PSOM Research Core Facilities

Lou Soslowsky, PhD
Associate Dean for Research Core Facilities

Perelman School of Medicine
University of Pennsylvania

Outline

- People and Cores
- Recent Activities
- Brief Summary of Each Core
- Core Project Examples
- Areas of Focus Going Forward

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Cores Committee

Perelman School of Medicine

Lou Soslowsky, Orthopaedic Surgery

Michael Beers, Medicine

Michael Betts, Microbiology

Maja Bucan, Genetics

Yale Goldman, Physiology

Warren Pear, Pathology

Celeste Simon, CDB

Nancy Speck, CDB

Admin Support:

Jenna Ruttkay & Jenna Roberg

CHOP

Harry Ischiropoulos

Wistar

Bill Wunner

Ex Officio

Jiju Mathew, Finance

Susan Passante, EVD/CSO

Danielle Rehr, Finance

Brian Wells, IT & Research Computing

First Year Activities

Area	REQUESTS / TO DO	Status
Advertising	website: update everyone's keywords	open
Advertising	brochure	in process
Central	Core Director Guide	open
Central	Discuss Penn Genomics Strucure	open
Central	Collect FY16 annual reports from Cores	in process
Central	review FY16 core requests/cost	open
Central	invite cores to present at committee meetings	open
Central	support faculty recruiting	ongoing
Central	Cores Statistics for PSOM use	on hold
Central	educational opportunity via Dan Kessler	on hold
Central	Business Plan for Centralization	in process - J. Mathew
Central	iLab - determine fiscal/admin plan for BA roles	in process - J. Mathew
Central	Penn CRISPR Cas 9 Targeting Core	in process
Central	High-Throughput Screening Growth	in process
Central	11/17 Cores Presentation	in process
Central	Winter 2016 Cores Newsletter	in process
Central	Mission Continuity	in process
Central	Cores Survey Email to PSOM	in process
Central	Cores Survey Updates	in process
Central	Recruit Committee Member to replace Y. Goldman starting Jan 17	open
Finance	obtain updated 5yr fiscal report from Finance	in process
Meetings	Agenda for 10/28 Cores Committee Meeting	in process
Advertising	Fall Cores newsletter	complete
Cores Fair	2016 Cores Fair	complete
Central	request FY16 annual reports	complete
Advertising	website - add grant proposal language	complete
Advertising	communicate opportunity for individual core videos	complete
Cores Fair	determing 2016 Cores Fair location and options	complete
Central	annual report responses	complete
Central	S10 communication process	complete
Cores Fair	determine 2016 Cores Fair date	complete
Cores Fair	determine 2016 Cores Fair date	complete
Advertising	website: welcome video	complete
Advertising	Website - transition to MODX	complete
Advertising	Website - add S10 request	complete
Central	Penn Box folder for Cores documents	complete
Central	SCDC meeting per Tom Hecker	complete
Central	announce new proteomics core	complete
Central	Core Community Faculty Meetings	complete
Central	Collect annual reports from Cores	complete
Central	Record Welcome Video from Lou	complete
Central	review FY16 core requests/cost	complete
Advertising	Website - add user survey	complete
Central	PGT - determine go-forward strategy	complete
Central	iLab - determine line of roles/responsibilities between iLab and CAMS	complete
Central	Make Directory and Map for Cores Fair	complete
Central	Hold 2015 Cores Fair Directors Meeting	complete
Central	2015 Cores Fair	complete
Central	Design ad for NERLSCD pamphlet	complete
Central	Sponsor NERLSCD meeting	complete
Central	Invite new members to Committee	complete
Advertising	website: Associated Cores search bar	closed
Central	Hold luncheon for Core Committee Members	complete
Central	Meet with Core Directors	complete
Central	Visit Wistar cores	complete
Central	Visit each PSOM core location	complete
Advertising	update EVD website re: cores information	complete
Advertising	website: hover blurbs	complete
Advertising	Website - add core committee members	complete
Central	CHOP member transition	complete
Central	review NGSC request	complete
Central	update policies	complete
Central	Ben Garcia: decision on stipend	complete
Central	look into S10 process w/ VPR; determine go-forward process	complete
Central	determine future of Proteomics Core	complete
Central	Welcome to Celeste Simon	complete
Finance	New Proteomics MOU	complete
Finance	review CCVPF equipment request	complete
Finance	PMI - New Service Center request	complete
Finance	HTS MOU	complete
Meetings	presentation of new proteomics core to Committee	complete
Meetings	agenda for 12/18 Committee meeting	complete
Meetings	agenda for 9/22 Cores Directors meeting	complete

Example Accomplishments

- Enhanced and diversified cores committee
- Site visits to each core
- One-on-one meetings with each core director
- One-on-one meetings with multiple chairs
- Campus-wide Research Core Facilities Fair
- MOUs for multiple cores
- Close/develop cores (recent and ongoing)
- Revamped and enhanced website
- Annual reports/reviews

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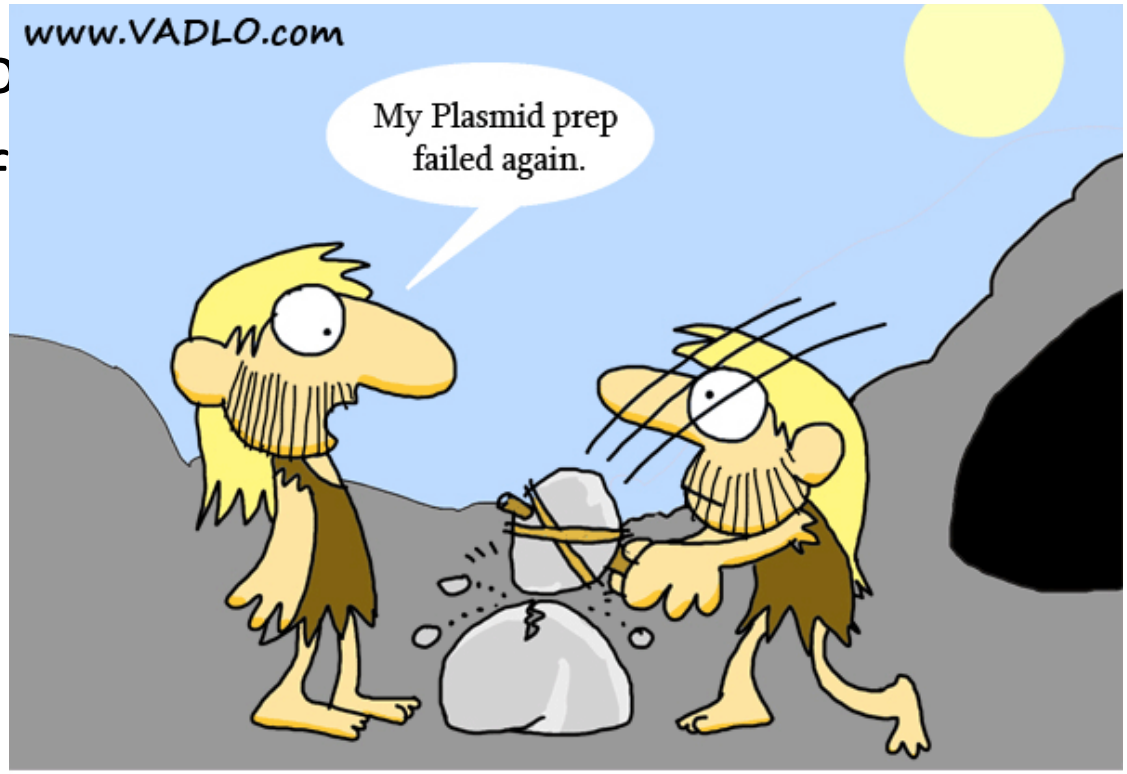
- Areas of



How it feels like working in an underfunded lab.

23 PSOM Resource Core Facilities

- Bioinformatics Core
- CDB Microscopy Core
- CRISPR Cas9 Mouse Targeting Core
- Cell Center Services Facility
- Cell Center Stockroom
- Clinical Cell and Vaccine Production Facility
- Clinical Research Computing Unit
- Electron Microscopy
- Flow Cytometry and Cell Sorting Facility
- High Throughput Screening
- Human Immunology Core
- Investigational Drug Service
- Neurobehavior Testing Core
- Next Generation Sequencing Core
- Penn Genomic Analysis Core
  - DNA Sequencing Facility
  - Molecular Profiling Facility
- Penn Gnotobiotic Mouse Facility
- Quantitative Proteomics Resource Core
- Research Instrumentation Shop
- Small Animal Imaging Facility
- Stem Cell and Xenograft Core
- Transgenic and Chimeric Mouse Facility
- Vector Core

What Our Cores Offer

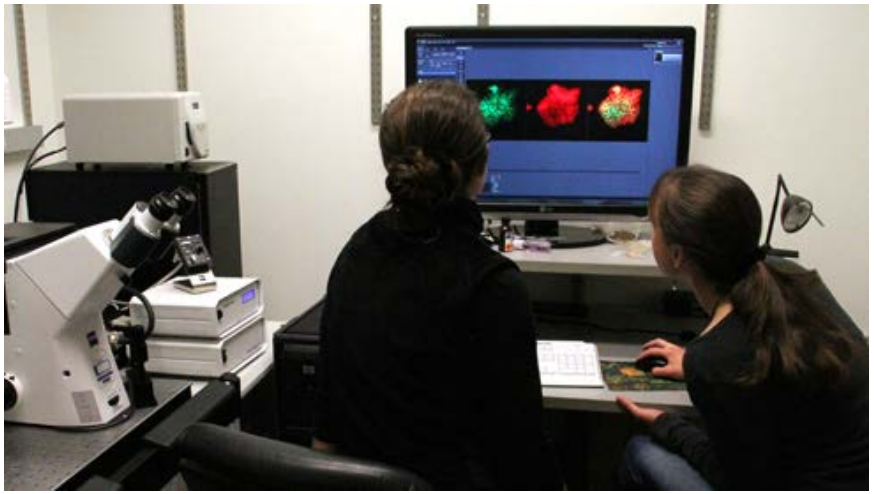
- State-of-the-art equipment
- Cutting-edge technical expertise and training
- Support and consultations for faculty and trainees
- Opportunities for scientific collaboration
- Interest groups, seminars, meetings

Bioinformatics Core

- Data analysis and consulting
- Pipelines to handle next-generation sequencing data analysis
- Support for grant applications
- Additional services:
  - Application Development
  - Database Development
  - Large Scale Biomedical Data Management

“CDB” Microscopy Core

- Five confocal and three widefield light microscopes, a scanning electron microscope
- Computers/software for image processing and analysis
- Personalized assistance on all aspects of imaging
  - sample preparation
  - microscope training
  - Image processing and analysis



Cell Center Services Facility

- Training/services in cell culture and associated procedures
- Large scale growth of hybridoma and other cell lines
- Preparation of specialized culture media, drosophila media, and reagents
- Additional services:
  - culture in Celline flask and hollow fiber bioreactor
  - hybridoma production/screening
  - transfection of mammalian cells

Cell Center Stockroom

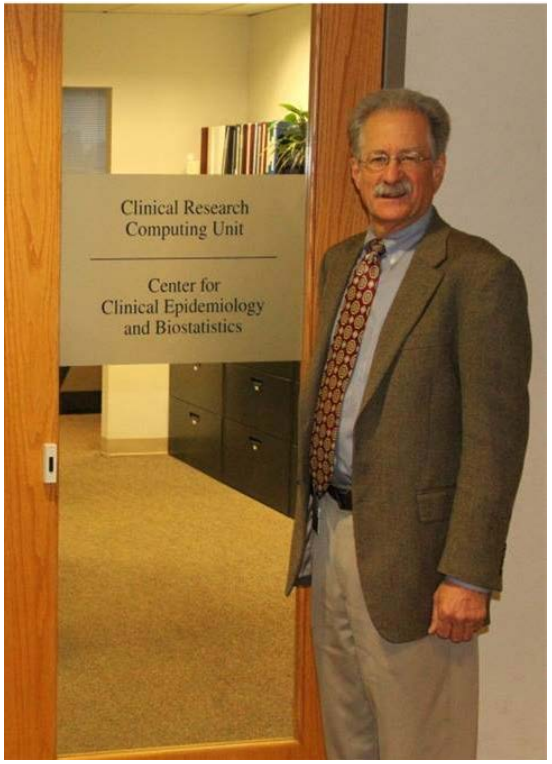
- Relationships with various suppliers of molecular biological research materials for bulk purchasing, negotiation of discounts and convenient delivery
- Over 1,400 products on-site for immediate delivery
- Special order from 24 bioreagent vendors with discounted pricing and overnight delivery

Clinical Cell & Vaccine Production Facility

- Scientific, technical and regulatory support for investigator initiated investigational new drug applications in cell and gene therapy in GMP facility
- Services include:
  - Washing and cryopreservation of pheresis product
  - Activation and expansion of donor or patient T lymphocytes
  - Transduction of activated T cells with retroviral or lentiviral vector
  - Generation of immature or mature dendritic cells

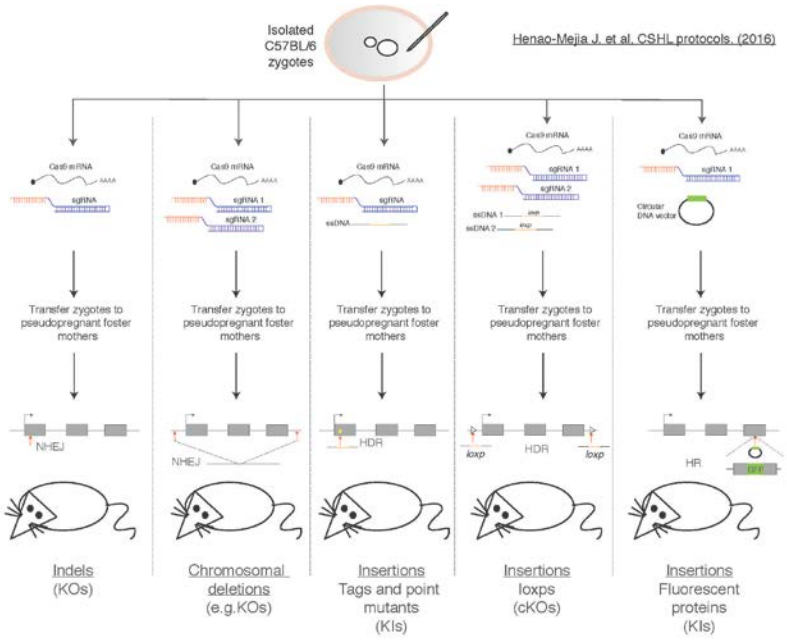
Clinical Research Computing Unit

- Provides expertise in all facets of research information management (biomedical, behavioral, clinical, translational), and by understanding the regulatory and cultural environments
- Services include:
  - project management
  - data management and research technology specializing in clinical research informatics
  - collaboration and research IT services



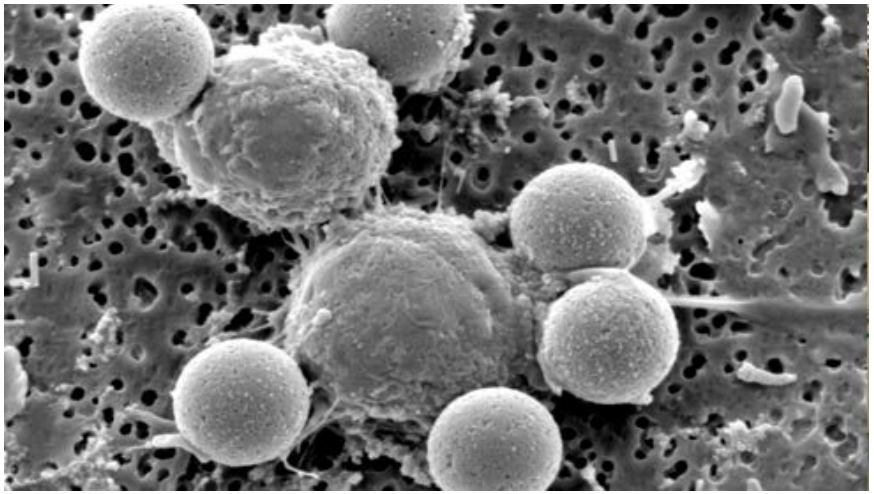
CRISPR Cas9 Mouse Targeting Core

- CRISPR/Cas9 technology reduces the time and cost required to generate genetically engineered mice.
- In partnership with the Transgenic and Chimeric Mouse Facility, the core offers a variety of services including:
  - targeting design
  - sgRNA preparation
  - Cas9 mRNA generation
  - DNA repair template preparation
  - microinjection
  - genotyping/screening for targeted mice



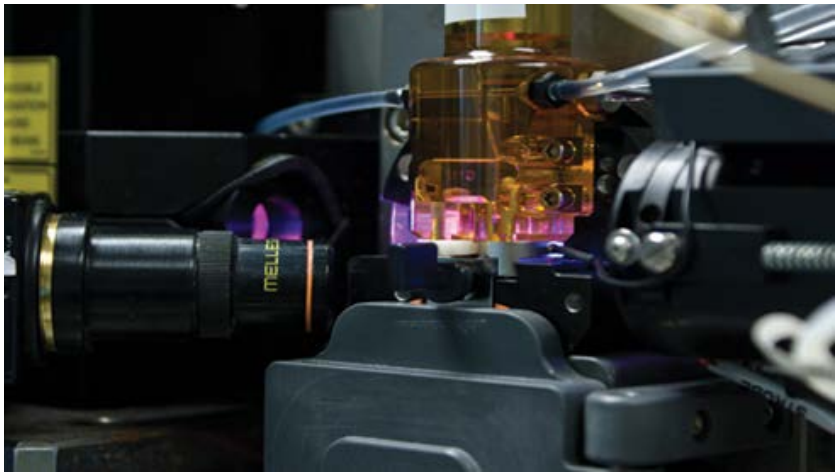
Electron Microscopy Resource Laboratory

- High quality ultrastructure analysis by electron microscopy (EM)
- Experienced staff and state-of-the-art preparation and imaging equipment
- Full spectrum of both transmission EM and scanning EM services



Flow Cytometry and Cell Sorting Facility

- Comprehensive flow cytometry (NCI laboratory of exceptional merit)
- Array of instrumentation, education, consultation
  - from high-speed multicolor to low-speed, large nozzle
  - cell analysis services (up to 20 parameters), from analog, tabletop analyzers to many-laser, many-color, high-speed, fully-digital instrumentation
  - 6 cell sorters and 19 analytical instruments
  - BSL3 suite (10 parameter)

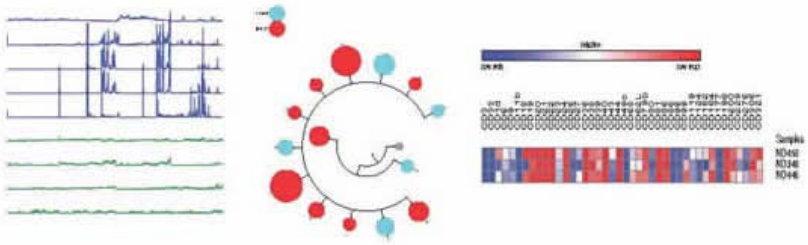


High-throughput Screening Core

- HTS screening to identify genes or organic small molecule modulators of signaling pathways, cellular phenotypes, and protein function staff will educate:
  - assay development, optimization, miniaturization, validation
  - libraries of siRNA, shRNA, cDNA, and FDA approved/ FDA-like organic small molecules
  - robotics infrastructure, including small screens of user defined libraries

Human Immunology Core

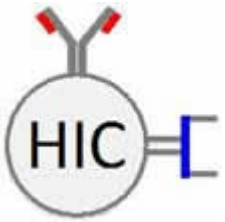
- Reagents/expertise/ consultations for study of immune function in humans
- Cell and tissue processing, generation of human blood cell products
- Cellular/molecular immune assays for early-phase trials
- Multicolor immuno-phenotyping, sequencing of T and B cell receptor genes, luminex, ELISPOTs, ELISA



Cytometric fingerprinting of flow data (with flow core)

Lineage tree analysis of high throughput BCR sequencing data

Analysis of flow cytometry data

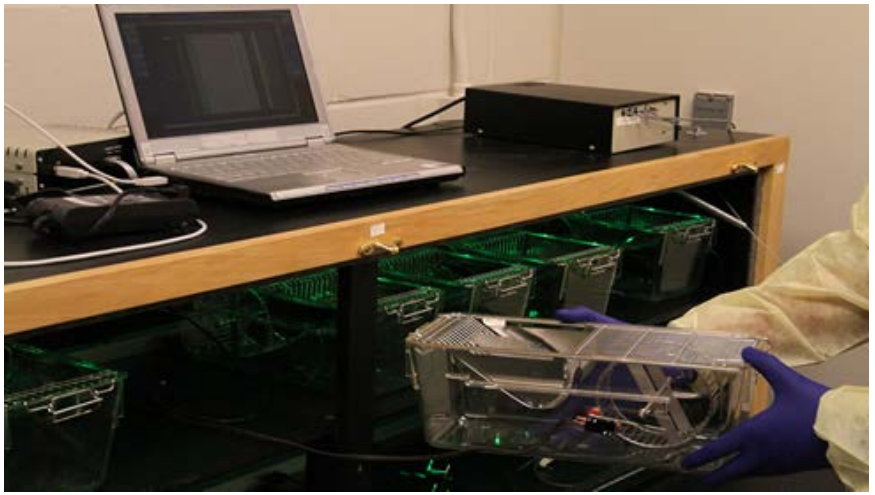


Investigational Drug Service

- Research pharmacy
  - management of research medications used in clinical drug trials
  - oversight of medication use in drug trials conducted throughout Penn and affiliates
- Agents overseen include drugs, natural products, vitamins, biologics and gene therapy agents

Neurobehavior Testing Core

- Consultation on study design (tests, mouse strains/lines, numbers, control groups, breeding, regulatory issues)
- Facilities and services to phenotype behaviors in mice
  - running wheels, beam splitters, EEG/EMG recording chambers, open field activity monitors and Rotarod, various mazes, light/dark chamber, passive/active avoidance chambers and hole poke arenas, object recognition tests



Next Generation Sequencing Core

- Library quality assessments, sequencing, and analysis including ChIP-seq, RNA-Seq, HITS-CLIP, miR-Seq, exome capture, and BIS-seq
- Advice on library preparation and limited library preparation services
- Two Illumina hiSeq2000s for large-scale sequencing and a MiSeq for sample evaluation or library testing

Penn Genomic Analysis Core: DNA Sequencing Facility

- Long read, automated Sanger sequencing with fast turnaround
- NGS on Ion Torrent Personal Genome Machine and Proton
- Genotyping and fragment analysis
- Molecular biologic services
- Data analysis

Penn Genomic Analysis Core: Molecular Profiling Facility

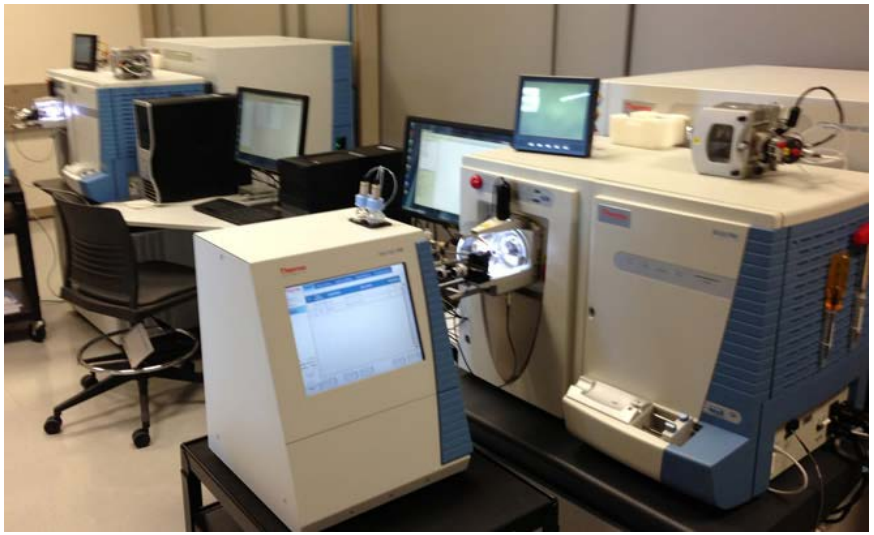
- DNA (Sequenom assays, and Illumina GoldenGate)
- RNA profiling (Affymetrix GeneChips, Illumina BeadChips, custom multiplex, Fluidigm, Luminex and deep sequencing)
- Whole-genome assays (Affymetrix SNP GeneChip and Illumina Infinium)
- Consultations, training, experimental design

Penn Gnotobiotic Mouse Facility

- Centralized germ-free and gnotobiotic mouse services
- Several common strains of germ-free mice and re-derivation services
- Isolators for germ-free and gnotobiotic mice for experimental procedures
- Technical support for procedures

Quantitative Proteomics Resource Core

- High resolution mass spectrometry-based proteomics to characterize and quantify proteins from complex biological samples
- Services include:
  - protein identification
  - protein post-translational modification analyses
  - global histone PTM analyses
  - global proteome and phosphoproteome analyses
  - experiments for custom proteomics analyses

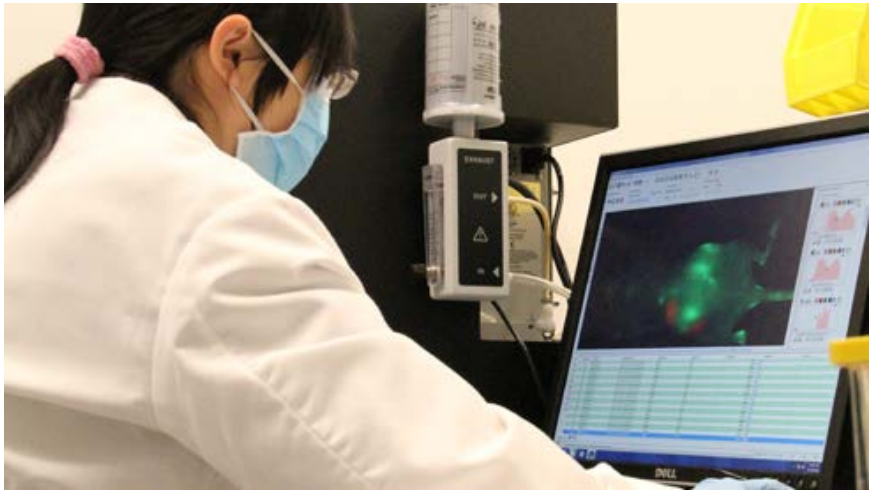


Research Instrumentation Shop

- Offers machine shop services including
  - making new mechanical parts
  - design assistance
  - packaging of electronics/optics
  - repairs of basic lab equipment

Small Animal Imaging Facility

- Comprehensive suite of imaging modalities for cells, tissues, and small animals including: MRI, MRS, optical imaging (bioluminescence and near IR), CT, PET, SPECT, and ultrasound
- Housing for mice and rats for longitudinal studies
- Ancillary facilities such as chemistry, radiochemistry, image analysis and tumor models

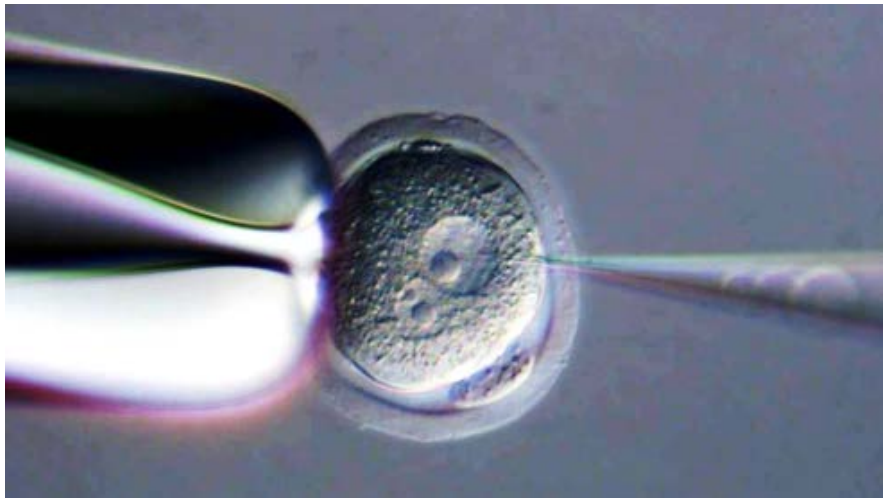


Stem Cell and Xenograft Core

- Integrates viable tissue bank of normal human hematopoietic cells and hematopoietic malignancies with a wide range of xenograft services
- Facilitates and promotes translational research involving viable primary human hematopoietic tissues through training or full-service

Transgenic & Chimeric Mouse Facility

- Produces transgenic founder, chimeric and genome-edited strains of mice carrying transgenes or "knockouts"
- Services include:
  - cytoplasmic RNA injection (for CRISPR technology)
  - ES cell injection into blastocysts
  - DNA pronuclear injection into fertilized oocytes
  - Embryo/sperm cryopreservation
  - in vitro fertilization
  - re-derivations of lines



Vector Core

- Resource for the use of vectors for gene transfer
- Vector technology for preclinical and other basic research applications
- Tools critical to the understanding of gene function and development of therapeutic vectors

Outline

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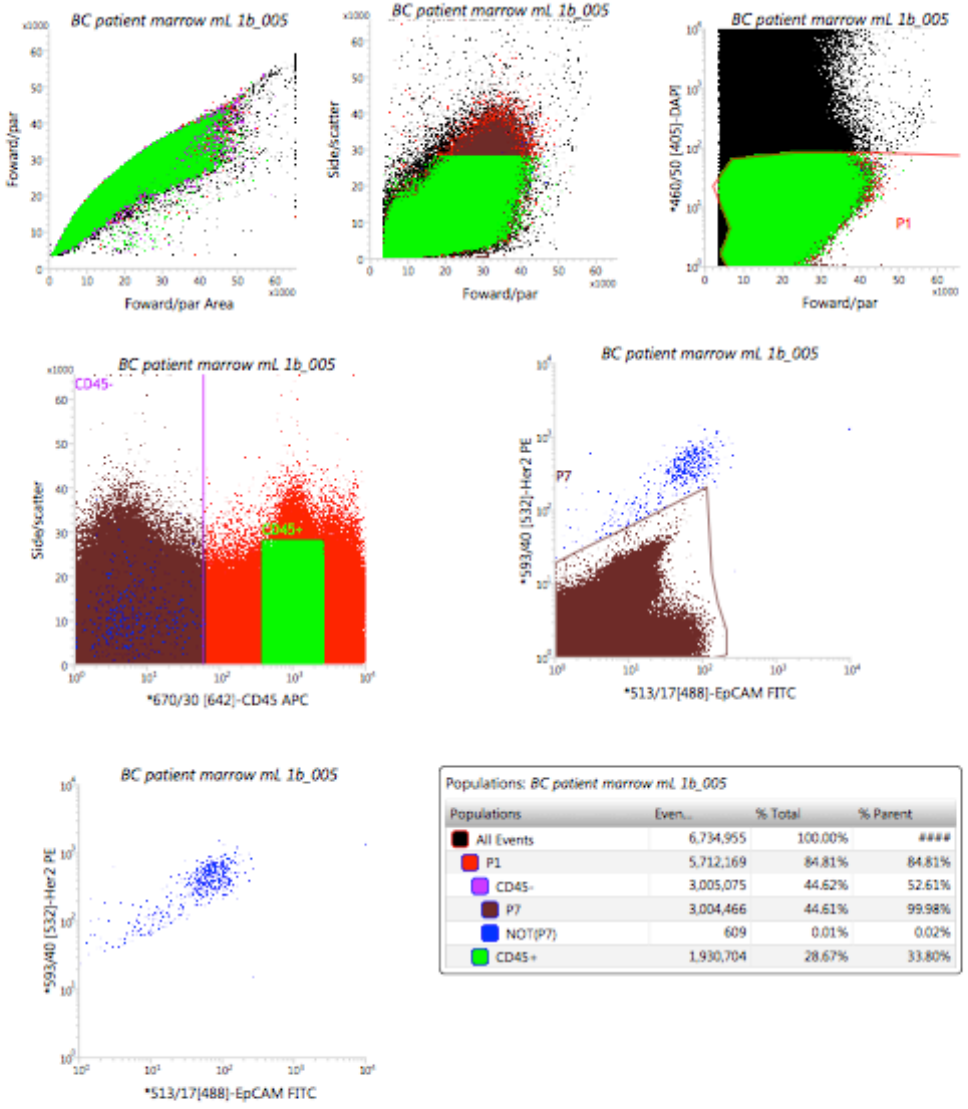
- Recent Activities
- Brief Summary of Each Core
- Core Project Examples
- Areas of Focus Going Forward

Flow Cytometry & Cell Sorting Resource Laboratory

- **Research Question:** To better understand the basic biology of disseminated breast cancer cells (DTCs) or circulating tumor cells (CTCs)
- **Method:** A novel application (the BD Biosciences FACS Focus) to identify and isolate these very rare cells in patients to allow early identification (1 in 10,000 or less)
- **Key Finding:** Success - the cells can be isolated!
- **Next step:** Assess gene expression (RNAseq) or mutations (WGA)
  - Understand the heterogeneity at the single cell level by sorting single cells into 96-well plates
- **Key implication:** Identify early, potential targets for cancer therapy

Some sample data from the Chodosh lab, sorting DTCs from the bone marrow of a breast cancer patient with metastatic disease: Sorted 609 Her2/EpCAM positive cells (in blue) from 6.7 million total events



Flow Cytometry & Cell Sorting Resource Laboratory

- **Opportunity:** a novel flow cytometer analyzer (the BD LSRFortessa X-50 – Beta test), using 5 lasers and up to 50 detectors, with novel electronics and software
  - Novel fluorescent probes are being identified
  - Involves sophisticated data analysis (the Initiative for Deep Phenotyping)
  - Identify novel immune cells relevant to a wide variety of diseases

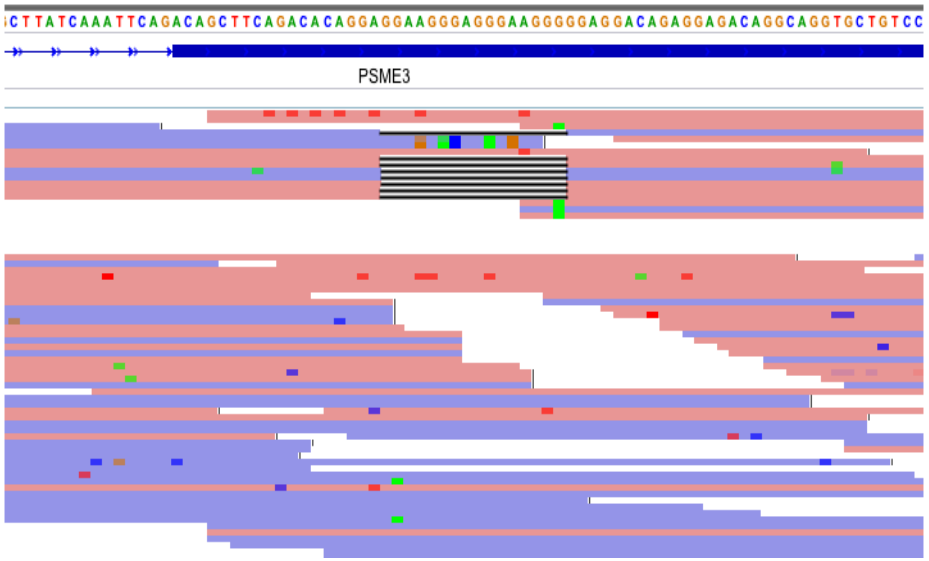
CDB Microscopy Core

- **Research Question:** When is Hopx (an atypical homeodomain protein) first expressed during cardiogenesis? Prior to the expression and assembly of sarcomeres, defining structural components of cardiac myocytes?
- **Method:** Document Hopx and sarcomere gene expression during early time points - using CDB Microscopy resources –in combination with RNA-expression analysis
- **Key Finding:** Hopx is expressed prior to sarcomere genes
- **Cover of Science, June 2015**

Integration of Bmp and Wnt signaling by Hopx specifies commitment of cardiomyoblasts. Jain R, Li, D.… Epstein JA. Science. 2015.

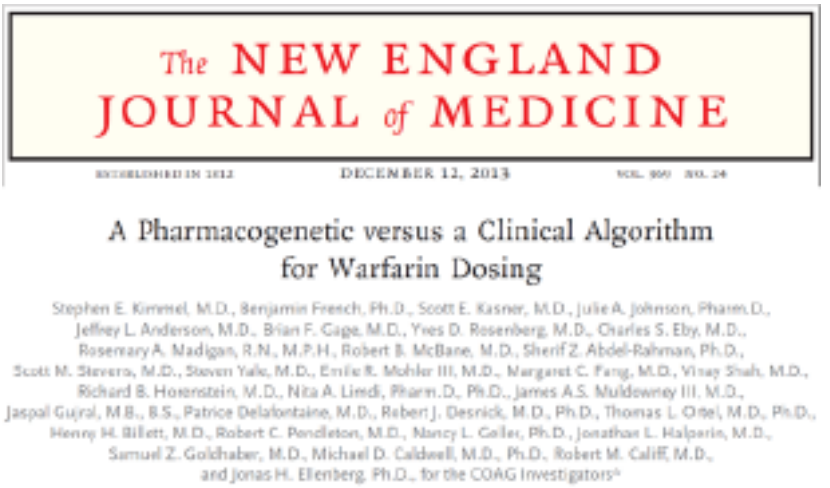
Next Generation Sequencing Core

- **Research Question:** Determine somatic sequence variants that might occur in beta-cells of diabetic patients
- **Method:** Because beta-cells do not proliferate and form clones, this had to be done on the single cell level (sequenced 39 libraries to establish feasibility)
- **Key Finding:** Enabled determination of genomic locations of microdeletions that result from NHEJ (pathway that repairs double-strand breaks in DNA)
    - figure shows a 16bp heterozygous deletion (black lines) found in a protein-coding exon which did not occur in any other cell that had coverage at this location
- **Key Implication:** Key data for $8M grant aimed at measuring the level of cellular damage on a single-cell level in old or T2D diabetic beta cells



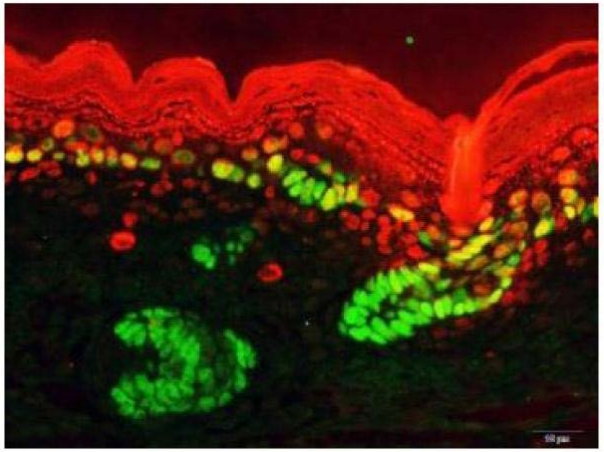
Clinical Research Computing Unit

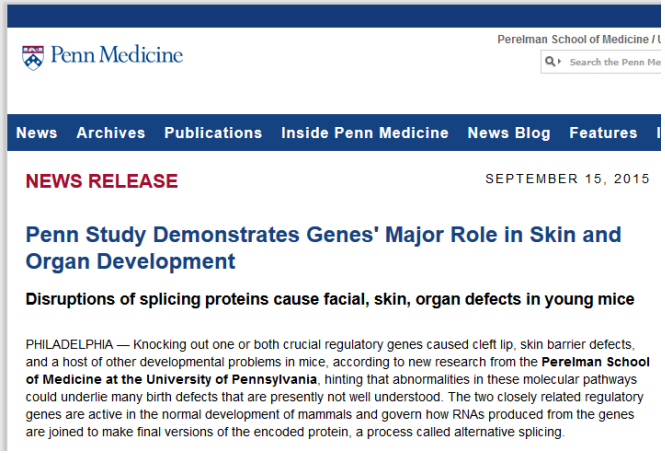
- **Research Question:** Is Pharmacogenetic versus Clinical Algorithm dosing of Warfarin more effective when initiating therapy?
- **Methods:** Double Blind RCT- subjects randomized to either standard of care (Clinical Algorithm dosing) or dosing based on genotype/clinical information for the first 5 days of therapy, followed for an additional 4 weeks
- **Key Finding:** Combining genetic data with clinical information to determine the initial dosage of the blood thinner warfarin was no more effective than using clinical information alone
  - In African-Americans, the genetics based approach had a slightly lower level of effectiveness
- **Next Step:** Understand the role genomics plays in determining optimal therapies



Transgenic and Chimeric Mouse Facility and Penn Gene Targeting Core

- **Research Question:** Is alternative splicing essential for posttranscriptional gene regulation, necessary for normal cellular function, patterning, and development
- **Methods:** Generated two conditional mouse knockout alleles, one complete knockout allele and a knockin mouse for two splicing factors discovered in Carstens lab (Esrp1 and Esrp2)
- **Key Finding:** Large scale programs for epithelial cell functions
- **Key Implication:** Mouse models formed the core of two new NIH R01 grants
- Publication *"The splicing regulators Esrp1 and Esrp2 direct an epithelial splicing program essential for mammalian development.", eLife, September 2015*





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Key Areas of Focus

- Marketing / advertising of resources
  - General, new faculty, BGS, BPP
  - Website, workshops, symposia
- Annual reporting/core requests/surveys
- Financial clarity
- Aid in faculty recruiting

Key Areas of Focus (cont'd)

- iLAB trial and evaluation
- Enhancement of core directors "community"
- Departments vs PSOM "home"
- Relationships/interactions with CHOP, Wistar, VA, etc

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