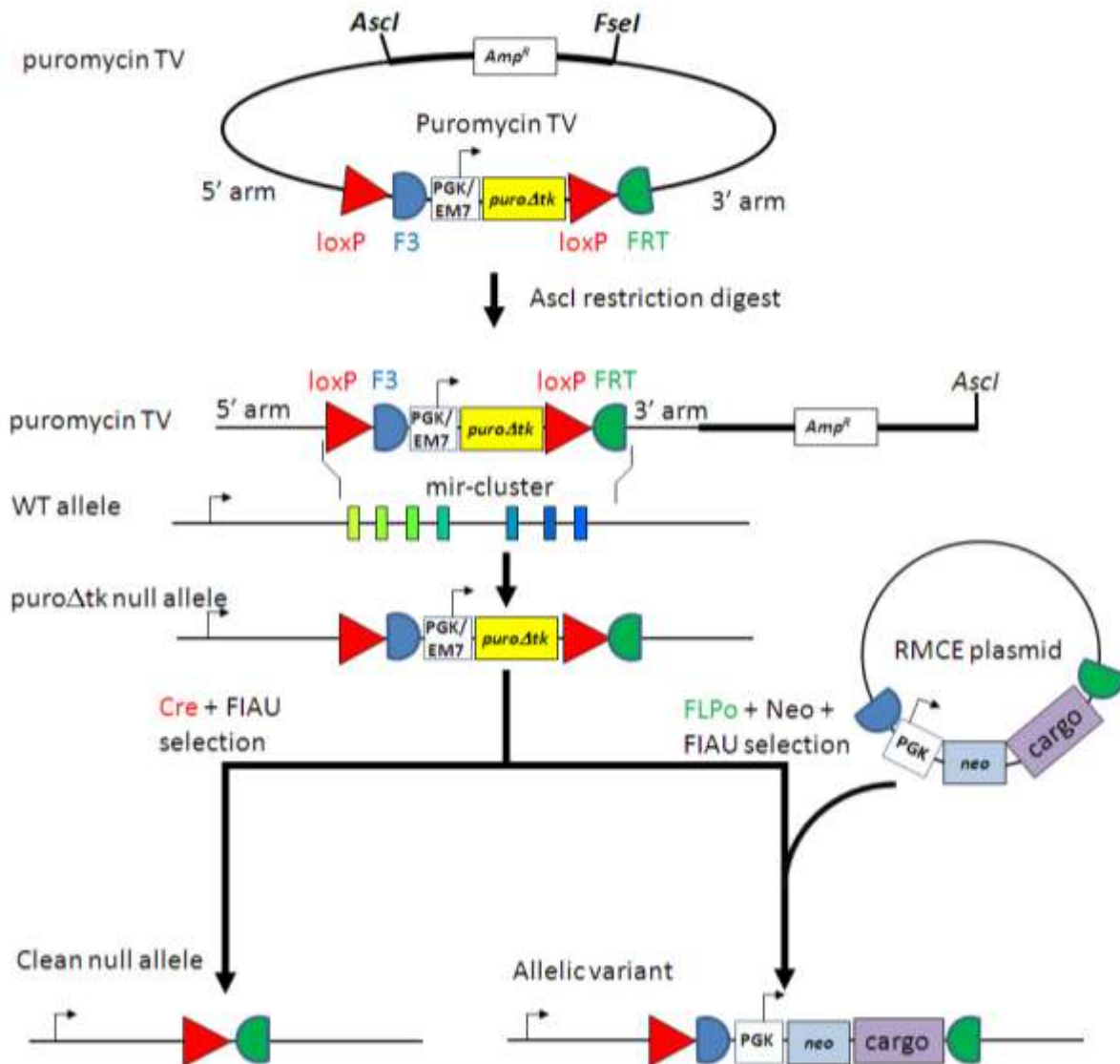


Schematic of mirKO targeting vector and alleles



The standard mirKO targeting vector contains a PGK-EM7 promoter *puroDtk* selection cassette flanked by recombinase sites. The vector is linearized using *Ascl* (or alternatively *FseI*) and transfected into JM8.A3 ES cells. Correct targeting replaces the mir gene(s), either a singleton or a cluster as depicted here, with the PGK-EM7 *puroDtk* selection cassette to create a “*puroDtk* null allele”. The *puroDtk* null allele is the version of the targeted ES cell clones that may be ordered from repositories. Upon receipt the targeted clones can be used in two alternative approaches. Either (i) the PGK-EM7 promoter *puroDtk* cassette can be removed by Cre transfection followed by selection against the tk gene with FIAU (or gancyclovir) to create a “clean” null allele or (ii) the *puroDtk* null allele can be converted using FLPo recombinase to a range of alternative allelic variants by the process of recombinase mediated cassette exchange (RMCE) followed by dual selection (e.g. G418 and FIAU).

Reference: “A resource of vectors and ES cells for targeted deletion of microRNAs in mice” H.M. Prosser, H. Koike-Yusa, J.D. Cooper, F.C. Law and A. Bradley. *Nature Biotechnology* 2011; Vol. 29 (no.9), 840-845