



*Fondazione per la Ricerca
sulla Fibrosi Cistica - ETS*
italian cystic fibrosis research foundation

**WEBINAR DI
PRESENTAZIONE**
15-17 GENNAIO 2024



**SERVIZI
ALLA RICERCA**



*Fondazione per la Ricerca
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**WEBINAR DI
PRESENTAZIONE**

15-17 GENNAIO 2024

MERCOLEDI' 17 GENNAIO 2024

SCP

(Servizio Colture Primarie)

Responsabile:

Valeria Capurro, PhD

Istituto Giannina Gaslini,

Genova

INSTITUTION:

The service born in 2012 from the collaboration between Cystic Fibrosis Research Foundation (FFC Research) and the Medical Genetics laboratory of the Giannina Gaslini Institute.

AIM:

The service aims to provide an important biological model of bronchial epithelium for studies related to Cystic Fibrosis (CF):

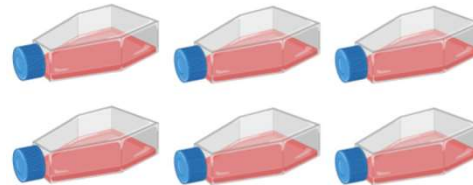
- 1) the physiology of the epithelium and the alterations caused by CFTR loss of function;
- 2) the efficacy of pharmacological and genetic therapies aiming at the correction of CF basic defect;
- 3) the interaction between bacteria and epithelial cells and the mechanisms associated with the inflammatory response.

Isolation of cells from bronchi



SERVICE:

Expansion



Cryopreservation



The facility provides human bronchial primary cells (HBECs) derived from both CF and non-CF bronchi to researchers of the FFC network and researchers with CF-related grants.

To get access to the facility, the researcher must provide, within the request form, a brief description of the experiments to be done on HBECs to address their technical feasibility.

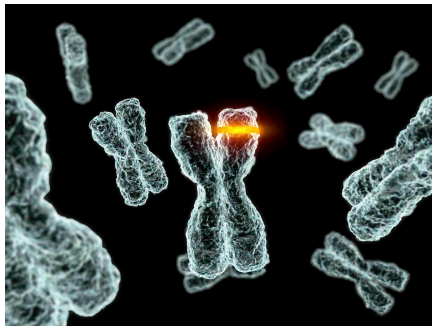
We provide to all users of the service:

- A protocol for the correct culture of the cells sent.
- The possibility for interested researchers to carry out a period of training at our laboratories.
- Our technical expertise.



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LIST OF GENOTYPES OF ISOLATED CF BRONCHIAL CELLS FROM THE SCP 2012/2024



| GENOTYPE | | |
|-------------------------|------------------------|-------------------------|
| F508del/F508del | F508del/3878delG | N1088D/G542X |
| F508del/G542X | F508del/1874insT+Y577F | N1303K/2183AA>G |
| F508del/R1162X | F508del/L927P | N1303K/711+5G>A |
| F508del/1717-1G>A | F508del/C276X | R1006C/M1V |
| F508del/N1303K | F508del/L1077P | R1158X/3849 +10KbC>T |
| F508del/R553X | F508del/2789+5G>A | R1162X/2789+5G>A |
| F508del/CFTRdelE 17A-18 | F508del/Q552X | R1162X/ 3849+10KbC>T |
| F508del/3849+10KbC>T | G542X/711+5G>A | del Ex 22-23-24/UK |
| F508del/62+1G>T | G542X/H609R | 1525-1G>A/G458R |
| F508del/G85E | G542X/1717-1G>A | 2789+5G>A/M1V |
| F508del/2184insA | I502T/N1303K | 2789+5G>A/R1070Q |
| F508del/1259insA | | |

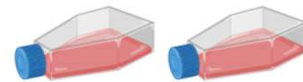


PROCEDURE:

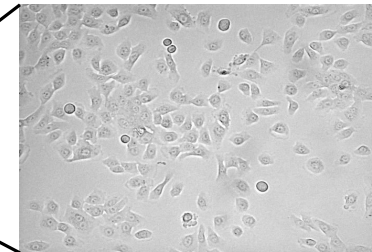
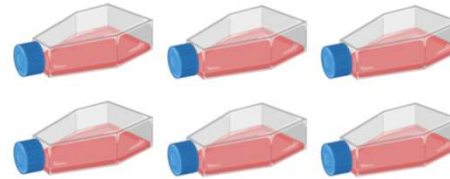
Cells and medium
are shipped to FFC laboratories



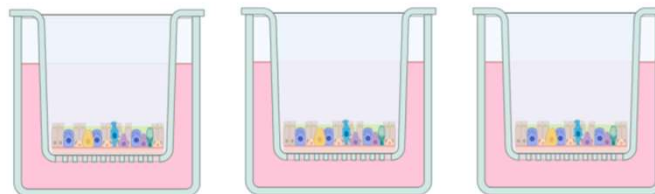
Thawing



First passage (70% confluence)



Plating on permeable supports

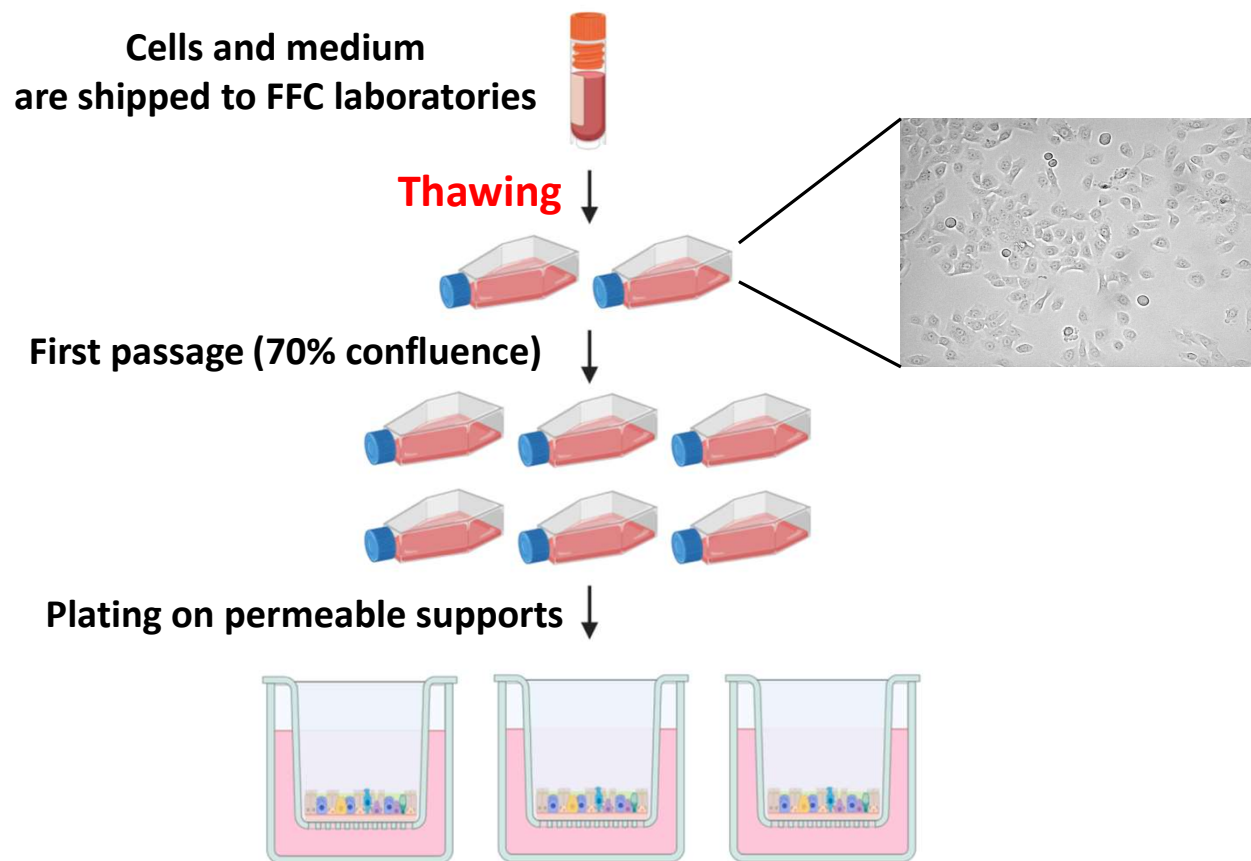


Cells are cultured on Snapwell or transwell supports
for 2-4 weeks to generate polarized epithelia

PROCEDURE:

THAWING

Cells are rapidly thawed at 37°C resuspended in serum-free medium, centrifuged and resuspended again in serum-free medium and sown in one or two flasks previously treated with collagen



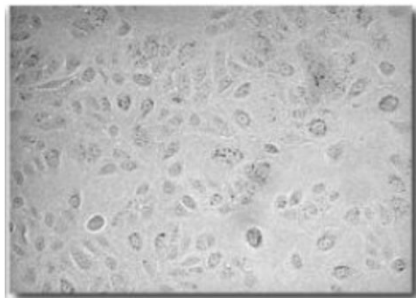
Cells are cultured on Snapwell or transwell supports for 2-4 weeks to generate polarized epithelia

PROCEDURE:

FIRST PASSAGE

During this first phase the cells must be checked carefully. It is best to trypsinize the cells before they reach 70% of confluence.

A higher density could limit cell growth and make cells unable to differentiate. Change the medium every two days.

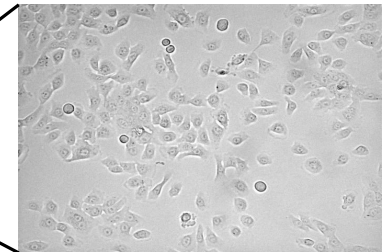
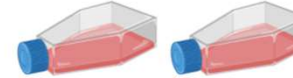


Higher density

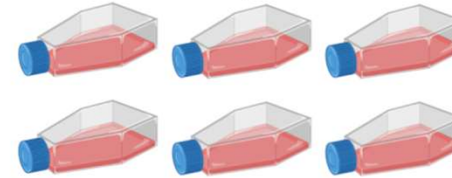
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are shipped to FFC laboratories



Thawing



First passage (70% confluence)



Plating on permeable supports



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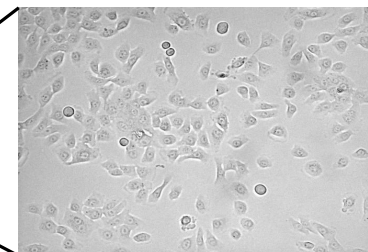
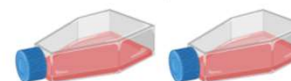
TRYPsinIZATION

The cells are trypsinized, centrifuged and the pellet is resuspended in medium LHC9/RPMI1640 in a volume sufficient to seed ~750.000 cells per flask T75 (13 ml per flask). Generally from 2 first flasks you get a sufficient number of cells for 6 flasks.

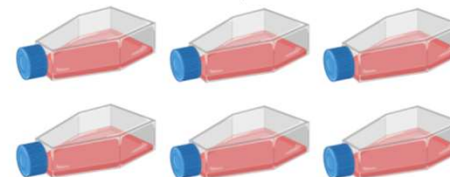
Cells and medium
are shipped to FFC laboratories



Thawing



First passage (70% confluence)



Plating on permeable supports



Cells are cultured on Snapwell or transwell supports
for 2-4 weeks to generate polarized epithelia

PROCEDURE:

PLATING ON PERMEABLE SUPPORTS

Before seeding on a porous support the cells can be allowed to grow to a greater density than those of the first phase.

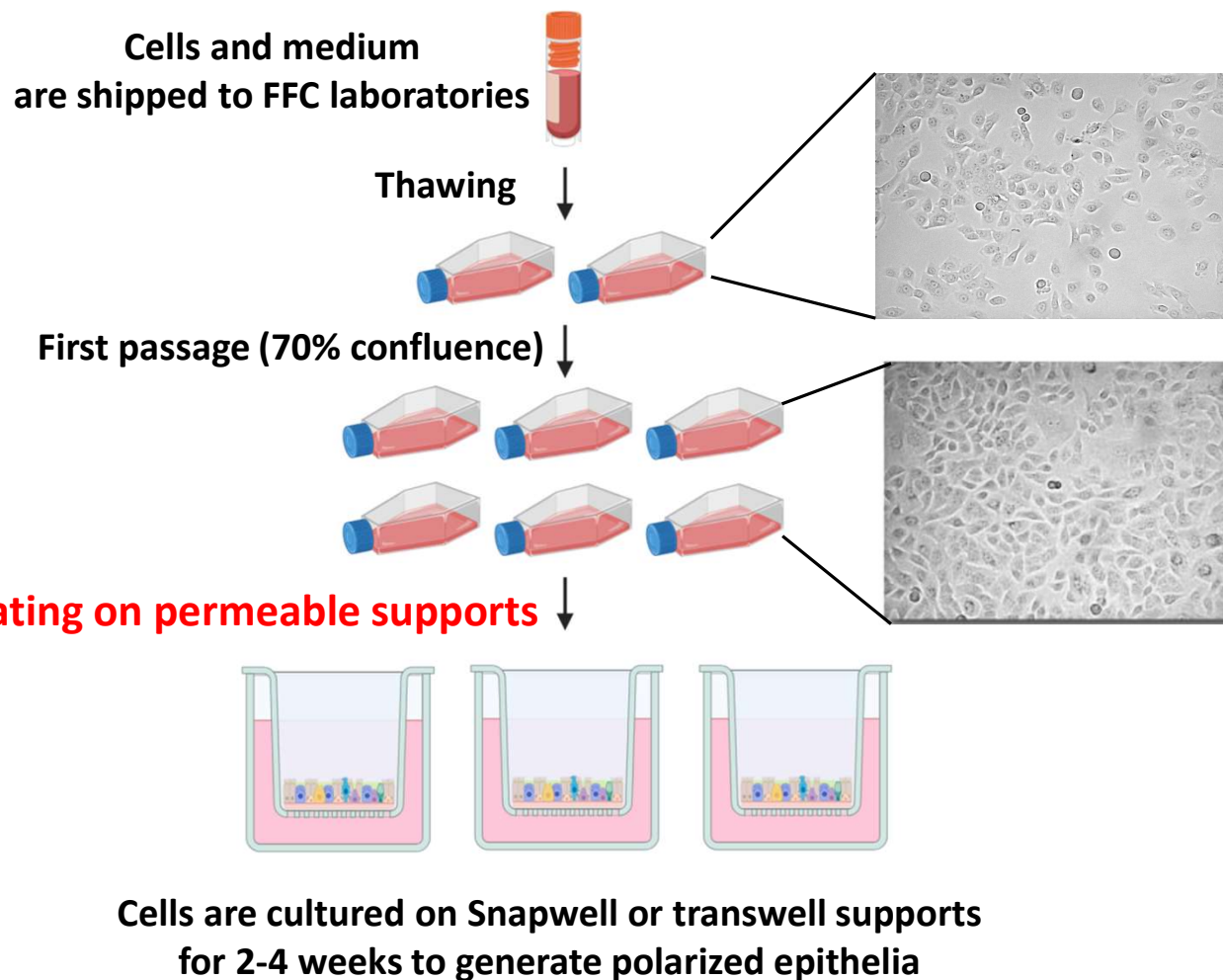
The cells are trypsinized and the suspension of cells is counted and centrifuged. The pellet is resuspended in LHC9/RPMI1640 medium and the cells are seeded onto the porous support.

Snapwell 3801 (1.33 cm²) 500.000 cells

Transwell 3450 (4.5 cm²) 2.5 million cells

HTS-Traswell 24 (0.33 cm²) 250.000 cells

24 hours after seeding the medium is replaced with DMEM/F12 with 2% UltrosorG (2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin).

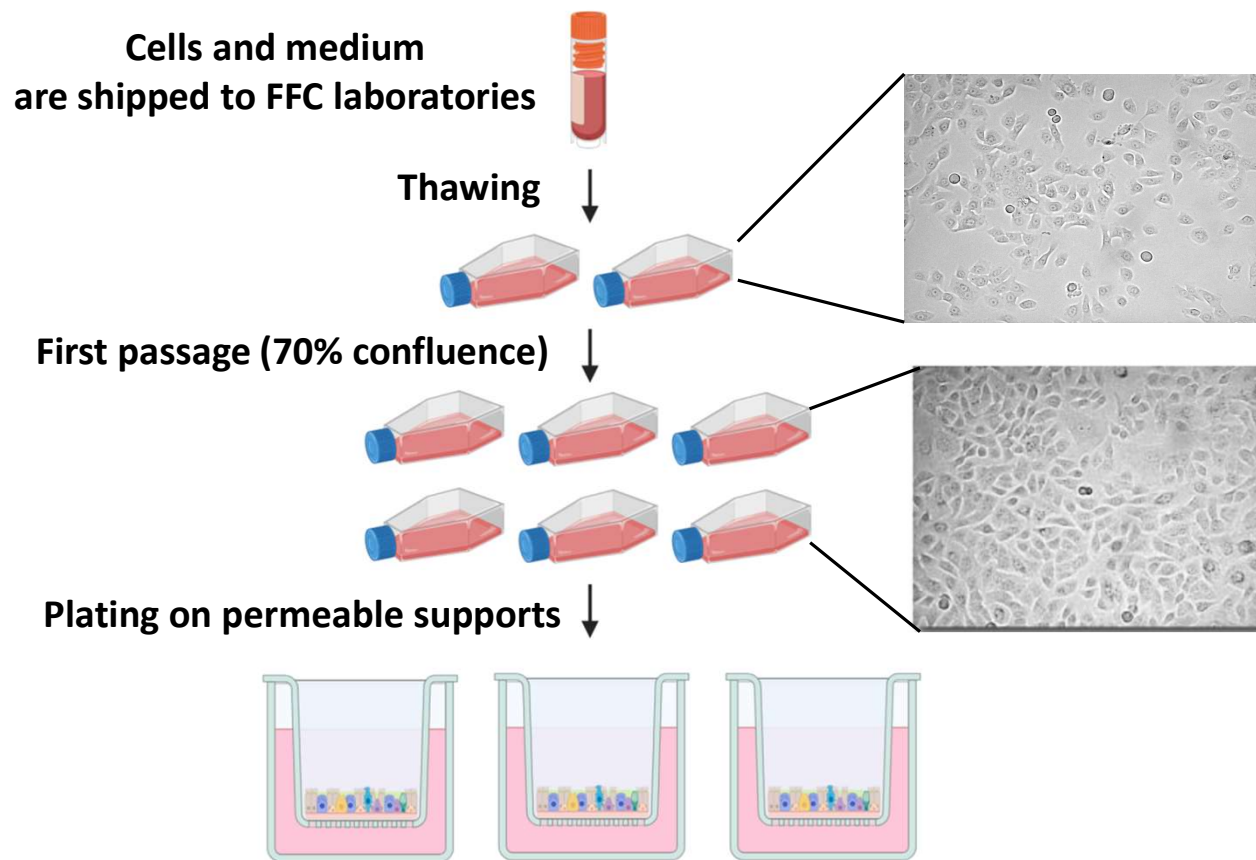


PROCEDURE:

DIFFERENTIATION

The medium is changed every 24 hours. On the fifth day only the basal medium is replaced, leaving the apical side dry (air liquid interface condition).

If the cells generate a high resistance the epithelium apical surface will remain dry. After 2/4 weeks the cells should show marked differentiation.



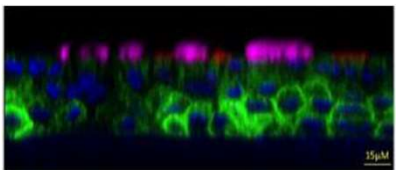
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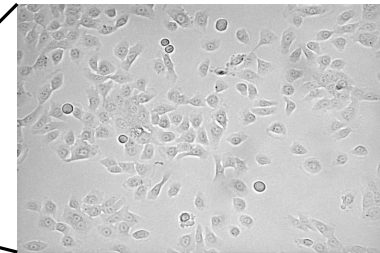
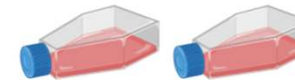
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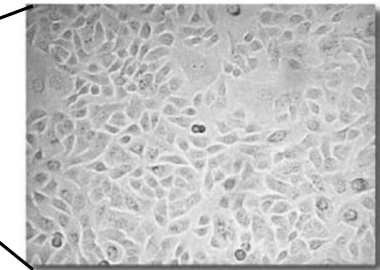
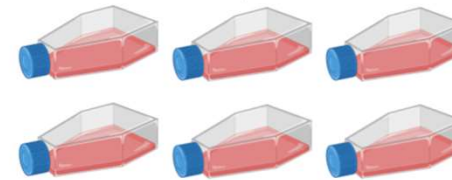
Example of differentiated bronchial epithelium observed under the confocal microscope. Cilia **magenta**, goblet cells **red**, apical membrane in **green**

Cells and medium are shipped to FFC laboratories

Thawing



First passage (70% confluence)



Plating on permeable supports



Cells are cultured on Snapwell or transwell supports for 2-4 weeks to generate polarized epithelia

PROCEDURE:

PLATING ON PERMEABLE SUPPORTS 2.0

Before seeding on a porous support the cells can be allowed to grow to a greater density than those of the first phase.

The cells are trypsinized and the suspension of cells is counted and centrifuged. The pellet is resuspended in **PNEUMACULT EX-PLUS** medium and the cells are seeded onto the porous support.

24 hours after seeding the basal medium is replaced with **PNEUMACULT ALI** medium, leaving the apical side dry (air liquid interface condition).

DIFFERENTIATION 2.0

The medium is changed every 48 hours.

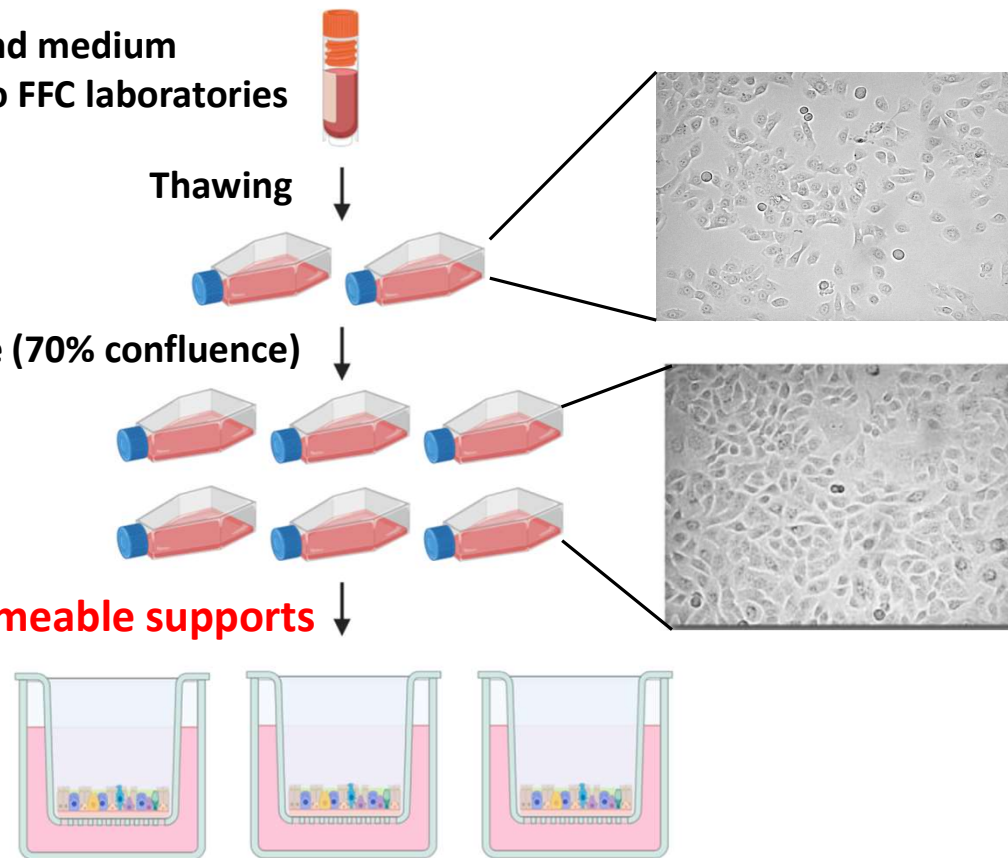
After 2/4 weeks the cells should show marked differentiation and mucus!!!

Cells and medium
are shipped to FFC laboratories

Thawing

First passage (70% confluence)

Plating on permeable supports



Cells are cultured on Snapwell or transwell supports
for 2-4 weeks to generate polarized epithelia

COMPONENTS SUPPLIED:

| SERVICE | ADVANCED SERVICE |
|--|--|
| Cryovial of frozen bronchial epithelial cells (each vial contains approximately 500.000 cells possibly at the third passage) | 24 differentiated epithelia (12-18 days of ALI condition) |
| Coating solution (25mg/ml) | Expertise |
| LHC9/RPMI1640 medium (frozen in 40 ml aliquots for 1 vial) | Scientific support |
| UltroserG | |



PRICES:

| SERVICE COSTS | |
|---|-------|
| 1 vial of cells with coating solution | € 125 |
| 1 vial of cells with coating solution plus LHC9/RPMI1640 | € 175 |
| UltroserG for preparation of 500 ml of differentiative medium | € 125 |
| LHC9/RPMI1640, 500 ml | € 150 |

ADVANCED SERVICE COSTS

| | |
|-----------------------------|--------|
| 24 differentiated epithelia | € 2000 |
|-----------------------------|--------|

OUR COSTS TO GENERATE 24 EPITHELIA

| | |
|---|--------|
| 1 vial of cells plus LHC9/RPMI1640 | € 175 |
| 24 porous supports | € 926 |
| PneumaCult ALI | € 464 |
| Other consumables (ExPlus, reagents, Lab Plasticware, electrophysiological validation) | € 435 |
| TOT | € 2000 |

APPLICATIONS of BRONCHIAL CELL CULTURES:

- Study transepithelial ion transport and the activity of CFTR and other channels/transporters using Ussing chamber or similar systems
- Study the expression of proteins by immunofluorescence or western blot
- Study gene expression by RNA extraction followed by RT-PCR or microarray analysis
- Study the effect of interactions between bacteria and epithelial cells
- Evaluate efficacy or possible side effects of new compounds on the bronchial epithelium

New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:

If you need **cells** and **medium** (**standard service**):

SCP should be listed as Service at **point 9** and the corresponding costs should be included in the budget under "Service"
(costs for cell vials and/or medium)

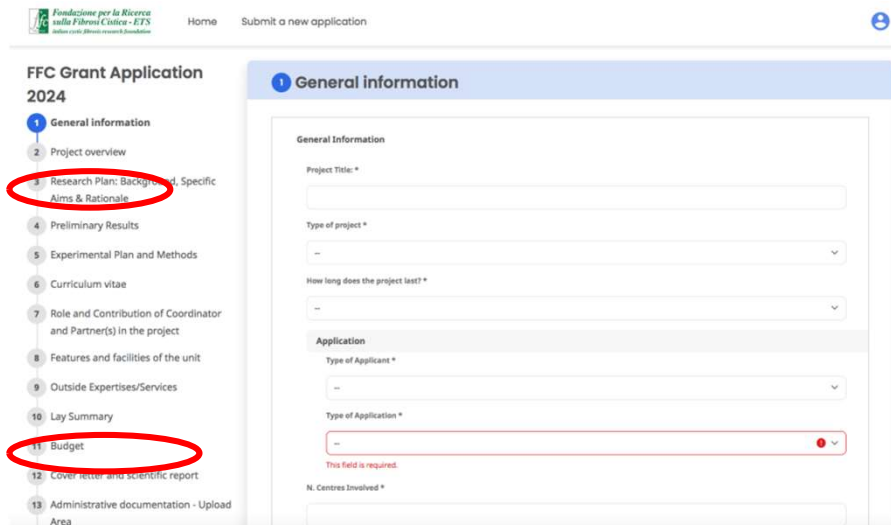
- 5 Experimental Plan and Methods
- 6 Curriculum vitae
- 7 Role and Contribution of Coordinator and Partner(s) in the project
- 8 Features and facilities of the unit
- 9 Outside Expertises/Services**
- 10 Lay Summary
- 11 Budget
- 12 Cover letter and scientific report
- 13 Administrative documentation - Upload Area

New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:

If you need **differentiated epithelia (advanced service)**:

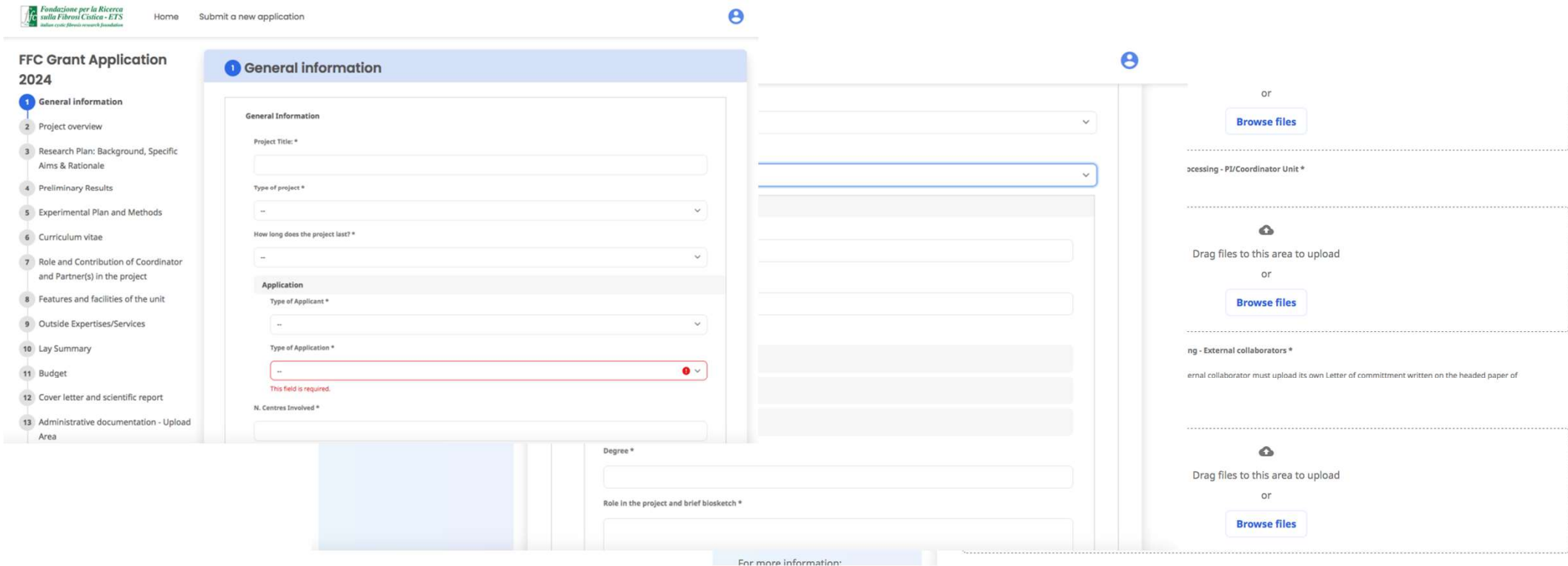
SCP should be listed:

- as Service at **point 9** and the corresponding costs should be included in the budget under "Service" (costs for epithelia preparation)
- as External Collaboration at **point 1** (1 SCP person) with the Collaboration Letter uploaded at **point 13**.



As a scientific collaboration, the SCP person should be listed as co-author in any publication

New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:



FFC Grant Application 2024

Home Submit a new application

- 1 General information
- 2 Project overview
- 3 Research Plan: Background, Specific Aims & Rationale
- 4 Preliminary Results
- 5 Experimental Plan and Methods
- 6 Curriculum vitae
- 7 Role and Contribution of Coordinator and Partner(s) in the project
- 8 Features and facilities of the unit
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1 General information

General Information

Project Title *

Type of project *

How long does the project last? *

Application

Type of Applicant *

Type of Application *

N. Centres Involved *

Degree *

Role in the project and brief biosketch *

Processing - PI/Coordinator Unit *

Drag files to this area to upload

or

Drag files to this area to upload

or

Drag files to this area to upload

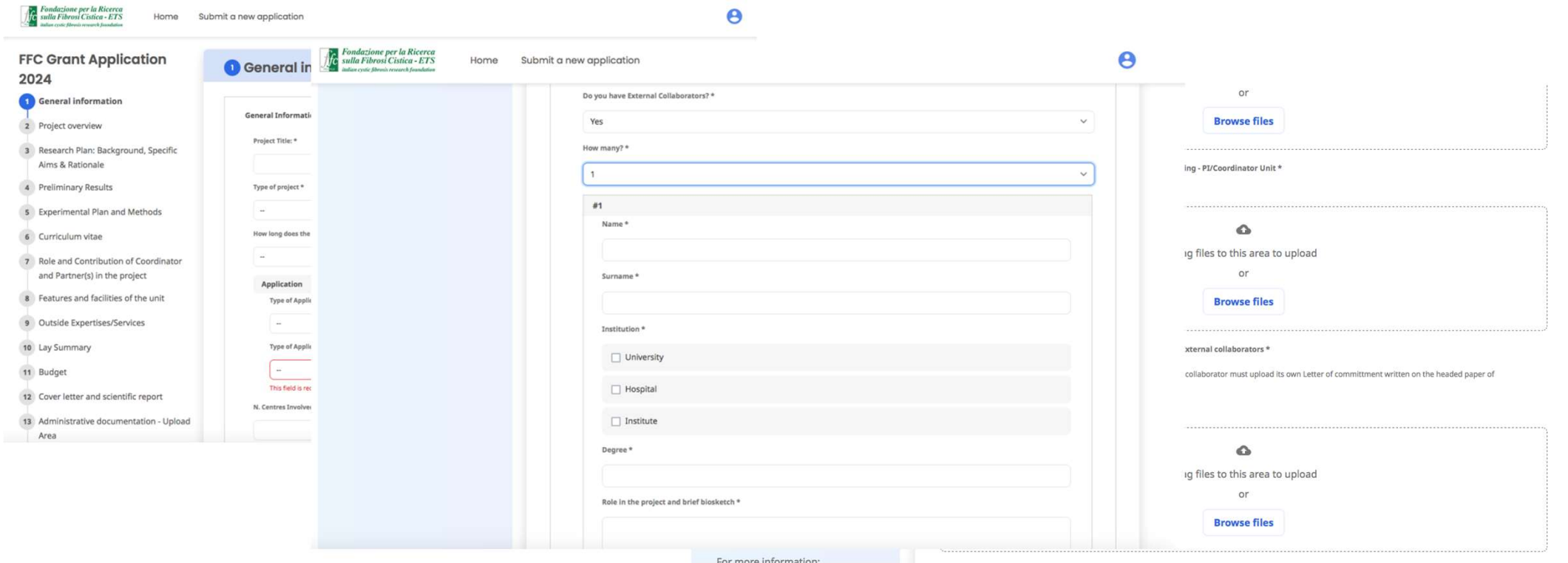
or

External collaborators *

External collaborator must upload its own Letter of commitment written on the headed paper of

For more information:

New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:

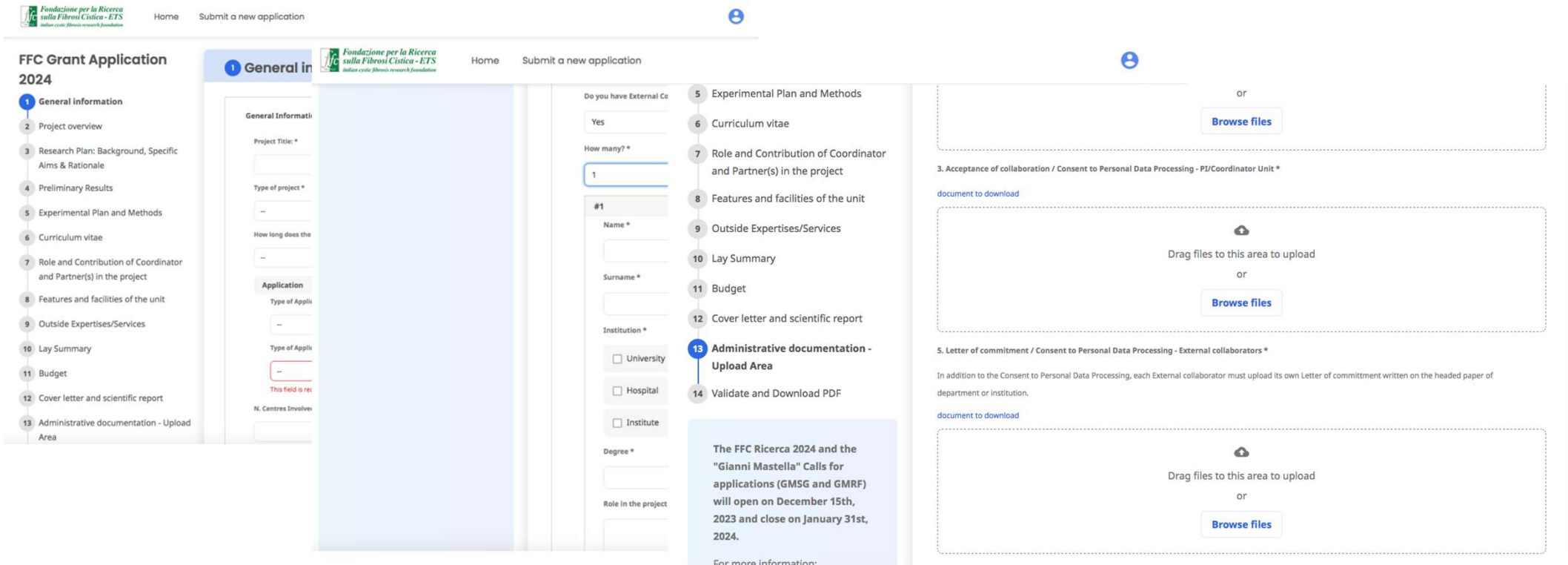


The screenshot displays the 'FFC Grant Application 2024' form, specifically the 'General information' section. The form is structured as follows:

- Navigation:** Home, Submit a new application
- Left Sidebar (Steps):**
 - 1 General information (selected)
 - 2 Project overview
 - 3 Research Plan: Background, Specific Aims & Rationale
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- Main Form Fields:**
 - General Information:**
 - Project Title: *
 - Type of project: *
 - How long does the project last: *
 - Application:**
 - Type of Application: *
 - Type of Applicant: *
 - External Collaborators:**
 - Do you have External Collaborators? * (Yes)
 - How many? * (1)
 - #1:**
 - Name *
 - Surname *
 - Institution *
 - University
 - Hospital
 - Institute
 - Degree *
 - Role in the project and brief biosketch *
- File Uploads:**
 - OR
 - [Browse files](#)
 - ing - PI/Coordinator Unit *
 - ig files to this area to upload
 - OR
 - [Browse files](#)
 - external collaborators *
 - collaborator must upload its own Letter of commitment written on the headed paper of
 - ig files to this area to upload
 - OR
 - [Browse files](#)

For more information:

New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:



Fondazione per la Ricerca sulla Fibrosi Cistica - ETS
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1 General information

General Information

Project Title: *

Type of project *

How long does the

Application

Type of Appli

Type of Appli

N. Centres Involve

Do you have External Co

Yes

How many? *

1

#1

Name *

Surname *

Institution *

University

Hospital

Institute

Degree *

Role in the project

- Experimental Plan and Methods
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- Administrative documentation - Upload Area**
- Validate and Download PDF

The FFC Ricerca 2024 and the "Gianni Mastella" Calls for applications (GMSG and GMRF) will open on December 15th, 2023 and close on January 31st, 2024.

For more information:

or

[Browse files](#)

3. Acceptance of collaboration / Consent to Personal Data Processing - PI/Coordinator Unit *

document to download

Drag files to this area to upload

or

[Browse files](#)

5. Letter of commitment / Consent to Personal Data Processing - External collaborators *

In addition to the Consent to Personal Data Processing, each External collaborator must upload its own Letter of commitment written on the headed paper of department or institution.

document to download

Drag files to this area to upload

or

[Browse files](#)

CONTACTS:

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THANKS



Nicoletta Pedemonte
Maria Teresa Lena
Cristina Pastorino
Emanuela Pesce
Valeria Tomati



Luis Galietta