Le MIAOU (**M**inimal **I**nformation **A**bout an **O**rganoid and its **U**se):
Eléments descriptifs permettant à l'homme de l'art de reproduire une expérience de fabrication, de caractérisation et d'étude fonctionnelle d'organoïdes

Le MIAOU sert à identifier les informations présentes (la réponse Oui/Non est la plus importante) et à évaluer la qualité de leur description pour la reproductibilité

A) SOURCE MATERIAL MATERIEL SOURCE

consentement adapté au but de la recherche	Yes
déclaration de collection- codecoh*	Yes
Prérequis pour biopsies : genre, âge, région anatomique, diagnostic, statut viral,	Yes
Pour patients : tableau clinique	Yes

<u>Starting cell line (indifferent origin, ATCC, IPSC, ESC ....)</u> Lignée cellulaire de départ (origine indifférente, ATCC, IPSC, ESC ….)

Genetic identity at arrival (example: DNA sequence, snips, digital PCR , STR, CGH array) (Identité génétique à réception)	Yes/no / if yes please specify
Genetic quality control (example : karyotype, STR, digital PCR)	Yes/no / if yes please specify
functional quality (example: differentiation test for pluripotency of IPSCs, permeability tests for intestinal epithelial cells...) (qualité fonctionnelle)	Yes/no / if yes please specify
Cell identity after X passages identité cellulaire après X passages	Yes/no / if yes please specify X
Cell type marker (example : marker name, detection method, target value) marqueur de type cellulaire	Yes/no / if yes please specify
Number of passages at arrival nombre de passages à la réception	Yes/no / if yes please specify
number of possible or required passages before genesis of organoids nombre de passages possibles ou requis avant genèse des organoïdes	Yes/no / if yes please specify
Storage conditions Protocole de conservation	Yes/no / if yes please specify (culture, freezing, thawing protocol, storage modalities)
Mutations if genetic disease mutations si maladie génétique	Yes/no
contamination tests (mycoplasma, bacteriological, fungal) tests contamination	Yes/no / if yes please specify

Primary cell of patient (and healthy subjects) and tumors **Cellule primaire de patient (et sujets sains) et tumeurs**

Genetic identity at arrival (example: DNA sequence, snips, digital PCR , STR, CGH array) (Identité génétique à réception)	no
Genetic quality control (example : Karyotype, STR, digital PCR) Contrôle de qualité génétique	no
functional quality (example: differentiation test for pluripotency of IPSCs, permeability tests for intestinal epithelial cells...) (qualité fonctionnelle)	no
Cell identity after X passages identité cellulaire après X passages	no
Cell type marker (example : marker name, detection method, target value) marqueur de type cellulaire	no
Number of passages at arrival nombre de passages à la réception	no
number of possible or required passages before genesis of organoids nombre de passages possibles ou requis avant genèse des organoïdes	none
Storage conditions Protocole de conservation	no
Mutations if genetic disease mutations si maladie génétique	no
contamination tests (mycoplasma, bacteriological, fungal) tests contamination	Yes Mycostrip test (InvivoGen)
method of tissue dissociation (production of single-cell material or tissue substructures - example: intestinal crypt) méthode de dissociation du tissu	Yes **Process culture in Drop** - Prepare a human tumour sample by trimming surgically resected specimens, or use endoscopic biopsy samples. - Keep the sample in 4 °C PBS or RPMI until processing.(The sample can be preserved overnight at 4 °C in DPBS or RPMI). - Before cells isolation, thaw Matrigel on ice and keep it cold. Prewarm a 48-well plate in a 37 °C incubator. Add 5 ml of FBS

	to 45 ml of basal medium to prepare 10% (vol/vol) FBS medium. - For a surgically resected specimen, strip the underlying muscle layer off using fine scissors under a stereomicroscope, and then cut the sample into 5-mm pieces on a Petri dish. (The dissected samples must be small enough to pass through the tip of a 10-ml pipette.) - Place the dissected pieces of sample or biopsy specimens into a 15-ml centrifuge tube containing 10 ml of cold DPBS. For the subsequent steps, coat the inner surface of every 10-ml pipette with 10% (vol/vol) FBS medium before use to avoid adherence of the samples on the pipette wall. - Wash the samples by pipetting with a 10-ml pipette at least ten times. -Centrifugation 1 min 1200rpm. Aspirate the supernatant with a 10-ml pipette and add 10 ml of cold DPBS. -Repeat Steps 2 times until the supernatant is free of debris. Thorough washing of the sample is crucial to avoid bacterial contamination. -Put in digestion buffer 20 min 37C (DMEM 2.5 % FBS, P/S, 75U/ml collagenase type IX and dispase) under agitation (x22) 1 mL DMEM 2.5% FBS+ 150 µl Collagenase 20%+ 1 mL Dispase -Add 12.5 µl DNAse I for 10 min at 37°C under agitation (100x) - Resuspend the mix with 10 ml PBS1X cold with a 25 ml pipet and mix inside the pipet to dissociate well all the piece of tumor -Fill until 50 ml PBS1X cold and centrifuge for 1200rpm – 5 min 4°C -Resuspend the pellet with around 20 ml PBS1X cold -Add 1 ml PBS through 70 µm filter and Pass cell suspension on 70 µm filter - Add 200 µl FBS -Take the 70µm filter and put in petri dish (35 mm) and add 2 ml trypLE + Y27632 (1/1000) on the top to dissociate the left over 5 min 37°C.

	-Block with 9 mL Complete AdDF medium + 2.5% FBS and mix it with the rest of the filtered tumor cells. -Add 1 ml PBS through 70 µm filter and Pass cell suspension on 40 µm filter - Fill until 50 ml PBS1X cold and Centrifuge again 1200rpm – 5 min 4C -Suspend pellet in 1 ml Complete AdDF medium +Y27632 (1/1000) and count cells -Dispatched 100 000 to 500 000 cells in 15-ml tube. -Add cold Complete AdDF medium to 10 ml into the tube and spin the cells at 4 °C at 1200 rpm for 5 min. Aspirate and discard the supernatant. -Suspend the cells with Corning Matrigel. Use a ratio of cells to Matrigel that will allow 1000 to 500 000 cells in 25 µl of Matrigel. - Dispense 25 µl of the crypt-Matrigel suspension into the center of each well of a 48-well plate using a 200-µl pipette. -Place the plate in a 37 °C incubator for 10 min to solidify the Matrigel. -Add 300 µl of organoid medium supplemented with 10 µM Y-27632 to each well (only 5 first days), and incubate the plate at 37°C, 5% CO_2.

Storage conditions of cells **Conditions de culture des cellules**

Composition of culture media, nature, origin and quantities of supplements used (e.g. glucose, serum, antibiotics, growth factors etc. ......) Composition des milieux	no
Nature and treatment of the supports Nature et traitement des supports	no
Seeding conditions Conditions d’ensemencement	no
Frequency of media changes Fréquence des changements de milieu	Yes The medium was changed every 2–3 days

CO2 / O2 Concentration	Yes 5%

Storage conditions of the lines or cells **Conditions de conservation des lignées ou cellules**

Master banks, (description of protocols, drift control) Banques mères	NA
Daughter banks(description of protocols, drift control) banques filles….	NA
Storage: freezing and thawing protocol Conservation : protocole de congélation et de décongélation	NA
Storage modalities modalités de conservation	NA

B) MANUFACTURING OF THE ORGANOID FABRICATION DE L'ORGANOIDE

Differenciation conditions **Conditions de différenciation (2D)**

Selon besoin, les cellules peuvent être différenciées avant d'entrer en culture 3D

Composition of culture media, nature, origin and quantities of supplements used (e.g. glucose, serum, antibiotics, growth factors etc. ......) Composition des milieux	N.A.
sequence and duration of treatments traitements	N.A.
Nature and treatment of the supports Nature et traitement des supports	N.A.
Seeding conditions Conditions d'ensemencement	N.A.
Frequency of media changes Fréquence des changements de milieu	N.A.
CO2 / O2 concentration	N.A.
quality control of differentiation process (e.g. morphology, mat homogeneity, max and min confluence, proliferation, functional test, monitoring of markers, possibly sorting, mortality rate) contrôle qualité de la différenciation	N.A.

Generation of organoids (3D): in general Génération des organoïdes (3D) : de manière générale

<table>
<tr><td>Composition of culture media, nature, origin and quantities of supplements used (e.g. glucose, serum, antibiotics, growth factors etc. ......)
Composition des milieux</td><td>Tumor medium
<table>
<tr><th>Medium component</th><th>Supplier</th><th>Catalogue number</th><th>Final concentration</th></tr>
<tr><td>Advanced DMEM/F12</td><td>Gibco™</td><td>12634010</td><td>1x</td></tr>
<tr><td>Glutamax 100x</td><td>Gibco™</td><td>35050061</td><td>1x</td></tr>
<tr><td>Hepes 100x</td><td>Gibco™</td><td>15630056</td><td>1x</td></tr>
<tr><td>Penicillin/Streptomycin 100x</td><td>Gibco™</td><td>15070063</td><td>1x</td></tr>
<tr><td>B27 supplement without vitamin A 50x</td><td>Gibco™</td><td>12587010</td><td>1x</td></tr>
<tr><td>N2 supplement 100x</td><td>Gibco™</td><td>17502048</td><td>1x</td></tr>
<tr><td>n-Acetyl Cysteine</td><td>Sigma</td><td>A9165</td><td>1,25 mM</td></tr>
<tr><td>EGF</td><td>Gibco™</td><td>PMG8043</td><td>50 ng/mL</td></tr>
<tr><td>IGF-1</td><td>Biolegend</td><td>590906</td><td>100 ng/mL</td></tr>
<tr><td>Prostaglandine E2</td><td>Tocris</td><td>2296</td><td>10 nM</td></tr>
<tr><td>Gastrin 1</td><td>Sigma</td><td>G9145</td><td>100 nM</td></tr>
<tr><td>A83-01</td><td>Tocris</td><td>2939</td><td>500 nM</td></tr>
<tr><td>SB202190</td><td>Sigma</td><td>S7067</td><td>3 µM</td></tr>
<tr><td>Primocin 500x</td><td>Invivogen</td><td>ant-pm-1</td><td>1x</td></tr>
<tr><td>LY27632</td><td>Sigma</td><td>Y0503</td><td>10 µM</td></tr>
</table></td></tr>
<tr><td>séquence et durée des traitements</td><td>N.A.</td></tr>
<tr><td>sequence and duration of treatments
traitements</td><td>N.A.</td></tr>
<tr><td>Nature and treatment of the supports
Nature et traitement des supports</td><td>N.A.</td></tr>
<tr><td>Seeding conditions
Conditions d’ensemencement</td><td>Yes
1000 to 500 000 dissociated cells in 25 µl of Matrigel.</td></tr>
<tr><td>Frequency of media changes
Fréquence des changements de milieu</td><td>The medium was changed every 2–3 days</td></tr>
<tr><td>Quality control of differentiation process (e.g. morphology, mat homogeneity, max and min confluence, proliferation, functional test, monitoring of</td><td>N.A.</td></tr>
</table>

markers, possibly sorting, mortality rate) contrôle qualité de la différenciation	

Generation of organoids (3D): specificities Génération des organoïdes (3D) : spécificités

Matrix culture Culture en matrice

nature of the matrix (matrigel, hydrogels, hyaluronic acid, human decellularized matrix etc. ....) nature de la matrice	Yes Matrigel corning ref 356255
Matrix concentration concentration de la matrice	Yes 5 mg/ml
preparation method (temperature, polymerization time, drop or layer structure, etc.) modalité de préparation	Yes Dispense 25 µl of the cells-Matrigel suspension into the center of each well of a 48-well plate using a 200-µl pipette. -Place the plate in a 37 °C incubator for 10 min to solidify the Matrigel.
Seeding density per matrix volume unit densité d'ensemencement	Yes 1000 to 500 000 cells in 25 µl of Matrigel.
volume and number of drops of matrix per unit area in the culture medium	Yes 1 drop 25µl matrigel / well in 48 wells plate
amount of medium depending on the size of the well quantité de milieu en fonction de la taille du puits	Yes 300 µl/well in 48 wells plate
Matrix dissociation method for organoid recovery méthode de dissociation de la matrice pour la récupération des organoïdes	Yes PBS 1h at 4°C
method of dissociation of organoids for their expansion méthode de dissociation des organoïdes pour leur expansion	Yes -Aspirate the medium and add 500 µl of TrypLE Express supplemented with 10 µM Y-27632 to each well. Scrape the Matrigel off of the bottom of the wells with a 1,000-µl pipette and collect the organoids, together with the Matrigel, into a 15-ml low-binding centrifuge tube. -Place the tube at 37 °C for 5 min. -Pipette it briefly with a 1,000-µl pipette. For maintenance passage, 10–15 rounds of pipetting is sufficient. Excessive dissociation results in diminished recovery of the organoids.

	-Add Complete AdDF medium up to 10 ml, and centrifuge it at 4 °C at 1200 rpm for 5 min. -Aspirate the supernatant and suspend the pellet with Matrigel.

Culture on solid 3D support (example: mineral support for bones, support for liquid-gas interfaces) *Culture sur support 3D solide*

Preparation method of the 3D solid support (composition of the medium to be freeze-dried, freeze-drying conditions) Méthode de préparation du support 3D solide	N.A.
Seeding method Méthode d'ensemencement	N.A.
nature of biocompatible materials (PDMS, COC, Silicon.....) nature des matériaux biocompatibles	N.A.
Chip design (provide a map) design de la puce	N.A.
physical characteritics caractéristiques physiques	N.A.

Suspension culture (self-organization) *Culture en suspension* (auto-organisation)

Type of container Type de contenant	N.A.
if applicable, the nature and protocol of the agitation nature et protocole de l'agitation	N.A.
nature and concentration of matrices (if applicable) nature et concentration des matrices	N.A.

Culture including multiple cell types *Culture incluant de multiples types cellulaires*

sequence of co-culturing and adaptation of co-culture media séquence des mises en co-culture	N.A.
proportion of cell types proportion des types cellulaires	N.A.

C) ORGANOID CHARACTERIZATION CARACTÉRISATION DES ORGANOIDES

The detailed characterization is project dependent, however some standards emerge

Morphology/structure	
Appearance, size, shape [circularity, tubularity, regularity of contour (budding)], Aspect, taille, forme	N.A.
opacity/réfringency opacité/réfringence	N.A.
intra and inter-organoid homogeneity, homogénéité	N.A.
Expected morphological, architectural and ultrastructural features, organization of cell types (identity, proportions, distribution). Particularités morphologiques	N.A.

Molecular Characterisation *Caractérisation moléculaire*	
elements of genomics, transcriptomics, metabolomics, proteomics, éléments de génomique, transcriptomique, métabolomique, protéomique,	No
expected specific molecular markers, epigenetic characteristics marqueurs moléculaires	N.A.

Function *Fonction*	Spécifique à chaque organoïde
Qualitative and (if possible) quantitative functional characteristic Caractéristique fonctionnelle	no
Response to treatments (pharmacological, chemical, physical, hormonal.....) the treatment protocol, and evaluation (quantitative or qualitative) of the response are described Réponse aux traitements	N.A.

Traceability, organoid drift *Traçabilité, dérive des organoïdes*	
Traceability of components (batches, suppliers etc... environments,	Yes

complements) Traçabilité des composants	
Traceability of conditioned media (drift of cells used for conditioning, control of lines as for those at the origin of the organoid), control of at least one of the growth factors) Traçabilité des milieux conditionnés	no
Drift criteria (morphological, structural, functional, molecular....) specific to each organoid. Specify indices if applicable Critères de dérive	N.A.

Methods of oragnoids cryopreservation *Méthodes de cryoconservation*	*(PARTIE RESTANT À VALIDER)*
preparation method (dissociation/harvesting) of the cells/organoids to be cryopreserved méthode de préparation	Yes/no / N.A. **Organoids Freezing** Organoids are frozen after enzymatic dissociation. -Thaw the 3DGro freeze medium and store in ice. -Aspirate the medium and add 500 µl of TrypLE Express supplemented with 10 µM Y-27632 to each well. Scrape the Matrigel off of the bottom of the wells with a 1,000-µl pipette and collect the organoids, together with the Matrigel, into a 15-ml low-binding centrifuge tube. -Place the tube at 37 °C for 5 min. -Pipette it briefly with a 1,000-µl pipette. -Add Complete AdDF medium +Y27632 (1/1000) up to 10 ml, count cells and centrifuge it at 4 °C at 1200 rpm for 5 min. -Aspirate the supernatant and suspend the pellet with 3DGro freezing medium 1 106 cells/ ml of freezing medium.
Cryopreservation media (composition, volume per number of cells) Milieux de cryopreservation	Yes 3D GRO cell freezing medium (SIGMA)
freezing procedure (possible successive temperatures, duration at each temperature, final storage temperature) procédure de congélation	Yes 2 days at -80°C Then Samples are stored liquid nitrogen
quantity of cells per vial and at which passage quantité de cellules	Yes 4 10^5 cells
Thawing procedures (see ROCKi? addition)	Yes

Procédures de decongélation	Organoïde thawing -Incubate freezing ampoule 1 min at 37°C. -Cells were resuspended in 1 ml Complete AdDF medium +Y27632 (1/1000) and transfered into a 15-ml low-binding centrifuge tube. -Add Complete AdDF medium up to 10 ml, and centrifuge it at 4 °C at 1200 rpm for 5 min. -Aspirate the supernatant and suspend the pellet with Matrigel. Typically, one well of grown organoids can be passaged into 4–8 wells. Dispense 25 µl of the organoid-Matrigel suspension into each well of a 48-well plate. -Place the plate in a 37 °C incubator for 10 min to solidify the Matrigel. -Add 300 µl of organoid medium supplemented with 10 µM Y-27632 (2 days) to each well, and incubate it in a 37 °C incubator.

D) USE OF ORGANOIDS UTILISATION DES ORGANOIDES

Organoid for basic research *Organoide en recherche fondamentale*

Bioproduction of organoids: in addition to the above, required certification in force, approval number) Bioproduction d'organoïdes	Yes MACROSWITCH-IPC 2021-083

Organoids for bioproduction *Organoïde en bioproduction*

Bioproduction from organoids Bioproduction à partir d'organoïdes (concept d'usinoïde)

In addition to the above, Technical Specifications (changes from the reference organoid) Spécifications techniques	N.A.

Organoid in preclinical research (pharmacology, toxicology, ...) *Organoïde en recherche préclinique (pharmacologie, toxicologie, ...)*

Functional similarity criterion between the organoid and the mimicked organ (battery of controls to be performed with target values) similarité fonctionnelle	N.A.
Number of usable passages Applicable for: Preclinical development of a drug candidate (IND file) using organoids nombre de passages exploitables	5 to 10
Number of usable passages Applicable for : Validation of a care protocol (specific patient) on a cohort: choice of a therapy Validation d'un Protocole de soin	Yes/no / N.A.

Organoid in clinic (personalized, predictive and regenerative medicine, transplantation)
Organoïde en clinique

GMP certification, total traceability of the components, qualification of the components for Domain 1: Care protocol (specific patient) (validation of the protocol of use of the organoid for the orientation of the therapeutic choice) - Criterion of similarity between the organoid and the biopsy Certification GMP	N.A.
GMP certification, total traceability of components, qualification of components for Domain 2: Use in regenerative medicine (same as cell and tissue therapies) - Functionality criteria, safety (Derivation of biological material and evaluation of the risk of cancer) Certification GMP	N.A. N.A.

* : gestion de la COnservation D'Éléments du COrps Humain