psiRNA-h7SKneo G1 Kit
A simple and innovative tool to create siRNAs
Catalog # ksiRNA3-n21
For research use only
Version # 14L08-MM

PRODUCT INFORMATION

Content:
- 20 µg of lyophilized psiRNA-h7SKneo G1 plasmid
- 20 µg of lyophilized psiRNA-h7SKnEGFP, a control plasmid expressing a siRNA targeting the EGFP gene
- siEGFP sequence: GCAAGCGTGGCAGGAAUCC
  uuCGUUGCACUGGCCUCAAGU

siLucGL3 Hairpin

GACUUACGCUAGACUCUUGCA
uuCUGAUGCGACUCAUGAAGCU

siEGFP Hairpin

GCAAGCGTGGCAGGAAUCC
uuCGUUGCACUGGCCUCAAGU

- 1 disk of lyophilized GT116 E. coli bacteria
  GT116 genotype: F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZM15 ΔlacX74 recA1 endA1 ΔsbcC-sbcD
- 10 µg of lyophilized OL559 primer (forward)
  OL599 sequence: 5’ CGATAAGTAACCTTGACCTAAGTG 3’ (23 mer)
  MW: 7072 g/mol. Tm: 57.1°C
- 10 µg of lyophilized OL408 primer (reverse)
  OL408 sequence: 5’ GCGTTACTAAGGGAACAC 3’ (20 mer)
  MW: 6141 g/mol. Tm: 55.3°C
- 4 pouches of Fast-Media® Kan XGal

Storage and stability:
- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20 °C. Avoid repeated freeze-thaw cycles.
- The disk of lyophilized GT116 E. coli bacteria should be stored at -20 °C.
- Store sequencing primers at -20 °C.
- Store Fast-Media® Kan XGal at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:
- Plasmid DNA was prepared using affinity column and lyophilized.
- Plasmid construct has been confirmed by restriction analysis.
- The sequence between Cla I and Xba I restriction sites has been verified by sequencing.

OVERVIEW
RNA interference (RNAi) has recently emerged as a powerful technology for studying gene functions in eukaryotes. RNAi is a post-transcriptional process triggered by the introduction of small interfering RNA (siRNA) which leads to gene silencing in a sequence-specific manner. siRNAs consist of 19-21 mer double-stranded RNA with 2 nt 3’ overhangs, or a 43-51 mer RNA molecule with a hairpin structure, called short hairpin RNA (shRNA) which are homologous to a region within the target gene. Introduction of siRNAs in mammalian cells induces strong and specific suppression of the gene of interest. However, this effect is only transient due to the short lifespan of synthetic RNAs, which severely limits the applications of siRNAs. To overcome this limitation, Invivogen has designed an efficient and simple-to-use vector, called psiRNA, that allows the production of siRNAs within the cells.

GENERAL PRODUCT USE
psiRNA is specifically designed for the cloning of small synthetic oligonucleotides (around 50-mer) that encode two complementary sequences of 21 nt, homologous to a segment of the gene of interest, separated by a short spacer region of 5-9 nt. The insert is cloned downstream of a RNA polymerase III promoter, the human 7SK or H1 promoter. It is transcribed into a short dsRNA with a hairpin structure (shRNA) consisting of a 21 bp double stranded region corresponding to the target sequence and a small loop formed by the spacer region.

psiRNA exploits the white-blue selection system. The cloning sites flank a bacterial lacZ α-peptide cassette allowing the discrimination between blue parental clones and white recombinant clones in E. coli. Although over 90% of the white clones have integrated a fragment, it is necessary to sequence the insert to verify the integrity of the sequence since a single base difference can lead to an inactive siRNA.

psiRNA-h7SK G1 plasmids offer two cloning options:
- Bbs I / Bbs I (although these sites are recognized by the same enzyme, they are different avoiding self-ligation of the plasmid)
- Acc 65I / Hind III

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
PLASMID FEATURES

- **7SK prom**: 7SK is an abundant and evolutionarily conserved small nuclear RNA discovered in the mid-70s. It is transcribed by RNA polymerase III from one or more genes belonging to a family of interspersed repeats in the mammalian genome. The high conservation and abundance of 7SK suggest an important physiological function of this RNA. Recent studies suggest that 7SK acts as a negative regulator of the RNA polymerase II elongation factor P-TEFb, and that this activity is influenced by stress response pathways. The human 7SK promoter presents a strong permissivity for the nucleotide in the +1 position and recognizes a cluster of 4 or more T residues as a termination signal. This promoter is ideal for the production of shRNAs as it can generate high amounts of shRNAs.
- **EM7-alpha-peptide**: The EM7-lacZ α-peptide cassette is a bacterial expression cassette enabling white/blue selection. Transformation of permisive bacterial strains, such as strains harboring the lacZαM15 allele, leads to alpha-complementation which results in the production of shRNAs as it can generate high amounts of shRNAs.
- **CMV enh / prom**: The human cytomegalovirus immediate-early gene 1 enhancer/promoter was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Neo**: The neo gene from Tn5 encodes an aminoglycoside 3'-phosphotransferase (3' APH II) that confers resistance to the antibiotics kanamycin in bacteria and G418 in mammalian cells. The neo gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **βGlo pAn**: The human beta-globin 3'UTR and polyadenylation sequence allow efficient arrest of the transgene transcription.
- **pMB1 Ori**: is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

**References**


**METHODS**

**Plasmid resuspension**

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 μg/μl, resuspend the DNA in 20 μl of sterile H2O. Store resuspended plasmid at -20°C.

**Plasmid amplification**

psRNA is a low yield plasmid DNA. In order to enhance the yield, we suggest to increase the quantity of DNA of your culture before using a column. To do so, prepare a larger culture than recommended in the protocol provided by the manufacturer (for example instead of preparing 250 ml, prepare 2 liters). Lyse the culture and precipitate the DNA with isopropanol. Resuspend the pellet in the volume indicated by the protocol and treat with RNase before using the column. Then follow the manufacturer’s protocol.

**Plasmid preparation**

- Cloning with Bbs I
  Digest psiRNA plasmid with Bbs I (NEB, 2 units enzyme/μg plasmid DNA). Elute the large fragment (2945 bp) using a 0.7% low-melting agarose gel and dilute the purified DNA fragment to obtain a solution at 0.1 μg/μl.
- Cloning with Acc 65I / Hind III
  Digest psiRNA plasmid with Acc 65I and Hind III (with NEB enzymes, use NEBuffer 2 + BSA). Elute the large fragment (2915 bp) using a 0.7% low-melting agarose gel and dilute the purified DNA fragment to obtain a solution at 0.1 μg/μl.

**siRNA sequence design**

- With the help of the siRNA Wizard (www.sirnawizard.com), choose a sequence of 19-21 nucleotides within the coding region of the gene of interest. The siRNA Wizard offers two search options: the “Standard Search” uses default criteria to analyze your gene of interest whereas the “Advanced Search” lets you manually set the selection criteria.
- Using the siRNA Wizard, design two complementary oligonucleotides compatible with either Bbs I/Bbs I or Acc 65I/Hind III (Figure 1).

**Cloning into psiRNA (Figure 2)**

1. **Annealing of siRNA insert**
   - Dissolve the oligonucleotides (forward and reverse) at a concentration of 100 μM.
   - Dilute to obtain each oligonucleotide solution at 25 μM.
   - Prepare the annealing solution by mixing the following components:

     - Forward oligonucleotide (25 μM) 2 μl
     - Reverse oligonucleotide (25 μM) 2 μl
     - 0.5 M NaCl 6 μl
     - H2O to a final volume of 30 μl

   - Incubate 2 minutes at 80 °C then stop the heating and maintain in water bath until the temperature reaches 35 °C.
   - Use annealed siRNA insert immediately or store at -20 °C for further use.

   **Note**: Purification of the annealed oligonucleotide solution is not necessary for efficient ligation of the siRNA insert.
2- Ligation of siRNA insert into psiRNA
- Prepare the ligation solution by mixing the following components:

- **Digested psiRNA**: 1 µl (100 ng)
- **Annealed siRNA insert**: 1 µl
- **T4 DNA Ligase**: 1 µl (1 unit)
- **10X ligation buffer**: 2 µl
- **H2O** to a final volume of 20 µl

- Incubate the mixture at 16 ºC overnight (or at 27 ºC for 2 hours).

Reconstitution of *E. coli* GT116 strain
Use sterile conditions to do the following:
- Reconstitute *E. coli* GT116 by adding 1 ml of LB medium in the tube containing the paper disk. Let sit for 5 minutes. Mix gently by vortexing for 1-2 minutes.
- Streak bacteria taken from this suspension on a LB agar plate.
- Place the plate in an incubator at 37 ºC overnight.
- Isolate a single colony and grow the bacteria in LB or TB medium.
- Prepare competent cells utilizing protocol of choice.

*Note:* Alternatively, we recommend the use of ChemiComp GT116, frozen chemically competent *E. coli* cells.

3- Transformation of GT116
We recommend using *E. coli* GT116 strain, a sbcCD deletion mutant, which is more compatible with hairpin harboring plasmids than standard laboratory strains which are sbcCD⁺.

- Place the appropriate number of competent cells on ice (100 µl per ligation or transformation reaction).

*Note:* If competent cells are frozen, allow the cells to thaw on ice for 2-5 minutes.
- Introduce 10 µl of ligation product (or 1 µg supercoiled plasmid DNA) in pre chilled 1.5 ml tubes and return tubes to ice.
- Gently flick the cells twice to homogenize and add 100 µl of cells to each DNA-containing tubes.
- Mix by tapping gently and place in ice immediately.
- Incubate the tubes in ice for 30 minutes.
- Incubate the tubes in a 42 ºC water bath for exactly 30 minutes, then place the tubes back in ice for 1-2 minutes.
- Add 900 µl of room temperature SOC (or LB) medium to each reaction. (Practice sterile techniques to avoid contamination.)
- Incubate tubes at 37 ºC for 1h30 with shaking at 250 rpm.
- Spread each transformation reaction (150 µl if using a ligation product or 100 µl of 10⁻¹ and 10⁻² dilutions if using a supercoiled plasmid) onto agar plate prepared with Fast-Media® Kan X-Gal, to take advantage of the white/blue selection.

*Notes:* The white/blue selection will allow you to discriminate between blue parental clones and white recombinant clones. To increase the number of transformants, spread the remaining transformed cells first concentrated by low speed centrifugation.
- Incubate plates at 37 ºC overnight.

*Note:* The blue/white selection is more visible after 24h incubation.

4- DNA extraction and sequencing of the siRNA insert
- Extract plasmid DNA from 5 white clones.
- Confirm the presence of the siRNA insert on a 3% agarose gel (NuSieve GTG FMC type) after digestion with SpeI (2 hours at 37°C).
- psirNA-h7SKneo G1 containing a siRNA insert will generate two bands: 2240 bp and 741 bp (for an siRNA insert consisting of a 21 bp stem and 7 bp loop).
- Verify the sequence of the siRNA insert of 3 positive clones using the sequencing primers provided: OL559 (forward primer) and OL408 (reverse primer).

Transfection of mammalian cells
- Transfect your cell line with a recombinant plasmid containing the expected siRNA insert using standard transfection procedures.

*Note:* We recommend using LyoVec⁺, a transfection reagent optimized for psirNA.
- Two days after transfection, add G418 at the appropriate concentration (typically 1 mg/ml). Stable transfectants are usually individualized after 1-2 weeks.

Analysis of siRNA-induced silencing
siRNA-induced silencing of your gene of interest can be determined by using different techniques, such as Northern analysis or quantitative RT-PCR at the RNA level, or Western Blot at the protein level.

RELATED PRODUCTS

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<td>LyoVec⁺</td>
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InvivoGen Europe: +33 (0) 5-62-71-69-39
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E-mail: info@invivogen.com

www.invivogen.com
1- Selection of siRNA sequence using the siRNA Wizard software

GTGGCTGTGGAGACAAAAATCTA  siRNA sequence
...TCAAGTTTCAGAACGCTGCTGCTGAGACAAAAATCTAAGCAGCAGGAACCTCT... Target gene

2- Synthesis of complementary oligonucleotides

5' ACCTC-GTGGCTGCTGAGACAAAAATCTA GTGGCTGTGGAGACAAATCTAAGAG TAGATTTGTCTCCACAGCAGCAGAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC
3' CAAAAA-GTGGCTGCTGAGACAAAAATCTAAGAG TAGATTTGTCTCCACAGCAGCAGAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC

Complementary ODNs

3- Annealing of complementary oligonucleotides

5' ACCTC-GTGGCTGCTGAGACAAAAATCTA GTGGCTGTGGAGACAAATCTAAGAG TAGATTTGTCTCCACAGCAGCAGAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC
3' CAAAAA-GTGGCTGCTGAGACAAAAATCTAAGAG TAGATTTGTCTCCACAGCAGCAGAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC

Complementary ODNs

4- Digestion of psiRNA plasmid with Bbs I

CGGT GCCATGGA GCCAAGAAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC

Bbs I-digested psiRNA

5- Ligation and transformation of E. coli GT116

CGGT GCCATGGA GCCAAGAAAGTCTT CTTCTTGGTTGACGTTCGGTTCATACAAAAC

Recombinant psiRNA

shRNA (short hairpin RNA)

Figur 1

Figur 2
psiRNA-h7SKneo

(3284 bp)
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**2901**

CTCGTGCTTTACCTGAGTTTTTCAAAAGTAGTTGACAATTAATCATCGCATAGTATATCGGCATAGTATAATAGCAGCTCAGTATAAGGACACCA

**3001**

GGACCTGTGTGTGCTGCAAAGGAGAGCTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCCTCCCTTTGGCTCTTTGGAGGAACCTCT

**3101**

GAGAGCCAGACAGACAGACAGAGAGCACTCGGTCTCAGTGACATAGGACGAGGCTGGTTGCCCTGCCCTGACCTGCTGCTT

**3201**

GGCTGGACTGGACCTCCAGAGCTGACCTGTGACCCCTGAGCTAGGAAGACTTTTGGAAAAGCCTAGACTT

**XbaI (3270)**

BbsI (3249) HindIII (3265)

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**3201**

GGCTGGACTGGACCTCCAGAGCTGACCTGTGACCCCTGAGCTAGGAAGACTTTTGGAAAAGCCTAGACTT
Fast-Media® Kan Agar X-Gal
Microwaveable media for selection and propagation of kanamycin resistant *E. coli*
Catalog # fas-kn-x
For research use only
Version # 13F26-MM

PRODUCT INFORMATION

Contents:
- 20 individual sealed pouches of Fast-Media® Kan Agar X-Gal. Each pouch contains the necessary amount of powder for the preparation of 200 ml of Lysogeny Broth (LB) based solid medium supplemented with kanamycin, IPTG and X-Gal. Lysogeny Broth is also known as Luria Broth.

Fast-Media® Kan Agar X-Gal are used for the detection of blue/white resistant colonies after transformation by a vector carrying the LacZ gene.

Effective concentration: Kanamycin 50 µg/ml, IPTG 100 µg/ml, X-Gal 100 µg/ml

Storage and stability:
- Fast-Media® Kan Agar X-Gal are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.
- When properly prepared, Fast-Media® Kan Agar X-Gal broths are stable for 4 weeks at 4°C, retaining sterility and selective properties.

Quality control:
The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the kanamycin resistance gene are used as positive controls for Fast-Media® Kan Agar X-Gal.

METHOD

For customer convenience, the following procedure is directly printed on each pouch.
1. Pour the pouch contents into a clean borosilicate glass bottle or flask.
2. Add 200 ml of distilled or deionized water.
3. Mix thoroughly by swirling the glass bottle or flask.
4. Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes). Do not heat in a closed container.
5. Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6. Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7. Allow the medium to cool to 50-55 ºC before use.

Caution: Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.

Note: Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.

SPECIAL HANDLING

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breathe the dust.

FAST-MEDIA® FEATURES

Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of sterile *E.coli* growth medium in about five minutes using a microwave instead of an autoclave.

Fast-Media® is supplied with a choice of antibiotics for selection (see table below), and chromogenic substrates, for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies. Fast-Media® Base is supplied without selective antibiotics.

Several Fast-Media® are available:
- **Fast-Media® TB**, Terrific Broth based liquid medium
- **Fast-Media® LB**, Lysogeny Broth (LB) based liquid medium
- **Fast-Media® Agar**, LB based solid medium
- **Fast-Media® Agar X-Gal**, LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc**, LB based solid medium containing X-Gluc.

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InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com