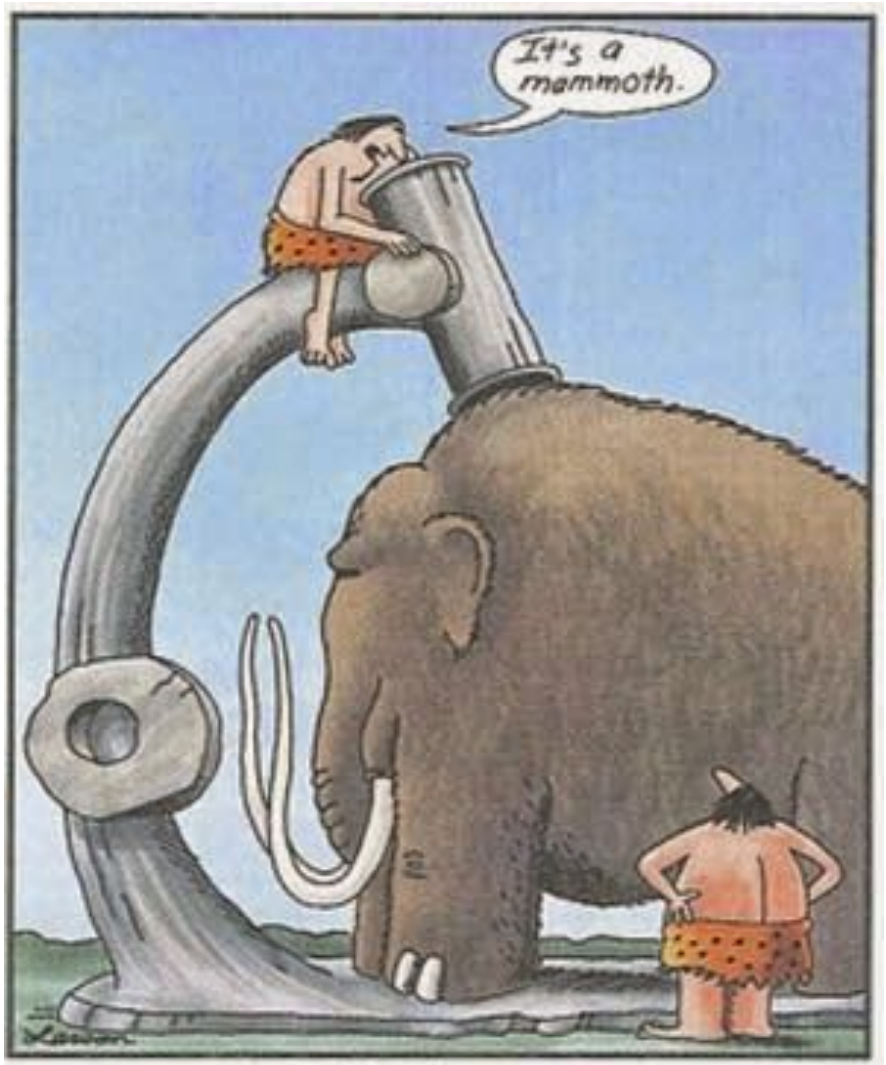
A great set of tools for cell analysis



Early microscope

université PARIS-SACLAY

FACULTÉ DE PHARMACIE

université PARIS-SACLAY
GRADUATE SCHOOL
Health and Drug Sciences

d2hp

D2HP, M1 International, Cancer Cell biology, TU n°05

Before becoming a drug blockbuster…

Complex, long, expensive and risked

AFM-Téléthon, Code de santé publique

Cells in culture

Primary cells : represent the tissue of origin

Difficult to culture and maintain, variability from donors

1917, aseptic and nutrients

Keratinocyte, enterocyte, endothelial cell, myocyte, fibroblast, hematopoietic stem cells ...

Transformation of primary cells in immortalized secondary cell line

Spontaneous / chemically or virally induced, easy to culture, no variability

HeLa, 1951, human cell line, derived from cervix cancer from Henrietta Lacks

Stem cells

Embryonic stem cells (ESCs) : (totipotent) pluripotent from blastocyst

1981 (mouse), 1998 (human) from blastocyst inner cell mass

Induced pluripotent stem cells (iPSCs)

2006 (from mouse, human fibroblasts)

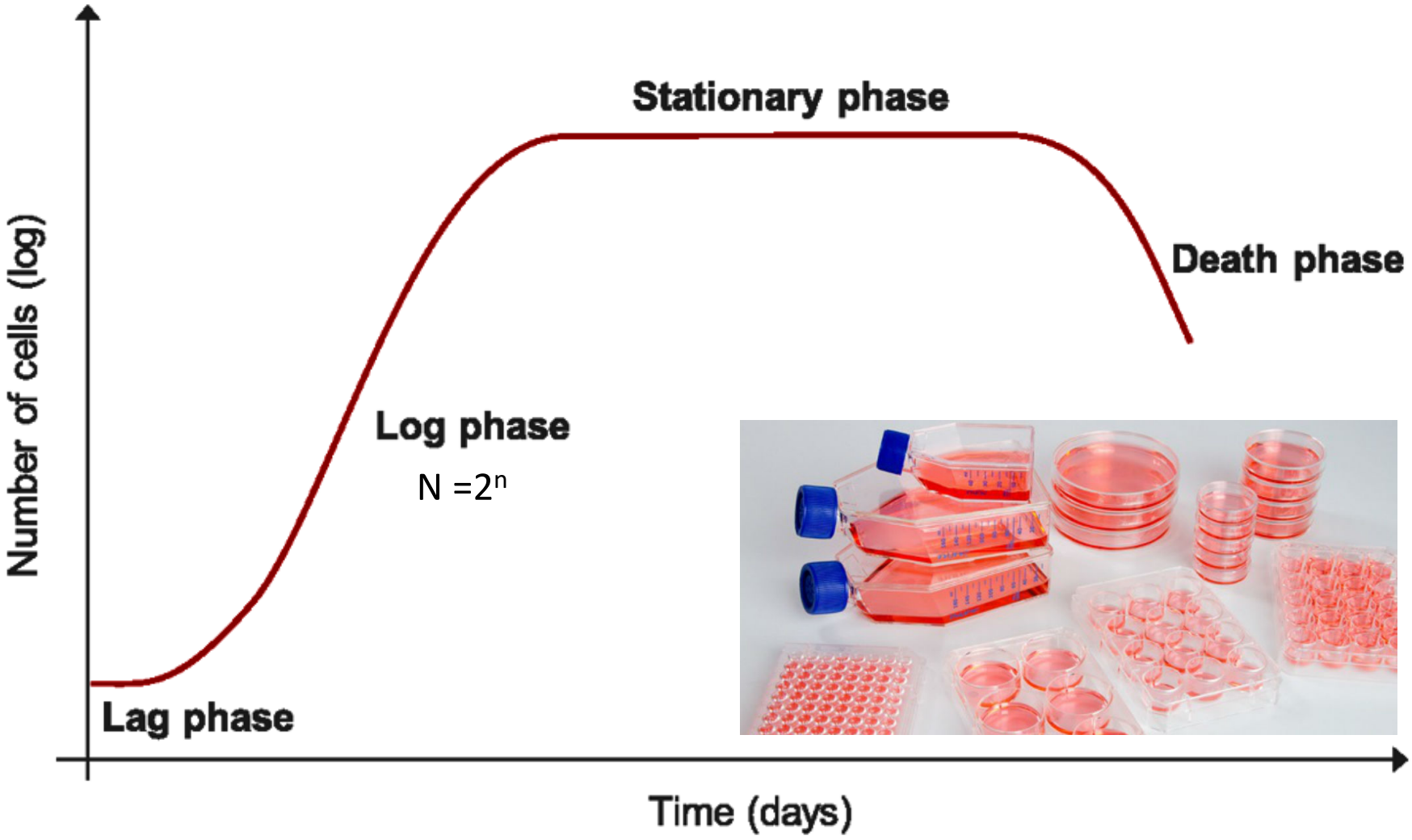
Nobel Prize in Physiology or Medicine 2012 John B. Gurdon & Shinya Yamanaka

Induced multipotent stem cells (ex : induced neural stem cell iNSCs)

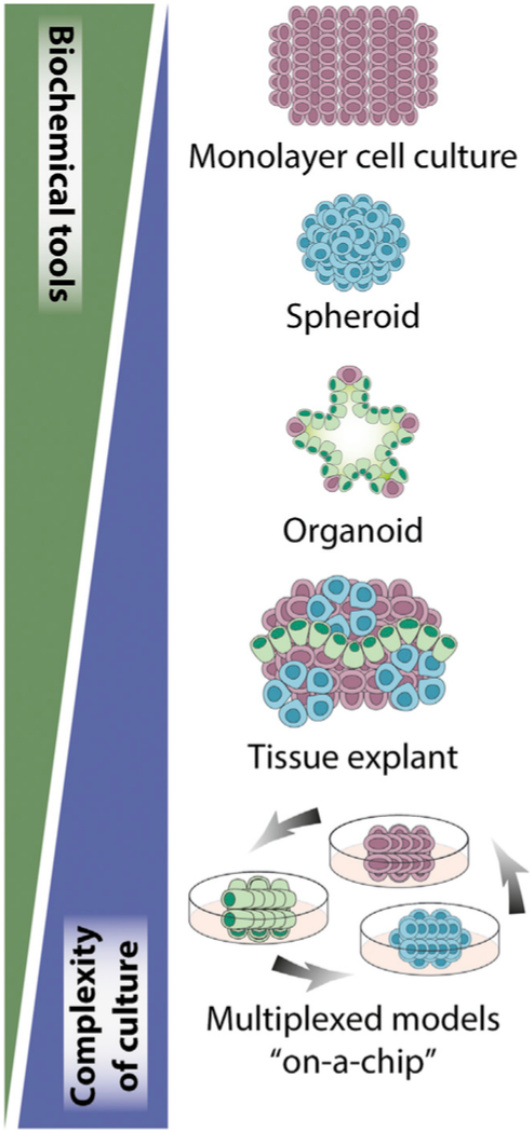
2012 (from fibroblasts). Reduced carcinogenic potential compared to iPSCs

Sakalem et al, Biotech. J., 2020

Cell line in 2D culture



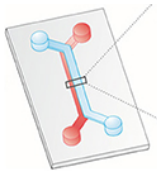
2D versus 3D cell culture



Primary cells
Immortal secondary cell lines
Stem cells

At least on cell type

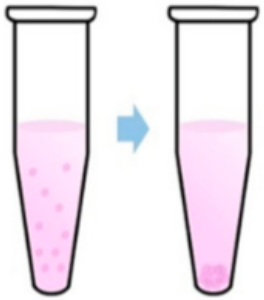
At least one epithelial and one mesenchymal cell type



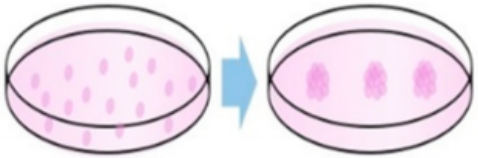
Yinet al, Cell Stem Cell, 2016

Spheroid/organoid scaffold-free culture methods

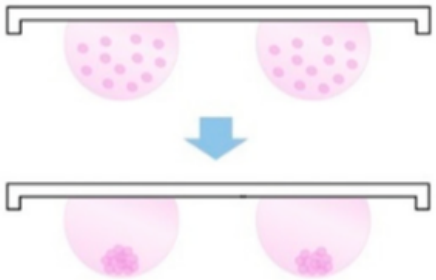
pellet



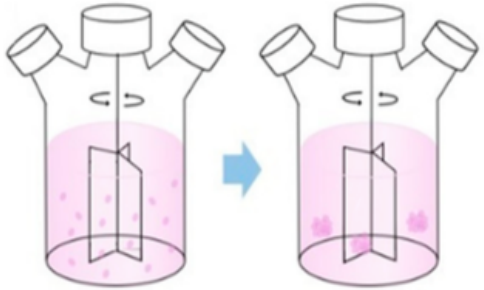
non adhesive flask

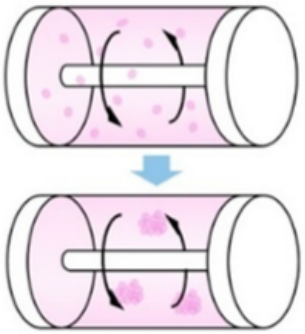


hanging drop

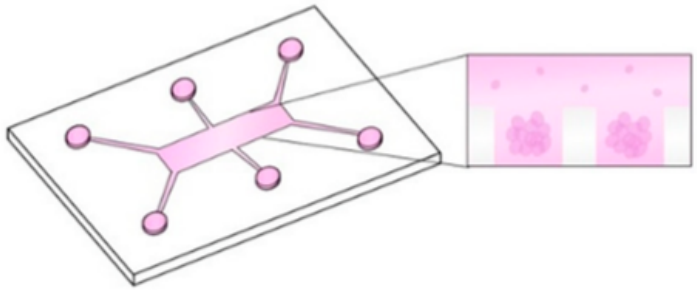


stirring vessel

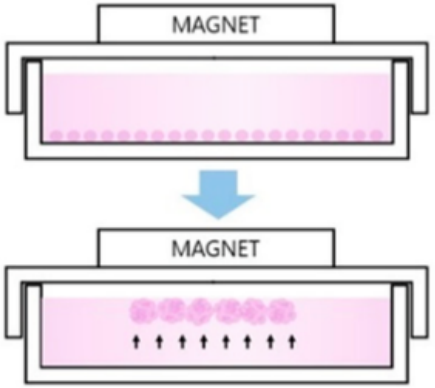




rotating vessel



Microfluidic
(organ on a chip)

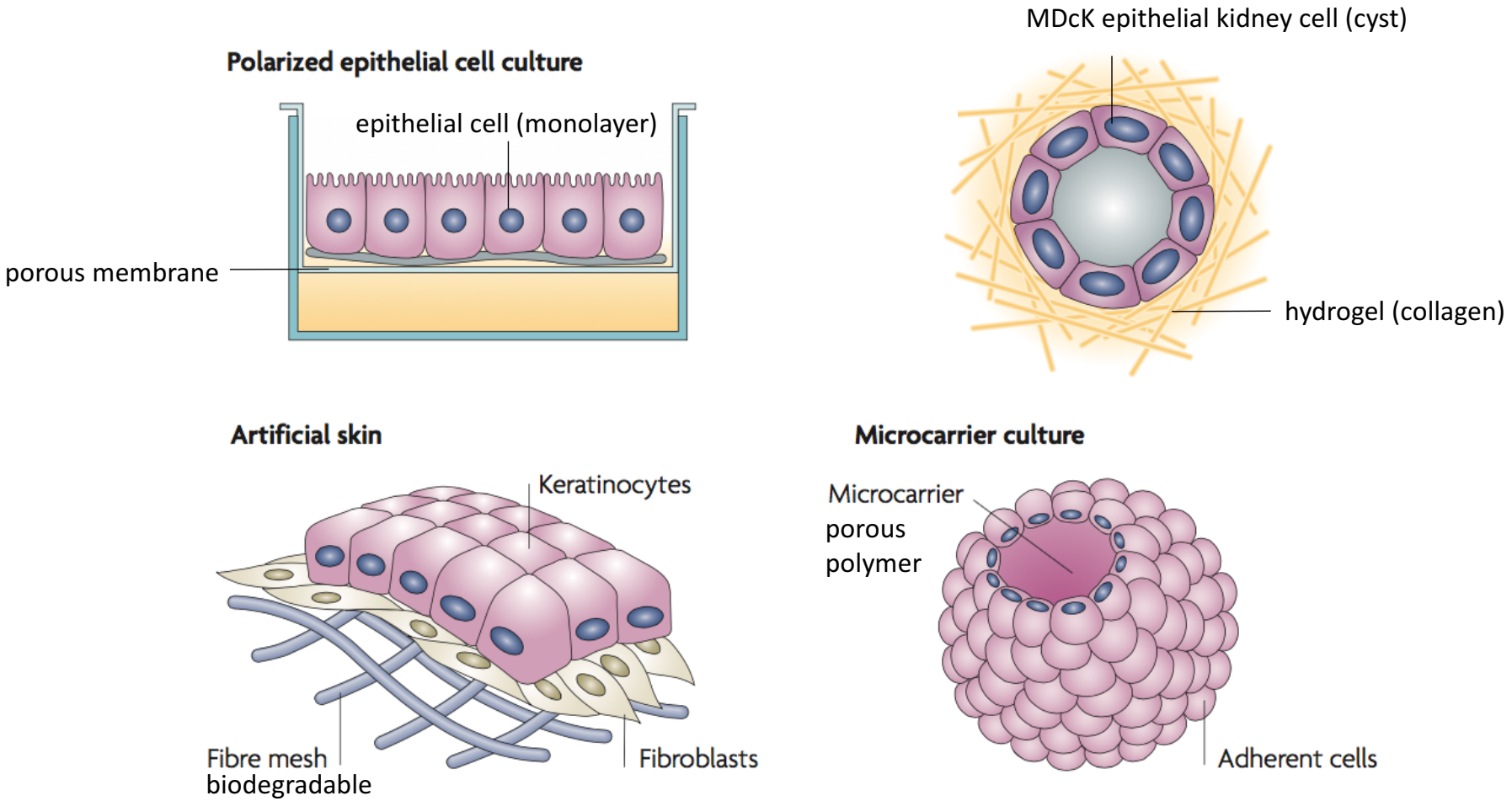


Magnetic levitation

Rely on cell self-assembly and prevention of cell adhesion to the flask

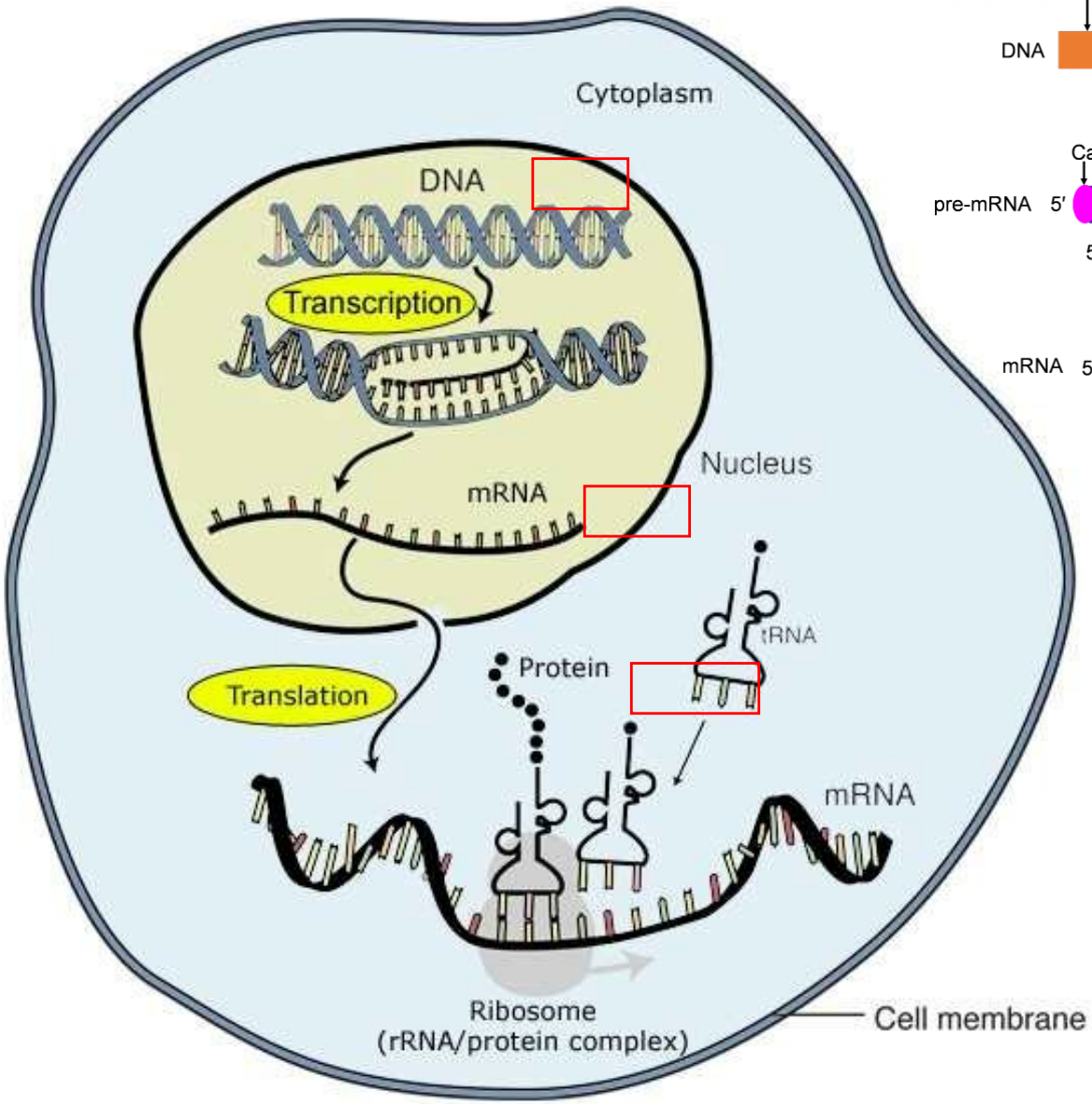
Ryu et al, Cells, 2019

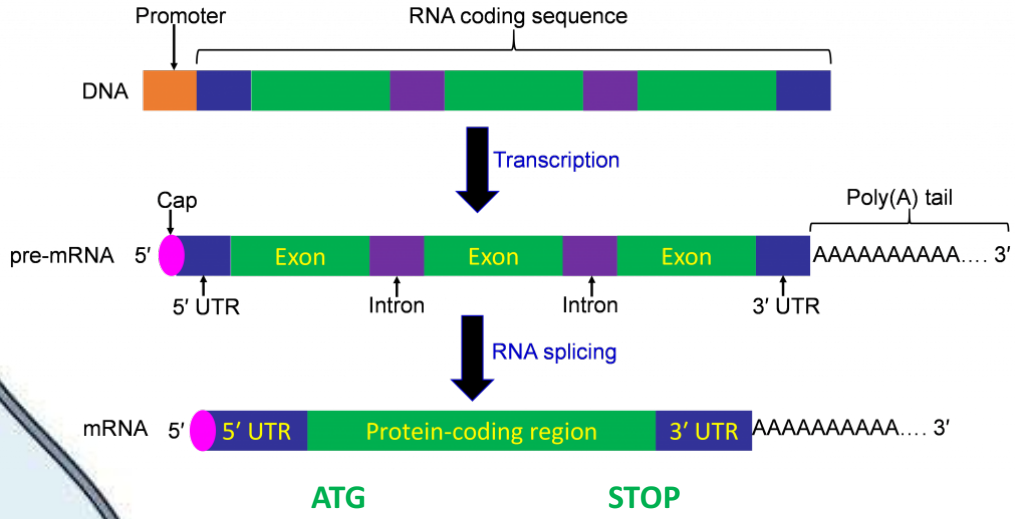
Scaffolds for 3D culture



Adapted from Pampaloni et al, Nat. Rev. Mol. Cell. Biol., 2007

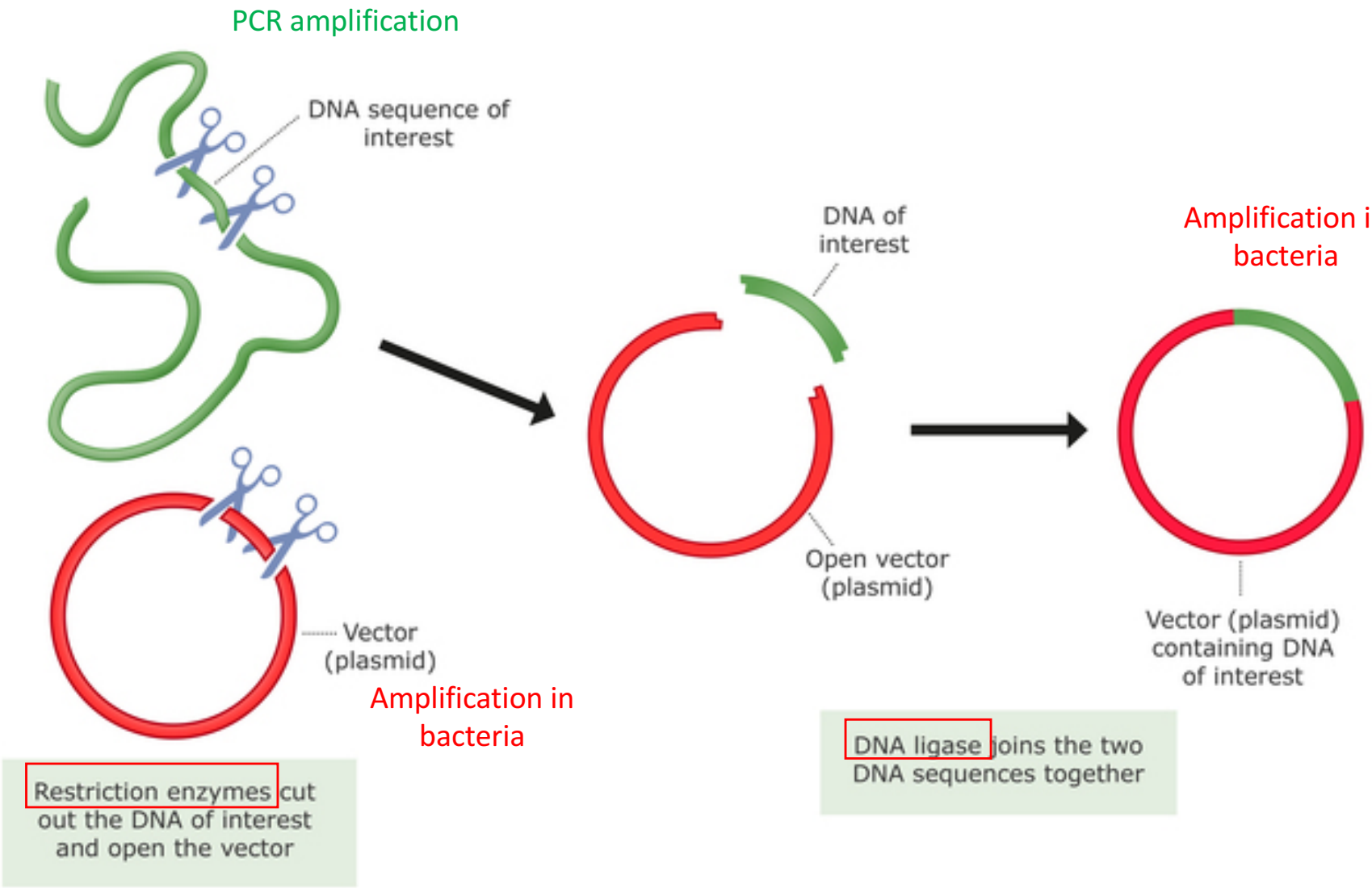
Gene expression





Allemeersch, 2006
https://bio.libretexts.org/

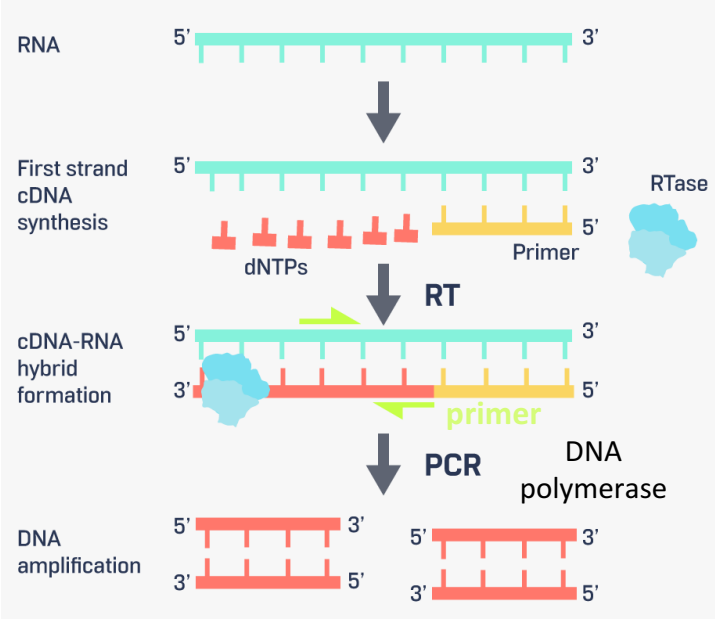
cloning



Restriction enzymes
Nobel Prize in Physiology or medicine 1978,
Arber, Nathans, Smith

RT-qPCR

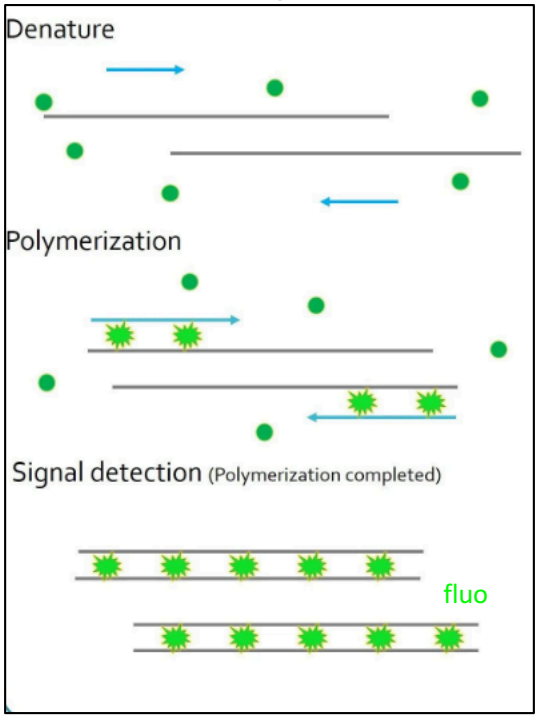
Reverse transcription (RT) + Polymerase chain reaction (PCR)



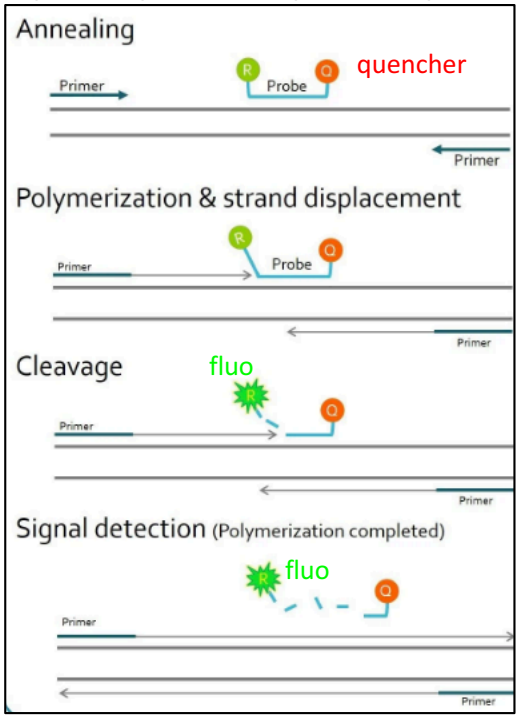
cDNA bank /cloning
Relative abundance of RNAs

Realtime quantitative PCR (qPCR)

SYBR green



TaqMan (probe sequence specific)

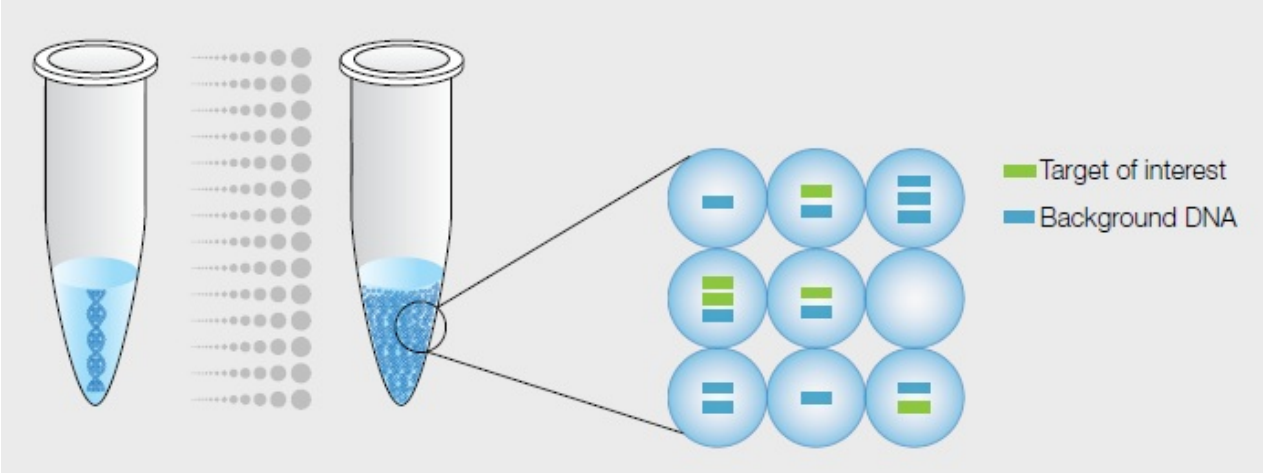


Relative abundance of RNAs

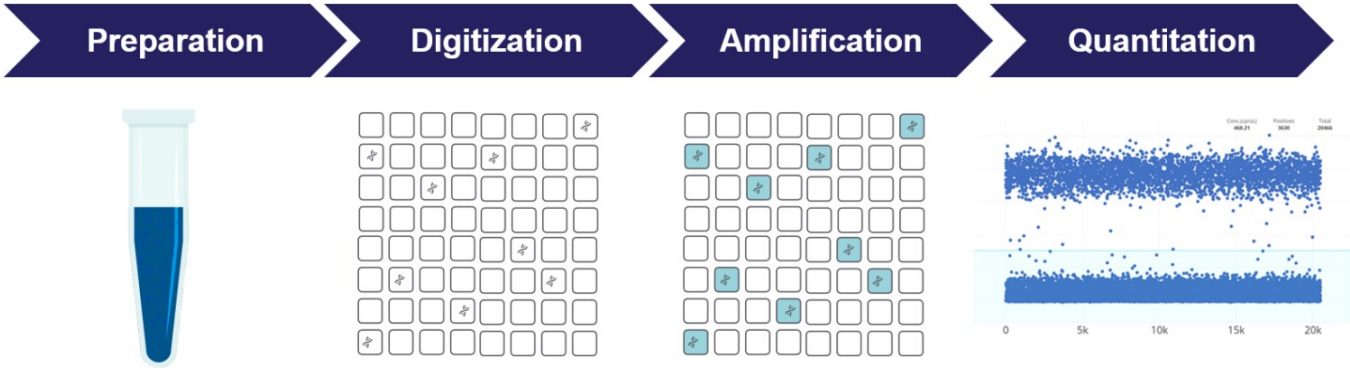
PCR, Nobel Prize in Chemistry 1993, Mullis & Smith

Biotech graphic
ThermoFisher Scientific

Third generation PCR : digital PCR



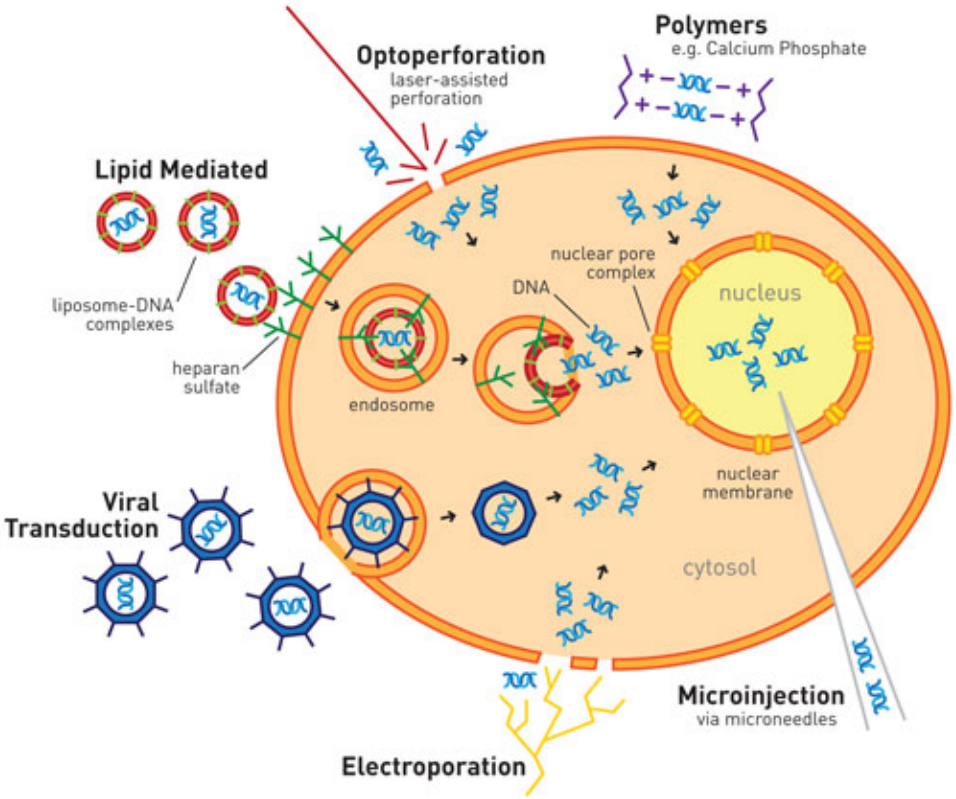
absolute quantification through partitioning the reaction



Biorad, thermofisher

Cell transfection, fluorescent-tagged protein

DNA transfection (transient / stable)



DNA plasmid → mRNA → protein
Can also do mRNA transfection

Sculpture of GFP
San Juan Island
(Washington)

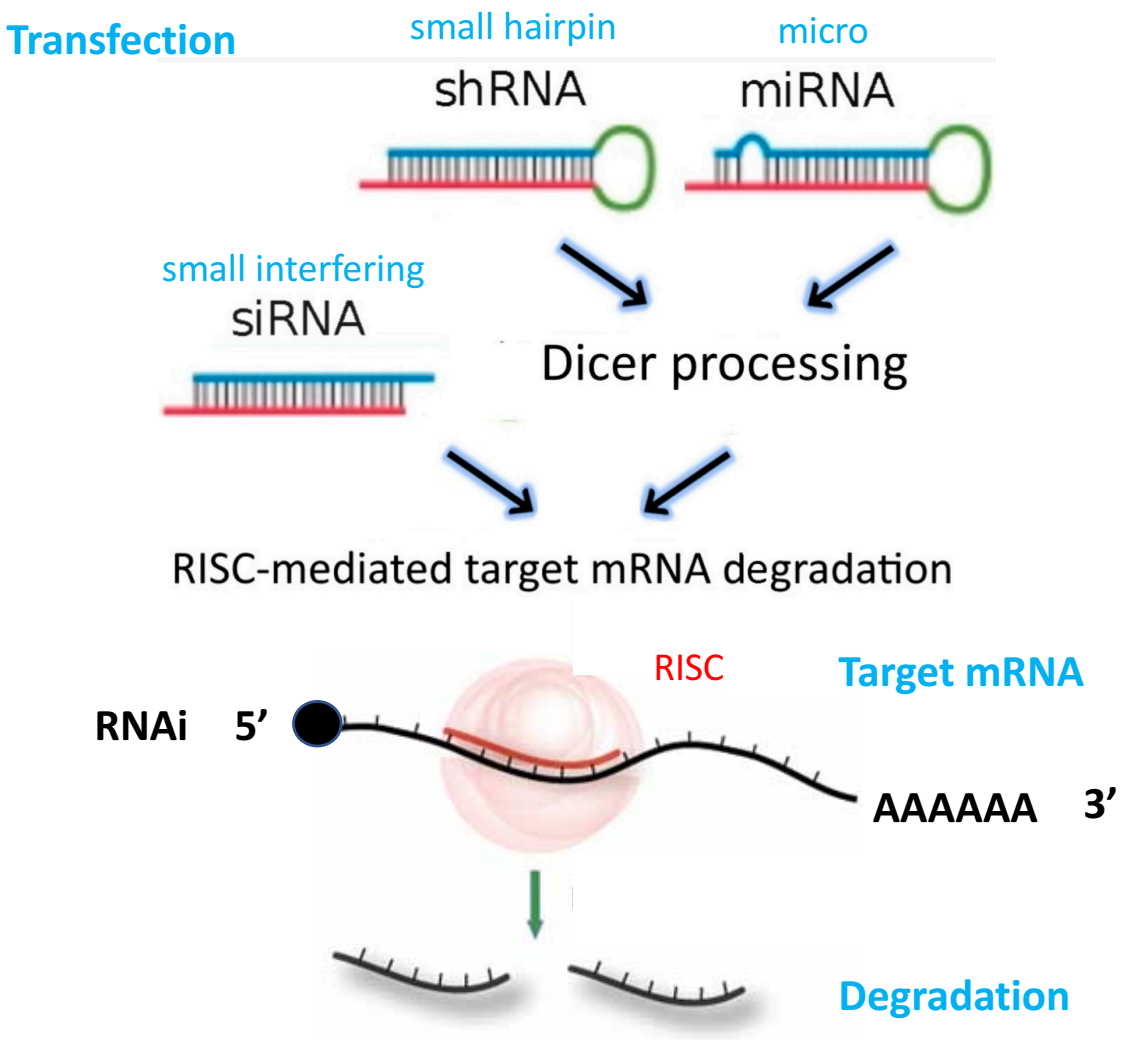
GFP **protein**

Fluorescent fusion protein

GFP, Nobel Prize in Chemistry 2008,
Shimomura, Chalfie & Tsien

Gene function and / or protein localization study
- Over-expression / rescue
- Expression Tagged protein

RNA interference (RNAi) : gene silencing



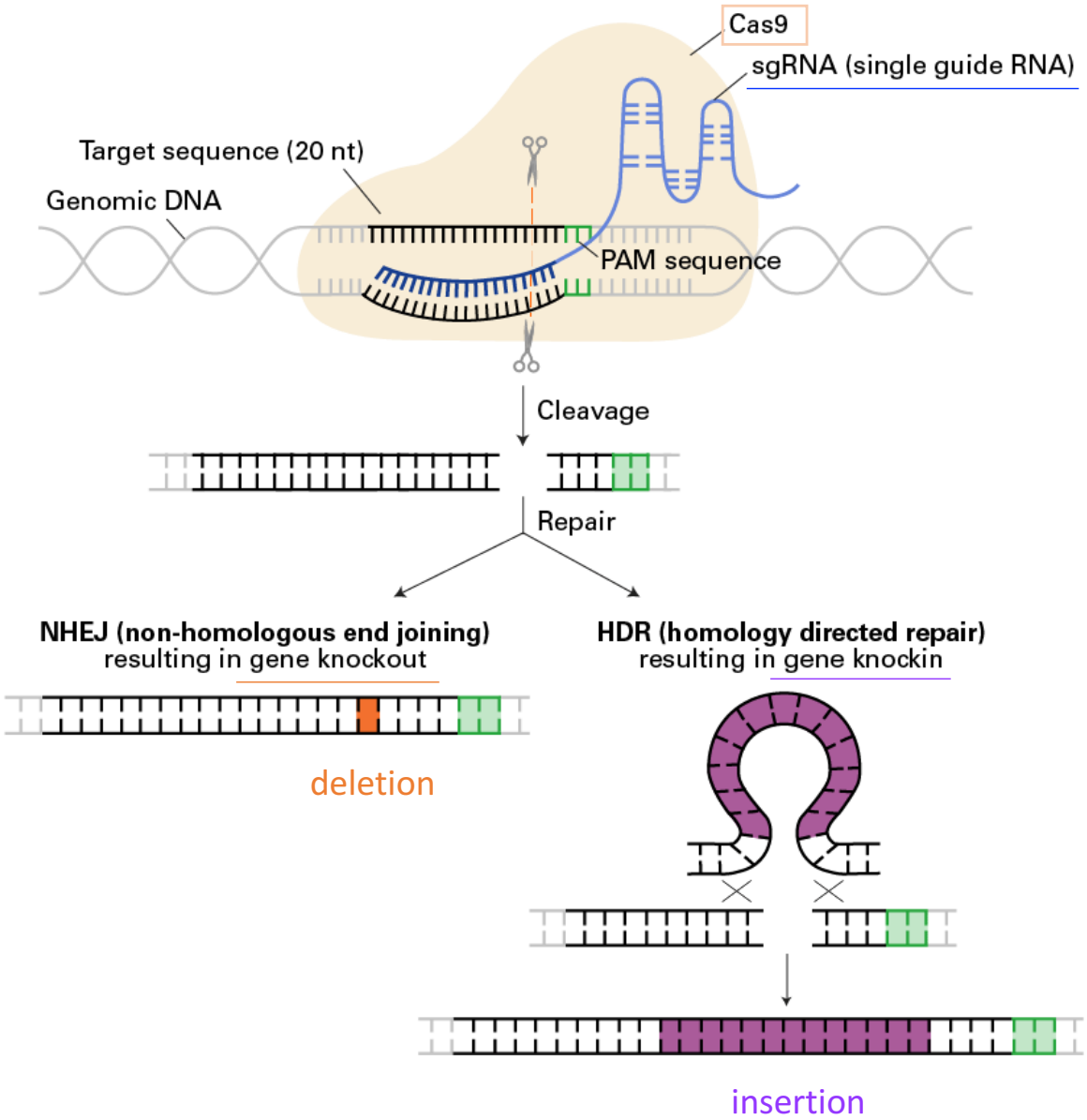
Discovered in worm
C. *elegans*

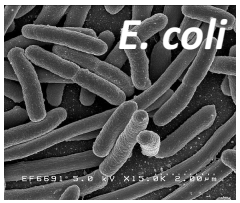
RNAi
Nobel Prize in Physiology or Medicine 2006,
Fire & Mello

Gene function study
Phenotype in absence of the gene

Adapted from Mainini & Eccles, Molecules, 2020
Kalavrizioti et al., Current Topics in Medicinal Chem., 2006

CRISPR - Cas9 : genomic molecular scissors



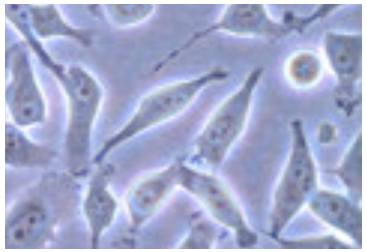


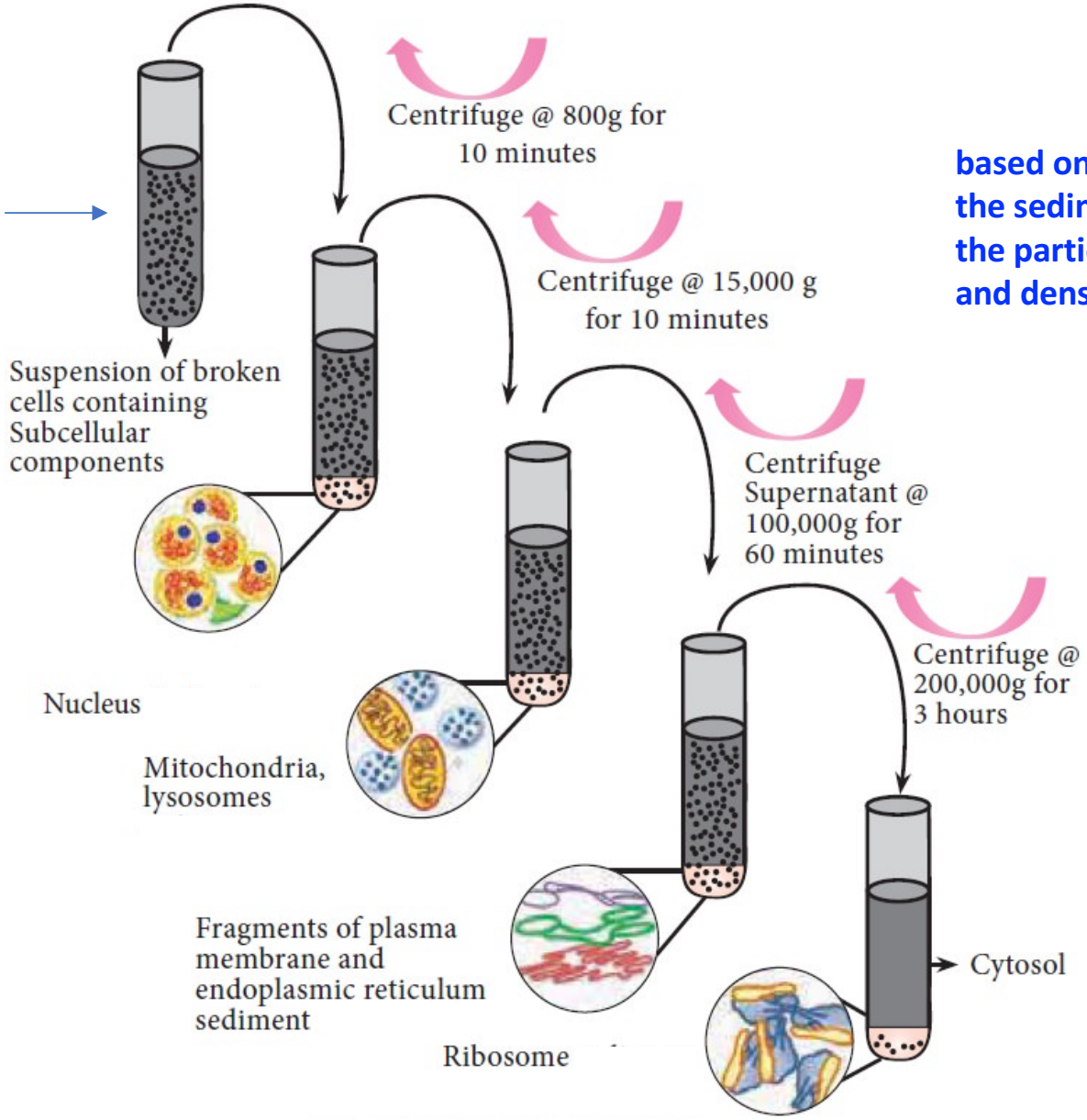
Discovered in bacteria

Nobel Prize in Chemistry 2020,
Charpentier & Doudna

Takara

Differential centrifugation : cell fraction

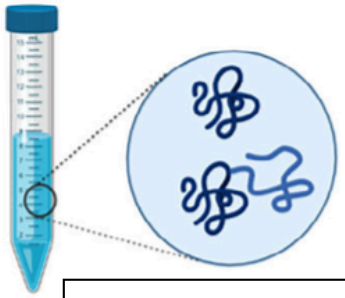




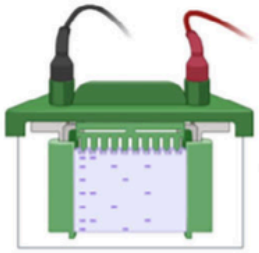
based on the difference in the sedimentation rate of the particles of different size and density

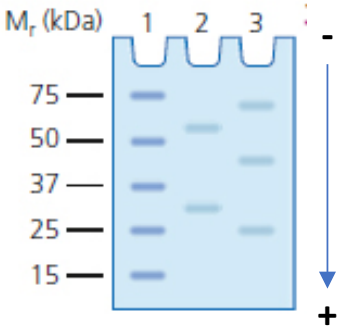
https://www.brainkart.com/article/Centrifugation-techniques

Western-blot : protein analysis



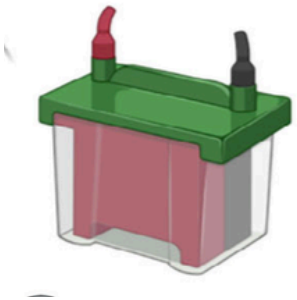
Protein extract

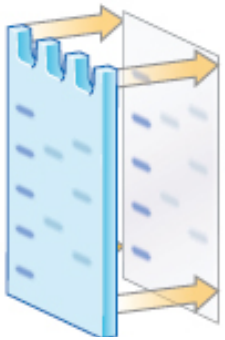




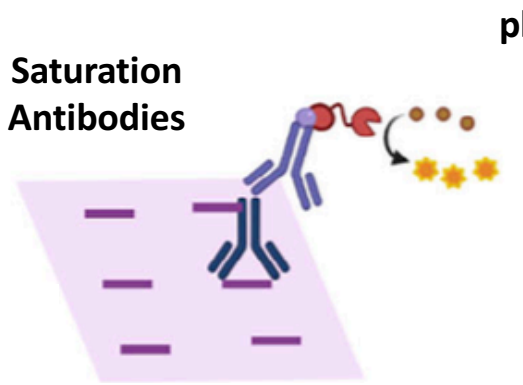
SDS-PAGE
Protein separation by size in acrylamide gel

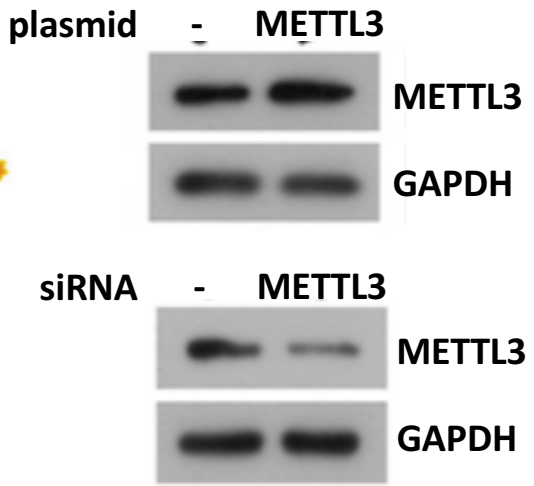
Blotting
Protein transferred on a membrane





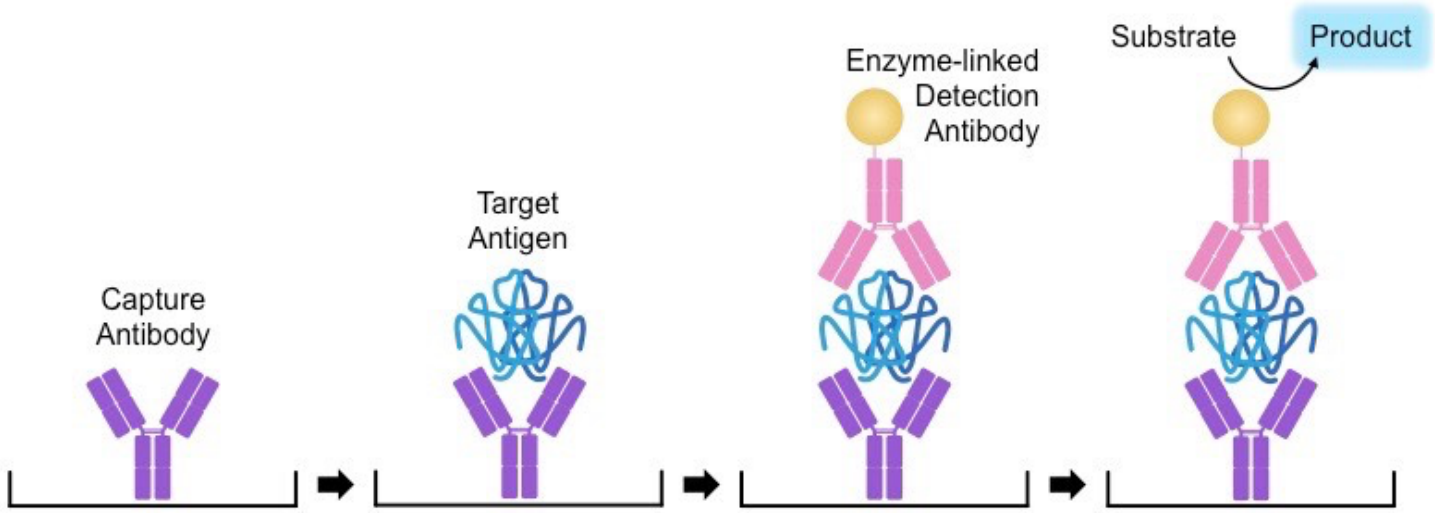
Immuno-revelation
Specific antibody for antigen recognition

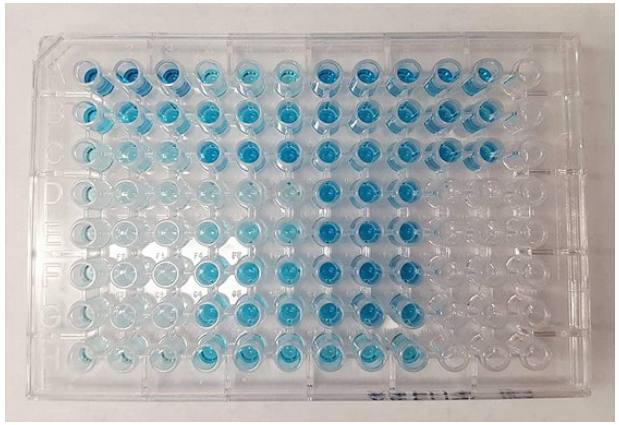




Adapted form Meftahi et al., 2020

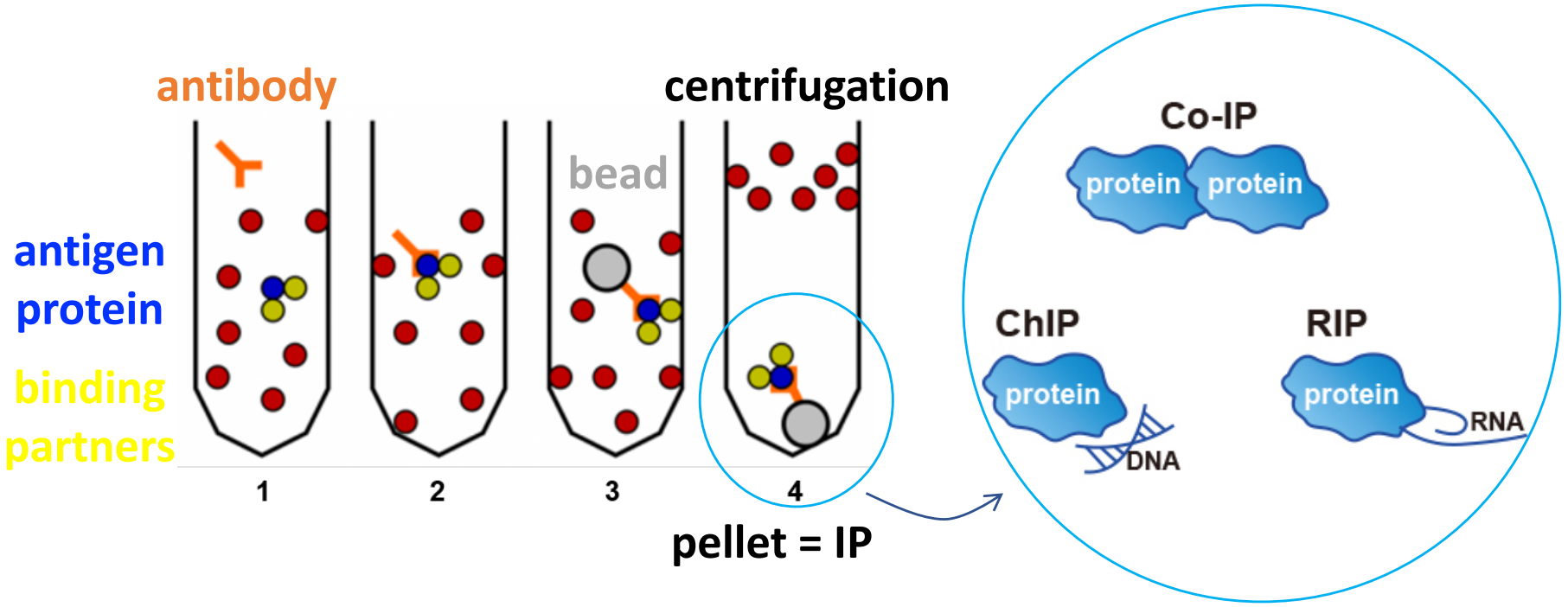
ELISA : immuno-enzymatic assay

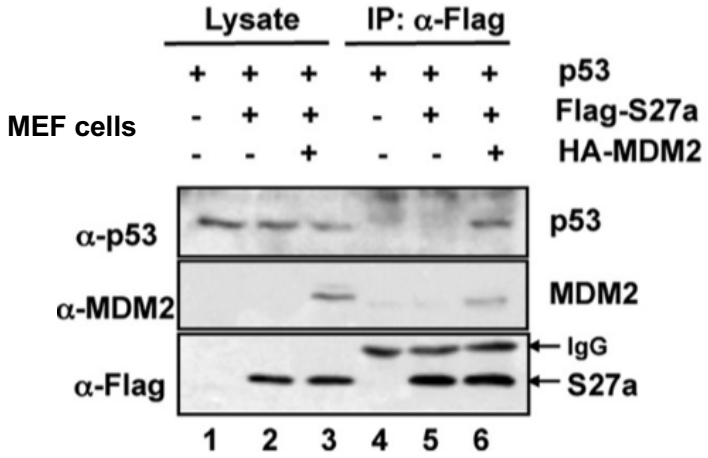




Medical lab analysis :
cytokine inflammation, COVID-19, VIH, IgE, hormones, tumor markers…

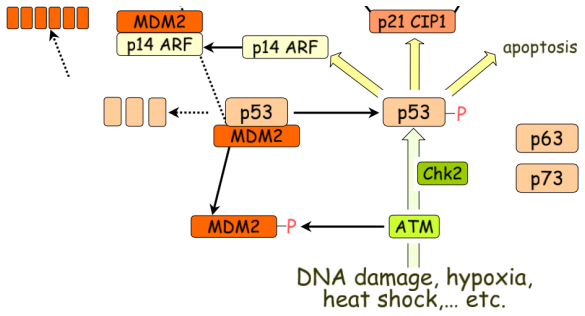
Immunoprecipitation (IP) : binding partners





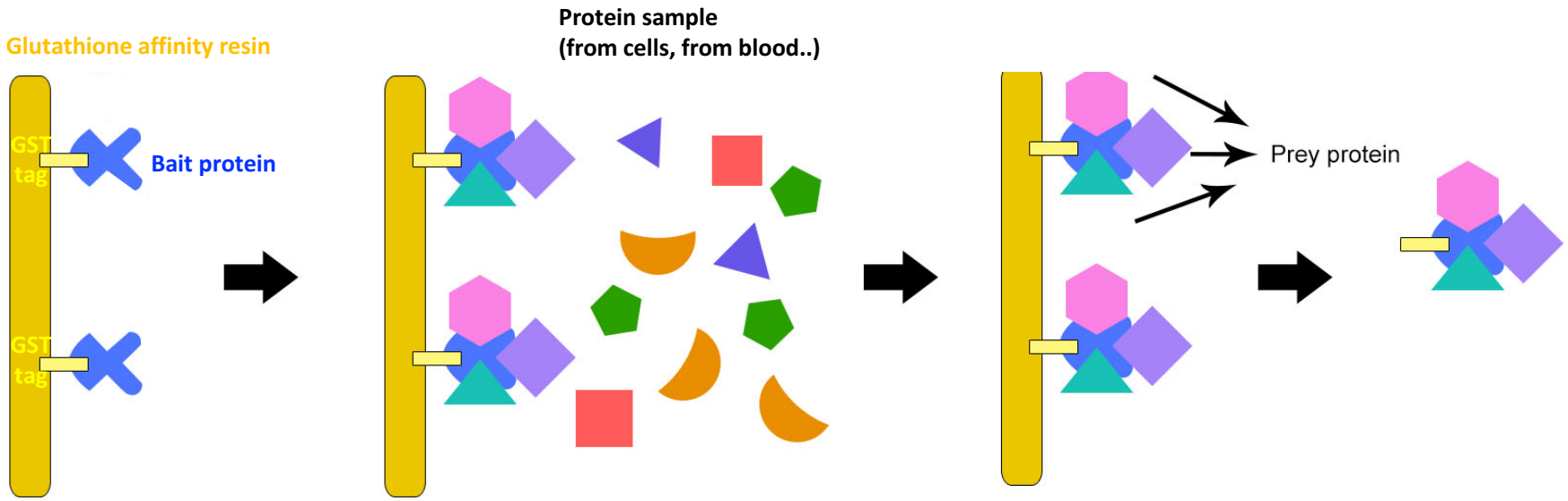
S27a (a ribosomal protein) interacts with p53 through MDM2

In case of ribosomal stress : suppresses MDM2-mediated p53 ubiquitination, leading to p53 activation and cell cycle arrest.

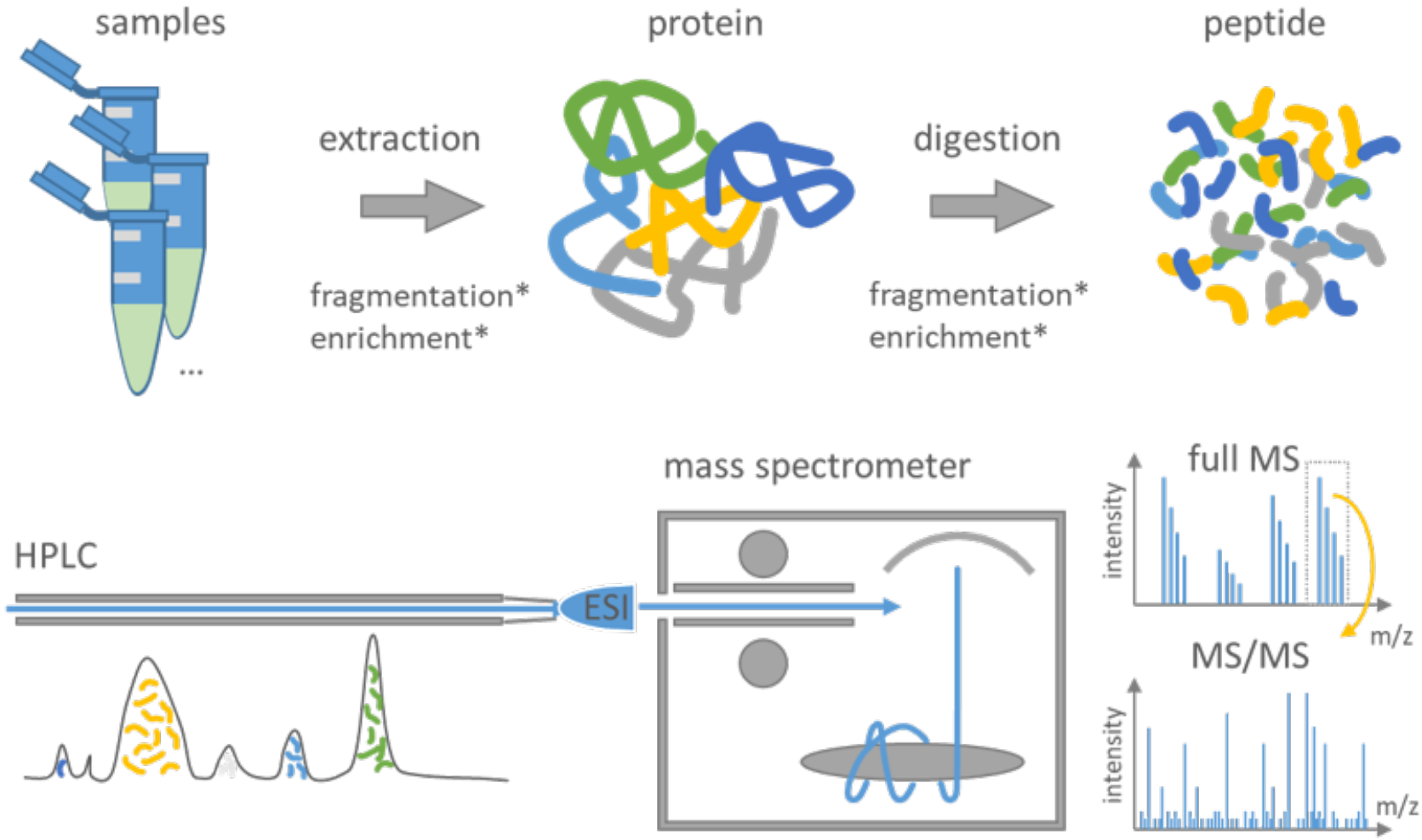


Sun et al., JBC, 2011

Pull-down assay: binding partners



Mass spectrometry

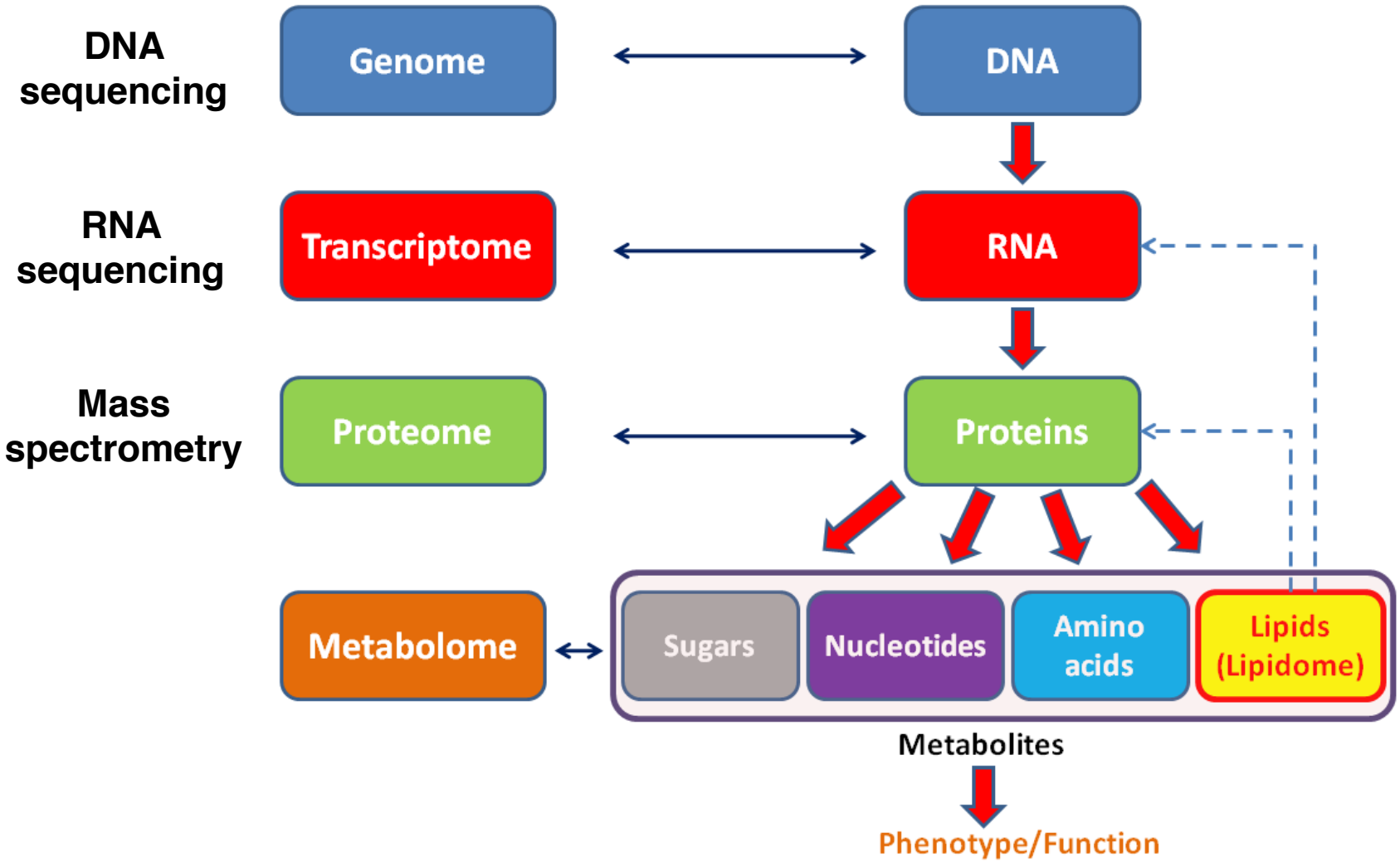


Mass / charge

Sequencing of peptides
Post-translational modifications

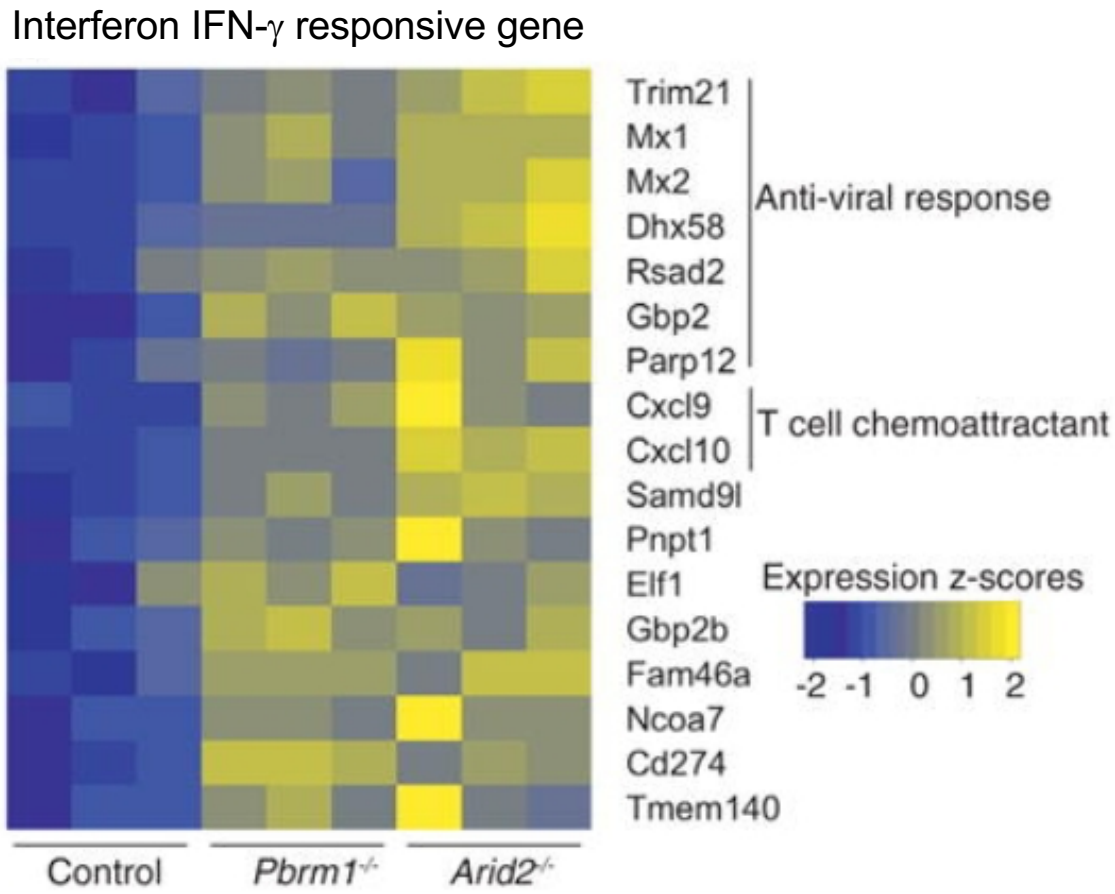
https://csbiology.github.io/BIO-BTE-06-L7/NB02a_Mass_spectrometry_based_proteomics.html

Omic approach (single cell possible)



https://entoxsimplified.com/2019/04/15/understandomics/

Heat map : graphical representation of data

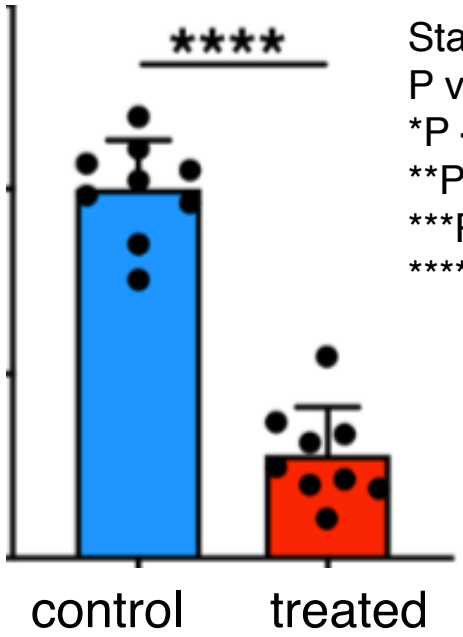


Magnitude of a phenomenon as color in two dimensions

Pan et al., PMC, 2018

Graphical representation of data & statistic

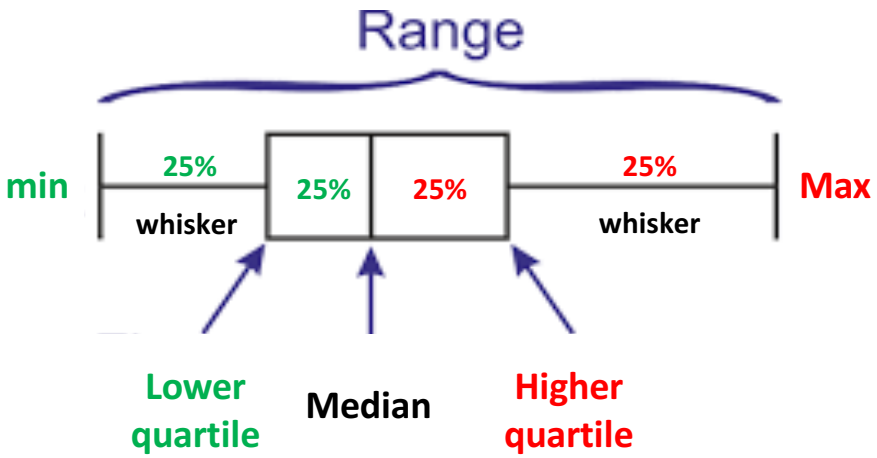
histogram



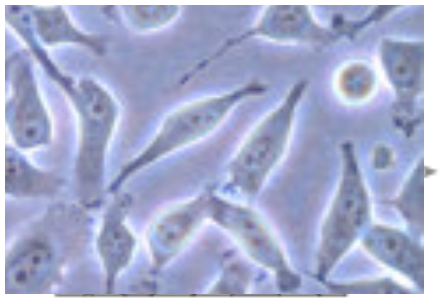
Statistical test
P value
*P < 0.05,
**P < 0.01
***P < 0.001
****P < 0.0001

● single data values
T Standard deviation

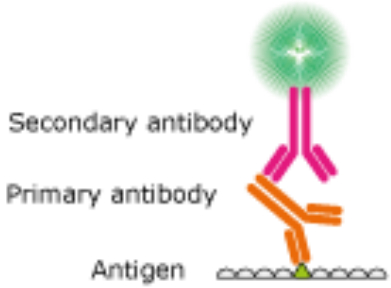
box plot



Immuno-staining, immuno-fluorescence

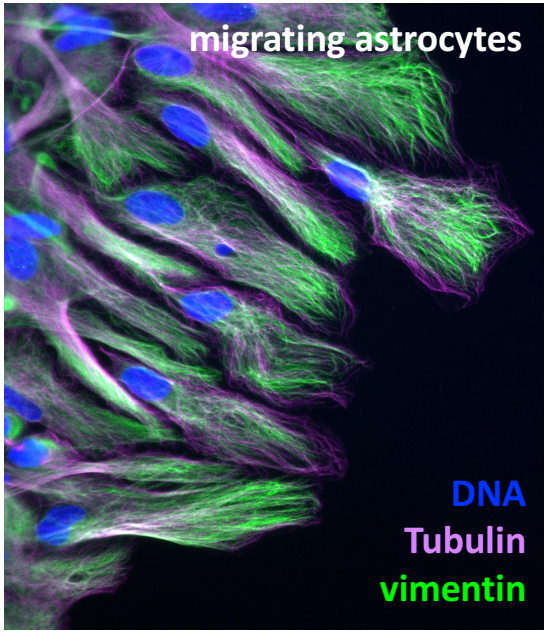


Cell culture



Fixation (alcohol, aldehyde)
Permeabilization (detergent)
Saturation (milk, BSA)
Antibodies (+ dyes for DNA)

imaging

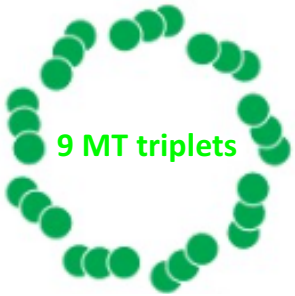


Pasteur

Monoclonal antibody,
Nobel Prize in Physiology or Medicine 1984,
Jerne, Köhler, Milstein

Super-Resolution / Electron Microscopy (SRM / EM)

centriole cross-section (~ Ø 250 nm)



Electronic

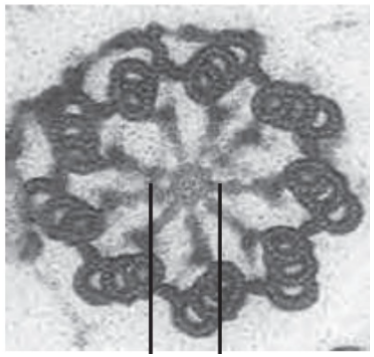
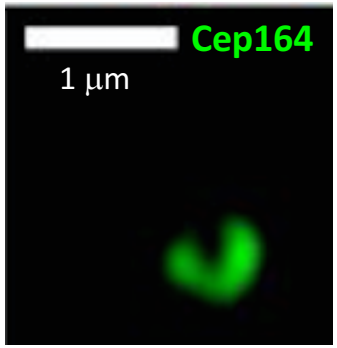


Figure 16-48 Molecular Biology of the Cell 6th

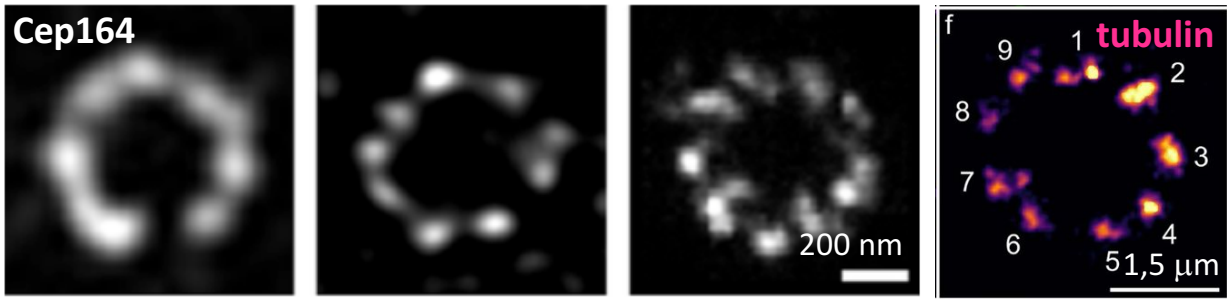
conventional optical



Joo et al., PNAS, 2012

Diffraction limit ~ 400 nm

3D-SIM | STED | STORM | expanded + STORM



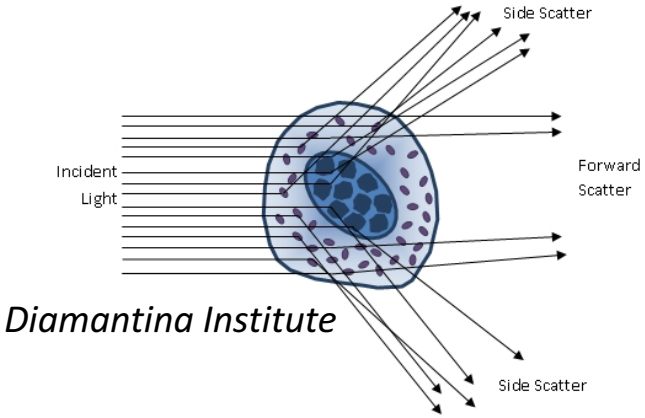
Fu & Zhang, Biophys. Report, 2019

Zwettler et al. BioRxiv 2020

SIM : structured illumination microscopy
STED : stimulated emission depletion microscopy
STORM : stochastic optical reconstruction microscopy

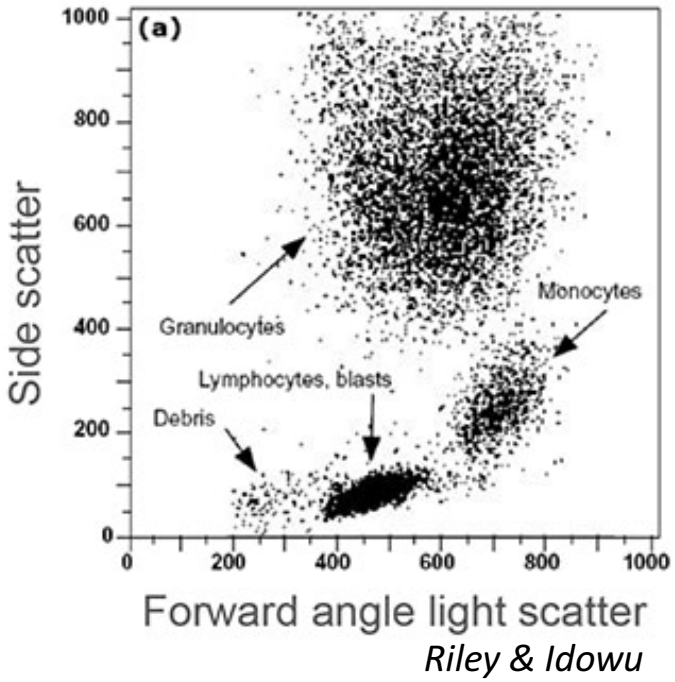
SRM, Nobel Prize in Chemistry 2014, Betzig, Hell, Moerner
Cryo-EM, Nobel Prize in Chemistry 2017, Dubochet, Frank, Henderson

Flow cytometry : analysis of a cell population



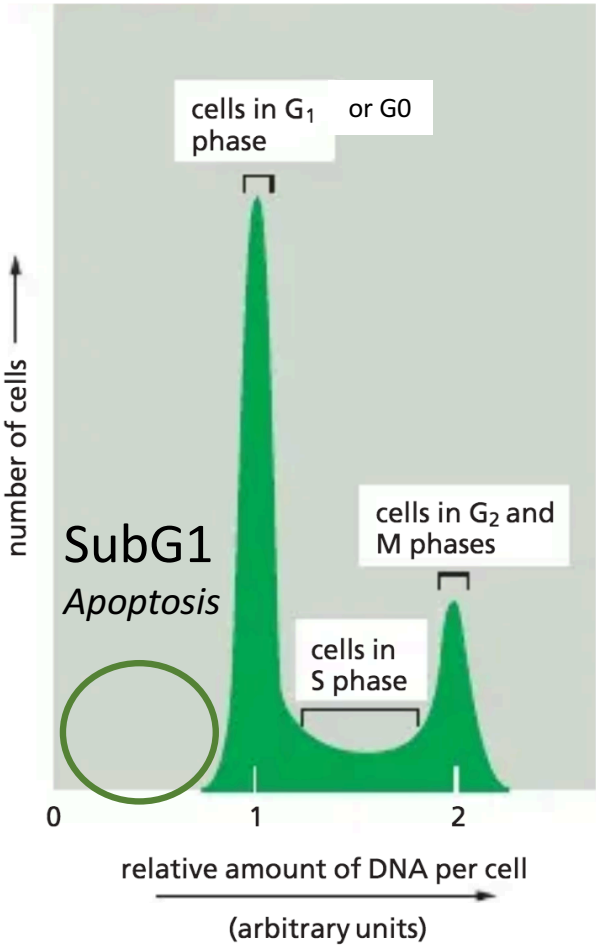
Diamantina Institute

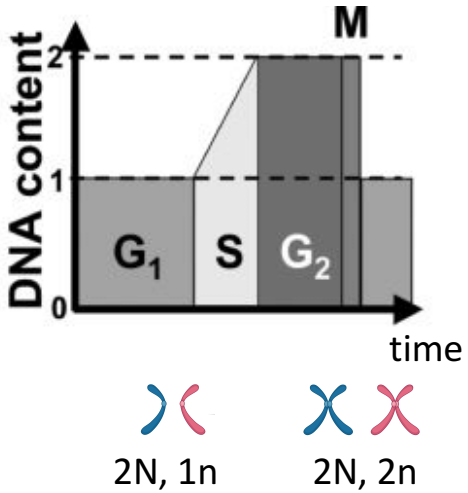
Cell size & granularity



Riley & Idowu

DNA content (fluorescent dyes)
(DAPI, Hoechst, propidium iodide)

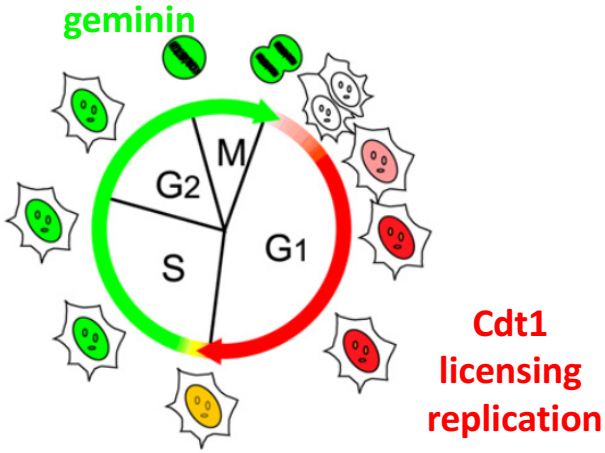




% of cell in each phase is proportional to the duration of each phase

Figure 17-8 Molecular Biology of the Cell 6th

Cell cycle markers and synchronization agents



Sakaue-Sawano et al., Cell, 2008

S
BrdU, EdU (nucleotide)
G2
Cyclin B expression (cytosol)
M
DNA condensation, P-H3, spindle MTs, nuclear cyclin B

G0 quiescence
Ki-67 negative

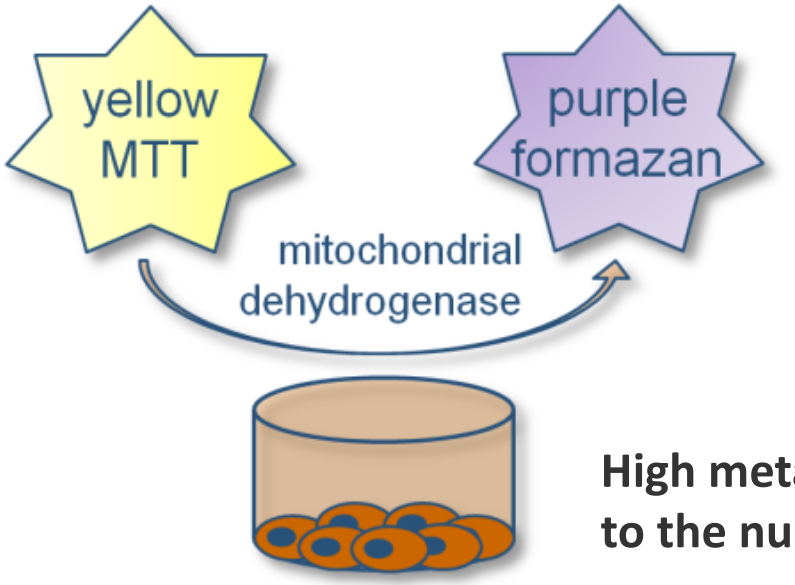
Senescence
SA-β-galactosidase
Ki-67 negative

Mitotic index
% of mitotic cells
to estimate M phase duration

Cell synchronization
- **Microtubule polymerization inhibitor nocodazole, taxol (M)**
- **mitotic shake-off (M)**
- **Double thymidine block (DNA synthesis G1/S)**
- **serum / growth factor starvation (G0)**

Cell viability : test MTT

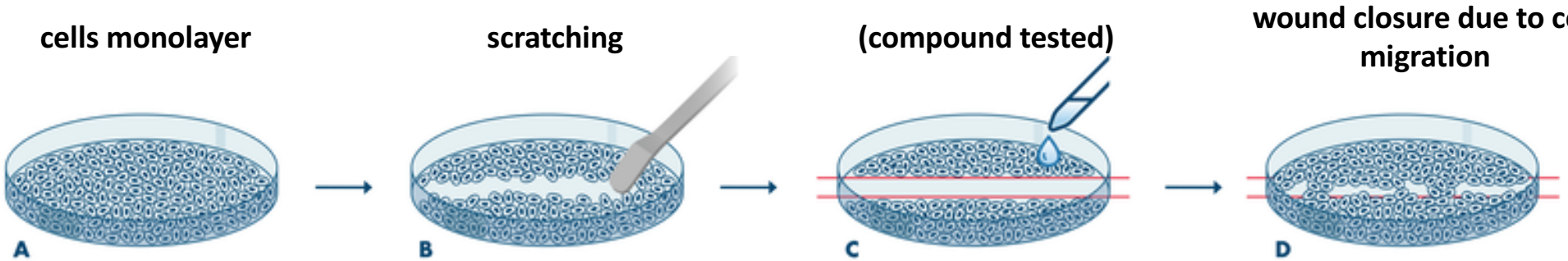
Viability of the cells is determined by a colorimetrical test :
Cleavage of the yellow tetrazolium salt MTT to form a violet formazan

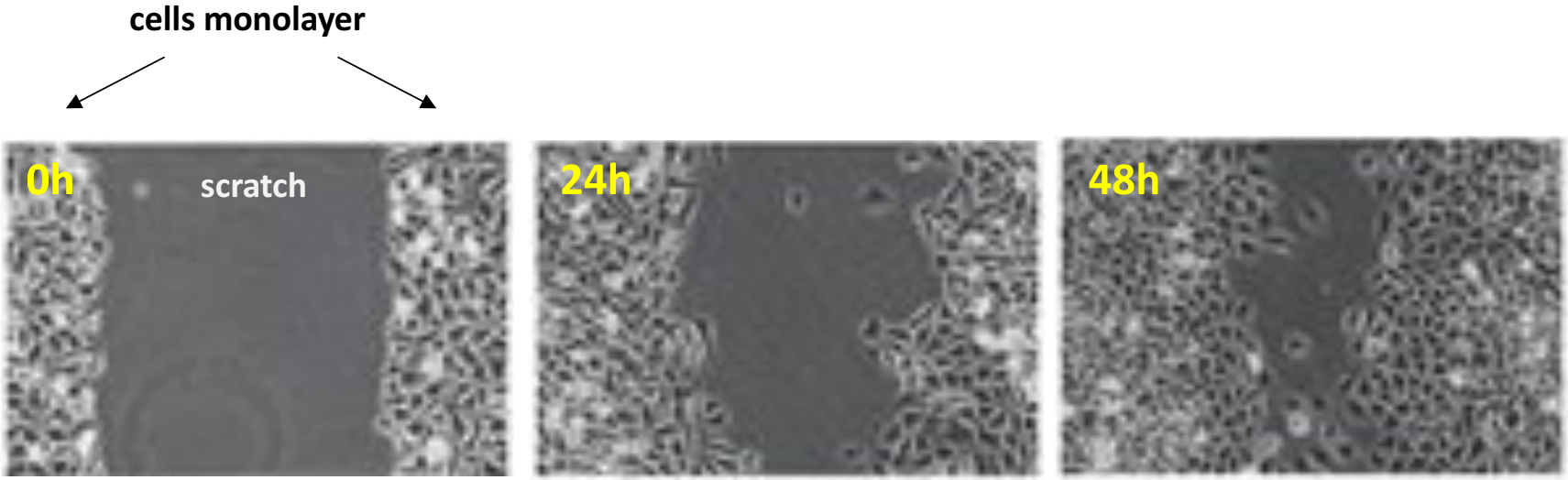


absorbance monitoring with a spectrometer

High metabolic activity proportional to the number of living cells

Migration : wound closure

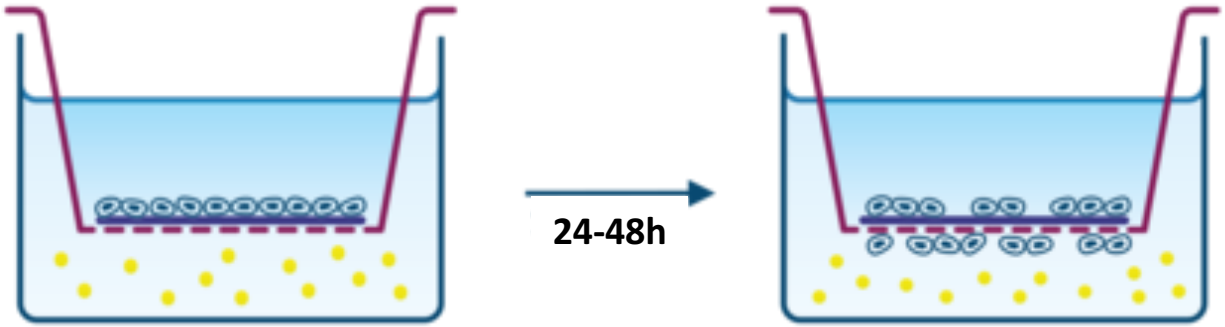


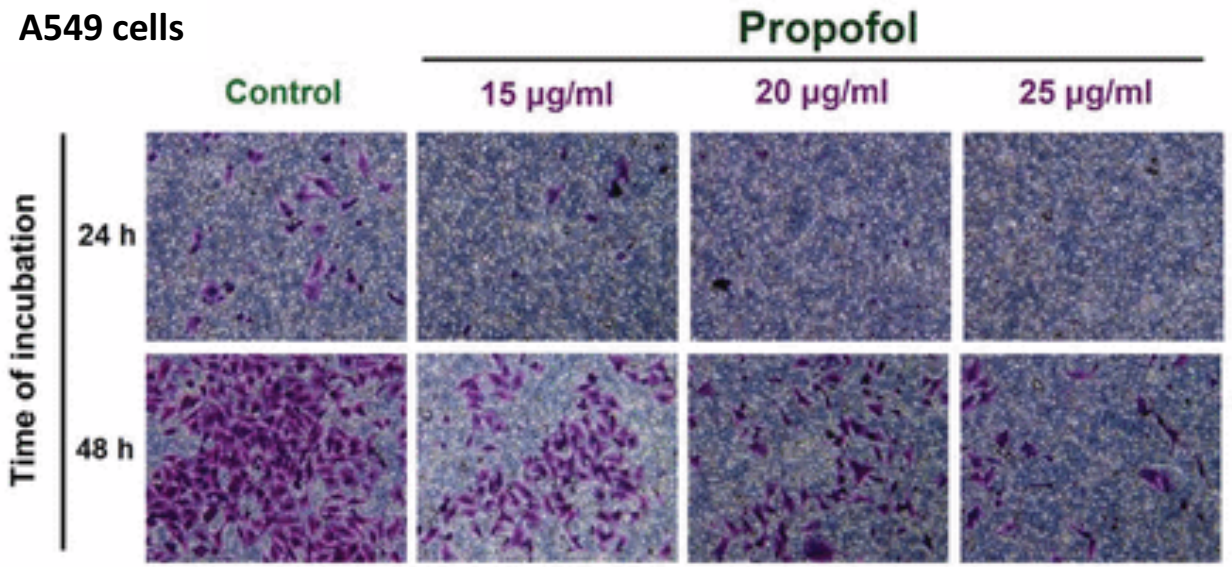


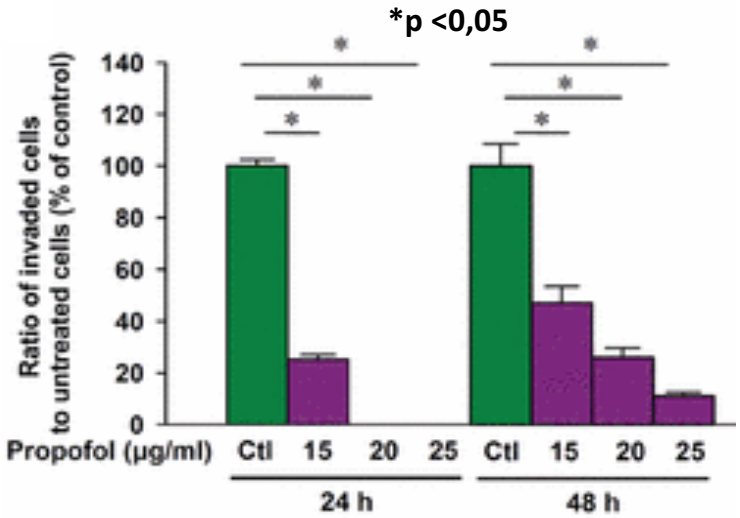
https://www.reactionbiology.com

Wu et al., Int J Oncology, 2019

Invasion : Boyden chamber





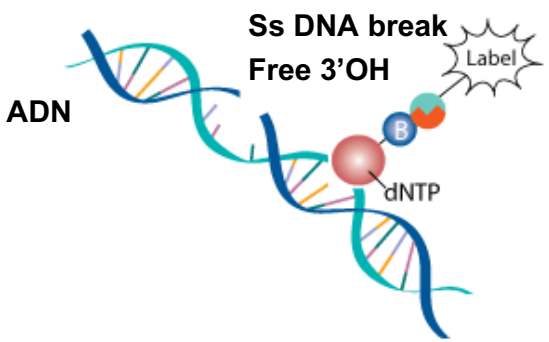


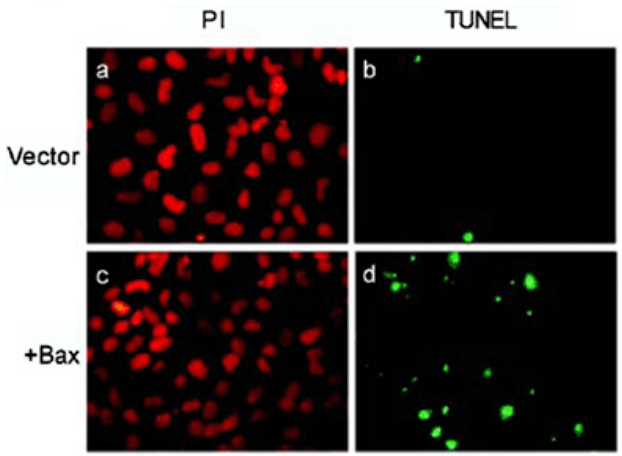
https://www.reactionbiology.com

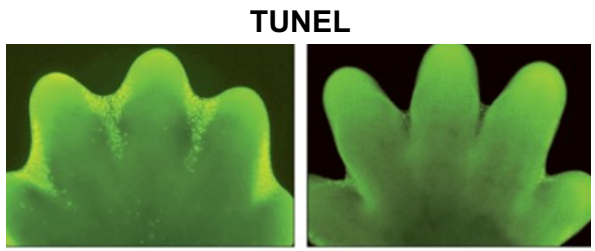
Wu et al., Anticancer Research, 2012

Apoptosis detection

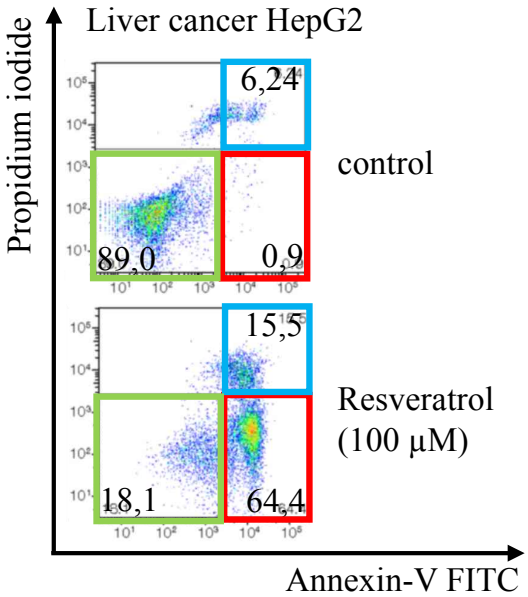
- **DNA break detection** (TUNEL Terminal deoxynucleotidyl transferase dUTP Nick End Labelling)
  (addition of labeled nucleotide on free 3'OH)

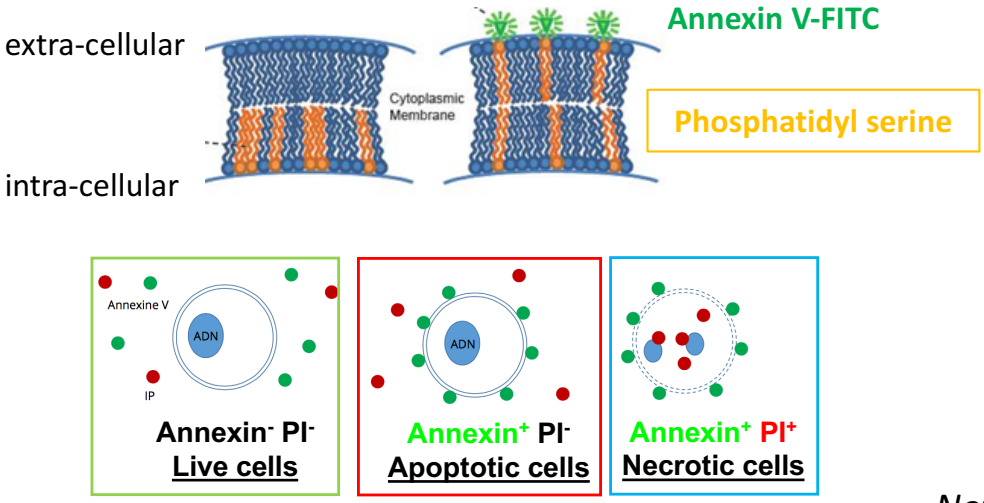






- **Propidium Iodide / Annexin-V-FITC staining + flow cytometry**

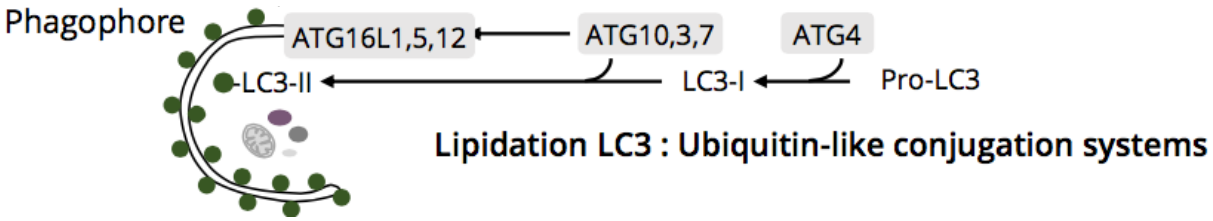




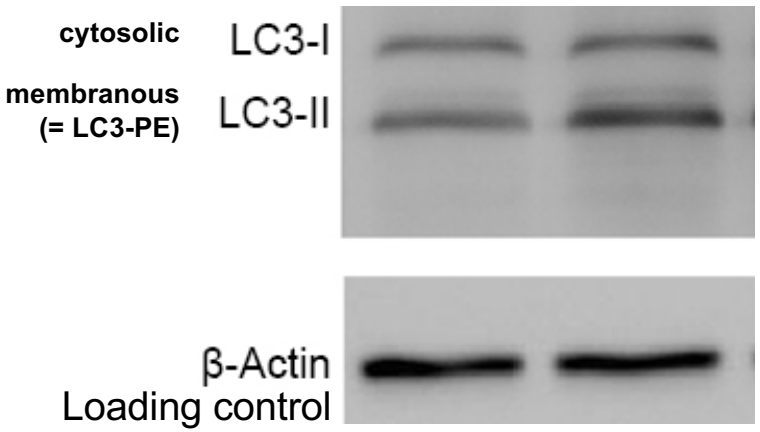
Novus biological
Lin et al., Cell Res, 2005
Mol Biol Cell of the Cell, 4th, 2000
Takashina et al., Inter J Onco, 2017

Autophagy detection

Hallmarks : LC3-II membranous isoform = cytosolic puncta corresponding to phagophores, autophagosomes, autophagolysosomes.



Western blot



immunofluorescence or live imaging