

Recombineered BAC Vectors to add Polymorphic V-regions to the Mouse Genome

1. Cassette Exchange — Replace existing V-regions

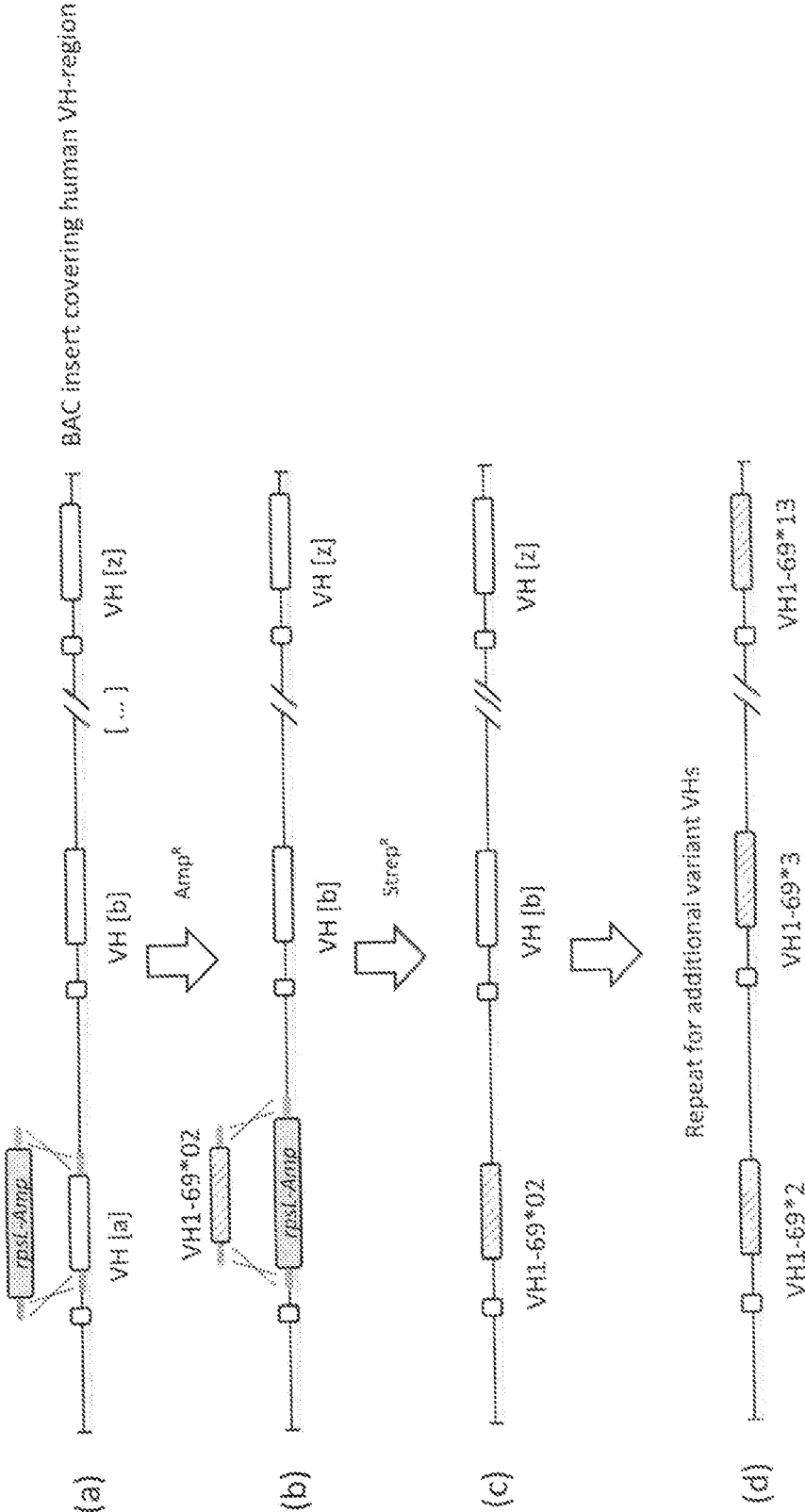


Figure 1

Recombineered BAC Vectors to add Heterogeneous V-regions to the Mouse Genome

2. Insertion of V-regions into Heterologous Genomic DNA

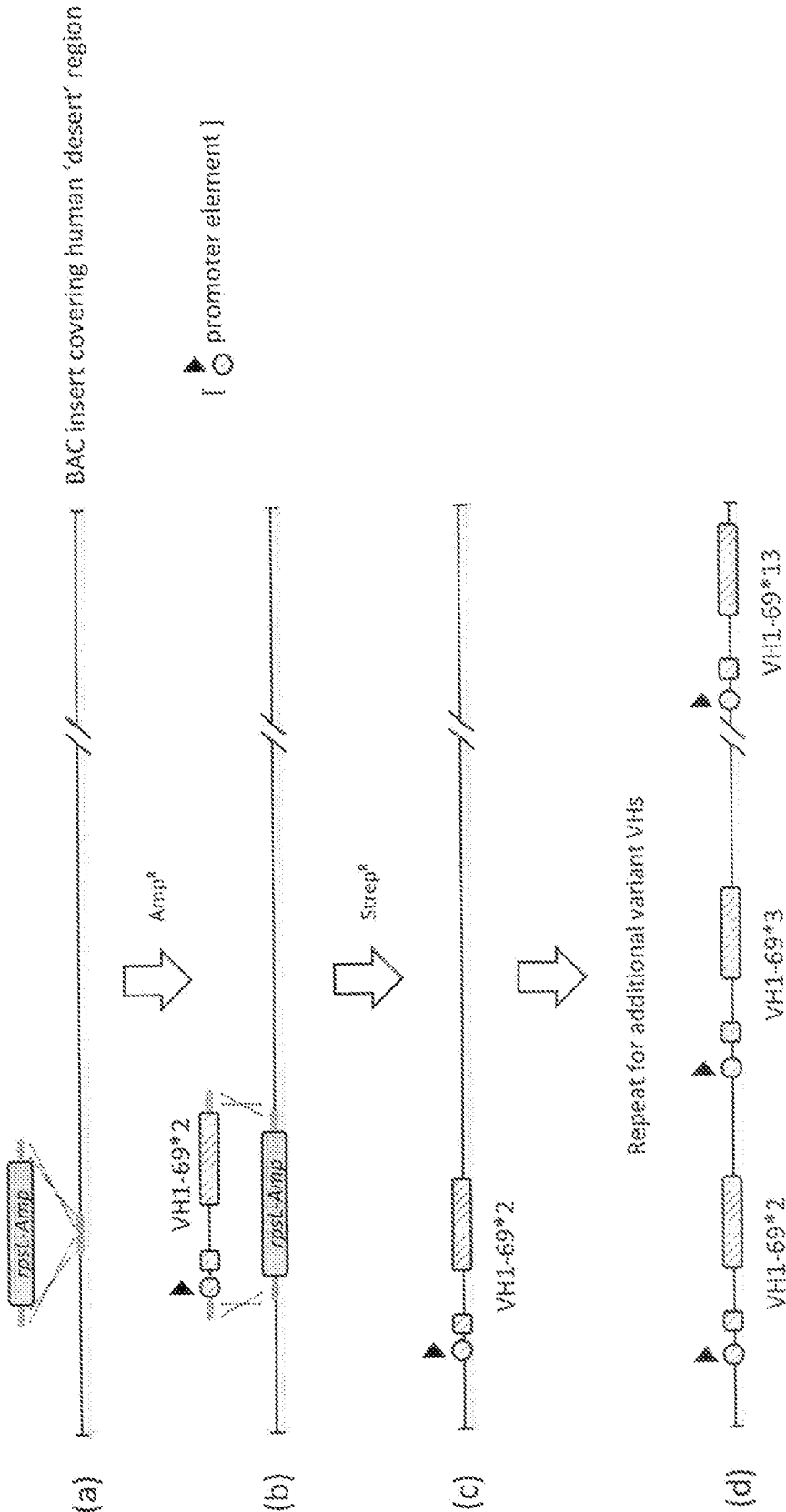


Figure 2

Recombineered BAC Vectors to add Heterogeneous V-regions to the Mouse Genome

3. Single Strand Nucleotide Changes

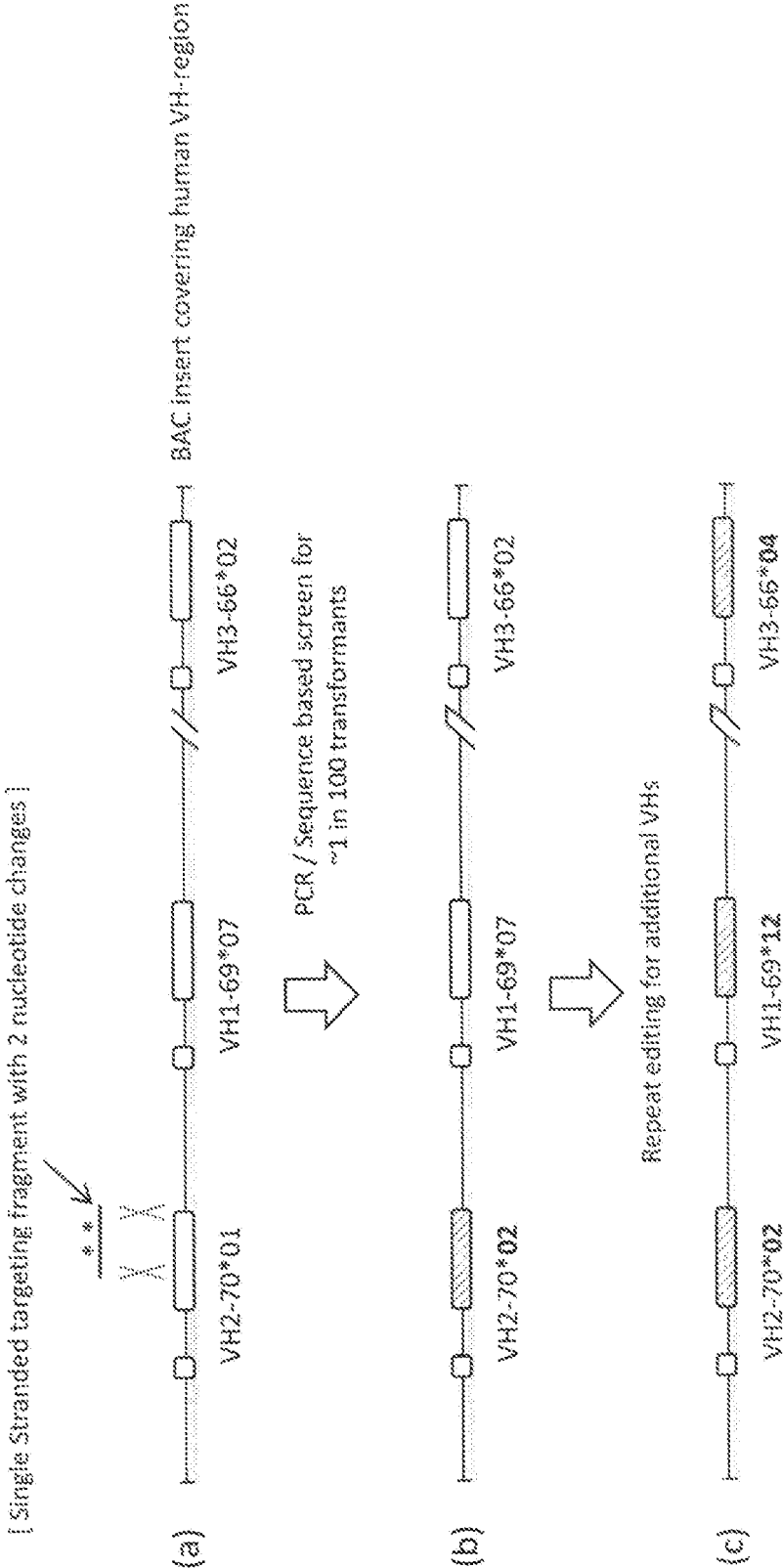


Figure 3

Adding Polymorphic V-regions to the Genome using SRMCE of Modified BACs

1. Insertion at a 'Landing Pad' site during SRMCE

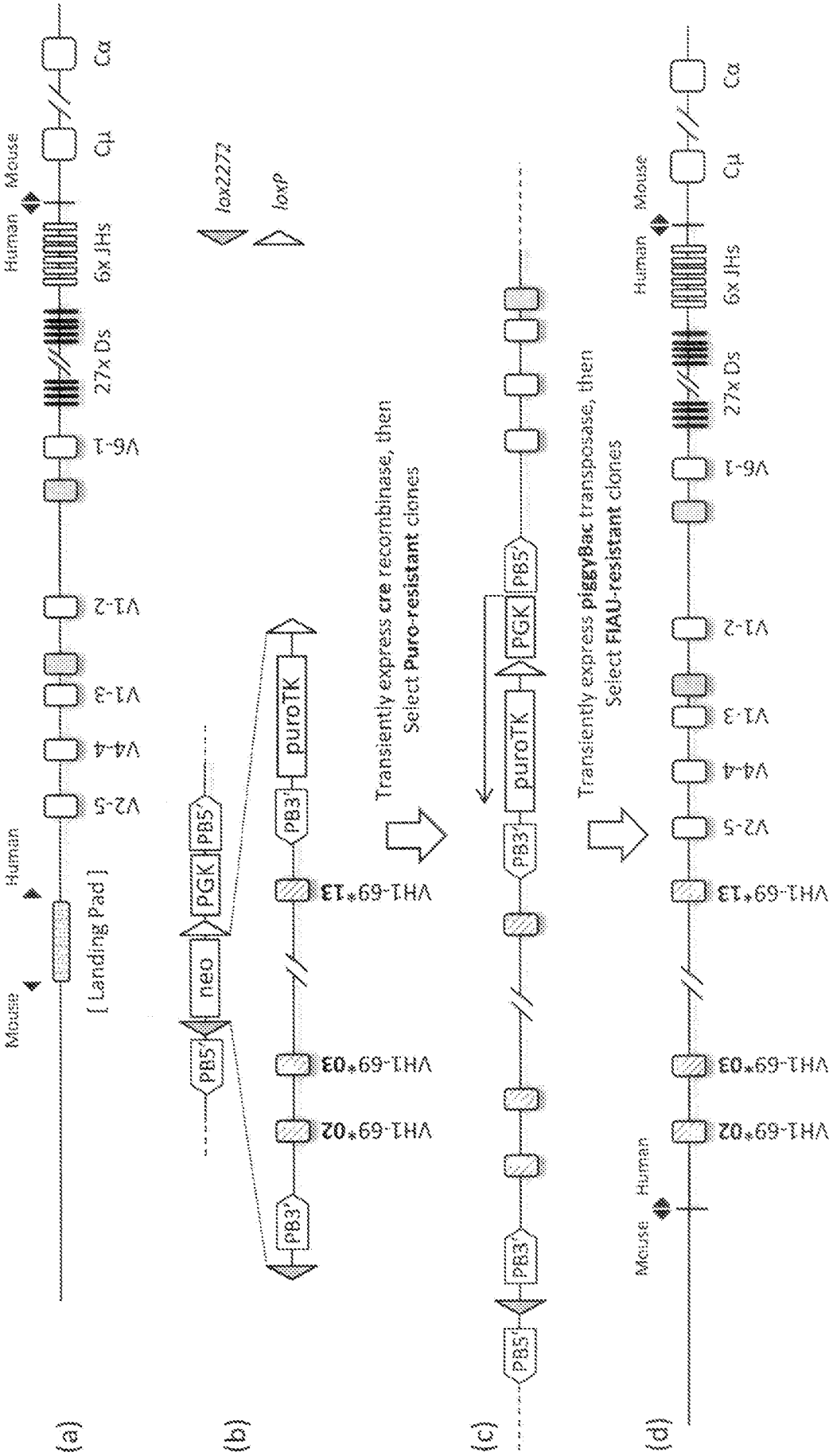


Figure 4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	Q	V	Q	L	V	Q	S	G	A	E	V	R	K	A	E	G	S	S	V	K	V	S	C	K	A	S
102582	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GCG	GCT	...	GAG	GTG	AAG	AAG	CCG	GCG	TCC	TGG	GTC	AAG	GTC	TGC	TGC	AAG	GCT	TCT
227505	IGHV1-69*01, hv1051	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
227505	IGHV1-69*02, y11846(YAC7)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Y92340	IGHV1-69*03, 5702FA8	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
M83132	IGHV1-69*04, hv1263	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
X67905	IGHV1-69*05, RR.VH1.2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
122583	IGHV1-69*06, hv1051K	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
229978	IGHV1-69*07, DA-2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14309	IGHV1-69*08	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14307	IGHV1-69*09	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14300	IGHV1-69*10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14296	IGHV1-69*11	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14301	IGHV1-69*12	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Z14214	IGHV1-69*13	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Alignment of 13 IGHV1-69 alleles showing the variable (V) coding region only. Nucleotides that differ from VH1-69 allele *01 are indicated at the appropriate position whereas identical nucleotides are marked with a dash. Where nucleotide changes result in amino acid differences, the encoded amino acid is shown above the corresponding triplet. Boxed regions correspond to CDR1, CDR2 and CDR3 as indicated.

Figure 5 (part 1 of 4)

	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
	G	G	T	F	S	S	S	A					I	S	W	V	R	Q	A	P	G	Q	G	L	E	W	N
	GGA	GGC	ACC	TTC	ACC	ACC	TAT	GCT					ATC	AGC	TGG	GTC	CGA	CAG	GCC	CCT	GGA	CAA	GCG	CCT	GAG	TGG	ATG
L22582 ,IGHV1-69*01, hv1051																											
227506 ,IGHV1-69*02, y1GH6(YAC7)								A																			
X92340 ,IGHV1-69*03, 57GHA8																											
M33133 ,IGHV1-69*04, hv1263																											
X67905 ,IGHV1-69*05, ER.VH1.2																											
L22563 ,IGHV1-69*06, hv1051K																											
Z20978 ,IGHV1-69*07, 5A-2								T																			
Z14309 ,IGHV1-69*08								A																			
Z14307 ,IGHV1-69*09																											
Z14300 ,IGHV1-69*10																											
Z14296 ,IGHV1-69*11																											
Z14301 ,IGHV1-69*12																											
Z14314 ,IGHV1-69*13																											

Alignment of 13 IGHV1-69 alleles showing the variable (V) coding region only. Nucleotides that differ from VH1-69 allele *01 are indicated at the appropriate position whereas identical nucleotides are marked with a dash. Where nucleotide changes result in amino acid differences, the encoded amino acid is shown above the corresponding triplet. Boxed regions correspond to CDR1, CDR2 and CDR3 as indicated.

Figure 5 (part 2 of 4)

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	CDR3
L22582	D	E	S	T	S	T	A	Y	N	E	L	S	S	L	K	S	E	D	T	A	V	Y	Y	C	A	K	
	GAC	GAA	TCC	ACG	ASC	ACA	GCC	TAC	ATG	GAG	CTG	AGC	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCC	GTC	TAT	TAC	TGT	GCG	AGA	GA
	K																										
Z22506	A																										
	YUHG(YAC7)																D										
X92340																											
	K																										
X93131	A																										
X67905																											
	K																										
L22583	A																										
ZC9976																											
	K																										
Z14309	A																										
	K																										
Z14307	A																										
	K																										
Z14300	A																										
Z14296																											
Z14301																											
Z14214																											

Alignment of 13 IGHV1-69 alleles showing the variable (V) coding region only. Nucleotides that differ from VH1-69 allele *01 are indicated at the appropriate position whereas identical nucleotides are marked with a dash. Where nucleotide changes result in amino acid differences, the encoded amino acid is shown above the corresponding triplet. Boxed regions correspond to CDR1, CDR2 and CDR3 as indicated.

Figure 5 (part 4 of 4)

Figure 6

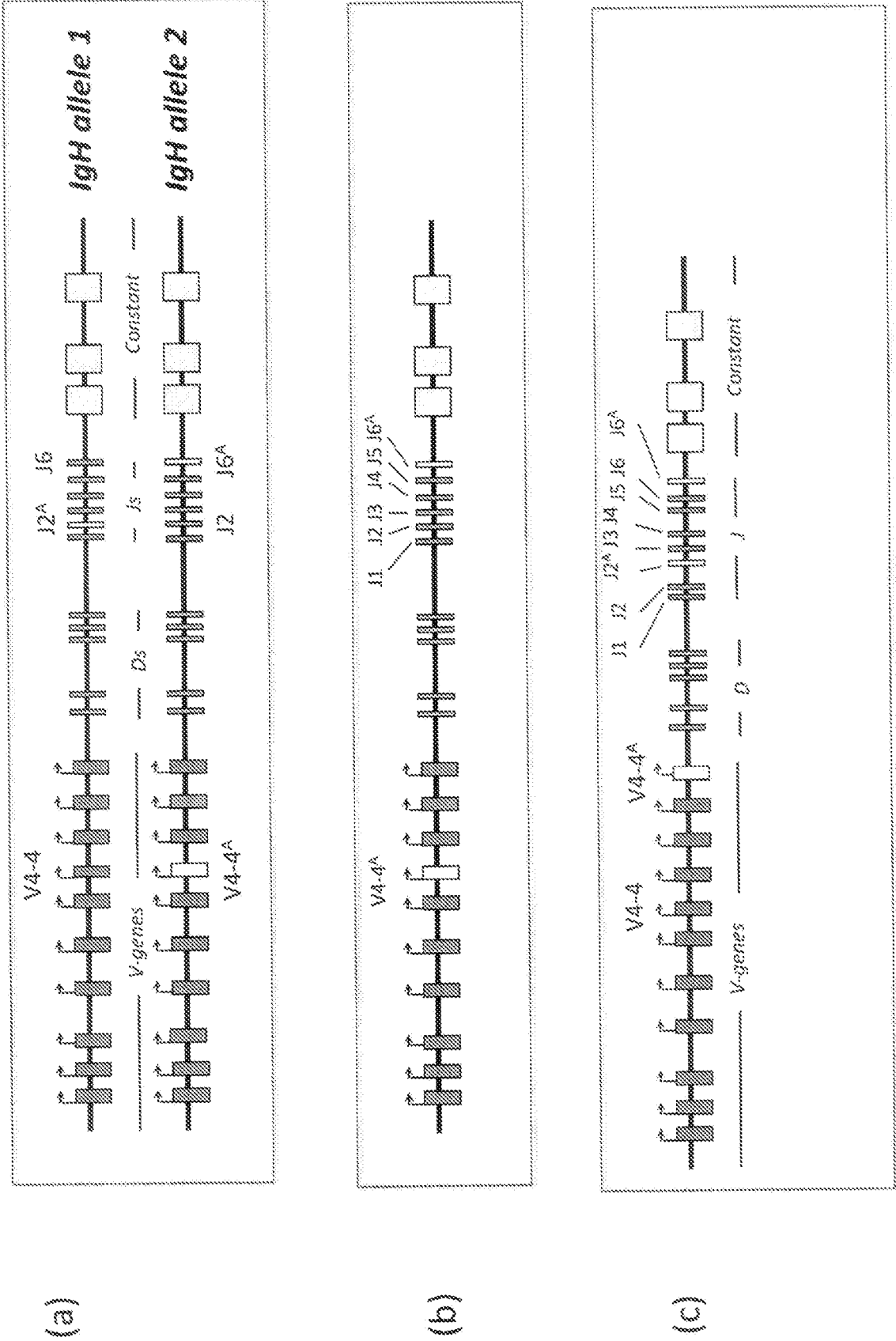


FIGURE 8

Rabbit	JH6	Y	Y	G	M	D	L
		at	tac	tac	ggc	atg	gac ctc
Sheep	JH6	Y	Y	G	V	D	V
		at	tac	tac	ggt	gta	gat gtc
Bovine	JH6	Y	Y	G	V	D	V
		at	tac	tac	ggt	gta	gat gtc
Dog	JH3	Y	Y	G	M	D	Y
		at	tac	tat	ggt	atg	gac tac
Human	JH6*02	Y	Y	Y	G	M	D
		at	tac	tac	tac	ggt	atg gac gtc

FIGURE 9

	T			C			A			G		
T	TTT	Phe	F	TCT	Ser	S	TAT	Tyr	Y	TGT	Cys	C
	TTC	Phe	F	TOC	Ser	S	TAC	Tyr	Y	TCC	Cys	C
	TTA	Leu	L	TCA	Ser	S	TAA	stop	*	TCA	stop	*
	TTG	Leu	L	TOG	Ser	S	TAG	stop	*	TGG	Trp	W
C	CTT	Leu	L	CCT	Pro	P	CAT	His	H	CGT	Arg	R
	CTC	Leu	L	CCC	Pro	P	CAC	His	H	CGC	Arg	R
	CTA	Leu	L	CCA	Pro	P	CAA	Gln	Q	CGA	Arg	R
	CTG	Leu	L	CCG	Pro	P	CAG	Gln	Q	CGG	Arg	R
A	ATT	Ile	I	ACT	Thr	T	AAT	Asn	N	AGT	Ser	S
	ATC	Ile	I	ACC	Thr	T	AAC	Asn	N	AGC	Ser	S
	ATA	Ile	I	ACA	Thr	T	AAA	Lys	K	AGA	Arg	R
	ATG	Met	M	ACG	Thr	T	AAG	Lys	K	AGG	Arg	R
G	GTT	Val	V	GCT	Ala	A	GAT	Asp	D	GGT	Gly	G
	GTC	Val	V	GCC	Ala	A	GAC	Asp	D	GGC	Gly	G
	GTA	Val	V	GCA	Ala	A	GAA	Glu	E	GGA	Gly	G
	GTG	Val	V	GCG	Ala	A	GAG	Glu	E	GGG	Gly	G

Figure 10

