openbiosystems.com info@openbiosystems.com
6705 Odyssey Drive /P/ 888.412.2225
Huntsville, AL 35806 /F/ 256.704.4849

Product: Expression Arrest™ eGFP control shRNA vector

Catalog #: RHS1702

Product Description

The laboratory of Dr. Greg Hannon at Cold Spring Harbor Laboratory (CSHL) has created an RNAi Clone Library comprised of multiple short-hairpin RNAs (shRNAs) specifically targeting annotated human and mouse genes. The shRNA Library permits rapid, cost efficient, loss-of-function genetic screens and rapid tests for genetic interactions to be performed in mammalian cells. Each shRNA has been cloned and sequence verified to ensure a match to the target gene.

The eGFP shRNA (Figure 1) is a positive control designed against the enhanced GFP reporter (BD Biosciences Clontech Cat. # 6085-1; GenBank Accession No: pEGFP U476561). This construct has been validated to produce ≥ 75% decrease in GFP fluorescence. The eGFP shRNA sequence is provided in pSM2, a murine stem cell viral and recombinational cloning vector from the laboratory of Dr. Steve Elledge (Harvard). The eGFP shRNA is expressed under the control of the U6 promoter.

Each vial of the eGFP shRNA vector is shipped at a concentration of 0.5μg/μl in a total volume of 20μl of Tris Buffer (pH 8.5), thus providing a total amount of 10μg vector DNA.

shRNA vector storage

The vector DNA is shipped in a microfuge tube at room temperature and should be stored at -20°C or -80°C.

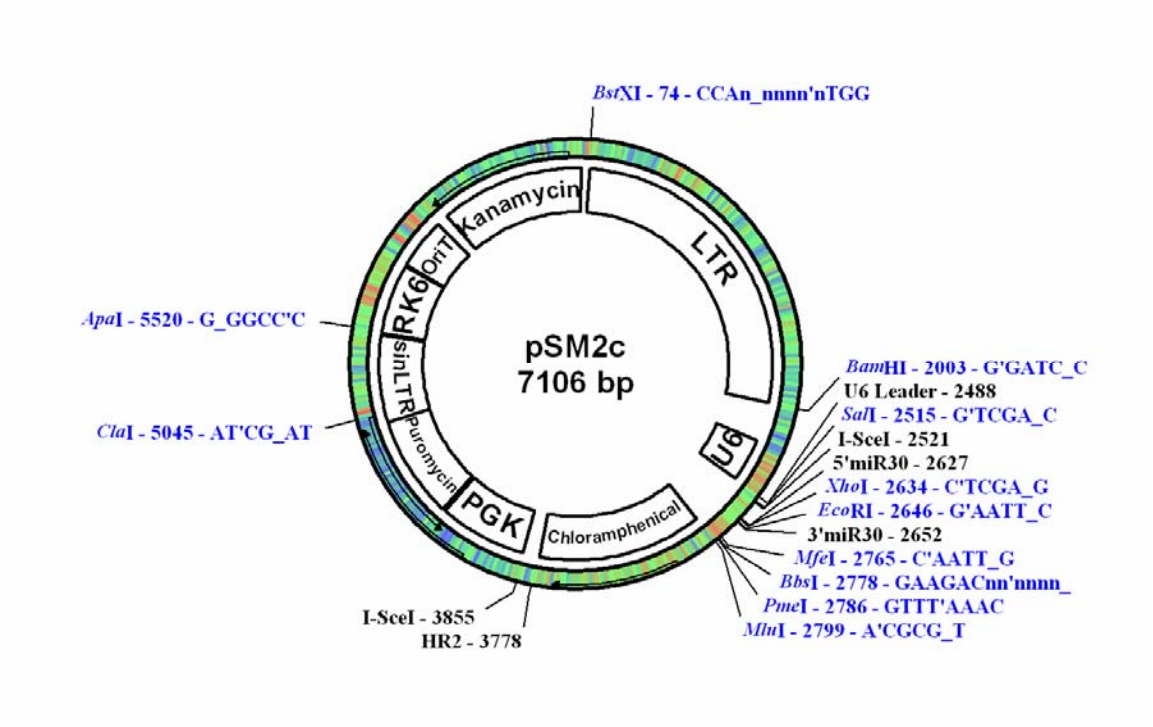


Figure 1: Vector Map of pSM2. The eGFP shRNA sequence was designed using unique microRNA based design rules the same as the shRNA Library and subsequently cloned into the XhoI and EcoRI restriction sites.

Quality Control of transfection ready plasmid DNA:

High purity (≥1.9 ratio 260/280 Abs) transfection ready plasmid DNA was prepared and resuspended in Tris buffer (pH 8.5). Following plasmid DNA preparation and restriction digest confirmation (see Figure 2) each construct was used in a transient transfection with Arrest-In™ transfection reagent to verify it's use as a control for silencing or as a negative control relative to the Non-silencing control shRNA or the pSM2c vector alone.

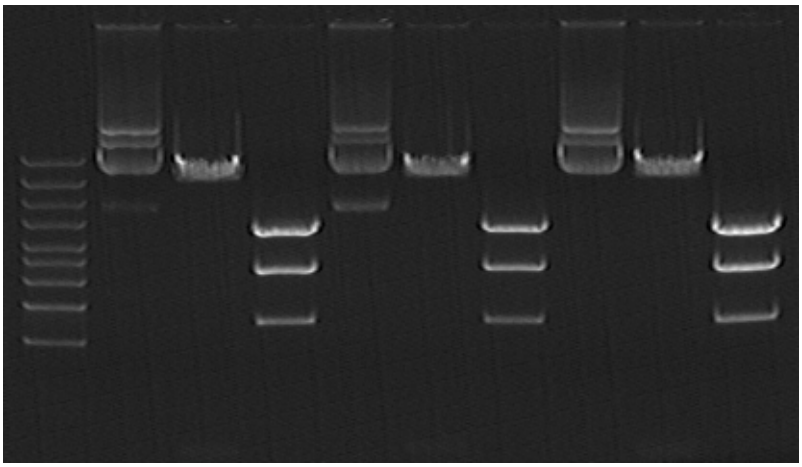
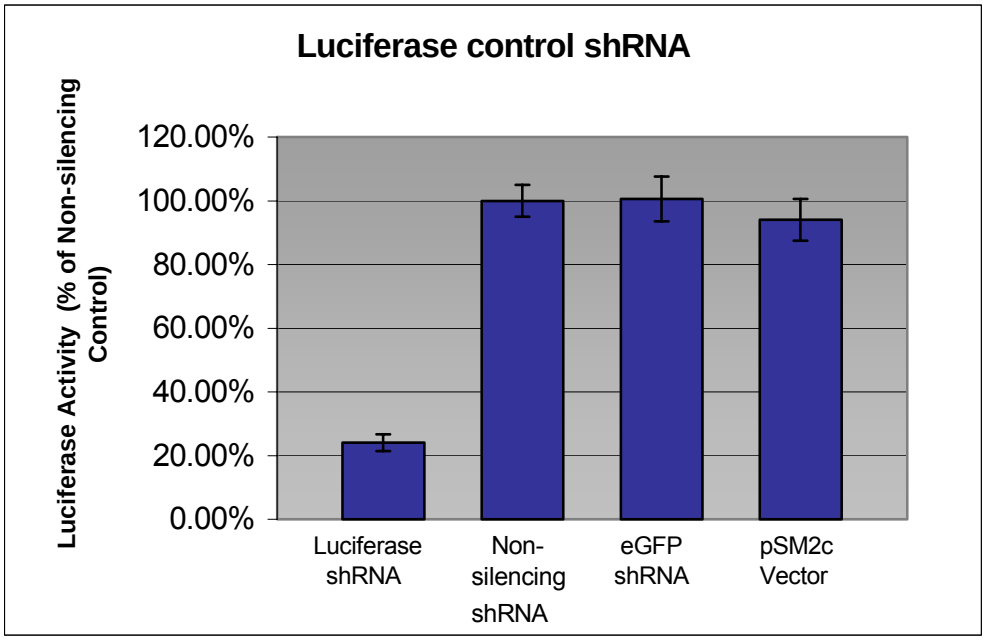
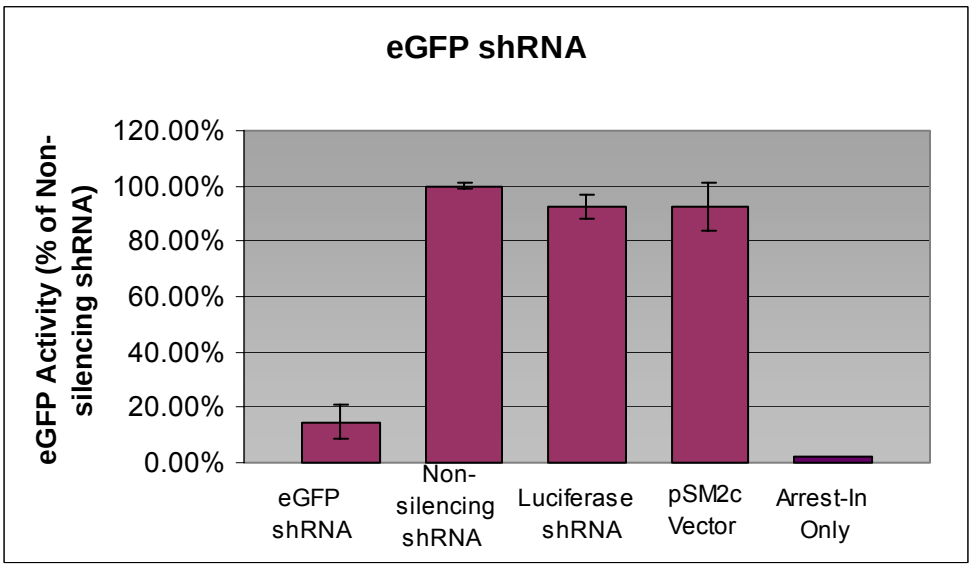


Figure 2: Agarose gel of shRNA control DNA. Lane 1 contains a 10kb ladder. The subsequent lanes contain uncut, Xho1/Ecor1 digested DNA, and XbaI/HINDIII digested DNA for the Non-silencing, eGFP, and Firefly Luciferase control shRNA's respectively.

Cos-1 cells were plated in a 24-well plate at a density of 2.5 x 10^4 one day prior to transfection. The following day shRNA constructs were transfected into the cells using Arrest-In™ reagent. Co-transfections were performed using a 10:1 ratio of shRNA to the reporter, and CMV-βgal was used as a transfection control. At 48 hours post-transfection the activity was measured and the results were normalized to the non-silencing shRNA (Figure 3).



A.



B.

Figure 3: (A) The data is shown as percent suppression of luciferase activity normalized to the non-silencing control. The firefly luciferase shRNA decreased luciferase activity by nearly 80% relative to the non-silencing control whereas the eGFP shRNA used as a negative control did not suppress activity. (B) The data is shown as eGFP activity normalized to the non-silencing control. The eGFP shRNA illustrated a high level of silencing relative to the non-silencing shRNA whereas the Luciferase shRNA as a negative control did not affect the activity.

Transformation

The plasmid DNA provided is of high purity and is validated transfection ready therefore it can be used immediately in RNAi assays. However, if a glycerol stock is made of the pSM2 control vectors they must be transformed into PIR1 competent bacteria. These plasmids harbor a conditional bacterial origin of replication, which requires the expression of the "pir1" gene for replication. PirPlus™ Competent cells from Open Biosystems (catalog# MBC1248, MBC1249) can be used for transformation as well as other commercially available pir+ lines.

Culturing and Antibiotic Resistance

The pSM2 vector contains 3 antibiotic resistance markers. (See Table 1). Following transformation the shRNA constructs are to be cultured in a rich medium such as 2X LB containing Chloramphenicol and Kanamycin.

Note: Due to the tendency of all viral vectors to recombine we recommend keeping the incubation times as short as possible and avoid subculturing. Return to your glycerol stock for each plasmid preparation.

Table 1: Antibiotic Resistance Conveyed by pSM2

Antibiotic	Concentration	Utility
Chloramphenicol	50 μg/ml	Bacterial selection marker
Kanamycin	optional	Bacterial selection marker
Puromycin	Dependent on cell line	Mammalian selectable marker

Production of Retrovirus using Viral Packaging Line

The pSM2 expression vector is a self-inactivating (SIN) MSCV retroviral vector, containing a NheI/XbaI deletion in the U3 region of the 3' LTR. Self-inactivating MSCV particles can be produced by transfection into LinX-A retroviral packaging line (LNX1500) or other commonly available retroviral packaging lines.

> *IMPORTANT SAFETY NOTE:*
> **Follow NIH guidelines regarding retroviral production and transduction; follow Biosafety Level 2 (BL2) laboratory criteria.**

Related Products:

Expression Arrest™ Luciferase shRNA, Expression Arrest™ eGFP shRNA, Expression Arrest™ Non-silencing shRNA (Catalog # RHS1701, RHS1702, RHS1703)

PirPlus™ Competent Cells (Catalog# MBC1248, MBC1249)

Arrest-In™ Transfection Reagent (Catalog # ATR1740, ATR1741, ATR1742, ATR1743)

Expression Arrest™ LinX-A Packaging Line (Catalog # LNX1500)

Useful References:

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