Supplemental Table 1. Alpha primers

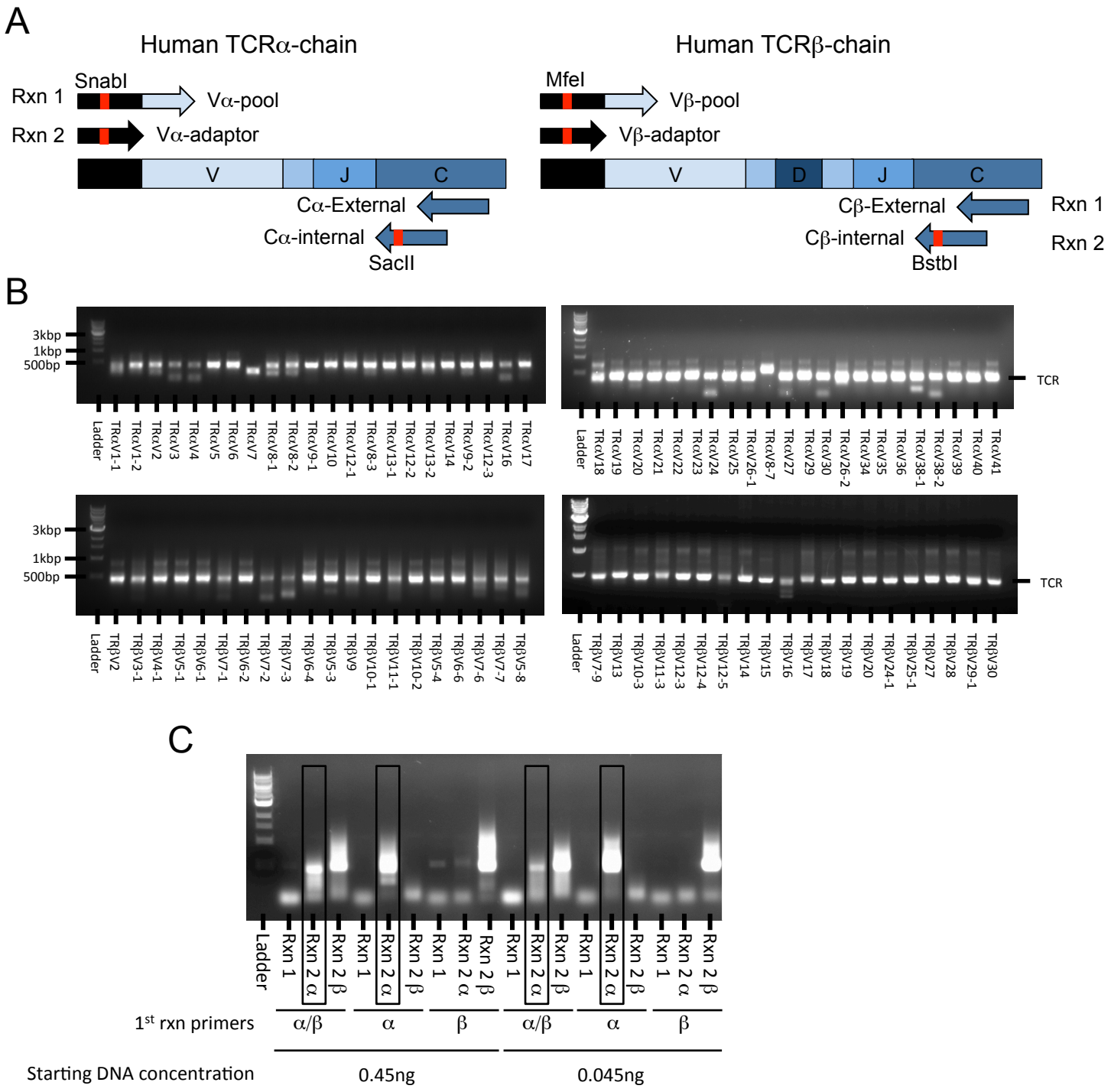
TARGET GENE	PRIMER ORIENTATION	SEQUENCE	RE CUT SITE	Tm
TRAV1-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**TGGGGAGCTTTCCTTCTCTATGTTT**	SnabI	71.6
TRAV1-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**TGGGGAGTTTTCCTTCTTTATGTTTC**	SnabI	70.7
TRAV2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GCTTTGCAGAGCACTCTGG**	SnabI	72.5
TRAV3	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GCCTCTGCACCCATCTCG**	SnabI	73.6
TRAV4	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AGGCAAGTGGCGAGAGTGATC**	SnabI	73.0
TRAV5	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAGACATTTGCTGGATTTTCGTTC**	SnabI	70.2
TRAV6	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGTCATTCCTGGGAGGTGTTT**	SnabI	72.0
TRAV7	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGAAGATGCGGAGACCTGTC**	SnabI	73.0
TRAV8-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTCCTGTTGCTCATACCAGTGC**	SnabI	72.9
TRAV8-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTCCTGCTGCTCGTCCC**	SnabI	73.8
TRAV8-3	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTCCTGGAGCTTATCCCACTG**	SnabI	73.0
TRAV8-7	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTCTTAGTGGTCATTCTGCTGCTT**	SnabI	71.8
TRAV9-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AATTCTTCTCCAGGACCAGCG**	SnabI	72.2
TRAV9-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AACTATTCTCCAGGCTTAGTATCTCTGATACTC**	SnabI	72.1
TRAV10	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAAAAGCATCTGACGACCTTCTTG**	SnabI	71.0
TRAV12-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ATATCCTTGAGAGTTTTACTGGTGATCC**	SnabI	71.3
TRAV12-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ATGAAATCCTTGAGAGTTTTACTAGTGATCC**	SnabI	70.9
TRAV12-3	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ATGAAATCCTTGAGAGTTTTACTGGTG**	SnabI	70.6
TRAV13-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ACATCCATTCGAGCTGTATTTATATTCC**	SnabI	70.5
TRAV13-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GCAGGCATTCGAGCTTTATTT**	SnabI	70.5
TRAV14	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**TCACTTTCTAGCCTGCTGAAGGTG**	SnabI	72.6
TRAV16	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAGCCCACCCTCATCTCAGTG**	SnabI	73.0
TRAV17	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAAACTCTCCTGGGAGTGTCTTTG**	SnabI	72.6
TRAV18	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTGTCTGCTTCCTGCTCAGG**	SnabI	73.2
TRAV19	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTGACTGCCAGCCTGTTGAG**	SnabI	73.2
TRAV20	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGAAAATGTTGGAGTGTGCATTC**	SnabI	71.0
TRAV21	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGACCCTCTTGGGCCTG**	SnabI	73.6
TRAV22	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAGAGGATATTGGGAGCTCTGCT**	SnabI	71.9
TRAV23	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GACAAGATCTTAGGAGCATCATTTTTAG**	SnabI	70.5
TRAV24	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGAAGAATCCTTTGGCAGCC**	SnabI	72.2
TRAV25	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTACTCATCACATCAATGTTGGTCTTAT**	SnabI	70.5
TRAV26-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AGGCTGGTGGCAAGAGTAACTG**	SnabI	72.9
TRAV26-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAGTTGGTGACAAGCATTACTGTACTCC**	SnabI	72.0
TRAV27	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GTCCTGAAATTCTCCGTGTCC**	SnabI	72.2
TRAV29	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GCCATGCTCCTGGGGG**	SnabI	74.0
TRAV30	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGACTCTCCTGAAAGTGCTTTCAG**	SnabI	72.4
TRAV34	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GAGACTGTTCTGCAAGTACTCCTAGG**	SnabI	73.1
TRAV35	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**CTCCTTGAACATTTATTAATAATCTTGTGG**	SnabI	69.6
TRAV36	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ATGAAGTGTCCACAGGCTTTACTAGC**	SnabI	72.3
TRAV38-1	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**ACACGAGTTAGCTTGCTGTGGG**	SnabI	72.9
TRAV38-2	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GCATGCCCTGGCTTCCT**	SnabI	72.9
TRAV39	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AAGAAGCTACTAGCAATGATTCTGTGG**	SnabI	71.4
TRAV40	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**AACTCCTCTCTGGACTTTCTAATTCTGA**	SnabI	71.3
TRAV41	Forward	CGGTTCAGCAGGAATGCCtacgtaATG**GTGAAGATCCGGCAATTTTTG**	SnabI	70.5
TRAC External	Reverse	CAGACAGACTTGTCACTGGATTTAGAGTCTC		61.6
TRAC Internal	Reverse	CAGCTGGTACAccgcggGGTCAGGGTTCTG	SacII	69.7
TRAV Adaptor	Forward	CGGTTCAGCAGGAATGCCtacgtaATG	SnabI	61.2

*Lower case letters within the primer sequences indicate the incorporated restriction enzyme cute sites

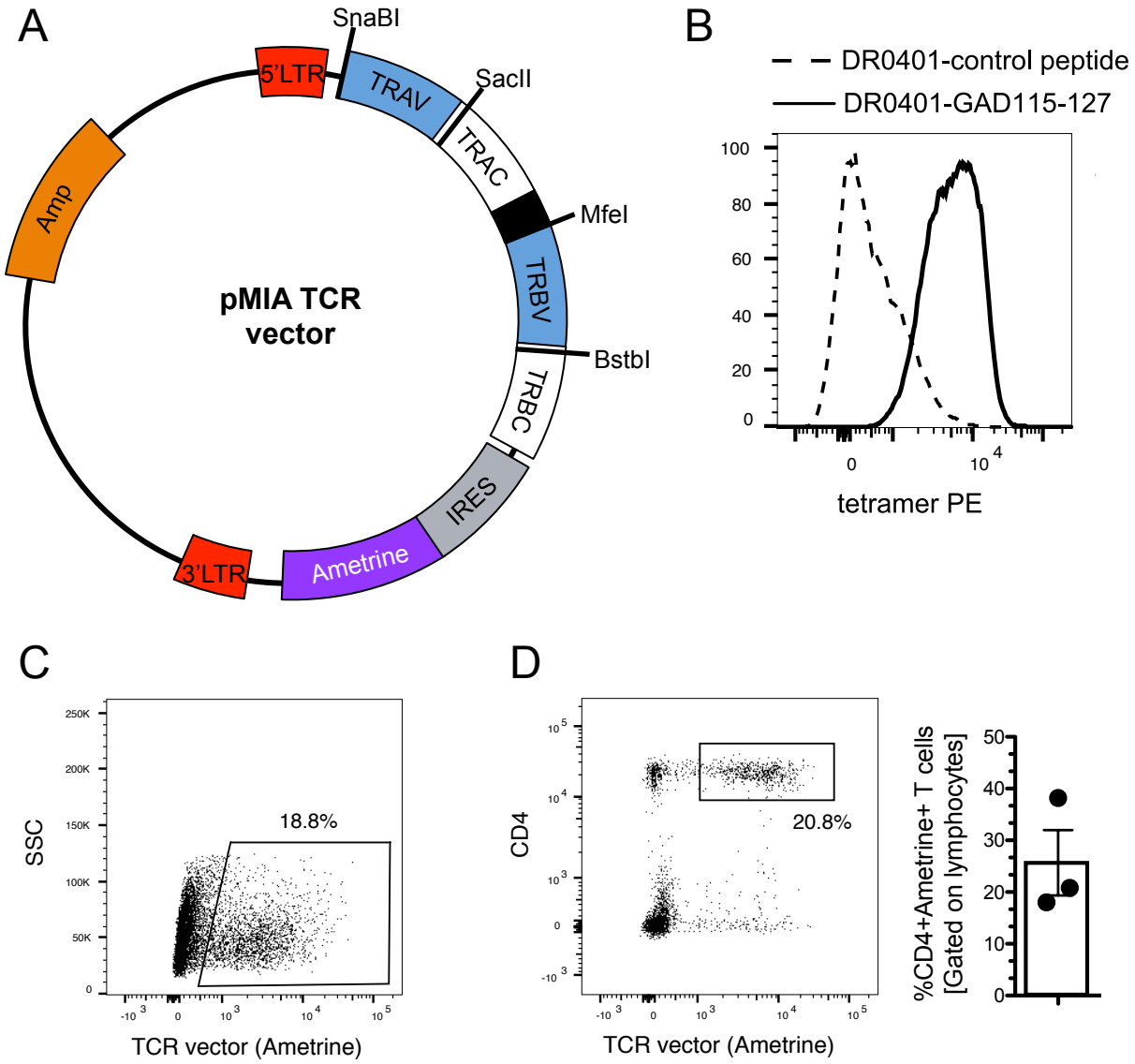
Supplemental Table 2. Beta primers/RT primers/HLA-typing primers

TARGET GENE	PRIMER ORIENTATION	SEQUENCE	RE CUT SITE	Tm
TRBV2	Forward	CAGAAGACGGCATACGAGATcaattgATG**GATACCTGGCTCGTATGCTGG**	MfeI	71.9
TRBV3-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCTGCAGGCTCCTCTG**	MfeI	72.5
TRBV4-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCTGCAGGCTGCTCTG**	MfeI	72.5
TRBV5-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCTCCAGGCTGCTCTGTT**	MfeI	72.2
TRBV5-3	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCCCCGGGCTCC**	MfeI	73.2
TRBV5-4	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCCCTGGGCTCCTCT**	MfeI	72.7
TRBV5-8	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGACCCAGGCTCCTCTTCT**	MfeI	72.2
TRBV6-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCATCGGGCTCCTGTGC**	MfeI	72.3
TRBV6-2	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCCTCGGGCTCCTGTG**	MfeI	72.5
TRBV6-4	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGAATCAGGCTCCTGTGCTGTG**	MfeI	71.8
TRBV6-6	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCATCAGCCTCCTGTGCTG**	MfeI	72.0
TRBV7-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACAAGGCTCCTCTGC**	MfeI	72.3
TRBV7-2	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACCAGGCTCCTCTTCT**	MfeI	72.2
TRBV7-3	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACCAGGCTCCTCTG**	MfeI	72.5
TRBV7-6	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACCAGTCTCCTATGCTG**	MfeI	72.0
TRBV7-7	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGTACCAGTCTCCTATGCTGGG**	MfeI	72.6
TRBV7-9	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACCAGCCTCCTCTG**	MfeI	72.5
TRBV9	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCTTCAGGCTCCTCTGCT**	MfeI	72.2
TRBV10-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACGAGGCTCTTCTTCTATG**	MfeI	71.8
TRBV10-2	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACCAGGCTCTTCTTCTATG**	MfeI	71.8
TRBV10-3	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCACAAGGTTGTTCTTCTATGTG**	MfeI	70.7
TRBV11-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCACCAGGCTTCTCTGCTG**	MfeI	72.0
TRBV11-3	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGTACCAGGCTCCTCTGCTG**	MfeI	72.9
TRBV12-3	Forward	CAGAAGACGGCATACGAGATcaattgATG**GACTCCTGGACCTTCTGCTGT**	MfeI	71.9
TRBV12-4	Forward	CAGAAGACGGCATACGAGATcaattgATG**GACTCCTGGACCCTCTGCTG**	MfeI	72.9
TRBV12-5	Forward	CAGAAGACGGCATACGAGATcaattgATG**GCCACCAGGCTCCTCTG**	MfeI	72.5
TRBV13	Forward	CAGAAGACGGCATACGAGATcaattgATG**CTTAGTCCTGACCTGCCTGACTC**	MfeI	72.4
TRBV14	Forward	CAGAAGACGGCATACGAGATcaattgATG**GTTTCCAGGCTTCTCAGTTTAGTGT**	MfeI	70.6
TRBV15	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGTCCTGGGCTTCTCCACT**	MfeI	72.2
TRBV16	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCCCAATATTCACCTGCATCA**	MfeI	70.2
TRBV17	Forward	CAGAAGACGGCATACGAGATcaattgATG**GATATCTGGCTCCTCTGCTGG**	MfeI	71.9
TRBV18	Forward	CAGAAGACGGCATACGAGATcaattgATG**GACACCAGAGTACTCTGCTGTGC**	MfeI	72.4
TRBV19	Forward	CAGAAGACGGCATACGAGATcaattgATG**AGCAACCAGGTGCTCTGCTG**	MfeI	72.0
TRBV20	Forward	CAGAAGACGGCATACGAGATcaattgATG**CTGCTGCTTCTGCTGCTTCT**	MfeI	71.2
TRBV24-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**GCCTCCCTGCTCTTCTTCTG**	MfeI	72.0
TRBV25-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**ACTATCAGGCTCCTCTGCTACATGG**	MfeI	72.1
TRBV27	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGCCCCCAGCTCCTTG**	MfeI	72.7
TRBV28	Forward	CAGAAGACGGCATACGAGATcaattgATG**GGAATCAGGCTCCTCTGTCG**	MfeI	72.0
TRBV29-1	Forward	CAGAAGACGGCATACGAGATcaattgATG**CTGAGTCTTCTGCTCCTTCTCCT**	MfeI	71.6
TRBV30	Forward	CAGAAGACGGCATACGAGATcaattgATG**CTCTGCTCTCTCCTTGCCCT**	MfeI	72.0
TRBC External	Reverse	GTGGCCAGGCACACCAGTGTG		60.1
TRBC Internal	Reverse	CTCTGCTTCTGATGGttcgaaCACAGCGACCTCGG	BstbI	69.0
TRBV Adaptor	Forward	CAGAAGACGGCATACGAGATcaattgATG	MfeI	60.0
TRAC cDNA	RT	AGCTGGACCACAGCCG		50.9
TRBC1 cDNA	RT	GAAATCCTTTCTCTTGACCATG		51.0
TRBC2 cDNA	RT	GCCTCTGGAATCCTTTCTCT		51.7
HLA SNP 1 rs3104413	Forward	CTGGGGCCCTAATAACCCTTT		55.2
HLA SNP 1 rs3104413	Reverse	CTTTGGGAATACATTAGGATATCTATTC		54.3
HLA SNP 2 rs2854275	Forward	TTTGCTGCTATGAGGATCAAGACTTA		55.1
HLA SNP 2 rs2854275	Reverse	GCCTCTTTCAGTCACTGGAAAATG		55.9
HLA SNP 3 rs2854275	Forward	CCCAAGGAGACCTAGTTCTCCAC		59.0
HLA SNP 3 rs2854275	Reverse	CACAGGACAAATGGCAGAGCTC		56.9

*Lower case letters within the primer sequences indicate the incorporated restriction enzyme cute sites



Supplemental Fig. 1: Primer validation and PCR optimization. (**A**) PCR amplification of paired TCR α- and β-chains. Rxn 1: multiplex variable region primer pools amplify the TCR variable region from the transcription start site. During rxn 1 an adaptor sequence including a restriction enzyme cute site is incorporated into the beginning of the TCR sequence. Rxn 2: nested primers amplify the entire variable region through the beginning of the constant region where a second restriction enzyme cut site is incorporated. (**B**) To verify the specificity and function of each Vα/Vβ primer, primers were tested individually for their ability to amplify a specific TCRα/β-chain from human PBMCs. RNA was isolated from human PBMCs using a TCR-specific reverse transcription (RT) reaction in which only TCR mRNA is reverse transcribed into cDNA. Polyclonal TCR cDNA was then used as a template for each individual variable region-specific primer. All 84 variable region-specific primers amplified a TCR sequence. About 80% of the primers amplified their specific TCR gene, and 20% resulted in amplification of a TCR gene similar in sequence to their target gene. (**C**) Initially the TCR α- and β-chains were amplified together during the multiplex PCR reaction (1st rxn primers α/β). Amplifying the TCR chains in separate multiplex reactions resulted in a significant increase in a PCR product yield, as shown by the black rectangles.



Supplemental Fig. 2: **T1D4-TCR specificity and hu-Rg mice generation**. (**A**) The TCR construct is encoded in a mouse stem cell virus (MSCV)-based retroviral vector containing an IRES-ametrine cassette for easy identification of construct+ cells. Human TCR variable regions (blue) were incorporated directly upstream to the corresponding mouse constant region (white). TCR α- and β-chains were linked by a P2A peptide to allow for stoichiometric expression of both chains. (**B**) T1D4 TCR specificity was verified by DRB1*0401-GAD115-127 tetramer staining of transfected HEK293T cells. (**C**) Bone marrow transduction efficiency prior to transfer into recipient mice. (**D**) Peripheral blood collected via tail vein bleed 6 weeks post-bone marrow transfer.