Thermo Scientific
SMARTchoice shRNA

customized lentiviral vectors

SMARTchoice shRNA

- Novel selection tool to easily identify best promoter
- Select the optimal SMARTchoice promoter and reporter for specific cells
- Increased expression improves shRNA functionality

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make the obvious choice for successful gene silencing

Thermo Scientific SMARTchoice shRNA

Effectual silencing of gene expression using vector-based RNAi depends on several factors, including the design of the shRNA, accurate processing to achieve the desired sequence and efficient loading of the guide strand into RISC. The level of shRNA vector expression once delivered into the cell is also a critical, but often underestimated, factor. Promoter activity can fluctuate from cell to cell, resulting in differing shRNA expression levels, thus varying the extent of gene knockdown that can be achieved (Figure 1).

To fully harness the utility of lentiviral vector approaches in shRNA-mediated gene silencing, careful attention must be paid to the choice of promoter controlling its expression. Inefficient promoter activity due to varying cellular and biological contexts results in sub-optimal knockdown and can be misinterpreted as poor shRNA functionality.

The Thermo Scientific SMARTchoice shRNA platform saves you time and money by allowing you to make informed decisions. First, evaluate activity of multiple promoters in the target cells of interest. Next, order Thermo Scientific SMARTvector 2.0 gene-specific lentiviral shRNA and controls containing the desired SMARTchoice promoter and reporter.

- SMARTchoice shRNA Promoter Selection Plate allows straightforward identification of the optimal promoter in the cells of interest.
- Flexibility to order gene-specific SMARTvector shRNA constructs with a choice of seven different SMARTchoice promoters and two fluorescent reporters.
- SMARTvector 2.0 targeting sequences are based on advanced microRNA-adapted shRNA designs for specific silencing and minimal off-target effects.

Strength of promoter activity is different from one biological context to another and can affect experimental outcomes.

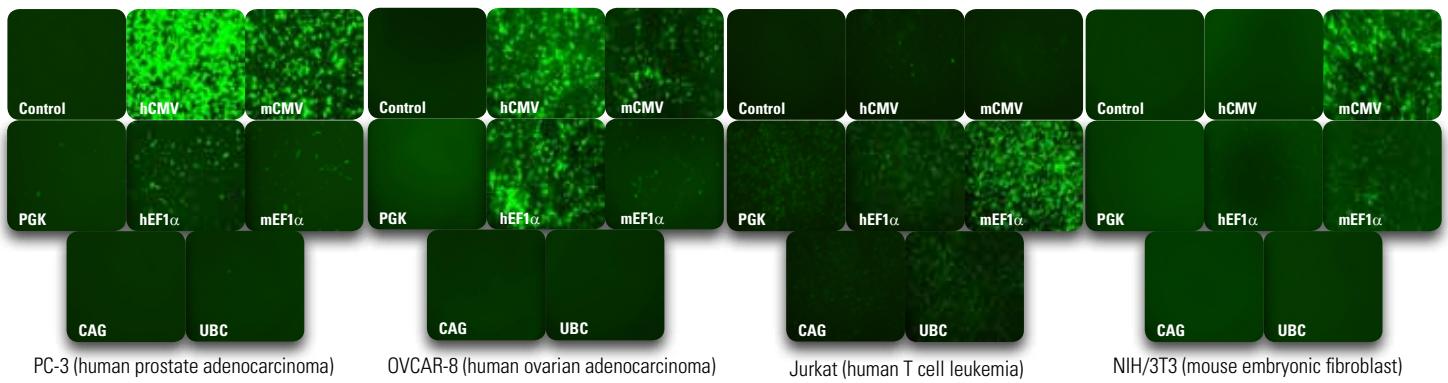


Figure 1. Promoters driving the expression of TurboGFP have differential activity from cell to cell.

Multiple cell lines were transduced with SMARTvector Non-targeting shRNA Control Particles which delivered vectors expressing TurboGFP driven by seven different cellular and viral promoters. Control wells contained untreated cells. Fluorescent images were obtained 72 hours post-transduction. Visual assessment of TurboGFP expression in human PC-3, human OVCAR-8, human Jurkat and mouse NIH/3T3 cells, shows a varying degree of promoter activity across cell lines.

Make informed decisions in the design of gene silencing experiments using the Thermo Scientific SMARTchoice shRNA Promoter Selection Plate.

Gene knockdown experiments can now be planned with practical observation of the optimal promoter choice for your cells. In a single matrixed experiment, the SMARTchoice Promoter Selection Plate enables the evaluation of seven different promoters at a range of multiplicities of infection (MOIs) in your cells of interest.

SMARTvector Non-targeting shRNA Controls (NTCs) with seven well-characterized cellular promoters driving the expression of TurboGFP (Evrogen, Moscow, Russia), are packaged into lentiviral particles and concentrated. High-titer NTC lentiviral particles are arrayed in a convenient 96-well format (Figure 2) in duplicate serial dilutions so that multiple MOIs can be tested.

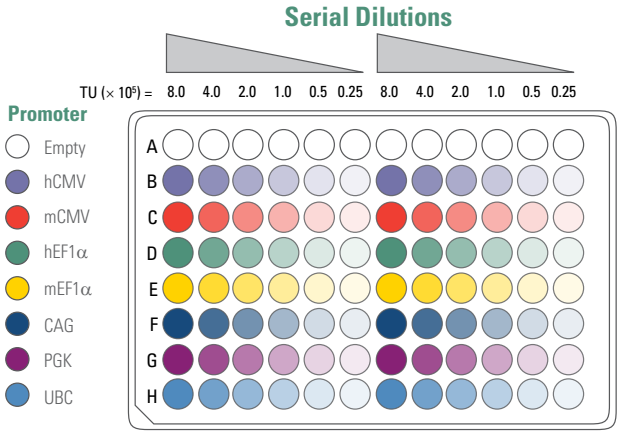


Figure 2. The 96-well Thermo Scientific SMARTchoice shRNA Promoter Selection Plate layout.

hCMV: human cytomegalovirus
mCMV: mouse cytomegalovirus
hEF1α: human elongation factor 1α
mEF1α: mouse elongation factor 1α
CAG: chicken β actin hybrid promoter
PGK: mouse phosphoglycerate kinase
UBC: human ubiquitin C

The SMARTchoice shRNA Promoter Selection Plate enables a straightforward qualitative assessment of promoters that actively drive expression.

After transduction of cells, wells can be quickly and visually evaluated for TurboGFP intensity using fluorescence microscopy, high-content imaging or detection using a microplate reader.

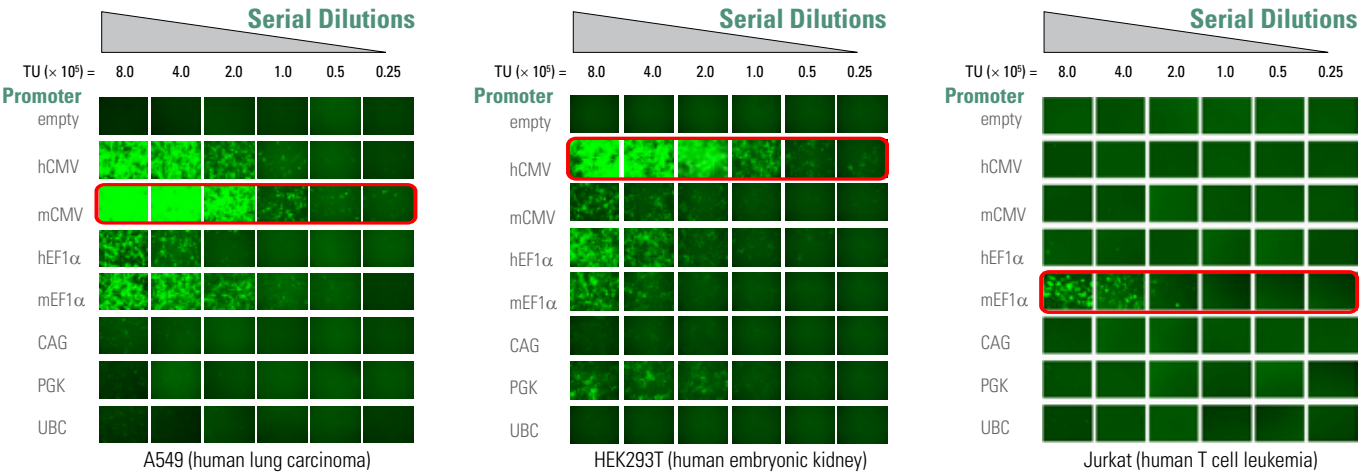


Figure 3. Human A549, HEK293T and Jurkat cells were transduced with concentrated lentiviral particles arrayed in the Thermo Scientific SMARTchoice Promoter Selection Plate.

TurboGFP expression was assessed by fluorescence microscopy 72 hours post-transduction. Images clearly demonstrate that the most functional promoter in A549 cells is mCMV, whereas the hCMV promoter is most active in HEK293T, and the mEF1α promoter is most active in Jurkat cells. TU = transducing unit.

Make the SMARTchoice and select the right promoter to increase gene silencing in biologically relevant models.

Identifying the optimal promoter for your cells of interest using the SMARTchoice shRNA Promoter Selection Plate allows for increased shRNA functionality and performance, resulting in successful knockdown of your target gene **in many difficult cells, including rodent, primary, embryonic stem and neuronal cells.**

Relative knockdown of shRNA-transduced NIH/3T3 cells.

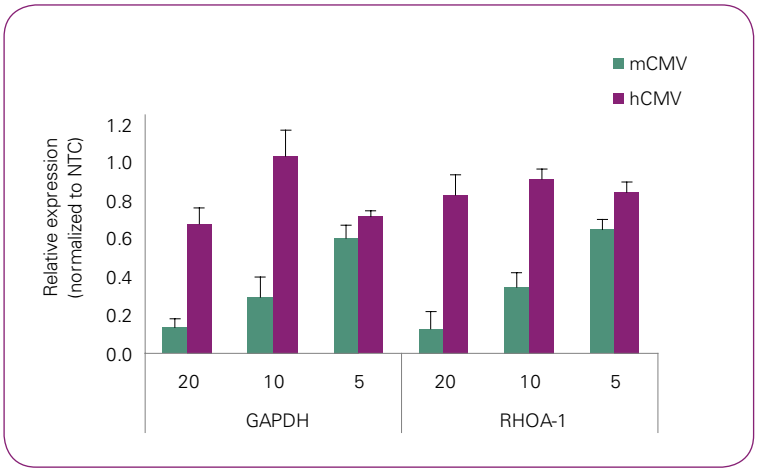


Figure 4. Selection of the mouse CMV promoter for mouse NIH/3T3 cells using the Thermo Scientific SMARTchoice Promoter Selection Plate results in greater gene silencing.

Gene silencing experiments targeting mouse *GAPDH* and *RHOA* genes demonstrate that shRNAs expressed by the mCMV promoter are significantly more effective at silencing the target gene in NIH/3T3 cells when compared to the same shRNAs expressed by the hCMV promoter. Cells were transduced with lentiviral particles at MOI = 20, 10 and 5; gene expression levels were detected and normalized to controls 72 hours post-transduction using Thermo Scientific Solaris qPCR Gene Expression Assays and Master Mix.

Relative knockdown of shRNA-transduced A549 cells.

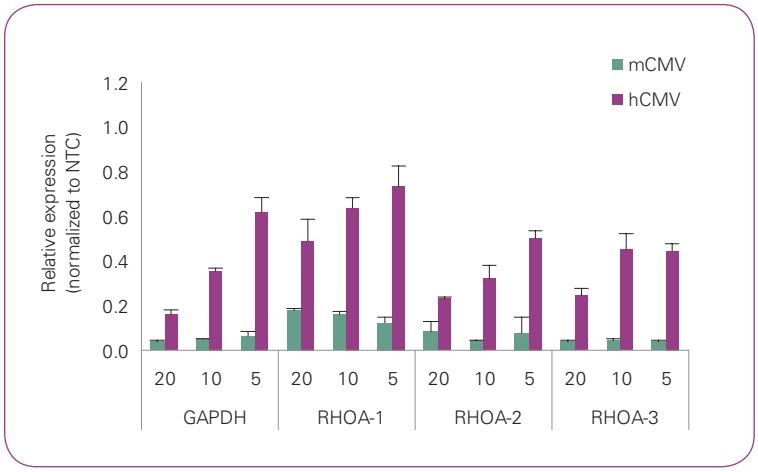


Figure 5. shRNAs expressed from the mouse CMV promoter result in significant gene silencing in human A549 cells.

One shRNA targeting human *GAPDH* and three shRNAs targeting human *RHOA* were delivered individually to A549 cells with expression driven by either the mCMV or hCMV promoter. Results demonstrate that all four shRNAs expressed from the mCMV promoter significantly silenced *GAPDH* or *RHOA* compared to the same shRNAs driven by the hCMV promoter. Cells were transduced with lentiviral particles at MOI = 20, 10 and 5; gene silencing was measured using Solaris™ qPCR Gene Expression reagents 72 hours post-transduction.

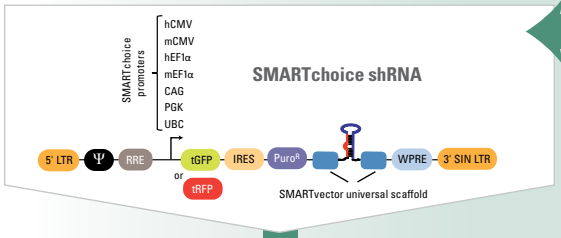
Begin by selecting the ideal promoter for your RNAi experiment using the SMARTchoice shRNA Promoter Selection Plate, then order gene-specific and control shRNAs as concentrated, transduction-ready lentiviral particles customized for your cells of interest.

Identify the optimal promoter for your cells of interest with the transduction-ready SMARTchoice Promoter Selection Plate. Next, place an online order for SMARTvector 2.0 shRNA constructs containing your choice of promoter and fluorescent reporter and save the extensive time, labor and money required for production of high-titer lentiviral particles. Benefit from our internal, quality-controlled process of cloning and packaging your customized vector into lentiviral particles using the Thermo Scientific Trans-Lentiviral packaging technology for enhanced biosafety.

Thermo Scientific Process

1. SMARTchoice Promoter Selection Plate
- Arrayed viral particles
- Normalized TUs

4. shRNA vector construction
- SMARTchoice promoter and fluorescent reporter of choice

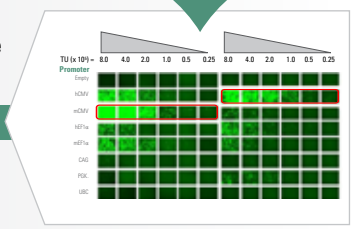


5. Lentiviral particle production
- High-titer particles
- Transduction-ready

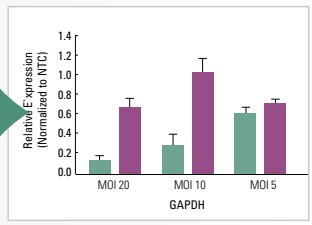
Researcher Experiment

2. Transduce cells of interest
- Cells specific to your application

3. Select optimal promoter
- Based on fluorescence intensity



6. Perform gene silencing experiments
- Utilizing matched controls



The SMARTchoice platform extends the advantages of the innovative SMARTvector 2.0 design for specificity and functionality by adding greater flexibility.

SMARTvector lentiviral shRNAs are designed using microRNA scaffold-specific attributes incorporating bioinformatic strategies to reduce off-target gene silencing events. SMARTvector shRNA designs are integrated into a lentiviral delivery system for efficient processing via the endogenous RNAi pathway.

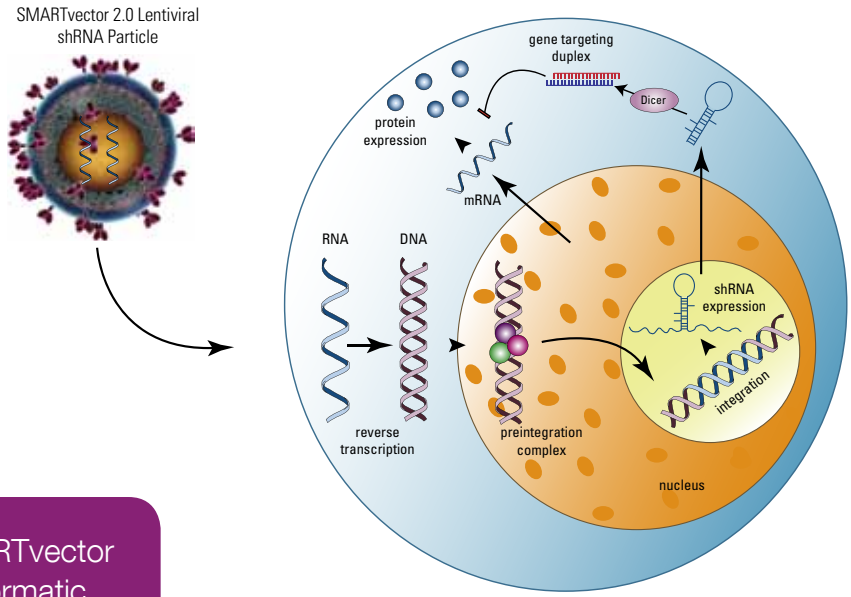


Figure 6. Lentiviral shRNA approaches rely on active and consistent expression of gene silencing triggers capable of entering the RNAi pathway.

Learn more about the advanced SMARTvector 2.0 shRNA design features and bioinformatic strategies to minimize off-target effects at:

www.thermoscientific.com/SMARTvector

For further information visit
www.thermoscientific.com/SMARTchoice

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