TRANSFEX - SUPERIOR GENE EXPRESSION FOR HARD-TO-TRANSFECT CELL TYPES

Kevin Grady
Product Line Business Manager
ASCB Vendor Showcase
Dec. 15, 2013

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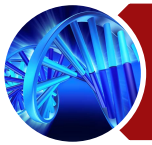
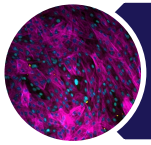
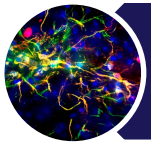
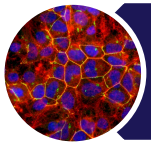
Outline

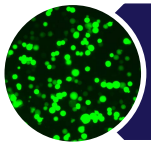

Overview of transfection


TransfeX


Primary/hTERT cell transfection


Stem cell transfection


Continuous cell line transfection


HEK*Plus* Protein Expression System

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Mechanisms of transfection

Physical

- Mechanical disruption of cell membrane opens “pores” in cell membrane
- Cell undergoes mitosis, plasmid DNA is captured by newly-formed nuclei of daughter cell
- **Common forms: Electroporation and Microinjection**

Viral

- Recombinant virus is generated by a multi-step process including gene cloning, amplification in packaging cells, and high titer viral particle purification
- Infect cells (containing viral specific receptor)
- **Common forms: Retrovirus (Lentivirus) and Adenovirus**

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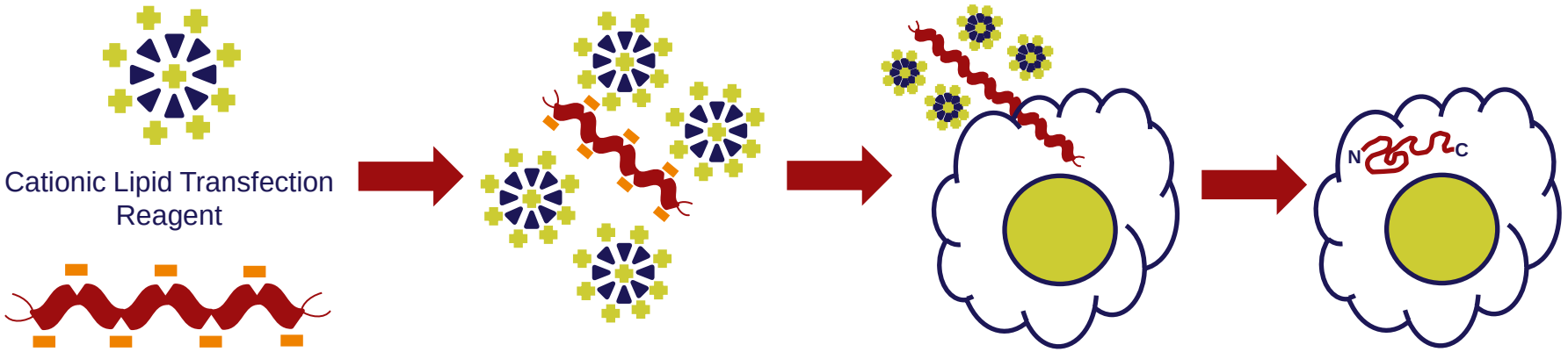
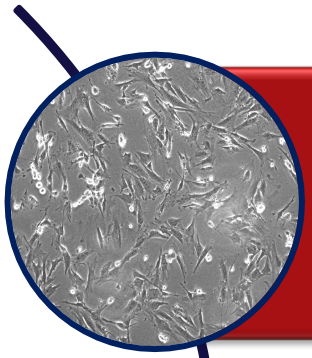
Mechanisms of transfection

Chemical

- DEAE-Dextran
- Calcium Phosphate
- **Cationic lipid reagent**
- Cationic non-lipid reagent

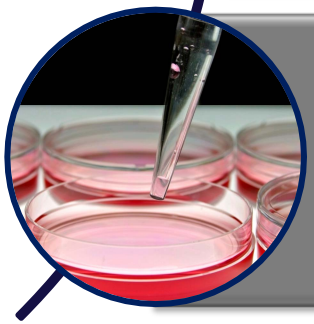


Factors affecting transfection efficiency



Cells
- Healthy and actively dividing
- Plating density and confluency at time of transfection
- Passage number

DNA
- High purity and endotoxin free
- Choice of promoter
- Ratio of DNA versus lipid
- Amount of DNA and lipid



Media
- Antibiotics
- Serum
- Polyanions

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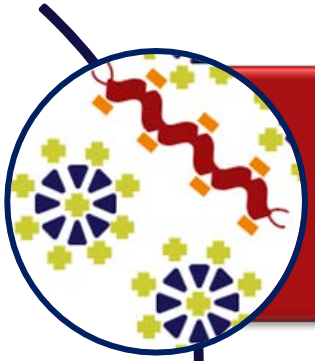
Factors affecting transfection efficiency



DNA to Reagent Ratio

- Must mask negative charge
- Determined empirically

Incubation Time

- Must balance penetration with viability
- Determined empirically

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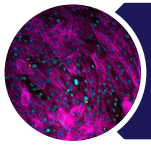
Outline


Overview of transfection


TransfeX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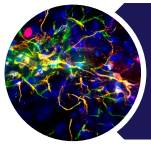
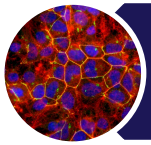
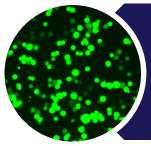

Stem cell transfection


Continuous cell line transfection


HEK*Plus* Protein Expression System

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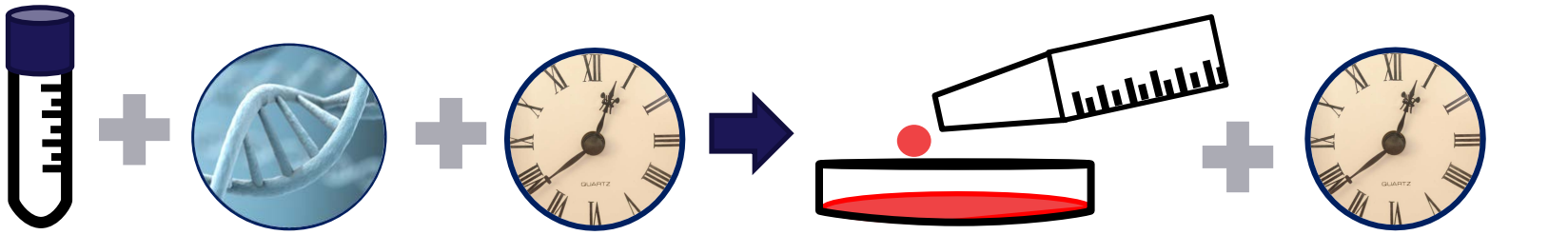
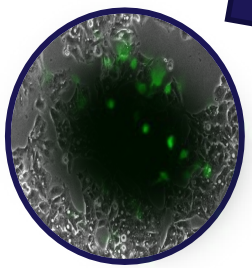
ATCC TransfeX transfection reagent (ATCC® ACS-4005™)

TransfeX Reagent:

- Designed for transfection of primary cells, stem cells, and hard-to-transfect cell lines
- Validated in many iPSCs, adult stem cells, primary cells, immortalized cell lines, and continuous cell lines
- Free from animal components
- Performance tested



TransfeX DNA 15 minutes 24 - 48 hours

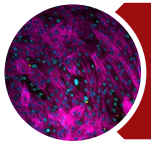


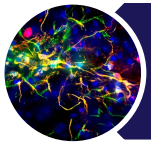
High efficiency transfection

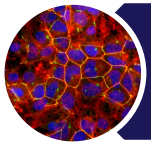
Outline

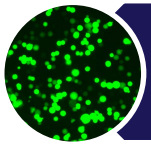

Overview of transfection


TransfeX


Primary/hTERT cell transfection


Stem cell transfection


Continuous cell line transfection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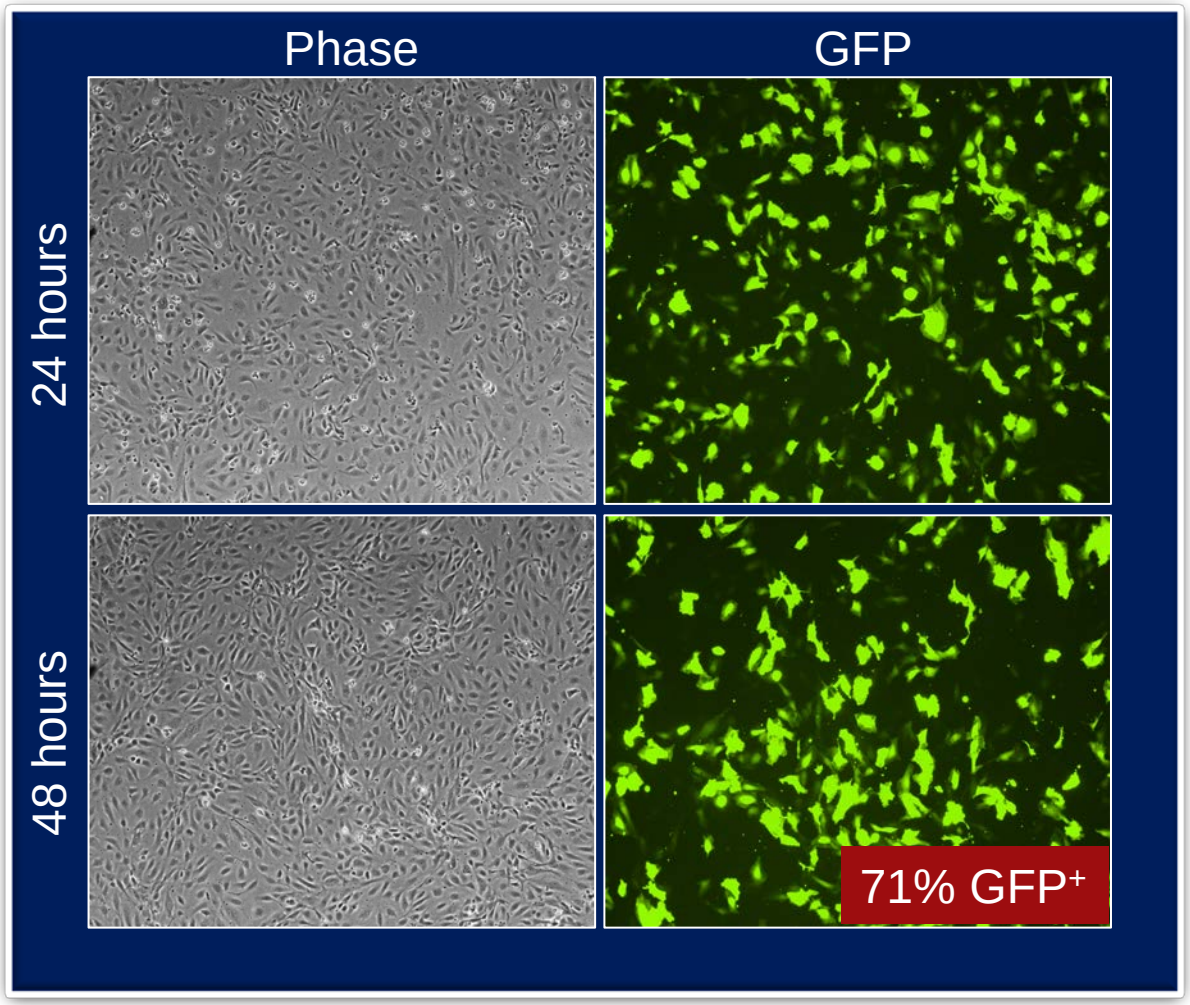
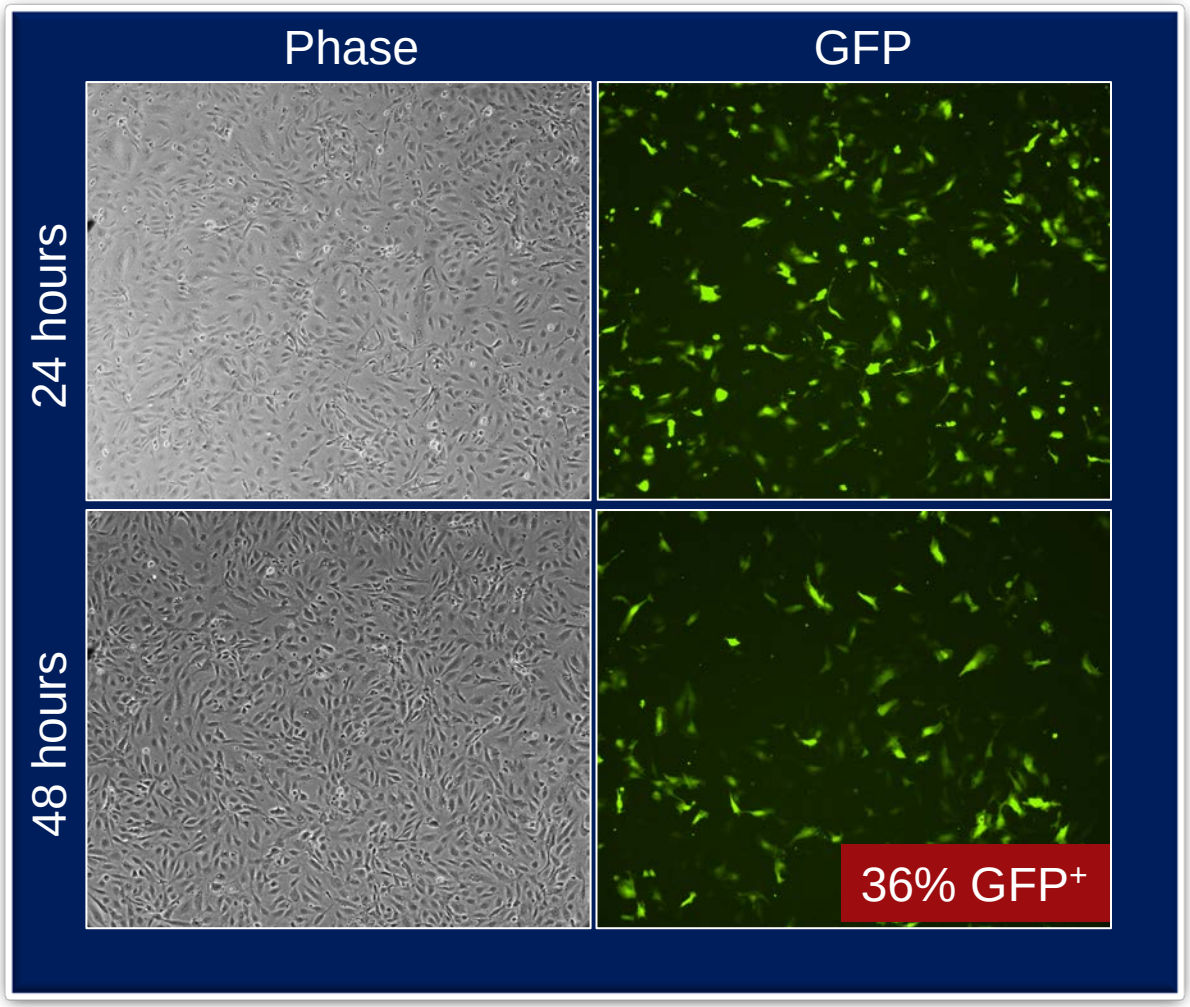
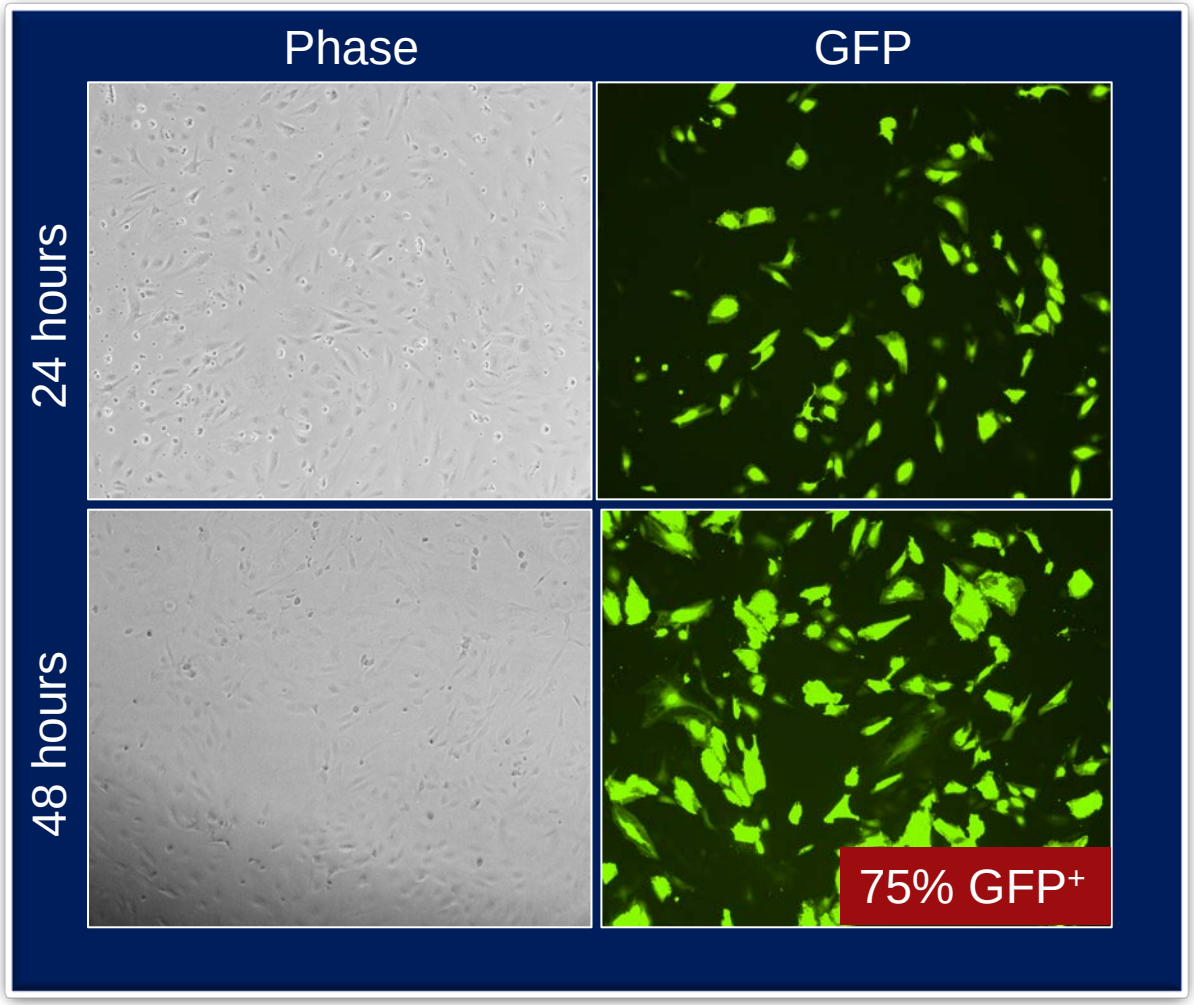
Transfection of HUVECs with TransfeX and EF1α-GFP vector



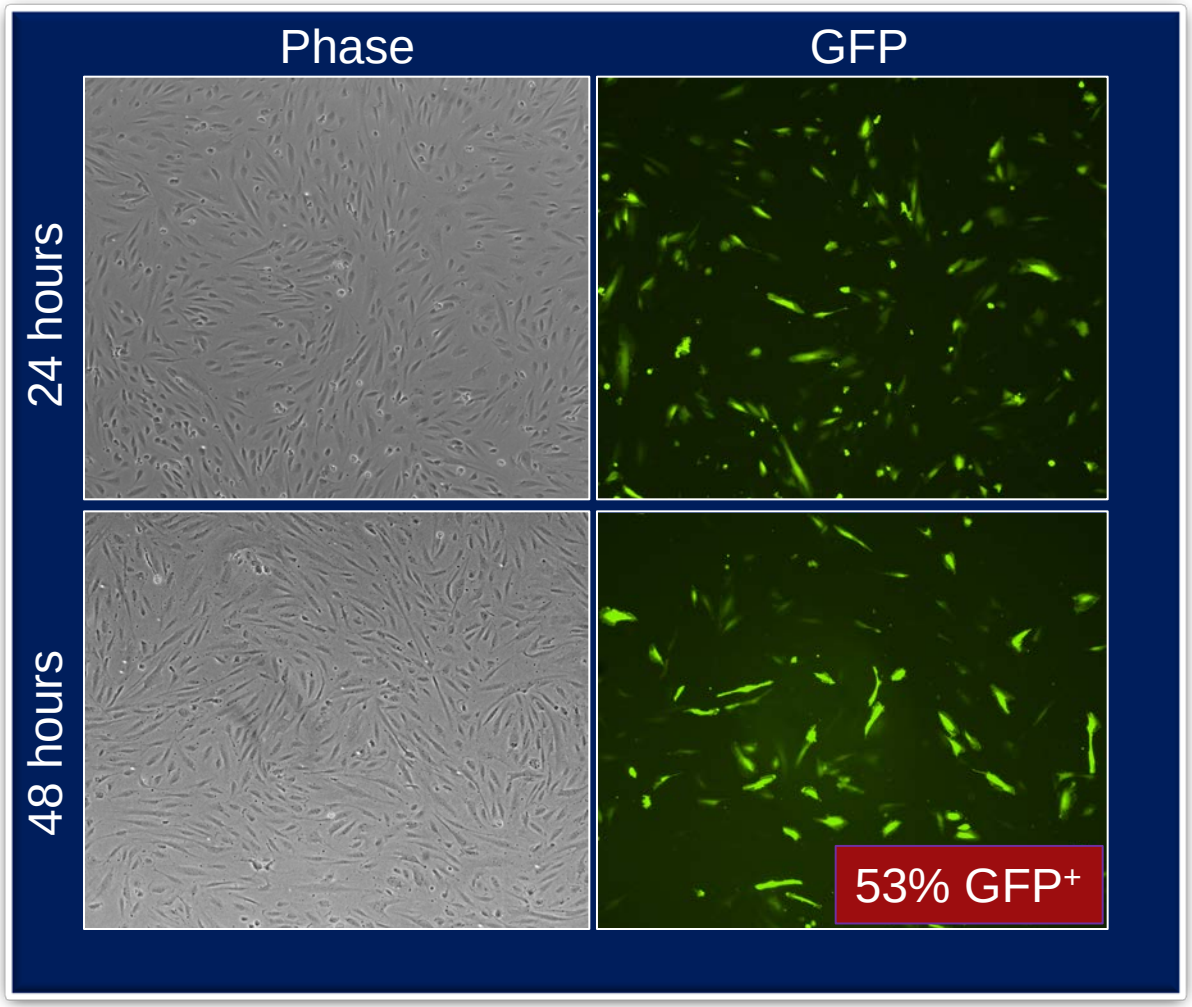
Transfection of HUVECs with alternate supplier reagent and EF1α-GFP vector



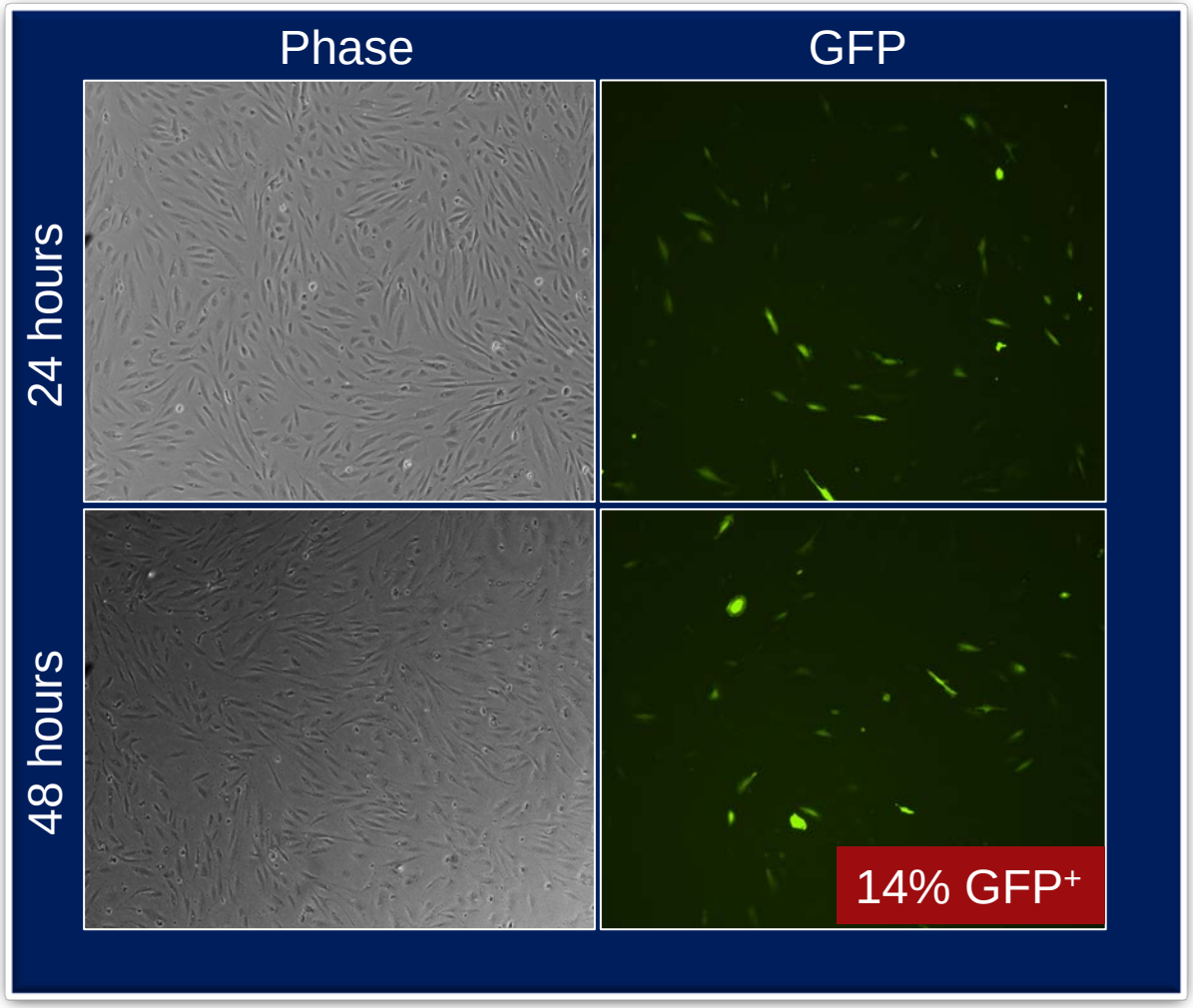
Transfection of primary dermal microvascular endothelial cells with TransfeX and EF1α-GFP vector



Transfection of immortalized human microvascular endothelial cells (TIME) with TransfeX and CMV-GFP vector



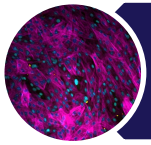
Transfection of immortalized human microvascular endothelial cells (TIME) with alternate supplier reagent and CMV-GFP vector

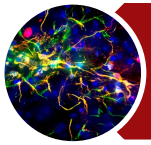


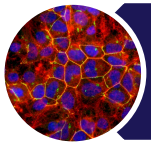
Outline

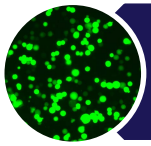

Overview of transfection


TransfeX


Primary/hTERT cell transfection


Stem cell transfection


Continuous cell line transfection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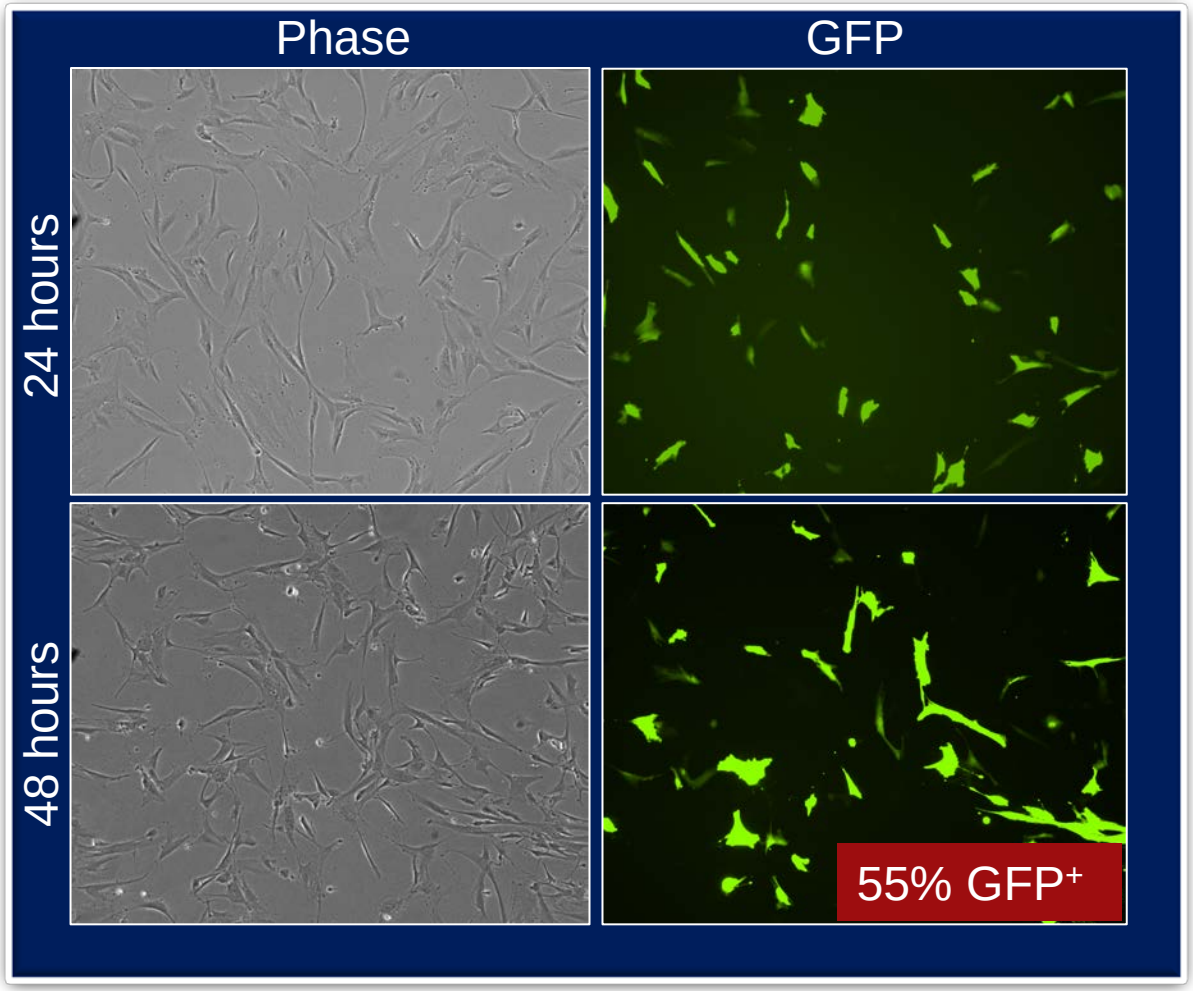
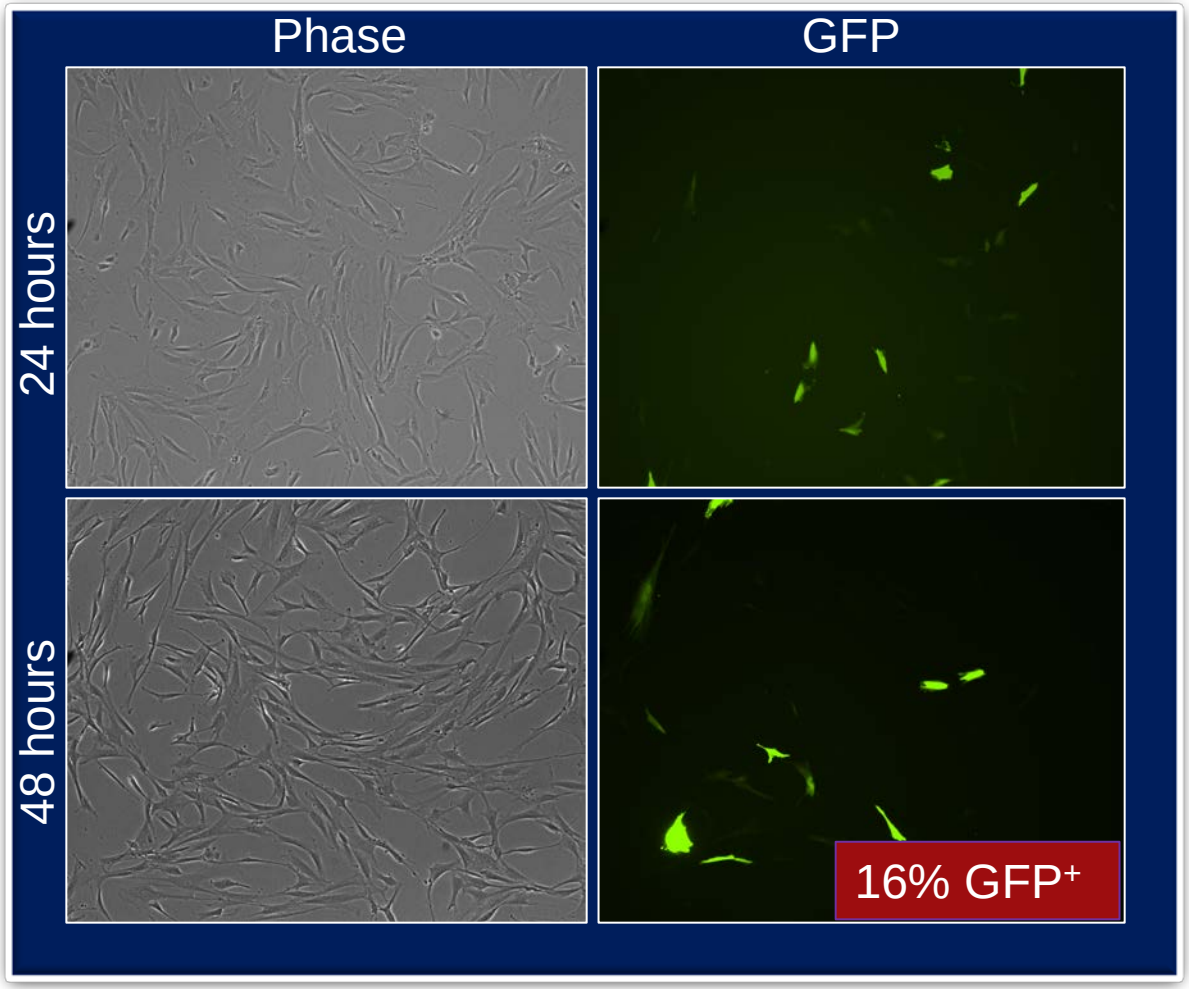
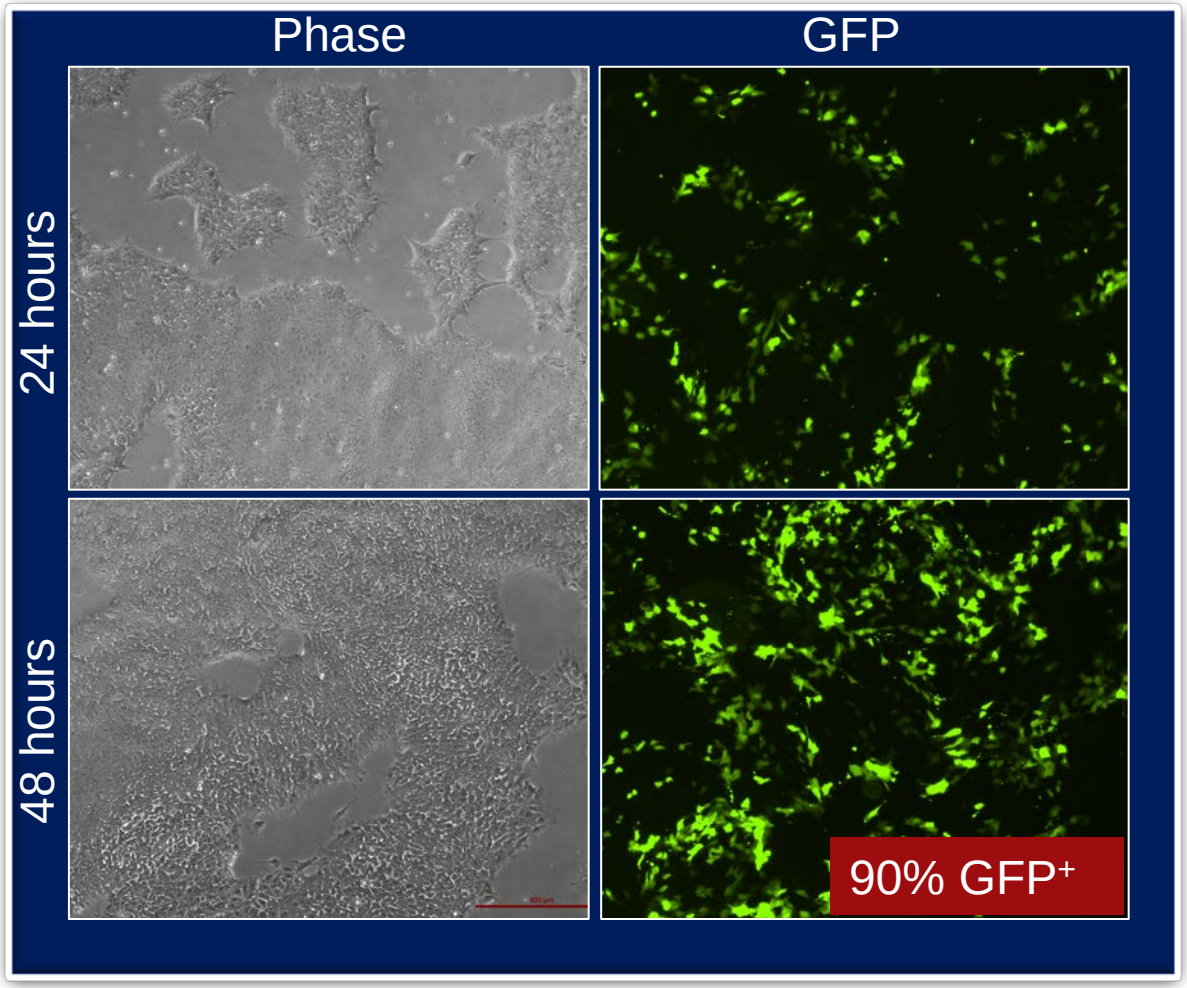
Transfection of BM-MSCs with TransfeX and EF1α-GFP vector



Transfection of BM-MSCs with alternate supplier reagent and EF1α-GFP vector



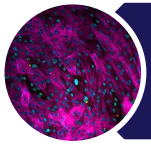
Transfection of hiPSCs with TransfeX and EF1α-GFP vector



Outline


Overview of transfection


TransfeX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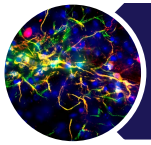
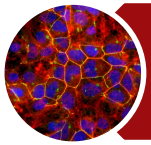
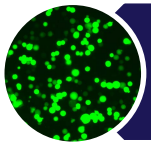

Stem cell transfection


Continuous cell line transfection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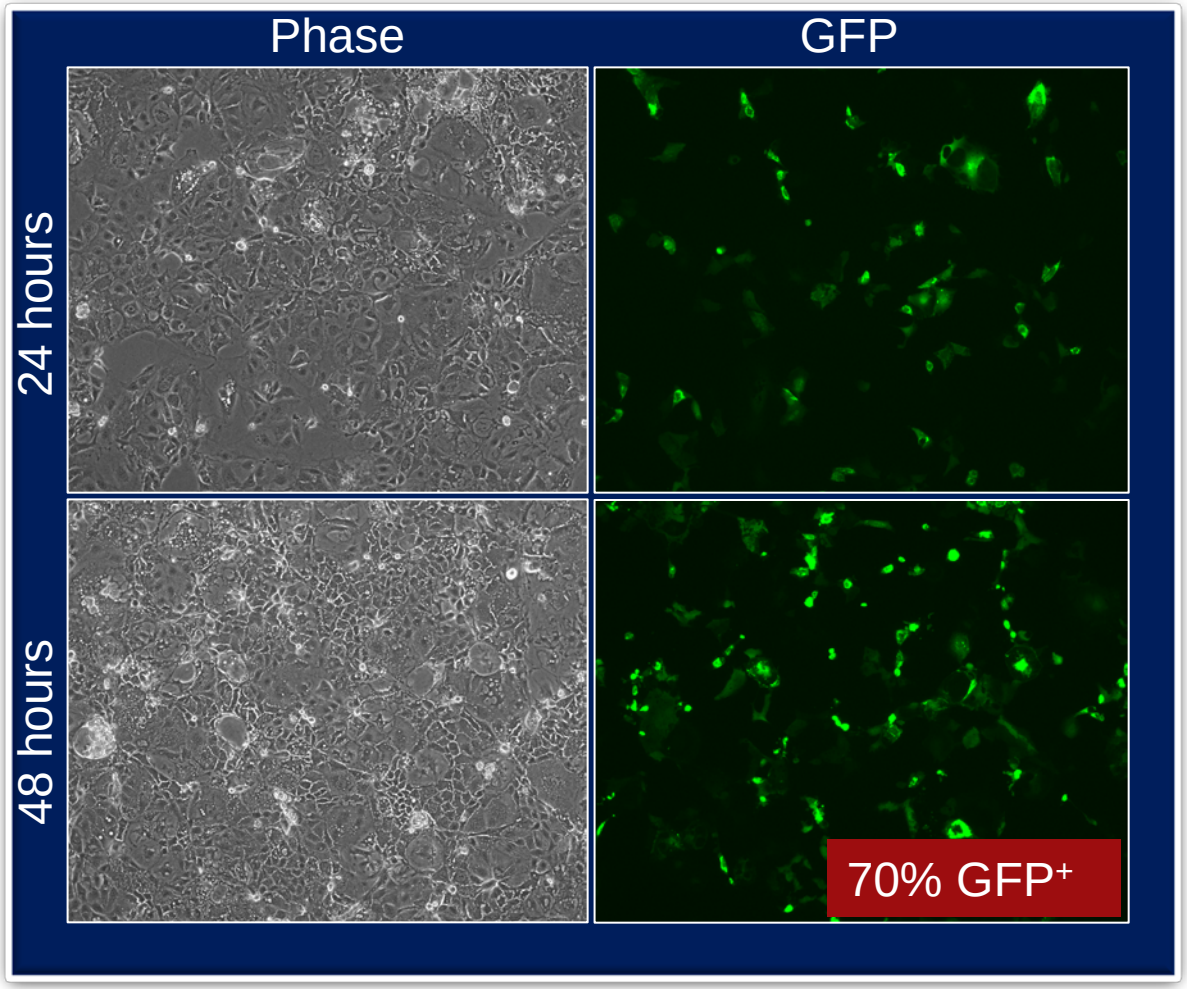
Transfection of Caco-2 cell line with TransfeX and EF1α-GFP vector



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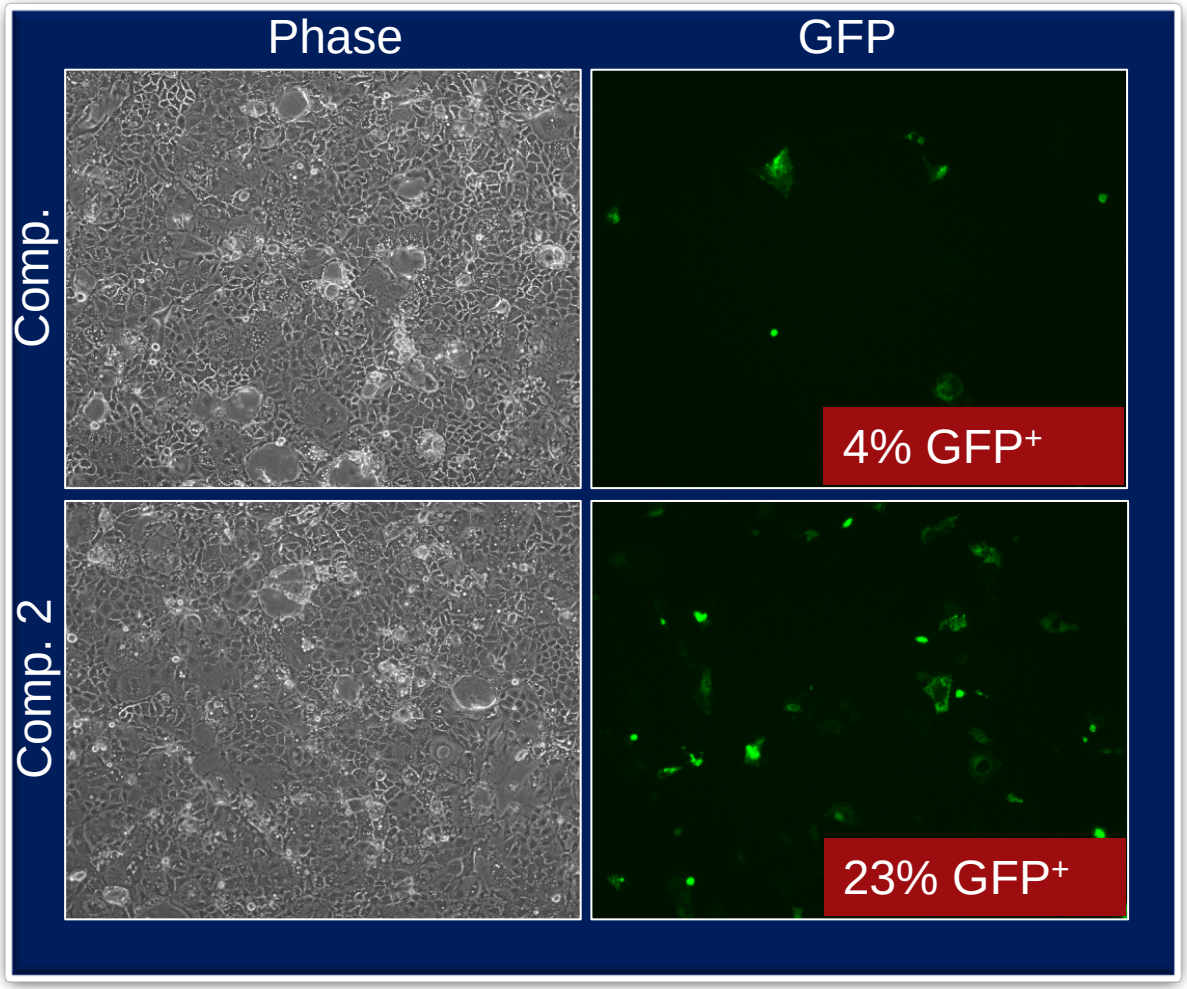
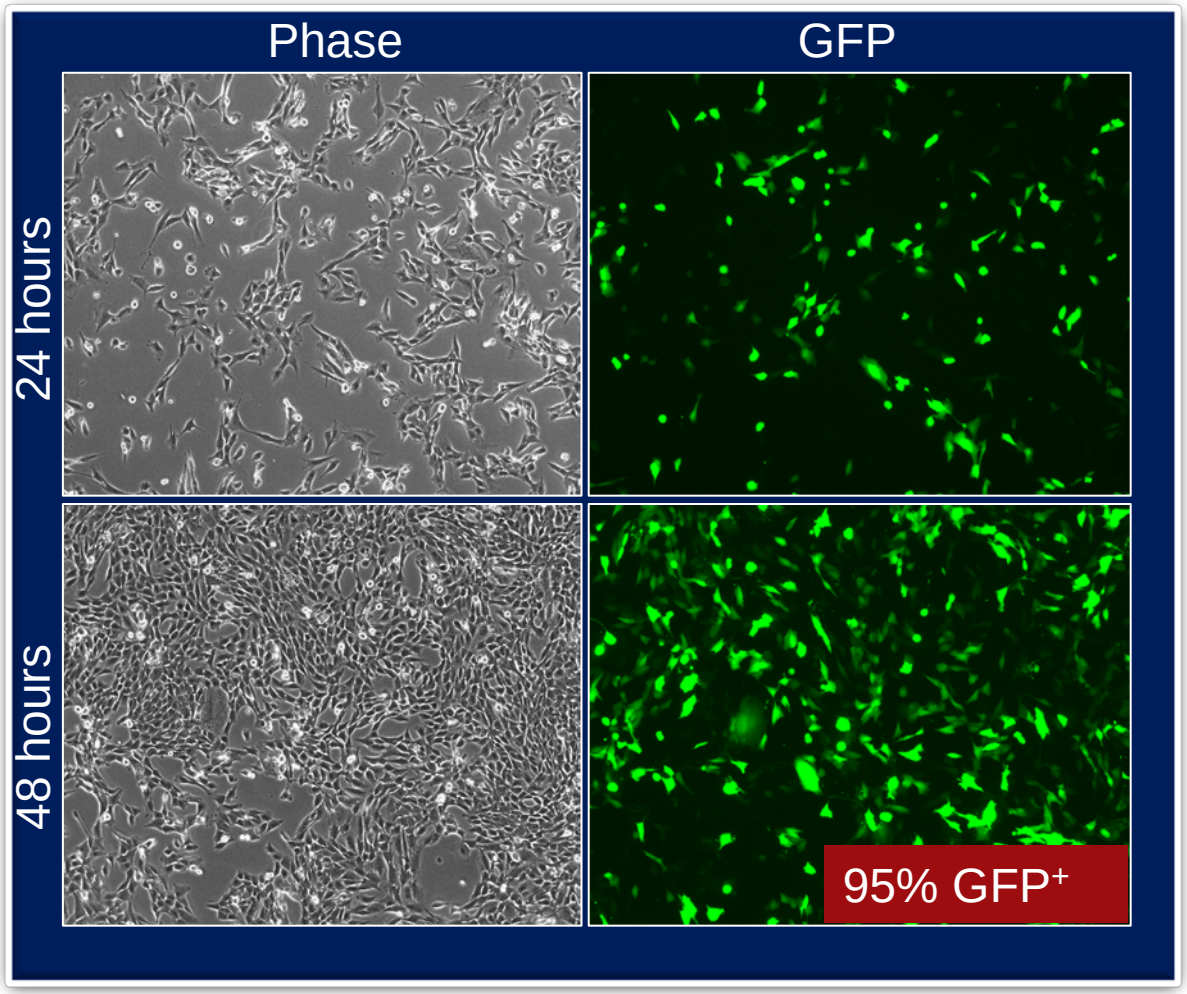
Transfection of Caco-2 cell line with alternate supplier reagent



Transfection of C2C12 cell line with TransfeX and EF1α-GFP vector



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ATCC TransfeX transfection guide

Protocols for using TransfeX to transfect . . .

Continuous	Stem	Primary
• LNCap • MDA-MB-231 • HepG2 • Caco-2 • C2C12 • 3T3-L1 • NuLi-1 • TIME • RPTEC-hTERT • hTERT-HME	• Bone-marrow derived MSCs • hiPSCs • BT-142	• Dermal Fibroblasts • Dermal Microvascular Endothelial Cells • HUVECs • RPTECs • Large Airway Epithelial Cells • hMECs

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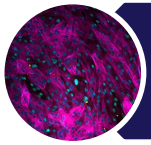
Contact Technical Service at tech@atcc.org

Outline


Overview of transfection


TransfeX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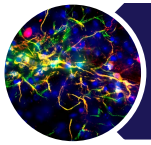
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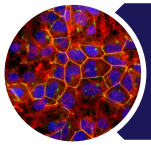
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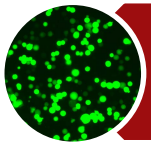
HEK*Plus* Protein Expression System

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HEKPlus Protein Expression System (ATCC® ACS-4800-K™)

A complete mammalian protein expression system using serum-free suspension cell cultures of HEK293 cells

Component	ATCC® No.
HEKPlus SF Suspension Cells	ACS-4500™
HEKPlus SFM Medium	ACS-4002™
HEKPlus Boost Solution	ACS-4003™
GeneX*Plus* Transfection Reagent	ACS-4004™
L-Alanyl-Glutamine, 200 mM	30-2115™

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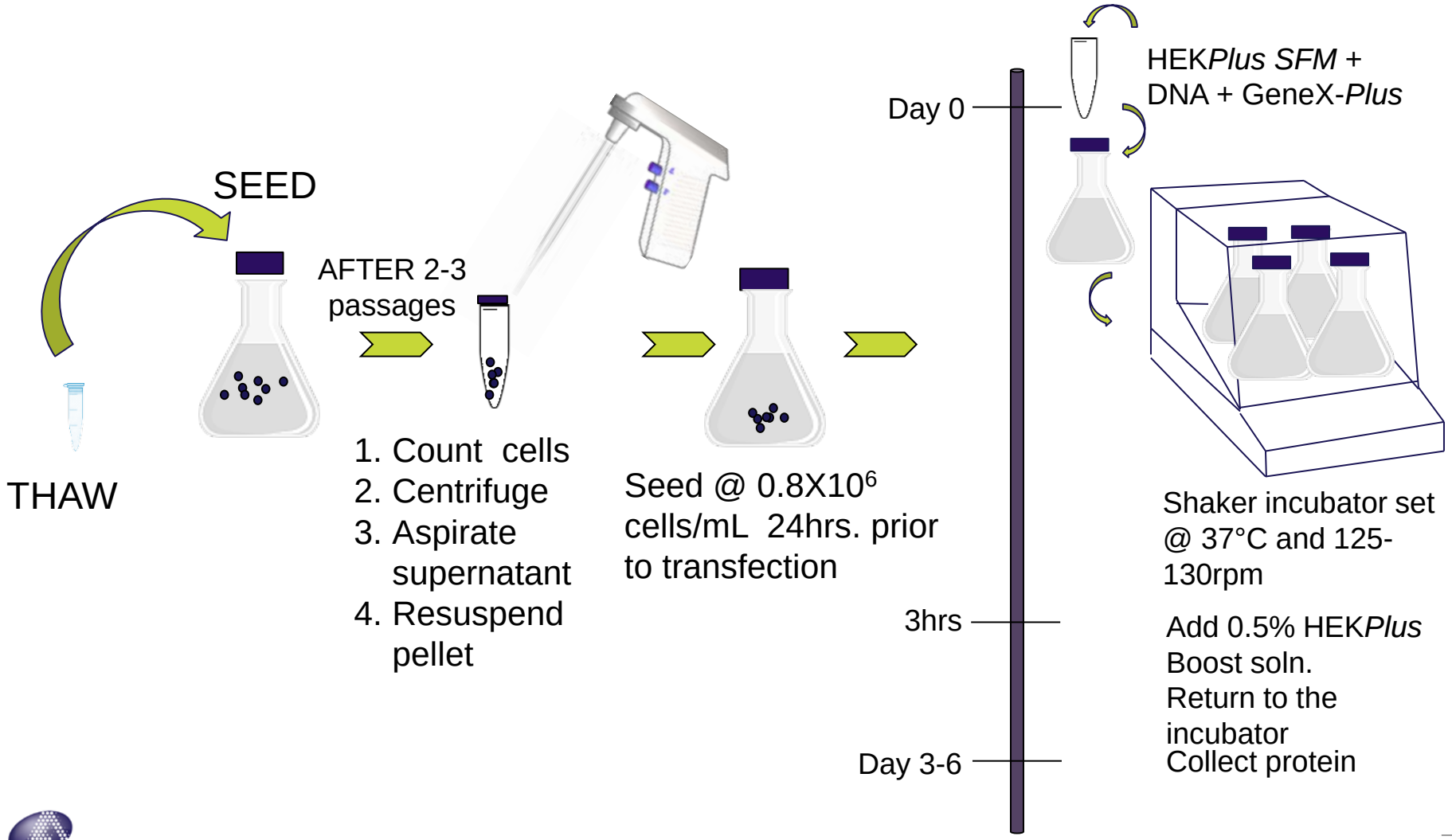
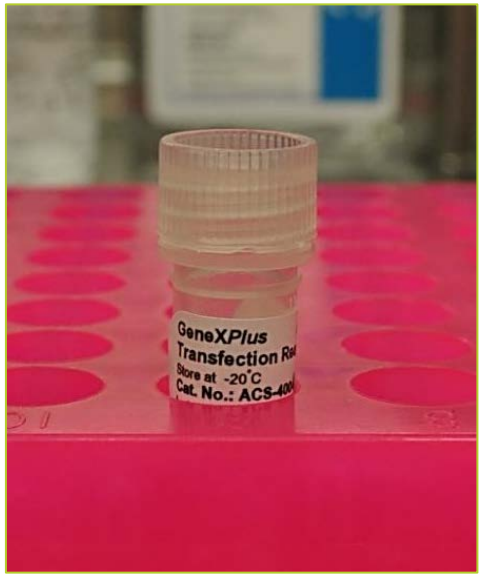
The kit components are optimized to work together



HEKPlus GeneXPlus Transfection Reagent

Gene*XPlus* Transfection Reagent (ACS-4004™)

- 1 mL
- Animal component free
- Used for transfection of plasmid DNA into mammalian cells
- Formulated for low cytotoxicity; optimized to balance cytotoxicity and potency
- Produces high levels of gene expression
- Suitable for both transient and stable transfection



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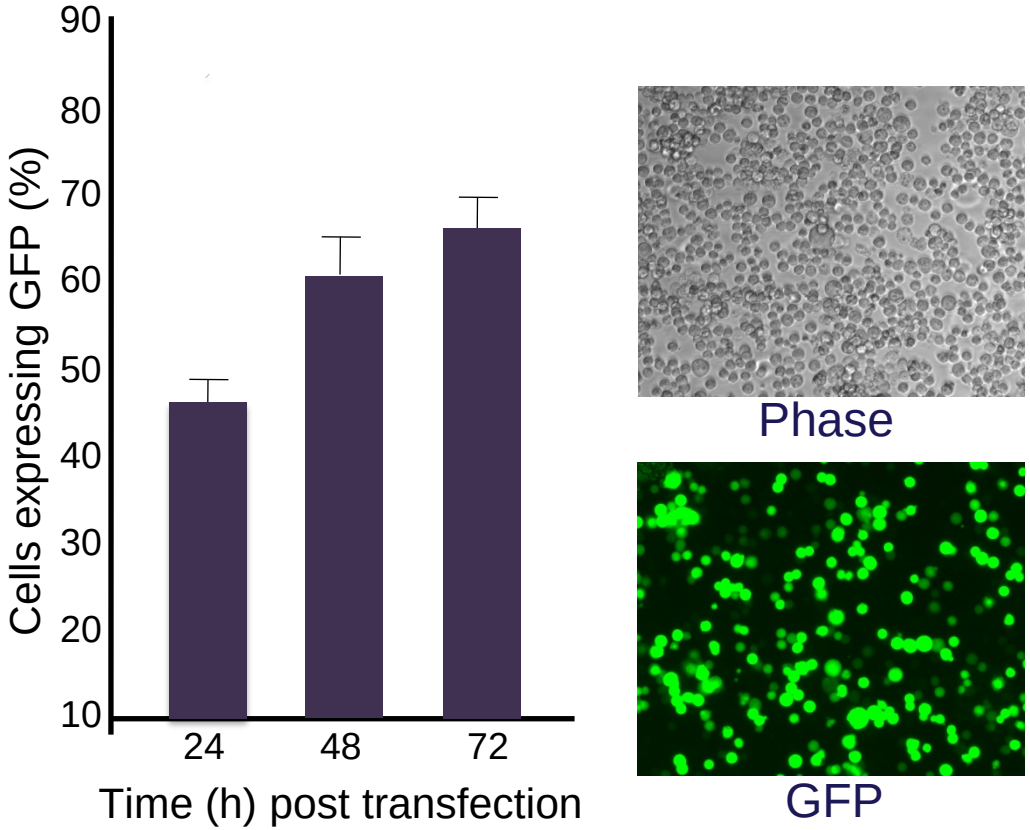
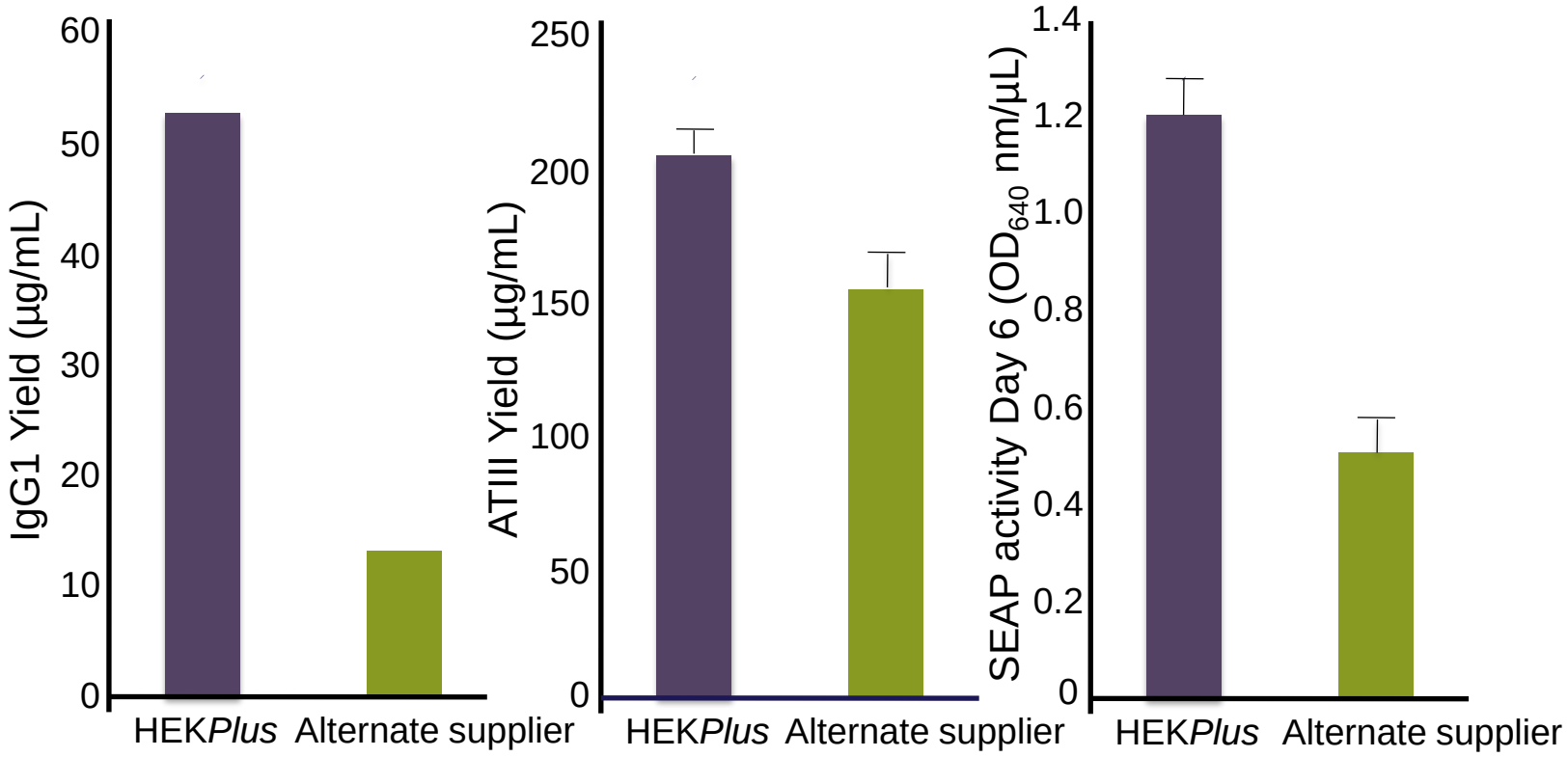
HEKPlus System: High transfection efficiency



The HEKPlus Expression system consistently achieves **high transfection efficiency**, with 65-70% of cells expressing the construct 48-72 hours after transfection

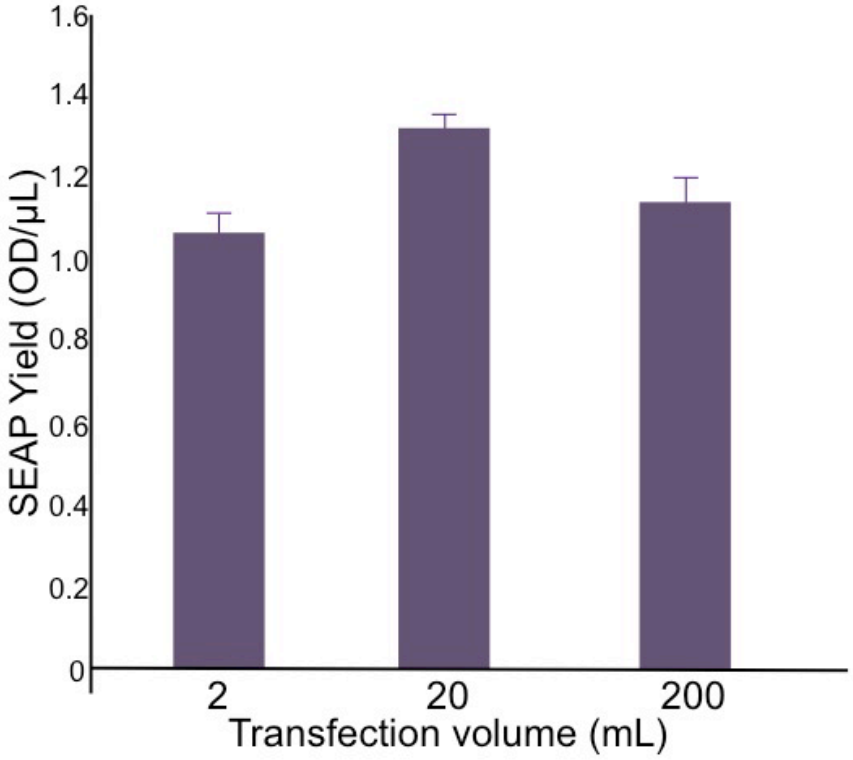
HEKPlus System: Better yield versus an alternate supplier



HEK*Plus* system results in **protein yields higher than the expression systems of an alternate supplier.**

SEAP was assayed using a phosphatase reaction, which suggests that the expressed protein is **functional.**

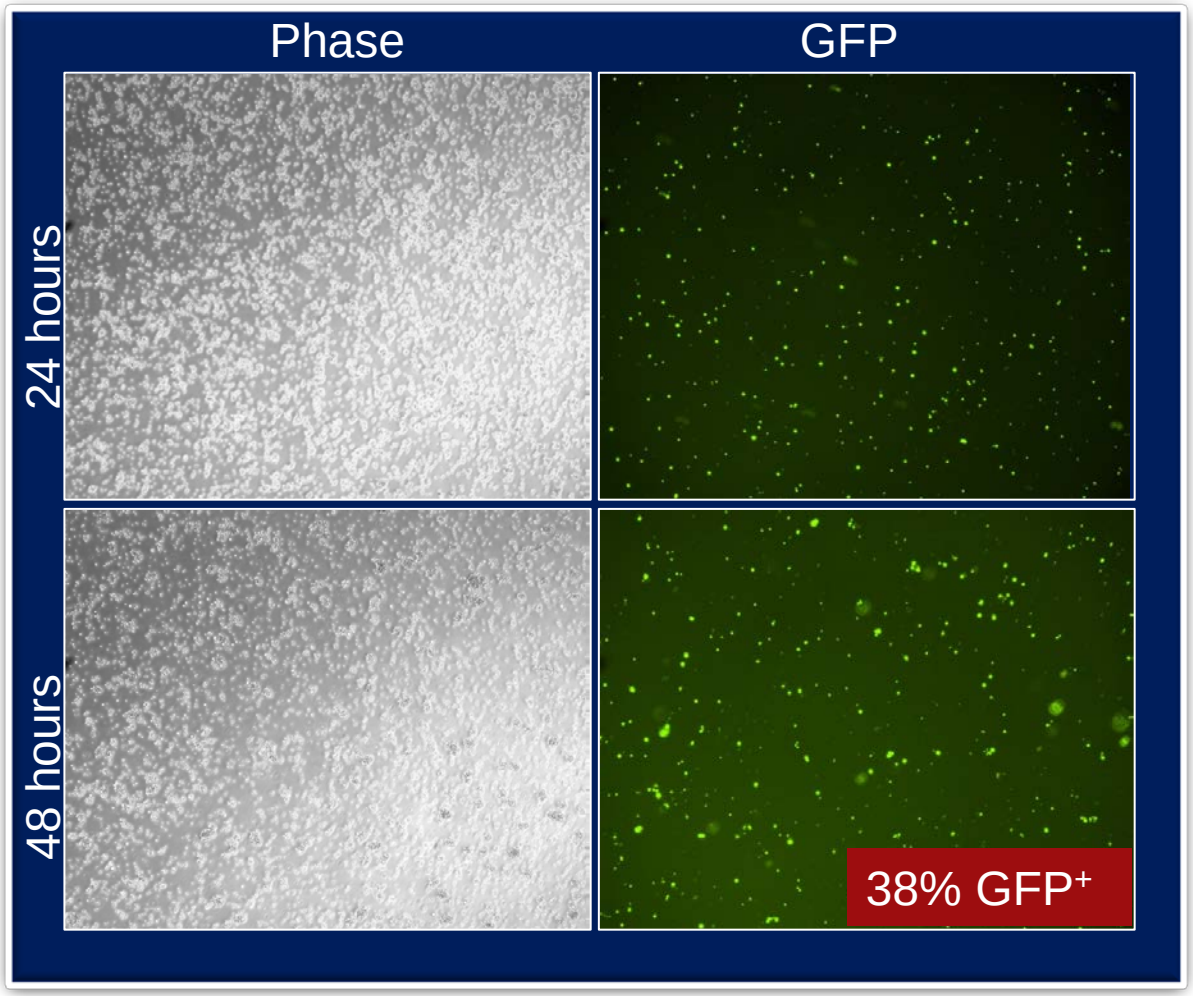
HEKPlus System: Cost-effective and scalable



Kit components offered as a **cost-effective complete system** or **individually** to meet the needs of the investigator.

The kit is **scalable**. It is tested to ensure a comparable yield of SEAP when either 2 mL or 200 mL of cells (1 x 10^6 cells/mL) are transfected.

Transfection of THP-1 cells with GeneX Plus and EF1α-GFP vector



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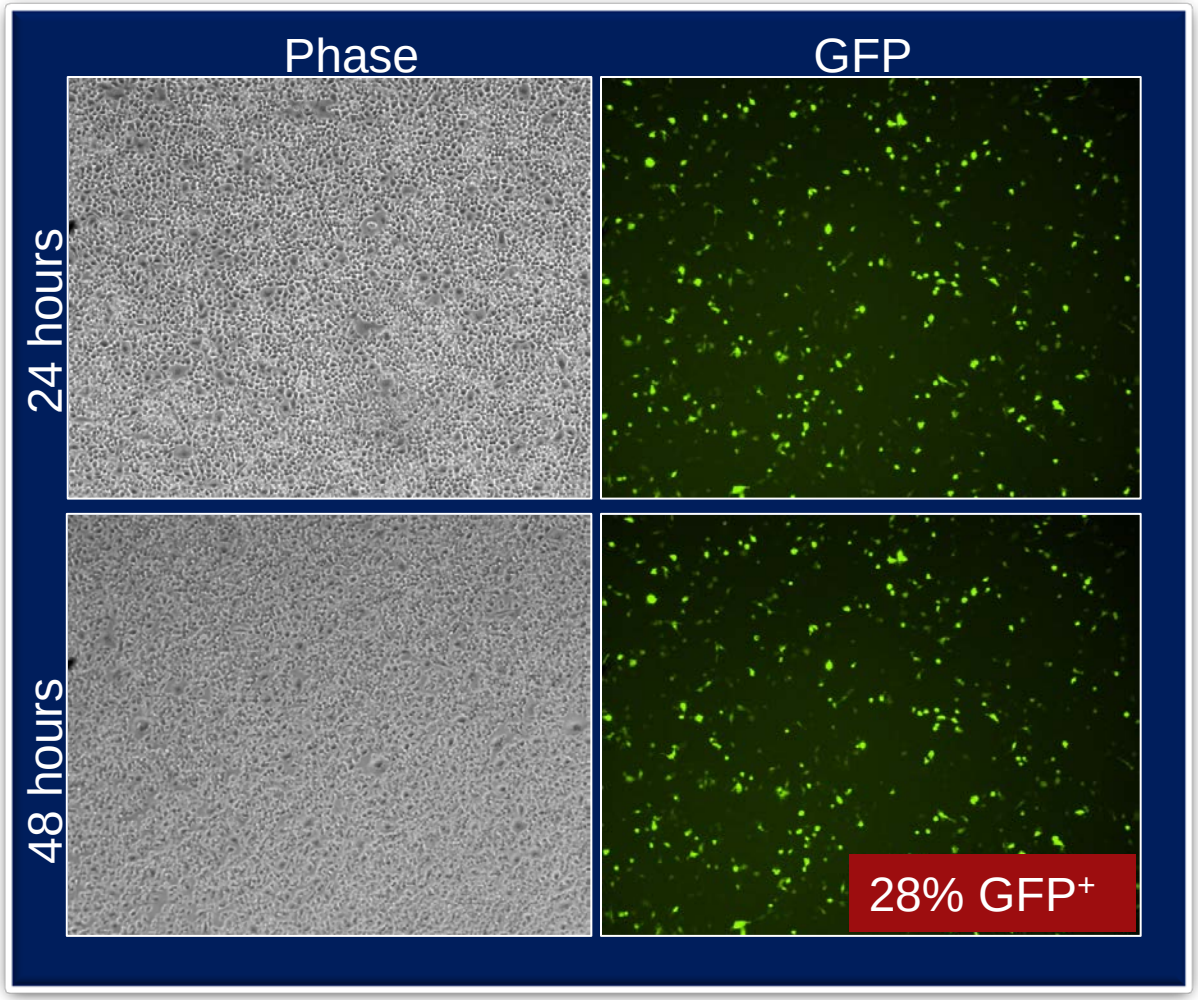
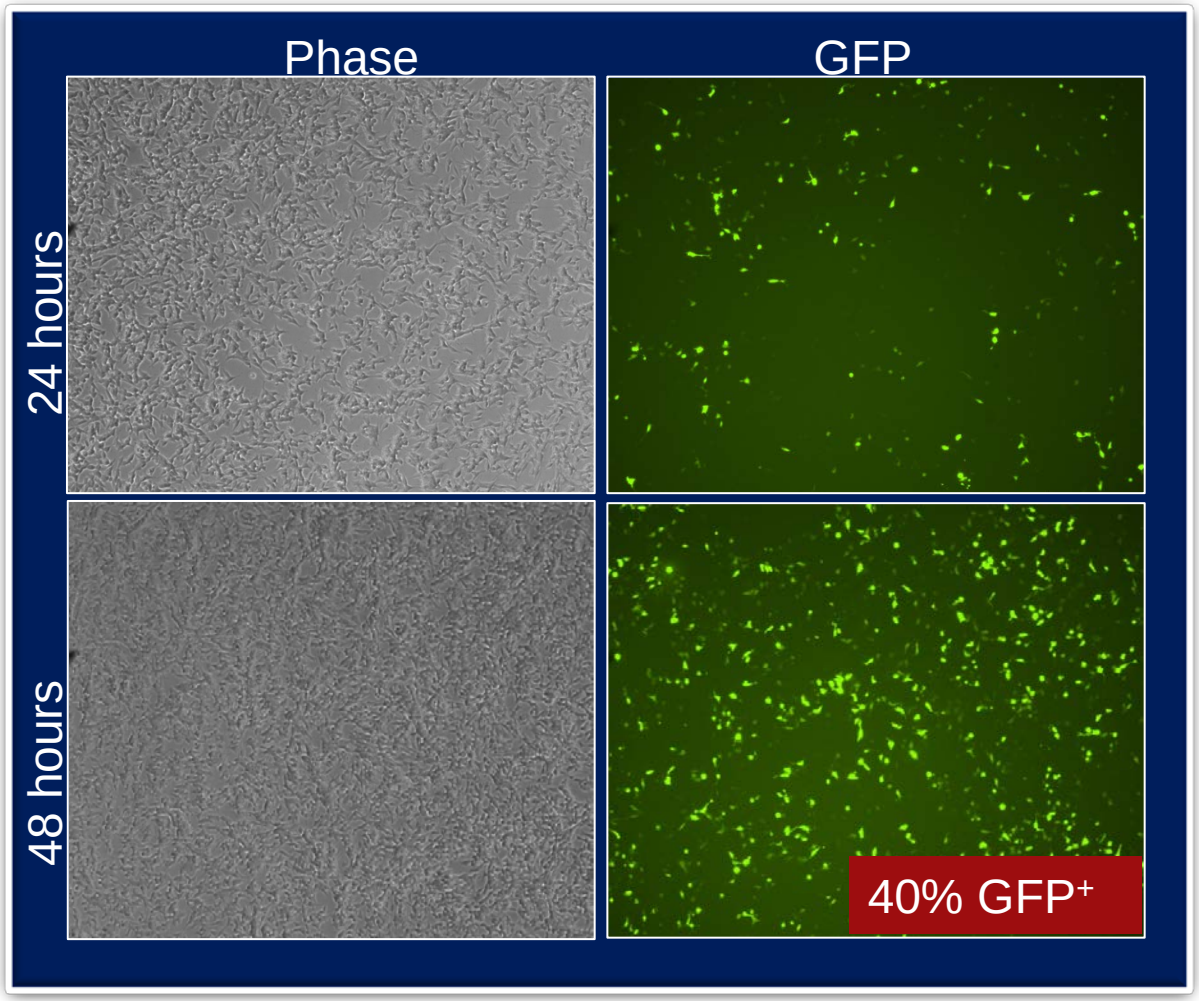
Transfection of Raw 264.7 cells with GeneX Plus and EF1α-GFP vector



Transfection of Raw 264.7 cells with GeneX Plus and EF1α-GFP vector



Summary: TransfeX and the HEKPlus Protein Expression System

TransfeX

- Universal transfection reagent that can be used to transfect difficult-to-transfect cells like stem and primary cells
- High efficiency and low cytotoxicity
- Cost effective and scalable

HEK*Plus*

- Serum-free, xeno-free fully optimized system simplifies purification and downstream processing.
- Efficient transfection reaction generates high-yield of functional protein.
- Kit is cost effective and scalable.
- GeneX Plus Reagent is also suitable for transfection of THP-1, Raw 264.7, SH-SY5Y, BM-MSCs, BJ-5ta, and HUVECs.

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