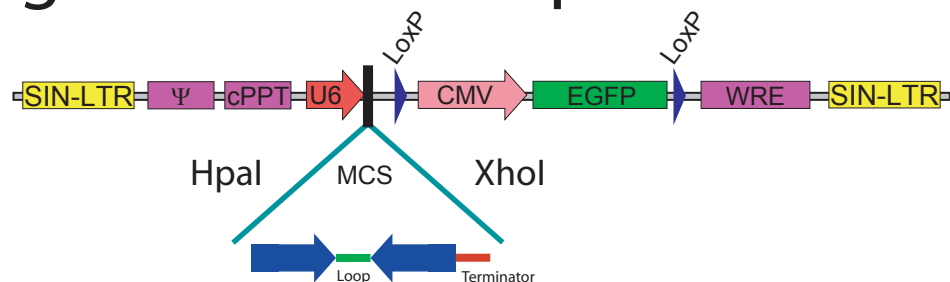


Creating RNAi stem loops for LentiLox 3.7



1. Find mRNA sequence and import into Vector NTi
2. Search sequence for AAG(N18) TT
 - Check G(N18) sequence for
 - o ~50% GC content
 - o Terminators sequences of = or > 4 A or T
 - o Blast sequence for homology to other genes
 - o Bizarre Looping

~~CGAAAACGAAGCCTACTAT~~ GCTACA ACTACTACATGAC

3. Add sequences for restriction sites and loop

GCTACA ACTACTACATGAC

Add T to the beginning of G(N18) to recreate -1 in U6 promoter

TGCTACA ACTACTACATGAC

Add loop sequence to end - TTCAAGAGA

TGCTACA ACTACTACATGACTTCAAGAGA

Add reverse complement of G(N18) to end - i.e. GAT then added ATC

TGCTACA ACTACTACATGACTTCAAGAGAGTCATGTAGTAGTTGTAGC

Add terminator sequence - 6 Ts

TGCTACA ACTACTACATGACTTCAAGAGAGTCATGTAGTAGTTGTAGCTTTTTT

Create antisense strand

TGCTACA ACTACTACATGACTTCAAGAGAGTCATGTAGTAGTTGTAGCTTTTTT

ACGATGTTGATGATGACTGAAGTTCTCTCAGTACATCATCAACATCGAAAAA

Add sticky end for Xho site

+1
 |
TGCTACA ACTACTACATGACTTCAAGAGAGTCATGTAGTAGTTGTAGCTTTTTT Loop Terminator
ACGATGTTGATGATGACTGAAGTTCTCTCAGTACATCATCAACATCGAAAAAGAGCT

4. Check sequence by inputting into Vector NTi and perform oligoduplex analysis.
5. Order oligos from IDT with 5' Phosphate and PAGE purification

