Protocols:

Plasmid DNA may be propagated in e. coli using standard methods. DNA may then be transfected into mammalian cells using lipid mediated transfection or electroporation. If co-transfected with a BxB1 recombinase expression plasmid and into the corresponding landing pad-containing cells, plasmids can be genomically integrated into the chromosomal landing pad using the appropriate protocol (Duportet et al. 2014, NAR).

miRNA target sites may be amplified by the primers listed below (or similar primers) followed by restriction ligation or Golden Gate assembly. In this case BsaI would be used to generate distinct overhangs (denoted as Q1-Q9) for ligation into a suitable backbone (eg. the low sensor backbone reverse [LSBr]).

miR-T\_LS\_Q1\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

GCTTCAGACGTCTCTGCTT

miR-T\_LS\_Q2\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

CAACCAGACGTCTCTGCTT

miR-T\_LS\_Q3\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

CAGACAGACGTCTCTGCTT

miR-T\_LS\_Q4\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

TGTGCAGACGTCTCTGCTT

miR-T\_LS\_Q5\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

GAGCCAGACGTCTCTGCTT

miR-T\_LS\_Q6\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

AACGCAGACGTCTCTGCTT

miR-T\_LS\_Q7\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

CTTCCAGACGTCTCTGCTT

miR-T\_LS\_Q8\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

AGACCAGACGTCTCTGCTT

miR-T\_LS\_Q9\_fwd CTACCACCCAGCTTTCTTGTACAAAGTGGTAGGTCTCA

AGGTCAGACGTCTCTGCTT

miR-T\_LS\_Q1\_rev CTGAGGAGTGAAGCACAGGTCTCTAAGCGTTG

miR-T\_LS\_Q2\_rev CTGAGGAGTGAAGCACAGGTCTCTGTTGGTTG

miR-T\_LS\_Q3\_rev CTGAGGAGTGAAGCACAGGTCTCTTCTGGTTG

miR-T\_LS\_Q4\_rev CTGAGGAGTGAAGCACAGGTCTCTCACAGTTG

miR-T\_LS\_Q5\_rev CTGAGGAGTGAAGCACAGGTCTCTGCTCGTTG

miR-T\_LS\_Q6\_rev CTGAGGAGTGAAGCACAGGTCTCTCGTTGTTG

miR-T\_LS\_Q7\_rev CTGAGGAGTGAAGCACAGGTCTCTGAAGGTTG

miR-T\_LS\_Q8\_rev CTGAGGAGTGAAGCACAGGTCTCTGTCTGTTG

miR-T\_LS\_Q9\_rev CTGAGGAGTGAAGCACAGGTCTCTACCTGTTG