A European infrastructure for farmed animal genotype to phenotype research

Deliverable 4.4

Standardised biobanking protocols adapted to species and material origin for input in WP3

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Table of Contents

1. Executive Summary

Background	The EuroFAANG INFRA-DEV project aims to streamline use of interdisciplinary capabilities for G2P (Genotype to Phenotype) research in terrestrial and aquatic farmed animals and provide transnational access to all of the relevant facilities, expertise and knowledge to European stakeholders. WP4 main aim is to facilitate the development and the sharing of cellular models for fundamental and pre-competitive G2P research in terrestrial and aquatic farmed species. This deliverable relies on the activities developed so far with the aim of setting a biobanking framework in Europe (T4.1: "Upgrade animal biobanking infrastructures to support G2P *in vitro* approaches") and to overcome the main barriers that preclude a widespread and harmonised use of *in vitro* cellular models (T4.2 "Enabling the use of *in vitro* animal models for G2P research").
Objectives	To establish the common descriptors of tissues and cells, and the operational and protocol standards to be shared among EU biorepositories to improve data integration, reproducibility, and interoperability.
Methods	This deliverable builds on the biobank survey, describing collection types and other biobank information, as well as the user interface of biobanking infrastructures previously developed and reported in D4.1 and D4.2. These references highlight the importance of metadata standardization as a fundamental step to make possible the exploration of biobank collections and facilitate user requests. For *in vitro* cellular models, it builds on i) the insights on advanced *in vitro* models gathered during the summer school (held in September, 2023), organized by INRAE, and ii) on the outputs gathered during the theoretical and practical training workshop "G2P-in-a-Dish" organized by the "In vitro systems" Think Tank of T4.2, on how best practices and standards that can be adopted by the community. The metadata standards and protocols shared for the practical sessions were used as templates to propose standardized biobanking protocols for EuroFAANG for further inputs into the FAANG data Portal.
Results & implications	The first rule sets and templates, as a first basis to further develop standardized biobanking protocols with defined metadata adapted to different species (terrestrial and aquatic), and to share biological materials (tissues, cells) stored by the network of EU national infrastructures - are available on the EuroFAANG data portal. Particularly the metadata rules for cells and organoids will be further expanded improved by further community's interactions in the future infrastructure. This will enable researchers accessing biobanking services for different types of biological samples to reuse and compare data more effectively across different labs.

2. Introduction

EuroFAANG started as a coordinated effort across six large EU RIA projects contributing to FAANG to standardize research processes, analysis pipelines and methods. Since 2023, the EuroFAANG INFRA-DEV project capitalizes on the progress made by a larger global FAANG community to make it sustainable. The project is leveraging global scientific and technical expertise, and predictive power through combining standardized data. The project is structured into eight work packages (WP) that explore the technical and conceptual feasibility of providing access to new or upgraded fully fledged user facilities and capabilities for G2P research in farmed animals, namely data access, biobanking, genome editing, and new breeding and genomic technologies, shared across partners in the consortia.

Standardizing biobanking protocols is essential for reducing variability, enabling data sharing and aligning with FAIR principles (Wilkinson et al., 2016). These protocols are guided by recognized best practices, including:

- ISBER best practices for Repositories (ISBER, 2023)
- OECD Guidelines Biobanks and Genetic Research Databases (OECD, 2009)
- FAANG Sample Metadata Checklist and submission workflows (FAANG 2021).

In biobanking, protocols are standardized procedures and guidelines for the collection, processing, storage, management, and distribution of biological samples (e.g., tissues, cells, body fluids, DNA, etc.…) and their associated metadata and data. These protocols ensure samples are handled in a consistent, high quality, and ethically complaint manner. For example, specific protocols have been developed for cancer research (Mendy et al., 2017) and emphasize pre-analytical variables such as sample collection, labelling, preservation, and storage, which affect biospecimen quality and research usability (ISBER, 2023).

The standardization process requires biobanks to precisely describe their protocols and standard operation procedures (SOPs) in detail. While common protocols are encouraged, adaptations maybe necessary depending on the sample type or research context, and must be clearly explained. Inter-biobanking further demands the use of shared ontologies and data exchange formats, so that, even if protocols differ, the accompanying metadata are consistent and interoperable. Moreover, harmonization, ensuring methodological compatibility across different biobanks supports the sharing and comparative analysis of biospecimens and data. This is particularly critical when integrating high throughput data, which relies on uniform operational details and platform consistency (Harris et al., 2012).

As biobanks expand their roles to include more complex specimens like advanced cell models that closely mimic physiological functions, ensuring consistency becomes more challenging. In many cases, *standards* (fixed, widely accepted methods) do not yet exist for these emerging methodologies. Instead, the focus on *best practices* (flexible, community-accepted procedures) such as transparent protocol sharing and reproducibility efforts can pave way for future standardization.

3. Metadata Standards and Ontologies - status

3.1 General definitions

Standards are formalised, uniform protocols aimed at ensuring reproducibility and comparability.

Best practices are evolving guidelines that support consistency and quality in areas where standards are not yet fully established.

Metadata is commonly defined as 'data about data'. Metadata provide descriptive information and context about research data, making it easier to understand, organise, and manage throughout its lifecycle. It serves as essential documentation that describes the various aspects of data, facilitating data discovery, interpretation, and reuse. In research on farm animals, metadata could include Species, Breed, date of birth, sex, geographic location, rearing system, physiological stage at sampling, date of sampling, sample type, etc.

Metadata standards are documented agreements that establish the structure and format of metadata, and sharing guidelines, procedures and protocols for sample collection, processing, and *in vitro* model development. They ensure that data is described and managed consistently. Metadata standards are important because they facilitate uniformity and interoperability, enabling effective data management.

Ontologies are formal, structured frameworks recommended to define, standardize and annotate biological samples and metadata, so that data is interoperable and searchable. Key part of standardisation is controlling terminology through standardised ontologies to make downstream search and analysis more powerful. This is important for *in vitro* systems too. These systems are often derived from tissues but require their own ontology classification. Most used ontologies are NCBItaxon for species, Livestock Breed Ontology for breeds, UBERON or BRENDA for tissue type… as can be found on the FAANG portal, illustrated below. A library of ontologies is available at https://bioportal.bioontology.org/ontologies.

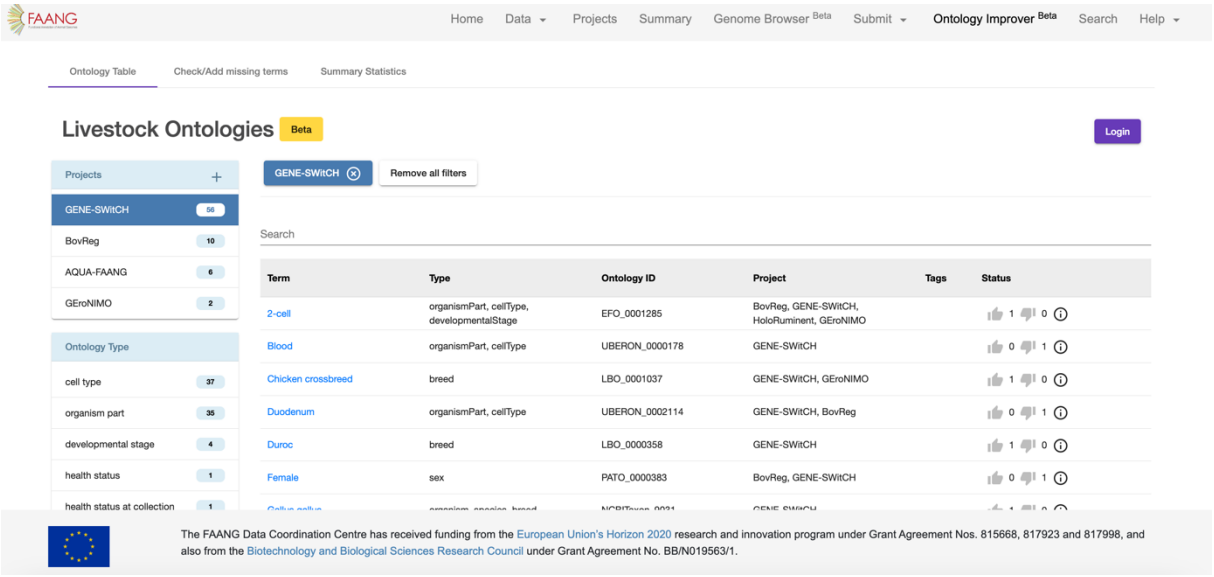

Figure 1: Ontology Improvement Service curation tool.

3.2 Structure and the current status of the FAANG Data Portal

The Functional Annotation of ANimal Genomes (FAANG) is a worldwide coordinated action to create high-quality functional annotation of farmed and companion animal genomes (https://www.faang.org).

The FAANG Data Portal (https://data.faang.org) developed by the EMBL-EBI FAANG Data Coordination Centre, provides open access to richly annotated sample, sequencing, and analysis data following FAIR principles (Findable, Accessible, Interoperable and Reusable). It employs metadata standards to ensure data consistency, reproducibility, and cross-laboratory usability, using structured and standardized terminology. To support ontology development for *in vitro* systems, the FAANG Ontology Improvement Service was created under H2020 projects like GENE-SWitCH, BovReg, and Aqua-FAANG, enabling expert contributions to improve livestock ontologies collaboratively.

The FAANG Data Portal offers advanced features including predictive search, protocol catalogues, predefined filters, dataset-linked publications, and direct download from linked archives to facilitate seamless, integrative access to FAANG data. The Data Portal plays a key role for FAANG by supporting high-quality functional annotation of animal genomes, through open FAIR sharing of data, complete with standardised rich metadata. The standards continue to evolve, under the guidance of the FAANG Metadata and Data Sharing Committee, as new technologies and experiment strategies are developed and employed by researchers to reflect the non-static nature of the field.

3.2.1. Structure of the FAANG Data Portal

The essential features of the Data Portal are described below:

The Landing/Home page: provides an introduction to FAANG objectives and the purpose of the portal. It also highlights other features of the portal. https://data.faang.org/home

The Organisms page enables you search for any organism in the FAANG data set. The term "organism" means any individual animal that has contributed a biological specimen. https://data.faang.org/organism?sortTerm=id_number&sortDirection=desc

The Specimens page lets you search for any specimen in the FAANG data set. The term "specimen" means any biological material originating from a particular organism. https://data.faang.org/specimen?sortTerm=id_number&sortDirection=desc

The Dataset page allows you search for any dataset in the FAANG data set. The term "dataset" t means the Study concept used in ENA (also referred as a Project). https://data.faang.org/dataset?sortTerm=accession&sortDirection=desc

The File page lets you search for any experiment files in the FAANG data set. The term "file" means any experiment assay file that has been submitted to a public archive. https://data.faang.org/file?sortTerm=fileName&sortDirection=desc

The Search interface permits you search across different types of data tables. It enables custom search allowing you to join tables and select specific columns. https://data.faang.org/graphql

Protocols Repository stores standardized lab and computational protocols https://data.faang.org/protocol/samples?sortTerm=protocolName&sortDirection=asc
Project pages dedicated pages for reaching participating project like EuroFAANG, GeneSwitch, BovReg, etc. https://data.faang.org/projects

Submission system page permits researchers to submit data via structured templates, with integrated metadata validation tools ensuring compliance with FAANG standards. It also supports submission to EMBL-EBI archives such as ENA and Biosamples.

3.2.2.1. Component of the Rule Set for "Organism", "Cell culture", "Purified cell", "Organoid" and "Cell line"

For Organism

- **Organism type:** NCBI taxon ID of organism.
- **Sex:** Animal sex, described using any child term of PATO_0000047.
- **Birthrate:** Birth date, in the format YYYY-MM-DD, or YYYY-MM where only the month is known. For embryo samples record 'not applicable'.

- **Breed:** Animal breed, described using the FAANG breed description guidelines (http://bit.ly/FAANGbreed). Should be considered mandatory for terrestrial species, for aquatic species record 'not applicable'.
- **Health status:** Healthy animals should have the term normal, otherwise use the as many disease terms as necessary from EFO.
- **Diet:** Organism diet summary, more detailed information will be recorded in the associated protocols. Particularly important for projects with controlled diet treatments. Free text field, but ensure standardisation within each study.
- **Birth location:** Name of the birth location.
- **Birth location latitude:** Latitude of the birth location in decimal degrees.
- **Birth location longitude:** Longitude of the birth location in decimal degrees.
- **Birthweight:** Birth weight, in kilograms or grams.
- **Placenta weight:** Placental weight, in kilograms or grams.
- **Pregnancy length:** Pregnancy length of time, in days, weeks or months.
- **Delivery timing:** Was pregnancy full-term. Early or delayed.
- **Delivery ease:** Did the delivery require assistance.
- **Pedigree:** A link to pedigree information for the animal.
- **Child of:** Sample name or BioSample ID for sire/dam. Required if related animals are part of FAANG, e.g. quads.

For cell culture

- **Sample source:** Sample name or BioSample ID for the sample the cell culture was derived from.
- **Culture description**: Cell type, Culture type,
- **Cell culture protocol:** Link to protocol describing how the cells were purified.
- **Experimental conditions**: Culture condition, brief description of incubation parameters, duration of culture and treatment conditions.
- **Number of passages**: Number of times the cell line has been re-plated and allowed to grow back to confluency or to some maximum density if using suspension cultures.

For purified cell:

- **Sample source:** Sample name or BioSample ID for a specimen record.
- **Cell description**: Provide a cell type from the Cell Line ontology.
- **Purification protocol:** Link to protocol describing how the cells were purified.
- **Markers**: Markers used to isolate and identify the cell type (e.g., for FACS sorted cells).

For Organoid:

- **Sample source:** Sample name or BioSample ID for a specimen or organoid record.
- **Organoid morphology**: General description of the organoid morphology. e.g. 'Epithelial monolayer with budding crypt-like domains' or 'Optic cup structure'.
- **Organoid culture type**: Whether the organoid culture is two dimensional or three dimensional.

- **Organoid culture and passage protocol:** Protocol for the culture and passage of organoids, growth environment (matrigel or other); incubation temperature and oxygen level are expected in this protocol
- **Experimental conditions**: growth environment in which the organoid is grown. e.g., 'matrigel', 'liquid suspension' or 'adherent', temperature of incubation in °Celsius.
- **Organoid passages**: Number of passages. Passage 0 is the plating of cells to create the organoid
- **Organ and organ part model**: Organ for which this organoid is a model system e.g., 'heart' or 'liver'. High level organ term. Organ part for which this organoid is a model system e.g., 'bone marrow' or 'islet of Langerhans'. More specific part of organ.
- **Freezing conditions**: Date that the organoid was frozen, method of freezing of organoid. Temperatures are in °Celsius. 'Frozen, vapor phase' refers to storing samples above liquid nitrogen in the vapor.
- **Freezing protocols**: A link to the protocol for freezing.
- **Number of frozen cells**: Number of organoids cells that were frozen.

For cell line:

- **Sample source/biomaterial provider:** Sample name or BioSample ID for the sample or animal the cell line was derived from, where this is known and can be described within the FAANG standards (optional).
- **Sex:** Animal sex, described using any child term of PATO_0000047, name of company or lab that supplied the cell line.
- **Cell line name**: Name of the cell line
- **Catalogue number**: identifier for the cell line in the suppliers' catalogue. E.g. 'ACC 701' for IPEC-J2 from DSMZ.
- **Cell type**: Cell type using a child term of either CL_0000000 or BTO_0000000.
- **Culture protocol:** Link to protocol describing the maintenance of the culture.
- **Culture conditions**: Brief description of culture conditions (e.g. 'on feeder cells', 'E8 media').
- **Number of passages**: The number of times the cell line has been re-plated and allowed to grow back to confluency or to some maximum density if using suspension cultures.
- **Disease**: disease child term of either PATO_0000461 or EFO_0000408.
- **Karyotype**: Karyotype of the cell line.
- **Breed**: Animal breed, described using the FAANG breed description guidelines (http://bit.ly/FAANGbreed).
- **Date established**: Date the line was established/re-established.
- **Publication**: Publication Digital Object Identifier (DOI) e.g. 'doi://10.1139/o09-005'. Publication where the cell line has been fully described and should include details such as doubling time and adhesion preference. You can use "pubmed" to find your DOI https://www.ncbi.nlm.nih.gov/pubmed.

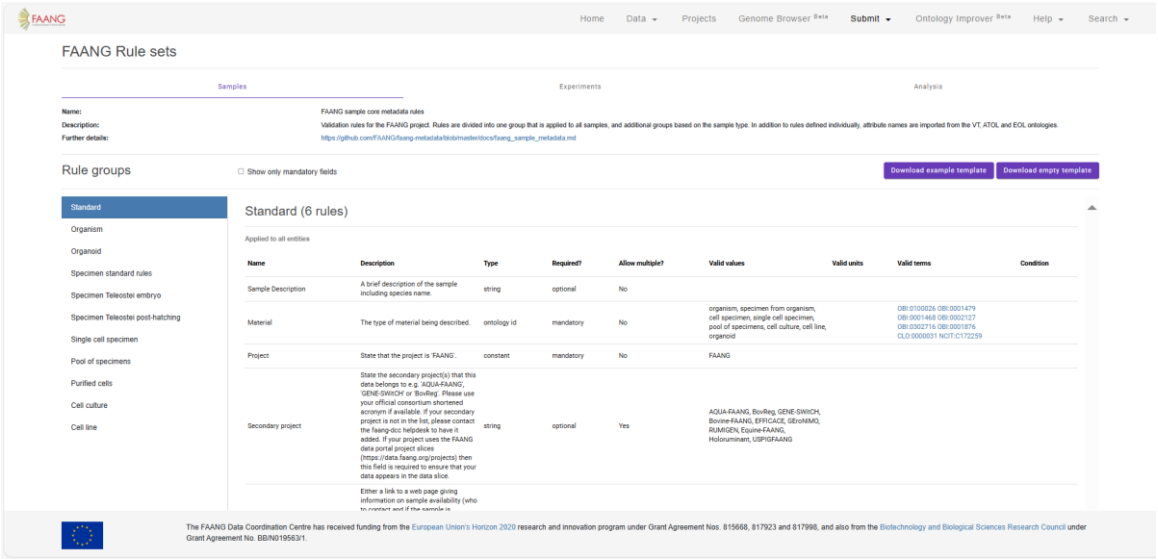

Figure 2: Snapshot of FAANG Metadata Rule Set for the first 4 (out of a total of 10) rules.

3.3 Examples from past projects contributing to biobanking developments in the FAANG Data Portal

3.3.1 Standardized tissue biobanking Protocols

In the frame of FR-AgEncode project (https://www.fragencode.org/), 21 protocols were uploaded between 2016 and 2018 on the FAANG data portal as part of INRAE's submissions (https://data.faang.org/protocol/samples?university_name=French%20National%20Institute%20for%20Agricultural%20Research%20(France)&sortTerm=protocolName&sortDirection=asc; Tixier-Boichard et al. 2021).

A number of protocols are available at national biobanks/infrastructures, such as CRB-ANim (see Annex 1). Many of these protocols could be shared in the near future in the FAANG data Portal as the users' community expands. Examples of these protocols are the isolation of chicken splenocytes and of genomic DNA extraction from liver provided in Annex 2 and Annex 3, respectively.

3.3.2 Organoids

The study PRJEB64165 provides an example of organoid metadata and RNA-seq data obtained from porcine organoids and the tissue (4 portions of the intestine) they derive from. The tissues were obtained from animal specimens collected in the frame of H2020 GENE-SWitCH, see Annex 4.

4. EuroFAANG actions for achieving and promoting standards for biobanking *in vitro* systems

In EuroFAANG, WP4 and the *in vitro* systems think tank (MS9) had as one aim to review and enhance the Organoid and Biobanking metadata rulesets.

4.1 Actions carried out by task 4.1, 4.2 and 4.3

Task 4.1 focused on the review of current practices in European biobanks which were interested in including *in vitro* models in their collections.
The biobank survey reported in D4.1 showed that the most commonly available information, whatever the collection types, was sample information, collection date and storage information. This is followed by collection location and sample ownership. Other information available in the database are:

- Collection method
- Sample quality /quality assessment method
- Conditioning/freezing protocol used
- Sample sanitary status

According to the survey results, biobanks currently store mostly semen, blood, DNA, embryo, whole blood, with a minute number of *in vitro* cellular models. This shows that the description of collections appeared still to be rather heterogenous, which underlines the need for a European coordination and to upgrade biobanking infrastructures. In order to facilitate this upgrade, the majority of biobanks have declared a strong interest and potential to set up biobanking services of cellular models with the provision of training, equipment, budget to cover cost and space. These services include: storage, stem cells and organoids production services, as well as access to cell culture lab.

The user interface of biobanking infrastructures was analyzed in D4.2, considering the French example of CRB-Anim and the European ESFRI landmarks BBMRI, EMBRC, MIRRI and INFRAFRONTIER. The standardization of metadata is key to make possible the exploration of collections and to allow the user putting a request.
Based on the summary of information extracted from the CRB-Anim portal and each of the RIs portal, the following steps involved in processing a request were identified (D4.2):

- Steps to be followed by users based on:
  - Identification
  - Ask for information
  - Searching of samples: on the basis of standard description
  - Purpose and Initiation of request; sample order and deposit requests

- Steps to be followed by the biobank to process request with joint agreement established by a MTA based on:
  - Assessment of request
  - Validation of request, including I.P. issues
  - Implementation of request

Task 4.2 focused on achieving adequate standardization of the whole process of production, biobanking and quality control, characterization and manipulation of *in vitro* models. To facilitate the establishment of a durable network, a committee (*In vitro* systems Think Tank) has been established to oversee the planned activities (MS9). The aim was to overcome the barriers that preclude a widespread and harmonized use of *in vitro* cellular models in animal species, as well as proposing new future actions for their further development.

The first (virtual) meeting of the committee was held on the 12 of June 2023 (13:00 – 14:45). The committee meet periodically (12 meetings including the WP4 regular meetings have been held so far) to advance on the planned activities. Following this establishment, a central resource has been created in form of a web portal known as the "*In vitro* models hub" (MS22). The aim is to map the main facilities and expertise across Europe to connect the community working with in vitro models and provide access to key resources for the community.

As part of these effort, a summer school titled "Innovative Cellular Models for Animal Science – Support the 3Rs through the use of organoids" was held in INRAE Jouy-en-Josas on September 11-15th, 2023, laying the foundation for promoting the use and sharing of standards. It gathered 23 participants from 6 countries with the aim to propose a state of-the-art overview of research on organoids/organ-on-chips, with a specific focus on farm animal species. The scope of the Summer school was to expand access and increasing uptake of the use of *in vitro* model systems in G2P research (MS10). Building on this, a training workshop on the potential of cellular systems and genome editing in farm animals to validate hypotheses and explore new ones while adhering to the ethical principles of the 3Rs was held in February 2025 to further advance these objectives (MS11).

Task 4.3 focused on the establishment of a connection with European organizations such as EUGENA (the European animal gene bank network, managed by the European Regional Focal point) (https://www.eugena-erfp.net/en/), and breeding companies that are EFFAB members with the aim to contribute to safeguarding genetic diversity through biobanking. There were exchanges between EuroFAANG and the EUGENA network, and the outcome of the exchanges showed that EUGENA members are very positive about their participation in EuroFAANG (D4.5).

The connection between EuroFAANG and breeding companies' members was advanced during the G2P workshop with a dedicated session titled *in vivo* vs *in vitro* G2P research and the links to the industry including a talk on incorporating functional annotation genomics data in animal breeding applications. This talk was delivered by Derek Bickhart from Hendrix Genetics, Netherlands.

To support Task 4.3, EFFAB organized a World Café in 2023 to foster dialogue between breeders and researchers on key issues related to animal breeding and reproduction techniques, with a particular focus on biobanking and genome editing. The session facilitated exchanges on the concept of dynamic biobanks, especially regarding data and sample sharing under clear rules. It also explored the differences between public and private biobanking models and the role of biobanks in safeguarding genetic diversity. This interactive format enabled open discussion and strengthened connections with breeding companies across Europe, in line with the task's

objective to promote stakeholder engagement and contribute to European biobanking efforts such as EUGENA.

In parallel, EFFAB disseminated a targeted survey as part of the EuroFAANG RI. The survey was designed to identify bottlenecks in the biobanking of *in vitro* cellular models—such as stem cells, genome-edited cell lines, and organoids—and to assess stakeholder needs for advancing G2P research on farm animal genetic resources. By collecting structured input from a diverse range of actors, the survey supports Task 4.3 by informing future infrastructure development, access frameworks, and collaboration models.

4.2 Workshop G2P-in-a-dish

In 2023, the summer school titled "Innovative Cellular Models for Animal Science – Support the 3Rs through the use of organoids" (held in INRAE Jouy-en-Josas on September 11-15th) was organised to gather the community on the topic of organoids and paved the initial foundation for promoting the use and sharing of standards.

This was further elaborated during the "G2P-in-a-Dish" training workshop (https://eurofaang.eu/g2p-in-a-dish) held on February 3-7th 2025, which brought together PhD students, post-docs early-career scientists, biobank managers, researchers, engineers and bioinformaticians. The scope of this workshop was to promote the use and sharing of standards and enlarge the EU community joining the *in vitro* systems hub to contribute to the development of shared standards and processes. The specific aim was to unlock the potential of cellular model systems and genome editing tools in farm animals research and applications, in line with the ethical principles of the 3Rs (reduce, refine, replace) for animal experiments. The workshop was co-organized with the Think Tank members (WP4, WP5 and WP6) of EuroFAANG and provided a collaborative space for detailed review and community engagement on standards (MS11, D6.1)

The talk titled "Developing metadata and standards for G2P research:Examples from the FAANG Consortium" by E. Clark and the practical session "FAANG Metadata standards", held by EMBL-EBI, introduced attendees to the concepts and practices of shared protocols and metadata harmonization. During this practical session, participants were trained on the steps to submit samples and experiments using the FAANG Submission System. Additionally, there was a session dedicated to biobanking and *in vitro* models' genome editing. This session includes a practical session titled "tissue processing for biobanking cell lines" where a protocol prepared for the French biobanking infrastructure for domestic animals 'CRB-Anim' was provided as an example (see annex 5).

There two roundtable discussions aimed at tackling several aspects linked to the use of *in vitro* systems and tools, like the need of achieving standards from biobanking to single-cell omic data production, analysis and sharing, but also on the high pace at which new technologies emerge, and the challenge ahead to bridge the gap between *in vitro* and *in vivo* studies. The second roundtable discussion (*In vitro* vs. *in vivo* G2P research, which perspectives?) focused on the challenges associated with reproducibility and standardization procedures involved in advancing *in vitro* models and how to overcome them. A key discussion point was to how the research

community can balance efforts between simpler *in vitro* models like immortalized cell lines and primary cells, and more complex systems such as organoids and Organ-on-Chip technologies. While complex models offer greater physiological relevance, advancing G2P research depends on standardizing and understanding simpler systems first.

To support this, the community must prioritise sharing best protocols, establishing clear metadata standards, and identifying reference procedures. This will achieve reproducibility, enable data integration, and support scalability across labs. In farmed animals, where complex models are still emerging, these foundational steps are essential for building robust, comparable *in vitro* systems (D6.1).

5. Conclusions and Perspectives

The development and standardization of biobanking protocols across species and biological materials is a central goal of EuroFAANG, particularly in ensuring reproducibility, traceability, and interoperability of biological samples. While fully established standards are still limited, the actions carried out in WP4 and current efforts have provided an essential framework and outlook toward more formalized and harmonized practices.

This deliverable has aimed at building a common foundation that encourages the sharing of protocols. This includes making available not only biobanked samples but also standard operating procedures (SOPs), online pipelines, and metadata structures that allow consistent and transparent sample handling. Although developing protocols and submitting related metadata to platforms like the FAANG Data Portal can be time-consuming, and the required human resources are often underestimated, this is a critical step toward building a sustainable and FAIR-compliant ecosystem.

Currently, national biobanks offer standardized biobanking protocols for collection, sampling and preservation methods of traditional samples types such as tissues, body fluids, and nucleic acids extracts for most livestock species. Cryopreservation is well advanced for reproductive materials like semen and embryos while the biobanking of cells and cell lines is still under-represented (e.g., see Annex 1). The development of standards for new cell lines and edited cell lines, and for complex cell systems such as organoids has been started in EuroFAANG through the actions carried out in T4.2. For such complex systems, consolidated standards are still rare, and a main item emerging is a basic need for the community to first achieve and consolidate best practices that will progressively lead to established protocol standards. The outcomes of the Summer School and of the G2P workshop have shown that the community's active contribution is necessary to propose and disseminate best practices as well as share the first achieved standards, and that this process can strongly benefit from the engagement of expert working groups specialised in using these systems in humans and classical model species.

In biobanking, standardization must be understood as a dynamic and iterative process, not a one-time effort. Protocols should be periodically reviewed and improved to reflect advancements in methodologies and technology to enable other laboratories to adopt or adapt protocols based on

validated benchmarks. An infrastructure-level mechanism for continuous improvement and protocol sharing and upgrading should be included in EuroFAANG's long-term strategy and service descriptions.

To support this goal, several key actions are recommended:

- Promotion of community engagement in protocol development, including calls for submission and curation of protocols by experts.
- Regular training workshops to build capacity and raise awareness around protocol standardization, metadata submission, and FAIR sample handling.
- Inclusion of protocols for sample distribution and retrieval methods on the FAANG data portal by identifying the "fit for purpose" for samples (ISO 21899, 2020).
- Integration with ELIXIR and similar platforms to align metadata and biological sample descriptions with broader European data infrastructures.
- Establishment of centralized or federated repositories for protocol sharing, ideally supporting DOI assignment (e.g., through Dataverse), to enhance discoverability and encourage citation.
- Incorporation of feedback loops and version control to keep protocols current and relevant to evolving research needs.

By embedding a culture of continuous refinement transparency, and collaboration into the protocol design process, EuroFAANG can ensure that biobanked samples are not only high-quality and well-documented but also interoperable and reusable across current and future initiatives.

6. Annex: Examples of Protocol and Metadata

Annex 1: List of protocols for CRB-Anim

List of CRB-Anim protocols - 2020

Procedure	Pillar/Domain	Species	Language	English Name	Biological material	file
Collecte/sampling	CRB-Anim /Animal	Ovis aries	French	Sheep	embryo	COLLECTE_embryon_Ovis aries_TJO_2017_FR.docx
Collecte/sampling	CRB-Anim /Animal	Coturnix japonica	French	Quail	semen	COLLECTE_semence_Coturnix japonica_IGR_2017_FR.docx
Collecte/sampling	CRB-Anim /Animal	Gallus gallus	French	Rooster	semen	COLLECTE_semence_Gallus gallus-IGR_29-04-2020_FR.docx
Collecte/sampling	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	semen	COLLECTE_semence_Oryctolagus cuniculus_TJO_2017_FR.docx
Collecte/sampling	CRB-Anim /Animal	Numida meleagris	French	Guinea fowl	semen	COLLECTE_semence_Numida meleagris_IGR_2017_FR.docx
Collecte/sampling	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	embryo	COLLECTE-embryon_Oryctolagus cuniculus_TJO_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Ovis aries	French	Sheep	embryo	CR CRYO LENTE_embryon_Ovis aries_TJO_2017_FR.docx
Vitrification	CRB-Anim /Animal	Ovis aries	French	Sheep	embryo	CRYO VITRIFICATION_embryon_Ovis aries_TJO_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	embryo	CRYO_embryon_Oryctolagus cuniculus_TJO_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Equus caballus	French	Horse	embryo	CRYO_Embryons_Equides_MCA_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	Ear biopsy	CRYO_oreille_Oryctolagus cuniculus_TJO_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Crassostrea gigas	French	Pacific oyster	Larvae	CRYO_Larves_Crassostrea gigas_CLA_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	Pluripotent stem cells	CRYO_PSC_Oryctolagus cuniculus_MAF_2018_FR.docx
Cryopreservation	CRB-Anim /Animal	Gallus gallus	French	Rooster	semen	CRYO_semence_Gallus gallus_IGR_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	semen	CRYO_semence_Oryctolagus cuniculus_TJO_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Numida meleagris	French	Guinea fowl	semen	CRYO_semence_Numida meleagris_IGR_2017_FR.docx
Cryopreservation and thawing	CRB-Anim /Animal	Gallus gallus Anas platyrhynchos Cairina moschata Taeniopygia guttata	French	Rooster Common duck Barbary duck Mandarin duck	iPSCs, fibroblastes	CRYO-DECONGELATION_cellules_Oiseaux_BPA_2018_FR.docx
Cryopreservation	CRB-Anim /Animal	Oncorhynchus mykiss	French	Rainbow trout	spermatogonia	CRYO-spermatogonieA_Oncorhynchus mykiss_CLA_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Pecten maximus	French	Scallop	semen	CRYO-Sperme_Pecten maximus_MSU_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Crassostrea gigas	French	Pacific oyster	semen	CRYO-Sperme_Crassostrea gigas_CLA_2017_FR.docx
Cryopreservation	CRB-Anim /Animal	Oncorhynchus mykiss	French	Rainbow trout	Testis/Germinal stem cells	CRYO_fragments testiculaires_Oncorhynchus mykiss_CLA_2022_FR
Thawing/transplant	CRB-Anim /Animal	Oncorhynchus mykiss	French	Rainbow trout	Testis/germinal stem cells	DECONG-GREFFE_fragments testiculaires_Oncorhynchus mykiss_CLA_2022_FR
Cryopreservation	CRB-Anim /Animal	Acipenser baerii	French	Siberian sturgeon	semen	CRYO_semence_Acipenser baerii_CLA_2022_FR
Cryopreservation	CRB-Anim /Animal	Equidés	French		semen	CRYO_Semence_Equides_MMA_2022_FR
Thawing	CRB-Anim /Animal	Gallus gallus	French	Rooster	semen	DECONGELATION_semence_Gallus gallus_IGR_2017_FR.docx
Thawing	CRB-Anim /Animal	Numida meleagris	French	Guinea fowl	semen	DECONGELATION_semence_Numida meleagris_IGR_2017_FR.docx
Cell preparation	CRB-Anim /Animal	Gallus gallus Anas platyrhynchos Cairina moschata Taeniopygia guttata	French	Rooster Common duck Barbary duck Mandarin duck	iPSC	ETABLISSEMENT_iPSCs_Oiseaux_BPA_2018_FR.docx
Insemination	CRB-Anim /Animal	Coturnix japonica	French	Quail	semen	INSEMINATION_semence_Coturnix japonica_IGR_2017_FR.docx
Insemination	CRB-Anim /Animal	Gallus gallus	French	Gallus gallus	semen	INSEMINATION_semence decongelee_Gallus gallus_IGR_2017_FR.docx

Insemination	CRB-Anim /Animal	Numida meleagris	French	Guinea fowl	semen	INSEMINATION_semence_Numida meleagris_IGR_2017_FR.docx
Cell preparation	CRB-Anim /Animal	Gallus gallus Anas platyrhynchos Cairina moschata Taeniopygia guttata	French	Rooster Common duck Muscovy duck Mandarin duck	fibroblastes	CULTURE_fibroblastes embryonnaires_oiseaux_BPA_2018_FR.docx
Cell media	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	semen	PREPARATION_milieux TCG SEGORBE_semence_Oryctolagus cuniculus_TJO_2017_FR.docx
Production	CRB-Anim /Animal	Ovis aries	French	Sheep	embryo	PRODUCTION_embryon_Ovis aries_TJO_2017_FR.docx
Production	CRB-Anim /Animal	Oryctolagus cuniculus	French	Rabbit	embryo	PRODUCTION_embryon_lapin_JOLY_2017.docx
Transfer	CRB-Anim /Animal	Ovis aries	French	Sheep	embryo	TRANSFERT_embryon_brebis_JOLY_2017.docx
	CRB-Anim /Animal		English			THAWING_semen-Gallus gallus_IGR EBL AVC_2020_EN.docx
	CRB-Anim /Animal					
	CRB-Anim /Animal					
		Procedures for characterizing collections				
Dosage ; methylation de l'ADN ; LUMA	CRB-Anim /Animal	Pisces	French	Fish	DNA	LUMA_sperme_poissons_ADE_2017_FR.docx

Annex 2: Isolation of chicken splenocytes

U.M.R. Infectiologie Santé Publique
Centre Val de Loire
Site de Tours

Operating mode

N° d'identification :
1282-MO-000N

Version : 01
Nb pages : 2

Isolation of chicken splenocytes

Date d'émission :	**Dernière mise à jour :**	**Date de retrait :**
11/06/15		
Rédigé par :	Revu par :	Approuvé par :
Hélène Marty et Pascale Quéré	English translation Michèle Tixier-Boichard	

Diffusion : FrAgEncode project

Key-words : *splenocytes*

CONTENT

1. Object

Isolation of splenocytes from birds: protocol used on chickens for Fr-AgEncode

2. Domain

Cell biology

3. Principle Splenocytes are separated from the whole spleen by density gradient using Histopaque 1.077 in order to remove nucleated erythrocytes

4. Reagents
-Histopaque 1.077 (SIGMA ALDRICH-10771)
-RPMI 1640 (FISHER 11554516)
-Fetal Calf Serum (FISHER 10309433)
-DPBS Ca- Mg- (FISHER 11590476)

5. Material and equipments
- metallic circular grids
- Cell Strainer 100µm for 50 mL tubes
- Scalpels, pliers, scissors
- 15mL & 50mL Falcon tubes
- petri dishes
- Centrifuge (for 15mL& 50mL tubes)
- Syringe 10mL
- a microscope, a Malassez or Thoma cell counter

6.Operating mode

NB histopaque 1.077 must be kept at room temperature at least 2h before use

I/ Tissue sampling
The spleen of a chicken is sampled after euthanasia (electronarcosis + bleeding, without anesthetic injection). The spleen is gently removed with a plier with smooth end, scissors and a scalpel, without touching other organs at proximity (liver, gall blader…).
Spleen is kept in sterile PBS in a 50 mL tube placed in crushed ice. It may be kept as such during 6 hours.

II/ Isolation of splenocytes from the spleen.
1- Spleen is placed in a sterile petri dish: the conjunctive capsula is removed with scissors and pliers, and the spleen is cut into small pieces with scissors
2- Add 10mL of RPMI in the dish.
3- Within the petri dish, crush the spleen on the metallic grid with the piston of a 10mL syringe then filter the crushed tissues on a 100 µm Cell Strainer on top of 50 mL Falcon tube. Do not hesitate to add RPMI1640 medium to dilute and rince the crushed tissue. Change the Cell Strainer if it gets clogged.

Adjust the final volume so that it is a multiple of 6.
4- In 15mL tubes, gently distribute 6mL of the material obtained in 3 on a 6mL pad of Histopaque 1.077.
5- Centrifuge 30 min at 700g at room temperature, (acceleration 1, and deceleration 1).
6- Collect the white cells at the interface in a 50mLtube and add DPBS for washing.
7- Centrifuge 10 min at 450-500g at room temperature (acceleration 9, deceleration 9)
8- Repeat 6 + 7 once.
9- After the second wash, collect the cell pellet in 1mL DPBS.
10- After counting, calculate the number of ml you need to sample according to the number of cells you wish to store per aliquot.

III/ Freezing the cells

Prepare the buffer = decomplemented FCS (56°c 30 minutes in a water bath) + 5% DMSO
Add 5 x 106 to 1 x 107 cells per ml of SVF-DMSO
Immediately store at -20°C ; keep for 24h at -20°C, then store in liquid nitrogen.

Annex 3: purification of genomic DNA from tissues

Authors: Hervé Acloque (INRAE) herve.acloque@inrae.fr

i. Sample collection: Required Reagents and Instruments

To perform the extraction of genomic DNA from liver, we followed the User manual of the "Chemagic Star" machine and the Chemagic Star DNA Cell 12m (CMG-1769).

- Chemagic Star DNA Cell12M (Perkin Elmer CMG-1769)
- Chemagic Star (Chemagen)
- Standard Sensitivity Genomic DNA Analysis Kit - 50Kb (Agilent ref: DNF-467-0500)
- Qubit 1X dsDNA BR assay kit (Q33265)
- Qubit device (ThermoFisher)
- 2 ml Tube and 2 ml centrifuge tubes
- Pipets and tips
- Fragment Analyzer (Agilent)
- Nanodrop spectrophotometer (Thermofischer)
- Multi-Therm shaker (Benchmark)
- Proteinase K (Qiagen 19131)
- Cryotable (or box of dry ice)
- Cryoprotection gloves
- Sterile disposable Petri dishes (100 mm and 60mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long
- Racks for 2 mL tubes
- A permanent marker to label the zip lock bags
- Paper towels
- Waste bag

ii. Preparatory stage

Before starting the purification of genomic DNA we weighted the adequate quantity of tissue and performed tissue homogenization. Working on a cryotable at - 25°C (or above a box of dry ice), for each sample, we excise 10 mg of liver tissue. Each piece of tissue is then placed into a 2ml centrifuge tube and stored at - 80°C before performing the homogenization step. Between each tissue, we took care to clean the forceps and scalpel with ethanol and to tare the pre-labelled centrifuge tube.
To perform tissue homogenization, we add 200µl of lysis buffer (from the Chemagic kit) and 6µl of Proteinase K to each microtube containing liver sample. The tube is then placed into the Multitherm shaker at 56°C and 700rpm overnight. The following day, the tubes are centrifugated at 4000g during 20 minutes at room temperature.
The lysate is then loaded into a deepWell 2ml plate (from the Chemagic kit) and processed using

the Chemagic Star workstation.

iii. Preservation, Storage, Quantification, and Determination of Quality of total RNA

Depending on the analysis, preservation methods include: DNAs are stored at - 20°C. Quantification is performed with a Qubit device and a Nanodrop to determine the concentration of each purification and the 260/230, 260/280 ratios. DNA Integrity Numbers (RINs) were determined using a Fragment Analyzer.

Annex 4: Metadata and RNA-seq. data obtained from porcine organoids and the tissue

Study name: PRJEB64165
Title: Pig Intestine Segments Tissus and their derived Organoids gene expression profiling by RNA-seq
Alias: SSC_INRAE_GUT_ORGANOID_RNAseq
Sub-project:
Secondary accession: ERP149302
Species Sus scrofa

Metadata Fields	Example Value
Name	SSC_INRAE_GUT_ORGANOID_100C
BioSample ID	SAMEA113988854
Release date	2023-07-05
Update date	2023-07-06
Sex	male
Organism BioSample Id	SAMEA111507626
Organism	*Sus scrofa*
Breed	Large White
Health status	normal
Standard met	FAANG
Project	FAANG
Secondary project	GENE-SWitCH
Organisation	INRAE(institution)
Material	specimen from organism
Derived from	SAMEA111507626
Specimen collection date	2019
Animal age at collection	5.0 month
Developmental stage	adult
Organism part	Colon
Specimen collection protocol	INRAE_SOP_generating_organoids_20230329.pdf
biosample id	SAMEA113988854
collection date	2019
derived from	SSC_INRAE_GS_WP4_100
geographic location (country and/or sea)	France
sample description	pig100_colon

sample name:	SSC_INRAE_GUT_ORGANOID_100C
submission description	UPDATE of Pig Intestine Segments Tissus for studying their derived Organoids
submission title	INRAE_SSC_GUT_ORGANOIDS

Annex 5: Protocol for primary culture of chicken embryo fibroblasts

Inserm
Institut national
de la santé et de la recherche médicale

Operating procedure Version n° : 1 As of: 26/07/2017	Page **22** of **26**

Obtaining and primary culturing Avian Embryonic Fibroblasts

Gallus gallus
Anas platyrhynchos
Cairina moschata
Taeniopygia guttata

This procedure is the result of research carried out in CRB-Anim WP2.2 (technological developments).
(this information may be omitted if not relevant, and/or supplemented by the names of other funding bodies)

Sanitary approval of collection, storage and installation operations

Depending on the species, collection, storage and/or placement operations can only be carried out in approved, i.e. officially inspected, establishments. Operators wishing to implement the present procedure are therefore advised to contact the relevant authorities beforehand, to take account of the regulatory constraints imposed by the activity in question.

Irrespective of these constraints, it should be remembered that the resources collected must be processed with the utmost care to guarantee the health of the samples, particularly with a view to their eventual national or international distribution.

DISCLAIMER: *Know-how is a very important parameter for the success of many of the procedures we offer in CRB-Anim. This know-how is acquired with experience and time. In most cases, it requires training from those responsible for the procedures. For this reason, we expressly recommend that future users of our methods contact the editors or their representatives before using them. Specific guidelines, or even training courses, will then be offered to future users to encourage effective transfer of the procedures.*

1. Objectives of the operating procedure and areas of application

- Obtain Primary Embryonic Fibroblasts from Chicken (CEF), Duck (DEF) and Zebra Finch (ZEF).

- Preservation of biological resources.

2. Bibliographic references

Fuet A, Pain B. (2017). Chicken Induced Pluripotent Stem Cells: Establishment and Characterization. Methods Mol Biol. 1650: 211-228.

3. Operating mode

3.1. Solutions

- Preparation of TPB (Tryptose Phosphate Broth): mix 14.75 g TPB + 500 mL H20 □ autoclave, aliquot and store at 4°C.

- Fibroblast medium:

Components	Suppliers	References	Quantities (%vol/vol)
DMEM/F12	Thermoscientific	21331-020	
TPB	BD	260300	10%
SVF (Fetal Calf Serum)	Thermoscientific	10270	5%
Penicillin/streptomycin	Thermoscientific	15140-122	1%

- Trypsin-EDTA 10X (Thermoscientific, Ref.15400-054)

3.2. Method of obtaining the sample

Eggs	Species	Supplier	Incubation
CNR, Leghorn, ...	Chicken, Gallus gallus	Various suppliers	11
Peking I37, I44	Duck, Anas platyrhynchos	UEPFG	13
Rouen	Duck, Anas platyrhynchos	UEPFG	13
Barbarie	Duck, Cairina moschata	UEPFG	15

3.3. Quality criteria of the sample before processing

- The first qualitative criterion is the development of the embryo. Only embryos at the correct stage of development on the indicated incubation day are kept for the preparation of fibroblasts, evaluated according to the usual tables for 'staging' the considered species Table HH and recent derivatives:

- Hamburger V & Hamilton HL. (1992). A series of normal stages in the development of the chick embryo. 1951. Dev. Dyn., 195, 231-72. PMID: 1304821 DOI.
- Li et al., (2019). Comparison of whole embryonic development in the duck (Anas platyrhynchos) and goose (Anser cygnoides) with the chicken (Gallus gallus). Poult. Sci. 98:3278–3291.
- the second criterion after the preparation of the cells is their apparent viability which must be above 95%, a value estimated during the cell counting in the presence of Trypan Blue, a viability indicator.
- The last criterion and the seeding of cells is the proliferation of the cells and their apparent doubling time (about 20 to 24 hours depending on the strains). Cell confluence is achieved between 4 and 5 days for the mentioned seedings.
- All observations are recorded and noted during the preparation of the sample in the laboratory notebooks.

3.4. Procedure

- Disinfect fertilized eggs with 70° ethanol
- Incubate the eggs (time indicated in the table) at 37.5°C with humidity and turning (SMA 360 incubator).
- Disinfect eggs incubated with 70° ethanol
- Open the eggs under a PSM, retrieve the embryos in a sterile Petri dish, and wash them with PBS.
- Transfer them to a new box either individually or together – as needed –
- The embryos are killed according to the rules in force at the time of collection. Rapid decapitation is the means used today.
- Remove the limbs and viscera with a scalpel blade.
- Slice the embryo carcass until you get a homogeneous fluid slurry.
- Transfer the shredded material to a 50 mL Falcon tube.
- Add 2 mL of 10X trypsin
- Immediately add PBS to the tube to achieve a final volume of 10 mL.
- Mix gently.
- Incubate for 5 minutes at room temperature, shaking occasionally.
- Allow to decant and filter the supernatant through a 40 µM cell strainer, placed over a 50 mL Falcon tube containing 10 mL of fibroblast medium.
- Repeat the operation once by adding PBS/trypsin to the pellet.
- After recovering the filtered supernatants, centrifuge for 5 minutes at 400g.
- Remove the supernatant and re-suspend the cell pellet in 10 mL of fibroblast medium.
- Count the cells using trypan blue, with a KOVA slide.
- Plate 2x10^6 for a 100 mm diameter dish (P100) in 12 mL of fibroblast medium incubate at 38°C with 10% CO2 and 60%-70% humidity.
- Wait 48 hours before changing the culture medium.
- Pass the cells in trypsin after 4 to 6 days of incubation:

    1- Retrieve the culture medium of each diameter in a 50 mL Falcon tube.

    2- Wash the cells with PBS.

    3- Add 2 mL of 1x trypsin to each diameter.

    4- Incubate at 37°C until the cells detach (less than 5 minutes).

    5- Inactivate the trypsin with 5 mL of culture medium retrieved from the 50 mL Falcon tube.

    6- Detach all the cells with the pipette, recover them in the tube, and thoroughly dissociate the cells with the pipette.

    7- Wash the dish with 10 mL of PBS and recover it in the 50 mL Falcon tube.

8- Take 50µL of cells for counting using trypan blue, on a KOVA slide.
9- Centrifuge the cells for 5 minutes at 800g.
10- Re-suspend the cells in culture medium, adjusting the concentration to 1x10^6 cells per mL of medium for cell maintenance or 5x10^6 cells per mL of freezing medium – see CRB Anim Protocol: Freezing and Thawing of Cells.
11-Sow 1x10^6 cells per P100 in 10 mL of medium if maintaining and caring for the cells.
12-Change the medium every 2 days before new dissociation at confluence.

4. Hygiene and safety

Trypan blue (CAS No. 72-57-1) is a CMR (label H350).

Wearing a lab coat and gloves is mandatory, and disposal of Trypan blue waste must follow the specific regulations for CMRs in effect.

Disposal of culture media after treatment with bleach for 12 hours and sending to the biological waste treatment facility.

Disposal of non-contaminated solid waste in regular industrial trash bins. Disposal of solid waste contaminated with cells in DASRI bins.

5. Traceability

Identification of samples with:

- Cell name, including passage, freezing date, initials of the person who performed the freezing
- Ex: CEF-p2, 26-07-2017 AF

6. Ethics and consideration of animal welfare

- The killing of embryos must be carried out with respect for the animal, according to the rules in effect at the time of the samples, and by personnel authorized by the competent authorities.

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Institute/Unit : U1208 INSERM, USC1361 INRAE

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