"ALEXANDER FLEMING"
Biomedical Sciences Research Center

InfrafrontierGR/PHENOTYPOS

InfrafrontierGR/Phenotypos Research Infrastructure
Trans-regional Phenotyping Call - June 2020

Free-of-charge mouse phenotyping services

Context and aim of the Call

InfrafrontierGR/Phenotypos (www.infrafrontier.gr) is the Greek Research Infrastructure for the Molecular, Behavioral and Phenotypic Analysis of Mouse Models for Human Chronic Degenerative Diseases. The infrastructure provides access to first-class expertise and tools for biomedical research, offering cutting edge technological platforms and standardized pipelines for disease-oriented mouse phenotyping, as well as the generation, archiving and distribution of mouse mutants through the Greek node of the European Mouse Mutant Archive (EMMA).

The objective of this Call is to **provide free access to state-of-the-art mouse phenotyping platforms** to the Greek biomedical research community, including standardized and customized assays used to monitor disease parameters and elucidate the molecular and genetic basis of pathologic processes involved in chronic degenerative diseases. **Four (4) phenotyping projects will be supported through this Call**.

InfrafrontierGR services offered through this Call include:

1. **Live imaging assays (colonoscopy, X-ray/bone density, bioluminescence imaging)**
2. **MicroCT imaging assays**
3. **Blood Analysis assays and Cell sorting**
4. **Histology assays**

Call information and application form

For more information, including eligibility criteria, selection procedure, application instructions and application form please see the **Full Call text below.**

Deadline for submissions: **15/9/2020**.

European Union
European Regional Development Fund

HELLENIC REPUBLIC
MINISTRY OF ECONOMY & DEVELOPMENT
SPECIAL SECRETARY FOR ERDF & CF
MANAGING AUTHORITY OF EPAnEK

EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS
ENTREPRENEURSHIP
INNOVATION

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement 2014 - 2020

Co-financed by Greece and the European Union

Full Call text

Trans-Regional Access (TA) activity of the InfrafrontierGR/Phenotypos project
Free-of-charge mouse phenotyping services

The InfrafrontierGR/Phenotypos project (www.infrafrontier.gr) supports eligible researchers with **free-of-charge mouse phenotyping services** implemented as a Trans-regional Access activity. A total of **four (4) projects** will be supported through this Call, **one (1) project per assay type** (i.e. 1 live imaging project, 1 microCT project, 1 blood analysis or cell sorting project, 1 histopathology project). Free access will be granted on the basis of scientific excellence and supports the development and in-depth characterisation of mouse models to investigate gene function and human pathophysiology.

The services offered will be run by BSRC Fleming's InfrafrontierGR Units and may involve **one of the following assays**:

1. **Live imaging assays (Fleming mouse facilities)**
   a. Colonoscopy assay for colitis/colorectal cancer mouse model assessment
   b. X-rays and bone density assays for osteoporosis/arthritis mouse model assessment
   c. Luminol bioluminescence imaging assay for acute inflammation assessment (arthritis, enteritis)
   d. Lucigenin bioluminescence imaging for chronic inflammation assessment (arthritis, enteritis)
2. **Micro CT imaging assays**
   a. Trabecular mouse femur analysis and 3D imaging
   b. Cortical mouse femur analysis and 3D imaging
   c. Calcaneus mouse bone analysis and 3D imaging
3. **Blood Analysis assays and Cell sorting**
   a. Complete Mouse Blood Count
   b. Biochemical analysis of mouse serum/plasma
   c. Basic Immunophenotyping of mouse blood
   d. Cell Sorting
4. **Histology assays**
   a. Standard mouse tissue processing for FFPE block generation
   b. Histology slide preparation
   c. Automated Hematoxylin/Eosin (H/E) slide staining and covering
   d. Multiple histochemical staining's (Masson Trichrome, Luxol Fast Blue, Periodic Acid Schiff)

(see Appendix at the end of this document for technical specifications)

- A collaboration agreement will be established between the selected applicants and BSRC Fleming.
- All assays will be performed based on InfrafrontierGR Standard Operating Procedures.
- Results will be made available to selected applicants within a maximum of 6 months following provision of all required information and material.

- The analysed mouse models and the generated phenotyping data **will be made available to the scientific community**. Upon request, an optional grace period of up to 1 year for phenotyping data may apply, with immediate release of data after expiry of the grace period.

Cost: Access to the InfrafrontierGR/Phenotypos mouse phenotyping services under this Call is free of charge, with the exception of shipment costs of the mouse mutant lines or other material to/from BSRC Fleming, which must be covered by the applicant.

Eligibility: Applications for this Call can be submitted by Researchers based anywhere in Greece. Members of the InfrafrontierGR/Phenotypos infrastructure are not eligible for applying.

Applications: Applications for the Call are made via the following **application form**, which must be sent electronically **to infrafrontierGR@fleming.gr by September 15, 2020**. The form includes a short description of the project focusing on research plans for utilising the assay results plus the impact of the mouse model under investigation.

Selection procedure: Proposals will be subject to a review procedure which will be initiated after the Call for applications is closed. Criteria for evaluation include scientific merit and soundness of the proposal, experience of the applicants, quality of preliminary data, feasibility of implementation, research plans and the impact/prospects for exploitation of the phenotyping data. A mixed panel of members of InfrafrontierGR/Phenotypos and potentially external evaluators will assess service requests supported by this activity. Applicants will be informed of the outcome of the evaluation within 6 weeks after the Call deadline. In a further step, experts of InfrafrontierGR Units will assess the technical feasibility of projects.
The technical evaluation of projects may require the provision of additional data such as:
- Information on the genetic modification of your mutant mouse line if applicable (e.g. affected gene, MGI ID of the gene, type of mutation, ES-cell line used, genetic background (e.g. number of backcross generations)
- Description of DNA modification (vector, remaining non-recipient DNA, donor organism)
- Mutant phenotype(s), special housing or care requirements
- Current sanitary status
- Intellectual property rights (who generated and who owns the mouse line)

Acknowledgements: Selected beneficiaries are obliged to acknowledge support under this scheme in all resulting publications using the following wording "*We acknowledge support of this work by project InfrafrontierGR-Phenotypos (MIS 5002135), which is implemented under the Action Reinforcement of the Research and Innovation Infrastructure, is funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation (NSRF 2014-2020) and is co-financed by Greece and the European Union (European Regional Development Fund)".*

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APPENDIX: Technical specifications

1. Live imaging assays

a. Colonoscopy assay for colitis/colorectal cancer model assessment

Endoscopic examination of **up to 16 living mice** will be carried out at a particular time point with the Mainz COLOVIEW System from Karl Storz. Mainz COLOVIEW System is suitable for mouse specific endoscopy in experimental models of colitis/colorectal cancer. During the endoscopic procedure, an evaluation of colonic inflammation progression (endoscopic colitis score) or tumor development (tumor size, tumor load) will take place.

b. X-rays and bone density assays for osteoporosis/arthritis model assessment

X-ray analysis of **up to 16 living mice** will be carried out at a particular time point with the In-Vivo Xtreme Imaging System from Bruker. In-Vivo Xtreme Imaging System is suitable for mouse specific bone density quantification during disease progression in experimental models of osteoporosis and arthritis through the evaluation of the inner and outer radius (units of cm) and the bone volume density (g/cm^3) of the femur.

c. Luminol bioluminescence imaging assay for acute inflammation assessment (arthritis, enteritis)

Bioluminescent imaging examination of **up to 16 living mice** will be carried out at a particular time point with the In-Vivo Xtreme Imaging System from Bruker. In-Vivo Xtreme Imaging System offers quantitative analysis of the acute phase of inflammation in experimental models of arthritis and enteritis through the detection of luminol-labeled biomolecules. The evaluation will involve the Sum (P/s) and Net (P/s) intensity of the bioluminescent signal.

d. Lucigenin bioluminescence imaging for chronic inflammation assessment (arthritis, enteritis)

Bioluminescent imaging examination of **up to 16 living mice** will be carried out at a particular time point with the In-Vivo Xtreme Imaging System from Bruker. In-Vivo Xtreme Imaging System offers quantitative analysis of the chronic phase of inflammation in experimental models of arthritis and enteritis through the detection of lucigenin-labeled biomolecules. The evaluation will involve the Sum (P/s) and Net (P/s) intensity of the bioluminescent signal.

2. Micro CT imaging assays

a. Trabecular mouse femur analysis and 3D imaging

Trabecular mouse femur analysis and 3D imaging of **up to 20 murine samples** will be performed on the instrument Skyscan 1172 and the MicroCT workstation (Bruker). The SkyScan 1172 scanner is a MicroCT intermediate-high resolution levels, with an innovative dynamically variable acquisition geometry that provides the shortest scan times possible at a big range of magnifications, and achieves high spatial resolution without compromising sample size, allowing high-quality imaging. Our standard method of quantitatively describing trabecular bone architecture could provide the calculation of the following morphometric indices: Bone Volume and Surface (BV and BS), Tissue Volume and surface (TV and TS), Bone Volume Density (BV/TV), Bone Surface Density (BS/TV), Bone Surface to Volume ratio (BS/BV), Trabecular Thickness, Trabecular Number, Trabecular Separation and systemic Bone Mineral Density (BMD).

b. Cortical mouse femur analysis and 3D imaging

Cortical mouse femur analysis and 3D imaging of **up to 20 murine samples** will be performed on the instrument Skyscan 1172 and the MicroCT workstation (Bruker). The SkyScan 1172 scanner is a MicroCT intermediate-high resolution levels, with an innovative dynamically variable acquisition geometry that provides the shortest scan times possible at a big range of magnifications, and achieves high spatial resolution without compromising sample size, allowing high-quality imaging. Our standard method of quantitatively describing cortical bone architecture could provide the calculation of the following morphometric indices: Bone Volume and Surface (BV and BS), Tissue Volume and surface (TV and TS), Bone Volume Density (BV/TV), Bone Surface Density (BS/TV), Bone Surface to Volume ratio (BS/BV), Cortical Thickness, Cortical Porosity and cortical Bone Mineral Density (BMD).

c. Calcaneus mouse bone analysis and 3D imaging

Trabecular mouse calcaneus analysis and 3D imaging of ankle joints of **up to 20 murine samples** will be performed on the instrument Skyscan 1172 and the MicroCT workstation (Bruker). The SkyScan 1172 scanner is a MicroCT intermediate-high resolution levels, with an innovative dynamically variable acquisition geometry that provides the shortest scan times possible at a big range of magnifications, and achieves high spatial resolution without compromising sample size, allowing high-quality imaging. Our standard method of quantitatively describing trabecular bone architecture could provide the calculation of the following morphometric indices: Bone Volume and Surface (BV and BS), Tissue Volume and surface (TV and TS), Bone Volume Density (BV/TV), Bone Surface Density (BS/TV), Bone Surface to Volume ratio (BS/BV), Trabecular Thickness, Trabecular Number and Trabecular Separation.

3. Blood Analysis Assays and Cell Sorting (FACS Unit)

a. Complete Mouse Blood Count

Complete blood count of up to **20 mouse blood samples** will be carried out with the Mindray BC5000Vet analyzer. BC5000Vet is suitable for mouse specific 5Diff WBC analysis and evaluation of 23 parameters (WBC, Neu%, Lymph%, Mon%, Eos%, Bas%, Neu#, Lymph#, Mon#, Eos#, Bas#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW and PCT). For each sample 50 to 100µl of anticoagulant treated whole blood is required.

b. Biochemical analysis of mouse serum/plasma

Clinical-biochemical analysis of up to **20 mouse serum/plasma samples** will be carried out with the Beckman Coulter AU480 analyzer. The analysis can involve the detection and quantification of common mouse serum/plasma electrolytes, metabolites and enzymes (Sodium, Potassium, Chlorine, Phosphorus, Iron, UIBC, Total protein, Albumin, Glucose, Urea, Cholesterol, Triglycerides, Creatinine, AST, ALT, ALP, LDH, Creatinine Kinase and Ferritin). For each sample 150µl of serum or heparin-plasma is required.

c. Basic Immunophenotyping of mouse blood

Flow cytometric immunophenotyping analysis of up to **20 mouse blood samples** will be carried out with BD FACS CANTO II analyzer. The assay utilizes a lyse-no-wash approach and two multicolour antibody panels to detect and quantify basic blood immune subpopulations (T cells, B cells, NK cells, Monocytes, Granulocytes, Eosinophils and Dendritic cells) and their activation status. For each sample 50 to 100µl of anticoagulant treated whole blood is required.

d. Cell Sorting

Cell sorting of mouse primary cells from lymphoid or non-lymphoid tissue will be carried out with BD FACS ARIA III for a maximum of **3 sessions**. The user should provide ready to be sorted samples by using their own optimized sample preparation and staining protocol. The Facility will provide bench-space for sample preparation if required, establishment of the sorting protocol, sample sorting, and all sorting consumables.

3. Histology assays

a. Standard mouse tissue processing for FFPE block generation

The tissue will be placed into cassettes using biopsy pads and transferred to a Leica TP 1020 tissue processor for standard tissue processing to serially dehydrate sections and allow embedding in paraffin wax (FFPE, Formalin Fixed Paraffin Embedded).

b. Histology slide preparation

Specimens will be embedded into paraffin blocks and cut at a thickness of about 4 µm using the microtome Microm HM 200 Ergostar.

c. Automated Hematoxylin/Eosin (H/E) slide staining and covering. The H/E staining and the covering of the slides will be carried out on a Leica Autostainer XL Automated slide stainer and Leica CV5030 Robotic Coverslipper, respectively, for high-quality staining with superior optical resolution for reliable long-term storage.

d. Multiple histochemical stainings

The Facility also performs manually multiple histochemical staining's, such as Masson Trichrome, Luxol Fast Blue, Periodic Acid Schiff.

The above assays will be performed on **up to two types** of the following mouse tissue: small/large intestine, spinal cord, brain, lungs, liver and kidneys, from **up to 20 animals**. The user should bring the samples ready for analysis in 1x PBS after fixation in neutral buffered formalin.

InfrafrontierGR/PHENOTYPOS

INFRAFRONTIER-GR:
AN OPEN ACCESS INFRASTRUCTURE FOR THE DISEASE-ORIENTED ANALYSES OF PRECISION MUTATIONS

WHAT WE DO

CREATE
Precision mutations in the mouse

ANALYZE
In disease settings for human health

STORE
in the European Biobank (EMMA)

SHIP
To a world of research

OUR MISSION

A Hellenic Infrastructure of Excellence in Biomedical Research that provides innovative mouse resources and tools to study gene function in human disease and evaluate new therapeutics

OUR PRINCIPLES

- Harmonisation with International Quality Standards
- Open Access services
- Data FAIRification
- 4Rs

DISEASE AREAS WE SCREEN

- **Inflammatory Bowel Disease**
- **Arthritis**
- **Infection**
- **Neurodegeneration**
- **Mental Disability**
- **Metabolic Disorders**
- **Cancer**

WHAT WE OFFER

Standardised Evaluation Procedures
- 10 Phenotyping & Metabolic Pipelines
- 80 SOPs

Validated Mouse Models

Non-invasive & Invasive Methods

Secondary to Tertiary Phenotyping

State-of-the art Infrastructure
- FACS
- μCT imaging
- Proteomics
- Ultrasound imaging
- Optical / X-ray Imaging
- PET/CT imaging
- Endoscope imaging
- Histopathology infrastructure
- Biochemical Analyser
- Hematology Analyser
- Gene Editing infrastructure

THE MOUSE AS A MODEL

- 30 Nobel prizes
- Preclinical Evaluation of Therapies
- Human and Mouse Pathophysiological Processes are well Correlated
- Straightforward Genetic Manipulation

THE PARTNERS

"ALEXANDER FLEMING"
Biomedical Sciences Research Center

HELLENIC REPUBLIC
National and Kapodistrian University of Athens

DEMOCRITUS UNIVERSITY OF THRACE

FORTH

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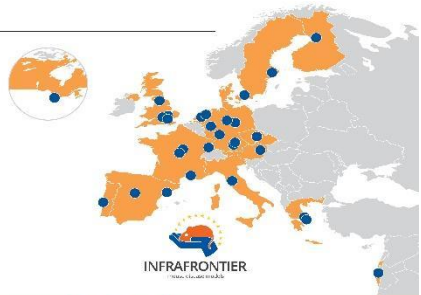

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ΕΠΑνΕΚ 2014–2020
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ΕΣΠΑ 2014-2020

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης