Nucleic Acids Research Group 2004 Study: Real-Time PCR Survey

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The Association of Biomolecular Resource Facilities — Research • Technology • Communication • Education

NUCLEIC ACIDS RESEARCH GROUP

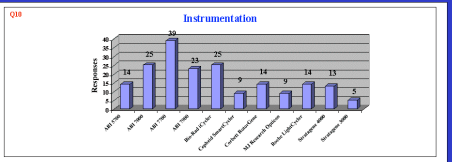
ABSTRACT

The Nucleic Acids Research Group (NARG) of the Association of Biomolecular Resource Facilities (ABRF) conducted an on-line survey designed to "take the pulse" of the real-time PCR international community. Respondents were asked questions concerning the type of facility, personnel, instrumentation, types of assays, templates, extraction methods, controls, analysis, etc. Additional questions for those that run core facilities that offer real-time PCR as a service were included. Participation was anonymous. Results provided insight into the way real-time PCR is being used in a variety of laboratory settings. Results will be freely available to all online.

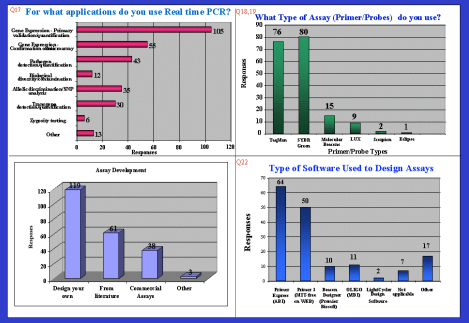
METHOD

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.

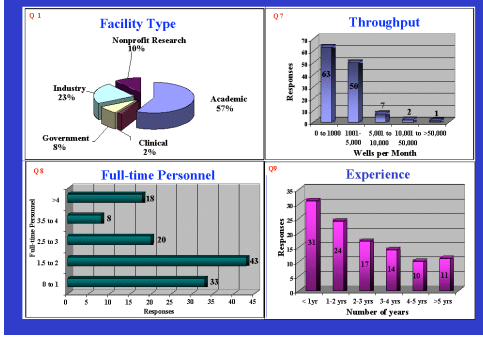
INSTRUMENTATION



ASSAY DEVELOPMENT



FACILITY



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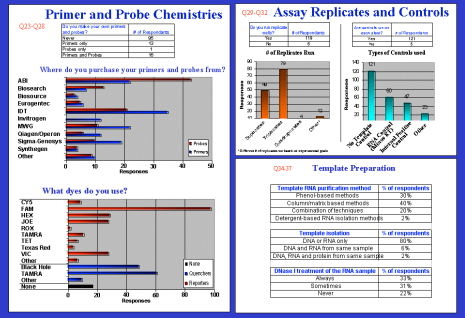
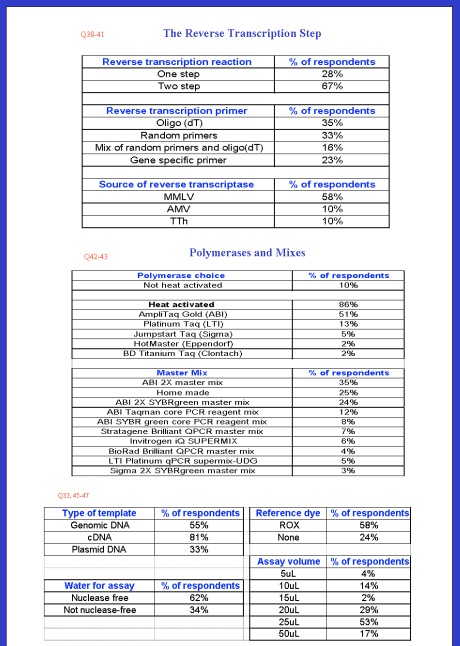
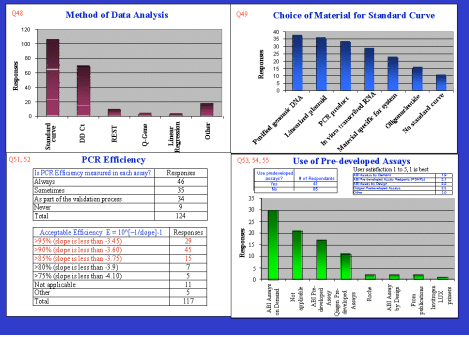


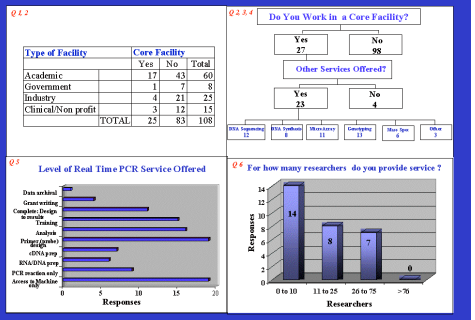
ASSAYS



ANALYSIS



CORE FACILITIES



SUMMARY

The Average Respondent

- works in academia and does not belong to a core facility
- runs 0-1000 wells/month and has less than 2 years of experience
- uses a 7700 and does not use robotics
- measures gene expression using SYBRgreen ® or Taqman
- designs his/her own assays using Primer Express
- orders his/her primers from IDT and his/her FAM/TAMRA probes from ABI
- validates his/her assays by determining PCR efficiency
- runs replicates and NTC controls
- uses cDNA as a template that was purified using a column
- uses two step reactions
- transcribes his/her RNA with a MMLV RT at 42°C using oligo dT or random primers.
- uses a heat-activated Taq and a commercial master mix with Amplitaq® Gold Taq
- uses ROX as a reference dye in a 25 ul 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 RNA
- validates PCR efficiency in every assay, considering an efficiency of >90% to be acceptable
- has not used a pre-developed assay.

ACKNOWLEDGEMENTS

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