International Journal of Cancer Management
Volume 18 · Issue 1 · e157228

OPEN PEER REVIEW

Integrative Analysis of Non-coding RNAs and Hippo Signaling Pathway in Hepatocellular Carcinoma Using Bioinformatics and Experimental Approaches

Farzin Mirzaei-Nasab, Mehrdad Hashemi, Yousef Seyedena, Nazanin Hosseinkhan, Ahmad Majd

SUBMITTED	REVISED	ACCEPTED	DOI
19 Oct 2024	30 Nov 2024	23 Dec 2024	10.5812/ijcm-157228

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Associate Editor 2
2 **Revision 1** Associate Editor 3
Authors' reply to reviewers 4
Reviewer and editor comments 13

1 Original submission

1 comment · 8 Nov 2024

Associate Editor

MAJOR REVISION 8 Nov 2024

Comments:
1- The sequence of the primers used produced nonspecific products with RNA of other genes. This can influence the results of the experiments.
2- Table 1 should be corrected. The authors should add the sequence of the reference gene to Table 1. Also, the authors should add the product size for each primer pair to the table.
3- The text of the manuscript needs to be edited by a person familiar with English.
4- There is no explanation for some data at the end of the manuscript (pages 28-33). They are unclear.
5- The title should be added to the X axis in Figure 4.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Associate Editor's Comments:

Associate Editor 1:
Comments:

1- The sequence of used primers produced the nonspecific products with RNA of other genes. This can influence on the results of experiments.

2- Table 1 should be corrected. The authors should add the sequence of reference gene to the Table 1. Also, the authors should add the size of product for each primer pairs to the table.

3- The text of manuscript needs to be edited by a person familiar with English.

4- There is no explanation for some data of the end of manuscript (pages 28-33). There are unclear.

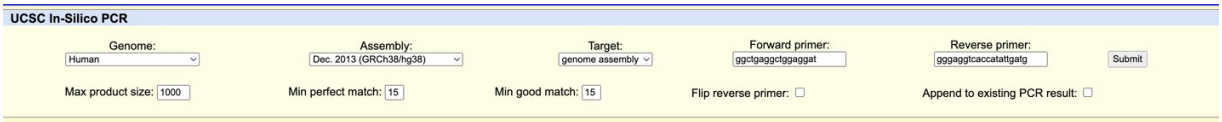
5- The title should be added to the X axis in Figure 4.

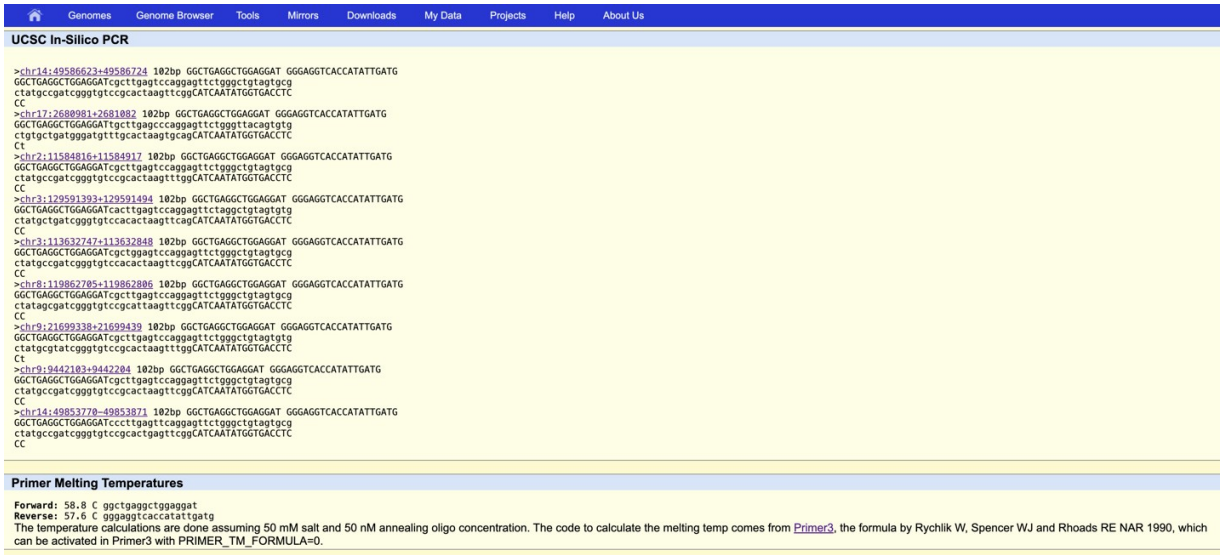
……..

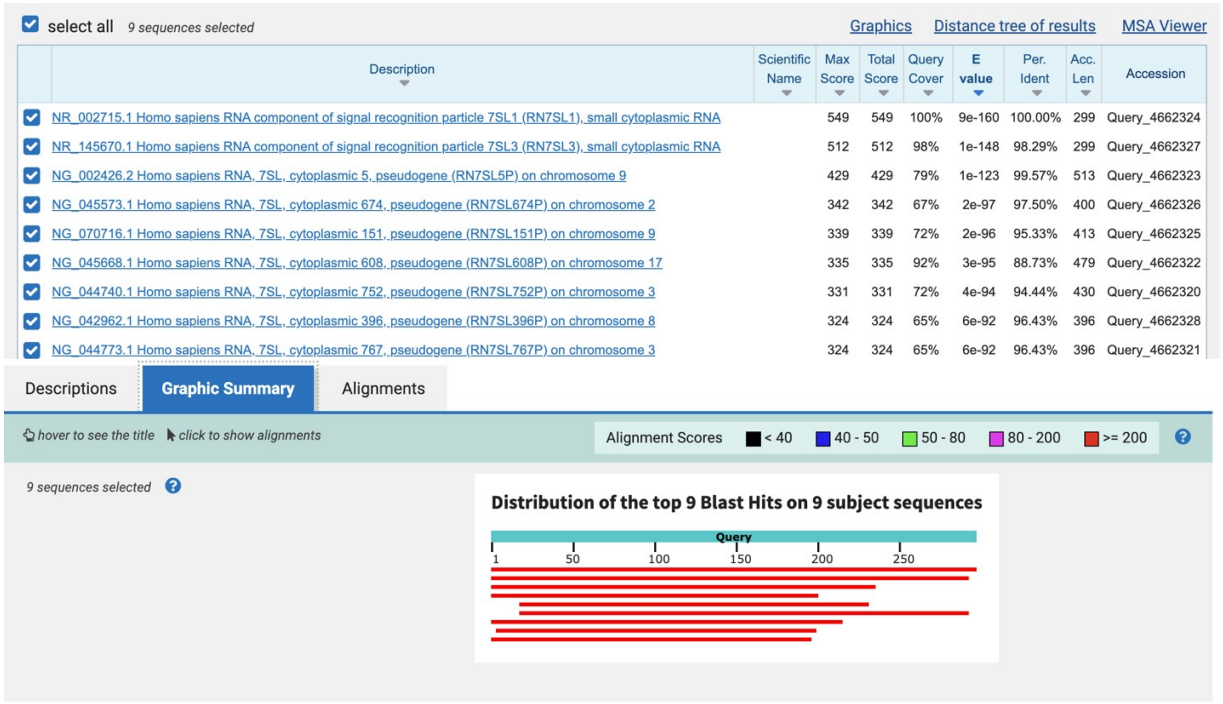
1- The sequence of used primers produced the nonspecific products with RNA of other genes. This can influence on the results of experiments.

Our response:

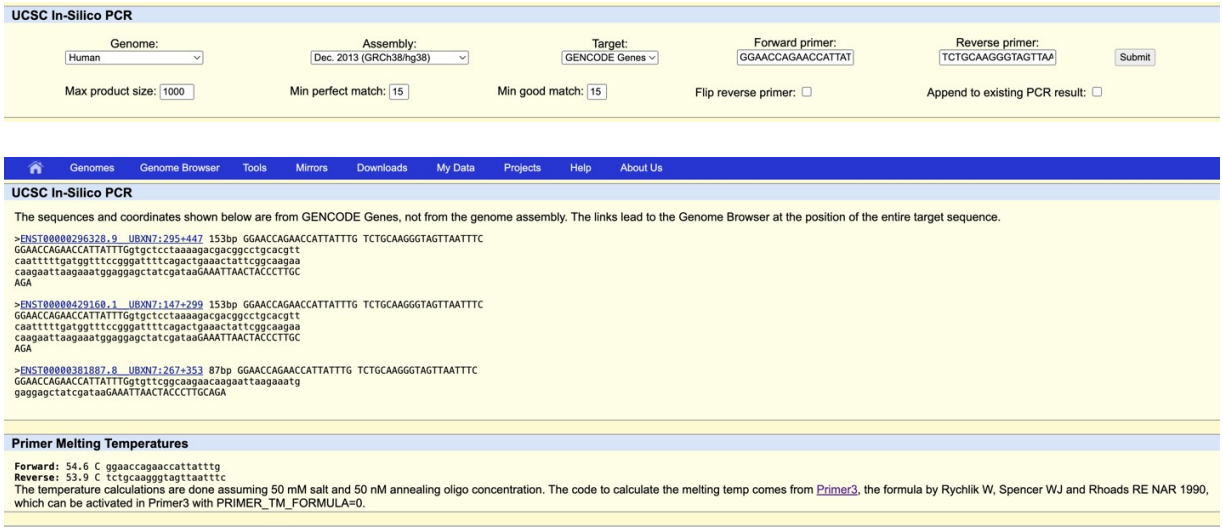
Regarding the *lnc-LRR1-1* gene, for which we designed primers, it is important to note that this transcript is an isoform of the *RN7SL1* gene. When we checked the designed primers using the PCR tool on the UCSC Genome Browser (https://genome.ucsc.edu/cgi-bin/hgPcr), we found that the primers produced results from 9 genes. These results correspond to different isoforms of the RN7SL1 gene, and a BLAST analysis of these sequences revealed that they share over 90% similarity. Therefore, these primers do not produce nonspecific products, as they amplify various isoforms of the same gene, as shown in the figures below.







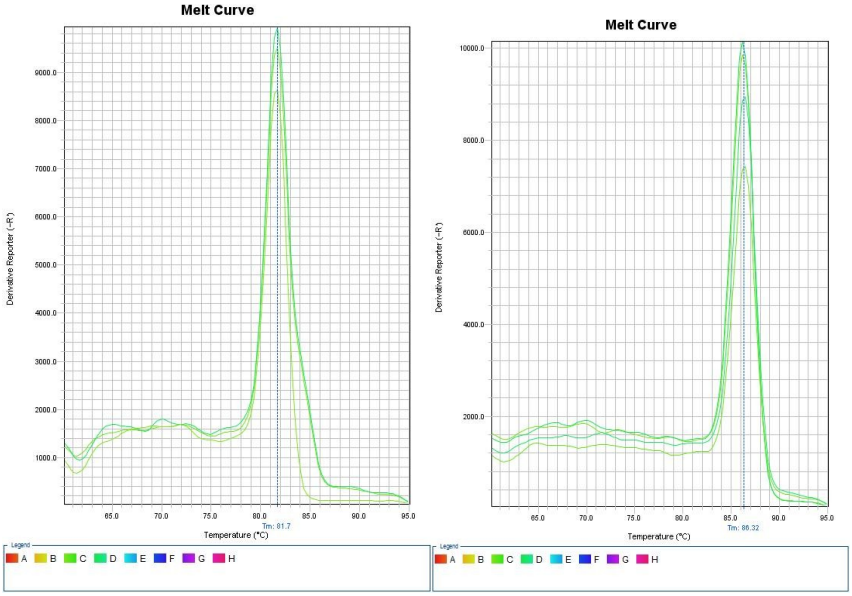
We reviewed the primer specificity for *hsa_circ_0001380* using the UCSC In-Silico PCR tool. The results show alignment with sequences from the host gene, *UBXN7*, rather than non-specific products from unrelated genes. This is expected, as the circRNA originates from *UBXN7* and shares sequence similarity. Therefore, the observed amplification aligns with the circRNA's genomic origin, not unintended off-target binding. We have clarified this in the manuscript to ensure transparency regarding primer design and expected results.



Additionally, we would like to present evidence demonstrating the high specificity of our primers:

1. The melting curve analysis from our Real-time PCR experiments shows single, sharp peaks for both genes (Tm: ~86.32°C and ~81.7°C respectively), indicating the amplification of specific products only. The absence of additional peaks or shoulders in the melting curves confirms that:
    - No non-specific products were amplified
    - No primer dimers were formed
    - Only specific target sequences were amplified
2. The clean, single-peaked melting curves demonstrate the high specificity of our primer pairs, validating the reliability of our gene expression results.

We have included the melting curve plots as supplementary evidence (Figure below) to demonstrate the specificity of our primers. These results strongly support the conclusion that our primers specifically amplified the intended target sequences without producing non-specific products."



Melt Curve *CIRC 0001380* Melt Curve *LNC-LRR1*

To improve clarity, this section has been revised and is now presented more explicitly in the manuscript.

In response to concerns regarding the specificity of the primers used for *lnc-LRR1-1* and *hsa_circ_0001380*, we conducted a thorough analysis to confirm specificity. The *lnc-LRR1-1* transcript is an isoform of the *RN7SL1* gene. Using the UCSC Genome Browser PCR tool, we observed that our designed primers amplified nine *RN7SL1* isoforms with over 90% sequence similarity, confirming that the primers target different isoforms of the same gene rather than nonspecific off-target sequences.

For *hsa_circ_0001380*, we verified specificity with UCSC In-Silico PCR, which showed alignment with the host gene *UBXN7*, from which the circRNA originates, not unrelated genes. Thus, the primers amplify the circRNA's genomic sequence as expected.

Additionally, melting curve analysis from our Real-time PCR experiments produced single, sharp peaks for both genes (*Tm*: ~86.32°C and ~81.7°C), indicating specific amplification without nonspecific products or primer-dimers. The single-peaked melting curves support our primer specificity and the reliability of the gene expression data. The melting curve plots are included as evidence of primer specificity.

2- Table 1 should be corrected. The authors should add the sequence of reference gene to the Table 1. Also, the authors should add the size of product for each primer pairs to the table.

We have revised Table 1 as requested. The table now includes the sequence of the reference gene, as well as the product size for each primer pair. The updated Table 1 appears as follows:

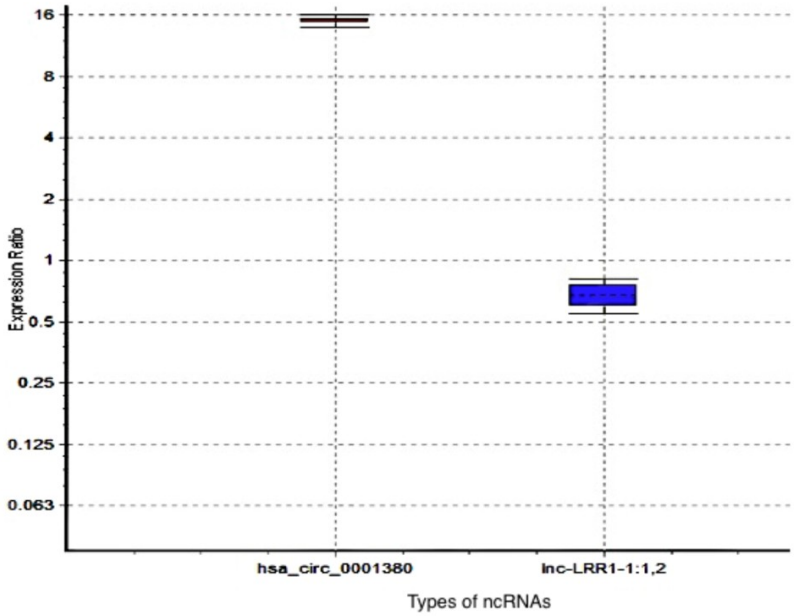
NO.	Name	Seq. (5'-3')	TM (°C)	size of gene	Product Size (bp)	Per 13 µl (total volume)
1	lnc-LRR1-1 -F	GGCTGAGGCTGGAGGAT	60	300	102	1 µl
	lnc-LRR1-1 -R	GGGAGGTCACCATATTGATG				
2	hsa_circ_0001380 -F	GGAACCAGAACCATTATTTG	60	247	87	1 µl
	hsa_circ_0001380 -R	TCTGCAAGGGTAGTTAATTTC				
3	Beta-actin -F	CCTGGCACCCAGCACAAT	60	5589	144	1 µl
	Beta-actin -R	GGGCCGGACTCGTCATAC				

3- The text of manuscript needs to be edited by a person familiar with English.

Thank you for highlighting this. We will have the manuscript thoroughly reviewed and edited by a native English speaker or a professional editor experienced with scientific language to improve clarity and fluency. Additionally, we have checked the text using Grammarly to address any grammatical and stylistic issues and ensure the language is polished.

4- There is no explanation for some data of the end of manuscript (pages 28-33). There are unclear.
It appears there might have been a misunderstanding regarding the page numbers, as my manuscript does not extend to pages 28-33. Could you please specify the data or section that requires further explanation? I would be glad to address any areas that need clarification.

5- The title should be added to the X axis in Figure 4.
I have addressed the issue by adding a title to the X-axis in Figure 4 as requested. Please let me know if there are any further adjustments needed to meet the standards.



Control of Tables: FAILED

Table 5 is not mentioned/referenced in the text correctly (or you have forgotten to mention one or more tables in the article body). Please mention/insert the Table(s) in the text consecutively.

You can read more about table design here (Direct Link: https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#tables.html)

Control of Figures: FAILED

Supplementary figure is mentioned in the text, but the figure is not available. Please upload the figure.
To read more about figures, refer here, and to read more about the formula, refer here.
https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#figures.html
https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#article_body.html

Dear Reviewer,

Thank you for your valuable feedback. We have addressed the issue as follows:

1. **Table 5 Reference**: The reference to Table 5 has been corrected in the manuscript. What was originally labeled as Table 4 has been updated to

Table 5, and this correction has been highlighted in green for your convenience.

2. **References to Tables 2 and 3**: We have also added references to Tables 2 and 3, which were previously missing. This addition has been made in the “4. Results” section under “Differential Gene Expression Analysis in HCC.” The new text, “The results of these analyses are presented in Tables 2 and 3,” has been highlighted in green.

Additionally, the supplementary files have been added in a separate Supplementary .jpg file.

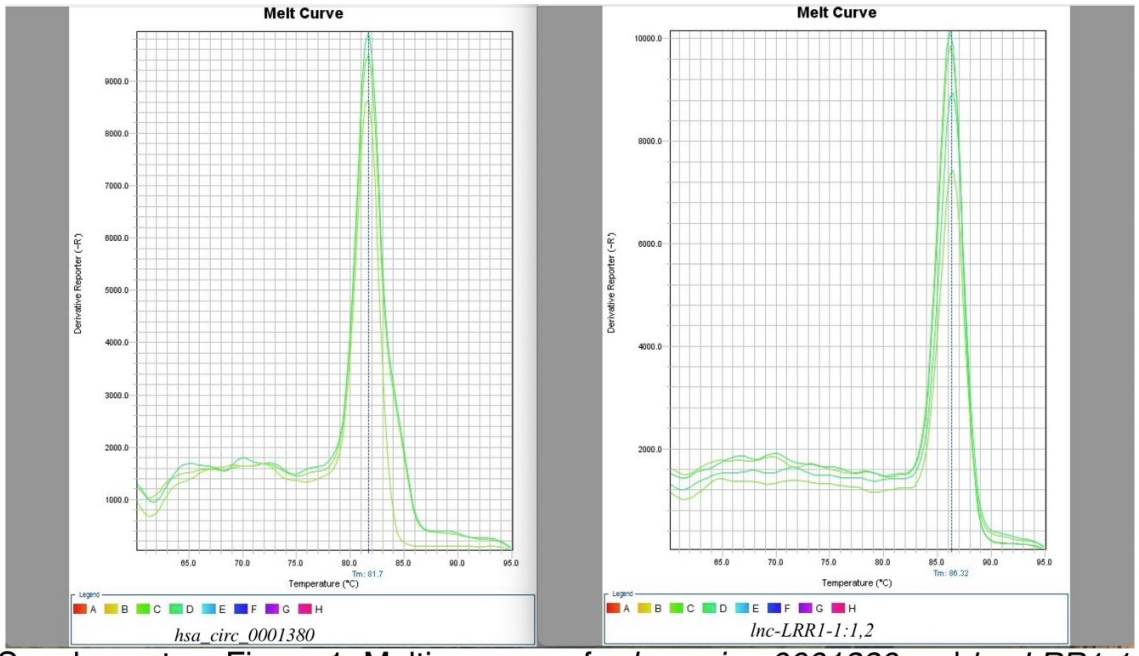
The previous versions of the response to reviewers and the full manuscript without authors have been removed and replaced with the updated versions.

We hope these changes meet your expectations, and we appreciate your assistance in improving our manuscript.

Best regards,

Control of Figures: FAILED Write a caption for Supplementary figure. To read more about figures, refer here, and to read more about the formula, refer here. https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#figures.html https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#article_body.html

We have addressed the concerns regarding the supplementary figure. The image now includes an appropriate caption. Additionally, a clarifying sentence has been added to the main text to ensure the supplementary figure is explicitly referenced, thereby enhancing the transparency and coherence of the information presented.



Supplementary Figure 1: Melting curves for *hsa_circ_0001380* and *lnc-LRR1-1*,2

Control of Revised Files: FAILED Highlight all revised text in your word file. You need to highlight any changes in your word file. You must reply to all reviewers' comments one by one with line number and numbering. Read more Here (Direct Link: https://brieflands.com/journals/international-journal-of-cancer-management/knowledgebase/category/tree.html#reply_to_reviewers.html). Upload a new file for "Reply to Reviewers." You should respond to Reviewers comments , not Screening e-mail.

We have addressed all the questions and issues raised. The revised document has been fully uploaded. In accordance with the guidelines, all revised text in the Word file has been highlighted. In response to each reviewer's comments, I have highlighted the relevant changes in the document.

Please note that we have attached a new file titled "Reply to Reviewers 1," which responds specifically to the reviewers' comments rather than the screening email.

Control of Authors' Contribution: FAILED In "Authors' Contribution," the first and last name of authors must be written only by capital letters of an abbreviation like S.M. Files Invalid

In response to your feedback regarding the "Authors' Contribution" section, we

have revised the document to ensure that the first and last names of the authors are now written using only the capital letters of their initials, as required. We appreciate your attention to detail and have made sure that this formatting is consistent throughout the section. Thank you for pointing this out, and please find the corrected version in the revised submission.

2 Revision 1

1 comment · 12 Dec 2024

Associate Editor

REJECT 12 Dec 2024

The sequences of the primers used produced nonspecific products with RNA from other genes. This can influence the results of the experiments. The authors' response is not convincing.