

Primer Sequence Disclosure: A Clarification of the MIQE Guidelines

To the Editor:

The publication of the minimum information for the publication of real-time quantitative PCR experiments (MIQE)¹ guidelines (1) has turned out to be a defining event in the maturing of quantitative real-time PCR (qPCR) technology. The response from instrument and reagent manufacturers has been universally positive. There has been extensive publicity in print, online, and at scientific meetings, and scientific journals are beginning to take note (2). Citations of the MIQE paper are accelerating, with 63 of the 169 citations (as of the end of January 2011) having appeared since September 2010. There is an enormous amount of good will toward this initiative, with many researchers keen to implement the different parameters within their own experimental protocols.

MIQE was never conceived with the intent of imposing an immutable edict, as in the spirit of a regulatory agency. The aim was to provide commonsense guidelines for enhancing the reproducibility and transparency of qPCR assays. MIQE, however, has become a marketing and selling argument (“MIQE compliance”), and this practice places a responsibility on the authors of the guidelines to assess whether the rapidly evolving technology demands refinement of the guidelines to acknowledge researchers’ uncertainty.

Most discussion has concerned the stipulation of primer sequence disclosure. Many commer-

cial qPCR assays are not supplied with the primer/probe sequences because most vendors consider such information commercially sensitive. In addition, there usually are no details provided regarding empirical validation of each individual assay. The increasing use of commercial qPCR assays is creating problems, because it leads to publications that cannot satisfy current MIQE requirements and limits the universal acceptance of MIQE. Consequently, we propose a pragmatic amendment of the original guidelines to require “**EITHER** primer sequences **OR** amplicon context sequence.” This proposal is based on our assessment that in the absence of full disclosure of primer sequence, it is possible to achieve an adequate level of transparency, but only if there is an appropriate level of background information and disclosure of validation results regarding the qPCR assay:

- Our key concern is that today’s reports must remain technically accessible in the medium to long term. For that reason, publications must not report assays without reference to sequence data, with invalid Web site references, or with resources obtained from vendors that no longer exist.
- We continue to affirm that full disclosure of the reagents used and validation of their performance are principal requirements for MIQE “compliance.”
- Full primer (and probe) sequence disclosure remains our ideal; however, it may be possible to obtain equivalent results from slightly different assays as long as they target the same region and splice variants and they take single-nucleotide polymorphisms and secondary structures into account.

Consequently, if primer sequences are not disclosed, a MIQE-

compliant publication should provide all of the following:

- The assay identification provided by the commercial vendor.
- The specific amplicon context sequence for the qPCR assay. Preferably, this information is obtained by sequencing the target PCR amplicon; alternatively, it could be supplied by the vendor or approximated by the authors (Fig. 1).
- The same validation criteria used for assays reporting primer/probe sequences. Specifically, when a precise -fold change for a transcript is reported, an essential requirement that remains is that the PCR efficiency, analytical sensitivity, and specificity of each individual assay be determined. Investigators should verify this information for the actual assay being reported under the laboratory conditions their personnel used in their laboratory; they should not extrapolate it from commercial assays validated by the vendors.

It is of paramount importance that commercial assay identification can continue to be traced, and it would be helpful to know why any assay was discontinued or replaced. Ideally, users should be able to order/use discontinued/replaced assays, either by the vendor providing them directly or by the vendor releasing primer and probe sequences for those qPCR assays. Vendors must also be more transparent about the bioinformatics efforts they use to validate their assays in silico.

MIQE aims to improve the transparency and hence the reproducibility of published qPCR assays by detailing minimum requirements. Crucially, “minimum” does not mean “ideal.” The original stipulation of primer sequence disclosure as “essential” remains our ideal, and is strongly

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¹ Nonstandard abbreviations: MIQE, minimum information for publication of quantitative real-time PCR experiments; qPCR, quantitative real-time PCR.

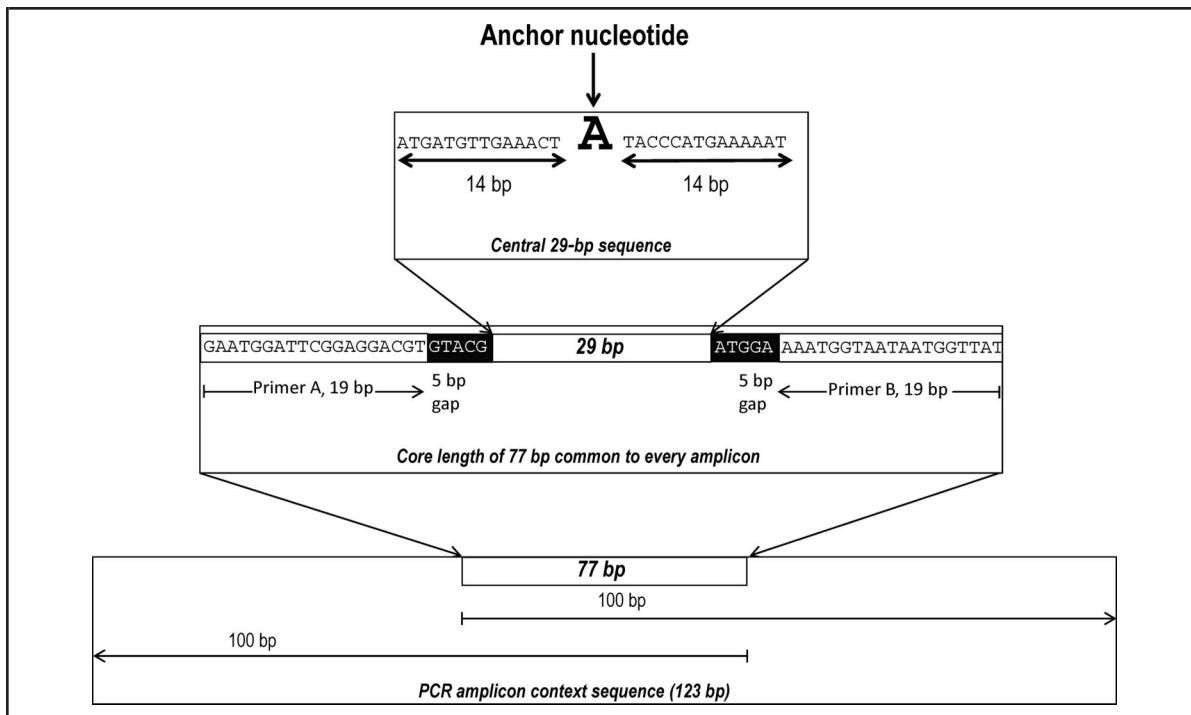


Fig. 1. An example of how to identify a PCR amplicon context sequence.

We introduce the concept of an “anchor nucleotide,” which we define as a nucleotide contained anywhere within the probe sequence. The probe and primers are assumed to be 15 bp and 19 bp, respectively, with a 5-bp gap between them. No assumptions are made with respect to optimal or suboptimal primer or probe sequences. The vendor publishes information regarding reference sequence, anchor nucleotide, and amplicon length, which in this example are NM_001145847.1, 2982, and 100 bp, respectively. A central 29-bp sequence centered on the anchor nucleotide delineates the maximum context sequence for the probe. A 77-bp core sequence consists of 24 bp (19 bp for the primer and 5 bp for the gap between the 3’ end of the primer and the 5’ end of the probe) added to either side of the central sequence. The amplicon context is obtained by adding 23 bp (100–77) to either side of the core sequence to demarcate the potential extent of the PCR amplicon. In this example, the 100-bp amplicon would have a 123-bp context sequence, which is what one would submit with the publication. For DNA dye-binding assays, we suggest that vendors provide an amplicon ± 20 bp of context sequence.

recommended that for precise measurements or for situations in which qPCR forms a major part of a published study. Greater transparency in scientific research is always better, and for qPCR that includes primer and probe sequences. Nevertheless, given the commercial reality, we felt it sensible to modify the minimum sequence requirements. We hope these “revised MIQE guidelines” will enhance their appeal and universality without compromising the importance of MIQE as a set of standards that is beginning to

achieve acceptance in the scientific community.

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Stephen A. Bustin^{2*}
Vladimir Benes³
Jeremy A. Garson⁴
Jan Hellemans^{5,6}
Jim Huggett^{7,8}
Mikael Kubista^{9,10}
Reinhold Mueller¹¹
Tania Nolan¹²
Michael W. Pfaffl¹³
Gregory L. Shipley¹⁴
Jo Vandesompele^{5,6}
Carl T. Wittwer^{15,16}

² *Barts and the London School of Medicine and Dentistry*
Queen Mary University of London
London, UK
³ *EMBL GeneCore*
Heidelberg, Germany
⁴ *Centre for Virology*
Research Department of Infection
University College Medical School
London, UK
⁵ *Ghent University*
Centre for Medical Genetics Ghent
Ghent University Hospital
Ghent, Belgium
⁶ *Biogazelle*
Zwijnaarde, Belgium
⁷ *LGC*
Teddington, UK
⁸ *University College London*
Centre for Infectious Diseases
London, UK
⁹ *TATAA Biocenter*
Gothenburg, Sweden
¹⁰ *Institute of Biotechnology*
Czech Academy of Sciences
Prague, Czech Republic
¹¹ *Sequenom*
San Diego, CA

¹² *Sigma-Aldrich*
Haverhill, UK
¹³ *Physiology Weihenstephan*
Life Science Center Weihenstephan
TUM
Munich, Germany
¹⁴ *University of Texas Health Science Center*
at Houston
Houston, TX
¹⁵ *Department of Pathology*
University of Utah
Salt Lake City, UT
¹⁶ *ARUP Institute for Clinical and*
Experimental Pathology
Salt Lake City, UT

AQ: F

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*Address correspondence to this author at:
 3rd Floor Alexandra Wing
 The Royal London Hospital
 London E1 1BB, UK
 Fax +44-0-20-7377-7283
 E-mail s.a.bustin@qmul.ac.uk

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