Reviewer #3:

Manuscript Summary:

This manuscript describes the development of a simple and efficient transient transformation procedure of protoplasts of Arabidopsis to examine the import of proteins into chloroplasts and its suitability for detecting protein-protein interactions using commercially available antibodies. Overall, the manuscript was concise and well written and would make a significant contribution to the research community. However, one major criticism is the lack of some specific technical details in a certain steps of the protocol. These details are critical to researchers who are not familiar with the process and that such information should be present considering the nature of manuscript. Below is a list of specific comments that highlight some of the missing details mentioned above.

Specific comments:

Page 2, line 75 "Before solidification" should read "Allow the medium to cool down to 55 °C,"

A : We corrected as you suggested.

Page 2, line 78 "… 70% ethanol" should be " … 70% (v/v).."

A : Corrected

Similarly, line 79 should also include "(v/v)" after "1% sodium hypochlorite solution"

A : Corrected

Page 3, lines 98, 99 and 103 insert "(w/v)" after "0.25% …, 1.0% …, 0.1% … and 21% …" respectively.

A : Corrected

Page 3, step 3.2, This step should include the number of 2-week old leaves that were used in 25 mL of enzyme solution as the ratio of tissue vs enzyme solution is extremely critical in obtaining a high yield of viable and healthy protoplasts. Also, the authors should put an emphasis here that intact leaves were used here instead of dissected leaf strips were used.

A : We corrected as you suggested.

Page 3, lines 118 and 122 please indicate the length of these centrifugation steps.

A : We added the centrifugation length

Page 3, line 129, should include the concentration of the PEG solution "40%" as the volume use by researchers vary

A : We added 40% for the concentration of the PEG solution

Page 4, lines 138 should indicate the amount of plasmid DNA use per "X" number of protoplasts. Thus, based on the above protoplast concentration, how much DNA would be required for 1.5 million protoplasts. For example, based on the protocol that was optimized in our lab, we found that 5 ug of plasmid DNA/20,000 protoplasts consistently gave the highest transformation rate.

A : No we cannot provide the highest transformation rate. If you increase the amount of DNA the transformation efficiency will increase with the amount of DNA. But at the same time, amount of proteins expressed in individual protoplasts would also increase. Then too much of protein expression in each protoplast may lead to unwanted overexpression-related problems. Therefore, we found that 10 ug of plasmid DNA with the size of around 3-4 kb in 1.5x106 protoplasts appears to be optimal condition for both the transformation efficiency and expression level.

Page 4, line 139, Also, the unit of protoplasts "300 mL" should be corrected to "μL"

A : Corrected

Page 4, line 144 "300 mL" should be corrected to "300 μL"

A : Corrected

Page 4, lines 156 and 159 "Fluorescent …." should be corrected to " Fluorescence ….".

A : Corrected

Page 4, line 170 "80 mL" should be corrected to " 80 μL".

A : Corrected

Page 4, line 171, specify the composition of the 5x SDS PAGE sample buffer.

A : The composition of the solution is described.

Page 7, lines 293-296 reference 15, please change the upper case letters to lower case in the article title.

A : Corrected