



Production and characterization of monoclonal antibodies to *Xenopus* proteins

Brett Horr, Ryan Kurtz, Ankit Pandey, Benjamin G. Hoffstrom, Elizabeth Schock, Carole LaBonne and Dominique Alfandari

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Original submission:	22 September 2022
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Original submission

First decision letter

MS ID#: DEVELOP/2022/201309

MS TITLE: Production and Characterization of Monoclonal antibodies to *Xenopus* proteins.

AUTHORS: Brett Horr, Ryan Kurtz, Ankit Pandey, Ben Hoffstrom, Elizabeth Schock, Carole La Bonne, and Dominique R Alfandari

Apologies for the delays in obtaining reviews and making a decision on your manuscript. I have now received all the referees' reports, discussed the manuscript with another editor and our executive editor and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The reviewers had mixed feelings about the suitability of your manuscript for publication in Development. Reviewer 2 sent comments directly to the editor indicating their opinion that the manuscript was not suitable for this journal as there was insufficient novelty in terms of methodology and that the resource was relatively modest as a community resource. The two other reviewers are more positive about the utility of the resource for the community. Reviewer 1 has relatively minor comments whereas Reviewer 3, in their review and in other comments, considers that the manuscript needs to be restructured/rewritten. I discussed the manuscript and reviews with my colleagues and we think that the study could be suitable as a Development paper but it would need to be condensed and restructured with a better separation of Methods and Results/Discussion sections. Much of the text on protocols could be moved to methods putting more focus on validation and characterisation of antibodies in the Results section. We think that much of the text in your current Conclusions and Future Directions could be cut and brief discussion points incorporated with the results in a Report format paper.

If you are able to revise the manuscript along the lines suggested, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round

of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee's comments, and we will look over this and provide further guidance.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This is a useful and necessary contribution to the Techniques and Resources section of DEVELOPMENT

Comments for the author

Major Point:

These days application of antibodies for ChIP should at least be discussed in the Conclusion and Future directions section

Minor Points:

- 1) in the Abstract, I wonder whether the authors would want to consider replacing 'rarely recognize' to 'unreliably recognize', since in a sizeable minority of instances antibodies against mammalian proteins do work in *Xenopus*, it is just unreliable and often affects at least some of the molecules that *Xenopus* researchers are working on.
- 2) on page 4, line 7, in order to avoid confusion, consider referring to four mice rather than to 'four animals', readers not already well versed in antibody production (which this target readership may not be) could be confused with the just previously mentioned *Xenopus*.
- 3) on page 4, line 11, at this stage the reference to spleens is unclear, consider either to explain here or refer to later on by inserting '(see below)' here.
- 4) on page 8 line 22, it is not clear which equipment you are referring to 'this equipment (not widely available)' only after reading figure legend for figure 4, could this possibly make sense.
- 5) on page 9, line 3, I don't think the abbreviation 'mab' has been introduced, maybe could be introduced in the Introduction section page 2.
- 6) on page 9, line 9, consider 'embryo extract' for 'embryos extract'
- 7) page 9, line 15, I think it is really not clear how the text relates to what is shown in the current Figure 7, was this from a previous draft?
- 8) A Table Legend would be really useful for Table 1, some of the headings and abbreviations only start to make some sense after carefully reading the result text, e.g. page 8 'Endogenous.'
- 9) consider whether Figures 5 and 6 could be combined into one figure.
- 10) again abbreviation Mab could be introduced.

Reviewer 2*Advance summary and potential significance to field*

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Comments for the author

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Reviewer 3*Advance summary and potential significance to field*

This is a useful resource paper that publicizes the production of a fairly large number of hybridomas raised against *Xenopus* proteins. The manuscript is useful to the field also in providing a conversational (and relatively grammar free) discussion of how the authors went about making the antibodies, which is certainly useful for the Developmental biology audience. There are good guidelines for those who would be interested in making antigens for immunizations, and processes for optimizing recovery of hybridomas. The distribution through DHSB is a major plus.

The manuscript will be well cited.

Comments for the author

Clearly the coauthors did not read the manuscript, and perhaps should do their due diligence, or be removed from authorship.

A few comments

Scientific: “between human and *Xenopus* is much lower resulting” give a percentage in conserved regions for mammal to mammal or mammal to frog? As provided, the statement is not enlightening.

Proofreading: Myleoma

Advances in genome annotation and genomic methods have allowed mRNA expression at most stages of embryo development. Should be “have quantified/detected mRNA expression”?

Run spellcheck? Lambruch-chromozomes Run on sentence- proofread? “Examples abound of a new lot of a commercial antibody that no longer recognizes a target recognized by prior lots or recognizing additional targets.”

sloppy wording. “The same construct could also be transfected in *Xenopus* XTC cells “ Imagine “infect” usage rather than the widely misused “transfect into”, and one rewords the sentence to “*Xenopus* XTC cells could also be transfected with the same construct”

“Once a positive well is identified, it is a race against time to identify the colony that produce the useful antibody.” What tense do the authors intend to use? Usually past tense for what was done, present tense for what is concluded. “Colony that produce” subject verb agreement.

“A typical well will have three colonies of various size growing.”

Plural hybridoma Hybridomas?

Rather than channel Roy Kent in further remarks about the rest of the manuscript, I will leave it to the authors to condense and rewrite more carefully in their revised draft.

First revisionAuthor response to reviewers' comments

I would like to thank the reviewer for their support of the article and constructive comments. The new draft has been significantly rewritten to incorporate all of the comments from both reviewers and editors.

Most notably,

The result and discussion have been merged and shortened.

The results concerning the use of bacterial fusion protein has been merged into subheading Antigen production, and the advantages and disadvantages of each are discussed early in the text (limitation of Hek293T production approach).

This re-organization and shortening has allowed us to make new figures (5 instead of 7) that flow better with the manuscript. The flow is much more natural now with antigen production, immunization, fusion, and screening.

Once re-structured, we have had 5 native English speakers proof read, we have ran multiple spell check and used Grammarly to correct. While I am sure there are still some errors I believe that most have been corrected.

Reviewer 1 Comments for the author

Major Point:

These days application of antibodies for ChIP should at least be discussed in the Conclusion and Future directions section

We have introduced the Chip IP in the results when discussing immunoprecipitating antibodies.

Minor Points:

1) in the Abstract, I wonder whether the authors would want to consider replacing 'rarely recognize' to 'unreliably recognize', since in a sizeable minority of instances antibodies against mammalian proteins do work in Xenopus, it is just unreliable and often affects at least some of the molecules that Xenopus researchers are working on.

We have changed the text with "often do not recognize" and have extended the reasoning why cross-reactivity is low.

2) on page 4, line 7, in order to avoid confusion, consider referring to four mice rather than to 'four animals', readers not already well versed in antibody production (which this target readership may not be) could be confused with the just previously mentioned Xenopus.

We changed the text

3) on page 4, line 11, at this stage the reference to spleens is unclear, consider either to explain here or refer to later on by inserting '(see below)' here.

The text has been significantly modified and this sentence was cut.

4) on page 8 line 22, it is not clear which equipment you are referring to 'this equipment (not widely available)' only after reading figure legend for figure 4, could this possibly make sense.

Line 10 of the same page started with the capillary system that was used. We have now eliminated that sentence in the new text.

5) on page 9, line 3, I don't think the abbreviation 'mab' has been introduced, maybe could be introduced in the Introduction section page 2.

mAb is now introduced at the beginning of the introduction

6) on page 9, line 9, consider 'embryo extract' for 'embryos extract'

We have fixed this everywhere in the text.

7) page 9, line 15, I think it is really not clear how the text relates to what is shown in the current Figure 7, was this from a previous draft?

We have significantly remodeled the text and only have 5 figures now. The original text was written based on the chronology of event but not in a fully logical way. This meant that the figure were called out of order. The new draft fixes all of these issues.

8) A Table Legend would be really useful for Table 1, some of the headings and abbreviations only start to make some sense after carefully reading the result text, e.g. page 8 'Endogenous.'
We have added a legend for table 1 that describes all of the column.

9) consider whether Figures 5 and 6 could be combined into one figure.
We have merged these figures.

10) again abbreviation Mab could be introduced.

-

Reviewer 3 Advance summary and potential significance to field

This is a useful resource paper that publicizes the production of a fairly large number of hybridomas raised against Xenopus proteins. The manuscript is useful to the field also in providing a conversational (and relatively grammar free) discussion of how the authors went about making the antibodies, which is certainly useful for the Developmental biology audience. There are good guidelines for those who would be interested in making antigens for immunizations, and processes for optimizing recovery of hybridomas. The distribution through DHSB is a major plus.

The manuscript will be well cited.

Reviewer 3 Comments for the author

Clearly the coauthors did not read the manuscript, and perhaps should do their due diligence, or be removed from authorship.

We apologize for the apparent lack of care of this first draft. As the lead author I take full responsibility for this and can only give as an excuse that I am not a native English speaker. Clearly the various software for spell and grammatical check are not to be relied upon.

I and the co-authors have spent a lot of time rewriting the manuscript dramatically and hope that it is now proper.

A few comments

Scientific: "between human and Xenopus is much lower resulting" give a percentage in conserved regions

We have introduced this data in the new manuscript, but this was not a trivial work. We found no publication or databases that provided these number and had to do our own proteome comparison.

for mammal to mammal or mammal to frog? As provided, the statement is not enlightening.

The text has been changed.

Proofreading: Myleoma
Corrected

Advances in genome annotation and genomic methods have allowed mRNA expression at most stages of embryo development. Should be "have quantified/detected mRNA expression"?

This has been changed to Advances in genome annotation and genomic methods have allowed quantification of mRNA expression at most stages of embryo development and in most cell types.

Run spellcheck? Lambruch-chromozomes
Sorry again

Run on sentence- proofread? "Examples abound of a new lot of a commercial antibody that no longer recognizes a target recognized by prior lots or recognizing additional targets."

Examples abound in which a new lot of a commercial antibody no longer recognizes the original, specific target protein or begins to recognize additional, nonspecific targets.

sloppy wording. “The same construct could also be transfected in Xenopus XTC cells “ Imagine “infect” usage rather than the widely misused “transfect into”, and one rewords the sentence to “Xenopus XTC cells could also be transfected with the same construct”

I am not sure that I understand the reviewer comment here. We use plasmid that are transfected. We do not use viral constructs that can infect cells.

I guess the cells are transfected with a plasmid as opposed to the plasmid transfected into cells?

Here is my attempt “Xenopus XTC cells were also transfected with the same constructs which enabled us to screen hybridomas by indirect immunofluorescence.”

“Once a positive well is identified, it is a race against time to identify the colony that produce the useful antibody.” What tense do the authors intend to use? Usually past tense for what was done, present tense

I believe that we have fixed all tense issues.

for what is concluded. “Colony that produce” subject verb agreement.

“A typical well will have three colonies of various size growing.”

Plural hybridoma Hybridomas?

All of the above mention issues have been corrected

Rather than channel Roy Kent in further remarks about the rest of the manuscript, I will leave it to the authors to condense and rewrite more carefully in their revised draft.

We have condensed the text and reorganize it so that the figure and logical flow is better.

Second decision letter

MS ID#: DEVELOP/2022/201309

MS TITLE: Production and Characterization of Monoclonal antibodies to Xenopus Proteins

AUTHORS: Brett Horr, Ryan Kurtz, Ankit Pandey, Ben Hoffstrom, Elizabeth Schock, Carole La Bonne, and Dominique R Alfandari

Apologies for the delay in obtaining reviews on your revised manuscript. You will be pleased to see that the two referees are happy with your revisions and we will be happy to publish your manuscript. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area. Before we proceed to publication, I would like you to consider whether Figure 3 is really necessary? Our executive editor and I have both read the paper and we both think that this figure is not needed and would prefer if it was removed (though this will not be a condition for publication) - normally we would suggest moving to supplemental data but you don't have any other supplemental data and so creating a supplemental data file just for this figure seems unnecessary. Please let me know your thoughts on this point.

Reviewer 1

Advance summary and potential significance to field

This revised manuscript looks ready for publication.

Comments for the author

This revised manuscript looks ready for publication

Reviewer 3

Advance summary and potential significance to field

This is an excellent revision, and I thank the authors for the clarity of writing. It reads well, and will be an important reference to this very valuable resource. I congratulate the authors for their effort and success.

If someone asks me about antibodies, I will direct them to this excellent review and reduction to practice of the methods and approaches.

Comments for the author

I apologize for asking for the % identities if it caused a lot of work.

I still suspect that the co-authors did not read the first iteration, and although the text is the responsibility of the senior and first authors, it is also surely the responsibility of all authors, some of whom claim English as a first language.

Also, I apologize for the pedantic view of the word transfect. The author has it exactly right in the response, it should be used in the same way as infect. One would never say, for example, that "SARS2 was infected into mice"

Only one grammatical correction to be noted: hybridomas libraries should be hybridoma libraries.

Many thanks for the opportunity to read this, and apologies for a review delayed by the season, and jury duty.

Second revision

Author response to reviewers' comments

We thank the reviewer and have corrected the Hybridoma Libraries.

Third decision letter

MS ID#: DEVELOP/2022/201309

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AUTHORS: Brett Horr, Ryan Kurtz, Ankit Pandey, Ben Hoffstrom, Elizabeth Schock, Carole La Bonne, and Dominique R Alfandari

ARTICLE TYPE: Techniques and Resources Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.