FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68732
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20963308

Title: Using Human Differentially Expressed Gene Lists to Perform Downstream Pathway Enrichment Analysis and Target Prioritization

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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? **Yes**

Current Protocol Length
Number of Steps: 05
Number of Shots: 11

Introduction

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

1.1. **Brett Pickett:** The aim of our research is to characterize the intracellular transcriptional response to various conditions, and to predict therapeutics and mechanistic/diagnostic markers for those conditions.

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

What are the current experimental challenges?

1.2. **Archarlie Chou:** Computational prediction gives candidates of drugs. Validating drugs effects is resource and time consuming, however, the in silico method could only improve with high quality experimental data.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Brett Pickett:** The advantage of this work is its ability to identify potential drug targets for repurposing within a signaling pathway, rather than just matching differentially expressed genes to known targets.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2*

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

Protocol

2. Executing SPIA and Pathway2Targets for Pathway Enrichment and Target Prioritization in RStudio

Demonstrator: Archarlie Chou

2.1. To run the SPIA pathway enrichment algorithm, first download the code on the computer system from GitHub **[1].** Open the SPIA_Code.Rmd *(S-P-I-A-Code-dot-R-M-D)* script in R Studio ~~by selecting the **File** menu and clicking **Open File**, then choosing script~~ **[2]**.

2.1.1. WIDE: Talent downloads R code from GitHub on the computer system.

2.1.2. SCREEN: 68732-2.1.2.mov 00:00-00:08

2.2. Select all lines of code then click the **Run** or **Run Selected Line(s)** *(lines)* button to execute the script **[1]**. Wait for the run to complete and verify that a similarly named .CSV *(dot-C-S-V)* file appears in the download directory **[2-TXT]**. Open the file as a spreadsheet to manually review and interpret the results **[3]**.

2.2.1. SCREEN: 68732-2.2.1.mov 00:05-00:15

2.2.2. SCREEN: 68732-2.2.2.mov. 00:00-00:11
TXT: This file contains the statistically significant intracellular signaling pathways

2.2.3. SCREEN: 68732-2.2.3.mov. 00:00-00:12

2.3. To run the target prioritization algorithm, open the Pathway2Targets.R script *(Pathway-2-Targets-dot-R-script)* in R Studio by selecting the **File** menu and clicking **Open File**, then choose the script name from the directory **[1]**. In the RStudio *(R-Studio)* code window, go to line 22 and replace the placeholder with the actual SPIA results filename **[2]**.

2.3.1. SCREEN: 68732-2.3.1.mov. 00:00-00:10

2.3.2. SCREEN: 68732-2.3.2.mov. 00:00-00:10

2.4. Select all lines of code and click the **Run** button to execute the algorithm **[1]**. Observe real-time progress messages appearing in the bottom-left panel of the screen **[2]**. After completion, check the download directory for a similarly named .TSV file which contains the prioritized targets **[3]**.

2.4.1. SCREEN: 68732-2.4.1.mov. 00:07-00:17

2.4.2. SCREEN: 68732-2.4.2.mov. 00:00-00:08

2.4.3. SCREEN: 68732-2.4.3.mov. 00:00-end

2.5. After generating the file with prioritized targets and their metrics, open it in a spreadsheet application to review **[1]**.

2.5.1. SCREEN: 68732-2.5.1.mov. 00:01-end

Results

3. Results

3.1. The SPIA *(S-P-I-A)* algorithm identified 10 statistically significant signaling pathways with an unadjusted p-value less than 0.05 **[1].**

3.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the columns " Gene.set" and "PVal"*

3.2. The Pathway2Targets *(Pathway-two-Targets)* algorithm identified multiple predicted targets **[1]**. The predicted therapeutic targets were consistent across both the ranked targets and ranked treatments outputs, including known colorectal cancer-related genes such as EGFR *(E-G-F-R)*, TP53 *(T-P-Fifty-Three)*, and AKT1 *(A-K-T-One)* **[2]**.

3.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the Column " Gene.set"*

3.2.2. LAB MEDIA: Table 3. *Video editor: Highlight the rows for "EGFR", "TP53", and "AKT1"*

Pronunciation Guide:
Transcriptional
Pronunciation link:
https://www.merriam-webster.com/dictionary/transcriptional
IPA: /trænˈskrɪpʃənəl/
Phonetic Spelling: tran-skrip-shuh-nuhl
Therapeutics
Pronunciation link:
https://www.merriam-webster.com/dictionary/therapeutic
IPA: /ˌθɛrəˈpjuːtɪks/
Phonetic Spelling: thair-uh-pyoo-tiks
Mechanistic
Pronunciation link:
https://www.merriam-webster.com/dictionary/mechanistic
IPA: /ˌmɛkəˈnɪstɪk/
Phonetic Spelling: meh-kuh-nis-tik
Computational
Pronunciation link:
https://www.merriam-webster.com/dictionary/computational
IPA: /ˌkɑːmpjʊˈteɪʃənəl/
Phonetic Spelling: com-pyoo-tay-shuh-nuhl
In silico
Pronunciation link:
https://www.merriam-webster.com/dictionary/in%20silico
IPA: /ɪn ˈsɪlɪˌkoʊ/
Phonetic Spelling: in sih-lih-koh
Signaling
Pronunciation link:
https://www.merriam-webster.com/dictionary/signaling
IPA: /ˈsɪgnəlɪŋ/
Phonetic Spelling: sig-nuh-ling
Algorithm
Pronunciation link:
https://www.merriam-webster.com/dictionary/algorithm
IPA: /ˈælgəˌrɪðəm/
Phonetic Spelling: al-guh-rith-uhm
GitHub
Pronunciation link:
https://www.merriam-webster.com/dictionary/github
IPA: /ˈgɪtˌhʌb/
Phonetic Spelling: git-hub
RStudio
Pronunciation link:

No confirmed link found
IPA: /ˈɑr ˌstuːdioʊ/
Phonetic Spelling: ar-stoo-dee-oh
CSV (Comma-Separated Values)
Pronunciation link:
https://www.merriam-webster.com/dictionary/CSV
IPA: /ˌsiː ˌɛs ˈviː/
Phonetic Spelling: see-ess-vee
TSV (Tab-Separated Values)
Pronunciation link:
No confirmed link found
IPA: /ˌtiː ˌɛs ˈviː/
Phonetic Spelling: tee-ess-vee
Enrichment
Pronunciation link:
https://www.merriam-webster.com/dictionary/enrichment
IPA: /ɪnˈrɪtʃmənt/
Phonetic Spelling: in-rich-muhnt
Colorectal
Pronunciation link:
https://www.merriam-webster.com/dictionary/colorectal
IPA: /ˌkoʊloʊˈrɛktəl/
Phonetic Spelling: koh-loh-rek-tuhl
EGFR (Epidermal Growth Factor Receptor)
Pronunciation link:
https://www.merriam-webster.com/dictionary/EGFR
IPA: /ˌiːˌdʒiːˌɛfˈɑr/
Phonetic Spelling: ee-jee-eff-ar
TP53
Pronunciation link:
No confirmed link found
IPA: /ˌtiːˌpiː ˌfɪfti ˈθriː/
Phonetic Spelling: tee-pee-fifty-three
AKT1
Pronunciation link:
No confirmed link found
IPA: /ˌeɪˌkeɪˌtiː ˈwʌn/
Phonetic Spelling: ay-kay-tee-one
Intracellular
Pronunciation link:
https://www.merriam-webster.com/dictionary/intracellular
IPA: /ˌɪntrəˈsɛljələr/
Phonetic Spelling: in-truh-sell-yuh-lur

Pathway2Targets (software tool)
Pronunciation link:
No confirmed link found
IPA: /ˈpæθweɪ tuː ˈtɑːrgɪts/
Phonetic Spelling: path-way too tar-gits
SPIA (Signaling Pathway Impact Analysis)
Pronunciation link:
No confirmed link found
IPA: /ˈspaɪə/
Phonetic Spelling: spy-uh