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Title: Biosensor-Based High Throughput Biopanning and Bioinformatics Analysis Strategy for the Global Validation of Drug-Protein Interactions

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

   Videographer: All screen captures provided, do not film

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **28**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Yoichi Takakusagi**: The identification of small molecule-protein interactions is essential for the research and development of drugs as well as for furthering our understanding of the pathological mechanisms underlying various diseases **[1]**.

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

REQUIRED:

1.2. **Yoichi Takakusagi**: This method facilitates the high throughput biopanning of drug-recognizing peptides and the global validation of drug-binding sites on proteins for small molecule drugs of interest **[1]**.

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

Protocol

2. Quartz Crystal Microbalance (QCM) Sensor Chip Preparation

2.1. To prepare a QCM (Q-C-M) sensor chip, attach a ceramic sensor chip onto the oscillator of a 27-megahertz QCM apparatus **[1]** and record the intrinsic frequency in the air phase before small molecule immobilization **[2]**.

2.1.1. WIDE: Talent attaching chip onto oscillator
2.1.2. SCREEN: screenshot_0: 00:02-00:45 *Video Editor: please speed up*

2.2. After the recording, detach the chip **[1]** and carefully add a 20-microliter drop of a 1-millmolar small molecule derivative solution in 70% ethanol to create a self-assembled monolayer on the gold electrode of the sensor chip **[2-TXT]**.

2.2.1. Talent detaching chip
2.2.2. Talent adding drop onto electrode, with small molecule derivative solution container visible in frame **TEXT: See text for all solution and buffer preparation details**

2.3. Place the chip into a Petri dish lined with moistened tissue protected from light for 1 hour at room temperature **[1]** before gently washing the electrode surface with ultrapure water **[2]**.

2.3.1. Talent placing chip into dish/covering dish with foil or similar
2.3.2. Electrode being washed

2.4. Dry the chip with a gentle application of air **[1]** and load the chip onto the QCM apparatus **[2]**.

2.4.1. Chip being dried
2.4.2. Talent loading chip onto apparatus

2.5. After 1 hour, record the reduction in frequency in the air phase to measure the amount of the small molecule that has been immobilized **[1]**.

2.5.1. SCREEN: screenshot_1: 00:02-00:35 *Video Editor: please speed up*

3. T7 Phage Library Biopanning

3.1. For T7 phage library biopanning, place a cuvette with a dedicated magnetic stirrer onto

the QCM biosensor set to 1000 revolutions per minute **[1]** and add 8 milliliters of reaction buffer to the cuvette **[2]**.

3.1.1. WIDE: Talent placing cuvette onto biosensor
3.1.2. Talent adding buffer to cuvette

3.2. While the buffer is being stirred **[1]**, attach the QCM sensor chip to the oscillator **[2]**.

3.2.1. Buffer being stirred
3.2.2. Talent attaching chip to oscillator

3.3. Pull down the arm of oscillator to immerse the chip into the buffer **[1]** and begin monitoring the QCM frequency **[2]**.

3.3.1. Talent pulling down arm
3.3.2. SCREEN: screenshot_2: 00:02-04:25 *Video Editor: please speed up*

3.4. When the sensorgram equilibrates to around 3 hertz/minute of frequency drift **[1]**, inject 8 microliters of a T7 phage library into the cuvette **[2]** and mark the injection point on the sensor **[3]**.

3.4.1. SCREEN: screenshot_3: 00:00-00:26 *Video Editor: please speed up*
3.4.2. Talent injecting library into cuvette **TEXT: i.e., 1-2 × 10^{10} pfu/mL**
3.4.3. Talent marking injection point

3.5. Monitor the frequency reduction caused by the T7 phages binding to the small molecule immobilized on the gold electrode surface **[1]**.

3.5.1. SCREEN: screenshot_3: 00:27-03:00 *Video Editor: please speed up*

3.6. After 10 minutes, stop the QCM frequency monitor and quickly lift the oscillator to remove the sensor chip from the batch. Detach the sensor chip from the oscillator and remove the buffer from the chip **[1]**.

3.6.1. Talent stopping monitoring, removing chip, detaching sensor, and removing buffer from chip *Videographer: Important/difficult step*

3.7. Place the dried sensor chip into a humid Petri dish **[1]** and add a 20-microliter drop of log phase *E. coli* host cells onto the gold electrode **[2]**.

3.7.1. Talent placing chip into dish
3.7.2. Drop being added to electrode

3.8. Incubate the dish onto a 96-well microplate mixer at 37 degrees Celsius and 1000-1500 revolutions per minute for 30 minutes protected from light to enhance the recovery of the bound T7 phages **[1]**.

3.8.1. Talent placing aluminum-covered dish onto mixer

3.9. At the end of the incubation, transfer the 20 microliters of *E. coli* suspension into 200 microliters of LB (L-B) medium **[1]** and use a 1% sodium dodecyl sulfate solution-soaked cotton swab to clean the electrode surface **[2]**.

3.9.1. Talent adding drop to medium, with dish with electrode visible in frame
3.9.2. Talent wiping electrode, with solution container visible in frame

3.10. Wash the gold surface with ultrapure water **[1]** and dry the electrode with air **[2]**.

3.10.1. Talent washing electrode *Videographer/Video Editor: shot will be used again*
3.10.2. Talent drying electrode *Videographer/Video Editor: shot will be used again*

3.11. Then treat the electrode surface **[0]** with 5 microliters of freshly prepared piranha solution for 5 minutes **[1]** followed by an ultrapure water wash **[2]** and air drying two times **[3]**.

3.11.0. Added shot: Talent wear a goggle

3.11.1. Piranha solution being added to electrode, with solution container visible in frame
3.11.2. Use 3.10.1. Electrode being washed
3.11.3. Use 3.10.2. Electrode being dried

4. Bioinformatics Analysis

4.1. To perform bioinformatics analysis using RELIC (relic), unzip the stand-alone RELIC program on a PC with a Windows operating system **[1-TXT]** and use the drug of interest to align the amino acid sequences of the affinity-selected 15-mer peptides **[2-TXT]**.

4.1.1. WIDE: Talent unzipping program, with monitor visible in frame **TEXT: RELIC: Receptor Ligand Contact** Author NOTE: The PC and the desktop when the talent was photographed was different from that used for the recording screenshot_4. Please edit to make the difference less obvious.
4.1.2. SCREEN: screenshot_4: 00:40-00:49 **TEXT: Alternative: randomly selected sequence from unscreened parent library**

4.2. Place the text files into the folder for running each RELIC program and enter the amino

acid sequence of single or multiple proteins in each text file with FAST-A (fast-A) format **[1-TXT]**.

4.2.1. SCREEN: screenshot_4: 00:52-01:39 *Video Editor: please speed up* **TEXT: Alternative: Download database text files in FASTA format from any protein database**, then files being added to folder

4.3. Click the executable file for AADIV (A-A-div), INFO (info), MOTIF (motif), MATCH (match), HETEROalign (hetero-align), FAST-A-con, and FAST-A-skan in the independent folder to open the Personal Version of FTN95 (F-T-N-ninety-five) **[1]** and enter the appropriate filename and extension in the command line to execute each program and to obtain the required text format file **[2]**.

4.3.1. SCREEN: screenshot_4: 01:41-02:08

4.3.2. Talent entering film name/executing program, with monitor visible in frame Author NOTE: The PC and the desktop when the talent was photographed was different from that used for the recording screenshot_4. Please edit to make the difference less obvious

4.4. Then export the resulting text file to a spreadsheet to generate a plot of information content or cumulative similarity scores calculated using a BLOSUM62 (blow-sum-sixty-two) **[1-TXT]**.

4.4.1. SCREEN: screenshot_5: 00:00-00:30 **TEXT: Contact tkksg@rs.noda.tus.ac.jp for stand-alone RELIC access**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.5., 3.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
2.5., 3.6.

Results

5. Results: Representative Global Validation of Drug-Protein Interactions

5.1. Using this strategy, single and multiple small molecule-binding sites on the target proteins have been successfully identified for six small molecule drugs **[1]**.

5.1.1. LAB MEDIA: Figure 4

5.2. For example, 29 peptides that recognized the clinically approved drug irinotecan **[1]** immobilized as a self-assembled monolayer were identified by QCM biosensor-based one-cycle biopanning **[2]**.

5.2.1. LAB MEDIA: Figure 3A drug formula
5.2.2. LAB MEDIA: Figure 3A drug formula and Table *Video Editor: please emphasize Table*

5.3. Subsequent pairwise alignment of the 29 peptides and acetylcholinesterase yielded maximal scores for specific amino acid residues **[1]** that were consistent with those making up the Irinotecan-binding site **[2]**.

5.3.1. LAB MEDIA: Figure 3A AChE graph *Video Editor: please emphasize Y121, Q225, F290, E327, H440, and Y442 peaks*
5.3.2. LAB MEDIA: Figure 3A AChE graph and 3D structure images *Video Editor: please emphasize Y121, Q225, F290, E327, H440, and Y442 texts in right 3D structure*

5.4. This same subset of peptides was also successfully identified in the vicinity of the catalytic triad in carboxylesterase **[1]**, indicating that these amino acids form a scaffold for irinotecan recognition during de-esterification **[2]**.

5.4.1. LAB MEDIA: Figure 3A CE graph *Video Editor: please emphasize E99, L100, L252, L305, I387, and V474 peaks*
5.4.2. LAB MEDIA: Figure 3A CE graph and 3d structure images *Video Editor: please emphasize E99, L100, L252, L305, I387, and V474 texts in right 3D structure*

5.5. The 27 peptides that recognized the anti-flu drug oseltamivir covering the QCM sensor chip gold electrode surface **[1]** successfully detected the oseltamivir-binding site in neuraminidase **[2]**.

5.5.1. LAB MEDIA: Figure 3B drug formula

5.5.2. LAB MEDIA: Figure 3B drug formula and Table *Video Editor: please emphasize red text in Table*

5.6. This binding site consists of unstructured peptide loops that potentially undergo dynamic movement while docking with oseltamivir **[1]**.

5.6.1. LAB MEDIA: Figure 3B graph and 3D structure images *Video Editor: please emphasize labeled peaks and labeled structure names in right 3D image*

Conclusion

6. Conclusion Interview Statements

6.1. **Yoichi Takakusagi**: Drugs, reagents, chemicals, recombinant bacteriophages, and bacteria are biological hazards and should be handled according to the Cartagena protocol. Remember to always wear gloves, goggles, and a lab coat for safety **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.2., 3.4., 3.6., 3.8., 3.110.)

6.2. **Yoichi Takakusagi**: Following this procedure, target proteins for various drugs can be globally validated in humans, pathological viruses, and even plants to understand the molecular mechanisms and potential therapeutic efficacy of drugs of interest **[1]**.

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