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

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NUCLEIC ACIDS RESEARCH GROUP

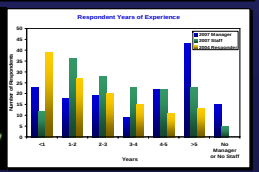
ABSTRACT

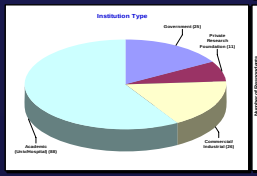
Real-time quantitative PCR (qPCR) has evolved to be one of the primary tools used by investigators to specifically detect and quantify nucleic acid levels. In an effort to follow the growth of this technology, the ABRF Nucleic Acids Research Group (NARG) has conducted periodic surveys designed to build a profile of a qPCR facility. The NARG conducted its first qPCR survey in 2004. The current 2007 survey was modeled after the previous survey. As such, comparisons will be made between the current 2007 and the previous 2004 surveys in an effort to illustrate changes in the way the qPCR technology has been used. Participation in the survey was requested from laboratories by posting instructions on qPCR-related electronic discussion groups. The survey was aimed at gathering information from academic, pharmaceutical, and commercial laboratories that offer realtime PCR technologies as a shared resource. Individual laboratories that have these technologies could also participate. A web-based survey was used to collect information on instrumentation, chemistries, staffing, and data analysis.

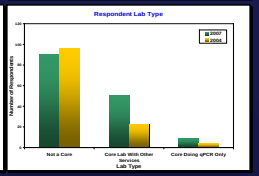
METHOD

Scientists from around the world were invited to participate by announcing the survey on the ABRF Discussion forum and the qPCR listserver at Yahoo.com. Submissions were anonymous and monitored only by IP address to eliminate duplicate entries. Participants submitted their answers via a webpage survey form created by phpESP and kindly hosted by Dr. Robert Lyons of University of Michigan. Results were exported to an Excel spreadsheet and analyzed by the members of the NARG. For some questions, more than one answer was permissible. A sampling of the results are presented in this poster. This poster and complete results (raw data) will be made available on the ABRF NARG web page.

DEMOGRAPHICS & FACILITY







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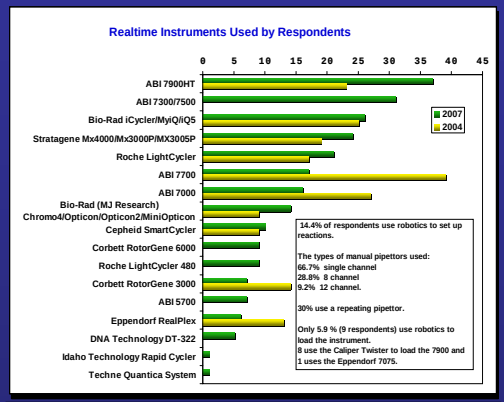

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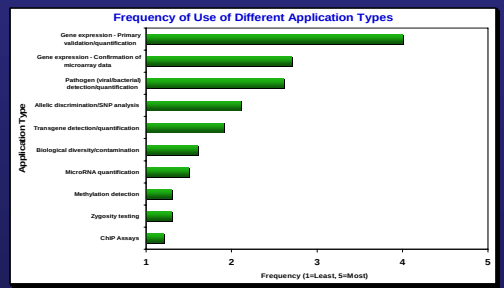

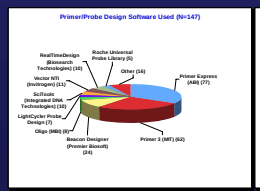

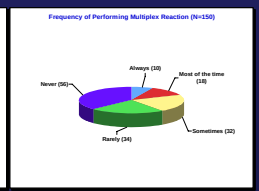

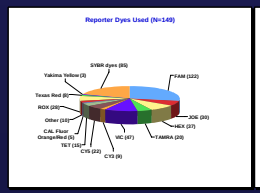

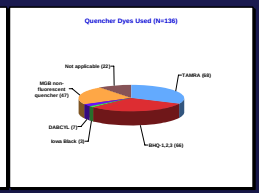

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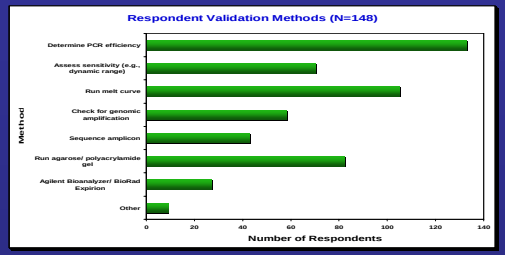
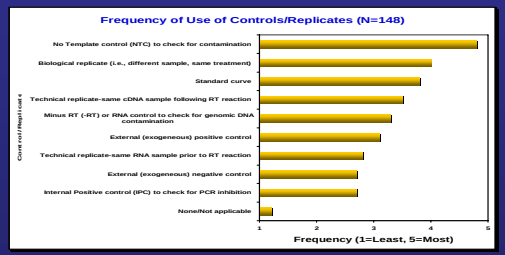
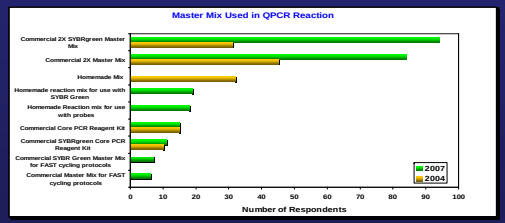
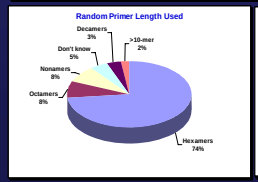
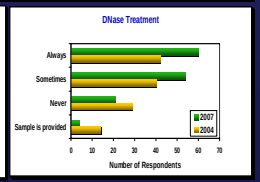


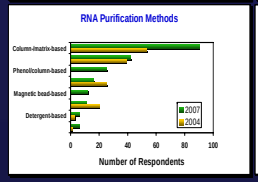


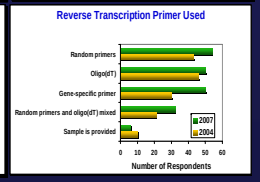
ASSAYS



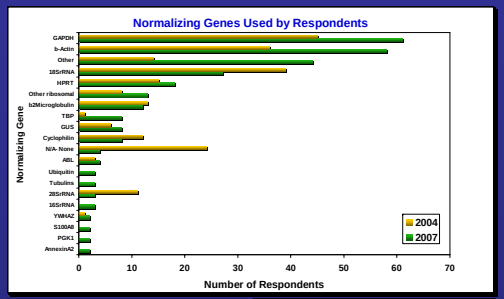


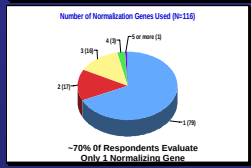


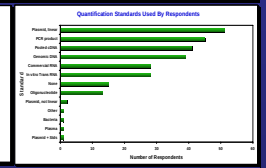


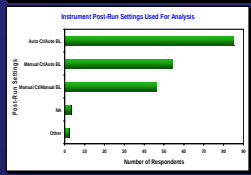


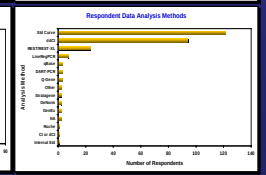
ANALYSIS











SUMMARY

The Average Respondent

- Works in academia in North America and not in a core facility
- Runs 0-1000 wells/month and has less than 2 years of experience and does not use robotics
- 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 RNA as a template that was purified using a column
- Uses two step reactions for transcript analysis
- Transcribes his/her RNA with a MMLV RT at 42^{0}C 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