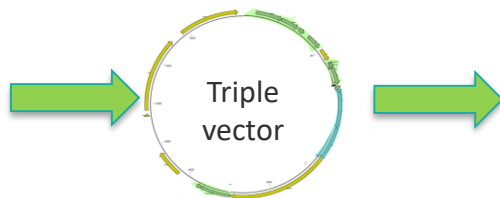


Case study XII

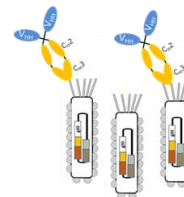
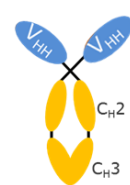
Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Generation of Llama VHH-huFc Naïve Library



Llama VHH-huFc Naïve Library ID	FJB Library ID	Library Size (cfu)	VHH-huFc Insert %
Llama Naïve pDCL5 pool Lib	FL0629	1.3E+09	99

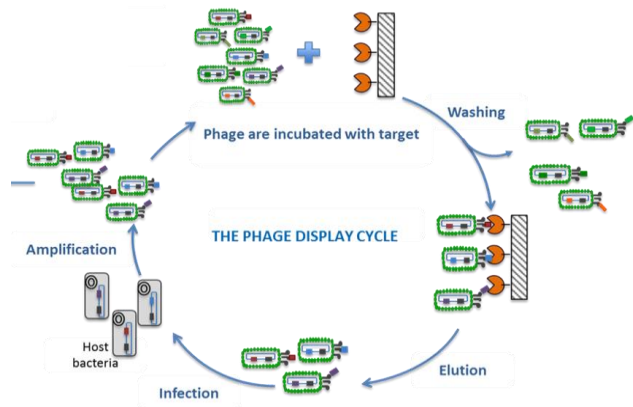
Non-immunized llamas



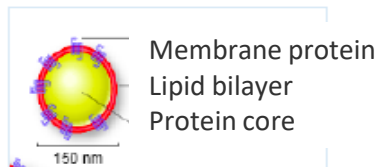
Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Panning on CXCR4 VLPs



Lipoparticles (VLPs)

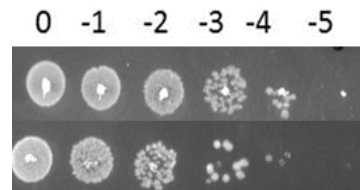


integral
molecular

Human CXCR4

20 U CXCR4

20 Null

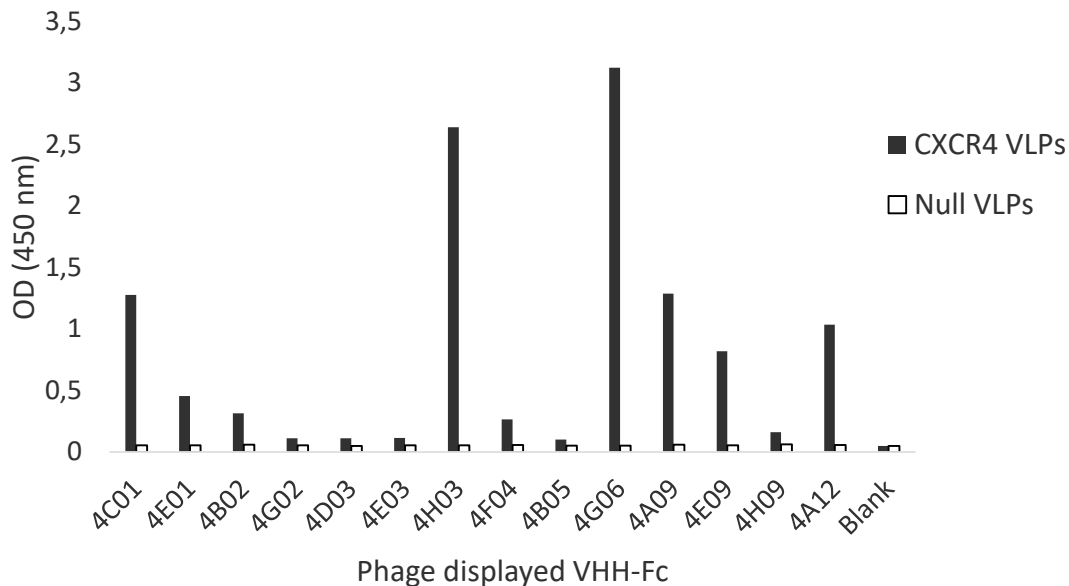


Titration of *E. coli* infected with phage from Naïve VHH-huFc
Llama Library selected after three rounds on huCXCR4 and Null VLPs

Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

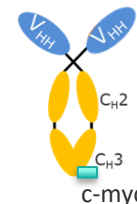
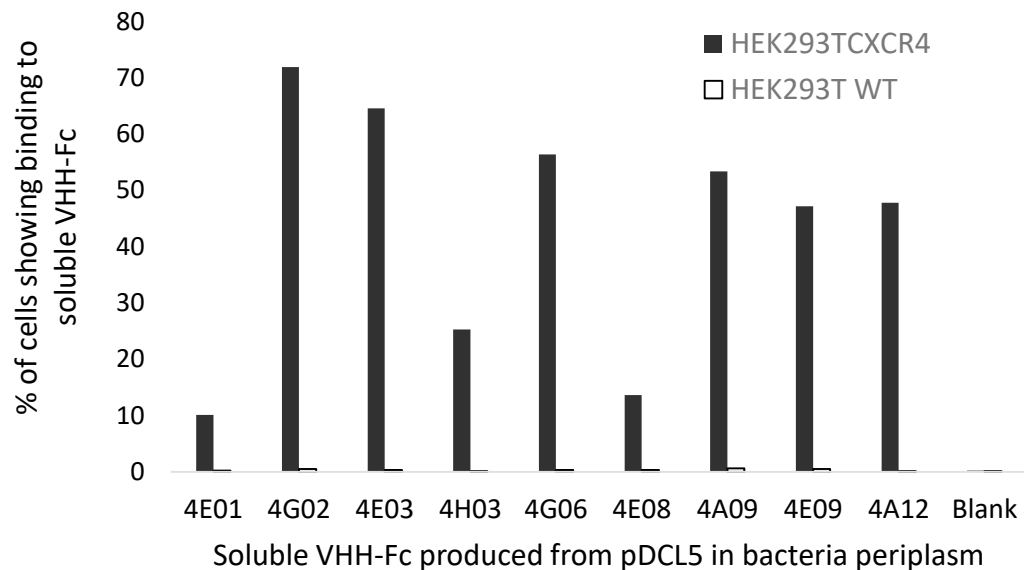
Binding ELISA on VLPs with phage selected after three rounds on CXCR4 VLPs from Llama VHH-huFc
Naïve Library



Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Binding FACS on HEK cells transfected with human CXCR4 and on HEK WT cells using P.E. produced from clones selected on CXCR4 VLPs from Llama VHH-huFc Naïve Library



Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Sequence diversity analysis of selected anti-human CXCR4 VHH-huFc

- Five different HCDR3 amino acid sequences in anti-huCXCR4 VHH-huFc selected clones

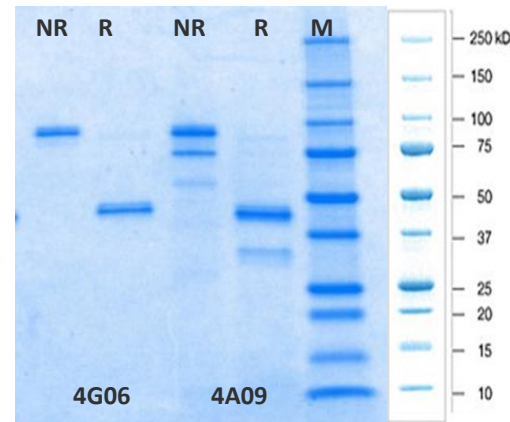
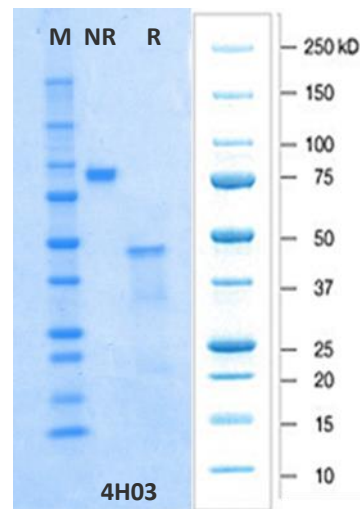
CDR3 amino acid sequence	Number of amino acids in CDR3	VHH-huFc Clone ID	CDR3 identified in VHH Naïve library
#1	18	4H03	No
#2	11	4E03, 4E09, 4A12	Yes
#3	17	4E01	Yes
#4	12	4G06	No
#5	12	4A09	No

Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Anti-huCXCR4 VHH-huFc molecules selected and expressed from triple vector in mammalian cells and purified by ProtA

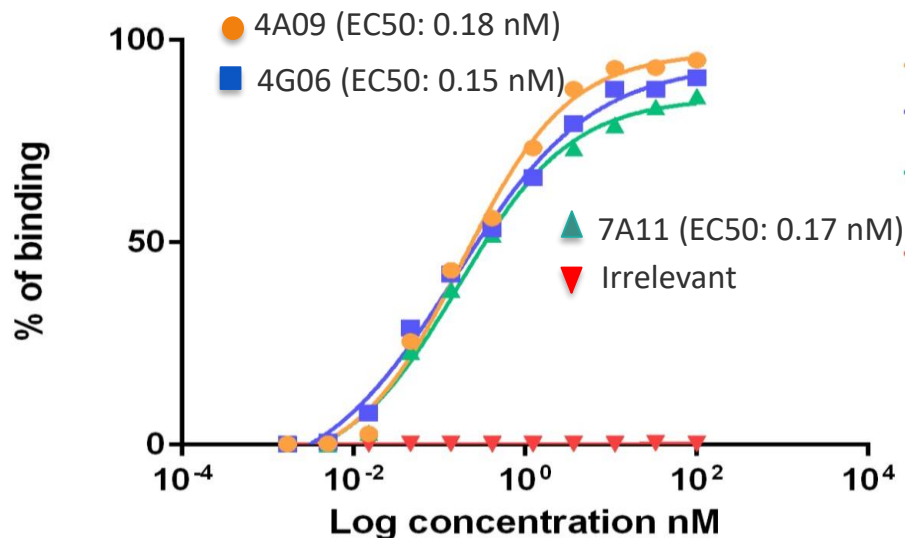
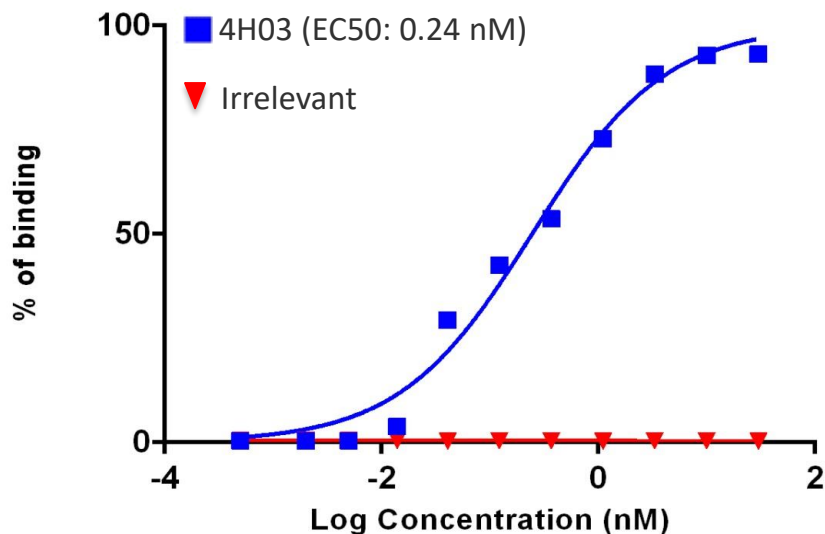
Clone ID	Mammalian cells	Final amount (µg)	Yield (mg/L)
4H03	HEK293FF	28	0.56
4G06	ExpiCHO	5900	120
4A09	ExpiCHO	1100	22



Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Binding to HEK293T-CXCR4 cells of anti-huCXCR4 VHH-huFc molecules selected and expressed from triple vector in mammalian cells



Case study XII

Generation of anti-huCXCR4 antibody molecules in full therapeutic format

Binding to FcRn of anti-huCXCR4 VHH-huFc molecules selected and expressed from triple vector in mammalian cells (SPR)

