FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69325
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21144173

Title: Optimized Quantitative Assessment of Enhancer RNA Stability in Mouse Embryonic Stem Cells

Authors and Affiliations:
Jiin Moon, Hyohi Lee, Yeonju Jang, Seung-Kyoon Kim

Department of Convergent Bioscience and Informatics, and Graduate School of Biological Sciences, Chungnam National University (CNU)

Corresponding Authors:
Seung-Kyoon Kim (sk.kim@cnu.ac.kr)

Email Addresses for All Authors:
Jiin Moon (answldls1004@naver.com)
Hyohi Lee (gygml3579@naver.com)
Yeonju Jang (yeonju2417@naver.com)
Seung-Kyoon Kim (sk.kim@cnu.ac.kr)

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: 05
Number of Shots: 10

Introduction

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

INTRODUCTION:

1.1. **Seung-Kyoon Kim:** Our lab studies epigenetics, focusing on RNA modification and enhancers at the molecular level.

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

1.2. **Jiin Moon:** We recently found that the RNA m5C writer enzyme NSUN2 is regulated by an intragenic enhancer in mouse embryonic stem cells.

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

CONCLUSION:

1.3. **Jiin Moon:** Our study provides a method to measure and normalize the short half-life of rapidly degrading enhancers.

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

1.4. **Jiin Moon:** We address the lack of clear methods for comparing intergenic and intragenic enhancer activity.

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

1.5. **Jiin Moon:** It simplifies data processing and allows fast calculation of enhancer decay using clear Prism steps.

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.

Protocol

2. Culturing Mouse Embryonic Stem Cells and Actinomycin D Treatment for Time-Course Analysis

Demonstrator: Jiin Moon

2.1. To begin, pre-coat the wells of a 6-well cell culture plates with 2 milliliters of gelatin per well **[1]**. Incubate the plates at 37 degrees Celsius for 10 minutes **[2]**.

2.1.1. WIDE: Talent pipetting 2 milliliters of 0.1 percent gelatin into each well of a 6-well plate.

2.1.2. Talent placing the 6-well plate into the incubator set to 37 degrees Celsius.

2.2. Aspirate the gelatin from each well **[1]**. Then wash each well once with 1 milliliter of PBS **[2]**. Sterilize the plates by placing them under ultraviolet light for at least 15 minutes **[3]**.

2.2.1. Talent aspirating gelatin from the wells using a vacuum aspirator.

2.2.2. Talent pipetting 1 milliliter of phosphate-buffered saline into each well and swirling gently.

2.2.3. Talent placing the 6-well plate under a UV sterilizer.

2.3. Seed mouse embryonic stem cells onto the gelatin-coated plates using 2 milliliters of complete cell culture medium per well **[1]**. Incubate the cells at 37 degrees Celsius in a humidified incubator with 5 percent carbon dioxide **[2]**.

2.3.1. Talent pipetting 2 milliliters of cell culture medium containing mouse embryonic stem cells into each well of the gelatin-coated plate.

2.3.2. Talent placing the 6-well plate into a humidified incubator set to 37 degrees Celsius with 5 percent carbon dioxide.

2.4. Next, dissolve actinomycin D in dimethyl sulfoxide to prepare a stock solution at a concentration of 1 milligram per milliliter **[1]**.

2.4.1. Talent pipetting dimethyl sulfoxide into a vial and adding actinomycin D to prepare the stock solution.

2.5. Two days after seeding the cells, or on the day of harvest, add the actinomycin D stock solution to the culture medium **[1-TXT]**. Collect samples at 0, 5, 10, 15, 20, and 30 minutes after treatment **[2]**.

2.5.1. Talent pipetting the actinomycin D stock solution into the wells to reach the final concentration. **TXT: Final concentration: 5 µg/mL**

2.5.2. Talent collecting cell samples at the specified time points after actinomycin D treatment into labeled tubes.

Results

3. Results

3.1. Following transcriptional arrest, the expression of enhancer RNAs decreased rapidly within 5 minutes **[1]**, while messenger RNAs declined more gradually and remained relatively stable over 30 minutes **[2]**.

3.1.1. LAB MEDIA: Figure 2A–C. *Video editor: Highlight the steep decline in the yellow, orange, and purple lines labeled as eRNAs within the first 5 minutes.*

3.1.2. LAB MEDIA: Figure 2A–C. *Video editor: Highlight the green, dark blue, and red lines labeled as mRNAs that show only a slight decrease or appear stable over the entire 30-minute timeline.*

3.2. Half-life analysis revealed that enhancer RNAs exhibited a half-life of approximately 2 to 3 minutes **[1]**, while messenger RNAs had a significantly longer half-life exceeding 60 minutes **[2]**.

3.2.1. LAB MEDIA: Figure 3A. *Video editor: Sequentially highlight the three bars labeled "Nsun2_eRNA", "Pou5f1_eRNA", and "Sox2_eRNA"*

3.2.2. LAB MEDIA: Figure 3B. *Video editor: Sequentially highlight the three bars labeled "Nsun2_mRNA", "Pou5f1_mRNA", and "Sox2_mRNA"*

3.3. For the *Pou5f1 (P-O-U-Five-F-One)* intergenic enhancer RNA, normalization to *Tbp (T-B-P)* messenger RNA or to the cycle threshold value at 0 minutes produced similar half-life estimates **[1]**. Nascent RNA sequencing data showed elevated RPKM *(R-P-K-M)* values at the Nsun2 *(N-sun-Two)* enhancer region compared to the negative control **[2]**.

3.3.1. LAB MEDIA: Figure 4A. *Video editor: Sequentially highlight the two bars*

3.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the blue bar labeled "Nsun2_Enhancer"*

3.4. RT-qPCR analysis confirmed higher normalized expression of Nsun2 intragenic enhancer RNA in the Nsun2 enhancer region compared to the negative control **[1]**.

3.4.1. LAB MEDIA: Figure 4C. Video editor: *Highlight the taller dark bar labeled "Nsun2_Enhancer"*

- gelatin

Pronunciation link: https://www.merriam-webster.com/dictionary/gelatin
IPA: /ˈdʒɛlətɪn/
Phonetic spelling: jel-uh-tin

- incubate
Pronunciation link: https://www.merriam-webster.com/dictionary/incubate
IPA: /ˈɪŋkjubeɪt/
Phonetic spelling: ing-kyoo-bayt

- aspirate
Pronunciation link: https://www.merriam-webster.com/dictionary/aspirate
IPA: /ˈæspəˌreɪt/
Phonetic spelling: as-puh-rayt

- phosphate
Pronunciation link: https://www.merriam-webster.com/dictionary/phosphate
IPA: /ˈfɑsˌfeɪt/
Phonetic spelling: fos-fayt

- buffered
Pronunciation link: https://www.merriam-webster.com/dictionary/buffered
IPA: /ˈbʌfərd/
Phonetic spelling: buf-erd

- saline
Pronunciation link: https://www.merriam-webster.com/dictionary/saline
IPA: /ˈseɪˌlaɪn/
Phonetic spelling: say-lyn

- dimethyl
Pronunciation link: https://www.merriam-webster.com/dictionary/dimethyl
IPA: /ˌdaɪˈmɛθəl/
Phonetic spelling: dye-meth-uhl

- sulfoxide
Pronunciation link: https://www.merriam-webster.com/dictionary/sulfoxide
IPA: /səlˈfɑksaɪd/
Phonetic spelling: sul-fok-syde

- actinomycin
Pronunciation link: https://www.merriam-webster.com/dictionary/actinomycin
IPA: /ˌæk.tɪˈnoʊmɪsɪn/
Phonetic spelling: ak-ti-noh-mi-sin

- embryonic
Pronunciation link: https://www.merriam-webster.com/dictionary/embryonic
IPA: /ˌɛm.briˈɑnɪk/
Phonetic spelling: em-bree-ah-nik

- transcriptional

Pronunciation link: https://www.merriam-webster.com/dictionary/transcriptional
IPA: /trænsˈkrɪpʃənəl/
Phonetic spelling: trans-krip-shuh-nul

- intragenic

Pronunciation link: https://www.merriam-webster.com/dictionary/intragenic
IPA: /ˌɪntrəˈdʒɛnɪk/
Phonetic spelling: in-truh-jen-ik

- nascent

Pronunciation link: https://www.merriam-webster.com/dictionary/nascent
IPA: /ˈneɪsənt/
Phonetic spelling: nay-suhnt

- sequencing

Pronunciation link: https://www.merriam-webster.com/dictionary/sequence
IPA: /ˈsiːkwəns/ for "sequence" — so "sequencing" => /ˈsiːkwənsɪŋ/
Phonetic spelling: see-kwens-ing

- normalization

Pronunciation link: https://www.merriam-webster.com/dictionary/normalization
IPA: /ˌnɔrmələˈzeɪʃən/
Phonetic spelling: nor-muh-luh-zay-shun

- RNA

Pronunciation link: https://www.merriam-webster.com/dictionary/RNA
IPA: /ˌɑːrɛnˈeɪ/
Phonetic spelling: ar-en-ay

- RPKM

(As an acronym, pronounce letter by letter)
Pronunciation link: No confirmed link found
Phonetic spelling: ar-pee-kay-em

- RT-qPCR

(As an acronym/initialism, pronounce letter-by-letter)
Pronunciation link: No confirmed link found
Phonetic spelling: ar-tee-cue-pee-see-ar