A Survey of Real -Time PCR

Nucleic Acids Research Group 2004

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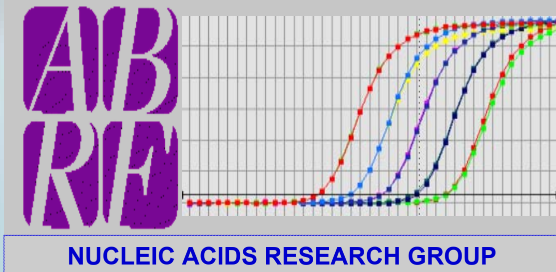
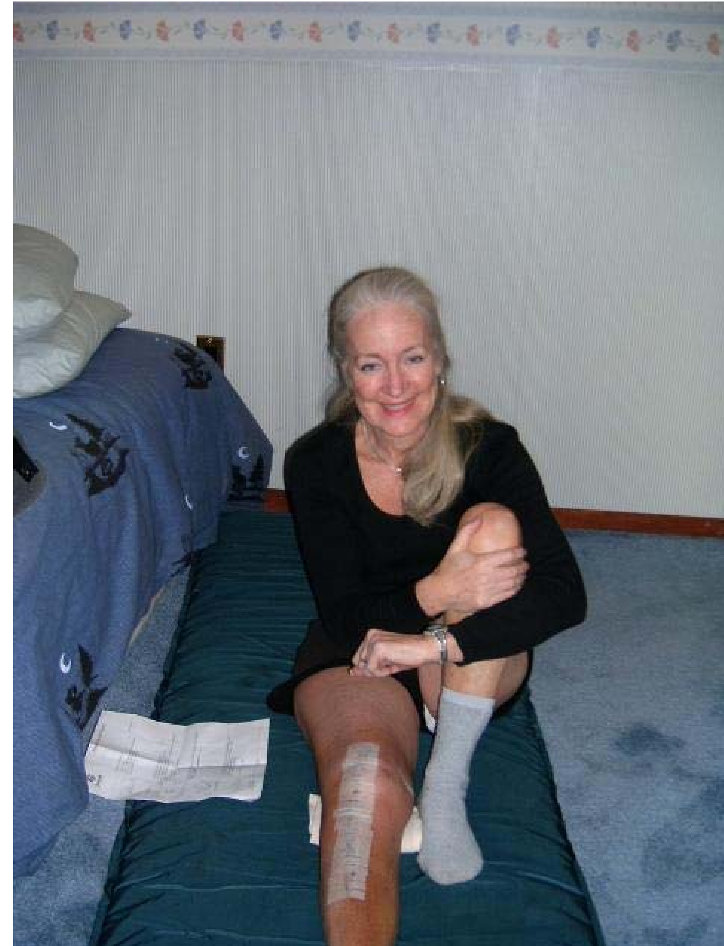


Survey Goals

- To survey laboratories through-out the world to determine the current status of real-time PCR technology.
- Emphasis placed on Core Facilities for survey discussion.



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Survey Basics

- Survey consisted of 58 questions
  - Not all questions were answered by each respondent
  - Several questions allowed multiple responses
- Survey posted on ABRF and qPCR listserver at Yahoo.com from November 2003 – January 2004
- Total of 125 respondents
- Survey results are posted on the ABRF Website:
  - http://www.abrf.org/index.cfm/group.show/NucleicAcids.32.htm

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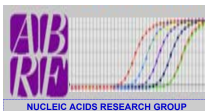

Facility Profile

Type of Facility	Core Facility		
	Yes	No	Total
Academic	17	54	71
Government	3	8	11
Industry	4	23	27
Clinical/Non profit	3	12	15
TOTALS	27	97	124

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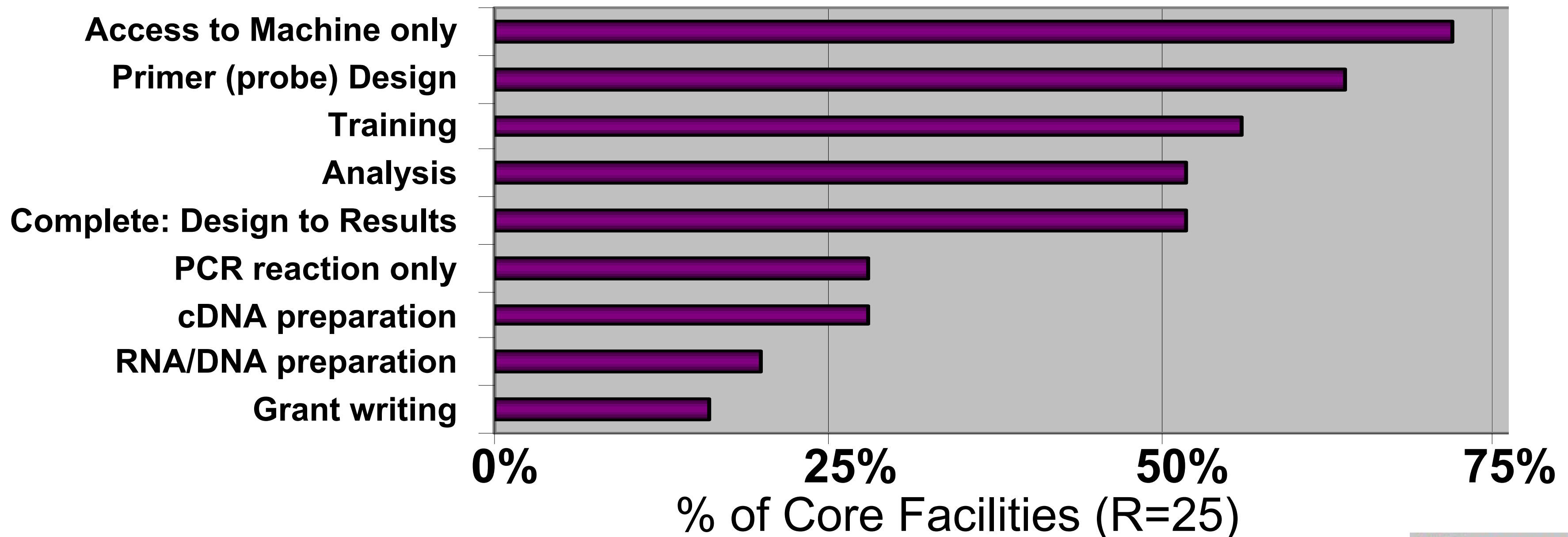
Level of Real Time PCR Support and Services offered by Core Facilities

Researchers Supported by Core Facilities (R=24)

0 – 10 Researchers	**48.28 %**
11 – 25 Researchers	**27.58 %**
26 – 75 Researchers	**17.24 %**



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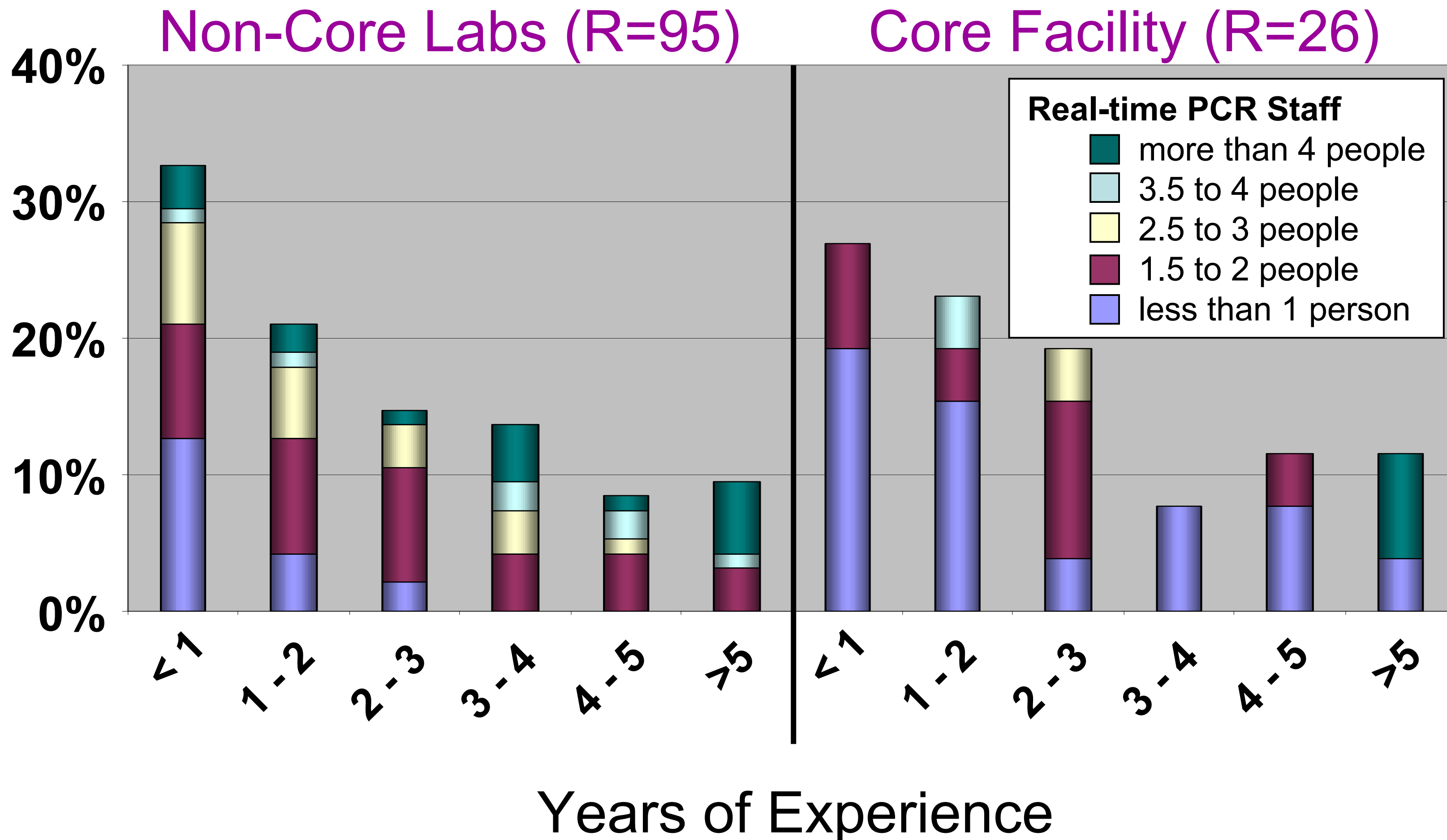
Staffing and Experience



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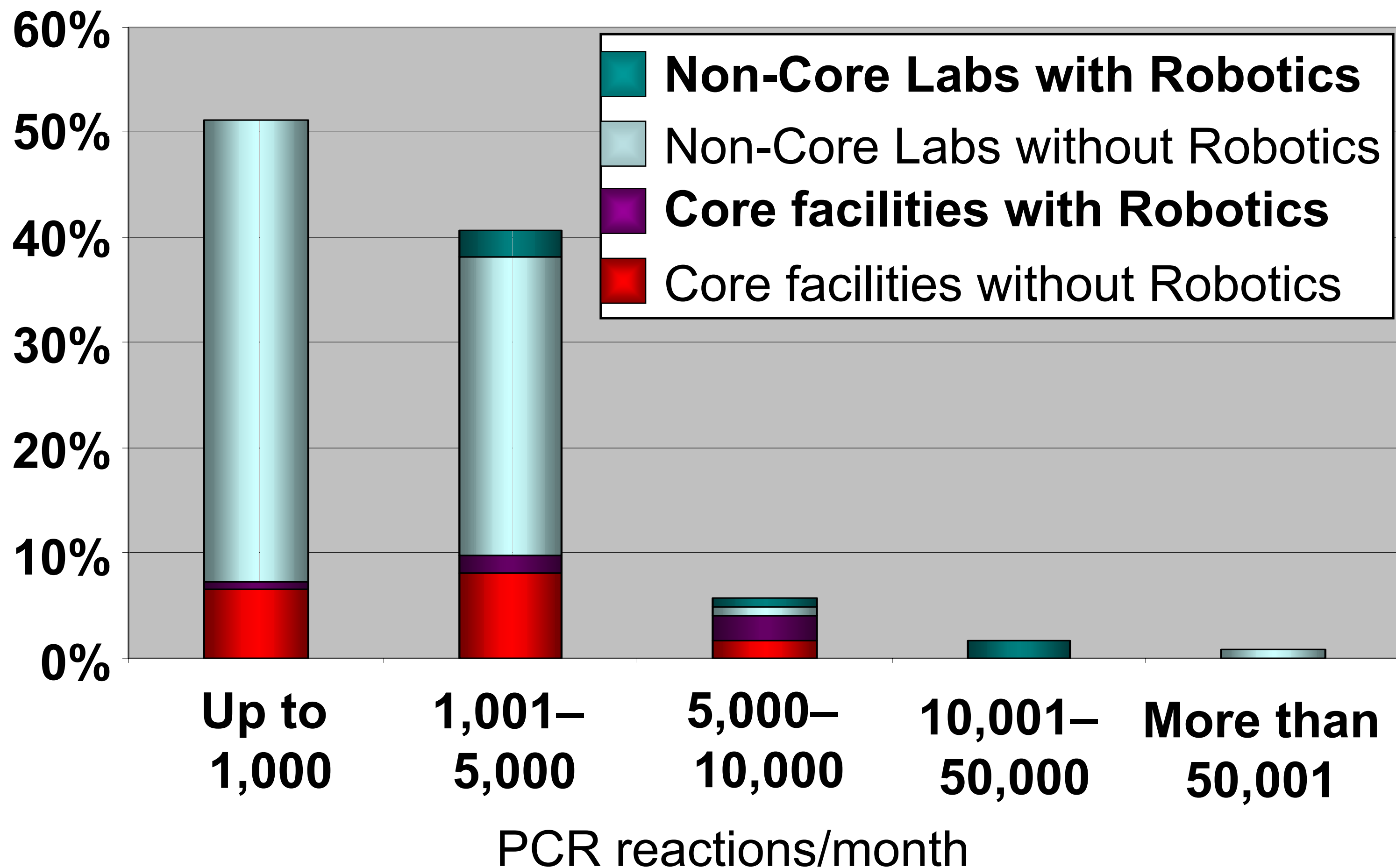
Real-time PCR reactions/month



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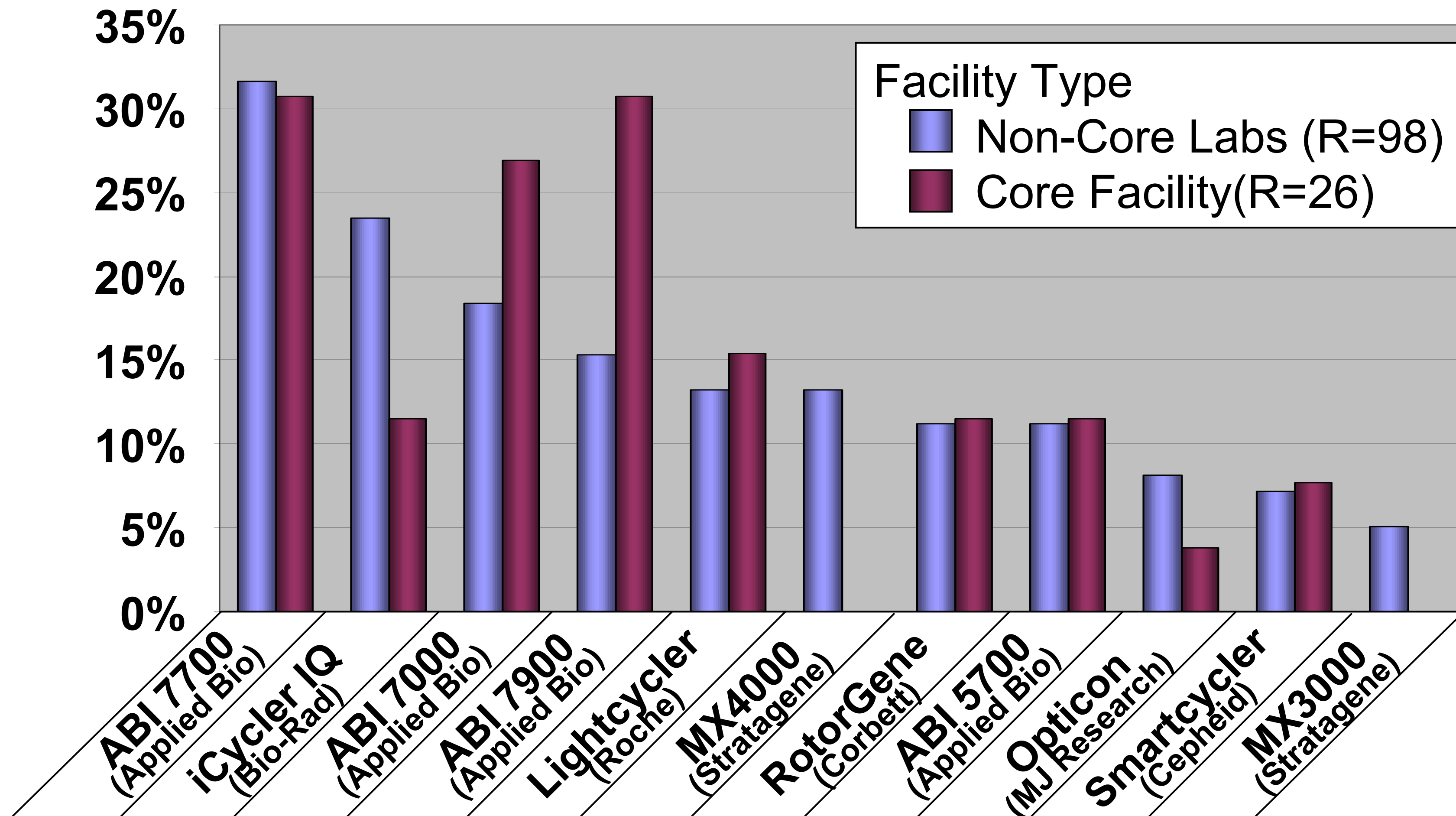
Real-time PCR Instrumentation



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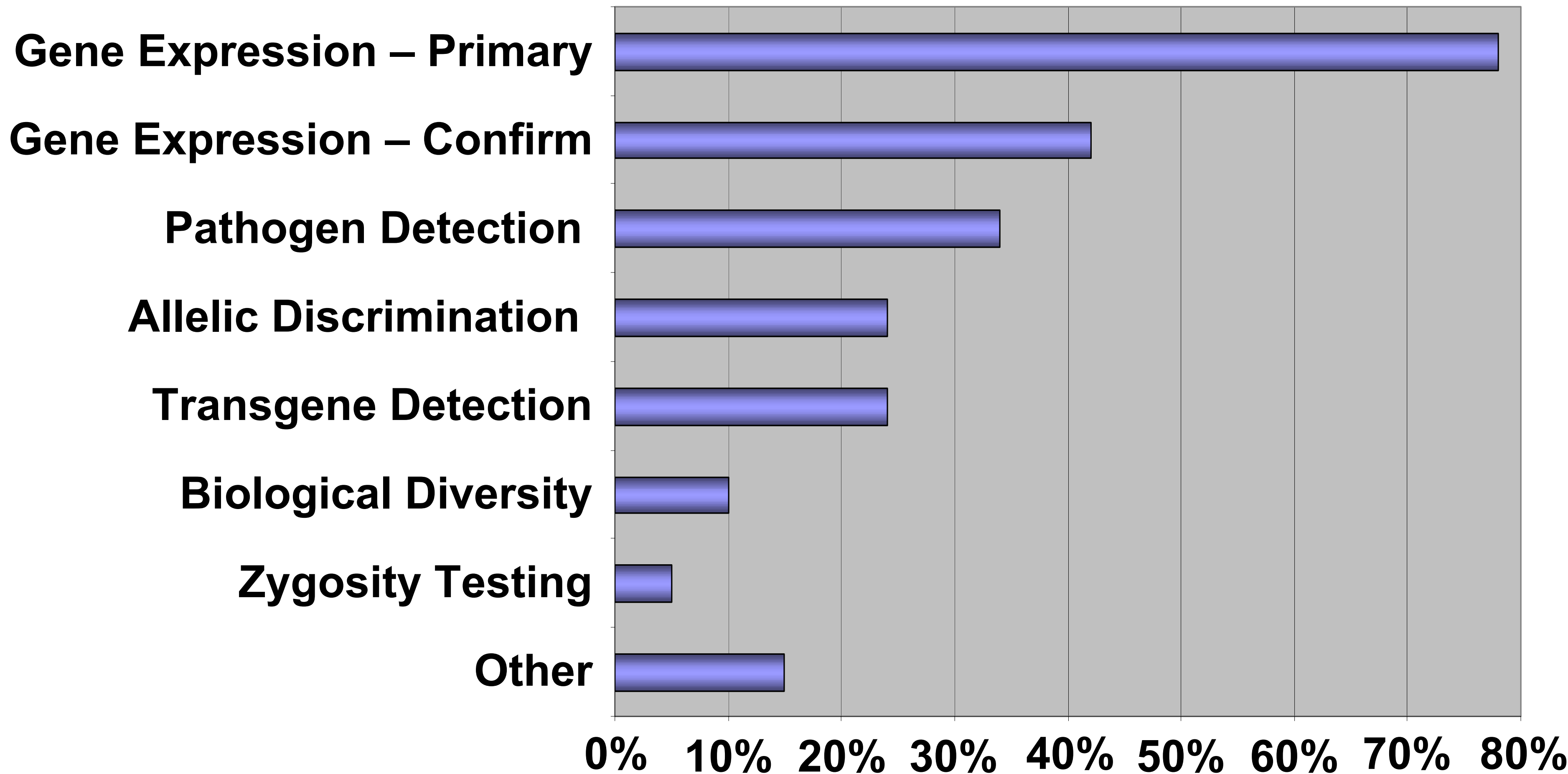
Real-time PCR Applications



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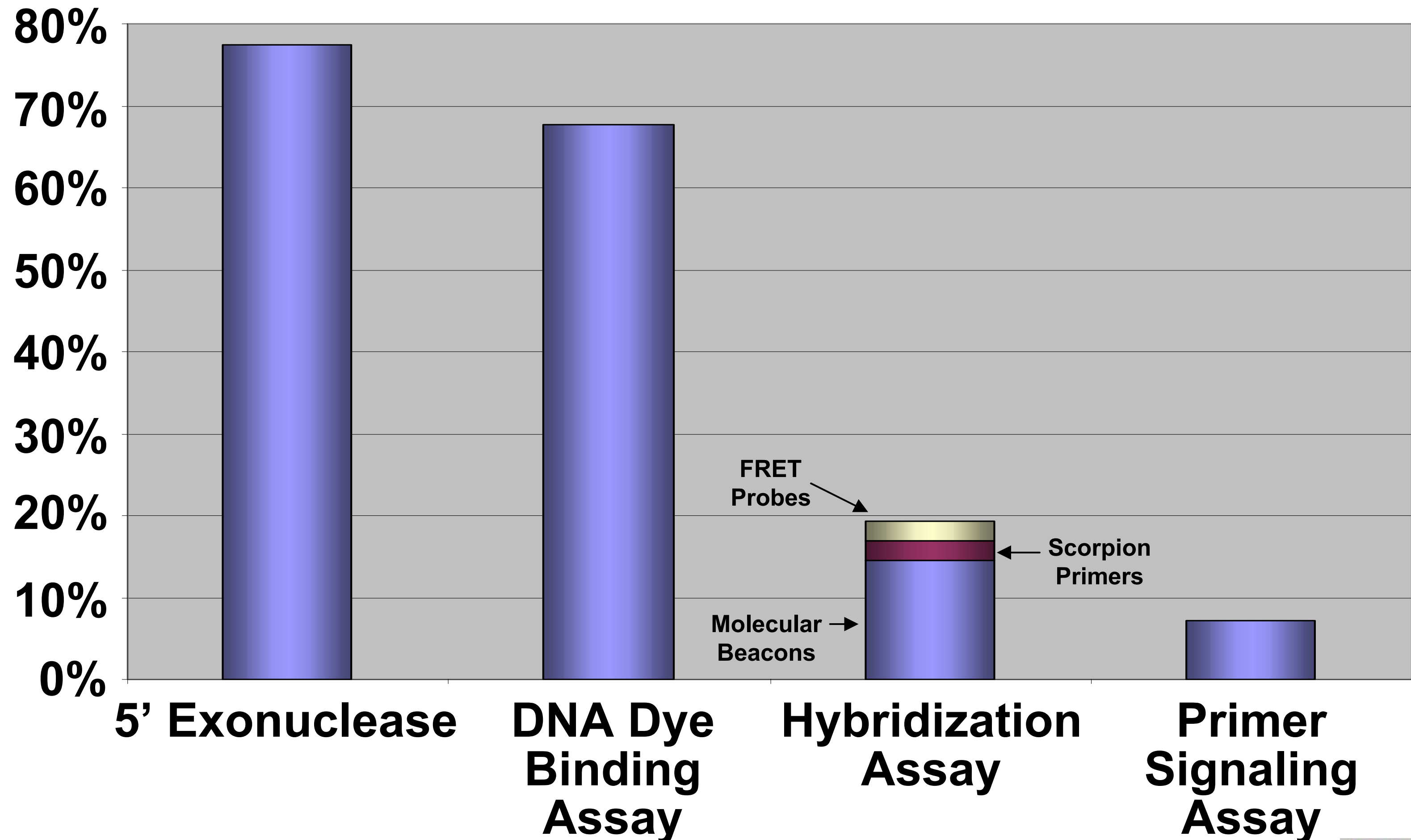
Types of Real-time Assays



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Assay Development

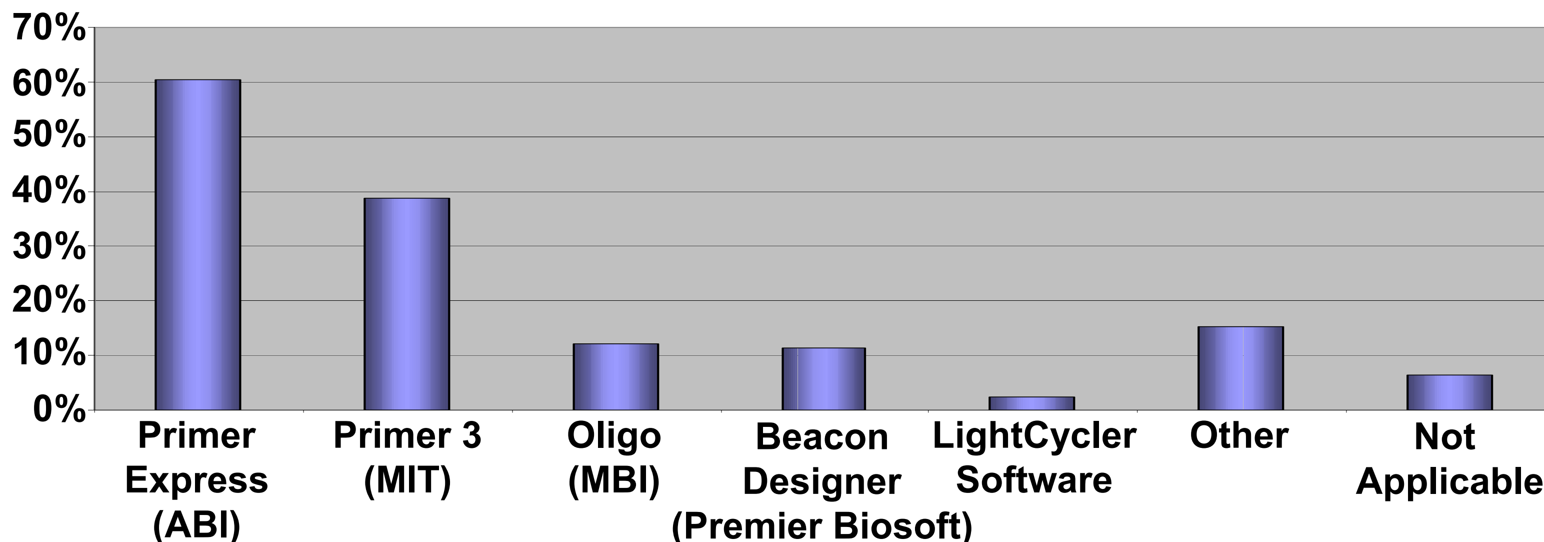
Method used for Assay Development

Design Your Own Assay	96.8%
Use Assay from Literature	49.2%
Use Commercial Assay	31.5%

Do you Multiplex Assays?

Always	5.4%
Sometimes	40.0%
Never	51.5%

Software used for Primer/Probe Design



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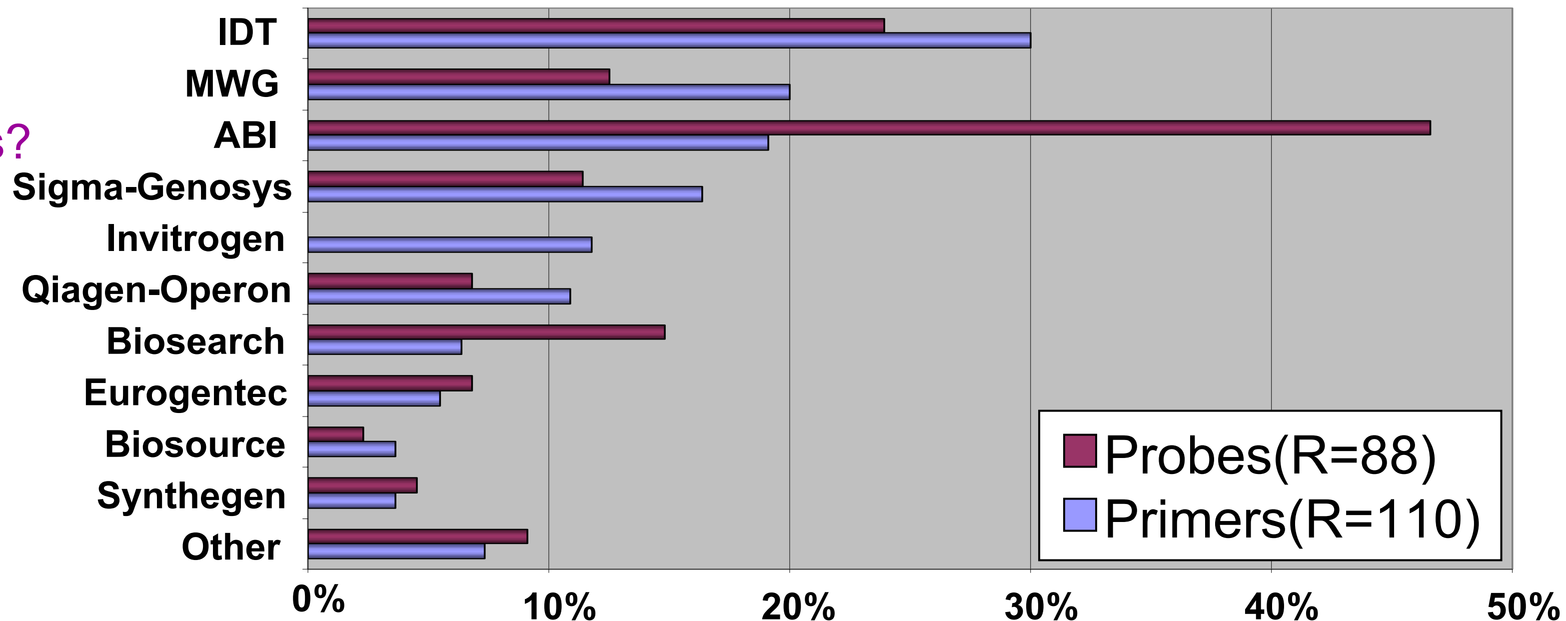
Primer and Probe Production

Do you make your own primers/probes?

	Core Facility(R=26)	Non-Core Labs (R=97)
Neither	69.2%	78.4%
Primers only	15.4%	9.2%
Probes only	3.8%	0.0%
Primers & Probes	11.5%	12.4%

Where do you order primers/probes?



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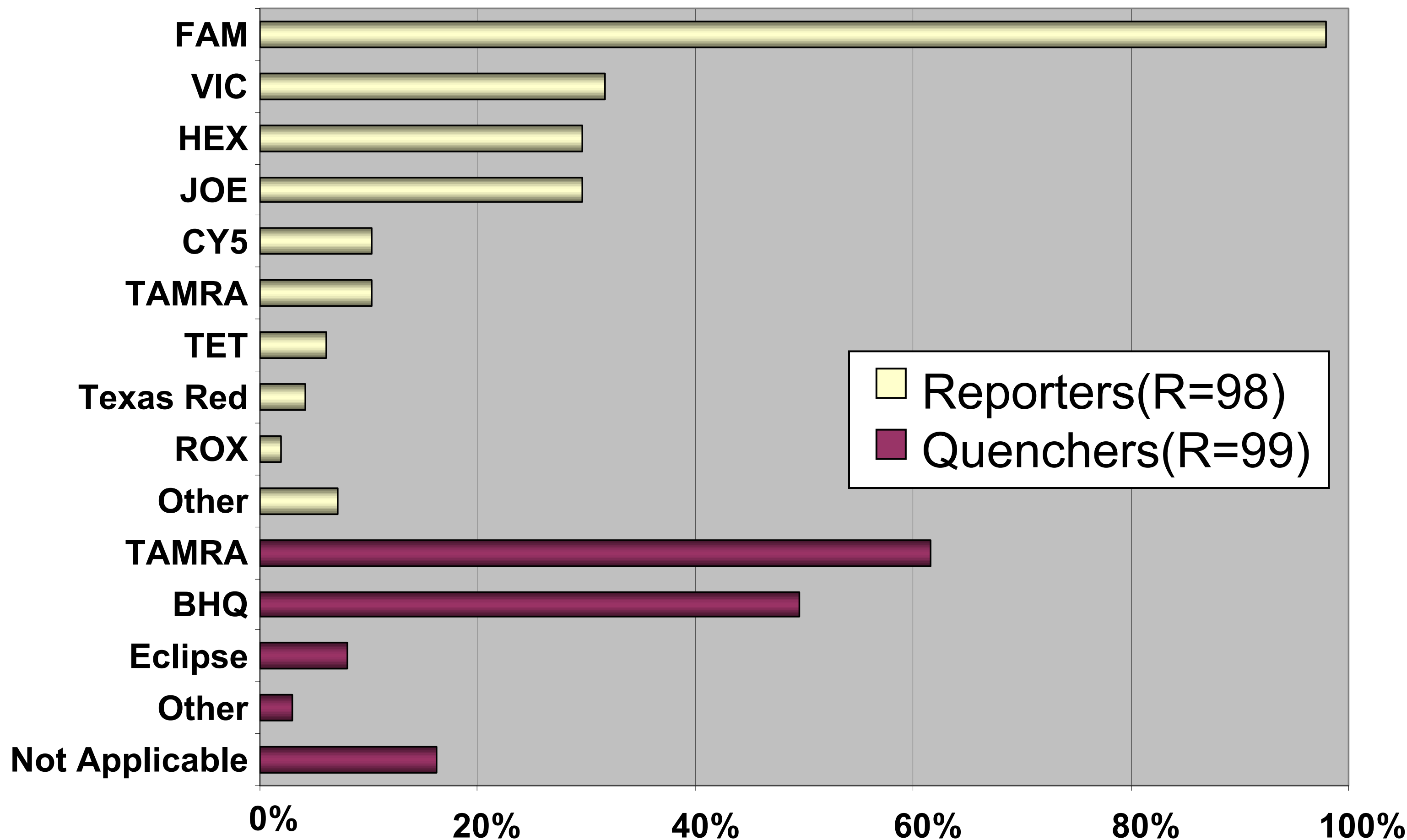
Reporters and Quenchers



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General Assay Conditions

# replicates per assay	% of respondents
Duplicates	38%
Triplicates	61%
>3 replicates	a few

Controls	% of respondents
No-template control	94%
Minus RT-control	46%
Positive control	46%

Type of template	% of respondents
Genomic DNA	55%
cDNA	81%
Plasmid DNA	33%

Reference dye	% of respondents
ROX	58%
None	24%

Assay volume	% of respondents
5uL	4%
10uL	14%
15uL	2%
20uL	29%
25uL	53%
50uL	17%

Water for assay	% of respondents
Nuclease free	62%
Not nuclease-free	34%

Q29-33, 45-47

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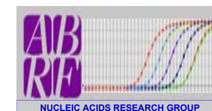

Template Preparation

Template RNA purification method	% of respondents
Phenol-based methods	30%
Column/matrix based methods	40%
Combination of techniques	20%
Detergent-based RNA isolation methods	2%

Template isolation	% of respondents
DNA or RNA only	80%
DNA and RNA from same sample	6%
DNA, RNA and protein from same sample	2%

Dnase I treatment of the RNA sample	% of respondents
Always	33%
Sometimes	31%
Never	22%

Q34-37

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Reverse Transcription

Reverse transcription reaction	% of respondents
One step	28%
Two step	67%

Reverse transcription primer	% of respondents
Oligo (dT)	35%
Random primers	33%
Mix of random primers and oligo(dT)	16%
Gene specific primer	23%

Source of reverse transcriptase	% of respondents
MMLV	58%
AMV	10%
TTh	10%

Q38-41

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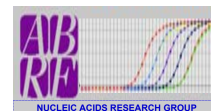

Polymerases and Mixes

Polymerase choice	% of respondents
Not heat activated	10%
Heat activated	86%
AmpliTaq Gold (ABI)	51%
Platinum Taq (LTI)	13%
Jumpstart Taq (Sigma)	5%
HotMaster (Eppendorf)	2%
BD Titanium Taq (Clontach)	2%

Master Mix	% of respondents
ABI 2X master mix	35%
Home made	25%
ABI 2X SYBRgreen master mix	24%
ABI Taqman core PCR reagent mix	12%
ABI SYBR green core PCR reagent mix	8%
Stratagene Brilliant QPCR master mix	7%
Invitrogen iQ SUPERMIX	6%
BioRad Brilliant QPCR master mix	4%
LTI Platinum qPCR supermix-UDG	5%
Sigma 2X SYBRgreen master mix	3%

Q42-43

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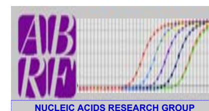
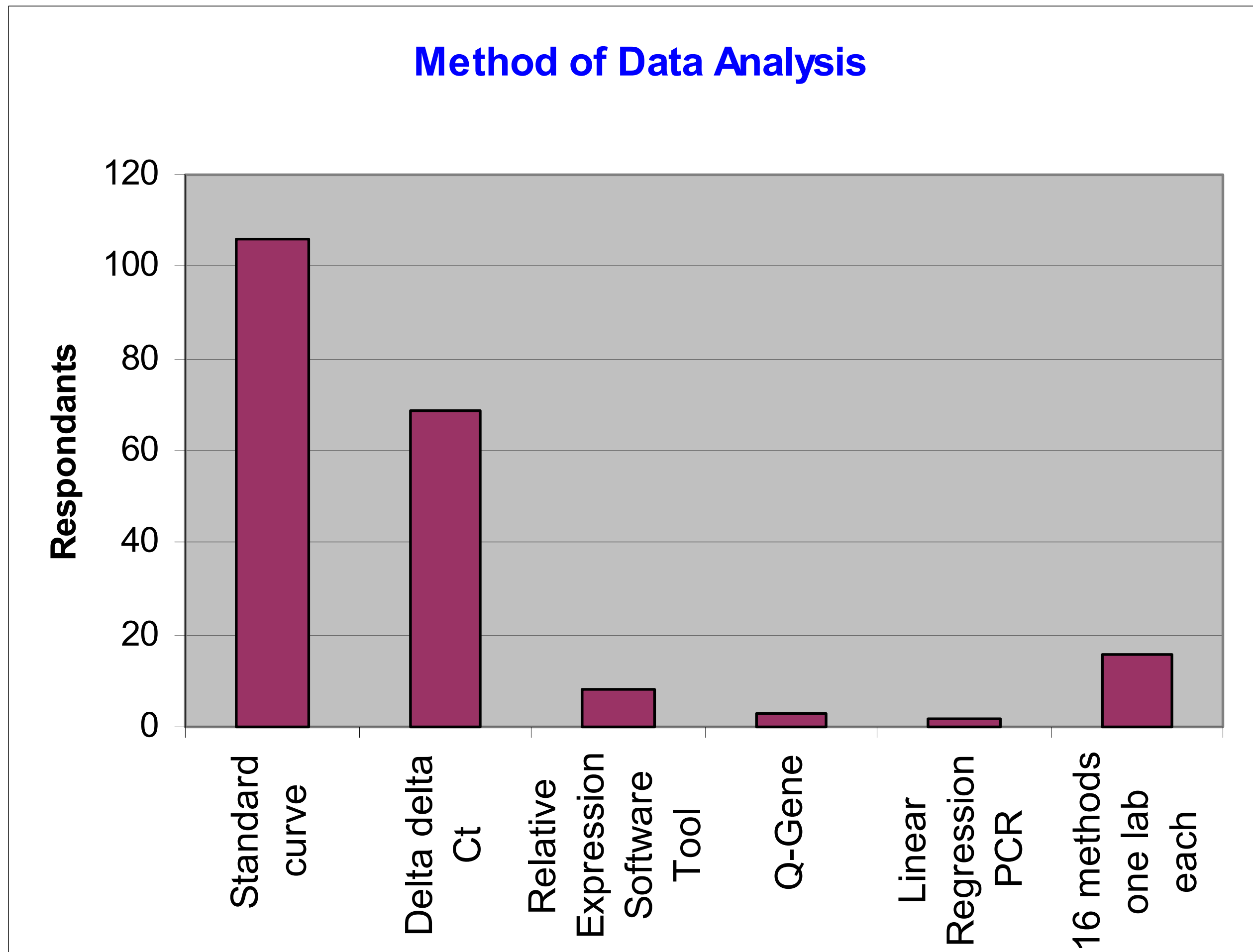


Method of Data Analysis



Q48

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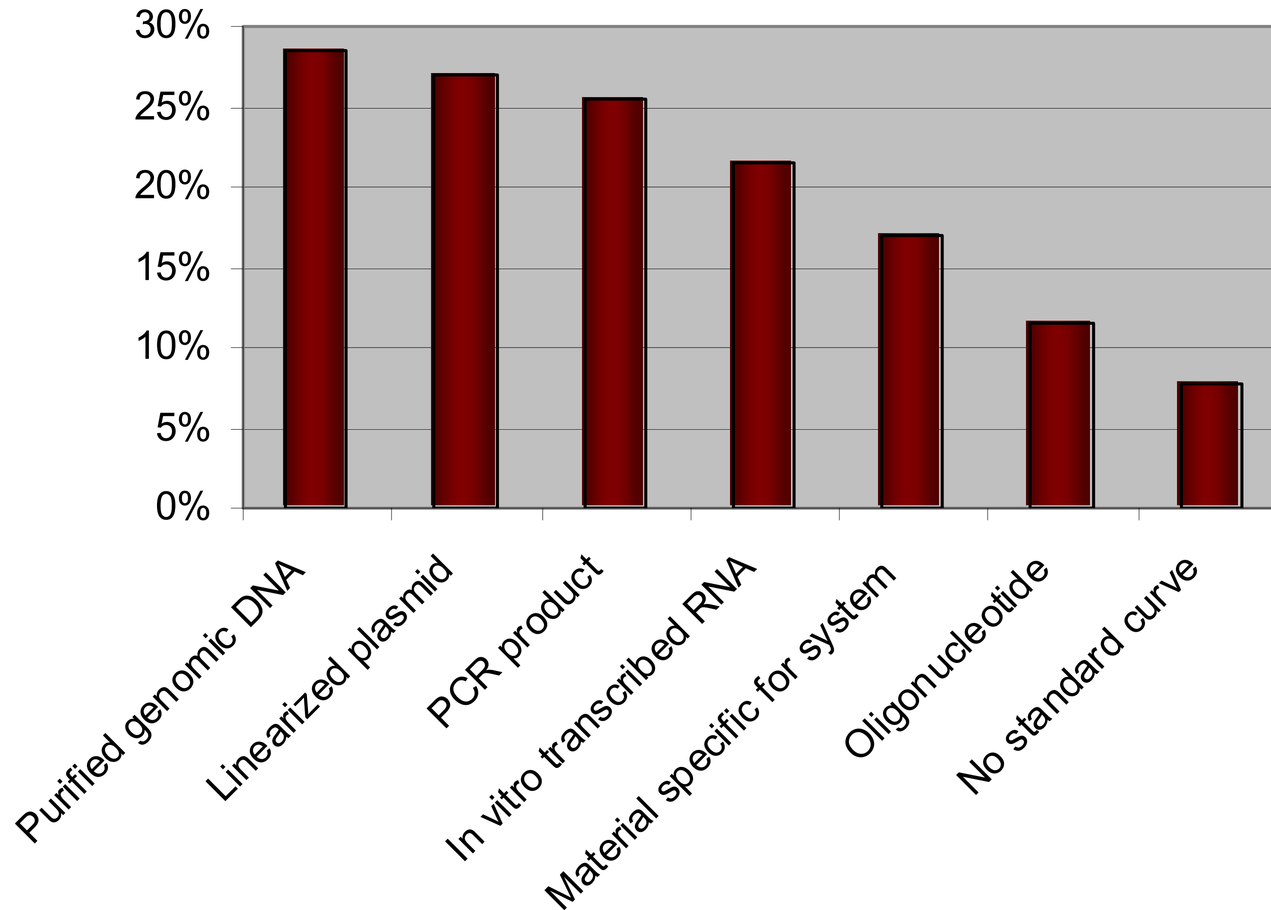
Material for Standard Curve



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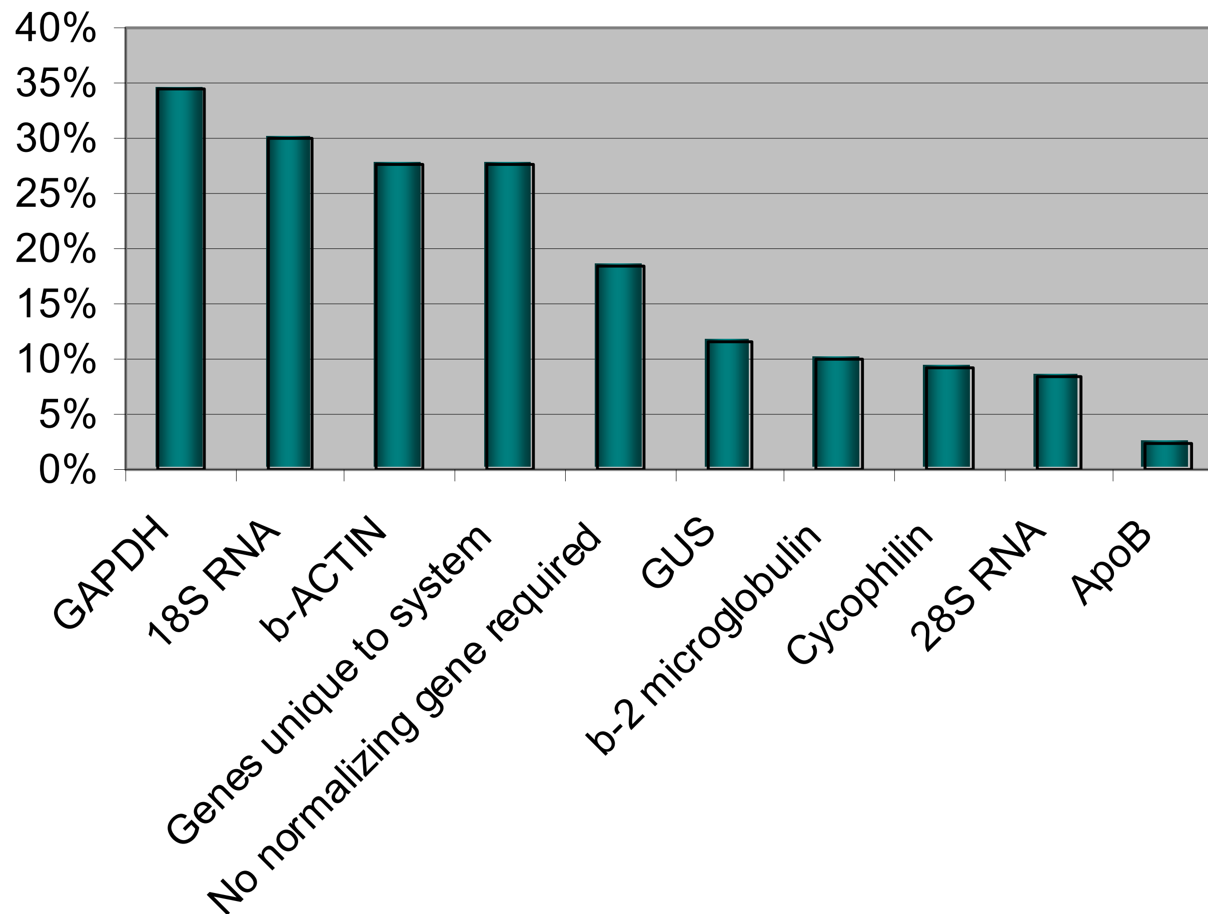
Normalization Reference Gene



Q50

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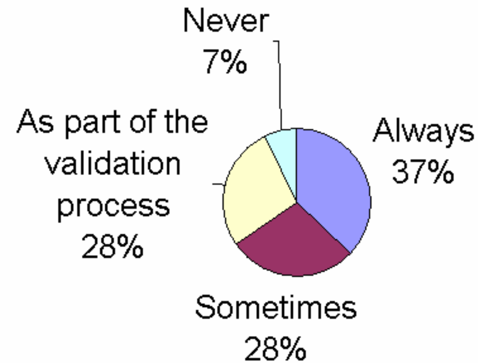
PCR Efficiency

Is PCR Efficiency Measured in Each Assay?



Acceptable slopes:

$E = 10^{[-1/slope]}-1$	% of Survey Participants	# of Respondents
>95% (slope is less than -3.45)	25	30
>90% (slope is less than -3.60)	35	45
>85% (slope is less than -3.75)	15	18
>80% (slope is less than -3.9)	5	7
>75% (slope is less than -4.10)	4	5
Not applicable	9	11
Other	3	3
Total	95	119

Q51, 52

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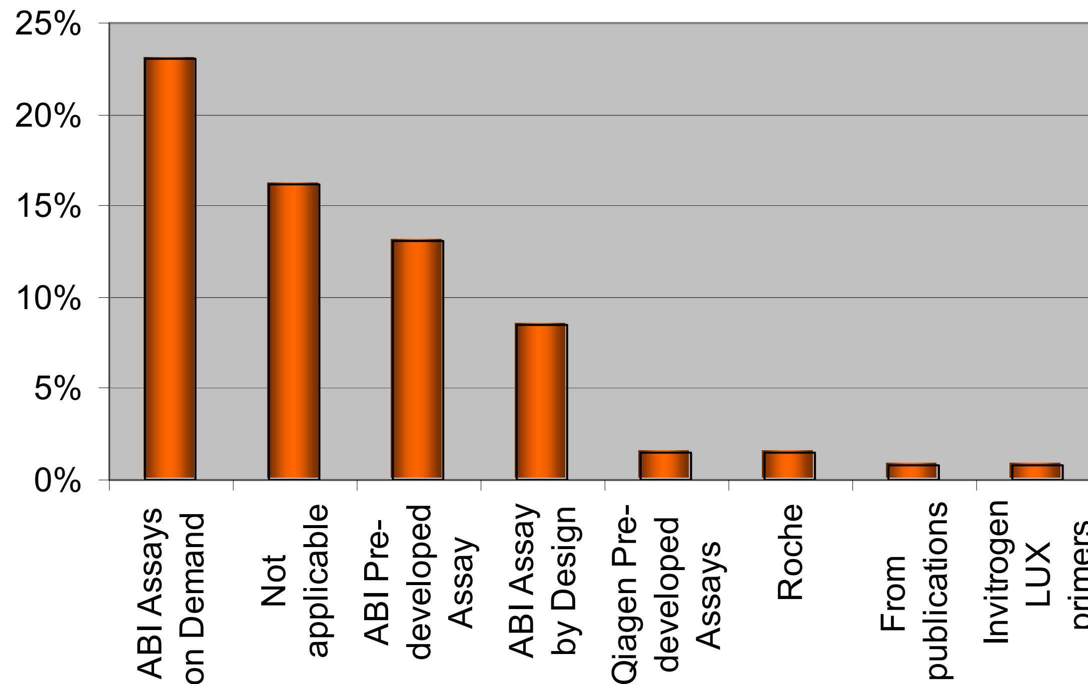
Pre-Developed Assays

User?	% of Respondents	# of Respondents
Yes	32	41
No	65	85
Total	96.9	126

User satisfaction 1 to 5, 1 is best

ABI Assays by Demand	1.9
ABI Pre developed Assay Reagents (PDAR's)	2.1
ABI Assay by Design	2.2
Qiagen Predeveloped Assays	2.5
Other	3.0



Q53, 54, 55

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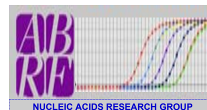

Other Comments and Suggestions

•More on how people are normalizing the data (genorm, stnd curve, rext xl, etc).

•How people are quantifying RNA/mRNA for RT (a260/280, pico green, etc), amplicon size

•Rating of qPCR machines. Equipment rated according to their world wide distribution.

A break down of what works "best" for a given organism and for what machines.

Use of TaqMan and other assay on a LightCycler platform

•Comparison of 96 well vs 384 well usage

•Housekeeping Genes! who's using what and why? Is there such a thing as a suitable houskeeping gene for ANY application!

More studies on housekeeping genes to use (maybe a website with a list of appropriate housekeeping genes for different biological experiment) and normalization procedures.

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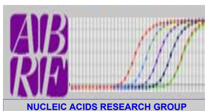

84% of respondents would like to see future surveys of real-time PCR.

•Comparison of data analysis software and the best method for interpreting data.

What data analysis approaches are being taken by others. There are many different published ways to analyze qPCR data, but there is little evaluation of the relative merits/demerits of each, much less any consensus of which is best. This problem seems particularly acute in the field of clinical diagnostics.

•Would like to have questions regarding how far is real-time pcr assay valid when it comes to confirmation of regulated genes from microarray experiments. Can we really believe what we see in this assay or do we have to go ahead and do Northern and other assays.

•Currently, there are no textbooks dedicated to real time PCR. It would be useful to 1st time users if a real time PCR textbook is available. I've been having trouble developing assays in Drosophila. I am curious as to whether other people have attempted to do the same and what worked best for them. Thanks for taking the time to make this survey!

•This survey is too biased towards expression analysis. We analyzes foods so no RT-PCR (no RNA available!) so use genomic targets. Use for GMO, species identification so outside the "norm" for qPCRers

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