



GUIDE TO SHARED RESOURCES

2021 - 2022



ROSWELL PARK





ROSWELL PARK MISSION

To eliminate cancer's grip on humanity by unlocking its secrets through personalized approaches and unleashing the healing power of hope.



A MESSAGE FROM THE PRESIDENT & CEO

As Roswell Park Comprehensive Cancer Center currently supports 23 scientific shared resources that provide our investigators with access to a broad range of sophisticated scientific instrumentation, cutting edge technical and analytical applications, comprehensive sample biorepositories and more. Our shared resources perform a highly valuable role in facilitating basic, clinical and translational scientific research at Roswell Park and are critical elements in accelerating the progress of our researchers and allowing them to successfully compete for peer-reviewed grant funding in an increasingly competitive scientific funding environment. The primary objectives of the Roswell Park shared resources are as follows:

- Provide institutional and regional access to high-end shared instrumentation.
- Provide technical services, which vary in degree of sophistication, but which usually cannot be readily performed in the investigator's laboratory in a timely and cost-effective manner.
- Assist staff in understanding the applications and limitations of various techniques to their research through consultation and discussion.

- Provide expertise toward the development of pilot feasibility studies, and referral to more capable or authoritative sources of information.
- Assess the technical needs of the staff in the context of the services offered by the resource, and develop the resource to assist investigators in accomplishing their research goals.
- Through surveys, research and staff requests, our resources work to anticipate future needs and help establish institutional directions for expanded technical and analytical services.

The 2021 - 2022 Guide to Shared Resources at Roswell Park is intended to orient institute faculty to the currently available scientific shared resources at Roswell Park. The services outlined are financially supported by a variety of mechanisms including an NCI Cancer Center Support Grant, chargebacks and, importantly, significant institutional support. This guide is updated regularly as new technologies and applications are introduced within the existing resources, and also as new resources are established to meet and address the changing needs of scientific and clinical research at the Institute. Our ultimate goal in providing access and necessary support to our institutional shared resources is to facilitate the natural progression of increased scientific interactions and accomplishments, enhanced peer-reviewed funding, and professional collaborations and partnerships among our scientific and clinical investigators.

CANDACE S. JOHNSON, PHD



President & CEO

Wallace Chair in Translational Research
Roswell Park Comprehensive Cancer Center

SHARED RESOURCE BUILDING LOCATIONS

#	BUILDING	SHARED RESOURCES
1	Science Building	Hematologic Procurement Nicotine & Tobacco Product Assessment Pathology Network Vector Development & Production
2	C&V Building	Health Communications Pathology Network
3	Ambulatory Service Building (ASB)	Investigational Drug Services Clinical Research Services
4	Hospital	
5	Ambulatory Center	
6	Grace Cancer Drug Center (GCDC)	Atlas Studios
7	Research Studies Center (RSC)	Bioinformatics Biomedical Research Informatics Biostatistics & Statistical Genomics
8	Vivarium	Laboratory Animals
9	Center for Genetics and Pharmacology (CGP)	Bioanalytics Metabolomics & Pharmacokinetics Gene Modulation Genomics and Onsite Supply Center Small Molecule Screening
10	Cancer Cell Center (CCC)	Flow & Image Cytometry Immune Analysis Therapeutic Cell Production Translational Imaging
11	Medical Research Complex (MRC)	Experimental Tumor Models Gene Targeting & Transgenic
12	Carlton House	Data Bank & BioRespiratory
13	901 Washington	
14	Clinical Services Center (CSC)	

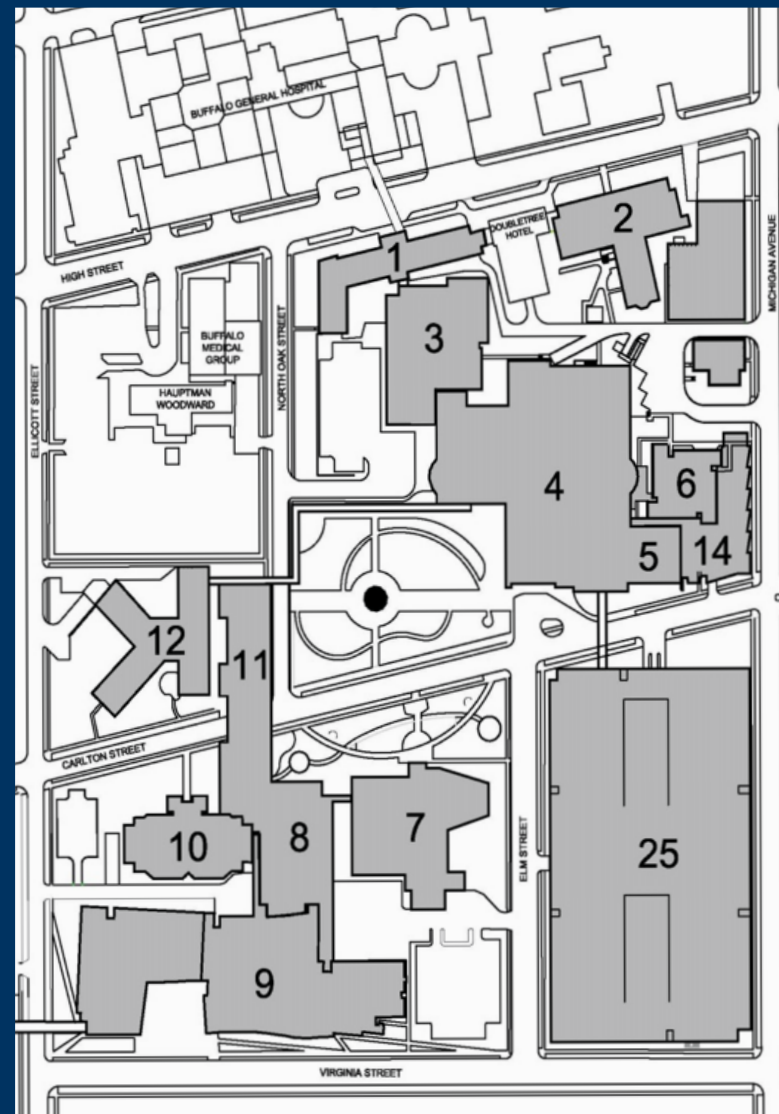


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Shared Resource**

Data Bank and BioRepository Shared Resource

Experimental Tumor Model Shared Resource

Flow and Image Cytometry Shared Resource

Gene Targeting and Transgenic Shared Resource

Gene Modulation Shared Resource

Genomics Shared Resource

Health Communications Shared Resource

Hematologic Procurement Shared Resource

Investigational Drug Services Shared Resource

Laboratory Animal Shared Resource

**Nicotine & Tobacco Product Assessment
Shared Resource**

Onsite Supply Center Shared Resource

Pathology Network Shared Resource

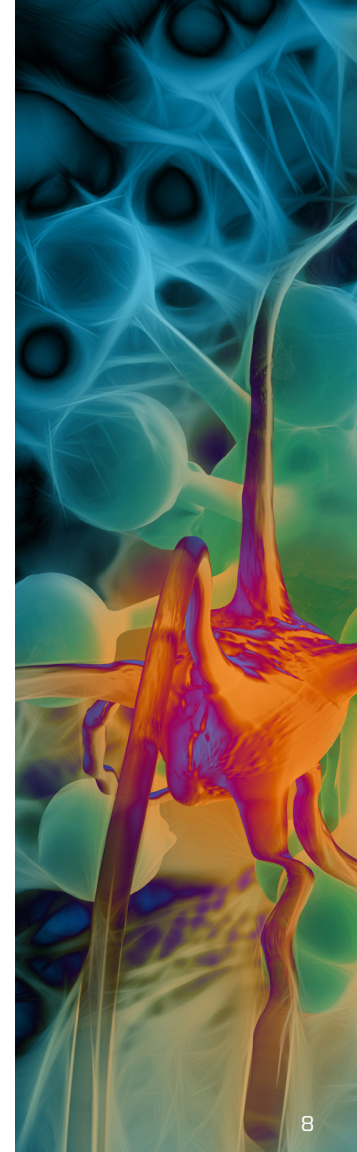
Small Molecule Screening Shared Resource

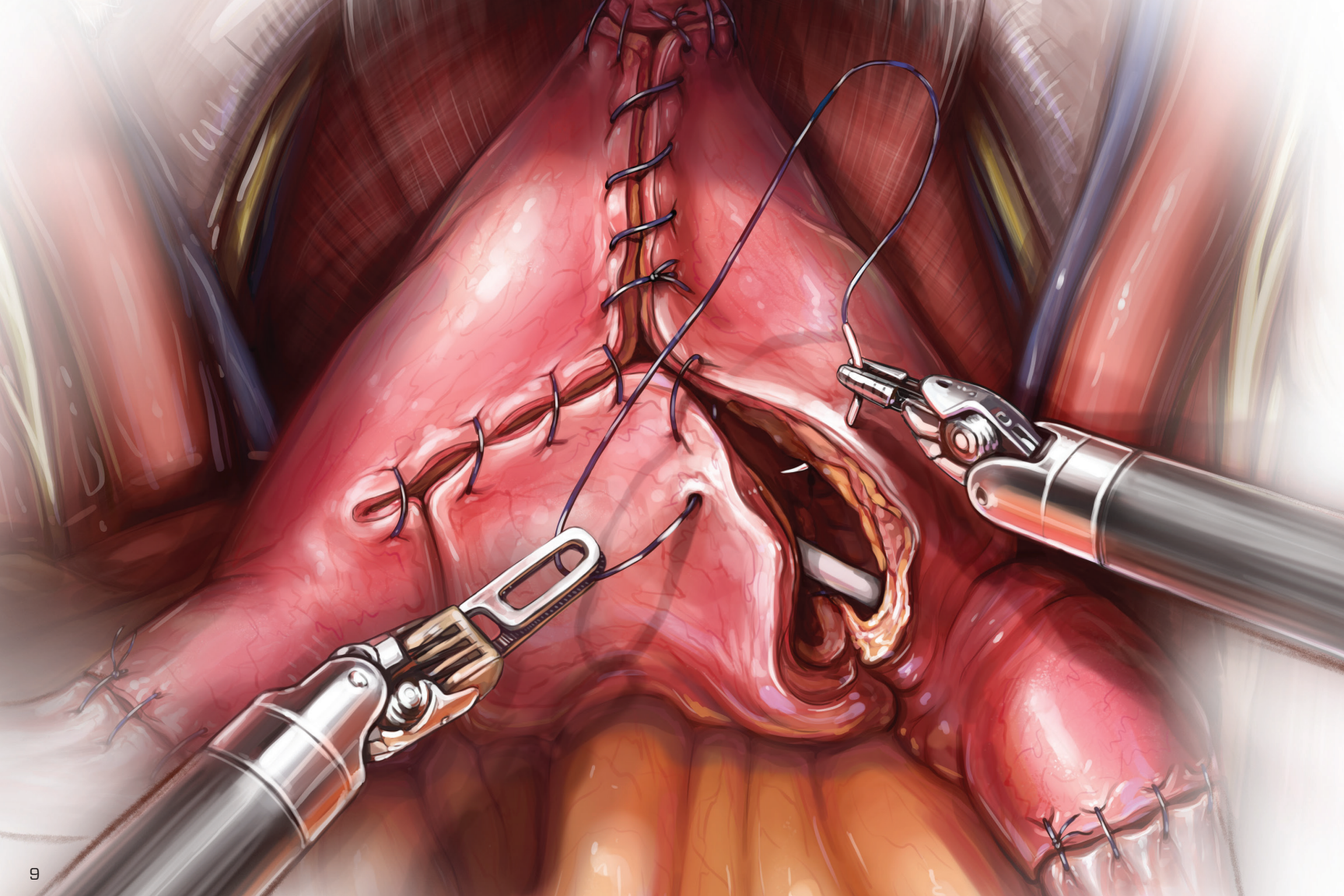
Translational Imaging Shared Resource

Therapeutic Cell Production Shared Resource

Vector Development and Production Facility

Immune Analysis Shared Resource





ATLAS STUDIOS
SHARED RESOURCE

ATLAS STUDIOS SHARED RESOURCE

OVERVIEW

ATLAS Studios is a Roswell Park Comprehensive Cancer Center Shared Resource specializing in medical and scientific illustration and animation, with a focus on cancer therapy and treatment. We create beautiful and accurate visual materials to clarify and communicate complex scientific research and concepts. Connecting people to science through visual stories is our goal. Our medical and scientific illustrations and animations can be used in academic publications, presentations, patient education materials, training materials, marketing, and more. We serve all Roswell Park researchers, physicians, and scientists, as well as external clients.

With a unique understanding of both science and art, we are able to communicate complex science clearly, accurately, and beautifully. Our rates depend on the complexity of the project. Please contact us to learn more!

USING THE RESOURCE

Investigators interested in discussing projects and quotes should contact Jenna Bizovi. For internal Roswell Park users, please submit an order form at: <https://i2.roswellpark.org/#/pages/read/f74eb1c7-aecb-4358-a931-d088f4d6b758>

ATLAS Studios is located in GCDC, 4th floor, 470. Hours of operation are weekdays, 8:00 AM – 5:00 PM.

Website: <https://www.roswellpark.org/shared-resources/atlas-studios>

FOR MORE INFORMATION ABOUT MEDICAL ILLUSTRATION: Association of Medical Illustrators <https://ami.org>

LEADERS

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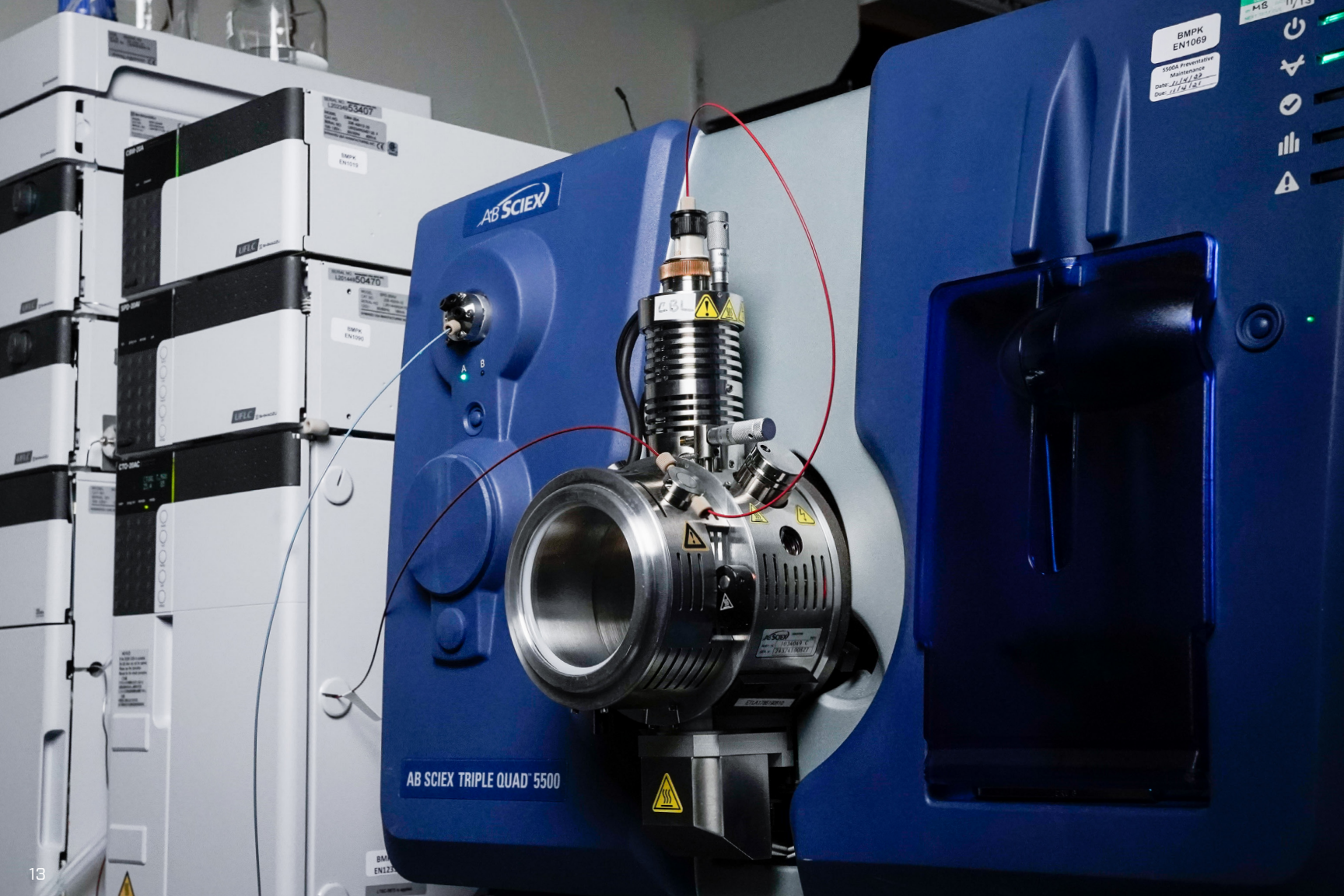
yana.hammond@roswellpark.org

SERVICES The resource provides a wide variety of services, including:

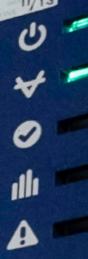
- **Illustrations for use in:**
 - PowerPoint presentations
 - Journals
 - Posters
 - Patient education materials
 - Surgical/Anatomy atlases
 - Books
 - Apps/Websites
- **Standard Animations for use in:**
 - PowerPoint presentations
 - Online journals
 - Online patient education materials
 - E-books
 - Apps/Websites
- **PowerPoint presentation:**
 - PowerPoint presentations
 - Online patient education materials
 - E-books
 - Apps/Websites
- **App Design**
- **3D illustrations**

SAMPLES OF WORK:

Behance Portfolio <https://www.behance.net/TheATLASstudiosRP>



BMPK
EN1069
5500A Preventative
Maintenance
Date: 11/24/22
Due: 1/14/23



AB SCIEX

AB SCIEX TRIPLE QUAD 5500



AB SCIEX
Model: 5500A
S/N: 17837110077

BIOANALYTICS,
METABOLOMICS &
PHARMACOKINETICS
SHARED RESOURCE

BIOANALYTICS, METABOLOMICS & PHARMACOKINETICS SHARED RESOURCE

OVERVIEW

The mission of the Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK) is to support basic research and pre-clinical/clinical drug development by providing broad-based bioanalytical analyses, PK/PD modeling, and consultation services to Roswell Park investigators and other academic institutions, cancer centers, and industry. The BMPK Shared Resource works closely with investigators at each stage of their project to provide feedback on PK/PD study design with recommendations on dose selection, timing of sample collections, sample handling, use of enzymatic inhibitors and other parameters to help optimize observations, data correlations and therapeutic outcomes.

The BMPK Shared Resource offers a wide array of analytical methods along with capabilities to develop and validate new assay methods to help achieve the required objectives from studies of pharmacological mechanisms, cancer therapeutics, and preventive agents. Using state-of-the-art techniques of LC-MS/MS, LC-MS, UPLC, HPLC, CMIA, ELISA, and atomic absorption spectroscopy, the BMPK provides

highly sensitive measurements for chemotherapeutic agents and their metabolites, biomarkers, other endogenous compounds, and targeted metabolomic pathways. Investigators are encouraged to consult with the BMPK Shared Resource as early as possible during the planning phases of their project or grant development to obtain a comprehensive understanding of the time commitment and cost associated with each phase of their project.

USING THE RESOURCE

Investigators who are interested in using the BMPK facility and its services should contact Dr. John Wilton or Dr. Andrew Goey to discuss shared resource capabilities, scheduling and pricing.

The BMPK Shared Resource is located on the 1st floor of the Center for Genetics and Pharmacology with the main laboratory located in room L1-140. Hours of operation are weekdays, 8:00 AM - 5:00 PM

Website: <https://www.roswellpark.org/shared-resources/bioanalytics-metabolomics-pharmacokinetics>

LEADERS

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SERVICES

The BMPK Shared Resource provides comprehensive services to investigators and sponsors ranging from study design consultation to analytical method development, assay validation/qualification, sample preparation, sample storage, analysis of study samples, and PK/PD data analysis. The services provided by BMPK are guided by more than 45 Standard Operating Procedures (SOPs), which are the framework for our resource policies, staff orientation and training, quality audits, metrology program, and bioanalytical guidelines. Routine preventative maintenance (PM) and calibration programs are in place to ensure the proper performance and functioning of laboratory equipment.

Bioanalytical Services

In addition to our existing methodologies, BMPK will develop new assay methods as needed by investigators to generate highly sensitive analytical measurements to meet their intended objectives in their in vitro, preclinical or clinical studies. To contain costs for investigators, the development and performance testing of analytical methods can range from simple, basic testing for a discovery method to much more thorough testing following the FDA Guidance, which is done for validated methods and required for all clinical trials. These methods have been developed for a wide range of matrices including, but not limited to plasma, serum, whole blood, a large variety of tissue samples, tumors, xenografts, bronchial alveolar lavage, cell pellets and media. When necessary, samples are treated with various enzymes to convert glucuronides and other metabolites back to their aglycone or parent compound.

ELISAs are routinely used to identify the presence of antibody, antigen or a particular drug or other substance in a biological fluid such as serum, plasma, or cell culture supernatants. In addition, BMPK is also equipped to perform elemental analysis using Graphite Furnace Atomic Absorption (GFAAS) for compounds containing metals such as platinum, selenium, and gold.

In addition to Multiple Reaction Monitoring (MRM) used in our quantitative analyses, some examples of other mass spectral (MS) techniques available for investigator research are:

- Q1 MS (Q1): A full mass scan using the 1st quadrupole (Q1) as the mass filter.
- Q3 MS (Q3): A full mass scan using the 3rd quadrupole (Q3) as the mass filter.
- Precursor Ion Scan: An MS/MS scan where different parent ions can be detected that have a common fragment ion (e.g., to detect all parent compounds containing a glucuronide).
- Product Ion Scan: An MS/MS scan where different fragment ions can be detected that have a common parent ion (e.g., to detect compounds having a common fragment such as sulfate).
- Neutral Loss (NL): An MS/MS scan where both 1st and 3rd quadrupoles are swept over a mass range using a fixed mass difference between them.
- MS/MS/MS (MS3): A scan in which product ions of a specified m/z that are generated in the 2nd quadrupole are collected in the linear ion trap (LIT) and further fragmented.

Examples of assays the Shared Resource provides include:

- **Hormones:** Testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone (DHEA), and androsterone (LC-MS/MS), and estrone and estradiol (LC-MS/MS)
- **Antimetabolites:** Gemcitabine and dFdU (LC-MS/MS), capecitabine (LC-MS/MS), and 5-fluorouracil (LC-MS/MS)
- **Taxanes:** Docetaxel (LC-MS/MS) and paclitaxel (LC-MS/MS)
- **Topoisomerase Agents:** Irinotecan (CPT-11), SN-38, SN-38G (UPLC and LC-MS/MS)
- **Anthracyclines:** Doxorubicin (UPLC) and daunorubicin (UPLC)
- **Targeted Agents:** Tivozanib (LC-MS/MS), enzalutamide and N-desmethylenzalutamide (LC-MS/MS), sorafenib and sorafenib-N-oxide (LC-MS/MS), carfilzomib (LC-MS/MS), and sunitinib (LC-MS/MS)
- **Platinum Based Compounds:** Oxaliplatin (atomic absorption), cisplatin (atomic absorption), and carboplatin (atomic absorption)
- **Selenium Based Compounds:** Selenomethionine and methylselenocysteine (atomic absorption)
- **Others:** Tryptophan/kynurenine (LC-MS/MS), sex hormone binding globulin (SHBG; CMIA), VEGFR2 (ELISA), lignans (enterodiol and enterolactone; LC-MS/MS) and eicosanoids (LC-MS/MS)
- **For a complete listing of our assays and additional details, please visit our website.**

Data Analysis and PK/PD Modeling

PK/PD modeling of bioanalytical results in conjunction with other demographic and longitudinal data is used to characterize relationships between the time course of drug concentrations and pharmacological effects. The BMPK resource is available to assist investigators in the design of clinical studies which incorporate clinical pharmacology or pharmacokinetic/pharmacodynamic objectives. PK/PD modeling and simulations are performed to gain insight into the mechanism of action of drugs, or combinations of drugs, and to assess inter-individual and random variability within a population. This information is crucial for optimal design of various aspects of a clinical

trial, including dosing strategy and the selection of patient populations. The software utilized is dependent on the information available and the type of modeling being requested. Dataset assembly and graphics are performed in a variety of ways to meet the needs of the investigator. Non-compartmental analyses are performed using Phoenix WinNonlin. PK/PD modeling and simulations are performed using ADAPT 5 or NONMEM.

Instrumentation and Software

The BMPK Shared Resource is equipped with state of the art instrumentation and PK/PD modeling software including:

INSTRUMENTATION

- Applied Biosystems 5500 QTrap triple quadrupole (ESI/APCI; LC-MS/MS)
- Applied Biosystems 5500 triple quadrupole (ESI/APCI;LC-MS/MS)
- Thermo Scientific TSQ Vantage triple quadrupole (ESI/APCI; LC-MS/MS)
- Applied Biosystems API3000 triple quadrupole (ESI; LC-MS/MS)
- Waters Ultra Performance Liquid Chromatography (UPLC) system with fluorescence and UV detection
- Waters BioAlliance High Performance Liquid Chromatography (HPLC) system with fluorescence and UV detection
- Abbott i1000SR Architect chemiluminescent magnetic microparticle immunoassay (CMIA)
- Perkin Elmer Analyst 600 graphite furnace atomic absorption
- Biotek Synergy 96-well plate reader providing fluorescence and UV detection
- Tomtec Quadra 4 robotic handling system

MODELING SOFTWARE

- Phoenix WinNonlin Professional: Noncompartmental and compartmental modeling
- ADAPT 5 and S ADAPT: Individual compartmental PK and PK/PD modeling
- NONMEN: Nonlinear mixed effect modeling software for population analysis to determine sources of variability in the PK and PD of a drug
- WinPOPT: Provides information for PK and /or PK/PS sampling strategies
- SAS 9.3: Used to manage and clean databases, and create graphics and tables

B I O I N F O R M A T I C S
S H A R E D R E S O U R C E

BIOINFORMATICS SHARED RESOURCE

OVERVIEW

The mission of the Bioinformatics Shared Resource (BIOINF) is to provide state-of-art bioinformatics expertise for the design, analysis, and interpretation of genomics, proteomics, and other high-resolution, high-throughput studies to better understand cancer biology and translate cancer omics discoveries to cancer treatment. BIOINF's strategy is to reduce the barrier for access to analytic expertise, improve CCSG investigator productivity, maintain a high standard for data collection and management, design and perform rigorous analytical strategies, and foster a collaborative and supportive research community. Services include: directing the design of high-throughput experiments; raw omics data processing, data analysis and interpretation; data mining and integration; assisting the design and deployment of appropriate informatics infrastructure for data sharing and management; reviewing and preparing grants and manuscripts; software evaluating, new methods and databases; and providing education and training. The BIOINF ensures that CCSG investigators have ready access to expert bioinformatics support and services to carry out basic translational, clinical, and population science research. BIOINF staff maintain regular contact with CCSG investigators to ensure the consistency and efficiency of procedures that will ultimately generate high-quality data and reproducible analytic results. Bioinformatics expertise provided by BIOINF personnel ensures that the yield of useful information from the scientific studies conducted is maximized while costs are minimized.

USING THE RESOURCE

To use the shared resource, users should first contact the listed core staff members or submit a job request via the LIMS system at <https://rpcilims.roswellpark.org>. The users will then be contacted by core staff regarding the project details and feasibility.

BIOINF is located on the fourth floor of the Research Studies Center. Approximately 1,300 sq. ft. of space consisting of offices, storage space, and general secure areas equipped with filing cabinets. Geographic proximity to the Genomics Shared Resource (GSR), located in an adjoining building

allows for daily interactions among members of both Shared Resources. Data produced at GSR are transferred easily to the BIOINF High Performance Computing infrastructure for analysis via secure File Transfer Protocol (FTP). BIOINF offices are located in close proximity to many research laboratories, which facilitates research collaboration and communication.

Website: <https://www.roswellpark.org/shared-resources>

LEADERS

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HOURS OF OPERATION ARE

Weekdays 8 AM-5 PM.

SERVICES

The BIOINF offers CCSG investigators a full spectrum of services. These include:

for our resource policies, staff orientation and training, quality audits, metrology program, and bioanalytical guidelines. Routine preventative maintenance (PM) and calibration programs are in place to ensure the proper performance and functioning of laboratory equipment.

...SERVICES CONTINUED

- **Project Design:** Resource personnel work closely with principal investigators and other members of the research team to define the nature and scope of relevant bioinformatics needs, as well as the type(s) of omics data to be collected and analyzed to achieve the study objectives.
- **Grant Development:** Resource personnel provide exploratory data-mining support for the development of the preliminary data section. They review--and, in general, develop--the bioinformatics analysis plan for the majority of CCSG and Roswell Park grant applications.
- **Data Processing, Integration and Interpretation:** Resource personnel perform bioinformatics analysis and data mining of various omics and other biological datasets. They assist investigators with the integration of omics data with clinical information; develop analytical models for these data based on the hypothesis of interest, and assist investigators with finding plausible biological and/or clinical interpretations of their respective results.
- **Manuscript Preparation:** Resource personnel, serving as scientific collaborators, review and write the bioinformatics analysis section of the manuscript of interest, and provide the relevant interpretation of the data models as they relate to the conclusions presented in the given manuscript.
- **Education & Training:** Resource personnel provide consultation, assistance, and hands-on training, when necessary, for investigators on the bioinformatics tools and resources needed to analyze their own data. Details regarding educational efforts are contained in the body of the text below.
- **Infrastructure Development:** In order to provide data warehousing and computing resources access for CCSG investigators for storing, managing, analyzing and sharing omics data and other types of data, the Resource contributes to the development of the underlying informatics infrastructure in close collaboration with the IT department.

- **Software Evaluation:** Resource personnel collect and test available bioinformatics products in order to help investigators select the appropriate tools for their specific studies.
- **Tool, Database, and Web Development:** Resource personnel develop customized bioinformatics tools, interactive web applications and underlying back-end databases, as necessary, when existing products are unavailable or do not meet the customized needs of CCSG investigators relative to answering specific study objectives. Whenever possible, we make these tools available to the broader community.

In addition to providing general collaborative and consulting services necessary for CCSG research and operations, the BIOINF provides unique and specialized services via its methodological research. As an example, the Bioconductor, led by Dr. Martin Morgan, is an open-source, open-development software project for the analysis and comprehension of high-throughput data in omics. It consists of over 100 software packages developed by the Bioconductor core team and by the national and international scientific community. Bioconductor is widely used (downloads to >330,000 unique IP addresses), highly cited (>21,000 PubMedCentral full text citations), and highly respected.





BIOMEDICAL RESEARCH
INFORMATICS
SHARED RESOURCE

BIOMEDICAL RESEARCH INFORMATICS SHARED RESOURCE

OVERVIEW

The Biomedical Data Science Shared Resource (BDS), formerly the Clinical Data Network (CDN), is a core resource that supports clinical and translational research at Roswell Park Comprehensive Cancer Center. The BDS provides full life cycle research computing support to Roswell investigators. BDS is a resource of the Department of Biostatistics and Bioinformatics.

MISSION

The mission of BDS is to advance the Roswell Park Comprehensive Cancer Center Data Science Strategic Plan, in alignment with the NIH Data Science Strategic Plan published in 2018.

These goals include:

- Enhance collaboration
- Facilitate data accessibility and sharing to maximize data standardization, use and re-use
- Enable high quality data capture and management through project-appropriate software development
- Provide highest quality data to biostatisticians for analytics
- Provide grant application co-authorship and support to increase Roswell Park grant funding

USING THE RESOURCE

Investigators may request BDS support via email: BDSOffice@roswellpark.org

Website: <https://www.roswellpark.org/shared-resources/biomedical-data-science>

LEADERS

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SERVICES

The BDS provides services to investigators and the research community in the five areas:

1. Grant Development

BDS supports funding applications through grant co-authorship, recommendation for required computing resources, and informatics and data management budgeting. BDS helps to ensure data plans for grant applications are strong and meet all federal requirements.

2. Project Triage

All new research projects start here for organization, software and data management direction and resources. BDS works with all investigators to guide new project development and coordinate resources.

3. Bio-curation

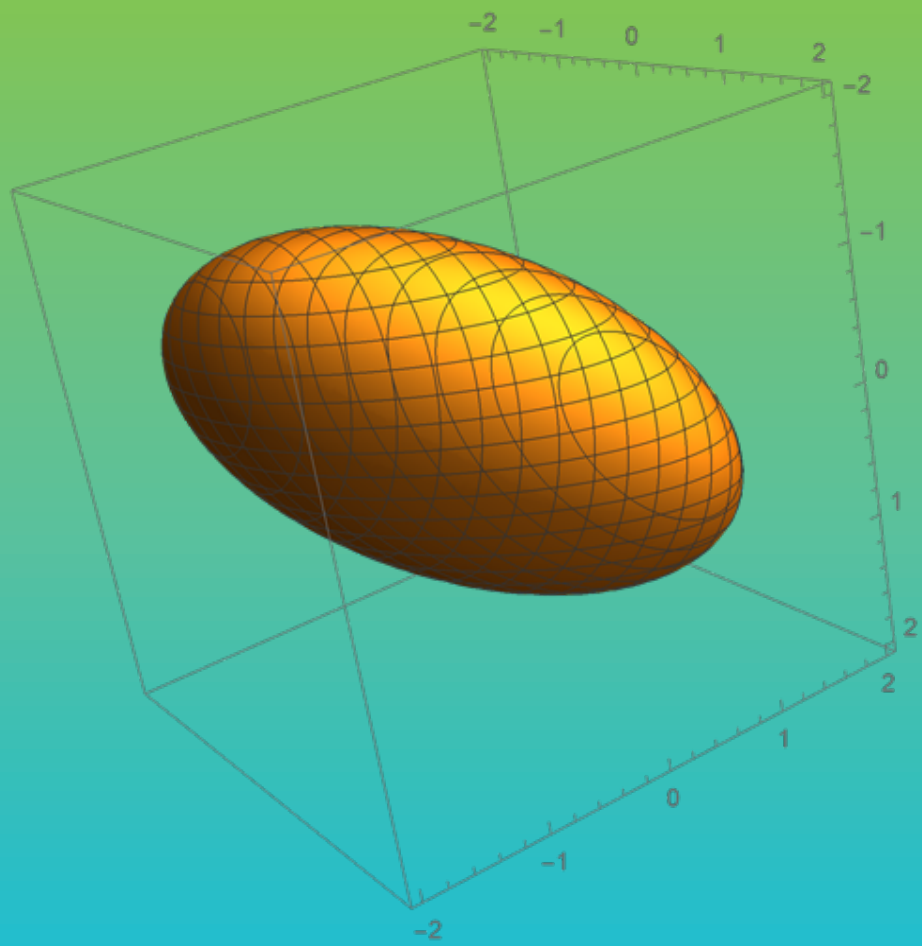
The BDS data management team provides annotation of data in Roswell repositories, extraction of data from repositories across the institute to answer research questions, and quality control and data cleaning. BDS bio-curators are also developing data standards for use across Roswell, moving to create interoperable data.

4. Data Standards and Ontology

BDS is developing and distributing NCI-consistent data standards in the form of Common Data Elements (CDEs) to support consistent research data capture. These are general, project-specific and cancer-specific standards.

5. Software Research and Development

The BDS team will research software to be used by research projects (commercial and custom) and develop custom open-source software (database, web applications) to support data capture, management and reporting for research protocols. Engineering of protocol processes, a critical step to ensure highest quality data capture, is part of this service.



BIOSTATISTICS
AND STATISTICAL
GENOMICS
SHARED RESOURCE

BIOSTATISTICS AND STATISTICAL GENOMICS SHARED RESOURCE

OVERVIEW

The Biostatistics and Statistical Genomics Resource (BSGR) offers collaborative and consulting services at the interface of biology, medicine, statistics, mathematics, and computer science. The staff has experience and expertise in the theory and application of biostatistics, biomathematics, statistical genetics, and computer simulation to all aspects of cancer research. Specialties include clinical trial design and analysis, statistical genetics modeling, including the analysis of microarray data and other high-throughput approaches, and epidemiological statistical methods. The resource has the ability to develop customized software and algorithms for specialized data analysis problems.

The Biostatistics and Statistical Genomics Resource provides well-planned designs and data collection instruments that are ethical, do not waste resources, and are cost-effective. The Biostatistics component of the resource provides statistical support for pre-clinical experiments, clinical trials, and observational studies. Clinical trials are monitored for data

collection and study conduct to help ensure patient safety. For all open and recently closed studies, written interim reports are provided to the PI and, if required, the Data and Safety Monitoring Committee (DSMC). Additionally, ClinicalTrials.gov reports are generated for all Phase I and II clinical trials where Roswell Park is responsible for the data and analysis; and other NIH or NCI funded intervention/non-intervention trials. The Statistical Genomics component of the resource applies and develops statistical and computational methods to address biological questions on human diseases and traits from genetics and genomics data. The data dealt by the team includes but not limited to candidate-gene studies, genome-wide association studies (GWAS), and next-generation sequencing (NGS) studies. The resource as a whole can provide support for the entire life-cycle of a project: from grant/proposal development, to data analysis, and through presentation of results (i.e. biostatistical sections of abstracts and manuscripts, and graphics development).

There are no formal direct charges for the services provided by the Biostatistics and

Statistical Genomics Resource. Collaborative joint grant proposals are encouraged. Funding for Biostatistical support must be included in grant proposals.

USING THE RESOURCE

Investigators may contact Drs. Attwood and Zhu for further information and details. Requests for collaboration should be made in our project tracking system, LIMS, at <https://rpcilims.roswellpark.org>.

Priority is given to CCSG members versus non-members, and investigators who have outside funding, an approved clinical trial, or are preparing protocols and/or grant applications. Statistical sections for abstracts and special presentations should be received at least two weeks in advance of a submission deadline. The Biostatistics and Statistical Genomics Resource is located in the Research Studies Center R-420. Hours of operations are weekdays, 8:30 AM – 5:00 PM.

Website: <https://www.roswellpark.edu/>

[Shared-Resources/Biostatistics](https://www.roswellpark.edu/Shared-Resources/Biostatistics)

LEADERS

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SERVICES

- Provide biostatistical support to basic, clinical, and population-oriented Roswell Park collaborators
- Scientific hypothesis formulation
- Exploratory data analysis
- Statistical methods sections for grants
- Protocol development (Linkage with IT and CRS)
- Study design (pre-clinical experiments, observational studies, and clinical trials)
- Fitting models to data (model rational, development, and fitting)
- Simulating data from models
- Developing customized data mining software and novel methods based on emerging technologies
- Statistical software development: developing novel statistical methodologies, software applications, quality control metrics, custom

Qianqian Zhu, PhD

Co-Director

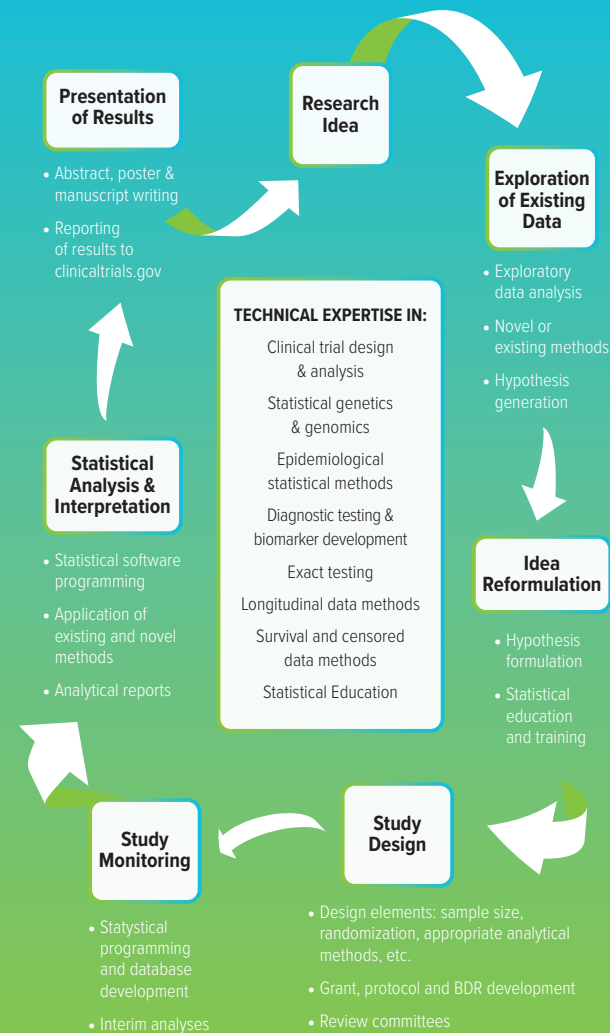
Department of Biostatistics and Bioinformatics

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- visualizations and custom diagnostics for high throughput technologies utilized at Roswell Park
- Statistical training and education
 - Assistance in abstract and manuscript writing
 - Statistical reviews across several Roswell Park committees (SRC, IRB, DSMC, Alliance Foundation)
 - Provides statistical expertise in proteomics, genomics, and epidemiology research within Roswell Park
 - Statistical Genetics and Genomics
 - Developing power analysis and sample size estimates, applying preprocessing methods to eliminate quality issues and account for inherent biases, deriving and applying statistical and machine learning algorithms for discovery in genetics, genomics and proteomics experiments, and employing disease related gene/pathway/network/system-biology analysis.





DATA BANK AND
BIOREPOSITORY
SHARED RESOURCE

DATA BANK AND BIOREPOSITORY SHARED RESOURCE

OVERVIEW

The mission of the DBBR is to reduce the burden for Investigators conducting translational research by providing a comprehensive bank of biospecimens and data. Services include biospecimen inventory evaluation and study feasibility, incorporation of DBBR services in grants, protocols and publications, and associated study data management services.

Data and biospecimens (primarily serum, plasma, DNA, buffy coat and red blood cells) are donated by Roswell Park patients who have cancer or who are at risk for cancer. Non-patients are also welcome to volunteer as healthy controls. Data and samples are made available to investigators with protocols approved by the Roswell Park Institutional Review Board (IRB) for studies related to cancer prevention, etiology, detection, treatment and prognosis.

LOCATION

DBBR Recruitment is located in the Main Hospital lobby and the Request Management Office is located in Carlton House. DBBR Pre-Admission Testing Services are located in the Main Hospital. Laboratory and Biospecimen Storage facilities are located in the Gratwick Basic Science Building. Hours of operation are weekdays 7:00 AM-5:30 PM.

Website: <https://www.roswellpark.edu/shared-resources/data-bank-and-biorepository>

USING THE RESOURCE

- 1. At the time of initial inquiry, investigators are asked to meet with or arrange a telephone call with DBBR operations director Annmarie Nowak to assess:**
 - Review existing inventory or determine need for study specific collection
 - Determine project time frame and develop cost estimate.
- 2. Submit a Biospecimen and Data Research (BDR) Protocol Application for the use of specimens and data.**
 - All samples and data provided by the DBBR are de-identified prior to release
 - The DBBR maintains the link between participants and their samples and data
 - Roswell Park Cancer Center Support Grant (CCSG) Members - members with peer-reviewed funding are given top priority for requests, followed by Roswell Park investigators with newly developing programs, then external academic investigators with federal funding and finally commercial investigators. Cost recovery fees are tiered for these different groups of investigators.

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SERVICES

1. Banked Biospecimens

The focus of the prospective bank is to collect blood samples from newly diagnosed patients prior to surgery or cytotoxic treatment. Patients who are post-treatment are also asked to participate based on identified future research needs. The DBBR recruitment staff work with outpatient clinical staff to identify and consent patients and coordinate sample collection for the bank. Blood samples are collected alongside existing clinical laboratory orders, and drawn by the phlebotomy service at Roswell Park. Family members and friends accompanying patients, as well as other visitors to Roswell Park are also asked to donate samples as controls. Control samples follow the same sample procurement and processing procedures as patient samples.

Every participant is asked to donate 30 mL of blood: one 10-ml non-heparinized red top tube (for serum and clot), and one 10-ml EDTA lavender top tube for plasma and buffy coat, and a second 10-ml EDTA lavender top tube (whole blood for DNA).. The phlebotomist prints sample barcode labels for the blood collection tubes using the Roswell Park laboratory management system. This procedure produces an alert signaling the pending arrival of specimens for processing in the biorepository processing laboratory. Samples are sent from phlebotomy to the sample processing laboratory via pneumatic tube delivery system. **The standard time from collection to freeze for DBBR blood samples is one hour.**

Specimens are centrifuged and the supernatant is automatically aliquoted using a MAPI robot from Cryobiosystem (parent company is IMV International corp., L'Aigle, France). The robotic system aliquots serum, plasma, buffy coat and red blood cells into inert plastic straws (0.5 mL each), seals each end and labels each straw with a barcode identifier. The straws are kept in a liquid nitrogen storage system developed by Cryobiosystem. In addition to the straws, whole blood, clot and buffy coat are aliquoted in 0.5mL barcoded cryovials and stored at -80.

DNA is readily available through upgrades in our processing using Autogen Flexstar DNA extractor which allows us to extract DNA from up to 80 samples per day, a DropSense 96 microplate reader from Caliper Life Sciences that facilitates high throughput quantification and evaluation of double stranded DNA and a Janus liquid handling system from Perkin Elmer Life Sciences which is used to automate DNA quantification and distribution.

2. Study-Specific Procurement

The DBBR can prospectively collect material in a custom format at single or multiple time points on a limited basis, if it can be accommodated within standard banking operations. Procurement, processing, and storage of additional sample type (PBMC, stool, urine, and other biospecimens) by request. Specific collection requests are carefully evaluated for feasibility and reviewed for approval by the DBBR Advisory Board and TRGs.

3. Clinical and Epidemiological Data

Clinical data from the Roswell Park Cancer Registry are reviewed against the donor's Electronic Medical Record (EMR) for context, and linked to each qualifying biospecimen donated by cancer patients where applicable, in addition to EMR abstraction for samples from high risk and benign

patients. This efficient annotation approach minimizes duplication of effort and allows the clinical data to be associated to the original sample collection and each individual aliquot and to any derivative material from that sample in the biorepository.

Basic Sample Annotation (Linked from Cancer Registry and Abstracted by the DBBR)

- Demographics – participant age, sex, race, ethnicity
- Personal History of Cancer – status and sites
- Diagnosis – site, topography, morphology, tumor size, nodes
- Staging – Collaborative Stage, AJCC Stage, SEER Stage
- Surgery – procedure of the primary site
- Benign diagnoses (for benign-only patients) at the time of sample collection
- Prior history of cancer at the time of sample collection
- Treatment status at the time of sample collection

Extended Sample Annotation (Extracted from Cancer Registry at the Time of Request to Ensure Timeliness)

- Site Specific Factors (tumor markers)
- Treatment history (chemotherapy, radiation, immunotherapy, hormone, hematologic/endocrine) and reason (first course, progression, recurrence, subsequent)
- Recurrence (type of recurrence, distant sites)
- Survival time
- Cancer status at follow up
- Patient status at follow up

Study Specific Data Collection (Abstracted from Medical Records by Request Only)

- Comorbidities at the time of sample collection
- Medications at the time of sample collection
- Other Data as designated by the Investigator

Epidemiologic Questionnaire Data: At the time of consent, all participants are asked to complete and return a self-administered scannable questionnaire (over 1,100 items). A five-point follow up schedule is used for missing surveys, participants are re-contacted to clarify inconsistencies, and customized data quality software is used for error checking. There is an overall 75% response rate and a complete data dictionary is available by request.

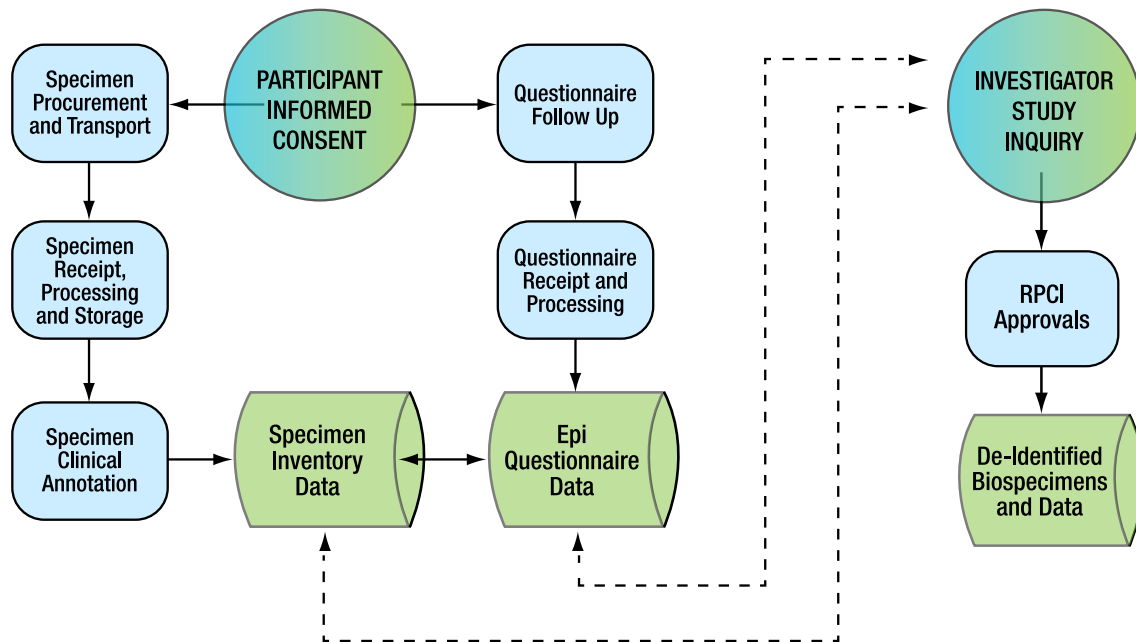
- Demographics
- Family history of cancer
- Medical history (screening, prior history of cancer, comorbidities, BMI history)
- Tobacco use history (lifetime)
- Medication use history (lifetime)
- Women's health history (lifetime)
- Food habits and food/beverages and alcohol frequency (last year)
- Exercise (past 10 years)
- Multivitamins, vitamins, supplements (past 10 years)
- Common supplements (lifetime)

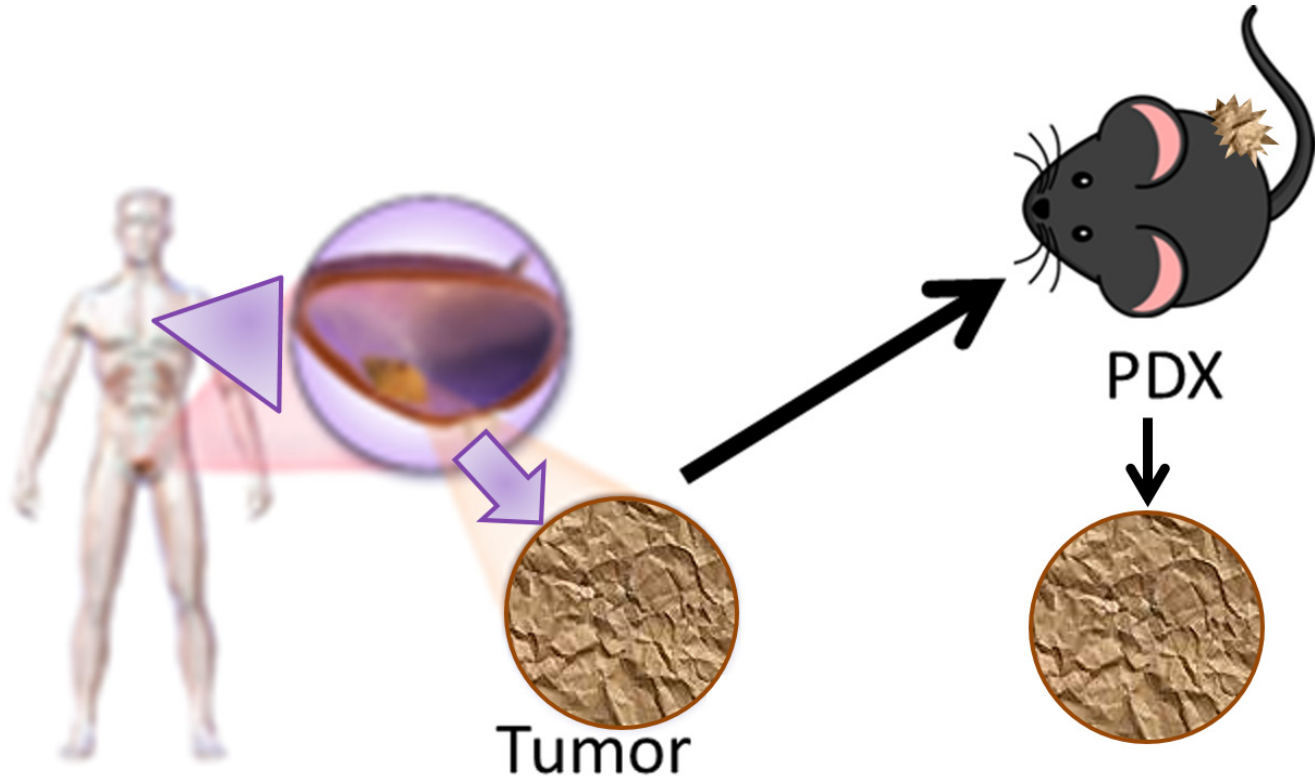
Linkage to Investigator Patient Data

With IRB approval and Data Use/Destruction Agreement, the DBBR will coordinate and link DBBR specimens and data to other data provided by Investigators. All final data sets are rendered to Investigators de-identified.

Variable and Analytic Data File Construction

The DBBR will construct variables needed for analysis by request (i.e., Age at Sample Collection, Time from Diagnosis to Sample Collection, Time from Sample Collection to Death, etc).





EXPERIMENTAL

TUMOR MODEL

SHARED RESOURCE

EXPERIMENTAL TUMOR MODEL SHARED RESOURCE

OVERVIEW

The Experimental Tumor Model Resource (ETM) was established to facilitate investigators in conducting preclinical animal studies. The staff of the ETM are highly trained and experienced in a wide range of small animal surgeries, procedures, and techniques in addition to histology processing and staining. The goal of the ETM is to provide full service support for animal studies to investigators. Services provided by ETM include in vivo experimental design for animal studies, treatment of animals, tissue procurement and processing, histological evaluation and animal colony management. The services available through the ETM include but are not limited to small animal surgery, castration, tumor resection, tumor inoculation (SQ, orthotopic, subrenal), tumor measurement, therapeutic treatments such as injection (IP, IV, sub-Q, intrafemoral, intracardiac), oral gavage, dietary manipulation, tissue procurement, tissue microdissection. ETM provides tissue processing services including: formalin fixed/paraffin embedding, cryofrozen in OCT, LN2 flash frozen, cryopreservation in DMSO and primary cell culture. Longitudinal animal studies can be conducted including tumor measurement, blood draw, and bioluminescent imaging. The ETM can manage your mouse colony including breeding, PCR screening and culling the colony for cost effective colony management. Specialized services include embryonic dissections, tissue recombinations, subrenal grafting and xenografting of human clinical samples into immunocompromised hosts. ETM also maintains several tumor models that are available to researchers for their studies. Tumor models available

through the ETM or in development include: TRAMP, CWR22, PB-Cre-4/Pten knockout, Hi-Myc, K-ras mutant sarcoma, K-ras mutant lung cancer, KPC pancreatic cancer, and PDX models of lung, bladder, colon, pancreas, and sarcoma. Experimental animals as well as flash frozen tissue and histological slides are available in the tumor bank. Tissue samples are available for disease progression (multiple timepoints and castration recurrent disease) for many of the models. The expertise of the ETM is available to assist with all of your preclinical animal studies.

USING THE RESOURCE

To initiate a project, investigators should contact Dr. Moser or Dr. Foster to obtain a description of the services offered and an explanation of the models available. Investigators meet with ETM personnel to discuss experimental design, treatment strategies, endpoints and data collection. The goal of these discussions is to develop an experimental plan with animal numbers and ages designated for all experiments. Next, the investigator needs to complete material transfer agreements, obtain IACUC approval or have the ETM added to the protocol as personnel, and provide a charge source.

If the investigator contracts with the ETM to maintain and provide experimental treatment, as well as, monitor and collect tissues and data, then the ETM will be added to the investigators Roswell Park IACUC protocol or the experiment will be performed under an ETM IACUC Core Protocol.

If the PI only needs the ETM to provide the animals needed for the experiments then the ETM initiates a breeding plan to obtain the needed number of animals. At weaning the experimental animals are transferred to the investigator's IACUC protocol and the animals are the responsibility of the investigator and all treatment and monitoring of the animals is performed under the investigator's approved IACUC protocol. At the time of tissue procurement, the ETM can assist with microdissection and tissue collection. This service is scheduled at the time of breeding so that scheduling of ETM personnel can be taken into account for the breeding strategy. Investigators using the Tumor Tissue Bank are required to schedule an initial meeting, complete MTAs and provide a charge source. Tumor models, PDX lines or cell lines are provided to investigators after MTAs are completed. Investigators are expected to expand the cell lines in their laboratories and freeze down sufficient aliquots for all necessary experiments.

The ETM is located in the Medical Research Complex, Room 452. Hours of operation are weekdays, 9:00 AM – 5:00 PM, and by appointment.

LEADERSHIP

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MRC 452

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Bryan Gillard

Animal Studies & Models

MRC 452

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SERVICES

Mouse models available:

- CWR22
- CWR22R
- Hi-Myc
- Pten null
- TRAMP
- p53/Kras sarcoma
- KPC
- Kras(G12D) lung
- Cre-luciferase

Surgical procedures:

- orthotopic implantation
- sub-renal implantation
- intrafemoral
- SQ and IV
- castration
- grafting clinical tissues

Other procedures:

- serial and terminal blood collection
- tissue microdissection
- gavage
- changing medicated or treated feed and water
- serial animal weights
- data collection and records maintenance
- primary cell culture
- tissue procurement
- snap freezing
- teratoma assay
- bioluminescence imaging
- high resolution X-ray
- embryonic dissection
- daily health monitoring
- tumor volume measurements
- organoid culture
- spheroid Culture

Histological procedures and services:

- CWR22
- CWR22R
- Hi-Myc
- Pten null
- TRAMP
- p53/Kras sarcoma
- KPC
- Kras(G12D) lung
- Cre-luciferase

Customized protocol development

The ETM provides scientific consultation/expertise on appropriate use of the models (strengths & limitations), experimental design (cohort design, timing, end-points and parameters) and tissue procurement. Additionally, for the tumor models available through the core the ETM maintains a tumor tissue bank representing tumor progression and corresponding normal tissues. The tissue bank contains the following types of samples: paraffin embedded, cryo-embedded and snap frozen. Contact Dr. Moser for a full list of samples available through the ETM tissue bank and the models currently supported through the ETM. Currently the ETM has established tumor banks for the following mouse models: TRAMP, CWR22 and CWR22R (castration recurrence), and Pten null, PKC (under development), and various PDX models within the ETM.

The purpose for the ETM arises from the multitude of animal models of cancer that have been established and characterized. Genetically modified mouse models of human cancers are increasingly being utilized for the study of cancer. These models have been used to identify molecular mechanisms of cancer initiation and progression, as well as for preclinical testing of new therapeutic compounds and approaches for the prevention and treatment of cancer. The appropriate use of transgenic and xenograft models require an in-depth working knowledge of the strengths and limitation of individual models. While such expertise is not readily available in all laboratories, this expertise is available within ETM for some of the widely used mouse tumor models. ETM provides a consolidated resource for maintenance of necessary breeding colonies, collection and archival of tumor samples in a tissue bank, consultation with experimental design, appropriateness of the model, breeding strategies and logistics, and technical expertise in tissue procurement. The shared costs

for services provided by the ETM results in reduced costs to the individual investigators at Roswell Park resulting from the resources conserved by maintaining only one colony per animal model, one maintenance xenograft line as well as capitalizing on the ETM technical and scientific expertise. The resulting savings are passed on to the Roswell Park investigator as reduced animal related costs for their research as well as access to mouse models that they would not normally have the technical and scientific expertise to utilize.

The ETM maintains homozygous breeding colonies of TRAMP animals (both C57BL/6 and FVB) and provides experimental animals for investigators. Dr. Barbara Foster is the ETM's scientific consultant for investigators using the transgenic prostate models in their research. The ETM also maintains the pten null model and a breeding colony for providing wildtype control mice (C57BL/6xFVB) since they are not available commercially. The ETM maintains the TRAMP pten null models in order to provide sufficient numbers of transgenic mice to Roswell Park investigators in a timely and efficient manner for both translational and basic research. In addition, the ETM maintains a tissue bank representative of disease progression for many of the models in ETM. The tumor bank consists of tissues for histology (paraffin embedded and cryosections), snap frozen tissue samples for molecular analysis and serum samples. The TRAMP tissue bank consists of prostates from at least 50 paraffin embedded prostates and associated tissues and controls (liver, kidney, lymph node, seminal vesicle and metastatic lesions; included in a 9 chamber cassette with the 4 lobes of the prostate), 50 specimens of snap-frozen prostate and associated tissue, 50 cryopreserved prostates for cryosectioning for each of the following stages of disease as well as matching serum samples. Banked tissue of the progression of the disease includes prostate and associated tissues from animals at 6, 8, 10, 12, 15, 20-25, and >35 weeks of age, castration

recurrent disease and metastasis. Prostates from wildtype mice at similar time points are also available with in the resource. The ETM also serves as a resource to Roswell Park investigators for stocks of parental and clonal cell lines established from the TRAMP tumor model. For all TRAMP mice, TRAMP tissue, and cell lines ETM requires that the PI obtain a material transfer agreement (MTA).

The CWR22 and CWR22R xenograft models maintained by ETM were a gift from their originator, Dr. Thomas G. Pretlow at Case Western Reserve University School of Medicine, hence the name of the xenografts. Dr. James Mohler works extensively with the CWR22 and CWR22R models and acts as a scientific consultant for this model. The ETM maintains by animal passage the CWR22 and CWR22R human xenograft lines in order to provide xenograft implanted mice and primary cells to Roswell Park investigators in a timely and efficient manner. ETM also maintains a tumor bank of CWR22 and CWR22R tumors (paraffin embedded, snap frozen, cryopreserved and matched serum) for use in preliminary studies by Roswell Park investigators.

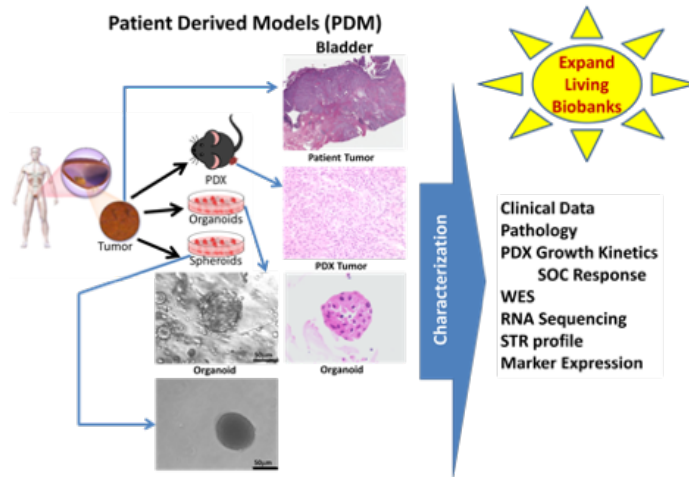
The full resources and specialized technical expertise of the ETM are available to Roswell Park investigators. This includes providing transgenic mice from the various models, aged matched wildtype mice, xenograft bearing experimental animals, and cell line implanted animals for projects as well as technical expertise in experimental design and appropriate use of these models. Technical services

available through the ETM include subrenal grafting and orthotopic grafting of tissues. ETM routinely implants human clinical samples into SCID mice subcutaneously and orthotopically. The success rate for ETM subcutaneous grafting of human prostate tumor tissue is >90%, However, take-rates for establishing PDX lines vary by the cancer type being grafted. In addition to establishing PDX models, ETM has experience establishing organoid and spheroid tissues from clinical samples. The ETM is also able to perform the embryonic dissection and tissue recombination allowing for rescue of tissue from embryonic lethal transgenic lines. Additional surgical services provided by the ETM include castration, subcutaneous and orthotopic implantation of xenografts and surgical samples, subcutaneous implantation of cells and tissues, intra femoral implantation, and implantation of testosterone and other drug impregnated pellets. Other services provided include collection of animal related data such as high resolution X-ray (Faxitron), in vitro imaging of luciferase using the Xenogen IVIS 50, bigenic breeding and screening, biweekly measurement of tumors, daily monitoring of health, serial blood sampling, and harvesting of tissue. ETM routinely performs tissue processing of formalin fixed tissues, paraffin embedding, and sectioning of paraffin and OTC fixed tissues. MTMT also provides H & E staining and immunohistochemical (IHC) staining for select markers. On request ETM can provide specialized biochemical and IHC staining of tissues. The ETM is dynamic and expands its models and technical services to accommodate the growing needs of investigators of the Institute.

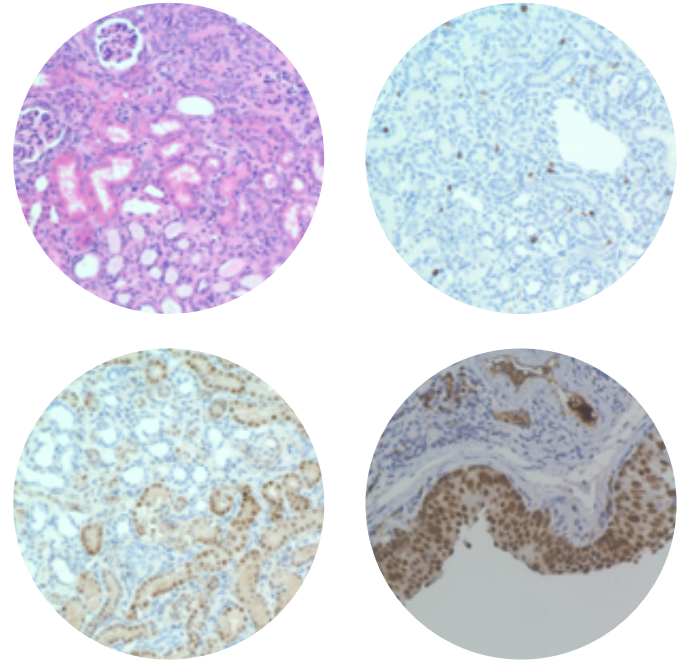
The ETM provides “fee for services” for specialized techniques as well provides personnel efforts for larger experiments that require extensive labor and considerable technically demanding procedures.

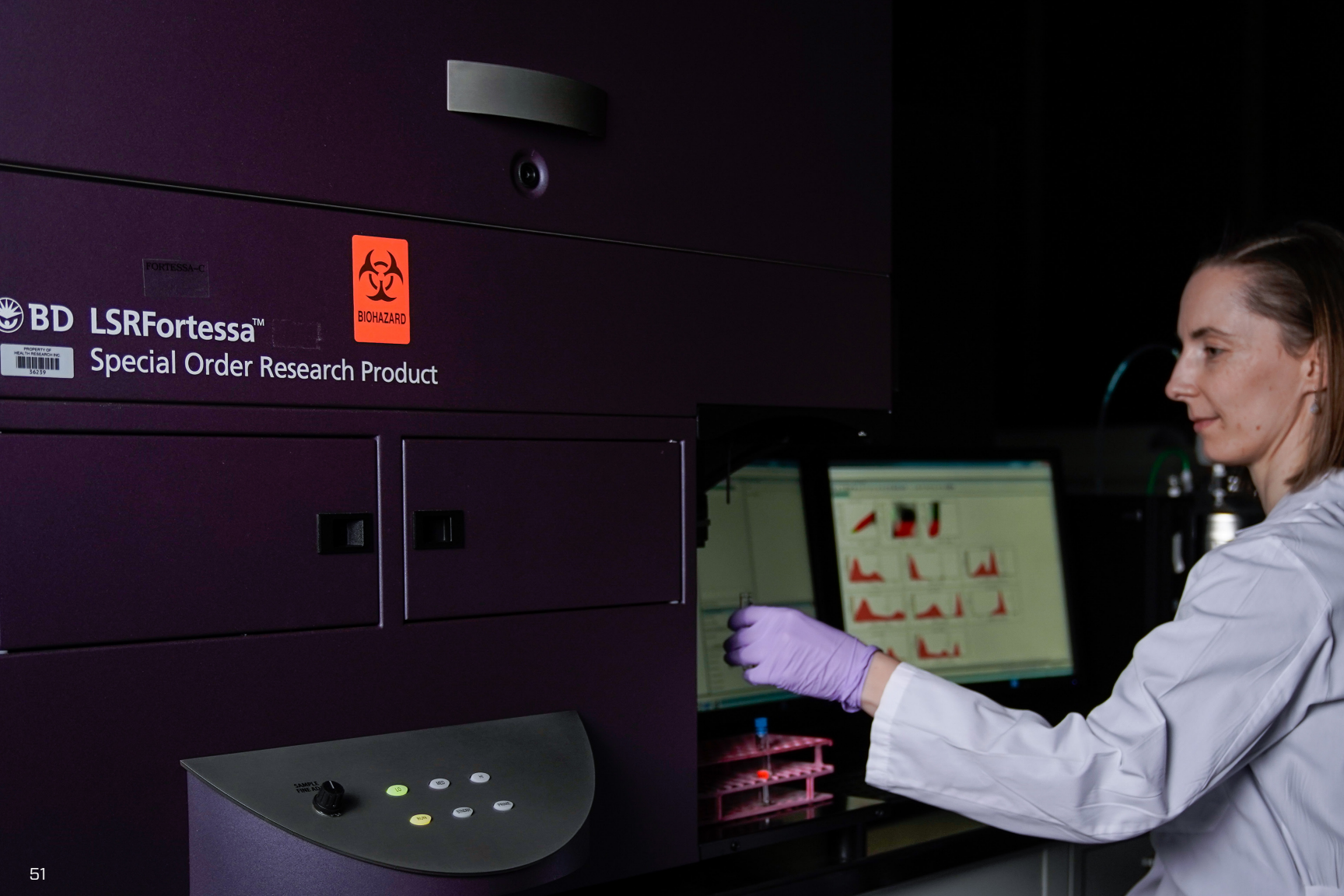
Equipment

The ETM has a Leica Tissue Processor (ASP300), a Leica microtome, cryostat, dissecting scope with image capturing capability, DAKO immunostainer (Autostainer Plus), 2 liquid nitrogen cryostorage system, ultralow freezers (Sanyo) and step-down freezer for controlled cryopreservation of living samples.



IHC STAINING OF MOUSE TUMORS






FORTESSA-C



BIOHAZARD

 **BD LSRFortessa™**
Special Order Research Product



SAMPLE
PANEL



FLOW & IMAGE
CYTOMETRY

SHARED RESOURCE

FLOW AND IMAGE CYTOMETRY SHARED RESOURCE

OVERVIEW

The Flow and Image Cytometry Shared Resource (FICSR) provides standard and advanced flow cytometry and imaging capabilities for research and clinical investigators at Roswell Park. The Resource is a licensed reference laboratory for classification of leukemia and lymphoma by immunophenotyping, for evaluating immunologically-based diseases, for monitoring transplant patients and for evaluating DNA in solid tumors. Clinical research support is provided for protocols requiring specific immunophenotyping follow-up to monitor the progress of therapy, and the Resource is committed to translational research through education and the development of new tests. Standard and advanced flow cytometry and imaging services are also provided to basic research scientists. After appropriate training, researchers have access to all research equipment. As a shared resource we are committed to education and offer one-on-one training on staining, acquisition and analysis techniques plus a variety of seminars to all users.

USING THE RESOURCE

Investigators are encouraged to contact the Shared Resource (the general research lab number: 845-3470) prior to initiating a flow or image cytometry project for appropriate training, to establish an account and to obtain access to instrumentation. For sorting services please visit <https://www.roswellpark.org/shared-resources/flow-and-image-cytometry/cell-sorting> where links can be found to required sort appointment request forms and sort information forms.

The Flow and Image Cytometry Resource is located in the Cancer Cell Center, 3rd Floor. Following the appropriate training, users have access to the research equipment 24/7. Regular hours of operation are weekdays, 8:00 AM - 6:00 PM

Website: <https://www.roswellpark.org/shared-resources/flow-and-image-cytometry>

LEADERS

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SERVICES

Clinical Applications: The Flow Cytometry Resource performs all required sample preparations and analysis on clinical samples, and provides reference laboratory services. Expedited service is available that provides results within four hours of receipt of the samples; otherwise, results are provided within 36 hours. A comprehensive group of panels, each containing up to 10 antibodies, has been designed that will identify myeloid and lymphoid subpopulations. Custom panels containing other antibodies of interest can be designed as required. Panels to enumerate circulating tumor and endothelial cells, T regulatory cells, myeloid derived suppressor cells (MDSC) and dendritic cells are examples of recently developed assays. The panels are designed to monitor response of cancer patients to therapy, evaluate patients with autoimmune disease and aid in the diagnosis and classification of hematopoietic malignancy. The measurement of DNA content by flow cytometry has been used to determine the degree of aneuploidy in malignant cells and to determine the percentage of cells in S phase. Antibodies to specific

cellular components such as cytokeratin, oncogene products and receptors also have been combined with DNA content measurements. These measurements provide additional information about neoplastic cells, leading to improved patient management and care.

Research Applications: The Flow and Image Cytometry Resource has the capability of analyzing up to eighteen colors of fluorescence simultaneously using conventional flow cytometry analyzers while 25+ fluorescent parameters can be simultaneously analyzed using the spectral flow cytometry analyzer. Both static and real-time measurements can be made. Nonsterile and sterile sorting is provided at a sorting rate of 5- 20 x 10⁶ cells per hour.

Investigators or their staff also can perform their own cytometry experiments. This hands-on approach provides the investigator with the know-how to fully use the capabilities of the resource. Also, experience with analyzing one's own data affords greater insight into its interpretation. Resource personnel are available to help the investigator in experimental design, data acquisition, analysis and

interpretation. In our experience, there are three types of research investigators who use the Flow and Image Cytometry Shared Resource. The level 1 users are those who are already proficient in the techniques and know the instruments' capabilities. For these investigators the laboratory provides oversight and consultation as necessary but mainly provides the instrumentation, data analysis systems, and instruction on how to use them, while the investigator and his/her technical staff perform the work. The level 2 user depends exclusively on the expertise of the Resource staff to provide sample preparation, data acquisition and analysis and interpretation. This includes all in-house and outside clinical research and clinical protocols as well as some basic research. Finally, the 3rd level of user includes investigators who have just learned about flow or image cytometry or who are just beginning to consider application of this technology to address their research questions. Here the Resource staff may perform pilot experiments with the user to determine feasibility and what would be a useful approach. Staff then work one-on-one to train the investigators on proper sample preparation, instrument operation and data analysis for their protocol. If successfully developed and applied, these investigators become proficient in the technique and attain level 1 status.

Multiplex Analyte Immunoassay: There are currently over 100 cytokines/chemokines whose coordinate or discordant regulation is of clinical interest. The optimal manner in which to correlate a specific disease process with changes in cytokine levels requires analyzing each sample for multiple cytokines. However, using conventional methodologies (ELISA), limited sample size and cost often precludes multiple cytokine analysis. We have pioneered soluble bead-based multiplex analysis with Luminex and currently offer customized multiplex assays to quantify different cytokines, chemokines, clinically important proteins and peptides, or DNA molecules in a single microtiter well. This multiplexing feature allows one to obtain a wealth of information using a minimum amount of sample. Multiplexing enables a comprehensive overview of complex biological systems and the inclusion of in-process controls. The Resource has been a long-time participant in the External Quality Assurance Program Oversight Laboratory (EQAPOL) Luminex program of the Duke Human Vaccine Institute's Immunology and Virology Quality Assessment Center which supports the development, implementation and oversight of external quality assurance programs that monitor laboratories performing the Luminex assays.

Imaging: The resource offers a high resolution confocal microscope for subcellular level imaging, a live cell imaging set up for time kinetic multiplex study of cell behavior under environmentally controlled conditions and the ImageStream platform for high throughput image analysis in immunophenotypically-defined cell populations. The ImageStream technology (Amnis Corp) is a flow cytometry-based image analysis platform that produces high resolution, spectrally separated but spatially correlated images of cells prepared in suspension. The innovation of this technology is that it can provide quantitative information not only on the prevalence of molecular targets in a heterogeneous cell population, but, unlike standard flow cytometry, also on their localization within the cell. The ability to do this with statistically meaningful cell numbers (1,000's of cells) sets this technology apart from alternative microscopy-based imaging technologies.

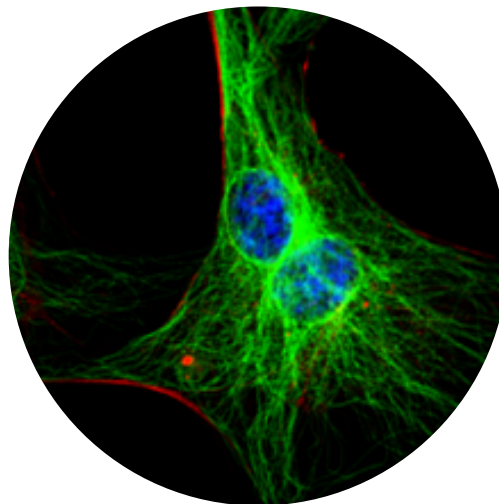
SERVICES

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Research Applications: The Flow and Image Cytometry Resource has the capability of analyzing up to eighteen colors of fluorescence simultaneously using conventional flow cytometry analyzers while 25+ fluorescent parameters can be simultaneously analyzed using

the spectral flow cytometry analyzer. Both static and real-time measurements can be made. Nonsterile and sterile sorting is provided at a sorting rate of 5- 20 x 10⁶ cells per hour.

Investigators or their staff also can perform their own cytometry experiments. This hands-on approach provides the investigator with the know-how to fully use the capabilities of the resource. Also, experience with analyzing one's own data affords greater insight into its interpretation. Resource personnel are available to help the investigator in experimental design, data acquisition, analysis and



INSTRUMENTATION

Flow Cytometry

- **BD Biosciences LSRII-A:** 355nm (100mW), 405nm (25mW), 488nm (20mW), 561nm (50mW), 640nm (40mW) Excitation Sources, 18 color detection + 2 scatters
- **BD Biosciences LSRII-B:** 405nm (100mW), 488nm (20mW), 561nm(50mW), 640nm (40mW) Excitation Sources, 16 color detection + 2 scatters
- **BD Biosciences LSR Fortessa A:** 405nm (50mW), 488nm (50mW), 640nm (40mW) Excitation Sources, 11 color detection + 2 scatters
- **BD Biosciences LSR Fortessa B:** 355 nm (60 mW), 405nm (50mW), 488nm (50mW), 640nm (40mW) Excitation Sources, 18 color detection + 2 scatters
- **BD Biosciences LSR Fortessa C:** 355 nm (60 mW), 405nm (50mW), 488nm (50mW), 640nm (40mW) Excitation Sources, 18 color detection + 2 scatters
- **BD Biosciences FacsCanto:** 405nm (30 mW), 488nm (20 mW), 633nm (40 mW) Excitation Sources, 10 color detection + 2 scatters.
- **Beckman Coulter Cytoflex:** 375nm (60mW), 405nm (80 mW), 488nm 50 mW), 561nm (30 mW), 638nm (50 mW) , 808nm (60 mW) Excitation Sources, 21 color detection + 2 scatters
- **Cytek Aurora (spectral analyzer):** 355 nm (20 mW), 405nm (100 mW), 488nm (50 mW), 638nm (80 mW) Excitation Sources, 54-detectors + 4 scatters

Sorting flow cytometers

- BD Biosciences FACS Aria-I: 405 nm(30mW), 488nm (15mW), 561nm (30mW), 633nm (17mW) Excitation Sources, 18 color detection + 2 scatters

- BD Biosciences FACS Aria-II: 355nm (100mW), 405 nm(50mW), 488nm (50mW), 640nm (17mW) Excitation Sources, 18 color detection + 2 scatters
- Sony MA900: 405 nm (optical fiber output 10 mW), 488nm, 561nm, 638nm (optical fiber outputs 36 mW), 12 fluorescence detectors + 2 scatters

Imaging

- Luminex/Amnis ImageStreamX-MKII-205: 405nm(100mW), 488nm (200mW), 561nm(350mW), 592nm (100mW), 642nm(100mW) Excitation Sources, 9 color detection + scatter + 2 brightfield, extended depth of field option, 20x, 40x, 60x objectives, autosampler
- Luminex/Amnis ImageStreamX-MKII-401: 405nm(100mW), 488nm (200mW), 561nm(350mW), 642nm(100mW) Excitation Sources, 9 color detection + scatter + 2 brightfield, 20x, 40x, 60x objectives.
- Leica SP8 laser scanning confocal microscope. 405nm, 488nm, 552nm and 638nm laser excitation lines, 3 detectors (1 PMT, 2 HyD), 10x, 20x and 63x objectives; stage incubator and motorized stage.
- Leica AF6000LX Live cell imager. Mercury short-arc reflector and monochromator (range 320nm-694nm) Excitation sources, RGB and YFP/CFP filter cubes

Other

- Miltenyi Biotec AutoMACS (immunomagnetic sorting)
- ParticleMetrix Zetaview (NTA nanoparticle sizing; 488nm or 405nm excitation options)
- Luminex 200 (multiplex bead array)

Software

- CellQuest (Flow Cytometry Data Acquisition)
- DiVA (Flow Cytometry Data Acquisition)
- FCS Express (Flow Cytometry Data Analysis)
- MultiCycle (Flow Cytometry DNA Cell Cycle Analysis)
- WinList (Flow Cytometry Data Analysis)
- GemStone (Flow Cytometry Probability State Modeling)
- ModFit (Flow Cytometry DNA Cell Cycle Analysis)
- FloJo (Flow Cytometry Data Analysis for Mac)
- IDEAS (Data analysis for ImageStream)
- ImagePro (Image Analysis)
- BeadView (Soluble Bead Array Analysis)
- ImageJ (Image Analysis)
- Immunophenotyping in flow cytometry
- DNA analysis in flow cytometry
- Introduction to confocal microscopy
- Introduction to ImageStream cytometry

Courses

Our facility organizes several courses, free of charge. Sign-up sheets for these courses are posted outside of the user room on the 3rd floor of the CCC building. The following courses are offered:

- Introduction to flow cytometry
- Introduction to DiVA acquisition software
- Compensation in flow cytometry

Notable Projects

The facility is nationally recognized and utilized for its immunophenotyping and immune monitoring service for clinical trials and protocols. In addition, the facility is recognized for the development and application of pre-clinical and clinical applications of the ImageStream platform, particularly in the field of signal transduction.

NOTE: You can analyze your data at your desk by using ModFit, WinList, GemStone, FCS Express and Multicycle which are available to any computer connected to the Roswell network. Additionally, our faculty frequently participates in national and international courses on flow and image cytometry.





GENE TARGETING
& TRANSGENIC

SHARED RESOURCE

GENE TARGETING & TRANSGENIC SHARED RESOURCE

OVERVIEW

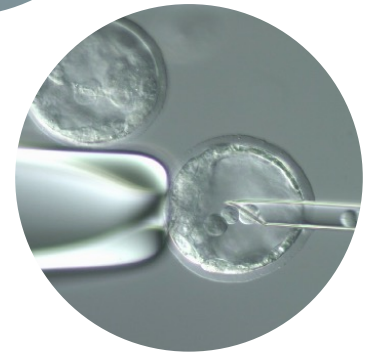
The **Gene Targeting and Transgenic Shared Resource (GeTT)** enables investigators to modify the murine genome for the systematic dissection of the genetic, molecular, cellular and physiological mechanisms underlying complex biological processes. Significant advances have been made in oncology, immunology, and developmental biology using this technology. This Core resource also provides investigators with mouse reproductive services to aid them in their research as well as generation of ES cell lines.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Aimee Stablewski to discuss scheduling and procedures.

The Gene Targeting and Transgenic Shared Resource is located in the Medical Research Complex. Hours of operation are weekdays, 6 AM – 5:30 PM, and variable weekend hours.

Website: <http://www.roswellpark.edu/shared-resources/gene-targeting-and-transgenics-resource>



LEADERS

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Director

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SERVICES

The Gene Targeting and Transgenic Shared Resource has the resources and skills necessary to construct mice with defined mutations at specific locations of the mouse genome. In addition, we provide ancillary mouse reproductive services such as IVF (in vitro fertilization) and cryopreservation.

Transgenic Mice:

Transgenic Injection (Per Day): (various strains may be used):

The GeTT will harvest and inject fertilized oocytes with DNA prepared and purified by the investigator or the GeTT facility (whichever is chosen). Surviving eggs will be implanted in pseudopregnant females, and pups born will undergo tail biopsy for isolation of DNA, and will also be identified by an ear tag. The GeTT guarantees that at least 100 eggs will be surgically transferred or three transgenic offspring will be produced (whichever comes first).

***Note: The expression of your gene is not guaranteed. ***

Chimeric Mice:

Blastocyst Microinjection with Your ES Cells Generated In-House or by a Collaborator (Per Day):

The Gene Targeting and Transgenic Shared Resource will harvest blastocysts from C57BL/6 mice and inject the appropriate number of ES cells/blastocyst (either provided by the investigator or generated in our shared resource (If not generated in our shared resource, you must submit a Mycoplasma Pathology Report with your cell line or we can submit your cell line to our Tissue Culture facility for testing, which will incur additional charges.)

Injected blastocysts will then be implanted into pseudopregnant females. The GeTT guarantees that at least 20 blastocysts will be successfully injected and implanted into foster moms, or three chimeric pups will be produced, whichever comes first.

KOMP/EUCOMM ES Cell Injections:

Same as Blastocyst Microinjection, however, please visit https://www.roswellpark.edu/sites/default/files/chimeric_mice.pdf to view a message from Aimee Stablewski regarding the KOMP/EUCOMM ES cell injections.

CRISPR SERVICE:

Background:

CRISPR/Cas9 is a powerful genome editing tool that is redefining the boundaries of biological research.

The GeTT at Roswell Park Comprehensive Cancer Center is pleased to introduce a FULL CRISPR-based gene editing platform to modify your mouse genome by global knockouts, small amino acid substitutions or other small tag knockins as well as larger gene fusions and conditional allele knockins.

We have had over 60 projects since 2013, and since 2017 with the inception of easi-CRISPR, we have had success in 99% of our projects.

Design and production of your guide RNA and oligos:

The GeTT will work with you to design the reagents needed for a successful project or design the entire project for you.

CRISPR Electroporation(used for KNOCKOUTs and small DNA insertions (base changes, amino acid substitutions (≤ 200 bp)):

The GeTT will harvest and inject fertilized oocytes with CRISPR reagents. RNAs and protein (sometimes DNA as well) are concurrently electroporated, and handled with the utmost care to prevent degradation.

Targeting DNA with homologous arms can be co-injected to direct integration/homologous recombination to that site. DNA and RNA are prepared and purified by the GeTT. Surviving eggs will be implanted in pseudopregnant females, and pups born will undergo tail biopsy for isolation of DNA, and will also be identified by an ear tag.

CRISPR One-cell Injection (typically used for larger DNA

insertions (>500bp):

The GeTT will harvest and inject fertilized oocytes with CRISPR reagents. RNAs and protein (sometimes DNA as well) are concurrently injected, and handled with the utmost care to prevent degradation.

Targeting DNA with homologous arms can be co-injected to direct integration/homologous recombination to that site. DNA and RNA are prepared and purified by the GeTT. Surviving eggs will be implanted in pseudopregnant females, and pups born will undergo tail biopsy for isolation of DNA, and will also be identified by an ear tag.

CRISPR Two-cell Injection (typically used for larger DNA insertions (>500bp):

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Targeting DNA with homologous arms can be co-injected to direct integration/homologous recombination to that site. DNA and RNA are prepared and purified by the GeTT. Surviving eggs will be implanted in pseudopregnant females, and pups born will undergo tail biopsy for isolation of DNA, and will also be identified by an ear tag.

According to a recent publication by the Rossant lab: they were able to achieve a more-than-tenfold increase in knock-in efficiency over standard methods using 2-cell injection. (Gu et al. Nature Biotechnology volume 36, pages 632–637 (2018))

The GeTT highly suggests getting targeted NGS done on the founder and F1 animals resulting from CRISPR injections/electroporations, as mosaicism is

always a concern.

****NOTE:** The expression of your gene is not guaranteed. **

REDERIVATION OF HORIZONTALLY-TRANSMITTED PATHOGENS:

Frozen or Fresh Embryos OR Sperm

The shared resource will rederive mouse strains from fresh or frozen embryos (or sperm) at the request of the investigator.

The GeTT will work with investigators to rederive mouse lines from dirty facilities into the clean facilities here at Roswell. In this situation, arrangements will need to be made with the facility regarding where the mice are currently housed and/or how the facility will receive embryos for transfer.

In Vitro Fertilization

Embryos will be created and then rederived as above. Please see IVF for details (below)

****NOTE:** After surgical transfers of washed embryos are done, per diem charges at the Gene Targeting and Transgenic Shared Resource's rate will be charged to investigators.

When pups are weaned, tailing can be done to check genotype.

Gene Targeting: Electroporation:

Upon receiving your lab's DNA construct, the Gene

Targeting and Transgenic Shared Resource will perform an electroporation of your DNA into W4 (129S6/SVEvTac), G4 (129/B6) or JM8A3.N1 (C57BL/6Tac) ES cells. Colonies will be selected with the appropriate drug, depending on your positive selectable marker. Colonies can also be negatively selected for enrichment purposes. Selection methods should be discussed with the facility prior to the electroporation date to prepare the necessary feeder cells.

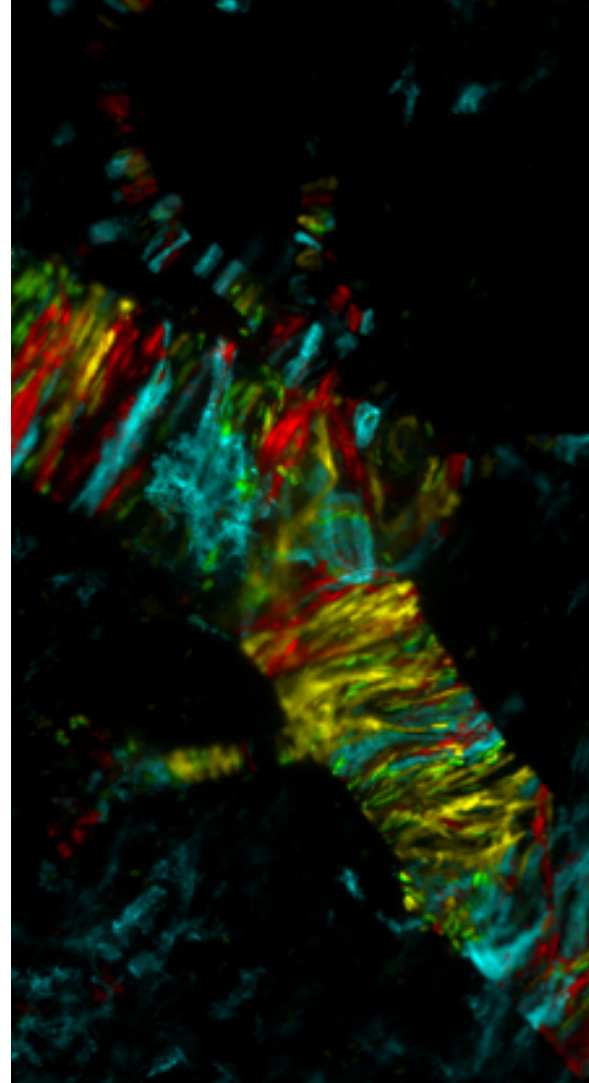
The facility will pick and freeze ~240 colonies, of which we will give the investigator's lab ~200 DNA preps. The investigator will screen these clones by Southern analysis, and/or PCR, and notify the facility of the positives for homologous recombination. The facility will expand five of these clones and give the investigator DNA again to reconfirm positives. When reconfirmed, the facility can begin Gene Targeting microinjections for the investigator's laboratory.

The entire electroporation process takes approximately one month to complete and the facility expects the investigator's lab to perform the positive confirmations in a timely fashion.

CRYOPRESERVATION OF MOUSE EMBRYOS AND SPERM:

Cryopreservation of Sperm:

1. 2-3 male mice which are between the ages of 10-16 weeks are needed to perform this service. We will freeze down 20 straws worth of sperm, and will store



them in liquid nitrogen. A post-thaw viability check and an in vitro fertilization (IVF) to the 2-cell stage will be performed on one of the straws.

2. Sperm cryopreservation presents the opportunity to substantially reduce the number of mice used to both store and re-establish a mouse line. Sperm collected from a single male can potentially give rise to large numbers of offspring following IVF of oocytes. The efficiencies of sperm cryopreservation are strain-dependent.
3. The disadvantage of sperm cryopreservation is that haploid genome is preserved. The sperm cryopreservation service does not guarantee recovery to live born by IVF and for some strains ICSI is required for recovery. Sperm counts, motility and morphology will be analyzed before cryopreservation.

Cryopreservation of Mouse Embryos:

1. Our professional staff will determine the quantity of embryos to cryopreserve, and the most efficient procedure for embryo collections. **In vitro quality control to indicate the success of cryopreservation is performed on every batch of embryos.**
2. Embryo collections can be done one of two ways:
 - **Traditional mating, collection and freezing:**
Traditional mating, collection and freezing: Specifically, stud males are mated weekly with egg donors per cryopreservation session to produce embryos for cryopreservation. On average, it takes 2-4 embryo collection sessions to freeze down enough embryos to guarantee recovery in this traditional way. If using homozygous males then fewer sessions will be needed. However, it may take

more sessions if the mouse strain has a low superovulation rate, the stud males have low fertility, or males are too old. Some strains cannot be successfully cryopreserved. The investigator is responsible for providing all stud males, embryo donors, and per diem costs for these animals. Females will be superovulated by the TG facility and mated with males, and embryos will be harvested and cryopreserved. This procedure will be repeated until a sufficient number of embryos are cryopreserved. Embryos are isolated and cryopreserved in straws and stored in liquid N₂.

• IVF, collection and freezing:

- Females will be superovulated by the TG facility and embryos will be created through IVF with frozen or fresh sperm. Embryos will be cryopreserved at the 2-cell stage. This procedure will be repeated until a sufficient number of embryos are cryopreserved. This usually should only take one session, but sometimes a second is needed.
- Embryos are cryopreserved in straws and stored in liquid N₂.

De Novo ES cell derivation:

The Gene Targeting and Transgenic Shared Resource will prepare new mouse ES cell lines from blastocysts or mice provided by investigators. Standard methods employing serum containing medium and MEK1 inhibitor are used. The success rate of this procedure is high as long as blastocysts can be obtained from the mouse strain in question. Mouse ES cells provide an endless supply of cells for in vitro studies because ES cells do not undergo senescence and cease division as do other cell types (e.g.

fibroblasts). In addition is possible to differentiate ES cells into different lineages to query gene function in cell culture systems instead of or in parallel to in vivo studies. The procedure requires intensive cell culture over an extended period of time by transgenic facility personnel. Advance notice should be given, preferably when the blastocyst-donors-to-be are born. The ideal age for of in-house blastocyst donors for superovulation response is 24-28 days.

In vitro fertilization (IVF):

IVF can be used to rapidly expand mouse lines from a few males that carry the desired genotype OR to maintain strains with poor breeding efficiency. The Gene Targeting and Transgenic Shared Resource will superovulate egg donors per session. We will perform IVF with sperm from your males and egg donors (or purchased).

After overnight culture, 2-cell embryos will be transferred to pseudopregnant females (unless specified for freezing). All weaned pups will be transferred to the investigator. We expect the investigator to genotype the pups and determine which pups have the desired genotype(s). IVF results vary according to genetic background and the quality of individual males used for IVF (Byers et al., 2006, Vergara et al., 1997). Thus, we cannot offer a guarantee that any given IVF procedure will produce large number of pups.

Subcloning:

In certain instances, ES cells are found to be aneuploid (greater than or less than 40 chromosomes). Aneuploid ES cells may generate chimeras; however they will never be transmitted through the germline. If you have an aneuploid ES cell clone, it may be possible to

rescue that clone by plating it at a low density and picking subclones that would be diploid, and thus transmit through the germline.

Mouse Colony Maintenance:

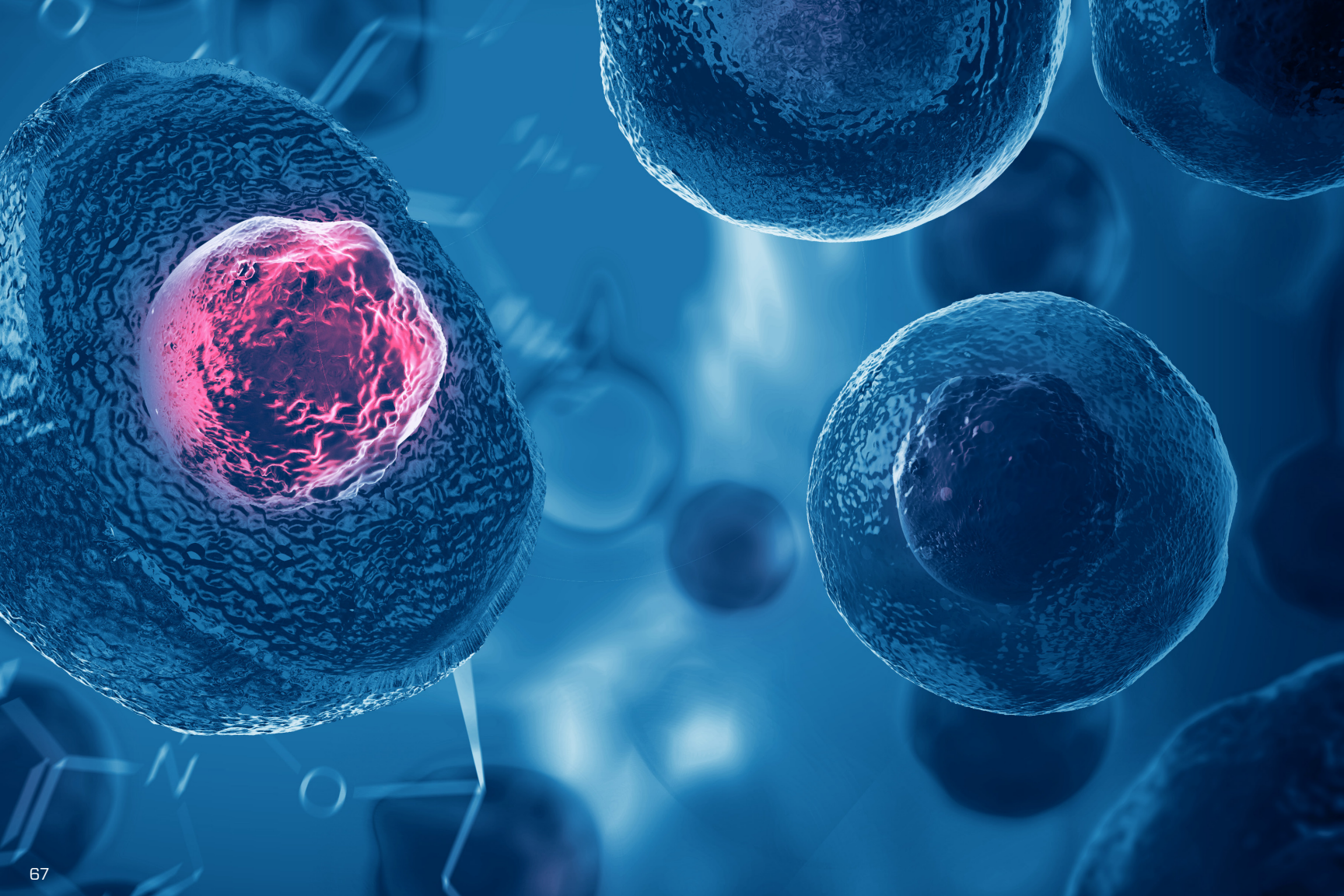
In this service, we have been helping people with weaning, tagging and tailing their genetically-engineered strains to deliver your needed cohorts of animals. We have a set fee schedule per strain, and in addition, we can add on IVF to rapidly expand the strains you need. Also, we in conjunction with MD Anderson can offer speed congenics to your lab if you need to move your mutation from one background to another.

Finally, we can also genotype your mice (see Genotyping below):

Genotyping:

Researchers using complex genetically modified animal models recognize that genotyping is tedious and time-consuming, with no room for error. Roswell Park's Genotyping Core Laboratory provides fast, accurate, convenient, and affordable genotyping technologies. We use conventional polymerase chain reaction (PCR) and gel electrophoresis methods. By pooling samples from a group of users, we can better ensure accuracy, cost-effectiveness, and quality control.

The GETT will work with your lab to identify the targeted mutations generated from any genome-editing platform. Our lab stores all of your information about your strain(s) including publications, primer sequences and amplification protocols.



GENE MODULATION
SHARED RESOURCE

GENE MODULATION SHARED RESOURCE

OVERVIEW

The Roswell Park Comprehensive Cancer Center / University at Buffalo Gene Modulation Shared Resource (GMSR) serves as the focal point of RNA interference and CRISPR (clustered regularly interspaced short palindromic repeat) repression and activation expertise for the Roswell Park and UB research communities. Researchers have access to a whole genome resource of individual shRNA constructs, pooled shRNA libraries, and the newly acquired CRISPRi and CRISPRa pooled libraries.

The Gene Modulation Shared Resource can also help with retroviral and lentiviral packaging of constructs provided by researchers and the subsequent infection and selection of target cells. The Resource also houses the ORFeome 8.1 library that contains ~13,000 full length human gene cDNAs in a Gateway-adapted lentivirus vector, originally produced by the Center for Cancer Systems Biology at Dana-Farber Cancer Institute. The Resource also makes available plasmids with gene markers (drug selection markers: G418, hygromycin, puromycin, bleomycin; cell markers: GFP, RFP, mCherry, luciferase, etc.), immortalization constructs (SV40-Tag, HPV16- E6, E7, hTERT),

and various regulated expression vector systems (e.g.- tetracycline-regulated).

In this way, the Resource provides a centralized service from which investigators interested in gene transfer or modulation, cell selection or immortalization, using viral vector technologies can order packaged, infectious yet replication-defective lenti- or retrovirus in a rapid and cost-efficient manner.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Renae Holtz to discuss available products, scheduling, procedures, or to obtain an order form. You may also contact Irwin Gelman for questions regarding experimental design.

The Gene Modulation Shared Resource is located in the CGP, 2nd Floor, L2-135. Hours of operation are weekdays, 8:30 AM - 5:00 PM

Website: <https://www.roswellpark.edu/shared-resources/genomics/services-and-fees/shrna>

LEADERS

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Rena Holtz

Research Technologist
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SERVICES

Individual shRNA constructs

The GE Dharmacon/Open Biosystems Expression Arrest™ Human pGIPZ lentiviral shRNA library and Expression Arrest™ Human and Mouse pSM2 retroviral shRNA library.

These libraries are available as renewable bacterial stocks, plasmid DNA, or infectious, replication-incompetent viral supernatants. The researcher's chosen target cells can also be infected and selected for antibiotic-resistant colonies in our BSLC2+ approved facility. After expansion, these cells will be provided back to the researcher and can be safely maintained following regular BSL2 guidelines.

- Each library consists of ~62,000 unique shRNA constructs targeting the entire human and mouse genomes (>25,000 genes) with an average of 2-3 constructs per gene.

Pooled shRNA libraries

GE Dharmacon/Open Biosystems Decode™ Human RNAi Viral

Screening Library and the COLLECTA DECIPHER™ Pooled shRNA Human and Mouse Lentiviral Libraries.

The pooled format of both screening libraries follow a simple protocol that does not require access to expensive high-throughput arrayed screen equipment and infrastructure, putting the genome-wide shRNA knockdown screens within reach of any researcher.

- Both the Decode™ and DECIPHER™ libraries are bar-coded lentiviral shRNA libraries optimized for RNAi Genetic Screens and are available as plasmid DNA or packaged infectious lentivirus.

Genomic Screens with CRISPR Pooled Libraries (targets human genes)

Activate or repress gene expression using the recently acquired CRISPRi and CRISPRa pooled libraries developed by Jonathon Weissman and made available through Addgene.

- The libraries use inactive dCas9 to activate (CRISPRa) or inhibit (CRISPRi) gene transcription in human cells. The CRISPRa sgRNA



library uses the sunCas9 system and contains 10 sgRNAs for each transcription start site in 15,977 human genes, and a set of 5,968 control sgRNAs for a total of 198,810 sgRNAs. The CRISPRi library contains 10 sgRNAs for each transcription start site in 15,977 human genes, and a set of 11,219 control sgRNAs for a total of 206,421 sgRNAs.

- Please visit the following website for more information: <https://www.addgene.org/crispr/libraries/>

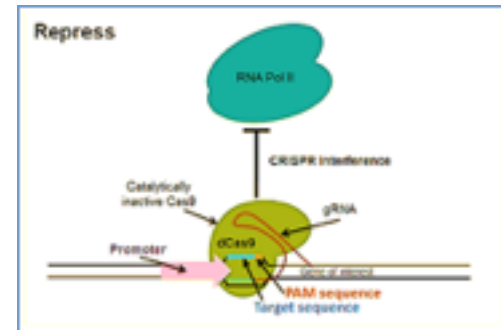
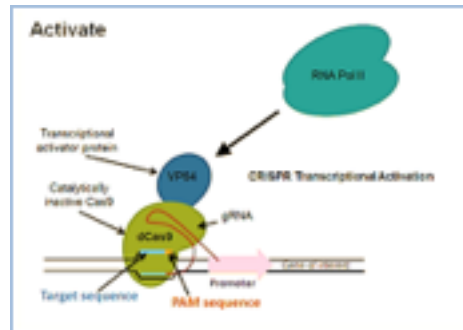
Additional Services

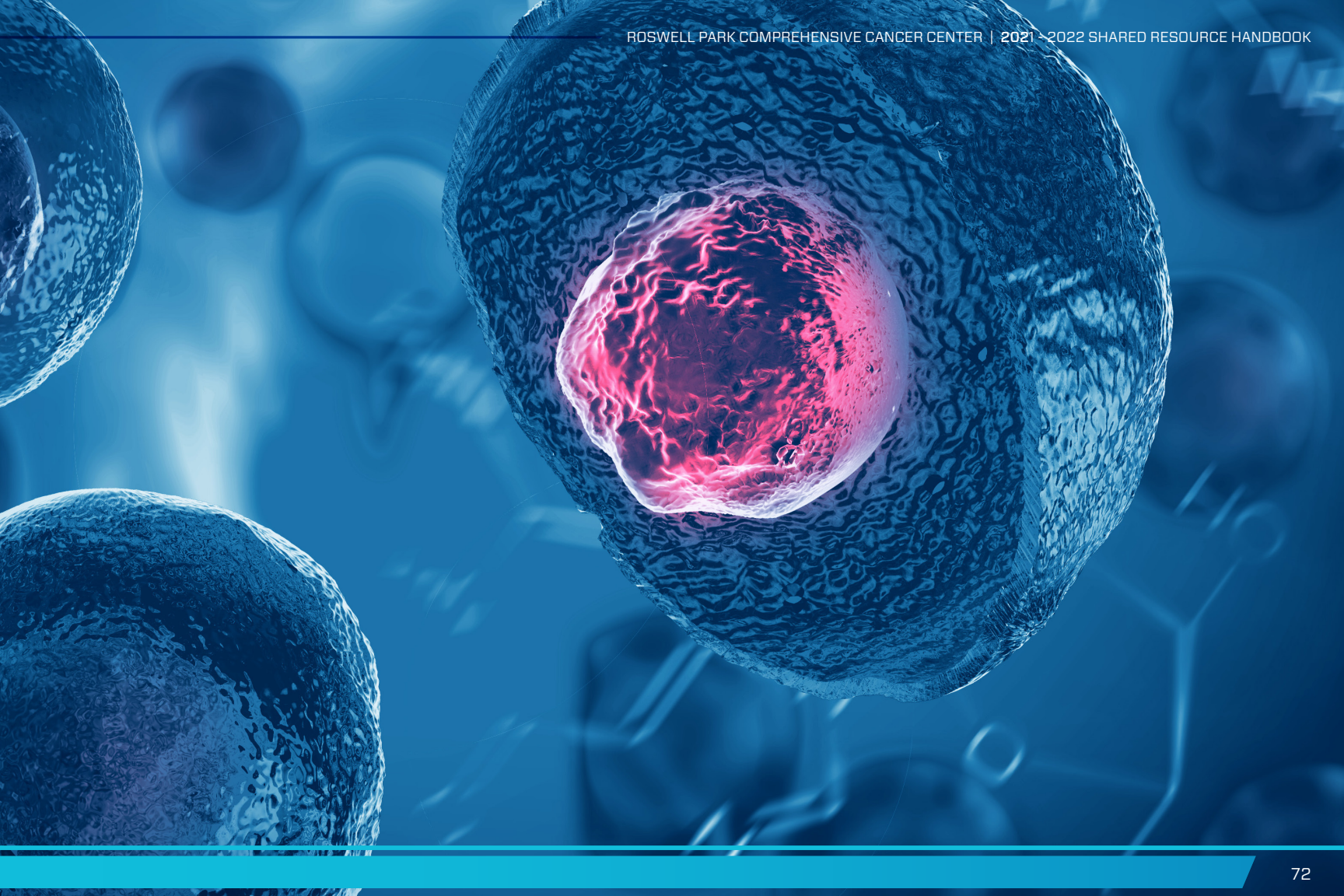
- **Packaging of researcher supplied retro- or lentiviral constructs**

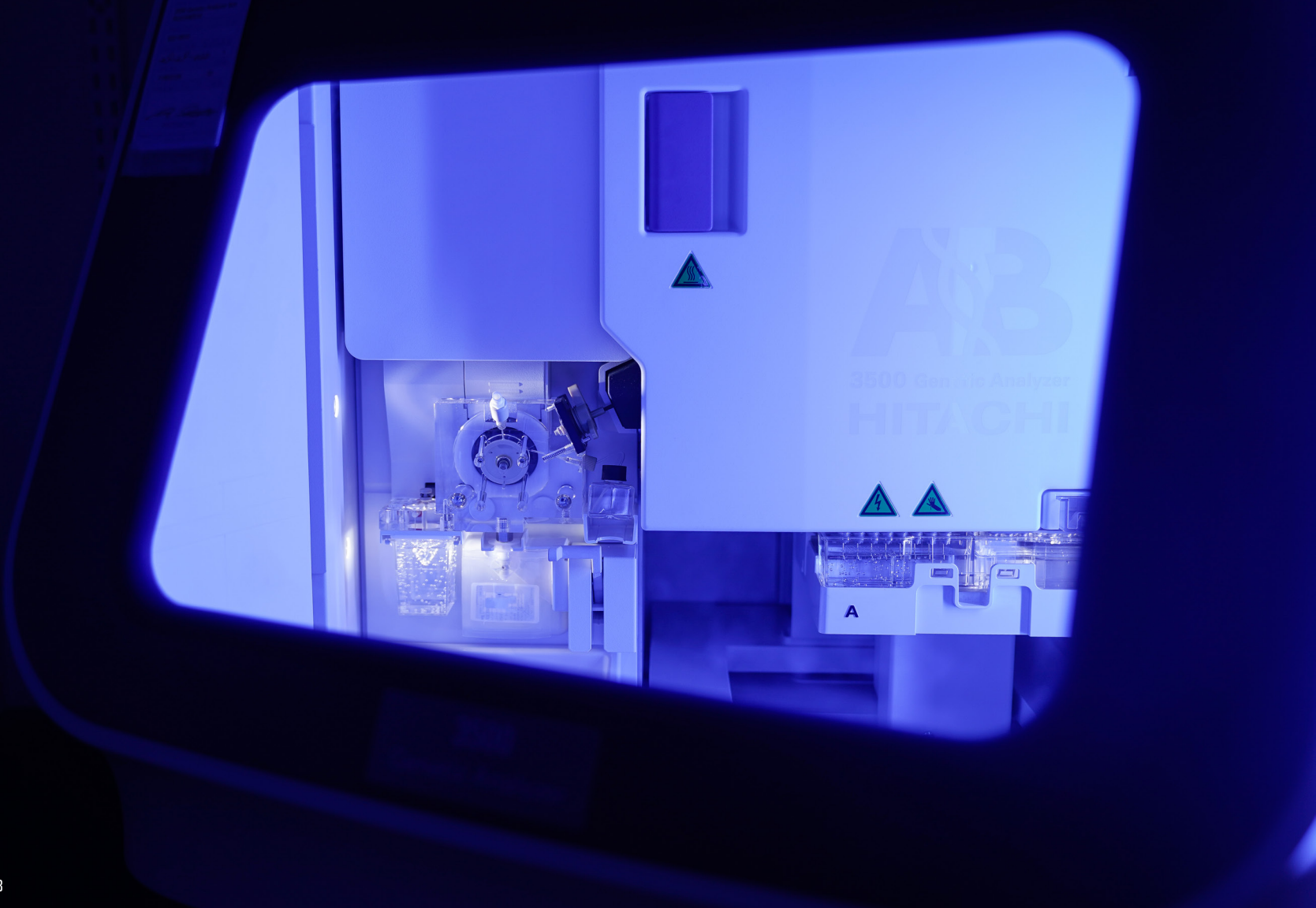
- The Roswell Park/UB shRNA Resource can help with all your retro- and lentivirus packaging. Viral supernatants can be produced from ecotropic, amphotropic, or polytropic retro- or lentiviral DNA constructs provided by the researcher.

- **Mycoplasma detection testing:**

- PCR based Mycoplasma testing using the Agilent Mycoplasma Plus PCR Primer Set is available for monitoring of cell cultures. The presence of contaminant mycoplasma can be detected accurately and rapidly in a cost-effective manner.







AB
3500 Gas-16 Analyzer
HITACHI

A

GENOMICS

SHARED RESOURCE

GENOMICS SHARED RESOURCE

OVERVIEW

The Genomics Shared Resource (GSR) offers sample-to-data services, with an expert technical staff performing all aspects of sample preparation, QC, assay design and analysis. The resource staff has been in place since 2000, offering a combined 70+ years' experience, and offer professional, timely and high quality service. The mission of the GSR is to provide state-of-the-art instrumentation and expertise that enables its users to acquire and analyze genomic data sets across basic, translational, clinical and population studies. Our long-standing history and contribution to the Human Genome Program through clone generation, high-throughput mapping, array technology development, and distribution of these resources worldwide provides a track record documenting our expertise that can be extended to the outside community. The GSR offers investigators a full spectrum of services, including Next Generation sequencing (NGS), genotyping (targeted and global), methylation (targeted and global), copy number and expression analysis (gene and miRNA), and Sanger sequencing. The facility houses

an Illumina iScan system, Agena Biosciences MassARRAY MALDI-TOF MS, two Applied Biosystems QuantStudio 6 Real-Time PCR System, two Applied Biosystems 3500 Genetic Analyzers, Caliper Sciclone NGS workstation, Fluidigm C1 and Access Array system, 10X Genomics Chromium system and Nanostring nCounter systems, as well as Illumina (NovaSeq, NextSeq and MiSeq) sequencers. The GSR provides a full complement of NGS services, including Whole Genome Sequencing (WGS), Whole Exome-seq (WES), RNA-seq, WGBis-seq (Methyl-seq), ChIP-Seq, targeted, and small RNA-seq. Single-cell RNA-seq and DNA applications can also be performed within the core using the 10X Genomics Chromium and Fluidigm C1 and Access Array system, allowing for resolution of genomic content down to a single cell. The GSR also provides methylation analysis of intact or compromised DNA samples using Agena Biosciences EpiTYPER (targeted assays) or the Illumina Infinium MethylationEPIC BeadChip (global) as well as full-service gene expression analysis and SNP/CNV (300K-4.5M SNPs) genotyping using Illumina BeadChip technology. The

core provides many levels of customizable validation technologies to fit any sized project, including custom Illumina BeadChips and Agena Biosciences Typer targeted SNP genotyping assays, qPCR, nCounter digital detection assays, and Sanger sequencing. The GSR is also a repository for the RP11 and RP23 BAC genomic clone libraries which are available for clone selection, characterization, FISH mapping, and distribution. The GSR is a state-of-the-art facility which utilizes a variety of platforms to accommodate any size request and monitors all processes using a LIMS that includes on-line requests, sample submission, workflow and sample tracking to ensure quality reproducible data generated efficiently. At an institutional level, the GSR interacts with other shared resources such as the Biostatistics and Bioinformatics Shared Resource, Data Bank and BioRepository Resource (DBBR), the Pathology Network Shared Resource (PNSR), and the Biomedical Data Science Shared Resource (BDS) to facilitate centralized high-throughput sample preparation from archival and prospective cohorts to better manage investigators projects and research questions.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Dr. Glenn or Dr. Singh to discuss scheduling and procedures.

For quotes, pricing and inquiries, please contact Dr. Sean Glenn or call 716-845-4012.

The GSR is located in the CGP, first floor, L1-110. Hours of operation are weekdays, 8:00 AM – 5:00 PM.

Website: <http://www.roswellpark.edu/shared-resources/genomics>

LEADERS

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SERVICES

The GSR provides a full spectrum of services for parallel analyses of genomes and their expression. The Resource works with the Bioinformatics Shared Resource to assist investigators in experimental design to minimize sample use and optimize efficiency. The Resource provides full-service (DNA/RNA to data) offerings as a fee for service, as well as access to state of the art instruments. Investigators are encouraged to consult the GSR for pricing, grant documents and manuscript material.

Next generation sequencing services: The GSR provides NGS library preparation, enrichment, and sequencing services using the Illumina NovaSeq, NextSeq and MiSeq platforms. We also validate NGS findings using Agena Biosciences, Illumina, Nanostring, qPCR and Sanger sequencing platforms.

Illumina Sequencing

- RNA-seq (Whole transcriptome and mRNA-seq)
- Small RNA
- Whole Genome
- Whole Exome
- ChIP-seq
- WGBis-seq (Methyl-seq)
- Targeted MethylCapture-seq
- RRBS
- Targeted

10X Genomics Single Cell sequencing applications

- Gene Expression Profiling
- Gene Expression CRISPR Screening
- Gene & Cell Surface Protein
- Immune Profiling
- Immune Profiling & Cell Surface Protein
- Immune Profiling & Antigen Specificity
- Chromatin Accessibility (ATAC-seq)

Fluidigm C1 Single-Cell Auto prep and Access Array Systems

- Single-cell cDNA prep. (RNA-seq)

- Single-cell WGA prep. (WGS and WES)
- Targeted DNA sequencing using Access Array system

Microarray services:

The GSR offers copy number, genotyping and global methylation microarray analyses.

DNA-based arrays

- Illumina HD BeadChips - Genotyping, CNV and LOH, 300K – 4.5M SNPs
- Illumina HumanmethylationEPIC BeadChips – global methylation of 800K CpG sites

Custom services:

The GSR offers targeted, custom assays for SNP genotyping, microarrays and methylation analysis.

- Agena Biosciences Typer - Targeted SNP Analysis for genotyping 50s - 1000s of samples for 10s-300s of SNPs.
- Agena Biosciences EpiTyper – Targeted Methylation Analysis for assaying 10s - 100s of samples for 10s-100s of CpG islands

Digital Detection Systems:

The GSR offers custom and off-the-shelf assays for quantitation of gene expression and DNA variants.

- NanoString nCounter – Digital detection and counting of hundreds of unique mRNAs, microRNAs, or DNA targets simultaneously in a single reaction. FFPE sample compatible.

Real time qPCR services:

The GSR offers real time qualitative PCR services for miRNA expression, gene expression, copy number, and SNP genotyping using the ABI Quant 6 real time PCR system.

- TaqMan assays - gene expression, SNP genotyping and miRNA expression assays from tumor biopsies, xenografts, and cell cultures using real time quantitative RT-PCR and end-point assays. Full service or ABI 7900HT usage available.
- SYBR green qPCR –gene expression and NGS library preparation quantitation.

Nucleic acid extraction and QC services:

The GSR prepares RNA and DNA from a variety of sample types, using several extraction protocols depending on the sample size and downstream application.

- Manual Extractions
- DNA (cells, tissues, FFPE): Genra Puregene, Qiagen DNeasy
- RNA (cells, tissues, FFPE): TRIzol, Qiagen RNeasy, PAXgene
- miRNA enriched: Qiagen miRNeasy
- Nucleic Acid Quality control
- NanoDrop: A260, A230, A280
- Fluorescent plate reader: picogreen, ribogreen quantitations
- Qubit
- Bioanalyzer 2100: RNA and DNA chips

Image and basic data analysis:

The GSR provides basic image analysis using platform-dependent software (GenomeStudio - Illumina, RQ Manager-ABI, MiSeq Reporter-Illumina).

Cell Line Authentication:

The GSR provides human cell line authentication using ThermoFisher Scientific AmpFLSTR® Identifier® Plus PCR Amplification Kit. Samples are run on Applied Biosystems 3500 Genetic Analyzer and analyzed with Applied Biosystems GeneMapper® v1.2 software.

INSTRUMENTATION**Next Generation Sequencing**

- Illumina NovaSeq 6000 (1)
- Illumina NextSeq 500 (1)
- Illumina MiSeq (1)
- Caliper Sciclone NGS workstation (1)

Single Cell and Circulating tumor cells:

- 10X Genomics Chromium System (1)
- Fluidigm C1 and Access Array System (1)
- Cynvenio Liquid Biopsy System (1)

Microarray

- Illumina iScan with Autoloader (1)

NanoString

- Nanostring nCounter (1)
- NanoString prep station (2)

Real Time PCR:

- ABI Quant 6 (2)
- Roche Lightcycler480 (1)

Nucleic acid extraction and QC:

- Next Advance Bullet Blender
- Agilent Bioanalyzer 2100 (3)
- Agilent TapeStation 4200 (1)
- Agilent Bioanalyzer chip vortexer (3)
- Life Technologies Qubit (2)
- GeminiXPS fluorimetric microplate reader (1)
- NanoDrop Spectrophotometer (1)

Sanger Sequencing:

- ABI 3500 Genetic analyzer (2)

Agena Bioscience:

- Agena Biosciences MassARRAY Compact (1)
- Agena Massarray Nanodispenser (1)

Thermocyclers:

- MJ Research Tetrad Thermocycler (1)
- BioRad C1000 Touch 96 well Thermocycler (6)
- BioRad C1000 Touch 384 well Thermocycler (3)
- ABI Veriti 96 well Thermocycler (3)

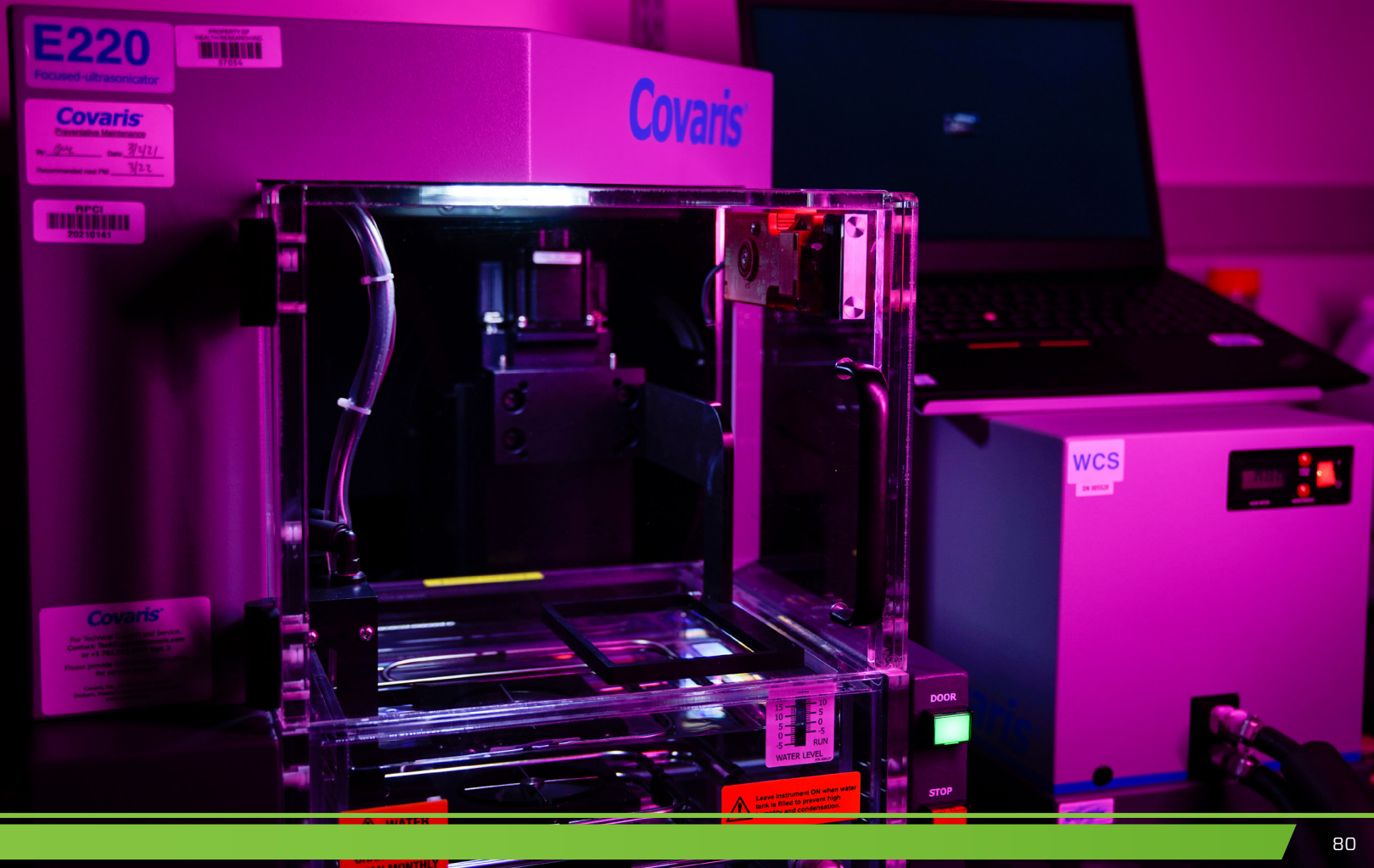
Centrifuges:

- Eppendorf 5415D centrifuge (1)
- Eppendorf 5415D microcentrifuge (4)
- Eppendorf 5810 centrifuge (1)
- Eppendorf Centrifuge 5810R (1)
- Eppendorf microcentrifuge 5415C (1)
- Legend RT tabletop centrifuge (2)
- Legend XTR tabletop Centrifuge (refrigerated) (1)
- Sorvall RT 6000D table top refrigerated centrifuge (1)

Other Equipment:

- Evos FL Auto 2 microscope
- Sage Pippet Prep (1)
- Agilent Rotisserie Hyb Oven
- Airclean PCR workstation

- Fisher Scientific Isotemp Oven
- Hybaid dual hyb oven
- IKA Orbital shaker
- Illumina Hyb Oven (2)
- Illumina Microplate Shaker (3)
- -80C Kelvinator large upright freezers (14)
- Laminar Flow Hoods (1)
- Eppendorf Vacufuge Plus SpeedVac (1)
- Savant SpeedVac (2)





HEALTH
COMMUNICATIONS
SHARED RESOURCE

HEALTH COMMUNICATIONS SHARED RESOURCE

OVERVIEW

The Health Communications Shared Resource, is a full service video and media production center, specializing in health communications. HCR helps investigators, institutions, community health advocates, and NGO's educate the public about important health-related matters, and promotion of health services. One area of focus is on understanding the many determinants that influence health behaviors, in order to reduce the burden of illness, and create effective, salient, and emotive media.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Paul Hage to discuss scheduling and procedures.

For quotes, pricing and inquiries, please contact Paul at 716-845-4778

The HCR Media Studio is located in the C&V Building, third floor, CV332.

Website: <https://vimeo.com/channels/healthcommunications>

LEADERSHIP

Paul J. Hage, MFA

Director, Health Communications Shared Resource

Department of Health Behavior C&V 332

Ph: 716-845-4012

paul.hage@roswellpark.org

SERVICES

We can create research/study highlight reels, facility overview videos, cancer prevention and public outreach campaigns, full documentary films, health conference media, and other health science related digital media. Being a non-profit organization, we are able to provide exceptional, professional media services that can accommodate most budgets.

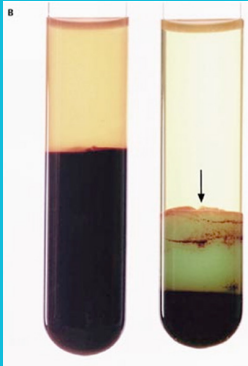
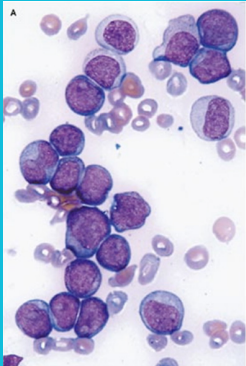
- **Pre-Production**
 - Media campaign planning and assistance
 - Housed in Roswell Park Comprehensive Cancer Center
 - Subject matter expertise in tobacco and cancer related topics
 - Scriptwriting or assistance
 - Storyboarding

- **Production**
 - 4K UHD Video Production
 - Can coordinate production crew anywhere in the world.
 - Conduct interviews, and record narration.
 - TV Studio-staging area for production
 - Green screen, multiple backdrops

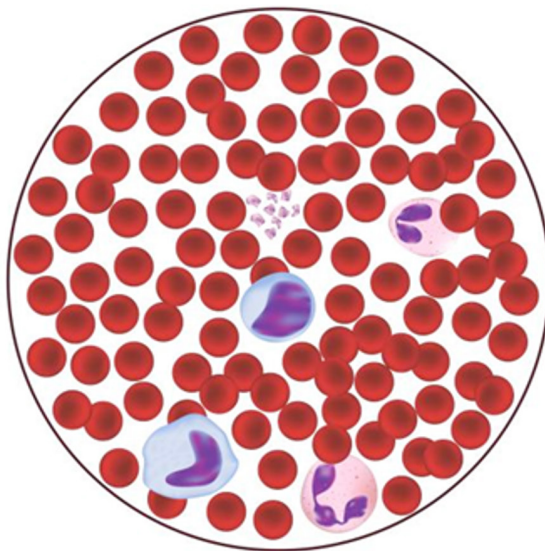
- **Post-Production**
 - Editing: Adobe Premiere Pro, Apple Final Cut Pro
 - Full Audio and Narration recording, Pro-Tools, Apple Logic Pro
 - Custom Music Composition and Arrangement
 - Digital Social Media assistance with multiple file conversion and uploading.

NOTABLE PROJECTS

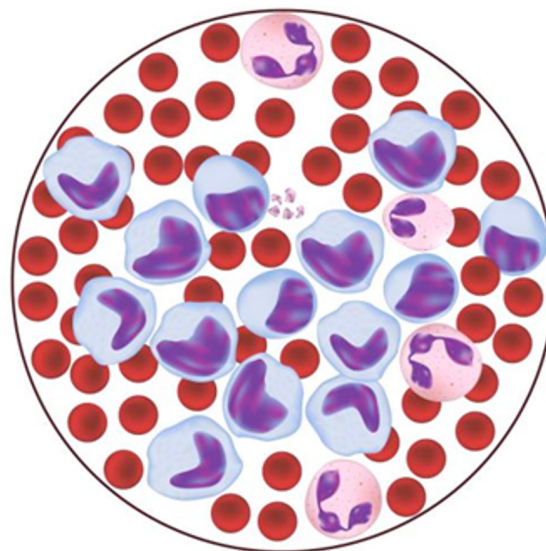
HCR has created videos utilized by the World Health Organization , World Cardiology Congress, International Tobacco Control Project, Global Smokefree Partnership, NCI SPRINT Initiative, NY State Department of Health, NY State Smokefree Coalitions, Roswell Park Cessation Services, NY State Smokers Quitline, Flight Attendant Medical Research Foundation, and Americans for Non-Smokers Rights Foundation.



Normal Blood



Leukemia



WISGEEK

HEMATOLOGICAL
PROCUREMENT

SHARED RESOURCE

HEMATOLOGICAL PROCUREMENT SHARED RESOURCE

OVERVIEW

The Roswell Park Hematologic Procurement Shared Resource (HPSR) serves two key functions at our institute: (1) procurement of hematologic samples for future research projects and (2) processing of correlative hematology samples from patients with any cancer enrolled on therapeutic cancer clinical trials.

In its first role, the HPSR successfully facilitates translation of basic research in hematological cancers to the clinical setting by providing basic researchers and clinical investigators with appropriately procured and cryopreserved samples of bone marrow and peripheral blood. This includes mononuclear cells, serum, and plasma samples from individual patients treated at our institute with hematological and other cancers. Currently the procurement bank contains a total of over 100,000 cryopreserved samples dating from the present back to 1991 and linked to a clinical database. On an annual basis, the resource procures over 5000 samples from patients with myeloid malignancies, acute and chronic leukemias, lymphoproliferative diseases, multiple myeloma, and other hematological

disorders. Prior academic and grant funded research collaborations have included projects with the Dana Farber Cancer Institute, Washington University, and University of Chicago.

In addition, the Hematologic Shared Resource staff also promotes cutting edge innovative clinical and translational research by providing around the clock (24/7) collection of samples from patients with any type of cancer enrolled on clinical trials at Roswell Park Comprehensive Cancer Center. A technician for sample processing is available nights, weekends and holidays on call to process patient samples.

We work closely with the Roswell Park Clinical Research Service (CRS) and pharmaceutical sponsors to accurately process peripheral blood, serum, and plasma samples based on individual protocol specifications from individuals with all cancer types receiving experimental therapy on clinical trials at our center. These correlative pharmacodynamic and pharmacokinetic samples are typically batch stored and shipped both to individual investigators and central laboratories on wet or dry ice for further analysis. The availability of technical staff to receive, process, and store peripheral blood

and other samples from patients on a 24/7 basis (including after hours and on weekends) is essential to the institute's commitment both to investigator initiated clinical research as well as our early phase clinical trials requiring intensive pharmacokinetic testing. The ability of laboratory staff to custom process and store samples ensures these valuable samples are not missed and ensures that patients can receive therapy in a timely fashion any day of the week. As an example, In calendar year 2017, the HPSR processed correlative samples for patients enrolled on at total of 57 clinical trials, including 20 investigator initiated and 37 pharmaceutical sponsored studies at Roswell Park Comprehensive Cancer Center. Pharmaceutical sponsors have included Abbvie, Amgen, Daiichi Sankyo, Astellas, Pfizer, and many other large and small companies involved in phase 1 through 3 cancer studies.

Current laboratory personnel include a physician-scientist facility director, three full-time laboratory technicians, and two clinical database managers (one full-time, one part-time). The square footage of the procurement lab where primary patient samples are being processed encompasses

approximately 500 square feet. The core laboratory contains fourteen large LN2 freezers, four -80° stand up freezers, one -20° stand up freezer, and one fridge/freezer. All freezers are

on the Rees system. The core also has two 6-foot hoods, two Coulter counters, three refrigerated centrifuges, and one non-refrigerated centrifuge. The core also has a water bath and micro-

centrifuge as well as desk space, computers, and monitors for all staff members. De-identified sample information is entered into an institute-wide LIMS system.

USING THE RESOURCE

Individuals interested in the use of the facility should first contact Linda Lutgen-Dunckley to discuss scheduling, procedures, pricing and other inquiries.

Discussion of research proposals including requests for letters of support for grant applications should be directed to Dr. Eunice Wang (Director).

Website: <https://www.roswellpark.org/shared-resources/pathology-network/services-and-fees/hematologic-procurement-bank>

LEADERS

Eunice S. Wang MD

Director, Chief, Leukemia Service Professor of Oncology
Department of Medicine, Clinical Sciences Center Rm 844
Ph: 716-845-3544
Eunice.wang@roswellpark.org

Linda Lutgen-Dunckley

Lab Manager
Department of Medicine, (GBSB) S-524
Ph: 716-845-8098
Linda.LutgenDunckley@roswellpark.org

SERVICES

Banked Samples

The main focus of this shared resource is to collect blood and bone marrow samples on patients at time of diagnosis of hematologic malignancies ideally prior to any therapeutic intervention as well as throughout the entire course of their disease. Patients must provide informed consent on an IRB approved non-interventional clinical protocol. All samples are collected at the same time as other existing clinical laboratory orders and procedures.

In general, samples from normal donors, patients with hematological malignancies and other malignancies, as well as other sources are processed by density ficoll gradient centrifugation for isolation of viable peripheral blood and/or bone marrow mononuclear cells. Peripheral blood samples are also centrifuged for collection of plasma and serum samples. Viable cells can also be processed for RNA and DNA extraction as requested by individual investigators. Additional samples processing for research purposes may also be possible following discussion with resource staff.

Study Specific Procurement

If a project can be accommodated within standard banking operations, the HPSR will prospectively collect samples at single or multiple time points for a limited amount of time. These samples can be prospectively banked under our IRB consent and obtained for future research following approval of study-specific biological data review and/or IRB protocols. The HPSR is more than happy to provide letters of support and can perform feasibility studies as needed in support of current and/or pending grant proposals after discussion with the HPSR director and laboratory manager and appropriate budgeting for services.

Clinical Data

We maintain an extensive clinical database linked to stored HPSR samples for certain hematological malignancies. Currently the HPSR employs a full-time dedicated clinical data manager who is tasked with abstraction of clinically relevant diagnostic, treatment, and pathological information from patients' medical records after informed consent. All samples are de-identified at the time of processing. Clinical data are handled by a dedicated data manager who functions in an honest broker capacity for all requests. Relevant information may include, but is not limited to, demographics such as age, sex, race and ethnicity, prior history of cancer, treatment history, comorbidities, medications, cytogenetics and molecular diagnostic findings. Other relevant patient data as designated by the Investigator can be obtained upon request and discussion with resource staff with appropriate resource allocation. Researchers are responsible for procuring and providing appropriate IRB-approved protocols in order for HPSR staff to release specimens linked to clinical data. Assistance with this process and discussion of construction of custom data sets can be provided upon request. All final data sets are distributed to Investigators de-identified.

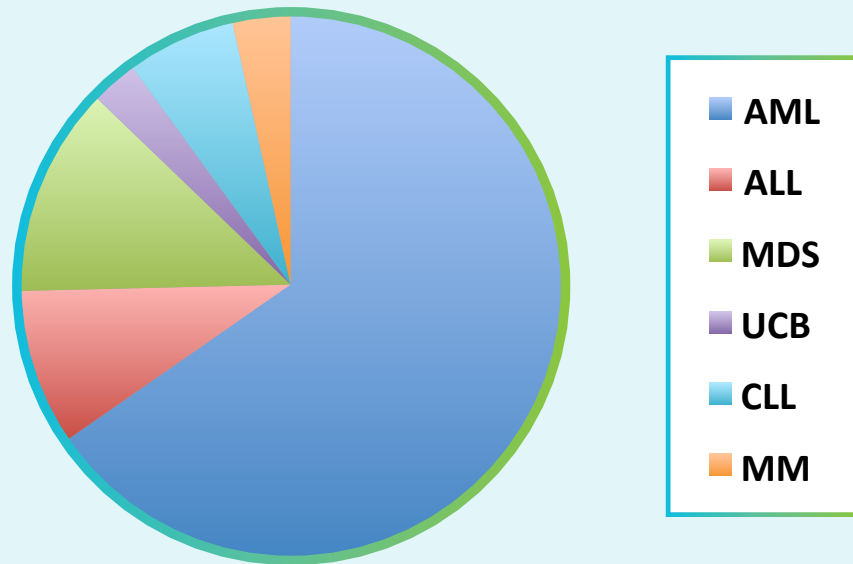
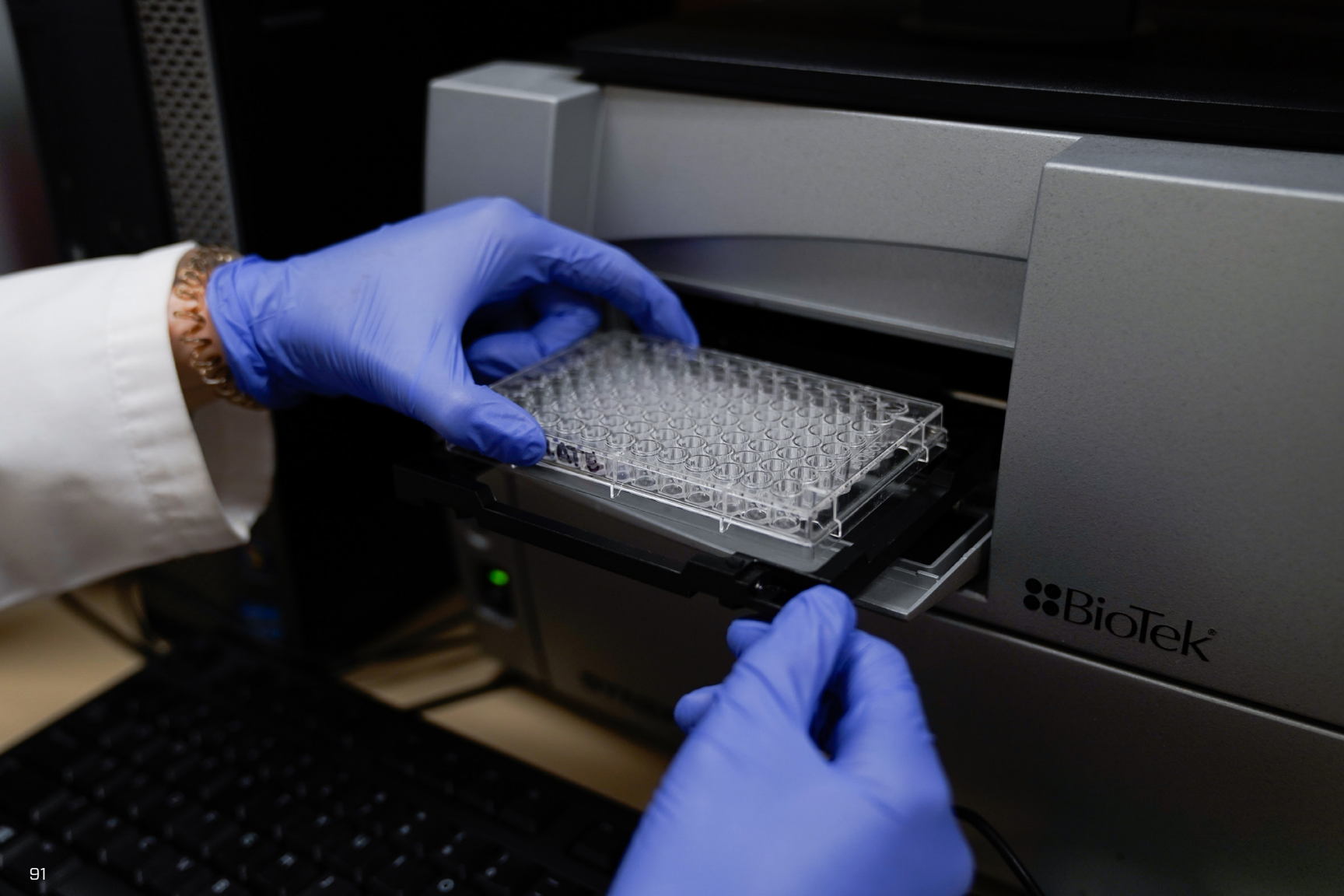


FIGURE: DIAGNOSES OF >100,000 PATIENT SAMPLES CURRENTLY STORED AT THE ROSWELL HPSR



IMMUNE ANALYSIS
SHARED RESOURCE

IMMUNE ANALYSIS SHARED RESOURCE

OVERVIEW

The Immune Analysis Shared Resource (IASR), part of the Center for Immunotherapy at Roswell Park Comprehensive Cancer Center, is a shared resource responsible for serial monitoring of immunologic functions in patients with cancer, those who are treated with biologic therapies, and those who participate in clinical trials or research protocols at Roswell Park and outside institutions/hospitals. The development of immune monitoring assays is essential to determine the immune responses in patients receiving novel immunotherapies and ultimately transitioning these therapies from the clinical trial phase to standard of care. The IASR makes available to its users a broad range of state-of-the-art immunologic assays, performed under a rigorous quality control program. In addition, as advances in immunobiology occur and new assays are requested by the users, the IASR performs pre-clinical evaluations of the assays and, when they become reliable and standardized, adds them to the available assay list. The ultimate goal of the facility is to continuously develop cutting-edge immunomonitoring technology.

The IASR offers consultation regarding optimal immunologic assessment and assay development for innovative approaches to evaluate immune

responses. We provide expert advice regarding the types of assays for immunomonitoring and data interpretation tailored to fit the endpoints of each specific clinical trial.

The IASR maintains extensive quality control (QC) and quality assurance (QA) programs to ensure the validity of test results. We participate in a worldwide immune monitoring consortium to standardize and validate immune assays and establish rigorous quality control standards. The IASR follows a fee-for-service schedule which gives priority to Roswell Park members. Interested investigators are asked to provide a summary of their clinical trial design, which will be reviewed by the IASR in order to design the appropriate methodology to successfully measure the endpoints of the study.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Dr. Matsuzaki to discuss scheduling and procedures.

The IASR is located in the Cancer Cell Center, 4th Floor, Room 416. Hours of operation are weekdays, 8:30 AM - 5:00 PM.

Website: <https://www.roswellpark.org/shared-resources/immune-analysis>

CONTACT FOR SCHEDULING

General IASR operation:

Courtney Ryan, MS, Research Associate
courtney.ryan@roswellpark.org

Seahorse:

Jessie Chiello, BS, Research Technologist
jessie.chiello@roswellpark.org

CyTOF:

Yuwen Zhang, PhD, HRI Scientist
yuwen.zhang@roswellpark.org

LEADERSHIP

Pawel Kalinski, MD, PhD

Director

Center for Genetics and Pharmacology

Ph: 716-845-7629

pawel.kalinski@roswellpark.org

SERVICES

- Separation and storage of human cells from blood and tumor tissues
- Measurement of soluble factors by ELISA, ELLA or MAGPIX
- Assessment of antigen-specific T cell frequency and reactivity by ELISPOT assay, tetramer staining or intracellular cytokine staining
- Live cell metabolic assays using Seahorse XF analyzer
- Phenotypic and functional analysis of cells by Flow Cytometry or Mass Cytometry
- Multi-spectra tissue analysis using Hyperion Imaging Mass Cytometry (under development)

Separation and Storage of human cells:

We separate, aliquot and cryopreserve peripheral blood mononuclear cells (PBMC) and serum/plasma from whole blood by Ficoll gradient centrifugation. Serum free cryopreservation medium is suitable for determining antigen-specific response following freeze/thaw and for long-term storage in liquid nitrogen freezer. Separation of mononuclear cells from any specimens, e.g. fluid, lymph node or spleen can be performed. Single cell suspension from solid tumor tissue is prepared by mechanical dissociation with and without enzyme digestion.

Enzyme-linked ImmunoSorbent Assay (ELISA):

This assay is used to measure the antibody titer specific to cancer antigens. Serially diluted serum or plasma from cancer patients are added to an ELISA plate coated with recombinant protein antigens. The antibodies bound to proteins are detected by utilizing enzyme-labeled anti-human IgG-specific antibody and fluorescent substrate. In addition to measurement of autoantibodies, commercially available kits can be used for the detection of cytokine and chemokine levels in serum, plasma, fluid or tissue culture supernatants.

ELLA:

The Ella is a next generation hands-free system for high-performance immunoassays, allowing researchers to acquire highly reproducible validated assay data with very few manual steps. This is the only self-contained testing platform for the simultaneous execution of multiple ELISA-based immunoassays. The assay volume required is 25 μ l or less for up to 4-plex customized assays and the data is ready in 2 hours from sample prep.

MAGPIX:

MAGPIX system is a new Luminex system, which is an affordable, compact and cost-effective fluorescent detection system suitable for medium-throughput multiplex immunoassays. The system immobilizes beads with magnetic force, excites them using light-emitting diodes (LEDs), and detects beads and analytes using a CCD camera. The system can read up to 50 unique magnetic bead regions simultaneously in each well of a 96-well plate.

Enzyme-Linked Immunospot Assay (ELISPOT):

This assay is used to detect and quantify the number of T cells that secrete a particular cytokine (e.g. Interferon- γ) upon recognition of a specific antigen.

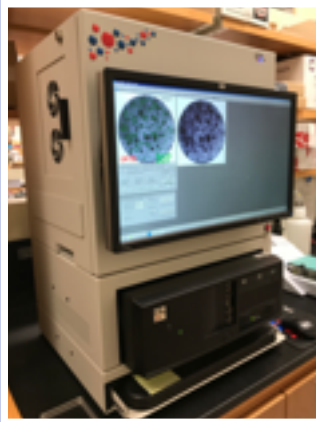
T cells are cultured with antigen-presenting cells in wells coated with an antibody recognizing a particular cytokine. The coating antibody captures the secreted cytokine and a second cytokine-specific antibody is coupled to a chromogenic substrate and used for detection. Results appear as spots, with each spot corresponding to one cytokine-secreting cell. The number of spots equals the number of cytokine-secreting cells for a specific antigen. However this assay does not determine the amount of cytokine secreted (ELISA/Ella described above can measure this). This assay is highly sensitive (1 cell in 100,000) and can be performed directly ex-vivo using relatively few T cells.

Peptide/MHC Tetramer Staining:

Tetramers of peptide/MHC complexes tagged with fluorescent dyes are used as TCR ligands to identify T cells with particular antigenic specificity. This permits detection of individual T cells by flow cytometry. T cell phenotype may also be determined by co-tagging the T cell with tetramer.

Intracellular Cytokine Staining (ICS):

This assay is used to detect T cell response to specific antigenic stimulation. Stimulation can be performed with autologous or HLA-matched EBV-transformed B cells, PHA-blasted T cells, dendritic cells, or tumor cells. Cells are treated with an inhibitor of the secretory pathway permitting accumulation of cytokines inside the cytoplasm. Cytokine production is determined by staining the fixed and permeabilized cells. By combining ICS with staining for phenotypic markers and tetramers, it is possible to determine the type of cells that produce the cytokine as well as the percentage of cytokine producing cells. This assay requires larger sample volumes than the ELISPOT. However, it permits the simultaneous determination of the phenotype of the cytokine-secreting cell as well as the identification of the type of cytokine being produced.



Metabolic assays using Seahorse XF analyzer:

Seahorse XFe96 extracellular flux analyzer tracks and records the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) that reflects mitochondrial respiration and glycolysis in real-time in 96-well plate format. Validated assays include the Glycolysis Stress test, Cell Mito Stress test, Mito Fuel Flex test, Glycolytic Rate assay, ATP Rate assay, Cell Energy Phenotype test and Fatty Acid Oxidation measurement, and custom-made assays are also available.

Mass Cytometry:

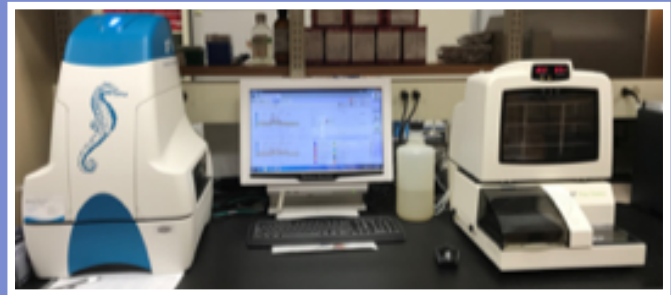
Helios/CyTOF, a time-of-flight mass cytometry is capable of acquiring single cell suspension labeled with transition metal isotopes-conjugated monoclonal antibodies. The main advantages of mass cytometry over conventional fluorescence-based flow cytometry are minimal spectral overlap of each parameter, thereby, the CyTOF can theoretically detect up to 135 isotopes without compensation. In addition, since the metal isotopes used for conjugation are rare in biological samples and the environment, it can detect the specific signal of each protein on individual cells in the absence of autofluorescence. Currently this system allows single cell analysis with up to 37 parameters for phenotype and function.

Imaging Mass Cytometry:

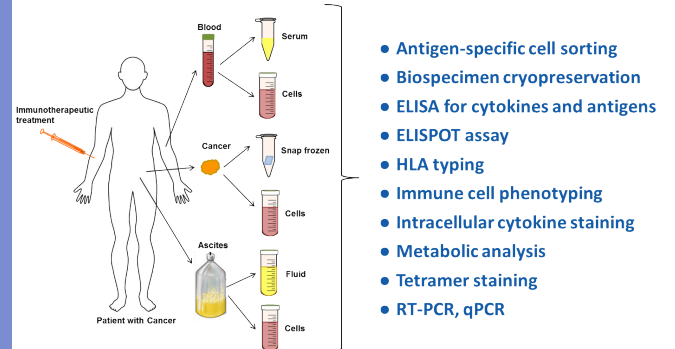
Hyperion Image Mass Cytometer (IMC) is a high-resolution laser ablation system for 1 m cellular resolution, allowing the most comprehensive analysis of the immune and non-immune contexture in the tumor microenvironment. The IMC module uses the CyTOF instrument to detect up to 40 markers simultaneously on a tissue slide and far exceeds the capabilities of any multi-spectral IHC system that currently exists, by measuring the abundance of metal isotopes tagged to antibodies. Sixty-eight antibodies for the IMC are now commercially available for immunocytometry and immunohistochemistry analyses. Staining tissue with antibodies will be conducted at Pathology Network Shared Resource or researcher's lab.

Mass Cytometry:

- Seahorse XFe96 Extracellular Flux Analyzer
- Helios, CyTOF system
- Hyperion, Imaging Mass Cytometer
- CTL Core 6 ELISPOT plate reader
- Bio-Tek Cytation 5
- Bio-Tek Synergy HT microplate reader
- ELLA, automated ELISA system
- MAGPIX, luminex system



state-of-the-art immune monitoring for clinical trials





INVESTIGATIONAL
DRUG SERVICES

SHARED RESOURCE

INVESTIGATIONAL DRUG SERVICES SHARED RESOURCE

OVERVIEW

The Investigational Drug Service (IDS) plays a critical role in Roswell Park research. IDS staff members are responsible for all aspects of investigational drug management, including accountability, ordering, receiving, destruction, returns, proper storage and dispensing. IDS pharmacists provide medication counseling for patients enrolled in clinical research studies. They also provide medication reconciliation for patients in screening for a research study, and this is documented in the electronic medical record (EMR). The number and complexity of research studies, especially Phase I studies, were the driving forces behind the creation of IDS by the Department of Pharmacy and the Clinical Protocol and Data Management (CPDM) office.

Responsibilities of IDS staff include study review for SRC and IRB submission, amendment review, review of amended investigator brochures, study implementation, dispensing and sterile products preparation, and clinical services such as medication review and patient counseling. An IDS staff member is also involved with implementation of Investigator-Initiated studies in the Roswell Park Clinical Research Network. IDS staff members provide expert consultation for each clinical research study utilizing pharmaceutical products.

USING THE RESOURCE

Investigators should contact Barbara Todaro for details. Utilization of IDS resources is prioritized as follows: First priority for use is given to peer-review-funded Roswell Park CCSG members; second priority to non-peer-review-funded CCSG members; third priority to non-members and academic collaborators; and last priority to external users. The Investigational Drug Services Shared Resource is located in the Clinical Research Center, 7 North. Hours of operations are weekdays, 7:30 AM – 4:30 PM.

Website: <http://www.roswellpark.edu/shared-resources/investigational-drug-service>

LEADERSHIP

Barbara Todaro, PharmD

Director

North Building, K-109B

Ph: 716-845-8676

barbara.todaro@roswellpark.org

SERVICES

• Pre-Production

- Medication reconciliation for study eligibility
- Medication review for possible interactions with study drug
- Patient counseling
- Inventory control and maintenance
- Review of proposed investigator initiated study prior to submission to ensure medication section is appropriate for implementation





LABORATORY ANIMAL
SHARED RESOURCE
SHARED RESOURCE

LABORATORY ANIMAL SHARED RESOURCE SHARED RESOURCE

OVERVIEW

The mission of the Laboratory Animal Shared Resource (LASR) is to promote the humane care and use of laboratory animals in support of basic and translational research conducted at Roswell Park. The facilities and veterinary program are both accredited by AAALAC International demonstrating the commitment to humane and responsible animal research and dedication to good science. The Laboratory Animal Shared Resource (LASR) is designed to offer the highest standards of animal care, with an expert technical staff performing all aspects of daily health observations, veterinary medical care, facility and equipment maintenance and surgical and technical services. The resource staff includes specialized veterinarians, licensed veterinary technicians (serving in different capacities within the resources) and a dedicated group of staff (many certified by the American Association of Laboratory Animal Science AALAS) that take pride in offering professional, timely and high quality service to support the research projects of cancer center members.

USING THE RESOURCE

Services by LASR to investigators are prioritized in the following order: 1) CCSG members with peer-reviewed funding; 2) CCSG members with non-peer-reviewed funding; 3) Non-CCSG members; 4) External academic collaborators; and 5) External industry collaborators. Principal Investigators are encouraged to contact Dr. Sexton or Ms. Spierto for operational information and are advised on the process to use the LASR. The submission of a protocol to the Institute Animal Care and Use Committee (IACUC) is the first step in the process followed by staff training and assistance with the animal procedures following standard barrier guidelines to ensure the quality of the research. Purchase requisitions for mice from approved commercial vendors should be submitted to LASR as well as a Request for Use (RFU) form. To obtain mice from LASR mouse production colonies, a Service Requisition (F-50) form should be submitted to LASR with all pertinent order information including an RFU number. Prior to initiating a lab animal research project, investigators are encouraged to discuss plans with LASR staff so that an appropriate animal care program, including suitable health surveillance, can be planned.

LEADERSHIP

The daily operations of the LASR are managed by a team lead by the Director, Dr. Sandra Sexton, who is responsible for the veterinary program and overall compliance of the resource. She is supported in management of the administrative matters by the LASR Administrator, Carol Spierto, in the veterinary program, by the Clinical Veterinarian Dr. Leslie Curtin and with facilities environmental controls and personnel by Mr. Justin Hartley, LASR Manager.

Sandra Sexton DVM, DACLAM

LASR Facility Director/ Attending Veterinarian

MRC Building, M269

Ph: 716-845-4463

sandra.sexton@roswellpark.org

Carol J. Spierto, MBA

LASR Administrator

Medical Research Complex (MRC), M261

Ph: 716-845-3160

carol.spierto@roswellpark.org

Leslie Curtin, DVM DACLAM

LASR Clinical Veterinarian

Medical Research Complex (MRC), M256

Ph: 716-845-7621

leslie.curtin@roswellpark.org

Justin Hartley, LVT

LASR Facility Manager

MRC Building, M254

Ph: 716-845-5732

justin.hartley@roswellpark.org

SERVICES

The LASR offices are located in the Medical Research Complex, M260. The laboratory animal housing facilities are located in the Medical Research Complex (MRC) and Cancer Cell Center (CCC) buildings.

Hours of operation are weekdays 7:30 AM – 4:00 PM.

Drs. Sexton and Curtin are on call for veterinary services during the weekend and Holidays.

Services include:

- Husbandry: Special arrangements can be made for most species, including large animals. All work involving animals must be approved by the IACUC. Care and use programs are developed to ensure that space, appropriate housing, nutrition, macro and micro environmental conditions, veterinary care procedures, decontamination and technical procedures are suited to the study. Barrier and isolation maintenance of rodents is provided for immune-deficient animals and in vivo use of Biohazardous agents.
- Veterinary and Surgical Services: Diagnostic services such as health surveillance and animals health profile, clinical pathology, necropsy and histopathology, are available to investigators. Other research support related to specialized techniques and procedures, as well as surgical techniques, anesthesia, analgesia and euthanasia are available.
- For information- Contact Dr. Sexton at ext. 4463 or Dr. Curtin at ext.7621
- Education and Training: LASR conducts an IACUC approved “Training Course in Responsible Care and Use of Animals in Research”. This course is mandatory for individuals who wish to work with animals.
- “The principles of Rodent Surgery” course is mandatory for those researchers performing surgical procedures in their laboratory rodents. Contact IACUC office at ext. 8853 for course schedule.
- Good Laboratory Practices (GLP). The recognized need to facilitate the FDA applications for test articles being developed at Roswell Park has given rise to an initiative to provide pre-clinical toxicologic testing non-GLP in the LASR and establishment of preferred provider agreements with GLP compliant providers for Roswell Park PI's.
- Animal Imports/Exports: LASR has developed specialized standard operating procedures to expedite researcher needs for animal transfer between institutions, nationally and internationally.
- Contact Dr. Sexton at ext. 4463 or Robyn Wilkins Import/Export coordinator at ext. 5914 for required procedures.
- Animal Receiving: All orders for animals from external sources must be processed through LAR. Please submit purchase requisitions to Melinda Zak (ext. 3397). Request for Use forms are to be submitted to Carol Spierito (ext. 3106) prior to submitting an animal order.
- Mouse Production: LASR technicians will manage mouse strains that cannot be purchased through commercial vendors for Principal Investigators. All Principal Investigators must have an approved protocol on file with the IACUC office justifying the requested breeding program. To set up a LASR managed mouse breeding colony contact Venessa Bazinet (ext.2368). A Request for Mouse Breeding Colony form should be on file with LASR. Please submit Request for Mouse Breeding Colony forms to Justin Hartley (ext.5732).
- Hematology Services: The Laboratory Animal Shared Resource (LASR) offers in house hematology services for laboratory animals.
 - ProCyte Dx® Hematology Analyzer - delivers an advanced five-part white blood cell differential, absolute reticulocyte count, and band neutrophil and nucleated red blood cell (nRBC) parameters.
 - Catalyst Dx® Chemistry Analyzer- Run the tests you need with complete testing flexibility. Preloaded CLIPs and 26 different tests. LAR offers renal panels, liver panels, and comprehensive panels according to the researcher's needs.

- Tissues/Blood: Various tissues, blood and serum products from a number of species of laboratory animals may be available through LAR with an approved IACUC protocol.

Instrumentation and Equipment

- State-of-the-art Rodent Micro isolation racks with individually ventilated cage system
- Reverse osmosis, computer-controlled, self-flushing, water distribution system
- 75 biosafety cabinets (BSCs) Class II Type II
- Isolation Cubicles
- The LASR houses an irradiation suite with Gamma and X-Ray irradiators.
- A state of the art necropsy suite with CO2 systems and downdraft dissection tables
- Two fully equipped large animal OR suites.
- Additional anesthesia system for large animals to equip one of the two operating rooms within the LASR.
- Two large Bulk autoclaves to process ~ 10,000 cages/week.
- IDEXX Hematology and Clinical Chemistry analyzers for diagnostics in multiple species.ABSL2/3 Suites
- Bio Bubble® Clean Rooms

Notable projects and capabilities

The LASR supports scientists that conduct in vivo studies in an AAALAC International accredited barrier facility. To further the success of the research programs at Roswell Park, the LASR is actively involved in refinement of procedures that usually become new methods or innovations to accomplish the goals of proposed animal studies. The LASR also actively participates in meetings focused on correlative sciences related to clinical trials and multi-PI program grants using laboratory animal models and tumor specific initiatives. International import and export of animals have broadened the scope of cancer research at Roswell Park.

At an institutional level, the LASR interacts with other shared resources that are also dedicated to providing specific services using laboratory animals including the Translational Imaging Shared Resource (TISR), the Gene Targeting and Transgenic Shared Resource (GeTT), and the Experimental Tumor Model Shared Resource (ETM). This interaction streamlines the management of the member's projects and promotes centralization of services.

LASR meet rigorous standards that demonstrate our commitment to humane and responsible animal research and our dedication to good science.



NICOTINE & TOBACCO
PRODUCT ASSESSMENT
SHARED RESOURCE

NICOTINE & TOBACCO PRODUCT ASSESSMENT **SHARED RESOURCE**

OVERVIEW

The Nicotine and Tobacco Product Assessment Shared Resource (NicoTAR) provides comprehensive testing of tobacco and alternative nicotine-containing products, including e-cigarettes and heat-not-burn products. Application examples include measuring concentrations of nicotine, as well as known carcinogens and flavorings. In addition to product testing, NicoTAR also provides analysis of biomarkers of tobacco use and exposure to secondhand and thirdhand tobacco smoke. Monitoring of indoor air pollution with tobacco smoke or e-cigarette aerosol is also provided. The NicoTAR facility is equipped with systems for controlled exposure of cell lines and small animals to tobacco smoke and e-cigarette emissions. Facility personnel also provide NicoTAR services such as user training, data acquisition, processing and interpretation.

USING THE RESOURCE

For those who wish to learn to run their own samples, training classes are available on an as-needed basis and can be arranged by contacting facility personnel (845-8603; NicoTAR@RoswellPark.org). To operate the instruments, a candidate will be instructed over several sessions by one of our operators. The successful candidate will then be allowed to operate under supervision until the operator deems him/her qualified to collect data alone.

NicoTAR director offices are located in Carlton House. Laboratory facilities are located in the Gratwick Basic Science Building.

Hours of operation are weekdays 7:00 AM-4:00 PM.

Website: <https://www.roswellpark.org/shared-resources/nicotine-tobacco-product-assessment>

LEADERS

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SERVICES

NicoTAR provides a full spectrum of services for analyses of nicotine and tobacco products.

This resource provides biomarker and tobacco product testing as a paid service, as well as access to state of the art instrumentation. Investigators are encouraged to consult NicoTAR for pricing, grant documents and manuscript material.

LC-MS/MS Assays:

NicoTAR offers analysis of biomarkers of tobacco exposure from urine, serum, plasma, and saliva samples.

- Cotinine and other nicotine metabolites
- NNAL – a tobacco-specific biomarker
- Metabolites of VOCs – biomarkers of exposure to toxic combustion byproducts
- TSNAs – tobacco-specific nitrosamines in tobacco products

GC-Q-TOF Assays:

- Identification and quantification of flavorings used in various tobacco products

GC-MS Assays:

- Propylene glycol (PG) and Vegetable Glycerin (VG) quantification
- Identification of additives used in various tobacco products

GC-NPD Assays:

- Nicotine content in tobacco products
- Nicotine yields in Mainstream (MS) and Sidestream (SS) smoke
- Nicotine in environmental samples (secondhand exposure from air and thirdhand exposure from surfaces)

Atomic Absorption Spectroscopy (AAS):

Analysis of metal content in tobacco products as well as urine for the following metals.

- Cadmium
- Chromium
- Lead
- Nickel

Analysis of combustible tobacco products, e-cigarettes, smokeless tobacco products, shisha, and Nicotine Replacement Therapy (NRT) products

- Generation of emissions using smoking machines under standardized or customized puffing testing regimens
- Measuring of cigarette filter ventilation, pressure drop and cigarette paper porosity

In vitro and In vivo Exposure Models:

- Cytotoxicity assays using air liquid interface (ALI) and established cell lines
- Inhalation toxicity of tobacco products using small animal exposure models

NicoTAR uses standardized methods (ISO, CORESTA) but we are fully prepared to adapt our routines to accommodate requests of our clients.

Instrumentation

- Waters Xevo TQ-XS Tandem Mass Spectrometer with ACQUITY UPLC I-Class Chromatography System (LC-MS/MS)
- Agilent Technologies 7890B Gas Chromatograph/ 7250 Quadrupole Time-of-Flight Mass Spectrometry (GC-QTOF)
- Agilent Technologies 7890B Gas Chromatograph/ 5977A Mass Spectrometer (GC-MS)
- Agilent Technologies 7890B Gas Chromatograph equipped with nitrogen-phosphorous detector (GC-NPD)
- Perkin-Elmer PinAAcle 900Z Graphite Furnace Atomic Absorption Spectrometer (AAS)
- Borgwaldt LX1 Single-Port Smoking Machines
- Borgwaldt S1000 Shisha Smoker
- Borgwaldt PV10 and KC-3 ventilation and pressure drop tester
- Cerulean PPM-1000 paper porosity tester
- 30-Port Automatic Cigarette and E-Cigarette Smoking Machine JB2090
- E-Cigarette Aerosol Generator ECAG





MT-10-013-CV

• DMEM

MT-10-013-CV
• DMEM-12
SOLAR MIX

MT-10-013-CV
• MEM MIX
ESSENTIAL

CORNING

CORNING

cellgro

ONSITE

SUPPLY CENTER

SHARED RESOURCE

ONSITE SUPPLY CENTER SHARED RESOURCE

OVERVIEW

The Onsite Supply Center Shared Resource at Roswell Park Comprehensive Cancer Center offers everyone the opportunity to get the best pricing and turnaround time on products required for their research. There are no shipping or dry ice charges when ordering through the Onsite Supply Center. The Supply Center Technician will track your orders upon request. There is a substantial amount of products on site, but non-stocked items may be ordered as well.

The Onsite Supply Center carries commonly used products from Bio Rad, Integrated DNA Technologies, Invitrogen, Roche, Promega, Thermo Fischer Scientific, HyClone, Gold Biotechnology, New England BioLabs, Krackeler Scientific, Sigma Aldrich, Corning, Qiagen and Tissue Culture Biologicals. Providing these supplies and reagents on site saves time, reduces shipping costs and increases production in your lab.

USING THE RESOURCE

Investigators or lab heads should contact Gina Blasko at the above numbers or e-mail her to establish a user account. Ordering procedure is easy.

Hours of operations are weekdays, 7:30 AM – 4:30 PM. Supplies from Roche and Promega may be accessed 24/7. The Onsite Supply Center is located in the Center for Genetics and Pharmacology (CGP) L1-218.

Website: <https://www.roswellpark.org/shared-resources/onsite-supply-center>

LEADERS

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CORNING



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PATHOLOGY
RESOURCE NETWORK
SHARED RESOURCE

PATHOLOGY NETWORK SHARED RESOURCE

OVERVIEW

The Pathology Network Shared Resource (PNSR) provides human specimens and laboratory services for basic and translational research to further the understanding of the cellular and molecular pathogenesis of human cancers. The overall mission of the PNSR is to facilitate access to human tissue for investigators with IRB approved protocols that have an emphasis on translational efforts.

USING THE RESOURCE

The Pathology Network Shared Resource Network is located in the Gratwick Basic Science Building, 6th floor. Hours of operation are weekdays, 7:00 AM – 4:30 PM.

Investigators interested in the use of the facility should contact Liz Brese to discuss scheduling and procedures for placing a work request. Please log into the LIMS with your regular Roswell Park network sign-on and password for online ordering: <https://rpcilims.roswellpark.org>.

Website: <http://www.roswellpark.edu/shared-resources/pathology-resource-network>

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Liz Brese

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SERVICES

APERIO SCANNING AND IMAGE ANALYSIS

Successful digital pathology depends upon the effective and timely creation of high-quality digitized glass slides. The Aperio Imaging and Analysis lab uses an Aperio® Scanscope XT slide scanner which scans conventional glass slides and creates a digital image. Aperio slide scanning permits investigators to easily access digital images of slides from anywhere using Spectrum™, a web-based digital pathology information management system. Annotation of the digital slides can be conducted via a digital platform, Aperio® ImageScope version 11.2.0.780. This allows researchers to create digital images for publication as well as facilitate inter-institutional collaboration by providing rapid and easy sharing of digital image information.

Digital slide scanning also permits automated image analysis, a service also provided by the Pathology Network Shared Resource. Aperio® algorithms can be tailored to fine tune the cellular, nuclear, and stain parameters, creating an optimized algorithm macro for each antibody target and tissue type to select the cells of interest. The original digital slide image is never modified. Rather, an annotation layer with the markup image and quantitative data is created and linked to the digital image.

BIOSPECIMENS AND TISSUE SERVICES

The PNSR functions to assist investigators with translational research by providing a variety of biospecimens.

DNA and RNA

DNA and RNA are isolated from tumor and matching non-tumor tissues in the biobank. DNA and RNA are primarily extracted from frozen tissue samples, but DNA isolation from formalin-fixed paraffin-embedded tissue is also performed upon request. All samples are QC'ed after extraction to confirm that high quality DNA

FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) TISSUE

Formalin-fixed paraffin-embedded blocks from surgical pathology tissues are archived and can be accessed for research purposes. The archive of paraffin tissues is extensive, having more than 200,000 cases that can be electronically searched and used for research purposes.

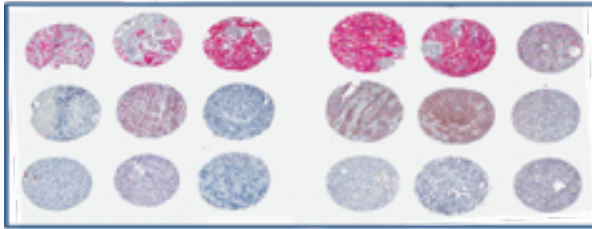
FRESH AND FROZEN TISSUE

Fresh and frozen remnant tissues are banked from surgical specimens and distributed for research purposes. Our biospecimens are procured by certified Pathologist Assistants and then stored in -80°C freezers that are protected 24/7 by state-of-the-art monitoring systems. Requests for fresh or frozen tissues are guided by the Laboratory Information Management System (LIMS) and can be integrated with other shared resource facilities (e.g. Genomics Shared Resource). The number of biospecimens available in the biobank varies by disease site and utilization, which is subject to DSRG approval and prioritization. Specific details for samples in the biobank including inventory, surgical events, tumor dimensions, and the numbers of tissues procured, banked, and distributed per disease site are available on the LIMS dashboard at <https://rpcilims.roswellpark.org/>



TISSUE MICROARRAYS

Tissue microarrays (TMA) are constructed by using a hollow needle to remove very small tissue cores from tumors of interest which are then inserted into a recipient paraffin block in an arrayed fashion. This format permits the screening of a large number of patient samples on a single slide. TMAs are an efficient and effective way to screen potential biomarkers and are typically used in conjunction with immunohistochemical staining or fluorescent in situ hybridization. The Pathology Network Shared Resource has constructed more than 125 different TMAs representing a wide variety of disease and tissue types. Currently, our TMA library contains more than 36 breast TMAs, 31 TMAs from gastrointestinal tissue, and 11 with prostate tissue. Other tissue types for which TMAs are constructed include lung, head and neck cancers, gynecological cancers, brain cancers, and melanoma.



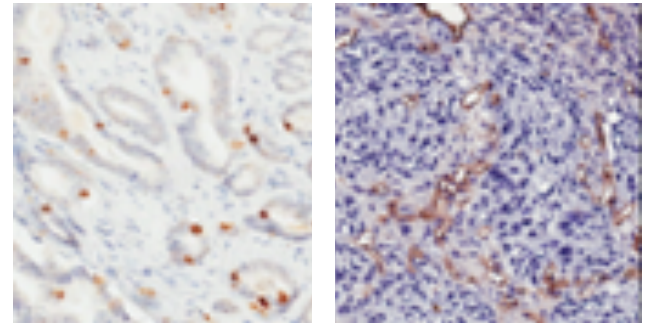
CLINICAL TRIALS PATHOLOGY OFFICE

The Correlative Science Pathology Office (CSPO) consists of a team of Laboratory professionals dedicated to managing all aspects of biospecimens related to clinical trials. CSPO coordinates tissue feasibility for clinical trials and provides services for processing, storing, and the shipment of specimens for clinical trials. All staff members are IATA certified to ship biological specimens and serve as a point of contact for addressing biospecimen related concerns.

Team members function in lab and office areas dedicated to clinical research activities. Additionally, CSPO staff facilitates specimen testing and reporting, provides budgets, and reviews studies for lab implementation for clinical trials at Roswell Park.

HISTOLOGY SERVICES

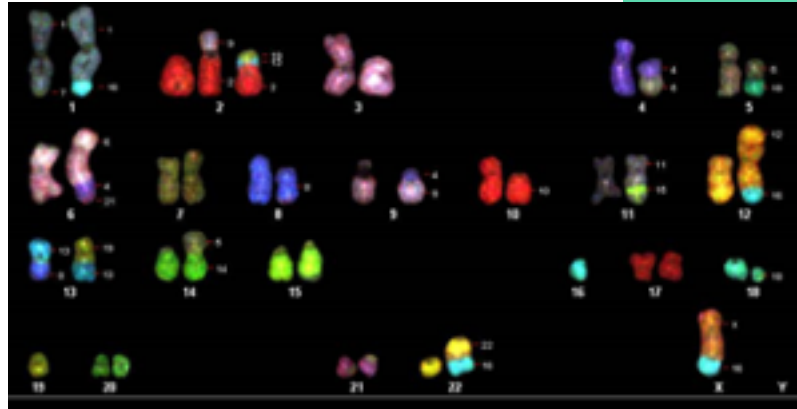
The Histology Facility is a centralized lab that provides services for standard tissue processing and embedding, cryotomy, microtomy, general histology staining, and antibody optimization and immunohistochemistry (IHC). We also perform laser microdissection and sterile needle coring to isolate DNA from tumor samples. We work closely with the Biomedical Data Science shared resource and other core labs within the Pathology Network Shared Resource to identify samples sets, and then perform the required staining for research studies that use IHC. Our lab contains two automated staining systems for consistent and high-throughput IHC staining. Antibody staining procedures and IHC slides are regularly reviewed by a pathologist for quality control. We continually work to expand our repertoire of antibody staining protocols and we currently have over 500 procedures.



SKY/ FISH SERVICES

The SKY/FISH facility provides cancer cytogenetics services. Services include conventional chromosome analysis such as G-banding and fragile site analysis as well as more specialized techniques such as Spectral karyotyping (SKY) and fluorescent in situ hybridization (FISH). These techniques are useful for identifying chromosomal abnormalities on a global scale or more specific gene level mutations in a variety of species such as human, mouse and rat.

- Spectral karyotyping (SKY) to identify chromosomes or chromosomal segments that are structurally and numerically altered in cells.
 - G-Banding analysis to identify chromosome number as well as chromosome aberrations including translocations, insertions, deletions or duplications in cells.
 - Fluorescence in-situ hybridization (FISH) analysis using up to three-fluorescent colors to detect and localize genes, gene sets, fusion genes, telomere-specific sequences, centromere-specific sequences and specific chromosomal regions.
 - Combined analysis by SKY and FISH to determine whether rearrangement, deletion or amplification occurs in genes encoding tumor suppressors, cell cycle factors, factors involved in immunological responses, DNA-damage responses, drug resistance, transgene expression, etc.
 - Quantification of gene copy number in transgene research material. This method is also useful for investigators who want to identify transgene locations.
- Chromosome aberration and fragile site analysis
 - Planning of inter-disciplinary projects utilizing SKY/FISH technology, data interpretation and technical education and guidance for standard cytogenetic protocols.





ROSTRA
DATA
SOLUTIONS

SMALL MOLECULE
SCREENING

SHARED RESOURCE

SMALL MOLECULE SCREENING SHARED RESOURCE

OVERVIEW

The Small Molecule Screening Share Resource (SMS) provides the expertise and infrastructure necessary for the screening of our libraries of small “drug-like” molecules for new research tools and prospective diagnostic and therapeutic compounds. Now researchers at Roswell Park Comprehensive Cancer Institute and other biomedical institutions in the Buffalo area can get assistance with the design and execution of chemical screenings in a variety of readout systems. Located on the Roswell Park campus, the SMS facility provides easy access to investigators during all stages of the screening project: design and adjustment of readout, high throughput screening, hit selection and confirmation, preliminary hit characterization and structure-activity relationship (SAR) analysis. The collection of chemicals at SMSSR totals more than 110,000 compounds. Sophisticated automated liquid handling equipment is used to ensure accurate delivery of this library in both the 96- and 384-well format, and our detection equipment allows for screening using either cell based or biochemical assays.

SMSSR capabilities are not only limited to screening related projects. We have significant experience with analysis of the activity of individual

bioactive compounds (IC50/CC50 experiments) and co-treatment experiments in different biological systems.

With the facility's recent addition of the BioTek Cytation 5, we have begun working with 3D cell culture models and live cell assays. This new technology allows for researchers to investigate wound healing, cell proliferation and mobility, and the levels and localization of fluorescent molecules in individual cells.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Dr. Chernov or Brian Buckley to discuss scheduling and procedures.

The SMS is located in the CGP, first floor, L1-215. Hours of operation are weekdays, 9:00 AM – 5:00 PM.

Website: <https://www.roswellpark.edu/shared-resources/small-molecule-screening-core-facility>

LEADERS

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Research Associate

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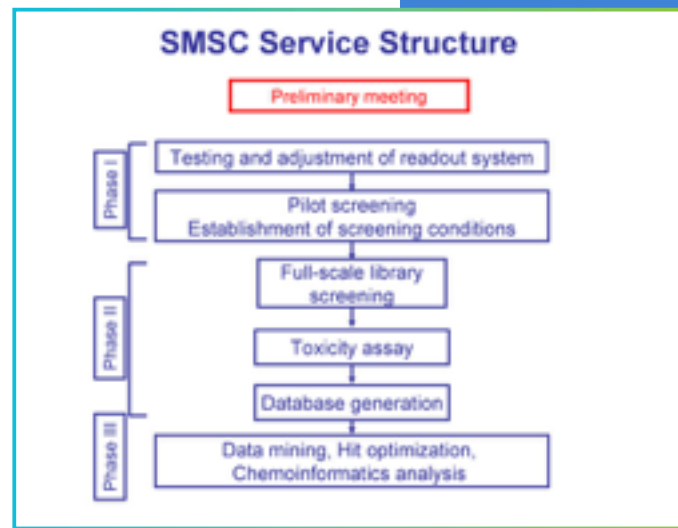
brian.buckley@roswellpark.org

SERVICES

The Service Structure flowchart illustrates the timeline of a typical chemical library screening project. It can be roughly divided into three phases: preparation, library screening, and data analysis/hit selection/pickup.

We recommend scheduling a preliminary meeting early during the planning phase of your project to discuss estimated costs, assay requirements, detection methods and controls for the high throughput format. By doing this early, you will save time and cost by avoiding unnecessary and repeated experiments later on. Core personnel will provide you general information about chemical screenings; requirements and evaluation of readouts to be used in high throughput screening and information about statistical analysis of readouts and HTS data. This information can be very helpful at the early stage of screening project planning.

Small Molecule Screening Shared Resource liquid handling and detection equipment can be used for non-screening projects by Roswell Park researchers for a minimal hourly fee. Our equipment is capable of running assays in high density format allowing investigators to minimize the use of rare or expensive reagents. Contact us for more details. Please note, that screening projects will have a priority in equipment use, so check the availability before planning your experiments.



CHEMICAL LIBRARIES AND INSTRUMENTATION

The three main components of the chemical screening facility are: chemical libraries, liquid handling equipment, and detection equipment.

Libraries

SMS owns three historical libraries created based on two different principles:

- EXPRESS-Pick™ by ChemBridge Corporation (San Diego, CA) – a diverse library of 55,230 compounds. This library consists of organic molecules with molecular weight in a range of 250 – 550, dissolved in DMSO at concentration 5mg/ml or 10-20 mM.
- HitDiscover by Maybridge (Part of Thermo Fisher) – 52,160 compounds dissolved in DMSO at 10mM concentration.
- LOPAC1280 by Sigma (1280 compounds), Spectrum by MDS, Inc (2000 compounds), Tocriscreen Total (1120 compounds), Tocriscreen Kinase Inhibitor Toolbox (80 kinase inhibitors) by Tocris Bioscience and FDA-approved Drugs Library (1508 compounds) by Selleckchem.com are the libraries of pharmacological compounds dissolved in DMSO, in 10 mM concentration (MW is < 500). These libraries include FDA and internationally approved drugs, bioactive and natural compounds with described biological activity.

Library Format

all libraries are available in both the 96- and 384-well format. Screening in the 384-well format is faster and less expensive, but some of the assays are incompatible with this high density format.

Liquid handling equipment

Equipment used for automated the delivery of liquid reagents and the transfer of compounds from the library to the assay plates in multi-well format.

Automated Liquid Handling Workstation JANUS by PerkinElmer

Using two pin-tools, library compounds can be delivered to 96- and 384-well plates in nl volumes.

Compact automated reagent dispensers

Compact automated reagent dispensers, such as MicroFill and MicroFlo by BioTek, are designed for accurate and fast distribution of solutions to 96- and 384-well plates.

Tecan D300e Digital Dispenser by Tecan

The D300 is a digital drug dispenser based on HP's ink jet technology. The D300 allows direct addition of liquids from compounds in DMSO to biomolecules in surfactant-containing aqueous solutions in picoliter-microliter range. It uses disposable dispensing chips in order to minimize dead volumes of these liquids. The D300 is perfect for setting up your PCR and qPCR reactions, generating enzyme profiles, drug combinatorial experiments, and dose response curves.

Detection equipment

Envision Excite multi label reader by PerkinElmer

This reader can be used for a wide range of fluorescence, luminescence and photometry based detection technologies:

- Fluorescence Intensity
- Fluorescence Polarization
- TRF – time-resolved fluorescence

- FRET – fluorescence resonance energy transfer
- Luminescence and enhanced luminescence
- Absorbance
- AlphaScreen

Cytation™ 5 Cell Imaging Multi-Mode Reader with automated mini incubator BioSpa manufactured by BioTek

Cytation™ 5 Cell Imaging Multi-Mode Reader manufactured in combination with the automated mini incubator BioSpa 8 by BioTek provides the unique opportunity to capture, store and analyze live cell images in bright field and fluorescence. Below are just a few examples of biological assays that can be performed using this equipment.

Label-free imaging and quantification of 3D spheroid-based tumor invasion assays

Live cell imaging of multi-parametric cell death using high contrast bright field and fluorescence imaging

Label-free imaging of 2D scratch wound healing assays

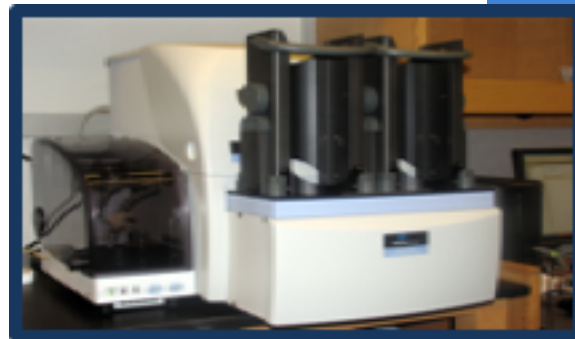
Imaging and cellular analysis of 2D and 3D T cell mediated cytotoxicity assays

Automated immunofluorescent imaging and dual-mask spot counting of γ H2AX Foci to determine DNA damage

Cell cycle analysis using DNA content and protein expression

TC complex

Dedicated tissue culture equipment simplifies the procedure of cell preparation for cell based readout systems and the plating of the cells in multiwell format.





THERAPEUTIC
CELL PRODUCTION
SHARED RESOURCE

THERAPEUTIC CELL PRODUCTION SHARED RESOURCE

OVERVIEW

The Therapeutic Cell Production Shared Resource (TCP) at the Center for Immunotherapy started with a single processing room cGMP facility in 2010. With increasing demand, the current larger 2,574 sq. ft. cGMP facility with four ISO7 processing rooms was built in 2014. With a proven track record, including active participation in internally developed, industry sponsored and collaborative group cell therapy trials, the TCP is well established to support investigators from academia and industry in the development and

execution of Phase I and II cell therapy product manufacturing. TCP recently received the FACT accreditation (Nov 5, 2018) for cellular therapy product processing with more than minimal manipulation. The mission of TCP is to provide manufacturing support for virtually any type 351 biologic and cell therapy product, where we have established a core expertise to efficiently and cost effectively translate novel and often complex laboratory processes to cGMP-compatible processes.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Dr. Chodon to discuss scheduling and procedures. The TCP cGMP facility is located in the Cancer Cell Center building, 4th floor, at the Center for Immunotherapy (CCC, Room C-404, Elm & Carlton Streets, Buffalo, NY 14263). General hours of operation are weekdays, 8:00 AM – 5:00 PM.

Website: <https://www.roswellpark.org/shared-resources/therapeutic-cell-production>

LEADERSHIP

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Joanna Stanson, MS

Operations Director,

Therapeutic Cell Production Shared Resource

SERVICES

The TCP provides a full spectrum of services to support investigators in the manufacture of clinical cellular therapy products for phase I and II clinical trials and works closely with the Transplant and Cellular Therapy (TCT) team at Roswell. Services range from protocol specific complete manufacturing of the cellular product starting from scheduling/communication with the clinical research coordinators/physicians, pick up of the apheresis product, cell purification/modification/expansion, cryopreservation or fresh, and the delivery of the cellular product to the bedside for administration, to simply shipping the starter product to the sponsor's central manufacturing site and receiving/storage of the cellular product until administration including any thawing/reconstitution/dilution required in preparation for administration (for company sponsored studies where the product is manufactured at the sponsor's central manufacturing site). TCP also provides help during clinical protocol development, CMC section for IND preparation, and validation runs.

Cell Processing-related Services

- Cell (bone marrow, mobilized and MNC apheresis products) purification/selection (ficoll, Elutra, CliniMACS, Prodigy)
- Retro- and Lenti-viral vector transduction of CD34+ cells, T cells
- Dendritic cell culture, DC-myeloma fusion cells
- Others (protocol specific per request)

Shipping/Receipt-related Services

- Media preparation (study specific) for specimen collection
- Shipment of tumor/apheresis product (domestic/international)
- Receipt/storage of cellular therapy product
- Receipt/storage of study-specific reagents

Cellular Product Administration-related Services

- Fresh product transport to bedside
- Cryopreserved product thaw/wash/dilute and transport to bedside
- Cryopreserved product, bedside thaw

Quality Control-related Services

- QC/QA/chain of custody
- In-process and release tests (in-house and outsourced, including Endotoxin, Mycoplasma, Gram stain, bacteria and fungal culture, cell identity/enumeration/transduction efficiency using flow cytometry)

INSTRUMENTATION

Each key card entry restricted Production room is equipped with the basic tools:

- 6 ft and 4 ft Class II biological safety cabinets
- Under-the-counter 4° C refrigerator and -20° C freezer
- Tabletop centrifuge(s), refrigerated
- Double stacked CO2 incubators
- Water bath, microscope, balance, Terumo sterile connecting device, tube sealer, vortex.

Specialized equipment:

- Milteyni cliniMacs
- Milteyni Prodigy
- Elutra cell separation device
- LOVO cell washer

Anteroom, Quarantine room:

- 4° C refrigerators
- -20° C freezer
- -80° C freezers

Freezer room:

- Controlled rate freezer
- LN2 freezer

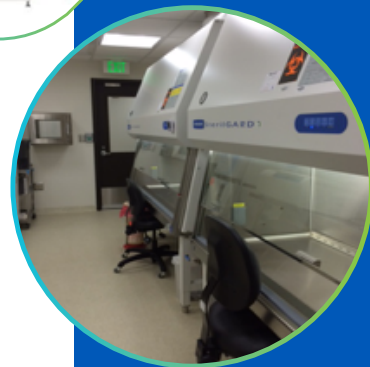
QC room:

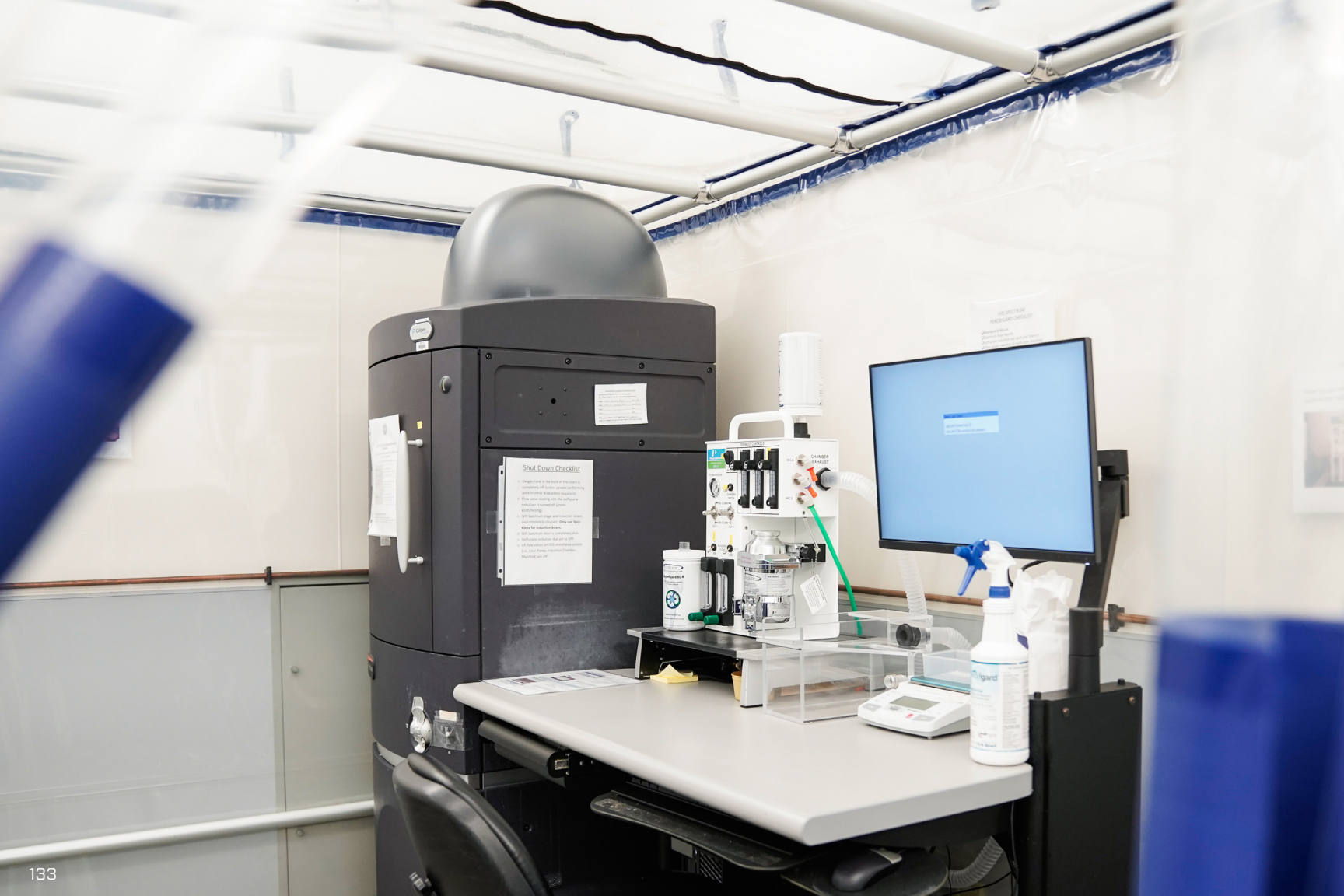
- Endosafe-PTS machine
- Mycoalert reader
- 3 ft Class II biological safety cabinet

Facility and equipment

24/7 monitoring:

- Isensix Guardian system
- Magnahelic gauges





Shut Down Checklist

- 1. Check that in the back of the chamber is completely off and the power is turned off.
- 2. Check that the door is closed and the chamber is locked.
- 3. Check that the door is closed and the chamber is locked.
- 4. Check that the door is closed and the chamber is locked.
- 5. Check that the door is closed and the chamber is locked.
- 6. Check that the door is closed and the chamber is locked.
- 7. Check that the door is closed and the chamber is locked.
- 8. Check that the door is closed and the chamber is locked.
- 9. Check that the door is closed and the chamber is locked.
- 10. Check that the door is closed and the chamber is locked.

**WEIGHTING
REQUIREMENT CHECKLIST**

Weighting & Balance
Calibration
Verification
Maintenance

TRANSLATIONAL
IMAGING

SHARED RESOURCE

TRANSLATIONAL IMAGING SHARED RESOURCE

OVERVIEW

The overall mission of the Translational Imaging Shared Resource (TISR) is to provide state-of-the-art preclinical and translational imaging services to investigators at Roswell Park Comprehensive Cancer Center in a time-efficient and cost-effective manner. The resource provides users access to advanced, non-invasive imaging modalities including magnetic resonance imaging (MRI), ultrasound (US), photoacoustic imaging (PAI), fluorescence and bioluminescence imaging (BLI). The resource is led by two PhD faculty with extensive experience in preclinical and clinical imaging, and is the only shared resource within 200 miles of Buffalo that allows for multimodal anatomic and functional imaging of small and large animal models of disease.

TISR currently supports the research of over 50 Roswell Park and University at Buffalo (UB) faculty who are conducting basic and translational investigations in oncology, radiology, tumor biology and cancer therapeutics.

The objectives of TISR are to:

AIM 1: Provide Roswell Park investigators with access to state-of-the-art in vivo imaging technologies.

AIM 2: Develop customized imaging protocols and quantitative image analysis schemes to conduct preclinical trials of experimental therapeutics in small and large animal models of cancer.

AIM 3: Establish a technology platform that facilitates clinical translation of imaging methods for improved disease detection and therapy monitoring in patients.

These objectives are accomplished through formal policies that have been established within the resource to create a structured environment that ensures prioritization of projects while providing equitable access to cancer center members. TISR staff members closely interact with investigators from the planning stages of projects to provide input into study design and provide continuous feedback during the conduct of research studies to ensure that reliable, high-quality scientific data is generated in a timely manner. The resource

provides cancer center members 24/7 access to and training in the application of non-invasive imaging technologies for their research needs.

USING THE RESOURCE

TISR directors meet with interested faculty, free of charge, to discuss project objectives. This consultation helps assure the use of appropriate imaging modalities, proper study design, and cost-effective use of resources.

The operational work flow for investigators interested in utilizing TISR services is as follows:

The investigator (or the personnel from his/her lab) directs initial inquiries to either Dr. Seshadri or Dr. Sperryak. A brief summary of the research goals of the project are provided for review. An initial meeting is held with the investigator during which the feasibility of the project and the overall research plan are discussed. The investigator is also notified of the resource service charges associated with the use of the imaging instrumentation. Once deemed feasible, a formal service request form that

includes all pertinent information (animal protocols, laboratory personnel dedicated for the project, grant/funding) is completed by the investigator. The investigator is requested to amend his/her IACUC protocol to include the resource service protocol for imaging procedures. Following IACUC/IRB approval, users are provided access (swipe card access to the resource, resource user account; access to resource schedule) to the resource and added to the user base. Lab personnel will undergo training on use of the instrumentation prior to the start of the actual experimental study. Protocols on data acquisition, SOPs on instrumentation and additional training modules are provided to users. Resource members interact closely with investigator/lab personnel throughout this process to ensure smooth and efficient workflow. Investigators interested in exploring the use of imaging for their research should direct their inquiries to Dr. Seshadri or Dr. Spernyak by email or phone.

Website: <https://www.roswellpark.org/shared-resources/translational-imaging>

LEADERS

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Scientific Director

MRC-232

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Cancer Cell Center (CCC), 114

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IMAGING SERVICES

Services provided by TISR to cancer center members can briefly be summarized as follows:

- Access to imaging technology
- Expert consultation for study design and execution of preclinical imaging studies
- Multimodal image acquisition and analysis
- Development of customized image analysis, image co-registration and visualization schemes
- Assistance with manuscript and grant preparation
- Assistance with IACUC protocol submission and approval
- Training and education.

ANATOMIC IMAGING

- Multimodality imaging of tumor growth in vivo (MRI,BLI, FI)
- Evaluation of metastatic tumors in vivo (liver, lungs, diaphragm, spleen) (MRI,BLI, FI)
- Assessment of chemotherapeutic efficacy (MRI - 3D tumor volume measurements)
- Genetic phenotyping (MRI)

FUNCTIONAL IMAGING

- Dynamic contrast enhanced (DCE) and macromolecular contrast media (MMCM) imaging for assessment of tumor blood flow and vascular permeability (MRI)
- Diffusion-weighted MR imaging of tumor cell kill following treatment (MRI)
- Heteronuclear in vivo MR spectroscopy and chemical shift imaging (MRI)
- MR Angiography (MRA) and quantitative flow measurements (MRI)
- Genetic expression via use of marker proteins, e.g. luciferase (BLI, FI)

ADDITIONAL SERVICES

- Development of labeled therapeutic and/or diagnostic agents (Gd-based, iron-oxide; 19F labeled as well as GFP, RFP labeled probes)
- Customized image processing and quantitative analysis of digital data
- Physiologic monitoring of tissue oxygenation, microcirculation and temperature measurements using solid state fiber optic probes

IMAGING TECHNOLOGIES/ INSTRUMENTATION

Presently, TISR's imaging armamentarium includes a 4.7T MR scanner (purchased through state and institutional funds), an ultrasound/photoacoustic imaging system (obtained through an S10 award in 2012), a Xenogen Spectrum bioluminescence and fluorescence imaging system (obtained through an S10 award in 2013), a IVIS 50 bioluminescence imaging system, and a cabinet X-ray system (both purchased through Roswell Park institutional funds). The major equipment includes:

- **Magnetic Resonance Imaging:** The "workhorse" for TISR is a 4.7T preclinical MRI scanner operating on the ParaVision

acquisition platform (v.4.0, Bruker Biospin, Billerica MA). It features a fixed set of Accustar® gradients (ID: 15 cm) for whole body mouse or rat imaging, and a 60 cm gradient insert (G060, Bruker Biospin) capable of high-resolution (<100 μm in-plane resolution) imaging of mice. Multiple 1H and 19F transceiver body coils are available ranging from 35 mm to 112 mm (ID), as well as several surface coils (7 mm to 20 mm diameter). The scanner is routinely used for phenotyping novel tumor models (Ku et al., Science, 2017; Adelaiye-Ogala et al., Can Res, 2017) and for functional imaging of tumor vascular and cellular response to chemotherapy, radiation and novel targeted anticancer therapies (Kalmuk et al., Oncotarget, 2015; Rich et al., Radiology, 2015).

- **An MR compatible animal monitoring & gating system** (Model 1025, SAIL Instruments, Stony Brook, NY), which allows for the monitoring of body temperature, heart rate, and respiratory rate of anesthetized animals, as well as providing warm air heating for anesthetized animals undergoing MR imaging.
- **Optical imaging systems:** TISR currently

operates multiple Xenogen systems for bioluminescence imaging of small animals. The first instrument was purchased using institutional funds and is a first-generation system that is capable of imaging 3 mice simultaneously. In 2013, an S10 Shared Instrumentation grant was awarded to TISR to purchase a state-of-the-art IVIS Spectrum, dual bioluminescence/fluorescence imaging system (Perkin-Elmer). The Spectrum system features a thermoelectrically-cooled charge-coupled device (CCD) camera capable of simultaneous imaging of 5 mice. A 24-position filter wheel featuring 18 emission filters (500-840 nm, 20 nm band pass) is situated in front of the camera for spectral unmixing capabilities. A laser galvanometer provides surface topography and alignment capabilities. Given the high-throughput nature of this technology, investigators routinely utilize BLI for longitudinal monitoring of tumor progression in genetically-engineered mouse models of cancer (Burdelya et al., Proc Natl Acad Sci U S A., 2013; Ebos et al., EMBO Mol Med, 2014; Eng et al., Nat Comm, 2015).

- **Ultrasound/Photoacoustic Imaging system:** The US/Photoacoustic Micro Imaging system (VevoLAZR; VisualSonics Inc. Toronto, Ontario) system is based on linear array technology developed by VisualSonics and enables collection of photoacoustic and ultrasound imaging datasets on the same plane, thereby enabling co-registration of structural and functional information on the tissues of interest. The VevoLAZR system was obtained through an S10 grant provides an integrated, compact and safe platform for in vivo imaging of morphology and microvascular dynamics of tumors in 2D and 3D. Combined use of PA mode imaging along with B-mode imaging gives both structural information on tumor morphology and functional information on tumor blood flow and oxygenation. Studies routinely utilize US for monitoring

tumor growth and for image-guided interventional procedures to establish tumors in orthotopic sites (e.g. lungs, salivary glands, prostate). In addition to using endogenous mechanisms of contrast (Rich et al. Theranostics 2018), PAI is also utilized for nanoparticle-mediated functional mapping of tumor hypoxia in animal models (Zhang et al. Nat Nanotechnology 2015) and multimodal theranostics (Shao et al., Small, 2017).

- **A fully digital, cabinet X-ray radiography system** TISR also oversees a cabinet digital X-ray radiography system (Faxitron MX-20) for animal imaging. This system allows for high-throughput monitoring animal models efficiently and affordably. X-RAY is the optimal imaging modality for bone-related studies such as osteoporosis, phenotyping and detection of bone metastases.
- **Software/Computational resources:** The resource has 8 64-bit multicore/multi-processor Windows-based graphics workstations all featuring at least 8 GB memory, including a workstation with 16 GB RAM for processing of large datasets. The resource maintains 3 network licenses of Analyze 7.0 (biomedical image processing software) that provides a versatile tool chest of medical image processing modules including: volumetric and intensity quantification of 2D/3D/4D image datasets, three-dimensional volume rendering, and object/cell counting. It also maintains an annual service contract for MATLAB (Mathworks) including the Image Processing and 6 additional toolboxes for programming of customized image processing routines, available on every computer within the facility. Lastly, the facility maintains 1 fixed-node license of visualization software Amira installed on a graphics workstation for 2D/3D quantification, visualization & animation.

SELECTED PUBLICATIONS

Zhang Y, Jeon M, Rich LJ, Hong H, Geng J, Zhang Y, Shi S, Barnhart TE, Alexandridis P, Huizinga JD, Seshadri M, Cai W, Kim C, Lovell JF. Non-invasive multimodal functional imaging of the intestine with frozen micellar naphthalocyanines. *Nat Nanotechnol*. 2014 Aug;9(8):631-8.

Gil M, Seshadri M, Komorowski MP, Abrams SI, Kozbor D. Targeting CXCL12/CXCR4 signaling with oncolytic virotherapy disrupts tumor vasculature and inhibits breast cancer metastases. *Proc Natl Acad Sci U S A*. 2013 Apr 2; 110(14):E1291-300. PMID: 23509246.

Seshadri M, Sacadura NT, Coulthard T. Monitoring antivasular therapy in head and neck cancer xenografts using contrast-enhanced MR and US imaging. *Angiogenesis*. 2011 Dec; 14(4):491-501 (Cover feature). PMID: 21901534.

Adelaiye-Ogala R, Budka J, Damayanti NP, Arrington J, Ferris M, Hsu CC, Chintala S, Orillion A, Miles KM, Shen L, Elbanna M, Ciamporcero E, Arisa S, Pettazzoni P, Draetta GF, Seshadri M, Hancock B, Radovich M, Kota J, Buck M, Keilhack H, McCarthy BP, Persohn SA, Territo PR, Zang Y, Irudayaraj J, Tao WA, Hollenhorst P, Pili R. EZH2 Modifies Sunitinib Resistance in Renal Cell Carcinoma by Kinome Reprogramming. *Cancer Res*. 2017 Dec 1; 77(23):6651-6666. PMID: PMC5712262.

Bothwell KD, Shaurova T, Merzianu M, Suresh A, Kuriakose MA, Johnson CS, Hershberger PA and Seshadri M. Impact of short-term 1,25-dihydroxyvitamin D3 on the chemopreventive efficacy of erlotinib against oral cancer. *Cancer Prev Res*. 2015. 8(9):765-76. PMID: PMC4560661. [Cover feature]

Ebos JM, Mastri M, Lee CR, Tracz A, Hudson JM, Attwood K, Cruz-Munoz WR, Jedeszko C, Burns P, Kerbel RS. Neoadjuvant antiangiogenic therapy reveals contrasts in primary and metastatic tumor efficacy. *EMBO Mol Med*. 2014 Oct 31;6(12):1561-76. PMID: PMC4287975.

Eng JW, Reed CB, Kokolus KM, Pitoniak R, Utley A, Bucsek MJ, Ma WW, Repasky EA, Hylander BL. Housing temperature-induced stress drives therapeutic resistance in murine tumour models through β 2-adrenergic receptor activation. *Nat Commun*. 2015 Mar 10; 6:6426. PMID: PMC4471870.

Folaron M, Kalmuk J, Lockwood JL, Frangou C, Vokes J, Turowski SG, Merzianu M, Rigual NR, Sullivan-Nasca M, Kuriakose MA, Hicks WL Jr, Singh AK, Seshadri M. Vascular priming enhances chemotherapeutic efficacy against head and neck cancer. *Oral Oncol*. 2013 Sep;49(9):893-902. PMID: PMC3772633. [Cover Feature]

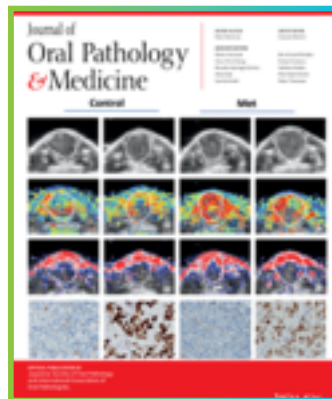
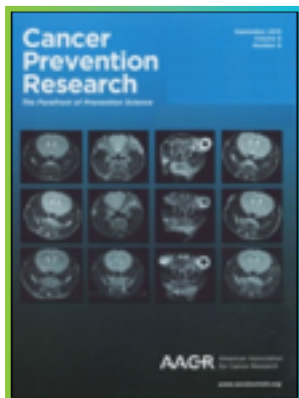
Ku SY, Rosario S, Wang Y, Mu P, Seshadri M, Goodrich ZW, Goodrich MM, Labbé DP, Gomez EC, Wang J, Long HW, Xu B, Brown M, Loda M, Sawyers CL, Ellis L, Goodrich DW. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science*. 2017 Jan; 355(6320):78-83. PMID: PMC5367887.

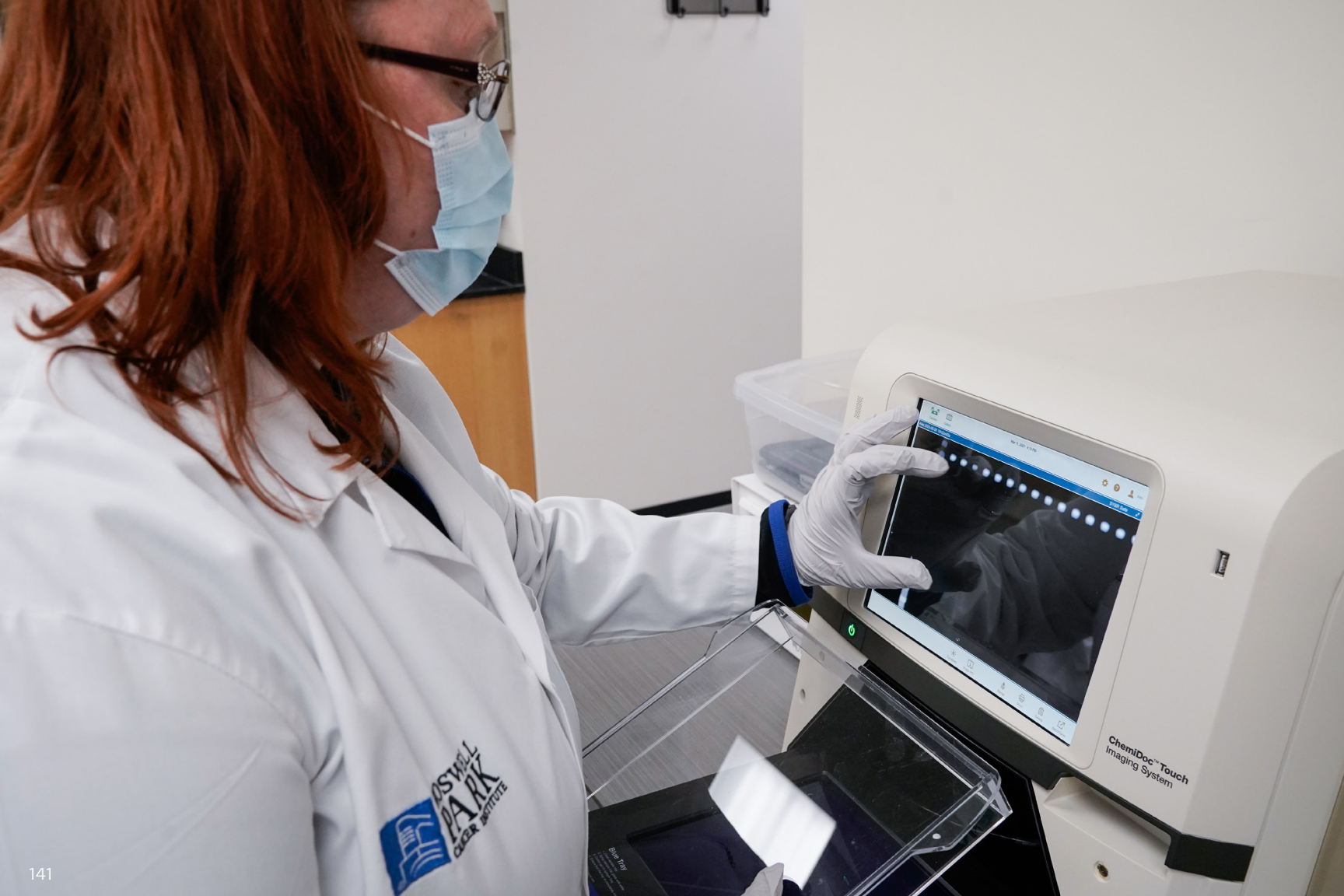
Rich L, Seshadri M. Photoacoustic Imaging of Vascular Hemodynamics: Validation with Blood Oxygenation Level-Dependent MR imaging. *Radiology*;2015; 275:110-118. PMID: PMC4455674.

Rich LJ, Miller A, Singh AK, Seshadri M. Photoacoustic imaging as a biomarker of radiation therapeutic efficacy in head and neck cancer. *Theranostics*. 2018. 8(8):2064-2078. PMID: PMC5928871.

Shao S, Do TN, Razi A, Chitgupi U, Geng J, Alsop RJ, Dzikovski BG, Rheinstädter MC, Ortega J, Karttunen M, Spornyak JA, Lovell JF. Design of Hydrated Porphyrin-Phospholipid Bilayers with Enhanced Magnetic Resonance Contrast. *Small*. 2017 Jan;13(1). PMID: PMC5209247

Verma A, Rich LJ, Vincent-Chong VK, Seshadri M. Visualizing the effects of Metformin on tumor growth, vascularity and metabolism in head and neck cancer. *J Oral Pathol Med*. 2018 May;47(5):484-491. PMID: 29573032





VECTOR DEVELOPMENT
PRODUCTION FACILITY
SHARED RESOURCE

VECTOR PRODUCTION DEVELOPMENT FACILITY

OVERVIEW

The Vector Development and Production Facility (VDPF) occupies 1331 sq. ft. of space on the 6th floor of Gratwick Basic Science Building (GBSB) and is part of the Center for Immunotherapy at Roswell Park Comprehensive Cancer Center. VDPF is a cGMP (current Good Manufacturing Procedure) facility dedicated for the manufacture of clinical grade lentiviral and retroviral vectors, following cGMP regulations, to be utilized to genetically modify cells from patients enrolled in clinical trials.

The facility follows cGMP, i.e. manufacturing products that meet specific requirements for identity, strength, quality, and purity in the pharmaceutical field, ensuring that the generated viral vector product is suitable for clinical trial use and compliant with FDA guidelines.

The VDPF is equipped to manufacture large scale viral vectors. The facility includes two class 10,000 cell-processing clean-rooms. It has a unidirectional design with gown-in, ante-room, production rooms, post-ante-room and gown-out. The facility also has a QC room for lot release tests, storage room and freezer rooms.

VDPF was designed following discussions with the FDA (Type C meeting). The clean room area has pre- and terminal HEPA filtration with air changes/hour meeting the ISO7 requirements (>50 changes /hour) in the 2 production rooms and ante-room 1 and ISO 8 requirements (>20 changes /hour) in the gown-in, ante-2 and gown out rooms. The biosafety cabinets are equipped with terminal hepa-filters. It has a uni-directional flow design to avoid contamination. cGMP Suite and entry to production rooms are controlled via an electronic card access security system and air and equipment are monitored continuously by a centralized system. Refrigerators and suite are locked at all times with access to only designated VDPF personnel. Vector manufacture will be done by only trained personnel following SOPs and using appropriate PPEs including

cleanroom coverall suit, booties, hair cover, mask, sterile sleeve covers, and nitrile gloves. All procedures will be done inside the biosafety cabinet or using closed system bioreactors. Closed system filtration (Tangential Flow Filtration system) will be used to concentrate the vector when required.

USING THE RESOURCE

Investigators interested in the use of the facility should contact Dr. Pawel Kalinski to discuss the project and services.

For quotes, pricing and inquiries, please contact Dr. Pawel Kalinski or call 716-845-1300 ext. 7629. The VDPF is located in the GBSB, sixth floor. Hours of operation are weekdays, 9:00 AM – 5:30 PM.

Website: <https://www.roswellpark.org/immunotherapy/facilities/vector-development-and-production-facility>

LEADERSHIP

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Joanna Stanson, MS

Operations Director, Vector Production

Development Facility

VECTOR DEVELOPMENT AND PRODUCTION FACILITY (VDPF) MANUFACTURING

The VDPF manufactures clinical grade retroviral and lentiviral vectors for Phase I and II studies. Manufacture of every batch of viral vector comprise of 3 phases:

1. Phase 1: Assessment (sponsor requests and confirmation of plasmid identity)
2. Phase 2: Small scale manufacturing, testing and optimization
3. Phase 3: Final product manufacture and release

Retroviral Vector Manufacturing Process

1. Establishing a master cell bank (MCB).
2. Master Cell bank certification.
3. Manufacture of cGMP retroviral vector supernatant.
4. Vector supernatant harvest and filtration.
5. Storage.
6. Certification of final product.

Lentiviral Vector Manufacturing Process

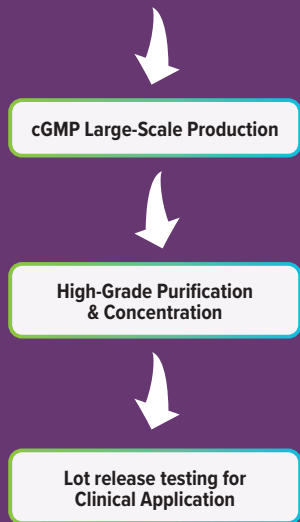
1. Cell Plating of Certified 293T cells.
2. Transfection.
3. Transfection media removal.
4. Vector supernatant harvest.

5. Purification and concentration
6. Vialing and Storage.
7. Certification of final product.

SERVICES

The VDPF provides comprehensive services for development and production of large-scale lentiviral and retroviral vector products suitable for use in clinical trials. Since each vector is a custom project, prospective customers should discuss with Dr. Kalinski for consultation of services and scale of production.

- Tangential flow filtration and Diafiltration System (KMPI KROSFLO M.KROS and KLOSFLO R Ilii)
- Automated Hollow Fiber Bioreactor System
- BIORAD ChemiDoc
- Electrophorator for cell transfection
- Synergy HTX Microplate absorbance reader
- PCR cycler instruments
- 6-foot Calss II biosafety cabinets
- Double stacked CO2 Heracell incubators
- SORVALL centrifuges
- Liquid Nitrogen storage containers
- -80 C, -20 C freezers
- Inverted Phase Contrast Microscope
- Terumo Sterile Connecting Device
- Tube Sealer
- Electronic Key Card Access system
- ISENSIX Advanced Remote Monitoring System
- Magnahelic Gauges





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