**Identification of Appropriate Reference Genes for qPCR Analyses of Placental Expression
of *SLC7A3* and Induction of *SLC5A1* in Porcine Endometrium**

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ABSTRACT

Introduction

Endometria and placentae undergo developmental changes that affect the stability of genes used as references for normalization of qPCR data. We identified genes that are stable within the porcine endometrium and placenta throughout pregnancy, and elucidated the temporal/spatial mRNA localization of the glucose and arginine transporters, *solute carrier family (SLC) 5A1* and *SLC7A3*, respectively.

Materials and Methods

qPCR was performed for 10 genes within porcine endometria from Days 5, 11, and 15 of the estrous cycle and 11, 15, 25, 40, 60, and 85 of pregnancy; and chorioallantois from Days 30, 35, 45, 50, 60, and 85. Gene stability was analyzed using GeNorm and NormFinder algorithms. qPCR and *in situ* hybridization determined temporal/spatial localization of *SLC5A1* and *SLC7A3* at the uterine-placental interface.

Results

The geometric mean of *TATA-binding protein (TBP)*, *hypoxanthine phosphoribosyl transferase 1 (HPRT1)*, and *tubulin alpha 1B (TUBA1B)* provides acceptable reference values for porcine placenta. The geometric mean of *TBP*, *beta actin (ACTB)*, and *succinate dehydrogenase complex subunit A flavoprotein (SDHA)* is acceptable for endometria. *SLC5A1* is induced by estrogen in endometrial luminal epithelium (LE) on Days 12 and 13 of pregnancy. *SLC7A3* is expressed in the chorion.

Discussion and Conclusion

Using appropriate reference genes resulted in complementary results between qPCR and *in situ* hybridization techniques for *SLC5A1* and *SLC7A3* mRNAs. *SLC5A1* is induced in uterine LE by estrogen of trophoderm origin when the blastocyst is free-floating and dependent on glucose from the endometrium, and *SLC7A3* is expressed by the established placenta to support fetal growth.

INTRODUCTION

Developmental changes occur in the porcine endometrium and placenta to facilitate exchange of gases, macromolecules, and micronutrients (including glucose and amino acids) necessary for growth and development of the conceptus (embryo/fetus and extraembryonic membranes). Pig blastocysts remain free-floating within the uterine lumen for an extended period of time while they undergo elongation prior to attaching to the uterine wall between Days 13 and 25 of pregnancy [1, 2]. Elongation of the trophoderm requires nutrient support from the endometrium and the endometrium undergoes energy-dependent restructuring to increase surface area for contact with the placenta [3, 4]. Estrogen, the maternal recognition of pregnancy signal in pigs, and cytokines secreted by the conceptus regulate transcription of genes in the endometrium postulated to mediate structural and physiological changes in the endometrium to accommodate implantation [2, 5-8]. Progesterone, the hormone of pregnancy, also affects genes in the placenta and endometrium [9, 10]. Pigs develop a true epitheliochorial placenta [11] which increases exponentially in weight from Day 20 to Day 70 while capillary bed volume increases until the end of gestation [12, 13]. The placenta is dynamic in terms of metabolism and structure.

Significant amounts of glucose and arginine are transported across the pig placental membranes [14, 15]. *SLC5A1* is a sodium-dependent glucose transporter that moves glucose

70 against its concentration gradient [13]. SLC7A3 is a cationic amino acid transporter that
71 transports arginine, which is used in protein synthesis and production of nitric oxide and
72 polyamines [14, 15].

73 Quantitative PCR is an efficient and sensitive method for quantifying gene transcripts in
74 tissues. However, rigorous and repeatable qPCR depends on the use of stable endogenous genes
75 as references to minimize variation arising from RNA extraction or cDNA synthesis efficiency
76 [16, 17]. Reference genes used widely include *glyceraldehydes-3-phosphate dehydrogenase*
77 (*GAPDH*), *β -actin* (*ACTB*), and *18S* rRNA, but these genes are involved in major cellular
78 processes and are not stable in all tissues [18, 19]. Beta-2-microglobulin (B2M), a commonly
79 used reference gene, increases in porcine endometria in response to interferons secreted by
80 conceptuses [20], and ACTB and tubulin alpha 1B (TUBA1B) proteins increase from Days 9 to
81 12 of pregnancy [21]. Use of either an inappropriate or a single reference gene can lead to
82 inaccurate assessment of target genes in any species or tissue [22]. A few studies with pigs have
83 determined stable reference genes for short periods of time or individual days of the estrous
84 cycle and pregnancy for endometria [19, 23], but not throughout pregnancy and not in the
85 placenta.

86 We identified three genes with stable expression in porcine endometria across the estrous
87 cycle and pregnancy and another three genes with stable expression in the placenta across
88 pregnancy for which their geometric means were acceptable endogenous references for qPCR
89 analysis. Finally, we compared assessment of a glucose transporter, *SLC5A1*, and a cationic
90 amino acid transporter, *SLC7A3*, by qPCR, and localization of their mRNAs by *in situ*
91 hybridization to validate use of the chosen qPCR reference genes.

MATERIALS AND METHODS

Animals and Tissue Collection

Eight-month-old crossbred gilts were observed for estrus (Day 0) and exhibited two estrous cycles before use in studies. All experimental/surgical procedures complied with the Guide for Care and Use of Agricultural Animals in Teaching and Research and were approved by the Institutional Animal Care and Use Committee of Texas A&M University.

In Study 1, gilts were assigned randomly to cyclic or pregnant status. Gilts in the pregnant group were bred to boars and ovariectomized on either Day 5, 9, 11, 12, 13, 15 or 17 of the estrous cycle or Day 9, 11, 12, 13, 14, 15, 17, 20, 25, 30, 40, 60, or 85 of pregnancy (n = 3 or 4 gilts/day/status). Several tissue cross-sections (uterus or uterus with attached placenta approximately 1 cm thick) from the middle of each uterine horn were fixed in 4% paraformaldehyde and embedded in Paraplast-Plus (Oxford Laboratory, St. Louis, MO). Endometrium was dissected from myometrium and stored at -80°C for RNA extraction. Chorioallantois was dissected from uteri and frozen similarly. Areolae and inter-areolar areas of the chorioallantois of Day 60 placentae were immediately evaluated for transport of arginine.

In Study 2, gilts were assigned randomly to receive daily intramuscular injections of 5 mg 17 β -estradiol benzoate in 5 ml corn oil (Sigma-Aldrich, St. Louis, MO; E2; n = 4) or 5 ml corn oil alone (CO; n = 4) on Days 11, 12, 13, and 14 of the estrous cycle. Gilts were ovariectomized on Day 15 post-estrus and endometrial tissues collected as described.

In Study 3 gilts were ovariectomized on Day 12 of the estrous cycle and assigned randomly to receive daily intramuscular injections of either P4 (200 mg in 4 ml CO) or CO on Days 12 through 39 post-estrus (n=3/treatment). Gilts were hysterectomized on Day 40 post-estrus and endometrial tissues collected as described.

RNA Extraction and cDNA Synthesis

Total RNA from endometrial and placental tissues was isolated using Trizol reagent (Life Technologies, Carlsbad, CA, USA), purity determined using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA), and first strand cDNA was synthesized using Superscript III First Strand Kit (Life Technologies).

Gene Selection and Primer Design

ACTB, *B2M*, glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), *HPRT1*, *peptidylprolyl isomerase A (PPIA)*, *ribosomal protein L7 (RPL7)*, *succinate dehydrogenase complex subunit A flavoprotein (SDHA)*, *TATA-binding protein (TBP)*, *TUBA1B*, and *tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ)* were selected based on common usage in porcine tissues. Primers for reference genes not present in the literature and for *SLC16A6*, *SLC5A1*, and *SLC7A3* were designed using Genbank sequences inserted into Primer3Plus software [24]. Primers were submitted to NCBI BLAST to confirm specificity against the porcine genome. All primer sequences and product lengths are presented in Table 1.

Quantitative PCR

All qPCR reactions were performed in triplicate, in 10 µl reactions using PerfeCta SYBR Green Mastermix (Quanta Biosciences, Gaithersburg, MD, USA) and 2.5 mM of each specific primer, on a Roche 480 Lightcycler (Roche) with approximately 75 ng of cDNA per reaction. The PCR program began with 5 min at 95°C followed by 40 cycles of 95°C denaturation for 10

sec and 60°C annealing/extension for 30 sec. A dissociation curve was produced with every run after the 40th cycle to verify a single gene-specific peak. Additionally, qPCR products were run in a 2.5% agarose electrophoresis gel to confirm primer specificity. Standard curves with 4-fold serial dilutions of pooled cDNA samples were run to determine primer efficiencies. All primer slopes were -3.30 to -3.48 with efficiencies of 97-100%.

Gene Stability

Analysis of the stability of candidate reference genes was performed by inputting the qPCR data into the NormFinder v20 [25] algorithm, accessed as an Excel Add-In, and the GeNorm [26] algorithm accessed as an Excel Macro. In addition to considering the algorithms when choosing the three genes to be included in the geometric mean, the abundance was also noted so that the abundances of the reference genes covered a range that would encompass the abundance of most target genes. Additionally, the function of the potential reference genes were considered in order to minimize the possibility that they be similarly regulated in the tissue [26].

In situ Hybridization Analysis

Localization of mRNA in sections of endometria and placentae was accomplished by radioactive *in situ* hybridization as described previously [27]. Digital images of representative fields on tissue sections were recorded under brightfield or darkfield illumination using an Axioplan 2 microscope (Carl Zeiss, Thornwood, NY) interfaced with an Axioplan HR digital camera and Axiovision 4.3 software. Photographic plates were assembled using Adobe Photoshop (version 6.0, Adobe Systems Inc., San Jose, CA).

Arginine Transport in Placenta

Arginine transport in placenta was determined using L-[U-¹⁴C]-arginine (American Radiolabeled Chemicals, St. Louis, MO) as described previously [28]. Placental samples (~200 mg of Day 60 areolar or inter-areolar chorioallantois; n=4 placentae) were incubated KHB buffer containing 0.05 µCi L-[U-¹⁴C]-arginine, and ¹⁴C radioactivity measured in a Packard 1900 liquid scintillation counter (Meriden, CT). The specific activity of L-[U-¹⁴C]-arginine was used to calculate arginine uptake by placental tissue.

Statistical Analyses

Fold changes for qPCR were determined using the $2^{-\Delta\Delta C_t}$ Method with raw Cq values normalized to either *B2M* or the geometric mean of *TBP*, *SDHA*, and *ACTB* for endometrial tissue, or the geometric mean of *TBP*, *HPRT1*, and *TUBA1B* for placental tissue [29]. The qPCR data for Study 1 were analyzed for effect of day, pregnancy status, and their interaction for Days 9, 11, 13, 15 and 17 by 2-way ANOVA via the General Linear Models (GLM) procedures of the Statistical Analysis System (SAS Institute, Cary, NC) using the least significant difference (LSD) multiple testing method. The qPCR data were also subjected to a sliding time window linear regression to determine effect of day of gestation on genes during three biologically relevant periods: beginning, middle, and end of the study period. Each window contained three or more days contingent on the regression model not detecting a decrease in significance in terms of r^2 and p-values of the F-statistics.

The data from Studies 2 and 3 were analyzed using the Students t-test. The arginine transport data were subjected to a least-squares regression analysis (ANOVA) using the GLM

procedures of the Statistical Analysis System. All data are presented as means $\pm$ standard error of the mean with significance set at $p < 0.05$.

RESULTS

Primer Specificity and Efficiency

All selected genes (*ACTB*, *B2M*, *GAPDH*, *HPRT1*, *PPIA*, *RPL7*, *SDHA*, *TBP*, *TUBA1B* and *YWHAZ*) were detected in endometrial and placental tissues with the expected amplicon size, exhibiting one band in agarose gels, and identified as a single peak in the dissociation curves (Supplemental Figure 1).

Gene Stability Analyses

TBP was the most stable gene based on both Normfinder and GeNorm algorithms (Supplemental Tables 1 and 2). The acceptable standard for calculation of normalization factors includes a geometric mean of the top three most stable genes [26, 30]. For endometria, the geometric mean of *TBP*, *SDHA*, and *ACTB* is recommended, while the geometric mean of *TBP*, *HPRT1*, and *TUBA1B* is recommended for placenta (Supplemental Table 3). Genes that were the least stable and would not be indicated for use as reference genes in either endometrium or placenta included *GAPDH*, *B2M*, and *YWHAZ*.

Influence of Reference Gene Choice

In order to demonstrate that conclusions made from qPCR analyses are profoundly impacted by choice of reference gene, qPCR was performed to measure mRNA for the monocarboxylate transporter *SLC16A6* in endometrial tissue and normalized using the geometric

mean of *TBP*, *SDHA*, and *ACTB* or the single gene, *B2M* (Figure 1). The data normalized to the trio of genes indicated no difference in *SLC16A6* mRNA between the estrous cycle and pregnancy (Figure 1a). The data normalized to *B2M* indicated more *SLC16A6* mRNA in endometria from cyclic compared to pregnant gilts on Days 13-17 (Figure 1b; $p<0.05$), but the reference gene was higher on Days 15-17 of pregnancy than the estrous cycle (Figure 1c), and this high reference gene value caused the value for the target gene to appear lower after normalization. Data from Figure 1a suggest the conceptus has no influence on endometrial *SLC16A6* mRNA, while Figure 1b suggests the conceptus may secrete estrogens or cytokines which suppress endometrial *SLC16A6*.

SLC5A1 mRNA in Porcine Endometria and Placentae

Using the geometric mean of *TBP*, *SDHA*, and *ACTB* for normalization of qPCR data, *SLC5A1* mRNA increased between Days 5 and 9, then decreased between Days 9 and 11 in endometria from both cyclic and pregnant gilts (Figure 2a). There was an increase in *SLC5A1* mRNA between Days 11 and 13 of pregnancy, but not during the estrous cycle (Figure 2a; $p<0.001$). After Day 13, *SLC5A1* mRNA decreased and remained low for the duration of pregnancy (Figure 2a; $p>0.05$; $r^2=0.173$). Analysis by qPCR using the geometric mean of *TBP*, *HPRT1*, and *TUBA1B* for normalization determined that *SLC5A1* mRNA did not change in placental tissue between Days 30 and 85 of pregnancy (Figure 2b; $p>0.05$; $r^2=0.008$).

To confirm that the geometric mean of *TBP*, *SDHA*, and *ACTB* is appropriate for qPCR normalization we performed *in situ* hybridization analysis for glucose transporter *SLC5A1* mRNA and compared those results with the above qPCR data. *In situ* hybridization localized *SLC5A1* mRNA to the uterine LE and GE of both cyclic and pregnant gilts on Day 9, then

hybridization appeared to decrease during the estrous cycle, but further increase during pregnancy between Days 9 and 12 (Figure 2C). *SLC5A1* mRNA then appeared to decrease from Days 13 to 14 of gestation and remained low thereafter.

Quantitative PCR analysis indicated more *SLC5A1* mRNA in endometria of E2-treated as compared to control gilts (Figure 3a; $p < 0.01$) and *SLC5A1* mRNA was not affected by long-term P4 treatment (Figure 3a,b; $p > 0.05$). *In situ* hybridization analysis localized *SLC5A1* mRNA to uterine LE of Day 15 E2-treated, but not control gilts (Figure 3c). Treatment of ovariectomized gilts with P4 did not induce *SLC5A1* mRNA in the endometrium (Figure 3c).

SLC7A3 mRNA in Porcine Endometria and Placentae

Analysis by qPCR, using the geometric mean of *TBP*, *HPRT1*, and *TUBA1B* for normalization, determined that *SLC7A3* mRNA increased in placental tissue from Days 30-40 ($p < 0.001$; $r^2 = 0.999$), then remained unchanged through Day 85 of pregnancy (Figure 4a; $p > 0.05$; $r^2 = 0.086$). *In situ* hybridization localized *SLC7A3* mRNA to the chorion on Days 25 through 85 (Figure 4b). *SLC7A3* mRNA appeared to increase in placental tissues through Day 85, and was present in both the areolae and non-areolar regions of the chorioallantois (Figure 4b). *SLC7A3* mRNA was not detected by qPCR or *in situ* hybridization in endometria from any day of the estrous cycle or pregnancy (Figure 4b).

Radiolabeled arginine was transported from culture medium into the porcine chorioallantoic cells from Day 60 pregnancy (Figure 4c). As predicted from results of *in situ* hybridization analysis, no significant difference in arginine transport was found between areolar and inter-areolar regions of the chorioallantois (Figure 4c; $p < 0.05$).

DISCUSSION

Rigorous and reproducible endometrial and placental gene quantification applied over the duration of the estrous cycle and pregnancy is a useful tool for understanding the biology of pregnancy. In pigs, protein analysis is restricted by the availability of antibodies that cross-react with porcine proteins, making qPCR one of the main tools for documenting gene changes in tissues. However, as pregnancy progresses, the endometrium and placenta undergo physical remodeling and physiological changes which may involve up- or down-regulation of traditional reference genes. Therefore, the physiology of the tissue being analyzed should be considered when choosing reference genes, and one must determine which genes are reliable and appropriate reference genes for qPCR [31].

Previous studies have identified optimal reference genes for use in porcine tissues other than endometria and placentae, including: *TBP*, *ACTB*, *HPRT1*, *YWHAZ*, *HSPCB* [31, 32]. A previous study determined *TBP*, *SDHA*, and *UBC* were optimal reference genes in porcine endometria from Days 15-60 of pregnancy, but tissues from cyclic gilts were not examined [32]. The present results indicate that *TBP* and *SDHA* are also stable reference genes for endometrial tissues from cycling gilts. Indeed, there were multiple genes in the present results that would be equally acceptable as they varied little in their stability values. It is those genes that had poor stability values (*YWHAZ*, *B2M* and *GAPDH*) that should not be used as reference genes in these tissues. The ability to compare tissue from pregnant and cycling gilts is important for understanding how the conceptus modifies gene endometrial expression during establishment of pregnancy. This is the first study to identify appropriate reference genes for porcine placenta.

To confirm that the reference genes chosen were appropriate, both qPCR and *in situ* hybridization analyses were performed for two genes over the duration of the estrous cycle and pregnancy in the pig. The qPCR analysis of *SLC5A1* mRNA correlated with results from *in situ* hybridization analysis, both indicating a peak of *SLC5A1* mRNA on Day 15 of pregnancy. The biological significance for this peak of *SLC5A2* mRNA was suggested by both qPCR and *in situ* hybridization data indicating up-regulation of this gene in the endometrial LE of E2-treated gilts. Indeed, conceptus-produced E2 induces or upregulates expression of a number of endometrial growth factors and cytokines that are released by uterine LE and GE into the uterine lumen to support development of free-floating conceptuses [3, 33]. We hypothesize that as porcine conceptuses begin to elongate, an energy-dependent event, they secrete E2 that increases the number of *SLC5A1* transporters in uterine LE, thereby increasing transport of glucose into the uterine lumen to nourish free-floating conceptuses.

The qPCR and *in situ* hybridization analyses also correlated with one-another concerning the cationic amino acid transporter *SLC7A3* mRNA. Together these techniques demonstrated that *SLC7A3* mRNA significantly increased between Days 30 and Day 60 in the chorion, coinciding with increased abundance of arginine in porcine allantoic fluid [34]. Further, when chorioallantois from Day 60 of pregnancy was isolated and areolae separated from inter-areolar regions of the placenta, both areolar and inter-areolar tissues exhibited comparable abilities to transport radioactive arginine into cells of those tissues. We hypothesize that as the attachment phase of implantation in pigs is completed and mature placentation proceeds between Days 25 and 40 of pregnancy, the number of *SLC7A3* transporters increase in the placental chorion for transport of arginine across the chorioallantois and into the fetal-placental vasculature and/or

allantoic fluid. This enables arginine to be used to support development and growth of the conceptus, particularly the fetus during the second and third trimesters of gestation in pigs.

In conclusion, for normalization of qPCR we recommend use of the geometric mean of *TBP*, *ACTB*, and *SDHA* for endometrial tissue and use of the geometric mean of *TBP*, *HPRT1*, and *TUBA1B* for placental tissue. We utilized those genes to identify *SLC5A1* and *SLC7A3* as excellent candidate genes for placental transport of glucose and arginine, respectively, during pregnancy to support growth and development of the porcine conceptus.

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FIGURE LEGENDS

Figure 1 Expression of *SLC16A6* mRNA in endometrial tissues normalized using optimal and suboptimal reference genes.

SL16A6 mRNA across the estrous cycle and pregnancy in endometria normalized to the geometric mean of *TBP*, *ACTB*, and *SDHA* (A) or to *B2M* (B). *B2M* mRNA normalized to the geometric mean of *TBP*, *ACTB*, and *SDHA* (C). Relative mRNA levels are expressed as a ratio to Day 5 of the estrous cycle. Differing superscript letters indicate significant differences ($p<0.05$). Values are presented as mean $\pm$ SEM.

Figure 2 Expression *SLC5A1* mRNA in endometria and placentae.

SLC5A1 mRNA was greater in endometria (A) from pregnant than cyclic gilts on Day 13 ($p<0.05$), but placental *SLC5A1* mRNA (B) showed no change from Days 30 to 85 of pregnancy ($p>0.05$). Corresponding bright-field and dark-field images from *in situ* hybridization (C) reveal maximal levels of *SLC5A1* mRNA in the uterine LE on Day 12 of pregnancy. The D20 sense panel is the negative control. Width of field is 870 μ m. Legend: LE, luminal epithelium; GE, glandular epithelium; Tr, trophoctoderm; CE, chorion.

Figure 3 Estradiol induces expression of *SLC5A1* mRNA in uterine LE.

SLC5A1 mRNA was greater in endometria from E2-treated as compared to control (CO) gilts (A; $p<0.05$), but was not different in endometria from P4-treated compared to control gilts (B; $p>0.05$). Corresponding bright-field and dark-field images from *in situ* hybridization (C) depict *SLC5A1* mRNA in the uterine LE and GE in response to E2, while P4 treatment had no detectable effect on *SLC5A1* mRNA. The D20 Sense panel in Figure 3.3 is the negative control. Legend: LE, luminal epithelium.

Figure 4 *SLC7A3* mRNA and arginine transport in placentae.

SLC7A3 mRNA in placentae (A) increased from Days 30 to 40 of pregnancy ($p<0.001$; $r^2=0.999$) and then remained unchanged through Day 85 of pregnancy ($p>0.05$; $r^2=0.086$). Corresponding bright-field and dark-field images from *in situ* hybridization (B) localize *SLC7A3* mRNA to the trophoctoderm, CE, and areolae. There was no difference in arginine transport between areolar and inter-areolar regions (C; $p>0.05$). The D60P Sense panel is the *in situ* hybridization negative control. Legend: LE, luminal epithelium; Tr, trophoctoderm; CE, chorion.

Supplemental Figure 1 PCR of candidate reference genes.

Agarose gels (2.5%) showing PCR products for candidate reference genes from endometrial and placental tissues. Amplicons were of expected sizes (*ACTB* 187bp, *B2M* 166bp, *GAPDH* 173bp; *HPRT1* 91b; *PPIA* 59bp; *RPL7* 171bp; *SDHA* 141bp; *TBP* 153bp; *TUBA1B* 116bp; *YWHAZ* 203bp). Legend: Endometrium (E); Placenta (P).

443 **Table 1 Primer information for qPCR and *in situ* hybridization analyses.**

<i>Gene Name</i>	<i>Genbank Reference Sequence</i>	<i>Sequence</i>	<i>Product Length</i>	<i>Reference</i>
<i>ACTB</i>	XM_003124280	5'-TCCCTGGAGAAGAGCTACGA-3' 5'-TGT TGG CGT AGA GGT CCT TC-3'	187	Designed
<i>B2M</i>	NM_213978	5'-TTCACACCGCTCCAGTAG-3' 5'-CCAGATACATAGCAGTTCAGG-3'	166	[19]
<i>GAPDH</i>	NM_001206359	5'-GGTGAAGGTCGGAGTGAACG-3' 5'-TGA CTGTGCCGTGGAATTTG-3'	173	Designed
<i>HPRT1</i>	NM_001032376	5'-GGACTTGAATCATGTTTGTG-3' 5'-CAGATGTTTCCAAACTCAAC-3'	91	[19]
<i>PPIA</i>	NM_214353	5'-CCAACACAAACGGTTCCCAG-3' 5'-GCCATCCAACCACTCAGTCT-3'	59	Designed
<i>RPL7</i>	NM_001113217	5'-AAGCCAAGCACTATCACAAGGAATACA-3' 5'-TGCAACACCTTTCTGACCTTTGG-3'	171	[35]
<i>SDHA</i>	XM_003362140	5'-CTACAAGGGGCAGGTTCTGA-3' 5'-AAGACAACGAGGTCCAGGAG-3'	141	[36]
<i>SLC5A1</i> (qPCR)	NM_001164021.1	5'-GGCTGGACGGAAGTATGGTGT-3' 5'-ACAACCACCCAAATCAGAGC-3'	158	Designed
<i>SLC5A1</i> (ISH)	NM_001164021.1	5'-TCTCATGAGCTCCCTGACCT-3' 5'-GAGACGACCAGGACGATGAT-3'	468	Designed
<i>SLC7A3</i> (qPCR)	NM_001130973.1	5'-CACTCAACTCCATCCCCACT-3' 5'-CTGTGGCTGTCTCCAGATGA-3'	206	Designed
<i>SLC7A3</i> (ISH)	NM_001130973.1	5'-CTGGAGGATTTGTGCCATTT-3' 5'-AAAGAGGAATGCCATGAACG-3'	479	Designed
<i>SLC16A6</i>	XM_005674551.1	5'-AGGAGTTTGGGTATGGCAGAAA-3' 5'-ACTTACTCCCTGGGTTGACATC-3'	143	Designed
<i>TBP</i>	XM_003361418	5'-AACAGTTCAGTAGTTATGAGCCAGA-3' 5'-AGATGTTCTCAAACGCTTCG-3'	153	[36]
<i>TUBA1B</i>	NM_001044544	5'-GCTGCCAATAACTATGCCCG-3' 5'-ACCAAGAAGCCCTGAAGACC-3'	116	Designed
<i>YWHAZ</i>	XM_001927228	5'-TGATGATAAGAAAGGGATTGTGG-3' 5'-GTT CAGCAATGGCTTCATCA-3'	203	[36]

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