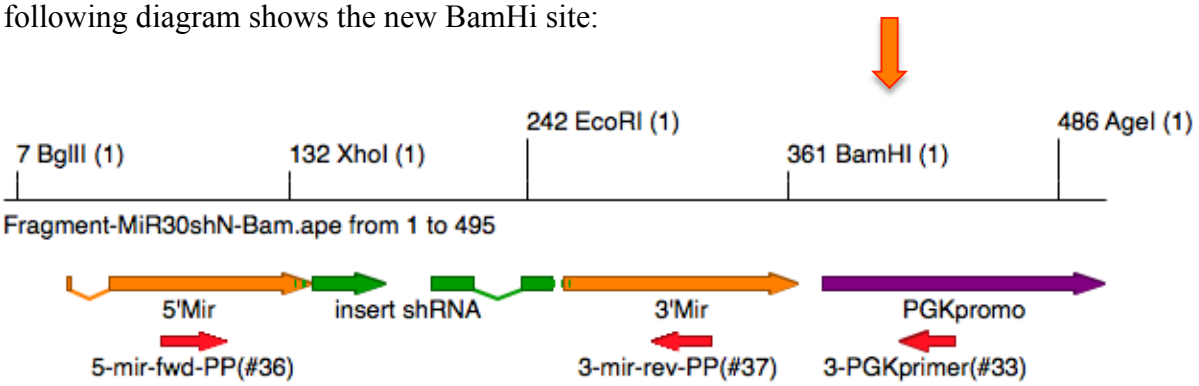
Combined shRNA expression vectors

Inspiration:
Chicas A, Wang X, Zhang C, McCurrach M, Zhao Z, Mert O, Dickins RA, Narita M, Zhang M, Lowe SW.
Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence.
Cancer Cell. 2010 Apr 13;17(4):376-87.
PMID: 20385362

We received MLP vectors from Scott Lowe lab expressing combinations of shRNAs against the pocket proteins used in the reference above (however, we did not receive their cloning systems). It does work nicely although combined shRNAs each are slightly less efficient than used independently probably because they compete for the available RISC complexes in the cell. In their paper, they described the cloning as follow:

> "Cloning of polycistronic shRNAs.
> The polycistronic shRNA vectors were cloned in two steps. First shRNAs were cloned into two intermediate vectors. One shRNA was cloned into the Xho/EcoR1 sites of LTR-CMV-Mir30-PIG (LCMP) while the other into the Xho/EcoR1 sites of a vector containing the MIR30 cassette with flanking BamH1 sites (BamH1-INT). The latter was cut with BamH1 and the mir30 cassette containing the shRNA was cloned into the BglII/BamH1 sites of LCMP to make the tandem (LMP TAN). Cloning into the BamH1 site of LMP TAN another MIR30 cassette from BamH1-INT makes the triple shRNA expressing vectors.

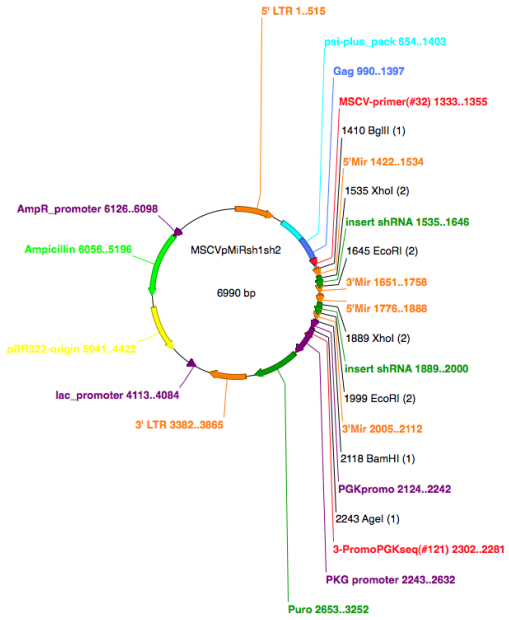
We did like the idea of their cloning system and decided to synthesize a variant of MLP with an added BamHI site after the miR30 context which I have called MLP(B). The following diagram shows the new BamHi site:



Thus shRNAs cloned in this variant can be excised with BglII-BamHI (isolating a miR30 5'-shRNA-miR30 3' fragment) that can be inserted in the BglII site of another MLP-shRNA vector (**cut and dephosphorylated**). It is thus the same principle has in Scott's lab but our variation of it.

The combination of the miR30 5'-shRNA-mir30 3' conserves the BglII and BamHI sites before and after the miR30 cassettes but does not conserve enzyme sites in between the two miR30 cassettes (BglII/BamHI are compatible in ligation without reforming a restriction site) if the insertion is in the right orientation. The double insert can easily be detected by restriction analysis (with double the size of single shRNA expression vectors). We also have a vector variant without IRES-GFP. Vector maps showing two inserts can be found bellow.

Please note that this vector system has not yet been published. I do not have an empty version of it but one can excise whatever shRNA in it with XhoI-EcoRI to clone its own before doing combinations.



If you are designing novel shRNAs, and are starting from the gene sequence, this is an old site we used in the past that was created by Greg Hannon – Cold Spring Harbour Laboratories: http://cancan.cshl.edu/RNAi_central/RNAi.cgi?type=shRNA.

If you are designing novel shRNAs and can start from publish siRNA or shRNA were the targeting sequence is given, this web site (from François Major's lab at IRIC)can tell you what oligo to order to clone in the miR30 context of MLP base on the method from Paddisson et al.: http://www.major.iric.ca/~dallaire/m2sh/m2sh_form.html

Paddison PJ, Cleary M, Silva JM, Chang K, Sheth N, Sachidanandam R, Hannon GJ.
Cloning of short hairpin RNAs for gene knockdown in mammalian cells.
Nat Methods. 2004 Nov;1(2):163-7. PubMed PMID: 16144086