

QUANTITATIVE MOLECULAR BIOLOGY (QMB)

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Introduction

The QMB module will facilitate the quantification of mRNA and DNA requirements for faculty and staff. This module also offers access and consultation to technologies designed to characterize and quantify changes in gene expression. The QMB module is located at the MSC and adjacent to the University of Wisconsin Biotechnology Center, which is the location of the facilities required for the microarray analysis.

Services

Primer design

Investigators unfamiliar with PCR primer design can consult with the QMB Core to design primers best suited for PCR needs. Primer design strategies to amplify homologous sequences from species where there may not be a published target sequence can also be utilized.

PCR optimization

PCR will be performed across a temperature gradient of 40-70°C using an Eppendorf eppgradient Mastercycler (acquired 2003). Products will be analyzed by standard agarose gel electrophoresis, followed by ethidium bromide staining, and a digital image will be provided to the user. The user will provide the primers and template DNA for this service unless “Full Service” is requested.

Quantitative real time PCR (Q-PCR)

Optimized qPCR will be performed on an ABI 7300 Real Time PCR machine (acquired 2004) using Taqman SYBR Green I (as the primary dye). Data generated from each run will be exported into an Excel Spreadsheet for use by the customer. The customer may also be able to utilize the ABI software on the 7300 machine for analysis provided there is no run ongoing. ABI analysis software is also available for use on remote computer stations, but this is not a service that is available at this time. The fragments generated in each qPCR reaction can also be independently analyzed by agarose gel electrophoresis upon request. Each qPCR run will also contain data on melt-curves for each reaction vial, which provides information on the specificity and integrity of the product bands in the sample (low stringency products, such as primer dimers, are detected using this feature). Although SYBR Green is the dye choice available for this service, the 7300 also has the capacity to detect multiple dyes allowing for multiplexing of PCR products. Users interested in multiplexing should be prepared to purchase their own reagents separately rather than use Core reagents for a fee. Three services for qPCR are available, including:

- Run Only, where the User prepares a plate or strip tubes of samples and reagents from his/her own laboratory.
- Set-up and Run, where the User brings in template DNA and primers. The Core facility will prepare each reaction accordingly and execute the PCR run.
- Full Service, where the User brings in sample RNA (and primers) only. The Core facility will prepare the template cDNA, optimize the PCR conditions, and execute the qPCR run.

Probe synthesis

RNA antisense (and sense control) probes (riboprobes) will be synthesized upon request. Probe synthesis can be made using radioisotopic incorporation, or non-radioactive probes can be synthesized using digoxigenin labeled nucleotides. Probe synthesis will be conducted using constructs of target sequences cloned into appropriate plasmid vectors that contain RNA polymerase binding sites flanking the target sequence. Plasmid should be provided (unless otherwise contracted) complete with a restriction map. The Core will linearize and purify the plasmid, and then make and purify riboprobes ready for use. The user has several options for this service including:

- Digoxigenin labeled probes – RNA probes will be made by incorporating 11-Dig-UTP into the reaction. Probes will be purified by eliminating template DNA and phenol chloroform extraction and precipitation. Purified probe will be assayed by formaldehyde-gel electrophoresis.
- Radioactive probe synthesis – RNA probes will be synthesized by incorporation of radioisotopes into the synthesis reaction. For ^{32}P -UTP labeled probes used for RNase protection assays, probes will be synthesized and purified by elution from urea polyacrylamide denaturing gels.
- Full service probe preparation – Full service preparation will include initial cloning (or subcloning) a suitable target sequence into a plasmid that can be used for riboprobe synthesis. This service can include PCR cloning of a region of a target molecule, or subcloning of a small fragment from a larger DNA sequence provided by the user. Cloning or subcloning strategies (to achieve the most optimum target sequence for riboprobes, depending on the application) will be initially conducted in consultation with the Core director.

Microarray services

The QMBC will provide technical support for use of the microarray facilities available through the University of Wisconsin Biotechnology Gene Expression Center (GEC). Services at the GEC include hybridization and reading of Affymetrix gene chips, and the printing, hybridization, and analysis of glass slides containing arrayed DNA spots. The technician will be an expert liaison between ophthalmology laboratories and the Biotechnology Center and either do the printing and analysis of arrays through the Center, or provide technical assistance to Investigators who wish to use the facility themselves. Detailed descriptions of services offered at the GEC, complete with pricing, are available at: www.biotech.wisc.edu/gec/Products_services.asp. Investigators using services at the GEC are responsible for the charges issued for these services. Technical assistance offered by the QMBC is not charged unless the QMB Core technician executes the GEC service for individual investigators.

Special Services

In special circumstances, the QMB Core can provide full services to quantify RNA samples using conventional methods such as Northern Blotting and RNase Protection Assays (RPA). Northern blotting services can be obtained in two formats, including a full service format where the QMBC will run formaldehyde gels of total RNA or polyA(+) mRNA (provided by the User), blot the RNA to GeneScreen Plus nylon membrane, and probe the membrane with probes made by the QMB Core. Probed filters will be exposed to X-ray film or PhosphorImager screens. A secondary service provided by the QMB Core will be to run the formaldehyde gels and blot the RNA to membrane. The membrane will then be provided to the User. RPA can also be contracted out using the QMB Core. RPA is done using total RNA provided by the User and probe prepared by the QMB Core. Digestions are done using a method employing RNase ONE as the digesting nuclease. The QMB Core will expose the

resulting digests to X-ray film and quantify the protected fragments using PhosphoImager analysis. Special services are provided only when there is available time in the QMB Core or in certain circumstances. Consultation with the QMB Core is strongly recommended if these services are desired.