FINAL SCRIPT: APPROVED FOR FILMING

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Project Page Link: https://review.jove.com/account/file-uploader?src=20876688

Title: Efficient Sampling of Genetically Encoded Biosensor Design Space Enabled with a Design of Experiments and Automation Workflow

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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**

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: 26
Number of Shots: 58

Introduction

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

Videographer's Note: The Authors told me they were unable to book a meeting room for the interview. We were kicked out of the first room we were in due to a booking. We had to look for another room after this for the interview section. This is the reason why the rooms look different in the headshots compared to the interview. The room I shot the headshots in was a lot nicer.

Phillip (Author) slightly altered the words he said in the interview compared to what was written on the script.

1.1. **Philip Le Roy :** The development of and optimization of biosensors for applications in biotechnology is the scope of my research. Specifically, we aim to understand how to engineer biosensors towards desired functions through modulation of their genetic elements.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.5*

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

1.2. **Philip Le Roy:** Intuition driven engineering choices through classical promotor characterization, as well as fluorescence activated cell sorting are technologies routinely applied in biosensor optimization and design.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 4.2*

What research gap are you addressing with your protocol?

1.3. **Philip Le Roy:** Design of experiment methodologies have not yet been widely adopted in genetic circuit design. Through this protocol we seek to encourage wider uptake of this technique in biosensor and genetic circuit design strategies.

1.3.1. 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

Videographer's Note: The date on the clapperboard is incorrect. I had a technical issue with the app when trying to adjust the date. Please disregard what it shows on the board. It should have shown 23rd September 2025

There is a small amount of vignette on some shots in the protocol. This due to a polariser filter I was using on my lens. Some shots required me to be shooting at my widest focal length, which is where the vignette appears. Apologies about this. It was a new filter I recently purchased and it was my first time using it. Hopefully a small amount of scaling in post production will be adequate to compensate for this.

2. Automated Culturing and Barcoding of a Variant Library Using Liquid Handling and Colony Picking Platforms

Demonstrator: Philip Le Roy

2.1. To begin, determine the theoretical library size and calculate the number of individual variants needed to ensure greater than ninety-five percent library coverage **[1]**. Calculate the required volume of antibiotic-supplemented lysogeny broth media according to the number of colonies that are to be screened **[2]**.

2.1.1. WIDE: Talent calculating theoretical library size and determining variant count using paper or computer.

2.1.2. SCREEN: 68448_Screenshot_1.mov 00:02-00:22

2.2. Open the liquid handler software and click on **Run** next to the **MTP Liquid Transfer** program **[1]**. Place the prepared media in the corresponding reservoir position **[2]**, then fill the deck with empty microtiter plates according to the layout **[3]**.

2.2.1. SCREEN: 68448_Screenshot_2.mp4 00:05-00:24

2.2.2. Talent placing media reservoir on liquid handler deck.

2.2.3. Talent loading empty microtiter plates onto deck as per layout.
Videographer's Note: 2.2.2 and 2.2.3 were shot together

Added shot: 2.4.4 : Shot of the machine operating
Videographer's Note: 2.2.4 is an extra shot to show machine operating since I couldn't get this in the previous shot. Take 2 is better

2.3. Set the program to dispense two hundred microliters of media **[1]**. Click **OK** to confirm program start **[2-TXT]**. Then seal the filled microtiter plates with a breathable membrane to maintain sterility **[3]**.

2.3.1. SCREEN: 68448_Screenshot_2.mp4. 00:25-00:33

2.3.2. SCREEN: 68448_Screenshot_2.mp4. 00:34-00:39 **TXT: Repeat program as**

many times as needed

2.3.3. Talent sealing plates with breathable membrane.
Videographer's Note: 2.3.3,2.4.1-2.4.2 were shot together. Clapperboard on this shot had incorrect information by showing 2.4.4 was included. But 2.4.4 was done seperately

2.4. Now, transfer filled microtiter plate wells to a colony picker platform and unseal **[1]**, and transfer square agar plates of *Pseudomonas putida* transformed with plasmid variant library DNA into the colony picker platform **[2]**. Use the colony picker to inoculate each prefilled well with a single colony from the transformant plates **[3]**.

2.4.1. Talent placing plates onto colony picker and unsealing them.

2.4.2. Shot of agar plates of transformed *Pseudomonas putida* being placed into the colony picker platform.

2.4.3. SCREEN: 68448_Screenshot_3.mp4. 03:45-04:01
Added shot: 2.4.4 : Shot of the machine operating

2.5. Reseal the inoculated plates **[1]** and transfer them to an offline shaking incubator **[2]**.

2.5.1. Talent sealing plates.

2.5.2. Talent placing plates in incubator. **TXT: Incubation: 30 °C, 800 rpm, 75% RH**

2.6. Post incubation, return the grown plates to the liquid handler platform and unseal **[1]**. Click **Run** next to the **Add glycerol to MTP** protocol, ensure that the plate layout on screen matches that of the liquid handler dock **[2]**, and sequentially click **OK** for the protocol to run **[3]**.

2.6.1. Talent transferring plates from incubator to the liquid handler platform.
Videographer's Note: 2.6.1 split into 2 parts. The clapperboard for the first part of this shot is dark due to the polariser I had on my lens. I made a mental note to make adjustments to the polariser filter for the following shots. Shot 2.6.1B is the second part to shot 2.6.1 because there wasn't enough space (physically) for me to capture the whole sequence

2.6.2. SCREEN: 68448_Screenshot_4.mp4. 00:02-00:27

2.6.3. SCREEN: 68448_Screenshot_4.mp4. 00:28-00:40
Added shot: 2.6.4 : Extra shot to show robot operating

2.7. Once finished, seal the plates and briefly mix them in an offline shaking incubator at eight hundred revolutions per minute for five minutes **[1]**. Barcode the plates and store at minus eighty degrees Celsius until needed **[2-TXT]**.

2.7.1. Talent sealing plates and placing them on a shaker.
Videographer's Note: Shot 2.7.1 was split into two shots (A & B). Due to the small footprint of space I had to work with.

2.7.2. Talent placing barcoded plates into minus eighty degrees Celsius storage. **TXT: Repeat protocol until all the MTPs have glycerol added, are barcoded and stored**

3. Automation of Multi-Step Assay Setup for Microbial Variant Library Screening

Demonstrator: Philip Le Roy

3.1. Calculate the required volume of antibiotic-supplemented media for deep well blocks **[1]**.

3.1.1. Talent calculating media volume requirement.

3.2. Click **Run** next to the **DWB Liquid Transfer** program **[1]**. Ensure that media is added to the correct reservoir, the empty deep well blocks are in correct layout positions, and that an adequate supply of tips is available **[2]**.

3.2.1. SCREEN : 68448_Screenshot_5.mp4. 00:02-00:10

3.2.2. Talent adding media into the correct reservoir, checking DWB position and tip quantity.

3.3. Set the program to dispense 495 microliters of media **[1]**. When ready, sequentially click **OK** to start the program **[2]**.

3.3.1. SCREEN: 68448_Screenshot_5.mp4. 00:27-00:37

3.3.2. SCREEN: 68448_Screenshot_5.mp4. 00:38-00:50
Added shot: 3.3.3 : Extra shot to show robot operating
Videographer's Note: Shot 3.3.3 is an extra shot to show the machine operating.

3.4. Now seal the filled deep well blocks with breathable membrane **[1]** and transfer to temporary storage at four degrees Celsius **[2-TXT]**.

3.4.1. Talent sealing DWBs.

3.4.2. Talent placing them into cold storage. **TXT: Repeat loading until required DWBs are filled**

3.5. Next, click **Run** next to the **Inoculate from Thawed MTP** program **[1]**. Ensure that MTP cryostocks and filled deep well blocks are transferred to the platform per layout and that sufficient tips are loaded **[2]**. Sequentially click **OK** to initialize **[3]**.

3.5.1. SCREEN: 68448_Screenshot_6.mp4. 00:02-00:10

3.5.2. Talent loading cryostocks, DWBs and tips.

3.5.3. SCREEN: 68448_Screenshot_6.mp4. 00:29-00:39
Added shot: 3.5.4 : Extra shot to show robot operating
Videographer's Note: Shot 3.5.4 is an extra shot to show the machine operating.

3.6. When the program finishes, seal the inoculated overnight deep well blocks with

breathable membrane **[1]**. Transfer them to an offline plate shaker incubator set for 16 hours at 30 degrees Celsius, 180 revolutions per minute, and 75 percent humidity **[2]**. Reseal, mix, and return cryostock microtiter plates to minus 80 degrees Celsius freezer **[3-TXT]**.

3.6.1. Talent sealing blocks with breathable membrane.
Videographer's Note: Use 3.4.2. This shot was not filmed since it is the same as 3.4.2

3.6.2. Talent placing blocks in incubator.

3.6.3. Talent sealing and mixing plates and transferring to -80 °C. **TXT: Repeat inoculation and incubation with required number of DWBs**
Videographer's Note: Shot 3.6.3 was broken up into 3 shots. A, B and C. However, shot 3.6.3 C was not filmed due to the Authors telling me it is the same shot as 2.7.2

3.7. Now, calculate the required volume of media supplemented with various effector and antibiotic concentrations for the overnight deep well blocks to be screened **[1]**.

3.7.1. SCREEN: 68448_Screenshot_7.mov. 00:00-00:20

3.8. Click **Run** next to the **DWB Liquid Transfer** program **[1]**. Then ensure effector-supplemented media reservoirs are correctly placed **[2]**.

3.8.1. SCREEN: 68448_Screenshot_8.mp4. 00:02-00:12

3.8.2. Shot of correctly placed media reservoirs.
Videographer's Note: Shots 3.8.2 & 3.9.1 was joined together. I was unable to fit all of this information on the clapperboard.

3.9. Add empty deep well blocks into the liquid handler platform **[1]**. When sufficient tips are available, sequentially click **OK** to start protocol to generate assay deep well blocks **[2]**.

3.9.1. Talent loading deep well blocks into the liquid handler platform.

3.9.2. SCREEN: 68448_Screenshot_8.mp4. 00:29-00:50
Added shot: 3.9.3 : Extra shot to show robot operating
Videographer's Note: Shot 3.9.3 is an extra shot to show the machine operating.

3.10. After filling, seal the assay deep well blocks with breathable membrane **[1-TXT].** Refill the liquid handler with empty plates **[2]**.Repeat inoculation until all assay blocks are filled **[3]**.

3.10.1. Talent sealing filled assay blocks. **TXT: Store at 4 °C**

3.10.2. Talent reloading handler for next batch.
Videographer's Note: Shot 3.10.2 & 3.10.3 was combined together

3.10.3. Shot of all loaded blocks.

3.11. Next, click **Run** next to the **Transfer to Assay DWB** program **[1]**. After unsealed assay deep well blocks with effector-supplemented media are placed correctly, transfer and unseal overnight deep well blocks containing grown *P. putida* per layout **[2]**. Sequentially click **OK** to start protocol **[3]**.

3.11.1. SCREEN: 68448_Screenshot_9.mp4. 00:02-00:14

3.11.2. Talent transferring DWBs with grown *P. putida* into the handler.
Videographer's Note: 3.11.2 and 3.11.4 were filmed together.

3.11.3. SCREEN: 68448_Screenshot_9.mp4. 000:23-00:30
Added shot: 3.11.4 : Extra shot to show robot operating
Videographer's Note: Shot 3.11.4 is an extra shot to show the machine operating.3.11.2 and 3.11.4 were filmed together. Shot 3.11.4 didn't go according to plan in the combined shot with 3.11.2. So we did shot 3.11.4 again but on its own.

3.12. When program has finished, seal transfer assay plates to offline incubator **[1-TXT]**. Discard the overnight deep well blocks after inoculation **[2-TXT]**.

3.12.1. Talent placing sealed plates into incubator. **TXT: Incubation: 30 °C, 75% humidity, 16 h, 180 rpm; Final volume: 500 µL**

3.12.2. Talent discarding used deep well blocks. **TXT: Repeat inoculation and incubation for all blocks**

3.13. Next, transfer the deep well blocks into a centrifuge **[1].** Pellet cells at 4000 *g* in an ice-controlled rotor at 18 degrees Celsius for 5 minutes **[2]**. After discarding the supernatant, place the centrifuged blocks onto the liquid handler platform **[3]**.

3.13.1. Talent moving plates from incubator to centrifuge.

3.13.2. Shot of the settings being input into the centrifuge.

3.13.3. Talent placing centrifuged blocks onto the liquid handler platform, after discarding the supernatant.
Videographer's Note: Shots 3.13.3 & 3.15.2 were combined together along with a new shot called 3.15.4. This new shot shows the machine operating. I couldn't fit all of this information on the clapperboard properly.

3.14. Calculate the volume of one-times PBS required based the number of deep well blocks to be screened **[1]**.

3.14.1. SCREEN: 68448_Screenshot_10.mov. 00:00-00:20

3.15. Click **Run** next to the **Assay Setup – PBS Resuspension (DWB)** *(Assay-Setup-P-B-S-Resuspension-D-W-B)* program, set the dispense volume to 500 microliters **[1]**. Then ensure PBS is added to correct reservoir and array the centrifuged plates according to layout **[2].** Click **OK** to start the program after confirming tip availability **[3]**.

3.15.1. SCREEN: 68448_Screenshot_11.mp4. 00:04-00:29

3.15.2. Talent adding PBS to the correct reservoir and positioning the centrifuged plates in the correct position.

3.15.3. SCREEN: 68448_Screenshot_11.mp4.. 00:30-00:45

3.16. Reseal and remove resuspended deep well blocks from the liquid handler **[1]**. Check the underside of the plate to ensure pellets are completely resuspended **[2-TXT]**.

3.16.1. Talent sealing and removing plates.

Videographer's Note: Shot 3.16.1 was split into two parts - 3.16.1 A & B. This shot was also combined with shot 3.16.2. So there are three shots combined into one. I was unable to fit all of this information on the clapperboard properly.

3.16.2. Talent inspecting plate underside. **TXT: Repeat resuspension until all plates are processed**

3.17. Click **Run** next to the **Assay Setup – Cells and PBS addition (MTP)** *(Assay-Setup-Cells-and-P-B-S-addition-M-T-P)* program **[1]**. Then transfer resuspended deep well blocks into the liquid handler according to layout **[2]**.

3.17.1. SCREEN: 68448_Screenshot_12.mp4. 00:02-00:15

3.17.2. Talent transferring resuspended DWBs into the liquid handler system.

Videographer's Note: Shots 3.17.2 & 3.18.1 were combined into one shot. A new shot called 3.18.3 was added to this shot as well. Which shows the machine operating. I was unable to fit all of this information on the clapperboard properly.

3.18. Load the empty microtiter plates according to layout **[1]** and set dispense volume to 200 microliters before clicking **OK [2]**.

3.18.1. Talent loafing the system with the empty MTPs.

3.18.2. SCREEN: 68448_Screenshot_12.mp4. 00:18-00:30

3.19. Transfer the filled microtiter plates to an offline multimode plate reader and measure the relative fluorescence and OD_{600} *(OD-Six-Hundred)* **[1-TXT]**.

3.19.1. Talent moving plates to multimode reader. **TXT: sfGFP lEx/lEm = 488/520; Repeat until all asap DWPs have been transferred to MTPs and measured**

Results

4. Results

4.1. Automated screening of 5,000 promoter variants identified top candidates showing greater than 3.6-fold activation **[1]**.

4.1.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the scatter plot (right most graph)*

4.2. The EC50 *(E-C-Fifty)* values for 100 unique variants were plotted to visualize the sensitivity distribution and identify robust candidates **[1]**. EC50 values were computed for 226 enriched variants and ranked using lin-log transformation to form a sensitivity-scaled library **[2]**.

4.2.1. LAB MEDIA: Figure 4 *Video editor: Highlight the B image*

4.2.2. LAB MEDIA: Figure 4 *Video editor: Highlight the D image*

4.3. A definitive screening design was constructed using +1, 0, and −1 level variants from four modules- transport, regulator, Pout, and output ribosome binding site **[1]**.

4.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the A image*

4.4. Model profiles revealed how changes in expression levels of the four modules non-linearly impacted EC50 *(E-C-Fifty)* and Hill's Coefficient , identifying optimal expression combinations **[1]**, with RBSout *(R-B-S-Out)* showing a strong positive effect on both sensitivity and slope **[2]**.

4.4.1. LAB MEDIA: Figure 5 *Video editor: Highlight the C image*

4.4.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the RBS-Out Panels (top and bottom)*

4.5. The globally optimized biosensor variant incorporating ideal module strengths demonstrated enhanced sensitivity and Hill coefficient compared to both the parental and DSD*(D-S-D)* -optimized versions **[1]**.

4.5.1. LAB MEDIA: Figure 5D. Video editor: Highlight the purple line

Pronunciation Guide:

1. **biosensor**
   Pronunciation link: https://www.merriam-webster.com/dictionary/biosensor
   IPA: /ˈbaɪ.oʊˌsɛn.sər/
   Phonetic spelling: *BY-oh-sen-ser*
2. **automated**
   Pronunciation link: https://www.merriam-webster.com/dictionary/automated
   IPA: /ˈɔː.təˌmeɪ.təd/ (or /ˈɑː.təˌmeɪ.təd/ in some dialects)
   Phonetic spelling: *AW-tuh-may-ted*
3. **promoter**
   Pronunciation link: https://www.merriam-webster.com/dictionary/promoter
   IPA: /prəˈmoʊ.tər/
   Phonetic spelling: *pruh-MOH-ter*
4. **fluorescence**
   Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
   IPA: /flʊˈrɛs.əns/
   Phonetic spelling: *floo-RESS-ence*
5. **ribosome**
   Pronunciation link: https://www.merriam-webster.com/dictionary/ribosome
   IPA: /ˈraɪ.bə.soʊm/
   Phonetic spelling: *RYE-buh-sohm*
6. **coefficient**
   Pronunciation link: https://www.merriam-webster.com/dictionary/coefficient
   IPA: /ˌkoʊ.ɪˈfɪʃ.ənt/
   Phonetic spelling: *koh-ih-FISH-uhnt*
7. **non-linearly**
   Pronunciation link: https://www.merriam-webster.com/dictionary/nonlinear (for "nonlinear")
   IPA: /nɑnˈlɪn.i.ər.li/
   Phonetic spelling: *non-LIN-ee-er-lee*
8. **optimization**
   Pronunciation link: https://www.merriam-webster.com/dictionary/optimization
   IPA: /ˌɑp.tə.məˈzeɪ.ʃən/
   Phonetic spelling: *op-tuh-muh-ZAY-shun*