National Cancer Institute

2009 CCR Mouse Research Models Mouse 101 Laboratory of Animal Sciences Program CORE Resources

Lionel Feigenbaum, Ph.D.
Director, Laboratory of Animal Sciences Program

November 2, 2009

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

SAIC Frederick

CORE resources

- Centralized Scientific Resources have been established to provide investigators access to new technologies and to complement and advance the NCI's research efforts.

Criteria

- Institutional Commitment
- High demand
- Current and developing technologies
- Time savings
- Cost Effective in relation to commercial providers and economies of scale

LASP CORE Programs

Mouse Model Development

Animal Care

Strain Preservation

In-vivo Imaging

Phenotyping-PHL

NCI-Repository

SAIC Frederick

Mouse Model Development

Transgenic mice

Generation of transgenic mice by pronuclear microinjection with:

- Plasmid
- Cosmid
- Bacterial artificial chromosome (BAC)
- P1 artificial chromosome (PAC)
- Lentiviral vectors

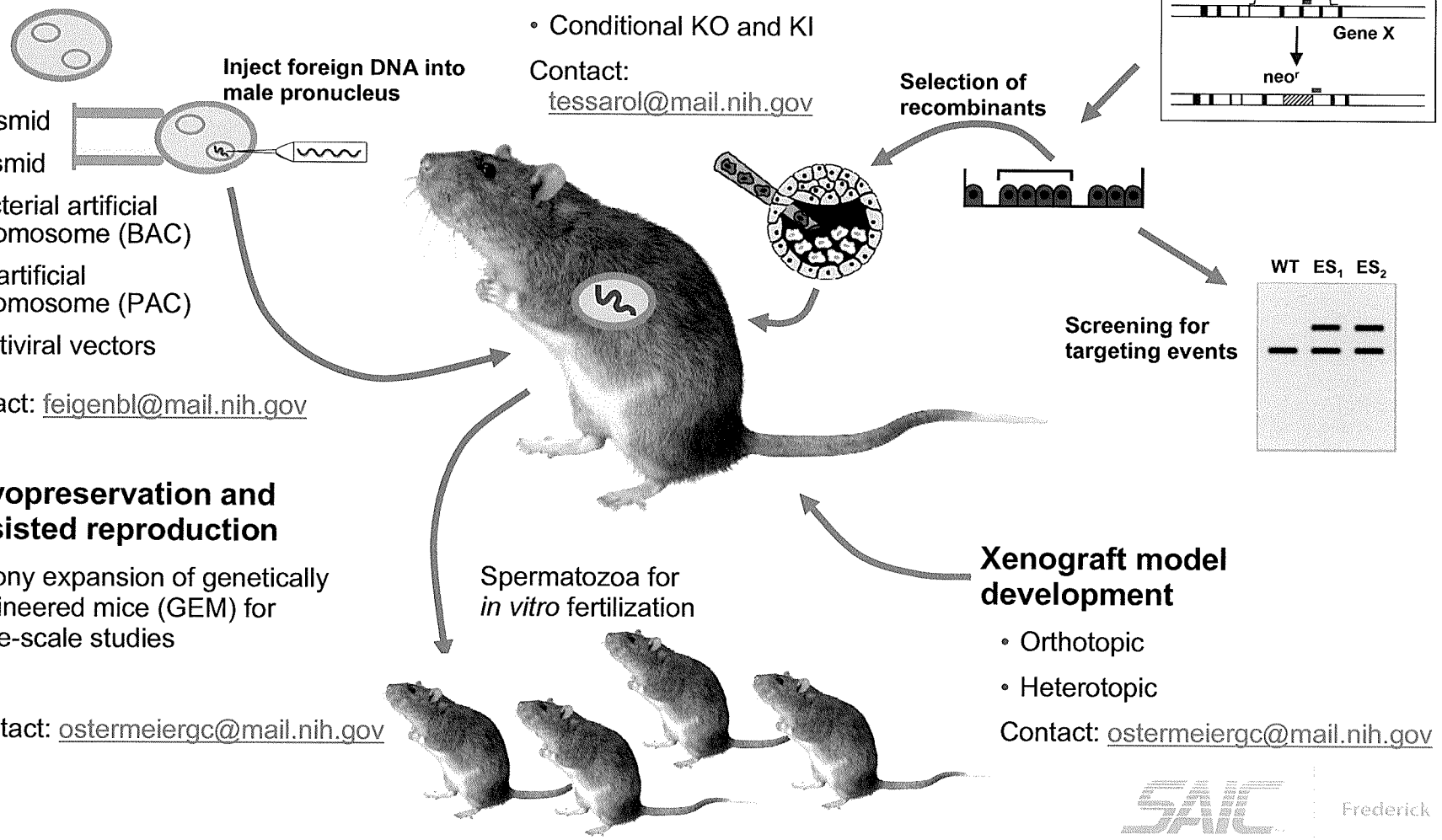
Contact: feigenbl@mail.nih.gov

Gene targeting

Production of:

- Knockout (KO)
- Knockin (KI)
- Conditional KO and KI

Contact: tessarol@mail.nih.gov



Cryopreservation and assisted reproduction

Colony expansion of genetically engineered mice (GEM) for large-scale studies

Contact: ostermeiergc@mail.nih.gov

Xenograft model development

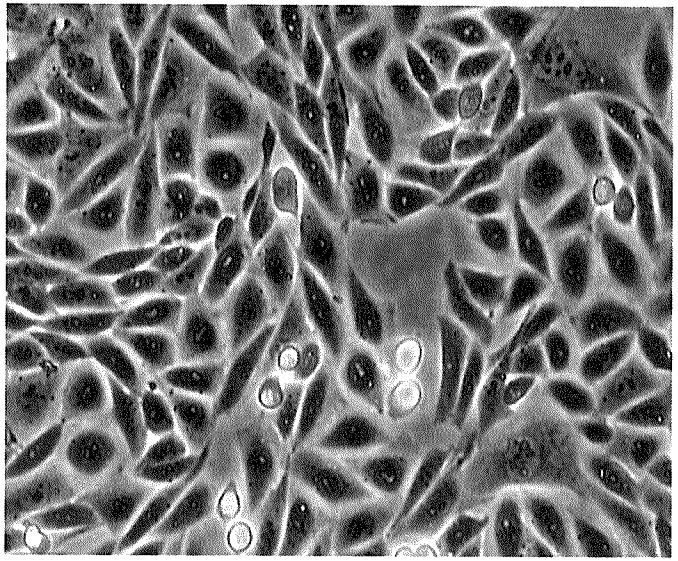
- Orthotopic
- Heterotopic

Contact: ostermeiergc@mail.nih.gov

SAIC Frederick

Cell Culture and Tumorigenicity Laboratory

- Centralized facility providing technical support for the maintenance and culture of cells for orthotopic and xenograft mouse studies.
  - Importation of cell lines into NCI and infectious agent testing.
  - Expansion and freezing of cell lines for long term storage.
  - Secure storage and tracking of frozen cell line stocks.
  - Recovery, expansion and preparation of cells for transplantation into mice.
  - Delivery of prepared cells to animal facility staff.



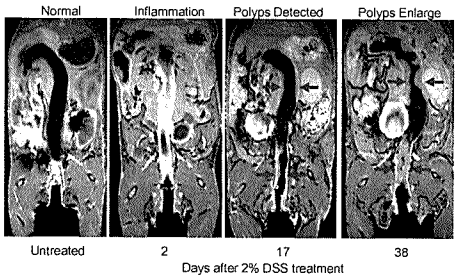
Contact: ostermeiergc@mail.nih.gov

Small Animal Imaging Program (SAIP) at NCI-Frederick

- Provide state-of-the-art in vivo imaging to the NCI community.
- Ability to understand and develop new mouse models, molecular markers, theragnostics and monitor tumors in vivo.

3T MRI

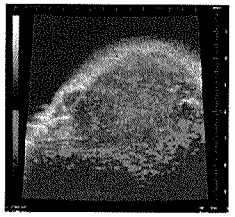
(Philips Medical Systems)



Imaging colon tumors.

ULTRASOUND IMAGING

(VisualSonics, Inc.)



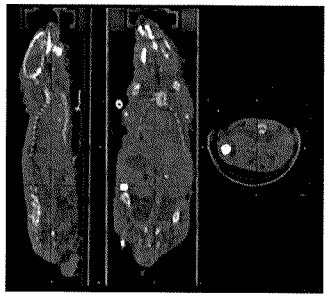
Imaging angiogenesis using microbubbles tagged with VEGFR2 antibodies.

Data Analysis

Biodistribution
Anatomical and Physiological Volume
Image Probe Uptake (Wash-In/Out)
Parametric Maps
Kinetic Modeling
Fluorescence Lifetime
Tumor Vasculature
Blood Flow

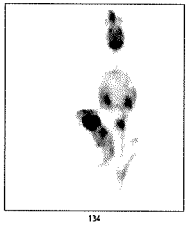
NUCLEAR IMAGING

NanoSPECT/CT
(Bioscan, Inc.)



Fused SPECT/CT

Inveon PET/CT
(Siemens Medical)



Tumor imaging using [18F]-deoxyglucose, a glucose analog, to image metabolic activity.

OPTICAL IMAGING

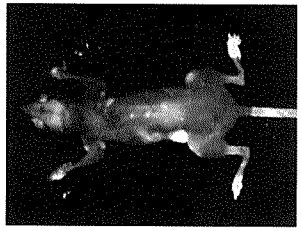
Bioluminescence

Xenogen IVIS SPECTRUM
(Caliper Life Sciences)

Cell Trafficking

Fluorescence

Maestro-GNIR
(Cambridge Research Institute)
OPTIX MX2
(Advanced Research Technologies)



Contact: kalenj@mail.nih.gov

SAIC Frederick

Pathology/Histotechnology Lab (PHL)

- Service core that integrates anatomic pathology, molecular histology and imaging.
- Work with other LASP cores to increase capabilities http://web.ncifcrf.gov/rtp/lasp/phl/

Functional units:

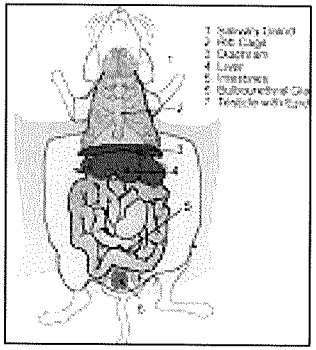
Anatomic Pathology: (Contact: anvermr@mail.nih.gov)

Histotechnology: (Contact: sternberglr@mail.nih.gov)

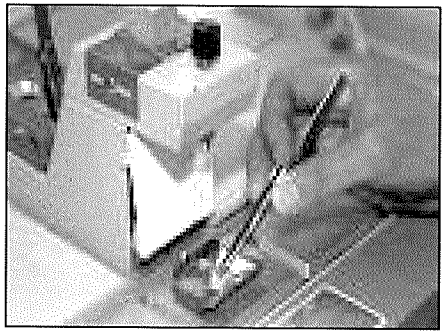
- Necropsy, Tissue Processing,
- Hematology, Clinical Chemistry
- Microtomy, Staining
- Molecular Histology (IHC,ISH)
- Nucleic Acid Isolation/LCM
- Image Capture and Analysis

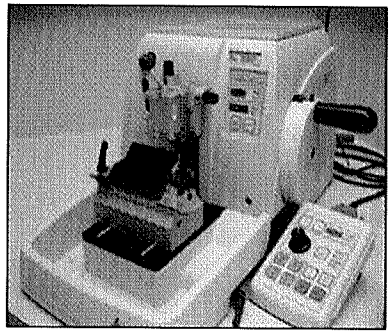
Histotechnology Services

- **Animal Necropsy**
  - Full Bioassay
  - Embryo Micro-dissection

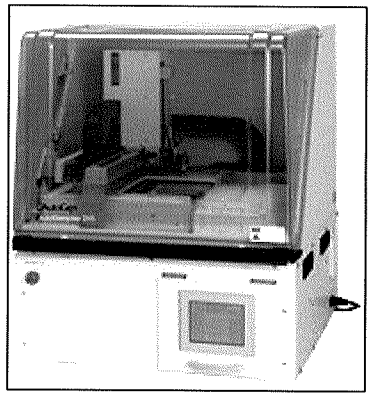


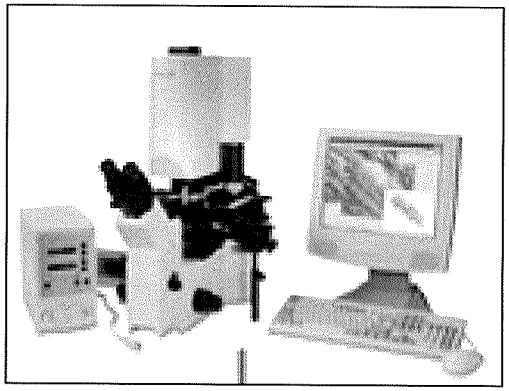
- **Tissue Processing**
  - Trim
  - Tissue Blocks
    - Paraffin, Frozen, Plastic
  - Microtomy/ Vibratome
  - Histological Staining



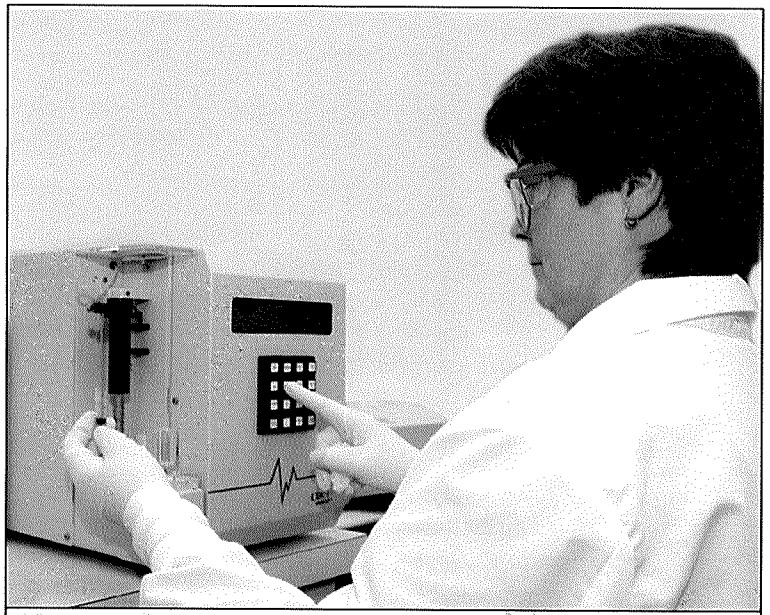


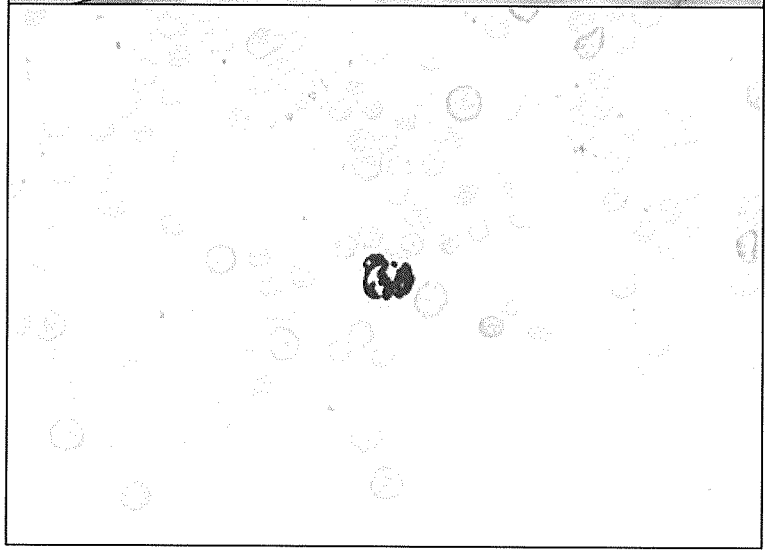
- **Tissue Derivatives**
  - DNA/ RNA/ Protein
  - LCM (Laser Capture)
    - Pathology Driven





Hematology and Clinical Chemistry





NCI - FREDERICK CANCER CENTER RESEARCH & DEVELOPMENT
Boyles Street * Frederick, MD. 21702-1201
(301) 846-5190

Patient: _____
Patient ID: PATIENT 00

Species: MOUSE
Test No: 15

Date: 12/14/01
Time: 15:22:10

MASCOT™ HEMATOLOGY PROFILE: **HEMAVET® 850**

Parameter(Units)		Results	Normal Range
Leukocytes:			
WBC	(K/µL)	4.14	1.8 - 10.7
NE	(K/µL)	2.30	0.1 - 2.4
LY	(K/µL)	1.54	0.9 - 9.3
MO	(K/µL)	0.04	0.0 - 0.4
EO	(K/µL)	0.21 H	0.0 - 0.2
BA	(K/µL)	0.06	0.0 - 0.2
NRBC	(K/µL)		RARE
NE	(%)	55.48 H	6.6 - 38.9
LY	(%)	37.21 L	55.8 - 91.6
MO	(%)	0.90	0.0 - 7.5
EO	(%)	5.02 H	0.0 - 3.9
BA	(%)	1.39	0.0 - 2.0
NRBC	(%)		RARE
Erythrocytes:			
RBC	(M/µL)	9.46 H	6.36 - 9.42
Hb	(g/dL)	12.7	11.0 - 15.1
HCT	(%)	42.9	35.1 - 45.4
MCV	(fL)	45.3 L	45.4 - 60.3
MCH	(pg)	13.4 L	14.1 - 19.3
MCHC	(g/dL)	29.6 L	30.2 - 34.2
RDW	(%)	19.1	12.4 - 27.0
RSD	(fL)		
Retics	(M/µL)		0.09 - 0.97
Retics	(%)		5.58 - 9.91
Thrombocytes:			
PLT	(K/µL)	636.	592. - 2972.
PCT	(%)		
MPV	(fL)	4.7 L	5.0 - 20.0
PDW	(%)		

HEMATOLOGICAL ABNORMALITIES

WBC: LOW <----------> HIGH
NORMAL
NORMAL
NORMAL
NORMAL
EOSINOPHILIA
NORMAL

LOW <----------> HIGH

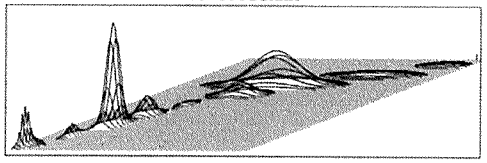
RBC: LOW <----------> HIGH
POLYCYTHEMIA
NORMAL
NORMAL
MICROCYTOSIS
LOW
LOW
NORMAL

PLT: LOW <----------> HIGH
NORMAL
LOW

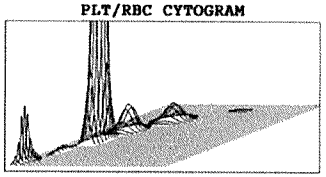
DIAGNOSTIC CONSIDERATIONS:

RECOMMENDED FOLLOW-UP:

WBC CYTOGRAM



PLT/RBC CYTOGRAM

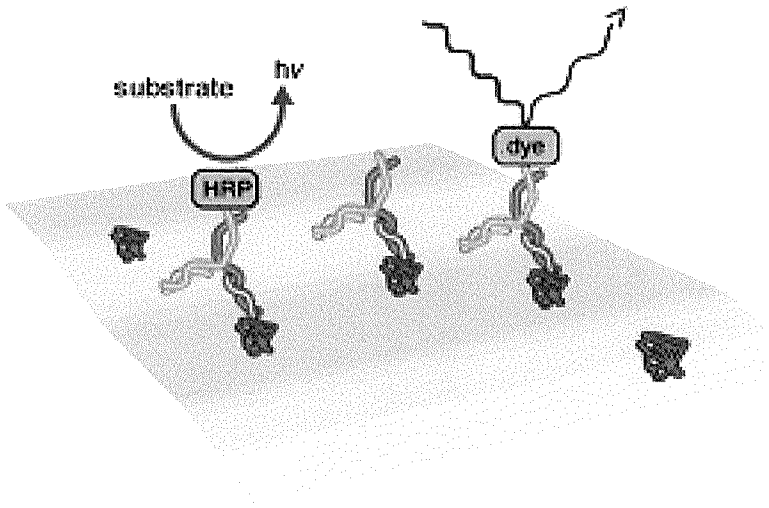


CDC Technologies Inc., Oxford, Connecticut 1-800-858-7331

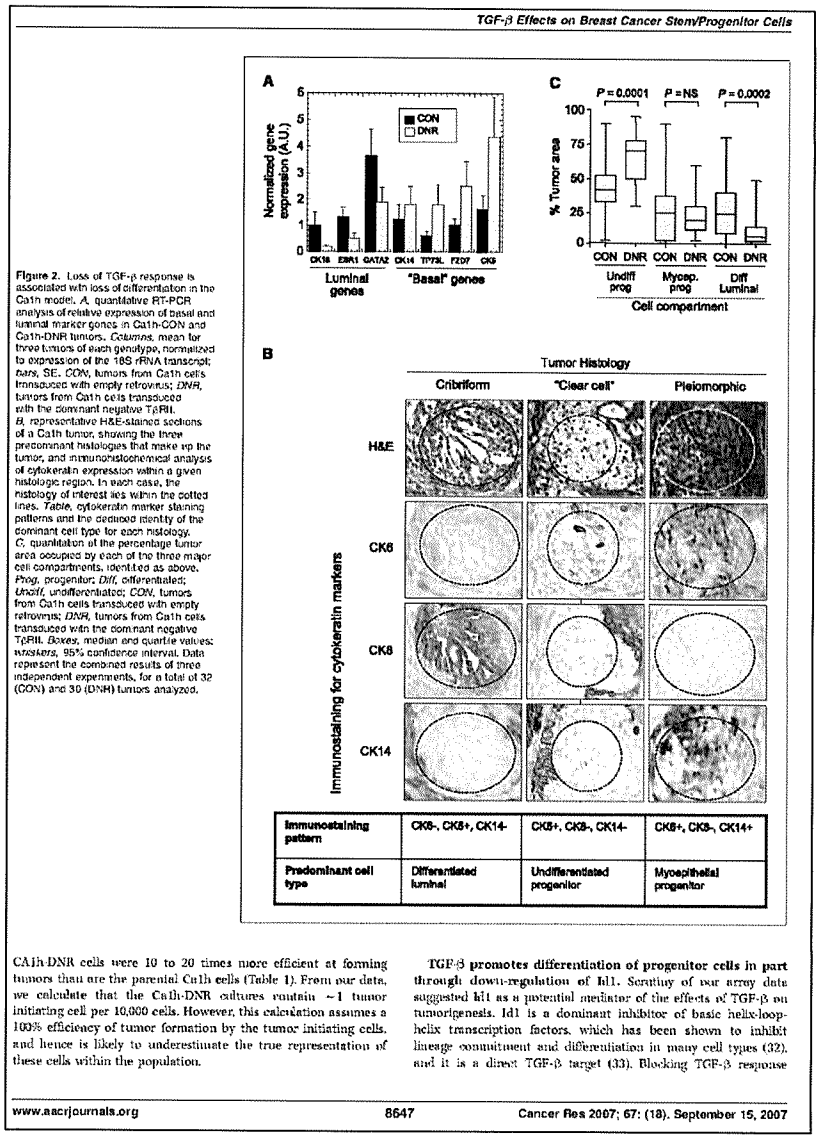
Immunohistochemistry

PHL IHC Database (400+ Protocols) http://www.ncifcrf.gov/rtp/lasp/phl/immuno/

- **Antigen** is recognized by a specific **Antibody**



- Uses in Veterinary Pathology
  - Confirm diagnosis
  - Detect micro-metastasis
  - Prognostic markers
  - Diagnosis of infectious disease



TGF-β Effects on Breast Cancer Stem/Progenitor Cells

Figure 2. Loss of TGF-β response is associated with loss of differentiation in the Ca1h model. *A*, quantitative RT-PCR analysis of relative expression of basal and luminal marker genes in Ca1h-CON and Ca1h-DNR tumors. *Columns*, mean for three tumors of each genotype, normalized to expression of the 18S rRNA transcript; *bars*, SE. *CON*, tumors from Ca1h cells transduced with empty retrovirus; *DNR*, tumors from Ca1h cells transduced with the dominant negative TβRII. *B*, representative H&E-stained sections of a Ca1h tumor, showing the three predominant histologies that make up the tumor, and immunohistochemical analysis of cytokeratin expression within a given histologic region. In each case, the histology of interest lies within the dotted lines. *Table*, cytokeratin marker staining patterns and the deduced identity of the dominant cell type for each histology. *C*, quantitation of the percentage tumor area occupied by each of the three major cell compartments, identified as above. *Prog*, progenitor; *Diff*, differentiated; *Undiff*, undifferentiated; *CON*, tumors from Ca1h cells transduced with empty retrovirus; *DNR*, tumors from Ca1h cells transduced with the dominant negative TβRII. *Boxes*, median and quartile values; *whiskers*, 95% confidence interval. Data represent the combined results of three independent experiments, for a total of 32 (CON) and 30 (DNR) tumors analyzed.

Immunostaining pattern	CK5-, CK8+, CK14-	CK5+, CK8-, CK14-	CK5+, CK8-, CK14+
Predominant cell type	Differentiated luminal	Undifferentiated progenitor	Myoepithelial progenitor

Ca1h-DNR cells were 10 to 20 times more efficient at forming tumors than are the parental Ca1h cells (Table 1). From our data, we calculate that the Ca1h-DNR cultures contain ~1 tumor initiating cell per 10,000 cells. However, this calculation assumes a 100% efficiency of tumor formation by the tumor initiating cells, and hence is likely to underestimate the true representation of these cells within the population.

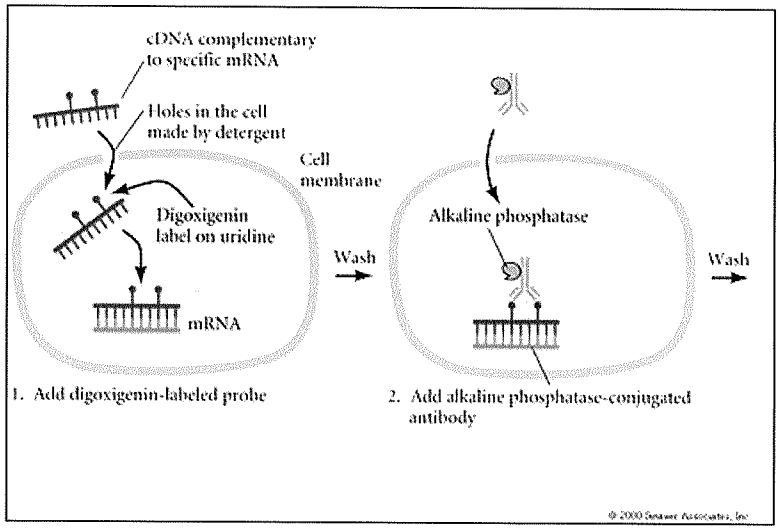
TGF-β promotes differentiation of progenitor cells in part through down-regulation of Id1. Scrutiny of our array data suggested Id1 as a potential mediator of the effects of TGF-β on tumorigenesis. Id1 is a dominant inhibitor of basic helix-loop-helix transcription factors, which has been shown to inhibit lineage commitment and differentiation in many cell types (32), and it is a direct TGF-β target (33). Blocking TGF-β response

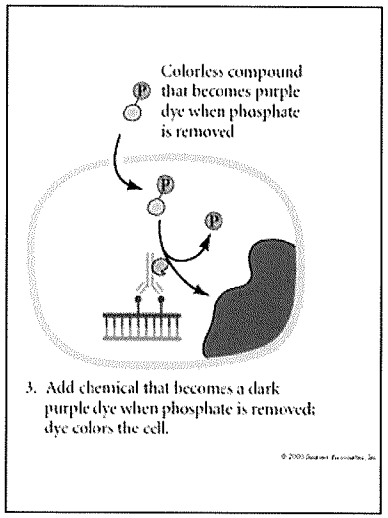
www.aacrjournals.org 8647 Cancer Res 2007; 67: (18). September 15, 2007

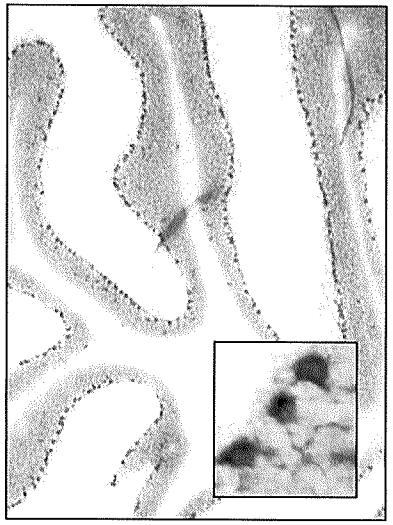
In Situ Hybridization (ISH)

Identifying the cellular source of a protein using mRNA as a proxy

- **Method utilizes a labeled "anti-sense" riboprobe to visualize corresponding target mRNA**
  - Custom Probe Design, Synthesis and Validation
  - Optimized Tissue Processing
  - RNAse-Free Microtomy
  - Novel Assay Development
  - Control Reagents
  - Intavis ProVs Robot Stainer
  - Digital Image Documentation
  - Pathology Interpretation

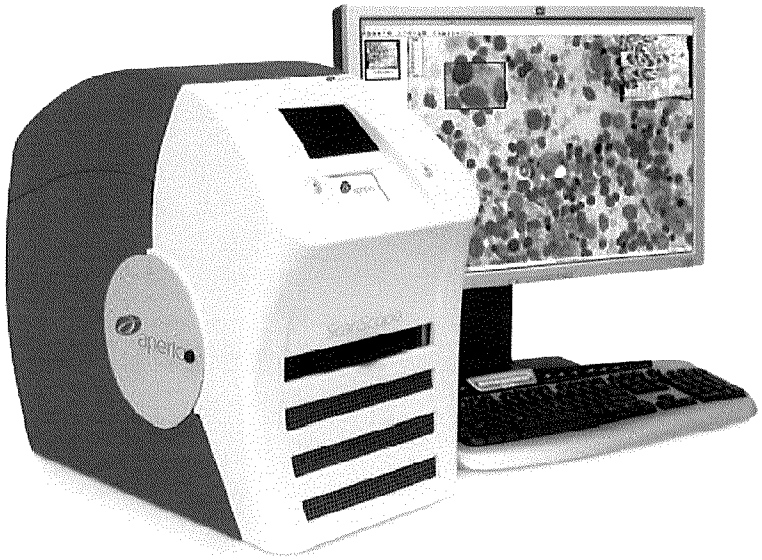




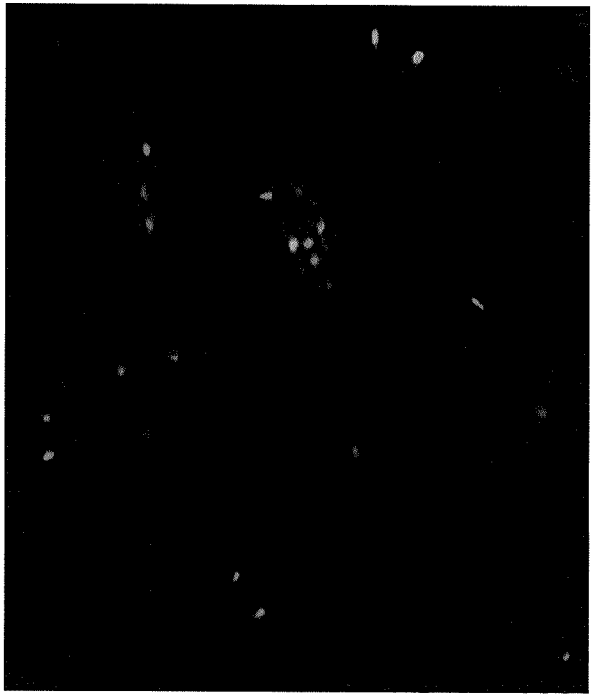


Digital Image Capture

- Digital Whole Slide Scanning (Aperio ScanScope)
  - "publication-ready" image files at 5-40x native resolution
- Digital Slide Conferencing ("tele-pathology"):
  - Secure Web-Access to Images and/or annotation prior to LCM

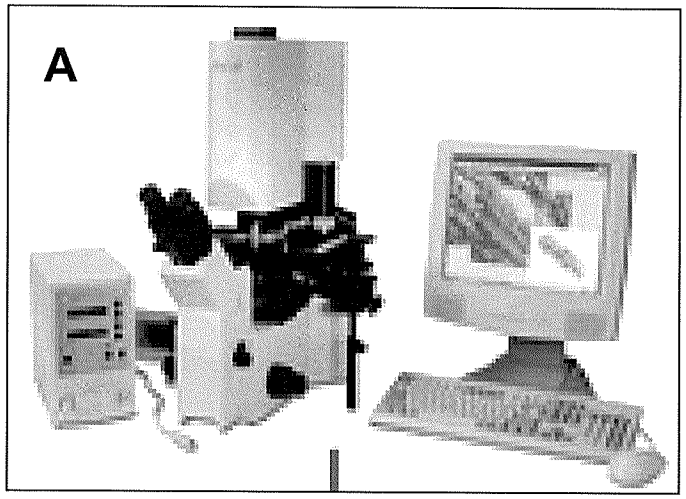


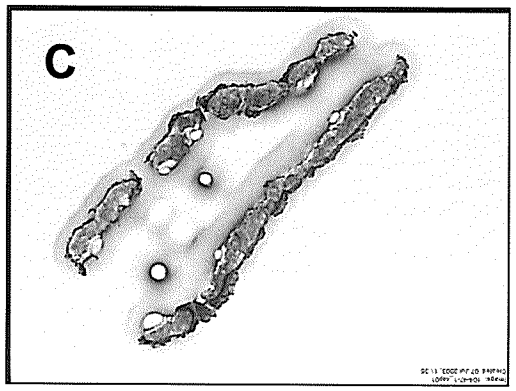
The ScanScope® OS allows you to create digital slides of blood smears, bone marrow, Gram stain, and other microscopic specimens using oil immersion.

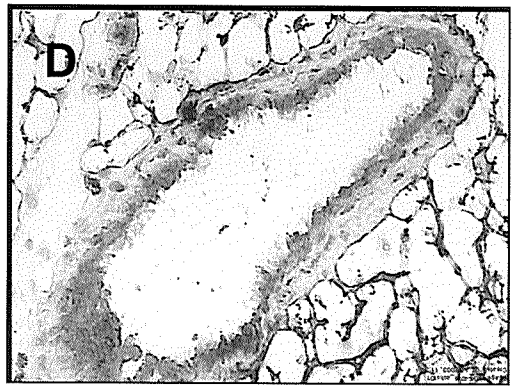


Laser Capture Microdissection and Laser Cutting

- Workflow:
  - Sample Preparation
  - Pathology Review (A)
  - LCM
    - Selection (B)
    - Capture (C)
    - Validation (D)

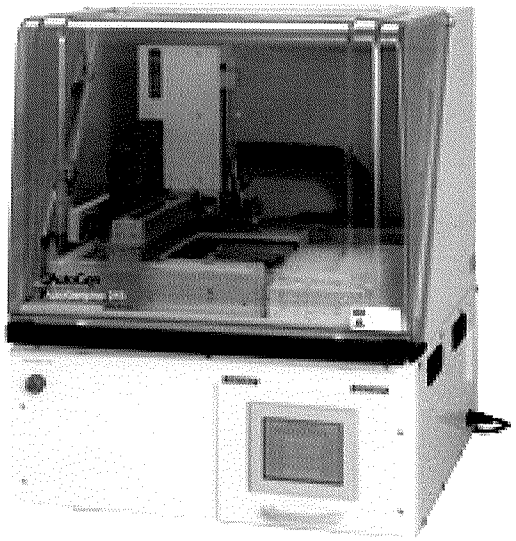






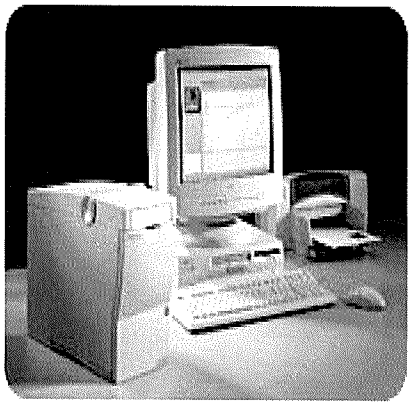
Nucleic Acid Isolation

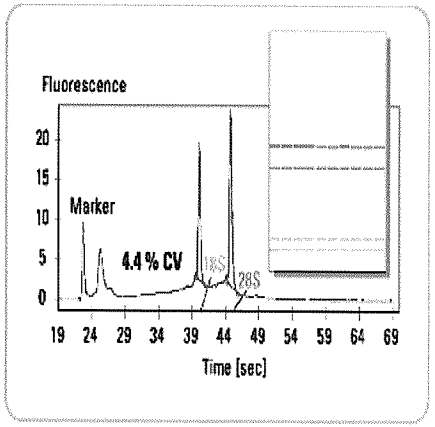
- **The Autogen 245 nucleic acid workstation automates and standardizes isolation of DNA and/or RNA** from 1-24 fresh, frozen, or fixed samples
  - Homogenization and inactivation of nucleases occur simultaneously.
  - Acid phenol extraction compatible with micro RNA studies.
  - Yield and purity check via spectrophotometer (NanoDrop)



AutoGen 245 Workstation

- **The Agilent 2100 Bioanalyzer capillary electrophoresis system simplifies quality analysis of RNA**
  - Analysis via NanoChip or PicoChip results in an accepted measure of RNA quality termed the "RNA Integrity Number (RIN)"
  - Material produced is suitable for CGH, sequencing, global gene expression profiling, qPCR, etc.

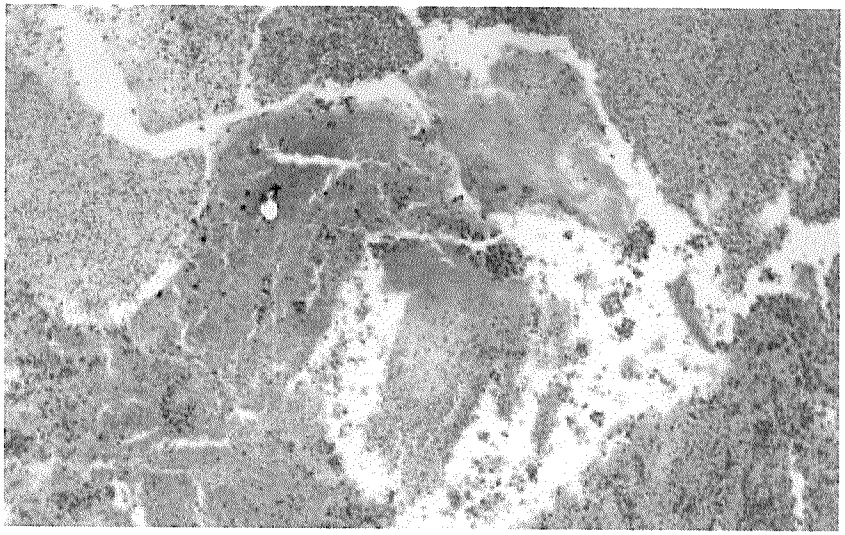




Agilent 2100 Bioanalyzer

Veterinary Pathology

- Study design
- Protocol development
- Data analysis
- Animal imaging validation



Address http://web.ncifcrf.gov/default.aspx

NCI-Frederick

ABOUT RESEARCH SERVICES CAREERS CAMPUS PHONE CONTACT

- Order Services
- Animal Resources
- Bioinformatics and Biostatistics
- Chemistry and Structural Biology
- Clinical Research Support
- Cytogenetics
- Services to Other Govt Agencies
- Services to Non-Govt Agencies
- Flow Cytometry
- Genetics, Genomics, DNA Sequencing
- Imaging/Microscopy
- Mass Spectrometry
- Microarray and Quantification of Gene Expression
- Protein Chemistry and Biophysics
- Protein Expression/Purification
- Proteomics
- Repository Services
- Research Donor Program
- Scientific Graphics and Media
- SAIC-F Customer Support

SEARCH

THIS MONTH AT N

Spotlight on Jean

Not many people ha
However, Jeanne He
Medicine (LAM) pro
more...

NEWS CENTER

The Influence of Variati
Chemistry (Chemist?)
HIV-1 Assembly, Relea
Foamy Virus (non)path
CANCELLED-Circulati

r Pool
Diversity Team
e 2007
k For Others Program

TOP LINKS

Order Services
Maps & Directions
Security & Safety
Shuttle Schedule
Scientific Library
Emergency Preparedness
Computer & Statistical Services
Fort Detrick

NCI-FREDERICK QUICKLINKS

Login / Create Account

- Yellow Task System
- ATP Accessions System (CSAS)
- Outreach & Special Programs
- Getting Started at NCI-Frederick

WHAT'S FOR LUNCH

NCI-Frederick Cafeteria

Intern

Address http://web.ncifcrf.gov/research/s_core_new.asp#animals

ABOUT RESEARCH SERVICES CAREERS CAMPUS PHONE CONTACT

RESEARCH

Research Overview
Scientists
Labs/Branches/Programs
Biotechnology Resources
Research Services Catalog
Faculties & Interest Groups
Center for Cancer Research
Develop. Therapeutics

Overview of Services
Access Methods

ORDER SERVICES

PHONEBOOK QUICK SEARCH

FIND

NCI-FREDERICK QUICKLINKS

1. Yellow Task System
2. ATP Accessions System (CSAS)
3. Center for Cancer Research (CCR)
4. Employee Diversity Team
5. NCI-Frederick Fitness Challenge
6. Outreach & Special Programs
7. Getting Started at NCI-Frederick
8. C&SS Helpdesk
9. Online Warehouse
10. ALS

Login / Create Account

Print this page. Quicklinks Login.

NCI-Frederick Biotechnology Available Services

Below is a list of available Research Support Services at the NCI-Frederick, along with the preferred access method. To learn more about a particular service, simply click on the service's name; investigators may also place requests directly by clicking on "access method" link. Each resource is listed under the discipline or technology that it supports. Availability of these resources may be on a open or limited basis.

Open – Open and available to all NCI intramural investigators.
Limited – Available on a limited basis to NCI investigators who are outside the lab/branch that sponsors this resource.

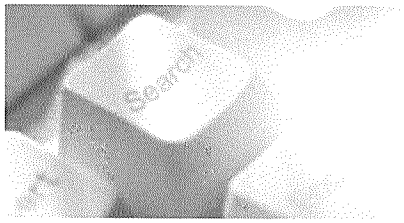
Jump to: Select a Service Area...

Service Area	Availability	Access Method
Animal Resources		
Comparative Molecular Pathology Unit, CCR	Open	Direct
Gene Targeting Facility, CCR	Open	Direct
Laboratory Animal Sciences Program, SAIC	Open	Direct

- Animal Technical and Veterinary Services (Bethesda/Frederick)
- Animal Care and Use Committee (Bethesda/Frederick)
- Transgenic/Knockout Services
- Speed Congenics
- Cryopreservation/Breeding
- Histotechnology/Veterinary Pathology
- Mouse Models of Human Cancers Repository
- Animal Health & Molecular Diagnostic Services
- Receiving & Quarantine Services
- In Vivo Imaging Services

http://web.ncifcrf.gov/rtp/lasp/intra/default.asp Go

Home Menu Forms Access LASP Services Contacts



Need to find something? Try the LASP *Search* Feature!

Target information specific to LASP for quick and accurate results by using the LASP Search. Advanced Search options allow you to narrow your search criteria, expand the search area, and modify how your results will be listed.

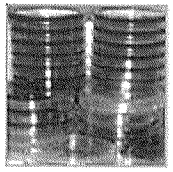
Enter search term [ ] search

NEW **Prevention and Detection of Adventitious Infections of Mice with Mouse Parvovirus (MPV) and Murine Norovirus (MNV) Lecture** [William Shek, DVM, PhD]

CHARLES RIVER LABORATORIES

Research Support Services

LASP provides scientific support services to assist investigators conducting animal-based research in Frederick and Bethesda



Animal Health & Diagnostics

AHDL is responsible for animal health monitoring by providing diagnostic services at both the Frederick and Bethesda holding locations. Online animal health reports are available here.

Animal Holding & Tech Support

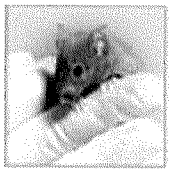
LASP assists investigators with housing and technical services in support of their animal-related research projects at both the Frederick and Bethesda campuses

Receiving & Quarantine

The R&Q facility located at the Frederick campus allows investigators to import strains from a variety of sources into Frederick and Bethesda facilities. Online request forms are available here.

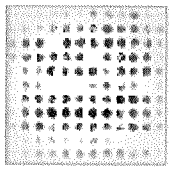
Veterinary Services

The LASP veterinary staff members provide clinical support and consultation services to ensure optimal health conditions for animals housed at both the Frederick and Bethesda campuses



Animal Care & Use Committee

LASP provides support to the ACUC at both the Frederick and Bethesda campuses to ensure compliance with Federal regulations governing the care and use of animals in biomedical research



Pathology & Histotechnology

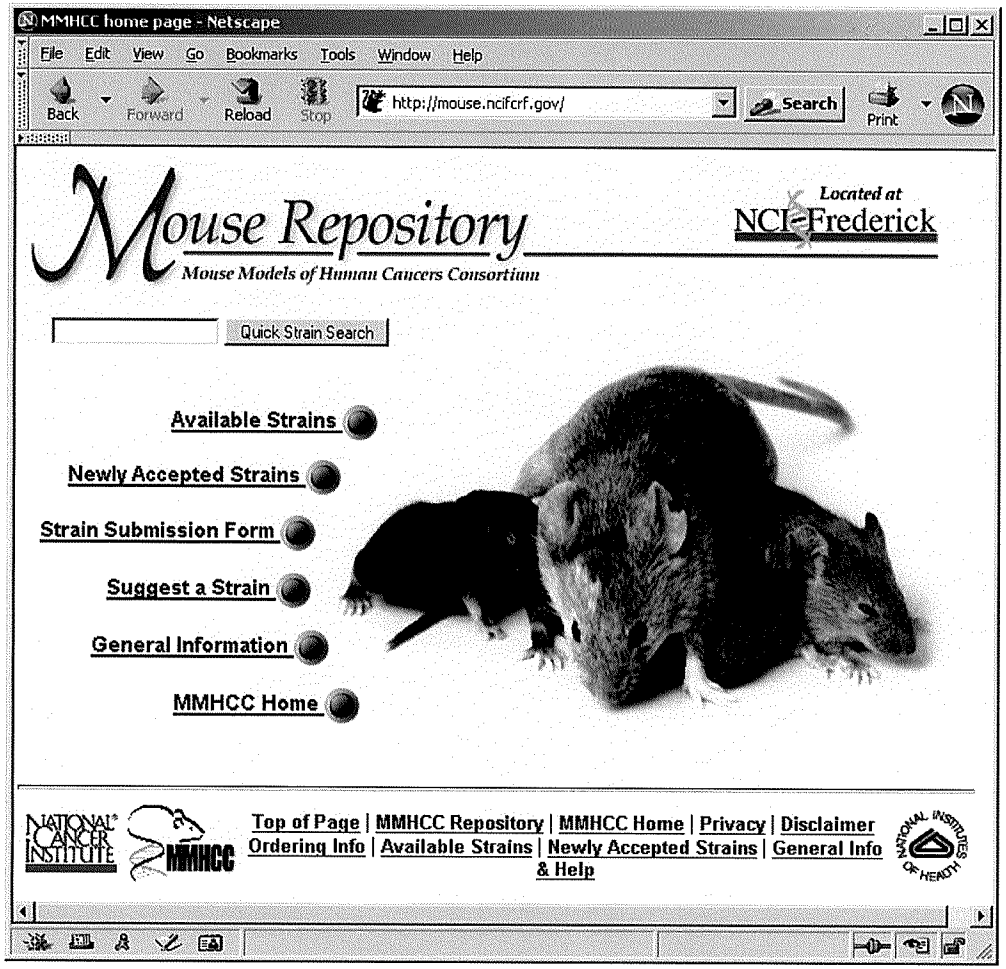
PHL provides collaborative support services to include experimental design assistance, animal necropsies, routine and special histological preparations, and histopathologic evaluations

General Info & Training

Access LASP services (Yellow Task/Accession System), lecture and web-based training opportunities, contact information, as well as general information

Obtaining Genetically Engineered Mice

- Generation:
  - Institutional core facilities
  - Commercial production facility
  - Individual laboratories
- Obtain already generated models:
  - Mutant mouse repositories-IMR, MMRRC, EMMA, NCI
  - Individual laboratories



The NCI Repository

- Resource generated by the NCI's Mouse Models of Human Cancers Consortium (MMHCC).
- Mission: Preservation, propagation, and distribution of mouse models for cancer research.
- Current Repository Volume
  - 120 strains being distributed
  - 1 strains in R&Q
  - 10 strains in the "pipeline"

AMDL performs genetic quality control for the MMHCC Repository

Useful Website Links

- **Laboratory Animal Sciences Program**

  http://web.ncifcrf.gov/rtp/lasp/intra/

- **NCI Repository**

  http://mouse.ncifcrf.gov/

International Mouse Strain Resources

The IMSR is a searchable online database of mouse strains and stocks available worldwide, including inbred, mutant, and genetically engineered mice.
http://www.jax.org/resources/mouse_resources.html

MMRRC-Mouse Mutant Regional Resource Centers

http://mouse.ncifcrf.gov/