

Nucleic Acids Research Group Presentation



ABRF 2007

April 3, 2007

2-3 PM

Agenda

**The ABRF NARG Real-Time PCR Survey 2007:
Taking the Pulse of the QPCR Field
Kevin Knudtson**

Round Table Discussion led by Timothy Hunter



**THE ABRF NARG REALTIME PCR SURVEY
2007: TAKING THE PULSE OF THE
QUANTITATIVE PCR FIELD**

Goals of the Survey

- **Capture the “current” state of the quantitative real-time PCR community**
 - **Instrumentation**
 - **Assays/Chemistries**
 - **Analysis**
- **Compare the results of the 2007 survey with the 2004 NARG survey**

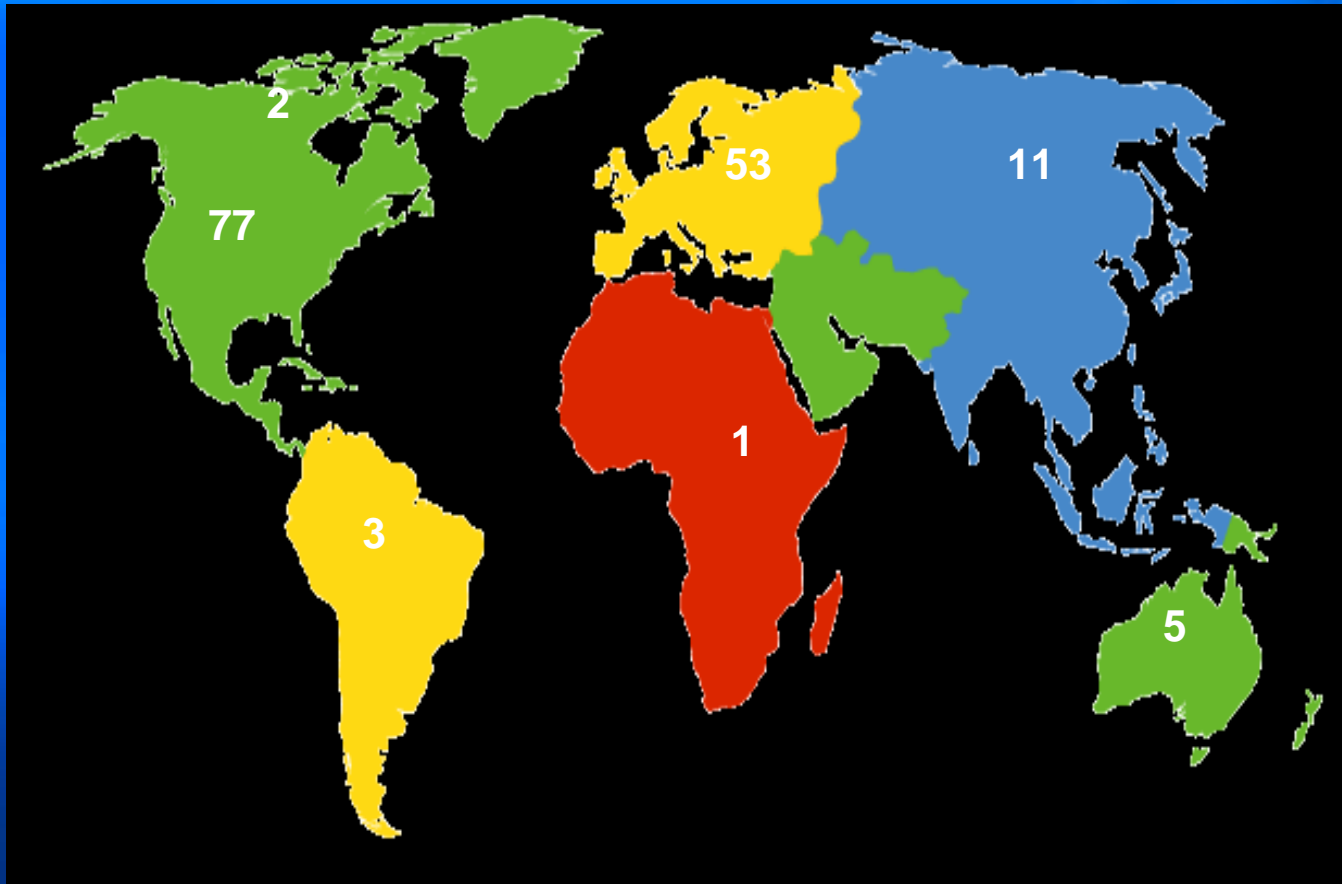
Method

- **Online survey consisting of 55 questions**
- **Survey was hosted by Dr. Robert Lyons at the University of Michigan**
- **Investigators were invited to participate by announcing the survey on the ABRF and the qPCR listservers**
- **Responses were stripped of any identifying information and exported to an Excel spreadsheet**
- **Participants were anonymous to the NARG**

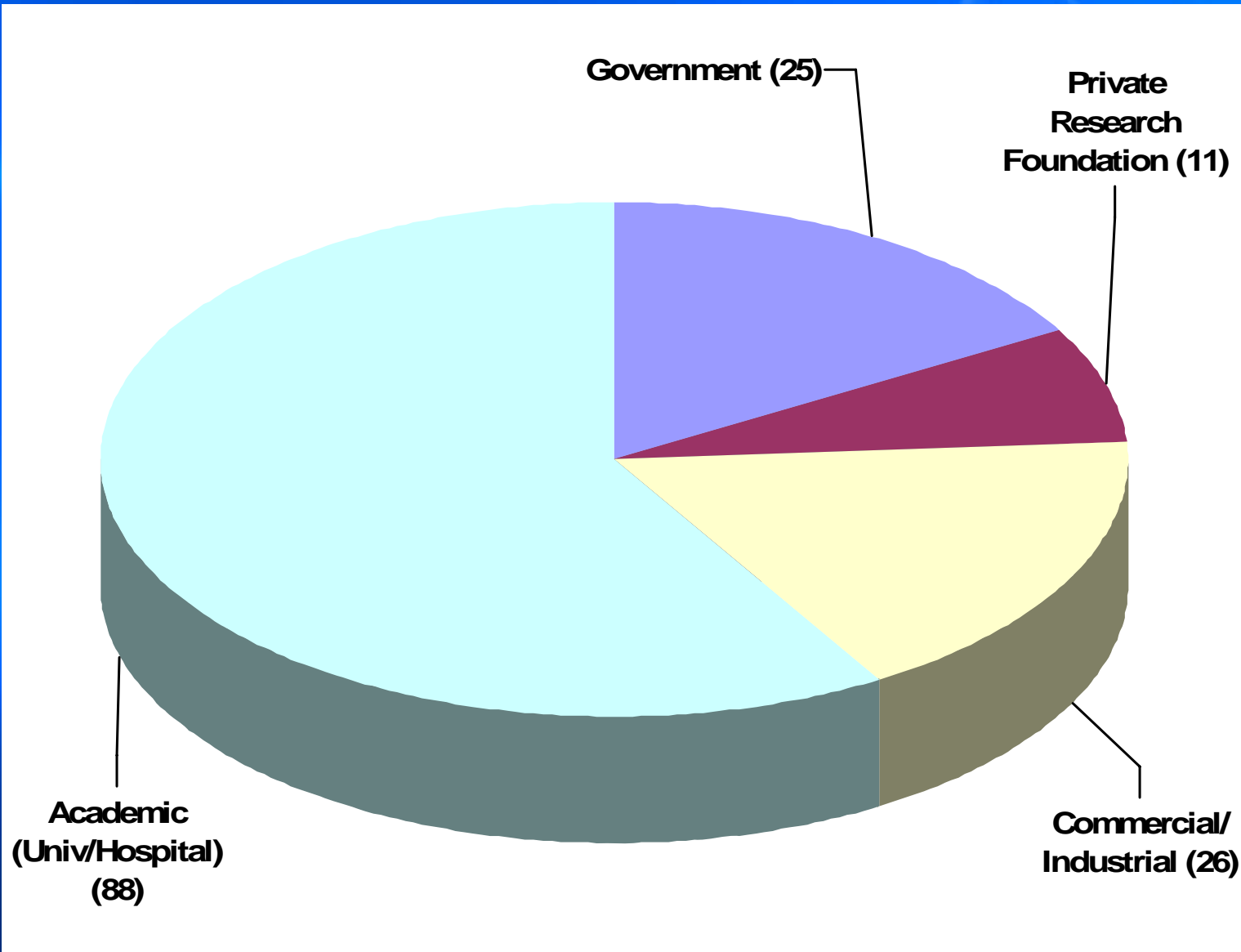


DEMOGRAPHICS AND FACILITY

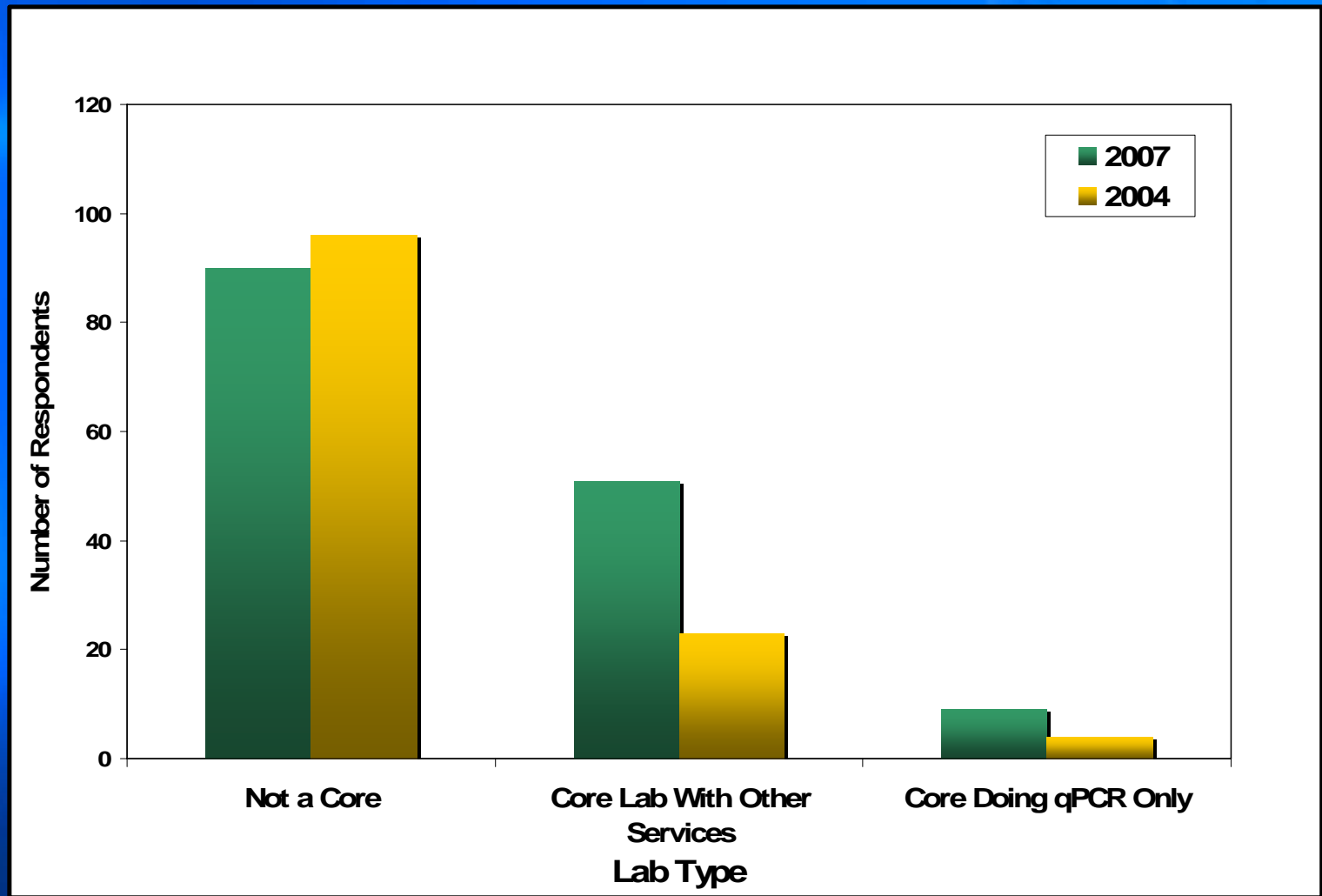
Geographical Location of Participants (N=152)



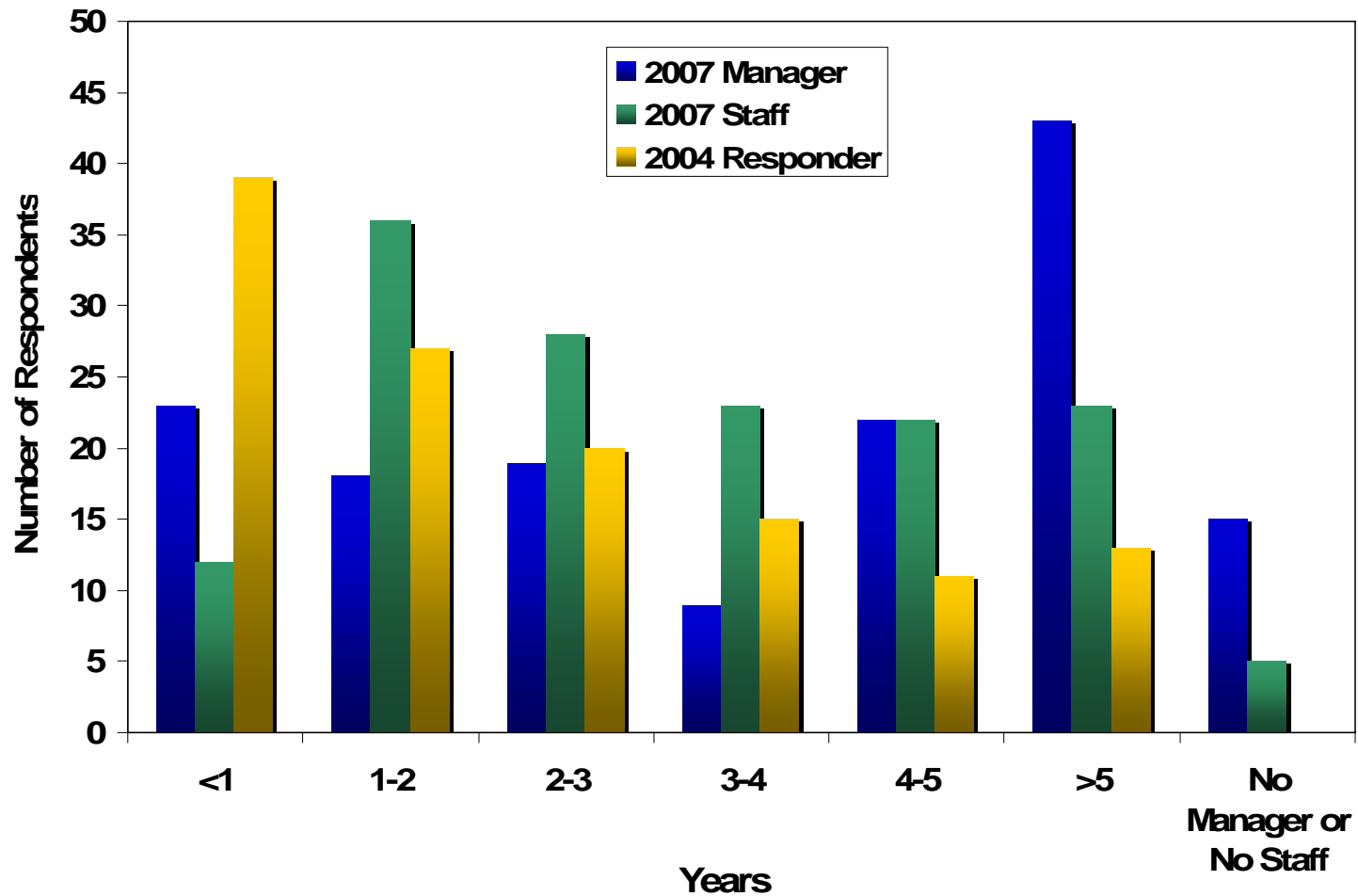
Institution Type (N=150)



Respondent Lab Type (N=150)



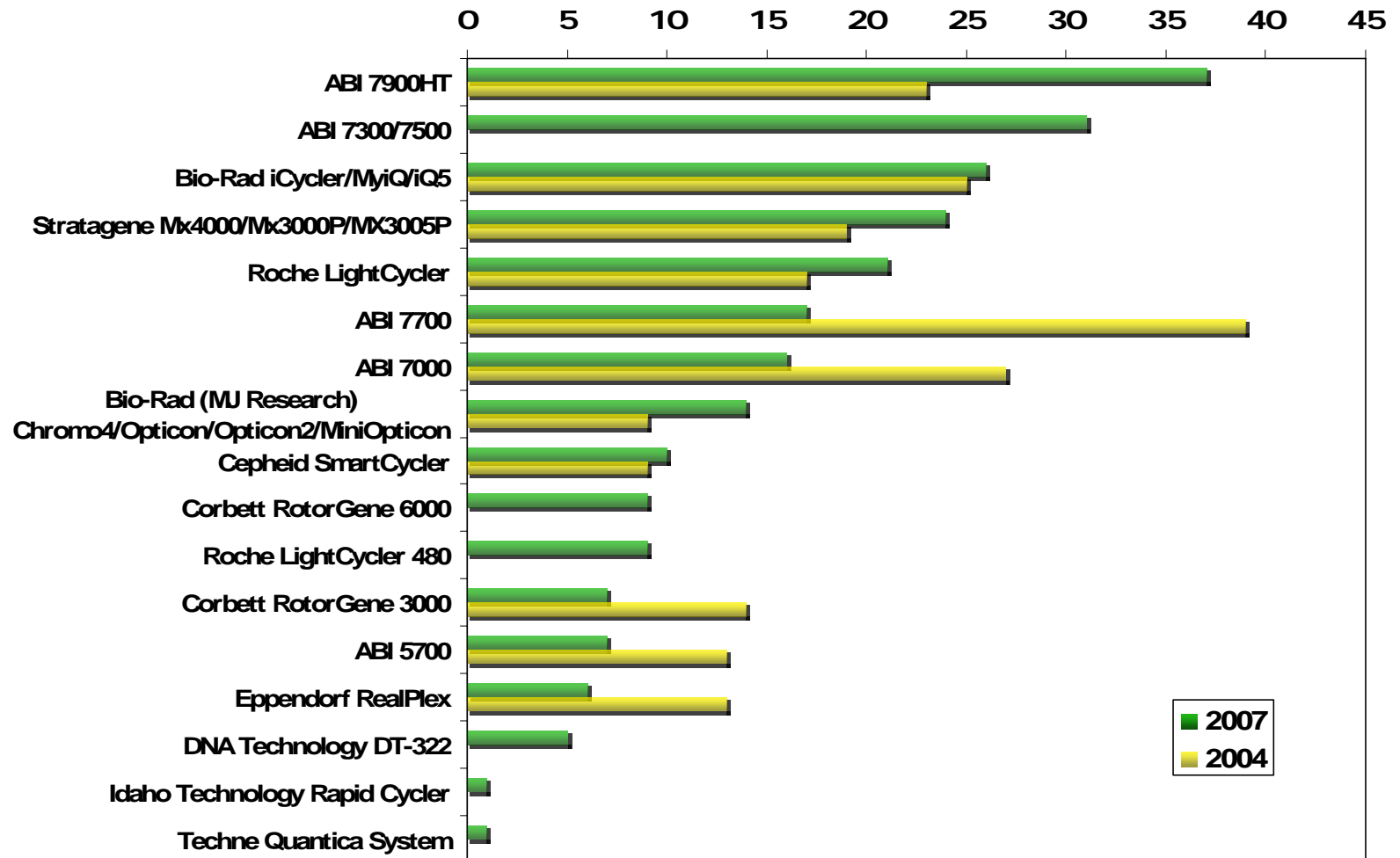
Years of Experience





INSTRUMENTATION

Real-time Instruments Used



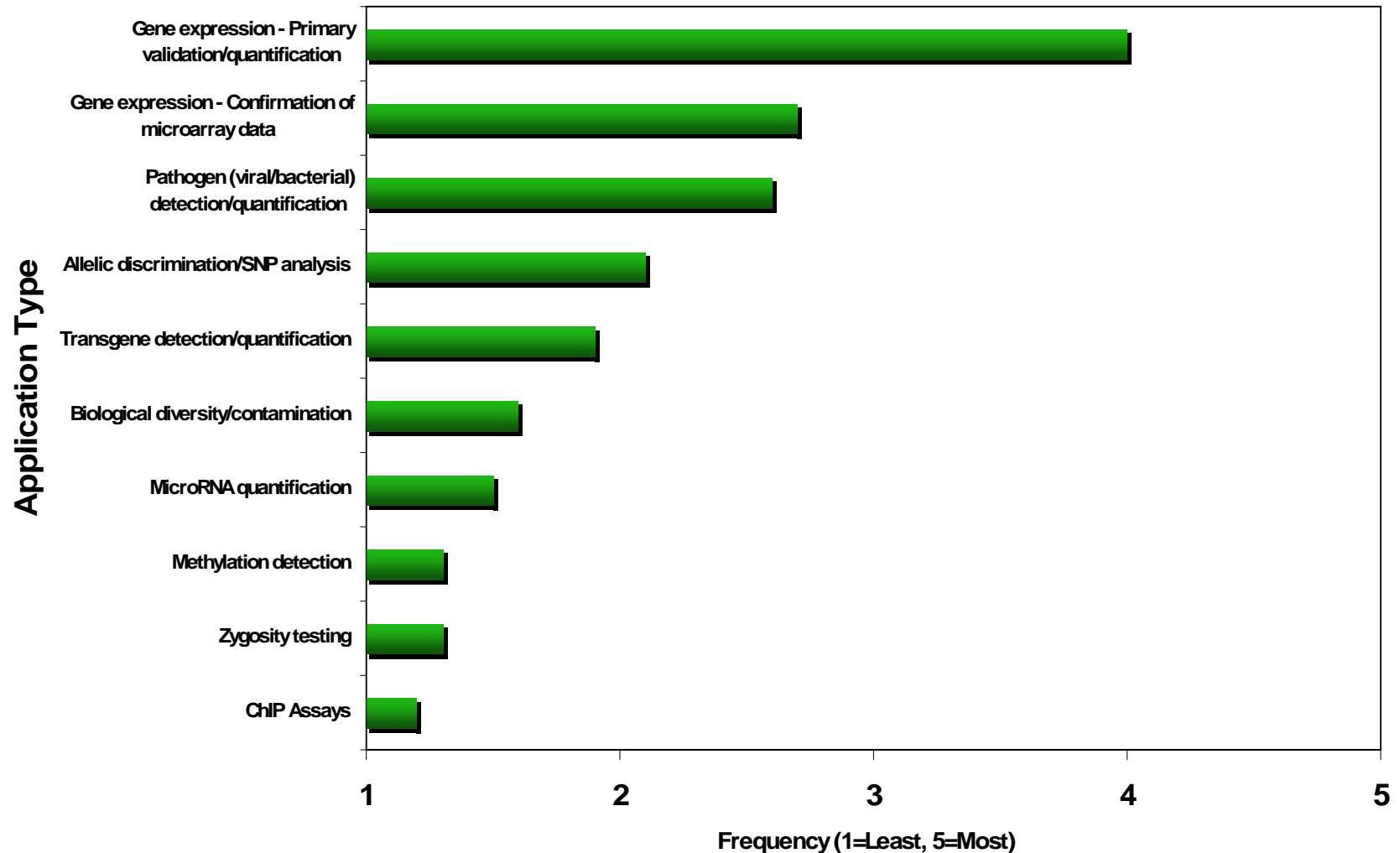
Other Instrumentation

- **14.4% of respondents use robotics to set up reactions**
- **The types of manual pipettors used:**
 - **66.7% single channel**
 - **28.8% 8 channel**
 - **9.2% 12 channel**
- **30% use a repeating pipettor**
- **Only 5.9 % (9 respondents) use robotics to load the instrument**
- **8 use the Caliper Twister to load the 7900 and 1 uses the Eppendorf 7075**



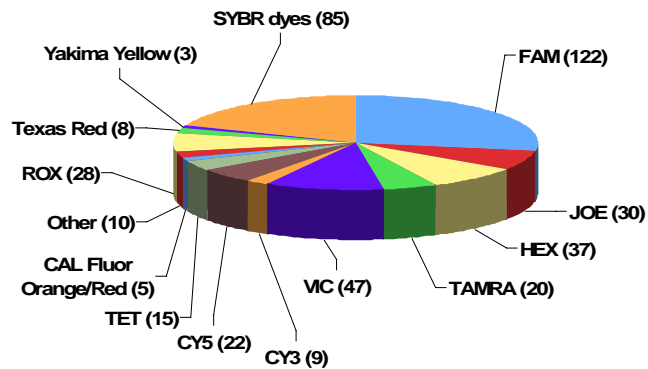
ASSAYS

Frequency of Use of Different Application Types

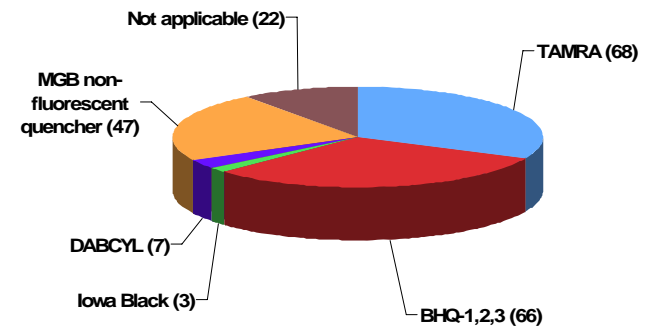


Reporter/Quencher Dyes Used

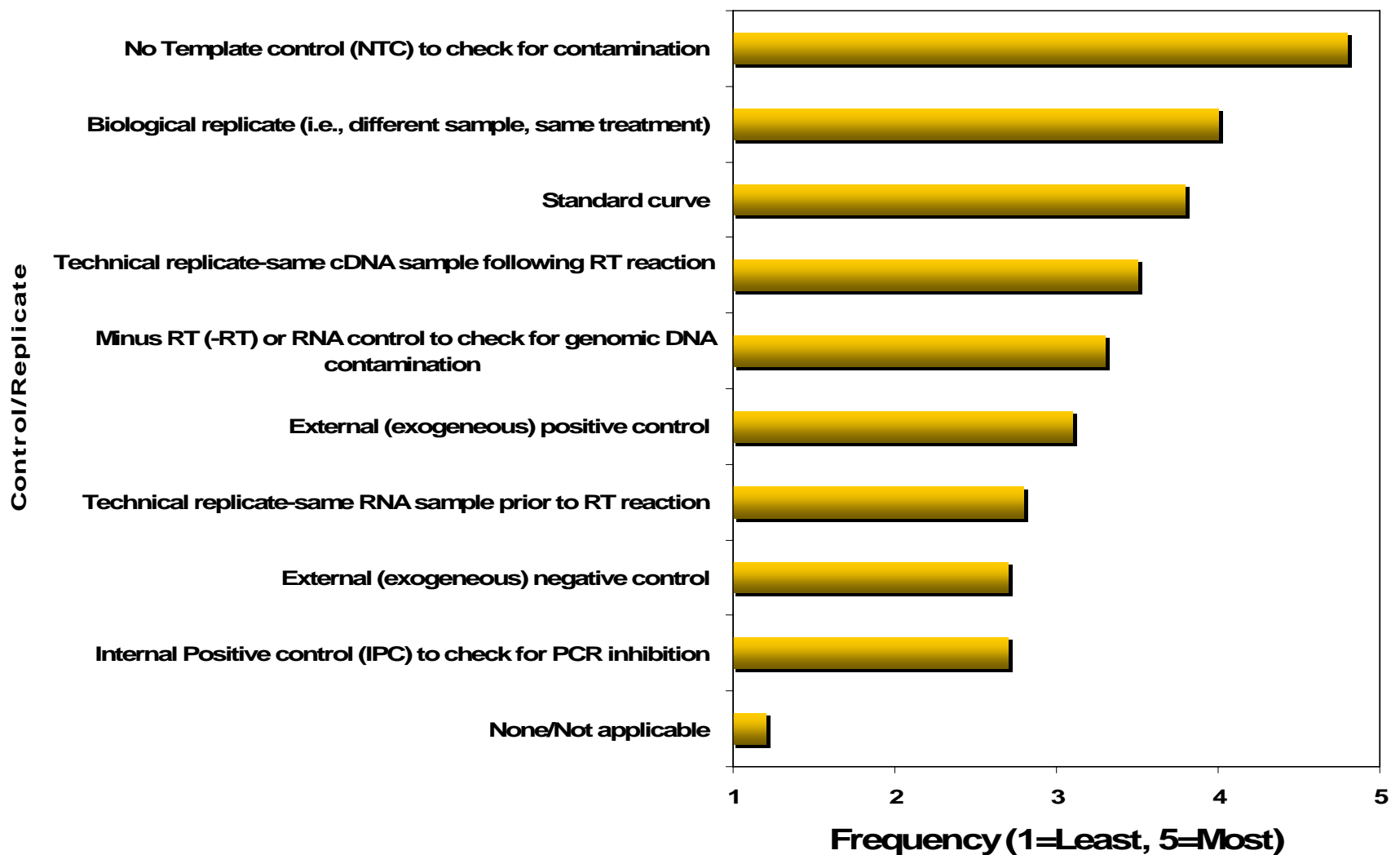
Reporter Dyes Used (N=149)



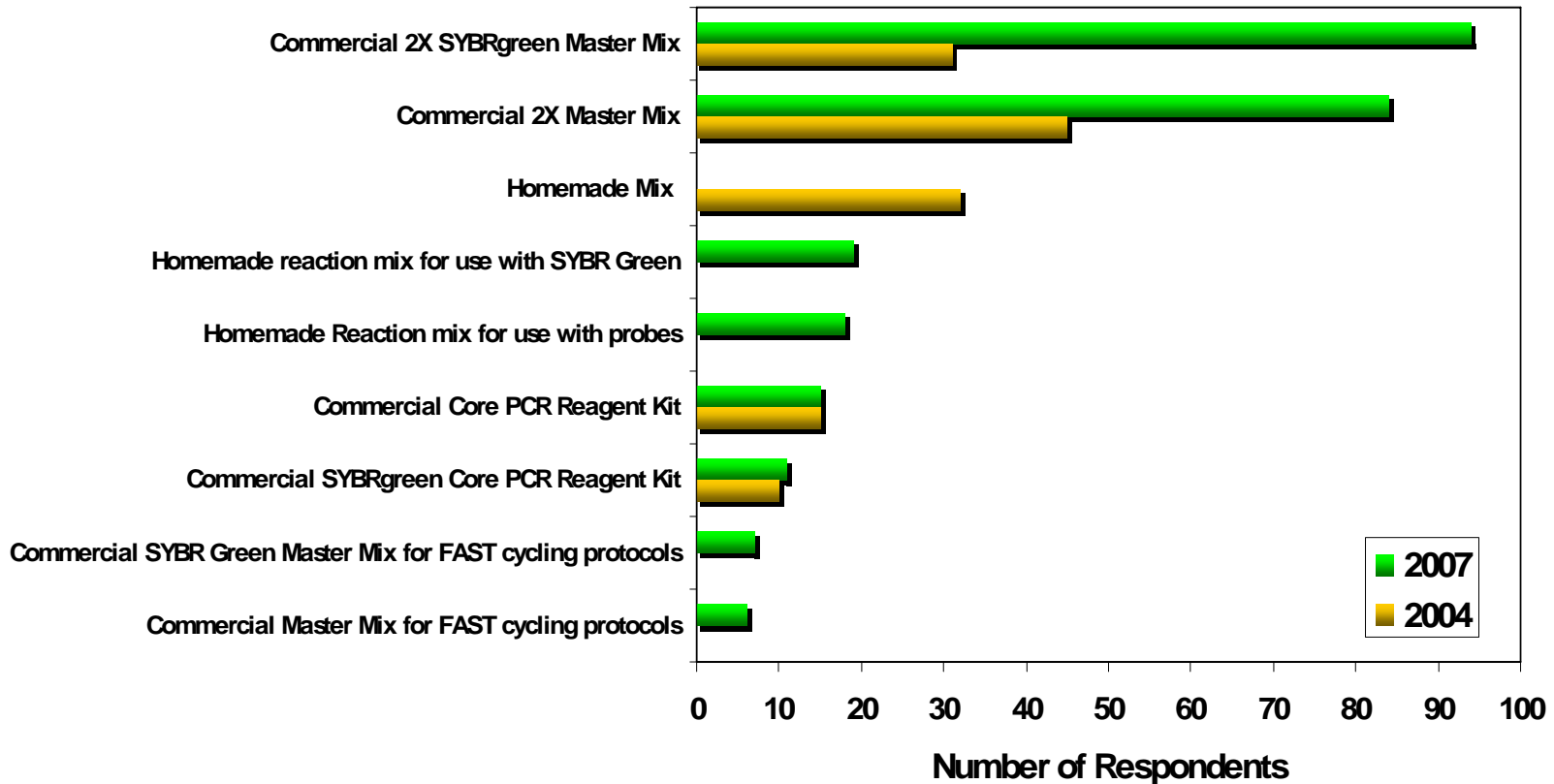
Quencher Dyes Used (N=136)



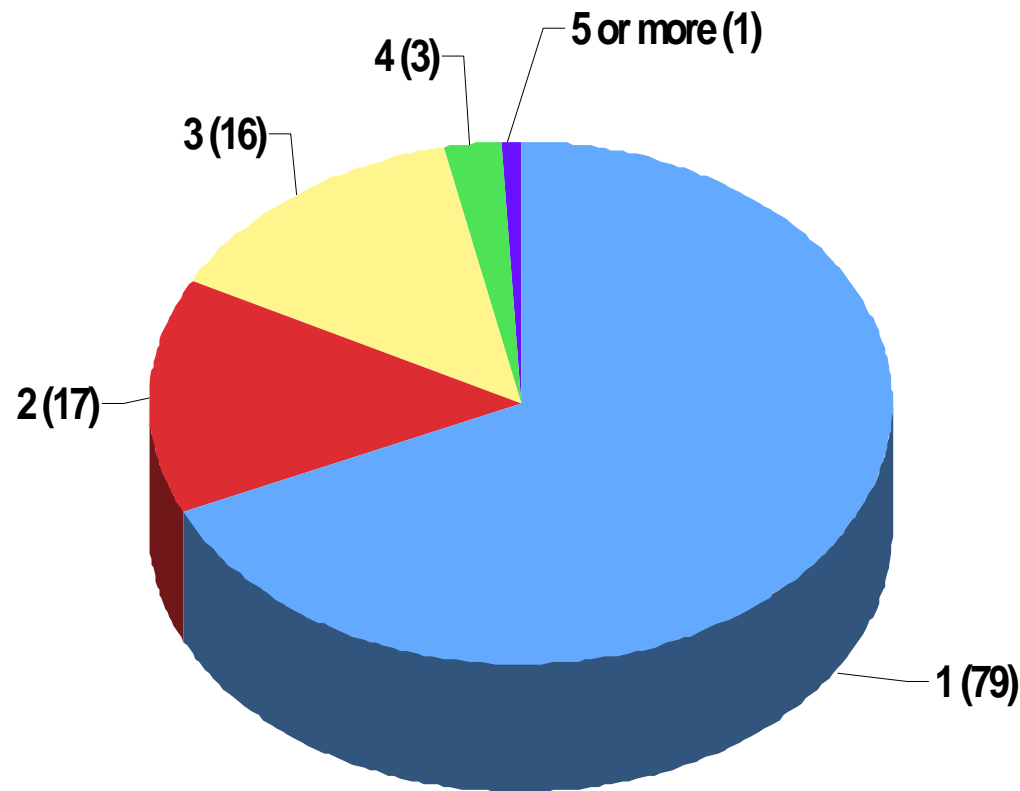
Frequency of Use of Controls/Replicates (N=148)



Master Mixes Used



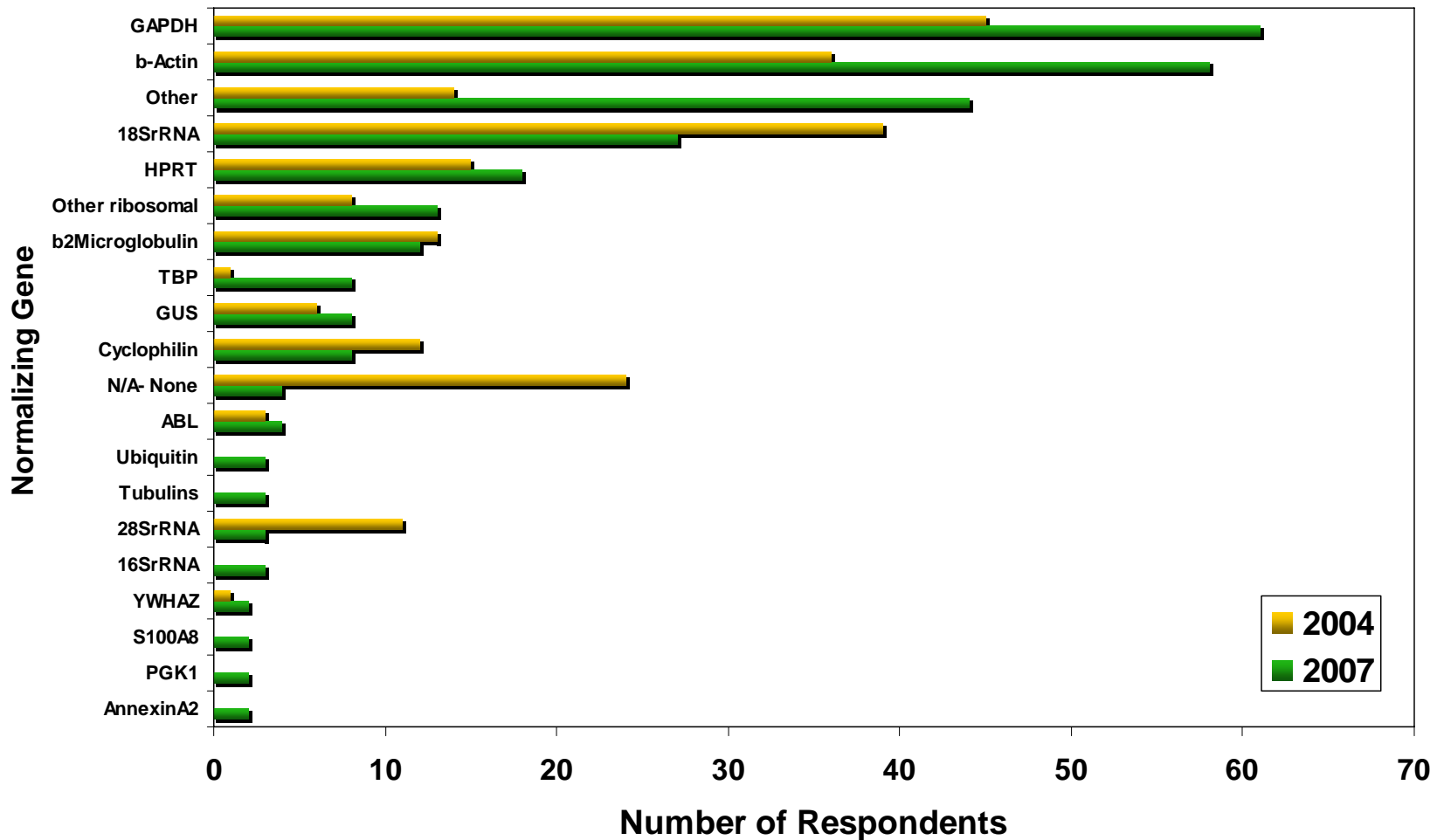
Number of Normalizing Genes Used



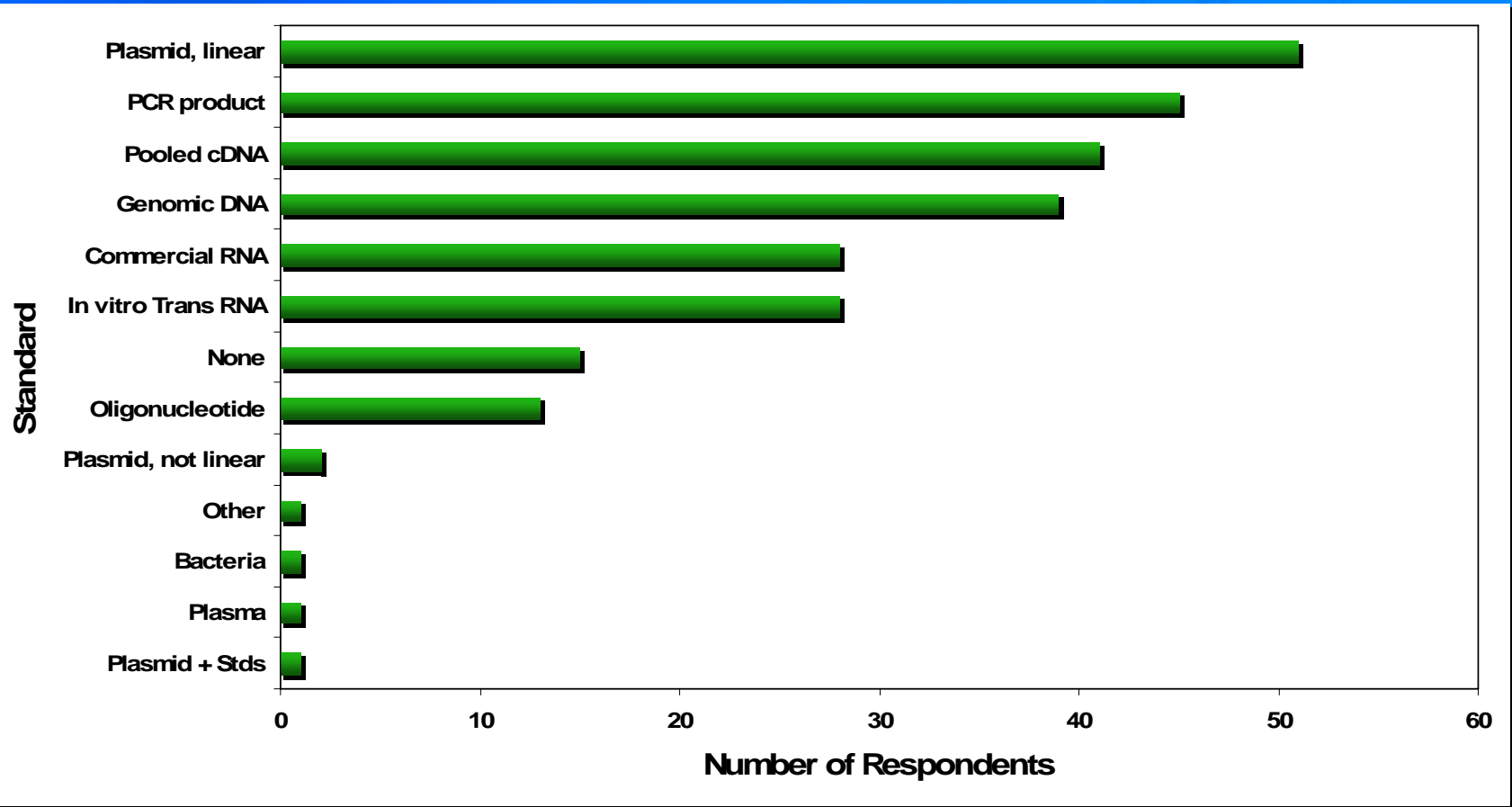
**~70% of Respondents Evaluate
Only 1 Normalizing Gene**

Normalizing Genes Used

Normalizing Genes Used by Respondents



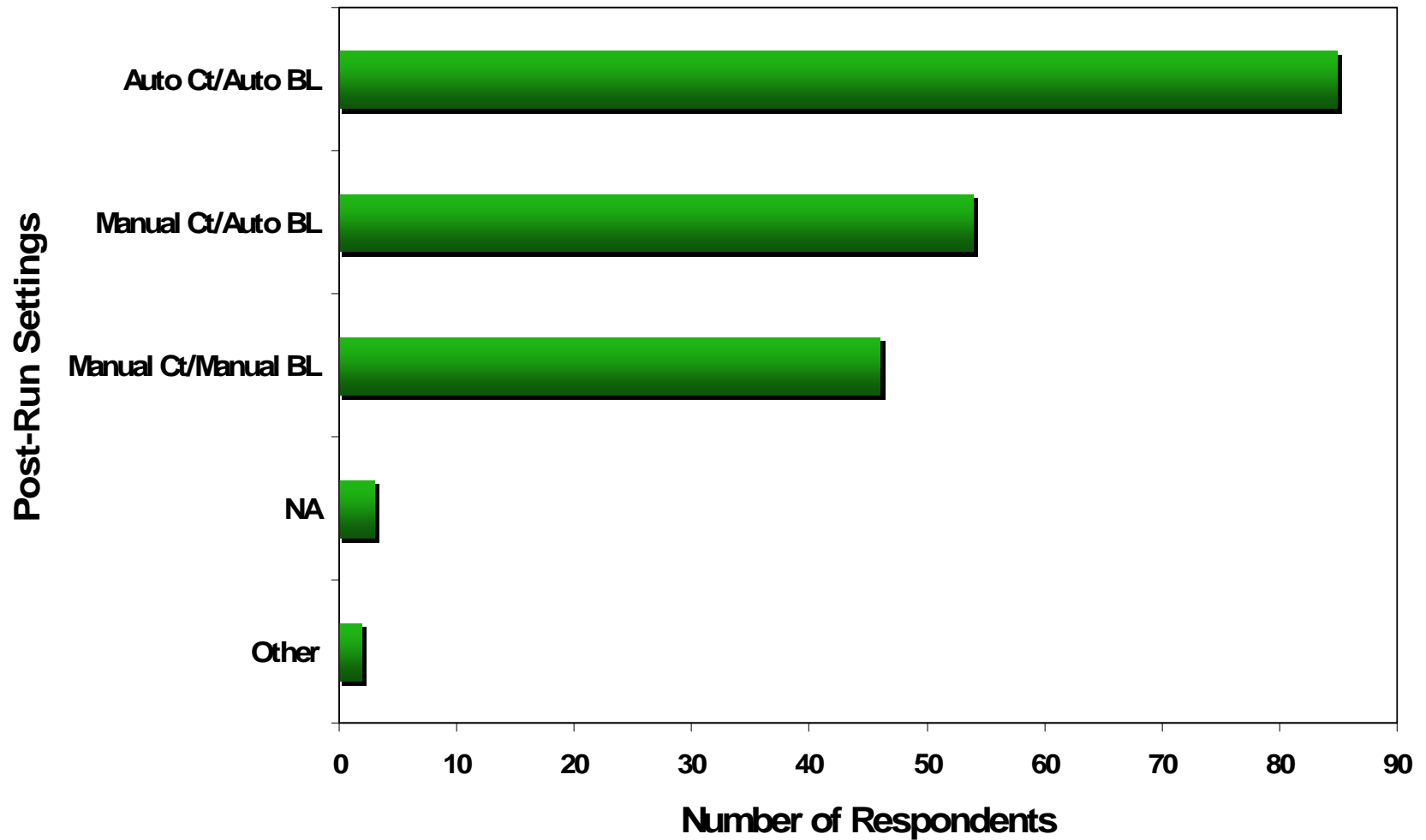
Quantification Standards



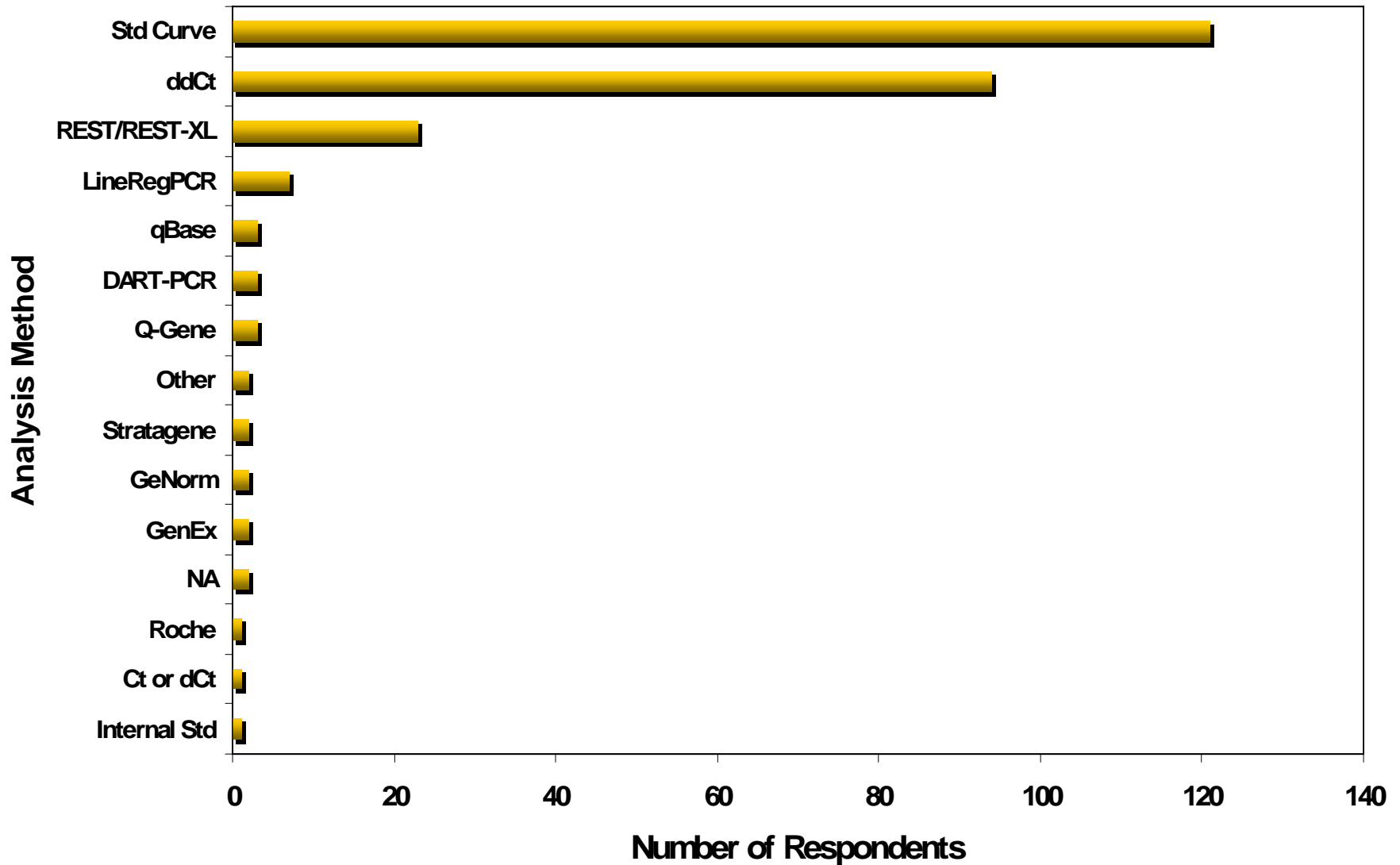


ANALYSIS

Instrument Post-Run Settings



Data Analysis Methods



Summary-Average Respondent

- Works in academia in North America and not in a core facility
- Measures gene expression using SYBRgreen ® or Taqman
- Designs his/her own assays
- Validates his/her assays by determining PCR efficiency
- Runs biological replicates and NTC controls
- Uses two step reactions for transcript analysis
- Transcribes his/her RNA with a MMLV RT at 42C using oligo dT or random primers
- Uses a heat-activated Taq and a commercial master mix
- ROX as a reference dye in a 25 µl reaction
- Analyzes his/her data using a standard curve obtained from a plasmid, PCR product, transcribed RNA, or genomic DNA
- Normalizes to GAPDH, β -actin or 18S rRNA
- Validates PCR efficiency in every assay, considering an efficiency of >90% to be acceptable

Nucleic Acids Research Group

- **Deborah S. Grove (Chair)** Penn State University
- **Deborah Hollingshead** University of Pittsburgh
- **Timothy Hunter** U. of Vermont
- **Kevin Knudtson** University of Iowa
- **Gregory L. Shipley** UTHSC- Houston
- **Pamela “Scottie” Adams** Trudeau Institute
(EB liaison)

<http://www.abrf.org/NARG>