

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

Association of Biomolecular Resource Facilities (ABRF)

INTRODUCTION: The purpose of this study is to provide an opportunity for participating laboratories to gain crucial information about the variability of the RT-step of the qPCR assay and about the comparability of qPCR results obtained using different cDNA priming strategies. In addition, the study will act as an audit for participating laboratories, who will be able to compare the results from their protocols, techniques and equipment with those from other laboratories around the world. The study is open for those who use Taqman® probe-based or SYBR Green I-based assay systems. Deadline for sample submission is Dec. 15, 2005. Data will be presented at the ABRF 2006 annual meeting in Long Beach, CA, February 11 - 14, 2006. We think it will be worth your time to participate in this study.

Please answer the following questions about how the study was performed in your laboratory. All fields are "required", except for your email address. If you have no answer, enter "Not done".

E-mail your results to Deb Grove at 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 at <http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm> for the preferred data presentation.

Questions marked with a * are required.

- * 1.** Please enter a 4 digit identification code, so you can identify your results. People often use last 4 digits of their social security number or telephone number. If more than one set of data is submitted, please use an a,b,c, etc. after the number.

***2.** What type of assay are these results from?

- Taqman®
- SYBR Green I

***3.** What type of real-time PCR instrument was used for this study?

- ABI 5700
- ABI 7000
- ABI 7300
- ABI 7500
- ABI 7700
- ABI 7900
- Bio-Rad iCycler
- Cepheid SmartCycler
- Corbett RotorGene
- MJ Research Opticon/MJ Research Chromo4
- Roche Light Cycler
- Stratagene MX3000
- Stratagene MX4000
- !Other: Please specify

***4.** How much RNA template (ng) was used in each RT reaction? E.g., 200 ng.

***5.** What volume (ul) was used for the RT portion of the assay? E.g., 10 ul.

***6.** What type of RT mix was used for the Reverse Transcription step?

- Commercial kit
- Homemade mix

- Combination
- !Other: Please explain

***7.** Which source of reverse transcriptase was used?

- AMV
- MMLV
- TTh
- !Other: Please specify

***8.** At what temperature was the RT reaction performed?

- 37 C
- 42 C
- 50 C
- 55 C
- 60 C
- !Other: Please specify

***9.** For how long was the RT reaction performed? (minutes)

***10.** What volume (ul) of the RT reaction was added to each PCR reaction. E.g., 2.5 ul was added to each well of the PCR reaction.

***11.** What total volume (ul) was used for the PCR portion of the assay? E.g., 25 ul.

***12. How were triplicate reactions performed?**

- 3 RT reactions were performed for each priming strategy
- 1 RT reaction was performed and triplicate PCR wells were done

Submission Instructions

Please press the "Submit Survey" button at the bottom left of this page one time to submit your entry.

Don't forget to email your data to Deb Grove at dsg4@email.psu.edu

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13. Please enter your email address if you would like to be contacted if we have questions about your entry.