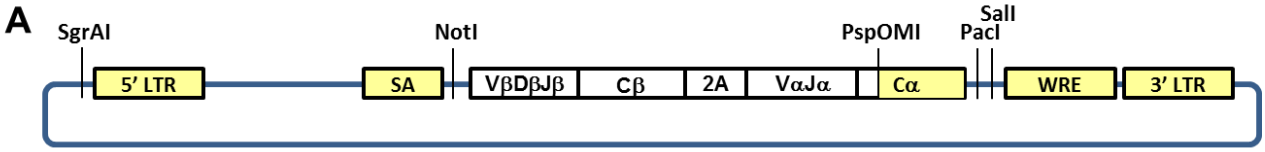

B

1. Comparison of Natural and PspOMI-modified Cα sequences

Natural TCR Cα sequence

5′-TGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACC-3′

Modified TCR Cα sequence (PspOMI sequence underlined)

5′-TGACCCTGCCGTGTACCAGCTGGGGCCCTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACC-3′

2. Double-strand DNA fragment before assemble reaction (complementary sequences underlined)

TCR fragment 3′ end

5′-TGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCT-3′

3′-ACTGGGACGGCACATGGTCGACTCTCTGAGATTTAGGTCACTGTTCAGACAGACGGA-5′

Vector 5′ end after PspOMI treatment

5′-GGCCCTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACC-3′

3′-GAGATTTAGGTCACTGTTCAGACAGACGGATAAGTGG-5′

3. Double-strand DNA fragment during assemble reaction (complementary sequences underlined)

TCR fragment 3′ end after 5′ Exonuclease reaction

5′-TGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCT-3′

3′-ACTGGGACGGCACATGGTCGACTCTCT-5′

Vector 5′ end after 5′ Exonuclease reaction

5′-ATTCACC-3′

3′-GAGATTTAGGTCACTGTTCAGACAGACGGATAAGTGG-5′

4. Double-strand DNA fragment after assemble reaction (complementary sequences are underlined)

5′-TGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACC-3′

3′-ACTGGGACGGCACATGGTCGACTCTCTGAGATTTAGGTCACTGTTCAGACAGACGGATAAGTGG-5′

Supplementary Figure S1

Construction of a destination plasmid for TCR gene cloning. **A**, Schematic representation of the destination plasmid vector. To create the destination plasmid, fragment from 5' Long Terminal Repeat (LTR) to the multiple cloning site (MCS) in a pDON-5 retroviral vectors was amplified by PCR using the sense primer: TGGCGCCGGTGATGTGAAAGACCCCACCTGTAG (SgrAI site underlined) and anti-sense primer: AATGTCGACTATGCGGCCGCAGATCCGAGCTCGGTACC (SalI and NotI sites underlined). The DNA fragment was column-purified and treated with SgrAI and SalI restriction enzymes and inserted into a Murine Stem Cell Virus (MSCV)-based retroviral plasmid pMIG-w (a gift from Luk Parijs (Addgene plasmid # 12282)). The plasmid contains TCR-expressing cassette as a stuffer gene fragment. In the stuffer TCR-expressing cassette, Cα region was modified to provide a PspOMI-recognition site for excision of the stuffer fragment together with NotI, leaving 3' part of Cα-coding region in the vector fragment. To create PspOMI-containing TCR-expressing cassette, Two DNA fragments containing PspOMI-recognizing site in the α chain constant region was prepared by using (1) sense primer: ACCAGCTGGGGCCCTCTAAATCCAGTGACAAGTCTGTCTGCC (PspOMI site underlined) and anti-sense primer: ATTGTCGAC TTAATTAATCAGCTGGACCACAGCCG (SalI and PacI sites underlined) and (2) vector-specific sense primer AATTGATCCGCGGCCGCCACCATG (NotI site underlined) and anti-sense primer: GAGGGCCCCAGCTGGTACACGGCAGGG (PspOMI site underlined). Two PCR fragments were fused by the overlap extension PCR. The fused fragment was introduced into the NotI and SalI site in the plasmid vector. Abbreviations used are: 5' LTR: 5' HCMV/MLV hybrid long terminal repeats; SA: the splice acceptor site from the human elongation factor 1a intron-exon junction; WRE; Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element; 3' LTR; MSCV LTR. **B**, Correction of PspOMI modification during assembling reaction. (1) TCR α chain constant region was modified to provide PspOMI-recognizing sequence; (2) Sequences of TCR VJα and restriction enzyme-treated and purified vector fragments before assembling; (3) During assembling reaction, both fragments were degraded by a 5'-to-3' exonuclease in the reaction mix, by which artificial PspOMI-recognizing sequence in the vector fragment is removed; and (4) After assembling, positive-strand DNA of TCR VJα fragment and negative-strand DNA of the vector fragment form the natural TCR Cα sequence.