

The Nucleic Acids Research Group (NARG) of the Association of Biological Resource Facilities (ABRF) invites you to participate in an empirical study to define the parameters required to make an optimal 5'-nuclease (Taqman®) real-time PCR assay. New assay design can be one of the major rate-limiting steps to those new to this technology in rapidly acquiring data from their genes of interest. Although a large number of pre-made assays can be purchased from Applied Biosystems and Qiagen, it is much more cost effective to make your own assays if a large number of samples need to be run with each assay. There are general guidelines available concerning assay design. However, exactly how important each of these parameters are has not been studied in an empirical manner. Further, there may be as yet unknown factors that should be taken into account during assay design.

The purpose of this study is to give a large number of investigators an opportunity to design what they feel will be an optimal primer/probe set for a common transcript and then have them tested empirically for efficiency. Each participant is asked to provide the sequence of a pair of primers and a probe within the coding region of the mouse IFN $\gamma$  transcript. Members of the NARG will then synthesize the primers and probe and test them using a plasmid containing the mouse IFN $\gamma$  cDNA clone as a template standard. The results for each assay will be posted on the ABRF web site and published. Entries are identified by a user designated code and are completely anonymous.

- Primer and probe sets will be tested using an ABI 7700 run at default conditions: 50°C 2, min 95°C 10 min; and 40 cycles of 95°C -15 sec and 60°C -30 sec. Primers should be designed with a T<sub>m</sub> of 55-60°C and probes accordingly.
- Primers will be run at 400 nM and the probe at 100 nM.
- Reactions will be run using ABI 2X Master Mix Duplicate wells will be run for each concentration of the template.
- Template will be run over a 5-log range: 2x10<sup>1</sup> – 2x10<sup>5</sup> copies + 2 NTCs.
- Reactions will be setup using a Biomek 2000 robot for maximal accuracy.

Effectiveness of primer/probe design will be judged by PCR efficiency (slope), delta R<sub>n</sub>, y-intercept and Ct.

We encourage all real-time investigators to participate, regardless of experience. This is not a contest. It is an opportunity to sharpen your skills, learn some new ones and help us demonstrate the principles of Taqman® primer/probe design.

Contact Scottie Adams ([sadams@northnet.org](mailto:sadams@northnet.org)) with questions.