Human Endogenous Control Gene Panel

Background

For all gene expression studies using quantitative PCR it is necessary to compensate for differences between samples due to material losses, differences in RT yields and PCR inhibition. Normalization should include an endogenous control gene, but can also be complemented by identical sample input amounts. The endogenous control gene should have constant expression in all the samples compared. There is no universal control gene, expressed at a constant level under all conditions and in all tissues.

The best way to choose the proper reference gene is by running a panel of potential genes on a number of representative test samples. The gene(s) most appropriate for normalization are chosen in each case.

The Human Endogenous Control Panel consists of 12 validated qPCR assays for the most common endogenous control genes for gene expression studies, and provides a rapid and cost efficient way to identify your control genes.

Endogenous Control Panel

The genes included in the Human Endogenous Control Panel are commonly used in gene expression studies. Genes with varying cellular function and expression level have been selected. Primers have been designed to be exon-spanning where possible and to give low levels of primer-dimers.

Gene	Full name
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
TUBB	Tubulin, beta polypeptide
PPIA	Cyclophilin A
ACTB	Actin, beta
YWHAZ	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide
RRN18S	18S rRNA
B2M	Beta-2-microglobulin
UBC	Ubiquitin C
TBP	TATAA-box binding protein
RPLP	60S acidic ribosomal protein P0
GUSB	Beta-glucuronidase
HPRT1	Hypoxanthine-guanine phosphoribosyltransferase

Materials included

- Primer sets for 12 candidate endogenous control genes. Enough for 100 rxns per gene. This is enough for several evaluations of suitable reference genes in representative samples
- Positive Control DNA.

The addition of real-time PCR reagents is necessary. Primer sets are validated for use with most commercially available mastermixes for qPCR containing SYBR Green I.

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Selecting the most appropriate endogenous control gene

A number of methods have been proposed how to select the most appropriate control gene(s). The Excel macro named GeNorm is available to determine the genes with most correlated expression in a set of samples.

An alternative is to normalize RNA input, and correlate expression of candidate reference genes to total RNA levels. Those with most constant expression among the samples are the appropriate normalization genes to use.

A number of other statistical methods to define the most appropriate reference genes from a panel, tested on a set of samples, are listed in the references below.

geNorm

Change Data	GAPD	ACTB	HPRT1	UBC	YHWAZ	Normalisation
FIB1	0,516257	0,499303	0,482906	0,447207	0,572560	**0,5020**
FIB2	0,287796	0,238713	0,313899	0,221805	0,351638	**0,2787**
FIB3	0,160974	0,262108	0,147588	0,177935	0,306647	**0,2024**
FIB4	0,462392	0,151078	0,284928	0,372176	0,221805	**0,2774**
FIB5	0,694914	0,678860	0,765572	0,572560	0,851906	**0,7066**
FIB6	0,001146	0,000160	0,000765	0,000377	0,000690	**0,0005**
FIB7	0,487767	0,574475	0,512821	0,418316	0,685694	**0,5285**
FIB8	0,192781	0,183976	0,151584	0,181536	0,169808	**0,1753**
FIB9	0,393914	0,281148	0,386101	0,411390	0,342369	**0,3597**
FIB10	0,011902	0,005503	0,009390	0,010310	0,012347	**0,0095**
FIB11	0,016844	0,008107	0,013740	0,022522	0,023837	**0,0159**
FIB12	0,011059	0,014301	0,011902	0,017709	0,015443	**0,0139**
FIB13	0,008438	0,007141	0,009676	0,010589	0,014206	**0,0097**
FIB14	0,593982	0,697238	0,624490	0,550071	0,708977	**0,6320**
FIB15	0,283978	0,196683	0,218133	0,445716	0,234760	**0,2637**
FIB16	0,572560	0,423941	0,544589	0,414147	0,528466	**0,4923**
FIB17	0,720913	0,990033	0,877896	0,880833	0,983443	**0,8850**
FIB18	0,514536	0,504330	0,533786	0,590028	0,467047	**0,5204**
FIB19	1,00E+00	1,00E+00	1,00E+00	1,00E+00	1,00E+00	**1,0000**
FIB20	0,399211	0,316002	0,283978	0,441273	0,349298	**0,3535**
M < 1.5	0.513	0.664	0.432	0.523	0.475	

Storage and instructions for use

Primer solutions are supplied as ready-to-use working solutions in water and are stable for 6 months at +4°C but should be stored at -20°C for long term storage. 1µl of primer solution per 25µl PCR is recommended. Recommended temperature protocol is 95°C 20s, 60°C 20s, 72°C 20s. Detection should be performed during/after elongation. Primer sets are experimentally validated for use with most commercially available mastermixes for qPCR containing SYBR Green I.

References

Vandesompele J. et al (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes *Genome Biology* 3(7) 0034.I - 0034.II

Szabo A. et al (2004) Statistical modeling for selecting housekeeper genes *Genome Biology* 5:R59

Andersen C.L. et al (2004) Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets *Cancer Research* 64, 5245–5250

Contact and ordering

The Human Endogenous Control Panel can be ordered from our distribution partners around the world. Please see www.tataa.com for a list of our partners or contact us on refpanel@tataa.com for more information.

TATAA Biocenter conducts commisioned research and training within molecular diagnostics and gene expression analysis using real-time PCR technology to specifically quantify nucleic acids. Our competence is based on knowledge and experience accumulated through years of research from research groups at Chalmers University of Technology, Sweden. Our services comprise the entire field of real-time PCR including commissioned research, hands-on training, and custom design of real-time PCR assays.

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