VH and VL germline gene cloning:

The germline genes corresponding to the selected heavy and light variable regions (VH1-2, VH1-18, VH1-69, VH3-30.3, VH3-48, VH3-23, VH5-51, VK1-33, VK1-39, VK3-11, VK3-15, VK3-20, VL1-44, VL1-51; nomenclature according to the IMGT database) were amplified using AccuPrime Pfx DNA polymerase (Invitrogen) and specific primers designed according to the sequences found in the IMGT database and cloned into the pGEM vector (Promega).

Generation of VH and VL Acceptor Frameworks

In order to generate the Acceptor Frameworks, each of the selected germline genes was amplified in multiple steps in order to add at the 3' end a DNA sequence containing two BsmBI restriction sites followed by a variable FR4 gene sequence. The first amplification step was performed using 50 ng of the pGEM plasmid containing a selected germline gene as template whereas the subsequent amplifications were performed using 5 µl of the previous PCR reaction. The PCR conditions were: 15 cycles (25 sec. at 94°C, 20 sec. at 55°C, 40 sec. at 68°C) using AccuPrime Pfx DNA polymerase (Invitrogen).

For the VH Acceptors four amplification steps were performed. For the first PCR, 5' and 3' primers specific for a germline family were used whereas for the three subsequent amplifications the germline specific 5' primer was used in combination with overlapping 3' extension primers. The same extension primers were used for all germline genes as the stuffer and the VH FR4 region added were the same. The 5' primers and final 3' extension primer contain NcoI and XhoI restriction sites (underlined), respectively. The VH primers are listed below:

5VH1	5'-CAGCCGGCCATGGCCCAGGTGCAGCTGGTGCAG-3'
5VH3-30.3	5'-CAGCCGGCCATGGCCCAGGTGCAGCTGGTGGAG-3'
5VH3-23	5'-CAGCCGGCCATGGCCGAGGTGCAGCTGTTGGAG-3'
5VH3-48	5'-CAGCCGGCCATGGCCGAGGTGCAGCTGGTGGAG-3'
5VH5-51	5'-CAGCCGGCCATGGCCGAGGTGCAGCTGGTGCAG-3'
3VH1/3	5'-CTTACCGTTATTCGTCTCATCTCGCACAGTAATACAC-3'
3VH3-23	5'-CTTACCGTTATTCGTCTCATTTCGCACAGTAATATAC-3'
3VH5-51	5'-CTTACCGTTATTCGTCTCATCTCGCACAGTAATACAT-3'
3VHext1	5'-CAATACGCGTTTAAACCTGGTAAACCGCCTTACCGTTATTCGTCTCA-3'
3VHext2	5'-GTTCCCTGGCCCCAAGAGACGCGCCTTCCCAATACGCGTTTAAACCTG-3'
3VHext3	5'-CCTCCACCGCTCGAGACTGTGACCAGGGTTCCCTGGCCCCAAGAG-3'

The same strategy was applied to generate the Vκ and Vλ acceptor frameworks. The 5' primers and final 3' extension primer contain SalI and NotI restriction sites (underlined), respectively. The VK and Vλ primers are listed below:

5VK1	5'-CGGGTCGACGGACATCCAGATGACCCAGTC-3'
5VK3-11	5'-CGGGTCGACGGAAATTGTGTTGACACAGTCTCCAGC-3'
5VK3-15	5'-CGGGTCGACGGAAATAGTGATGACGCAGTCTCCAGC-3'
5VK3-20	5'-CGGGTCGACGGAAATTGTGTTGACGCAGTCTCCAGG-3'
3VK1-33	5'-CCTTACCGTTATTCGTCTCGCTGCTGACAGTAATATGTTGCAATA-3'

3VK1-39 5′-CCTTACCGTTATTCGTCTCGCTGCTGACAGTAGTAAGTTGCAAAA-3′

3VK3 5′-CCTTACCGTTATTCGTCTCGCTGCTGACAGTAATAAACTGCAAAATC-3′

3VKext1 5′-CCAATACGCGTTTAAACCTGGTAAACCGCCTTACCGTTATTCGTCTC-3′

3VKext2 5′-GGTCCCTTGGCCGAATGAGACGCGCCTTCCCAATACGCGTTTAAAC-3′

3VKext3 5′-GTGCGGCCGCCCGTTTGATTTCCACCTTGGTCCCTTGGCCGAATG-3′

5VL1-44 5′-CGGGTCGACGCAGTCTGTGCTGACTCAGCCAC-3′

5VL1-51 5′-CGGGTCGACGCAGTCTGTGTTGACGCAGCCGC-3′

3VL1-44 5′-CCTTACCGTTATTCGTCTCCTGCTGCACAGTAATAATC-3′

3VL1-51 5′-CCTTACCGTTATTCGTCTCCTGTTCCGCAGTAATAATC-3′

3VLext1 5′-CCAATACGCGTTTAAACCTGGTAAACCGCCTTACCGTTATTCGTCTC-3′

3VLext2 5′-CCCTCCGCCGAACACAGAGACGCGCCTTCCCAATACGCGTTTAAAC-3′

3VLext3 5′-GTGCGGCCGCCCCTAGGACGGTCAGCTTGGTCCCTCCGCCGAACACAGA-3′

The products of each final PCR assembly were purified on 1.2% agarose E-gel (Invitrogen). All the VK and Vλ Acceptor Frameworks were cloned SalI/NotI into a pNDS1 vector containing a fixed functional VH3-23. Conversely, the VH Acceptor Frameworks were cloned NcoI/XhoI into a pNDS1 vector containing a fixed functional VK1-39. These cloning steps resulted in the generation of 7 vectors containing each a VH Acceptor Framework in combination with a VK1-39 light chain and 7 vectors containing each a Vκ or Vλ Acceptor Framework in combination with a VH3-23 heavy chain. These 14 vectors were used to generate libraries by replacing the stuffer region with diversified DNA sequences using the Type IIS restriction enzyme BsmBI.

Synthetic CDR3 diversity:

The synthetic diversity was generated by PCR assembly using an equimolar mixture of three primers. The 5' and 3' primers were biotinylated and had affixed sequence whereas the central primers differed in length and contained diversity using different randomization strategies NNS, DVK, NVT or DVT depending on the size of the randomized sequence. The assembly was performed using 5 PCR cycles (25 sec. at 94°C, 15 sec. at 65°C, 15 sec. at 68°C) using AccuPrime Pfx DNA polymerase (Invitrogen). The assembly products were purified on Wizard SV Gel and PCR Clean-Up System spin columns (Promega).

5H3_biot 5′-biot-ATGATGCTGCTGGCACGTCTCCGAGA-3′

3H3_biot 5′-biot-CCACGTCATCCGATCCGTCTCCCCCAATAATCAAA-3′

H3nns 5′-GCTGGCACGTCTCCGAGA(NNS)$_{4-6}$ TTTGATTATTGGGGGAGACG-3′

H3dvk 5′-GCTGGCACGTCTCCGAGA(DVK)$_{6-7}$ TTTGATTATTGGGGGAGACG-3′

H3nvt 5′-GCTGGCACGTCTCCGAGA(NVT)$_{7-9}$ TTTGATTATTGGGGGAGACG-3′

H3dvt 5′-GCTGGCACGTCTCCGAGA(DVT)$_{9-10}$TTTGATTATTGGGGGAGACG-3′

5KL3_biot 5′-biot-CCGGTGTAGCGAAGGCGTCTCAGCAG-3′

3KL3_biot 5′-biot-TAGGGTCGCCTTGATCGTCTCCCGAAGGTCGG-3′

K4nns 5′-GAAGGCGTCTCAGCAG(NNS)$_4$ CCGACCTTCGGGAGACG-3′

K56nns 5′-GAAGGCGTCTCAGCAG(NNS)$_{5-6}$CCGNNSACCTTCGGGAGACG-3′

5L44_biot 5′-biot-CGGTCAGTCGCAATACGTCTCCAGCATATGAT-3′

5L51_biot 5′-biot-CGGTCAGTCGCAATACGTCTCGAACATATGAT-3′

3L_biot 5′-biot-CAGGACCAGTCTCGTGAGGATCGTCTCAACAC-3′

L44nns 5′-CGTCTCCAGCATATGAT$(NNS)_{4-6}$ GTGTTGAGACGATCCTC-3′

L51nns 5′-CGTCTCGAACATATGAT$(NNS)_{4-6}$ GTGTTGAGACGATCCTC-3′

Digestion and ligation :

The diversified CDR3 assembly products and the 14 pNDS1 vectors, containing the different Acceptor Frameworks, were digested at 55°C with BsmBI restriction enzyme (NEB) for 5 hrs. The digested vectors were purified on ChromaSpin TE-1000 columns (Clontech) according to the manufacturer's protocol. The digested inserts were purified in three steps. First, all biotinylated DNA fragments were captured using streptavidin coated beads (Dynal) according to the manufacturer's protocol. This step allowed for the removal of digested extremities as well as not fully digested products. The bead supernatants containing the digested inserts were extracted using Phenol/Chloroform/Isoamyl alcohol (25 :24 :1, v/v) (Invitrogen). The inserts were then precipitated and resuspended in water. Ligations were performed using Rapid DNA ligation kit (Roche). The ligation products were purified by Phenol/Chloroform/Isoamyl alcohol extraction and precipitation with Sodium acetate/Ethanol/Glycogen.

AE1 library generation:

In order to get the final library AE1 with diversity in both CDR3 VH and VL, we recombined the previous seven VH with the seven VL libraries.
The VH libraries were digested with XhoI/NotI for 6 hrs and SwaI, that cuts in the middle of the fixed VK1-39 chain, for 3 hrs and purified on ChromaSpin TE-1000 columns according to the manufacturer's protocol to remove the whole VK1-39 chain. The VL libraries were digested with XhoI/NotI for 6 hrs and the VL fragments purified on 1.2% agarose E-Gel. Ligations were performed using Rapid DNA ligation kit (Roche). The ligation products were purified by Phenol/Chloroform/Isoamyl alcohol extraction and precipitation with Sodium acetate/Ethanol/Glycogen. The ligation product was electroporated into TG1 cells.