HUMAN TECHNOPOLE

NATIONAL FACILITY FOR DATA HANDLING AND ANALYSIS

CALL FOR ACCESS

25-DHA-ROUND1

Table of Contents

1. INTRODUCTION

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

Services offered by NFs are available through regular open calls for Access that are published yearly on the HT website (link) and are free of charge for the project (or aspects of the project) approved for Access.

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

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

1.1 Access modalities

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

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

2. TERMS AND DEFINITIONS

2.1 Access

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

2.2 Researcher

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

2.3 Principal Investigator

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

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

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

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

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

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

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

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

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

2.4 Applicant

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

2.5 User

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

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

3. APPLICATION TYPE

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

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

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

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

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

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

4. ELIGIBILITY AND ADMISSIBILITY

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

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

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

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

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

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

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

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

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

Public research entities: this category includes:

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

Eligible Institutions/ Institutes are strongly encouraged to limit the number of applications submitted to this call for Access to the very best two, with at least 50% coming from Junior PIs.

Such indication does not represent an eligibility criterion but rather a guideline aimed at ensuring the widest distribution of Access among Institutions in the Country.

Applicants shall declare that they have not received funding to perform the submitted project (limited to the aspects included for Access to the NF) in their own laboratory, home Institution or elsewhere. Applicants shall confirm the economic and scientific feasibility for the aspects of the project to be performed outside the NFs.

Applicants cannot request Access for the same service if an approved Access is ongoing. Before submitting a new application for the same service, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the next evaluation round. A clear motivation for the request must be provided.

A PI submitting an application to this call for Access cannot request access to other NFs (i.e., cannot participate to other 2025 - ROUND 1 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 application before receiving notification of the results.

Applications must be written in English and must be complete (i.e., consist of all the requested elements and information) and respect all administrative and technical requirements (e.g., proposal or CV format, mandatory declarations, technical requirements of the services, sample availability, sample requirements, including number of samples to be analysed). Incomplete applications or applications that do not meet the requirements will be considered not admissible and will be rejected at the administrative review stage.

5. APPLICATION CONTENT AND FORMAT

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

1. **Applicant's general information**.
2. **Justification for requesting Access to the NF**.
3. **Abstract** to be inserted in the dedicated section on the application portal (Max 1500 characters including spaces).
4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application portal, shall include the following sections:
   a. *Title*
   b. *Significance.*
   c. *Innovation.*
   d. *Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
   e. *Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

   Below, the mandatory format for the proposal:

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

   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

Proposal template is available in Annex II of this call.

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

5. **Applicant's CV in NIH biosketch format**. The CV, to be uploaded in PDF, shall be drafted in English, using the template available at this link and following the mandatory format: max 4 pages, page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom. For support in drafting the CV, please refer to NIH website: Create Biosketches | NIAID: National Institute of Allergy and Infectious Diseases (nih.gov).

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

6. **Letter of Institutional Endorsement**, addressing the following points:

   a. *Confirmation of the Applicant's role at their Institution, and their eligibility under the category of PI (see section 2.3).*

   b. *Confirmation that relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained no later than two (2) months after Access approval, in order to process samples and data through the NFs.*

   c. *Justification of the request for Access – including a statement on why the project cannot be performed at the Applicant's Institution.*

   d. *Confirmation that the Applicant has not received funding for performing the submitted project, for the aspects to be performed at the NFs, in their own laboratory, home Institution, or elsewhere.*

   e. *Confirmation of the project's economic and scientific feasibility for the aspects to be performed at the host Institution.*

   f. *Acceptance of NF Access Rules.*

   The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section on the application portal, shall be drafted using the facsimile available as Annex I of this call.

7. **Technical information**, to be filled in in the dedicated section(s) of the application portal, indicatively including:

   a. *Requested service(s), as described in Annex III of this call.*

   b. *Sample technical information.*

   c. *Requested preliminary data for technical feasibility analysis (if applicable).*

   d. *Whether the entire sample set is already available (otherwise indicate the date of availability of the entire sample set).* ***It is mandatory that samples and relevant authorisations are available at the moment of application or no later than two (2) months from receiving Access approval****.*

   e. *Resources and expertise to receive and process the products – data (e.g. Cryo-EM micrographs) or reagents (e.g. human iPSCs) – generated by the NF.*

   f. *Research data management plan and bioinformatics support for data analysis, specifying (mandatory when the project output includes research data - e.g.,* *genomics or proteomics data, bioimages from microscopy services, among other):*

i. *How the bioinformatics analysis of the data generated by the NF will be performed (if such analysis is not provided by the NF for Data Handling and Analysis).*
ii. *How the data generated by the NF will be handled during and after the end of the project.*
iii. *Whether and how the data will be shared/ made Open Access.*
iv. *How data will be curated and preserved, including after the end of the project.*

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

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

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

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

6. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

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

This call for Access (Call ID: 25-DHA-ROUND 1) will open on the 15th of February 2025 (13:00 CET) and will close on the 31st of May 2025 (13:00 CET).

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

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

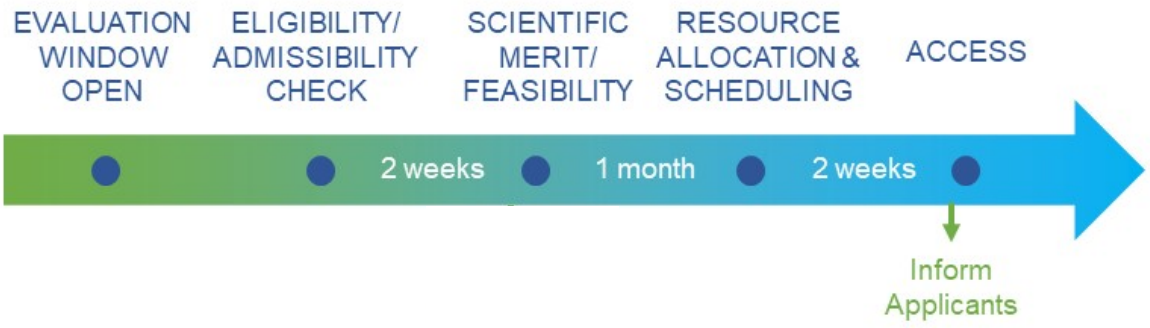
Samples as well as relevant authorisation for their use, shall be available at the moment of submitting the application or not later than two (2) months after Access approval. When the project foresees the analysis of more than one batch of samples, the first batch shall be available when the application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

7. EVALUATION OF APPLICATION

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

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

Below is a scheme describing the evaluation steps and timeline.


EVALUATION WINDOW OPEN
ELIGIBILITY/ ADMISSIBILITY CHECK
SCIENTIFIC MERIT/ FEASIBILITY
RESOURCE ALLOCATION & SCHEDULING
ACCESS
2 weeks
1 month
2 weeks
Inform Applicants

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.

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

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

Triage criteria will include:

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

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

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

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

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

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

7.1 Evaluation criteria

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

- **Significance**: Overall scientific merit of the proposed research. If all the experiments proposed are successful, how will the resulting knowledge advance the field?
- **Innovation**: Degree of innovation (conceptual and/ or technological), and ambition of the proposed study compared to the state-of-the-art in the relevant field.
- **Approach**: Appropriateness of proposed methodology, preliminary data in support of proposed experiments, and project feasibility.
- **Environment**: Facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).
- **Justification for requesting Access to the NF**: Explanation on why the service cannot be performed at the host Institution, at a cost which is deemed affordable for the applicant.
- **Applicant**: PI's scientific background and expertise.

7.2 Scoring system

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

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

7.3 Technical feasibility analysis

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

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

7.4 Evaluation results and Access approval

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

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

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

8. AFTER ACCESS HAS BEEN APPROVED

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

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

9. AFTER ACCESS HAS BEEN COMPLETED

At the end of the activities carried out at the NF, and not later than 3 months thereafter, if not differently agreed with the NF User Access Office, the Applicant must submit a short report on the results obtained and the impact of the service on their research. Moreover, a final report to be published on the NFs website and describing the impact of the Access to the NF on the research project for which the service has been requested, shall be provided upon publication of the relevant results. Applicants who will not be able to demonstrate the consistency and relevance of the activities carried out at the NF with the research project for which Access was requested will be considered not eligible to participate in the subsequent calls for Access.

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

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

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

10. CONTACTS

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

11. REFERENCES

NF Access Workflow_Convenzione (link)

NF Access Rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

12. CHANGES TO THE CALL

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

ANNEX I: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE

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

Endorsement letter of the host Institution

To whom it may concern:

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

Declare

- That the host Institution is among those eligible to participate in the call for Access as it belongs to the following eligible category: (select among University, IRCSS, Public Research Entities);
- That the Applicant, Dr ……….. (*Applicant's first name and surname*) is an independent group leader (Principal Investigator) affiliated with a primary appointment at the host Institution and that they meet the eligibility criteria as indicated in the call;
- That the Applicant has not received funding for performing elsewhere, the aspects of the project for which they are seeking here support from or Access to Human Technopole National Facilities;
- That the services requested here cannot be performed by the Applicant at the host Institution, at a cost which is deemed affordable for them;
- That relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained within two (2) months after the approval of the Access in order to process samples and data through Human Technopole;
- That, if applicable, biological specimens have been obtained with the corresponding approval of the Bioethics Committee and appropriately signed 'informed consent', both for their collection and their use, including conservation, manipulation, derivation and processing to be carried out by Human Technopole National Facilities;
- That, if samples were obtained from subjects who signed an 'informed consent', said informed consent allows that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorised third parties;

and is committed

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

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

Date ………………….

Name and Title …………………. ; ………………….

Email and Signature of legal representative or delegated person

…………………. ; …………………………….

ANNEX II: PROJECT PROPOSAL TEMPLATE

Mandatory proposal format

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

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*

PLEASE REMOVE THE INFORMATION ABOVE BEFORE SUBMITTING

Proposal content:

1. TITLE

2. SIGNIFICANCE

3. INNOVATION

4. APPROACH

5. ENVIRONMENT

6. REFERENCES (Optional)

ANNEX III: SERVICE LIST

HUMAN TECHNOPOLE

NATIONAL FACILITY FOR DATA HANDLING AND ANALYSIS

CALL FOR ACCESS

25-DHA-ROUND1

SERVICE LIST

Table of contents

1. INTRODUCTION

The mission of the National Facility for Data Handling and Analysis (NF-DATA) at Human Technopole (HT) is to support the national research community by providing state-of-the-art analysis of biological datasets generated by high-throughput genomic and imaging technologies. The main objective of this National Facility is to provide bioinformatics and bioimage analysis expertise for the interpretation of complex, large-scale biomedical datasets.

The National Facility for Data Handling and Analysis includes three Infrastructural Units:

Bioimage Analysis (IU1) - This unit provides high-quality image analysis solutions, including Quality Control (QC), denoising and image restoration, segmentation, and basic quantification for imaging data. It also develops and maintains new open-source image analysis tools that are released to the broader community and included in the service portfolio.

Omics Analysis (IU2) - this unit performs the analysis of "omics" data generated by other Facilities or by external sources. Basic services include QC, alignment to reference genomes, basic quantification and/or variant calling. In-depth analysis can be provided on a project-specific basis.

Technology Development, DevOps and Web development (IU3) - this unit focuses on providing installable and containerised versions of analysis tools and pipelines, as well as creating and maintaining user-friendly WebApps providing services to the scientific community.

All three units of the National Facility for Data Handling and Analysis engage in technology development, creating re-usable research software for use within the Facility, and enabling the deployment of novel resources that users will be able to "take home" to their own institutions. Technology development ensures that the Facility remains state-of-the-art and adapts organically to the needs of the research community.

Another main pillar of the Facility is the provision of training for the users through workshops and in-depth courses, allowing users to take the acquired knowledge back to their home institutions and creating national awareness of our service portfolio.

Finally, the National Facility for Data Handling and Analysis supports users in the management of the data produced by the National Facilities, assisting them with identifying proper storage for the data and the most effective data transfer methods.

The National Facility for Data Handling and Analysis is supported by a large Data Centre and Scientific Computing infrastructure initially composed of an HPC system (over 100 compute nodes, 30 GPU nodes, 20 PB of storage space) combined with access to cloud-based resources.

NF-DATA services can be accessed under two modalities:

- Access to facility service: the facility will perform the analysis autonomously and will deliver data, results, and a full analysis report to the users. This is the standard modality, available for all services.
- Access to facility service including training: the facility will host up to two members of the user's group, and analysis will be performed collaboratively, allowing hosted scientists will learn how to perform it. This modality is only available for a subset of services.

2. SERVICE LIST

2.1 IU1 service list

IU1 offers the following services:

Category	Service code	Service name
Light Microscopy	NF61.01.01	Light microscopy analysis
Cryo-Electron Microscopy	NF61.02.01	Cryo-electron microscopy analysis
Volume-Electron Microscopy	NF61.03.01	Volume electron microscopy analysis

A detailed description of each IU1 service is found in the remainder of this section.

NF61.01.01 Light Microscopy Analysis

Service description

Light microscopy analysis encompasses the analysis of data generated by any light microscopy modality (ie, brightfield, phase contrast, widefield epi-fluorescence, confocal, lightsheet, etc) and across any sample type.

The services we provide include, but are not necessarily limited to, the following use-cases:

- **Image restoration and denoising**: Removal of pixel-independent noise from images to increase signal-to-noise ratio (SNR).
- **Semantic and Instance segmentation**: Identification and segmentation of objects in an image, generation of image masks.
- **Quantitative Image Analysis**: Quantification of intensity levels in images or segmented objects.
- **Morphometric Analysis**: Analysis of shape and morphology of segmented objects.
- **Custom pipeline development**: Construction of an analysis pipeline combining two or more individual steps.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with users to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the data and the desired form of the analysis result; the precise details of the analysis will be discussed with the users upon selection of the project.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

For this service, we require a detailed project description outlining the analysis goals, and the data to be analyzed. A full analysis plan will be developed in collaboration with successful applicants as the project proceeds. A detailed list of required information (ie number of images, resolution) can be found in the application. Upon successful application, Users will be requested to transfer all raw data to Human Technopole for analysis.

Technical requirements

Applicants must ensure that the dataset is available at sufficient quantity and quality (for example resolution and signal-to-noise ratio) before the closing date of the application period. This will be assessed on example data submitted during the application phase. Applicants are responsible for uploading their image data to Human Technopole file servers at the initiation of the project, and for downloading the final results at the conclusion of the project.

Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs. In addition, we will provide whatever software, code, and support is required for the User to reproduce the analysis at their home institute. The form will depend on the specifics of the project and the needs of the Users, but we anticipate delivery in the form of Python scripts and/or ImageJ macros. To reduce the burden of Access for our Users, we will use open-source software tools during the NF projects.

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

Combined services

This service can be combined with the following services offered by the National Facility for Light Imaging:

NF50 – All services

NF55.006 - Ion imaging assisted experiment

NF52.07.07 - Zeiss Axioscan Z.1 automated slide scanner

To access the combined services, please submit an application to the National Facility for Light Imaging requesting data analysis.

NF61.02.01 Cryo-Electron Microscopy Analysis

Service description

Cryo-Electron microscopy analysis encompasses the analysis of cryo-electron microscopy data, both single particle and tomographic reconstruction. This service includes, but is not necessarily limited to, the following use-cases:

- **Single-particle analysis (SPA)**: Development of image processing pipelines for the reconstruction of single particle 3D density maps, starting from cryoEM raw datasets or pre-processed micrographs/particles. Map validation.
- **Atomic Model Building**: De novo model building from reconstructed 3D density maps, fitting of existing atomic structures and refining of atomic models. Model validation.

- **Analysis of Flexibility and Heterogeneity**: Development of image processing pipelines for local reconstruction and refinement of flexible regions and evaluation of the conformational heterogeneity landscape of the macromolecules.
- **Tomography reconstruction**: Development of image processing pipelines for the reconstruction and analysis of tomograms, starting from tilt-series containing fiducial markers or fiducial less. Segmentation of the tomograms and sub-tomogram averaging (STA).
- **Custom pipeline development**: Construction of a computational pipeline combining two or more individual steps.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with successful Applicants to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our Users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the data and the desired form of the analysis result; the precise details of the analysis will be discussed with the Applicants upon selection of the project.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

For feasibility assessment, uploading a set of at least 10 movies/micrographs as part of the application process is required. It is important that these images accurately reflect the diversity of the data in the dataset (i.e., not the best set of possible images). Please ensure to include any applicable metadata. If CTF estimation is available, please include this as part of the application. Upon successful application, Users will be requested to transfer all raw data to Human Technopole for analysis.

Technical requirements

Applicants must ensure that the dataset is available at sufficient quantity and quality (for example resolution, contrast and signal-to-noise ratio) before the closing date of the application period. This will be assessed on example data submitted during the application phase. Applicants are responsible for uploading their image data to Human Technopole file servers at the initiation of the project, and for downloading the final results at the conclusion of the project.

Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs (typically .mrc or .pdb, but other formats or intermediate files may be delivered depending on User preferences). In addition, we will provide whatever software, code, and support is required for the User to reproduce the analysis at their home institute.

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

Combined services

This service can be combined with the following services offered by the National Facility for Structural Biology:

SB-IU1-C – High-resolution Cryo-TEM Imaging

To access the combined services, please submit an application to the National Facility for Structural Biology requesting data analysis.

NF61.03.01 Volumetric EM Analysis

Service description

The Volumetric EM service provides segmentation and analysis of structures in serial EM section data. This service is restricted to segmentation and analysis of a finite number of structures per image (ie, mitochondria, organelles, vesicles, other similar objects of study). The proposal must include specific examples of the structure(s) of interest in order to judge feasibility. This service includes, but is not necessarily limited to, the following use-cases:

- **Data pre-processing**: de-streaking, alignment, contrast adjustment.
- **Data annotation**: generating dense labels covering structures of interest for the purpose of training AI algorithms.
- **Model Development**: Training and deployment of AI segmentation algorithms specific to the research question.
- **Downstream Analysis**: Analysis of segmented structures, morphology, number, distribution.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with successful Applicants to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our Users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the data and the desired form of the analysis result; the precise details of the analysis will be discussed with the Applicants upon selection of the project.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

For this service, we require a detailed project description outlining the analysis goals, and the data to be analyzed. A full analysis plan will be developed in collaboration with successful applicants as the project proceeds. A detailed list of required information (ie number of images, image dimensions, pixel size) can be found in the application. Upon successful application, Users will be requested to transfer all raw data to Human Technopole for analysis.

Technical requirements

Applicants must ensure that the dataset is available at sufficient quantity and quality (for example resolution and signal-to-noise ratio) before the closing date of the application period. This will be assessed on example data submitted during the application phase. Applicants are responsible for uploading their image data to Human Technopole file servers at the initiation of the project, and for downloading the final results at the conclusion of the project.

Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs. In addition, we will provide whatever software, code, models, and support is required for the User to reproduce the analysis at their home institute. The form will depend on the specifics of the project and the needs of the Users. To reduce the burden of access for our Users, we will use open-source software tools during the NF projects.

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

Combined services

This service can be combined with the following services offered by the National Facility for Structural Biology:

SB-IU1-D – Volume Electron Microscopy

To access the combined services, please submit an application to the National Facility for Structural Biology requesting data analysis.

2.2 IU2 service list

IU2 offers the following services:

Category	Service code	Service name
RNA	NF62.01.01	Bulk RNA-Seq analysis
	NF62.01.02	miRNA analysis
DNA	NF62.02.01	WGS analysis
	NF62.02.02	WES analysis
	NF62.02.03	Microbiome analysis
Single-cell / spatial	NF62.03.01	scRNA-Seq analysis
	NF62.03.02	scATAC-Seq analysis
	NF62.03.03	Single-cell immune profiling (VDJ)
	NF62.03.04	Single-cell multiome (ATAC + gene expression)
	NF62.03.05	Spatial transcriptomics (10X Visium platform)

A detailed description of each IU2 service is found in the remainder of this section.

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.

Advanced (optional) analysis steps include the following:

1. **Functional enrichment and pathway analysis of significant genes**: An over-representation analysis is performed to test the enrichment of the list of differentially expressed genes against Gene Ontology and the main pathway collections (e.g. KEGG, Reactome, Biocarta, Hallmark, IPA).
2. **Alternative Splicing Analysis**: Mapped reads are analyzed to identify splicing isoforms and novel splice variants. Observed alternative splicing events are summarized and annotated.
3. **Identification of gene fusions events**: Gene fusions, resulting from the joining of two separate genes, have been found in various tumor types, leading to the overexpression and constitutive activation of genes not normally expressed.
4. **Variant calling**: RNA-seq datasets can be analyzed to identify variants in coding regions. Although an exact assessment of frequencies is not possible, this analysis may identify variants with a high potential for functional effects.

Access modality available

- Access to facility service

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. For differential gene expression analysis, users should make sure to specify the conditions to be compared. See the example provided in Appendix 3.1. Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same preparation kit, and sequencing must have been performed with the same pairing modality for all the samples (paired-end/single-end), ideally in the same sequencing run. We recommend a minimum sequencing depth of 30 million reads per sample for mammalian-sized genomes (this limit can be reduced in the case of smaller genomes), and a Q30 cutoff of 80%.

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 and normalized count matrices containing expression values for each gene in each sample.
4. Tables of differentially expressed genes with statistical significance information.
5. 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.
6. 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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-005 - mRNA sequencing from standard and low input

G-006 - totalRNA from standard input

G-024 - Sequencing only with NovaSeq 6000 (Illumina)

G-025 - Sequencing only with NextSeq 2000 (Illumina)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.01.02 miRNA analysis

Service description

Small RNA sequencing is a specialized technique designed to analyze and profile small RNA molecules present in a biological sample. It is widely used to study the expression profiles of miRNAs and other small RNAs, providing valuable insights into their roles in gene regulation, development, and disease. Small RNAs are polymeric ribonucleic acid molecules with a length lower than 200 nucleotides, comprising microRNA (miRNA), PIWI-interacting RNA (piRNA), small interfering RNA (siRNA), and tRNA-derived small RNA (tsRNA).

miRNAs are the most studied type of small RNAs, constituted by 20 to 25 nucleotides. They participate in several processes and can regulate gene expression at a posttranscriptional level. miRNAs can also act as transcription factors by binding the seed sequence within 3'UTR of target genes, leading to a variety of cell activities at different levels.

The standard bioinformatics analysis for miRNA datasets comprises the following steps:

1. **Quality check of the raw sequence data**: Sequencing quality of the raw reads is evaluated. It assesses the data quality distribution across reads, per-base content, and adapter contamination.
2. **UMI extraction and trimming**: Low-quality reads, UMI sequences and adapter contamination will be removed and excluded from the analysis. QC is performed again on the trimmed reads.
3. **Filtering for miRNA**: Filtering reads according to length and assessing their nature with respect to other types of small RNAs.
4. **Mapping**: Trimmed reads will be mapped against the reference genome, and mature miRNAs and precursors (hairpins) will be obtained from miRBase.
5. **Expression quantification**: Uniquely mapped reads are assigned to the corresponding features (mature miRNAs and miRNA precursors (hairpins)). A counts matrix is produced that summarizes the inferred expression level for each known miRNA in each sample.
6. **Quality metrics collection**: Quality metrics from sequencing, trimming, alignment and quantification are collected and summarized in a complete, interactive report.
7. **Normalization and filtering**: Expression levels are normalized to account for the different library sizes across samples. Non-expressed miRNAs are filtered out.
8. **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 sample characteristics. If applicable and necessary, batch correction is performed using regression models. The expression of selected project-specific targets (if applicable, e.g. knocked-out genes, tissue markers, etc.) is evaluated across all samples.
9. **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 miRNAs are identified by setting cutoffs on the obtained p-values and log2FoldChanges.

Advanced (optional) analysis steps include the following:

1. **Known and novel miRNA identification**: Canonical and non-canonical miRNAs are identified. An interactive report is produced with an overview of all detected miRNAs.
2. **Isomir identification**: BAM files are parsed, and a mirGFF3 file is created with the information about miRNAs and isomirs. Results will indicate unique isomirs for each miRNA, isomir sequences highlighting canonical sequences, and additions/deletions at 5' or 3' ends. Count matrices summarize total isomirs detected, reference sequence (miRBase) and number of miRNAs detected overall, and after filtering for the isomirs present in all samples.
3. **miRNA-targets identification**: miRNA-targets are obtained from external databases containing predicted (DIANA-microT-CDS, MicroCosm, miRanda, miRDB, PicTar, and

TargetScan) or experimentally validated (miRecords, miRTarBase, and TarBase) miRNA-target interactions.

4. **Functional enrichment and pathway analysis of significant genes**: An over-representation analysis is performed to test the enrichment of the list of differentially expressed miRNAs and/or their target genes.

Access modality available

- Access to facility service

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. For differential gene expression analysis, users should make sure to specify the conditions to be compared. See the example provided in Appendix 0. Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same preparation kit, and sequencing must have been performed with the same pairing modality for all the samples (paired-end or single-end), ideally in the same sequencing run. We recommend a minimum sequencing depth of 5 million reads per sample for mammalian-sized genomes (this limit can be reduced in the case of smaller genomes), and a Q30 cutoff of 80%.

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 and normalized count matrices containing expression values for each miRNA in each sample.
4. Tables of differentially expressed miRNAs with statistical significance information.
5. 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, PCA/MDS) and tables included to the report will also be provided as separate files.
6. 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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-007 – Small RNA sequencing.

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

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.
4. **Cancer-specific analysis**: Identification of tumor-Specific Signature Analysis; Actionable Mutation Identification (e.g., KRAS, BRCA1/2, BRAF, EGFR); Tumor Mutational Burden (TMB); Microsatellite Instability (MSI) and Mismatch Repair (MMR) Deficiency.
5. **Differential analysis**: Based on the experimental design, we can apply different statistical analyses to interpret genomic differences between the variants of the groups under examination

Access modality available

- Access to facility service

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 0.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same preparation kit, and sequencing must have been performed with the same pairing modality for all the samples (paired-end/single-end), ideally in the same sequencing run. We recommend a minimum average coverage of 30X (for germline variants) or 100X (for somatic variants), and a Q30 cutoff of 80%.

Somatic Variant Calling requires a panel of normal (PON) to perform the analysis. GATK recommends aiming for a minimum of 40 samples to create a PON[1].

For **CNV analysis** we recommend a reference set of at least 20 samples to ensure adequate representation of natural variation. It is best to include samples that are as similar as possible to the cases you are analyzing in terms of tissue type and other relevant characteristics.

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.

[1] https://gatk.broadinstitute.org/hc/en-us/articles/360035890631-Panel-of-Normals-PON

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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-001 - Whole Genome Sequencing

G-024 - Sequencing only with NovaSeq 6000 (Illumina)

G-025 - Sequencing only with NextSeq 2000 (Illumina)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.02.02 WES analysis

Service description

Exome sequencing is an application of DNA sequencing (see NF62.02.01) that focuses on preferentially sequencing the exons, or protein-coding regions, which make up about 1-2% of the genome and are more likely to harbor disease-causing mutations. It is used to study genetic variations that affect protein function, thus particularly in disease research. The focus is on identifying genetic variants (e.g., single nucleotide variants, insertions, deletions, copy-number variation). 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 require specialized methods to account for tissue purity and heterogeneity to detect mutations acquired in specific tissues which are present only in a subset of cells.

The standard bioinformatics analysis for WES 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.

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 classes:
    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 duplicated or deleted.

Advanced (optional) analysis steps include the following:

1. **Annotation and gene-level interpretation**: For all the different classes of standard analysis (SNPs, indels, and CNVs) 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, OMIM, 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.
4. **Cancer-specific analysis**: Tumor-Specific Signature Analysis; Actionable Mutation Identification (e.g., KRAS, BRCA1/2, BRAF, EGFR); Tumor Mutational Burden (TMB); Microsatellite Instability (MSI) and Mismatch Repair (MMR) Deficiency.
5. **Differential Analysis**: Based on the experimental design, we can apply different statistical analyses to interpret genomic differences between the variants of the groups under examination.

Access modality available

- Access to facility service

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 0.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same preparation kit, and sequencing must have been performed with the same pairing modality for all the samples (paired-end/single-end), ideally in the same sequencing run. We recommend a minimum average coverage of 30X (for germline variants) or 100X (for somatic variants), and a Q30 cutoff of 80%.

Somatic Variant Calling requires a panel of normal (PON) to perform the analysis. GATK recommends aiming for a minimum of 40 samples to create a PON[2].

For **CNV analysis** we recommend a reference set of at least 20 samples to ensure adequate representation of natural variation. It is best to include samples that are as similar as possible to the cases analyzed in terms of tissue type and other relevant characteristics.

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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-002 - Whole Exome Sequencing

G-024 - Sequencing only with NovaSeq 6000 (Illumina)

G-025 - Sequencing only with NextSeq 2000 (Illumina)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.02.03 Microbiome Analysis

Service description

Microbiome analysis using 16S and ITS amplicon sequencing is a widely used technique to study the composition and diversity of microbial communities, particularly bacteria and fungi. The 16S ribosomal RNA (rRNA) gene is a molecular marker found in the genomes of bacteria and archaea, and its variable regions are commonly used for taxonomic classification, while ITS is used to profile fungal communities.

Microbiome analysis using 16S and ITS amplicon sequencing is valuable in a range of fields, including environmental science, human health, and agriculture. It provides a cost-effective way to characterize microbial communities and understand their roles in various ecosystems or host-associated environments.

[2] https://gatk.broadinstitute.org/hc/en-us/articles/360035890631-Panel-of-Normals-PON

The standard bioinformatics analysis for microbiome datasets of variable regions of the 16S rRNA (V3-V5 regions) or ITS comprises the following steps:

1. **Quality check of the raw sequence data**: Evaluating sequencing quality of raw reads, 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. **Amplicon Sequence Variants (ASVs) inference**: Inferring ASVs from amplicon data by computing an error model on the sequencing reads. Dereplicating sequences via quality filtering, denoising, read pair merging (for paired end Illumina reads only) and PCR chimera removal. Removing mitochondrial and chloroplast sequences in order to focus exclusively on the microbial community.
4. **Taxonomic classification**: Clustering reads into operational taxonomic units (OTUs) or ASVs, referring to the SILVA database for 16S and the UNITE database for ITS.
5. **Abundance and relative abundance**: Calculating abundance based on the computed ASVs and taxonomic classification. Calculating relative abundance based on TSS (Total Sum Scaling normalization) for several taxonomic levels for each sample and reporting in tabular format.
6. **Diversity and Community Analysis (Alpha and Beta diversity)**: Assessing richness, evenness, and composition of the microbial communities using the alpha diversity (within-sample) and beta diversity (between-sample) measures.

Advanced (optional) analysis steps include the following:

1. **Differential abundance**: Differential abundance analysis identifies relative abundance from microbial features across sample groups using ANCOM statistical framework.
2. **Alpha diversity rarefaction curves**: Produce rarefaction plots displaying alpha diversity indices that determine samples richness.
3. **Functional abundances**: Functional abundances are predicted based on marker gene sequences. Enzyme Classification numbers and KEGG orthologs will be predicted for each sample.

Access modality available

- Access to facility services

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 3.1 Metadata file example.

If differential abundance analysis is requested, users should provide the list of conditions to be compared.

Technical requirements

All FASTQ files associated with all the samples must be provided, including the sequences of the amplicons, together with the corresponding md5 checksum files (unless sequencing is performed by the National Facility for Genomics).

The libraries for all the samples must have been prepared using the same preparation kit, and sequencing must have been performed with the same pairing modality for all the samples (paired-end or single-end), ideally in the same sequencing run. We recommend a minimum sequencing depth of 5 million reads per sample, and a Q30 cutoff of 80%.

The user shall provide a table listing all biological conditions in the experiment and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. An example is provided in Appendix 0.

Results

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

1. Abundance, taxonomic, and ASV tables for each sample.
2. 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.* taxonomic abundance barplots, phylogenetic trees, *etc.*) and tables included to the report will also be provided as separate files.
3. Phyloseq R object 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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-003 - Amplicon sequencing for microbiome analysis (16S-ITS)

G-026 - Sequencing only with MiSeq (Illumina)

G-025 - Sequencing only with NextSeq 2000 (Illumina)

G-024 - Sequencing only with NovaSeq 6000 (Illumina)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.03.01 scRNA-seq analysis

Service description

Single-cell RNA sequencing (scRNA-seq) is a technique used to analyse gene expression at the individual cell level, making it possible to resolve cellular heterogeneity within a biological sample. Unlike bulk RNA sequencing, which averages gene expression across many cells, scRNA-seq enables the identification of distinct cell types, states, and subpopulations.

This approach is crucial for understanding complex tissues, developmental processes, and disease progression, as it reveals how gene expression varies from cell to cell, and it is widely applied in biomedical research to advance personalized medicine, immunology, cancer research, and tissue regeneration studies.

The standard bioinformatics analysis for a scRNA-seq dataset 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. **Cell barcode identification and extraction**: Identifying unique cell barcodes corresponding to true cells using the number of associated transcripts as a proxy for their vitality. Extracting transcripts associated to these cells from sequencing reads.
3. **Mapping to the reference genome**: Aligning reads to a reference genome, generating a BAM file. Quantifying the number of reads mapped to each gene in order to measure gene expression per cell.
4. **Doublet detection and quality filtering on individual samples**: Identification of potential doublets (i.e. two or more cells mistakenly captured as one) based on the number and consistency of their expressed genes. Marking corresponding barcodes as potentially derived from multiplets, without initially excluding from the analysis. Applying other quality filters to exclude low-quality, stressed, or damaged cells.
5. **Dataset integration**: Integrating expression data from all sequenced samples into one single dataset on which the overall analysis is performed. Grouping of the samples into integrated datasets will depend on the experimental design and project requirements (e.g. in the case of samples derived from different species and/or tissues, etc.).
6. **Normalization and batch correction**: Normalizing expression values according to different library sizes and subsequent scaling. Identification of most variable genes within each dataset for use in subsequent steps. Evaluation and correction of "batch effect" variability related to the different samples of origin via data harmonization algorithms. Assessment of other potential sources of intrinsic variability, such as the cell cycle.
7. **Analysis of cell populations within the integrated datasets**: Analysis of the cellular composition of each integrated dataset using a standard workflow based on dimensionality reduction techniques (e.g., PCA, UMAP or t-SNE) and clustering algorithms to identify distinct groups of cells. Performing differential gene expression analysis (DGE) between these groups to detect marker genes specifically expressed by certain populations. These marker genes could be used to infer the identity of each cell type. If samples from different conditions were pooled together, a DGE could also be performed to compare the expression profiles of cell populations across conditions.

Advanced (optional) analysis steps include the following:

1. **Automatic cell type annotation**: Inferring the identity of each cell population using automated tools based on the lists of previously identified marker genes and known cell type-specific signatures.
1. **Advanced DGE models using pseudo-bulk**: In case of complex design, application of pseudo-bulk approaches to compare the expression profiles of specific cell populations across different conditions, while adjusting for biological and technical variables.
2. **Differential abundance analysis**: Testing whether the proportions of specific cell types vary across different types of samples.
3. **Variational autoencoders**: Employing these advanced analytical tools for various purposes, such as cleaning up noisy data, filling in missing information, combining datasets, or transferring labels between datasets.

4. **Cell-cell interactions**: Inspecting communication across different cell types through cell type-specific expression of signaling molecules such as ligands, receptors, and their downstream signaling pathways.
5. **Trajectory analysis**: Inferring transcriptional changes related to developmental processes, cell proliferation or response to simuli, via a pseudotime trajectory.

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 0.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Access modality available

- Access to facility service
- Access to facility service including training

Technical requirements

The libraries for all the samples must have been prepared using the same kit and barcoding strategy, ideally in the same sequencing run. We recommend at least 1000 cells per sample and a minimum of 50.000 reads per cell, with a Q30 cutoff of 80%.

Results

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

1. Raw and filtered fastq files for each sample.
2. BAM files for each sample.
3. Count matrices containing expression values for each gene in each cell.
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. UMAP, dotplots, volcano plots, feature plots, etc.) and tables included to the report will also be provided as separate files.
5. Python objects (.h5ad) containing the processed data.
6. 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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-008/G-012 - Single-cell 3'RNAsequencing or Single-cell gene Expression Flex

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.03.02 scATAC-seq analysis

Service description

Single-cell ATAC sequencing (scATAC-seq) is a powerful molecular biology technique used to profile the chromatin accessibility of individual cells/nuclei at a high resolution. Chromatin accessibility refers to the degree to which DNA within chromatin is accessible by cellular machinery, particularly those parts involved in transcription, such as transcription factors and RNA polymerase.

Unlike bulk ATAC sequencing, which cannot determine the chromatin states of individual subpopulations of cells within a sample, scATAC-seq is widely used to provide valuable insights into chromatin accessibility, transcription factor binding, epigenetic modifications, and gene regulation. This technology is particularly useful in studying various processes and biological mechanisms including developmental processes, tumorigenesis, and immunological memory establishment.

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

1. **Quality check of the raw sequence data**: Evaluating sequencing quality of raw reads, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.
2. **Mapping to the reference genome and peak calling**: Aligning good quality sequencing reads to the reference genome. Quantifying the number of fragments mapped to coding and non-coding regions (i.e promoters, enhancers) to identify accessible chromatin peaks.
3. **Barcode counting**: Identifying cell barcodes corresponding to true cells using the number of fragments overlapping peaks.
4. **Quantification of chromatin accessibility**: Summarizing the inferred chromatin accessibility level for each peak in each cell in a count matrix.
5. **Cell-level and sample-level QC metrics collection**: Identifying low quality cells based on several metrics including transcription start site (TSS) enrichment score, nucleosome signal, and the ratio of fragments in genomic blacklist regions. Evaluate sample-level quality through other quality filters such as the fraction of fragments in peak (FRIP).
6. **Normalization and dimensionality reduction**: Normalizing peak-cell matrices according to different library sizes and/or across peaks (e.g. frequency-inverse document frequency (TF-IDF) normalization) to emphasize most informative features. Using the most variable features within each dataset for dimensionality reduction (e.g. SVD, PCA, UMAP).

Advanced (optional) analysis steps include the following:

1. **Differential accessibility analysis**: Differential accessibility region (DAR) analysis is performed to detect differences in chromatin accessibility across sample conditions.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 3.1.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same kit and barcoding strategy, ideally in the same sequencing run. We recommend at least 1000 cells per sample and a minimum of 50.000 reads per cell, with a Q30 cutoff of 80%.

Results

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

1. Raw and filtered fastq files for each sample (if not already available).
2. BAM files for each sample.
3. Raw and normalized peak-by-cell matrices containing peaks for each region of the genome in each cell.
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. motif enrichment plots, etc.) and tables included to the report will also be provided as separate files.
5. Python objects (.h5ad) containing the processed data.
6. 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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-011/G-013 - Single-cell ATAC sequencing (10X Genomics)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.03.03 Single-cell Immune profiling-V(D)J

Service description

Single-cell immune profiling-V(D)J is a powerful molecular biology technique used to profile both 5’ gene expression and T-cell and/or B-cell receptors of individual cells at a high resolution allowing the characterization of cellular heterogeneity and clonal expansion within a biological sample.

Unlike bulk RNA and T/B-cell receptor (TCR/BCR) sequencing, which allow to study gene expression and TCR/BCR repertoires across many cells, single-cell immune profiling-V(D)J enables the identification of distinct cell types, states, and subpopulations both in terms of transcriptional profile (GEX data) and TCR/BCR repertoires (V(D)J data). This approach is crucial for understanding complex tissues, developmental progression, tumorigenesis, and tracking clonal expansion and immune responses. It is widely applied in biomedical research to advance personalized medicine, immunology, cancer immunotherapy, autoimmune disease and infection disease.

The Single-cell Immune profiling-V(D)J datasets include two modalities: gene expression (GEX) and TCR/BCR (V(D)J).

The standard bioinformatics analysis for Single-cell Immune profiling-V(D)J datasets comprises the following steps:

Regarding the GEX data analysis:

1. **Quality check of the original sequences**: Evaluating raw read sequencing quality, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.
2. **Cell barcodes identification and extraction**: Identifying unique cell barcodes corresponding to true cells using the number of associated transcripts as a proxy for their vitality. Extracting transcripts associated to these cells from sequencing reads.
3. **Mapping to the reference genome and quantification of gene expression**: Aligning sequencing reads to the reference genome. Quantifying the number of reads mapped to each gene as a proxy for gene expression per cell.
4. **Doublet detection and quality filtering on individual samples**: Identification of potential doublets (i.e. two or more cells mistakenly captured as one) based on the number and consistency of their expressed genes. Marking corresponding barcodes as potentially derived from multiplets, without initially excluding from the analysis. Applying other quality filters to exclude low-quality, stressed, or damaged cells.
5. **Dataset integration**: Integrating expression data from all sequenced samples into one single dataset on which the overall analysis is performed. Grouping of the samples into integrated datasets will depend on the experimental design and project requirements, (e.g. in the case of samples derived from different species and/or tissues, etc.).
6. **Normalization and batch correction**: Normalizing expression values according to different library sizes and subsequent scaling. Identifying the most variable genes within each dataset are identified for further analysis steps. Evaluating and correcting "batch effect" variability related to the different samples of origin using data harmonization algorithms. Assessing other potential sources of intrinsic variability, such as cell cycle stage.
7. **Analysis of cell populations within integrated datasets**: Analyzing the cellular composition of each integrated dataset using a standard workflow based on dimensionality reduction techniques (e.g., PCA, UMAP or t-SNE) and clustering algorithms to identify distinct groups of cells. Detecting marker genes specifically expressed by certain populations via differential gene expression analysis (DGE) between these groups. Inferring cell type identity via marker genes uncovered in groups. If samples from different conditions were pooled together, performing a DGE to compare the expression profiles of cell populations also across conditions.

Regarding the V(D)J data analysis:

1. **Quality check of the original sequences**: Evaluating raw read sequencing quality, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.

2. **Mapping to the V(D)J reference transcriptome**: Aligning sequencing reads to the reference genome. Quantifying the number of reads mapped to constant and complementarity determining regions (CDRs) of TCR/BCR.
3. **Contig assembly and annotation**: Assembling reads into longer contigs to reconstruct the full TCR/BCR sequence. Annotating contigs by aligning them to V, D and J segments, and by identifying the CD3R sequences.
4. **T and B cell barcode identification and extraction**: Selecting unique cell barcodes corresponding to productive and confident contigs, indeed only T and B cells produce fully rearranged transcripts that contain both a V and a C segments.
5. **Clonotype generation**: Cells with minimal CDR3 sequence mutations are labeled as belonging to the same clonotype by assigning them a unique clonotype ID.
6. **Mapping of clonotypes**: Mapping the identified clonotypes onto dimensionality reduced space generated from GEX modality (e.g., PCA, UMAP or t-SNE) to facilitate the characterization of their transcriptional profile and clustering within immune cell populations.

Advanced (optional) analysis steps regarding the V(D)J data analysis include the following:

7. **Identification of expanded clones**: Identified clonotypes with the same clonotype ID are grouped together to define clonal cells within the same subjects and/or across multiple subjects. Clone size is measured by the number of cells sharing the same clonotype.
8. **Clonotypes characterization**: Identified clonotypes are characterized within the same subjects and/or across multiple subjects based on V and J segment usage vectors, CDR3 length, repertoire overlap and diversity.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 3.1.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same kit and barcoding strategy, ideally in the same sequencing run. We recommend at least 1000 cells per sample and a minimum of 50.000 reads per cell, with a Q30 cutoff of 80% for GEX libraries and at a minimum of 5.000 reads per cell, with a Q30 cutoff of 80% for V(D)J libraries.

Results

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

1. Raw and filtered fastq files for each sample and modality.
2. BAM files for each sample and modality.

3. Count matrices containing expression values for each gene in each cell (in .h5ad).
4. Tables containing high-level description of each clonotype for each cell (in .csv).
5. 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. alluvial plot, Circos plots, etc.) and tables included to the report will also be provided as separate files.
6. Python objects (.h5ad) containing the processed data.
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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-009/012/014 - Single-cell Immune profiling-V(D)J (10X Genomics)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.03.04 Single-cell multiome (ATAC + gene expression)

Service description

Single-cell multiome sequencing (scRNA-seq + scATAC-seq) is molecular biology technique used to analyze both gene expression and chromatin accessibility of individual cells/nuclei at a high resolution, allowing the resolution of cellular heterogeneity within a biological sample.

Unlike bulk RNA and ATAC sequencing, which averages gene expression and chromatin accessibility across many cells, multiome sequencing enables the identification of distinct cell types, states, and subpopulations both in terms of transcriptional and epigenetic profiles. This technology is particularly useful in studying various processes and biological mechanisms including developmental processes, tumorigenesis, and immunological memory establishment. It is widely applied in biomedical research to advance personalized medicine, immunology, cancer research, and tissue regeneration studies.

Multiome datasets include two different assays: gene expression (GEX) and chromatin accessibility (ATAC).

The standard bioinformatics analysis for multiome datasets comprises the following steps:

1. For the GEX modality, refer to
2.
3. NF62.03.01 scRNA-seq analysis.
4. For the ATAC modality, refer to NF62.03.02 scATAC-seq analysis.

Advanced (optional) analysis steps on single-cell multiome data include the following:

1. **Dataset integration**: Integrating data from both modalities into a single dataset on which the overall analysis is performed. Grouping of the two modalities into integrated datasets will depend on the experimental design and project requirements.

Access modality available

- Access to facility service
- Access to facility service including training

Requested inputs from users

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 3.1.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

Technical requirements

The libraries for all the samples must have been prepared using the same kit and barcoding strategy, ideally in the same sequencing run. We recommend at least 1000 cells per sample and a minimum of 50.000 reads per cell for both modalities, with a Q30 cutoff of 80%.

Results

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

1. Raw and filtered fastq files for each sample and modality.
2. BAM files for each sample and modality.
3. Count matrices containing expression values for each gene in each cell.
4. Raw and normalized peak-by-cell matrices containing peaks for each region of the genome in each cell.
5. 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, PCA/MDS) and tables included to the report will also be provided as separate files.
6. Python objects (.h5ad) containing the processed data.
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*.

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:

G-010/012/013 – Single-cell multiome ATAC + Gene expression (10X Genomics)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

NF62.03.05 Spatial transcriptomics (10X Visum platform)

Services description

The Visium Spatial Gene Expression solution from 10X Genomics enables spatial profiling of gene expression within intact tissue sections. This protocol allows for the analysis of gene expression while preserving the spatial context of cells within a tissue sample. It provides valuable insights into spatially distinct gene expression patterns and cell-type localization, facilitating a deeper understanding of tissue organization, disease progression, and cellular microenvironments.

The standard bioinformatics analysis for a Visium Spatial Gene Expression dataset comprises the following steps:

1. **Quality check of the original sequences**: Evaluating sequencing quality of raw reads, assessing nucleotide composition of the reads, K-mer over-representation, duplication rate, and adapter content.
2. **Image segmentation**: Processing and segmenting the high-resolution images of tissue slices to consider only Visium spots covered by tissue. Only the reads derived from those spots are retained in the analysis.
3. **Cell barcodes identification and extraction**: Identifying cell barcodes associated to the tissue-covered spots, extracting transcripts associated to these cells from sequencing reads to undergo subsequent analysis.
4. **Mapping to the reference genome and quantification of gene expression**: Aligning reads to a reference genome, generating a BAM file. Quantifying the number of reads mapped to each gene in order to measure gene expression per cell.
5. **Normalization**: Normalizing expression data to account for technical artifacts while preserving biological variance, such as heterogeneous cell density across various parts of the tissue. The most variable genes are identified and used for further steps of the analysis.
6. **Dimensionality reduction and clustering analysis**: Applying the standard single-cell analysis workflow based on dimensionality reduction techniques (e.g., PCA and UMAP) and unsupervised clustering algorithms to each sample to identify distinct groups of spots. Performing differential gene expression analysis (DGE) between these groups to detect marker genes specifically expressed in certain clusters. Coloring the tissue image according to the clusters assigned to each spot, thus highlighting the spatial distribution of the different groups of spots within the slide.

Advanced (optional) analysis steps include the following:

1. **Multi-samples integration**: If multiple samples are processed, their expression data could be integrated into one single gene expression dataset on which the standard single-cell analysis workflow is applied. In this case, dimensionality reduction and unsupervised clustering are performed on this integrated dataset, and the obtained clusters are projected to each individual tissue slide.
2. **Spot deconvolution and/or signatures evaluation**: Given than each spot of the 10X Visium platform usually embeds more than one single cell, the cell type proportions composing each spot could be inferred by disentangling its mixed gene expression signals. This could be done using a matched single-cell RNA-seq dataset produced in the same experimental conditions (recommended) or relying on publicly available data. Expression of specific genes and signatures could also be evaluated and the spatial distribution of the corresponding scores is correlated with the identified clusters.

Access modality available

- Access to facility service
- Access to facility service including training

Technical requirements

All fastq files associated to all the samples must be provided, together with the corresponding md5 files (unless sequencing is performed by the National Facility for Genomics).

The user shall provide a table listing all biological and experimental conditions in the study and all samples belonging to each condition, ensuring that the sample names exactly match the names of the provided fastq files. See the example provided in Appendix 3.1.

Unless the organism under study is human or a model organism, the user shall provide a reference to the annotated reference genome to be used for analysis.

High-resolution images of each considered tissue slice must also be provided in .tiff format.

The preparation of all the samples must have been performed using the 10X Visium or Visium HD platform starting from Fresh Frozen or FFPE tissues.

Results

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

1. fastq files for each sample.
2. BAM files for each sample.
3. Count matrix containing expression values for each gene in each spot.
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. spatial feature plots, UMAP, violin plots) and tables included to the report will also be provided as separate files.
5. Pipeline and scripts used to perform the analysis.

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

Combined services

This service can be combined with the following services offered by the National Facility for Genomics:G-015/016 – Visium Spatial gene expression from Fresh-Frozen or FFPE tissues (10X Genomics)

To access the combined services, please submit an application to the National Facility for Genomics requesting data analysis.

2.3 IU3 service list

IU3 offers the following services:

Category	Service code	Service name
Scientific software development	NF63.01.01	Web application and web service development
Scientific software maintenance	NF63.02.01	Pipeline containerization and code maintenance

A detailed description of each IU3 service is found in the remainder of this section.

NF63.01.01 – Web Application and Web Service Development

Service description

This service allows for the creation of web applications and web services that are of interest to the scientific community. Web based applications often represent better solutions compared to desktop applications, the latter entailing manual software installation, software copyright and licensing, software updates, operating systems compatibility, and finally dealing with system requirements. Many of these issues are solved by the adoption of client-server architectures where users can access services hosted on a remote machine. This includes both full-fledged web applications, available through a web browser interface, as well as lower-level services such as APIs.

Example of common applications are web portals with interactive charts to inspect data stored on databases, or application wrappers around bioinformatic packages that enable scientists with limited computer programming skills to use them through easy-to-use interfaces.

The service includes three main areas, which can be combined to achieve the goals of the proposed application:

1. Frontend application development
2. Backend application development
3. Data layer design and implementation

Area 1 includes the design of the user side of the application, which includes the GUI (graphical user interface) and the optional data retrieval logic for getting data from an external resource, such as the application backend or a third-party API. The final output is a web page running within a web browser, which can involve a greatly varying amount of application logic, ranging from plain static web pages (e.g. promotional websites and portfolios) to complex applications that resemble standard desktop applications.

Our unit provides the expertise to build websites and web applications taking advantage of technologies such as Single Page Applications (SPA), Server Side Rendered Applications (SSR), and Static Sites Generators (SSG), taking care of the design of the user interface, responsive layouts to fit multiple screen sizes, SEO friendliness and accessibility. Main technologies and languages in this area include dynamic HTML, CSS, JS as well as very popular frameworks such as Vue.js and Astro.js.

Area 2 is focused on everything running on the server side, called the backend. Backend services span a plethora of different use cases, fulfilling many different needs. These services provide means to store, distribute, and process data, execute code, authenticating users (in combination with other actions), and more. Applicants can request the development of backend services taking advantage of state-of-the-art technologies, best practices and frameworks.

Area 3 is directly linked to area 2 and deals with how to model, store and retrieve data. This involves data storage strategies, as well as relational and non-relational databases. An important activity is relational database design, where databases are engineered to optimize queries, ensure data normalization and provide robust relational constraints.

Such services can be combined to build web applications that wrap already existing bioinformatic packages developed by the applicants, for which a graphical user interface and simplified usage are required.

Requested inputs from users

Application proposals will be evaluated according to innovativeness and scientific impact, measured in terms of potential audience, number of users, and society benefits. Applicants are encouraged to conduct a survey on already existing solutions, either free or paid, that address similar issues. If similar resources already exist, the applicant should clarify what is the added value and innovation component of their proposal.

Users must provide a comprehensive description of the application, accurately describing the desired inputs, outputs and desirable controls. In case of user interfaces, users are encouraged to provide any useful information to ease the GUI design phase such as user interface sketches, user-session workflow, etc.

Data, biological algorithms and specific code implementations / packages which need to be encapsulated in the application must be provided, in compliance with legal licensing assessment performed during the evaluation phase of the proposal. Every file and dataset uploaded by the users will be treated according to standard GDPR legislation.

Users may also request support in deploying the application on an appropriate hosting infrastructure. Hosting on Human Technopole servers is not currently offered.

Access modality available

- Access to facility service

Technical requirements

For this service, there are no strict technical requirements to be fulfilled. On the other hand, the National Facility reserves the right to select the most appropriate technology for implementing the applications, using industry standard and well-established open-source technologies such as the ones listed below.

- Frontend: available frameworks are Vue.js, Nuxt.js, Astro.js. Styling is provided by custom developed CSS or prebuilt systems such as Material Design or utility libraries like Tailwind.
- Backend: available languages are python and JS / TS (Node.js). Available backend frameworks are FastAPI (Python), Express.js (Node.js), Fastify (Node.js).
- Data layer: file storage through S3 buckets / local storage. Relational Databases: MySQL, MariaDB, Postgres. Non-relational databases: MongoDB, neo4j.

Specific technological needs must be documented and justified in the application proposal.

Results

The users will receive the requested software application and all related source code, assets, containerized images, and other artifacts. The application will be released under an appropriate open-source license. Developed code will be shared through dedicated remote repositories (such as GitHub or GitLab). Distributable artifacts, such as code bundles and docker images, will be shared in dedicated indexes and registries. Full documentation of code and data models will be provided as well.

Implemented services will be provided in containerized format through docker images, hosted on online accessible registries. The National Facility may also be able to assist users in the deployment of the application on a dedicated infrastructure (either cloud-based or on-premises) owned or controlled by the applicants. Feasibility of this step will be discussed with the applicants during the technical evaluation phase.

Combined services

This service may be combined with all analysis services offered by the National Facility for Data Handling and Analysis.

NF63.02.01 – Pipeline Containerization and Code Maintenance

Service description

This service provides support for the code maintenance lifecycle for scientific software which has already reached a sufficiently mature development stage. The general aim is to provide best tools and practices to achieve better quality of code, software reproducibility and efficiency, making it a high value product and ensuring its long-term survival.

The service focuses on two main areas:

1. Containerization, standardization and improvement of bioinformatics pipelines;
2. Maintenance of general software for the life sciences.

The first area involves procedures to make existing bioinformatics pipelines more efficient and reproducible. Additional features such as data reporting can also be developed. The target output is a Nextflow pipeline composed of containerized modules, with a focus on code execution efficiency and reproducibility.

The second area encompasses the broader category of software that needs to be improved and/or updated to avoid obsolescence.

Both areas involve partially overlapping possible activities, highly dependent on the specific project and starting pipeline / software. Main activities include, but are not limited to, the ones described here below:

- **Code refactoring and optimization:** concurrency and parallelism analysis, SW readability and maintainability, error management, application of design patterns.
- **Dependency update and management:** managing dependencies and solving incompatibilities or outdated software issues, dependency version freeze for increased reproducibility and time robustness.
- **Testing and monitoring:** unit/integration tests, implementation of logging and monitoring systems.

- **Extra features development:** plots, charts and report integration.
- **Containerization**: whenever possible, maintained SW will be containerized to make its compatibility and execution easier.

Requested inputs from users

Users must provide full accessibility to the already developed codebase through a remote repository (e.g: GitHub), granting full access role-based authentication to NF Data IU3 members involved in the project. SW licensing must be compliant with the possibility of acquiring / modifying code without any legal implication (e.g. MIT, BSD, GNU GPL). Further legal aspects will be discussed during the evaluation phase of the selection process to assess the legal feasibility of the project, also exploring GDPR rules about application data treatment.

A thorough explanation of the expected output from the project must be provided, which should clarify the main areas of improvement and intervention on the current codebase, describing the high-level characteristics of the expected output. In addition, sufficient context and details about the code must be provided, whenever not sufficiently documented.

A survey on similar technologies should also be provided by the applicants to compare their software with similar already available commercial / free solutions, if any, with the purpose of identifying the added value of the proposed software to the available state of the art.

Access modality available

- Access to facility service

Technical requirements

Applications will be evaluated according to the following technical requirements:

- Supported languages: R (version 4.0 or above), Python (version 3.7 or above), bash. Code written with older versions of these languages will be evaluated to determine the feasibility of updating it to an appropriate long-term supported version.
- For pipeline projects, supported frameworks are Nextflow and Snakemake. The output format will be determined by the National Facility according to the technical requirements of the project.
- Supported operating system targets: Unix / Linux.
- Output containerization format: Docker / Singularity.
- Software dependencies must be actively maintained. Conversely, working versions of dismissed dependency projects must be retrievable and must prove to work in the current codebase.

For pipeline containerization projects, the applicant must also provide details about the target hosting infrastructure where the pipeline needs to be run, defining its technical specs (CPUs, GPUs, memory, architecture). Hosting on Human Technopole servers is not currently offered.

Technical debt and obsolescence will be a pivotal point during the evaluation process, privileging the choice for modern and promising software over hard to update / maintain older codebases, with smaller technological and scientific impact.

Results

Developed code will be made available on a remote repository, either with public or private access. Additional artifacts such as container images, documentation, Software packages, will be made available for download by the applicants through designated remote indexes, pages and registries, either public or private. Documentation on how to run the software will be provided, as well as optional training for installing the software and operate it on target machines or computational infrastructure. The National Facility may also be able to assist users in the deployment of the application on a dedicated infrastructure (either cloud-based or on-premises) owned or controlled by the applicants. Feasibility of this step will be discussed with the applicants during the technical evaluation phase.

3. APPENDIX

3.1 Metadata file example

Metadata about sequenced samples should be provided in a table (in tab-delimited or Excel format) with the following structure:

Condition	Sample	FASTQ1	FASTQ2	Var1	Var2	Var…
control	sample1	sample1_R1.fastq.gz	sample1_R2.fastq.gz			
control	sample2	sample2_R1.fastq.gz	sample2_R2.fastq.gz			
treatment	sample3	sample3_R1.fastq.gz	sample3_R2.fastq.gz			
treatment	sample4	sample4_R1.fastq.gz	sample4_R2.fastq.gz			

- The first three columns are required and should be named Condition, Sample, and FASTQ1 respectively.
- The fourth column can be omitted in the case of single-end sequencing. If present, it should be named FASTQ2.
- Condition names and sample names should only contain letters, digits, and the underscore character. Please do not include spaces, symbols, or special characters.
- Additional variables associated with each sample can be added to the table, and will be included in the final reports.

For metagenomic projects, please add the primers used to amplify the target regions, as in the following example:

Condition	Sample	FASTQ1	Forward Primer	Reverse Primer
control	sample1	sample1_R1.fastq.gz	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT
control	sample2	sample2_R1.fastq.gz	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT
treatment	sample3	sample3_R1.fastq.gz	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT
treatment	sample4	sample4_R1.fastq.gz	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT

3.2 Glossary of terms

Section	Term	Definition
0	Signal-to-noise ratio (SNR)	Measure of how well the signal of interest coming from the sample can be distinguished from noise on the microscope detector.
0	Image restoration	Process of improving image quality by removing random noise, enhancing the signal-to-noise ratio (SNR).
0	Semantic and Instance Segmentation	Techniques to identify and separate distinct objects in an image, generating precise object boundaries (masks).
0	Morphometric Analysis	Measurement and analysis of shapes, sizes, and structures in biological images.
0	Single-Particle Analysis (SPA)	Computational technique for reconstructing 3D structures of macromolecules from 2D images obtained by cryo-electron microscopy.
0	Sub-tomogram Averaging (STA)	Method for enhancing resolution in 3D reconstructions of structures from cryo-electron tomography data by averaging multiple similar regions.
0	CTF	Contrast Transfer Function.
0	Splice-aware aligner	Tool that aligns RNA-seq reads, accounting for exon-exon junctions (e.g., STAR, HISAT2).
0	Pseudoalignment	Assigns reads to transcripts without full alignment (e.g., used by Kallisto, Salmon).
0	Q30	Quality score indicating a 1 in 1000 error rate in sequencing (99.9% accuracy).
05	Amplicon Sequence Variants (ASVs)	Unique single-nucleotide precision DNA sequences from amplicon data (e.g., 16S rRNA), alternative to traditional OTUs for microbial diversity analysis.
0	Alpha Diversity	Measure of species diversity within a single sample, considering species richness (number of species) and evenness (distribution of species).
0	Beta Diversity	Measure of species diversity between samples
06	Pseudo-bulk	Data aggregation technique where cell-level data are grouped to create virtual bulk samples for statistical analysis.
0	Peak calling	Identifying regions in the genome where sequencing reads are highly concentrated, indicating active or accessible DNA sites.

0	Blacklist region	Genomic regions known to produce unreliable or artifact signals in sequencing experiments, typically excluded from analysis to avoid misinterpretation of data.
0	Spot deconvolution	Estimating the proportions of different cell types within a single spatial transcriptomics data spot, as spots often contain multiple cells.

3.3 Cited databases

Section	**Database name**	**Database description**	**URL**
0	KEGG	Linking genes and proteins to metabolic and disease-related functions	https://www.kegg.jp/
0	Reactome	Detailed gene-protein relationships and cross-pathway interactions	https://reactome.org/
0	Biocarta	Molecular mechanisms underlying well-known biological pathways	https://maayanlab.cloud/Harmonizome/dataset/Biocarta+Pathways
0	Hallmark	Gene sets of high-level biological states or processes	https://www.gsea-msigdb.org/gsea/msigdb/
0	IPA	Manually curated pathways to predict causal relationships and identify regulatory networks	Provided as software
0	miRbase	Repository for miRNA sequences and annotations	https://www.mirbase.org/
0	DIANA-microT-CDS	Predicts miRNA targets in CDS and 3' UTRs.	https://dianalab.e-ce.uth.gr/microt_webserver/
0	MicroCosm	miRNA target identification and annotation.	https://tools4mirs.org/software/mirna_databases/microcosm-targets/
0	miRanda	miRNA target prediction using sequence analysis.	https://bioweb.pasteur.fr/packages/pack@miRanda@3.3a
0	miRDB	Predicts miRNA targets using machine learning.	http://www.mirdb.org/
0	PicTar	Conserved miRNA target prediction in animals.	https://pictar.mdc-berlin.de/

0	TargetScan	miRNA target prediction using conservation.	https://www.targetscan.org/
0	miRecords	Curated database of miRNA-target interactions.	http://c1.accurascience.com/miRecords/
0	miRTarBase	Experimentally validated miRNA targets.	https://mirtarbase.cuhk.edu.cn/ https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2019/php/index.php
0	TarBase	Manually curated miRNA-target interactions.	https://dianalab.e-ce.uth.gr/tarbasev9
0	Clinvar	Reports of human variants classified for diseases and drug responses.	https://www.ncbi.nlm.nih.gov/clinvar/
0	OMIM	Catalog of human genes and genetic disorders.	https://www.omim.org/
0	ACMG	Genetic variant classification guidelines.	https://www.acmg.net/
0	COSMIC	Catalog of somatic mutations in cancer.	https://cancer.sanger.ac.uk/cosmic
0	Civic	Clinical evidence for cancer variants.	https://civicdb.org/
0	OncoKB	Precision oncology knowledge base.	https://www.oncokb.org/
0	AMP	Molecular testing guidelines for cancer.	https://www.amp.org/
0	SILVA	rRNA sequences for taxonomy and phylogeny	https://www.arb-silva.de/
0	UNITE	Fungal ITS sequences for taxonomy	https://unite.ut.ee/

3.4 Tools used

The following table shows examples of software tools that may be used in our analysis pipelines. The specific tools used in a project will be listed in the final analysis report.

Tool	Purpose	Service
ImageJ	Image processing and quantification for microscopy data.	Spatial transcriptomics
FastQC	Quality control of sequencing reads, checking for adapter content and base quality.	Bulk RNA-Seq, scRNA-Seq, scATAC-Seq, WGS, WES, ChIP-Seq, Methyl-Seq, Microbiome analysis, miRNA analysis
MultiQC	Aggregating QC metrics for different pipeline steps.	Bulk RNA-Seq, scRNA-Seq, scATAC-Seq, WGS, WES, ChIP-Seq, Methyl-Seq, Microbiome analysis, miRNA analysis
TrimGalore!	Trimming low-quality bases and adapters from sequencing reads.	Bulk RNA-Seq, scRNA-Seq, scATAC-Seq, WGS, WES, ChIP-Seq, Methyl-Seq
FastP	Trimming low-quality bases and adapters from sequencing reads.	WGS, WES, miRNA analysis
Cutadapt	Trimming low-quality bases and adapters from sequencing reads.	Microbiome analysis
SAMtools	Manipulating and analyzing BAM/CRAM files from sequencing data.	All sequencing analyses (general-purpose tool)
BEDTools	Tools to analyze BAM/BED files from sequencing data.	ChIP-Seq, scATAC-Seq, WGS, WES, Methyl-Seq
Picard	Tools for BAM file manipulation and quality assessment.	WGS, WES, Bulk RNA-Seq
Bowtie2	Alignment of short reads to a reference genome.	WGS, WES, ChIP-Seq, scATAC-Seq
Bowtie	Alignment of short reads to mature miRNAs and miRNA precursors (hairpins).	mirRNA analysis
BWA-MEM2	Alignment of short reads to a reference genome.	WGS, WES, ChIP-Seq, scATAC-Seq
STAR	Splice-aware alignment of RNA-seq reads to the reference genome.	Bulk RNA-Seq, scRNA-Seq
Salmon	Pseudoalignment and quantification of transcript abundance.	Bulk RNA-Seq, scRNA-Seq

DESeq2	Differential gene expression analysis for RNA-seq count data.	Bulk RNA-Seq, scRNA-Seq, miRNA analysis
edgeR	Differential expression and count-based RNA-seq analysis.	Bulk RNA-Seq, scRNA-Seq, miRNA analysis
enrichR	Functional enrichment of GO and pathway data.	Bulk RNA-Seq, scRNA-Seq, ChIP-Seq, scATAC-Seq
ClusterProfiler	Statistical analysis of GO and pathway data.	Bulk RNA-Seq, scRNA-Seq, ChIP-Seq, scATAC-Seq
CellRanger	Preprocessing, alignment, and quantification of RNA-seq, ATAC-seq, and TCR-seq at the single-cell level.	scRNA-Seq, scATAC-Seq, Single-cell immune profiling, Single-cell multiome
Scanpy	Analysis and visualization of single-cell and spatial RNA-seq data.	scRNA-Seq, Spatial transcriptomics
muon	Analysis and filtering of single-cell ATAC-seq data for visualization of quality metrics.	scATAC-Seq, Single-cell multiome
Scrublet	Doublet detection in single-cell sequencing.	scRNA-Seq, scATAC-Seq, Single-cell multiome
DoubletFinder	Doublet detection in single-cell sequencing.	scRNA-Seq, scATAC-Seq, Single-cell multiome
SCVI	Integrating multiple samples, layers, and modes in single-cell data.	scRNA-Seq, scATAC-Seq, Single-cell multiome
Harmony	Integration of multiple samples in single-cell datasets.	scRNA-Seq, scATAC-Seq, Single-cell multiome
Space Ranger	Preprocessing, alignment, and quantification of spatially resolved RNA-seq data.	Spatial transcriptomics
Loupe Browser	Manual segmentation of spatial transcriptomics images and data visualization.	Spatial transcriptomics
HaplotypeCaller	Germline variant calling (SNP/indel).	WGS, WES
Mutect2	Somatic variant calling (SNP/indel).	WGS, WES
Strelka	Somatic variant calling (SNP/indel).	WGS, WES
Manta	Structural variant detection.	WGS, WES
DeepVariant	Germline variant calling (SNP/indel).	WGS, WES
MACS2	Peak calling for ChIP-seq, ATAC-seq, and single-cell ATAC-seq data.	ChIP-Seq, scATAC-Seq

SEACR	Peak calling for low-background assays like CUT&RUN.	CUT&RUN
HOMER	Motif discovery and annotation of regulatory regions in genomic datasets.	ChIP-Seq, ATAC-Seq, scATAC-Seq
Scirpy	Analysis and visualization of TCR/BCR data for immune profiling at the single-cell level.	Single-cell immune profiling (VDJ)
IGV	Interactive visualization of genomic data.	WGS, WES, Bulk RNA-Seq, scRNA-Seq, scATAC-Seq, ChIP-Seq, Methyl-Seq, Spatial transcriptomics
DADA2	Microbiome data analysis and Amplicon Sequence Variant (ASV) inference from microbiome data.	Microbiome analysis
QIIME2	Microbiome data analysis, including taxonomic classification and diversity analysis.	Microbiome analysis
Phyloseq	R object for working microbiome data.	Microbiome analysis
ANCOM	Analysis of composition of microbiomes.	Microbiome analysis
PICRUSt2	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States.	Microbiome analysis
miRDeep2	Identification of novel and known miRNAs.	miRNA analysis
miRTrace	Quality control for small RNA-seq data.	miRNA analysis