FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68483
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20887363

Title: Split Hybridization Probe Utilizing a DNA Fluorescent Light-up Aptamer as a Signal Reporter for Sequence-Specific Nucleic Acid Analysis

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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? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 15
Number of Shots: 32

Introduction

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

REQUIRED:

1.1. **Yulia Gerasimova:** We are developing new types of fluorescent hybridization probes for sequence-specific nucleic acid analysis.
   1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9.1*

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

1.2. **Justine Monsalve:** Most recently, hybridization analysis tools have expanded with assays that take advantage of CRISPR-Cas systems.
   1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Sug*
   1.2.2.

What technologies are currently used to advance research in your field?

1.3. **Justine Monsalve:** Currently, such state-of-the art hybridization probes as TaqMan and Molecular Beacon probes are most used to analyze nucleic acid targets.
   1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.6.3*

What are the current experimental challenges?

1.4. **Justine Monsalve:** Accurate detection of single-nucleotide substitutions in nucleic acid targets is still practically challenging.
   1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.*3

What research gap are you addressing with your protocol?

1.5. **Yulia Gerasimova:** Our protocol ensures the required level of selectivity, while providing a label-free fluorescent signal readout.
   1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.4*

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

Testimonial Questions:

Videographer:

- ***Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***
- ***Also, kindly note that testimonial statements will be presented live by the authors, offering their spontaneous perspectives.***

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Yulia Gerasimova, Associate Professor**: (authors will present their testimonial statements live).

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

Protocol

2. Construction and In Silico Validation of SLAS Strands
Demonstrator: Yulia Gerasimova

2.1. To begin, design the sequences of two unmodified DNA oligonucleotide strands constituting SLAS **[1-TXT].** Use a fragment containing nucleotides 4, 6 to 39 of DAP-10-42 *(D-A-P-Ten-Forty-Two)* **[2]**.

2.1.1. WIDE: Talent launching program for DNA strand design. **TXT: SLAS: Split Light-up Aptamer Sensor**

2.1.2. SCREEN: 68483_2.1-2.2.mp4. 00:00-00:06

2.2. Convert stem 1 consisting of nucleotides 1 to 8 and 36 to 42 into stem 1 prime by removing a bulging thymine at position 5, shortening the stem to 4 base pairs and adding a terminal cytosine-guanine base pair **[1]**. Include nucleotides 9 to 29 in one SLAS strand and nucleotides 30 to 35 in the other **[2]**.

2.2.1. SCREEN: 68483_2.1-2.2.mp4. 00:07-00:25
Video Editor: Please highlight blue stem labelled stem 1

2.2.2. SCREEN: 68483_2.1-2.2.mp4. 00:26-00:33

2.3. Extend the 3-prime terminal sequence of the fragment with nucleotides 9 to 29 by adding d(GGTCAT) *(D-G-G-T-C-A-T)* **[1]**. Then extend the 5-prime terminal sequence of the 30 to 35-fragment with d(ATGACC) *(D-A-T-G-A-C-C)* to form a 6-base pair stem 2 **[2]**.

2.3.1. SCREEN: 68483_2.3-2.4.mp4 00:00-00:09

2.3.2. SCREEN: 68483_2.3-2.4.mp4. 00:10-00:19

2.4. Now extend stem 1 prime on both strands with d(TT) *(D-T-T)* linkers and DNA sequences complementary to the nucleic acid target **[1]**. This yields the sequences of strands SLAS-U *(See-lus-U)* and SLAS-S *(See-lus-S)* constituting the probe **[2].** NOTE: The VO has been slightly edited.

2.4.1. SCREEN: 68483_2.3-2.4.mp4. 00:20-00:26

2.4.2. SCREEN: 68483_2.3-2.4.mp4. 00:27-00:32

2.5. Make SLAS-S complementary to a 7 to 10 nucleotide region that includes the single-nucleotide substitution site **[1]**. Then ensure that the target-binding arm of SLAS-U is complementary to a 15 to 25 nucleotide fragment adjacent to the SLAS-S region **[2]**.

2.5.1. SCREEN: 68483_2.5.mp4. 00:00-00:09

2.5.2. SCREEN: 68483_2.5.mp4. 00:10-00:19

2.6. Assess the melting temperatures of the target-binding duplexes using the UNAFold *(U-Na-Fold)* web server **[1]**. Click on the **DINAMelt** *(Die-Na-Melt)* tab, go to **Applications**,

and select **Two State Melting Hybridization [2]**.

2.6.1. SCREEN: 68483_2.6-2.7.mp4. 00:01
Video Editor: Please freeze frame here

2.6.2. SCREEN: 68483_2.6-2.7.mp4. 00:02-00:06

2.7. Enter the interacting sequences in 5-prime to 3-prime order in the left and right boxes, **[1].** Then adjust the assay temperature to 22 degrees Celsius and input the monovalent and divalent cation concentrations to 20 millimolar and 25 millimolar ions, respectively **[2-TXT]**. Press **Submit** and review the Gibbs energy change, enthalpy, entropy, and melting temperature values for the corresponding duplexes **[3]**.

2.7.1. SCREEN: 68483_2.6-2.7.mp4. 00:07-00:29

2.7.2. SCREEN:68483_2.6-2.7.mp4. 00:47-00:56
TXT: Indicate concentration of interacting sequences

2.7.3. SCREEN: 68483_2.6-2.7.mp4. 00:57-01:04

2.8. Confirm that the melting temperatures for perfectly matched targets and the target-binding arms of SLAS-S and SLAS-U are above the assay temperature, 22 degrees Celsius **[1]**. Ensure that the duplex between SLAS-S and a mismatched target yields melting temperatures below the assay temperature to maintain specificity **[2-TXT]**. If necessary, adjust the lengths of target-binding arms to meet these conditions **[3]**.

2.8.1. SCREEN: 68483_2.8_updated.mp4.00:00-00:10

2.8.2. SCREEN: 68483_2.8_updated.mp4. 00:11-00:15
TXT: This prevents AO fluorogen from signaling the presence of the targeted nucleic acid analyte if it contains an SNS in the SLAS-binding fragment

2.8.3. Talent modifying sequence lengths in the design interface to optimize melting temperatures.

2.9. Obtain the finalized SLAS-U and SLAS-S oligonucleotide strands from a commercial DNA supplier or synthesize them in-house using an automated DNA synthesizer **[1]**.

2.9.1. Shot of labelled vials containing synthesized SLAS-U and SLAS DNA oligonucleotide strands.

3. SLAS Assay Setup and Fluorescence Measurement
Demonstrator: Justine Monsalve

3.1. Prepare stock solutions of auramine O, SLAS-S, SLAS-U, and assay buffer **[1]**. NOTE: The VO has been slightly edited.

3.1.1. Talent labeling the stock tubes of auramine O, SLAS-S, SLAS-U, and assay buffer.
AND
TEXT ON PLAIN BACKGROUND:

Stock Solutions:
Auramine O: 0.1 mM in DMSO, 10x
SLAS-S: 10 µM in nuclease-free water, 10x
SLAS-U: 10 µM in nuclease-free water, 10x
4x assay buffer: 80 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 80 mM KCl
Video Editor: Please play both shots side by side

3.2. Prepare the master mix containing all assay components but the target **[1]**. Add nuclease-free water to the final five by sixths volume of the master mix **[2].**

3.2.1. Talent preparing master mix. **TXT: Multiply the sample volume by the number of samples plus one, for master mix volume**

3.2.2. Shot of nuclease free water being added to the master mix.

3.3. Then vortex and spin the master mix **[1]**. Dispense 50 microliters into each sample tube **[2]**.

3.3.1. Talent placing the vortexed prepared mix in a centrifuge.

3.3.2. Talent pipetting 50 microliters of master mix into individual tubes.

3.4. Next, label one sample as a no-target blank and one as a positive control **[1]**. Add 10 microliters of target-containing sample to a tube containing the master mix to make a 60-microliter sample **[2-TXT]**.

3.4.1. Talent labelling a no-target blank tube and a positive control tube.

3.4.2. Talent adding target sample to reaction tubes. **TXT: Final volume: 60 µL with 10 - 1000 nM target**

3.5. For the blank, add 10 microliters of nuclease-free water **[1].** Then, pipette 10 microliters of synthetic DNA oligonucleotide containing the target sequence into the positive control tube **[2].** NOTE: The VO has been edited.

3.5.1. Shot of 10 µL nuclease-free water being pipetted into the blank.

3.5.2. Talent adding 10 µL synthetic DNA oligonucleotide containing the target sequence into the positive control tube

~~3.5.3. Talent adding 10 microliters of the 0.6 to 3 micromolar control to the positive control tube.~~ NOTE: This shot was not filmed during the shoot.

3.6. Mix all samples and centrifuge briefly using a microcentrifuge **[1]**. Then incubate the tubes at 22 degrees Celsius for 10 to 60 minutes **[2]**. Measure fluorescence at 540 nanometers upon excitation at 475 nanometers using a fluorescence spectrophotometer **[3]**.

3.6.1. Talent spinning down tubes in a microcentrifuge.

3.6.2. Talent placing the tubes in a 22-degree incubator.

3.6.3. Talent placing the tubes into a cuvette and running the fluorescence read on the spectrophotometer.

Results

4. Results

4.1. SLAS was tailored to target a specific fragment of the *NANOGP8 (Nano-G-P-Eight)* gene **[1].** Target M was fully complementary to the SLAS-S strand **[2],** while target MM *(M-M)* had a cytosine at nucleotide position 1423, introducing a mismatch with SLAS-S **[3].**

4.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the row labeled "DAP-10-42"*

4.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the row for "M"*

4.1.3. LAB MEDIA: Table 1. *Video editor: Highlight the row for "MM" sequence*

4.2. Upon addition of the fully complementary target M, fluorescence increased steadily and plateaued after 45 to 50 minutes **[1]**. However, a clear signal was detectable within 10 minutes with a signal-to-blank ratio of 10 **[2]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the sharp rise in fluorescence curve following the arrow marked "target M added*".

4.2.2. LAB MEDIA: Figure 3. *Video editor: Please highlight the curve between 0 to 10*

4.3. SLAS showed high fluorescence signal for fully matched targets, but not for mismatched targets or blanks **[1]**.

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the "M" bars across all three color groups*

4.4. Fluorescence output increased linearly with target concentration up to 500 nanomolar, enabling quantification and determination of detection limits **[1]**.

4.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight the increasing line till 500 nM*

4.5. PCR-amplified samples showed varying levels of signal, with only sample 2 exceeding the fluorescence threshold value of 2 **[1]**. Based on the calibration curve, the concentration of NANOGP8 amplicon in sample 2 was estimated at 124 plus or minus 13 nanomolar **[2]**. NOTE: The VO has been slightly edited.

4.5.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the green "Sample 2" trace*

4.5.2. LAB MEDIA: Figure 6B. *Video editor: Emphasize the tall bar labeled "2"*

4.6. Fluorescence detection of SLAS signal was consistent across both a benchtop spectrophotometer and a portable fluorometer **[1]**. A signal-to-blank ratio above 20 was also visually observed using UV light **[2]**.

4.6.1. LAB MEDIA: Figure 7A and 7B. *Video editor: Highlight green and blue bars*

labeled "M" on both plots

4.6.2. LAB MEDIA: Figure 7C. *Video editor: Zoom in on the glowing PCR tube labeled "M"*

Pronunciation guides:

1. oligonucleotide
Pronunciation link:
https://www.merriam-webster.com/dictionary/oligonucleotide
IPA: /ˌɑlɨgoʊˌnuːˈkliːəˌtaɪd/
Phonetic Spelling: ol-i-go-noo-klee-uh-tyde
2. centrifuge
Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛntrəˌfjudʒ/
Phonetic Spelling: sen-truh-fyooj
3. centrifugation
Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifugation
IPA: /ˌsɛntrəˌfjuːˈgeɪʃən/
Phonetic Spelling: sen-truh-fyoo-gay-shuhn
4. spectrophotometer
Pronunciation link: No confirmed link found
IPA: /ˌspɛk.troʊˌfoʊˈtɑː.mə.tər/
Phonetic Spelling: spek-tro-fo-TAH-muh-ter
5. aptamer
Pronunciation link: No confirmed link found
IPA: /ˈæptəmər/
Phonetic Spelling: AHP-tuh-mur
6. oligoribonucleotide
Pronunciation link:
https://www.merriam-webster.com/medical/oligoribonucleotide
IPA: /ˌɑlɨgoʊˌraɪboʊˌnuːˈkliːəˌtaɪd/
Phonetic Spelling: ol-i-go-rye-bo noo-klee-uh-tyde
7. enthalpy
Pronunciation link: No confirmed link found
IPA: /ˈɛnθəlpi/
Phonetic Spelling: EN-thuhl-pee
8. Gibbs (as in Gibbs energy)

Pronunciation link: No confirmed link found
IPA: /gɪbz/
Phonetic Spelling: gibs
9. fluorogen
Pronunciation link: No confirmed link found
IPA: /ˈflʊərəʊdʒən/
Phonetic Spelling: FLU-uh-ro-jen
10. auramine
Pronunciation link: No confirmed link found
IPA: /ˈɔːrəˌmiːn/
Phonetic Spelling: OR-uh-meen