

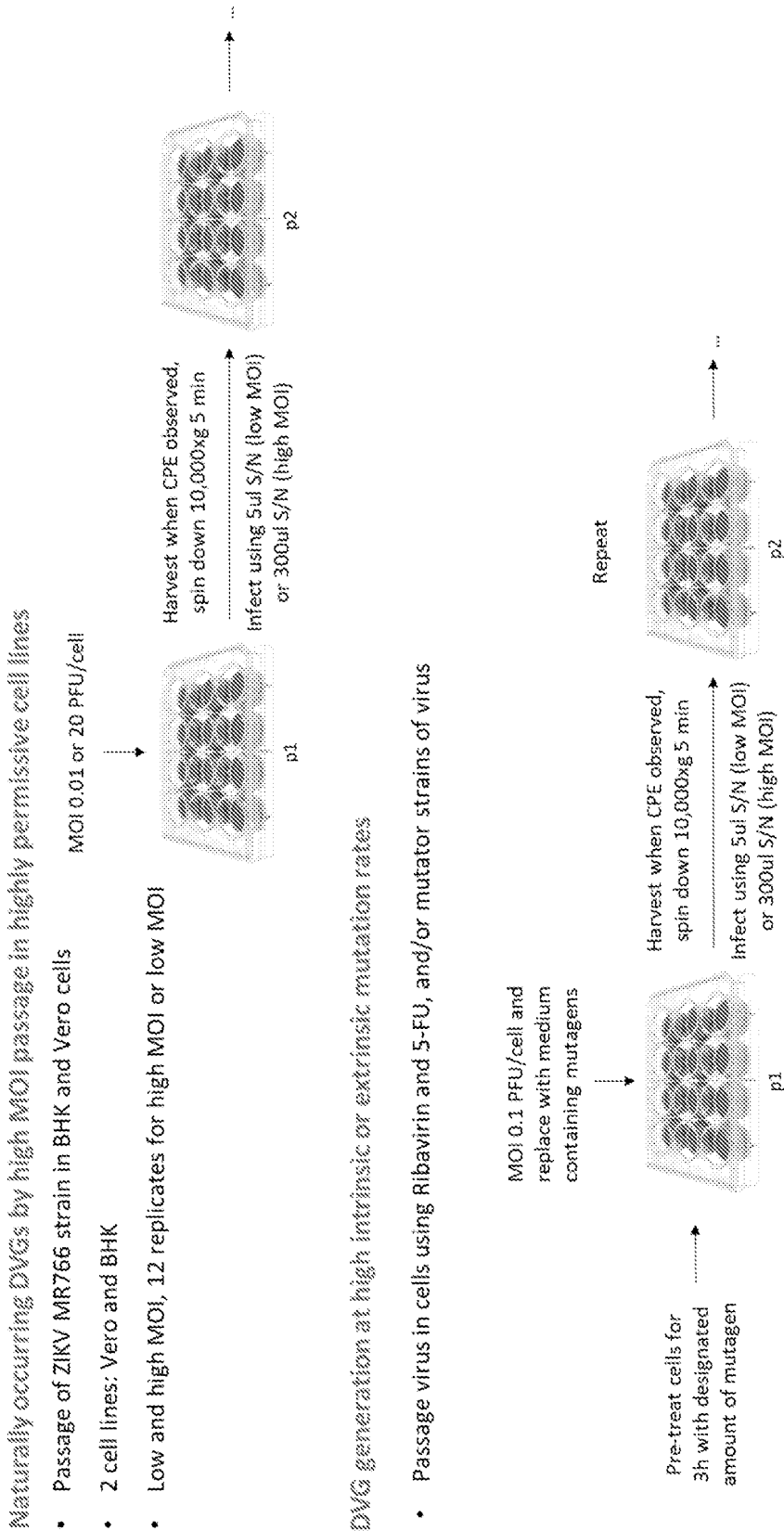


Figure 1

Detection of deletions in short-read sequences (BBTools)

- Inputs raw reads files
1. BBDOUK : remove bad quality bases and adapter sequences
 2. CLUMPIFY discard duplicate reads (optical duplicates)
 3. BBMAP : map reads on reference genome
 4. CALLVARIANT : call for substitutions, insertions, deletions
- Outputs variants coordinates (SUB, INS, DEL)

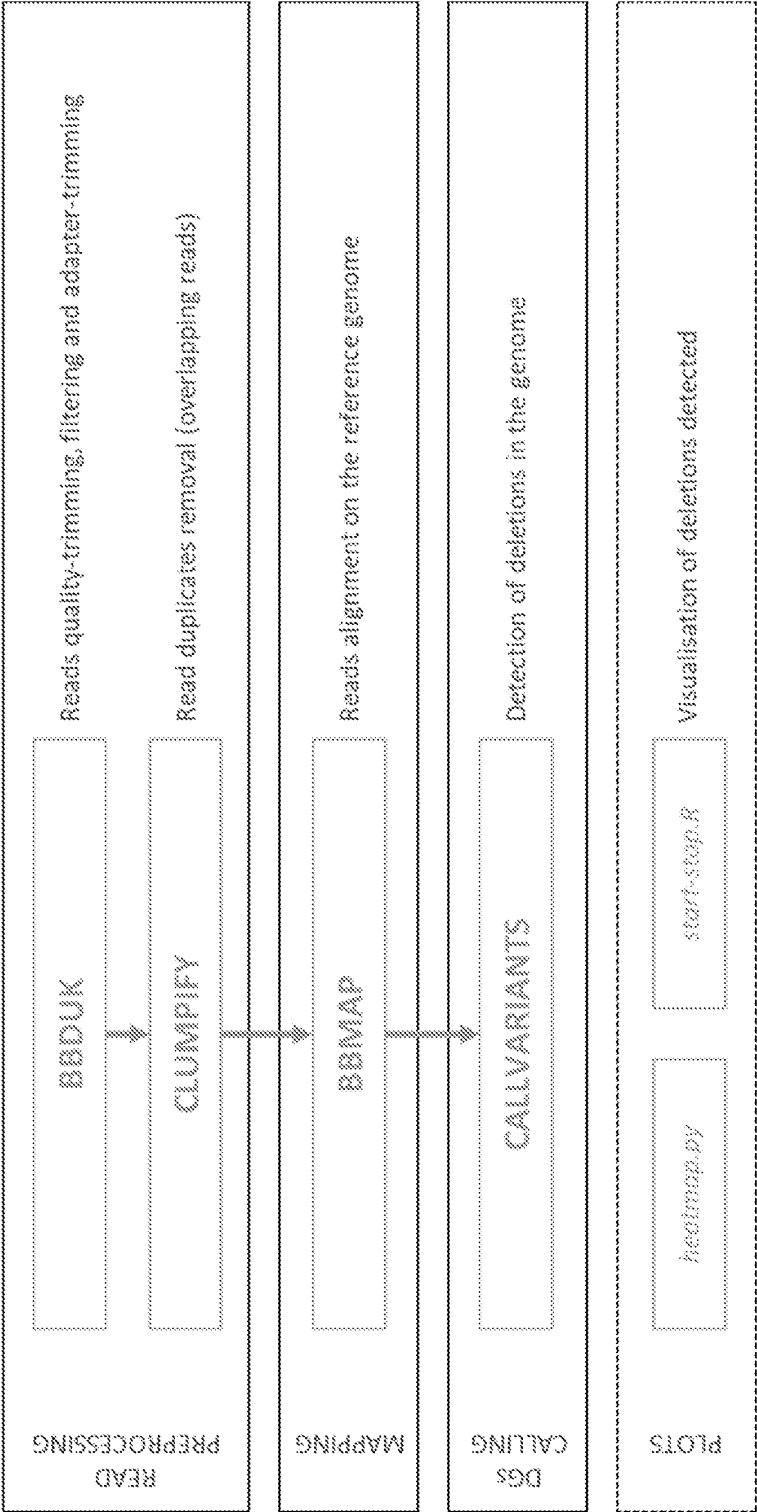


Figure 2

ZIKA virus deletion hotspots – Vero cells, high volume, individual replicates (across passages)

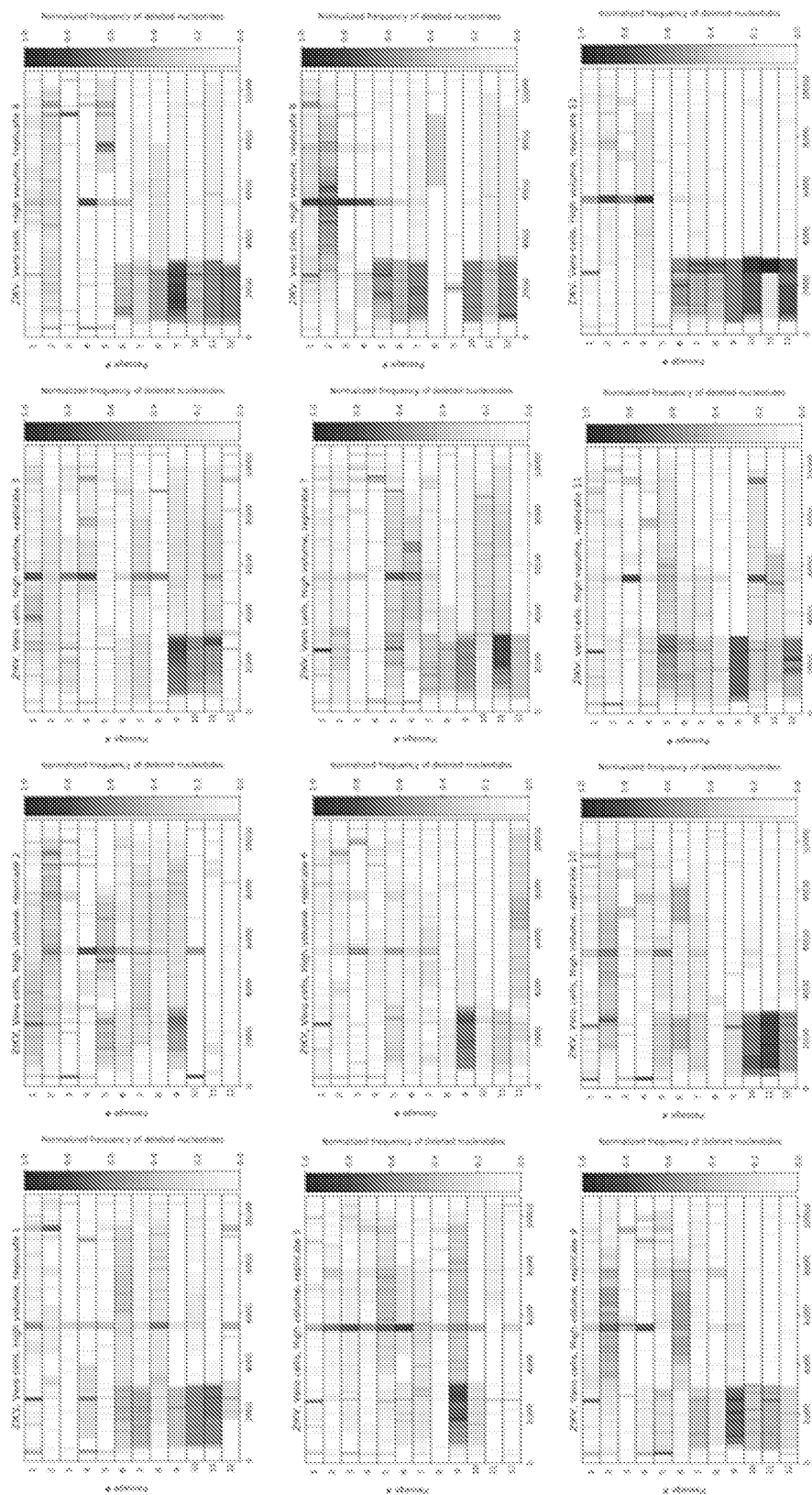
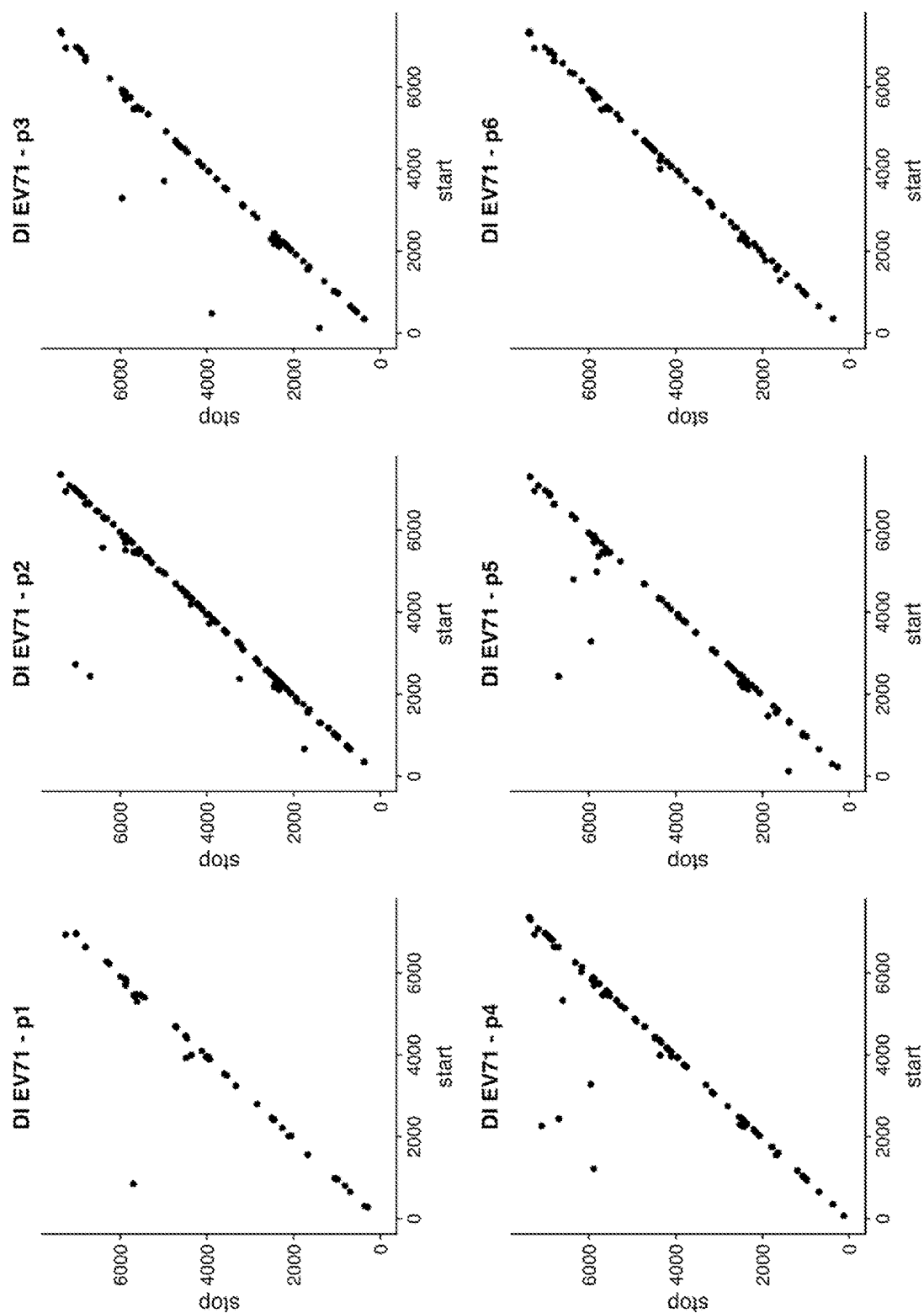


Figure 3

**Figure 4**

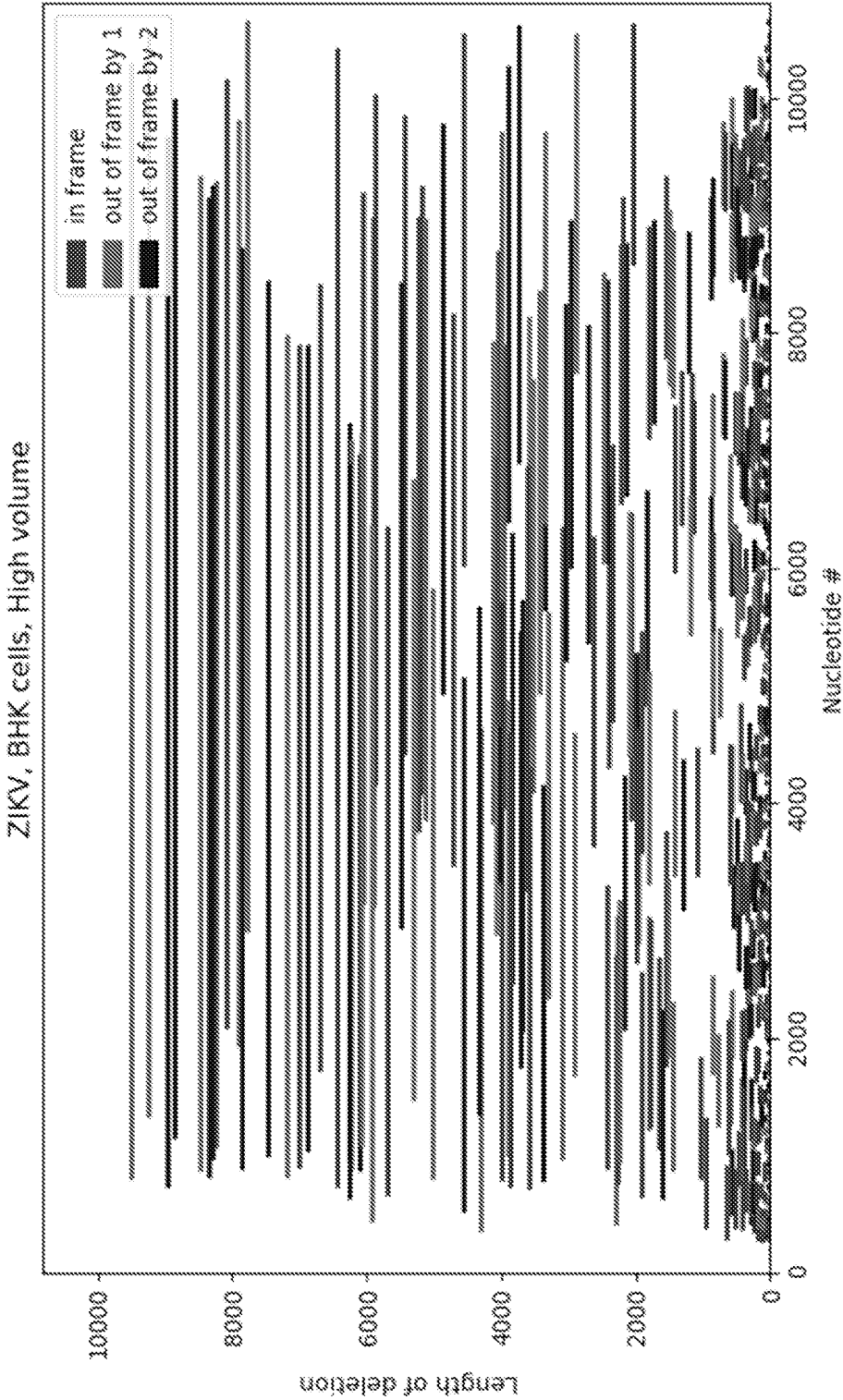
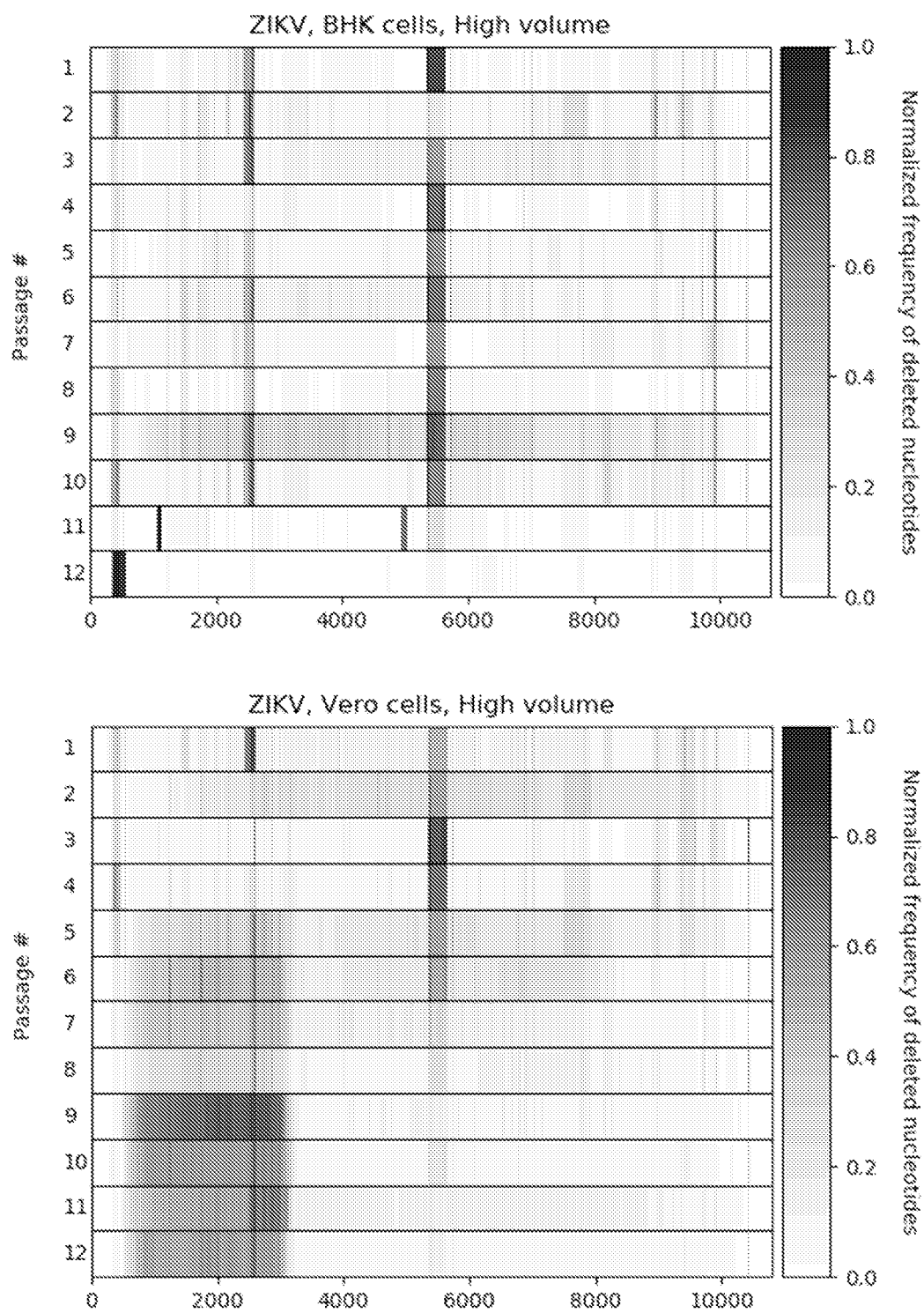


Figure 5

**Figure 6**

ZIKV DVG generation at high intrinsic or extrinsic mutation rates

Sequenced p2 by RNAseq to look for DVGs:

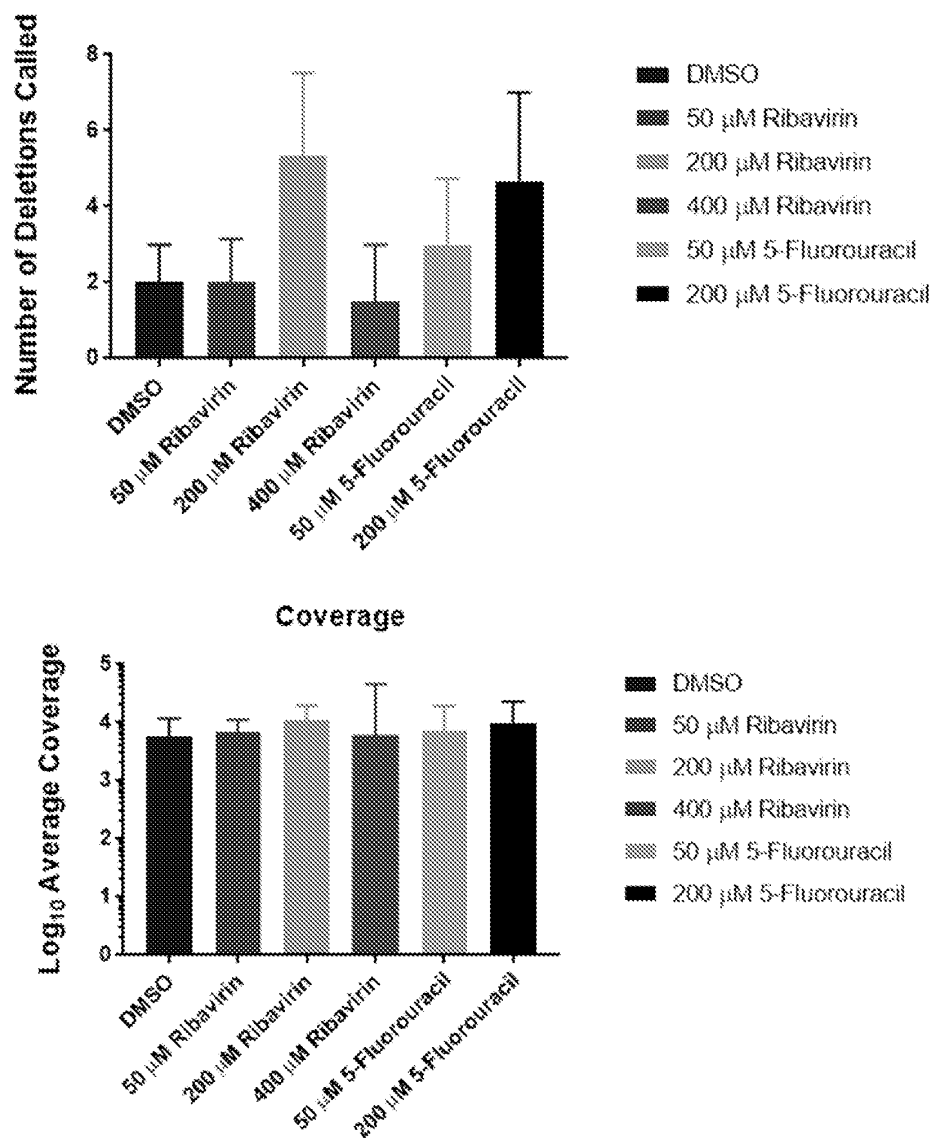
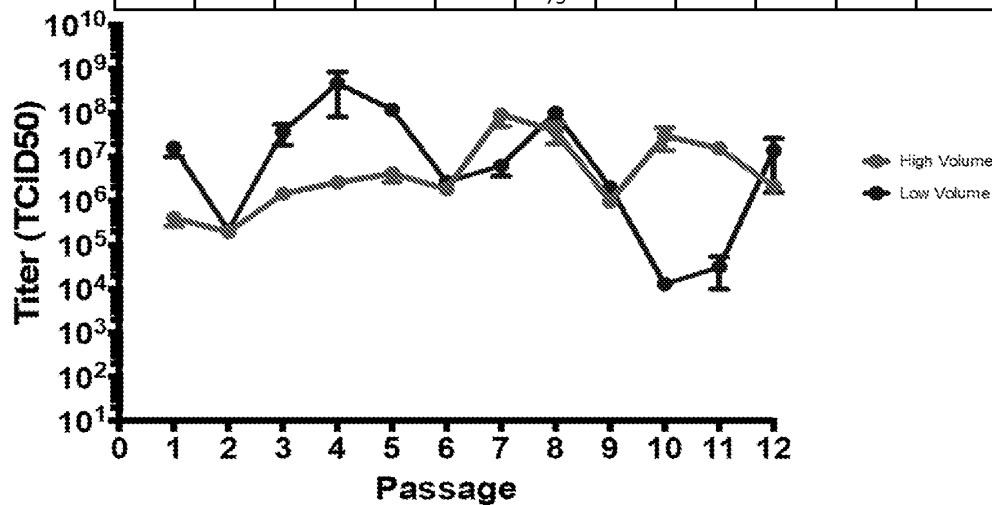


Figure 7

Sample Generation – Serial Passaging
Vero

Input	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
MOI	0,5	0,024	0,03	0,188	0,38	0,6	0,12	2,37	2,37	0,3	2,37	1,5
High		-	-	-	-	-	-	-	-	-	-	-
		1,5	0,24	2,37	3,76	14,96	2,99	375,9	188	1,5	149,6	29,86
Low	0,005	0,00024	0,0006	0,0119	0,024	0,0299	0,006	0,0188	0,03	0,0075	0,00000	0,00000
		-	-	-	-	-	-	-	-	-	75	47
		0,3759	0,00237	0,299	23,72	1,88391	0,03	0,188	1,88	0,038	0,00018	0,00119
						75						



RD

Input	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
MOI	0,5	0,3	0,095	0,0019	0,0047	0,095	0,188	11,9	0,0047	0,06	0,0047	0,00075
High		-	-	-	-	-	-	-	-	-	-	-
		2,99	0,24	1,88	0,038	1,5	0,94	375,9	0,0047	1,19	0,0047	0,0047
Low	0,005	0,015	0,000094	0,00004	0,00018	0,00004	0,00004	0,000004	0,0002	0,000004	0,0000075	
		-	-	7	8	7	7	7	9	7	-	
		0,038	0,0038	0,00038	0,0015	0,0075	0,00075	0,000047	0,0119	0,000075	0,00012	

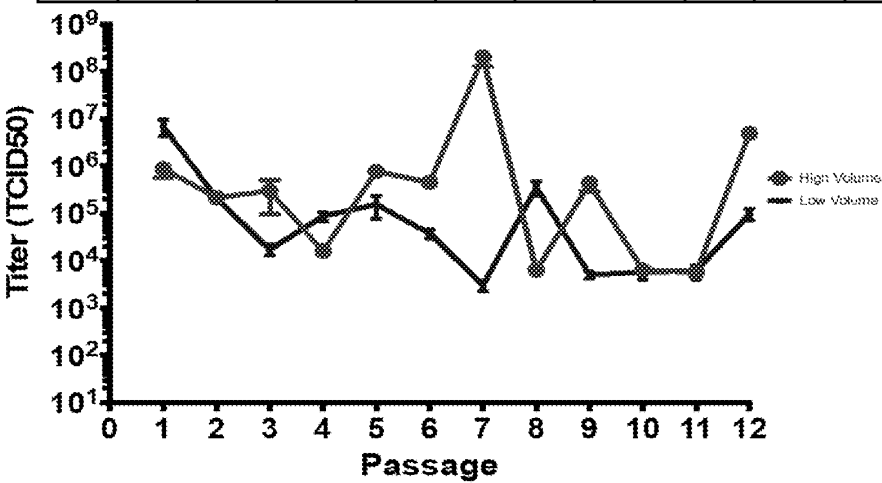


Figure 8

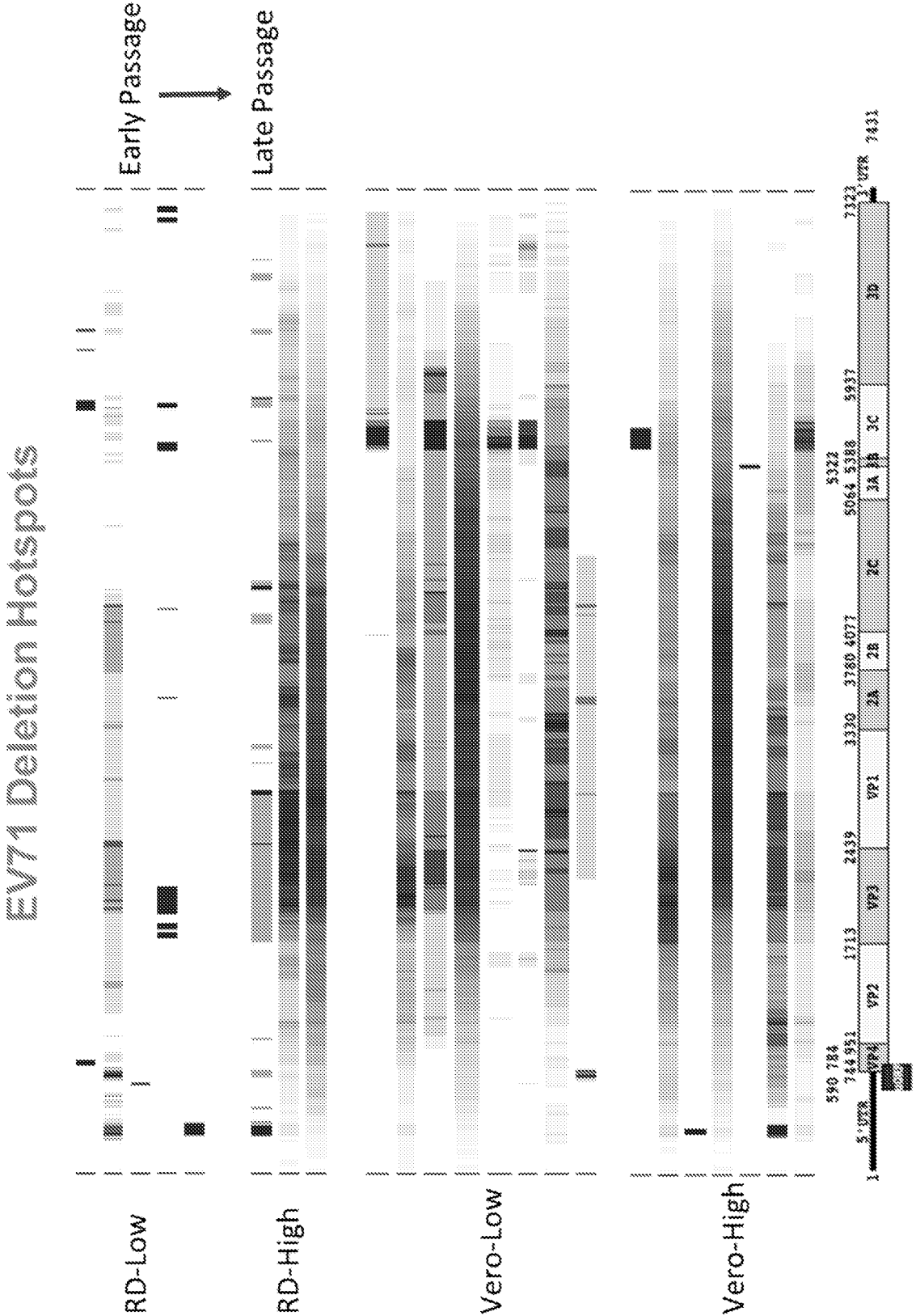


Figure 9

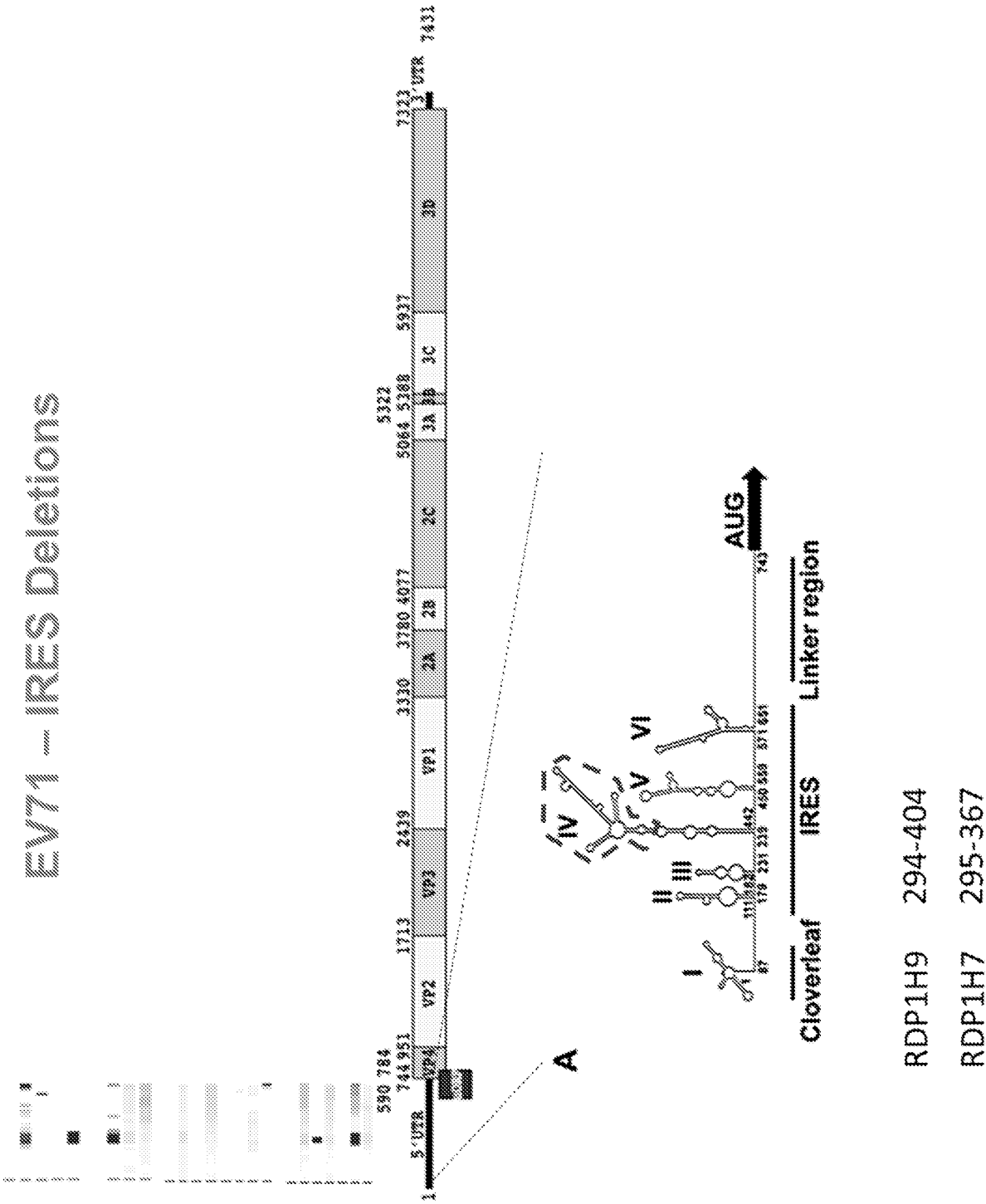


Figure 10

10/67

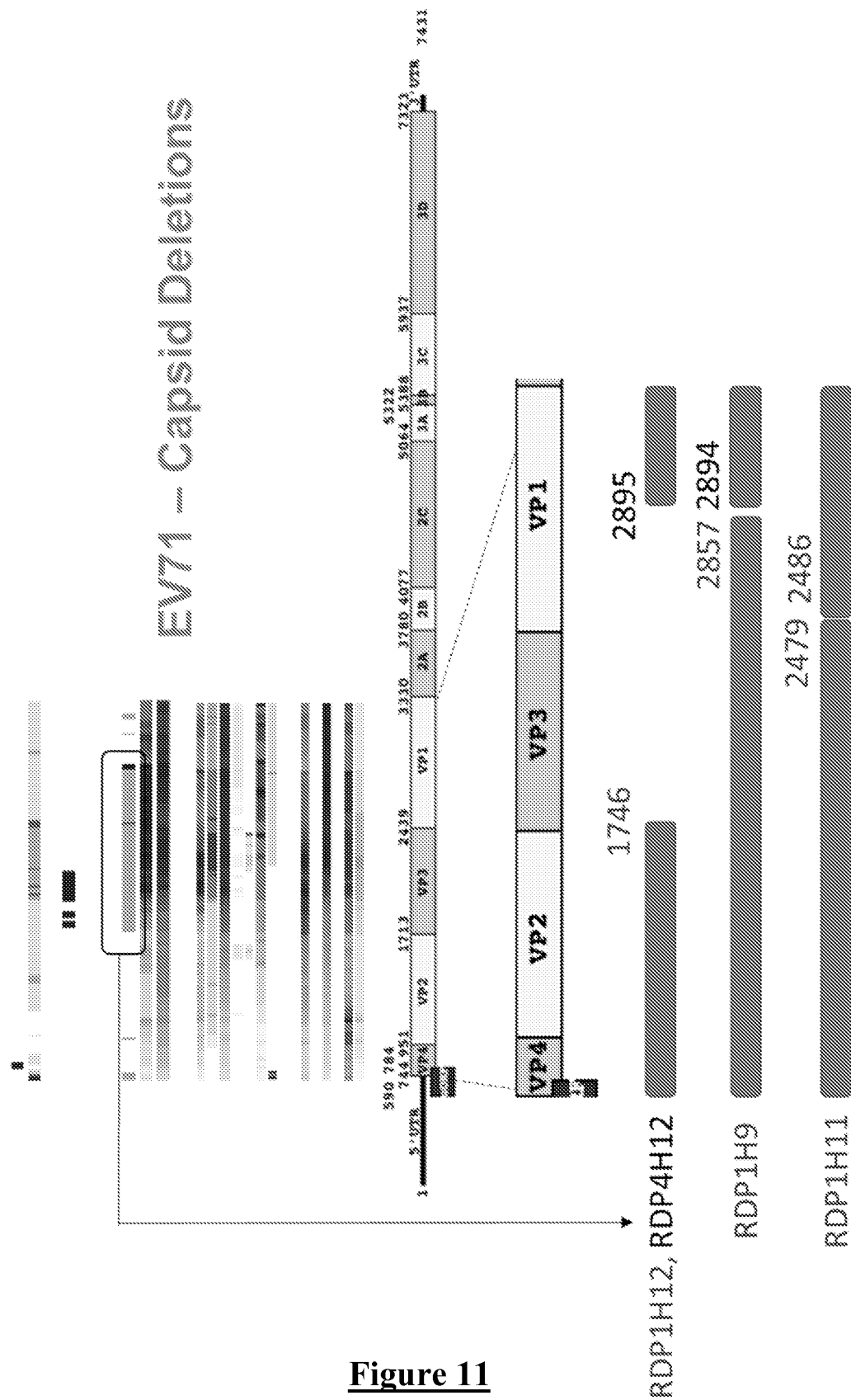
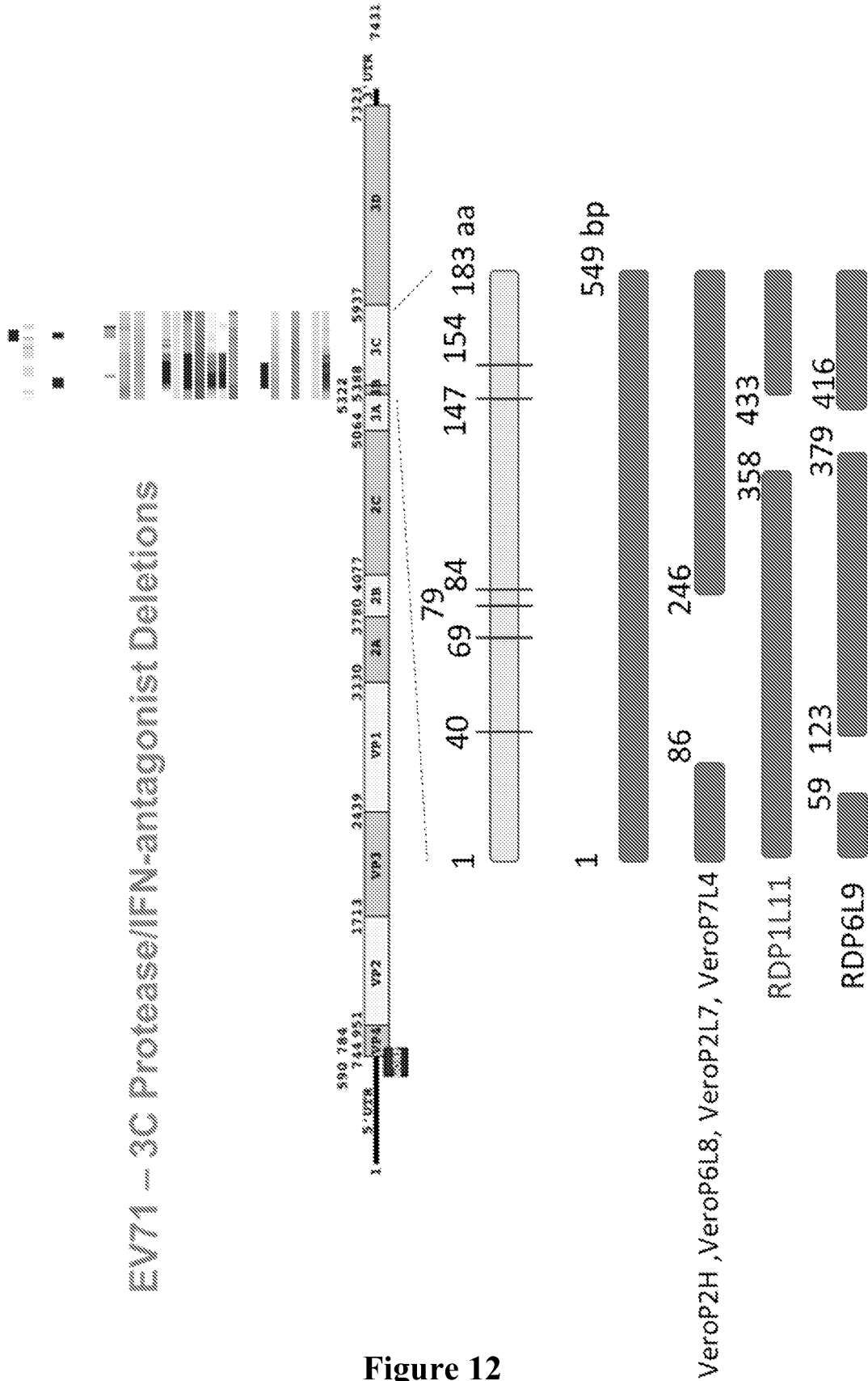


Figure 11

EV71 – 3C Protease/IFN-antagonist Deletions



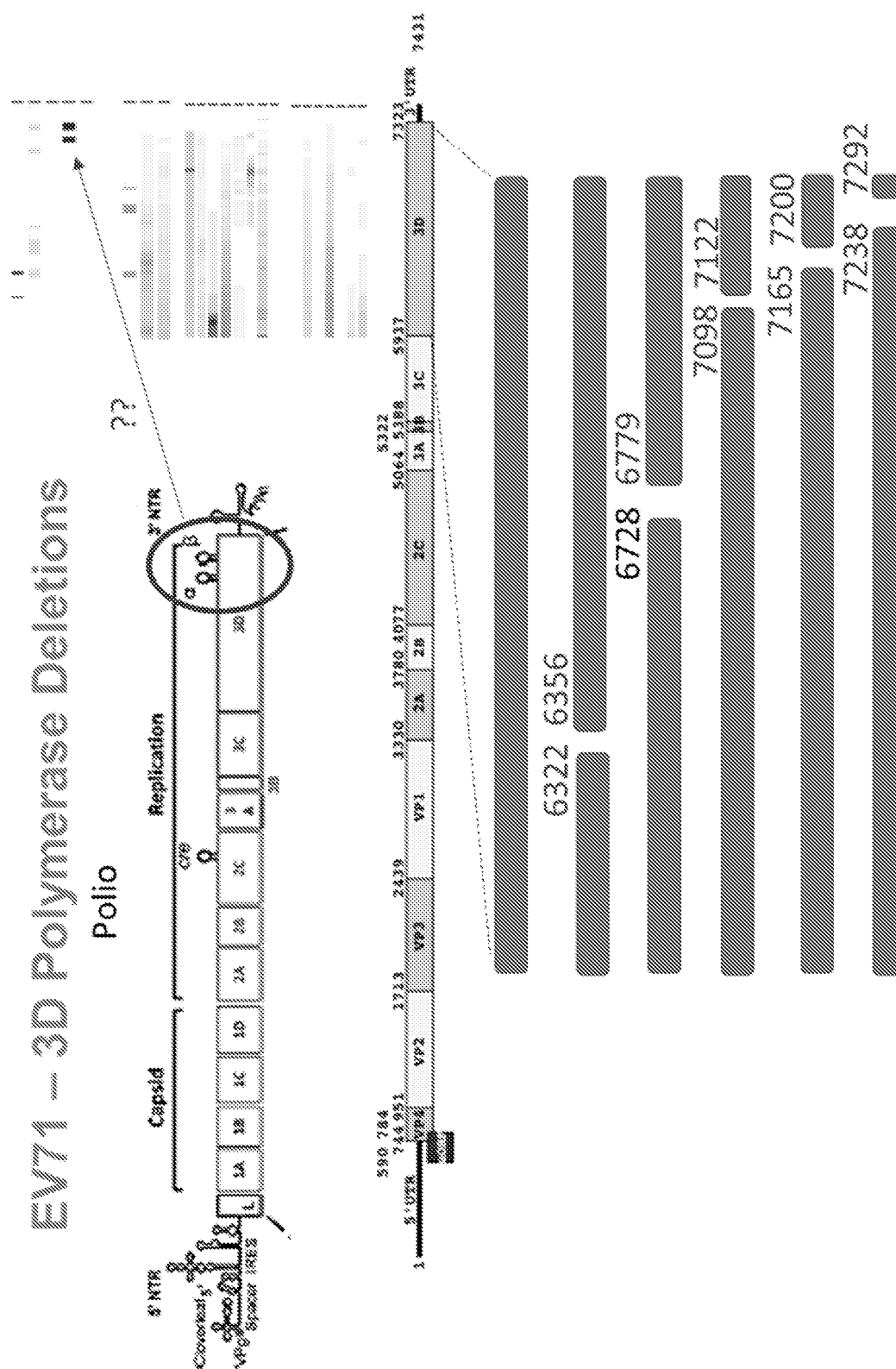


Figure 13

EV71 Complementarity after Breakpoint

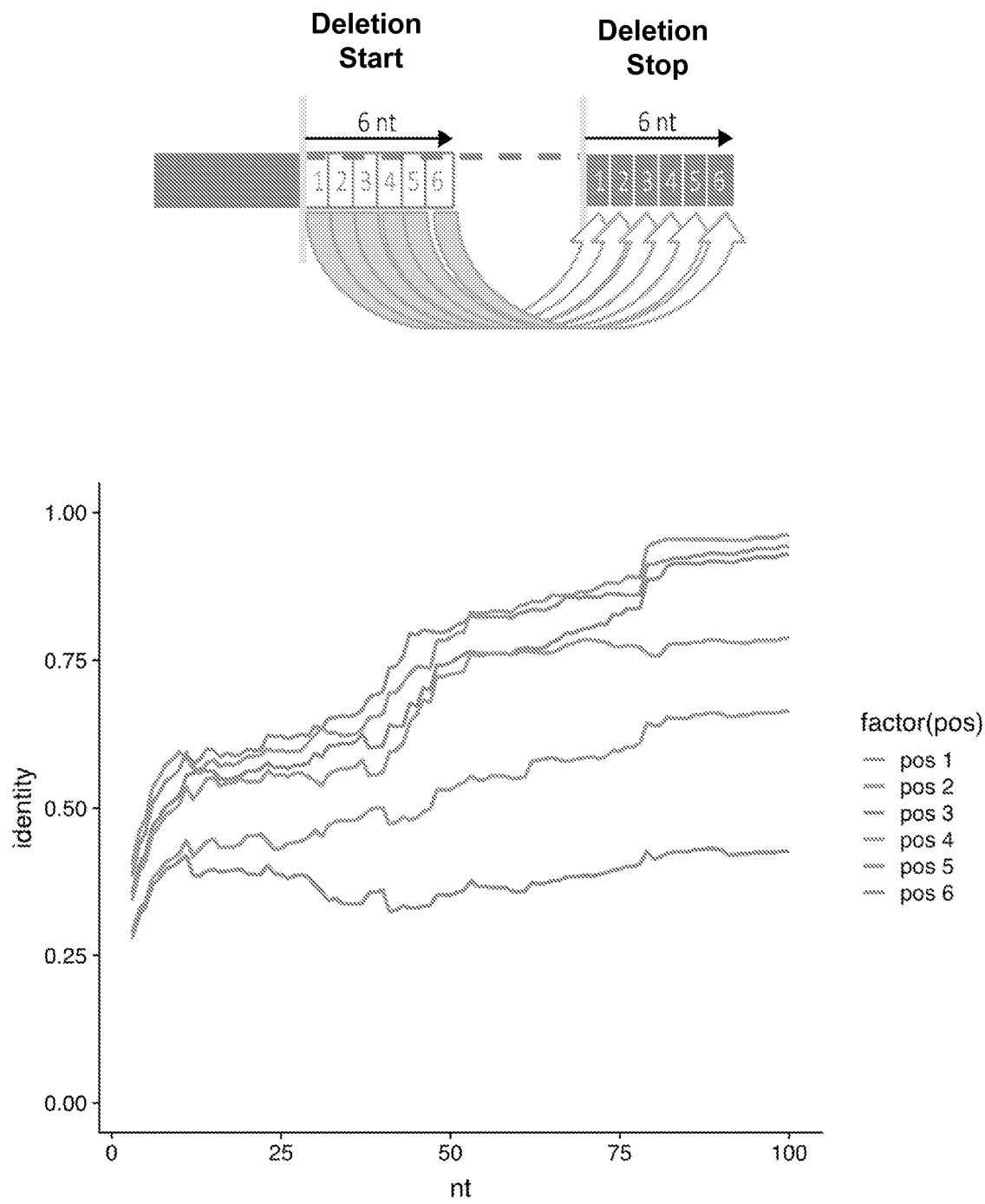


Figure 14

Complementarity around Breakpoints across different Viruses

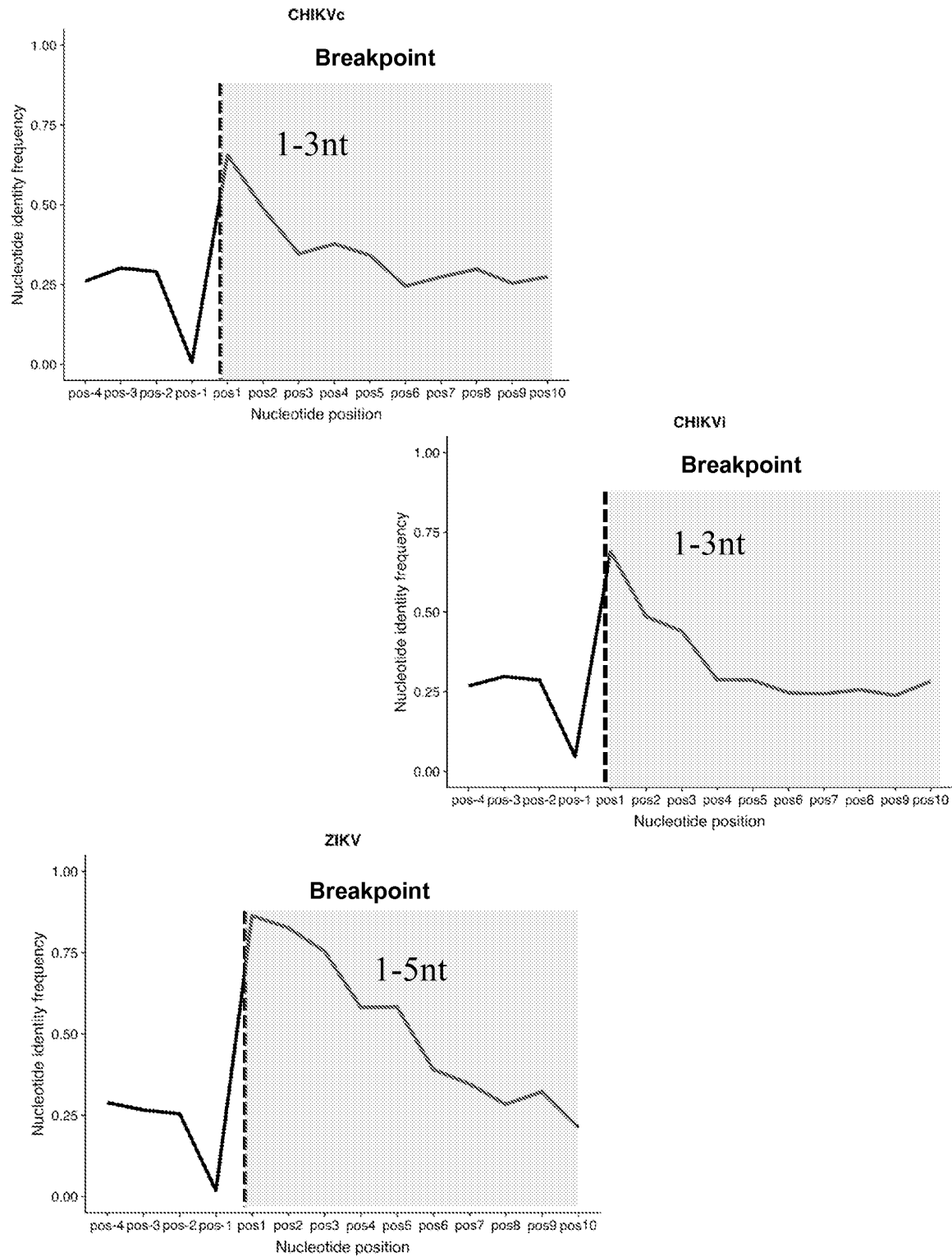


Figure 15 (start)

Complementarity around Breakpoints across different Viruses

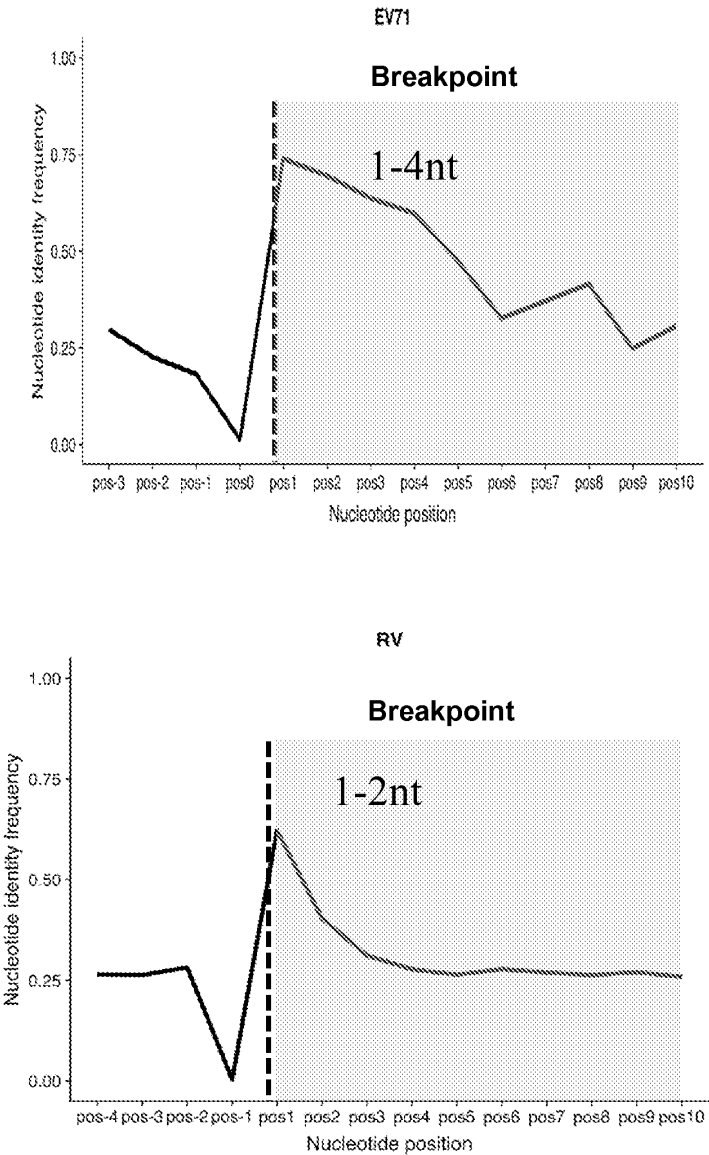


Figure 15 (end)

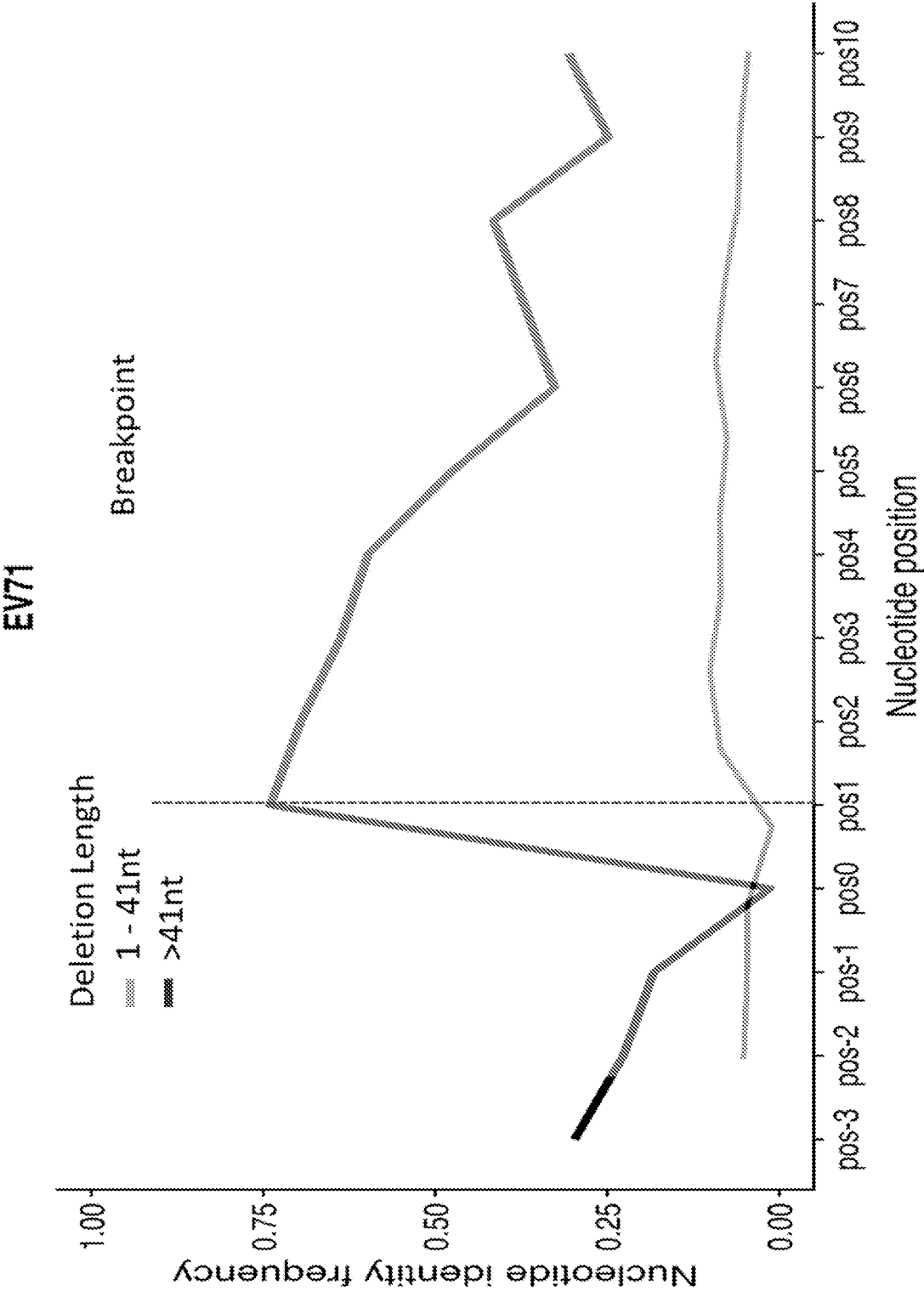


Figure 16

EV71 - Examples of Complementary Sequences around Breakpoints

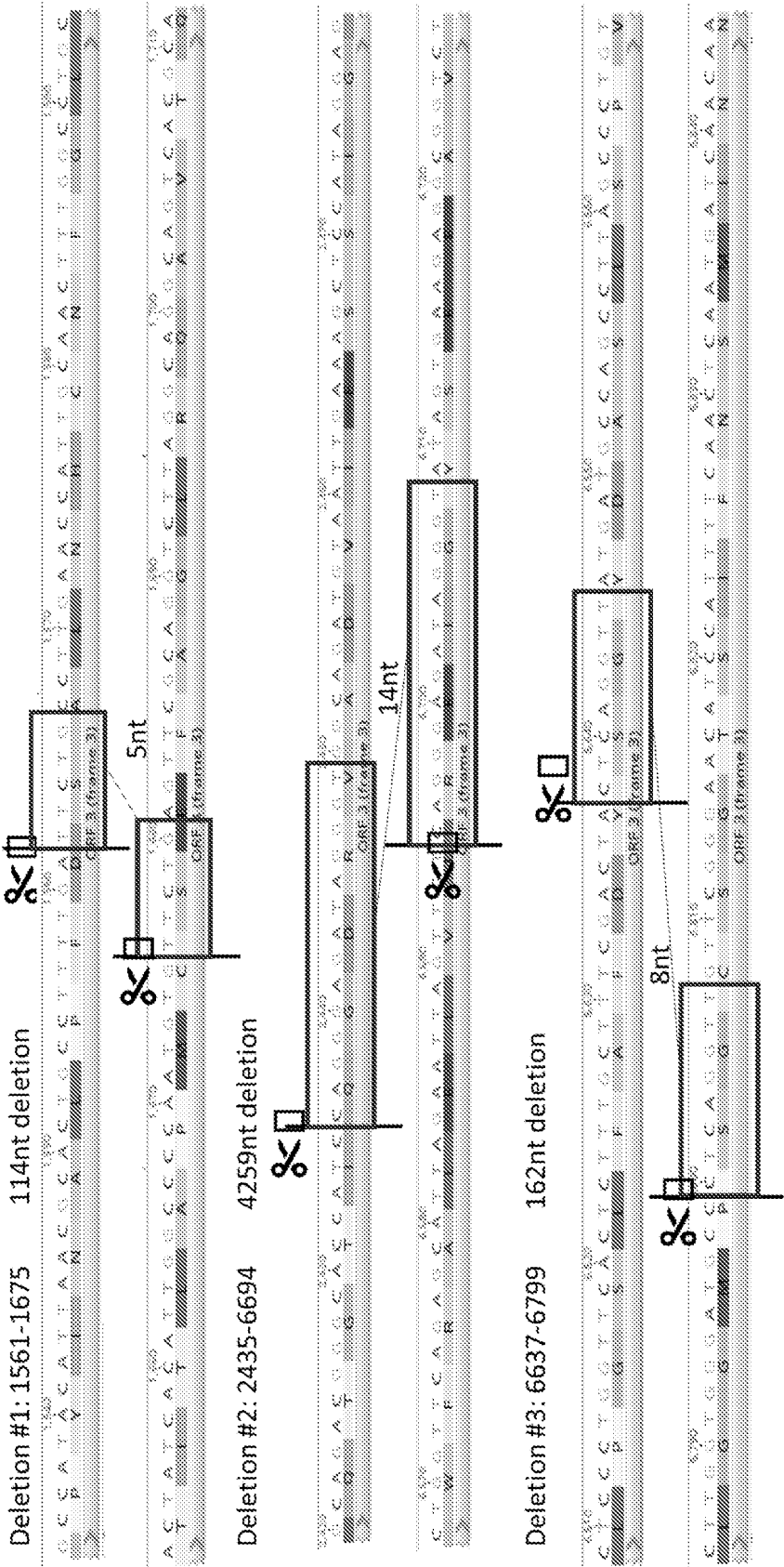
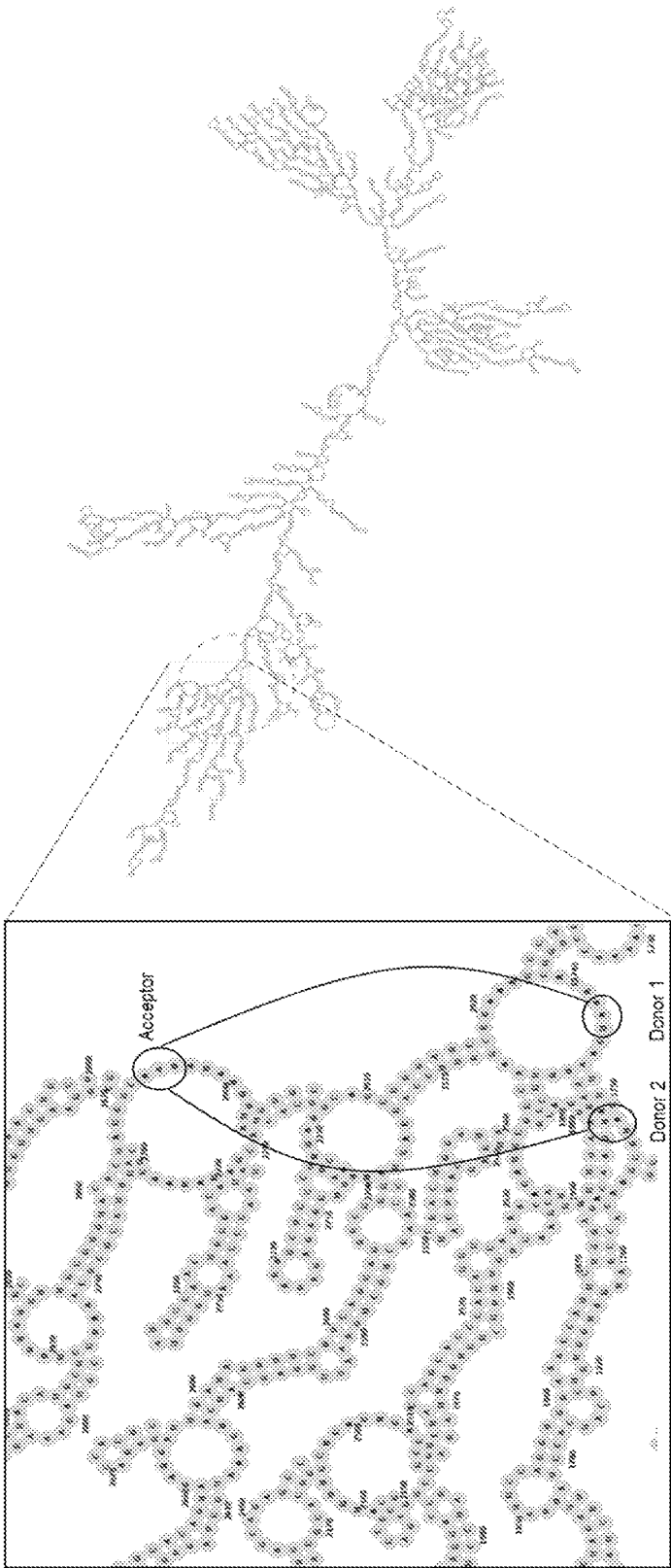


Figure 17



**EV71 – Example of RNA Secondary Structure
Involvement around Complementary Breakpoints**

Figure 18

EV71 – DI Candidates

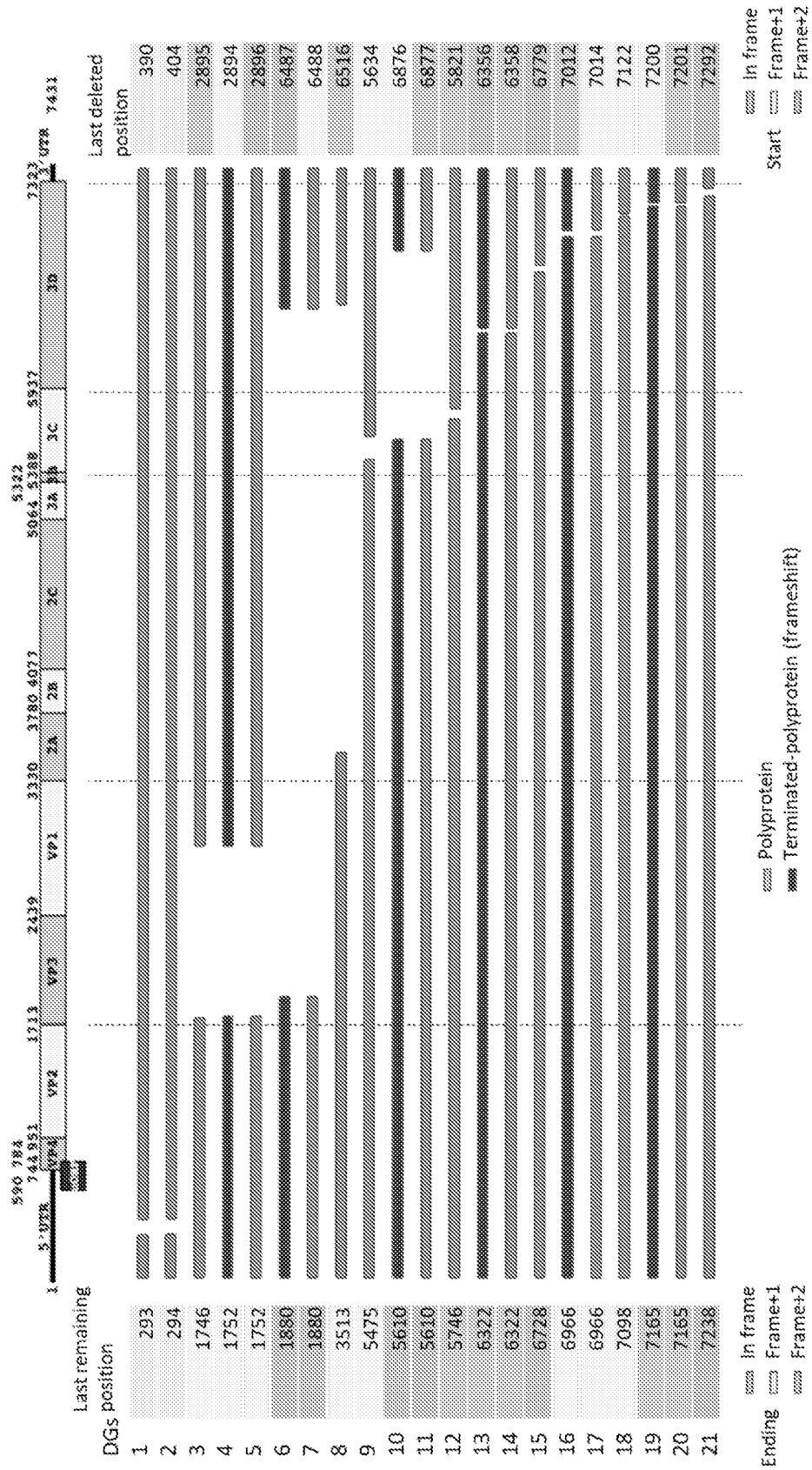


Figure 19

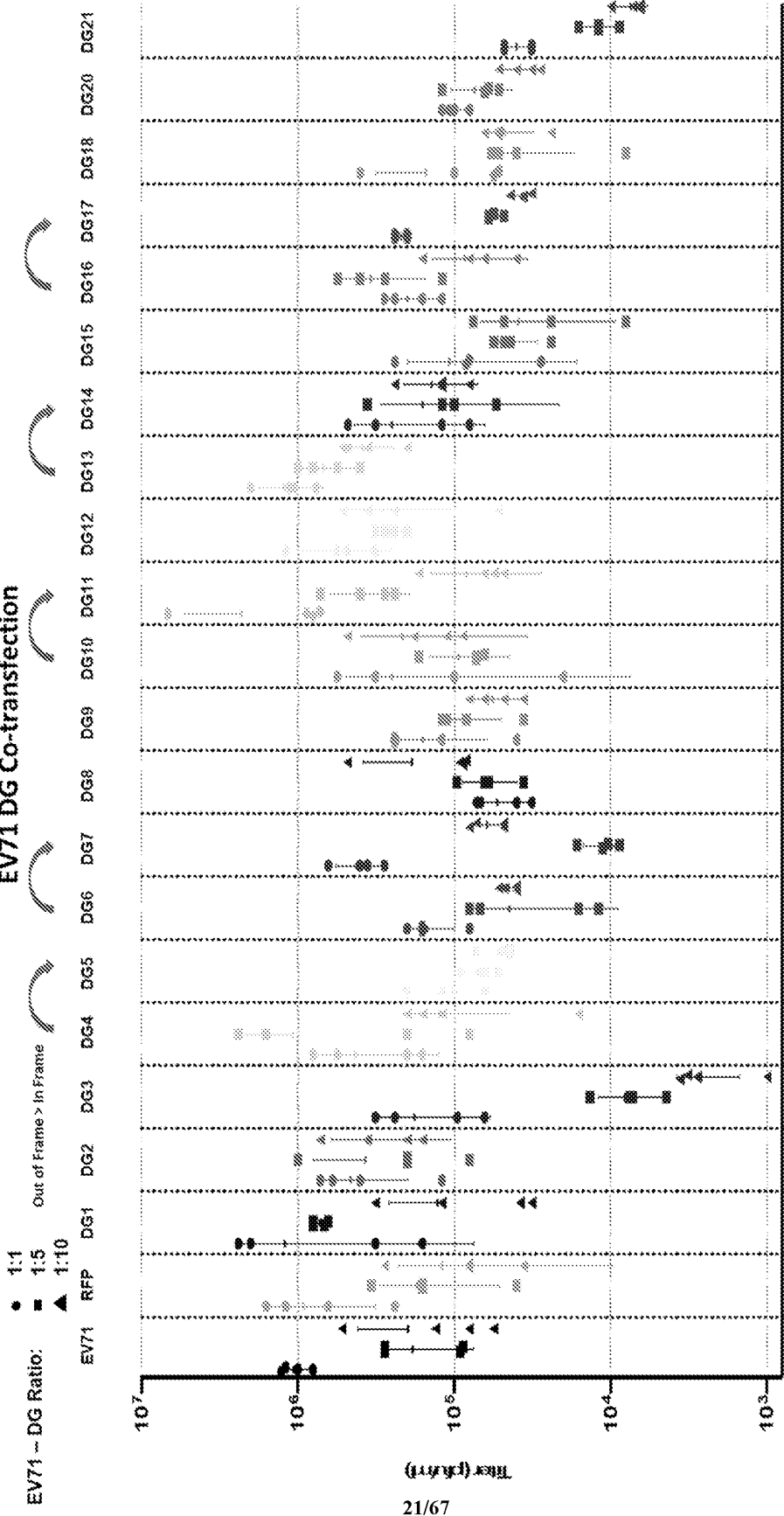


Figure 20

DVG identification in BHK-21 cells

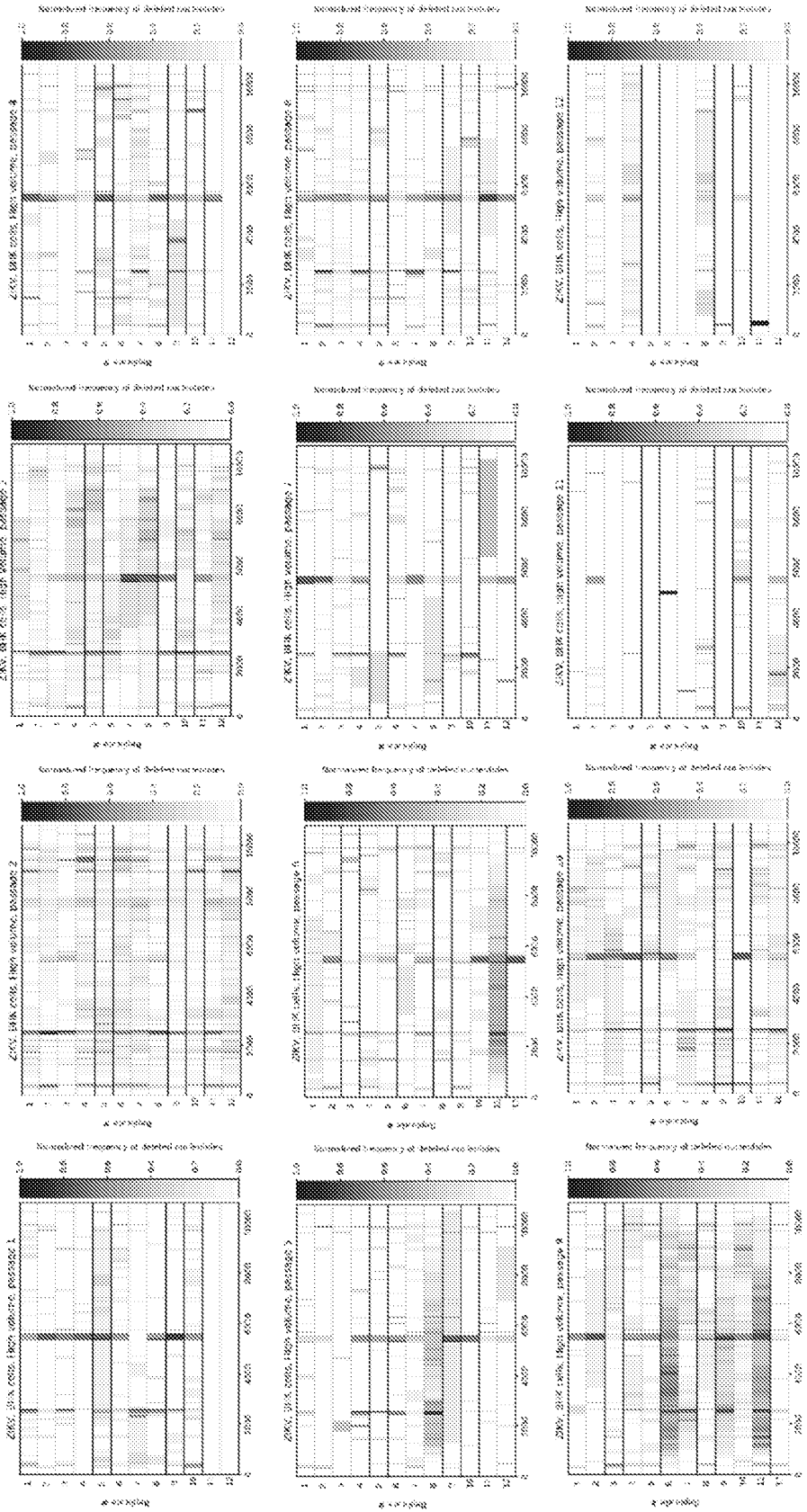


Figure 21

DI Identification — Vero cells

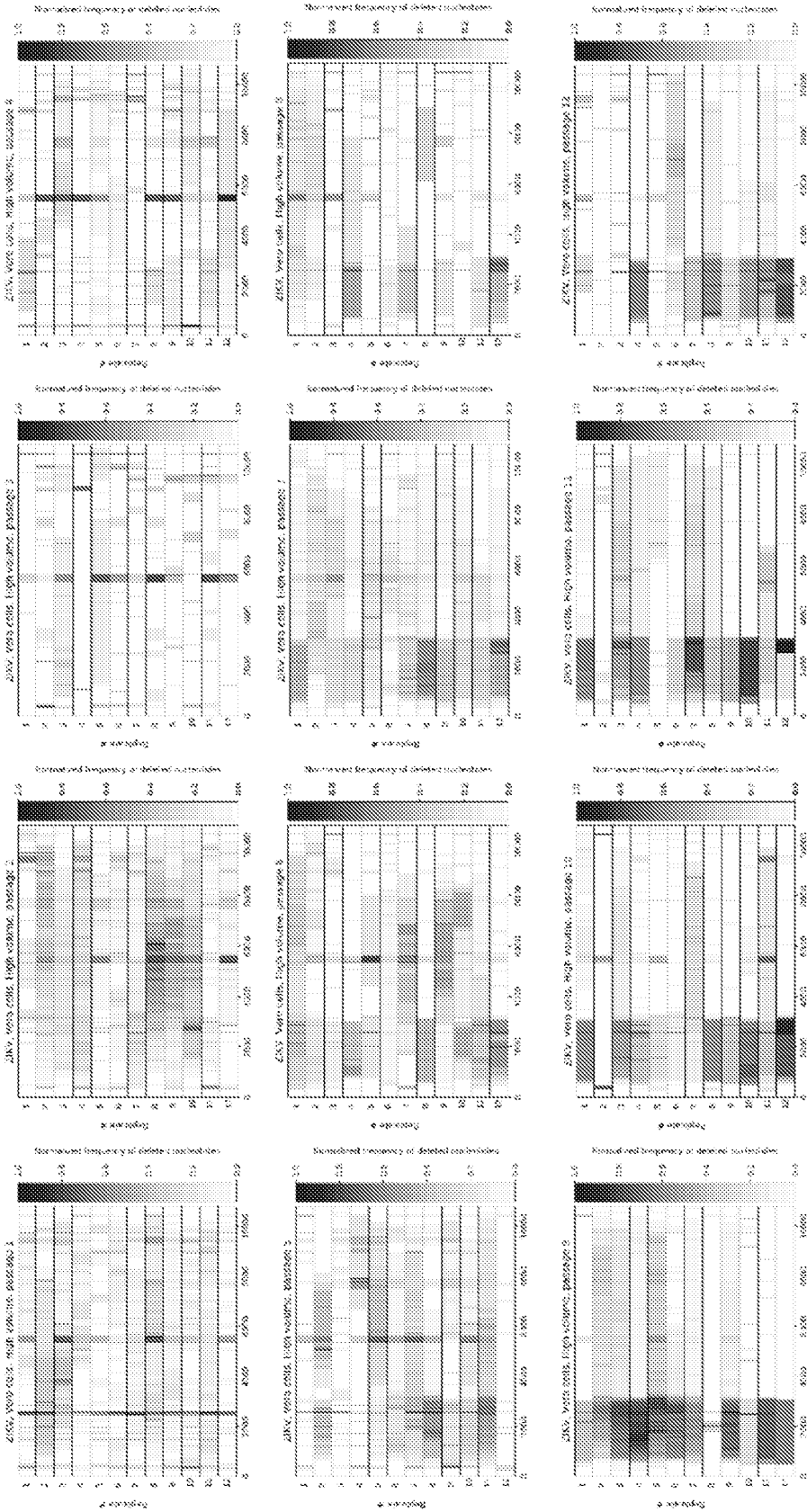


Figure 22

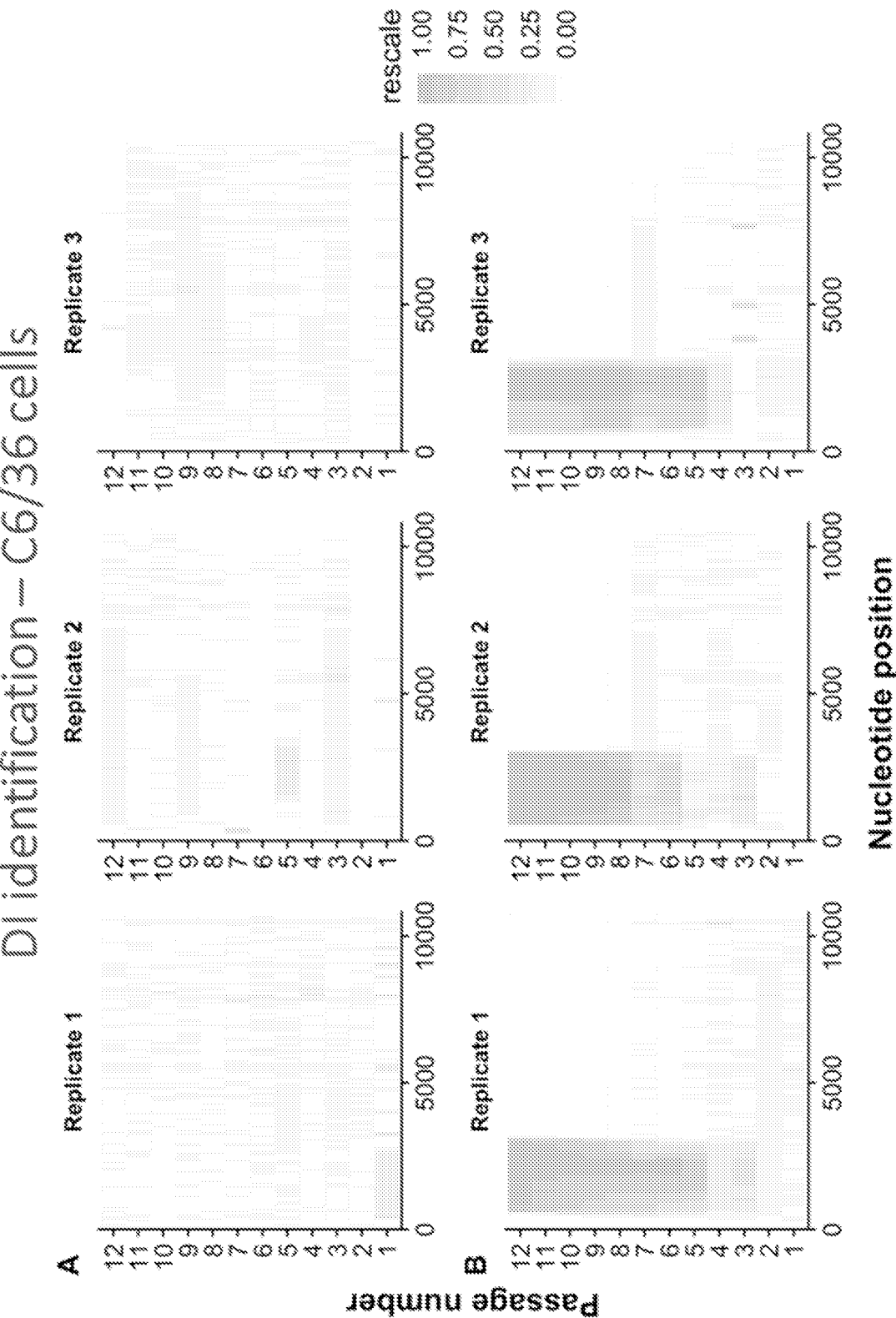


Figure 23

Identification of ZIKV deletion ‘hotspots’

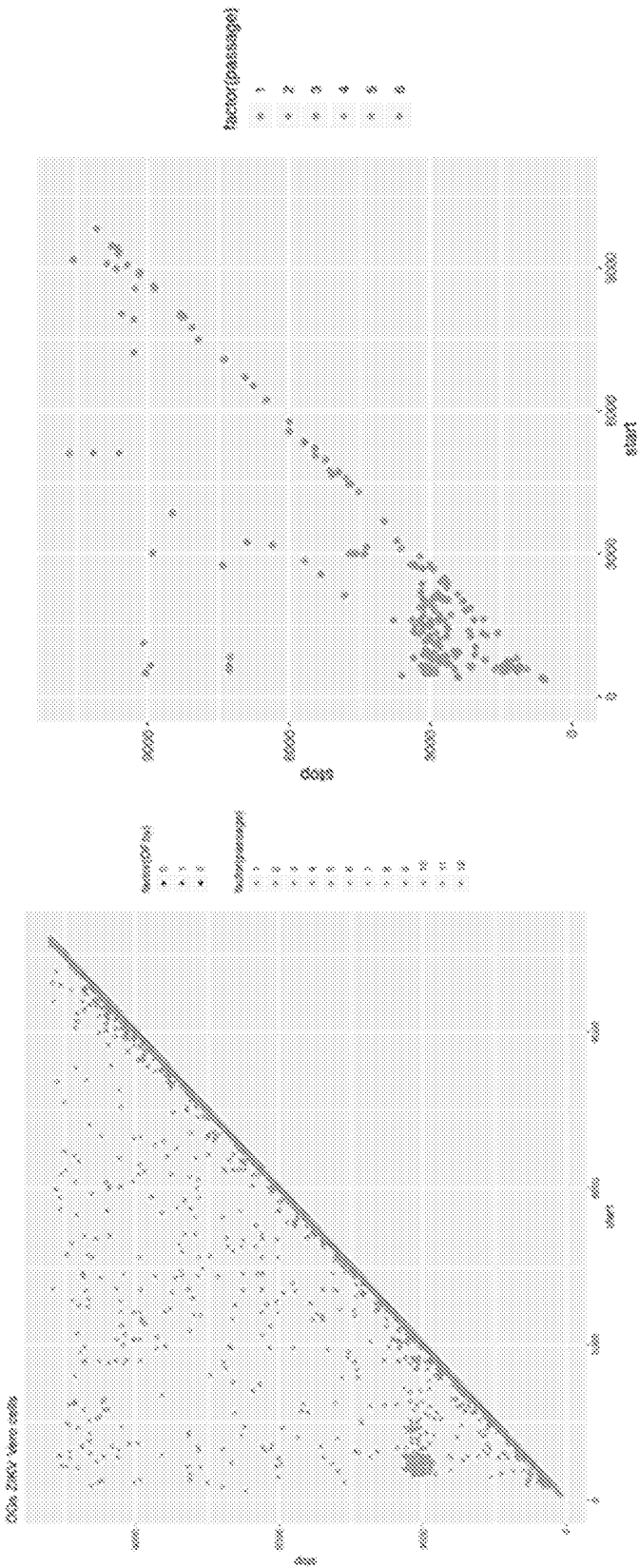


Figure 24

Candidate DVGs

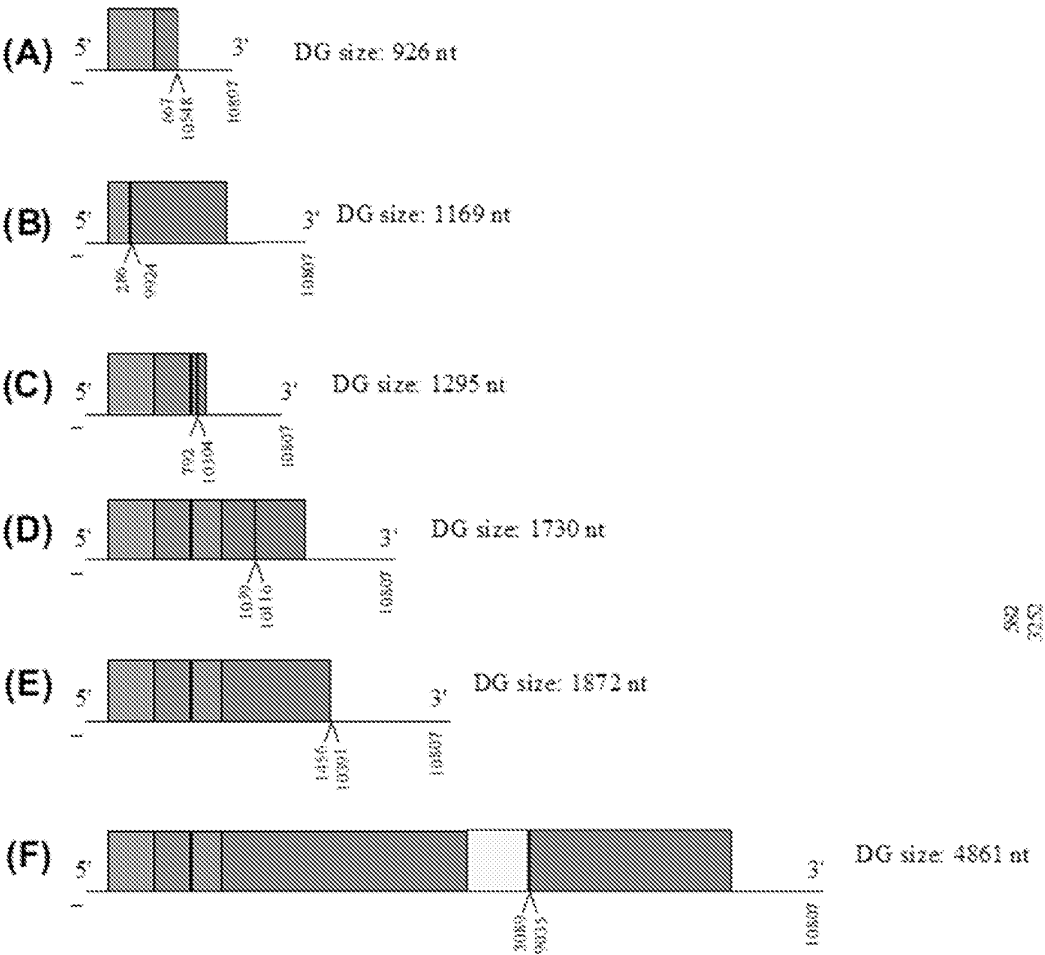


Figure 25

ZIKV replication inhibition by DVGs *in vitro*

DGs generation: 293T 96h p.t.

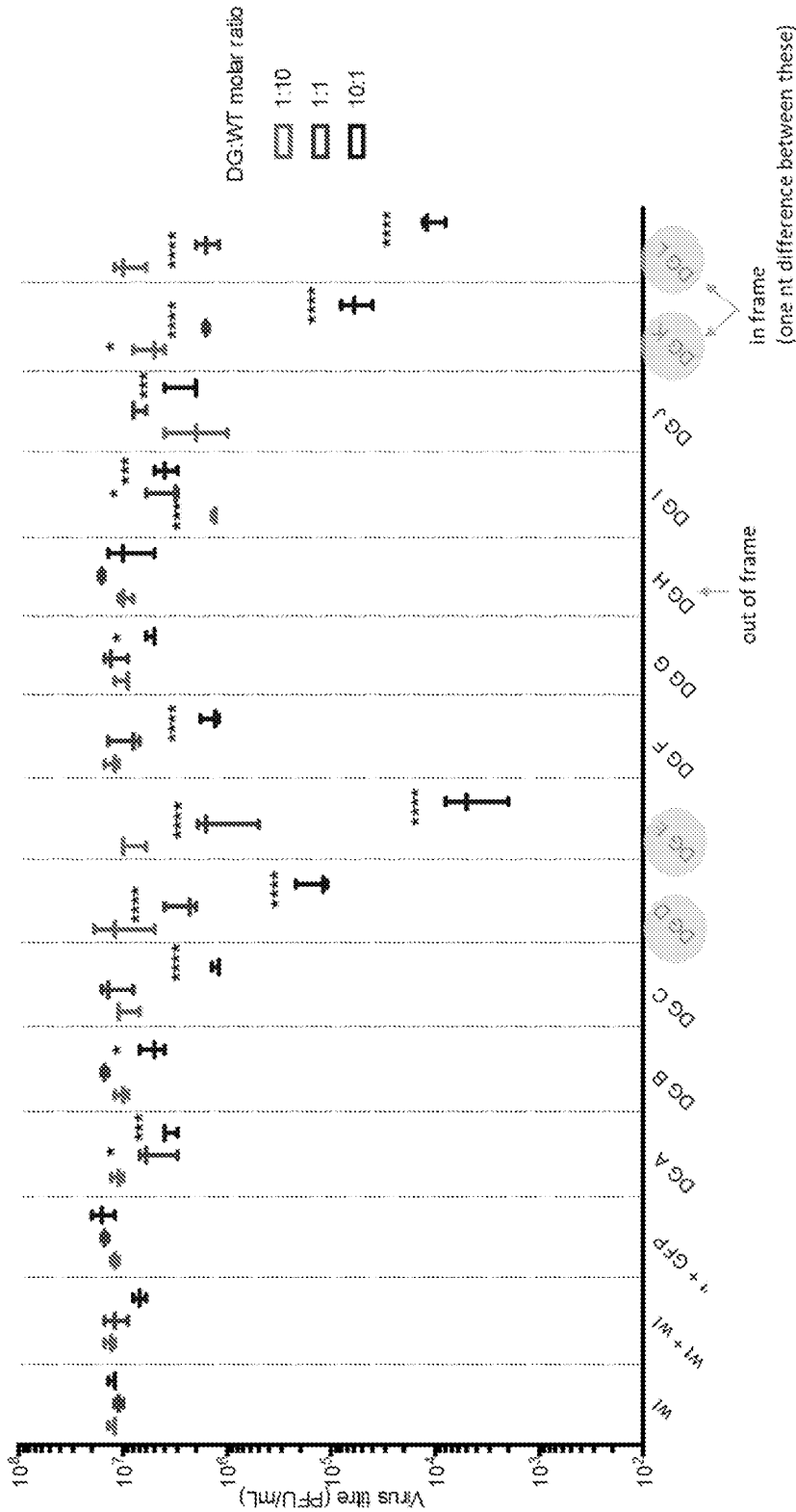


Figure 26

Candidate DVG “K” is non-replicative *per se*

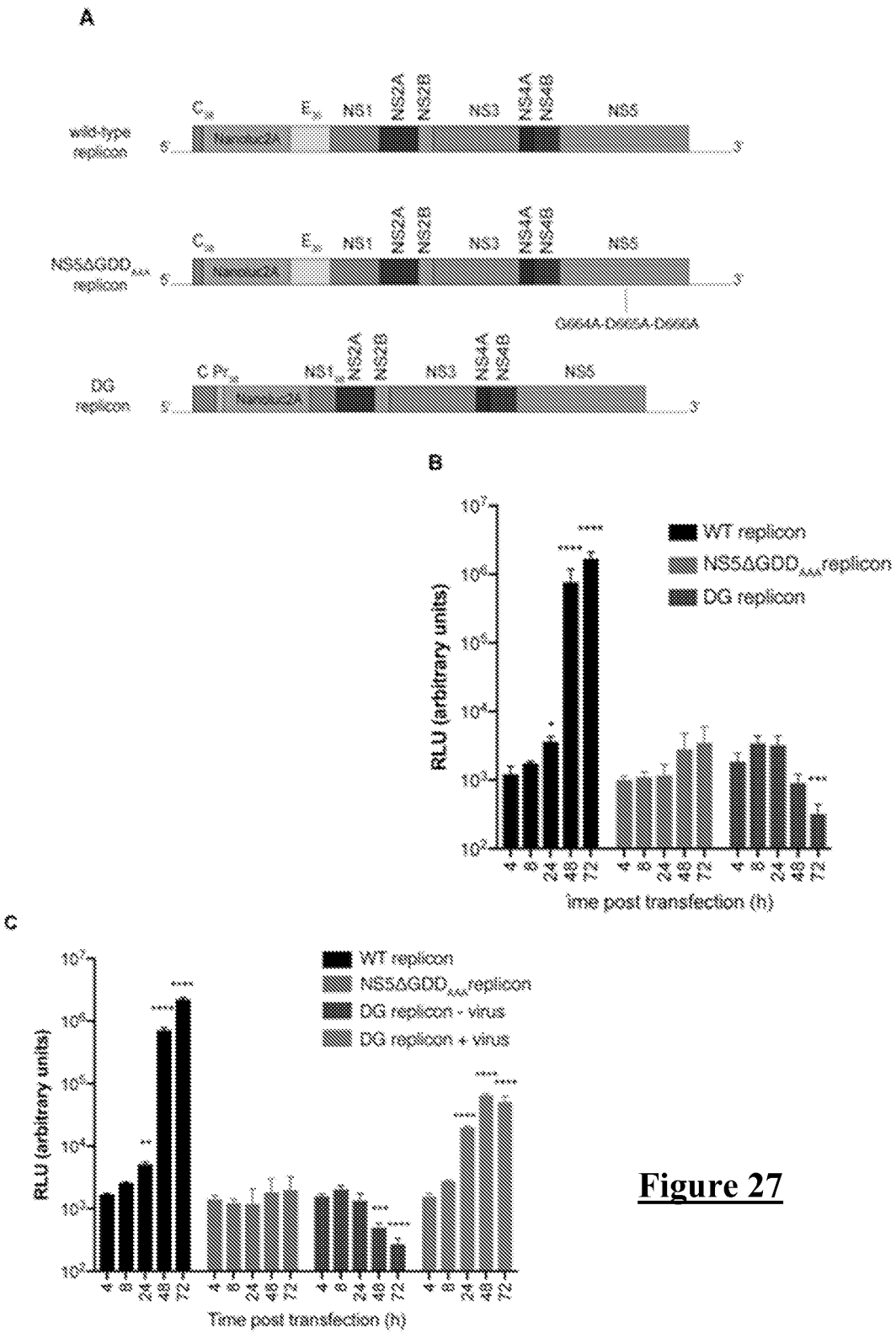


Figure 27

ZIKV replication inhibition by DGS *in vivo*

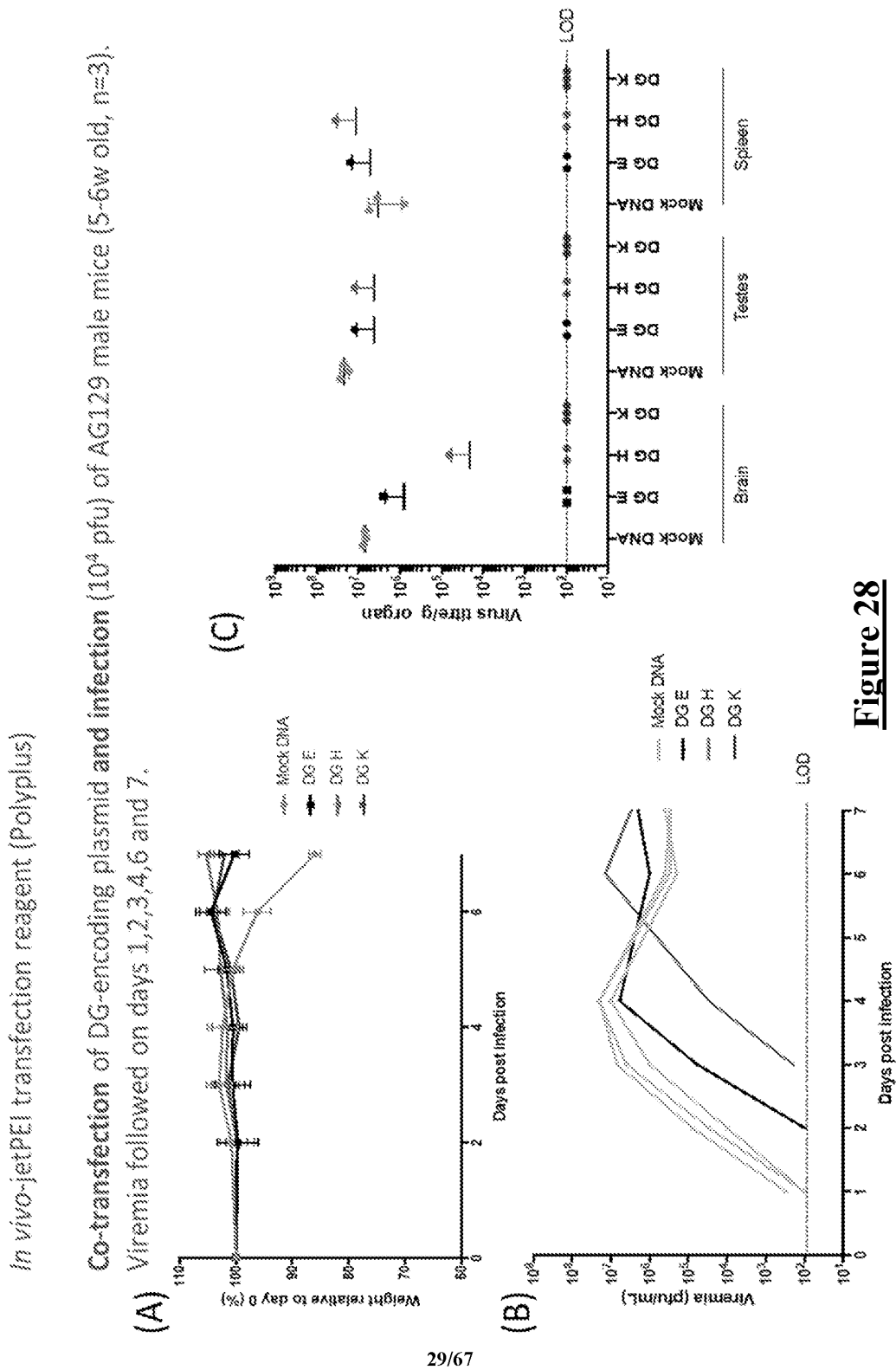


Figure 28

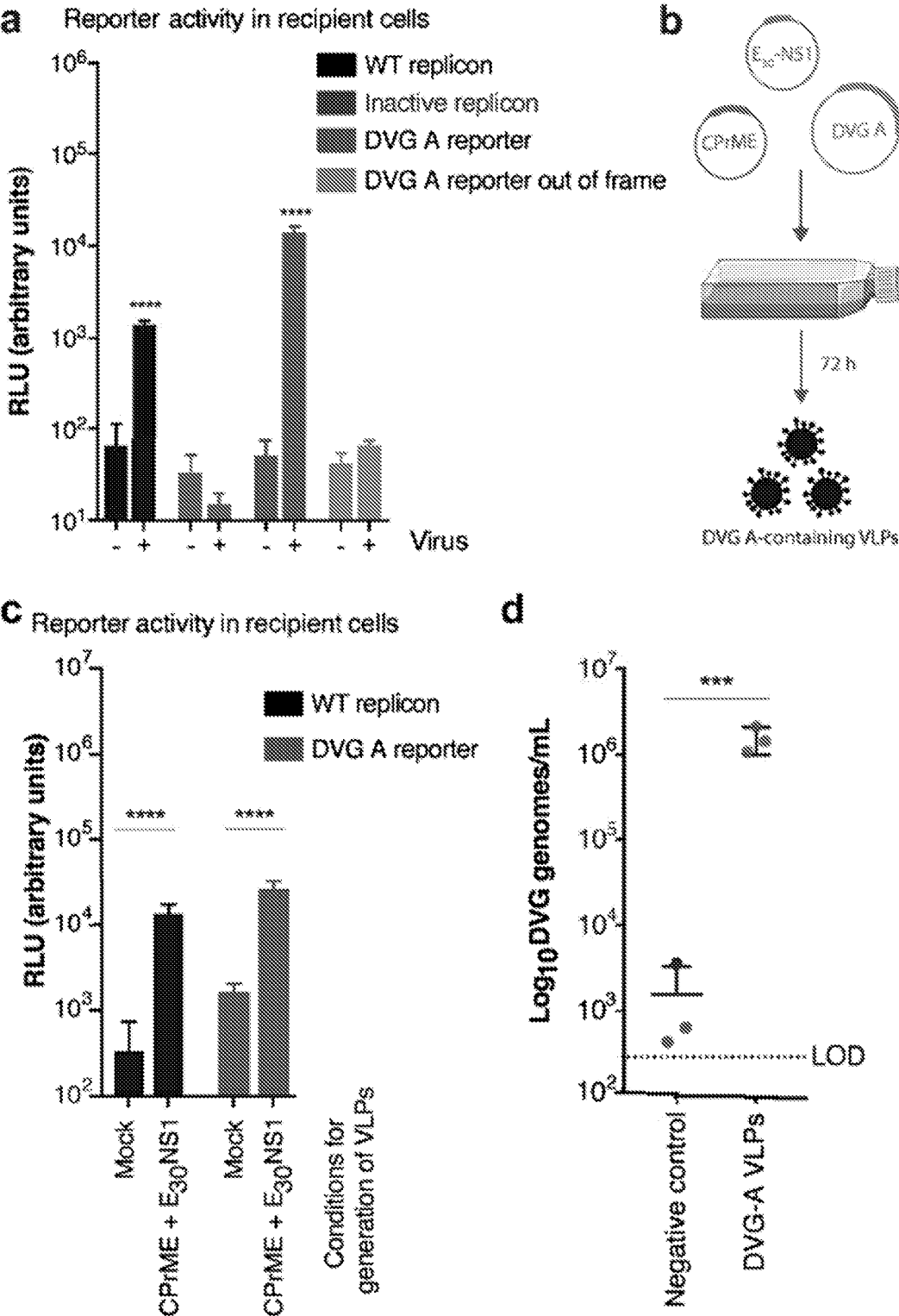


Figure 29

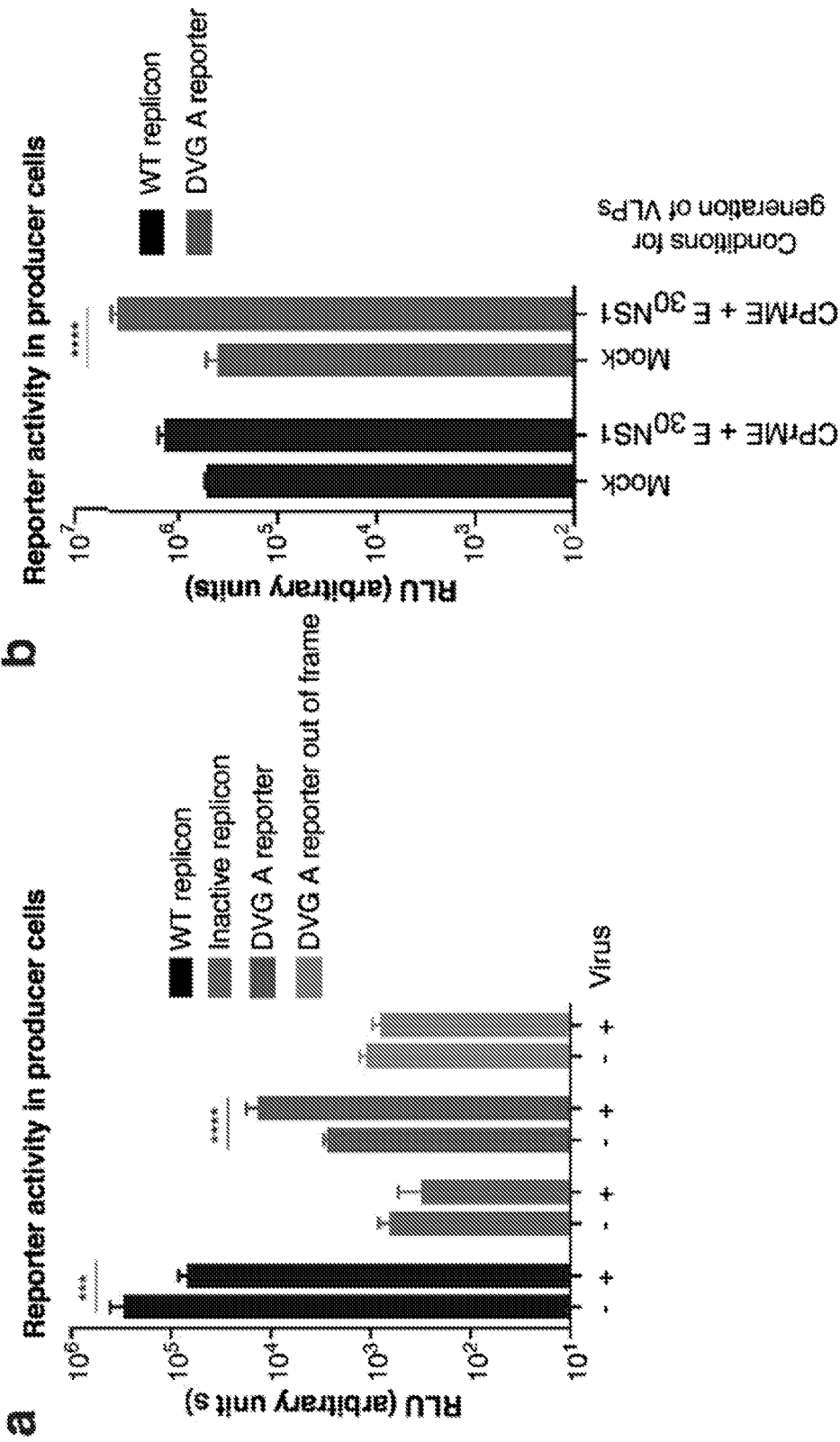


Figure 30

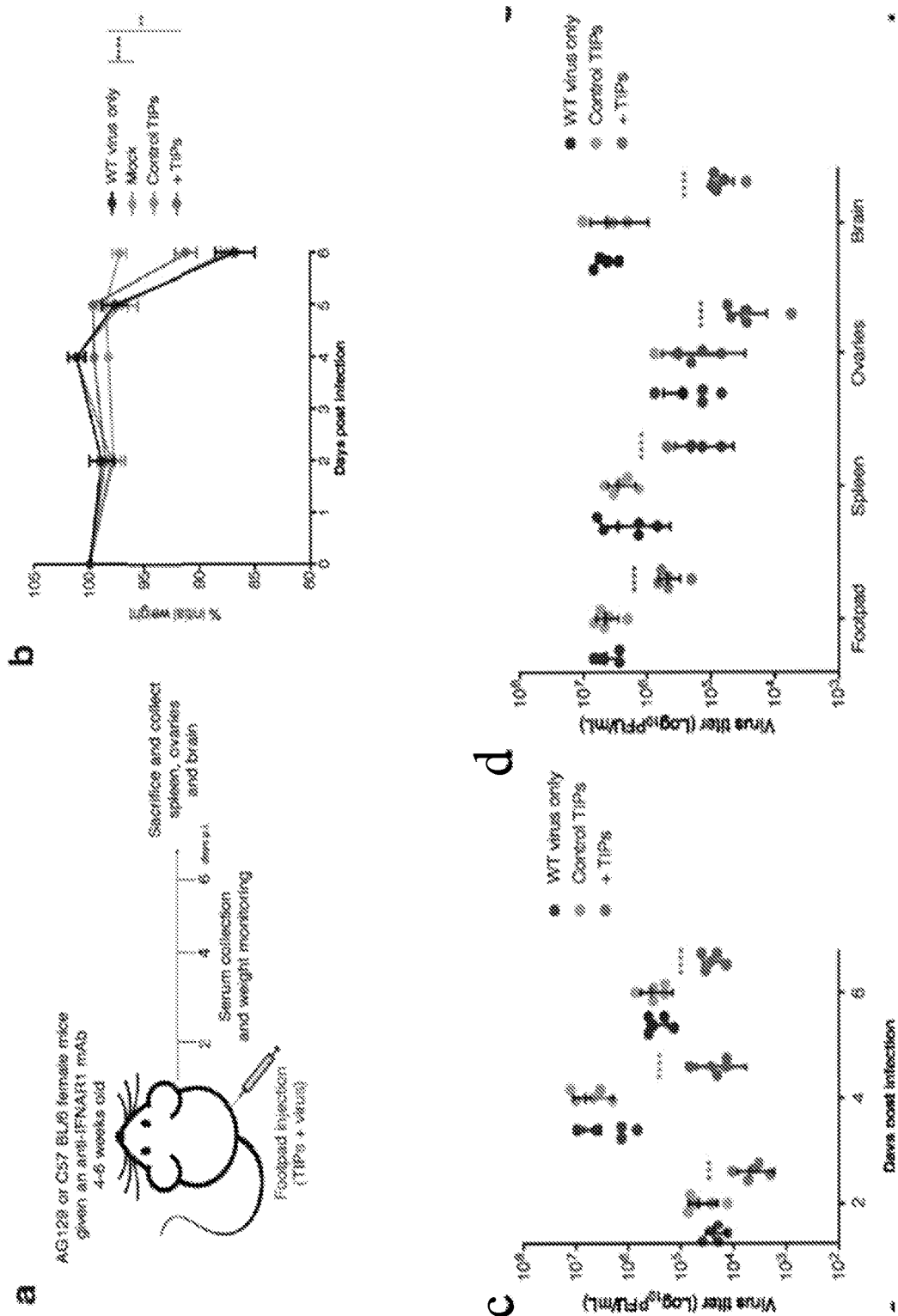


Figure 31 (start)

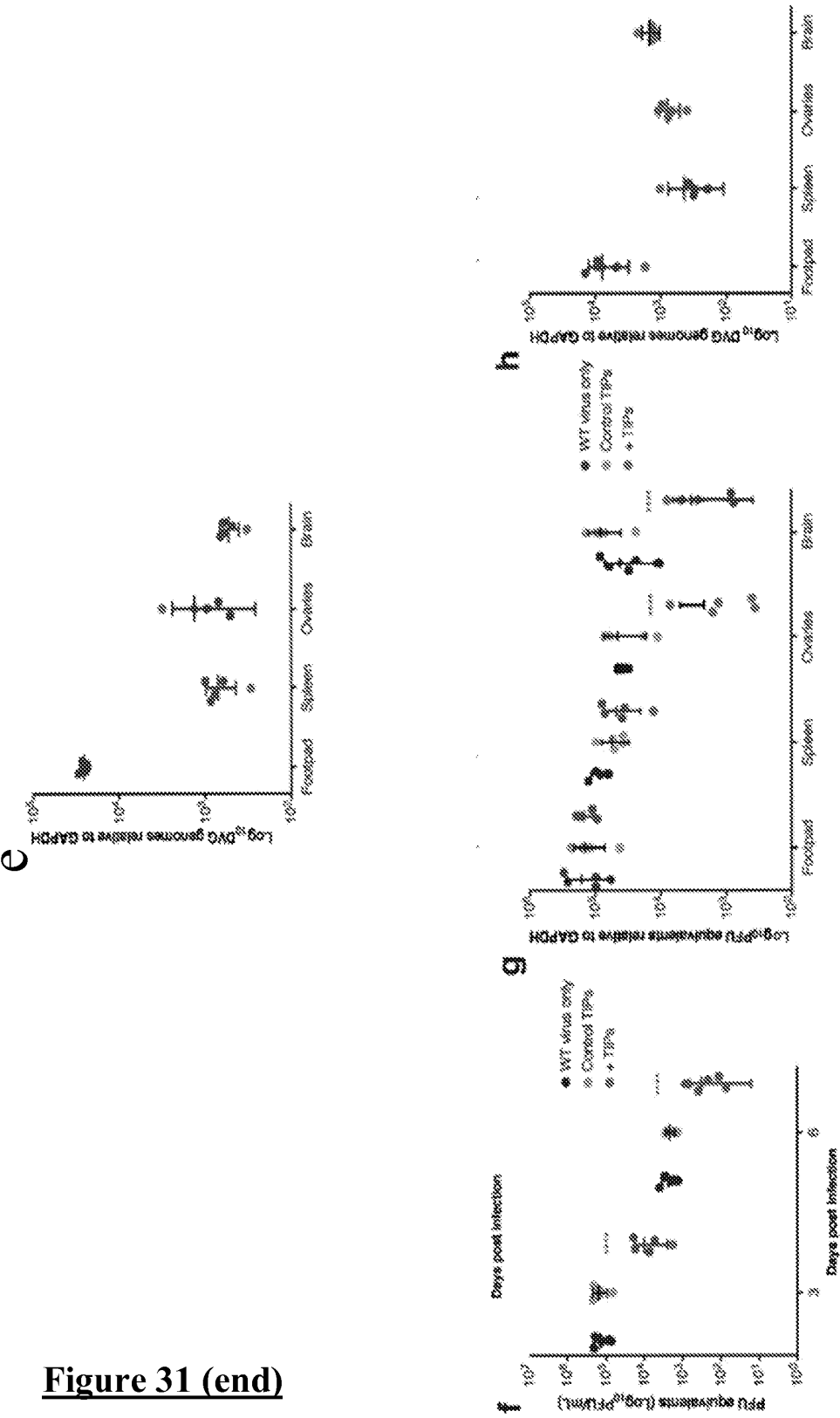


Figure 31 (end)

ZIKV replication inhibition by DGS “K” *in vivo* (mosquitoes)

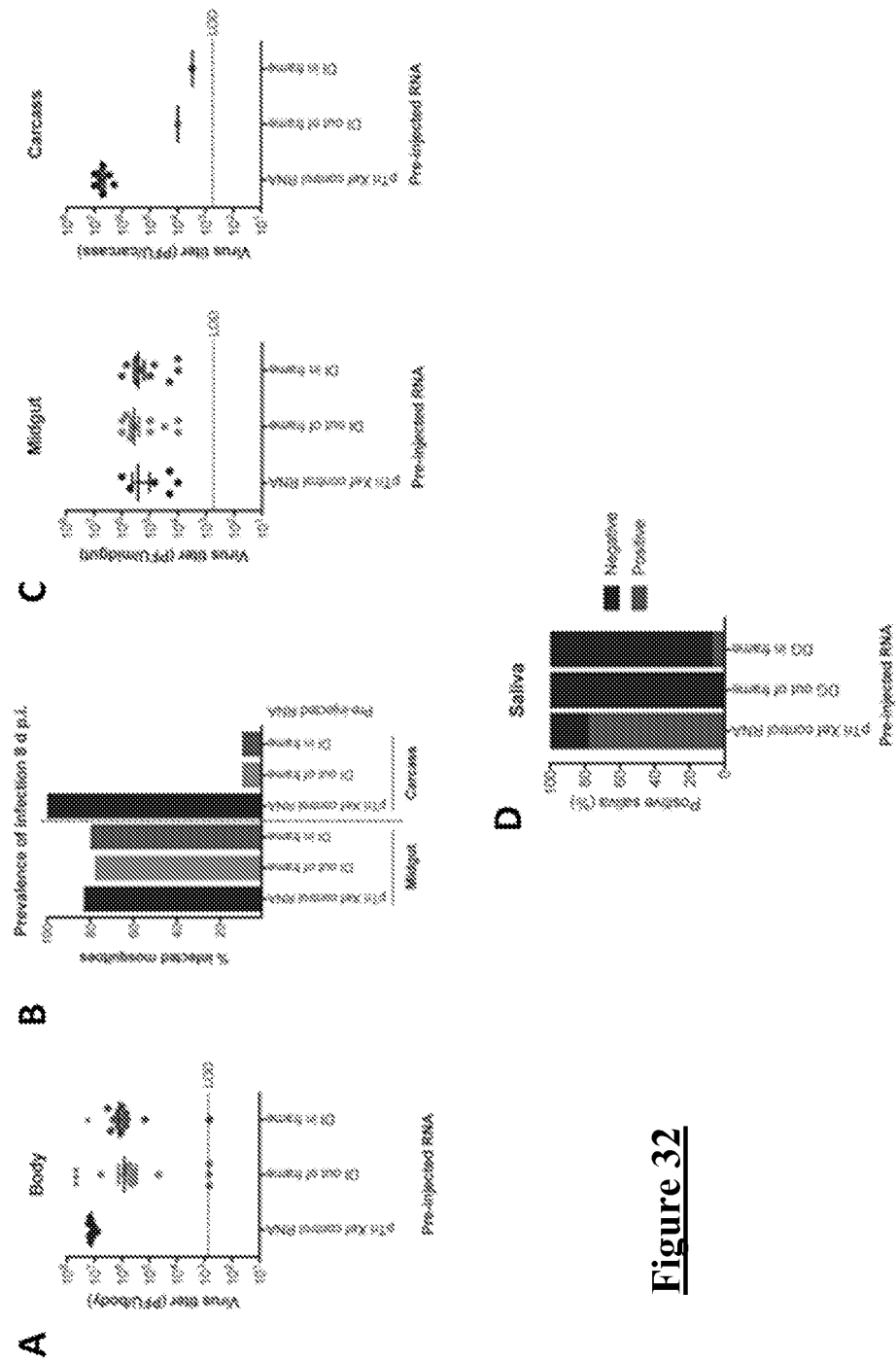
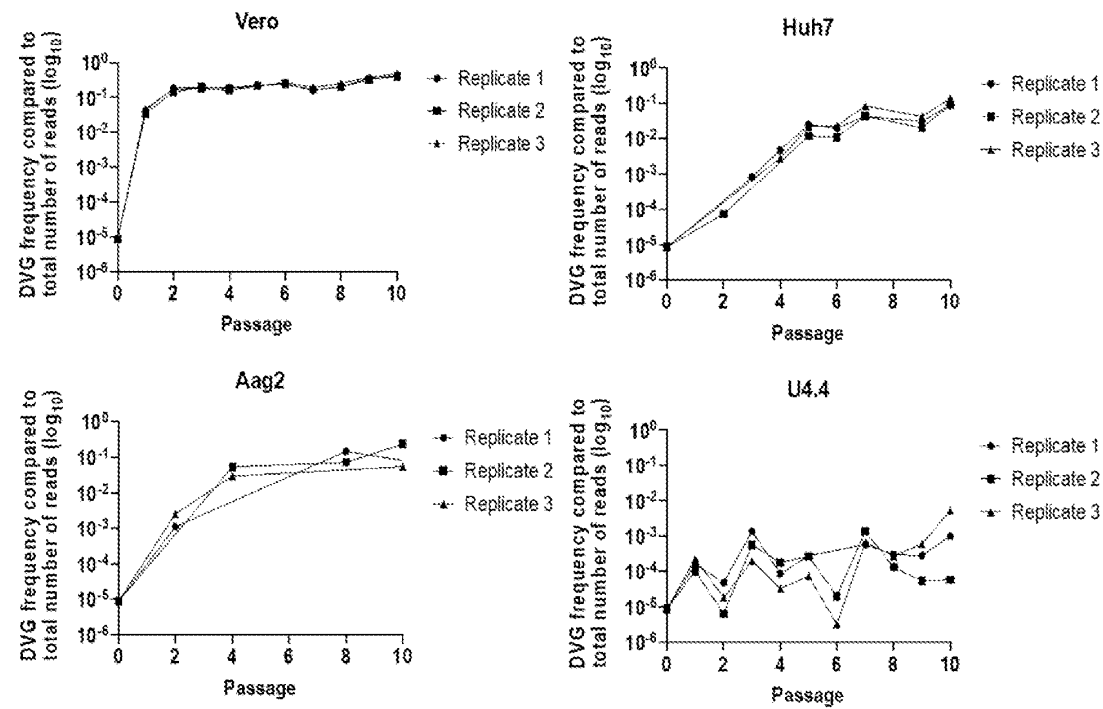


Figure 32

A



B

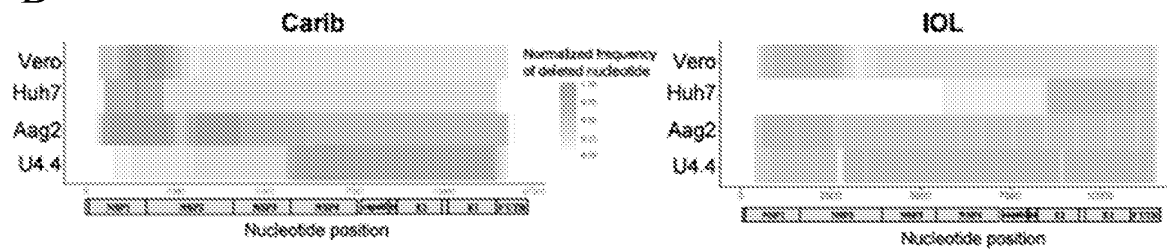


Figure 33 (start)

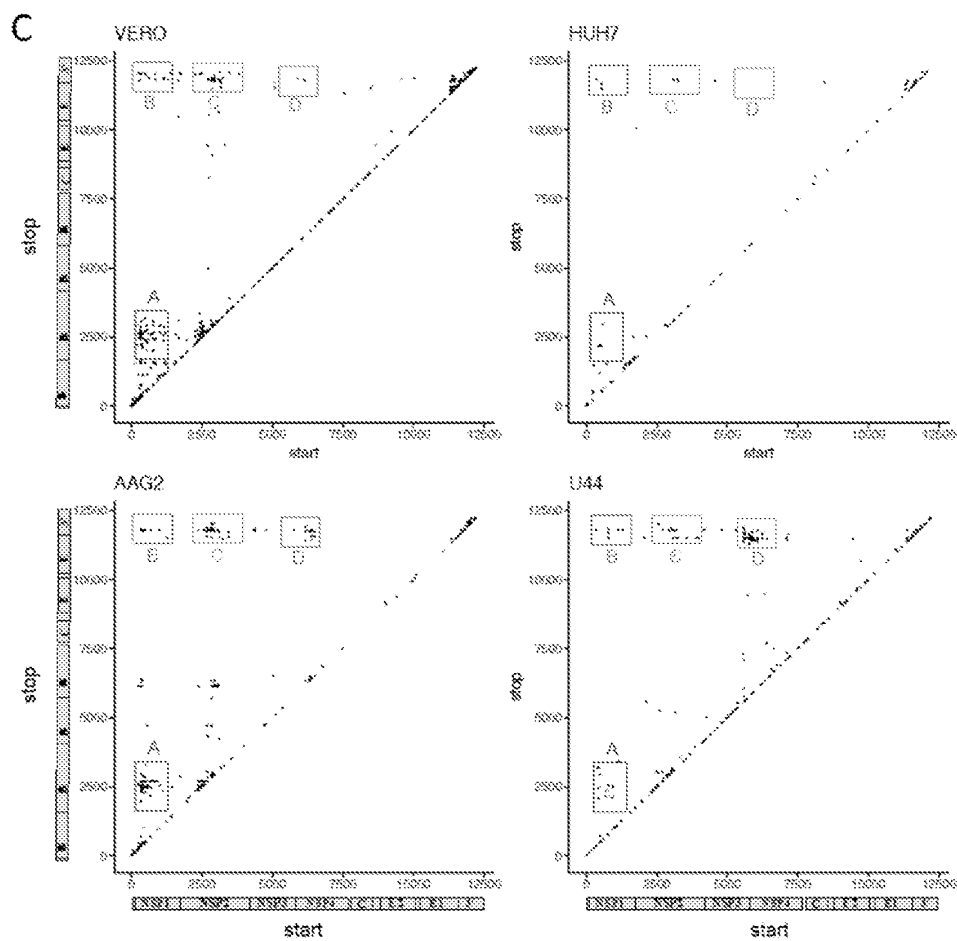


Figure 33 (end)

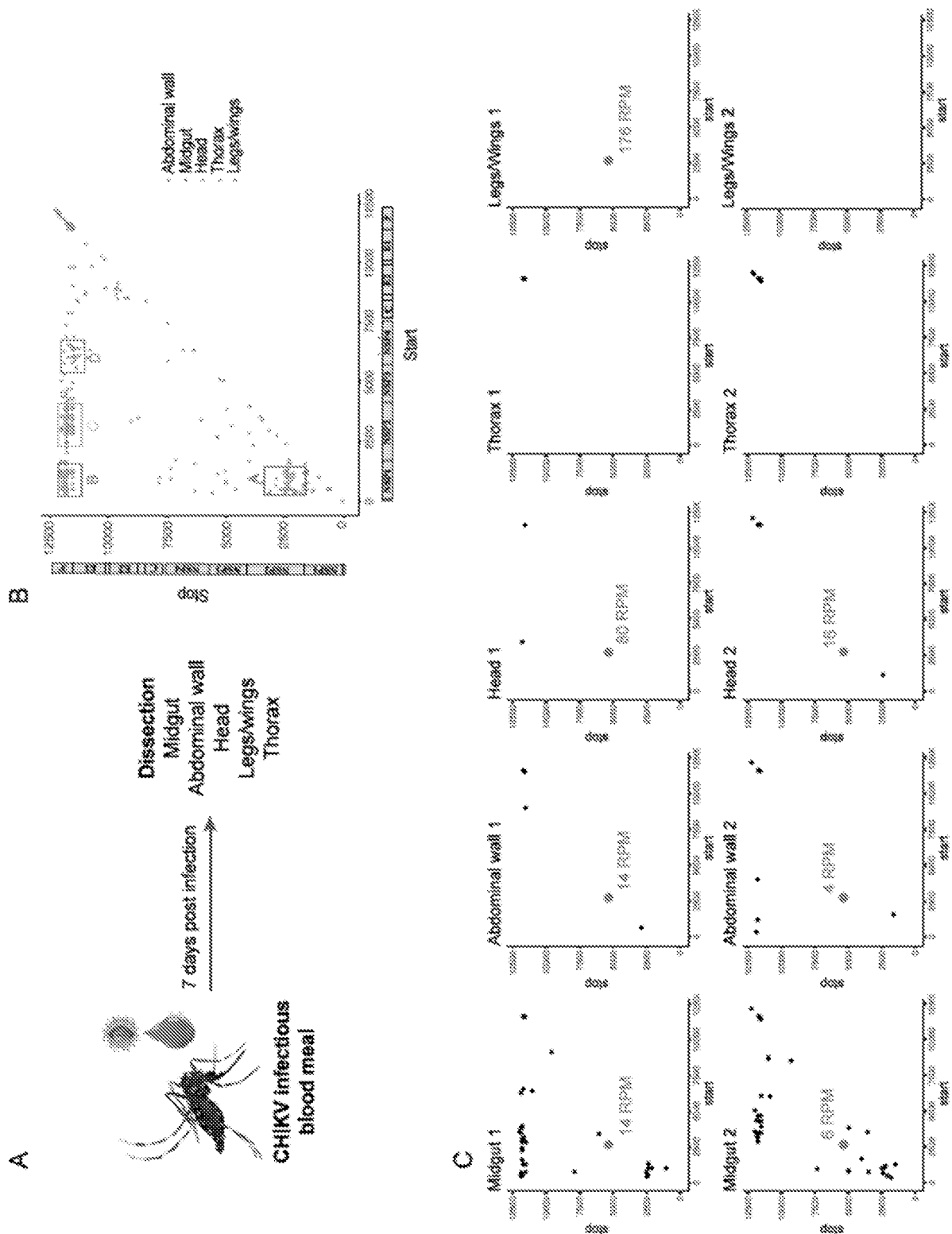


Figure 34

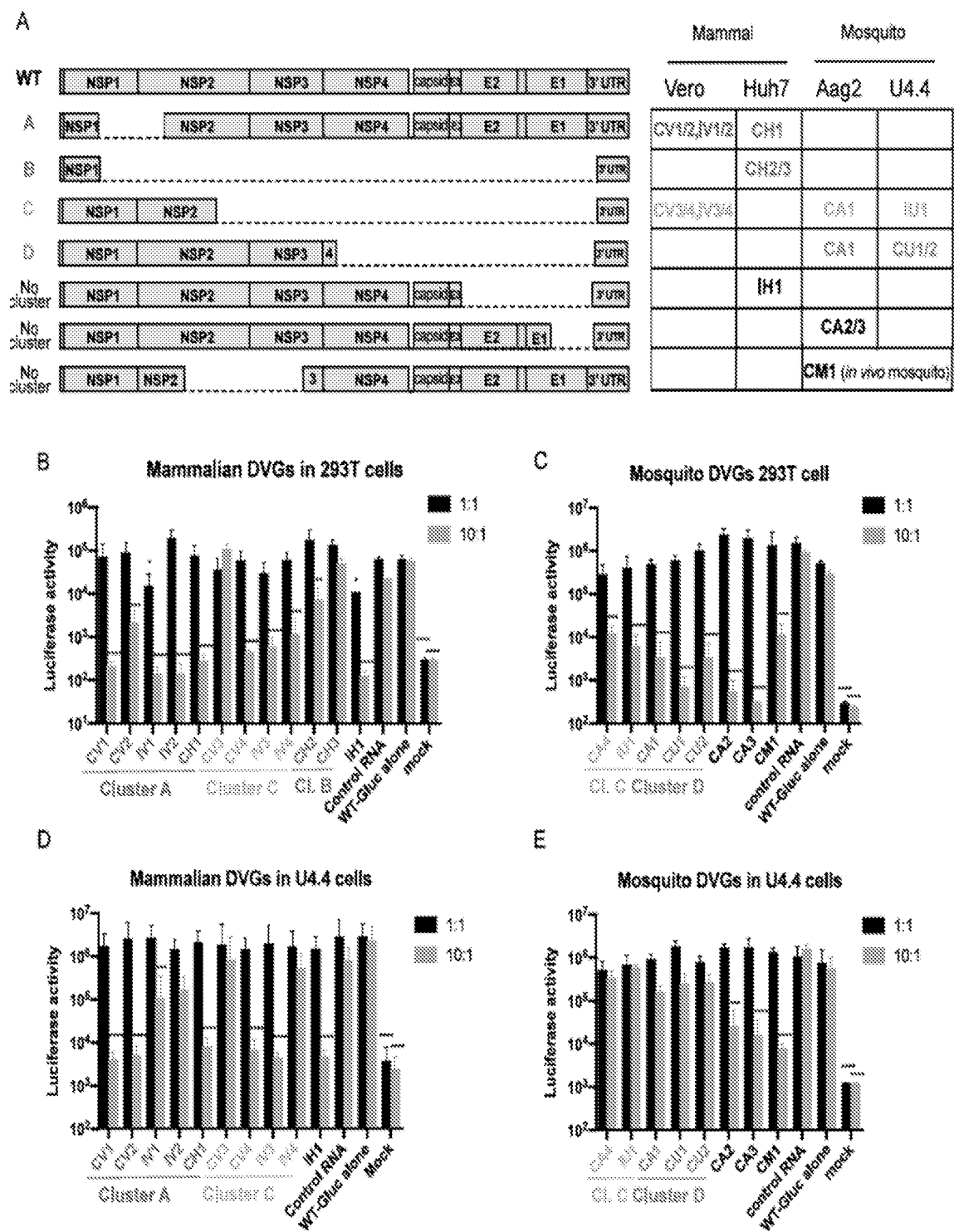


Figure 35

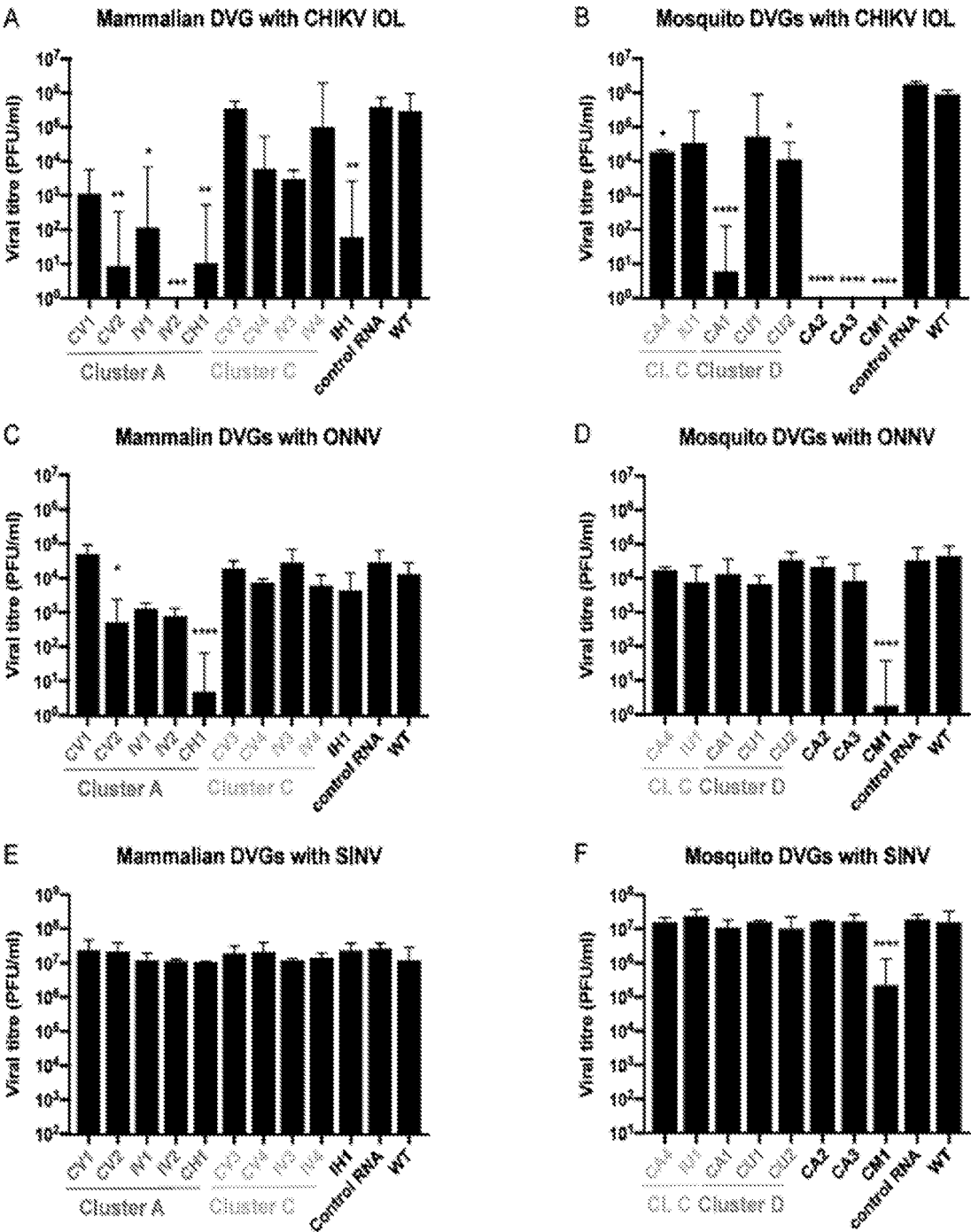


Figure 36

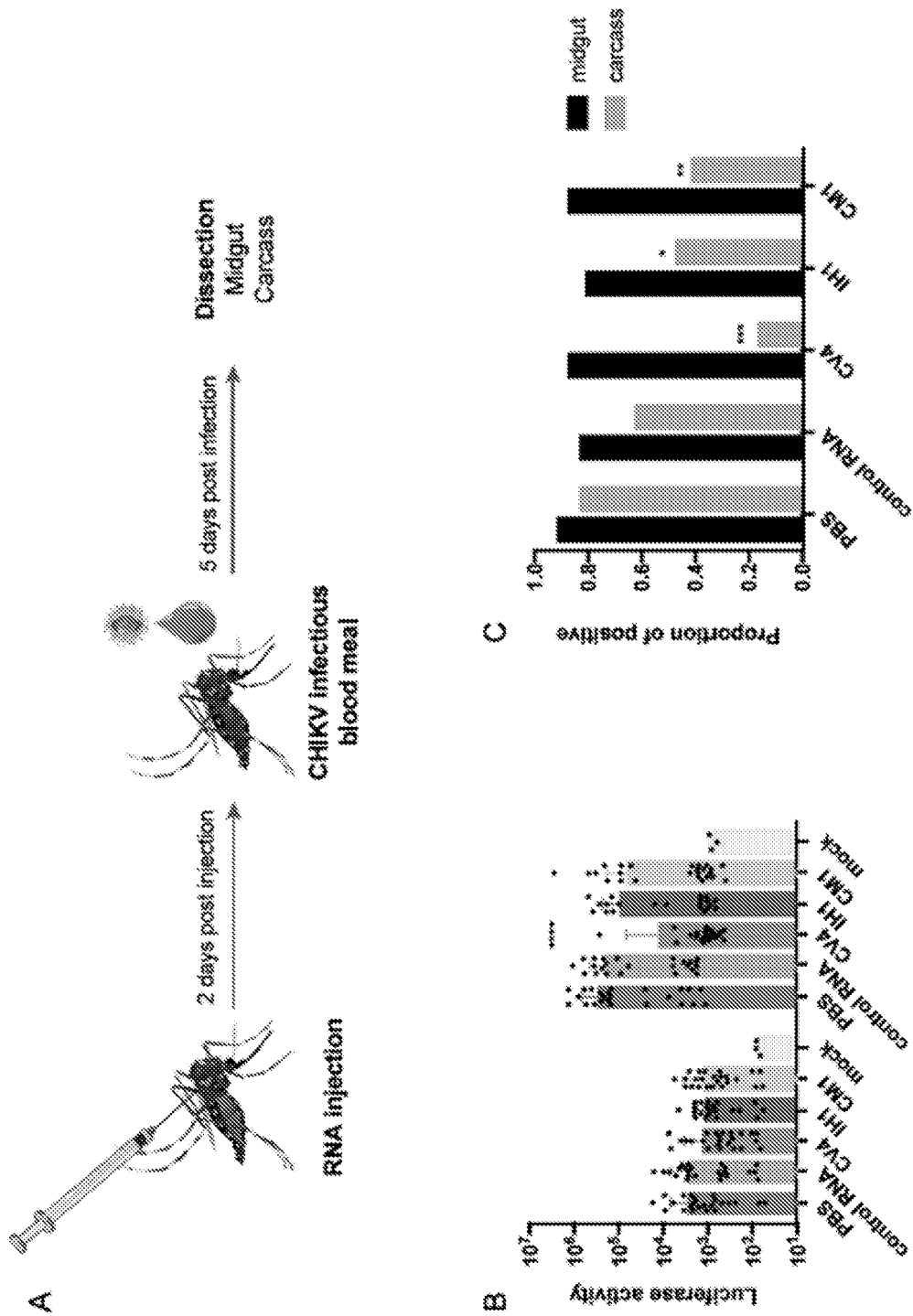


Figure 37

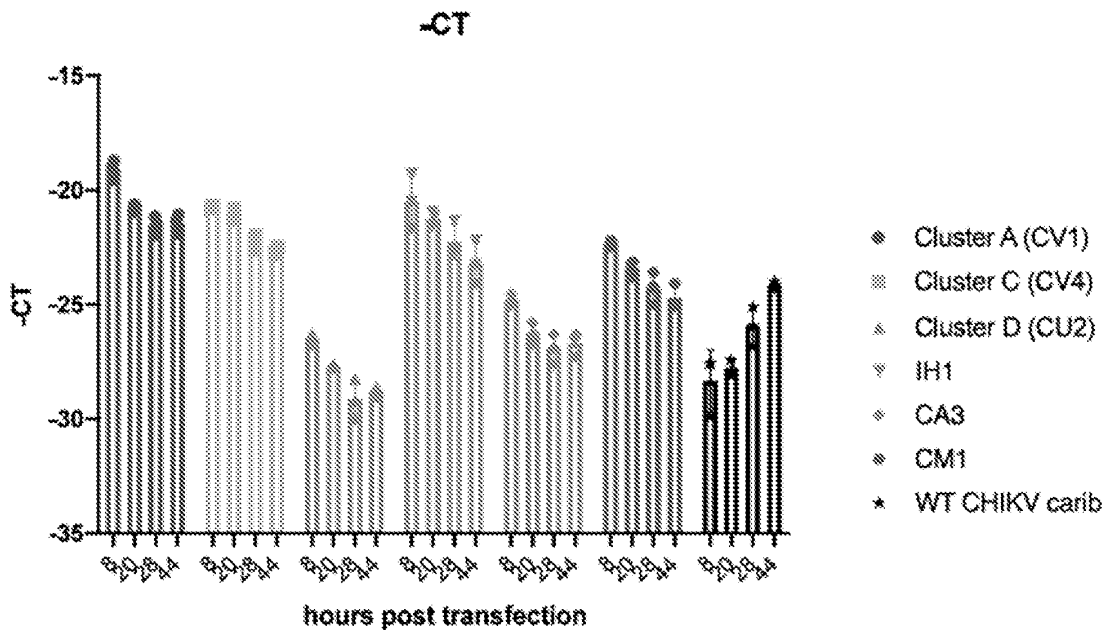


Figure 38

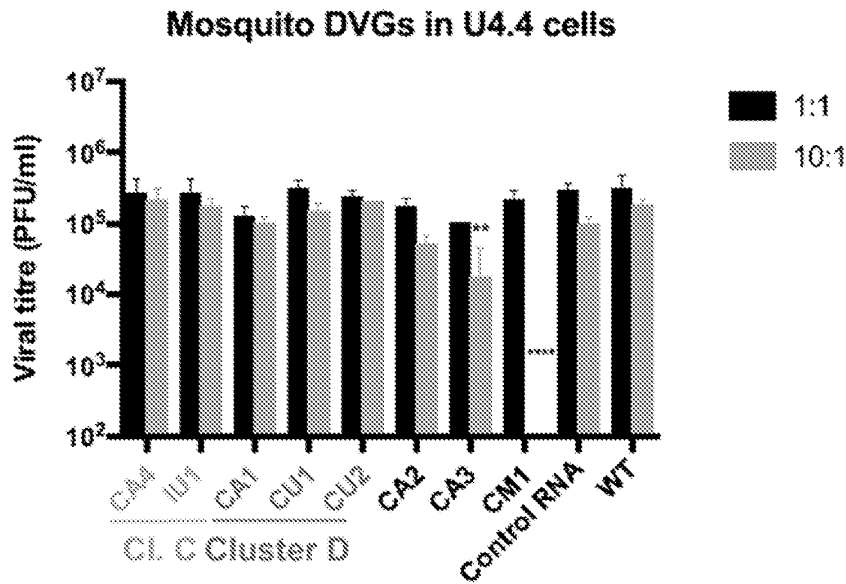


Figure 39

SEQ ID NO	Virus	Name	Start position*	Stop position**	Deletion size	DVG size	Mutation	ORF
22	ZIKV	A	668	10548	9880	927N/A		Out of frame by 1 nt
23	ZIKV	B	287	9924	9637	1170N/A		Out of frame by 1 nt
24	ZIKV	C	793	10304	9511	1296N/A		Out of frame by 1 nt
25	ZIKV	D	1040	10116	9076	1731N/A		Out of frame by 1 nt
26	ZIKV	E	1457	10391	8934	1873N/A		In frame
27	ZIKV	F	3090	9035	5945	4862N/A		Out of frame by 2 nt
28	ZIKV	G	349	4643	4294	6513N/A		Out of frame by 1 nt
29	ZIKV	H	581	3252	2671	8136N/A		Out of frame by 1 nt
30	ZIKV	I	8434	9017	583	10224N/A		Out of frame by 1 nt
31	ZIKV	J	5845	6380	535	10272N/A		Out of frame by 1 nt
32	ZIKV	K	581	3251	2670	8137N/A		In frame
33	ZIKV	L	582	3252	2670	8137N/A		In frame

Figure 40

SEQ ID NO	Virus	Name	Start position	Stop position	Deletion size	DVG size	Mutation	ORF
34	CHIKV IOL	IV1	435	3123	2689	9163	0	F+1
35	CHIKV IOL	IV2	663	2878	2216	9636	0	F+2
36	CHIKV IOL	IV3	3892	11508	7617	4235	0	N/A
37	CHIKV IOL	IV4	3850	11629	7780	4072	0	N/A
38	CHIKV IOL	IH1	8495	11593	3099	4072	0	F
39	CHIKV IOL	IU1	3570	11509	7940	3912	0	N/A
40	CHIKV carib	CV1	435	3123	2689	9541	0	F+1
41	CHIKV carib	CV2	663	2878	2216	10014	0	F+2
42	CHIKV carib	CV3	3893	11527	7635	4595	0	N/A
43	CHIKV carib	CV4	3893	11704	7812	4418	0	N/A
44	CHIKV carib	CH1	508	2188	1681	10549	0	F+1
45	CHIKV carib	CH2	555	11501	10947	1283	0	N/A
46	CHIKV carib	CH3	555	11678	11124	1106	0	N/A
47	CHIKV carib	CM1	2695	5358	2664	9566	0	F
48	CHIKV carib	CU1	5628	11468	5841	6389	0	N/A
49	CHIKV carib	CU2	5897	11528	5632	6598	0	N/A
50	CHIKV carib	CA1	6183	11784	5602	6628	0	N/A
51	CHIKV carib	CA2	3050	6141	3092	9138	0	F+2
52	CHIKV carib	CA3	373	2344	1972	10258	0	F+1
53	CHIKV carib	CA4	2698	11790	9093	3137	0	N/A

Figure 41

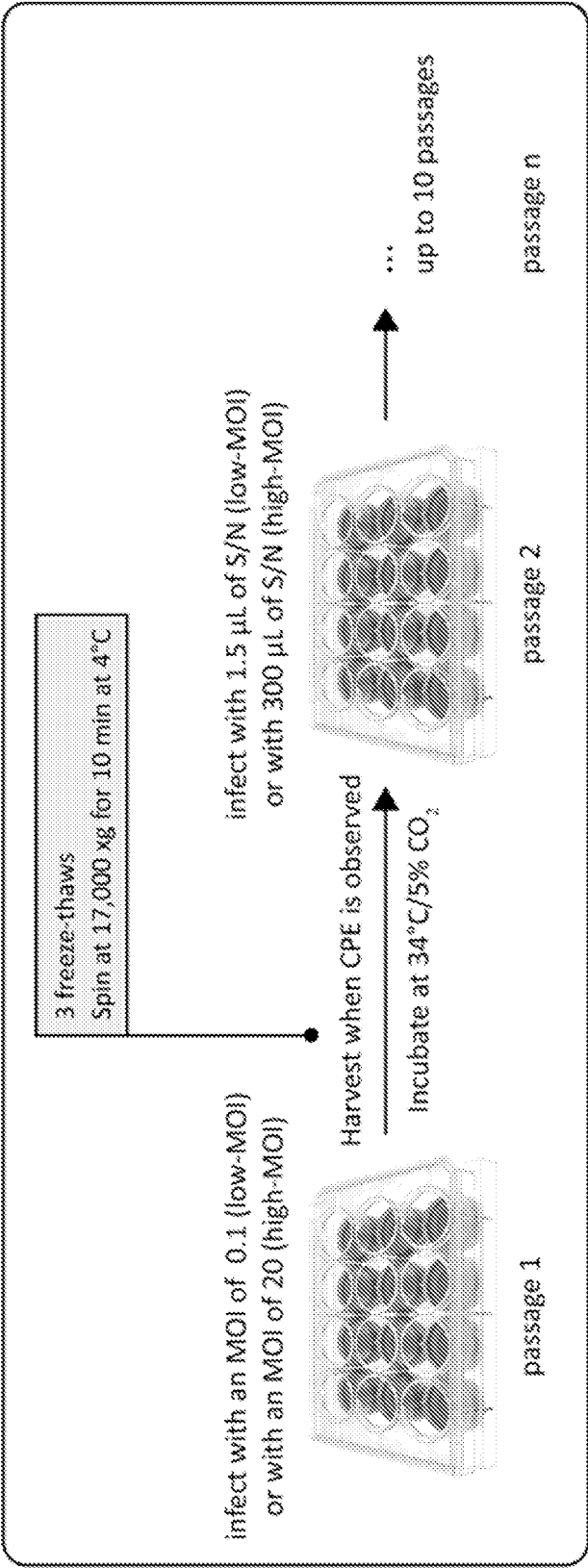


Figure 42

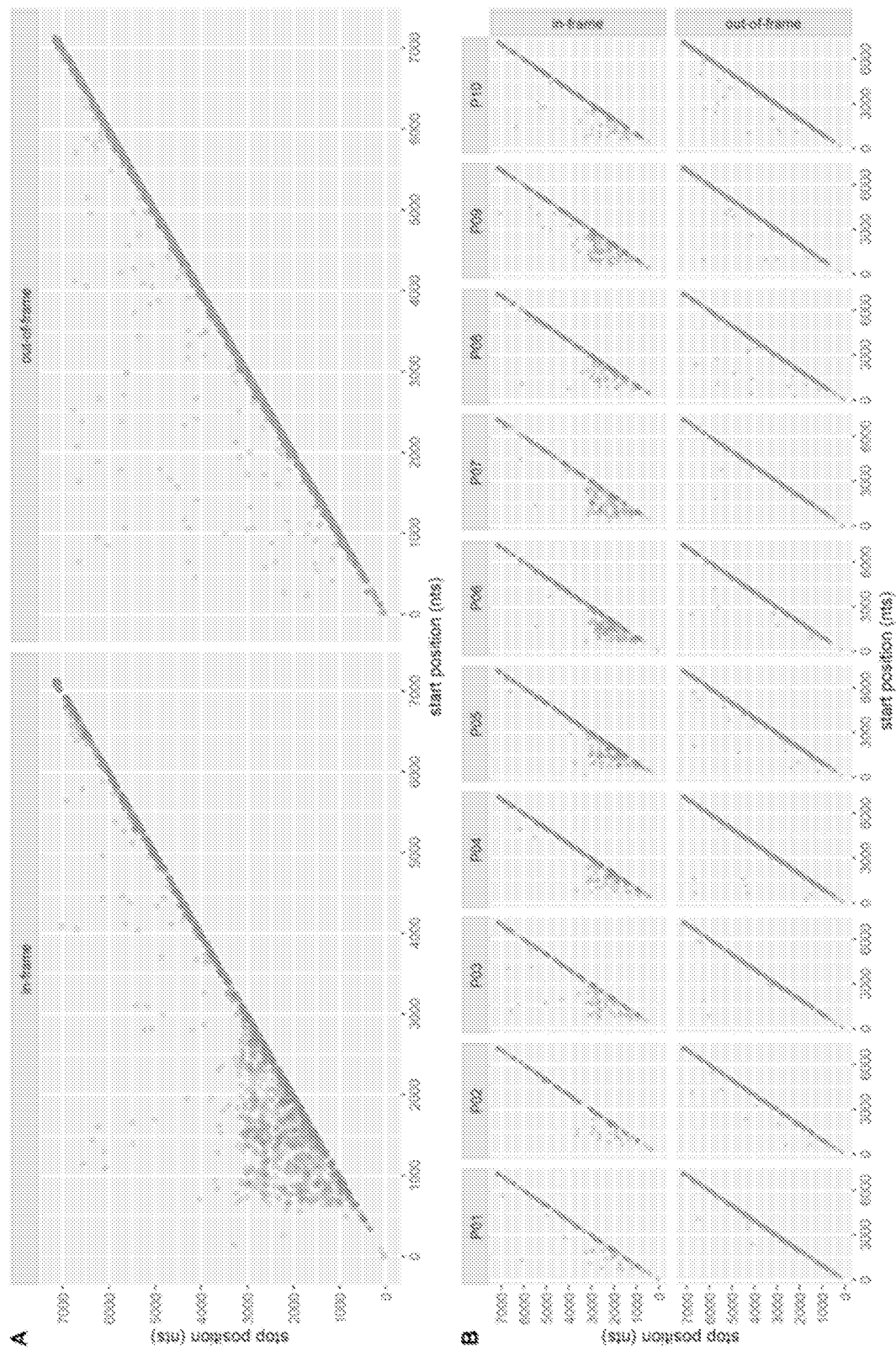


Figure 43

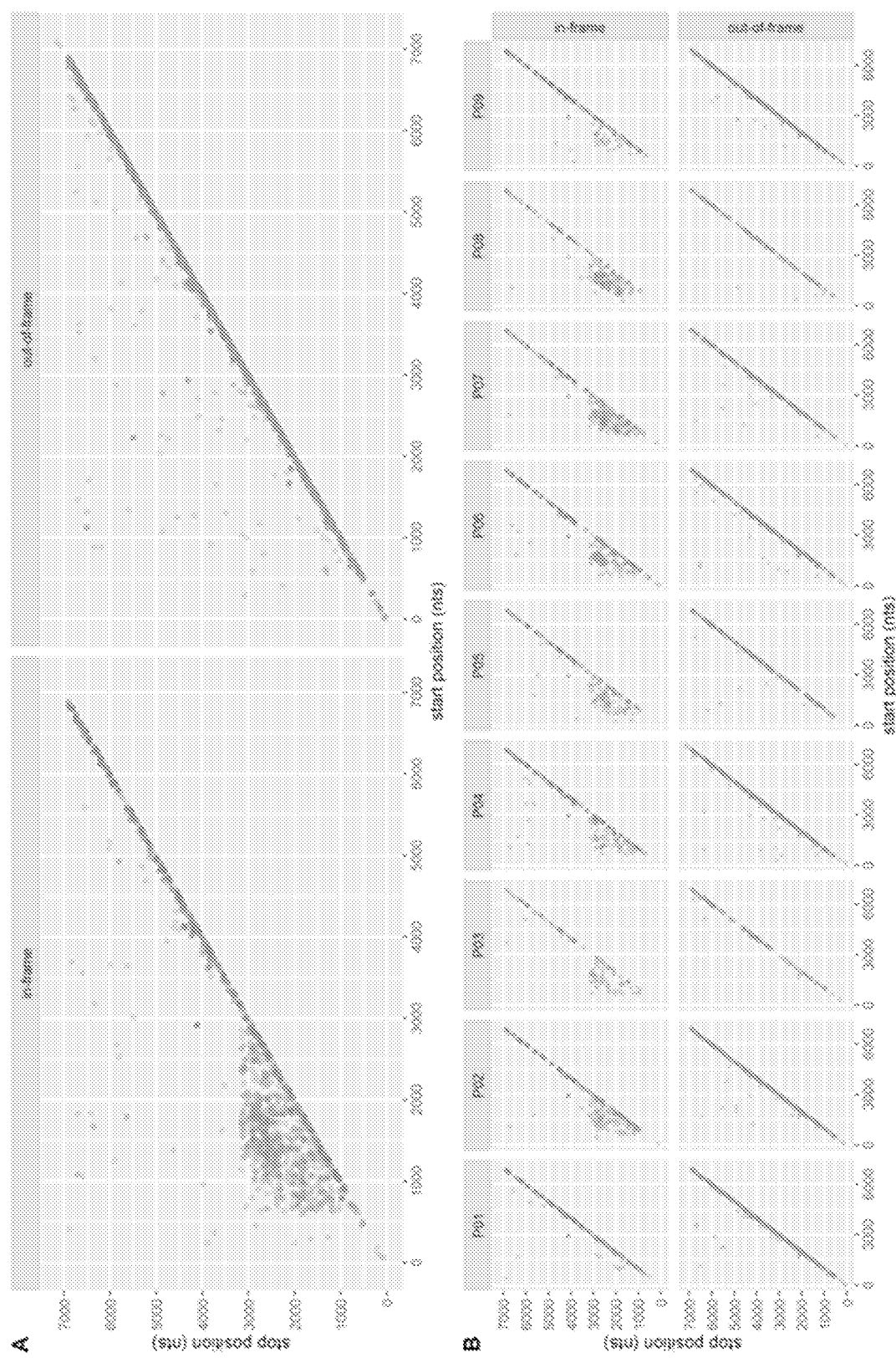


Figure 44

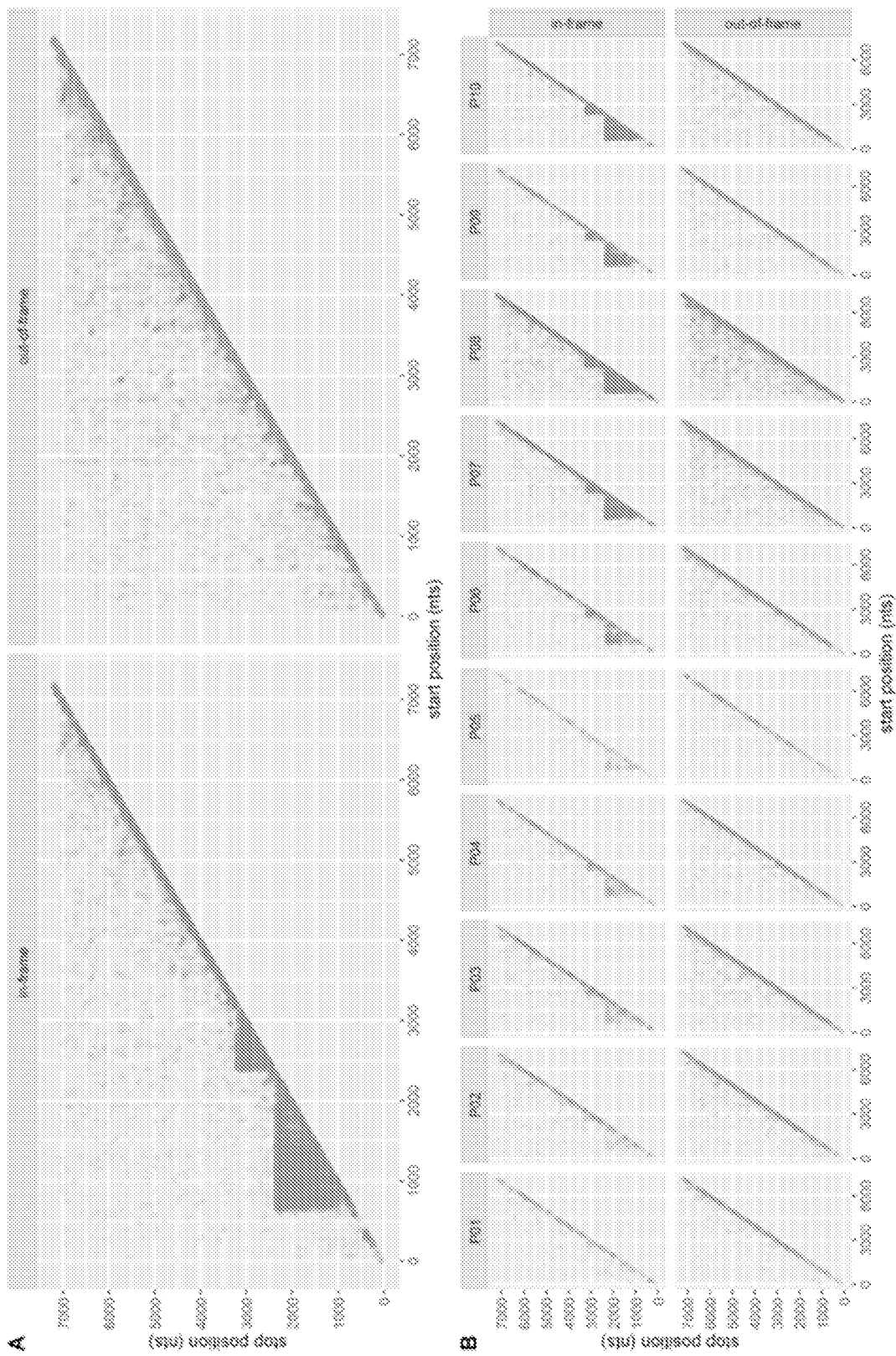


Figure 45

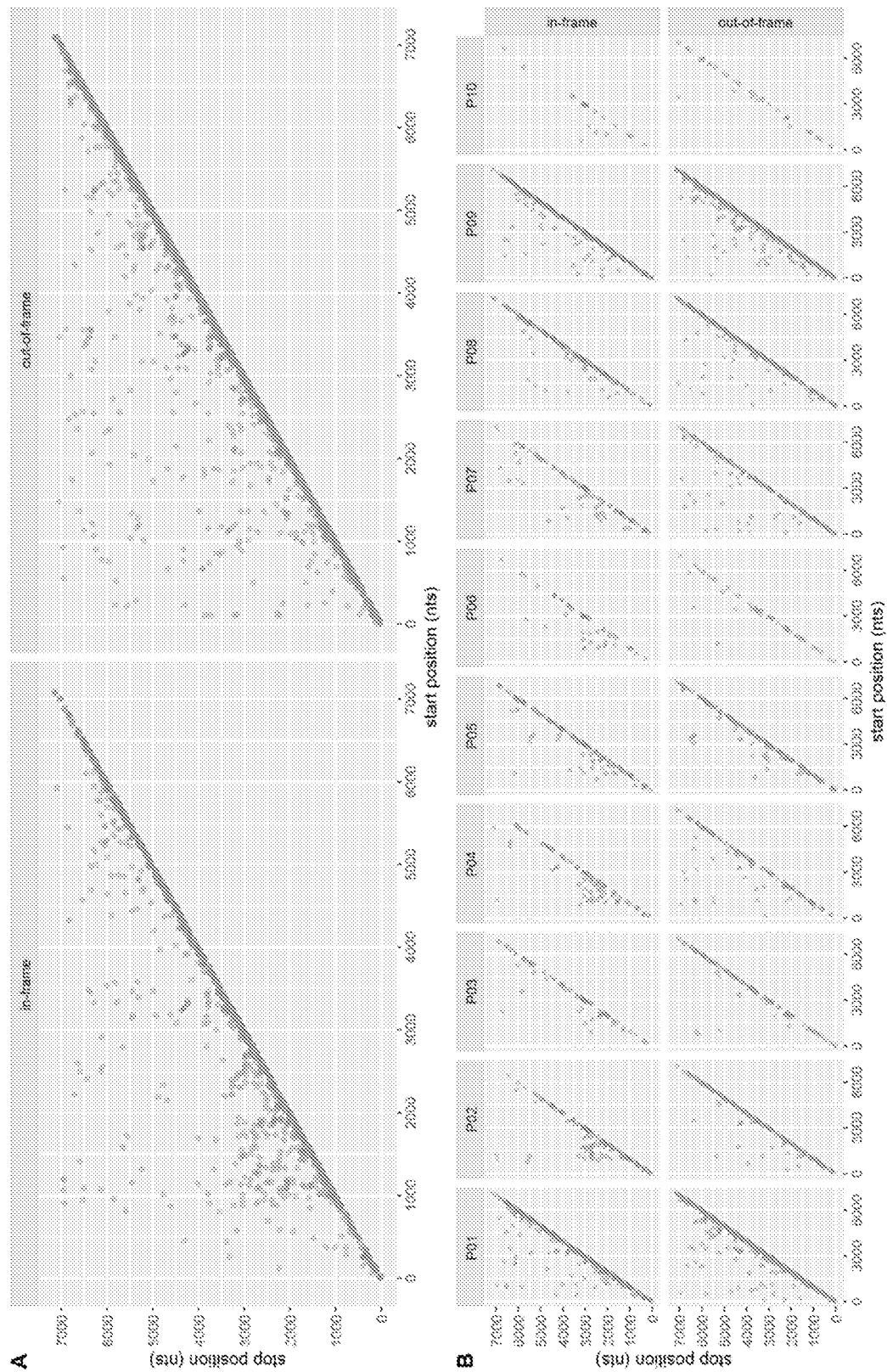


Figure 46

TIP ID	START	STOP	DEL LENGTH	MUTATION	ORF	TIP ID	START	STOP	DEL LENGTH	MUTATION	ORF
B14-TIP-01	1063	2772	1209	-	in-frame	A01a-TIP-01	1914	2109	195	-	in-frame
B14-TIP-02	1063	2273	1210	-	out-of-frame	A01a-TIP-02	1914	2110	196	-	out-of-frame
B14-TIP-03	2692	3148	456	-	in-frame	A01a-TIP-03	3001	3236	237	-	in-frame
B14-TIP-04	2692	3149	457	-	out-of-frame	A01a-TIP-04	3484	3565	81	-	in-frame
B14-TIP-05	2692	3148	456	3C (R12L)	in-frame	A01a-TIP-05	5001	5082	81	-	in-frame
B14-TIP-06	2692	3148	456	3C (K13L)	in-frame	A01a-TIP-06	5820	5832	12	-	in-frame
B14-TIP-07	2692	3148	456	3C (R84L)	in-frame						
B14-TIP-08	2692	3148	456	3D (I445D, R454D)	in-frame	A16-TIP-01	1866	2076	210	-	in-frame
B14-TIP-09	2692	3148	456	3D (D327A, D328A)	in-frame	A16-TIP-02	1866	2077	211	-	out-of-frame
B14-TIP-10	1063	2772	1209	-	in-frame	A16-TIP-03	3162	3207	45	-	in-frame
	2692	3148	456	-	in-frame	A16-TIP-04	2916	4107	1191	-	in-frame
B14-TIP-11	1063	2272	1209	-	out-of-frame	A16-TIP-05	4137	4294	157	-	out-of-frame
	2692	3149	457	-	in-frame						
B14-TIP-12	3178	3253	75	-	in-frame	C15-TIP-01	2874	2943	69	-	in-frame
B14-TIP-13	3464	3516	52	-	out-of-frame	C15-TIP-02	3256	3451	195	-	in-frame
B14-TIP-14	3810	3868	58	-	out-of-frame	C15-TIP-03	4706	4758	52	-	out-of-frame
B14-TIP-15	3943	3985	42	-	in-frame						
B14-TIP-16	5066	5102	36	-	in-frame						
B14-TIP-17	5421	5462	41	-	out-of-frame						
B14-TIP-18	6126	6173	47	-	out-of-frame						
B14-TIP-19	6444	6993	549	-	in-frame						
	628	2272	1644	-	in-frame						
B14-TIP-20	2692	7165	4473	-	in-frame						

Figure 48

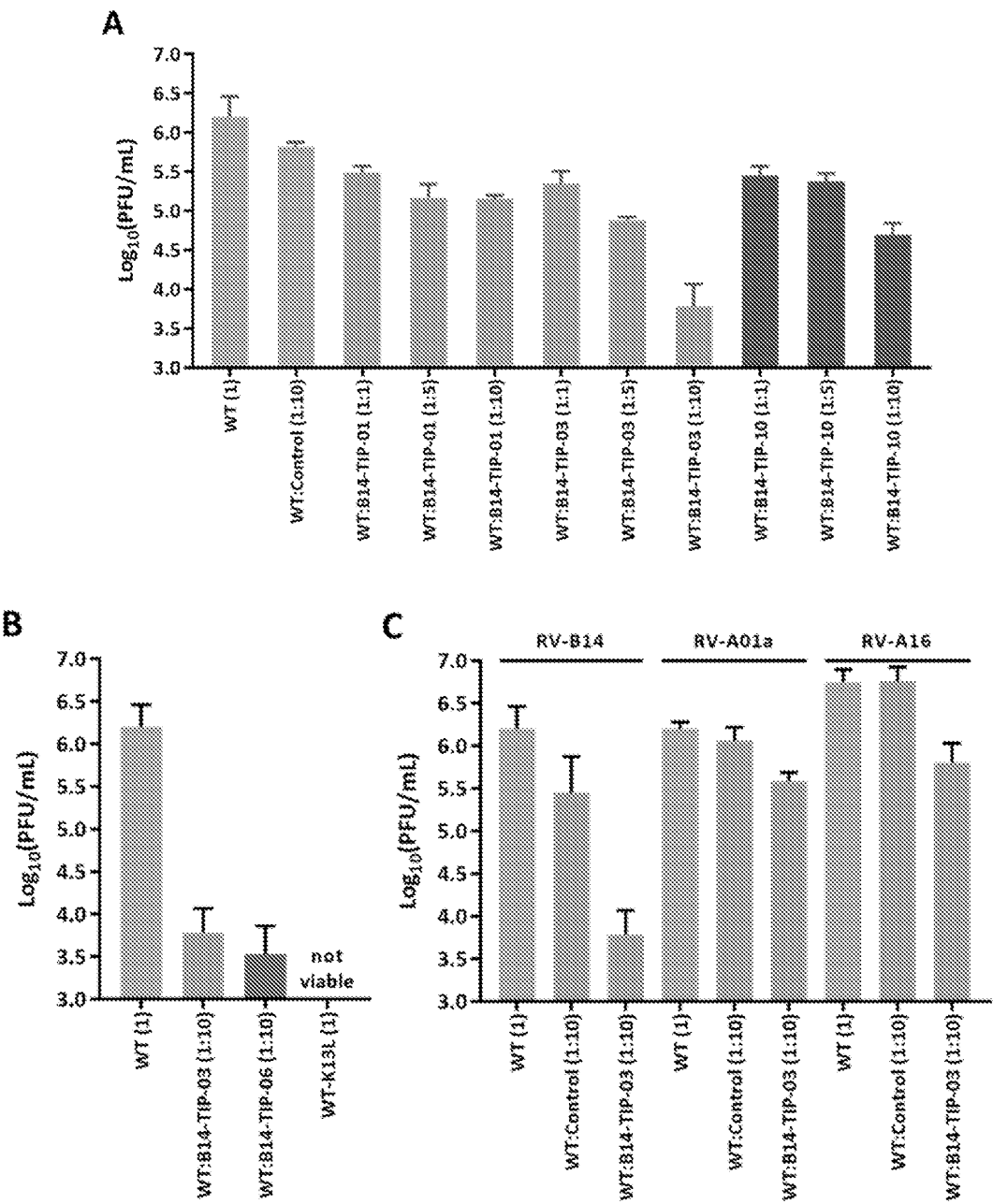


Figure 49

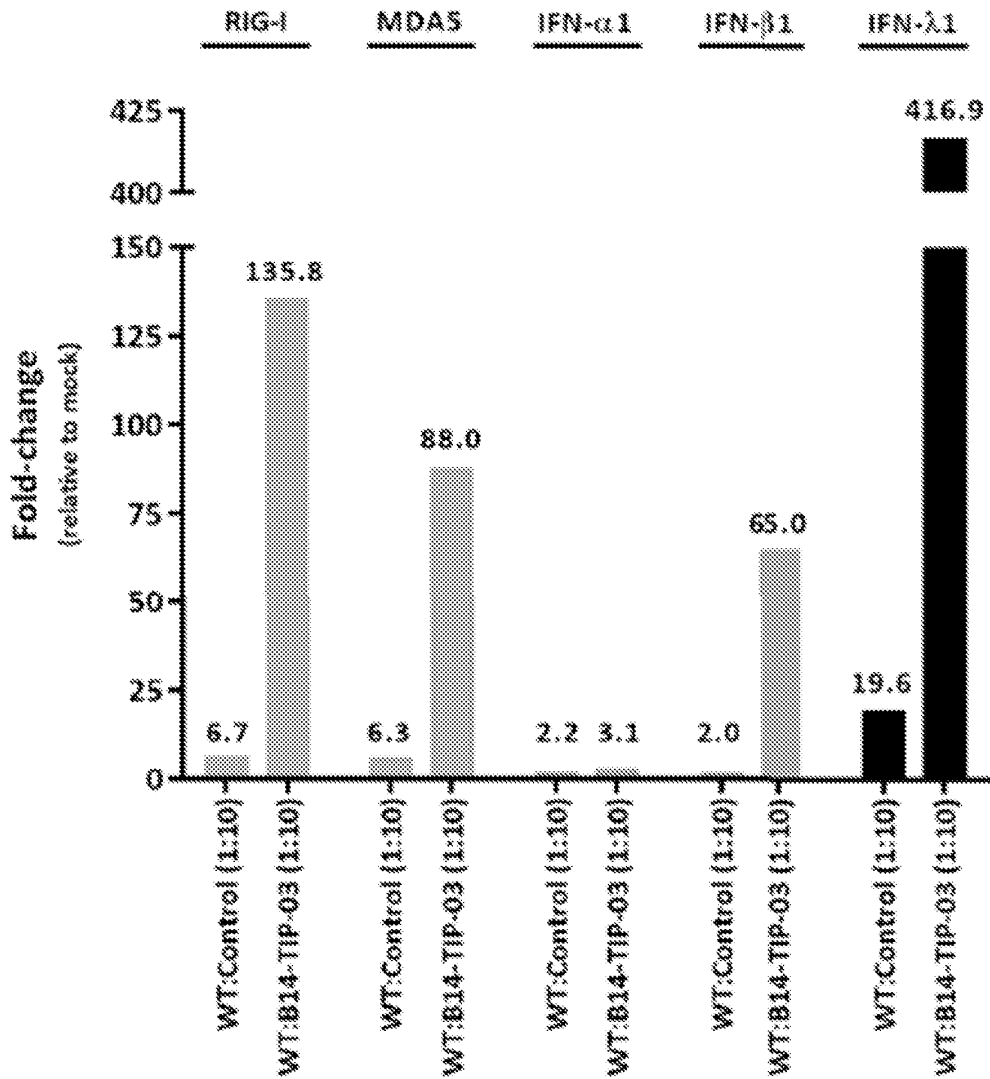


Figure 50

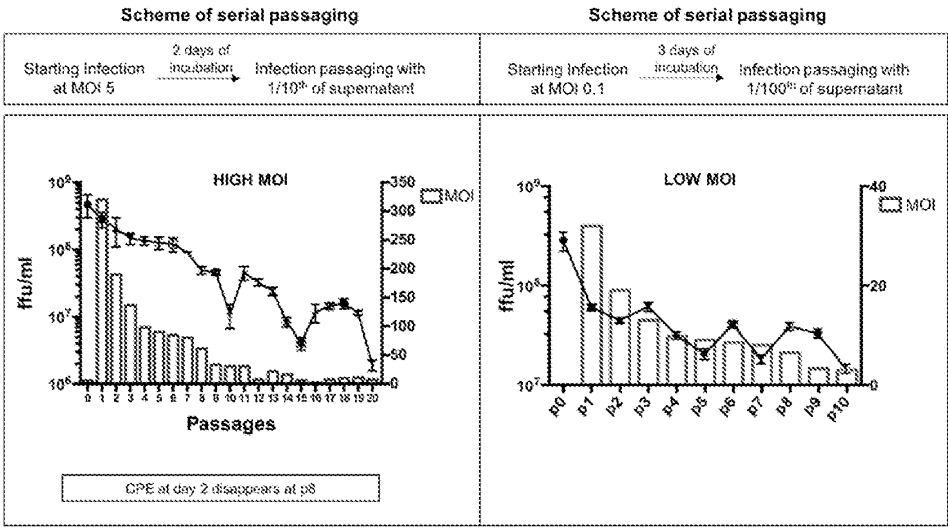
SEQ ID NO	Virus	Name	Start position	Stop position	Deletion size	Mutation	ORF
54	RV-A01a	A01a-TIP-00-WT					
55	RV-A01a	A01a-TIP-01	1914	2109	195		in-frame
56	RV	A01a-TIP-02	1914	2110	196		out-of-frame
57	RV-A01a	A01a-TIP-03	3001	3238	237		in-frame
58	RV-A01a	A01a-TIP-04	3484	3565	81		in-frame
59	RV-A01a	A01a-TIP-05	5001	5082	81		in-frame
60	RV-A01a	A01a-TIP-06	5820	5832	12		in-frame
61	RV-A16	A16-TIP-00-WT					
62	RV-A16	A16-TIP-01	1866	2076	210		in-frame
63	RV-A16	A16-TIP-02	1866	2077	211		out-of-frame
64	RV-A16	A16-TIP-03	3162	3207	45		in-frame
65	RV-A16	A16-TIP-04	2916	4107	1191		in-frame
66	RV-A16	A16-TIP-05	4137	4294	157		out-of-frame
67	RV-B14	B14-TIP-00-WT					
68	RV-B14	B14-TIP-01	1063	2272	1209		in-frame
69	RV-B14	B14-TIP-02	1063	2273	1210		out-of-frame
70	RV-B14	B14-TIP-03	2692	3148	456		in-frame
71	RV-B14	B14-TIP-04	2692	3149	457		out-of-frame
72	RV-B14	B14-TIP-05	2692	3148	456	3C (R12L)	in-frame

Figure 51

73	RV-B14	73	RV-B14	3148	456	3C(K13L)	in-frame
74	RV-B14	74	RV-B14	3148	456	3C(R84I)	in-frame
75	RV-B14	75	RV-B14	3148	456	3D(I445D, R454D)	in-frame
76	RV-B14	B14-TIP-09	2692	3148	456	3D(D3327A, D328A)	in-frame
77	RV-B14	B14-TIP-10	1063	2272	1209	in-frame	in-frame
	RV-B14	B14-TIP-10	2692	3148	456	in-frame	in-frame
78	RV-B14	B14-TIP-11	1063	2272	1209	out-of-frame	out-of-frame
	RV-B14	B14-TIP-11	2692	3149	457	out-of-frame	out-of-frame
79	RV-B14	B14-TIP-12	3178	3253	75	in-frame	in-frame
80	RV-B14	B14-TIP-13	3464	3516	52	out-of-frame	out-of-frame
81	RV-B14	B14-TIP-14	3810	3868	58	out-of-frame	out-of-frame
82	RV-B14	B14-TIP-15	3943	3985	42	in-frame	in-frame
83	RV-B14	B14-TIP-16	5066	5102	36	in-frame	in-frame
84	RV-B14	B14-TIP-17	5421	5462	41	out-of-frame	out-of-frame
85	RV-B14	B14-TIP-18	6126	6173	47	out-of-frame	out-of-frame
86	RV-B14	B14-TIP-19	6444	6993	549	in-frame	in-frame
87	RV-B14	B14-TIP-20	628	2272	1644	in-frame	in-frame
	RV-B14	B14-TIP-20	2692	7165	4473	in-frame	in-frame
88	RV-C15	C15-TIP-00-WT					
89	RV-C15	C15-TIP-01	2874	2943	69	in-frame	in-frame
90	RV-C15	C15-TIP-02	3256	3451	195	in-frame	in-frame
91	RV-C15	C15-TIP-03	4706	4758	52	out-of-frame	out-of-frame
92	RV-C15	C15-TIP-04					

Figure 52

YF17D/SW13



YF17D/VeroE6

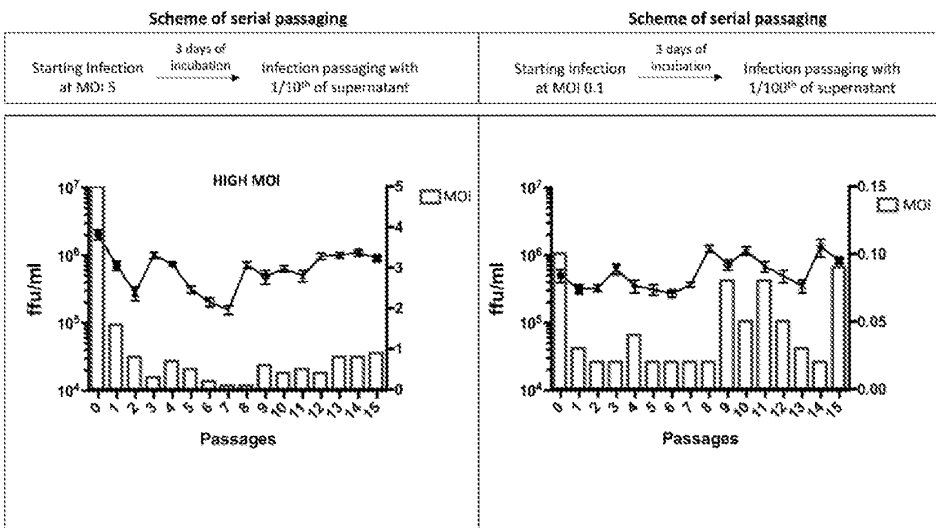
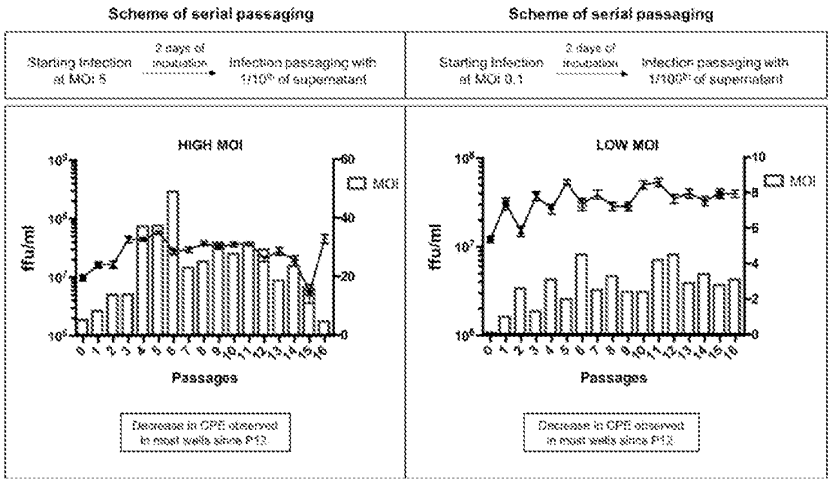
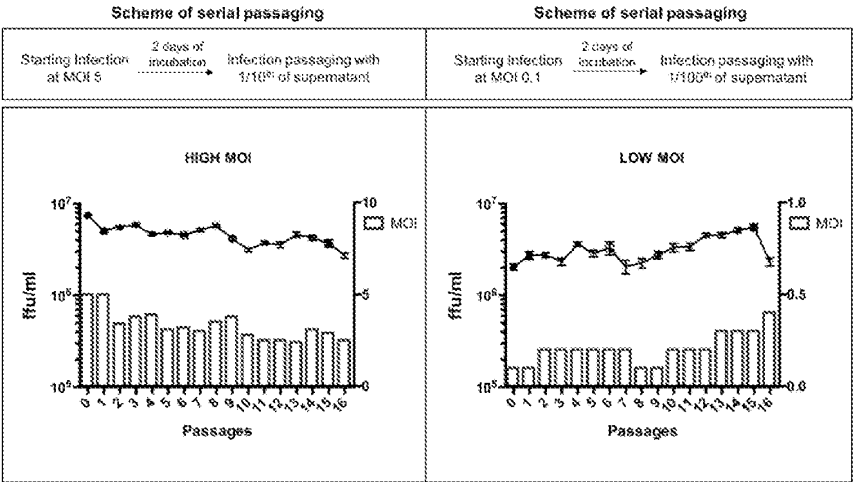


Figure 53 (start)

YF-Asibi/SW13



YF-Asibi/C6-36



YF-Asibi/VeroE6

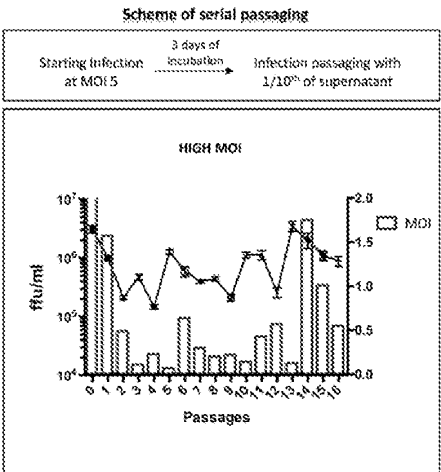


Figure 53 (end)

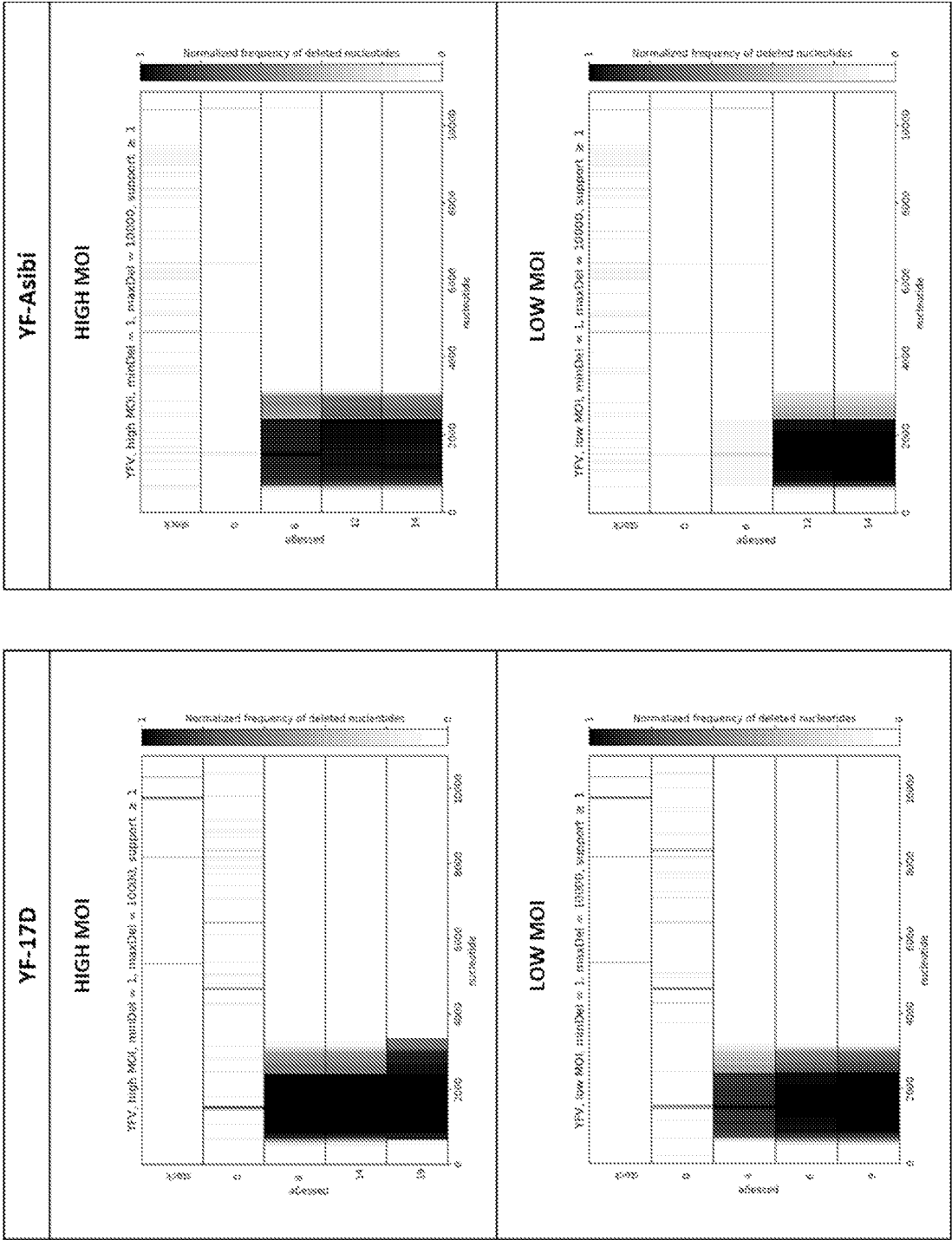


Figure 54

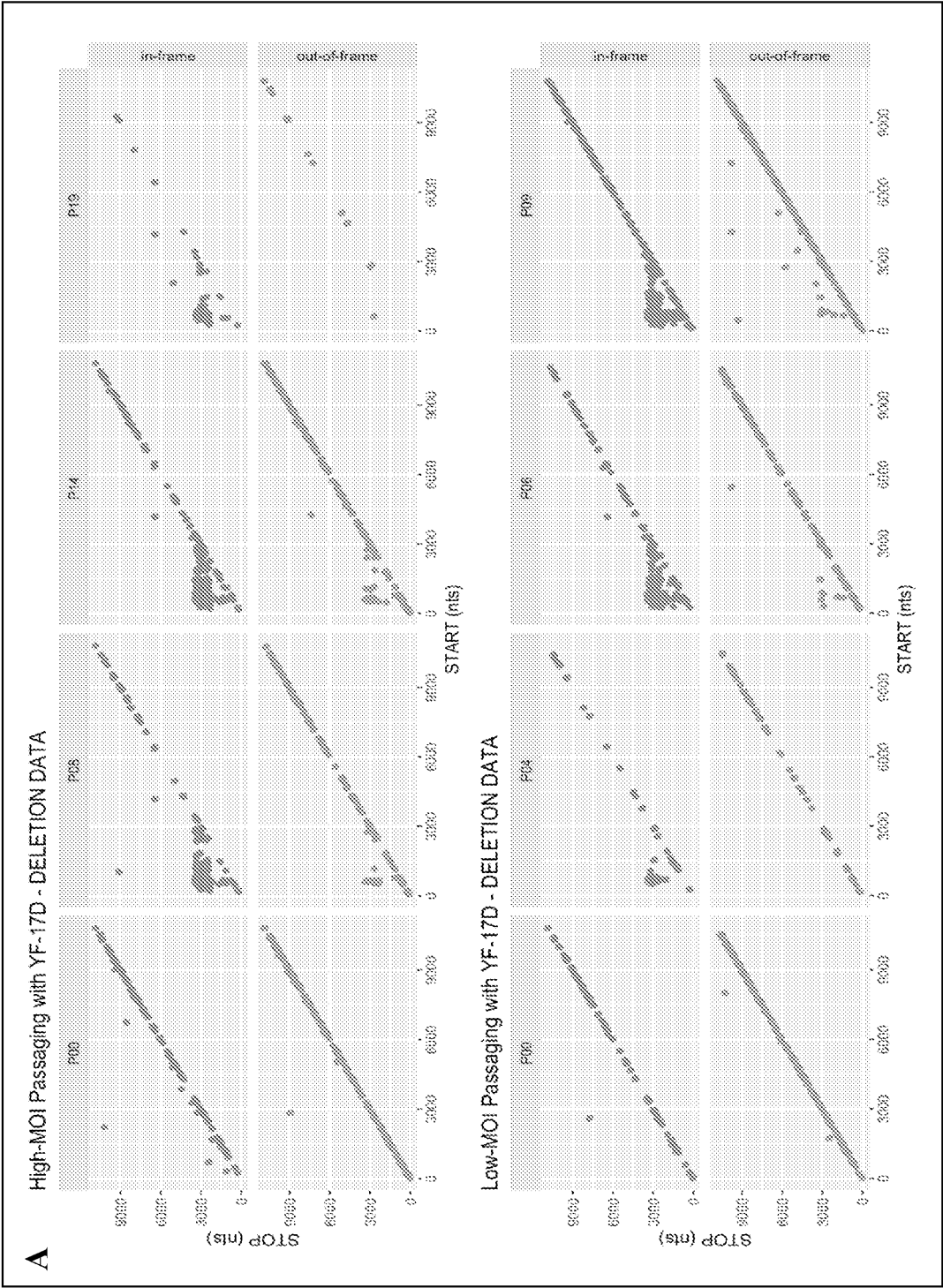


Figure 55 (start)

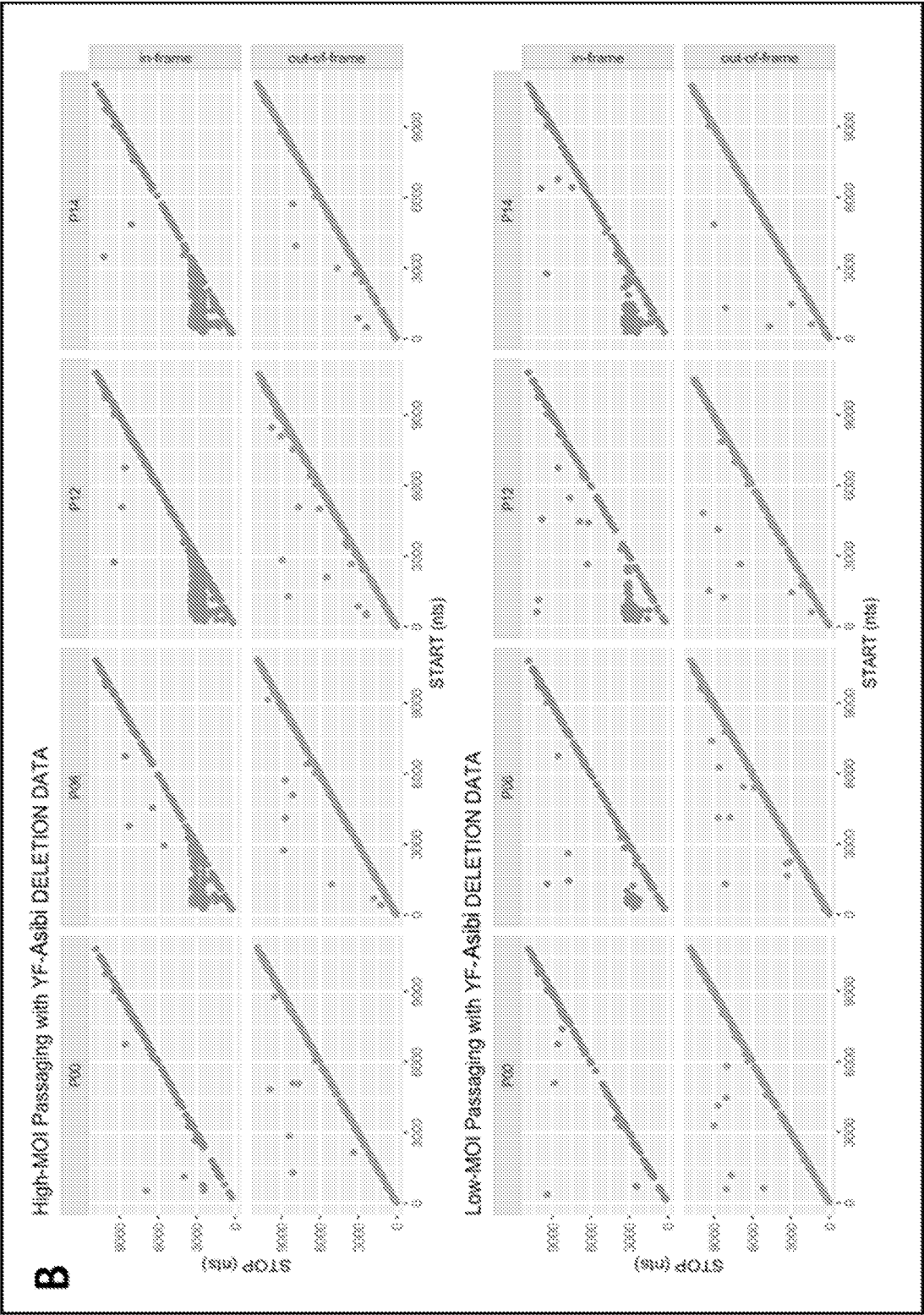


Figure 55 (continued)

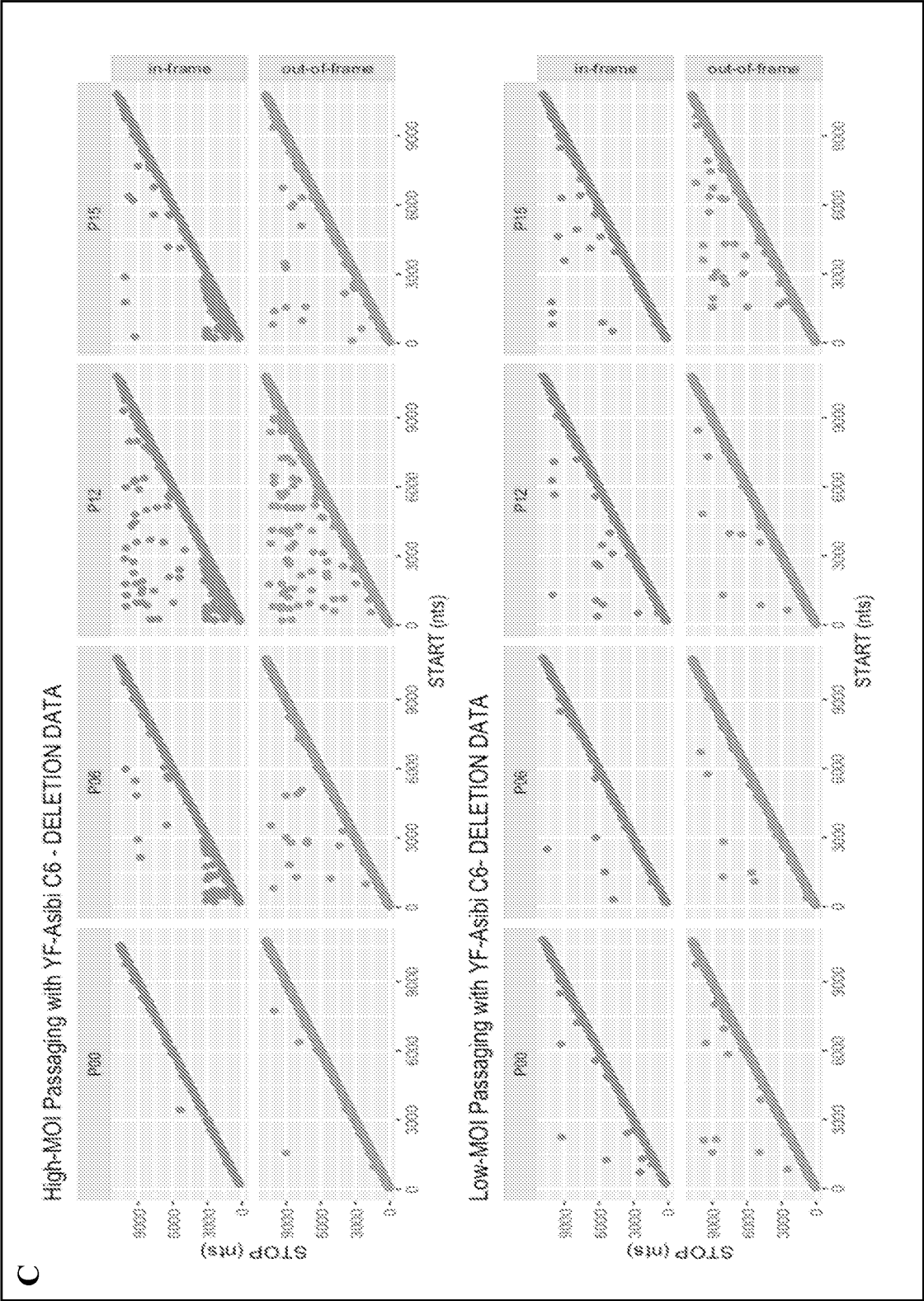


Figure 55 (end)

High fitness DVGs YF / SW13

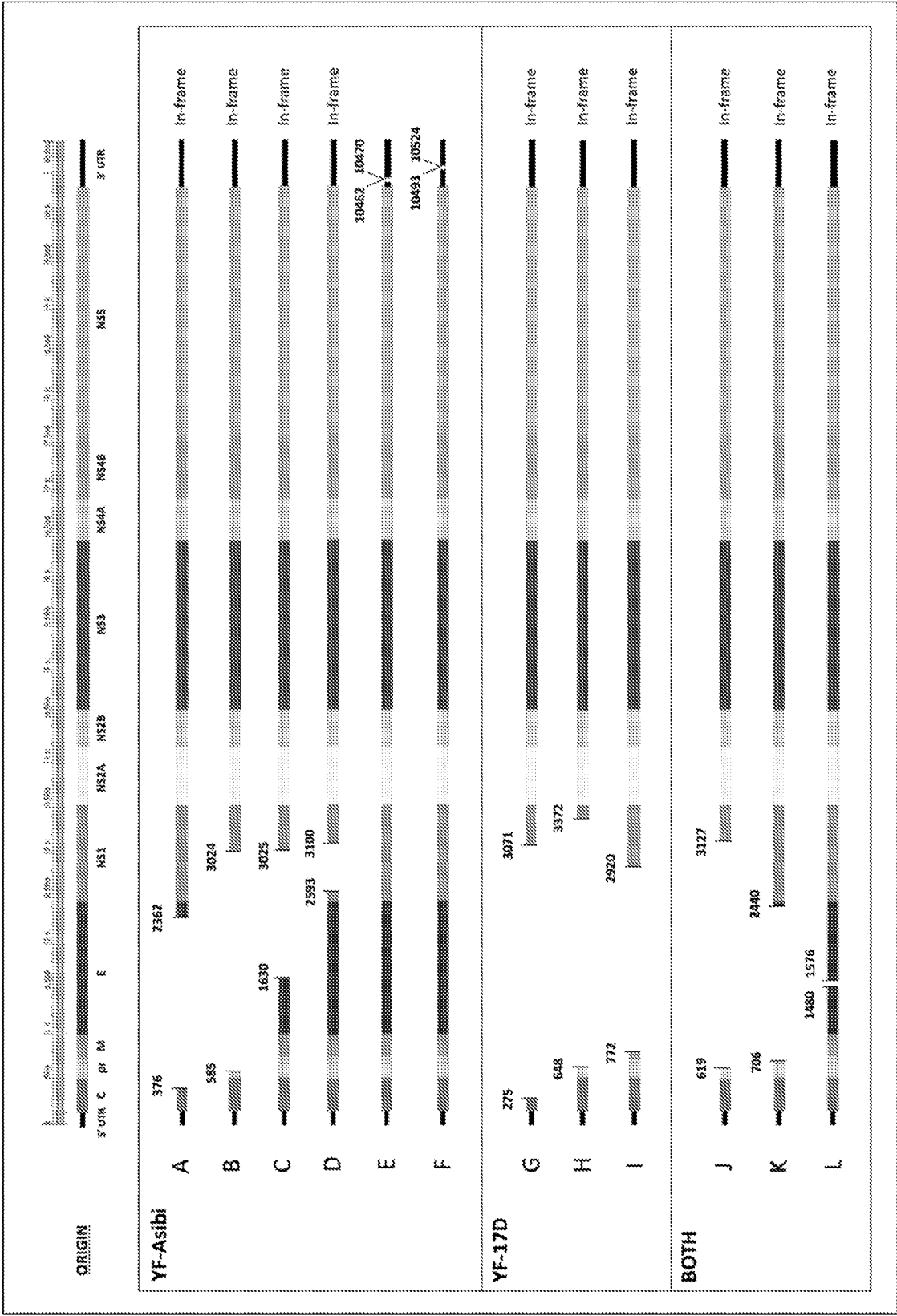


Figure 56

Sample generation-Serial passaging

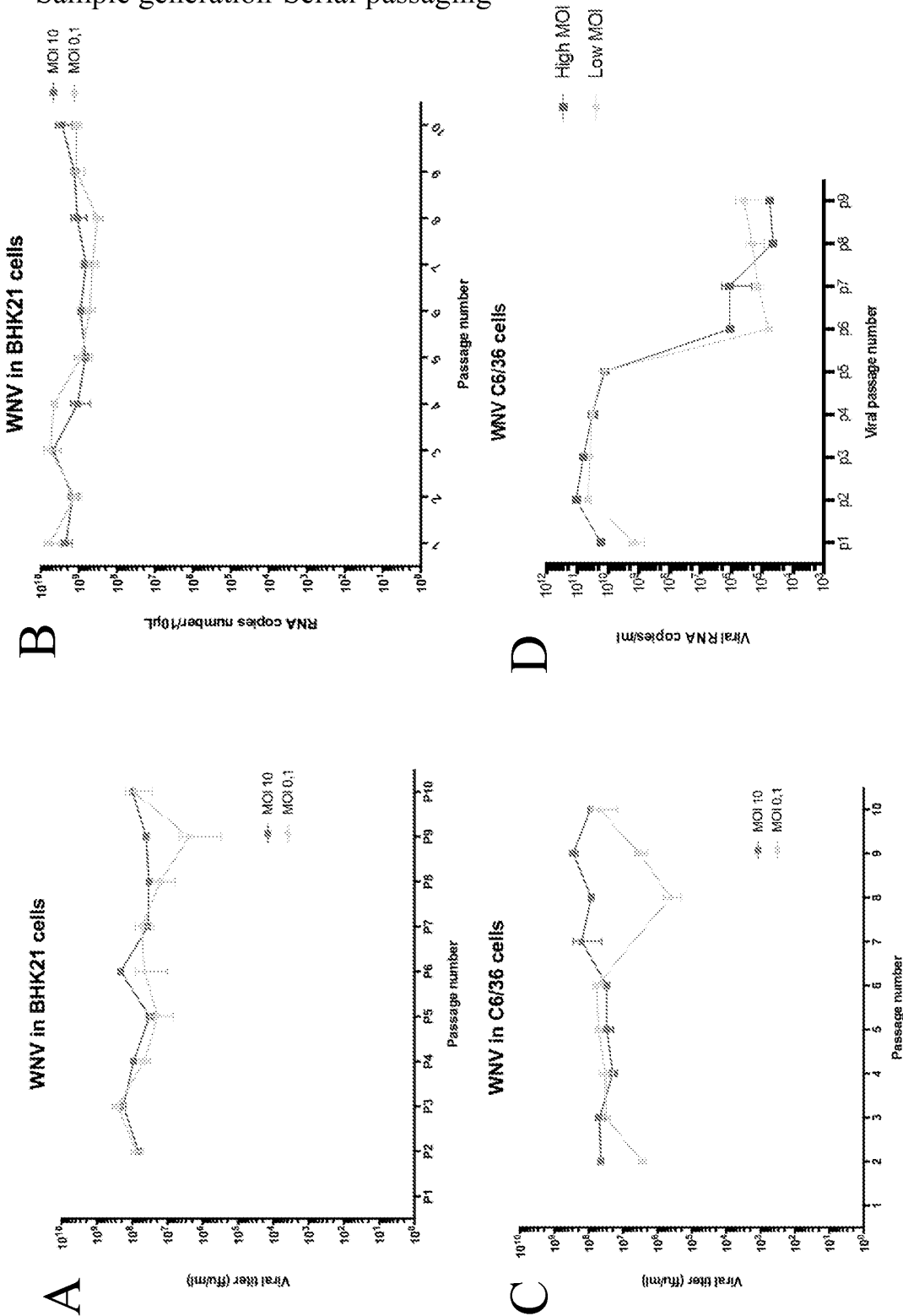


Figure 57
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Deletions in WNV genome after 10 passages

	5'-UTR	C	PrM	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5	3'-UTR	Total
WNV BHK21	1	1	5	19	26	13	4	31	11	17	54	26	208
WNV C6/36	1	1	2	10	14	6	2	15	5	5	35	8	104

Figure 58

Deletion heatmaps of WNV passaged in BHK21 or C6/36 cells

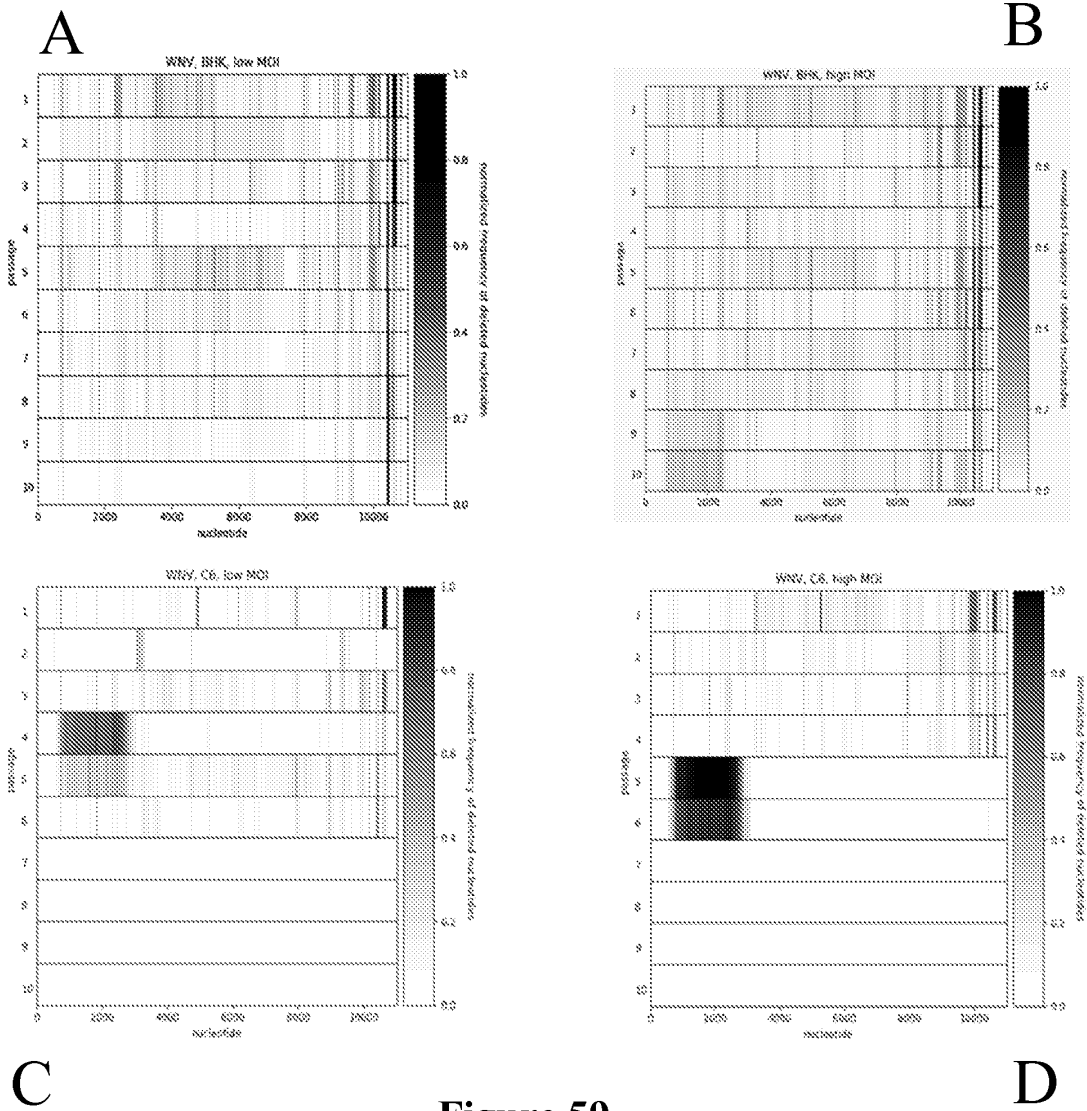


Figure 59

Identification of deletion hotspots in C6/36 cells

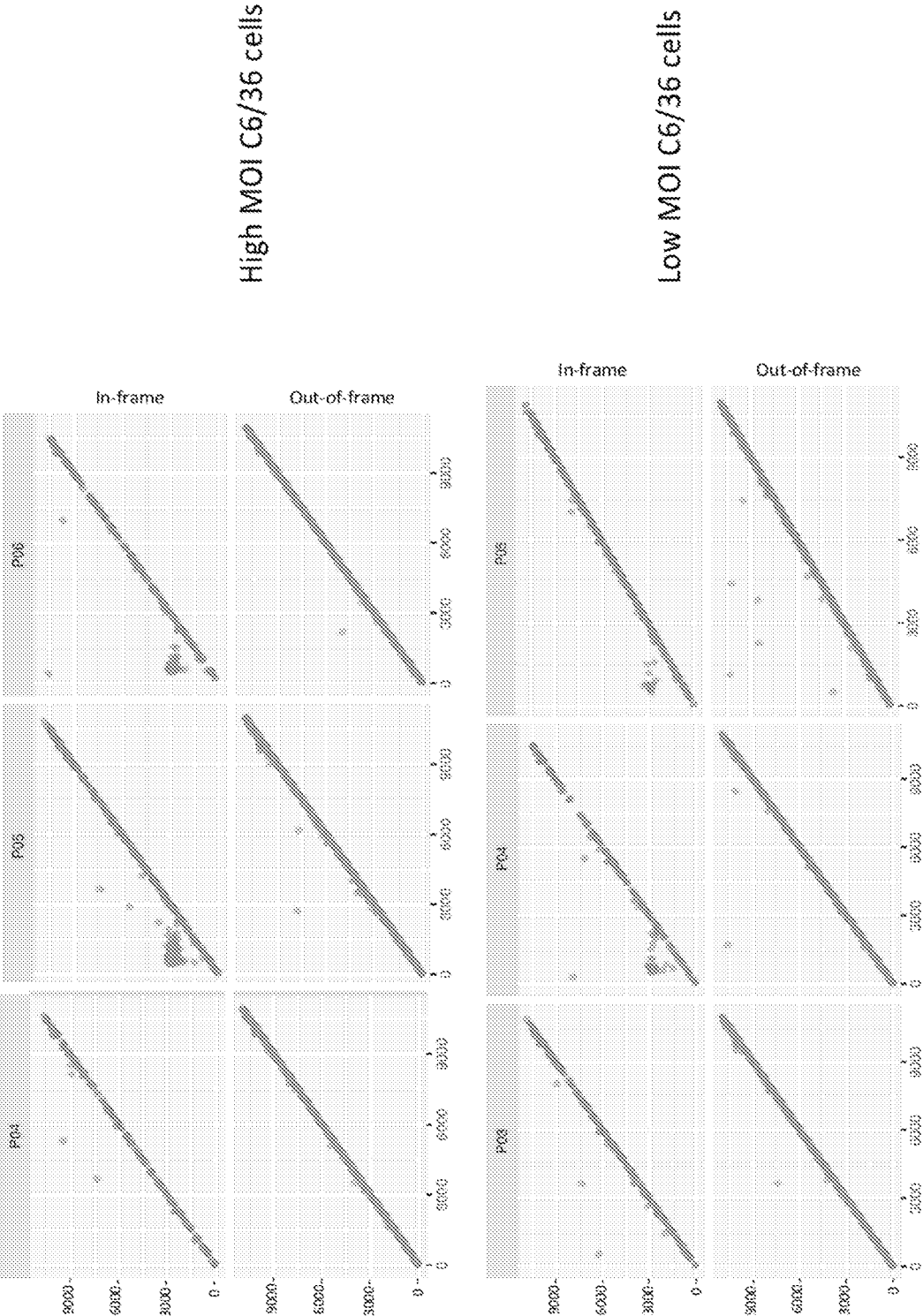


Figure 60

Schematic representation of candidate WNV DVGs

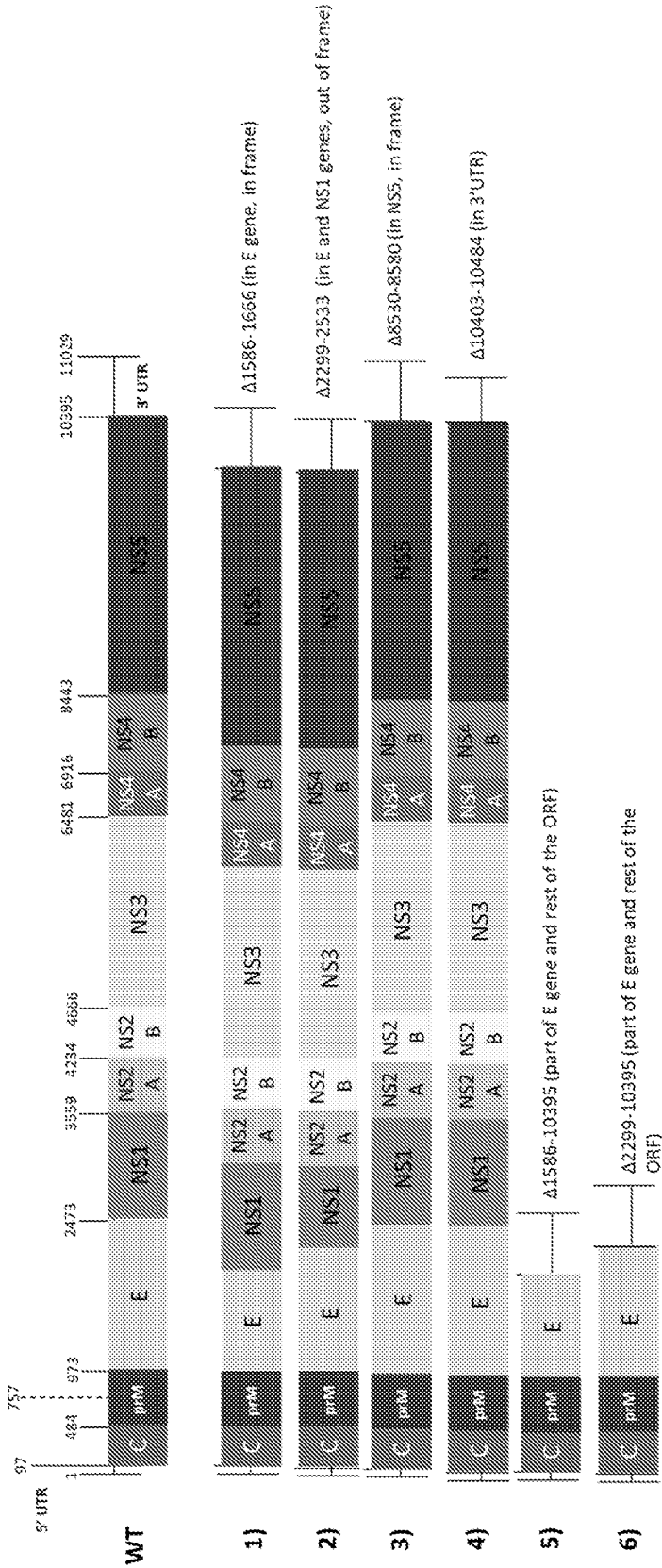
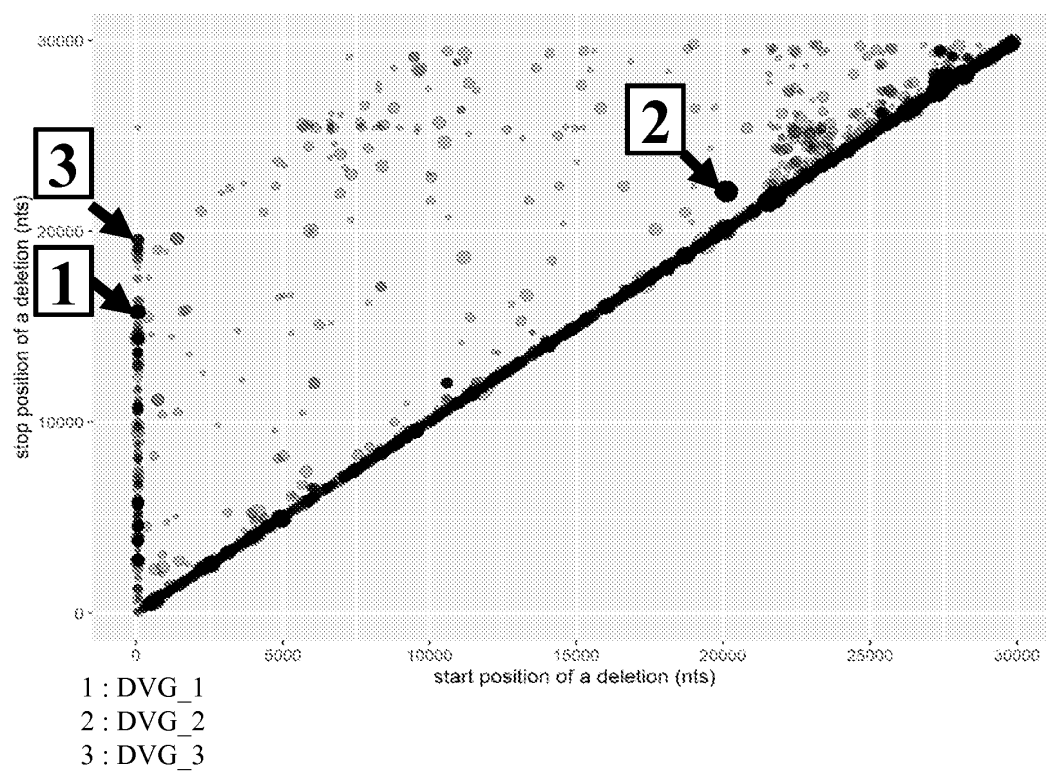


Figure 61

**Figure 62**

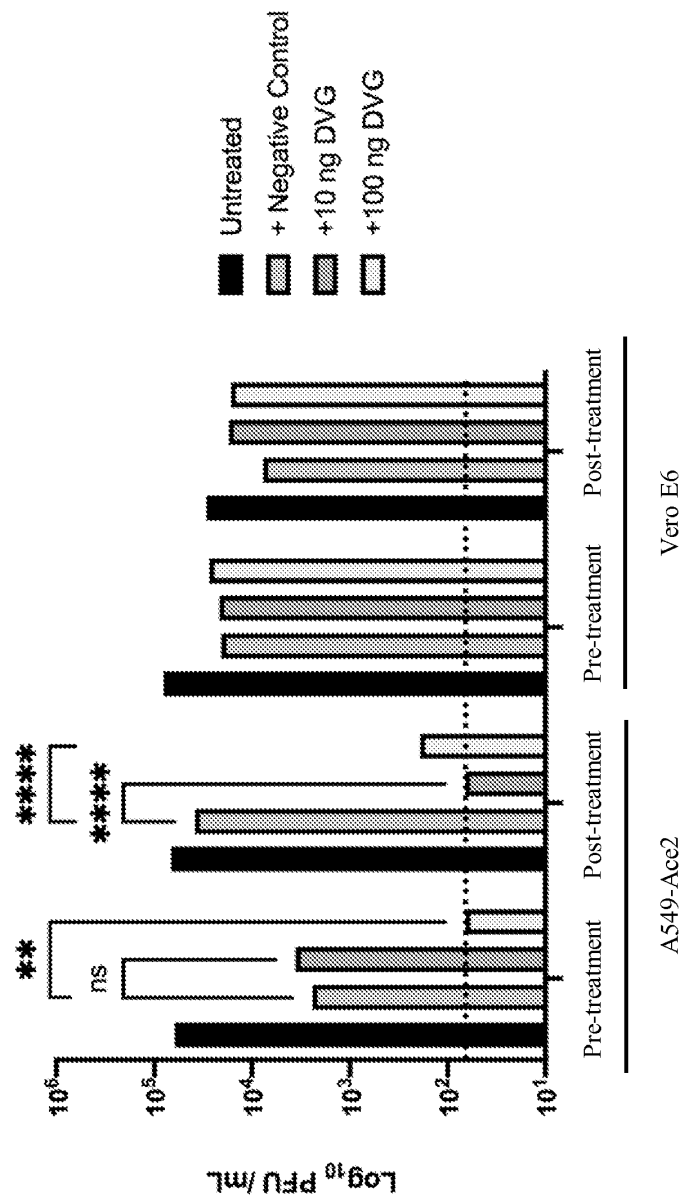


Figure 63