“Force measurement during contraction to assess muscle function in zebrafish larvae” by Darcée D. Sloboda, Dennis R. Claflin, James J. Dowling, and Susan V. Brooks

Editorial comments:  
  
\* Please remove all trademark symbols and use of the word "we" from the protocol text. We removed the trademark symbol and the use of the word “we” from the protocol text.  
  
\* If any material is added to the protocol text, please check to make sure the highlighted portion is under 3 pages. We removed highlighting over a small portion of the protocol (muscle measurements) to keep the highlighted portion under 3 pages. The muscle measurements portion of the protocol is sufficiently described through the text and the corresponding figure.  
  
Reviewers' comments:  
  
Reviewer #1:   
  
*Minor Concerns:*  
\* Did not see how the zebrafish larva were anesthetized. Tricaine, included in the testing solution, is the anesthetic. We altered the protocol text (step 3.5) to clarify this point for the readers.  
\* Are the electrodes described? Are they just the Aurora platinum electrodes that are used for EDLs? Are they modified in any way (e.g., size)? Our testing system actually pre-dates the Aurora system. The electrodes are described as “platinum parallel plate electrodes” in the Figure 2 legend. We added dimensions to the description.  
\* Step 6.2 suggests taking measurements at an anatomical landmark (e.g., urogential opening). The legend for Figure 4 states "Red bars indicate measurement locations". Is this the urogential opening that was used in this example (Fig 4)? Yes. We modified the protocol text in step 6.2 and the Figure 4 legend to clarify this point.  
\* Authors do a nice job reporting optimal current and representative forces. I was going to ask if force-frequency curves ever been generated for the zebrafish larva, but I presume one can only examine twitch? What happens if a tetanic contraction is attempted (e.g. a 200 ms train)? Tetanic contractions (e.g. 200 ms trains) can be elicited and a force-frequency curve can be generated. However, the maximum force generated during a twitch or at lower frequencies is not much different than the maximum force generated during a tetanic contraction (e.g. 85-95% of maximum force with stimuli delivered at 50 Hz vs. 100% of maximum force with stimuli delivered at 200 Hz). We use twitches as a measure of muscle strength since there is less risk of the larvae ripping or slipping at the suture ties during a twitch compared with a tetanic contraction. We added a paragraph at the beginning of the discussion section to address this point.

Reviewer #2:   
  
*Minor Concerns:*  
i) I would like to see more information on the "video sarcomere length system" - it is unclear how this works. Text was added to the protocol (step 5.5) to provide more detail, describing how the system works. Use of the system will be shown on the video and this system is commercially available (indicated in the equipment list) so readers/viewers can also refer to the manufacturer for more information.   
ii) There needs to be a good reason for performing the analysis at a non-standard temperature (ie 25 rather than 28.5 degrees). A good reason is not given. We recognize that 28.5 °C is the standard housing temperature for zebrafish. However, the slightly colder temperature does not alter force and allows the method to be used more broadly, by researchers who may not have temperature control on their force testing system. We did alter the protocol text (step 5.2) and added a sentence in the discussion section to let the readers know that measurements can be collected at 28.5 °C and that zebrafish should be maintained at 28.5 °C prior to testing for accurate staging.  
iii) The classic model of zebrafish muscle disease was the development of the dmd mutant (Bassett et al 2003, Development 130(23):5851-60.). This should be referenced on p2 in the introduction. This reference was added and the numbering of other references was changed.