Children's Hospital LOS ANGELES
THE SABAN RESEARCH INSTITUTE
USC University of Southern California

October 1, 2016

Dear Colleagues,

We are pleased to announce an expansion of our program for the acquisition of unfunded pilot data using TSRI cores. Our objective with this expansion is to increase the number and quality of publications coming from CHLA and the number of funded grant applications for CHLA investigators.

This expanded pilot program is open to the following investigators:

- Faculty of any rank at CHLA or USC for the purposes of collecting data that are not included in the budget of a funded grant (or, if clinical research, that are not covered in standard of care), and that will be included as preliminary data in a future grant application.
- Graduate students and postdoctoral fellows under the supervision of a research faculty member for the purpose of collecting data that are not included in the budget of a funded grant, and that will be included as preliminary studies in a future grant or fellowship application.

Written proposals for pilot research should be sent to the respective core directors (listed below). Each proposal will be reviewed on a rolling basis by a committee to assess its scientific merit and to ensure that the proposed use of core resources will be productive. Please note that the scheduling of funded studies will always take priority over unfunded ones. Also, note that this pilot data program does not apply to the TSRI Biostatistics Core, which has its own specific pilot award program.

The proposal should be brief (generally 2 pages or less) and must contain the following information:

1. Specific aims and hypotheses, and statement of scientific significance
2. The proposed number of unfunded pilot hours for equipment use, technician time, training, and/or support of the core director(s), as applicable
3. Study design, including proposed assessments and measures
4. If indicated, consultation with experienced researchers in study design, data acquisition, analysis, and interpretation of findings. Co-investigators who will perform these functions should be identified. Consider inviting the collaboration of the core directors if this assistance is needed.
5. An assurance from the Principal Investigator that the costs for the non-core portions of the study will be covered, and the means for doing so should be identified.
6. An assurance from the Principal Investigator that the pilot data will generate a publication and submission of a funding application, including a description of how the pilot data will complete or advance a publication or grant application, and with timeline/milestones to publication and grant application clearly specified. Approval of future requests for pilot data acquisition will depend in part on the track record for publication and funding from prior pilot studies, if any.
7. If any prior pilot studies have been authorized, a separate addendum should be included that details progress made on analysis of the previously collected data, as well as funding applications and papers that each pilot study has generated
8. Written assurance that, if the pilot data produce a successful grant application, research of the same nature in the funded application will continue to be acquired within the TSRI core that generated the pilot data, and the funded applicant will pay the full standard fees for use of the core.
9. To foster interdisciplinary collaborations, awardees must commit to presenting their research in one of the TSRI seminars.

Please note that if funds are required for reagents, antibodies, or other materials needed for use of core instrumentation, this should be detailed for review in the initial application, but their support will be at the discretion of the TSRI Director based on the availability of funds once the pilot study has been reviewed and approved by the relevant

TSRI core. The demonstration of financial need will be a foremost consideration in review of this component of the application.

Contact information for Core directors can be found at http://www.chla.org/core-facilities.

The Cores and examples of their cutting-edge technologies include:

Cellular Imaging Core provides equipment and expert guidance for imaging cells and tissues using various light microscopy techniques, including fluorescence, confocal, and lightsheet microscopy. The core includes training for independent use of microscopes and software for visualization and quantitative analysis of 3D images -- Esteban Fernandez

- Lightsheet Microscope (LaVision Biotec UltraMicroscope II) Specializes in imaging large specimens, such as whole mouse organs and embryos, rapidly and at high resolution. Compatible with organic solvents to achieve superb specimen clarity and high-quality images deep inside tissue.
- 3D/4D Image Analysis Software (Arivis Vision4D) Uses the latest software technology to display and manipulate the largest 3D datasets, such as lightsheet and confocal images, fluidly and without hang-ups. Includes analysis functions to extract quantitative measures such as object count, volume and surface area from images, and it performs 3D object tracking over time.

Fluorescence Activation Cell Sorting (FACS) Core Analysis and quantification of heterogeneous cell populations and for cell purification/separation by physical sorting of living or fixed cells, including single cell separation based on up to 19 parameters -- Hisham Abdel-Azim, Bob Seeger, Michael Sheard

- Yellow-Green (561nm) Laser Capabilities include:
  - tdTomato detection (fluorescent protein is 283% brighter than eGFP)
  - MitoTracker Orange (accumulation depends on membrane potential, and the dye is well-retained after aldehyde fixation)
  - CRISPR-Cas9, an emerging gene-editing ("genome surgery") tool that frequently uses mCherry reporters. A yellow-green laser is required for optimal excitation of this fluorescent protein
  - Up to 4-fold increase in the stain index with antibodies conjugated with PE and PE-based tandem dyes
  - Avoids spectral overlap and spill-over, leading to more accurate detection of complex multicolour experiments
- Image Stream X Mark II: Imaging flow cytometer that combines standard multi-color flow cytometry with brightfield, darkfield, and fluorescent markers microscope imaging, including a Mutli-mag feature that allows magnification of individual cell images, and an Extended Depth of Field that allows crisply-focused image at different sub-cellular structures. Applications include visualization of exosomes, cell signaling, co-localization, cell-cell interactions, morphology, internalization, DNA damage and repair
- Brilliant Ultraviolet (BUV) fluorochrome optics: Simultaneous detection of BUV395, BUV496, and BUV737
- Brilliant Violet (BV) optics: Simultaneous detection of BV421, BV510, BV605, BV711 and BV786. These and other fluorochromes enable 16-color analysis of each cell
- Luminex-200: Multiplexed measurement of cytokines, chemokines, and other proteins in 25 µl of biologic fluid (serum, plasma, supernatant, etc.)

Human 3T MRI Core Provides state-of-the-art MR images in across the lifespan -- Bradley Peterson, Marvin Nelson

- Philips Achieva 3.0T dStream MRI: The scanner has an X-series Quasar dual gradient system, dynamic 1st and 2nd order shimming, 32-channel head and torso coils, and active shielding. The scanner was upgraded in September 2016 to include:
  - dStream architecture, providing improved image quality and faster scan times
  - DirectDigital RF receive technology, providing improved MR signal and ultra-short TRs and TEs, real-time motion correction, and high-resolution diffusion (including multi-band DTI)
  - Parallel RF transmission and reception to enhance signal and image contrast uniformity

Mi Next Generation Science (MiNGS) Core provides access to a variety of cutting-edge technologies in biomedical research and bioinformatics support -- Jeff Bender, Shahab Asgharzadeh

- CyTOF: Mass cytometry (Helios, a CyTOF System, Fluidigm) combines time-of-flight mass spectrometry with metal-labeling technology for comprehensive functional profiling applications, with 135 available detection channels to study functional complexity of biological systems at the single-cell level, including phosphoprotein signaling analysis on subpopulations of cells.
- C1: Automated single-cell platform (C1, Fluidigm) for mRNA sequencing, DNA sequencing, epigenetics, or miRNA expression, with the new 800 individual cell chips for large-scale whole transcriptome studies to survey heterogeneity and discover novel cell populations.
- Next Seq and MiSeq: Massively parallel sequencers (NextSeq and MiSeq, Illumina) to analyze single-cell genomics, RNA sequencing, microbiomes (bacterial and fungal), shotgun metagenomics, targeted gene analysis, and microbial whole genomes.
- Biomark HD: High-throughput, low volume real-time PCR (Biomark HD, Fluidigm) for up to 96 targets in 96 samples in a single run with minimal input DNA.
- QiaCube: Fully automated sample preparation (QiaCube, Qiagen) for standardized DNA, RNA, and protein extraction from all sample types.
- Bioinformatics: Bioinformatics resources and expertise to analyze data generated with the Core's equipment.

Mouse Genome Core Generates genetically modified mouse models -- Di Tian

- Crispr-mediated genome editing technology
- BAC transgenic technology

Neuropsychology Core Provides state-of-the-art measures of cognitive, behavioral, and psychological functions to inform studies about CNS health and injury. Assists with grant proposals, study design, data collection and interpretation, and manuscript writing -- Sharon O'Neil

Rodent Metabolism Core Provides standardized, metabolic phenotyping of rodents -- Sebastien Bouret

- Metabolic Homecage System (TSE LabMaster/PhenoMaster): Automated and continuous assessment of energy expenditure, food intake, and activity in both rats and mice
- Body Composition (ECHO MRI 700 Whole Body Resonance Analyzer): Rapid, accurate, small animal measurement of total body fat mass, lean mass, and fluids
- Glucose CLAMP: Whole body assessment of endogenous glucose production and insulin sensitivity, can be adapted for various glycemic reactions, the addition of tracers allows for tissue specificity

Small Animal Imaging Core Long-term and repeated imaging studies, specializing in pre-clinical trials and mechanistic studies -- Rex Moats, Gevorg Karapetyan

- 7 Tesla Bruker Pharma Scan Magnetic Resonance Imager: Non-ionizing, 3-D anatomical imaging of samples up to 6 cm in diameter
- 1172 SkyScan microCT: Micron-resolution 3-D imaging of specimens up to 2 cm diameter
- Xenogen Bioluminescence/ fluorescence imager: Molecular and cellular imaging in live rodents

Stem Cell Analytics Core Provides tissue culture and other equipment support for pluripotent stem cell (PSC) research -- David Cobrinik, Jennifer Aparicio, Narine Harutyunyan

- Provides human pluripotent stem cell (hESC and iPSC) lines, reagents, tissue culture facilities, and equipment to enable modeling of human organotypic development and disease
- Cell line authentication and banking
- Shared equipment, including two ABI 7900 HT qPCR machines, nucleofector, microplate reader, and dissecting microscopes for hESC passaging and differentiation

Vector Core Advanced design and construction of viral vectors -- Rusty Lansford, Hedvika Davis

- Viral vectors include retroviral, lentiviral adenovirus, adeno-associated virus, avian adeno-associated virus, herpes simplex virus, and the packaging of developed vectors for research that does not involve use in vivo in human subjects
- Basic cloning with sequencing or PCR, immediate cloning with sequencing or PCR, and custom cloning
- Endotoxin-free maxipreps and packaging retroviral vectors
- Design, generation, and testing of CRISPR reagents

Biostatistics Core is jointly supported by The Saban Research Institute and the Southern California Clinical and Translational Science Institute. It has its own pilot program – please contact Interim Director Carolyn Wong for details: cawong@chla.usc.edu

If you have questions about this pilot program, please do not hesitate to contact us.

Sincerely,

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