

# NARG 2005 Study: Validation of Your Real-Time PCR Technique

## Association of Biomolecular Resource Facilities (ABRF)

**INTRODUCTION:** The purpose of this study is to give investigators an opportunity to test their reverse transcription real-time PCR technique and to gather information about the performance of various platforms, variations due to reagents and how people analyze their data. The study is open for those who use both Taqman® type systems and SYBR green systems. A table showing (anonymously) how the individual participants fared in their assay efforts will be posted on this page. Data will be presented at the ABRF 2005 annual meeting in Savannah, GA, March 5 - March 8, 2005. This study will be open until December 15, 2004. We think it will be worth your time to participate in this study.

Submit a separate survey for each "assay". E.g., if you run the two standard curves on 2 different platforms or using 2 different Master mixes. See Submission Instructions at the end of the questionnaire for the easiest way to do this.

**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 you plan to submit more than one set of data use an a,b,c, etc. after the number.

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

Taqman®

SYBR green I

!Other: Please specify

**\*3. What type of instrument did you use 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. Did you do a 1 step (RT and PCR in same reaction) or 2 step Assay (separate RT and PCR steps) ?****\*5. If you did a 2 step assay, did you perform the RT and the PCR in the same tube or well?**

Yes

No

**\*6. Did you dilute the RNA and DNA standards in the diluent provided?**

Yes

No

**7. If not, what did you use?****\*8. Did you treat the DNA sample exactly as you did the RNA sample? (Did you subject the DNA to a RT reaction?)**

Yes  
No

**\*9.** Did you use robotics to set up any part of the assay?

Yes  
No

**\*10.** Which source of reverse transcriptase do you use?

AMV  
MMLV  
TTh  
!Other: Please specify

**\*11.** At what temperature did you run the RT reaction?

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

**\*12.** For how long did you run the RT reaction? (minutes)

**\*13.** What type of "master mix" did you use for your real-time PCR?

ABI 2X Master Mix  
ABI TaqMan Core PCR Reagent Mix  
ABI 2X SYBRgreen Master Mix  
ABI SYBRgreen Core PCR Reagent Mix  
Bio-Rad iQ Supermix (for probes)  
Bio-Rad iQ SYBR Green Supermix  
Bio-Rad iTaq Supermix with ROX (for probes)  
Bio-Rad iTaq<sup>TM</sup> SYBR Green Supermix with ROX

Eurogentec 2X assay mix  
LTI Platinum Quantitative PCR SuperMix-UDG  
Sigma 2X SYBRgreen Master Mix  
Stratagene Brilliant® QPCR Master Mix  
"Homemade"  
!Other: Please specify

**\*14.** Did you use a passive reference dye (e.g. ROX) in your PCR reaction?

Yes  
No

**\*15.** Did you use UNG in your PCR reaction?

Yes  
No

**\*16.** What volume did you use for the PCR portion of the assay? (ul)

**\*17.** Please list the PCR cycling profile used. E.g., 95C, 10 min. Then 40 cycles of 95C, 15 sec; 60C, 60sec.

#### ANALYSIS (DNA curves)

**\*18.** What baseline setting was used for the DNA curve? E.g., cycles 3-10, adaptive, machine default.

**\*19.** What was your threshold setting for the DNA curve? E.g., 0.05.

**\*20.** What was the highest "normalized" fluorescent value for the DNA standards? E.g., deltaRn was 1.25.

**\*21.** What was the average Ct (Cp) for the first point on the DNA standard curve? Please use one decimal point. E.g., 13.2.

**\*22.** What was the slope of the DNA standard curve?? E.g., -03.45.

**\*23.** What was the y-intercept of the DNA standard curve? E.g., 37.4.

**\*24.** What was the r2 (coefficient of correlation) for the DNA standard curve? E.g., 0.9986

#### ANALYSIS (RNA curves)

**\*25.** What baseline setting was used for the RNA curve? E.g., cycles 3-10, adaptive, machine default.

**\*26.** What was your threshold setting for the RNA curve? E.g., 0.05.

**\*27.** What was the highest "normalized" fluorescent value for the RNA standards? E.g., deltaRn was 1.25.

- \*28.** What was the average Ct (Cp) for the first point on the RNA standard curve? Please use one decimal point. E.g., 13.2.

- \*29.** What was the slope of the RNA standard curve? E.g., -3.25.

- \*30.** What was the y-intercept of the RNA standard curve? E.g., 39.2.

- \*31.** What was the r<sup>2</sup> (coefficient of correlation) for the RNA standard curve? E.g., 0.9234.

### Submission Instructions

Please fill out a survey for every different condition that you use for the 2 standard curves . E.g., if you ran a Taqman Assay and a SYBR green assay. It is easy to submit more than 1 assay. After submitting the first assay, use the **BACK** button on your browser. It will take you back to the form which you just filled out and then you need only change the variable(s) which might be different and add an a,b,c etc after your ID number. If you click on the return link on the thank you page, then it will return you to a blank survey and you will have to refill all the answers.

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

Don't forget to email your data to "Gregory L Shipley" Gregory.L.Shipley@uth.tmc.edu

A copy of you exported data labeled with your 4 digit ID code and exportdata. E.g., 1234exportdata. The export data should contain (minimally) the Ct for each well, whether there was DNA or RNA in the well, the amount of DNA or RNA in the well

A screen capture( pdf or jpg) of the amplification curves for DNA labeled 1234DNAamp

A screen capture( pdf or jpg) of the amplification curves for RNA labeled 1234RNAamp

A screen capture( pdf or jpg) of the Standard curve for DNA labeled 1234DNAstd

**A screen capture( pdf or jpg) of the Standard curve for RNA labeled 1234RNAsd**

**32.** Please enter your email address if you would like to be contacted if we have questions about your entry and/or you would like some feedback on your data.

Submit Survey