Small Ruminant Research 184 (2020) 106050

Contents lists available at ScienceDirect

Small Ruminant Research

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

Short communication

Evaluation of global expression of selected genes as potential candidates for internal normalizing control during transcriptome analysis in dromedary camel (*camelus dromedarius*)

Marwa A. Ibrahim[a], Moustafa I. Radwan[b], Hyoung Kyu Kim[c], Jin Han[c], Mohamad Warda[a,*]

[a] Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt
[b] National Lab for Veterinary Quality Control on Poultry Production, Dokki, Giza, Egypt
[c] Department of Physiology, BK21 Plus Project Team, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, Republic of Korea

ARTICLE INFO

Keywords:
Camelus dromedarius
Housekeeping gene
HKG
geNorm
Expression stability
GAPDH
ACTB
H-Ras
c-Myc
TUBB: ΔCt
Normfinder

ABSTRACT

Background: Selection of the stable normalizing genes is critical for quantification of gene expression. Unfortunately, till date, no report on systematic assessment of reference genes is known for dromedary camel (Camelus dromedaries). Therefore, this is the first study to validate the stability of five potential reference genes in different tissues of Camel.

Results: The expression levels of the glyceraldehyde-3P-dehydrogenase (GAPDH), TUBB (β-tubulin), H-Ras protein, c-Myc and ACTB (β-actin) transcripts were quantified in the liver, brain, heart, spleen, kidney, adipose tissue, and skeletal muscle. Based on the stability ranking, performed with the geNorm VBA applet for Microsoft Excel, Normfinder and ΔCt method, the examined genes were arranged as follows GAPDH > ACTB > TUBB > H-Ras > c-Myc.

Conclusion: Our findings suggest that the *GAPDH* and *ACTB* genes can be used as stable reference genes for accurate normalization of gene expression data using qRT-PCR in the *Camelus dromedarius*.

1. Introduction

Gene-expression quantification is an increasingly principal step in numerous fields of scientific research. Identification of gene expression patterns provides insight into the complicated regulatory systems of the studied genes (Kamel et al., 2018a). Gene expression analysis resolves the complicated associations at the transcriptomic level (Yassin et al., 2016).

For reliable results, normalization of the RT-qPCR data should be done using the proper housekeeping genes (HKGs) in order to quench the errors that can be assimilated during the RNA extraction and cDNA synthesis (Jain et al., 2018).

To date, the internal control genes should be expressed persistently among various tissues, at all developmental stages of an organism and should not be affected by the experimental treatment (Warzybok and Migocka, 2013). However, many reports showed that although HKGs expressions are occasionally steady in a particular tissue or experimental condition, they can vary substantially (Walker et al., 2009). Numerous literatures assumed that there is no single universal gene with a stable level of expression among all experimental circumstances and/or developmental stages (Aitha and Rajeswari, 2015; Moein et al., 2017). The best choice of reference gene to use as an endogenous control varies, depending on the tissues of interest and the species of the experiment. Picking of the appropriate reference genes for the qRT-PCR analysis got a major concern in plants, animals, and human researches (Chen et al., 2012; Torres et al., 2013; Ibrahem and Ibrahim, 2014; Hassanen et al., 2019). The selected genes belonged to various biological groups to avoid co-regulation and a similar expression pattern (Aggarwal et al., 2013).

Dromedary camel, on the other hand, is especially adapted animal to cope with arid and famine habitat of Arab peninsula with tropical and subtropical extensions. For 14 centuries, the dromedary stands as a singular marvel creature (Warda et al., 2014) having the ability to store water and energy. The red blood cells of camel has less osmotic fragility among mammals and can withstand extreme osmotic variation during prolonged dehydration as well as sudden rehydration possibly due to **specific** membrane phospholipid distribution (Warda and Zeisig, 2000). As pseudo-ruminant, dromedary represents unique distinguished digestive tract morphology. It differs than other ruminants in having comparable higher blood sugar level with strong anti-insulin attribute

* Corresponding author.
E-mail address: maawarda@scu.eg (M. Warda).

https://doi.org/10.1016/j.smallrumres.2020.106050
Received 7 June 2019; Received in revised form 8 January 2020; Accepted 12 January 2020
Available online 13 January 2020
0921-4488/

Table 1
Gene symbol, description, cellular localization, and biological functions for the five candidate genes assessed as housekeeping genes in the current study.

Gene symbol	Description	Cellular localization	Biological function
ACTB	β-actin	Cytoplasm	Cytoskeletal structural protein
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase	Plasma membrane	Glycolytic enzyme
c-Myc	Myelocytomatosis	Nucleolus	Cellular proliferation, growth, differentiation, and apoptosis.
H-Ras	Transforming protein	Plasma membrane	Regulator of signal pathways.
TUBB	β-Tubulin	Plasma membrane	Polymerize into mircrotubules

(Abdel-Fattah et al., 1999).

Our previous study on resolving camel proteome (Warda et al., 2014) disclosed the localized peculiarity of dromedary to cope with hemoconcentration–hemodilution episodes with high cellular regenerative capacity evidenced by over-expression of certain cytoskeleton and heat shock proteins in dromedary organs above that of mammalian model. The rest of this proteome analysis elucidated another peculiarity in energy homeostasis related elements. Moreover, it is recently proved that the cultured camel heat shock protein has an efficient glycosylation mechanism that guarantees more stability with superior capability against stressful conditions (Hoter et al., 2018).

These finding turn our eyes to expand our search to puzzle out the most reliable candidate genes with global stability to be selected as the best internal control during transcriptome analysis in this unique mammal. The aim of the study was to perform an extensive assessment of five commonly used HKGs in seven different camel tissues (Table 1). The geNorm VBA applet for Microsoft Excel, Normfinder and ΔCt method were then used to evaluate the stability of these genes and to outline accurate, stable, and reliable normalizer for m-RNA gene-expression data in the dromedary camel.

2. Materials and methods

2.1. Tissue collection

Fresh liver, brain, heart, spleen, kidney, adipose tissue, and skeletal muscle were obtained from ten apparent healthy adult male of 3–4 years old dromedary camels in the slaughterhouse at Cairo. The tissue samples (15 ± 5 gm each) were immediately flash freezed in liquid nitrogen and stored at −80 °C. The Institutional Animal Care and Use Committee (IACUC) approved all the experimental procedures. The consent for this research has been assigned as (CU II F C 5 19).

2.2. Primer designing and validation

All the primer sets were designed using Primer 3.0 software (Table 2). To check the specificity of the primer sequences, the primer sets were aligned against the BLAST database using BLASTN at NCBI.

2.3. Isolation of total RNA and cDNA synthesis

Total RNA was isolated using RNeasy Mini Kit (Qiagen Cat No./ID: 74,104) according to the provided guidelines. Both the concentration and purity of the extracted RNA were assessed by a Nanodrop (ND-1000). The integrity of the isolated RNA was checked by agarose gel electrophoresis.The c-DNA synthesis was done using Viva 2-steps RT-PCR Kit (Vivantis) according to the manufacturer's instructions (Kamel et al., 2018b).

2.4. qReal time PCR

The RT-qPCR reaction was prepared as follows: 10 µl of 2 × SYBR Green SuperMix-UDG (Life technologies, USA), 2.5 µl of template cDNA, 0.2 µM of gene-specific primer and the final volume was 20 µl. The following conditions were applied: UDG activation at 55OC for 2 min, denaturation at 95OC for 2 min, and 45 cycles of denaturation for 10 s at 95OC, primer annealing for 10 s and final extension for 1 min at 72OC. The melting curve was generated to test the reaction specificity for each amplicon as follows: 15 s at 95OC, 1 min at 60OC followed by ramping to 95OC and 15 s at 95OC. All samples were run in triplicates and a `no template' control was included in each RT-qPCR run (**Kamel et al., 2018a**).

2.5. Data analysis

The transcript stability of the five HKGs under investigation was determined by the cycle threshold (Ct) values using geNorm software VBA applet for Microsoft Excel (version 3.5) (Vandesompele et al., 2002). The gene stability measures (M-value) were calculated according to the average pairwise variation of a specific reference gene with all other control genes. The expression stability (M-value < 1.5) was considered acceptable. The rank of the examined candidate genes was compared using Normfinder and ΔCt method as well for the overall ranking.

3. Results

High-quality RNA samples with A260/280 ratio ~ 2 were selected. The specificity of each primer set of the five genes under study was confirmed by a prominent single band in the agarose gel and the dissociation curve (not shown). The average cycle threshold (Ct) values for the studied HKGs ranged from 17.0 (*ACTB*) to 29.6 (*c-Myc*). The features of each gene based on its Ct value in the different tissues were shown as box-whisker plot in Fig. (1).

The mean Ct values were used to estimate the stability of the candidate genes using the three algorithms: GeNORM, NormFinder, and ΔCt. The five tested genes appeared to be stable with little difference in their expression values among tissues (Fig. 2).

Table 2
Primer sequences for the reference genes evaluated in the study.

Gene	Forward sequence	Reverse sequence	Accession Number
GAPDH	5′ TGGGAAGCTAACTGGCATGG 3′	5′ TGAAGTCGCAGGAGACAACC 3′	EU331417
ACTB	5́ AGGCCAACCGTGAGAAGATG 3′	5́ AGTCCATCACGATGCCAGTG 3′	AB270711
H-Ras	5́ CGAAACCTCAGCCAAGACCA 3′	5′ TTACATCACCACACACGGCA 3′	XM_010980715.1
C-Myc	5́ CAACTGCCTCTGGAAGGGC 3′	5́ TTTCACTCCGGATCTCCCCT 3′	XM_010988786.1
TUBB	5′AGCTGTGTGAGTGTCGTCTG 3′	5′ TGTCAAAGCGCATACTGGGT3′	XM_010987837.1

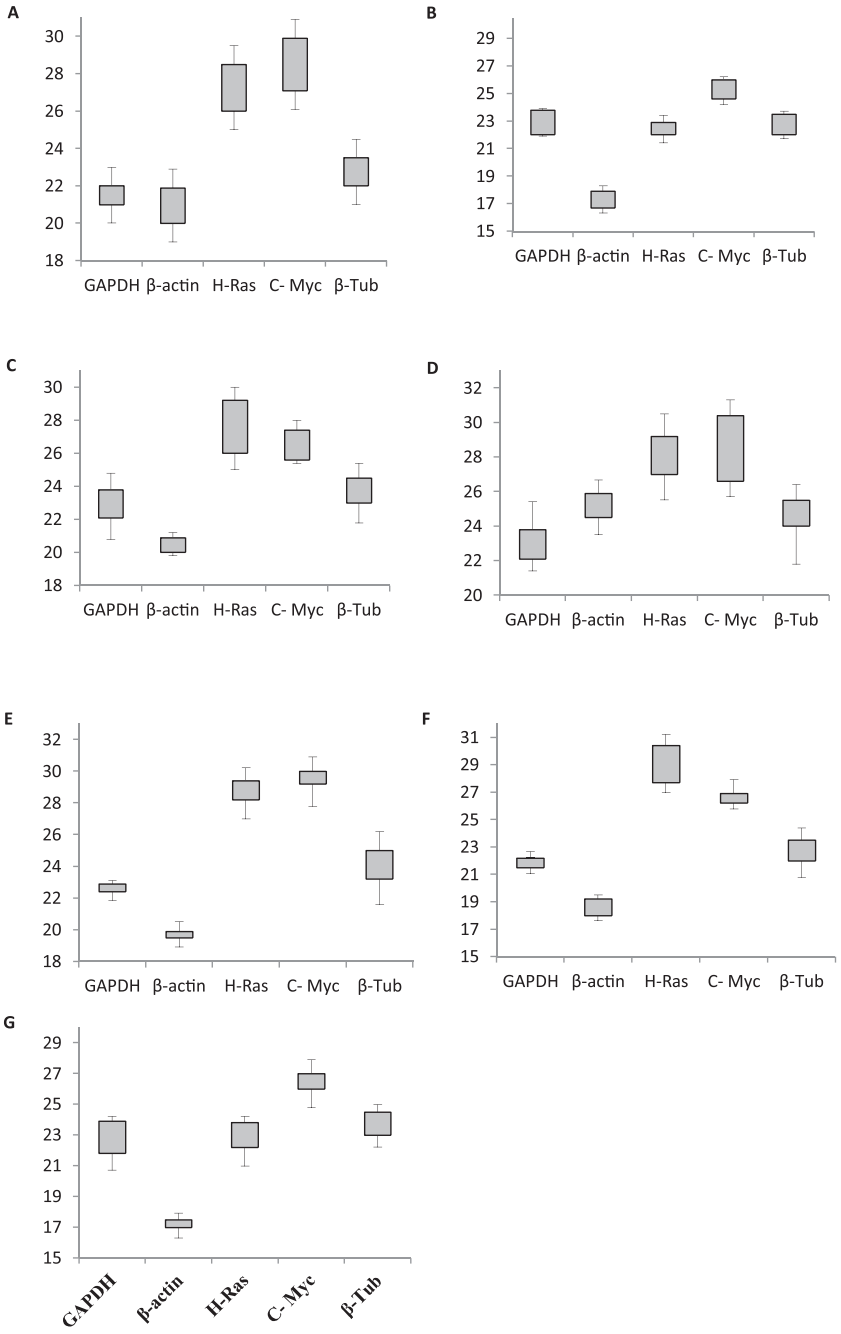

Fig. 1. Overall expression pattern of the evaluated genes among different tissues in the Arabian camel. A: liver; B: heart; C: kidney; D: spleen; E: brain; F: adipose tissue; G: skeletal muscle. The data are represented as cycle threshold (Ct) values of each gene in the box and whisker diagram. The boxes represent the median the 25th and 75th percentile ranges and the whiskers indicate maximum and minimum values.

The geNorm, Normfinder and ΔCt algorithms used for ranking of the studied genes among the selected tissues, revealed that the expression stability values were within the acceptable range (< 1.5). The ranking of the five HKGs is depicted in Fig. (2) according to their stability values. The genes were ranked from the lowest stability value (the most stable) to the highest stability value (the least stable), as follows: *GAPDH* > *ACTB* > TUBB > *H-Ras* > *c-Myc*.

The comparisons of the candidate genes revealed that GAPDH was the most stable HKG followed by *ACTB*, TUBB, *H–Ras* and *c-Myc*, respectively.Thus, the c-myc was classified as the least suitable HKGs for normalization of the gene expression in the Arabian camel, if compared to the *GAPDH* and *ACTB* in all tissues (Fig. 3).

4. Discussion

The relative mRNA quantification has increasingly become the method of choice for precise gene profiling. However, selection of the appropriate HKG is critical for normalization of the qRT-PCR results. To the best of our knowledge, there are no comprehensive reports for the validation and identification of consistent and reliable reference genes for *Camelus dromedarius*.

In the current study, we evaluated the expression stabilities of five candidate housekeeping genes among seven tissues in the one-humped camel. Those five reference genes were selected by referring to available research reports in mammals: *GAPDH* (Barber et al., 2005; Shaydurov et al., 2018), *ACTB* (Caracausi et al., 2017; Veres-Székely et al., 2017), *TUBB* (Bittermann et al., 2019), *H-Ras* and *c-Myc*

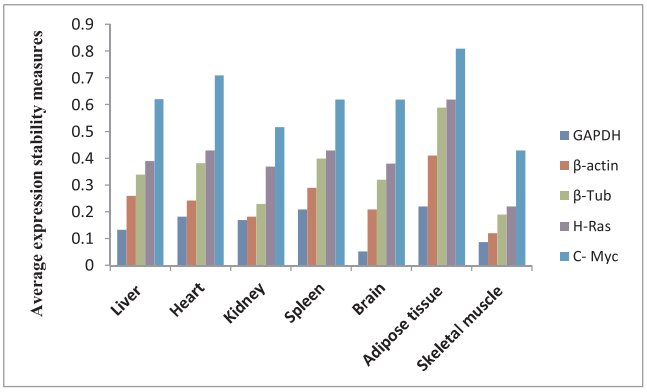

Fig. 2. Comparison of the expression stability values for the studied genes among the different tissues.

(Eisenberg and Levanon, 2003**).**

We analyzed our data using three algorithms to identify and rank the stably expressed genes using expression stability value of each method (Aggarwal et al., 2013; Yang et al., 2017; Tsotetsi et al., 2018). The five candidate reference genes exhibited different stability among different tissues of the camel. However, all the examined genes showed stability values < 1.5, indicating that all of them are highly stable. Consequently, the five genes could be used as potential internal control for normalization of the qRT-PCR data. As a central point of cellular energy homeostasis the results revealed that the *GAPDH* is the most reliable HKG among the examined genes in all studied tissues, particularly the brain. GAPDH is basic catalytic enzyme of glycolysis that plays a major role in cellular metabolism. *GAPDH*is among the chief HKGs (Kozera and Rapacz, 2013) and is often used to normalize gene expression data in camel (Yassin et al., 2016; Kamel et al., 2018a, b). However, the *GAPDH*transcript levels greatly varied according to the organism, tissue, and diseases (Zhu et al., 2012).

In consistent to our previous proteomic data (Warda et al., 2014), our results showed that *ACTB* – as a cytoskeleton related gene- is the most stable gene expressed among the different camel's tissues mainly in the skeletal muscle and kidney. ACTB is one of the cytoskeletal actin isoforms **(**Spence and Soderling, 2015). *ACTB*has been used extensively as a reliable HKG in camel (Soman and Tinson, 2016; Manee et al., 2017). The current finding may disagree with others reported data in other mammalian species (Zhu et al., 2012; Wang et al., 2016).

Tubulins are ultimate sturcture cellular blocks that are assembled in the form of dynamic microtubules which are crucial in mitotic division, cellular differentiation and shape (Xu et al., 2019). Although the TUBB is highly conserved with stable expression, variations in its expression was recorded in different types of cancer **(** Parker et al., 2014**).** The TUBB overexpression was associated with resistant tumors to the anti-cancer therapies **(**Yang et al., 2016**).** In camel tissue TUBB was the third gene in its expression stability compared to all the studied genes.

Away from the energy homeostasis GAPDH gene (Morgan et al., 2017, Abdel Aziz et al., 2018) and cytoskeleton ACTB gene (Manee et al., 2017), here we further screened the expression of two oncogenes that potentially represent housekeeping elements in other mammals **(**Eisenberg and Levanon, 2003**).**

The *H-Ras* is one of the most common oncogenes. It codes for the H-Ras protein which is a regulator in cell division through relaying the signals from extracellular to the nucleus. It is ranked here as the fourth stable gene. The least stability value for the *H-Ras* gene was recorded in the skeletal muscle. *H-Ras* is a regulatory protein involved in cell signaling and growth with recorded variation between its transcriptome and proteome values (Kim et al., 2006**).**

Our study showed that *c-Myc* is the least stable gene among the evaluated HKGs. The *c-Myc* is another proto-oncogene encoding a transactivating factor that controls cell proliferation, differentiation, and apoptosis (Eisenberg and Levanon, 2003**).** Myc proteins are

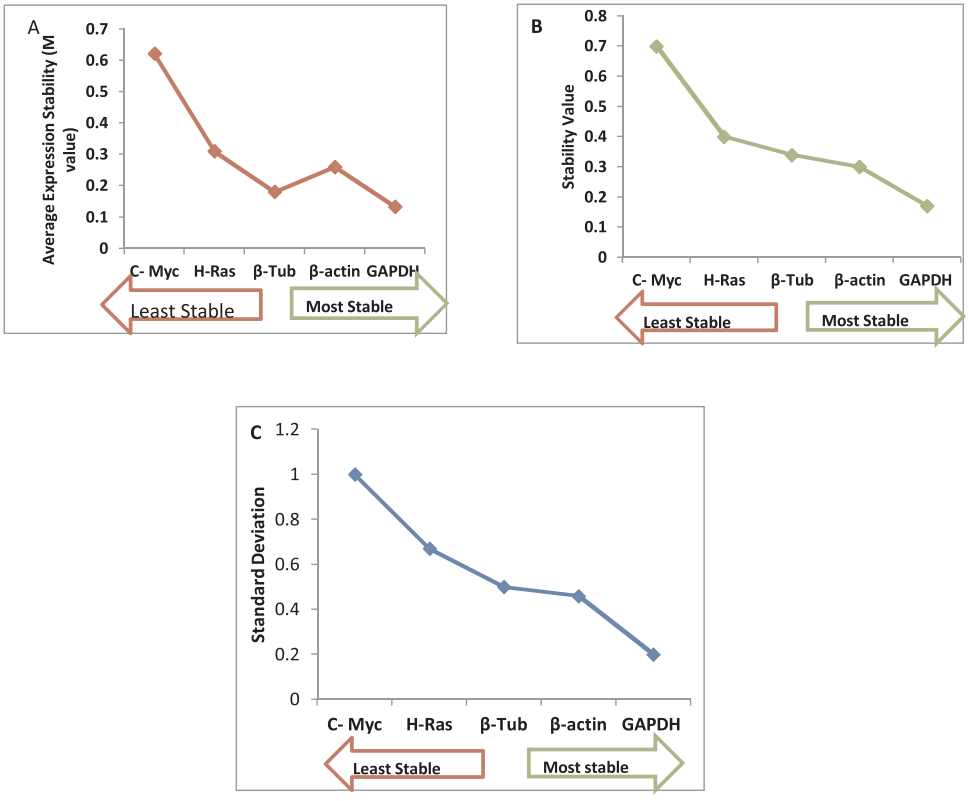

Fig. 3. Expression stability analysis and ranking of the house-keeping genes by the three algorithms used in this study. (**A**) GeNORM,based on average stability value (M), (**B**) NormFinder,based on the stability values calculated by the software, and (**C**) ΔCt, based on the standard deviation values.

transcription factors that trigger the expression of numerous pro-proliferative genes (Rahl and Young, 2014). *C-Myc* expression is recently used as a potential marker of stem cells in the ovarian cells of *Camelus dromedarius* (Saadeldin et al., 2018). Overexpression of *c-Myc* was implicated in renal tumors (Tang et al., 2009) and apoptosis (Dang et al., 2006).

5. Conclusion

The five examined reference genes are found to be stable, despite slight differences found among different tissues. *GAPDH* was most consistent across the studied organs of Camelus dromedarius. On the other hand, *c-Myc* was the least stable gene in all tissues. For accurate results, we suggest that at least, two reliable internal controls should be used to normalize the expression data in a particular tissue.

Availability of data and material

There is no restriction on the availability of any materials and data upon request.

Declaration of Competing Interest

The authors report no conflict of interest in this work.

References

Abdel Aziz, R.L., Abdel-Wahab, A., Abo El-Ela, F.I., Hassan, N.E.Y., El-Nahass, E.S., Ibrahim, M.A., Khalil, A.A.Y., 2018. Dose- dependent ameliorative effects of quercetin and l-Carnitine against atrazine- induced reproductive toxicity in adult male Albino rats. Biomed. Pharmacother. 2018 (June (102)), 855–864.

Abdel-Fattah, M., Amer, H., Ghoneim, M.A., Warda, M., Megahed, Y., 1999. Response of one-humped camel (*Camelus dromedarius*) to intravenous glucagon injection and to infusion of glucose and volatile fatty acids, and the kinetics of glucagon disappearance from the blood. Zentralbl Veterinarmed. A (46), 473–481.

Aggarwal, J., Sharma, A., Kishore, A., Mishra, B.P., Yadav, A., Mohanty, A., Sodhi, M., Kataria, R.S., Malakar, D., Mukesh, M., 2013. Identification of suitable housekeeping genes for normalization of quantitative real-time PCR data during different physiological stages of mammary gland in riverine buffaloes (Bubalus bubalis). J Anim Physiol Anim Nutr (Berl). 97 (December (6)), 1132–1141.

Aitha, M., Rajeswari, N., 2015. Validation of housekeeping genes for gene expression analysis in glioblastoma using quantitative real-time polymerase chain reaction. Brain Tumor Res. Treat. 3 (April (1)), 24–29.

Barber, R.D., Harmer, D.W., Coleman, R.A., Clark, B.J., 2005. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol. Genomics 21 (3), 389–395 May 11.

Bittermann, E., Abdelhamed, Z., Liegel, R.P., Menke, C., Timms, A., Beier, D.R., 2019. Stottmann RW Differential requirements of tubulin genes in mammalian forebrain development. PLoS Genet. 15 (8), e1008243 August 6.

Caracausi, M., Piovesan, A., Antonaros, F., Strippoli, P., Vitale, L., Pelleri, M.C., 2017. Systematic identification of human housekeeping genes possibly useful as references in gene expression studies. Mol. Med. Rep. 16 (September (3)), 2397–2410.

Chen, Y., Zhang, B., Feng, H., Shu, W., Chen, G.Y., Zhong, J.F., 2012. An automated microfluidic device for assessment of mammalian cell genetic stability. Lab Chip 12 (20), 3930–3935 October 21.

Dang, C.V., O'Donnell, K.A., Zeller, K.I., Nguyen, T., Osthus, R.C., Li, F., 2006. The c-Myc target gene network. Semin. Cancer Biol. 16 (August (4)), 253–264.

Eisenberg, E., Levanon, E.Y., 2003. Human housekeeping genes are compact. Trends Genet. 19 (July (7)), 362–365 2003.

Hassanen, E.I., Tohamy, A.F., Issa, M.Y., Ibrahim, M.A., Farroh, K.Y., Hassan, A.M., 2019. Pomegranate juice diminishes the mitochondria-dependent cell death and NF-kB signaling pathway induced by copper oxide nanoparticles on liver and kidneys of rats. Int. J. Nanomedicine 15 (November (14)), 8905–8922.

Hoter, A., Amiri, M., Prince, A., Amer, H., Warda, M., Naim, H.Y., 2018. Differential glycosylation and modulation of camel and human HSP isoforms in response to thermal and hypoxic stresses. Int. J. Mol. Sci. 30 (19) January (2).

Ibrahem, M.D., Ibrahim, A.M., 2014. The potential effects of spirulina platensis (arthrospira platensis) on tissue protection of nile tilapia (oreochromis niloticus) through estimation of p53 level. J. Adv. Res. 5 (January (1)), 133–136.

Jain, N., Vergish, S., Khurana, J.P., 2018. Validation of house keeping genes for normalization of gene expression dataduring diurnal/circadian studies in rice by RT- RT-qPCR. Sci. Rep. 8 (1), 3203 February 16.

Kamel, S., Ibrahim, M., Awad, E.T., El-Hindi, H.M.A., Abdel-Aziz, S.A., 2018a. Differential expression of CYP2j2 gene and protein in Camelus dromedarius. J. Biol. Regul. Homeost. Agents 32 (6), 1473–1477 NOV-DEC.

Kamel, S., Ibrahim, M.A., Awad, E.T., El-Hindi, H.M.A., 2018b. Abdel-Aziz SA.MOlecular cloning and characterization of the novel CYP2J2 in dromedary camels (Camelus dromedarius). Int. J. Biol. Macromol. 120 (December (Pt B)), 1770–1776.

Kim, N., Kim, H., Youm, J.B., Park, W.S., Warda, M., Ko, J.H., Han, J., 2006. Site specific differential activation of ras/raf/ERK signaling in rabbit isoproterenol-induced left ventricular hypertrophy. Biochim. Biophys. Acta 1763 (October (10)), 1067–1075.

Kozera, B., Rapacz, M., 2013. Reference genes in real-time PCR. J Appl Genetics 54, 391–406.

Manee, M.M., Alharbi, S.N., Algarni, A.T., Alghamdi, W.M., Altammami, M.A., Alkhrayef, M.N., Alnafjan, B.M., 2017. Molecular cloning, bioinformatics analysis, and expression of small heat shockprotein beta-1 from Camelus dromedarius, Arabian camel. PLoS One 12 (12), e0189905 December 29.

Moein, S., Javanmard, S.H., Abedi, M., Izadpanahi, M.H., Gheisari, Y., 2017. Identification of appropriate housekeeping genes for gene expression analysisin long-term hypoxia-treated kidney cells. Adv. Biomed. Res. 22 (February (6)), 15.

Morgan, A.M., Ibrahim, M.A., Noshy, P.A., 2017. Reproductive toxicity provoked by titanium dioxide nanoparticles and the ameliorative role of Tiron in adult male rats. Biochem. Biophysiol. Res. Commun. 486 (2), 595–600.

Parker, A.L., Kavallaris, M., McCarroll, J.A., 2014. Microtubules and their role in cellular stress in cancer. Front. Oncol. 4, 153.

Rahl, P.B., Young, R.A., 2014. MYC and transcription elongation. Cold Spring Harb. Perspect. Med. 4 (1), a020990 January 1.

Saadeldin, I.M., Swelum, A.A., Elsafadi, M., Mahmood, A., Alfayez, M., Alowaimer, A.N., 2018. Cumulus cells of camel (Camelus dromedarius) antral follicles are multipotent stem cells. Theriogenology 15 (September (118)), 233–242.

Shaydurov, V.A., Kasianov, A., Bolshakov, A.P., 2018. Analysis of housekeeping genes for accurate normalization of RT-qPCRData during early postnatal brain development. J. Mol. Neurosci. 64 (March (3)), 431–439.

Soman, S.S., Tinson, A., 2016. Development and evaluation of a simple and effective real time PCR assay for mitochondrial quantification in racing camels. Mol. Cell. Probes 30 (October (5)), 326–330.

Spence, E.F., Soderling, S.H., 2015. Actin out: regulation of the synaptic cytoskeleton. J. Biol. Chem. 290, 28613–28622. https://doi.org/10.1074/jbc.R115.655118.

Tang, S.W., Chang, W.H., Su, Y.C., Chen, Y.C., Lai, Y.H., Wu, P.T., Hsu, C.I., Lin, W.C., Lai, M.K., JY, Lin, 2009. MYC pathway is activated in clear cell renal cell carcinoma and essential for proliferation of clear cell renal cell carcinoma cells. Cancer Lett. 273 (1), 35–43.

Torres, A., Torres, K., Wdowiak, P., Paszkowski, T., 2013. Maciejewski R.SElection and validation of endogenous controls for microRNA expression studies in endometrioid endometrial cancer tissues. Gynecol. Oncol. 130 (September (3)), 588–594.

Tsotetsi, T.N., Collins, N.E., Oosthuizen, M.C., 2018. Sibeko-Matjila KPSelection and evaluation of housekeeping genes as endogenous controls for quantification of mRNA transcripts in Theileria parva using quantitative real-time polymerase chain reaction (qPCR). PLoS One 13 (5) e0196715. May 4.

Vandesompele, J., DePreter, K., Pattyn, F., Poppe, B., VanRoy, N., DePaepe, A., et al., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3, 1–11.

Veres-Székely, A., Pap, D., Szikszi, E., Jávorszky, E., Rokonay, R., Lippai, R., Tory, K., Fekete, A., Tulassay, T., Szabó, A.J., 2017. Vannay ÁSelective measurement of α smooth muscle actin: why β-actin can not be used as a housekeeping gene when tissue fibrosis occurs. BMC Mol. Biol. 18 (1), 12 April 27.

Walker, C.G., Meier, S., Mitchell, M.D., Roche, J.R., 2009. Littlejohn M.EValuation of real-time PCR endogenous control genes for analysis of geneexpression in bovine endometrium. BMC Mol. Biol. 10 (100) November 1.

Wang, S., Guo, C., Zhou, L., Zhong, Z., Zhu, W., Huang, Y., et al., 2016. Effects of dietary supplementation with epidermal growth factor-expressing Saccharomyces cerevisiae on duodenal development in weaned piglets. Br. J. Nutr. 115, 1509–1520.

Warda, M., Prince, A., Kim, H.K., Khafaga, N., Scholkamy, L., Jin, H., 2014. Proteomics of old world camelid (Camelus dromedarius): better understanding the interplay between homeostasis and desert environment. J. Adv. Res. 5 (March (2)), 219–242.

Warda, M., Zeisig, R., 2000. Phospholipid- and fatty acid-composition in the erythrocyte membrane of the one-humped camel [*Camelus dromedarius*] and its influence on vesicle properties prepared from these lipids. Tierarztl. Wochenschr. 107, 368–373.

Warzybok, A., Migocka, M., 2013. Reliable reference genes for normalization of gene expression in cucumber grownunder different nitrogen nutrition. PLoS One 8 (9), e72887 September 13.

Xu, X., Shang, D., Cheng, H., Klionsky, D.J., Zhou, R., 2019. Gene essentiality of Tubgcp4: dosage effect and autophagy regulation in retinal photoreceptors.AUtophagy. Oct 15 (10), 1834–1837.

Yang, C., Yap, E., Xiao, H., Fiser, A., Horwitz, S., 2016. 2-(m-Azidobenzoyl)taxol binds differentially to distinct beta-tubulin isotypes. Proc. Natl. Acad. Sci. U.S.A. 113, 11294–11299.

Yang, Y., Li, Z., Cao, J., Li, Y., Li, H., Yang, Q., Zhang, Q., Liu, X., 2017. Identification and evaluation of suitable reference genes for normalization of MicroRNA expression in Helicoverpa armigera (Lepidoptera: noctuidae) Using quantitative real-time PCR. J. Insect Sci. 17 (2) January 1.

Yassin, A.M., Hamid, M.I.A., Farid, O.A., Amer, H., Warda, M., 2016. Dromedary milk exosomes as mammary transcriptome nano-vehicle: their isolation, vesicular and phospholipidomic characterizations. J. Adv. Res. 7 (5), 749–756.

Zhu, X., Li, X., ChenW, Chen J., LuW, Chen L., Fu, D., 2012. Evaluation of new reference genes in papaya for accurate transcript normalization under different experimental conditions. PLoS One 7 (8) e44405.