

Core Facilities for Trainees:

Departmental Cores

Three Core Laboratories within the Department of Human Genetics were established with Institutional funds provided by the Dean, and have markedly enhanced the research environment for our MRDD trainees. These include: 1) a DNA Core that provides oligonucleotide synthesis, PCR amplification, DNA sequencing and sequence analysis, and special automated resources for protein purification; 2) A Tissue Culture Core that routinely establishes and maintains human fibroblast and lymphoblast cell lines, as well as other primary cell cultures, if needed; and 3) A "Knock-Out" Core Laboratory that provides a facility for the generation of knockout/knockin mice. These are dedicated core facilities, each with designated space, a Director and a technician. Dr. David Bishop is Director of the DNA/Sequencing Core, Dr. Peter Warburton directs the Tissue Culture Core, and Drs. Douglas Forrest and Jon Gordon Co-Direct the Knock-Out Core. All predoctoral and postdoctoral trainees have access to these facilities and to the expertise of the Core Directors and their technical staff. In addition, our MRDD trainees have full access to the "Vector Core and GMP Production Facility" and "Morphology & Assessment Core," housed within the Institute for Gene Therapy. A brief description of each core is provided below.

1. DNA/Protein Core (Director: Dr. David Bishop)

The DNA/Protein Core Laboratory is housed on the 14th floor of the East Building in room 14-59. This 600 sq. ft. laboratory contains an Applied Biosystems Model 3700 and an ABI Model 3100 capillary array sequencer, an ABI Model 377 vertical gel DNA sequencer, two ABI Catalyst 877 Turbo Thermocycling Robotic Workstations, an ABI Model 394 oligonucleotide synthesizer, a Transgenomics WAVE[®] denaturing HPLC with autosampler, an Applied Biosystems 9600 and an ABI 9700 96-well format thermocycler, a SUN Microsystems SparcStation 20 for networked DNA Sequence Analysis, and dedicated support equipment including the Savant OligoPrep, the Savant SpeedVac, centrifuges, balance, Macintosh and NT computers, printers, refrigerator/freezer, etc. Dr. Bishop supervises three full-time technicians who carry out the daily tasks of the laboratory. The Core provides the following services: 1) Oligonucleotide synthesis: Standard oligonucleotides (from 40 nanomole to 1 μ mole scale and up to 120 nucleotides in length), biotinylated oligos, sulfurized oligos, fluorescent oligos and other derivatives, as needed, are routinely synthesized and delivered on the next day or within two days of request. The next day turn around has proven very efficient for the trainees, particularly those involved in sequencing projects. 2) PCR amplification: The Core Laboratory has two 96-well PCR machines for use by trainees. PCR is carried out in a special room within the Laboratory which houses the thermocyclers. A computer program, Oligo 4.0, is available for the design of their PCR amplification and sequencing primers. 3) DNA sequencing: The Core offers automated DNA sequencing using dye-terminator chemistries. The appropriate software programs for sequence analysis functions are available. The trainee provides the Core with the appropriate primer(s) and an adequate amount of DNA template purified by the Core's protocol. DNA sequencing is performed and the trainee receives a disk with the sequence (up to 800 nucleotides/run). In addition, the raw electrophoretograms are available on the networked SUN workstation for further remote analysis. The availability of this service with a turn-around of two days already has proven to be a great value to our trainees who are pursuing gene sequencing, positional cloning, mutagenesis, mutation detection, gene regulation or gene therapy projects. 4) DNA genotyping: The ABI Model 3100 and 377 sequencers are also used for genotyping for rapid large scale marker screens for linkage analyses. The Transgenomics WAVE[®] dHPLC instrument facilitates rapid screening of candidate exons for single missense mutations with a nearly 100% capture efficiency. 5) Protein purification: Our Director, Dr. Bishop, and several of

our Faculty (Desnick, Ioannou, McGovern, and Schuchman) have extensive experience in protein purification. To facilitate purification efforts in the Department, we have a state-of-the-art perfusion chromatographic system, the Perceptive Biosystems BioCad 80. This instrument fully automates the laborious chore of method development in protein purification. It is a computer driven workstation which has a built in autosampler, dual column support, automated valve switching, UV, visible, pH, and conductivity monitoring, and automated sample fraction collection. The rapid flow rate perfusion chromatography columns permit runs as short as 2-5 min. Thus, systematic exploration of pH, salt, flow rate and sample loading can be accomplished in hours instead of weeks. This facility already has proven its value for the optimization and scaled-up purification of several native and recombinant proteins provided by our trainees.

2. Tissue Culture Core (Director: Dr. Peter Warburton):

The Tissue Culture Facility is housed in East Building 14 (600 sq. ft.) and contains cell culture transfer rooms, walk-in cold and warm rooms, and a media kitchen. The Core provides the following services: 1) Tissue culture and establishment of cell lines: The cell culture facility establishes cultured fibroblast cell lines from skin biopsies and cultured lymphoid cell lines from venous blood samples. Permanent lymphoblast cell lines are established with a greater than 90% success rate. These transformed cells are particularly useful for molecular biology experiments since large quantities can be grown for DNA and/or RNA isolation. Dr. Warburton supervises a full-time technician who establishes, maintains, freezes-down, and stores the various lines used by our Faculty and trainees. She also prepares the different media required for growth of the different cell types, establishes primary cultures, grows the cells and performs the transfers to produce the cells required for each trainee's research studies. The Core provides laminar flow hood facilities, and a backup -135 °C freezer and liquid nitrogen storage. 2) Human gene mapping: The human gene mapping facility, a component of the Tissue Culture Core, is also under the direction of Dr. Warburton. This facility provides somatic cell hybrid panels and/or DNA isolated from these panels for the chromosomal mapping of human genes. Southern blots are prepared containing various restriction digests of each hybrid panel for subsequent hybridization with species-specific cDNA and/or oligonucleotide probes. In addition, PCR analyses are performed on the hybrid panels for gene assignment. Single chromosome hybrid cells and cells containing specific gene rearrangements also are available for most human chromosomes and are useful for regional chromosomal assignments. In addition, fluorescence *in situ* hybridization (FISH) techniques are ongoing under Dr. Astrin's supervision.

3. Murine Knock-Out/ Knock-In Core (Director: Drs. John Gordon and Douglas Forrest):

This Departmental Core currently is housed in East Building 14-32 and 14-30 (600 sq. ft.) and contains the necessary equipment for the generation of knockout or transgenic mice. For the generation of knock-out mice, embryonic stem cells are grown and screened in the Core and consultation is available for the design of constructs for targeted disruption. Once a gene-disrupted or modified cell line is generated and confirmed, the Core generates and injects blastocysts and implants them into pseudopregnant mice, in conjunction with the microinjection facility in the East Building rooms of the Center for Comparative Surgery and Medicine at Mount Sinai. In addition, the Core assists with the animal husbandry, genotyping, histology and *in situ* hybridization analyses of mutant mouse strains, as necessary.

Institute for Gene Therapy Core Facilities

1. Vector Core and GMP Production Facility (Director: Marcia Meseck, M.S., L.L.P.)

The Vector Core and GMP Production Facility plays a central role at the Carl C. Icahn Institute for Gene Therapy and Molecular Medicine by providing viral vectors for preclinical and clinical studies. The Facility is located on the 17th floor of the Annenberg building, in close proximity to many of the laboratories performing basic science, translational, and clinical research. There are two main areas within the production facility. The first is the Class 10,000 area which has multiple tissue culture rooms, storage areas, and a general laboratory for performing preclinical production and quality control functions, such as, testing, receipt, and storage of raw materials and final product. The Class 10,000 GMP production area has five self-contained tissue culture suites that will allow for the concurrent production of up to five different vectors. There are two additional self-contained production suites under temperature control that are suitable for adaptation to scale-up procedures.

2. Morphology and Assessment Core (Director: Swan N. Thung, M.D.)

This core offers assistance or collaboration to investigators whose basic or clinical research projects require light microscopic and molecular pathologic analyses. The core staff routinely processes tissue for formalin fixed, paraffin embedded sections and fresh frozen sections, perform routine histological, histochemical (special stains) and immunohistochemical stainings for the detection of various antigens on paraffin and frozen sections. Proper positive and negative controls are included in each run to assure reliability of the results and comparability of results from different runs. The Core staff routinely handles frozen and fixed tissue, as well as fluid for polymerase chain reaction (PCR) and RT-PCR procedures to detect various genes and their mRNA. The staff will work with investigators in selecting the primers and interpretation of the results. The Core is a fully equipped laboratory including light and UV microscopes, cameras, a tissue processor, a tissue embedding machine, a cryotome and microtome. All PCR and RT-PCR assays are then conducted in the Molecular Diagnostic laboratory in the department of Pathology labs. The laboratory is under Dr. David Zhang's supervision. This laboratory provides molecular testing including: HCV RT-PCR, quantitative HIV-1 RT-PCR and T cell receptor gene rearrangement by PCR. Several other molecular testing methods, such as bacterial genotyping by restriction fragment length polymorphism and pulse field gel electrophoresis, are performed routinely.