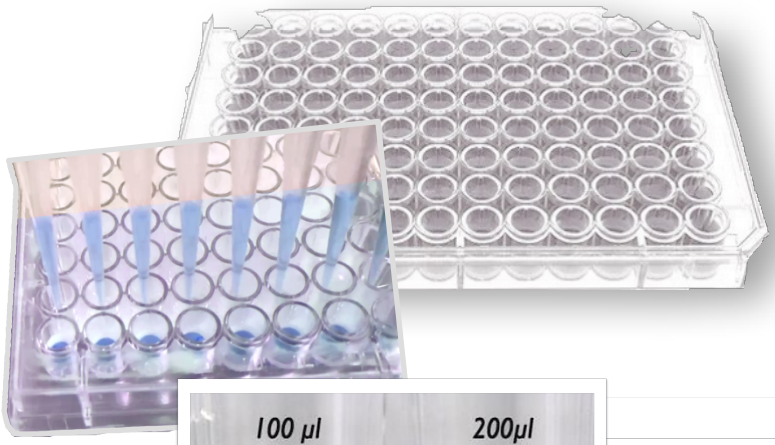
Pearl BioSystem SAS - France - www.pearlbiosystem.com
Product for research use only. Not for diagnostic.

An innovative methodology to optimize in vivo testing the 96-well Universal 3D *in perlo*™ Plate.

in silico → *in vitro* → ***in perlo***™ → *ex vivo* → *in vivo*



Fully aligned with 3R guidance, ***in perlo***™ enhances and optimizes candidate selection rapidly, securely, and reliably.

Pearl Bio System

Transforming Cellular Research with Groundbreaking 3D Culture Innovations!

An Innovative *in vitro* 3D Cell Culture Technique of Tumor Development

- **Accurate Cancer Modeling:** Replicates tumoroid shapes for better research accuracy.
- **Accelerate Drug Discovery:** speeds up the development of new drugs.
- **Better Translational Research:** Bridges the gap between bench and bedside.

Why Choose *in perlo*™ ?

- **Advanced 3D Model:** mimics the tumor shapes, outperforming traditional 2D and 3D gel-based methods.
- **Enhanced Predictability:** Provides reliable data for drug testing.
- **Cost-Effective & Scalable:** Fits into any lab's workflow with ease.

Limitless Applications:

- **Drug screening** tailored to individual patients.
- **Studying tumor biology** and monitoring **metastatic escape.**
- **Immuno-oncology** with **easy co-culture** applications**.**

Key Features:

- **Easy-to-follow protocols,** for both **manual** and **automated** processes.
- **Highly reproducible** for consistent results**.**
- **Supports various endpoint analyses.**

Any Cell Phenotype

Compatible with a wide range of cell types for diverse studies.

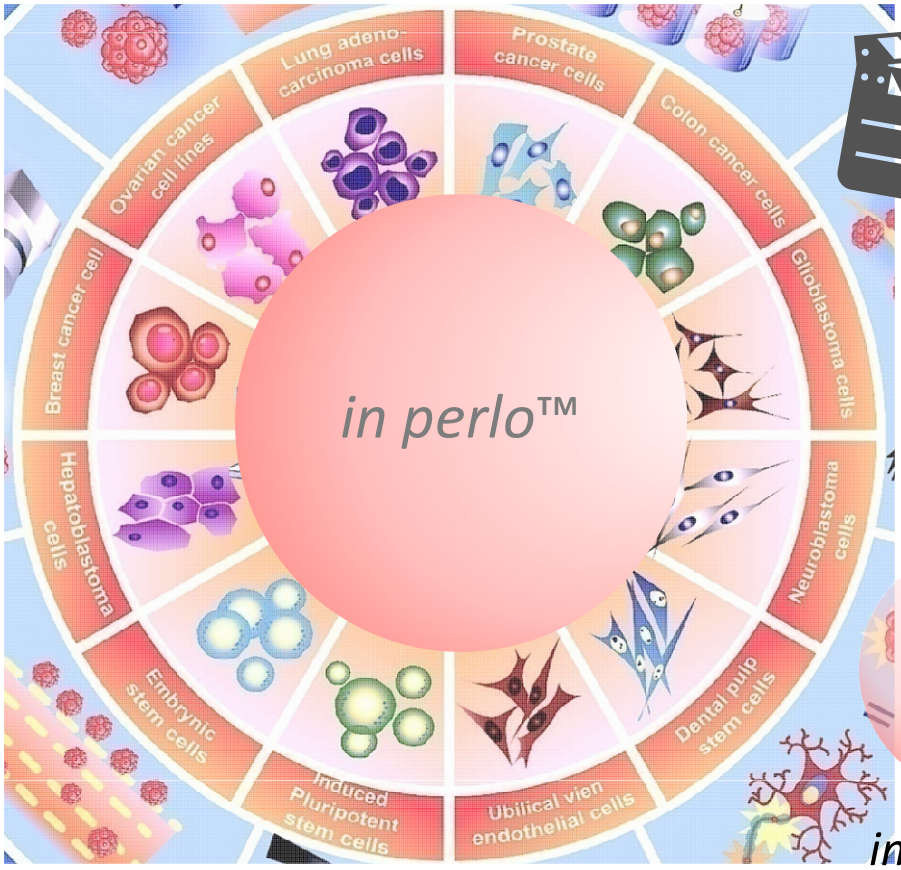
Any Molecular Type

Adaptable to investigate various molecular pathways and markers.

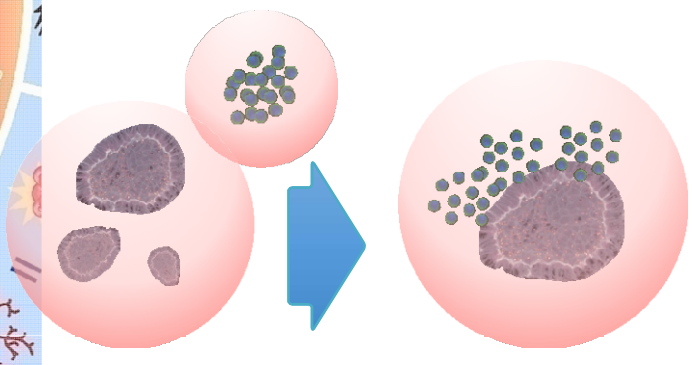
Any Application

Versatile for drug screening, tumor biology, immuno-oncology, and more.

This flexibility ensures *in perlo™* fits seamlessly into your research, no matter the focus.



in addition THE special
in perlo™
genuine feature
PEARL - FUSION



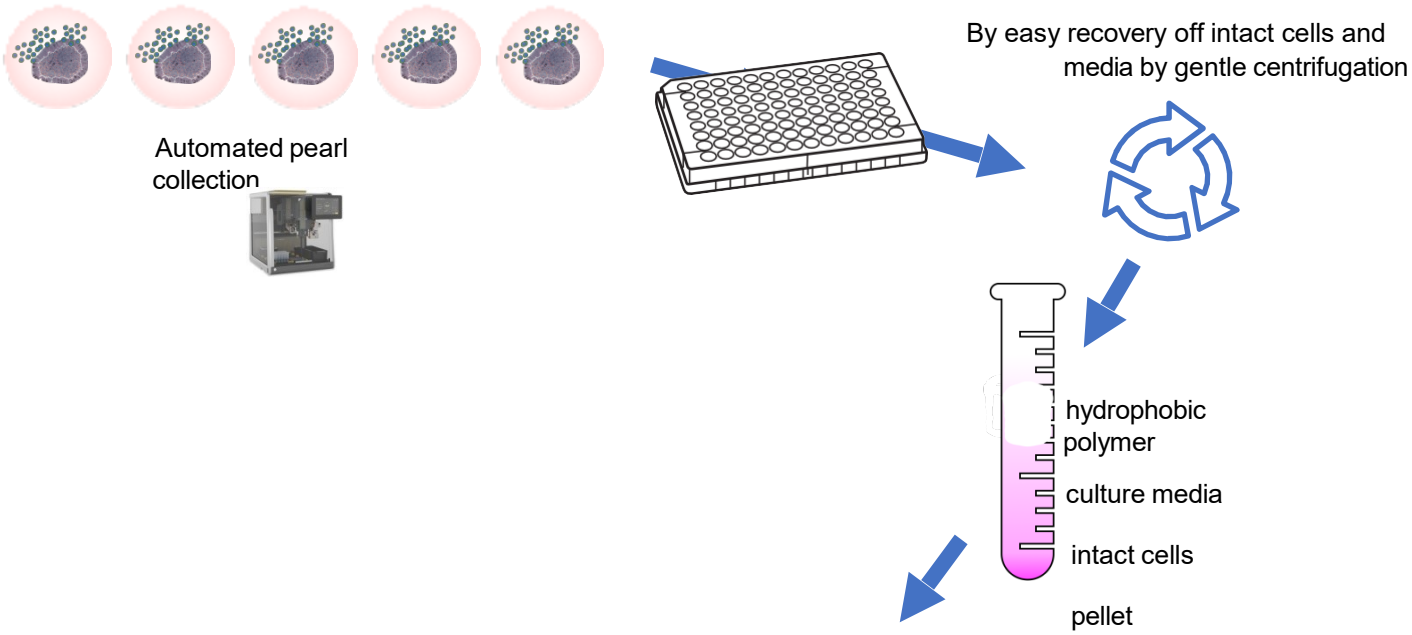
in perlo™ co-culture generation

≠ cell line phenotypes

- Cell-cell interaction
- Molecule injection
- Combination of molecules
- Co-culture and fusion

- Antibodies
- DNA
- RNA
- Synthetic molecules (biomimetics and peptides)
- Your natural extracts under development.

Multiple end-points possibilities after *in perlo*™ assay



Molecular analysis to be performed on the intact cells
i.e. mass spectra, FPLC, western blot, ELISA, Immunocytochemistry etc…

1. Genomics
2. Transcriptomics
3. Epigenomics
4. Proteomics
5. Metabolomics
6. Lipidomics
7. Glycomics
8. Secretomics (Secretome Analysis)
9. Phosphoproteomics
10. Cellular Imaging-Based Analysis
11. Functional Genomics
12. Post-translational Modifications

Product Catalog for the *in perlo*™ assay

Our Tailored Solutions

We have developed a **ready-to-use kit** that enables analysis using a 96-well plate format. It allows testing of either **4 molecules on a single cellular phenotype or 2 molecules across two phenotypes**, as outlined below.

Our technology provides **an efficient and cost-effective alternative** for screening your promising molecules during the preclinical phase, offering a competitive tool before committing to expensive clinical trials.

You could also use our ***in perlo***™ kit in parallel of your current experiment goal (tox…) to collect more raw materials (cells) to do **more multiple-end-point read-out.**

STERILE for Multiple Analysis	Quantity	Reference
Universal 3D cell culture in perlo assay plate - 96W - Sterile	1	U3DCC-96W-ST-1
Universal 3D cell culture in perlo assay plate - 96W - Sterile	5	U3DCC-96W-ST-5
Universal 3D cell culture in perlo assay plate - 96W - Sterile	10	U3DCC-96W-ST-10
Universal 3D cell culture in perlo assay plate - 96W - Sterile	25	U3DCC-96W-ST-25
Universal 3D cell culture in perlo assay plate - 96W - Sterile	50	U3DCC-96W-ST-50

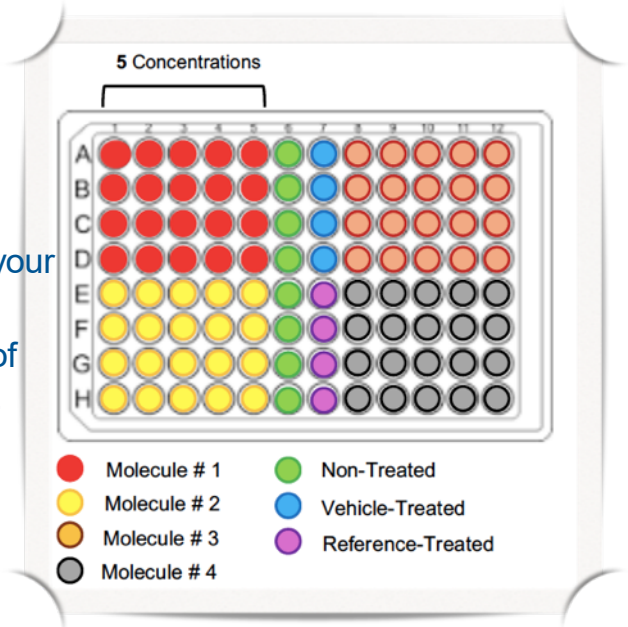
To plan your experiments, determine the number of *in perlo*™ kits needed based on:

M(i): number of molecules to be tested.
P(i): number of phenotypes to be analyzed.

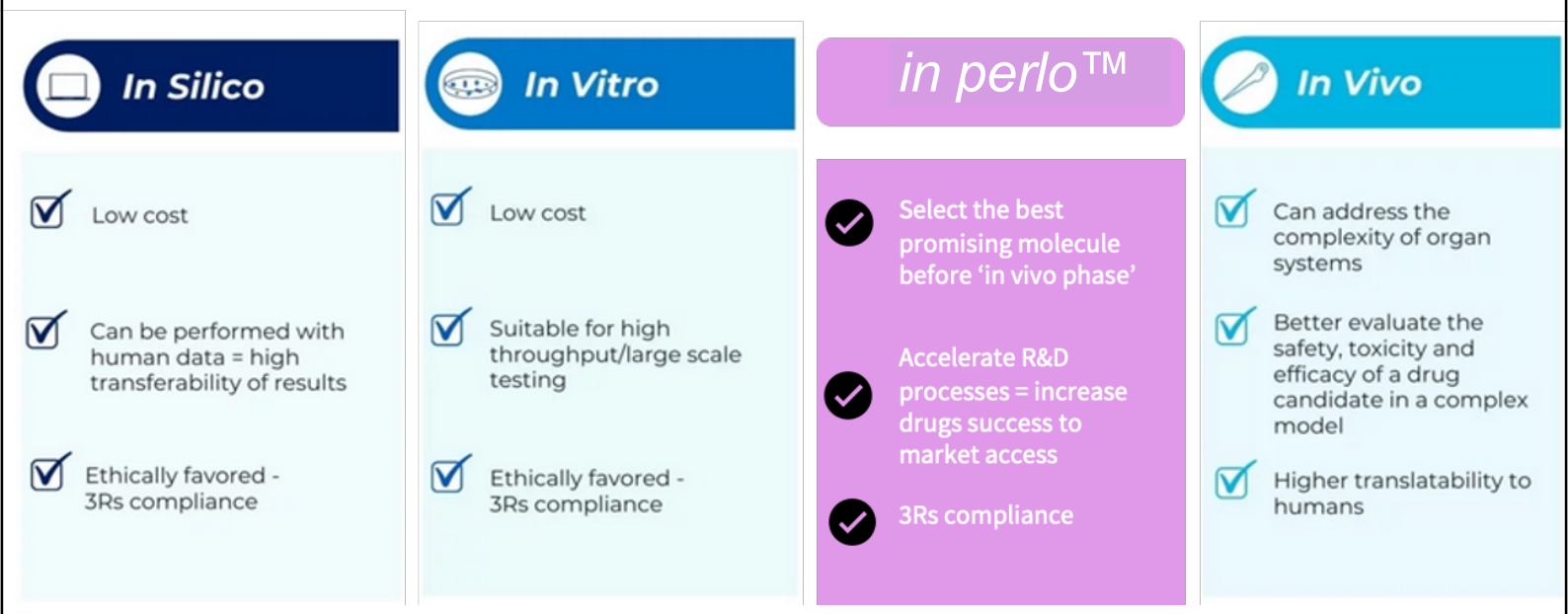
This will ensure you have the right amount of kits to meet your experimental requirements efficiently.

For multiple endpoint readouts, let us know the volume **V** of samples required as each pearl provides 100 µL of sample.

Calculate the total number of *in perlo*™ plate needed:
number kits = **M(i)**/4 x **P(i)** x (**V** sample of 100 µL)



in perlo™ positioning for compound screening

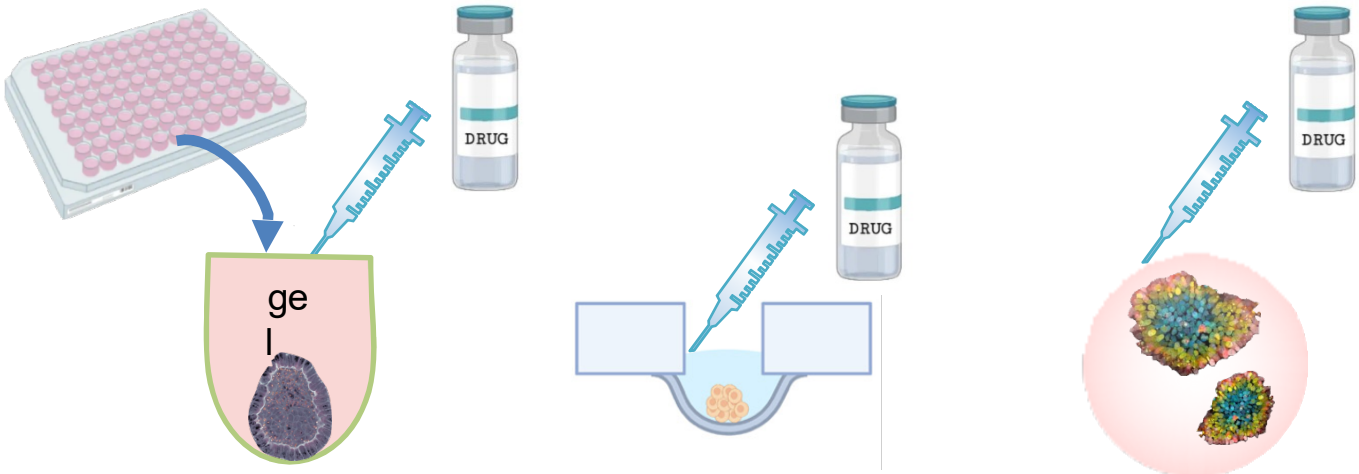


In perlo™ features	*In perlo*™ benefits	Scientist benefits
Use only liquid media	same as the one used in classic 2D culture	Secure the scientist to easily convert his current phenotype model
Pearl are optically transparent	Compatible with any reverse microscope devices for HCS	Easier integration of the *In Perlo*™ read-out
Possibility to recover treatment molecules	for translational analysis such as proteomics	More insights molecule metabolism
Better diffusion of the molecules to the cells	excellent bioavailability	Closest to the real in-vivo conditions
No need of enzymes to dissociate the scaffold from the tumoroid	Reduces steps in the *In perlo*™ protocol	Automation faster
Only 0.001 g compound vs 15 g	No possibility to characterize the molecule & Samples easy to ship	Secure the confidentiality of the molecules (truly in Pharma - Biotech - CRO - CDMO)
Compatible with hydrophobic and hydrophilic compounds	Multiple applications	Global market approach : DD – Dermo-cosmetic – NutriSciences… Theragnostic
No gel	No need to control the temperature during the 3Dculture process	Automation steps easier – lower cost per assay phase development
Only 3 steps to generate the pearl	Faster assay phase development at lower cost	Increased throughput rate and help to select earlier most promising compounds

Pearl Bio System

Pearl BioSystem Technology: a boost for your R&D funding

Many 3D Culture Systems Exist
What Makes *in perlo*™ Truly Revolutionary?



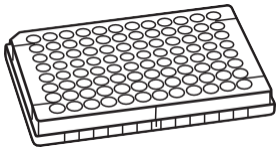
Low adhesion wells

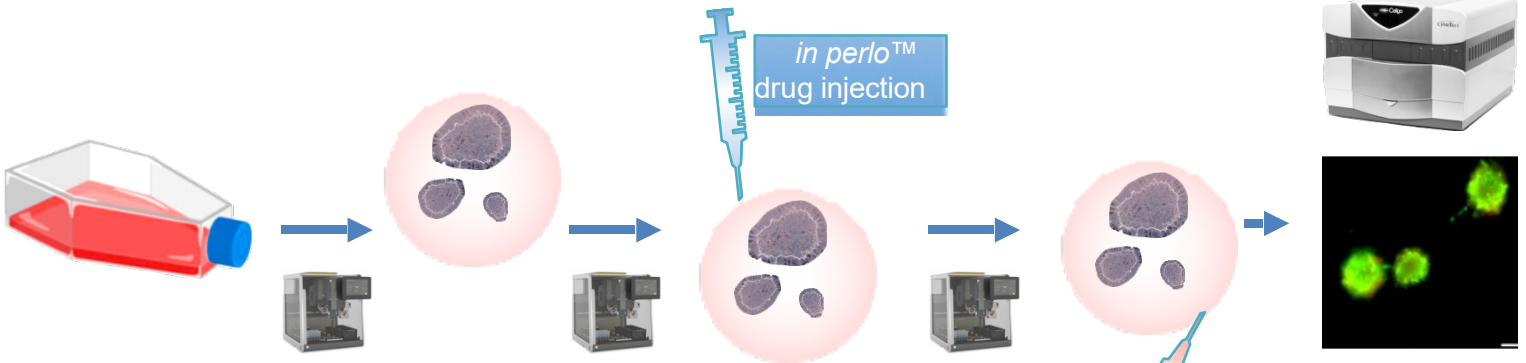
hanging drop

in perlo™

3D culture screening techniques

Automated workflow for screening





human cancer cell lines

in perlo™ tumoroid formation

in perlo™ tumoroid culture

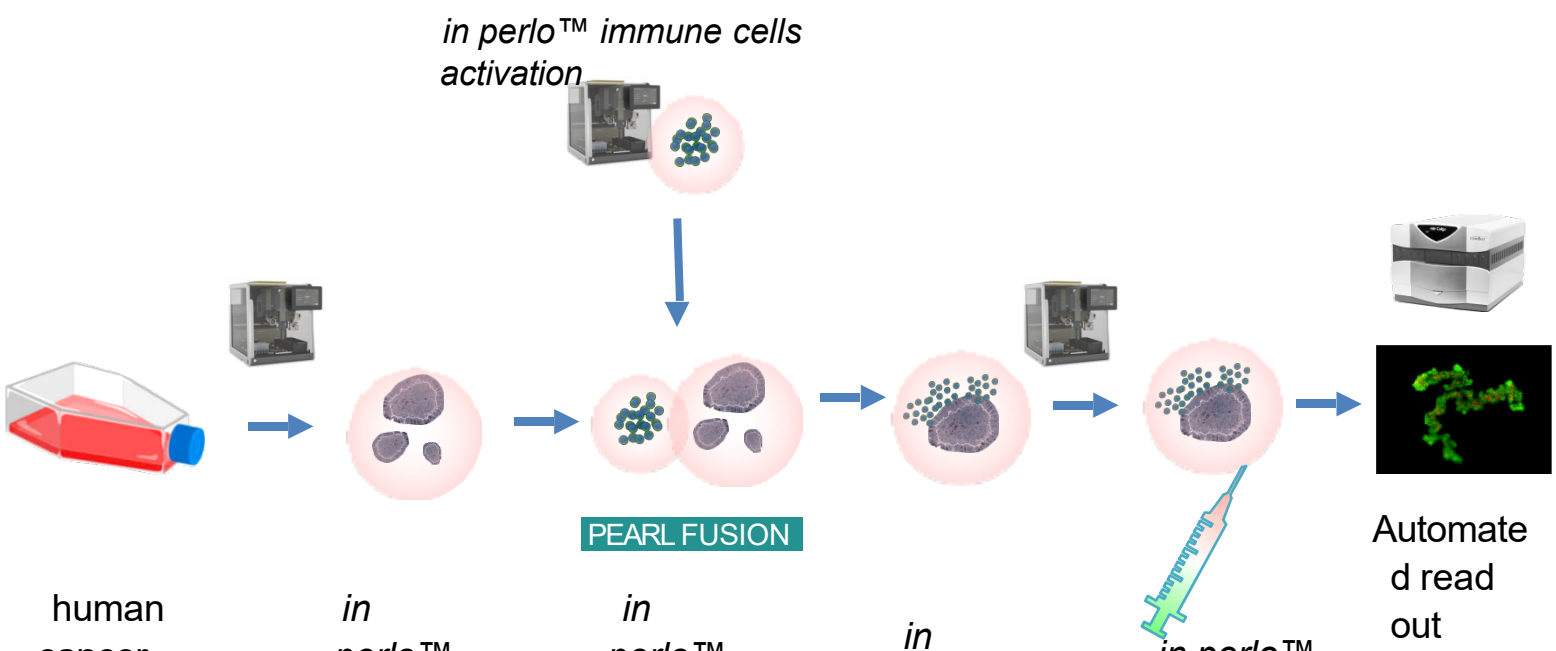
in perlo™ fluo labelling

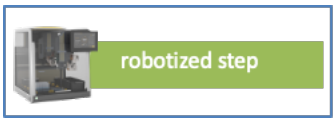
Automated read out

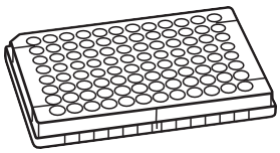
robotized step

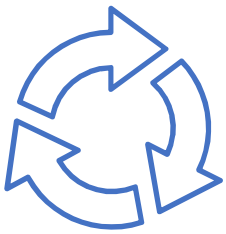
Easy recovery off intact cells and media by gentle centrifugation

Workflow for immune stimulation Horizontal co-culture



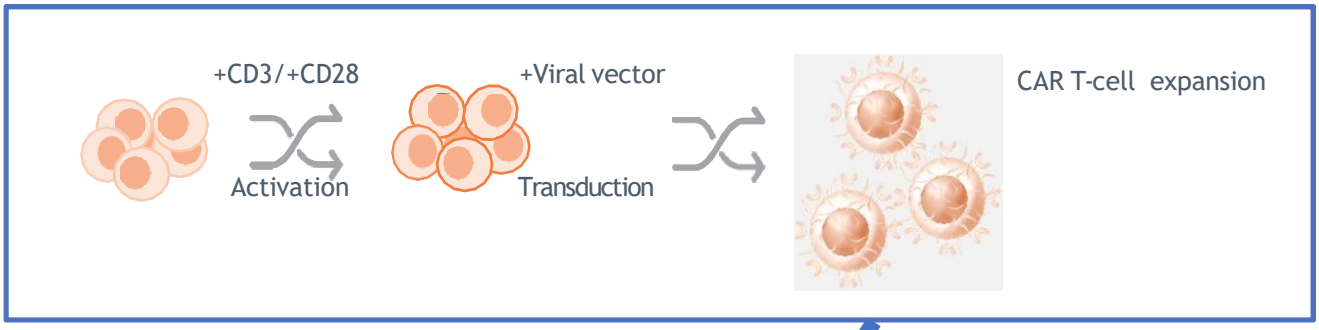


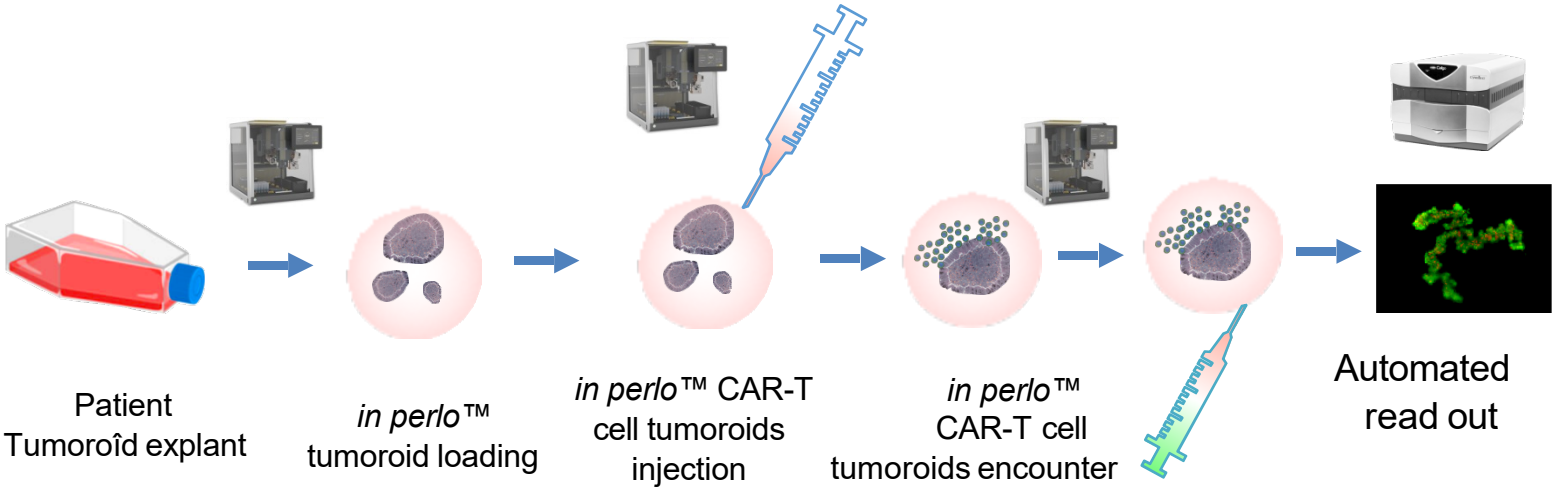




Easy recovery off intact cells and media by gentle centrifugation

Workflow for immune stimulation Vertical co-culture applied to Car-T cell





Patient Tumoroîd explant

in perlo™ tumoroid loading

in perlo™ CAR-T cell tumoroids injection

in perlo™ CAR-T cell tumoroids encounter

in perlo™ fluo labelling

Automated read out

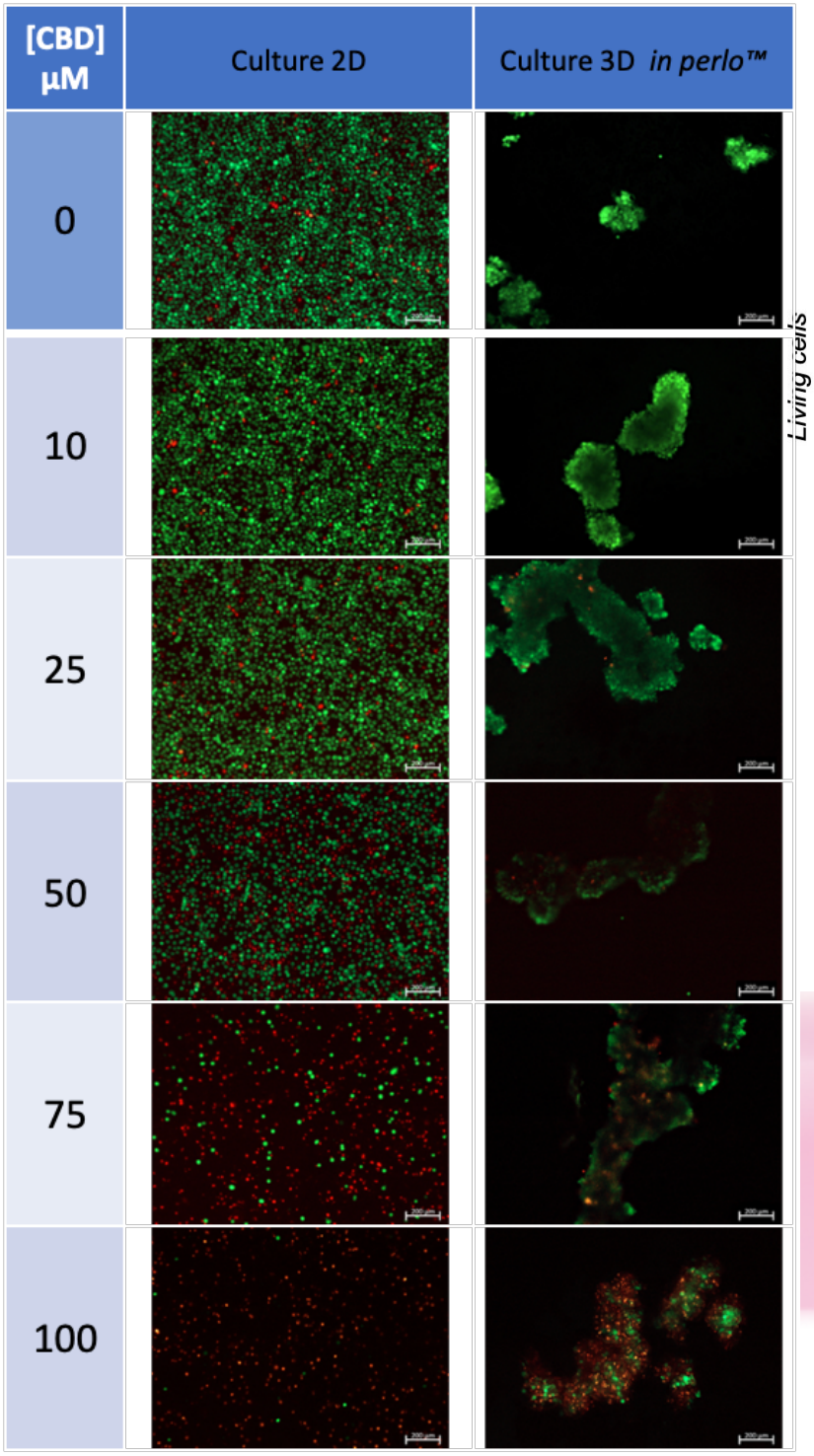
robotized step

Easy recovery off intact cells and media by gentle centrifugation

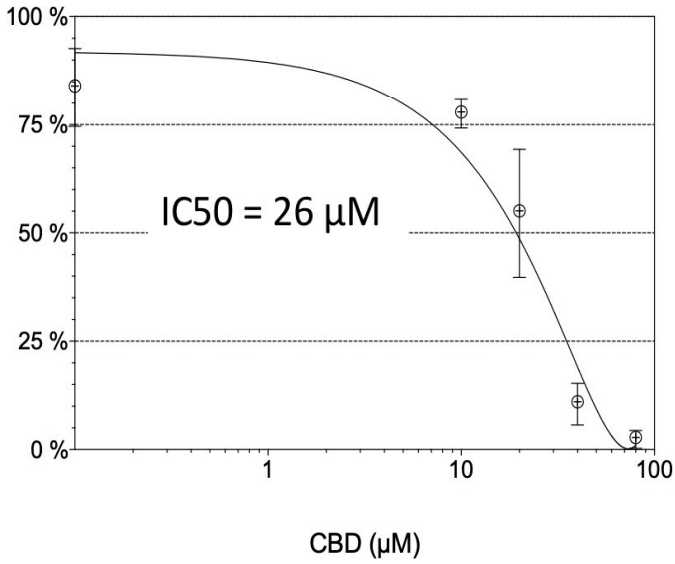
Molecular analysis to be performed on the intact cells
i.e. mass spectra, FPLC, western blot,
ELISA, Immunocytochemistry etc…

3D *in perlo*™ vs 2D direct comparison
Medicinal cannabis *in perlo*™ testing

In recent years, interest in *Cannabis sativa L.* has been rising as legislation is finally moving in the right direction.



IC_{50} of CBD on human chemo resistant **pancreatic** cancer cells AsPC-1 (ATCC® CRL-168)

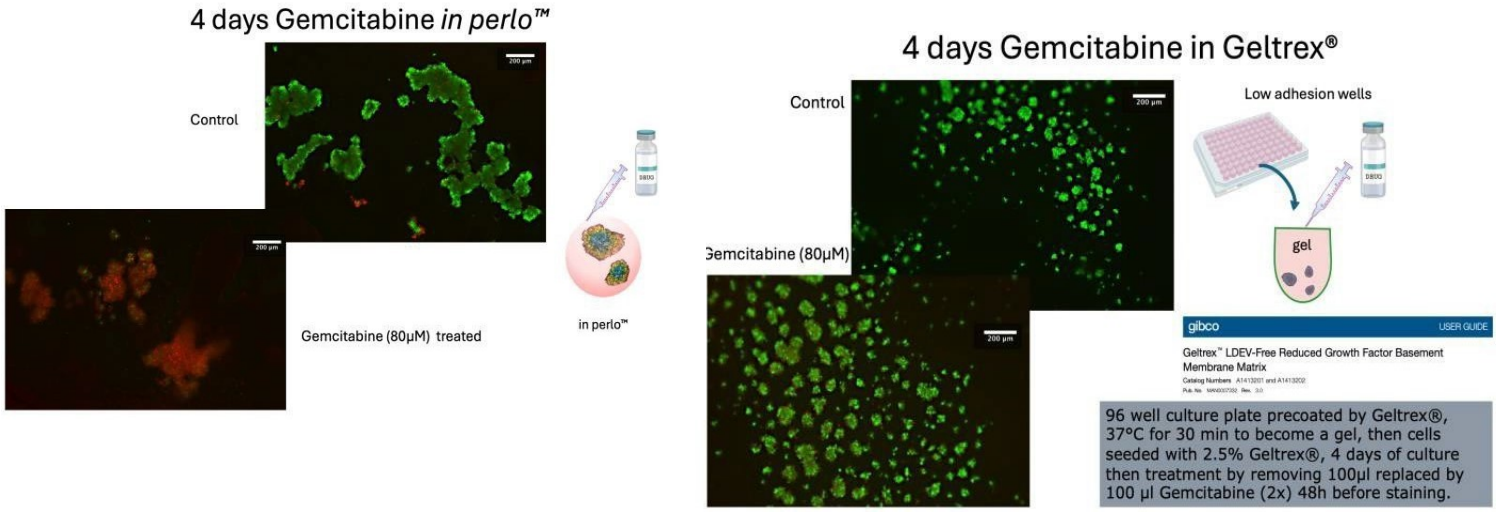


Our ***in perlo™*** data, recently , recently published in *Molecules*, supports the notion that CBD is the most effective bioactive molecule for anticancer activity among the components of this plant. **Molecules 2022,27,1214** https://doi.org/ 10.3390/molecules27041214

Dose-dependent cell viability after 72H CBD treatment in 2D and 3D culture on chemo resistant **colorectal** cancer cells

3D *in perlo™* vs. 3D Geltrex®
A Direct Evaluation

Most 3D cell culture models available are matrigels, microfluidic systems, aggregates such as "organ on a chip", or other polymers. These 3D models demonstrate various issues with performance, reproducibility, validation failures, bioavailability limitations, lengthy processes, and prohibitive costs.



Pancreatic cancerous tumoroids, generated for 24 h in liquid pearls, respond to 80µM treatment, after 48 hours incubation, equivalent to 80µM Gemcitabine used in therapy as a first-line treatment alone for pancreatic cancer.

The in *perlo*™ model method requires a minimal amount of liquid added to a hydrophobic powder that propagates spontaneously at the liquid-air interface. A typical seeding volume is 10-20µl in a single 70µl preformed pearl. The *in perlo*™ is optically transparent permitting fluorescence microscopy. We have significantly evaluated co-culture using our model and performed fusion of different phenotype cells originated from adherent or suspension type culture. Multiple drugs or treatment rounds are easily injected inside the *in perlo*™ model. We have demonstrated that no enzymatic recovery method is required for extraction so to proceed to any preferred measurement endpoint by simple centrifugation.

In 2017; Dubois and collaborators determined when comparing 2D versus 3D cell culture, that viability in the presence of increasing concentrations of chemotherapeutic agents i.e., cisplatin, docetaxel and epirubicin, was different, as Geltrex® spheroids were clearly less sensitive than monolayer cell cultures (4). The data shared here definitively demonstrate that in perloTM cultures are significantly more sensitive than in gel cultured spheroids. While 3D cultures and screening methods have enormous potential, certain elements still limit their use: the complexity of implementing these systems, administration costs, requisite culture duration, or the challenges of co-cultures effectuation.

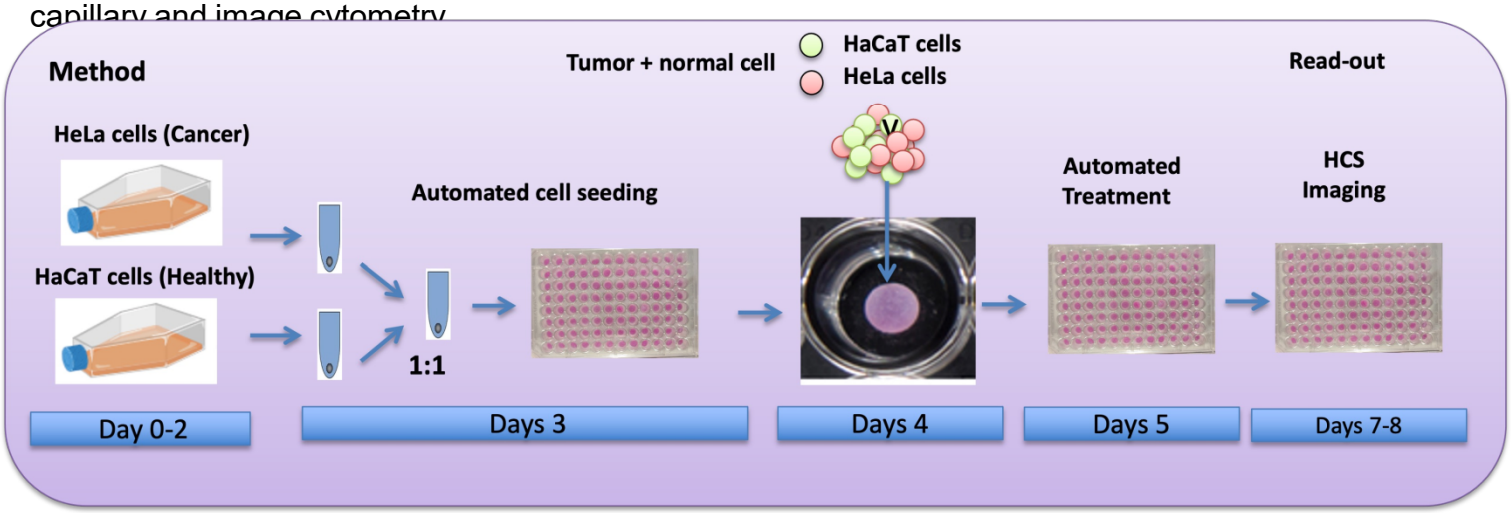
Moreover, in the context of 3D screening techniques, the bioavailability of molecules inserted into artificial gelatinous mediums is rarely satisfactory due to their evolution and sufficient downstream measurements are further negatively impacted by artificial enzymatic extraction buffers or physical processes. The use of the endlessly versatile *in perlo*™ culture model and method overcomes these limitations without exception.

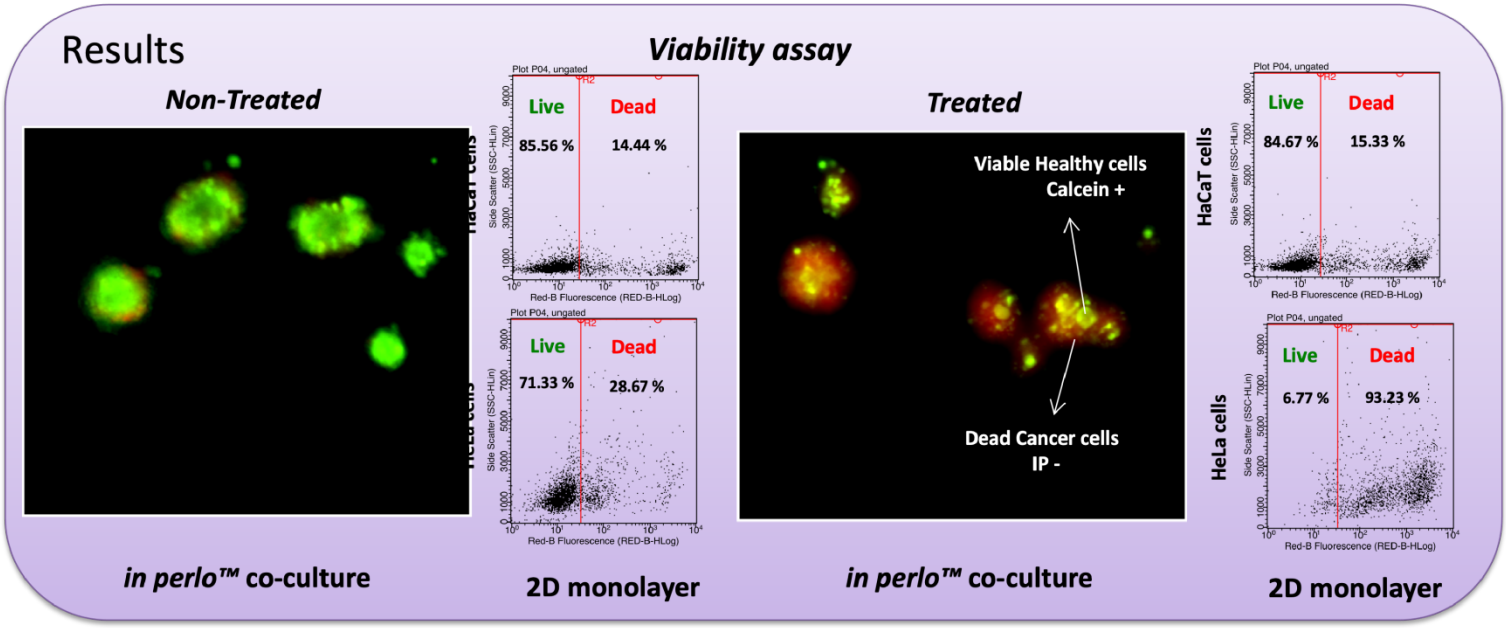
In perlo™ co cultures: healthy & cancerous cells

Experimental conditions

Healthy HaCaT keratinocyte cells and cancerous HeLa epithelial cells were used to explore their capacity to grow together even issued for two different culture media. Potent and selective anticancer molecules were applied to validate the suitability of the *in perlo*™ method for use in high-content screening (HCS) under co- culture conditions.

The Base medium for HaCaT and HeLa cells were EMEM and DMEM high glucose respectively. For co culture HaCaT and HeLa cells were mixed (1:1 ration) in medium consisting of a 1:1 mixture of both culture medium. After 4days of incubation followed by 2days of treatment, the viability assay were measured using Calcein AM and Propidium iodide. End-points were analyzed by capillary and image cytometry.





Conclusion

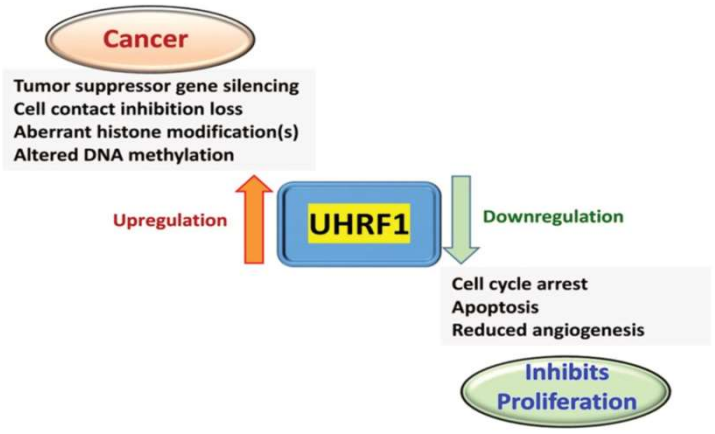
We here fully demonstrate the feasibility of co-culturing HaCaT and HeLa cells in a mixed medium, preserving the viability of both cell types over the incubation and treatment periods. **The application of a selective anticancer molecule under co-culture conditions effectively validates the *in perlo*™ method as a promising tool for high-content screening (HCS).** Our results provide significant insights into the behavior of healthy and cancerous cells **in shared environments**, supporting future advancements in cancer drug discovery and cell-based assays.

Finally, since the cellular populations and media can be easily recovered through centrifugation, **various other read-out endpoints can be performed**, such as genomics, proteomics, secretome analysis, and more.

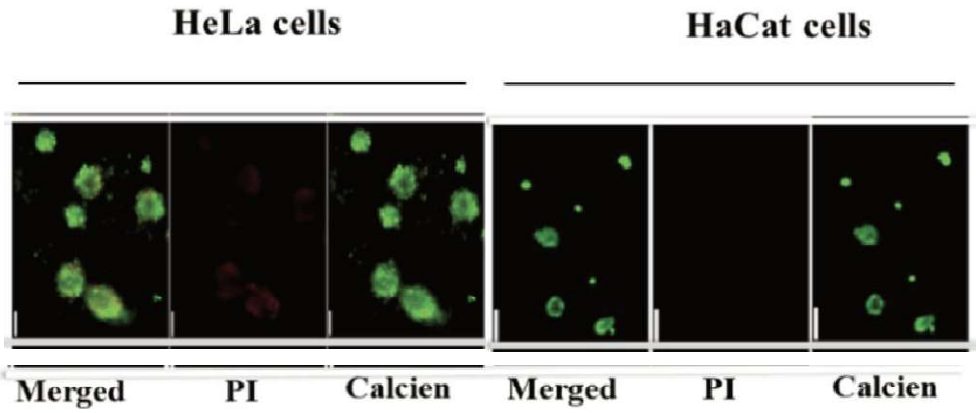
Example of 2 Different Readouts after in 3D *in perlo*™ culture

- UHRF1 inhibitors that target SRA domain decrease global DNA methylation levels in cancer cells.

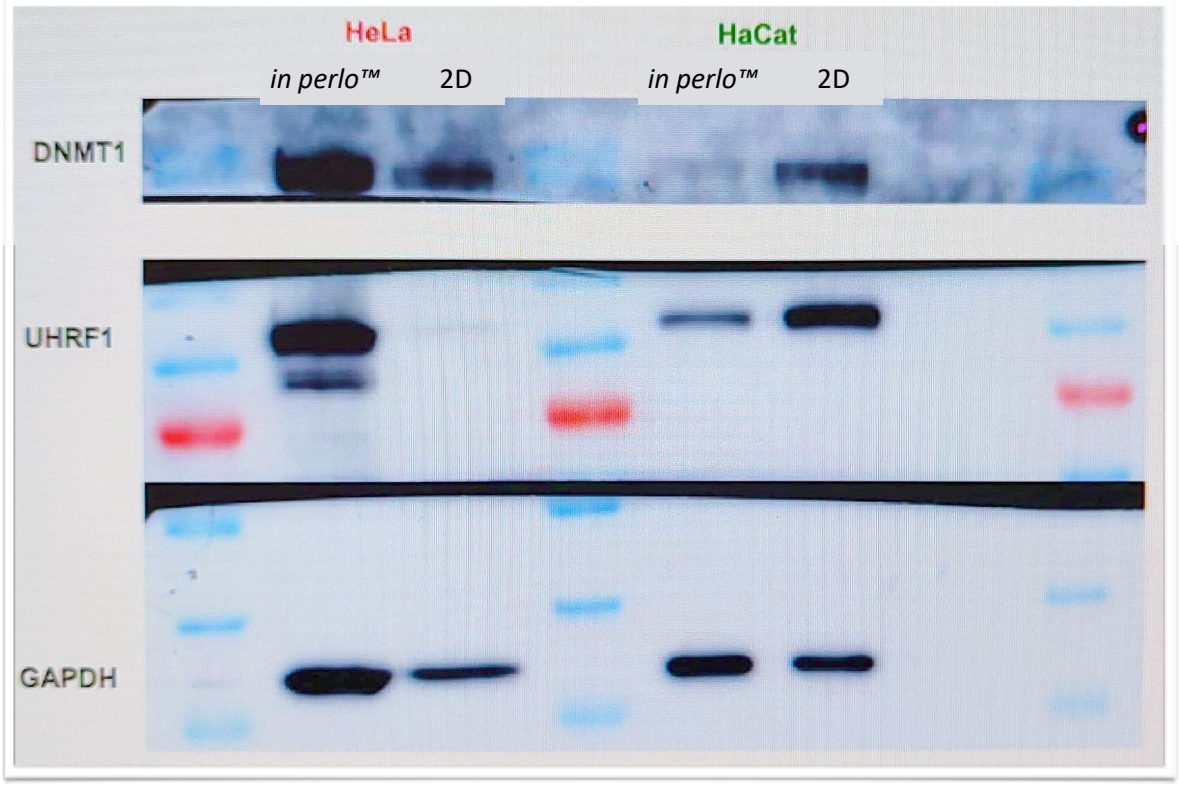
- Screening for UHRF1-SRA inhibitors will produce interesting leads towards anticancer therapeutics research as they can facilitate the design of selective anticancer drugs fostering high efficacy and low side effects in clinical settings.



- Fluorescent microscopy performed after *in perlo*™ cell culture (living cells green /dead cells red):



- Western Blot experiments performed by **pulling 10 pearls** per sample:



Conclusion. UHRF1 is a factor highly expressed in cancer cells grown *in perlo*™ but absent in 2D cultures for cancer cells only.