Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Non-invasive Chromosome Screening (NICS) of human preimplantation embryos: sample collection and chromosomal ploidy analysis by MALBAC-NGS” (ID: JoVE56125).

Those comments are all valuable and very helpful for revising and improving our pap-er, as well as the important guiding significance to our researches. We have studied  comments carefully and have made correction which we hope will meet with approval.

The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the editorial comments：

1. We have removed all the embedded figures and tables in the text and prepared all the figures in the pdf format.
2. All the commercial language has been removed and rearranged the table of materials/reagent which including all the material used in the protocol.
3. All format and phrases errors in the text have been corrected according editorial requirement.
4. The abbreviations of IM, GM, BM have been defined in step 1.1.
5. The approach has received the agreement of Institutional Review Board in the ethic statement section.
6. For step 1.2 and step 1.3. “provide a reference for generating fire polished tips” we performed the preparation fully base on our experience. The method of fire polishing can be trained and exercised.
7. The stepwise details have been added for each step mainly clarified how to perform.
8. For step 3.5 and 3.7, we provided the manufacture’s website to download the guide for user in reference.
9. For the data analysis part, we provided a simply application for user to upload and analysis data in Figure 3. And the software used in the analysis procedure have been clarified.
10. Results, figure legend and discussion have been enriched base on the editorial suggestion which can also been read in the responds to reviewer’s comments.
11. All the filmable content has been highlighted in yellow although it is little more than three pages, cause our NICS technology continuously including the preparation of culture medium, whole genome amplification (WGA), NGS library preparation and data analysis four sections. We not only want to show how to collection medium but also want to present the final CNV results of data analysis. So we sincerely hope editors can understand and support our opinion, and it will only take a little more minutes to make the video. Thanks a lot!

Responds to the reviewer’s comments: Reviewer #1:

Comment 1: This approach still need to generate a small hole to release the fluid from blastocyst，so strictly it is not 100% Non-invasive method. Did the authors ever try to use the culture medium without releasing the fluid in blastocyst?

Response: We indeed performed the NICS assay without Artificial shrinkage (AS) before. And the result is still available. The reason why we add this step is AS can increase the DNA content in the medium, improve the success rate of amplification, get a more stable result. It also has been demonstrated that too much blastocoele fluid may disturb the efficacy of vitrification. Previous studies have shown that AS of blastocoeles before vitrification can increase the survival rate of vitrified blastocysts and improve the IVF-ET clinical outcome. AS is the regular operation before cryopreservation, and it is not the additional specific step for NICS.

Comment 2: What do the authors use for positive control? (line 216)

Response: The positive control is the human genomic DNA, and the concentration is 10 ng/µL. We have added the details in step 3.1.1. of the manuscript. The final DNA content in this reaction range from 5 to 10 pg.

Comment 3: Can the authors please figure out the detection efficiency and accuracy of this approach?

Response: We presented the data in the published PNAS journal. The negative predictive value (NPV) of chromosomal abnormalities with the NICS assay is 91.3%, which is substantially higher than the positive predictive value (PPV) (78.9%) of the assay18.

Comment 4: Some hatched blastocysts would release aneuploidy cells into media, how to exclude these cells?

Response: Actually, we are not sure whether these cells are aneuploid cells or not and have no way to exclude aneuploid cells. Right now, we can calculate the mosaic rate of the gDNA at spent culture media and assess whether there are aneuploid cells released into the culture media. In the operation procedure, we always centrifuge the collection medium before added into the lysis buffer to avoid the interference of aneuploidy cells.

Comment 5 and 6:"allcumulus-corona" should be "all cumulus-corona". (line 385), Company "Axcygen" should be "Axygen". (Table of Materials/Equipment)

Response: Thanks for your patient review. We have corrected the spell error in the text.

Responds to the reviewer’s comments: Reviewer #2:

Comment 1: If the authors simply explain criteria or a threshold between the results of figure A-E (abnormal) and F (normal) quantitatively based on the data, it would be helpful for readers.

Response: Thanks for your suggestion. We have explained the karyotype of all the 6 NICS results in result section and figure legends. Such as 46, XN, +1p (pter→p21.2,×3), -18 (q21.32→qter,×1) is long arm of chromosome 18 q21.32→qter region deletion and short arm of chromosome 1 pter→p21.2 region duplication caused by the parents’ balanced translocation.

Comment 2: The meaning of PGS should be explained in the main text though it is in the keywords.

Response: We add the meaning of PGS in introduction section in line 78 according some references. Such as “Preimplantation genetic screening (PGS) with comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages”

Comment 3,4,5: The figure legend does not have enough information, the meaning of gray dots (not read and blue) should be explained, the meaning of each figure title (e.g, 45,XN,-18(×1)) should be explained.

Response: The previous figure legend indeed can’t explain the figure clearly, we have fully clarified the karyotype of every NICS result in result section. The gray dots are the ruler scale of copy number response each bin window.

We appreciate for Editors and Reviewers’ warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.