

GGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAAATTAATTTGACTGTAAACACAAAGATATTAGT

Sequence primer

pUC origin

Human U6 promoter

ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAAATATGTTTTAAAAATGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCTTGGCTTTATATCTTGTGGAAAG

Human U6 promoter

GACGAAAACACGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAAAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGT

gRNA cloning site

chimeric guide RNA scaffold

U6 terminator

Human U6 promoter

AAATAAGGCTAGTCCGTTTTAGCGCGTGCGCCAATCTGCAGACAAATGGCTCTAGAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAATTGGAAATTAATTTGA

XbaI

Human U6 promoter

CTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAAATATGTTTTAAAAATGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCTTG

Human U6 promoter

GCTTTATATATCTTGTGGAAAGGACGAAACACCGCATCGTACGCGTACGTGTTGTTTTAGAGCTAGAAATAGCAAGTTAAAAAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGT

PITCh-gRNA#3

chimeric guide RNA scaffold

U6 terminator

Human U6 promoter

TTTAGAGCTAGAAATAGCAAGTTAAAAAAGGCTAGTCCGTTTTAGCGCGTGCGCCAATCTGCAGACAAATGGCGGTACCCGTTACATAACTTACGGTAAATGGCCCGCCTG

KpnI

CBh