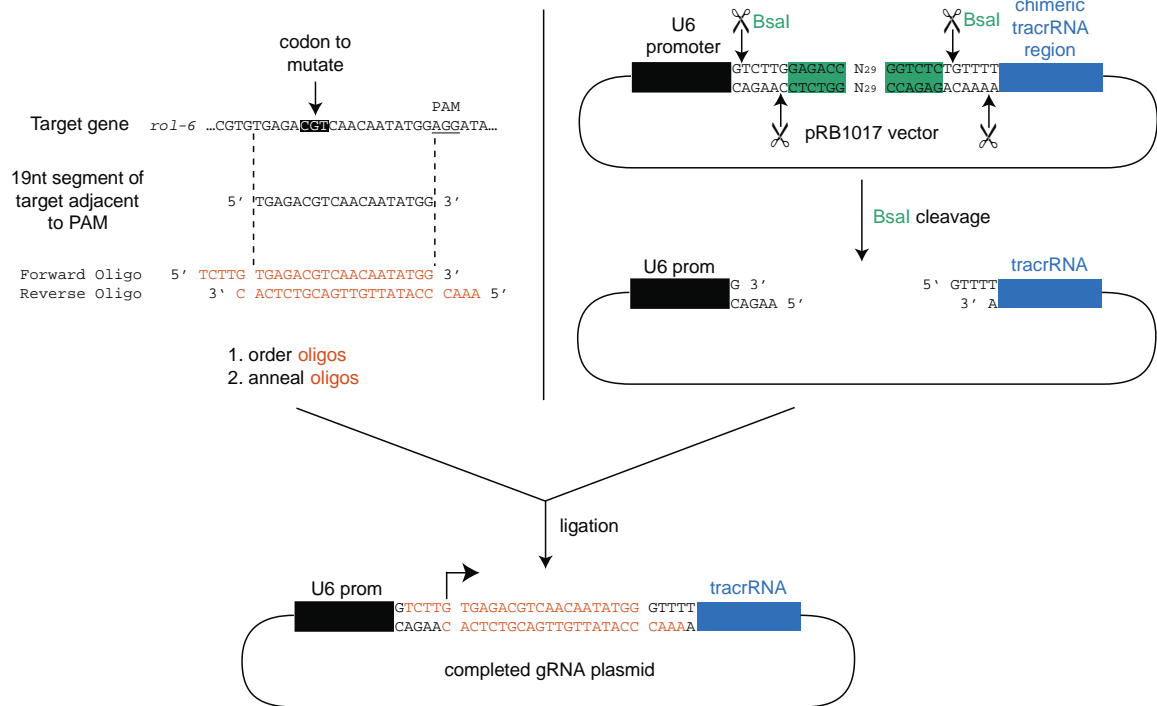


### Guide to using the gRNA expression cassette (pRB1017) vector



**Figure S3** Guide RNA cloning strategy

First, the base to mutate and a nearby PAM are identified. 19 nucleotides upstream of the NGG PAM constitutes the guide RNA target sequence, and forward and reverse oligonucleotides with additional 5' and/or 3' nucleotides ends are ordered (left). The recipient guide RNA vector (pRB1017) is cut with Bsal (sites boxed in green), generating 4 nt overhangs complimentary to the oligonucleotide ends. Oligonucleotides are annealed, then ligated into the Bsal-cut vector. The guanosine where U6 initiates transcription is indicated with an arrow.