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Optimization of a protocol for gene expression using biofilm cells from *S. epidermidis*

Ana Isabel Freitas[1], Ana Filipa Henriques[1], Ângela França[1], Carlos Vasconcelos[2], Manuel Vilanova[3], Nuno Cerca[1]

[1] Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal; [2]Hospital Santo António, Centro Hospitalar do Porto, Porto, Portugal; [3]ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Portugal

Gene expression assays are one of the most common tools used nowadays to evaluate the importance of genes in many different life sciences areas, namely, in clinical microbiology. Since most gene expression kits for qPCR have been optimized for assays with planktonic cells it is important to also optimize protocols for this type of assays, to be used with biofilms. Biofilms are communities of bacteria that grow attached to a surface and embedded in an extracellular matrix, what poses some difficulties to RNA extraction. Proper RNA quality is of the upmost importance during all the downstream processes, namely cDNA synthesis and qPCR quantification. The aim of this work was to optimize a protocol for gene quantification from biofilm samples of *S. epidermidis*, a known biofilm forming nosocomial pathogen.This optimization was made in many different steps, from the RNA extraction (a crucial step) to complementary DNA (cDNA) synthesis and qPCR reactions, using growth conditions well described in the literature, so that the results obtained could be anticipated beforehand. The expression of the *icaA* gene was tested from RNA extracted with a custom made protocol and then quantified using a combination of 4 commercial kits of cDNA synthesis and 4 commercial kits of qPCR quantification. Furthermore, the volumes of reaction were either the volume recommended by the manufacturer (20 µl) or half that volume.From our results, we conclude that there were no significant differences of *icaA* expression when using any of the qPCR kits used in this study. However, using different cDNA synthesis kits, a statistical difference was found in the results obtained using one of the kits, with an *icaA* expression near 4-fold different than that obtained using the other kits. Interestingly, the 10 µl reaction generally resulted in higher *icaA* expression than when using the 20 µl reaction volume, but within the expected range of values, indicating that any of the two volumes could be used for quantification studies. Excluding the cDNA kit with low *icaA* levels expression, the average of *icaA* expression induced by glucose was similar in both cDNA and qPCR optimization steps (9.5 and 9.4 fold, respectively).The obtained protocol provides reliable results, comparable to the ones in literature, with the advantage of saving reagents. Furthermore, our results confirm that cDNA synthesis is a more crucial step that previous thought.

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