pLKO.1 U6-sgRNA cloning template

CACCGGTGT**GCAGGT**GAGTGATCAAGA**ACCTGC**TGACGTTTTAGAGCTAGAAATAGCAA

GTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

TTGAATTC

The pLKO.1 U6 promoter sgRNA template DNA sequence is shown where the U6 promoter transcription start site is indicated in magenta and the constant portion of the sgRNA sequence is highlighted in yellow (Mali et al., 2013). The type IIS BfuAI recognition sites utilized for cloning annealed oligonucleotides encoding the single-guide RNA sequence are indicated in bold letters, where the cleavage positions on the Crick and Watson strands are respectively indicated above and below the sequence yielding 4 base pair 5’ overhangs.