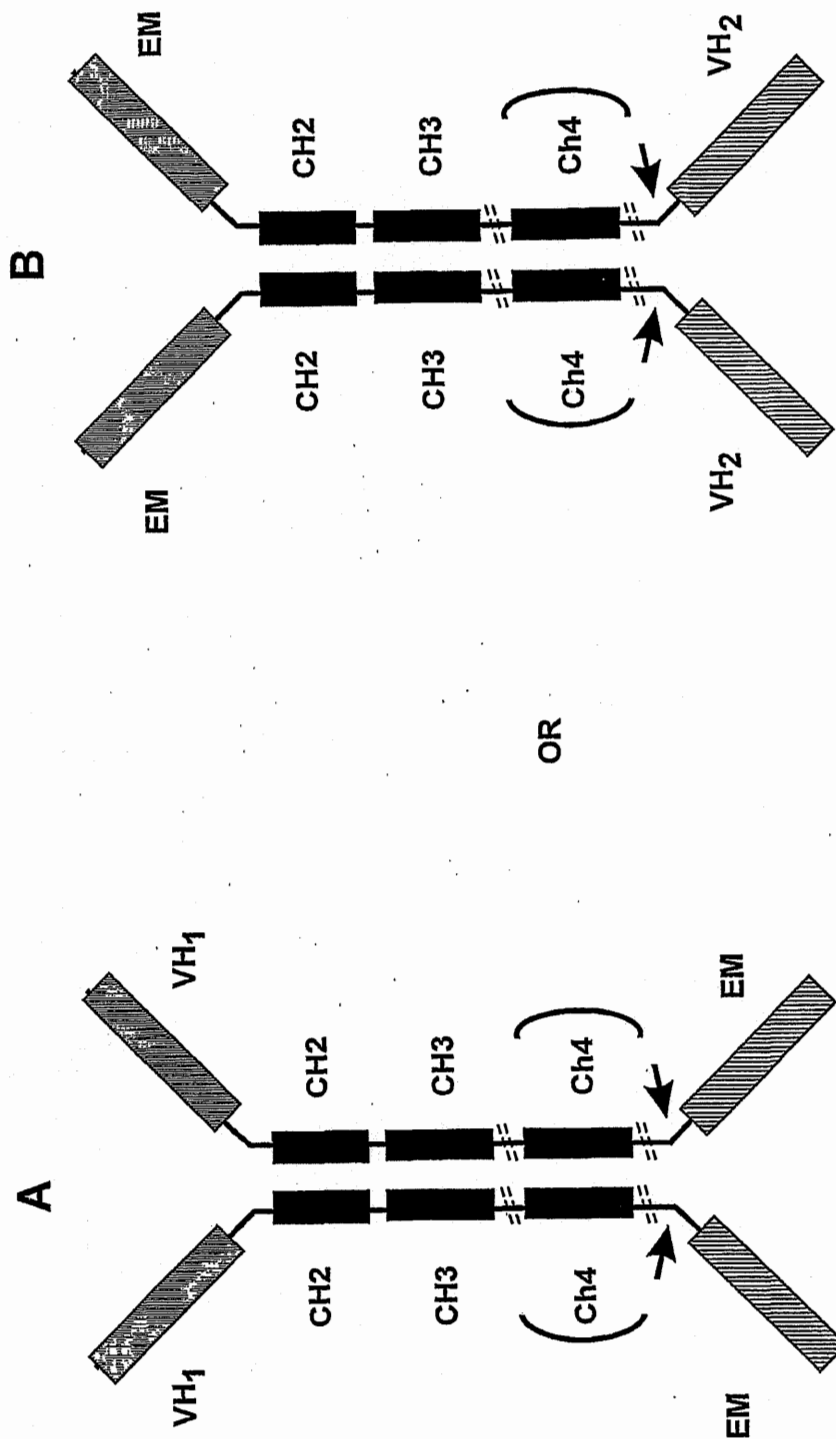


FIG. 1



Plus variations of A and B

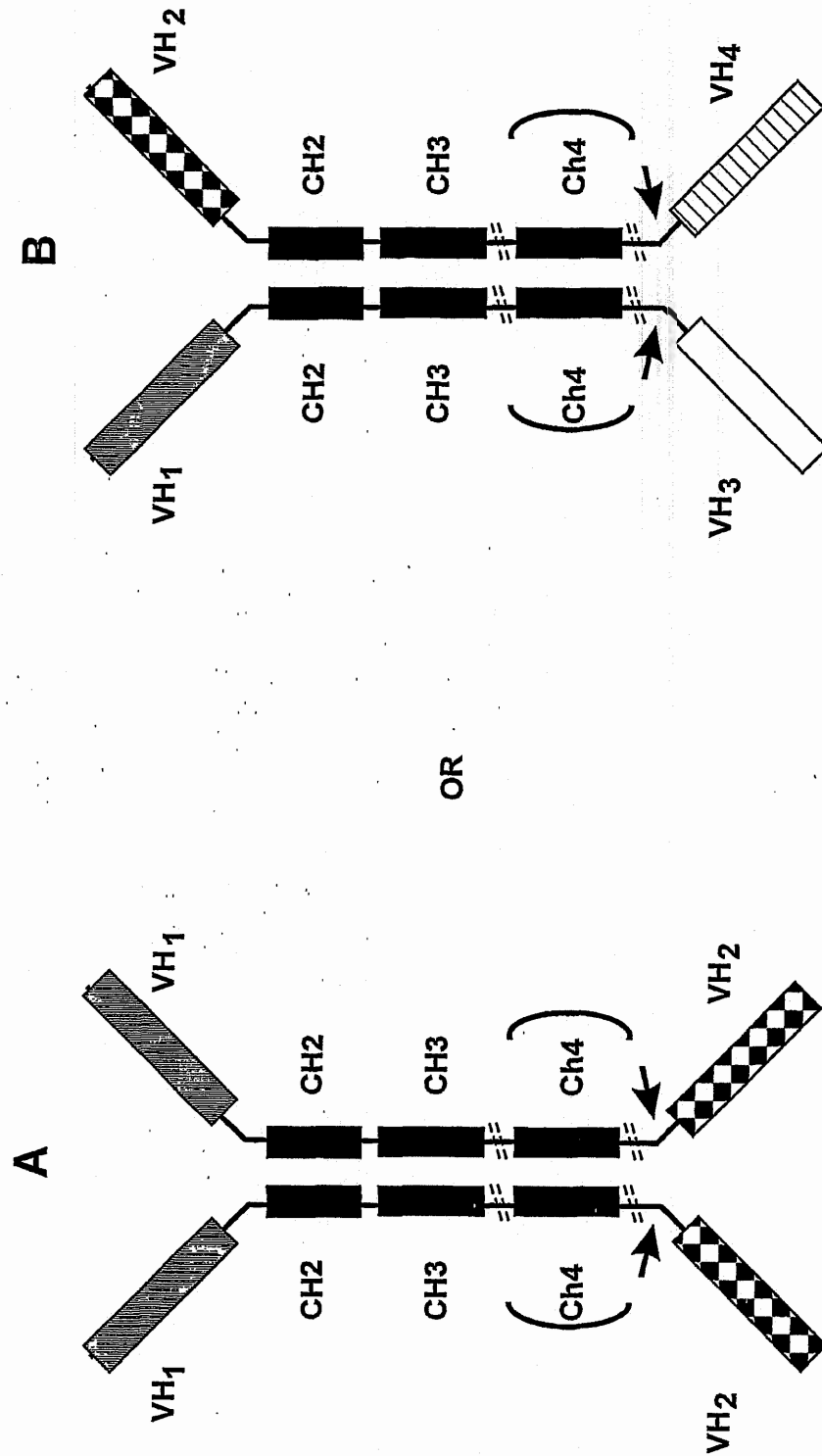
FIG. 2

FIG. 3

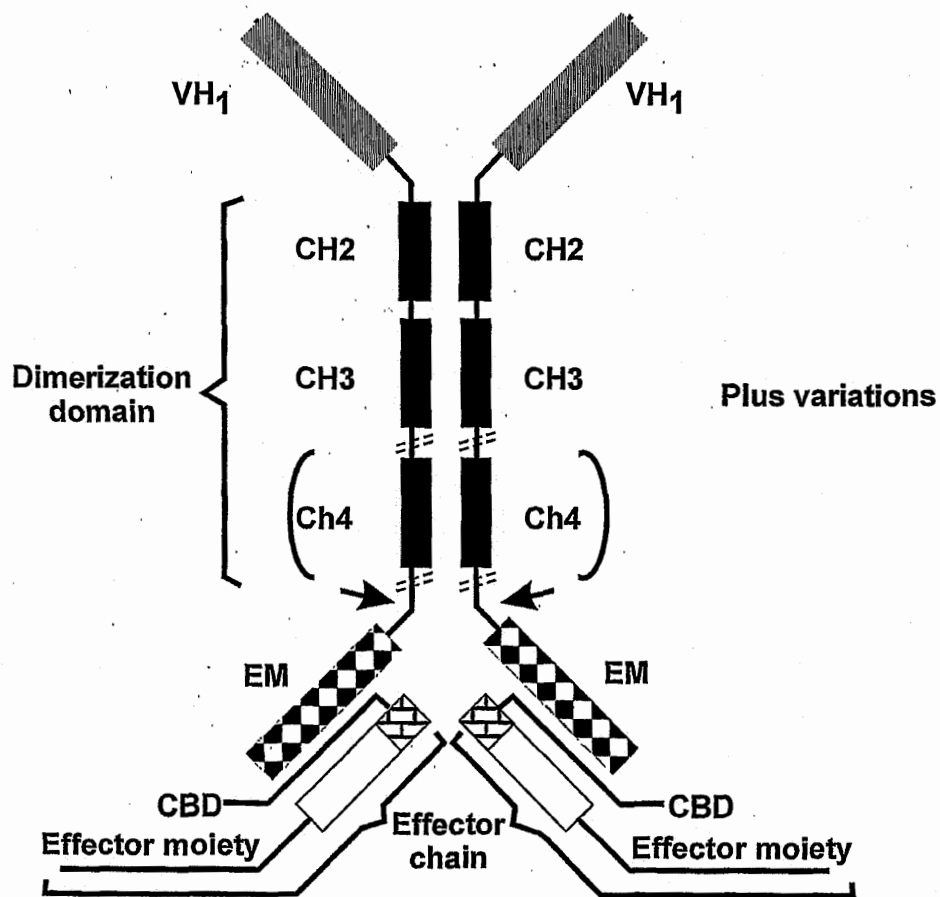
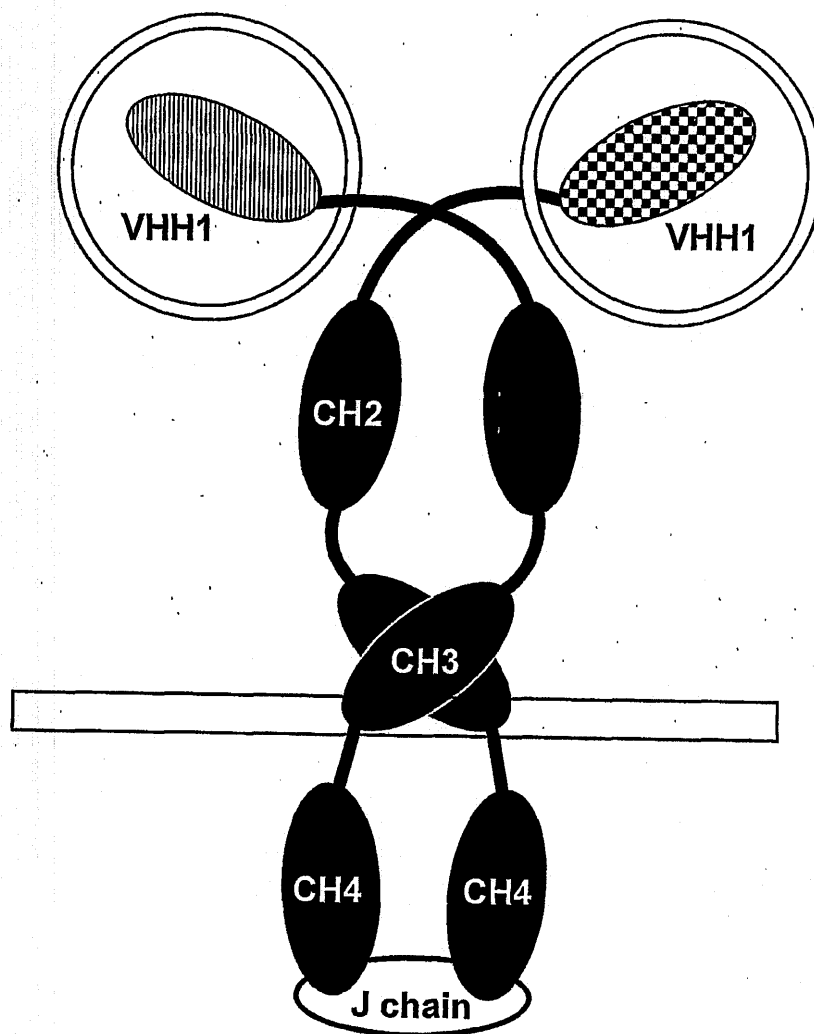


FIG. 4

Bivalent secretory IgA.



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FIG. 5
Multivalent IgM like

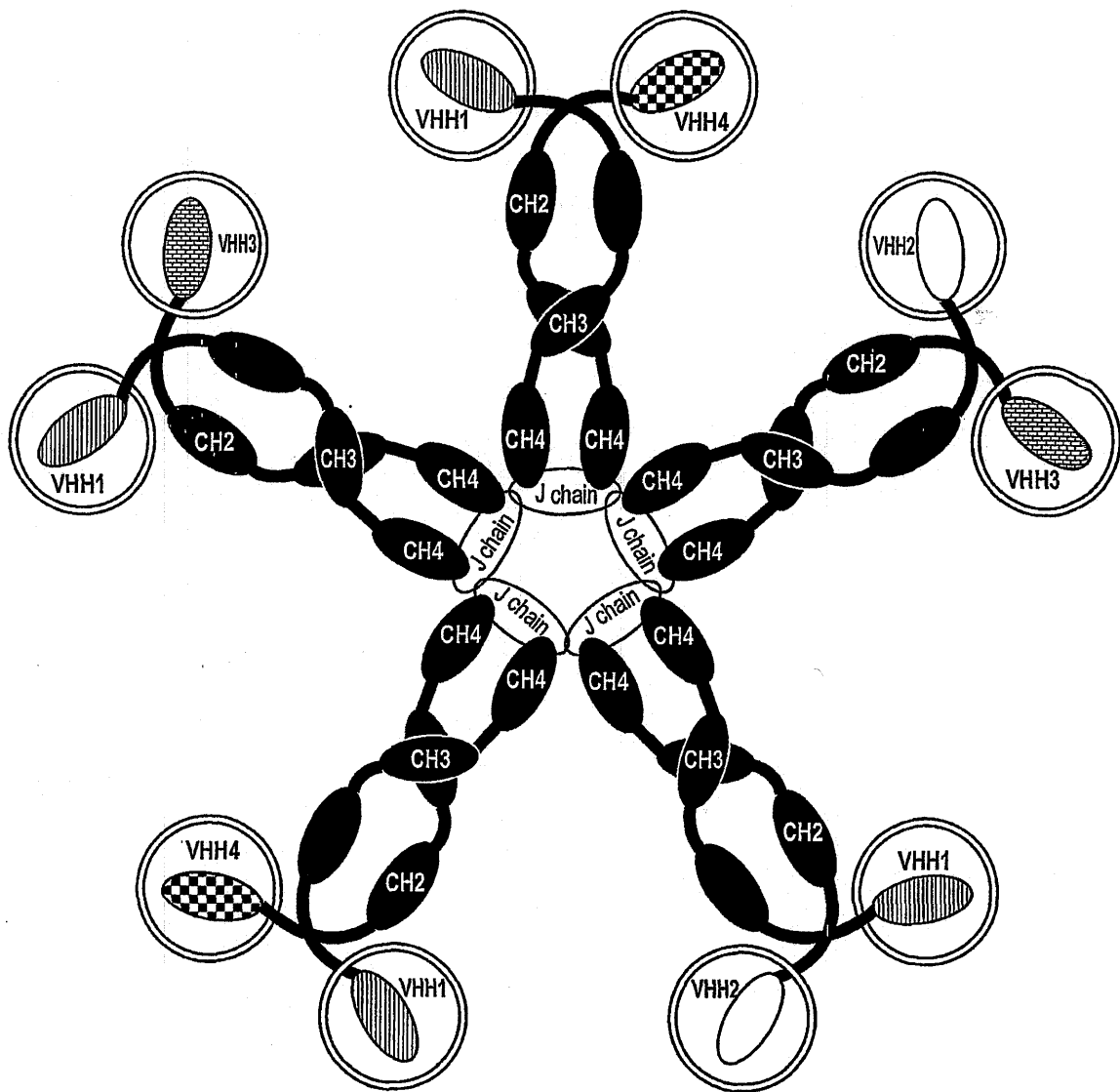


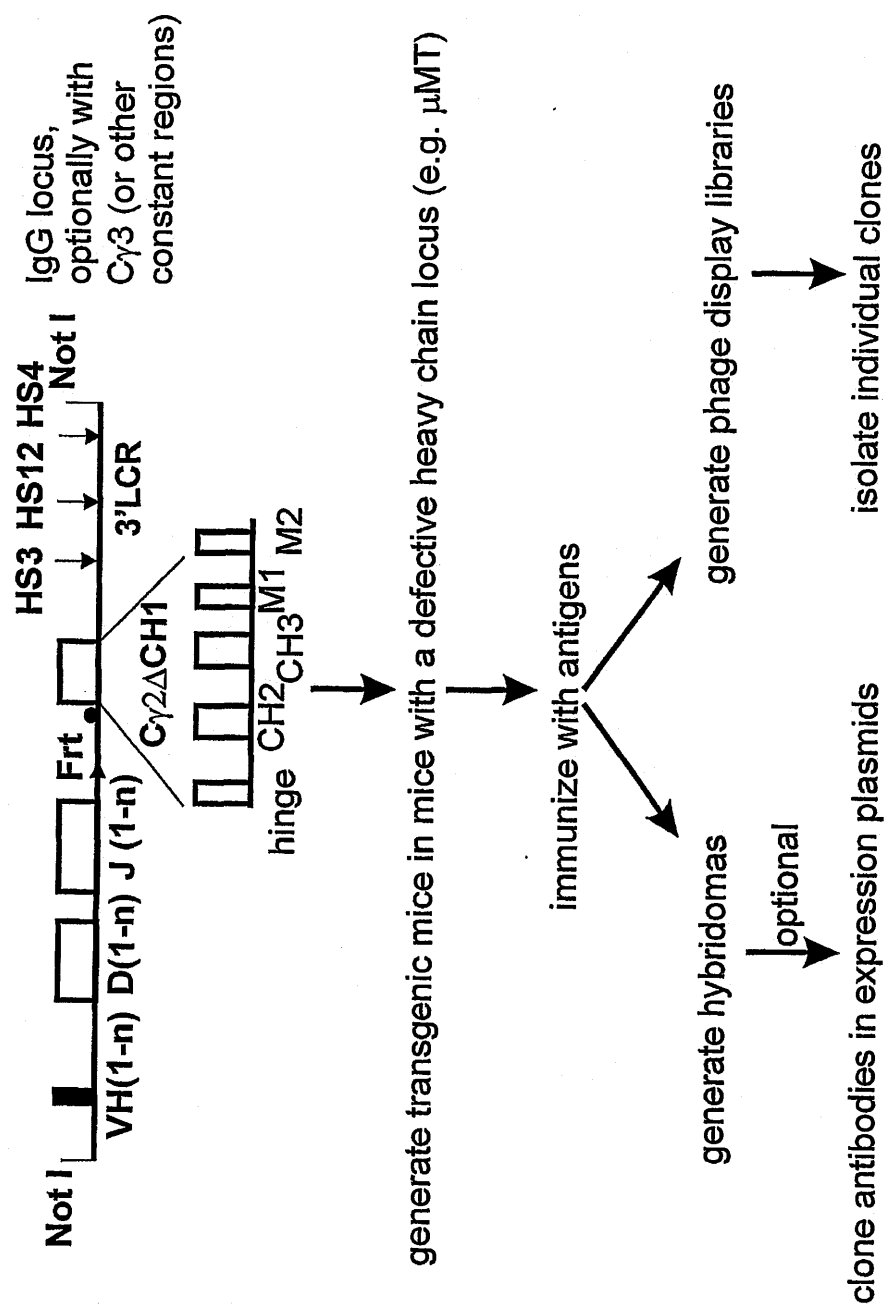
FIG. 6**Generation of heavy chain only IgG**

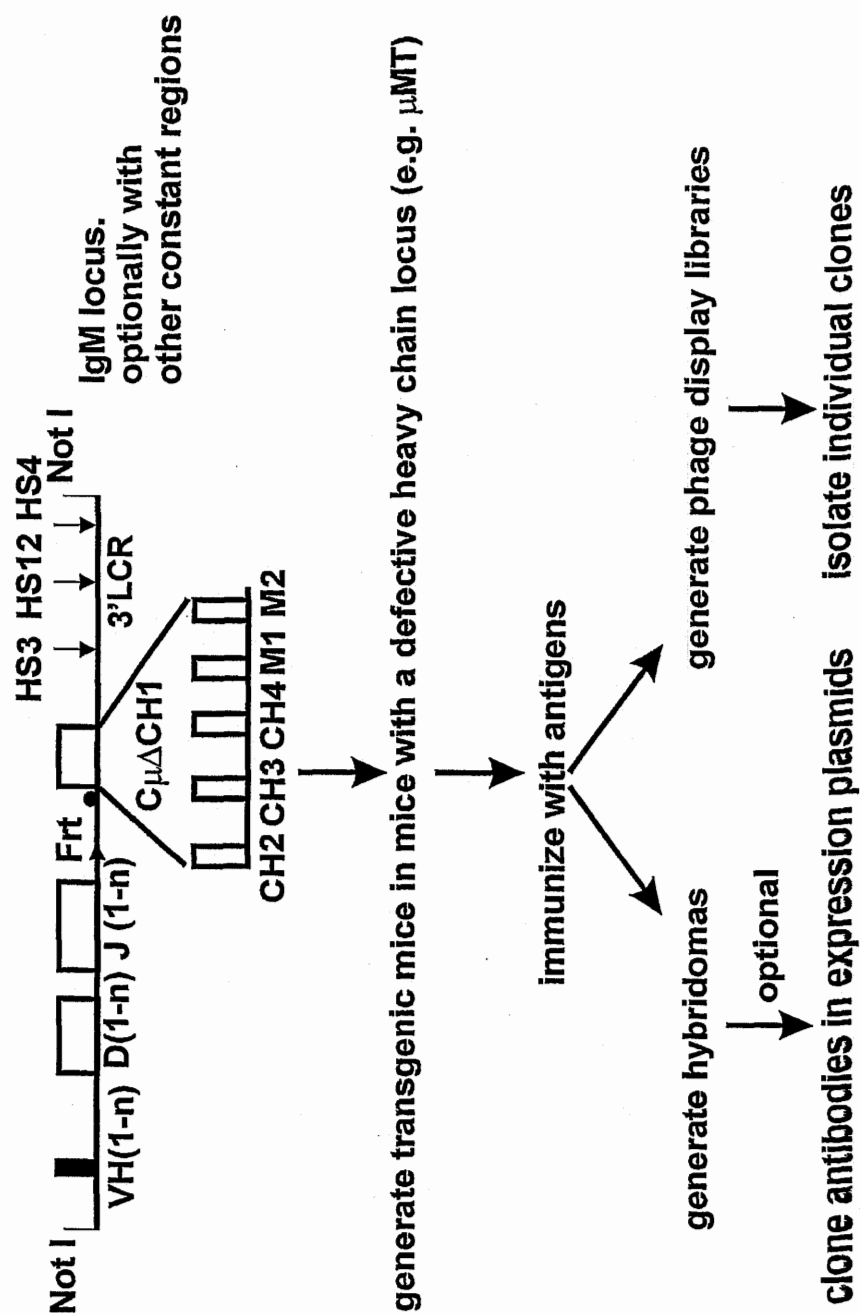
FIG. 7**Generation of heavy chain only IgM**

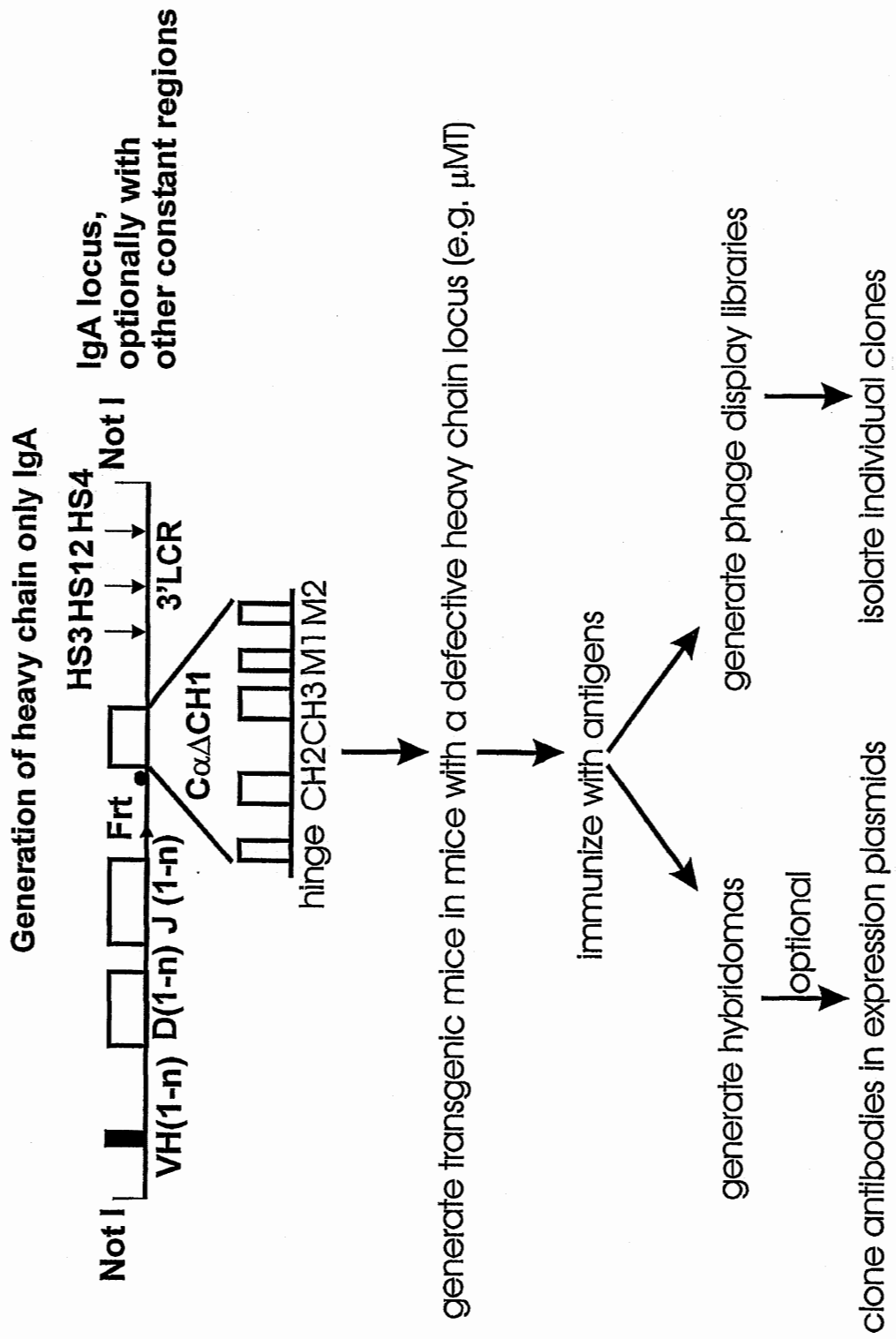
FIG. 8

FIG. 9

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	VHH	D	J
VDJg*1	GACACGGCCGTGTAGTATCTGTAAGGCAGATGG.....GGTAGTACTATGGTTCG....GGGA.		
VDJg*2	GAC.....ATTCCCACATTCGATC....T....		
VDJg*3	GACACGGCCGTCTATTACTGTAATGCCACTACG.....ATATTTTGAATGGTTAT....TATA.		
VDJg*4	GACACGGCCGTCCAATCGGATACAG.....CTATGGTTACGTACTTT		
VDJg*5	GACACGGCCGTCTATTACTGTAATGCAGATGTATTACTATGGTTCGGGGAGCCTATAGCCTTACTACTACGGTATG.		

	J	CH1
VDJg*1	GTCCACCACTGCGGCTAGAGGGGCCAGGCAACACTGGTCCGCGTGTCAATCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*2	CTGGGGCCGTGGCACCCTGGTCACCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*3	GAC.....GCTACTGGGGCCAGGCAACCTGGTCACCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*4	GACTA.....CTGGGGCCAGGCAACCTGGTCACCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*5	GACGT.....CTGGGGCCAGGCAACCTGGTCACCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	

	CH1
VDJg*1	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
VDJg*2	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
VDJg*3	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
VDJg*4	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
VDJg*5	CCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG

	CH1
VDJg*1	TGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCAGCTGTCTACAGTCTCAGGACTCTAC
VDJg*2	TGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCAGCTGTCTACAGTCTCAGGACTCTAC
VDJg*3	TGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCAGCTGTCTACAGTCTCAGGACTCTAC
VDJg*4	TGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCAGCTGTCTACAGTCTCAGGACTCTAC
VDJg*5	TGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCAGCTGTCTACAGTCTCAGGACTCTAC

	CH1
VDJg*1	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
VDJg*2	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
VDJg*3	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
VDJg*4	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCAGACCTACACCTGCAACGTAGATCACAAGCCCAG
VDJg*5	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCAGACCTACACCTGCAACGTAGATCACAAGCCCAG

	CH1	Hinge
VDJg*1	CAACACCAAGAGCGCAAATGTTGTGTCGAG	
VDJg*2	CAACACCAAGAGCGCAAATGTTGTGTCGAG	
VDJg*3	CAACACCAAGAGCGCAAATGTTGTGTCGAG	
VDJg*4	CAACACCAAGAGCGCAAATGTTGTGTCGAG	
VDJg*5	CAACACCAAGAGCGCAAATGTTGTGTCGAG	

◆ GGTGGACAAGACAGTT

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FIG. 10

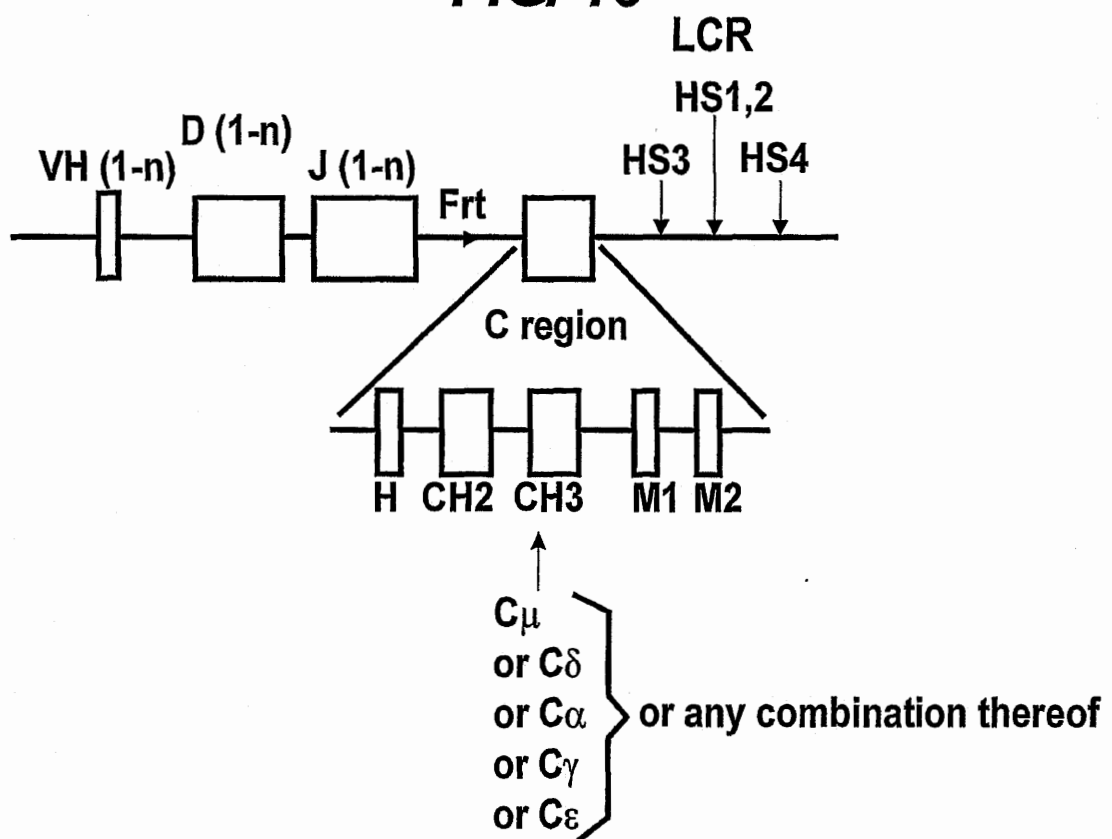
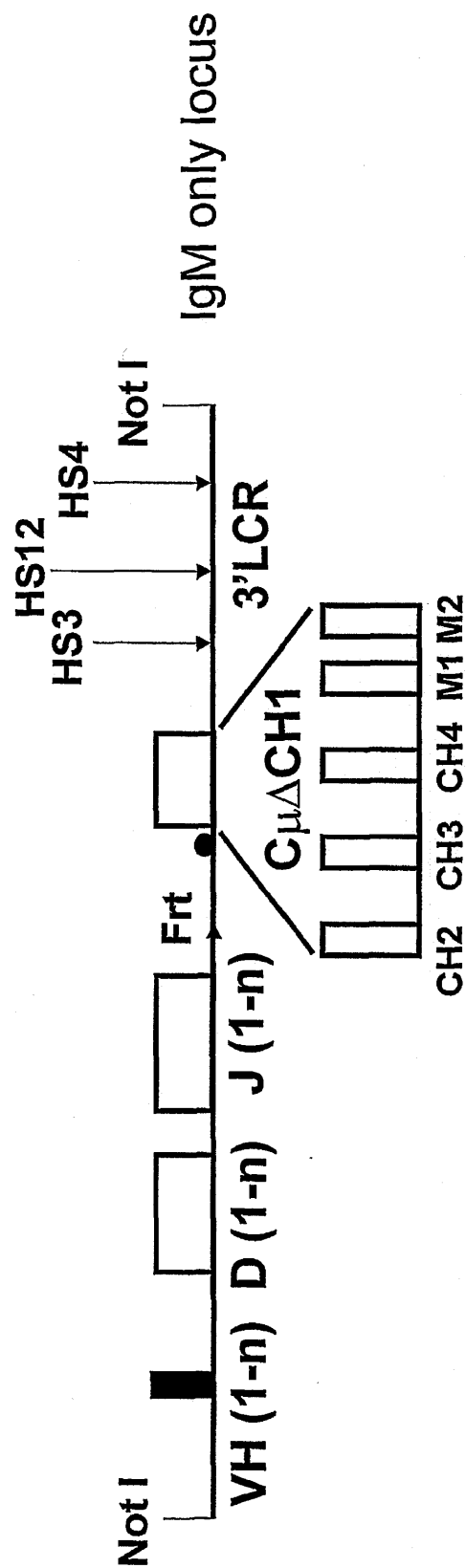


FIG. 11





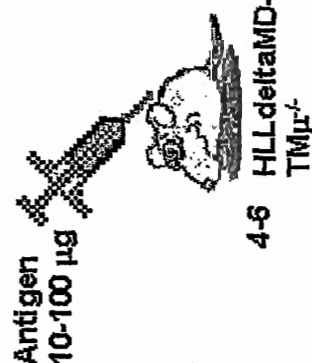
IgM plus IgG locus, C_δ is optional

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



Spleen cells

Myeloma cells Sp2-0-Ag14

Fusion + HAT

Hybrids

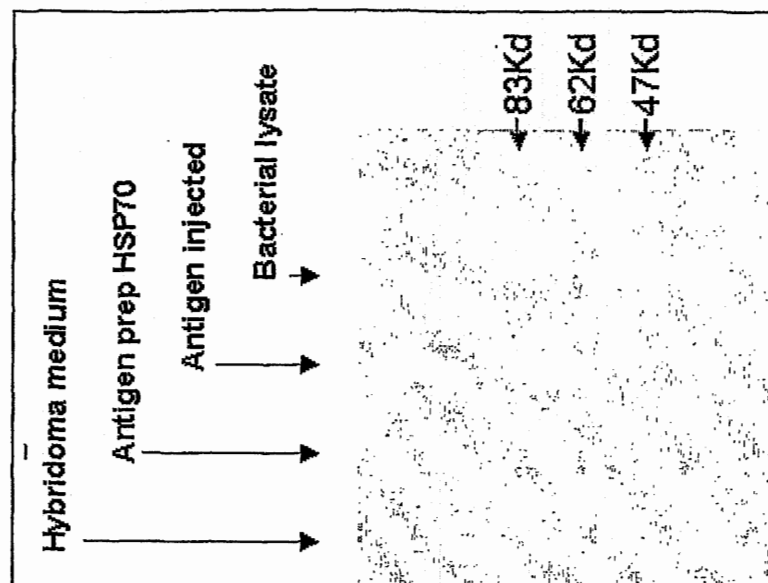
Culture in HAT

Screening

Cloning

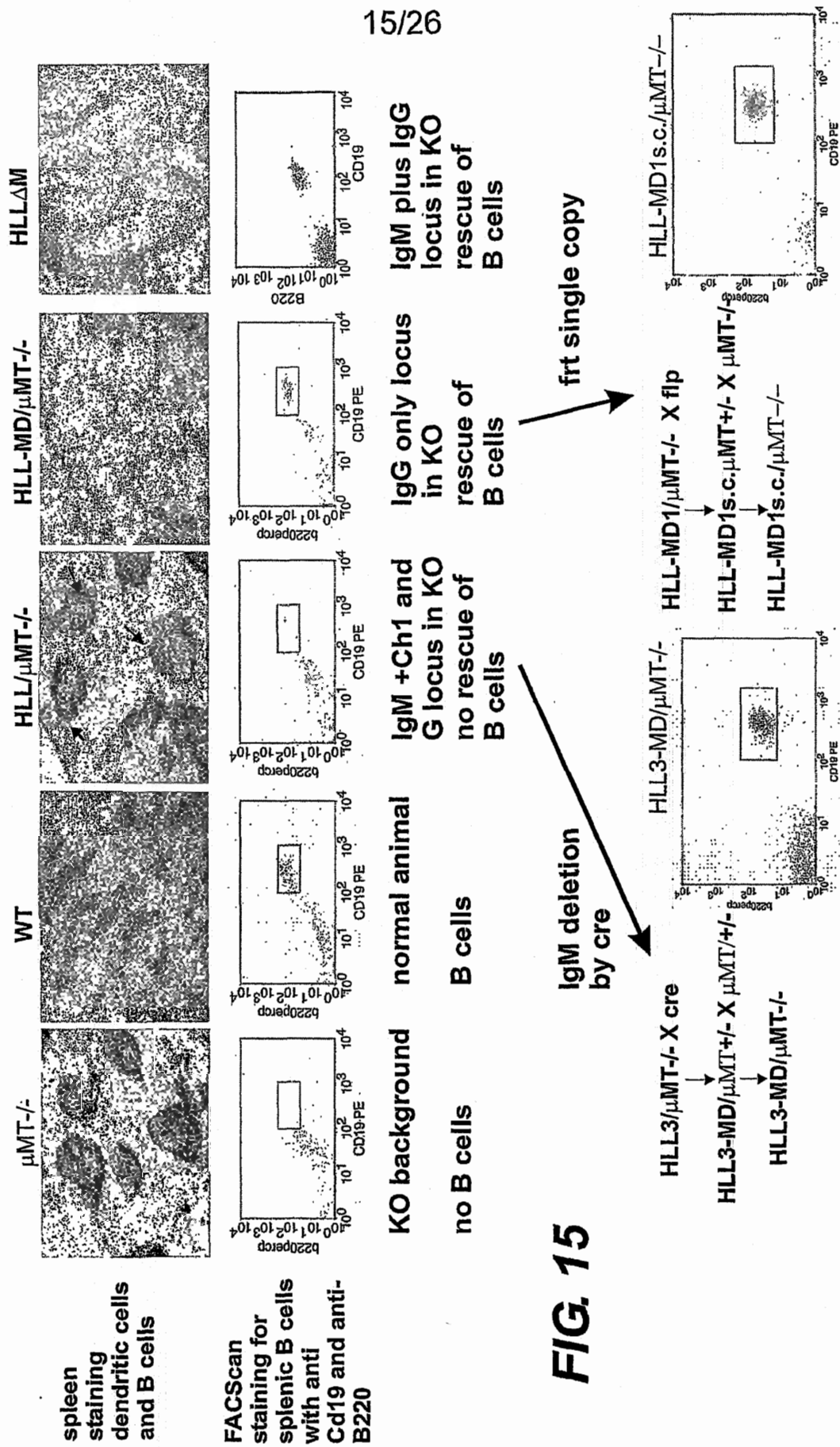


FIG. 14



G18 HCab (IgG2 class)
VH2

RLSCAASGSIFSNAWGWSRQAPGKQRELVAITSGGSTNYADSVKGRFTISRDNAKN...

**FIG. 15**

ELISA of VH
against pertussis
antigen

fingerprint by
restriction digestion
of positive ELISA clones



FIG. 16

α-B. Pertussis

CDR3

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKGPITHVRG
2 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITHIRG
3 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAARTPTVVRG
4 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISFDKAKNTVYLQMNSLKPEDSAVYYCNAKRTGPITHVRG
5 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNDADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAESPITKVRG

CDR3

HINGE

....VHYWGQGTTLTVSSERKCCV...
....VHHWGQGTTLTVSSERKCCV...
....VHYWGQGTTLTVSSERKCCV...
....VDYWGRTTLTVSSERKCCV...
....VSYWGQGTTLTVSSERKCCV...

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

ne VHH1 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKN
 Clone 1 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKN
 Clone 2 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKN
 Clone 3 RLSCAASGFTLDYYAIGWFRQAEGKEREGVSCISSSDGSTYYADSVKGRFTISRDNAKN

CDR1 CDR2

ne VHH2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKN
 Clone 1 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKN
 Clone 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKN
 Clone 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKN

CDR1 CDR2

CDR1

CDR2

VHH2 germline VHH2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCN.....

CDR3

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKGPITHVRG
 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITHIRG
 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITVVRG
 4 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITHVRG
 5 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAESPITKVRG

α-B.*Pertussis*

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAERAGDP.....
 2 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCGVLWFGELSD..
 3 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITVVRG..
 4 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITHVRG

α-Tetanus

α-hsp70 RLSCAASGSIFSINAMGWYRQAPGKQRELVA ITSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAKTPITVVRGVII

CDR3

HINGE

1....VHYWGQGLTVTVSSERKCCV...
 2....VHHWGQGLTVTVSSERKCCV...
 3....VHYWGQGLTVTVSSERKCCV...
 4....VDYWGQGLTVTVSSERKCCV...
 5....VSYWGQGLTVTVSSERKCCV...

α-B.*Pertussis*

1....FDYWGQGLTVTVSSERKCCV...
 2....WFDYWGQGLTVTVSSERKCCV...
 3....YFDHYWGQGLTVTVSSERKCCV...
 4....LPFDYWGQGLTVTVSSERKCCV...

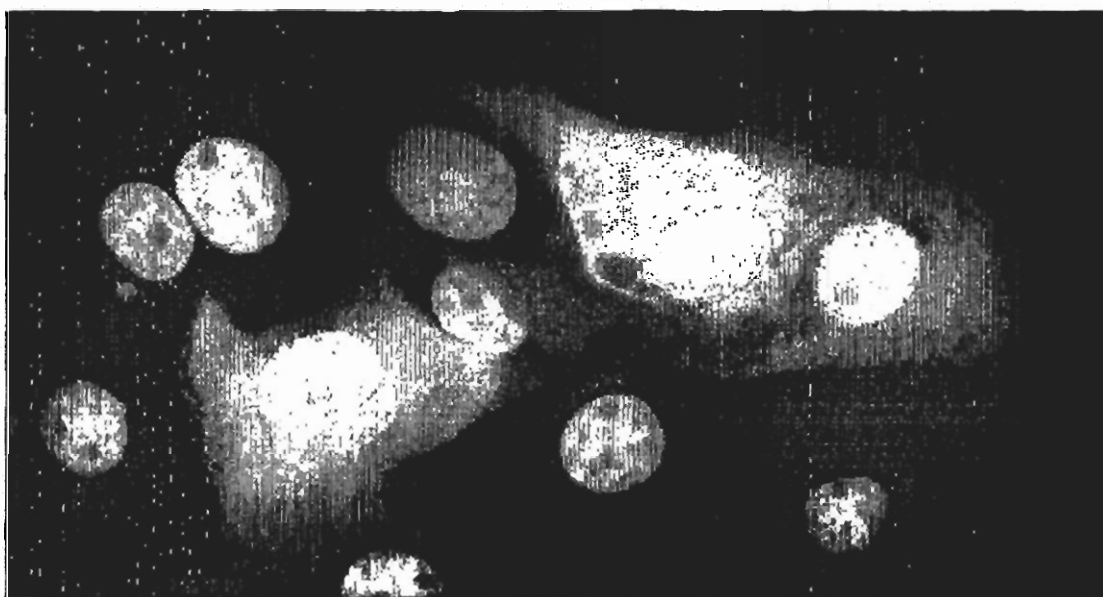
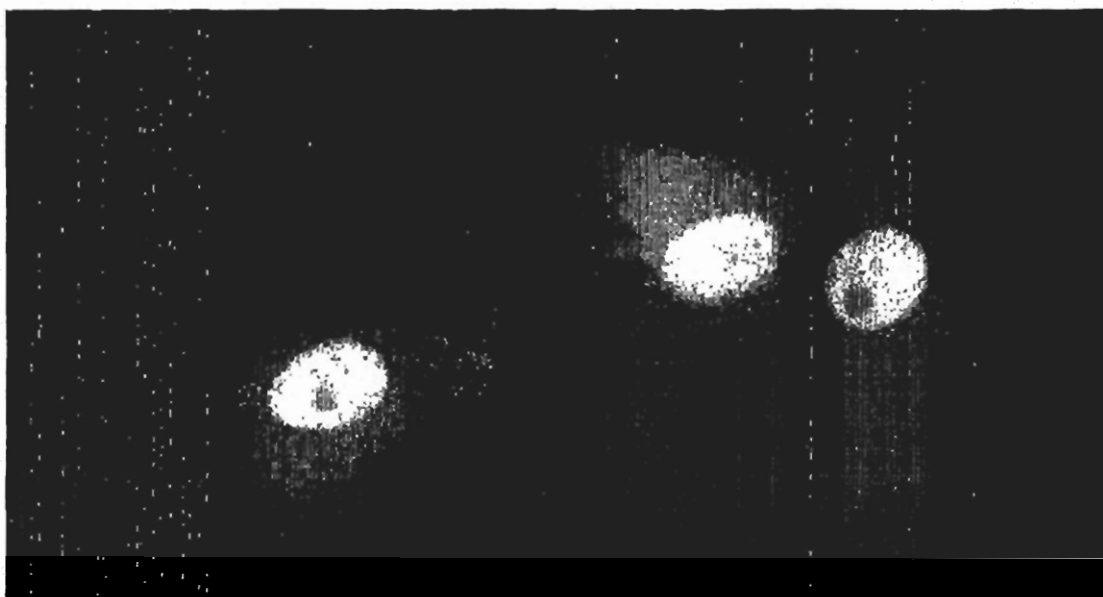
α-Tetanus

α-hsp70 KYRFDYWGQGLTVTVSSERKCCV...

FIG. 17

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FIG. 18



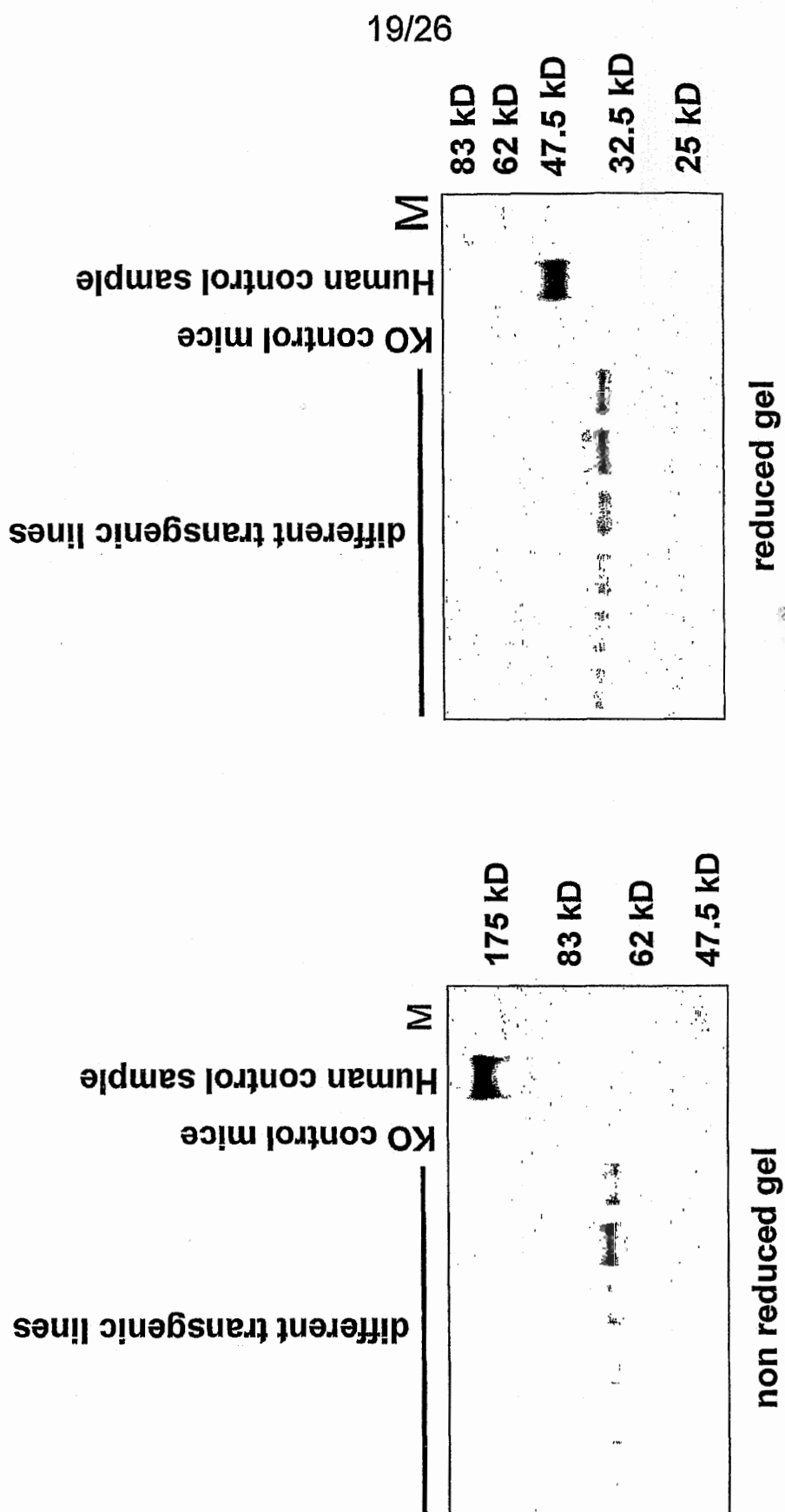
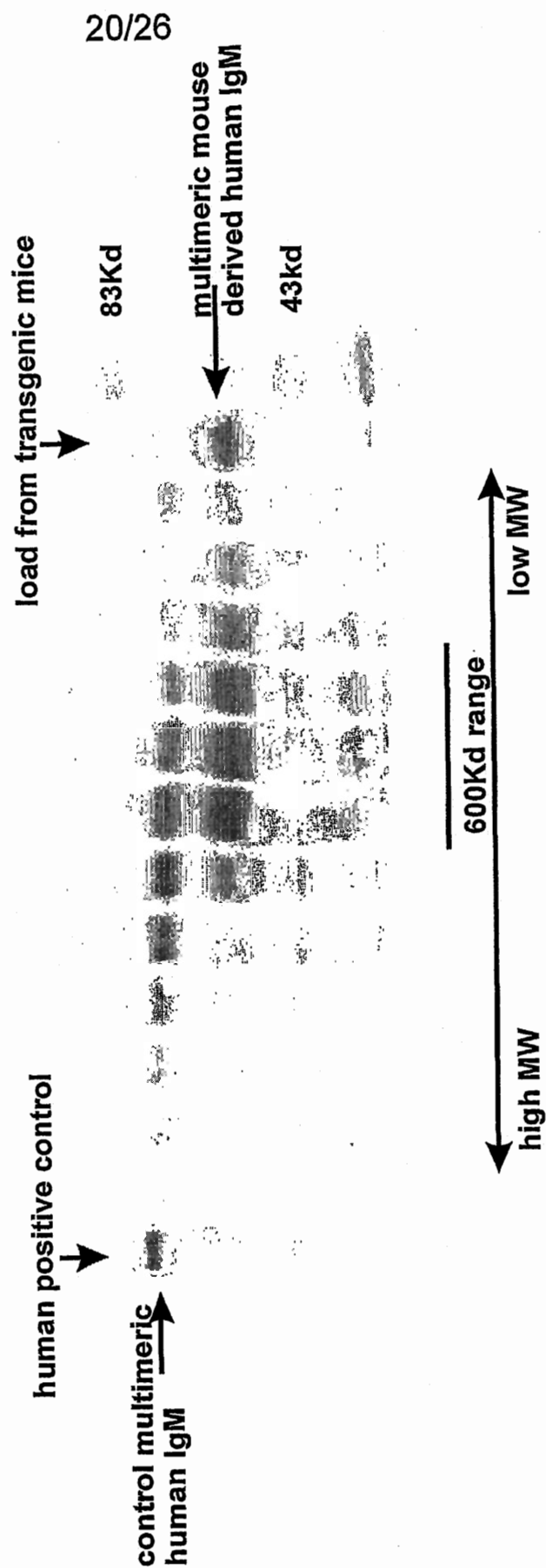


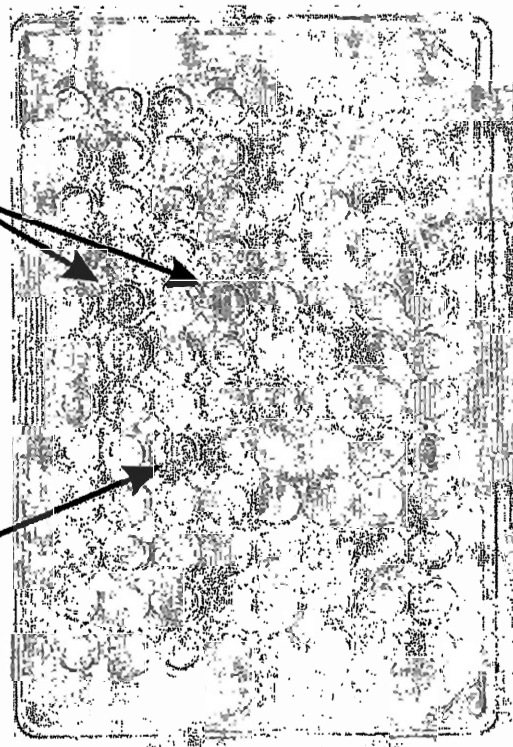
FIG. 19

FIG. 20

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



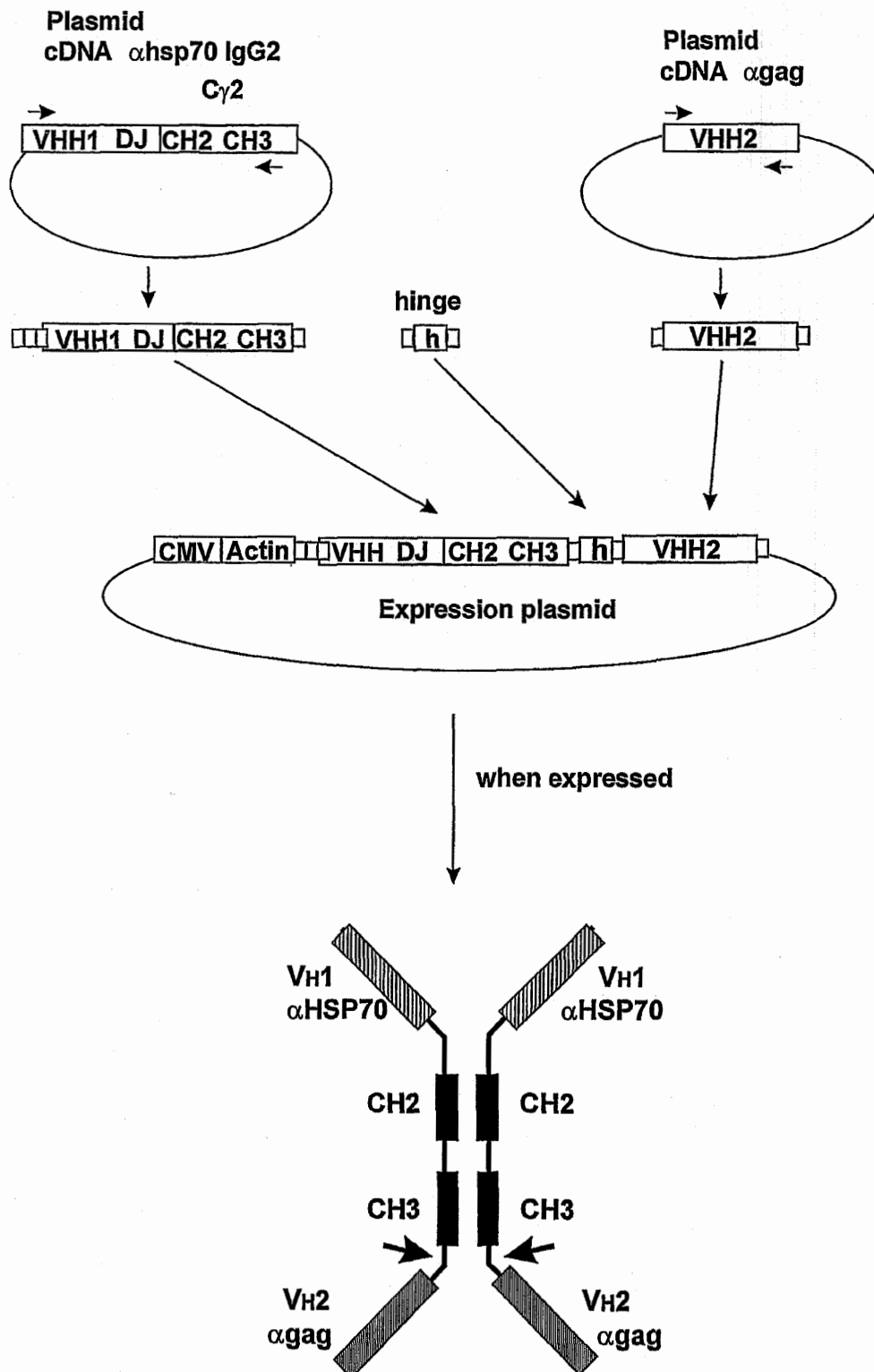
ELISA of single chain IgM and IgG antibodies raised against human TNF α



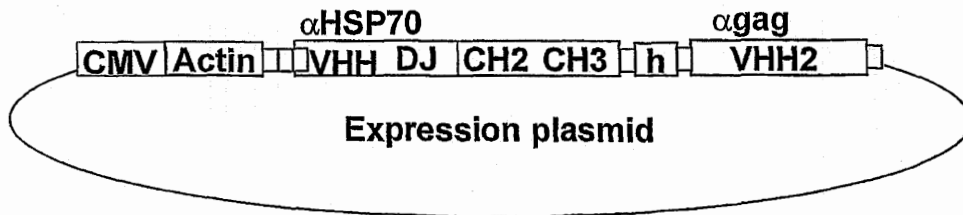
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FIG. 22

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

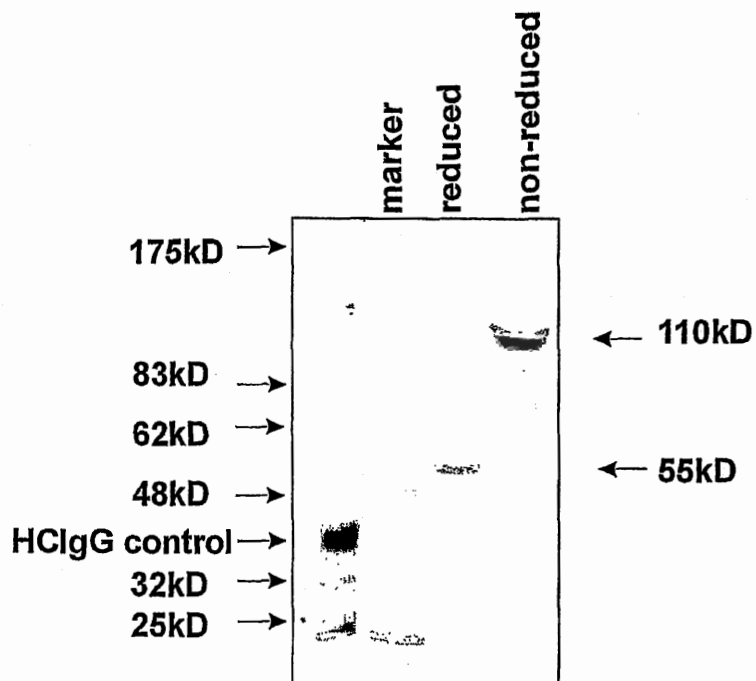
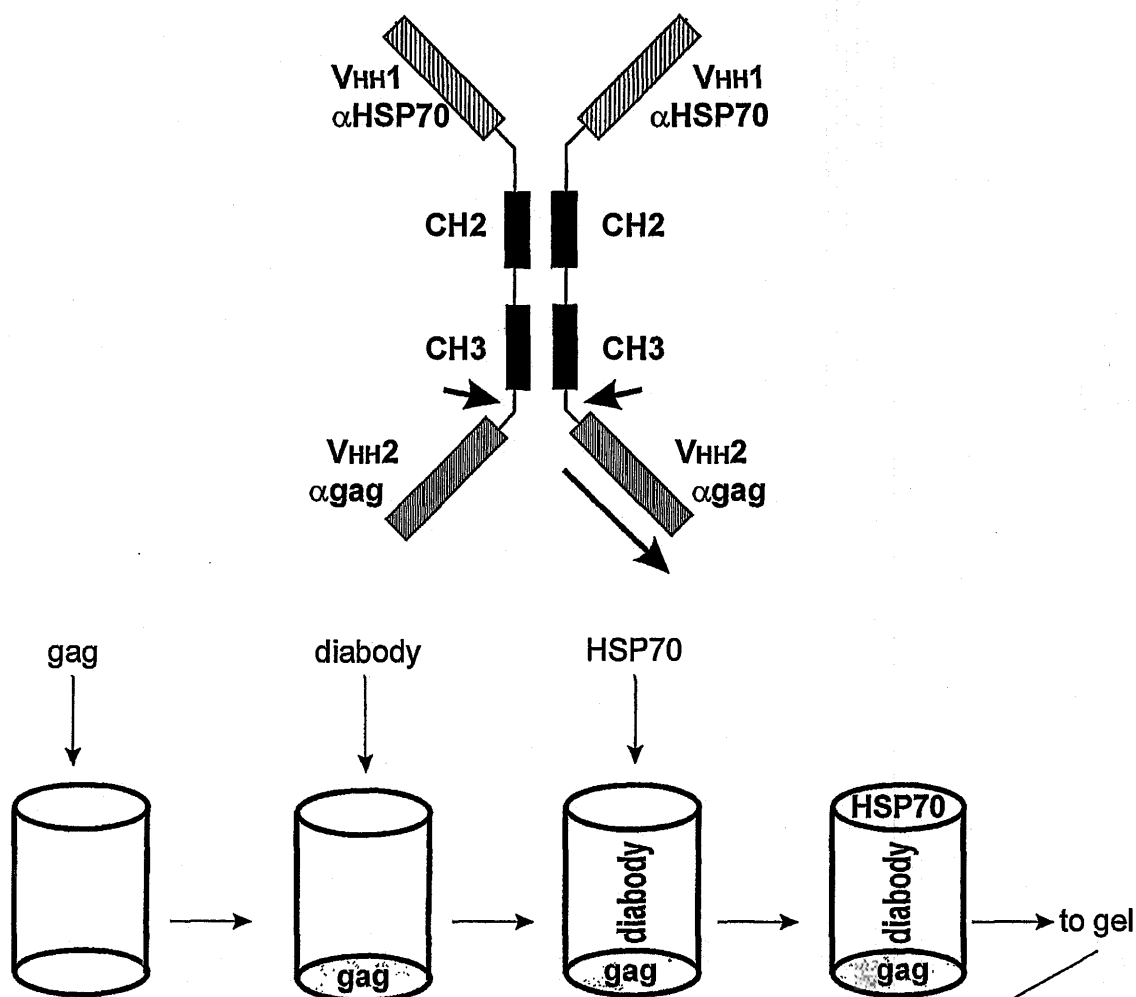


FIG. 23

detection by α human IgG-HRP

FIG. 24

Characterisation of the diabody



gel electrophoresis and Western blot analysis

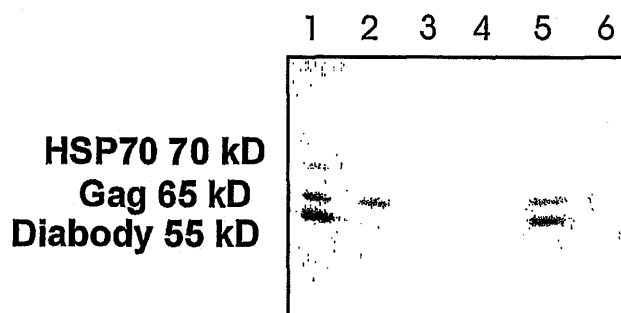
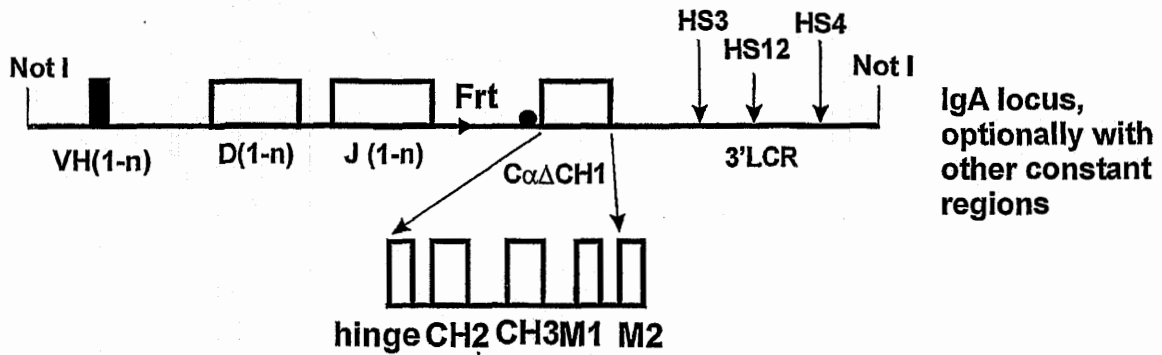


FIG. 25**Generation of heavy chain only IgA**

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

immunize with antigens

generate hybridomas

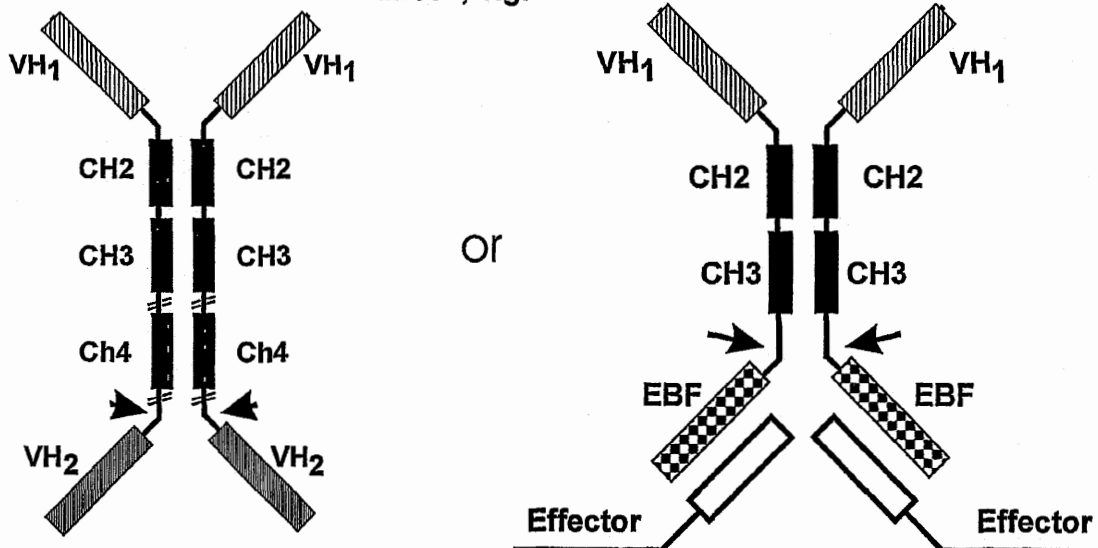
generate phage display libraries

optional

clone antibodies in expression plasmids

isolate individual clones

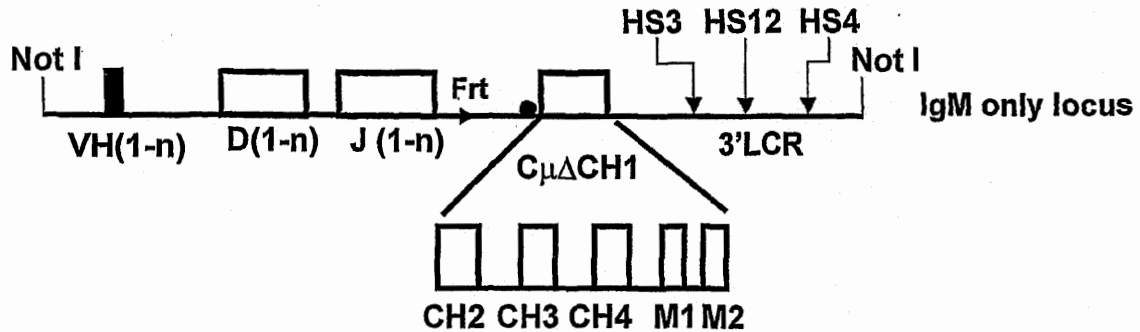
engineer by recombinant DNA technology bispecific bivalent antibodies as shown in figures above, e.g.



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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.

