Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Distinct Capabilities of the Laboratory Animal Sciences Program

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Director; Laboratory Animal Sciences Program

October 24, 2019

DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

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Laboratory Animal Sciences Program - an Overview

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

LASP is one of the five Science and Technology Directorates within FNLCR.
We operate the NCI animal facilities and provide routine and specialized animal husbandry services for NCI Investigators on the Bethesda (NIH) and Frederick (Fort Detrick) campuses.

Animal Care and Facility Management

Management of 27 rodent and 1 non-human primate vivaria.

Maintenance of ~350,000 animals occupying 40,000+ cages.

Operates *Receiving and Quarantine*, coordinates animal shipments.

Support of 231 investigators with 587 active animal study protocols.

Provides support for the Frederick ACUC and Bethesda ACUC.

Provides board-certified veterinary care for all research animals.

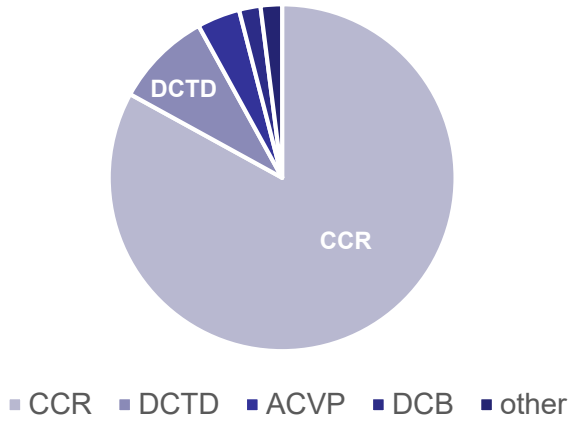
Drs. Jatinder Gulani, Julie Stephens-DeValle, Joshua Kramer, Matthew Breed, & Melody Roelke-Parker

Laboratory Animal Sciences Program - an Overview

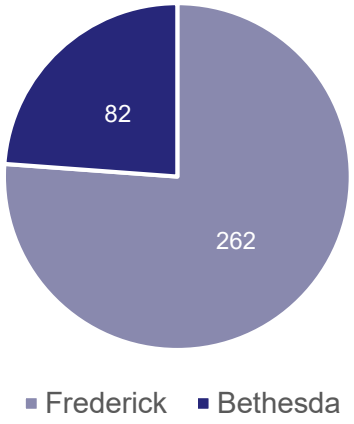
Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

LASP provides support to the NCI Center for Cancer Research (CCR), the Division of Cancer Treatment and Detection (DCTD), the Division of Cancer Biology (DCB), and other Institutes (NHGRI, NIAMS, NIDCD, NIDDK, NIMH, NIA). In addition, LASP supports the FNLCR *AIDS and Cancer Virus Program* (ACVP), and offers occasional support to other federal agencies through interagency agreements.

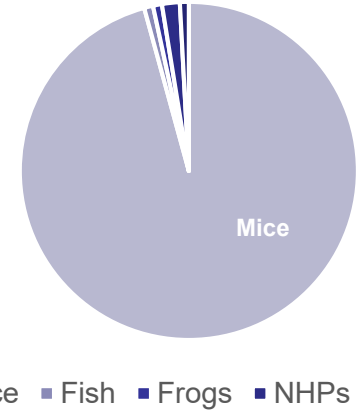
Support



Staffing

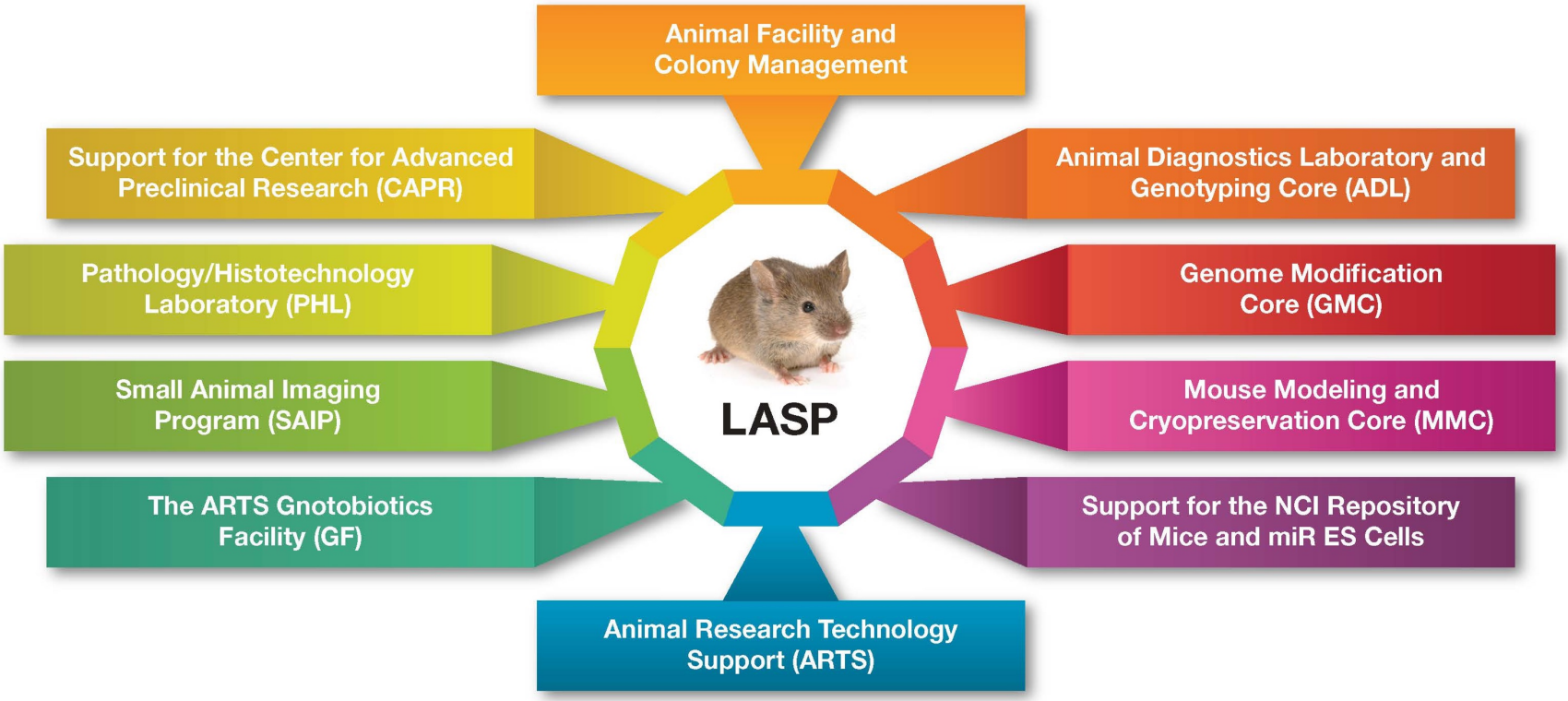


Species (protocols)



LASP also provides many state-of-the-art cores or facilities to assist NCI Investigators in performing their animal-based research of cancer and AIDS.

LASP – Cores and Facilities



In addition to operating Cores utilized by many NCI and FNLCR investigators, LASP undertakes "Technology Development" Projects with funding provided by the NCI Office of the Director (OD)-Office of Scientific Operations (OSO). This research seek to improve the efficiency of ongoing assays and foster the development of new services and new resources.

Thus, many LASP cores have rather distinctive capabilities.

LASP – Animal Diagnostic Lab (ADL)

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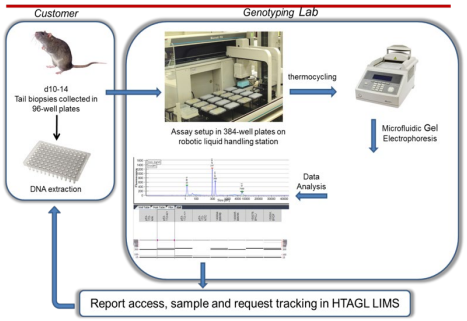
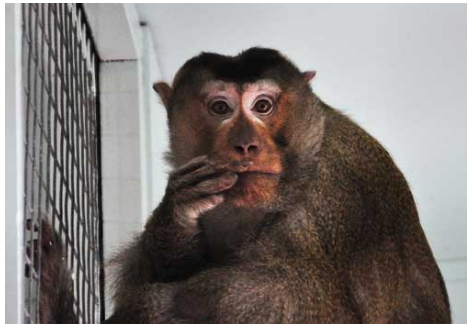
1. Animal Health Diagnostics

Viral serology, bacteriology, and parasitology diagnostic services for rodents and non-human primates.

2. Molecular-based Diagnostics and High-throughput Genotyping

Assists in health monitoring and provides many other advanced molecular services, including the monitoring of pathogens in cultured cells and tumor fragments, DNA fingerprinting technologies to evaluate genetic backgrounds, and high-throughput genotyping strategies and services.





Dr. Wang-Ting Hsieh

LASP – Animal Diagnostic Lab (ADL)

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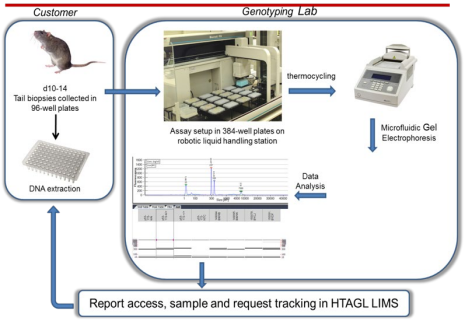
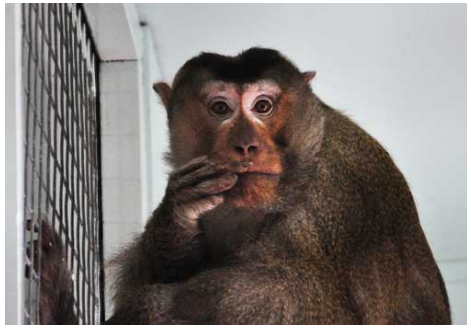
Recent Technology Development projects in ADL have produced molecular assays for most rodent pathogens, allowed us to switch the NCI to more robust "direct-colony tests" and reduce reliance on sentinel animals. The results: improved testing in our colonies, the detection of opportunistic pathogens in the immunocompromised colonies (*S. muris*, MKPV, *C. bovis*, etc.), the discontinuing of inter-building transfer testing, and reduced the time animals spend in quarantine when entering the Bethesda or Frederick facilities.





Dr. Wang-Ting Hsieh

LASP – Genome Modification Core (GMC)

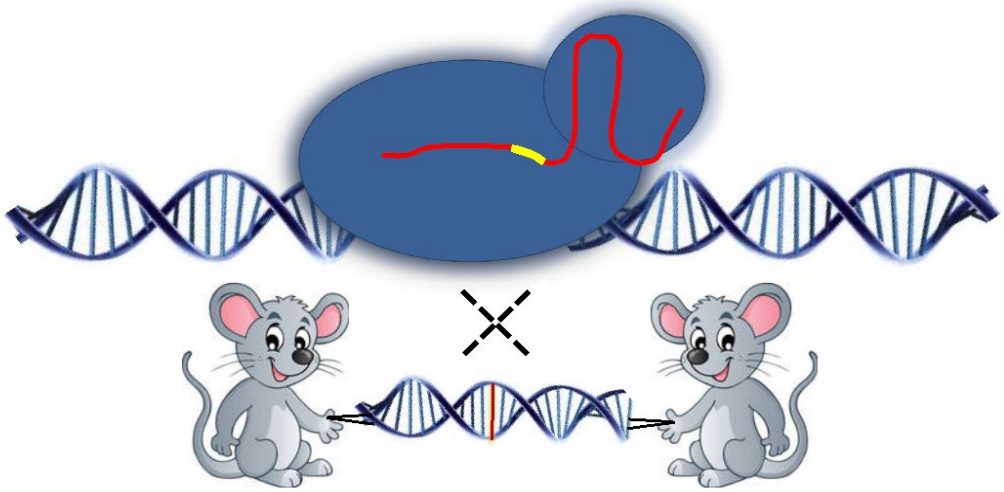
The Genome Modification Core (2017) provides to NCI investigators-

(1) Expert guidance on editing procedures to create precise genetic and epigenetic modifications.

(2) The generation and validation of nuclease (Cas-9 and Cpf-1) reagents.

(3) Various pooled guide libraries useful in performing genetic screens.

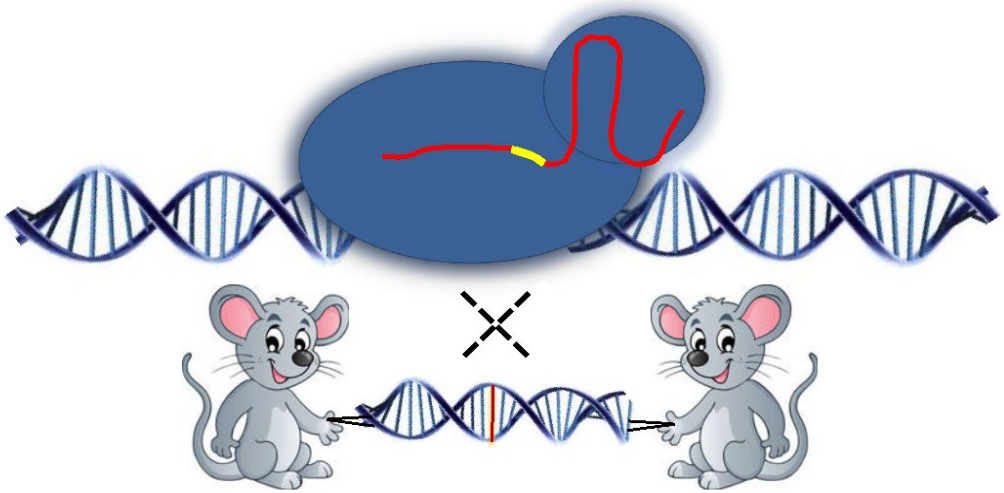
The GMC offers technical advice and expertise for gene-editing in primary cells and cell lines, and interacts closely with the LASP Mouse Modeling Core to facilitate the generation of CRISPR-mediated mouse models.



Dr. Raj Chari

LASP – Genome Modification Core (GMC)

Ongoing tech-Dev projects include the development of next generation CRISPR libraries for *in vitro* and *in vivo* screening to provide CRISPR-a and CRISPR-i reagents with fewer off-target effects, and the evaluation of sgRNA prediction algorithms using endogenous targeting data generated in human and mouse cells to determine the best algorithm to use for guide RNA designs.



Dr. Raj Chari

LASP – Mouse Modeling & Cryopreservation Core (MMC)

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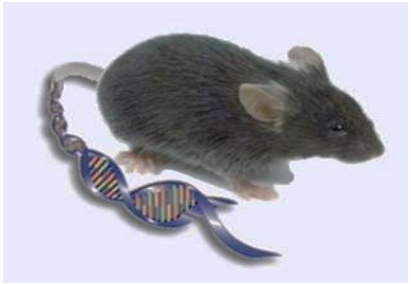
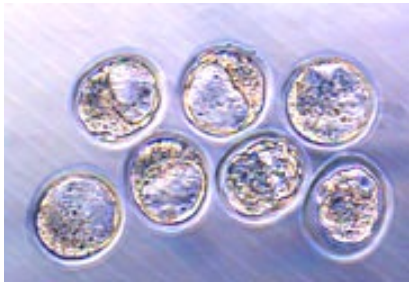
Mouse Model Development

- Generation of transgenic and CRISPR-modified mice (pronuclear injection).
- Gene targeting in mouse ES cells and subsequent generation of mice (blastocyst or morulae injections).
- 50-100 models generated annually for NCI, NIAID, and other Institutes.



Cryopreservation & Regeneration

- Cryopreservation of mouse strains and assisted reproduction (IVF).
- Regeneration of frozen mouse sperm or embryos to live mice.



The NCI Mouse Repository

- Supported by **Division of Cancer Biology** (extramural research community).
- Cryopreservation and distribution of frozen sperm or embryos for >150 popular mouse models for cancer research.
- Expansion and distribution of 1500+ validated ES cell clones with conditional-expression of miRNA constructs.

Roackie Awasthi, MS.

LASP – Mouse Modeling & Cryopreservation Core (MMC)

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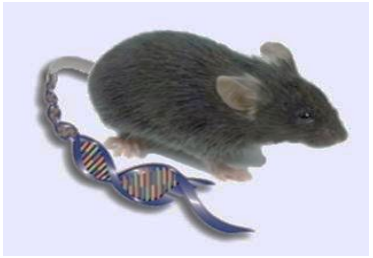
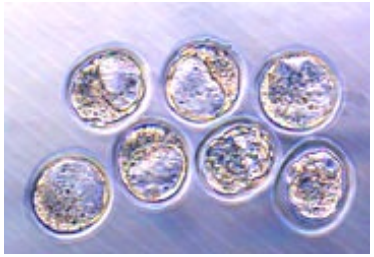
Technology Development Projects in the MMC have led to the development of new mouse models for biosensor expression detection by intravital microscopy, which involves the imaging of cells in a live animal through an window implanted into the animal tissue. In addition, the MMC has an ongoing Tech-Dev project that explores electroporation of CRISPR reagents into mouse embryos to accelerate the generation of new cancer models. The MMC has also recently expanded its capabilities to culture cells from tumors and to facilitate PDX work.





Roackie Awasthi, MS.

LASP – Animal Research Technical Support (ARTS)

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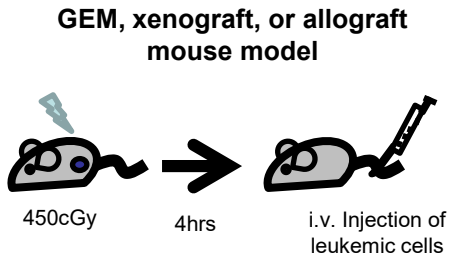
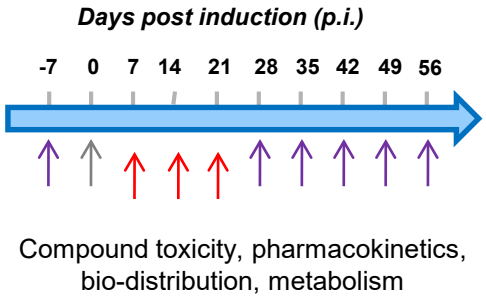
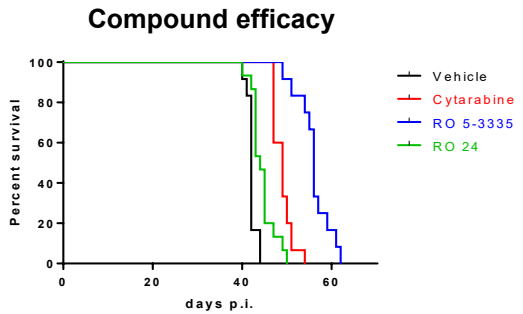
Animal Research Technical Support (2017):

- A team of 16 scientists who assist Investigators by generating and characterizing tumors in preclinical animal models.
- Assist Investigators in immunizations and the assessment of immune functions by transplantations.
- Perform metabolism studies, food and water consumption, and tissue and fluid collections.
- Perform survival surgery procedures, including tumor excision, removal of ovaries, thymus, spleen, liver (partial), colon resection, vasectomy, and the implantation of osmotic pumps and slow-release devices.







Dr. Simone Difilippantonio

A Technology-Development project can be shared across multiple LASP cores.
Example: generating a mouse model of pediatric glioblastoma

LASP core Tech-Dev in pre-clinical cancer research

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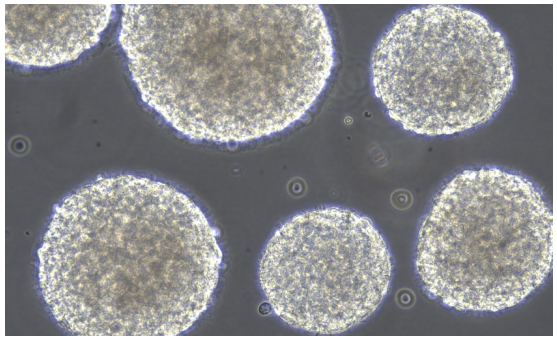
Example Project: Establishment of a Preclinical Model for Pediatric Brain Cancer

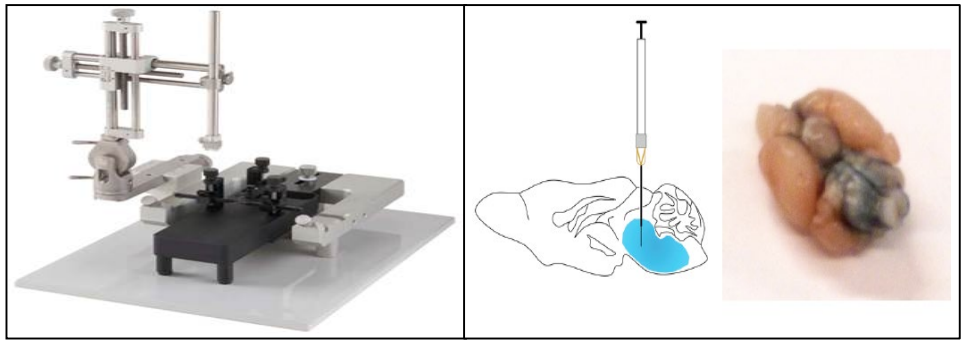
Brain tumors are the most common cause of childhood death from cancer. Diffuse intrinsic pontine glioma (DIPG) is the primary cause of brain tumor-related death in children, and there has been no improvement in patient treatment in over 40 years. Dr. Kathy Warren (Pediatric Oncology Branch) wished to establish a comprehensive DIPG treatment center and required an animal model on which to test potential therapies.

Cell culture performed by the MMC



DIPG patient cells grown as neurospheres; infected with recombinant luciferase virus.

Neurosphere injections into mice cranium performed by ARTS



Intra-cranial injections of marked neurospheres into brain stem (pons) of five-week immuno-deficient mice using a stereotaxic injection apparatus.

Leveraging LASP Cores to perform cancer research

Frederick National Laboratory for Cancer Research
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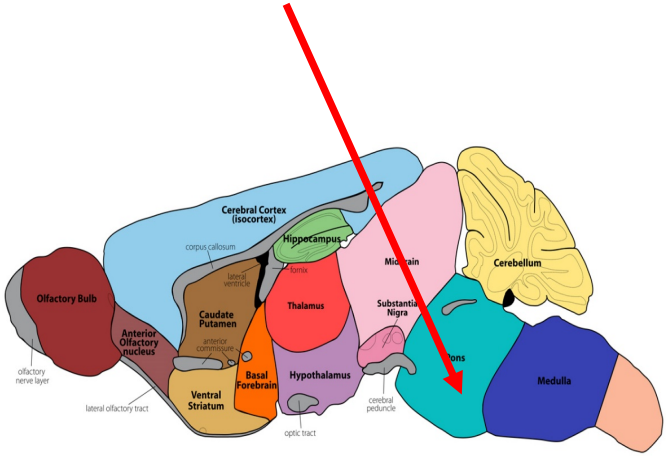
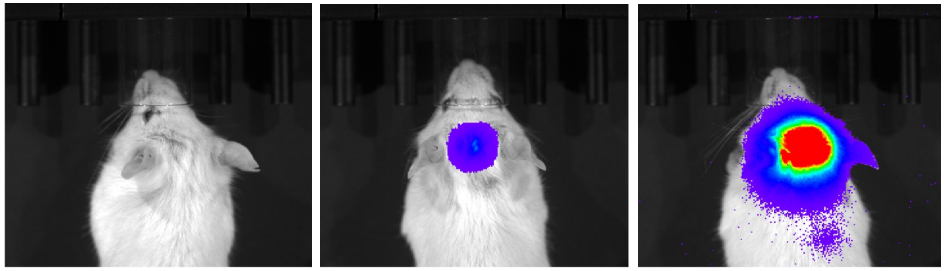
Developing a preclinical mouse model for human pediatric brain cancer

Site of cell injection by ARTS

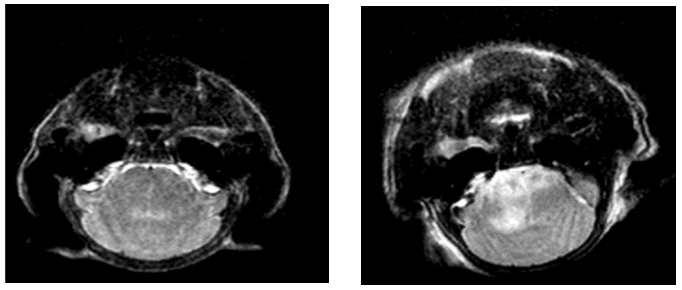


Bioluminescence of luciferase-marked DIPG cells performed by SAIP



Mock injection ***2 weeks*** ***4 weeks***

MRI of resulting tumors performed by the SAIP



Mock injection ***DIPG injection***

LASP – Small Animal Imaging Program (SAIP)

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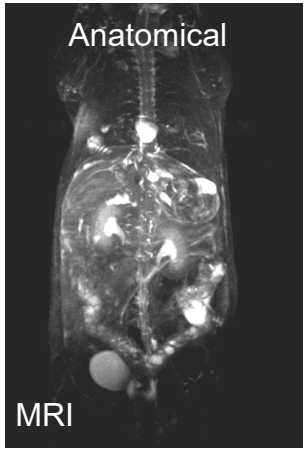
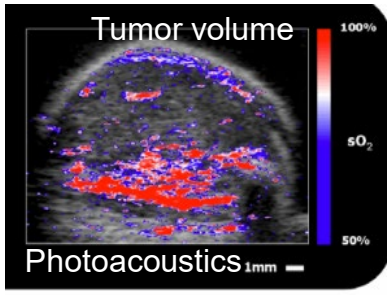
Small Animal Imaging Program collaborates with NCI investigators in:

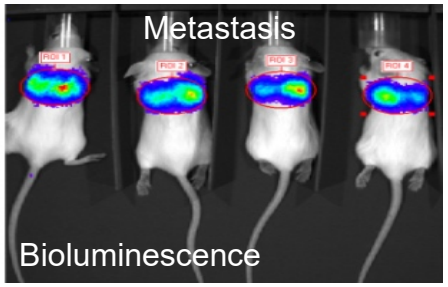
- The monitoring of mouse models of cancer utilizing non-invasive, *in vivo* imaging techniques.
- The development of new molecular imaging probes.

Assays:

Perfusion (**Ultrasound** + dynamic susceptibility contrast MRI)
Anatomical volumes (Ultrasound and **MRI**)
Angiogenesis (Ultrasound using tagged microbubbles)
Glucose metabolism [^{18}F]FDG (**PET/CT**)
Permeability (Dynamic Contrast Enhanced DCE-MRI)
Hypoxia (Oxygen saturation) (**Photo-acoustics**)
Cell Proliferation [^{18}F]FLT (PET/CT)
Cell Trafficking (**Fluorescence, Bioluminescence**)
Metastasis (Bioluminescence and MRI)
Cardiac Function and Blood Flow (Ultrasound and Doppler)
Probe bio-distribution (Gamma-well counter)



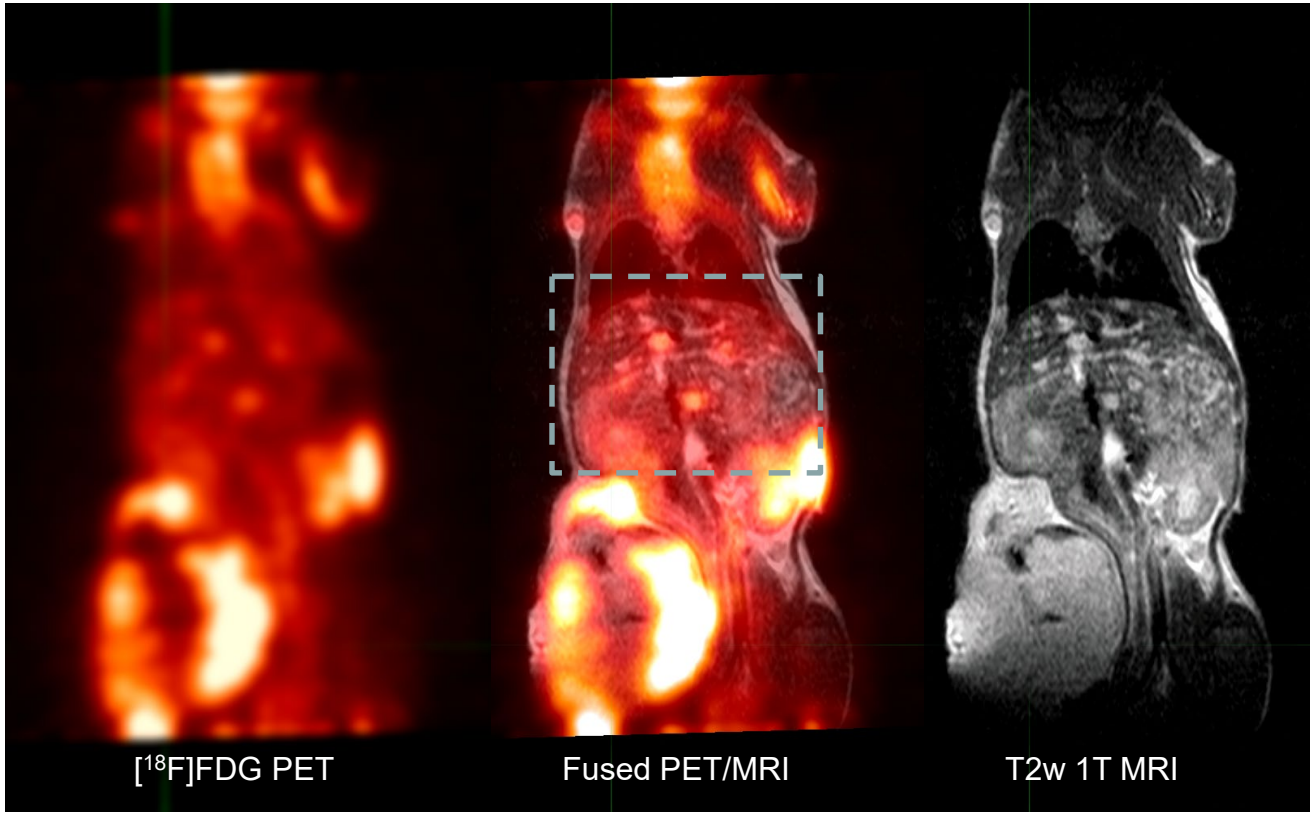




Dr. Joseph Kalen

Distinctive capabilities of the SAIP

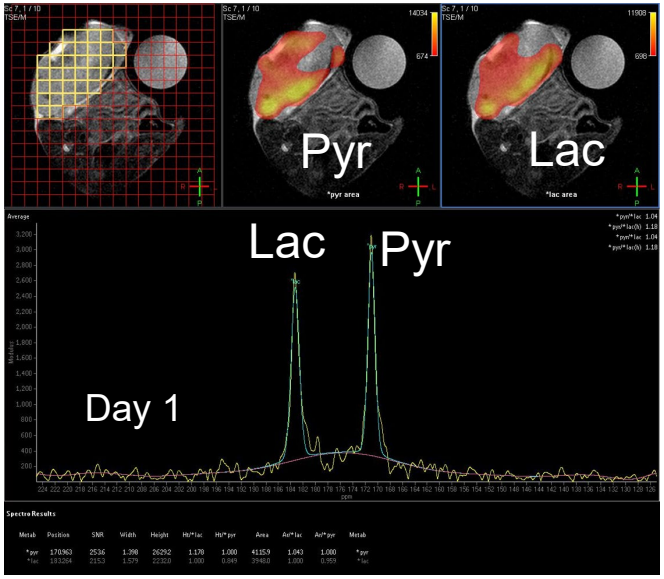
MRI/PET Fusion: enhancement for metabolic metastastic analysis



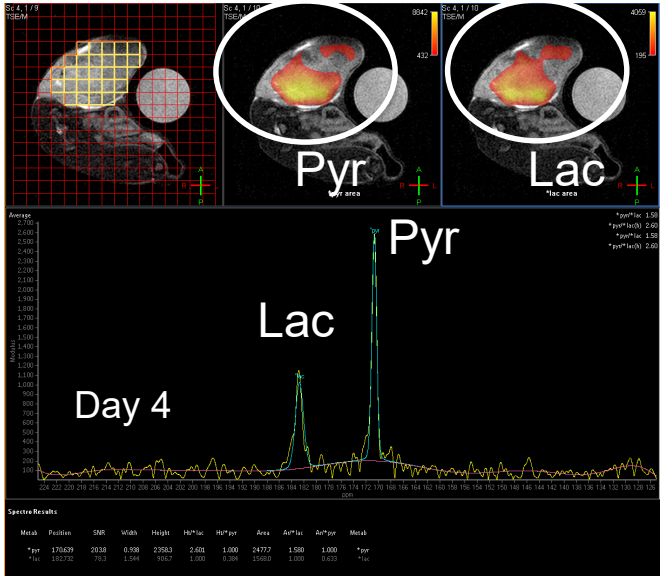
Dr. Joseph Kalen

Distinctive capabilities of the SAIP

Hyperpolarized [^{13}C]-pyruvate magnetic resonance study in a treatment model



Averaged spectrum in the tumor



	Tumor	Muscle	Blood	Tumor/Muscle	Tumor/Blood
Lac/Pyr	0.959	0.491	0.066	1.95	14.6
Lac/(Lac+Pyr)	0.490	0.329	0.062	1.49	7.9

	Tumor	Muscle	Blood	Tumor/Muscle	Tumor/Blood
Lac/Pyr	0.633	0.935	0.041	0.68	15.5
Lac/(Lac+Pyr)	0.388	0.483	0.039	0.80	9.9

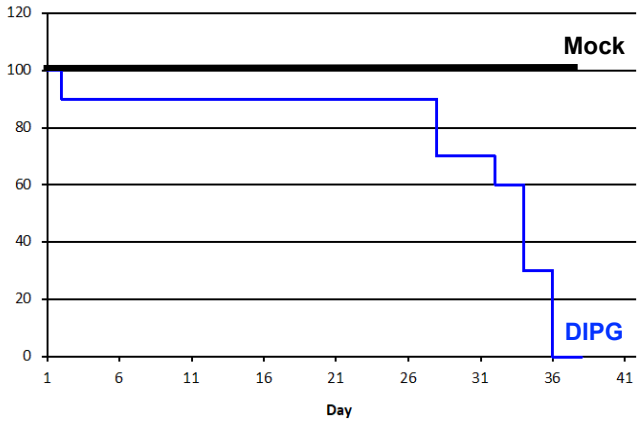
Bladder cancer (PDX), Conversion of pyruvate to lactate in tumor is reduced by Temozolomide.

Dr. Joseph Kalen

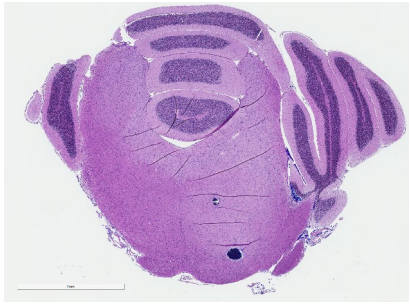
Leveraging LASP Cores to perform cancer research

In the DIPG study, histology & pathology were performed by the MHL group

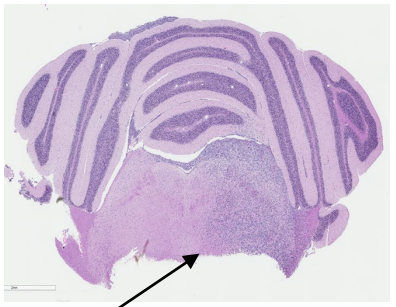
Survival post-injection



Mock injection



DIPG injection



Diffuse tumor invasion of the brain stem with leptomenegeal spread

We now have a useful (high penetrance, rapid presentation) DIPG model to test various treatments for pediatric glioma. LASP will use this mouse model to study the effectiveness of the HDAC inhibitor *panbinostat* in treating DIPG (in conjunction with the National Center for Advancing Translational Sciences)

LASP – Molecular Histopathology Lab (MHL)

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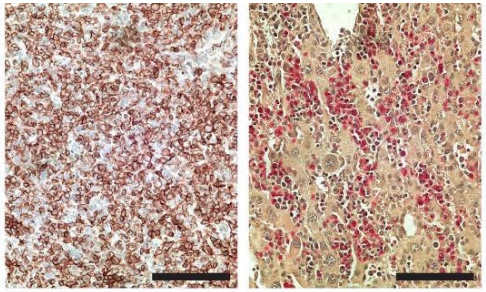
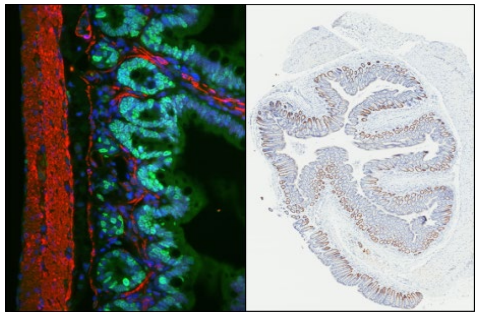
Histotechnology:

- Animal necropsies & processing of wet tissue (fixed and/or frozen).
- Generation of tissue slides with physical and enzymatic staining.
- Molecular histology assay development: immuno-staining.
- *In situ* hybridization for gene expression using nucleic acid probes.
- Laser-capture tissue micro-dissection and nucleic acid isolation.
- Hematology and blood chemistry.



Veterinary Pathology:

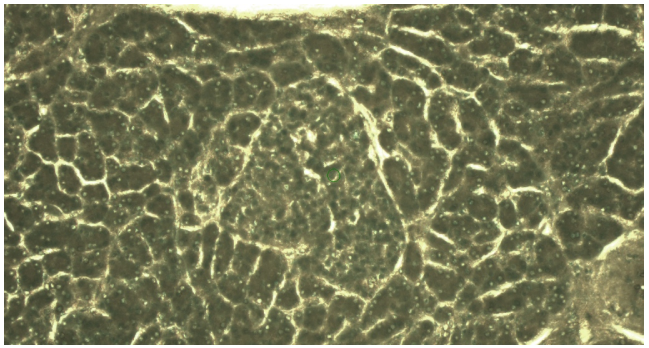
- Digital imaging and annotation, including quantitative molecular analysis.
- Phenotypic and toxicologic analysis of animal models.
- Embryology and development studies.
- Specimen classifications for micro-dissection and micro-array.
- Assistance with protocol development and study design.
- Consultations throughout the study.
- New Vet-Path fellowship program (intensive 1-year training).



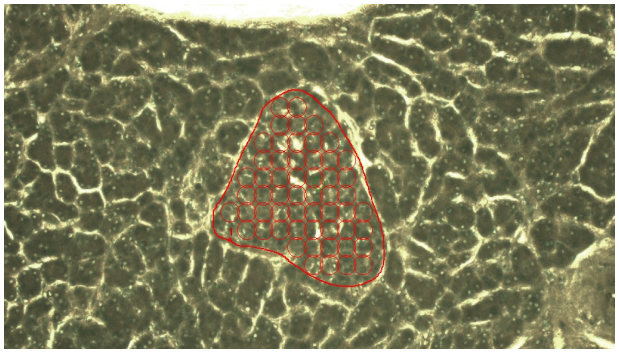
Drs. Larry Sternberg & Baktiar Karim

Distinctive capabilities of the MHL

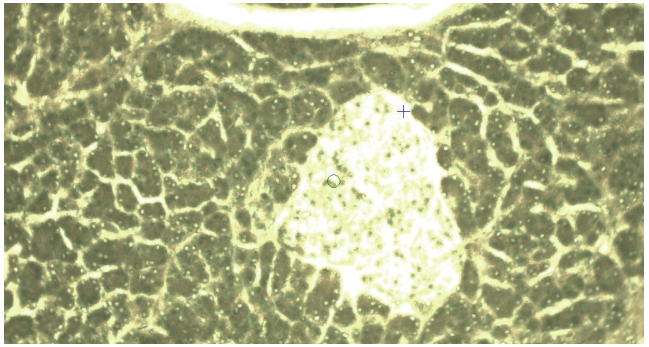
Laser Capture Microscopy



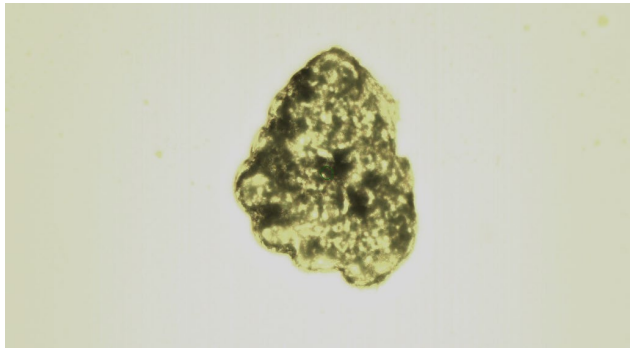
Pancreas islets before LCM



Pancreas islets annotated for LCM



Pancreas islets after LCM

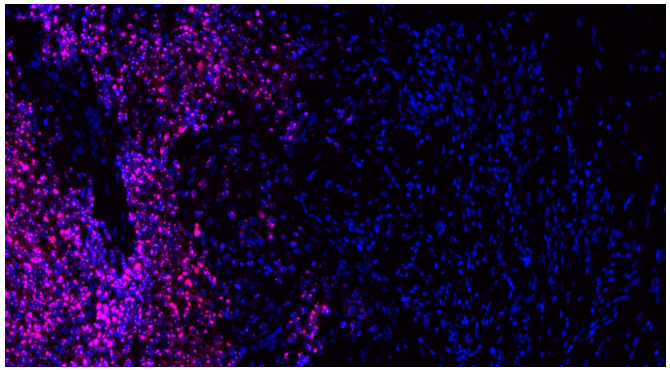


Pancreas islets on LCM cap

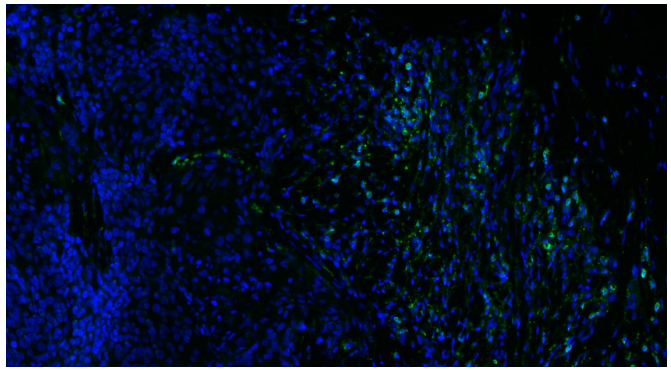
Baktiar Karim and Elijah Edmondson

Distinctive capabilities of the MHL

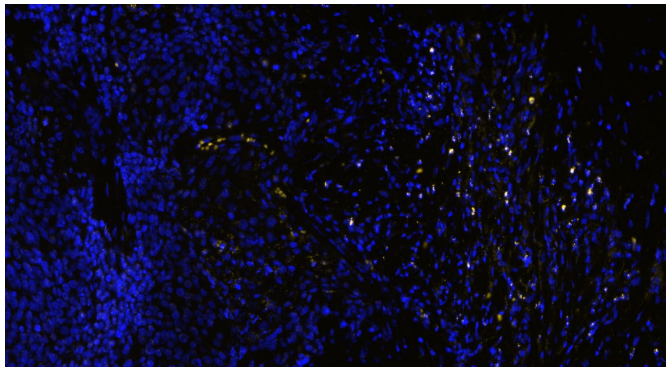
Combined immunofluorescent and *In situ* hybridization (RNAscope)



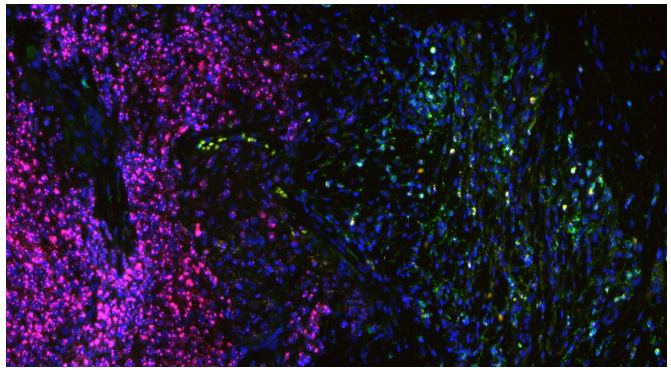
Vulvar tumor (patient) HPV-E7 ISH (20X)



Anti-CD3 (autologous, modified T cells) IHC (20X)



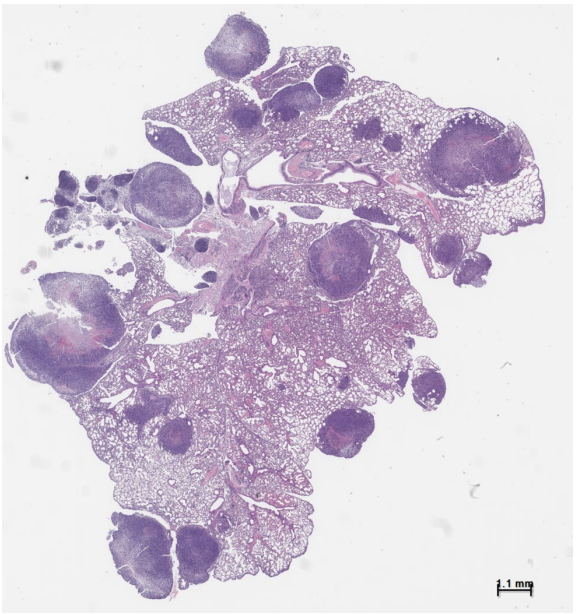
RNAscope- transduced tumor antigen receptor ISH (20X)

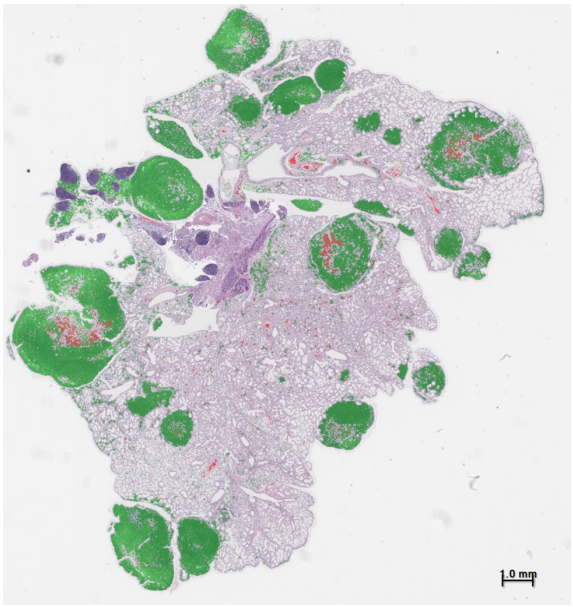


HPV patient tumor - triplex (20X)

Distinctive capabilities of the MHL

Digital Pathology (quantification of metastatic tumor burden)





Classified Area (mm²)	184.33866
Tumor Area (mm²) *green*	43.04716
Normal Area (mm²)	139.14902
Hemorrhage Area (mm²)	0.20154
necrosis Area (mm²)	1.94094

Larry Sternberg & Baktiar Karim

Aperio scan of lung section analyzed with *Halo* software

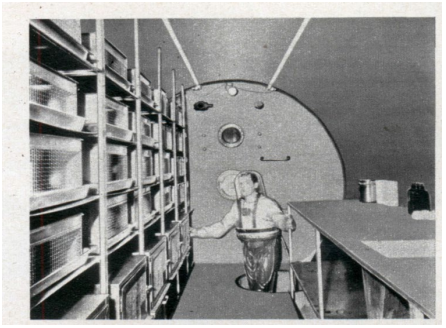
LASP – The ARTS Gnotobiotic Facility (GF)

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

Gnotobiosis (*gnostos* "known" and *bios* "life"); all life forms present within an organism are known.

Typically gnotobiotic organisms are germ-free (axenic) or gnotophoric (having only one biologic agent), though reconstitution of an axenic mouse with the microbiome of a wild mouse or with a patient microbiome (i.e. a *humanized* mouse) is becoming common.

standard mouse facility



1950's Gnotobiotics

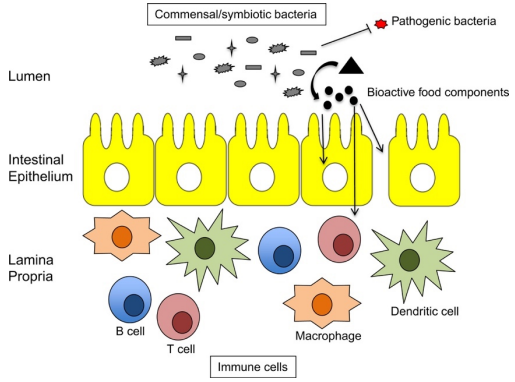
Present day- LASP Gnotobiotic Facility

Dr. Simone Difilippantonio

LASP – The Gnotobiotic Facility (GF)

The **Gnotobiotic Facility** works with NCI investigators to:

- Development and monitor germ-free mouse colonies.
- Explore the role of the microbiota in inflammation and cancer.
- Examine effects of microbiota on mouse models of human cancer.



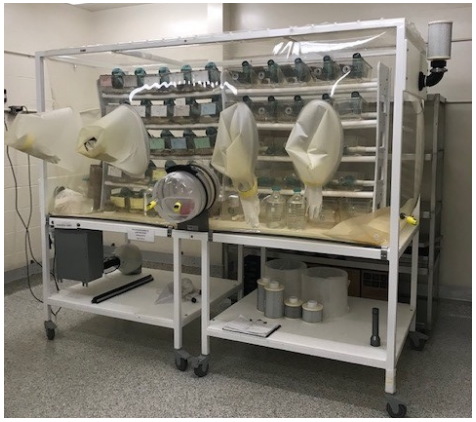
Technology Development projects in the Gnotobiotic Facility have supported the creation (2015) and expansion (2016) of the GF, the axenic rederivation of multiple GEM strains, defined the safety requirements for performing ABSL-2 experimental procedures in bio-isolators, and facilitated the use of micro-isolator racks and cages to undertake microbial and fecal transplants into germ-free mice. GF services now include association studies of human (transplanted) microbiota with inflammation and cancer treatment responses.

LASP – The Gnotobiotic Facility (GF)

10 breeder isolators (18 cages)

Staffing is dedicated to the GF and performs husbandry, isolator and equipment maintenance, colony management and experimental study support.

- 1 Research Associate II
- 1 Senior Lab Animal Technician
- 3 Lab Animal Technicians
- 1 Animal Caretaker
- *Several other technical staff members from ARTS have been cross-trained in GF procedures*



Large breeder isolator (50 cage)

40 experimental isolators (3-5 cages)

LASP – The Gnotobiotic Facility (GF)

Four bio-containment racks
(48 cages each)

- HEPA-filtered air in/out at the rack level.
- HEPA-filtered air in/out at the cage level.
- Suitable for association studies with bacteria and human fecal samples.
- ABSL2 compliant (negative pressure)

GF is located in a dedicated vivarium (B550) with own cage/rack wash and autoclave

LASP – The Gnotobiotic Facility (GF)

- Germ-free animals require a high degree of pre-project planning and requisite equipment is brought into the bio-isolator at the start of the experiment.
- Supplies are autoclaved or sprayed and brought in via transfer cylinder.
- Animal transfers from breeder to experimental isolators occurs via transfer sleeve.
- Diagnostic tests are performed whenever animals are moved or a port is opened.

Molecular diagnostic tests and bacterial/fungal culture tests performed by LASP-ADL

Distinctive capabilities of the Gnotobiotic Facility

- Germ-free strain rederivation and colony expansion
- Disease induction via various routes
- Characterization of germ-free mouse models
- Administration of investigational compounds via various routes
- Evaluation of anti-cancer therapeutics
- Microbial or fecal transplant association studies
- Compound formulation and administration
- Tissue and specimen collection in all phases of the study
- Assessment of immunologic and metabolic function during disease progression
- The GF has performed ~150 studies since 2016 using mice bred in-house.

Distinctive capabilities of LASP cores

Various technologies and services offered by LASP Cores may also be present at various academic institutes or NCI-supported cancer centers (or accessible via commercial entities). However, few cores offer the wide range of services found within an individual LASP core.

Expertise within LASP cores allows assays to be modified as projects progress. Thus, initial findings can (and often do) instruct the latter part of a study. For example an MHL histologist or pathologist can modify IHC staining or necropsy protocols to further or better evaluate the initial experimental results. Or an SAIP scientist may switch imaging modalities if the initial findings appear non-informative.

Importantly, the LASP cores often work together to further scientific goals. For example, utilizing CRISPR gene-targeting in mice involves the seamless interaction of the GMC, MMC, and ADL cores. Generating a DIPG mouse model is another example, and involved the MMC, ARTS, SAIP, and MHL cores.

Finally, there are some aspects of LASP cores are **highly distinctive** (relatively unique in the cancer research community), such as the ARTS- Gnotobiotics Facility, the digital imaging capabilities within MHL, or the nuclear imaging capabilities present in SAIP.

Laboratory Animal Sciences Program – Our Mission

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

LASP serves as a comprehensive resource for FNLCR and NCI scientists performing animal-based pre-clinical research by providing the highest level of animal care, by offering robust and cutting edge scientific support for animal studies, and by ensuring that all Investigators' animals are cared for and studied in a humane and highly professional manner (and in full accordance with regulatory guidelines).

The FNLCR - Laboratory Animal Sciences Program

Frederick National Laboratory for Cancer Research
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