Supporting Information (SI)

Isolation of common light chain antibodies from immunized chickens using yeast biopanning and fluorescence-activated cell sorting

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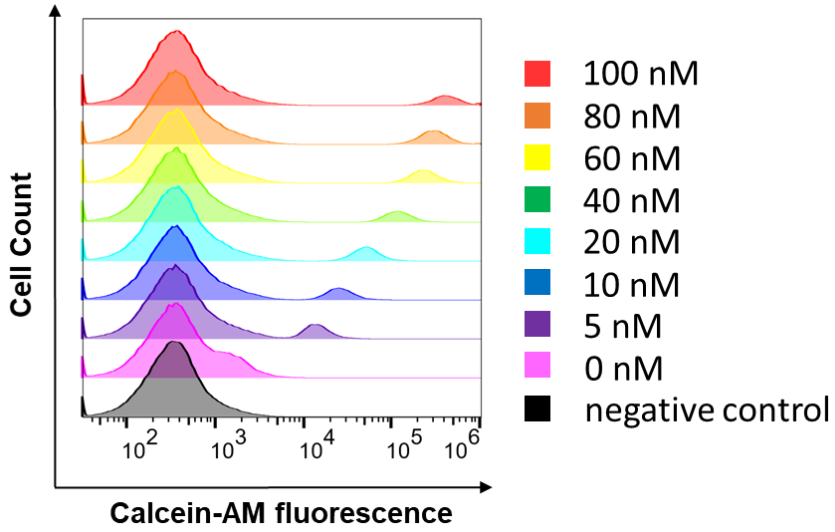

Figure S1. Determination of the optimal Calcein-AM concentration for staining of A431 cells. Up to 5×10^5 A431 cells were stained with 5-100nM Calcein-AM for 20 min and subsequently washed. A431 cells were mixed with a tenfold excess of non-presenting yeast cells and analyzed by flow cytometry. To find the appropriate Calcein-AM concentration, the differences in fluorescence intensity between both populations were analyzed. The left population represent yeast cells, the right population viable A431 cells after staining with different concentrations of Calcein-AM. While 5 nM Calcein-AM staining showed no baseline separation, 100 nM Calcein-AM hit the detector threshold. Therefor 80 nM Calcein-AM was chosen for following experiments.

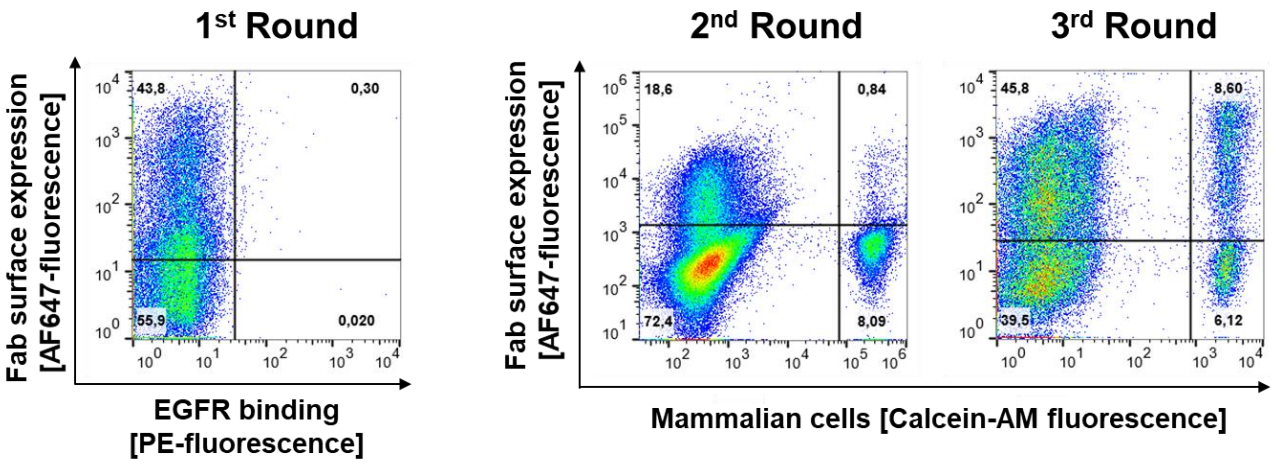

Figure S2. Sorting rounds with soluble EGFR and biopanning using A431 cells. Surface presentation of Fab-fragments was verified by utilizing the goat anti-human Kappa-Alexa Fluor® 647 (SouthernBiotech) antibody and is displayed on the y-axis. On the x-axis, target binding is indicated. For the first round, soluble EGFR-Fc chimera was utilized, target detection is performed using the goat anti-human IgG-Fc-PE conjugate (Fisher Scientific, 1:50) antibody. For round two and three Calcein-AM stained A431 cells were utilized. Calcein-AM fluorescence is plotted on the x-axis. In the lower left quadrant, yeast cells are depicted. In the upper right quadrant, yeast cells expressing a Fab-fragment are shown. In the lower right quadrant, Calcein-AM stained mammalian cells are shown. Double positive events (right upper quadrant) indicate enrichment of yeast clones that can bind to viable stained A431 cells. The percentage of events per gate is indicated. For sorting purposes, this gate was chosen. Between 50.000 and 100.000 events are plotted.

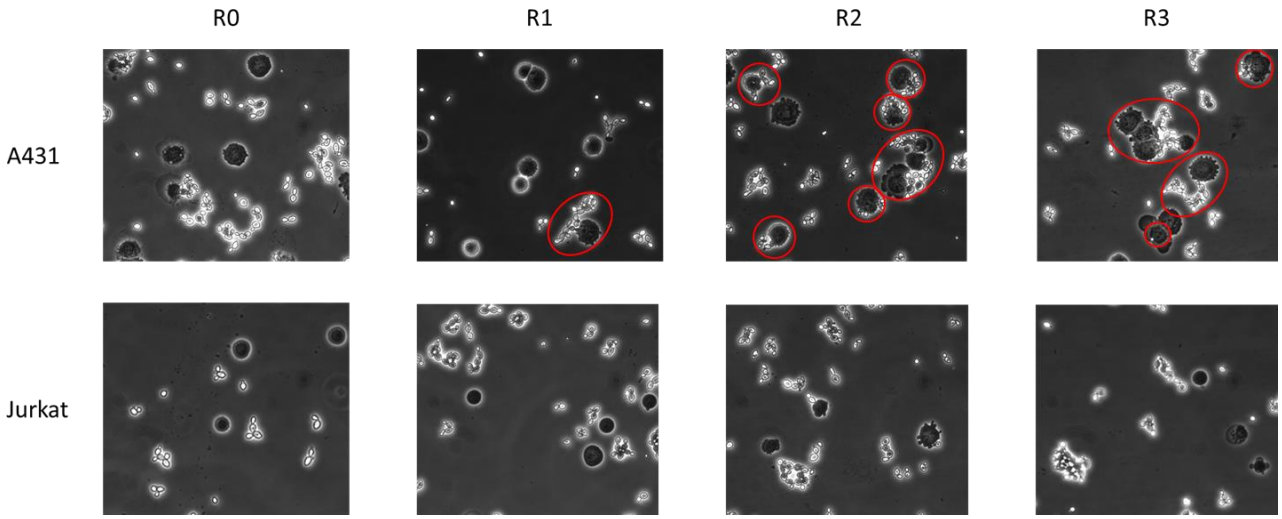

Figure S3. Microscopic analysis of sorting rounds with A431 and Jurkat cells. Induced yeast cells from each round were mixed with A431 or Jurkat cells in a 20:1 ratio as described. The mixture was analyzed using the Axio Vert.A1 microscope (Carl Zeiss AG). While the initial library showed no aggregation of yeast and mammalian cells on either cell line, subsequent rounds showed complexes (red circles) for A431 cells, but not for Jurkat cells.

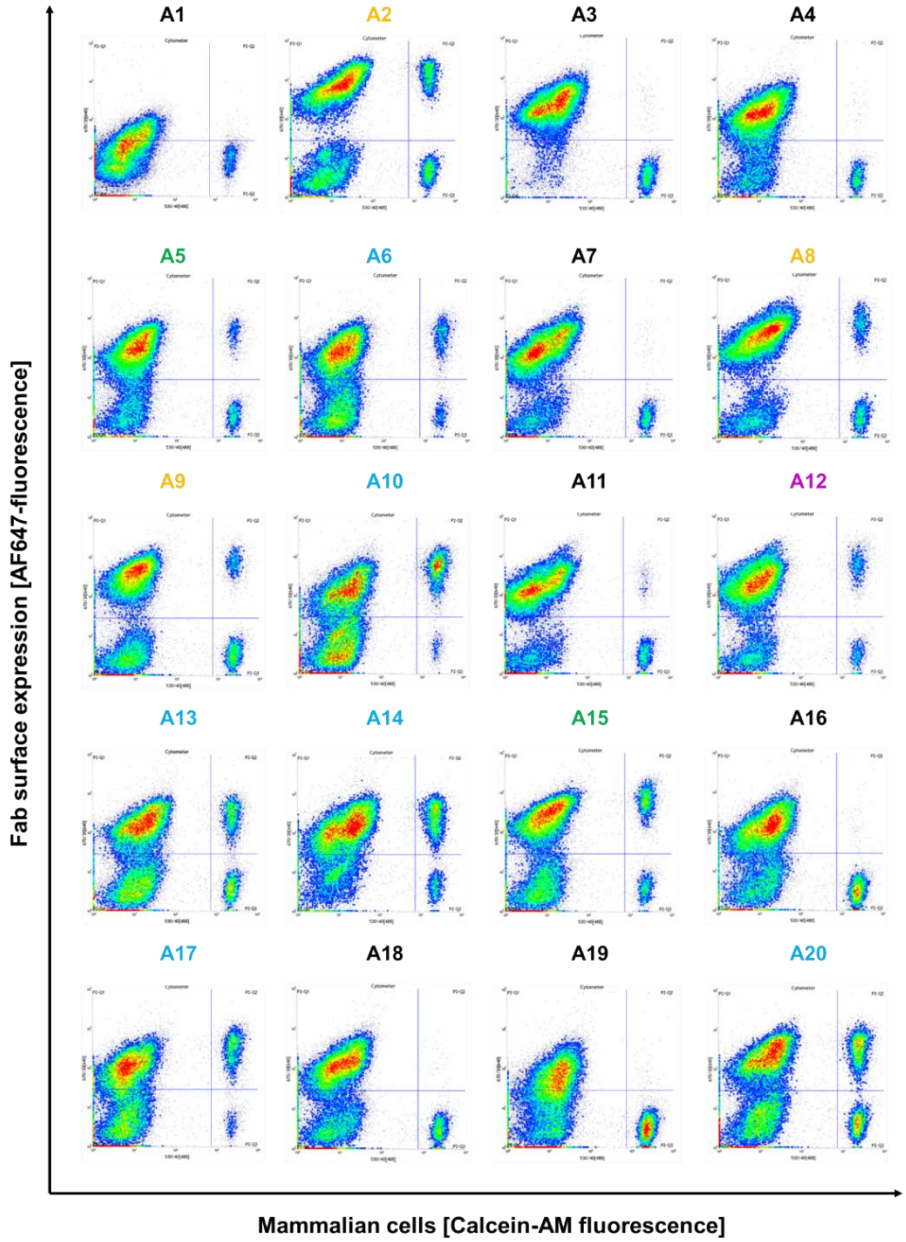

Figure S4. Binding to A431 cells of 20 randomly chosen clones. Surface presentation is displayed on the y-axis. On the x-axis, the fluorescence intensity of Calcein-AM is depicted referring to stained EGFR-positive A431 cells. In the lower left quadrant, yeast cells are depicted. In the upper right quadrant, yeast cells expressing a Fab-fragment are shown. In the lower right quadrant, Calcein-AM stained mammalian cells are shown. Double positive events (right upper quadrant) represent Fab variants on yeast cells that are able to bind to viable mammalian target cells. 50.000 events are plotted. Yeast clones exhibiting the genes for identical Fab fragments are depicted in the same color colors. Yeast clones with black labels were not sequenced due to their inability to bind A431 cells.

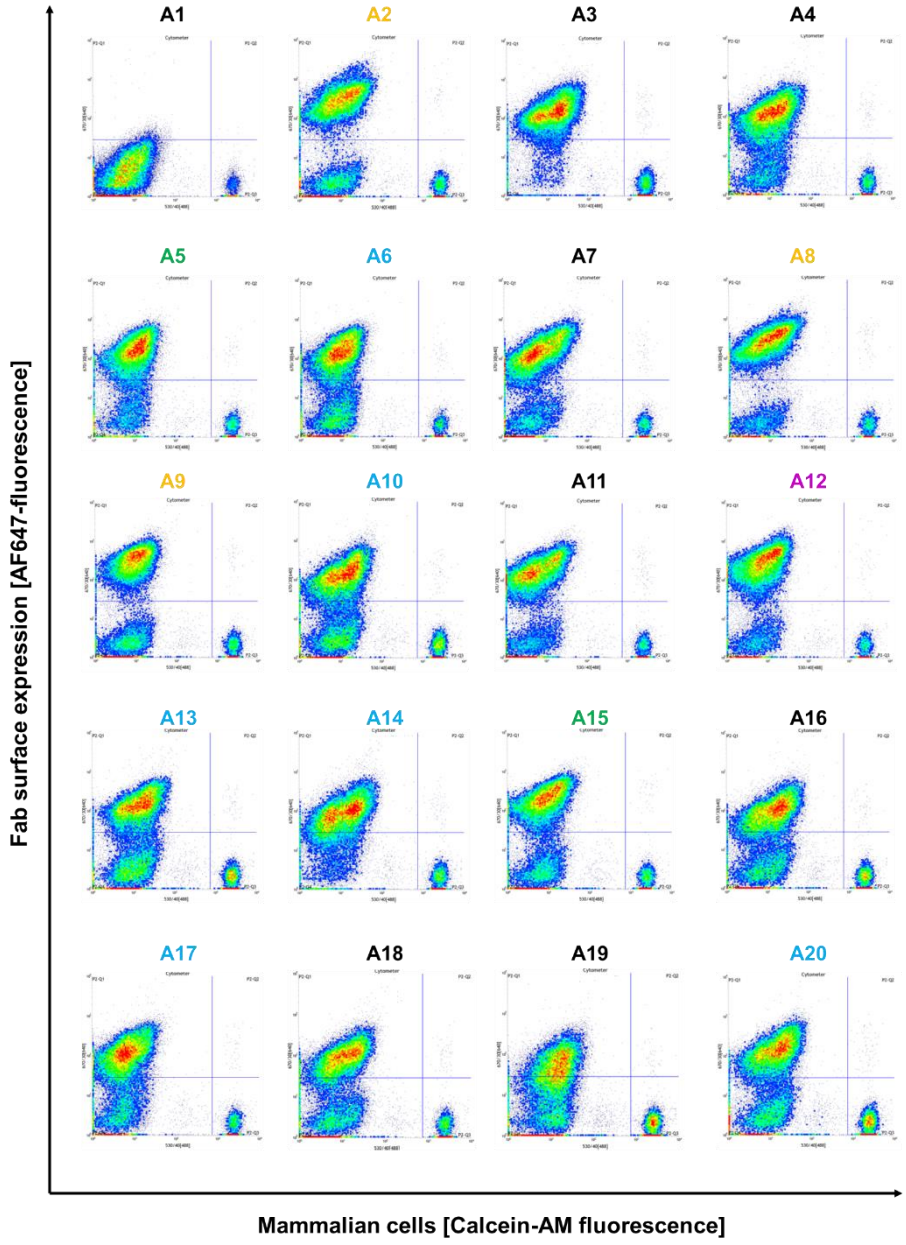

Figure S5. Binding to Jurkat cells of 20 randomly chosen clones. Surface presentation is displayed on the y-axis. On the x-axis, the fluorescence intensity of Calcein-AM is depicted referring to stained EGFR-negative Jurkat cells. In the lower left quadrant, yeast cells are depicted. In the upper right quadrant, yeast cells expressing a Fab-fragment are shown. In the lower right quadrant, Calcein-AM stained mammalian cells are shown. Double positive events (right upper quadrant) represent Fab variants on yeast cells that are able to bind to viable mammalian target cells. 50.000 events are plotted. Yeast clones exhibiting the genes for identical Fab fragments are depicted in the same color colors. Yeast clones with black labels were not sequenced due to their inability to bind A431 cells.

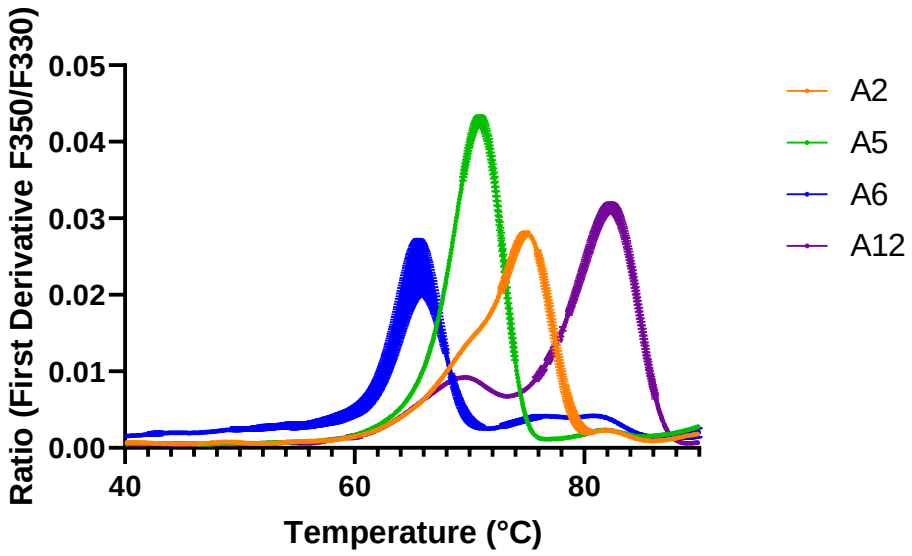

Figure S6. Analysis of thermal stability of isolated mAbs. The thermal stability was analyzed using the Prometheus NT.48 nanoDSF device (NanoTemper Technologies GmbH). With a heating rate of 1°C/min, the ratio of integrated fluorescence at 350 nm/330 nm was calculated. The corresponding software calculated the melting temperature at the inflection point. Error bars are indicated (n=4).

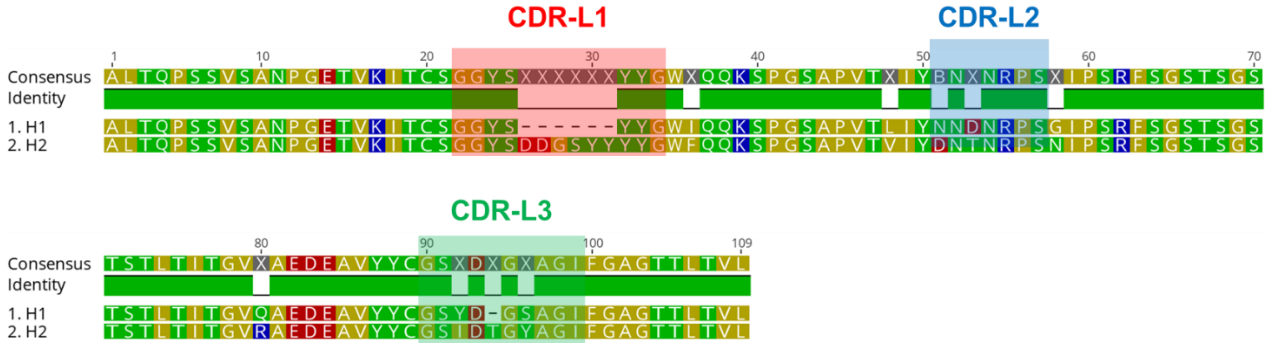

Figure S7. Sequence alignment of H1 and H2 VL domains.

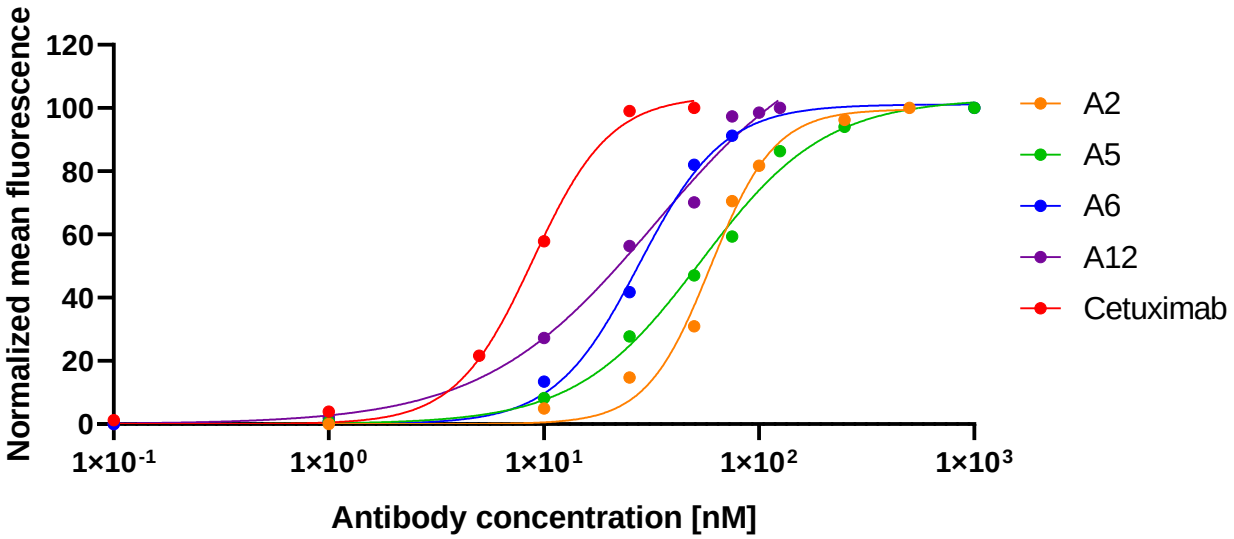

Figure S8. Affinity determination isolated antibodies by flow cytometry. 3×10^5 A431 cells were stained with defined concentration of the respective antibodies (0.1 nM – 1 µM) for 30 min on ice. For detection of cell binding, staining was performed using a goat anti-human Fc PE-conjugated secondary antibody (1:50 dilution) in PBSB for 15 min at 4 °C. The BD Influx flow cytometer was used to calculate the mean fluorescence, which was plotted against the logarithmic antibody concentrations. Data points were fitted using GraphPad Prism 8 for determination of EC50 values: Cetuximab: 8.9 nM; A2: 59.5 nM; A5: 53.7 nM, A6: 27.2 nM; A12: 32.8 nM.

pTT5 – Destination Vector

5’-
AAACGGATCTCTAGCGAATTCGCCACCATGAAACTTCCAGTCAGGCTCCTCGTCTTGATGTTTTGGATACCGGCATCTC
TCAGTAGAAGAGCTGCCATAGCATGACTTGAAGCCGTCTACACCTAGCTCTTCAGGATCCCCCGACCTCGACCTCTG-3’

Signal Peptide
SapI site
Stuffer
Partial pTT5 vector

pYD1 – Heavy Chain Entry Vector

5’-CATTTTCAATTAAGACCATGAGATTTCCTTCAATTTTTACGCTCTTCTGCC
-CH1-CH2-CH3-
TGATAATGGAAGAAGAGCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAGGCCGCAGCCGAACAGAAG
TTGATTTC-3’

Partial pYD1 vector
SapI site
Partial CH1
Stop codon

pYD1 – Lambda CL Entry Vector

5’-CATTTTCAATTAAGACCATGAGATTTCCTTCAATTTTTACGCTCTTCTGGG
-Lambda CL-
TAATAGTGGAAGAAGAGCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAGGCCGCAGCCGAACAGAAG
TTGATTTC-3’

Partial pYD1 vector
SapI site
Partial Lambda CL
Stop codon

Figure S9. Sequences of Entry and Destination vectors. Only changes to the original plasmids are indicated, but flanking homologues sequences are depicted. During the Golden Gate Assembly, the stuffer sequence of the pTT5 vector, the encoding sequences of the constant IgG domains on the pYD1 vectors and the flanking sites of the PCR product are hydrolyzed via SapI and are ligated into the linearized pTT5 vector, resulting in a complete heavy chain or light chain coding sequence, respectively.

Table S1. Sequences of primers used in this study. Underlined sequences harbor either homologous overhangs for gap repair in yeast or are overhangs exhibiting SapI sites for Golden Gate subcloning.

Primer Name	Sequence 5'-3'
VH library for	CCATGCAGTTACTTCGCTGTTTTTCAATATTTTCTGTTATTGCTAGCGTTTTAGCAGGGGCCGTGACGTTGGACGAG
VH library rev	GGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTACTAGCGGAGGAGACGATGACTTCGGT
VL library for	GCATTGCTGCTAAAGAAGAAGGGGTACAACTCGATAAAAGAGCGCTGACTCAGCCGTCCTCG
VL library rev	GATGGCGGGAAGATGAAGACAGATGGTGCAGCCACAGTTCGTAGGACGGTCAGGGTTGTCCC
Chicken VH to pTT5 SapI GGA for	AAAAAGCTCTTCAAGTGCCGTGACGTTGGACGAG
Chicken VH to pTT5 CH1 SapI GGA rev	TTTTTTGCTCTTCTGGCGGAGGAGACGATGACTTCGGT
Chicken VL to pTT5 SapI GGA for	AAAAAGCTCTTCAAGTGCGCTGACTCAGCCGTCCTCG
Chick Lam VL to pTT5 SapI GGA rev	TTTTTTGCTCTTCACCCTAGGACGGTCAGGGTTGTCCC