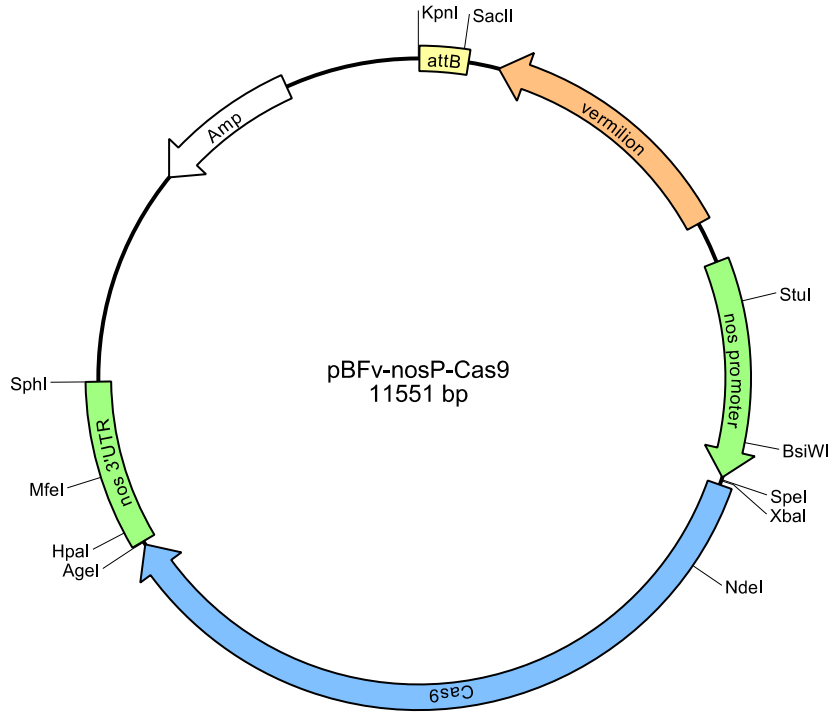


**pBFv-nosP-Cas9**

attB: 7-290  
 vermilion: 469-1962  
 nos promoter: 2207-3470  
 Cas9: 3516-7655,  
 nos 3'UTR: 7677-8641  
 Amp<sup>R</sup>: 9911-10771



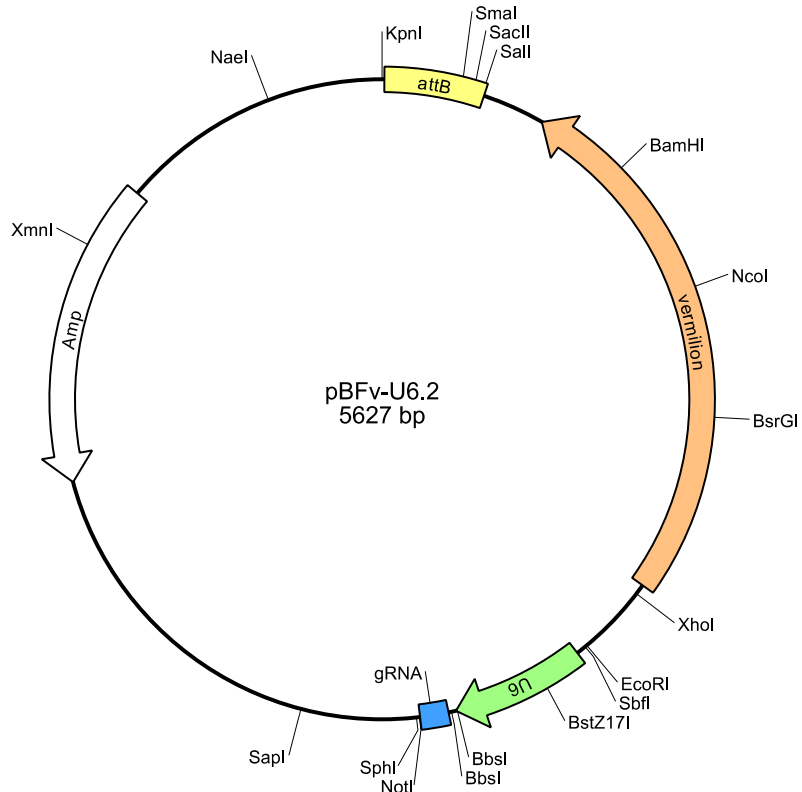
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**pBFv-U6.2**

attB: 7-290  
 vermilion: 469-1962  
 U6 promoter: 2207-2605  
 gRNA: 2627-2707  
 Amp<sup>R</sup>: 3987-4847



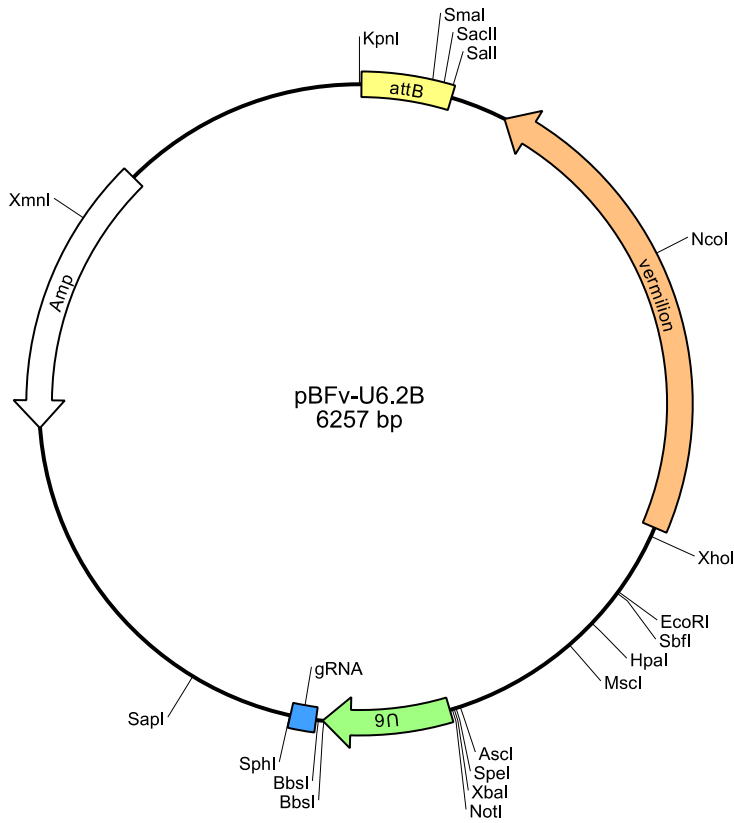
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CATCTTTTACTTTACCCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATA  
CTCTTCTTTTCAATATATGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGTTCC  
GCGCACATTTCCCGAAAAGTGCCACCTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAAAATCAGCTCATTTTTAAACCAATA  
GGCCGAAATCGGCAAAATCCCTTATAAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAACGTGG  
ACTCCAACGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAGCA  
CTAAATCGGAACCTAAAGGGAGCCCGGATTTAGAGCTTGACGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGC  
TAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACCCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCAGGCTGC  
GCAACTGTTGGGAAGGGCGATCGGTGCGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAACCCAGG  
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**pBFv-U6.2B**

attB: 7-290  
 vermilion: 469-1962  
 U6 promoter: 2845-3243  
 gRNA: 3265-3345  
 Amp<sup>R</sup>: 4617-5477



>pBFv-U6.2B

**GGTACC**TCGACATGCCCGCGTGACCGTCGAGAACCCCGTGACGCTGCCCGCGTATCCGCACCCGCCGCGCTCGCACGTCCCGTGCTCACCGTGACC  
 ACCGCGCCAGCGGTTTCGAGGGCGAGGGCTTCCCGGTGCGCCGCGCGTTCGCGGGGATCAACTACCGCCACCTCGACCCGTTTCATCATGATGGACAGAT  
 GGGTGAGGTGGAGTACGCGCCCGGGGAGCCAAAGGGCACGCCCTGGCACCCGCGCTTCGAGACCGTGACTACATCGTTCGAC**AAGCTT**GGATTTA  
 TTTTGTATGTTATATGATATATATGTCAGACATAAAGAAAAGGAACACATCAAAATGTGATAACAAAGACTAAACAAGTAATTTTATACACCAAAACGCAC  
 AAAACAGTAGGCAGAACAAACAACGCATAGCCAAACATTGACGAATTGGATACCCTGCCGATTT**TCAGACACTTTTGTGTGATCAGTTCTTTCGCAATGGCT**  
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 AGGGAGAACAGTTTGAGTTCATTGATCCCGGATATAATCACATCTGCGATGATCACCTGAGAGTGGAGCGCAGATATTGATATCCAGACGAGCCACCGATG  
 CCAACTGTTGGGATCCAATCATGCGTTGCACCATGATCAGCTATTGCTCGCGCGGGAATAGAAAAGTATTGGTTAGGAAAACAGTCTTAAACATAAG  
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 CTGAACCTGGGTTTCATCCCTATAGAAGGTGATCATGATGGCTCCCTGAAGGGCACGATGGCTAAACCCGGCGATCCCCACGACCGCACCCAGTGCATCGTGCAC  
 TGCCGGATCAAAGATGGAGCGATACACCTCGCGTTCCTCAATGTCCATGAGGCGGTAGTTTTTCGCTTCTCCACGGGCTCTCCATGGCGCTCTGTGA  
 CCTGCGCTCCAGGAATCGATCGACGCTCTCCTGAAACTTGGCCAGAAGTGAAGCCACTCTCCTCCAGTCCGGGCGCTCTCCAGCCATCGCTGCAC  
 AGTCCAGTAGCGAGGGATCTTCTCCGAGTTGCGAATCGAATCGAGTTCGCGCTCCTCGTCTGATAAAGACATCCGAGTACTCTGGTTGTATCTCACCCGCTG  
 CTCTGTGCAACTCCAGCTTGTCTCGATCAAACGGAATGCAGCGACTGAAAACAGATGCGGGTGCCAGGTACTTCCGGAAGTCCATGAAGTCTAGCG  
 GGTCTAGTGTCCAGAAATGGGCACTTGGTCCACAGGAGCTGTACAAGGAAGTTATAACCGGATTTGGTAAGAGATTGAAAGCACTCACTTTTAGA  
 ATCAGAACTCCGTTTTCAGTTCGTTGACAATCTCCAGGCTTCCAGCTTCTGGTTTTCGATGGTTGAATCGCAGAACTGAAAGTTCGATGGCATTTCTGGCAG  
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 TGAACAGATGCTCATCGTGCACGGGTCGTTGTCTCCTCGACAGCATAACACTGGGCTCCAGCAGTTTGTCCAGCATGATCACTTCCATAGATTTTG  
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 TCCGCCAGCGGACCGGAGATGAGACCCAGCGAACCAGATAACAGAGCGAGAGAGCTCCAGTTCGACTGATGCAACGCTGGTGTCTGGCGATGGGCAC  
 TGCCAGATAGGCTGGGAATATCAATCACTTGAAGTGAAGTGGCGGCACACAAATCC**AAGCTT**GATATC**GAATCCCTGCAGG**CGAGTTTGGTTGGCAT  
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 AAATGGCGAGAGATAAAAATCCGGCGTCGGCAAAGTAGACAAAAAATCAGTATACCATTTAGCTACCTCTCTCACTCGACCGAGTCCCGGCTCAAGT  
 TGGGCGCGCTCTGCAATTCGATTTTCTTGGGTGTGTAACTAATCATCCGTTTCCCTTCCCTCATCCACAGCTGAAAGCGCGCGGATCCACTA  
 GTTCTAGA**CGGCGCGC**TT**CGACTTGCAGCCTGAAATACGGCACGAGTAGGAAAAGCCGAGTCAAATGCCGAATGCAGAGTCTCATTACAGCACAATCAACT**  
**CAAGAAAACCTCGACACTTTTTTACCATTTGCACCTTAAATCCTTTTTTATCTGTTATGTAATACTTTTTTGGTCCCTAACCAAAACCAAACTCTCT**  
**TAGTCGTGCTCTATATTTAAAACATCAATTTTATATAGTCAATAAATCGAACTGTGTTTTCAACAAAACGAAACATAGGACACTTTGATTCTAAAGGAAA**  
**TTTTGAAAATCTTAAGCAGAGGTTCTTAAGACCATTTGCCAATCTTATAATCTCAACTGCTTTTTCTGATGTTGATCATTTATATAGTATGTTTTTC**  
**CTCAATCTTCGGTCTTCGAGTT**GAAGACTT**TTTTAGAGCTAGAAAATAGCAAGTTAAAATAAGCTAGTCCGTTATCAACTGAAAAGTTGGCCAG**  
**TCGGTGTCTTTTT**GCATG**CACTTGGCTTTCCACCGTTGGTATCGAATCTCTGGGACGATGAGTTCGAGCTCCAGCTTTTTGTTCCCTTAGTGAGGGTTAAT**  
 TCGCGCTTGGCGTAAATCATGGTATAGTGTGTTTCTGTTGAAATGTTATCCGCTCACAATCCACACAACATACGAGCCGGAAGCATAAAGGTAAAG  
 CCTGGGTGCCTAATGAGTGAGCTAACTACATTAATTCGTTTCGCTGACTGCGCGCTTCCAGTCCGGAAACCTGTGCTGCGGAAACCCGCAAGCAGGATCA  
 GGCCAACCGCGGGGAGAGGGGTTTGGCTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTGGGCTCGGTCGTTTCGGCTGCGGGAGCGGATC  
 AGTCACTCAAAGCGGTAATACCGTTATCCACAGAAATCAGGGGATAACCGGAAAGAAAGATGTGAGCAAAAGGCCAGAAAAGCCGGAACCGTAAAG  
 AGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCAAGTCCAGAGGTGGCGAAACCCGCAAGCAGGATCA  
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 GTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG  
 AAAAAGAGTTGGTAGCTTTGATCCGGCAACAAACACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACCGCGCAAAAAAAGGATCTC

AAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACC  
TAGATCCTTTTAAATTAATAAATGAAGTTTAAATCAATCAAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTAT  
CTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA  
TGATACCGCGAGACCCACGCTCACGGCTCCAGATTTATCAGCAATAAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTTCAACTTTTATCCGCC  
TCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTTCGCCAGTTAATAGTTTGCGCCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCTC  
ACGCTCGTCTGTTTGGTATGGCTTCATTCAGTCCGGTTCCCAACGATCAAGGGCAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCG  
GTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAATCTCTTACTGTCATGCCATCCGTAAGATGC  
TTTTCTGTGACTGGTGAAGTACTCAACCAAGTCAATCTGAGAATAGTGTATGCGGGCAGCCGAGTTGCTCTTGCCCGGCTCAATACGGGATAATACCGCGCC  
ACATAGCAGAACTTTAAAAGTGTCTATCATTTGGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA  
CTCGTGCAACCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCG  
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GAAAAATAAACAAATAGGGTTCCGCGCACATTTCCCGGAAAAGTGCCACCTAAATGTAAAGCGTAAATATTTTGTAAAAATTCGCGTTAAATTTTGTTA  
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AGAGTCCACTATTAAGAACGTTGACTCCAACGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTT  
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GAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACACCCGCGCGCTTAATGCGCCGCTACAGGGCG  
CGTCCCATTGCGCAATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGG  
CGATTAAGTTGGGTAACGCCAGGGTTTTTCCAGTACAGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTAATACGACTACTATAGGGCGAATTTG

**Figure S1** Plasmid maps and sequences. Full sequences of the germline Cas9 expression vector pBFv-nosP-Cas9 and the gRNA expression vectors pBFv-U6.2 and pBFv-U6.2B are shown along with their maps. Useful restriction enzyme sites are underlined.