Cleveland Clinic Lerner College of Medicine
of
Case Western Reserve University
Department of Molecular Medicine

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University Center for Innovation in Teaching and Education
Allen Building 101
Case Western Reserve University

NORD Grant Application for initiating a laboratory component of the Molecular Methods and Tools Course for first year graduate students in the CWRU School of Medicine, Molecular Medicine Ph.D. Program

Charles Tannenbaum

Dear NORD Grant Committee,

For the last several years, first year students in the Molecular Medicine Ph.D. Program have begun their studies by taking an intensive, 6 week course reviewing the basic laboratory techniques employed in the performance of modern biological research. The purpose of this "tools class" is to prepare the students for their laboratory rotations, journal club, subsequent courses, and eventual long term research projects, by providing them with an overview and understanding of the methodologies they are likely to encounter or utilize while pursuing their goals. Heretofore the course has consisted solely of daily, two hour seminars focusing on specific molecular techniques, emphasizing their underlying principles, their uses, and in very general terms, how they are carried out, controlled, and analyzed. Conversations with students and faculty alike now suggest that the sessions would be greatly enhanced by incorporating a laboratory component. This stems from the realization that in spite of their hours of didactic classroom training, the students acknowledge that upon completion of the course they arrive at their laboratories without ever having actually performed many of the techniques they heard and read about. The purpose of this NORD Grant application is to request the funds needed to develop a laboratory component of the tools course that will complement and parallel what the students learn in lecture. A gene cloning project carried out during the 6 weeks of this class, using many of the techniques discussed in lecture, should provide the students with hands-on experience in the methodologies they'll need to perform both their rotations and thesis projects independently and with greater insight and confidence.

For the laboratory component of the tools course, each student will be assigned his or her own cytokine or chemokine gene to clone. This task will introduce the students to a number of computer-based, on-line analytical programs, such as those for DNA Blasting and sequence determination, identification of open reading frames and signal sequences, restriction analysis, translation, and primer selection. Bench techniques learned and employed for this project will include DNA and RNA isolation and quantification, RT/PCR, agarose gel electrophoresis, gel extraction, DNA cloning and subcloning, minipreps, restriction enzyme analysis and digestion, transfection, cell culture, Real Time PCR, western analysis, immunoprecipitation and ELISA. Second, third and fourth year graduate students also stand to gain pedagogical experience from this new laboratory component, as it will afford them the opportunity to be teaching assistants for those sessions utilizing techniques at which they're specifically skilled. A

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list of the reagents needed for the laboratory sessions is appended below, and together come to approximately $4,000. While some will need to be purchased on a yearly basis, many will last for multiple years, thus significantly reducing the future annual cost of supporting the program. Gel boxes, power packs, pipetmen, pipet aids and other necessary hardware will be loaned to the class as needed from various laboratories within the Lerner Research Institute for the several hours they're needed, further reducing the costs of the project.

Details of the Laboratory Component

The students will first design the appropriate primers for cloning their specific cytokine gene by first consulting GenBank for the protein's coding sequence. It should be noted that the primers to be used for cloning each cytokine have already been purchased and validated; thus once the students design their 5' and 3' primers, they will be given an aliquot of each. Total RNA will be isolated from a cell line known to express the molecule, and will be converted to cDNA using reverse transcriptase and an oligo dt primer. PCR of this product using the relevant cytokine-specific primers will generate a full length coding sequence with A overhangs, which will be ligated into a TA cloning vector containing its multiple cloning site within an encoded beta galactosidase gene. Competent, XL-1 blue bacteria will be transformed with the constructs, and recombinants will be identified by their ability to grow as white colonies on x-gal-coated, ampicillin-containing agarose plates. Amp-resistant colonies will be grown in culture, and plasmids will be isolated from each using mini-prep columns. The isolated, hypothetically recombinant vectors will then be cut with the appropriate restriction enzymes and run on an agarose gel against molecular weight standards to ensure the cloned insert is present and of the correct length. TA cloning vectors determined to contain a correctly-sized insert will then be sequenced by the core facility to verify its identity and complete homology to the wild type gene, after which the insert will be cut out of the vector with restriction enzymes, run on a gel, and isolated for subcloning into a eukaryotic expression vector using a gel-extraction kit. Purified cytokine-encoding inserts will be ligated into Pbabe puro that has been cut with the corresponding restriction enzymes and treated with Calf-intestinal alkaline phosphatase to prevent re-ligation of non-recombinant vectors. Following ligation, transformation of competent cells, and isolation of ampicillin-resistant colonies, plasmids isolated from these minipreps will be assessed for inserts, and then sequenced to insure correct orientation in the expression vector. 3T3 fibroblasts will be transfected with the eukaryotic expression vectors, and treated 24h later with puromycin to kill cells that didn't take up the plasmid. Confluent, puromycin-resistant 3T3 cells will then be assessed for specific cytokine expression by Real Time RT/PCR, ELISA, Western analysis and immunoprecipitation. To save money on antibodies, all students will perform the immunoprecipitation on just one of the successfully overexpressed cytokines.

Note that each student will be provided with a notebook containing protocols outlining each step of the cloning experiment for current and future reference. Evaluation will be based on students' participation, their contribution to the group's learning, and successful cloning of his/her assigned cytokine. Though no difficulties are anticipated for any of the steps through transfection of cytokines into mammalian cells, any of the redundant methods for demonstrating overexpression (northern analysis, real time PCR, ELISA, immunoprecipitation) that prove to be an ineffective use of time or money will be deleted from next year's curriculum.

Thank you for considering this request.

Sincerely,

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