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Title: Clinical Variant Interpretation with the Integrative Genomics Viewer (IGV) for Molecular Pathologists

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FINAL SCRIPT: APPROVED FOR FILMING



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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? No

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? YES, done

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **Week of 8/3/26 or 8/10/26**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 7

Number of Shots: 18

Interviews

Authors: The statements are edited for length and clarity. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

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

- 1.1. **Eitan Halper-Stromberg:** We aim to provide foundational skills for using the IGV to correctly find and report somatic mutations in cancer patients.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Eitan Halper-Stromberg:** There is limited guidance on using IGV in clinical laboratories. This protocol addresses the gap with practical, curated clinical vignettes
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

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

- 1.3. **Eitan Halper-Stromberg:** The key consideration when performing this protocol is correctly recognizing and interpreting how different types of somatic mutations appear in IGV.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Eitan Halper-Stromberg:** While this protocol focuses on human cancer mutations, the same approach can be applied to other sequencing studies and mutation types.
 - 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. **Eitan Halper-Stromberg**: Future studies may explore methods to enhance the IGV with code and routines that interact with the IGV.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Software Setup and Workflow Vignette to Assess EGFR Alterations

Demonstrator: Eitan Halper-Stromberg

2.1. To begin, download and install the latest version of the Integrative Genomics Viewer software for Windows, with Java included [1]. Next, download the clinical vignette files from the GitHub repository [2]. In IGV (*I-G-V*) software overhead menu, verify that **Show coverage track** is enabled [3]. To detect clinically significant low-level variants, set the **Coverage allele-fraction** threshold to 0.01 [4]. Finally, enable **Show soft-clipped bases** from the overhead menu to display partially aligned reads with clipped ends [5].

2.1.1. WIDE: Talent sitting at the computer to install the IGV software

2.1.2. SCREEN: download-igv-824 00:00:05-00:35-00:39

2.1.3. SCREEN: download-igv-824: 0:49 – 0:59

2.1.4. SCREEN: download-igv-824: 1:08 – 1:18

2.1.5. SCREEN: download-igv-824: 1:28 – 1:38

NOTE to video editor: Please zoom into or highlight the places where the cursor is moving and where actions are happening in the entire script

2.2. Review case-vignette details like patient age, affliction, and NGS (*N-G-S*) results before starting the workflow [1]. In the genome dropdown menu at the top left, set the reference genome to **Hg38** (*H-G-thirty-eight*) [2]. Then, drag and drop the proband or patient and comparator sample segment files into the viewer [3].

NOTE to video editor: Please zoom into or highlight the places where the cursor is moving

2.2.1. SCREEN: egfr-amp-824 0:04 – 0:15

2.2.2. SCREEN: egfr-amp-824 0:32-0:45

2.2.3. SCREEN: egfr_amp_824 0:53- 1:00, 1:02-1:12

2.3. Next, navigate to Chr 7 (*Chromosome 7*) and locate the epidermal growth factor receptor locus [4]. Now, compare relative color intensity in the heat map, between

the patient sample and other samples C1 to C9, to distinguish true copy number changes from artifacts [5].

2.3.1. SCREEN: egfr_amp_824 1:33-1:44

2.3.2. SCREEN: egfr_amp_824 2:20-2:29, and 3:45-3:59

2.4. Review case vignette details once again [1]. Then in IGV, switch to the **Hg19** (*H-G-nineteen*) genome as reference using the genome dropdown window at the top left [2]. Now drag and drop the proband or patient BAM (*bam*) file, and BAM index file into the viewer [3].

2.4.1. SCREEN: egfr-vIII-824: 0:07 – 0:19

2.4.2. SCREEN: egfr-vIII-824: 0:26 – 0:35

2.4.3. SCREEN: egfr-vIII-824: 0:45 – 0:55, 0:56 – 1:04

NOTE to video editor: Please zoom into or highlight the places where the cursor is moving

2.5. Now, navigate to Chr 7 and locate the EGFR (*E-G-F-R*) gene [1]. Examine breakpoints upstream of exon 2 and downstream of exon 7 for EGFRvIII (*E-G-F-R-Vee-three*) by performing **sort-by-base** [2] and visualizing soft-clipped reads [3]. Right-click a soft-clipped sequence and select BLAT (*blat*) sequence [4]. Then, select the first result and examine the BLAT track displayed at the bottom of the IGV window to visualize structural rearrangements [5].

2.5.1. SCREEN: egfr-vIII-824 1:20 – 1:27 and 2:52 – 2:58

2.5.2. SCREEN: egfr-vIII-824: 3:10 – 3:14 and 3:34- 3:43

2.5.3. SCREEN: egfr-vIII-824: 3:34- 3:43 and 3:50 – 3:54

2.5.4. SCREEN: egfr-vIII-824: 4:07 – 4:16 and 4:28 – 4:34

2.5.5. SCREEN: egfr-vIII-824: 4:36 – 4:45 and 5:50 - 6:00

NOTE to video editor: Please zoom into or highlight the places where the cursor is moving

Results

3. Results

3.1. Mapping the EGFR locus in patient, also referred to as proband, and random samples to the Hg38 reference genome revealed an EGFR copy number gain in the patient [1] At the EGFR locus, the patient sample showed EGFR amplification beyond background [2], whereas the random samples showed little [3] or no amplification [4].

3.1.1. LAB MEDIA: Figure 5C *Video Editor: Please highlight with the small black square in the proband row at the bottom of the picture. The file name is Figure 5C marked in the update section on Monday.com.*

3.1.2. LAB MEDIA: Figure 5D *Video Editor: Please highlight the bright red bar at the end corresponding to proband.*

3.1.3. LAB MEDIA: Figure 5D *Video Editor: Please highlight the light blue and dark blue bars on top corresponding to C1 to C2*

3.1.4. LAB MEDIA: Figure 5D *Video Editor: Please highlight the white region in the middle corresponding to C3 to C8*

3.2. The BLAT tool identified genomic locations matching the soft-clipped sequence and assigned alignment scores [1]. Selecting the first result navigated to the corresponding genomic location, confirming the structural rearrangement. [2].

3.2.1. LAB MEDIA: Figure 6C *Video Editor: Please highlight the “BLAT result for query sequence” Box entirely and in the bottom panel where the table is seen, there, emphasize score and 1000 value*

3.2.2. LAB MEDIA: Figure 6D *Video Editor: Please highlight the area inside the small black box with an arrow to show structural variant. The file name is Figure 6D marked in the update section on Monday.com.*

1. Integrative

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

IPA: /'ɪntəˌɡreɪtɪv/

Phonetic Spelling: in·tuh·gray·tiv

2. Genomics

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

IPA: /dʒiˈnoʊmɪks/, /dʒəˈnoʊmɪks/

Phonetic Spelling: jee·noh·miks / juh·noh·miks

3. Vignette

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

- IPA: /vɪnˈjet/, /viːnˈjet/
Phonetic Spelling: vin·yet / veen·yet
4. Allele
Pronunciation link: <https://www.merriam-webster.com/dictionary/allele>
IPA: /əˈli:l/
Phonetic Spelling: uh·leel
 5. Affliction
Pronunciation link: <https://www.merriam-webster.com/dictionary/affliction>
IPA: /əˈflɪkʃən/
Phonetic Spelling: uh·flik·shuhn
 6. Proband
Pronunciation link: <https://www.merriam-webster.com/dictionary/proband>
IPA: /ˈproʊˌbænd/, /proʊˈbænd/
Phonetic Spelling: proh·band / proh·BAND
 7. Comparator
Pronunciation link: <https://www.merriam-webster.com/dictionary/comparator>
IPA: /kəmˈpærətər/, /kəmˈpærətər/
Phonetic Spelling: kuhm·PAIR·uh·ter / kuhm·PAR·uh·ter
 8. Chromosome
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromosome>
IPA: /ˈkroʊməˌsoʊm/, /ˈkroʊməˌzoʊm/
Phonetic Spelling: kroh·muh·sohm / kroh·muh·zohm
 9. Epidermal
Pronunciation link: <https://www.merriam-webster.com/dictionary/epidermal>
IPA: /ˌɛpəˈdɜːməl/
Phonetic Spelling: ep·uh·DER·muhl
 10. Locus
Pronunciation link: <https://www.merriam-webster.com/dictionary/locus>
IPA: /ˈloʊkəs/
Phonetic Spelling: loh·kuhs
 11. Exon
Pronunciation link: <https://www.merriam-webster.com/dictionary/exon>
IPA: /ˈɛkˌsoːn/
Phonetic Spelling: ek·sahn
 12. Alignment
Pronunciation link: <https://www.merriam-webster.com/dictionary/alignment>
IPA: /əˈlaɪnmənt/
Phonetic Spelling: uh·line·muhnt
 13. Rearrangements
Pronunciation link: <https://www.merriam-webster.com/dictionary/rearrangement>
IPA: /ˌriːəˈreɪndʒmənts/
Phonetic Spelling: ree·uh·RAYNJ·muhnts
 14. Genomic
Pronunciation link: <https://www.merriam-webster.com/dictionary/genomic>

IPA: /dʒɪˈnoʊmɪk/, /dʒɪˈnɑːmɪk/

Phonetic Spelling: jɪh·NOH·mɪk / jɪh·NAH·mɪk

15. Amplification

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

IPA: /ˌæmpləfəˈkeɪʃən/

Phonetic Spelling: am·pluh·fuh·KAY·shuhn