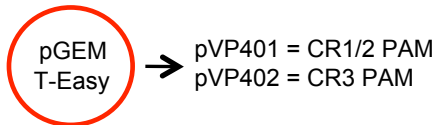


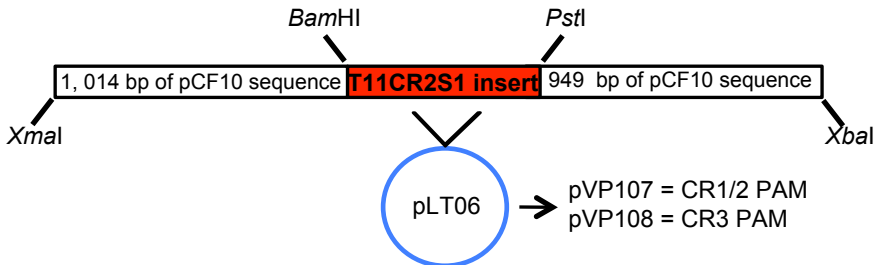
Complementary oligos containing **T11CR2S1**, **PAM** and pCF10 *uvrB* sequence were annealed:

pVP501 GGATCCGATTTTGAACCTTTGGAAACAAAAGACGCTAAACTTCCGCATAGGTATCTTTTCTTACCTTGGGGTGCAGTAGGAATTGCTTTTGGTGGATCC
CCTAGGCATAAACTTGAACCTTGTCTGCGATTTGAAGGCTATCCATAGAAAAGAATGGTACCACACGTATCCTTTAACGAAAAAACGACCTAGG

Annealed oligos were ligated into pGEM T-Easy:



pVP401 and pVP402 were used as templates to change restriction site for generation of pLT06 constructs:



pVP107 and pVP108 were electroporated into competent OG1SSp pCF10 cells and subjected to temperature shift and counter-selection to incorporate the new sequence into pCF10:

pVP501 AACTTCCGCATAGGTATCTTTTCTTACCTTGG
pVP502 AACTTCCGCATAGGTATCTTTTCTTACCTTGTGA

