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 sulla Fibrosi Cistica - ETS
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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

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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:

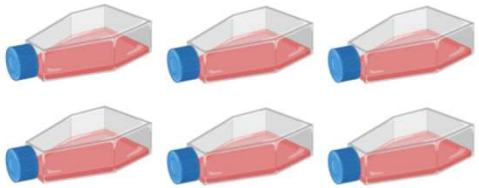
The service aims to provide an important biological model of bronchial epithelium for studies related to Cystic Fibrosis (CF):

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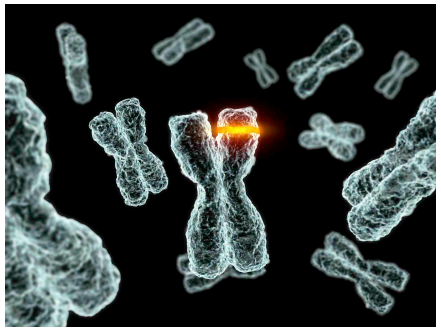
LIST OF GENOTYPES OF ISOLATED CF BRONCHIAL CELLS FROM THE SCP 2012/2024



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		

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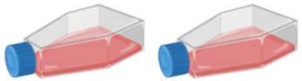
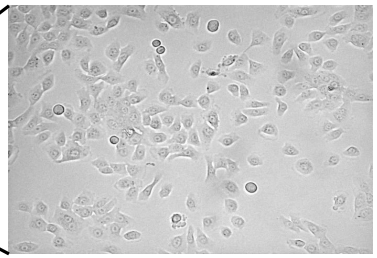
PROCEDURE:

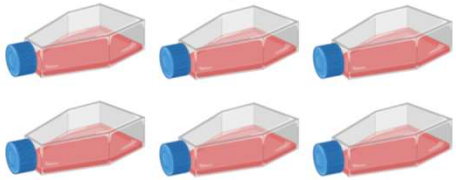
Cells and medium
are shipped to FFC laboratories

Thawing ↓

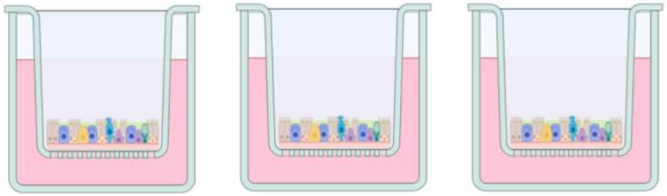




First passage (70% confluence) ↓



Plating on permeable supports ↓



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

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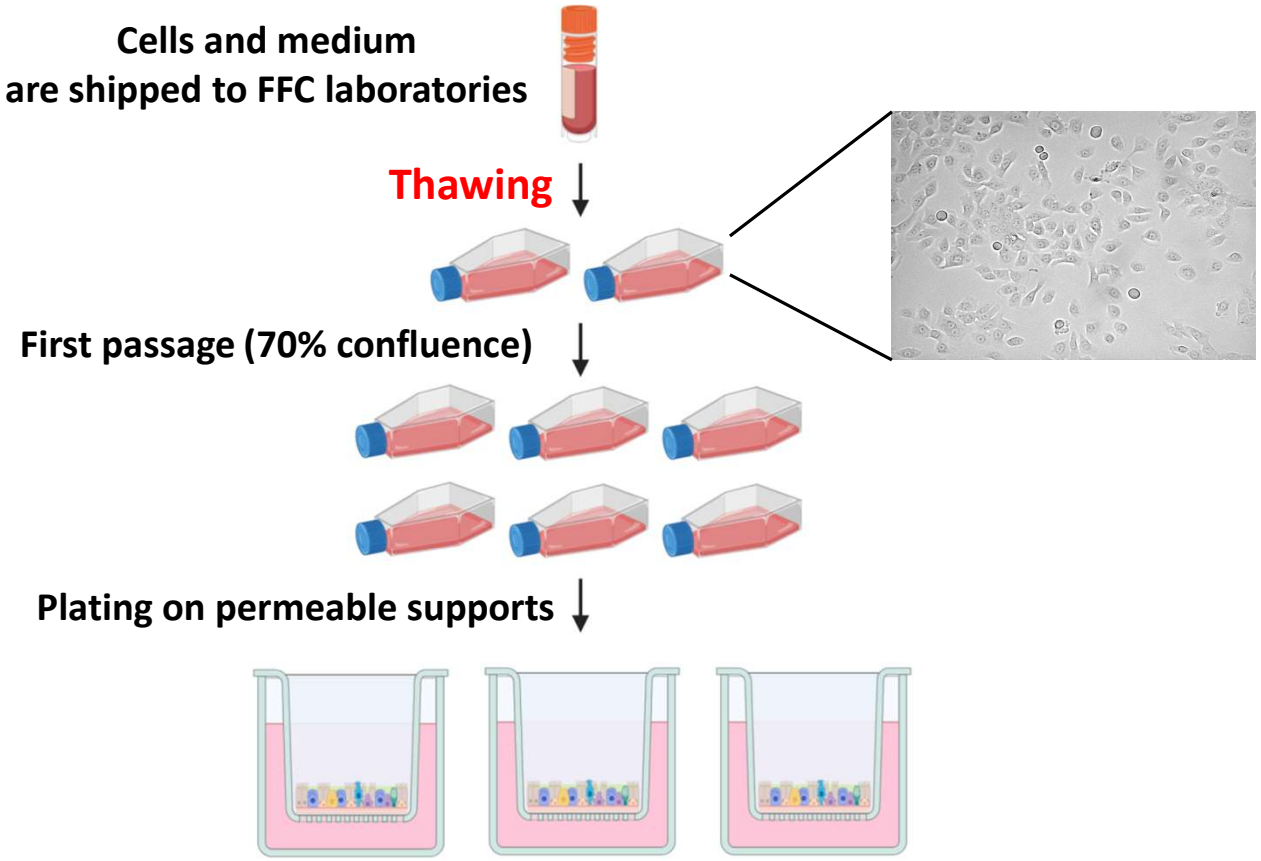
PROCEDURE:

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



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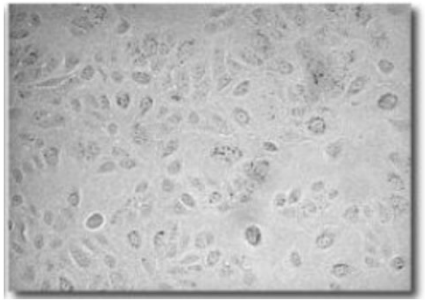
PROCEDURE:

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.

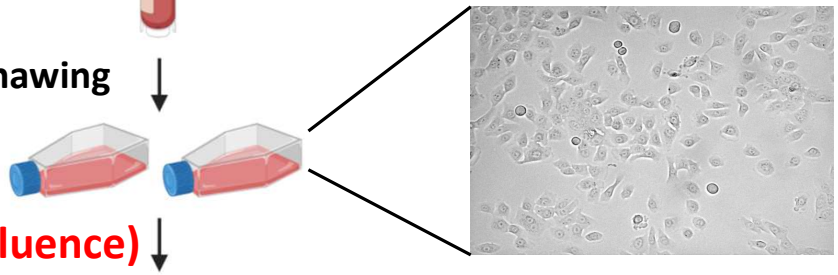
A higher density could limit cell growth and make cells unable to differentiate. Change the medium every two days.



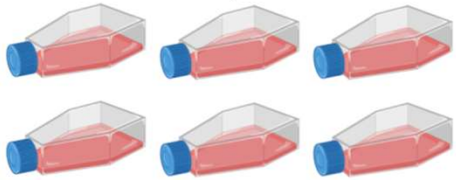
Higher density

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

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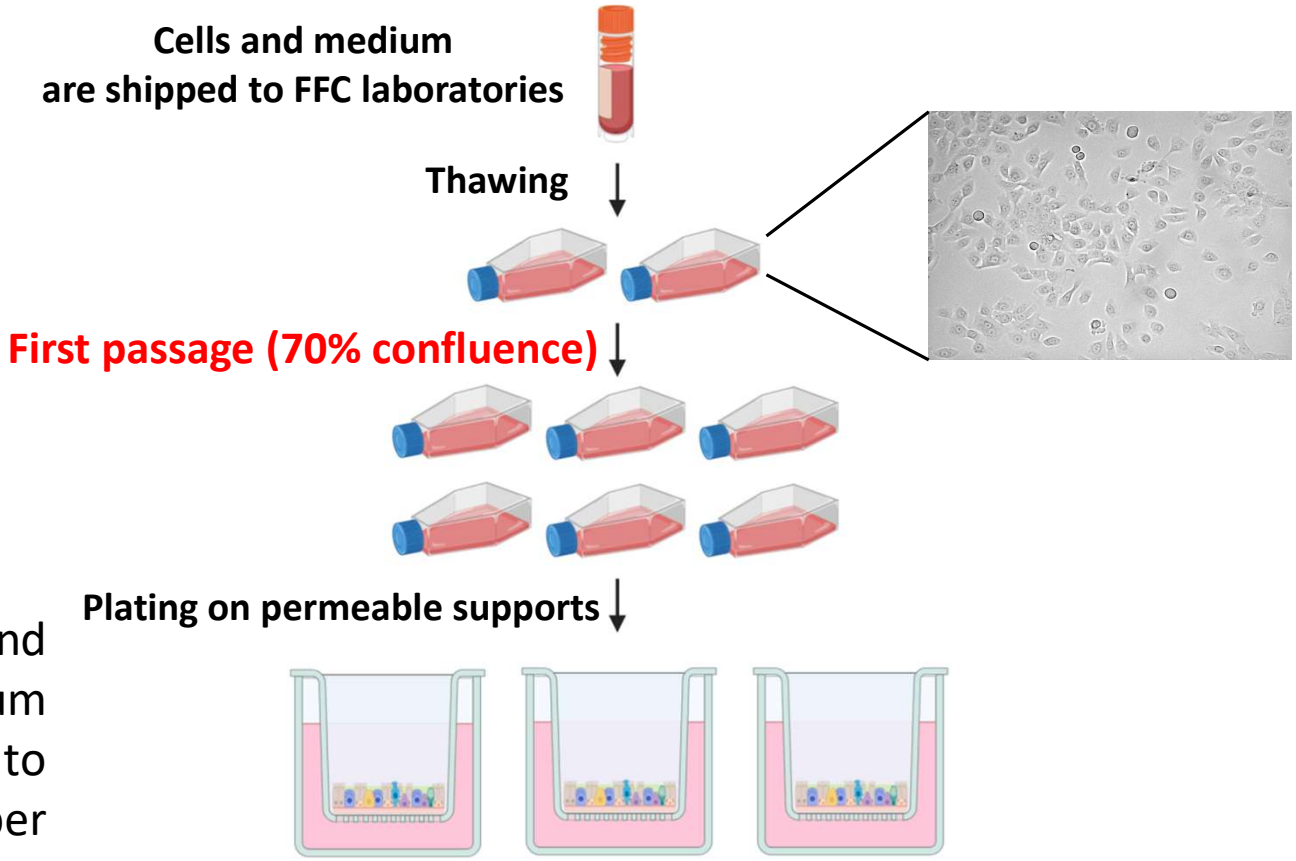
PROCEDURE:

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.

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.



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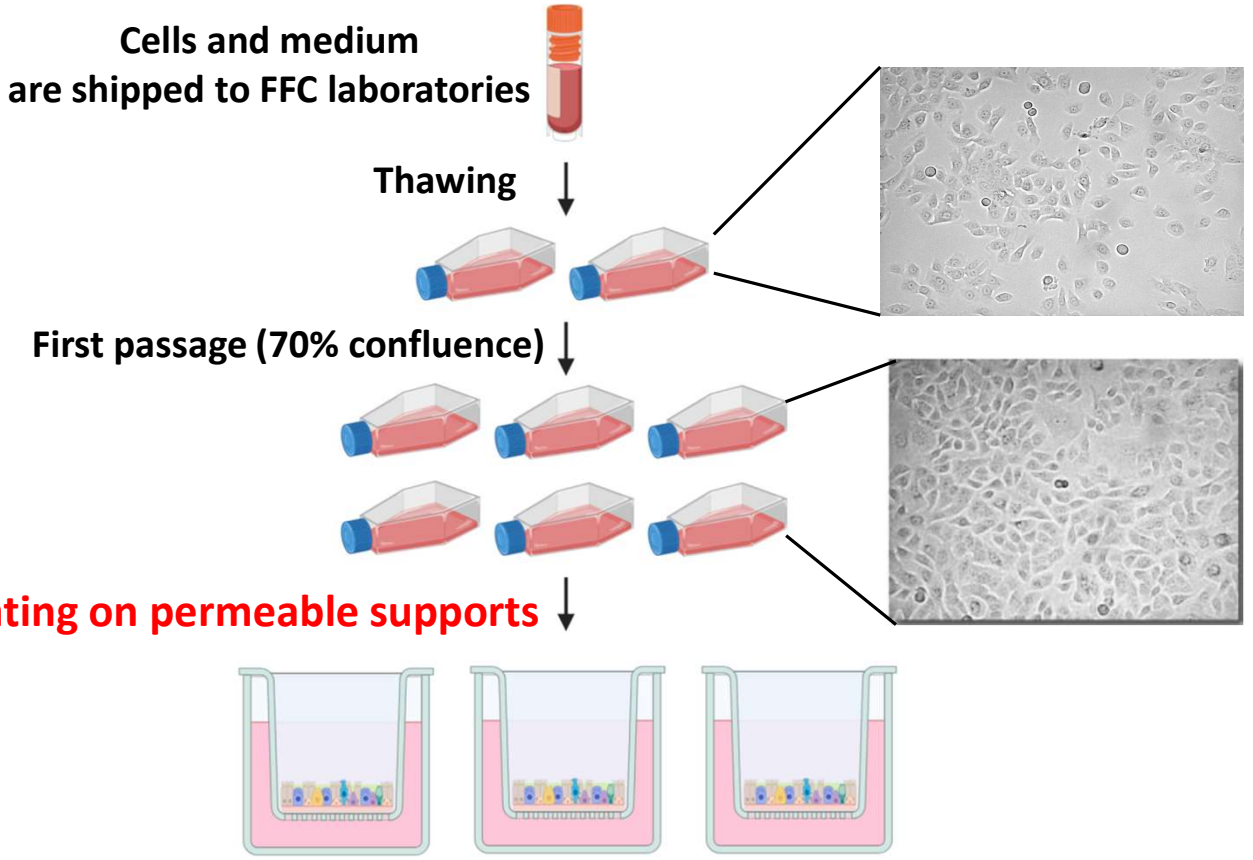
PROCEDURE:

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 cm^2) 500.000 cells
Transwell 3450 (4.5 cm^2) 2.5 million cells
HTS-Traswell 24 (0.33 cm^2) 250.000 cells

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



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

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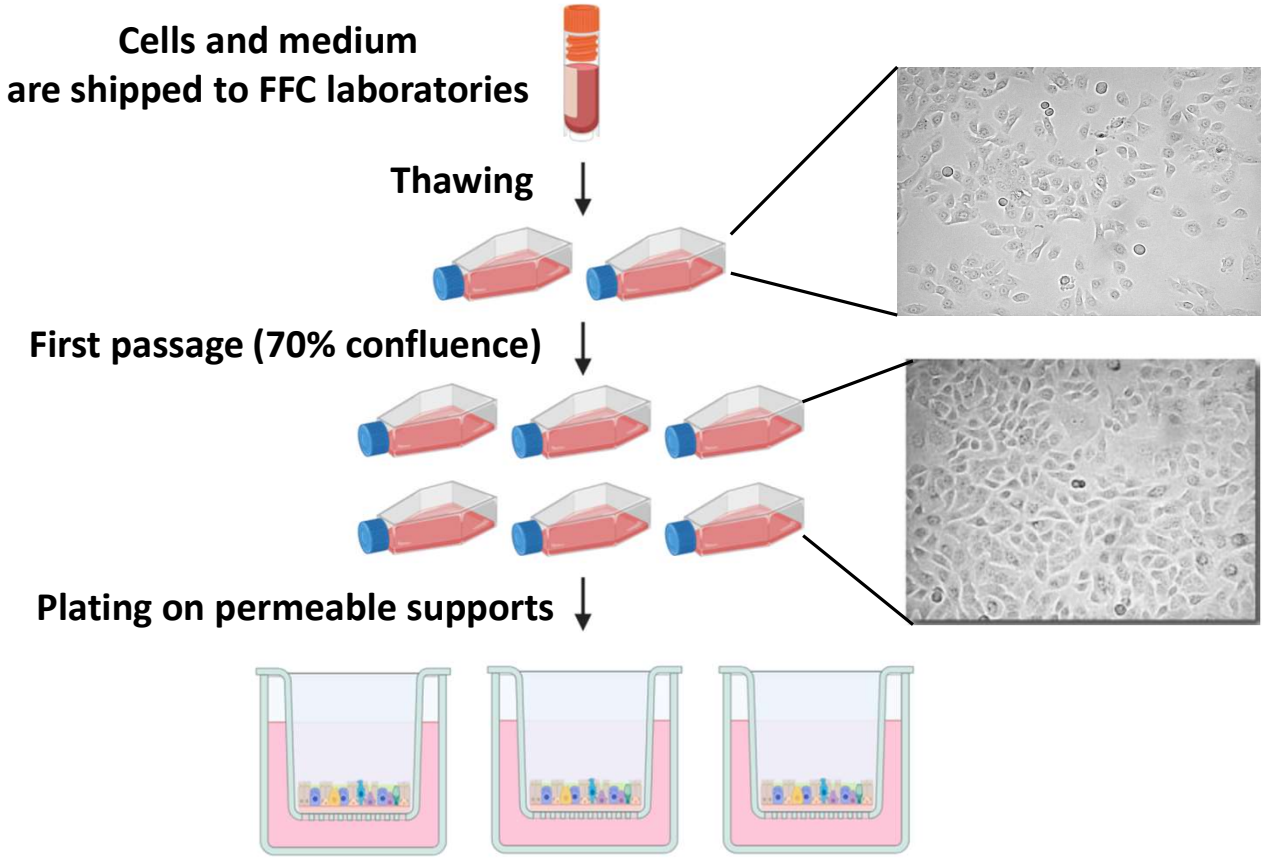
PROCEDURE:

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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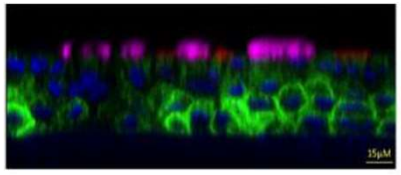
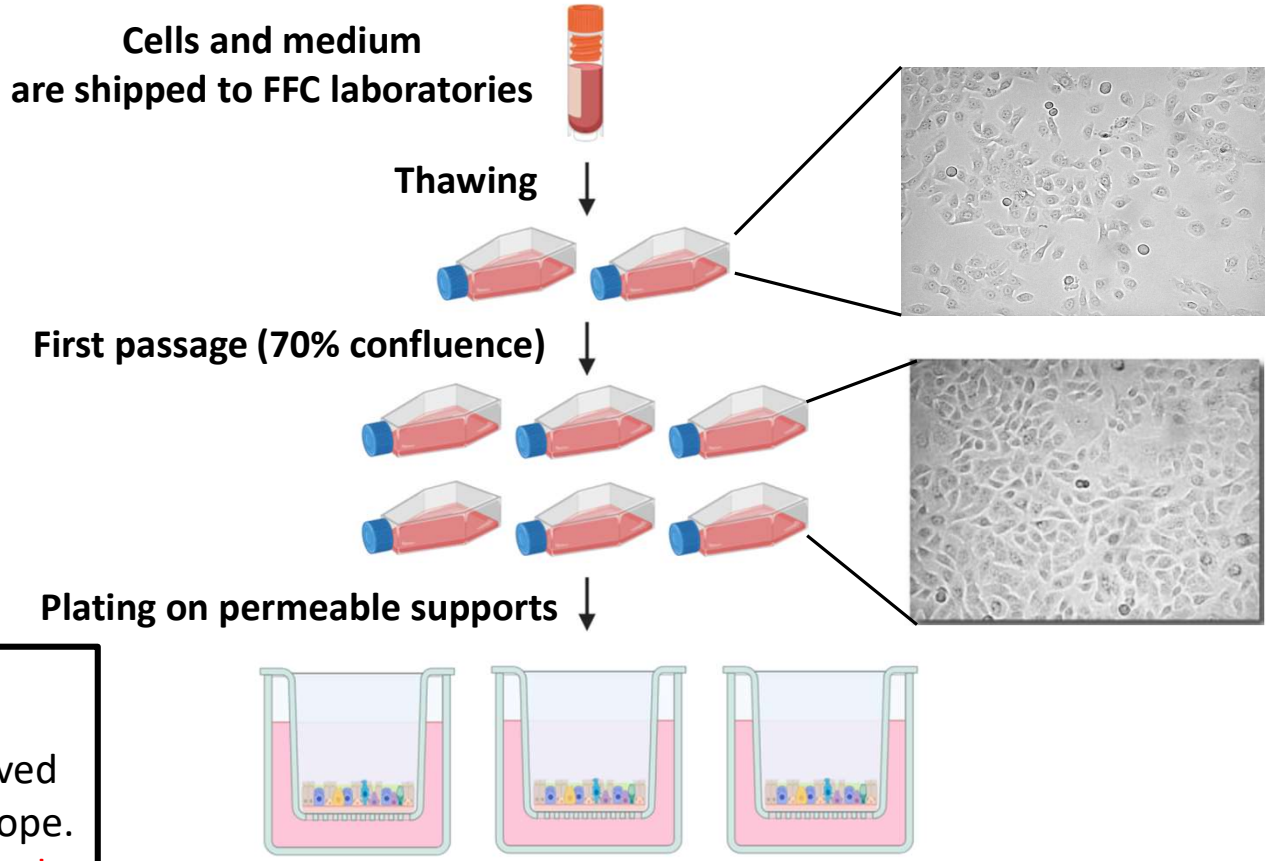


Example of differentiated bronchial epithelium observed under the confocal microscope. Cilia magenta, goblet cells red, apical membrane in green



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

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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.

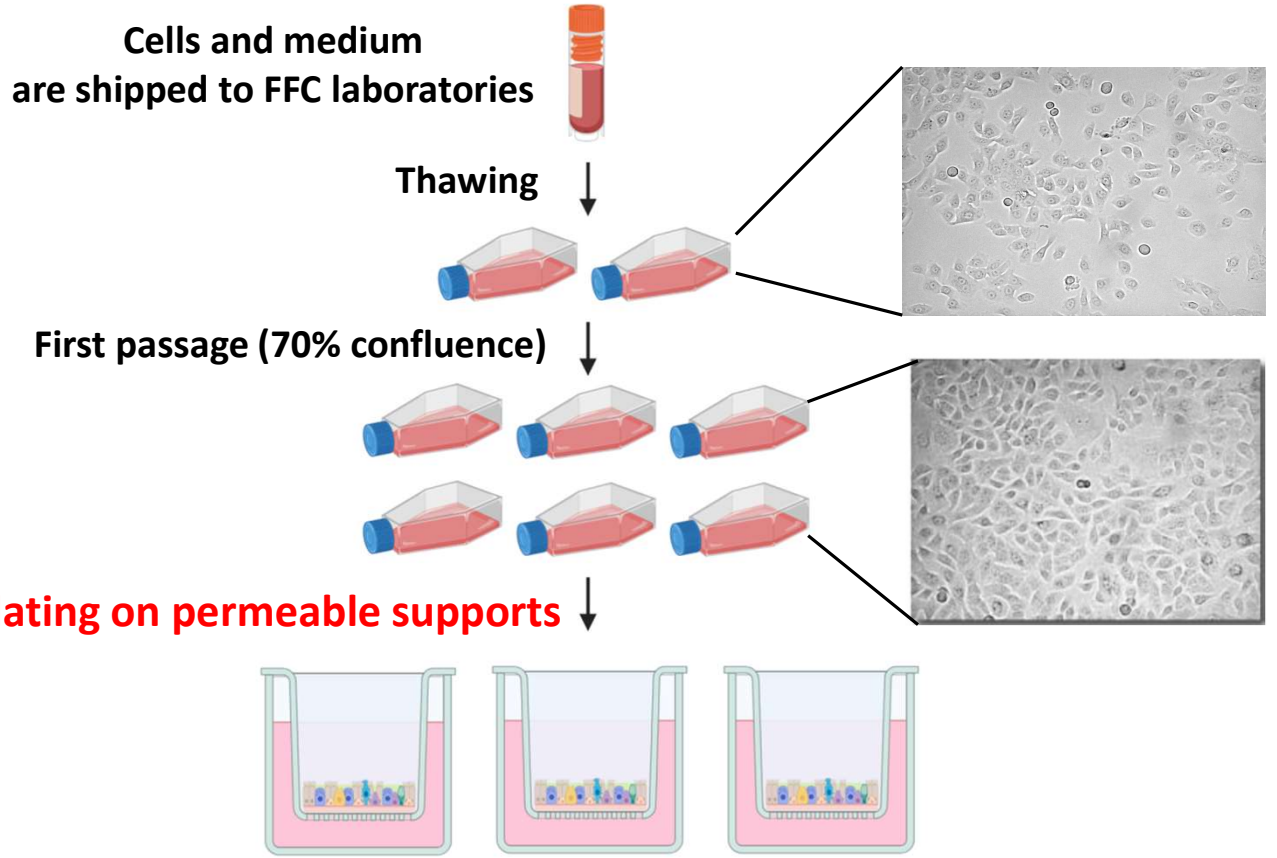
The cells are trypsinized and the suspension of cells is counted and centrifuged. The pellet is resuspended in **PNEUMACULT EX-PLUS** medium and the cells are seeded onto the porous support.

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

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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	

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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

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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

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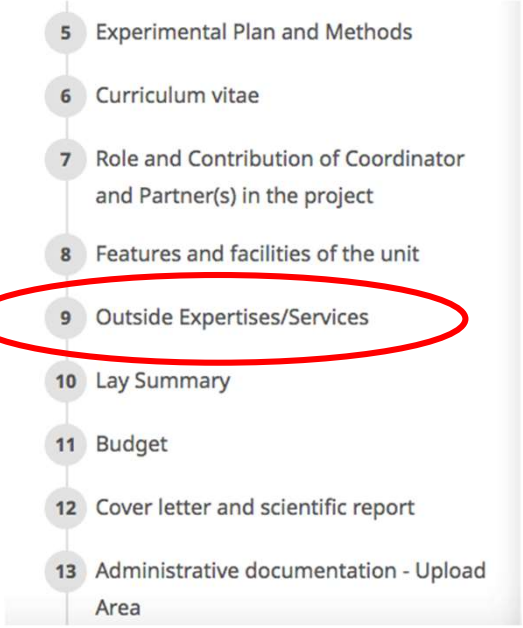
New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:

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)



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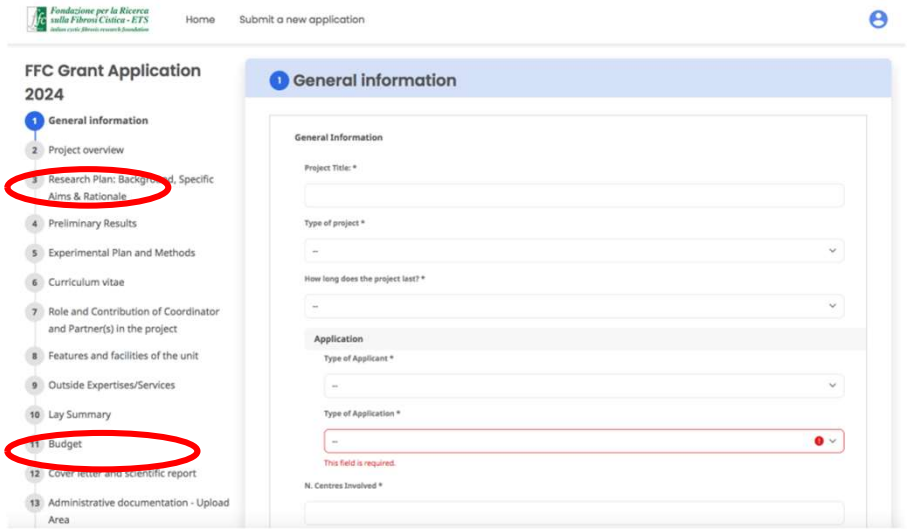
New call for projects for Cystic Fibrosis Research (FFC Research), how to involve the Service:

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 pubblication

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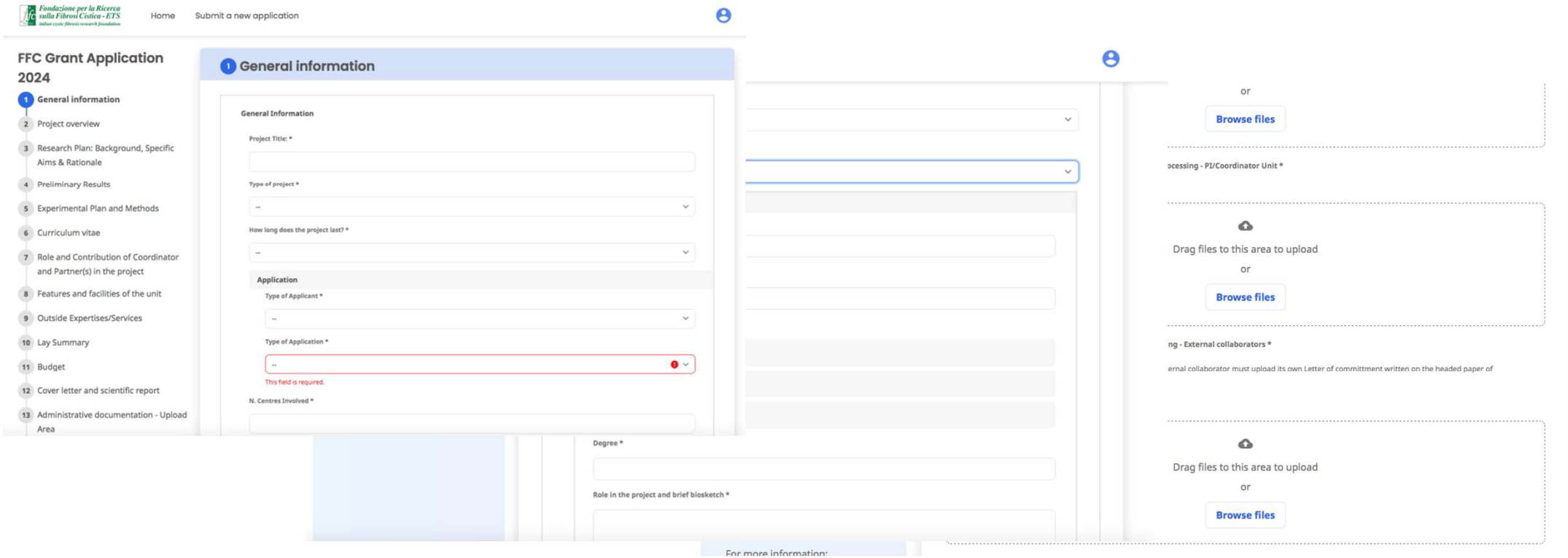

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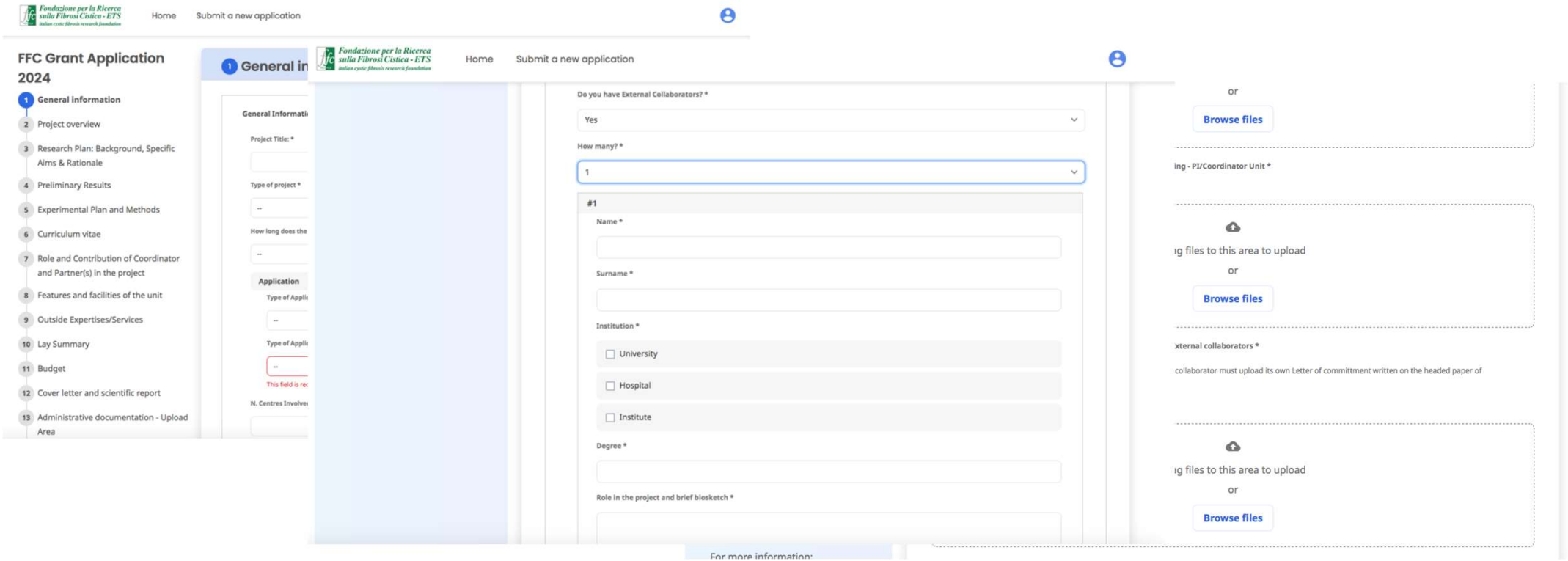

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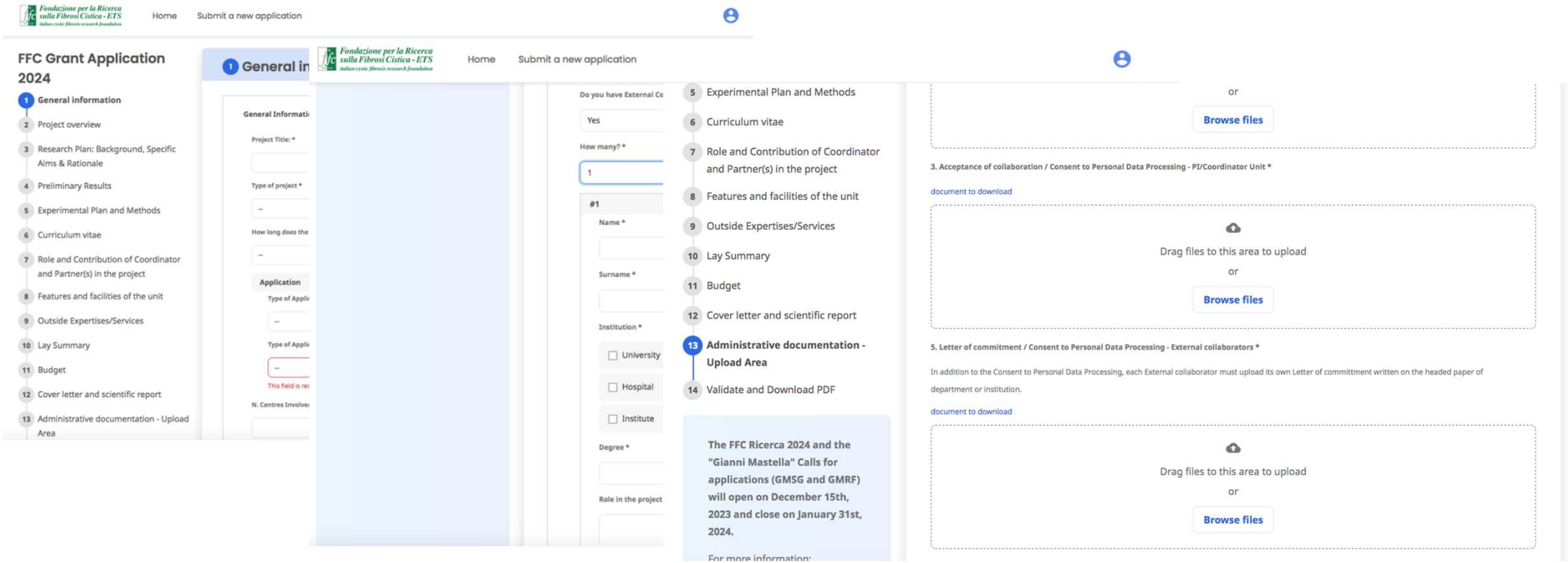

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WEBINAR DI PRESENTAZIONE

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THANKS

Nicoletta Pedemonte
Maria Teresa Lena
Cristina Pastorino
Emanuela Pesce
Valeria Tomati

Luis Galietta