

Validation of your Reverse Transcription Real-Time PCR Technique

NARG 2005 Research Study

Dear Colleagues,

The Association for Biomolecular Resource Facilities (ABRF) Nucleic Acid Research Group's (NARG) research project for this year is designed with several goals in mind:

- To provide members of the real-time PCR community with an opportunity to test their technique.
- To gather comparative information about the many real-time platforms available today.
- To investigate potential variation due to different reagents.
- To determine how people analyze their data.

The study is open for those who use either Taqman® probes or SYBR green I detection systems. This is a great opportunity, in total anonymity, to test your skills in reverse transcription and quantitative real-time PCR and contribute to shared knowledge. The members of NARG will provide you with sufficient primers, probe (if applicable), an *in vitro* transcribed RNA template, a synthetic oligonucleotide DNA template and a dilution buffer for five 6-log standard curves. There should be enough materials provided for both SYBR green I and Taqman® probe assays to be run by each lab if they wish to compare assay types. Examples of detailed protocols for setting up the experiment are available. We ask each contributing group to run the assays and use the assay mixes, conditions and a real-time machine in their laboratory. Submission of results by email and filling out a simple web-form questionnaire about how you ran the assay should provide sufficient data to summarize and compare results across laboratories. Your participation in performing these assays will help us measure multiple aspects important in running real-time experiments:

- Both the RNA and DNA standards should have the same slope and values for each of the 6-log dilutions. The dilutions will be made by the receiving lab, thereby allowing pipetting accuracy to be measured.
- Because one standard is RNA and the other DNA, there will be differential sensitivities to handling. How well each laboratory handles these standards is a measure of how well the lab handles each type of template, in general.
- Each lab will use the reagents they normally use, whether a kit or homemade master mixes. Thus, the study will provide data on how different reagents compare in performing this task.
- Similarly, each lab will use the analytical platform they have in the lab. Information from the study will allow some comparison of different real-time PCR platforms.
- Each lab will submit analyzed results which should allow a measure of whether the data is being analyzed at optimum conditions.

The results of the study will be presented at the ABRF 2005 meeting in Savannah, GA and posted on the ABRF web site. This study should give each participant valuable feedback concerning their laboratory methodologies.

To learn more and/or participate in this interesting study, please go to (<http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm> and look for “**Validation of your Reverse Transcription Real-Time PCR Technique**” link.

All results must be received by December 15, 2004.

Contact Greg Shipley (Gregory.I.Shipley@uth.tmc.edu) to request a sample or with any questions.

The ABRF Nucleic Acid Research Group

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