Biomolecular Detection and Quantification 11 (2017) 21–30

Contents lists available at ScienceDirect

Biomolecular Detection and Quantification

journal homepage: www.elsevier.com/locate/bdq

Research paper

Measuring *E. coli* and bacteriophage DNA in cell sonicates to evaluate the CAL1 reaction as a synthetic biology standard for qPCR

Alexander Templar, Desmond M. Schofield, Darren N. Nesbeth*

The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, WC1E 6BT, UK

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form
29 September 2016
Accepted 1 December 2016
Available online 29 December 2016
Handled by Jim Huggett

Keywords:
Pcr
Synthetic biology
Standard curve
Standardisation
Linear regression
Efficiency

ABSTRACT

We measured the impact of the presence of total *Escherichia coli* (*E. coli*) cellular material on the performance of the Linear Regression of Efficiency (LRE) method of absolute quantitative PCR (LRE qPCR), which features the putatively universal CAL1 calibration reaction, which we propose as a synthetic biology standard. We firstly used a qPCR reaction in which a sequence present in the lone genomic BirA locus is amplified. Amplification efficiency for this reaction, a key metric for many quantitative qPCR methods, was inhibited by cellular material from bioreactor cultivation to a greater extent than material from shake flask cultivation. We then compared LRE qPCR to the Standard Curve method of absolute qPCR (SC qPCR). LRE qPCR method matched the performance of the SC qPCR when used to measure 417–4.17×10^7 copies of the BirA target sequence present in a shake flask-derived cell sonicates sample, and for 97–9.7×10^5 copies in the equivalent bioreactor-derived sample. A plasmid-encoded T7 bacteriophage sequence was next used to compare the methods. In the presence of cell sonicates from samples of up to $OD_{600} = 160$, LRE qPCR outperformed SC qPCR in the range of 1.54×10^8–1.54×10^{10} copies of the T7 target sequence and matched SC qPCR over 1.54×10^4–1.54×10^7 copies. These data suggest the CAL1 standard, combined with the LRE qPCR method, represents an attractive choice as a synthetic biology qPCR standard that performs well even when unpurified industrial samples are used as the source of template material.

1. Introduction

1.1. LRE qPCR and the CAL1 reaction as a synthetic biology standard for qPCR

Perhaps the most widely known use of real time PCR, also known as quantitative PCR (qPCR), is as a tool to measure the relative abundance of a given messenger RNA (mRNA) transcript. In this 'relative qPCR' approach reverse transcriptase (RT) is used to convert a population of mRNA molecules to single stranded complimentary DNA (cDNA) molecules. A bespoke primer pair is then used to amplify cDNA corresponding to an mRNA whose abundance is well characterised. Further primer pairs are used to amplify cDNA corresponding to mRNA molecules whose abundance is unknown. Ideally, primers for qPCR will be designed in accordance with the 'Minimum Information for Publication of Quantitative Real-Time PCR Experiments' or 'MIQE' guidelines proposed by Bustin et al. [5].

The amount of PCR product (amplicon) present during each reaction cycle can be determined by measuring in real time [9] the light emission from a fluorescent reporter dye that binds to double-stranded DNA (dsDNA). The kinetics of the appearance of fluorescence over time can then be used to infer the amount of template cDNA that was initially present [20]. A key metric for this procedure is the number of cycles required for fluorescence to exceed a set threshold. This cycle number is known as the quantification cycle (Cq). The less cycles required for fluorescence to reach Cq, the more template was present in the starting material. For relative qPCR the principle data gathered is the fold-difference in abundance of the well-characterised, reference mRNA in comparison to the mRNA of unknown abundance.

While relative qPCR has been immensely valuable in helping researchers gain fundamental biological insights, it is arguably less well suited to the aim of synthetic biology, which is to render biological systems more amenable to rigorous engineering methods. Fortunately for synthetic biologists it is possible to derive absolute measurements using qPCR [23]. Serially diluted standards of known concentration produce a linear relationship between the Cq value and the logarithm of the initial amount of total template DNA

Abbreviations: PCR, Polymerase chain reaction; HCD, high cell density; qPCR, quantitative PCR; SF, shake flask; wcw, wet cell weight; WCB, working cell bank; LRE, linear regression of efficiency; OCF, optical calibration factor.

* Corresponding author.
E-mail address: d.nesbeth@ucl.ac.uk (D.N. Nesbeth).

http://dx.doi.org/10.1016/j.bdq.2016.12.001

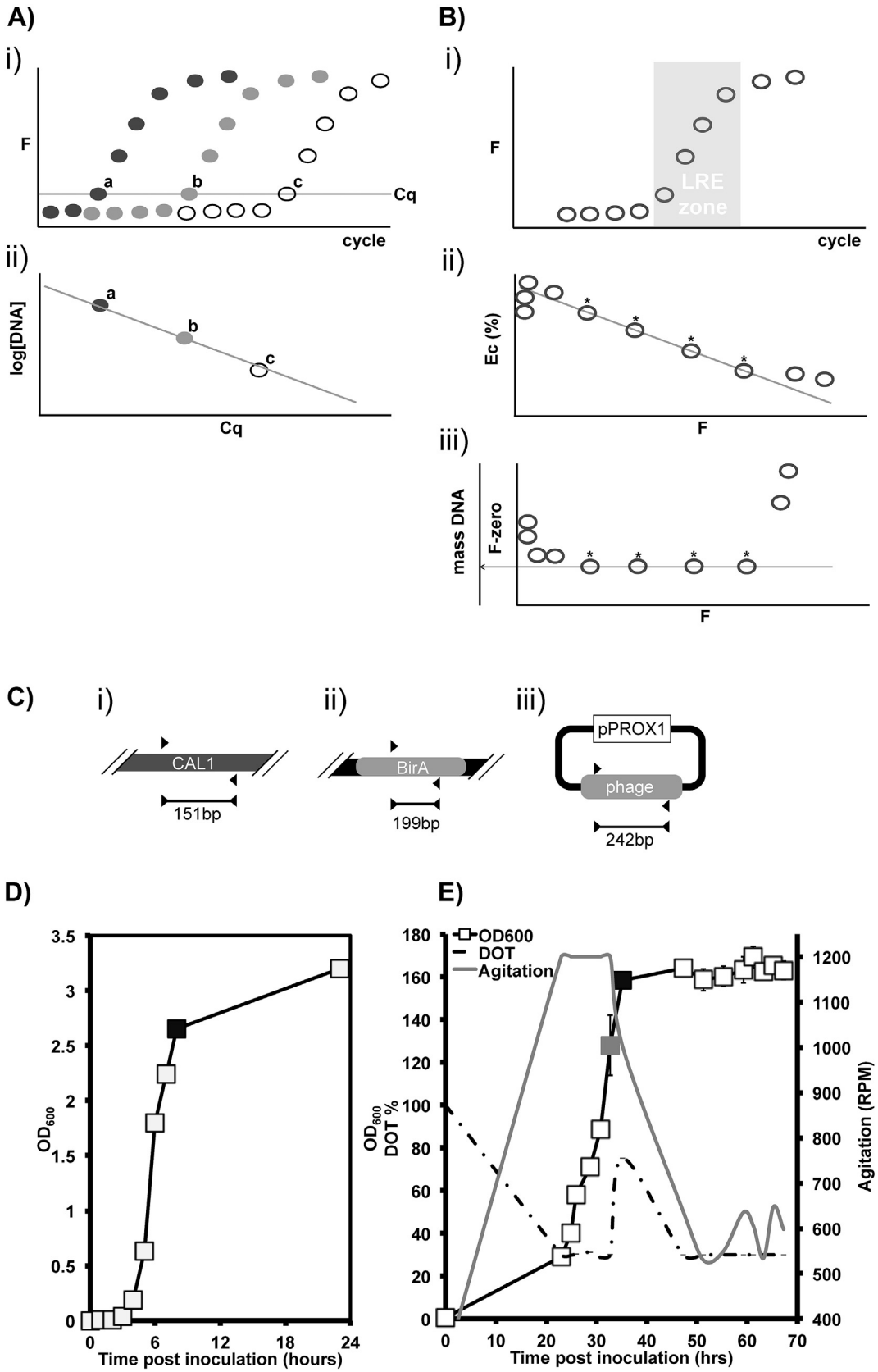

Fig. 1. PCR approaches and cell cultivation. A) i) Illustration of the fluorescence data profile for a conventional qPCR experiment. Serial dilutions of template are made and real time appearance of fluorescence plotted as a function of cycle number. Fluorescent data points for three dilutions of template are indicated by black, grey and white (with black border) data points to convey increasing template dilution. Typically four or more are used in actual qPCR experiments. The point at which each fluorescence signal reaches the Cq is logged (cycles a, b and c). ii) Cq number is proportional to the log of the DNA concentration in purified target sequence samples of known concentration. This data set provides a standard curve for calibration of Cq data gathered from samples of unknown target sequence concentration. B) i) For LRE qPCR these is no inherent requirement to perform a template dilution series or set a Cq threshold. Instead the flourescence data set is analysed as a classic Boltzmann sigmoid function such that a