gRNA Synthesis Protocol

STEP 1: Find all 23bp genomic sites of the form 5'-N_{20}NGG-3' near your intended target site (ideally ±50bp). These may reside on the + or – strand.

PAM

5'…NNNNN NNNNN NNNNN NNNNN NGG…3'

STEP 2: Using NCBI blast, select sequences for which none or very few sequences of the form 5'-NNNNN NNBBB BBBBB BBBBB NGG-3' exist at any other location in the human genome (here the B's represent the actual bases at the target genomic location).

Option A

STEP 3: Incorporate 19bp of the selected target sequence as highlighted here: 5'-NNNNN NNNNN NNNNN NNNNN NGG-3' into the DNA fragment as indicated below:

TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACC
AAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATA
CGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAG
ATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCA
GTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAG
TATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG*GNNNNN*
*NNNNNNNNNNNNNNN*GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC
CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCTAGACCCAGC
TTTCTTGTACAAAGTTGGCATTA

Step 4: This 455bp fragment bears all components necessary for gRNA expression, namely: U6 promoter + target sequence + guide RNA scaffold + termination signal. Synthesize this as a gBlock from IDT (http://www.idtdna.com/pages/products/genes/gblocks-gene-fragments)

Step 5: Clone the synthesized gBlock into an empty backbone vector such as pCR-Blunt II-TOPO from Invitrogen (http://products.invitrogen.com/ivgn/product/K280020), or directly pcr amplify this fragment (*gRNA_F*: TGTACAAAAAAGCAGGCTTTAAAG, *gRNA_R*: TAATGCCAACTTTGTACAAGAAAG) for transfection and gRNA expression.

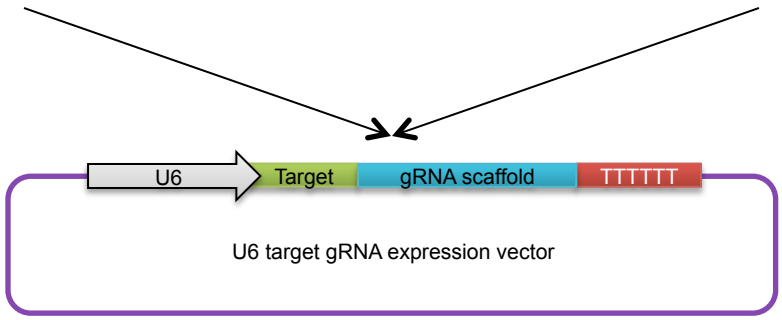
Option B

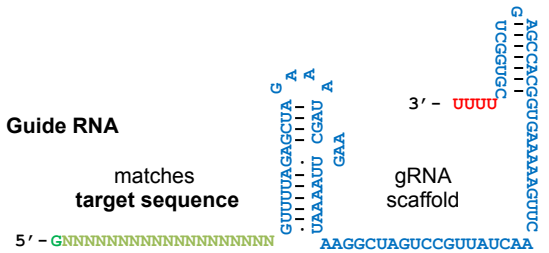
STEP 3: Incorporate 19bp of the selected target sequence as highlighted here: 5'-NNNNN NNNNN NNNNN NNNNN NGG-3' into two 60mer oligonucleotides as indicated below (sequences are 5' to 3', and the regions marked in green and red are reverse complements of each other):

Insert_F:
TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC*GNNNNNNNNNNNNN*
NNNNNNN
Insert_R:
GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC*NNNNNNNNNNNNNN*
NNNNNNC

Step 4: Anneal the two oligos and extend these to make a 100bp double stranded DNA fragment using Phusion polymerase (http://www.neb.com/nebecomm/products/productm0530.asp).

Step 5: Linearize the gRNA cloning vector (http://www.addgene.org/41824/) using *AflII* and incorporate the 100bp DNA fragment from Step 4 above into it using Gibson assembly (http://www.neb.com/nebecomm/products/productE2611.asp). The resulting vector is the desired gRNA expression vector. **Note:** This synthesis strategy is amenable to construction of multiple gRNA expression vectors, and even large gRNA libraries using oligonucleotide pools synthesized using custom DNA arrays (refer Supplementary Fig. 11: http://www.sciencemag.org/content/suppl/2013/01/03/science.1232033.DC1/Mali.SM.pdf).





Prashant Mali (Church Lab), Version: 01-14-2013