

T R U D E A U I N S T I T U T E
Molecular Biology Core Facility
(MBCF)

ESTABLISHED SERVICES

Class I Tetramers

Production of Class I tetramers is performed on a routine basis. To date, 702 batches of successful murine MHC Class I tetramers have been produced representing 48 different tetramers. Staining reagents can be supplied as monomers or labeled with PE or APC. Plans are in progress to provide Pacific Blue in the future. De novo tetramers can be prepared to suit the researcher using the Db, Kb, Kd and Dd alleles. Recently, the MBCF has generated combinatorial tetramers for staining of limited samples with 5 different epitopes, using a combination of PE, APC and PECy7. Class I tetramers are generally available within 24 hours of request. De novo tetramers require approximately two weeks to generate. Tetramers are traditionally used in FACS analysis but have been successfully used in In Situ Histochemistry (ISH). The MBCF is internationally recognized as a provider of quality Class I tetramers and provides tetramers as part of collaborations to researchers throughout the United States, Canada, Europe and Japan.

Class II Multimers/Tetramers

The MBCF, in collaboration with Dario Vignali of St. Jude Children's Research Hospital has successfully prepared 33 different Class II dimers using the Invitrogen Drosophila Expression system (DES). All unlabeled Class II multimers could be successfully used for plate-bound in vitro stimulation. However, only the SenHN(419-433)/A^b multimer labeled with AlexaFluor 647 and multimerized with Protein A has been used extensively by the Woodland and Mohrs labs for staining CD4⁺ T cells in a Sendai infection. Recently, the MBCF has developed an IAb/Mtb Esat-6 tetramer obtained from the Marc Jenkins laboratory and prepared with the cooperation of Bill Reiley and Gary Winslow and the Wadsworth Expression Core. The IAb/Mtb Esat-6 tetramer is currently produced entirely in house. The MBCF plans to generate other Class II tetramers using the Jenkins approach.

DNA Sequencing, Genotyping, Fragment Analysis, Spectratyping

The MBCF currently has an Applied Biosystems Model 310 automated fluorescent DNA sequencer. There are two levels of DNA sequencing service available: (1) The user provides the DNA (and primers, if necessary) and the MBCF will perform the sequencing reaction, cleanup the samples and run them on the sequencing gel. (2) The user does the sequencing reactions and sample cleanup and the MBCF runs these samples on a sequencing gel. The latest version of Sequencher software for Macintosh computers by Genecodes can be installed on any Mac using OSX. A one-user license is currently in force. The MBCF has optimized the system to allow DNA sequencing or Spectratyping/Fragment Analysis/Genotyping to be run interchangeably with no down time. GeneScan analysis software for spectratype analysis is available to be installed on any Mac computer which can run OS9. DNA sequencing and spectratyping are performed on a routine basis. Turnaround time is generally 24 hours.

Quantitative RT-PCR (Taqman)

The MBCF contains two (2) Applied Biosystems instruments for quantitative real time fluorescent PCR (RT-qPCR), the Model 7700 and the new Model 7500 with a FAST block. The MBCF has designed, tested, and verified the specificity of a panel of more than 100 primers/probes for the most common chemokine/cytokine/receptors which are available for general use, including an assay for the PA gene of the Influenza PR8 virus and the NP gene of the Sendai virus. SuperArray PCR arrays are available through the MBCF and a Taqman assay for any murine gene is available from Applied Biosystems at a discounted price through the MBCF. Reverse transcription kits and 2X Master mix to run the assays are stocked by the MBCF along with the >100 pre-made assays. The MBCF will, under certain circumstances, run your Taqman assays for you. The MBCF can design any new assays that are required, including those for splice variants that may not be available commercially. Analysis software for the 7700 can be installed on any Mac which can run OS9 and analysis software for the 7500 can be installed on any PC using Windows XP. Melt curve analyses are available for both instruments. Instructional courses, consultation, equipment maintenance and reagents are provided by the MBCF. Twice yearly update seminars on quantitative real time PCR are organized by the MBCF. Routine testing of new reagents to lower expenses to the end user is performed.

RNA/Protein "Lab on a Chip" analysis

The MBCF has a Bio-Rad Experion for RNA and protein analysis using the latest "chip" technology. Validation of RNA quality can be performed using as little as 0.1 ng/ul RNA in 2 hours. Results are returned as individual chromatograms of each sample, a gel image and a RQI (RNA Quality Index). The 28s/18s ratio is computed and quantitative values are generated for sample in the 25-500 ng/ul range. Turnaround time is generally 24 hours.

Recombinant Protein Production

The MBCF will produce recombinant proteins as a special project request. A variety of vectors are available. FPLC equipment is located in a refrigerated cabinet and size exclusion, affinity and ion exchange columns are in-house.

Primer/Probe Design and Production

The MBCF will design primers and/or probes for any application, including probes for ISH, methylation assays and Taqman assays. The MBCF will prepare RNA/DNA probes for use in ISH, methylation assays, Southern blots, RPAs, etc. It is not necessary to have a plasmid containing the DNA of interest. The MBCF can use PCR techniques to add T3 /T7 promoter sequences on the ends for transcription. The MBCF can make biotinylated probes.

Primer/Peptide ordering

The MBCF will order your primers/peptides for you or give you the information on how to order your own. We have made special arrangements with New England Peptide to get peptides at a reduced price. Similar discounts are available through IDT for regular primers, fluorescently labeled primers (for spectratyping) and phosphorthiolated CpG ODNs.

Reagents (Taq Enzyme)

The MBCF is always willing to provide Molecular Biology reagents to other labs, which are charged back at cost. The MBCF prepares standard Taq enzyme appropriate for use in viral Limiting Dilution Assays (LDA) and PCR screening of mice. MBCF Taq is a fraction of the cost of commercial Taqs.

Transgenic Mouse Screening

The MBCF is available to help out with transgenic mouse screening on a limited basis when a laboratory is short on personnel. The MBCF will extract the DNA, quantify the DNA and perform the PCR reactions or any combinations of steps. The MBCF can assist with assay design, troubleshooting and multiplexing.

Consulting

The personnel in the MBCF are always available to answer your questions and provide advice.

Monthly Accounting Reports

The MBCF provides each investigator with a monthly statement detailing charge-backs for each service /reagent provided by the MBCF. Records of every MBCF transaction are available at the end of the month on the Shared server.

Webpage

An Intranet (in-house) Webpage has been developed by the MBCF in conjunction with the IT department. It features current information on the types of services offered by the MBCF, web order forms and other useful information on Molecular Biology techniques, protocols, search sites, etc.

SHARED EQUIPMENT

NanoDrop Spectrophotometer (1 ul required, DNA/RNA/Protein quantification)

Bio-Rad ChemiDoc system (DNA/RNA/Protein/ gel/ Western blot documentation)

Bio-Rad Molecular Imager (densitometry, image analysis)

Fisher Sonic Dismembrator (large probe for large scale cell disruption)

Applied Biosystems Model 7700 Real time PCR machine

Applied Biosystems Model 7500 FAST Real time PCR machine

Qiagen TissueLyser II (high throughput homogenization of tissue samples)