HUMAN TECHNOPOLE

NATIONAL FACILITY FOR GENOME ENGINEERING AND DISEASE MODELLING

CALL FOR ACCESS

25 – GEDM – ROUND 2

Table of Contents

1. INTRODUCTION

The Access of Researchers affiliated with Universities, *Istituti di Ricovero e Cura a Carattere Scientifico* (IRCCS), and Public Research Entities to Fondazione Human Technopole (HT) National Facilities (NFs) is regulated by the NF Access rules available on the NFs dedicated webpage (link).

Services offered by the NFs are available through regular open calls for Access that are published yearly on the HT website (link) and are entirely subsidized by HT through an indirect financing system for the project (or aspects of the project) approved for Access.

The open call for Access is aimed at supporting Access to the technologies offered by the NFs and it is not meant to provide direct funding to the Applicant. The costs for the activities to be performed at the NFs will be fully subsidized. This includes shipment of relevant material from and to the Applicant's laboratory as well as travel and accommodation for the Applicant and/ or Applicant's team member(s) (User) while accessing the NF. Project-related costs (personnel, consumables, and other costs) at the Applicant's laboratory are not funded.

The User Access workflow comprises different steps, spanning from the initial submission of the application to evaluation and Access approval, Access to the performance of the service(s) and Access conclusion. A detailed description of the workflow is available on the NFs dedicated webpage (link).

1.1 Access modalities

Three different Access modalities can be requested. Their availability will vary, based on the service specifics of each NF:

- **"Simple" Access to NF or individual instruments thereof**: This modality is intended for Users involved in projects requiring technologies that are available at the NF for **direct Access by User**. This Access modality requires prior expertise with the technology of interest. After an initial introductory training aimed at defining the level of expertise of the User, **the use of the instrument with limited supervision by NF staff is authorised**. For defined NFs/ instruments/ services this Access modality may be restricted or not available.
- **Access to NF services**: This procedure entails the provision of **services performed by NF staff on behalf of the User**. NF services may include both standard services as well as, when foreseen by the technology development specifics of each NF, bespoke services conceived and discussed with the User. To allow the NF staff to best align the experimental activity to the research objective, the User may be invited, if needed, to assist the NF staff while performing the project or aspects of it.
- **Access to NF services including training**: This procedure **entails training by NF staff** to provide Users, in addition to or alternatively to the services described in the previous modality, with training courses and/or programs, aimed at transferring the expertise necessary for the independent use of the specific technology. In this case, technical and/or experimental activities are conducted with the active participation of the User. Training can be provided by NF staff while performing the service(s) or in a dedicated session. This type of Access is also aimed at researchers who want to acquire expertise for subsequent independent use of a specific technology in other laboratories.

2. TERMS AND DEFINITIONS

2.1 Access

"Access" refers to the authorised use of the NF and of the services offered. Such Access can be granted for sample preparation, set-up, execution and dismantling of experiments, education and training, expert support and analytical services, among others. Access to the NFs includes all infrastructural, logistical, technical and scientific support (including training) that is necessary to perform the aspects of the project approved for Access.

2.2 Researcher

"Researcher" is a professional engaged in the conception or creation of scientific knowledge. They conduct research and improve or develop concepts, theories, models, techniques, instrumentation, software or operational methods.

2.3 Principal Investigator

"Principal Investigator" (PI) is the Researcher affiliated with an eligible Institution with the role of independent Group Leader, who is responsible for coordinating the research activities conducted within the framework of the submitted project.

The PI shall hold a primary appointment as Group Leader at an eligible Institution, with the following requisites:

- Coordinate an independent research team.
- Have a supervisory role towards junior and/ or senior Researchers.
- Their Group has an autonomous budget sufficient to cover their current research expenses.
- Be the recipient of independent research funding as PI or co-PI.

Junior PI: Up to 6 years from their first appointment in an independent Group Leader position.

The period specified above may be extended beyond 6 years in the event of adequately documented career breaks, occurring before the submission of the application and resulting from:

i. Maternity leave: The time limit is increased by 18 months for each child born after their first appointment in an independent group leader position; if the Applicant is able to document a longer total maternity leave, the period of eligibility will be extended by a period equal to the documented leave, taken before the submission of the application. Maternity status must be documented by submitting the birth certificate of the child or children.

ii. Paternity leave: The time limit is increased by the actual amount of paternity leave taken before the application submission deadline for each child born after their first appointment in an independent group leader position. Paternity status must be documented by submitting the birth certificate of the child or children.

iii. Long-term illness of more than 90 days, or national service: The time limit is increased, for each eligible event occurring after their first appointment in an independent group leader position, by the actual amount of leave from which the Applicant has benefited prior to the application submission deadline.

Established PI: More than 6 years from their first appointment in an independent group leader position.

2.4 Applicant

"Applicant" is the Principal Investigator who applies to a NF open call for Access and who is responsible for the submitted project. They can be of any nationality and must be affiliated with an eligible Italian Institution, as detailed in section 4.

2.5 User

A "User" is intended as a Researcher affiliated with an eligible Institution who accesses, physically or remotely, the NFs to perform the approved activities or to support the National Facility staff while performing the approved service.

If requested by the Applicant, the User of the NF can also be a separate member of their research team.

3. APPLICATION TYPE

Applicants shall select the type of application they want to submit, choosing between two options:

a. **Standard** application for projects that are technically mature.

b. **Proof-of-concept** application for:

i. Projects with high scientific potential but with insufficient technical maturity or preliminary data.

ii. Projects aimed at setting up the experimental conditions required for a standard project, including methods or technology development projects.

iii. Time-limited Access projects (e.g., to acquire data to complete a manuscript, or preliminary data needed for a grant application, or single microscopy session).

4. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in section 2.3 of this call, affiliated with an eligible Institution are eligible to apply. The Applicant's role as a PI shall be confirmed by their Institution in a mandatory letter of Institutional endorsement (Template available in Annex I).

Applications from Researchers who are not independent should be submitted by their Group Leader. Applicants are strongly encouraged to support NF Access by young Researchers (R1 and R2 profiles of the European Framework for Research Careers, link) who are part of their group. In this case, the Applicant shall indicate in the application form that the NF User is a member of their group, specifying User's career stage.

Below are the links to the relevant lists of eligible Institutions:

Universities: This category includes Institutions recognized by the Ministry of University and Research (link). In detail:

i. State funded public universities, listed under the following link.

ii. Specialized superior graduate schools or Institutions, listed under the following link.

iii. Legally recognized non-public universities, listed under the following link.

iv. On-line universities, listed under the following link.

Istituti di Ricerca e Cura a Carattere Scientifico (IRCCS): this category includes Institutions recognized by the Ministry of Health and listed at the following link.

Public research entities: this category includes:

a) Institutions recognized by the Ministry of University and Research and listed at the following link;
b) Area di Ricerca Scientifica e Tecnologica di Trieste - Area Science Park;
c) Agenzia Spaziale Italiana - ASI;
d) Consiglio Nazionale delle Ricerche - CNR;
e) Istituto Italiano di Studi Germanici;
f) Istituto Nazionale di Astrofisica - INAF;
g) Istituto Nazionale di Alta Matematica "Francesco Severi" - INDAM;
h) Istituto Nazionale di Fisica Nucleare - INFN;
i) Istituto Nazionale di Geofisica e Vulcanologia - INGV;
j) Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS;
k) Istituto Nazionale di Ricerca Metrologica - INRIM;
l) Museo Storico della Fisica e Centro Studi e Ricerche "Enrico Fermi";
m) Stazione Zoologica "Anton Dohrn";
n) Istituto Nazionale per la Valutazione del Sistema Educativo di Istruzione e di
o) Formazione - INVALSI;
p) Istituto Nazionale di Documentazione, Innovazione e Ricerca Educativa - INDIRE;
q) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CREA;
r) Agenzia Nazionale per le Nuove Tecnologie, l'energia e lo Sviluppo Sostenibile - ENEA;
s) Istituto per lo Sviluppo della Formazione Professionale dei Lavoratori - ISFOL (a decorrere dal 1° dicembre 2016 denominato Istituto nazionale per l'analisi delle politiche pubbliche - INAPP);
t) Istituto Nazionale di Statistica - ISTAT;
u) Istituto Superiore di Sanità - ISS;
v) Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, ferme restando le disposizioni di cui alla legge 28 giugno 2016 n.132;
w) Istituto nazionale per l'assicurazione contro gli infortuni sul lavoro – INAIL.

Applicants shall declare that they have **not received funding to perform the submitted project (limited to the aspects included for Access to the NF)** in their own laboratory, host Institution or elsewhere.

Applicants shall confirm the **economic and scientific feasibility** for the aspects of the project to be performed outside the NFs.

Applicants will need to certify that **samples and relevant authorisations are available at the moment of application or no later than two (2) months** from receiving Access approval. **If samples and/ or relevant ethical and legal authorisation(s) for their use will not be**

provided within this time frame, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.

Applicants **cannot request Access for the same service** if an approved Access is ongoing (i.e., Access that has been granted in a previous call for Access and is not yet completed). Before submitting a new application for the same service, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the next evaluation round. A **clear motivation for the request must be provided** in the dedicated section of the application portal.

A PI submitting an application to this call for Access **cannot request access to other NFs** (i.e., cannot participate to other 2025 - ROUND 2 calls for Access). If more than one application is submitted, **ALL will be rejected** during administrative review.

Applicants who have an **application under evaluation** are not allowed to submit another one before receiving notification of the results. If an application is erroneously submitted, this will be rejected at the administrative review stage.

Applications must be **written in English**, they must be **complete** (i.e., consist of all the requested elements and information) and **abide to all administrative and technical requirements** (e.g., proposal and/or CV format, mandatory declarations, technical requirements for the services, sample availability, sample requirements, including but not limited to number of samples to be analysed, and research data management plan).

Incomplete applications or applications that do not meet the requirements will be considered not admissible and will be rejected at the administrative review stage.

5. APPLICATION CONTENT AND FORMAT

The application, to be submitted through the online portal PICA (link) consists of six components:

1. **Applicant's general information**.
2. **Justification for requesting Access to the NF**. The Applicant is requested to select the one that best applies from these four options:

   1. The requested service/ technology is not available at the host Institution;

   2. The requested service/ technology cannot be performed at the host Institution or elsewhere at an affordable cost;

   3. The requested service/ technology is available at the host Institution but the necessary expertise is lacking;

   4. The requested service/ technology is available at the host Institution but the service cannot be performed in a timeframe or scale compatible with the experimental requirements.

   The Standing Independent Evaluation Committee, in charge of the evaluation procedure, may reserve the option to contact the host Institution and its core facilities to confirm the justification provided.
3. **Abstract** to be inserted in the dedicated section on the application portal (Max 1500 characters including spaces).

4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application portal, shall include the following sections:
    a. *Title*
    b. *Significance.*
    c. *Innovation.*
    d. *Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
    e. *Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

    Below, the mandatory format for the proposal:

    Standard application: Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname (e.g., ID123456_PROPOSAL_Rossi)

    Proof-of-Concept application: Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname (e.g., ID123456_PROPOSAL_Rossi)

    Proposal template is available in Annex II of this call.

    Applications that do not meet the format requirements will be considered not admissible and will be rejected at the initial administrative review stage.

5. **Applicant's CV in NIH biosketch format**. The CV, to be uploaded in PDF, shall be drafted in English, using the template available at this link and following the mandatory format: max 4-5 pages, page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom. For support in drafting the CV, please refer to NIH website: Create Biosketches | NIAID: National Institute of Allergy and Infectious Diseases (nih.gov). Please note that eRA COMMONS USER NAME is NOT mandatory..

    Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_CV_Surname (e.g., ID123456_CV_Rossi).

    Applications that do not meet the format requirements will be considered not admissible and will be rejected at the administrative review stage.

6. **Letter of Institutional Endorsement**, addressing the following points:
    a. *Confirmation of the Applicant's role at their Institution, and their eligibility under the category of PI (see section 2.3).*
    b. *Confirmation that relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained no later than two (2) months after Access approval, in order to process samples and data through the NFs.*
    c. *Justification of the request for Access – include a statement on why the project cannot be performed at the Applicant's Institution. Such statement shall confirm Applicant's justification provided (see point 2 above).*

   d. *Confirmation that the Applicant has not received funding for performing the submitted project, for the aspects to be performed at the NFs, in their own laboratory, host Institution, or elsewhere.*
   e. *Confirmation of the project's economic and scientific feasibility for the aspects to be performed at the host Institution.*
   f. *Acceptance of NF Access Rules.*

   The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section on the application portal, shall be drafted using the facsimile available as Annex I of this call. Name the file as APPLICATION ID_ENDORSEMENT_Surname (e.g., ID123456_ENDORSEMENT_Rossi).

7. **Technical information**, to be filled in in the dedicated section(s) of the application portal, indicatively including:
   a. *Requested service(s), as described in Annex III of this call.*
   b. *Sample technical information.*
   c. *Requested preliminary data for technical feasibility analysis (if applicable).*
   d. *Whether the entire sample set is already available, or will be available no later than two (2) months from receiving Access approval.* ***Please note that if samples and/ or relevant ethical and legal authorisation(s) for their use will not be provided within this time frame, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call****.*
   e. *Resources and expertise to receive and process the output – data (e.g. Cryo-EM micrographs) or reagents (e.g. human iPSCs) – generated by the NF.*
   f. *Research data management plan and bioinformatics support for data analysis, specifying (****mandatory when the project output includes research data*** *- e.g., genomics or proteomics data, bioimages from microscopy services, among other):*
      i. *How the bioinformatics analysis of the data generated by the NF will be performed (if such analysis is not provided by the NF for Data Handling and Analysis).*
      ii. *How the data generated by the NF will be handled during and after the end of the project.*
      iii. *Whether and how the data will be shared/ made Open Access.*
      iv. *How data will be curated and preserved, including after the end of the project.*

   Details and format of the technical information to be provided are available in the dedicated section of the application portal.

Information provided in sections 1 and 6 are used for the eligibility and admissibility check.

Information provided in section 7 is used for assessing the technical feasibility of the aspects of the project to be performed at the NF.

The entire application is evaluated by the Standing Independent Evaluation Committee (SIEC) to assess its scientific merit.

6. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

Applications shall be submitted exclusively through the application portal PICA managed by CINECA and accessible at this link, according to the indicated terms and methods.

This call for Access (Call ID: 25-GEDM-ROUND2) will open on the 1st of June 2025 (13:00 CET) and will close on the 30th of September 2025 (13:00 CET).

A comprehensive list of services, available equipment and the technical requirements for Access as well as terms and conditions are available on the dedicated NFs webpage (link).

The complete list of offered services and technical requirements are available in the Annex III of this call.

Samples as well as relevant authorisation for their use, **shall ideally be available by when the application is submitted, but categorically not later than two (2) months after Access approval**. When the project foresees the analysis of more than one batch of samples, similarly, the first batch should be available when the application is submitted or not later than two (2) months after Access approval.

7. EVALUATION OF APPLICATION

The evaluation procedure is conducted by the SIEC that is supported by a Panel of independent external Reviewers (Review Panel) selected by the SIEC on the basis of their scientific expertise.

Each Review Panel is composed of two (2) SIEC members, who will act as Chairs, plus 10 appointed external Reviewers, with the relevant expertise.

Below is a scheme describing the evaluation steps and the **indicative timeline for the process**.

There are four application categories that are evaluated and ranked separately:

- Junior PI – Standard application
- Established PI – Standard application

- Junior PI – Proof of Concept application
- Established PI – Proof of Concept application

The NF User Access Office first performs an administrative review of the application to ensure that all the requested components have been provided, and that all eligibility criteria have been met. Incomplete applications or applications that do not meet all the requirements will be considered not admissible and will be rejected at the administrative review stage.

7.1 Triage

If the number of applications exceeds by a factor of three (3) the estimated capacity of the NF, a triage will be applied within each application category.

Triage criteria will include:

a. Justification for requesting Access to the NF: priority will be given to researchers who do not have direct access to the service/ technology at their home institute.

b. Ongoing and previous support received by the NFs: priority will be giving to researchers who do not have any ongoing Access to the NFs or who have never benefit form NF Access.

Should the number of proposals still exceed the allowable estimated limit after having applied the triage, as a tool of last resort, a lottery will be applied.

To ensure broader access for all institutes across Italy, proposals submitted by a single Institution that are sent for evaluation should not exceed the 10% of the total for any given career-based category.

7.2 Evaluation procedure and criteria

The application is then sent to the Review Panel for assessing technical feasibility and scientific merit. A comprehensive analysis of the technical feasibility of the project, which is performed by the NF staff, is provided as supporting documentation.

The application will remain confidential throughout the entire evaluation process. Reviewers will be asked to declare that they do not have any conflict of interest, and they will be bound by a Confidentiality Agreement.

The application will be individually evaluated by three Reviewers who are part of the relevant Review Panel.

Proposals will be evaluated and ranked based on their average score, within each category.

An on-line meeting of the Review Panel may be requested by the Chairs if deemed necessary (for example to discuss proposals with highly discrepant scores).

At least 50% of the available Access will be allocated to applications from Junior PIs.

The scientific merit of the project is assessed based on the following criteria:

- **Significance**: Overall scientific merit of the proposed research. If all the experiments proposed are successful, how will the resulting knowledge advance the field?
- **Innovation**: Degree of innovation (conceptual and/ or technological), and ambition of the proposed study compared to the state-of-the-art in the relevant field.

- **Approach**: Appropriateness of proposed methodology, preliminary data in support of proposed experiments, and project feasibility.
- **Environment**: Facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).
- **Justification for requesting Access to the NF**: Explanation on why the service cannot be performed at the host Institution, at a cost which is deemed affordable for the applicant.
- **Applicant**: PI's scientific background and expertise.

7.3 Scoring system

A numeric score between 1 (exceptional) and 9 (poor) is provided for each of the six evaluation criteria. Moreover, an overall project score including a short descriptive comment is provided as feedback to the Applicant.

- **HIGH**:
  - **Score 1 (Outstanding)** – The proposal successfully addresses all relevant aspects of the criterion. There are no weaknesses.
  - **Score 2-3 (Excellent - Very Good)** – The proposal addresses the criterion exceptionally well, aside from a small number of minor weaknesses.
- **MEDIUM**:
  - **Score 4-6 (Very good - Good)** – The proposal addresses the criterion well, but a number of weaknesses are present.
- **LOW**:
  - **Score 7-8 (Fair - Poor)** – The proposal broadly addresses the criterion, but there are significant weaknesses.
  - **Score 9 (Poor)** – The criterion is inadequately addressed, or there are serious inherent weaknesses.

7.4 Technical feasibility analysis

During the evaluation, the SIEC Chairs as well as the Reviewers will receive a report from NF staff who will perform a comprehensive analysis of the proposed project's technical feasibility. Technical feasibility also includes an evaluation of the fulfilment of the technical requirements in terms of capacity to receive and process the research data generated by the NF, as described in the research data management plan. This latter evaluation is performed in consultation with the NF for Data Handling and Analysis.

Based on the technical maturity of the project, the application can be assessed as Feasible/ Not Feasible/ Proof-of-Concept study required.

At this stage, the NF staff provides the SIEC Chairs with information on the resources needed (cost and time) to perform the proposed projects.

7.5 Evaluation results and Access approval

Applications with the highest scientific score that fulfil all technical requirements are approved for Access by the SIEC, based on the capacity of the NF.

At least 50% of the overall capacity of each requested service is guaranteed for projects submitted by Junior PIs, provided that a sufficient number of qualified proposals are received.

In case of comparable scores, for applicants that are at the same career level category, the SIEC Chairs will have the authority to rank the applications based on secondary parameters such as number of applications per Institution, budgetary considerations and geographical distribution.

Evaluation results – Access granted, Access conditionally granted, Reserve list, Access not granted – are communicated to the Applicant through the Access portal.

A selected number of applications may be placed on a reserve list (i.e., waiting list in case of cancellations of Access granted projects). Applicants whose applications are placed on this list will receive additional information advising whether the project can be Access approved or should be resubmitted within the subsequent application window (see below).

8. RESUBMISSION OF RESERVE LIST PROJECTS

Reserve list projects that are not accommodated in the round they have been evaluated for will be granted two options:

a. To be included in the new evaluation round maintaining the same project and review score as their initial application. Here, the application cannot be updated and is not sent out again for evaluation. **This option is provided only once, for the subsequent round only**.

   To choose this option, the Applicant shall submit the request through the dedicated application dedicated call (Application for Reserve List Projects) accessible at this link, indicating the call and the ID of the reserve list project.

b. To resubmit an updated version of the proposal (for example including new preliminary data and/or taking into account Reviewer's comments).

   To choose this option, the Applicant shall submit a new application which will be sent out for evaluation, indicating that it is a resubmission of a reserve list project and specifying the project ID.

9. AFTER ACCESS HAS BEEN APPROVED

A kick-off meeting is organised after Access approval, in which the Applicant is invited to meet NF staff to discuss the experimental design of the project and to finalize the project plan.

Once the project plan has been agreed and the relevant ethical and legal authorisation(s) for the use of the samples has(have) been provided, the NF User Access Office coordinates the signature of the required formal Agreements (e.g., Access Agreement, Collaboration Agreement, other), when required, and the project can commence.

If samples and/ or relevant ethical and legal authorisation(s) for their use are not available within two (2) months after Access approval, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.

10. AFTER ACCESS HAS BEEN COMPLETED

At the end of the activities carried out at the NF, and not later than three (3) months thereafter, if not differently agreed with the NF User Access Office, the Applicant must submit a short report to be published on the NFs website on the results obtained and the impact of the service on their research. Moreover, a final report describing the impact of the Access to the NF on the research project for which the service has been requested, shall be provided upon publication of the relevant results. The NF User Access Office will provide a template for the requested reports including the information required (activities performed, outcomes, impact on PI's research, plan for data sharing with scientific community, among others). Applicants who will not be able to demonstrate the consistency and relevance of the activities carried out at the NF with the research project for which Access was requested will be considered not eligible to participate in the subsequent calls for Access.

Moreover, the Applicant will be asked to fill in a brief, mandatory survey regarding their experience, providing feedback and suggestions for further service improvement.

The Applicant must communicate to the NF User Access Office (via email to national.facilities@fht.org) any publication acknowledging the NF.

Research data obtained during Access shall be made available to the scientific community following the FAIR principles. Applicant must inform the NF User Access Office (via email to national.facilities@fht.org) when and how the data are made public.

11. CONTACTS

Requests for information and/or clarifications concerning the application procedure may be sent to the dedicated e-mail address national.facilities@fht.org, indicating the call ID in the subject line.

11. REFERENCES

NF Access Workflow_Convenzione (link)

NF Access Rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

12. CHANGES TO THE CALL

Any changes or additions to this notice will be communicated through publication on the NFs website (link).

ANNEX I: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE

(Print on paper bearing the official letterhead of the host Institution)

Endorsement letter of the host Institution

To whom it may concern:

I, the undersigned, …….. (*name of legal representative or special attorney*), born in ……. (*city*) on ……..(*date*), as legal representative *(or special attorney, by means of special power of attorney identified by ……...*) and on behalf of ……(*name of the host Institution*), legal residence in *(referred to the host Institution*) ……(*city*), address ……., regarding the project (*Title*)……., presented by ……(*Applicants's first name and surname*), as Principal Investigator on the call for Access to Human Technopole National Facilities…..(ID *of the call*),

Declare

- That the host Institution is among those eligible to participate in the call for Access as it belongs to the following eligible category: **(*please select the one that applies: University, IRCSS, Public Research Entities*);**
- That the Applicant, Dr …… (*Applicant's first name and surname*) is an independent group leader (Principal Investigator) affiliated with a primary appointment at the host Institution and that they meet the eligibility criteria as indicated in the call;
- That the Applicant has not received funding for performing elsewhere, the aspects of the project for which they are seeking here support from or Access to Human Technopole National Facilities;
- That Applicant's request to Access the National Facilities is justified for the following reason (***please select the one that applies or best fits this application***):

  1. The requested service/ technology is not available at the Host Institution;

  2. The requested service/ technology cannot be performed at the Host Institution or elsewhere at an affordable cost;

  3. The requested service/ technology is available at the Host Institution but the necessary expertise is lacking;

  4. The requested service/ technology is available at the Host Institution but the service cannot be performed in a timeframe or scale compatible with the experimental requirements.

- That relevant authorisations, declarations and accreditations from the competent authority(ies) have been obtained in order to process samples and data through Human Technopole OR that, if relevant authorisations, declarations and accreditations

from the competent authority(ies) have not been obtained yet, they will be available before the starting date of the project, and not later than 2 months after Access approval;

- That, if applicable, biological specimens have been obtained or will be obtained with the corresponding approval of the Bioethics Committee and appropriately signed 'informed consent', both for their collection and their use, including conservation, manipulation, derivation and processing to be carried out by Human Technopole National Facilities;
- That, if samples were obtained or will be obtained from subjects who signed an 'informed consent', said informed consent allows or will allow that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorized third parties;
- That, if applicable, copy of the relevant authorisations, declarations and accreditations will be provided at the moment of the application or not later than 2 months after Access approval;
- That, in case Physical Access to the National Facilities is requested, Applicant and/ or the team member(s) who will Access the National Facility have comprehensive insurance coverage for accidents and third-party liability, encompassing all their activities during their stay at HT and ensure that HT is recognized as third party. Name of the insurance company, insurance policy number and expiration date will be provided to HT before physical Access.

and is committed

To accept the terms and conditions to Access Human Technopole National Facilities as described in the National Facilities Access Rules (link) and, when applicable, the Access Agreement and its annexes (link).

For the host Institution (Applicant legal entity/beneficiary):

Date …….

Name and Title ……. ; …….

Email and Signature of **legal representative or delegated person (e.g., Head of Department)**

……. ; …….

ANNEX II: PROJECT PROPOSAL TEMPLATE

Mandatory proposal format

Standard application*: Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname* (e.g., ID123456_PROPOSAL_Rossi)

Proof-of-Concept application*: Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID_PROPOSAL_Surname* (e.g., ID123456_PROPOSAL_Rossi)

PLEASE REMOVE THE INFORMATION ABOVE BEFORE SUBMITTING

Proposal content:

1. TITLE

2. SIGNIFICANCE

3. INNOVATION

4. APPROACH

5. ENVIRONMENT

6. REFERENCES (Optional)

ANNEX III: SERVICE LIST

HUMAN TECHNOPOLE

NF FOR GENOME ENGINEERING AND DISEASE MODELLING

CALL FOR ACCESS

25-GEDM-ROUND2

SERVICE LIST

Table of Contents

1. INTRODUCTION

The mission of the NF for Genome Engineering and Disease Modelling is to provide a multidisciplinary portfolio of services entailing state-of-the-art technologies in pluripotent stem cells and genomic engineering.

The NF offers fully validated pipelines for *in vitro* physio-pathological modelling of human biology.

The services offered in this call for Access include the reprogramming of somatic cells into iPSCs and genome engineering of both stem cells and cell lines.

Project proposals can be submitted for either individual services or a combination of multiple/sequential services.

2. GENERAL TECHNICAL REQUIREMENTS

- The NF for Genome Engineering and Disease Modelling can accept biological samples of biosafety containment levels (BSL) 1 or 2.
- Applicants must ensure that the samples are available at sufficient quantity and quality before the closing date of the application period (see the specifics below).
- Applicants must ensure compliance with all ethical requirements relevant to their project and, if applicable, have an appropriate Material Transfer Agreement (MTA) in place to regulate the handling and use of the proposed cell line.
- Applicants must ensure that cell line samples have undergone Mycoplasma testing within the two months prior to shipment to Human Technopole. While Mycoplasma testing is not mandatory for primary samples, it should be conducted if it does not compromise sample integrity and availability.
- If relevant, applicants and/or the personnel working in the applicant's laboratory should have proficiency in PSC culturing techniques.

3. DEFINITIONS

PBMCs: Peripheral Blood Mononuclear Cells

PSCs: Pluripotent Stem Cells

iPSCs: induced Pluripotent Stem Cells

STR: Short Tandem Repeat

CNV: Copy Number Variation

LPS: Low Pass Sequencing

DSBs: Double Strand Breaks

InDels: Insertion and/or Deletion of nucleotides into genomic DNA

gRNA: guide RNA

ddPCR: digital droplet PCR

NHEJ: Non-homologous end joining

HDR: Homology-Directed Repair

IVS: Intervening sequence

4. SERVICE LIST

The following section provides a detailed list of services offered by the facility. The NF-GEDM offers two main categories of services: (i) somatic cell reprogramming and (ii) genome engineering. When submitting a proposal, Users may request Access to either a single service category or a combination/sequential use of both, provided that the experimental need for such a selection is clearly justified within the proposal.

4.1 REPROGRAMMING OF SOMATIC CELLS TO iPSCs

Description: Direct reprogramming of human primary cells into iPSCs.

iPSCs are generated through non-integrating reprogramming techniques, mitigating the risk of genomic integrations of reprogramming factors and preventing insertional mutagenesis. This approach also addresses residual expression of reprogramming factors in iPSCs and their derivatives.

iPSC clones are thoroughly characterized through a comprehensive panel of assays and are delivered as a cryopreserved Master Cell Bank. Full details of the characterization process are available in each specific service description.

Please note: Applicants may request a maximum of three reprogramming services in total. If both PBMC and fibroblast reprogramming are requested, the combined number of patient cells across both services must not exceed three (i.e., not three per service, but three overall).

4.1.1 Reprogramming of PBMC

SID: NF20.001 - Reprogramming of PBMCs

Service: Reprogramming of human Peripheral Blood Mononuclear Cells (PBMCs) sample into iPSCs.

Cells are seeded and transduced with vectors from the *CytoTune-iPS 2.0 Sendai Reprogramming Kit* (ThermoFisher Scientific). Transduced cells are then reseeded at clonal density onto Laminin-511 and cultured for 20 to 28 days until the emergence of iPSC colonies. Colonies are then picked and passaged in 24-well plates until complete viral clearance and stabilisation of the culture.

Technical requirements: Applicants may submit a maximum of 3 patients in total across both reprogramming services. Projects involving larger cohorts may optionally be described in the written proposal. These requests will be evaluated on a case-by-case basis and considered

only in exceptional circumstances, depending on the Facility's available capacity and the overall ranking of the project.

PBMCs should be submitted in a cryopreserved state: > 1 x 10^6 cells/cryovial.

Method: Non-integrating Sendai viral vectors.

Characterization: Every iPSC clone undergoes a comprehensive assay characterization panel and is accompanied by a Certificate of Analysis (CoA):

(please refer to appendix 1 for details)

- Undetectable mycoplasma and bacterial contamination.
- Transgene clearance.
- Post-thaw viability.
- Expression of markers for undifferentiated PSCs.
- *In vitro* three-germ layers differentiation.
- Cell identity: STR profiling of primary cells and derived clone.
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (G or Q-banding).

Deliverables: We generate a maximum of 3 clones from each PBMC sample. The exact number of delivered clones may vary based on experimental outcomes. The cell lines are expanded and cryopreserved in a Master Cell Bank, with 10 to 12 cryovials (1 well of a 6-well plate/cryovial) allocated for each clone. The cryovials from the Master Cell Bank will be shipped in dry ice.

Delivery Time: iPSC derivation service from PBMCs requires 6 months for its completion.

Important note: The quality of PBMC culture is influenced by both the efficiency of the isolation process and the state of cryopreservation, which affects the efficiency of reprogramming. Upon receipt, the cells will undergo thorough testing for viability, mycoplasma and bacterial contamination. If the quality falls below the required standards, the cells will be discarded.

Access modality available: Access to NF services.

4.1.2 Reprogramming of Fibroblasts

SID: NF20.002 - Reprogramming of Fibroblasts

Service: Direct reprogramming of human fibroblast sample into induced pluripotent stem cells (iPSCs).

Fibroblasts are plated onto Laminin-511-coated plates and transfected with an nm-RNA cocktail daily (day 1-3) overnight. On day 4, the reprogramming plate's medium is replaced with fresh NutriStem™ Medium. Once colonies reach an adequate size (10 to 15 days), they are selected and transferred to individual wells of a 96-well plate coated with iMatrix-511. Individual clones are subsequently passaged in 24-well plates until they meet the defined standards for pluripotency.

Technical requirements: Applicants may submit a maximum of 3 samples in total across both reprogramming services. Projects involving larger cohorts may optionally be described in the written proposal. These requests will be evaluated on a case-by-case basis and considered only in exceptional circumstances, depending on the Facility's available capacity and the overall ranking of the project.

Fibroblasts should be submitted in a cryopreserved state (>5 × 10^5 cells per cryovial) at a low passage number (P2–P6) to ensure optimal reprogramming efficiency. Cells at higher passage numbers (>P7) can still be submitted and will undergo reprogramming; however, the efficiency may be significantly reduced, often dropping below 1%.

Information to be provided in the application: please provide the passage number of each sample to be processed.

Method: nm-RNA transfection.

Characterization: Every cell line (clone) undergoes a comprehensive assay characterization panel and is accompanied by a Certificate of Analysis (CoA):

(please refer to appendix 1 for details)

- Undetectable mycoplasma and bacterial contamination.
- Post-thaw viability.
- Expression of markers for undifferentiated hPSCs.
- *In vitro* three-germ layers differentiation.
- Cell identity: STR profiling of primary cells and derived clone.
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (G or Q-banding).

Deliverables: We generate a maximum of 3 clones from each Fibroblast sample. The cell lines are expanded and cryopreserved in a Master Cell Bank, with 10 to 12 cryovials (1 well of a 6-well plate/cryovial) allocated for each clone. The cryovials from the Master Cell Bank will be shipped in dry ice.

Delivery Time: iPSC derivation service from Fibroblasts requires 3 - 4 months for its completion.

Important note: The quality of fibroblast culture is influenced by both the state of cryopreservation and passage number (see *Technical Requirements)*, which affects the efficiency of reprogramming. Upon receipt, the cells will undergo thorough testing for viability, mycoplasma and bacterial contamination. If the quality falls below the required standards, the cells will be discarded.

Access modality available: Access to NF services.

4.2 GENOME ENGINEERING

Description: We provide gene editing services for the generation of engineered PSCs or immortalized/cancer cell lines. Please note that primary cells are not eligible for this type of service.

The following diagram illustrates our workflow:

Figure 1: Overview of NF-GEDM Genome Editing Workflow.

Genome engineering services include the following experimental phases:

1. **Strategy Design**
   We begin with a detailed consultation to develop a customized genome editing strategy tailored to the User's specific needs.
2. **Incoming procedures**
   The cells of interest are thawed, tested for mycoplasma contamination, and adapted to our internal cell culture conditions to ensure compatibility.
3. **Genome editing**
   Genome editing reagents are delivered into the target cell line, ensuring precise execution of the editing strategy.
4. **Bulk Validation**
   The edited bulk cell population is screened and validated to confirm successful genome modification.
5. **Clonal screening**
   Edited cells are seeded at clonal density to enable the isolation of single-cell-derived colonies. Clones are subsequently expanded and sequence-validated to confirm the presence of the intended genome edits, including the identification of monoallelic and biallelic modifications.
6. **Quality controls**
   Comprehensive quality control assays are conducted to thoroughly characterize the genetically modified cell product.
7. **Freezing & delivery**
   Expanded and validated clones are banked to establish a Master Cell Bank and prepared for delivery to the User, along with a Certificate of Analysis detailing the quality control results, genome editing validation, and relevant culture conditions.

Our standard services fall into three main subcategories:

(i) Target Gene Knock-Out.

(ii) Knock-In.

(iii) Base Editing.

The details for each of these services are provided in the following paragraphs.

For customized modifications (not covered by the NF20.005-020 single gene services) targeting multiple genes or gene families simultaneously and for projects demanding tailored approaches, please submit a Gene Editing Custom Project application (refer to the details below, SID: NF20.CustomEditing).

Disclaimer: A successful editing depends on various experimental factors, making the outcome of the project uncertain. In such instances, when technically feasible, we will endeavour to make a secondary attempt, agreeing in advance on potential alternative experimental methods.

4.2.1 General requirements and information

For each submitted proposal, a maximum of two genome editing services can be requested. For example, this could include the establishment of knock-out (KO) cell lines from two different hiPSC lines, or the tagging of gene 1 with GFP and gene 2 with RFP within the same cancer cell line. Monoallelic and biallelic clones generated from the same editing event will be considered as a single service.

NOTE: Projects involving more than 2 samples may optionally be described in the written proposal. These requests will be evaluated on a case-by-case basis and considered only in exceptional circumstances, depending on the Facility's available capacity and the overall ranking of the project.

Technical requirements: We provide the service for PSCs and immortalized/cancer cell lines. Cell lines should be submitted in a cryopreserved state: >1 x 10^6 cells/cryovial, along with a detailed protocol for their culture. The applicant is required to present documentation confirming the absence of Mycoplasma within the two months preceding the shipment to Human Technopole.

Information to be provided in the application:

- Has your genome engineering strategy already been published? If so, please add the reference(s).
- Do you need to target a particular region within the locus of interest? Provide relevant information (e.g., exon 1/2, IVS, etc.) including coordinates in the genome (current major release of the human reference assembly).
- Has your gene(s) of interest nearly identical paralogs?
- Is the gene of interest expressed in the required cell line?
- Do you need bi-allelic and/or mono-allelic engineered cells?
- Are genome integrity data available for the target cell line?

Characterization: Every engineered cell line undergoes a comprehensive assay characterization panel and is accompanied by a Certificate of Analysis (CoA):

(please refer to appendix 1 for details)

- *In silico* evaluation of potential off-targets caused by the gRNA.

- Experimentally determined confirmation of desired editing.
- Undetectable mycoplasma and bacterial contamination.
- Post-thaw viability.
- Cell identity: STR profiling of primary cells and derived clone (only for human cells).
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (G-banding - only for human cells).

Delivery Time: Full genome editing services, including single-cell clone isolation and complete quality control, generally require up to 5 months from the start of the project. Bulk genome editing services, involving only editing and bulk validation without clonal screening, typically require approximately 3 months to complete.

Important note: Upon receipt, the cells will undergo thorough testing for mycoplasma contamination and viability. If the quality falls below the required standards, the cells will be discarded.

Access modality available: Access to NF services.

4.2.2 Bulk option

During the proposal submission phase, applicants are asked to indicate whether they are willing and technically equipped to allow their project to be converted from a full service to a bulk service, **in case full-service capacity is exceeded** (see description of bulk below). This declaration does not imply the automatic conversion of the request to a bulk service but enables flexibility during project selection.

Once proposals are evaluated and ranked based on scientific merit, those exceeding the facility's full-service capacity may be redirected to the Bulk Genome Editing Service. Applicant shall demonstrate that they have Access to the necessary infrastructure and expertise (e.g., single-cell cloning, genotyping assays, data analysis) to perform clonal screening and quality control steps at their home institution. This mechanism ensures that high-quality projects can still proceed, even if full-service slots have reached saturation.

***Figure 2: Overview of the service offered through the Bulk Option**. The NF-GEDM Facility is responsible for designing the editing strategy, performing the genome editing procedure, and validating the bulk cell population. Clonal selection and quality control steps are the responsibility of the User. To support these downstream processes, NF-GEDM staff will provide assistance through dedicated video consultations.*

Description: The Bulk Genome Editing Service is a streamlined project in which NF-GEDI staff designs the genome editing strategy, performs the editing experiment, and validates the editing efficiency at the bulk population level using targeted assays (e.g., PCR, ddPCR, or Sanger sequencing on pooled cells). Once the editing efficiency in the pool is measured and

deemed sufficient, clonal isolation, screening, and quality control activities are carried out in the User's laboratory under de guidance of the NF-GEDI Staff. To support this process, NF-GEDI provides a customized screening protocol along with focused consultancy to assist with troubleshooting and strategic guidance.

This service is designed to expand facility capacity while empowering proficient users with the internal capabilities to manage clonal workflows, all while benefiting from expert design and editing execution provided by the NF-GEDI Facility.

Deliverables: At the completion of the Bulk Genome Editing Service, NF-GEDI will provide:

- Up to four cryovials for each editing strategy employed (e.g., each unique sgRNA or donor template used) containing the genome-edited bulk cell population, validated for editing efficiency at the population level.
- A comprehensive report summarizing the genome editing strategy, including details of the targeted locus, the gRNA sequences employed, and donor template information if applicable. Results of the editing validation at the bulk population level, obtained through PCR, ddPCR, or Sanger sequencing of pooled cells, will be presented. Additionally, an *in silico* analysis of potential off-target sites for all gRNAs utilized in the project will be included
- A written custom screening protocol tailored to the specific project will be provided, offering detailed guidelines for single-cell cloning methods, such as limiting dilution or FACS-based sorting. The protocol will also include recommendations for appropriate screening assays to validate clonal editing events, as well as suggested genotyping primers and a workflow designed to facilitate efficient downstream analysis.
- Scientific consultancy to support the interpretation of bulk editing validation results, advise on clonal screening strategies, and assist with troubleshooting for downstream applications. The number of remote consulting session and their time is set upon valuation of each single project in order to protect time of the NF-GEDI staff, contain costs but at the same time guarantee the successful competition of the project.

4.2.3 Target Gene Knock-Out

SID: NF20.005: Knock-out hPSCs

SID: NF20.007: Knock-out Cell Lines

SID: NF20.011: Large knock-out hPSCs

SID: NF20.012: Large knock-out Cell Lines

Description: Our Target Knock-Out service provides flexible genome editing solutions for iPSCs and established cell lines using high-fidelity Cas9 (Hi-Fi SpCas9) technology. For classic knock-out applications, we introduce targeted insertions or deletions (InDels) at coding exons, transcription factor binding sites, or splicing junctions, disrupting gene function or regulatory elements via non-homologous end joining (NHEJ). For large knock-out strategies, we design and deliver two guide RNAs (gRNAs) flanking the target region, enabling precise excision of larger genomic segments such as entire exons, promoters, or non-coding regulatory regions. The efficiency of large fragment deletion is quantitatively assessed by droplet digital PCR (ddPCR). Edited clones from both strategies undergo rigorous validation by Sanger sequencing to confirm on-target editing events.

Monoallelic and biallelic modifications can be generated according to User specifications, and an *in silico* off-target prediction analysis is included to ensure specificity. This service is ideal for generating complete gene knock-outs, dissecting regulatory elements, and developing custom cell models.

Deliverables: For each requested clone, we provide a Master Cell Bank consisting of 10 cryopreserved vials. Each Master Bank is accompanied by a Certificate of Analysis (CoA), which details the molecular editing strategy, the experimentally confirmed genotype, and the results of a comprehensive quality control panel. This panel includes assessments of genome integrity, chromosomal stability, cell line identity, and verification of the absence of mycoplasma contamination. In addition, an *in silico* prediction of potential off-target events is performed and reported to provide an additional layer of confidence in genome editing specificity. A full description of each quality control assay is provided in section 4.2.6.

4.2.4 Target Gene Knock-In

SID: NF20.006: Knock-in hPSCs

SID: NF20.008: Knock-in Cell Lines

SID: NF20.013: Large knock-in hPSCs

SID: NF20.014: Large knock-in Cell Lines

Description: Our Targeted Knock-In service enables the precise introduction of specific genetic modifications into iPSCs and established cell lines using high-fidelity Cas9 (Hi-Fi SpCas9) in combination with optimized donor templates. For small knock-ins (insertions <200 bp), such as point mutations, epitope tags, or short functional elements, we employ single-stranded oligodeoxynucleotides (ssODNs) as homology donors to achieve efficient and scarless integration via homology-directed repair (HDR).

For larger insertions (>200 bp), including fluorescent tags (e.g., GFP, mCherry) or selection cassettes, we utilize plasmid-based DNA donors with longer homology arms to support robust targeted integration. Each engineered clone is validated by Sanger sequencing or by digital drop PCR (ddPCR) to confirm precise on-target editing.

Monoallelic or biallelic knock-in events can be generated based on User specifications. This service is ideally suited for disease modeling, live-cell imaging applications, lineage tracing, and functional genomics studies.

Deliverables: For each requested clone, we provide a Master Cell Bank consisting of 10 cryopreserved vials. Each Master Bank is accompanied by a Certificate of Analysis (CoA), which details the molecular editing strategy, the experimentally confirmed genotype, and the results of a comprehensive quality control panel. This panel includes assessments of genome integrity, chromosomal stability, cell line identity, and verification of the absence of mycoplasma contamination. In addition, an *in silico* prediction of potential off-target events is performed and reported to provide an additional layer of confidence in genome editing specificity. A full description of each quality control assay is provided in section 4.2.6.

4.2.5 Base editing

SID: NF20.009: Base editing hPSCs

SID: NF20.010: Base editing Cell Lines

Description: Our Base Editing service offers a next-generation genome engineering platform that enables precise, single-nucleotide conversions without inducing double-strand breaks (DSBs), minimizing the risk of unintended genomic alterations. We utilize advanced cytidine base editors (CBEs) and adenine base editors (ABEs), based on Cas9 nickase fused to a deaminase domain, to efficiently install C→T or A→G substitutions at predefined genomic sites.

This technology is particularly suited for generating disease-relevant point mutations, correcting pathogenic alleles, or modulating regulatory elements with high precision. Special emphasis is placed on editing safety, including rigorous design and validation steps to minimize bystander edits (unintended modifications of adjacent nucleotides) and to maximize editing specificity. Proper target site selection is critical and must satisfy key criteria such as the presence of an appropriate PAM sequence, optimal spacer positioning, and minimal predicted off-target activity. Each base-edited clone is validated by Sanger sequencing to confirm the precise nucleotide change, and the absence of unwanted InDels.

Monoallelic or biallelic base edits can be generated according to User-defined requirements. This service is ideally suited for the generation of precise disease models, functional studies, and therapeutic research applications where genome integrity and specificity are paramount.

Deliverables: For each requested clone, we provide a Master Cell Bank consisting of 10 cryopreserved vials. Each Master Bank is accompanied by a Certificate of Analysis (CoA), which details the molecular editing strategy, the experimentally confirmed genotype, and the results of a comprehensive quality control panel. This panel includes assessments of genome integrity, chromosomal stability, cell line identity, and verification of the absence of mycoplasma contamination. In addition, an *in silico* prediction of potential off-target events is performed and reported to provide an additional layer of confidence in genome editing specificity. A full description of each quality control assay is provided in section 4.2.6.

4.2.6 Custom Services

SID: NF20.CustomEditing

Description: For projects that do not fit within our standard Knock-Out, Knock-In, or Base Editing platforms (e.g. targeting multiple genes or gene families simultaneously), we offer a fully customized genome engineering service. Our team of experts at NF-GEDI will work closely with the User to assess project feasibility and design a tailored editing strategy based on the specific scientific objectives and technical requirements. Depending on the project, this may involve the use of alternative CRISPR systems, hybrid editing approaches, multiplex editing, targeted inversions, complex large-scale genomic rearrangements, or other specialized genome modifications.

Each custom project undergoes a thorough technical evaluation, including target site analysis, feasibility assessment, risk analysis (e.g., off-target potential), and a proposed experimental

workflow. If deemed feasible, a detailed project plan and timeline will be provided. Validation strategies, including Sanger sequencing, and additional functional assays if needed, will be incorporated to ensure delivery of high-quality edited clones. Projects are handled on a case-by-case basis to maximize innovation while maintaining the highest standards of genome integrity and safety.

4.3 COMBINATION/SEQUENTIAL SERVICES

Project proposals may include requests for either individual services or a combination of multiple services, depending on the scientific objectives. Applicants have the flexibility to design multi-step projects that integrate up to two services from each of the two main categories offered by the NF for Genome Engineering and Disease Modelling: i) somatic cell reprogramming and ii) genome engineering.

Combined service applications must be justified by a clear experimental rationale and detailed in the project proposal. The sequencing of services should reflect the logical progression of the research goals, and the overall timeline will be defined based on the complexity of the requested workflow.

Illustrative Example: Multi Service Application

- *Project Scope*: The applicant presents a proposal for a multi-service project that incorporates two services of the NF for Genome Engineering and Disease Modelling services: e.g. generation of iPSC combined with gene editing of selected clones.
- *Project Execution*: The PBMCs will be dispatched to the Human Technopole for iPSC derivation, characterization, and banking. The derived iPSC will thus be employed to generate isogenic cell line.
- *Project completion*: Project completion timeline is estimated to be +12 months.

5. ADDITIONAL SERVICES

Description: This section encompasses supplementary services offered in partnership with other NFs and, in this call, only available in combination with one or more of the services described above. The additional services may allow further analyses of the samples generated by the NF for Genome Engineering and Disease Modelling, when essential for achieving the proposal's objectives.

SID: G-001 – Whole Genome Sequencing (WGS)

Services description:

Whole Genome Sequencing (WGS) is a comprehensive and high-throughput technique that enables the complete DNA sequence of an organism's entire genome. Whole Genome Sequencing is a powerful tool with applications in various fields, including genomics research, personalized medicine, and clinical diagnostics. It provides a comprehensive view of an organism's genetic makeup, enabling a deeper understanding of genetic variations, evolution, and the genetic basis of diseases.

Bioinformatic analysis of WGS data can be provided as a combined service by the NF for Data Handling and Analysis. SID: NF62.001.

Library preparation protocol:

Libraries will be prepared by following the protocol:

Illumina DNA PCR-Free Library Prep Reference Guide (1000000086922)

Illumina DNA PCR-Free offers a unique combination of benefits from on-bead tagmentation and PCR-free chemistry steps. On-bead tagmentation supports bead-based normalization, easy volume-based library pooling, and elimination of pre- and post-library quantification steps. The PCR-free workflow simplifies and reduces the overall workflow time while providing highly uniform coverage across repetitive or uneven genome regions. For sensitive applications such as human WGS, de novo assembly of microbial genomes, or tumour–normal variant calling, Illumina DNA PCR-Free delivers uniform coverage, and high-accuracy data.

Libraries sequencing and NGS coverage: Libraries will be sequenced using the NovaSeq 6000 system (Illumina) by generating 150 bp Paired End reads.

Next-generation sequencing (NGS) coverage describes the average number of reads that align to, or "cover," known reference bases. Sequencing coverage requirements vary by application, as noted below. At higher levels of coverage, each base is covered by a greater number of aligned sequences reads, so base calls can be made with a higher degree of confidence.

Results that will be delivered to the Users:

The NF for Genomics will deliver to the Users the following files for every sample sequenced:

- FASTQ files
- QC report

- Mapping metrics (if reference genome/transcriptome is available)

For projects in which the bioinformatic analysis of WGS data will be requested as a combined service from the NF for Data Handling and Analysis, the following files will be delivered to the Users:

- Alignment (BAM files)
- Mapping metrics
- Identified variants (VCF files)
- Analysis QC reports

SID: G-006 – totalRNA sequencing from standard input

Services description:

Total RNA sequencing is a powerful and widely used molecular biology technique that aims to analyse and quantify the entire transcriptome of a biological sample. The transcriptome represents the complete set of RNA molecules, including messenger RNA (mRNA), and non-coding RNAs, present in a cell or tissue. Total RNA sequencing provides a comprehensive view of the transcriptome, allowing researchers to gain insights into gene expression patterns, identify novel transcripts, and understand the regulatory mechanisms underlying various biological processes.

Bioinformatic analysis of totalRNAseq data can be provided as a combined service by the NF for Data Handling and Analysis. SID: NF62.001

Library preparation protocol:

Libraries will be prepared by following the protocol:

Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus Reference Guide

Illumina Total RNA Prep with Ribo-Zero Plus supports a broad range of RNA inputs. It's compatible with various sample types, including formalin-fixed paraffin-embedded (FFPE) and other low-quality samples. The included Ribo-Zero Plus or Ribo-Zero Plus Microbiome removes abundant RNA from multiple species, including human, mouse, rat, bacteria, and epidemiology samples or complex microbial samples, including stool samples, for meta-transcriptomic studies.

Libraries sequencing and NGS coverage:

Libraries will be sequenced using either the NextSeq 2000 system or the NovaSeq 6000 system (Illumina) by generating 100 bp Paired End reads.

On average 80 million reads pairs (40 million clusters 100bp PE) per sample will be generated for species with reference genomes and 160 million reads pairs (80 million clusters 100bp PE) will be generated for species without reference genomes or for meta-transcriptomics studies.

Results that will be delivered to the Users:

The NF for Genomics will deliver to the Users the following files for every sample sequenced:

- FASTQ files
- QC report

For projects in which the bioinformatic analysis of WGS data will be requested as a combined service from the NF for Data Handling and Analysis, the following files will be delivered to the Users:

- Transcriptome alignment (BAM files)
- Mapping metrics
- Raw and normalized expression matrix
- Differential expression analysis
- Analysis QC reports

APPENDIX 1: CELL LINE CHARACTERISATION

Mycoplasma contamination detection

Cell lines undergo mycoplasma contamination testing utilizing an isothermal PCR-based assay (MycosStrip™, Invivogen, Cat. No. rep-mys-10) or a colorimetric assay (PlasmoTest™ - Mycoplasma Detection Kit, Catalog #: rep-pt1, Invivogen). Following thawing, the medium is replaced the next day, and cells are maintained for up to 5 days without antibiotics. At this point, conditioned medium is collected and subjected to testing.

Transgene Clearance of reprogrammed iPSC clones

At passage 10, RNA is extracted and subjected to retro-transcription. The presence or absence of the Sendai genome is examined by assaying >5ng of cDNA in a qPCR run with 40 cycles.

Positive (RNA from iPSCs containing Sendai vectors) and negative controls (NTC and NRT) are included in each run.

Post-thaw viability

2+ weeks post freezing date, a vial from the master bank is thawed to monitor mycoplasma contamination and assess viability. The medium is replaced one day after thawing, and the cells are kept in culture until confluency is achieved.

Genome Integrity

a. Low-Pass Sequencing

DNAs from parental and derived cells are subjected to whole-genome sequencing (WGS) data at a 1x coverage (Low Pass Sequencing, LPS). The reads are aligned to the human GRCh38 reference assembly using BWA (version v0.7.17). Next, duplicated reads are removed using Picard (version 2.26.10). Finally, a read depth segmentation analysis is performed using ControlFREEC (version v11.6) to compare the coverage of the cell line sample with that of a WGS 1x of the parental cells. The workflow allows to identify significant deviations (CNVs) in coverage in bins of 100k bases.
Obtained results exclude the presence of large aneuploidies, chromosomal abnormalities or clinically relevant deletions or duplications. LPS, as other comparable techniques (aCGH and SNPs array), does not detect balanced translocations, inversions, or any other balanced alterations in chromosome structure, low-grade mosaicism, or polyploidy.

b. Karyotyping

QFQ-banding assay detects genomic abnormalities (>5-10 Mb): Inversions, duplications/deletions, balanced and unbalanced translocations, aneuploidies. Q-banding is a reversible fluorescent-technique in which quinacrine is used for the staining. After UV light exposure, AT-rich regions enhance the fluorescence signal while GC-rich regions quench the fluorescence, showing a final pattern

equivalent to the one obtained in G-banding. Karyotyping is conducted as a standard metaphase karyotype (300–400 bands), useful for identifying extra or missing chromosomes. Our service guarantees the analysis of 50 metaphases, which excludes 6% mosaicism with 95% confidence.

Expression of markers for undifferentiated hPSCs

hPSCs colonies are fixed and stained for the presence of markers for undifferentiated hPSCs (see Table 1). Co-expression of OCT4 and SSEA-4, and NANOG and TRA-1-60, expression SOX2 and lack of SSEA-1 expression are used to determine hPSCs state.

Target protein	Provider	Cat.no.	Dilution
NANOG	abcam	ab109250	1:500
OCT3/4	abcam	ab19857	1:500
SOX2	Sigma/Merck Millipore	AB5603	1:500
SSEA-1	Biolegend	125602	1:500
SSEA-4	Biolegend	330402	1:500
TRA-1-60	Biolegend	339692	1:500

Table 1 List of antibodies used for the assay

Evaluation of differentiation potential for hPSCs

Cells are detached using 0,5 mM EDTA and seeded as aggregates in ultra-low attachment wells in E8 medium supplemented with Rock Inhibitor to allow embryoid bodies generation. One day after the seeding, the aggregates are switched to spontaneous differentiation conditions and cultured for seven days in suspension in Embryoid Bodies medium (containing DMEM-F12, 20% KnockOut™ Serum (Cat. no. 10828-028, Thermo Fisher), 2mM GlutaMAX™ (Cat. No. 35050-061, Thermo Fisher), 0,1mM Non-Essential Amino Acids (Cat. no. 11140-076, Thermo Fisher), 0.1mM b-Mercaptoethanol).

After 7 days, EBs are moved onto 0.1% gelatin-coated wells and maintained in culture for further seven days. EBs are fixed and stained with antibodies for three-germ layers markers (see the following table). Expression of at least one marker per germ layer is considered indicative of pluripotency.

Target protein	Provider	Cat.no.	Dilution
FOXA2	R&D Systems	AF2400	1:60
SOX17	Millipore	09-038-I	1:300
SMA	Sigma/Merck Millipore	A5228-200UL	1:500
NESTIN	R&D Systems	MAB1259	1:300
PAX6	Biolegend	901301	1:200

Table 2 List of antibodies used for the assay

Cell Line Authentication

Cell line authentication is performed on a Spectrum Compact CE System (Promega) using *GenePrint*® 10 System (Promega, Cat. No. B9510).

GenePrint® 10 provides co-amplification and four-color detection of ten loci, TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818 plus Amelogenin and D21S11.

The analysis is generated with the software Package: SoftGenetics GeneMarker_HID V3.0.

Time-course imaging

Live cells are imaged in phase contrast at specific time points using an Axio Zeiss Observer microscope equipped with a Zeiss Axiocam 807 mono, and a motorized scanning stage. Whole-well images or field of views of specific objects over time are available depending on the service.