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JUNE 29, 2023 | DR. LAURA LLERAS FORERO AND INES KRISTINA HARTMANN

Good Cell Culture Practice

How to Improve the Reproducibility of Your Experiments

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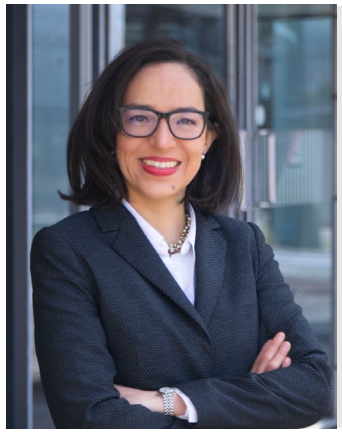
Your Experts Today



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Application Specialist, Cell Handling
Eppendorf SE

Agenda

01 Introduction

02 Cell Passage, Confluency, and Cell Count

03 Cell Seeding

04 Cell Incubation

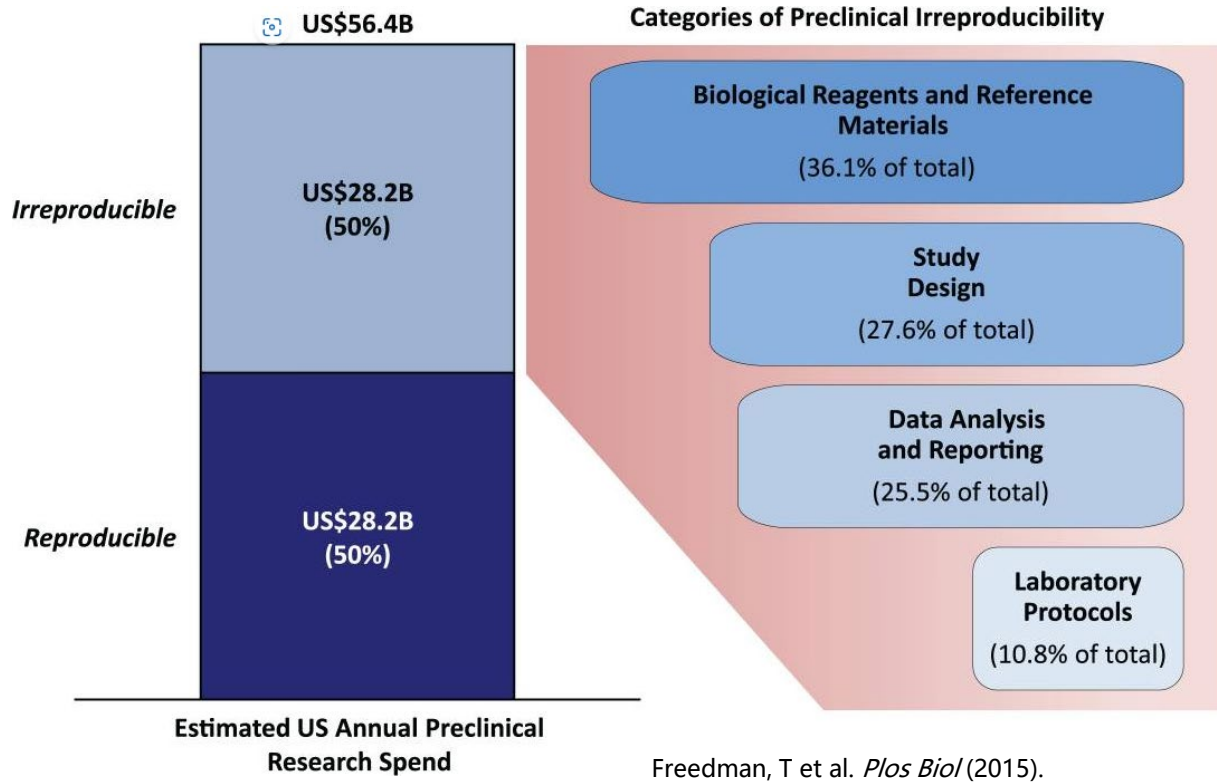
05 Conclusions and Recommendations

01

Introduction

What Is Reproducibility?

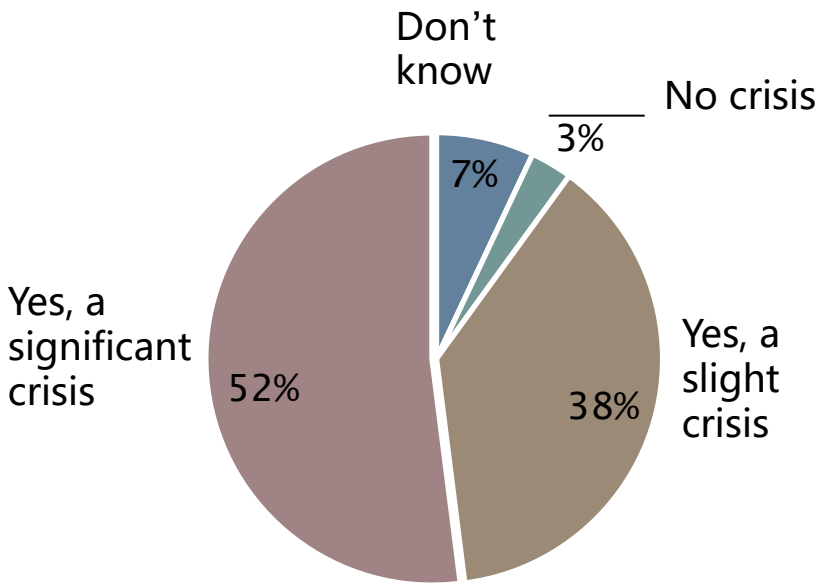
1. The ability to replicate the results from a published study using the same methods and materials.
2. Are the methodology and results presented in sufficient detail to enable replication?



Freedman, T et al. *Plos Biol* (2015).

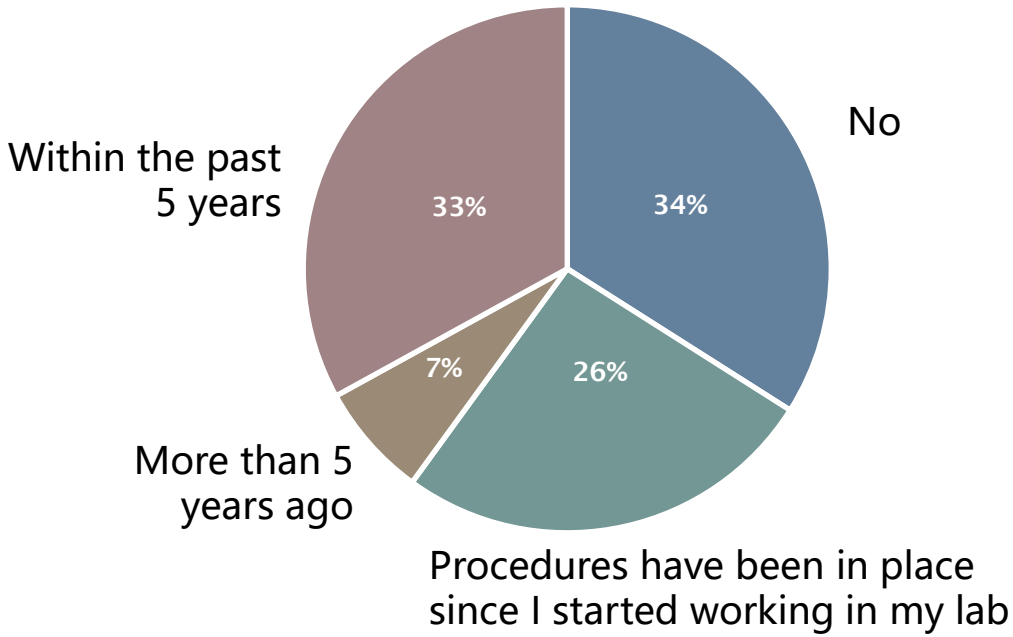
Reproducibility in Science

Is there a reproducibility crisis?*



***1,576 researchers surveyed**

Have you ever established procedures for reproducibility?*



Source: **1,500 scientists lift the lid on reproducibility**. Baker M. 2016, *Nature* 533(7604): 452-4

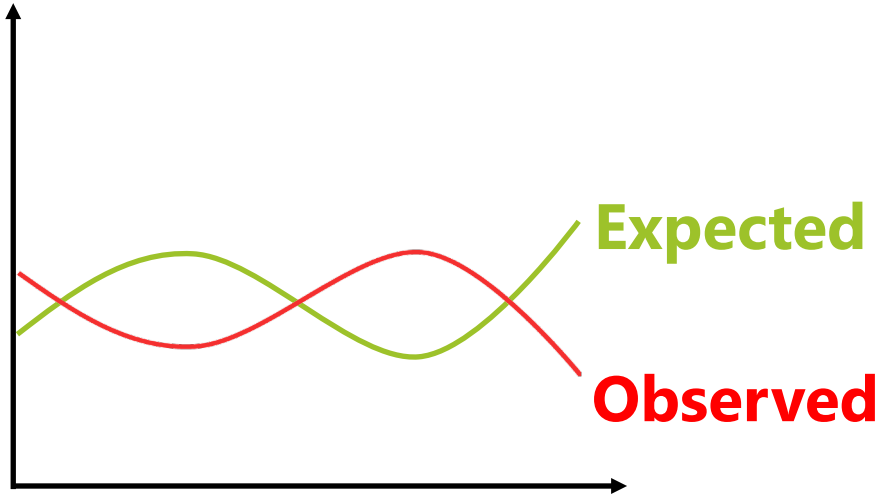
Cultured Cells as Model Systems

Benefits:

- Cells retain many functions and properties of their parental tissue
- Cells serve as a model system to test new therapeutic approaches
- Renewable resource of cell material

Risks:

- Contamination with microorganisms
- Cross-contamination of different cell lines
- Genetic drift during cultivation
- Response to different cultivation conditions



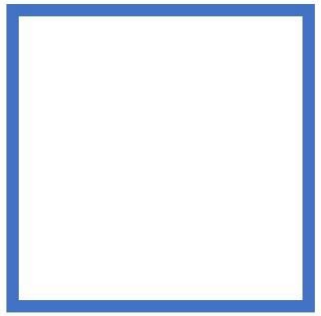
“Cell culture sometimes feels like a black art, with everyone having their own preferred method”

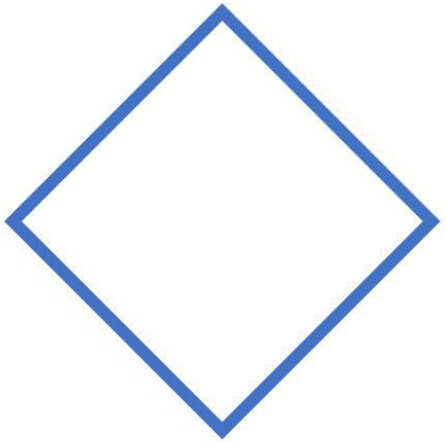
- Dr. Delcassian, MIT (Mass., USA). Technology Networks Cell Science 2018

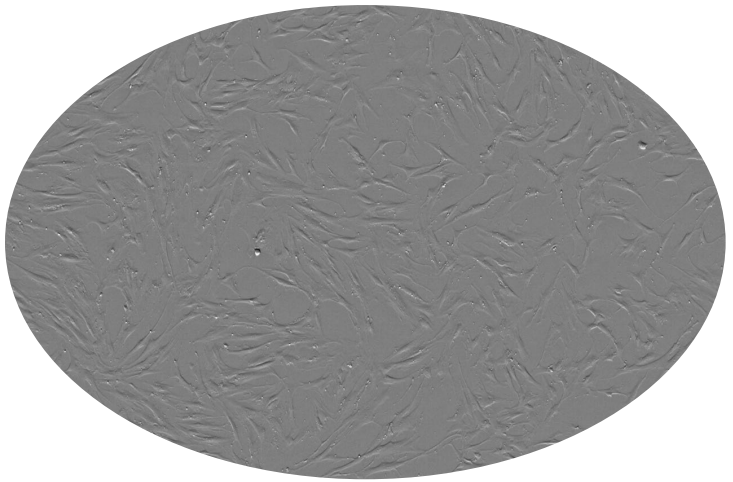
02

Cell Passage, Confluency, and Cell Count

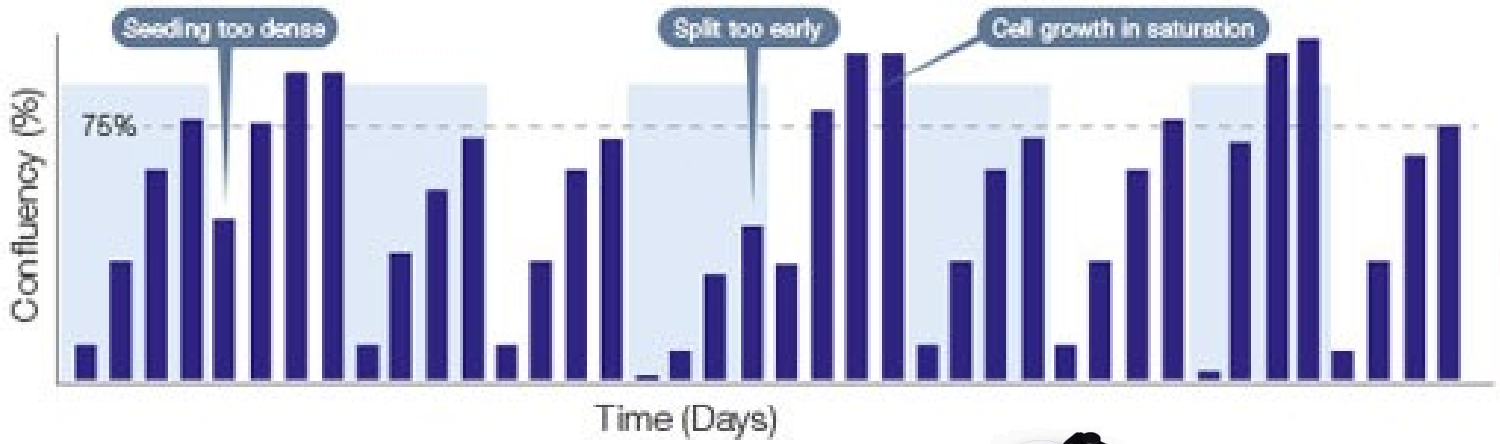
The Human Eye Is Not Quantitative



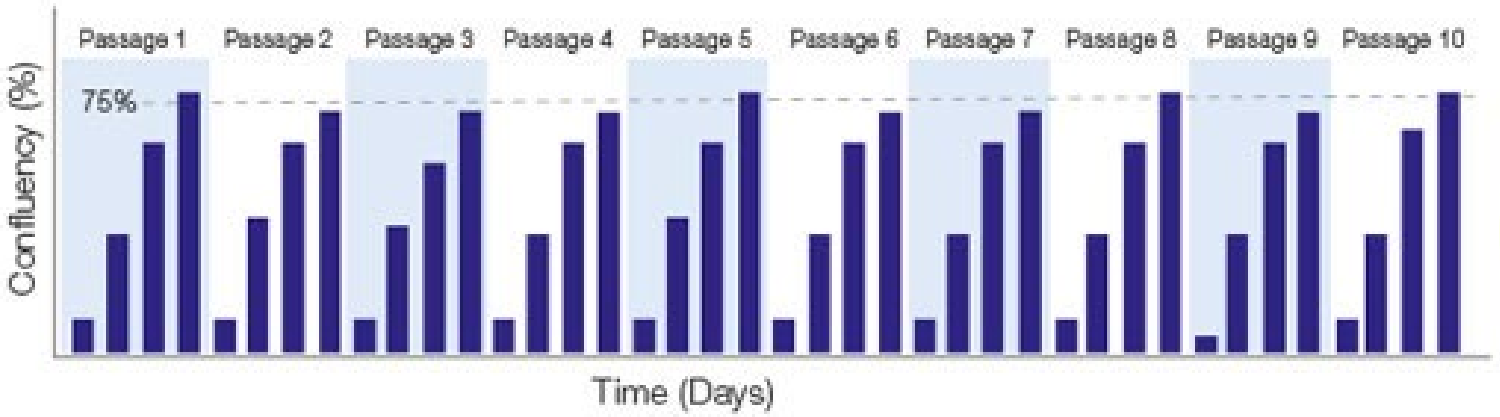




Irregular Cell Culture Process



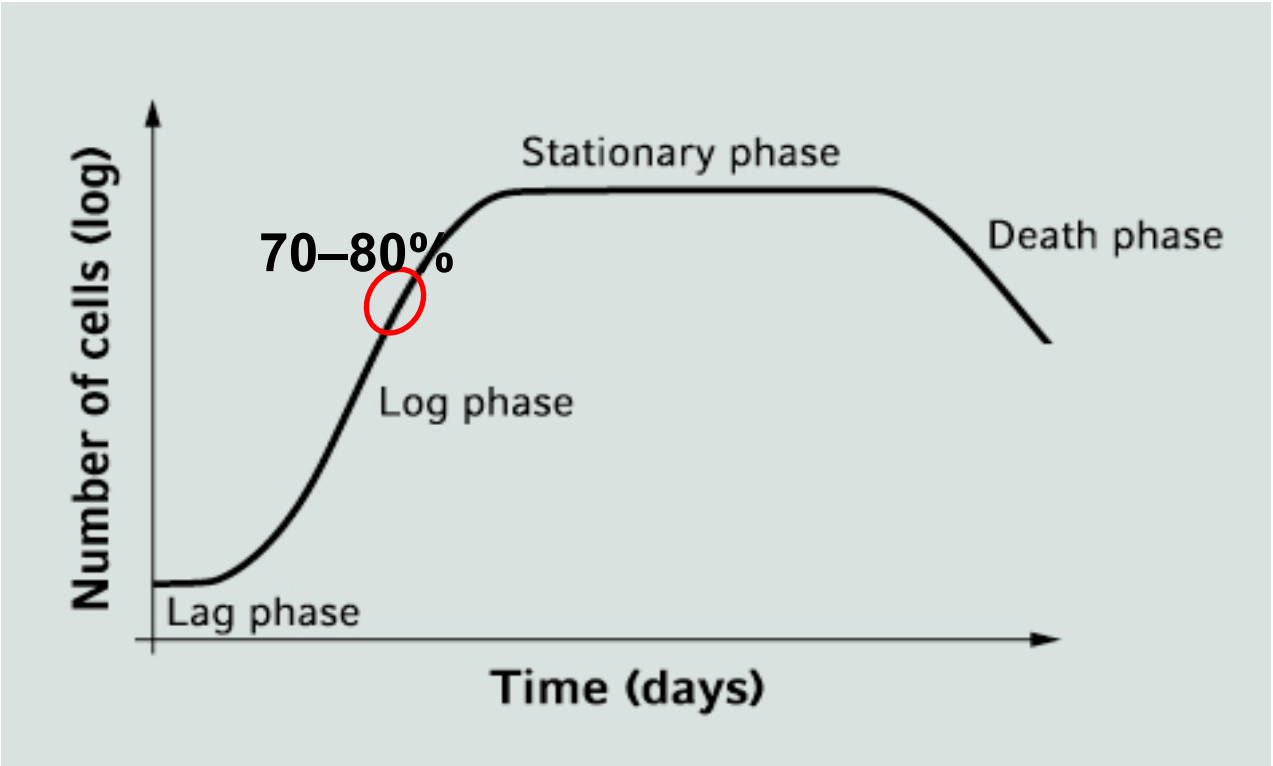
Regular Cell Culture Process



Optimal Timepoint for Passaging

Optimizing cell growth

- Plot a cell growth curve
- Don’t let the cultures overgrow
- Check cell viability
- Do not use cultures with <80% viability

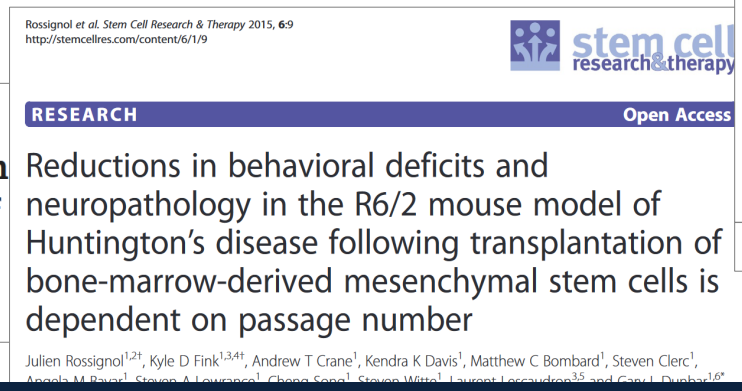


How many passages are too many?


Proc Soc Exp Biol Med. 1997 Mar;214(3):248-57. doi: 10.3181/00379727-214-44093.

The influence of culture time and passage number on the morphological and physiological development of Caco-2 cells

M J Briske-Anderson [1], J W Finley, S M Newman

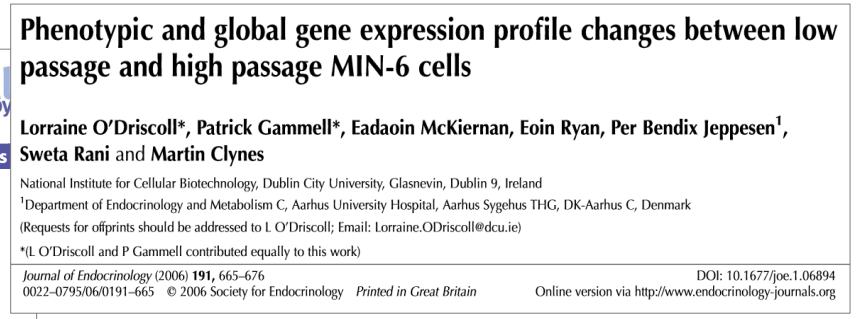

Rossignol *et al. Stem Cell Research & Therapy* 2015, **6**:9
http://stemcellres.com/content/6/1/9

stem cell research&therapy

RESEARCH Open Access

Reductions in behavioral deficits and neuropathology in the R6/2 mouse model of Huntington's disease following transplantation of bone-marrow-derived mesenchymal stem cells is dependent on passage number

Julien Rossignol[1,2†], Kyle D Fink[1,3,4†], Andrew T Crane[1], Kendra K Davis[1], Matthew C Bombard[1], Steven Clerc[1],


Phenotypic and global gene expression profile changes between low passage and high passage MIN-6 cells

Lorraine O'Driscoll*, Patrick Gammell*, Eadaoin McKiernan, Eoin Ryan, Per Bendix Jeppesen[1], Sweta Rani and **Martin Clynes**

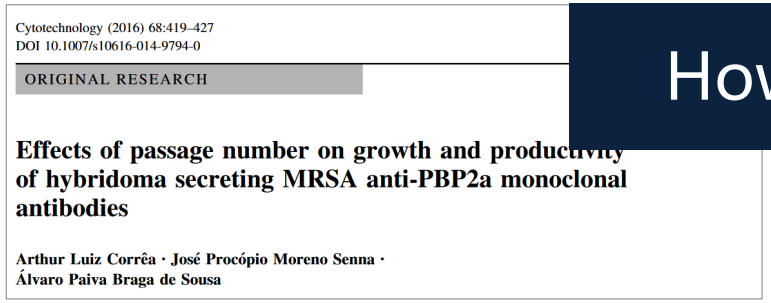
National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

[1]Department of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus Sygehus THG, DK-Aarhus C, Denmark

(Requests for offprints should be addressed to L O'Driscoll; Email: Lorraine.ODriscoll@dcu.ie)

*(L O'Driscoll and P Gammell contributed equally to this work)

Journal of Endocrinology (2006) **191**, 665–676 DOI: 10.1677/joe.1.06894
0022–0795/06/0191–665 © 2006 Society for Endocrinology *Printed in Great Britain* Online version via http://www.endocrinology-journals.org


Cytotechnology (2016) 68:419–427
DOI 10.1007/s10616-014-9794-0

ORIGINAL RESEARCH

Effects of passage number on growth and productivity of hybridoma secreting MRSA anti-PBP2a monoclonal antibodies

Arthur Luiz Corrêa · José Procópio Moreno Senna · Álvaro Paiva Braga de Sousa

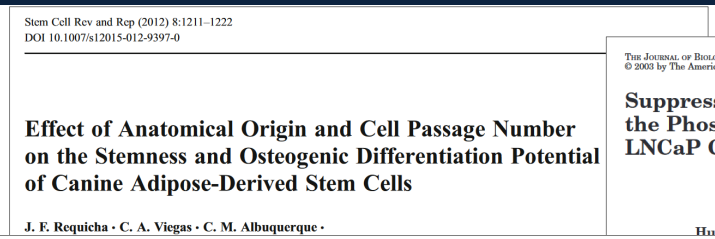

BIOTECHNOLOGY PROGRESS

Society for Biological Engineering AIChE

Conditioned Medium Factors and Passage Number on ysiology and Productivity

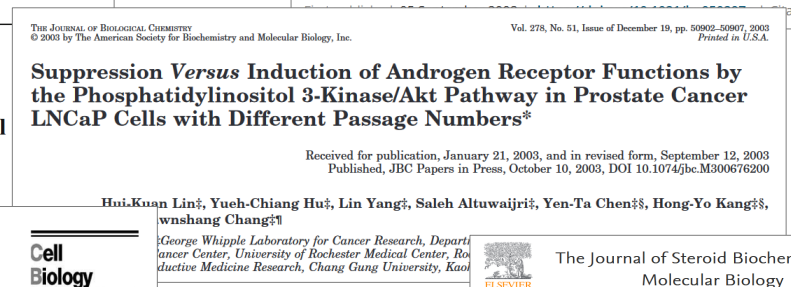
Karin Calles, Ingrid Svensson, Eva Lindskog, Lena Häggström

ations: 19


Stem Cell Rev and Rep (2012) 8:1211–1222
DOI 10.1007/s12015-012-9397-0

Effect of Anatomical Origin and Cell Passage Number on the Stemness and Osteogenic Differentiation Potential of Canine Adipose-Derived Stem Cells

J. F. Requicha · C. A. Viegas · C. M. Albuquerque ·


THE JOURNAL OF BIOLOGICAL CHEMISTRY
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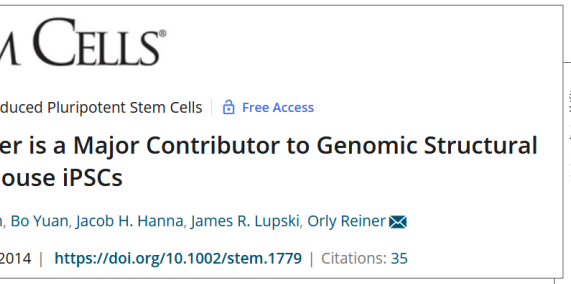
Vol. 278, No. 51, Issue of December 19, pp. 50902–50907, 2003
Printed in U.S.A.

Suppression *Versus* Induction of Androgen Receptor Functions by the Phosphatidylinositol 3-Kinase/Akt Pathway in Prostate Cancer LNCaP Cells with Different Passage Numbers*

Received for publication, January 21, 2003, and in revised form, September 12, 2003
Published, JBC Papers in Press, October 10, 2003, DOI 10.1074/jbc.M300676200

Hui-Kuan Lin‡, Yueh-Chiang Hu‡, Lin Yang‡, Saleh Altuwaijri‡, Yen-Ta Chen‡§, Hong-Yo Kang‡§, wnshang Chang‡¶

George Whipple Laboratory for Cancer Research, Departi ancer Center, University of Rochester Medical Center, Ro ductive Medicine Research, Chang Gung University, Kao

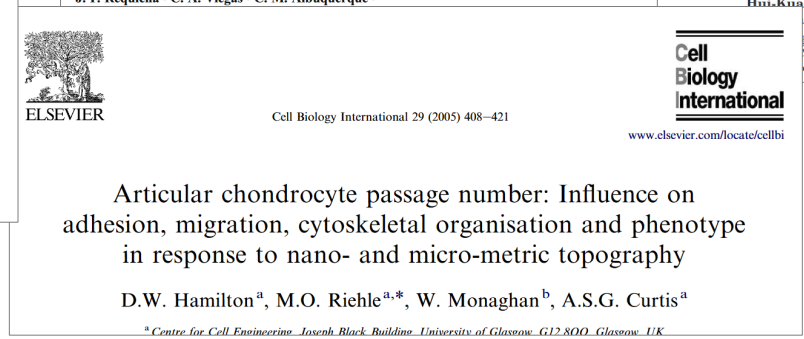

STEM CELLS

Embryonic Stem Cells/Induced Pluripotent Stem Cells | Free Access

Passage Number is a Major Contributor to Genomic Structural Variations in Mouse iPSCs

Pengfei Liu, Anna Kaplan, Bo Yuan, Jacob H. Hanna, James R. Lupski, Orly Reiner

First published: 26 June 2014 | https://doi.org/10.1002/stem.1779 | Citations: 35


ELSEVIER

Cell Biology International 29 (2005) 408–421

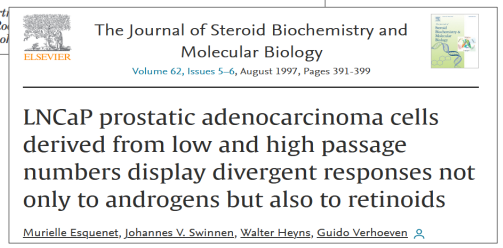
Cell Biology International

www.elsevier.com/locate/cellbi

Articular chondrocyte passage number: Influence on adhesion, migration, cytoskeletal organisation and phenotype in response to nano- and micro-metric topography

D.W. Hamilton[a], M.O. Riehle[a,*], W. Monaghan[b], A.S.G. Curtis[a]

[a] *Centre for Cell Engineering, Joseph Black Building, University of Glasgow, G12 8QQ, Glasgow, UK*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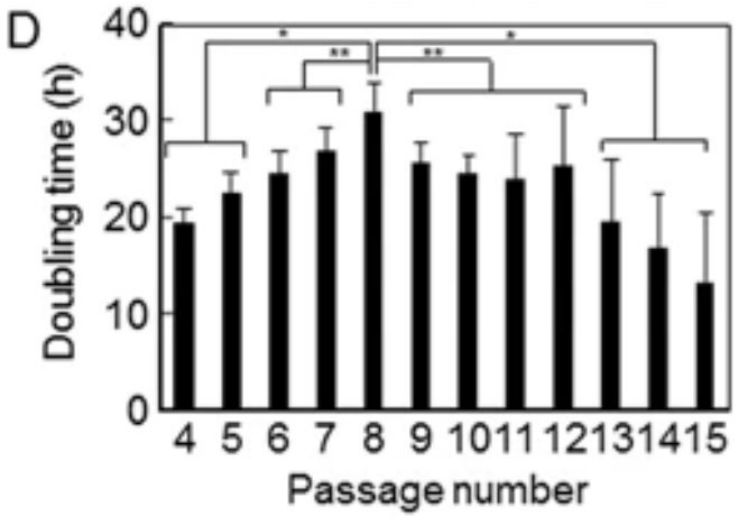
The Journal of Steroid Biochemistry and Molecular Biology

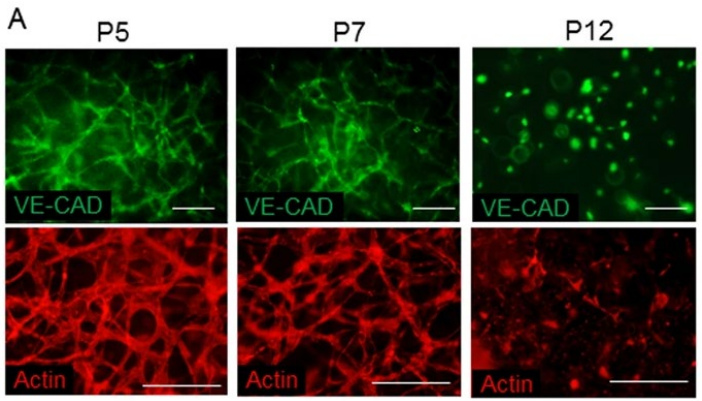
Volume 62, Issues 5–6, August 1997, Pages 391-399

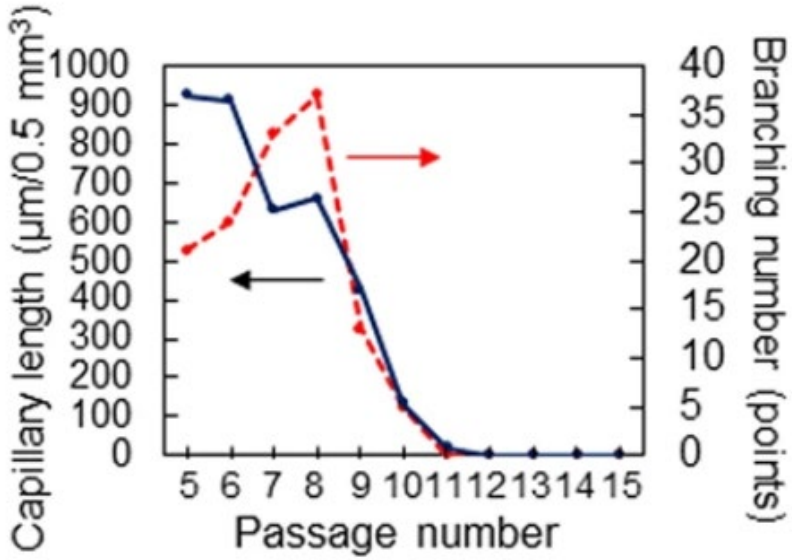
LNCaP prostatic adenocarcinoma cells derived from low and high passage numbers display divergent responses not only to androgens but also to retinoids

Murielle Esquenet, Johannes V. Swinnen, Walter Heyns, Guido Verhoeven

Cell Passage Influences Cell Culture Reproducibility

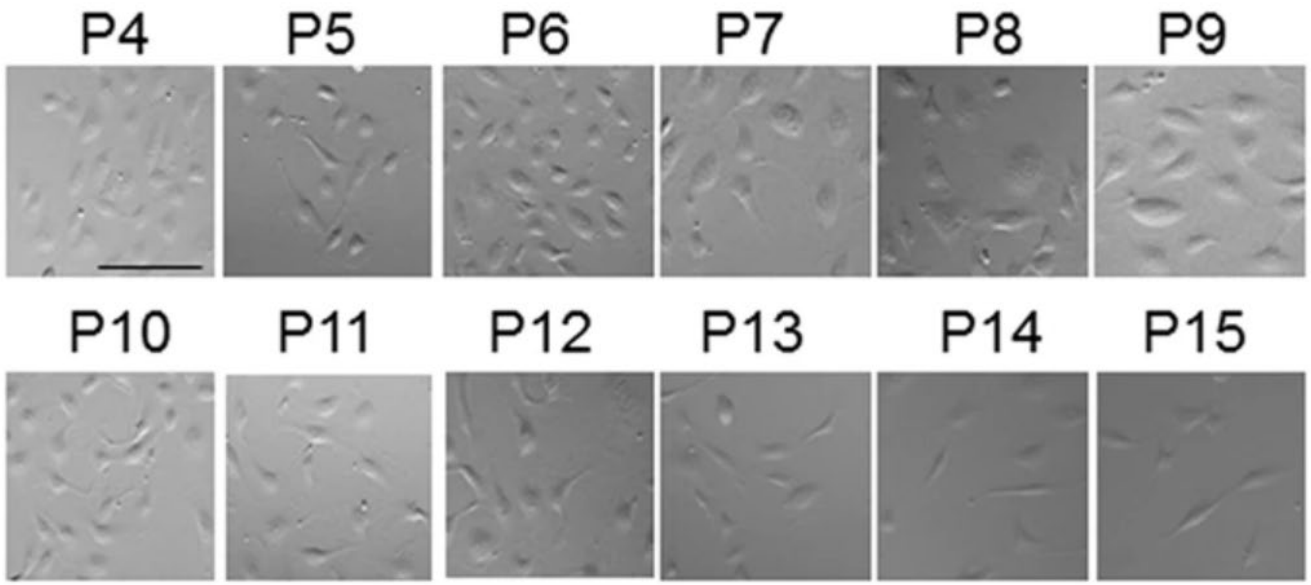


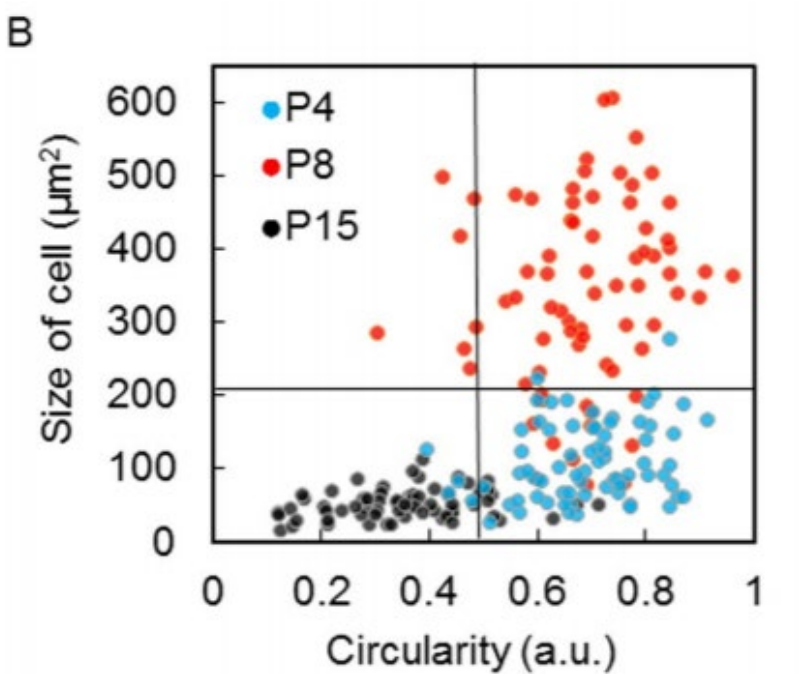




Osaki, T et al. *Sci Rep* 7, 1897 (2017).

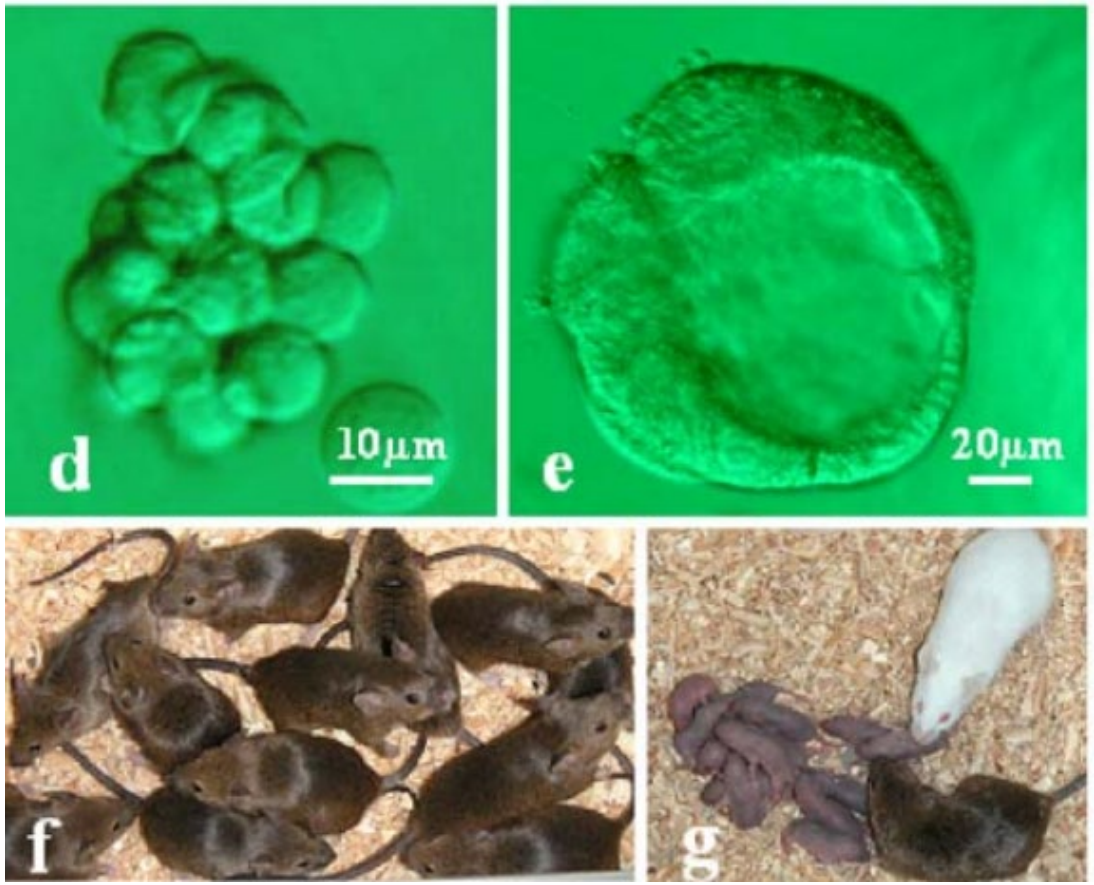
Cell Morphology Changes with Different Passages





Osaki, T et al. *Sci Rep* 7, 1897 (2017).

Cell Morphology Changes with Different Passages

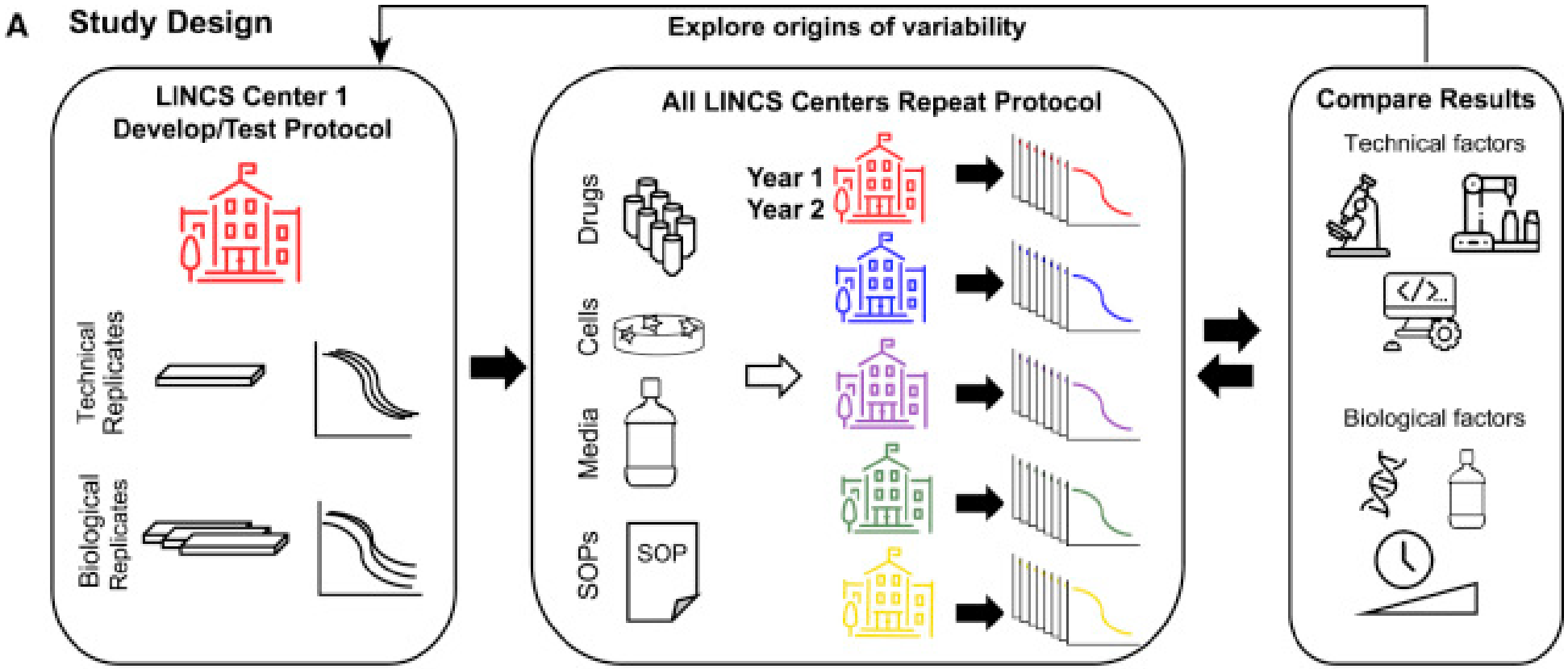


Li et al. *Cell Tissue Res* (2007).

Avoid Prolonged Passaging

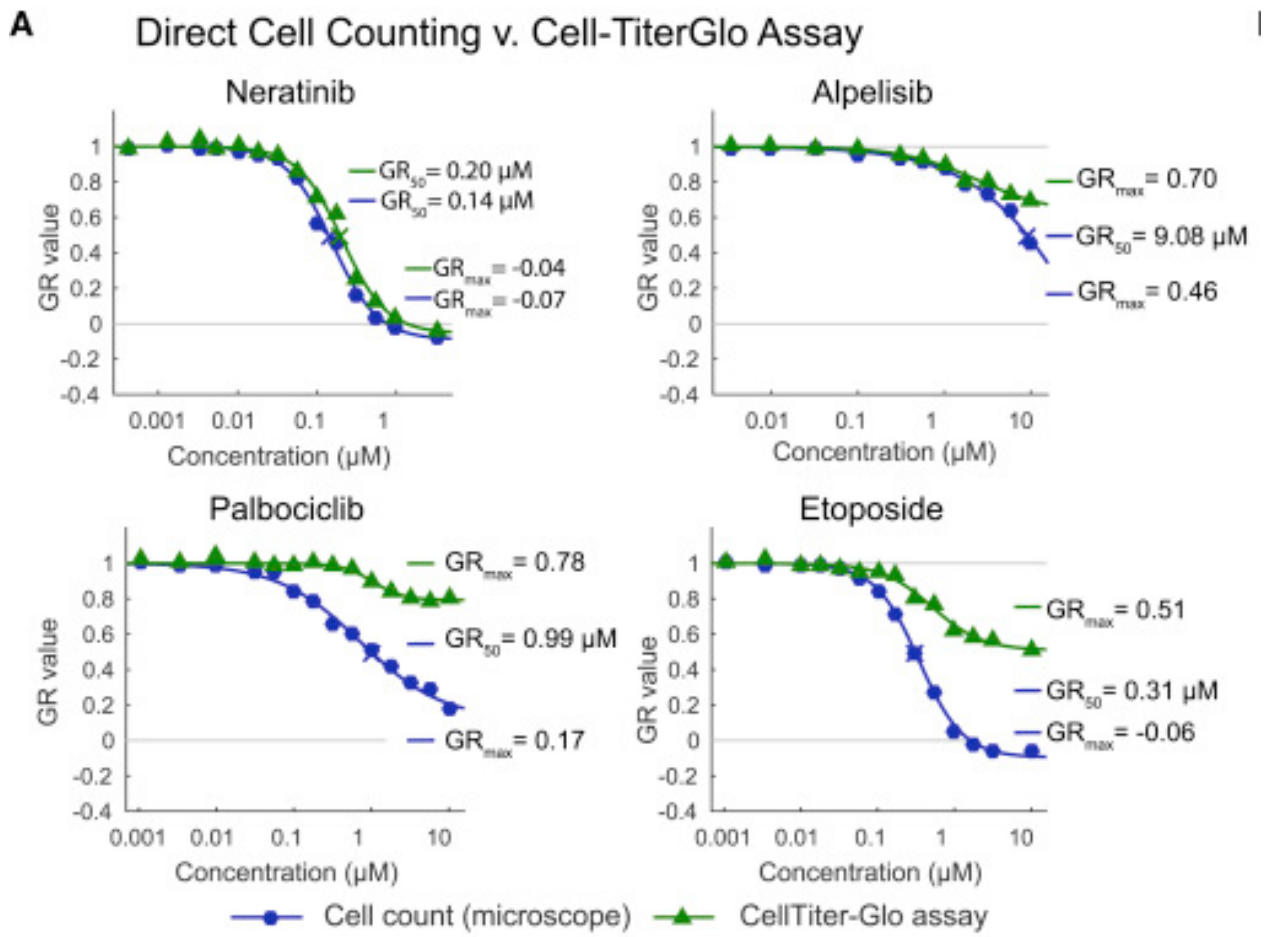
- Use high-quality cells as a starting point
- Establish a reference cell stock
- Determine the safe passage number by establishing baselines
  - Expression/presence of proteins of interest
  - Proliferation rates
- Monitor morphology and document culture maintenance
- Stop active cultivation of cells that are not used

Cell Count Is Essential for Experimental Reproducibility



Niepel et al. *Cell Systems* (2019)

Cell Count Affects the End Experimental Result

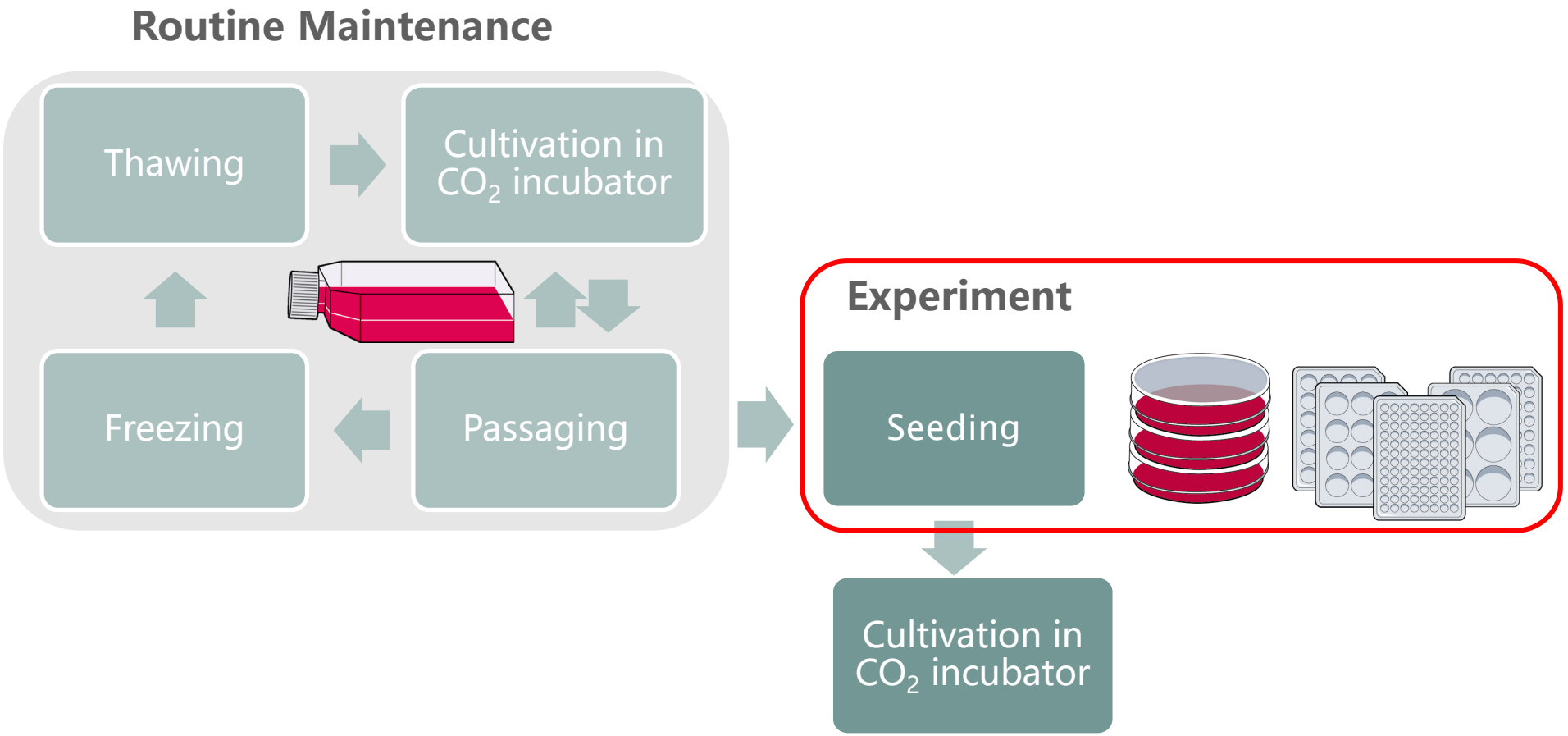


Niepel et al. *Cell Systems* (2019)

03

Cell Seeding

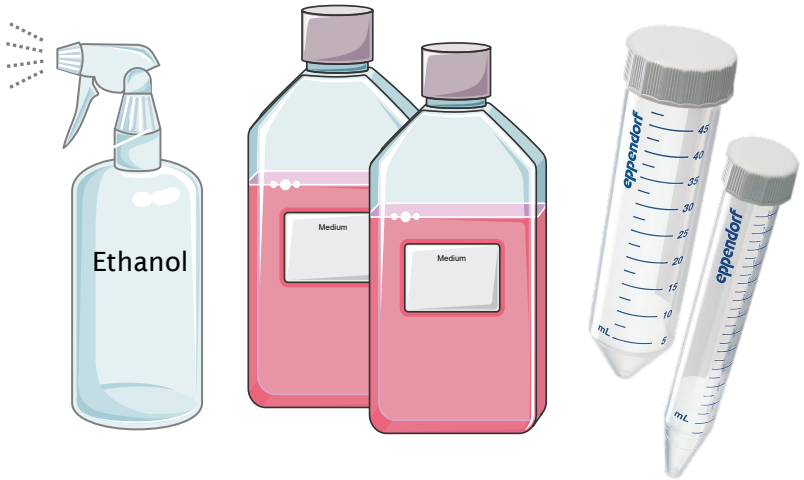
Cell Culture Workflow

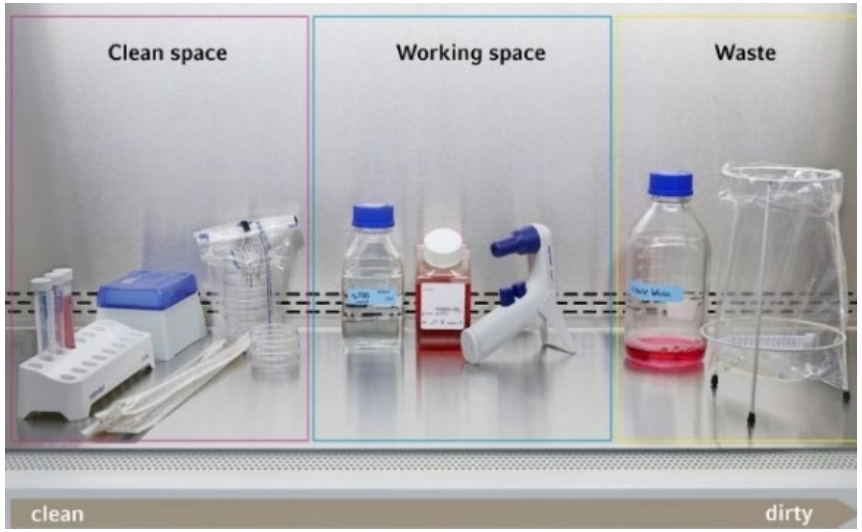


Prepare Your Workspace

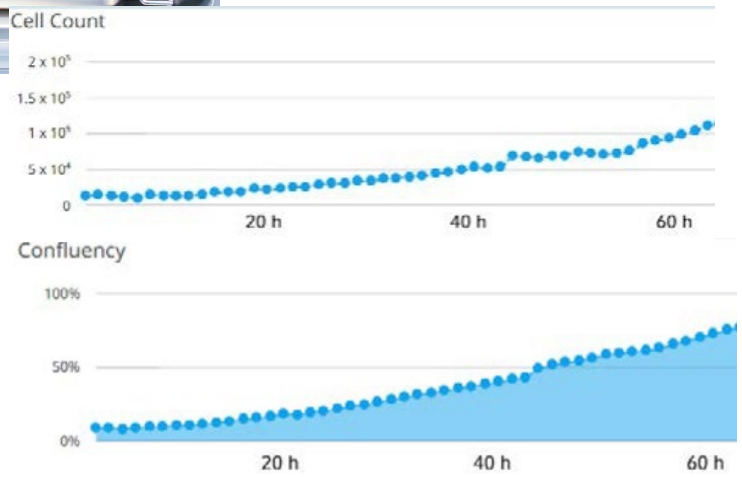
If you handle different cell lines:

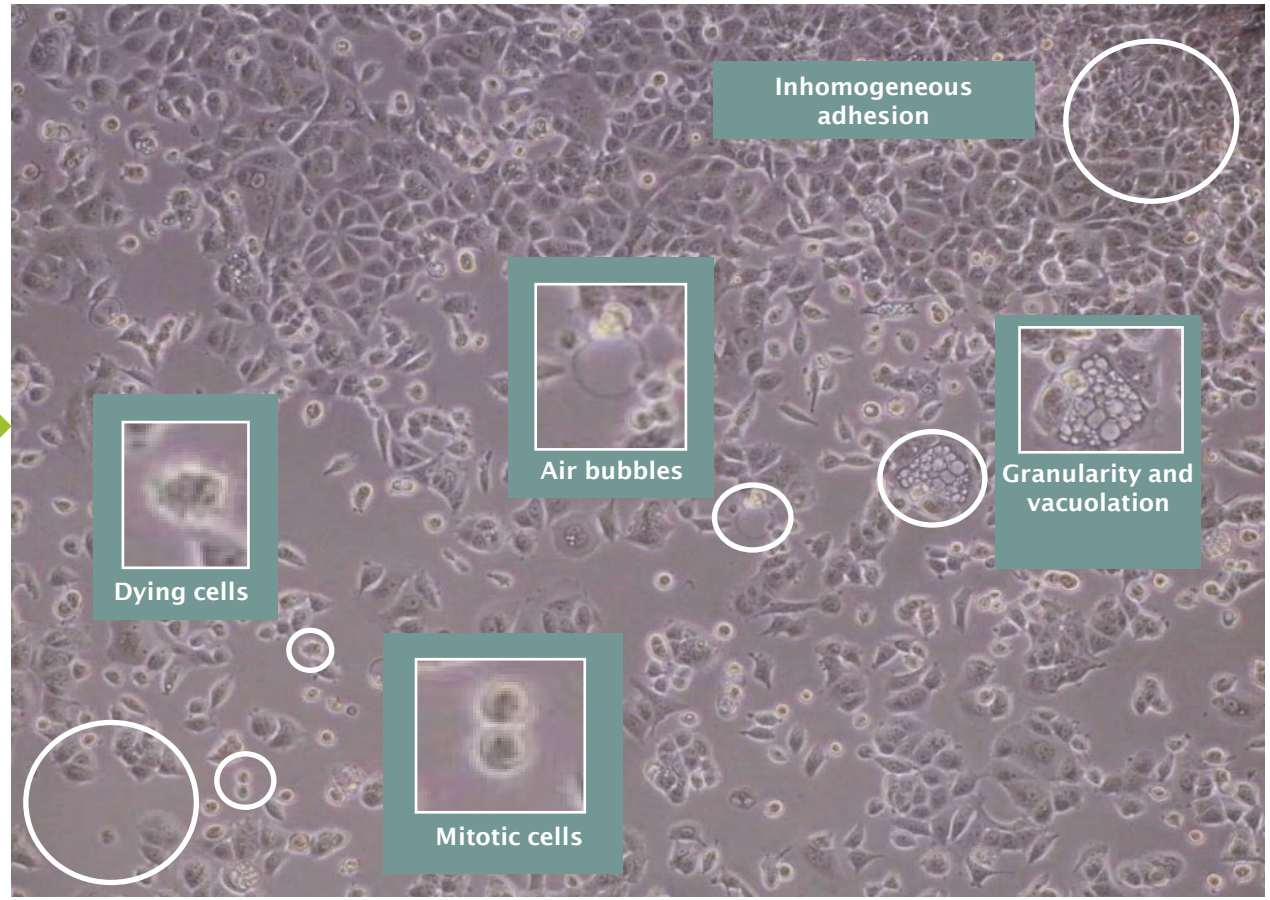
- Work with one cell line at a time
- Disinfect the workspace in between
- Dedicate aliquots/media bottles for each cell line



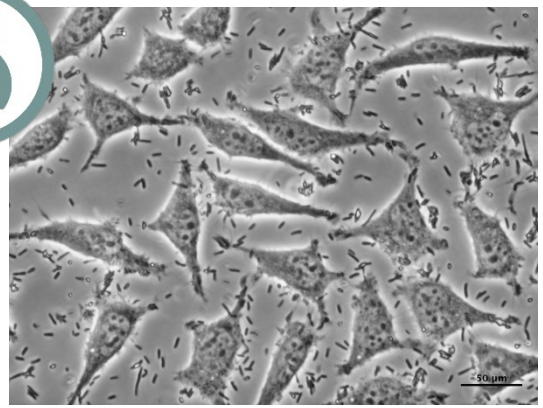


Know Your Cells' Growth Behavior and Morphology

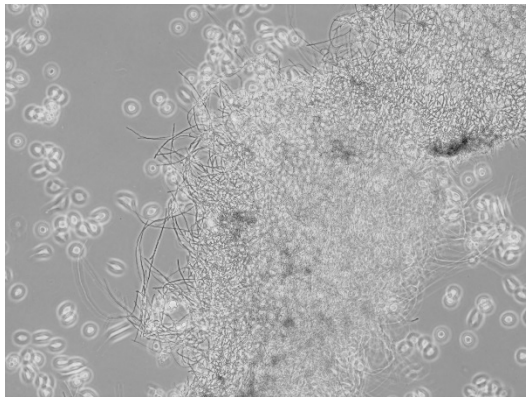




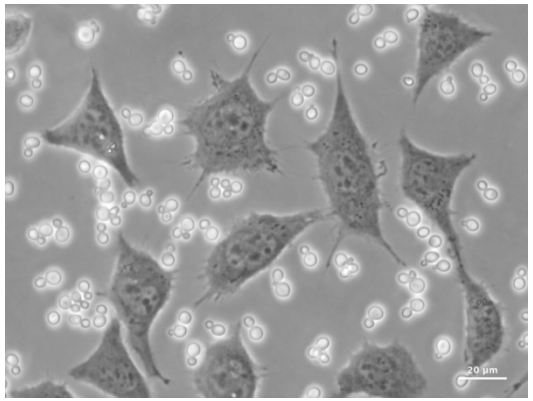
Check for Contamination



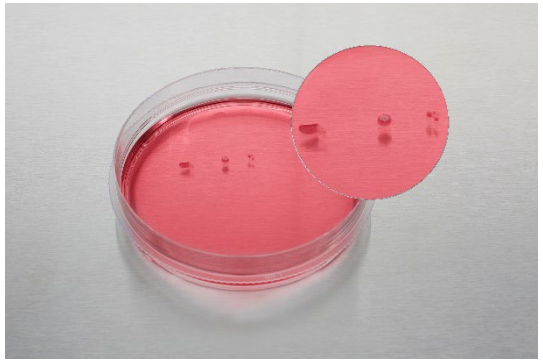
Bacteria



Fungus



Yeast

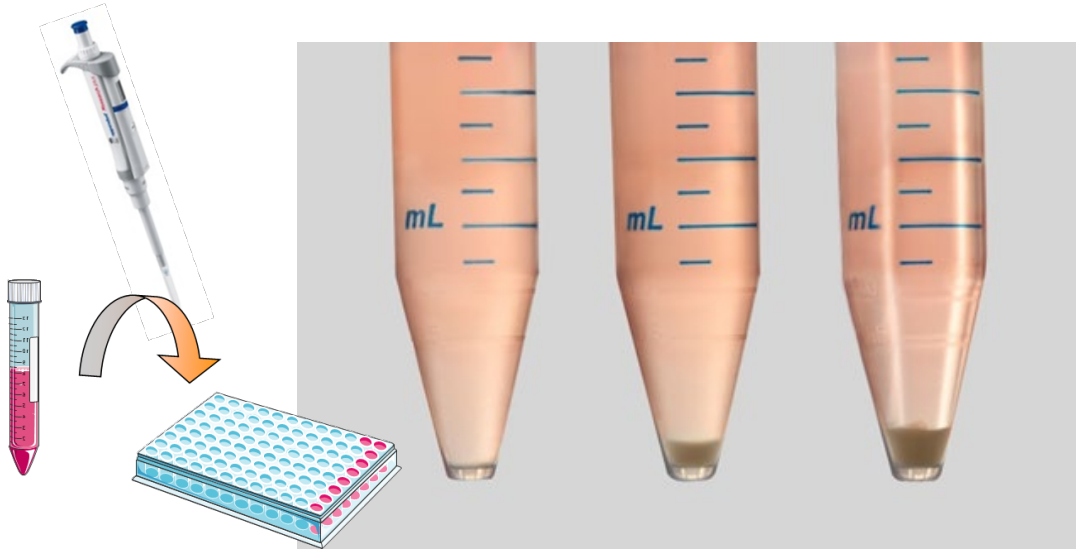


Cell cultures should be tested for mycoplasma contamination regularly.

Seeding Cells

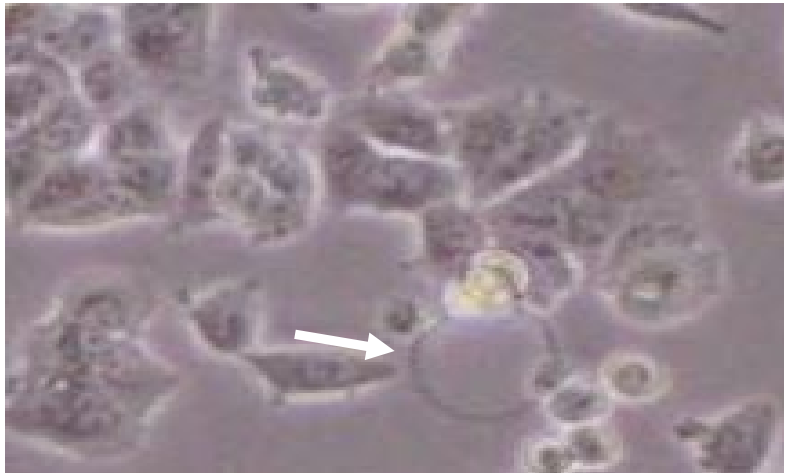
Filling a plate is time consuming.

- Cell sedimentation in the tube is quite fast
- The longer the seeding process, the higher the decrease in cell number
- **Make sure to resuspend in between**



The formation of air bubbles.

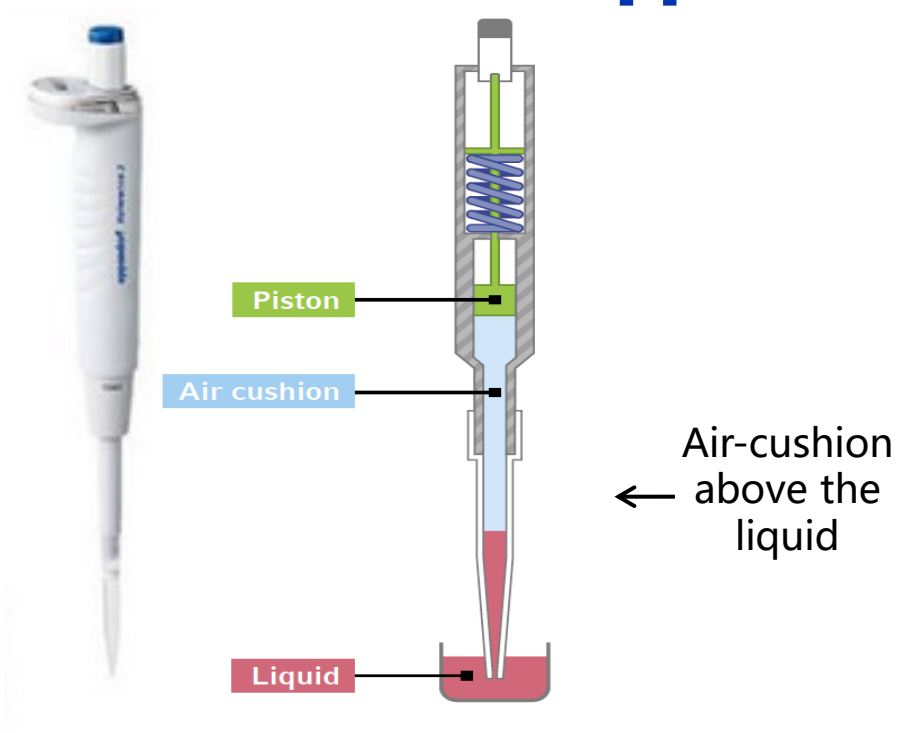
- Culture medium tends to foam
- Air bubbles can hinder cell attachment
- **Avoid harsh and fast pipetting**

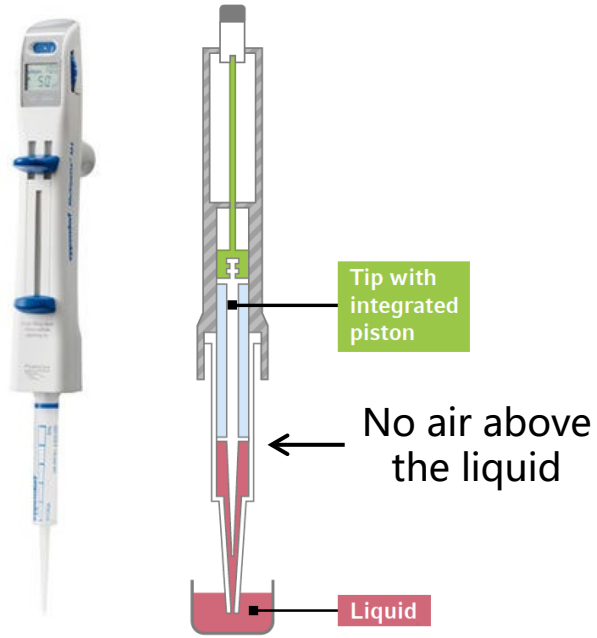


Influence of Pipetting Systems

Air-cushion

- Variable influencing factors
- Optimal for aqueous solutions
- Prone to contamination by aerosols (-> filter tips)





Positive displacement

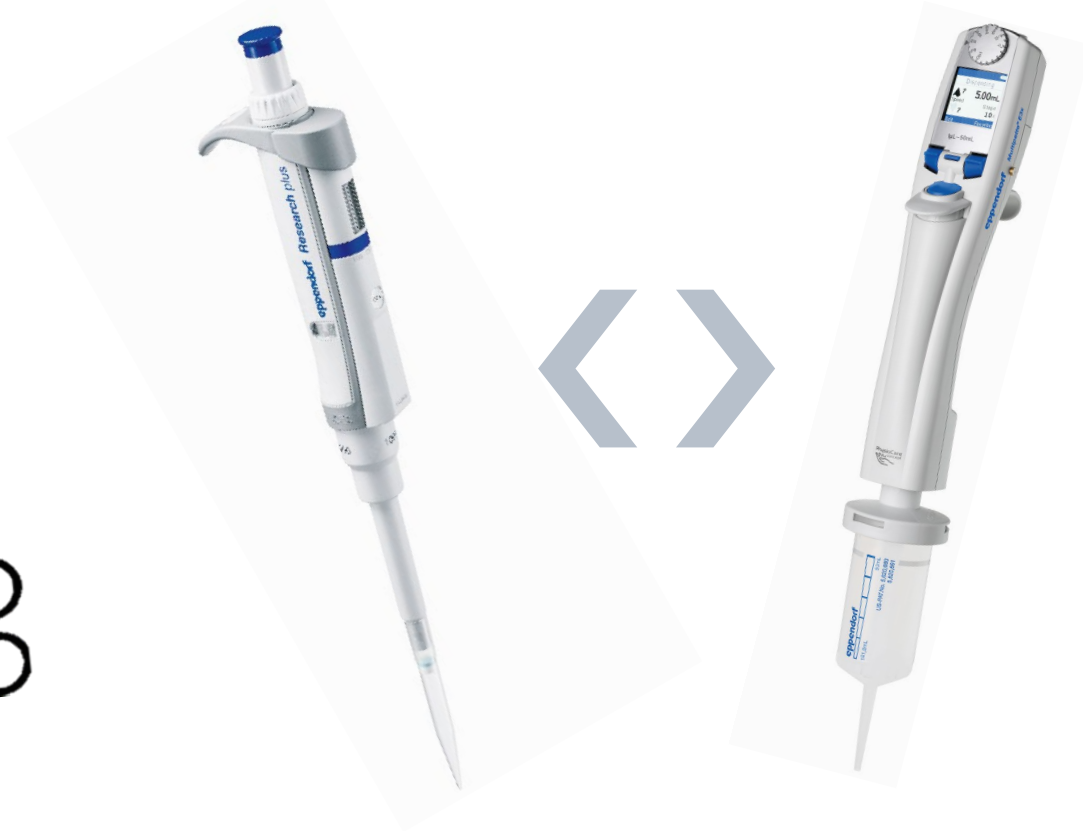
- Unaffected by physical properties of liquid
- Suitable for “problematic” liquids
- Contamination free

Pipetting Different Liquids

Common dissolvents can be problematic

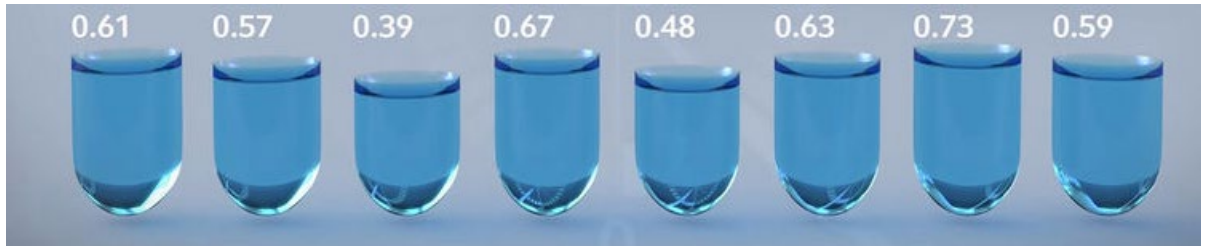
- Ethanol (volatile liquid)
- DMSO (viscous liquid)

Air-cushion vs. positive displacement



Good Pipetting Practice (Air-Cushion)

- **Choose the smallest pipette** (for 100 µL, a 10–100 µL is prefered rather than a 100–1000 µL)
- **Hold** the pipette **vertical when aspirating** liquid
- Only slightly immerse the tip in the liquid
- Keep a constant immersion depth
- While **dispensing, keep a constant angle** of 45°



Liquid pipetted with a varying angle during dispensing

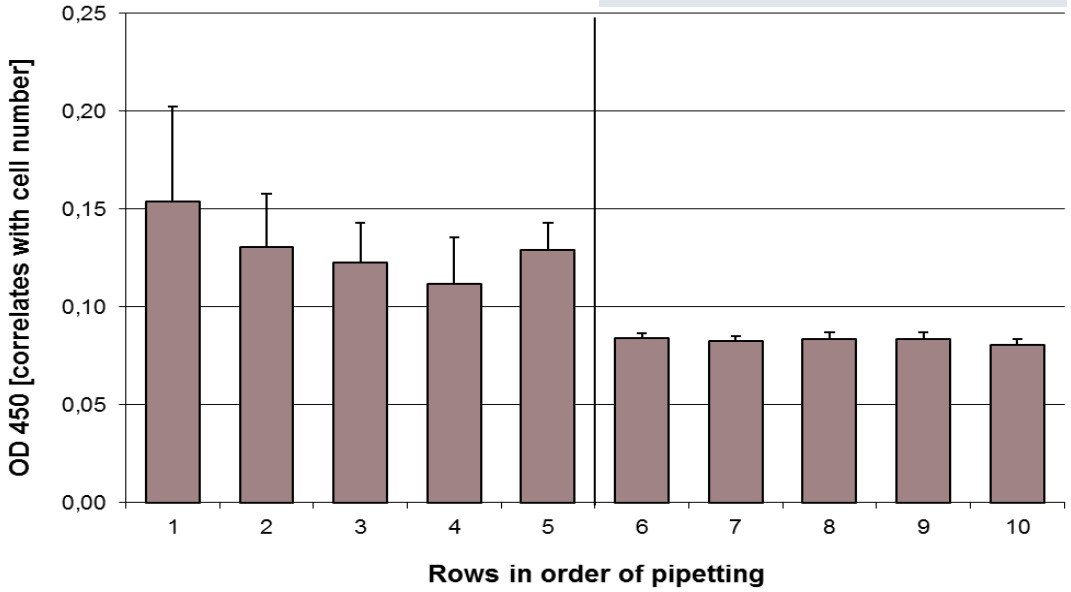
Aspirating **Dispensing**

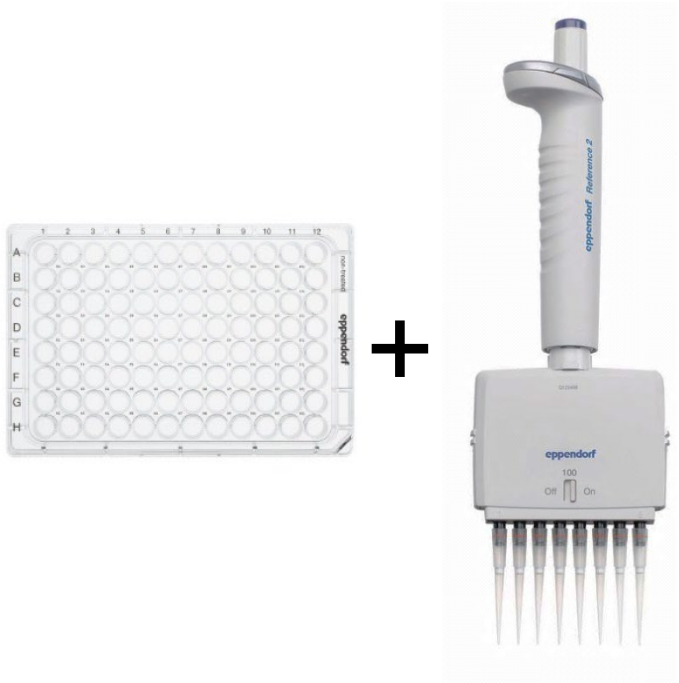
Mixing and Pipetting Technique

Pipetting can affect assay results.

- No mixing between rows
- Different aspiration angles and heights

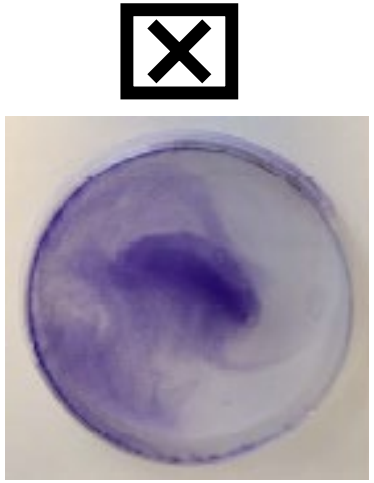
- Mixing before pipetting each row
- Upright and constant aspiration height

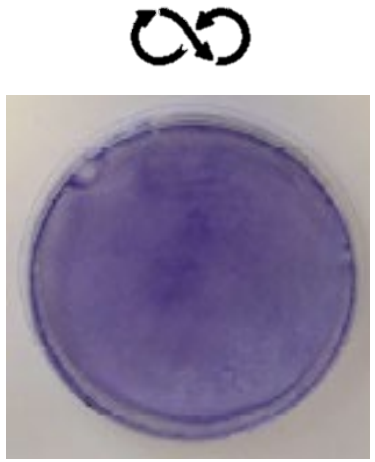


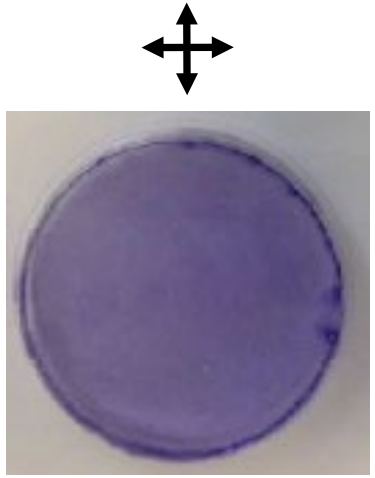


Source: InCelligence

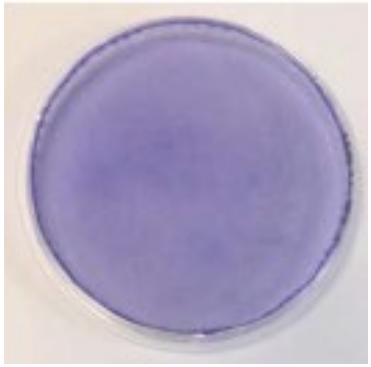
How to Achieve Homogeneous Cell Adhesion



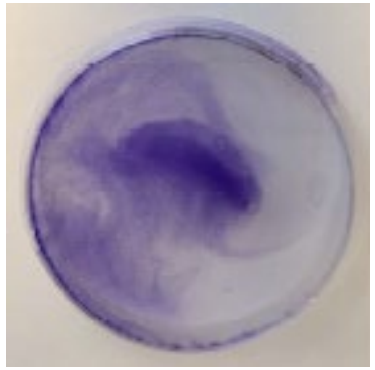


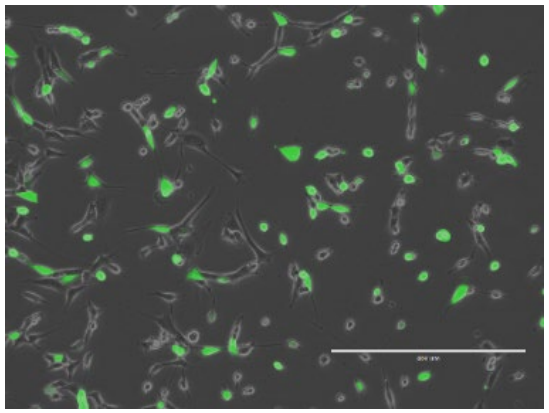


‘Mastermix’

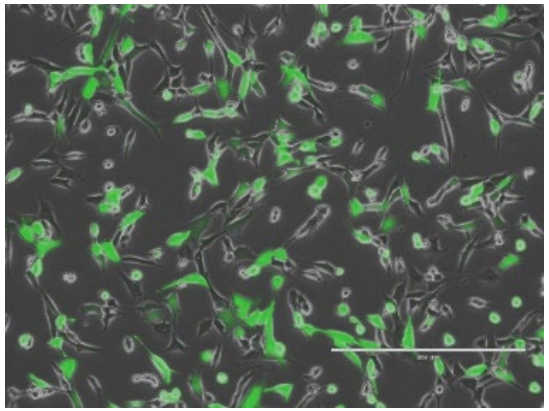


Density-Dependent Cell Behavior

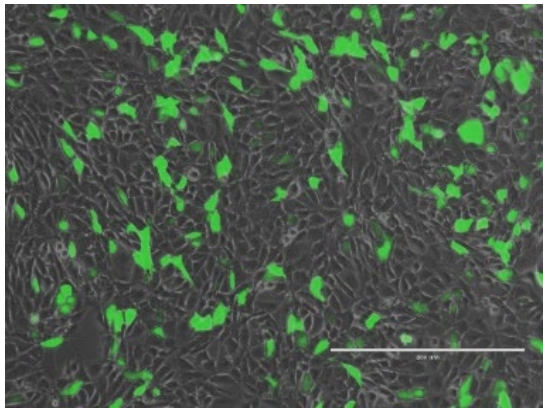




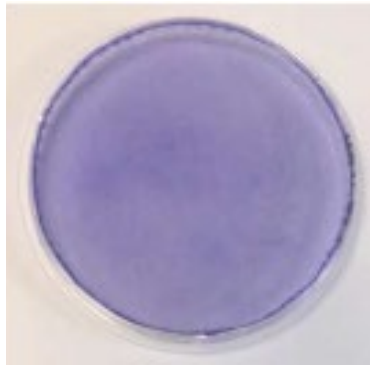
Low density

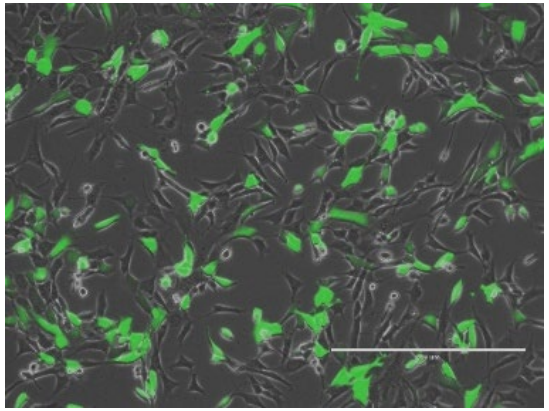


Medium density

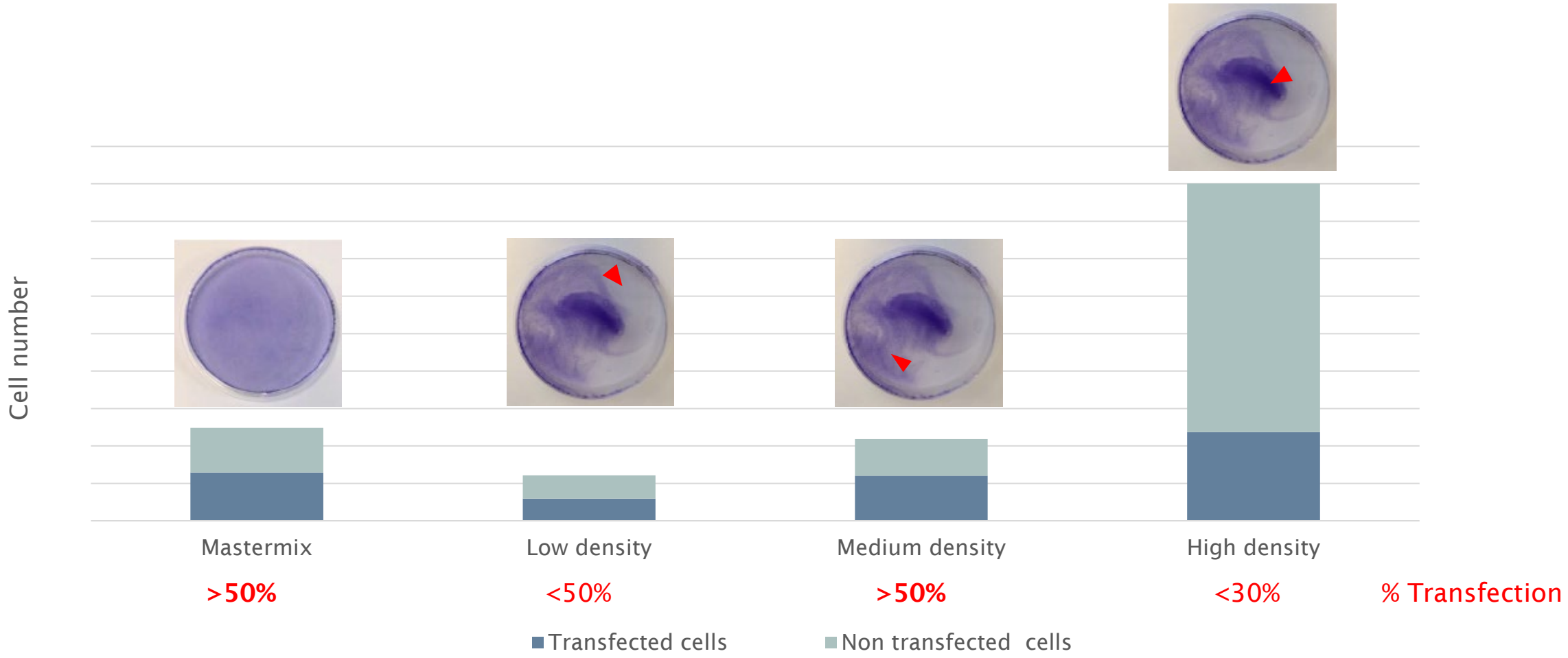


High density



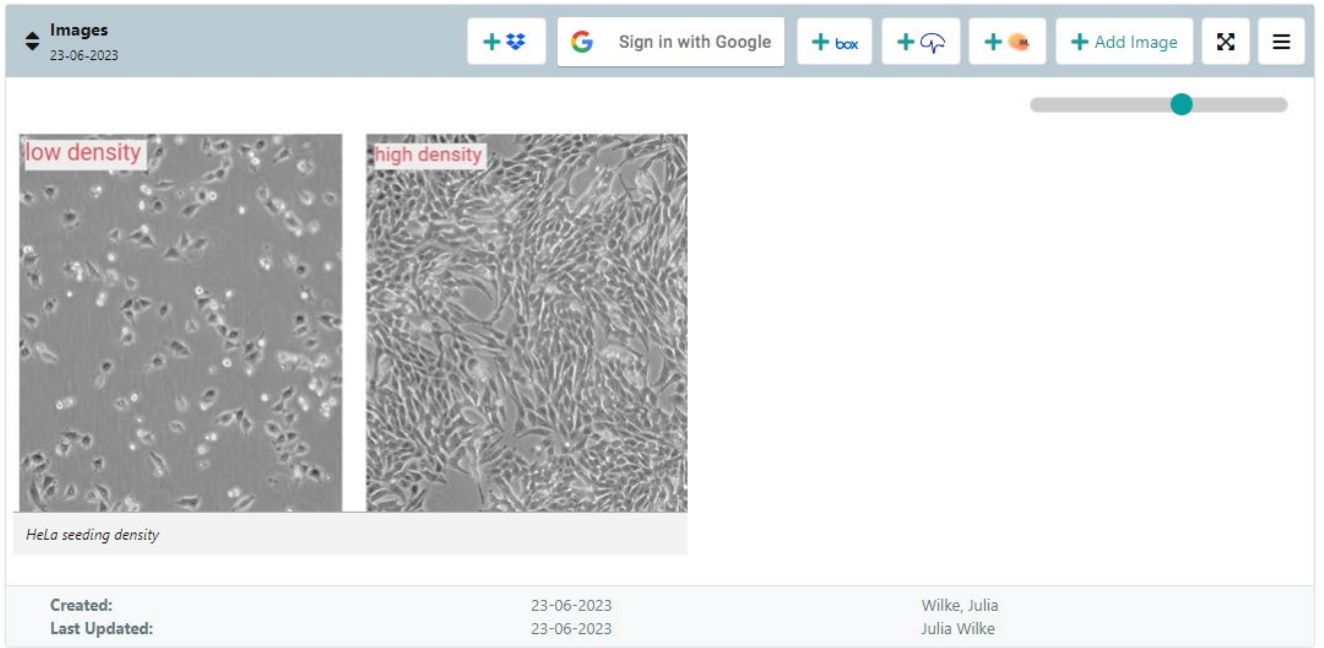


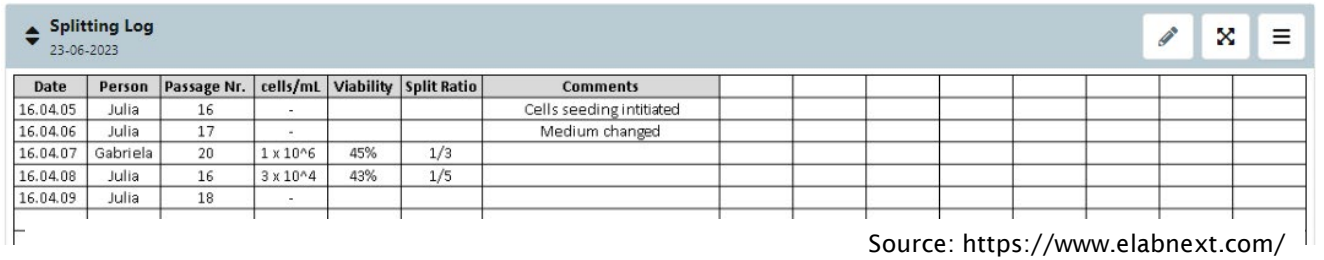
Transfection Efficiency



Constant Documentation

- Clear and traceable cell identification
- Culture conditions and procedures
- Reference images
- Everyday culture practice





Date	Person	Passage Nr.	cells/mL	Viability	Split Ratio	Comments
16.04.05	Julia	16	-			Cells seeding intitiated
16.04.06	Julia	17	-			Medium changed
16.04.07	Gabriela	20	1 x 10^6	45%	1/3	
16.04.08	Julia	16	3 x 10^4	43%	1/5	
16.04.09	Julia	18	-			

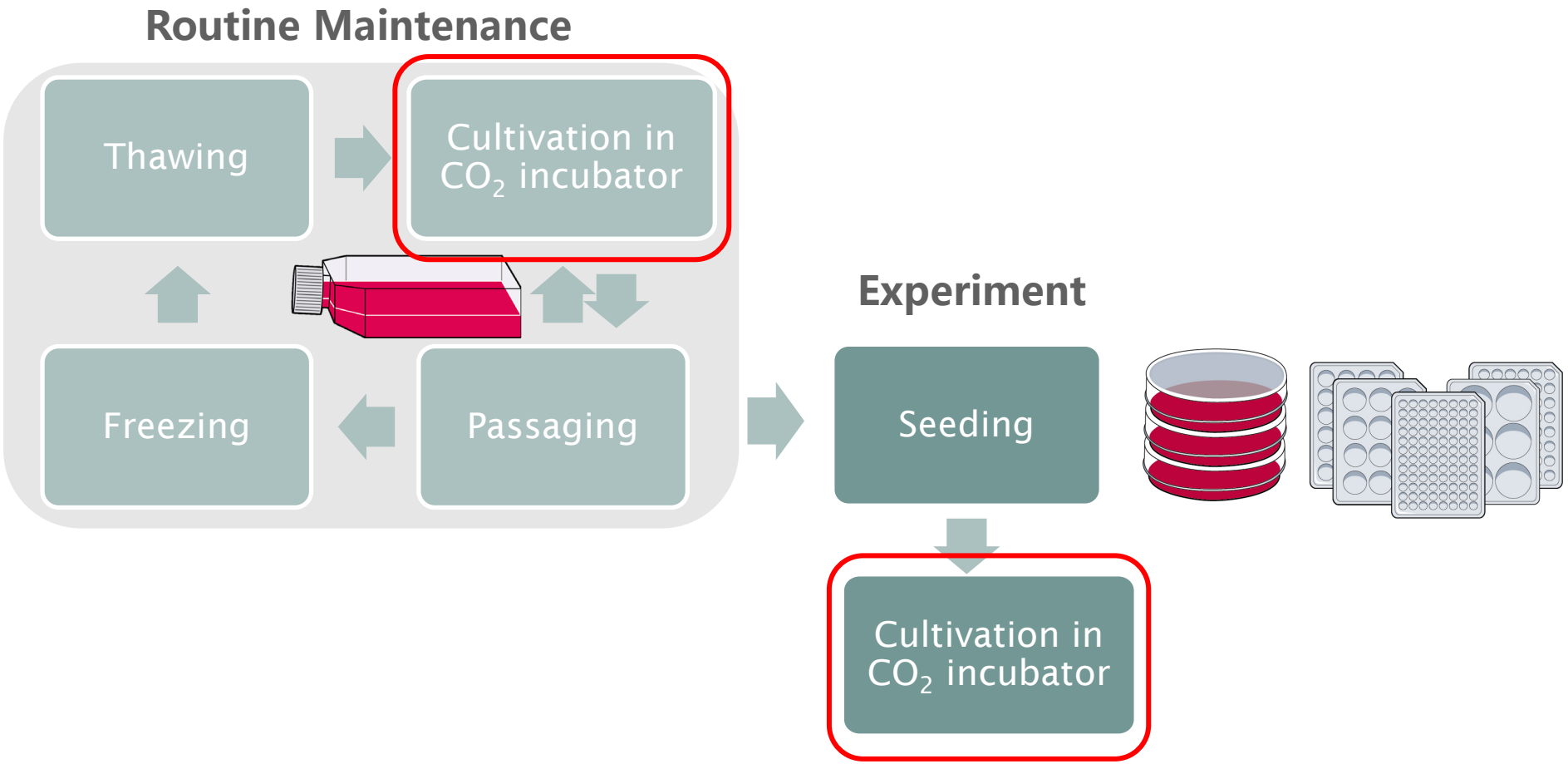
Source: https://www.elabnext.com/

Increase reproducibility by minimizing user-dependent variations

04

Cell Incubation

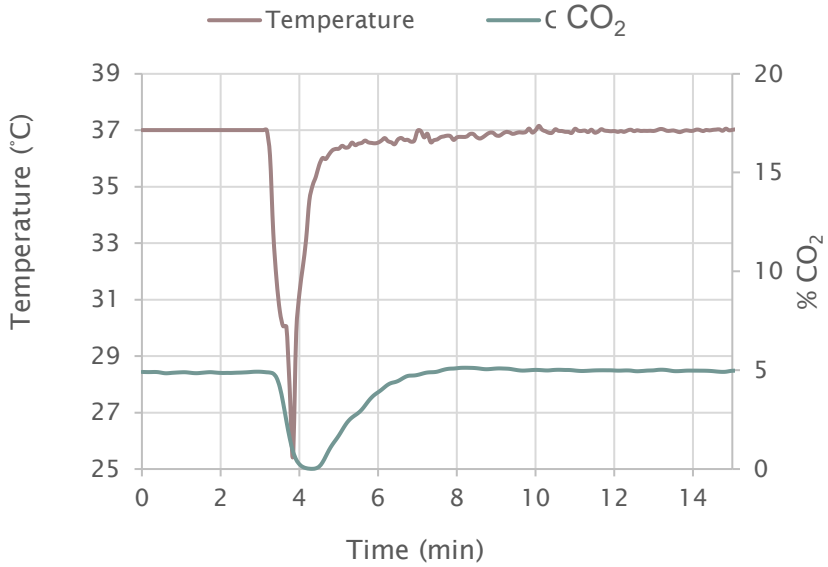
Cell Culture Workflow



Provide Stable Incubation Conditions

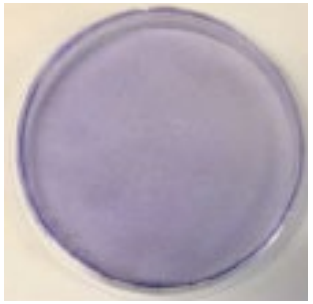
Reduce traffic in and out:

- Incubators with segmented inner doors
- Recovery of temperature and CO_2 (O_2) level
- No set point overshoot
- Separate incubators for maintenance and experiment, etc.

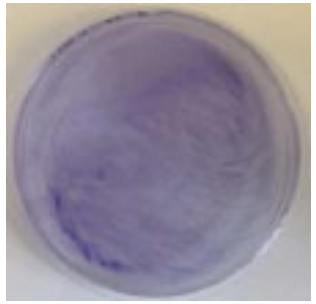


Avoid Vibrations

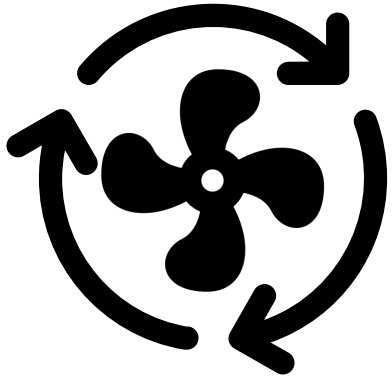
Incubator door openings



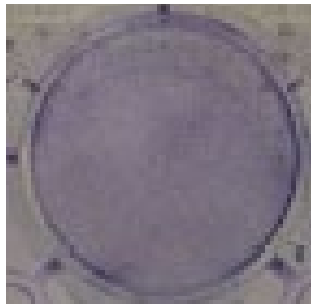
Closed door



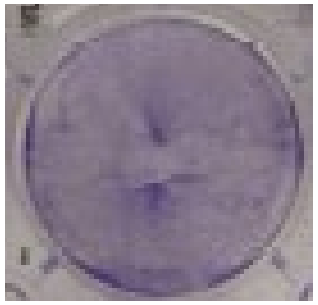
Frequent door openings



Moving culture vessels after seeding



Not moving



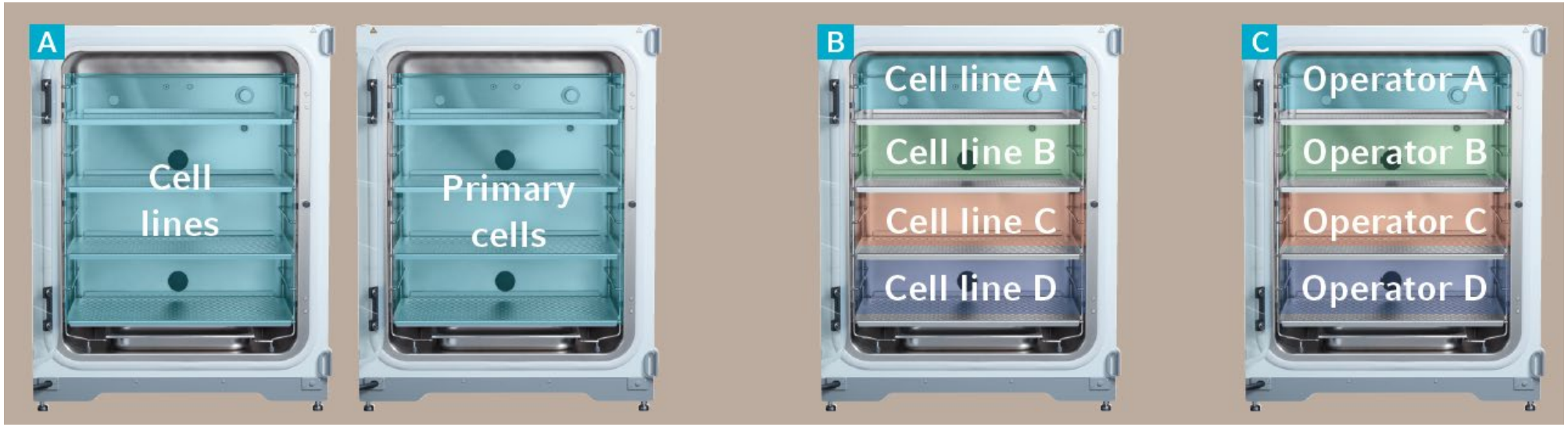
Manual moving

Other vibration sources in the lab:

- Centrifuges, freezers, air conditioners
- CO_2 incubators with a fan

Minimize Disturbances—Stay Organized

Organize to reduce the number and duration of incubator door openings.

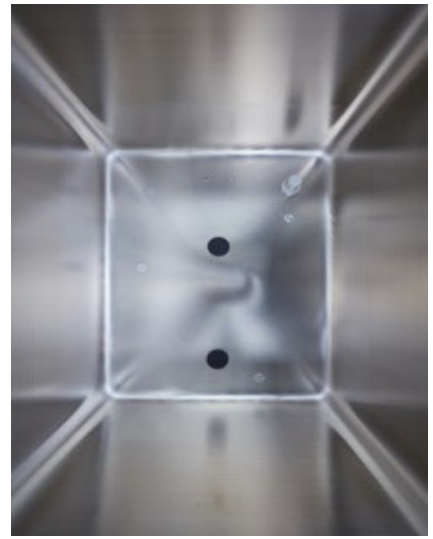


- Optimal incubator content organization depends on lab routines
- Segmented inner doors decrease recovery times

Avoid Contamination Risks

Clean and decontaminate on a regular basis.

- Use incubators with a seamless chamber
- Disassemble and clean inner parts
- Clean/disinfect inner walls
- Run high disinfection routine (optional)



Eppendorf CellXPert

Monitor Incubation Parameters

Reproducible culture conditions

- Check for door openings during incubation
- Download performance data for documentation

05

Conclusions and Recommendations

Conclusions and Recommendations

1. To increase reproducibility in your cell count and confluency measurements, include an impartial measurement method not based on personal estimation

2. Track population doubling times and profile growth curve characteristics periodically

3. When receiving a new batch of cells, test for mycoplasma, authenticate the cells, and make a frozen aliquot

4. Establish quality control protocols in your laboratory

5. Properly describe everything in the methodology and perform statistical analysis

Conclusions and Recommendations

Reproducible experiments and culture conditions:

- For seeding cells, choose the proper pipette and pipetting technique
- For experiments, seed the cells from a pre-diluted master mix and do not let the cells sediment
- Apply continuous proper documentation that can be shared (e.g., with an electronic lab notebook)
- Avoid vibrations and minimize disturbances in your incubation environment (fan-less design, organized incubators, split doors)
- Check the data log on your device to track device performance and download it if needed

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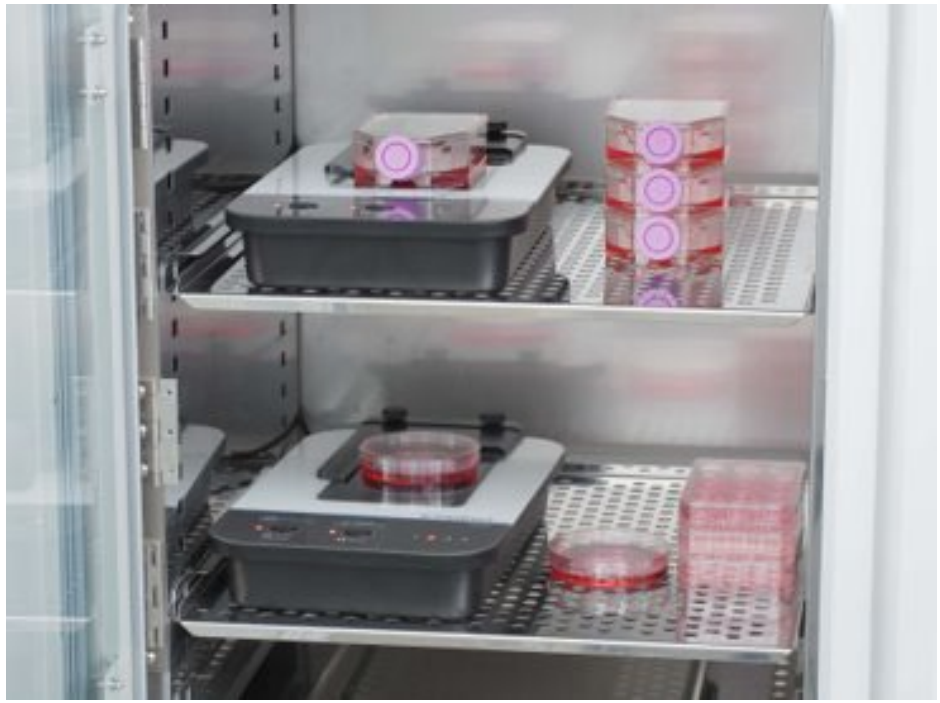
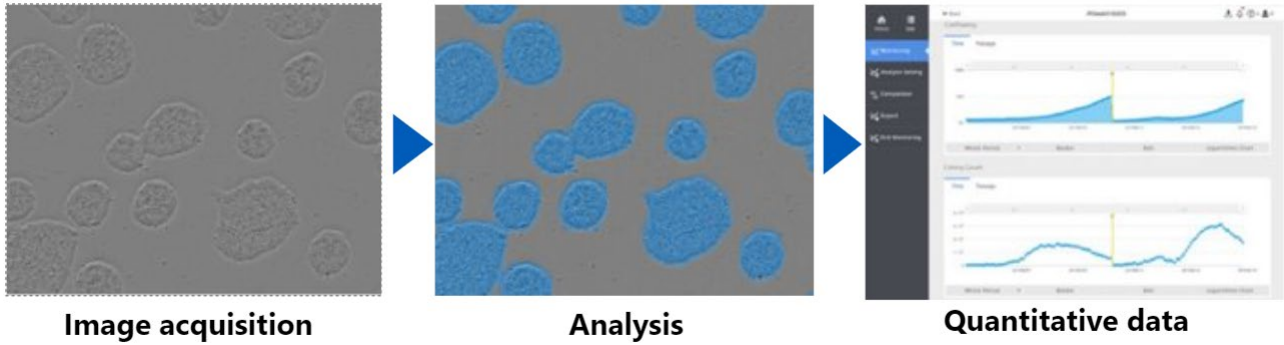
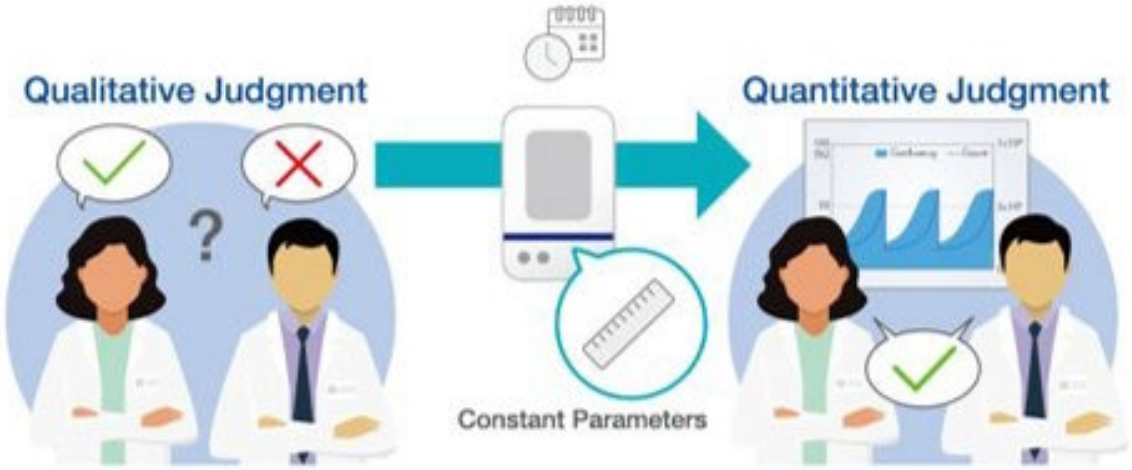
SEEING IS SOLVING

Thank you for your attention

The CM30 Incubation Monitoring Systems

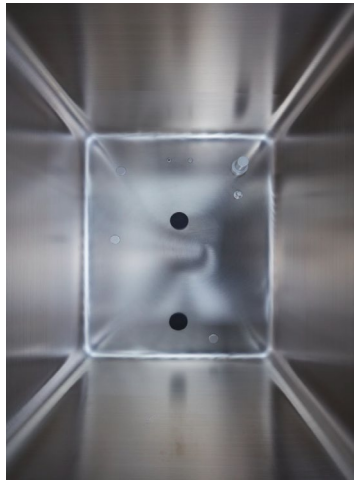


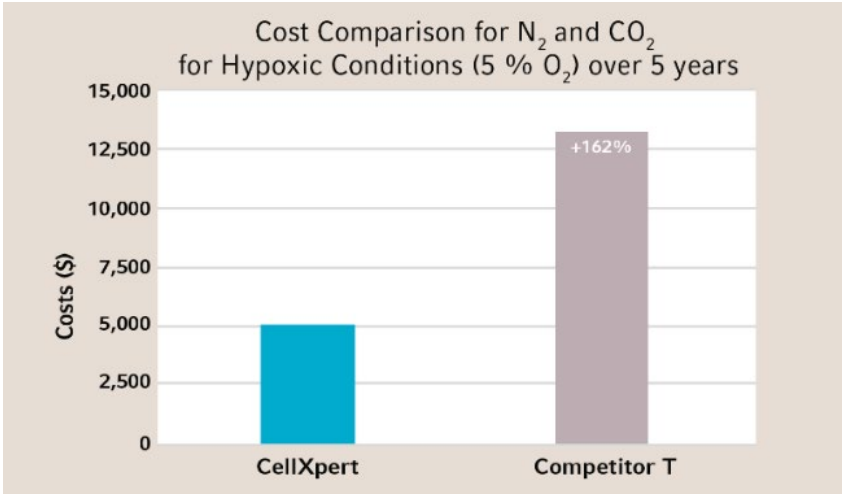




CellXpert® CO_2 Incubator Family

More than a safe home for your cells

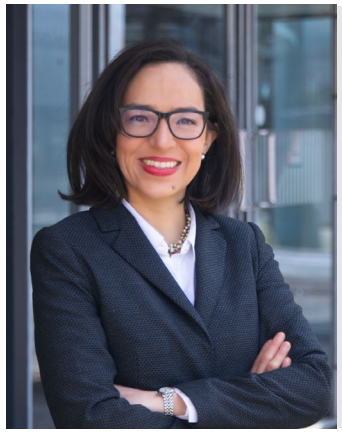




- ✓ Easy to clean, fast atmosphere recovery, no fan, 180 °C (365 °F) disinfection, and more
- ✓ Saves up to 8,300€/$9,000 over five years
- ✓ Up to 25% more usable space

Eppendorf.com/co2-incubators

ASK THE EXPERTS



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