Cell Cultures

REGE

Summary

- Cell culture equipment: what do we need to work with cells?
- Different types of cell cultures
- What can we do with cells?
- Organoids and 3D cultures

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- Different types of cell cultures
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Cell culture equipment: what do we need to work with cells?

- BASIC EQUIPMENT:

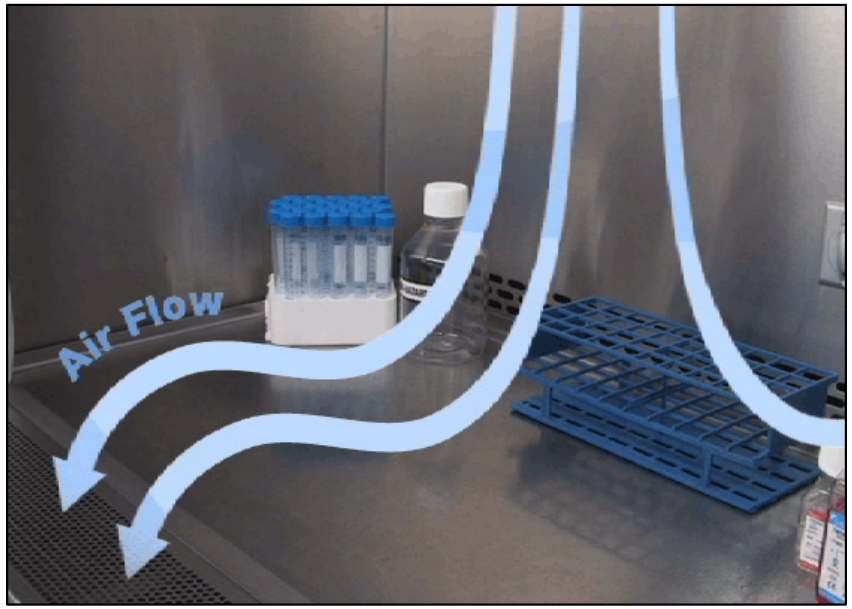
1. Cell culture hood (i.e., laminar-flow hood)
2. Incubator
3. Microscope
4. Cell counter
5. Sterilizer
6. Cryogenic storage
7. Culture media

Cell culture equipment:
what do we need to work with cells?

- BASIC EQUIPMENT:

1. Cell culture hood (i.e., laminar-flow hood)

The laminar flow hood provides an aseptic work area while allowing the containment of infectious splashes or aerosols generated while we are working.



A constant and unidirectional flow of **filtered air** is maintained over the work area.

The horizontal flow provides protection to the culture (if the air flowing towards the user) or to the user (if the air is drawn in through the front of the cabinet by negative air pressure inside).

The vertical flow (blowing from the top of the cabinet onto the work surface) provides protection to the user and the cell culture

https://www.thermofisher.com/it/en/home/references/gibco-cell-culture-basics/cell-culture-equipment/laminar-flow-hood.html

Cell culture equipment:
what do we need to work with cells?

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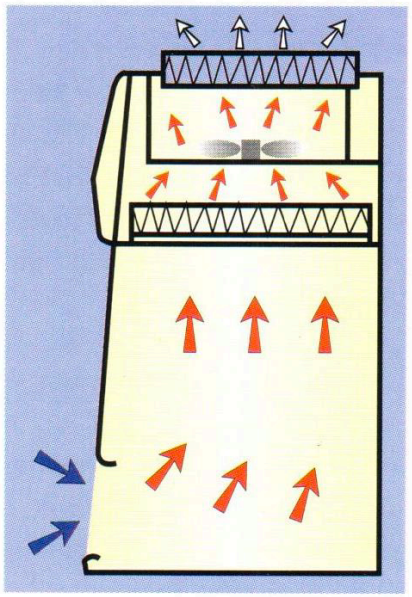

Class I

Class I hoods offer protection to the **worker** and to the **environment**, but they do not provide cultures protection from contamination.

They are similar in design and air flow characteristics to chemical fume hoods.

https://www.thermofisher.com/it/en/home/references/gibco-cell-culture-basics/cell-culture-equipment/laminar-flow-hood.html

Cell culture equipment: what do we need to work with cells?

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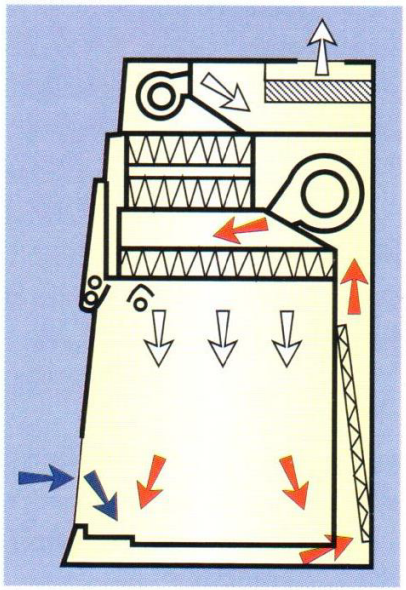

Class II

Class II hoods are designed for working with **BSL-1, 2, and 3 materials**, and they also provide an **aseptic** environment for cell cultures.

They allow the handling of potentially hazardous materials, such as primate-derived cultures, virally infected cultures, toxic reagents.

https://www.thermofisher.com/it/en/home/references/gibco-cell-culture-basics/cell-culture-equipment/laminar-flow-hood.html

Cell culture equipment:
what do we need to work with cells?

- BASIC EQUIPMENT:

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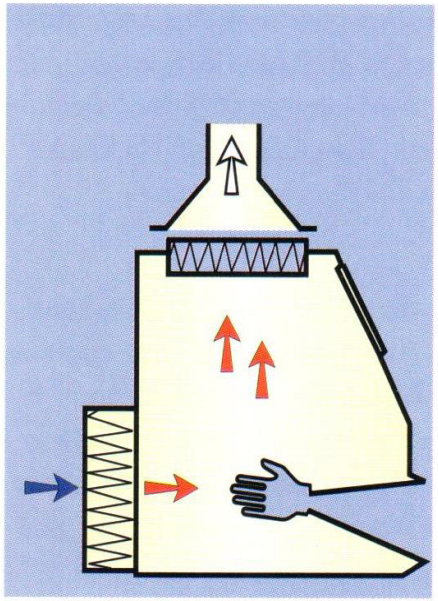


Class III

Class III hoods are gas-tight, and they provide the **highest protection** to **personnel** and the **environment**.

A Class III biosafety cabinet is required for work involving **BSL-4** materials.

https://www.thermofisher.com/it/en/home/references/gibco-cell-culture-basics/cell-culture-equipment/laminar-flow-hood.html

Cell culture equipment:
what do we need to work with cells?

DIFFERENT TYPES OF CONTAMINANTS

Operator (hair, hands, clothes)
Environment

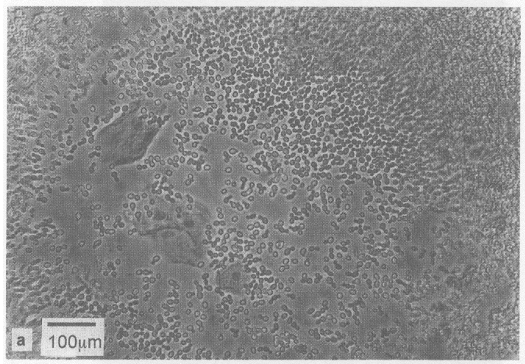
Microbial contamination:
Bacteria Micoplasma (bacterium) Fungi Yeast

Other biological materials: tissues, other cell lines

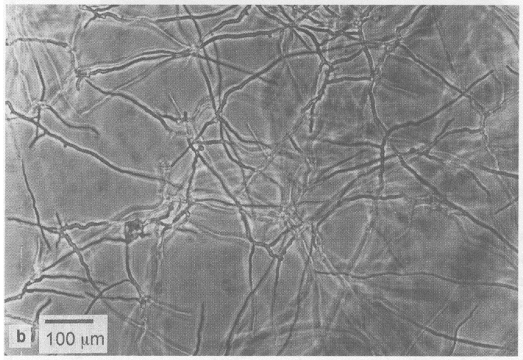
Cell culture equipment:
what do we need to work with cells?

DIFFERENT TYPES OF CONTAMINANTS

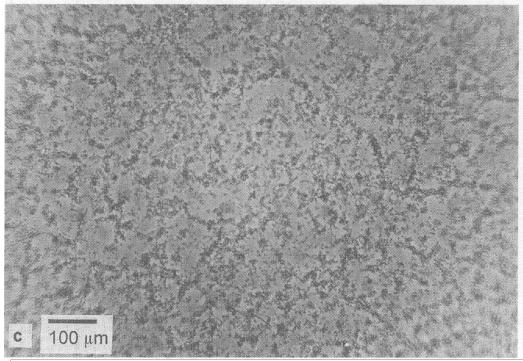
Yeast

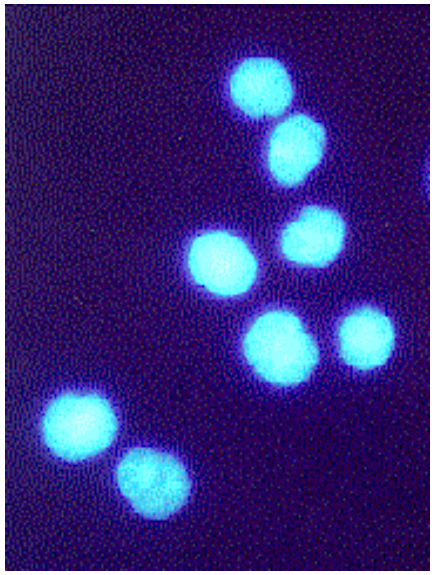


Fungi

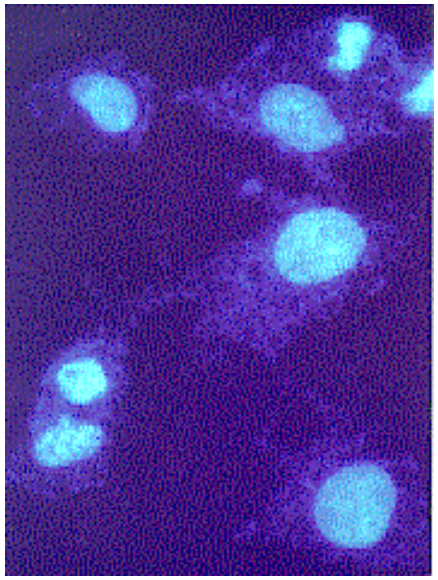


Bacteria





Micoplasma-free



Micoplasma contamination

Cell culture equipment: what do we need to work with cells?

- BASIC EQUIPMENT:

2. Incubator

37°C (±0.2°C),
5% CO_2

The incubator provides the **appropriate environment** for cell growth.

- **Dry incubators:** economical, but require the cell cultures to be incubated in sealed flasks to prevent evaporation.

- **Humid CO_2 incubators:** more expensive, but allow superior control of culture conditions. They can be used to incubate cells cultured in Petri dishes or multiwell plates.

https://www.thermofisher.com/it/en/home/references/gibco-cell-culture-basics/cell-culture-equipment/basic-laboratory-equipment.html

Cell culture equipment:
what do we need to work with cells?

- BASIC EQUIPMENT:

2. Microscope

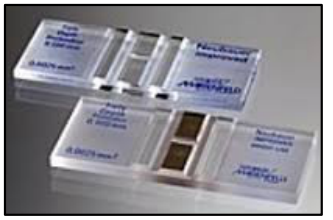
Inverted microscope

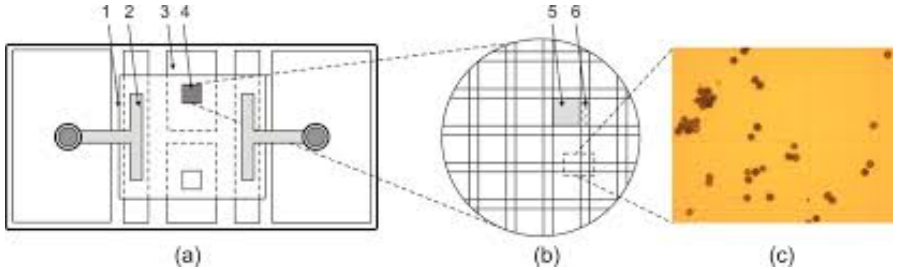
3. Cell counter

A cell counter is essential for quantitative growth kinetics, to count cells before plating…

- Automated cell counters

- Burker chamber





Cell culture equipment:
what do we need to work with cells?

- BASIC EQUIPMENT:

4. Sterilizer

Dry heat:
180°C for 3 hours -> glassware

Autoclave (humid heat):
1 atm, 121°C for 20 minutes
-> solutions

0.22 µm filters:
For culture media, organic solutions…

Gamma radiations:
For plastic materials

Cell culture equipment: what do we need to work with cells?

- BASIC EQUIPMENT:

5. Cryogenic storage: how to freeze/thaw cells

Freezing:

Very slow

Store cells in **liquid nitrogen** in complete medium in the presence of a **cryoprotective agent** such as dimethylsulfoxide (DMSO) -> reduces the freezing point of the medium and also allow a slower cooling rate, reducing the risk of ice **crystal formation**, which can damage cells and cause cell death.

Thawing:

Fast

Move cells from liquid nitrogen directly to a 37°C water bath.

Pellet cells to remove freezing medium, then resuspend in new medium and plate.

Cell culture equipment:
what do we need to work with cells?

- BASIC EQUIPMENT:

5. Culture media

Basic culture media: RPMI 1640, IMDM, McCoy's, Alpha MEM and DMEM

Serum: i.e. FBS (Fetal Bovin Serum)

[**Growth factors:** G-CSF, GM-CSF, IL-2, IL-3, IL-6, SCF, EGF, FGF a e b, NGF, etc.]

Glutamine and Antibiotics (i.e. Penicillin/Streptomycin)

Summary

- Cell culture equipment: what do we need to work with cells?
- Different types of cell cultures
- What can we do with cells?
- Organoids and 3D cultures

Different types of cell cultures

TISSUE CULTURE

Culture of non-disaggregated tissue fragments

ORGAN CULTURE

Culture of non-disaggregated tissue fragments that maintain 3D architecture and others characteristics of the organ they come from

CELL CULTURE

Culture of disaggregated cells, derived from a tissue, a primary culture, a cell line…

HISTOTYPIC CULTURE

Culture of cells that have been re-aggregated in vitro to create a 3D structure similar to the tissue they come from

Different types of cell cultures

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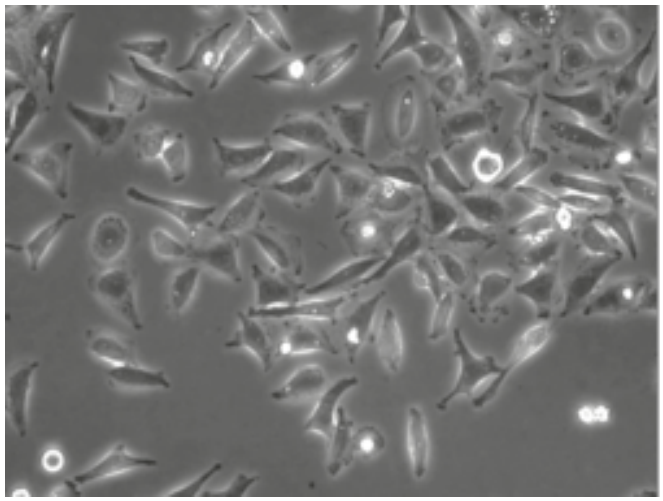
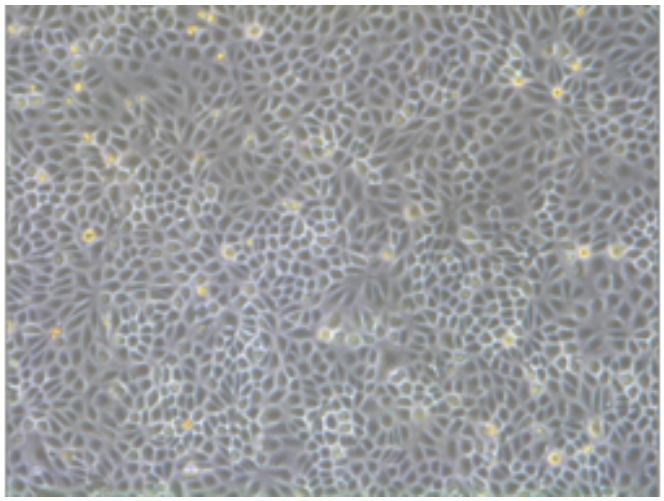
Different types of cell cultures

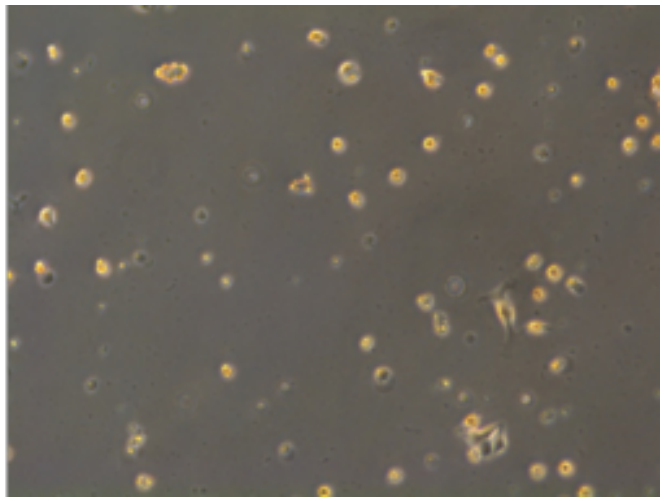
Cultured cells can be divided into three basic categories based on their **morphology**:



Fibroblast-like cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate



Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches



Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface

http://www.leica-microsystems.com/science-lab/introduction-to-mammalian-cell-culture/

Different types of cell cultures

- Primary cultures
- Immortalized cell lines
- [Stem cells]

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- Primary cultures 
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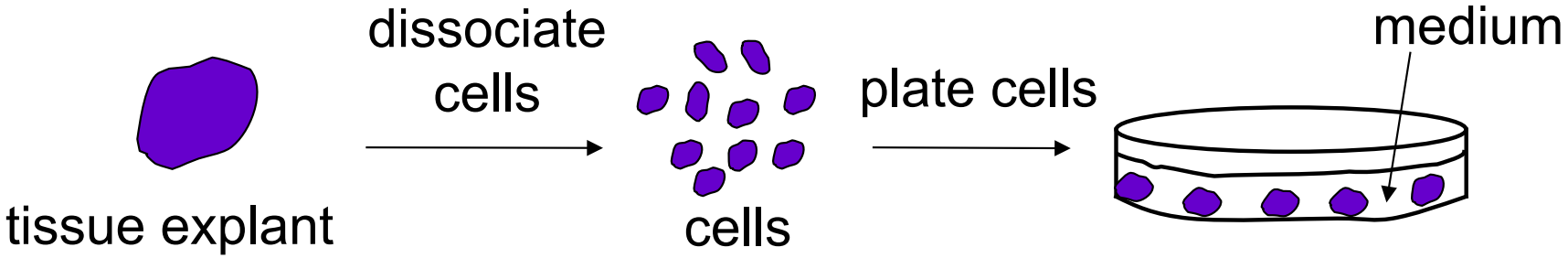
Different types of cell cultures

PRIMARY CULTURES:

They derive from tissue explants:

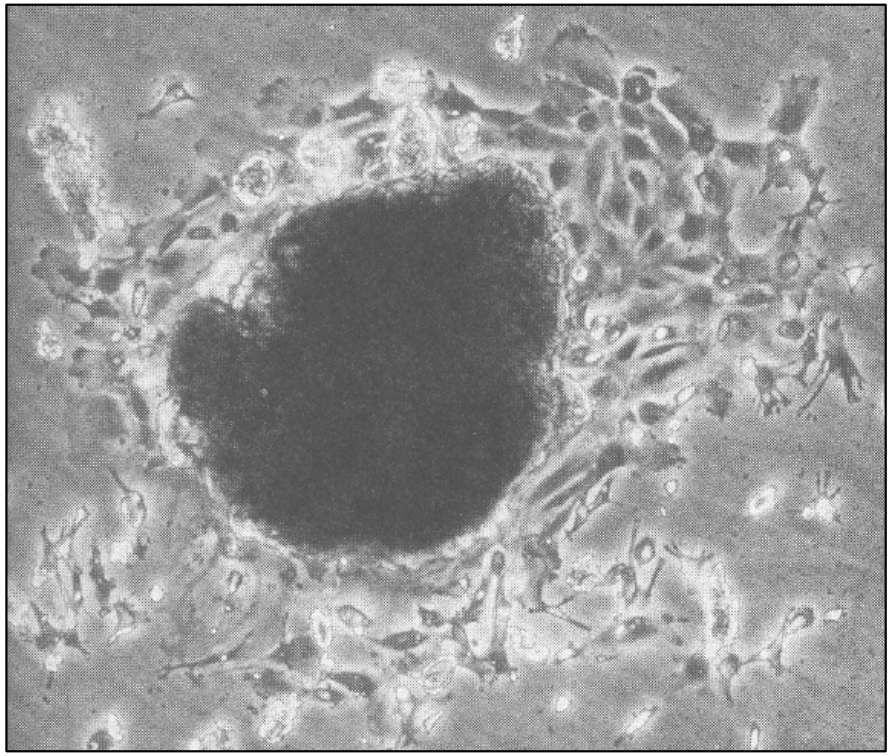


Primary cells proliferate under the appropriate conditions until they reach **confluence**. Then the cells have to be **subcultured** by transferring them to a new plate with fresh growth medium to provide more room for continued growth.

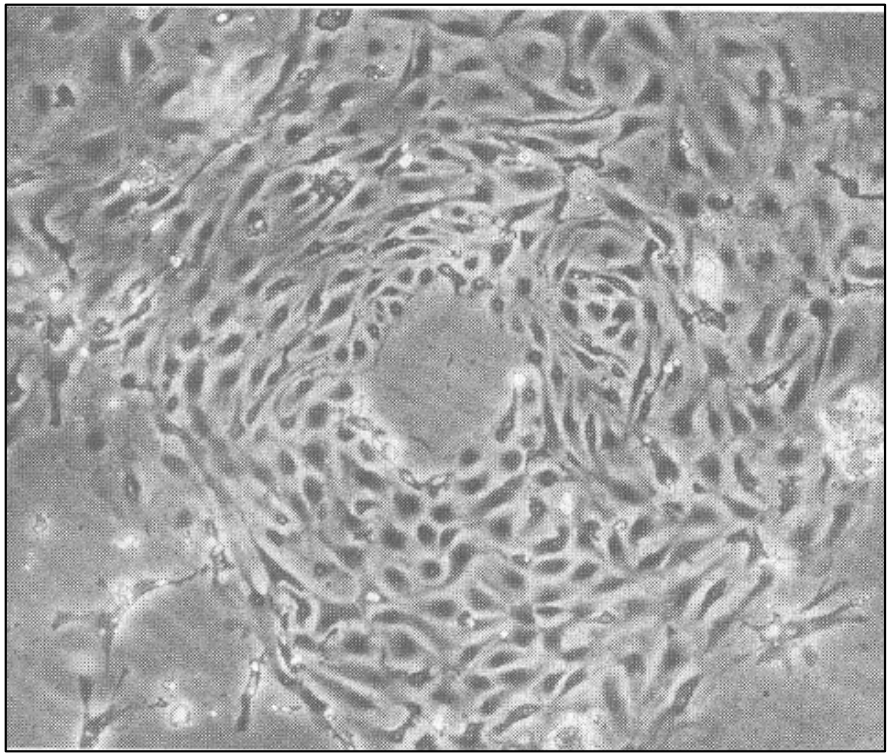
They undergo apoptosis after 50-100 divisions

Different types of cell cultures

PRIMARY CULTURES:



3-day culture



7-day culture, after the explant has been removed

Different types of cell cultures

- Primary cultures
- Immortalized cell lines 
- [Stem cells]

Different types of cell cultures

IMMORTALIZED CELL LINE:

An **immortalized** cell line is a population of cells which, **thanks to mutation**, have evaded normal cellular senescence and instead can keep proliferating.

The mutations can occur naturally or be intentionally induced.

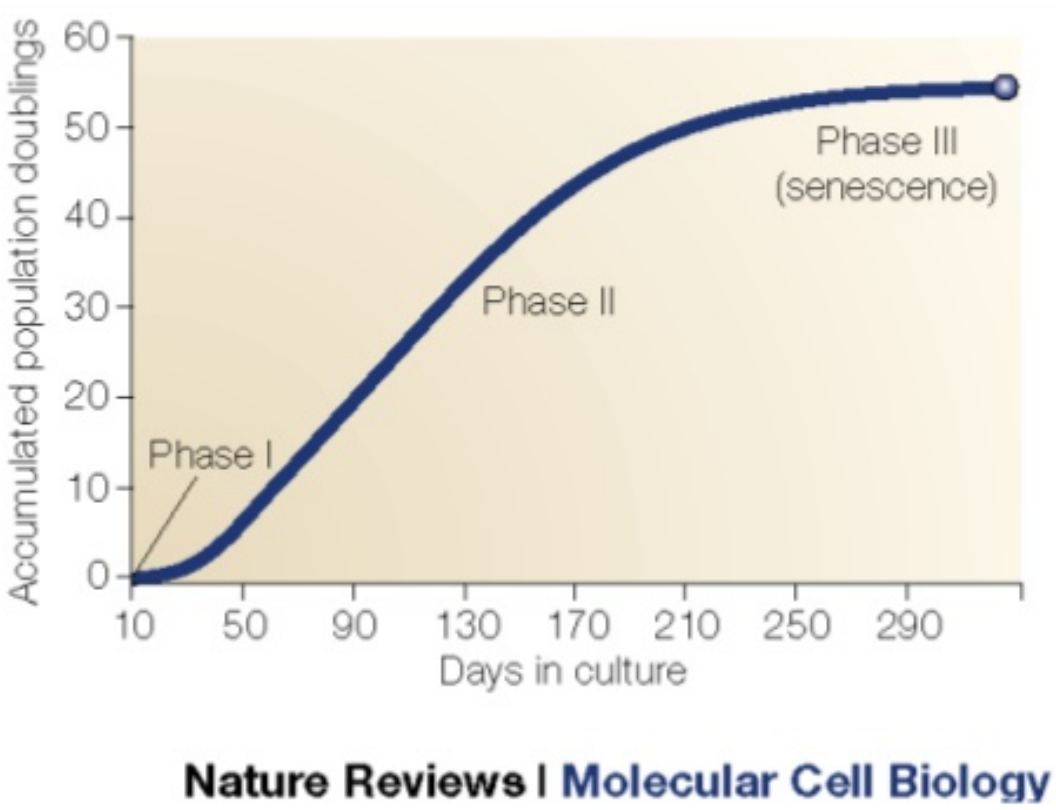
This cells can be keep in culture in vitro for a long time.

Immortal cell lines are a very important tool for research -> very good “model system”

Different types of cell cultures

The HAYFLICK'S LIMIT

Upon each cell division, telomeres shorten more and more.
The maximum number of divisions that a cell can undergo is called **Hayflick's limit**.
When this limit is reached, replicative senescence is induced.
Cells can bypass this limit thanks to the aberrant activations of genes such as **p53**.
At this point thay can reach another proliferation block, or they can keep proliferating thanks to the activaction of the **telomerase** gene.



Different types of cell cultures

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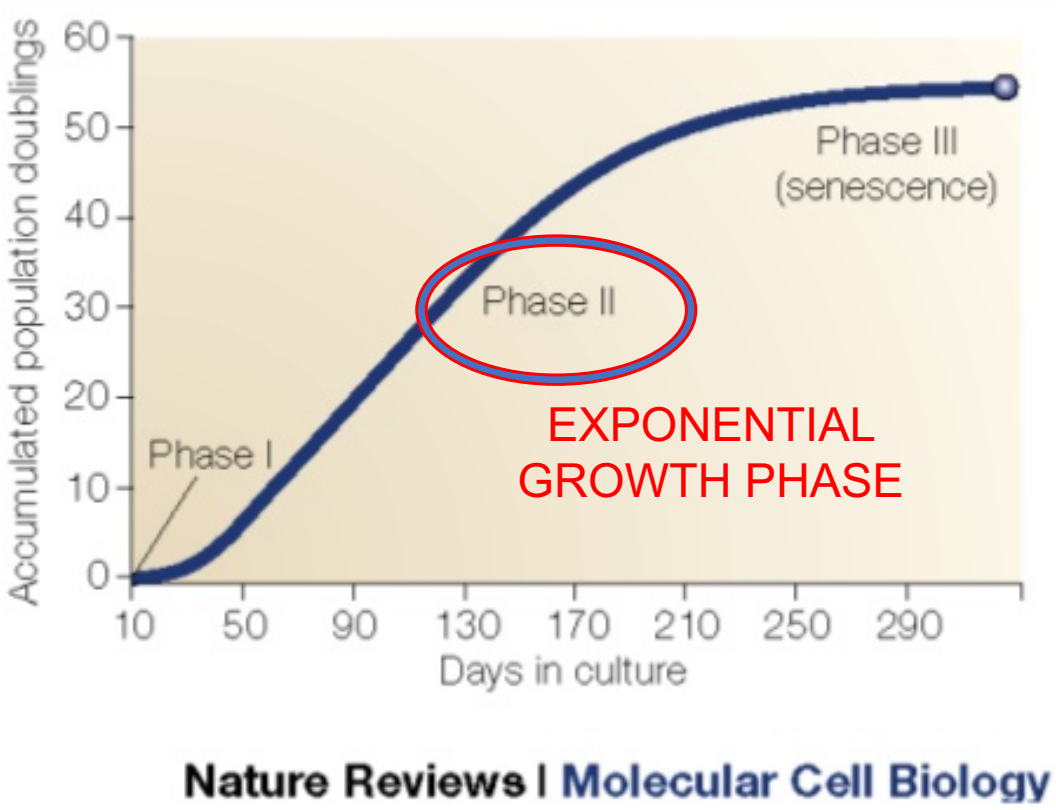


Different types of cell cultures

IMMORTALIZED CELL LINE:

We can derive an immortalized cell line from:

1. **Primary cultures (“normal cells”)**

They grow until they contact adiacent cells (CONTACT INHIBITION)

They do not form tumors if injected in nude mice

Thay can be “immortalized”, either artificially, or thanks to rare genetics mutations that naturally occur (es. BSC-1 cell line, derived from African Green Monkey kidney)

Different types of cell cultures

IMMORTALIZED CELL LINE:

We can derive an immortalized cell line from:

2. Transformed cells

They have more genetics mutations induced by radiations, chemical agents, virus integrations…

They have **lost** conctact inhibition and proliferation control

They have **abnormal caryotypes**

They form tumors if injected into nude mice

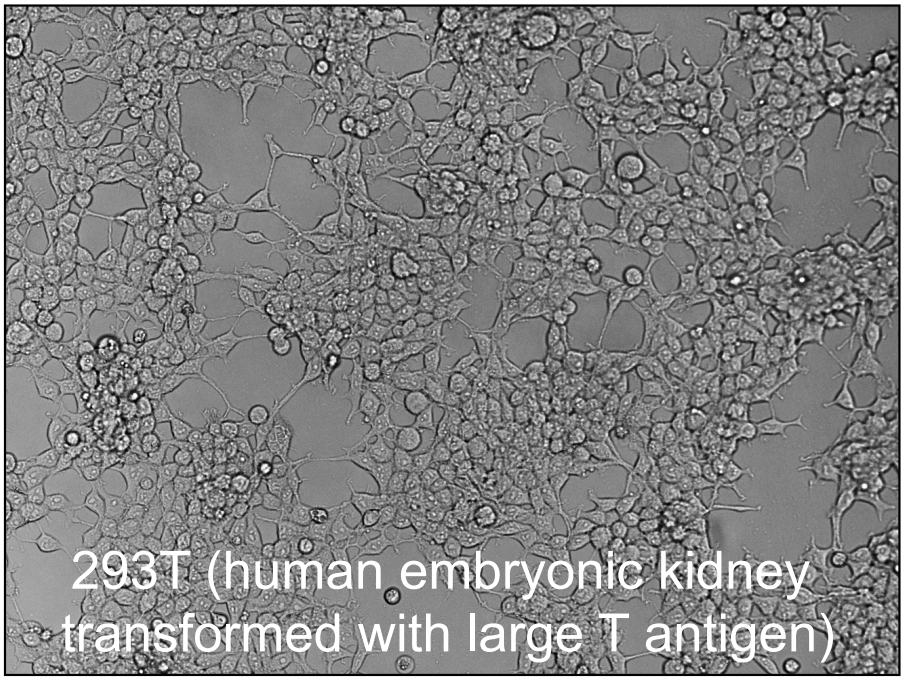
Different types of cell cultures

IMMORTALIZED CELL LINE:

We can derive an immortalized cell line from:

2. Transformed cells

3T3 mouse embryo fibroblasts



293T (human embryonic kidney transformed with large T antigen)

Different types of cell cultures

IMMORTALIZED CELL LINE:

We can derive an immortalized cell line from:

3. **Cancer cells**

They show uncontrolled proliferation -> **unlimited** replication potential

They have **lost** conctact inhibition

They have **abnormal caryotypes**

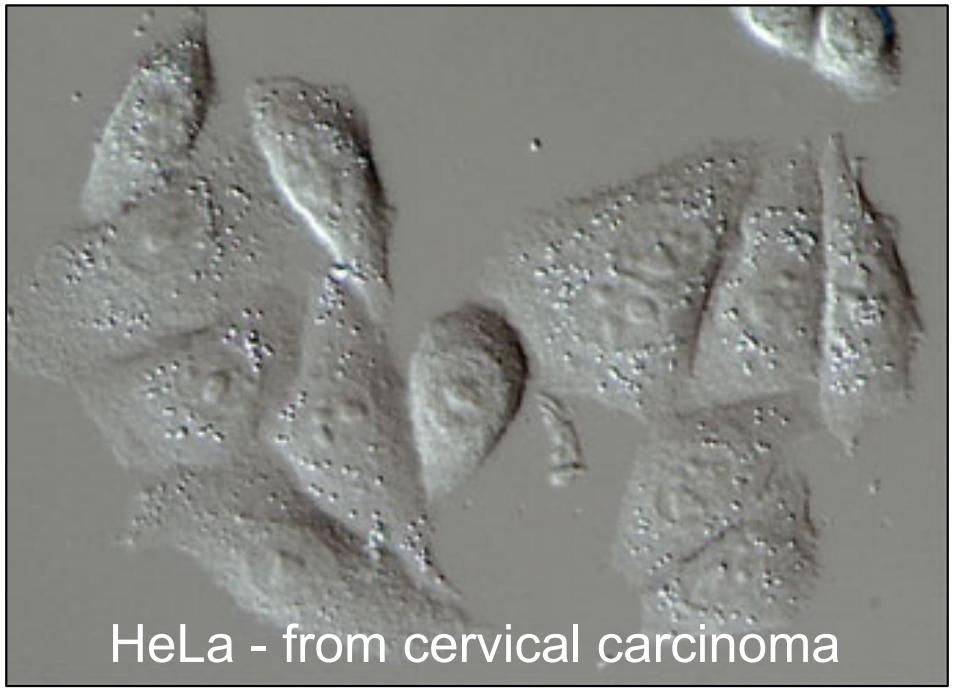
They form tumors if injected into nude mice and they are able to form metastasis

Different types of cell cultures

IMMORTALIZED CELL LINE:

We can derive an immortalized cell line from:

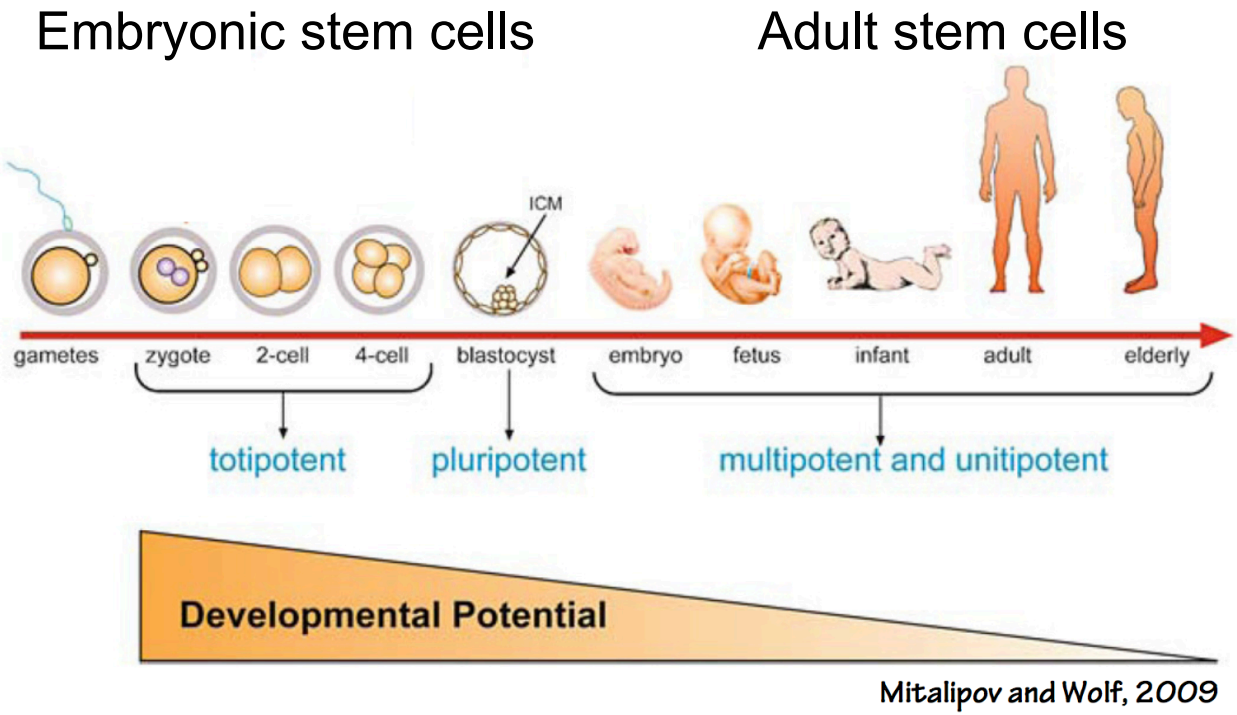
3. Cancer cells



Different types of cell cultures

STEM CELLS:

They are able to **self-renew** and **differentiate** in different cell types



Summary

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- Different types of cell cultures
- What can we do with cells?
- Organoids and 3D cultures

What can we do with cells?

MANY DIFFERENT EXPERIMENTS!!

FOR EXAMPLE:

- GENE EXPRESSION
- STUDY CELLULAR PROCESSES -> Differentiation mechanisms

What can we do with cells?

- GENE EXPRESSION

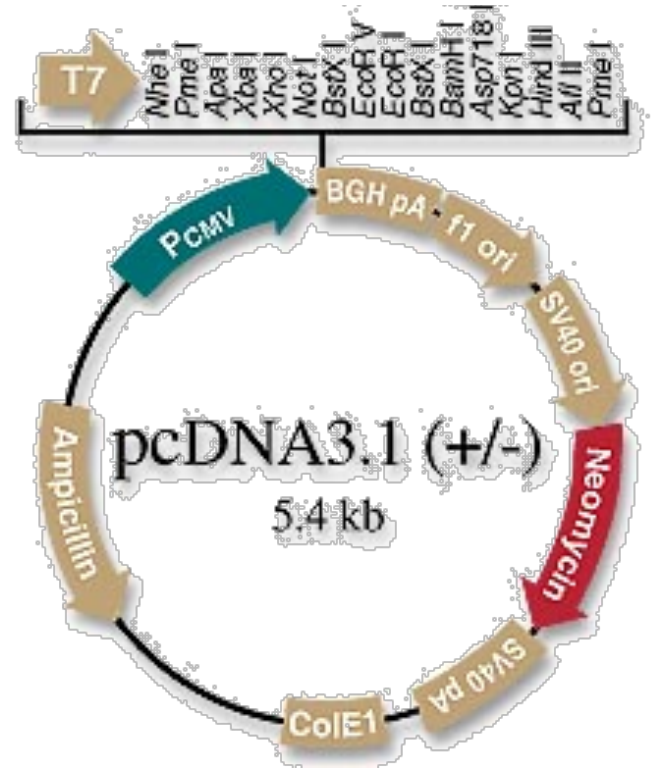
Expression vectors for mammalian gene expression should have:

• Bacterial replication origin and selection marker

• MCS (multiple cloning site) to insert our DNA sequence of interest

• Eukaryotic promoter and poly-A signal that work in mammalian cells

• A reporter gene or a selection marker to identify/select cells that have been transfected



What can we do with cells?

- GENE EXPRESSION

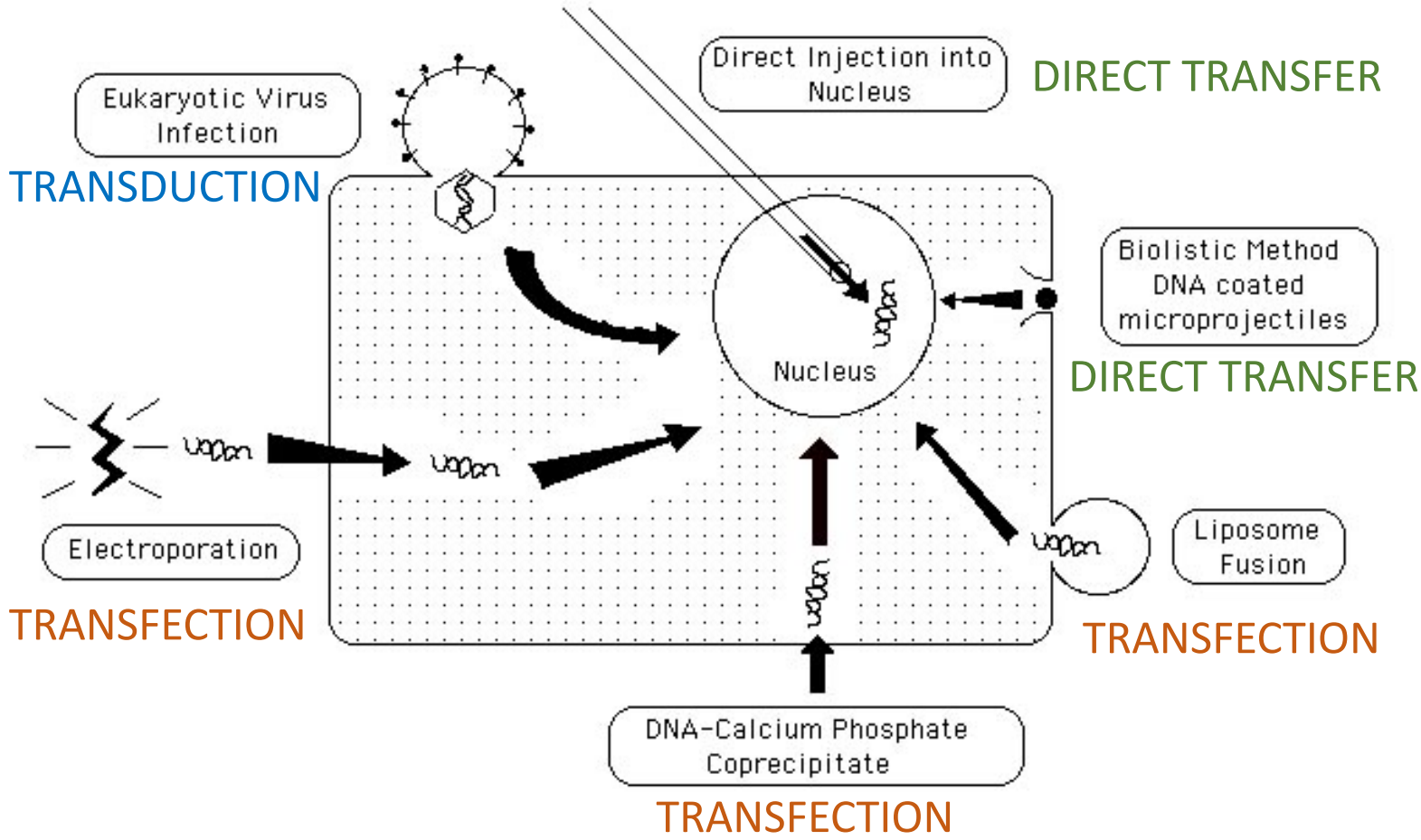
Promoters for mammalian gene expression can be:

Promotor type	Expression level
CONSTITUTIVE	High/Low expression
CELL TYPE SPECIFIC	Cell type-dependent expression level
INDUCIBLE	Low -> High High -> Low

What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cells?

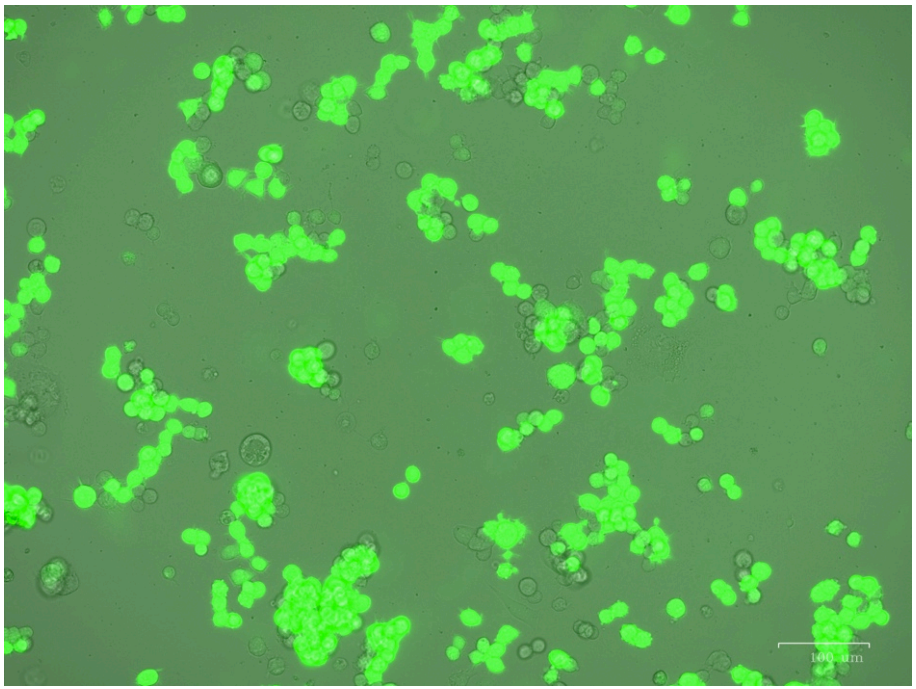


What can we do with cells?

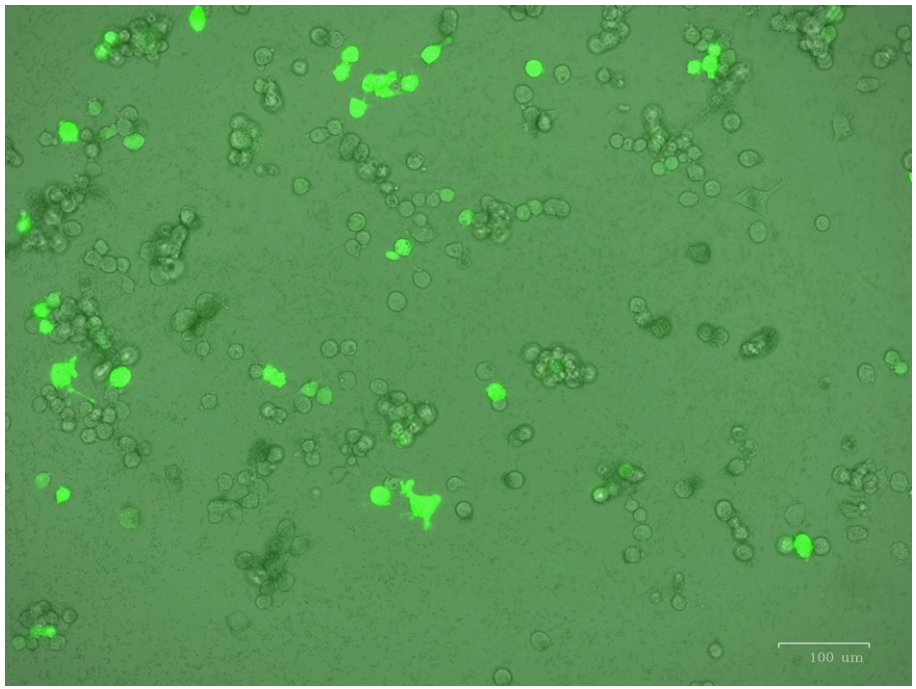
- GENE EXPRESSION

Comparing the **efficiency** of different methods of transient transfection in different cell lines

N2A, 24h



Lipofectamine 2000



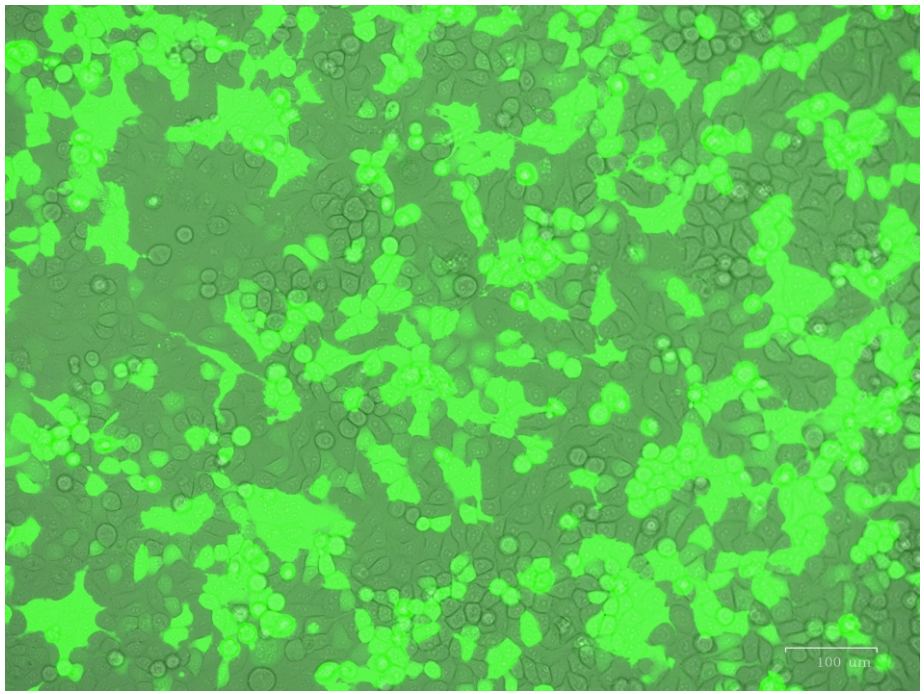
Calcium-phosphate

What can we do with cells?

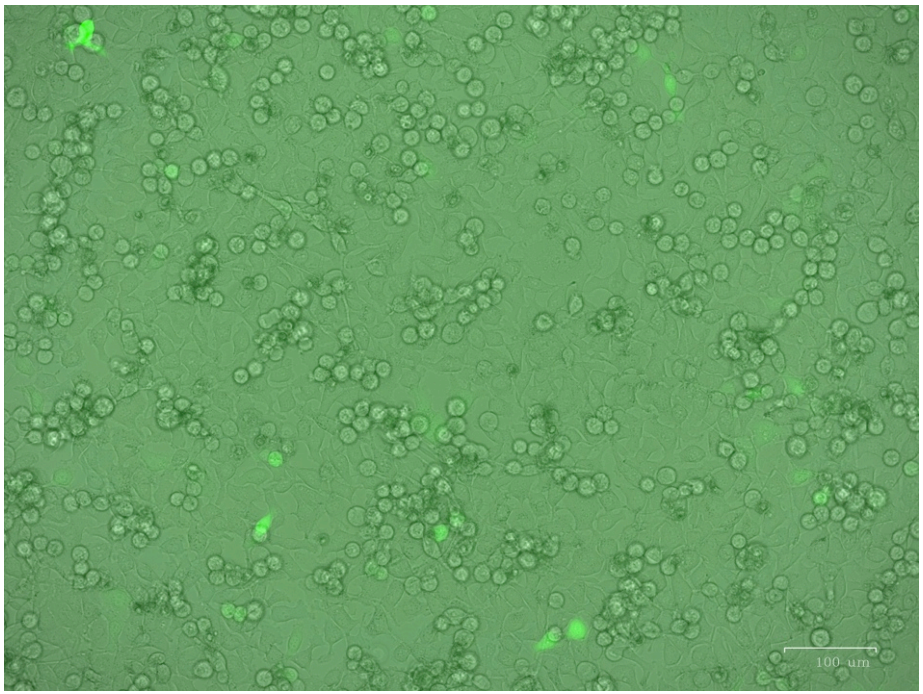
- GENE EXPRESSION

Comparing the **efficiency** of different methods of transient transfection in different cell lines

HeLa, 24h



Lipofectamine 2000



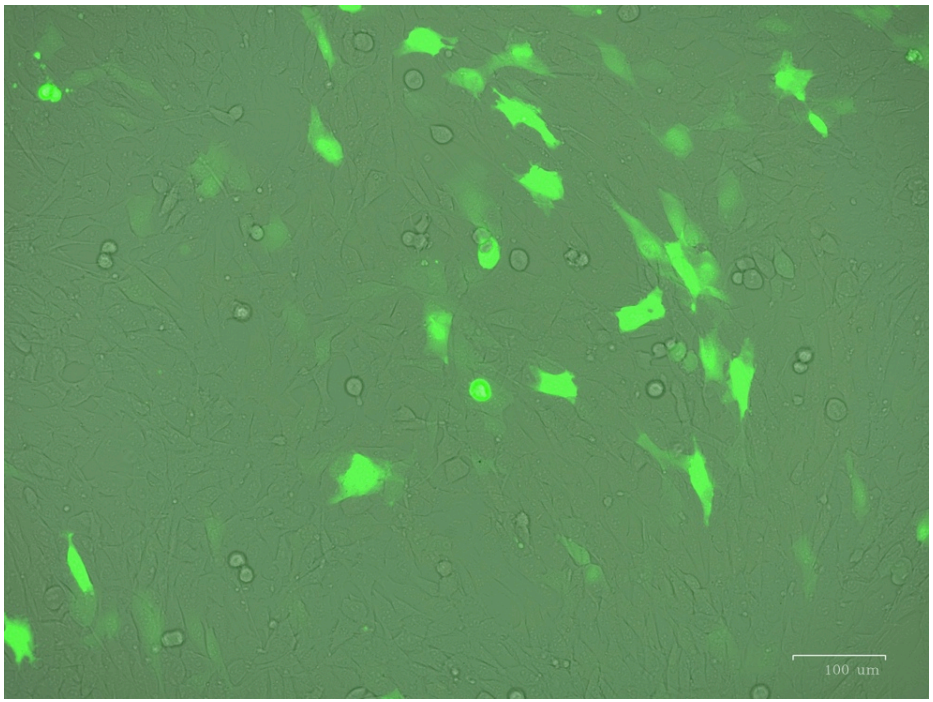
Calcium-phosphate

What can we do with cells?

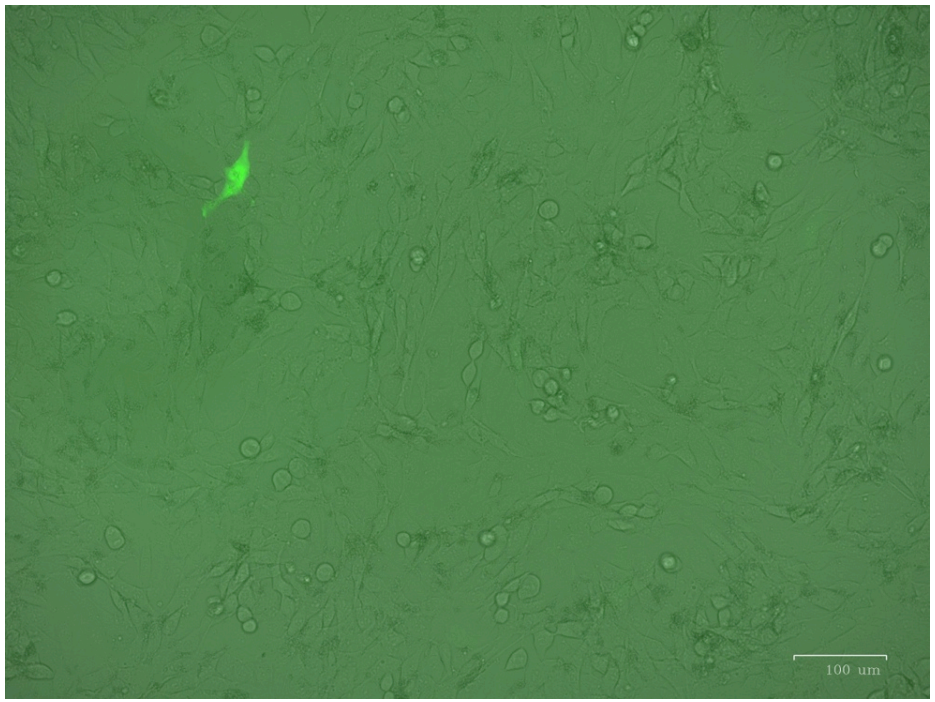
- GENE EXPRESSION

Comparing the **efficiency** of different methods of transient transfection in different cell lines

C2C12, 24h



Lipofectamine 2000



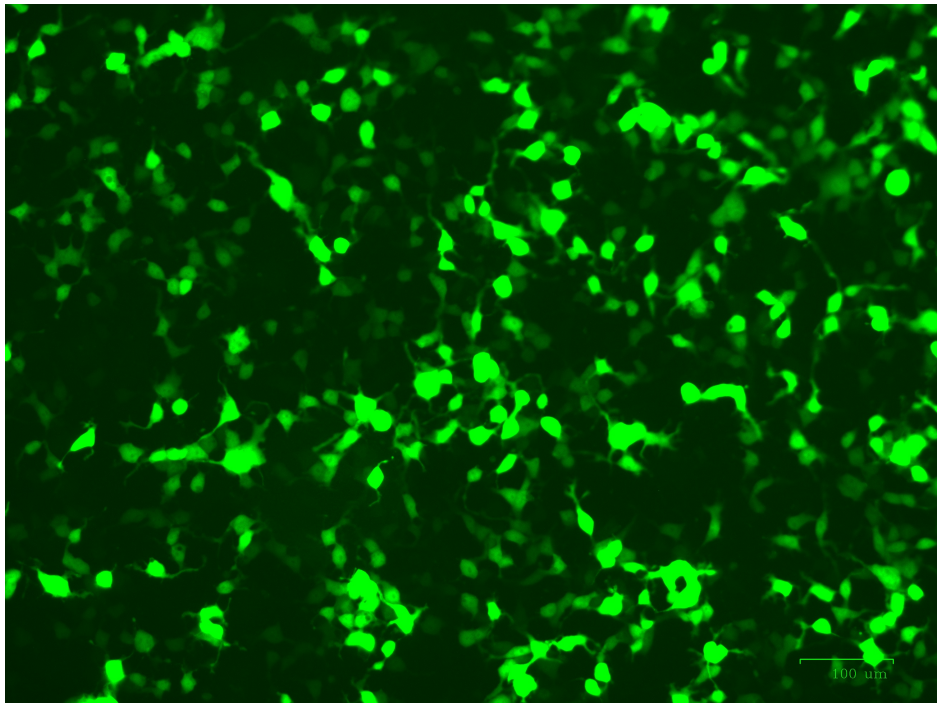
Calcium-phosphate

What can we do with cells?

- GENE EXPRESSION

Comparing the **efficiency** of different methods of transient transfection in different cell lines

293T cells



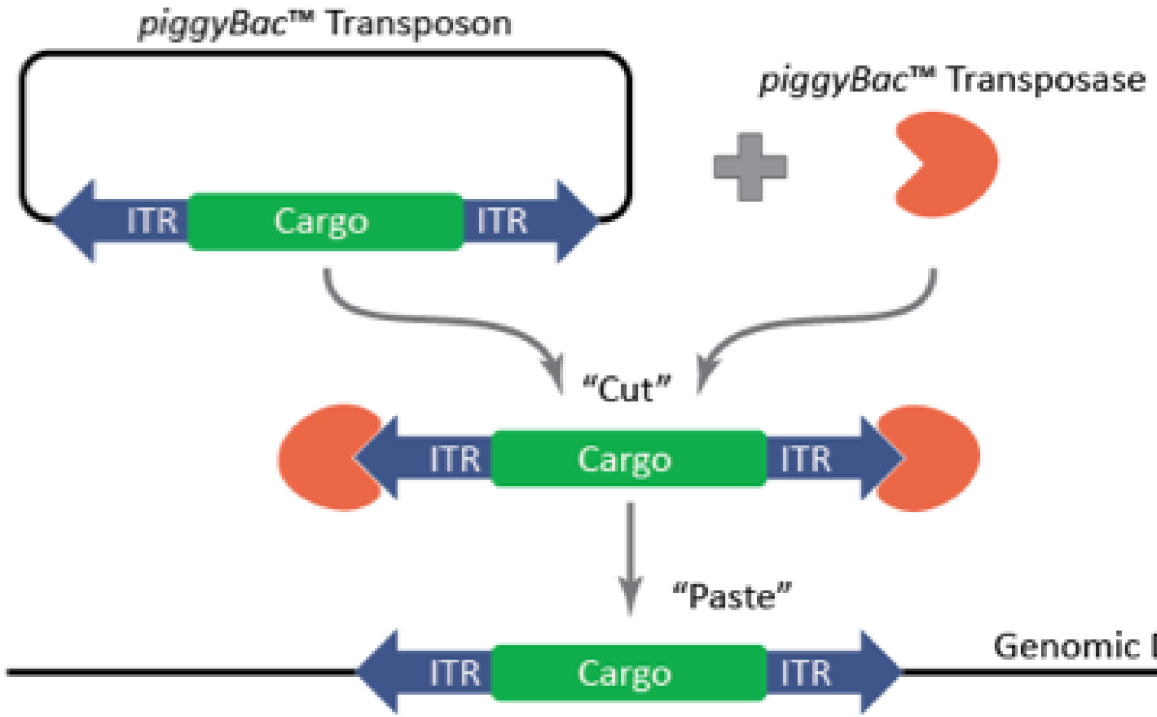
Calcium-phosphate

What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

1. Trasposons



RANDOM INTEGRATION!!!

http://blog.addgene.org/piggybac-ing-through-the-genome-editing-field

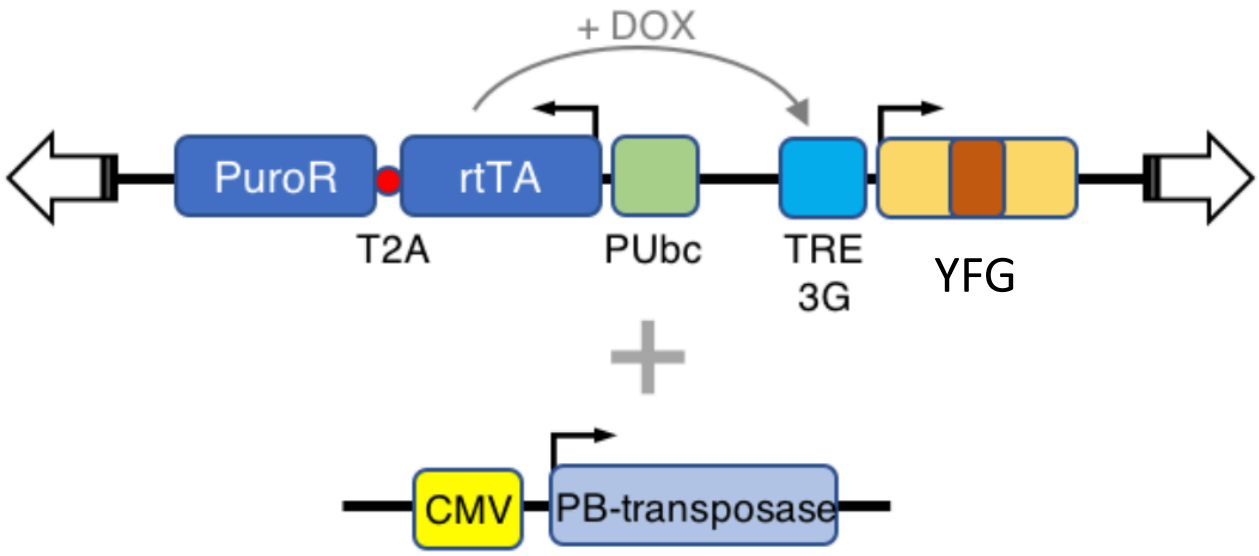
What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

1. Trasposons

ES. Transposon with Doxycycline inducible promoter and selection marker



p-PB-transposase (Helper vector)

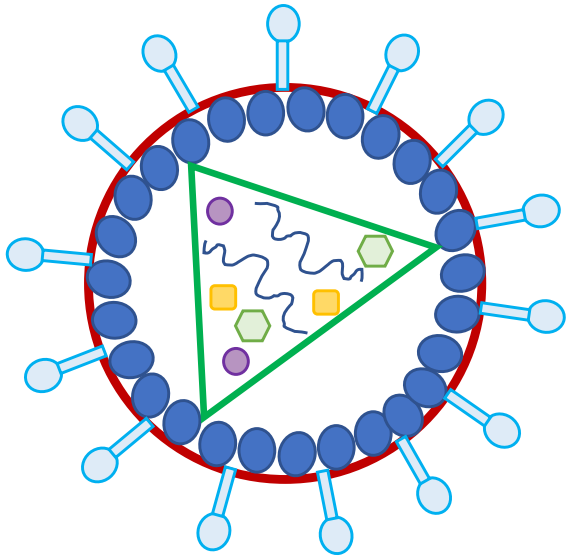
What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

2. Viral vectors: RETROVIRIDAE

- Members of the Retroviridae family: γ-retroviruses (simple) and lentiviruses (complex)
- Retrotranscribe their RNA genome into a cDNA copy, which is then stably integrated into the host cell genome.
- The viral particles of both groups contain 2 copies of RNA (+) with an associated viral reverse transcriptase (RT) located within an internal core. Also located within this compartment are structural and enzymatic proteins, including the nucleocapsid (NC), capsid (CA), integrase (IN), and protease (PR).
- Enveloped viruses



What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

2. Viral vectors: LENTIVIRUS

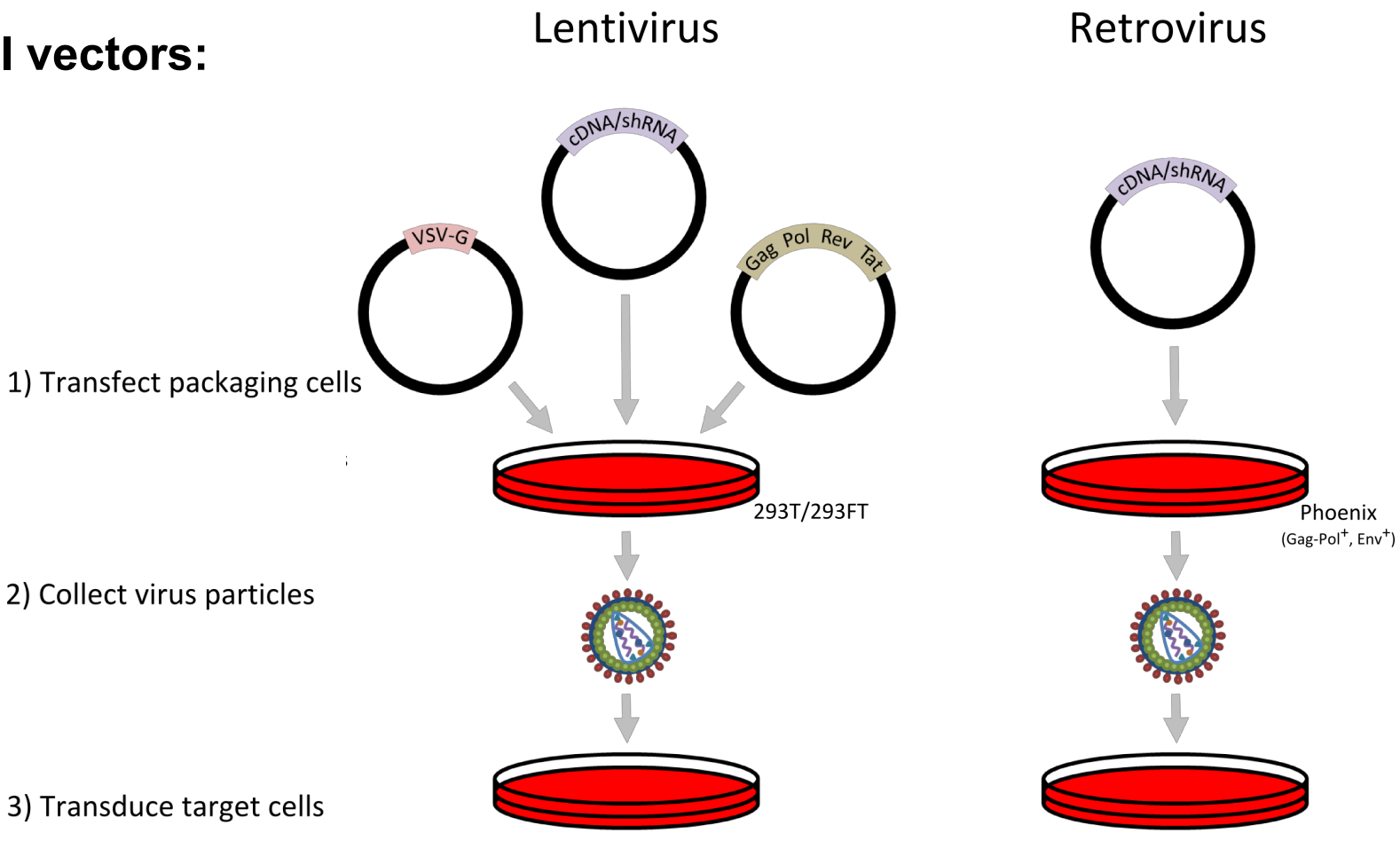
- The main feature of complex retroviral genomes distinguishing them from those of simple retroviruses is the presence of a set of accessory genes whose products are involved in the regulation of transcription, RNA transport, gene expression, and assembly.
- They can infect non-proliferating cells too, while retroviruses can infect only actively proliferating cells.

What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

2. Viral vectors:



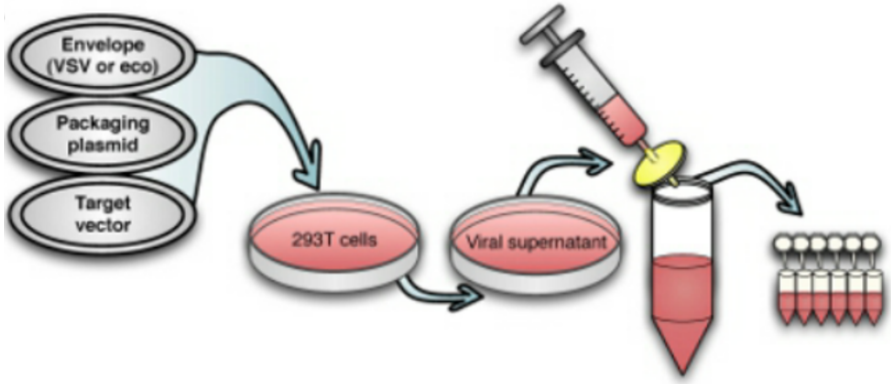
What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

2. Viral vectors:

- After 1-2 days from transformation of packaging cells, viruses are collected by filtering the growth medium, which contains virus particles.



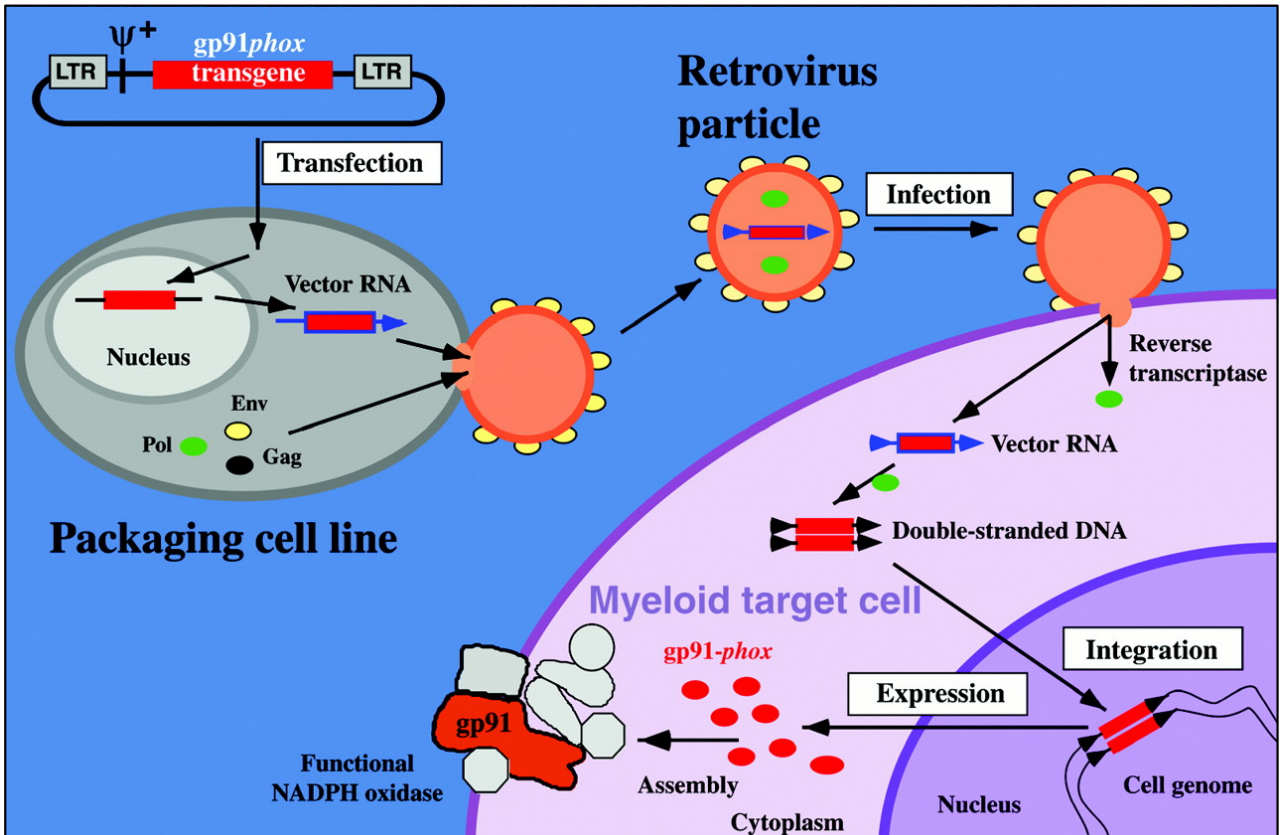
- These particles are used to infect cells
- After 24h cells correctly infected, in which the integration has occurred, are selected

What can we do with cells?

- GENE EXPRESSION

How can we “insert” an exogenous gene into the cell in a STABLE way?

2. Viral vectors:



What can we do with cells?

- GENE EXPRESSION

Selecting transfected cells expressing fluorescent markers: FACS SORTING

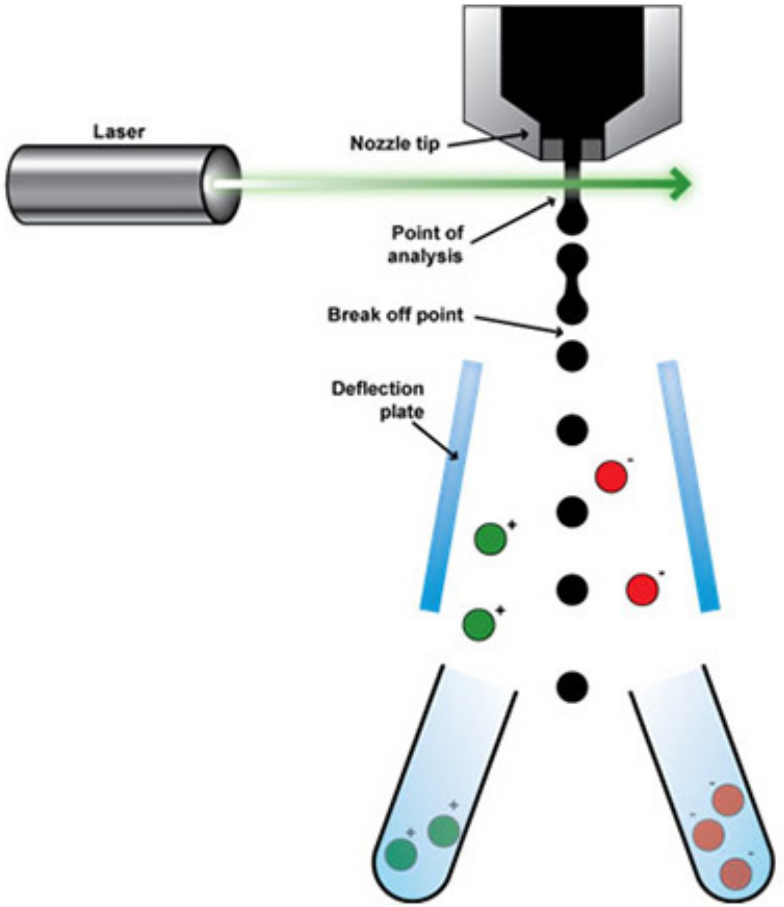
• Individual cells are "interrogated" by the laser

• The machine is set up so that each individual cell then enters a single droplet. This drop is given an electronic charge, depending on the fluorescence of the cell inside the drop.

• Deflection plates attract or repel the cells accordingly into collection tubes.

• Sorted cell populations are then analyzed to ensure successful cell sorting.

• Sorted cells can then be cultured.



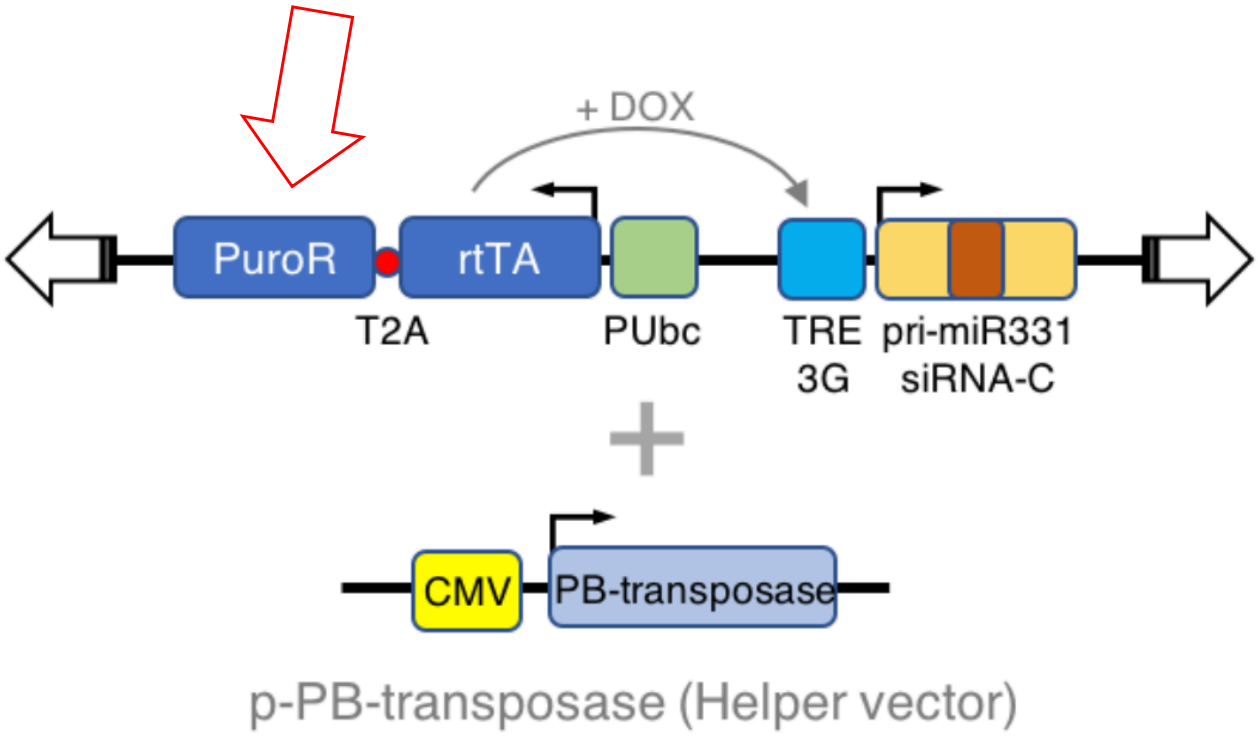
What can we do with cells?

- GENE EXPRESSION

Selecting transfected cells:

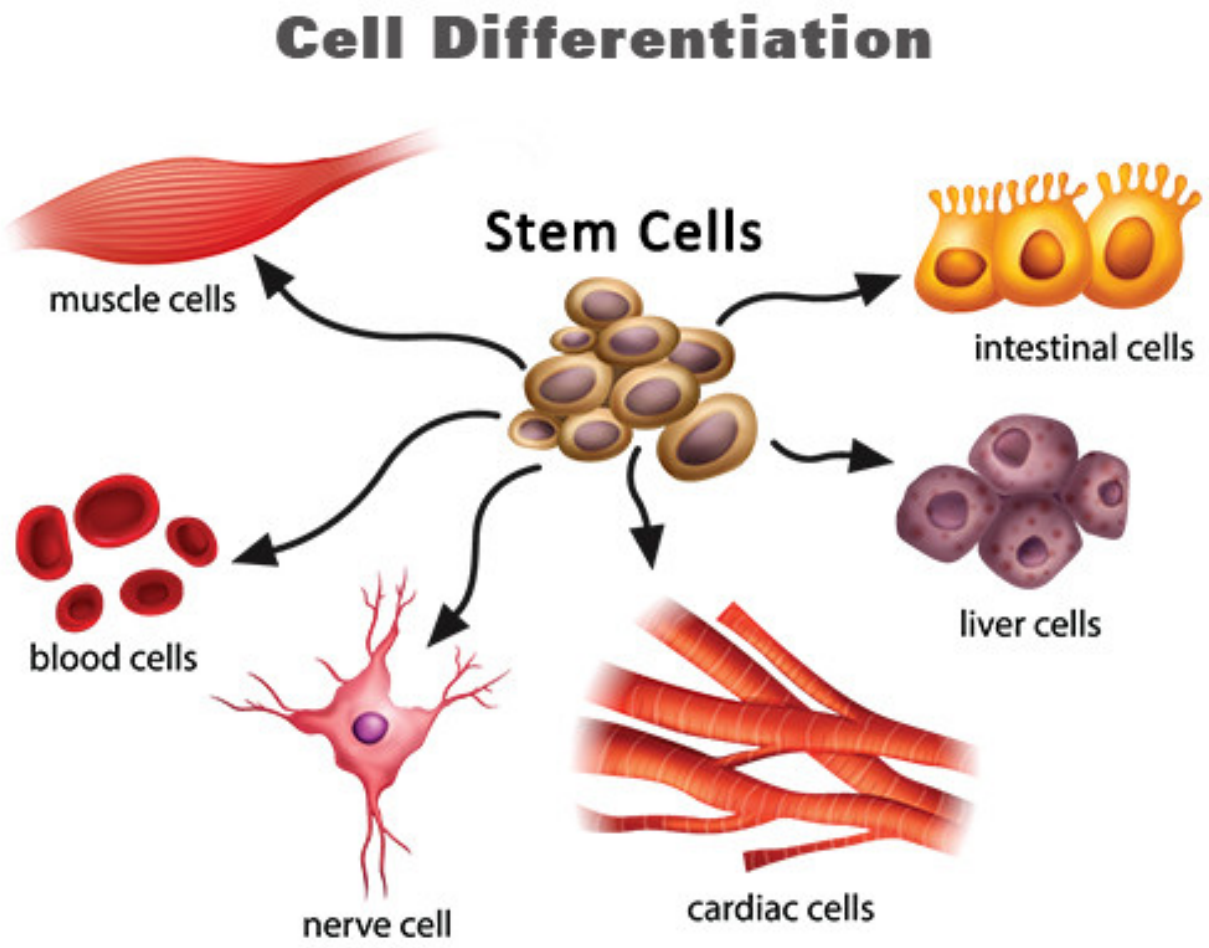
RESISTANCE TO ANTIBIOTICS

Puromycin
Neomycin
…



What can we do with cells?

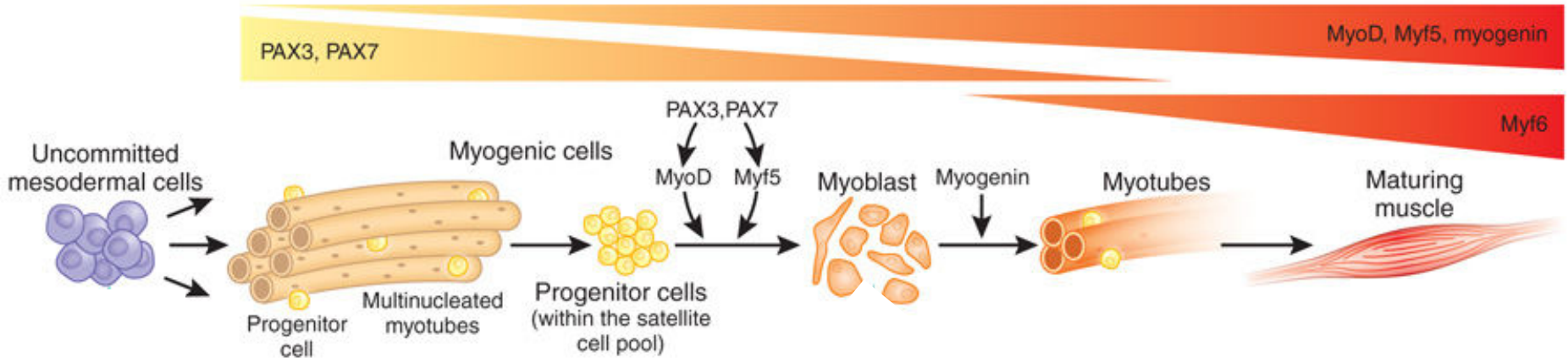
- STUDY BIOLOGICAL PROCESSES -> DIFFERENTIATION



What can we do with cells?

- STUDY BIOLOGICAL PROCESSES -> DIFFERENTIATION

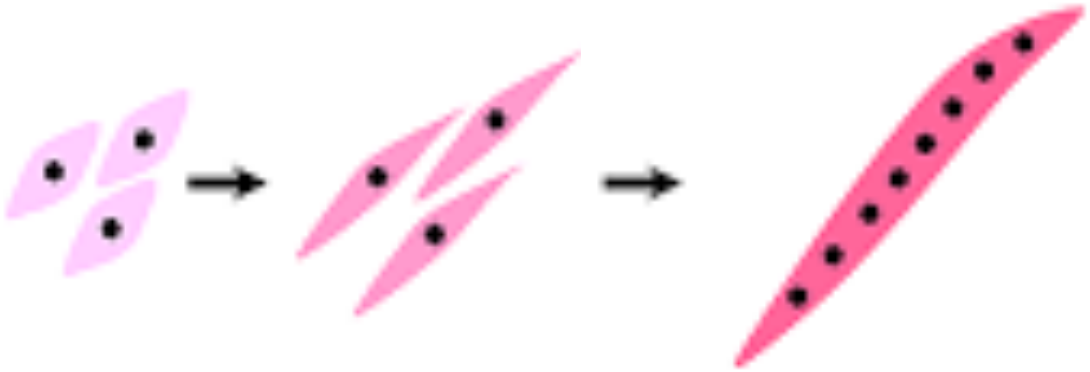
Es. Myogenic differentiation



What can we do with cells?

- STUDY BIOLOGICAL PROCESSES -> **Myogenic differentiation**

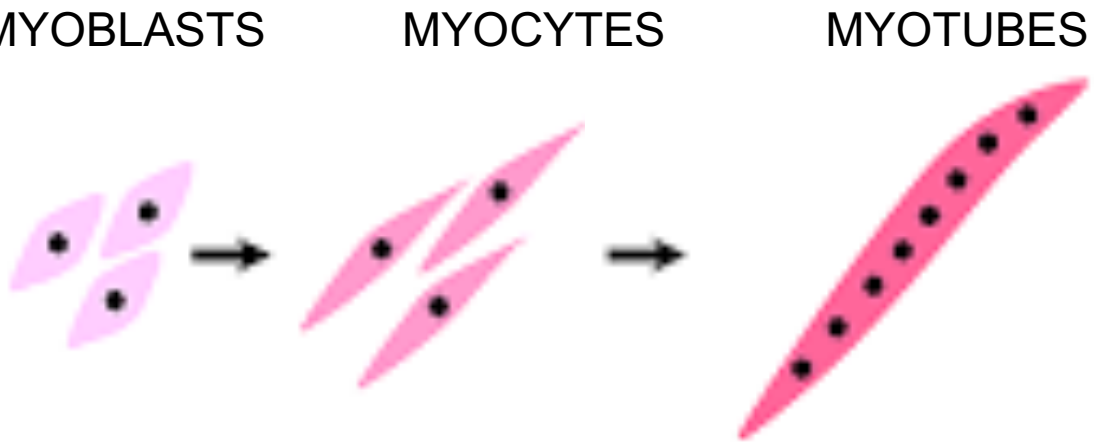
MYOBLASTS MYOCYTES MYOTUBES



SERUM DEPRIVATION
+ CELL-CELL CONTACT ORIENTATION FUSION

What can we do with cells?

- STUDY BIOLOGICAL PROCESSES -> **Myogenic differentiation**



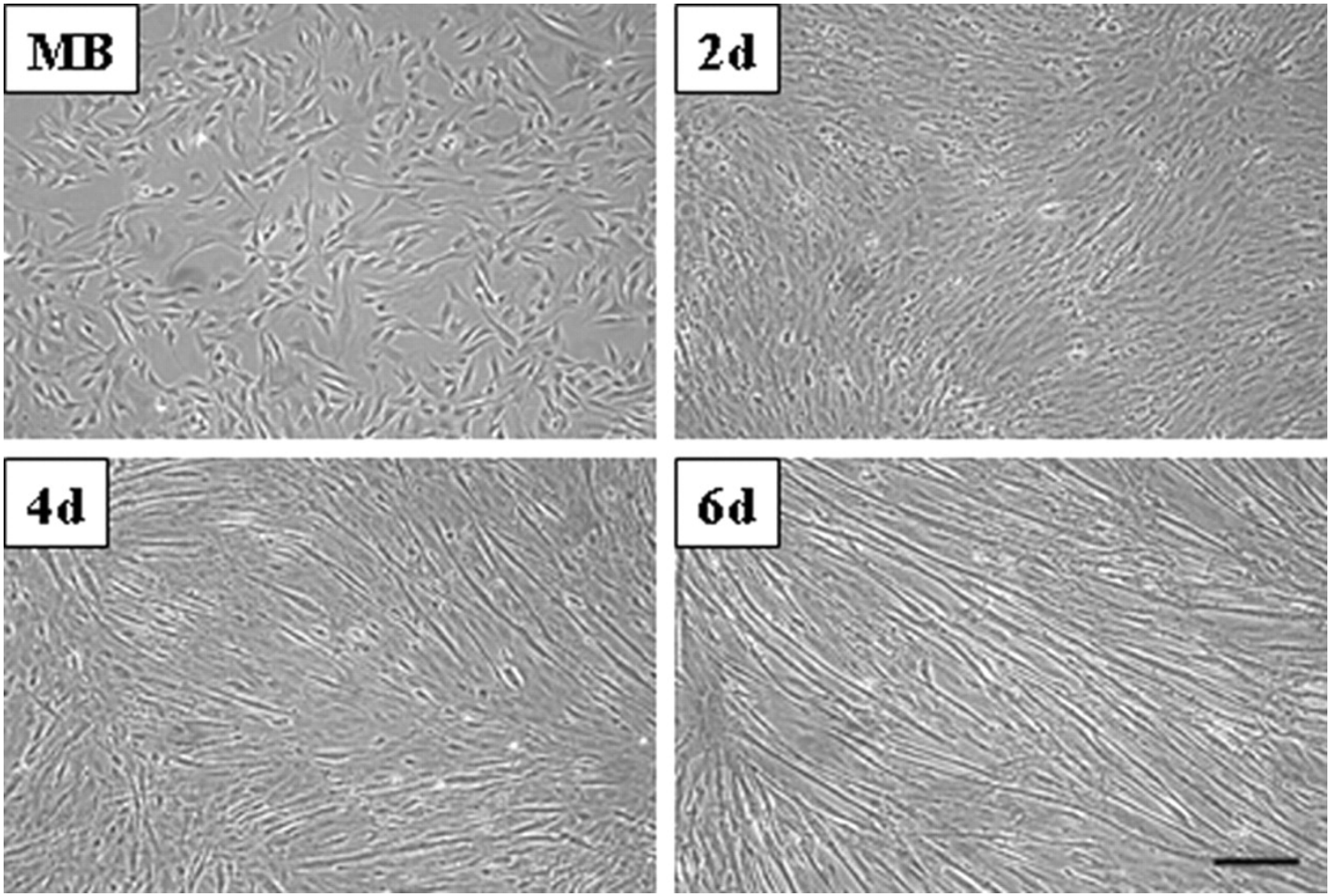
C2C12: mouse myoblasts

growth conditions: DMEM + **10%** or **20**% FBS

at confluence -> switch to **differentiation medium**: DMEM + **0.5**% or **2%** FBS

What can we do with cells?

- STUDY BIOLOGICAL PROCESSES -> **Myogenic differentiation**



Summary

- Cell culture equipment: what do we need to work with cells?
- Different types of cell cultures
- What can we do with cells?
- Organoids and 3D cultures

Organoids and 3D cultures

"[…] we define an organoid as an *in vitro* 3D cellular cluster derived exclusively from primary tissue, ESCs or iPSCs, capable of self-renewal and self-organization, and exhibiting similar organ functionality as the tissue of origin.

Most of the documented organoid cultures contain functional tissue units that lack the mesenchymal, stromal, immune and neural cells that intersperse the tissue *in vivo.*

These organoids rely on artificial extracellular matrices (ECM) to facilitate their self-organization into structures that resemble native tissue architecture."

Fatehullah *et al*., Nature Cell Biology, 2016

Organoids and 3D cultures

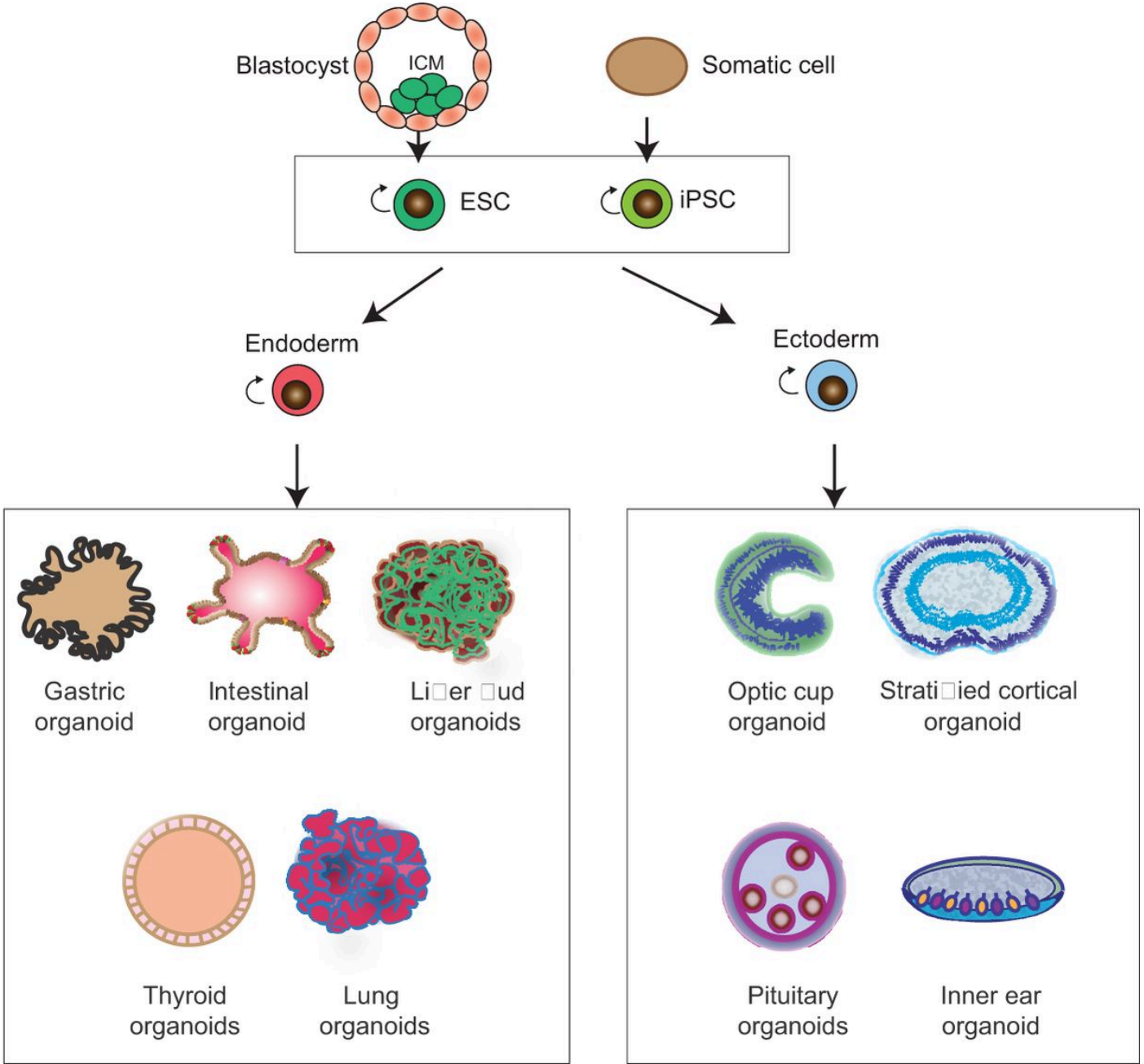
Advantages
Near-physiological model system for studying adult stem cells and tissues in a variety of contexts
Adult stem cells can be propagated in organoids, and specific tissue lineages can be cultured in high purity with minimal contributions from other cell types (for example, fibroblasts and endothelial cells)
Can be propagated for a long time (years) without genomic alterations
Amenable to a wide variety of established experimental techniques
Can be derived from multiple sources: adult and foetal tissues, ESCs and iPSCs
Can generate organoids encompassing a broad range of tissues
Limited amounts of starting material can be expanded for numerous applications
Human diseases that are difficult to model in animals can be studied with patient-derived organoids
Possibility of generating isogenic adult tissue for transplantation in regenerative procedures

Limitations	Possible solution(s)
Lack of native microenvironment precludes studies about interaction of stem cells with their niches, immune cells, etc.	Complement with organotypic culture system, or co-culture with other cell types such as stromal cells[15] or immune cells
Limited use in modelling inflammatory responses to infection or drugs due to absence of immune cells in culture system	
Unable to mimic *in vivo* growth factor/signalling gradients in Matrigel matrix	Apply microfluidic technologies to generate concentration gradients
Unable to mimic biomechanical forces that stem cells encounter *in vivo*	Novel substrates and ECM factors are being identified to model such interactions *in vitro*[50–52]
Relatively rigid ECM could limit drug penetration, hence hampering the use of organoids in drug screens	Devise ways to vary physical attributes of ECM such as composition, porosity and stiffness
Challenging to culture organoids from tissues whose niche factors are not well understood (for example, the ovary)	Screen for small-molecule modulators of key signalling pathways and specific hormones as potential culture components
Organoids in the same culture are heterogeneous in terms of viability, size and shape, impeding phenotype screens	Organoids can be tracked individually by live or time-lapse imaging
Organoid cultures depend on mouse-sarcoma-derived Matrigel, which precludes transplantation of organoids into humans	More defined ECMs that support organoid growth are being developed to comply with regulations for transplantation into humans

Fatehullah *et al.*, Nature Cell Biology, 2016

Organoids and 3D cultures

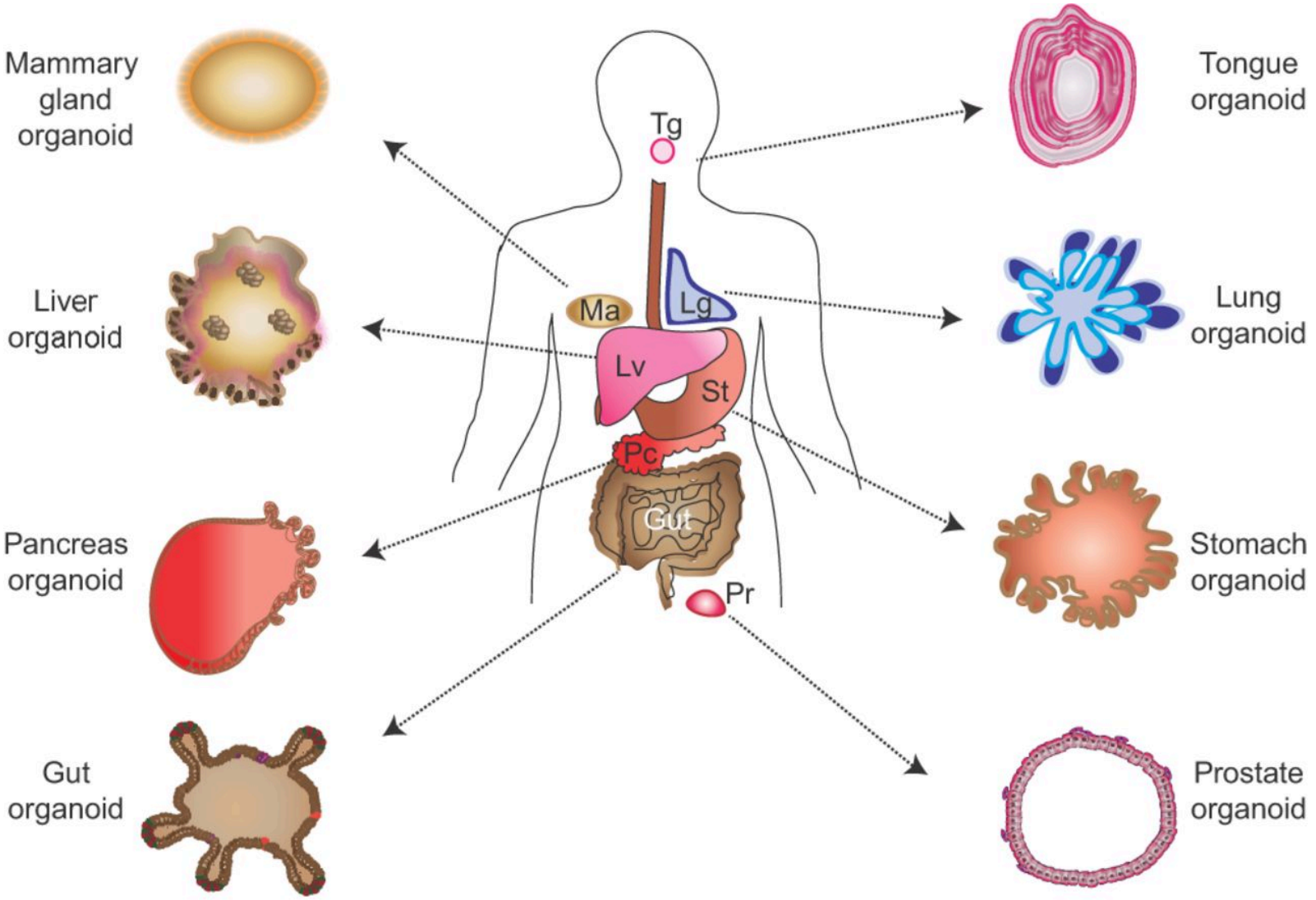
Pluripotent stem cell (PSC)-derived organoids



Huch and Koo, Development, 2015

Organoids and 3D cultures

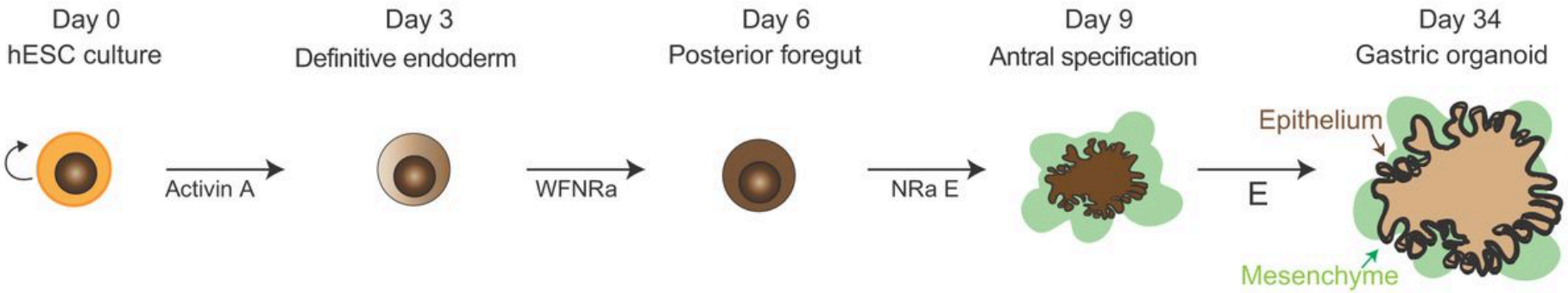
Adult Stem Cell-derived organoids



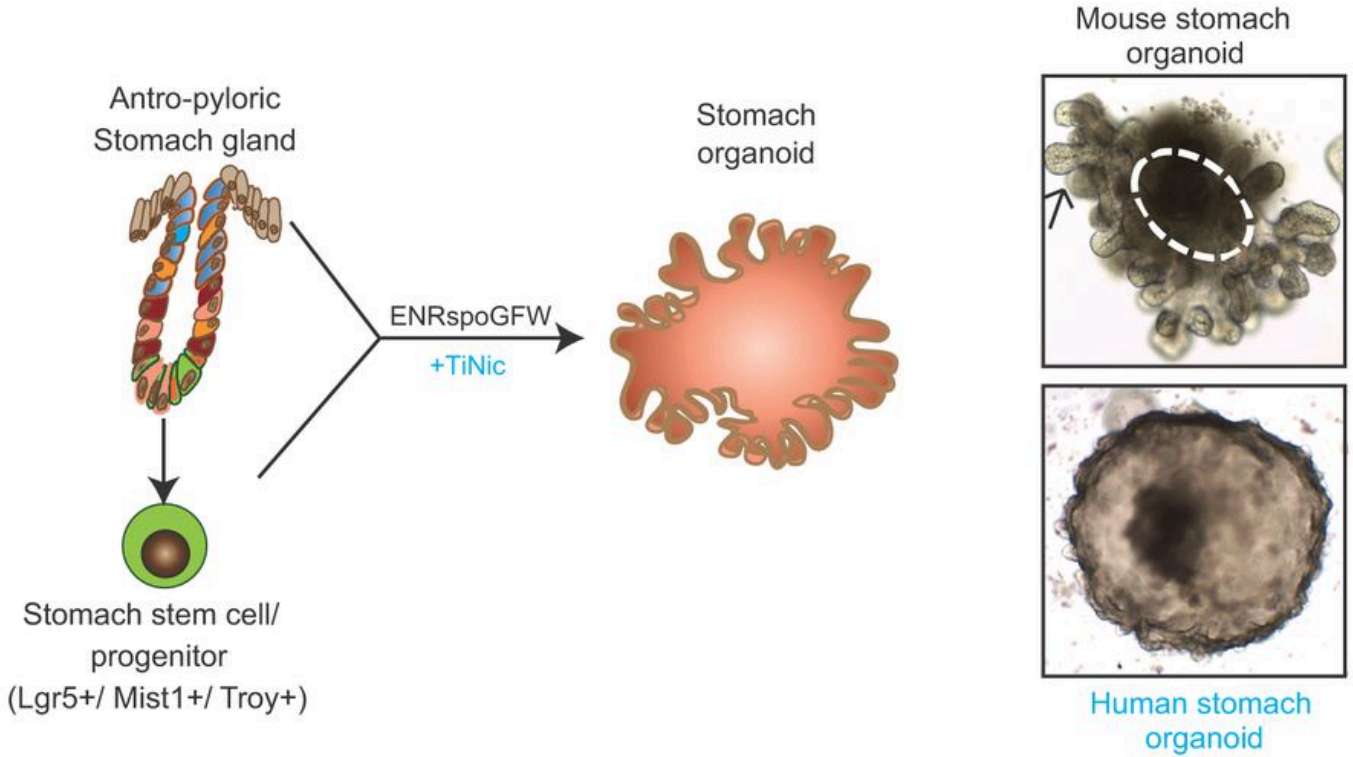
Huch and Koo, Development, 2015

Organoids and 3D cultures

Pluripotent stem cell (PSC)-derived organoids



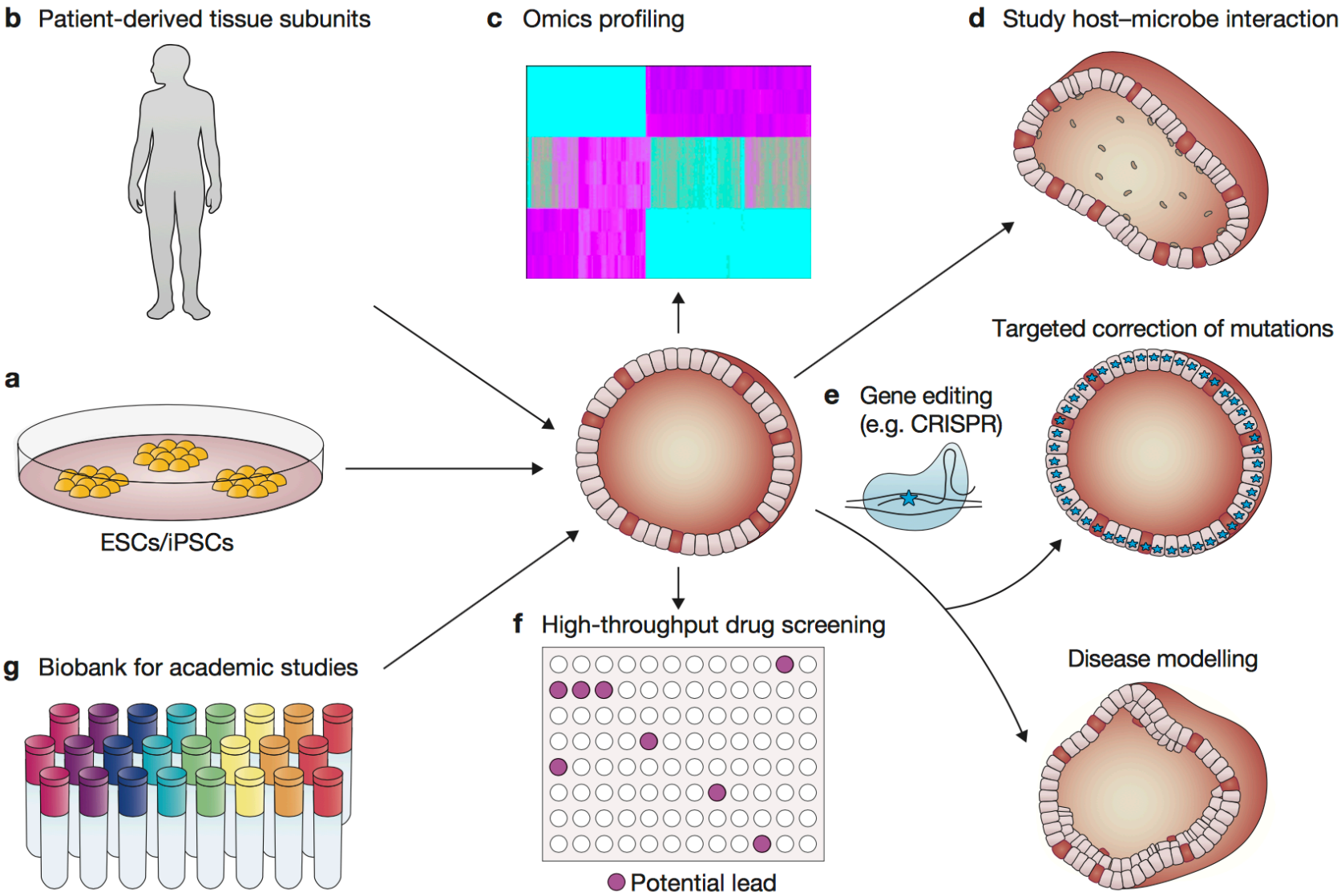
Adult Stem Cell-derived organoids



Adapted from Huch and Koo, Development, 2015

Organoids and 3D cultures

Applications of organoid technology for studying development, homeostasis and diseases



Fatehullah *et al.*, Nature Cell Biology, 2016