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IGKV1-39*01	-----FR1-IMGT----- CDR1 -----FR2-IMGT--- C -----
A	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQKPKAPKLLIYAASSLQSGVPS
B	F.....T H I.....V.....YT.....
C	A.....I.....NF.....G.YN.....
D	 HN .NN.....H.....N.....
E	A.....T.....N.....
F	N.....N.....D.....
G	A.L.....N.....N.....D.....
H	A.....N.N..... H D.....F.....
I	A.L.....
J	A.....
K	A.L.....M.....

IGKV1-39*01	-----FR3-IMGT----- CDR3-	SEQ ID NO:1
A	RFGSGSGTDFTLTISSLPEDFATYQCQSYST-P-----	SEQ ID NO:136
B	...A.....T.....NI...FTFGPGTKVDIKR	SEQ ID NO:137
C	I..... H -----LTFGGGTKVEIKR	SEQ ID NO:138
D	Y.....T.H.....GN.....SMYTFGQGTQLEIKR	SEQ ID NO:139
E	 H I.....ITFGQGTTRLEIKR	SEQ ID NO:140
F	T.H..... H ITFGQGTTRLEIKR	SEQ ID NO:141
G	T..... H ITFGQGTTRLEIKR	SEQ ID NO:142
H	P.ITFGQGTTRVEIKR	SEQ ID NO:143
I	...K.....I H .ITFGQGTTRLEIKR	SEQ ID NO:144
J	 H .IP.ITFGQGTTRLEIK.	SEQ ID NO:145
K	...T.....R..... H ..P.ITFGQGTTRLEIKR	SEQ ID NO:146

FIG. 1

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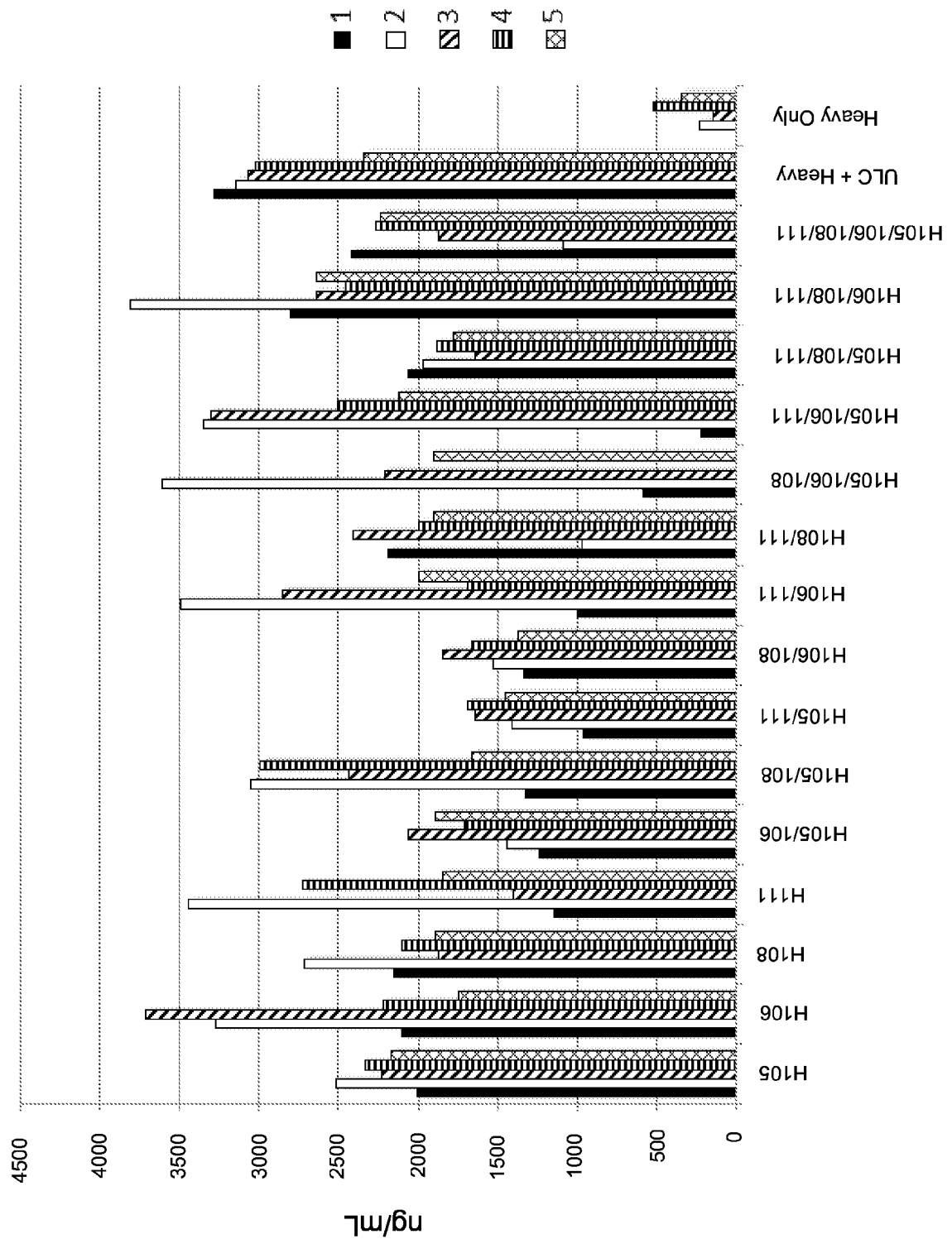


FIG. 3

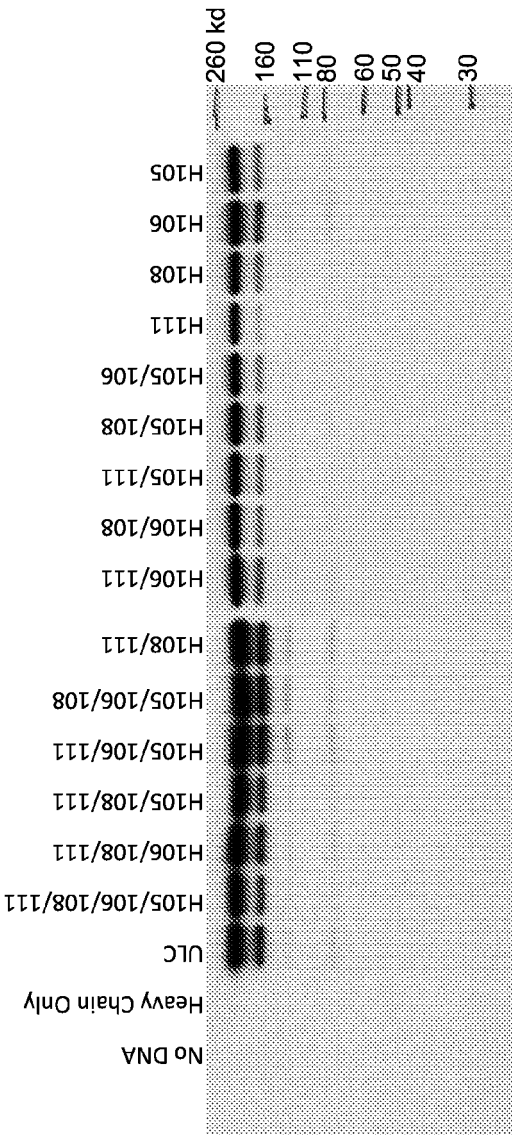


FIG. 4

Heavy Chain	Light Chain Mutant	Kinetics at pH 7.4						Kinetics at pH 5.75						Ratio of low pH / neutral pH		
		mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	ka	kd	KD
1	H105	488	47.2	3.52E+05	9.30E-03	2.64E-08	1.2	378	37.8	4.24E+05	1.38E-02	3.24E-08	0.8	1.2	1.5	1.2
	H106	620	73.7	3.22E+05	3.67E-03	1.14E-08	3.1	505	65.4	3.66E+05	4.86E-03	1.33E-08	2.4	1.1	1.3	1.2
	H108	648	99.0	3.17E+05	2.07E-03	6.54E-09	5.6	496	80.7	3.59E+05	3.02E-03	8.41E-09	3.8	1.1	1.5	1.3
	H111	669	81.0	3.01E+05	3.81E-03	1.27E-08	3.0	536	62.8	3.32E+05	5.41E-03	1.63E-08	2.1	1.1	1.4	1.3
	H105/106	492	55.6	3.74E+05	6.44E-03	1.72E-08	1.8	404	45.7	4.30E+05	1.01E-02	2.34E-08	1.1	1.2	1.6	1.4
	H105/108	538	75.2	3.28E+05	4.17E-03	1.27E-08	2.8	416	56.3	3.87E+05	7.43E-03	1.92E-08	1.6	1.2	1.8	1.5
	H105/111	501	44.6	3.44E+05	9.77E-03	2.84E-08	1.2	402	31.9	4.06E+05	1.70E-02	4.19E-08	0.7	1.2	1.7	1.5
	H106/108	494	64.9	3.36E+05	4.14E-03	1.23E-08	2.8	407	43.0	4.07E+05	1.17E-02	2.86E-08	1.0	1.2	2.8	2.3
	H106/111	536	78.2	3.09E+05	3.10E-03	1.00E-08	3.7	423	61.4	3.62E+05	4.68E-03	1.29E-08	2.5	1.2	1.5	1.3
	H108/111	584	78.5	3.11E+05	3.80E-03	1.22E-08	3.0	473	59.0	3.50E+05	6.13E-03	1.75E-08	1.9	1.1	1.6	1.4
	H105/106/108	442	51.2	3.72E+05	6.38E-03	1.71E-08	1.8	370	28.4	4.26E+05	2.13E-02	5.00E-08	0.5	1.1	3.3	2.9
	H105/106/111	473	62.8	3.40E+05	4.55E-03	1.34E-08	2.5	378	47.6	4.07E+05	7.42E-03	1.82E-08	1.6	1.2	1.6	1.4
	H105/108/111	433	49.5	3.57E+05	7.42E-03	2.08E-08	1.6	354	31.3	4.09E+05	1.73E-02	4.23E-08	0.7	1.1	2.3	2.0
	H106/108/111	491	66.6	3.44E+05	3.82E-03	1.11E-08	3.0	415	43.3	3.91E+05	1.06E-02	2.72E-08	1.1	1.1	2.8	2.5
	H105/106/108/111	454	57.9	3.51E+05	5.77E-03	1.64E-08	2.0	368	36.8	4.06E+05	1.35E-02	3.32E-08	0.9	1.2	2.3	2.0
	ULC + Heavy	586	76.0	3.16E+05	3.85E-03	1.22E-08	3.0	483	66.5	3.45E+05	4.58E-03	1.33E-08	2.5	1.1	1.2	1.1
	Heavy only	141	-1.3	NB	NB	NB	NB	107	-6.7	NB	NB	NB	NB	Negative Control		
	No DNA	60	-1.5	NB	NB	NB	NB	26	-7.2	NB	NB	NB	NB			

FIG. 5A

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		Kinetics at pH 7.4						Kinetics at pH 5.75						Ratio of low pH / neutral pH		
Heavy Chain	Light Chain Mutant	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t½ (min)	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t½ (min)	ka	kd	KD
	H105	720	33.7	1.02E+05	9.10E-04	8.89E-09	12.7	595	0.3	NB	NB	NB	NB	No binding at Low pH		
	H106	735	32.9	9.11E+04	4.47E-04	4.91E-09	25.8	623	4.3	2.27E+04	6.41E-03	2.82E-07	1.8	0.2	14.3	57.4
	H108	728	32.2	8.48E+04	2.67E-04	3.14E-09	43.3	598	3.6	1.16E+03	3.41E-03	2.93E-06	3.4	0.0	12.8	933.1
	H111	802	42.4	1.17E+05	1.15E-04	9.88E-10	100.3	670	14.1	3.01E+04	7.08E-04	2.35E-08	16.3	0.3	6.1	23.8
	H105/106	570	24.9	8.51E+04	2.92E-04	3.43E-09	39.5	484	2.4	1.14E+04	6.47E-03	5.69E-07	1.8	0.1	22.1	165.9
	H105/108	723	28.4	8.73E+04	1.30E-03	1.49E-08	8.9	603	-2.6	NB	NB	NB	NB	No binding at Low pH		
	H105/111	627	28.5	8.82E+04	3.60E-04	4.08E-09	32.1	519	2.3	1.02E+04	1.30E-02	1.27E-06	0.9	0.1	36.2	311.3
	H106/108	550	21.1	6.75E+04	9.12E-04	1.35E-08	12.7	470	-1.9	NB	NB	NB	NB	No binding at Low pH		
	H106/111	808	32.7	9.01E+04	6.78E-04	7.52E-09	17.0	678	0.9	NB	NB	NB	NB	No binding at Low pH		
2	H108/111	801	40.5	1.17E+05	9.80E-05	8.40E-10	117.8	676	10.6	1.87E+04	5.84E-04	3.12E-08	19.8	0.2	6.0	37.1
	H105/106/108	409	13.0	8.21E+04	4.29E-04	5.22E-09	26.9	345	-4.4	NB	NB	NB	NB	No binding at Low pH		
	H105/106/111	767	9.3	4.84E+04	1.25E-03	2.58E-08	9.3	645	-6.1	NB	NB	NB	NB	No binding at Low pH		
	H105/108/111	578	24.5	8.99E+04	2.87E-04	3.19E-09	40.2	479	-0.7	NB	NB	NB	NB	No binding at Low pH		
	H106/108/111	765	15.1	5.04E+04	1.65E-03	3.27E-08	7.0	661	-5.9	NB	NB	NB	NB	No binding at Low pH		
	H105/106/108/111	723	0.3	NB	NB	NB	NB	611	-7.0	NB	NB	NB	NB	No binding at either pH tested		
	ULC + Heavy	781	39.8	1.13E+05	1.78E-04	1.57E-09	64.9	665	11.7	2.04E+04	1.22E-03	6.00E-08	9.4	0.2	6.9	38.2
	Heavy only	143	-1.8	NB	NB	NB	NB	115	-6.1	NB	NB	NB	NB	Negative Control		
	No DNA	63	-2.1	NB	NB	NB	NB	35	-6.4	NB	NB	NB	NB	Negative Control		

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FIG. 5B

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Heavy Chain	Light Chain Mutant	Kinetics at pH 7.4						Kinetics at pH 5.75						Ratio of low pH / neutral pH		
		mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	ka	kd	KD
3	H105	574	12.4	5.64E+04	1.21E-03	2.15E-08	9.5	496	0.1	NB	NB	NB	NB	No binding at Low pH		
	H106	662	16.1	7.37E+04	5.36E-04	7.27E-09	21.5	584	3.0	3.00E+04	4.79E-03	1.58E-07	2.4	0.4	8.9	21.7
	H108	518	12.3	4.82E+04	2.15E-04	4.46E-09	53.7	440	2.2	3.70E+04	1.25E-03	3.40E-08	9.3	0.8	5.8	7.6
	H111	480	9.1	1.82E+04	4.16E-04	2.28E-08	27.7	406	0.4	NB	NB	NB	NB	No binding at Low pH		
	H105/106	487	7.4	1.82E+04	7.45E-03	4.10E-07	1.6	423	-4.1	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/108	548	11.8	3.25E+04	5.25E-04	1.62E-08	22.0	468	0.0	NB	NB	NB	NB	No binding at Low pH		
	H105/111	504	7.9	1.06E+04	1.66E-03	1.57E-07	7.0	430	-1.6	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106/108	487	10.7	6.64E+04	2.91E-04	4.38E-09	39.7	424	-4.2	NB	NB	NB	NB	No binding at Low pH		
	H106/111	563	10.5	2.75E+04	3.71E-04	1.35E-08	31.2	482	-1.9	NB	NB	NB	NB	No binding at Low pH		
	H108/111	528	10.1	1.10E+04	8.01E-05	7.27E-09	144.2	449	-0.5	NB	NB	NB	NB	No binding at Low pH		
	H105/106/108	470	6.4	2.64E+04	5.40E-03	2.04E-07	2.1	412	-5.3	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/106/111	539	0.8	NB	NB	NB	NB	462	-5.6	NB	NB	NB	NB	No binding at either pH tested		
	H105/108/111	419	6.5	1.76E+04	4.01E-04	2.27E-08	28.8	354	-3.8	NB	NB	NB	NB	No binding at Low pH		
	H106/108/111	509	3.3	5.47E+04	9.07E-04	1.66E-08	12.7	443	-5.9	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/106/108/111	450	-1.1	NB	NB	NB	NB	382	-6.4	NB	NB	NB	NB	No binding at either pH tested		
	ULC + Heavy	688	16.9	3.54E+04	3.57E-04	1.01E-08	32.3	598	5.0	1.16E+04	1.76E-03	1.52E-07	6.6	0.3	4.9	15.0
	Heavy only	152	-1.6	NB	NB	NB	NB	127	-5.4	NB	NB	NB	NB	Negative Control		
	No DNA	54	-1.7	NB	NB	NB	NB	30	-5.6	NB	NB	NB	NB			

FIG. 5C

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Heavy Chain	Light Chain Mutant	Kinetics at pH 7.4						Kinetics at pH 5.75						Ratio of low pH / neutral pH		
		mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	ka	kd	KD
4	H105	598	4.9	2.80E+04	1.38E-03	4.91E-08	8.4	512	-0.2	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106	569	7.1	1.49E+04	9.67E-04	6.49E-08	11.9	494	1.2	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H108	587	6.3	2.25E+04	2.02E-03	8.96E-08	5.7	500	-0.9	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H111	606	5.6	3.46E+04	2.19E-03	6.33E-08	5.3	521	-0.9	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/106	517	3.1	2.00E+04	2.05E-03	1.03E-07	5.6	460	-1.1	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/108	581	2.5	1.61E+05	2.30E-03	1.43E-08	5.0	506	-2.7	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/111	575	3.8	1.01E+04	1.70E-03	1.68E-07	6.8	504	-2.0	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106/108	498	2.5	4.01E+04	6.07E-04	1.51E-08	19.0	447	-3.9	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106/111	587	3.0	7.85E+04	9.20E-04	1.17E-08	12.6	509	-2.6	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H108/111	599	4.0	9.68E+04	2.02E-03	2.09E-08	5.7	528	-2.6	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/106/108	64	-1.7	NB	NB	NB	NB	46	-5.4	NB	NB	NB	NB	No binding at either pH tested		
	H105/106/111	561	1.1	NB	NB	NB	NB	493	-2.8	NB	NB	NB	NB	No binding at either pH tested		
	H105/108/111	521	2.7	7.66E+04	1.29E-03	1.68E-08	9.0	461	-3.2	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106/108/111	577	1.3	NB	NB	NB	NB	514	-4.2	NB	NB	NB	NB	No binding at either pH tested		
	H105/106/108/111	554	0.6	NB	NB	NB	NB	495	-4.8	NB	NB	NB	NB	No binding at either pH tested		
	ULC + Heavy	585	7.4	5.69E+04	2.01E-03	3.53E-08	5.7	527	1.9	NB	NB	NB	NB	No binding at Low pH		
	Heavy only	187	-2.4	NB	NB	NB	NB	161	-5.4	NB	NB	NB	NB	Negative Control		
	No DNA	49	-2.6	NB	NB	NB	NB	25	-5.6	NB	NB	NB	NB			

FIG. 5D

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Heavy Chain	Light Chain Mutant	Kinetics at pH 7.4						Kinetics at pH 5.75						Ratio of low pH / neutral pH		
		mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	mAb captured (RU)	50nM Antigen Binding (RU)	ka (1/Ms)	kd (1/s)	KD (M)	t _{1/2} (min)	ka	kd	KD
5	H105	522	3.2	5.05E+04	9.48E-03	1.88E-07	1.2	463	-4.3	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H106	496	0.3	NB	NB	NB	NB	437	-5.2	NB	NB	NB	NB	No binding at either pH tested		
	H108	475	-0.1	NB	NB	NB	NB	415	-5.7	NB	NB	NB	NB	No binding at either pH tested		
	H111	523	1.6	NB	NB	NB	NB	463	-4.7	NB	NB	NB	NB	No binding at either pH tested		
	H105/106	534	-0.6	NB	NB	NB	NB	472	-5.5	NB	NB	NB	NB	No binding at either pH tested		
	H105/108	499	2.0	5.80E+04	1.16E-02	1.99E-07	1.0	433	-5.5	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/111	489	0.1	NB	NB	NB	NB	426	-4.7	NB	NB	NB	NB	No binding at either pH tested		
	H106/108	441	-0.5	NB	NB	NB	NB	386	-4.2	NB	NB	NB	NB	No binding at either pH tested		
	H106/111	529	-0.8	NB	NB	NB	NB	461	-6.3	NB	NB	NB	NB	No binding at either pH tested		
	H108/111	495	4.1	1.73E+05	1.06E-02	6.11E-08	1.1	435	-4.0	NB	NB	NB	NB	Poor binding at Neutral, No binding at Low pH		
	H105/106/108	505	-1.1	NB	NB	NB	NB	444	-5.5	NB	NB	NB	NB	No binding at either pH tested		
	H105/106/111	499	-1.7	NB	NB	NB	NB	438	-5.8	NB	NB	NB	NB	No binding at either pH tested		
	H105/108/111	436	0.8	NB	NB	NB	NB	388	-3.7	NB	NB	NB	NB	No binding at either pH tested		
	H106/108/111	539	-1.4	NB	NB	NB	NB	477	-4.0	NB	NB	NB	NB	No binding at either pH tested		
	H105/106/108/111	556	-1.6	NB	NB	NB	NB	490	-4.4	NB	NB	NB	NB	No binding at either pH tested		
	ULC + Heavy	516	0.6	NB	NB	NB	NB	451	-5.0	NB	NB	NB	NB	No binding at either pH tested		
	Heavy only	144	-1.3	NB	NB	NB	NB	122	-5.7	NB	NB	NB	NB	No binding at either pH tested		
	No DNA	47	-1.4	NB	NB	NB	NB	25	-5.8	NB	NB	NB	NB	Negative Control		

FIG. 5E

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V _H	V _K	K _D at pH7.4 (nM)	K _D at pH5.75 (nM)	T _{1/2} at pH7.4 (min)	T _{1/2} at pH5.75 (min)
2	Parental ULC	1.6	60	65	9.4
2	ULC (His105,106)	3.4	570	39	1.8
2	ULC (His105,111)	4.1	1270	32	0.9
2	ULC (His105,108,111)	3.2	NB	40	NB
2	ULC (His105,106,108)	5.2	NB	27	NB
3	Parental ULC	10.1	152	32	6.6
3	ULC (His106,108)	4.4	NB	40	NB
3	ULC (His108,111)	7.3	NB	144	NB
6	Parental ULC	3.0	2.4	10	6
6	ULC (His105,111)	3.5	NB	9	NB
6	ULC (His106, 108,111)	2.0	NB	16	NB

FIG. 6

Site-directed mutagenesis primers for Histidine substitutions in CDR3 of Universal Light Chain of hVK1-39JK5

Primer name	Sequence	%GC	N	%mismatch	Tm
GERMLINE hVH1-39/JK5	CAACTTACTACTGTCAAACAGAGTTACAGTACCCCTCCGATCACCTTCGGC				
1633-H106/108/111 F	CTTACTACTGTCAACA T AGTCACAGTACCC A TCCGATCACCTTCG	47.0	45	6.7	79.0
1633-H105/106/108/111 F	CAACTTACTACTGTCA C CA T AGTCACAGTACCC A TCCGATCACCTTCGGC	50.0	50	8.0	80.5
1633-H106/108/111 R	CGAAGGTGATCGGA T GGGTACTGTGACT A TGTTGACAGTAGTAAG	47.0	45	6.7	79.0
1633-H105/106/108/111 R	GCCGAAGGTGATCGGA T GGGTACTGTGACT A TGTTGACAGTAGTAAGTTG	50.0	50	8.0	80.5

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Primer	SEQ ID NO
GERMLINE hVK1-39/JK5	81
1633-H106/108/111 F	34
1633-H105/106/108/111 F	35
1633-H106/108/111 R	36
1633-H105/106/108/111 R	37

FIG. 7

Construction of VK1-39 HULC LTVECs



FIG. 8A

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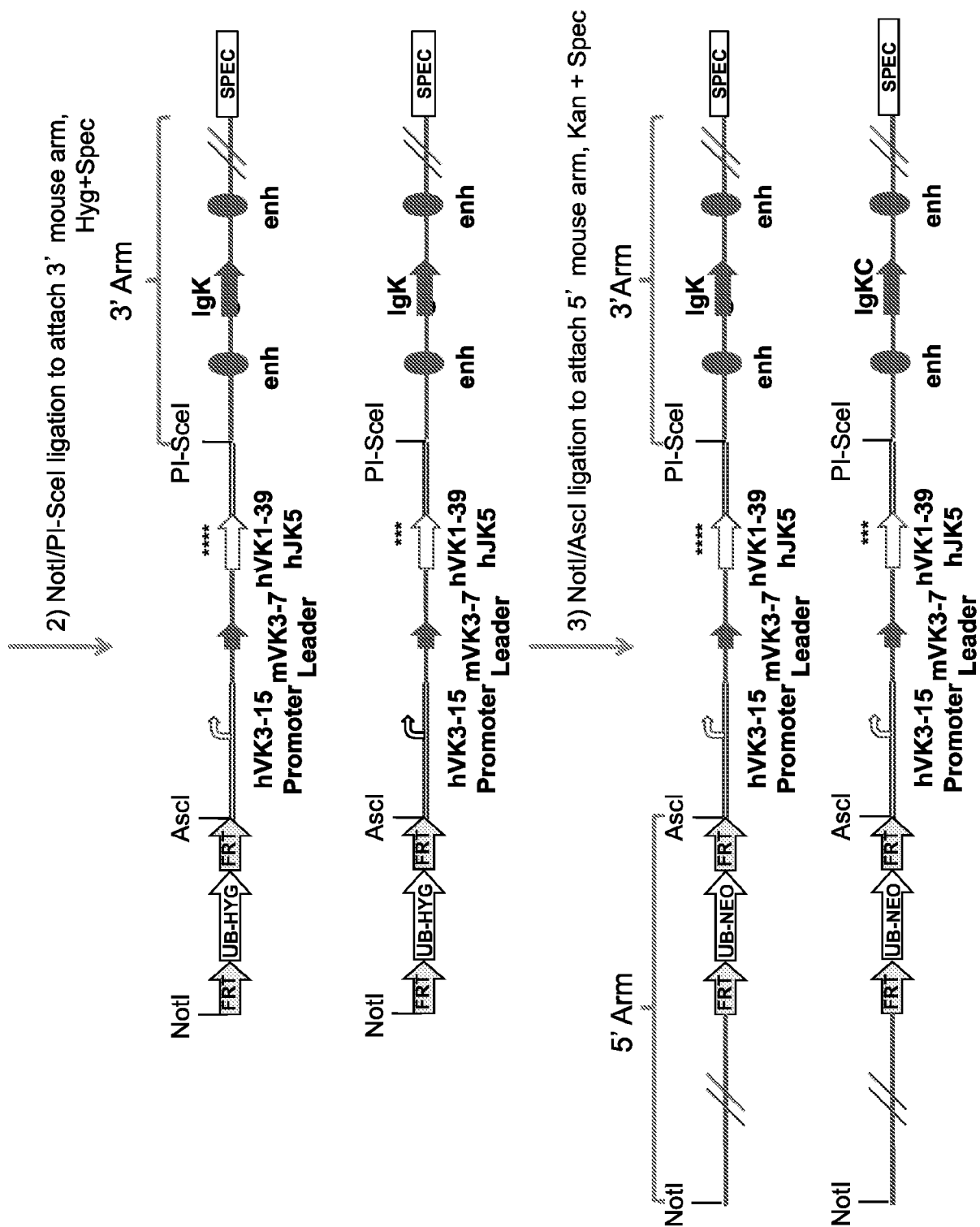


FIG. 8B

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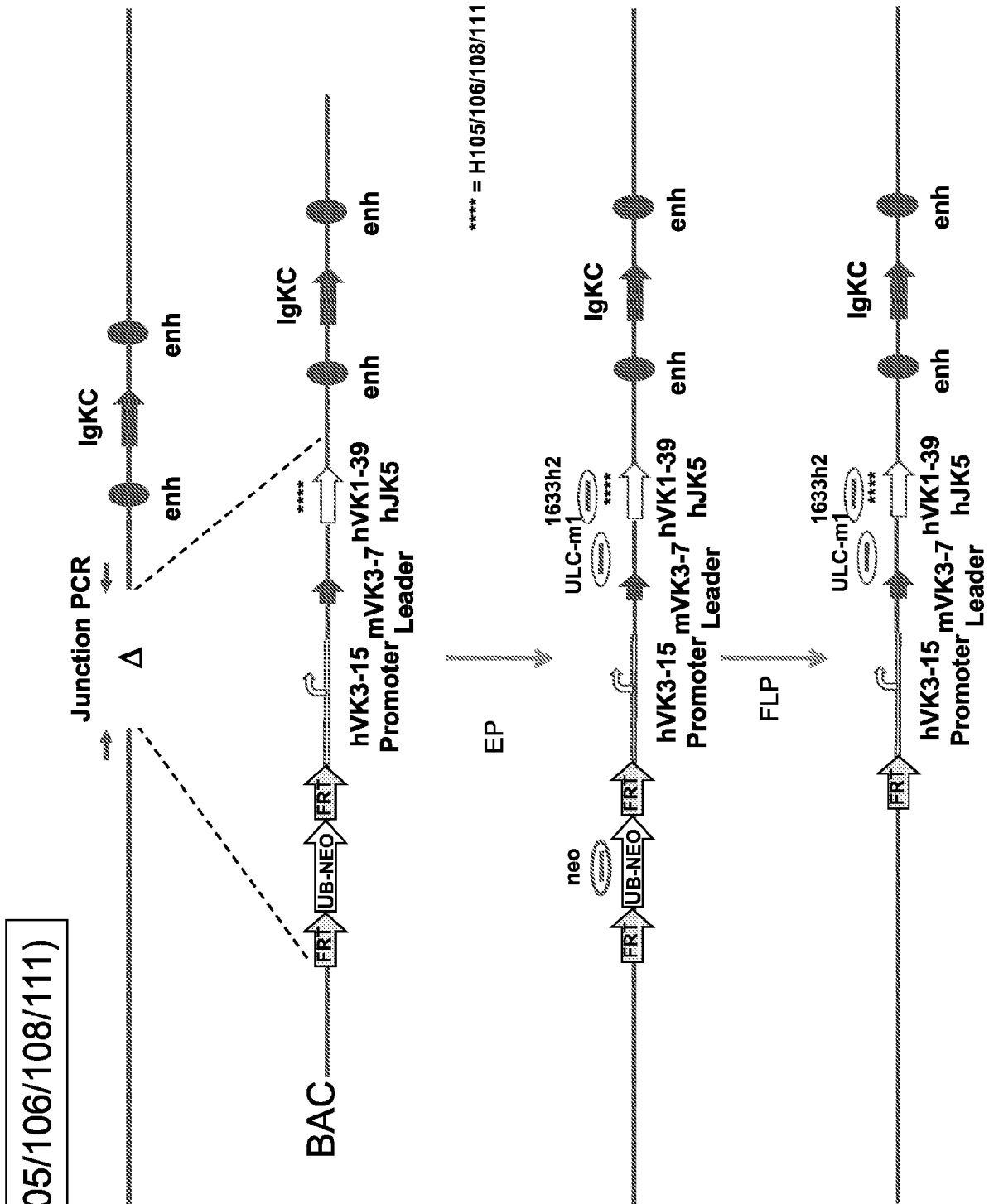


FIG. 8C

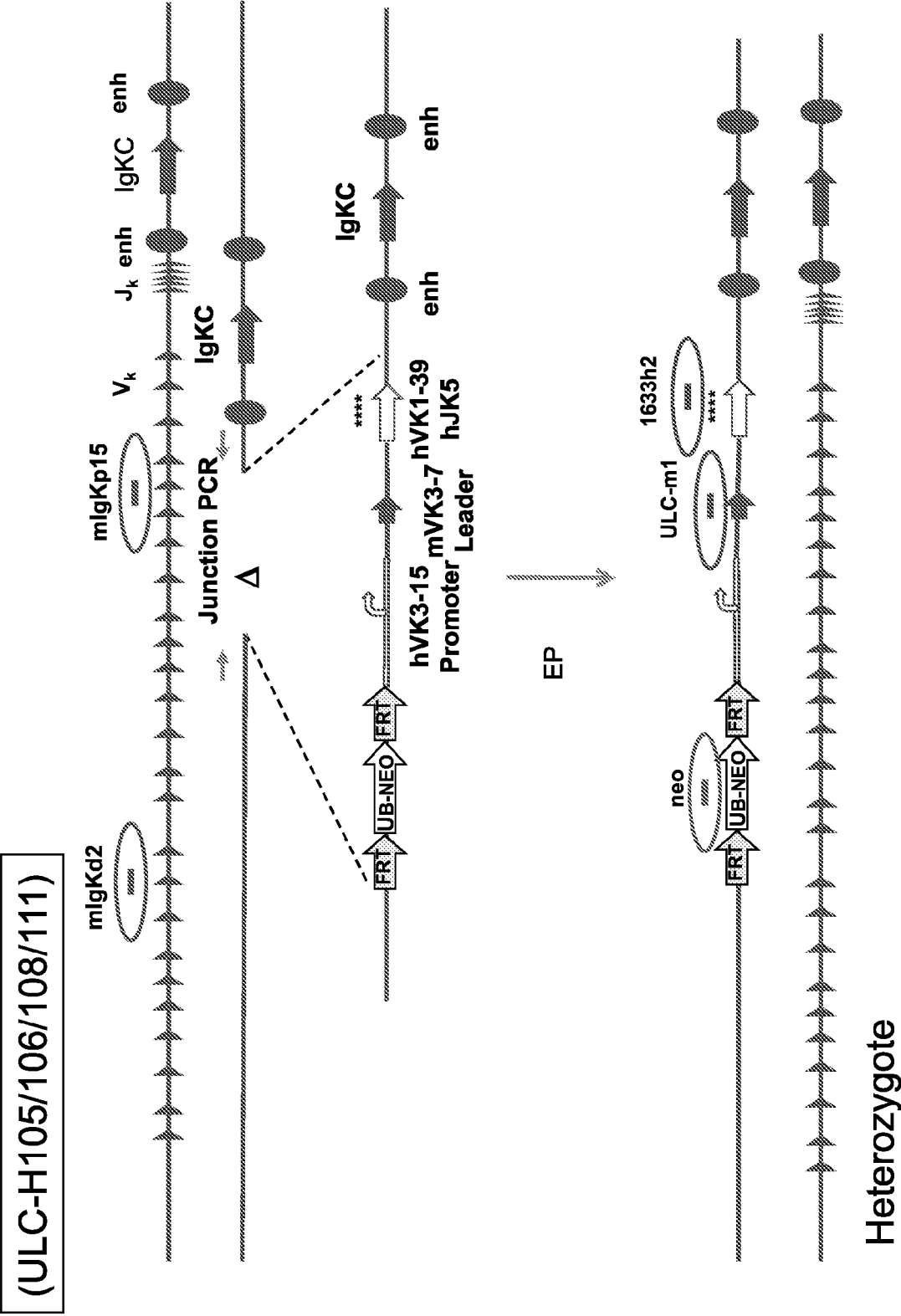


FIG. 8D



FIG. 8E

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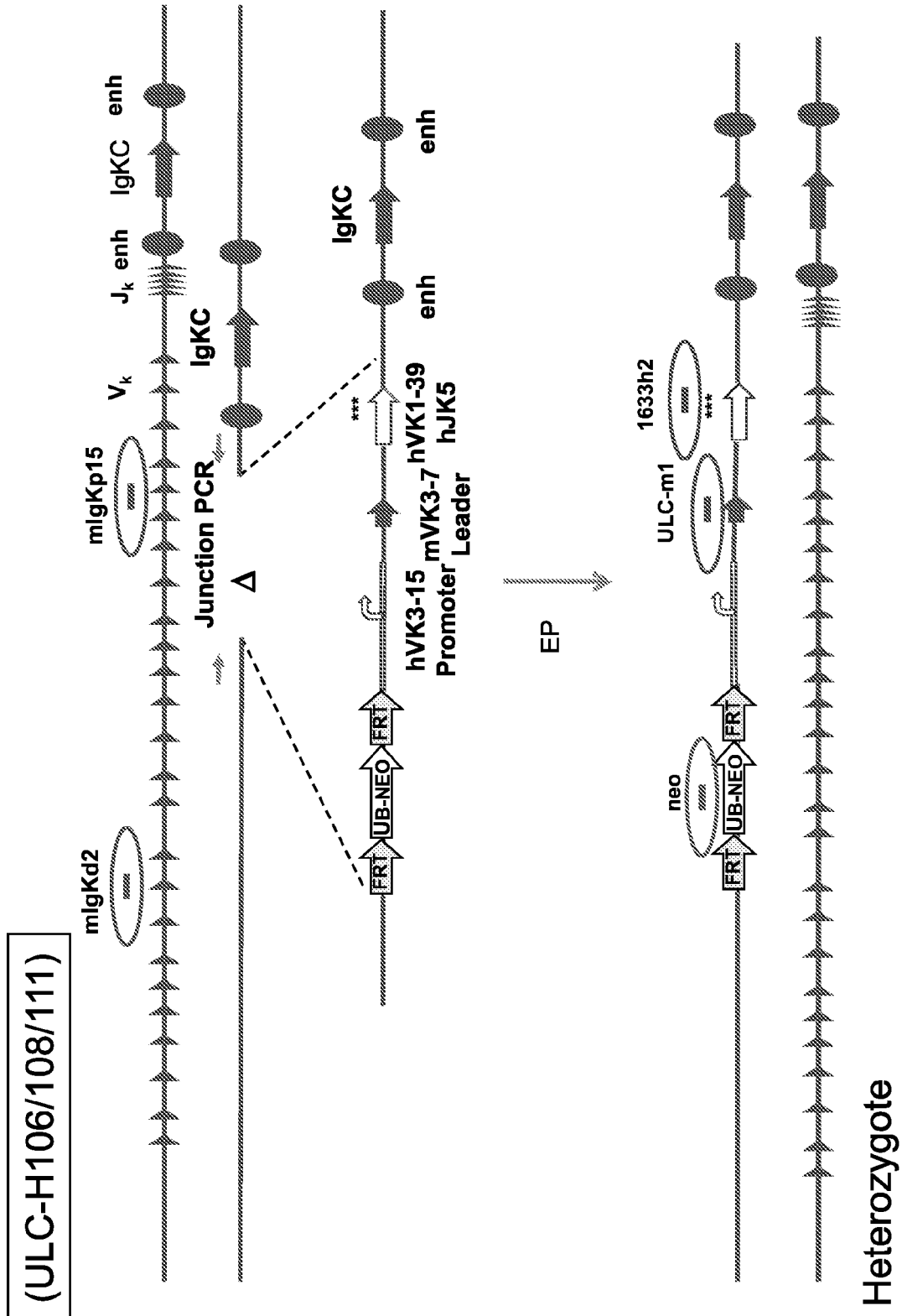
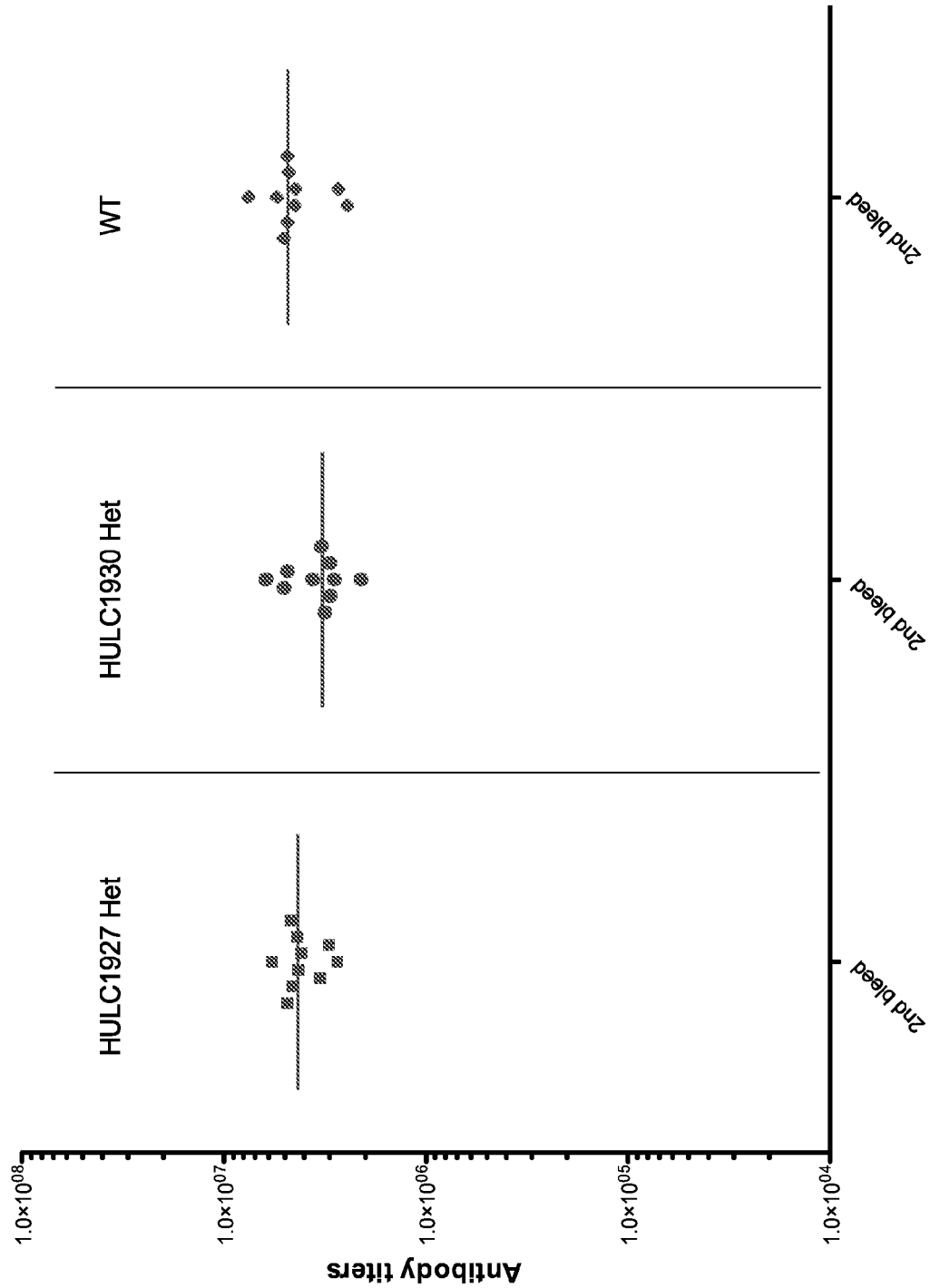


FIG. 8F

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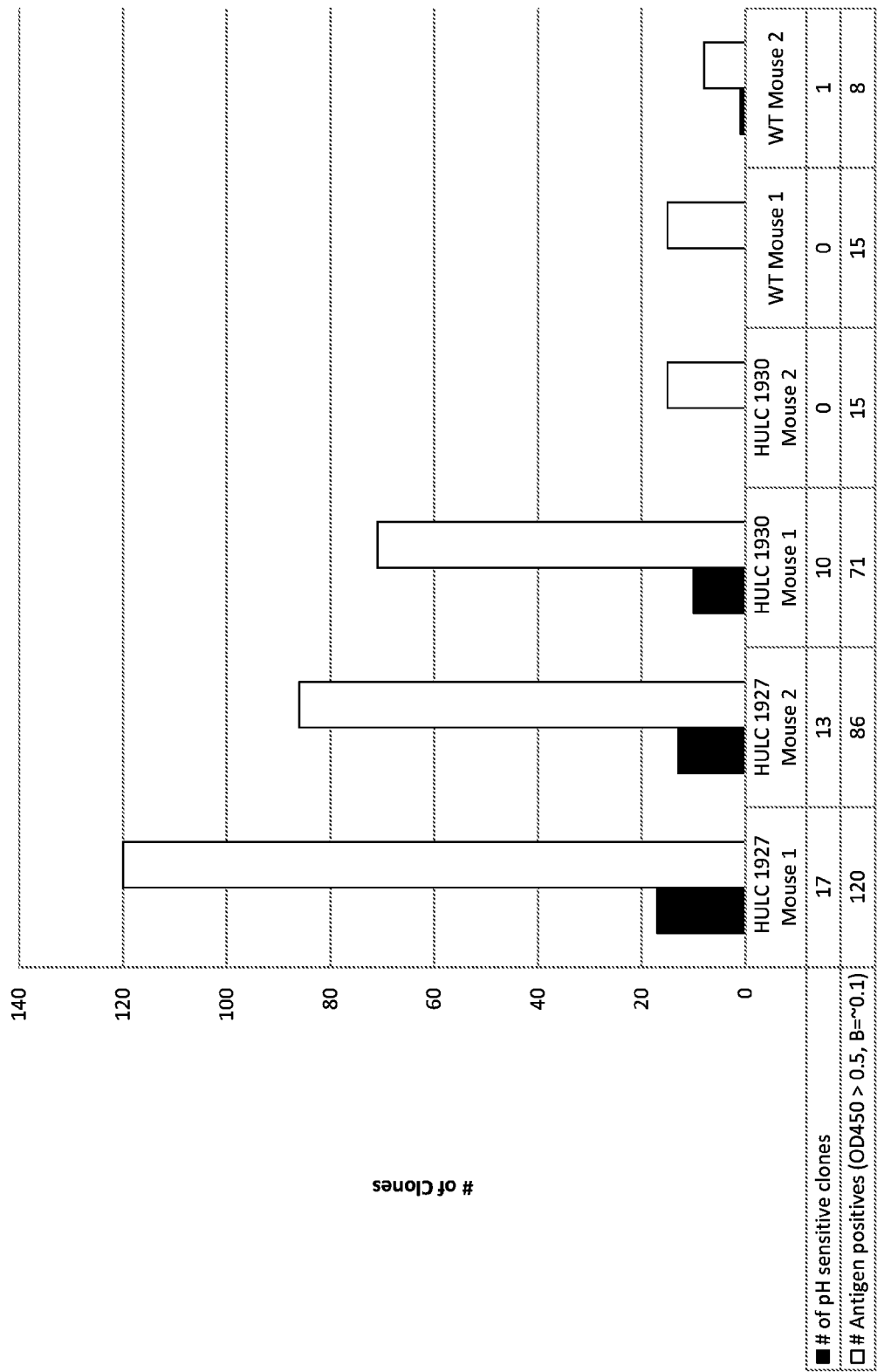


FIG. 10

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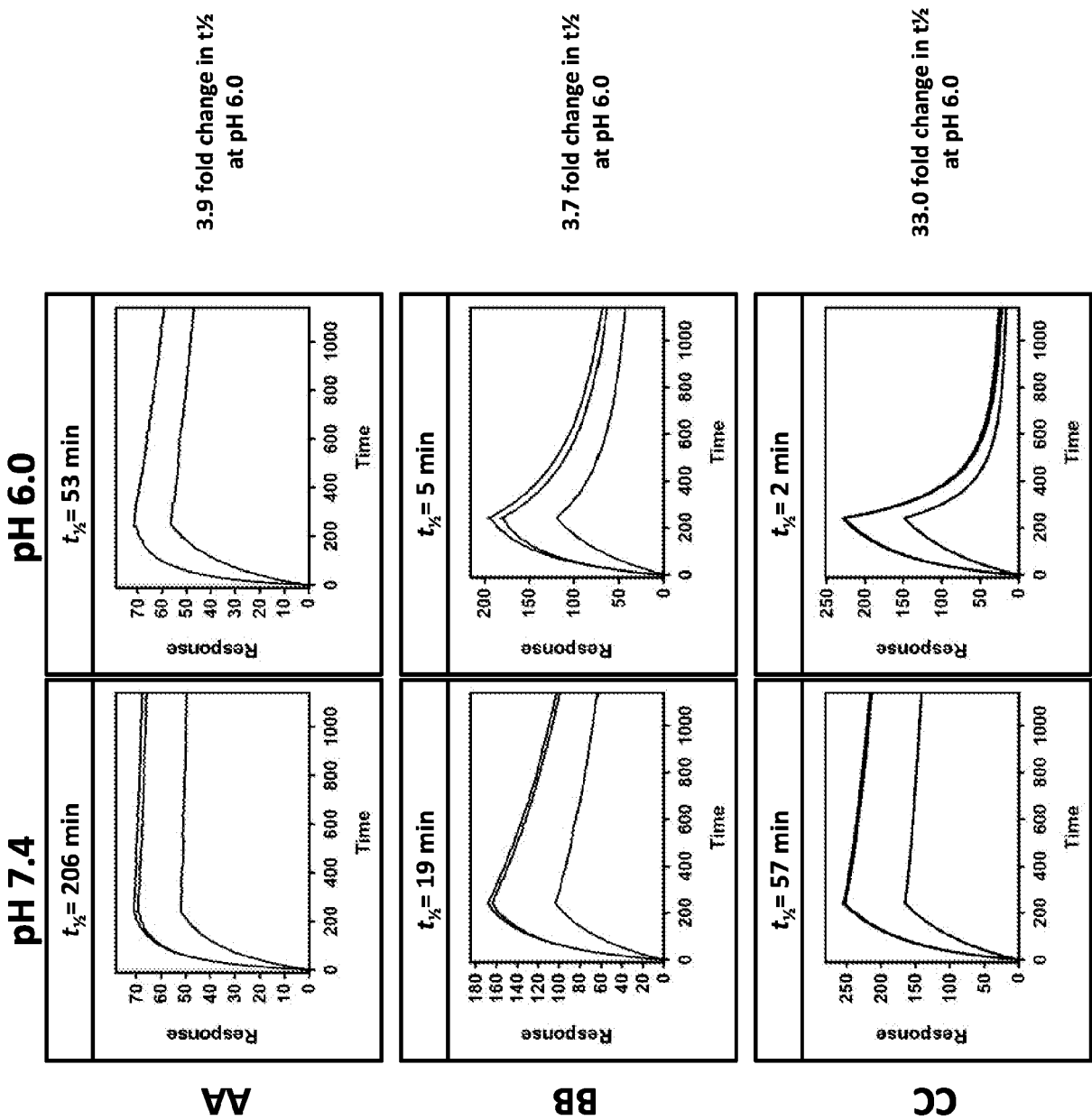


FIG. 11A

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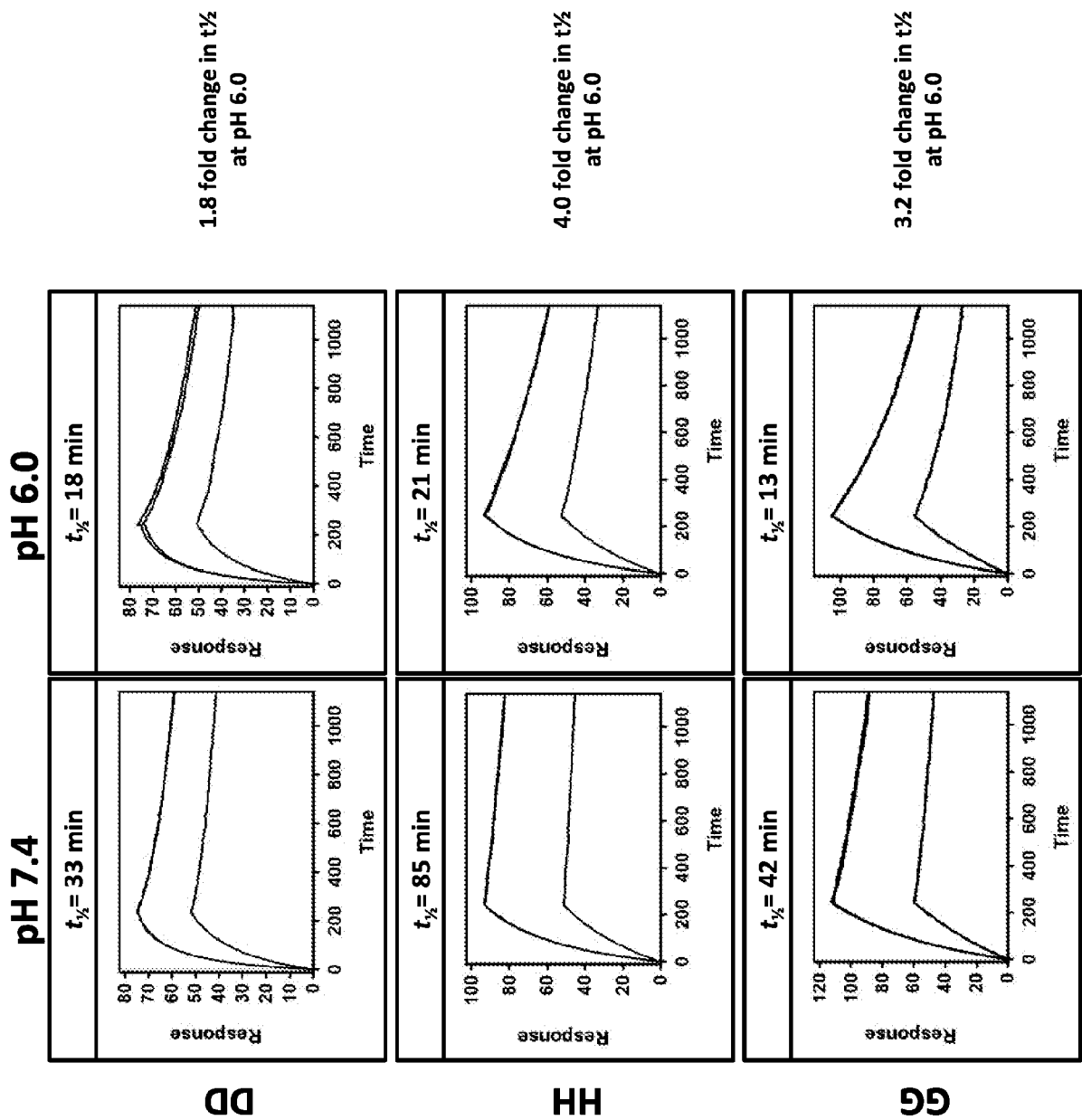


FIG. 11B

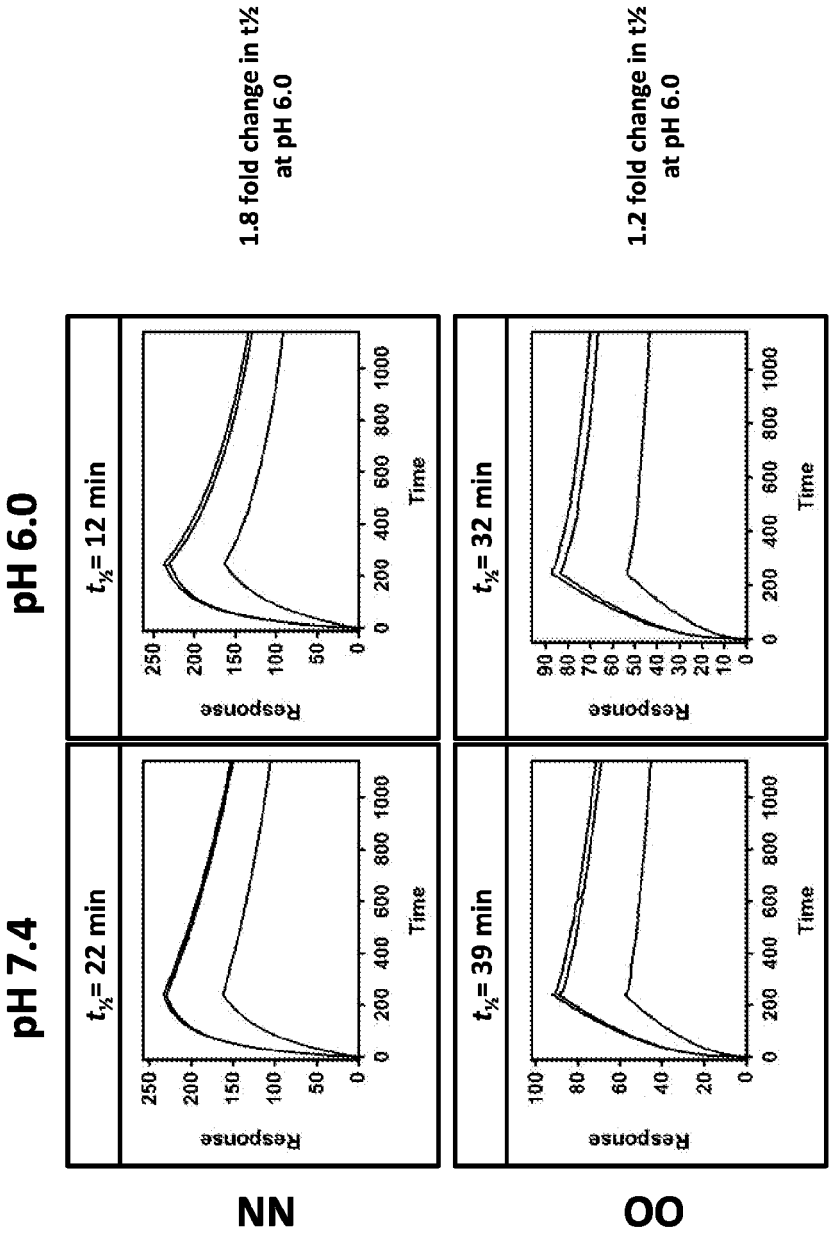


FIG. 11C

	105	106	107	108	109	110	111	
WT	Q	Q	Y	G	S	S	P	SEQ ID NO:75
	CAG	CAG	TAT	GGT	AGC	TCA	CCT	SEQ ID NO:74
H105/106/107/109	H	H	H	G	H	S	P	SEQ ID NO:77
	CAT	CAC	CAT	GGT	CAC	TCA	CCT	SEQ ID NO:76
H105/106/109	H	H	Y	G	H	S	P	SEQ ID NO:79
	CAT	CAC	TAT	GGT	CAC	TCA	CCT	SEQ ID NO:78

FIG. 12

Site-directed mutagenesis primers for Histidine substitutions in CDR3 of VK3-20JK1 ULC plasmid

Primer name	Sequence	%GC	N	mismatches	Tm
GERMLINE hVK3-20	GATTTTGCAGTGTATTACTGTCA GCAGTATGGTAGCTCACCTTGGACGTTTCGGC				
hVK3-20 (H105/106/107/109) F	GATTTTGCAGTGTATTACTGTCA TCA CCATGGT CA CTCACCTTGGACGTTTCGGC	48	54	5	79.5
hVK3-20 (H105/106/107/109) R	GCCGAACGTCCAAGGTGAG TG ACCAT GG TGATGACAGTAATACACTGCAAAATC	48	54	5	79.5
hVK3-20 (H105/106/109) F	GCAGTGTATTACTGTCA TCA CTATGGT CA CTCACCTTGGACGTTTCGG	49	47	4	78.7
hVK3-20 (H105/106/109) R	CCGAACGTCCAAAGGTGAG TG ACCATA GTGA TGACAGTAATACACTGC	49	47	4	79.7

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Primer	SEQ ID NO
GERMLINE hVK3-20/JK1	60
hVK3-20 H105/106/107/109 F	61
hVK3-20 H105/106/107/109 R	62
hVK3-20 H105/106/109 F	63
hVK3-20 H105/106/109 R	64

FIG. 13

Construction of VK3-20 HULC LTVECs

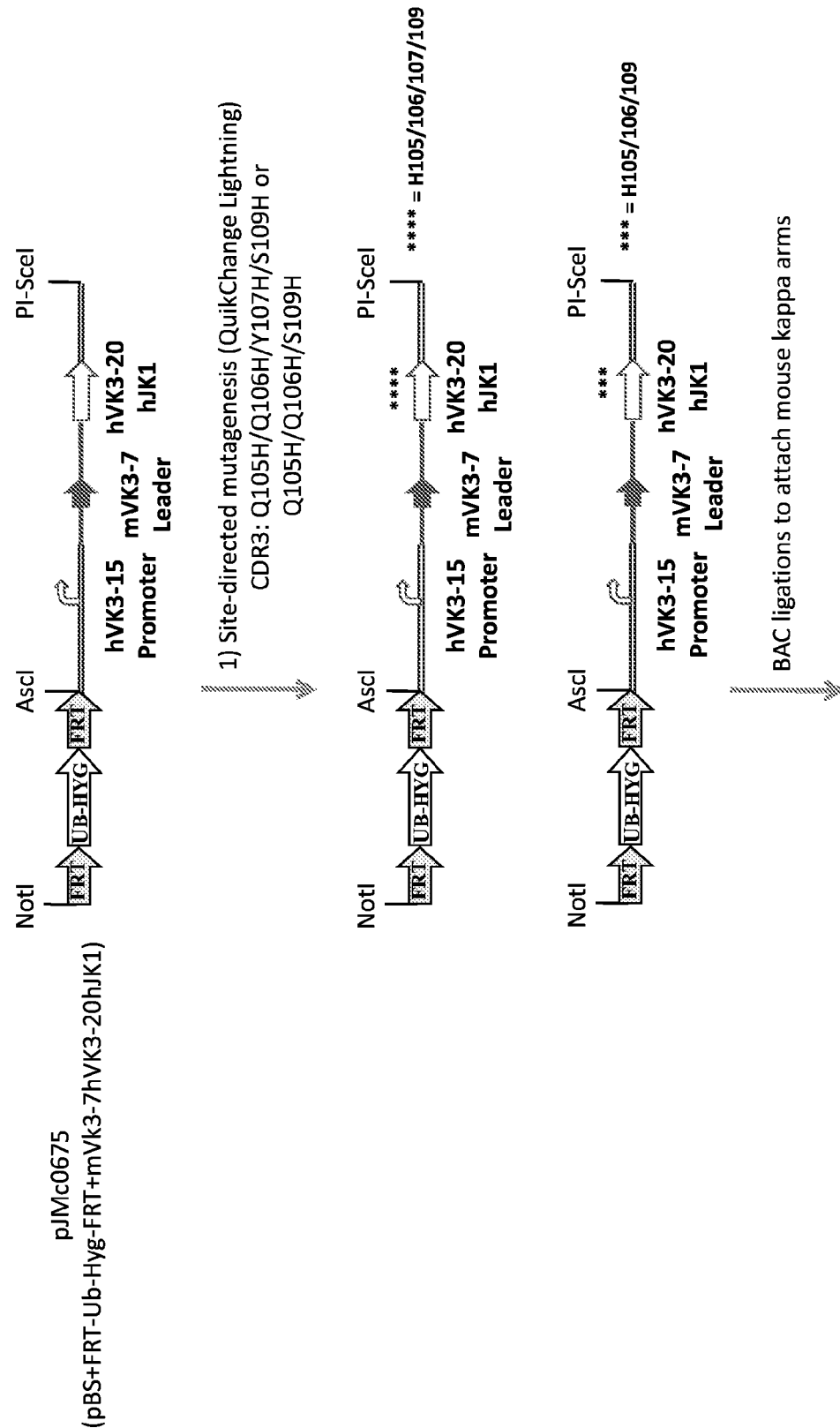


FIG. 14A

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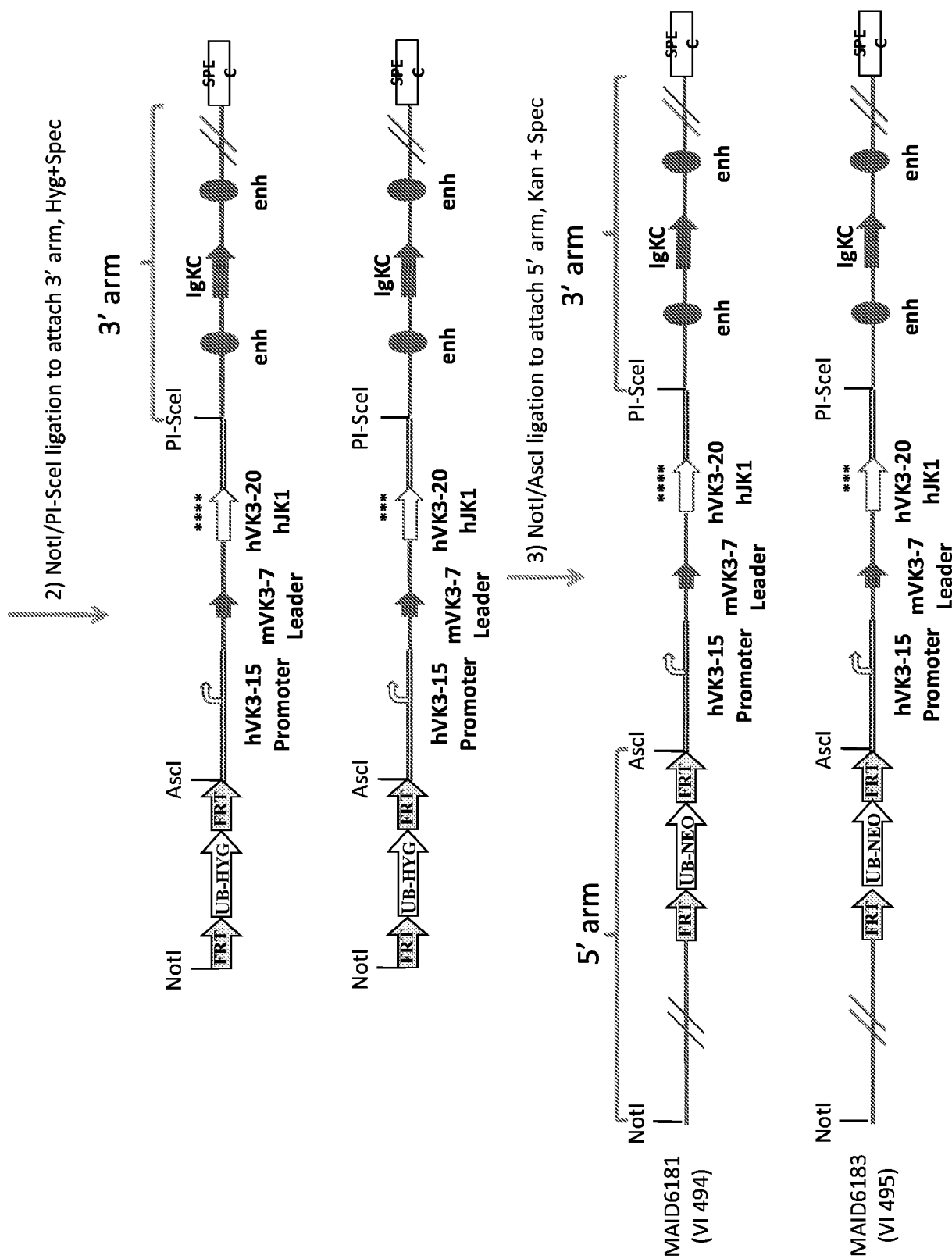


FIG. 14B

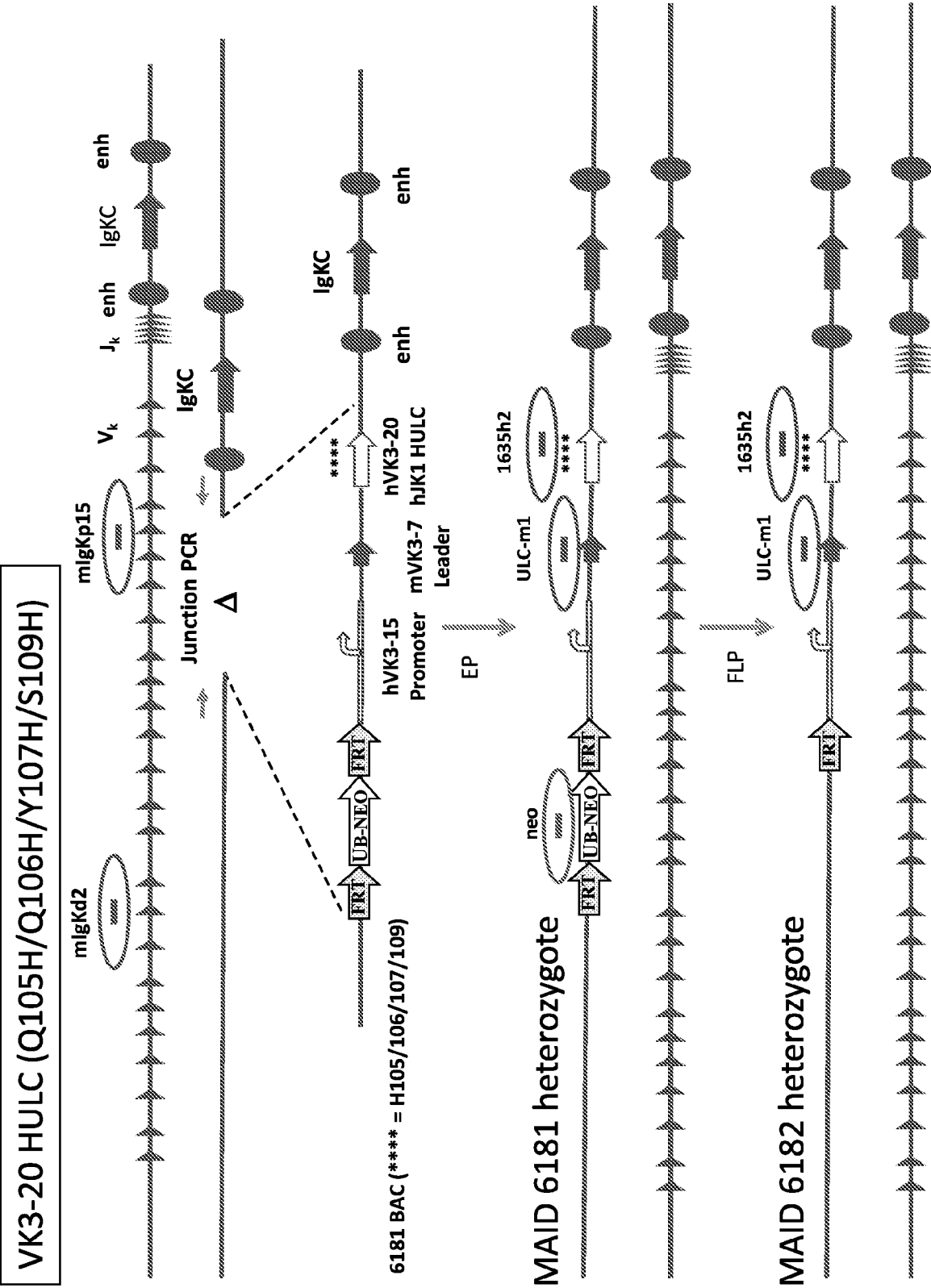


FIG. 14C

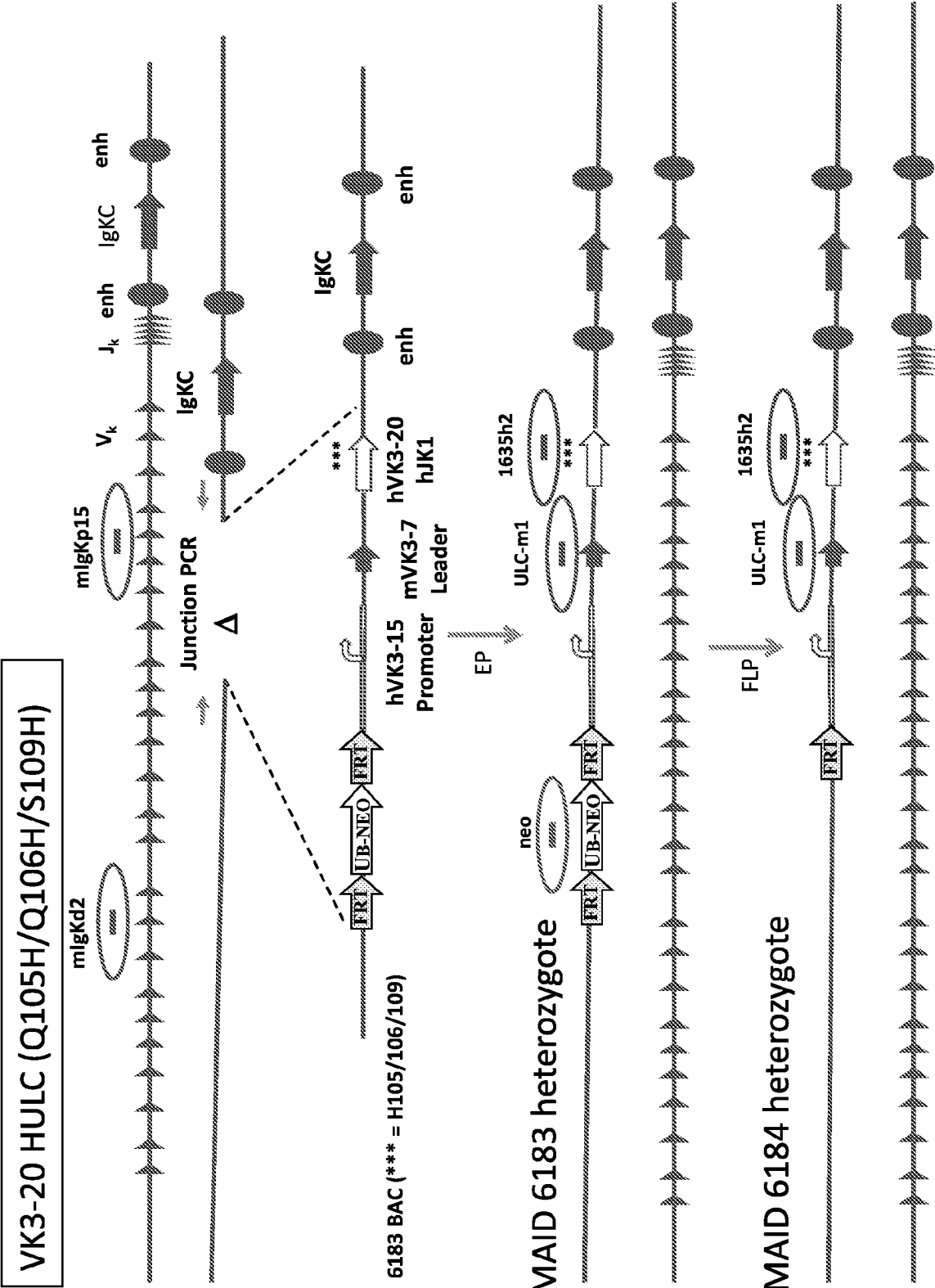


FIG. 14D

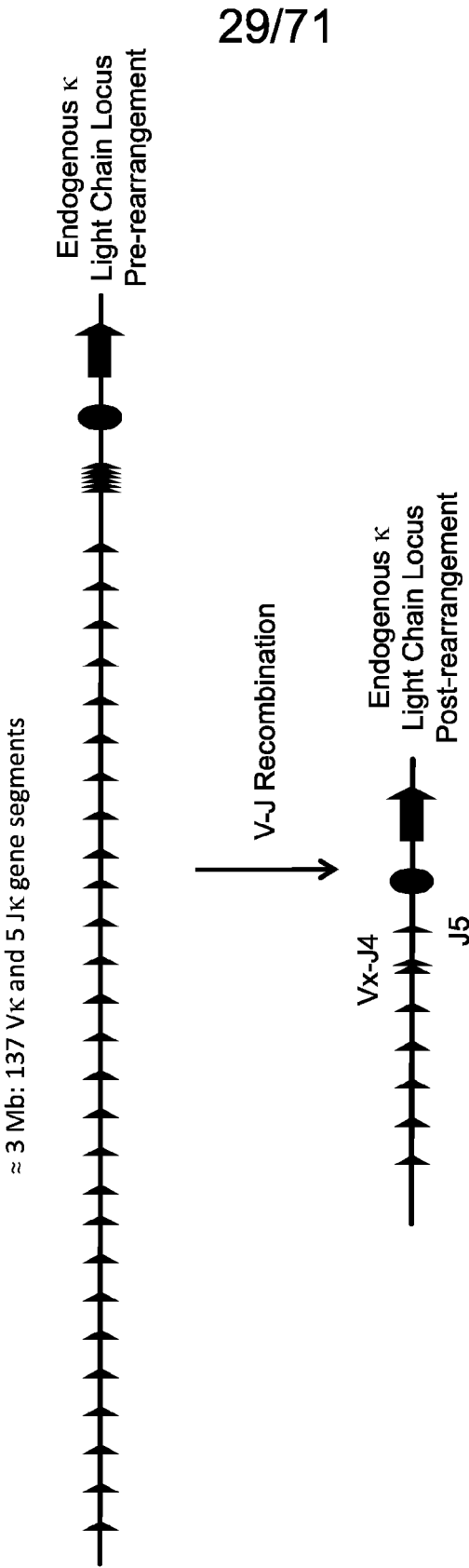


FIG. 15

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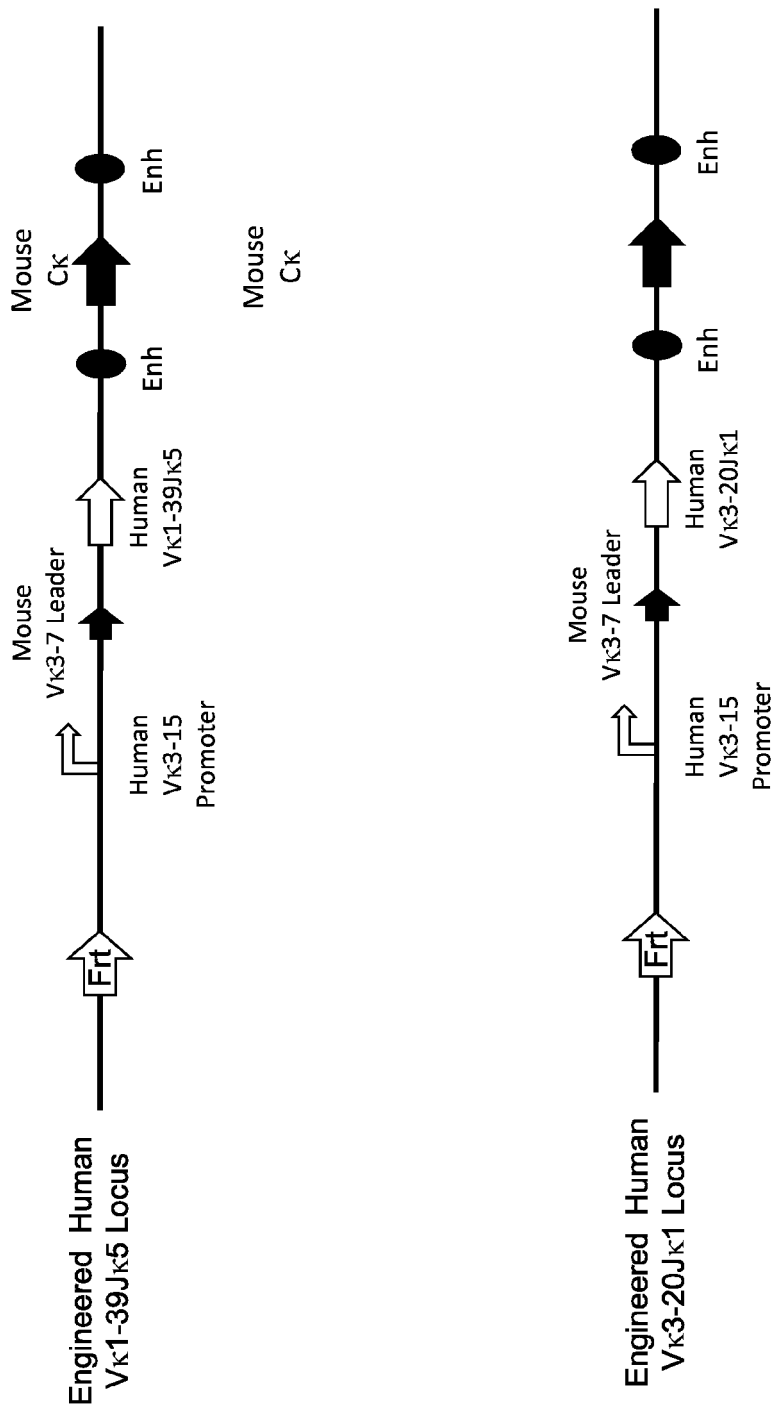
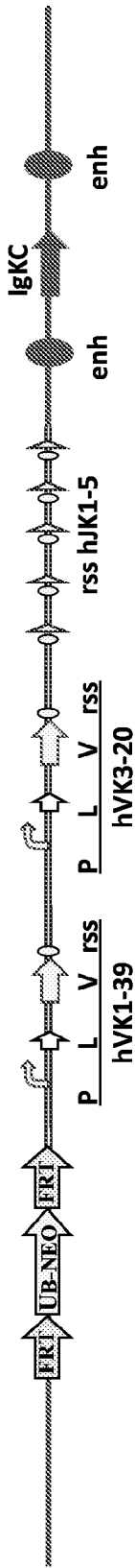


FIG. 16A

DLC-5J Locus



DLC-1J Locus

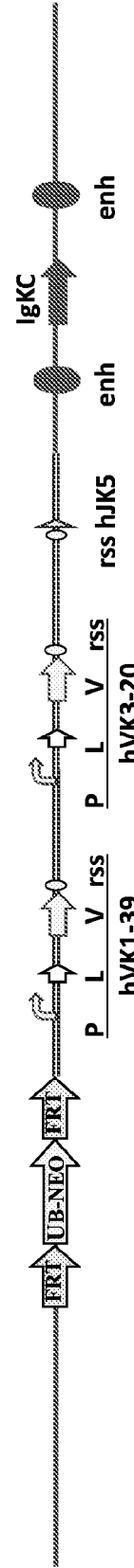


FIG. 16B

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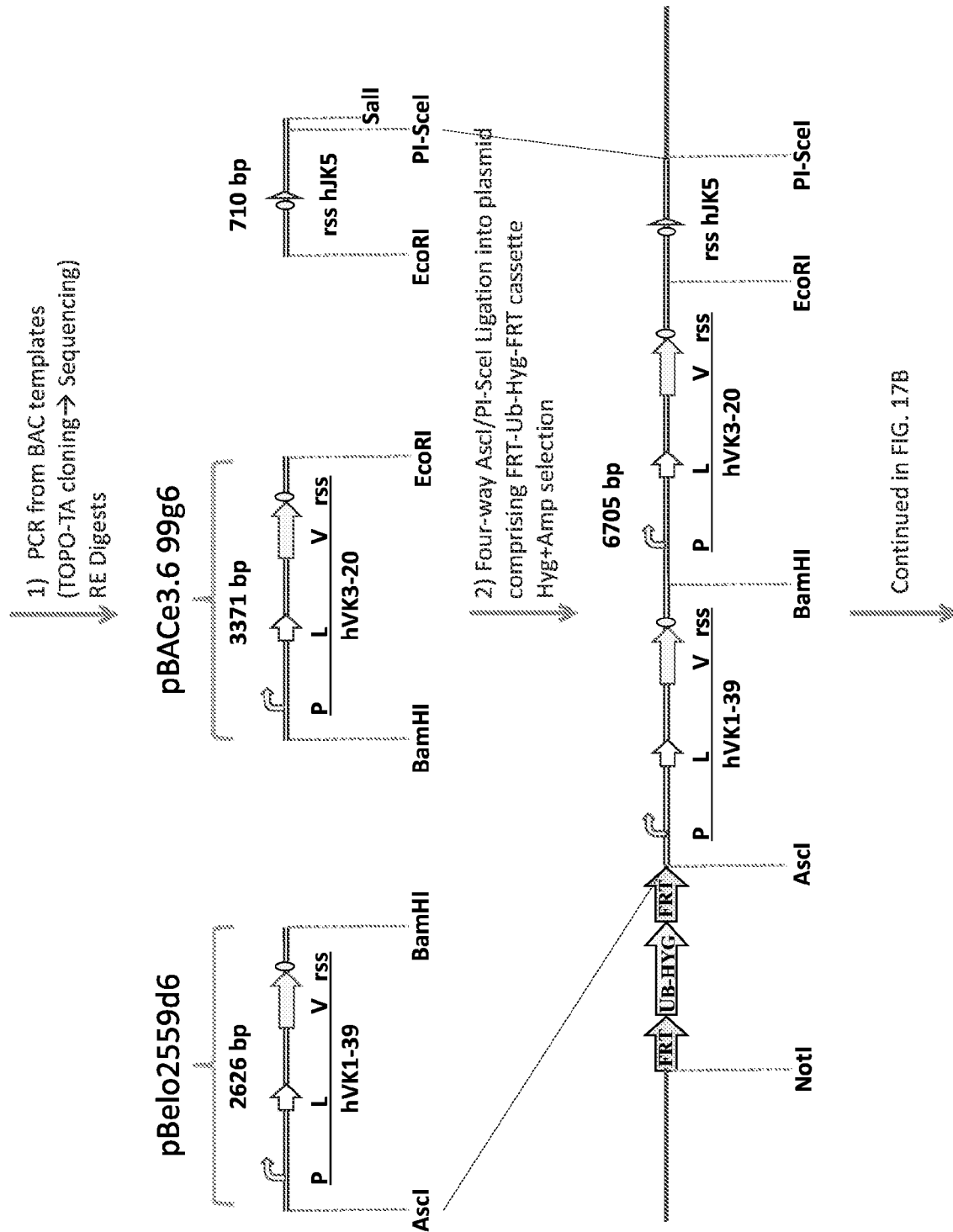


FIG. 17A

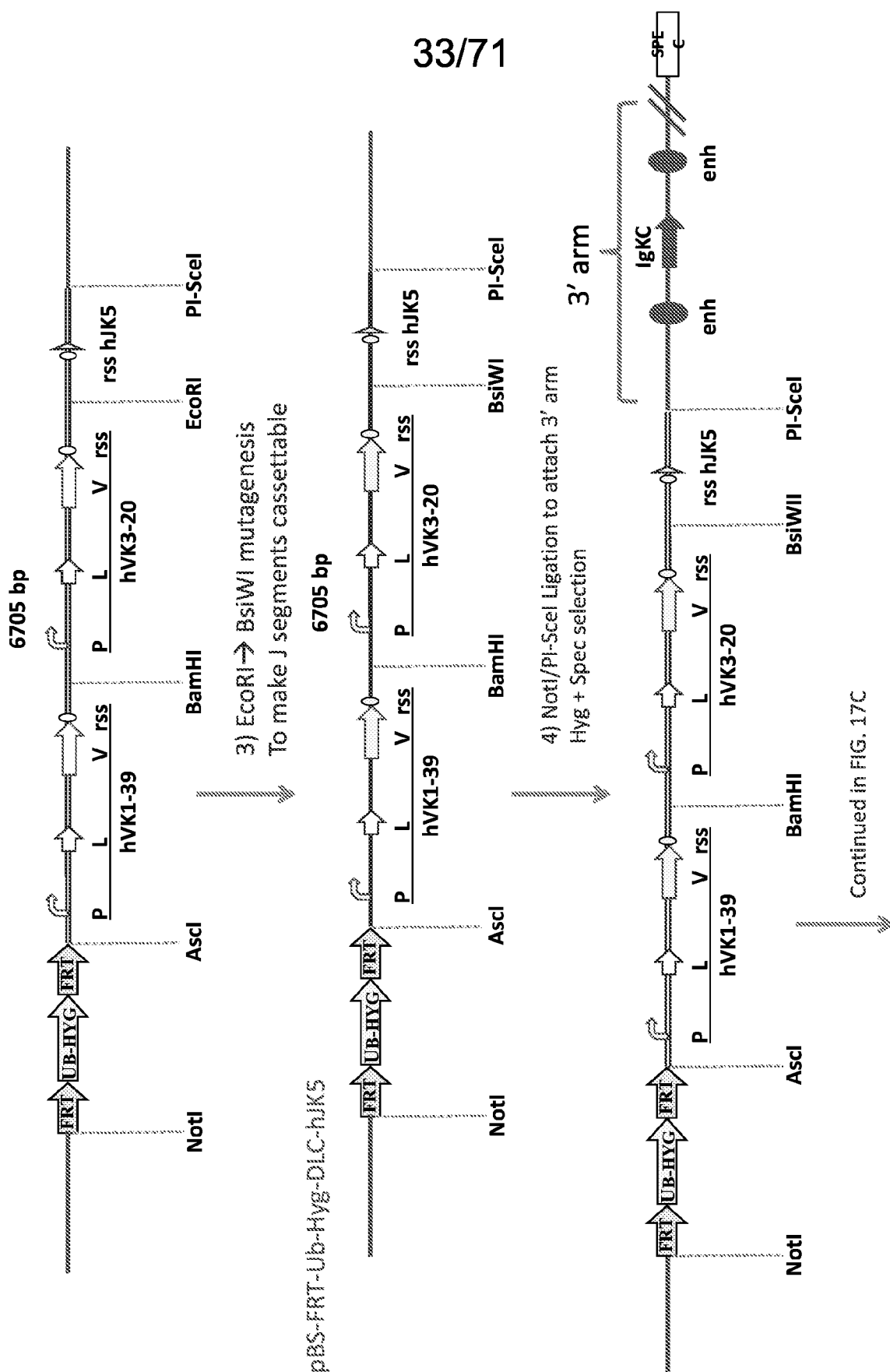


FIG. 17B

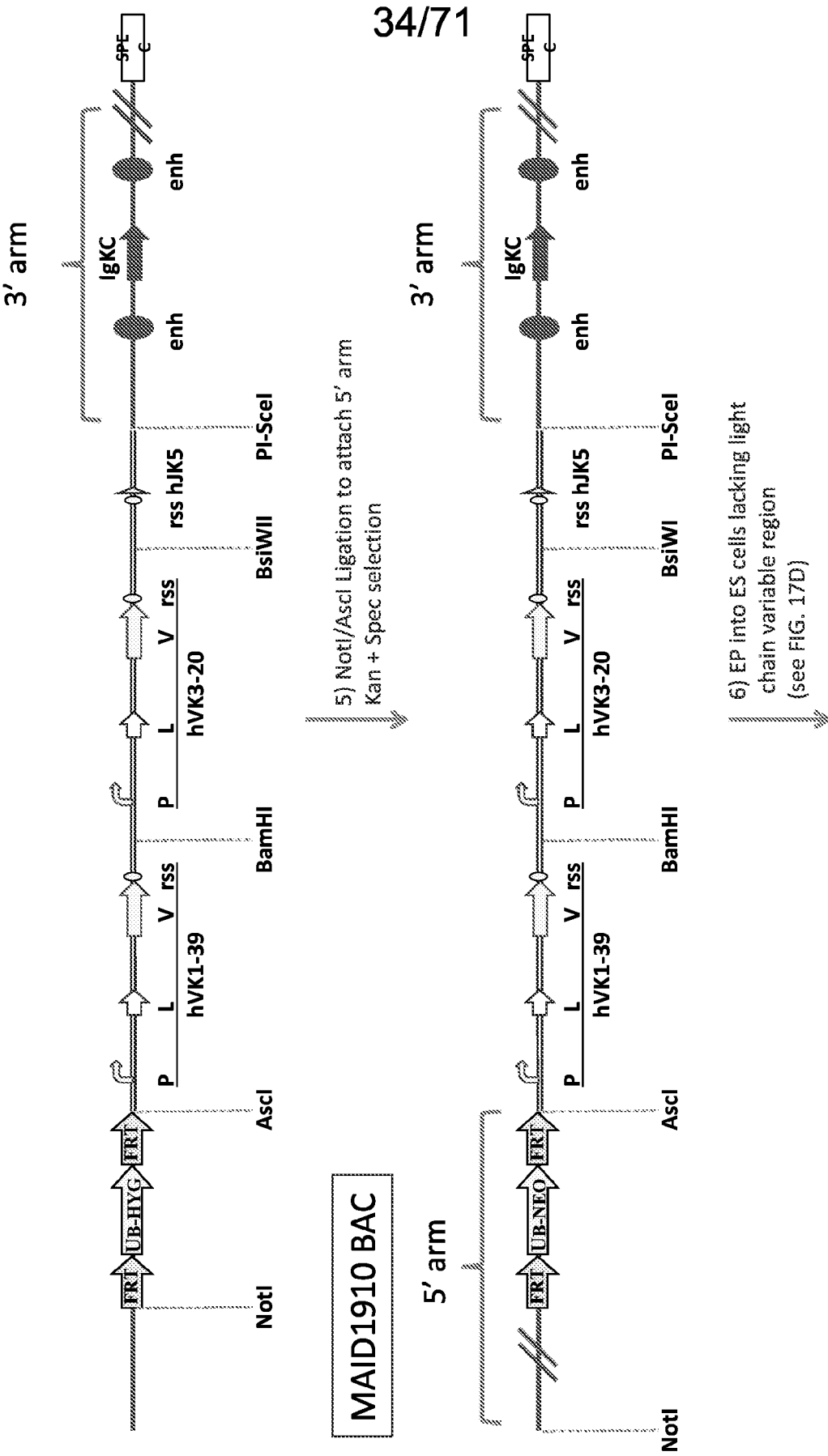


FIG. 17C

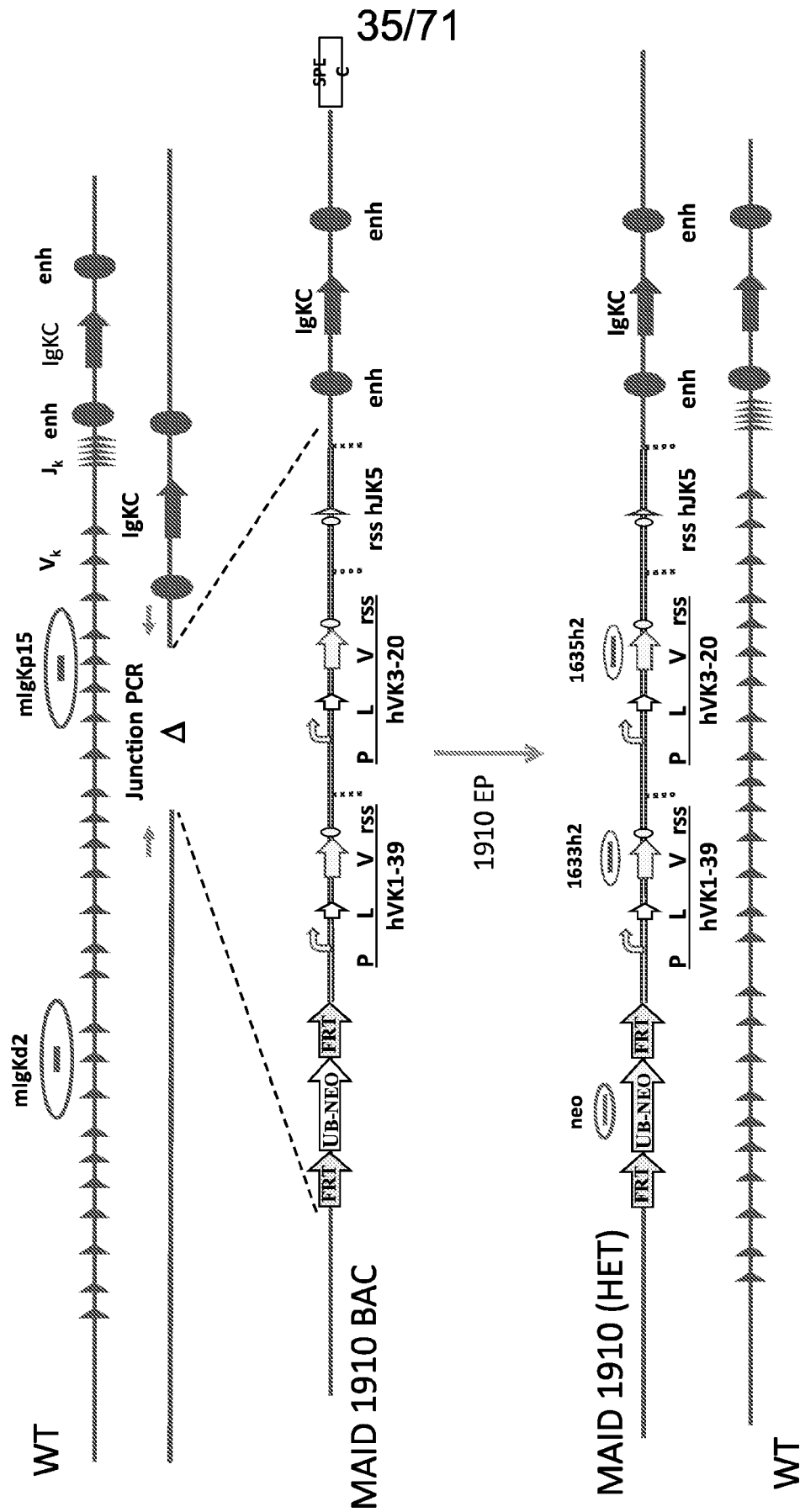


FIG. 17D

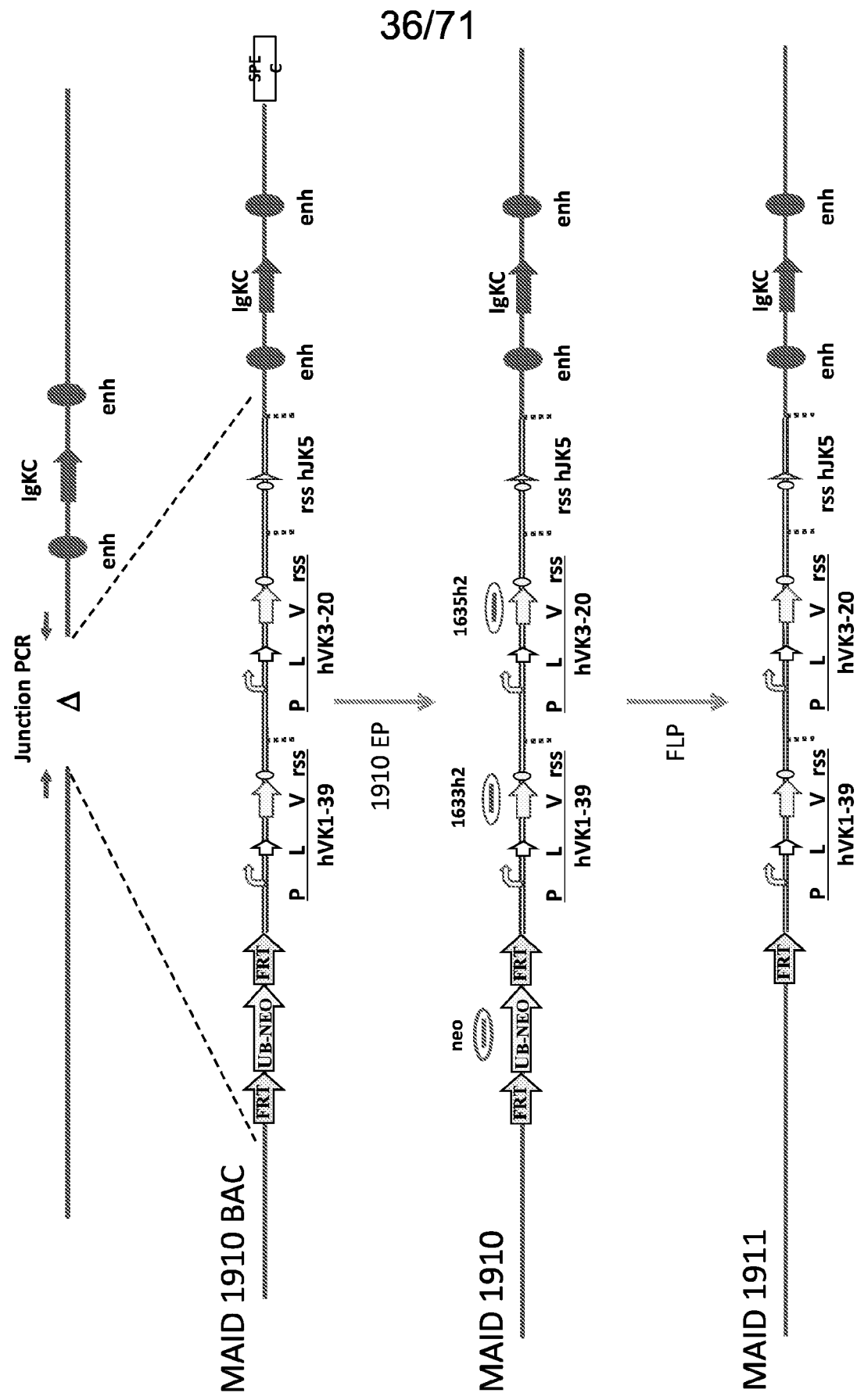


FIG. 17E

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DLC-1J
SEQ ID NO:82

GCACCACTTCGTCGCAGCGCAGGCTTTGGTCTCCCTTGTCCGTGCGGGGACAGCCACCGAGTTTACGCACCCAGCACACCGAGGTCCTGGTTGGTACCG
 AAGTTCCTATTCCGAGGTTCCCTATTCTCTAGAAAGTATAGGAACCTTCGCGCGTCTGGCTCCGAGGCCCTCCGCGCGGGTTTGGCGCCTCCCGCG
 GCGCCGCCCTCCTCCTACGGCGAGCGCTGCCACGTACAGCAAGAGCGGCGAGGAGGCTCTGATCCTTCGCGCGCGAGCGCTCAGAACAGCGCGCGCGCTG
 CTCATTAAGACTCGCGCTTAGAACCCCACTATACAGCAAGAGCAATTTAGGACGGGACTTGGGAGACTCTAGGGCACTGGTTTCTTCCAGAGAGCG
 GAACAGCGGAGGAAGAAGTAGTCCCTTCGCGGAGTCTCCGTGGGCGGTGAGACCGCGATGATATATATAAGGACGCGCGGGTGTG
 GCACAGCTAGTTCGTCGACGCGGATTTGGTCGCGGTCTTGTGTGGATCCTCTGTGAFCGTCACTTGTGATAGTACCGGCTGCTGGGCTGGCTGGC
 GGGCTTTCTGTCGCGCGCGCTCGGTGGACGGAAGCGTGTGGAGAGACCGCCAAAGGCTGTAGTCTGGGTCCGCGAGCAAGGTTCGCCCTGAAC
 TGGGGTTGGGGAGCGCAGCAAAATGGCGCTGTTCGCGAGCTTGAATGAAGACGCTGTGAGAGCGGCTGTGAGGCTGTGAAACAAAGGTGGG
 CGGCATGCTGGCGCGCAAGAACCAAGCTCTGAGCCCTTCGCAATCGCGAAAGCTCTTATTCGCGGTGAGATGGCTCGGCAACCAATCTCGGCAAC
 CTGACGTGAAGTTGTACGTGACTGGAGAACTCGGTTGTGCTGTTCGCGGGCGCGCAATATGGCGGTGCGGTGGCAGTGGCACCCGTACCTTT
 GGGAGCGCGCGCTCGTGTGTCGTGACCTACCGCTTCGTGTGGCTTATAATGACGGGTGGGGCCACCTGCGCGTAGGTGTGCGGTAGGCTTTTCT
 CCGTCGCAAGCAGCGAGGTTCCGGCTAGGTAAGCTCTCCGAAATCGACAGCGCGCGACCTCTGCTGAGGGAGGGATAAATGAGGCGTACAGTTTC
 TTGGTCGGTTTATGTACCTATCTTTAAGTAGCTGAAGCTCCGGTTTGAACATAAGCTCGGCTCGGGGTGGCGAGTGTGTCTTGTGAAGTTTCTTAG
 GCACCTTTTGAATGAATCATTTGGGTCAATATGTAATTTACAGTTTAGACTAGTAAATATGKCCGTAATATCTGCGCTTTTGGCTTTTGTGT
 AGACGTGAGCTCTAGATTGGAAACCGCGCTCTCGAATGTGTGACAATTAATCATCGCAATAGTATATCGCAATAGTATAATACGACAAAGGTGAGG
 AACTAAACCATCCACCATGATTGAACAAGATGGATTGCACGCGGTTCTCGCGCGCTTGGSHGAGAGGCTATTCGGTATATGACTHGGCACAAACAG
 CAATCGGCTGCTGTGATGCGCGGTGTTCCGGCTGTACGGCAGGGGCGCGCTCTTTTGTGCAAGACCGGACCTGTCGGTCCCTGAAATGAACATG
 CAGGACGAGGACGCGGCTATCGTGGCTGGCCACGACGGGCTTCTTGGCAGCTGTGCTCGACGTTGTCTACCTGAAGCGGAGGACTGGCTGCT
 ATTGGCGAAGTCCCGGCGAGGATCTCTCTGTATCTACCTTCCTCTGCGAGAAATATCCATCATGGCTGAGTGAAGCAATCGGCGGCTGCTATACG
 TTGATCCGGCTACCTGCGCATTCGACCAACCAAGGAAACATCGCATCGAGGACGCTACTCGGATGGAAGCGGCTCTTGTGATCAGGATGATCTG
 GAGGAAGACATCAGGGCTCGCGCCAGCGAACTGTTCGCGAGGCTCAAGGCGGCGCATGCGCGAGCGGAGGATCTGCTGTAAGCGGATGCGGATGCG
 CTGCTTGCAGATATCATGGTGGAAATGGCGCTTTCTGGATTCTATCGACTGTGGCGGCTGGGCGGACCGCTATCAGGACATAGCGTTGG
 CTACCGCTGATATTGCTGAAGACTTGGCGGGAATGGCGGACCGCTCTCTGCTGTCTACGGTATCGCGCTCCCGATTCGAGCGCATCGCGCTTC
 TATCGCTTCTTGACGAGTTCTTCTGAAGATCCGCTGTAACTCTGCAGAAATTTGATGATCTATTAACAATAAAGATGTCCACTAAATGGAAGTTT
 TCCGTCTACTTTGTTAAGAAGGTTGAGAACAGAGTACCTACATTTTGAATGGAAGGATTTGGAGCTACGGGTGCGGGTGGGGTGGGATTAAGATAA
 ATGCTGCTCTTTACTGAAGCTCTTACTATTGCTTTATGATAAGTTTCTATAGTTGGATATFCAATAATTTAAACAACCAAACTTAAGGGCCA
 GCTCATTCCTCCACTCATGATCTATAGATCTCTCGTGGGACATTTGTTTCTCTGATTCCTCACTTCCGCTTGTGGTCTTAAGTACTGTGGT
 TCCAAATGTCTAGTTTCAATAGCTTGAAGAACGAGATCAGGCGCTGTTCTCCACATACACTTCATCTCAGTATTTGTTTGGCAAGTCTAATTCGA
 TCAGACCTCGACCTCGAGCCCTHAGAGAAAGTTCCTAATCCGAAGTCTCTATCTCTAGAAAGTATAGGAACCTCTCTAGGGTTTCAACCGGTGGCGCGCC
 GAGAGAGGAAGAGAGAGCGGATACCGAAATGTCTCAGCGAGAAGCTACCAAGAGGATGAATGGAGATCAAGGCCACGTGGAAACATGGGAAAT
 GTCTCAGTATTTTCCACCTAAGAGCGGAGGATCGGGTATCTATACACCTCCCTCTCTCACTGATTGAGGGCTTCCGACAGGATGCTCATTC
 AGGTGCTGTGATAGCCCATGTGTACAGGCAAGGCTGCTCTCTCCAGCTTCAGATAGACAGTGAAGAAAGATATGGCCATGGGGGTGACAGAGT
 ACAGCAAGGGGAAAGGGTAGCAAGAGTGAACAATATATCAACCCCCCACACACACACACACGAAATTTGTGTATTGCAATCCAG
 AACTGCTTCTCTCTGAACCTAAATCTTAGCAAGCAGTTTACCAGTAACTGCGCTTGAAATTCAGGCCCCCTGGAAAGGACGAGGGGTGTGTACAGGC

FIG. 18A

DLC-1J
SEQ ID NO:82 continued

[illegible]

VK1-39

SEQ ID NO:82 continued

VK3-20

JK5

FIG. 18C

DLC-1J
SEQ ID NO:82 continued

CCCTTTTGTTCATAAATTATTATTGTCAGACAACAATAAATAAATCAATAGCACGCCCTAAGATCTAGATGCATGCTCGAGTGGCATTTCATTACCTCTT
TCTCCGCACCCGACATAGATAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCCGTGGCATACAGTGTACAGATTTCTGTTTATCAAG
CTAGTGAGATTAGGGGCAAAA

Sequences of Vk1-39, Vk3-20, and Jk5 are in large font and boxed
Mouse sequences italicized
Human sequences in regular font
Restriction enzyme sites in bold
Neo Cassette sequences underlined
FRT sites double underlined

FIG. 18D

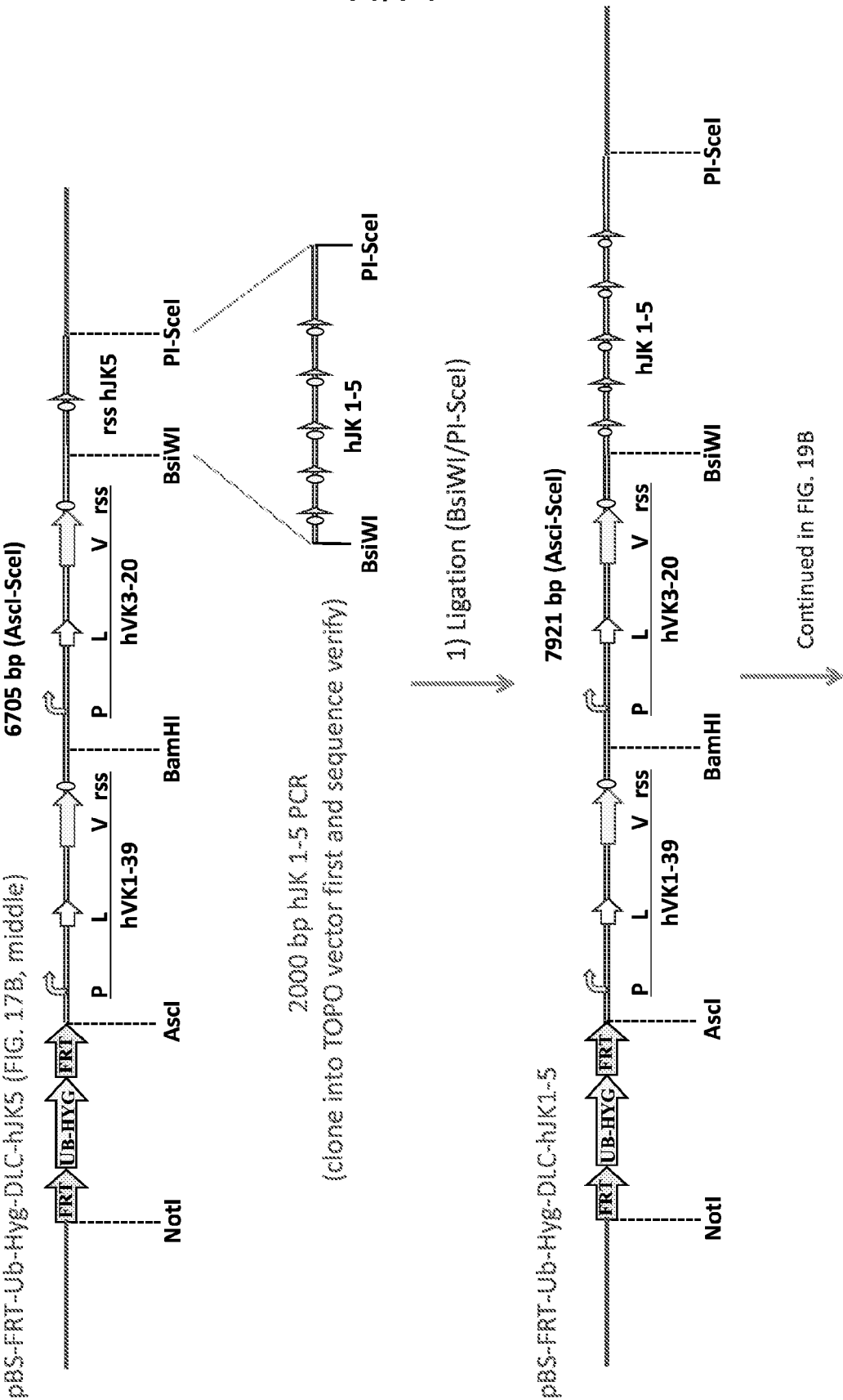


FIG. 19A

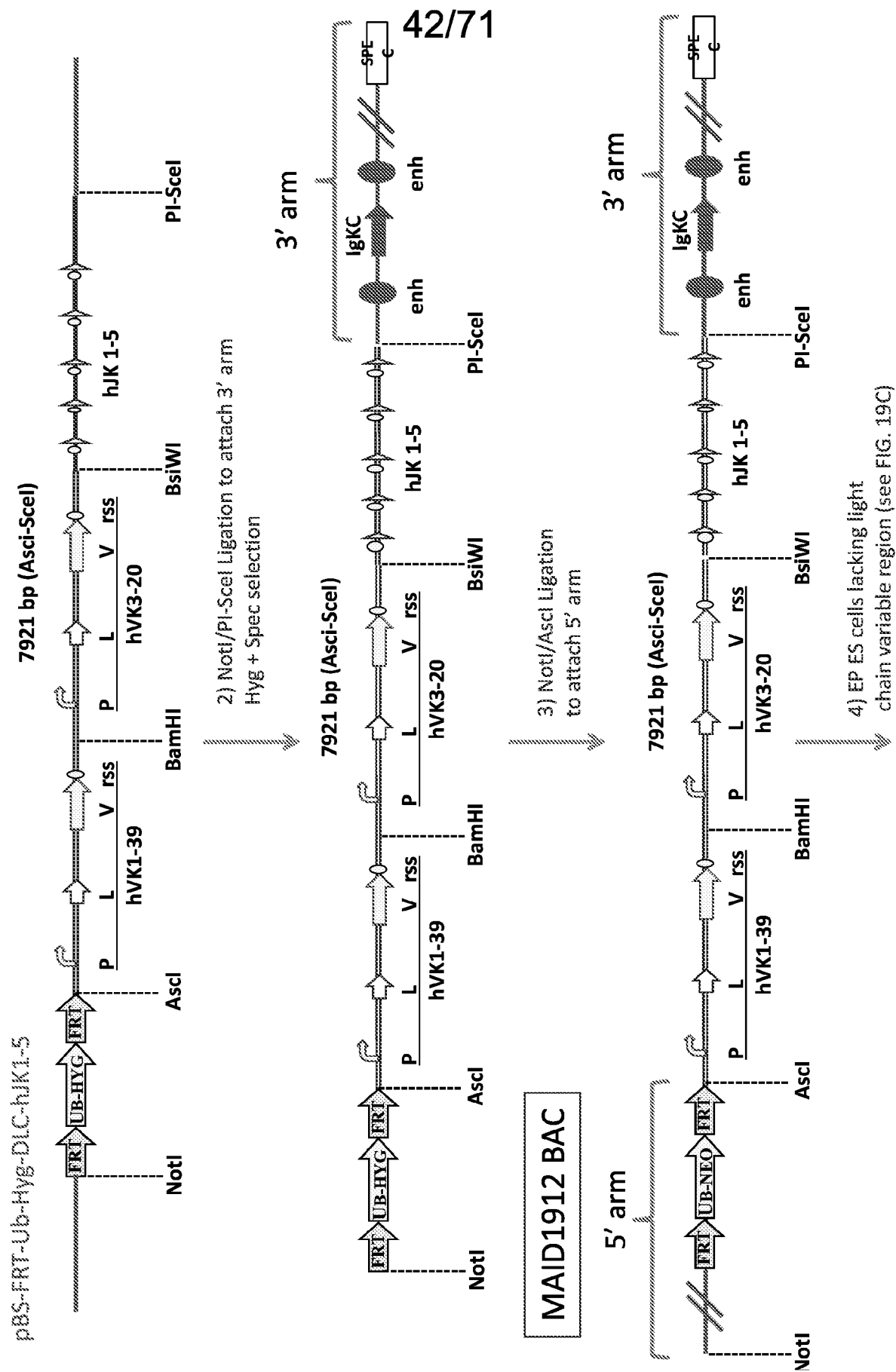


FIG. 19B

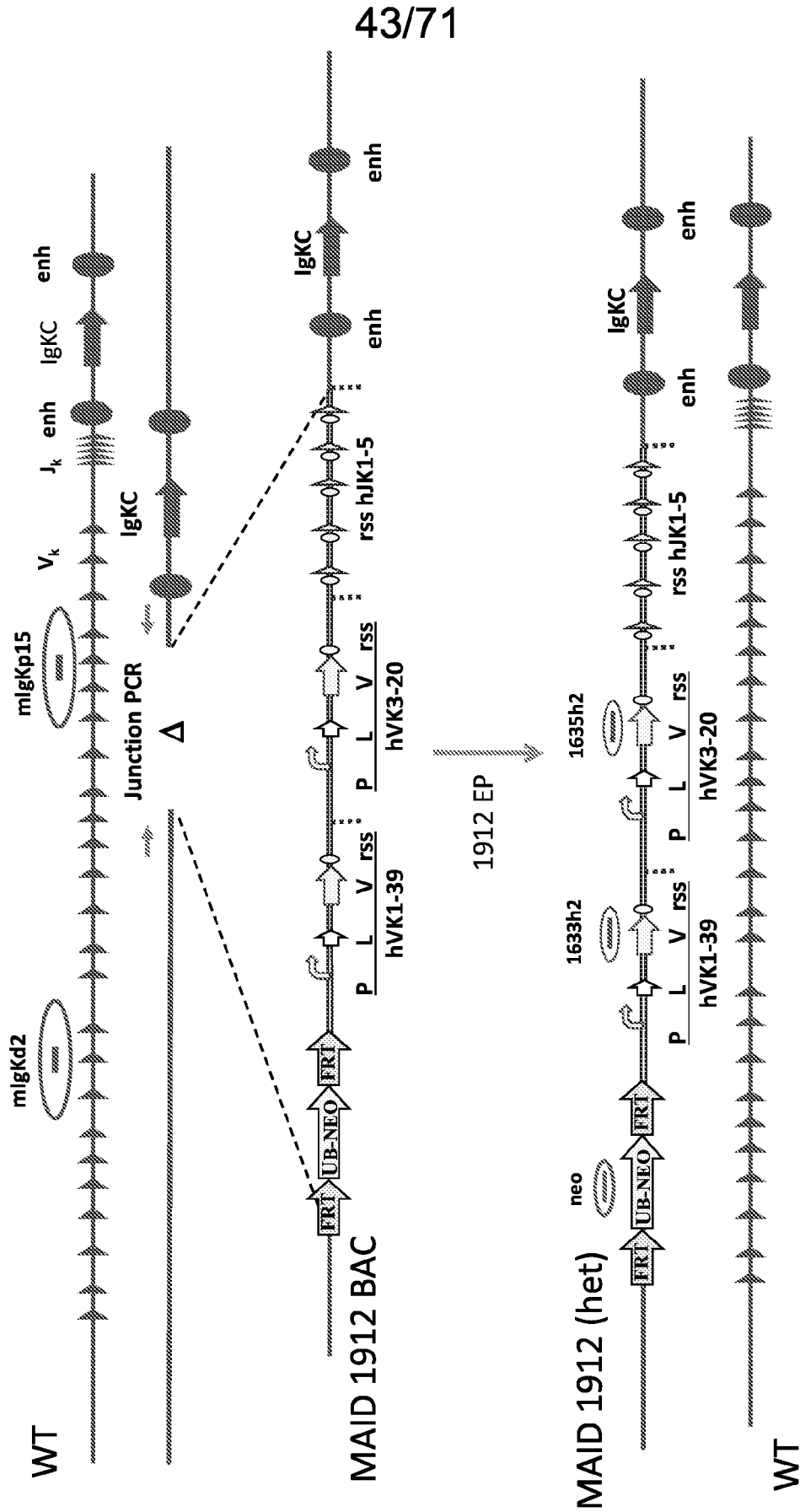


FIG. 19C

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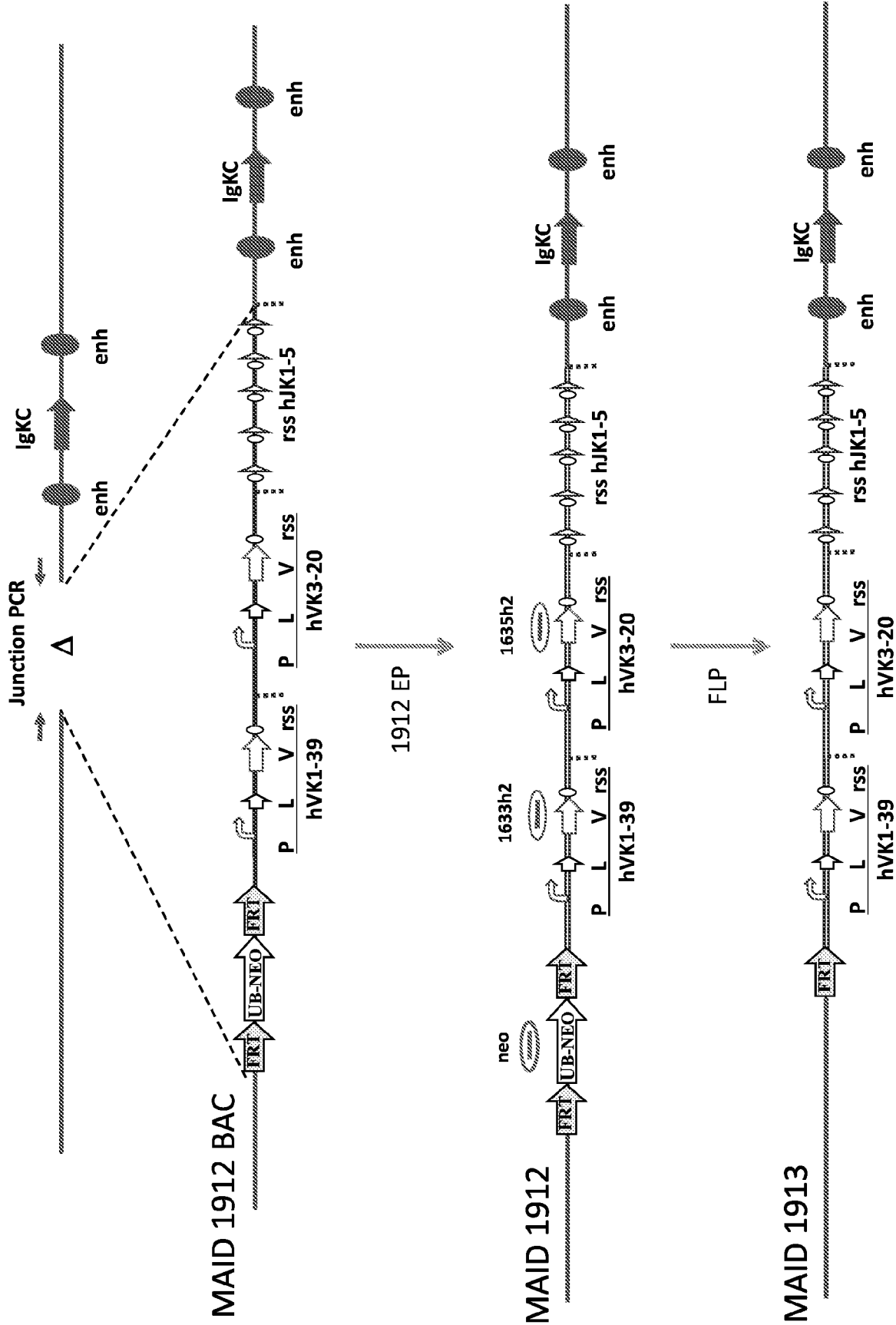


FIG. 19D

DLC-5J
SEQ ID NO:83

FIG. 20A

DLC-5J
SEQ ID NO:83 continued

GGGTGATGACGTTATCAGACATGCTCAATTTCTACAGTACTTGACCTGCCCTCTAGAACTGCACAAACGAGGGTGAAAGGCACTGCATCAAGCAACAA
TGGCAATGGGTGCCAAAGAGGTAGTAGTCTCAGAAACAGGGAGAGACAGACTGAGTCCCTCCACTAACTCAGCCCAACACCTGTGGATGAGTAGGGGA
TGCCCTGAGATCCTGGGTGGATGGGAGGTGGGCACCTGATCTGTCAATCTGCTTTTCTTCAAGGATCAGGCAGCAGAGACCCAGAGCTTCAATGTCT
TTGTAAAGGCTCTTCCAAAGCCAAACACAGATAAATTTGACAAACACACTGATCTGCATCCAGACCTCAACCTCTCACAGACTGAAGTGGTTGTTTCATCC
TACTAAAGGTAAACTATACCAGCTATACAGAAATAAAGACTGGATAGTTTAGAGGATCACCCAGAATAAGTTCTTTTGGCTGCATGGACAAACACAT
CTTCTGCTTTTAGGAAAATGGTATCACTACCTCAGCAATTTGGAGCCAGGCTCACTTATCTCTGCAGTTGTGAAACCTCCTCACACCCACAGCTGCTG
AGGTTAATTTGAATGTACTCTTTTAAATTTCTGCAAGAAATGAGACAGCTTCTGGACCTTCAGGAAGATCACTAACAAAGTAAATAACAAGTATATCCGG
AAGATAAAGTTGTAATAGACTCTTCTTCAACTGTATCCATCATGCAATTTAGGGAGCTGACTGGGCACAAGTTGGAGCAGAAAGAGAAAAATGAAC
CACAGCTTCTATTTTGTCTTAAACAGACTTGTACCAACATTTCTGTGGCTCAATCTAGGTGATGTTGAGACAAGAGGACACAGGGGTTAAATTTCTGT
GGCCGAGGGGAGAACTCTACCTCAGACTAGCCCAACGGGCTTTTCTGGCCCTGATCACTGGCATGGGCTGCTGAGACGCAAAAGGGGAGGCGAGA
TTGTCTCTGCAGCTGCAAGCCAGCACCCGCCAGCTGCTTGGCATGTCCCTCCAGCCGCTCTGAGTCCAGAGCCCATATCAATGCCCTGGGTCAG
AGCTCTGGAGAAAGCTGCTCAGTTAGCACCCAGAGGGAACCATGGAAACCCCAAGCGGAGCTTCTCTCTCCTGCTACTCTGGCTCCAGGTCAGGS
GAACAATGGGATGGTTTTCATGTGATGTGAAACCTCTCAAGTCTTGTTTACCTGGCAACTCTGCTCACTCAATACAAATTAATTAAGCTCAATATAAAG
CAATAATCTGGCTCTCTCTGGGAAGACAAATGGGTTTGATTAGATTACATGGGTGACTTTTCTGTTTATTTTCCAAATCTCAGATACCACCGGA

GAA

ATTGTGTTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCCAGGGAAAGAGCCACCCTCTCTCTGCAGGGCCAGTCA

GAGTGTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCTATGTTGTCAT

CCAGCAGGGCCACTGGCATCCAGACAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTCACCTCACCATCAGCAGAGA

CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCC

CCCTGCAAGACCTTCAATTTGTTTACTAGATTATACAGGCTGCTTCTTTACAGATAGCTGTCAATGACAACTCAATTTTAGCATCTCTTCTCTCTGCT
TGGGCATTTTGGGGATCTTAAAAAAGTAATCCCTTGATATATTTTGGACTCTGATTCCTGCAATTTTCTCAGACCAGATGGACAGCCAGGTTTAAAG
CACAGTTTCACTAATGGCCACTGGATCAGATTACATCAGTGGATGTCAAGTAAGTCCCAACAGAGCCATAAGGCARCAACAATAGCAACAATA
AATCAAAATTTGAAAAGAAATTAAGCTGTCAATAATTCACACTGATGAAGGATTTGTGTGCAGATAAAATTCAAAATTTTGTCTACAGAGAAACTACTAAA
ATTGACATGAGAAAATAGAAAAATCATTAGATTCAAGATCAATTTATTAAATTCATAGATTCAAAAATCAATTTCTGCAATATAAANAATGCTTAA
AATTAAATATTATAAAACAAACACACCATTTACAAAACATCAAGTCAAAATTAATTAATAAGTATCAAAATACACTGACGCTCCAGAAATATTA
TTTTGAAAATAAAGAAAACCTAAGTAAATAGAAAATTCAGTCCAAAGCACTGAATGTCTCAGTACTATAAAAATGTCAATTTCTTCAAGATTAATA
TTGATTACATATAAGAAAATCAAAATCTTAAAGTATACCTCAATTTAAATTAAGAAAGCTAATCTAAATATTATATGGGAATGTCAAGGATGTAGAAAT
AGCCACATGAACTGAAGAAACACCAAAATGAGAACTTCCAGTCCCTGAATACCTGGAAATATAGTGTGGGTGGCAGTATGTTGATGGTGCAGATCAGA
AGTTTAAAAAATTTGCAAAACGTGCTTATTTTGGAAATAATCACTACGCAGATGTAGCCAAACCTCTTCAACTGTGGCGGTACGGCACCTGCTCGAAAG
GGAGTTGAGCTTTCAGCAGCTGACCCAGGACTCTGTTCCTCTTGGTGAGAAAGGTTTTTTGTTCAGCAAGACAATGGAGAGCTCTCACTGTCTGTGGA

GTGGA

CGTTGGCCCAAGGACCAAGGTGGAATCAAAAC

CTATGAAGTGATCTATAAGCTGACTCTGCAATCAGCCTCTGATATCTTCAGGGAAAGATAAAGATAAGTCTGTAGTCAAACTCGAGAAATTGATTCG

A

VK3-20

JK1

 γ_2

FIG. 20C

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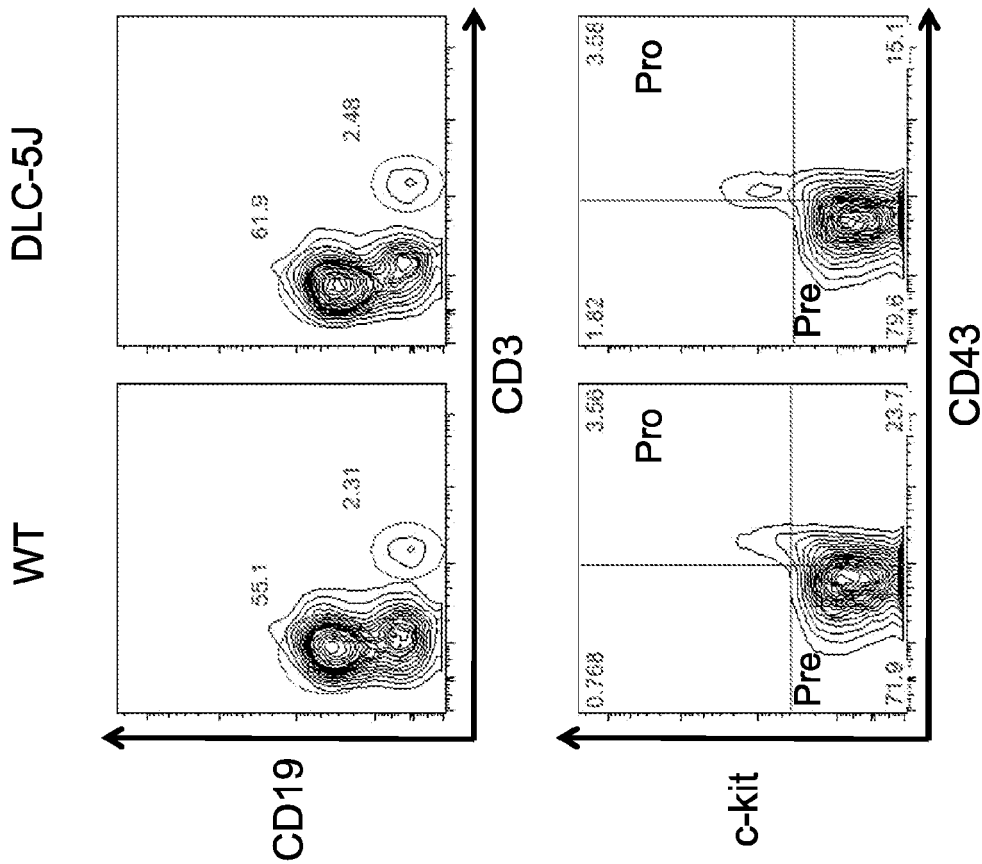


FIG. 21A

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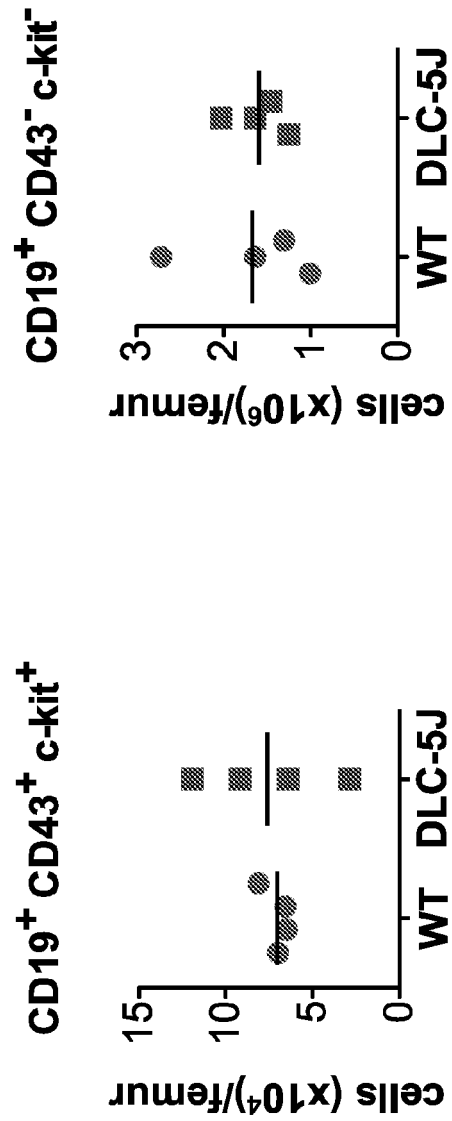


FIG. 21B

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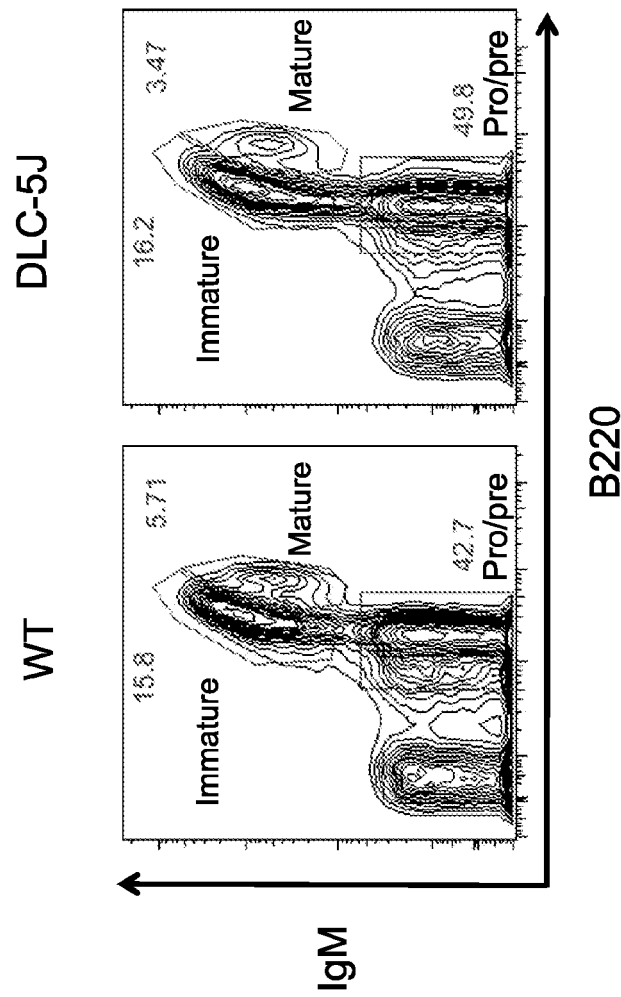


FIG. 22A

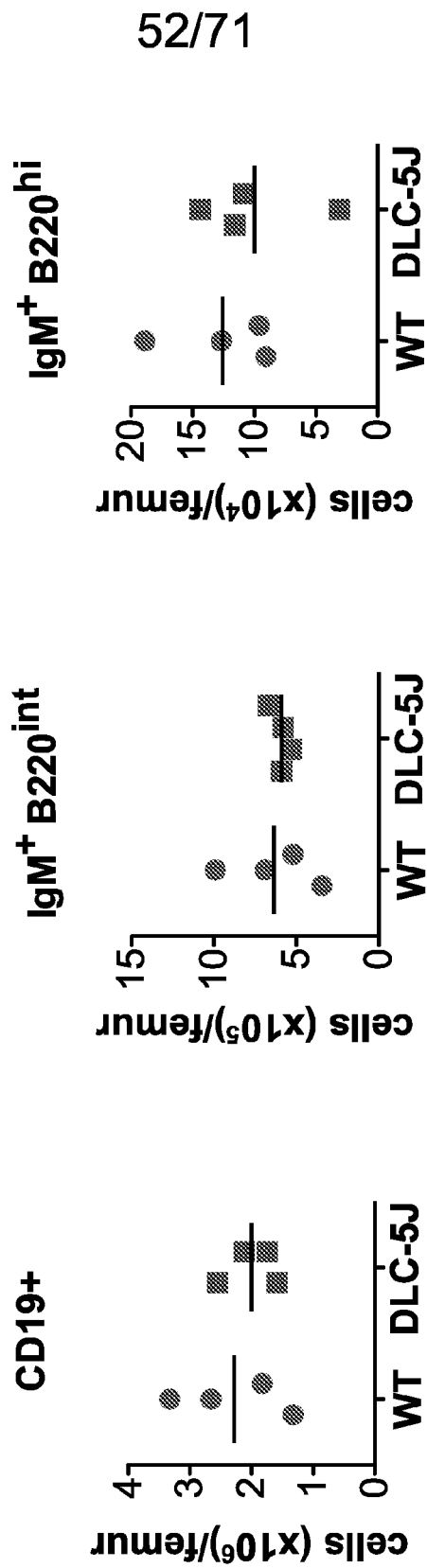


FIG. 22B

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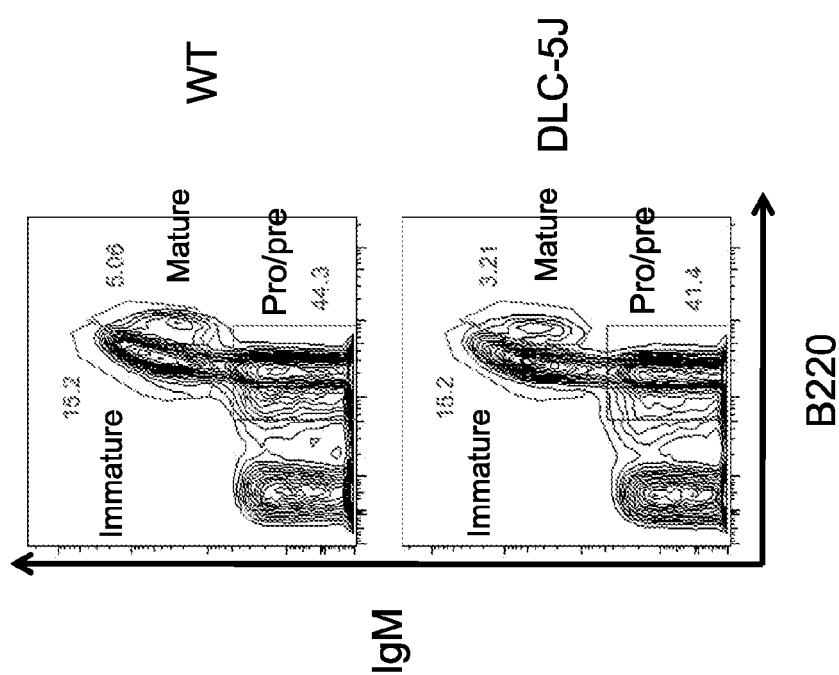


FIG. 23A

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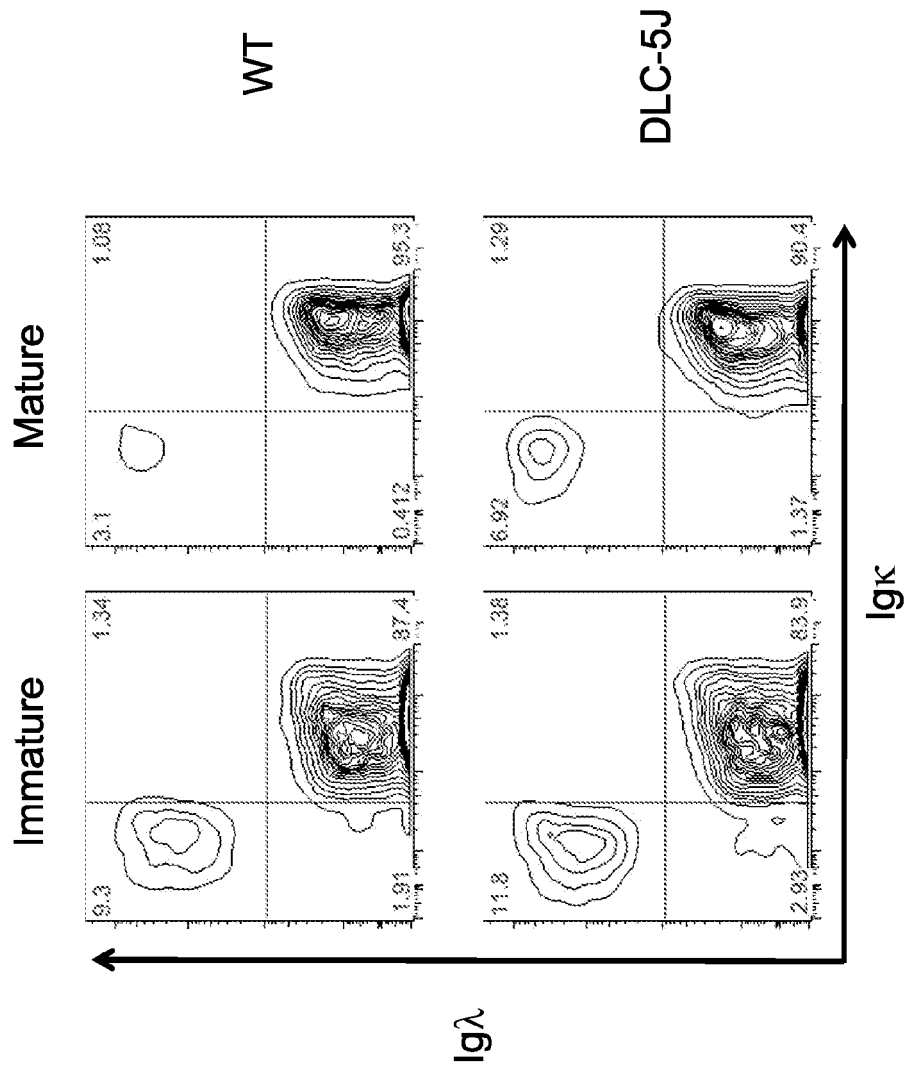


FIG. 23B

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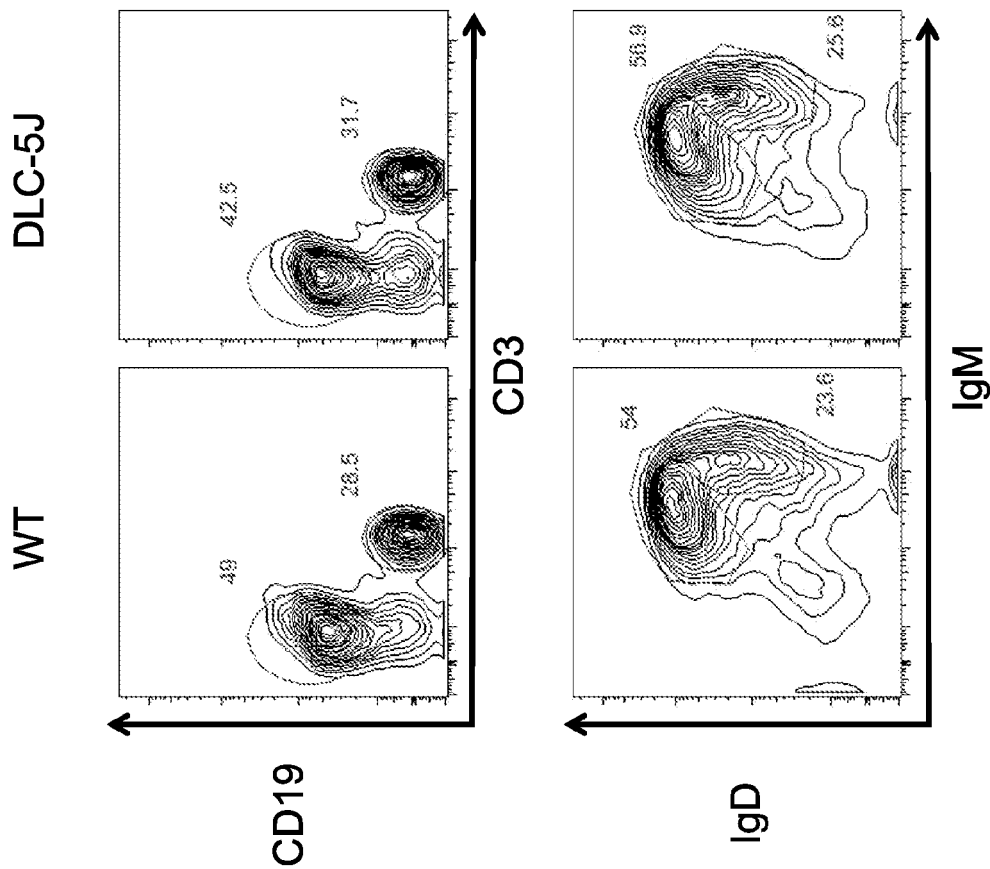


FIG. 24A

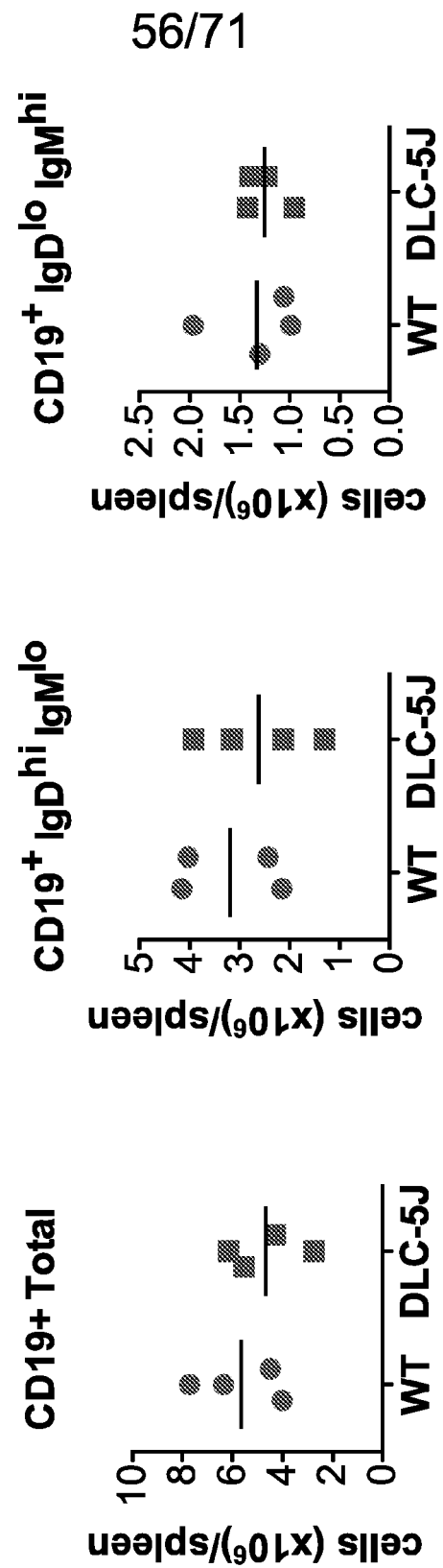


FIG. 24B

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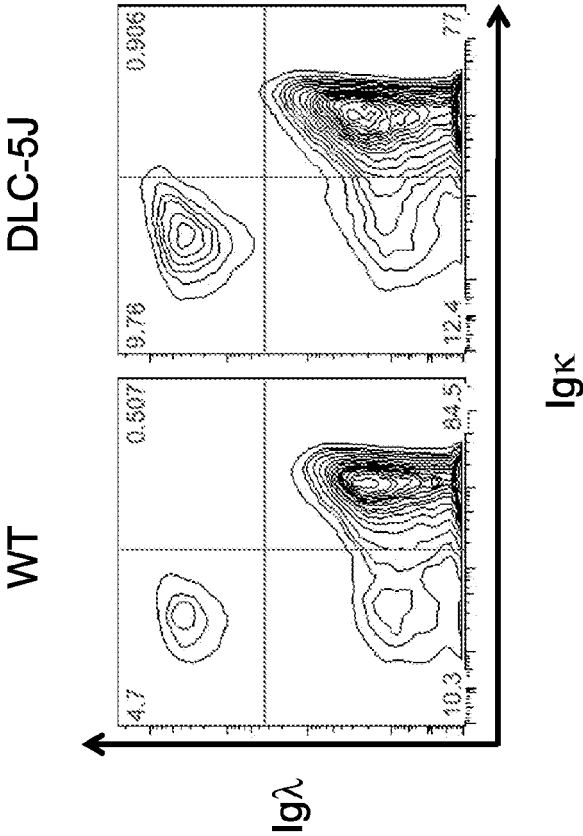


FIG. 25A

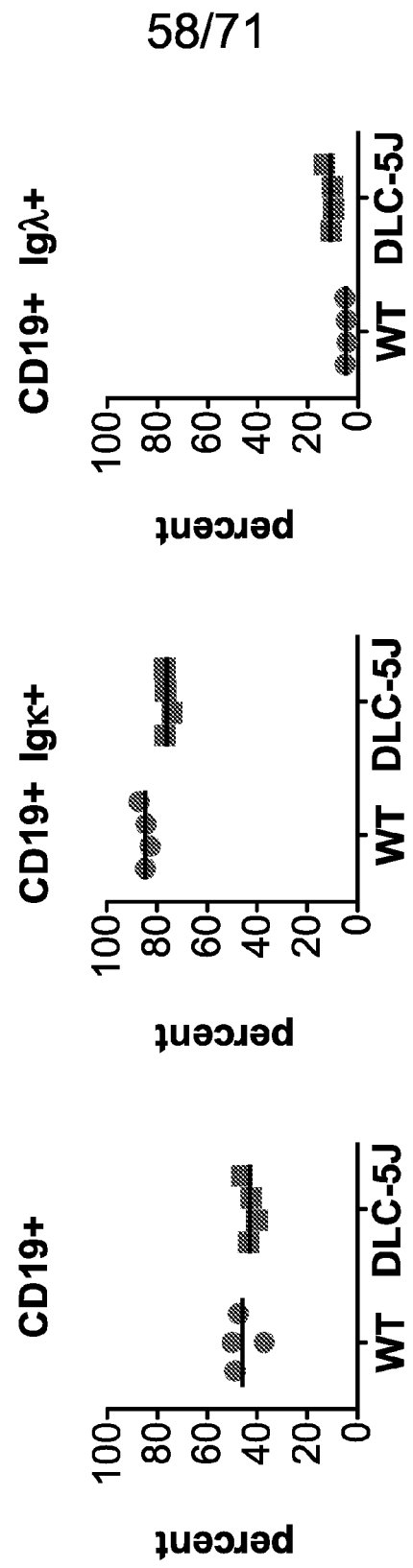


FIG. 25B

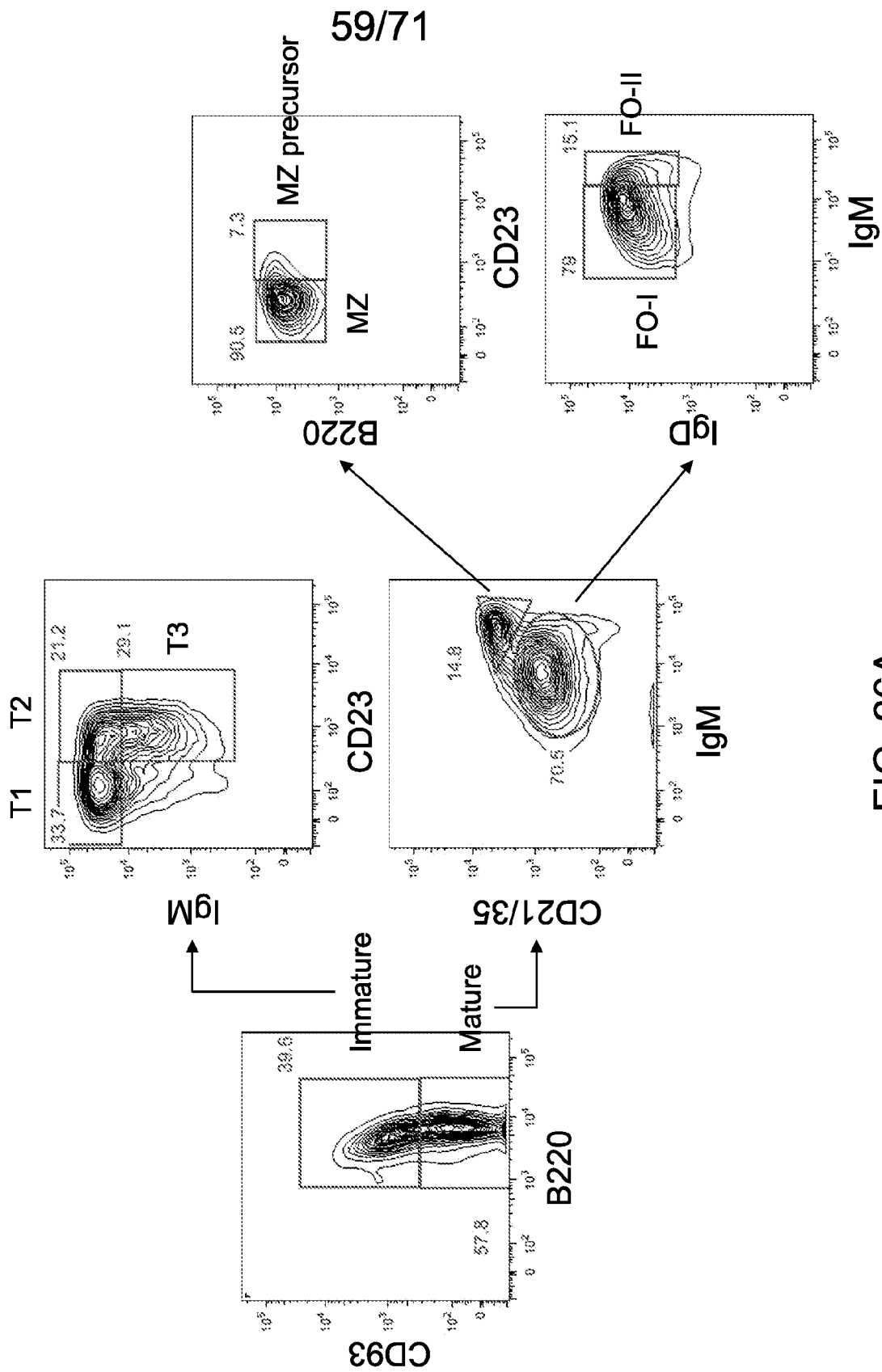


FIG. 26A

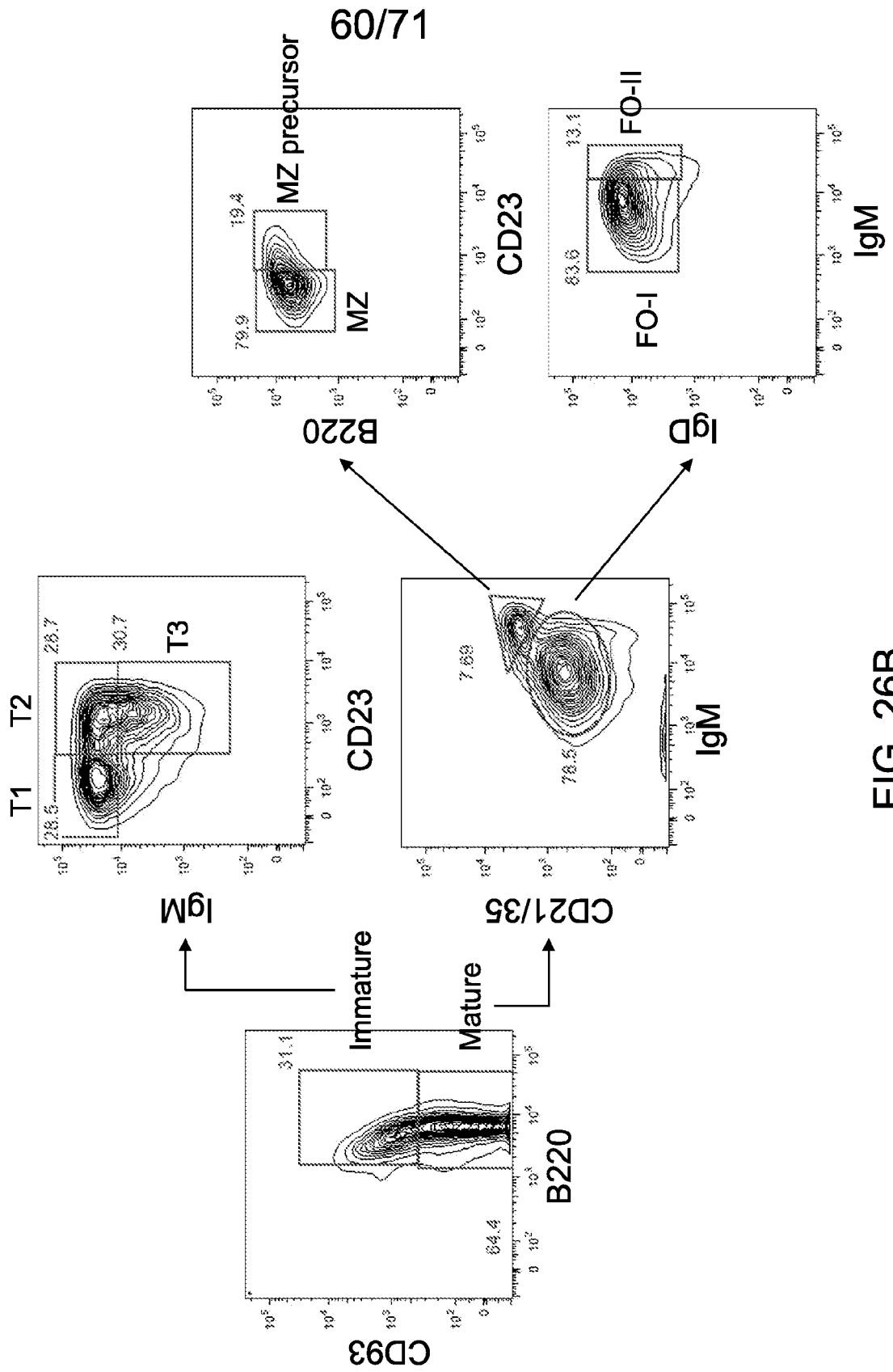


FIG. 26B

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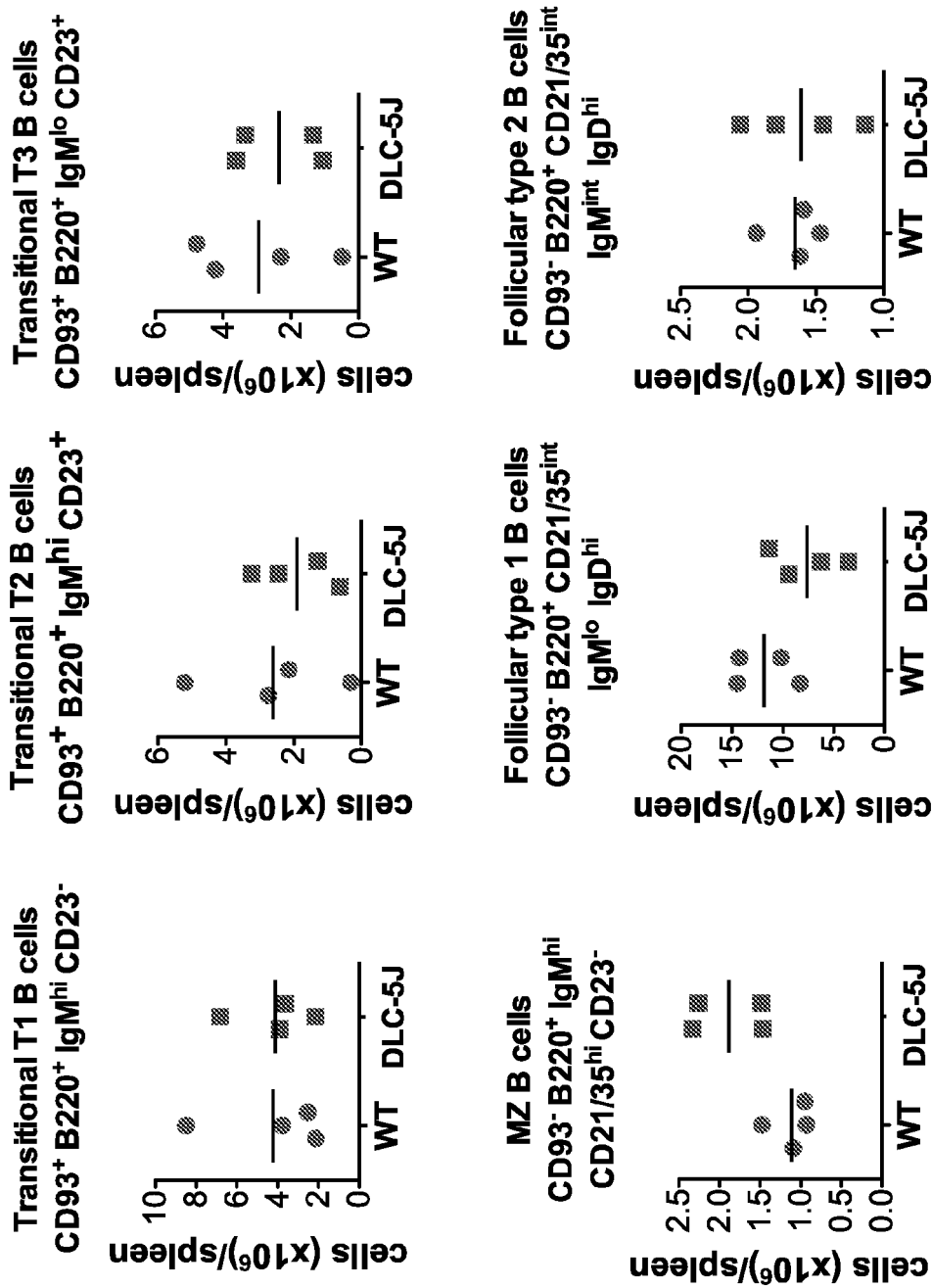


FIG. 27

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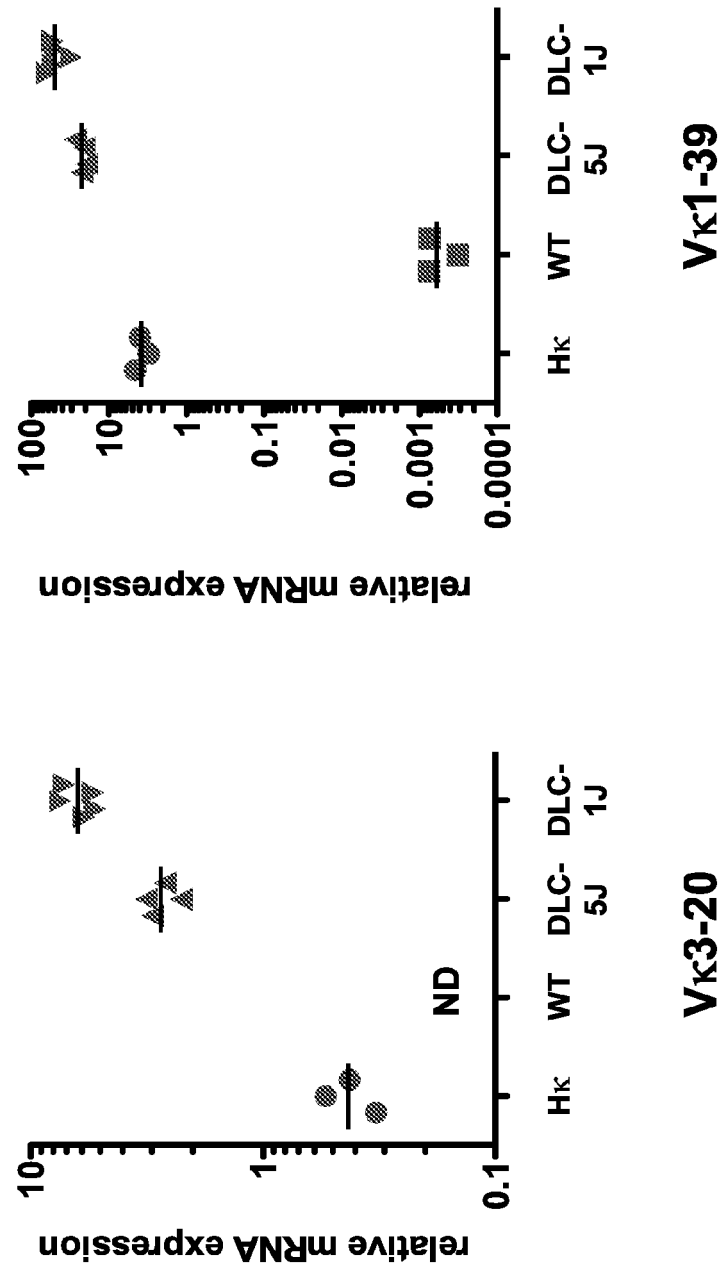


FIG. 28

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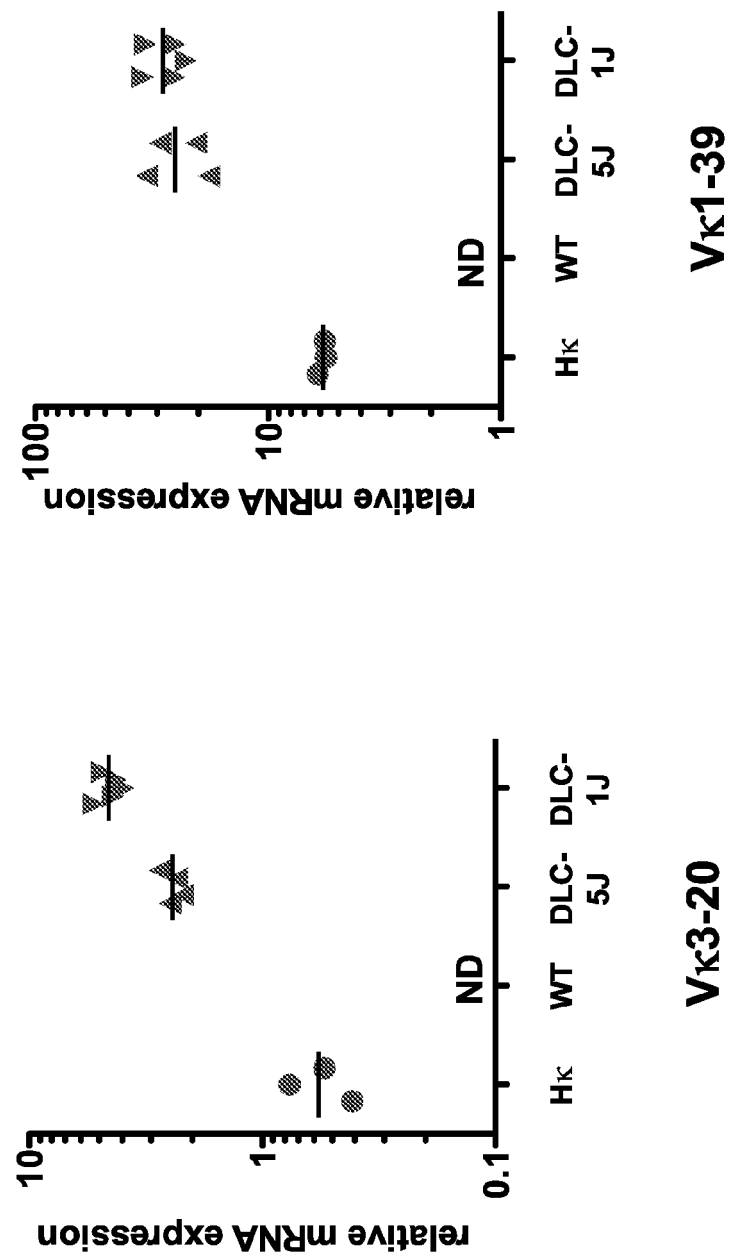


FIG. 29

Site-directed Mutagenesis Primers for 4 Histidine Substitutions in CDR3s of each VK of DLC-JK1-5 Plasmid

Primer name	Sequence	%GC	N	mis- matches	Tm
GERMLINE hVK1-39	CAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCACACAGTGTACAAG				
hVK1-39					
HDLC(Q105H/Q106H/Y108H/P111H) F	CAACTTACTACTGTCAACCATAGTCACAGTACCCATCCACACAGTGTACAAG	45	51	4	79
hVK1-39					
HDLC(Q105H/Q106H/Y108H/P111H) R	CTTGTAACACTGTGGGATGGGTACTGTGACTATGGTGACAGTAGTAAGTTG	45	51	4	79
GERMLINE hVK3-20	GCAGTGTATTACTGTCAAGCAGTATGGTAGCTCACCTCCAC				
hVK3-20					
HDLC(Q105H/Q106H/Y107H/S109H) F	AGATTTTGAGTGTATTACTGTCAACCATCATGGTCACTCACCTCCACAGTATTCAGCT	45	60	5	82
hVK3-20					
HDLC(Q105H/Q106H/Y107H/S109H) R	AGCTGAATCACTGTGGGAGGTGAGTGACCATGATGGTGACAGTAATACACTGCAAAATCT	45	60	5	82

Primer	SEQ ID NO
GERMLINE hVK1-39	105
hVK1-39 HDLC {Q105H/Q106H/Y108H/P111H} F	106
hVK1-39 HDLC {Q105H/Q106H/Y108H/P111H} R	107
GERMLINE hVK3-20	108
hVK3-20 {Q105H/Q106H/Y107H/S109H} F	109
hVK3-20 {Q105H/Q106H/Y107H/S109H} R	110

FIG. 30

MAID 6185 (HDLC with 4 HIS modifications in each VK)

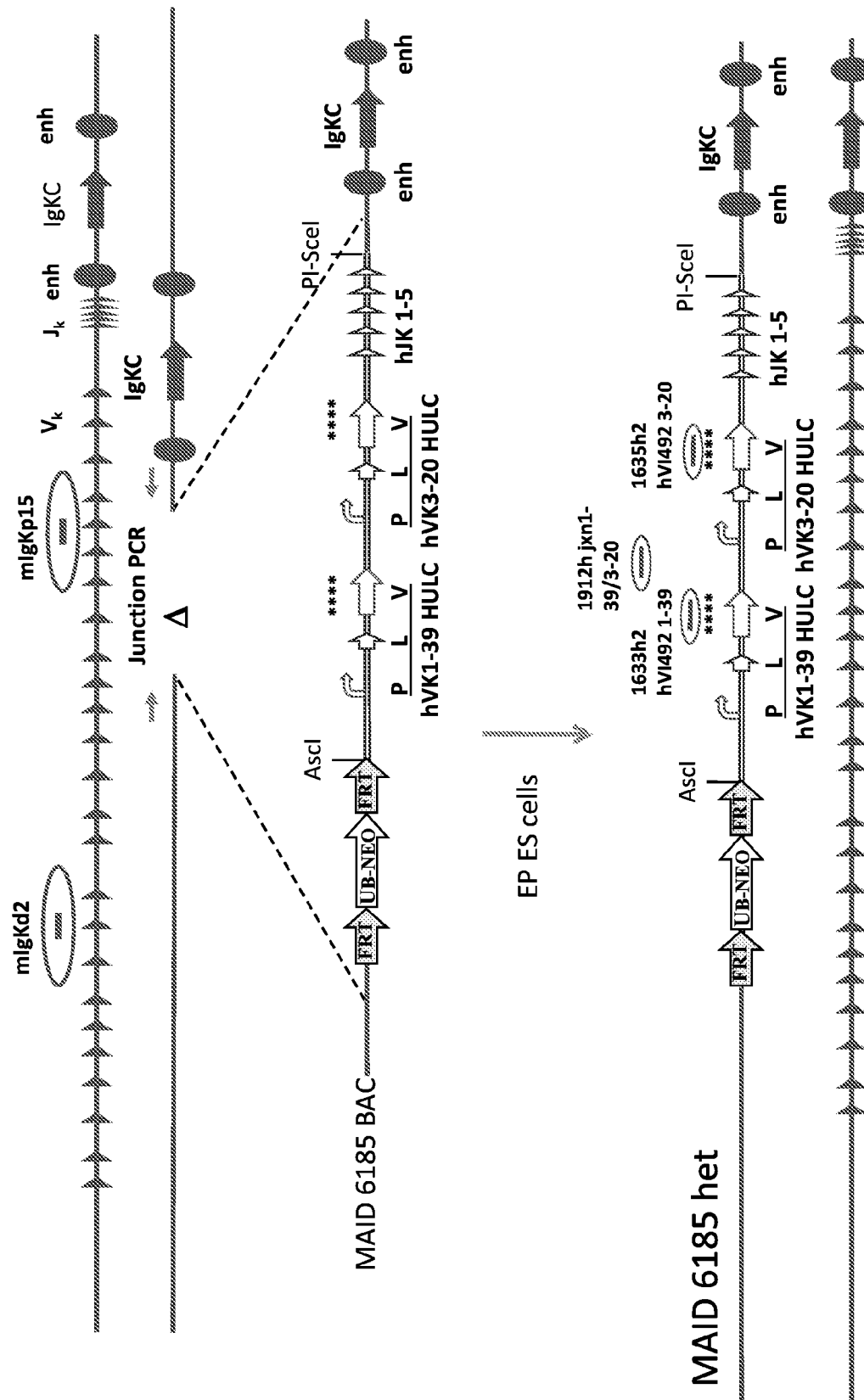


FIG. 31A

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MAID 6186 (HDLC with 4 HIS modifications in each VK), selection cassette deleted

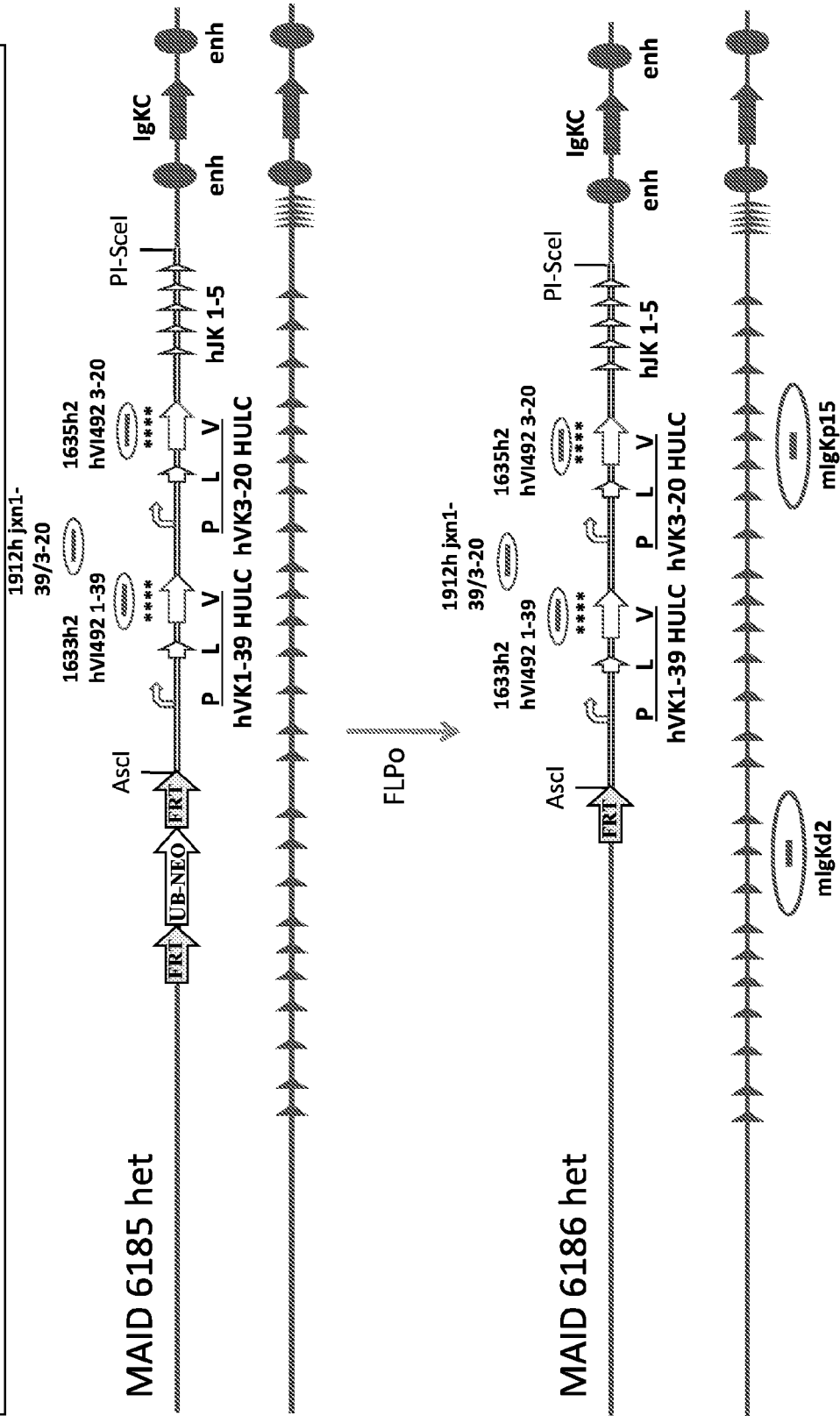


FIG. 31B

Site-Directed Mutagenesis Primers for 3 Histidine Substitutions in CDR3s of each VK of DLC-JK1-5 plasmid

Primer name	Sequence	%GC	N	mismatches	Tm
GERMLINE hVK1-39	CAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCACACAGTGTACAAG				
hVK1-39					
HDLC(Q106H/Y108H/P111H) F	ATTTTGCAACTTACTACTGTCAACATAGTCACAGTACCCATCCACACAGTGTTC	40.7	54	3	73
hVK1-39					
HDLC(Q106H/Y108H/P111H) R	GTAACACTGTGGGATGGGTACTGTGACTATGTTGACAGTAGTAAGTTGCAAAAT	40.7	54	3	73
GERMLINE hVK3-20	GCAGTGATTACTGTGAGCAGTAAGGTAGCTCACCTCCAC				
hVK3-20					
HDLC(Q105H/Q106H/S109H) F	ATTTTGCAAGTGATTACTGTGTCACCATTTAGTCACTCACCTCCACAGTGATTTCAG	42.9	56	4	75
hVK3-20					
HDLC(Q105H/Q106H/S109H) R	CTGAATCACTGTGGGAGGTGAGTGACCATATAATGGTGACAGTAATACACTGCAAAAT	42.9	56	4	75

Primer	SEQ ID NO
GERMLINE hVK1-39	117
hVK1-39 HDLC (Q106H/Y108H/P111H) F	118
hVK1-39 HDLC (Q106H/Y108H/P111H) R	119
GERMLINE hVK3-20	120
hVK3-20 (Q105H/Q106H/S109H) F	121
hVK3-20 (Q105H/Q106H/S109H) R	122

FIG. 32

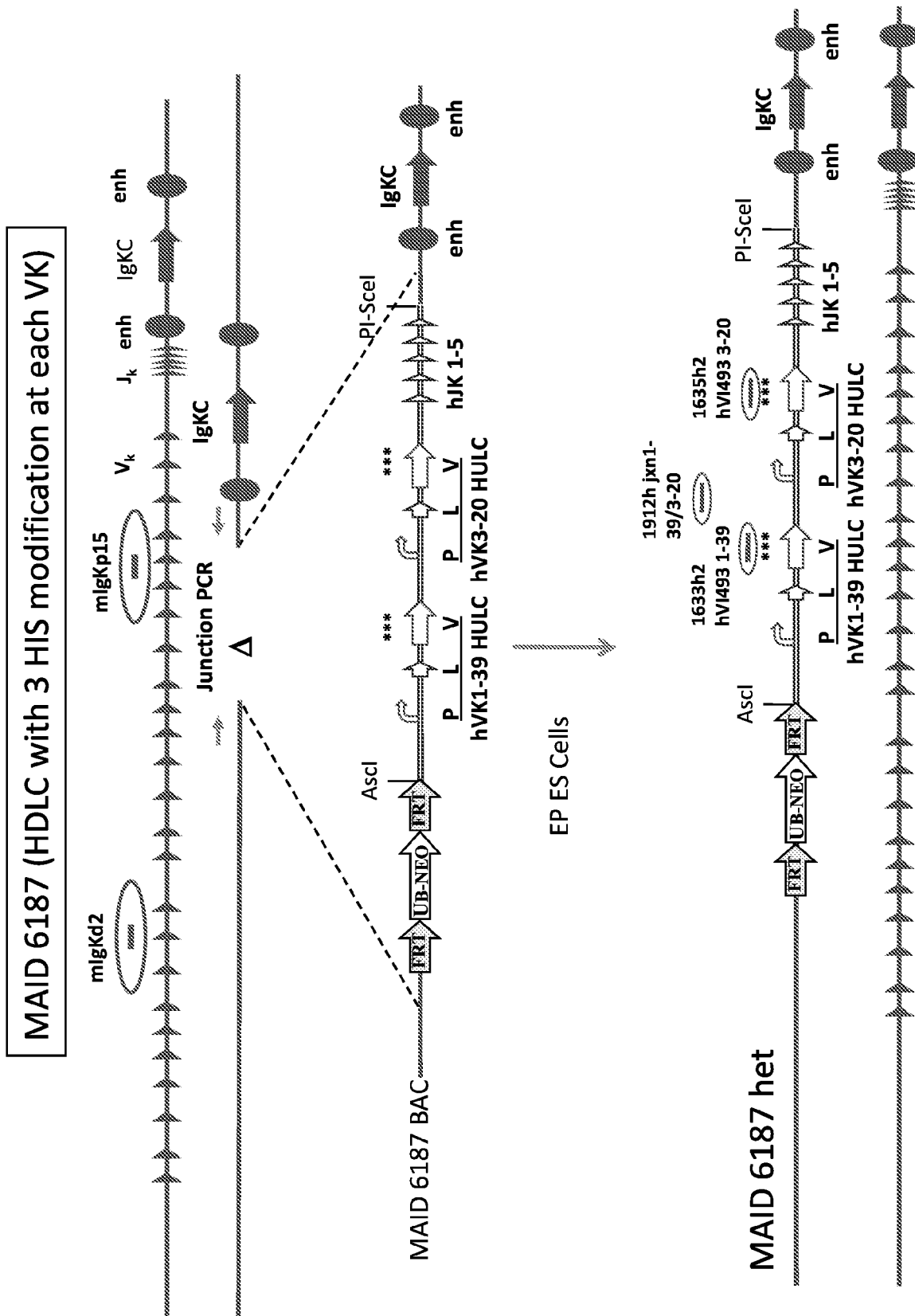
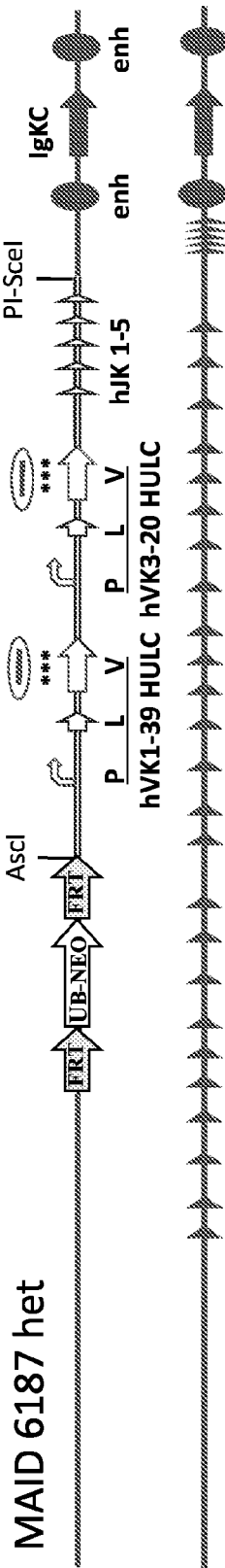


FIG. 33A

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MAID 6188 (HDLC with 3 HIS modification at each VK), selection cassette deleted



FLPo

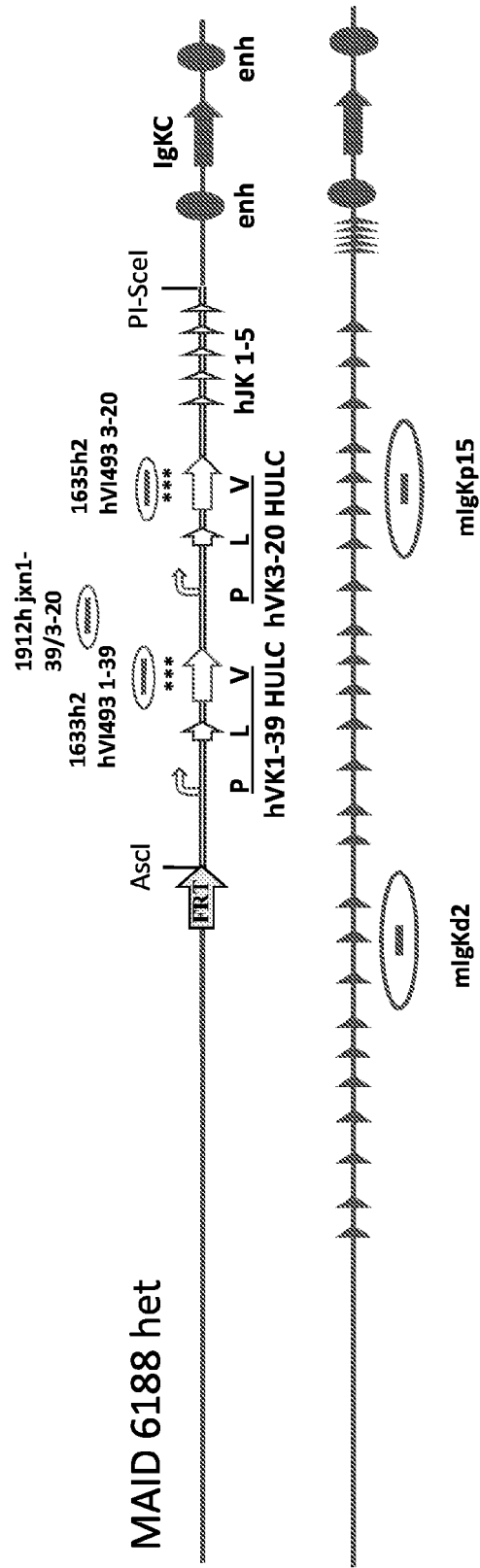


FIG. 33B

	FR1	CDR1	FR2	CDR2
IGKV3-20*01 F	EIVLTQSPGTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIY	GAS
	EIVLTQSPGTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIY	GAS
	FR3			CDR3
IGKV3-20*01 F	SRATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYC	HHYGH	SEQ ID NO:131	
	SRATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYC	QQYGSSP	SEQ ID NO:132	
		HH H		

FIG. 34A

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	FR1	CDR1	FR2	CDR2
	<u>DIQMTQSPSSLSASVGDRVTITCRAS</u>	<u>QSISSY</u>	<u>LNWYQQKPGKAPKLLIY</u>	<u>AAS</u>
IGKV1-39*01 F	DIQMTQSPSSLSASVGDRVTITCRAS	QSISSY	LNWYQQKPGKAPKLLIY	AAS
			CDR3	
	<u>SLQSGVPSRFSGSGGTDFTLTIS</u>	<u>SLQPEDFATYYC</u>	<u>QHSHSTH</u>	<u>SEQ ID NO:133</u>
IGKV1-39*01 F	SLQSGVPSRFSGSGGTDFTLTIS	SLQPEDFATYYC	QQSYSTP	SEQ ID NO:134
			H H H	

	FR1	CDR1	FR2	CDR2
	<u>DIQMTQSPSSLSASVGDRVTITCRAS</u>	<u>QSISSY</u>	<u>LNWYQQKPGKAPKLLIY</u>	<u>AAS</u>
IGKV1-39*01 F	DIQMTQSPSSLSASVGDRVTITCRAS	QSISSY	LNWYQQKPGKAPKLLIY	AAS
			CDR3	
	<u>SLQSGVPSRFSGSGGTDFTLTIS</u>	<u>SLQPEDFATYYC</u>	<u>QHSHSTK</u>	<u>SEQ ID NO:135</u>
IGKV1-39*01 F	SLQSGVPSRFSGSGGTDFTLTIS	SLQPEDFATYYC	QQSYSTP	SEQ ID NO:134
			H H	

FIG. 34B