

[Help](#)

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 57 questions. The survey should take less than 15 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)	 56.2%	(86)
Government	 16.3%	(25)
Commercial/Industrial	 17.0%	(26)
Private Research Foundation	 4.6%	(7)
!Other Contract Research Organization	 0.7%	(1)
!Other Non-profit making member funded research association	 0.7%	(1)
!Other Non-profit research Institute	 0.7%	(1)
!Other Private Hospital Lab	 0.7%	(1)
!Other Public Hospital	 0.7%	(1)
!Other research institute	 0.7%	(1)

TOTAL		98.0%	153
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2. Geographic location?

Africa		0.7%	(1)
Asia		7.2%	(11)
Australia/New Zealand		3.3%	(5)
Europe		34.6%	(53)
Mexico/South America		2.0%	(3)
Canada		1.3%	(2)
USA		50.3%	(77)
TOTAL		99.3%	153

3. Are you a member of a core facility?

Yes		39.2%	(60)
No		58.8%	(90)
TOTAL		98.0%	153

4. If "no", proceed to Question 7. If "yes", do you offer services other than real-time qPCR?

Yes		33.3%	(51)
No		5.2%	(8)
TOTAL		38.6%	153

5. If "yes" to question 4, what service(s) do you provide beside real-time qPCR? Check all that apply.







DNA synthesis		3.9%	(6)
DNA sequencing		19.6%	(30)
Microarray		13.1%	(20)
Genotyping (Fragment analysis, SNP, STR)		19.0%	(29)
Mass spec		5.9%	(9)
!Other Analysis of nucleic acid integrity		0.7%	(1)
!Other cell sorting, confocal, HCS		0.7%	(1)
!Other cell sorting, confocal, high content screening		0.7%	(1)
!Other DNA Extraction, banking, quantitation		0.7%	(1)
!Other DNA purification		0.7%	(1)
!Other electrophoresis (2D), transfers, gel and blot imaging, spectroscopy,		0.7%	(1)
!Other end-point PCR		0.7%	(1)
!Other Flow cytometry		0.7%	(1)
!Other IHC, LCM		0.7%	(1)

!Other imaging, DHPLC, nucleic acid extractions, experimental design consultation	0.7%	(1)
!Other PCR	0.7%	(1)
!Other PCR and semi-quantitative PCR	0.7%	(1)
!Other Protein Analysis	0.7%	(1)
!Other proteomics	0.7%	(1)
!Other Recombinant Protein production	0.7%	(1)
!Other RNA bioanalyzer	0.7%	(1)
!Other RNA Extractions, RNA QC with Bioanalyzer	0.7%	(1)
!Other RNA/DNA extraction	0.7%	(1)
!Other robotic liquid handling; Bioanalyzer; pyrosequencing	0.7%	(1)
!Other Tissue Microarray	0.7%	(1)
!Other transgenics, flow cytometry	0.7%	(1)
!Other We don't provide services, only equipment and training to use it.	1.3%	(2)


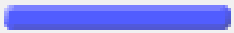



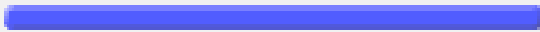
6. What level of real-time PCR service do you offer? Check all that apply.

Access to Machine only	22.2%	(34)
PCR reaction only	11.1%	(17)
RNA/DNA prep	17.6%	(27)
cDNA prep	17.6%	(27)
Primer (probe) design	24.2%	(37)
Analysis	26.8%	(41)
Set up and run RT-PCR or PCR	25.5%	(39)
Complete RT-PCR from design to results	25.5%	(39)
Training	27.5%	(42)
Grant writing	7.2%	(11)
!Other clinical routine diagnostics	0.7%	(1)
!Other collaboration	0.7%	(1)
!Other Software	0.7%	(1)


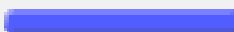



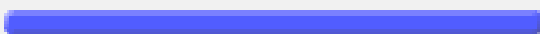
7. For how many researchers have you provided service in the past year?

0 to 10		47.7%	(73)
11 to 25		18.3%	(28)
26 to 75		11.8%	(18)
76-100		1.3%	(2)
>100		3.3%	(5)
TOTAL		82.4%	153






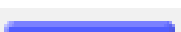


8. How many "wells" do you run monthly? Please supply an average number. E.g., if you run 100 - 96 well plates/month, the answer would be 5001-10,000.

0 to 1000		41.2%	(63)
1001-5,000		38.6%	(59)
5,001 to 10,000		9.8%	(15)
10,001 to 50,000		6.5%	(10)
>50,000		1.3%	(2)
TOTAL		97.4%	153




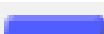




9. How many people work in your lab performing real-time PCR? Please answer in terms of full time equivalents.

0 to 1		28.8%	(44)
1.5 to 2		40.5%	(62)
2.5 to 3		14.4%	(22)
3.5 to 4		3.3%	(5)
>4		10.5%	(16)
TOTAL		97.4%	153

10. How many years of experience does the lab manager/director have performing real-time qPCR?





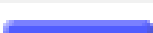
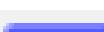













less than 1 year		15.0%	(23)
1 to 2 years		11.8%	(18)
2 to 3 years		12.4%	(19)
3 to 4 years		5.9%	(9)
4 to 5 years		14.4%	(22)
>5 years		28.1%	(43)
No Lab Manager/Director		9.8%	(15)
TOTAL		97.4%	153

11. On average, how many years of experience do you or the support staff have performing real-time qPCR?

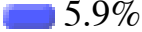


less than 1 year	 7.8%	(12)
1 to 2 years	 23.5%	(36)
2 to 3 years	 18.3%	(28)
3 to 4 years	 15.0%	(23)
4 to 5 years	 14.4%	(22)
>5 years	 15.0%	(23)
No support staff	 3.3%	(5)
TOTAL	 97.4%	153

INSTRUMENTATION

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

ABI 5700	 3.9%	(6)
ABI 7000	 10.5%	(16)
ABI 7300/7500	 20.3%	(31)
ABI 7700	 11.1%	(17)
ABI 7900HT	 24.2%	(37)
Bio-Rad iCycler/MyiQ/iQ5	 17.0%	(26)
Bio-Rad (MJ Research) Chromo4/Opticon/Opticon2/MiniOpticon	 9.2%	(14)
BioGene InSyte/Synchron		(0)
Cepheid SmartCycler	 6.5%	(10)
Corbett RotorGene 6000	 5.9%	(9)
DNA Technology DT-322	 0.7%	(1)
Eppendorf RealPlex	 3.3%	(5)
Exicycler		(0)
Idaho Technology Rapid Cyclor	 0.7%	(1)
Roche LightCycler	 13.7%	(21)
Roche LightCycler 480	 5.9%	(9)
Stratagene Mx4000/Mx3000P/MX3005P	 15.7%	(24)
Techne Quantica System	 0.7%	(1)
!Other Corbett RotorGene 3000	 3.3%	(5)
!Other Corbett rotorgene 3000	 0.7%	(1)
!Other Rotor Gene 3000	 0.7%	(1)




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

Yes		5.9%	(9)
No		92.2%	(141)
TOTAL		98.0%	153


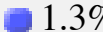



14. If "yes", manufacturer of robot?

Caliper (Zymark) Twister (ABI 7900HT, LC480 loader)		5.2%	(8)
Corbett Research			(0)
!Other eppendorf 7075		0.7%	(1)

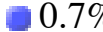
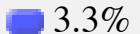
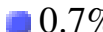









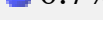

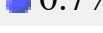
15. Do you use liquid handling robots to dispense reagents (set up reactions)?

Yes		14.4%	(22)
No		79.7%	(122)
TOTAL		94.1%	153

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

Disposable		18.3%	(28)
Fixed (non-disposable)		1.3%	(2)
!Other (Describe) both		0.7%	(1)
!Other (Describe) Both disposable & fixed		0.7%	(1)
TOTAL		20.9%	153

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


ABI 6700		0.7%	(1)
Beckman Biomek Series		3.3%	(5)
MWG		0.7%	(1)
Tecan		2.0%	(3)
!Other Caliper ALH3000		0.7%	(1)
!Other corbett		0.7%	(1)
!Other Corbett Research		0.7%	(1)
!Other Corbett robotics		0.7%	(1)
!Other eppendorf		1.3%	(2)
!Other Eppendorf		0.7%	(1)
!Other Eppendorf EpMotion		0.7%	(1)
!Other Hamilton		0.7%	(1)
!Other Matrix		0.7%	(1)
!Other packard		0.7%	(1)
!Other Perkin Elmer		0.7%	(1)

!Other Perkin Elmer Multi-Probe HT2 Low Volume (nL)		0.7%	(1)
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!Other Qiagen		0.7%	(1)
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18. If you don't use robotics for dispensing reagents, what type of manual pipettor do you use?

8 channel		28.8%	(44)
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12 channel		9.2%	(14)
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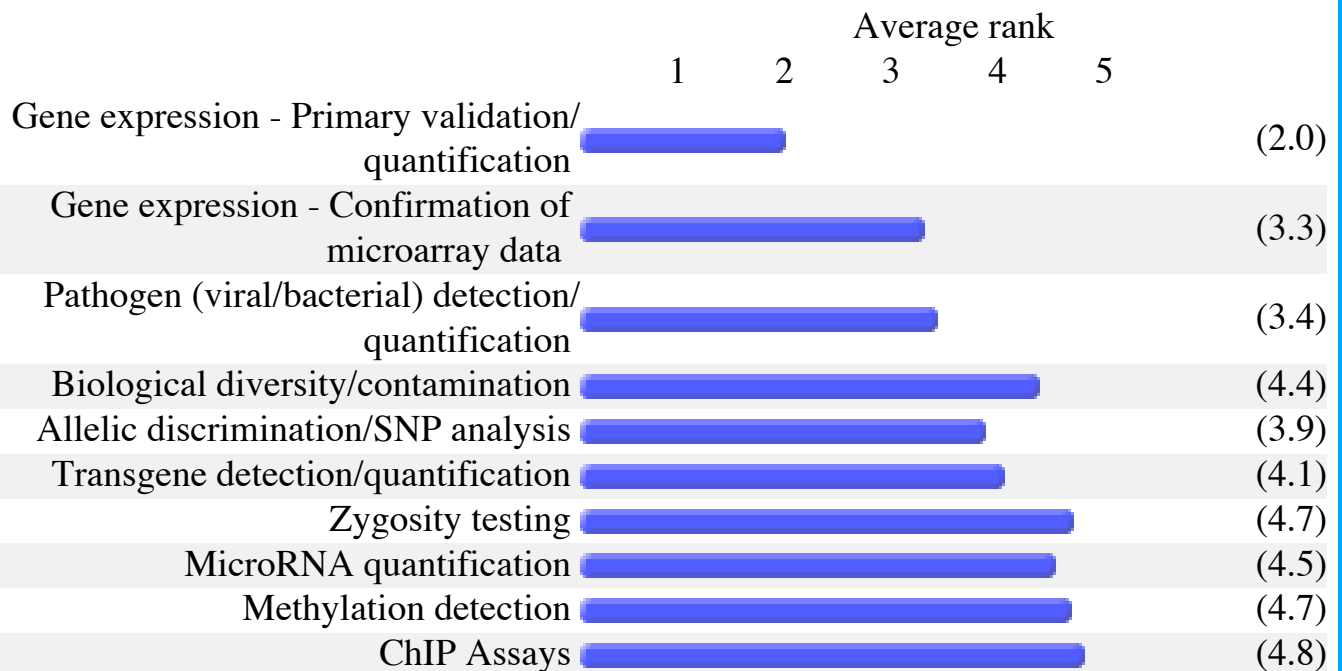
Single channel		66.7%	(102)
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Repeating pipettor		29.4%	(45)
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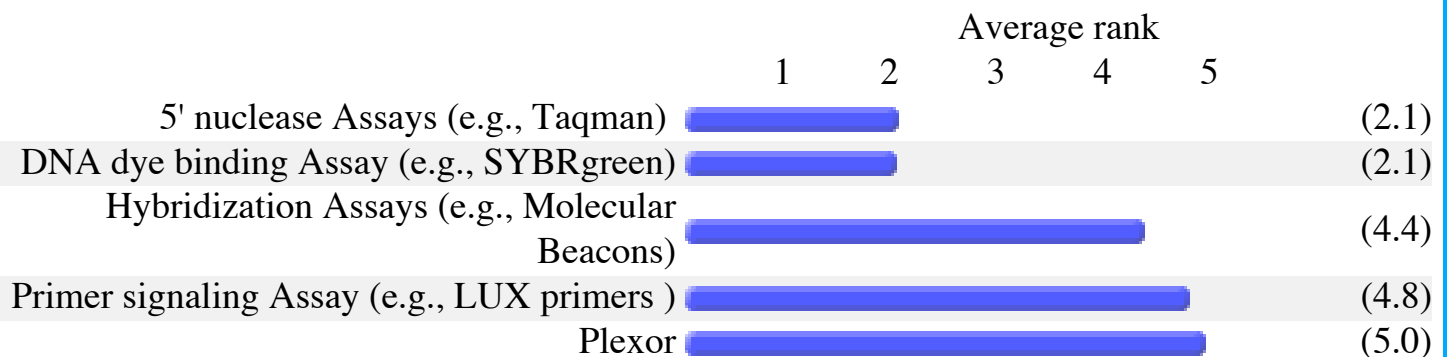
!Other for cDNA synthesis		0.7%	(1)
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ASSAY DEVELOPMENT

19. When you perform real-time PCR, with what frequency do you perform each application listed below ? Frequency is defined as the relative number of assays (runs) and not the number of wells. 1=Most frequent, 2=Frequently, 3=Occasionally, 4=Rarely, and 5=Never.



20. When you perform realtime PCR, with what frequency do you perform each assay type listed below ? Frequency is defined as the relative number of assays (runs) and not the number of wells. 1=Most frequent, 2=Frequently, 3=Occasionally, 4=Rarely, and 5=Never.



Scorpions		(4.9)
Amplifluor		(4.9)
Eclipse		(4.9)
LNA (e.g., Roche Universal Probe Library)		(4.5)

21. When you need to develop an assay, with what frequency do you use the following methods? Frequency is defined as the relative number of assays. 1=Most frequent, 2=Frequently, 3=Occasionally, 4=Rarely, and 5=Never.

	Average rank					
	1	2	3	4	5	
Design your own assays (primer and/or probe sets)						(1.7)
Use primer and/or probe sets from literature						(2.8)
Use commercial assays (pre-designed)						(3.2)
Use manufacturer's design from sequence you provide						(4.2)

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

Primer Express (ABI)		50.3%	(77)
Primer 3 (MIT- free on the web)		40.5%	(62)
Beacon Designer (Premier Biosoft)		15.7%	(24)
Oligo (MBI)		5.2%	(8)
LightCycler Probe Design Software		4.6%	(7)
SciTools (Integrated DNA Technologies)		6.5%	(10)
Vector NTI (Invitrogen)		7.2%	(11)
RealTimeDesign (Biosearch Technologies)		6.5%	(10)
DesignMyProbe.com (Sigma-Genosys)		1.3%	(2)
Not applicable		4.6%	(7)
!Other "by hand" without software		0.7%	(1)
!Other AlleleID		0.7%	(1)
!Other amplify		0.7%	(1)
!Other DNSstar		0.7%	(1)
!Other DS Gene		0.7%	(1)
!Other HUSAR EMBL		0.7%	(1)
!Other IDT website, Oligotech		0.7%	(1)
!Other MacVector (Accelrys)		0.7%	(1)
!Other mfold web site especially for unimolecular scorpion probe		0.7%	(1)
!Other Net Primer		0.7%	(1)

!Other oligo explorer	0.7%	(1)
!Other oligo, jellyfish, internet	0.7%	(1)
!Other PerlPrimer	0.7%	(1)
!Other Primer Premire	0.7%	(1)
!Other probe finder for universal probe library	0.7%	(1)
!Other probelibrary design in Roche	0.7%	(1)
!Other Roche universal probe lib	0.7%	(1)
!Other Roche Universal Probe Library software (https://www.roche-applied-science.com/sis/rtPCR/upl/index.jsp)	0.7%	(1)
!Other Several Linux Apps	0.7%	(1)
!Other universal probe library	0.7%	(1)

23. How often do you perform multiplex assays?

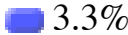





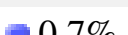

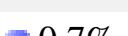

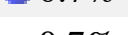
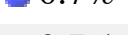
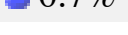
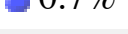
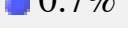
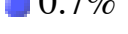
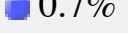
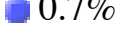
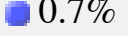
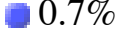
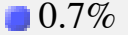
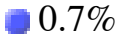
Always	6.5%	(10)
Most of the time	11.8%	(18)
Sometime	20.9%	(32)
Rarely	22.2%	(34)
Never	36.6%	(56)
TOTAL	98.0%	153

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






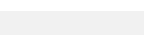




Neither	83.7%	(128)
Primers only	5.2%	(8)
Probes only		(0)
Primers and Probes	7.8%	(12)
TOTAL	96.7%	153

25. If you do not make all your own primers, from whom do you currently order your primers?

ABI	17.0%	(26)
Biosearch	3.3%	(5)
IDT	26.8%	(41)
MWG	18.3%	(28)
Sigma-Genosys	19.6%	(30)
Synthegen		(0)
Invitrogen	15.0%	(23)
Operon	10.5%	(16)

Proligo	 3.3%	(5)
Eurogentec	 4.6%	(7)
In house core facility	 5.2%	(8)
!Other Biomers	 0.7%	(1)
!Other DNA technology Denmark	 0.7%	(1)
!Other DNA Technology, Denmark	 0.7%	(1)
!Other DNA Technology, TAGC	 0.7%	(1)
!Other Fluorescentric, SimpliGen	 0.7%	(1)
!Other Generi Biotech	 0.7%	(1)
!Other Germany based smaller companies	 0.7%	(1)
!Other isogen	 0.7%	(1)
!Other Local supplier	 0.7%	(1)
!Other Metabion	 0.7%	(1)
!Other Oligomer, Finland	 0.7%	(1)
!Other PRIMM	 0.7%	(1)
!Other TCGA or Microsynth	 0.7%	(1)
!Other Thermo Scientific	 0.7%	(1)
!Other TIB MOLBIOL	 0.7%	(1)
!Other TIB MolBiol	 0.7%	(1)
!Other TIB/IT BioChem	 0.7%	(1)
!Other tibmolbiol	 0.7%	(1)
!Other VBC Genomics	 0.7%	(1)

26. If you do not make all your own probes, from whom do you currently order your probes?

ABI	 36.6%	(56)
Biosearch	 10.5%	(16)
IDT	 13.7%	(21)
MWG	 9.8%	(15)
Sigma-Genosys	 10.5%	(16)
Synthegen		(0)
Invitrogen	 6.5%	(10)
Operon	 7.8%	(12)
Eurogentec	 4.6%	(7)
In house core facility	 2.6%	(4)
!Other Biolegio	 0.7%	(1)

!Other CHEMICON	0.7%	(1)
!Other DNA technology; TIB MOLBIOL	0.7%	(1)
!Other don't use at this time	0.7%	(1)
!Other Fluorescentric	0.7%	(1)
!Other Fluoresentric, SimpliGen	0.7%	(1)
!Other Generi Biotech	0.7%	(1)
!Other METABION	0.7%	(1)
!Other Microsynth	0.7%	(1)
!Other N/A	0.7%	(1)
!Other no probe design	0.7%	(1)
!Other PROLIGO	0.7%	(1)
!Other Proligo scorpion	0.7%	(1)
!Other Qiagen Quantiprobos	0.7%	(1)
!Other Roce uni probe lib	0.7%	(1)
!Other Roche universal probe library	0.7%	(1)
!Other ROCHE UPL	0.7%	(1)
!Other Roche UPL	0.7%	(1)
!Other Same as above	0.7%	(1)
!Other TIB & IT BioChem	0.7%	(1)
!Other tib molbiol	0.7%	(1)
!Other TIB MOLBIOL	0.7%	(1)
!Other TIB MolBiol	1.3%	(2)
!Other tibmolbiol	0.7%	(1)
!Other VBC Genomics	0.7%	(1)

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

FAM	79.7%	(122)
JOE	19.6%	(30)
HEX	24.2%	(37)
TAMRA	13.1%	(20)
VIC	30.7%	(47)
CY3	5.9%	(9)
CY5	14.4%	(22)
TET	9.8%	(15)
CAL Fluor Orange/Red	3.3%	(5)
Oregon Green		(0)

ROX	18.3%	(28)
Texas Red	5.2%	(8)
Yakima Yellow	2.0%	(3)
SYBR dyes	55.6%	(85)
!Other Alexa 546	0.7%	(1)
!Other LC Red Dyes	0.7%	(1)
!Other Lightcycler Red 705 Fluorescein	0.7%	(1)
!Other NED	1.3%	(2)
!Other Ned	0.7%	(1)
!Other PULSAR	0.7%	(1)
!Other Pulsar 650	0.7%	(1)
!Other Quasar 570, Quasar 670	0.7%	(1)
!Other SYTO9	0.7%	(1)

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

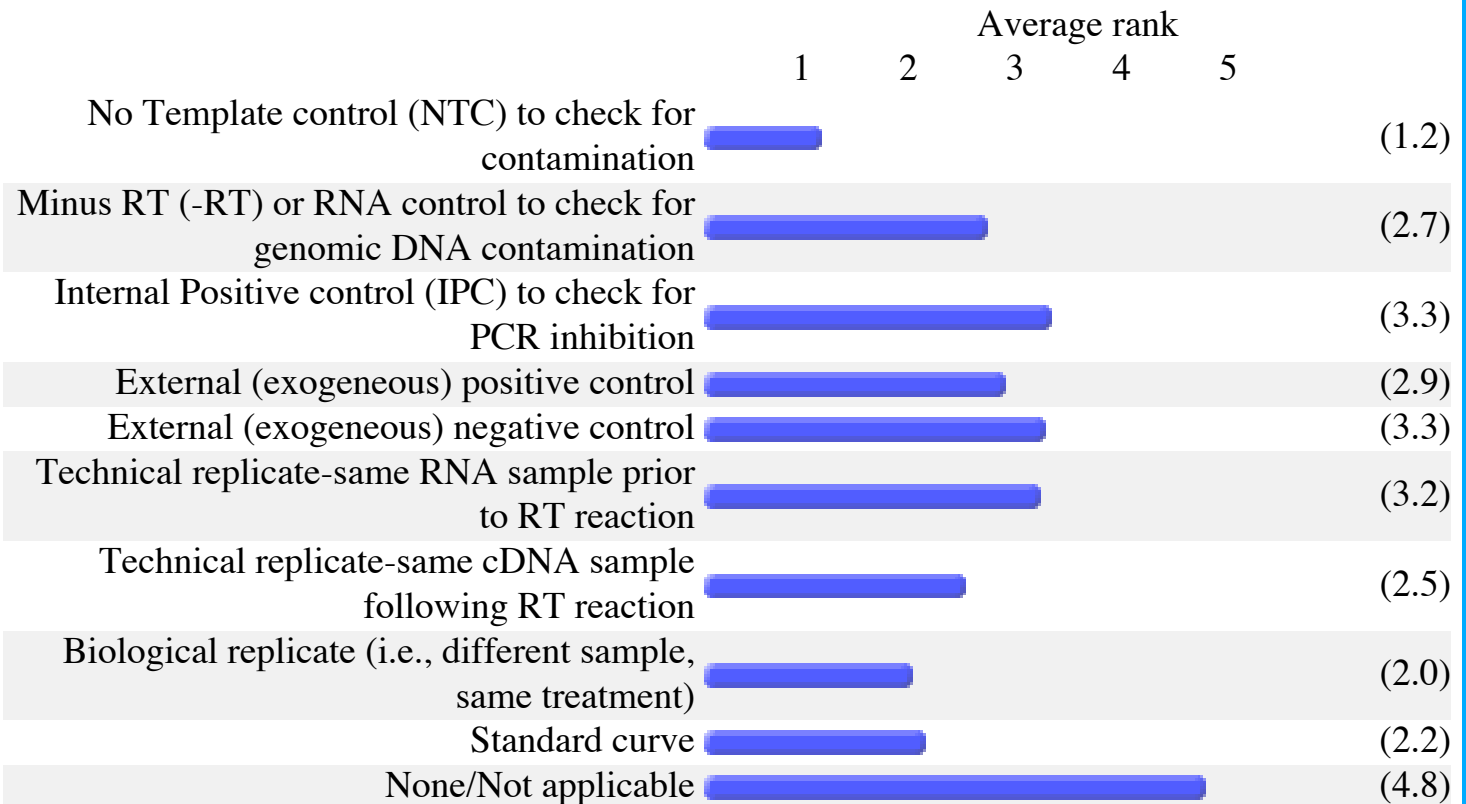
TAMRA	44.4%	(68)
BHQ-1,2,3	43.1%	(66)
QSY		(0)
Iowa Black	2.0%	(3)
DABCYL	4.6%	(7)
MGB non-fluorescent quencher	30.7%	(47)
Not applicable	14.4%	(22)
!Other ?	0.7%	(1)
!Other dark quencher in probe library	0.7%	(1)

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

Determine PCR efficiency	86.9%	(133)
Run agarose/polyacrylamide gel	53.6%	(82)
Run melt curve	68.6%	(105)
Sequence amplicon	28.1%	(43)
Check for genomic amplification	37.9%	(58)
Agilent Bioanalyzer/BioRad Expirion	17.6%	(27)
Assess sensitivity (e.g., dynamic range)	45.8%	(70)
Not applicable	1.3%	(2)
!Other as per ICH Q2R	0.7%	(1)

!Other check raw amplification curves using known, quality, gDNA samples for end-point bi-allelic snp assays (only)	0.7%	(1)
!Other check specificity	0.7%	(1)
!Other check specificity; check for PCR inhibition	0.7%	(1)
!Other Fluorescent Validation Service	0.7%	(1)
!Other Interference	0.7%	(1)
!Other intra/interassay variability; extensive testing for the appropriate reference gene, testing of preanalytical conditions (e.g. storage time and -temperature of blood specimens)	0.7%	(1)
!Other RNA transcripts for sensitivity	0.7%	(1)
!Other sequence specificity against non-human genomes	0.7%	(1)

30. When performing a realtime qPCR assay, with what frequency do you (or your users) include the following controls/replicates? Frequency is defined as the relative number of assays. 1=Always, 2=Most of the time, 3=Occasionally, 4=Rarely, and 5=Never.



ASSAYS

31. What method do you use, or recommend your users to use, to purify RNA for real-time PCR assays?

Phenol-based isolation method	27.5%	(42)
Column-/matrix-based isolation method	58.8%	(90)
Detergent-based isolation method	3.9%	(6)
Magnetic bead-based method	7.8%	(12)
DNA/RNA is provided	7.2%	(11)
Phenol- followed by column-based method	16.3%	(25)
Combination of techniques	10.5%	(16)
!Other dont use RNA	0.7%	(1)
!Other no experience in this field	0.7%	(1)
!Other no gene expression done at our lab	0.7%	(1)
!Other not applicable	0.7%	(1)
!Other trizol	0.7%	(1)
!Other ZenLys	0.7%	(1)

32. Are the RNA samples DNase I treated?

Always	39.2%	(60)
Sometimes	35.3%	(54)
Never	13.7%	(21)
Sample is provided	2.6%	(4)
TOTAL	90.8%	153

33. What method do you use, or recommend your users to use, to purify DNA for real-time PCR assays?

Phenol-based isolation method	12.4%	(19)
Column/matrix based isolation method	60.1%	(92)
Detergent based isolation method	7.8%	(12)
Magnetic bead-based method	7.2%	(11)
DNA/RNA is provided	4.6%	(7)
Phenol- followed by column-based method	6.5%	(10)
Combination of techniques	9.8%	(15)
!Other DNazol	0.7%	(1)
!Other Do not use DNA	0.7%	(1)
!Other don't do this	0.7%	(1)
!Other don't use DNA	0.7%	(1)
!Other EtOH Pptn	0.7%	(1)

!Other Gentra Kit	0.7%	(1)
!Other NA	0.7%	(1)
!Other no experience in this field	0.7%	(1)
!Other No need to purify	0.7%	(1)
!Other None	0.7%	(1)
!Other PROTEINASE K	0.7%	(1)
!Other Proteinase K	0.7%	(1)
!Other Wako DNA extractor	0.7%	(1)

34. When isolating templates, what do you isolate?

DNA or RNA only	81.7%	(125)
DNA and RNA together	15.7%	(24)
DNA and/or RNA and protein	6.5%	(10)
DNA/RNA is provided	6.5%	(10)
!Other DNA only	0.7%	(1)
!Other do not perform this step	0.7%	(1)
!Other done by customer	0.7%	(1)
!Other NA	0.7%	(1)
!Other protein	0.7%	(1)
!Other we don't isolate templates.	0.7%	(1)











35. 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	30.1%	(46)
Two step	75.8%	(116)
Not applicable	6.5%	(10)






















36. What do you use for a reverse transcription primer?

Oligo(dT)	32.7%	(50)
Random primers	35.3%	(54)
Random primers and oligo(dT) mixed	20.9%	(32)
Gene-specific primer	32.7%	(50)
Sample is provided	3.9%	(6)




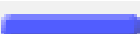










37. If you use random primers, what length do you use?

Hexamers		41.2%	(63)
Octamers		4.6%	(7)
Nonamers		4.6%	(7)
Decamers		2.0%	(3)
>10-mer		1.3%	(2)
!Other ABI mix		0.7%	(1)
!Other don't know (contained in iScript mix)		0.7%	(1)
!Other polyT		0.7%	(1)
!Other vendor supplied mix		0.7%	(1)
!Other whatever is supplied in iScript kit		0.7%	(1)




38. Which source of reverse transcriptase do you use?

MMLV		62.7%	(96)
AMV		13.7%	(21)
TTh		3.9%	(6)
Not applicable		4.6%	(7)
!Other BioRad Kit		0.7%	(1)
!Other iScript (bioRad)		0.7%	(1)
!Other kit		0.7%	(1)
!Other mix		0.7%	(1)
!Other MuLV in kit		0.7%	(1)
!Other Omniscript		2.0%	(3)
!Other omniscript		0.7%	(1)
!Other qiagen		0.7%	(1)
!Other Qiagen Omniscript		0.7%	(1)
!Other recombinant E. coli Roche Transcriptor		0.7%	(1)
!Other recombinant RTase from E. coli		0.7%	(1)
!Other sensiscript Qiagen		0.7%	(1)
!Other superscript		1.3%	(2)
!Other superscript 3		0.7%	(1)
!Other Superscript II		0.7%	(1)
!Other superscript III		1.3%	(2)
!Other superscript III which is an MMLV		0.7%	(1)









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

37 degrees C		20.9%	(32)
42 degrees C		41.2%	(63)
48 degrees C		6.5%	(10)
50 degrees C		22.2%	(34)
55 degrees C		7.8%	(12)
60 degrees C		5.9%	(9)
65 degrees C or greater		3.3%	(5)
Not applicable		2.6%	(4)
!Other 39 degrees C		0.7%	(1)
!Other 44		0.7%	(1)
!Other 45		0.7%	(1)
!Other 5 degrees C lower than Tm		0.7%	(1)
!Other 70deg		0.7%	(1)
!Other depending on assay		0.7%	(1)









40. Do you use a "Hot Start" DNA polymerase (Taq) enzyme in your real-time PCR reaction?

Yes		84.3%	(129)
No		11.1%	(17)
TOTAL		95.4%	153

41. What type of "master mix" do you use for real-time PCR? Check all that apply.

Commercial 2X Master Mix		54.9%	(84)
Commercial Core PCR Reagent Kit		9.8%	(15)
Commercial 2X SYBRgreen Master Mix		61.4%	(94)
Commercial SYBRgreen Core PCR Reagent Kit		7.2%	(11)
Homemade Reaction mix for use with probes		11.8%	(18)
Homemade reaction mix for use with SYBR Green		12.4%	(19)
Commercial Master Mix for FAST cycling protocols		3.9%	(6)
Commercial SYBR Green Master Mix for FAST cycling protocols		4.6%	(7)

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

ROX		60.1%	(92)
Blue 636			(0)
No reference dye used		24.2%	(37)
Not applicable		9.2%	(14)
!Other FAM		0.7%	(1)
!Other Fluoresceine		0.7%	(1)
!Other Fluoresceine on the iCycler, no dye on LC480		0.7%	(1)
!Other if one is used: Rox, mostly none reference dye is used		0.7%	(1)
TOTAL		96.1%	153

43. What reaction volume(s), in microliters, do you usually use when performing a real-time PCR assay using a 96-well plate? (Please separate multiple responses with a comma.)

#	Response
4	10
2	10, 15
2	10, 20
1	10,25
2	15
1	15,25
1	2
27	20
1	20 microl Lightcycler capillaries
2	20, 25
1	20, 50
1	20,25
1	20,25,50
1	20uL
1	20ul, 10ul
1	24
64	25
1	25 in rotorgene rotor!
1	25 microliter
1	25 or 12.5ul

1	25, 20
1	25, 22.5
2	25, 50
1	25,0
1	25,15,12.5
1	25,20
2	25,50
2	25ul
2	30
7	50
1	do not use 96 plates
2	N/A
1	Not applicable

44. What reaction volume(s), in microliters, do you usually use when performing a real-time PCR assay using a 384-well plate? (Please separate multiple responses with a comma.)

#	Response
1	-
1	1
13	10
1	10,20
1	10,5
2	10 μ l
1	12.5
2	15
8	20
1	20uL
1	25
1	4,5
2	5
1	5, 10
2	5,10
1	5,20
1	5.0
1	5ul

1	5ul, 10ul, 20ul
1	7
1	7.5
11	n/a
5	NA
3	not applicable
1	we do not use 384 well plate

ANALYSIS

45. What post-run instrument settings do you usually use to analyze the your real-time qPCR data?

Automatic Ct/Automatic baseline	55.6%	(85)
Manual Ct/Automatic baseline	35.3%	(54)
Manual Ct/Manual baseline	30.1%	(46)
Not applicable	2.0%	(3)
!Other Comparative quantitation analysis	0.7%	(1)
!Other SDM, CalQplex, own methods	0.7%	(1)

46. How do you analyze your data. Check all that apply.

Standard Curve	79.1%	(121)
Delta delta Ct method	61.4%	(94)
Relative Expression Software Tool (REST/REST-XL)	15.0%	(23)
Q-Gene	2.0%	(3)
LinRegPCR (Ramakers et al, Neurosci Lett. 2003)	4.6%	(7)
DART-PCR (Pierson et al, NAR 2003)	2.0%	(3)
Not applicable	1.3%	(2)
!Other Biorad Genex	0.7%	(1)
!Other comparative analysis	0.7%	(1)
!Other GenEx Professional	0.7%	(1)
!Other genorm	0.7%	(1)
!Other geNorm	0.7%	(1)
!Other home software	0.7%	(1)
!Other Liu and Saint, BBRC 2002	0.7%	(1)
!Other Measure signal from sample relative to signal from known copies of internal standard	0.7%	(1)

!Other mostly just CT or delta CT	<input type="checkbox"/> 0.7%	(1)
!Other MX PRO software	<input type="checkbox"/> 0.7%	(1)
!Other MX3000P build-in software	<input type="checkbox"/> 0.7%	(1)
!Other qBASE	<input type="checkbox"/> 0.7%	(1)
!Other qBase (Hellemans et al., in press)	<input type="checkbox"/> 0.7%	(1)
!Other qBase software	<input type="checkbox"/> 0.7%	(1)
!Other Roche Rel Quant	<input type="checkbox"/> 0.7%	(1)
!Other Specific Excel spreadsheets	<input type="checkbox"/> 0.7%	(1)

47. What do you use as a standard to generate your standard curves? Check all that apply.

Oligonucleotide	<input checked="" type="checkbox"/> 8.5%	(13)
PCR product	<input checked="" type="checkbox"/> 29.4%	(45)
Plasmid, linearized	<input checked="" type="checkbox"/> 33.3%	(51)
In vitro transcribed RNA	<input checked="" type="checkbox"/> 18.3%	(28)
Purified genomic DNA	<input checked="" type="checkbox"/> 25.5%	(39)
Pooled cDNA	<input checked="" type="checkbox"/> 26.8%	(41)
Commercial RNA	<input checked="" type="checkbox"/> 18.3%	(28)
No standard curve is run	<input checked="" type="checkbox"/> 9.8%	(15)
!Other calibrator ³	<input type="checkbox"/> 0.7%	(1)
!Other cDNA with arbitrary assigned values/ dilution curve	<input type="checkbox"/> 0.7%	(1)
!Other commercial protein	<input type="checkbox"/> 0.7%	(1)
!Other Dilution of bacteria	<input type="checkbox"/> 0.7%	(1)
!Other plasmid	<input type="checkbox"/> 0.7%	(1)
!Other plasmid, not linearized	<input type="checkbox"/> 0.7%	(1)
!Other Use linearized plasmid in standardized mixture of internal standards	<input type="checkbox"/> 0.7%	(1)
!Other virus positive plasma	<input type="checkbox"/> 0.7%	(1)

48. How many normalizing genes do you typically evaluate when designing your assays? (Please enter a numeric value)

#	Response
32	1
5	10
1	11
1	12
1	15
18	2
35	3
5	4
8	5
1	50
4	6
4	8

49. How many normalizing genes do you typically use for each assay? (Please enter a numeric value)

#	Response
79	1
17	2
1	200
16	3
3	4
1	5

50. Please list (up to 3, separated by comma) the genes you frequently use to normalize your real-time qPCR assays.


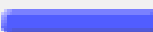

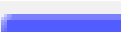

#	Response
1	-
1	16s rRNA
1	18S
1	18S RNA, b-actin, gapdh
1	18s rRNA, actin, GADPH, L4 ribosomal protein
2	18s rRNA, actin, GAPDH, but looking into others
1	18S, 28S, GAPDH, 28S, B2M, 16S, AMA1, EFT2
1	18S, Actin, Gapdh
1	18s, B2Microglobulin, HPRT

3 18s, GAPDH, b-actin
1 18s, gapdh, bactin
1 18s, gaph, cyclophilin
1 18S,B-actin,gapdh
1 18s,B2m,gapdh,GUS,B-actin
1 18s,hgprrt,gapdh
1 18SrRNA, b-Actin, Cyclophilin
1 20,20,20
1 5S, U6
1 ABL,
1 ABL, b2M, GUS
2 ABL,GAPDH
1 acpP (PA01)
1 ACTB, GAPDH
1 actb, gapdh, 16s
1 ACTB, TBP, GUSB, HPRT1
1 ACTB,GAPD,PO
4 ACTIN
1 actin, 18S
2 actin, GAPDH
1 actin, gapdh, ubiquitin
1 actin, RNA polymerase II, GHPDH
1 alpha tubulin, actin, beta tubulin
1 apt, pdf2, ubc9
1 at5g46630,At1g58050, At1g62930
1 B-actin TBP
1 B-actin, gapdh, 18s
1 B-actin, HPRT
1 B2M
1 bActin, GAPDH,18S
1 BCR, b-actin, GUS
1 beta acten, gapdh,
1 beta actin
1 beta actin, b2m
1 beta actin, HPRT

1 beta-actin
1 beta-actin, 18S rRNA, GAPDH
1 beta-actin, 36B4, GAPDH
1 beta-actin, gapdh, rubisco
1 cyclophilin
1 Cyclophilin B, 36B4 (ribosomal phosphoprotein P0), Ywhaz
1 Cyclophilin, Elongation factor, Ubiquitin
1 Cyclophilin, GAPDH, 18s
1 cyclophillin, 28S rRNA
1 ERV3
2 GAPDH
1 gapdh, 28s, genes from microarray data
1 GAPDH, ACTB
1 gapdh, actb, 6gpdh
1 GAPDH, Actin
1 GAPDH, b actin, CYPB
1 GAPDH, b'act
1 gapdh, b-actin, acid ribosomal protein
1 GAPDH, beta-actin, ribosomal rna's
1 gapdh, g6pd, b actin
1 GAPDH, Histone, beta-actin, ubiquitin
1 GAPDH, HPRT
1 gapdh, hpert, actin
1 GAPDH, PGK, HPRT
1 GAPDH, PGK1, TFRC
1 GAPDH, RS8, YWHAZ
1 gapdh, tbp, b2m
1 gapdh, tubulin, cyclophilin
1 GAPDH, UBC, b-Actin
1 gapdh,beta-actin,rp13a
1 GAPDH. B2m, HPRT
1 GPDH, B2M, ACT
1 gus, hprti1, hmbs
1 gus, hprti1, hmbs, ppia
2 gusB

- 1 HIV genes
- 1 HPRT, beta-actin
- 1 HPRT, GAPDH, Microglobulin
- 1 hprt, tbp, bactin
- 1 hrpt, gadph, 18s
- 1 human PO, Titin, Albumin, 18SRNA, TFIID
- 1 L32, b-actin
- 1 MDH1, ATP5b, B2M
- 1 mGAPDH
- 3 N/A
- 1 NA
- 1 PGK1, LaminB
- 1 PIP2, HPRT
- 1 polR2F, TBP, RPS6
- 1 RP49
- 1 RP49(drosophila), ACTB(human), GAPDH(human)
- 1 RPL13A, 18S rRNA, GAPDH
- 1 RPLP0, GAPDH
- 1 rRNA, beta-actin, GAPDH
- 1 S100A8, ANXA2, RPL37, B-Actin, GAPDH
- 1 S100A8, BACT, ANXA2
- 1 SA0098
- 1 TBP
- 1 TBP, HPRT, GAPD
- 1 TFRC, TBP, B2M
- 1 UBC, GAPDH, HPRT1
- 1 varies with experiment, scamp2, itga5, plcg2
- 1 YWHAZ, 18S, HMBS, HPRT

51. Do you measure PCR efficiency in each assay?

Always		44.4%	(68)
Sometimes		26.1%	(40)
Never		3.3%	(5)
As part of the validation process		20.9%	(32)
TOTAL		94.8%	153

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

>95% (slope is greater than -3.45)	26.8%	(41)
>93% (slope is greater than -3.50)	15.7%	(24)
>90% (slope is greater than -3.60)	28.8%	(44)
>85% (slope is greater than -3.75)	6.5%	(10)
>80% (slope is greater than -3.9)	5.9%	(9)
>75% (slope is greater than -4.10)	1.3%	(2)
Not applicable	4.6%	(7)
!Other any value can be useful provided that you use that efficiency value in your calculations	0.7%	(1)
!Other Don't mind too much as long as it matches normaliser efficiency	0.7%	(1)
!Other Measure relative to internal standard, ensuring lower detection threshold of 10 molecules in 35 cycles	0.7%	(1)
!Other RT-PCR efficacy: >70%, PCR efficiency >90%	0.7%	(1)
TOTAL	92.2%	153

GENERAL

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

Yes	41.2%	(63)
No	54.9%	(84)
TOTAL	96.1%	153

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

Assays-on-Demand (ABI)		26.1%	(40)
Assays-by-Design (ABI)		12.4%	(19)
TaqMan Low Density Arrays (ABI)		7.2%	(11)
Qiagen Pre-developed Assays		7.8%	(12)
RT2 Profiler PCR Array (SuperArray)		3.9%	(6)
Roche Universal Probe Library		8.5%	(13)
Not applicable		7.8%	(12)
!Other commercial kits for viral detection		0.7%	(1)
!Other Fluorescentic, SimpliGen		0.7%	(1)
!Other Food analysis kits (TaqMan based)		0.7%	(1)
!Other invitrogen		0.7%	(1)
!Other mirVana by Ambion		0.7%	(1)
!Other Roche Kits		0.7%	(1)
!Other USDA provided		0.7%	(1)

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

55. What other questions should have been asked?

#	Response
1	# of replicates used
1	1) What did you find to be the most difficult aspect to qPCR when you initially learned and performed the method. 2) Please describe the environment you work in: A) environmental testing, B) human diagnostics, C) veterinary diagnostics, D) food testing, E) academic service, F) academic research, G) agricultural biotechnology... etc.
1	absolute Quantification
1	Are you satisfied with your choice of machine? Are you satisfied with the results you get?
1	automation of RNA extraction
1	Do you amplify your RT product? What procedure do you use for amplification?
1	Do you consider primer dimers to invalidate data?
1	Do you use a commercially available reverse transcription kit? If yes, which one?
1	Do your assays give reproducible results, upto what extend?
1	For how long are you doing Real Time PCR in your core facility?
1	Funding?

- 1 How do you check the efficacy of your DNase treatment? How do you assess the stability of your reference genes? How do you do statistical evaluation of your results (confidence intervals, parametric test, non-parametric test, exploratory analysis (e.g. clustering))? What experimental layout do you most often design (sample maximization or gene maximization)?
- 1 How do you determine PCR efficiency? Might want to clarify RT priming method for one-step vs two-step RT-PCR. I assumed you were referring to two-step as GSP should be the standard for one-step RT-PCR (but you never know).
- 1 how do you quantitate your standard to generate standard curve (O.D. 260nm, picogreen, qPCR assay...)
- 1 How/Is RNA quantified prior to RT? How/Is RNA quality checked prior to RT? Do you continue to use/publish a particular normaliser even if you have some doubts/evidence about it's suitability? If you're in a rush, do you sometime bypass some of the validation/optimisation expts? If using commercial assays with comparative Ct method, do you confirm equal PCR efficiencies every time, or do you sometimes presume they'll be ok because they usually are? Do you add equal amounts of RNA to a RT rxn? Have you ever looked at/worried about the effect of adding different amounts of RNA to RTs & then making comparisons? Which do you do your stats on, the Ct, the linear value, the one which gives the answer you want? How old is the oldest cDNA you use? Do you aliquot samples/oligos for long-term storage? Are you worried about multiple freeze/thaw cycles? Do you ever compare abundances across genes?
- 1 No mention of GenEx software for data analysis The question on hot-start, sometimes I do and sometime I don't depending on the complexity of the template, no choices here
- 1 Problems with primer dimers?
- 1 Questions about specific brands of reagent mixes used
- 1 test design: preanalytical steps need to be considered and validated, especially with respect to stability of reference gene/ target gene
- 1 the difference of the Cts between your reference gene and target gene.
- 1 What do people report in publications? Statistics, slopes, validation experiments?
- 1 What sample types do you analyse? Do you use qPCR for qualitative detection?
- 1 what species do you evaluate (i.e. we do mainly cat, so our housekeeping genes are limited at the moment by seequence, murine and human commercially available, but lots 'o veterinary researchers out there:-))
- 1 You might ask whether anyone quantifies relative to internal standards, and if so why they don't simply quantify at endpoint and bypass the expense and trouble of real-time analysis.

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

#	Response
1	- inter assay variability on different platforms
1	4-Dimension Hybridizations
1	absolute Quantification
1	comparisons of probes. mgb, scorpion, etc
1	Do studies with internal standards within standardized mixtures of internal standards and determine whether this eliminates the need for real-time analysis. Vast amounts of data clearly indicate this is the case. Probably more than 80% of the problems people on the qPCR listserv have will be eliminated by using this approach.
1	For detection and quantification assays using biological samples I would be interested to know what max. Ct(s) real-time PCR users determined to be a positive or suspect sample and what Cts were generally accepted as a negative sample, and how this was determined.
1	how many primers do you design and test before choosing a pair for your assay?, other questions about primer design about specificity thresholds (by Blast) and lowest allowable deltaG energies for primer dimers and homo dimers; which parameter takes precedence?. When picking from an optimization assay, which do you pick first or go by: lowest CT or max increase in fluorescence?
1	measure run-to-run variation
1	The RT reaction is under studied
1	We need more studies regards on standard curve and cRNA standards preparation.
1	What are researchers and core labs looking for in choosing outside vendor kits, mixes, etc.
1	What is the right method? Is there a definite one type of method to follow while performing real time pcr and analyze (is that possible)?
1	working on standard quantification (development of reference materials) to generate standard curve

57. Other Comments:

#	Response
1	Great Survey
1	I could not answer a couple of questions and there was no N/A option, e.g. RT-PCR questions. We do not work with RNA.
1	I'd like to be able to use multiple reference (normalization) genes in every qRT-PCR. It's fine in theory, and do-able in a research setting. However, in clinical diagnostic work, one is often faced with very limiting amounts of precious patient specimens: often one recovers just enough RNA to supply the bare minimum number of wells (e.g., NTC, normalization gene, and target gene replicated 2 or 3 times if possible. I just don't see how clinical qPCR tests can be done routinely with more than one normalization gene because opportunities to multiplex these reactions is severely limited. I'd love it if the qPCR community could give more advice for those of us in the clinical testing arena about selecting, validating and using normalization genes.
1	Maybe there should be one universal method for doing and analyzing real time pcr so that some papers do not get rejected based on their real time pcr method not being the one that the reviewer knows of.
1	Our ABI 7900 is currently used only for bi-allelic snp genotyping (end-point fluorescence detection).
1	Please send me info on this meeting, I would like to attend.
1	question 21 is hard to answer when you answer LNA in q20 q54 Roche uni probe lib is predesigned but I often modify the primers to be used, to get a better assay
1	Thank you for considering my comments.
1	Thanks guys, great job
1	These questionnaires are terrific! thank you! I love to see what others are doing and "keep up with the Joneses"
1	We are a new company from Argentina, that's why we don't have many clients right now. And we are setting up most of the reactions.
1	Would be nice to see web based training for those of us who work in remote areas. Many of us have limited access to training courses and workshops that are available in Europe and USA

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

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