Figure 1

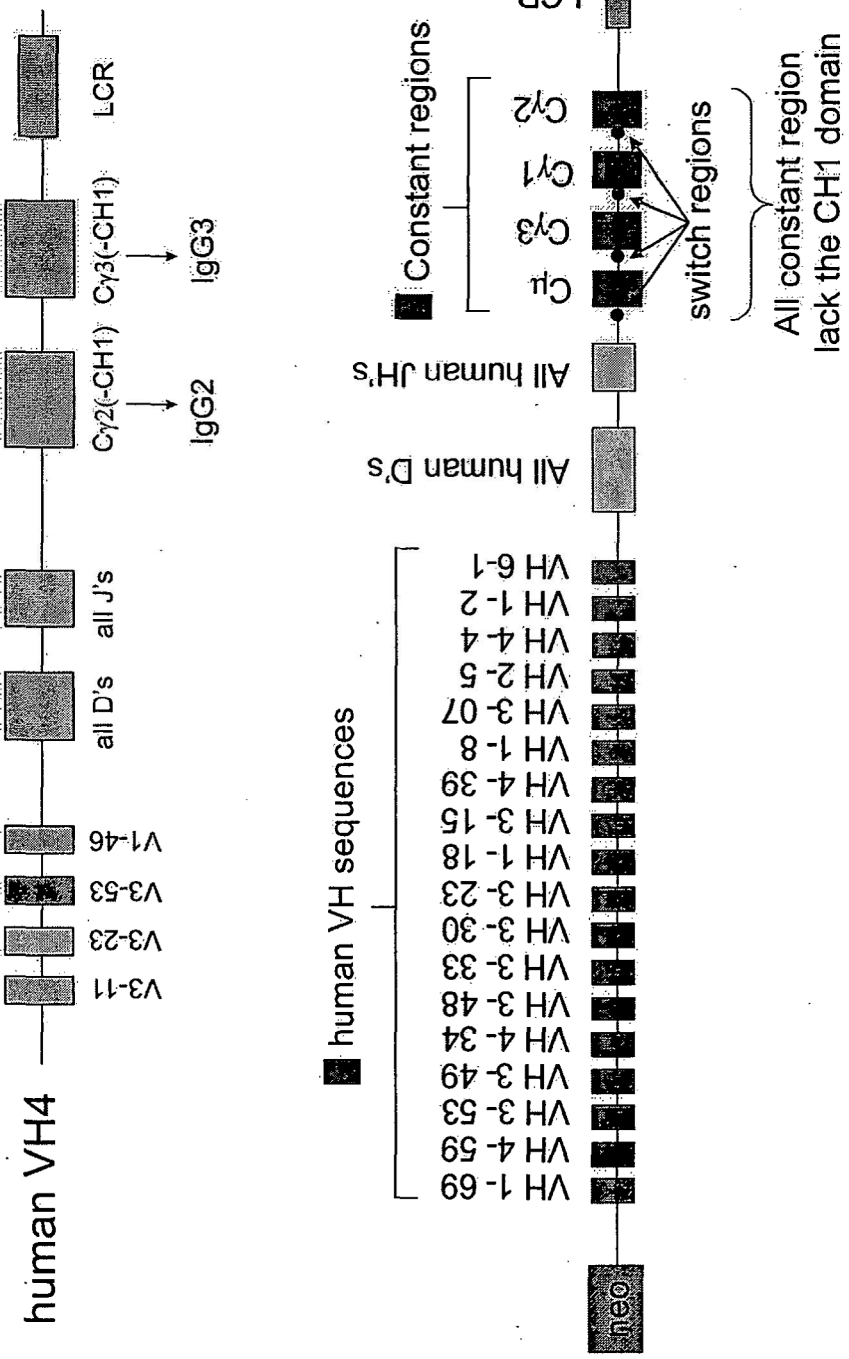

human VH4
V3-11
V3-23
V3-53
V1-46
all D's
all J's
Cγ2(-CH1)
IgG2
Cγ3(-CH1)
IgG3
LCR
human VH sequences
VH 6-1
VH 1-2
VH 4-4
VH 2-5
VH 3-07
VH 1-8
VH 4-39
VH 3-15
VH 1-18
VH 3-23
VH 3-30
VH 3-33
VH 4-34
VH 3-48
VH 3-49
VH 3-53
VH 4-59
VH 1-69
All human D's
All human JH's
Constant regions
Cμ
Cγ3
Cγ1
Cγ2
switch regions
All constant region
lack the CH1 domain
LCR
neo

Figure 2

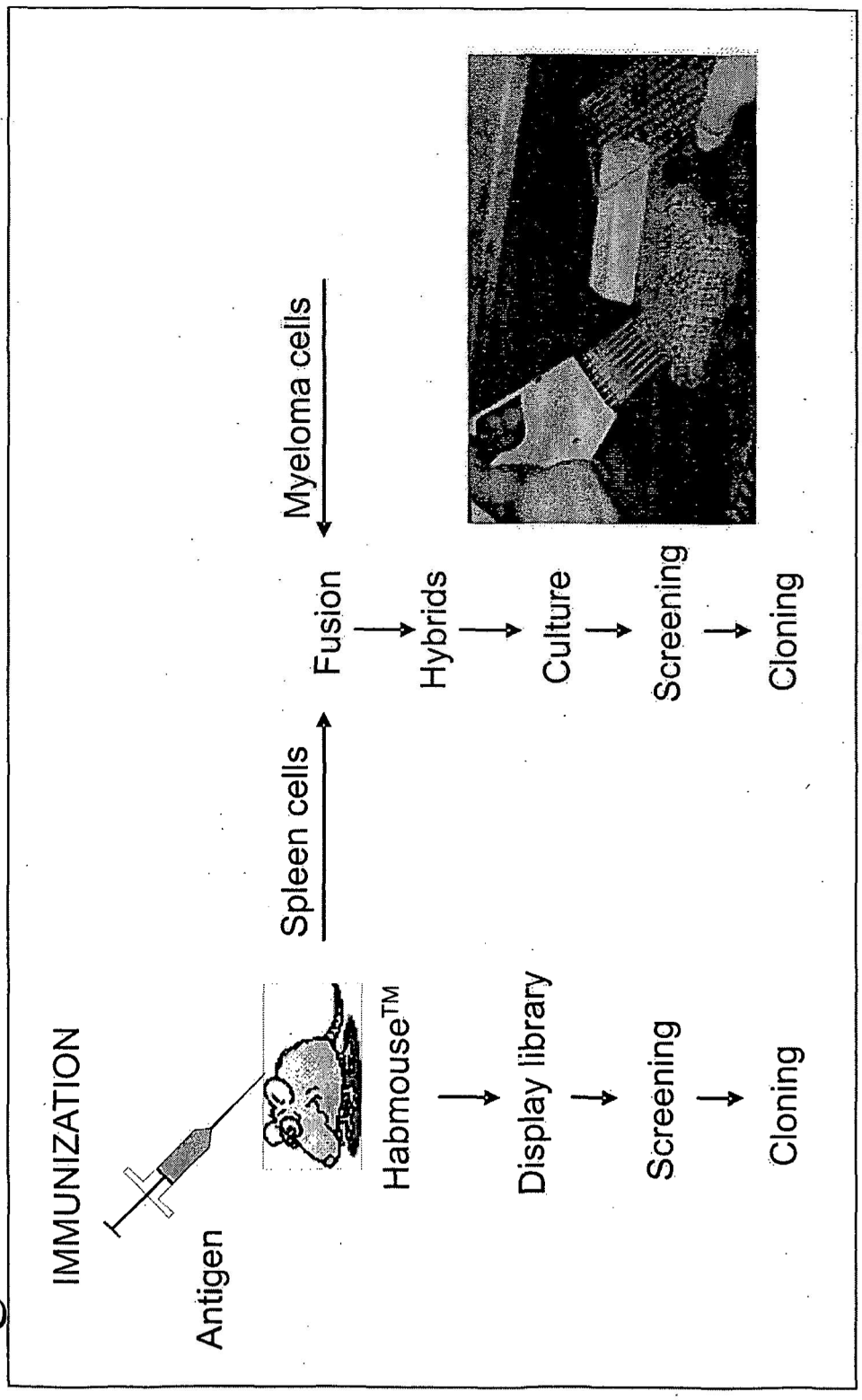

IMMUNIZATION
Antigen
Habmouse™
Display library
Screening
Cloning
Spleen cells
Myeloma cells
Fusion
Hybrids
Culture
Screening
Cloning

Figure 3

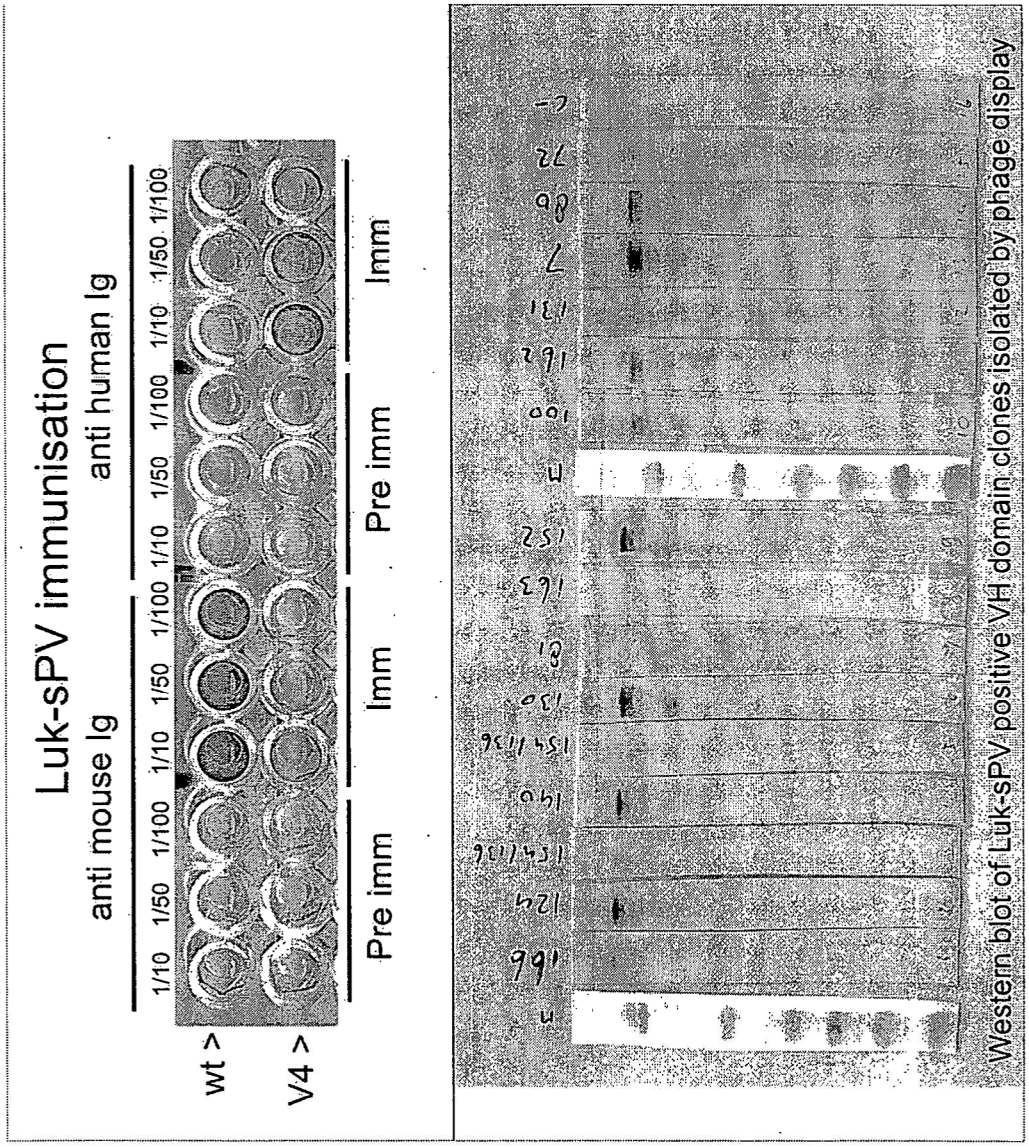
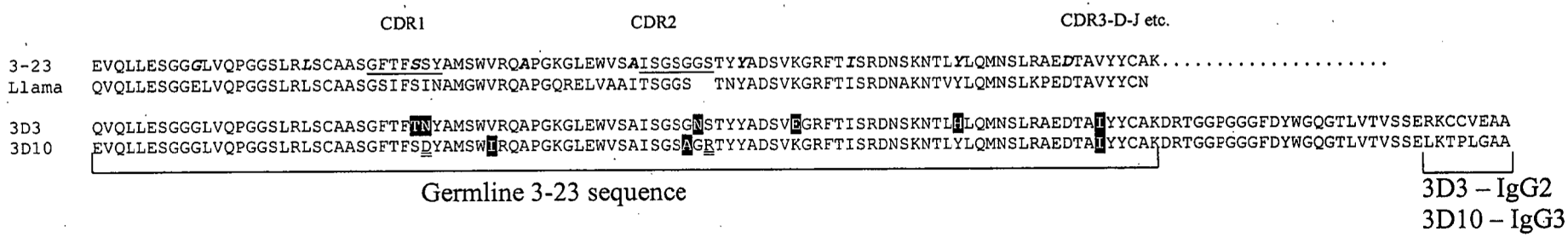

Luk-sPV immunisation
anti mouse Ig
anti human Ig
1/10 1/50 1/100 1/10 1/50 1/100 1/10 1/50 1/100 1/10 1/50 1/100
wt >
V4 >
Pre imm
Imm
Pre imm
Imm
Western blot of Luk-sPV positive VH domain clones isolated by phage display

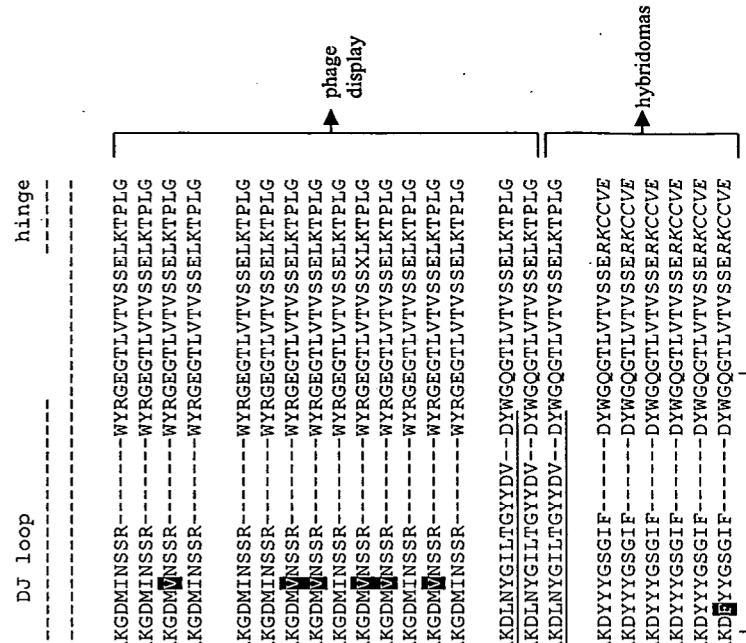
Figure 4



The first two amino acids of 3D3 are to be ignored. Changes are likely due to degenerate primer.
Amino acids highlighted in black are mutations different from 3-23 or between clones.
Bold italicised amino acids are every 10 amino acids.
Double underlined residues are differences between clones with the same VDJ rearrangement.
Single underlined CDR1, CDR2 and CDR3 etc.

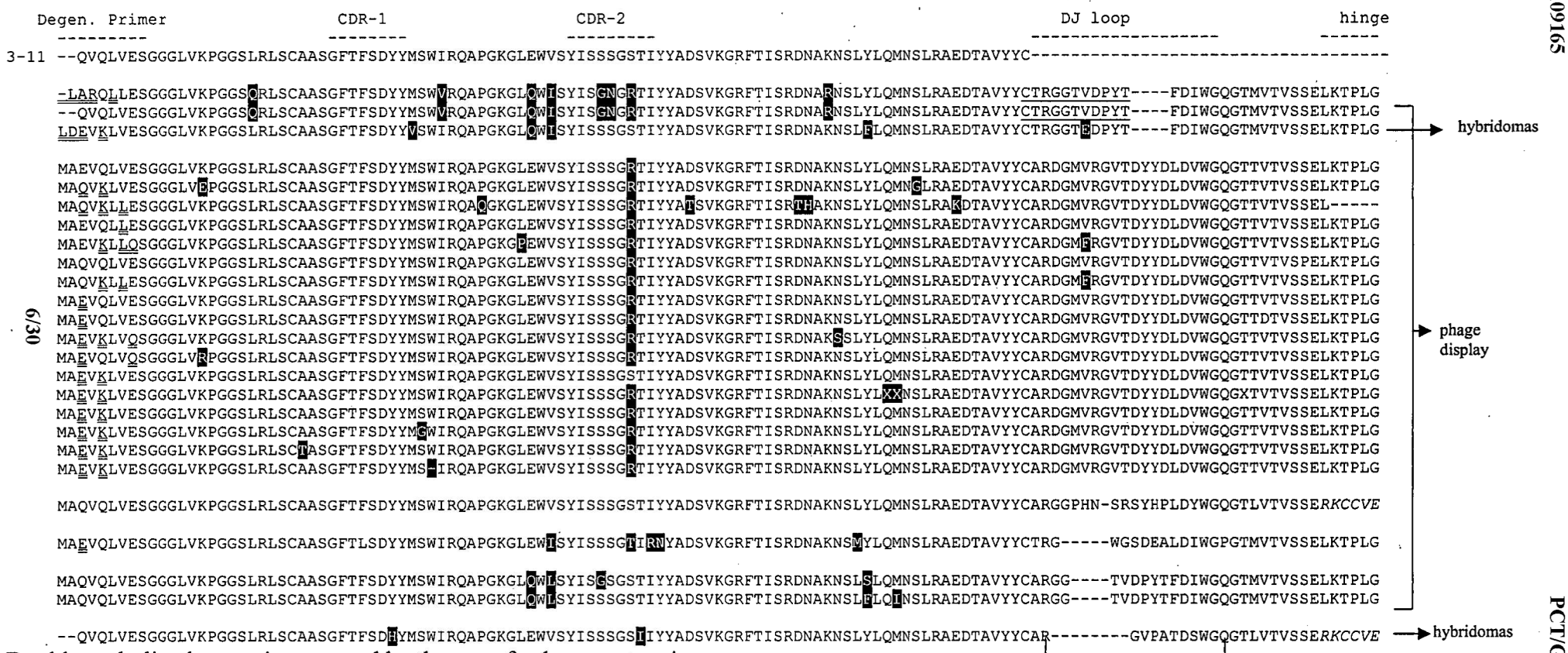
SUBSTITUTE SHEET (RULE 26)

Figure 5



Double underlined: mutations caused by the use of a degenerate primer
Highlighted in black: mutations
Groupings: different VDJ rearrangements
Italicised (bottom group): IgG2, while the rest is IgG3.

Figure 6



Double underlined: mutations caused by the use of a degenerate primer
Highlighted in black: mutations
Groupings: different VDJ rearrangements
Italicised: IgG2, while the rest is IgG3

SUBSTITUTE SHEET (RULE 26)

6/30

Figure 7

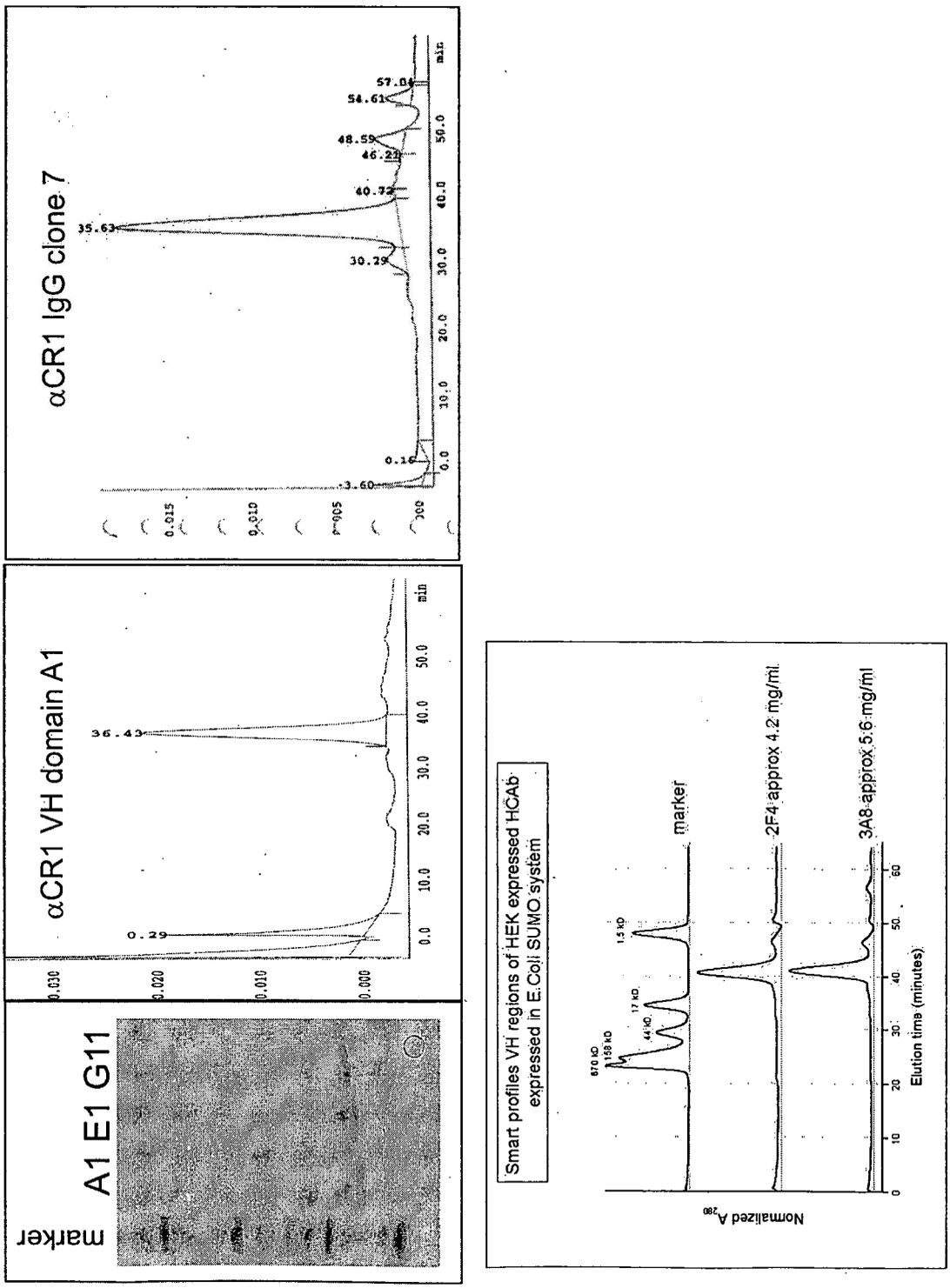

αCR1 IgG clone 7
35.63
30.29
40.72
48.59
54.61
57.86
0.16
3.60
min
αCR1 VH domain A1
36.43
0.29
min
marker
A1 E1 G11
Smart profiles VH regions of HEK expressed HCAb expressed in E.Coli SUMO system
marker
2F4 approx 4.2 mg/ml
3A8 approx 5.6 mg/ml
Normalized A280
Elution time (minutes)

Figure 8

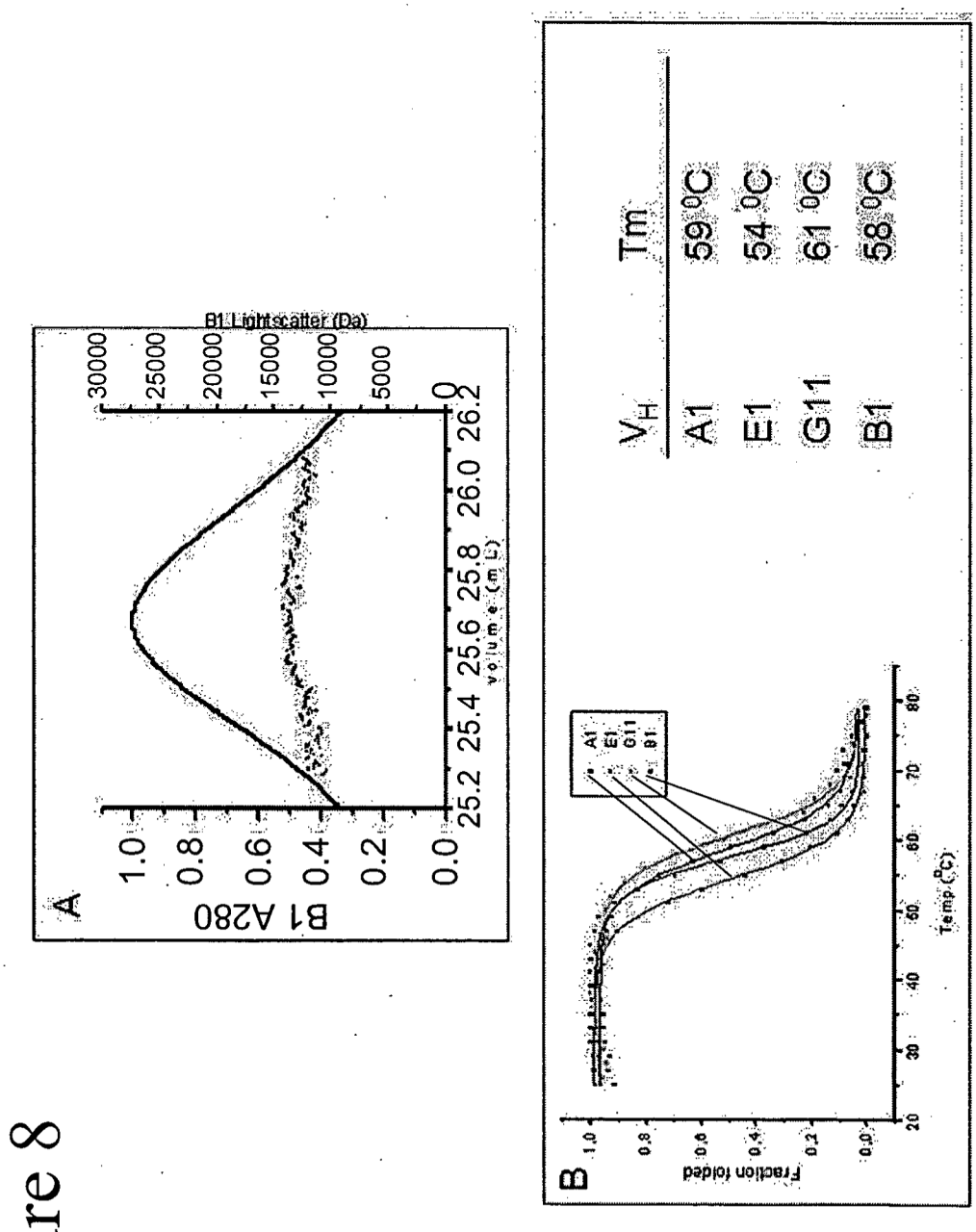

A
B1 A280
B1 Lightscatter (Da)
volume (mL)
B
Fraction folded
Temp (°C)
A1
E1
G11
B1
V_H
Tm
A1 59 °C
E1 54 °C
G11 61 °C
B1 58 °C

Figure 9

BiaCore T100*

Octet

K_D of CR1 heavy chain only antibodies	Purified Ab	Purified antibody		Crude hybridoma medium	
		Global fit	Local fit	Global fit	Local fit
124	$1.0x10^{-10}$	ND	ND	$8.1x10^{-10}$	$5.9x10^{-10}$
152	$1.3x10^{-8}$	$3.7x10^{-10}$	$6.5x10^{-10}$	$7.9x10^{-10}$	$6.5x10^{-10}$
5E5	$2.4x10^{-9}$	ND	$1.8x10^{-9}$	rejected	$5.9x10^{-9}$
7	$8.5x10^{-11}$	$3.8x10^{-11}$	$9.2x10^{-11}$	$3.5x10^{-11}$	$5.2x10^{-11}$

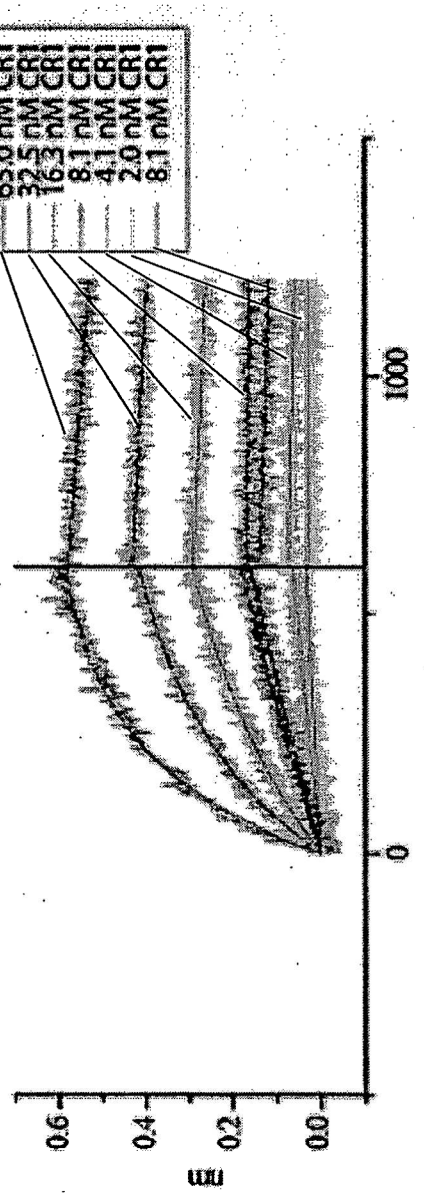


Figure 10

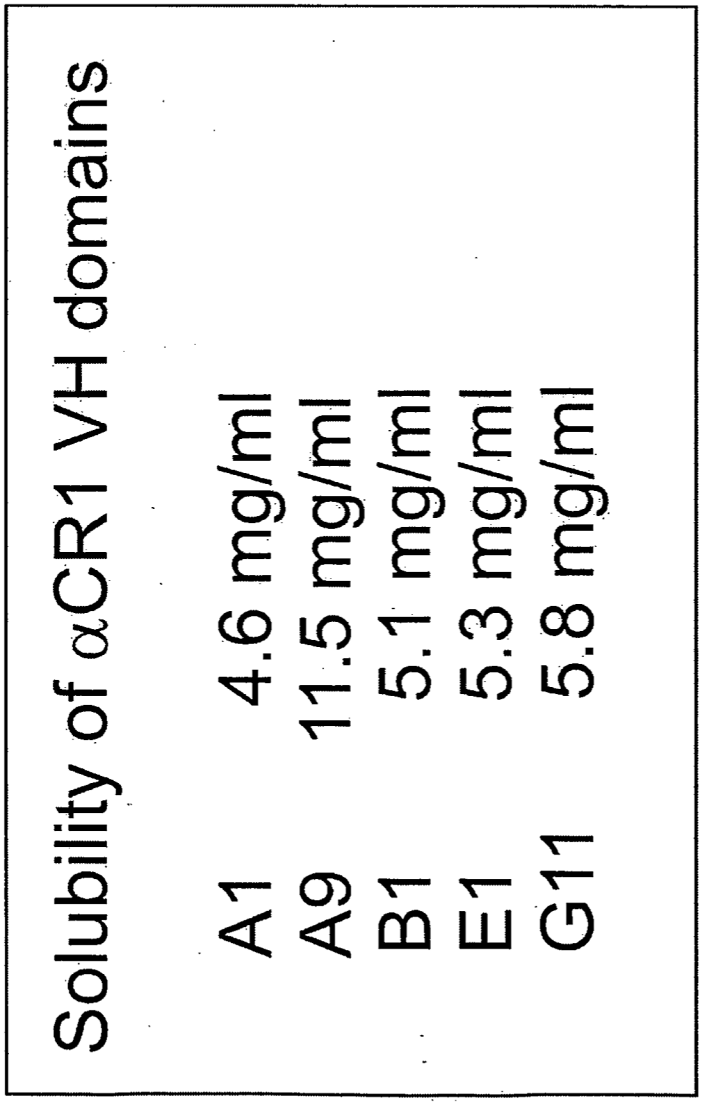
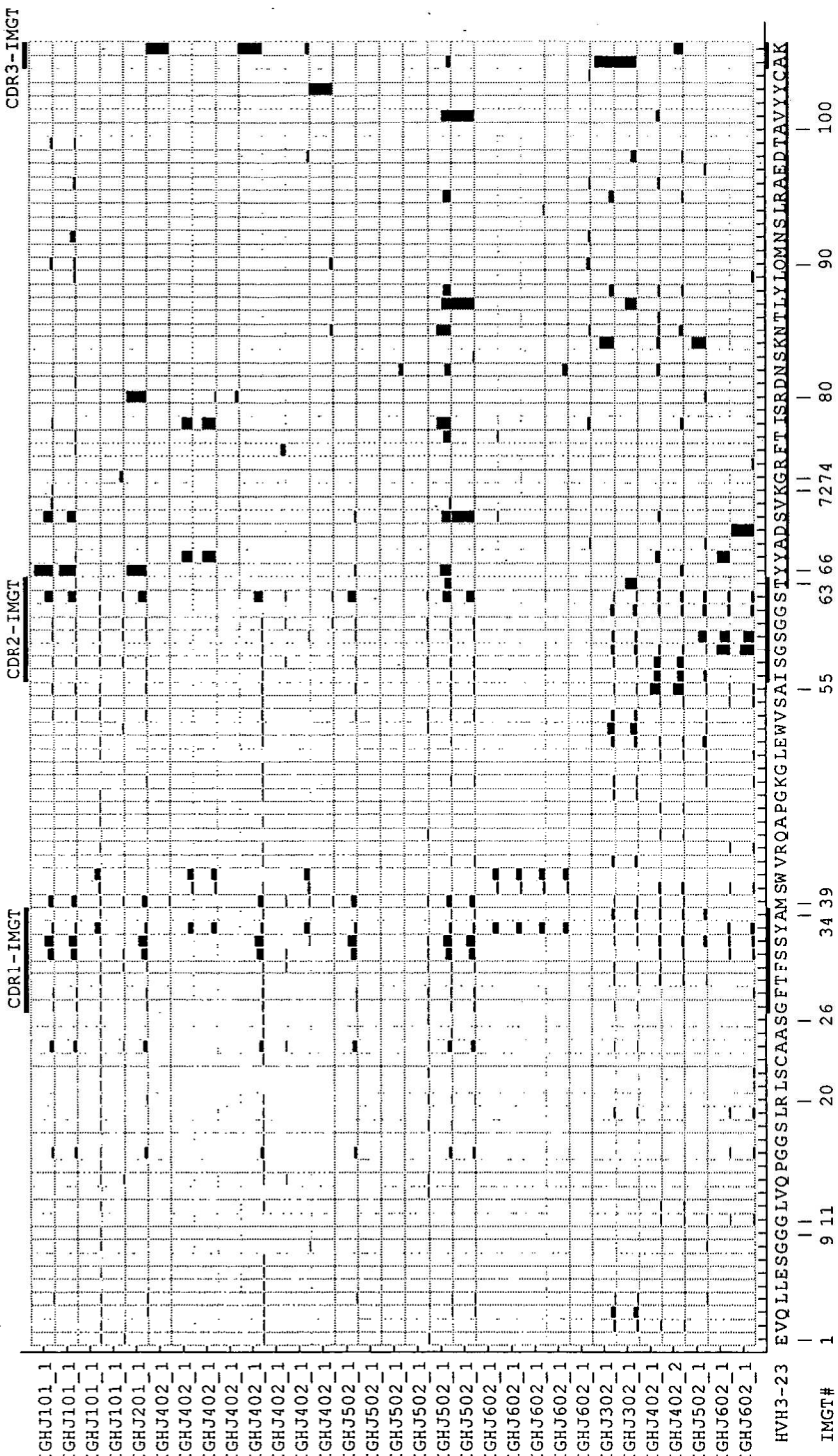

Solubility of αCR1 VH domains
A1 4.6 mg/ml
A9 11.5 mg/ml
B1 5.1 mg/ml
E1 5.3 mg/ml
G11 5.8 mg/ml

Figure 11



The sequences were obtained by forward (amino acids 1 to 62) and reverse (amino acids 63 to 106) sequencing.
The sequences have been classified by D and J regions (left numbering, e.g. D31 or J1 in the top sequence).
The mutations indicated are averages of mutations occurring at a particular position.
White indicates no mutations, while an increase in the size of the black bar indicates an increase of mutation frequency at that position. Immediately apparent in addition to the expected CDR1, 2 and 3 region mutations are the framework mutations. Numbering is according to the IMGT standard.

Figure 12

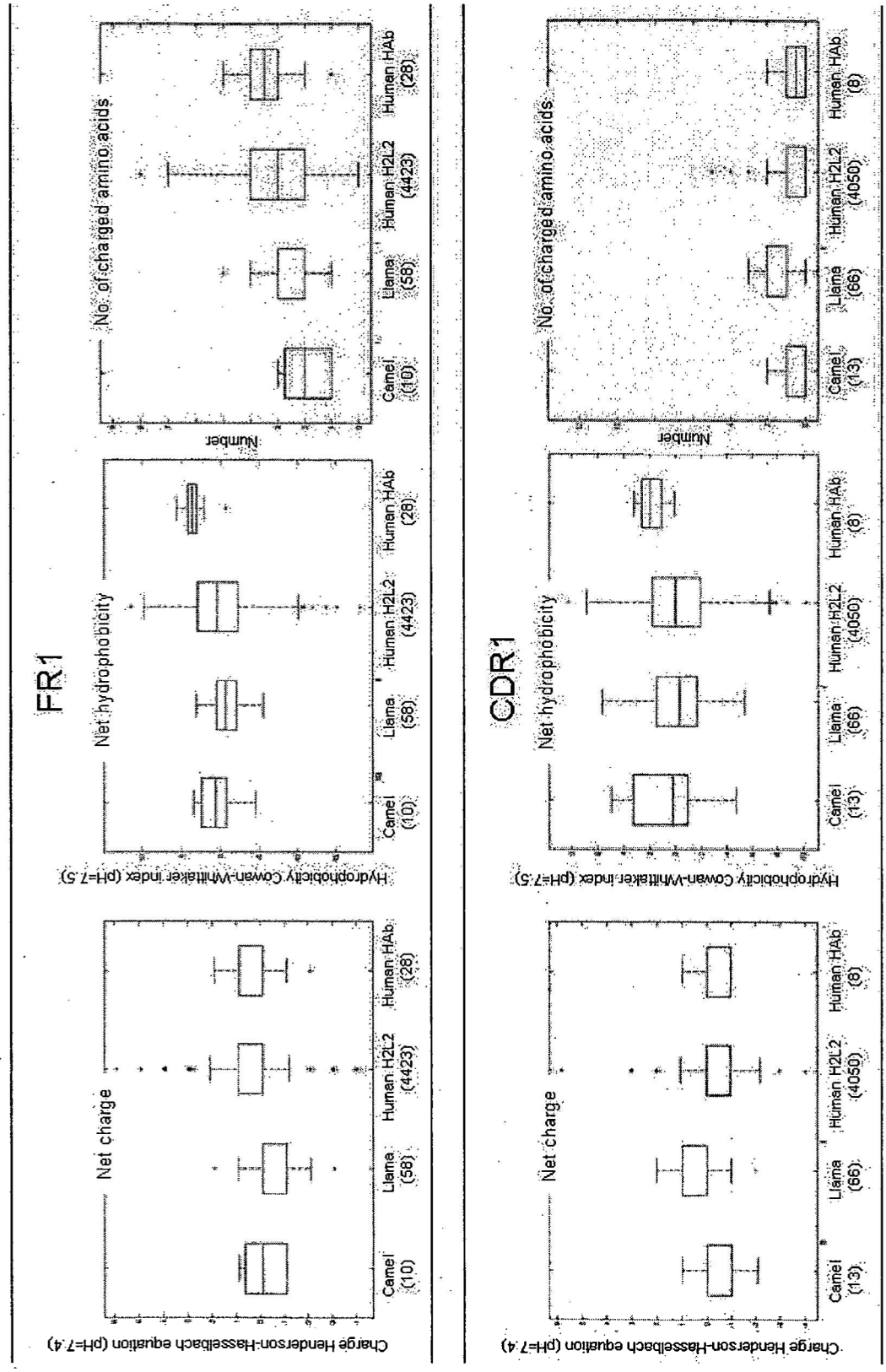

FR1
CDR1
Net charge
Net hydrophobicity
No. of charged amino acids
Charge Henderson-Hasselbach equation (pH=7.4)
Hydrophobicity Cowan-Whittaker index (pH=7.5)
Number
Camel (10)
Llama (58)
Human H2L2 (4423)
Human HAb (28)
Camel (13)
Llama (66)
Human H2L2 (4050)
Human HAb (8)

Figure 13

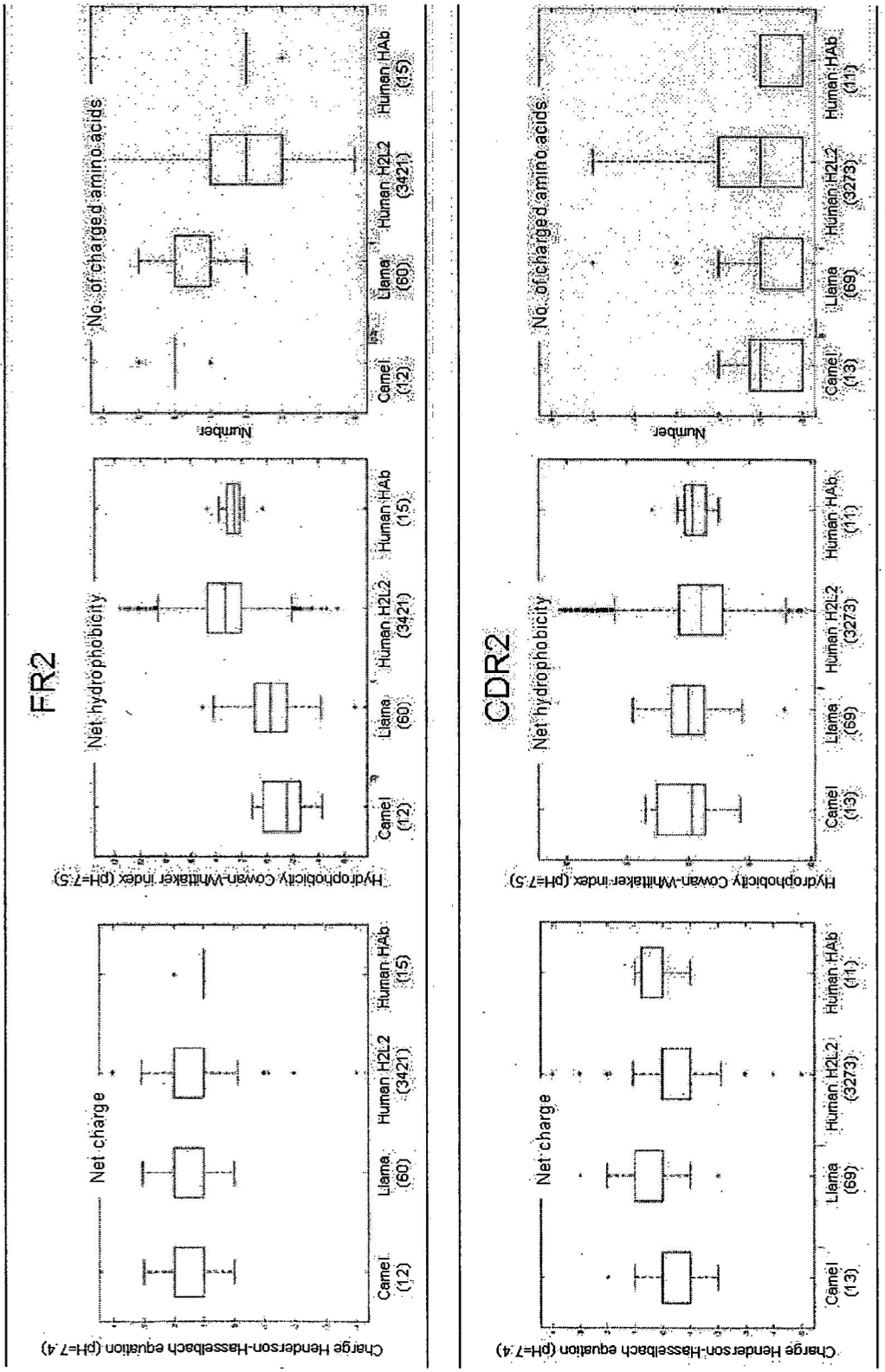

FR2
CDR2
Charge Henderson-Hasselbach equation (pH=7.4)
Net charge
Hydrophobicity Cowan-Whittaker index (pH=7.5)
Net hydrophobicity
No. of charged amino acids
Number
Camel (12)
Llama (60)
Human H2L2 (3421)
Human HAb (15)
Camel (13)
Llama (69)
Human H2L2 (3273)
Human HAb (11)

Figure 14

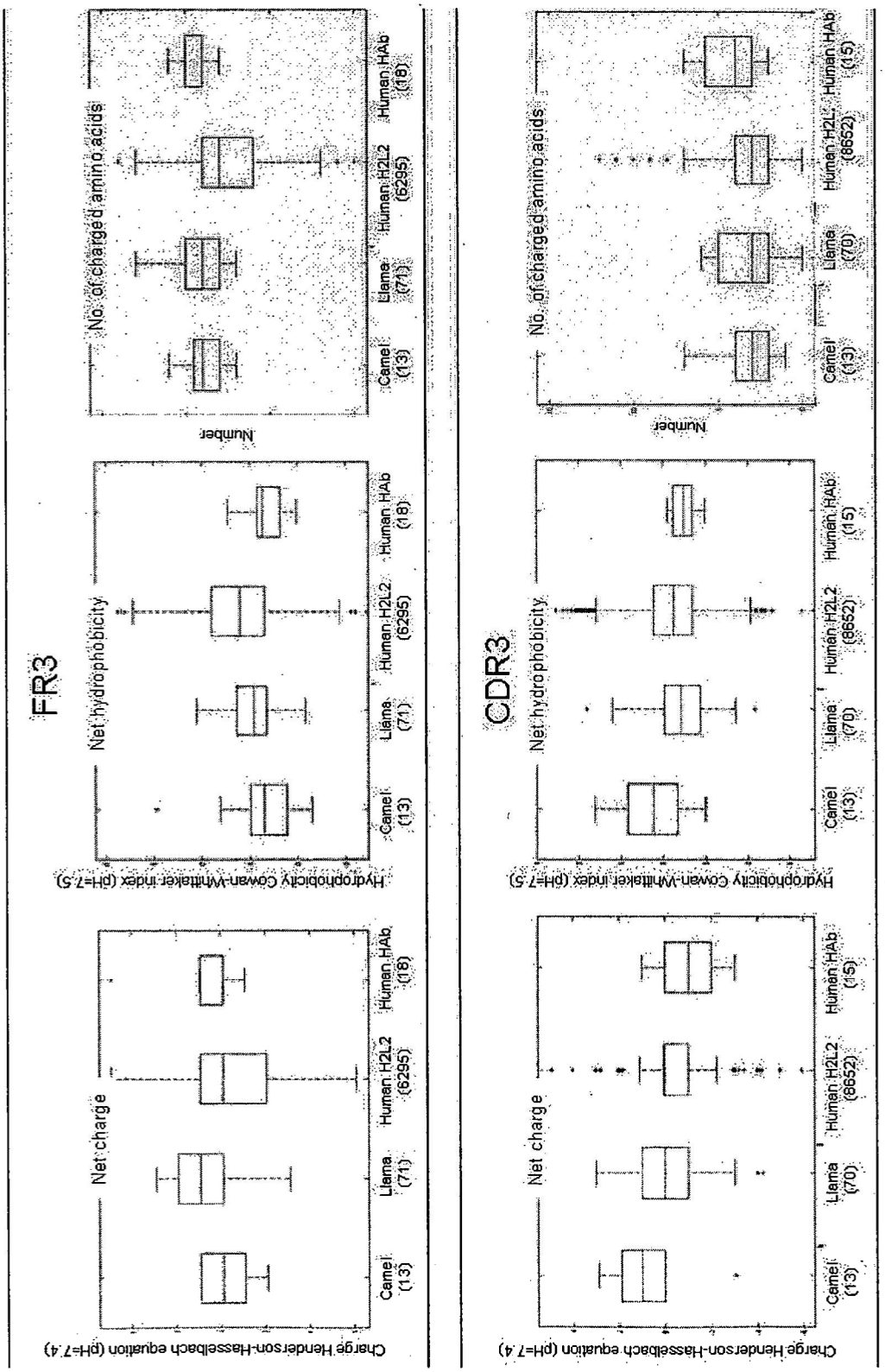

FR3
CDR3
Net charge
Net hydrophobicity
No. of charged amino acids
Charge Henderson-Hasselbach equation (pH=7.4)
Hydrophobicity Cowan-Whittaker index (pH=7.5)
Number
Camel (13)
Llama (71)
Llama (70)
Human H2L2 (6295)
Human H2L2 (8652)
Human HAb (18)
Human HAb (15)

Figure 15

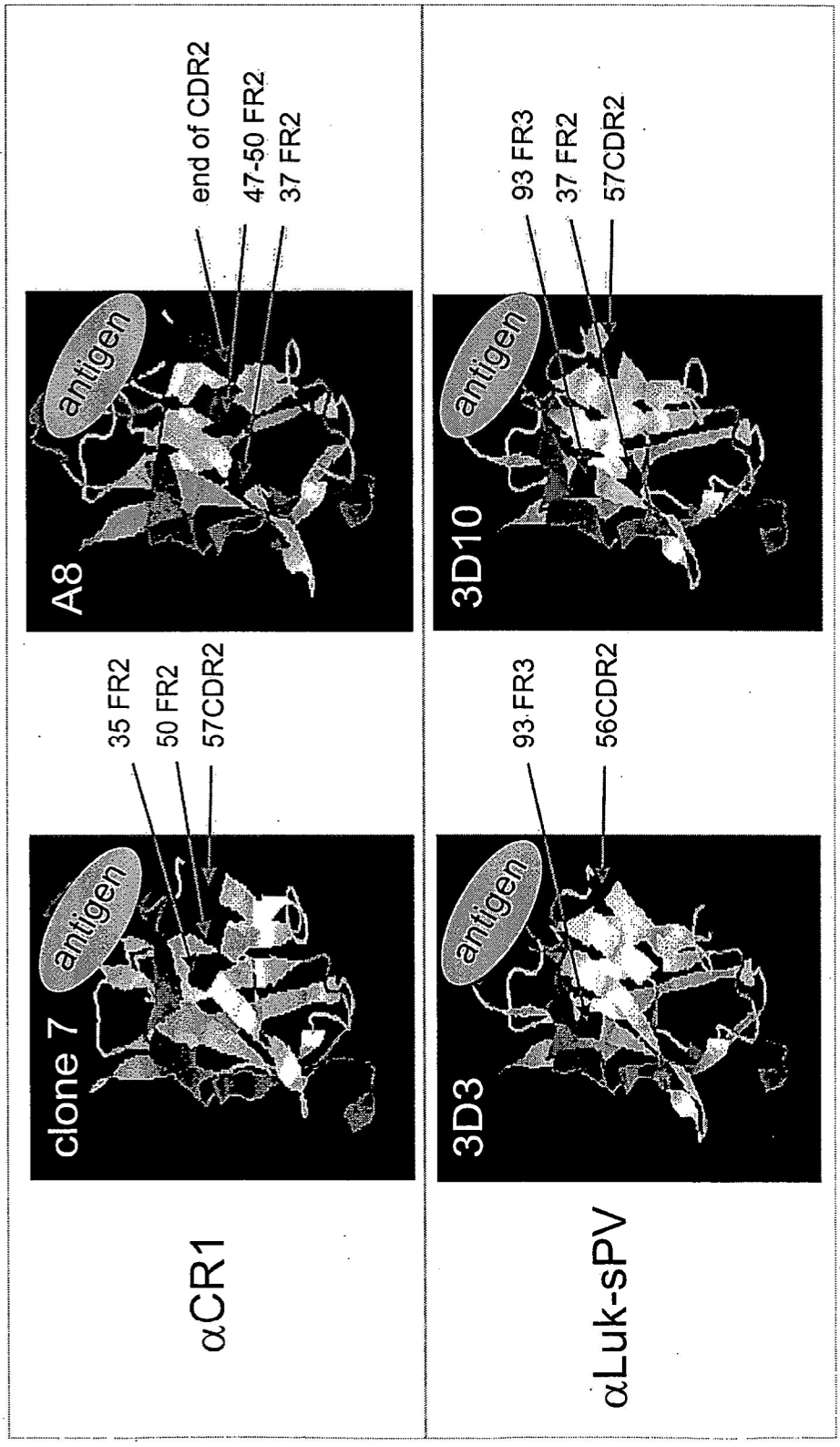

clone 7
antigen
35 FR2
50 FR2
57CDR2
A8
antigen
end of CDR2
47-50 FR2
37 FR2
αCR1
3D3
antigen
93 FR3
56CDR2
3D10
antigen
93 FR3
37 FR2
57CDR2
αLuk-sPV

Figure 16

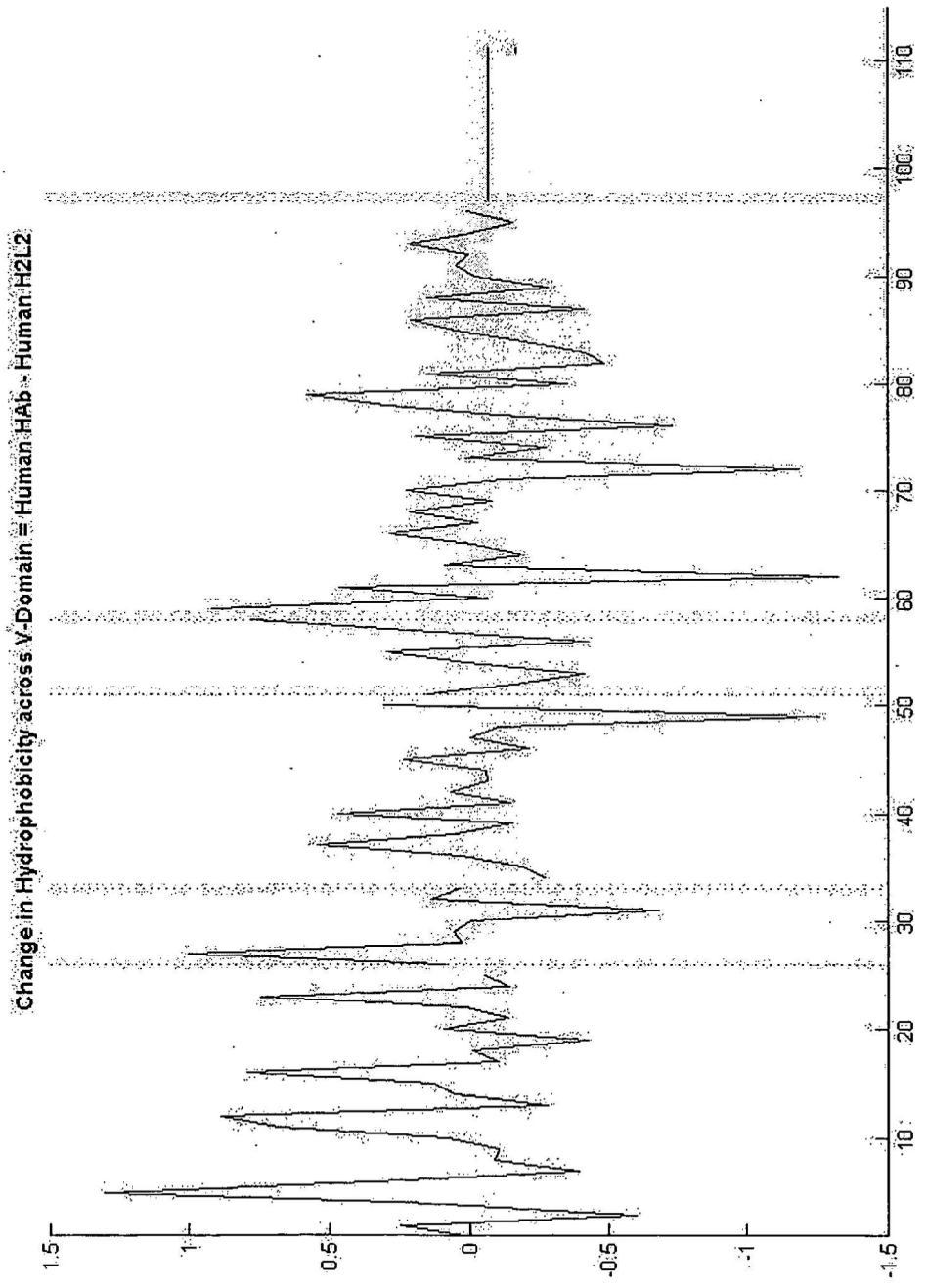

Change in Hydrophobicity across V-Domain = Human HAb - Human H2L2
1.5
1
0.5
0
-0.5
-1
-1.5
10
20
30
40
50
60
70
80
90
100
110

Figure 17

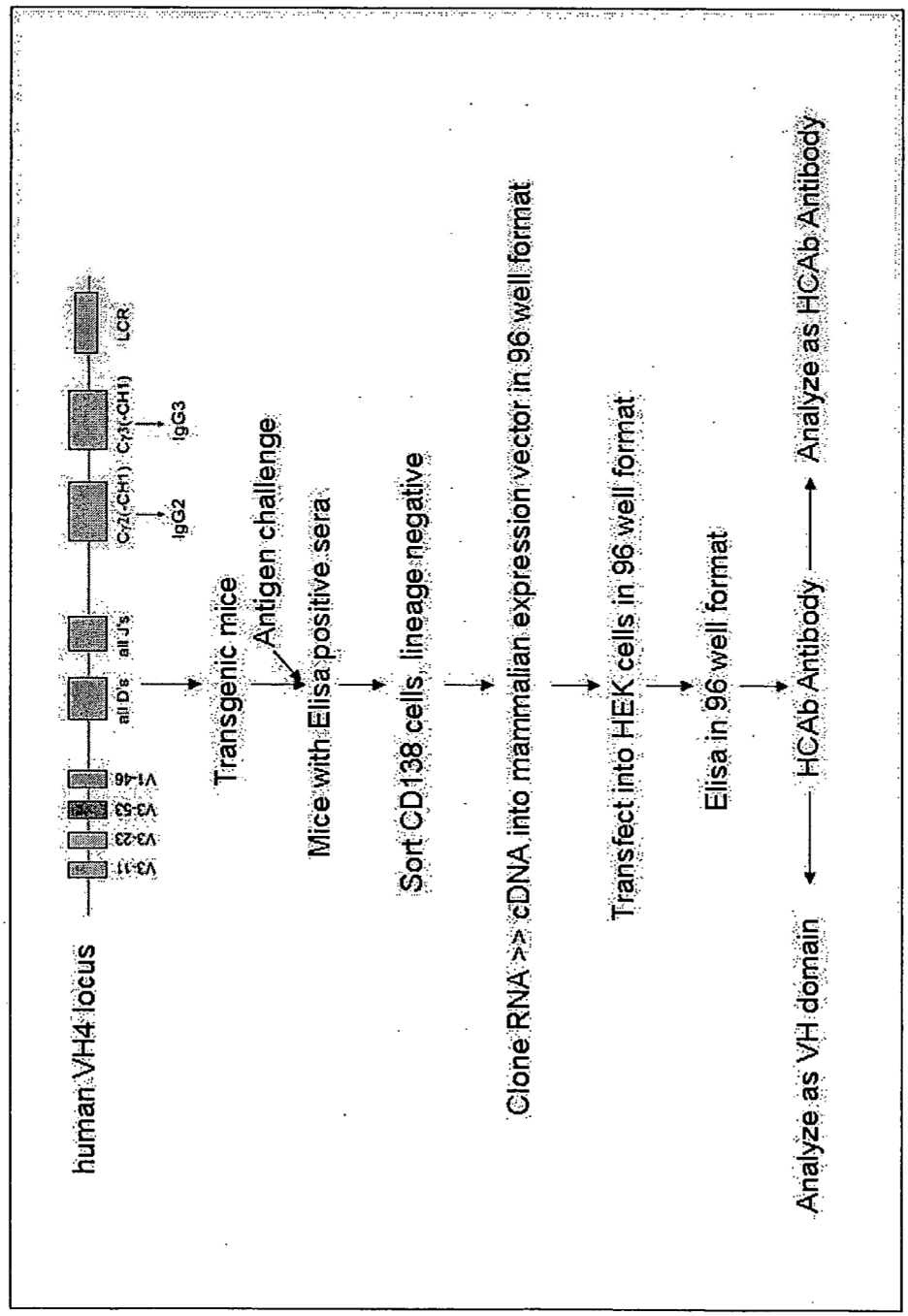

human VH4 locus
V3-11
V3-23
V3-53
V1-46
all D's
all J's
Cγ2(-CH1)
IgG2
Cγ3(-CH1)
IgG3
LCR
Transgenic mice
Antigen challenge
Mice with Elisa positive sera
Sort CD138 cells, lineage negative
Clone RNA >> cDNA into mammalian expression vector in 96 well format
Transfect into HEK cells in 96 well format
Elisa in 96 well format
HCAb Antibody
Analyze as HCAb Antibody
Analyze as VH domain

Figure 18

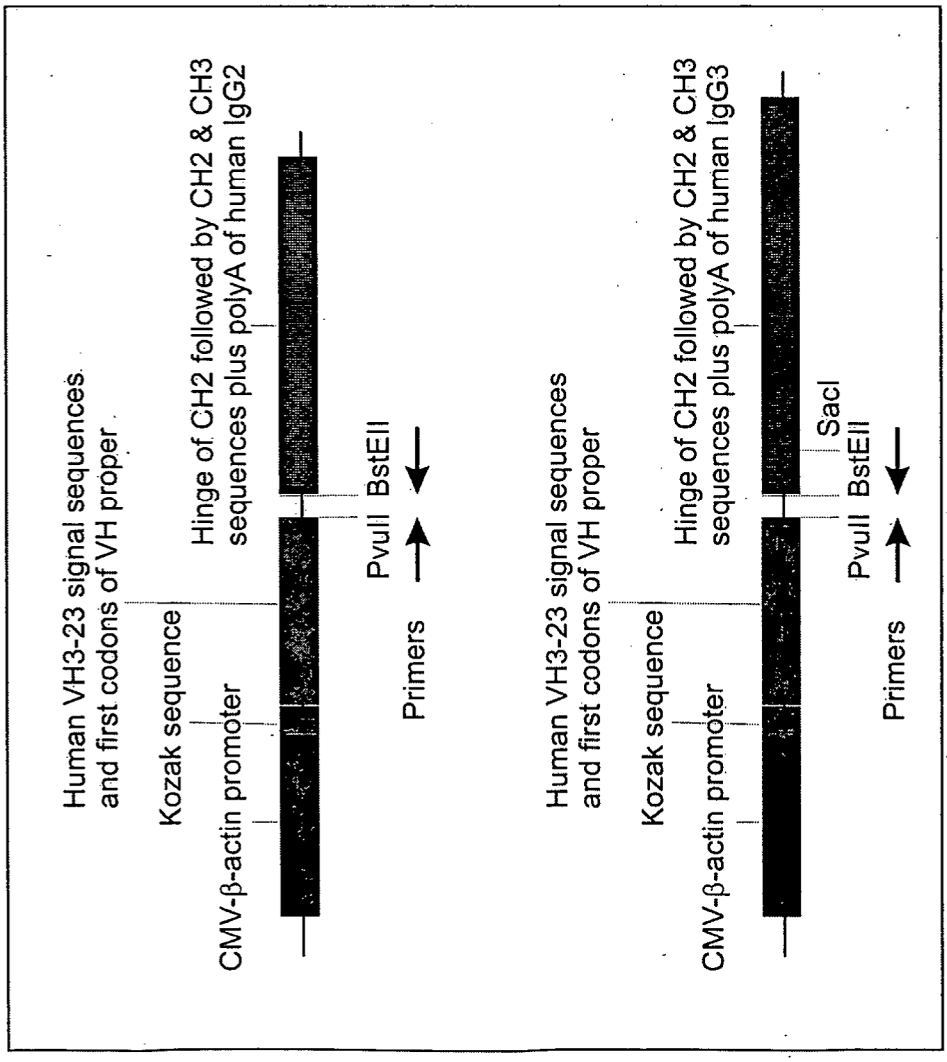

Human VH3-23 signal sequences and first codons of VH proper
Kozak sequence
CMV-β-actin promoter
Hinge of CH2 followed by CH2 & CH3 sequences plus polyA of human IgG2
PvuII BstEII
Primers
Human VH3-23 signal sequences and first codons of VH proper
Kozak sequence
CMV-β-actin promoter
Hinge of CH2 followed by CH2 & CH3 sequences plus polyA of human IgG3
SacI
PvuII BstEII
Primers

Figure 19

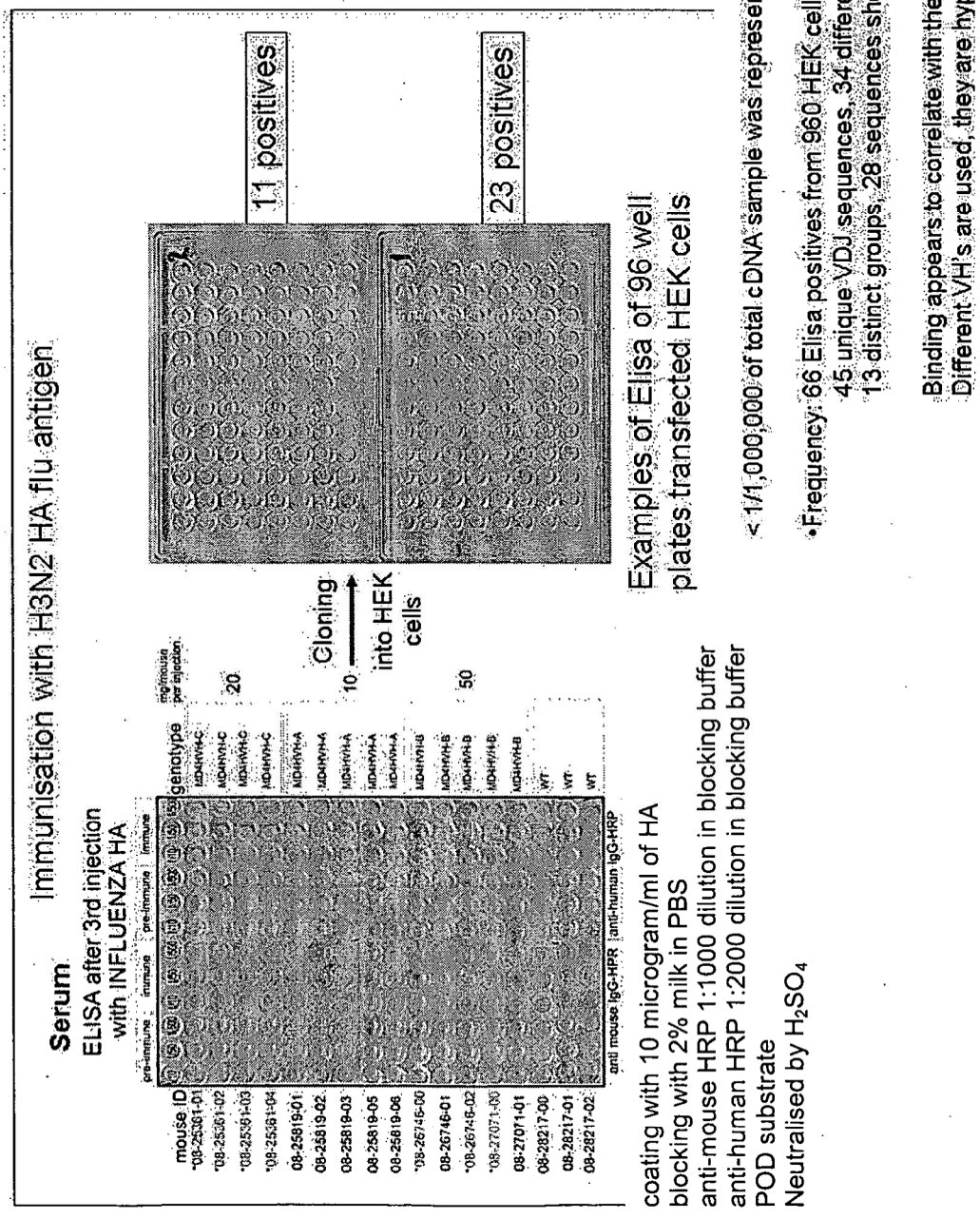

Serum
Immunisation with H3N2 HA flu antigen
ELISA after 3rd injection with INFLUENZA HA
pre-immune
immune
genotype
mg/mouse per injection
mouse ID
08-25361-01
08-25361-02
08-25361-03
08-25361-04
08-25819-01
08-25819-02
08-25819-03
08-25819-05
08-25819-06
08-26746-00
08-26746-01
08-26746-02
08-27071-00
08-27071-01
08-28217-00
08-28217-01
08-28217-02
MDHVH-C
MDHVH-A
MDHVH-B
WT
20
10
50
Cloning
into HEK cells
11 positives
23 positives
Examples of Elisa of 96 well plates transfected HEK cells
anti mouse IgG-HRP
anti-human IgG-HRP
coating with 10 microgram/ml of HA
blocking with 2% milk in PBS
anti-mouse HRP 1:1000 dilution in blocking buffer
anti-human HRP 1:2000 dilution in blocking buffer
POD substrate
Neutralised by H_2SO_4
< 1/1,000,000 of total cDNA sample was represented by 960 wells
•Frequency: 66 Elisa positives from 960 HEK cell clones
45 unique VDJ sequences, 34 different DJ regions
13 distinct groups, 28 sequences showed H3N2 binding on the Octet
Binding appears to correlate with the DJ sequence
Different VH's are used, they are hypermutated and class switched

Figure 20

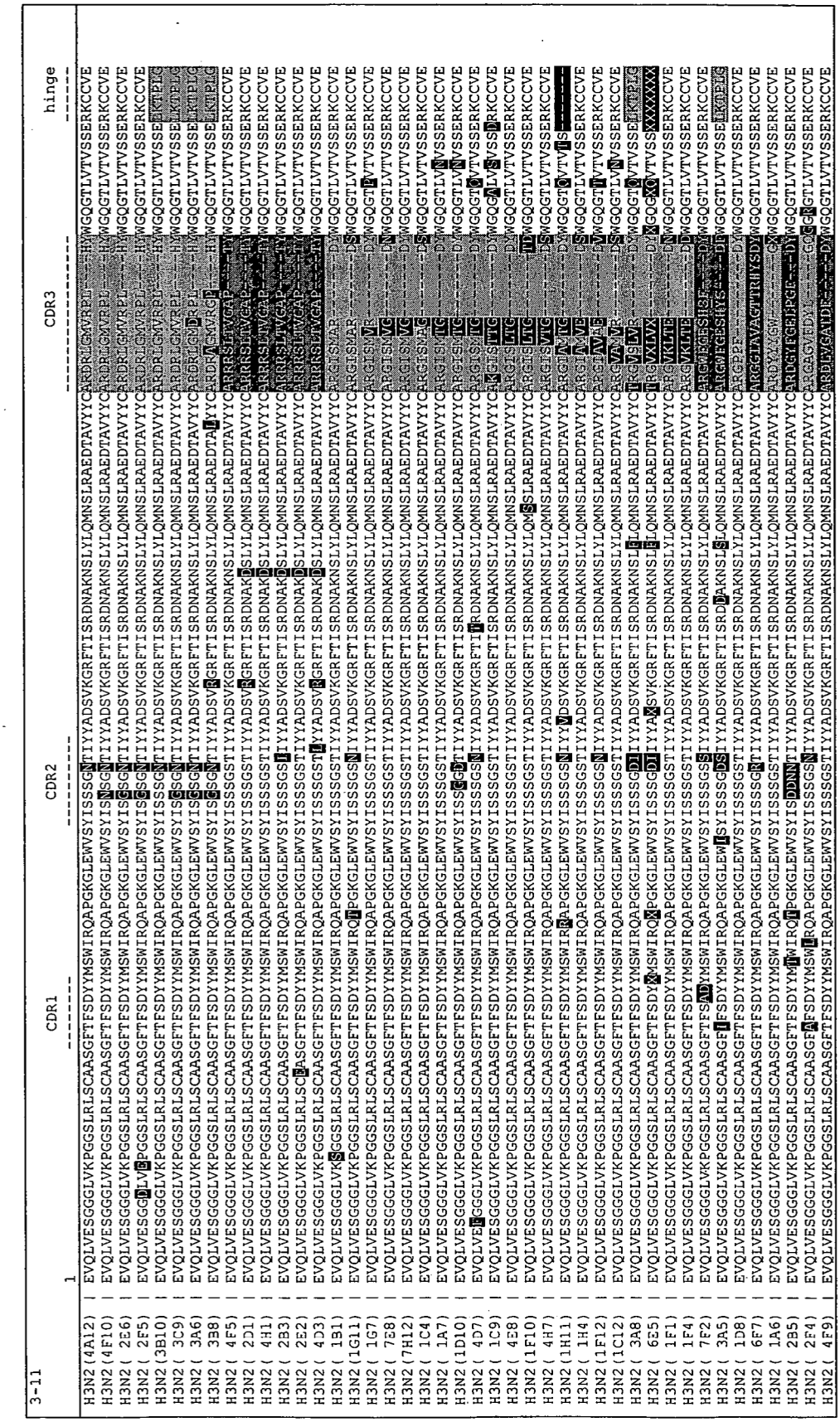


Figure 21

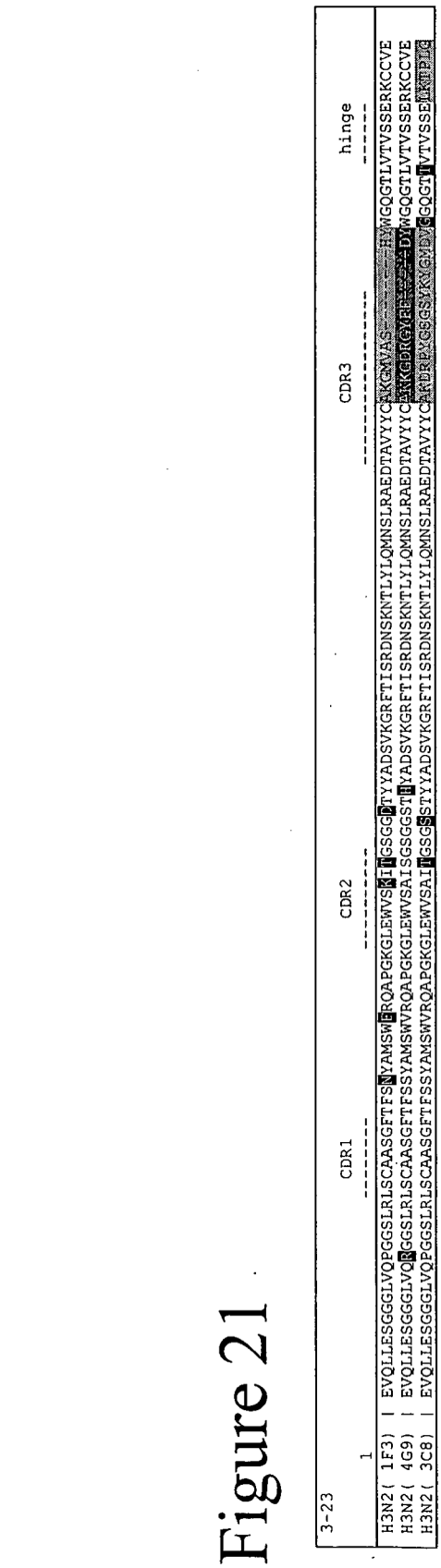


Figure 22

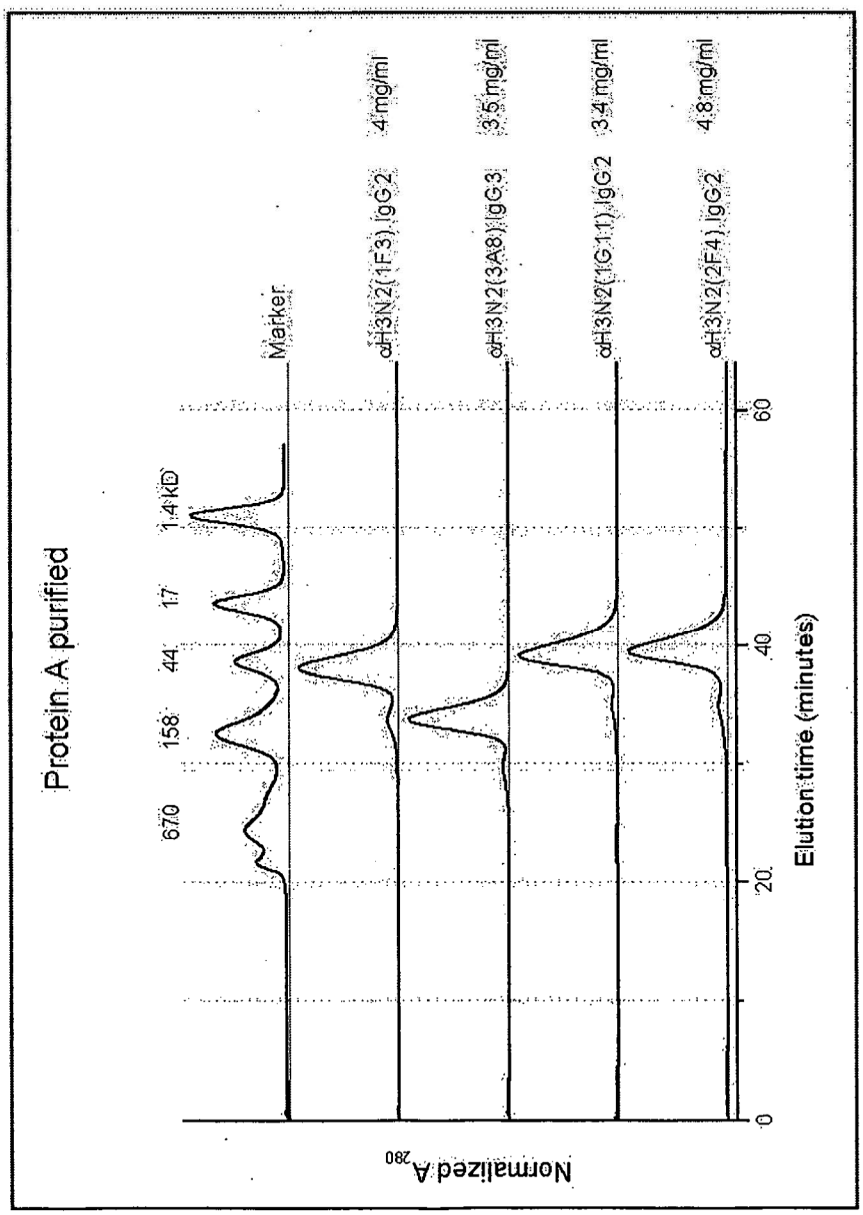

Protein A purified
670
158
44
17
1.4 kD
Marker
αH3N2(1F3) IgG2
4 mg/ml
αH3N2(3A8) IgG3
3.5 mg/ml
αH3N2(1G11) IgG2
3.4 mg/ml
αH3N2(2F4) IgG2
4.8 mg/ml
Normalized A280
0
20
40
60
Elution time (minutes)

Figure 23a

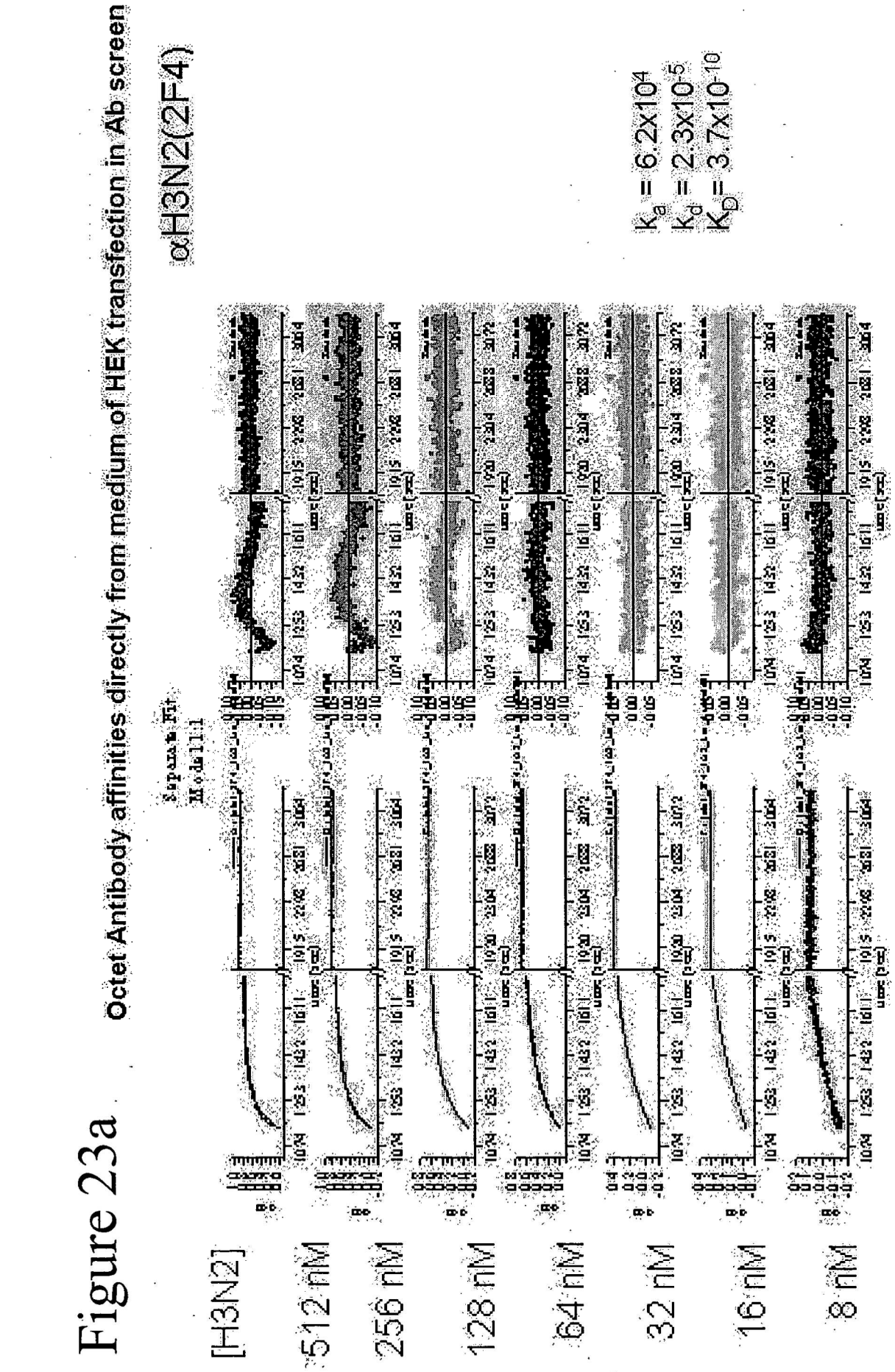

Octet Antibody affinities directly from medium of HEK transfection in Ab screen
αH3N2(2F4)
Separate Fit
Model 1:1
[H3N2]
512 nM
256 nM
128 nM
64 nM
32 nM
16 nM
8 nM
K_a = 6.2X10^4
K_d = 2.3X10^{-5}
K_D = 3.7X10^{-10}

Figure 23b

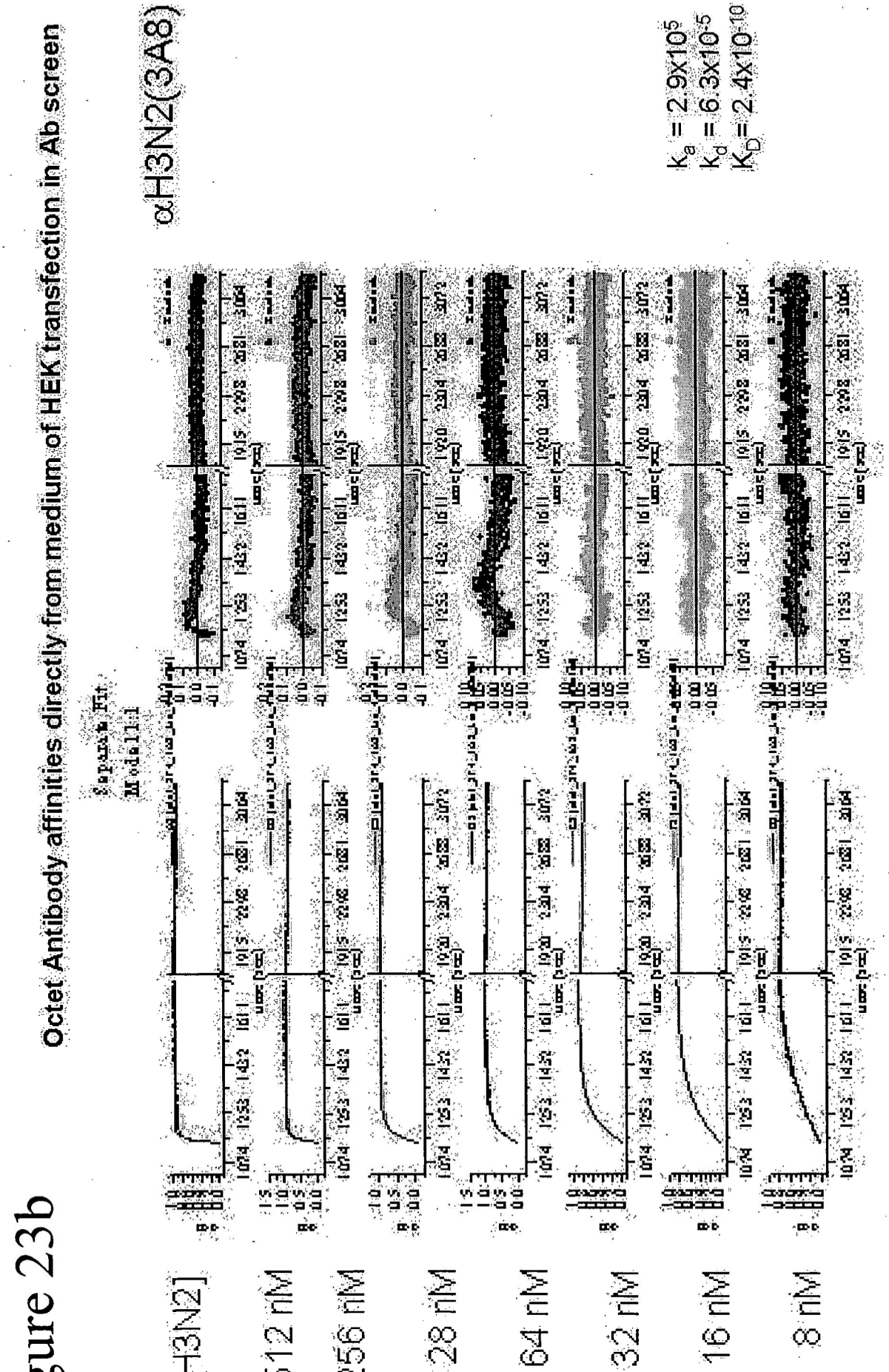

Octet Antibody affinities directly from medium of HEK transfection in Ab screen
αH3N2(3A8)
k_a = 2.9X10^5
k_d = 6.3X10^{-5}
K_D = 2.4X10^{-10}
[H3N2]
512 nM
256 nM
128 nM
64 nM
32 nM
16 nM
8 nM

Figure 24a

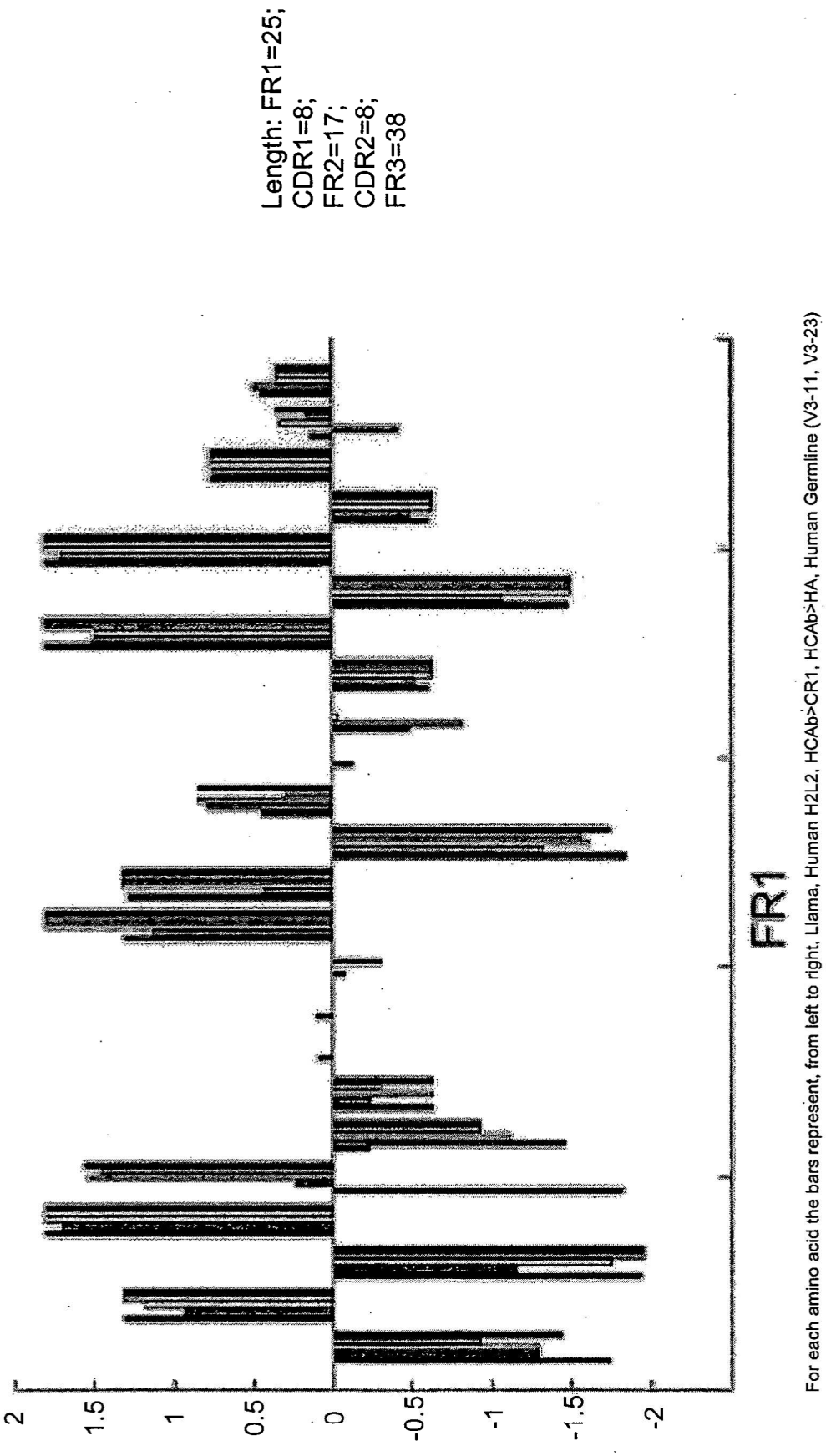

Length: FR1=25;
CDR1=8;
FR2=17;
CDR2=8;
FR3=38
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
FR1
For each amino acid the bars represent, from left to right, Llama, Human H2L2, HCAb>CR1, HCAb>HA, Human Germline (V3-11, V3-23)

Figure 24b

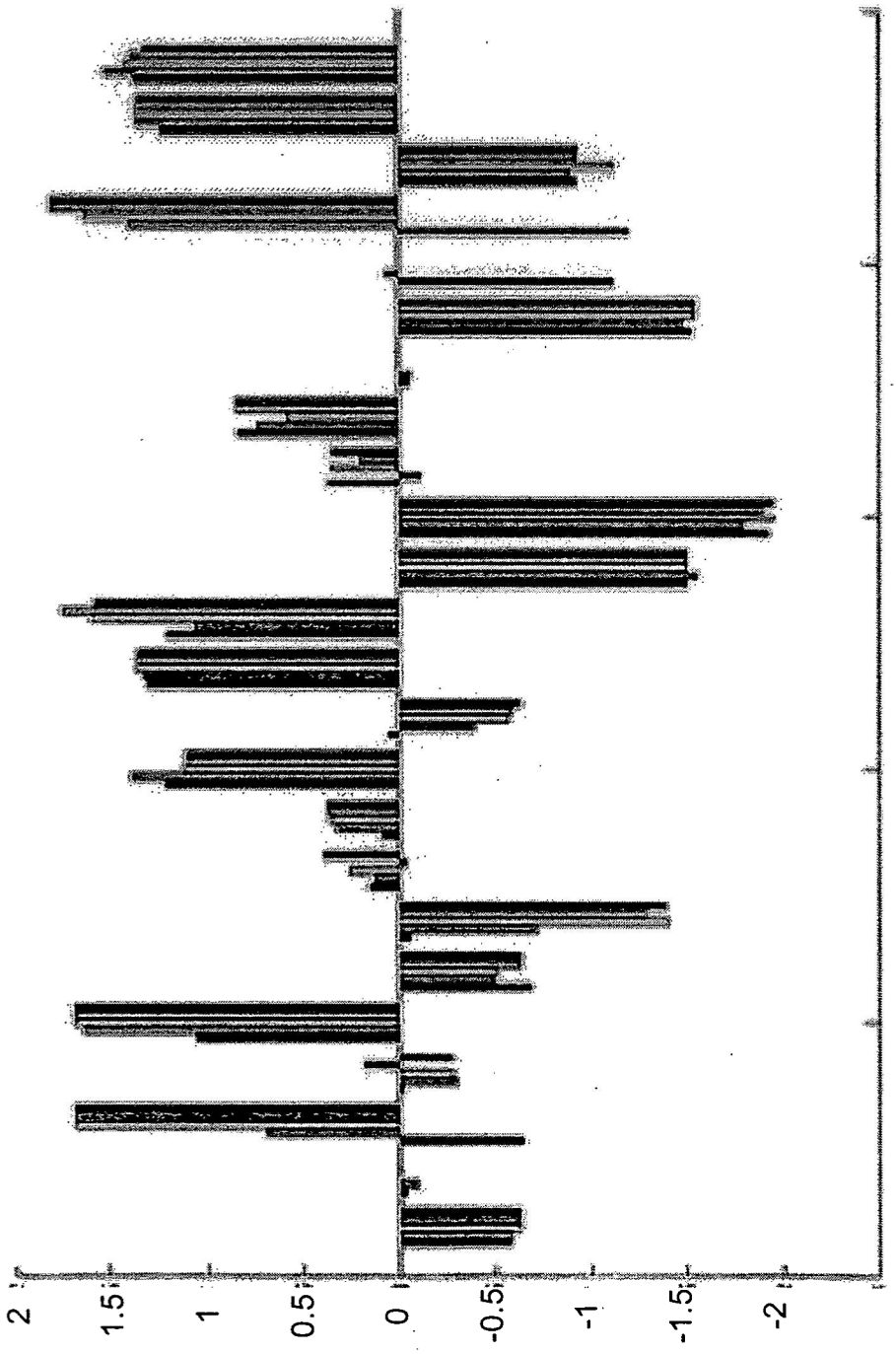

Length: FR1=25;
CDR1=8;
FR2=17;
CDR2=8;
FR3=38
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
CDR1
FR2
For each amino acid the bars represent, from left to right, Llama, Human H2L2, HCAb>CR1, HCAb>HA, Human Germline (V3-11, V3-23)

Figure 24c

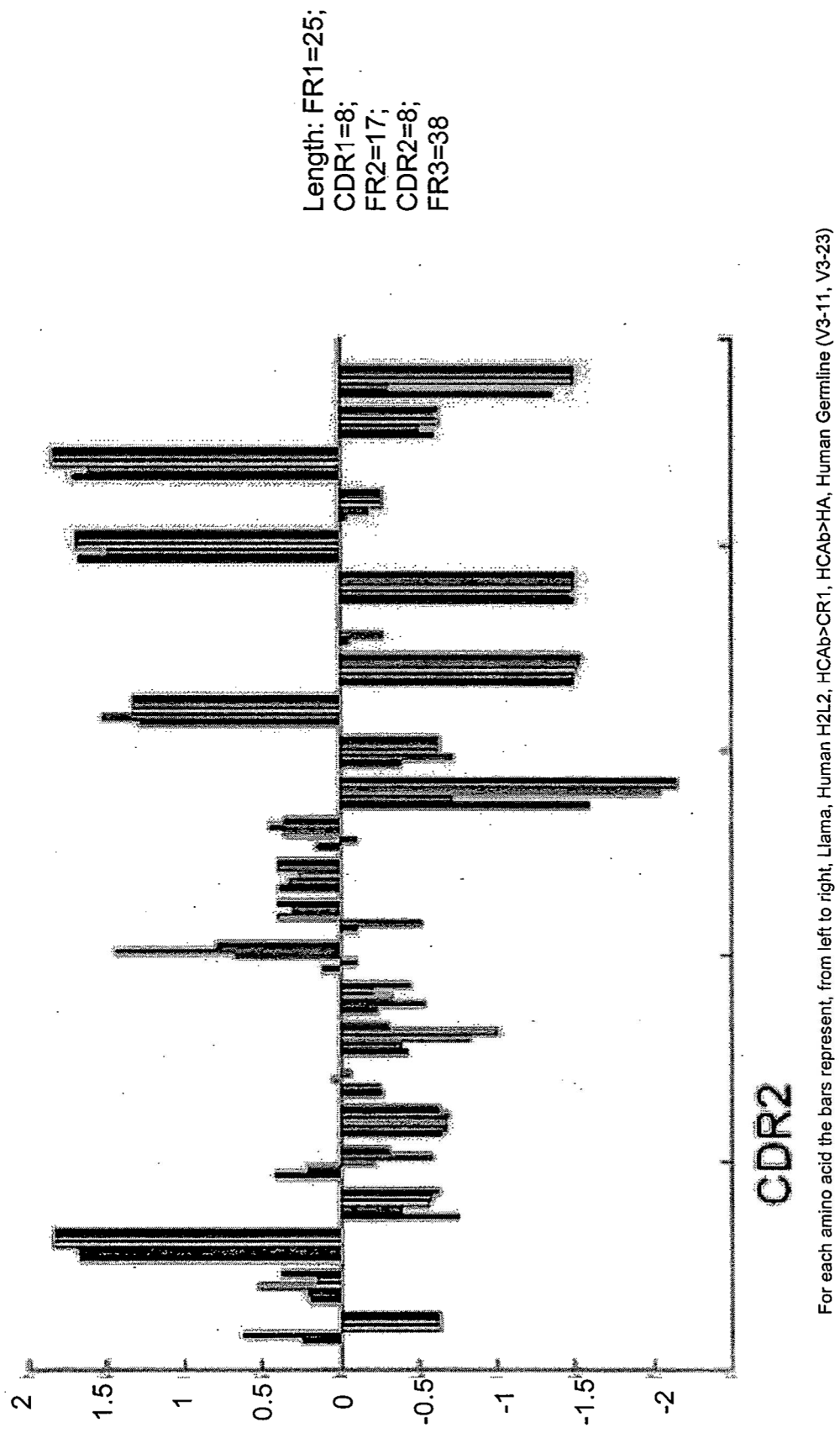

Length: FR1=25;
CDR1=8;
FR2=17;
CDR2=8;
FR3=38
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
CDR2
For each amino acid the bars represent, from left to right, Llama, Human H2L2, HCAb>CR1, HCAb>HA, Human Germline (V3-11, V3-23)

Figure 24d

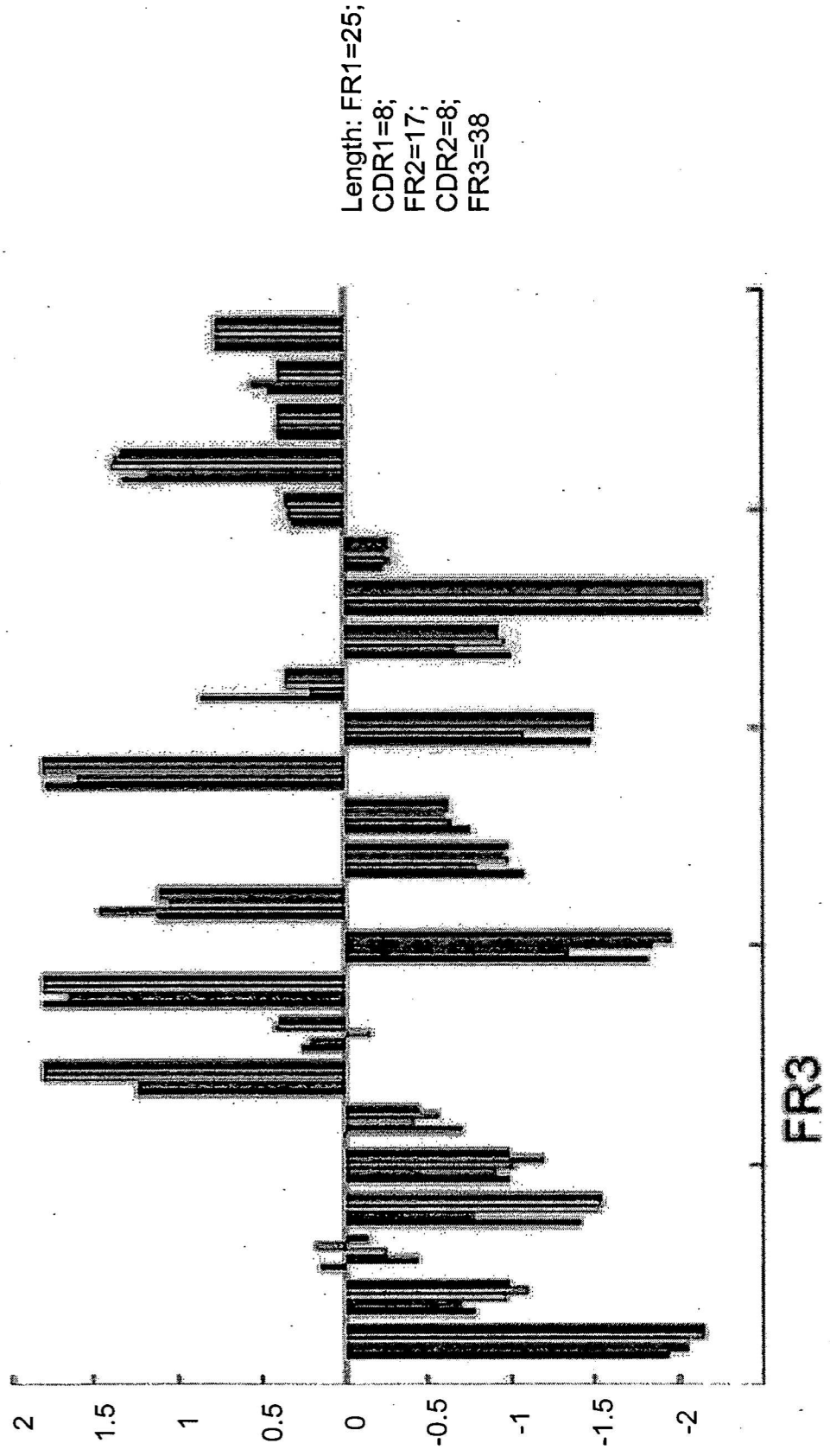

Length: FR1=25;
CDR1=8;
FR2=17;
CDR2=8;
FR3=38
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
FR3
For each amino acid the bars represent, from left to right, Llama, Human H2L2, HCAb>CR1, HCAb>HA, Human Germline (V3-11, V3-23)

Figure 25

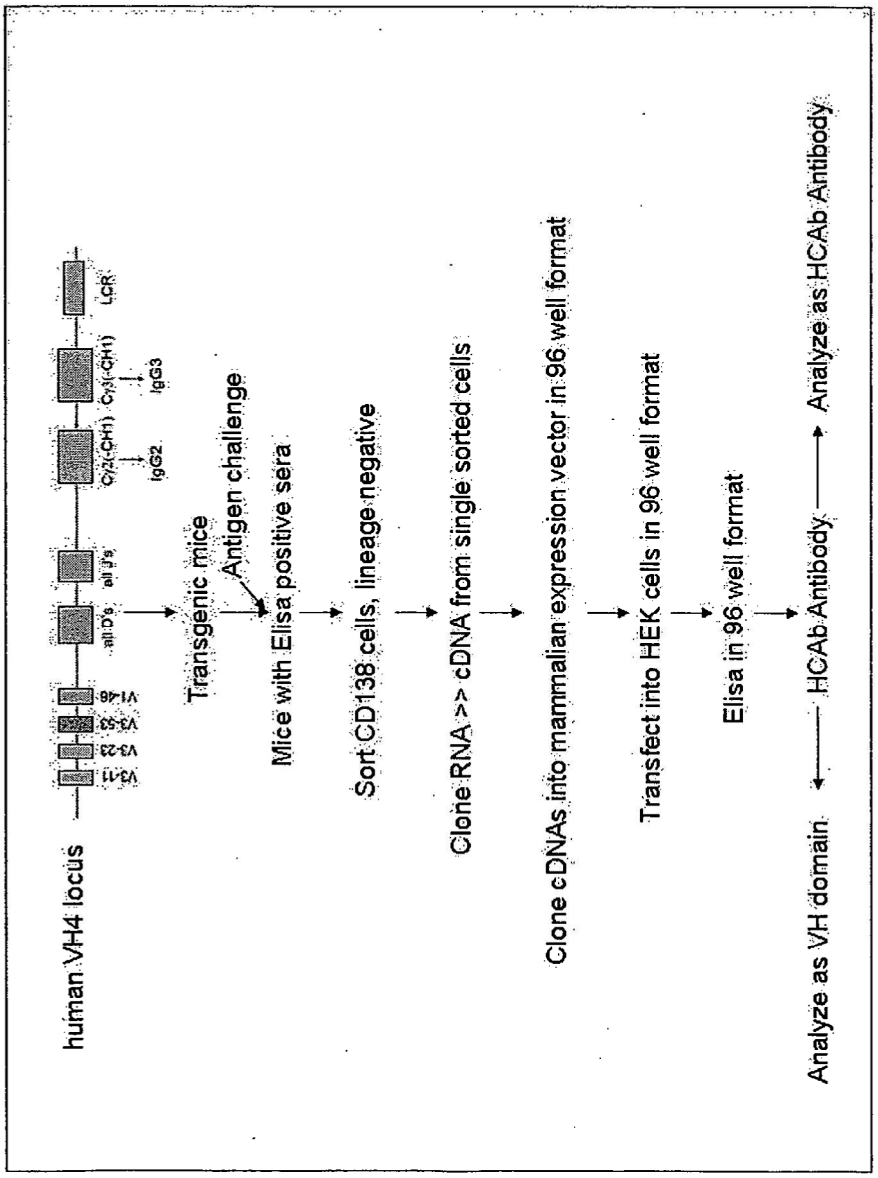
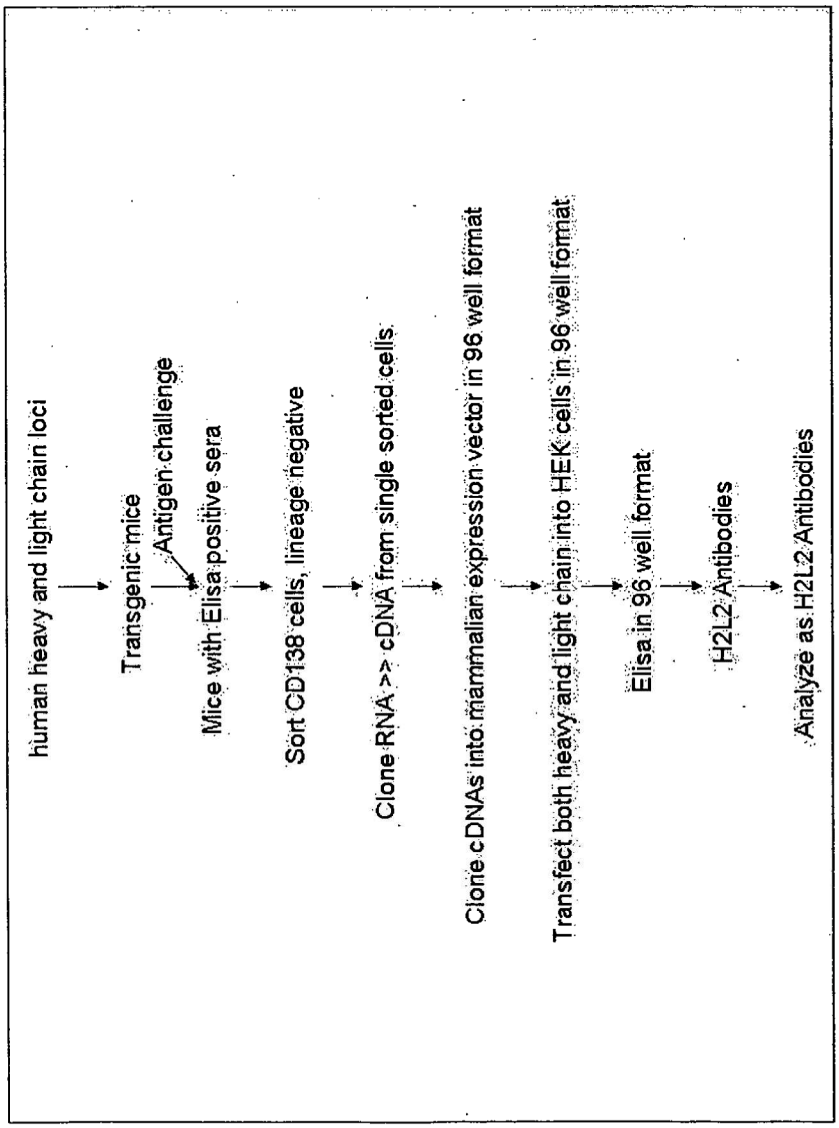

human VH4 locus
Transgenic mice
Antigen challenge
Mice with Elisa positive sera
Sort CD138 cells, lineage negative
Clone RNA >> cDNA from single sorted cells
Clone cDNAs into mammalian expression vector in 96 well format
Transfect into HEK cells in 96 well format
Elisa in 96 well format
HCAb Antibody
Analyze as HCAb Antibody
Analyze as VH domain

Figure 26


human heavy and light chain loci
Transgenic mice
Antigen challenge
Mice with Elisa positive sera
Sort CD138 cells, lineage negative
Clone RNA >> cDNA from single sorted cells
Clone cDNAs into mammalian expression vector in 96 well format
Transfect both heavy and light chain into HEK cells in 96 well format
Elisa in 96 well format
H2L2 Antibodies
Analyze as H2L2 Antibodies