

Construction of pAAneo adeno-associated virus vector

pAAneo (the same with pccAAneo, or pAAccneo)

The pAV1 plasmid, which contains the human AAV genomic DNA was obtained from ATCC. The DraIII sites of the pAV1 were exchanged with either BamHI, ClaI, EcoRI, or XhoI site, by blunting the ends of the DraIII-digested pAV1, followed by the respective linkers ligation, d(pCGGATCCG), d(pCATCGATG), d(pGGAATTCC), or d(pCCTCGAGG), resulting in the plasmids, pAV1db, pAV1dc, pAV1de, and pAV1dx(XhoI), respectively.

The AAV genomic cassette was cut out from the pAV1de by BglII digestion, blunted by T4 DNA polymerase, and subcloned into the blunted SpeI/PstI sites of the pCAcc plasmid DNA (Yoshida et al.), resulting in pccAAdeR and pccAAdeL (the reverse orientation of the pccAAdeR) plasmids.

pccAAneo was constructed as follows:

The neo resistant gene fragment was cut out from the pPNT plasmid (Wakimoto et al. JJCR 1997) by XhoI (blunted) and EcoRI digestion, and subcloned into the SnaBI/EcoRI sites of the pccAAdeR, resulting in the pccAAneo (the same with the pAAneo).

References:

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