

TCTTGTGGAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNNTTTTGTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG

PCR

TCTTGTGGAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNNTTTTGTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
AGAACACCTTCTGCTTTGTGCGNNNNNNNNNNNNNNNNNNNCAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCAGG

+

BfuAI Digested Vector

U6 Promoter

ATATATCTTGTGGAAGGACGAAA
TATATAGAACACCTTCTGCTTTGTGG

sgRNA Scaffold

GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA
TCTCGATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTT

5' Exonuclease } Gibson Assmby
DNA Polymerase } Master Mix
DNA Ligase }

ATATATCTTGTGGAAGGACGAAAACCGNNNNNNNNNNNNNNNNNNNTTTTGTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA
TATATAGAACACCTTCTGCTTTGTGCGNNNNNNNNNNNNNNNNNNNCAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTT

FIGURE S1. Diagram of Gibson assembly. The plate synthesized sgRNA oligos are PCR amplified using primers which bind to the adaptor sequences. The PCR amplified dsDNA and BfuAI digested pLX-sgRNA-BfuAI-2k vector were combined and the Gibson assembly reaction performed. This inserts the sgRNA targeting sequence between the U6 primer and sgRNA scaffold.