**Patrick Lajoie**

Assistant Professor

Department of Anatomy and Cell Biology

Schulich School of Medicine and Dentistry

Western University

London, Ontario, CANADA

August 30th 2018,

Editor *JOVE*

Dear Editor,

We are pleased to resubmit our manuscript titled “Assessing the effect of fluorescent proteins on fusion partners using polyglutamine toxicity assays in yeast -58748\_R1” by Yuwei Jiang, *et al*. for consideration as a video protocol in *JOVE*.

We believe that we have addressed all the reviewers’ comments and that our manuscript is now acceptable for publication in *JOVE*.

Best regards,



Patrick Lajoie, Ph.D.

**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**We have proof read the manuscript.**

2. Please specify the primers for PCR reactions.

**The primers would be the one generated in step 1.1. We have specified so in the text.**

3. Figure 1. Please add a scale bar to the Fluorescence Spectra.

**Done.**

4. Figure 2A: Please add scale bars.

**There is no fluorescent micrograph in this figure.**

5. Step 1.1: Please specify how to design the primers. Please add more details. For steps that are done using software, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step.  
**We have added details about the composition of the different primers. No software is required.**

6. 1.9: Please specify the condition for growing the colonies.

**We have included the temperature.**

7. 3.1: If you want to film this step, Step 2.1-2.5 must be highlighted.  
**We have highlighted steps 2.1-2.5.**

8. 3.6: How to create? What software is used?  
**We now mention the software and how to use it.**

9. Please specify the antibodies used in the protocol.  
**We now mention explicitly the anti-FLAG and appropriate secondary antibody.**

10. Please use standard SI unit symbols and prefixes such as µL, mL, L, g, m, etc.  
**Done.**

11. Please use a single space between numerical values and their units.  
**Done.**

12. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).  
**Done.**

13. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

**We now mention critical steps in the protocol, namely the induction in galactose media and the need to use a RNQ1+ strain.**

b) Any modifications and troubleshooting of the technique

**We mention that the assay can be adapted to other yeast strains but that it may require additional modifications to the protocols that are discussed.**

c) Any limitations of the technique

**Limitations are acknowledged. Mainly the inability of the assays to discriminate between oligomeric and monomeric species of GFP variants and the fact that FPs can shows different behavior in yeast compared to other organisms.**

d) The significance with respect to existing methods

**We now mention that the assay has the advantage of being easily adaptable to high content screens compared to other methods.**

e) Any future applications of the technique

**We mention that the assay can be used to test the impact of the next generation of FPs, or to screen mutants of existing FPs. In addition, it could be employed to test the effects of other tags, such as SNAP tag.**