HUMAN TECHNOPOLE
NATIONAL FACILITY FOR
GENOME ENGINEERING AND DISEASE MODELLING
CALL FOR ACCESS
26-GEDM-ROUND-3

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1. SUMMARY OF CHANGES TO THIS CALL FOR ACCESS

Compared to previous rounds, the following changes have been introduced in this call:

- *Specific procedures for the **resubmission** of proposals already evaluated in previous calls, and for the submission of **project continuation** (formerly known as "follow-up"), i.e. research proposals that build on the findings and results of a previously approved Access, have been implemented in the application process (section 9 and section 10).*
- ***Changes in the available services for this call (Annex III).***
- *Rules for the "Responsible use of generative Artificial Intelligence tools in proposal preparation and evaluation" (link to the document).*
- *Eligibility and admissibility (section 5).*
- *Application content and Accountability of Applicants and host institutions (section 6).*

We recommend that the applicants read the present document and the guidelines (link) carefully before applying to the current call for Access.

2. 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).

2.1 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 (Physical Access)**: 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 (Remote Access)**: 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. In this case, physical Access may be approved.

- **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. **Note that in the application, a motivation underlying the request for "Access with training" must be specified.**

3. TERMS AND DEFINITIONS

3.1 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.

3.1.1. Physical Access (or in-person Access)

"Physical Access" applies when a User accesses a NF or a service by being physically present at the selected NF. In order to obtain physical Access, the User must complete all necessary security and health, safety and environment (HSE) checks, complete all required training and be provided with all the necessary insurances. Physical Access is also required for on-site training.

3.1.2. Remote Access

"Remote Access" means Access to a NF or service without the User being physically present at the NF. Typically, NF staff perform the experimental protocols entailed by the service and return the Data and results produced to the User together with any resulting samples (when appropriate). If applicable, "remote Access" can also include remote analysis of the samples by the User after sending them to the NF.

IMPORTANT - Additional information and details about **Access** are reported in the NF Access rules available on the NFs dedicated webpage (link).

3.2 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.

3.3 Principal Investigator, Junior and Established PI

"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.

3.4 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 5.

3.5 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.

4. 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, substantiated by robust preliminary data and/or requesting a standard service based on existing, well validated experimental conditions and/or protocol(s).

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).

Additional details related to the application contents are provided in section 6.

5. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in section 3.3 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.

Applicants are highly encouraged to contact the staff of the facility for a consultation before submitting their application, to discuss any technical aspects of the project that may affect its feasibility.

All applications submitted to this call, regardless of whether a consultation has taken place, must undergo a technical feasibility assessment. Applicants choosing not to consult with the facility staff acknowledge that their application may be considered not feasible following the technical feasibility analysis.

To get in touch, please send an inquiry to NF.gedi@fht.org, indicating the name of the requested service(s) in the email subject line.

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:
  - State funded public universities, listed under the following link.
  - Specialized superior graduate schools or institutions, listed under the following link.
  - Legally recognized non-public universities, listed under the following link.
  - 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 Formazione - INVALSI;
o) Istituto Nazionale di Documentazione, Innovazione e Ricerca Educativa - INDIRE;
p) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CREA;
q) Agenzia Nazionale per le Nuove Tecnologie, l'energia e lo Sviluppo Sostenibile - ENEA;
r) 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);
s) Istituto Nazionale di Statistica - ISTAT;
t) Istituto Superiore di Sanità - ISS;
u) Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, ferme restando le disposizioni di cui alla legge 28 giugno 2016 n.132;
v) 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/ data and relevant authorisations are available at the moment of application or no later than two (2) months** from receiving Access approval (refer to section 8). **If samples/ data 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 form.

A PI submitting an application to this call for Access **cannot request Access to other NFs** (i.e., cannot participate to other 26-ROUND-3 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, samples/ data availability, samples/ data 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 call requirements will be considered not admissible and will be rejected at the administrative review stage.

6. APPLICATION

6.1 Application content and format

All applications must be submitted through the online portal PICA (link), following carefully the guidelines (link) and filling all mandatory fields of the application form. Applicants should also consider the information provided in section 7 before initiating the submission process.

The main contents of the application form are:

1. **Applicant's general information**.
2. **Justification for requesting Access to the NF,** explaining why the project cannot be performed at the Applicant's institution.

   We recommend that the Applicant contacts the institute representative, i.e., the individual authorized to sign the Letter of Institutional Endorsement (LoE) to ensure that the most appropriate justification is provided and that it aligns with the one included in the LoE (point 6.c, below).

   If the justification provided by the Applicant does not match the one provided by the host institution through the LoE, the application will be rejected during the administrative review stage.

   The Applicant must choose the option that best applies and must provide further details supporting the choice:

   - The requested service/ technology is not available at the host institution.
   - The requested service/ technology cannot be performed at the host institution or elsewhere at an affordable cost.
   - The requested service/ technology is available at the host institution, but the necessary expertise is lacking.
   - 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.

   NOTE: The Standing Independent Evaluation Committee (SIEC), in charge of the evaluation procedure, may reserve the option to contact the host Institution and its core facilities to confirm the justification provided. **Read carefully information in section 6.2**.

3. **Project Title, Abstract** and **Area of Research**, to be inserted in the dedicated section on the application form (Max 1500 characters including spaces).

IMPORTANT - Do not share any confidential information: project title and abstract will be published on HT website as provided for by the Convenzione, Art 5, Comma 5 (for more information, the Convenzione is available at this link or as pdf).

4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application form, shall include the following (see also Annex II):

   - Title
   - Significance
   - Innovation
   - Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results
   - Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF)

   The **use of generative AI tools** in the preparation of proposals is governed by the rules and recommendations outlined in the "Rules for the Responsible Use of Generative Artificial Intelligence in Proposal Preparation and Evaluation for Calls for Access to National Facilities" (link to the document), which is an integral part of this call. Applicants are also required to declare any use of generative AI by responding to the relevant questions in the application form.

   Below are the **mandatory formats** for the proposal, also based on the type of application (see sections 4, 9 and 10 for additional details):

   a. **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)

   b. **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)

   c. For **Resubmissions** (section 9), a **Resubmission cover page** must be included in the proposal. The total length of the application is hence increased by one page with respect to the format illustrated above. Refer to section 9 for important details on Resubmissions.

   d. For **Project Continuation** (section 10), the **Prior Results and Research Advancement cover page** must be included in the proposal. The total length of the application is hence increased by one page with respect to the format illustrated above. Refer to section 10 for important details on Project continuation.

   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 as PDF file, shall be in English and produced by using the **NIH biosketch template available in Annex II or at this link.** For support in drafting the CV, you can still refer to the NIH website Biographical Sketch Common Form (nih.gov). Please note that having an eRA COMMONS USERNAME is NOT required.

   Biosketch 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.

Accepted file format: 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 3.3).*
   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 (refer to section 8), in order to process samples and data through the NFs.*
   c. *Justification for requesting Access to the NF explaining why the project cannot be performed at the Applicant's institution. We recommend that the Applicant contacts the institute representative, i.e., the individual authorized to sign the Letter of Institutional Endorsement (LoE) to ensure that the most appropriate justification is provided and that it aligns with the one included in the LoE.*

      If the justification provided by the Applicant does not match the one provided by the host institution, the application will be rejected during the administrative review stage.

      *The host institution must select **only one option**, from the four listed below — please do not alter the statements:*

      - *The requested service/ technology is not available at the host institution.*
      - *The requested service/ technology cannot be performed at the host institution or elsewhere at an affordable cost.*
      - *The requested service/ technology is available at the host institution, but the necessary expertise is lacking.*
      - *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.*

      ***NOTE**: The SIEC, in charge of the evaluation procedure, may reserve the option to contact the host institution and its core facilities to confirm the justification provided.* **Read carefully information in section 6.2**.
   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* (link).

The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section of the application form, 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).

IMPORTANT: *Do not modify, add or remove any part of the Letter of Endorsement and fill in all the required information. Please, make sure that the signature of the letter is done in compliance with rules and procedures of your host institution.*

7. **Technical information**, to be filled in in the dedicated section(s) of the application form, indicatively including:
    a. *Requested service(s), as described in Annex III of this call.*
    b. *Samples technical information.*
    c. *Additional data, useful for technical feasibility analysis (if applicable).*
    d. *Whether the entire sample/ data set is already available, or will be available no later than two (2) months from receiving Access approval (refer to section 8).* ***Please note that if samples/ data 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 form.

Information provided in points 1 to 6 (application contents) is used for the **eligibility and admissibility check.**

Information provided in point 7 (technical information of the application) 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 SIEC to assess its scientific merit, according to the evaluation process illustrated in section 8.

6.2 Accountability of Applicants and host institutions

The Applicant and the host institution are solely responsible for the accuracy, completeness, and truthfulness of all data and information provided in the application. By submitting the application, they declare that all statements and supporting documentation are true and correct, acknowledging the liability and the sanctions stated in art. 75 and 76 of

the DPR 445/2000 for false affirmations and mendacious declarations, including the rejection of the application and/ or the loss of any granted benefits.

In case of Letters of Endorsement deemed not compliant or presenting inconsistencies in the submitted information (through the application), the User Access Office can conduct, on behalf of the SIEC, further verifications and/or to reject the application at the administrative review stage. This information can be also taken into consideration by the SIEC during the evaluation process and final approval decision.

Furthermore, acting on behalf of the SIEC, UAO may conduct **audits of participating institutions** to assess availability of any given or all services and ensure that these match the information submitted in their submitted applications.

In the event of violations and/or inaccurate or false declarations, the SIEC reserves the right to ban the Applicant and/or the host institution from participating in future Calls for Access for a period of up to twenty-four (24) months.

7. 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.

Application guidelines containing important information related to the submission procedure are available at this link.

This call for Access (Call ID: 26-GEDM-ROUND-3) will open on the 1st of October 2026 (13:00 CET) and will close on the 31st of January 2027 (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 webpages (call for Access; services).

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

Samples/ data 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** (refer to section 8). When the project foresees the analysis of more than one batch of samples/ data, similarly, the first batch should be available when the application is submitted or not later than two (2) months after Access approval.

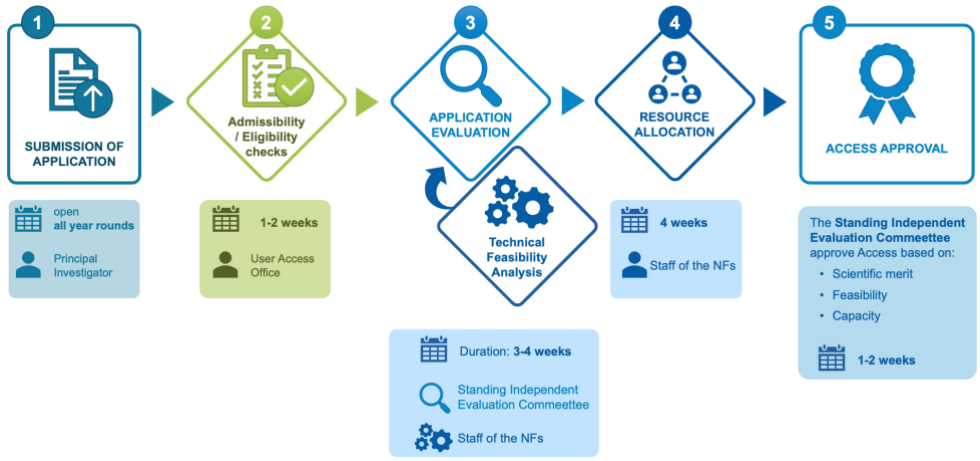
8. EVALUATION OF APPLICATIONS

Overview

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, along with a variable number of appointed external Reviewers selected according to the number of submitted proposals and their corresponding areas of expertise.

Below is a scheme describing the evaluation steps and the **indicative timeline for the process**. Evaluation results will be communicated through the PICA portal within 8 to 12 weeks after the closing of the call for Access.



The table below reports the indicative timeline for this call.

Opening	Closure	Evaluation	Access Approval
01.10.2026	31.01.2027	February – April 2027	By April 2027

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.

8.1 Triage

Based on the number of applications, if the requested services exceed 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:

1. **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 institution. For the Triage, the Justification provided in the Letter of Endorsement (section 6, point 6.c) will be taken into consideration.
2. **Ongoing and previous support received by the NFs**: priority will be given to researchers who do not have ongoing Access to the same NF they are applying for, and/ or who have not been granted Access to it in the two calls preceding the current one *(e.g. in the call 26-GEDM-ROUND-3 will be given priority to researchers who do not*

have any ongoing Access to the NF for Genomics and/ or who have not been granted Access in the calls 26-GEDM-ROUND-1 and 25-GEDM-ROUND-2).

Applicant career stage (Established and Junior PIs) will be considered during the triage phase to ensure alignment with the requirements of the evaluation procedure (section 8.2).

Should the number of requested services 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 institutions across Italy, proposals submitted by a single Institution/Institute that are sent for evaluation should not exceed the 10% of the total for any given career-based category.

8.2 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 (section 8.4).

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

The SIEC commits to allocating at least 50% of the available Access to applications from Junior PIs, while maintaining the Scientific merit as the primary criterion.

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.

8.3 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.

The use of **generative AI tools** in the review and evaluation of proposals is governed by the rules and recommendations outlined in the " Rules for the Responsible Use of Generative Artificial Intelligence in Proposal Preparation and Evaluation for Calls for Access to National Facilities" (link to the document), which is an integral part of this call. Reviewers are also required to declare any use of generative AI by responding to the relevant questions in the PICA portal.

8.4 Technical feasibility analysis

During the evaluation, the SIEC Chairs as well as the Reviewers will receive a report from the 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.

8.5 Evaluation results and Access approval

Applications with the highest scientific score that fulfil all technical requirements are approved for Access by the SIEC, subject to the capacity of the NF to host and execute the projects.

The SIEC commits to allocating at least 50% of the available Access to applications from Junior PIs, while maintaining the Scientific merit as the primary criterion, 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, previous Access, research outputs (e.g., publications) originated by previous Access, budgetary considerations and geographical distribution.

Evaluation results – Access granted, Access conditionally granted, Reserve list, Access not granted, Rejected (not eligible or not feasible proposals), Excluded during triage – are communicated to the Applicant through the portal PICA.

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 as resubmissions (see section 9).

9. RESUBMISSION OF PROPOSALS PREVIOUSLY EVALUATED AS "RESERVE LIST" OR "ACCESS NOT GRANTED"

9.1 Resubmission of a proposal evaluated as "Reserve list" in the previous round

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 this new evaluation round, maintaining the same project and review score as their initial application. Here, the application cannot be modified and is not sent out again for evaluation.

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

This option is provided only once, for the subsequent round only. An exception applies when the requested service is not available in the subsequent round. In such cases, the Reserve list application can be submitted to the first upcoming round of availability of the service *(e.g., the proposal has been submitted in the call 26-ROUND-1 and the evaluation outcome is "Reserve list"; the requested service is not available in the subsequent call 26-ROUND-2 but it is in the call 26-ROUND-3: the applicant can participate to the 26-Round-3 applying to the "Application for Reserve List Projects" call).*

b. To resubmit an updated version of the proposal (for example, including new preliminary data and/or taking into account Reviewer's comments), the Applicant should follow **the resubmission procedure described below** (section 9.2).

9.2 Resubmission

Applications that have been evaluated in a previous call, but that were not granted Access, or Reserve list projects without further Access being granted (Applicants that opt for option b above), can be submitted as a "**Resubmission**". Resubmission is not allowed for applications that were rejected (i.e., applications submitted to a previous round that were not eligible or not feasible) or excluded during triage in a previous evaluation round.

Of note, a **resubmission** is a proposal that has been **substantially improved** by adding new preliminary data, and/or by implementing Reviewers' suggestions and/or addressing concerns, etc. Applicants choosing the resubmission option shall include, in the proposal, the "Resubmission Cover page". **The Resubmission cover page must clearly describe how the proposal has been improved compared to the original version**.

IMPORTANT – The Resubmission Cover page must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: *Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Resubmissions that do not meet these requirements will be considered not admissible and the application will be rejected at the initial administrative review stage.

Resubmissions will enter the standard competitive evaluation procedure along with all proposals submitted to the current round (section 8).

Resubmissions are allowed only once, regardless of the round. A project proposal submitted multiple times (i.e., more than one) as a resubmission will be considered not eligible and will be rejected at the initial administrative review stage.

Applicants can participate to future calls for Access submitting a new application.

10. PROJECT CONTINUATION APPLICATIONS

Applicants that would like to request Access for continuing a project that already benefited of an Access to the National Facilities, shall submit a "**Project continuation application**". These are research proposals that build on the findings and results of a previously approved Access, with the aim of further developing the research and advancing knowledge in the field.

The previously approved project has been conducted in any of the National Facilities, and it is ongoing or it has been completed (End of Access) at the time of the submission of the project continuation proposal. Please consider that, if the previously approved project is ongoing applicants cannot request **Access for the same service**. Before submitting an application requesting the same service of an ongoing Access, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the evaluation round (refer also to section 5).

Applicants choosing to submit a project continuation application shall include, at the beginning of the proposal, the summary of **Prior Results and Research Advancement**. This summary must clearly outline the main achievements of the previously approved Access, citing any resulting publication (where applicable), and must explain how this project continuation proposal builds on and differs from the previously approved one, highlighting how it advances research knowledge.

IMPORTANT – The summary of "Prior Results and Research Advancement" must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: *Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Project continuation applications that do not meet these requirements will be considered not admissible and the application will be rejected at the initial administrative review stage.

Project continuation applications will enter the standard competitive evaluation procedure along with all proposals submitted to the current round (section 8).

11. AFTER ACCESS HAS BEEN APPROVED

For requests where access is granted, Applicants accept the National Facilities Access rules. **Access rules** are detailed in the document: "NF Access Rules_Convenzione" (link) **which constitutes an integral part of this call**.

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/ data 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.

Of note, no biological material and/or experimental support material may be transferred from Human Technopole to the PI or from the PI to HT prior to the project having officially started (refer to the Access rules for additional details).

If samples/ data and/ or relevant ethical and legal authorisation(s) for their use are not available within two (2) months after Access approval (refer to section 8), the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.

12. AFTER ACCESS HAS BEEN COMPLETED

12.1 Reporting and feedback

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. **Refer to the Access rules document (paragraph 15) for additional details.**

12.2 Ownership of results and research outputs

Research data obtained during Access shall be made available to the scientific community following the FAIR principles. It is requested that Applicants make raw data available to the scientific community by publishing them in an appropriate public repository. Applicant must inform the NF User Access Office (via email to national.facilities@fht.org) when and how the data are made public, providing a citable identifier.

In particular, the Applicant must communicate to the NF User Access Office (via email to national.facilities@fht.org) any **publication** acknowledging the NF and/or in which any member of the NF staff is listed as a co-author. Additional details about **ownership of the data, publication of the results and intellectual property rights** are reported in the Access rules document (paragraphs 13, 14).

13. CONTACTS

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

14. REFERENCES

NF Access Workflow_Convenzione (link)

NF Access Rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

NF Application Guidelines_PICA portal (link)

15. 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 - please, do not modify, add or remove any section of the letter and fill in all the required information)

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 mark the one that applies or best fits this application; only one choice is permitted***):

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

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

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

  ☐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 (refer to section 8 of the call text);

- 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 and/ or data 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 (refer to section 8 of the call text);
- 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;
- That the information provided in the Application is true and accurate, being aware of the accountability implications set out in section 6.2 of the call text;
- That the above data are true, being aware of the liability and the penal sanctions stated in art. 75 and 76 of the DPR 445/2000 for false affirmations and mendacious declarations that will involve the loss of the granted benefits.

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

A. Project proposal formats and template

Mandatory proposal formats

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)

Resubmission Cover page: *must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Project Continuation Cover page: *must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Proposal content:

- Resubmission cover page - applies to Resubmissions only (see section 9 for details)
- Project continuation cover page - applies to Project continuation applications only (see section 10 for details)
- Sections:
  1. TITLE
  2. SIGNIFICANCE
  3. INNOVATION
  4. APPROACH - including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results
  5. ENVIRONMENT - including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF)
  6. REFERENCES (Optional)

NOTE: The cover page (where required), together with Sections 1 through 6, constitutes the content of the proposal PDF to be uploaded as part of the application form.

B. Biosketch template

A **Biosketch template** can be downloaded from this link.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME:

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE:

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY

A. Personal Statement

B. Positions, Scientific Appointments, and Honors

C. Contributions to Science

ANNEX III: SERVICE LIST

HUMAN TECHNOPOLE

NATIONAL FACILITY FOR GENOME ENGINEERING AND DISEASE MODELLING

SERVICE LIST

26-GEDM-ROUND-3

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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, genomic engineering, and differentiation.

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, genome engineering of both stem cells and cell lines, and direct differentiation of hPSCs.

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

MEA: Multi Electrode Array

4. SERVICE LIST

The following section provides a detailed list of services offered by the facility. The National Facility for Genome Engineering and Disease Modelling (GEDM) offers three main categories of services:

- somatic cell reprogramming;
- genome engineering;
- differentiation.

Within the same application to this call, applicants may request either differentiation services or reprogramming and/or genome engineering services.

Specifically, reprogramming and genome engineering services may be requested **individually** or in **combination**, provided that the request is justified by the research objectives, and that the required starting material for each requested service is already available at the time of application and in compliance with the time specified in this call (see sections 5 and 7).

Differentiation services cannot currently be requested in combination with reprogramming and/or genome engineering services.

Applicants are highly encouraged to contact the staff of the facility for a consultation before submitting their application, to discuss any **technical aspects** of the project that may affect its feasibility (nf.gedi@fht.org).

4.1 REPROGRAMMING OF SOMATIC CELLS TO iPSCs

Description: Somatic cell 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. 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.

NOTE: While no formal limit is imposed on the **number of reprogramming services** that may be requested within a single application, applicants must justify the necessity of the requested number of services indicating, in the relevant section of application form, whether it could be reduced (scale down) while preserving the scientific objectives and overall significance of the project. The requests will be evaluated and considered based on the scientific merit of the proposal and the available capacity of the GEDM facility.

This applies to reprogramming services requested either individually or in combination with genome engineering services.

4.1.1 Reprogramming of PBMC

SID: NF20.001 - Reprogramming of PBMCs

Service: Reprogramming of human Peripheral Blood Mononuclear Cells (PBMCs) sample into induced Pluripotent Stem Cell (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 iMatrix-511 (Biogems) 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: For proposals involving large cohorts and depending on their significance and the Facility's available capacity, the Evaluation Committee may approve the project only for a portion of the requested services.

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 (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 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: Somatic cell reprogramming of human fibroblast sample into induced pluripotent stem cells (iPSCs).

Fibroblasts are plated onto iMatrix-511-coated plates and subjected to three consecutive overnight transfections with an NM-RNA cocktail (days 1–3) (StemRNA™ 3rd Gen Reprogramming Kit, REPROCELL). Each morning following transfection, the medium is replaced with fresh NutriStem™ Medium. After the third transfection, NutriStem™ Medium is replaced daily until colony picking. Once colonies reach an adequate size (typically 10–15 days), they are selected and transferred to individual wells of a 96-well plate coated with iMatrix-511. Individual clones are subsequently passaged into 24-well plates and maintained in culture until passage 8.

Technical requirements: For proposals involving large cohorts and depending on their significance and the Facility's available capacity, the Evaluation Committee may approve the project only for a portion of the requested services.

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 (StemRNA™ 3rd Gen Reprogramming Kit, REPROCELL)

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 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 Fibroblast sample. The cell lines are expanded and cryopreserved in a Master Cell Bank, with 10 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 5-6 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 application, 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 services/samples may optionally be described in the written proposal. To this end, applicants requesting more than two genome engineering services must justify the necessity of the proposed services in the application form.

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 scientific merit of the project.

This applies to genome engineering services requested either individually or in combination with reprogramming services.

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.

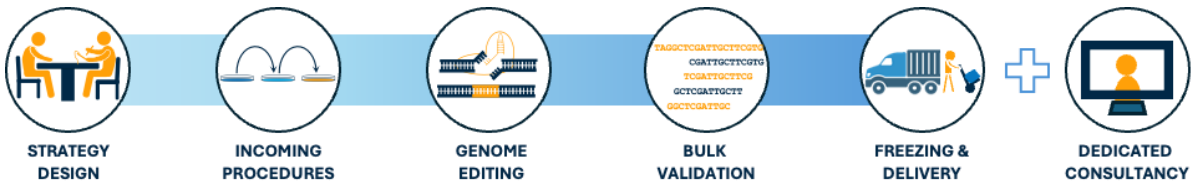

Figure 2: Overview of the service offered through the Bulk Option. *The NF-GEDM Facility is responsible for designing the editing strategy, performing the genome editing procedure, and validating the bulk cell population. Clonal selection and quality control steps are the responsibility of the User. 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: NF20.034 Gene Editing - Custom Project

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 DIRECTED DIFFERENTIATION OF hPSCs

We provide directed differentiation services to generate specific cell and tissue types from hPSCs. These services support projects requiring human cell-based models to investigate disease mechanism, gene function, and cellular phenotypes.

Depending on the scientific question, hPSCs can be differentiated into defined cell populations or three-dimensional organoid models, which reproduce tissue heterogeneity, architecture, and cell-cell interaction. The facility currently provides validated differentiation models covering neural, cardiac, and haematopoietic lineages.

Applicants seeking for guidance on the most appropriate model for their scientific question are encouraged to contact the facility before submission. Projects involving differentiation protocols not listed in the service catalogue may also be considered.

Please contact the facility to discuss any technical aspect that might affect project feasibility (nf.gedi@fht.org).

Research area	Available models
Neural biology	Forebrain and cerebellar organoids
Neuromuscular biology	Neuromuscular organoids
Cardiovascular biology	Cardiomyocytes, cardiac organoids
Haematological biology	Erythroblasts, Megakaryocytes

Differentiation services require applicants to provide suitable hPSC lines. If you already have suitable hPSC lines you may apply directly for differentiation. Alternatively, the facility also provides somatic cell reprogramming services (NF20.001 and NF20.002). Please consult section 4.1 and contact the facility (nf.gedi@fht.org) to discuss the appropriate access route.

4.3.1 General requirements and information

Applicants may submit proposals for a single directed differentiation model, including up to four biological replicates. Information on the number of hPSC lines, technical replicates, and sampling time points is provided in the table associated with each service.

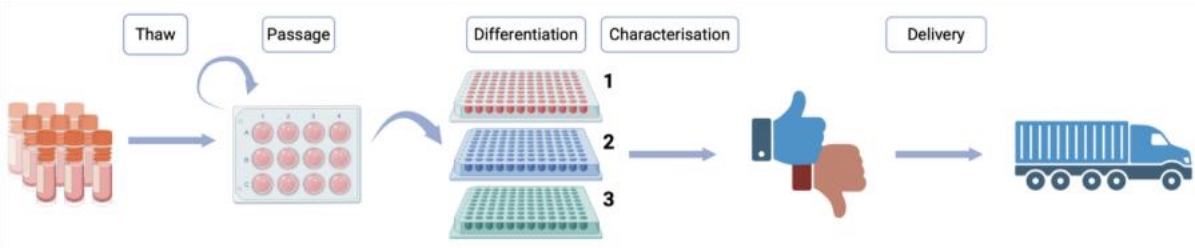

Figure 1: Overview of the general pipeline of differentiation and validation.

Important note: the success of hPSCs differentiation is influenced by several factors, including the intrinsic propensity of each cell line to respond to differentiation cues, variability

between lines, differences across reagent batches, and culture-related technical factors. For these reasons, outcomes may vary and cannot be fully guaranteed. Whenever feasible, we will perform a secondary attempt, with potential alternative approaches agreed upon in advance with the User. All the differentiations will be performed in parallel with our internal control line(s) to confirm the technical validity of each procedure.

4.3.2 Directed differentiation of hPSCs into tissue-specific 3D-organoids

SID: NF20.033 Differentiation - Custom Project

Service: Standard tissue-specific generation from hPSCs.

hPSC lines are seeded as single cells and allowed to form aggregates in culture medium supplemented with a pro-survival cocktail. Homogeneous aggregation is favoured by using u-, v-, or pyramidal- bottom anti-adhesion plates. Upon aggregation, hPSC cell-fate commitment, regionalisation, and maturation are driven by either permissive or directed culture condition depending on the protocol applied.

Protocols available in our facility include the derivation of organoids having features of the following tissues:

- Dorsal forebrain
- Cerebellum
- Neuromuscular junction
- Heart

Applicants interested in differentiation protocols or tissues not listed in the service catalogue, a larger cohort of hPSC lines and/or number of organoids, or multiple differentiation fates are **strongly encouraged to contact the facility** before the call deadline. Acceptance is contingent upon existing expertise, available resources, and demonstrated feasibility.

Item	Max number
Protocols	1
hPSC lines	10
Biological replicates	4
Technical replicates	30 per time point
Time points	4
Days *In Vitro*	120

Table 1. Maximum number of items allowed within a standard service.

Service: Large-scale organoid generation and experimental perturbation.

For projects requiring increased experimental scale with respect with the numbers reported in table 1, selected organoid models can be generated using the Facility's automated culture platform. This is an experimental service performed in collaboration with the NF GEDI Technology Development staff. Depending on project feasibility and compatibility with the selected organoid model, the automated platform can support the generation and

maintenance of larger numbers of organoids and the application of user-defined experimental perturbations.

The scope of the service is limited to generation of the selected organoid model and execution of the agreed experimental perturbation. Perturbations may include compounds or other reagents compatible with the automated workflow. Perturbation libraries are generally limited to approximately 100 individual compounds or perturbations per project. The final experimental scale, including the number of concentrations, replicates, time points and control conditions, is subject to feasibility assessment and available capacity. Arrayed CRISPR screening is not currently offered as part of this service.

Applicants must provide all compounds or other treatment reagents required for the experiment. It is applicant's responsibility to provide validated and/or characterised treatment reagents. The Facility's responsibility is limited to the generation of the selected organoid model and the execution of the agreed perturbation workflow. Treatment conditions, number of experimental groups, replicates, time points, labware format, and sampling strategy will be agreed with the Facility before project submission.

The service includes organoid generation, execution of the agreed perturbation scheme, and collection and delivery of samples at the defined experimental endpoints. Brightfield images will be acquired during the experiment and shared with the users. Downstream analytical assays, phenotypic readouts, and analysis of treated samples are not included. Applicants must describe in the proposal the analytical strategy that will be used to assess the experimental outcomes and demonstrate access to the required analytical methods and expertise.

Technical requirements:

hPSC lines accepted: NF-GEDI internal lines are currently not eligible for experimental use, and they will only serve as internal control of differentiation. Users must provide their own hPSC lines.

hPSC lines must be shipped cryopreserved (> 2 x 10^5 cells/cryovial).

Sterility: Upon thawing, cells will be tested for mycoplasma contamination and only included in the study if results are negative.

Genomic abnormalities: If the cells have not been tested within the last six months or eight passages, they will undergo evaluation for karyotypic abnormalities and/or copy number variations upon thawing. Cells that do not meet chromosomal integrity standards will be excluded from the differentiation protocol and discarded.

Characterisation

Every hPSC lines and selected derivatives will be compared with internal controls in targeted characterisation assays for:

- Undetectable mycoplasma and bacteria contamination
- Cell identity verification via STR profiling (undifferentiated cultures and endpoint derivatives)
- Expression of differentiation markers at relevant time points

Deliverables

We generate up to 30 organoids per time point per line (Standard service). Actual number may vary depending on experimental outcomes.

The delivery conditions will be defined in agreement with the User, based on the experimental plan and the selected differentiation protocol. Live organoids cannot currently be shipped and

are available for hand delivery only. Frozen pellets, fixed samples, and lysates can be shipped.

Functional characterization of neural organoids

Depending on the protocol, neural organoids may be eligible for the additional services listed below (NF55.006 and NF55.003/004/010/014). Please note that protocols that have never been tested previously will require a pilot experiment before full eligibility can be confirmed.

Functional characterization can be performed by using either a Calcium Imaging Assay or a Multi Electrode Array (MEA) recording (only one assay can be selected). If eligible, Users may request direct delivery of organoids to the National Facility for Light Imaging Microscopy – IU5 Ion Imaging for further processing.

The characterizations focus on the basal activity of the organoids. For MEA recordings, detectable activity will be validated pharmacologically via synaptic transmission blocker(s).

Up to 36 organoids can be analysed within a 10-day window. Exact sample numbers and experimental settings will be determined in consultation with the facility staff.

The functional characterization will be performed at a predefined time window upon advance agreement with the User. If <50% of biological samples show activity, the assay will be repeated once at a later time point (up to 120 days of differentiation).

For full details on the additional services, refer to paragraph 4.5 below.

4.3.3 Directed differentiation of hPSCs in 2D-specific cell types

SID: NF20.033 Differentiation - Custom Project

Service: Directed differentiation of hPSCs into defined cell types.

Upon seeding, hPSC fate commitment, regionalisation, and maturation are driven by either permissive or directed culture condition depending on the protocol applied. Cells are treated as adherent or floating cultures depending on the tissue. Protocols available in our facility include the derivation of the following cell types:

- Cardiomyocytes
- Megakaryocytes
- Erythroblasts

Applicants interested in differentiation protocols or tissues not listed in the service catalogue, a larger cohort of hPSC lines, or multiple differentiation fates are encouraged to contact the facility before the call deadline. Acceptance is contingent upon existing expertise, available resources, and demonstrated feasibility.

Item	Max number
Protocols	1
hPSC lines	4
Biological replicates	4
Technical replicates	Depends on protocol

Time points	3
Days *In Vitro*	60

Table 2. Maximum number of items allowed within a standard service.

Technical requirements:

hPSC lines accepted: NF-GEDI internal lines are currently not eligible for experimental use and they will only serve as internal control of differentiation. Users must provide their own hPSC lines.

hPSC lines must be shipped cryopreserved (> 2 x 10^5 cells/cryovial).

Sterility: Upon thawing, cells will be tested for mycoplasma contamination and only included in the study if results are negative.

Genomic abnormalities: If the cells have not been tested within the last six months or eight passages, they will undergo evaluation for karyotypic abnormalities and/or copy number variations upon thawing. Cells that do not meet chromosomal integrity standards will be discarded.

Characterisation

Every hPSC lines and selected derivatives will be compared with internal controls in targeted characterisation assays for:

- Undetectable mycoplasma and bacteria contamination
- Cell identity verification via STR profiling (undifferentiated cultures and endpoint derivatives)
- Expression of differentiation markers at relevant time points

Deliverables

We generate up to 4 technical replicates per time point per line. Actual number may vary depending on experimental outcomes.

The delivery conditions will be defined in agreement with the User, based on the experimental plan and the selected differentiation protocol. Live cells cannot currently be shipped and are available for hand delivery only. Frozen pellets, fixed samples, and lysates can be shipped.

Important note: upon receipt, all the lines are tested for viability, mycoplasma and bacterial contamination. Lines failing quality criteria will be discarded.

4.4 ADDITIONAL SERVICES

This section encompasses additional 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.

All technical and experimental details related to any additional service will be managed by the relevant NF (NF-Genomics and NF-Data Handling and Analysis).

List of available additional services for the Reprogramming and Genome Engineering services:

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

NF62.02.01 WGS analysis (can be combined with G-001)

SID: G-006 – totalRNA sequencing from standard input

NF62.01.01 Bulk RNA-seq analysis (can be combined with G-006)

List of available additional services for the Directed Differentiation services:

SID: NF55. 003/004/010/014 – Microelectrode Arrays (MEAs) assays OR

SID: NF55.006 - Ion imaging assisted experiment

A detailed justification including the goals of any additional analysis requested shall be provided at the moment of the application.

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

This service can be requested in combination with cell reprogramming or genome engineering services.

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.

Library preparation protocol:

Libraries will be prepared by following the protocol:

Illumina DNA PCR-Free Library Prep

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 either the NovaSeq 6000 or NovaSeq X Plus systems (Illumina) by generating 150 bp Paired End reads.

This service can be combined with the following service: **NF62.02.01** WGS analysis, offered by the National Facility for Data Handling and Analysis.

4.5.2 SID: G-006 – totalRNA sequencing from standard input

This service can be requested in combination with cell reprogramming or genome engineering services.

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.

Library preparation protocol:

Libraries will be prepared by following the protocol:

Stranded Total RNA Prep, Ligation kit with Ribo-Zero Plus or Ribo-Zero Plus Microbiome

Or

TruSeq Stranded Total RNA

Or

Watchmaker RNA Library Prep Kit with Polaris Depletion

Or

QIAseq Fast Select Epidemiology Kits

Illumina Total RNA Prep with Ribo-Zero Plus supports a broad range of RNA inputs. It's compatible with different sample types, including low-quality samples such as formalin-fixed paraffin-embedded (FFPE). The included Ribo-Zero Plus or Ribo-Zero Plus Microbiome Depletion kit 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.

The Illumina TruSeq Stranded Total RNA kit allows whole-transcriptome analysis of a broad range of RNA inputs. The optimization of fragmentation conditions allows to process degraded-RNA and formalin-fixed paraffin embedded (FFPE) samples. The kit can be combined with Ribo-Zero Gold that depletes samples of both cytoplasmic and mitochondrial rRNA or Ribo-Zero Plant that targets cytoplasmic and chloroplast rRNA.

Please check if your species of interest are included in the following table: Stranded Total RNA Species Compatibility Tables.

The Watchmaker RNA Library Prep Kit with Polaris Depletion enables rapid generation of stranded whole-transcriptome libraries by combining RNA library prep with rRNA and globin depletion in a single workflow. It supports low-input and degraded samples (including FFPE), improving sensitivity and transcript coverage across coding and non-coding RNA species. An engineered reverse transcriptase enhances cDNA synthesis efficiency, yielding high-complexity libraries suitable for diverse RNA-seq applications. The streamlined Polaris Depletion module depletes highly abundant rRNA in human, mouse, and rat samples, and human globin transcripts. For other species, dedicated ribodepletion probe pools can be applied (see https://www.sitoolsbiotech.com/products/ribopools/rrna-depletion#c5432)

QIAseq FastSelect Epidemiology Kits are developed to support dual-sequencing (dual-seq) and host–pathogen RNA-seq library preparation workflows. During library construction, these kits efficiently deplete bacterial 5S, 16S, and 23S ribosomal RNA together with cytoplasmic and mitochondrial ribosomal RNA from human, mouse, and rat samples.

The kits are compatible with input RNA quantities ranging from 100 ng to 1 µg while maintaining reliable performance across this range. They also perform effectively with a variety of RNA sample qualities, including low-quality, highly fragmented RNA as well as high-quality, intact full-length RNA.

Libraries sequencing and NGS coverage:

Libraries will be sequenced using the NovaSeq X Plus systems (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.

4.5.3 NF62.02.01 WGS analysis

Service description: DNA sequencing is critical for genetic research, evolutionary studies, and personalized medicine, where it helps to uncover the genetic basis of diseases, track hereditary conditions, and guide targeted therapies. It provides a detailed understanding of an organism's complete genetic makeup, offering insights into complex biological processes and evolutionary relationships.

Whole-Genome Sequencing (WGS) involves sequencing the entire genome, including both coding and non-coding regions. WGS provides the most comprehensive view of an organism's genetic information, as the focus is not only on identifying genetic variants (e.g., single nucleotide variants, insertions, deletions, copy-number variation), but also on identifying rare variants, structural variations, and novel mutations in both coding and non-coding regions. Different algorithms will be applied for germline or somatic samples – the former algorithms are designed to identify inherited variants present in all cells, whereas the latter algorithms focus on detecting mutations acquired in specific tissues which are present only in a subset of cells thus requiring specialized methods to account for tissue purity and heterogeneity.

The standard bioinformatics analysis for WGS projects comprises the following steps:

1. **Quality check of the raw sequence data**: Evaluating raw read sequencing quality, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.
2. **Trimming**: Trimming reads according to base quality, excluding excessively short reads and those with low average quality from the analysis. Trimming adapters and other technical residuals. Performing a second QC step on the trimmed reads.

3. **Mapping to the reference genome**: Aligning high-quality reads to a reference genome, generating a BAM file. Removing PCR duplicates post-alignment to reduce bias, applying quality score recalibration to correct sequencing errors. Indel realignment may also be performed to refine alignment accuracy around insertion-deletions, ensuring reliable variant calling in downstream analysis.
4. **Variant calling**: Variant calling is the process by which algorithms scan the aligned reads for deviations from the reference genome, marking potential variant sites. Depending on the scientific purpose of the analysis we will identify different variant families:
    a. **Single Nucleotide Polymorphisms** (SNPs) are single-base changes in the DNA sequence, which may or may not affect gene function.
    b. **Insertion–deletion mutations** refer to small insertions or deletions (of less than 50 bases) in the genome.
    c. **Copy Number Variants** (CNVs) are structural variations in the genome, typically spanning kilobases to megabases, where segments of DNA are either duplicated or deleted.
    d. **Structural Variants** (SVs) are large-scale changes in the genome structure, such as inversions, translocations, duplications, or large insertions/deletions (more than 50 bases).

Advanced (optional) analysis steps include the following:

1. **Annotation and gene-level interpretation**: For all the different classes of standard analysis (SNPs, indels, CNV and SV) we will provide basic information on the genes and regulatory elements affected by the variation, which can reveal potential disease associations (functional impact, consequence on protein, pathogenicity predictors, population frequency data).
2. **Disease association analysis**: Based on the experiment we can provide specific annotations for germline (e.g. Clinvar, ACMG) or somatic variants (e.g. COSMIC, Civic, OncoKB, AMP).
3. **Trio analysis**: Identifies variants by inheritance patterns: de novo, autosomal recessive, autosomal dominant, or compound heterozygosity.

Results

The National Facility for Data Handling and Analysis will deliver the following to the Users:

1. Trimmed and filtered fastq files for each sample.
2. BAM files for each sample.
3. Raw VCF files for all samples, In case of advanced analysis, we will also provide filtered and annotated VCF files for all samples, and genotypes in tabular format.
4. Complete reports (in interactive HTML and publication-ready PDF formats) describing the quality of the data, all the analysis performed on the dataset, and their results. Plots (e.g. heatmaps, Circos plots, Manhattan plots) and tables included to the report will also be provided as separate files.
5. Pipeline and scripts used to perform the analysis, if applicable.

The facility will also assist the User in submitting raw data to public repositories, as stipulated in the *National Facilities Access Rules*.

4.5.4 NF62.01.01 Bulk RNA-seq analysis

Service description: RNA sequencing is a powerful molecular biology technique used to analyse the transcriptome of a biological sample. The transcriptome refers to the complete set of RNA molecules, in particular messenger RNA (for mRNA sequencing) and/or non-coding RNAs (for total RNA sequencing), in a cell or tissue.

RNA sequencing is widely used in genomics research, functional genomics, and clinical studies to understand gene expression patterns, identify novel transcripts, and investigate how gene expression varies under different conditions. In addition, total RNA sequencing provides an insight also on the regulatory mechanisms underlying various biological processes.

The standard bioinformatics analysis for RNA-seq datasets comprises the following steps:

1. **Quality check of the raw sequence data**: Evaluating raw read sequencing quality, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.
2. **Trimming**: Reads are trimmed according to base quality, and reads having low average quality, as well as reads that are too short, are excluded from analysis. This step also trims adapters and other technical residuals. Quality control is performed again on the trimmed reads.
3. **Mapping to the reference genome**: The surviving good-quality reads are mapped to the reference genome using a splice-aware aligner (e.g. STAR). In parallel, pseudoalignment is performed too.
4. **Quantification of gene expression**: Uniquely mapped reads are assigned to the corresponding genomic features (i.e. exons, transcripts or genes). A count matrix is produced that summarizes the inferred expression level for each gene in each sample.
5. **Quality metrics collection**: Quality metrics from sequencing, trimming, alignment and quantification are collected and summarized in a complete, interactive report.
6. **Normalization and filtering**: Expression levels are normalized to account for the different library size across samples and/or the lengths of different genes. Non-expressed genes are filtered out.
7. **Exploratory analysis on expression data**: Principal Component Analysis (PCA) and Multi-Dimensional Scaling (MDS) are performed to inspect the variability structure of the data and its possible relationship with samples characteristics. If applicable and necessary, batch correction is performed using regression models. The expression of selected housekeeping genes can be evaluated across all samples, as well as the expression of gender-specific genes (for human datasets) and project-specific genes (if applicable, e.g. knocked out genes, tissue markers etc.).
8. **Differential expression analysis**: Expression levels are compared between different groups of samples, using statistical models based on the experimental design (e.g. paired models, regression of covariates etc.). Differentially expressed transcripts are identified by setting cutoffs on the obtained p-values and log2FoldChanges.
9. **Automatic functional enrichment analysis**: For human and mouse datasets, an over-representation analysis (ORA) is performed to test the enrichment of the lists of differentially expressed genes against Gene Ontology and the main pathway collections (e.g. KEGG, Reactome, BioCarta, Hallmark). A pre-ranked Gene Set Enrichment Analysis (GSEA) is also performed on the same pathway collections. For other species, the two

analyses are performed only when suitable Gene Ontology annotations and/or pathway collections are available for the corresponding organism.

Advanced (optional) analysis steps include the following:

1. **Advanced pathway analysis of significant genes**: Advanced pathway analysis can be performed using industry standard tools. Significant differentially expressed genes are analyzed to identify enriched canonical pathways, upstream regulators, causal networks, biological functions, and disease associations. This analysis is available for human, mouse, and rat datasets. Support for other species will be discussed during the project kickoff meeting.

Results

The National Facility for Data Handling and Analysis will deliver the following to the Users:

1. BAM files for each sample.
2. Raw and normalized count matrices containing expression values for each gene in each sample.
3. Results from exploratory analysis: PCA and MDS scores and plots, table with sample-to-sample correlation values and the corresponding heatmap, plots showing the expression levels of control genes.
4. Tables of differentially expressed genes with statistical significance information, and associated diagnostic plots (volcano plots, histograms of nominal pvalues).
5. Results from functional enrichment: tables of enriched pathways with statistical significance information, GSEA enrichment curves.
6. Complete reports (in interactive HTML and publication-ready PDF formats) describing the quality of the data, all the analysis performed on the dataset, and their results. Plots (e.g. heatmaps, volcano plots, dotplots, PCA/MDS) and tables included to the report will also be provided as separate files.
7. Pipeline and scripts used to perform the analysis, if applicable.

The facility will also assist the User in submitting raw data to public repositories, as stipulated in the *National Facilities Access Rules*.

4.5.5 NF55. 003/004/010/014 – Microelectrode Arrays (MEAs) assays

Description: High-resolution, optics-free functional imaging based on high-density MEA electronic interfaces measuring extracellular voltage potentials. The assay is suitable for the characterization of functional phenotypes in cell networks and for compound screening.

The systems available:

- BioCAM DupleX and **planar MEAs** electronic chips equipped with 4,096 simultaneously recording electrodes (3BRAIN AG, Pfäffikon SZ, Switzerland). The MEAs chip is the CorePlate™ 1W38/60 (NF55.003_MEA chip testing and provision/ NF55.004_MEA chip rental) with an active sensing area of 3.8 mm x 3.8 mm; each electrode measures 21 mm x 21 mm with a 60 mm pitch. The system is particularly suitable for accessing the electrical phenotype of brain and cardiac tissue.
- BioCAM DupleX and **3D MEAs** electronic chips equipped with 4,096 simultaneously recording electrodes (3BRAIN AG, Pfäffikon SZ, Switzerland). The MEAs chip is the

CorePlate™ 1W-3D 38/60/90-X (NF55.014_MEA single well 3D) with an active sensing area of 3.8 mm x 3.8 mm, each electrode height is approximately 90 mm, with a section at the tip measuring approximately 14 mm x 14 mm with a 60 mm pitch. This system is highly recommended for acute recording of tissue or organoids.

- MaxTwo (MaxWell Biosystems) and **6-Well Plate** (NF55.010_MEA multiwell system). Each well has a 3.85 x 2.10 mm^2 active sensing area equipped with 26,400 electrodes with 17.5 mm pitch. Each well can simultaneously record from 1,020 electrodes. The system is ideal for neuronal cultures and tracking changes in neuronal arborization over time.

Applications: brain and cardiac cell models in 2- and 3D. Different biological models will be evaluated as custom services during the feasibility study of the project

Typical investigations include:

- Longitudinal recording, by means of multiple recording at different time point to follow the development of the electrical activity. It is applicable on 2- and 3D models cultured onto the MEA chip.
- Acute recording (only for 3D models)
- Characterization of spontaneous or pharmacologically triggered activity. The assay can also be used to investigate the cellular network and for compound screening

Limit: MEA services are available for proof-of-concept or small-scale projects. The maximum capacity per batch is 30 planar MEAs, 10 3D MEAs, and 10 6-well chips, but the project scale should be discussed with the NF manager.

Information to be provided in the technical information section of the application:

- Biosafety level and Mycoplasma test
- Type of sample (culture / organoids / assembloids)
- Number of samples
- Number of technical replicates
- Type of recording (Acute/ Culture / Longitudinal Recording)
- Number of Time Points to be evaluated
- Pharmacological validation of recorded activity (i.e.TTX)
- Culturing conditions for the sample (temperature, CO_2, medium composition)

Procedures: After a standard quality control to verify that the cell/organoid lines express the cell types of interest, samples will be prepared as cell/organoid cultures on MEAs electronic chips by the Facility 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 (i.e. sampling rate, filters) established by the user and within the range of the instrument's capability.

Data provided: The user will receive raw data from MEA recordings in a HDF5 format, and will be responsible for processing, analysis, and interpretation. Optionally, the user may request basic processing of the raw data using the proprietary software Brainwave5 (3BRAIN AG) or MaxLab Live (MaxWell) to detect electrophysiological events of interest. When applicable, the user will be responsible for selecting processing parameters (such as high-, low- or bandpass filters and detection thresholds) which are examples and not limited

to these options, depending on the software's capabilities. Processed data will be delivered to the user in a HDF5 format.

Access modality available: Access to NF service or Access with training

4.5.6 NF55.006 - Ion imaging assisted experiment

Description: Fluorescence-based time-lapse recordings of intracellular ion oscillations, based on confocal or epifluorescence microscopy.

- Imaging on Nikon Ti2 spinning disk with four cameras, TIRF and FRAP module (SID: NF50.007 or equivalent system suggested by the NF staff).

Applications: Calcium imaging based on chemical or genetically encoded sensors. Available and ready-to-use sensors are the chemical, cell permeable calcium dyes such as Fluo-4 AM or Rhod-3. Genetically encoded sensors should be expressed and tested in the biological model of interest before entering the NF (the applicant is responsible for this task). Different ions (e.g., chloride) and voltage imaging protocols can be developed upon request.

Typical investigations include:

- Characterization of spontaneous or pharmacologically induced ion oscillations across cell networks, in 2- and 3-dimensional cell culture models.
- Characterization of functional connectivity and signaling within cell networks, in 2- and 3-dimensional cell culture models.

Technical requirements:

- Only BSL 1 or BSL 2 samples.
- The method of shipment of the living sample will be discussed with the NF manager during the kick-off meeting.
- Applicants will prepare the samples in accordance with the technical requirements of the microscope and of time-lapse imaging approaches (e.g. using microscopy-grade dishes for adherent cell cultures).
- Applicants will provide the NF staff with the protocols for culturing and the media culture recipe to maintain living samples within the NF for the total duration of the experiments.
- The samples tested with this approach must be in a developmental stage (i.e., days in vitro) sufficient to express the phenomena of interest. This aspect strongly depends on the biology of the model. Therefore, the timing of experimental procedures will be discussed within the context of the specific project. The Applicant will deliver the samples to the Light Imaging NF at their estimated time of maturity for ion imaging experiments.

Information to be provided in the technical information section of the application:

For samples submitted for ion imaging services, please specify the following characteristics or conditions:

- Type of sample (2D culture / 3D culture) and number of samples
- Number of technical replicates
- Recording conditions (Basal only, One drug/ compound, More than one drug/compound)
- Number of Time Points

- BSL1/ BSL2
- Viability tested
- Mycoplasma tested
- Estimation of time (Hours) needed to complete the Access
- For adherent cells (2D culture), sample already tested for attachment on microscopy-grade dishes/glass coverslips (Yes/No)
- Culturing conditions for the sample (temperature, CO_2, O_2, medium composition)

Data provided: The Applicant will receive raw data from ion imaging recording in the format of the acquisition software used.

Access modality available: Access to NF service or Access with training.

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

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 mycoplsma 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

Q-banding assay detects genomic abnormalities (>5-10 Mb): Inversions, duplications/deletions, balanced and unbalanced translocations, aneuploidies. Our service guarantees the analysis of 20 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.