

Lullaby – siRNA[®] Transfection Reagent - Results

OZ Biosciences is delighted to announce the launching of a new siRNA transfection reagent: **Lullaby-siRNA[®]**. This lipid based transfection reagent is specifically designed for siRNA application. The cationic lipids formulation protects siRNA from extracellular degradation, transport them across cell membranes and efficiently release the siRNA into cells thanks to a triggered endosomal escape mechanism. In this way, **Lullaby[®]** efficacy allows studying gene silencing at very low dose of siRNA (< 10 nM) in a wide variety of cells, saving materials and time. **Lullaby[®]** formulation gives reliable higher gene silencing efficiencies in numerous cell types than any other transfection reagent.

Main **Lullaby[®]** features are:

1. Exceptional siRNA delivery efficiency and gene silencing (>90%)
2. Effective gene silencing at multiple siRNA concentrations including with low doses (<10 nM) – minimize the risk of off-target effects
3. Reliable and reproducible gene knockdown results
4. Powerful across a broad spectrum of cell lines and confluency (from 20 to 90%)
5. Outperform all other transfection reagents tested
6. Suitable for all siRNA applications: co-transfection, endogenous gene silencing & reverse transfection
7. Successful for siRNA, shRNA, dsRNA...
8. Compatible with and without serum-containing media & antibiotics
9. High cell viability - No cytotoxicity (biodegradable lipids)
10. Rapid and straightforward procedure (3-step)
11. Highly adapted to high-throughput siRNA screening

siRNA Mediated Gene Silencing

Lullaby- siRNA[®] Transfection Reagent is suitable for **siRNA**: small interfering RNA, **shRNA**: small hairpin RNA and **dsRNA**: double strand RNA. RNA interference is a powerful technique to knockdown gene expression in cells and organisms. This silencing effect constitutes a very helpful tool to study gene's function and a promising approach for new therapeutic treatments. Short RNA duplexes (siRNA, shRNA and dsRNA) are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein production. **Lullaby- siRNA[®]** Transfection Reagent introduces the siRNA duplexes in a variety of cells with a very high efficiency leading to exceptional knockdown effects with low doses of siRNA.

Cell Types

Lullaby- siRNA[®] Transfection Reagent is applicable and has been tested successfully on a variety of cells (see Table 1 page 2). If a particular cell type is not listed, this does not imply that **Lullaby[®]** is not going to work. An updated list of cells successfully transfected is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

Targeted Genes

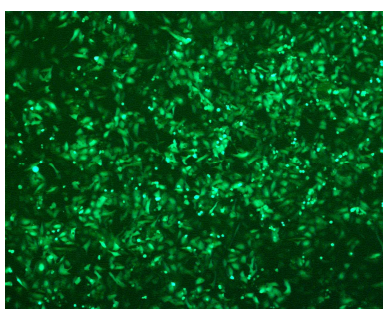
Gene knockdown mediated **Lullaby- siRNA[®]** Transfection Reagent has been tested successfully towards various targets. Gene silencing efficiency has been demonstrated in co-transfection (targeted genes: GFP and Lac Z), in stably transfected cells (targeted genes: GFP and Luciferase) and with endogenous targets such as GAPDH, Lamin and a kinase. Please consult our updated results available on the website: www.ozbiosciences.com.

Table 1: Example of cells successfully transfected with Lullaby- siRNA[®] Transfection Reagent.

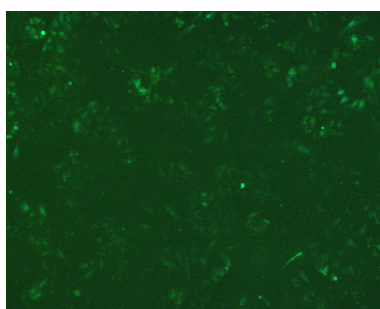
<i>Cell Lines</i>	<i>Cell Type</i>	<i>Species</i>
1207	Bladder carcinoma	Human
293, 293T	Kidney	Human
3T6	Embryonic Fibroblasts	Mouse
A549	Non-small cell lung carcinoma	Human
B16-F10	Melanoma	Mouse
BEAS-2B	Bronchial Epithelial	Human
BHK-21	Kidney	Hamster
CHO, CHO-K1	Ovary (epithelial like)	Chinese Hamster
COS-1	Kidney	Green Monkey
COS-7	Kidney	Green Monkey
CV-1	Fibroblast-like (Kidney)	Monkey
H441	Lung epithelial	Human
HEK293	Kidney	Human
HeLa, HeLa-S3	Cervix carcinoma	Human
M1	Kidney epithelial	Mouse
MCF-7	Breast adenocarcinoma	Human
MDCK	Kidney	Dog
MiaPaCa	Pancreatic carcinoma	Human
N2A	Neuroblastoma	Mouse
NIH-3T3	Fibroblasts	Mouse
PC-12	Pheochromocytoma (Adrenal)	Rat
U87	Glioma	Human
Vero	Kidney	Green Monkey

High Gene Silencing Efficiency Mediated by Lullaby[®]/ siRNA Complexes

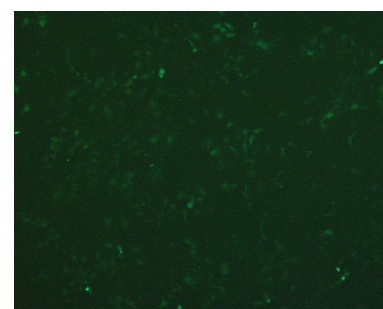
HeLa Cells stably transfected with GFP were treated with **Lullaby[®]** transfection reagent and siRNA (targeting GFP) as described in the instruction manual. Complexes were prepared with 1 μ L of **Lullaby[®]** and 5 nM (33.75 ng) or 3 μ L of **Lullaby[®]** and 25 nM (168.75 ng) siRNA. Cells seeded in 24-well plate were then transfected in 500 μ L of culture medium. GFP expression was monitored 72 h post-transfection by fluorescence microscopy.



Control



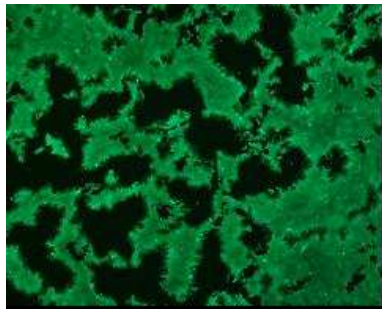
Lullaby[®] / 5nM siRNA



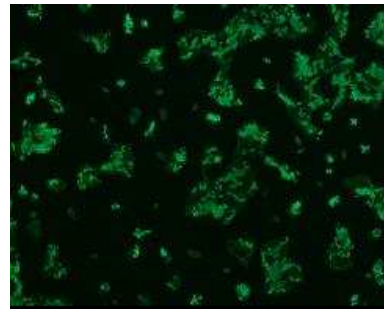
Lullaby[®] / 25nM siRNA

MDCK, 293T and CHO cells stably transfected with GFP were treated with **Lullaby[®]** transfection reagent and siRNA (targeting GFP) as described in the instruction manual. Complexes were prepared with 3 μ L of **Lullaby[®]** and 20 or 25 nM (168.75 ng) siRNA. Cells seeded in 24-well plate were then transfected in 500 μ L culture medium. GFP expression was monitored 72 h post-transfection by fluorescence microscopy.

MDCK

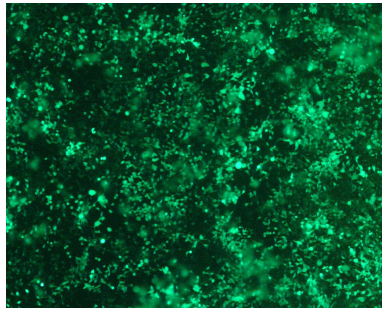


Control

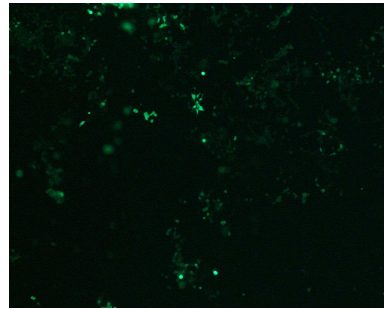


Lullaby® / 25nM siRNA

293T

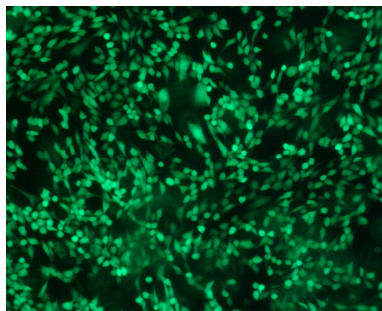


Control

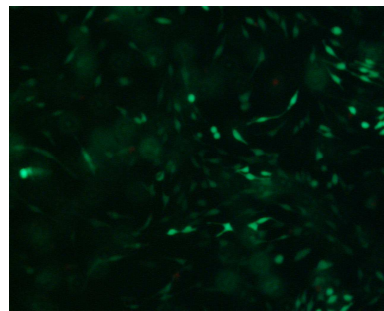


Lullaby® / 20nM siRNA

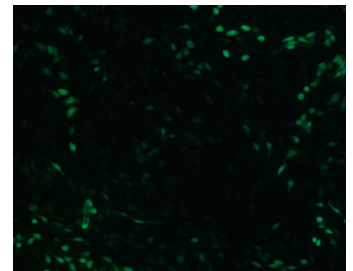
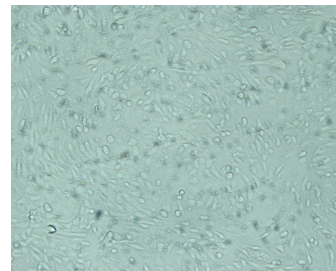
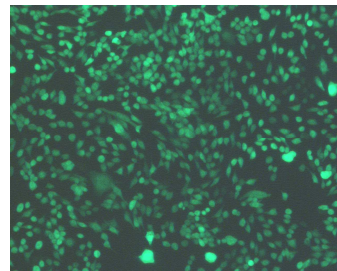
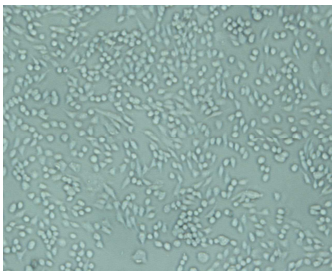
CHO



Control



Lullaby® / 20nM siRNA

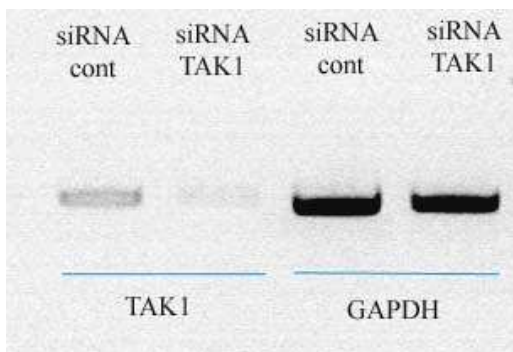


CHO Control

Control

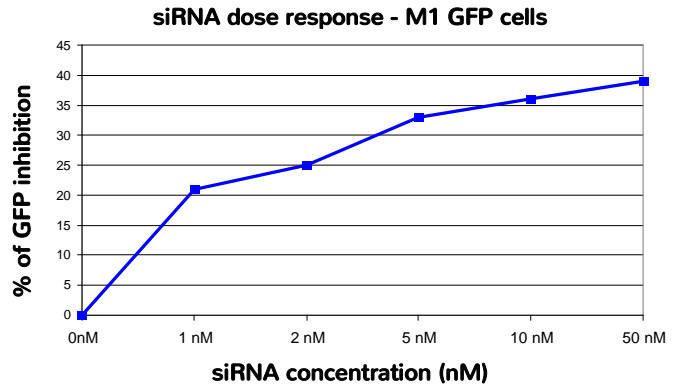
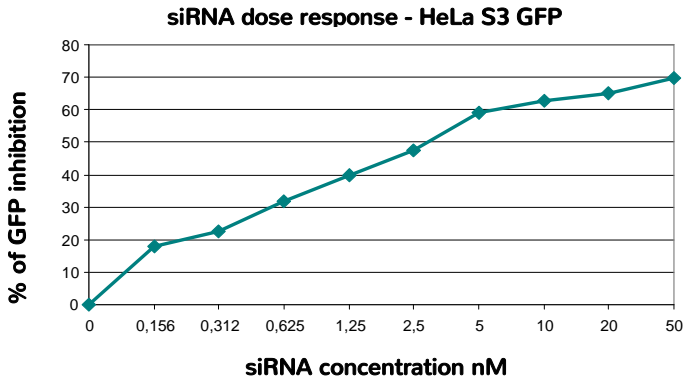
Lullaby® / 20nM siRNA

Lullaby® / 20nM siRNA



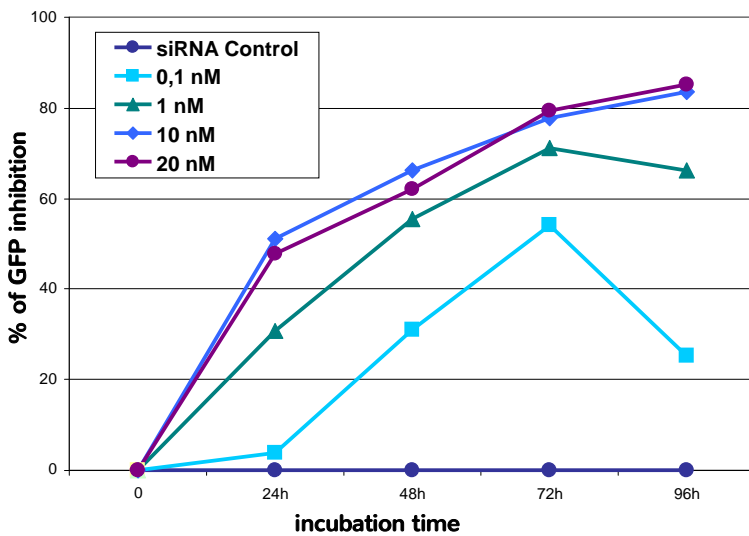
MIA PaCa-2 (Human pancreatic carcinoma cells) were assayed with **Lullaby®** transfection reagent and siRNA (targeting TAK1: TGF- activated kinase or control siRNA: GFP and GAPDH) as described in the instruction manual. Complexes were prepared with 8 μ L of **Lullaby®** and 50 nM siRNA. Cells seeded in 6-well plate were then transfected in 2 mL culture medium. TAK1 expression was monitored 72 h post-transfection by western blot using GAPDH as control. We are very grateful to Dr. V Giroux (INSERM-Marseille) for kindly sharing this result.

siRNA Dose Response



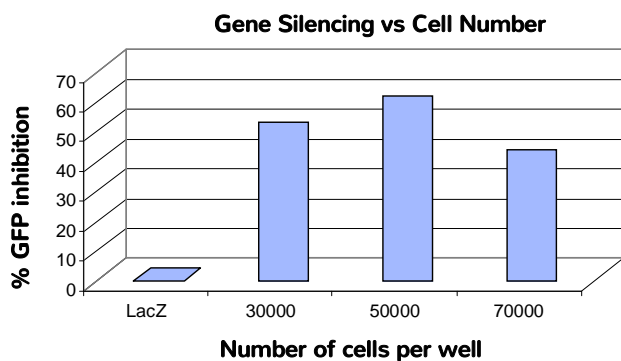
GFP-stably transfected **HeLa** and **M1 cells** were assayed with **Lullaby- siRNA[®]** transfection reagent and siRNA (targeting GFP) as described in the instruction manual. Cells seeded in 24-well plate were then transfected in 500 μ L culture medium. GFP expression was monitored 72 h post-transfection. Results show percentage of GFP inhibition.

Gene Silencing Time Course



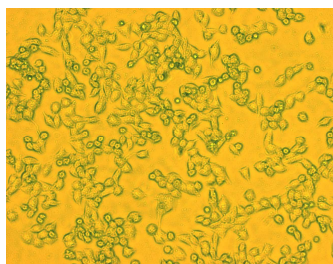
GFP-stably transfected **HeLa cells** were assayed with **Lullaby[®]** siRNA transfection reagent and siRNA as described in the instruction manual. Four siRNA concentrations, 0.1 nM, 1 nM, 10 nM and 20 nM, were monitored in function of time. Cells seeded in 24-well plate were then transfected in 500 μ L culture medium. GFP expression was recorded in function of post-transfection time.

Gene Silencing in Function of Cell Number

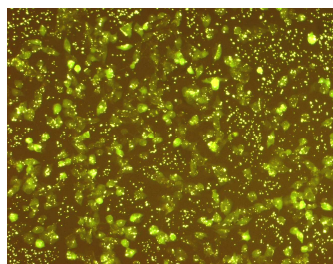


Various number of GFP-stably transfected **HeLa** cells per well were transfected in 24-well plates with 10 nM of siRNA associated with 2 μ L of **Lullaby[®]**. GFP expression was monitored 72h post-transfection.

siRNA-FITC Cellular Uptake



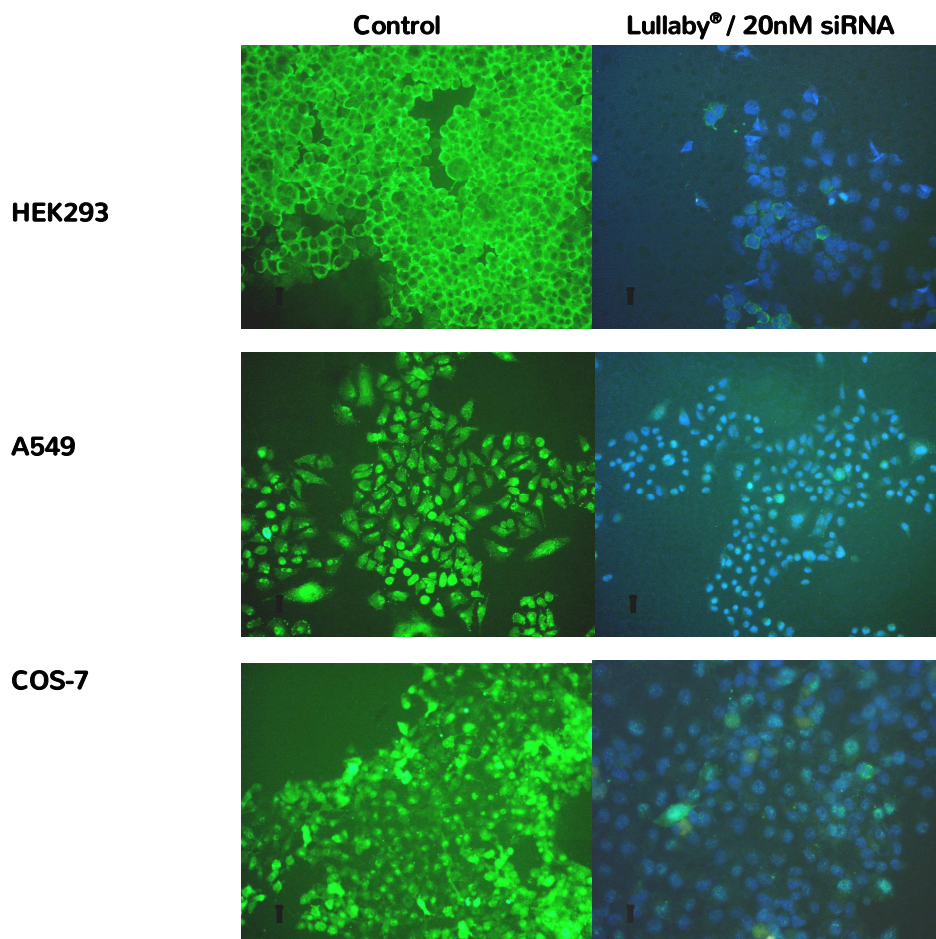
Control



siRNA-FITC uptake

MIA PaCa-2 (Human pancreatic carcinoma cells) were assayed with **Lullaby**[®] transfection reagent and fluorescently labeled siRNA. Cellular uptake was monitored 24 h post-transfection. We are very grateful to Dr. V. Giroux (INSERM-Marseille) for kindly sharing this result.

Lullaby[®] Efficiency to Target Endogenous Gene in various cells: GAPDH



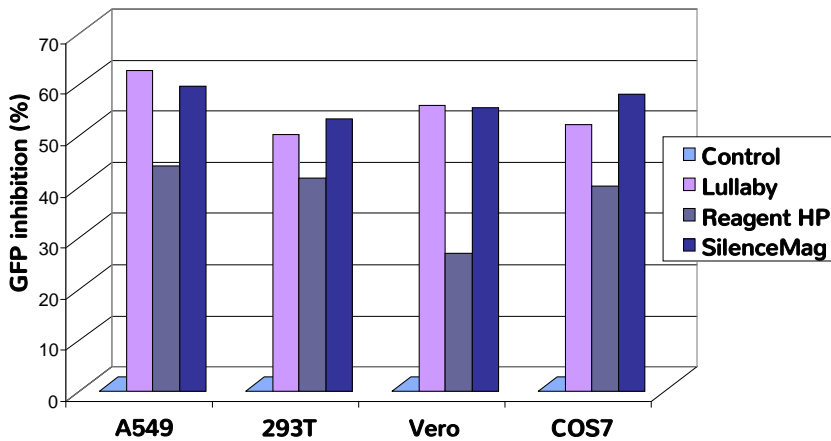
HEK293 (top), **A549** (middle) and **COS7** (bottom) cells were treated with **Lullaby**[®] siRNA transfection reagent and siRNA (targeting GAPDH gene). Complexes of **Lullaby**[®] and siRNA were performed as described in the instruction manual – [3 μ L of **Lullaby**[®] and 20 nM (135 ng) siRNA]. Cells were transfected in 24-well plate with a transfection volume of 0.5 mL. GAPDH expression was monitored 48 to 72 h post-transfection by immunocytochemistry. FITC-anti-GAPDH antibody and DAPI (label nucleus in blue) were detected by fluorescent microscopy.

Efficiency of **Lullaby- siRNA**® transfection reagent was also demonstrated by targeting other endogenous genes such as:

- TAK1 (TGF- activated kinase); gene knockdown results are shown above by western blot.
- GFP and luciferase in stably transfected cells.

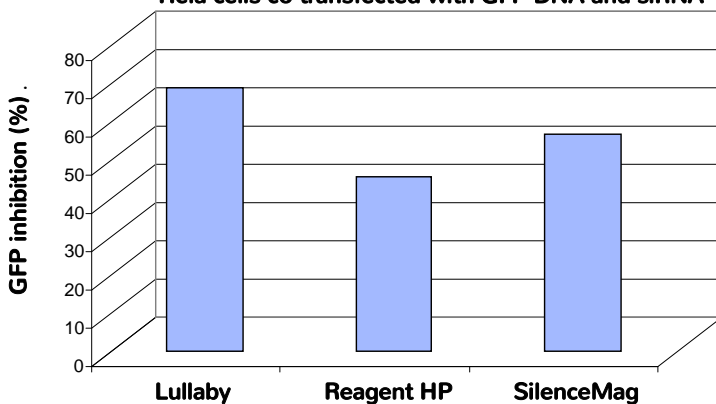
Lullaby® Efficiency in Various Cells Co-Transfected with Lac Z or GFP Gene

Lullaby Efficiency in Various Cells



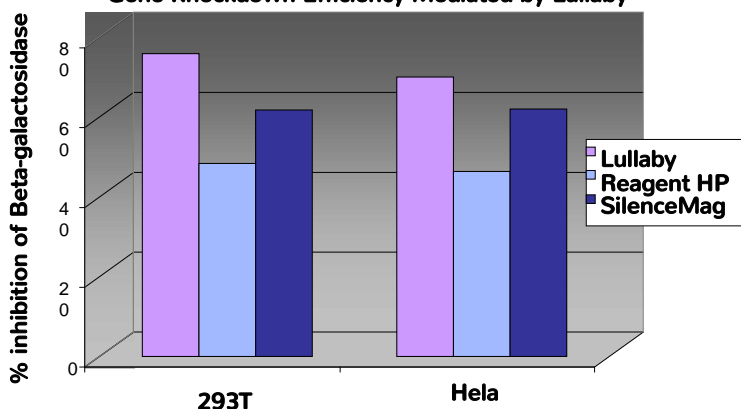
Various cells (A549, 293T, Vero and COS7) were transfected in 24-well plates with 20 nM of siRNA associated with 3 µL of **Lullaby**® or **SilenceMag** (cat # **SM11000**). Reagent HP was used according manufacturer's protocol. 4 hours later, cells were transfected with 0.5 µg of pGFP plasmid DNA complexed to 0.5 µL of **PolyMag** transfection reagent (cat # **PN30200**). GFP expression was monitored 72h post-transfection.

HeLa cells co-transfected with GFP-DNA and siRNA



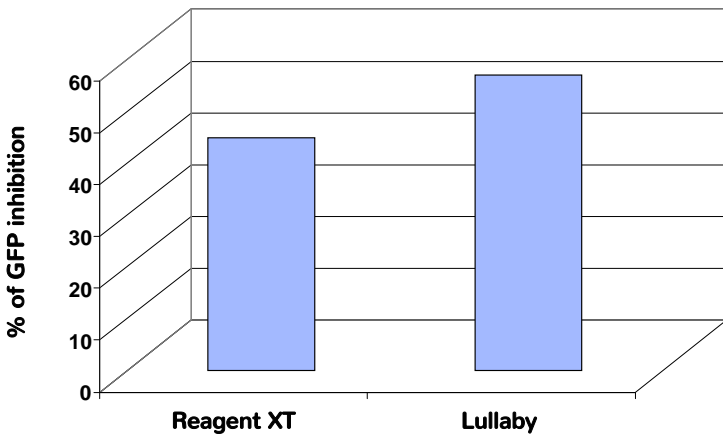
HeLa cells were co-transfected in 24-well plates with 1 µg of pGFP plasmid DNA complexed to 2 µL of **DreamFect** transfection reagent (**OZ Biosciences cat # DF41000**) and with 20 nM of siRNA associated with 3 µL of **Lullaby**® or **SilenceMag** (**OZ Biosciences cat # SM11000**) as described in the protocols. Reagent HP was used according manufacturer's protocol. GFP expression was monitored 48h post-transfection

Gene Knockdown Efficiency Mediated by Lullaby



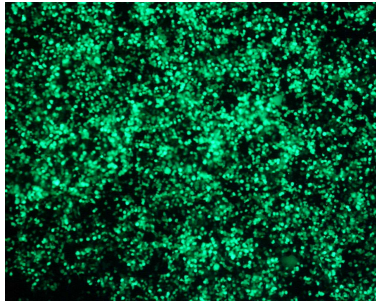
293T and HeLa cells were co-transfected in 96-well plates with 100 ng of pLacZ plasmid complexed to 0.2 µL of **DreamFect** transfection reagent (cat # **DF41000**) and with 10 nM of siRNA associated with 0.5 µL of **Lullaby**® or 0.5 µL of **SilenceMag** (cat # **SM11000**) as described in the protocols. Reagent HP was used according manufacturer's protocol. -Galactosidase expression was monitored 72h post-transfection using OZ Biosciences' -Galactosidase assay kit (catalog # **GO-10001**).

Cotransfection of pEGFP and siRNA in U87 cells

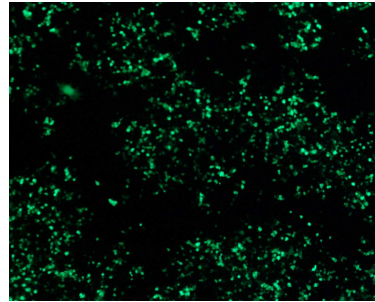


U87 cells (Human glioma) were assayed with **Lullaby[®]** reagent and siRNA (targeting GFP) as described in the instruction manual. Reagent XT was used according manufacturer's protocol. We are very grateful to Dr. Beclin (IBDM-Marseille) for kindly providing this result.

Control



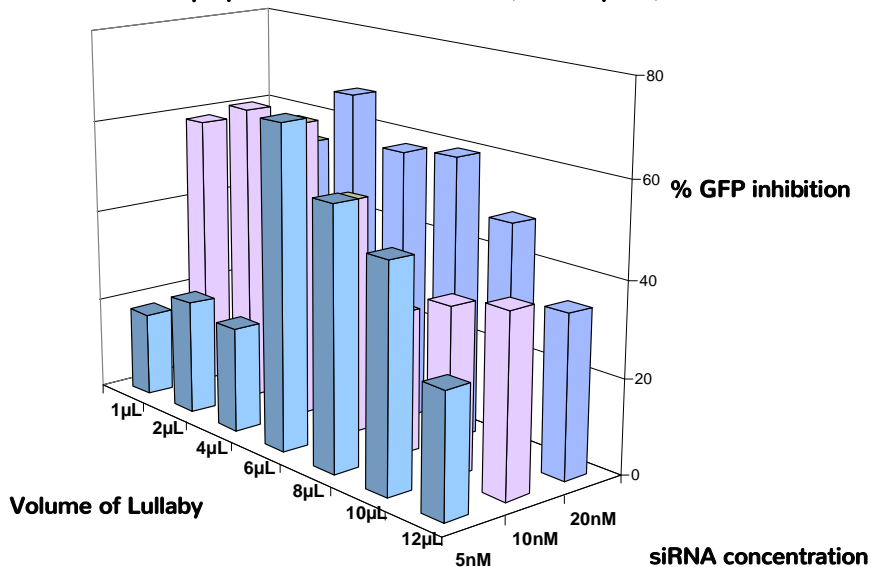
Lullaby[®] / 20nM siRNA



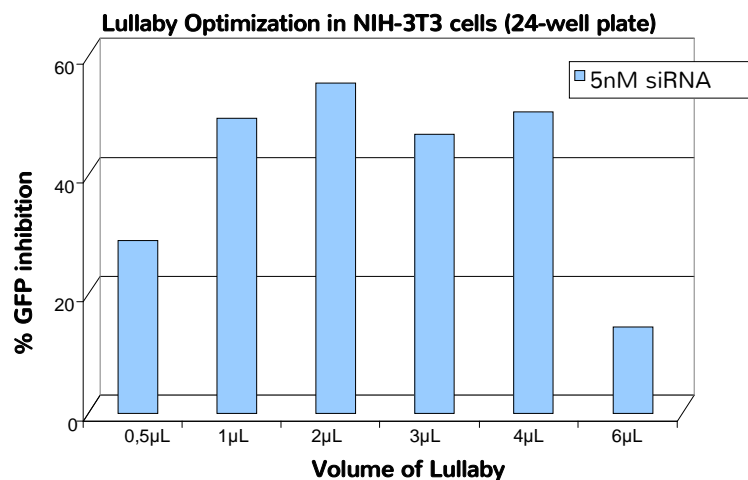
293T cells were co-transfected in 24-well plates with 1 µg of pGFP plasmid DNA complexed to 1 µL of **PolyMag** transfection reagent (*OZ Biosciences cat # PN30200*). 24 hours later, cells were treated with 20 nM of siRNA associated with 3 µL of **Lullaby[®]**. GFP expression was monitored 72h post-transfection.

Lullaby[®] Optimization in 12-well Plate

Lullaby Optimization in MDCK cells (12 well plate)

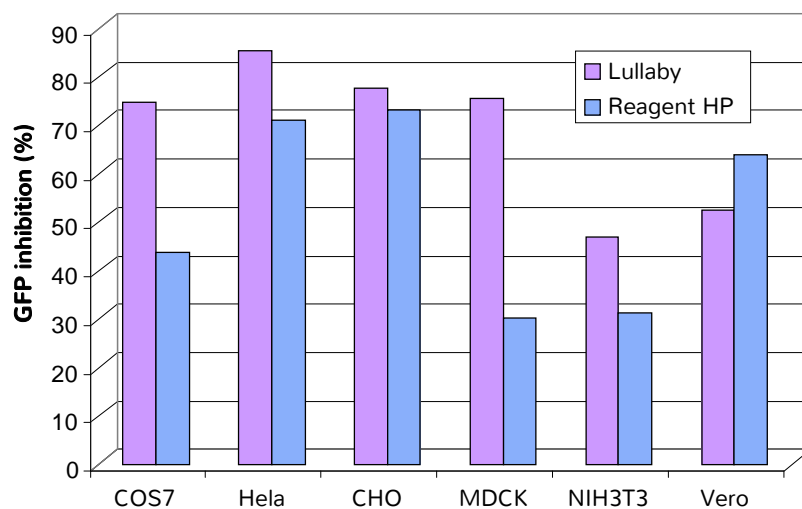


Lullaby® Optimization in 24-well Plate

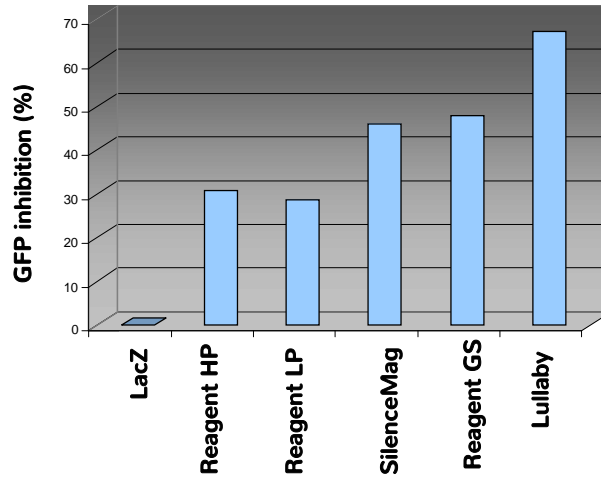


Comparison of Lullaby® Gene Silencing Efficiency with Other siRNA Reagents

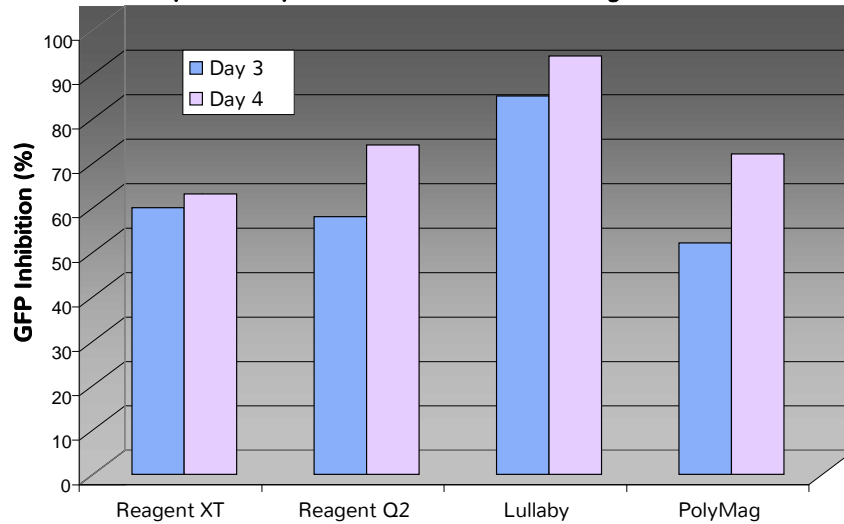
Various GFP-stably transfected cells (COS7, HeLa, CHO, MDCK, NIH3T3, U87, Vero, M1 and H441) were transfected in 24-well plates with 10nM of siRNA associated with 2µL of **Lullaby®** or **SilenceMag (cat # SM11000)** or **PolyMag cat # PN30200**. Reagents HP, LP, GS, XT, and Q2 were used according manufacturer's protocol. GFP expression was monitored 72h post-transfection.



Comparison of gene knockdown on HeLa cells

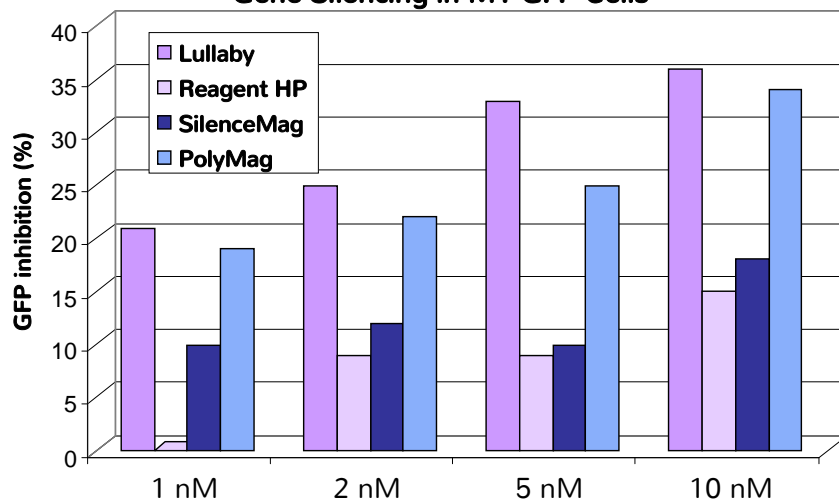


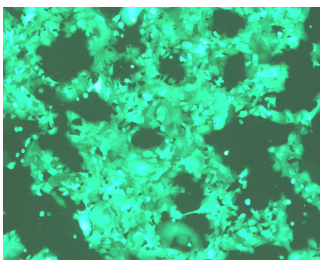
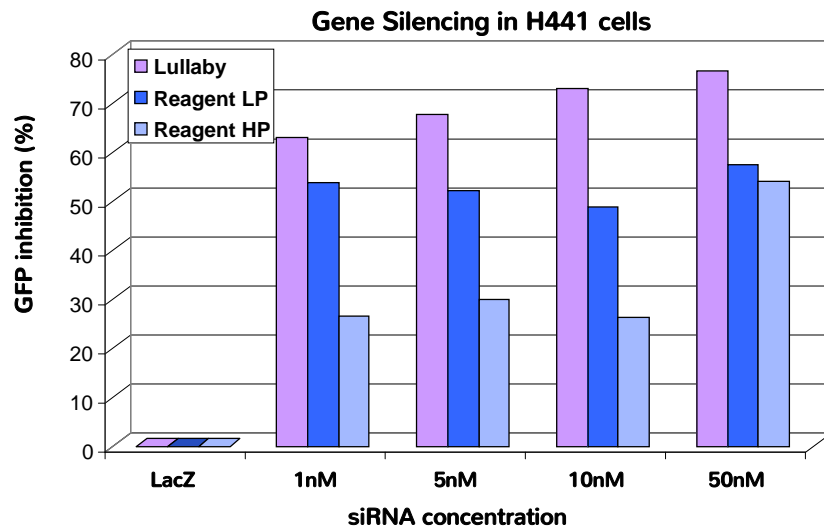
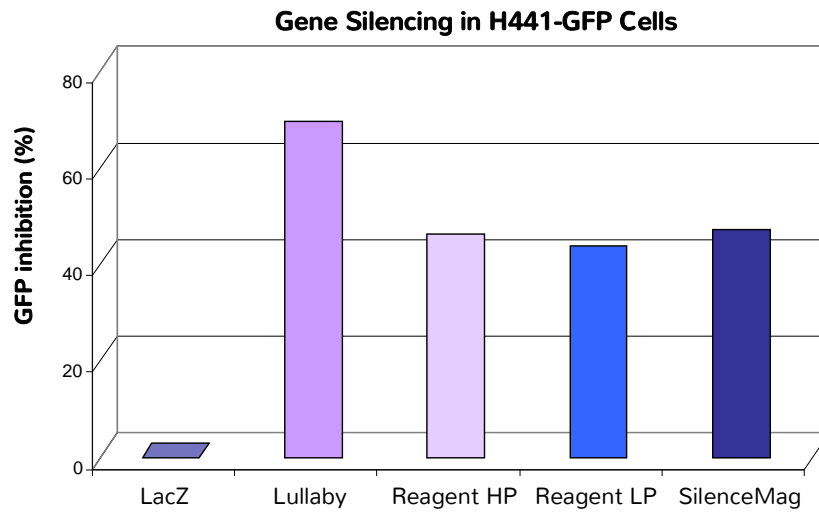
Lullaby efficiency versus other transfection reagents in U87 cells



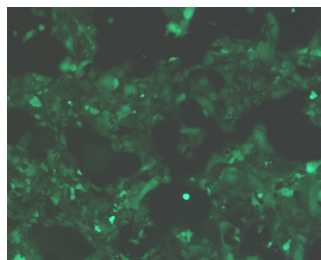
We are very grateful to Dr.Beclin (IBDM-Marseille) for kindly providing this result.

Gene Silencing in M1-GFP Cells

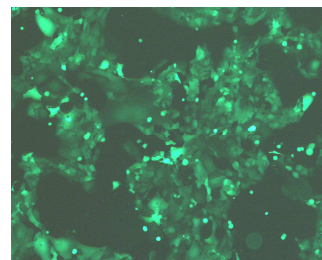




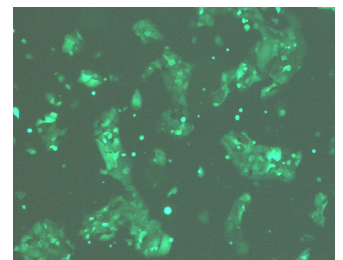
Control



Lullaby®

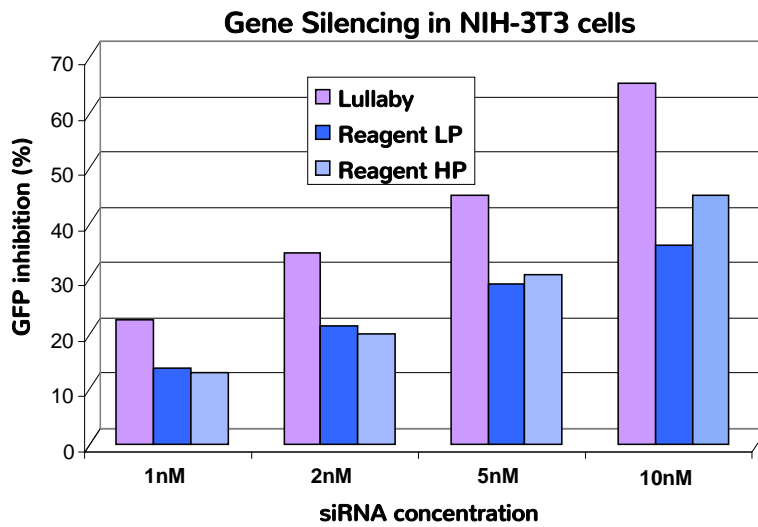


Reagent LP



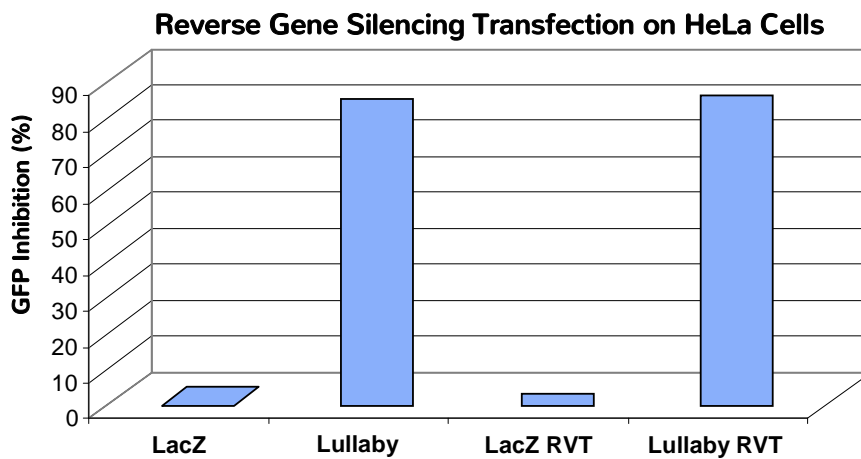
Reagent HP

H441-GFP cells were transfected in 24-well plates with 20 nM of siRNA and with 3µL of **Lullaby®** or other transfection reagents. Reagents HP and LP were used according manufacturer's protocol. GFP expression was monitored 72h post-transfection.



Lullaby® Gene Silencing by reverse transfection

HeLa-GFP cells were reverse transfected in 24-well plates with 10nM of siRNA associated with 2 μ L of **Lullaby®**. GFP expression was monitored 48h post-transfection by FACS. Briefly, siRNA / Lullaby complexes were added directly in an empty well of a 24-well plate in serum-free medium as described in the instruction manual. Then, 50,000 cells prepared in culture medium containing serum and antibiotics were added per well onto the preformed complexes.



RVT means reverse transfection

Bibliographic References

Please consult our list of references available on the website: www.ozbiosciences.com.