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Vidhya Iyer, Ph.D.
Reviewing Editor
JoVE
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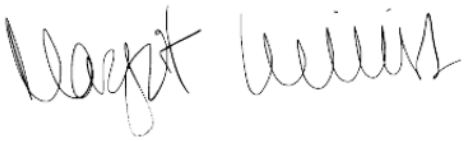
Dear Dr. Iyer,

We would like to thank yourself and the anonymous Reviewers for the thoughtful evaluation of our manuscript entitled "*Generation of naïve blastoderm explants from zebrafish embryos*" (JoVE62797). We found the Reviewers' critiques to be both thorough and fair, and we have done our very best to revise our article in accordance with both Reviewer and Editorial comments. On the next page, we provide a point-by-point response to these comments and describe the steps we have taken to address them.

We were pleased that the Reviewers feel our protocol will be of great utility to the Developmental Biology community, and we are especially grateful for their eagle-eyed proof-reading that identified typographic errors and improper font conversions that altered our units as presented. Notably, we also added sample numbers to our figure panels and included two new data panels demonstrating that Nodal-deficient explants – like uninjected WT explants – fail to express markers of mesoderm or neuroectoderm tissues. We feel these revisions have improved the quality of our manuscript and enhanced the clarity of our protocol. We anticipate that these changes will satisfy the concerns of both the Reviewers and Editors, and we look forward to your decision.

Thank you for your time and consideration.

With warm regards,



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We thank the Editor and Reviewers again for their careful review of our manuscript. Below, we detail our responses to their comments, and hope that we have addressed these concerns to the satisfaction of all Reviewers and Editors. Original comments are shown in blue, our responses below in black.

Response to Editorial comments

1. Thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

Both authors proofread the manuscript to ensure that spelling and grammar issues are addressed as well as abbreviations defined upon first use.

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.
And
13. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source (ITALICS). Volume (BOLD) (Issue), FirstPage–LastPage (YEAR).] For 6 and more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate the journal names.

We have revised the reference style, including in-text citations and bibliography, in accordance with these requirements.

3. Please consider providing solution composition as Tables in separate .xls or .xlsx files uploaded to your Editorial Manager account. These tables can then be referenced in the protocol text.

An .xls file has been created entitled “Solution Composition table”. These solution recipes are referenced in the protocol as follows: (Solution Composition: **Solution #**)

4. Please revise the following lines to avoid overlap with previously published work: 377-380

These lines comprise the legend of Figure 2, which was modified from Williams and Solnica-Krezel, *eLife*, 2020. The source publication was cited in the title of this figure, but it is now revised to: “Figure and legend modified from [Williams and Solnica-Krezel, *eLife*, 2020]”.

5. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials, e.g., Instant Ocean sea salts; MilliQ; Pico-pump; pronase etc

To our knowledge, all commercial language has been removed. However, it was our impression that “pronase” is the name of an enzyme rather than a commercial name. If we are mistaken, we will gladly make this change.

6. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

Numbering in the protocol has been revised.

7. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Personal pronouns have been removed from the manuscript.

8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.).

The manuscript has been edited to use the imperative tense throughout the protocol.

10. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

We have highlighted the portions of the protocol to be included in the corresponding video.

11. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique (the troubleshooting section of the protocol can be moved here with appropriate adjustments)
c) Any limitations of the technique (discussed well)
d) The significance with respect to existing methods
e) Any future applications of the technique

We have modified our Discussion section to explicitly cover these areas.

12. Please include a Disclosures section, providing information regarding the authors’ competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

We have added a Disclosure section stating that the authors declare no competing financial interests.

14. Please sort the Materials Table alphabetically by the name of the material.

We have reorganized the Materials Table in alphabetical order.

Response to Reviewer comments

We have corrected many small errors in our manuscript, including spaces between numbers and units and typographical errors. We thank the Reviewers for catching these, particularly the issues with units (mg vs. μg, for example) which resulted from improper conversions of fonts between Word and PDF formats. These have been corrected, and we have included a PDF version of the manuscript as well in the event of further issues with converted file formats.

Reviewer #3:

1. The authors described that explants are viable until at least 24 hours post fertilization (hpf), but maximum extension is usually achieved by 12-14 hpf. How did the authors know these explants are viable or not?

Our explant methods are largely based on those described in Xu et al, *Science*, 2014, in which the authors demonstrate viability of explants for up to 24 hours. We have now cited this article in our reference to explant viability at 24 hpf (in the Discussion section, page 11).

2. For explant media, why did the authors use DMEM/F12 and Newborn Calf Serum? Since the explants can only survive for 24 hours, I am wondering whether this medium can be improved. For example, Calf serum can be replaced by FBS or fish serum. DMEM/F12 can be replaced by L15.

A very similar media recipe was described in Xu et al, *Science*, 2014. Because it has suited our lab's needs well, we have made only minor modifications. For example, we have made our explant media with either FBS or NCS and found that it made no difference in explant growth or viability. We therefore continued to use the less expensive option: NCS. It is our impression that failure of explant viability after 24 hpf is likely not the result of suboptimal culture conditions, but rather a limit of the explants themselves (likely due to their lack of yolk). While it certainly may be possible to extend their viability by altering media composition, we feel that testing this is outside the scope of this article.

3. In this manuscript, the authors only test the utility of explants by studying the Nodal signaling. Is it possible to use other signaling to confirm such utility?

Xu and colleagues (*Science*, 2014) generated similar explants from embryos injected with RNAs encoding both Nodal and BMP to demonstrate that this combination of molecules is sufficient to recapitulate much of zebrafish axis formation. It is our sincerest hope that members of the developmental biology community will use these methods to test a variety of additional signaling molecules!

Reviewer #4:

First, I would like to see numbers for the data shown in Figures 2 and 3.

We have modified Figures 2 and 3 (and their legends) to depict sample numbers for the explants shown.

Second, the authors should show whether the MZoep mutant explants express Sox2 (and tbxta) alone, or in combination with wildtype, uninjected explants.

We added two new panels to Figure 3 (see the new panel D, below) depicting single-embryo explants from MZ*oep*-/- embryos injected with *ndr2* RNA to demonstrate that, even upon exposure to Nodal ligands, they express markers of neither mesoderm nor neuroectoderm.