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, 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 to 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 to 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?
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
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
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
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 \( E = 10^{(-1/slope)} - 1 \)
- >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