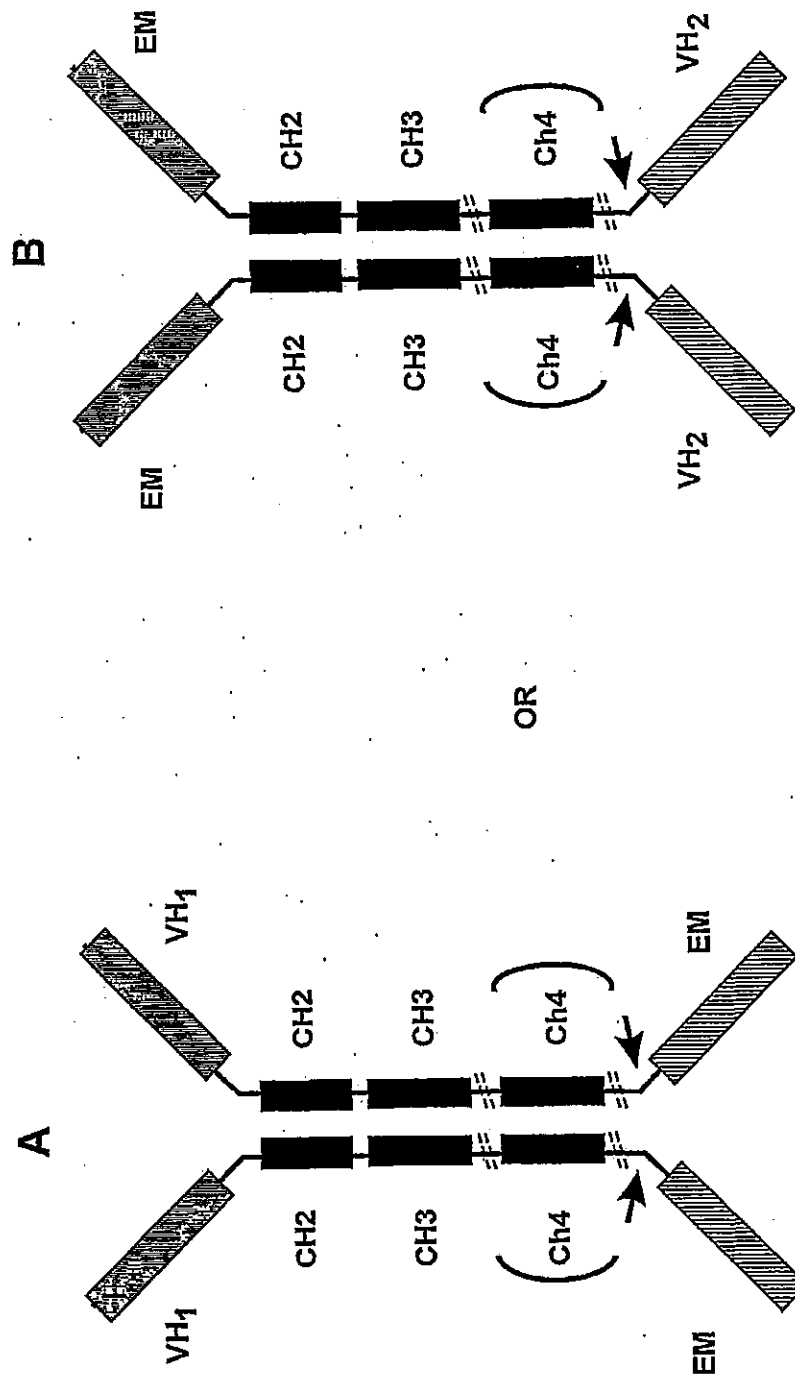


FIG. 1

Plus variations of A and B

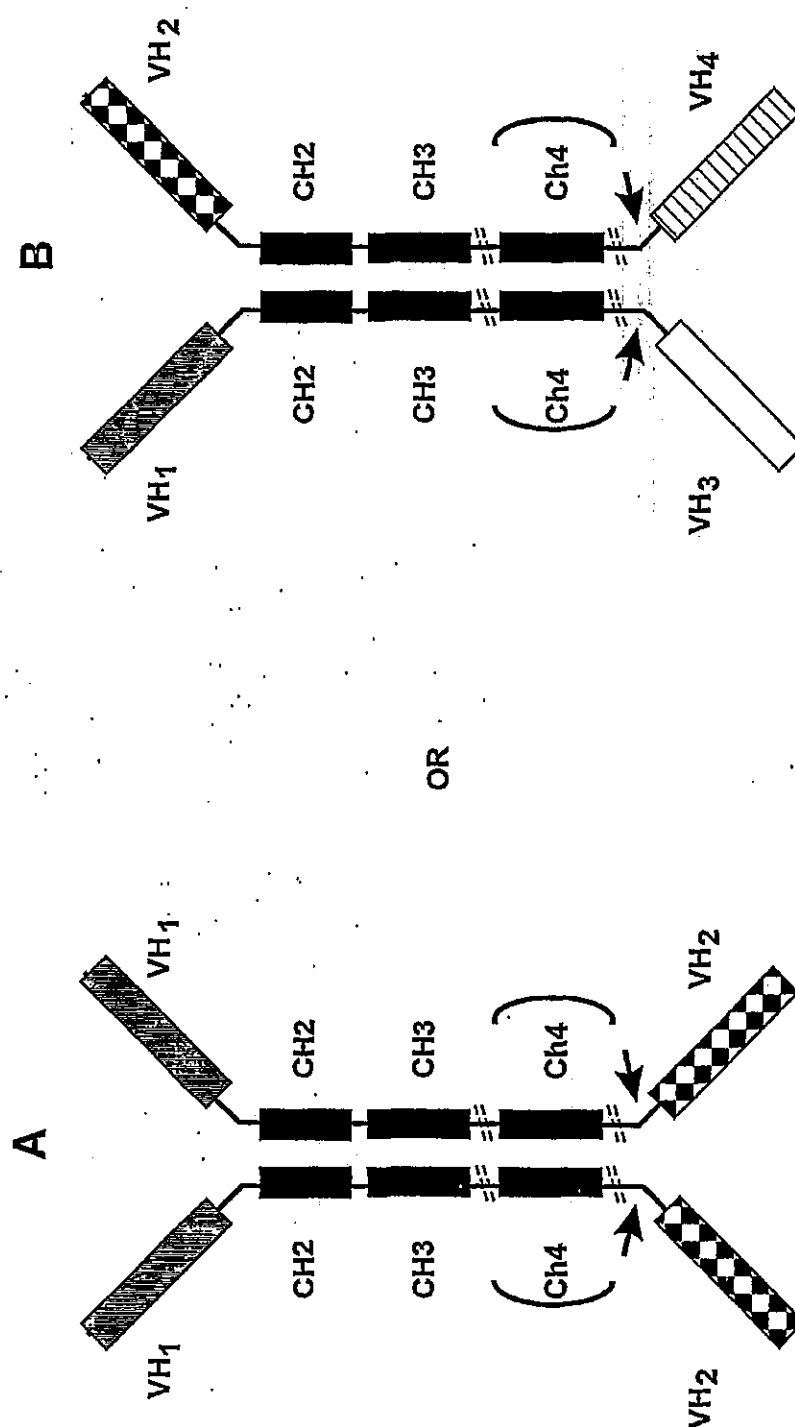
FIG. 2

FIG. 3

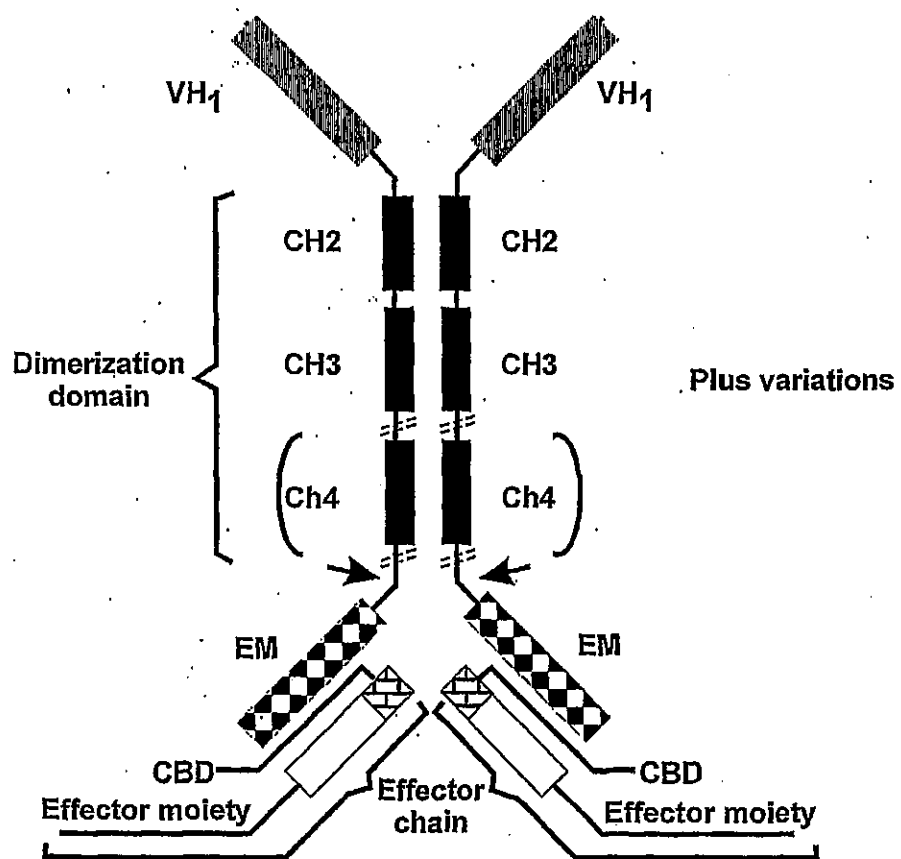


FIG. 4

Bivalent secretory IgA.

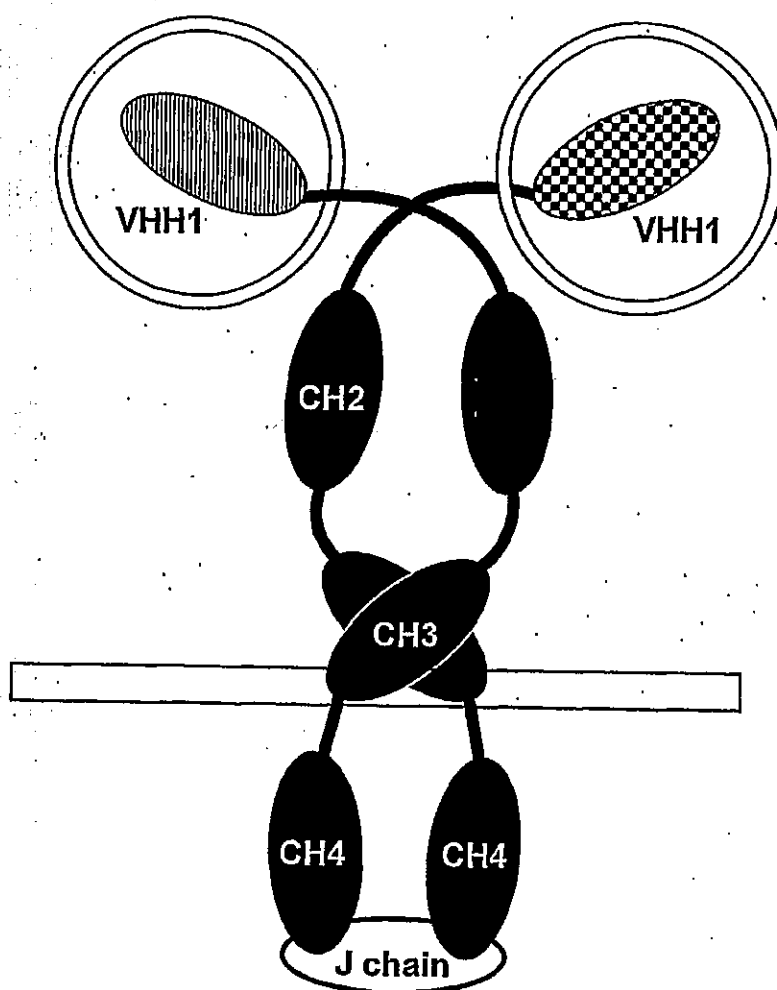


FIG. 5
Multivalent IgM like

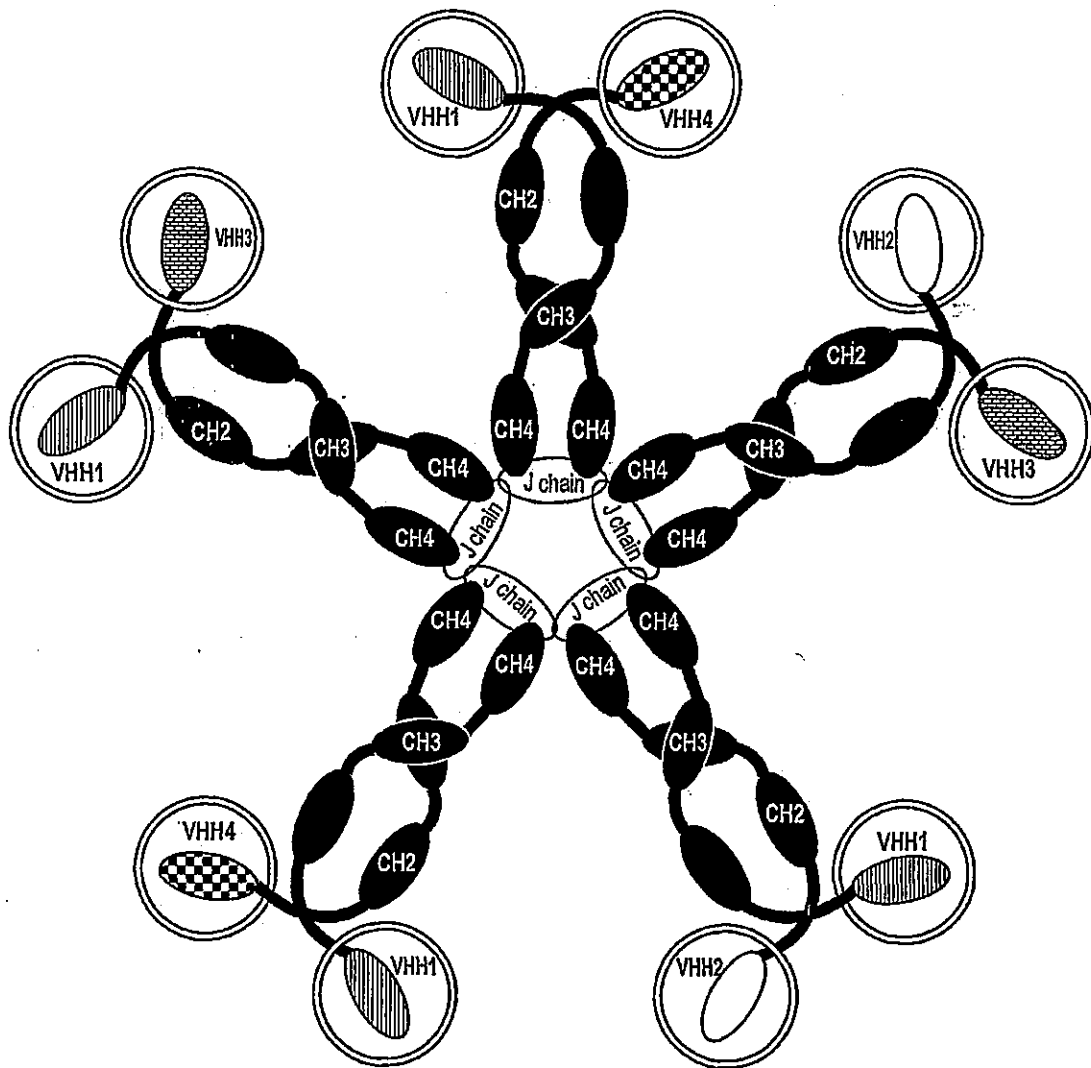


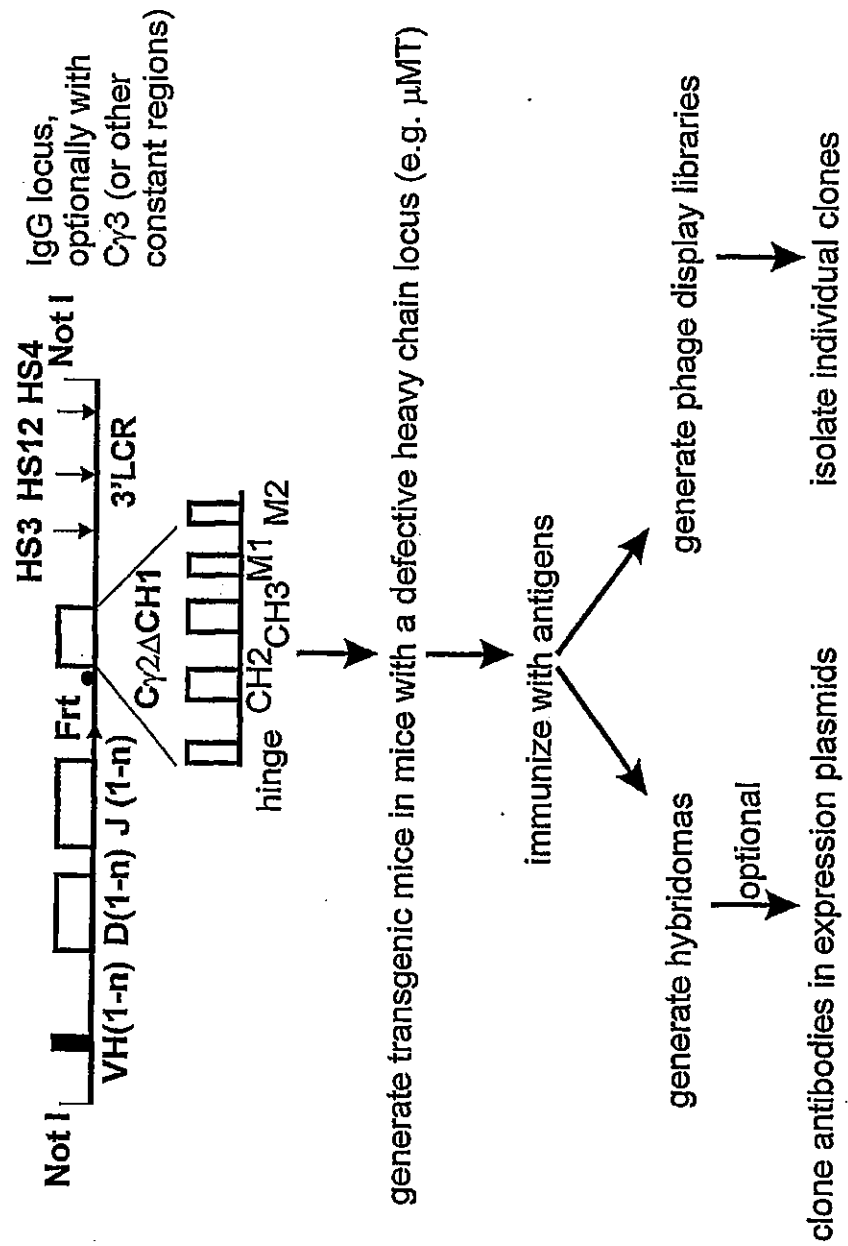
FIG. 6**Generation of heavy chain only IgG**

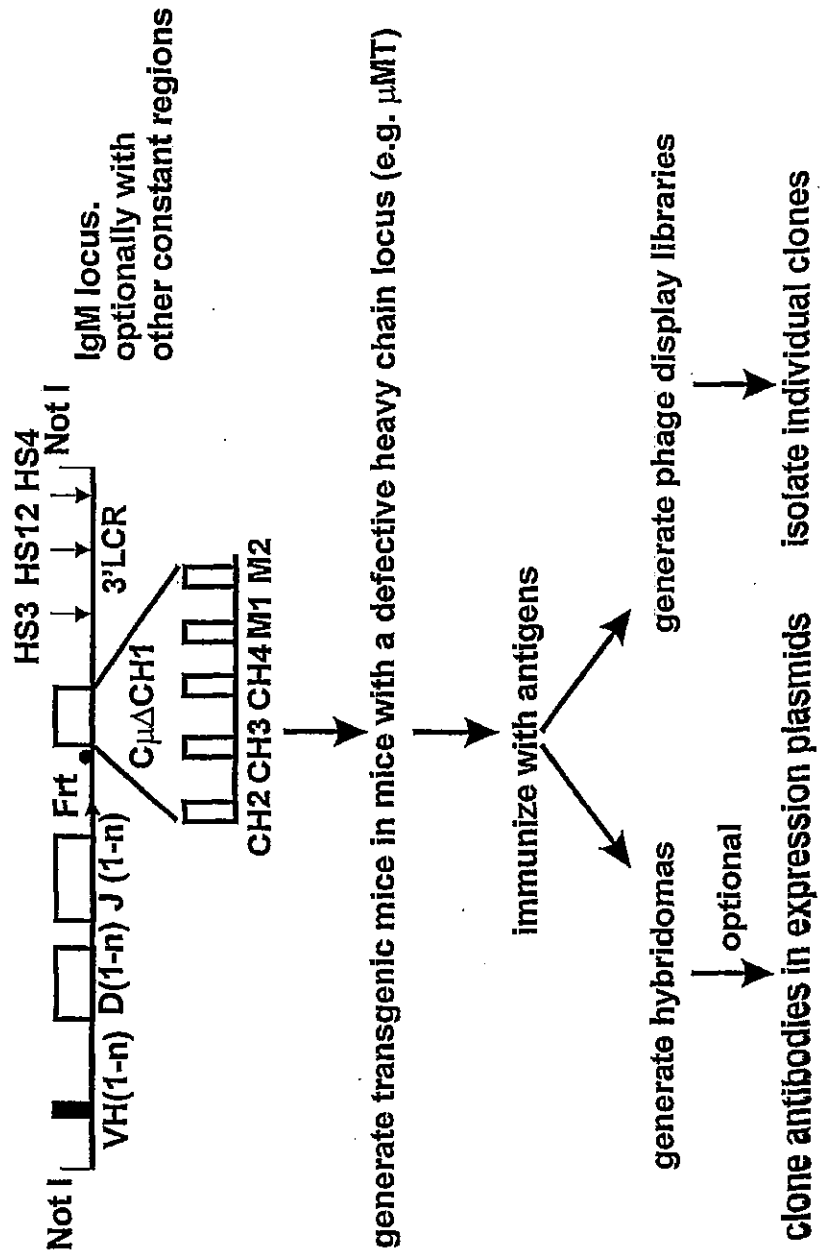
FIG. 7**Generation of heavy chain only IgM**

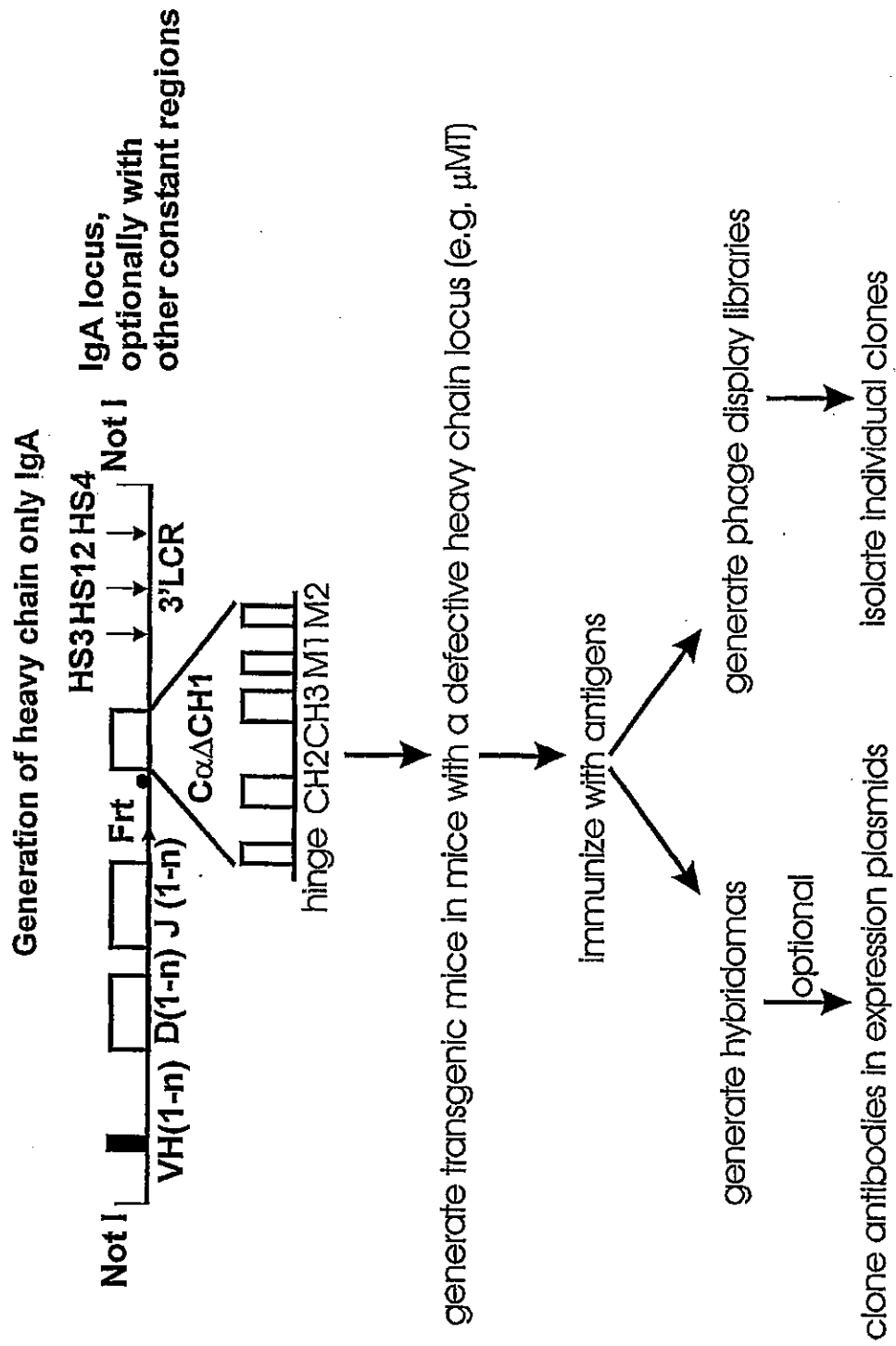
FIG. 8

FIG. 9

9/26

	VHH	D	J
VDJg*1	GACACGGCCGTAGTATCTGTAAGGCAGATGG.....GGTAGTACTATGGTTCG...GGGA.		
VDJg*2	GAC.....ATTCCCACTTCGATC...T...		
VDJg*3	GACACGGCCGTCTATTACTGTAATGCCACTAGC.....ATATTTGACTGGTTAT...TATA.		
VDJg*4	GACACGGCCGTCCAATCGGATACAG.....CTATGGTTACGTACTTT		
VDJg*5	GACACGGCCGTCTATTACTGTAATGCAGATGTATTACTATGGTTCGGGGAGCCTATAGCCTTACTACTACGGTATG.		
		J	CH1
VDJg*1	GTCCACCACTGCCGCTAGAGGGGCGAGGCAACACTGGTCGCGGTGTCATCAGCCTCCACCAAGGGCCCATCGGTCTTCCC		
VDJg*2	CTGGGGCGGTGGCAACCTGGTCACCTGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC		
VDJg*3	GAC.....GCTACTGGGGCGAGGCAACCTGGTCACCTGTCTCCTCAGCCTCCGCAAGGGCCCATCGGTCTTCCC		
VDJg*4	GACTA.....CTGGGGCGAGGCAACCTGGTCACCTGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC		
VDJg*5	GACGT.....CTGGGGCGAGGCAACCTGGTCACCTGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC		
		CH1	
VDJg*1	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG		
VDJg*2	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG		
VDJg*3	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG		
VDJg*4	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG		
VDJg*5	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG		
		CH1	
VDJg*1	TGACGGTGTGCTGGAACCTCAGCGCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCAGGACTCTAC		
VDJg*2	TGACGGTGTGCTGGAACCTCAGCGCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCAGGACTCTAC		
VDJg*3	TGACGGTGTGCTGGAACCTCAGCGCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCAGGACTCTAC		
VDJg*4	TGACGGTGTGCTGGAACCTCAGCGCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCAGGACTCTAC		
VDJg*5	TGACGGTGTGCTGGAACCTCAGCGCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCAGGACTCTAC		
		CH1	
VDJg*1	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG		
VDJg*2	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG		
VDJg*3	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG		
VDJg*4	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG		
VDJg*5	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG		
	CH1	Hinge	
VDJg*1	CAACACCAAGAGCGCAAATGTTGTGTCGAG		
VDJg*2	CAACACCAAGAGCGCAAATGTTGTGTCGAG		
VDJg*3	CAACACCAAGAGCGCAAATGTTGTGTCGAG		
VDJg*4	CAACACCAAGAGCGCAAATGTTGTGTCGAG		
VDJg*5	CAACACCAAGAGCGCAAATGTTGTGTCGAG		
		GGTGGACAAGACAGTT	

10/26

FIG. 10

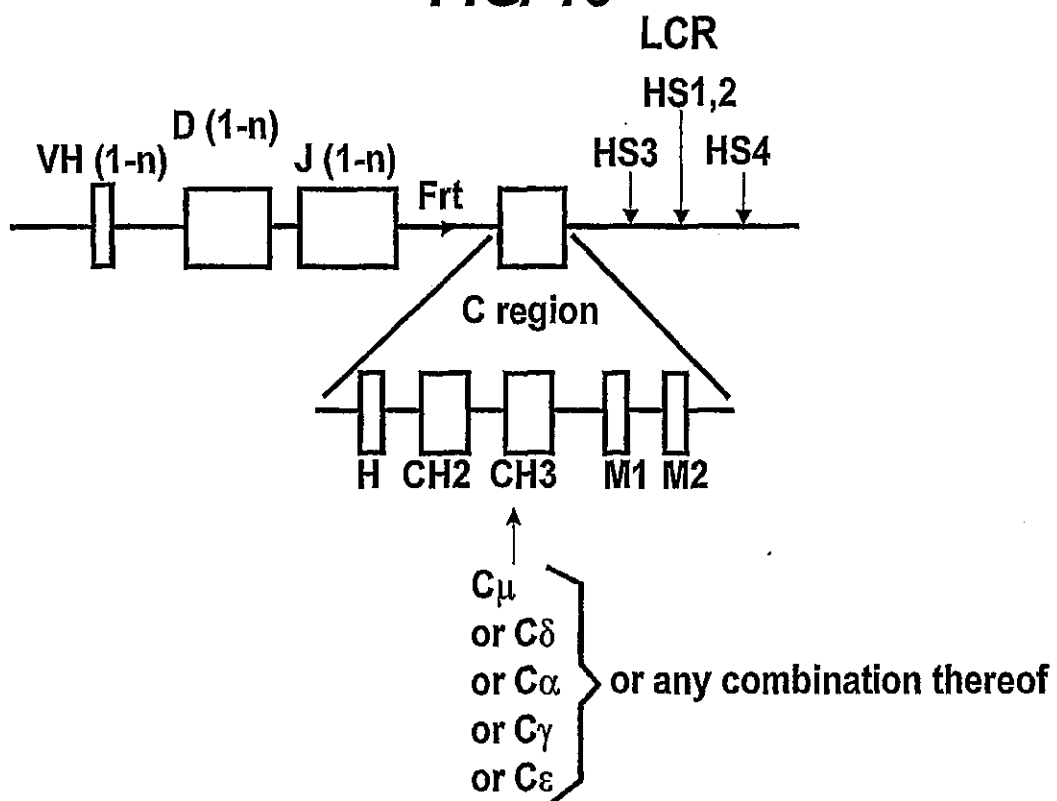
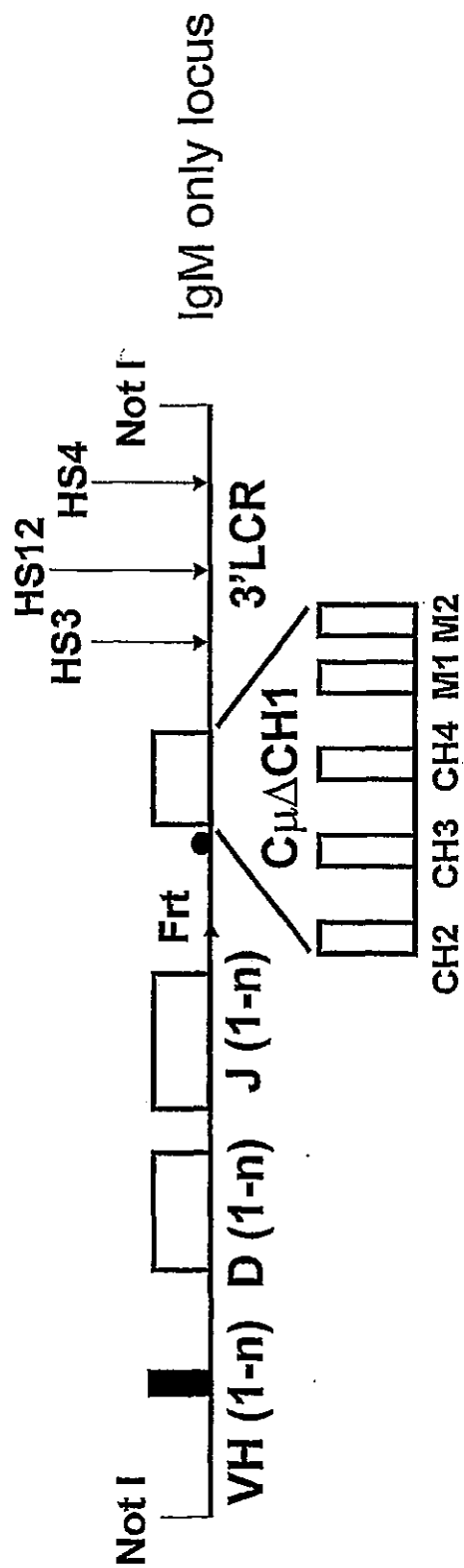
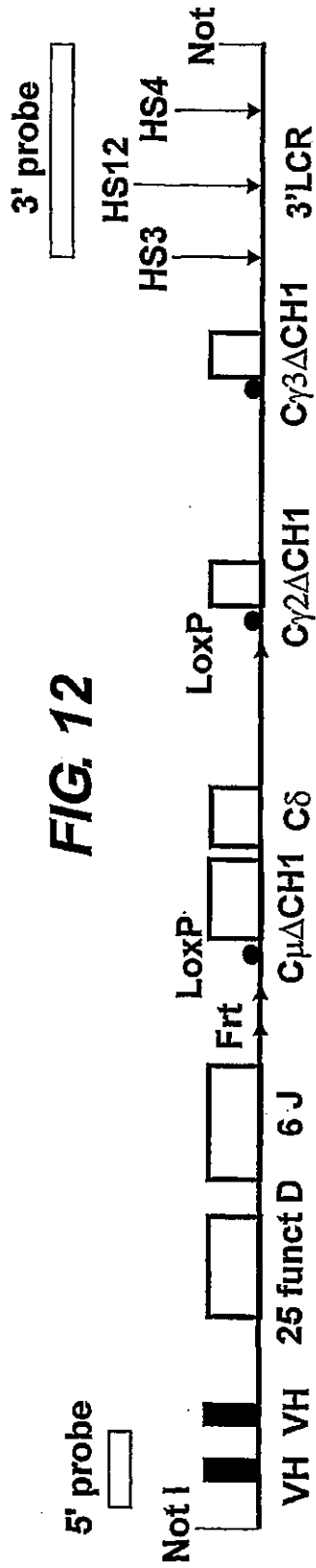


FIG. 11





IgM plus IgG locus, C δ is optional

FIG. 13

IgM plus IgG locus, Cδ is optional

IMMUNIZATION SCHEDULE

s.c. injections at days 0, 14, 28, 42
i.p or i.v. injection at day 50
day 56

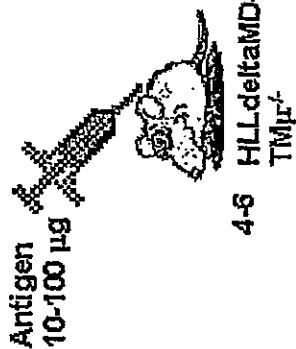
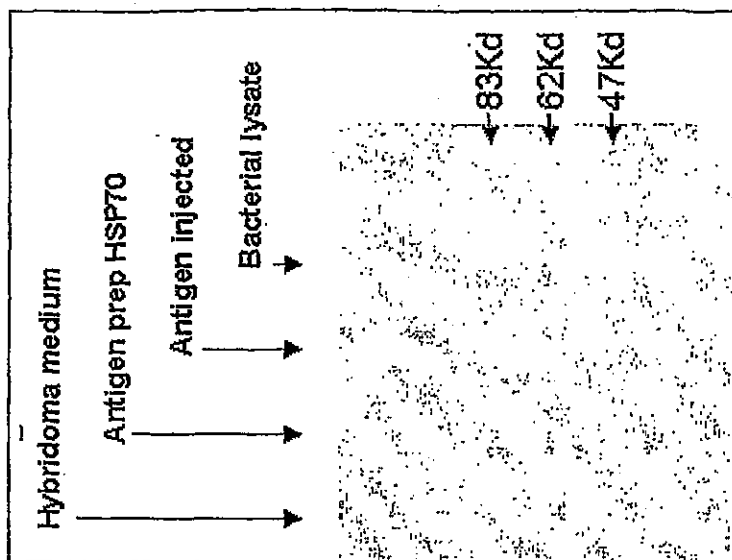
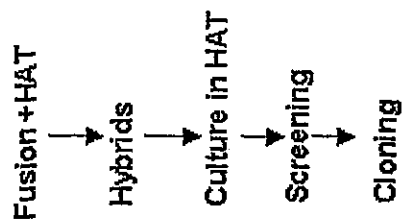


FIG. 14

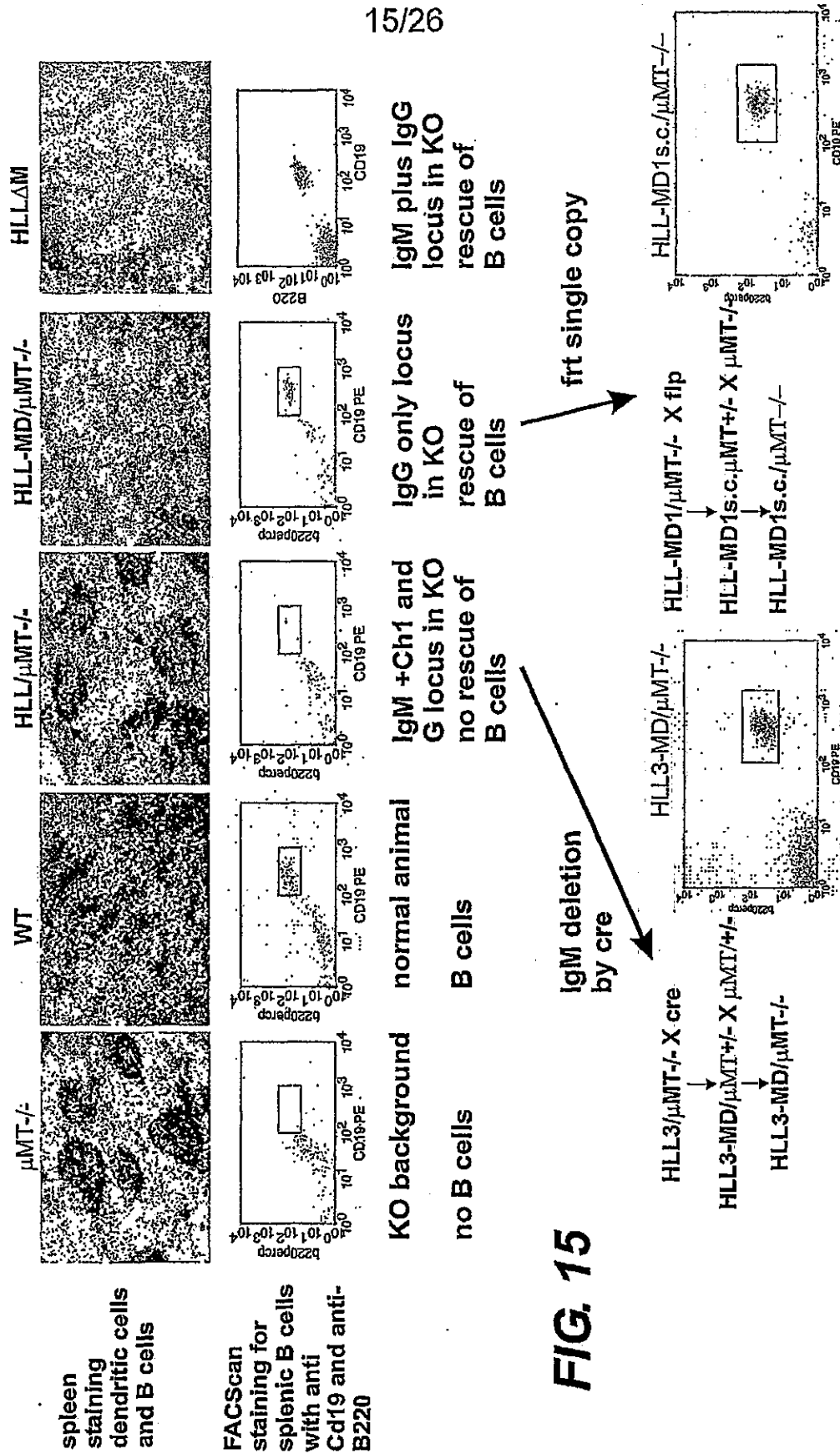
Myeloma cells Sp2-O-Ag14



G18 HCab (IgG2 class)

VHH2

RLSCAAGSIFSINAMGWSRGAPGKQRELVAATSGGSTNYADSVKGRFTISRDNKN...



ELISA of VH
against pertussis
antigen

fingerprint by
restriction digestion
of positive ELISA clones

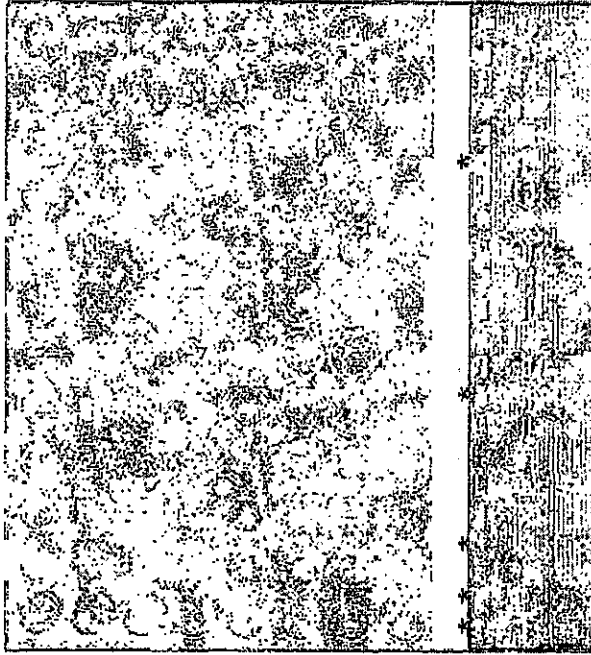


FIG. 16

CDR3

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNLSLKPEDTAVYYCNAKGPITHVRG
2 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNLSLKPEDTAVYYCNAKTPITHIRG
3 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNLSLKPEDTAVYYCNAKTPITHVRG
4 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNLSLKPEDTAVYYCNAKTPITHVRG
5 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNLSLKPEDTAVYYCNAESPITKVRG

α-B. Pertussis

CDR3

HINGE

....VHYWGQGTTLTVSSERKCCV...
....VHHWGQGTTLTVSSERKCCV...
....VHYWGQGTTLTVSSERKCCV...
....VDYWGRGTLTVSSERKCCV...
....VSYWGQGTTLTVSSERKCCV...

Sequence of clones indicated by an asterisk in the fingerprint,
note the short CDR3 region

ne VHH1 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSGTYADSVKGRFTISRDNNAKN
 Clone 1 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSGTYADSVKGRFTISRDNNAKN
 Clone 2 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSGTYADSVKGRFTISRDNNAKN
 Clone 3 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSGTYADSVKGRFTISRDNNAKN

CDR1 CDR2

ne VHH2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNNAKN
 Clone 1 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNNAKN
 Clone 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNNAKN
 Clone 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNNAKN

CDR1 CDR2

VHH2 germline VHH2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCN.....
 CDR1 CDR2 CDR3

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAKGPITHVRG
 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAKTPITHIRG
 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAKTPITVVRG
 4 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAKTPITHVRG
 5 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAESPITKVRG

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAERAGDP.....
 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCGVWFGELSD..
 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAECWGSRW..
 4 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAETSPRYFDW

α-hsp70 RLSCAASGSIFSINAMGWYRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNNAKNVYLMNSLKPEDTAVYYCNAAGNTMVRGVII

CDR3 HINGE
 1....VHYWGQGLTVTSSEKCCV...
 2....VHHWGQGLTVTSSEKCCV...
 3....VHYWGQGLTVTSSEKCCV...
 4....VDYWGQGLTVTSSEKCCV...
 5....VSYWGQGLTVTSSEKCCV...

α-B.Pertussis

1.....FDYWGQGLTVTSSEKCCV...
 2...WFDYWGQGLTVTSSEKCCV...
 3...YFDHYWGQGLTVTSSEKCCV...
 4..LPFDYWGQGLTVTSSEKCCV...

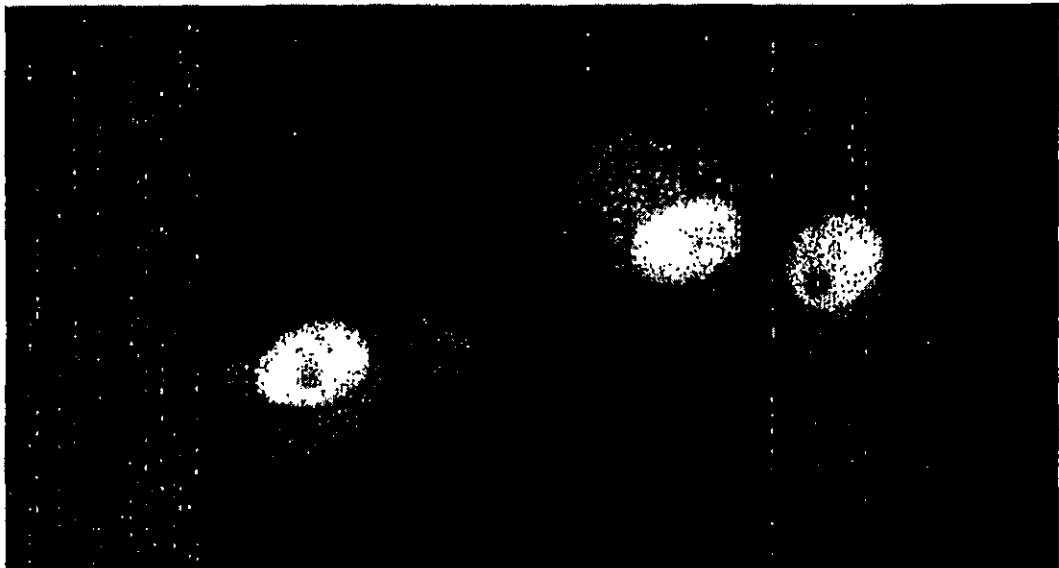
α-Tetanus

α-hsp70 KYRFDYWGQGLTVTSSEKCCV...

FIG. 17

18/26

FIG. 18



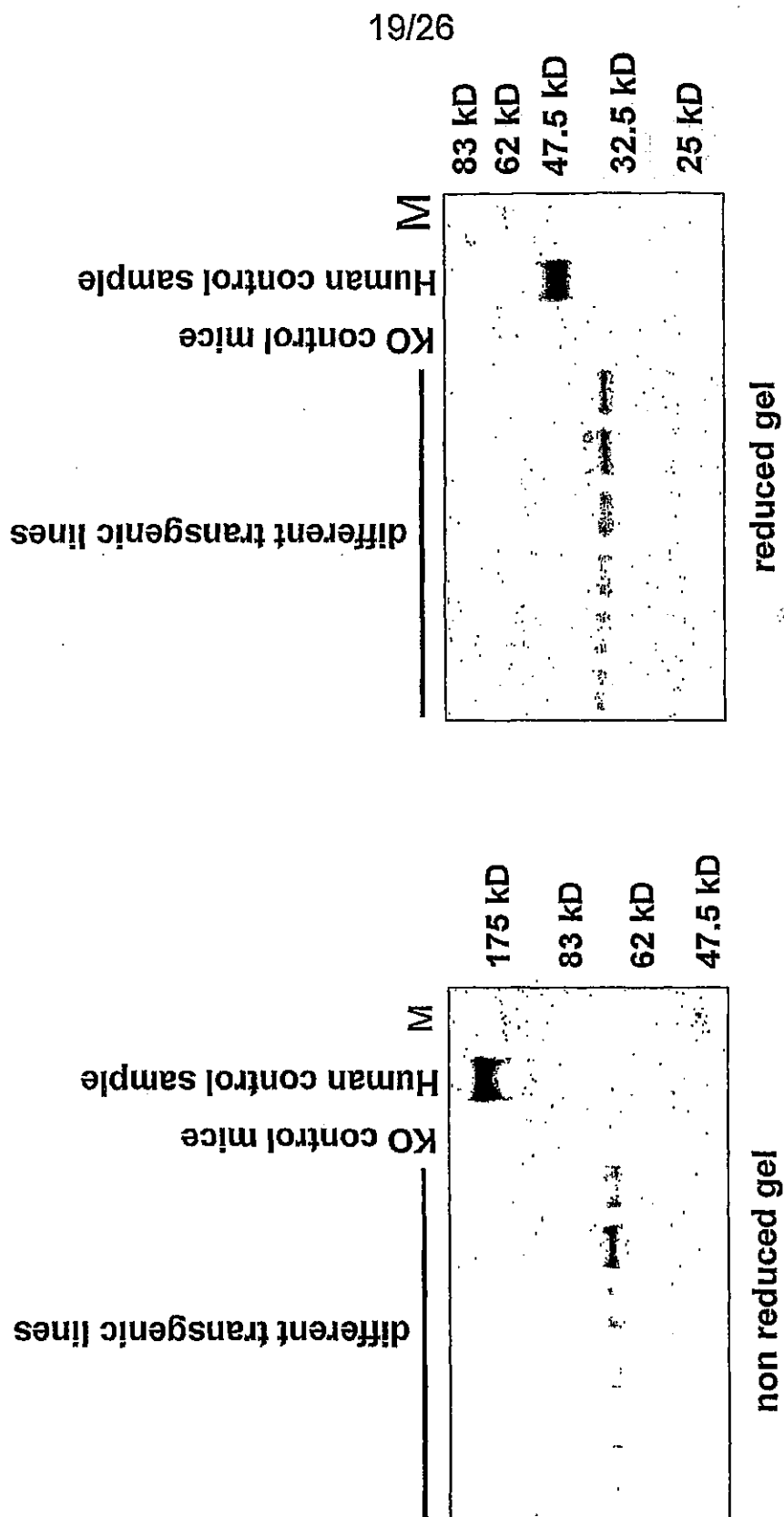


FIG. 19

FIG. 20

Size fractionation of human IgM mixed with human single chain IgM
produced by the IgM plus IgG locus mice

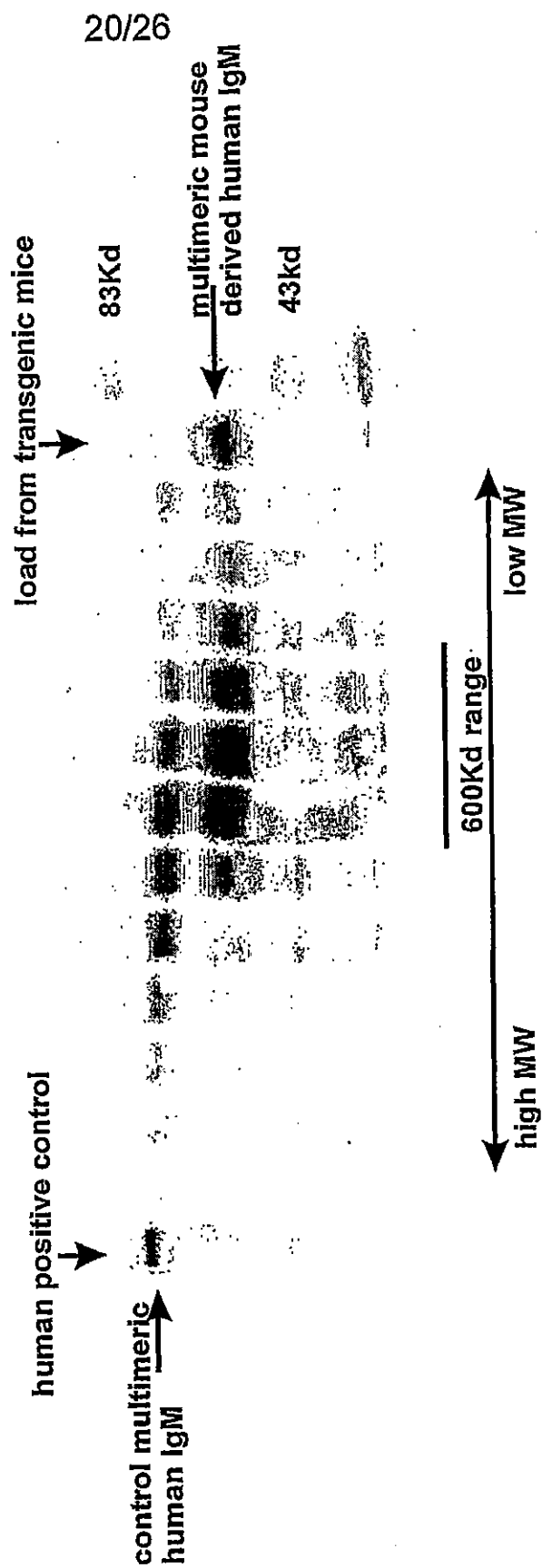


FIG. 21

ELISA of single chain IgM and IgG antibodies raised against human $\text{TNF}\alpha$

positive hybridoma serum of the immunised mouse

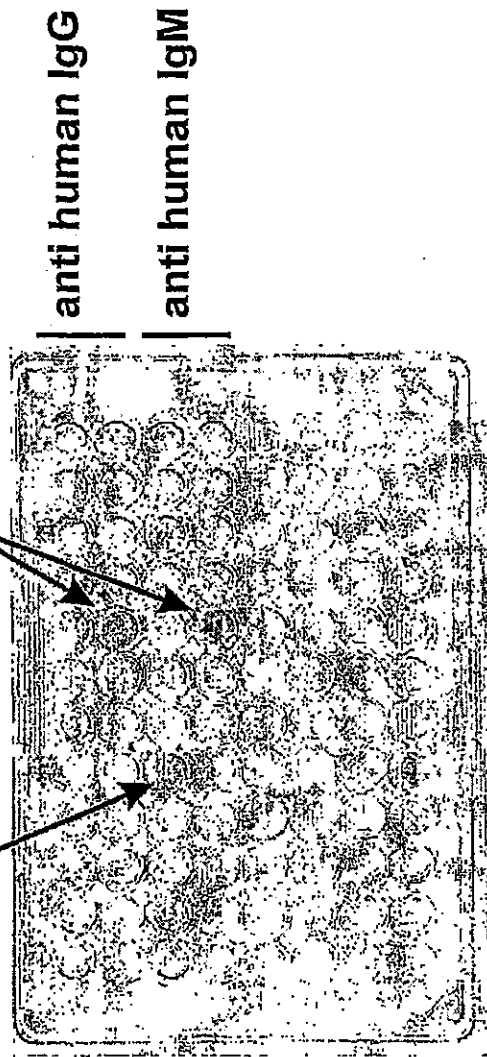
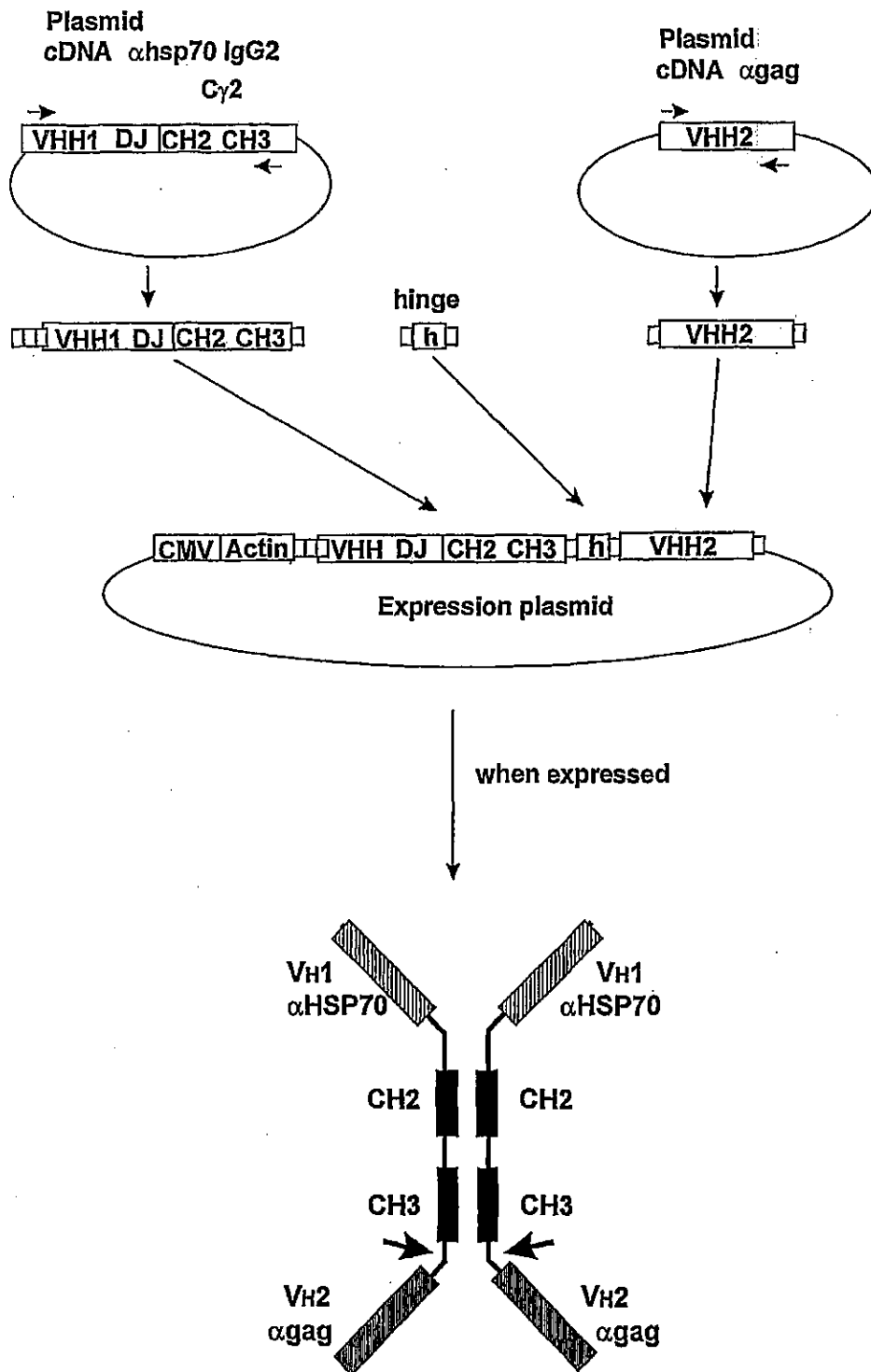
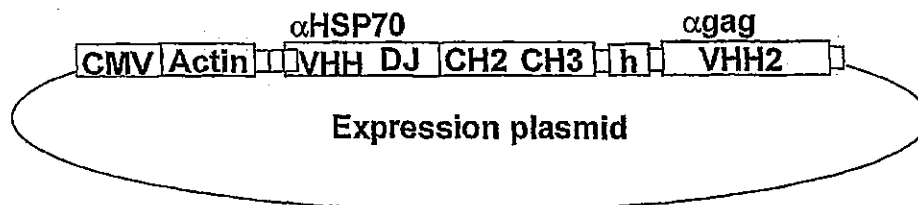


FIG. 22

Generation of the diabody expression plasmid



Expression of HClgm in CHO cells

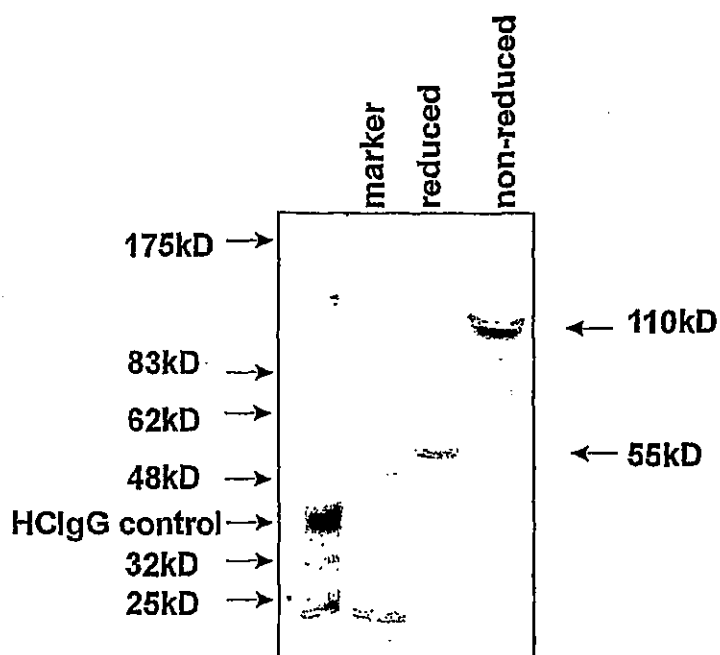


superfect into CHO cells
and select expressing cells
in hygromycin

Test individual clones by ELISA

Western blots

Western blots of clone 1



detection by α human IgG-HRP

FIG. 23

FIG. 24

Characterisation of the diabody

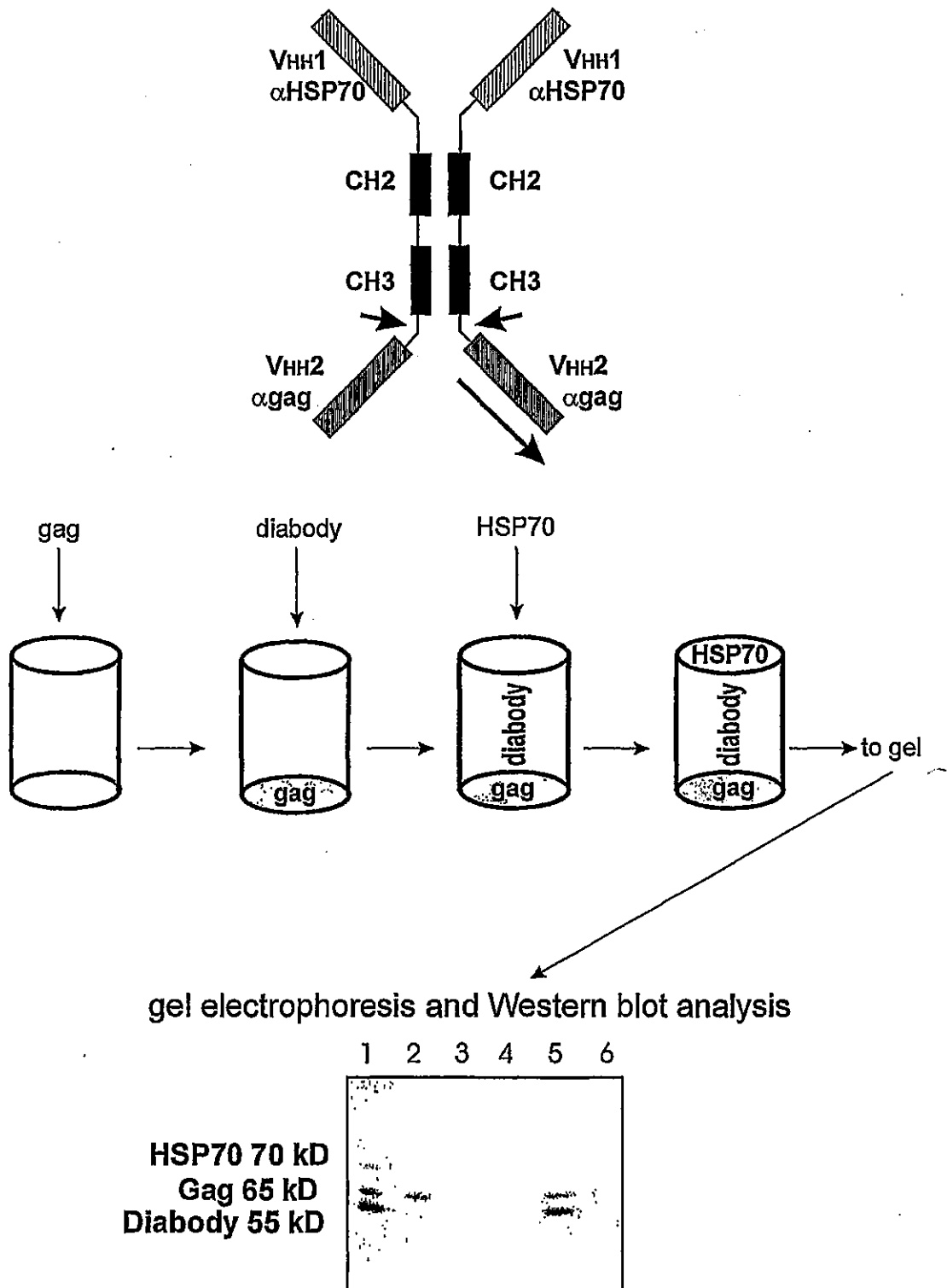
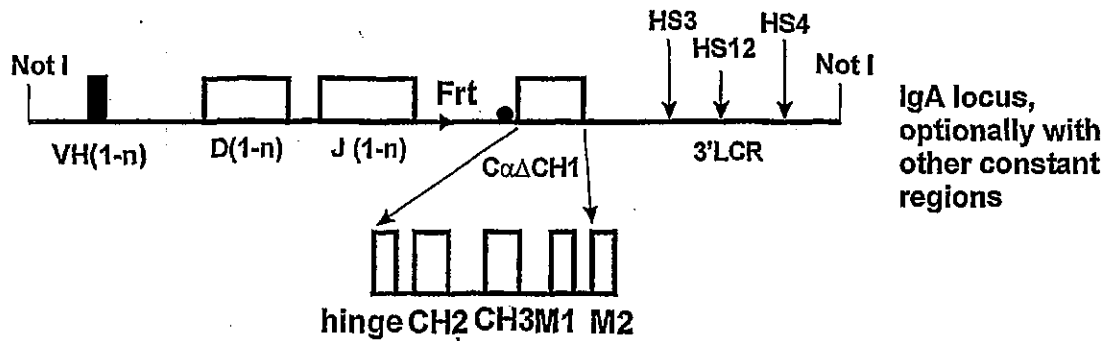


FIG. 25

Generation of heavy chain only IgA

generate transgenic mice in mice with a defective heavy chain locus (e.g. μMT)

immunize with antigens

generate hybridomas

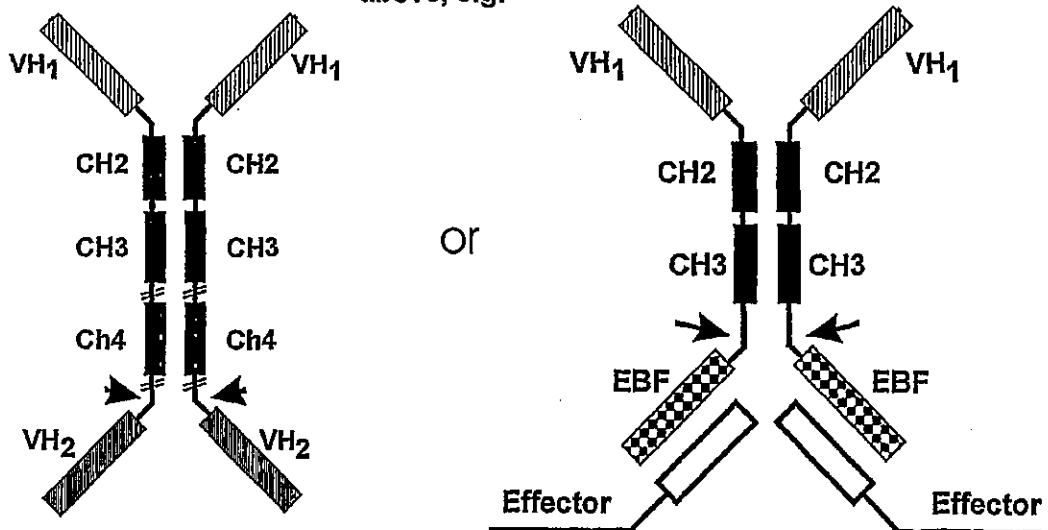
generate phage display libraries

optional

clone antibodies in expression plasmids

isolate individual clones

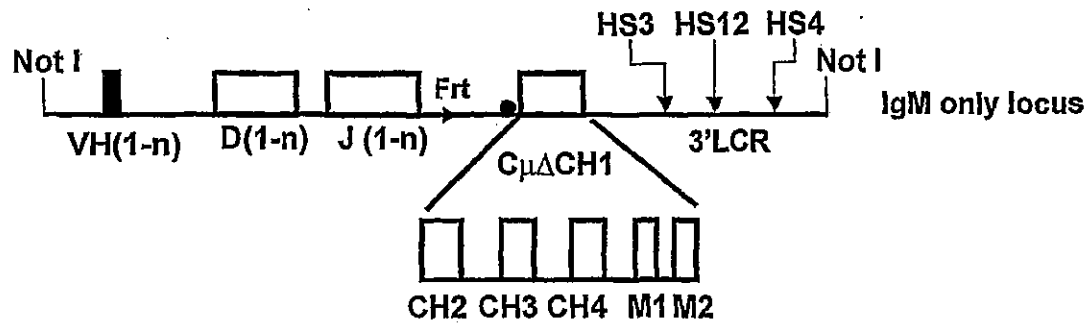
engineer by recombinant DNA technology bispecific bivalent antibodies as shown in figures above, e.g.



26/26

FIG. 26

Generation of multivalent IgM only antibodies



generate transgenic mice in mice with a defective heavy chain locus (e.g. μ MT)

immunize with antigens

generate hybridomas

generate phage display libraries

clone antibodies in plasmids

isolate individual clones

co-express different specificity IgM
e.g. different VH sol

engineer by recombinant DNA
technology bi specific bivalent
antibodies as shown in figures
above, e.g.

