Step 1.
Using the siRNA design online tool to pick up the 19mer shRNA;

http://biosettia.com/support/shrna-designer

Step 2. Design the hairpin primer pair to be synthesized

Template of the hairpin pair:

Top: 19mer-TTCAAGAGA-19mer Reverse Complement-TTTTTTGT
Bottom: CTAGACAAAAAA-19mer- TCTCTTGAA-19mer Reverse Complement

For example: GGTACAAGTTCTATCTGAA as the 19mer

Hairpin pair:

Top:
GGTACAAGTTCTATCTGAATTCAAGAGATTCAGATAGAACTTGTACCTTTTTTT
GT
Bottom:
CTAGACAAAAAAGGTACAAGTTCTATCTGAATCTCTTGAATTCAGATAGAACT
TGTACC

Step3. Order the above hairpin pair via IDT or other places. Dissolve the hairpin pair into H2O at 100pM final concentration.

Step4. Primer annealing
Add 41ul of H2O, 5ul of 10X PCR buffer, and 2ul of each primer into a PCR tube, run following program with a PCR machine:
95^{o}C for 10mins;
Step down to 4^{o}C at a rate of 1^{o}C per minute;

Step5. Harvest fragments of the FUGW-H1 plasmid

Digestion A):
XbaI/AgeI in 20ul reaction volume
2ug of FUW-H1
NEB digestion buffer
1.5 ul of XbaI
1.5 ul of AgeI
Add H2O to 20ul final volume, digest at 37 ^{o}C for 3 hrs

This gives two bands in gel electrophoresis: one large band (10-12kb) and one small band (1.5kb).

Digestion B):
SmaI/AgeI in 20ul reaction volume
2ug of FUW-H1
NEB digestion buffer
1.5 ul of SmaI
Add H2O to 20ul final volume, digest at room temperature or 25 ^{o}C for 1.5 hrs
Add 1.5 ul of AgeI to the above reaction, digest at 37 ^{o}C for another 1.5 hrs.

This gives three bands in gel electrophoresis. The smallest band is around 1.5 kb.

Run 1% Agarose gel for both digestion products, cut the largest band from the XbaI/AgeI digestion and the smallest band from the SmaI/AgeI digestion. Gel extraction both fragments.

Step 6. Ligation of the shRNA Oligos and the Fugw-H1 fragments

20ul total volume:
1 ul of the fragment from SmaI/AgeI digestion
1ul of the fragment from XbaI/AgeI digestion
1ul of the annealed oligos from step 4
Ligase buffer
T4 ligase
Add H2O to 20ul final volume

Anneal overnight at 16^{o}C with regular T4 ligation kit or RT for 5mins if using a quick ligation kit.

Step 7. Transformation with Stbl2 cells and pick clones for Miniprep

- Using Amp+ plate and Amp+ LB broth

Step 8. Sequencing to verify successful insertion
Sequencing primer: ACAGCAGAGATCCAGTTTG