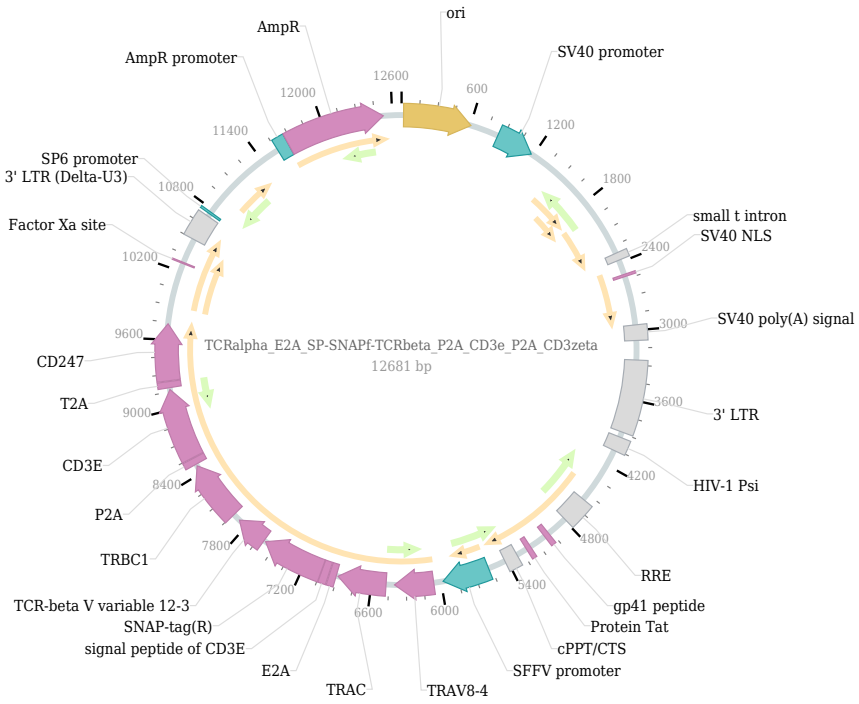
TCRalpha_E2A_SP-SNAPf-TCRbeta_P2A_CD3e_P2A_CD3zeta

载体编号 Vector ID	V017124
抗性 Antibiotic Resistance	Ampicillin
长度 Length	12681 bp
载体类型 Vector Type	Protein expression
宿主 Host	Mammalian cells, Lentivirus

产品信息

复制子 Replication Origin	ori
启动子 Promoter	SFFV
培养菌株 Growth Strain(s)	Stbl3
培养温度 Growth Temperature	37℃
备注 Comment	This multicistronic lentiviral vector (pHR backbone) was constructed in the 2017 Cell study by Taylor et al. to generate full-length DNA-TCR (DNA-CARTCR) in TCR-deficient JRT3 Jurkat cells. Driven by a single promoter, it uses three distinct self-cleaving 2A peptides (E2A, P2A, T2A) to co-express four separate TCR subunits stoichiometrically from one mRNA: Native human TCRα chain; SP-SNAPf-TCRβ: TCRβ with an extracellular SNAPf tag (replaces native pMHC binding domains to bind complementary DNA oligonucleotides) and a signal peptide (SP) for membrane trafficking; CD3ε; CD3ζ. The plasmid solves a critical experimental limitation: sole expression of SNAPf-TCRβ fails to form intact, surface-localized TCR complexes in JRT3 cells due to insufficient endogenous TCRα and CD3 subunits. Co-expression of all required subunits via this construct restores wild-type levels of full TCR complexes on the plasma membrane, which can be fluorescently labeled via SNAP-Surface dyes for FACS sorting and single-molecule TIRF imaging. Functionally, this DNA-CARTCR system mirrors the complete native TCR signaling cascade, serving as a physiological counterpart to the minimal DNA-CARζ construct. It validated that receptor clustering drives robust, stable ZAP70 recruitment and ligand discrimination—this kinetic proofreading mechanism holds true for full TCR complexes, not just truncated CD3ζ-only chimeric receptors.

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.