

## NARG Real-Time PCR Probe Synthesis Sample Survey

### INTRODUCTION:

This sample survey is to be used to answer questions about each FRET (dual-labeled) probe which you submit to the NARG for analysis. Please check the appropriate box to the left of your answer or fill in "Other". Your answers will help us to correlate conditions with quality.

### Identification Number of sample

(4 digit lab ID + Sample # + (C)rude or (P)urified: E.g., 3303-1-C

### Instrumentation

1. What instrument was used for oligonucleotide synthesis

ABI 380B  ABI 392  ABI 394  Expedite  Other Specify:

### Reagents

2. What 3' Quencher cpg column was used for this synthesis?

BHQ-1  TAMRA  Eclipse  Other Specify:

3. Where was the 3' Quencher cpg column obtained?

Biosearch Technologies  Glenn Research  Other Specify:

4. What scale was the 3' Quencher cpg column?

0.05 umole  0.2 umole  1 umole  Other Specify

5. Where was the 5' reporter phosphoramidite (FAM) reagent obtained?

Biosearch Technologies  Glenn Research  Other Specify

6. How was the 5' reporter phosphoramidite (FAM) stored?

Room temp  Room temp with dessicant  Other Specify

7. What A,C,G,T phosphoramidites were used for this synthesis ?

A(Bz) C(Bz) G(DMF) T  A(Bz) C(Bz) G(ibu)  Other Specify

8. What concentration of the regular (A,C,G,T) phosphoramidites was used:

0.1 M  0.05M  Other Specify

9. What concentration of the regular 5' reporter phosphoramidite (FAM) reagent was used:

0.1 M  0.05M  Other Specify

10. When was the 5' reporter phosphoramidite (FAM) reagent dissolved and placed on the machine? E.g, immediately prior to synthesis? Immediately prior to coupling?

### Synthesis

11. What protocol (synthesis cycle) was used ?

0.05 umole  0.2 umole  1 umole  Custom

Please describe custom:

12. What was the coupling time for the 5' reporter phosphoramidite (FAM) reagent

minutes

### Cleavage and Deprotection

13. Was the cleavage performed on or off the machine?

ON  OFF

14. What reagent was used to deprotect the sample?

concentrated NH<sub>4</sub>OH  TAMRA cocktail  Other Specify:

TAMRA Cocktail if different than that recommended:

15. What temperature was the sample deprotected at?

°C

16. For how long was the sample deprotected?

hours

17. How was sample was dried down??

in a vacuum centrifuge with heat  in a vacuum centrifuge with no heat

Other Specify

**Purification**

18. How were these probes purified?

None  HPLC -1X  HPLC -2X  RP (OPC) column  Gel  
 Other Specify

**Assessment of synthesis:**

19. Did you find the FRET probe synthesis easy?

Yes  No

20. Do you routinely make FRET probes?

Yes  No

21. If so, do you normally do quality control on the FRET probes?

Yes  No

22. If so, what is the method of quality control?

HPLC  Mass spec  Gel  CE  DHPLC  
 Other Specify

23. Do you normally purify the FRET probes for your customers?

Yes  No

24. If so, how do you normally purify your probes?

None  HPLC -1X  HPLC -2X  RP (OPC) column  Gel  
 Other Specify

25. Please share your recommendations for how to make a great FRET probes!

Attach separate sheet

If we have any questions about your sample. may we contact you??

Email Address:  (not required)

Thank you for your participation!!!!