

Region	Forward	Probe	Reverse	Loc <sup>1</sup>
<b>Igk locus</b>				
V <sub>κ</sub> h24	5'-TGGTTTGTTCCTTGTCTGGA-3'	5'-TCAACATGGCAAATTGAAGCCATTCT-3'	5'-TCCCATTTCCTAGGGATTCA-3'	25H
V <sub>κ</sub> X24	5'-AGTGGCCAGTGATTTGATTG-3'	5'-CAGTATTCCACACAGGGTGCTCTGC-3'	5'-CAAGGATGCAGAGGACATTG-3'	41H
V <sub>κ</sub> 12-38	5'-AGTGGGAGCACAGGTTGC-3'	5'-CCAGCTGCTCCTTACGATGTCTCCA-3'	5'-TCACTCTCAACAGCTCAGCA-3'	17H
V <sub>κ</sub> 21-3	5'-GCTCCAGGGCTGAACAAA-3'	5'-CCTCAGTGAGCTGCAACTCCAGGA-3'	5'-AGCTCTGTGTTGAGATGTCAGAG-3'	X
V <sub>κ</sub> 220	5'-TATGCAACATCCAACTGGCTCT-3'	5'-TCCCWGCTCGCTTCAGTGGCAGT-3'	5'-CCATGCTGCTGATTGTGAGAGAG-3'	53H
V <sub>κ</sub> (degen)	5'-TCCATGCTGCTGATTATGAGAGART-3'	5'-TCCCWGCTCGCTTCAGTGGCAGT-3'	5'-GCAATCCAACCTGGCTTCt-3'	≈50H
J <sub>κ</sub> 1	5'-TTGTACAGCCAGACAGTGGAG-3'	5'-TGGTGCCTCCACCGAACGTC-3'	5'-GCCACAGACATAGACAACGG-3'	X
J <sub>κ</sub> 2	5'-CAGATTCTGGCACTCTCCAA-3'	5'-CAAAGAAGCAGGGTAGCCTGCC-3'	5'-ACTGAGCATGGTCTGAGCAC-3'	X
J <sub>κ</sub> 4	5'-AGTGTGAAAGCTGAGCGAAA-3'	5'-CCTGCCTGTGAAGCCAGTCCA-3'	5'-CACAGTGAGGACTATGACATGC-3'	12H
J <sub>κ</sub> 5	5'-CATAGTCCTCACTGTGGCTCA-3'	5'-CTTGGTCCCAGCACCGAACG-3'	5'-AGTGTACTTACGTTTCAGCTCCA-3'	X
C <sub>κ</sub>	5'-GTGGAAGATTGATGGCAGTG-3'	5'-CAGTCCAAGTTCAGGACGCCA-3'	5'-GCTCATGCTGTAGGTGCTGT-3'	
<b>Igh locus</b>				
Vh558-77	5'-AAATCCTCCAGCACAGCCTA-3'	5'-CATGCAGCTCAGCAGCCTGACA-3'	5'-TAGACCGCAGAGTCCTCAGA-3'	12H
Vh558-72	5'-ATCTGAGGACTCTGCGGTCT-3'	5'-CTGTGCAAGACACAGTGTTGCAACC-3'	5'-CCTCCAGGGTTTCTGACT-3'	X
Vh558-47	5'-GGAGCTCAGCCGATTAACAT-3'	5'-TTGCAACACTGTGCCTTGACACA-3'	5'-TTGACACACACAGGATGTGG-3'	X
Vh558-31	5'-CATGGAATATCCTCGATTGG-3'	5'-TCAATGTGGCCTTGCCCTTGA-3'	5'-GCTCCATGTAGGCTGTGCTA	46H
Vh558-18	5'-AAGGCCACATTGACTGTTGA-3'	5'-TCCTCCAGCACAGCCTACATGGA-3'	5'-CCTCAGATGTCAGGCTGTTG-3'	25H
VhSM7	5'-TCTGAGGACACTGCCGTCTA-3'	5'-ACTGTGCTAGACACAGTGTTGCAACCA-3'	5'-TTTCTGACACGCTCAGGATG-3'	X
VhS107	5'-CAGAGACACTTCCCAAAGCA-3'	5'-CCCTGAGAGCTGAGGACACTGCC-3'	5'-GTGTCTGGGCTCACAATGAC-3'	X
Vh81x	5'-GACCCTGTACCTGCAAATGA-3'	5'-CAAGGCTGTGTCTCAGACCTCAGA-3'	5'-TTGCTCATTGTGTGCTTGC-3'	X
DFL16.1	5'-CCTGCAAACAGAGCTTACCA-3'	5'-TGTGTGCCAATCACATTCCCA-3'	5'-TCCAGTACTGCTGCTTGGTC-3'	X
DSP2	5'-CTCTGCACTGCTACCTCTGG-3'	5'-CCAGACAATGTTCTGCAGAACCTG-3'	5'-AAATCCCTGCCAAGTAAGGTA-3'	X
DQ52	5'-CATTGGTCCCTGACTCAAGA-3'	5'-CCCTGTGGTCTCTGACTGGTGCA-3'	5'-TCCCAGTTAGCACTGTGGTG-3'	200N-5'
Jh1	5'-TGCTACTGGTACTTCGATGTCTG-3'	5'-CGCAGGGACCACGGTCACC-3'	5'-GCCAGCTTACCTGAGGAGAC-3'	X
Jh2	5'-CAGTCTCCTCAGGTGAGTCCT-3'	5'-CCCAAGGTGTCCCTAGTCCTTCATGA-3'	5'-CCCAATGACCCTTTCTGACT-3'	X
Jh3	5'-TGTGCCTGGTTTGCTTACTG-3'	5'-AGTGACCAGAGTCCCTTGGCCC-3'	5'-GCAGAGAATCTTGGTCCTG-3'	X
Jh4	5'-TGTGATTACTATGCTATGGACTACTGG-3'	5'-TCAAGGAACCTCAGTCACCGTCTCC-3'	5'-GGAGAGGCCATTCTTACCTG-3'	X
C <sub>μ</sub>	5'-CCATTTCTTCACCTGGAAC-3'	5'-CCAGAACAACACTGAAGTCATCCAGGG-3'	5'-TCAGTGTTGGGAAGGTTCTG-3'	
<b>Tcra locus</b>				
TRAV1	5'-TCTGCCTCATACCTCTGTGC-3'	5'-TGCTAACCTCTGCAGCTGCACAGA-3'	5'-ACTCCTGCTGTGTCCCTCTT-3'	X
TRAV16	5'-TCCATCGGACTCATCAC-3'	5'-CGCCACACAGATTGAGGACTCAGC-3'	5'-GTGCCCTCTCTCATAGCACA-3'	X
TRAV17	5'-CGTCACGCTCTATGAAGGAG-3'	5'-TGGCCAGGATACTGCACGTACCA-3'	5'-TTGAGGAGAACTGGAGGCT-3'	137H

TRAV19	5'-TCCTGATATCCGTACGCTCA-3'	5'-TCACTGCACATCACAGCCTCCC-3'	5'-CACTGCTGTGTCTTCAGGCT-3'	18H
TRAV21	5'-CCTTAGGTATTGCGGGTGAT-3'	5'-TGCCTTGTAGCCACGCCACA-3'	5'-CTGAAGAGGAACCTGTCCGGT-3'	150H
TEA	5'-GAGCTCACTATTCACCAGTCA-3'	5'-TCTCTTATCCTTGAAGCCCGTCTCTT-3'	5'-ATCCACTTCTCTGGGTCTCTTAG-3'	
TRAJ61	5'-GGTGTCTACTTCTGTGGGTA-3'	5'-TGAGTCTTCCAGTCATGGCTCCAAA-3'	5'-TCAGATGTTACTTGCTGAGTTTCA-3'	X
TRAJ58	5'-GTTTCAGTTGATGCCACGAGT-3'	5'-CCCTTCAGTGCAGTGTGCAGCA-3'	5'-ACAGCTTAGACCCAGTGCCT-3'	X
TRAJ56	5'-GGGAGGTTTGGACAACACTGAG-3'	5'-TCCAGTAGCCACACAATGACACGG-3'	5'-CTCAGAACGGTTCCTTGACC-3'	X
TRAJ55	5'-TGGGAAGAGTCCTCTGGAGT-3'	5'-CCAAACCCGTAGCCAGGCCA-3'	5'-TCAACATCCCTCATCTCCCT-3'	X
TRAJ53	5'-CAGGCTGTGGGAACAGTG-3'	5'-CCCAAATGTCAGTTTGTAAATTGCTGCC-3'	5'-TGGAGTCACAGTTAAGAGAGTTCC-3'	X
TRAJ52	5'-TAAAGGCCCGTGTTACAGTG-3'	5'-CCGTGTCCAAACGTGAGCTTTCC-3'	5'-GATGGACCCTAAGGATGGTG-3'	X
TRAJ49	5'-AGCTCTGTCTTCTGCTGGTTT-3'	5'-TCTGGTAACCCGTGTTCCACTGTGA-3'	5'-TCAAACCTGTTCTTTCCCA-3'	X
TRAJ48	5'-ACACTGTGGCCAACACTATGGA-3'	5'-CCCTAGTCAGGAGACCAGCCGC-3'	5'-AAATCCCTAAGCCTGCGT-3'	X
TRAJ45	5'-TATGAGCTGTGCTGCCATTT-3'	5'-TCACACCCTGAGGCAAGCCTTG-3'	5'-AGCTGAGTTCCTTTCCAAA-3'	X
TRAJ37	5'-TTGTAAAGTGCAGCATTGGG-3'	5'-CCTTCAGACCCACCTGGTTGCA-3'	5'-AATGAGCATAAAGCGACAGC-3'	X
TRAJ16	5'-GGATTCCAAATTTGATTCATTG-3'	5'-CCATTCATCCAGACATCACGGGA-3'	5'-ATGAGAGGGCACCTTGCT-3'	80H
TRAC	5'-CCCAGATTTGTGAGGAAGGT-3'	5'-TAAGCACTGCTGCCGCACCC-3'	5'-CTTCAGATCCAGGAGAAGCC-3'	
<b><i>Tcrβ</i> locus</b>				
TRBV1	5'-CCAGGGCAGAACCTTGTACT-3'	5'-TCTGCAGGCGCATTGAAACAAA-3'	5'-GCAGGGTAGTCTGTGTGTGG-3'	X
TRBV16	5'-GCGGTGTATCTTTGTGCAAG-3'	5'-TCTGCTGAGGCAGAAACCATACGC-3'	5'-GGAGGTGGACAGGAGAGG-3'	X
TRBD1	5'-GCCACGGTGATTCAATTCTA-3'	5'-TGTCCCAAGGCCACAGTCCT-3'	5'-GCGACCCAGGAGAAGAGTAG	X
TRBJ1-1	5'-CTAGATACTAGCTGGGAAGAGCCT-3'	5'-TCCTCATCCTATGGCACTGTGCAA-3'	5'-TGAGTCTGGTTCCTTTACCAA-3'	X
TRBJ1-6	5'-CAGCTGTGTTCCCTATAATTCGC-3'	5'-AGGCACCCGGCTCACTGTGA-3'	5'-ACCATGGTCATCCAACACAG-3'	X
TRBC1	5'-CCCAAGAAGCTAAACAAGGC-3'	5'-CCAGATCCTCATTCCGGAACCC-3'	5'-TACAAGGAGCATGGAATGGA-3'	
TRBD2	5'-GTGAAAGGCTATCCAGGGAA-3'	5'-TGGGACCACCATTGAGCCCTT-3'	5'-ACGGATACTTTGCTGATGGTT-3'	129N-3'
TRBJ2-1	5'-TATGCTGAGCAGTTCCTTCGG-3'	5'-CAGGGACACGACTCACCGTCCT-3'	5'-CCTACCTTCTCTCCACCTG-3'	69H
TRBJ2-5	5'-TGTGAACCAAGACACCCAGT-3'	5'-CCGAGTGCCTGGCCCAAAGT-3'	5'-CAGCTCACCTAACACGAGGA-3'	X
TRBJ2-7	5'-TGAGCCTCTGTGCTCCTATG-3'	5'-CGGCACCAGGCTCACGGTTT-3'	5'-GTGGAAGCGAGAGATGTGAA-3'	X
TRBC2	5'-GTGGCTTTCTTTACCCAAAT-3'	5'-TCCCTCTCCTTTCTTTGAGACTGTGGA-3'	5'-TTTCAAAGCCAAGACCACAG-3'	
TRBV31	5'-GTGTGAGACTCCAGGCACAG-3'	5'-TTCCAGGCCGAAGGACGACC-3'	5'-GTGCAACTGAACCTCTCAGC-3'	X
<b>Non antigen receptor genes</b>				
$\alpha$ -actin	5'-GGCACGTGACACTCTTGTCT-3'	5'-ATCGGTGGCTCCATCCTGGC-3'	5'-GTCGTA CTCTGCTTGGTGA-3'	
$\beta$ -actin	5'-AGTGTGACGTTGACATCCGT-3'	5'-TGCATCCTGTCAGCAATGCCTG-3'	5'-TGCTAGGAGCCAGAGCAGTA-3'	
$\beta$ -globin	5'-GCTGCTGGTTGTCTACCCTT-3'	5'-TCCAAAGCTATCAAAGTACCGCTGGG-3'	5'-GCAGAGGCAGAGGATAGGTC-3'	
$\gamma$ -actin	5'-CCTCCTCCAATAAAGGGACA-3'	5'-AGAAGTGGTACCCTCTCCACG-3'	5'-GCCATCACATCCAGTCA-3'	
Elp4	5'-AGAGTTCCGATTGGGTCATC-3'	5'-AGCTCCCGGTCTGTGGCGTC-3'	5'-TGCCCTTCTCTGGAAACTA-3'	

Rars2	5'-CCTTAGACACGGAGGAGGTG-3'	5'-CATGCCTCCCTTTCCCGCAC-3'	5'-GACCTACGAAAGCCACACG-3'	
6430706D22Rik	5'-GTTTCCACCGGAAGTGCT-3'	5'-AACCGATCCTGCCCGCGTT-3'	5'-GCGCGCTAATAGGGTCTTT-3'	
D030013I16Rik	5'-GGACCACAGATTGGAAGCA-3'	5'-TCCTGTCCACGACCAGTGACTGC-3'	5'-GAGATGGCCGGAGAGAGTC-3'	
Mrps5	5'-GCTGACGACGAACTTGTGAC-3'	5'-TCCTCACCGCCTGCAGCAAC-3'	5'-AGCTACACAATGCAGGGAGA-3'	
Fahd1	5'-AGAGACCTTTCGCTGACCTC-3'	5'-TCTGGAGGCCGCCGTAGTCC-3'	5'-GGTCATGTGACCACCGACT-3'	
<b>Retroviral recombination substrates (pINV-12/23 and pINV-0)</b>				
12a	5'-CCTCTAGACTGCCGGATCTC-3'	5'-TCCCTCCTCCAAGCCCTGGA-3'	5'-CATAAGCCTGCTGGTTCAGA-3'	X
12b	5'-ACGCGTCACAGAGAAATGAA-3'	5'-TCCCTCCTCCAAGCCCTGGA-3'	5'-CATAAGCCTGCTGGTTCAGA-3'	69H
23c	5'-AGGCTGAACTCATGCTGGAT-3'	5'-CCAGCCTGACAGCCTGCCTG-3'	5'-TCTCAGTCTTGCAGGTGTCC-3'	109N
23d	5'-GCAAGACTGAGAGCAGGAGA-3'	5'-CCGTCCTCGAGGCACCATGA-3'	5'-ACACCGGCCTTATTCAA-3'	X

Table S1. List of oligonucleotide primers and probes used for sequence detection. Oligonucleotides are listed 5' to 3'. Primers were obtained from Invitrogen. Hydrolysis probes were labeled at the 5' end with the reporter dye 6-carboxyfluorescein (FAM) and at the 3' end with the quencher dye Black Hole Quencher (BHQ) (Biosearch Technologies).

<sup>1</sup>Location of PCR product relative to the RSS for gene segments containing RSSs. X indicates that the PCR product spans the RSS, whereas numbers indicate the distance in bp from the either the heptamer end (H) or nonamer end (N) of the RSS. For example, "69H" indicates that the PCR product is 69 bp from the first residue of the heptamer, while "109N" indicates a distance of 109 bp from the terminal residue of the nonamer. For D elements, 3' or 5' is included to indicate the RSS 3' or 5' or the D segment.

<b>Genotype of thymocytes</b>	<b>Antibody used for IP</b>	<b>Total sequencing reads passing quality filter</b>	<b>Uniquely aligned sequencing reads</b>	<b>Percentage aligned reads</b>
Wild type	RAG2	15,836,506	7,566,851	48%
RAG2 <sup>-/-</sup>	RAG2	15,066,878	7,140,058	47%
D708A-R1 <sup>-/-</sup> β	RAG2	15,369,687	8,417,638	55%
D708A-R1 <sup>-/-</sup> β	H3K4me3	14,838,753	12,130,218	82%
Wild type	H3K4me3	11,921,334	7,612,684	64%

Table S2. Sequencing data for ChIP-sequencing analysis of total thymocytes.