Dear Dr. Henderson,

We appreciate that each of the reviewers found our protocol clear and informative. We have addressed all the points raised be each of the reviewers (see detailed changes below). We hope that our article is now suitable for publication in JoVE.

Please contact me if you need any additional information.

Sincerely,

Mike Blower  
  
Editorial comments:  
\*Editor modifed the formatting of the manuscript to comply with JoVE instructions for authors, please maintain the current formatting throughout the manuscript. You can find the updated manuscript under "file inventory" and download the microsoft word document.

We have used the editor modified version of the text to make our changes.  
  
\*Slight modifications need to be made to the references section of the manuscript, only use "et al" if there are more than 6 authors. There also may be a glitch in the reference manager because some of the reference text appears on a separate line.

We have reformatted the references. We are using the endnote file for JoVE to format the references.  
  
Reviewers' comments:  
  
Reviewer #1:   
*Summary:*   
This is a nice methods description and there are no major concerns. Some minor cosmetic revision is recommended.   
  
*Minor Concerns:*  
1.) Introduction, line 11 - are/is

We have corrected the error.

2.) Introduction, paragraph 4 - Xenopus does not benefit. The benefit of using Xenopus...

We have corrected the error. The topic sentence now reads: “For decades *Xenopus laevis* frogs have been a powerful system for the study of developmental and cell biology, owing to the large oocyte size and robust external development17.

3.) Introduction, paragraph 4 - "the availability of a sequenced genome makes it an attractive model"

We have changed the word “this” to “it” in paragraph 4.

4.) Introduction, paragraph 5 - define CSF first.

The sentence now reads: “In this report we describe a method to make Meiosis II, cytostatic factor-arrested extracts (CSF) from *X. tropicalis*19.”

5.) Introduction, paragraph 5 - "This provides a powerful method to detect mRNA localization on a genome-wide scale." I have a problem with this statement. The genome-wide scale is OK, but the method only allows to determine, which RNAs are associated with MTs. It does not determine RNA localization in general as the sentence suggests.

We have modified the sentence to read: “This provides a powerful method to detect microtubule-targeted mRNA localization on a genome-wide scale.”

6.) Protocol 1 - I would add "egg harvesting" to the title. Or change it to something like: Generation of Xenopus tropicalis eggs.

We have changed the title to “Generation of X. tropicalis eggs.”

7.) Protocol 1.1 - "injected with hCG at on three successive days" eliminate 'at'

We have made the suggested change.

8.) Protocol 1.4 - What is tankwater? does it contain any salts or is it simply DI water?

We have included our recipe for frog tankwater in the protocol.

9.) Protocol 2.1 - "editing of eggs" What is this?

We have changed “editing” to read: “removal of activated eggs.” In Protocol 2.3, we explain: “In the first XB wash, remove eggs that have escaped CSF arrest by removing lysed, puffy, white, and pseudocleavage eggs.

10.) Protocol 3.2 - be consistent with only rcf or both rcf and rpm throughout manuscript.

We have converted all examples of rpm units to rcf in order to be consistent.

11.) Equipment - adapters are missing.

We have included the part in the equipment list and modified the text replacing “Corex” tubes with glass centrifuge tubes.

12.) Discussion, paragraph 1 - be consistent with abbreviations throughout, hCG vs HCG.

We have made the suggested change.

13.) Discussion, paragraph 2 - "Alternatives could include polymerization using GTP-induced polymerization (a classic technique) or using Ran-GTP as a nmicrotubule polymerizer to mimic the microtubules induced by chromatin-driven spindle assembly." Last line of paragraph should be 'and/or'.

We have made both corrections.   
  
Reviewer #2:   
*Summary:*   
In this methods paper Sharp and Blower describe the preparation of Xenopus tropicalis egg extract arrested in meiosis II and the purification of microtubule-associated RNAs. Extracts from Xenopus laevis eggs have been described a long time ago, are well established and have an important place in both historical as well as modern developmental and cell biology. However, Xl does not have a fully sequenced genome while Xt has.  
The preparation of Xt egg extract was pioneered by Brown and colleagues in the Heald lab. However, a detailed protocol was never published, hence this manuscript will be of high interest.  
The overall structure of the protocol is clear and easy to follow. Therefore, I only have a few, very specific comments and one more general.  
  
*Reviewer Concerns:*  
Specific comments:  
(1) Although never having worked with Xt, I remember temperature being crucial not only for the housing and care of the Xt frogs but also for the egg / extract quality. The authors should comment / be more specific about this.

(2) The classic Xl egg extract is spun at 17 000 g, starting the centrifugation at room temperature and cooling down to 4°C. In (2.7.) the eggs are spun at 20°C and not cooled down. Is this because of the nature of the tropicalis eggs or because of the subsequent use of the extract? In (3.2.) any temperature information is missing.

We have modified Protocol sections 1, 2.10, and 3.2 to include more specific temperature information for housing of the frogs and experimental manipulation of the extract. In addition, we have added text to the first paragraph of the discussion to highlight this point as a major difference between extract preparation methods (see below).   
  
(3) Extracts of Xl eggs are usually sensitive to mechanistic shearing. Do the authors recommend cutting the pipette tips,??

We have not observed mechanistic shearing of the extract when passed through an 18g needle or titurating with a 1 mL pipet tip. We have added text to clarify this point in Protocol section 2.10.   
  
General comment:  
This protocol will be particularly interesting for scientists that have so far worked with Xl. Thus, it would be very helpful to the write this protocol in a very analogous manner to the original Xl egg extract protocol. This has been realized for most parts, however, using terms like "packing", ? or particularly pointing out what the main differences / similarities are would improve the manuscript.

The first paragraph of the discussion highlights the main differences between the technical details of preparing egg extracts from *X. tropicalis* versus *X. laevis* frogs. One of the most significant and challenging aspects to working with *X. tropicalis* frogs is to achieve optimal egg laying conditions such that egg extracts still possess biochemical activity. We have added text to elaborate upon this point with regard to egg laying conditions and temperature. In addition, we have added text to clarify what is meant by “packing” of the eggs when the jelly coat is removed.

With regard to format, we have written this manuscript according to the suggested format compliant with JoVE editorial guidelines. In addition, we have taken particular care to provide sufficient detail such that the text, when supplemented with videography, will allow the reader with access to the proper equipment to duplicate the methods precisely.