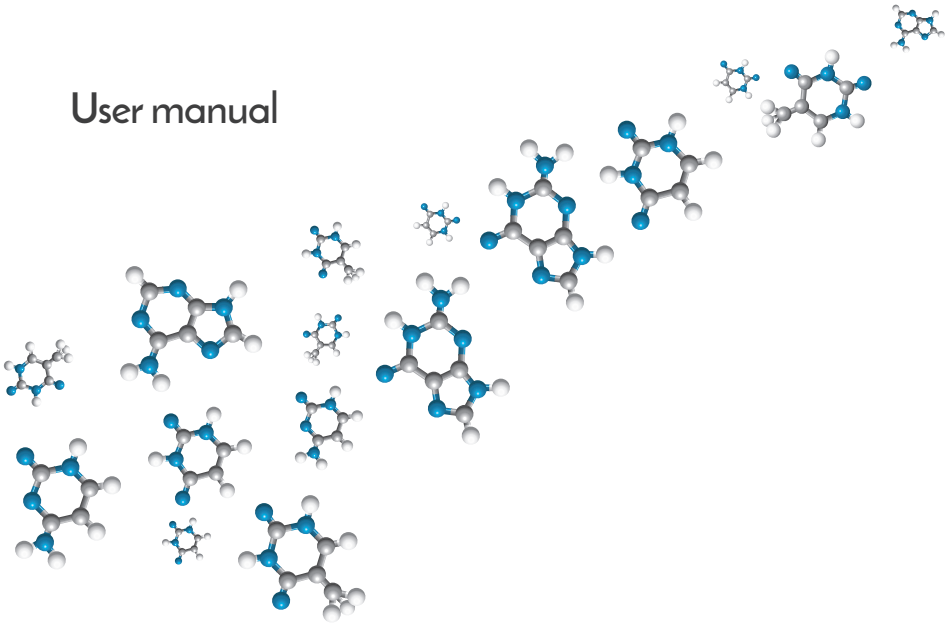
User manual



Reference Gene Panel

Rat

Version 1.3 - October 2017

SYBR® protocol

Order #:

Rat Reference Gene Panel: A103

tataabiocenter

Table of contents

Background

For accurate gene expression measurements it is necessary to normalise results of the expression of target genes to a reference that is not affected by the parameters studied in the specific study. There exists to our knowledge no reference gene that is unaffected in all conditions. Therefore it is necessary to find the optimal reference gene for your conditions and to validate that this gene is indeed non-regulated.

For this reason, we have developed the Reference Gene Panel Rat, which contains primer sets for 12 potential reference genes in rat. The expression of these genes should be measured on a number of representative samples, and from the results the most constantly expressed gene(s) can be determined. These genes can then be used for normalisation of target gene expression.

Assays included in the panel

The primers are designed to span exon-boundaries and to minimise primer dimer formation. Adequate efficiency of amplification was verified for each assay with commercial master mixes using SYBR Green I.

Gene	Full Name	Expression Level	Product Size
ACTB	Actin, beta	High	104 bp
B2M	Beta-2-microglobulin	Medium	142 bp
GAPDH	Glyceraldehyde-3-phospate dehydrogenase	High	104 bp
GUSB	Beta-glucuronidase	Low	158 bp
HPRT1	Hypoxanthine-guanine phosphoribsyltransferase	Low	99 bp
PGK1	Phosphoglycerate kinase 1	Low	146 bp
PPIA*	Peptidylpropyl isomerase A, Cyclophilin A	Low	128 bp
RPLP	60S acidic ribosomal protein P0	Medium	245 bp
RRN18S*	18S rRNA	Very High	120 bp
TBP	TATAA-box Binding Protein	Medium	133 bp
TUBB5	Tubulin, beta 5 class I	High	243 bp
YWHAZ	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide	Low	222 bp

*The 18S rRNA and PPIA assays are designed within an exon and may amplify genomic DNA.

Contents

- Positive Control DNA
- Primer solutions for 12 potential rat reference genes - 100 rxn (100 µl of primer mix, c=10 µM per primer)

Additionally required materials and devices

- **qPCR instrumentation**

This kit has been validated on several different instrument platforms. It is suitable for use on all qPCR instruments.

- **Master mix or master mix components**

This kit has been validated using a variety of commercially available master mixes with good results. For best results in SYBR® based qPCR TATAA Biocenter recommends to use the TATAA SYBR® GrandMaster® Mix.

- **Pipettes and tips**
- **Vortex and centrifuge**
- **Sample cDNA**

It is recommended to use cDNA of high quality. RNA integrity should be assessed prior to cDNA synthesis.

Storage

The contents of this kit can be stored for 1 month at +4°C. For long term storage -20°C is recommended. Repeated freeze-thaw cycles should be avoided. Vortex thoroughly and spin down before use.

Amplification protocol

The amplification protocol below is recommended. Optionally a 2-step amplification protocol may be used. We then recommend the protocol: 95°C for 5 seconds followed by 60°C for 30 seconds, in 40 cycles.

UNG step (optional)	according to mix instructions		
Enzyme activation	according to mix instructions		
Cycling (x40)	denaturation	5s	95°C
	annealing	30s	60°C
	elongation	10s	72°C

After amplification it is recommended to perform dissociation curve analysis from 65°C to 95°C. This should be programmed according to the instrument manufacturer's instructions.

Pipetting protocol

We recommend that each sample is quantified in duplicate or triplicate for each gene in the panel. Approximately 5-10 samples of each type (healthy/sick, treated/non-treated etc.) should be evaluated for determination of the most constantly expressed gene. Equal numbers of samples from each sample type should be included in the test.

Prepare master mixes for each gene according to the protocol to the right. Select the final volume that fits your wells. Prepare at least 1 reaction extra so that you do not run out of master mix.

Component	1 rxn	1 rxn	1 rxn
PCR-grade water	3.6 µl	7.2 µl	9.5 µl
Primer mix	0.4 µl	0.8 µl	1.0 µl
Master mix (2x)	5.0 µl	10.0 µl	12.5 µl
cDNA	1.0 µl	2.0 µl	2.0 µl
Final volume	10 µl	20 µl	25 µl

Larger volumes of cDNA may be added. In this case, decrease the volume of water accordingly. As an additional control of the PCR reaction, a sample containing Positive Control DNA instead of cDNA as template should be added for each gene. A negative control (NTC) with water instead of cDNA should always be included for each gene as a control of contamination of the reagents.

Selecting the most appropriate reference gene using GenEx Standard

A number of methods have been proposed for how to select the most appropriate reference gene(s). With the GenEx Standard software you can easily determine the most constantly expressed gene(s) by using two commonly used methods, GeNorm and Normfinder. Both these methods are included in the software. GenEx Standard also includes easy-to-use pre-processing tools to convert your data from Cq values to relative quantities, normalisation with reference genes or total RNA, the possibility to classify your samples in different groups (i.e. treated/non-treated) and much more. 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 section in the back of this manual. To import your data directly from the instrument into the GenEx software you can use the Instrument export instruction that is found in the Help section. Before or after pre-processing, the data can be visualised using a number of built in functions, e.g. box plot.

A free license for GenEx Enterprise is available for download from www.multid.se and provides the fully functional analysis software for a trial period of 14 days. To purchase GenEx licenses or for qPCR data analysis services, contact us at order@tataa.com.

Reorder Information

The Reference Gene Panel Rat SYBR has product number A103. When the optimal reference genes for your study has been determined, those genes can be ordered individually (product no qA-03-0125S to qA-03-0136S) from our webshop on www.tataa.com or by e-mail to order@tataa.com.

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.

Stålberg A. et al., (2005) Quantiative real-time PCR for cancer detection: the lymphoma case. Exp. Rev. Mol. Diagn. 5(2).

Contact

For more information about the product and reference genes please contact info@tataa.com.

License information

Troubleshooting

I do not get any amplification/signal?

The instrument may not have been programmed correctly or there may be a problem with your master mix. Evaluate if the problem is in the detection or the amplification by running the PCR-product on a gel. Run a new test using the positive control DNA provided with the kit.

My negative controls are amplified?

Since the assays are based on SYBR Green detection, all dsDNA is detected, including primer dimers. Depending on which mix is used, varying amounts of primer dimers are formed. Typically negative controls give Cq values well above 35 cycles. Evaluate whether amplification is due to primer dimers or contamination by using dissociation curve analysis.

My samples have same/higher Cq-value than my no template controls?

This indicates that you have added too little cDNA. Add more cDNA and try again. The cDNA may be of low quality. Check the quality of the RNA before performing cDNA synthesis. If your no template controls are positive, this indicates contamination of the water or the master mix.

My replicates are not very tight?

With good quality cDNA and good pipetting technique, very high reproducibility is expected. Low amounts of cDNA can lead to higher variation. Also, low quality cDNA can lead to differences between replicates. Check the accuracy and reproducibility of your pipettes.

The signal for 18S rRNA amplifies very early and does not have a good baseline?

In some cases the amount of 18S is too high, which can result in erroneous background subtraction. Try diluting the cDNA sample.

I get a positive amplification from genomic DNA

Where possible, the assays in the Reference Gene Panel have been designed to span exon boundaries. However, when intronless pseudogenes are present in the genome, genomic DNA may still give a positive amplification. Try removing DNA contamination by treating the RNA sample with DNase.

qPCR training courses at TATAA Biocenter

TATAA Biocenter is a leading organizer of hands-on training in qPCR and related technologies. For comprehensive training program please visit www.tataa.com.

Other products from TATAA

ValidPrime™ - mouse, human and other vertebrates

ValidPrime™ is an assay to test for the presence of gDNA in test samples and when combined with a gDNA control sample, replaces all RT(-) controls. ValidPrime™ is highly optimized and specific to a non-transcribed locus of gDNA that is present in exactly one copy per haploid normal genome. The kit also contains a gDNA standard that can be used to test the sensitivity of RT-qPCR assays for gDNA background. ValidPrime™ replaces the need to perform RT(-) controls or all reactions and makes RT-qPCR profiling easier and substantially cheaper.

(HL-)dsDNase

New generation DNase that is specific to double stranded DNA and can be efficiently inactivate by heating at 55 °C. It can be added to your RT reaction to efficiently remove any gDNA, without degrading single-stranded cDNA. It is completely inactivated by the PCR and does not degrade the double stranded PCR product.

GenEx software

GenEx provides the appropriate tools to analyze real-time PCR gene expression data and to extract valuable information from the measurements.

TATAA Interplate Calibrator - Variation Compensation

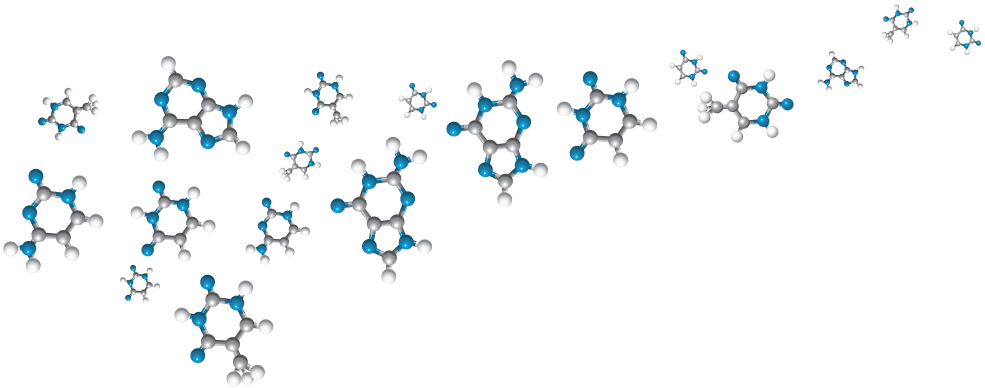
For practical reasons many qPCR studies involve the use of samples that are processed in more than a single batch or in which the sample set is extended over time. Even over a short time period, variation between qPCR processing runs is observed due to different baseline subtractions and threshold settings. The TATAA Interplate Calibrator (IPC) is used to compensate for the variatio between qPCR runs.

CelluLyser™ - for rapid and easy lysis and cDNA synthesis

The CelluLyser™ Lysis and cDNA Synthesis Kit enables you to generate cDNA from small samples with minimal losses and hands-on time. It is particularly useful for single cell analysis. By using CelluLyser™, the entire workflow from cell lysis to RT and qPCR can be performed without washing steps, thus eliminating material loss.

VisiBlue™ mastermix colorant

The VisiBlue™ mastermix colorant enables you to quickly color your favourite qPCR mastermix to easily visualize where the reagent has been added to your plates and tubes. VisiBlue™ is very easy to use by a simple addition to your favorite master mix.



Express your genius

TATAA Biocenter, with offices in Gothenburg, Sweden, and Prague, Czech Republic, is the leading provider of quantitative real-time PCR and NGS services and the prime organiser of quantitative real-time PCR and NGS workshops globally. TATAA Biocenter conducts commissioned research and training within the field of molecular diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.

tataabiocenter

TATAA Biocenter AB
Odinsgatan 28, 411 03 Göteborg
Tel: +46 31 761 57 00, Fax: +46 31 15 28 90
E-mail: info@tataa.com, Website: www.tataa.com