

Dear All:

The Nucleic Acids Research Group (NARG) of the ABRF would like to invite you to participate in their 2003 study of the synthesis of Real-Time PCR probes. Please contribute to this simple and useful study!

Please make the following probe sequence for beta actin: [hb-Actin-1020(+)]

5' FAM-ATC AAG ATC ATT GCT CCT CCT GAG CGC-TAMRA or BHQ 3'

- The 3' quencher can be BHQ1, TAMRA, or another one of your choice.
- We recommend that you use 3' quencher cpg columns to do the synthesis because it is so much easier and cheaper than post-synthesis labeling methods.

E.g. BHQ-1 CPG Synthesis Column; 200 nmol for ABI394 Cat # CG1-5041-2
See http://www.biosearchtech.com/products/display_products.asp?id=106

OR TAMRA CPG Synthesis Column; 200 nmol for ABI394 Cat # CG5-5012-2
http://www.biosearchtech.com/products/display_products.asp?id=18

Also

E.g.: 3'-TAMRA Quencher CPG 0.2 umol ABI 394/Expedite cat # 20-5910-42
See <http://www.glenresearch.com/ProductFiles/20-5910.html>

- Our preliminary data suggests that the most important point to quality probe synthesis is to use adequate coupling time for the reporter-coupling step. Even generic protocols of synthesis, cleavage and deprotection in use in most labs should be adequate for making good Real-Time PCR probes.
- NARG suggested protocols and related files can be found at NARG web sites at either <http://www.ABRF.org> under Research Groups/ Nucleic Acids/Studies or at <http://web-apps.fccc.edu/fccc/narg/> under NARG2003 study.
- Please use the sample survey pdf file provided at the ABRF web site to record pertinent parameters of your synthesis and mail it with your probes. Please read the sample survey before you start the synthesis to familiarize yourself with the questions we would like answered.
- Please mail the dried crude synthesis product at room temperature via Fed Ex. to Glenn Miller of Tony Yeung's lab. Purified probes may also be sent as an option if you do not mind the work. You are welcomed to send more than one probe to test the results of different synthesis protocols.
- If you have any questions, please contact Tony Yeung at at_yeung@fccc.edu.

Please label and code your oligos as:

XXXX -Y-Z

Where XXXX is the 4 letter lab code you assign, such as last 4 digits of your social security number.

Y = synthesis 1, or 2, or 3, etc.

Z = C (crude) or P (purified)

Please FedEx to:

Glenn Miller
DNA synthesis manager
Fox Chase Cancer Center
7701 Burholme Ave
Philadelphia PA 19111

Phone: 215-728-2858

Email: oligo@fccc.edu

Quality analyses:

- NARG will perform quality analysis on the probes with CE, DHPLC (WAVE), ESI-MS.
- NARG will do real time PCR experiments over four log range of template concentration to test efficiency, Ct, slope, sensitivity, etc.
- Data, identified only with lab codes, will be posted at our web site to allow comparison with other participants for improvements to be made.

Rules:

- Participants should use 6-FAM phosphoramidite as reporter, quencher cpg columns of any company and either regular phosphoramidites or dG^{dmf} phosphoramidites for the internal nucleotides.
- Participant may use reagents of any company they choose. E.g.: Biosearch Tech and Glen Research both sell quencher columns and reporter phosphoramidites.
- If you are not an ABRF member, you may still participate in this study. We encourage you to become an ABRF member. You can apply for membership at <http://www.ABRF.org>.