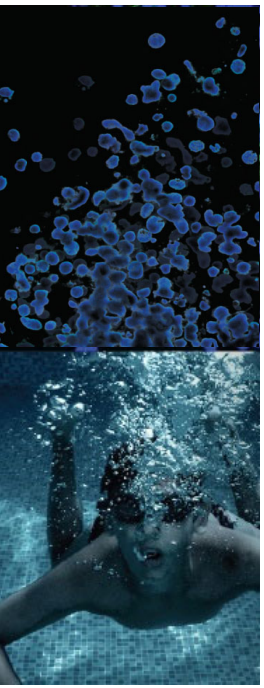
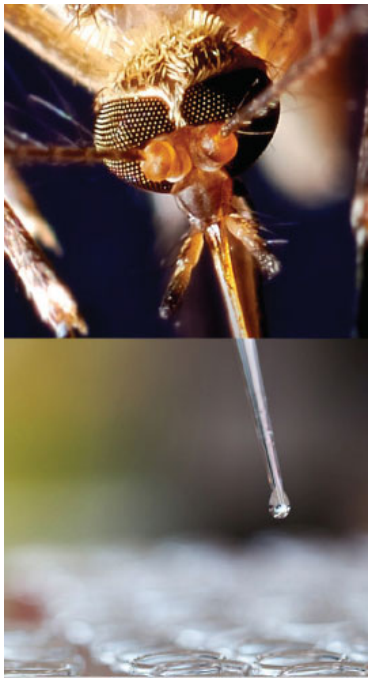
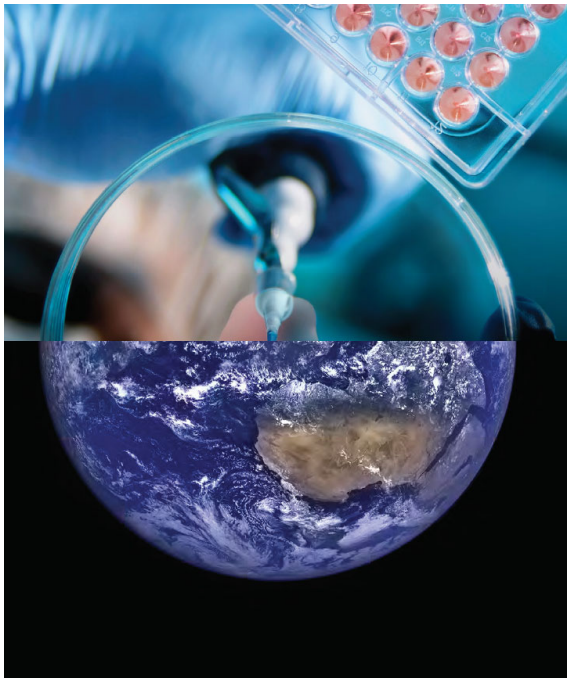
Best Practices for Cell Culture

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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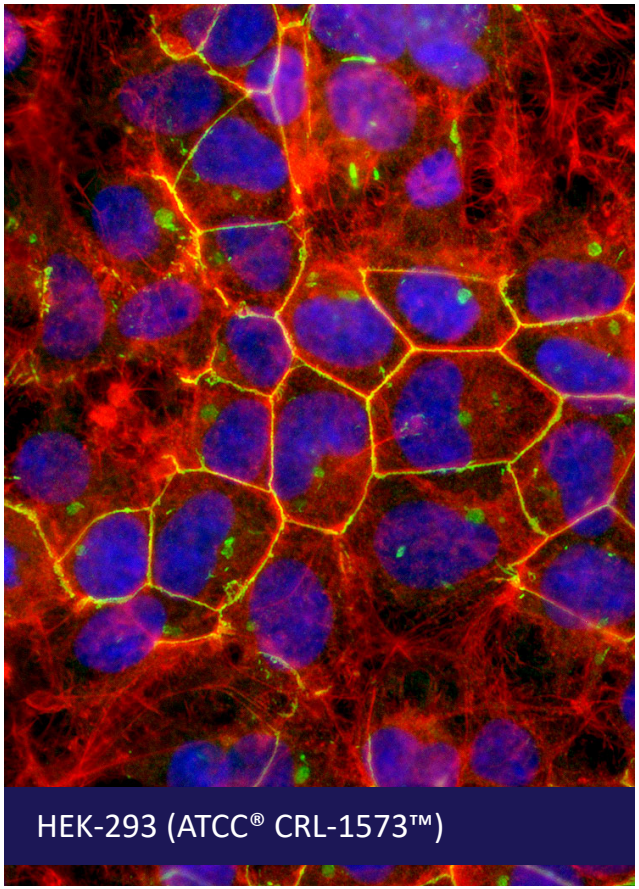
Agenda

- 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

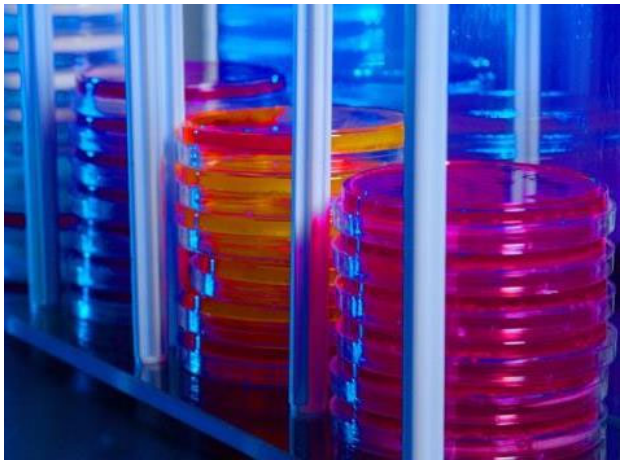
When bringing out of liquid nitrogen, thaw as quickly as possible

***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

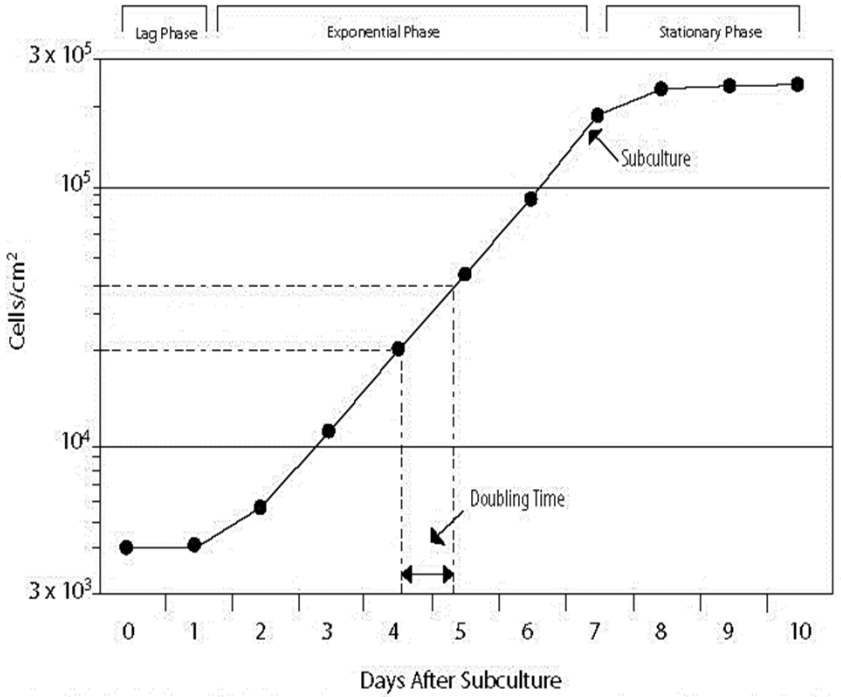


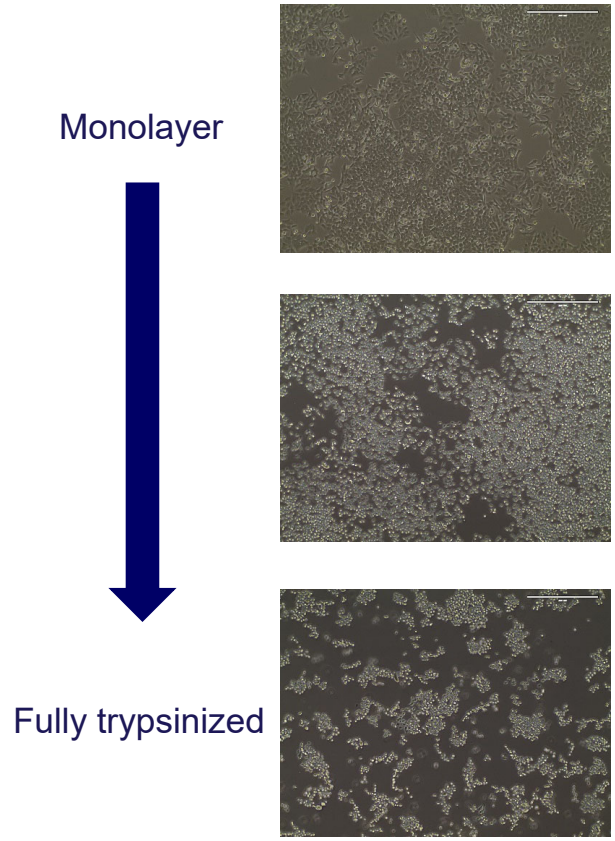
Figure 1. Growth curve for cells grown in culture. Cells should be subcultured while still in the exponential phase.

ATCC Animal Cell Culture Guide, 2014.

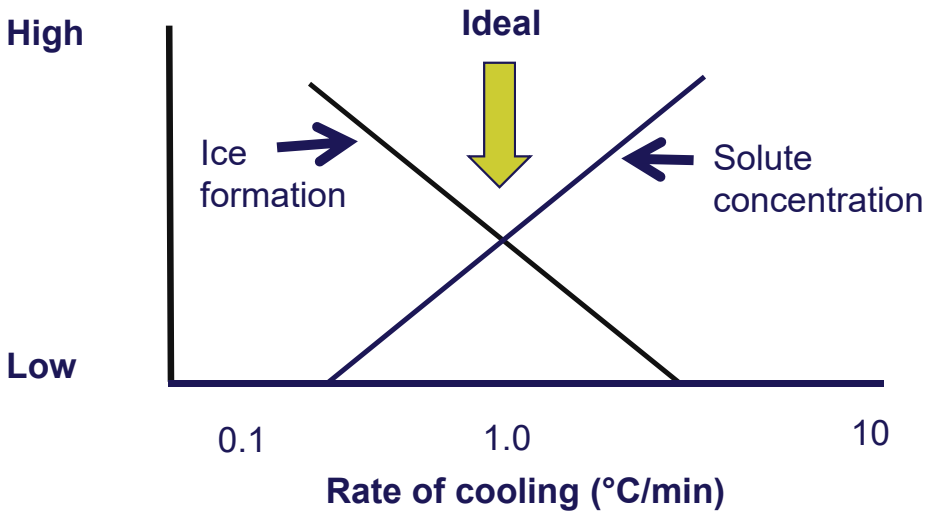
Trypsinization

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

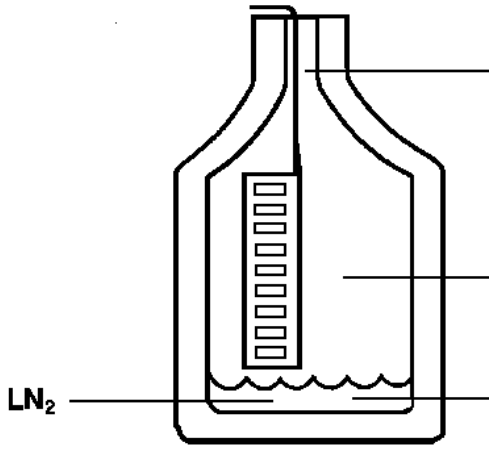
Low temperature storage

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**



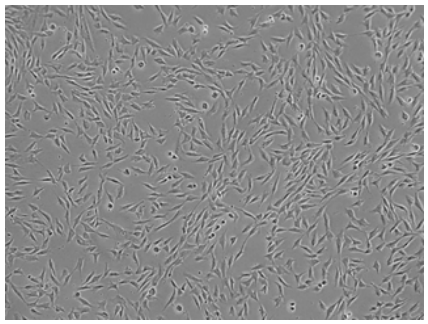
Cell characterization

Characterizing cells

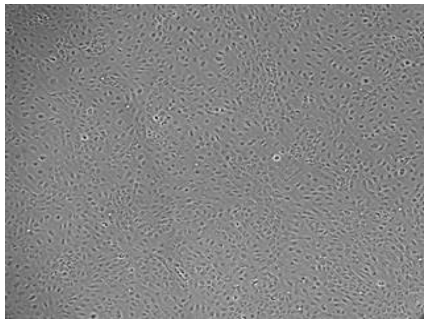
- Cell count before plating
  - Calculating % viability

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

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



Fibroblasts



HUVEC

Contamination in cultures

Sources

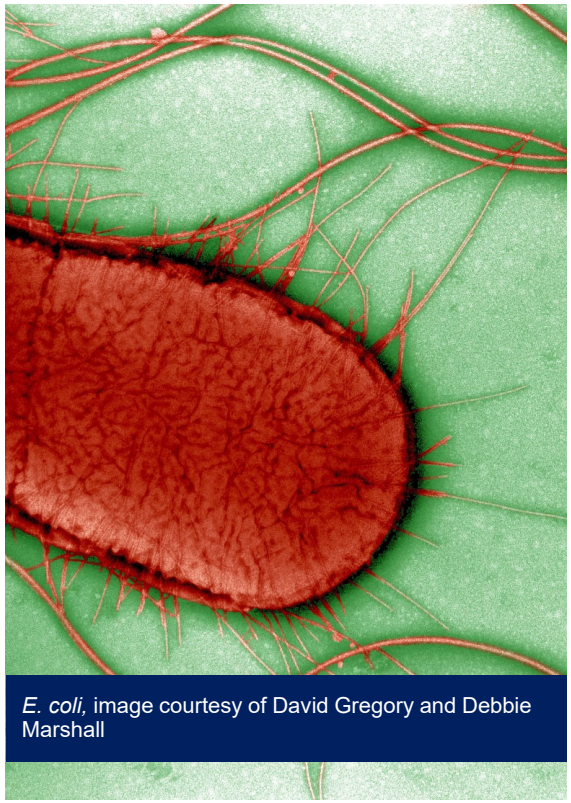
- Contaminated cell lines
- Improper aseptic technique

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

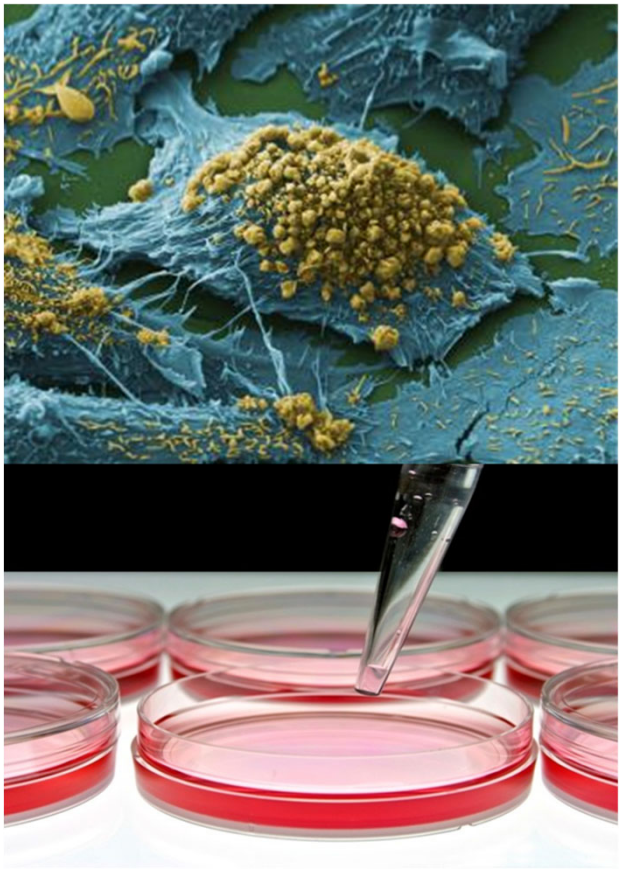
- Poor culturing practices
- Dust and aerosol

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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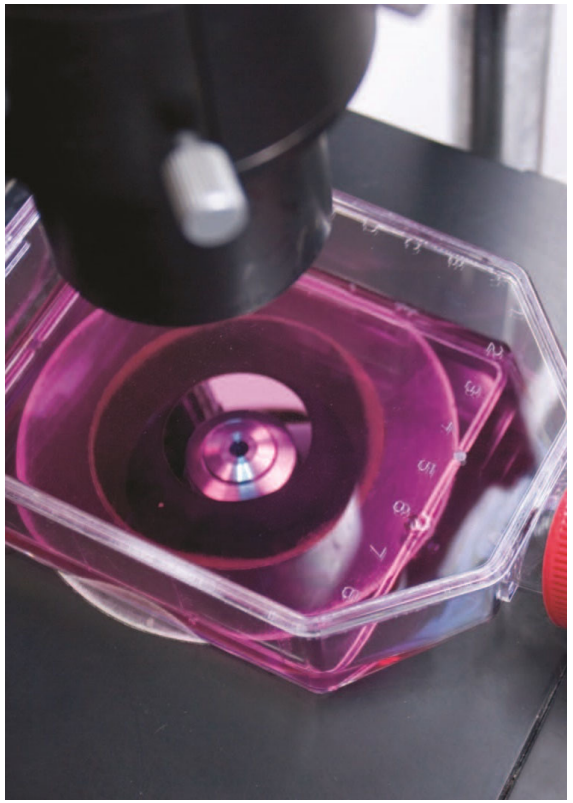
Mycoplasma contamination

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

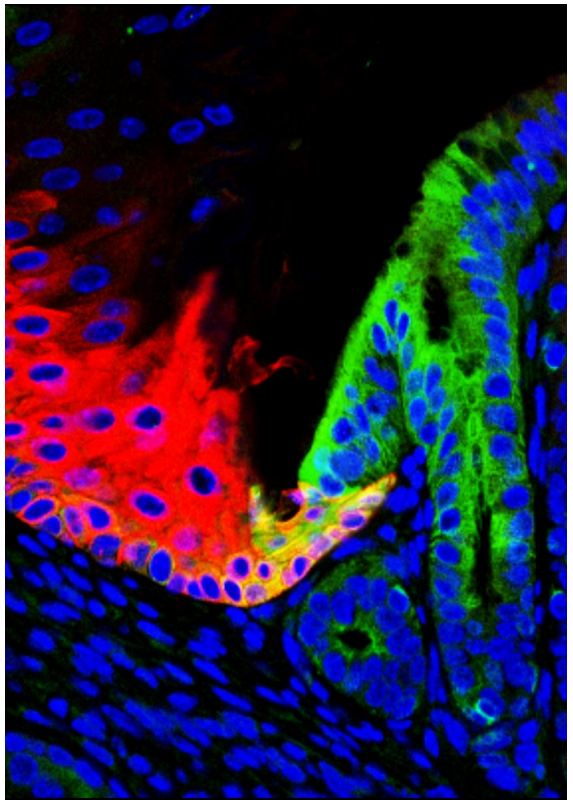
Leads to the replacement of the original cell line with the contaminant

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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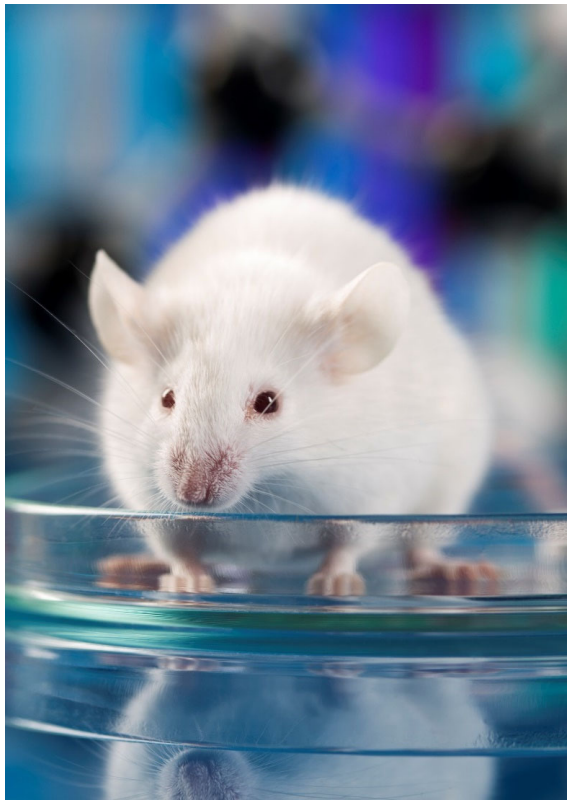
Short Tandem Repeats (STR)

- 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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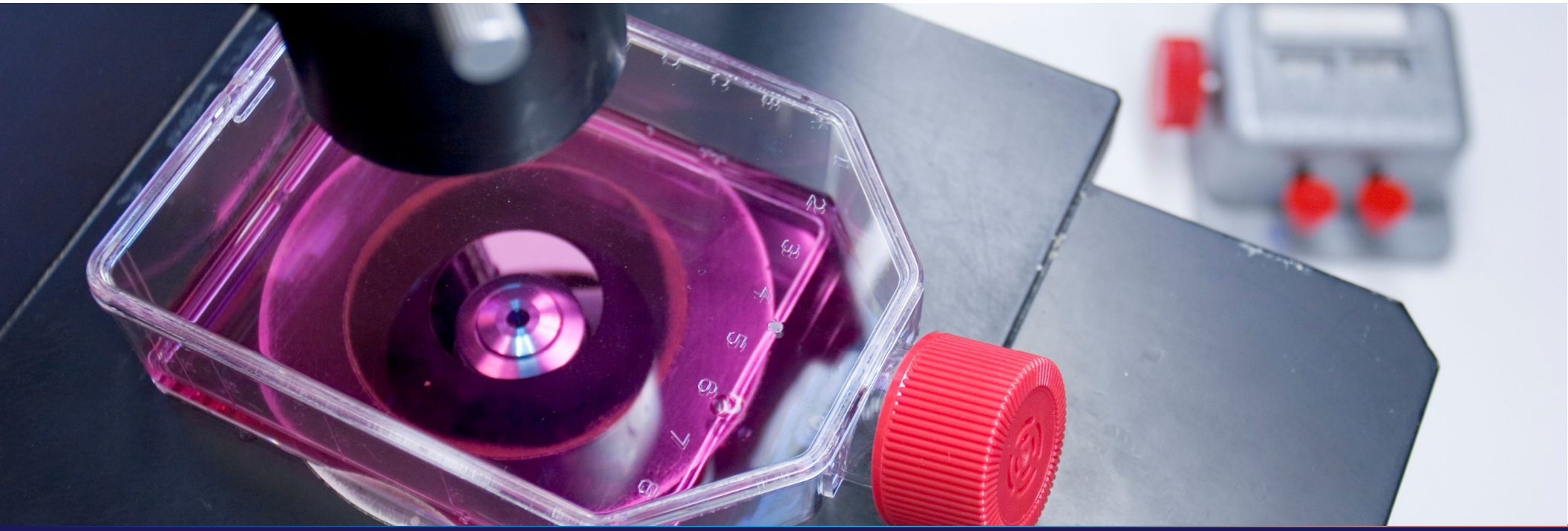

Workflow and how to choose the best model

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Media choices

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

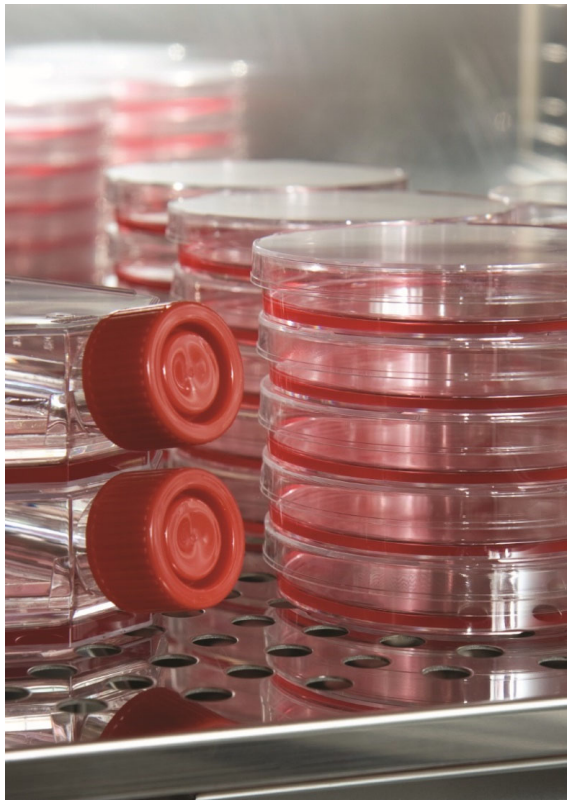
Specialized cell types (primary cells and stem cells require their own specially formulated media, specific to each cell type

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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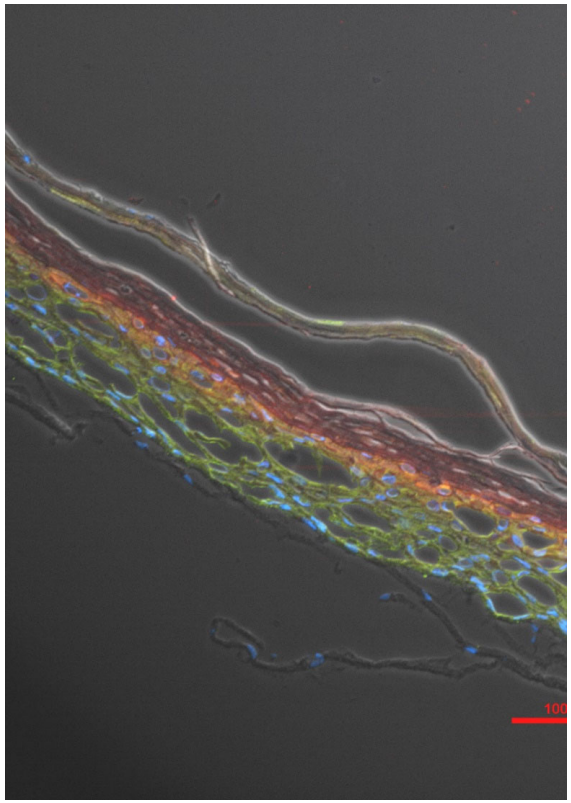
Primary cells as a control

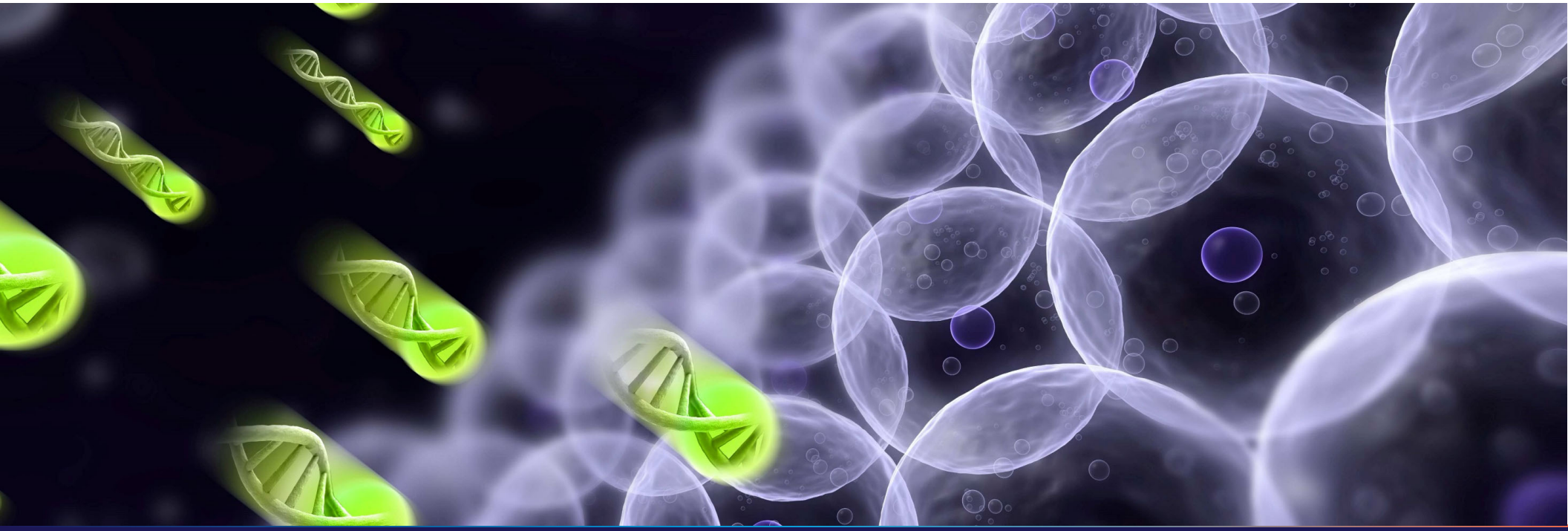
Primary neonatal keratinocytes (ATCC® PCS-201-010™) differentiated into physiological epidermis

- 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

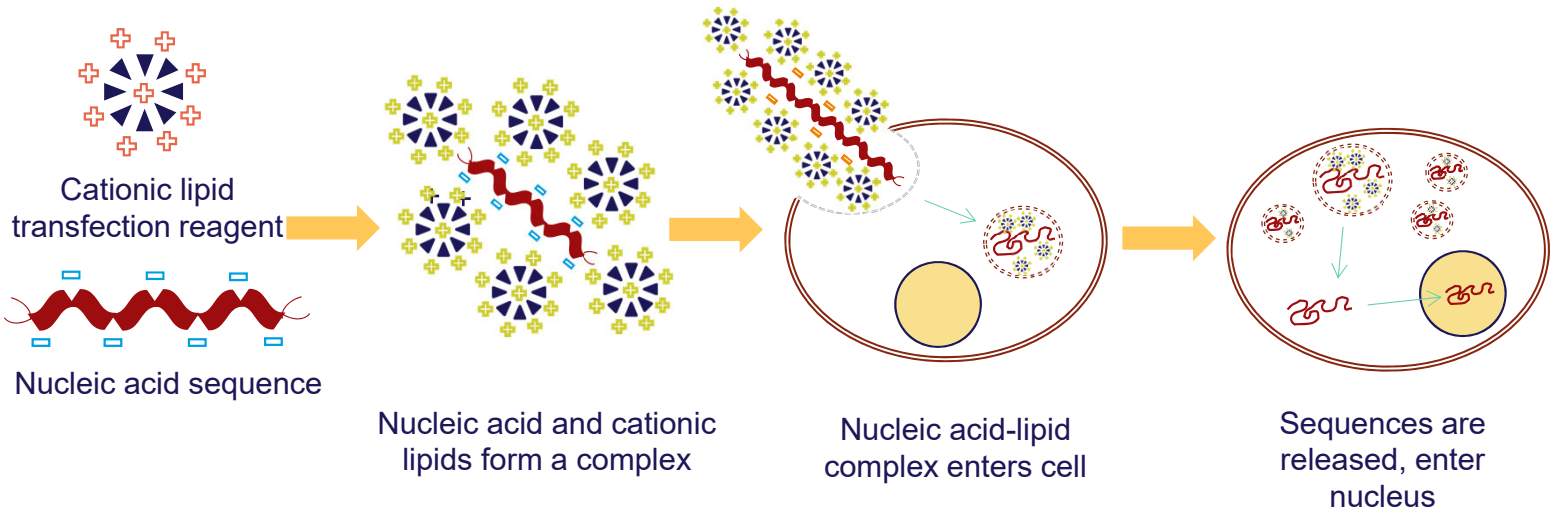
In every continuous cell line experiment, primary cells should be used as one of the controls





Transfection

Mechanism of lipid-based transfection



ATCC transfection reagents:

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

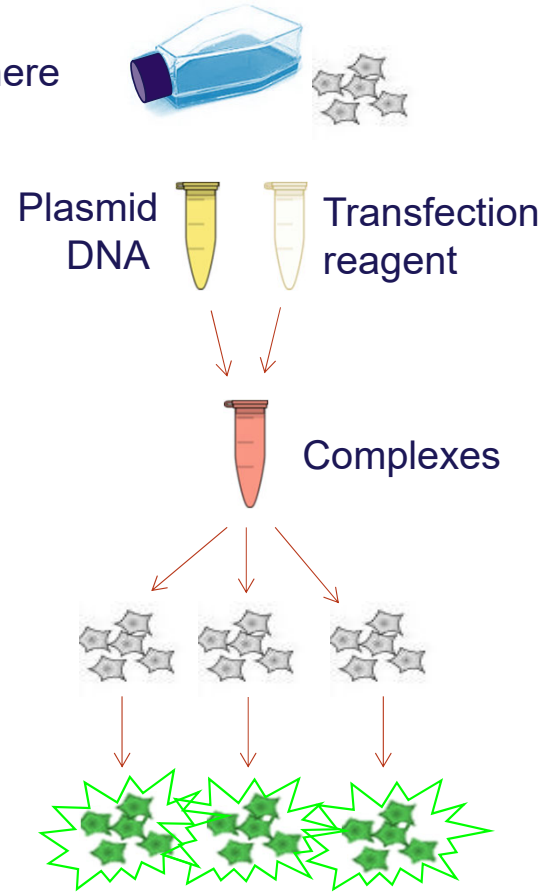
Typical transfection workflow

Day -1 Collect and seed cells into vessel where transfection will be performed

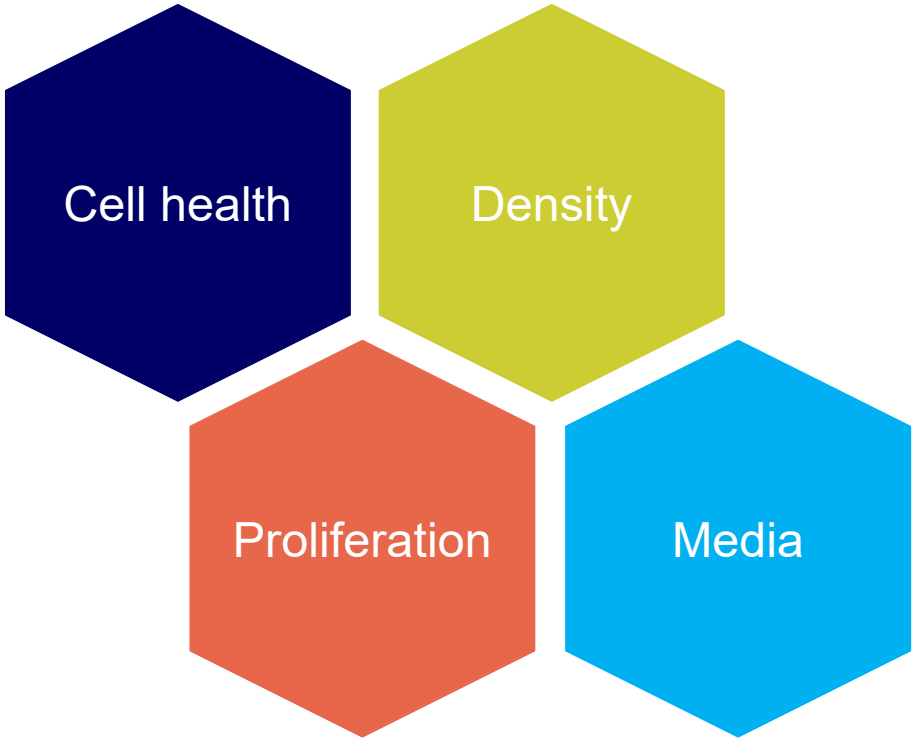
Day 0 Form transfection complexes by combining nucleic acid sequences and transfection reagent

Add transfection complexes to cells

Days 1+ Assess transfection



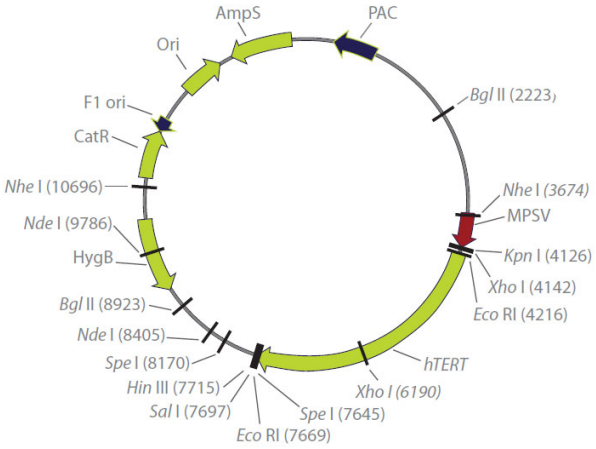
Cell culture conditions



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

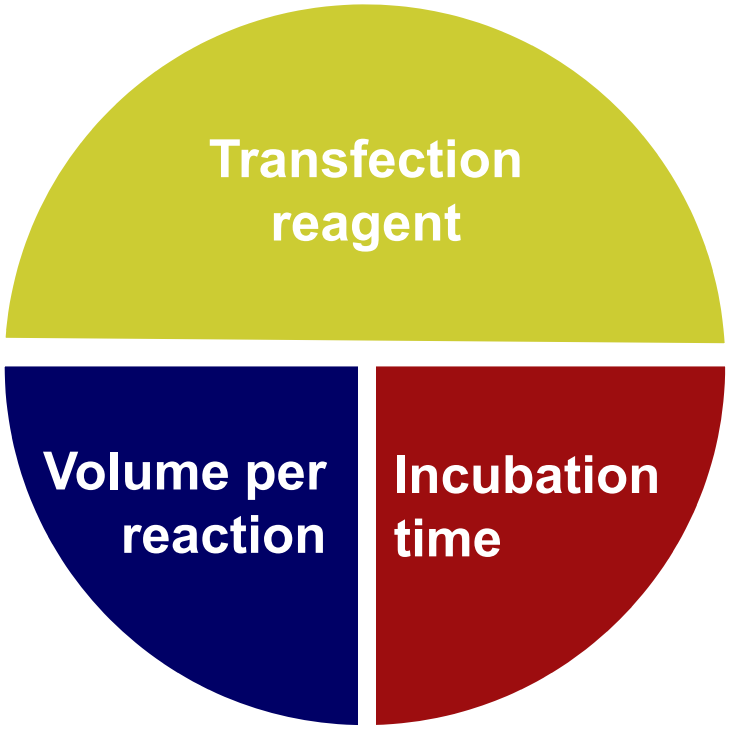
Protein

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

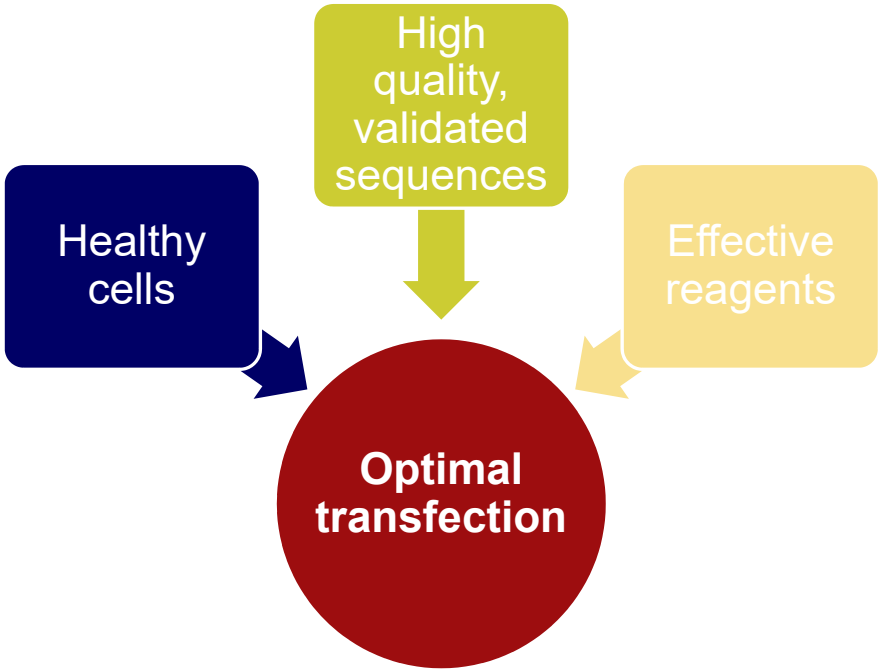
Other

- Morphology
- Functional

Transfection reagents

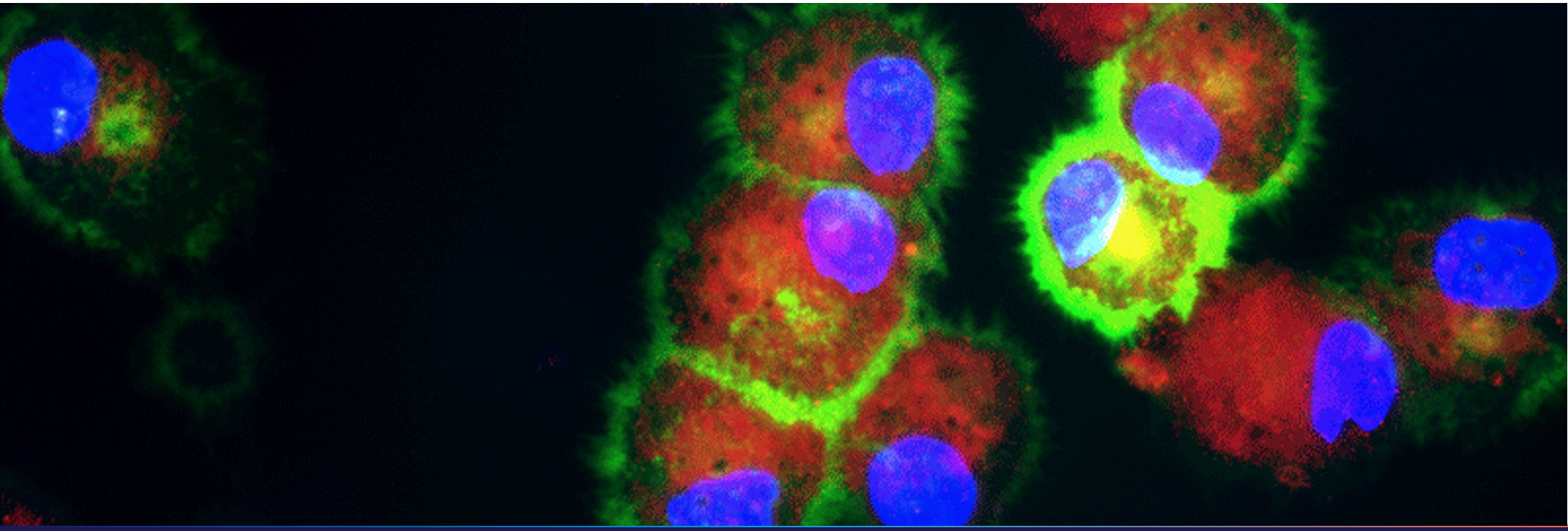


Best practices summary



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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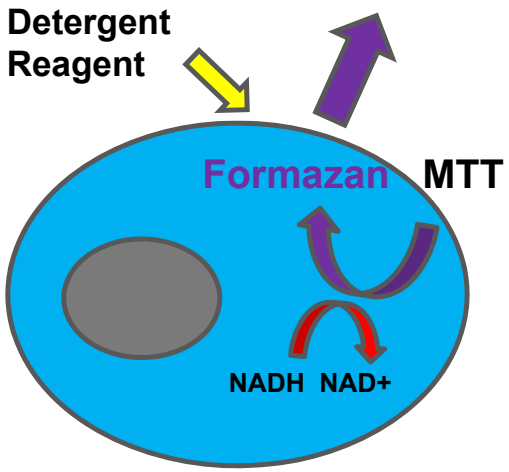
Viability assays

MTT Reaction

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

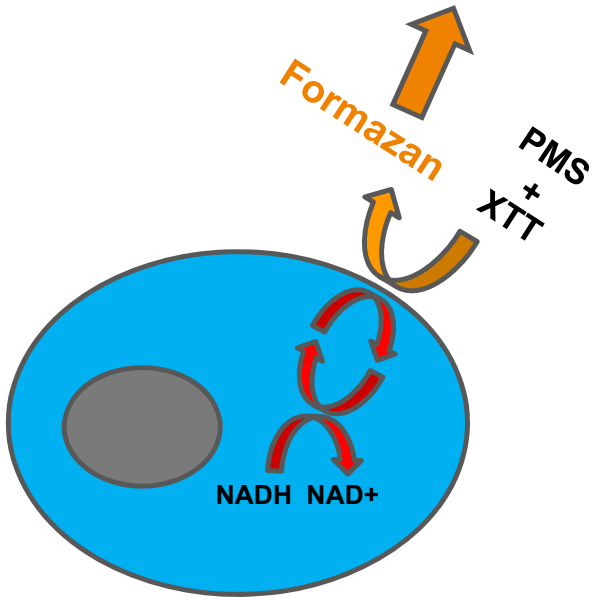
Media turns **PURPLE**



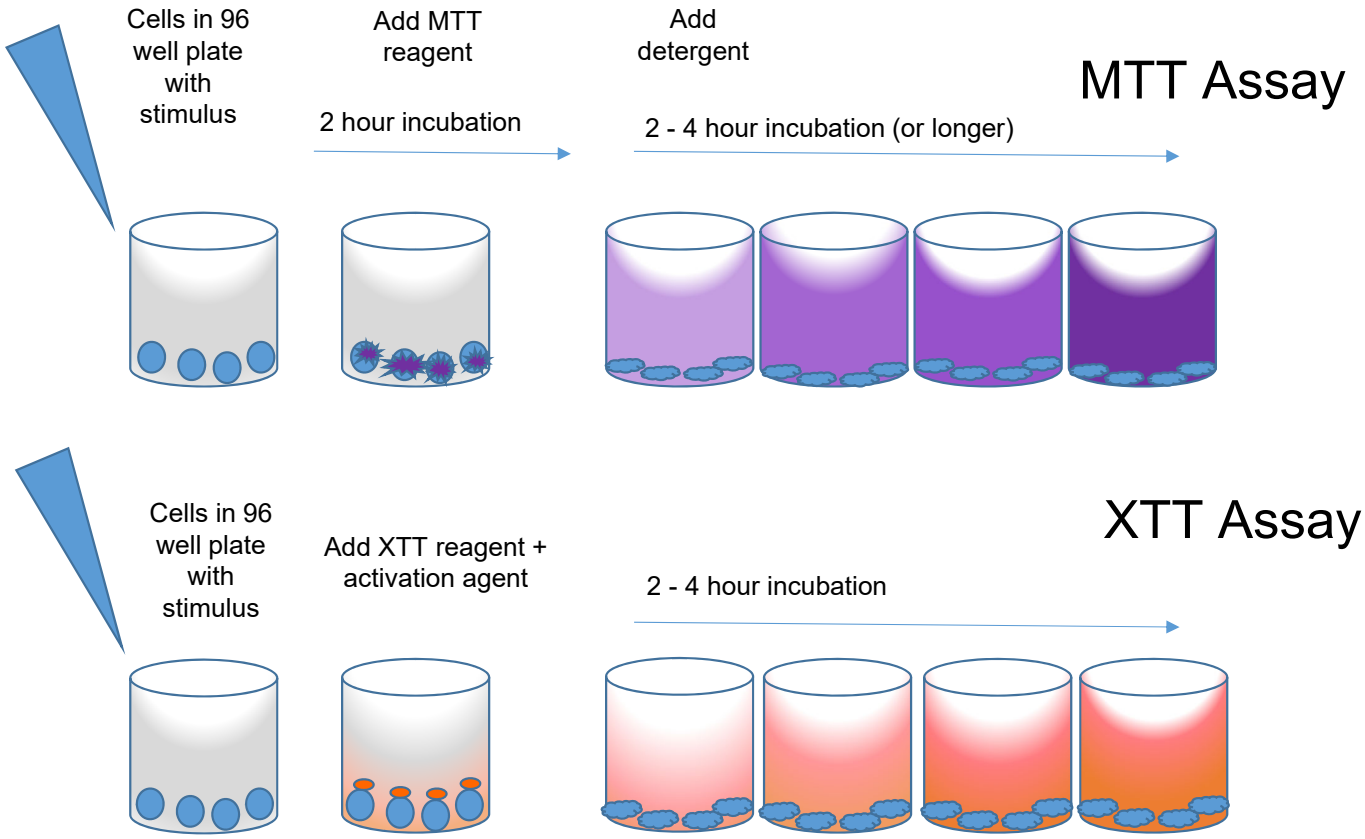
XTT Reaction

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

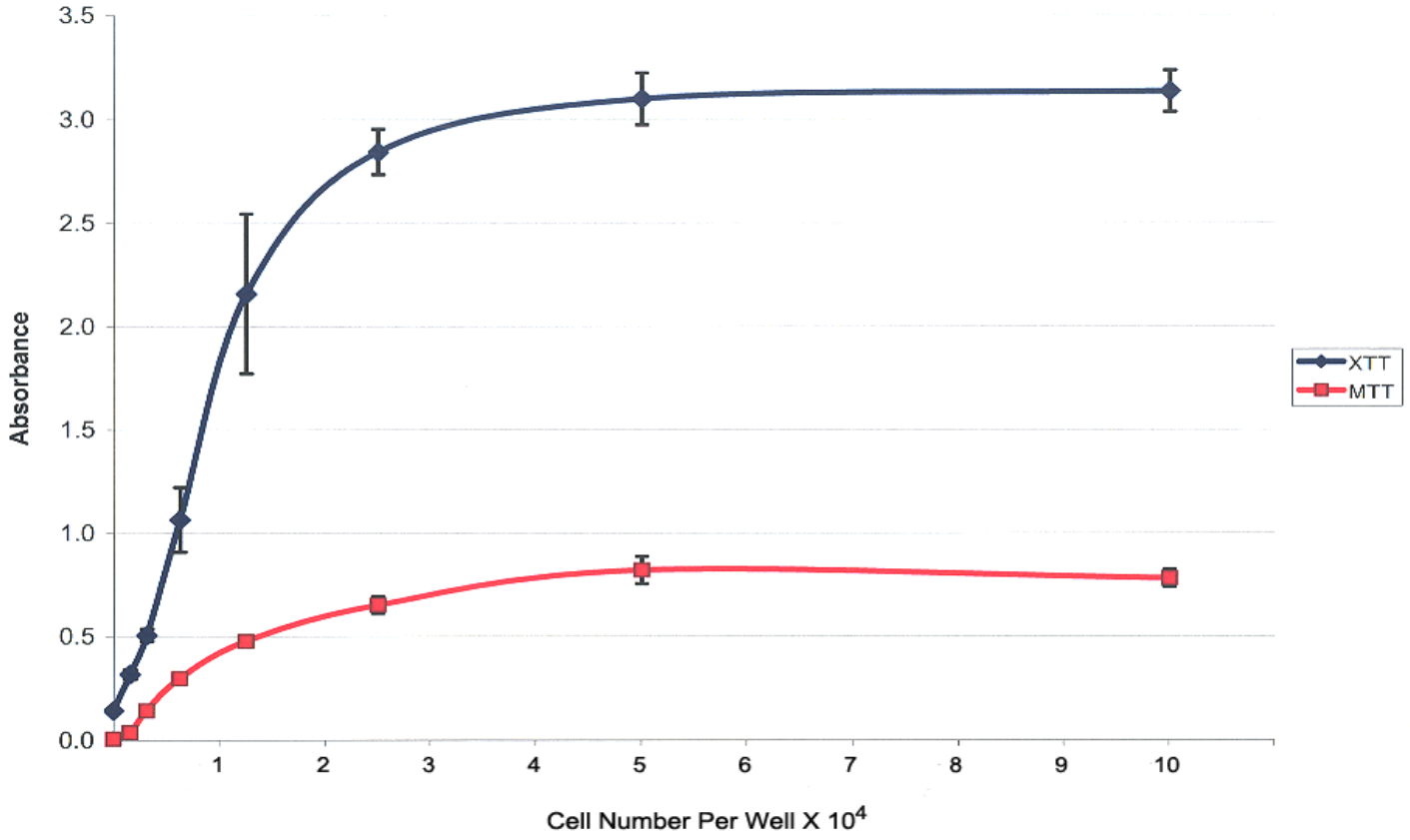
Media turns **ORANGE**



Viability assays



Viability assays



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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More webinars, coming soon!

iPSC-derived Primary Cells: Expand Your Cell-based Assays with an Unlimited, Biologically Relevant Source

Presented by Brian Shapiro, Ph.D.

12 ET, June 4, 2020

Understanding COVID-19: A Global Pandemic

Presented by Britany Tang

12 ET, June 11, 2020

Viral Metagenomics and the Use of Standards: From Biology to Clinical Applications

Presented by Tasha M. Santiago-Rodriguez, Ph.D.

12 ET, June 25, 2020

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