

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

1. Cassette Exchange -- Replace existing V-regions

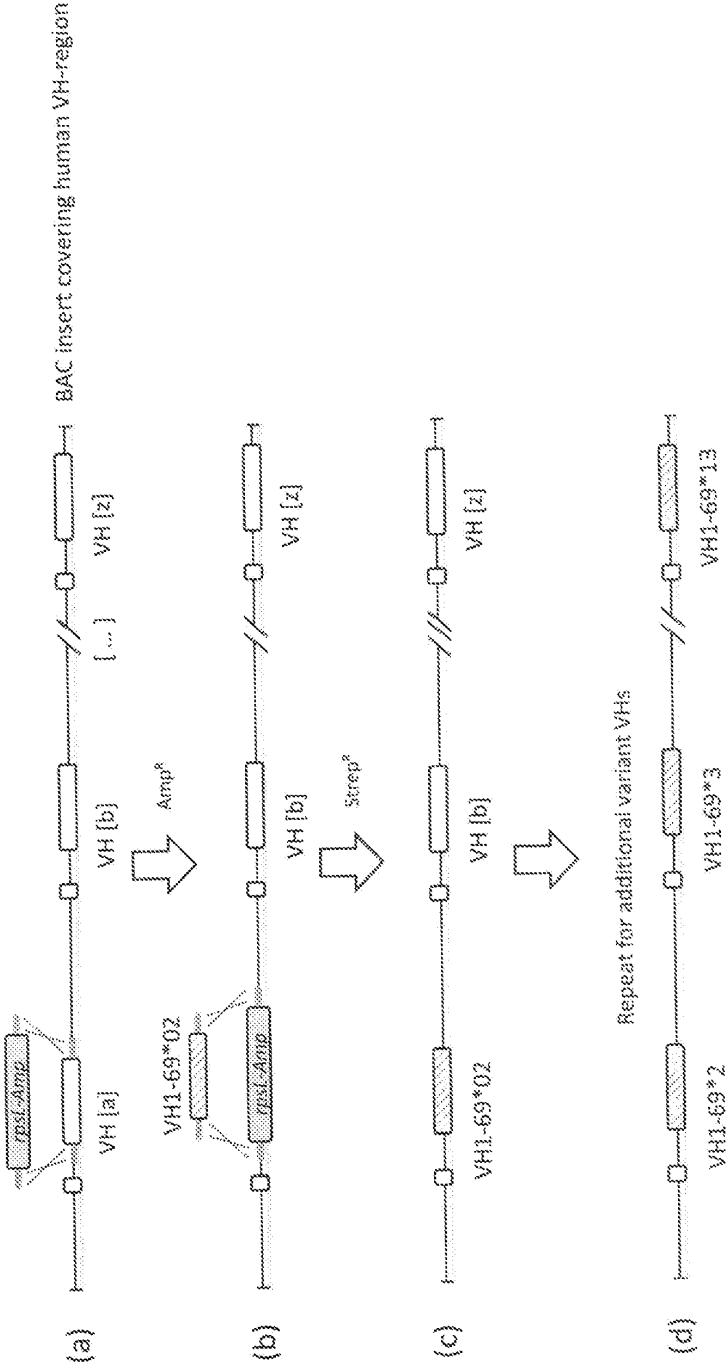


Figure 1

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

2. Insertion of V-regions into Heterologous Genomic DNA

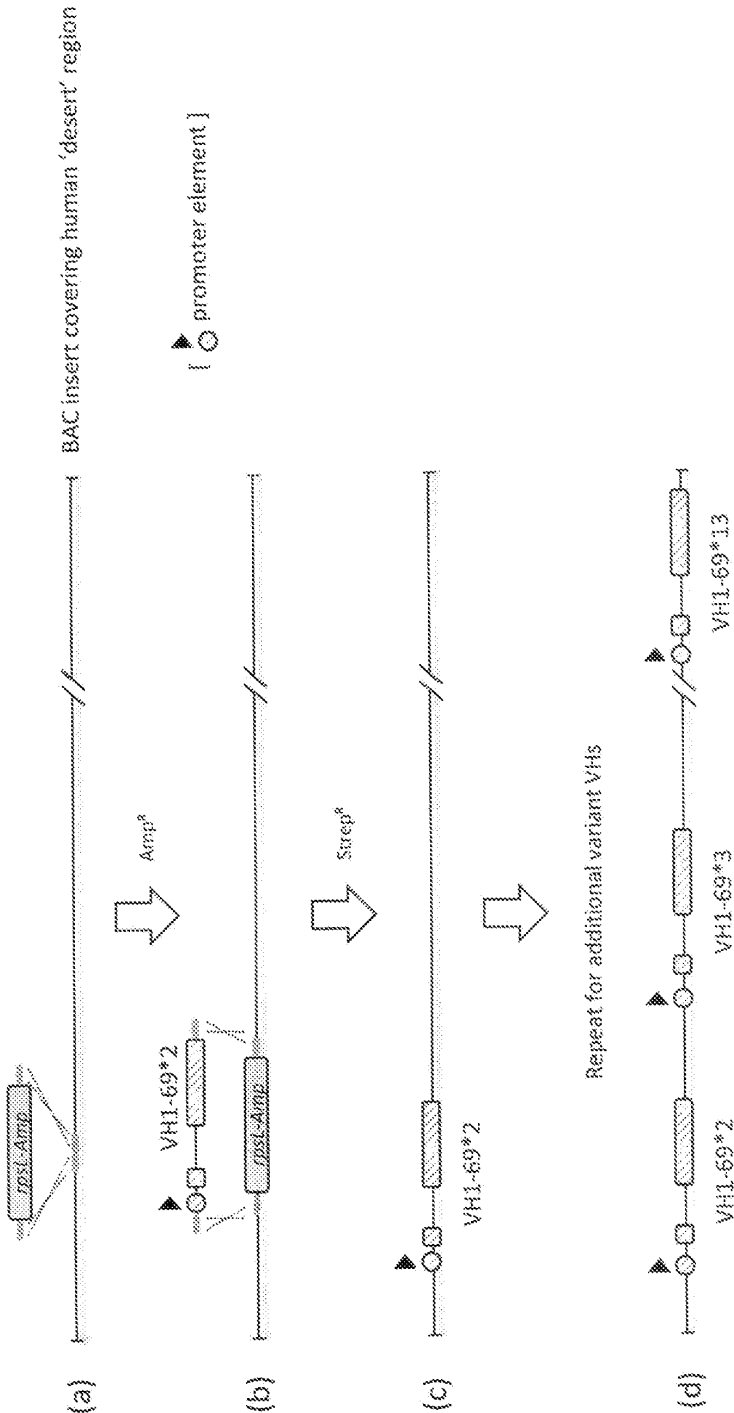


Figure 2

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

3. Single Strand Nucleotide Changes

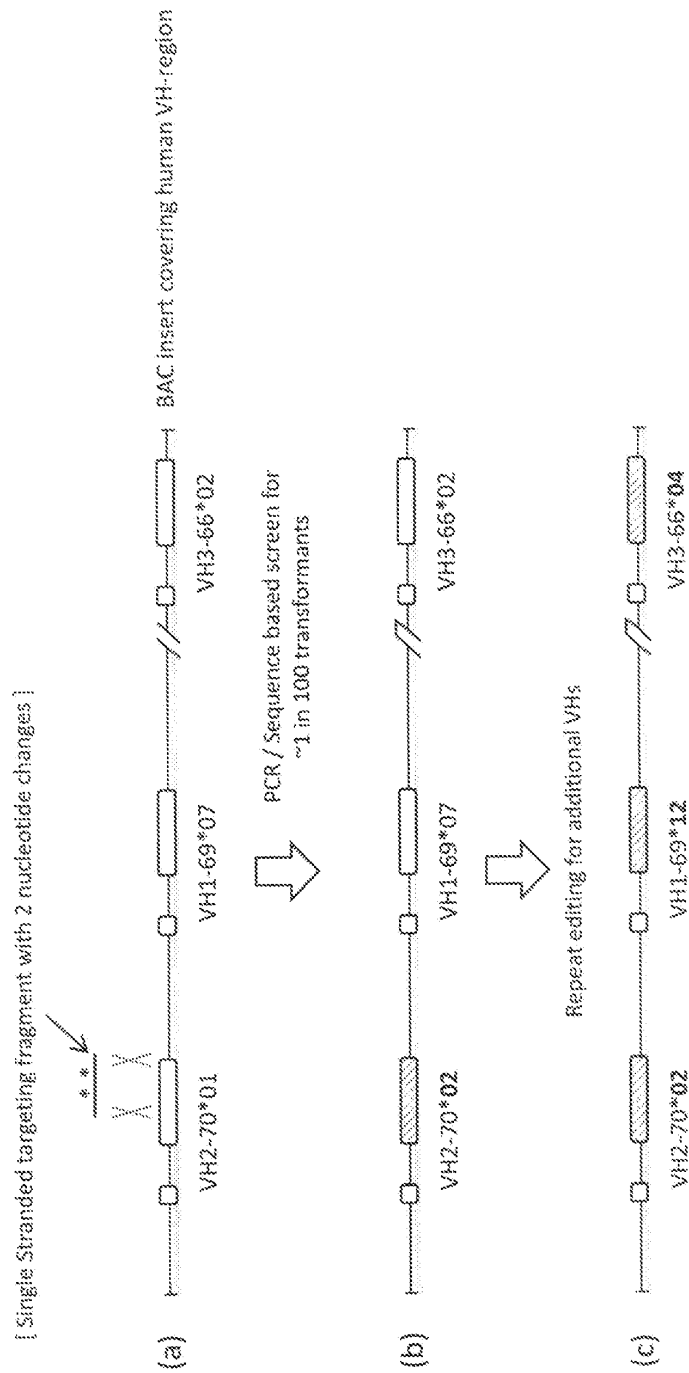


Figure 3

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

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

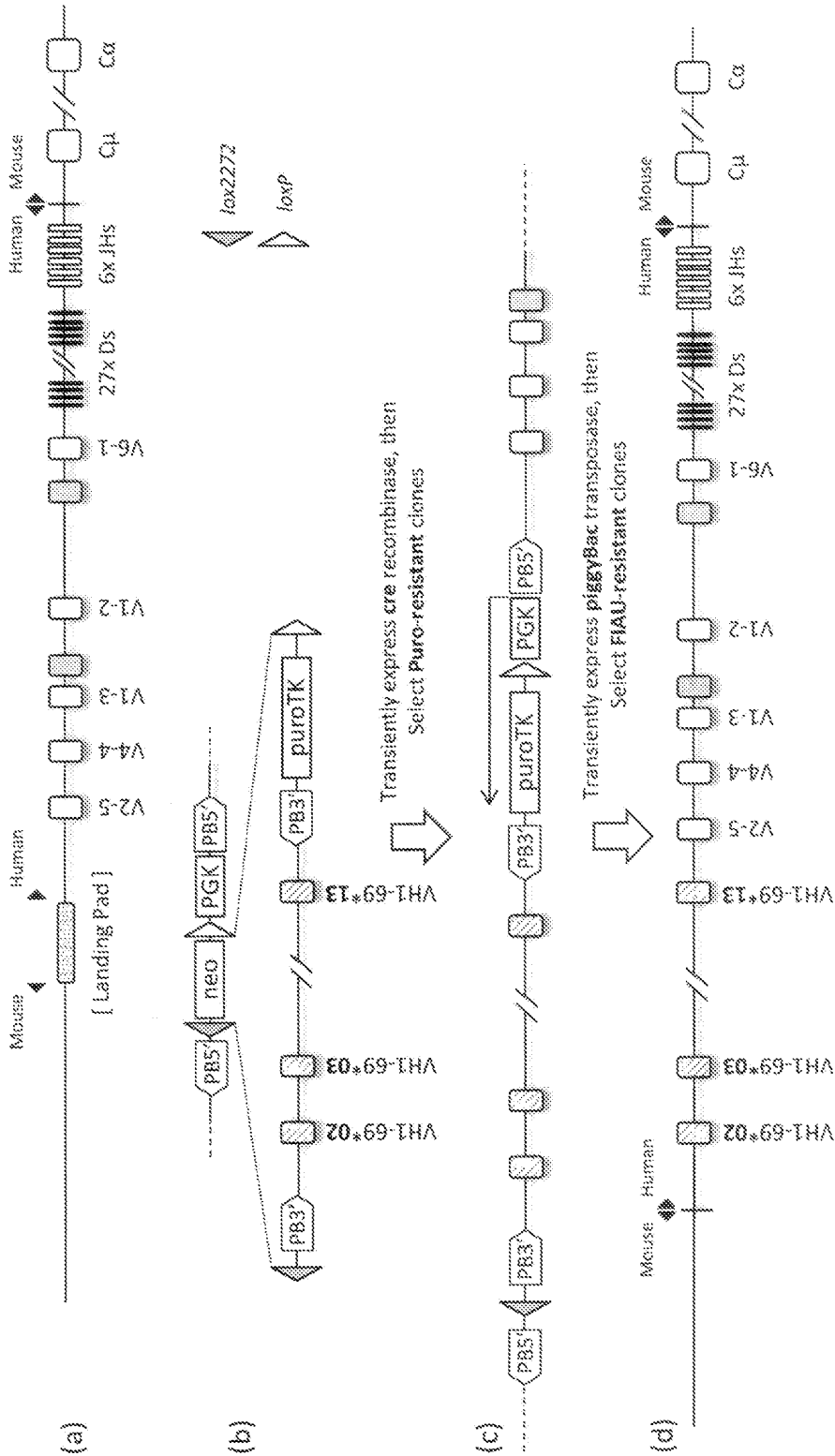


Figure 4

	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	K	K	A	P	G	S	S	Q	K	V	S	C	K	A	S
102582 ,IGHV1-69*01, hV1051	CAG	GTG	CAG	CTG	GTT	CAG	TCT	GAG	GTT	...	GAG	GTG	AAG	AAG	CCT	GAG	TCC	TCC	ATG	AAG	GTC	TCC	TCC	AAG	GCT	TCT
227505 ,IGHV1-69*02, yIGH6(YAC7)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
X02340 ,IGHV1-69*03, 578TA8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
X83132 ,IGHV1-69*04, hV1263	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
X67905 ,IGHV1-69*05, RR.VH1.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
122553 ,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	V	A					I	S	W	V	R	Q	A	P	G	Q	G	L	E	M	M
L22582 ,IGHV1-69*01, hv1051	GGA	GGC	ACC	TTT	ACC	AGC	TAT	GCT					ATC	AGC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	CAG	GCG	CTT	GAG	TGG	ATG
Z27506 ,IGHV1-69*02, y13H6(YAC?)								A																			
X92340 ,IGHV1-69*03, 57GHX6																											
M83133 ,IGHV1-69*04, hv1263																											
X67905 ,IGHV1-69*05, ER.VH1.2																											
L22563 ,IGHV1-69*06, hv1051N																											
Z22978 ,IGHV1-69*07, ER-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																											

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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	F	I	F	G	T	A			N	Y	A	Q	R	F	Q	G	G	R	V	T	I	T	A
LC2562	,IGHV1-69*01, hv1051	GGA	GAG	ATC	ATC	CCT	ATC	ATC	TTT	GCT	ACA	GCA	...	AAC	TAC	GCA	CAG	AAG	TTT	CAG	...	GGC	AGA	GTC	ACG	ATT	ACC	GCG
207506	,IGHV1-69*02, vIGH6(YAC7	R					L		C		I																	
392340	,IGHV1-69*03, 573TA9																											
883132	,IGHV1-69*04, hv1263	R					I			I																		
867905	,IGHV1-69*05, RR.VH1.2																											T
122583	,IGHV1-69*06, hv1051K	R																										
229978	,IGHV1-69*07, OA-2	R																										
214309	,IGHV1-69*08	R																										
214387	,IGHV1-69*09	R																										
214300	,IGHV1-69*10	R																										
214296	,IGHV1-69*11	R																										
214301	,IGHV1-69*12																											
214214	,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 3 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	
	D	E	S	T	S	T	A	X	N	E	L	S	S	L	K	S	E	D	T	A	V	Y	Y	C	A	K	
L22882 ,IGHV1-69*01, hv1051	GAC	GAA	TCC	ACG	AGC	ACA	GCC	TAC	ATG	GAG	CTG	AGC	AGC	CTG	ACA	TCT	GAG	GAC	ACG	GCC	GTG	TAT	TAC	TGT	GCG	AGA	GA
	K																										
227506 ,IGHV1-69*02, YIGH6(YAC7)	K																D										
X92340 ,IGHV1-69*03, 57GTA8																											
	K																										
M83131 ,IGHV1-69*04, hv1263																											
	K																										
X67905 ,IGHV1-69*05, ER-VH1.2																											
	K																										
L22563 ,IGHV1-69*06, hv1051K																											
	K																										
228978 ,IGHV1-69*07, DA-2																											
	K																										
214309 ,IGHV1-69*08																											
	K																										
214307 ,IGHV1-69*09																											
	K																										
214300 ,IGHV1-69*10																											
	K																										
214296 ,IGHV1-69*11																											
	K																										
214301 ,IGHV1-69*12																											
	K																										
214214 ,IGHV1-69*13																											
	K																										

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

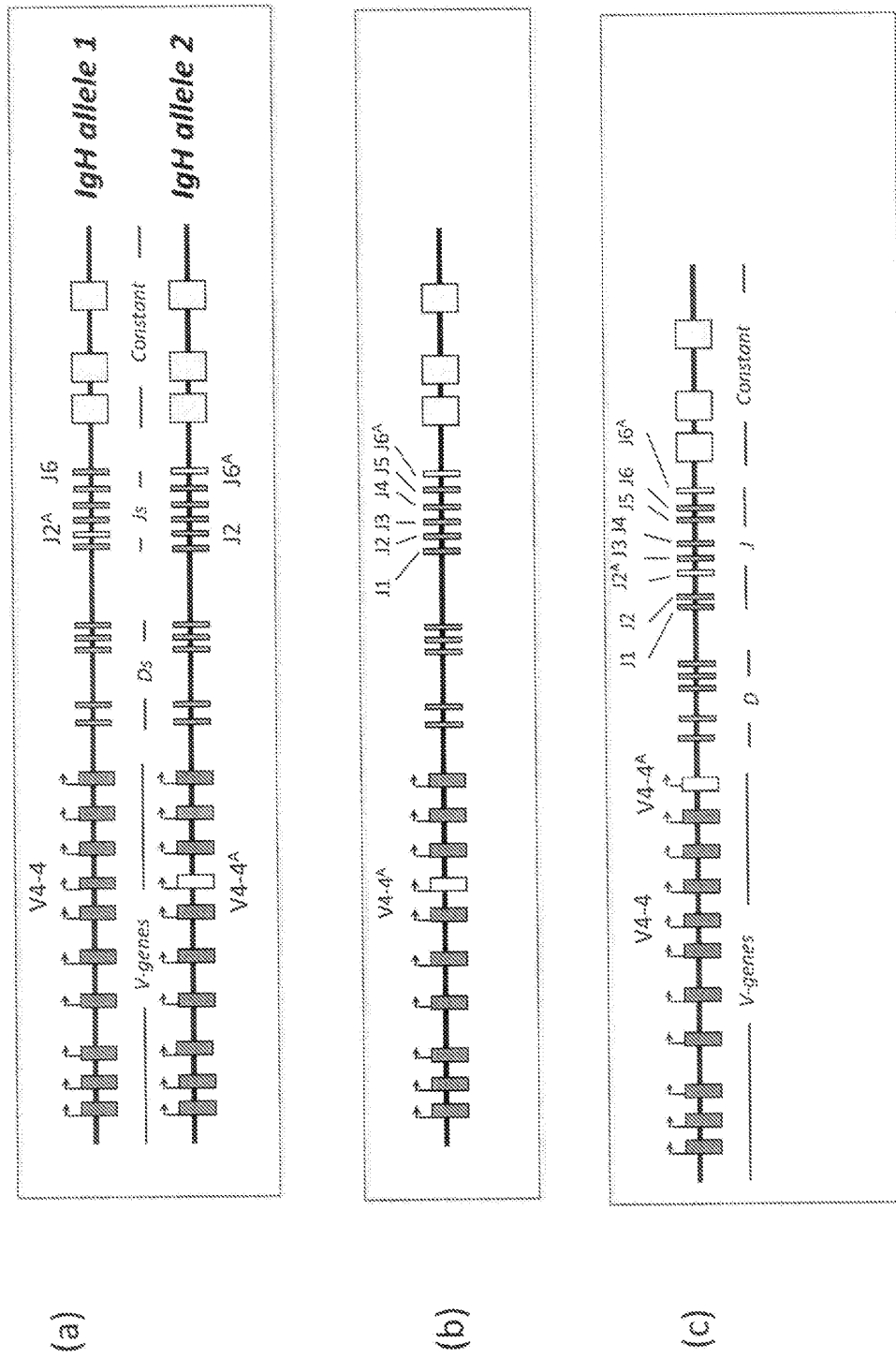


FIGURE 7

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

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	V
		at	tac	tac	tac	ggt	atg	gac
			Y	Y				gtc
			tac	tac				

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

