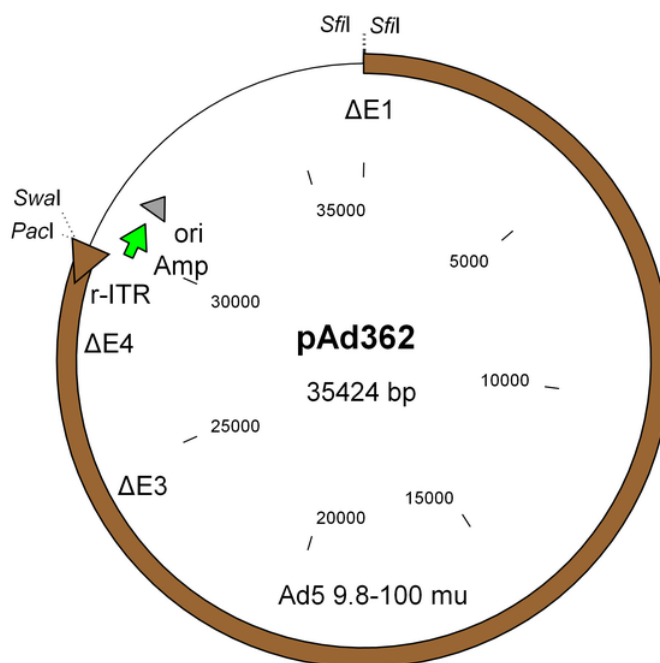


pAd362

pAd362 is a 35.4 kb plasmid that contains the sequence encompassing bp 3504-right end (9.8–100 mu) of the Ad5 genome. The two *Sfi*I sites naturally present in WT Ad5 DNA were mutated by substituting A for G and C at positions 16291 and 16294 in the Ad5 genome, and C and G for respectively G and C at positions 23001 and 23004 in the Ad5 genome, introducing silent mutations in the adenovirus pVII and DNA-binding protein coding sequences. Two *Sfi*I sites that allow for directional cloning replace the E1 region. The E3 region contains a 2.7 kb deletion that corresponds to bp 28137 to 30819 in the Ad5 genome. The E4 region contains a 1.2 kb deletion that encompasses ORF 1-4, and corresponds to bp 34119-35355 in the WT Ad5 genome. The right ITR is flanked by *Pac*I and *Swa*I sites. pAd362 is used in combination with shuttle plasmid pE1.1 and derivatives to construct “second-generation” replication-deficient adenoviruses containing transgenes in place of the E1 region. These recombinant adenoviruses are able to grow in 293 cells without E4 trans-complementation. Their maximum cargo capacity is 8.9 kb.



Sequence

PAD362.TXT (35.7 KB)