

# **Nucleic Acid Research Group Real-Time PCR Survey**

## **Association of Biomolecular Resource Facilities (ABRF)**

INTRODUCTION: This survey is designed to determine the current status of real-time PCR technology in laboratories around the world, particularly Core laboratories. Your answers will help us "take the pulse" of the real-time PCR community. Submissions are anonymous and results will be freely available via a "web poster". This survey will be "open" until January 15, 2004. Results will be presented at the ABRF 2004 annual meeting in Portland, OR, Feb 28-Mar 2, 2004 and will be available "on line" by April 1, 2004. We think it will be worth your time to participate in this study.

**Instructions: Please select the answer(s) that best applies to your situation. There are 58 questions. The survey should take less than 10 minutes to complete. If you submit a partial survey, you can still submit the remainder later and make a note in the comments box that this submission is a continuation. Contact "Scottie Adams" [sadams@northnet.org](mailto:sadams@northnet.org), if you have any questions or problems.**

## FACILITY

**1. At what type of facility are you located?**

- Academic
- Government
- Industry
- Private Research Foundation
- !Other

**2. Are you a member of a core facility?**

- Yes
- No

**3. If "no", proceed to Question 7. If "yes", do you offer other services?**

- Yes
- No

**4. If "yes", what other services do you provide other than real-time PCR? Check all that apply.**

- DNA synthesis
- DNA sequencing
- Microarray
- Genotyping (Fragment analysis)
- Mass spec
- !Other

**5. What level of real-time PCR service do you offer? Check all that apply.**

- Access to Machine only
- PCR reaction only
- RNA/DNA prep
- cDNA prep
- Primer (probe) design
- Analysis
- Training
- Complete RT-PCR from design to results
- Grant writing
- !Other

**6. For how many researchers have you provided service in the past year?**

- 0 to 10
- 11 to 25
- 26 to 75
- 76-100
- > 100

**7. How many "wells" do you run monthly? Please supply an average number. E.g., if you run 100 - 96 well plates/month, the answer would be 5001-10,000.**

- 0 to 1000
- 1001-5,000
- 5,001 to 10,000
- 10,001 to 50,000
- > 50,000

**8. How many people work in your lab performing real-time PCR? Please answer in terms of full time equivalents.**

- 0 to 1
- 1.5 to 2
- 2.5 to 3
- 3.5 to 4
- >4

**9. How many years of experience do you have doing real-time PCR?**

- less than 1 year
- 1 to 2 years
- 2 to 3 years
- 3 to 4 years
- 4 to 5 years
- > 5 years

## INSTRUMENTATION

**10.** What instrument(s) do you use for real-time PCR? Check all that apply.

- ABI 5700
- ABI 7000
- ABI 7700
- ABI 7900
- Bio-Rad iCycler
- Cepheid SmartCycler
- Corbett RotorGene
- MJ Research Opticon
- Roche LightCycler
- Stratagene MX 3000
- Stratagene MX 4000
- !Other

**11.** Do you use robotics?

- Yes
- No

**12.** If "no", proceed to question 16. If "yes", to load the plates into the instrument?

- Yes
- No

**13.** If "yes", manufacturer of robot?

- Zymark Twister (ABI)
- Corbett Research

!Other

**14. Do you use robotics to dispense reagents (set up reactions)?**

- Yes  
 No

**15. If "yes", manufacturer of robot? Check all that apply.**

- ABI 6700  
 BioMek  
 MWG  
 Tecan  
 !Other

**16. If you don't use robotics for dispensing reagents, what type of manual pipettor do you use?**

- 8 channel  
 12 channel  
 Single channel  
 Repeating pipettor  
 !Other

#### ASSAY DEVELOPMENT

**17. For what applications do you use real-time PCR? Check all that apply.**

- Gene expression - Primary validation/quantification  
 Gene expression - Confirmation of microarray data  
 Pathogen (viral/bacterial) detection/quantification  
 Biological diversity/contamination  
 Allelic discrimination/SNP analysis  
 Transgene detection/quantification  
 Zygosity testing  
 !Other

**18.** What type of assay do you use? Check all that apply.

- 5' nuclease Assays (E.g., Taqman)
- DNA dye binding Assay (E.g., SYBRgreen)
- Hybridization Assays (E.g., Molecular Beacons)
- Primer signaling Assay (E.g., LUX primers )
- !Other

**19.** What kind of primer/probes do you use? Check all that apply.

- Taqman
- SYBRgreen
- Molecular Beacons
- Scorpion
- LUX primers
- !Other

**20.** When you need to develop an assay, what method(s) do you use? Check all that apply.

- Design your own assays (primer and/or probe sets)
- Use primer and/or probe sets from literature
- Use commercial assays
- !Other:

**21.** Do you use multiplex assays?

- Always
- Sometimes
- Never

**22.** What type of software do you use to design your real-time PCR assays? Check all that apply.

- Primer Express (ABI)
- Primer 3 (MIT- free on the web)
- Beacon Designer (Premier Biosoft)
- Oligo (MBI)
- LightCycler Probe Design Software
- Not applicable

!Other

**23.** Do you ever make your own primers and/or probes for real-time PCR assays?

- Neither
- Primers only
- Probes only
- Primers and Probes

**24.** If you do not make all your own primers, from whom do you usually order your primers?

- ABI
- Biosearch
- Biosource
- IDT
- MWG
- Sigma-Genosys
- Synthegen
- !Other

**25.** If you do not make all your own probes, from whom do you usually order your probes?

- ABI
- Biosearch
- Biosource
- IDT
- MWG
- Sigma-Genosys
- Synthegen
- !Other

**26.** What dye(s) do you use for a reporter ? Check all that apply.

- FAM
- JOE
- HEX
- TAMRA

!Other

**27.** What quencher(s) do you use ? Check all that apply.

- TAMRA  
 BHQ-1,2,3  
 QSY  
 Not applicable  
 !Other

**28.** How do you validate the real-time PCR assays that you design? Check all that apply.

- Determine PCR efficiency  
 Run agarose gel  
 Run SYBRgreen Melt curve  
 Sequence amplicon  
 Check for genomic amplification  
 Not applicable  
 !Other

**29.** Do you run replicate wells/sample?

- Yes  
 No

**30.** If "yes", how many replicates do you run?

- Duplicates  
 Triplicates  
 Not applicable  
 !Other

**31.** Do you include controls on each plate?

- Yes  
 No

**32. What type of controls do you use? Check all that apply.**

- No Template control (NTC) to check for contamination
- Minus RT (-RT) or RNA control to check for genomic DNA contamination
- Internal Positive control (IPC) to check for PCR inhibition
- Not applicable
- !Other

## ASSAYS

**33. What type of template(s) do you use? Check all that apply.**

- Genomic DNA
- cDNA (from RNA)
- Plasmid DNA
- Sample is provided
- !Other

**34. How do you purify RNA for real-time PCR assays?**

- Phenol-based isolation method
- Column/matrix based isolation method
- Detergent based isolation method
- Combination of techniques
- DNA/RNA is provided
- !Other

**35. How do you purify your DNA for real-time PCR assays?**

- Phenol-based isolation method
- Column/matrix based isolation method
- Detergent based isolation method
- Combination of techniques
- DNA/RNA is provided
- !Other

**36.** When isolating templates, what do you isolate?

- DNA or RNA only
- DNA and RNA together
- DNA and/or RNA and protein
- DNA/RNA is provided
- !Other

**37.** Are the samples DNase I treated?

- Always
- Sometimes
- Never
- Sample is provided

**38.** Do you do your RT/PCR in one reaction (one-step) or sequentially in separate master mixes (two-step)? Check both if applicable.

- One Step
- Two step
- Not applicable

**39.** What do you use for a reverse transcription primer?

- Oligo (dT)
- Random primers
- Random primers and oligo(dT) mixed
- Gene-specific primer
- Sample is provided

**40.** Which source of reverse transcriptase do you use?

- MMLV
- AMV
- TTh
- Not applicable
- !Other

**41. At what temperature(s) do you run the RT reaction? Check all that apply.**

- 37 degrees C
- 42 degrees C
- 50 degrees C
- 55 degrees C
- 60 degrees C
- Not applicable
- !Other

**42. Do you use a heat activated Taq enzyme in your real-time PCR reaction?**

- Yes
- No

**43. What type of "master mix" do you use for real-time PCR?**

- ABI 2X Master Mix
- ABI TaqMan Core PCR Reagent Mix
- ABI 2X SYBRgreen Master Mix
- ABI SYBRgreen Core PCR Reagent Mix
- LTI Platinum Quantitative PCR SuperMix-UDG
- Invitrogen iQ SUPERMIX
- Bio-Rad Brilliant® QPCR Master Mix
- Stratagene Brilliant® QPCR Master Mix
- Sigma 2X SYBRgreen Master Mix
- "Homemade"
- !Other

**44. What Taq enzyme do you use in your real-time PCR reactions?**

- AmpliTaq Gold™ (ABI)
- Platinum Taq™ (LTI)
- HotMaster™ (Eppendorf)
- Jumpstart Taq™ (Sigma)
- TaKaRa Ex Taq™ (Takara)
- BD TITANIUM™ Taq DNA (Clontech)
- !Other

**45. What reference dye do you use in the real-time PCR reaction?**

- ROX
- Blue 636
- No reference dye used
- Not applicable
- !Other

**46. What volume/well do you use for your real-time PCR reactions?**

- 5 ul
- 10 ul
- 15 ul
- 20 ul
- 25 ul
- 50 ul
- !Other

**47. Do you purchase nuclease-free water for your assays?**

- Yes
- No

#### ANALYSIS

**48. How do you analyze your data. Check all that apply.**

- Standard Curve
- Delta delta Ct method
- Relative Expression Software Tool (REST/REST-XL)
- Q-Gene
- Not applicable
- !Other

**49. What do you use as a standard for your standard curves? Check all that apply.**

- Oligonucleotide
- PCR product

- Plasmid, linearized
- In vitro transcribed RNA
- Purified genomic DNA
- No standard curve is run
- !Other

**50.** What do you use for a normalization gene(s)? Check all that apply.

- 18S rRNA
- 28S rRNA
- GAPDH
- B-actin
- B-2 microglobulin
- GUS
- HPRT
- Cyclophilin
- ApoB
- No normalization gene is used
- !Other

**51.** Do you measure PCR efficiency in each assay?

- Always
- Sometimes
- Never
- As part of the validation process

**52.** What range of PCR efficiency is considered acceptable? PCR efficiency  $E = 10^{[-1/\text{slope}] - 1}$

- >95% (slope is less than -3.45)
- >90% (slope is less than -3.60)
- >85% (slope is less than -3.75)
- >80% (slope is less than -3.9)
- >75% (slope is less than -4.10)
- Not applicable
- !Other

**GENERAL**

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