1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
We have proofread the manuscript.

2. Please ensure the Introduction includes all of the following with citations:
a) A clear statement of the overall goal of this method
b) The rationale behind the development and/or use of this technique
c) The advantages over alternative techniques with applicable references to previous studies
d) A description of the context of the technique in the wider body of literature
e) Information to help readers to determine whether the method is appropriate for their application
The introduction contains all this information.

3. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Zymo Research yeast plasmid miniprep, PEG 3350, etc.
We have removed the Zymo and Thermo references from the protocol.

4. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
We have confirmed the protocol is written in this format.

5. The Protocol should contain only action items that direct the reader to do something.
We have confirmed the protocol is written in this format.

6. Please revise the protocol text to avoid the use of any personal pronouns in the protocol (e.g., "we", "you", "our" etc.).
We have confirmed the protocol is written in this format.

7. Please ensure that individual steps of the protocol should only contain 2-3 actions per step.
We have confirmed the protocol is written in this format.

8. Please ensure that you include all specific details associated with your protocol in a step by step manner. Please use complete sentences throughout.
We have confirmed the protocol is written in this format.

9. Please bring out the link between different subheadings of the protocol.
We have brought out the link between different subheadings in the protocol.

10. Please ensure you answer the “how” question, i.e., how is the step performed?
We have confirmed the protocol is written in this format.

11. 1: Please include what gene was amplified in your case, where was the nucleic acid obtained for amplification, what are the primers used, reaction set up and program used for amplification. Please also include a step by step protocol to show how the cloning was performed in the MCS of the plasmid used. Please include a short note stating why this plasmid was used. After cloning, did you perform restriction digestion, colony pcr, etc.
We have updated the protocol to include this information.

12. 2.3: Do you check the O.D in this case?
No

13. 2.3.6: What is the transformation mix- the mix obtained in the step 2.3.4? The plasmid DNA is from step 1? Please include this for clarity. Do you add the culture medium at this stage? Do you perform the incubations in the water bath?
Additional details have been added.

14. 3.1.2: Do you measure the O.D in this case?
No

15. 3.1.6: importance of pYep13?
pYep13 is the yeast genomic library. This information has been added to the protocol.

16. 3.1.7: Why is DMSO added at this stage?
The addition of DMSO significantly increases the transformation efficiency. This information has been added to the protocol.

17. 4.1.3: If this step needs filming, please describe the actions associated with it in brief.
This step does not need filming.

18. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Please adjust the highlights accordingly.
Highlights have been adjusted accordingly.

19. Please include representative results for all the sections in the protocol. For example, PCR results, positive clone screen is not included.
Representative results have been added.

20. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”
All figures are new, no copyright permission is needed.

21. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
We have confirmed the discussion contains this information and have shortened it to 6 paragraphs.

22. Please sort the materials table in alphabetical order.
The materials are now listed in alphabetical order.

Reviewers' comments:
Reviewer #1:

Manuscript Summary:
This manuscript provides a detailed explanation of how to use a yeast toxicity-suppressor screen to elucidate eukaryotic pathways that may be targeted by bacterial effector proteins. The protocol is timely as it has recently been used in several high profile publications that have reported function of novel effectors.

Major Concerns:
No major concerns

Minor Concerns:
* the protocol makes no mention of checking for expression of the effector protein in yeast although this is mentioned in the discussion. This should be included in the protocol. Authors mention a His-tag - is it routine to include a tag to detect the effector? N- or C- terminal? Incorporated into the oligonucleotide for amplification of the effector?
The His-tag is on the plasmid and thus is not incorporated into the oligos for effector amplification. We have incorporated that effector expression should be confirmed in the protocol.

* The protocol refers to "agar" and "broth" in vague ways. I think the yeast media being used should be more clearly defined (can a recipe be included?). I assume it is a Yeast minimal media with other amino acids supplemented?
We have added a section that includes the recipes for all the media and reagents.

* what are the oligonucleotides used to sequence recovered pYep13 clones?
The oligonucleotides used for sequencing have been added.

Reviewer #2:
Manuscript Summary:
This manuscript by Faris and Weber describes a protocol to identify bacterial effectors toxic to eukaryotic cells via yeast viability and subsequently identify yeast proteins/pathways targeted by the toxic effector using an over-expression suppressor screen. Yeast are powerful model organisms to investigate bacterial effector protein function and this protocol will be of immense value to the scientific community. Minor comments are listed below.

Major Concerns:
None.

Minor Concerns:
1. Line 126: Is pYesNTA-Kan available commercially or from the authors upon request? Are there alternative plasmids that can be used?
We have added a note in the protocol. The pYesNTA-Kan is available from our laboratory and other commercially available plasmids can be used for the screen.

2. Are the restriction sites in frame with the 6xHis tag? Please revise Figure 1 so that the MCS shows the 6xHis codons.
The figure has been revised to include the 6X His-tag codons.

3. Step 2.3.1 describes a centrifugation step at 4˚C. Are subsequent incubations/centrifugation steps also done at the this temperature? No temperature is specified.
Temperature for all centrifugation steps have been added.

4. Lines 158-169: fix numbering for steps and highlighting.
The numbering has been corrected.

5. Line 210: do you mean the growth of yeast harboring the toxic effector?
Yes, we have clarified this in the text.

6. Line 219: typo: manufacturers
The type has been corrected.

7. The use of idioms such as "pipe-dream" and "foolhardy" may decrease readability for non-native English readers. Consider revising verbiage.
We have revised the verbiage.