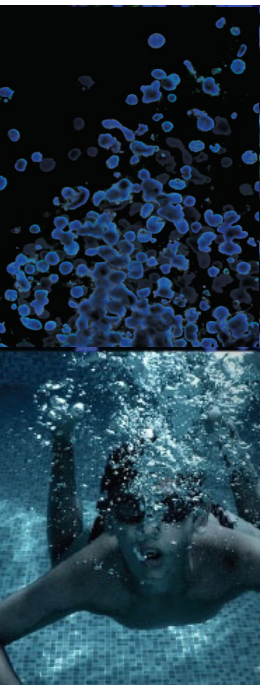
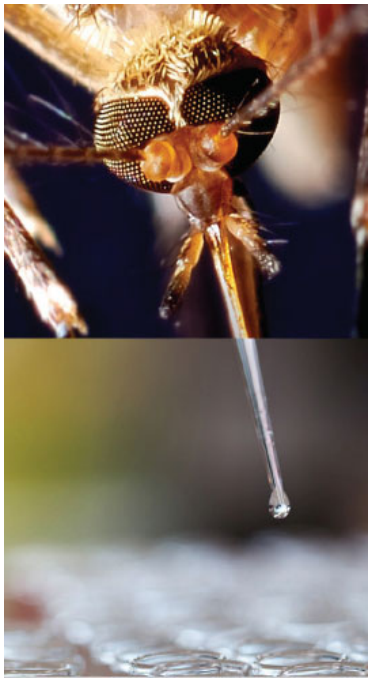
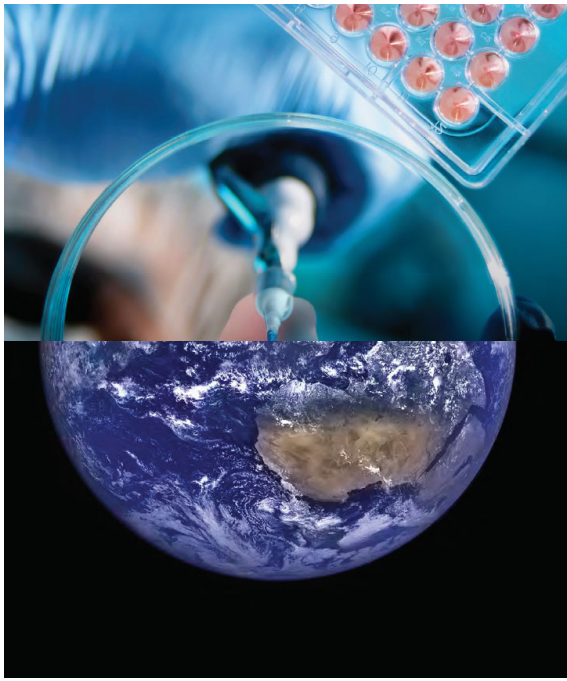
# Cell Culture Basics: Initiate, Expand, Authenticate, and Cryopreserve Your Cells with Confidence

Steven Budd, MS, MBA
*Product Line Business Specialist*, ATCC

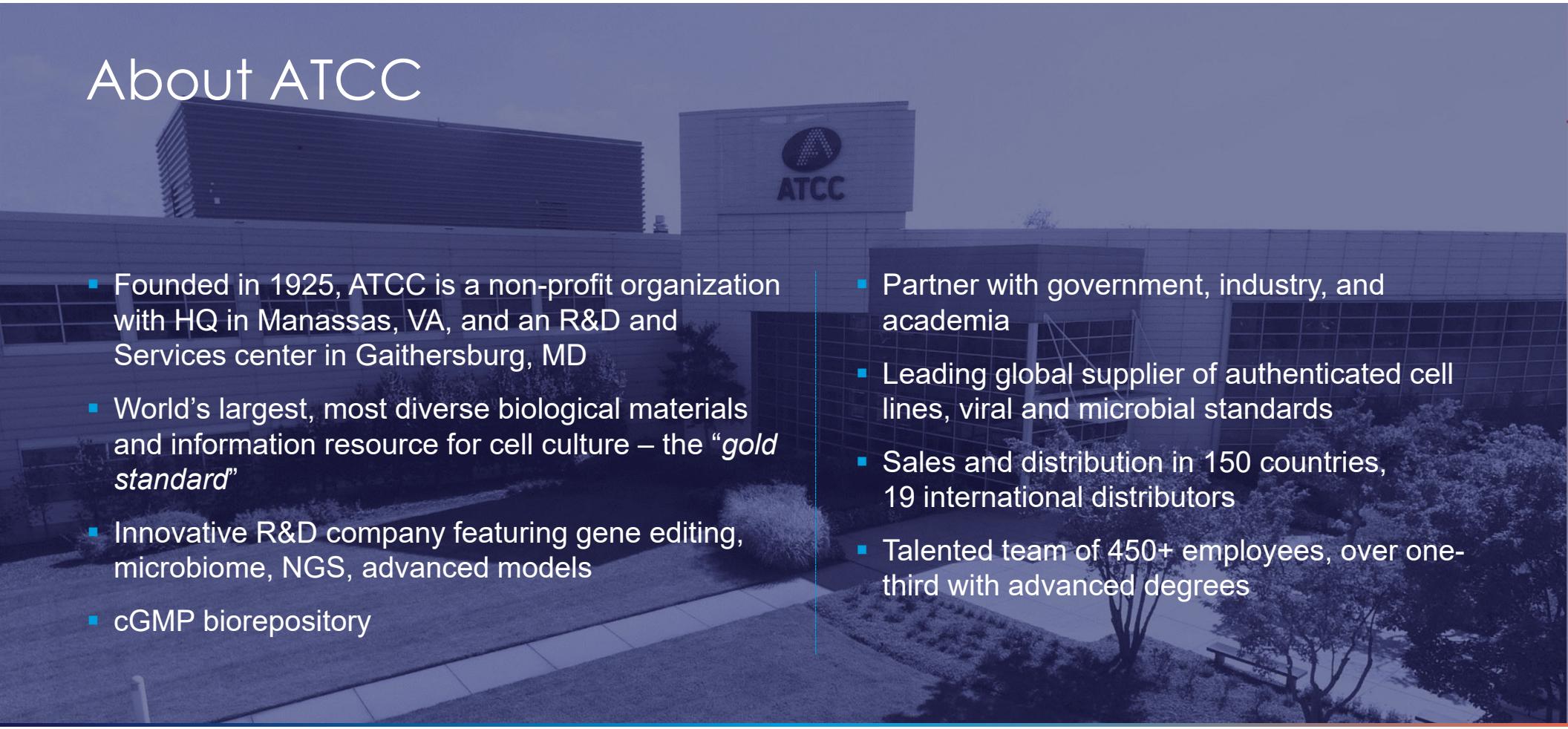
Credible Leads to Incredible™







# About ATCC




- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees



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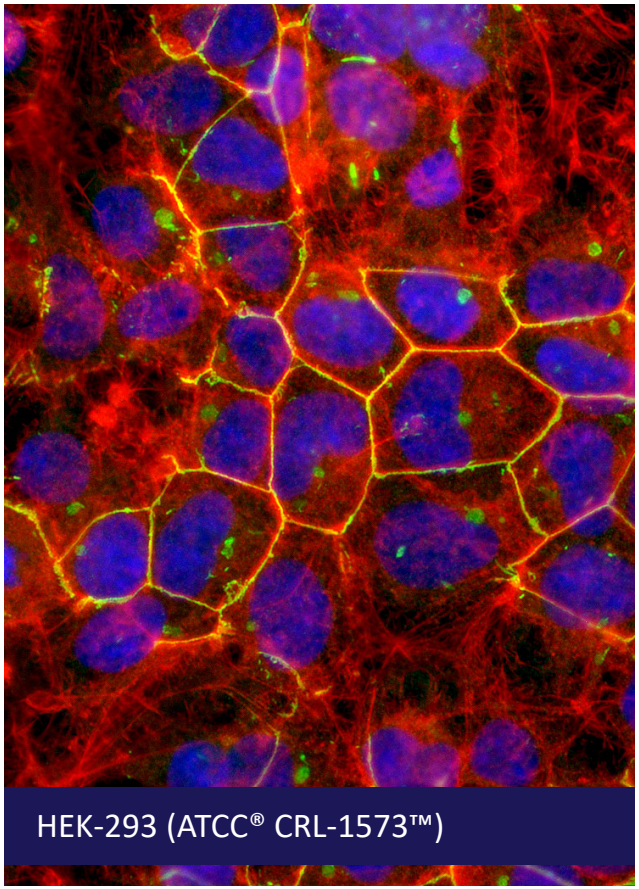
# Agenda

- Cell and Media handling
  - Cryopreservation
  - Aseptic technique and contamination
  - Authentication
  - Mycoplasma – effects and detection
- Workflow and how to choose the best model
- Applications
  - Transfection
  - Cell viability testing



HEK-293 (ATCC® CRL-1573™)

# Cell handling/media handling

# Thawing cells

- Thaw in 37°C water bath for approximately 2 minutes with gentle agitation
- Spray  vial with 70% ethanol
- Transfer to 10 mL centrifuge tube with 9 mL of appropriate growth media (10% FBS)
- *Centrifuge, resuspend in 2 mL of growth media
- Transfer to cell culture vessel

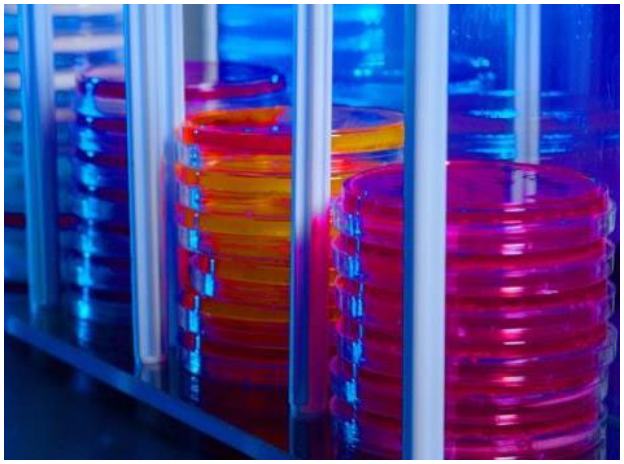
**When bringing out of liquid nitrogen, thaw as quickly as possible**

***For certain primary cells, centrifugation may be detrimental, refer to specific protocol**

# Cell expansion

- After thawing, cells should be plated in an appropriate cell culture vessel with complete media
- 24 hours after seeding, check for confluency

- **Note, primary cells may take up to several days to reach 80% confluency for subculturing**



# Cell expansion

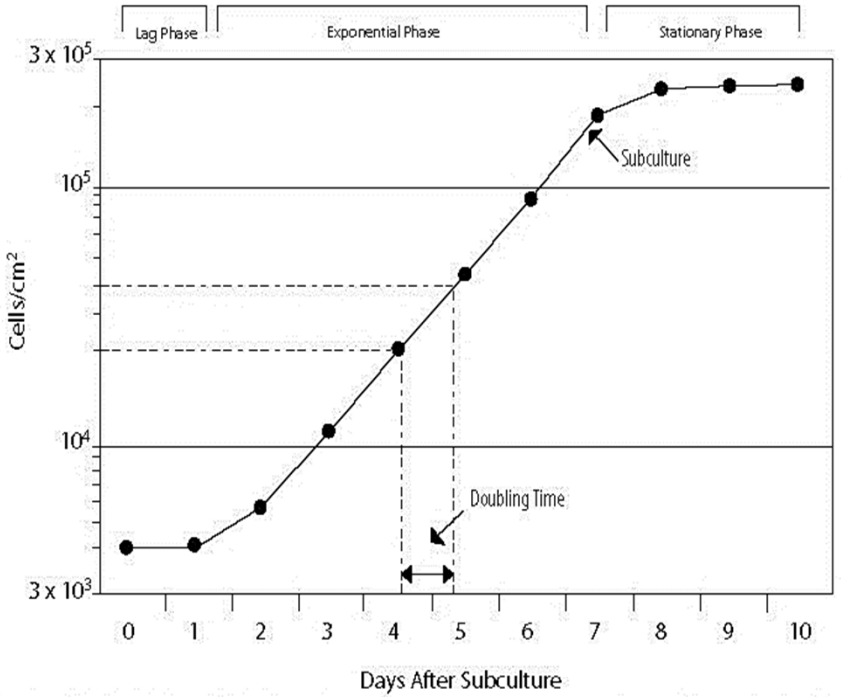


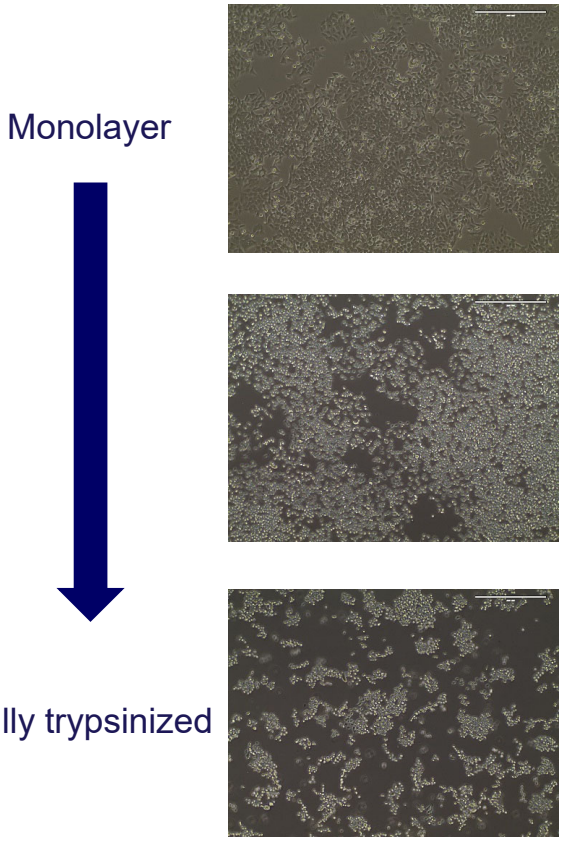

**Figure 1**. Growth curve for cells grown in culture. Cells should be subcultured while still in the exponential phase.

*ATCC Animal Cell Culture Guide, 2014.*

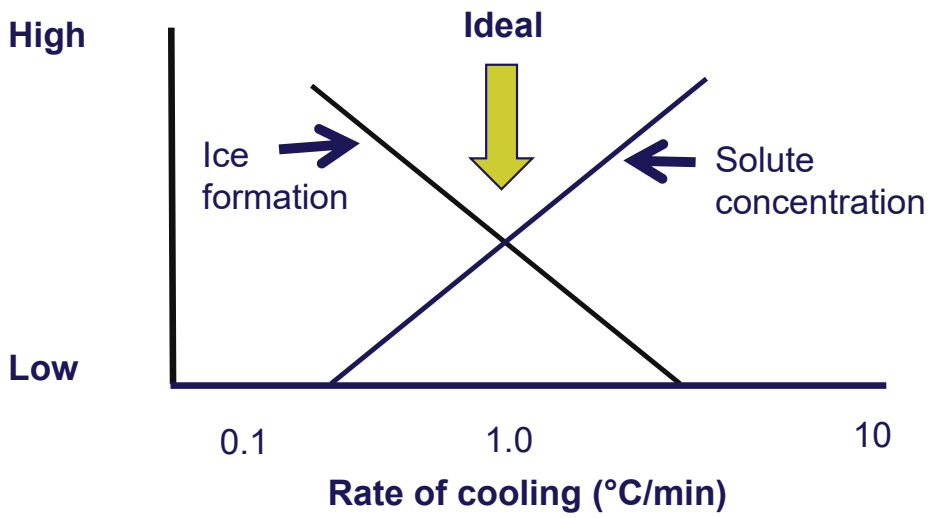
# Trypsinization

**At 80% confluency (primary cells), cells can be passed using Trypsin-EDTA**

- Using warm trypsin-EDTA for about 3-5 minutes, cells will detach with gentle agitation
- ***Trypsin-EDTA for Primary Cells (ATCC® PCS-999-003™) is a low concentration formula (.05% Trypsin and .002% EDTA) – necessary for primary cell survival***
- A Trypsin Soybean Neutralizing Solution (ATCC® 30-2104™) is also needed to prevent cell damage



# Cryopreservation




- High levels of ice formation and increased solute concentration have a negative impact on cell viability
- Optimal cooling rate for cell viability is 1 to 3°C/min



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# Freezing down cells

**-70°C**

- Controlled-rate freeze chamber
- -1°C/min cooling rate
- A few hours to 24 hours

**-140°C**

- Liquid nitrogen tank

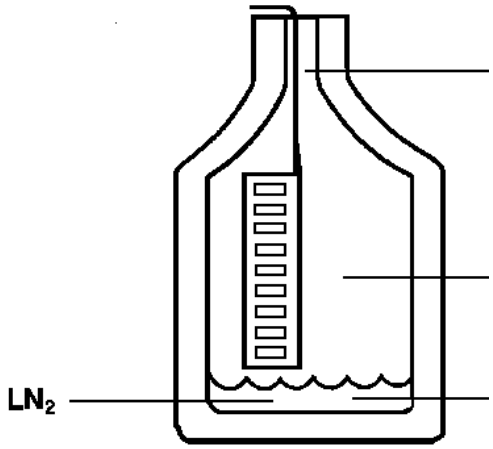
# Low temperature storage

**For the best security, always store your cells in liquid nitrogen freezers**

# Low temperature storage

**Mammalian cells**

- Long-term storage should be below -140°C
- Vials should be stored in a liquid nitrogen unit **above** the volume of liquid at the bottom of the tank
- This temperature should be between **-140°C** and **-180°C**



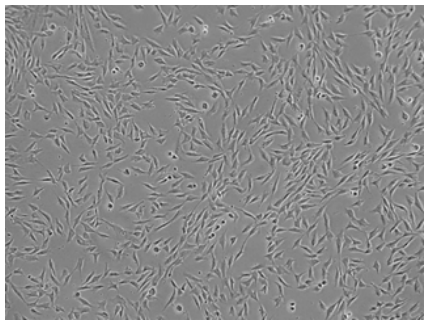

# Cell characterization

**Characterizing cells**

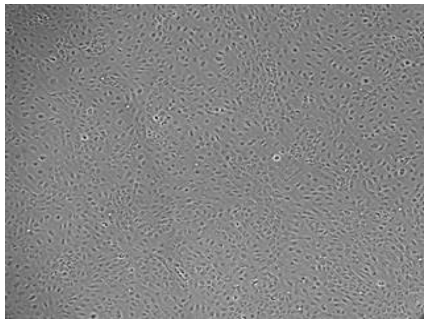
- Cell count before plating
  - Calculating % viability

- **Morphology**
  - Make sure the morphology is consistent with cell type

- **Doubling time**
  - Contamination from other cell types can affect growth rate



Fibroblasts



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# Contamination in cultures

**Sources**

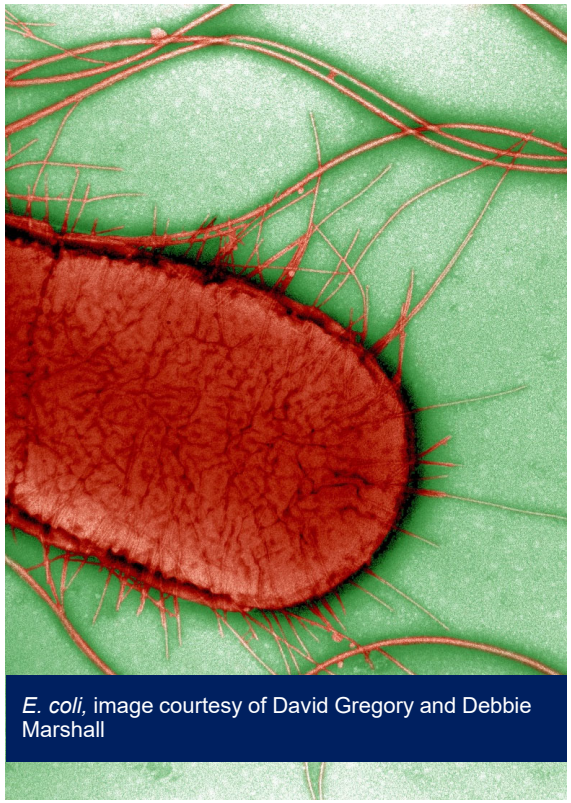
- Contaminated cell lines
- Improper aseptic technique

**Types**

- Microbial – bacteria, mycoplasma, fungi, viruses
- Cellular – cross contamination

**Signs**

- Turbid media
- Rapid decline in pH – color change
- Morphological changes
- Filamentous structures
- Changes in physiological reponses



*E. coli,* image courtesy of David Gregory and Debbie Marshall



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# Bacterial and Fungal Contamination

**Personnel and equipment**

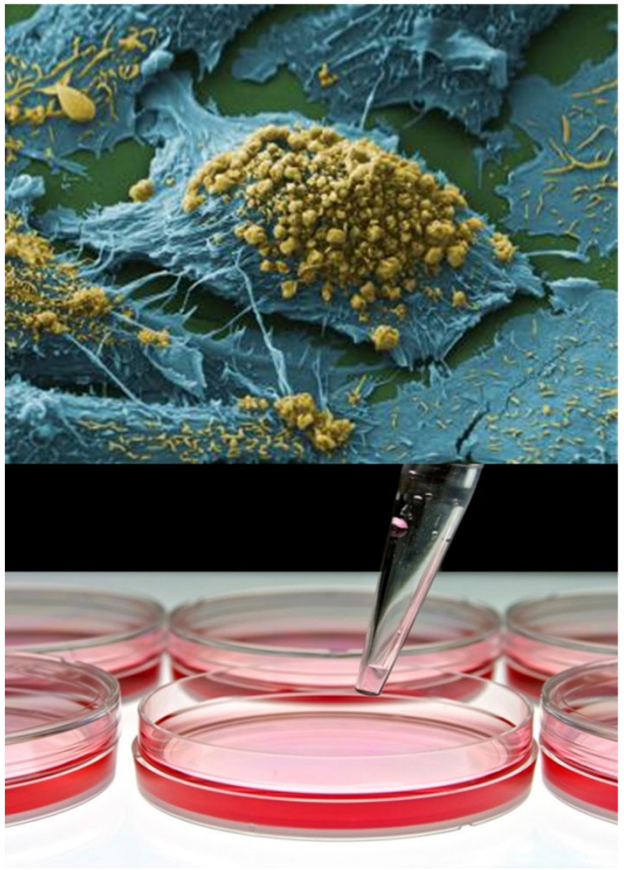
- Poor culturing practices
- Dust and aerosol

**Contamination**

- Aerosol dispersion of contaminated cell cultures
- Faulty laminar flow

**Culture reagents**

- Sera
- Media
- Reagents





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# Contamination prevention and aseptic technique

**Good aseptic technique**

- Make it difficult for microorganisms to invade culture vessels
  - Sealed cultured vessels
  - Vented cap flasks
- Disposable aspirators
  - Cell culture hoods with good laminar flow
  - Do not use as a storage area!
- Spray media bottles/reagents with alcohol



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# Contamination prevention and aseptic technique

**Use small volumes of reagents at a time**

- Aliquot stock solutions and reagents

**Always wear clean lab coats and protective clothing**

**Use seed stocks**

- Create master stocks

**Avoid using antibiotics in media!**

- Can contribute to chronic contamination
- Rarely prevents contamination
- Toxic to cells



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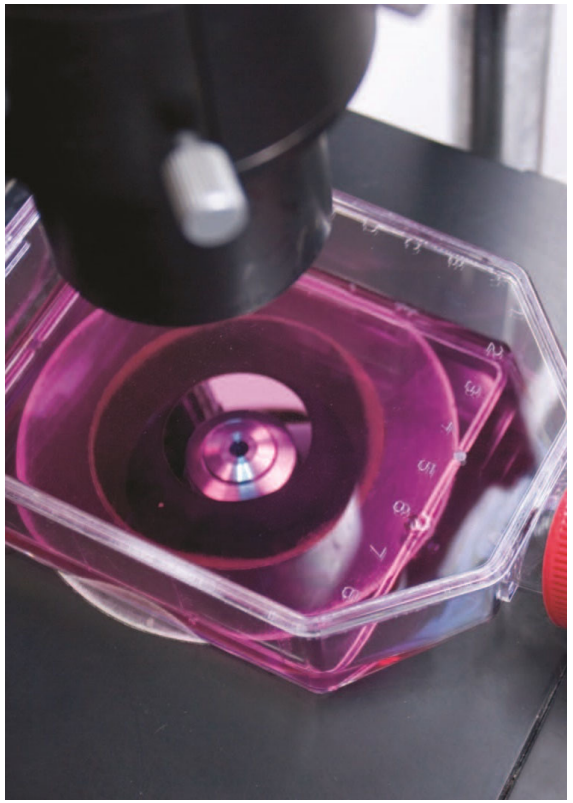
# Mycoplasma contamination

**Not easily detected**

- Does not cause media turbidity
- Does not alter the pH of the media
- Few metabolic byproducts
- Cannot be detected by microscopy

**Results in a number of deleterious effects**

- Chromosomal aberrations
- Disruption of nucleic acid synthesis
- Changes in membrane antigenicity
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Changes in gene expression profiles
- Affects virus production
- Cell death





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# Mycoplasma Detection Kit

**PCR-based kit**

**Detects any of the 60 most common mycoplasmas**


**Kit Components/Procedure**

- Buffer
- Mix of primers specific to mycoplasma
- Positive control
  - DNA from mycoplasma

**Collect/pellet cells → Cell lysis → Touchdown PCR → Run gel/stain**



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# Cell cross-contamination

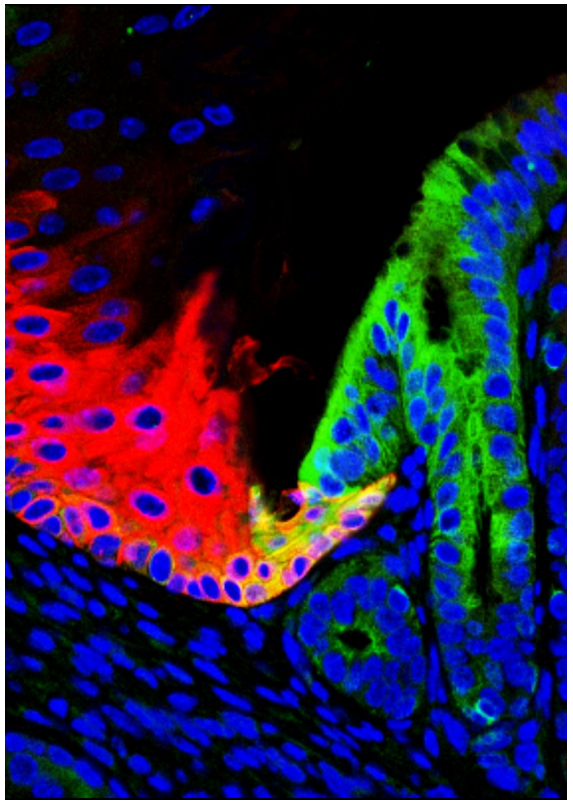
**Leads to the replacement of the original cell line with the contaminant**

Causes

- Multiple cell lines under the hood at the same time
- Failure to change out pipettes
- Receiving cell lines from other labs

**20% of scientific publications include misidentified cultures**

**50% of preclinical research is not reproducible**



*Nat Rev Cancer 10(6):441-8, 2010.*



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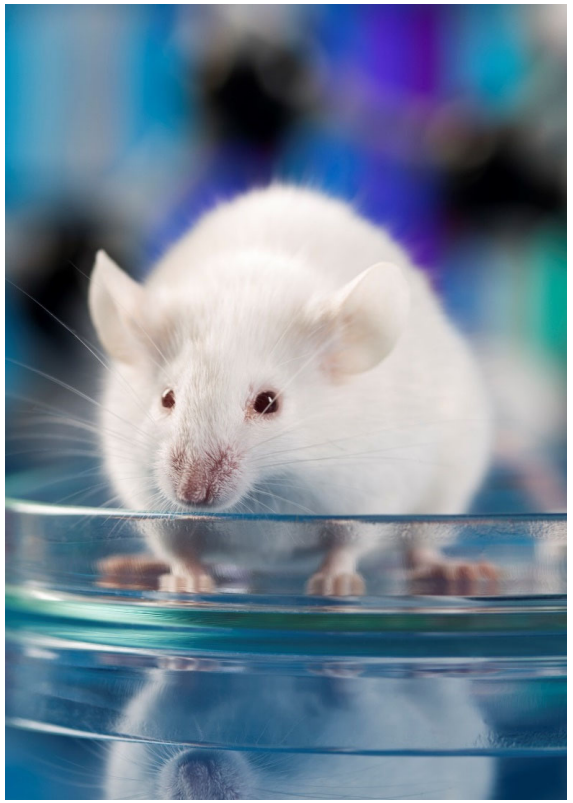
# Short Tandem Repeats (STR)

- ATCC worked with NIST to pioneer STR profiling for human and mouse cell lines
- ATCC authentication services are simple and inexpensive, after placing your order:
  - Spot
  - Dry
  - Mail
  - Receive you results in three to five days

- **Report includes:**
  - Submitted & Matched Allele Calls
  - Contamination check
  - Comparative output for database comparison
  - PDF of the submitted sample profile





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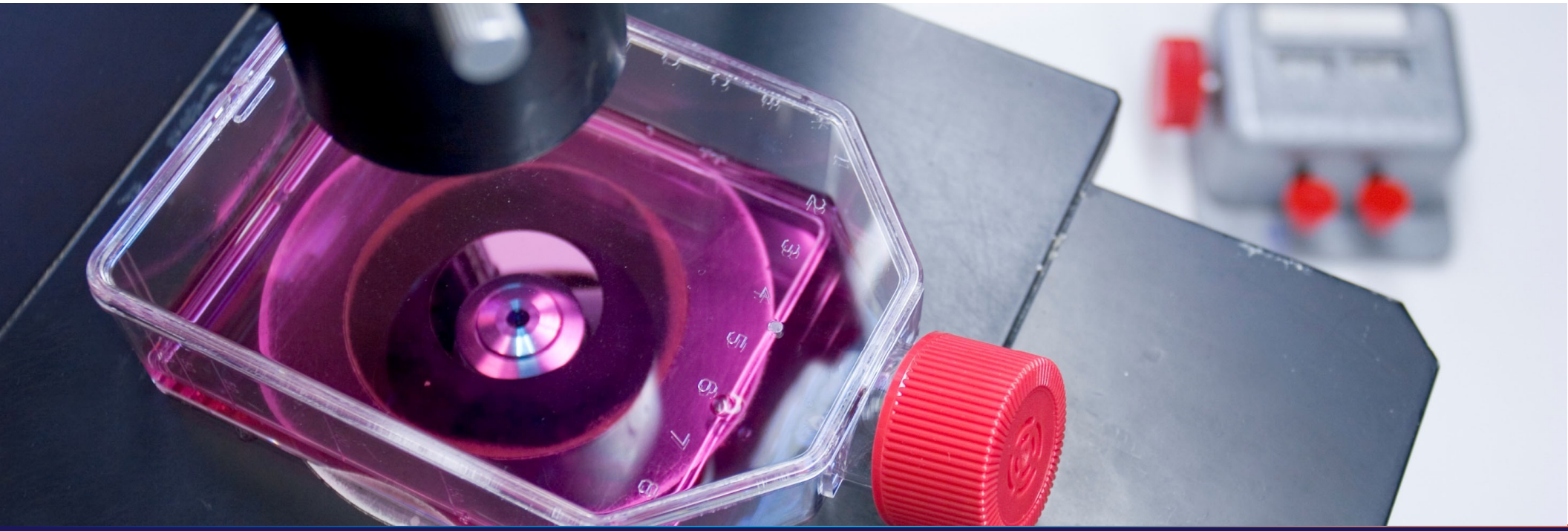

# Workflow and how to choose the best model



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# Media choices

**Continuous (human and animal) cell lines – generally require a classical medium + 10% FBS**

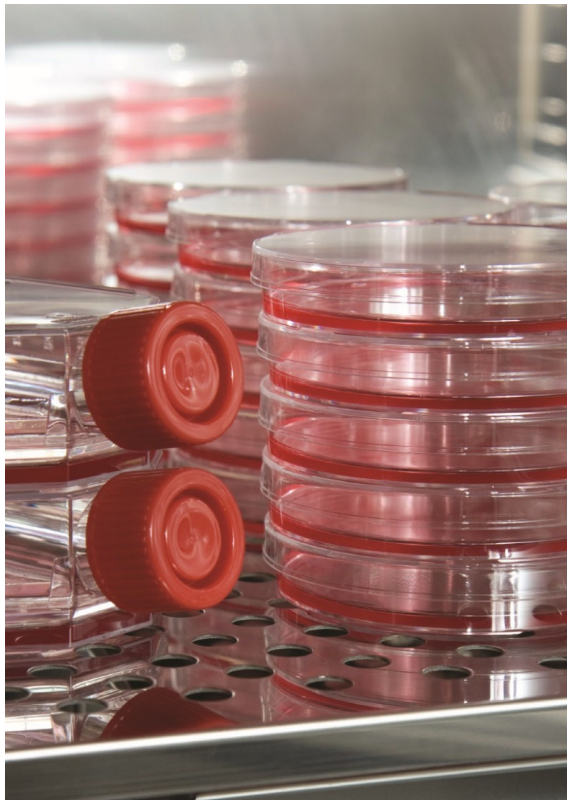
**Specialized cell types (primary cells and stem cells require their own specially formulated media, specific to each cell type**

**Generally maintain cells in the recommended medium**

**If an application requires a different medium formulation, to transfer to new media:**

- Use 1:1 mix (50% old, 50% new media)
- 1:2 mix
- 1:3 mix
- 1:7 mix

**Heat inactivation of FBS? Not recommended**





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# Pros and cons of different cell models

| | Primary Cells | iPSC-derived | hTERT Immortalized | Continuous Cell Lines |
|---|---|---|---|---|
| Mimic *in vivo* tissue type | ++++ | ++ | +++ | + |
| Genotypic stability | Diploid | Diploid | Diploid/ Near Diploid | Aneuploid |
| Proliferative capacity | + | + | +++ | +++ |
| Supply | + | +++ | +++ | +++ |
| Inter-experimental reproducibility | + | +++ | +++ | +++ |
| Cost | + | + | ++ | +++ |
| Ease-of-use | + | ++ | ++ | +++ |
| Predictability in studies | +++ | ++ | +++ | + |



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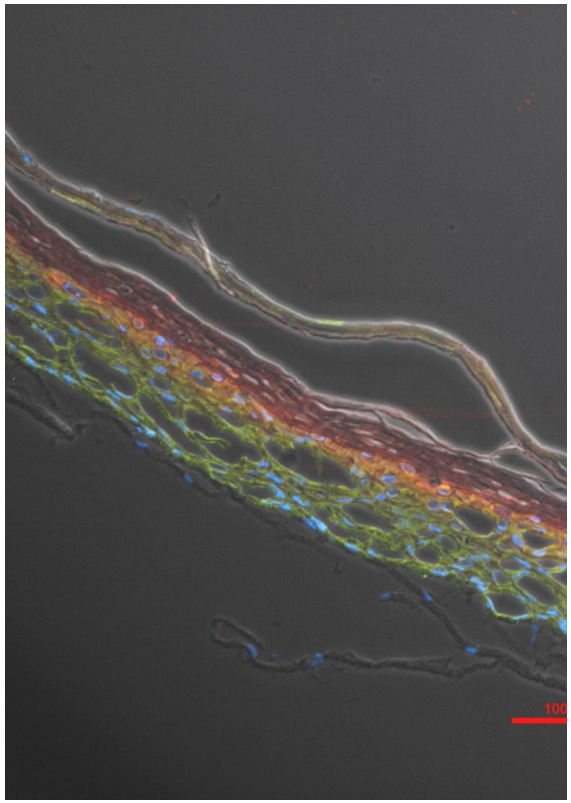
# Primary cells as a control

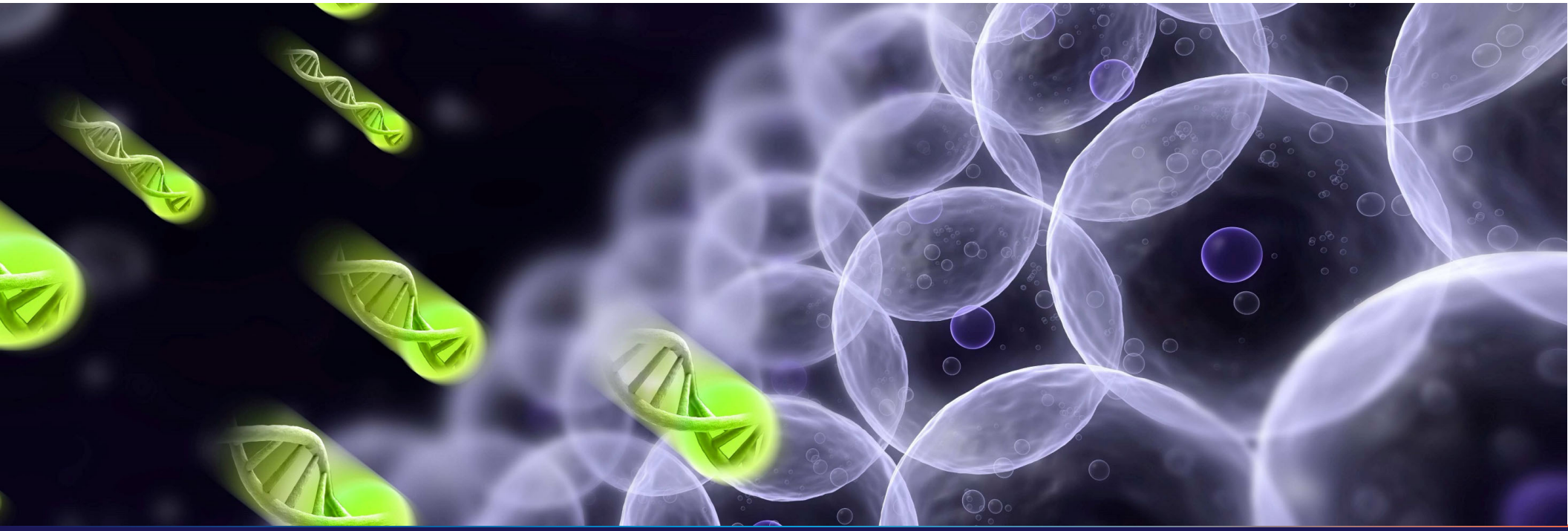
*Primary neonatal keratinocytes (ATCC® PCS-201-010™) differentiated into physiological epidermis*

- Continuous cell lines are cells isolated from primary tissue (often a tumor) that have mutated to survive a "crisis"

- Continuous cell lines have deviated from original source

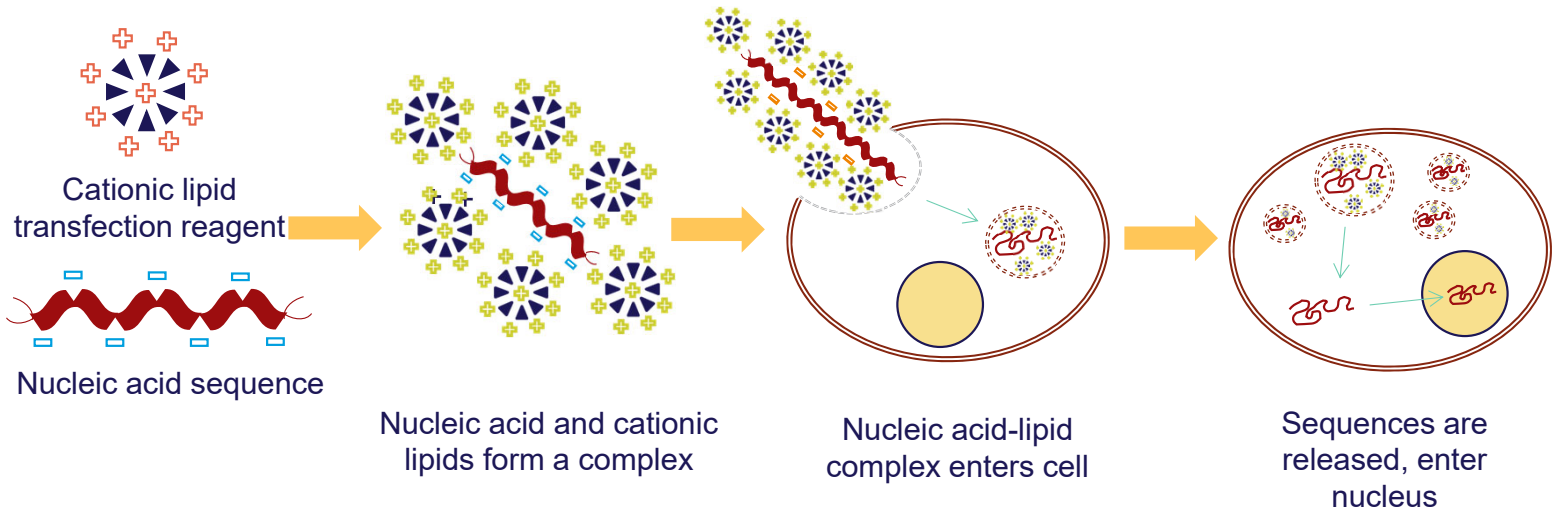
**In every continuous cell line experiment, primary cells should be used as one of the controls**





# Transfection

# Mechanism of lipid-based transfection




ATCC transfection reagents:

- GeneX*Plus* (ATCC® ACS-4004™)
- TransfeX™ (ATCC® ACS-4005™)

# Typical transfection workflow

**Day -1**

Collect and seed cells into vessel where transfection will be performed

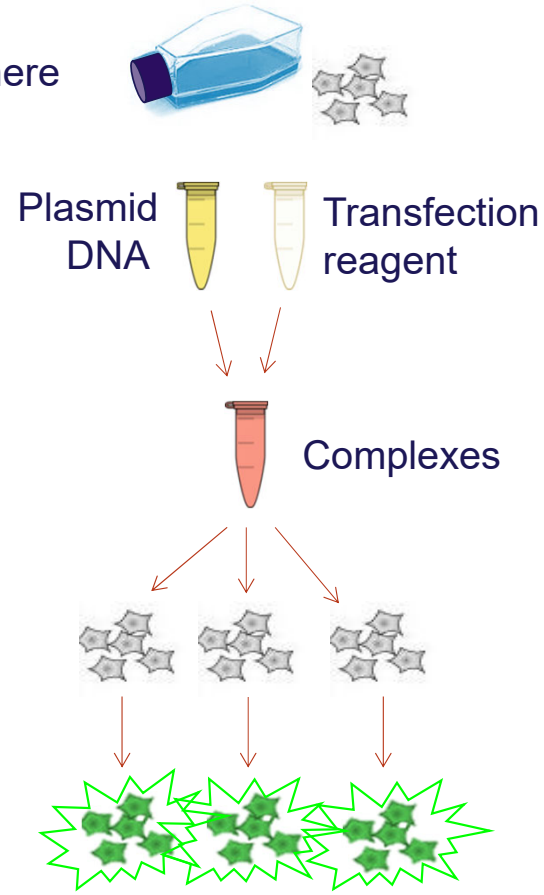
**Day 0**

Form transfection complexes by combining nucleic acid sequences and transfection reagent

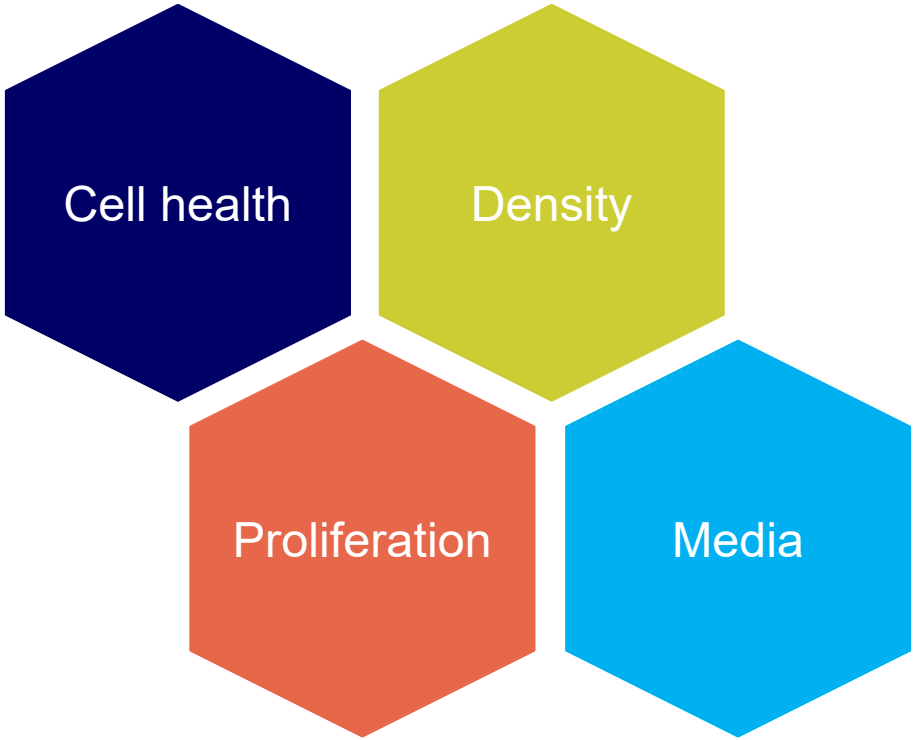
Add transfection complexes to cells

**Days 1+**

Assess transfection



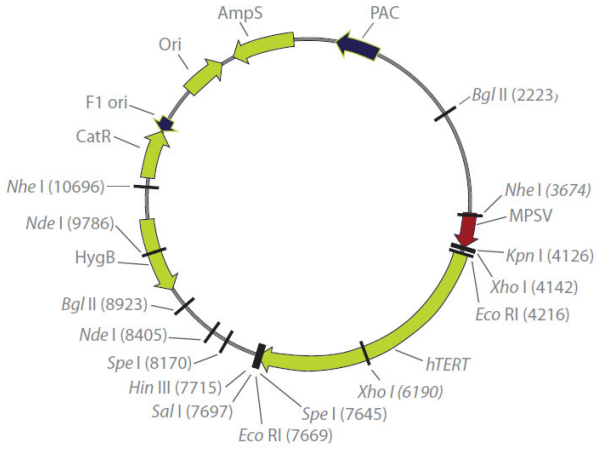
# Cell culture conditions



# Nucleic acids

## All nucleic acids

- High purity
- Endotoxin free
- Validated




## Plasmid DNA

- Promoter
- Plasmid size
- Conformation

# Transfection best practices – assay methods

**mRNA**

- Real time RT-PCR

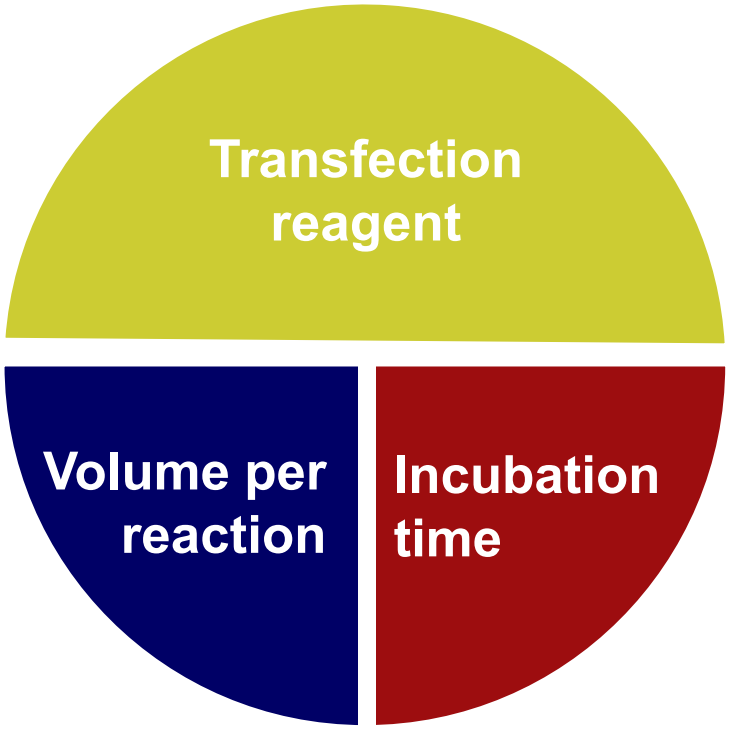
**Protein**

- Indirect (*e.g.*, enzymatic assays)
- Reporter assays
- Western blots
- Immunocytochemistry
- ELISA

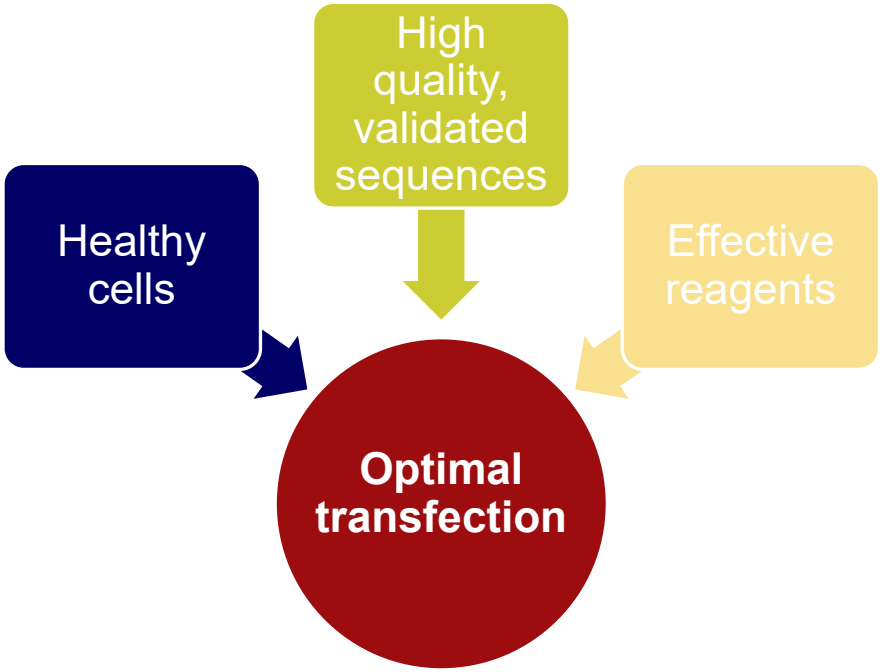
**Other**

- Morphology
- Functional

# Transfection reagents

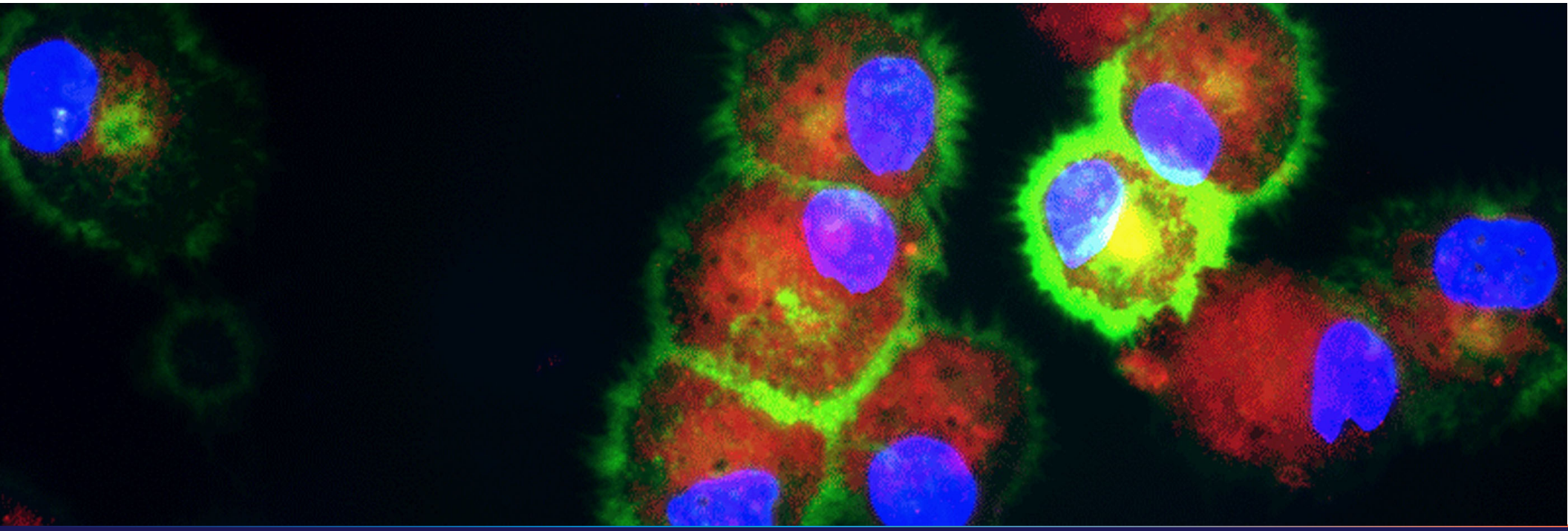


# Best practices summary




**Minimum optimization**

1. Seeding density
2. Volume of transfection reagent
3. Amount of DNA/RNA



# Viability assays

# Viability assays

**Quantitative evaluation of cell proliferation rate and response to external factors that affect cell viability**

- Commonly used for cytoxicity, high-throughput screening (e.g., drug development)
- Uses tetrazolium salts in a colorimetric method for evaluating cell populations

**MTT Cell Proliferation Assay (ATCC® 30-1010K™)**

- Tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

**XTT Cell Proliferation Assay (ATCC® 30- 1011K™)**

- Tetrazolium XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium



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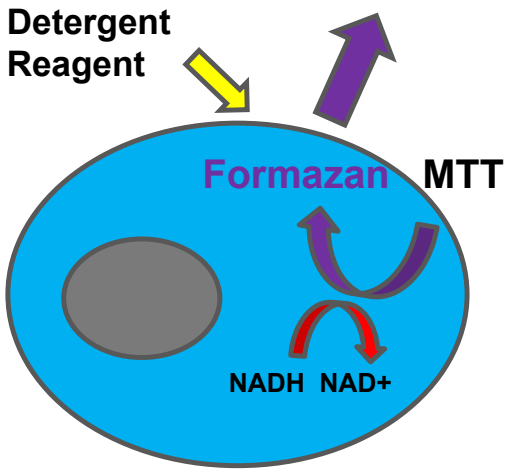
# Viability assays

## MTT Reaction

MTT salt is **reduced** within cellular matrix to Formazan, lysed with detergent to solubilize crystals

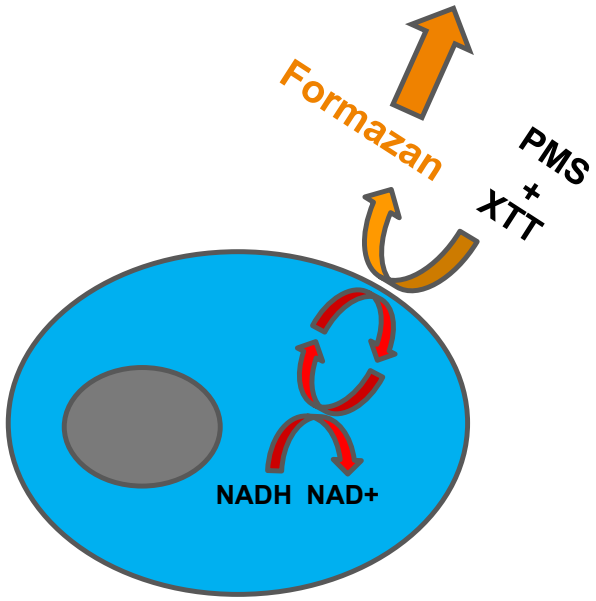
Media turns **PURPLE**




## XTT Reaction

XTT salt is **reduced** at cell membrane with PMS agent

Media turns **ORANGE**



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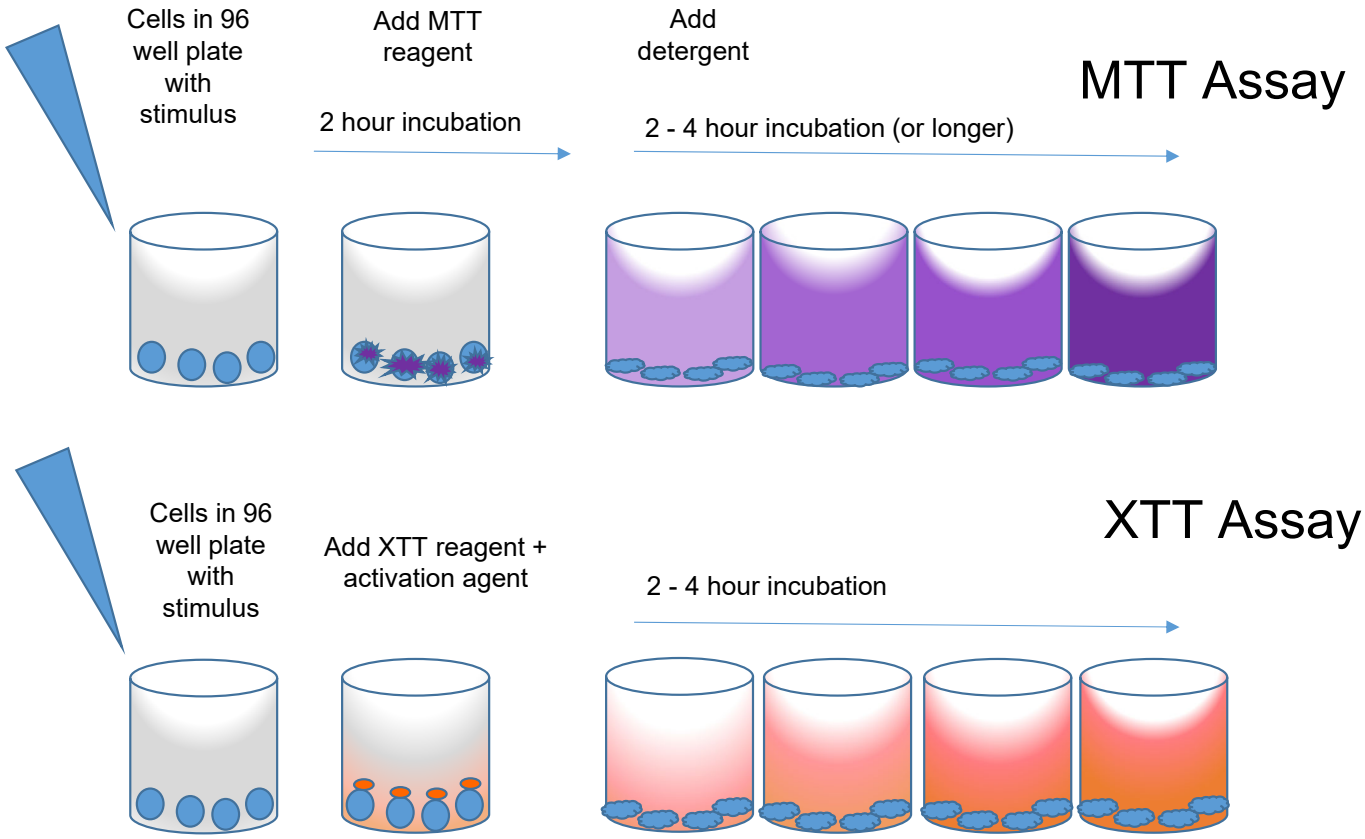

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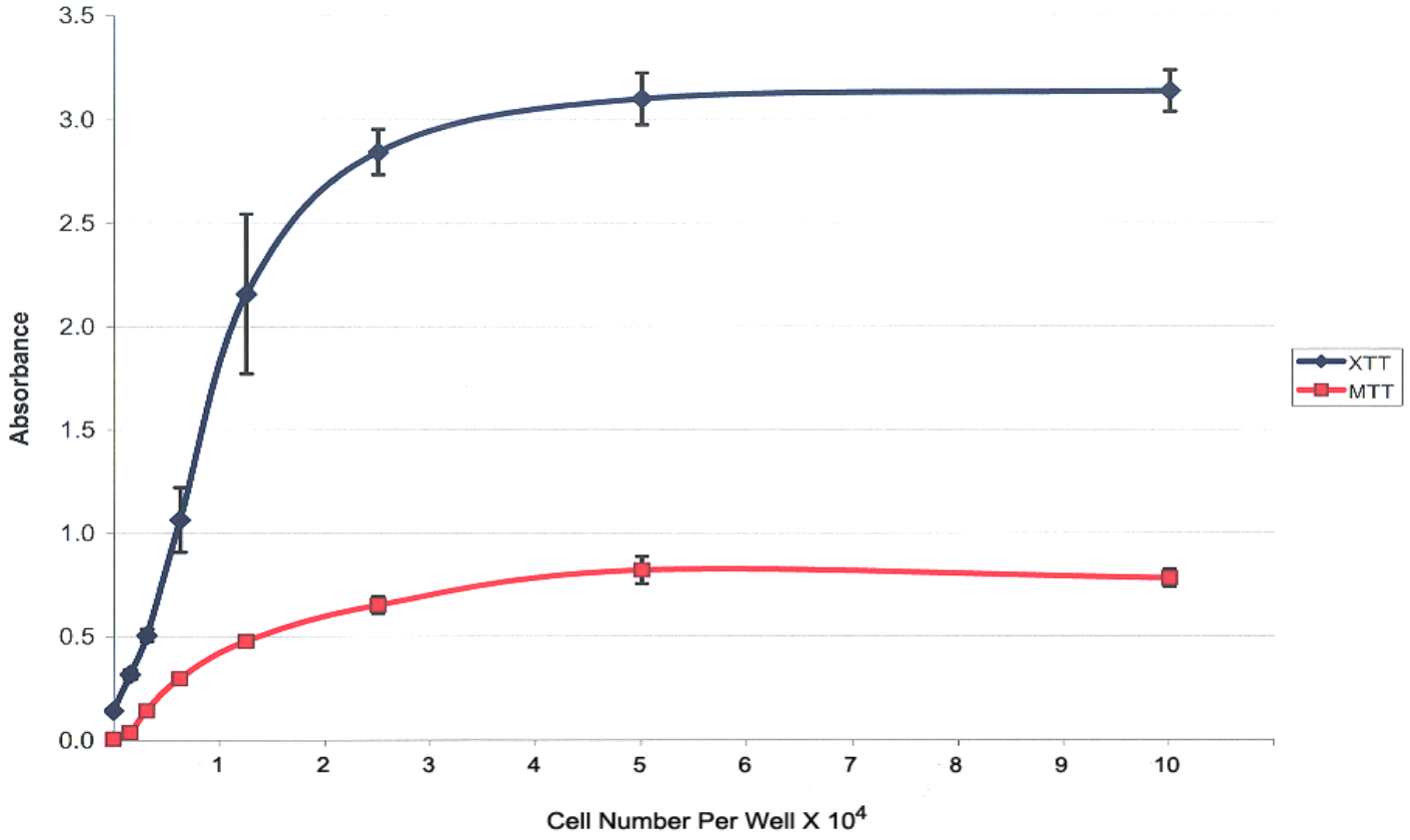

# Summary

# Summary

**Cell Handling/media handling**

- Be sure to employ best practices to eliminate contamination and ensure optimal growth and storage
- Routinely authenticate your cells to ensure reliable results

**Cell culture workflow**

- Use cell lines for standardization and confirmation of each experiment; use primary cells after standardization to further validate the results with a biologically relevant model
- hTERT-immortalized cells provide continuous growth with near-primary cell performance

**Transfection**

- Effective lipid transfection starts with healthy cells, purified substrate, and optimal ratio of substrate and lipid

**Viability assays**

- MTT, XTT assays can confirm cell growth characteristics



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# Learn more: **www.atcc.org/webinars**

Coming soon!

**Organoid Growth Media: Techniques to Help You Streamline Culture**
Presenters: Steve Budd, MS, MBA and James Clinton, PhD
September 29, 12:00 ET

**Evaluating the Differentiation Potential of Primary Airway Cells in 3-D Models**
Presenter: Kevin Tyo, PhD
October 6, 12:00 ET

**Luciferase Reporter Cancer Cell Lines: Facilitate Your CAR-T Development**
Presenter: John Foulke, MS
October 13, 12:00 ET

**Does Differentiation Matter? Comparing the Toxicological Response Between Airway Epithelial Models**
Presenter: Kevin Tyo, PhD
November 3, 12:00 ET