**Submission ID #: 68290**
**Scriptwriter Name: Poornima G**
**Project Page Link: https://review.jove.com/account/file-uploader?src=20828498**

# Title: In Ovo Xenografting of Patient-Derived Acute Lymphoblastic Leukemia (ALL) Cells (PDX-ALL)

**Authors and Affiliations:**

**Jung Kwon Lee[1], Lian Willetts[2], Jan Storek[3], Karl Riabowol[4], Myung Yung Jeong[5], Ki-Young Lee[2]**

[1]Department of Biochemistry & Molecular Biology, Arnie Charbonneau Cancer and Alberta Children's Hospital Research Institutes, University of Calgary
[2]Department of Cell Biology & Anatomy, Arnie Charbonneau Cancer and Alberta Children's Hospital Research Institutes, University of Calgary
[3]Department of Medicine, Oncology, and Microbiology/Immunology/Infectious Diseases, Arnie Charbonneau Cancer and Alberta Children's Hospital Research Institutes, University of Calgary
[4]Department of Biochemistry & Molecular Biology and Oncology, Arnie Charbonneau Cancer and Alberta Children's Hospital Research Institutes, University of Calgary
[5]Cogno-Mechatronics Engineering, Pusan National University

**Corresponding Authors:**
Ki-Young Lee kyleecal1957@gmail.com

**Email Addresses for All Authors:**
Jung Kwon Lee jungkwon.lee@ucalgary.ca
Lian Willetts lian.willetts@ucalgary.ca
Jan Storek jstorek@ucalgary.ca
Karl Riabowol karl@ucalgary.ca
Myung Yung Jeong myjeong@pusan.ac.kr
Ki-Young Lee kyleecal1957@gmail.com

# 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 the interviews here: **MM/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

**Current Protocol Length**

Number of Steps: 19
Number of Shots: 30

# Introduction

**NOTE to VO team**: Please record the Introduction statements.

**NOTE to editor:** The interview questions and answers are edited to remove personal pronouns, as our team will record them.

1.1. The scope of this research is cancer biology, with a focus on identifying therapeutic strategies to treat cancer **[1].**

1.1.1. *2.3.1*

What are the current experimental challenges?

1.2. The In Ovo patient-derived xenograft-acute lymphoblastic leukemia model is constrained by a relatively short experimental window of around 10 days, limiting long-term studies on acute lymphoblastic leukemia progression or drug response beyond this timeframe **[1].**

1.2.1. *2.5.1*

What significant findings have been established in this field?

1.3. This protocol establishes a rapid, cost-effective method for In Ovo xenografting of patient-derived acute lymphoblastic leukemia cells, including B-cell and T-cell lineages **[1].**

1.3.1. *3.5.1*

How will the findings advance research in the field?

1.4. This protocol represents a promising tool for preclinical drug screening, mechanistic studies, and potentially personalized medicine approaches in leukemia research **[1].**

1.4.1. *4.2.1*

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

1.5. The feasibility of using the established In Ovo xenograft model for preclinical applications will be explored **[1].**

1.5.1. *3.4.1*

# Protocol

**NOTE to VO team**: Please record the Introduction statements also.

## 2. Preparation of Egg and Cell Samples

**Demonstrator:** Jungkwon Lee

2.1. To begin, transfer the procured eggs into a humidified rolling incubator set at approximately 50 to 60 percent humidity and a temperature of 39 degrees Celsius **[1]**. Incubate the eggs in this incubator for 10 days post-fertilization **[2]**.

2.1.1. LAB MEDIA: Jove 68290-2 00:00-00:10.

2.1.2. LAB MEDIA: Jove 68290-3.

2.2. Transfer the blood samples into a sterile 50 milliliter centrifuge tube and dilute each sample three times using two volumes of PBS **[1]**.

2.2.1. LAB MEDIA: Jove 68290-5 00:08-00:16.

2.3. After centrifuging the sample along with a density gradient medium, collect the buffy coat layer of mononuclear cells from the interface **[1]** and transfer it to a new sterile 15-milliliter tube **[2]**. Add 10 milliliters of PBS *(P-B-S)* to the tube and centrifuge the tubes at 300 *g* for 5 minutes to remove remaining serum components **[3]**.

2.3.1. LAB MEDIA: Jove 68290-6.

2.3.2. LAB MEDIA: Jove 68290-7 00:10-00:20

2.3.3. LAB MEDIA: Jove 68290-9.

2.4. Resuspend the pelleted cells in RPMI 1640 *(R-P-M-I-sixteen-forty)* medium supplemented with 10 percent FBS *(F-B-S)* **[1]**. Use a hemocytometer to count the number of cells **[2]**.

2.4.1. LAB MEDIA: Jove 68290-8 00:05-00:09.

2.4.2. LAB MEDIA: Jove 68290-11

2.5. After resuspending the cells in freezing medium consisting of 10 percent DMSO *(D-M-S-O)* in FBS **[1].** Assess cell viability using the Trypan Blue Exclusion assay **[2]** and freeze the cells at minus 80 degrees Celsius and store them in liquid nitrogen **[3]**.

2.5.1. LAB MEDIA: Jove 68290-12 00:04-00:07

2.5.2. LAB MEDIA: Jove 68290-10

2.5.3. LAB MEDIA: Jove 68290-13

2.6. Next, co-transfect 1.5 million HEK293T *(Heck-Two-Nine-Three-T)* cells with the desired plasmid vector using Lipofectamine 3000 and incubate the transfected cells for 2 days **[1]**.

2.6.1. LAB MEDIA: Jove 68290-17.

2.7. Harvest the supernatant containing lentivirus from the wells **[1]** and filter it through a 0.45 micrometer filter to remove debris **[2]**.

2.7.1. LAB MEDIA: Jove 68290-19 00:00-00:20.

2.7.2. LAB MEDIA: Jove 68290-20 00:00-00:10.

2.8. Transfer the filtered viral supernatant into centrifuge tubes and centrifuge at 20,000 *g* for 2 hours at 4 degrees Celsius **[1-TXT]**.

2.8.1. LAB MEDIA: Jove 68290-21 00:00-00:10. **TXT: Resuspend the viral pellet in 100 µL PBS; Perform qPCR**

2.9. For labelling, plate 1 million cells per milliliter of B-cell precursor SEM *(sem)* and T-cell MOLT3 *(molt-3)* cell lines, as well as patient-derived B- or T-acute lymphoblastic leukemia cells **[1]**, into 6-well plates containing RPMI 1640 with 10% FBS **[2]**.

2.9.1. LAB MEDIA: Jove 68290-24 00:06-00:25

2.9.2. TEXT ON PLAIN BACKGROUND:

Infect the cells with the lentiviral preparation

Incubate at 37 °C with 5% $CO_2$ for 2 days

Add 1 µg/mL puromycin to the culture medium for selection

2.10. Visualize mCherry*(M-Cherry)*-labeled cells under a fluorescence microscope **[1]**.

2.10.1. LAB MEDIA: Jove 68290-25 and Jove 68290-26 side by side.

## 3. In Ovo Xenografting and Imaging

3.1. On day 10, examine the vasculature of the chick embryos under light to assess embryo viability **[1]**. Gently wash the surface of each egg with 70 percent ethanol **[2]**.

3.1.1. LAB MEDIA: Jove 68290-28 00:10-00:15

3.1.2. LAB MEDIA: Jove 68290-28 00:40-00:48

3.2. Using a hand drill, drill into the air cell of each egg to create a small window approximately 2 centimeters in diameter **[1]**.

3.2.1. LAB MEDIA: Jove 68290-28 01:05-01:15

3.3. Seal the created window with transparent adhesive tape **[1]** and return the eggs to a 5 percent carbon dioxide incubator **[2]**.

3.3.1. LAB MEDIA: Jove 68290-29

3.3.2. LAB MEDIA: Jove 68290-30 00:10-00:16

3.4. On day 11, inject 10 million mCherry-labeled acute lymphoblastic leukemia cells into the blood vessels of the developing embryos using 34-gauge needles **[1]**.

3.4.1. LAB MEDIA: Jove 68290-32

3.5. After sealing the window, return the eggs to a 5 percent carbon dioxide incubator, and monitor embryo viability daily **[1]**.

3.5.1. LAB MEDIA: Jove 68290-30 00:17-00:22

3.6. On day 15, dissect the blood vessels out from the embryos, place them on glass slides **[1]**, and photograph successful xenografts using a dissecting microscope equipped with fluorescence capabilities **[2]**.

3.6.1. LAB MEDIA: Jove 68290-39

3.6.2. LAB MEDIA: Jove 68290-42.

3.7. Collect blood from the vasculature of chicken embryos using a sterile syringe with a 32-gauge needle **[1]**. Transfer the blood into a 1.5 milliliter sterile tube containing heparin

and centrifuge at 226 g for 10 minutes at 4 degrees Celsius **[2]**.

3.7.1. LAB MEDIA: Jove 68290-43 00:16-00:23

3.7.2. LAB MEDIA: Jove 68290-45 00:20-00:30

3.8. Label B-cell acute lymphoblastic leukemia cells with human FITC *(fit-C)*-CD10 *(C-D-Ten)* and human PE *(P-E)*-CD19 *(C-D-Nineteen)* antibodies, and T-cell acute lymphoblastic leukemia cells with human FITC-CD4 *(C-D-Four)* and human PE-CD8 *(C-D-Eight)* antibodies at 4 degrees Celsius in the dark for 30 minutes **[1]**.

3.8.1. LAB MEDIA: Jove 68290-46.

3.9. Wash the labeled cells twice using PBS containing 1 percent BSA *(B-S-A)* and analyze using flow cytometry **[1]**.

3.9.1. LAB MEDIA: Jove 68290-47

# Results

## 4. Results

4.1. Vascular colonization by SEM and MOLT3 cell lines was clearly visible 4 days post-injection, confirming successful engraftment in the developing chicken embryo vasculature **[1]**.

4.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the SEM and MOLT3 panels in the bottom row*

4.2. Patient-derived B-ALL *(B-all)* and T-ALL *(T-all)* cells showed strong fluorescent signals in the blood vessels at 4 days post-injection, indicating active colonization and proliferation in the vasculature **[1]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the B-all and T-all images in the bottom row*

4.3. Flow cytometry revealed a significant increase in CD10-CD19-positive cells in embryos injected with SEM and patient-derived B-ALL cells at 4 days post-injection **[1]**, compared to controls collected 6 hours post-injection **[2]**.

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight '4 days after injection' panel for B-ALL row*

4.3.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the "6 hours after injection" panel B-ALL row*

4.4. Similarly, CD4-CD8-positive cells were significantly higher in embryos injected with MOLT3 and patient-derived T-ALL cells at 4 days post-injection **[1]**.

4.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the 4 days after injection' panel for T-ALL row*

**Pronunciation Guide**:

**Mononuclear**
Pronunciation link: https://www.merriam-webster.com/dictionary/mononuclear
IPA: /ˌmɑː.noʊˈnuː.kli.ɚ/
Phonetic Spelling: mah-noh-noo-klee-er

**Hemocytometer**
Pronunciation link: https://www.merriam-webster.com/dictionary/hemocytometer
IPA: /ˌhiː.moʊ.saɪˈtɑː.mɪ.tɚ/
Phonetic Spelling: hee-moh-sy-tah-muh-ter

**Trypan Blue**
Pronunciation link: https://www.howtopronounce.com/trypan
IPA: /ˈtraɪ.pæn bluː/
Phonetic Spelling: try-pan bloo

**Lipofectamine**
Pronunciation link: No confirmed link found
IPA: /ˌlaɪ.poʊˈfɛk.tə.miːn/
Phonetic Spelling: ly-poh-fek-tuh-meen

**Lentivirus**
Pronunciation link: https://www.merriam-webster.com/dictionary/lentivirus
IPA: /ˈlɛn.tiˌvaɪ.rəs/
Phonetic Spelling: len-tee-vy-ruhs

**Micrometer** (used for filter size)
Pronunciation link: https://www.merriam-webster.com/dictionary/micrometer
IPA: /maɪˈkrɑː.mɪ.tɚ/
Phonetic Spelling: my-krah-mi-ter

**Precursor**
Pronunciation link: https://www.merriam-webster.com/dictionary/precursor
IPA: /ˈpriˌkɝː.sɚ/
Phonetic Spelling: pree-kur-ser

**Leukemia**
Pronunciation link: https://www.merriam-webster.com/dictionary/leukemia
IPA: /luˈkiː.mi.ə/
Phonetic Spelling: loo-kee-mee-uh

**Xenografting**
Pronunciation link: https://www.merriam-webster.com/dictionary/xenograft
IPA: /ˈzɛn.əˌgræf.tɪŋ/
Phonetic Spelling: zen-uh-graft-ing

**Vasculature**
Pronunciation link: https://www.merriam-webster.com/dictionary/vasculature
IPA: /ˈvæs.kjə.ləˌtʃɚ/
Phonetic Spelling: vas-kyuh-luh-chur

**Puromycin**
Pronunciation link: https://www.merriam-webster.com/dictionary/puromycin
IPA: /pjʊˈroʊ.məˌsɪn/
Phonetic Spelling: pyoo-roh-muh-sin

**Fluorescence**
Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
IPA: /flʊˈrɛs.əns/ or /flɔːˈrɛs.əns/
Phonetic Spelling: floo-res-ens

**Dissect**
Pronunciation link: https://www.merriam-webster.com/dictionary/dissect
IPA: /daɪˈsɛkt/ or /dɪˈsɛkt/
Phonetic Spelling: dye-sekt or dih-sekt

**Heparin**
Pronunciation link: https://www.merriam-webster.com/dictionary/heparin
IPA: /ˈhɛp.ə.rɪn/
Phonetic Spelling: hep-uh-rin

**Cytometry** (from flow cytometry)
Pronunciation link: https://www.merriam-webster.com/dictionary/cytometry
IPA: /saɪˈtɑː.mə.tri/
Phonetic Spelling: sy-tah-muh-tree

**Colonization**
Pronunciation link: https://www.merriam-webster.com/dictionary/colonization
IPA: /ˌkɑː.lə.nəˈzeɪ.ʃən/
Phonetic Spelling: kah-luh-nuh-zay-shun

**Engraftment**
Pronunciation link: https://www.merriam-webster.com/dictionary/engraft

IPA: /ɪnˈgræft.mənt/
Phonetic Spelling: in-graft-ment

**Proliferation**
Pronunciation link: https://www.merriam-webster.com/dictionary/proliferation
IPA: /prəˌlɪf.əˈreɪ.ʃən/
Phonetic Spelling: pruh-lif-uh-ray-shun