**Editorial comments:**  
  
• Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. **Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions**. The updated manuscript is also attached.  
  
• Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

- The manuscript has been proofread.

• JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

- DOIs have been added to missing references, except reference 3, in which one is not available.

•Grammar: 4.4 – “an tube”

- The phrase an tube has been changed to a tube.   
  
•Visualization: The data analysis section does not contain enough information to be included in the video and should not be highlighted for filming.

- The data analysis section in the protocol has been un-highlighted.   
  
•Results: What is the difference between panels D & E? Please specify in the figure legend.

- The difference between panels D and E have been specified. Please see figure caption (legend) 2.   
  
•Discussion: Please discuss the advantages with respect to alternative multiplexing methods and include independent citations.

- Advantages of using cPILOT with respect to alternative multiplexing methods have been added to the discussion section. Please see lines 347 – 351.

**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
In this protocol, the authors described step-by-step methods for cPILOT, a quantitative proteomics strategy for multiplexing analysis. Overall, the experimental procedure has been described in great details and very easy to follow. It should serve as an important resource for the proteomics community who is interested in the application of cPILOT and multiplexing quantitative proteomics analysis. There are a few minor suggestions:  
  
*Major Concerns:*  
None  
  
*Minor Concerns:*  
1. Line 110, the author mentioned homogenizing tissues in PBS buffer with 8M urea. It would be better to note whether it's necessary to add some protease or phosphatase inhibitors.

- This issue has been addressed by talking about the addition of protease or phosphatase inhibitors. See lines 111 and 112.

2. Line 127, the author recommended adding DTT at a molar ratio of 40:1 to the sample, but on line 125, the author mentioned adding 100 μg of proteins. It would be hard to calculate how much DTT should actually be used.

- This issue has been addressed by prefacing how the calculations are made. Also, an example calculation was added. Please see lines 129 – 143.

3. Line 130, alkylation with iodoacetamide on ice. Why is it important to perform the reaction on ice?

* This has been addressed by stating the importance of doing this step on ice. Please see lines 145 and 146.

4. Line 140, the author mentioned adding 0.1% FA to re-acidify the sample. Does it actually mean adding FA till a final concentration of 0.1%?

* This has been re-phrased to state that the final concentration is 0.1% FA. Please see lines 156 and 157.

5. Line 194, the author mentioned a filtering step after reconstituting the peptides. It's necessary to provide some details about this step.

- Steps detailing how to filter reconstituted peptides have been added. As additional text was added, Line 194 has shifted. Please see lines 213 – 218.   
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
The manuscript entitled "Enhanced Sample Multiplexing of Tissues using Combined Precursor Isotopic Labeling and Isobaric Tagging" describes a method to perform quantitative proteomics on multiple samples at once. The ability to multiplex samples through isotopic labeling and isobaric tagging decreases experimental time and costs. The manuscript is well written and the procedure and materials are adequately described. The authors also do a good job of describing multiple uses for the method.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
I recommend the manuscript be published with a minor revision. For figure 2D and E the authors label which peaks are from which tissue. It may also be useful, either on the figure or in the figure legend to label which peaks are from the WT and AD samples for each tissue.

- Identification markers indicating the origin of the peptide represented by the reporter ion intensity (either WT or AD) have been added. Please see figure 2D and 2E.   
  
*Additional Comments to Authors:*  
N/A