



OZ BIOSCIENCES
The art of delivery systems

Eurobiomed 2010

Silencing Gene expression by siRNA delivery



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OZ Biosciences

Company
Overview

Focus

Create, produce and commercialize research reagents based on molecular delivery systems

Product Lines

Technologies:

Magnetofection

Lipofection

Polyfection

Applications:

DNA transfection

siRNA transfection

Viral application

Protein delivery

Introduction

dsRNA (>200 bps):

Long Double strand RNA insert directly into cytoplasm for Dicer cleavage into 20-25 nucleotide- double-stranded RNA before RISC complexe activation

Small interfering RNA (siRNA) (20-25 bps):

20-25 nucleotide double-stranded RNA insert into cytoplasm for direct activation of RISC complexe

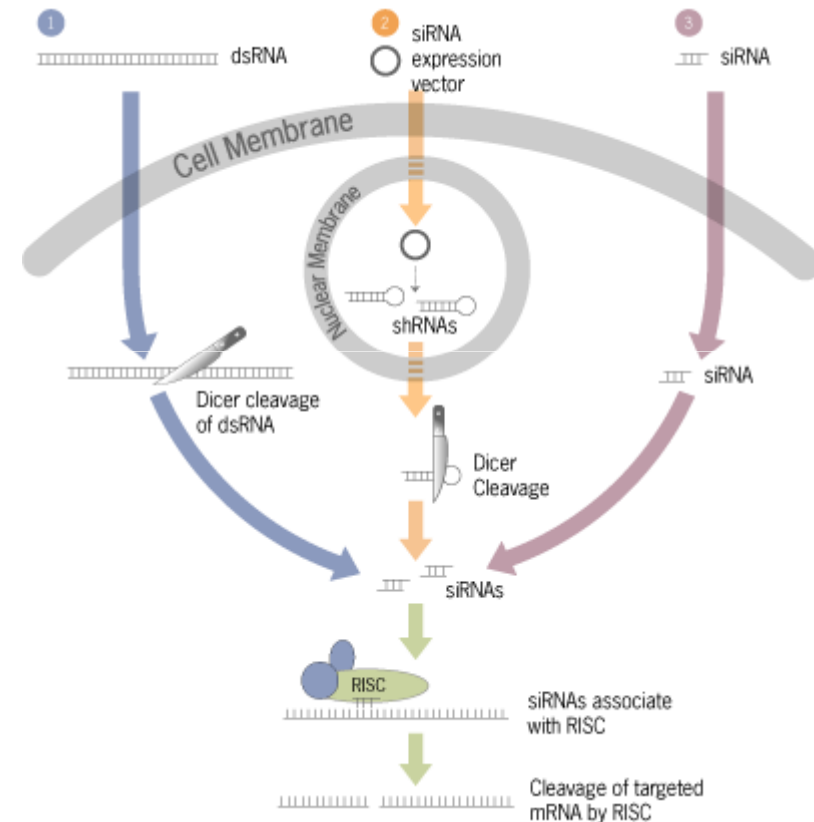
Strong but transient down-regulation

shRNA expression vectors (> 2Kb):

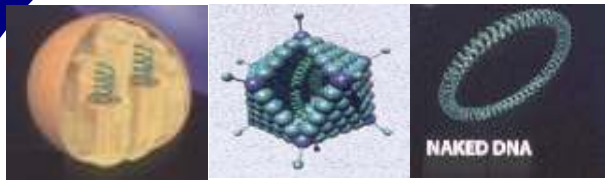
Adeno-Lenti-Retrovirus or plasmid DNA encoding for shRNA.

Required nucleus insertion for shRNA trascription followed by cleavage into 20-25 nucleotide- double-stranded RNA before RISC complexe activation

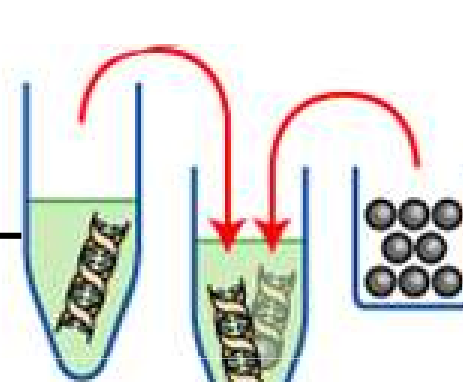
Long-term down regulation



Necessity of intracellular transporter in adequation with the construction used



dsRNA, siRNA
plasmid or virus encoding shRNA



Magnetic Plates

Magnetic complexes
on cells



Incubation on
magnetic plate



Assay (12/72h)



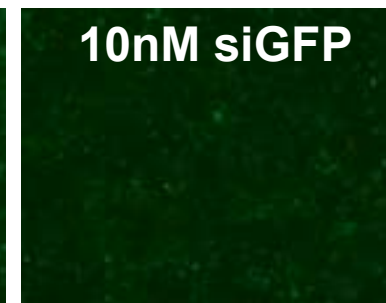
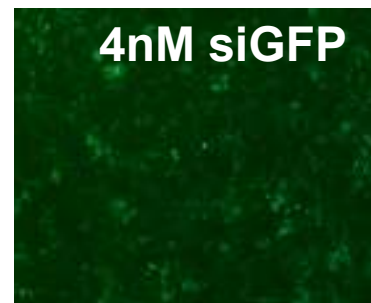
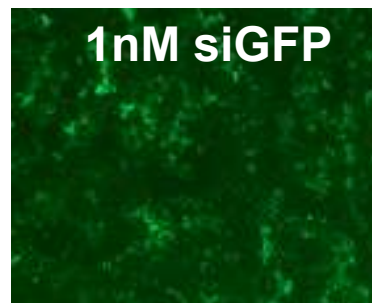
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SilenceMag

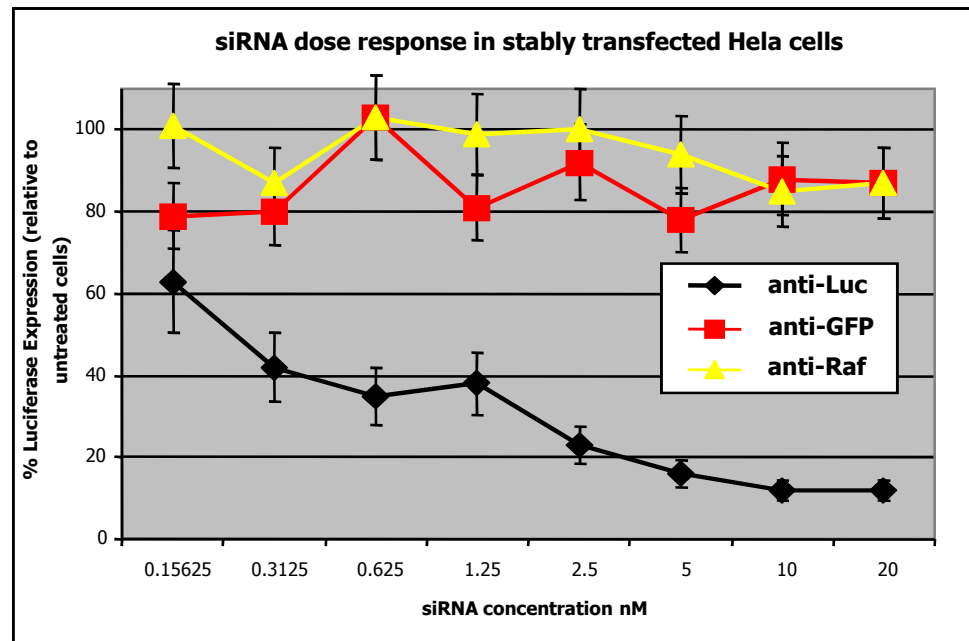
Results

siRNA Delivery

HeLa: Stably Transfected with GFP



HeLa: Stably Transfected with Luciferase



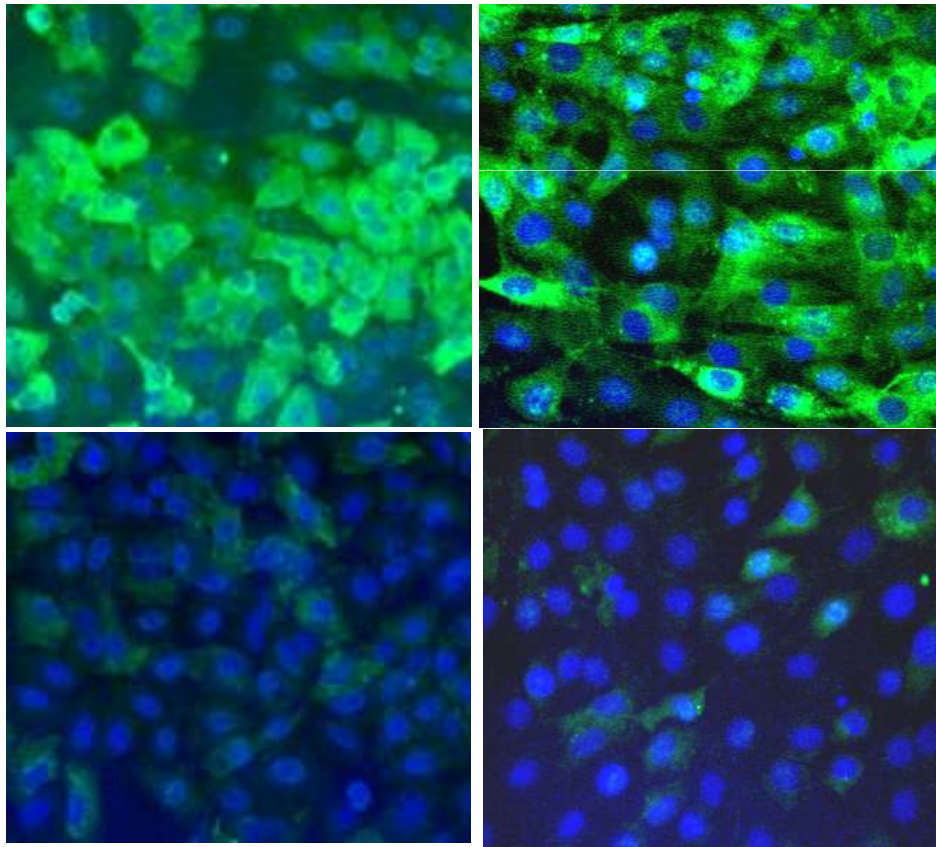
siRNADelivery

Targeted Gene: endogen GAPDH

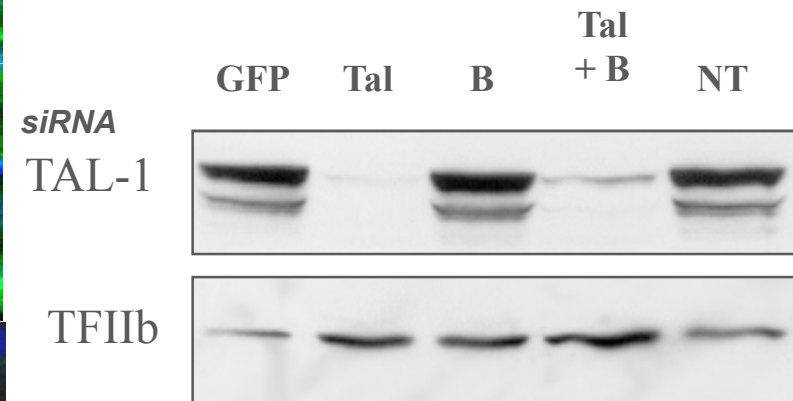
Cell lines:

A549 Human Carcinoma

NiH3T3 Mouse Fibroblast

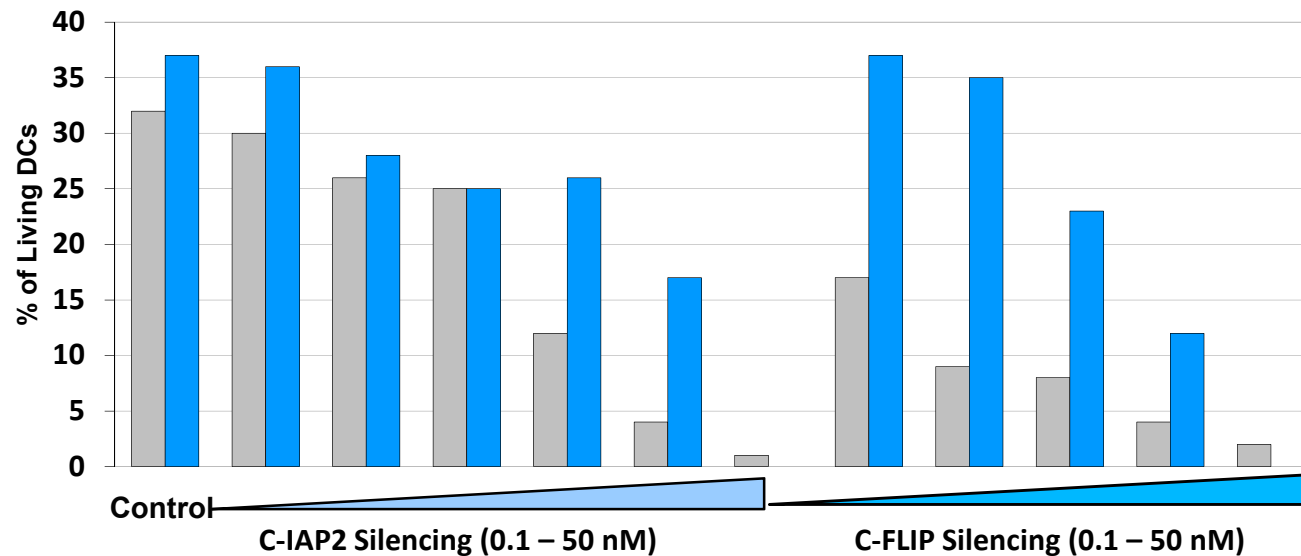


Primary HUVEC
Targeted Gene:
Transcription Factors



Dr. D. Matthieu, Montpellier, France

Magnetofection-based silencing experiment in primary dendritic cells



c-IAP2 and c-FLIP silencing using Magnetofection.

iDCs (grey bars) or DCHIV (blue bars) were transfected with control siRNA or increasing concentrations (0.1-50nM) of c-FLIP and c-IAP2 specific siRNA coupled to Magnetofection particles.



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Magnetofection™

RNAi

Silencing protein expression in **cell lines** with **Magnetofection™**

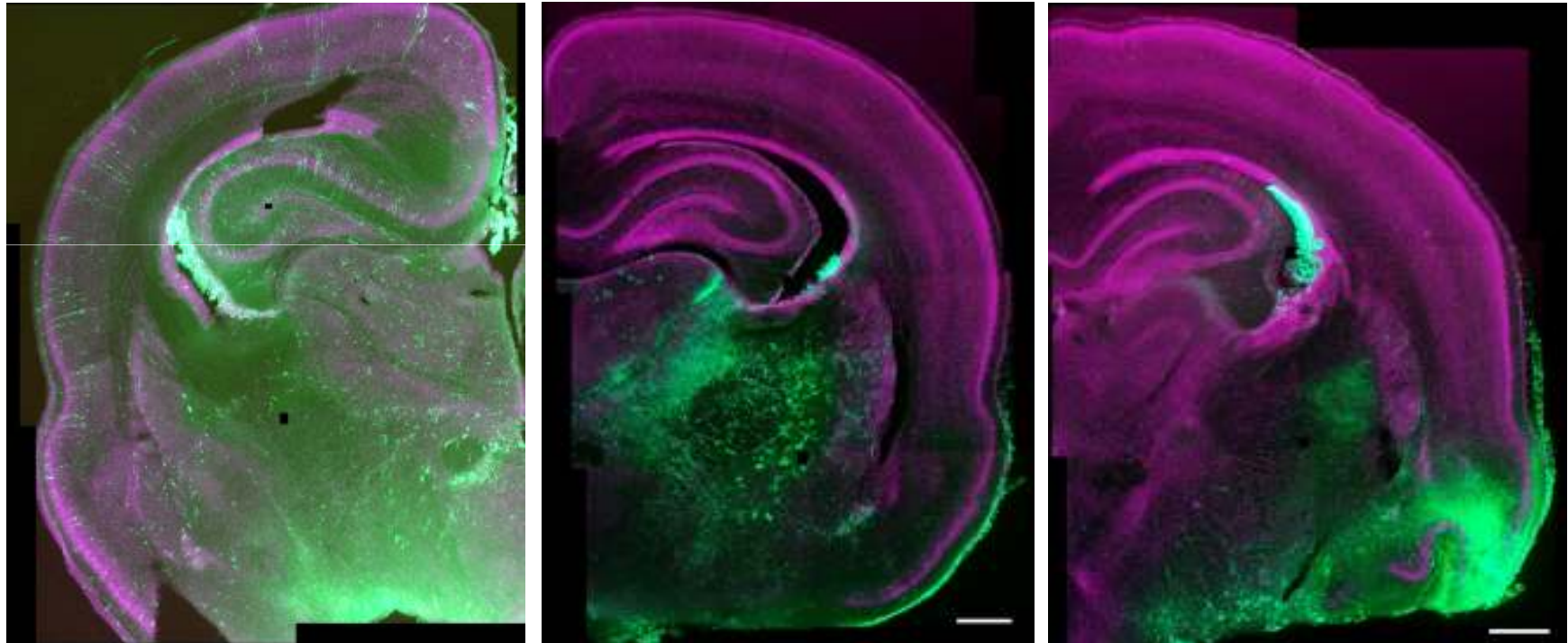
Origin	Cell line	description
Human	293, EBNA, T	embryonic
	A-293	Transformed human
	A431	carcinoma
	A549	carcinoma
	BEAS2B	epithelial
	H441	epithelial carcinoma
	HaCaT	keratinocyte
	HBL100	adenocarcinoma
	HCAEC	coronary artery endothelial
	HCT15, 116	adenocarcinoma
	HeLa	epithelial carcinoma
	Hep3B	carcinoma
	HepG2	hepatoma
	HLMVEC	lung microvascular endothelial
	HMEC-1	endotheliale
	HPAE	pulmonary artery endothelial
	HT1080	fibrosarcoma
	HT29	adenocarcinoma
	Jurkat	acute T lymphoma
	LNCaP	carcinoma
	MCF7	adenocarcinoma
	MeWo	melanoma
	Molt-4	T cell leukemia
	NCIH292, 82	carcinoma
	PC3	adenocarcinoma
	SaOS, SaOS-2	osteosarcoma
	SHSY5Y	neuroblastoma
	SUIT-2, S2-CP8	pancreatic adenocarcinoma
	T84	intestinal

Origin	Cell line	description
Mouse	3T6	fibroblast
	B16F10	melanoma
	C2C12	myoblast
	HT22	hippocampal
	L929	fibroblast
	M-1	renal cortical
	NIH-3T3	fibroblast
	NS20Y	neuroblastoma
	N2A	neuroblastoma
	MC3T3-E1	calvarial
Rat	3Y1	fibroblast
	A7r5	embryonic aorta smooth muscle
	C6	glioma
	L6	myoblast
	PC12	Pheochromocytoma
	RIE1-α5	intestinal epithelial
Hamster	BHK21	fibroblast
	CHO	epithelial
Feline	CRFK	epithelial
Monkey	CV1	fibroblast
Canine	MDCK	epithelial
Bovine	PT11	fibroblast

Silencing protein expression in primary cells with Magnetofection™

Origin	description
Human	Airway epithelium
	Aortic Endothelial (PAEC)
	Chondrocyte
	dendritic
	corneal epithelia
	Gastric Epithelial
	Fibroblast
	Gastric myofibroblast
	hematopoietic stem cells (from BM, LSK)
	Micro vascular endothelial (HMEC)
	Umbilical vein endothelial (HUVEC)
	Keratinocytes
	Myofibroblasts
	Nasal airway epithelium
	Stroma Endotrium
Mouse	Epithelial
	Fibroblast
	Hippocampal neurons
	Keratinocytes
Rat	Hippocampal neurons
	Umbilical vein endothelial
	vagal afferent neurons
Bovine	Aortic Endothelial (PAEC)
	Carotid Artery Smooth Muscle
Porcine	Airway epithelium
	Chondrocyte
Rabitt	Chondrocyte

- Unique solution to control, promote, assist and target viral transduction in vitro and in vivo (brain of rat embryos)



Lipophilic part:
Hydrophobic properties

Biodegradable linker:
Efficiency release
Low toxicity

Charged polyamine head:
Biomolecules interaction
Endosome buffering capacity

Chimera molecules
Tee-Technology
Triggered Endosomal Escape

Compaction of DNA in nanoparticles efficiently internalized by cells
Protection of nucleic acids against nucleases degradation

New & Innovative
transfection reagents

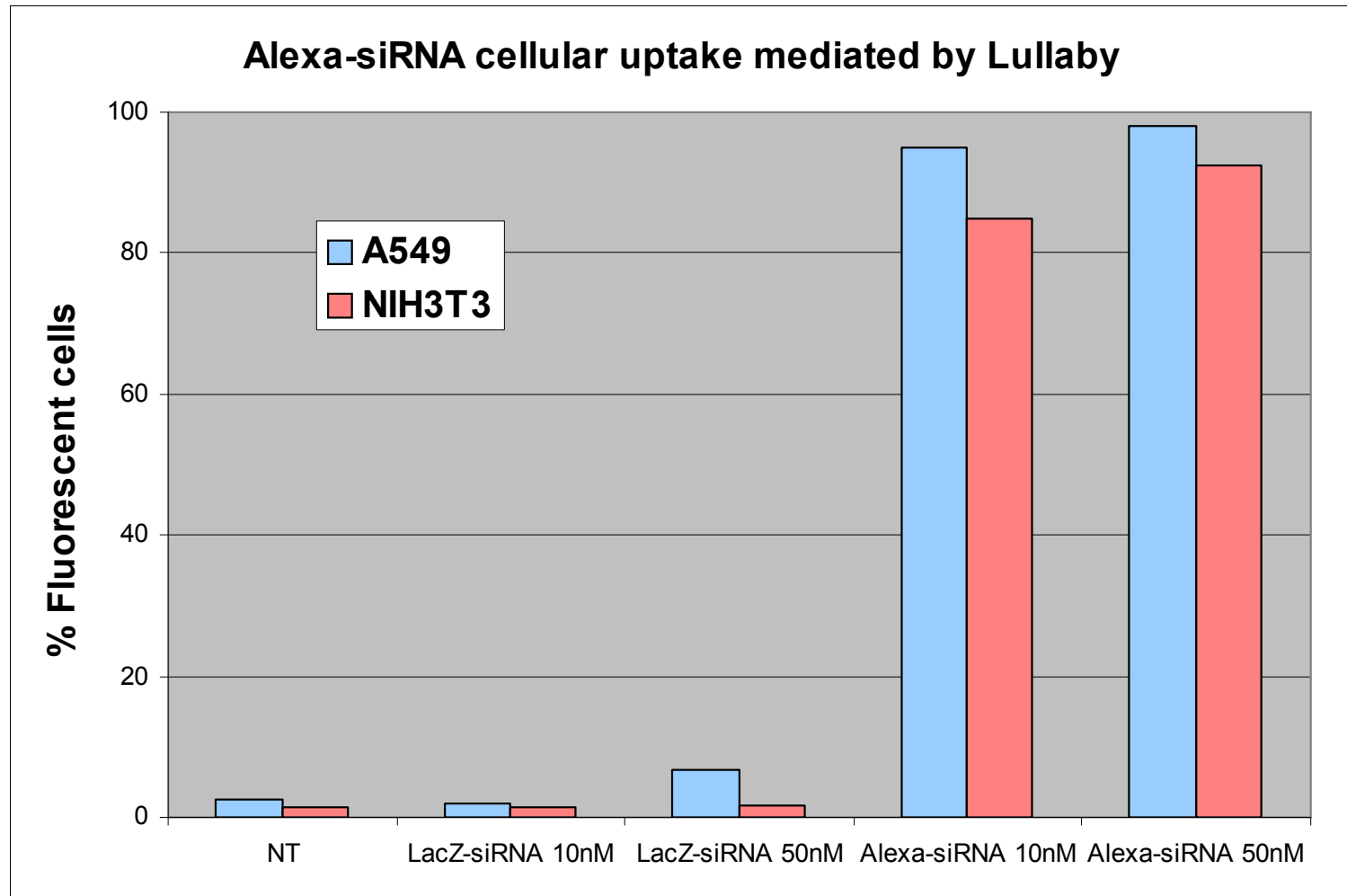


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Lullaby™

Results

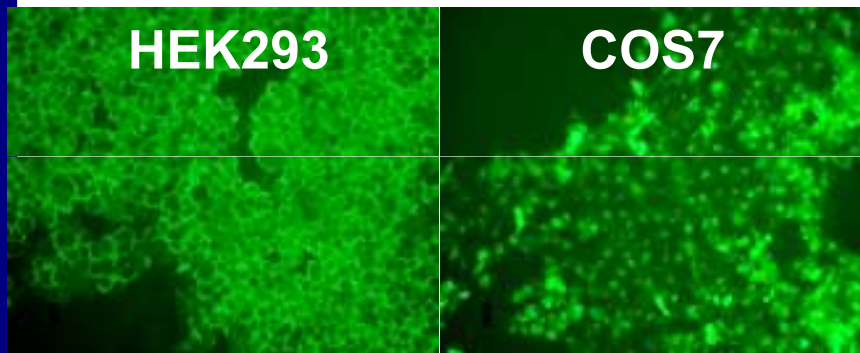
siRNA Delivery



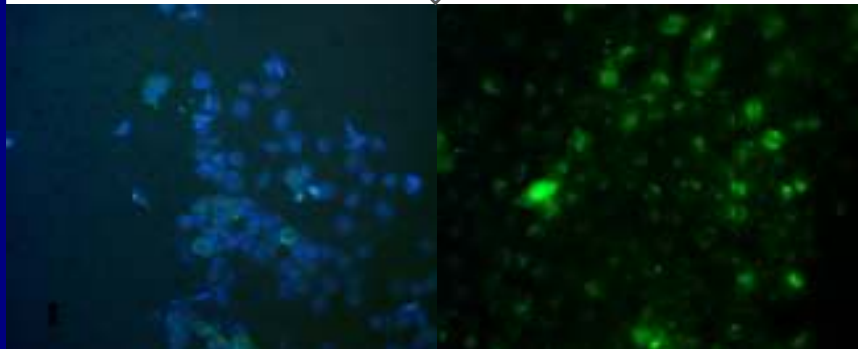
siRNA Delivery

Endogenous Target:

Anti GAPDH FITC

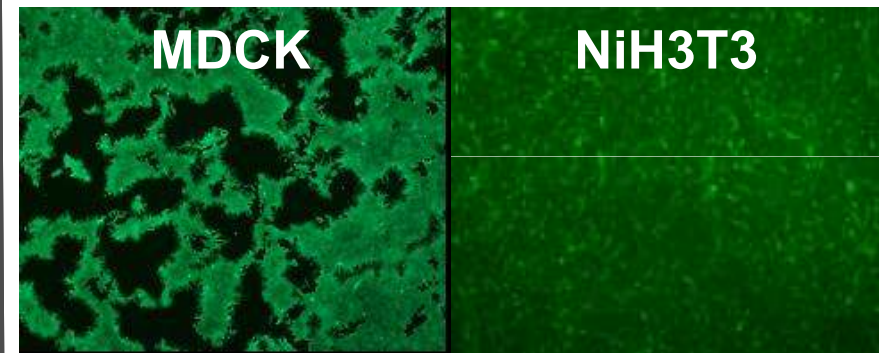


siGAPDH-20nM plus lullaby

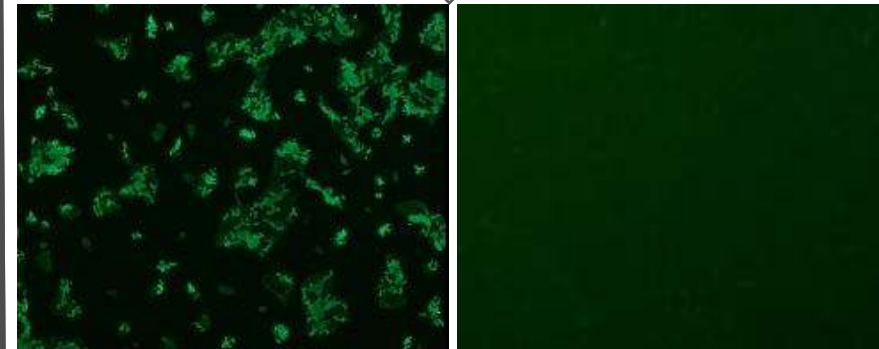


Exogenous Target:

Stably GFP transfected

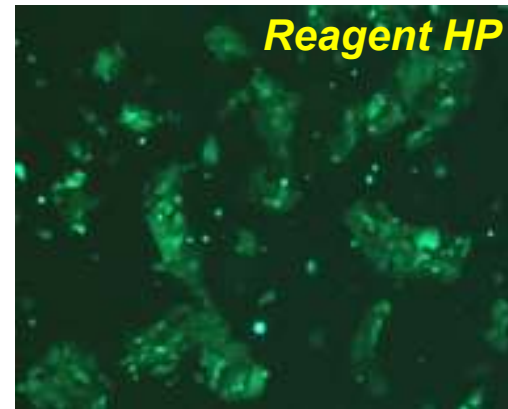
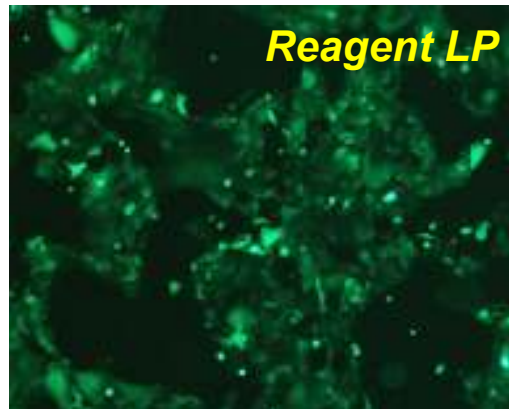
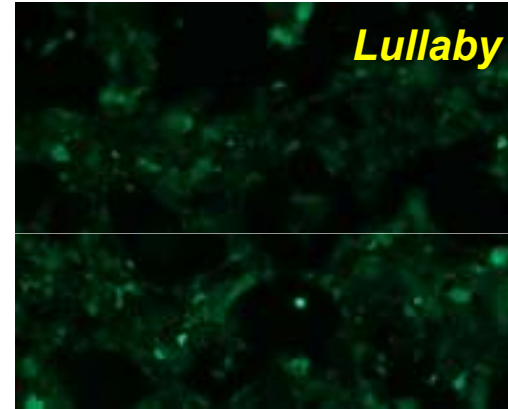
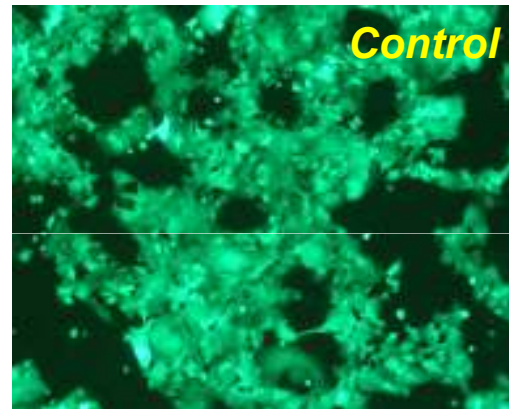


siGFP-20nM plus lullaby



Comparison of Gene Silencing in H441 cells

Targeted Gene: GFP – 20nM siRNA

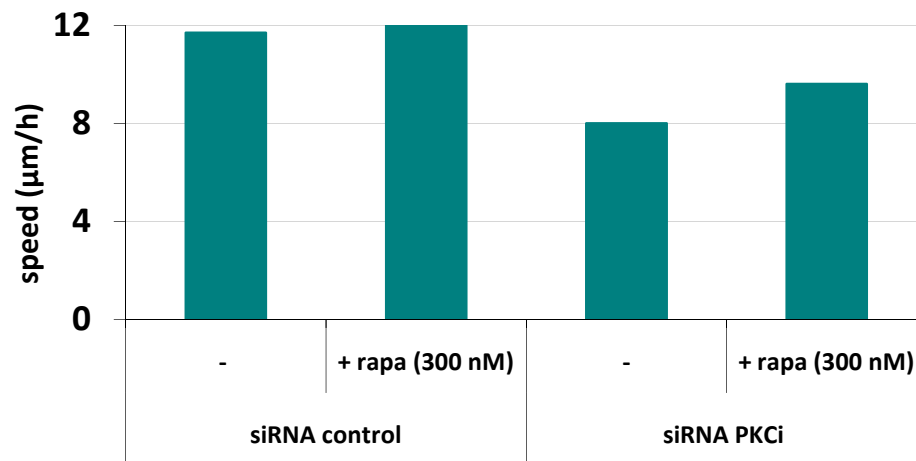


Successful transfection of locked nucleic acid with Lullaby in human embryonic stem cells

		LNA scramble	LNA-miRNA-145
Level of miRNA-145 at 144h		~20	~3.5
Percentage of differentiation at 96h		65	50
Relative mRNA level	OCT4	1	1.5
	KLF4	1	2

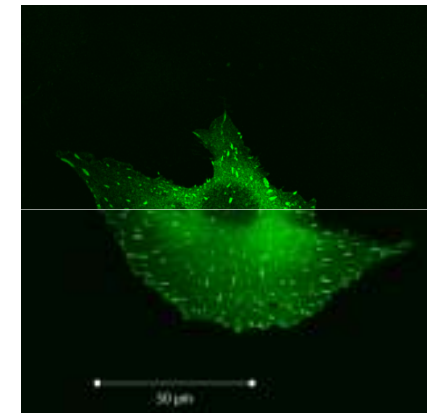
hESCs cells were transfected with the inhibitor LNA-miR-145 or the control using the Lullaby
 LNA-scr highlighted the necessity of miR-145 during hESC differentiation: i) LNA-miR-145 inhibition decreased endogenous miR-145 upregulation during differentiation. ii) miR-145 depletion by LNA-miR-145 delayed hESC differentiation as determined by qRT-PCR. iii) LNA-miR-145 elevated the target gene expression of OCT4 and KLF4.

Singel cell velocity in scratch-wound healing



Quantification of cell velocity in a scratch-wound healing.

NRK cells transfected with control siRNA or siPKCi in association with Lullaby were wounded 72h post-transfection and treated with 300 nM rapalogue. Healing was followed over 12h and single cell velocity in scratch-wound healing was measured.



NRK cell transfected with Lullaby and Paxillin construct
We are grateful to Professor Peter Parker for kindly providing this picture.

Silencing protein expression in various cell models with Lipofection

Origine	Cell line	description
Human	1207	carcinoma
	A-293	Transformed human
	A549	carcinoma
	BEAS2B	epithelial
	BHK-21	fibroblast
	H441	epithelial carcinoma
	HEK293, T, EBNA	ebryonic
	HeLa	epithelial carcinoma
	HepG2	Liver carcinoma
	H441	carcinoma
	Jurkat	acute T lymphoma
	K562	Myelogenous leukemia
	MCF7	adenocarcinoma
	MDAMB435	Breast Cancer
	MIA PaCa-2	carcinoma
	MRC5	Lung Fibroblasts
	NCI-H441	Lung adenocarcinoma
	SHSY5Y	neuroblastoma
	THP-1	promonocytic leukemia
	U2-OS	Osteosarcoma
U251	Glioblastoma	
U87	Glioma	

Origine	Cell line	description
Mouse	3T6	fibroblast
	B16F10, OVA	melanoma
	IC21	Macrophage
	M-1	renal cortical
	MEF	Fibroblast
	MIN6	pancreatic insulinoma
	N2A	neuroblastoma
	NIH-3T3	fibroblast
	Pre-B	lymphoma
	Rat	LM(tk-tTA)
NRK		fibroblast
PC12		Pheochromocytoma
Hamster	BHK21	fibroblast
	CHO, CHO-K1	epithelial
Monkey	COS-1, COS-7	fibroblast
	CV1	fibroblast
Canine	Vero E6	epithelial
Canine	MDCK	epithelial

Origine	Primary cell description
Human	Dermal Fibroblast
	Embryonic stem cells (hESC)
Mouse	Dermal Fibroblast
Rat	Biliary epithelial (BECs)
	Dorsal Root Ganglia neurons



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Thank you for your attention !



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