome blade. **TXT: Replace tools and tube between samples to prevent contamination**

2.3. Then, perform routine hematoxylin and eosin staining to evaluate the tumor cell content **[1]**.

2.3.1. Talent placing the tissue sections into staining containers.

2.4. For nucleic acid extraction, using a microtome, section the formalin-fixed paraffin-embedded block to obtain a ribbon **[1]** and place the ribbon into a 1.5-milliliter microcentrifuge tube **[2]**. Transfer the tube to the designated sample input location on the automated extraction instrument **[3]**.

2.4.1. Talent cutting a ribbon section from the paraffin block using a microtome.

2.4.2. Talent placing the ribbon into a 1.5 milliliter microcentrifuge tube.

2.4.3. Talent positioning the tube at the sample input area of the automated extraction instrument.

2.5. Next, use an automatic extraction instrument with an automatic extraction kit **[1]**. Replace manual pipetting steps with an automatic magnetic bead nucleic acid extraction system **[2]**.

2.5.1. Talent operating the automatic extraction instrument with the extraction kit loaded.

2.5.2. Show the automated magnetic bead nucleic acid extraction process being initiated.

2.6. Then, select the **C1102** *(C-One-One-Zero-Two)* **Program** on the instrument **[1]**. Choose the sample dewaxing incubation time as 16 hours and set the elution volume to 100 microliters **[2]**.

2.6.1. Show the software interface with the **C1102 Program** being selected from the program list.

2.6.2. Show the option for dewaxing incubation time being set to 16 hours and the elution volume field being edited to enter 100 microliters.

2.7. Use spectrophotometry to verify DNA purity **[1-TXT]**. Then, use a detection kit with sequence-specific fluorescent dyes to perform a fluorometric assay for precise DNA concentration measurement **[2]**.

2.7.1. Talent placing the DNA sample into the spectrophotometer and initiating measurement. **TXT: OD260/OD280 > 1.8; OD260/OD230 > 2.0**

2.7.2. Talent pipetting the reagents from the fluorometric assay kit to the sample.

3. Fully Automated Library Preparation

3.1. To prepare fragmented genomic DNA using ultrasonication, mix 200 nanograms of genomic DNA in 50 microliters of low Tris-EDTA buffer **[1]**. Place the sample tube in a pre-cooled sample rack maintained at 8 degrees Celsius **[2]**.

3.1.1. Talent pipetting 200 nanograms of genomic DNA into 50 microliters of low Tris-EDTA buffer.

3.1.2. Talent placing the sample tube into the pre-cooled rack.

3.2. Set the instrument parameters according to the protocol **[1]** and initiate DNA fragmentation **[2]**.

3.2.1. LAB MEDIA: Table 4

3.2.2. The DNA fragmentation process is started.

3.3. To start automated library preparation, press the **Power** button to activate the system and wait for the initialization to complete **[1]**. Navigate to **Program Setting**, select **Run Protocol**, and choose **Protocol: Burning Rock HS** *(Protocol-Burning-Rock-H-S)* **[2]**.

3.3.1. Talent pressing the power button on the instrument to activate the system.

3.3.2. Show the instrument menu where Program Setting is selected, followed by Run

Protocol, and then Protocol: Burning Rock HS.

3.4. Set the **Sample Type** to **High Quality DNA**, **Input Amount (nanograms)** to **50**, **Pre PCR Cycles** to **12**, and **Post PCR Cycles** to **12**. Then, click **RUN** to proceed **[1]**.

3.4.1. Show user selecting each QC parameter and clicking **RUN** to initiate the process.

3.5. Then, insert the prepackaged reagent cartridge into the designated slot of the instrument, which will automatically validate reagent placement and status **[1]**.

3.5.1. Talent inserting the reagent cartridge into the instrument slot.

3.6. After loading the samples, press **Start** to initiate the autonomous run **[1]**. Observe the LED indicators on the system to monitor the progress **[2]**.

3.6.1. Talent pressing the Start button on the instrument.

3.6.2. Close-up of LED indicators changing color or pattern to indicate the system's progress.

3.7. After the program ends, click **OK** to confirm **[1],** and the system will automatically transfer the final library into the library tube, completing library preparation **[2]**.

3.7.1. Show the user clicking OK.

3.7.2. Display the system transferring the final library into the output tube.

3.8. Measure the concentration of both pre-library and total library using fluorometry **[1]**.

3.8.1. Talent using a fluorometric device to measure pre-library and total library concentrations.

3.9. Dilute the sequencing library to 1.6 picomolar and 1300 microliters for sequencing operations **[1]**.

3.9.1. Talent pipetting the solution to the sample tube.

3.10. Control the sequencing environment by maintaining an indoor temperature and indoor humidity **[1]**. Complete quality control of the instrument output data by verifying base quality above Q30 *(Q-Thirty)* and cluster density passing filter **[2]**.

3.10.1. Talent checking environmental control panel and adjusting temperature and humidity settings as needed.

3.10.2. Show the instrument display with QC metrics including base quality above Q30 and passing filter cluster density.

Results

4. Results

4.1. The optimized detection workflow reliably identified several clinically actionable genomic alterations in representative tumor samples **[1].**

4.1.1. LAB MEDIA: Figure 3

4.2. The sequencing results demonstrated a high-frequency EGFR *(E-G-F-R)* p.L858R *(P-L-Eight-Five-Eight-R)* missense mutation **[1],** accompanied by a significant EGFR gene copy number amplification **[2].**

4.2.1. LAB MEDIA: Table 6. *Video editor: Highlight the row for "EGFR" with mutation type "missense variant" and description "p.L858R", showing allele frequency 89.25%*

4.2.2. LAB MEDIA: Table 6. *Video editor: Highlight the row for "EGFR" with mutation type "cn_amp" and description "cn_amp", showing copy number 14.7*

4.3. Moderate amplification of the MET *(Met)* gene was observed **[1],** and low-level amplification of the BRAF *(B-raf)* gene was observed **[2].**

4.3.1. LAB MEDIA: Table 6. *Video editor: Highlight the row for "MET" with mutation type "cn_amp" and copy number 5.0*

4.3.2. LAB MEDIA: Table 6. *Video editor: Highlight the row for "BRAF" with mutation type "cn_amp" and copy number 3.8*

4.4. The sample exhibited an intermediate tumor mutation burden **[1]** and microsatellite stable status **[2].**

4.4.1. LAB MEDIA: Table 6. *Video editor: Highlight the row for "TMB (tumor mutation burden)" with the value "9.0 mutations/Mb"*

4.4.2. LAB MEDIA: Table 6. *Video editor: Highlight the row for "MSI (microsatellite instability)" showing the value "MSS"*

4.5. All targeted gene loci, including ALK *(A-L-K)*, BRAF, KRAS *(K-Ras)*, and ROS1 *(Ros-One)*, achieved a 100% detection rate across four replicate experiments **[1].**

4.5.1. LAB MEDIA: Table 7. *Video editor: Highlight the rows for "ALK", "BRAF", "KRAS", and "ROS1" showing "100%" in the "compliance rate" column*

4.6. Each mutation was consistently detected in all four repeated experiments, with expected abundance values remaining within the normal fluctuation range **[1].**

4.6.1. LAB MEDIA: Table 7. *Video editor: Highlight the "Number of detections" and "Expected abundance" columns for all rows, showing values of 4 and 0.40% respectively*

Pronunciation Guide:

- Formalin-fixed paraffin-embedded

Pronunciation link: https://www.merriam-webster.com/dictionary/formalin JoVE
IPA: /fɔːrˈmælɪn fɪkst pəˈræfɪn ɛmˈbɛdɪd/
Phonetic: for-MAL-in fixt puh-RAFF-in em-BEDid

- Dewaxing

Pronunciation link: https://www.merriam-webster.com/dictionary/dewaxing JoVE
IPA: /diːˈwæk.sɪŋ/
Phonetic: dee-WAK-sing

- Elution

Pronunciation link: https://www.merriam-webster.com/dictionary/elution JoVE
IPA: /ɪˈluːʃən/
Phonetic: ih-LOO-shun

- Nanogram

Pronunciation link: https://www.merriam-webster.com/dictionary/nanogram JoVE
IPA: /ˈnænəˌgræm/
Phonetic: NAN-oh-gram

- Microlitre

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter JoVE
IPA: /ˈmaɪ.krəˌliːtər/
Phonetic: MY-kroh-lee-ter

- Fluorometric

Pronunciation link: No confirmed link found
IPA: /ˌflʊərəʊˈmɛtrɪk/
Phonetic: FLUOR-oh-MET-rik

- Ultrasonication

Pronunciation link: No confirmed link found
IPA: /ˌʌltrəˌsoʊnɪˈkeɪʃən/
Phonetic: UL-truh-soh-ni-KAY-shun

- Cluster density

Pronunciation link: No confirmed link found (for this exact phrase)
IPA: /ˈklʌstər ˈdɛnsɪti/
Phonetic: CLUS-ter DEN-suh-tee

- Microsatellite instability

Pronunciation link: https://www.merriam-webster.com/dictionary/microsatellite JoVE
IPA for “microsatellite”: /ˌmaɪ.kroʊˈsætə.laɪt/
IPA full: /ˌmaɪ.kroʊˈsætəlaɪt ɪnˌstæbɪˈlɪti/
Phonetic: MY-kroh-SAT-uh-lyte in-stab-i-LIT-ee

- Amplification (gene copy number amplification)

Pronunciation link: https://www.merriam-webster.com/dictionary/amplification JoVE
IPA: /ˌæmpləfɪˈkeɪʃən/
Phonetic: AM-pluh-fi-KAY-shun