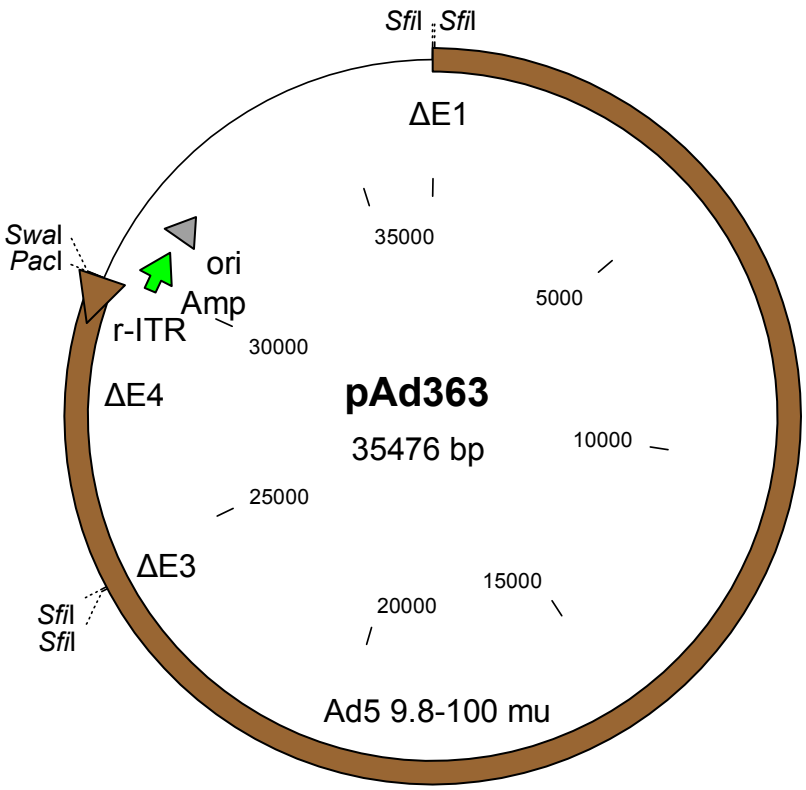
O.D.260 Inc.

pAd363

35,476 base pairs
Sequence available upon request to info@od260.com

pAd363 is a 35.5 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 pairs of *Sfi*I sites that allow for directional cloning replace the E1 and E3 regions. The E3 region contains a 2.7 kb deletion that corresponds to bp 28,137 to 30,819 in the Ad5 genome. The E4 region contains a 1.2 kb deletion that encompasses ORF 1-4, and corresponds to bp 34,119-35,355 in the WT Ad5 genome. The right ITR is flanked by *Pac*I and *Swa*I sites. pAd363 is used in combination with the shuttle vectors pE1.2 and pE3.1 to construct "second-generation" bipartite replication-deficient adenoviruses containing transgenes in place of the E1 and E3 regions. These recombinant adenoviruses are able to grow in 293 cells without E4 trans-complementation. The maximum combined transgene capacity is 8.7 kb

Feature	Coordinates	Source
9.8-100 mu	6-28,578	Ad5
E3 deletion	24,637-24,699	Ad5
E4 deletion	27,998	Ad5
Right ITR	28,476-28,578	Ad5
origin of replication	29,761-30,348	pUC19
Amp^r	28,729-29,589	*Tn3*



Relevant restriction sites only