

Dear Editors

We are sincerely thankful for thorough review and for suggesting modifications that would improve the quality of the article. The suggested modifications have now been included in the revised version. Please find below line by line response to each comment.

Comment 1: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Response 1: Manuscript has been proofread for spelling or grammatical errors.

Protocol Language Comments:

Comment 2: Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

Response 2: The language of the protocol has been checked for imperative voice.

Comment 3: Lines 142-145, 162-164, 207-214 etc should be NOTES.

Response 3: The above mentioned lines have been now included as NOTES in the manuscript.

Comment 4: Section 5 must be rewritten as steps in the imperative voice.

Response 4: As suggested, we have edited section 5.

Protocol Detail Comments:

Comment 5: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Response 5: As suggested, we have added these details. Please refer to revised sections 4.3.11 and 5 of the revised manuscript.

Comment 6: 1.1: unclear what the "same ethanol gradient and xylene" is.

Response 6: We have now included details on these steps in revised version.

Comment 7: 1.4: Is any reagent used? Unclear why the tip is cut and how the collection of dry sample is performed? Is only cancerous tissue collected?

Response 7: To collect the tissues, no reagent is used. Rather, the electrostatically charged tip attracts the dissected tissue and allows for maximum tissue recovery after dissection. Once the tissue sticks to the charged tip, the tip is cut into an empty 2 mL collection tube. This description has been included in revised version. Cancerous tissue and/or normal adjacent areas are collected.

Comment 8: Section 2: What kind of serum is this?

Response 8: These are patient-derived serum samples isolated from metastatic CRPC cases with adenocarcinoma or NE differentiation. Serum samples were collected from same set of patients as those used for microdissected tissues to allow comparison between corresponding tissues and serum-derived EVs. The section has been modified in the manuscript for clarification.

Comment 9: 3.2.1: What are the compositions of lysis buffer A and B?

Response 9: Buffer A and B were part of exosomal RNA isolation kit. A line on using kit based method has been included for better understanding.

Comment 10: 3.2.4, What is the composition of elution buffer?

Response 10: Elution buffer was part of exosomal RNA isolation kit. A line on using kit based method has been included for better understanding.

Comment 11: 4.2.1, 4.2.4, 4.2.8, 4.2.10, 4.2.12: "Keep on pre-heated PCR" appears incomplete. Please check.

Response 11: The line has been modified in the manuscript as suggested.

Comment 12: 4.1: List all primers in the table of materials.

Response 12: We have added list of primers in 'Table of Materials'. Please refer to sheet 2 'list of primers' under the revised table.

Protocol Numbering Comment:

Comment 13: Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. Please add a one-line space after each protocol step.

Response 13: We have modified and numbered the protocols according to the required format as suggested by the Editor.

Protocol Highlight comment:

Comment 14: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

- 1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
- 2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
- 3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
- 4) Notes cannot be filmed and should be excluded from highlighting.

Response 14: The manuscript has been accordingly highlighted, taking in to consideration the above mentioned comments.

Discussion comment

Comment 15: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Response 15: Discussion has been reviewed and checked to fit the format description as suggested by the Editor.

Figures comment

Comment 16: Fig 1: The numerous panels on the right hand side of this figure are too small to read. Please make this a separate figure.

Response 16: Figure 1 has been separated to Fig1, 2 and Fig. S1 for better clarity and ai files for each figure have been provided

Comment 17: Figure/Table Legends: 1) Fig 2: define error bars.

Response 17: The error bars are standard deviations for the error rates for PhiX alignment over different cycles. The line has been modified and included in the figure legend for the corresponding figure.

References comments:

Comment 18: Please edit your references to comply with JoVE instructions for authors. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then et al.): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, doi:DOI (YEAR).]

Response 18: As suggested, we have edited references.

Comment 19: Please spell out journal names.

Response 19: We have spelled out full journal names for all the references.

Commercial Language comment:

Comment 20: JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are nanosight, nanodrop, mini-spin column, Qubit, (Illumina, PCR block, geldoc, bioanalyzer 1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

Response 20: As suggested, all the commercial sounding words as mentioned above have been replaced to more generic names in the manuscript.

Other comments:

Comment 21: Please define all abbreviations at first use.

Response 21: Abbreviations have been defined in the manuscript at first use.

Comment 22: If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Response 22: All figures and tables have not been published previously.

Again, we sincerely thank the editor for helpful comments. We hope that we have satisfactorily addressed all the raised concerns in the revision and hope for a favorable consideration.

Thank you!

Sincerely

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