

## Supplemental Information

### **FAMSi: A Synthetic Biology Approach to the Fast Assembly of Multiplex siRNAs for Silencing Gene Expression in Mammalian Cells**

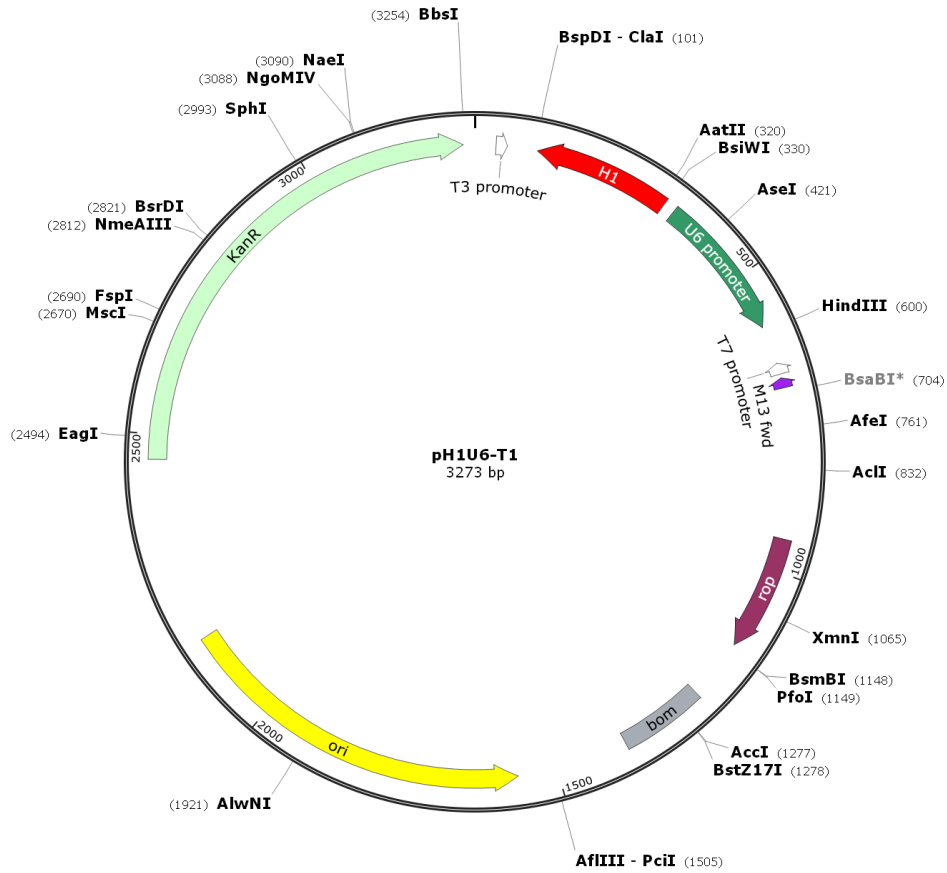
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**Table S1. List of Oligonucleotides Used in the Study**

Supplemental Table 1. List of Oligonucleotides Used in the Study

Gene	Forward	Use
<i>Gapdh</i>	GCCTCGTCCCGTAGACAAAA TTCCCATTTCTCGGCCTTGAC	qPCR
<i>Alp</i>	CCCCATGTGATGGCGTAT CGGTAGGGAGAGCACAGC	
<i>Runx2</i>	CCGGTCTCCTTCCAGGAT GGGAAGTCTGTGGCTTC	
<i>Opn</i>	CCTCCCGGTGAAAGTGAC CTGTGGCGCAAGGAGATT	
<i>Sox9</i>	GCAAGCAAAGGAGACCAAAA CGCTGGTATTTCAGGGAGGTA	
<i>Osx</i>	GAAGTCCAATGGGGATCTGA AGAATCCCTTTCCCTCTCCA	
<i>Ppary</i>	TTTTCAAGGGTGCCAGTTTC AATCCTTGGCCCTCTGAGAT	
<i>Smad1</i>	AAGCTGTGGACGCTTTGG ATCCAGGGAGCGAGGAAT	
<i>Smad4</i>	GCGAGGTTGCACATAGGC GGCCAGCTTCTCTGTCCA	
<i>Smad5</i>	TTCCATCCCCTGCCAGTA GCCTCTCGGTGCTCTCTG	
<i>Smad8</i>	ACCAGGAGGCACATGGGA AGCCGTGCTGGTAGTTGC	
simSmad4-A Fwd	aaaaaGCAATTGAGAGTTTGGTAAtttttAGAGTGGTCT	
simSmad4-A Fwd w/ Bsal-Bbs1-A	ggtGGTCTCGggcaaaaaGCAATTGAGAGTTTGGTAA	
simSmad4-B Rev	aaaaaATCCTGAGAGATCAATTCctttttTTCGTCCTTTC	
simSmad4-B Rev w/ Bsal-B	ggtGGTCTCGcgttAaaaaATCCTGAGAGATCAATTCC	
siRNA-2 Fwd w/ Bsal-B (Common)	ggtGGTCTCGaacyTtttttGTCTCATAACAGAACTTATAA	
simSmad4-C Rev	aaaaaATGATGGTAAGTAGCTGGctttttTTCGTCCTTT	
simSmad4-C Rev w/ Bsal-Bbs1-R	ggtGGTCTCGgccaaaaaaATGATGGTAAGTAGCTGGC	
simSmad1 Rev	aaaaaAAAGAGTCTGGGAACGTGGttttttTTCGTCCTTT	
simSmad1-A Rev w/ Bsal-C	ggtGGTCTCGcgaaaaaaAAAGAGTCTGGGAACGTGG	
siRNA-3 Fwd w/ Bsal-C (Common)	ggtGGTCTCGtccgTtttttGTCTCATAACAGAACTTATAA	
simSmad5 Rev	aaaaaTAATACTGGAGGTAAGACTttttttTTCGTCCTTT	
simSmad5 Rev w/ Bsal-D	ggtGGTCTCGacgcaaaaaTAATACTGGAGGTAAGACT	
siRNA-4 Fwd w/ Bsal-D (Common)	ggtGGTCTCGcgtTtttttGTCTCATAACAGAACTTATAA	
simSmad8 Rev	aaaaaTAATGGTATGGGTTGATGctttttTTCGTCCTTT	
simSmad8 Rev w/ Bsal-Bbs1-R	ggtGGTCTCGgccaaaaaaTAATGGTATGGGTTGATGC	

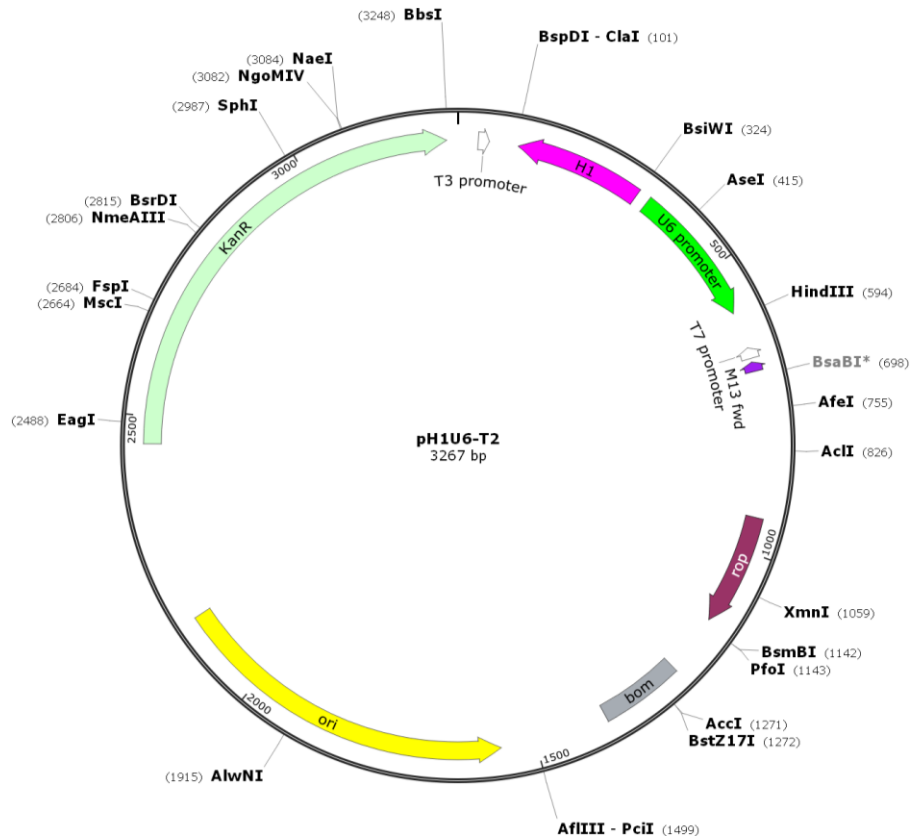
**Figure S1. pH1U6-T1 (Template-1) Vector Map and Sequence**



**pH1U6-T1 (Template-1) Full-length Sequence**

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CCTCACACGTTCCAGTAACCGGGCATGTTcATCATCAGTAACCCGATTCGTGAGCATCCCTCTCGTTTTcATCCGTATCATACCCCATGAACAGAAATCC  
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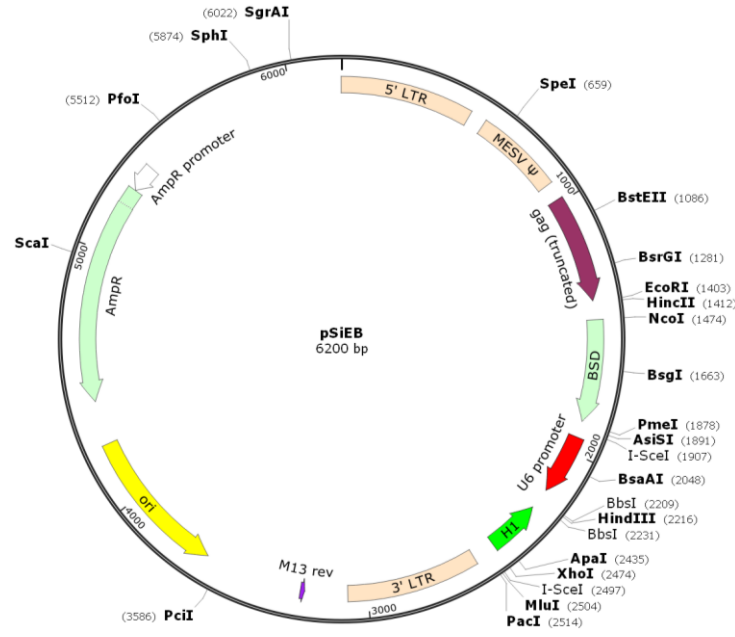
**Figure S2. pH1U6-T2 (Template-2) Vector Map and Sequence**



**pH1U6-T2 (Template-2) Full-length Sequence**

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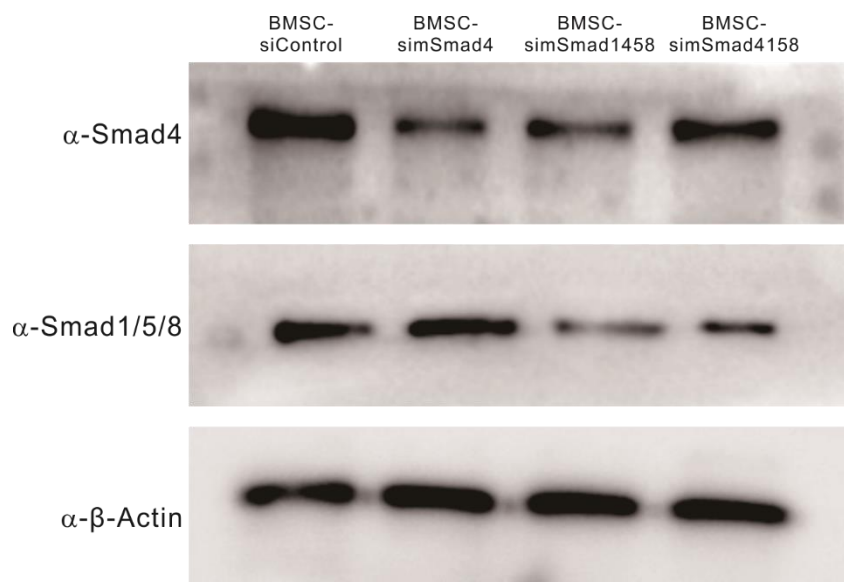
**Figure S3. pSiEB Vector Map and Sequence**



**pSiEB Full-length Sequence**

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### Figure S4. Western Blotting Confirmation of siRNA Silencing Efficacy



**Figure S4. Western blotting analysis of simSmad silencing efficiency in BMSC cells.** Subconfluent BMSC-siControl, BMSC-simSmad4, BMSC-simSmad1458, BMSC-simSmad4158 cells were lysed in 2x Laemmli Sample Buffer and subjected to SDS-PAGE. The immunoblots were probed with the Smad4 and Smad1/5/8 antibodies, respectively. Loading controls were assessed by probing the immunoblots with a  $\beta$ -actin antibody. All antibodies were from Santa Cruz Biotechnology. The presence of proteins of interest was visualized with the SuperSignal West Femto Maximum Sensitivity Substrate/Enhanced Chemiluminescence (ECL) kit (ThermoFisher Scientific).