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A Note on the Classification and Confirmation of Reference Genes

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Abstract

Normalization, which is typically accomplished by comparing the abundance of the gene of interest to that of an endogenous reference gene, is an essential step in gene expression analysis for obtaining useful data from reverse transcription quantitative PCR (RT qPCR) assays. It's not easy to find these reference genes, which should be stable when expressed in multiple tissue samples and under different experimental conditions. A set of genes has been identified and evaluated in this work that could serve as a reference for gene expression studies on water buffalo. The first step involves downloading a Bos taurusexpressed sequence tags database from the TIGR Gene Index and mining it with simple frequency algorithms to determine which tentative consensuses are more suitable for inclusion in a starter set of candidate reference genes because they are present in a remarkable number of different cDNA libraries. An RT qPCR analysis, in which the expression stability of these genes was evaluated on a panel of buffalo tissues and organs, was carried out in order to validate the potential of such candidates for their use as normalizers in buffalo gene expression analysis. According to our findings, gene expression levels in buffalo tissues and organs can be compared using normalizers based on ribosomal proteins L4 and L5 and Gek proteins.

Keywords: Gene expression; Reference genes; Normalization; RT qPCR

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Introduction

In biological, biomedical, and veterinary research, real-time reverse transcription PCR (RT-PCR) is widely used for gene expression evaluation [1]. However, there are a number of technical considerations that have a significant impact on the assay [2]. Pre-analytical steps like sampling technologies, nucleic acid extraction and purification, and RT, as well as data analysis, normalization, optimization, and standardization of the qPCR reaction, are all essential for producing quantitative data that can be trusted and replicated. Data normalization is an essential step in obtaining useful RT qPCR assay results that means to compare the results to the transcript of a reference gene. According to Schmittgen and Zakrajsek (2000), the use of single housekeepers for normalization cannot guarantee unbiased results because it has become clear that no single gene is constitutively expressed in all cell types and under all experimental conditions in several organisms [3]. However, it is difficult to identify an appropriate panel of reference genes for data normalization and up until this point, these genes have been chosen from those that are commonly used or adapted from the literature or by starting with data from microarrays [4, 5]. Expressed Grouping Tag (EST) information for a nimble recognizable proof of reference qualities reasonable for natural circumstances. Therefore, we should be able to identify potentially reference genes that are constitutively expressed in a wide range of different tissues, organs, and physiological conditions if we select the transcripts that are present in a remarkable number of different cDNA libraries. In order to accomplish this, we have concentrated on ESTs derived from cDNA libraries of Bos Taurus that have been collected in the TIGR Gene Indices database (http://compbio.dfci.harvard.edu/tgi/) and prepared from a variety of tissues and physiological conditions. These Indices are species-specific public databases based on ESTs. They are made by clustering and then putting together ESTs and annotated gene sequences to make a set of one-of-a-kind, high-fidelity virtual transcripts (Tentative Consensus, or TC) [6]. The absence of a similar database for

Bubalus bubalis and comparative genomic studies that have demonstrated the usefulness of genomic tools developed for cattle when applied to related species like water buffaloes (Ritz et al.,) support the use of a ESTs database from Bos Taurus [7].

Methods

In silicon selection of potential reference genes a freely accessible the Bos taurus ESTs database was downloaded and mined using some simple frequency algorithms to determine which TCs are present in a remarkable number of different cDNA libraries and, thus, more suitable to be included in a starter set of candidate reference genes [8]. More specifically, the TIGR Cattle Gene Index (BtGI), Release 12.0 (http:// compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/T release.pl?gudb5 cattle) was screened with a Pyton-based script to identify the TCs present in a remarkable number of different libraries and to order them by frequency (number of hosting libraries/total number of libraries). The 15 most common TCs (as listed in with their preliminary announcements) have been chosen for further investigation.

Discussion

Two buffaloes were selected at random in a slaughterhouse and samples of their liver, spleen, kidney, heart, skeletal muscle, lymph node, udder, and lung were taken as the first step of the work. Six buffaloes were selected at random in a slaughterhouse and samples of their liver, kidney, skeletal muscle, lymph node, udder, and lung were taken in the second step [9].

The samples were immediately transported to the sampling laboratory in clean nylon bags suspended in ice.

To prevent contamination, the slaughter and sampling rooms were separated but close together. To get a sample of the inner part of each organ, the outermost layer of tissue was taken out for the same reason. Before being used for RNA extraction, three 25 mg aliquots from each sample were immediately stored in liquid nitrogen at 2808C.

Special care was taken during the sampling procedure to ensure low temperatures and prevent accidental environmental contamination. Using Trizol (Invitrogen, CA, USA), total RNA was extracted from the samples in accordance with the manufacturer's instructions. Following the RNA cleanup protocol, the resulting RNAs were purified with Qiagen RNeasy columns (Qiagen GmbH, Hilden, Germany).

Standard optical density measurements were used to quantify the purified RNAs, and a fluorescent-based solution assay using Rybo-Green dye (Molecular Probes Inc., Eugene, OR, USA) was used to confirm the results (Jones et al., 1998), in accordance with manufacturer guidelines. Based on microcapillary electrophoresis, the 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA) was used to further assess the quantity and quality of RNAs. With this innovation, electro-pherograms and gel-like pictures can be outwardly assessed and a specialist programming can produce a RNA honesty number (RIN), a client free evaluation of RNA uprightness. All of the extracted samples had a mean RIN of 7.1 6 0.9. The total RNA samples were reverse transcribed in triplicate using the TaqMan One Step RT-PCR protocol (Applied Biosystems, Foster City, CA, USA). This value is greater than 6, which is the dividing line between RNA of high and low quality. The following thermal profile was applied to 100 ng of purified RNA containing 0.25 U/ml MultiScribe Reverse Transcriptase (Applied Biosystems), 0.4 U/ml Rnase Inhibitor (Applied Biosystems), 150 nM forward and 150 nM reverse primers in a final volume of 50 ml of SYBR Green PCR Master Mix 1X (Applied Biosystems): 40 cycles with a denaturation step at 958C for 15 seconds and an annealing/ extension step at 608C for 1 minute, followed by one step at 488C for 30 minutes and one step at 958C for 10 minutes [10].

Using MicroAmp optical tubes and caps, PCRs were carried out in the PE Biosystems GeneAmp 7300 Sequence Detection System (Applied Biosystems). Applied Biosystems' Primer Express 2.0 software was used to design the primers, and the assays were then optimized and evaluated for specific product amplification. contains a list of the primer sequences utilized in real-time RT-PCR. Two approaches were used to assess the amplified products' specificity. The list of TCs that were examined in this work for their expression in buffalo tissues. These TCs are remembered for TIGR Steers Quality Record, a freely accessible information base created by the Foundation for Genomic Exploration (Rockville, MD, USA) that gathers ESTs, halfway, single-pass successions from one or the flip side of a cDNA clone. The current rendition of Steers Quality Record incorporates 1051687 ESTs from a few cDNA libraries created by global Bos Taurus EST sequencing and quality examination projects. 90392 TC sequences were created by assembling these ESTs into virtual transcripts. We have ordered all TCs according to their frequency, which is calculated as the number of hosting libraries divided by the total number of libraries included in the TIGR Cattle Gene Index, with the intention of screening for reference genes in water buffalo. We have selected a panel of 15 TCs based on the most common ones. In response to the requirement that a maintenance gene must be constitutively expressed in order to ensure cellular, genes that are expressed in multiple tissues, organs, and conditions have been identified through this selection procedure.

Conclusion

A two-step laboratory bench-based test, in which the expression stability of all the TCs is reported in Table 1 and evaluated on a panel of RNA samples extracted from tissues or organs, was carried out in order to evaluate the potential of such candidates for their use as normalizers in buffalo gene expression analysis. The absence of multiple peaks in melt curve analysis and the sequencing of the amplified products, which was followed by annotation, ensured the specificity of each RT qPCR reaction.

In a first screening step, the articulation levels of the 15 not entirely settled in RNA tests separated from liver, spleen, kidney, heart, skeletal muscle, lymph hub, udder furthermore, lung of two creatures haphazardly chose in a slaugh-terhouse. Information have been assessed by both geNorm also, NormFinder programming projects (Vandesompele et al., 2002; Andersen and others, 2004) and graphically detailed in Figure 1 for the entire arrangement of inspected tissues. TC302447 and TC339084, according to both software, are the best genes for data

normalization. Additionally, differences in the TCs' expression stability among the tissues were evaluated using NormFinder analysis. The intergroup variation that reflects the gene's stability across various tissues is presented in for each TC.

Acknowledgement

None

Conflict of Interest

None

References

1 Matevossian E, Snopok Y, Altomonte J, Sinicina I, Thorban S, et al. (2009) Surgeon Yurii Voronoy (1895-1961)-a pioneer in the history of clinical transplantation: in memoriam at the 75th anniversary of the first human kidney transplantation. Transplant International 22: 1132-1139.

2 Bernat James L, Capron Alexander M, Bleck Thomas P, Blosser Sandralee, Bratton Susan L, Childress James F, DeVita Michael A, Fulda Gerard J, Gries Cynthia J (2010) The circulatory–respiratory determination of death in organ donation. Critical Care Medicine 38: 963-970.

3 Alexander GC, Sehgal AR (1998) Barriers to cadaveric renal transplantation among blacks, women, and the poor. JAMA 280: 1148-1152.

4 Tom Treasure (2007) The Falun Gong, organ transplantation, the holocaust and ourselves. J R Soc Med 100: 119-121.

5 White SL, Domínguez-Gil B, Delmonico FL Noel L, Chapman J, Matesanz R, et al. (2014) the global diffusion of organ transplantation: trends, drivers and policy implications. Bull World Health Organ 92: 826-835.

6 Cyranoski David, Gaind Nisha, Gibney Elizabeth, Masood Ehsan, Maxmen Amy, et al. (2019) Nature's 10: Ten people who mattered in science in 2019. Nature 576: 361-372.

7 Fickl S, Zuhr O, Wachtel H, Stappert CF, Hürzeler MB, et al. (2008) Dimensional changes of the alveolar ridge contour after different socket preservation techniques. J Clin Periodontol 35: 906-913.

8 Ramos E, Santamaría J, Santamaría G, Barbier L, Arteagoitia I, et al. (2016) Do systemic antibiotics prevent dry socket and infection after third molar extraction? A systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 122: 403-425.

9 McCormick NJ, Moore UJ, Meechan JG (2014) Haemostasis Part 1: The management of post-extraction haemorrhage. Dental Update 41 (4): 294-296.

10 Susarla SM, Blaeser BF, Magalnick D (2003) Third molar surgery and associated complications. Oral Maxillofac Surg Clin North Am 15: 177-186.