siRNA Cloning Kit

Description

The siRNA Cloning Kit is designed for inducing small interference RNA(siRNA) in mammalian cells driven by a humanU6 RNA polymerase III promoter. For gene silencing, a small DNA oligonucleotide insert is cloned into the vector downstream of the U6 promoter. Once transfected into mammalian cells, the insert-containing vector directs the transcription of a short hairpin RNA, which hybridizes to the target RNA and directs target degradation by enzymatic cleavage. The PsiRNA vector in the kit is pre-linearized to facilitate directional cloning. Oligonucleotides encoding siRNA sequences with complementary overhangs can be readily ligated into the vectors and used to transform *E. coli* to generate plasmids for siRNA studies. Each kit offers enough components for 20 ligations.

Features

- **Directional cloning of synthesized oligonucleotides into the vector**
- **No restriction enzymes needed**
- **No PCR needed to avoid mutations**
- **Low non-recombinant background; >95% recombinants**
- **Simple protocol**
- **Transient expression of siRNA in mammalian cells for quick cell function assays or**
- **Very fast establish of stable siRNA expression cell lines for long term cell function studies**

Components

Pre-linearized PsiRNA vector (20 ligations)
siRNA control insert (20 ligations)

Strategy

1. Choose siRNA target sequence

GCATGAACCGGAGGCCCAT

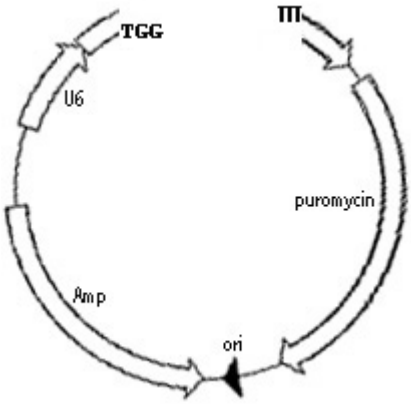
2. Synthesize oligonucleotides (5' phosphorylation is not necessary)

```
5'ACCGCATGAACCGGAGGCCCATCTTCCTGTCAATGGGCCTCCGGTTCATGC 3'
                                 +
   3'CGTACTTGGCCTCCGGGTAGAAGGACAGTTACCCGGAGGCCAAGTACGAAA 5'
```

3. Anneal oligonucleotides

ACCGCATGAACCGGAGGCCCATCTTCCTGTCAATGGGCCTCCGGTTCATGC
CGTACTTGGCCTCCGGGTAGAAGGACAGTTACCCGGAGGCCAAGTACGAAA

4. Ligate into the pre-linearized PsiRNA vector



5. Transformation into competent cells

6. Plasmid purification

7. Verify recombinants

8. Transfect recombinants into mammalian cells

9. Transcription by RNA pol III

GCAUGAACCGGAGGCCCAUCUUCCUGUCAAUGGGCCUCCGGUUCAUGCUUU

10. Formation of hairpin siRNA

```
                              CUUC
      GCAUGAACCGGAGGCCCAU       C
UUUCGUACUUGGCCUCCGGGUA          U
                              ACUG
```

11. Transient expression or select stable transfected cells

12. Assay target gene silence

PROTOCAL

1. Cloning

1. Assemble the following reaction:

5 ul	PsiRNA vector
14 ul (0.5pm)	Annealed oligonucleotide
1 ul	T4 DNA ligase (1 U)
20 ul	total volume

   Mix gently and quick spin
2. Incubate the reaction at room temperature for 30 min or at 4 degree Overnight
3. Transformation
4. Follow standard protocols to verify insert and purify plasmid

2. Transient Transfection

1. PsiRNA vector can be transfected into mammalian cells with a variety of methods and reagents. Mirus offers a broad range of Transfection Reagent. Please follow manufacturer's protocols.

2. Harvest the cells at appropriate days after transfection and assay for the target gene silencing

3. Stable Transfection

A. Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing siRNA from siRNA products, you need to determine the minimum concentration of puromycin required to kill your untransfected host cell line. Typically, concentrations ranging from 1 to 10 ug/ml puromycin are sufficient to kill most untransfected mammalian cell lines. We recommend that you test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your host cell line.

1. Culture cells in 96 well plate and let them to grow overnight to reach 50% confluent.
2. The next day, change culture medium with medium containing varying concentrations of puromycin (0, 2, 4, 6, 8, 10 ug/ml).
3. Count the number of viable cells at regular intervals to determine the appropriate concentration of puromycin that kills untransfected cell within 1 to 2 days after addition of puromycin.

B. Stable Cell line Selection

Once you have determined the appropriate puromycin concentration to use for selection in your host cell line, you can generate a stable cell line expressing your siRNA.

1. 48 hours after transfection, split the cells into fresh medium containing puromycin at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
2. Feed the cells with selective medium every 3–4 days until puromycin-resistant foci can be identified.
3. Pick and expand colonies in 96 well plate or pool the colonies.
4. Harvest the cells and assay for the target gene silencing with Vicgen's Gene Expression Quantitative Kits or Western blot