

## 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)<sup>1</sup> 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-

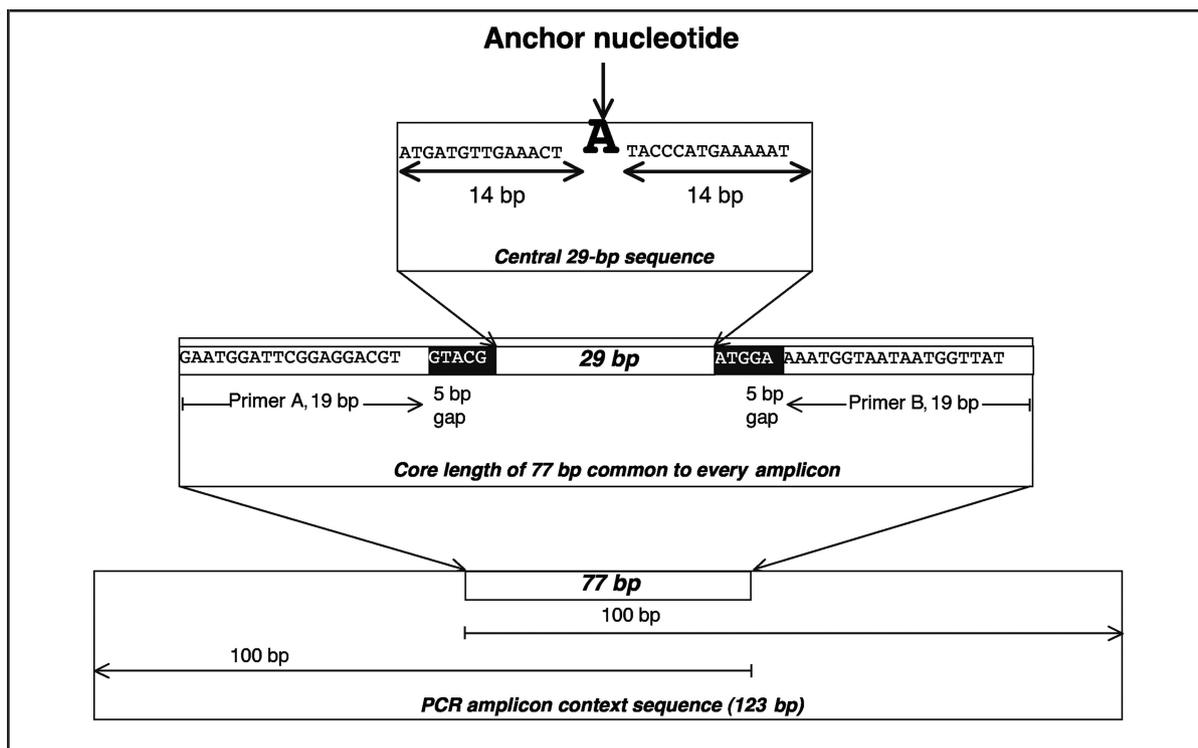
compliant publication should provide all of the following:

- 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 we strongly recommend that for precise mea-

<sup>1</sup> 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  $\pm 20$  bp of context sequence.

surements 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.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures or Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the Disclosures of

Potential Conflict of Interest form. Potential conflicts of interest:

**Employment or Leadership:** C.T. Wittwer, Idaho Technology.

**Consultant or Advisory Role:** G.L. Shipley, Applied Biosystems.

**Stock Ownership:** M. Kubista, TATAA Bio-center; R. Mueller, C.T. Wittwer, Idaho Technology.

**Honoraria:** None declared.

**Research Funding:** C.T. Wittwer, Idaho Technology.

**Expert Testimony:** None declared.

**Other Remuneration:** G.L. Shipley, Cambridge Healthtech Institute, Science Webinar, and qPCR Symposium (travel, room, and registration expenses).

**Role of Sponsor:** The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

## References

1. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009;55:611–22.
2. Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FS, Olsvik PA, et al. MIQE précis: practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Mol Biol* 2010;11:74.

**Stephen A. Bustin**<sup>2\*</sup>  
**Vladimir Benes**<sup>3</sup>  
**Jeremy A. Garson**<sup>4</sup>  
**Jan Hellemans**<sup>5,6</sup>  
**Jim Huggett**<sup>7,8</sup>  
**Mikael Kubista**<sup>9,10</sup>  
**Reinhold Mueller**<sup>11</sup>  
**Tania Nolan**<sup>12</sup>  
**Michael W. Pfaffl**<sup>13</sup>  
**Gregory L. Shipley**<sup>14</sup>

**Jo Vandesompele**<sup>5,6</sup>  
**Carl T. Wittwer**<sup>15,16</sup>

<sup>2</sup> *Barts and the London School of Medicine and Dentistry  
Queen Mary University of London  
London, UK*

<sup>3</sup> *EMBL GeneCore  
Heidelberg, Germany*

<sup>4</sup> *Centre for Virology  
Research Department of Infection  
University College Medical School  
London, UK*

<sup>5</sup> *Ghent University  
Ghent, Belgium*

<sup>6</sup> *Biogazelle  
Zwijnaarde, Belgium*

<sup>7</sup> *LGC  
Teddington, UK*

<sup>8</sup> *University College London  
London, UK*

<sup>9</sup> *TATAA Biocenter  
Gothenburg, Sweden*

<sup>10</sup> *Institute of Biotechnology  
Czech Academy of Sciences  
Prague, Czech Republic*

<sup>11</sup> *Sequenom  
San Diego, CA*

<sup>12</sup> *Sigma-Aldrich  
Haverhill, UK*

<sup>13</sup> *Physiology Weihenstephan  
Life Science Center Weihenstephan  
TUM*

*Munich, Germany*

<sup>14</sup> *University of Texas Health Science Center  
at Houston  
Houston, TX*

<sup>15</sup> *Department of Pathology  
University of Utah  
Salt Lake City, UT*

<sup>16</sup> *ARUP Institute for Clinical and  
Experimental Pathology  
Salt Lake City, UT*

---

\*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

---

Previously published online at  
DOI: 10.1373/clinchem.2011.162958

---