

## Survey Design

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# Nucleic Acid Research Group 2007 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 February 2, 2007. Results will be presented at the ABRF 2007 annual meeting in Tampa Bay, FL, Mar 31-Apr 3, 2007 and will be available "on line" by May 1, 2007. 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 Kevin Knudtson (kevin-knudtson@uiowa.edu), if you have any questions or problems.

FACILITY

**1. Which of the following best describes your institution?**

- Academic (University/Hospital)
- Government
- Commercial/Industrial
- Private Research Foundation
- !Other

**2. Geograpic location?**

- Africa
- Asia
- Australia/New Zealand
- Europe
- South America
- USA/Canada

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

- Yes
- No

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

- Yes
- No

**5. 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

**6.** 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

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

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

**8.** 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

**9.** 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

**10.** 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**

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

- ABI 5700
- ABI 7000/7300/7500
- ABI 7700
- ABI 7900HT
- Bio-Rad iCycler/MyiQ/iQ5
- Bio-Rad (MJ Research) Chromo4/Opticon/Opticon2/MiniOpticon
- BioGene InSyte/Synchron
- Cepheid SmartCycler
- Corbett RotorGene 6000
- DNA Technology DT-322
- Exicycler
- Eppendorf RealPlex
- Idaho Technology Rapid Cyclor
- Roche LightCycler
- Roche LightCycler 480
- Stratagene Mx4000/Mx3000P/MX3005P
- Techne Quantica System
- !Other

**12.** Do you use robotics to load plates into the instrument?

- Yes  
 No

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

- Caliper (Zymark) Twister (ABI 9700HT loader)  
 Corbett Research  
 !Other

**14.** Do you use liquid handling robots to dispense reagents (set up reactions)?

- Yes  
 No

**15.** If "yes", what type of tips do you primarily use to set up your reactions?

- Disposable  
 Fixed (non-disposable)  
 !Other (Describe)

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

- ABI 6700  
 Beckam BioMek Series  
 MWG  
 Tecan  
 !Other

**17.** 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

**18.** 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
- Zygoty testing
- MicroRNA quantification
- Methylation detection
- ChIP Assays
- !Other

**19.** 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 )
- Plexor
- Scorpions
- Amplifluor
- !Other

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

- Taqman (hydrolysis) probes
- DNA intercalators (E.g., SYBRgreen)
- Molecular Beacons
- Scorpion
- LUX primers
- Plexor
- Ampliflour
- LNA (E.g., Roche Universal Probe Library)
- !Other:

**21.** 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:

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

- Always
- Sometimes
- Never

**23.** 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
- SciTools (Integrated DNA Technologies)
- Vector NTI (Invitrogen)
- RealTimeDesign (Biosearch Technologies)
- Not applicable
- !Other:

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

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

**25.** 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
- Invitrogen
- Operon
- Prologo
- In house core facility
- !Other

**26.** 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
- Invitrogen
- Operon
- Eurogentec
- !Other

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

- FAM
- JOE
- HEX
- TAMRA
- VIC
- CY3
- CY5
- TET
- CAL Fluor Orange/Red
- Oregon Green
- ROX
- Texas Red
- Yakima Yellow
- SYBR dyes
- !Other

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

- TAMRA
- BHQ-1,2,3
- QSY
- Iowa Black
- DABCYL
- MGB non-fluorescent quencher
- Not applicable
- !Other

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

- Determine PCR efficiency
- Run agarose gel
- Run melt curve
- Sequence amplicon
- Check for genomic amplification
- Agilent Bioanalyzer/BioRad Expirion

- Assess sensitivity and specificity (E.g., dynamic range)
- Not applicable
- !Other

**30.** Do you run technical replicate wells/sample?

- Yes
- No

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

- Duplicates
- Triplicates
- Not applicable
- !Other

**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
- External (exogenous) positive control
- External (exogenous) negative control
- None/Not applicable
- !Other

#### ASSAYS

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

- Phenol-based isolation method
- Column/matrix based isolation method
- Detergent based isolation method

- Magnetic bead-based method
- DNA/RNA is provided
- Combination of techniques
- !Other

**34.** Are the RNA samples DNase I treated?

- Always
- Sometimes
- Never
- Sample is provided

**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
- Magnetic bead-based method
- DNA/RNA is provided
- Combination of techniques
- !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.** 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

**38.** 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

**39.** If you use random primers, what length do you use?

- Hexamers
- Septamers
- Octamers
- Nonamers
- Decamers
- Mixture
- !Other

**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
- 48 degrees C

- 50 degrees C
- 55 degrees C
- 60 degrees C
- 70 degrees C or greater
- 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 when using 96-well plates?

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

**47.** What volume/well do you use for your real-time reactions when using 384-well plates?

- 2 ul
- 4 ul
- 5 ul
- 8 ul
- 10 ul
- 15 ul
- 20 ul
- 25 ul
- 50 ul
- !Other

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
- LinRegPCR (Ramakers et al, Neurosci Lett. 2003)
- DART-PCR (Pierson et al, NAR 2003)
- 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
- Pooled cDNA
- Commercial RNA
- 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 do you consider 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**

**53.** Do you use any type of pre-developed assay for real-time PCR?

- Yes
- No

**54.** If "yes", what assays have you used? Check all that apply.

- Assays-on-Demand (ABI)
- Assays-by-Design (ABI)
- TaqMan Low Density Arrays (ABI)
- Qiagen Pre-developed Assays
- RT2 Profiler PCR Array (SuperArray)
- Roche Universal Probe Library
- Not applicable
- !Other

THANK YOU!!!! We appreciate your participation in this survey.

**55.** Do you have any suggestions about what questions should have been asked?

**56.** Do you have any suggestions for future studies about real-time PCR? Please list them.

**57.** Other Comments:

You are finished with the survey. Please click one time on submit.

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