

## NARG 2006 Study information

The Nucleic Acid Research Group of the ABRF would like to thank you for agreeing to participate in this year's study on "Priming Strategies for Real-Time RT-PCR".

Two human genes will be assayed using a commercial reference RNA:  $\beta$ -glucuronidase (hGUS) and TATA-box-binding protein (hTBP). The test kit contains sufficient reagents for testing two genes with 5 different priming strategies. The cDNA produced in the 5 different reverse transcriptase reactions should be amplified in a Taqman® probe-based and/or SYBR GREEN 1 assay.

A copy of this information is also available at

<http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm>

Look for: NARG 2006 Study: Priming Strategies for Real-Time RT-PCR

Before performing the study, please read the questions you will need to answer.

[http://seqcore.brcf.med.umich.edu/phpESP/public/survey.php?name=NARG06\\_3](http://seqcore.brcf.med.umich.edu/phpESP/public/survey.php?name=NARG06_3).

The kit contains the following as dry pellets: Resuspend in nuclease free water in the amounts indicated.

Reagent	Amt/tube	unit	Resuspend (ul)	Conc	unit
hReference RNA Template	4000	ng	40	100	ng/ul
Random Primer	125	pmole	50	2.5	uM
Oligo dT	125	pmole	50	2.5	uM
hGUS R primer for RT	125	pmole	50	2.5	uM
h GUS F primer	1000	pmole	50	20	uM
hGUS R primer	1000	pmole	50	20	uM
hGUS Probe 5'FAM 3' BHQ1	200	pmole	10	20	uM
hTBP R primer for RT	125	pmole	50	2.5	uM
hTBP F primer	1000	pmole	50	20	uM
hTBP R primer	1000	pmole	50	20	uM
hTBP Probe 5'FAM 3' BHQ1	200	pmole	10	20	uM

All other reagents provided by user.

There should be enough reagents to do triplicates of each priming strategy. Each RT reaction should yield enough material for 2 + wells for the PCR reaction.

Set up triplicate reverse transcription reactions for each priming strategy in a volume of 10 ul at a concentration of 20 ng/ul. The stock RNA is reconstituted to 100 ng/ul, therefore, adding 2 ul of RNA in an RT of 10 ul, gives a final concentration of RNA of 20 ng/ul and a total of 200 ng RNA being transcribed/well (tube). Use the reverse transcription reagents currently in use in your laboratory. Triplicate RT reactions are being used rather than triplicate PCR reactions because the variation in the RT step is being measured. A minus reverse transcriptase reaction, to check for genomic DNA contamination is not required, since the RNA is a commercial template.

Fifty (50) ng of the cDNA (based on the RNA input) (2.5 ul) from the RT should be added to each well for the final PCR reaction. Please include triplicate NTC (all PCR reagents, but 2.5 ul of water instead of an RT generated template) wells.

Use the PCR reagents, protocols and real-time hardware currently in use in your laboratory.

An example of an experimental protocol, setup and result report is included and can also be found at [http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm#R\\_4](http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm#R_4)

Go to the following Web page to fill out a Web-based questionnaire that will give NARG some details about your protocol.

[http://seqcore.brcf.med.umich.edu/phpESP/public/survey.php?name=NARG06\\_3](http://seqcore.brcf.med.umich.edu/phpESP/public/survey.php?name=NARG06_3).

E-mail your results to Deb Grove at [dsg4@email.psu.edu](mailto:dsg4@email.psu.edu) as a copy of your exported data in an Excel spreadsheet labeled with your 4 digit ID code. The exported data should contain (minimally) the Ct for each well, the gene being assayed and the RT primer used. Import a picture (jpg) into the spreadsheet of the amplification curves for each gene; e.g, all 5 priming methods for hGUS in one “picture” and all 5 priming methods for hTBP in another “picture”. See NARG06 Examples for the preferred data presentation.