

Constructing self-regulatable silencing construct

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1. Synthesize DNA oligoes encoding the miRNA as shown below:

```
KpnI                                     shRNA sense (use your seq)
5' -CTGCT GTTGACAGTG AGCGAATCCT GGTCAGTTCT ATTAATTGTG
3' -CATGGACGA CAACTGTCAC TCGCTTAGGA CCAGTCAAGA TAATTAACAC
```

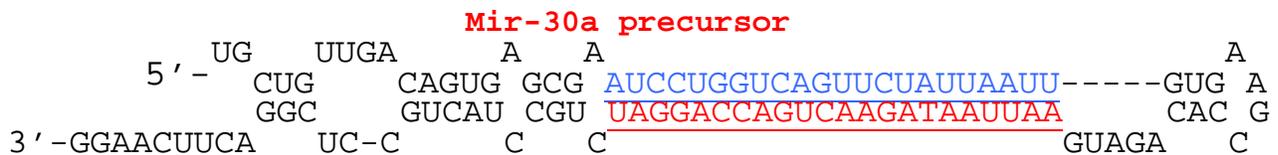
```
AAGCCACAGA TGAATTTAAT AGAACTGACC AGGATCTGCC TACTGCCTCG
TTCGGTGTCT ACTTAAATTA TCTTGACTGG TCCTAGACGG ATGACGGAGC
shRNA antisense (use your seq)
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```
GACTTCAAGG G-3'
CTGAAGTTCC CTTAA-5'
EcoRI
```

Replace the blue seq with the sense strand seq of your shRNA.

Replace the red seq with the antisense strand of your shRNA.

The transcript of the above fragment is predicted to spontaneously form a secondary structure as shown below.



2. Anneal the two DNA oligoes as follows:

A, dissolve the single strand in H₂O to a final concentration of 40uM

B, mix 20ul of each strand in a PCR tube at equal amount

C, denature the mixed DNA oligos at 95°C for 10 min

D, reduce the temperature by degrees, stay at each degree for 1min until cooling down to 25°C (for example, stay at 94C for 1min, then at 93C for another min)

3. Digest UbC-TRE-miSOD2-tTS-IRES-EGFP vector with BamHI, purify the large fragment and religate it. Transform and obtain a miniprep DNA. This step will eliminate the EcoRI and Kpn I sites in the polylinker region.

4. Digest UbC-TRE-miSOD2-tTS-IRES-EGFP vector sequentially using KpnI and EcoRI to cut out the existing hp that is in the plasmid (see the sequence and map below).

5. Use quick ligase (New England Biolab) to ligate the annealed oligo with the digested pUbC-multi-shRNA vector at a ratio of 8:1 (insert: carrier vector).

6. Transform bacteria and grow the colonies.

7. Pick 3-4 colonies and sequence to check whether they contain the hairpin and whether the hairpin sequence is correct.

