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Title: Subcutaneous Injection of Human Colorectal Cancer Cells in Athymic Nude Mice to Evaluate Antitumor Efficacy

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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? **NO**

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: **31/12/2025**

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

Current Protocol Length

Number of Steps: 28

Number of Shots: **47**

Introduction

NOTE to VO: Please record introductory and conclusion interview statements.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. ~~**Silvia Montoro García:**~~ This research presents a reproducible preclinical protocol using a subcutaneous colorectal cancer mouse model to assess antitumor effects of bioactive compounds.

1.1.1. [3.5.1.](#)

~~What are the most recent developments in your field of research?~~

- 1.2. ~~**Begoña Albuquerque Gonzalez:**~~ Recent developments include refined xenograft techniques, improved animal handling, and advanced imaging methods that enhance tumor monitoring and reproducibility in preclinical cancer studies.

1.2.1. [4.4.2](#)

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. ~~**Clara Noguera Navarro:**~~ This protocol enables non-invasive IVIS tumor monitoring, offering a controlled, reproducible platform that reduces animal use and minimizes experimental variability.

1.3.1. [4.3.1](#)

~~What research questions will your laboratory focus on in the future?~~

- 1.4. ~~**Andreia Filipa Cruz Santos:**~~ Future research will develop human-relevant preclinical models, refine protocols for reproducibility, prioritize animal welfare, and optimize metastasis monitoring.

1.4.1. [4.6.1](#)

Ethics Title Card

This research has been approved by the Ethical Committee at the *Instituto Murciano de Investigación Biosanitaria* (IMIB, Murcia)

Protocol

2. Preparation and Handling of Bioluminescent HCT-116 Cells for Injection

Demonstrator: Andreia Filipa Cruz Santos

NOTE: Script drafted from Author Provided Footage

2.1. To begin, transfer a working solution of D-Luciferin Potassium Salt Bioluminescent Substrate through a 0.2-micrometer syringe filter for sterilization [1]. Place the extracellular matrix on ice and [2], using a prechilled P1000 micropipette, aliquot 200 microliters of extracellular matrix into sterile tubes [1].

2.1.1. LAB MEDIA: 3-Intraperitoneal injection of luciferin.MP4	00:08–00:30
2.1.2. LAB MEDIA: Resuspending cells in matrigel.MP4	00:33–00:47
2.1.3. LAB MEDIA: Resuspending cells in matrigel.MP4	01:29–01:41

2.2. Aspirate the culture medium carefully to avoid disturbing adherent HCT-116 (*H-C-T-One-One-Six*) bioluminescent cells [1]. Next, wash the cells with 8 milliliters of sterile PBS prewarmed at 30 degrees Celsius to remove residual serum [2].

2.2.1. LAB MEDIA: 1-Trypsinize cells.mp4	00:16–00:33
2.2.2. LAB MEDIA: 1-Trypsinize cells.mp4	01:15–01:38

2.3. Add 2 milliliters per flask of warm Trypsin-EDTA at 0.05 percent and incubate at 37 degrees Celsius for 4 to 5 minutes [1]. Monitor cell detachment under an inverted microscope [2-TXT].

2.3.1. LAB MEDIA: 1-Trypsinize cells.mp4	02:00–02:21
2.3.2. LAB MEDIA: 2-Visualize detaching cells.MP4	00:00-full
TXT: If cells remain attached, gently tap the side of the flask	

2.4. Next, add 10 milliliters of complete growth medium containing fetal bovine serum to neutralize trypsin [1]. Then transfer the cells into a 15-milliliter conical tube using a 10-milliliter serological pipette [2-TXT].

2.4.1. LAB MEDIA: 3-add medium and centrifuge cells.MP4	00:12–00:28
2.4.2. LAB MEDIA: 3-add medium and centrifuge cells.MP4	00:31–00:47
TXT: Repeat rinse once more and transfer to the tube	

2.5. Now, centrifuge the cell suspension at 200 *g* for 5 minutes at room temperature [1]. Carefully aspirate the supernatant without disturbing the cell pellet [2].

2.5.1. LAB MEDIA: 3-add medium and centrifuge cells.MP4 01:05–01:20

2.5.2. LAB MEDIA: 4-remove trypsin.MP4 00:13–00:23

2.6. Gently resuspend the cell pellet in 3 milliliters of seeding medium to achieve a homogeneous suspension [1]. Count the cells in a Neubauer chamber or an automatic cell counter [2]. Determine cell viability by mixing 10 microliters of the cell suspension with 10 microliters of Trypan blue and counting viable cells under an inverted microscope [3].

2.6.1. LAB MEDIA: 4-remove trypsin.MP4 01:16–01:31

2.6.2. LAB MEDIA: 5-Count cells.MP4 00:00–00:20

2.6.3. LAB MEDIA: 5-Count cells.MP4 00:42–00:50,01:11–01:17,01:24–01:30, 01:40–01:42, 02:52–03:00

2.7. Resuspend the cells in sterile PBS, prepare 200-microliter aliquots [1-TXT].

2.7.1. LAB MEDIA: 6-Alicuot cells.MP4 00:00–end

TXT: Final cell concentration: 2×10^7 cells/mL

3. Subcutaneous Injection of Bioluminescent Colorectal Cancer Cells in Mice

Demonstrator: Begoña Albuquerque Gonzalez

3.1. Identify and safely restrain each mouse [1].

3.1.1. LAB MEDIA: Resuspending cells in Matrigel.MP4 00:00–00:10

3.2. Mix 200 microliters of the prepared bioluminescent colorectal cancer cell aliquots with 200 microliters of extracellular matrix in a cryovial inside a biosafety cabinet [1]. Gently pipette with a P1000 pipette to avoid air bubble formation and dissociation [2].

3.2.1. LAB MEDIA: Resuspending cells in matrigel.MP4 01:03–01:05,01:11–01:24

3.2.2. LAB MEDIA: Resuspending cells in matrigel.MP4 00:52–01:01

3.3. Transfer the mixture to a 1-milliliter syringe [1], remove air bubbles [2], and proceed immediately to injection [3].

3.3.1. LAB MEDIA: Injection 4.MP4 00:07–00:23

3.4. Remove the mouse from the chamber once it is anesthetized and place the animal's snout in an outlet supplying the same anesthetic [1-TXT].

3.4.1. LAB MEDIA: Injection 2.MP4 00:00–00:15 **TXT: Anesthesia: 2% isoflurane in oxygen inhalation**

- 3.5. Place the mouse on its right side on a flat surface [1-TXT]. Using the tips of the fingers of the non-dominant hand, gently pinch and lift the animal's skin in the lower left quadrant [2]. Insert the needle bevel-up, two-thirds into the lifted skin, parallel to the body [3].

- 3.5.1. LAB MEDIA: Inyection 2.MP4 01:07–01:15

- 3.5.2. LAB MEDIA: Inyection 2.MP4 01:25–01:28

- 3.5.3. LAB MEDIA: Inyection 2.MP4 01:28–01:33

- 3.6. Inject the contents of the syringe into the lifted skin, right between the two fingers [1]. Hold briefly before withdrawing the needle slowly [2-TXT].

- 3.6.1. LAB MEDIA: Inyection 2.MP4 01:33–01:42

- 3.6.2. LAB MEDIA: Inyection 2.MP4 01:42-01:46 **TXT: Discard needle into a Sharps container**

4. Intraperitoneal Injection of D-Luciferin and *In Vivo* Imaging of Tumor-Bearing Mice

- 4.1. Manually restrain the mouse in dorsal recumbence, abdomen side up, with the cranial end of the animal pointed down to allow the intestinal contents to move downward [1].

- 4.1.1. LAB MEDIA: 3-Intraperitoneal Injection of luciferin.MP4 01:02–01:06

- 4.2. Keeping the needle bevel-side up and angled at 15 to 20 degrees, push the needle into the abdominal cavity so the tip just penetrates the abdominal wall of the left lower abdominal quadrant [1]. Slowly inject the D-luciferin into the intraperitoneal cavity, then remove the needle [2-TXT].

- 4.2.1. LAB MEDIA: 3-Intraperitoneal Inyection of luciferin.MP4 01:07–01:14

- 4.2.2. LAB MEDIA: 3-Intraperitoneal Inyection of luciferin.MP4 01:14–01:23
TXT: Transfer the mouse post-injection into the anesthesia chamber

- 4.3. Place the anesthetized animal in the chamber of the imaging system, ensuring proper alignment to capture the tumor area [1].

- 4.3.1. LAB MEDIA: 1-taking mice inside the IVIS.MP4 00:00-00:10, 00:15-00:21

- 4.4. Open the linked software [1]. Choose the visual or photographic imaging mode to confirm the position of the animal [2]. Save the data and change the folder's name [3].

- 4.4.1. LAB MEDIA: 4-Confirming position.MP4 00:00–00:06

- 4.4.2. LAB MEDIA: 4-Confirming position.MP4 00:07–00:18

- 4.4.3. LAB MEDIA: 4-Confirming position.MP4 00:32–end

- 4.5. Set up a Region of Interest around the tumor for analysis and copy it for further analyses. Measure the light of the Region of Interest and export the results [1]. Then paste previously created and copied Regions of Interest of the same size for subsequent

tumors [3].

4.5.1. LAB MEDIA: 5-Measuring RO and light.MP4 00:05–01:02

4.5.2. LAB MEDIA: 5-Measuring RO and light.MP4 01:10–01:31

4.6. Use a calibrated digital caliper to measure tumor length, width, and depth [1].

4.6.1. LAB MEDIA: 5-Caliper measurment.MP4 full 00:08-00:23

Results

5. Results

5.1. Bioluminescence imaging enables sensitive real-time imaging of small tumor lesions [1], with homogeneous signals [2]. A linear correlation was observed between tumor volume and total light flux, confirming that bioluminescence accurately reflects tumor size [3].

5.1.1. LAB MEDIA: Figure 1A.

5.1.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the plotted triangle points aligning along the diagonal line on the graph.*

5.1.3. LAB MEDIA: Figure 1C. *Video editor: Highlight the black dots*

5.2. Tumor volume estimates calculated with Formula 1 significantly overestimated actual volume compared to Formula 2 [1].

5.2.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the taller Formula 1 column*

1. Bioluminescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/bioluminescent>
IPA: /ˌbaɪ.luː.məˈnes.ənt/
Phonetic Spelling: bye·oh·loo·muh·nes·uhnt
2. Luciferin
Pronunciation link: <https://www.howtopronounce.com/luciferin>
IPA: /luːˈsɪf.ə.ɪn/
Phonetic Spelling: loo·sif·er·in
3. Potassium
Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium>
IPA: /pəˈtæs.i.əm/
Phonetic Spelling: puh·tas·ee·uhm
4. Substrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/substrate>

- IPA: /'sʌb.streɪt/
Phonetic Spelling: sub-strayt
5. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪ'krɑː.mə.tər/
Phonetic Spelling: my-krah-muh-ter
6. Extracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/extracellular>
IPA: /,ek.strə'sel.jə.lə/
Phonetic Spelling: ek-struh-sel-yuh-ler
7. Micropipette
Pronunciation link: <https://www.howtopronounce.com/micropipette>
IPA: /,maɪ.kroʊ.pɪ'pet/
Phonetic Spelling: my-kroh-pih-pet
8. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'æl.i.kwɑːt/
Phonetic Spelling: al-ih-kwot
9. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æs.pə,et/
Phonetic Spelling: as-puh-rayt
10. Adherent
Pronunciation link: <https://www.merriam-webster.com/dictionary/adherent>
IPA: /əd'hɪr.ənt/
Phonetic Spelling: ad-heer-uhnt
11. Residual
Pronunciation link: <https://www.merriam-webster.com/dictionary/residual>
IPA: /rɪ'zɪdʒ.u.əl/
Phonetic Spelling: ri-zij-oo-uhl
12. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪp.sɪn/
Phonetic Spelling: trip-sin
13. EDTA
Pronunciation link: <https://www.howtopronounce.com/edta>
IPA: /,iː.diː.ti:'eɪ/
Phonetic Spelling: ee-dee-tee-ay
14. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪn.kjuː,bet/
Phonetic Spelling: in-kyoo-bayt
15. Detachment
Pronunciation link: <https://www.merriam-webster.com/dictionary/detachment>
IPA: /dɪ'tætʃ.mənt/
Phonetic Spelling: dih-tach-muhnt

16. Inverted
Pronunciation link: <https://www.merriam-webster.com/dictionary/invert>
IPA: /ɪnˈvɜː.tɪd/
Phonetic Spelling: in-vur-tuhd
17. Neutralize
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralize>
IPA: /ˈnuː.trəˌlaɪz/, /ˈnjuː.trəˌlaɪz/
Phonetic Spelling: noo-truh-lyze; nyoo-truh-lyze
18. Serological
Pronunciation link: <https://www.merriam-webster.com/dictionary/serological>
IPA: /ˌsɪr.əˈlɑː.dʒɪ.kəl/
Phonetic Spelling: seer-uh-loj-ih-kuhl
19. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen.trəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj
20. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /səˈspen.ʃən/
Phonetic Spelling: suh-spen-shuhn
21. Homogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /ˌhoʊ.məˈdʒiː.ni.əs/
Phonetic Spelling: hoh-muh-jee-nee-uhs
22. Neubauer
Pronunciation link: <https://www.howtopronounce.com/neubauer>
IPA: /ˈnoʊ.baʊ.ə/
Phonetic Spelling: noy-bow-er
23. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /ˌvaɪ.əˈbɪl.ə.ti/
Phonetic Spelling: vy-uh-bil-uh-tee
24. Trypan
Pronunciation link: <https://www.howtopronounce.com/trypan>
IPA: /ˈtraɪ.pæn/
Phonetic Spelling: try-pan
25. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /ˌbaɪ.oʊˈseɪf.ti/
Phonetic Spelling: bye-oh-sayf-tee
26. Cryovial
Pronunciation link: <https://www.howtopronounce.com/cryovial>
IPA: /ˈkraɪ.oʊˌvaɪ.əl/
Phonetic Spelling: cry-oh-vy-uhl
27. Dissociation
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>

IPA: /dɪ.sʊʊ.si'eɪ.fən/

Phonetic Spelling: dih·soh·see·ay·shuhn

28. Anesthetic

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

IPA: /,æn.əs'θet.ɪk/

Phonetic Spelling: an·uhs·thet·ik

29. Intraperitoneal

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

IPA: /,ɪn.trə.per.ɪ.tə'ni:.əl/

Phonetic Spelling: in·truh·per·ih·tuh·nee·uhl

30. Recumbence

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

IPA: /rɪ'kʌm.bəns/

Phonetic Spelling: ri·kum·buhns

31. Cranial

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

IPA: /'kreɪ.ni.əl/

Phonetic Spelling: kray·nee·uhl

32. Abdominal

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

IPA: /æb'dɑ:.mə.nəl/

Phonetic Spelling: ab·dom·uh·nuhl

33. Caliper

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

IPA: /'kæl.ə.pə/

Phonetic Spelling: kal·uh·per