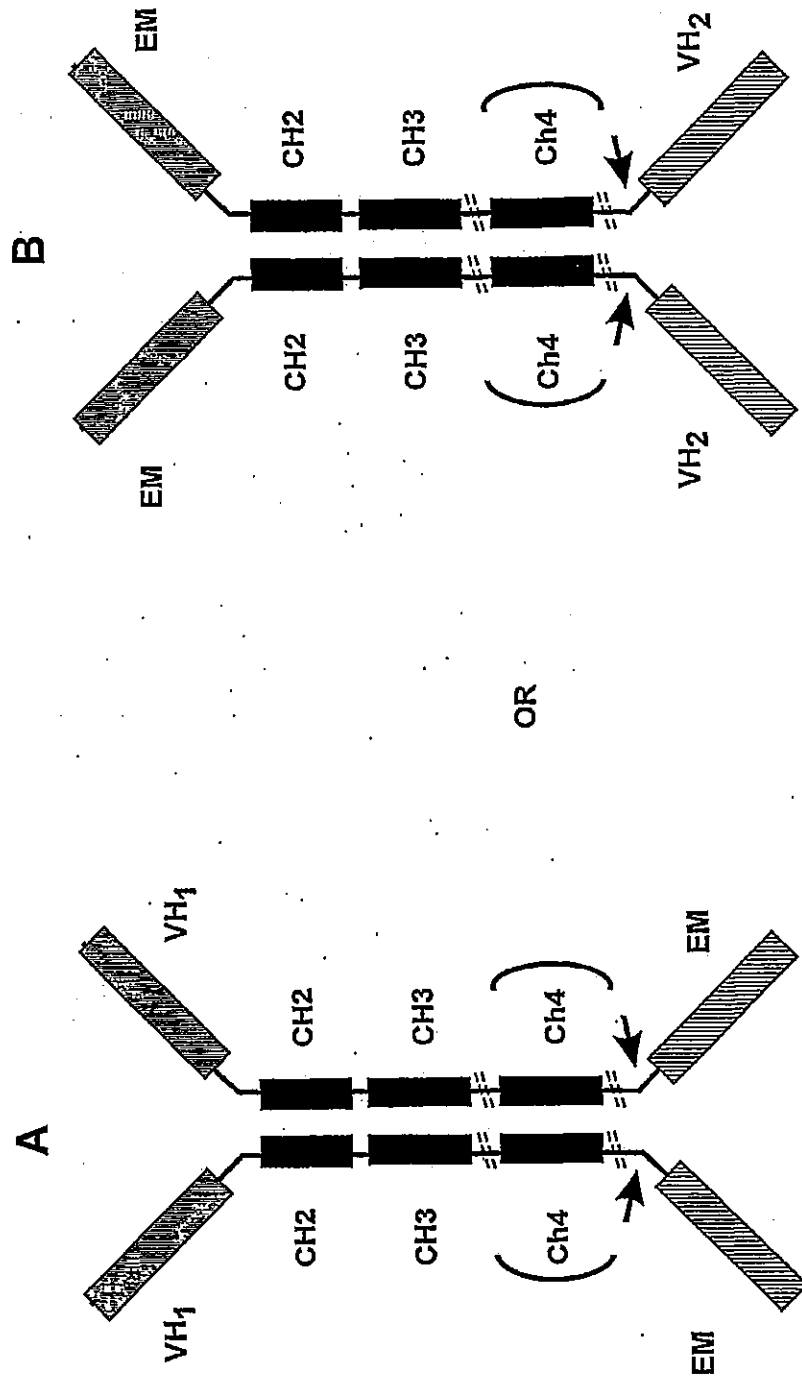
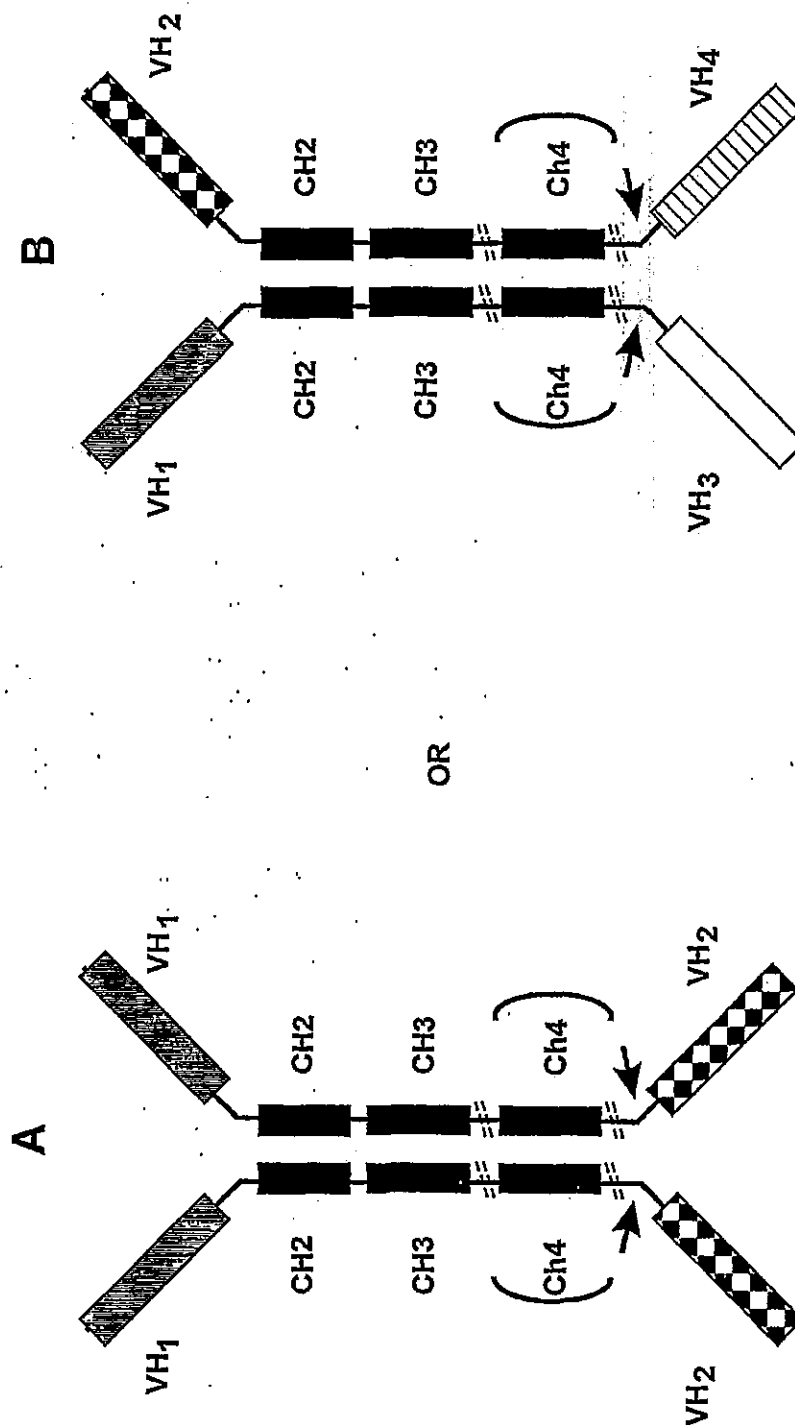


FIG. 1



Plus variations of A and B

FIG. 2



Plus variations of examples shown in A or B

FIG. 3

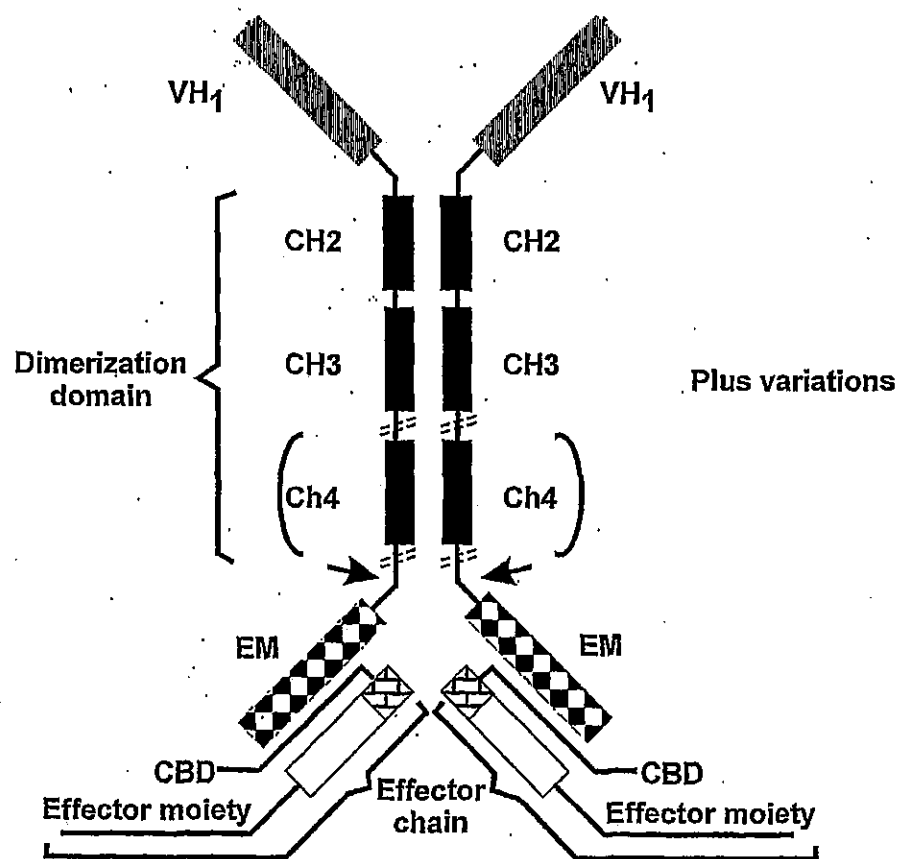
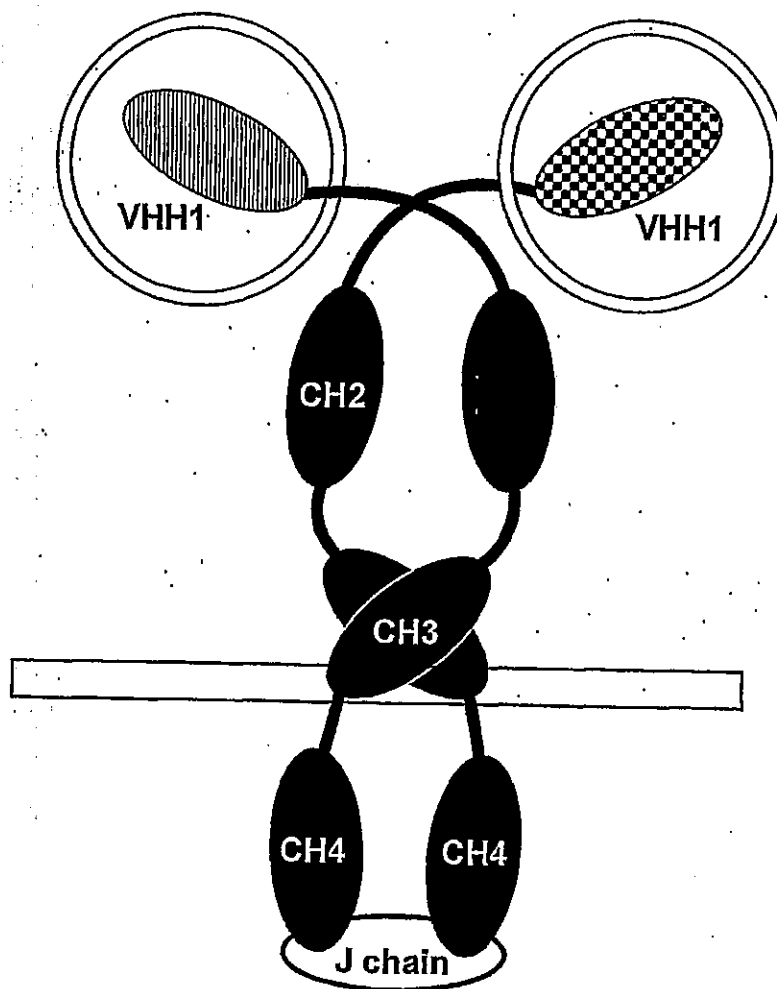


FIG. 4**Bivalent secretory IgA.**

5/26

FIG. 5
Multivalent IgM like

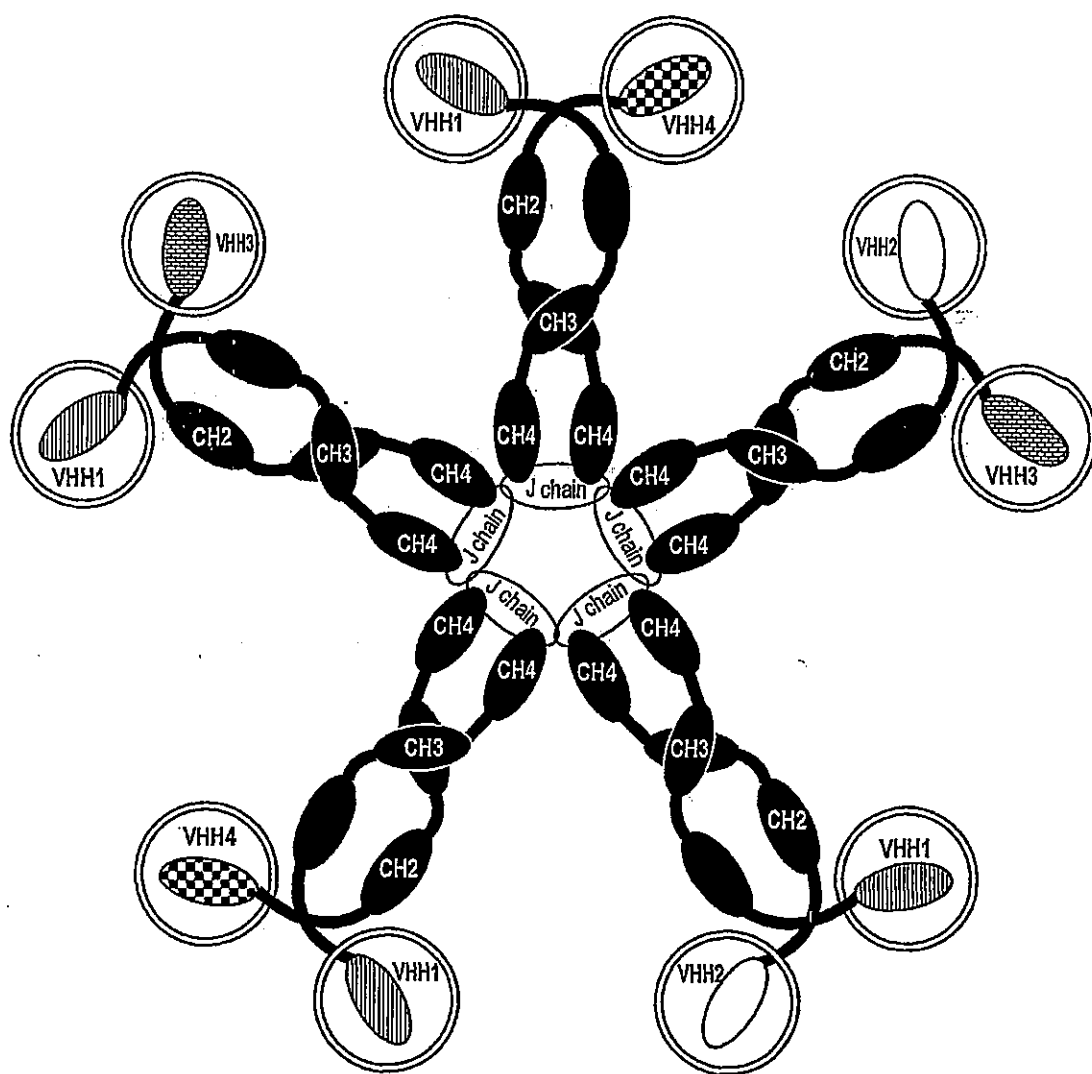


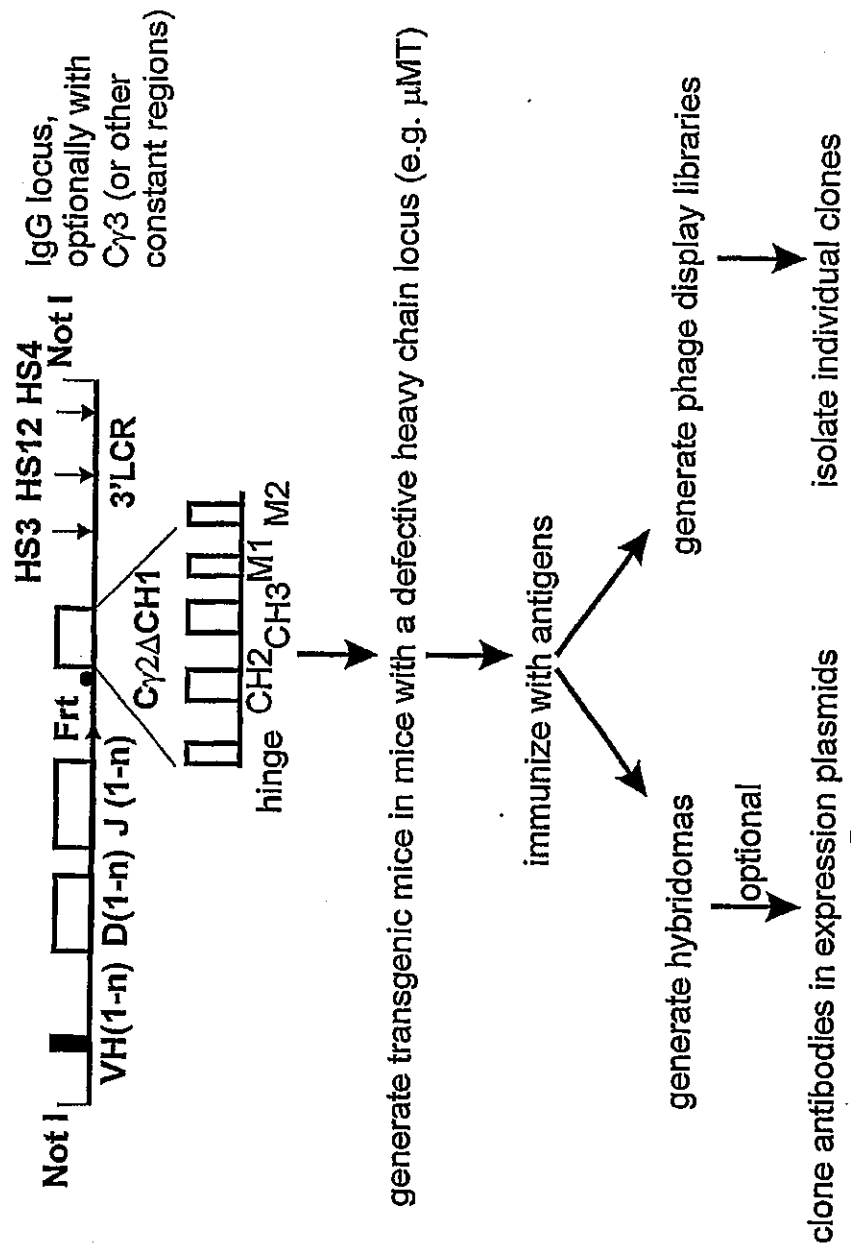
FIG. 6**Generation of heavy chain only IgG**

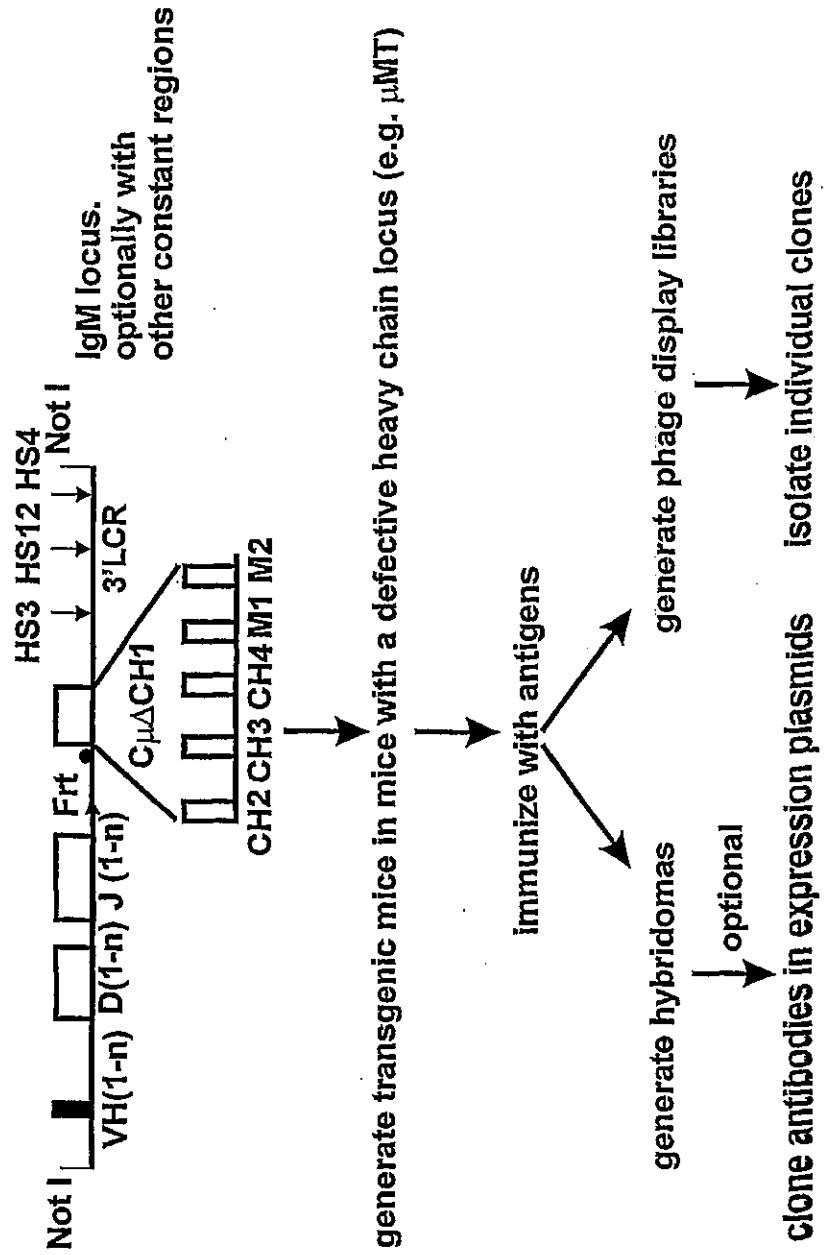
FIG. 7**Generation of heavy chain only IgM**

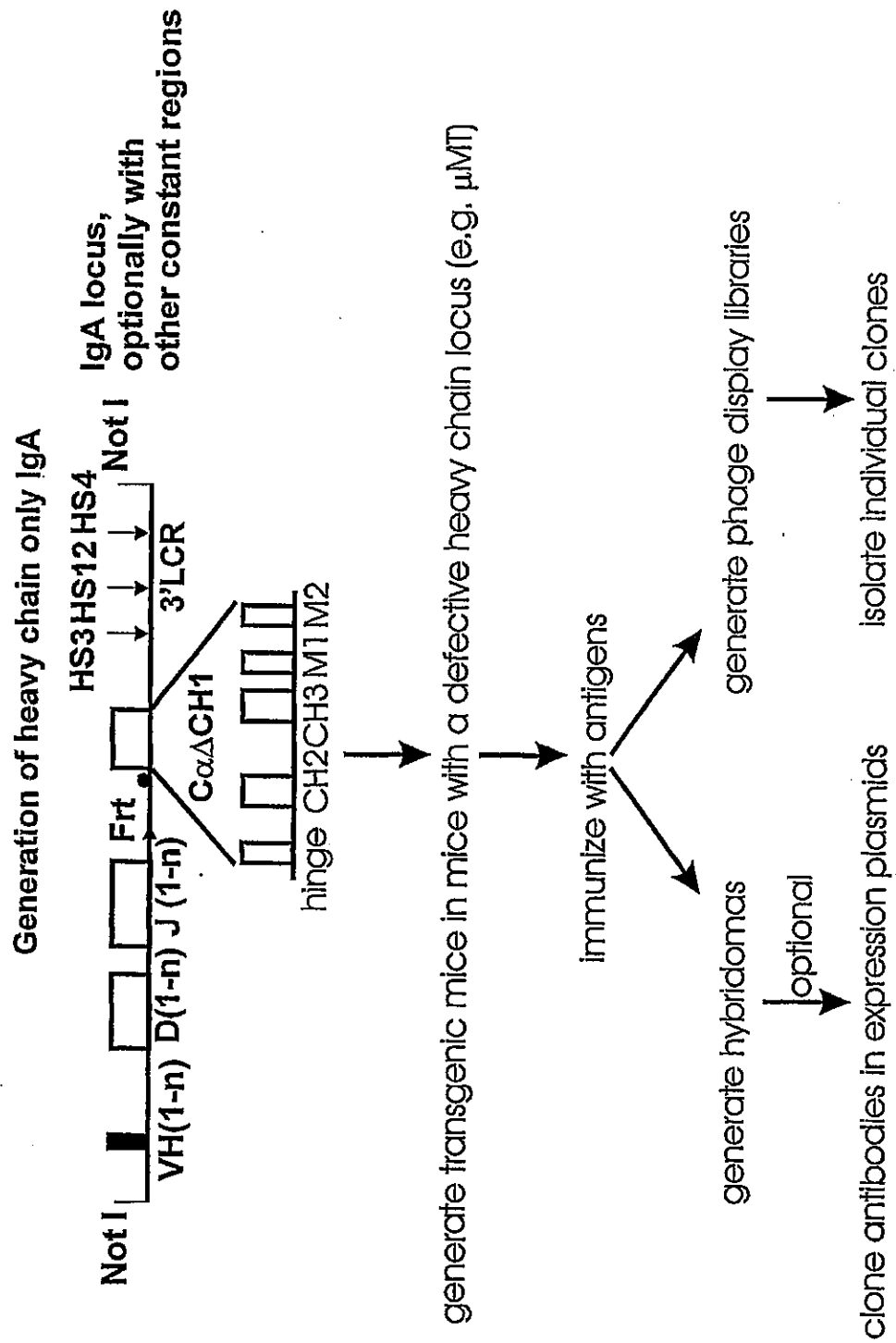
FIG. 8

FIG. 9

9/26

	VHH	D	J
VDJg*1	GACACGGCCGTGTAGTATCTGTAAGGCAGATGG.....GGTAGTACTATGGTTCG....GGGA.		
VDJg*2	GAC.....ATTCACACTTCGATC....T....		
VDJg*3	GACACGGCCGTCTATTACTGTAATGCCACTACG.....ATATTTTCACTGGTTAT....TATA.		
VDJg*4	GACACGGCCGTCCAATCGGATACAG.....CATGGTTACGTACTTT		
VDJg*5	GACACGGCCGTCTATTACTGTAATGCAGATGTATTACTATGGTTCGGGGAGCCTATAGCCTTACTACTACGGTATG.		

	J	CH1
VDJg*1	GTCCACCACTGCGGCTAGAGGGCCAGGCAACACTGGTCGCGGTGTCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*2	CTGGGGCCGTGGACCCCTGGTCACCTGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*3	GAC.....GCTACTGGGGCCAGGCAACCCCTGGTCACCGTCTCCTCAGCCTCCGCCAAGGGCCCATCGGTCTTCCC	
VDJg*4	GACTA.....CTGGGGCCAGGCAACCCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	
VDJg*5	GACGT.....CTGGGGCCAGGCAACCCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCC	

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

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

	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

FIG. 10

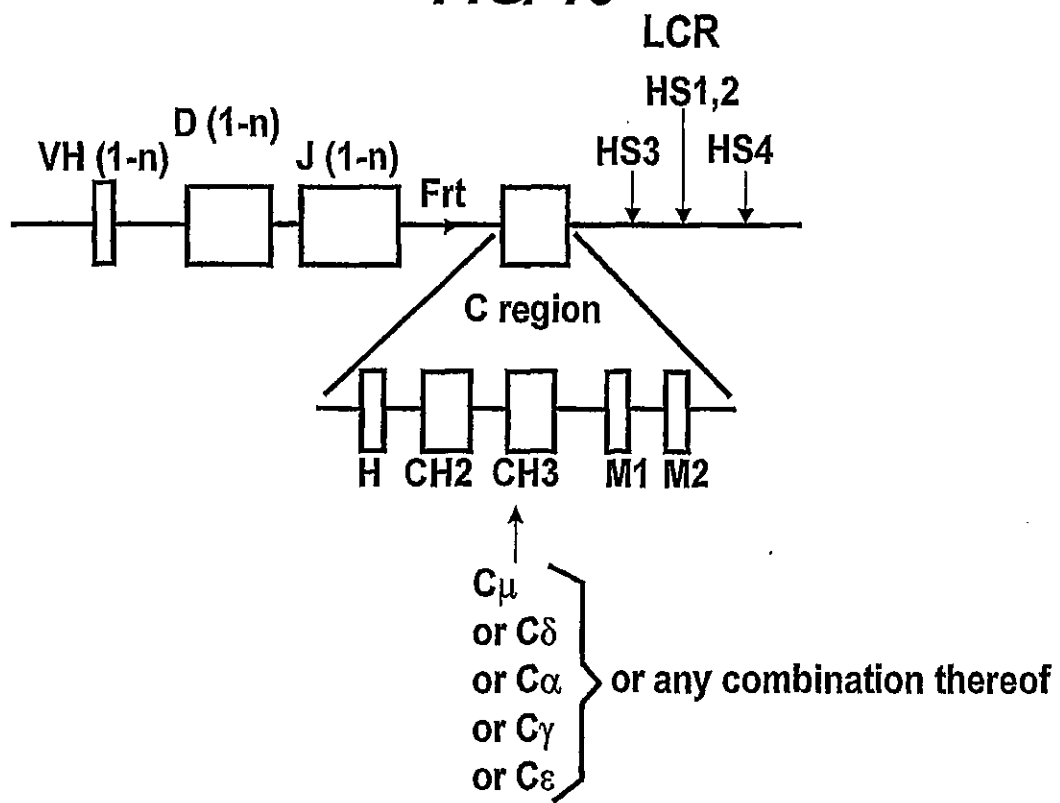
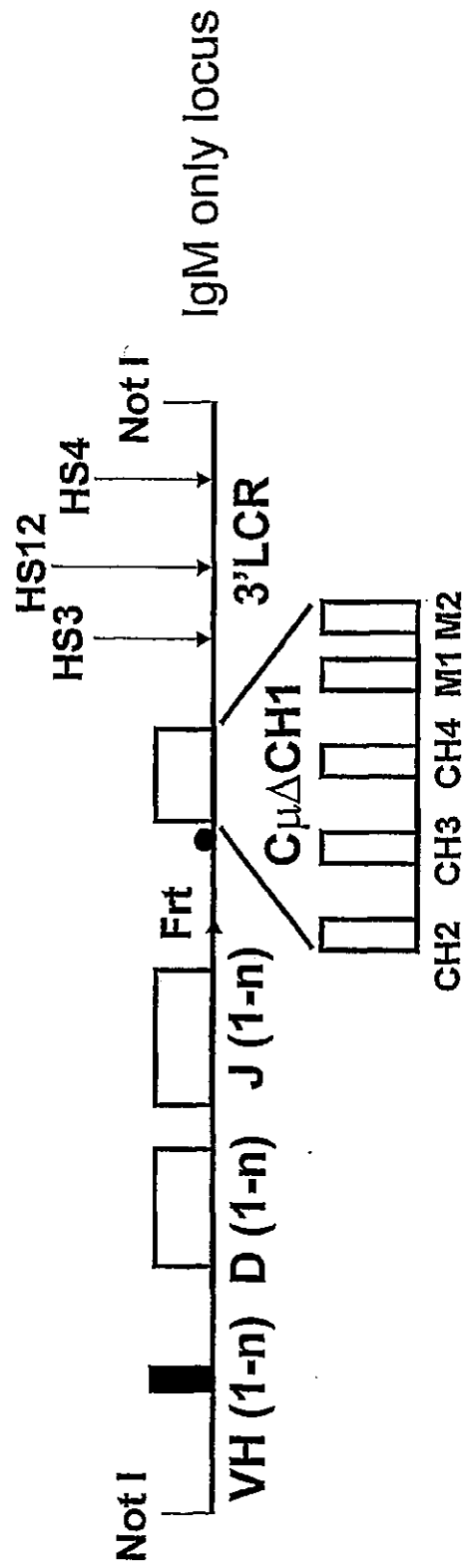
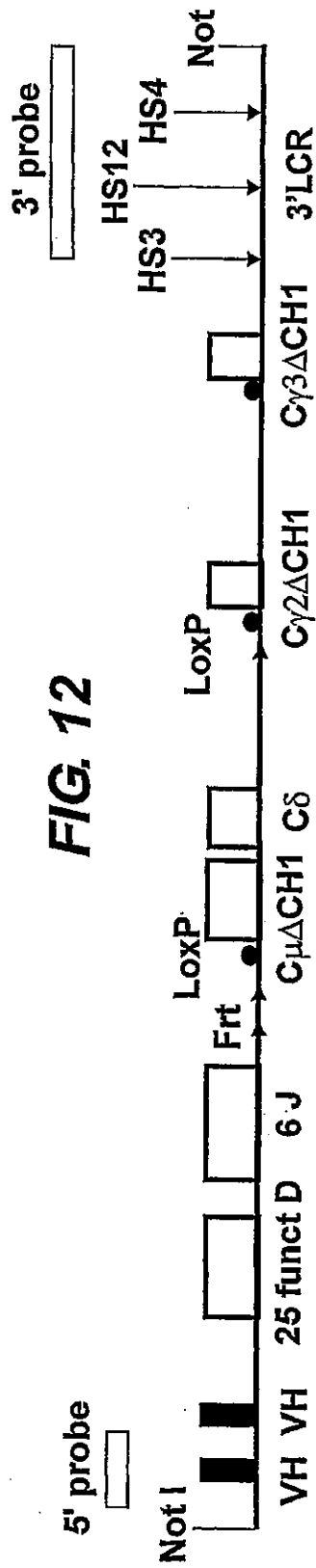
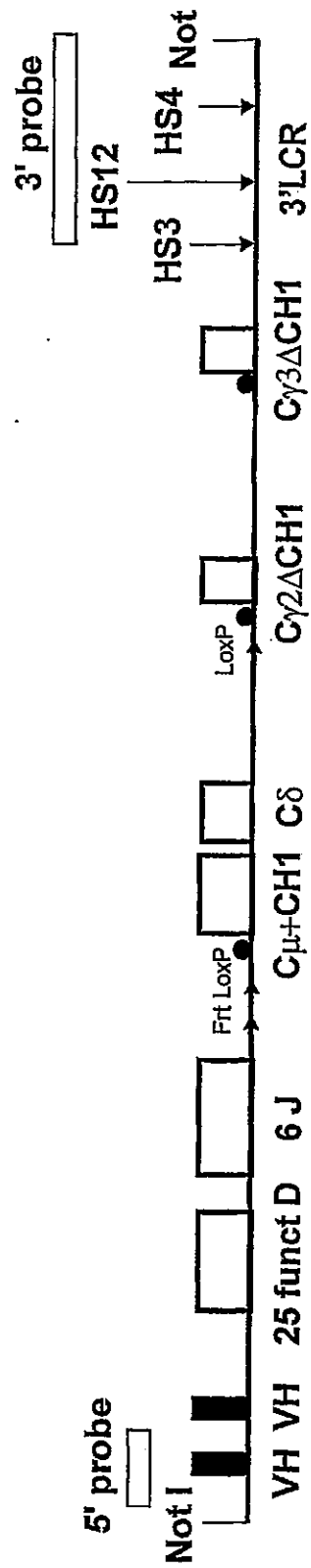


FIG. 11



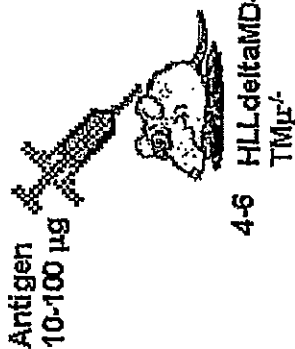
IgM plus IgG locus, C δ is optional

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

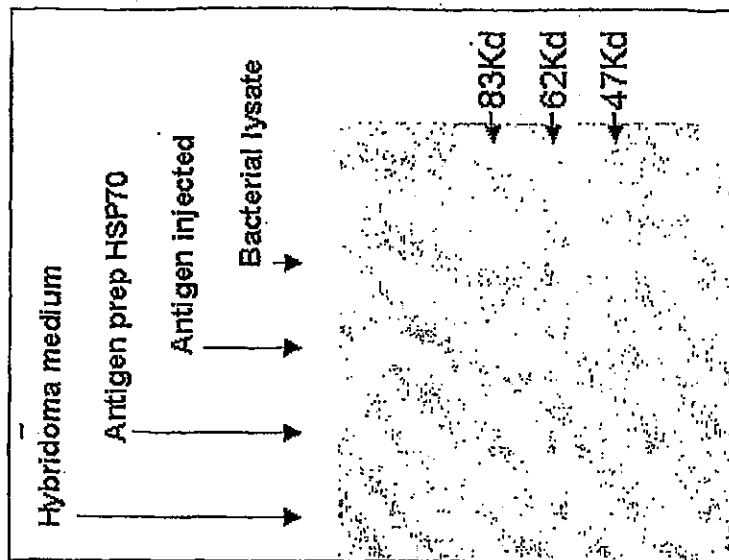


G18 HCab (IgG2 class)

VHH2

RLSCAAGSIFSNAMGWSRGAPGKGRELVAATSGGSTNYADSVKGRFTISRDNKN...

FIG. 14



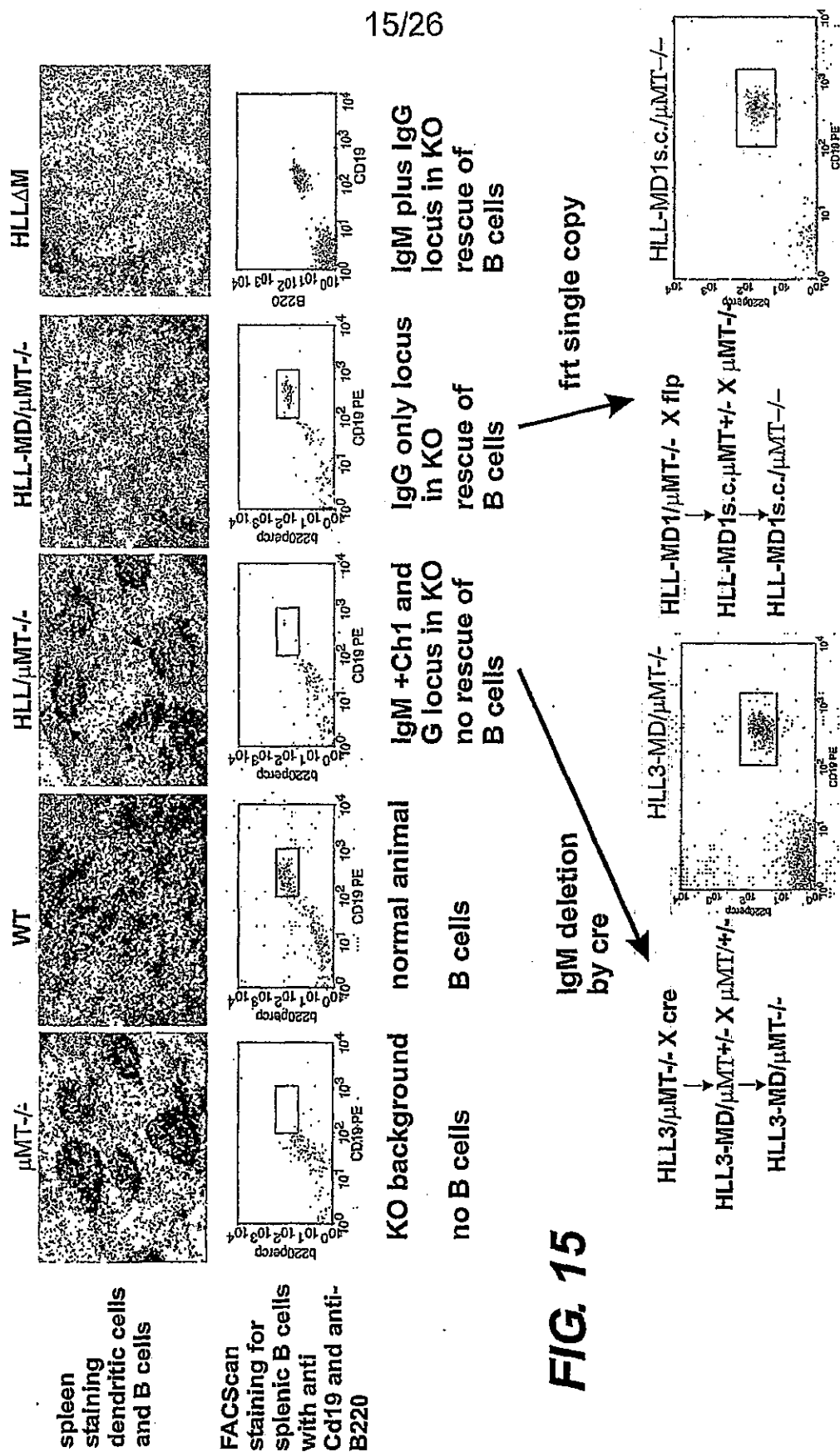


FIG. 15

ELISA of VH
against pertussis
antigen

fingerprint by
restriction digestion
of positive ELISA clones

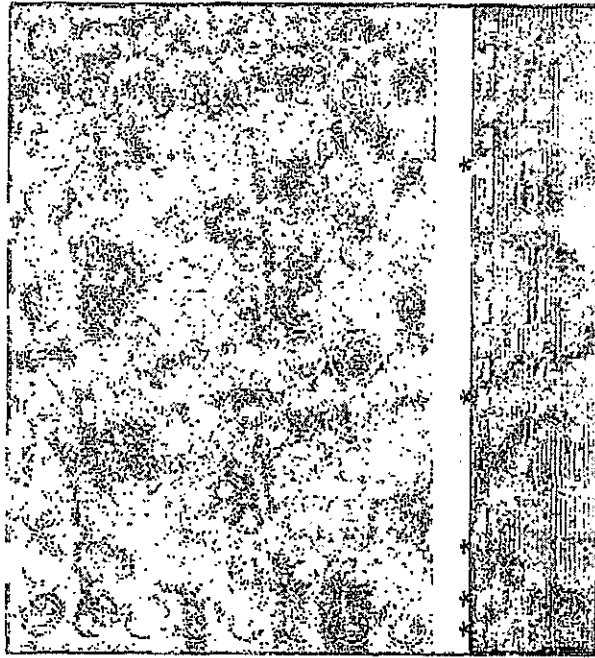


FIG. 16

α-B. Pertussis

1 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNKNTVYLQMNLSLKPEDTAVYYCNAKGPITHVRG
2 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNKNTVYLQMNLSLKPEDTAVYYCNAKTPITHIRG
3 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNKNTVYLQMNLSLKPEDTAVYYCNAKTPITVVRG
4 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDKAKNTVYLQMNLSLKPEDSAVYYCNRTPITHVRG
5 RLSCAASGSIFSINAMGWYRQAPGKQRELVAAITSGGSTNYADSVKGRFTISRDNKNTVYLQMNLSLKPEDTAVYYCNAESPITKVRG

CDR3

CDR3 HINGE
....VHYWGQGTTLTVSSERKCCV...
....VHHWGQGTTLTVSSERKCCV...
....VHYWGQGTTLTVSSERKCCV...
....VDYWGRTTLTVSSERKCCV...
....VSYWGQGTTLTVSSERKCCV...

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

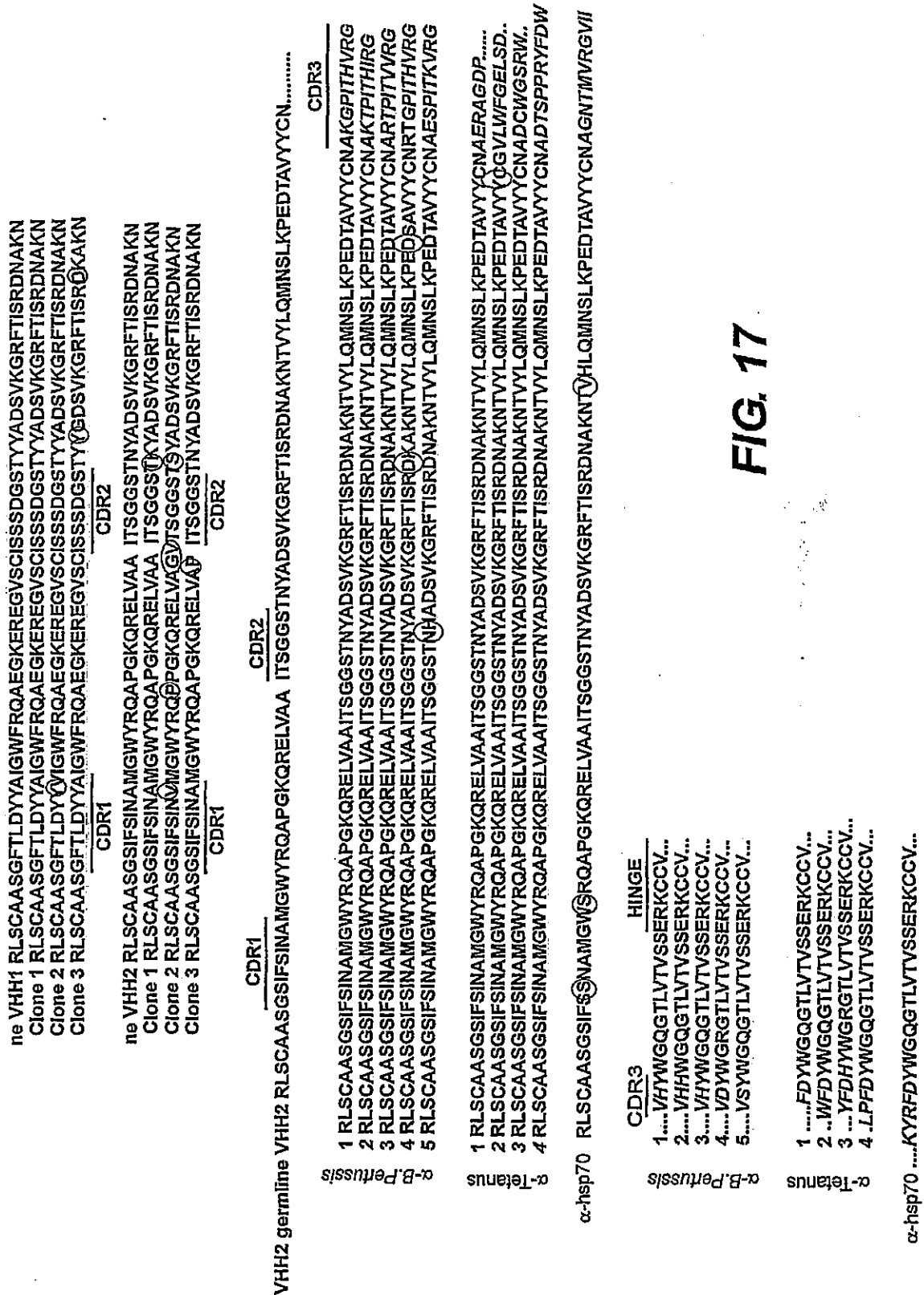
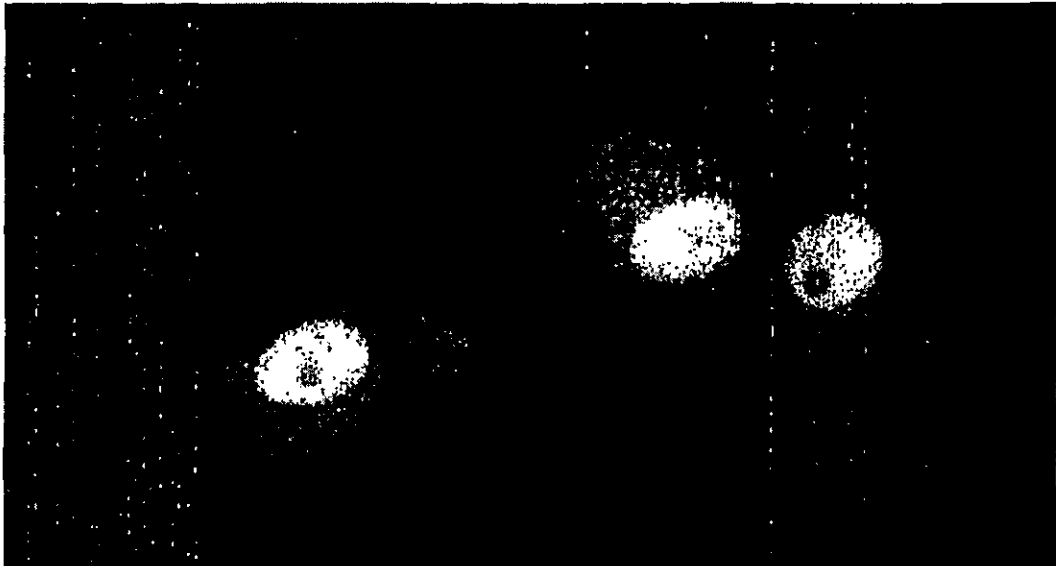


FIG. 17

18/26

FIG. 18



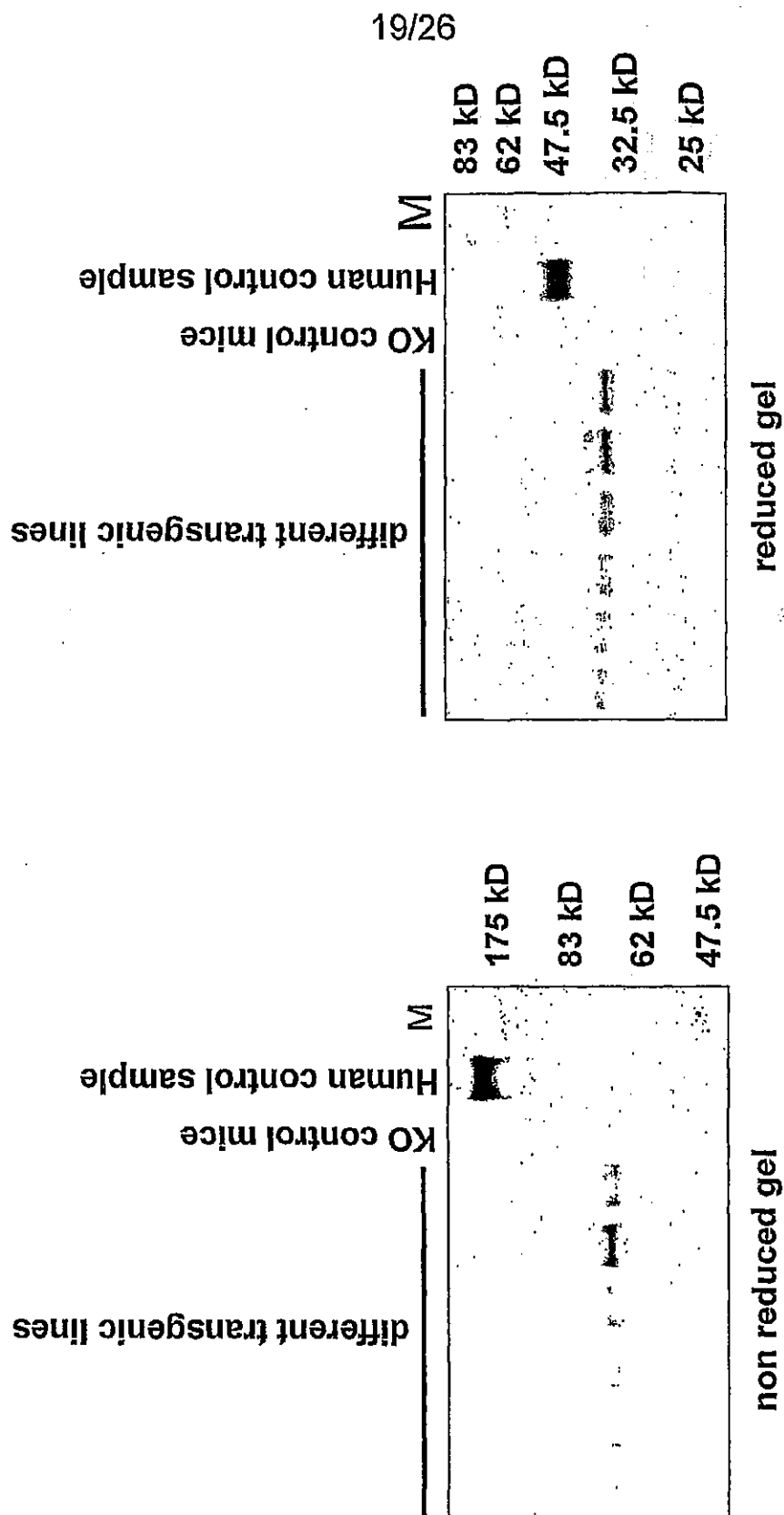


FIG. 19

FIG. 20

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

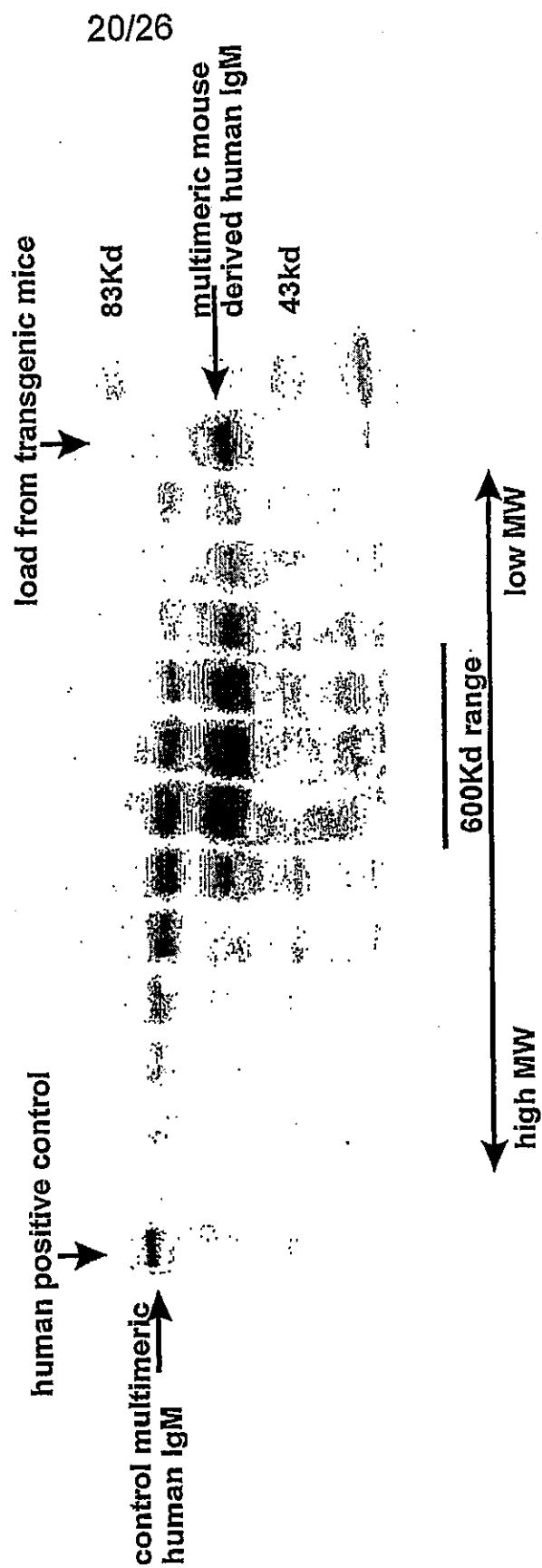


FIG. 21

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

positive hybridoma serum of the immunised mouse

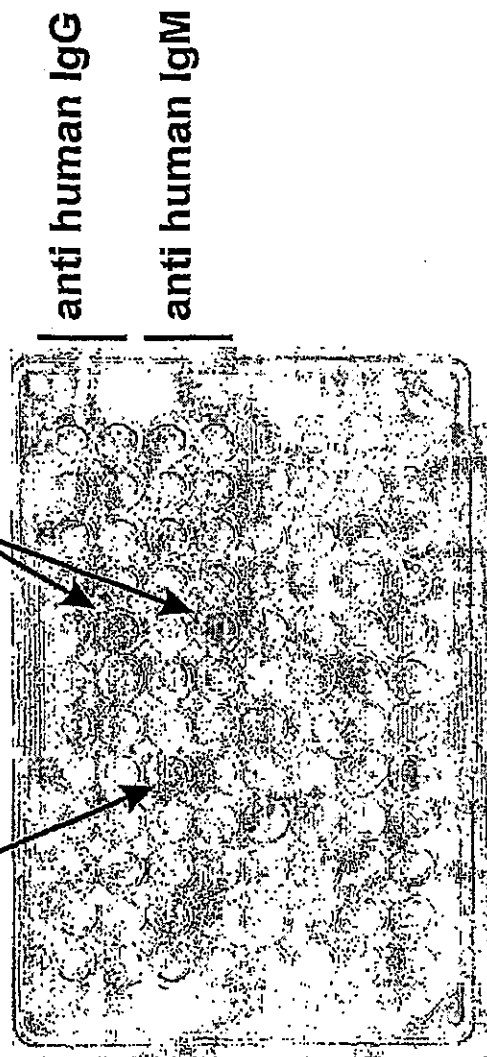
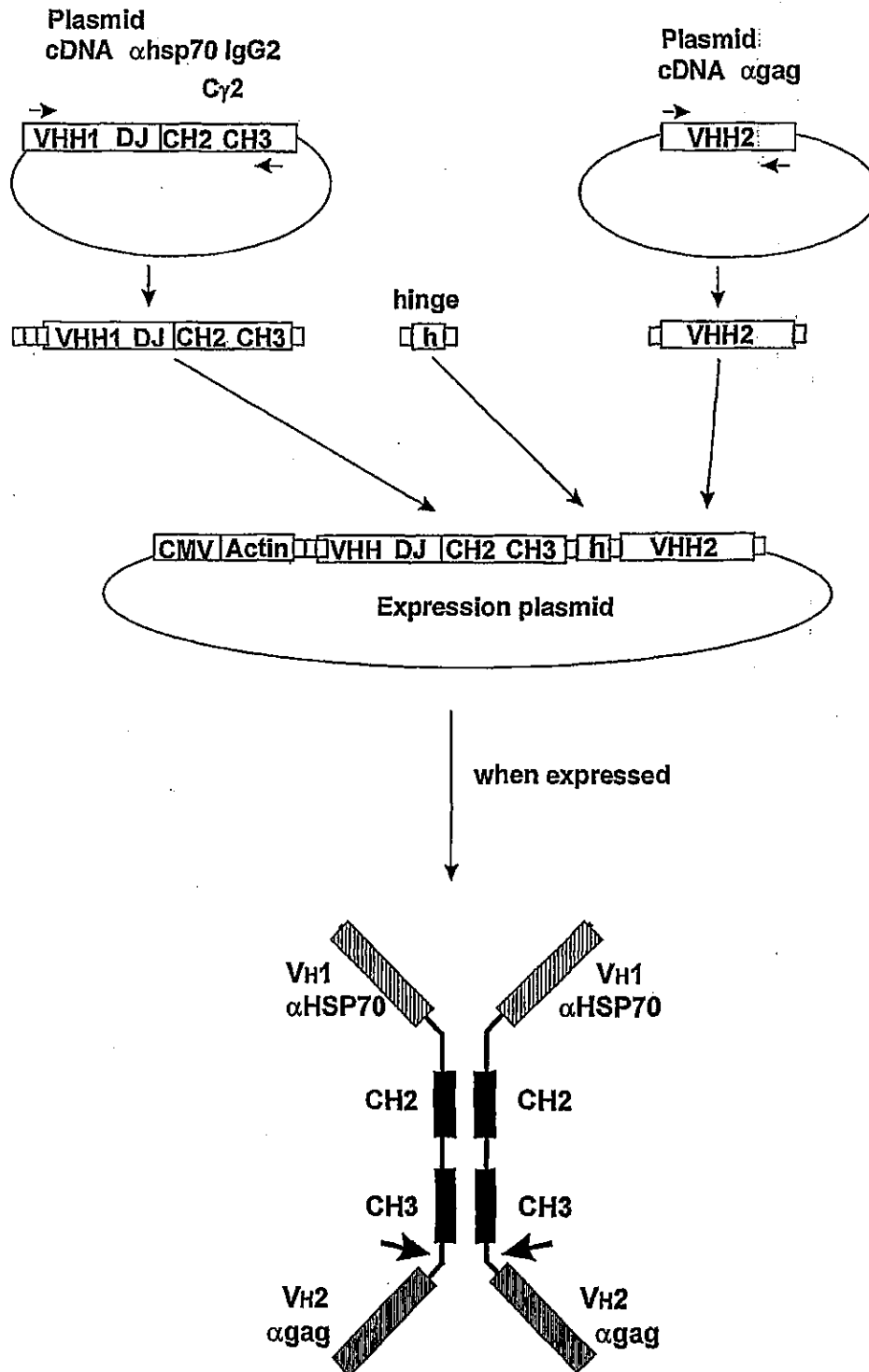
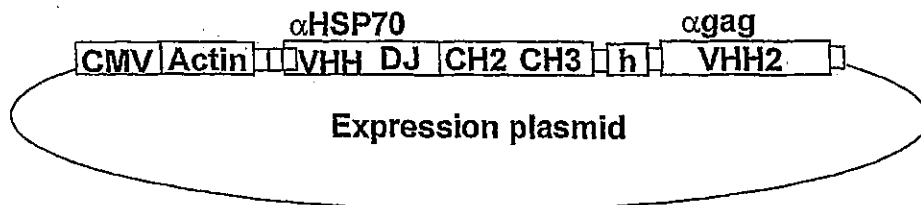


FIG. 22

Generation of the diabody expression plasmid



Expression of HClgm in CHO cells

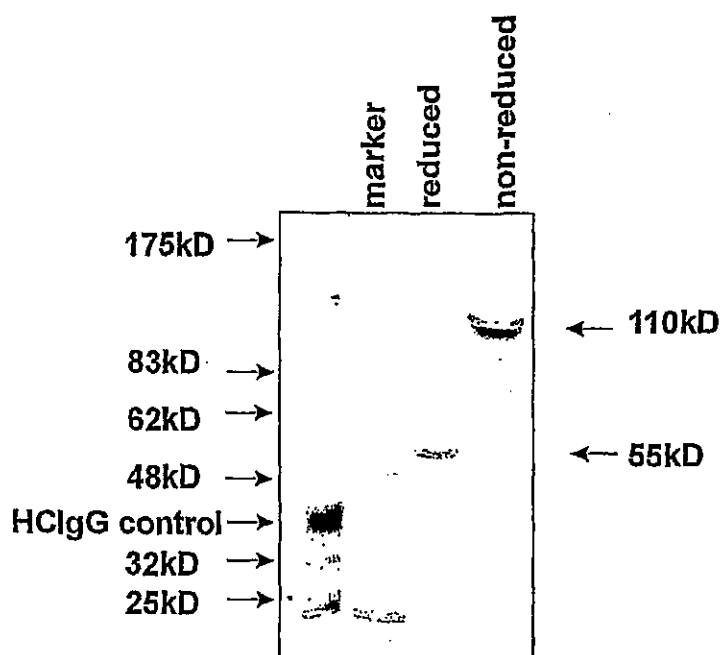


superfect into CHO cells
and select expressing cells
in hygromycin

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

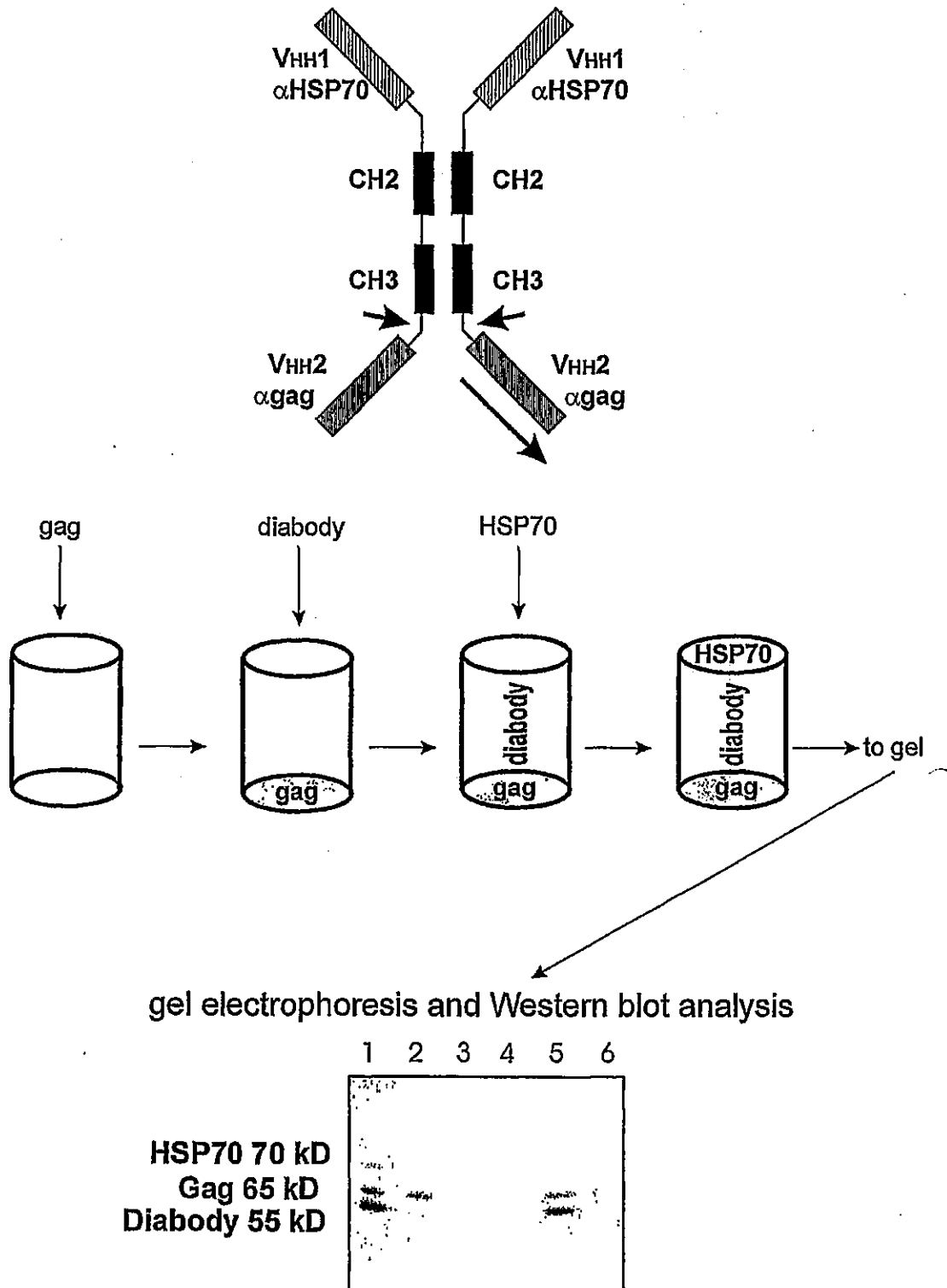
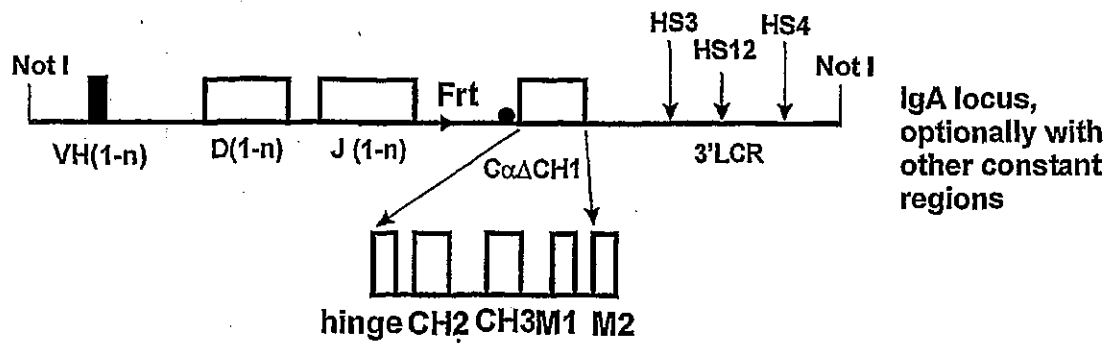


FIG. 25

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

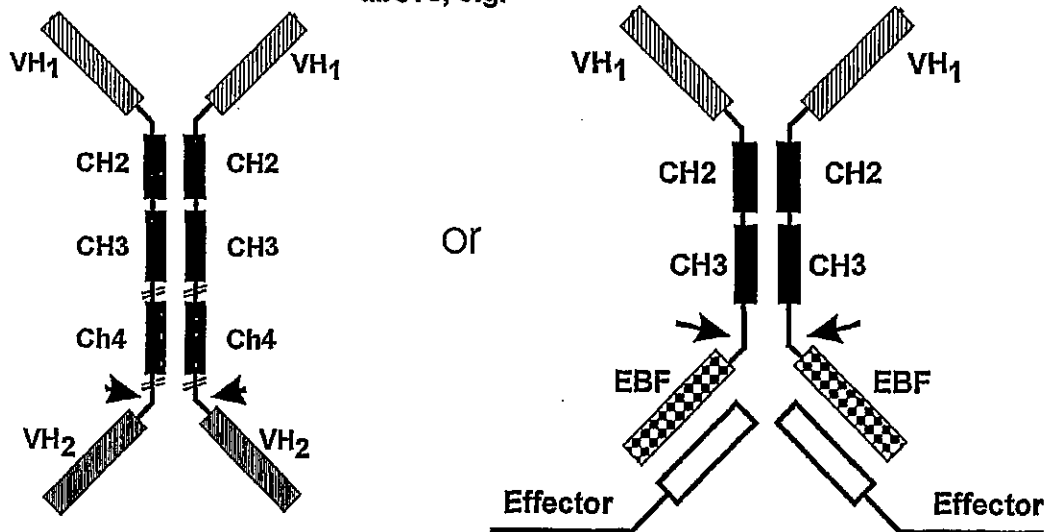
generate phage display libraries

optional

clone antibodies in expression plasmids

isolate individual clones

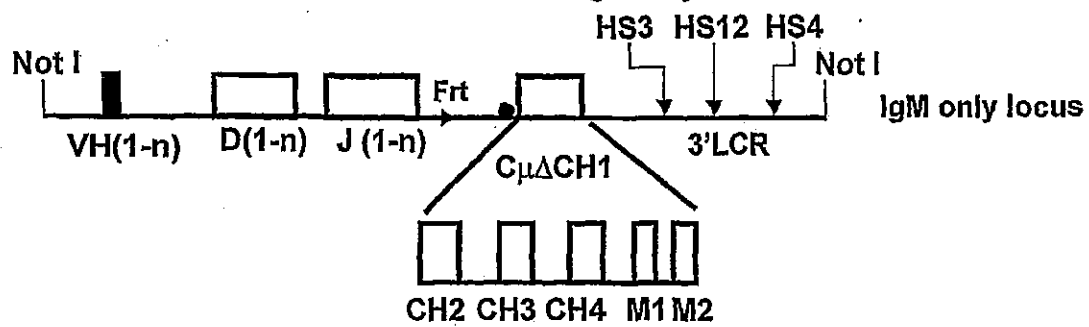
engineer by recombinant DNA technology bispecific bivalent antibodies as shown in figures above, e.g.



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.

