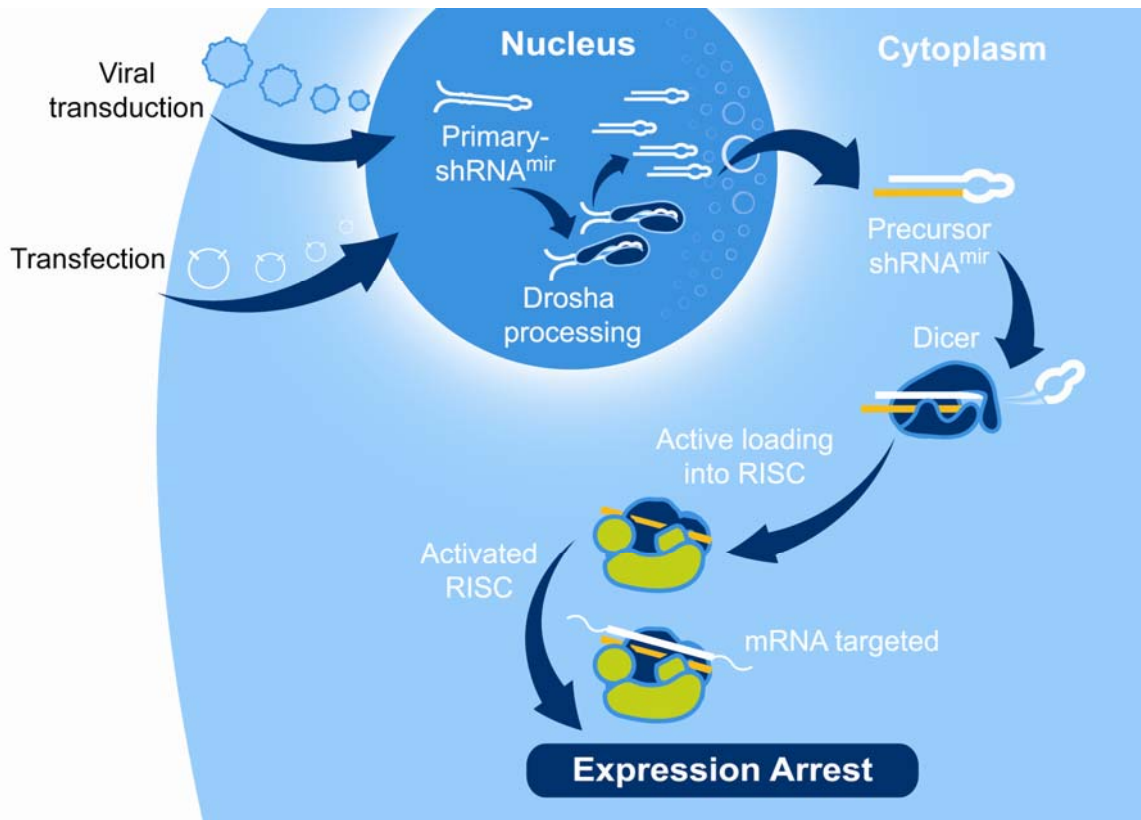


# RNAintro™ GIPZ lentiviral shRNAmir starter kits

RHS4287



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**RNAintro™ GIPZ lentiviral shRNAmir starter kit  
RHS4287**

<b>Kit Contents:</b>	<b>Catalog#</b>
GIPZ lentiviral shRNAmir constructs	RHS4430
GAPDH lentiviral shRNAmir positive control	RHS4371
EG5 lentiviral shRNAmir positive control	RHS4480
Non-Silencing control shRNAmir-Glycerol	RHS4346
Arrest-In™ Transfection Reagent	ATR1740

**Shipping and Storage Conditions:**

The following catalog items ship on ice packs and are to be stored long term at -80°C.

RHS4430

RHS4346

RHS4480

RHS4371

Arrest-In transfection reagent (Item ATR1740) is shipped on ice packs but separated from direct contact with the ice pack via packing material in order to avoid freezing. This item is to be stored at 4°C upon arrival.

The pGIPZ lentiviral shRNAmir vectors are shipped as bacterial cultures of *E. coli* (prime+) in LB-Lennox (low salt) broth with 8% glycerol, carbenicillin (100ug/ml) and zeocin (25ug/ml). Individual vials are shipped on wet ice. Open Biosystems checks all cultures for growth prior to shipment. Store at -80°C

## **Introduction to RNAi *in vitro* shRNA mir starter kits**

The use of vector-based RNAi for gene silencing is a powerful and versatile tool. Successful gene silencing *in vitro* is dependent on several variables including 1) The target cell line being studied 2) Transfection and transduction efficiency 3) Abundance of the mRNA or protein of interest in the target cell line 4) Half life of the protein 5) Robust experimental protocols. For all these reasons it is very important to run controlled experiments where the transfection and transduction efficiencies are as high as possible and measurable.

Controls are a critical part of a gene silencing experiment. They enable accurate representation of knockdown data and provide confidence in the specificity of the response. Changes in the mRNA or protein levels in cells treated with negative or non-silencing controls reflect non-specific responses in cells and can be used as a baseline against which specific knockdown can be measured. Positive controls are useful to demonstrate that your experimental system is functional and your shRNA construct is successfully activating the RNAi pathway.

RNAi *in vitro* GIPZ lentiviral shRNA mir starter kits provide optimized reagents and validated controls necessary for a gene silencing experiment. Each kit includes:

- Three GIPZ lentiviral shRNA mir constructs
- Positive controls: EG5 and GAPDH lentiviral shRNA mir-Glycerol
- Negative control: Non-silencing shRNA mir-Glycerol
- Arrest-In Transfection Reagent for shRNA delivery

### **Advantages of shRNA mir design**

- Replaced mature microRNA sequence in human microRNA 30 (mir-30) with gene specific duplexes
- Adding mir-30 loop and context sequences adds endogenous processing by Drosha which increases subsequent Dicer recognition and specificity
- Dicer processing promotes active loading into the RISC complex
- Rules-based design includes destabilizing the 5' end of the antisense strand for strand specific incorporation into RISC

***Increased Drosha/Dicer processing=More siRNA=Greater knockdown***



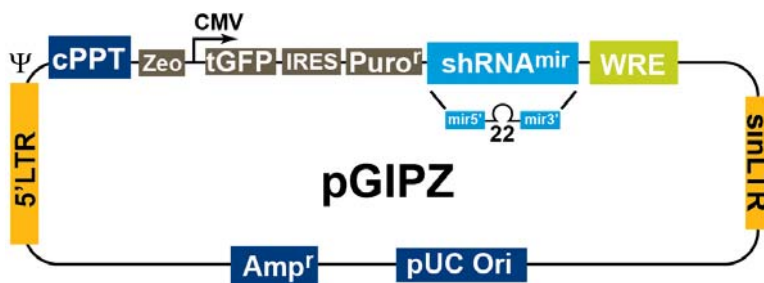


Figure 2: pGIPZ lentiviral vector

Table 1: Features of the pGIPZ Vector

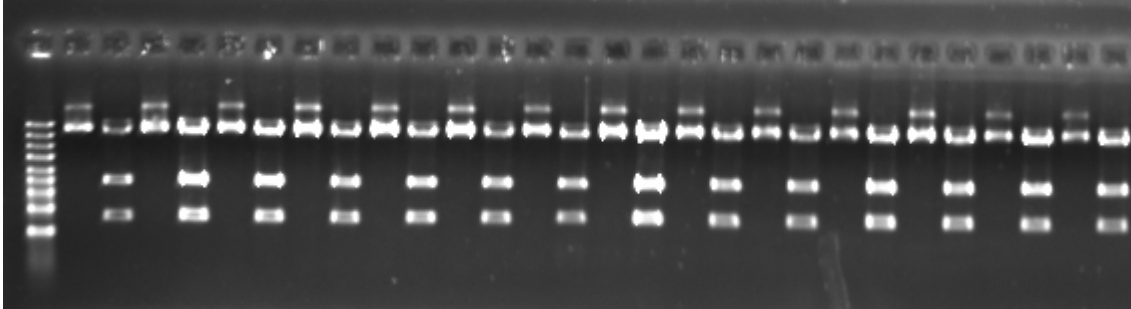
Vector Element	Utility
CMV Promoter	RNA Polymerase II promoter
cPPT	Central Polypurine tract helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
tGFP	Marker to track shRNAmir expression
IRES-Puro	Mammalian selectable marker
AMP <sup>r</sup>	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in <i>E.coli</i>
SIN-LTR	3' Self inactivating long terminal repeat
RRE	Rev response element
ZEO <sup>r</sup>	Bacterial selectable marker

Table 2: Antibiotic Resistances Conveyed by pGIPZ

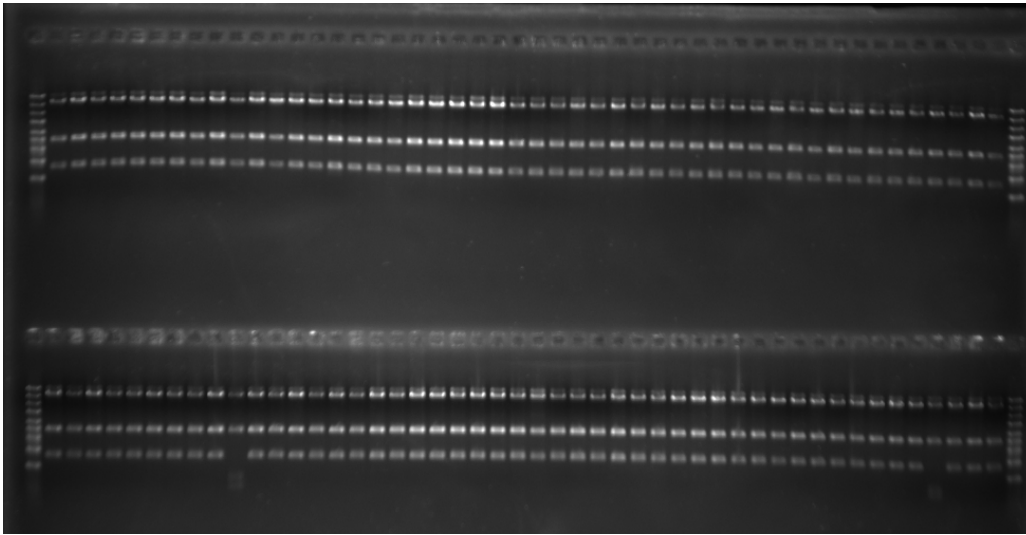
Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100µg/ml	Bacterial selection marker
Zeocin	25µg/ml	Bacterial selection marker (vector)
Puromycin	variable	Mammalian selectable marker

## Culturing protocols and maintenance of pGIPZ

It is well known that viral vectors have a tendency to recombine producing background recombinants. Recombination occurs at the long terminal repeat regions (LTR's). The LTR recombination, which results in loss of most of the plasmid, can confer a growth advantage on the cells. It is therefore critical to maintain careful growth conditions when culturing viral vectors in *E.coli* in order to reduce the number and abundance of background recombinants. The GIPZ lentiviral shRNAmir library has passed through internal QC processes to ensure high quality and low recombination.



**Figure 3.** Representative shRNAmir containing pGIPZ lentiviral clones grown for 16 hours at 30° C and the plasmid isolated and normalized to a standard concentration. Clones were then digested with *Sac*I and run out on a gel. The expected band sizes are (bp)= **1259, 2502, 7927**. No recombinant products are visible. 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb)



**Figure 4.** Gel image of a single plate from the GIPZ library cultured for 10 successive generations in an attempt to determine the tendency of the pGIPZ vector to recombine. Each generation was thawed, replicated and incubated O/N for 16 hours at 30° C then frozen, thawed and replicated. This process was repeated for 10 growth cycles. After the 10th growth cycle, plasmid was isolated and normalized to a standard concentration. Clones were then digested with *Sac*I and run on a gel. Expected band sizes (bp) = **1259, 2502, 7927**. 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb). The pGIPZ vector appears stable without showing any recombination.

### **Culture conditions for individual plasmid preparations**

Most plasmid mini-prep kits recommend a culture volume of 1–10ml for good yield. For shRNAmir constructs, 5ml of culture can be used for one plasmid mini-prep generally producing 5–10µg of plasmid DNA.

1. Upon receiving your glycerol stock(s) containing the shRNAmir of interest store at –80°C until ready to begin.
2. To prepare plasmid DNA first thaw your glycerol stock culture and pulse vortex to resuspend any *E. coli* that may have settled to the bottom of the tube.
3. Take a 10µl inoculum from the glycerol stock into 3-5ml of LB (low salt) with 100µg/ml carbenicillin and 25ug/ml zeocin. Incubate at 37°C for 16 hours with vigorous shaking. Return the glycerol stock(s) to -80°C. If a larger culture volume is desired, use the 3-5ml overnight culture as a starter inoculum. Incubate at 37°C for 16 hrs with vigorous shaking.
4. Pellet the 3-5ml culture and begin preparation of plasmid DNA.
5. Run 3-5µl of the plasmid DNA on a 1% agarose gel. pGIPZ with shRNAmir is 11744bp.

*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 original glycerol stock or the colony glycerol stock for each plasmid preparation.*

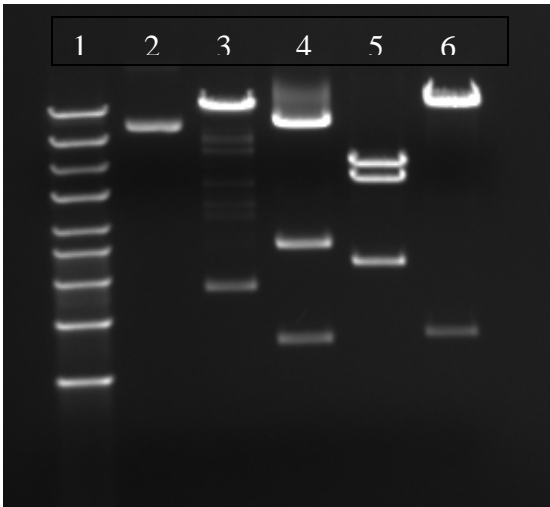
### **Restriction Digests of pGIPZ**

The following is a sample protocol for restriction enzyme digestion using *KpnI*, *SacII*, *Sall*, *XhoI* and/or *NotI* for diagnostic quality control of pGIPZ lentiviral vectors.

1. Using filtered pipette tips and sterile conditions add the following components, in the order stated, to a sterile PCR thin-wall tube.

Sterile, nuclease-free water	X µl
Restriction enzyme 10X buffer	1µl
BSA (10X, 10mg/ml) if required	1µl
DNA sample 80 -240ng, in water or TE buffer	X µl
<u>Restriction enzyme 20U</u>	<u>0.25µl</u>
<b>Final volume</b>	<b>10µl</b>

2. Mix gently by pipetting.
3. Incubate in a thermalcycler at 37°C for 2 hours to digest
4. Load the gel with 10µl of each of the digested samples (*KpnI*, *SacII*, *Sall*, *XhoI* and/or *NotI*) on a 1% agarose gel. Run uncut sample alongside the digested samples.



**Figure 5:** Restriction digests with pGIPZ. Lane 1– 10kb molecular weight ladder (10kb, 7kb, 5kb, 4kb, 3kb, 2.5kb, 2kb, 1.5kb, 1kb). Lane2 -Uncut pGIPZ vector. Lane 3 - *KpnI* digested pGIPZ produces 2 bands at 1750bp and 9860bp. Lane4- *SacII* digest produces 3 bands at 1178bp, 2502bp and 7930bp. Lane 5 -*SalI* produces 3 bands at 2188bp, 4298bp and 5124bp. Lane 6 – *XhoI* digest produces 2 bands at 1210bp and 10400bp.

### **Culture conditions for 96-well plasmid preparation**

Inoculate 96-well bio-block containing 1ml per well of the above media with 1µl of the culture. Incubate at 37°C with shaking (~170-200 RPM). We have observed that incubation times from 16 hours produces good plasmid yield. For plasmid preparation, follow the kit protocols recommended by the manufacturer.

*Note: The cells can be grown at 37°C for purposes of template preparation or sequencing. For archive replication, grow all pGIPZ clones at 30°C in LB-Lennox (low salt) media plus 25ug/ml zeocin and 100ug/ml carbenicillin in order to provide maximum stability of the clones.*

### **Materials Required**

LB-Lennox Broth (low salt) – VWR item# EM1.00547.0500  
 Glycerol – VWR item# EM-4760  
 Carbenicillin or Ampicillin – VWR item# EM-2200 or 80030-956  
 Zeocin – Invivogen item# ant-zn-5p  
 96-well microplates – VWR item# 62407-174  
 Aluminum seals – VWR item# 73520-056  
 Disposable replicators – Genetix item# X5054

## CaPO<sub>4</sub> Transfection Protocol for pGIPZ Lentiviral packaging

(100-mm dish format)

1. Approximately 24 hours before transfection, seed  $6.0 \times 10^6$  293T cells in 14-ml of complete media (Dulbecco's modified Eagle's medium (DMEM), 10% FBS, 2 mM L-glutamine, 1X Pen-Strep).
2. Incubate at 37°C, 5% CO<sub>2</sub> overnight. Transfection should begin when cells are approximately 90% confluent.
3. The following describes the preparation of DNA-CaPO<sub>4</sub> mixture and the protocol for performing 1 transfection (one 100-mm dish). Transfection reactions are carried out in a 5-ml polystyrene round-bottom tube (Falcon catalog # 352058)

### DNA Preparation

DNA to be co-transfected, add volume to 945 µl with sterile water:

- |  |        |
|--|--------|
| 1. Transgene (gene transfer vector):                     | 21µg   |
| 2. pCMV-Gag-Pol (2 <sup>nd</sup> Generation eg. psPAX2): | 21µg   |
| 3. pCMV-VSV-G-poly A (e.g pMD2.G)                        | 10.5µg |

Note: The number of transfection reactions is scalable. For example, if transfecting numerous 100-mm dishes to generate larger volumes or higher titers of the same vector stock, a master mix of the DNA-water stock is made and aliquoted into 50-ml polystyrene tubes. A maximum of seven 100-mm dishes can be transfected from one 50-ml tube. For seven transfections, pipette 6615 µl of the DNA-water mix maintaining the same ratio of each of the vector plasmids as well as DNA to water.

4. The following describes the CaPO<sub>4</sub> precipitation reaction in both one and seven 100-mm dish formats.

#### One 100-mm dish:

In one 5-ml snap cap polystyrene tube mix:

- a. DNA plus sterile water to final volume of 945 µl.
- b. Add 105 µl of 2.5 M CaCl<sub>2</sub>.
- c. While vortexing tube, add dropwise 1050 µl of 2X HBSS (2100 µl total volume). Make sure vortexer is set so that the contents mix thoroughly without spilling over.

#### For seven 100-mm dishes:

In one 50-ml polystyrene tube mix:

- a. DNA plus sterile water to final volume of 6615 µl.

- b. Add 735  $\mu$ l of 2.5 M  $\text{CaCl}_2$ .
  - c. While vortexing tube, add dropwise 7350  $\mu$ l of 2X HBSS (14,700  $\mu$ l total volume). Make sure vortexer is set so that the contents mix thoroughly without spilling over.
5. Incubate at room temperature for 3 minutes. A chalky white precipitate should be visible in the tube. If no precipitate is noticeable, allow the incubation to continue at room temperature until it is visible.
6. Following incubation, vortex contents of the tube a few seconds, and pipette 2100  $\mu$ l of the transfection mixture dropwise into one well. Do not add the transfection mixture to only one area of the well but instead spread the drops over the entire surface of well.
7. Incubate at 37°C, 5%  $\text{CO}_2$  for 12-16 hours.
8. Remove media from each plate and slowly pipette 14 ml of DMEM, 5% FBS, 2 mM L-glutamine, 1X Pen-Strep) to each well. DO NOT WASH cells. 5% FBS is used to decrease the amount of serum proteins pelleted with the Vector stock during ultracentrifugation.
9. Incubate at 37°C, 5%  $\text{CO}_2$  for an additional 48 hours.
10. Harvest virus-containing supernatant. Pellet cells/debris by low-speed centrifugation (1600 x g for 10 min).
11. Aliquot virus and store at -80°C.
12. Virus can be concentrated by ultracentrifugation (SW28, 23,000rpm, 1.5h @ 4°C).

#### Reagents:

##### **2.5 M $\text{CaCl}_2$**

(For 100 ml):

36.75 g  $\text{CaCl}_2$  (Sigma, Cat. No. C-7902)

Add sterile  $\text{dH}_2\text{O}$  to 100 ml

Filter-sterilize through 0.22  $\mu$ m filter flask (Millipore)

##### **2X HBSS** (Hepes Buffered Saline Solution)

50 mM Hepes (pH 7.1)

280 mM NaCl

1.5 mM Sodium Phosphate

**The final pH should be 7.1**

(For 1 liter):

11.915 g	Hepes (Sigma, Cat. No. H-3375)
16.363 g	NaCl (Sigma, Cat. No. S-3014)
0.090 g	NaH <sub>2</sub> PO <sub>4</sub> (Sigma, Cat. No. S-3139)
0.107 g	Na <sub>2</sub> HPO <sub>4</sub> (Sigma, Cat. No. S-3264)

Add sterile dH<sub>2</sub>O to 990 ml  
pH to 7.1 by dropwise adding 10 N NaOH

## **Arrest-In Transfection reagent for delivery of shRNA**

Gene silencing using RNAi is critically dependent on highly efficient delivery of shRNA or siRNAs into cells. Arrest-In™ transfection reagent is a proprietary polymeric formulation, developed and optimized for transfection of shRNA plasmid DNA into the nucleus of cultured eukaryotic cells. It is well known that polymers, **but not cationic lipids**, protect DNA in the cytoplasm and promote entry into the nucleus of transfected cells (Pollard *et al* 1998).

Arrest-In transfection reagent also provides an enhanced uptake efficiency of the shRNA plasmid DNA into cells. Once in the cells, Arrest-In™ promotes the entry of the shRNA containing plasmid into the nucleus where it is transcribed into a hairpin, enters the cytoplasm and is processed by the endogenous RNAi machinery into functional siRNAs.

Arrest-In™ is easy to use, robust, and exhibits very low toxicity. One milliliter is sufficient for approximately 100 transfections on 35-mm tissue culture dishes using 2µg of DNA. Simply mix diluted shRNA plasmid with diluted Arrest-In in the recommended ratios (See Table 1), add to cells and assay 48–96 hrs later.

### **Transfection Protocols with Arrest-In:**

*It is preferable that transfection be carried out in medium that is serum- free and antibiotic free. However it is possible to carry out successful transfections with serum present, you will have to optimize according to your needs.*

*The protocol below is optimized for transfection of the shRNA construct into Hek293T cells in a 24-well plate. If a different culture dish is used adjust the number of cells, volumes and reagent quantities in proportion to the change in surface area (see Table 3).*

*Warm Arrest-In™ reagent to ambient temperature (approximately 10-15 minutes at room temperature) prior to use. Always mix well by vortex or inversion prior to use.*

*Maintain sterile working conditions with the DNA and Arrest-In™ mixtures as they will be added to you cells.*

**Table 3** - Suggested amounts of DNA, medium and Arrest-In™ reagent for transfection of shRNA constructs into adherent cells

Tissue Culture Dish	Surface area per well (cm <sup>2</sup> )	Total serum free medium volume per well (ml)	shRNA plasmid DNA (µg)*	Arrest-In™ (µg)**
60 mm	20	2	4	21
35 mm	8	1	2	10
6-well	9.4	1	2	10
12-well	3.8	0.5	1	5
24-well	1.9	0.25	0.5	2.5
96-well	0.3	0.1	0.1–0.2	0.5–1

\*Recommended starting amount of DNA. May need to be optimized for the highest efficiency

\*\*Recommended starting amounts of Arrest-In™ reagent. See Transfection Optimization.

**1. The day before transfection, plate the cells at a density between 5–8 x 10<sup>4</sup> cells/well of a 24-well plate.**

*You will still be using full medium (i.e. with serum and antibiotics) at this stage.*

**2. On the day of transfection form the DNA/Arrest-In™ transfection complexes.**

*The principle is to prepare your shRNA DNA and Arrest-In™ dilutions in an equal amount of serum-free medium in two separate tubes. These two mixtures (viz. the DNA and the Arrest-In™) will be added to each other and incubated for 10-15 minutes prior to addition to your cells. This enables the DNA/ Arrest-In™ complexes to form.*

**Dilute 500ng shRNA DNA into 50µl of serum-free medium in a microfuge tube. Dilute 2.5µg (2.5µl) of the Arrest-In™ into 50µl of serum-free medium.**

*This will give you a 1:5 DNA:Arrest-In™ ratio which is recommended for successful transfection. Optimization of these quantities and ratios may be required for your cells.*

**Set up all your desired controls in a similar fashion. The table (Table 4) below will**

aid you in this.

**Table 4:** Relative quantities of DNA for transfection experiments

	shRNA experiment (pGIPZ)	Transfection efficiency of reporter	Control for knockdown efficiency of reporter	Knockdown efficiency of reporter	Non-silencing control
shRNA DNA (ng)	500	0	0	450-500	500
Reporter** (ng)	0	500	50	50	
Carrier DNA* (ng)	0	0	450-500	0	
Serum-free medium (final volume in $\mu$ l)	50	50	50	50	50

\* Carrier DNA is required to increase the total DNA quantity for the formation adequate DNA/Arrest-In™ complexes. Recommended carriers are pUC19 or pBluescript plasmids.

\*\* It is not necessary to transfect a GFP reporter into your cells as your shRNA construct (pGIPZ) already has a tGFP (turbo GFP) for the convenient estimation of transfection efficiency of your pGIPZ construct. Other recommended reporters should you choose; include  $\beta$ -gal (X-gal staining and/or ONPG assays) and luciferase.

For each well in transfection, add 2.5 $\mu$ g (2.5 $\mu$ l) Arrest-In™ to 47.5 $\mu$ l serum-free medium. You may make a mastermix of the Arrest-In™ dilution to cover all your transfections (i.e. 5 x mastermix for the above table).

It is advisable to include the following controls in addition to the above-mentioned ones: an Arrest-In™- only control, a reporter DNA-only control, and a “nothing-added” control. Additionally it is recommended that all experimental points get carried out in duplicate.

**Add the 50 $\mu$ l of medium containing the diluted Arrest-In™ to the 50 $\mu$ l diluted DNA, mix well and incubate at room temperature for 10-15 minutes to form the transfection complexes.**

*Your total volume will be 100 $\mu$ l at this stage.*

**3. Aspirate the growth medium from the cells. Add an additional 150 $\mu$ l of serum-free medium to each of the tubes containing the transfection complexes, mix gently, then overlay onto the cells. Return the cells to the CO<sub>2</sub> incubator at 37°C for 3-6 hrs.**

*Your total volume will be 250µl at this stage.*

**4. Following the 3-6 hour incubation, add an equal volume of growth medium (250µl) containing twice the amount of normal serum to the cells (i.e. to bring the overall concentration of serum to what is typical for your cell line). Alternatively the transfection mixture can be aspirated and replaced with standard culture medium or you can add 1ml standard culture medium to you 250µl reaction mixture. Return the cells to the CO<sub>2</sub> incubator at 37°C.**

*Arrest-In™ has displayed low toxicity in the cell lines tested therefore removal of transfection reagent is not required for many cell lines. In our hands higher transfection efficiencies have been achieved if the transfection medium is not removed. However, if toxicity is a problem, aspirate the transfection mixture after 3-6 hrs and replace with fresh growth medium. Additionally, fresh growth medium should be replenished as required for continued cell growth.*

**5. After 48-96 hours of incubation examine the cells microscopically for the presence of tGFP expression as this will be your first indication as to the efficiency of your transfection. Then assay the cells for reduction in gene or reporter activity; compared to reporter alone, non-silencing shRNA, or other negative controls by quantitative/real-time RT-PCR, western blot or the appropriate functional assay.**

*The optimal length of incubation from the start of transfection to analysis is dependent on cell type, your gene of interest and the stability of the mRNA and/or protein being analyzed; we recommend 48 hours for the reporter assay. The visualization of tGFP expression should preferably be done 48-96 hours after transfection.*

*Factors affecting transfection efficiency are not limited to but include purity of plasmid DNA, health of transfected cells, inconsistencies in number of cells plated, insufficient mixing of transfection complexes.*

**6. If selecting for stably transfected cells (optional), transfer the cells to medium containing puromycin for selection. It is important to wait at least 48 hours before beginning selection.**

*The working concentration of puromycin varies between cell lines. We recommend you determine the optimal concentration of antibiotic required to kill your host cell line prior to selection for stable shRNA transfectants. Typically the working concentration ranges from 1–10 µg/ml.*

### **Cells Grown in Suspension**

*Transfection of cells in suspension would follow all the above principles and the protocol would largely remain the same, except that the DNA/Arrest-In™ mixture should be added to cells that are either resuspended in 250µl of medium with serum (no*

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antibiotics) or cells resuspended in 250µl serum-free medium.

#### **Determining Puromycin Dose-Response**

*In order to generate stable cell lines expressing the shRNA of interest, it is important to determine the minimum amount of puromycin required to kill non-transfected cells. A simple procedure to quickly test this is as follows:*

1. Plate cells at a 25% confluency in 14 wells of a 24-well plate. Allow them to incubate overnight under proper conditions for your cells.
2. Label the wells to reflect the concentration of antibiotic to be applied (in duplicate). Prepare medium containing 0, 1, 2, 4, 6, 8, 10 µg/ml puromycin.
3. Aspirate the growth medium from the cells.
4. Apply the medium containing the dilutions of the antibiotic to the appropriate well.
5. Return the plate to the proper conditions for your cells.
6. Every 3 days aspirate the old medium and replace with freshly prepared selective medium.
7. Monitor the cells daily and observe the percentage of surviving cells. Optimum effectiveness should be reached in 3–10 days with puromycin.
8. The minimum antibiotic concentration to use is the lowest concentration that kills 100% of the cells in 5–10 days from the start of antibiotic selection.

#### **Transfection Optimization using Arrest-In™**

*It is essential to optimize transfection conditions to achieve the highest transfection efficiencies and lowest toxicity with your cells. The most important parameters for optimization are transfection reagent to DNA ratio, DNA concentrations and cell confluency. We recommend that you initially begin with 5–8 x 10<sup>4</sup> cells/well of a 24-well plate, and with the Arrest-In™ and DNA amount indicated in Table 2.*

#### **Additional Factors Influencing Successful Transfection:**

1. **Concentration and purity of nucleic acids** – Determine the concentration of your DNA using 260nm absorbance. Avoid cytotoxic effects by using pure preparations of nucleic acids.
2. **Transfection in serum containing or serum-free medium** – Our studies indicate that Arrest-In™/DNA complexes should always be formed in the absence of serum. In the cell lines tested we found that the highest transfection efficiencies can be obtained if the cells are exposed to the transfection complexes in serum free conditions followed by the addition of medium containing twice the amount of normal serum to the complex medium 3–6 hours post transfection (leaving the complexes on the cells). However, the transfection medium can be replaced with normal growth medium if high toxicity is observed.
3. **Presence of antibiotics in transfection medium** – The presence of antibiotics can adversely affect the transfection efficiency and lead to increased toxicity levels in some cell types. It is recommended that these additives be initially excluded until optimized conditions are achieved, then these components can be added, and the cells can be monitored for any changes in the transfection results.
4. **Cell history, density, and passage number**—It is very important to use healthy

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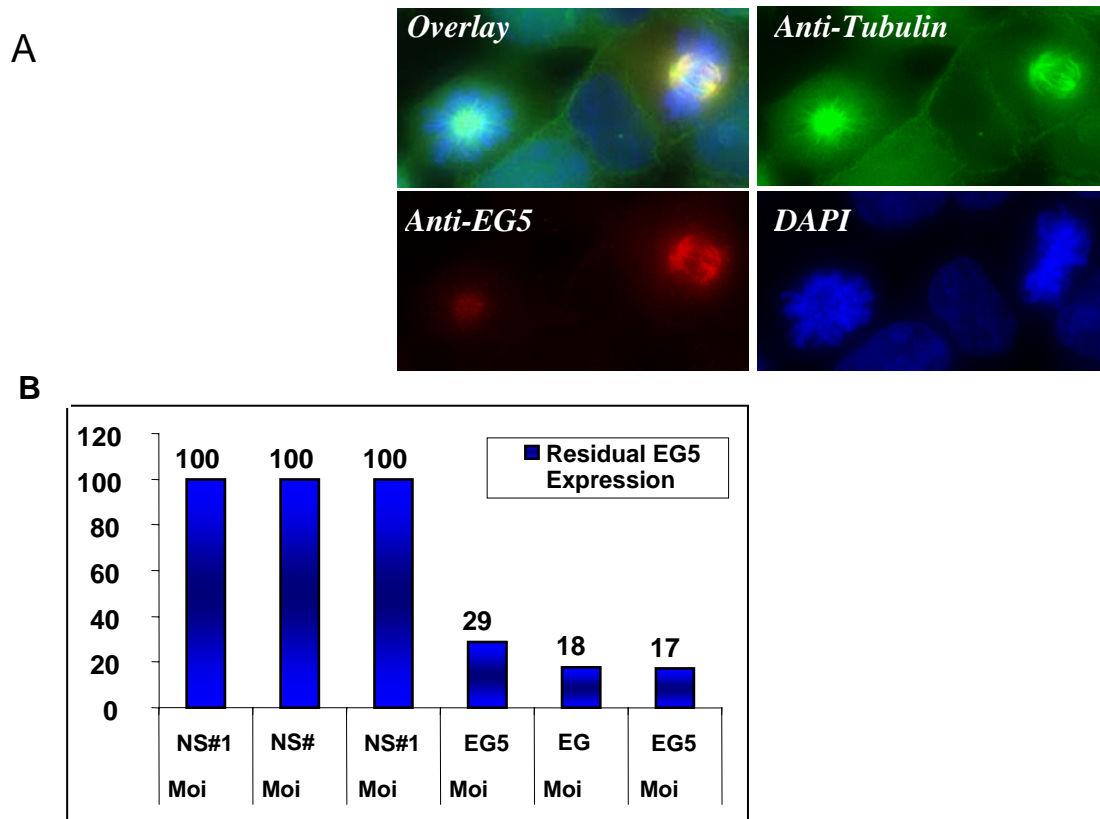
*cells that are regularly passaged and in growth phase. The highest transfection efficiencies are achieved if cells are plated the day before. However, adequate time should be given to allow the cells to recover from the passaging (generally >12 hours). Plate cells at a consistent density to minimize experimental variation. If transfection efficiencies are low or reduction occurs over time, thawing a new batch of cells or using cells with a lower passage number may improve the results.*

### Transduction based validation studies

Hek 293T cells were trypsinized from a healthy, growing cultures, seeded into 24-well plates at  $5-8 \times 10^4$  per well and allowed to adhere for 24 hours in DMEM with 10% FCS. DMEM containing serum was replaced with 200 $\mu$ l serum-free media and lentiviral particles containing GAPDH or EG5 shRNAmir, non-silencing or non-transduced controls were added to the appropriate wells at three different multiplicity of infections (MOI) and incubated for 6 hours. DMEM containing serum was then added and the transduced cells were further incubated for a total of 48 hours.

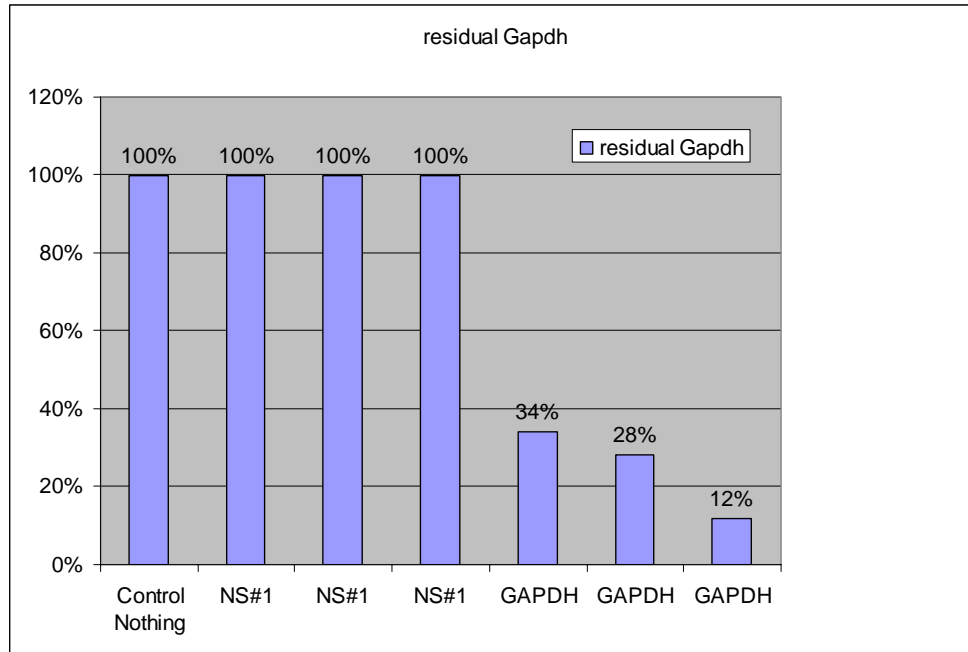
### RNA Extraction and Validation

At 48 hours post-transduction, transduced cells were lysed and total RNA was extracted using the Qiagen RNAeasy Kit (cat# 74104). The RNA was converted to cDNA using the ABI- High Capacity cDNA RT Kit (cat#4368813), using 500ng total RNA in a 100 $\mu$ l reaction. A 1/100 dilution of the cDNA was used in Real Time Quantitative PCR (QPCR). Each gene was validated in triplicate, standardized to a 18s endogenous control and compared to non-silencing or non-transduced experimental controls. Knockdown was calculated as the percentage remaining gene expression normalized to the relevant non-silencing control.

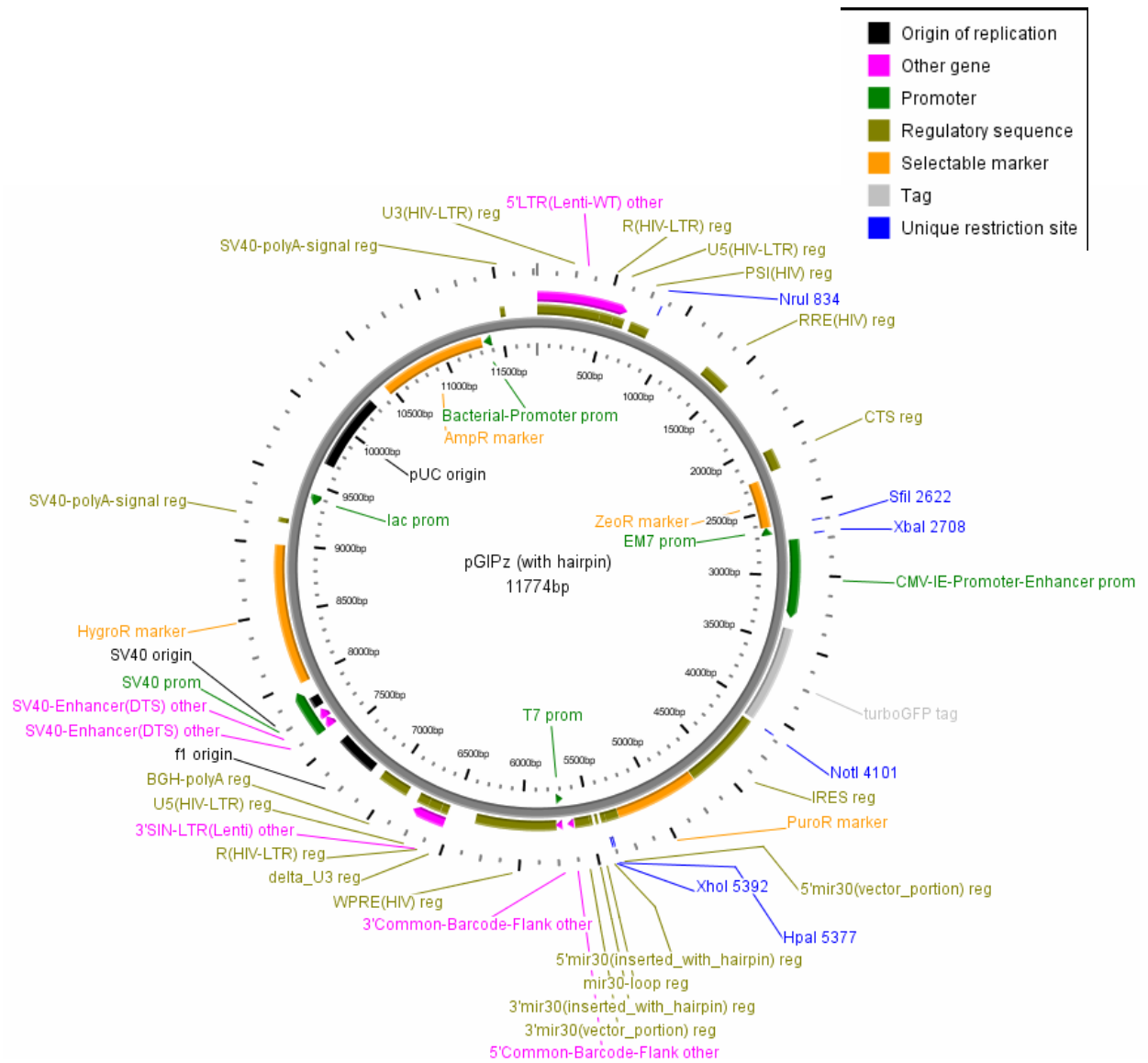


**Figure 6.** Knockdown of the EG5 (KIF11) gene allowed evaluation of phenotypic evidence of RNAi as well as its molecular manifestation. A. The characteristic phenotype observed by the targeting of the EG5 (KIF11) gene results in the formation of half spindles, mitotic arrest and monoastrial microtubular arrays (green, see the cell on the left). By contrast, normal cells show

bipolar spindles and microtubule networks in mitosis and in interphase (see the cell on the right). The comparative expression of EG5 (red) between the cell on the left and the right shows the extensive knockdown of EG5 in the cell displaying the phenotype (left). The cells were visualized at 100x magnification using a Leica DMIRB fluorescence microscope. HEK293T cells were stained for tubulin (anti-tubulin, green), DNA (DAPI, blue) and EG5 (anti-EG5, red). B. HEK293T cells were transduced with lentiviral particles expressing EG5 or non-silencing shRNAmir at MOIs of 3.5, 8.5 and 17. The graph depicts the residual levels of EG5 relative to its non-silencing control.



**Figure 7.** HEK293T cells were transduced with lentiviral particles expressing GAPDH or non-silencing shRNAmir at variable MOIs ranging from 9-48. The graph depicts the residual levels of GAPDH relative to its non-silencing control.



**Figure 8: Detailed Vector Map of pGIPZ.**

## Sequence of pGIPZ lentiviral vector (11774bp)

```
5'LTR(Lenti-WT) other(1,635)>>>
|
U3(HIV-LTR) reg(1,455)>>>
|
1   tggaagggctaattcactcccaaagaagacaagatatccttgatctgtggatctaccaca 60
    ACCTTCCCGATTAAGTGAGGGTTTCTTCTGTTCTATAGGAACTAGACACCTAGATGGTGT
61   cacaaggctacttccctgattagcagaactacacaccagggccaggggtcagatatccac 120
    GTGTTCCGATGAAGGGACTAATCGTCTTGATGTGTGGTCCCGGTCCCCAGTCTATAGGTG
121  tgacctttggatggtgctacaagctagtagcagttgagccagataaggtagaagaggcca 180
    ACTGGAAACCTACCACGATGTTTCGATCATGGTCAACTCGGTCTATTCCATCTTCTCCGGT
181  ataaaggagagaacaccagcttgttacaccctgtgagcctgcatgggatggatgaccggy 240
    TATTTCTCTCTTGTGGTTCGAACAATGTGGGACACTCGGACGTACCCTACCTACTGGGCC
241  agagagaagtgttagagtggaggtttgacagccgcctagcatttcatcacgtggcccgag 300
    TCTCTCTTCACAATCTCACCTCCAAACTGTGCGCGGATCGTAAAGTAGTGCACCGGGCTC
301  agctgcatccggagtagtacttcaagaactgctgatatcgagcttgctacaagggactttccg 360
    TCGACGTAGGCCTCATGAAGTTCTTGACGACTATAGCTCGAACGATGTTCCCTGAAAGGC
361  ctggggactttccagggaggcgtggcctgggcgggactggggagtggcgagccctcagat 420
    GACCCCTGAAAGGTCCCTCCGCACCGGACCCGCCCTGACCCCTCACCGCTCGGGAGTCTA
                                     R(HIV-LTR) reg(456,550)>>>
                                     |
421  cctgcatataagcagctgctttttgctgtactgggtctctctggttagaccagatctga 480
    GGACGTATATTCGTTCGACGAAAAACGGACATGACCCAGAGAGACCAATCTGGTCTAGACT
481  gcctgggagctctctggctaactagggaaaccactgcttaagcctcaataaagcttgct 540
    CGGACCCCTCGAGAGACCGATTGATCCCTTGGGTGACGAATTCGGAGTTATTTTGAACGGA
                                     U5(HIV-LTR) reg(551,635)>>>
                                     |
541  tgagtgcttcaagtagtgtgtgcccgtctgttgtgtgactctggtaactagagatccctc 600
    ACTCACGAAGTTCATCACACACGGGCAGACAACACACTGAGACCATTGATCTCTAGGGAG
601  agacccttttagtcagtggtgaaaatctctagcagtgggcggccgaacagggacttgaaag 660
    TCTGGGAAAATCAGTCACACCTTTTAGAGATCGTCACCGCGGGCTTGTCCCTGAACTTTC
                                     PSI(HIV) reg(685,822)>>>
                                     |
661  cgaaagggaaaccagaggagctctctcgacgcaggactcggcttgctgaagcgcgcacgg 720
    GCTTTCCCTTTGGTCTCCTCGAGAGAGCTGCGTCTGAGCCGAACGACTTCGCGCGTGCC
721  caagagggcagggggcggcgactggtgagtacgcaaaaattttgactagcggaggctaga 780
    GTTCTCCGCTCCCCGCCGCTGACCACTCATGCGGTTTTTAAACTGATCGCCTCCGATCT
```

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NruI  
|

781   aggagagagatgggtgagagagcgtcagtattaagcgggggagaattagatcgcgatggg 840  
 TCCTCTCTCTACCCACGCTCTCGCAGTCATAATTCGCCCCCTCTTAATCTAGCGCTACCC

841   aaaaaattcgggtaaggccagggggaaagaaaaaatataaattaaaacatatagtatggg 900  
 TTTTTTAAGCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATACCC

901   caagcagggagctagaacgattcgcagttaatcctggcctgtagaaacatcagaaggct 960  
 GTTCGTCCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTTCCGA

961   gtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagat 1020  
 CATCTGTTTATGACCCTGTCGATGTTGGTAGGGAAGTCTGTCCTAGTCTTCTTGAATCTA

1021   cattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaaagaca 1080  
 GTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCTATCTCTATTTTCTGT

1081   ccaaggaagctttagacaagatagaggaagagcaaaacaaaagtaagaccaccgcacagc 1140  
 GGTTCCCTTCGAAATCTGTTCTATCTCCTTCTCGTTTTGTTTTTCATTCTGGTGGCGTGTCC

1141   aagcggccggccgctgatcttcagacctggaggaggagatatgagggacaattggagaag 1200  
 TTCGCCGGCCGGCGACTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTTAACCTCTTC

1201   tgaattatataaatataaaagtagtaaaaattgaaccattaggagtagcaccaccaaggc 1260  
 ACTTAATATATTTATATTTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTTCCG

RRE (HIV)

reg(1314,1518)>>>

|

1261   aaagagaagagtgggtgagagagaaaaagagcagtggggaataggagctttgttccttgg 1320  
 TTTCTTTCTCACCCAGTCTCTTTTTTTCTCGTCACCCTTATCCTCGAAACAAGGAACC

1321   gttccttgggagcagcaggaagcactatgggagcagcgtcaatgacgctgacggtacaggc 1380  
 CAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGTTACTGCGACTGCCATGTCCG

1381   cagacaattattgtctggtatagtgagcagcagacaacaatttgctgagggctattgaggc 1440  
 GTCTGTTAATAACAGACCATATCACGTCGTCGTCCTTGTTAAACGACTCCCGATAACTCCG

1441   gcaacagcatctggtgcaactcacagtctggggcatcaagcagctccaggcaagaatcct 1500  
 CGTTGTCGTAGACAACGTTGAGTGTGACACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGA

1501   ggctgtggaaagatacctaaaggatcaacagctcctggggatttgggggtgctctggaaa 1560  
 CCGACACCTTTCTATGGATTTCTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTT

1561   actcatttgcaccactgctgtgccttggaaatgctagttggagtaataaatctctggaaca 1620  
 TGAGTAAACGTGGTGACGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGT

1621   gatttggaaatcacacgacctggatggagtgggacagagaaattaacaattacacaagctt 1680  
 CTAAACCTTAGTGTGCTGGACCTACCTCACCTGTCTCTTTAATTGTTAATGTGTTTCGAA

1681 aatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattatt 1740  
TTATGTGAGGAATTAACCTTCTTAGCGTTTTGGTCGTTCTTTTCTTACTTGTTCTTAATAA

1741 ggaattagataaatgggcaagtttgtggaattggtttaacataacaaattggctgtggta 1800  
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1801 tataaaaattattcataatgatagtaggaggttggttaggtttaagaatagtttttgcgtgt 1860  
ATATTTTAATAAGTATTACTATCATCTCCGAACCATCCAAATTCTTATCAAAAACGACA

1861 acttttctatagtgaatagagtttaggcagggatattcaccattatcgtttcagaccacct 1920  
TGAAAGATATCACTTATCTCAATCCGTCCTATAAGTGGTAATAGCAAAGTCTGGGTGGA

1921 cccaacccccgaggggacccgacagggcccgaaggaatagaagaagaaggtggagagagaga 1980  
GGGTTGGGGCTCCCCTGGGCTGTCCGGGCTTCCTTATCTTCTTCCACCTCTCTCTCT

1981 cagagacagatccattcgattagtgaacggatcggcactgctgcgccaattctgcagac 2040  
GTCTCTGTCTAGGTAAGCTAATCACTTGCCTAGCCGTGACGCACGCGGTTAAGACGTCTG

CTS reg(2064,2214)>>>

|

2041 aaatggcagttattcatccacaattttaaaagaaaaggggggattggggggtacagtgcgag 2100  
TTTACCGTCATAAGTAGGTGTTAAAATTTTCTTTTCCCCCTAACCCCATGTCACGTC

2101 gggaaagaatagtagacataatagcaacagacatacaaaactaaagaattacaaaaacaaa 2160  
CCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTTGATTTCTTAATGTTTTTGT

2161 ttacaaaaattcaaaattttcgggtttattacagggacagcagagatccagtttggttag 2220  
AATGTTTTTAAGTTTTAAAGCCCAAATAATGTCCCTGTCTCTAGGTCAAACCAATC

ZeoR marker(2245,2619)<<<

|

2221 taccgggcccgtctagtcggaatcagtcctgctcctcggccacgaagtgcacgcagtt 2280  
ATGGCCCGGGCGAGATCAGGCCTTAGTCAGGACGAGGAGCCGGTGCTTCACGTGCGTCAA

2281 gccggccgggtcgcgagggcgaactcccggcccccacggctgctcgccgatctcggtcat 2340  
CGGCCGGCCAGCGCGTCCCGCTTGAGGGCGGGGTGCCGACGAGCGGCTAGAGCCAGTA

2341 ggccggccgggagggcgtcccgggaagttcgtggacacgacctccgaccactcggcgtacag 2400  
CCGGCCGGCCCTCCGAGGGCCTTCAAGCACCTGTGCTGGAGGCTGGTGAGCCGCATGTC

2401 ctcgtccaggccgcgacccacacccaggccaggggtgttgcggccaccacctggctcctg 2460  
GAGCAGGTCCGGCGCGTGGGTGTGGGTCCCGTCCCACAACAGGCCGTGGTGGACCAGGAC

2461 gaccgctgatgaacaggggtcacgtcgtcccggaccacaccggcgaagtcgtcctccac 2520  
CTGGCGGACTACTTGTCCCAGTGCAGCAGGGCCTGGTGTGGCCGCTTACAGCAGGAGGTG

2521 gaagtcccgggagaacccgagccgggtcggtccagaactcgaccgctccggcgacgtcgcg 2580  
CTTCAGGGCCCTCTTGGGCTCGGCCAGCCAGGTCTTGAGCTGGCGAGGCCGCTGCAGCGC



turboGFP tag(3390,4088)>>>

3361 acaccgactctactagaggatctgccaccatggagagcgacgagagcggcctgcccgcca 3420  
TGTGGCTGAGATGATCTCCTAGACGGTGGTACCTCTCGCTGCTCTCGCCGGACGGGCGGT  
3421 tggagatcgagtgccgcatcaccggcaccctgaacggcgtggagttcgagctggtgggcg 3480  
ACCTCTAGCTCACGGCGTAGTGGCCGTGGGACTTGCCGCACCTCAAGCTCGACCACCCGC  
3481 gcgagaggggacccccgagcagggccgcatgaccaacaagatgaagagcaccaaaggcg 3540  
CGCCTCTCCCGTGGGGGCTCGTCCCGGCGTACTGGTTGTTCTACTTCTCGTGGTTTCCGC  
3541 ccctgaccttcagcccctacctgctgagccacgtgatgggctacggcttctaccacttcg 3600  
GGGACTGGAAGTCGGGGATGGACGACTCGGTGCACTACCCGATGCCGAAGATGGTGAAGC  
3601 gcacctaccccagcggctacgagaacccttccctgcacgcatcaacaacggcggctaca 3660  
CGTGGATGGGGTCCCGATGCTCTTGGGAAGGACGTGCGGTAGTTGTTGCCGCCGATGT  
3661 ccaacaccgcatcgagaagtagcaggacggcggcgtgctgcacgtgagcttcagctacc 3720  
GGTTGTGGGCGTAGCTCTTCATGCTCCTGCCGCCGACGACGTGCACTCGAAGTCGATGG  
3721 gctacgaggccggccgctgatcggcgacttcaaggtgatgggaccggcttccccgagg 3780  
CGATGCTCCGGCCGGCGCACTAGCCGCTGAAGTTCCACTACCCGTGGCCGAAGGGGCTCC  
3781 acagcgtgatcttcaccgacaagatcatccgcagcaacgccaccgctggagcacctgcacc 3840  
TGTCGCACTAGAAGTGGCTGTTCTAGTAGGCGTCGTTGCGGTGGCACCTCGTGGACGTGG  
3841 ccatgggcgataaacgatctggatggcagcttcaccgcaccttcagcctgcgcgacggcg 3900  
GGTACCCGCTATTGCTAGACCTACCGTCGAAGTGGGCGTGGAAGTCGGACGCGCTGCCGC  
3901 gctactacagctccgtggtggacagccacatgcacttcaagagcgccatccaccccagca 3960  
CGATGATGTCGAGGCACCACCTGTCCGGTGTACGTGAAGTTCTCGCGGTAGGTGGGGTCTG  
3961 tcctgcagaacgggggccccatgttcgcttccgcgcgctggaggaggatcacagcaaca 4020  
AGGACGTCTTGCCCCGGGGTACAAGCGGAAGGCGGCGCACCTCCTCCTAGTGTGTTGT  
4021 ccgagctgggcatcgtggagtaccagcacgccttcaagaccccggatgcagatgccgggtg 4080  
GGCTCGACCCGTAGCACCTCATGGTCGTGCGGAAGTTCTGGGGCCTACGTCTACGGCCAC

NotI IRES reg(4114,4689)>>>

4081 aagaataatgtacaagtagcggccgcaaattccgccccctctccctccccccccctaacg 4140  
TTCTTATTACATGTTTCATCGCCGGCGTTTAAAGCGGGGAGAGGGAGGGGGGGGATTGC  
4141 ttactggccgaagccgcttgggaataaggccgggtgtgctgttctatatgttattttcca 4200  
AATGACCGGCTTCGGCGAACCTTATTCCGGCCACACGCAAACAGATATAACAATAAAAGGT  
4201 ccatattgcccgtcttttggcaatgtgagggcccgaaacctggccctgtcttcttgacga 4260  
GGTATAACGGCAGAAAACCGTTACTCCCGGGCCTTTGGACCGGGACAGAAGAAGTCTGCT  
4261 gcattcctaggggtctttccccctctcgccaaaggaatgcaaggtctgttgaatgtcgtga 4320  
CGTAAGGATCCCCAGAAAGGGGAGAGCGGTTTTCCTTACGTTCCAGACAACCTTACAGCACT

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4321 aggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgaccctttgca 4380  
TCCTTCGTCAAGGAGACCTTCGAAGAACTTCTGTTTGTTCAGACATCGCTGGGAAACGT

4381 ggcagcggaacccccacctggcgacaggtgcctctgcgccaaaagccacgtgtataag 4440  
CCGTGCGCTTGGGGGGTGGACCGCTGTCCACGGAGACGCCGGTTTTTCGGTGCACATATTC

4441 atacacctgcaaaggcggcacaacccccagtgccacgttgtgagttggatagttgtggaaa 4500  
TATGTGGACGTTTTCCGCCGTGTTGGGGTACGGTGAACACTCAACCTATCAACACCTTT

4501 gagtcaaattggctctcctcaagcgtattcaacaaggggctgaaggatgccagaaggtac 4560  
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4561 cccattgtatgggatctgatctggggcctcgggtgcacatgctttacatgtgttttagtga 4620  
GGGTAACATAACCCTAGACTAGACCCCGGAGCCACGTGTACGAAATGTACACAAATCAGCT

4621 ggttaaaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaaac 4680  
CCAATTTTTTTTGCAGATCCGGGGGGCTTGGTGGCCCTGCACCAAAGGAAACTTTTTGTG

PuroR marker (4696, 5292) >>>  
|

4681 gataataccatggccaccgagtacaagcccacgggtgcgccctcgccaccgagcagcgtc 4740  
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4741 ccccgggccgtacgcaccctcgccgcccgcgttcgcccactaccccgccacgcgccacacc 4800  
GGGGCCCCGCATGCGTGGGAGCGGGCGCAAGCGGCTGATGGGGCGGTGCGCGGTGTGG

4801 gtcgaccggaccgccacatcgagcgggtcaccgagctgcaagaactcttcctcacgcgc 4860  
CAGCTGGGCCTGGCGGTGTAGCTCGCCCAGTGGCTCGACGTTCTTGAGAAGGAGTGC GCG

4861 gtcgggctcgacatcggcaaggtgtgggtcgcgacgacggcgcccggtggcggtctgg 4920  
CAGCCCCGAGCTGTAGCCGTTCCACACCCAGCGCCTGCTGCCGCGGCCACCGCCAGACC

4921 accacgcccggagagcgtcgaagcggggcggtgttcgcccagatcggctcgcgcatggcc 4980  
TGGTGCGGCCTCTCGCAGCTTCGCCCCCGCCACAAGCGGCTCTAGCCGAGCGCGTACCGG

4981 gagttgagcgggttcccggctggccgcgagcaacagatggaaggcctcctggcgccgcac 5040  
CTCAACTCGCCAAGGGCCGACCGGCGCGTCTGTTGTCTACCTTCCGGAGGACCGCGGCGTG

5041 cggcccaaggagcccgcgtgggttctggccaccgtcggcgtctcgcccgaccaccagggc 5100  
GCCGGGTTCTCGGGCGCACCAAGGACCGGTGGCAGCCGAGAGCGGGCTGGTGGTCCCC

5101 aagggtctgggcagcgcctcgtgctccccggagtgaggcggccgagcgcgctgggggtg 5160  
TTCCCAGACCCGTCGCGGCAGCACGAGGGGCCTCACCTCCGCCGGCTCGCGGACCCAC

5161 cccgccttctggagacctccgcgccccgcaacctccccttctacgagcgggtcggcttc 5220  
GGGCGGAAGGACCTCTGGAGGCGCGGGGCGTTGGAGGGGAAGATGCTCGCCGAGCCGAAG

5221 accgtcaccgcccagcgtcgaggtgccgaaggaccgcgacactggtgcatgaccgcaag 5280  
TGGCAGTGGCGGCTGCAGCTCCACGGGCTTCTGGCGCGTGGACCACGTA TGGGCGTTC



```

5821  tgtggatacgtgcttttaaatgcctttgtatcatgctattgcttcccgtatggcttttcatt 5880
      ACACCTATGCGACGAAATTACGGAAACATAGTACGATAACGAAGGGCATAACCGAAAGTAA

5881  ttctcctccttgtataaatcctgggtgctgtctctttatgaggagtgtgtggcccgttgtc 5940
      AAGAGGAGGAACATATTTAGGACCAACGACAGAGAAATACTCCTCAACACCGGGCAACAG

5941  aggcaacgtggcgtggtgtgactgtgtttgctgacgcaacccccactggttggggcatt 6000
      TCCGTTGCACCGCACCACACGTGACACAAACGACTGCGTTGGGGGTGACCAACCCCGTAA

6001  gccaccacctgtcagctcctttccgggactttcgtttccccctccctattgccacggcg 6060
      CCGTGGTGGACAGTCGAGGAAAGGCCCTGAAAGCGAAAGGGGGAGGGATAACGGTGCCGC

6061  gaactcatcgccgcctgccttgcccgtgctggacaggggctcggctggtgggactgac 6120
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6121  aattccgtggtggtgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgcc 6180
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6181  acctggattctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggac 6240
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6241  cttccttcccgcggcctgctgcccgtctgcccctcttccgcgtcttcgccttcgcct 6300
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6301  cagacgagtcggatctccctttgggcccctccccgcctggaattaattctgcagtcgag 6360
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6361  acctagaaaaacatggagcaatcacaagtagcaatacagcagctaccaatgctgattgtg 6420
      TGGATCTTTTTGTACCTCGTTAGTGTTTCATCGTTATGTCTGTCGATGGTTACGACTAACAC

6421  cctggctagaagcacaagaggaggagggtgggttttccagtcacacctcaggtacctt 6480
      GGACCGATCTTCGTGTTCTCCTCCTCCTCCACCCAAAAGGTCAGTGTGGAGTCCATGGAA

6481  taagaccaatgacttacaaggcagctgtagatcttagccactttttaaaagaaaagaggg 6540
      ATTCTGGTTACTGAATGTTCCGTCGACATCTAGAATCGGTGAAAAATTTTCTTTTCTCCC

      3'SIN-LTR(Lenti) other(6544,6779)>>>
      |
      delta_U3 reg(6544,6596)>>>
LTR) reg(6599,6693)>>>
      |
      |
6541  gactggaagggctaattcactcccaacgaagacaagatctgctttttgcttgtactgggt 6600
      CTGACCTTCCCGATTAAGTGAGGGTTGCTTCTGTTCTAGACGAAAAACGAACATGACCCA

6601  ctctctggtttagaccagatctgagcctgggagctctctgggtaactagggaaacctgc 6660
      GAGAGACCAATCTGGTCTAGACTCGGACCCTCGAGAGACCGATTGATCCCTTGGGTGACG

      U5(HIV-LTR) reg(6694,6778)>>>
      |
6661  ttaagcctcaataaagcttgcttgagtgcttcaagtagtggtgcccgtctgttggtg 6720
      AATTCCGAGTTATTTTGAACGGAACCTCACGAAGTTCATCACACACGGGCAGACAACACAC

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6721 actctggtaactagagatccctcagacccttttagtcagtggtggaaaatctctagcagta 6780
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6781 gtagttcatgtcatcttattattcagatatttataaacttgcaaagaaatgaatatcagaga 6840
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                                BGH-polyA reg(6868,7091)>>>
                                |
6841 gtgagaggccttgacattgtttaaaccgcgctgatcagcctcgactgtgccttctagttgc 6900
CACTCTCCGGAAGTGAACAAATTTGGGCGACTAGTCGGAGCTGACACGGAAGATCAACG

6901 cagccatctgttgtttgcccctccccgcgcttccttgaccctggaaggtgccactccc 6960
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TGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGA

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7081 catgctgggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctct 7140
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                                f1 origin(7171,7477)>>>
                                |
7141 aggggggatccccacgcgcctgtagcggcgcatthaagcgcggcggggtgtggtggttacg 7200
TCCCCCATAGGGGTGCGCGGGACATCGCCGCGTAATTCGCGCCGCCACACCACCAATGC

7201 cgcagcgtgaccgctacacttgccagcgccttagcgcggcctcctttcgctttcttcct 7260
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7261 tcctttctcgccacgcttcgcccgtttccccgctcaagctctaaatcgggggctcccttta 7320
AGGAAAGAGCGGTGCAAGCGGCCGAAAGGGGCAGTTCGAGATTTAGCCCCGAGGGAAAT

7321 gggttccgatttagtgctttacggcacctcgacccccaaaaaacttgattaggggtgatggt 7380
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7441 ttctttaatagtgactcctgttccaaactggaacaacactcaaccctatctcggctctat 7500
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7501 tcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatt 7560
AGAAAATAAATATTCCTAAAACGGCTAAAGCCGGATAACCAATTTTTTACTCGACTAA

```

```

Enhancer(DTS) other(7609,7680)>>>

```

```
SV40-
```

```
prom(7609,7930)>>>
```

```
|
SV40
```

```
|
```

```

7561 taacaaaaatttaacgcgaattaattctgtggaatgtgtgtcagttagggtgtggaaagt 7620
ATTGTTTTTAAATTGCGCTTAATTAAGACACCTTACACACAGTCAATCCCACACCTTTCA

7621 ccccaggctcccagcagggcagaagatgcaaagcatgcatctcaattagtcagcaacca 7680
GGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGT

SV40-Enhancer(DTS) other(7681,7752)>>>
|
7681 ggtgtggaaagtcggcaggctcccagcagggcagaagatgcaaagcatgcatctcaatt 7740
CCACACCTTTCAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAA

SV40 origin(7776,7853)>>>
|
7741 agtcagcaaccatagtcggccccaactccgcccataccgcccctaactccgcccagtt 7800
TCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAA

7801 ccgcccattctccgccccatggctgactaattttttttatgtatgcagagggccgaggccg 7860
GGCGGGTAAGAGGCGGGGTACCGACTGATTAATAAATAAATACGTCTCCGGCTCCGGC

7861 cctctgcctctgagctattccagaagtagtgaggaggcttttttgaggcctaggctttt 7920
GGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAACCTCCGGATCCGAAAA

HygroR
marker(7979,8996)>>>
|
7921 gcaaaaagctcccgggagcttgtatatccattttcggatctgatcagcacgtgatgaaaa 7980
CGTTTTTCGAGGGCCCTCGAACATATAGGTAAAAGCCTAGACTAGTCGTGCACTACTTTT

7981 agcctgaactcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtct 8040
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8041 ccgacctgatgcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggag 8100
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8101 ggcgtggatagtcctgccccgtaaatagctgccccgatggtttctacaaagatcgttatg 8160
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8161 tttatcggcactttgcatcggccgcgctcccgatccggaagtgcttgacattggggaaat 8220
AAATAGCCGTGAAACGTAGCCGGCGGAGGGCTAAGGCCTTACGAACTGTAACCCCTTA

8221 tcagcgagagcctgacctattgcatctcccgccgtgcacaggggtgtcacggttgcaagacc 8280
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8281 tgcctgaaaccgaactgccccgctgttctgcagccggctcgcggaggccatggatgcatcg 8340
ACGGACTTTGGCTTGACGGGCGACAAGACGTCCGCCAGCGCCTCCGGTACCTACGCTAGC

8341 ctgccccgatcttagccagacgagcgggttcggcccattcggaccgcaaggaatcggtc 8400
GACGCCGGCTAGAATCGGTCTGCTCGCCCAAGCCGGGTAAGCCTGGCGTTCCTTAGCCAG

8401 aatacactacatggcgtgatttcatatgcgcgattgctgatccccatgtgtatcactggc 8460
TTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCG

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8461 aaactgtgatggacgacaccgtcagtgcggtccgtcgcgccaggctctcgatgagctgatgc 8520  
 TTTGACTACTGCTGTGGCAGTCACGCAGGCAGCGCTCCGAGAGCTACTCGACTACG

8521 tttgggcccaggactgccccgaagtccggcacctcgtgcacgaggatttcgggtccaaca 8580  
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8581 atgtcctgacggacaatggccgcataacagcgggtcattgactggagcggagcggatggttcg 8640  
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8701 agcagcagacgcgctacttcgagcggaggcatccggagcttgaggatcgccgaggctcc 8760  
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8761 gggcgatatatgctccgcattggctcttgaccaactctatcagagcttggttgacggcaatt 8820  
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8821 tcgatgatgcagcttgggcccagggtcgatgacgacgcaatcgtccgatccggagccggga 8880  
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8881 ctgtcgggcgtagacacaaatcgcccgcagaagcgcggccgtctggaccgatggctgtgtag 8940  
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8941 aagtactcgccgatagtggaaccgacgccccagcactcgtccgagggcaaaggaatagc 9000  
 TTCATGAGCGGCTATCACCTTTGGCTGCGGGGTCGTGAGCAGGCTCCCGTTTCCTTATCG

9001 acgtgctacgagatthtcgattccaccgcccgccttctatgaaaggttgggcttcggaatcg 9060  
 TGCACGATGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGC

9061 ttttcggggacgcccggctggatgatcctccagcgcggggatctcatgctggagttcttcg 9120  
 AAAAGGCCCTGCGGCCGACCTACTAGGAGGTGCGGCCCTAGAGTACGACCTCAAGAAGC

SV40-polyA-signal

reg(9160,9194)>>>

9121 cccaccccaacttgthttattgcagcttataatggttacaaataaagcaatagcatcacia 9180  
 GGGTGGGGTTGAACAAATAACGTCGAATATTACCAATGTTTATTTTCGTTATCGTAGTGT

9181 atttcaciaaataaagcatttttttactgcatctagttgtggthttgtccaaactcatca 9240  
 TAAAGTGTTHATTTTCGTAATAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGT

9241 atgtatcttatcatgtctgtataaccgctcgacctctagctagagcttggcgtaatcatggt 9300  
 TACATAGAATAGTACAGACATATGGCAGCTGGAGATCGATCTCGAACCGCATTAGTACCA

lac prom(9342,9425)<<<

9301 catagctgtttcctgtgtgaaattgthtccgctcacaattccacacaacatacagagccg 9360  
 GTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGC

9361 gaagcataaagtgtaaagcctgggggtgcctaataagagtgagctaaactcacattaattgct 9420  
 CTTCTGATTTTACATTTTCGGACCCACGGATTACTCACTCGATTGAGTGTAATTAACGCA

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9421 tgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcg 9480  
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9481 gccaacgcgcggggagagggcggtttgcgatttggcgctcttccgcttcctcgctcactg 9540  
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9541 actcgctgcgctcggtcggttcggctgcccgcgagcggtatcagctcactcaaaggcggtaa 9600  
TGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTCCGCCATT

9601 tacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc 9660  
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pUC origin(9686,10305)<<<  
|

9661 aaaaggccaggaaccgtaaaaaggccgcttgcgtggcgctttttccataggctccgcccc 9720  
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9721 ctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactat 9780  
GACTGCTCGTAGTGTCTTTTAGCTGCGAGTTTCACTCTCCACCGCTTTGGGCTGTCTGATA

9781 aaagataccaggcggtttccccctggaagctccctcgtgcgctctcctggtccgaccctgc 9840  
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9841 cgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagct 9900  
GCGAATGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTATCGA

9901 cacgctgtaggtatctcagttcgggtgtaggtcgcttcgctccaagctgggctgtgtgcacg 9960  
GTGCGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTCCGACCCGACACACGTGC

9961 aaccccccgttcagcccagccgctgcgcccttatccggtaactatcgtcttgagtccaacc  
10020 TTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGG

10021 cggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga  
10080 GCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCT

10081 ggtatgtaggcggtgctacagagttcttgaagtggcctaactacggctacactagaa  
10140 CCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTT

10141 gaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggta  
10200 CTTGTCATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTTTCTCAACCAT

10201 gctcttgatccggcaaaaccaccgctggtagcggtggttttttggtttgcaagcagc  
10260 CGAGAACTAGGCCGTTTGTGTTGGTGGCGACCATCGCCACCAAAAAACAAACGTTTCGTCG

10261 agattacgcgcagaaaaaaggatctcaagaagatcctttgatcttttctacggggctcg  
10320 TCTAATGCGCGTCTTTTTTTTCTAGAGTTCTTCTAGGAACTAGAAAAGATGCCCCAGAC

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10321 acgctcagtggaacgaaaactcacgttaagggatTTTGGTcatgagattatcaaaaagga  
 10380 TCGGAGTCACCTTGCTTTTGGAGTGCAATTCCTAAAACAGTACTCTAATAGTTTTTCCT  
  
 10381 tcttcacctagatccttttaaatataaaatgaagtttttaaatcaatctaaagtatatatg  
 10440 AGAAGTGGATCTAGGAAAATTTAATTTTTACTTCAAATTTAGTTAGATTTTCATATATAC  
  
 AmpR marker(10460,11320)<<<  
 |  
 10441 agtaaacttggctctgacagttaccaatgcttaatcagtgaggcacctatctcagcgcgct  
 10500 TCATTTGAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGA  
  
 10501 gtctatTTTcgTtcatccatagttgcctgactccccgTcgtgtagataactacgatacggg  
 10560 CAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGATGCTATGCC  
  
 10561 agggcttaccatctggccccagtgctgcaatgataccgagagaccacgctcaccggctc  
 10620 TCCCGAATGGTAGACCGGGTACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAG  
  
 10621 cagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggctcctgcaa  
 10680 GTCTAAATAGTCGTTATTTGGTCGGTCGGCCTTCCCGCTCGCGTCTTACCAGGACGTT  
  
  
 10681 ctttatccgcctccatccagctctattaattgTtgccgggaagctagagtaagtagttcgc  
 10740 GAAATAGGCGGAGGTAGGTACAGATAATTAACAACGGCCCTTCGATCTCATTTCATCAAGCG  
  
 10741 cagTtaatagtttgcgcaacgTtgTtgccattgctacaggcatcgtggTgtcacgctcgt  
 10800 GTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACCACAGTGCAGCA  
  
 10801 cgTttggTatggcttcattcagctccggttcccaacgatcaaggcagttacatgatccc  
 10860 GCAAACCATACCGAAGTAAGTCGAGGCCAAGGGTTGCTAGTTCGCTCAATGTACTAGGG  
  
 10861 ccatgTtgTgcaaaaaagcggttagctccttcggtcctccgatcgtTgtcagaagtaagt  
 10920 GGTACAACACGTTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCA  
  
 10921 tggccgcagtgTtatcactcatggttatggcagcactgcataattctcttactgtcatgc  
 10980 ACCGGCGTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACG  
  
 10981 catccgtaagatgcttttctgtgactggTgagtactcaaccaagtcatctgagaatagt  
 11040 GTAGGCATTCTACGAAAAGACACTGACCACTCATGAGTTGGTTCAGTAAGACTCTTATCA

11041 gtatgcgggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacata  
 11100 CATACGCCGCTGGCTCAACGAGAACGGGCCGAGTTATGCCCTATTATGGCGCGGTGTAT  
  
 11101 gcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaagga  
 11160 CGTCTTGAAATTTTCACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCCT  
  
 11161 tcttaccgctggttgagatccagttcgatgtaaccactcgtgcacccaactgatcttcag  
 11220 AGAATGGCGACAACCTCTAGGTCAAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTC  
  
 11221 catcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaa  
 11280 GTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTTGTCCTTCCGTTTTACGGCGTT  
  
 11281 aaaaggaataagggcgacacggaaatggtgaatactcatactcttcctttttcaatatt  
 11340 TTTTCCCTTATTCCCGCTGTGCCTTTACAACCTTATGAGTATGAGAAGGAAAAAGTTATAA  
  

Bacterial-Promoter prom(11362,11400)<<<  
 |

 11341 attgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttaga  
 11400 TAACTTCGTAAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCT  
  
 11401 aaaataaaciaaataggggttccgcgcacatttccccgaaaagtgccacctgacgtcgacg  
 11460 TTTTATTTGTTTATCCCCAAGGCGCGTGTAAAGGGGCTTTTCACGGTGGACTGCAGCTGC  
  

SV40-polyA-signal

 reg(11504,11538)>>>  
  

|

 11461 gatcgggagatcaacttgtttattgcagcttataatggttacaaataaagcaatagcatc  
 11520 CTAGCCCTCTAGTTGAACAAATAACGTGCAATATTACCAATGTTTATTTTCGTTATCGTAG  
  
 11521 acaaatttcacaaataaagcatttttttactgcattctagttgtgggttgtccaaactc  
 11580 TGTTTAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAG  
  
 11581 atcaatgtatcttatcatgtctggatcaactggataactcaagctaaccaaaatcatccc  
 11640 TAGTTACATAGAATAGTACAGACCTAGTTGACCTATTGAGTTCGATTGGTTTTAGTAGGG  
  
 11641 aaacttcccacccataccctattaccactgccaattacctgtggtttcatttactctaa  
 11700 TTTGAAGGGTGGGGTATGGGATAATGGTGACGGTTAATGGACACCAAAGTAAATGAGATT  
  
 11701 acctgtgattcctctgaattatctttcatttttaagaaattgtatttgtaaatatgtact  
 11760 TGGACACTAAGGAGACTTAATAAAAGTAAATTTCTTTAACATAAACAATTTATACATGA

11761 acaaacttagtagt 11774  
TGTTTGAATCATCA

Restriction analysis of pGIPZ lentiviral vector

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**AhdI** (GACnn\_n'nnGTC) [Eam1105I,AspEI,DriI,EclHKI]  
Cuts 1 time.  
Cuts at position 10533.  
Fragment sizes 10533, 1241.

**AleI** (CACnn'nnGTG) [OliI]  
Cuts 1 time.  
Cuts at position 1577.  
Fragment sizes 1577, 10197.

**AlOI** (GAACnnnnnnTCCnnnnnnn\_nnnnn')  
Cuts 1 time.  
Cuts at position 7423.  
Fragment sizes 7423, 4351.

**AlOI** (GGAnnnnnnGTTcnnnnnnn\_nnnnn')  
Cuts 1 time.  
Cuts at position 7455.  
Fragment sizes 7455, 4319.

**AsiSI** (GCG\_AT'CGC) [SgfI]  
Cuts 1 time.  
Cuts at position 8338.  
Fragment sizes 8338, 3436.

**BbvCI** (CC'TCA\_GC)  
Cuts 1 time.  
Cuts at position 1424.  
Fragment sizes 1424, 10350.

**BlpI** (GC'TnA\_GC) [Bpu1102I,Bsp1720I,CelIII]  
Cuts 1 time.  
Cuts at position 3564.  
Fragment sizes 3564, 8210.

**Bpu10I** (CC'TnA\_GC)  
Cuts 1 time.  
Cuts at position 1424.  
Fragment sizes 1424, 10350.

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**BsaBI** (GATnn'nnATC) [Bse8I,BseJI,MamI]  
[dam methylated]  
Cuts 1 time.  
Cuts at position [3853].  
Fragment sizes 3853, 7921.

**BsiWI** (C'GTAC\_G) [Pfl123II,PspLI,SunI]  
Cuts 1 time.  
Cuts at position 4749.  
Fragment sizes 4749, 7025.

**BsrGI** (T'GTAC\_A) [Bsp1407I,BstAUI,SspBI]  
Cuts 1 time.  
Cuts at position 4089.  
Fragment sizes 4089, 7685.

**BstEII** (G'GTnAC\_C) [BstPI,Eco91I,EcoO65I,PspEI]  
Cuts 1 time.  
Cuts at position 4827.  
Fragment sizes 4827, 6947.

**BstZ17I** (GTA'TAC) [BssNAI,Bst1107I]  
Cuts 1 time.  
Cuts at position 9261.  
Fragment sizes 9261, 2513.

**Bsu36I** (CC'TnA\_GG) [AxyI,Bse21I,Eco81I]  
Cuts 1 time.  
Cuts at position 6469.  
Fragment sizes 6469, 5305.

**CspCI** (CAAnnnnnGTGGnnnnnnnnnn\_nn')  
Cuts 1 time.  
Cuts at position 3141.  
Fragment sizes 3141, 8633.

**CspCI** (CCACnnnnnTTGnnnnnnnnnn\_nn')  
Cuts 1 time.  
Cuts at position 3106.  
Fragment sizes 3106, 8668.

**EcoNI** (CCTnn'n\_nnAGG) [BstENI,XagI]  
Cuts 1 time.  
Cuts at position 1170.  
Fragment sizes 1170, 10604.

**FspI** (TGC'GCA) [Acc16I,AviIII,NsbI]  
Cuts 1 time.  
Cuts at position 10755.  
Fragment sizes 10755, 1019.

**HpaI** (GTT'AAC) [KspAI]  
Cuts 1 time.  
Cuts at position 5376.  
Fragment sizes 5376, 6398.

**MluI** (A'CGCG\_T)  
Cuts 1 time.  
Cuts at position 5736.  
Fragment sizes 5736, 6038.

**NotI** (GC'GGCC\_GC) [CciNI]  
Cuts 1 time.  
Cuts at position 4100.  
Fragment sizes 4100, 7674.

**NruI** (TCG'CGA) [Bsp68I]  
[dam methylated]  
Cuts 1 time.  
Cuts at position [833].  
Fragment sizes 833, 10941.

**PmeI** (GTTT'AAAC) [MssI]  
Cuts 1 time.  
Cuts at position 6862.  
Fragment sizes 6862, 4912.

**PpuMI** (rG'GwC\_Cy) [PpuXI,Psp5II,PspPPI]  
[dcm methylated]  
Cuts 1 time.  
Cuts at position 1934.  
Fragment sizes 1934, 9840.

**PshAI** (GACnn'nnGTC) [BoxI,BstPAI]  
Cuts 1 time.  
Cuts at position 8001.  
Fragment sizes 8001, 3773.

**SanDI** (GG'GwC\_CC)  
Cuts 1 time.  
Cuts at position 1934.  
Fragment sizes 1934, 9840.

**SfiI** (GGCCn\_nnn'nGGCC)

[dcm methylated]

Cuts 1 time.

Cuts at position 2621.

Fragment sizes 2621, 9153.

**SgrAI** (Cr'CCGG\_yG)

Cuts 1 time.

Cuts at position 2500.

Fragment sizes 2500, 9274.

**SnaBI** (TAC'GTA) [BstSNI,Eco105I]

Cuts 1 time.

Cuts at position 3070.

Fragment sizes 3070, 8704.

**SspI** (AAT'ATT)

Cuts 1 time.

Cuts at position 11337.

Fragment sizes 11337, 437.

**XbaI** (T'CTAG\_A)

[dam methylated]

Cuts 1 time.

Cuts at position 2707.

Fragment sizes 2707, 9067.

**XhoI** (C'TCGA\_G) [BssHI,Paer7I,Sfr274I,SlaI,StrI,TliI]

Cuts 1 time.

Cuts at position 5391.

Fragment sizes 5391, 6383.

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