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# Single cell analysis for intrinsic stemness potential of cancer

Bernadette Neve\*, Aude Sivery°, Isabelle van Seuningen\*, Anthony Treizebre°, Audrey Vincent\*

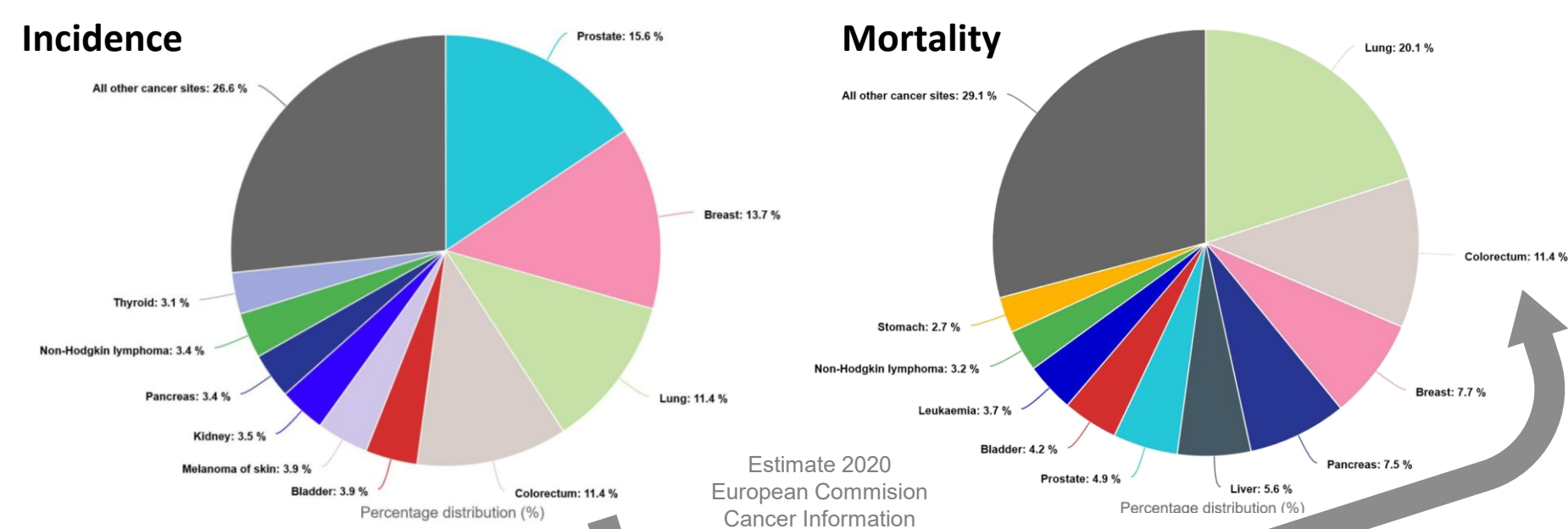
\*Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277

CANTHER – Cancer Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France

°Univ. Lille, CNRS, Centrale Lille Institut Junia, Univ. Polytechnique Hauts-de-France  
IEMN UMR CNRS 8520, 59652 Villeneuve d'Ascq, France.



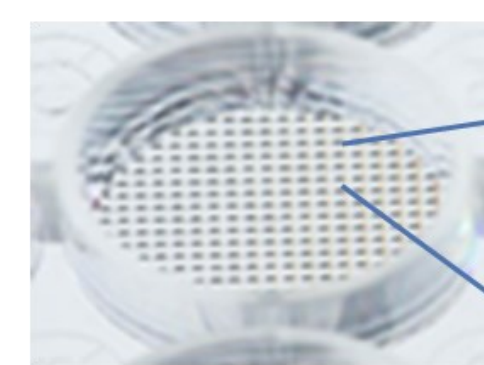
## Cancer prevalence in France



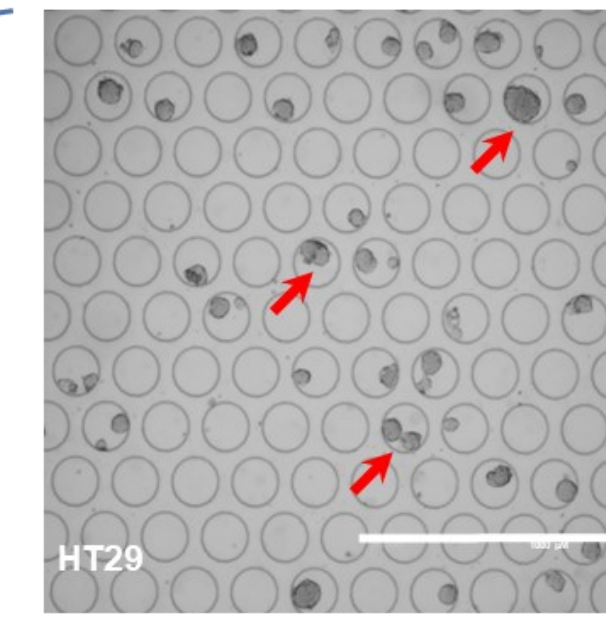
## Colorectal cancers (CRC)

- Poor survival rates are largely due to resistance to chemotherapeutic treatments and the development of metastatic lesions.
- More than 15% of patients have metastatic lesions at diagnosis and another 20% develop them. Metastatic CRC has a poor 5-year survival rate (14%)
- CRC is treated with targeted therapy, immunotherapy and/or surgery combined with chemotherapy (e.g. FOLFIRINOX: 5FU, folic acid, oxaliplatin and irinotecan)

## HT29 cells in custom micro-wells plates



7d culture



Individual cell ~15 µm  
Spheres ~ 150 µm

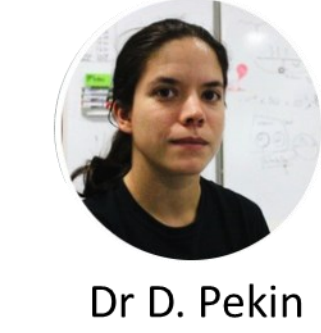
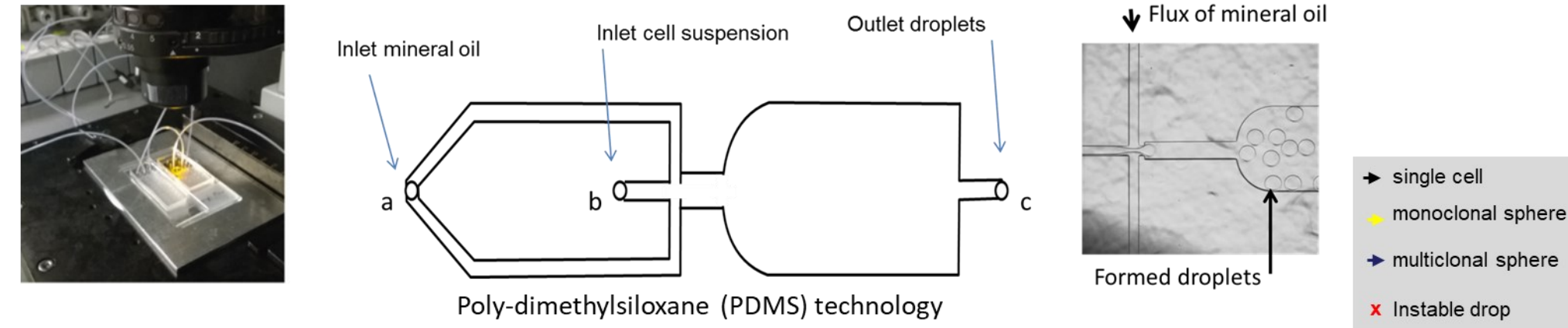
µwell = 175 µm

Seeding of a (diluted) cell suspension  
In a 6-well plate with a custom µwell  
Stencil.

- ❖ More than 1 cell / well, no single cell analysis
- ❖ Cell distribution sensible to vibrations

## HT29 cells in microfluidic droplets

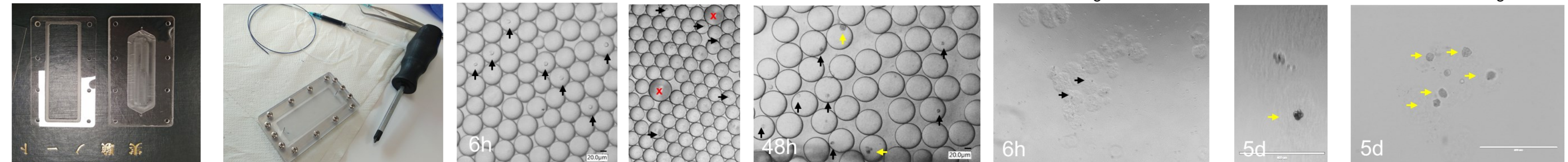
Separation of individual cells in medium-containing droplets followed by tumorsphere culture



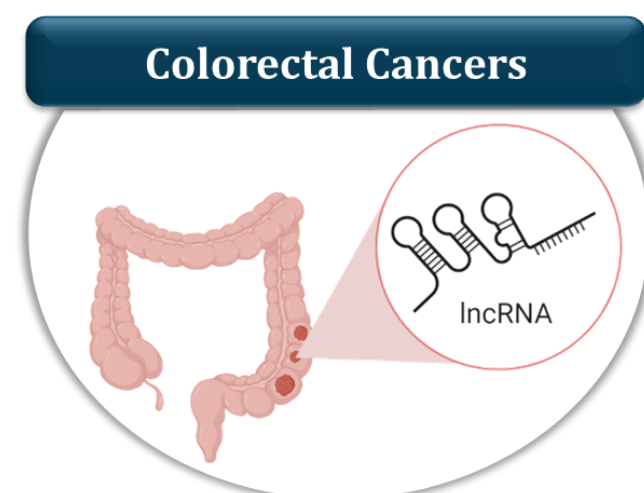
Dr D. Pekin

- ❖ Microfluidics setup in culture room
- ❖ Matrigel to stabilize drops
- ❖ Each experiment needed fine-tuning of flux, difficult to standardize using matrigel

Culture Incubation chamber

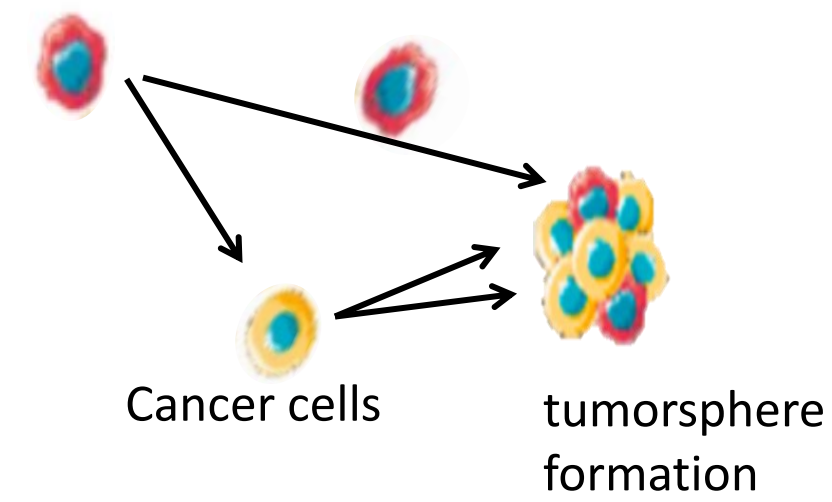


## Role of lncRNAs in cancer persistent cells

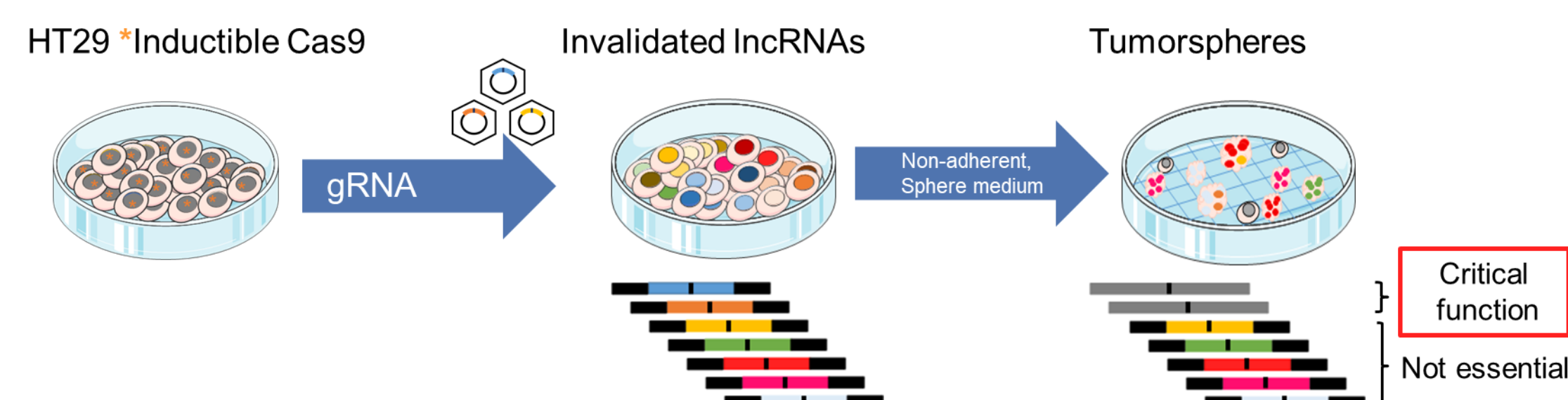


lncRNA	Fold-Change
GASS	4.1
LINC01133	-3.6
Linc-UBC1	2.9
CRNDE	3.6
H19	2.7
UCA1	2.6
MALAT1	2.0

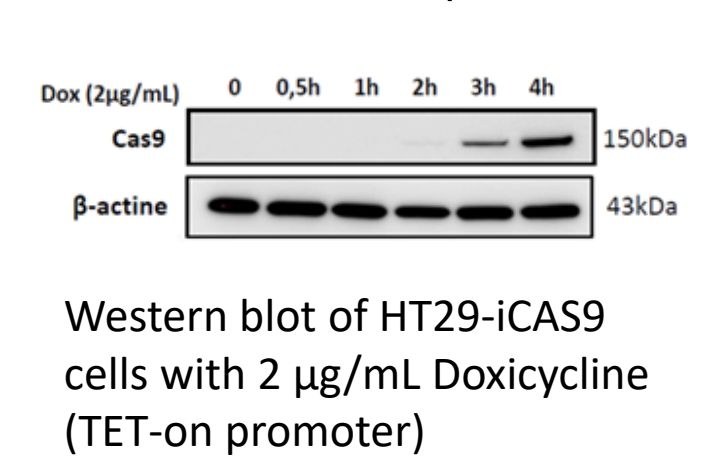
Cancer stem cells



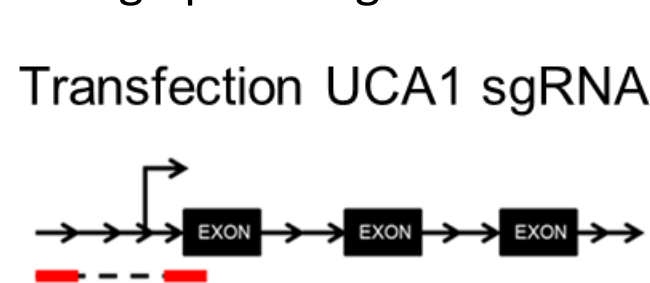
## Lentiviral CRISPR-Cas9 library screen



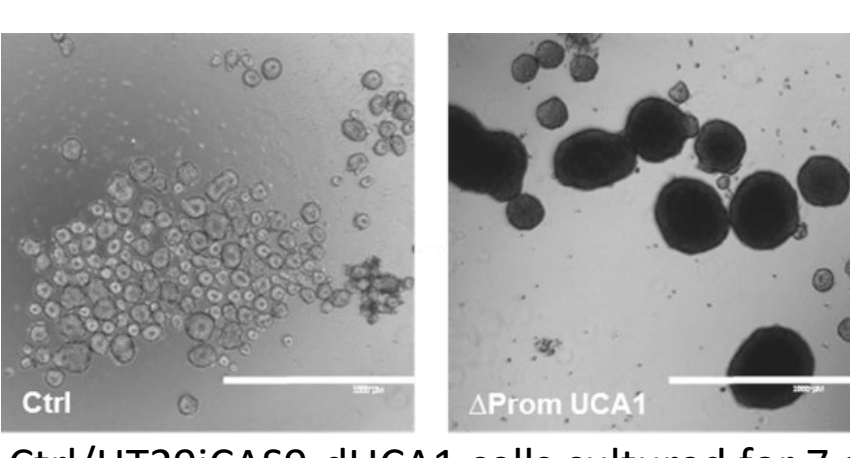
Induction of Cas9 protein



Design paired sgRNAs

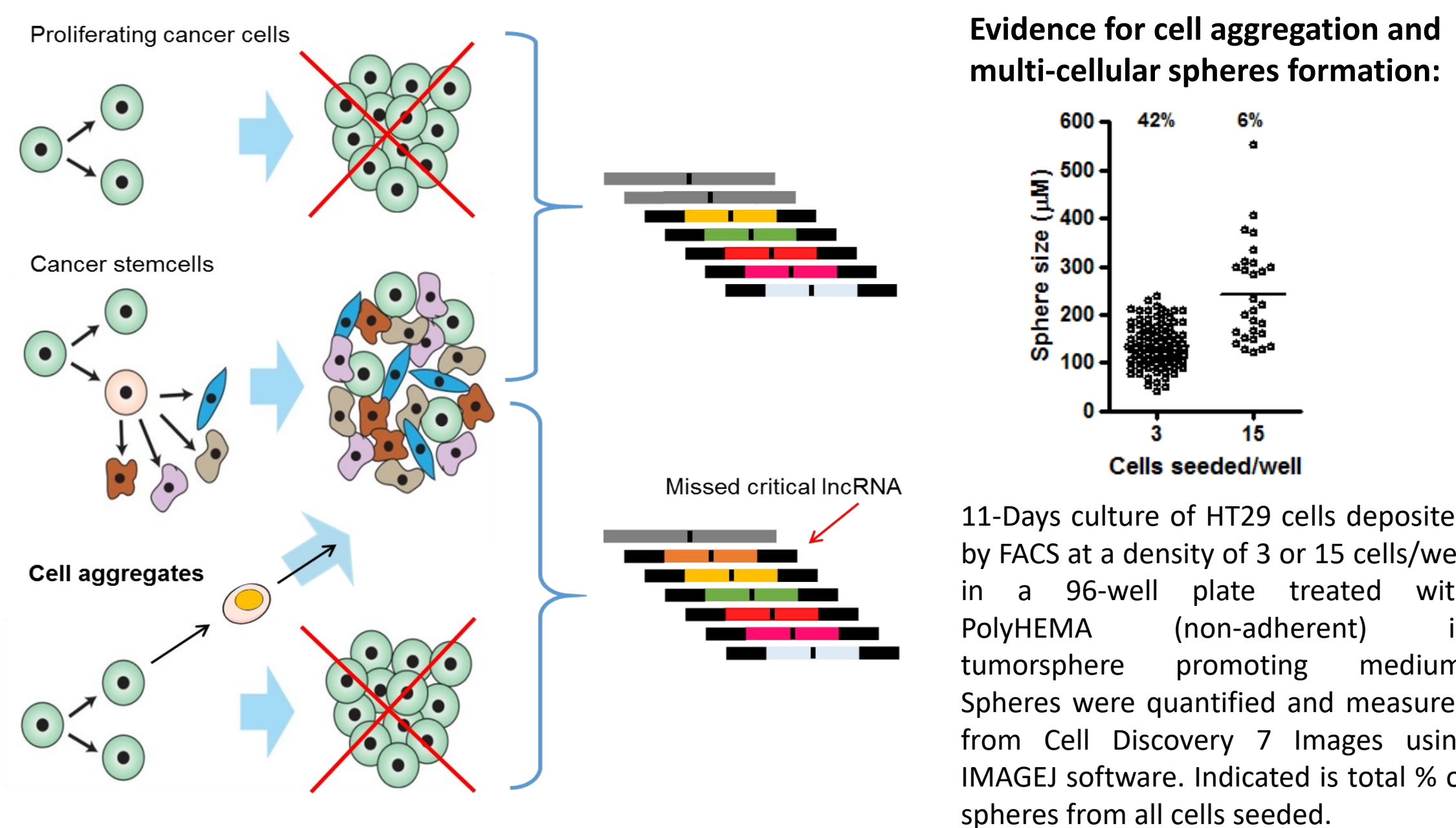


Non-adherent cell culture

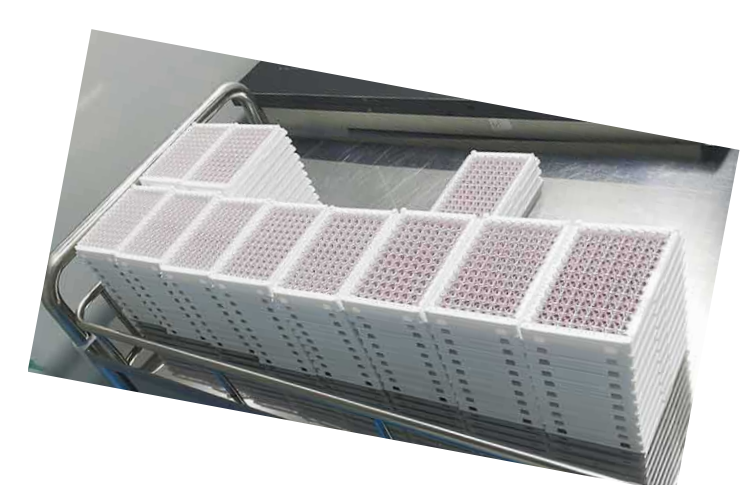


Ctrl/HT29iCAS9-dUCA1 cells cultured for 7 days in tumorsphere promoting conditions

## ➢ Need for single cell analysis

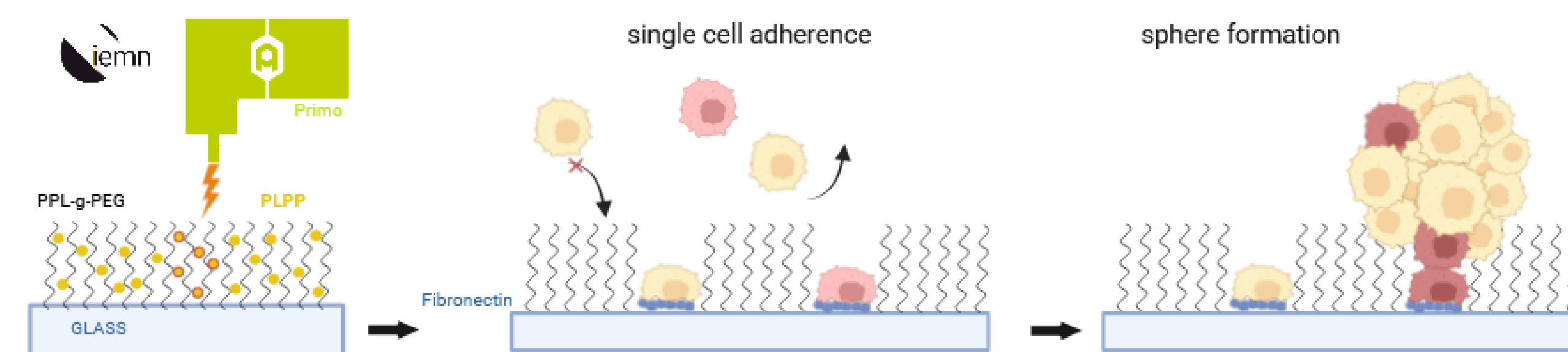


## ➢ Need for accessible high-throughput readout assay

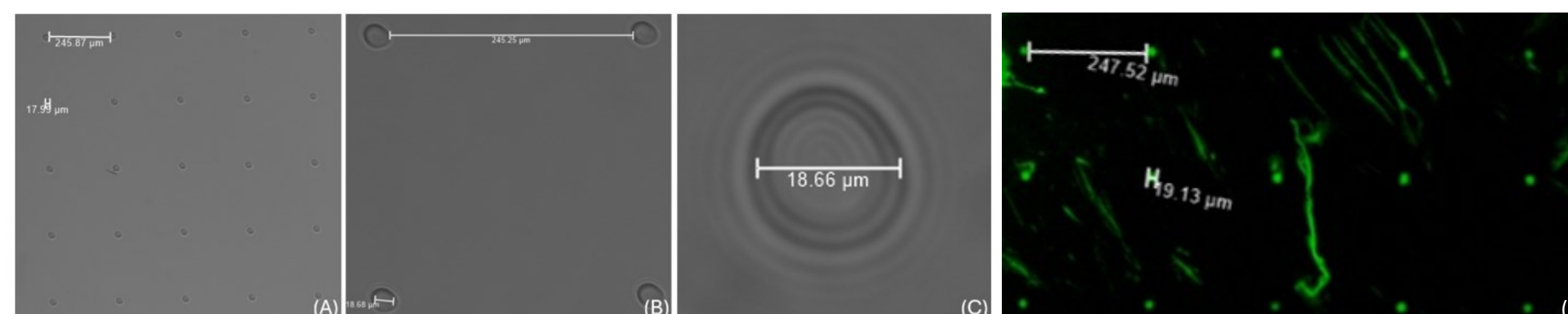


- FACS sorting 1 cell / well
- One sgRNA-pair / cell => low MOI, more cells to infect
- Negative screen: each sgRNA 1000x represented in library
- RNA analysis of all spheres: 96-well format not practical

## HT29 cells in single cell adherence plates followed by tumorsphere culture



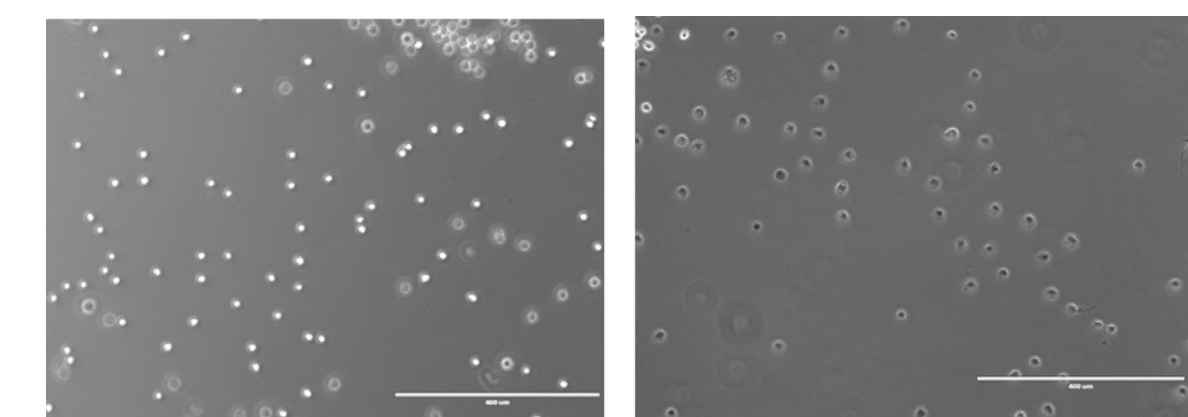
Photopatterning Primo  
bioengineering technology (Alvéole, France)



Images from Léo Wurthlin (M1): Photopatterning realized with a dose of 30 mJ/mm<sup>2</sup> to obtain a matrix of 50 x 50 circles with a diameter of 18 µm separated by 250 µm. (A-C) Images with different magnification acquired by a DMI8 inverted microscope, after the photopattern was visualized by DWS LAB Vitra™ 429 ink. (D) DMI8-acquired image of a green-fluorescent Alexa Fluor® 488 fibrinogen conjugate deposited in a 6-well glass bottom plate after photopatterning.

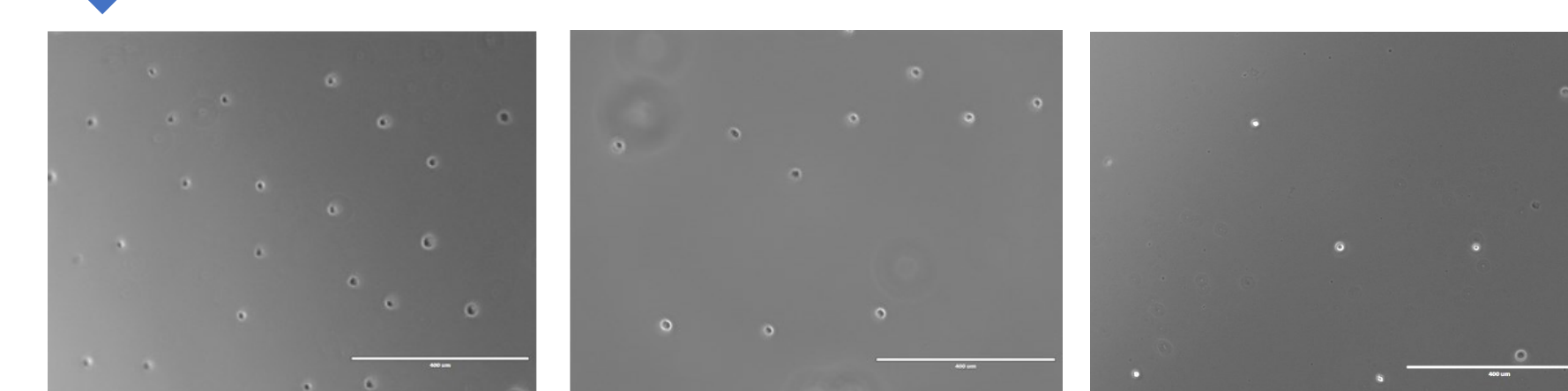


Seeding of HT29 in a Primo-treated well



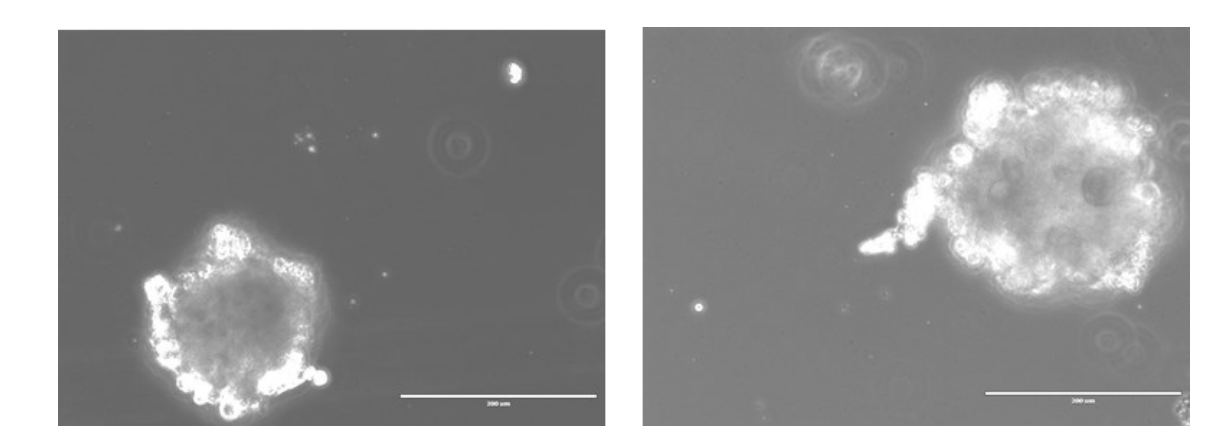
HT29 cells incubated at 37C in standard culture medium for at least 2h. O/N culture results in inert cells

Washes with PBS to remove cells not in adherence spots

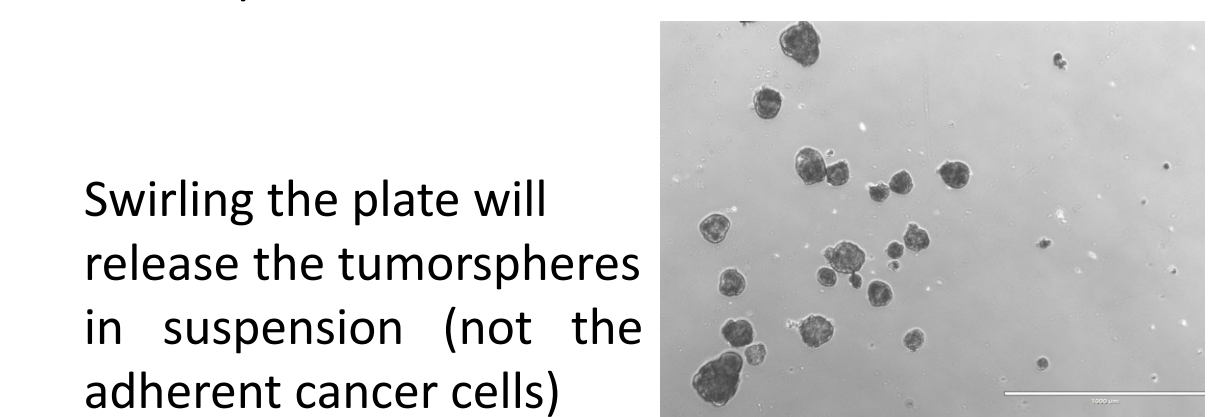


HT29 cells seeded at 200K cells/well adhere in an individual cell pattern. Primo-treated plates are best used within 1-2 days for best results.

- Easy transport of 6-well plate to (lentiviral) culture room
- Seeding of a cell suspension, but individual cell culture



After adherence HT29 cells were cultured in 3D Tumorsphere Medium XF (Promocell). Only stem-like cells will proliferate and develop tumorspheres



Swirling the plate will release the tumorspheres in suspension (not the adherent cancer cells)

- This method is suitable as high-throughput readout assay for library screens
- Single-cell originated tumorspheres can easily be recolted for RNAseq analyses assessing sgRNA expression and transcriptomes

