Evaluation of the integration potential of a theraputic hemophilia AAV vector in canines

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Genomic DNA samples were sonically sheared after which adapter sequence with sample specific barcodes were appended. Genomic fragments possessing remnants of AAV ITR sequences were preferentially amplified with a nested PCR protocol where primer sequences bound to the interior edge of ITR sequences and sheared adapter sequences. Amplified fragments were sequenced with the Illuminia MiSeq platform and analyzed with software developed specifically for this investigation. Genomic fragment read pairs which met a number of criteria (read quality, presence of an interior ITR sequence, proper paired-end read alignment to canine genome, ect.) were accepted and used to identify AAV incorporation locations and gauge the number of cells harboring each incorporations by using the number of unique sonic breaks associated with each position as an lower end estimate for abundance. Incorporations within 100 nucleotides of one another with reads aligning to opposite strands were considered the duel detection of a single incorporation where the interior edges of both 5' and 3' ITR sequences were present.

Figure 1. Sheared genomic fragment sequencing strategy. A single sequencing primer binds to the interior edge of AAV 5' and 3' ITR sequences and sequences outward towards genomic sequences. A second set of primers sequence inward from sheared genomic break points. The 5' and 3' ITR sequencing reactions yield different ITR sequences: reverse compliment of A, C', C, B, B', A' and A, B, B', C', C, A' respectively. The difference in observed ITR sequences suggests a double stranded AAV intermediate with breaks within its ITR structures.
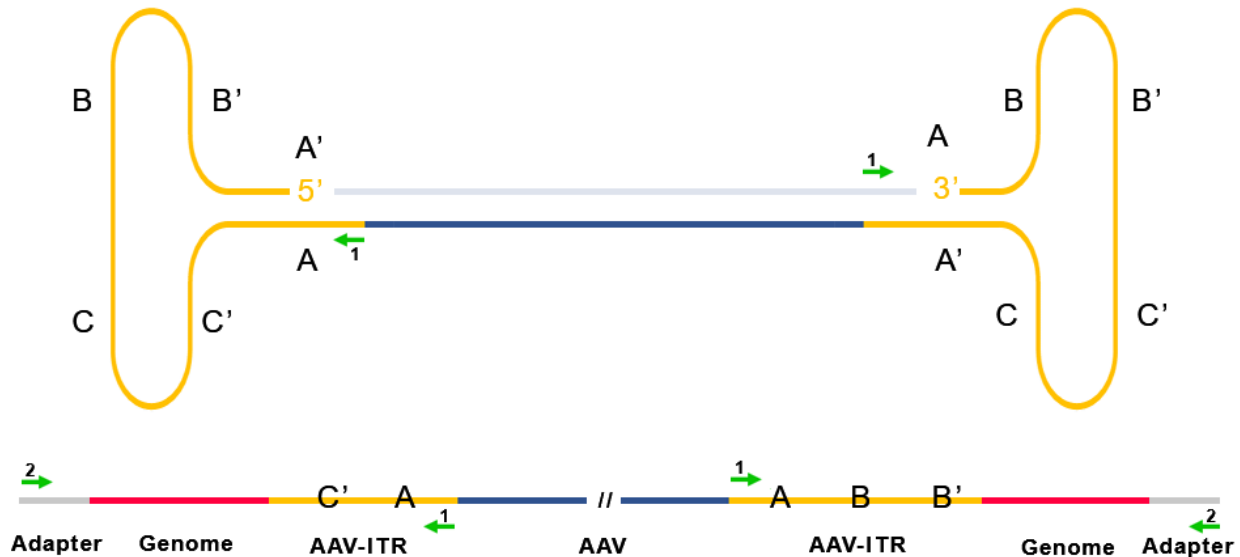
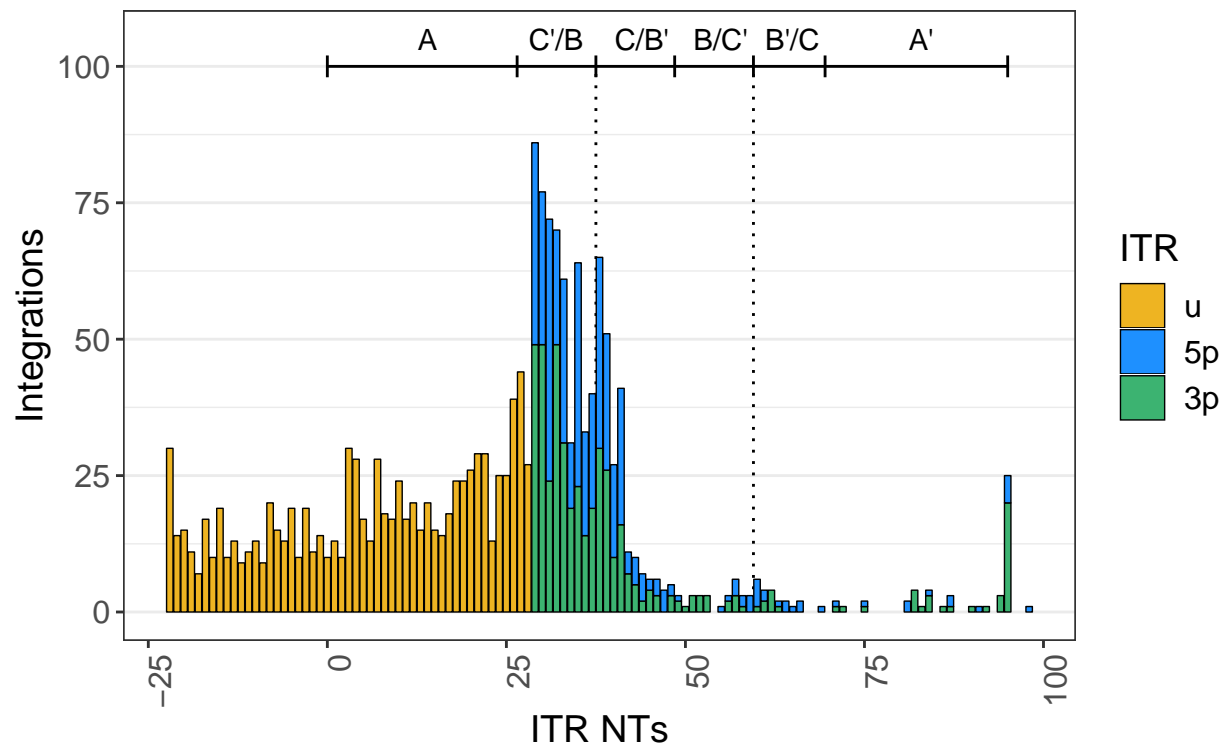Figure 2. Distribtion of ITR break points colored by (A) inferred sequencing direction and (B) clonal abundance.

A.

B.

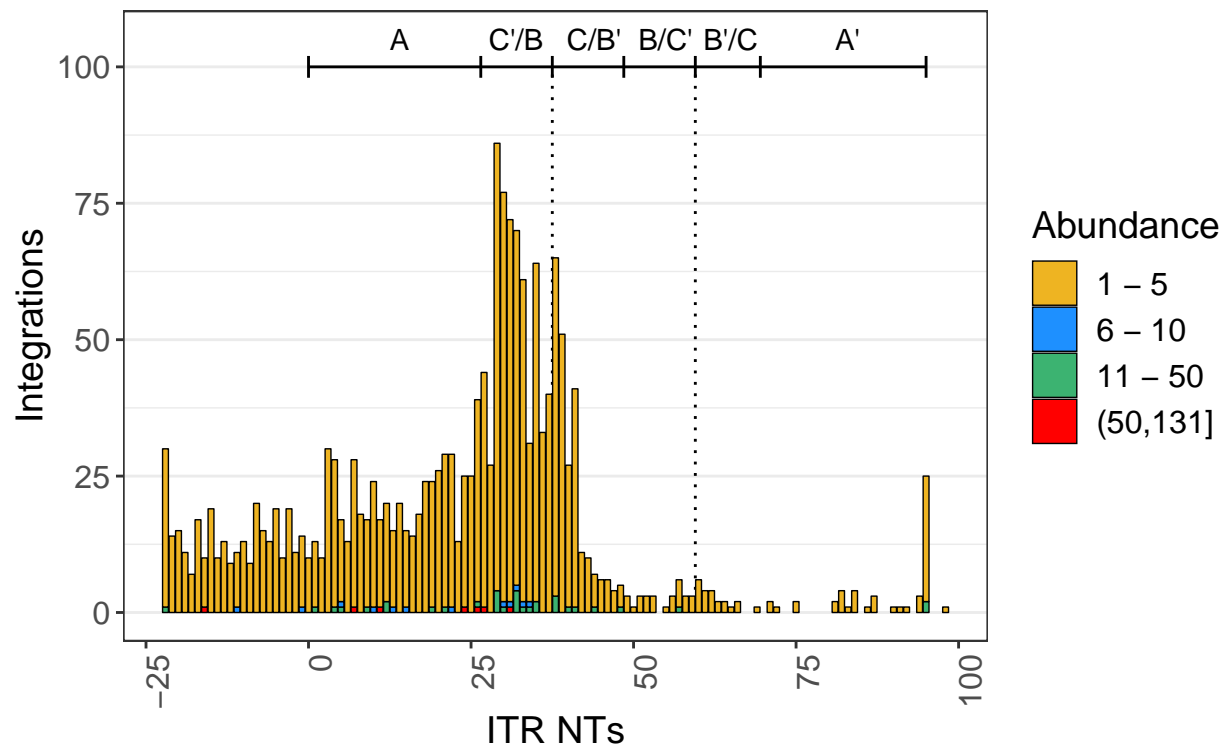Figure 3. Clones with abundances ≥ 5 cells (only most abundant 15 clones shown from each dog) where the sampled liver lobe number is shown at the end of each bar. Positions where alignment strands are denoted with an asterisk, i.e., chr25*34591227, denote dually detected incorporations
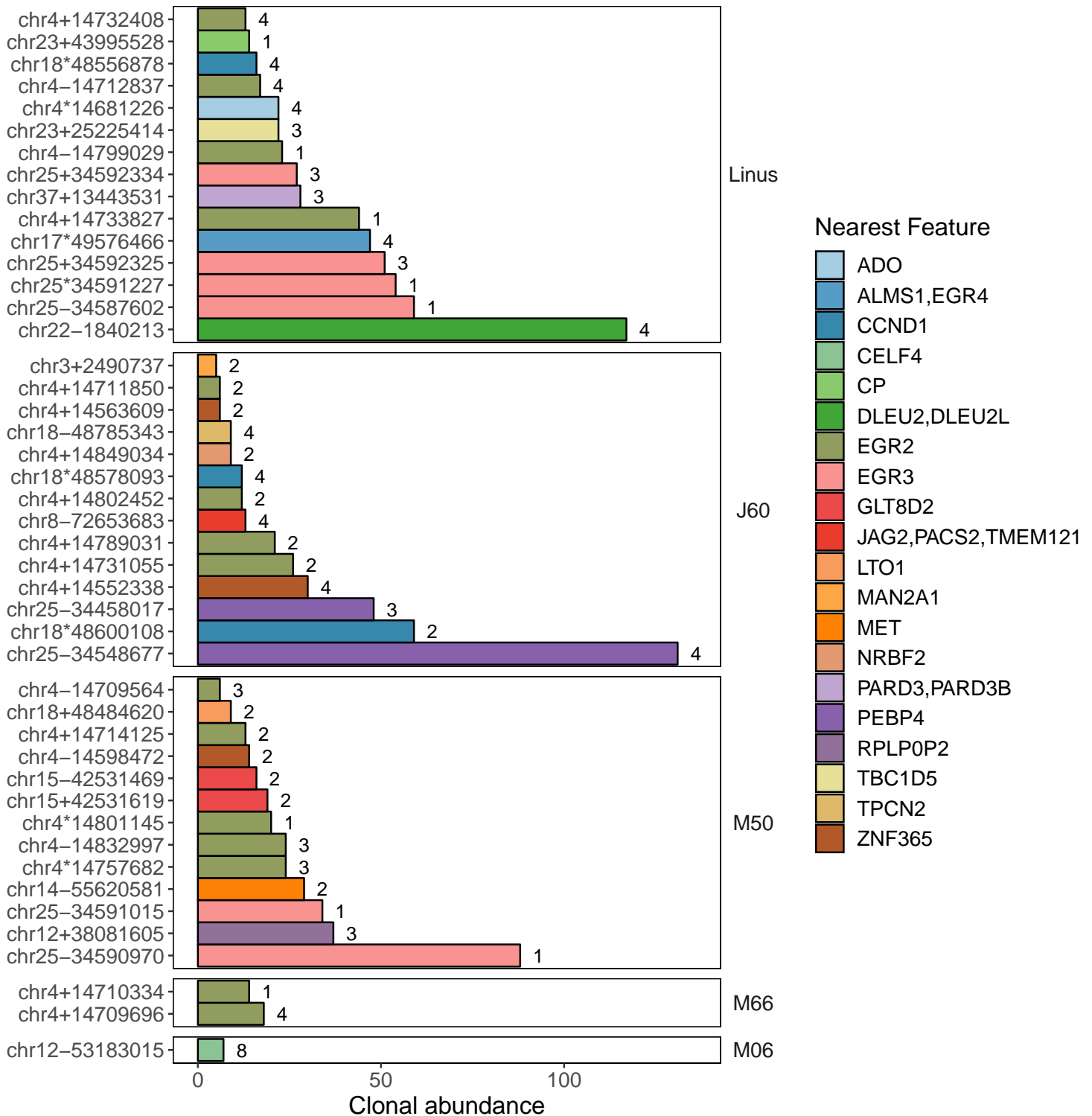
Figure 4. Schematic of incorperated AAV fragments identified vis PCR with flanking genomic primers and Nextera sequencing.
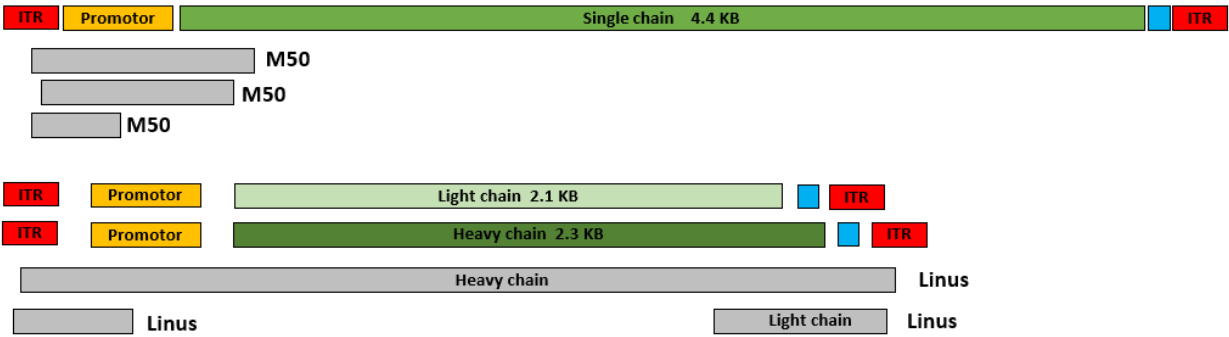
Figure 5. Comparisons of 1 million randomly drawn sites to experimental sites (sites within Factor VIII excluded). A. Distribution of percentages of randomly drawn sites found within transcription units where the percentage of experimental sites is shown with a blue dot (p-value: 0). B. Percentage of randomly drawn sites found within oncogenes where the experimental value bin is highlighted blue (p-value: 0.00315).

A.

B.
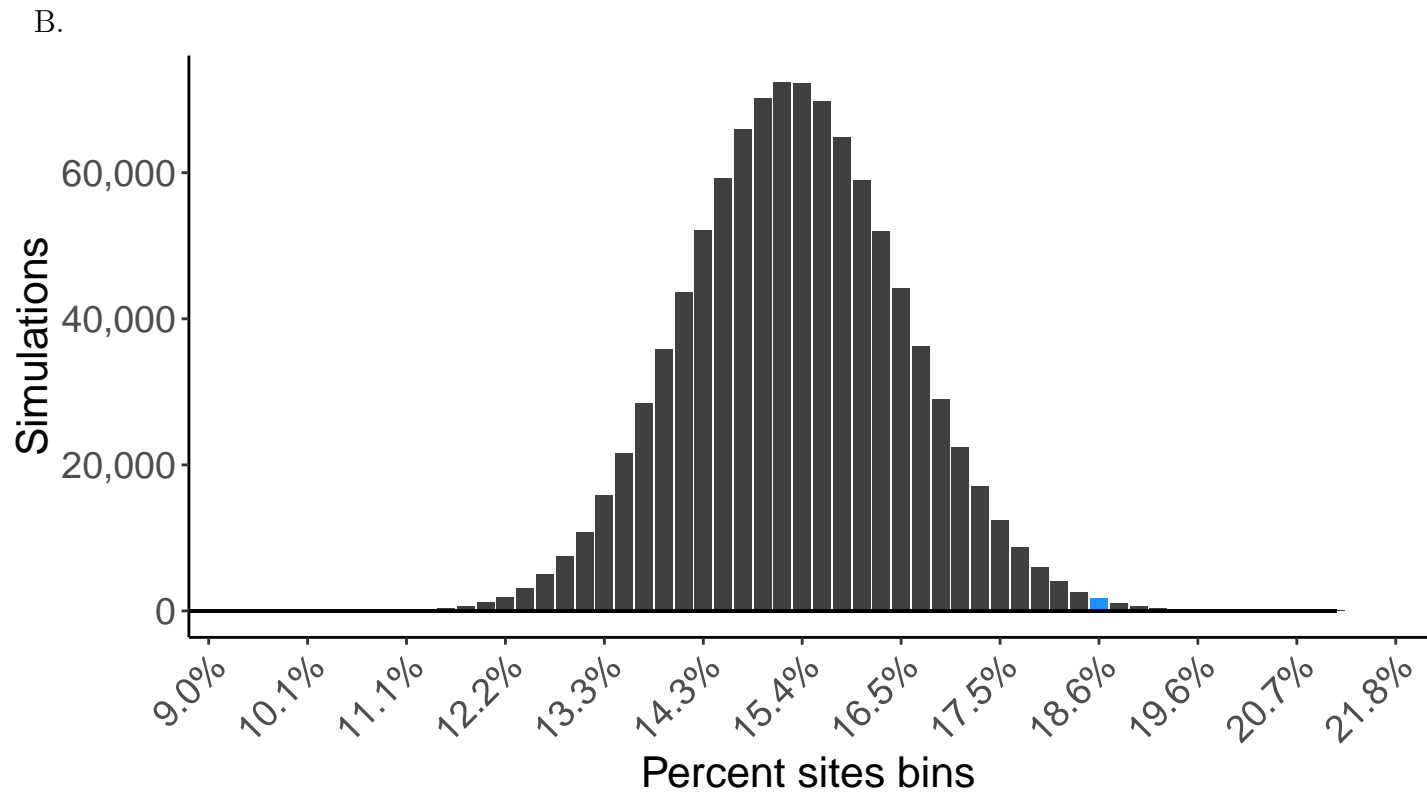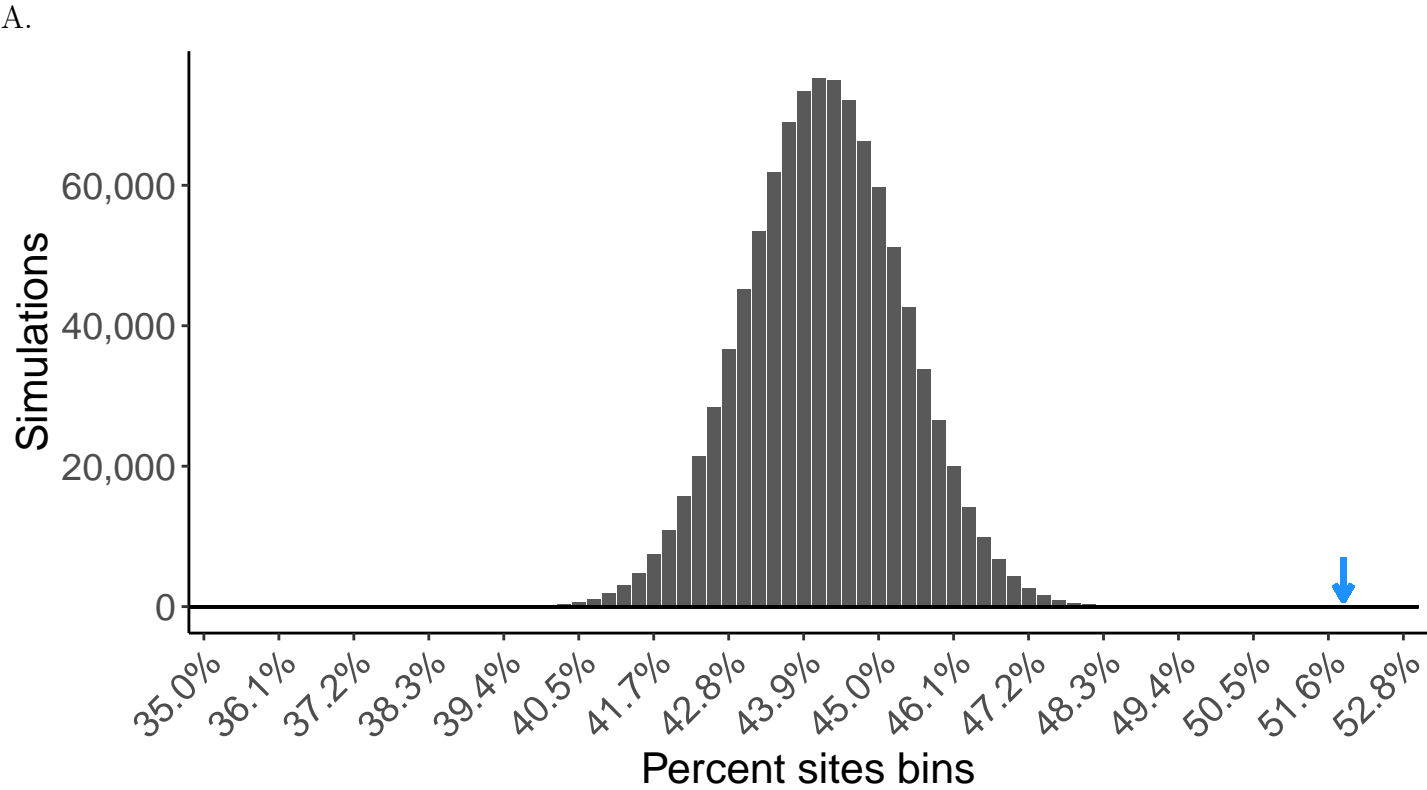
Figure 6. Correlation between VCN and number of sites recovered. Sites within Factor VIII were included because VCN and sample masses are whole sample parameters (R^2: 0.683, p-value: 2.39e-05).
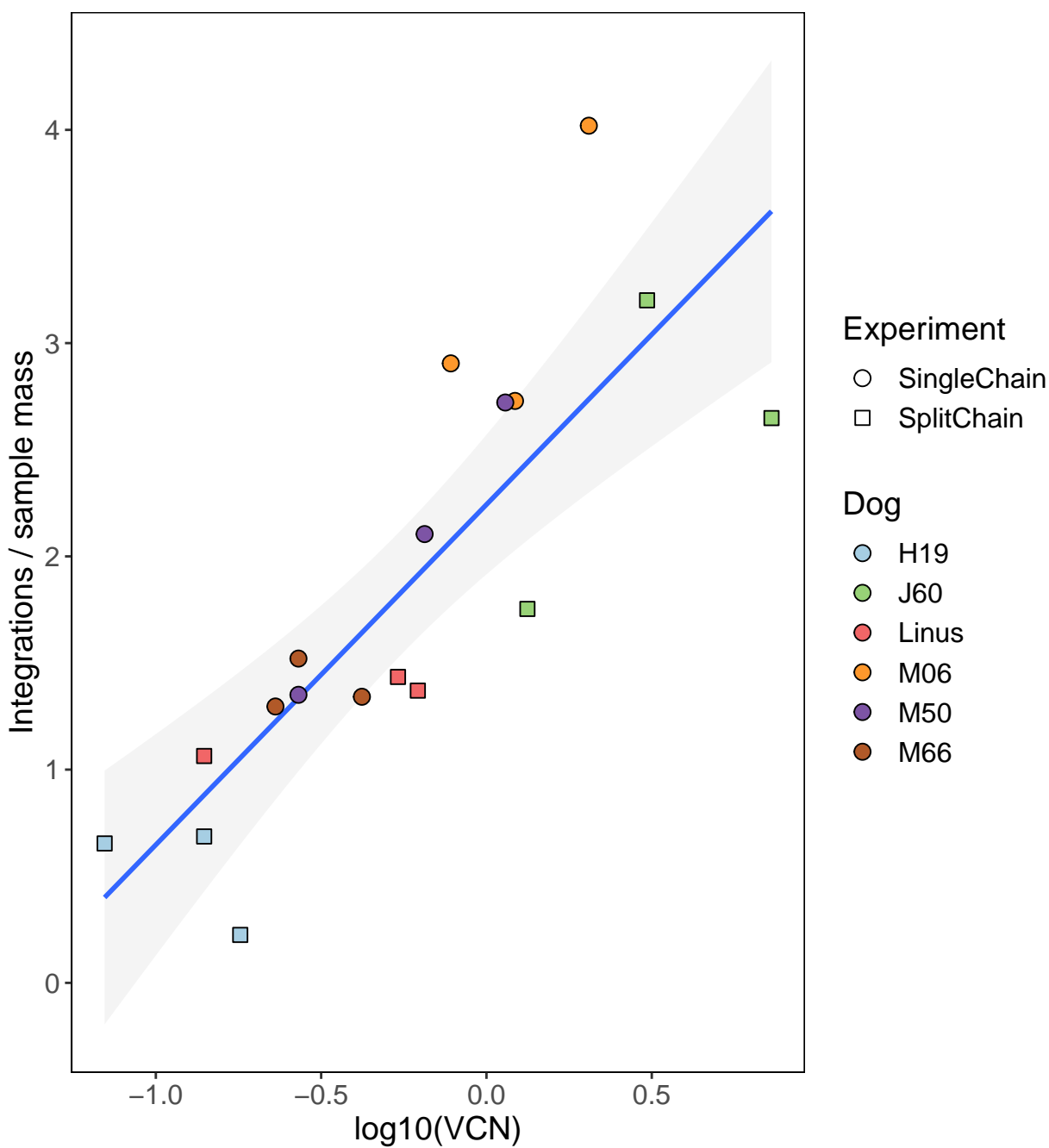
Figure 7. Incorperations within Factor VIII. All incorperations (data points are randomly jittered vertically for clarity, colored by dog with different shapes denoting forward vs. reverse alignments to the genome, tick marks placed every 50NT) are within exons. Sequenced genomic fragments do not extend into Factor VIII introns. This strongly suggests that these detections are from multiply self-incorporated AAVs which may or may not be incorporated into the host genome. Exon 14 shows no incorperations within its center because this region of the exon was exluded from the transgene.
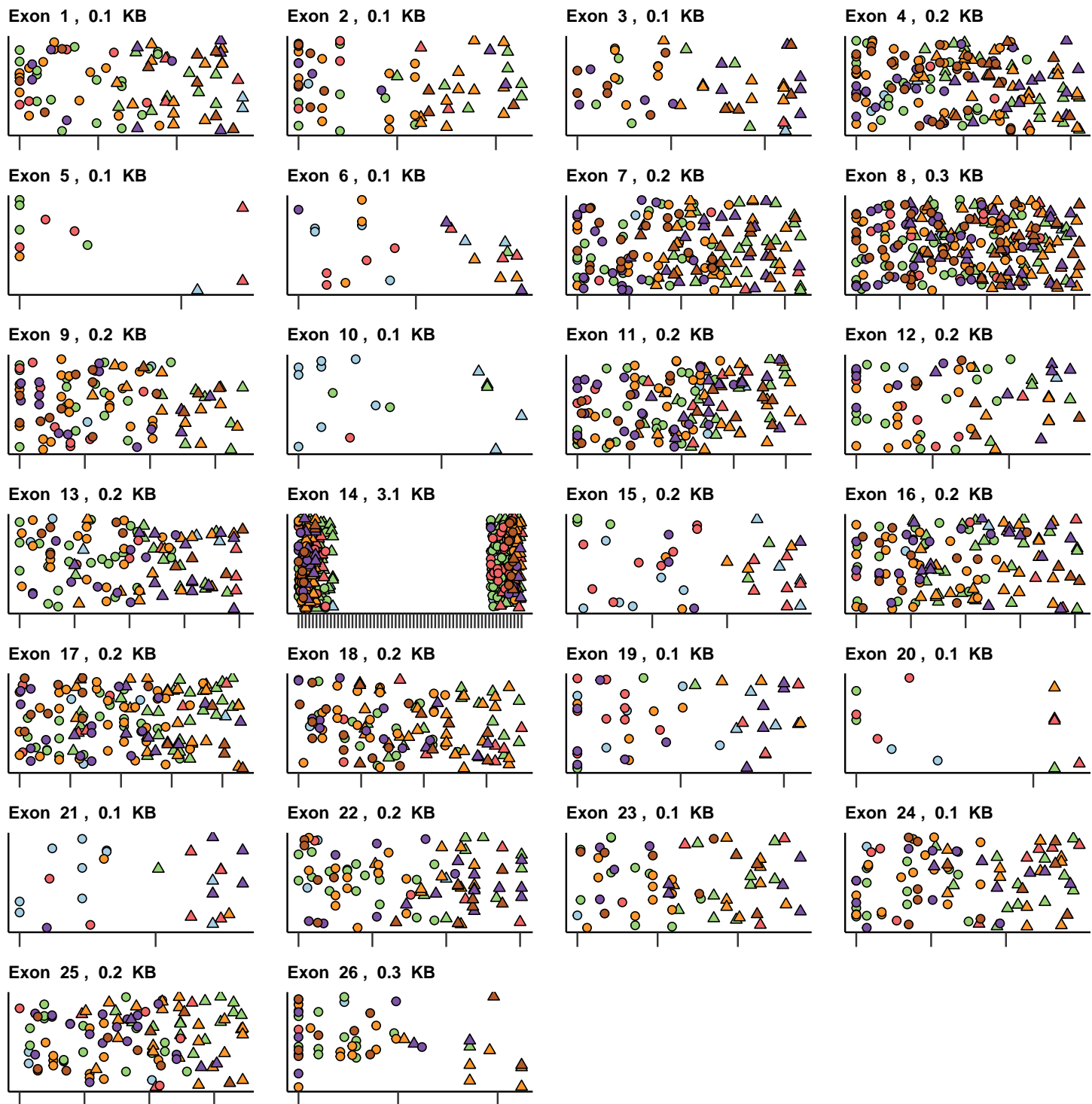
Figure 8. Genomic heatmap of incorporations exluding F8 sites.

(This analysis differs from the random draw test in two ways: the heat map uses the sparsely annotated canFam3 genome annotations rather than the canFam3-hg38 xenoref annotations and random positions are not checked to see if they are 'N'.)
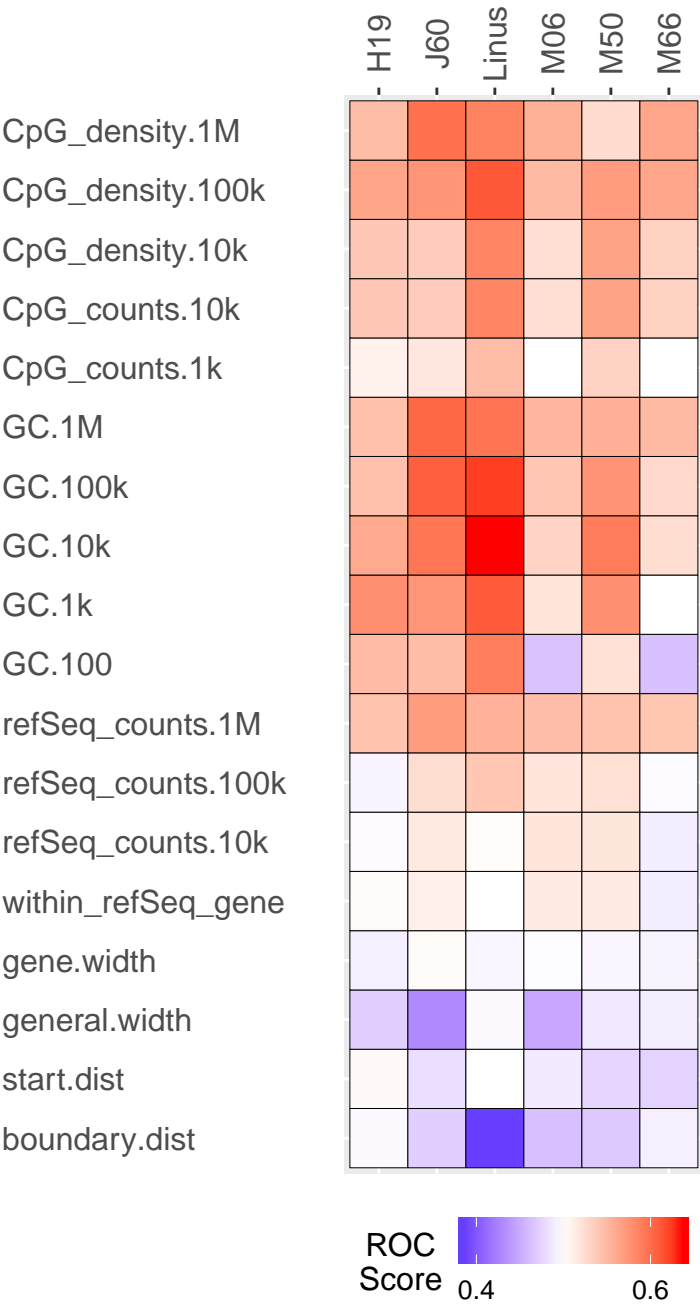
Figure 9. Visulization of incorperation locations.
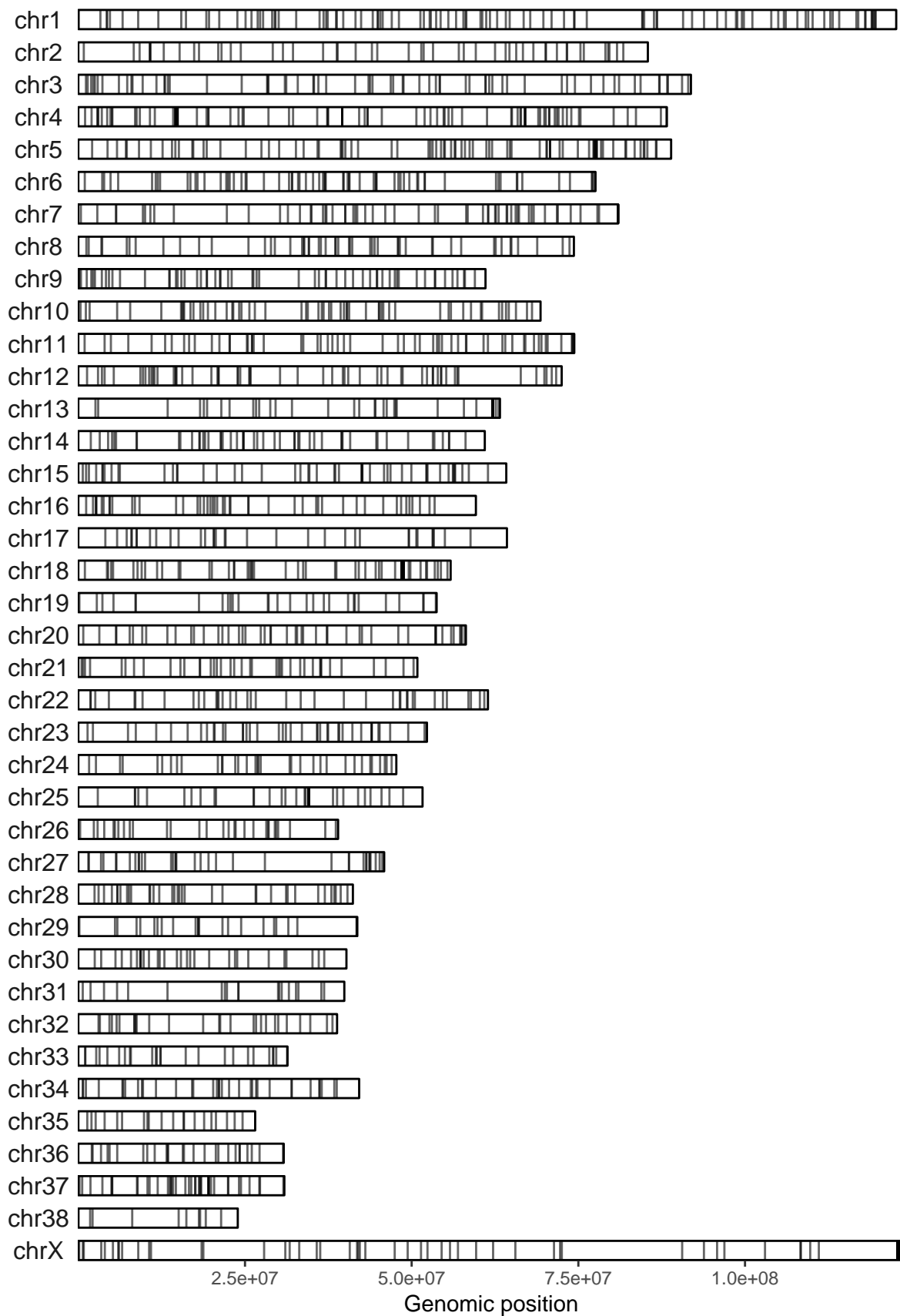
Incorperations within AAV

Incorperations within AAV itself have the tell-tale alignment structure: *expected ITR remnant, additional vector NTs which do not align to the genome followed by NTs which do align to the genome* (Figure 9).

Figure 10. Example of an incorporation within AAV within the poly-A signal sequence.
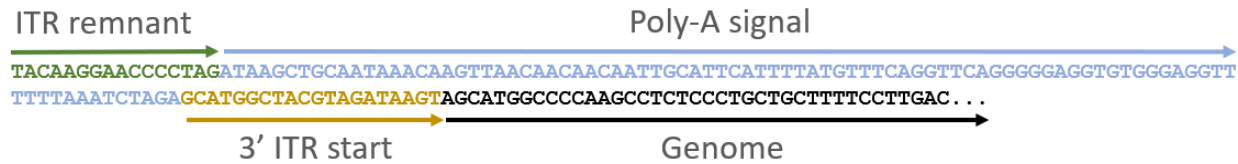
The large majority of incorporations have little to no additional NTs between ITR sequence remnants and genomic sequences (Figure 10) though there are a notable number of incorporations which do possess a number of additional NTs.

Figure 11. Instances of additional NTs between ITR sequences and genomic sequences.
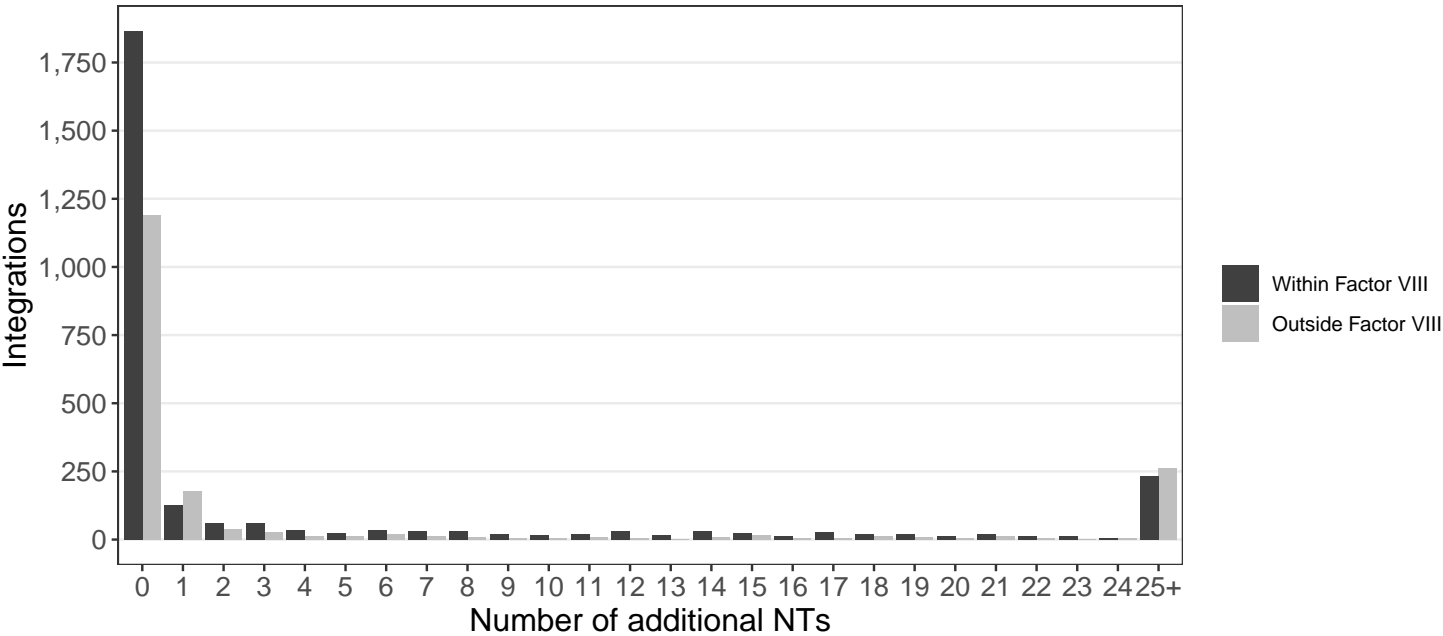
These additional NT sequences can be clustered and the top 15 cluster representative sequences are shown in Tables 1 and 2. Incorporations which map to Factor VIII are interesting in that the additional NTs are often Factor VIII exon boundaries. These NTs are called as additional NTs because sequence aligners expect intron sequences which are not present in the vector transgene. Occasionally a common genome locus is the source of additional NTs for distant incorporation events (Figure 11).

Figure 11. Example of additional NTs where the additional NTs are from a common genome locus yet map to distant parts of the genome.

ITR remnant   chr25:34548608...   Genome
AACCCCTAGTG...TCTGCGCGGCCTGGGGCTACCTCGTGAGGAAGAAGGAAGGAGTAAAGGAAGGATGGAAGGAAGG**AAGGGTCCCTGGGTGGCGC**...
AACCCCTAGTG...TCTGCGCGGCCTGGGGCTACCTCGTGAGGAAGAAGGAAGGAGTAAAGGAAGAATGGAAGG**CCAACCTCTCTGGTTCCCACCAC**...
AACCCCTAGTG...TCTGCGCGGCCTGGGGCTACCTCGTGAGGAAGAAGGAAGGAGTAAAGGAAGGATGGAAGGAAGGAAGGTGGGTGGGA**TAACCC**...

Table 1. Common additional NT sequences associated with incorporation *within Factor VIII*.

n Sites	Rep. cluster seq	Desc.
6	GGCCTCAGTGAGCGA... +10	Region of ITR before first dumbbell tip
4	AGCTTATAATGGTTA... +45	Vector poly-A signal
4	CTCCCTCTCTGCGCG... +11	End of promotor, start of F8
4	ATATCCCTTCCCATCAACAT	Region of ITR before first dumbbell tip
3	ATAGGCACCTATTGG... +74	F8
3	GTAAATATAAAATTT... +65	F8
3	ACTGCATTCTAGTTG... +46	Vector (between promotor and F8)
3	CCTCAGTGAGCGAGC... +14	Vector poly-A signal
3	CTTTTAGGTTTAAAC... +6	Region of ITR after second dumbbell tip
3	GCACCCTGTTTCTGG... +2	F8
3	GAGTCCCGGTTTCCCTGGAAG	F8
3	AGGTTCTCCCAGAATCCACCCGTG	F8
3	GCCACTCTTTTGGGTTATTTG	F8
3	ATTTCCACAGTCTCAAAAAC	F8
3	ACCCCAGAACCCACAG	F8

Table 2. Common additional NT sequences associated with incorporation *outside Factor VIII*.

n Sites	Rep. cluster seq	Desc.
15	CAAGGTCAGCAGGCA... +69	Internal edge of 5' ITR + promotor
9	TGCATGGCTACGTAGATAAGT	Internal edge of 5' ITR
6	CTGGACTTTTGACTG... +41	HC/LC vector between 5' ITR and promotor
6	AACCTCTACAAATGT... +37	HC/LC - end of vector poly-A signal + start of 3' ITR
6	ACTCCCTCTCTGCGC... +17	Full - end of vector poly-A signal + start of 3' ITR
5	ATAAGCTGCAATAAA... +86	Region of ITR before first dumbbell tip
5	TCAGTGAGCGAGCGA... +29	Internal edge of ITRs
5	TAGAGCATGGCTACGTAGATAAGT	Unknown
5	GCAGTCTTAATTGCGATCGTGT	Region of ITR before first dumbbell tip
4	GGCCTCAGTGAGCGA... +15	Region of ITR before first dumbbell tip
4	CCCATTTTAGGGGTC... +14	Internal edge of 5' ITR + promotor
3	GCCTGGGGCTACCTC... +31	chr25:34548608-34548676
3	CTTTGGCCACTCCCT... +17	Region of ITR before first dumbbell tip
3	GAGAGGCAGAGACAT... +10	Region of ITR before first dumbbell tip
3	TAGAGCATGGCTACGTAGATAA	chrX:72159400-72159435

Table 3. 203 incorporations with two or more unqiue clones identified. A total of 1,801 unique incorporations excluding incorperations within Factor VIII were identified. Positions where alignment strands are denoted with an asterisk, i.e., chr25*34591227, denote dually detected incorporations. The highest level of validation for each incorporation is provided:

Short PCR: presence of an appropriate size PCR product when amplifying from the internal edge of an ITR to flanking genomic DNA. **Sanger**: successful sequencing and sequence conformation of the short PCR test product. **Long PCR**: presence of a considerably larger PCR product then expected when amplifying a region of the genome with PCR primers flanking a suspected incorporation site. **Nextera**: successful sequencing and sequence conformation of the long PCR test product.

Dog	Timepoint	Position	Abundance	ITR Remnant length	Nearest Gene	Distance	Nearest oncogene	Distance	Validated
J60	y9.5	chr25-34548677	131	39	PEBP4	0	EGR3	-42611	Sanger/Long PCR
Linus	y10	chr22-1840213	117	16	DLEU2,DLEU2L	0	DLEU2	0	Nextera
M50	y7	chr25-34590970	88	59	EGR3	-318	EGR3	-318	Nextera
J60	y9.5	chr18*48600108	59	63	CCND1	-90384	CCND1	-90384	Sanger/Long PCR
Linus	y10	chr25-34587602	59	58	EGR3	-3686	EGR3	-3686	Nextera
Linus	y10	chr25*34591227	54	43	EGR3	-100	EGR3	-100	NA
Linus	y10	chr25+34592325	51	56	EGR3	0	EGR3	0	NA
J60	y9.5	chr25-34458017	48	64	PEBP4	0	RHOBTB2	-128882	Sanger/Long PCR
Linus	y10	chr17*49576466	47	76	ALMS1,EGR4	0	LOXL3	965375	NA
Linus	y10	chr4+14733827	44	37	EGR2	-21855	RHOBTB1	-1645118	Sanger
M50	y7	chr12+38081605	37	44	RPLP0P2	674	PHIP	1816387	Short PCR
M50	y7	chr25-34591015	34	127	EGR3	-273	EGR3	-273	NA
J60	y9.5	chr4+14552338	30	67	ZNF365	0	RHOBTB1	-1463629	Sanger/Long PCR
M50	y7	chr14-55620581	29	62	MET	-5864	MET	-5864	Sanger
Linus	y10	chr37+13443531	28	41	PARD3,PARD3B	0	RAPH1	-1097001	Nextera
Linus	y10	chr25+34592334	27	65	EGR3	0	EGR3	0	NA
J60	y9.5	chr4+14731055	26	72	EGR2	-19083	RHOBTB1	-1642346	NA
M50	y7	chr4*14757682	24	127	EGR2	-45706	RHOBTB1	-1668969	Nextera
M50	y7	chr4-14832997	24	67	EGR2	-121025	RHOBTB1	-1744288	Sanger
Linus	y10	chr4-14799029	23	53	EGR2	-87057	RHOBTB1	-1710320	NA
Linus	y10	chr23+25225414	22	64	TBC1D5	0	SATB1	530445	NA
Linus	y10	chr4*14681226	22	10	ADO	-17015	RHOBTB1	-1592540	Failed
J60	y9.5	chr4+14789031	21	61	EGR2	-77059	RHOBTB1	-1700322	NA
M50	y7	chr4*14801145	20	51	EGR2	-89160	RHOBTB1	-1712423	Pending
M50	y7	chr15+42531619	19	44	GLT8D2	0	HSP90B1	57500	NA
M66	y6	chr4+14709696	18	61	EGR2	0	RHOBTB1	-1620987	Long PCR
Linus	y10	chr4-14712837	17	80	EGR2	-865	RHOBTB1	-1624128	NA
Linus	y10	chr18+48556878	16	89	CCND1	-47154	CCND1	-47154	Pending
M50	y7	chr15-42531469	16	66	GLT8D2	0	HSP90B1	57350	Nextera
Linus	y10	chr23+43995528	14	70	CP	0	HLTF	-76924	NA
M50	y7	chr4-14598472	14	61	ZNF365	13629	RHOBTB1	-1509763	NA
M66	y6	chr4+14710334	14	64	EGR2	0	RHOBTB1	-1621625	NA
J60	y9.5	chr8-72653683	13	58	JAG2,PACS2...	0	JAG2	0	NA
Linus	y10	chr4+14732408	13	70	EGR2	-20436	RHOBTB1	-1643699	NA
M50	y7	chr4+14714125	13	64	EGR2	-2153	RHOBTB1	-1625416	NA
J60	y9.5	chr4+14802452	12	73	EGR2	-90480	RHOBTB1	-1713743	NA
J60	y9.5	chr18*48578093	12	70	CCND1	-68377	CCND1	-68377	Failed
Linus	y10	chr4+14759642	12	36	EGR2	-47670	RHOBTB1	-1670933	NA
Linus	y10	chr18+48493167	11	33	LTO1	3195	CCND1	5806	Long PCR
Linus	y10	chr5-78404628	11	61	ZFHX3	46069	ZFHX3	46069	NA
Linus	y10	chr25+34591807	10	37	EGR3	0	EGR3	0	NA
Linus	y10	chr21-39487582	10	45	PLEKHA7	0	MYOD1	-770244	NA
J60	y9.5	chr4+14849034	9	63	NRBF2	-109158	RHOBTB1	-1760325	NA
J60	y9.5	chr18-48785343	9	64	TPCN2	167424	CCND1	-275635	NA
Linus	y10	chr18-48501832	9	54	CCND1	0	CCND1	0	NA
M50	y7	chr18+48484620	9	21	LTO1	0	CCND1	14353	NA
Linus	y10	chr25+34591962	8	42	EGR3	0	EGR3	0	NA
Linus	y10	chr18-48760360	7	66	TPCN2	192407	CCND1	-250652	NA
M06	y2	chr12-53183015	7	62	CELF4	0	EPHA7	-877840	NA
J60	y9.5	chr4+14563609	6	47	ZNF365	0	RHOBTB1	-1474900	NA
J60	y9.5	chr4+14711850	6	65	EGR2	0	RHOBTB1	-1623141	NA
M50	y7	chr4-14709564	6	31	EGR2	0	RHOBTB1	-1620855	NA
J60	y9.5	chr3+2490737	5	72	MAN2A1	-13287	APC	-2110233	NA
Linus	y10	chr18+48493174	5	33	LTO1	3202	CCND1	5799	NA
H19	y8.5	chr32-4667479	4	71	CFAP299	0	FGF5	134689	NA

Dog	Timepoint	Position	Abundance	ITR Remnant length	Nearest Gene	Distance	Nearest oncogene	Distance	Validated
Linus	y10	chr18+48501970	4	71	CCND1	0	CCND1	0	NA
Linus	y10	chr13-21450894	4	41	DERL1	0	RNF139	-1108984	NA
Linus	y10	chr4-14709639	4	43	EGR2	0	RHOBTB1	-1620930	NA
M50	y7	chr7+67593155	4	58	YES1	0	YES1	0	NA
M50	y7	chr18-48543799	4	47	CCND1	-34091	CCND1	-34091	NA
M50	y7	chr18-48623683	4	36	CCND1	-113975	CCND1	-113975	NA
J60	y9.5	chr18+48736726	3	64	TPCN2	216041	CCND1	-227018	NA
J60	y9.5	chr11*26044659	3	64	EGR1	-1288	EGR1	-1288	NA
J60	y9.5	chr15+6230997	3	50	AGO3	0	THRAP3	-208604	NA
J60	y9.5	chr18-48573130	3	16	CCND1	-63422	CCND1	-63422	NA
J60	y9.5	chr18+48500622	3	71	CCND1	0	CCND1	0	NA
J60	y9.5	chr2*32199118	3	69	PITRM1	0	KLF6	535042	NA
J60	y9.5	chr4-14789791	3	54	EGR2	-77819	RHOBTB1	-1701082	NA
J60	y9.5	chr25-34548671	3	39	PEBP4	0	EGR3	-42617	NA
Linus	y10	chr18-48645201	3	16	CCND1	-135493	CCND1	-135493	NA
Linus	y10	chr4-14785464	3	26	EGR2	-73492	RHOBTB1	-1696755	NA
Linus	y10	chr4-14713726	3	65	EGR2	-1754	RHOBTB1	-1625017	NA
Linus	y10	chr18+48584297	3	52	CCND1	-74589	CCND1	-74589	NA
Linus	y10	chr18*48722018	3	67	CCND1	-212308	CCND1	-212308	NA
Linus	y10	chr18+48584456	3	69	CCND1	-74748	CCND1	-74748	NA
Linus	y10	chr25-30507351	3	32	STMN4	155046	CLU	506629	NA
Linus	y10	chr4+14782170	3	55	EGR2	-70198	RHOBTB1	-1693461	NA
M50	y7	chr21-6506179	3	64	MRE11	0	MRE11	0	NA
M50	y7	chr25-34590999	3	40	EGR3	-289	EGR3	-289	NA
M50	y7	chr2-66688859	3	70	SIAH1	-16203	ZNF423	908202	NA
M50	y7	chr18-48513636	3	41	CCND1	-3928	CCND1	-3928	NA
M50	y7	chr4-14788834	3	71	EGR2	-76862	RHOBTB1	-1700125	NA
M66	y6	chr4*14722170	3	22	EGR2	-10148	RHOBTB1	-1633411	NA
H19	y8.5	chr1-45651422	2	62	LOC105378068	398922	ARID1B	-719139	NA
H19	y8.5	chr10-45097655	2	35	FAM178B	0	CNNM4	59781	NA
H19	y8.5	chr15+39094941	2	74	ANKS1B	0	APAF1	977914	NA
H19	y8.5	chr30-23830049	2	21	MINDY2	2558	GCNT3	-654693	NA
H19	y8.5	chr33+11774631	2	67	CBLB	-302183	CBLB	-302183	NA
H19	y8.5	chr36-24205150	2	63	UBE2E3,UBE2E4P	0	PDE11A	-2475504	NA
H19	y8.5	chr9-36088961	2	28	C17orf64	-6674	USP32	-79132	NA
H19	y8.5	chr1+116987164	2	46	RBM42	-1439	FXYD5	425710	NA
H19	y8.5	chr10+1129800	2	76	SDR9C7	-9069	NAB2	-96754	NA
H19	y8.5	chr2+73264837	2	65	GPN2	3291	ZDHHC18	9346	NA
H19	y8.5	chr34+21096771	2	50	LPP	0	LPP	0	NA
H19	y8.5	chr5+61633275	2	73	ERRFI1	0	ERRFI1	0	NA
H19	y8.5	chr7-40039717	2	13	NVL	-2038	TP53BP2	-256559	NA
H19	y8.5	chr7+61474770	2	69	CHST9	-96960	SS18	-1016075	NA
H19	y8.5	chr8-7267761	2	68	AK3	-161625	FOXG1	-1010286	NA
H19	y8.5	chr9+4143400	2	49	ST6GALNAC2	-14478	ST6GALNAC1	19437	NA
H19	y8.5	chr1+38773155	2	38	SAMD5	203006	LATS1	1656972	NA
J60	y9.5	chr1-86823525	2	66	TRPM3	0	HMGN2P46	-373233	NA
J60	y9.5	chr10-331748	2	69	SUOX	0	ERBB3	-8201	NA
J60	y9.5	chr18+48736654	2	41	TPCN2	216113	CCND1	-226946	NA
J60	y9.5	chr29+21622936	2	67	LDHA	-4909	NCOA2	-2174534	NA
J60	y9.5	chr34-38763204	2	27	NAALADL2	0	ECT2	1803894	NA
J60	y9.5	chr36-9749057	2	61	GRB14	0	LRP2	4161391	NA
J60	y9.5	chr4*14701825	2	76	ADO	0	RHOBTB1	-1613112	NA
J60	y9.5	chr4-14924592	2	62	NRBF2	-33600	RHOBTB1	-1835883	NA
J60	y9.5	chr4+14715301	2	62	EGR2	-3329	RHOBTB1	-1626592	NA
J60	y9.5	chr13+62168428	2	61	ALB	0	RASSF6	130134	NA
J60	y9.5	chr18-44600867	2	58	CD82	57628	CD82	57628	NA
J60	y9.5	chr18-48821243	2	75	TPCN2	131524	MAP2K1	258795	NA
J60	y9.5	chr4*14641819	2	65	ADO	-56469	RHOBTB1	-1553086	NA
J60	y9.5	chr4-14713052	2	59	EGR2	-1080	RHOBTB1	-1624343	NA
J60	y9.5	chr4-14841073	2	27	NRBF2	-117119	RHOBTB1	-1752364	NA
J60	y9.5	chr4+14805710	2	53	EGR2	-93738	RHOBTB1	-1717001	NA
J60	y9.5	chr8-3461644	2	72	SLC7A8	0	BCL2L2	-107759	NA
Linus	y10	chr10+34424210	2	27	SH3RF3	0	RANBP2	662940	NA
Linus	y10	chr2-20988823	2	63	MINDY3	0	CUBN	947319	NA
Linus	y10	chr2+59618697	2	64	MT3	6153	AMFR	-149364	NA
Linus	y10	chr22+2544527	2	68	FNDC3A	0	RB1	517092	NA
Linus	y10	chr4+14733834	2	37	EGR2	-21862	RHOBTB1	-1645125	NA
Linus	y10	chr12-15583942	2	64	CD2AP	0	RUNX2	1740306	NA
Linus	y10	chr14-15091597	2	65	ZNF804B	0	SRI	-817304	NA
Linus	y10	chr18+48491590	2	54	LTO1	1618	CCND1	7383	NA
Linus	y10	chr25+34592383	2	40	EGR3	0	EGR3	0	NA
Linus	y10	chr1-118074774	2	44	LSM14A	0	FXYD5	-650607	NA
Linus	y10	chr11-26058613	2	61	EGR1	8808	EGR1	8808	NA
Linus	y10	chr16+3513239	2	10	CNTNAP2	0	CUL1	-1460378	NA

Dog	Timepoint	Position	Abundance	ITR Remnant length	Nearest Gene	Distance	Nearest oncogene	Distance	Validated
Linus	y10	chr18*48522347	2	55	CCND1	-12627	CCND1	-12627	NA
Linus	y10	chr18+48771203	2	29	TPCN2	181564	CCND1	-261495	NA
Linus	y10	chr22-1840015	2	38	DLEU2,DLEU2L	0	DLEU2	0	NA
Linus	y10	chr30+2461957	2	59	GJD2	15390	GREM1	-230128	NA
Linus	y10	chr4+14753192	2	75	EGR2	-41220	RHOBTB1	-1664483	NA
M06	y2	chr1-110141910	2	65	ERCC1,GPR68	0	ERCC1,GPR68	0	NA
M06	y2	chr12-25761444	2	61	KHDRBS2	0	DST	-1566251	NA
M06	y2	chr15+35765032	2	73	HAL	1609	ELK3	-185502	NA
M06	y2	chr18-9500676	2	62	SUGCT	0	RALA	521817	NA
M06	y2	chr21-999882	2	75	LYAR	-28458	MAML2	-3823223	NA
M06	y2	chr26-13854842	2	107	NOS1	0	PEBP1	-667262	NA
M06	y2	chr28-15948142	2	61	SH3PXD2A	0	SUFU	832642	NA
M06	y2	chr3+74490062	2	39	RELL1	-3132	KLF3	-826168	NA
M06	y2	chr34+17067867	2	53	DVL3	0	PSMD2	-112224	NA
M06	y2	chr37-18405952	2	83	CPS1	0	ERBB4	623748	NA
M06	y2	chr4-39589745	2	62	DUSP1	0	MIXL1	-165200	NA
M06	y2	chr4-39589762	2	56	DUSP1	0	MIXL1	-165217	NA
M06	y2	chr4+39589807	2	58	DUSP1	0	MIXL1	-165262	NA
M06	y2	chr4+73281440	2	59	PRLR	0	PRLR	0	NA
M06	y2	chr13-18262892	2	69	LOC101927513	17962	TNFRSF11B	-97856	NA
M06	y2	chr14-21392553	2	52	SLC25A13	0	TAC1	-1239456	NA
M06	y2	chr15+24557055	2	63	TMTC2	-72116	PAWR	-2403627	NA
M06	y2	chr17-58827471	2	66	PDZK1P1	0	PIAS3	-166716	NA
M06	y2	chr26+31747628	2	62	NEB	-343531	MAPK1	-614608	NA
M06	y2	chr28+10665763	2	45	RRP12	6319	FRAT1	27302	NA
M06	y2	chr31+29963191	2	74	RCAN1	0	RUNX1	203171	NA
M06	y2	chr4-14746313	2	59	EGR2	-34341	RHOBTB1	-1657604	NA
M06	y2	chr4+39589807	2	61	DUSP1	0	MIXL1	-165262	NA
M06	y2	chr7-33241851	2	26	EXO1	0	FH	-314393	NA
M06	y2	chr1-42417078	2	67	SYNE1	0	SYNE1	0	NA
M06	y2	chr10-60378614	2	25	MIR4432HG	124107	BCL11A	209642	NA
M06	y2	chr14-35388428	2	45	SP4	0	ABCB5	627021	NA
M06	y2	chr15-54263444	2	62	PDGFC	-23558	FBXW7	-3846235	NA
M06	y2	chr15+3625821	2	69	MACF1	0	MACF1	0	NA
M06	y2	chr20+17342340	2	67	AIMP1	-165032	CHL1	-357318	NA
M06	y2	chr23+8622032	2	23	SCN11A	0	XIRP1	227676	NA
M06	y2	chr28-5033380	2	65	KIF20B	362621	RET	-1039184	NA
M06	y2	chr32+10633798	2	59	AFF1	2387	AFF1	2387	NA
M06	y2	chr4+39589807	2	51	DUSP1	0	MIXL1	-165262	NA
M50	y7	chr20-54608016	2	72	PTPRS	0	RFX2	496323	NA
M50	y7	chr22+50166197	2	61	PCCA	0	FGF14	1213603	NA
M50	y7	chr32-29986493	2	69	CFI	-622	EGF	-87256	NA
M50	y7	chr33+25455439	2	50	CASR	21486	CSTA	-22619	NA
M50	y7	chr4-17787487	2	20	CTNNA3	0	SIRT1	-1401120	NA
M50	y7	chr4+14711818	2	70	EGR2	0	RHOBTB1	-1623109	NA
M50	y7	chr6-65773858	2	67	ADGRL2	0	ADGRL2	0	NA
M50	y7	chrX+71350380	2	19	ACTN1	230697	HNRNPK	-1711394	NA
M50	y7	chr14+22874727	2	127	C1GALT1	0	COL28A1	118443	NA
M50	y7	chr25+34601125	2	70	EGR3	3980	EGR3	3980	NA
M50	y7	chr33+29230184	2	44	TFRC	0	TFRC	0	NA
M50	y7	chr38-1772649	2	17	TMCC2	0	RBBP5	-126482	NA
M50	y7	chr5-78694771	2	80	HCCAT5	-27367	ZFHX3	-84743	NA
M50	y7	chr6+17876405	2	65	PRRT2	0	MVP	-9712	NA
M50	y7	chr10-24124521	2	56	XPNPEP3,EP300	0	EP300	0	NA
M50	y7	chr14-1871659	2	25	OR2L5,OR2L3,OR2L8...	179566	WNT3A	-963029	NA
M50	y7	chr2+64665745	2	67	NOD2	0	CYLD	-36930	NA
M50	y7	chr27+40595108	2	23	CCND2-AS1	0	CCND2-AS1	0	NA
M50	y7	chr28+15144698	2	43	ARL3	5147	SUFU	29198	NA
M50	y7	chr34-32001200	2	67	ZBBX	169637	MECOM	1746286	NA
M50	y7	chr4-14713093	2	62	EGR2	-1121	RHOBTB1	-1624384	NA
M50	y7	chr4+39589852	2	38	DUSP1	0	MIXL1	-165307	NA
M50	y7	chr6-5975701	2	73	GTF2IRD1	-11819	LIMK1	287670	NA
M50	y7	chr8-38607187	2	73	SYNE2	0	SYNE2	0	NA
M66	y6	chr10-43140250	2	71	AFF3	25562	AFF3	25562	NA
M66	y6	chr15-56522259	2	67	HOXD-AS2	22810	EEF1A1	2330802	NA
M66	y6	chr16-18834023	2	67	SHH	-170782	SHH	-170782	NA
M66	y6	chr23+43994847	2	61	CP	0	HLTF	-76243	NA
M66	y6	chr3+48777081	2	62	SV2B	0	AKAP13	-214358	NA
M66	y6	chr16+45738385	2	65	ACSL1	-14656	SORBS2	675110	NA
M66	y6	chr37+13896954	2	57	PARD3,PARD3B	0	ADAM23	-1099909	NA
M66	y6	chr37+169848	2	50	WDR75	0	KDM3A	45767	NA
M66	y6	chr5+41957696	2	21	PEMT	0	EWSR1	-182944	NA
M66	y6	chr15-42495602	2	42	TDG	0	HSP90B1	21483	NA
M66	y6	chr25-43821788	2	31	DIS3L2	0	PTMA	253801	NA

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M66	y6	chr28+35981345	2	17	DOCK1	0	ADAM12	-496135	NA
M66	y6	chr8+33570819	2	45	ACTR10	-4588	ARID4A	-90734	NA

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