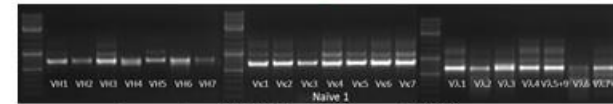
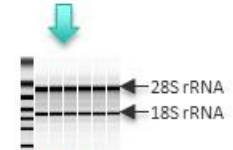
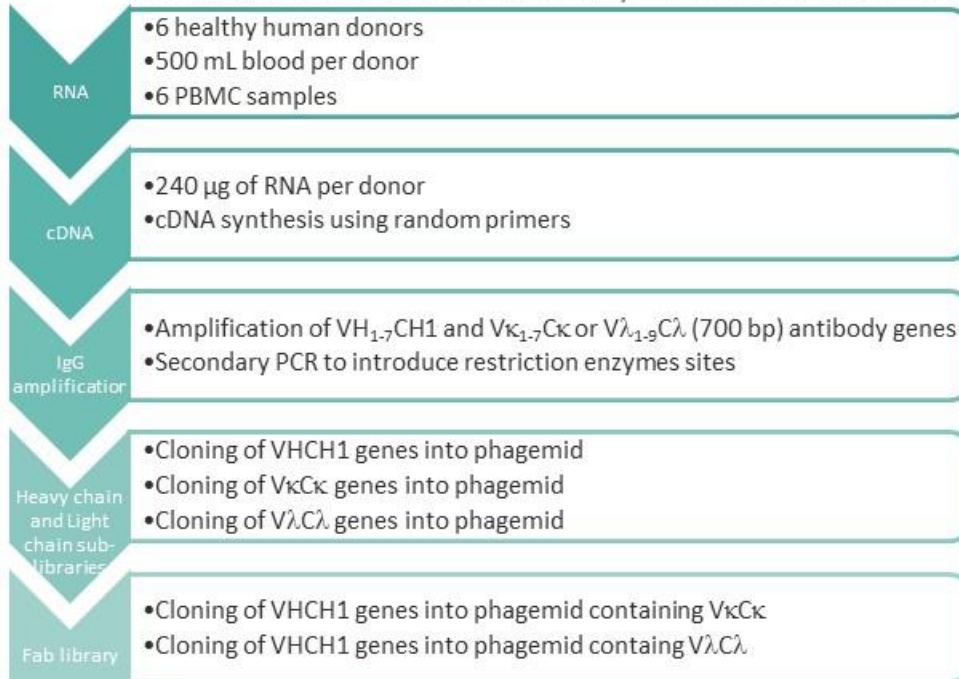


Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library



Human naïve Fab library H002-H007 construction



Example of $VHCH1$, $V\kappa C\kappa$ and $V\lambda C\lambda$ genes

Case study I

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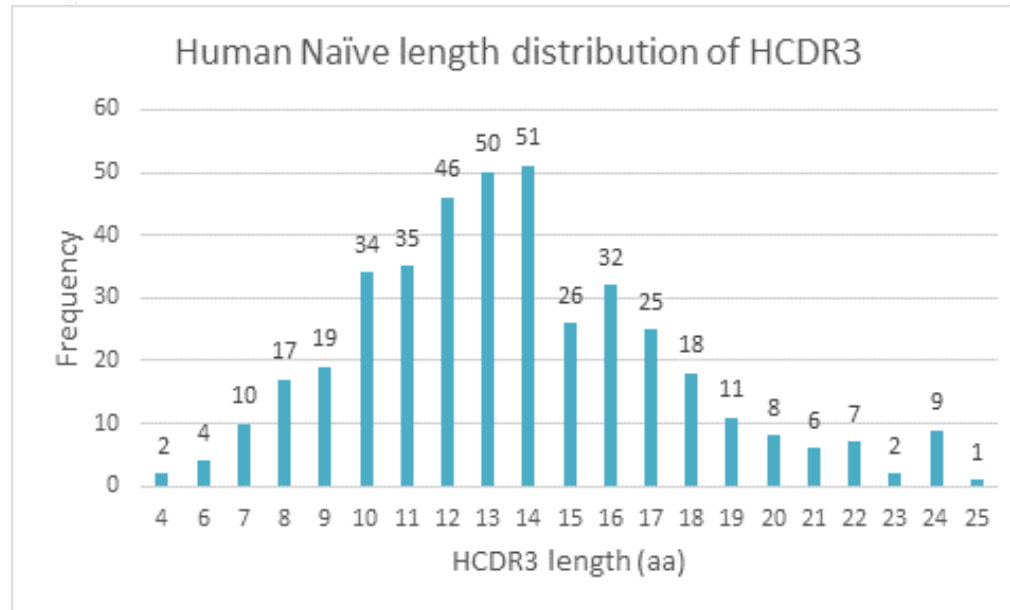
Human Fab library ID	Library Size (cfu)	Fab Insert %	Human Fab library ID	Library Size (cfu)	Fab Insert %
H002 κ	8,4E+08	100 %	H002 λ	5,4E+08	100 %
H003 κ	7,8E+08	100 %	H003 λ	6,0E+08	91 %
H004 κ	4,8E+08	100 %	H004 λ	7,2E+08	86 %
H005 κ	7,2E+08	91 %	H005 λ	8,4E+08	100 %
H006 κ	9,6E+07	100 %	H006 λ	9,6E+07	100 %
H007 κ	9,0E+08	100 %	H007 λ	1,4E+09	91 %

Human Fab library ID	Library Size (cfu)		Fab Insert %	% Non-redundant Sequences	Number of different HCDR3	% ORF
FJB Naïve Fab Pool Lib (Kappa)	3,8E+09	7,8E+09	99 %	97 %	197	63 %
FJB Naïve Fab Pool Lib (Lambda)	4,0E+09		95 %	99 %	200	50 %

Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library

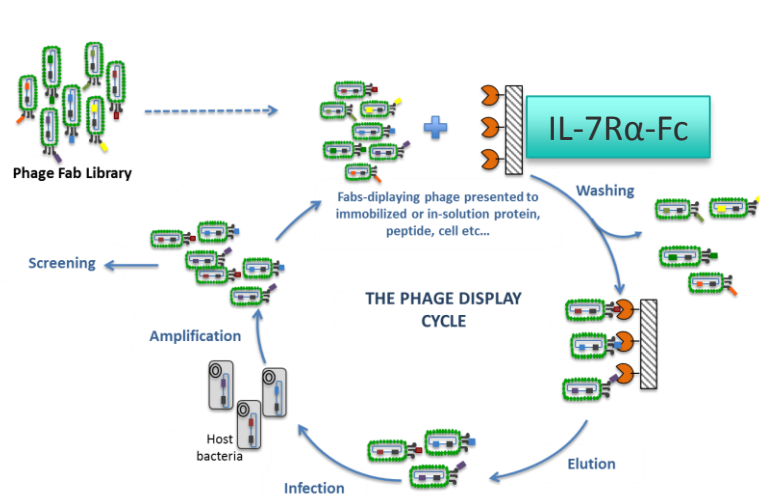
Human naïve Fab library H002-H007 construction



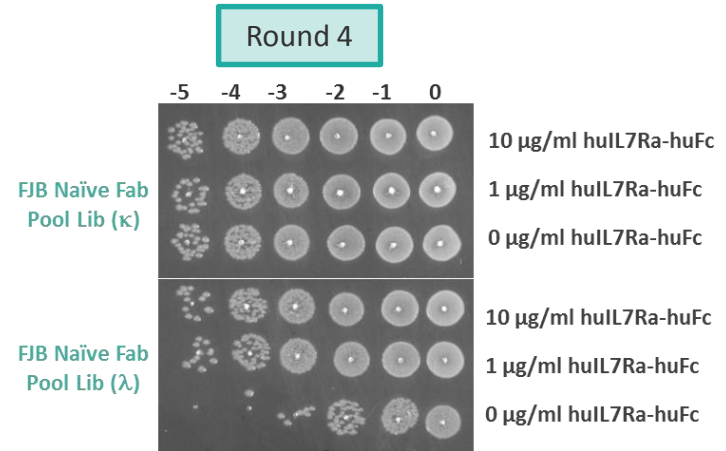
Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library

Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007



4 rounds of panning on huIL-7R α
(counter selection with total human IgG)



Titration of *E. coli* infected with phage from FJB Naïve human Fab phage display library selected after four rounds on 10 or 1 μ g/ml of human IL-7R α

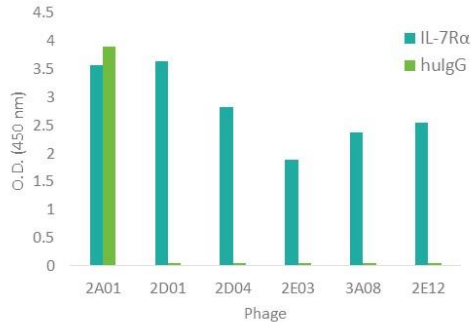
Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library
Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

Phage ELISA

Human Fab library ID	Phage ELISA Hit-rate*
FJB Naïve Fab Pool Lib (Kappa)	14 % (n=94)
FJB Naïve Fab Pool Lib (Lambda)	73 % (n=94)

* - OD450nm 5 fold ELISA background (PBS)



Sequence diversity

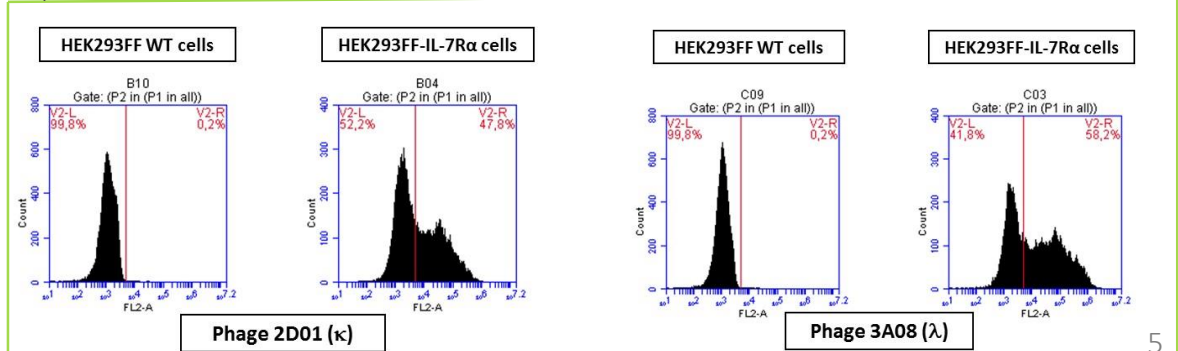
Human Fab library ID	Nº of IL-7R α specific HCDR3 families **
FJB Naïve Fab Pool Lib (Kappa)	7 (n=13)
FJB Naïve Fab Pool Lib (Lambda)	6 (n=73)

** - 16 different clones based on differences in LC and HC sequences

Cell binding phage FACS

Human Fab library ID	Phage FACS Hit rate ***
FJB Naïve Fab Pool Lib (Kappa)	75 % (n=8)
FJB Naïve Fab Pool Lib (Lambda)	94 % (n=13)

*** - At least 5% of binding and 3 times the MFI on the WT cells



Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library
Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

Periplasmic extract (P.E.) FACS

Off-rate analysis

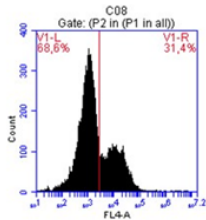
IL-7R α /IL-7 blocking

Soluble Fab in P.E.

Soluble Fab in P.E.

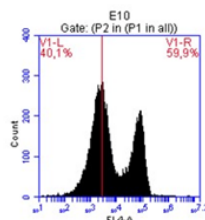
Human Fab library ID	P.E. Binding FACS Hit rate *
FJB Naïve Fab Pool Lib (Kappa)	87.5% (n=8)
FJB Naïve Fab Pool Lib (Lambda)	70 % (n=13)

HEK293FF-IL-7R α cells



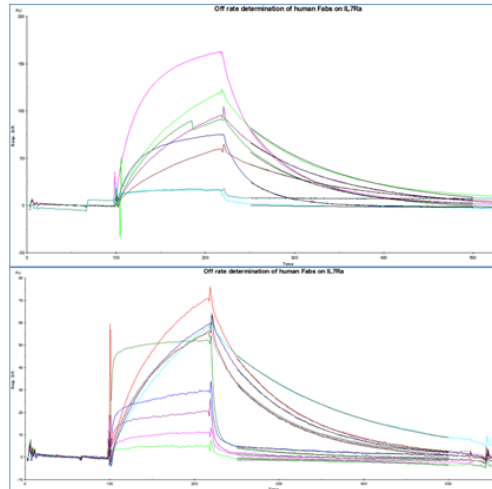
P.E. 2D01 (κ)

HEK293FF-IL-7R α cells

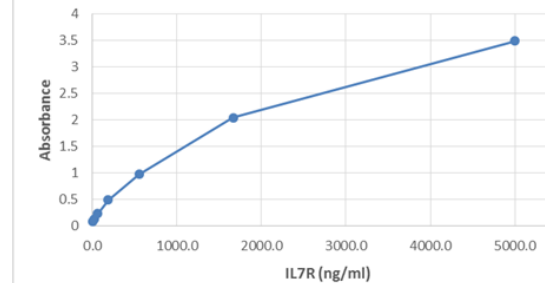


P.E. 3A08 (λ)

Off-rates in the 10^{-2} to 10^{-3} sec $^{-1}$



IL7R binding to IL7 QC ELISA



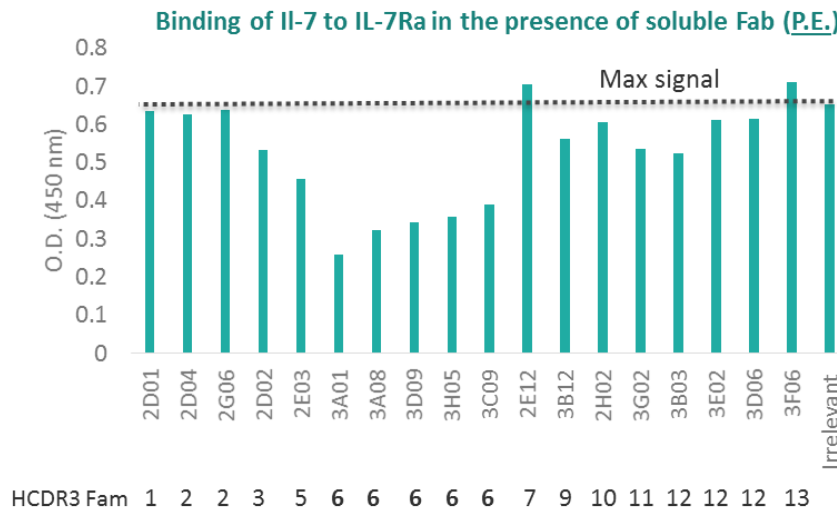
Results of blocking assay in next slide

Case study I

Isolation of anti human IL-7R α human IgGs from Human naïve Fab library

Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

IL-7R α /IL-7 blocking



Clone	HCDR3 Fam	inhibition
2D01	1	3.0%
2D04	2	4.0%
2G06	2	2.6%
2D02	3	18.5%
2E03	5	30.3%
3A01*	6	60.6%
3A08	6	50.6%
3D09	6	47.4%
3H05*	6	45.4%
3C09*	6	40.5%

Clone	HCDR3 Fam	inhibition
2E12	7	-7.9%
3B12	9	14.1%
2H02	10	7.2%
3G02	11	18.4%
3B03	12	19.6%
3E02	12	6.6%
3D06	12	6.0%
3F06	13	-8.9%
Irrelev	-	0

* - Same VH and VL AA sequences

Case study I

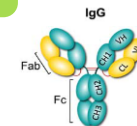
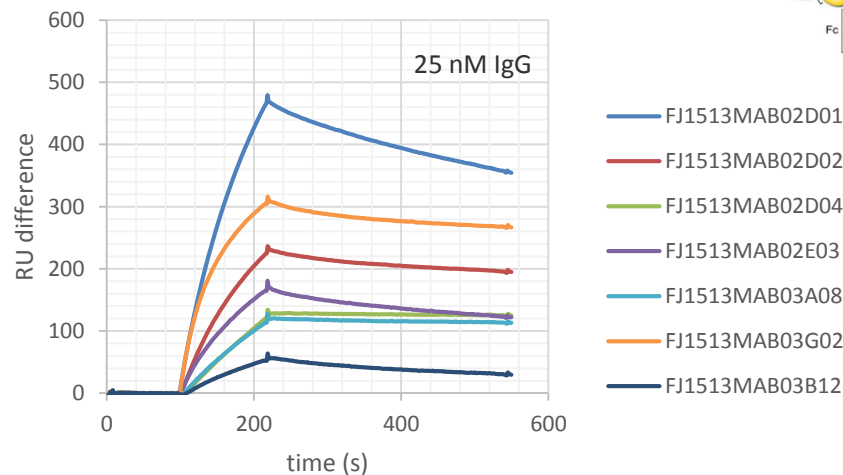
Isolation of anti human IL-7R α human IgGs from Human naïve Fab library

Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

Conversion to human IgG1 and Affinity assessment of anti-huIL-7R α human IgG1s

IgG	k_a (1/Ms)	k_d (1/s)	Rmax (RU)	KD (nM)
FJ1513MAB02D01	4.34E+05	1.01E-03	684	2.3
FJ1513MAB02D02	4.65E+05	6.16E-04	319	1.3
FJ1513MAB02D04	1.49E+05	1.23E-04	371	0.8
FJ1513MAB02E03	4.38E+05	1.10E-03	245	2.5
FJ1513MAB03A08	1.82E+05	2.66E-04	293	1.5
FJ1513MAB03G02	1.04E+06	4.29E-04	316	0.4
FJ1513MAB03B12	2.42E+05	2.29E-03	130	9.4

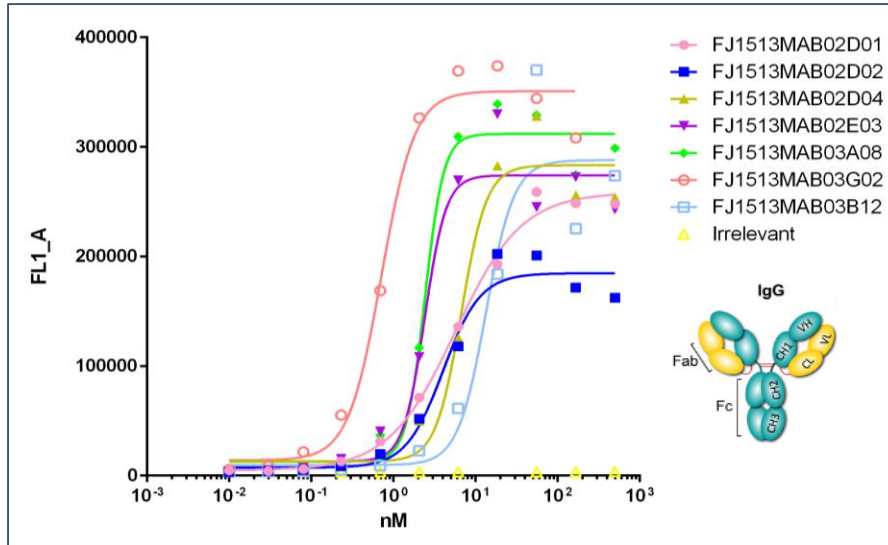
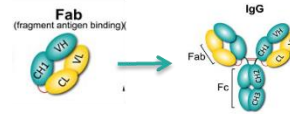
IL7-R α immobilized on a CM5 chip



Human naïve Fab phage display libraries

Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

Conversion to human IgG1 and binding to HEK293FF-IL-7R α cells



Human IgGs bind to cells expressing IL-7R α with one-digit nM and sub nM EC₅₀ values

IgG	EC ₅₀ (nM)
FJ1513MAB02D01	5.6
FJ1513MAB02D02	4.0
FJ1513MAB02D04	6.7
FJ1513MAB02E03	2.4
FJ1513MAB03A08	2.6
FJ1513MAB03G02	0.7
FJ1513MAB03B12	13.4

Human naïve Fab phage display libraries

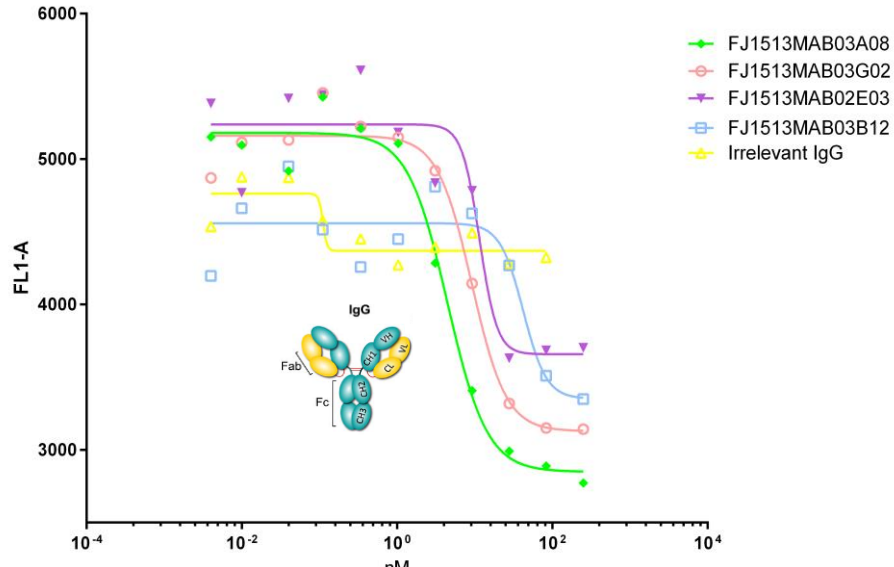


Anti-human IL-7R α Fabs isolated from human naïve Fab library H002-H007

Blocking of IL-7 binding to HEK293FF-IL-7R α cells with various different IL-7R α human IgG1

Various epitopes coverage

Competition with 12 nM of bio-rhIL7
in IL7R α HEK293T transfected cells



Competition with 12 nM of bio-rhIL7
in IL7R α HEK293T transfected cells

