

**HUMAN TECHNOPOLE  
NATIONAL FACILITY FOR GENOME ENGINEERING  
AND DISEASE MODELLING  
CALL FOR ACCESS  
24-GEDM-PILOT**

## Table of Contents

<b>1. INTRODUCTION</b> .....	<b>3</b>
1.1 Access modalities .....	3
<b>2. TERMS AND DEFINITIONS</b> .....	<b>4</b>
2.1 Access .....	4
2.2 Researcher .....	4
2.3 Principal Investigator .....	4
2.4 Applicant.....	5
2.5 User .....	5
<b>3. APPLICATION TYPE</b> .....	<b>5</b>
<b>4. ELIGIBILITY AND ADMISSIBILITY</b> .....	<b>5</b>
<b>5. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS</b> .....	<b>8</b>
<b>6. EVALUATION OF APPLICATION</b> .....	<b>8</b>
6.1 Evaluation criteria .....	9
6.2 Scoring system .....	10
6.3 Technical feasibility analysis .....	10
6.4 Evaluation results and Access approval.....	10
<b>7. AFTER ACCESS HAS BEEN APPROVED</b> .....	<b>11</b>
<b>8. AFTER ACCESS HAS BEEN COMPLETED</b> .....	<b>11</b>
<b>9. CONTACTS</b> .....	<b>11</b>
<b>10. REFERENCES</b> .....	<b>11</b>
<b>11. CHANGES TO THE CALL</b> .....	<b>11</b>
<b>ANNEX I: NATIONAL FACILITY FOR GENOME ENGINEERING AND DISEASE MODELLING: SERVICE LIST</b> .....	<b>12</b>
<b>ANNEX II: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE</b> .....	<b>31</b>

## 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 NFs are available through regular open calls for Access that are published yearly on the HT website ([link](#)) and are free of charge 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 User. The costs for the activities to be performed at the NFs will be fully covered, including shipment of relevant material from and to the User's laboratory as well as travel and accommodation for the User while accessing the NF. Project-related costs (personnel, consumables, and other costs) at the User 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. 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 authorized 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 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.

**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 Institutions recognized by the Ministry of University and Research and listed at the following [link](#).

**Eligible Institutions are strongly encouraged to limit the number of applications to the very best 2/ NF, with at least 50% coming from Junior PIs.**

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, home Institution or elsewhere. Applicants shall confirm the economic and scientific feasibility for the aspects of the project to be performed outside the NFs.

Applicants cannot request Access for the same service if an approved Access is ongoing. 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.

During the same application window, a PI can submit only one application to the NFs (i.e., participate to only one call 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 application before receiving notification of the results.

Applications must be written in English and must be complete (i.e., consist of all the requested elements and information). Incomplete applications will be considered not eligible and will be rejected at the administrative review stage.

The application form consists of six components:

1. Applicant's general information.
2. Justification for requesting Access to the NF.
3. Abstract.
4. Project proposal, including:
  - a. *Significance.*
  - b. *Innovation.*
  - c. *Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
  - d. *Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

5. Applicant's CV in NIH biosketch format.
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 in order to process samples and data through the NFs.*
  - c. *Justification of the request for Access – including a statement on why the project cannot be performed at the Applicant's Institution.*
  - 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, home 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 terms and conditions.*

The facsimile available as [Annex II](#) of this call shall be used as template.

7. Technical information, including:
  - a. *Requested service(s), as described in [Annex I](#) 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 (otherwise indicate the date of availability of the entire sample set).*
  - e. *Resources and expertise to receive and process the products – 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:*
    - 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.*

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) for assessing scientific merit.

## 5. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

Applications shall be submitted exclusively through the web-based procedure managed by CINECA and accessible at this [link](#), according to the terms and methods there indicated.

Applications are accepted throughout the year and assessed in three evaluation rounds per year. The time between one evaluation round and the next one is considered an application window.

- Evaluation round 1: applications submitted between the 1<sup>st</sup> of January and the 30<sup>th</sup> of April will enter the first evaluation round (May/ June).
- Evaluation round 2: applications submitted between the 1<sup>st</sup> of May and the 31<sup>st</sup> of August will enter the second evaluation round (September/ October).
- Evaluation round 3: applications submitted between the 1<sup>st</sup> of September and the 31<sup>st</sup> of December will enter the third evaluation round (January/ February).

This first call for Access (Call ID: 2024-GEDM-Pilot) will be **open from the 10<sup>th</sup> of June 2024 (1 pm) to the 31<sup>st</sup> of December 2024 (1 pm)** and will involve 2 rounds of evaluation (September 2024 and January 2025).

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 I](#) of this call.

## 6. 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 2 SIEC members, who will act as Chairs, plus 10 appointed external Reviewers, with the relevant expertise.

Below is a scheme describing evaluation steps and timeline.



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.

The application is then sent to the Review Panel for assessing scientific merit and technical feasibility.

If the number of applications exceeds by a factor of 4 the estimated capacity of the NF, a triage will be applied within each application category by the relevant Review Panel.

Triage criteria will include:

- a. Justification for requesting Access to the NF.
- b. Field-Weighted Citation Impact (FWCI).
- c. Track record in securing research funding.

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 the two Junior PI categories.

### 6.1 Evaluation criteria

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.

## 6.2 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.

## 6.3 Technical feasibility analysis

During the evaluation, the relevant experts from SIEC 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/ Pilot study required (switch from Standard to "Proof-of-Concept application track).

## 6.4 Evaluation results and Access approval

NF staff provides the SIEC with information on the resources needed (cost and time) to perform the highest ranked projects. The most positively evaluated applications that fulfil all technical requirements are approved for Access by SIEC, based on the capacity of the NF. NF staff schedules Access. A selected number of applications may be placed on a waiting list (in case of cancellations).

Evaluation results – Access approved, Access waitlisted, Access not approved – are communicated to the Applicant through the Access portal.

Applicants whose applications are placed on the waiting list will receive additional information advising whether the project can be Access approved or should be resubmitted within the subsequent application window.

## 7. AFTER ACCESS HAS BEEN APPROVED

After Access approval, a kick-off meeting is organized by the NF User Access Office and 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, the NF User Access Office coordinates the signature of the required formal Agreements (e.g., Access Agreement, Collaboration Agreement, other) and the project can start.

## 8. AFTER ACCESS HAS BEEN COMPLETED

At the end of the activities carried out at the NF, and not later than 3 months thereafter if not differently agreed with the NF User Access Office, the User must submit a short report on the results obtained and the impact of the service on their research. Moreover, a final report to be published on the NFs website and 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. Users 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 User will be asked to fill in a brief, mandatory survey regarding their experience, providing feedback and suggestions for further service improvement.

The User must communicate to the NF User Access Office (via email to [national.facilities@fht.org](mailto: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. User must inform the NF User Access Office (via email to [national.facilities@fht.org](mailto:national.facilities@fht.org)) when and how the data are made public.

## 9. CONTACTS

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

## 10. REFERENCES

NF Access workflow\_Convenzione ([link](#))

NF Access rules\_Convenzione ([link](#))

NF Access Agreement\_Convenzione ([link](#))

## 11. CHANGES TO THE CALL

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

**ANNEX I: NATIONAL FACILITY FOR GENOME ENGINEERING AND  
DISEASE MODELLING: SERVICE LIST**

**HUMAN TECHNOPOLE  
NF FOR GENOME ENGINEERING AND DISEASE  
MODELLING  
CALL FOR ACCESS  
24-GEDM-PILOT  
SERVICE LIST**

## Table of Contents

<b>1. INTRODUCTION.....</b>	<b>14</b>
<b>2. GENERAL TECHNICAL REQUIREMENTS .....</b>	<b>14</b>
<b>3. DEFINITIONS .....</b>	<b>14</b>
<b>4. SEQUENTIAL SERVICES.....</b>	<b>14</b>
<b>5. SERVICE LIST.....</b>	<b>15</b>
5.1 REPROGRAMMING OF SOMATIC CELLS.....	15
SID: NF20.001 - Reprogramming of PBMCs.....	16
SID: NF20.002 - Reprogramming of Fibroblasts .....	17
5.2 GENOME ENGINEERING .....	18
5.2.1 Target Gene Knock-Out .....	18
SID: NF20.004: Knock-out hPSCs.....	18
SID: NF20.007: Knock-out Cell Lines .....	18
5.2.2 Knock-In/Point Mutations .....	19
SID: NF20.005: Knock-In/Point Mutations hPSCs.....	19
SID: NF20.008: Knock-In/Point Mutations Cell lines .....	19
5.2.3 Custom Projects .....	21
SID: NF20.CustomEditing: Custom Gene Editing Projects .....	21
<b>6. ADDITIONAL SERVICES .....</b>	<b>22</b>
SID: NF55.003/004/005 - Extracellular electrophysiology - Microelectrode Array (MEA) assays .....	22
SID: NF53.001 – Flow Cytometry Cell Sorting Services.....	23
SID: NF53.002/003 Flow Cytometry Analysis/ Assisted Flow Cytometry Analysis.....	23
SID: NF53.004 Consultation session: Sample Preparation/Panel Design/Data Analysis .....	24
SID: G-001 – Whole Genome Sequencing (WGS).....	24
SID: G-006 – totalRNA sequencing from standard input.....	26
<b>APPENDIX 1: CELL LINE CHARACTERISATION .....</b>	<b>28</b>

## 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 pilot call for Access include the reprogramming of somatic cells into iPSCs and genome engineering of both stem cells and cell lines.

## 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.
- 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

INDEL: INsertion and/or DEletion of nucleotides into genomic DNA

HDR: Homology-Directed Repair

MEA: Microelectrode Array

## 4. SEQUENTIAL SERVICES

Project proposals can be submitted for either individual services or a combination of multiple sequential services, as illustrated in the figure below.



### Illustrative Examples:

#### Single Service Application:

- *Project Scope:* The applicant proposes the generation of iPSC from Peripheral Blood Mononuclear Cells (PBMCs) sourced from a cohort of 5 subjects.
- *Project Execution:* The PBMCs will be dispatched to the Human Technopole for iPSC derivation, characterization, and banking, with an anticipated completion timeline of 8 months.
  - *Scenario one: Project Completion.* iPSC lines are shipped to the User and the project is closed.
  - *Scenario two: Project Continuation.* Prior to the project's conclusion, the applicant has the option to submit a new request for Access for project continuation, focusing on the generation of gene-edited isogenic cell lines from the original samples.

#### Multi Service Application:

- *Project Scope:* The applicant presents a proposal for a multi-service project that incorporates two or more 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. SERVICE LIST

### 5.1 REPROGRAMMING OF SOMATIC CELLS

**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 characterized through a comprehensive panel of assays and delivered in a cryopreserved Master Bank (details can be found in each service description).

#### SID: NF20.001 - Reprogramming of PBMCs

**Service:** Direct reprogramming of one 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 25 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:** The maximum allowable number of samples is 6. Proposals for projects involving reprogramming larger cohorts will be assessed on a case-by-case basis, taking into consideration the NF's available capacity.

PBMCs should be submitted in a cryopreserved state:  $> 5 \times 10^5$  cells/cryovial.

**Method:** Non-integrating Sendai viral vectors.

**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.
- Transgene clearance.
- 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 (optional, G-banding).

**Deliverables:** We generate a maximum of 3 clones from each PBMC 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 PBMCs requires 3 - 4 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 mycoplasma contamination and viability. If the quality falls below the required standards, the cells will be discarded.

**Access modality available:** Access to NF services.



## SID: NF20.002 - Reprogramming of Fibroblasts

**Service:** Direct reprogramming of one 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 1-4) overnight. On day 5, 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 24-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:** The maximum allowable number of samples is 6. Proposals for projects involving reprogramming larger cohorts will be assessed on a case-by-case basis, taking into consideration the NF's available capacity.

Fibroblasts should be submitted in a cryopreserved state ( $>5 \times 10^5$  cells/cryovial) at low passage number (P2-P5). Reprogramming efficiencies after passage 7 can drop 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 (optional, G-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.

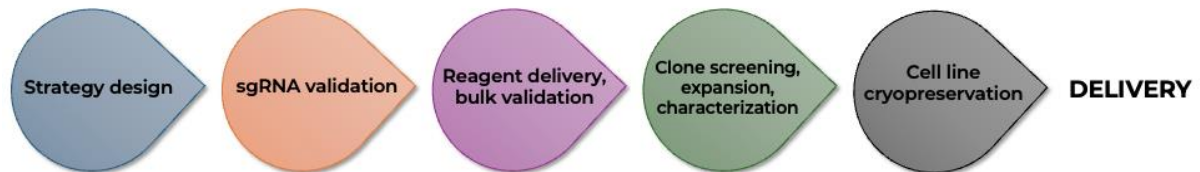
**Important note:** The quality of fibroblast culture is influenced by both the state of cryopreservation and passage number (see *Technical Requirements*), which affects the efficiency of reprogramming. Upon receipt, the cells will undergo thorough testing for mycoplasma contamination and viability.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

**Access modality available:** Access to NF services.

## 5.2 GENOME ENGINEERING

**Description:** We provide gene editing services for the generation of engineered PSCs or immortalized/cancer cell lines.

The following diagram illustrates our workflow:



All genome engineering services include:

- i. Initial consultation to discuss project requirements.
- ii. Design and validation of gRNA.
- iii. Generation of donor template for Knock-In/Point Mutations.
- iv. Delivery of the reagents into one designated cell line.
- v. Pre-screening of bulk cell pool.
- vi. Clonal isolation and sequence validation of up to 3 modified clones.
- vii. Establishment of a Master Bank of expanded clones.

For customized modifications (not covered by the NF20.004/ NF20.005/ NF20.007/ NF20.008 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:** The project outcome and the editing required hinge on numerous experimental variables, making success uncertain. In such instances, where feasible, we will endeavour to make a secondary attempt, agreeing in advance on potential alternative experimental methods.

### 5.2.1 Target Gene Knock-Out

SID: NF20.004: Knock-out hPSCs

SID: NF20.007: Knock-out Cell Lines

**Service:** Introduction of INDELS (frame-shift mutations) to generate gene knock-out lines. Each service includes the KO of a single gene in a single hPSCs or immortalized/cancer cell line.

**Technical requirements:** We provide the service for hPSCs and immortalized/cancer cell lines. Cell lines should be submitted in a cryopreserved state:  $>1 \times 10^6$  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.
- Sanger sequencing confirmation of desired editing.
- Undetectable mycoplasma and bacterial contamination.
- Post-thaw viability.
- Cell identity: STR profiling of primary cells and derived clone.
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (optional, G-banding).
- *For hPSCs only, optional:* Expression of markers for undifferentiated hPSCs.
- *For hPSCs only, optional:* *In vitro* three-germ layers differentiation.

**Deliverables:** We generate up to 3 homozygous knock-out clones. Heterozygous KO clones can be supplied following an agreement with the applicant. If technically feasible, clones will be analysed for the occurrence of allelic dropout.

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:** The target gene KO service requires 2 - 3 months for its completion.

**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.

### 5.2.2 Knock-In/Point Mutations

SID: NF20.005: Knock-In/Point Mutations hPSCs

SID: NF20.008: Knock-In/Point Mutations Cell lines

**Service:** Endogenous gene tagging; generation of reporter cell lines; safe harbor insertions; point mutations.

Each service comprises the introduction of one Knock-In (KI) based on the selected strategy into a single hPSCs or immortalized/cancer cell line.

**Technical requirements:** We provide the service for hPSCs and immortalized/cancer cell lines. Cell lines should be submitted in a cryopreserved state:  $>1 \times 10^6$  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).
- 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.
- Sanger sequencing confirmation of desired editing.
- Undetectable mycoplasma and bacterial contamination.
- Post-thaw viability.
- If applicable: Imaging of fluorescent reporter activity.
- Cell identity: STR profiling of primary cells and derived clone.
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (optional, G-banding).
- *For hPSCs only, optional:* Expression of markers for undifferentiated hPSCs.
- *For hPSCs only, optional:* *In vitro* three-germ layers differentiation.

**Deliverables:** We generate up to 3 knock-in clones.

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:** The KI service requires 2 - 4 months for its completion.

**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.

### 5.2.3 Custom Projects

SID: NF20.CustomEditing: Custom Gene Editing Projects

**Service:** Development and execution of custom gene editing projects.

This service is designed to offer expertise and technical support for the implementation of genome engineering projects that demand customized approaches not presently covered by off-the-shelf editing services (NF20.004/ NF20.005/ NF20.007/ NF20.008).

The proposal should provide a thorough explanation of the rationale, goals, and expected outcomes to facilitate a comprehensive evaluation.

Tailored gene editing projects involve completing sequential checkpoints before moving on to the implementation of the proposed experiment (Phase 3). Throughout these phases, significant interaction with the applicant is expected.

**Phase 1 (Strategy design):** A tailored strategy will be designed following preliminary meetings and discussed with the applicant before proceeding to the next phase.

**Phase 2 (Strategy validation):** The designed approach will undergo testing in the relevant cell line to assess its effectiveness (efficiency, locus accessibility, ...).

**Phase 3 (Target editing generation):** Upon successful completion of Phase 2, the validated approach will be applied to generate the desired cell line.

**Technical requirements:** We provide the service for hPSCs and immortalized/cancer cell lines. Cell lines should be submitted in a cryopreserved state:  $>1 \times 10^6$  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).
- 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.
- Sanger sequencing confirmation of desired editing.
- Undetectable mycoplasma and bacterial contamination.
- Post-thaw viability.
- If applicable: Imaging of fluorescent reporter activity.

- Cell identity: STR profiling of primary cells and derived clone.
- Genome Integrity: CNVs detection using Low-Pass Sequencing (LPS) and Karyotype (optional, G-banding).
- *For hPSCs only, optional:* Expression of markers for undifferentiated hPSCs.
- *For hPSCs only, optional:* *In vitro* three-germ layers differentiation.

Additional characterization will be incorporated as per the requirements of the User.

**Deliverables:** based on project requirements.

If available, cell lines will be 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:** based on project requirements.

**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.

## 6. 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: NF55.003/004/005 - Extracellular electrophysiology - Microelectrode Array \(MEA\) assays](#)

**Service:** High resolution, optics-free functional imaging based on high density MEA electronic interfaces measuring extracellular voltage potentials. The system available is the multichannel amplifier BioCAM Duplex and MEAs electronic chips equipped with 4,096 electrodes (21 x 21 µm, 60 µm pitch; 3BRAIN AG). The assay is suitable for the characterization of functional phenotypes in cell networks, and for compound screening. The high density of electrodes allows to collect a large amount of data within a single recording, and it is particularly suitable for accessing the electrical phenotype of brain and cardiac organoids.

**Technical requirements:** standard *in vitro* models handled and accepted by the Ion Imaging Infrastructural Unit are the ones provided by GEDI (the maximum allowable number of samples is 30).

**Information to be provided in the application:** please specify if the biological model is known to be electrogenic and if so, is the electrical activity is spontaneous or induced.

**Method:** Samples will be prepared as cell/organoid cultures on MEAs electronic chips by the Ion Imaging Infrastructural Unit staff *when they reach the proper developmental stage for functional characterization* (this timing depends on the biological model and will be discussed

during the feasibility study of the project). Recordings from MEA cultures will be acquired with the parameters suitable for detecting the event of interest.

**Deliverables:** The User will receive raw data from MEA recordings in hdf5 format and will be responsible for their processing, analysis, and interpretation. Optionally, the User can ask to perform a basic processing of raw data using the 3BRAIN AG proprietary software (Brainwave) for the detection of the electrophysiological events of interest. Processed data will be delivered to the User in hdf5 format.

**Delivery Time:** depends on the User's specified time points and requirements.

#### SID: NF53.001 – Flow Cytometry Cell Sorting Services

Full-service sorting of rare populations from heterogeneous samples, cell cloning (single cell deposition into multi-well plates), particle enrichment, and high purity bulk sorts.

High-recovery and indexed single-cell sorting for sequencing.

Cell sorting of many cell types including:

- immune cell and hematopoietic stem cell subsets.
- mesenchymal stem cells.
- viable cytokine producing cells.
- General cell sorting approaches for cell lines and transfected cells.

#### Sorting Technical Details

Capable of standard and high-speed sorting of up to 6 populations simultaneously. Sorted cells may be recovered in numerous tube and plate types including:

- 6, 12, 24, 28, 96, 384, 1536 well plates & 96 deep well plates.
- Custom vessels may also be programmed.
- 0.2, 0.5, 1.2, 1.5, 2, 5 mL tubes for 6-way sorting.
- 15, 50 mL tubes for 2- or 3-way sorting.
- Slides or Ibidi vessels.

The sorter is equipped with 6 lasers having the following emissions: 355nm, 405nm, 488nm, 560nm, 592nm, 645nm.

Proper controls for each session must be included with the analysis samples. For example:

- Unstained, unlabelled or other cellular controls.
- Spectral overlap compensation controls.
- FMO/FMX controls when appropriate.

#### SID: NF53.002/003 Flow Cytometry Analysis/ Assisted Flow Cytometry Analysis

**Description:** Autonomous Flow Cytometry Analysis (after completion of a training session) or Operator Assisted Flow Cytometry Analysis.

The following are examples of analyses we offer:

- Prepared immune cell and hematopoietic stem cell subsets.
- Prepared and labelled samples of mesenchymal stem cells.
- Analysis of cytokine producing cells.
- Multi-color extracellular and intracellular stained samples.
- DNA content analysis (single color or multi-color).
- Cell proliferation analysis.
- Apoptosis analysis.
- Functional and metabolic assays (mitochondrial function, ROS production, lipid metabolism).
- Analysis of bead assays (eg. Cytokine/chemokine bead assays).

**Analyzer Technical Details:**

- Integrated absolute count in the analysis.
- Plate loader supporting 96 wells plate and 96 deep wells plate. U, V or Flat bottom.
- Different sample injection modes, manual or automatic.
- Wide sample flow rate and minimal dead sample volume (about 20ul).

Analysers are equipped with 5 or 6 lasers having the following emissions: 355nm, 405nm, 488nm, 561nm, 640nm and 808nm. SSC parameter is available for the 488 and the 405 lasers lines.

- Capable of complex polychromatic panels.
- Advice in experimental design and dedicated training sessions are also available.

Proper controls for each session must be included with the analysis samples. For example:

- Unstained, unlabelled or other cellular controls.
- Spectral overlap compensation controls.
- FMO/FMX controls when appropriate.

A consultation session (SID: NF53.004) can be added to this service.

[SID: NF53.004 Consultation session: Sample Preparation/Panel Design/Data Analysis](#)

User consultation is an essential first step in assuring high-quality flow cytometric data. Our staff are available to guide new and current Users to meet each projects' needs, addressing all critical steps between the conception of the flow cytometry experiment, the appropriate steps for sample preparation including the required experimental controls, the acquisition of samples and the analysis of the data generated.

[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**

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.

A karyotyping service is available upon request and will be performed in outsourcing with G- or Q-banding staining and analysis of 50 metaphases.

### **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	Biologend	125602	1:500
SSEA-4	Biologend	330402	1:500
TRA-1-60	Biologend	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	Biologend	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.

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

Each guide RNA used in Genome Engineering services undergoes assessment using available online tools which predicts the corresponding on-target and off-target scores. The tool generates a list of potential off-target sites, considering mismatches ranging from 0 to 4 within the protospacer sequence, for each guide RNA.

**Sanger sequencing confirmation of desired editing**

The desired edit is verified through PCR amplification of the genomic target site, followed by Sanger sequencing of the PCR product. The resulting chromatogram is analysed using available online tools capable of deconvolving Sanger sequencing data to quantify the observed edits.

**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.

**Growth-curve**

The images captured throughout the differentiation process undergo analysis for morphometric parameters utilizing image analysis workflows developed by the NF for Data Handling and Image Analysis.

**Imaging of fluorescent reporter activity**

Live cells are imaged using an Axio Zeiss Observer epifluorescent microscope equipped with a Zeiss Axiocam 807 mono. This is applicable in case the fluorescent reporter generated by the Knock-in service or the Custom Gene editing service is expressed in standard cell culture conditions (i.e. the same condition for maintaining the cells in culture) or requires minimal modifications to obtain the target cell type.

## ANNEX II: 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: (select among 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 the services requested here cannot be performed by the Applicant at the host Institution, at a cost which is deemed affordable for them.
- That relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained in order to process samples and data through Human Technopole;
- That, if applicable, biological specimens have been 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 from subjects who signed an 'informed consent', said informed consent allows that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorized third parties;

**and is committed**

- To accept the terms and conditions to Access Human Technopole National Facilities as described in the National Facilities Access rules ([link](#));
- To sign the Access Agreement should the project be approved ([link](#))

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

Date .....

Name and Title ..... ; .....

Email and Signature of legal representative or delegated person

..... ; .....