1 Transgenic Mouse Facility

UCI Chao Family Comprehensive Cancer Center

LEADERSHIP & MISSION

Grant MacGregor, DPhil
Director

Shimako Kawauchi, PhD
Manager

TMF facilitates use of the mouse as a mammalian experimental system to investigate mechanisms of oncogenesis and testing of cancer therapeutics

- Advises investigators wishing to use genetically engineered mouse models (GEMMs) in their research program, on experimental design and analysis, helps write grant proposals and manuscripts and provides letters of support
- Provides access to specialized expertise and equipment to develop GEMMs, provides technical support, and sources additional reagents required to manipulate the mouse genome and analyze the consequences thereof
- Communicates awareness of novel mouse-related resources, facilitates their acquisition, and provides practical assistance with their use
- Assists researchers by importing, or helping to develop, new experimental approaches necessary to address specific experimental questions in their research

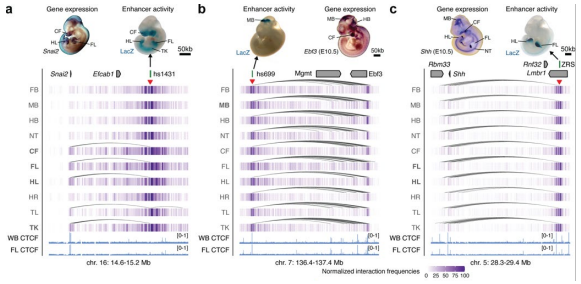
SERVICES, TECHNOLOGIES & EQUIPMENT

Services cover design, development, re-derivation, cryopreservation, and re-animation of GEMMs in an efficient and cost-effective manner, including:

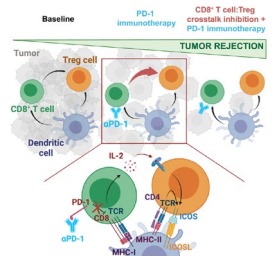
- Consultation, at **no cost to PI**, on strategies to engineer the mouse genome
- Design and targeted engineering of loci in mouse zygotes via CRISPR (>300 projects completed to date)
- Targeted transgenesis at the *Hipp11* and *ROSA26* loci
- Targeted engineering of endogenous loci in mES cells including CRISPR-mediated humanized gene replacement
- Southern analysis of targeted loci in ES cells and animals including PFGE
- Insertion of conventional multi-copy transgenes and bacterial artificial chromosomes (BAC) at random loci via pronuclear injection of DNA
- Development of RT-PCR genotyping assays
- High-throughput analysis of standard PCR assays using Fragment Analyzer
- Production of large cohorts of genetically defined mice
- Content and figures for grant proposals and manuscripts, letters of support, etc. at **no cost to PI**

RESEARCH HIGHLIGHTS

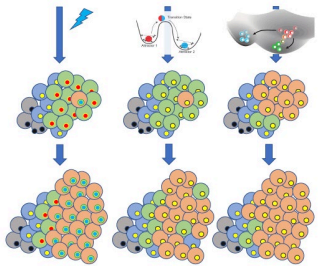
Systematic analysis of enhancer-promoter interaction

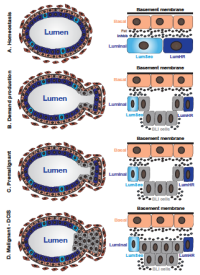


Overcoming limits to immunotherapy in melanoma



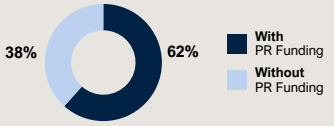
NEW MPI P01: Tipping Points in Cancer





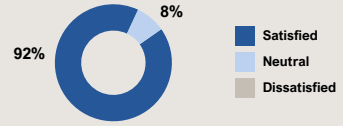
IMPACT & KEY METRICS CY2024

CFCCC MEMBER UTILIZATION



Used by **14** Unique CFCCC Member Labs
6% Overall Usage by CFCCC Members

CFCCC USER SATISFACTION

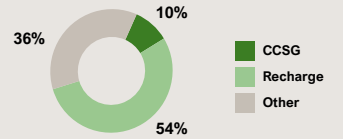


43% Response Rate

$3.5M Supported CFCCC Members Received **4** New Cancer-relevant Grants (Total Direct Costs)

5 Support Led to **2** New Cancer-Relevant Publications **(75%)** in IF ≥ 10 Journals

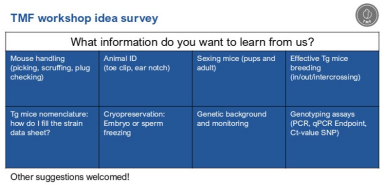
BASE SR FUNDING



$868,145 Annual Budget

TRAINING

- Personalized training via e-mail and meetings
- Annual lecture on GEMM and genome engineering
- Provision of GEMM and cancer-related resources via TMF website
- Recorded tutorials via TMF website



FUTURE PLANS

- In person workshops to provide training in best practices for mouse handling, ID, genotyping, breeding, nomenclature, cryopreservation, genetic background and monitoring, genotyping assays

Internal Advisory Committee

Extensive advocation for CFCCC Membership

Aimee Edinger, VMD, PhD
Deputy Associate Director
Basic Science, CFCCC

Evgeny Kvon, PhD
Assistant Professor
Development & Cell Biology

Marcus Seldin, PhD
Assistant Professor
Biological Chemistry

Claire Lindsell, PhD, BVSc
Director, ULAR

MEMBERS

- The internal advisory committee includes experts in cancer, metabolism systems genetics, mouse biology, mouse genetics and transgenesis
- **Member Responsibilities:** Review TMF technology, operations, priorities, efficiencies. Provide ideas about GEMM-related emerging technologies of potential broad use by CFCCC membership
- **Selection Process:** Identified and petitioned by TMF Scientific Director, using following criteria: CFCCC membership; expertise in use of genetically modified mice in biomedical research; expertise in mouse biology and health, expertise in administrative oversight
- **Appointment Terms:** Renewed on continuous basis

FREQUENCY
Normally annually

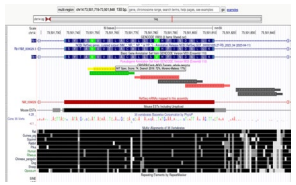
FUNCTION
Independent feedback on TMF services and activities

AUTHORITY
Advise on strategic goals, identify opportunities and address challenges

MAIN

Services, Technologies & Equipment

Design, development, import, re-derivation, cryopreservation, re-animation and production of GEMMs



- Bioinformatic analyses of mouse and human genomics to design strategies for genome engineering

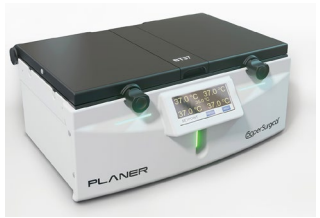
- TaqMan, rhAMP based genotyping via two Bio-Rad RT-PCR systems

- High-throughput (3 x 96-well tray) analysis of standard PCR reactions using Agilent capillary array Fragment Analyzers (two instruments)

- Microinjection, electroporation and culture of zygotes / preimplantation embryos (two systems)

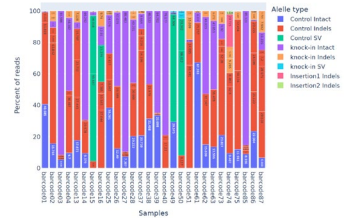
- Freezing, cryogenic storage and reanimation of sperm, embryos, mES cell lines (multiple freezers with duplicate storage in two buildings)

- PFGE and Southern analysis using Bio-Rad CHEF Mapper



- IVF-based mouse production (multiple incubators) for rapid allele assembly at multiple loci and cohort development of experimental and control animals

- Multiple animal holding rooms with ventilated cage racks and sterile caging



- Deeper and faster CRIPSR modification analysis with ONT sequencing and DAJIN2 software

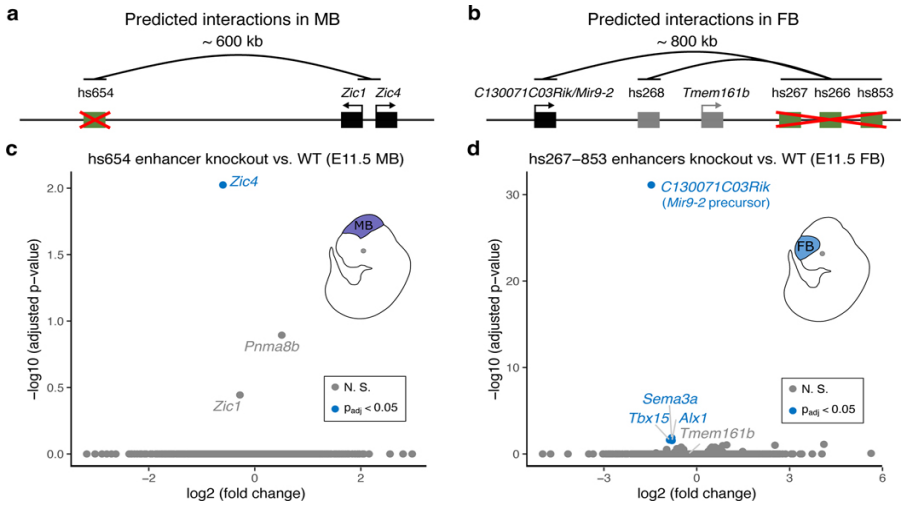
- Tissue culture suite with incubators, hoods and electroporation apparatus (not shown)

MAIN

Systematic analysis of enhancer-promoter interaction in vivo

Functional analysis of tissue-specific enhancer-promoter interactions

- Deregulated gene expression can drive oncogenesis- e.g. loss of expression of tumor suppressors or gain of function of oncogenes
- Dynamics and mechanisms of enhancer-promoter interactions regulating gene expression are poorly understood
- Systematic Hi-C analysis of ~1000 promoter-enhancer interactions in multiple organs during mouse development reveals nearest promoters are frequently bypassed and active enhancers contact each other in clusters



CRISPR-mediated knockout analysis of hs654 and hs267/hs266/hs853 enhancers in mice. a,b, Predicted chromatin interactions between enhancers (green boxes) and target genes (black boxes). c,d, Transcriptome-wide mRNA expression changes in the midbrain of hs654-knockout mice (c) and forebrain of hs267/hs266/hs853-knockout mice (d) relative to wild-type mice

TMF generated numerous lines of KO and KI mice for project via CRISPR

CATCHMENT AREA RELEVANCE

Investigators

Kvon, PhD

CFCCC Investments

SHARED RESOURCE

PROGRAMS

Outcomes

PUBLICATION

Kvon, Nature Genetics, 2024, PMC11203181

GRANTS DP2 GM149555

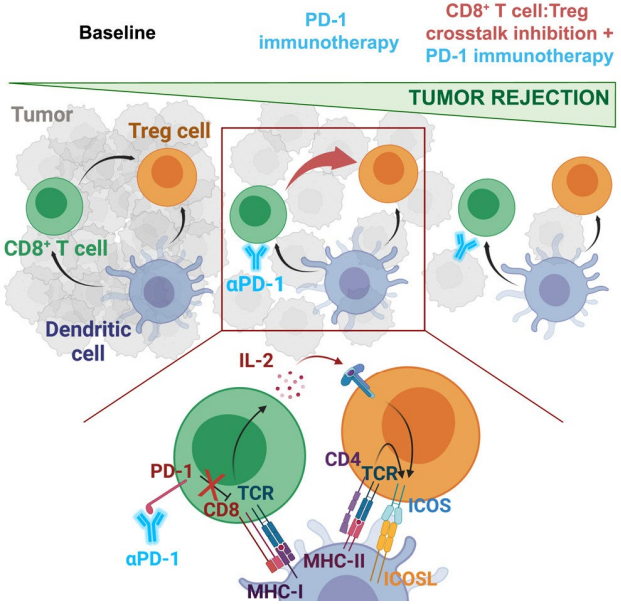
IMPACT | Systematic mapping of *cis*-regulatory elements and 3D structures affecting gene expression *in vivo*

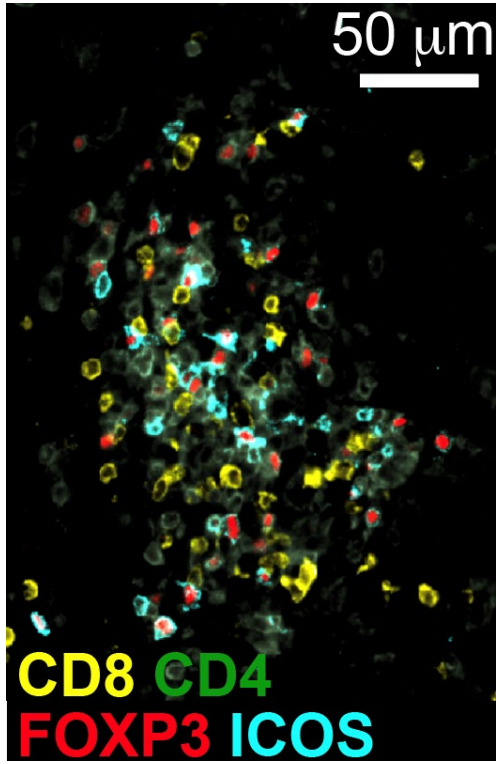
MAIN

Disrupting CD8 and T signaling enhances immunotherapy

Cutting the phone cable between CD8 and T regulatory cells

- PD-1 blockade expands Tregs in melanoma and limits the efficacy of immunotherapy
- Treg-intrinsic PD-1 inhibition does not cause tumor Treg accumulations
- Anti-PD-1 increases Treg numbers via an intratumor CD8+ T cell/ IL-2/ ICOS axis
- Inhibition of the CD8 + T cell: Treg crosstalk by anti-ICOSL synergizes with anti-PD-1 therapy
- Risk/SDH- This work shows one mechanism of resistance to PD-1 immunotherapy. PD-1 blockade causes the accumulation of tumor Tregs, and restricts its own antitumor efficacy, Inhibition of this mechanism ameliorates PD-1 immunotherapy





TMF imported strains of mice infected with MVM from investigator in Japan, rederived these, and used assisted reproduction to accelerate development of *Pdcd1* ^{loxP}; *Foxp3* ^{CreERT2} and other models for study

CATCHMENT AREA RELEVANCE

Investigators


Marangoni, PhD


Ganesan, MD, PhD


Othy, PhD


Nie, PhD

CFCCC Investments

SHARED RESOURCE

DOT

FUNDING
2020, 2022
2023, 2024

PROGRAMS

Outcomes

PUBLICATION
Marangoni, Cancer Cell, 2024, PMC11285091

GRANTS MRA 929155
DOD ME220176P1

IMPACT | Identified mechanism to improve immunotherapy in melanoma

MAIN

Use of assisted reproduction to accelerate research using GEMMS

Examining Basel-luminal progenitor cell expansion in breast tumors

GEMMs - minimum time requried for genotype assembly and cohort production via conventional breeding vs assisted reproduction - e.g. four alleles *Brca1*$^{loxP/loxP}$; *Trp53*$^{loxP/loxP}$; *Fst*$^{loxP/loxP}$; ± *Wap-Cre Tg*

Starting strain genotypes	*Chr*
Brca1$^{loxP/+}$ (Jax cryorecovery)	11, 70Mb
Trp53$^{loxP/loxP}$ (Jax)	11, 101Mb
Fst$^{loxP/loxP}$ (import)	13, 115Mb
Wap-cre +ve (Jax cryorecovery)	?

	Conventional breeding, *min.* crosses & time	Assisted Reproduction *min.* "crosses" & time
Genotype assembly *Brca1*$^{loxP/loxP}$, *Trp53*$^{loxP/loxP}$; *Fst*$^{loxP/}$*loxP*; *Wap-cre* + *Brca1*$^{loxP/loxP}$, *Trp53*$^{loxP/loxP}$; *Fst*$^{loxP/loxP}$	6 cross x 3 mo = 18 mo 5 cross x 3 mo = 15 mo (B6J first litters often lost)	6 IVF x 1.5 - 2.5 mo = 9-15 mo 5 IVF x 1.5 - 2.5 mo = 7.5-12.5 mo
Genotype expansion	3 cross x 3 mo = 9 mo (B6J first litters often lost)	3 IVF x 1.5 - 2.5 mo = 4.5 -7 mo
Cohort Production (c. 50 females ea) *Brca1*$^{loxP/loxP}$, *Trp53*$^{loxP/loxP}$; *Fst*$^{loxP/}$*loxP*; *Wap-cre* + & *Brca1*$^{loxP/loxP}$, *Trp53*$^{loxP/loxP}$; *Fst*$^{loxP/loxP}$	2 -4 gen x 3 mo = ~9 mo (B6J first litters often lost)	3 IVF = 2 mo
Total time estimate	**18 + 9 + 9 = 36 mo**	**15 + 7 + 2 = 24 mo**

Generation of cohorts of GEMMs with complex genotypes via assisted reprodution saves time and animals

CATCHMENT AREA RELEVANCE

Investigators


Lawson, PhD


Lander, MD, PhD


Lowengrub, PhD


Van Etten, MD, PhD

CFCCC Investments

SHARED RESOURCE

DOT

FUNDING 2020, 2022 2023

PROGRAMS

Outcomes

PUBLICATION
In Process

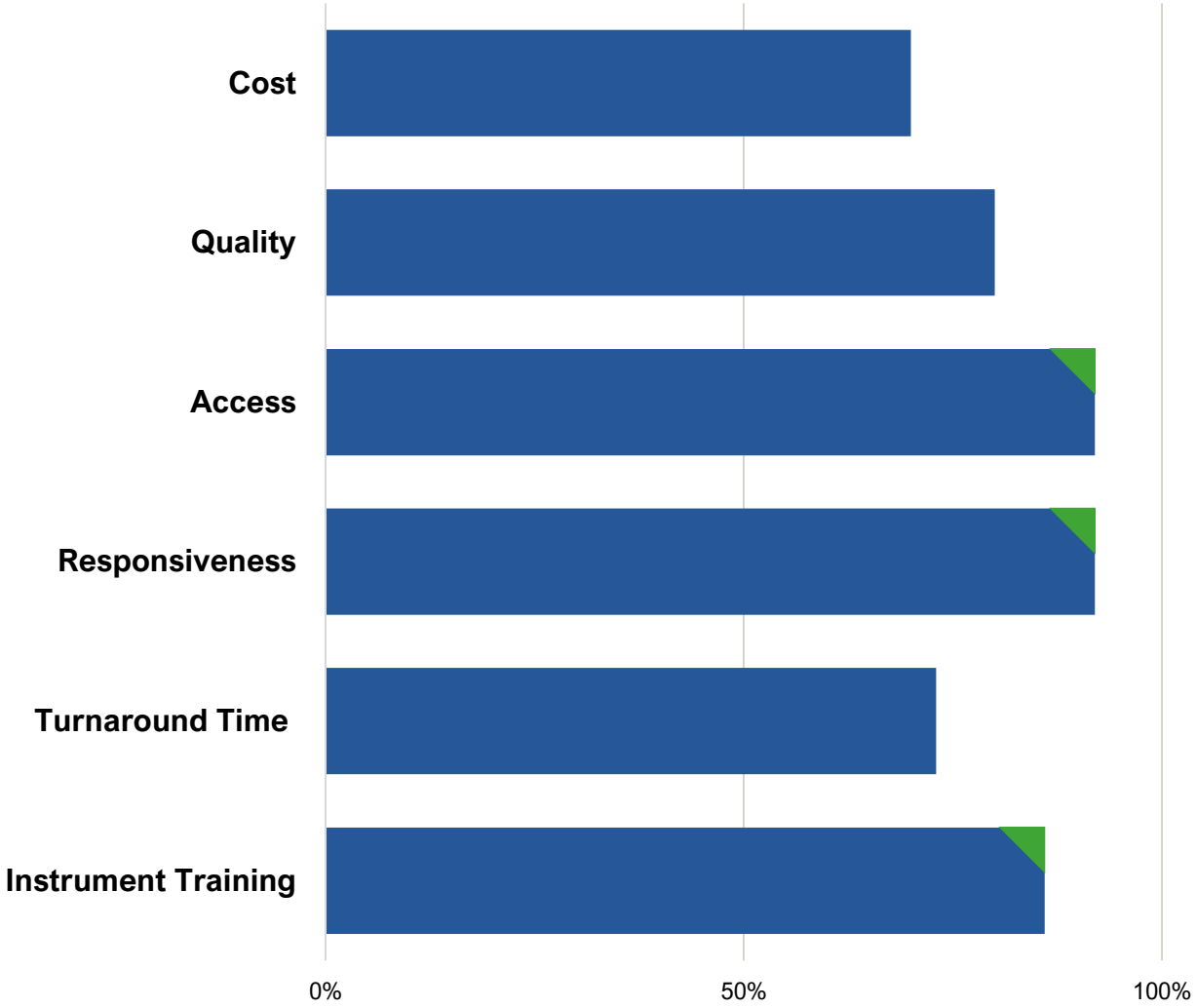
GRANTS P01 Tipping Points in Cancer
Pending NOA

IMPACT | Efficient GEMM production accelerates cancer research, improving study power, rigor, and reproducibility

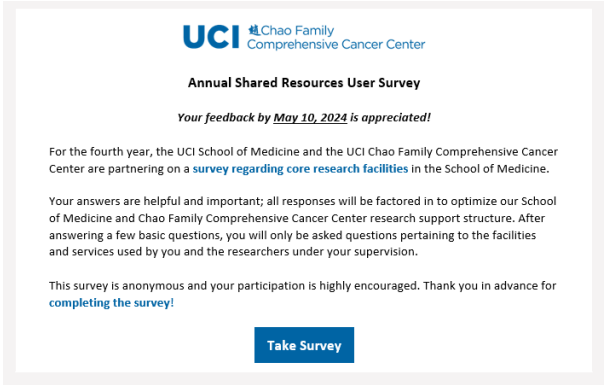
MAIN

2024 Annual Shared Resource Survey

Excellent + Good (No scores below average received) Improved since 2021



SURVEY PROMOTION



UCI Chao Family Comprehensive Cancer Center

Annual Shared Resources User Survey

Your feedback by May 10, 2024 is appreciated!

For the fourth year, the UCI School of Medicine and the UCI Chao Family Comprehensive Cancer Center are partnering on a **survey regarding core research facilities** in the School of Medicine.

Your answers are helpful and important; all responses will be factored in to optimize our School of Medicine and Chao Family Comprehensive Cancer Center research support structure. After answering a few basic questions, you will only be asked questions pertaining to the facilities and services used by you and the researchers under your supervision.

This survey is anonymous and your participation is highly encouraged. Thank you in advance for **completing the survey!**

Take Survey

2024 Core Facilities Survey

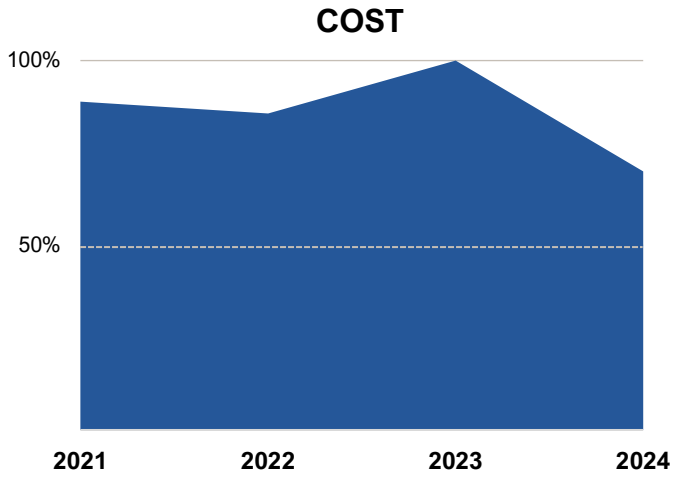
UCI School of Medicine and the UCI Chao Family Comprehensive Cancer Center are partnering on a survey regarding core research facilities in the School of Medicine. Your answers are helpful and important; all responses will be factored in to optimize our research support structure. After answering a few basic questions, you will only be asked questions pertaining to the facilities and services used by you and the researchers under your supervision. This survey is anonymous. For questions, contact **Claire Brainard Draper**. Please complete the survey by **May 10, 2024**.

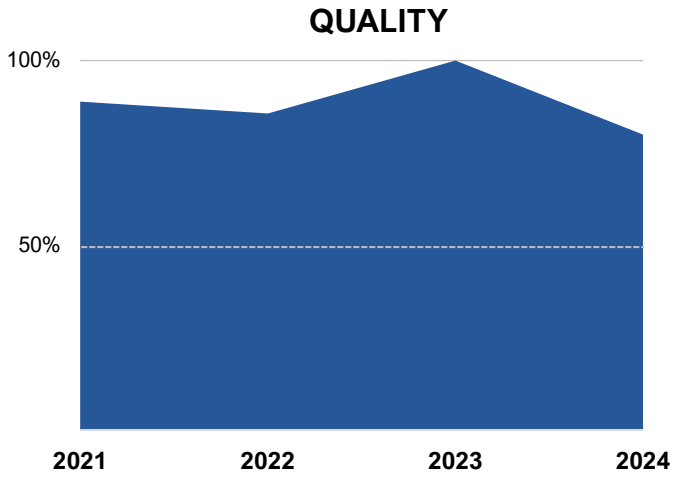
Complete Survey

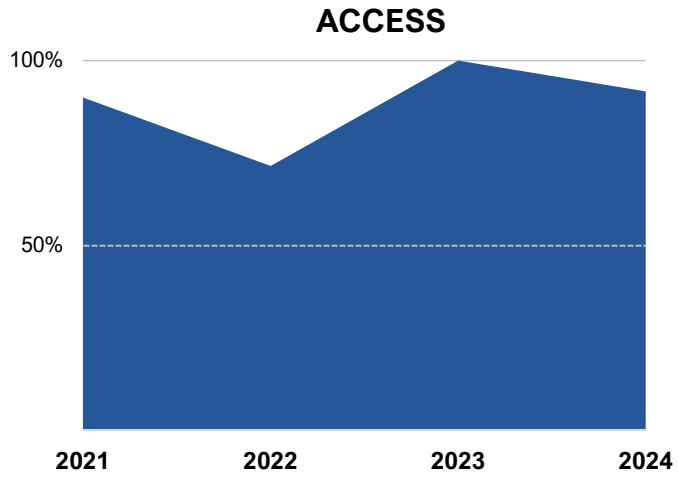
NEXT

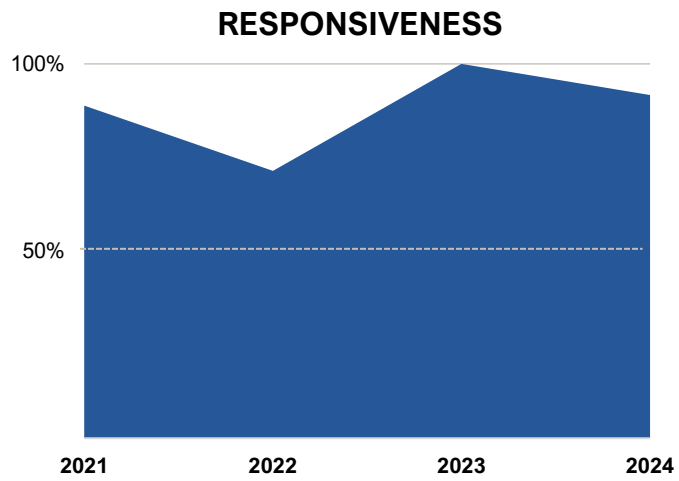
Annual Core Research Facilities Survey

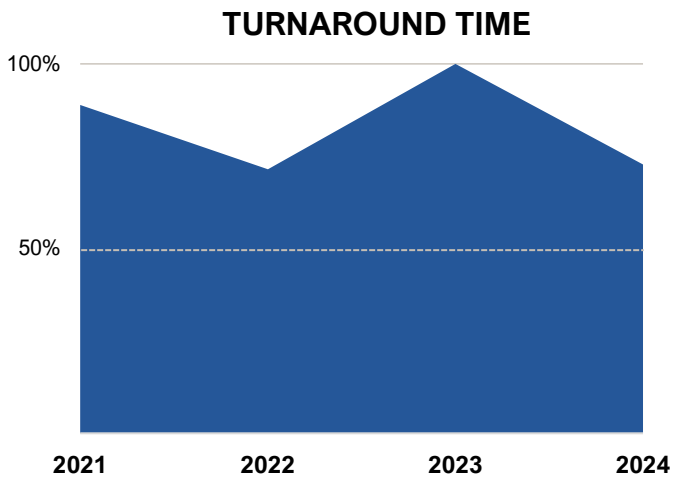
Excellent + Good

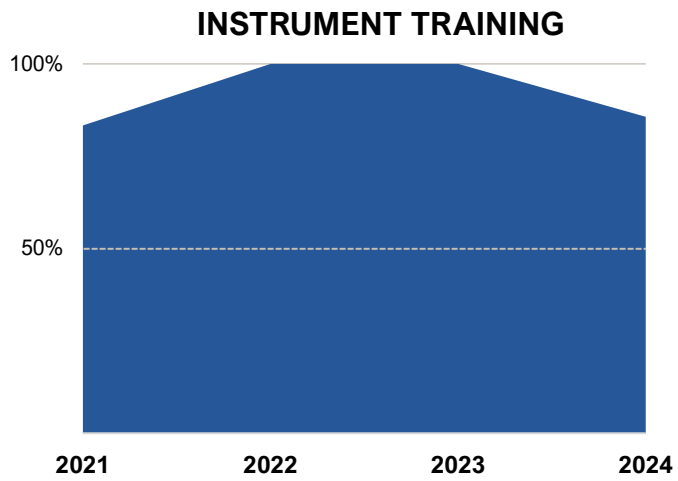












MAIN

Selected 2024 Publications

CFCCC INVESTIGATOR(S)	PROGRAM	JOURNAL	YEAR
Francesco Marangoni, PhD Qing Nie, PhD Anand Ganesan, MD, PhD	SPT SPT BIDD	Cancer Cell	2024
Evgeny Kvon, PhD	SPT	Nature Genetics	2024
Qing Nie, PhD Maksim Plikus, PhD Evgeny Kvon, PhD	SPT	Nature	2024
Evgeny Kvon, PhD	SPT	bioRxiv	2024
Grant MacGregor, DPhil	SPT	Proc. Natl Acad Sci	*in press* 2024

MAIN