

Comments for “Molecular Detection Mapping and Analysis Platform (MDMAP)...” Yu, Young, Deeth, and Hanner

## **Basic Reporting**

The authors have met the editorial criteria for this section. The manuscript is well written, clear, and descriptive. The explanation of the need for the product is well described and many examples are given regarding the areas of study that could use the Platform. The figures are examples from each step of the process during which data are entered into the platform and are clear. A few comments regarding areas that could use a bit more detail are in the Reviewer Comments document.

## **Experimental Design**

This work is original research and appears to fall within the scope of the journal. They have clearly outlined its relevance to the qPCR field of study. I am particularly appreciative of the fact that the authors have made the various settings adjustable (i.e. Ct cutoff, etc.), recognizing the inherent variability from assay to assay, thus making the Platform more broadly applicable. The data fields chosen for the metadata are useful for secondary analysis of qPCR results. However, I wonder if the authors considered MIQE reporting for qPCR assays for the metadata (Bustin, et al 2009. <https://academic.oup.com/clinchem/article/55/4/611/5631762>).

## **Validity of the Findings**

This is a novel tool for compiling and standardizing qPCR data and its associated metadata. I feel tools such as this are very much in need in the field, and I congratulate the authors on producing it. The authors give good examples as to the potential uses of the tool. That being said, the primary functionality is the ability to pull raw fluorescence data from qPCR outputs and calculate a Ct value, then inserting the 2 columns at the end of a metadata table the user needs to fill out. The Platform’s strength is in this first step, which is limited to use in 3 qPCR systems (2 from the same company), none of which are broadly used yet in the field of qPCR research (understanding the irony that the Biomeme systems are in fact more broadly used in the actual “field”, being a portable system!). Therefore, a bit more focus on the planned development of R scripts to allow the upload of additional qPCR fluorescence data formats is warranted and would be encouraging to the reader.

## **Abstract**

**Keywords:** Generally, do not need keywords that are in your title (qPCR).

## **Introduction:**

Line 39: I suggest caution using the term “molecular diagnostics” with qPCR as much of the use for qPCR to date has included gene expression studies, which that term aptly describes, but the lean towards eDNA you lend in your manuscript, while appropriate as a growing field using qPCR, does not meet the traditional concept of the term “molecular diagnostics”. Effectively, your tool crosses over nicely with medical and field research, so using a term generally assigned to the former overshadows the latter. Remember, “diagnostics” help to diagnose something, as in a disease or condition, and does not apply to areas such as eDNA necessarily.

Line 41: Context of term “real time” – while successfully described in the caption of Box 1, my interpretation of your reference to “real time” in this sentence itself was less clear. For example, in eDNA studies, that DNA may have been present for some time before its presence is detected. Perhaps use a different term, like “quickly and with high sensitivity”.

Line 78: Use of word “diagnostics”, in my opinion, relates more to the example of food safety (generally) than it does the resource management or conservation planning references. Perhaps use a more neutral term?

## **Methods and Results**

Lines 167-170: Signal intensity values: where did these come from? Is this based on a reference? Assigning a signal intensity carries a lot of weight in the interpretation of the results.

## **Discussion**

Line 322: This sentence needs some work – Suggest “MDMAP could help to...” and the part “either assay or species specific of geographically, over time” is confusing.

Paragraph 325-333: Suggest word-smithing the pp. Line 328 has the word conclusions twice, one word apart, and should be “regarded as a source...”. Last sentence is a repeat of the first.

Line 336: Perhaps be more specific here? Did you look into other data formats? Which ones? Readers may be expecting the more common systems are used. Also, all outputs from every major system are in .csv or .xlsx these days, so clarification as to what aspects are proprietary would also help here.

Line 341: You have referenced many articles that look at standards for reporting (i.e. Klymus, et al 2019), and more are out there (i.e. the Bustin 2009 I reference in the Experimental Design comments). Can you address why these were not incorporated (such as LOD, threshold values, etc.)?

## **Conclusion**

Line 362: Geographical? Not sure if this is correct.

Line 364: Please check grammar, or comma usage “the availability and quality and reliability improvements of...” It’s a bit unclear.

Line 365: “addressing” used twice in the sentence, recommend replacing one with another word for clarity.

## **Reference**

General: Check formatting. Some titles are first word capitalized, some are all lower case, and some are first word in sentence capitalized.

Line 397: XML in title should be all caps

Line 452: Remove “( )” from 2015

## **Supplemental file 1**

verbatimElevation – Description should indicate “meters”

verbatimDepth – Description should indicate units (meters, cm, inches, etc)

decimalLatitude, decimalLongitude – Description should indicate which datum is to be used (NAD, WGS, etc).