

Research Article

Transcriptome Analysis of *Spermophilus lateralis* and *Spermophilus tridecemlineatus* Liver Does Not Suggest the Presence of *Spermophilus*-Liver-Specific Reference Genes

Bryan M. H. Keng¹ Oliver Y. W. Chan,¹ Sean S. J. Heng¹ and Maurice H. T. Ling^{2,3}

¹Ra es Institution, One Ra es Institution Lane, Singapore

²Department of Zoology, e University of Melbourne, Genetics Lane, Parkville, VIC , Australia

³Department of Mathematics and Statistics, South Dakota State University, SD , USA

Correspondence should be addressed to Maurice H. T. Ling; mauriceling@acm.org

Received March ; Accepted April

Academic Editors: A. Bolshoy and D. Labudde

Copyright © Bryan M. H. Keng et al. is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gene expressions of reference genes used in gene expression studies are assumed to be stable under most circumstances. However, studies had demonstrated that genes assumed to be stably expressed in a species are not necessarily stably expressed in other organisms. This study aims to evaluate the likelihood of genus-specific reference genes for liver using comparable microarray datasets from *Spermophilus lateralis* and *Spermophilus tridecemlineatus*. The coefficient of variance (CV) of each probe was calculated and there were probes common between the lowest % CV of both datasets ($n = 1258$). All lists were analysed by NormFinder. Our results suggest that the most invariant probe for *S. tridecemlineatus* was *actb*, while that for *S. lateralis* was *actb*. However, our results showed that Probes *actb* and *actb* are ranked 1st and 2nd in terms of invariability for *S. lateralis* and *S. tridecemlineatus* respectively. This suggests the lack of common liver-specific reference probes for both *S. lateralis* and *S. tridecemlineatus*. Given that *S. lateralis* and *S. tridecemlineatus* are closely related species and the datasets are comparable, our results do not support the presence of genus-specific reference genes.

1. Introduction

Gene expression analysis is examining the variations in gene expression by measuring DNA expression levels over time.

These variations may be a result of many factors, such as environmental, developmental, and metabolic changes, or treatments. Quantitative real-time polymerase chain reaction (qRT-PCR) is one such used technique to quantify and analyse gene expressions [1, 2]. However, qRT-PCR requires a stably expressed gene under a wide variety of conditions [3, 4], known as a reference gene, as a standard to produce accurate and reliable results on transcriptional differences of various genes of interest.

Candidate reference genes, which are commonly assumed to be invariant, can be identified using statistically based algorithms, such as geNorm [5], NormFinder [6], and Best-Keeper [7], or descriptive statistics, such as regression [8].

Microarrays, which usually contain thousands of probes, present a good source of data for identifying reference genes [9]. Reference genes had been successfully identified from microarrays in a number of studies [10, 11].

However, several studies had refuted the possibility of universal reference genes [12, 13] that can be used in every organ in every organism. This corroborates several studies demonstrating that genes commonly considered to be expressionally invariable may vary under different experimental conditions [14, 15]. Some studies had verified the applicability of commonly used reference genes such as *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) [16] or *UBQ* (ubiquitinone) [17]. However, other studies had demonstrated that the expressions of *GAPDH* [18] and *UBQ* [19] vary in some conditions. *Polr* has been suggested to be stably expressed in mouse heart [20], but Mamo et al. [21] had shown that *Polr* is not stably expressed in mouse

