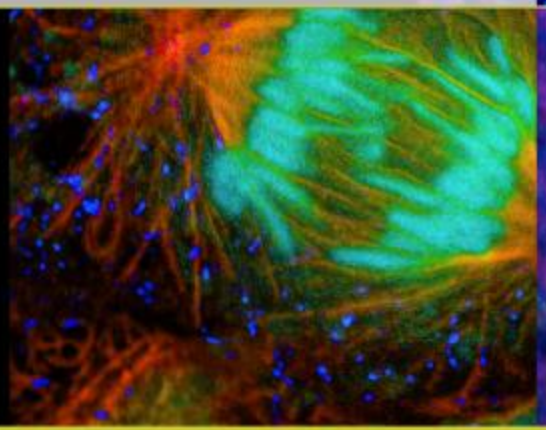
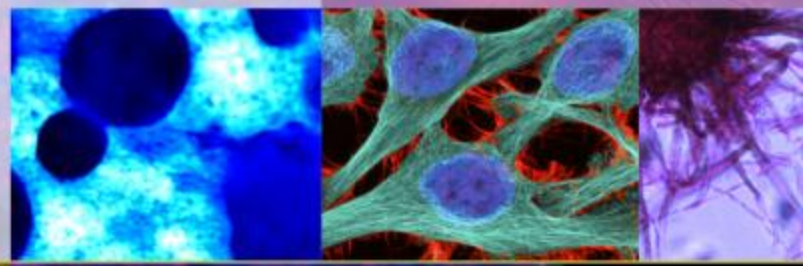
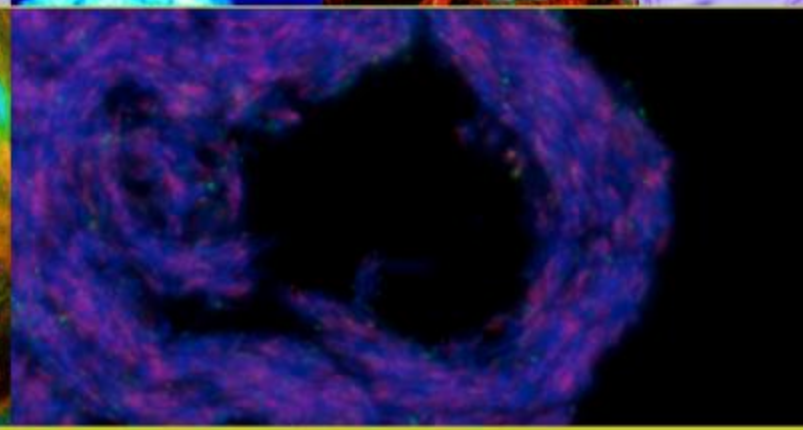
TRANSFECTION REAGENTS; POWERFUL TOOLS TO ENABLE GENETIC MANIPULATION

James Clinton, Ph.D.
Scientist, ATCC
April 02, 2015

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees
- Broad range of biomaterials
  - Cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, yeasts, protists, and viruses
  - Tumor cell panels
  - Media, sera, and reagents

Established partner to global researchers and scientists

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Introduction to Transfection

Best Practices

ATCC Transfection Reagents

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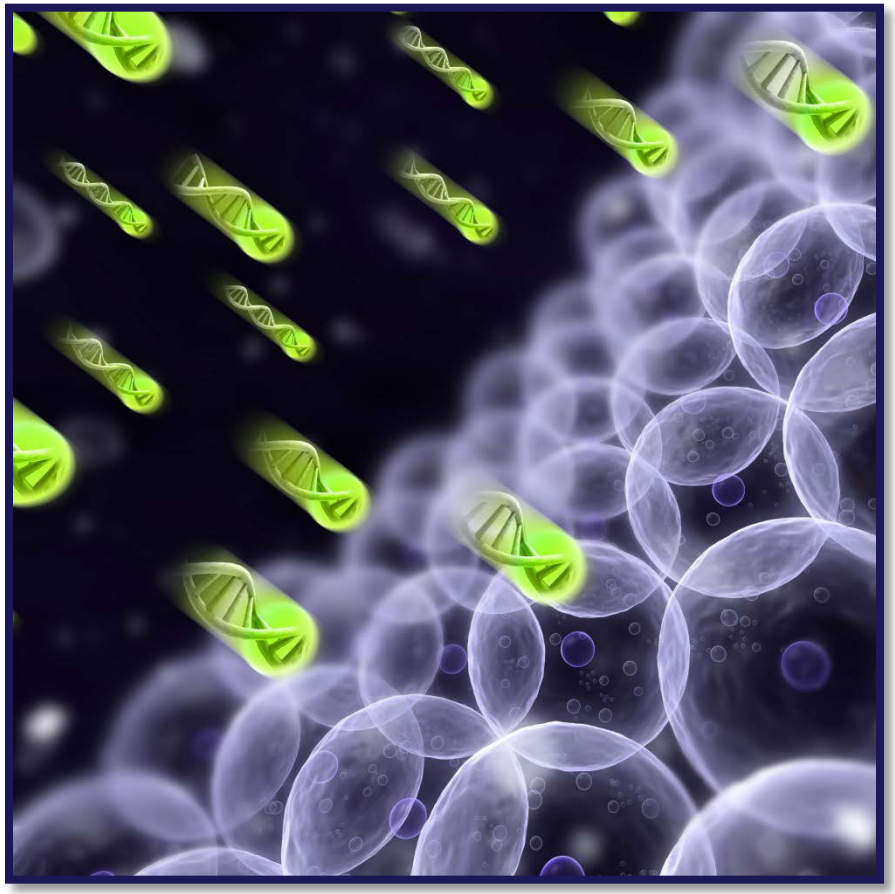
Introduction to transfection

- Method for introducing exogenous nucleic acid sequences into mammalian cells
- Widely used technique that has made expressing DNA or RNA in most types of cells relatively easy
- A variety of approaches have been developed for use across a range of applications
- No single approach will work for all conditions/cell types/applications



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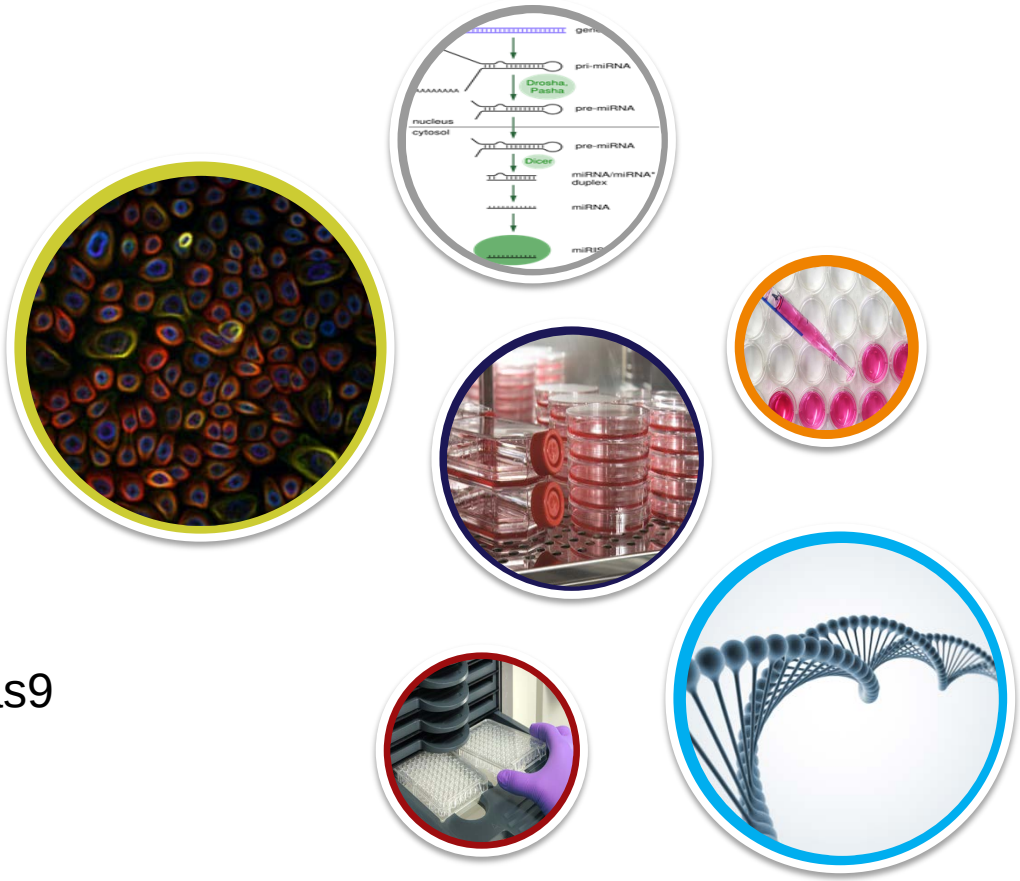
Applications

- Gene function
- RNAi gene silencing
- Pathway analysis
- Functional screening
- Virus production
- Protein production
- Generation of stable cell lines
- Stem cell reprogramming
- Cell differentiation
- Genome editing with CRISPR/Cas9



Transfection methods

Lipid	• Easy, most common method • Variable efficiencies • Will not work with all cell types
Viral	• Will transfect non-dividing cells • Technically challenging, expensive • Safety issues, immune response, mutagenesis
Electroporation	• Requires specialized equipment • Cells must be in suspension • Toxicity can be an issue
Physical	• Technically challenging, expensive • Requires specialized equipment • Works with non-nucleic acids; single cell transfection
Other	• Not common, may be technically challenging • Non-lipid based chemicals • Nanoparticles/ Laser/ Ultrasound/ Magnetic

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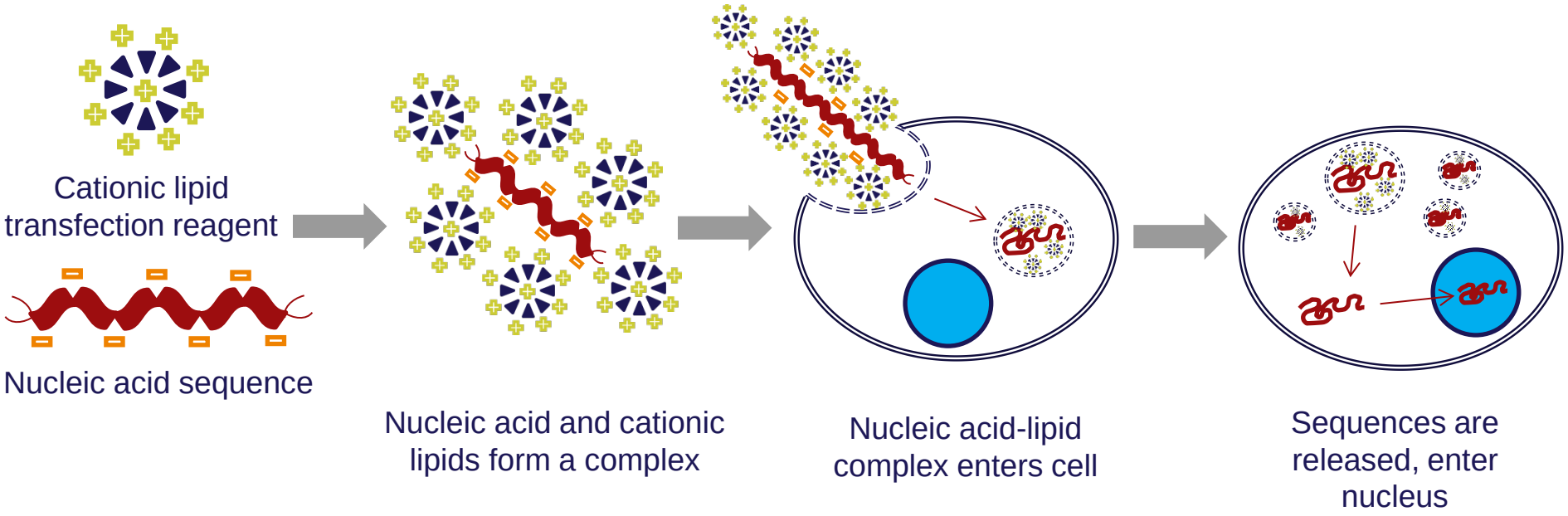
Mechanism of lipid based transfection



Typical transfection workflow

Day -1

Collect and seed into vessel where transfection will be performed

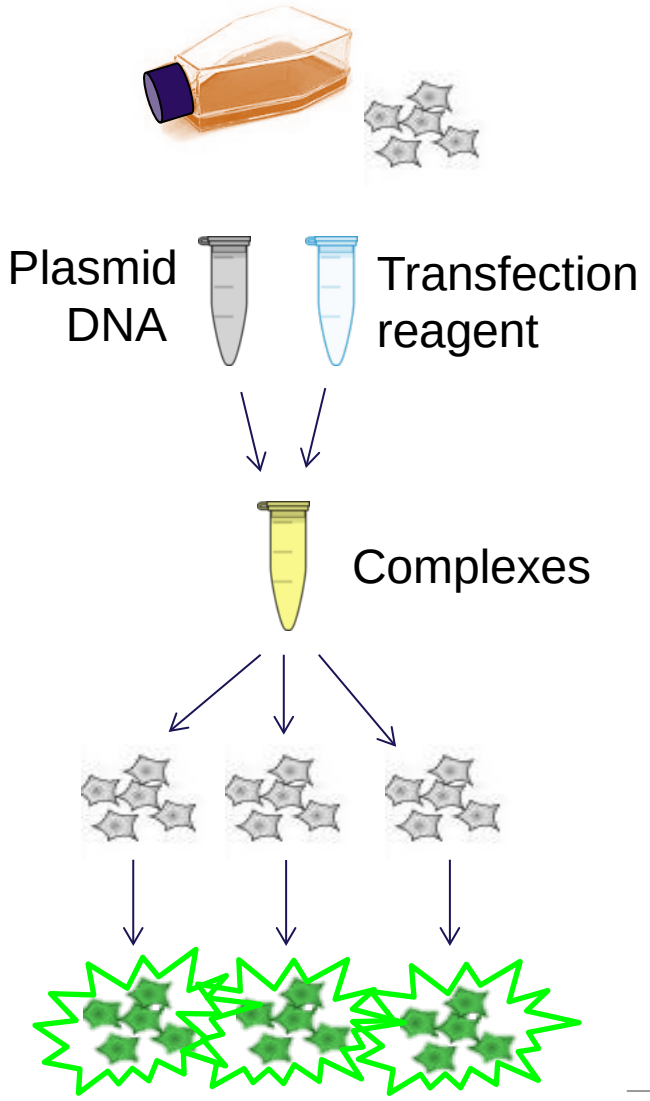
Day 0

Form transfection complexes by combining nucleic acid sequences and transfection reagent

Add transection complexes to cells

Days 1+

Assess transfection



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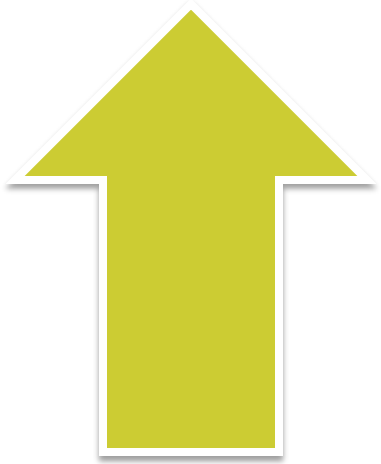
Overexpression vs. knockdown



Introduce foreign plasmid DNA/mRNA to induce expression of a desired transcript/protein

Utilize RNAi pathway to degrade or inhibit translation of mRNA transcripts and subsequently reduce the amount of protein

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Transient vs. stable transfection

Transient

- Foreign gene not integrated into genome
- Expression persists for limited time
- Foreign gene lost due to cell division, degradation, or other factors

Stable

- Initially a transient transfection
- Use co-expressed selection markers
- Long-term, only cells that have integrated the foreign gene persist

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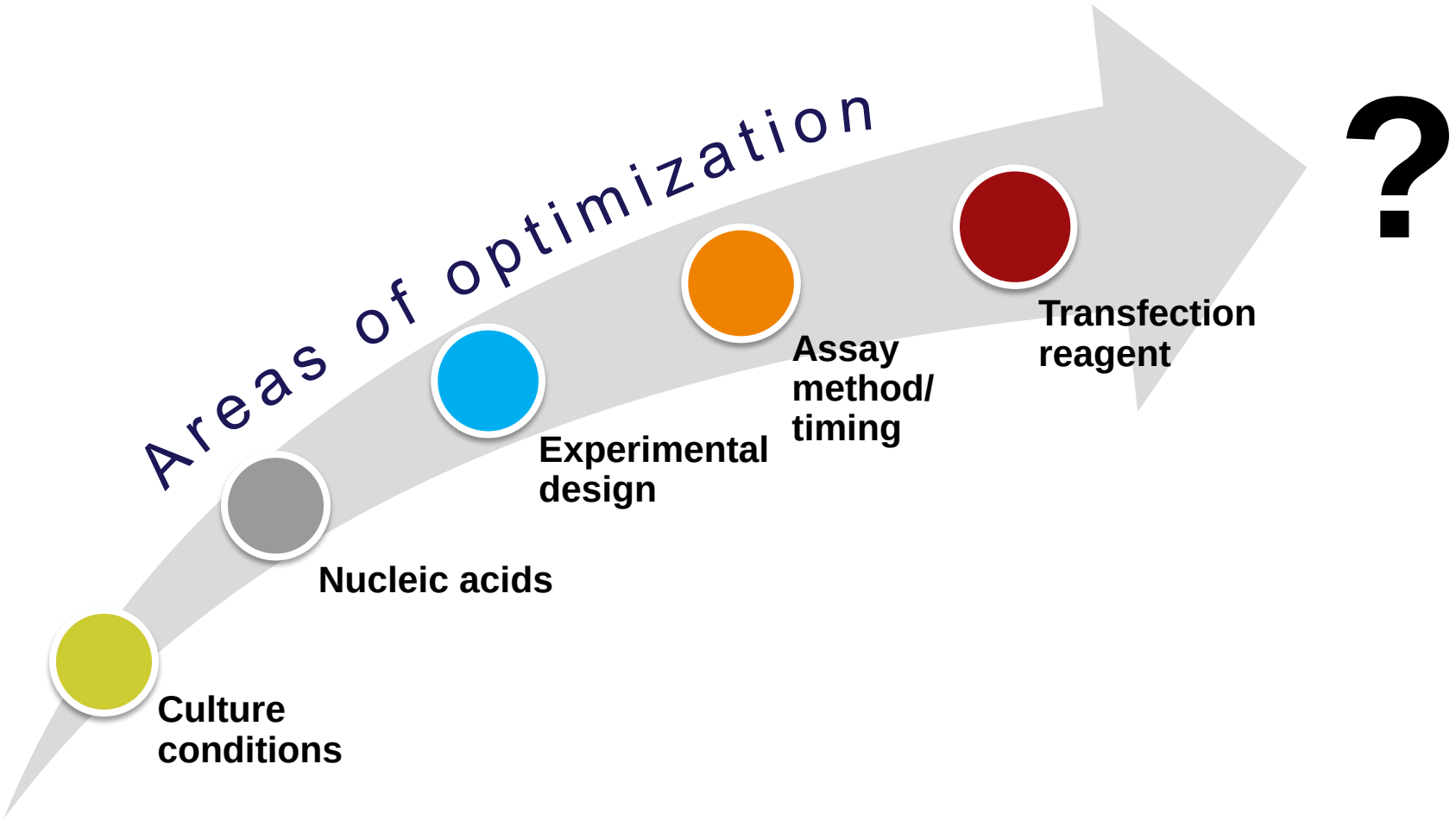
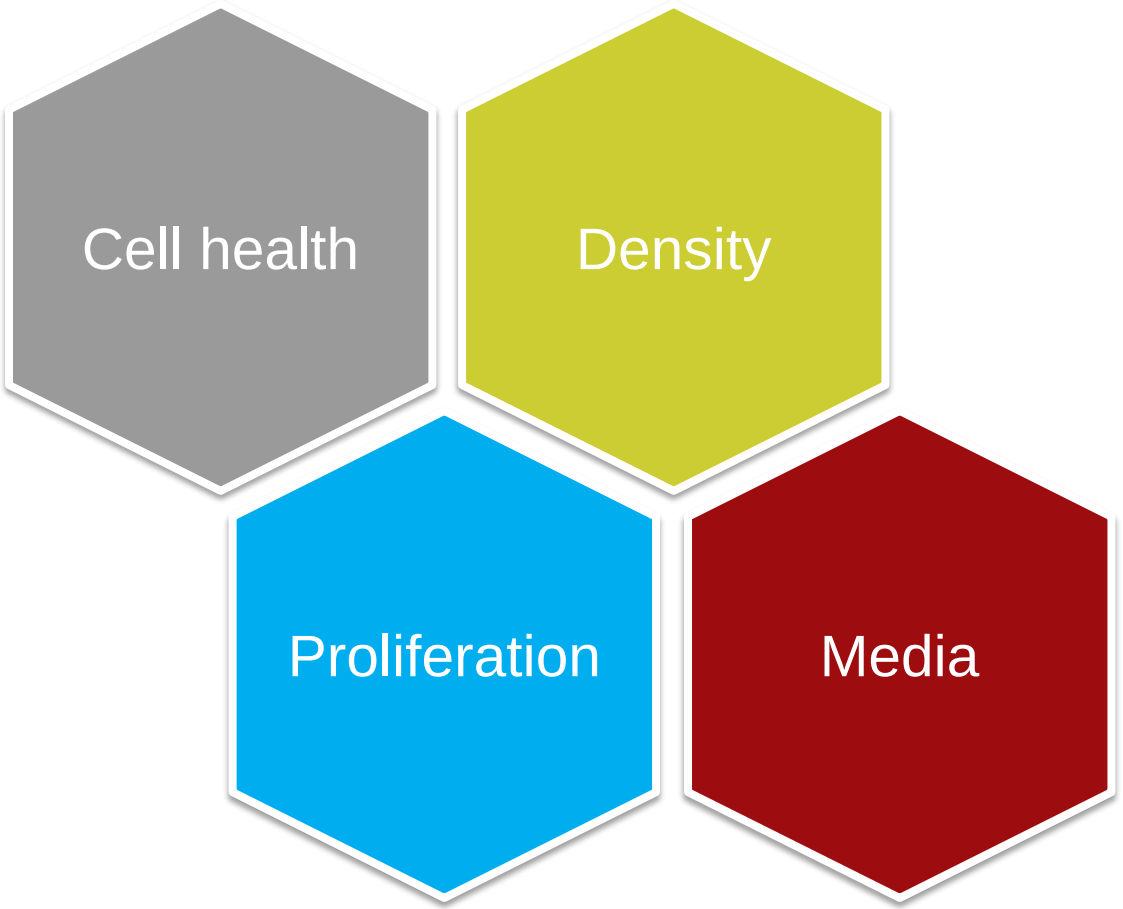
Transfection: best practices



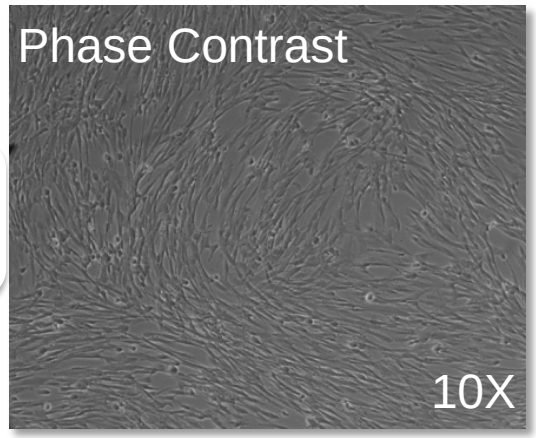
Cell culture conditions

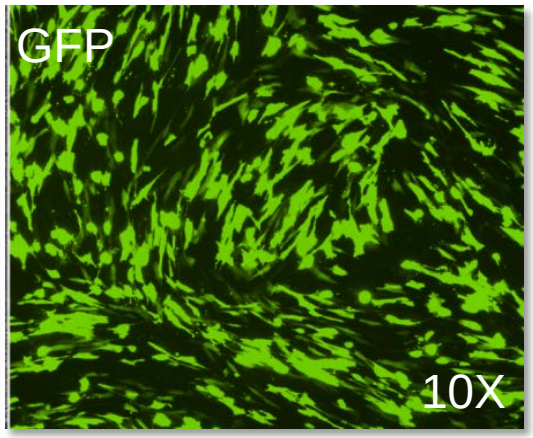


Example: culture conditions can be critical

Primary Uterine Smooth Muscle Cells (SMCs; ATCC® No. PCS-460-011)

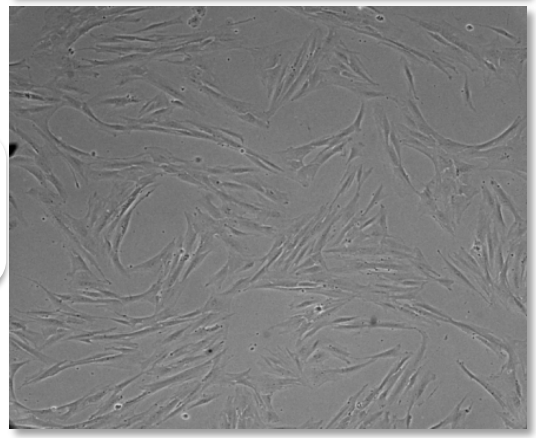
Transfected in complete growth media

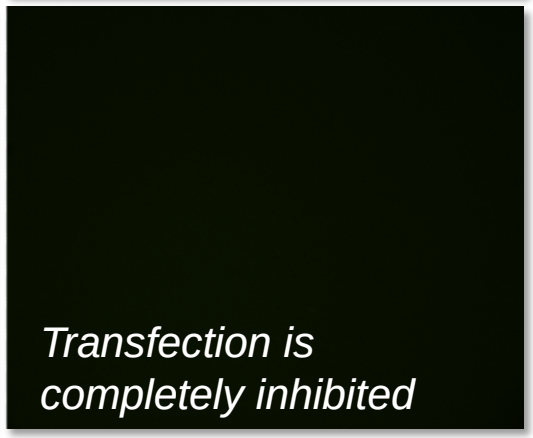




Transfected in differentiation media

Contains heparin sulfate





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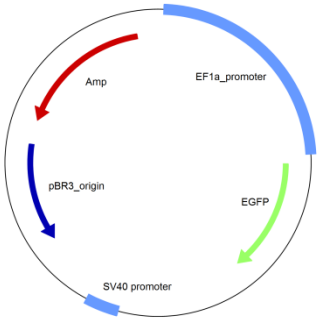
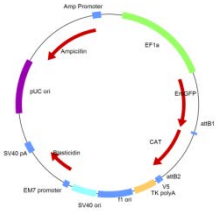
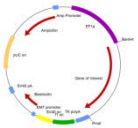
Nucleic acids

In general

- High purity
- Endotoxin free
- Validated







Plasmid DNA

- Promoter
- Plasmid size
- Conformation

RNA

- Chemical modifications
- Pooled siRNAs

Experimental design & execution

Transfection Protocol	• Use master mixes • Distribute complexes evenly • Store DNA/RNA properly
Proper Controls	• Positive and negative control • Transfected and un-transfected controls
Monitor Toxicity/ Off-target Effects	• Morphological changes • Presence of vacuoles • Changes in proliferation
Validate Results	• Multiple assays • For siRNA: test multiple sequences • For miRNA: increase & suppress

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Assay methods

mRNA

- Real time RT-PCR

Protein

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

Other

- Morphology
- Functional

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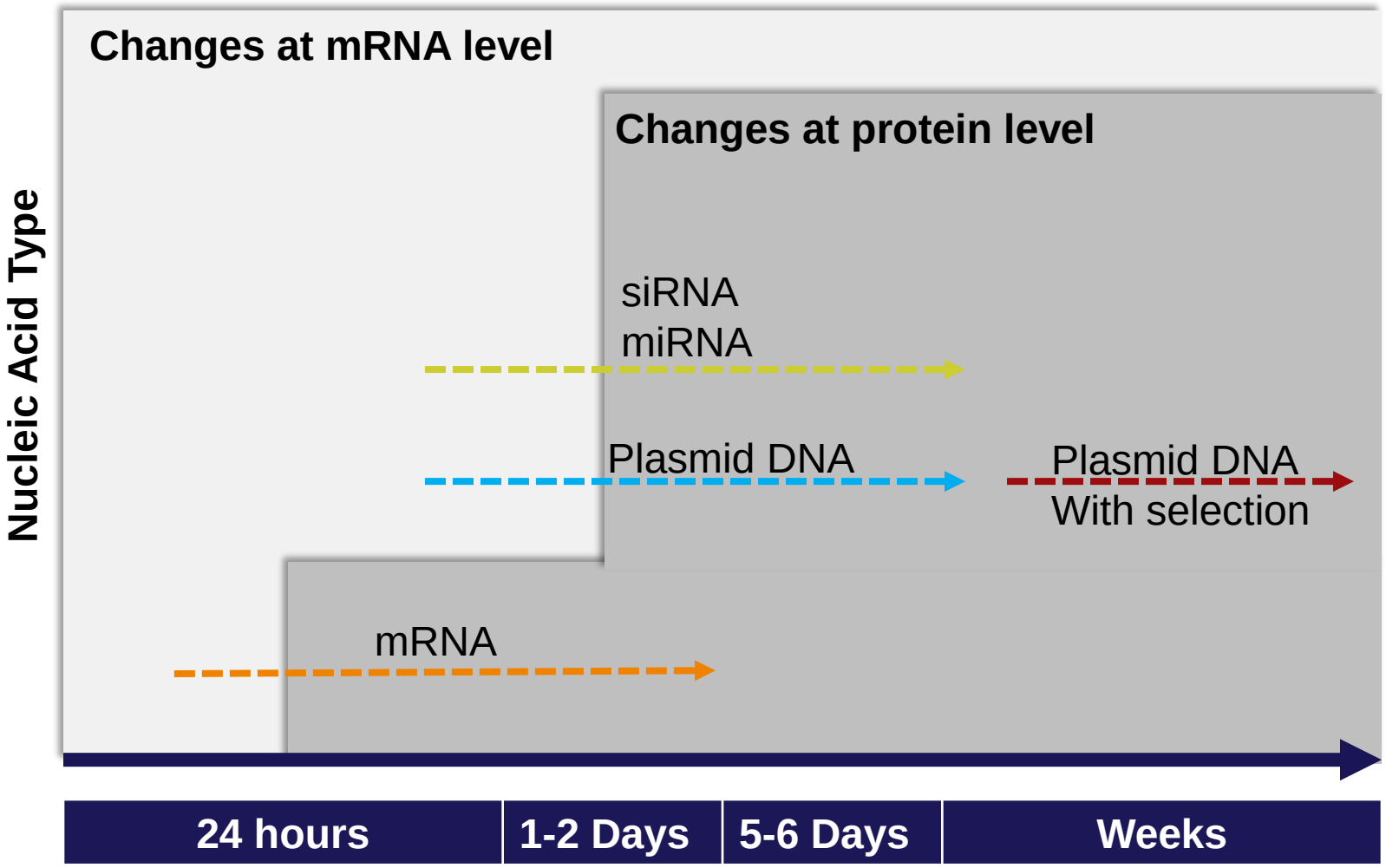
Assay timing



Transfection reagents considerations

Ideal reagent

- Effective in all cell types
- No optimization necessary
- No cytotoxicity

Reality

- Effective in your cell type of interest
- Broad activity across culture conditions and protocols
- Minimal cytotoxicity
- An optimized protocol delivers desired expression

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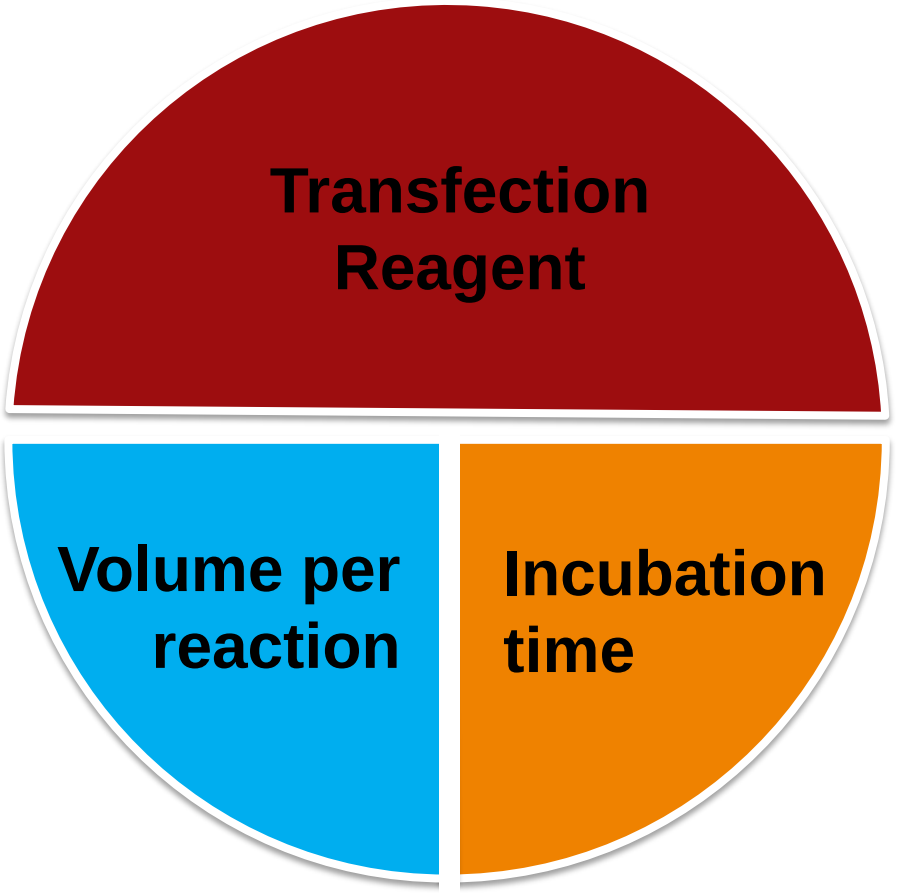
Transfection reagents



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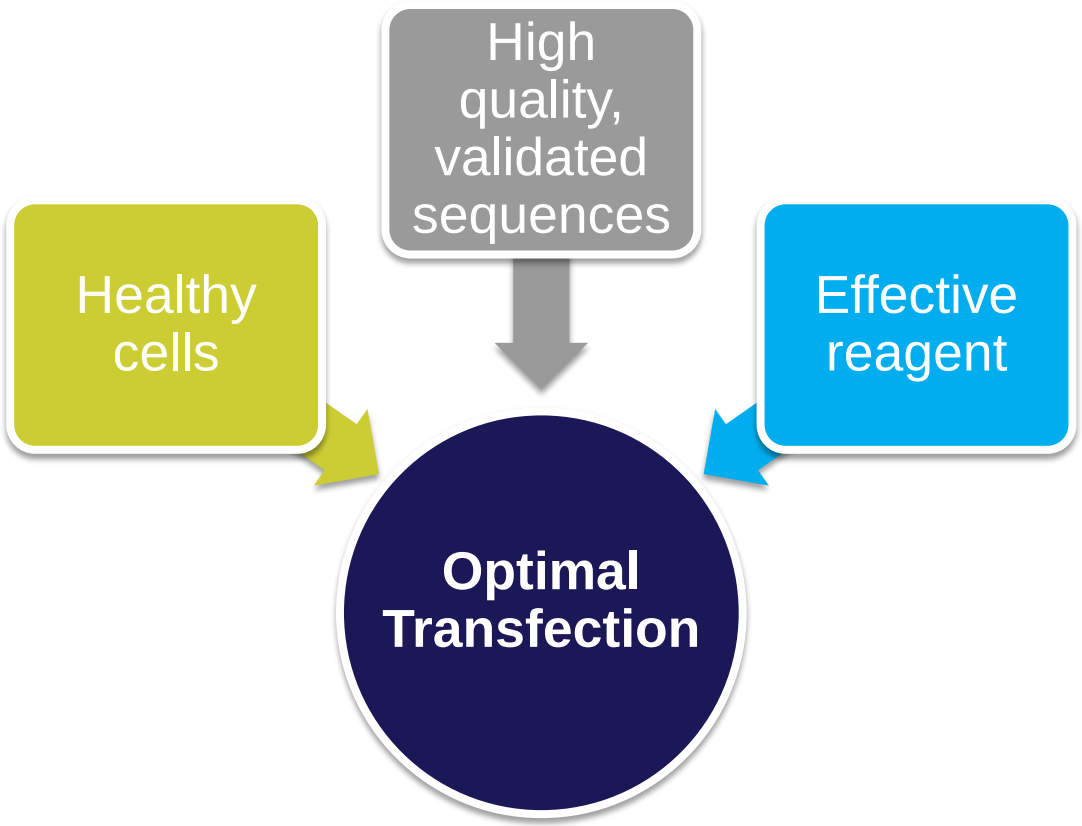
Best practices summary



Minimum Optimization

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

Introduction to Transfection

Best Practices

ATCC Transfection Reagents

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ATCC transfection reagents overview

- Used for transfection of mammalian adherent and suspension cells
- Formulated for low cytotoxicity and high efficiency
- Produces high levels of gene expression (or inhibition)
- Suitable for both transient and stable transfection
- Sterility, purity, and performance tested
- Animal component-free

Reagent	ATCC® No.	Volume	Storage
GeneX*Plus* Transfection Reagent	ACS-4004	1 mL	-20ºC
TransfeX™ Transfection Reagent	ACS-4005	500 µL	4ºC
siFEX™ RNAi Transfection Reagent	ACS-4006	500 µL	4ºC

For more information on our transfection reagents: www.atcc.org/transfection

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ATCC transfection reagents selection guide

Reagent	Plasmid DNA	mRNA	siRNA & miRNA	Suspension	Hard to Transfect Cells
GeneX*Plus*	✓✓✓			✓✓	
TransfeX™	✓✓✓	✓✓		✓	✓✓✓

Newly released in 2015:

siFEX™			✓✓✓	✓	

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Available GeneX*Plus* optimized protocols

Continuous	Stem	Primary
• THP-1 • RAW 264.7 • BJ-5ta • SH-SY5Y • MRC-5 • HEK 293T/SF	• Bone marrow-derived mesenchymal stem cells (MSCs)	• Dermal microvascular endothelial cells

Optimized protocol list as of April 2015

- Find the current list of available protocols at www.atcc.org/transfection
- Contact Technical Service at tech@atcc.org

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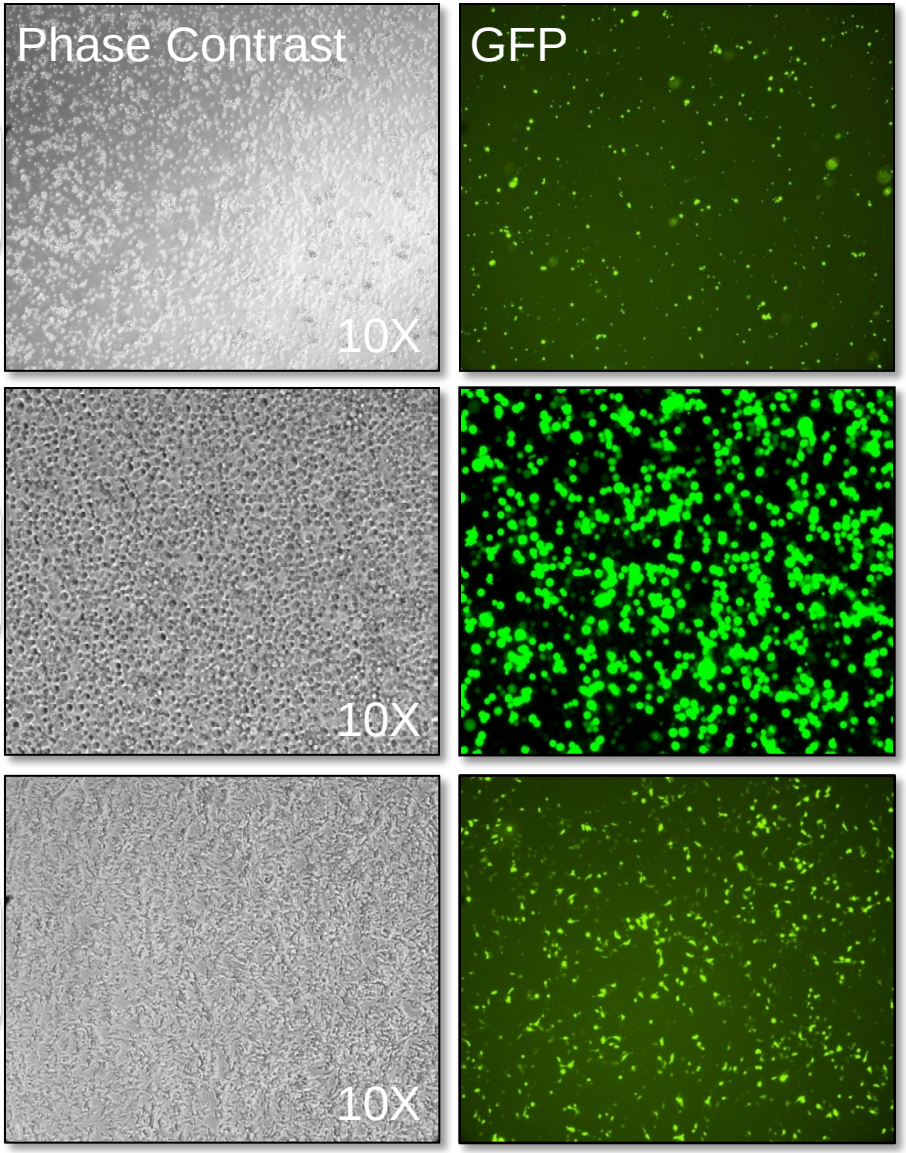
Transfection of suspension cells with GeneX*Plus*

THP-1
(ATCC® No. TIB-202™)

HEK 293T SF
(ATCC® No. ACS-4500™)

SH-SY5Y
(ATCC® No. CRL-2266™)



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Available TransfeX™ optimized protocols

Continuous

- A549
- HeLa
- LNCap
- MDA-MB-231
- HepG2
- Caco-2
- C2C12
- 3T3-L1
- CHO-K1
- HEK293
- HUV-EC-C
- MCF7
- NuLi-1
- TIME
- BT-142

Stem

- Bone marrow-derived MSCs
- Adipose tissue-derived MSCs
- Cord blood-derived MSCs
- Induced pluripotent stem cells (iPSCs)

Primary

- Dermal fibroblasts (DFs)
- DMEC
- HEMCs
- HUVECs
- RPTECs
- Large airway epithelial cells
- Large airway SMCs
- Uterine fibroblasts
- Uterine SMCs
- Prostate epithelial cells

Optimized protocol list as of April 2015

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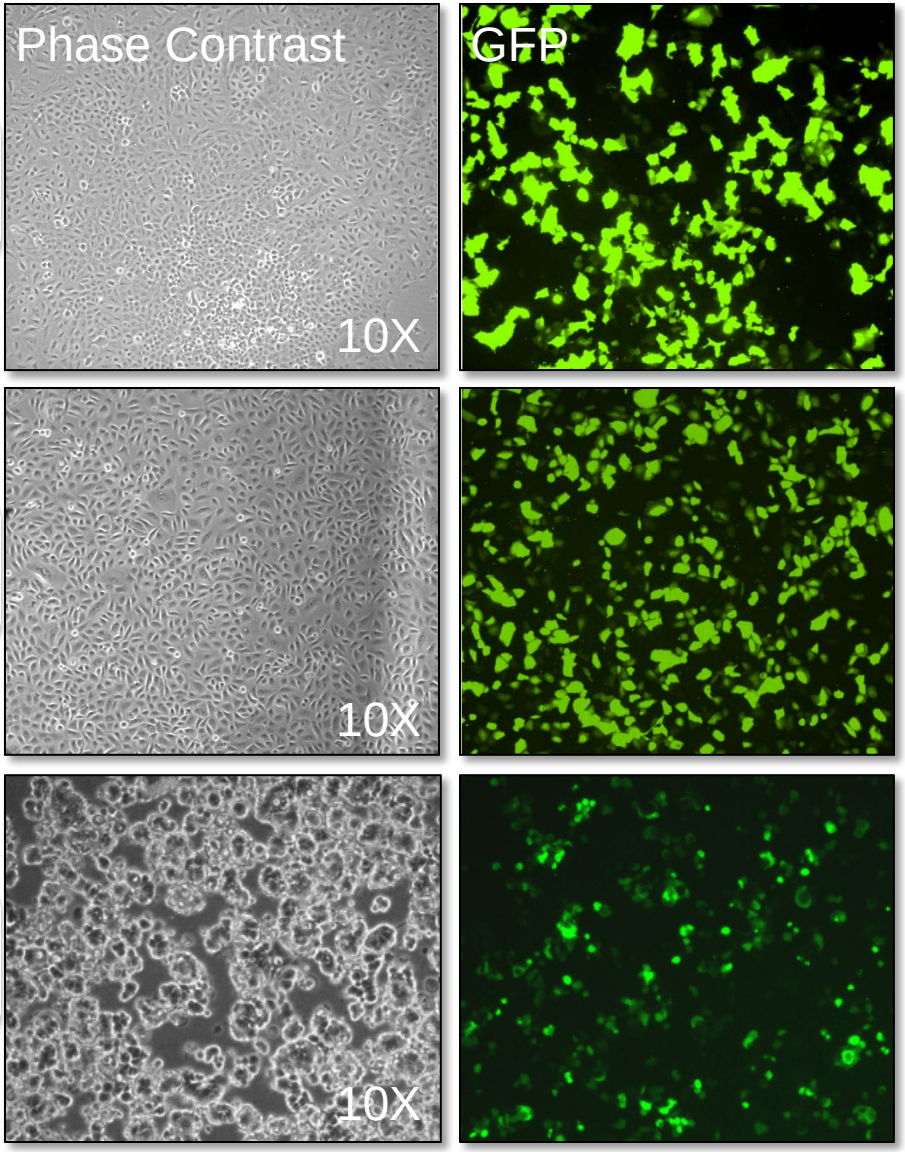
Transfect continuous cells with TransfeX™

A549
(ATCC® No. CCL-185™)

NuLi-1
(ATCC® No. CRL-4011™)

MCF-7
(ATCC® No. HTB-22™)



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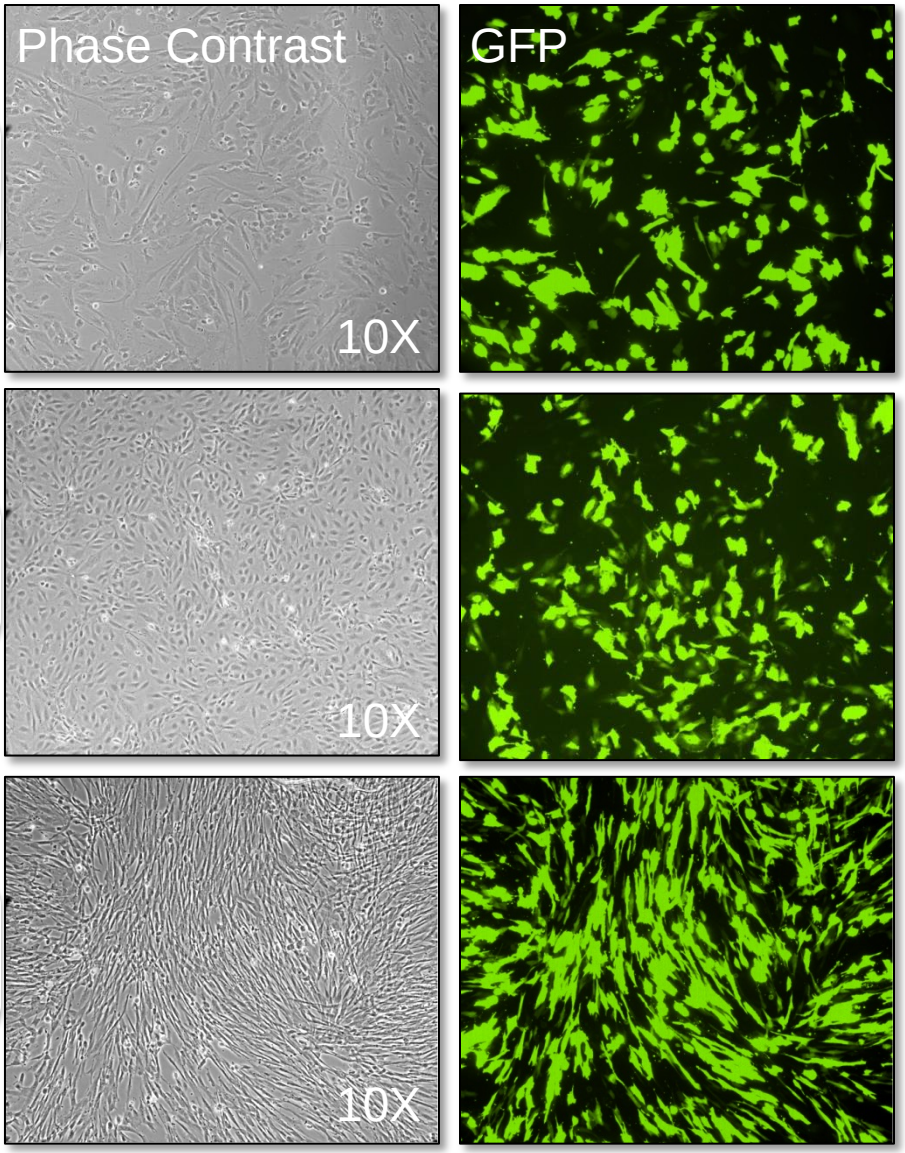
Transfect primary cells with TransfeX™

Primary DFs
(ATCC® No. PCS-100-012)

Primary HUVECs
(ATCC® No. PCS-100-010)

Primary Uterine SMCs
(ATCC® No. PCS-460-011)



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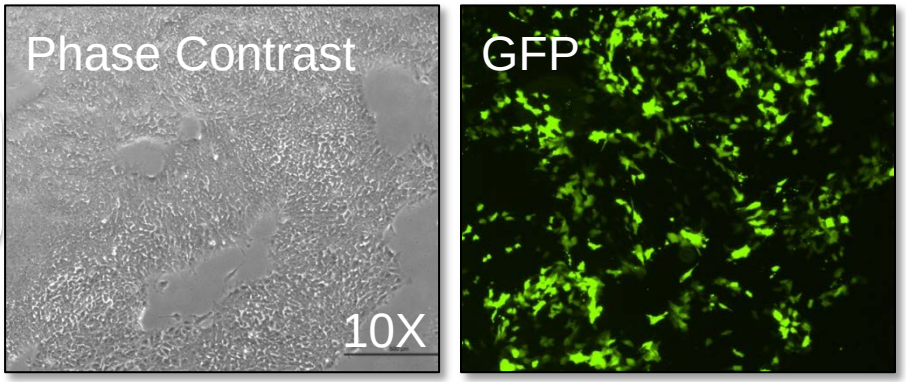
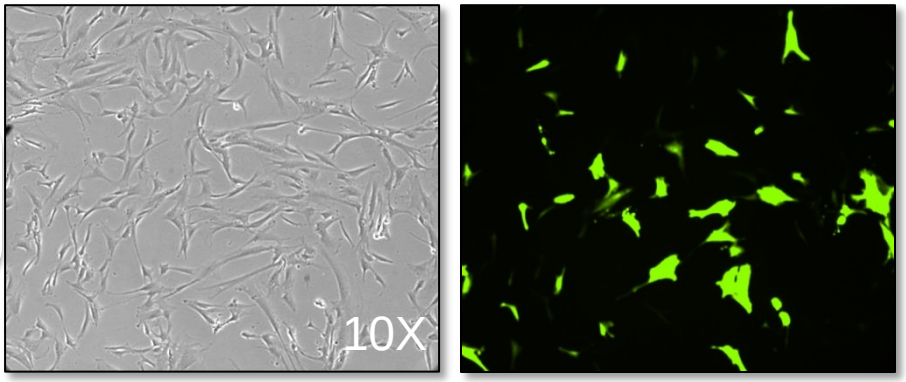
Transfect stem cells with TransfeX™

iPSCs
(ATCC® No. ACS-1012™)



Bone Marrow-derived MSCs
(ATCC® No. PCS-500-012)



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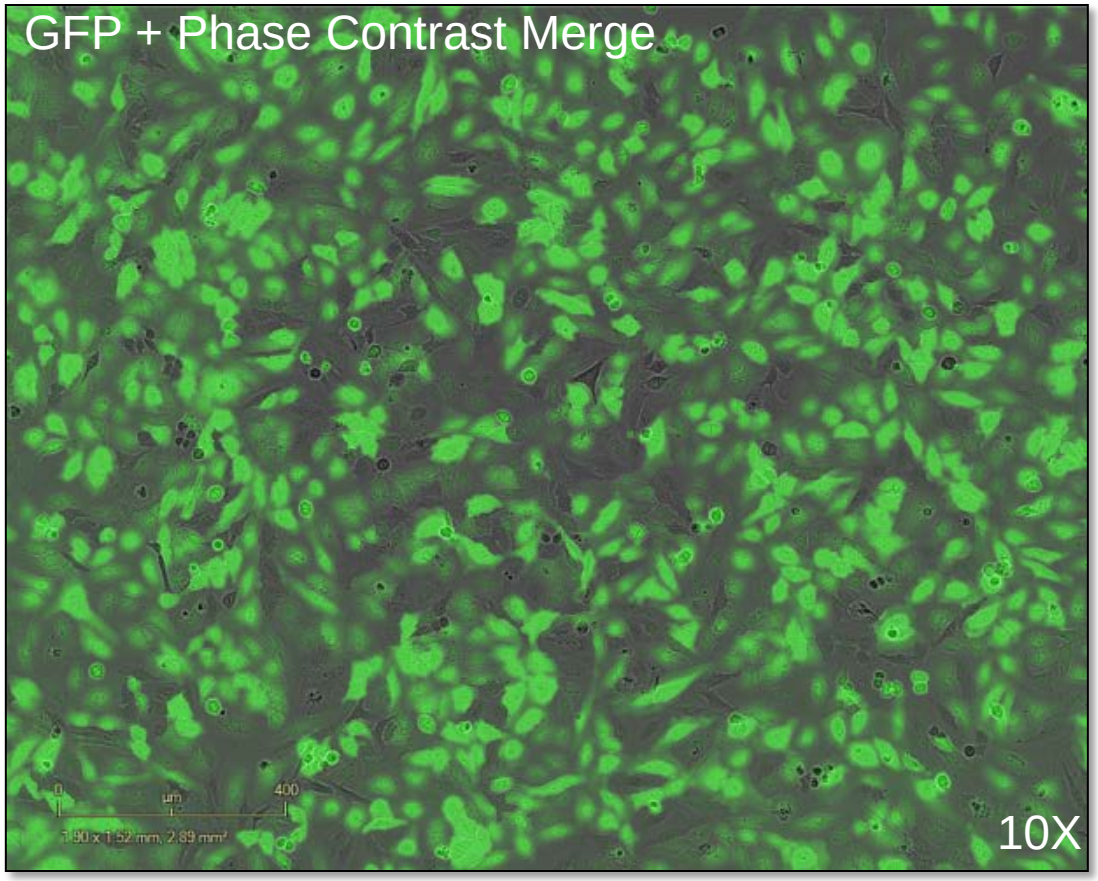
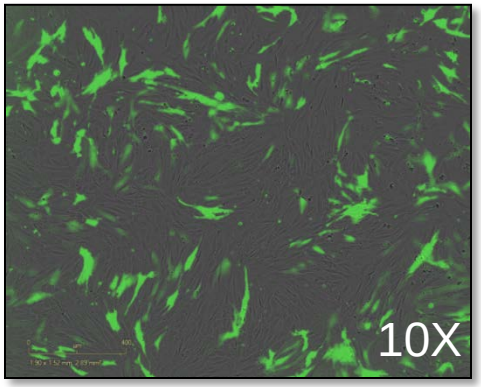
Transfection of mRNA with TransfeX™

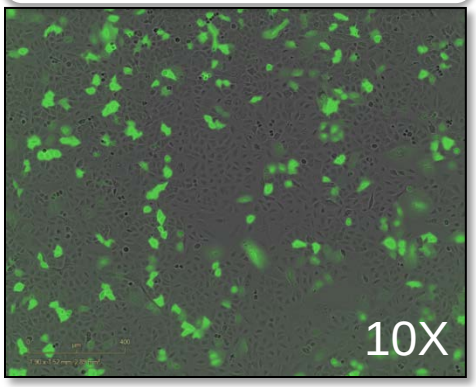
HeLa (ATCC® No. CCL-2™)



Primary DF



A549



Available siFEX optimized protocols

Continuous

- TeloHAEC-GFP
- HepG2
- MCF-7
- A549
- MDA-MB-231
- HeLa
- MRC-5
- HEK293T/17
- HUV-EC-C
- 3T3-L1
- C2C12
- Caco2
- LNCap

Stem

- Adipose tissue derived MSCs
- Cord blood-derived MSCs

Primary

- Primary DFs
- Primary prostate epithelial cells

Optimized protocol list as of April 2015

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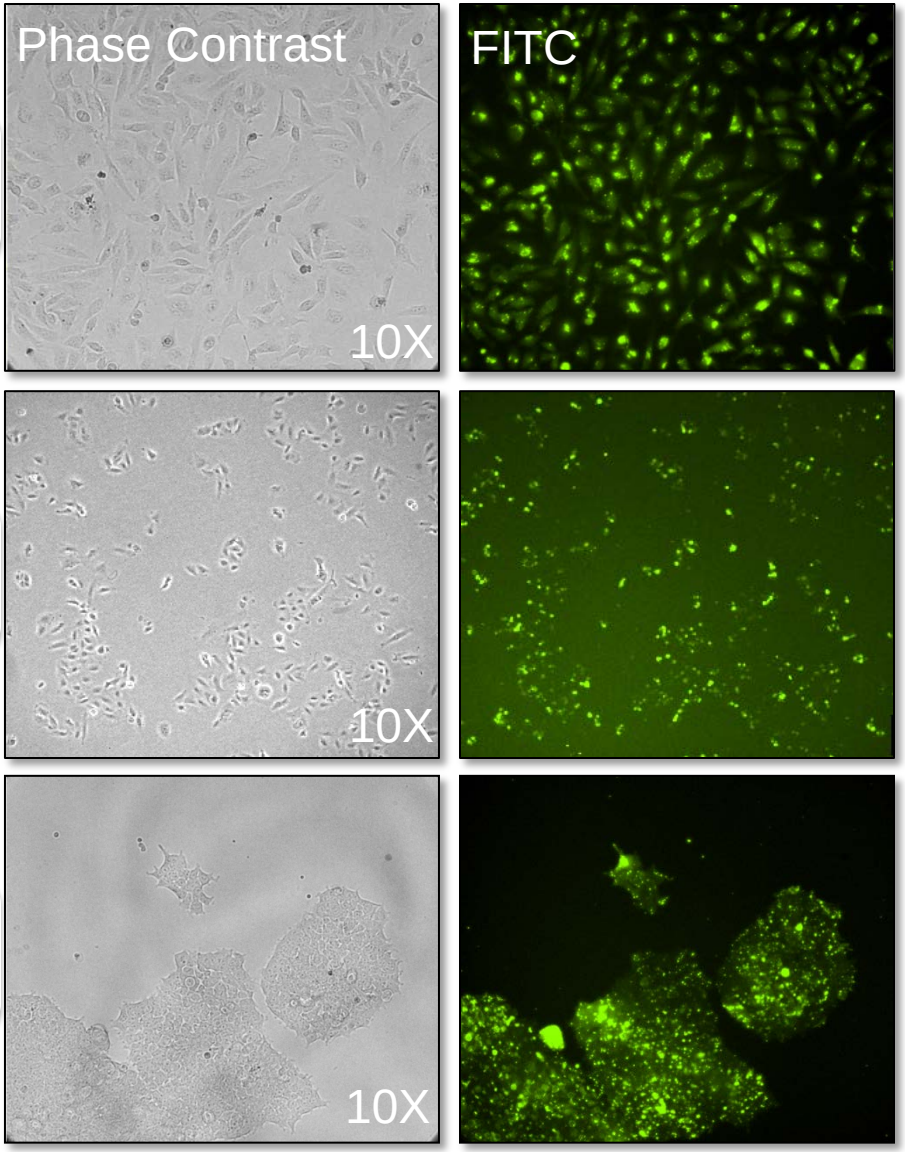
Transfection of fluorescently labeled siRNA

HeLa
ATCC® No. CCL-2™)

A549
(ATCC® No. CCL-185™)

HEK293T/17
(ATCC® No. CRL-11268™)



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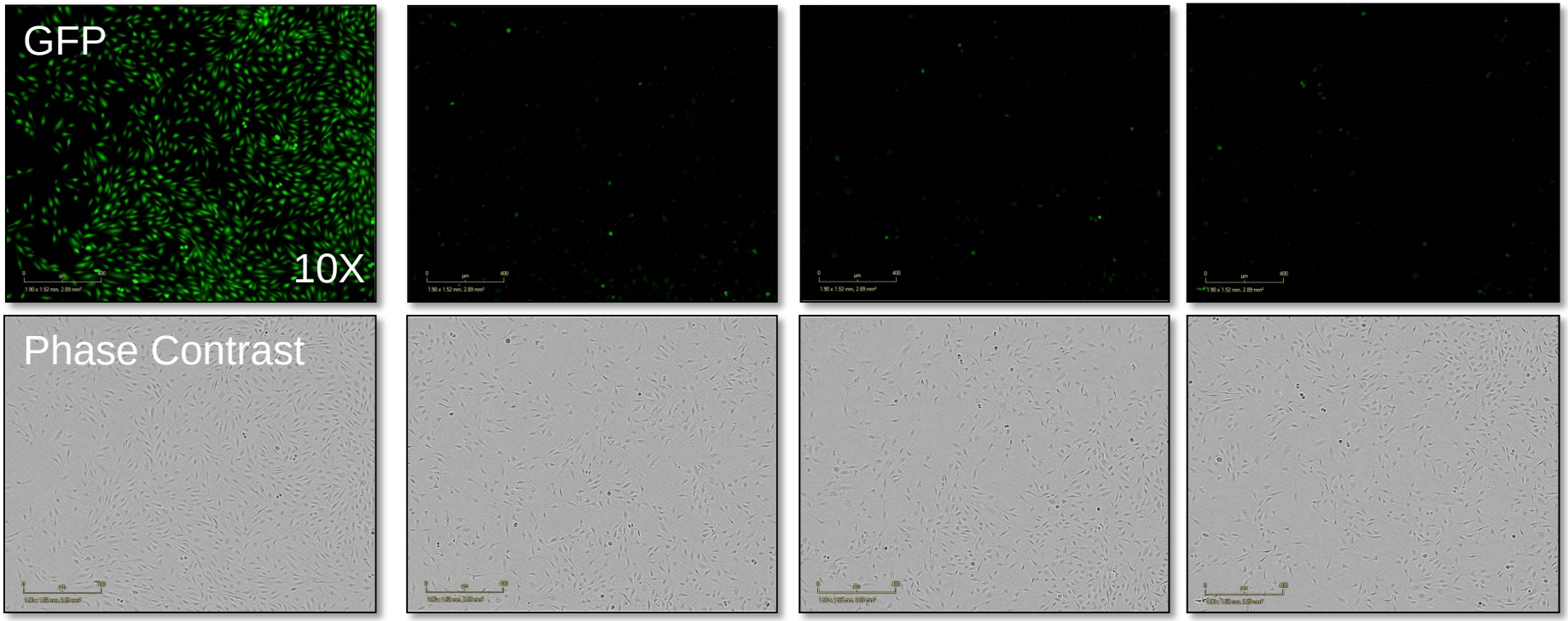
Knockdown of constitutive GFP expression

TeloHAEC-GFP (ATCC® No. CRL-4054™)

Untransfected

+Anti-GFP siRNA



For more information on TeloHAEC and TeloHAEC-GFP cells, visit the ATCC® Research page in our Learning Center at www.atcc.org/LearningCenter

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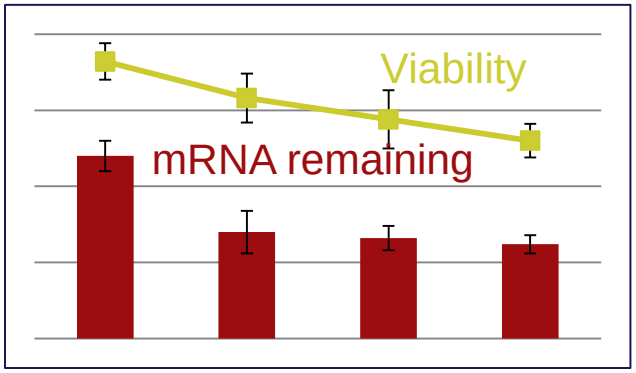
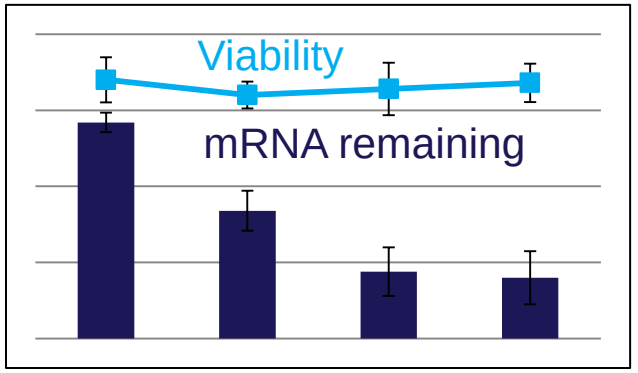
siFEX: performance tested

HeLa

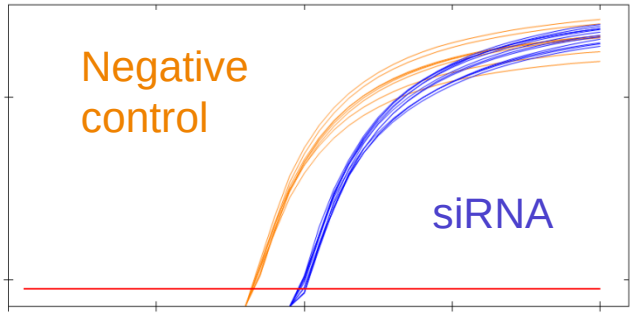
A549

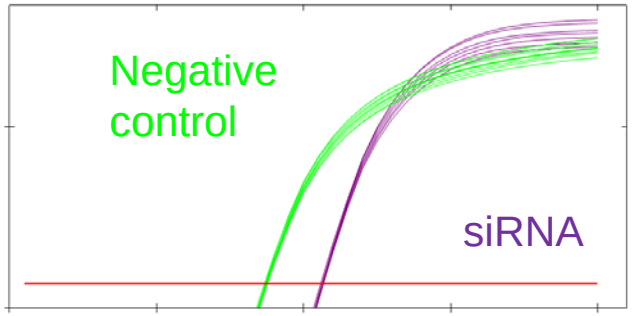
Colormetric assay



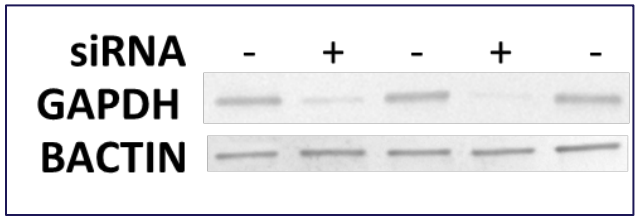


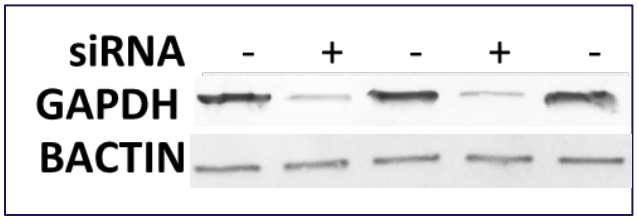
RT-PCR





Western blot





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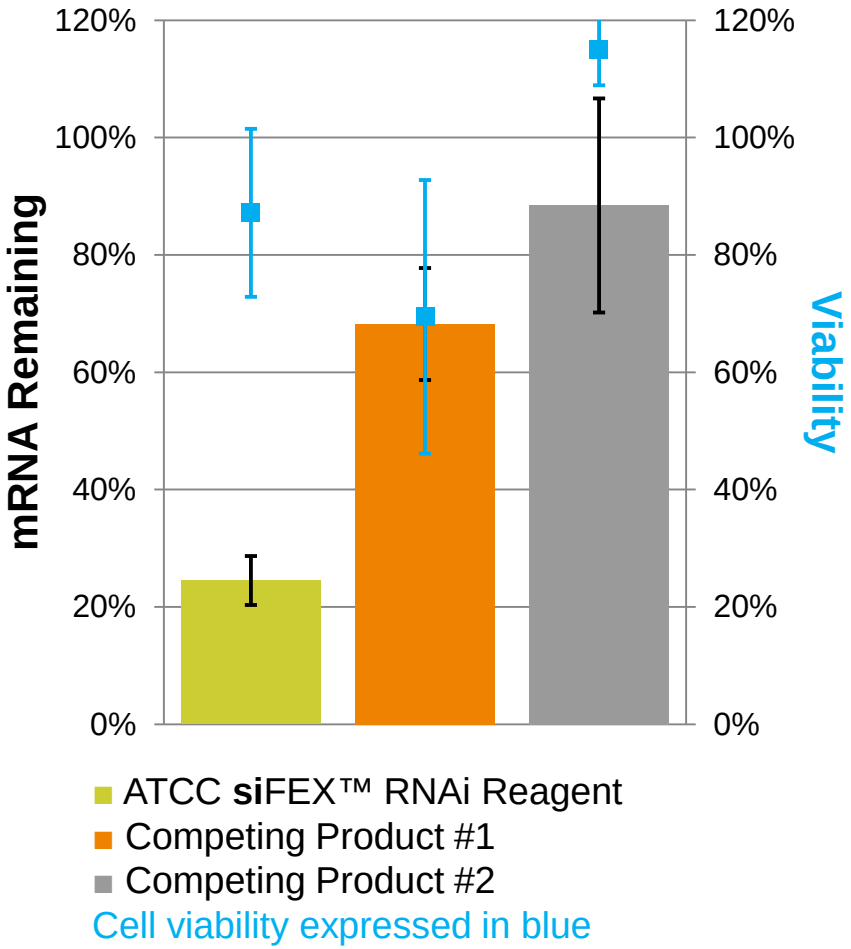
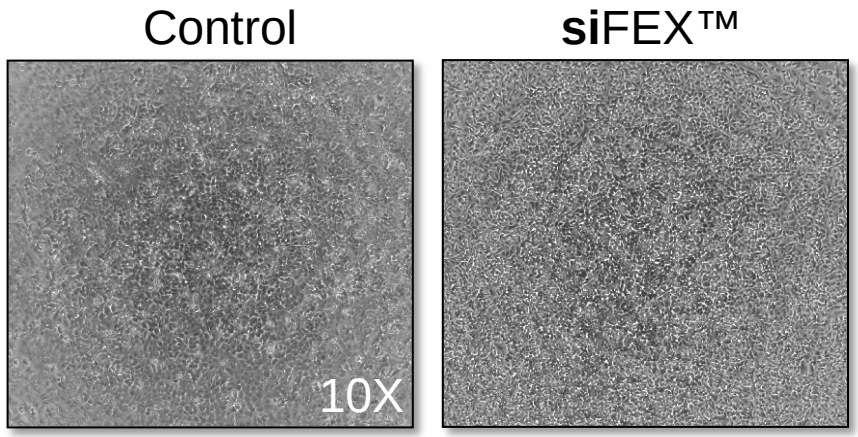
Transfection of pre-miRNA with siFEX





- HeLa cells in 24-well plate.
- Transfected with 20 nM hsa-miR-1 pre-miRNA
- Expression of PTK9 mRNA assessed 48 h post-transfection via RT-qPCR
- Results were calculated via ΔΔCT method, n=6 transfections, mean ± STD

Summary

- Lipid-mediated transfection is a powerful tool and useful in a variety of applications
- ATCC offers a variety of transfection reagents suitable for the transfection of plasmid DNA, mRNA, and dsRNA into a variety of cell types
- ATCC transfection reagents have been performance tested to deliver high efficiency and low cytotoxicity
- ATCC offers optimized transfection protocols for dozens of cell types to help you achieve results faster
  - Continuous cell lines
  - Primary cells
  - Stem cells
  - Adding new protocols all the time

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Thank you!

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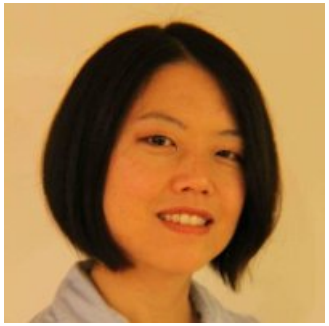

May 21, 2015
10:00 AM ET or 3:00 PM ET
Jodie Lee, M.S., *Lead Biologist,* ATCC
Seeing is Believing – Reporter Labeled Microbial Control Strains

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Thank you for joining today!
Please send additional questions to tech@atcc.org

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