2019 Call 'Development of experimental models for rare diseases'

French Foundation for Rare Diseases (Fondation Maladies Rares)

A – Context and aim of the call

Genome editing and advances in associated methodologies represent a technological revolution that extend opportunities in rare diseases research and new avenues towards addressing pathophysiological and therapeutic approaches. This call aims to give a significant boost to the **development of new experimental models of rare diseases**, in order to:

- improve the understanding of molecular and cellular mechanisms leading to pathological conditions related to a rare disease,
- provide evidence for therapeutic proofs of principle that may eventually lead to treatment.

The project must directly rely on the study of one/several rare disease(s). The rationale of the proposed model must be robust enough for a better understanding of the pathophysiological mechanisms or for answering a key objective in the development of a therapeutic strategy related to the targeted pathology. Prior to developing the model, functional studies should have been performed to demonstrate the pathogenicity of the mutation involved or the networks linking the causative gene to disease.

New collaborations between rare diseases research teams and groups with extensive experience with a particular animal model are encouraged to help the development of interesting and innovative models and the related functional studies, which would lead to significant advances in rare diseases research.

Choosing an appropriate and reliable experimental model is of particular importance and must be guided by obtaining results that best mimic human pathology. In some cases, it can be advantageous using several complementary models to cover different aspects of the same disease (transversal/integrative approaches) and/or reach distinct objectives. In addition, the use of one or more experimental models is essential to validate a proof of concept in order to bridge the translational gap between preclinical and clinical research.

Mouse models are widely and the most commonly used for the study of human diseases and have already demonstrated the extent of their impact. However, in some cases, the **rat model** offers better clinical characterization approaches than the mouse model; its larger size allows to perform several experiments and surgical procedures and to monitor

physiological parameters. Because rats share many physiological similarities with humans, the rat has long been a model favoured by physiologists, pharmacologists, cardiologists and neuroscientists.

Some **other small animal models** could alternatively be used because they may present some close physiological similarities and better mimic a disease. They may furthermore provide a number of advantages, such as to obtain quicker results, better monitoring and modelling of physiopathological processes that can only be followed *in vivo* and to perform integrated studies (-omics) and screening of phenotypic changes in response to genetic alterations (such as pangenomic screen) or small molecules.

New technologies contribute to the respect of the 3R rules which aim at reducing the number of used animals and at replacing them when suitable and are now leading to new solutions to optimize the application of the rules.

Disease-specific **induced pluripotent stem cells (iPSC)** can be generated from patients with rare diseases, providing an effective approach for disease modelling and drug discovery and the opportunity to study a cellular response in a closed system, where the experimental conditions are maintained. The iPSCs provide a unique way for observing associated phenotypes and therapeutic screening to different genetic variants. The development of in vitro models as substitutes to animal models may allow optimising the reproduction of physiological/pathophysiological processes in vivo (ex: 2D, 3D, iPSc, organoids...)

In order to achieve the objective of **generating new experimental models dedicated to rare diseases,** the French Foundation for Rare Diseases has set up partnerships with:

- CELPHEDIA (Creation, Breeding, Phenotyping, Distribution and Archiving of model organisms), the French infrastructure that promotes innovative services and tools on model organisms dedicated to the generation of disease models,
- experienced national platforms including the ones giving support for the generation and characterization of models in the nematode *C. elegans* (Biology of *C. elegans* and *C. elegans* functional genomics) and the rabbit (Transgenic Rabbit Model),
- the iPSC Nantes platform, for the development of disease-specific induced pluripotent stem cell (iPSC) lines.

All these partners offer an outstanding range of expertise, skills and services, provide advise to select **the most appropriate experimental model**, and answer the common objective to develop **model resources** that will be made available to the scientific community.

B – Content of the call

The project must be based on scientifically validated preliminary data and the choice of the experimental model must be clearly justified. The call is dedicated only to generating new experimental models for rare diseases (including conditions for archiving lines). Any other request (breeding, phenotyping, advanced imaging etc.) is not eligible.

Successful applicants will have a facilitated access to tools proposed by experienced platforms in order to develop new experimental models of rare diseases.
The precise type of model development (knock-out, knock-in, humanized model, transgenic…), will be specific to each organism, but will rely on the latest improvements and most appropriate techniques of genome editing (ZFNs, TALEN, CRISPR/CAS9, etc.) or more classical transgenic approaches (such as DNA microinjection or lentiviral infection).
For the development of *in vitro* models based on induced pluripotent stem cell (iPSC) lines, the best suited approach will be designed for each project. Please pay attention that only projects for which agreements of lineage derivation from iPS of patients have already been obtained will be considered.

Only one project per research team can be funded for the current call.

In the scope of this call, **animal models** will be developed with the support of the following national platforms, most of them being part of CELPHEDIA, the French infrastructure that promotes innovative services and tool on model organisms:
Non-mammalian models:
- Worm, Biology of *C. elegans* IGF-Lyon and the *C. elegans* functional genomics CIML;
Mammalian models:
- Rabbit, transgenic rabbit models INRA Jouy-en-Josas;
- Rat, TRIP-Nantes and ICS Strasbourg (*CELPHEDIA*);
- Mouse, PHENOMIN infrastructure (*CELPHEDIA*).

For animal models, if specific needs are not covered by partner platforms, please contact the Foundation at **aap-bio@fondation-maladiesrares.com** in order to evaluate eligibility of the proposed model and conditions of services. In any case, technical issues must have been discussed with platforms to ensure feasibility of the project. The model will be developed with the support of a platform.

Human induced pluripotent stem cell (iPSC) lines will be developed with the support of the Platform iPSC Nantes.

Technologies and applications used by each platform, links to websites and contacts for each platform are listed in Annex 1.

Principal investigators must contact platforms for a detailed description of services that could fit the objectives of their project and to obtain assistance in optimizing the technical design. Technical eligibility of the project must be checked and approved by the platform before submission. This procedure should be planned early in the process in order to ensure timely submission of the project.
An additional delay of 4 weeks is planned after the submission, in order to allow discussions between the PI and the platform, in order to optimize if necessary, the experimental design and to receive **technical validation of the project by the platform, which is mandatory.**

Please confer to each platform to be informed of model distribution rules to make models available to the whole scientific community.

C – Evaluation

C1. Eligibility
The principal investigator of the project must belong to a **French academic research team** working in universities, other higher education institutions or research institutes, and/or to clinical/public health sector (university hospitals/public health organizations).
For the development of iPS cell lines, please pay attention that only projects for which agreements of lineage derivation from iPS of patients have already been obtained will be considered.

C2. Evaluation criteria
The following elements will be particularly considered in the evaluation of the project:

- Originality of the project;
- Relevance of preliminary data justifying the development of the model;
- Adequacy of the proposed model for the human disease;
- Clarity of objectives and outcomes of the project;
- Prospects in terms of disease knowledge and expected therapeutic benefits;
- Detailed description and timetable of the research program proposed;
- Quality of the team;
- Integration of the project in the research program of the applicant;
- Team experience and complementary/synergy of associated partners in model exploration;
- Positioning of the project in the national and international context.

C3. Selection
Proposals will be evaluated by at least two external, national and international, academic referees with a recognized expertise on the model. Projects will then be selected by a

scientific committee composed of experimental models experts and members of the Scientific Advisory Board of the French Foundation for Rare Diseases.

D – Funding

The French Foundation for Rare Diseases will provide financial support only to cover costs of **services provided by the platform** and will not cover equipment, running costs or personnel costs in the researcher's laboratory.

E – Proposal submission and schedule of the call

To complete and submit an application form, please access to the portal "**Applicant portal**".

Requirements for full proposals

* *Applicants resubmitting projects are required to provide a detailed answer to the comments provided by the FFRD Scientific Committee at a previous session and highlight changes in the revised version.*
* *Applicants who were previously funded by the FFRD are required to provide a detailed report on the results and impacts of all ended projects. For ongoing projects, a detailed progress and / or preliminary data report is required. This reporting (free format) is mandatory.*

Submission deadline for proposals: **April 2, 2019 (5:00 pm)**.
The provisional schedule of the call is the following:

February, 2019	Launch of the call
April 2, 2019	Submission deadline for proposals
April 30, 2019	Technical validation by platform
April-June 2019	Evaluation by two external referees
July 2019	Selection by the committee

The title of the selected projects and name of their principal investigator will be published on the website of the French Foundation for Rare Diseases by the end of July 2019. The summary written for a general audience may be used for communication purposes by the Foundation.

Results and Intellectual Property data resulting from projects funded through the call will be owned by the researcher's organizations.

Acknowledgement Policy: **It is required that projects funded acknowledge the French Foundation for Rare Diseases in all publications and communications.** Reference(s) of the publication(s) must be sent to the Foundation.

IRDiRC policies and guidelines: the project partners are expected to follow IRDiRC policies and guidelines. For more information see http://www.irdirc.org

ANNEX 1: PARTNER PLATFORMS OF THE CALL

"Caenorhabditis elegans Biology" Facility	
localization	CNRS UMS3421, Université Claude Bernard - Lyon I, Faculté de Médecine et de Pharmacie, 8 avenue Rockefeller, 69008 Lyon; Centre d'Immunologie de Marseille-Luminy, 163 Avenue de Luminy, 13009 Marseille
website	https://www.univ-lyon1.fr/recherche/entites-de-recherche/biologie-de-c-aelig-norhabditis-elegans-618137.kjsp http://www.ciml.univ-mrs.fr/fr/technologie/g-nomique-fonctionnelle-de-c-elegans
contacts	Maite Carre-Pierrat : maite.carre-pierrat@univ-lyon1.fr Jonathan Ewbank : ewbank@ciml.univ-mrs.fr
phone	04 26 68 82 84 ; 04 91 26 94 72
organism(s)	*Caenorhabditis elegans*
applications	KO and KI strain generation Mos-engineered mutant strains (KO, KI, tag) development Pangenomic screen (C. elegans functional genomics facility)
technologies	CRISPR/Cas 9 MosSCI, MosTIC, MosDEL COPAS Biosort worm sorter RNAi libraries

PHENOMIN	
localization	Institut Clinique de la Souris (ICS, Illkirch), Centre for Immunophenomics (CIPHE, Marseille)
website	http://www.phenomin.fr
contacts	contact@phenomin.fr
organism(s)	*Mus musculus* *Rattus norvegicus*
applications	Knock-Out constitutive mice and rats, conditional mice Knock-In mice and rats Transgenic mice Cell engineering Colony expansion and cryopreservation
technologies	from ES cells derived from the international IMPC resource CRISPR/Cas9 technology, ES-based methods injections of a single transgene PCR, qPCR and digital PCR genotyping phenotyping platforms

TRIP-Nantes

localization	INSERM UMR 1064, 30, boulevard Jean Monnet, 44093 Nantes
website	http://www.tgr.nantes.inserm.fr
contacts	Ignacio Anegon: Ignacio.Anegon@univ-nantes.fr Séverine Ménoret: severine.menoret@univ-nantes.fr
phone	02 40 08 74 15
organism(s)	*Rattus norvegicus (with inbred or outbred strains)*
applications	1) Generation of transgenic by small or large (BAC) DNA microinjectionn 2) Generation of KO and KI by exon exchange with ssODN or by insertion of DNA construct
technologies	DNA, ZFNs, TALENs, CRISPR/Cas9, PiggyBac transposon and lentiviral vectors microinjection Embryo freezing Q-PCR genotyping (zygosity animals) Identification of transgenes integration site (LM-PCR) Electroporation of embryos Production of animal with conventional health status or with SPF status

Atelier de modification génétique chez les mammifères non rongeurs

localization	INRA UMR 1198 Biologie du Développement et Reproduction 78350 Jouy en Josas
website	http://www6.jouy.inra.fr/bdr/Services-communs/Ateliers
contacts	Geneviève Jolivet genevieve.jolivet@jouy.inra.fr
phone	01 34 65 25 44
organism(s)	*Oryctolagus cuniculus* (New Zealand rabbits, NZ 1077)
applications	Production of transgenic rabbits (additive transgenesis) KO, KI, siRNA mediated knock down Design of transgene, TALEN, gRNA Phenotyping investigation
technologies	DNA, RNA injection, BAC DNA injection TALEN, ZFN, CRISPR technologies Genotyping (PCR, cloning of transgene integration sites) Ultrasound analysis, iDEXA -Dual X-ray Absorptiometry

PF iPSC Nantes	
localization	SFR-SANTE, Université de Nantes, UMR INSERM 1064, UMR CNRS 3556, CHU Nantes
website	https://sfrsante.univ-nantes.fr/plates-formes/modeles-cellulaires-et-geniques/plate-forme-ipsc-nantes-cellules-souches-pluripotentes-induites-1101640.kjsp?RH=1232979193469
contacts	Laurent David laurent.david@univ-nantes.fr ; pf-ipsc@univ-nantes.fr
phone	02 28 08 01 46
organism(s)	*Human*
applications	*Production of pluripotent cells from samples of patients with rare diseases, then differentiation into the cell type of interest to mimic the disease*
technologies	*Preparation of PBMC from blood samples* *Reprogramming somatic cells of patients by transcription factors*

PHENOMIN

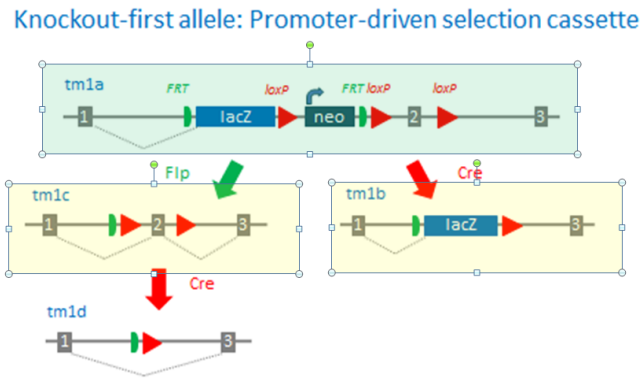
1. Generation of mouse models

a. Knock-Out constitutive, conditional mice:

Knock-Out models will be generated either:

1) from ES cells derived from the international IMPC resource (www.knockoutmouse.org). Models will be generated on a C57BL/6N genetic background. Knock-Out models with conditional potential (KO-first allele, tm1a) will be first produced; Knock-out by disruption of a critical exon (knock-out tm1b allele) and Conditional Knock-Outs (cKO tm1c allele) can then be provided by using Cre/LoxP and Flp/FRT systems.

KO-first allele, tm1a is provided, tm1c or tm1b alleles are provided on request:



2) In case of ES clones unavailability from the IMPC resource,

- **constitutive Knock-Out** models will be generated by the CRISPR/Cas9 nuclease technology (C57BL/6N genetic background only),
- **conditional Knock-Out** models will be generated *de novo* by ES-based methods (C57BL/6N genetic background only)).

b. Knock-In mice:

Knock-In mice [reporter gene, point mutation, humanization, targeted transgenesis (ROSA26), but excluding complex modifications] will be generated *de novo* by the PHENOMIN infrastructure in C57BL/6N genetic background.

c. Transgenic mice:

Transgenic mice (overexpression by pronuclear injection in C57BL/6N fertilized oocytes, but excluding complex constructs) will be generated *de novo* by the PHENOMIN infrastructure.

2. Models generation and availability to the investigator and to the scientific community

Models generated from the IMPC resource will be sent to the principal investigator of the project within 8 to 16 months after obtaining necessary information and materials to start the project. The majority of projects generated with IMPC clones are completed within 12 months following the initiation of the project. More time may be necessary in a few cases, depending on the time needed to obtain the IMPC clones or to achieve the targeting constructs.

Models generated *de novo* will be provided to the principal investigator within an average of 18 months after initiation of the project (minimum period: 12 months).

According to the IMPC consortium rules, **Knock-Out first models (with conditional potential) generated from this resource** and **knock-out models generated by CRISPR/Cas9 genome editing technology** will be made available to the principal investigator of the project and to any interested group, as soon as germline transmission is confirmed. Similarly, phenotypic data will be made available to the scientific community through a single database accessible on the web. The IMPC program provides a single web-based database referencing all resources. Any model already completed or underway in the world is thus made available to any interested group almost in real time.

Models generated *de novo* by the PHENOMIN infrastructure will be provided first to the principal investigator. Models will be preserved and archived and will then be made available to the whole scientific community within 24 months.

3. Technical eligibility check

Technical eligibility of the project must be checked and approved by the platform.

1. **Knock-Out constitutive, conditional models:**

   Several cases are possible.

- *Case 1: The model is already available or is developed by another IMPC resource center.*
  In this case, the proposal is not eligible. The principal investigator will be informed of the corresponding center.

- *Case 2: Three ES mutant clones with conditional inactivation of the gene are available.*
  These clones are referred to as "clones with conditional potential" on the IMPC website (www.knockoutmouse.org).
  In this case, only the recombinant ES clones generated by the consortium IMPC will be used for successful applications.

- *Case 3: For projects that do not match any of the two afore mentioned cases.*
  The *de novo* creation of the Knock-Out [constitutive, conditional] model (based on information available) will be evaluated specifically.

2. **Knock-In models:**

- Models will be generated by CRISPR/Cas9 (point mutation only) or ES cells for more complex design.
- For complex designs feasibility will be assessed.
- Only C57BL/6N genetic background is available.

3. **Transgenic models:**

- Only injections of a single transgene will be made.
- Generation of more than two lines and overexpression of the transgene cannot be guaranteed.
- Only C57BL/6N genetic background.

Transgenesis Rat ImmunoPhenomic (TRIP) platform

1. Generation of transgenic rats by embryonic microinjection of DNA fragments, BACs or lentiviral vectors.

2. Generation of KO / KI rats by embryonic microinjection or electroporation of gene-specific nucleases (Zinc Finger Nucleases, TALE Nucleases, meganucleases, CRISPR-Cas9).

Generation of KO rats with small deletions or deletions of several hundred Kb.

Generation of KI rats: nucleotide exchanges, cell lineage reporter genes by insertion of reporter genes under the control of endogenous promoters, loxP insertions, generation of transgenic rats by insertion of expression cassettes in the Rosa26 locus.

KOs or KIs can be constitutive or conditional using the Cre-loxP system.

All of these experiments can be performed in outbred strain rats (Sprague-Dawley, Wistar or Long Evans) or inbred rat strains.

We can also perform this type of experiment with a conventional or SPF health status.

All these services will be the subject of a detailed estimate depending on the service requested, the strains of rats used and the health status of the animals generated.

3. Genotyping (PCR, qPCR, Southern blot, LAM-PCR)

4. Cryopreservation of transgenic embryos / KO / KI.

TRIP is ISO 9001 (norm 2015) certified

Representative publications :

Ménoret S, Ouisse LH, Tesson L, Delbos F, Garnier D, Remy S, Usal C, Concordet JP, Giovannangeli C, Chenouard V, Brusselle L, Merieau E, Nerrière-Daguin V, Duteille F, Bellier-Waast F, Fraichard A, Nguyen TH, Anegon I. Generation of Immunodeficient Rats With Rag1 and Il2rg Gene Deletions and Human Tissue Grafting Models. Transplantation. 2018 Aug;102(8):1271-1278.

Charpentier M, Khedher AHY, Menoret S, Brion A, Lamribet K, Dardillac E, Boix C, Perrouault L, Tesson L, Geny S, De Cian A, Itier JM, Anegon I, Lopez B, Giovannangeli C, Concordet JP. CtIP fusion to Cas9 enhances transgene integration by homology-dependent repair. Nat Commun. 2018 Mar 19;9(1):1133.

Remy S, Chenouard V, Tesson L, Usal C, Ménoret S, Brusselle L, Heslan JM, Nguyen TH, Bellien J, Merot J, De Cian A, Giovannangeli C, Concordet JP, Anegon I. Generation of gene-edited rats by delivery of CRISPR/Cas9 protein and donor DNA into intact zygotes using electroporation. Sci Rep. 2017 Nov 29;7(1):16554.

Renaud JB, Boix C, Charpentier M, De Cian A, Cochennec J, Duvernois-Berthet E, Perrouault L, Tesson L, Edouard J, Thinard R, Cherifi Y, Menoret S, Fontanière S, de Crozé N, Fraichard A, Sohm F, Anegon I,

Concordet JP, Giovannangeli C. Improved Genome Editing Efficiency and Flexibility Using Modified Oligonucleotides with TALEN and CRISPR-Cas9 Nucleases. Cell Rep. 2016 Mar 8;14(9):2263-72.

Jung CJ, Ménoret S, Brusselle L, Tesson L, Usal C, Chenouard V, Remy S, Ouisse LH, Poirier N, Vanhove B, de Jong PJ, Anegon I. Comparative Analysis of piggyBac, CRISPR/Cas9 and TALEN Mediated BAC Transgenesis in the Zygote for the Generation of Humanized SIRPA Rats. Sci Rep. 2016 Aug 17;6:31455.

Ménoret S, De Cian A, Tesson L, Remy S, Usal C, Boulé JB, Boix C, Fontanière S, Crénéguy A, Nguyen TH, Brusselle L, Thinard R, Gauguier D, Concordet JP, Cherifi Y, Fraichard A, Giovannangeli C, Anegon I. Homology-directed repair in rodent zygotes using Cas9 and TALEN engineered proteins. Sci Rep. 2015 Oct 7;5:14410.

Larcher T, Lafoux A, Tesson L, Remy S, Thepenier V, François V, Le Guiner C,Goubin H, Dutilleul M, Guigand L, Toumaniantz G, De Cian A, Boix C, Renaud JB, Cherel Y, Giovannangeli C, Concordet JP, Anegon I, Huchet C. Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. PLoS One. 2014 Oct 13;9(10):e110371.

Tesson L, Usal C, Ménoret S, Leung E, Niles BJ, Remy S, Santiago Y, Vincent AI, Meng X, Zhang L, Gregory PD, Anegon I, Cost GJ. Knockout rats generated by embryo microinjection of TALENs. Nat Biotechnol. 2011 Aug 5;29(8):695-6. doi: 10.1038/nbt.1940.

A. M. Geurts, G. J. Cost, J. C. Miller, Y. Freyvert, B. Zeitler, V. M. Choi, S. S. Jenkins, A. Wood, X. Cui, X. Meng, A. Vincent, S. Lam, R. C. DeKelver, M. Michalkiewicz, R. Schilling, J. Foeckler, S. Kalloway, H. Weiler, S. Ménoret, I. Anegon, G. D. Davis, P. Sullivan, L. Zhang, E. J. Rebar, P. D. Gregory, F. D. Urnov, H. J. Jacob, and R. Buelow. Knockout rats produced via embryo microinjection of designed Zinc Finger Nucleases. 2009. Science, 325:433.