

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

1. Cassette Exchange – Replace existing V-regions

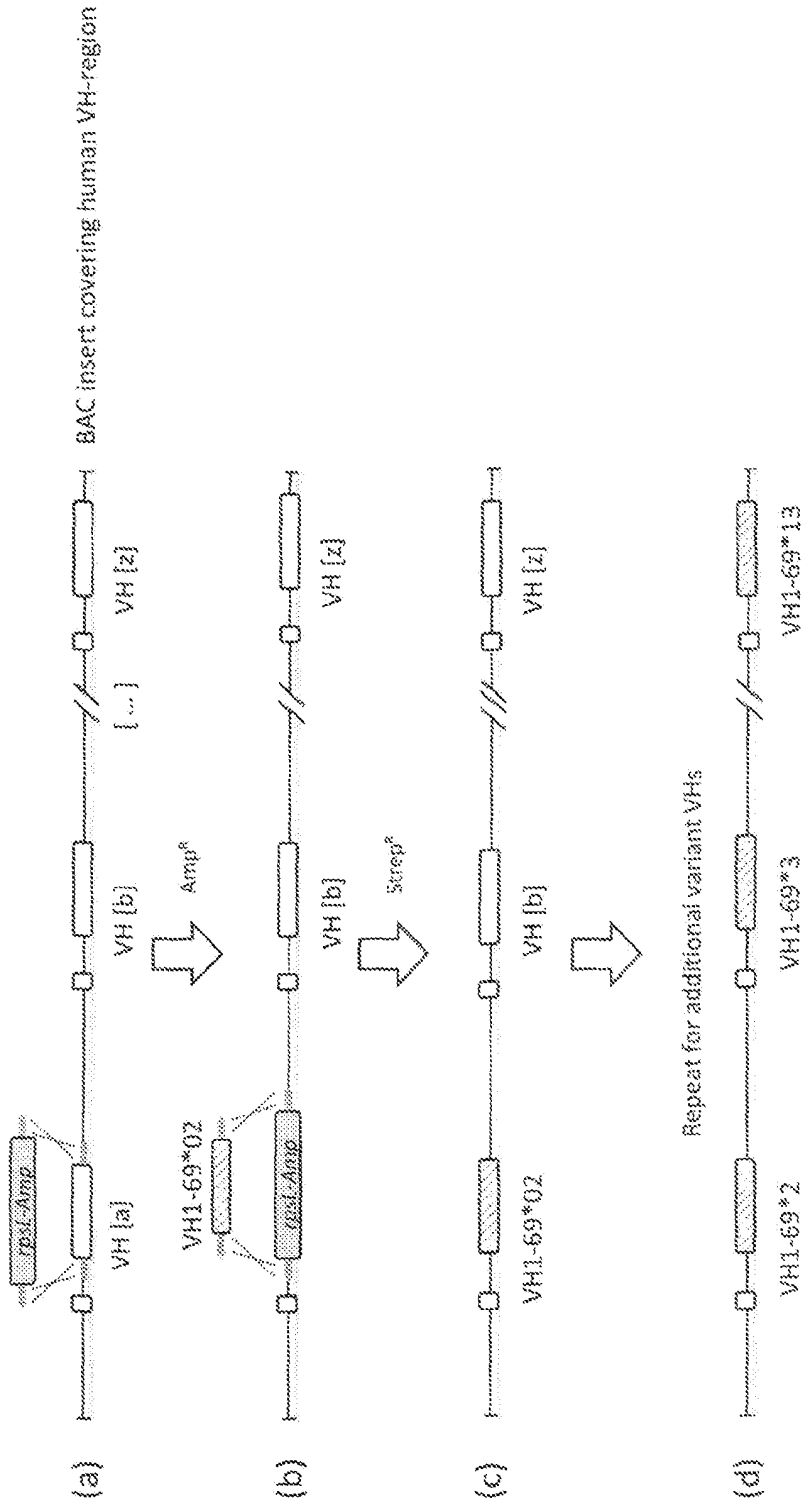


Figure 1

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

2. Insertion of V-regions into Heterologous Genomic DNA

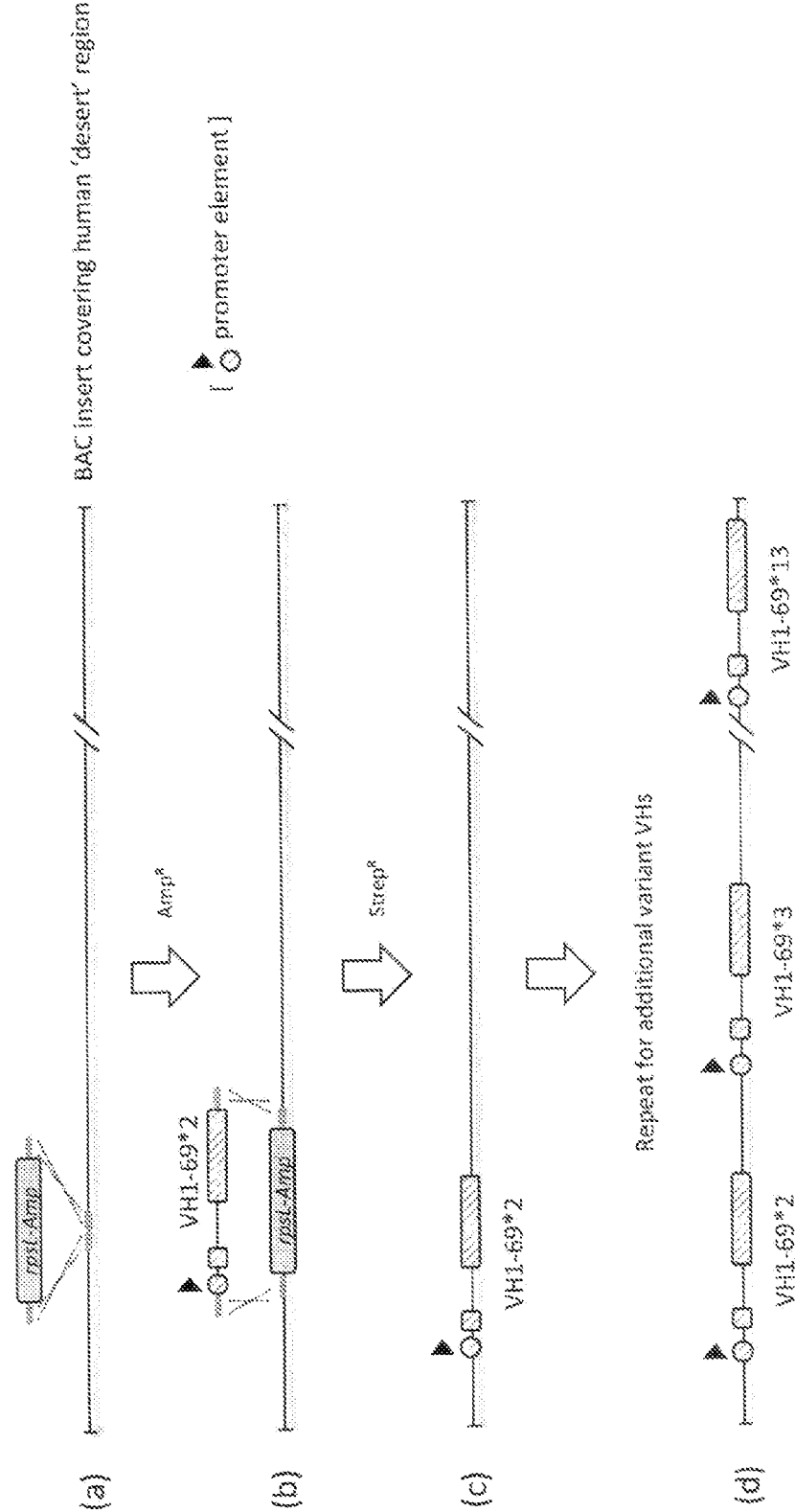


Figure 2

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

3. Single Strand Nucleotide Changes

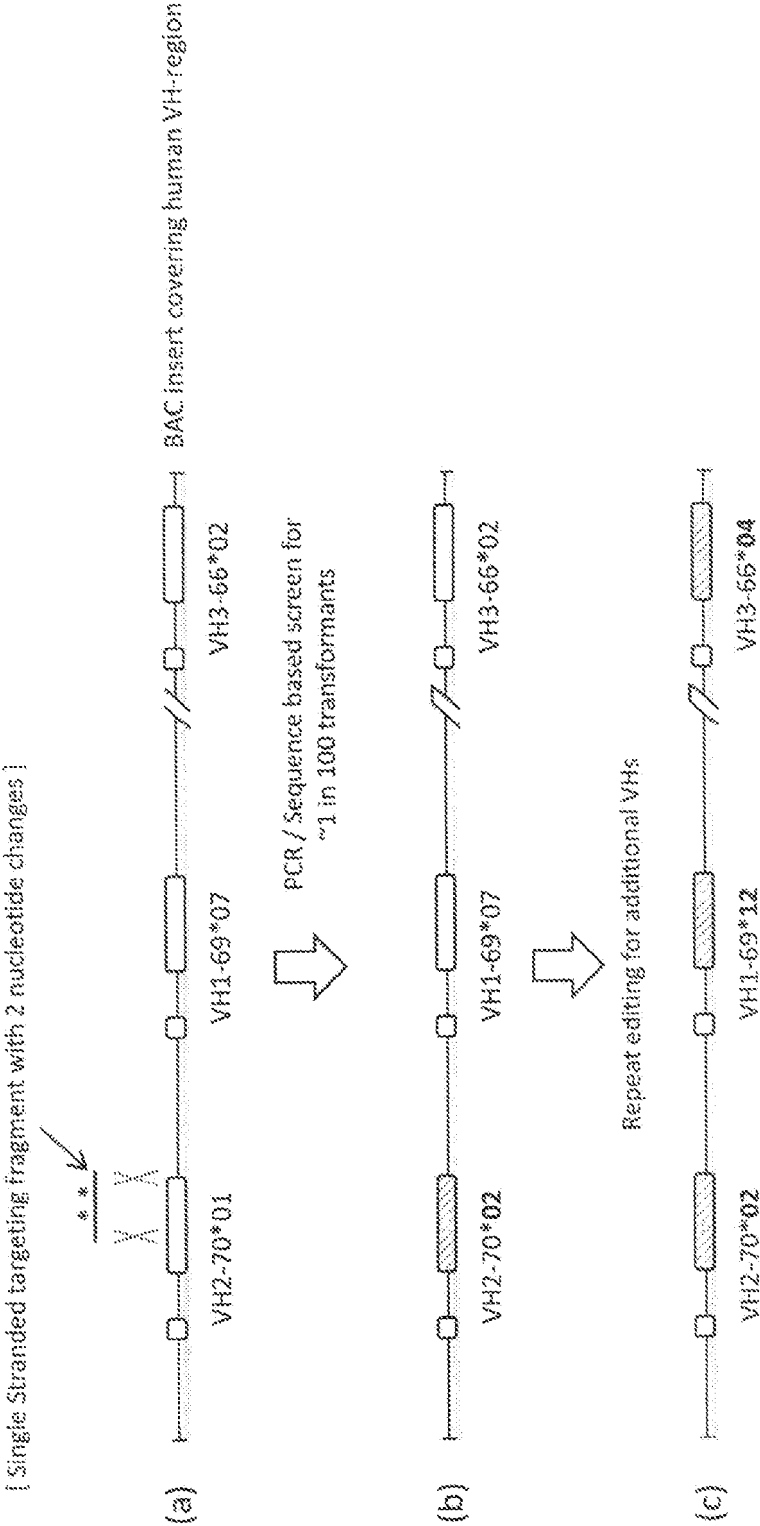
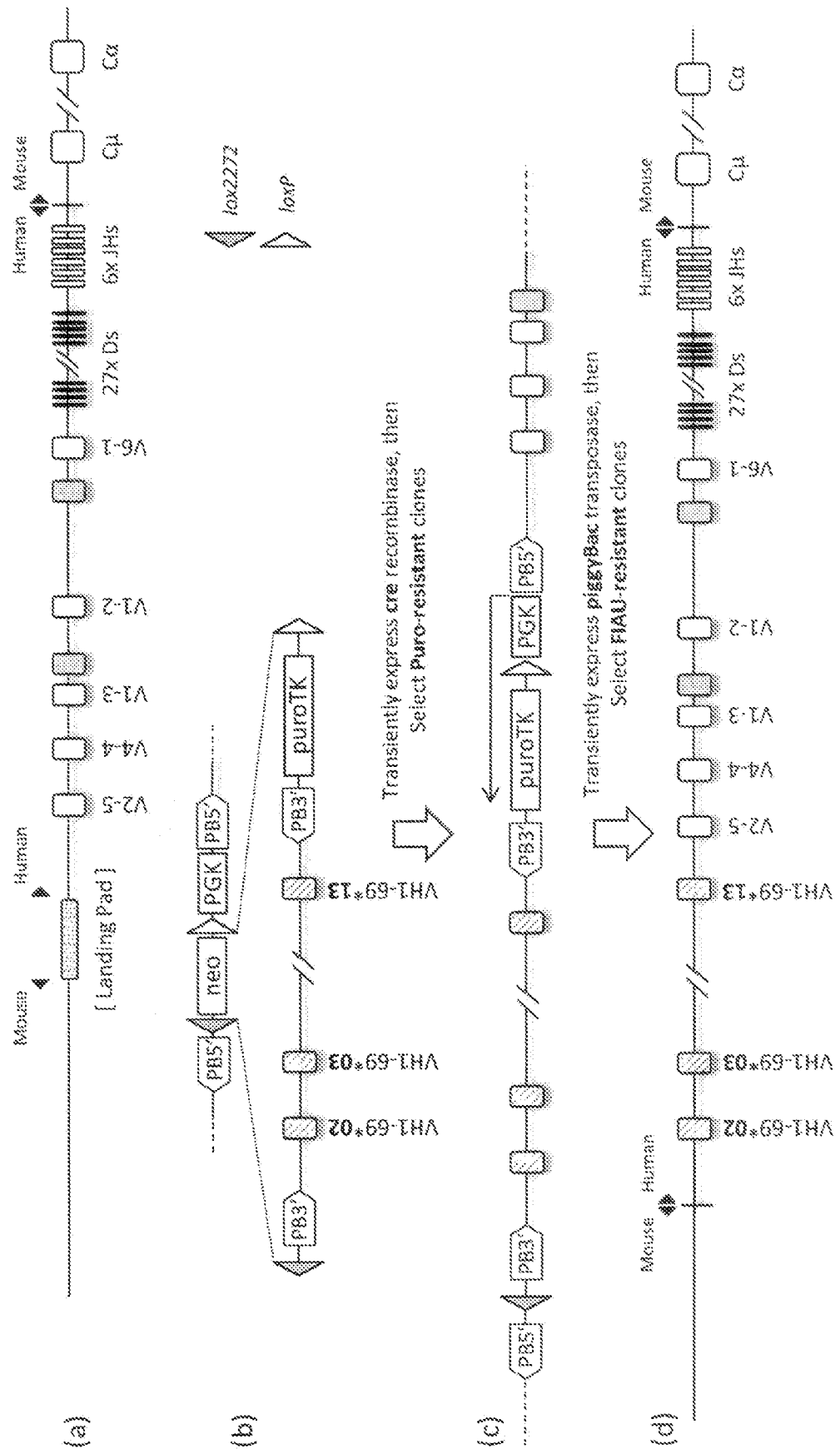


Figure 3

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

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



2000

	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	E	G	A	E	V	K	K	R	F	G	S	S	V	K	V	S	C	K	A	S
1L2582	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCT	...	GAG	GTG	AAG	AAG	ACT	GGG	TCC	TCC	GTG	AAG	GAC	TCC	TGC	ATG	GCT	TCT
217505	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Y92340	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
M83132	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
X67405	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
U22553	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
229978	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214309	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214307	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214300	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214296	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214301	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
214219	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

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	V	A					I	S	W	V	R	Q	A	P	G	Q	G	L	E	W	N
L22582 ,IGHV1-69*01, hv1051	GGA	GGC	ACC	TTC	AGC	AGC	TAT	GCT					ATC	AGC	TGG	GTC	CGA	CAG	GCC	GCT	GGA	CBA	GCG	GTT	GAG	TGG	ATG
227506 ,IGHV1-69*02, y1GH6(YAC7)								A																			
X92340 ,IGHV1-69*03, 57GHX8																											
M83131 ,IGHV1-69*04, hv1263																											
X67905 ,IGHV1-69*05, BK.VHL.2																											
L22563 ,IGHV1-69*06, hv1051N																											
X26979 ,IGHV1-69*07, EA-2																											
Z14309 ,IGHV1-69*08																											
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)

	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
	G	G	I	I	P	I	F	G	T	A			R	Y	A	G	K	F	Q	G	G	R	V	T	I	I	A
122582	CGA	GGG	ATC	ATC	CCT	ATC	TTT	GGT	ACA	GCA	...	...	ATC	TAC	SCA	CAG	AGG	TTC	CAG	...	GGC	AGA	GTC	ACG	ATT	ACC	GCG
227506	R																										
	A																										
832340																											
883132	R																										
	A																										
867905																											
122563	R																										
	A																										
229978																											
	A																										
214309	R																										
	A																										
214307	R																										
	A																										
214300																											
	R																										
214296	A																										
	A																										
214301																											
214314																											

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 3 of 4)

	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	CDR3
L22582, IGHV1-69*01, hV1051	D GAC GAA	E TCC	S ACC	T ACG	S AGC	T ACA	A GCC	T AAC	M ATG	E GAG	L CTG	S AGC	S AGC	L CTG	E GAG	T TGT	105 106
Z27506, IGHV1-69*02, YUSH6(YAC7)	K A																
X92340, IGHV1-69*03, 575TA8																	
X89332, IGHV1-69*04, hV1263	K A																
X67905, IGHV1-69*05, BR.VH1.2																	
Z22583, IGHV1-69*06, hV1051K	K A																
Z29978, IGHV1-69*07, DA-2																	
Z14309, IGHV1-69*08	K A																
Z14307, IGHV1-69*09	K A																
Z14300, IGHV1-69*10	K A																
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 4 of 4)

Figure 6

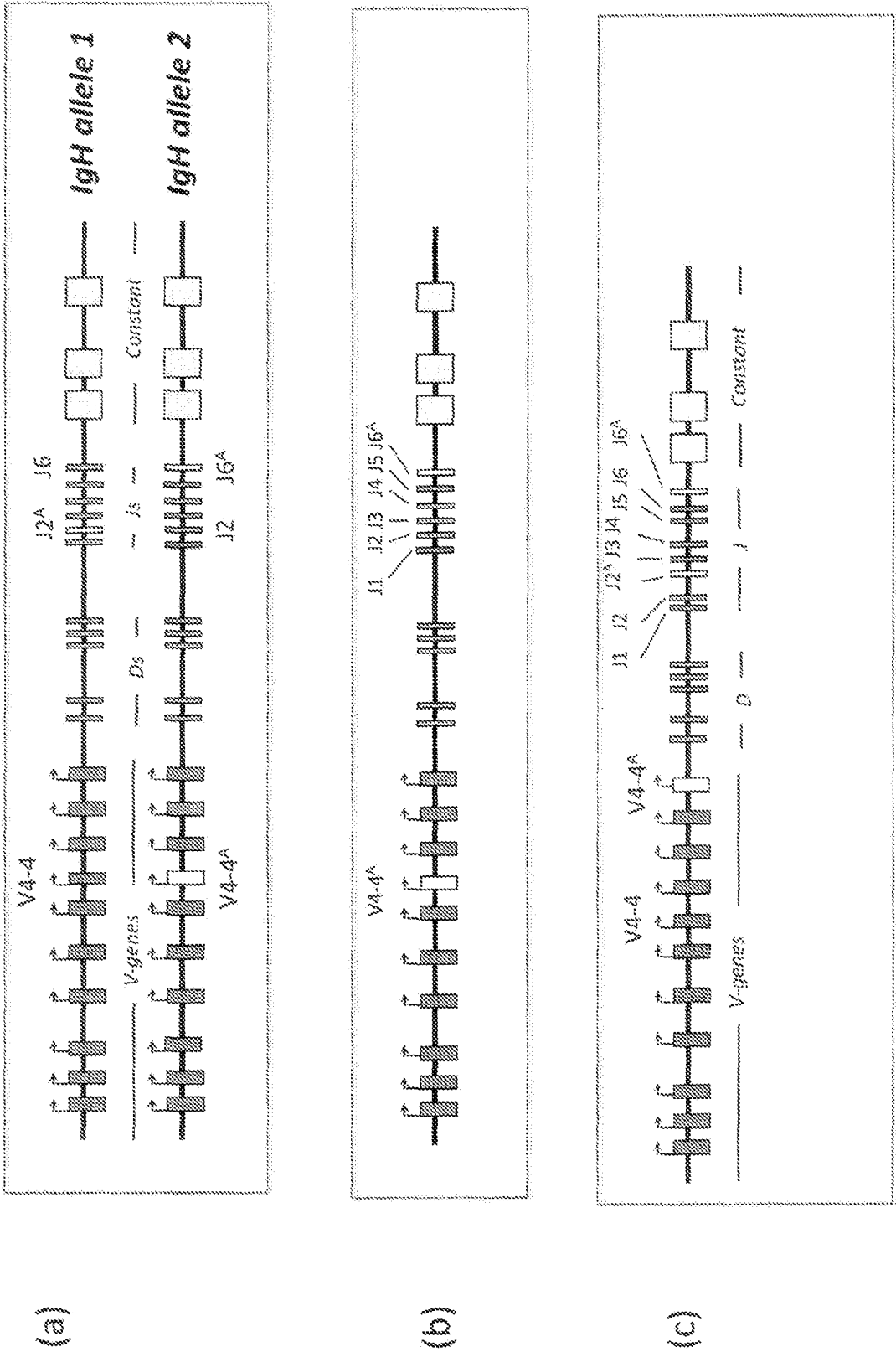


FIGURE 7

J00256, IGHJ6*01	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Y	Y	Y	Y	Y	G	M	D	V	W	G	Q	G	T	T	V	T	V	S	S
	AT	TAC	TAC	TAC	TAC	GGT	ATG	GAC	GTC	TGG	GGG	CAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	TCA
X86355, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
X86357, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
X86358, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
M63031, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
X97051, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
M25625, IGHJ6*02	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	---	---	---	---
X86356, IGHJ6*03	---	---	---	---	---	Y TAC	---	---	---	---	C	K A	---	---	---	---	---	---	---	---
X86359, IGHJ6*03	---	---	---	---	---	Y TAC	---	---	---	---	C	K A	---	---	---	---	---	---	---	---
M63030, IGHJ6*03	---	---	---	---	---	Y TAC	---	---	---	---	C	K A	---	---	---	---	---	---	---	---
AJ879487, IGHJ6*04	---	---	---	---	---	---	---	---	---	---	C	K A	---	---	---	---	---	---	---	---

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	Y	G	M D V
		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	TCC	Ser	S	TAC	Tyr	Y	TGC	Cys	C
	TTA	Leu	L	TCA	Ser	S	TAA	stop	*	TGA	stop	*
	TTG	Leu	L	TCG	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

