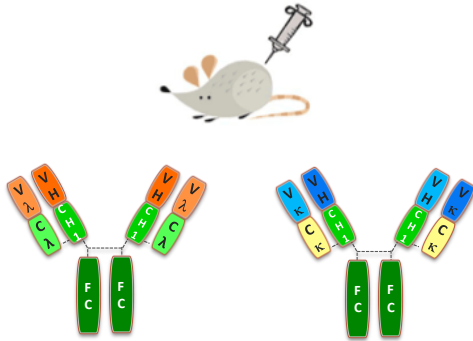


Case study VI

Generation of mouse mAbs against different domains of llama IgG

- Aim: To generate mAbs against different domains of llama IgG1
 - Anti IgG (anti Fc)
 - Anti light chain specific isotypes (anti C λ and anti C κ)
 - Anti Fabs (anti CH1)
- Technology: Mice immunizations + phage display



Immunizations with llama IgGs (lambda + kappa)

Phage display Fab libraries

Library	Library size	Fab Insert %
M001	2.4×10^8	90.9 %
M002	1.3×10^8	86.4 %

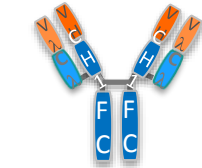
Case study VI

Generation of mouse mAbs against different domains of llama IgG

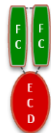
- Phage display selections to isolate Fabs against llama IgG Anti IgG (anti Fc)

Counter selections:

- Incubation of biotinylated llama gG + Phage library +/- Chimera IgG (Human Cs & llama Vs) +/- Human ECD-llama Fc
- Capturing of complex in neutravidin plates
- Elution by trypsin after several washing steps

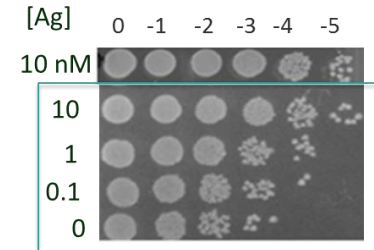
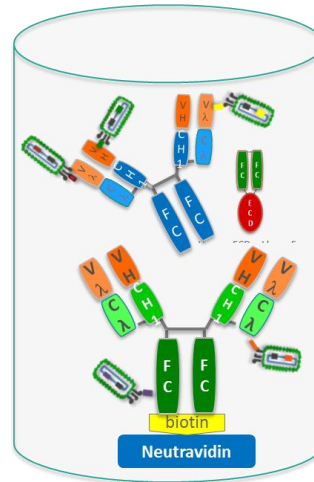


Chimera human
Cs / llama Vs



Chimera human
ECD -llama Fc

Counter
antigens



Counter selections

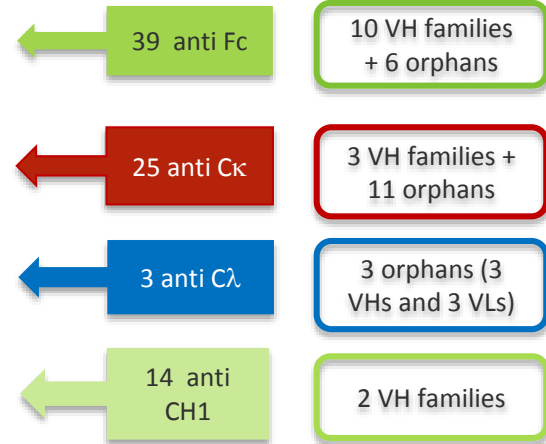
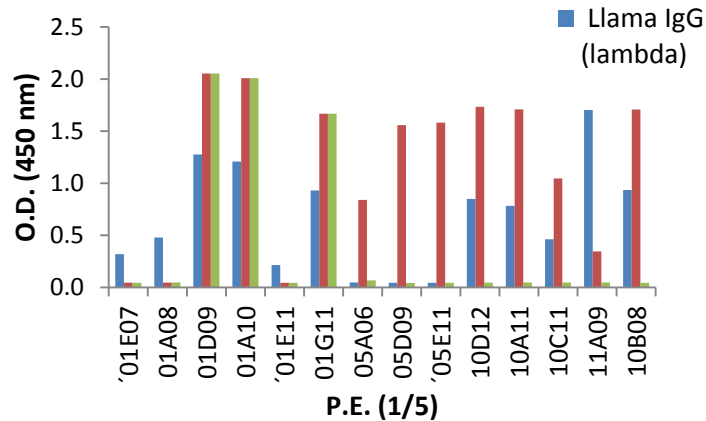
Titration of *E. coli* infected with phage from a Fab immune library selected after two rounds on llama IgG in the presence or absence of counter antigens

Case study VI

Generation of mouse mAbs against different domains of llama IgG

- ELISA & sequencing reveal specificity and diversity

- Neutravidin coated plates
- Biotinylated ECD-llama Fc/ Lambda or kappa llama IgG
- Fab containing-Periplasmic extract (P.E)
- Anti c-myc-HRP antibody

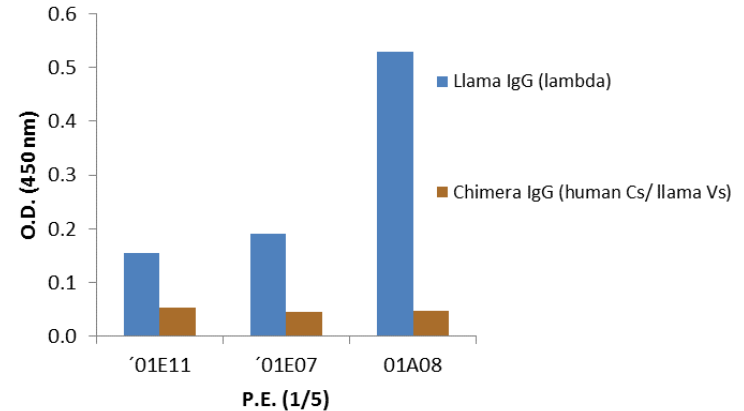
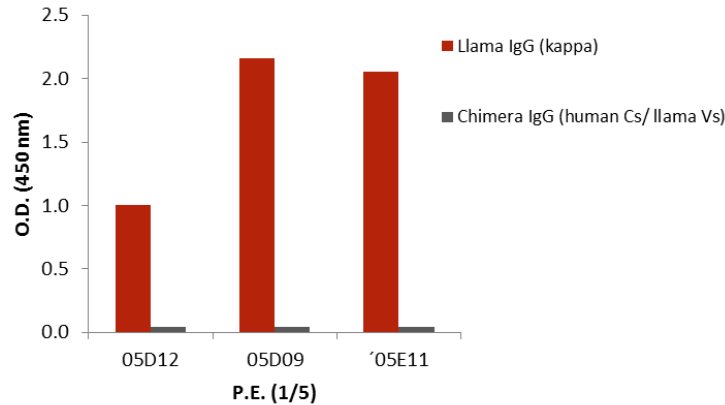


Case study VI

Generation of mouse mAbs against different domains of llama IgG

- ELISA & sequencing reveal specificity and diversity

- Neutravidin coated plates + Lambda or kappa llama IgG
- Chimera IgG (human Cs & Llama Vs) coated plates
- Fab containing-Periplasmic extract (P.E)
- Anti c-myc-HRP antibody

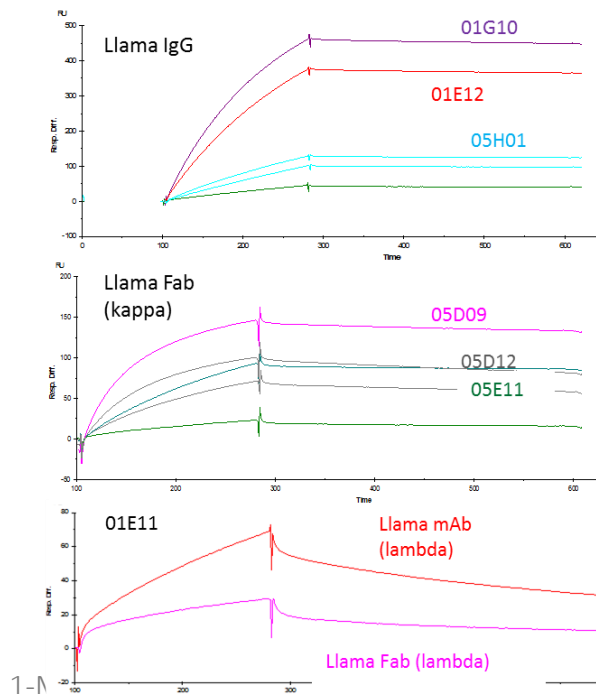


Case study VI

Generation of mouse mAbs against different domains of llama IgG

- SPR confirms specificity and affinity

— Chip coated with llama IgG (Lambda), Llama Fabs (kappa and lambda)

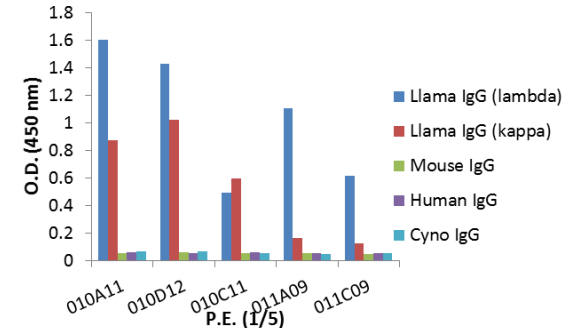
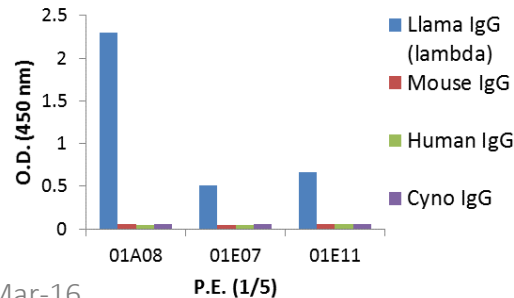
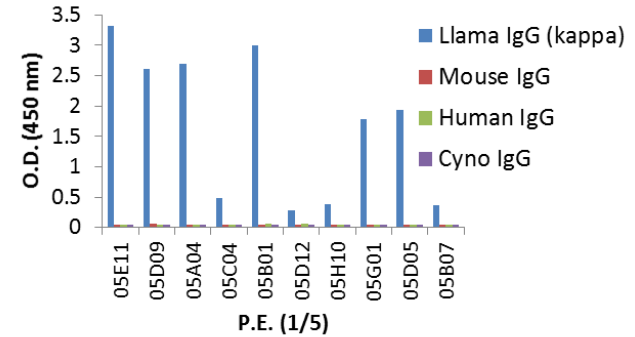
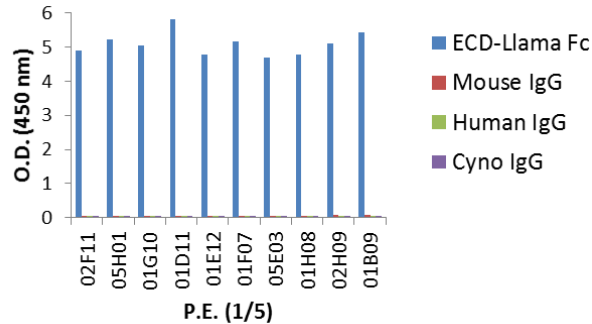


Fab Clone	Specificity/Domain	Off-rate (s^{-1})
01G10	Anti Fc	6.6×10^{-5}
01E12	Anti Fc	7.1×10^{-5}
05H01	Anti Fc	6.0×10^{-5}
05D09	Anti C κ	1.7×10^{-4}
05D12	Anti C κ	5.0×10^{-4}
05E11	Anti C κ	1.0×10^{-4}
01E11	Anti C λ	1.2×10^{-3}
10A11	Anti CH1	5.1×10^{-4}

Case study VI

Generation of mouse mAbs against different domains of llama IgG

- Fabs bind IgG of llama and not of other species
 - ELISA on llama, Human, Mouse and Cyno IgG and on ECD-llama Fc



Case study VI

Generation of mouse mAbs against different domains of llama IgG

- Mouse Fabs formatted to mouse IgG
 - Specificity and affinity (M)



mAb	Immobilized Ag (K_D M)			Binding specificity
	Llama mAb	Llama Fab lambda	Llama Fab kappa	
mAb 01E11	1.86×10^{-8}	2.86×10^{-8}	-	Llama C-lambda
mAb 11C09	6.34×10^{-12}	3.12×10^{-11}	6.87×10^{-9}	Llama CH1
mAb 11A09	3.13×10^{-12}	1.72×10^{-10}	9.07×10^{-9}	Llama CH1
mAb 05D09	-	-	1.39×10^{-12}	Llama C-kappa
mAb 05C04	-	-	2.42×10^{-13}	Llama C-kappa
mAb 10D12	5.70×10^{-12}	9.68×10^{-13}	5.66×10^{-11}	Llama CH1
mAb 04F12	1.34×10^{-12}	-	-	Llama Fc
mAb 01H08	1.02×10^{-12}	-	-	Llama Fc

Case study VI



Generation of mouse mAbs against different domains of llama IgG

- Conclusions

Immunizations of two mice and Phage display-based counter selections allowed the identification of mAbs that bind specifically (with no other specie cross-reactivity) to:

- Any IgG1 from llama (anti Fc and anti CH1)
- Any Fab from llama species (anti CH1)
- Llama kappa IgG isotypes (anti C κ)
- Llama lambda IgG isotypes (anti C λ)