

## Construction of retroviral vectors

pMFGneo (the same with pU8neo)

The retrovirus expression cassette was cut out from the pMFG vector (Ref. Dranoff et al. PNAS 1993; Wakimoto et al., Cancer Res. 1996) by HindIII/EcoRI digestion, and subcloned into the HindIII/EcoRI sites of the pUC8 plasmid, resulting in pU8(MFG) retroviral expression plasmids. The EcoRI site at the end of the 3' flanking genomic DNA was exchanged with a SalI site by blunting the EcoRI-digested end followed by a SalI linker d(pGGTCGACC) ligation, resulting in the pU8(MFG)s retroviral expression plasmids. The NcoI/BamHI fragment from the pPGKneo plasmid (Ref. Wakimoto et al., Cancer Res. 1996; Wakimoto et al. JJCR 1997), which contains the whole coding region for the neo-resistant gene, was subcloned into the NcoI/BamHI sites of the pU8(MFG)s retroviral expression plasmid, resulting in the plasmid, pMFGneo (the same with pU8neo).

pRx-nZiresNeo

The pRx-nZ, and pRx-nZiresNeo (abbreviated as pRx-nZiN) plasmids for recombinant retrovirus vectors are described previously (Wakimoto et al., JJCR 1997).

### References:

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2. Dranoff G., Jaffee E., Lazenby A., Golumbek P., Levitsky H., Brose K., Jackson V., Hamada H., Pardoll D. and Mulligan R.C. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc.*

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