Thank you for the guidance on revising our manuscript.

We have now fully incorporated all of the suggestions in the submitted revised manuscript as specified below. Those changes are highlighted in the text.

-Rob Piper

**Editorial comments:**  
Changes to be made by the Author(s):  
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.  
2. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.  
3. 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.  
4. Please provide reaction recipes throughout: Step 2.1 digestion, etc.  
5. Please provide gel conditions: V/cm, etc.  
6. Please specify plating densities: 2.3, etc.  
7. 3.1.1: What is a small amount here? Please quantitate.  
8. 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.  
9. There is concern that once the longer paragraphs of protocol text are properly broken up into sub-steps, the highlighted protocol text would be too long.  
10. Please use SI abbreviations for time: h, min, s in Figure 1, etc.  
  
  
  
**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
This protocol is very detailed and describe an approach that will be of interest to a wide array of cell and molecular biologists as a way of defining new protein-protein interactions. It is an exciting new quantitative approach based on yeast two hybrid that leverages new high throughput sequencing to facilitate identification of protein interactions. Importantly, this approach should capture weak associations between proteins as well as robust interactions. The authors have done an admirable job updating the protocol.  
  
  
**Reviewer #2:**  
This is a fantastic method paper that provides rigorous details on DEEPN, the millennial update of a classic methodology that is truly making two-hybrids great again. The revisions made have strengthened the paper, and I think the decision to split into two separate methods papers (one on the experimental approaches and the accompanying manuscript on the computational analysis) is wise. Overall, the current manuscript is well-suited for publication in JoVE and I have only very minor suggestions relating to the grammar:  
  
\* Line 86: the sentence beginning "The resulting workflow…" is convoluted and possibly a run-on.  
\* Line 120: "that" should be "than"  
\* Line 545: "…for a many" - delete the "a"  
\* Line 673: I think this refers to Figure 3 (not Figure 2).