quicR: An R Library for Streamlined Data Handling of Real-Time Quaking Induced Conversion Assays
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Response to Reviews
Reviewers comments in black font — Our responses in blue

We appreciate the comments and suggestions made by both reviewers, they have improved our manuscript. We have made edits aligning with the reviewers' suggestions. We strongly feel that the R package presented in the manuscript will greatly facilitate diagnostic reproducibility in RT-QuIC data. We provide our responses below.

Reviewer #1: The manuscript introduces quicR, an R package designed to streamline RT-QuIC data analysis, addressing a critical need for standardization in a rapidly evolving diagnostic field. The tool's architecture and workflows are well-structured, with clear integration of data handling, metric calculation, and visualization. The inclusion of metadata management and compatibility with BMG Labtech's MARS software demonstrates practical utility. However, certain areas require refinement to enhance reproducibility, accessibility, and alignment with best practices in software documentation.

1. The manuscript does not compare quicR's performance to other RT-QuIC analysis tools, such as manual thresholding in GraphPad Prism or scripts from Li et al. [33]. It is recommended to include a table or paragraph benchmarking computation speed, accuracy, and ease of use against established methods.

We agree that comparing the performance across other RT-QuIC analysis tools is important. We have made comparisons across the available packages and have added a new subsection 2.3 Comparable Methods (lines 138–165) as well as a table highlighting the comparisons (Table 2).

2. Inconsistent use of "RT-QuIC" vs. "Real-time quaking induced conversion." Use "RT-QuIC" after first full mention and update abbreviations list.

We have made this change.

3. The manuscript currently lacks explicit statements regarding code maintenance protocols (e.g., version update plans) and user engagement mechanisms. To strengthen long-term reproducibility and community adoption, the authors are advised to incorporate the following in either the "Software Description" section or "Discussion": (1) Commitments to sustainable maintenance (e.g., update frequency, dependency management), (2) Clearly defined issue reporting channels (e.g., GitHub Issues, dedicated email), (3) Roadmap for community-driven development (e.g., contributor guidelines, plugin architecture).

We agree and have added a CONTRIBUTING.md in the GitHub repository as well as subsection 2.4 Maintenance and Community Involvement (lines 178–187).

4. While the manuscript emphasizes 'reproducibility' as a key methodological feature, it lacks empirical validation through critical stress tests such as cross-platform consistency checks or inter-user variability assessments. To substantiate the claimed reproducibility, we strongly recommend supplementing the analysis with a dedicated reproducibility study. This should involve multiple independent operators analyzing identical datasets across heterogeneous R environments (e.g., Docker containers vs. native installations), with quantitative comparisons of result concordance rates.

We have conducted a reproducibility study and included the following subsection 2.4 Reproducibility (166–177) as well as a table (Table 3).

Reviewer #2: As a wet lab researcher who went from analyzing this kind of data semi-manually in Excel and then figuring out R and writing their own code, I commend authors for this initiative! Unfortunately, I could not figure out how to try the code, as the package did not install for me (R version 4.2.2). Based on the description of functions, the calculations and visualizations that the package allows are highly relevant.

We have independently confirmed that users operating in multiple R environments, OSs, and machines are able to download and execute the package functions (see lines 166–177). The following code should work to install the package:

install.packages("quicR")
library(quicR)

If this is unsuccessful, we recommend trying to install the development version from GitHub:

install.packages("devtools")
devtools::install_github("https://github.com/gage1145/quicR")
library(quicR)

The accompanying manual is not very easy to understand, it would be amazing if authors could make a little walkthrough video or at least provide code with dependent files for less experienced users to understand the whole process better. (if you already did, please forgive me and point me towards it)

We have added an additional step in the example script which highlights how to install and load the package (lines 195–197). We plan on developing educational content, such as a video walkthrough, in the coming months.

Time to threshold is also known as lag time.

We have added this clarification in the first instance of the term on page 2, line 25.

RT-QuIC is also known as PMCA and SAA. At least in PD research the consensus name is SAA, authors could mention that to increase the visibility of their package.

RT-QuIC and PMCA are within the family of seed amplification assays, but the two are not interchangeable. This package specifically focuses on RT-QuIC because PMCA does not have a real-time data component and relies on Western blots for analysis. We have added a clarification that RT-QuIC is an SAA in the "Motivation and Significance" section of the manuscript (lines 3–4).