topoDefault

June 20, 2024

Contents

Introduction

The attached report describes the results of integration site analysis for samples from gene therapy trials. Cellular DNA was processed as described in Sherman et al. 2017 (doi: 10.1016/j.omtm.2016.11.002.) and analyzed using the analysis software AAVengeR (https://github.com/helixscript/AAVengeR).

PI Summary

No Summary Given

Sequencing and Integration Summary Table

Presented below are summary details of each sample. We estimate the number of cell clones sampled using the SonicLength method (Berry, 2012); this is summarized in the column "Inferred cells". Relative abundance was not measured from read counts, which are known to be inaccurate, but from marks introduced into DNA specimens prior to PCR amplification using the SonicLength method PMID:22238265.

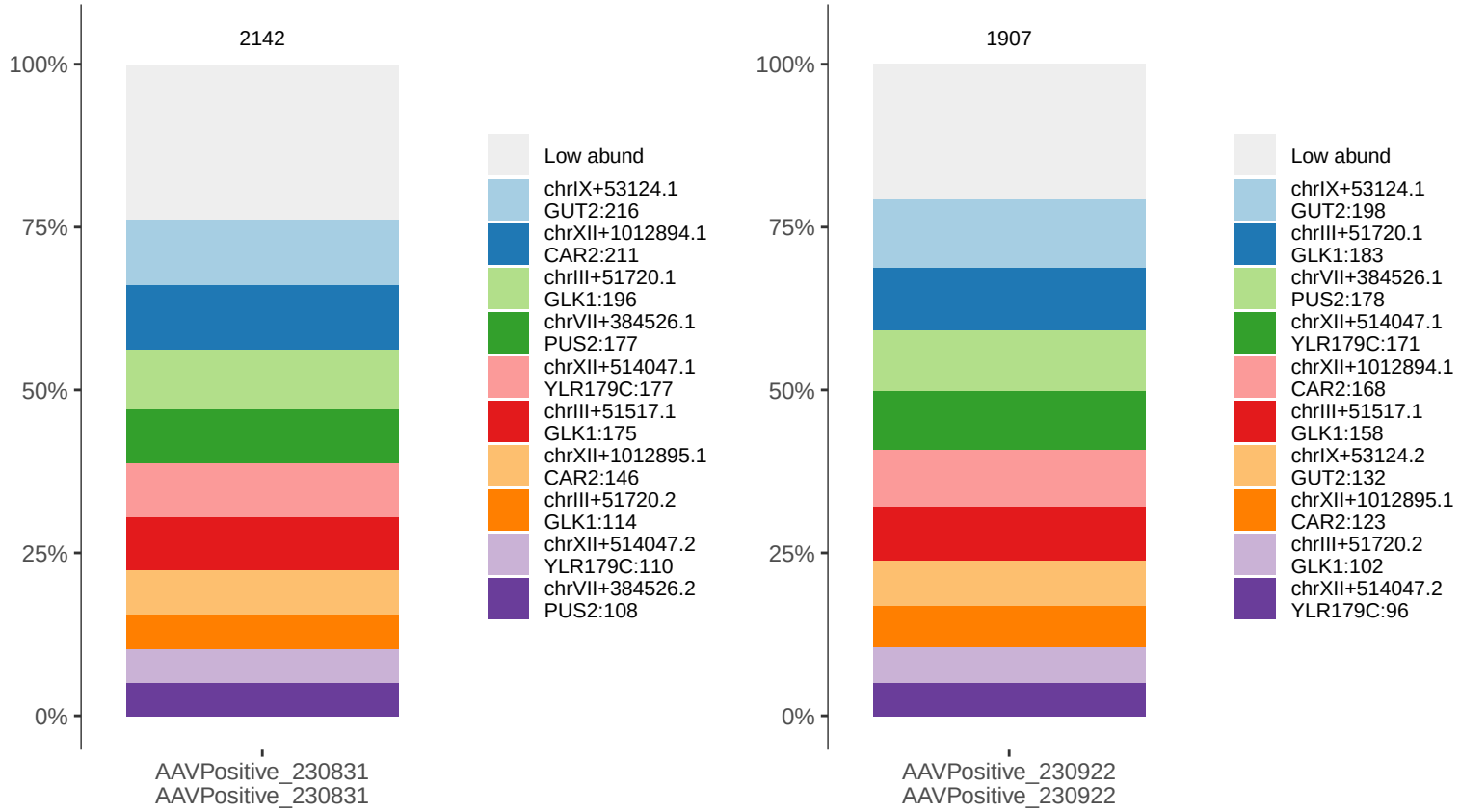
Under most circumstances, only a subset of sites will be sampled. We thus include an estimate of sample size based on the frequency of isolation information from the SonicLength method (Berry, 2012). The 'S.chao1' column denotes the estimated lower bound for population size derived using Chao estimate (Chao, 1987).

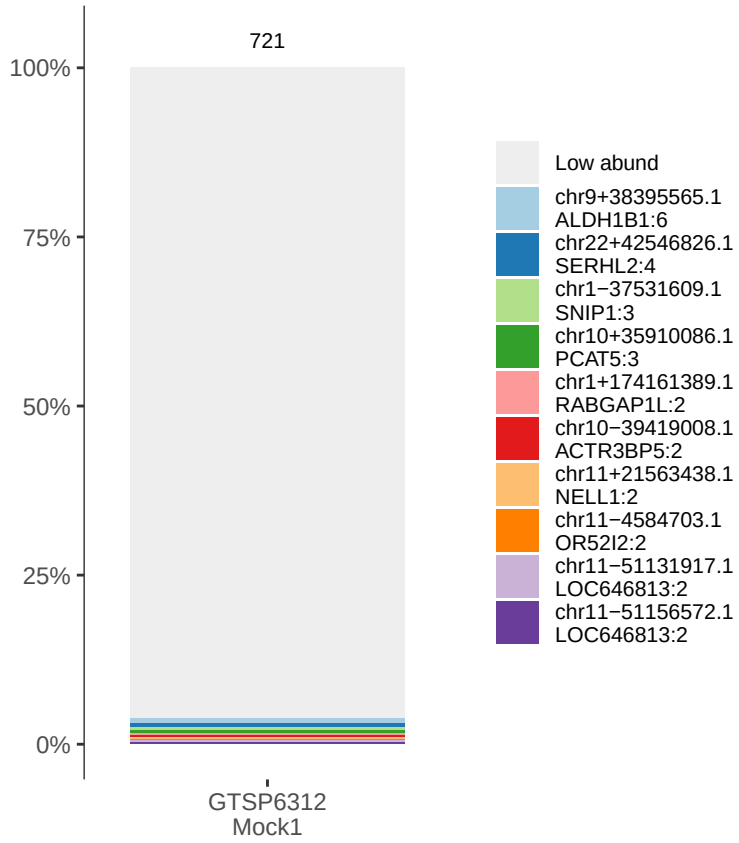
Table 1: integration summary table

sample	patientID	total reads	Unique Sites	inferred cell	Chao1	info
AAVPositive_230831	AAVPositive_230831	319719	29	2142	31.500	AAVPositive_230831
AAVPositive_230922	AAVPositive_230922	208373	31	1907	32.000	AAVPositive_230922
GTSP6312	Mock1	79003	679	721	7378.677	Mock1
GTSP6313	Mock2	138471	794	858	10583.000	Mock2
GTSP6314	Mock3	176922	978	1093	7066.727	Mock3
GTSP6315	Mock4	130581	719	751	8342.484	Mock4
GTSP6316	Camptothecin1	113110	1209	1337	8035.511	Camptothecin1
GTSP6317	Camptothecin2	91920	1018	1108	9335.200	Camptothecin2
GTSP6318	Camptothecin3	164269	1272	1394	9400.235	Camptothecin3
GTSP6319	Camptothecin4	107577	1007	1064	12234.317	Camptothecin4
GTSP6320	Doxorubicin1	155678	2854	3192	18743.834	Doxorubicin1
GTSP6321	Doxorubicin2	146721	2388	2609	21292.008	Doxorubicin2
GTSP6322	Doxorubicin3	133409	2452	2735	15041.444	Doxorubicin3
GTSP6323	Doxorubicin4	126152	2026	2229	14083.750	Doxorubicin4
GTSP6324	Etoposide1	166807	2263	2523	16419.414	Etoposide1
GTSP6325	Etoposide2	128478	2246	2493	16943.103	Etoposide2
GTSP6326	Etoposide3	179336	1945	2162	13148.922	Etoposide3
GTSP6327	Etoposide4	202713	1750	1862	18024.179	Etoposide4

Abundance Frequency Plots

Stacked bar plots indicate clonal abundance frequency. Only the top 10 clonal types are being plotted, and the rest will be ploted in grey as "low abundance". The number above the stacked bar plots indicates the total clonal types. The legend in each plot correspond to the clonal type integration sites. The number after colon indicates the count for that specific clonal type.

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SERHL2:4
chr1−37531609.1
SNIP1:3
chr10+35910086.1
PCAT5:3
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RABGAP1L:2
chr10−39419008.1
ACTR3BP5:2
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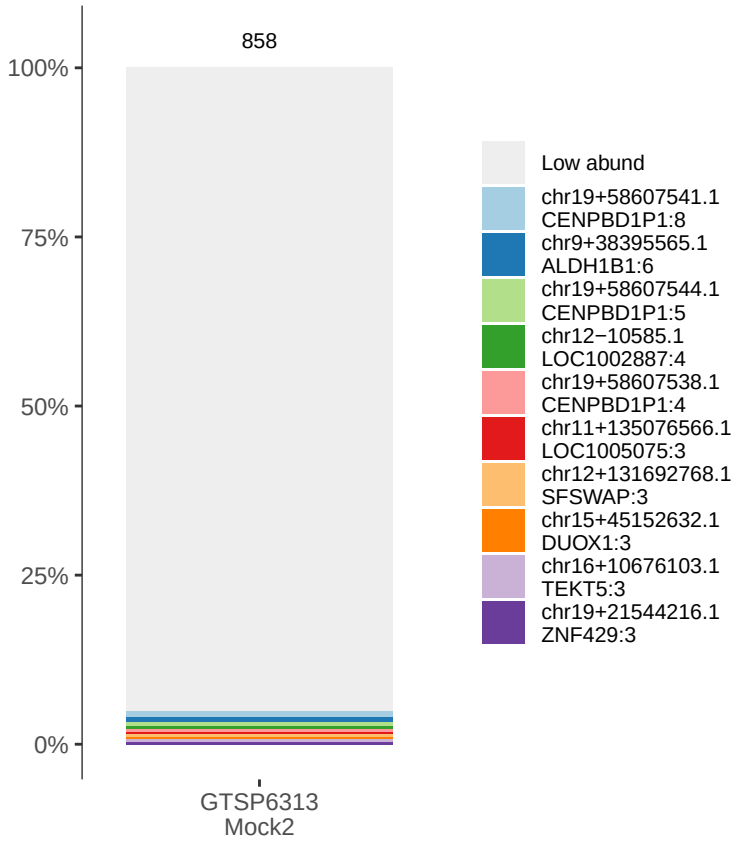
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chr11+135076566.1
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chr12+131692768.1
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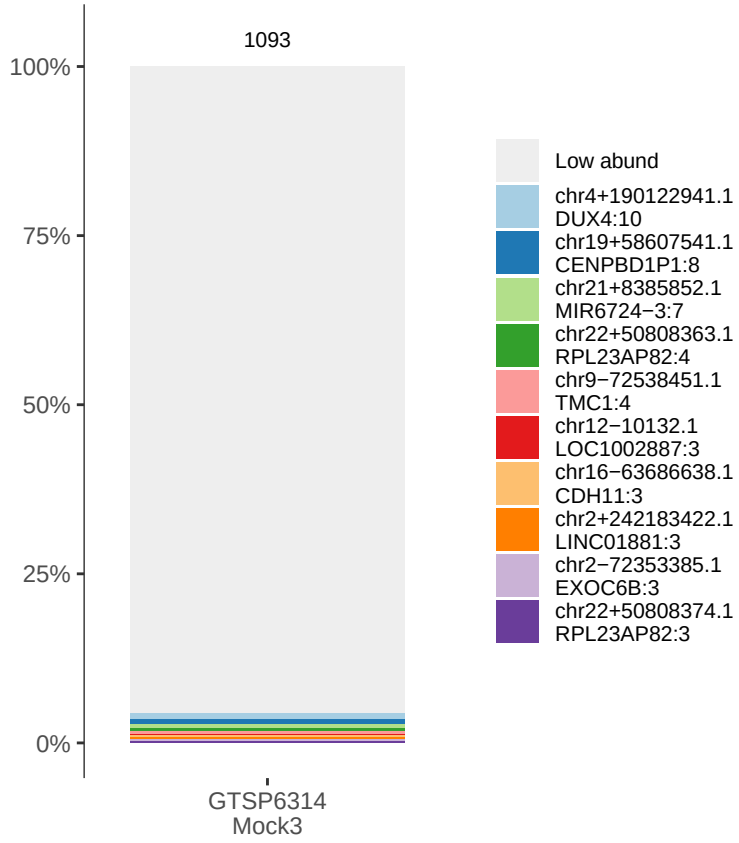
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RPL23AP82:4
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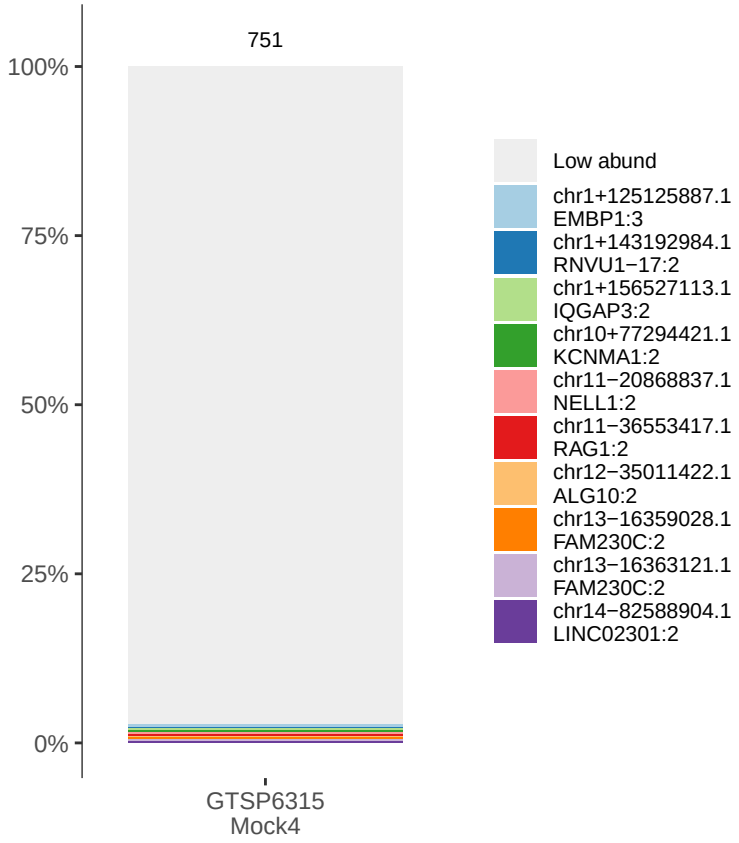
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IQGAP3:2
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chr11−20868837.1
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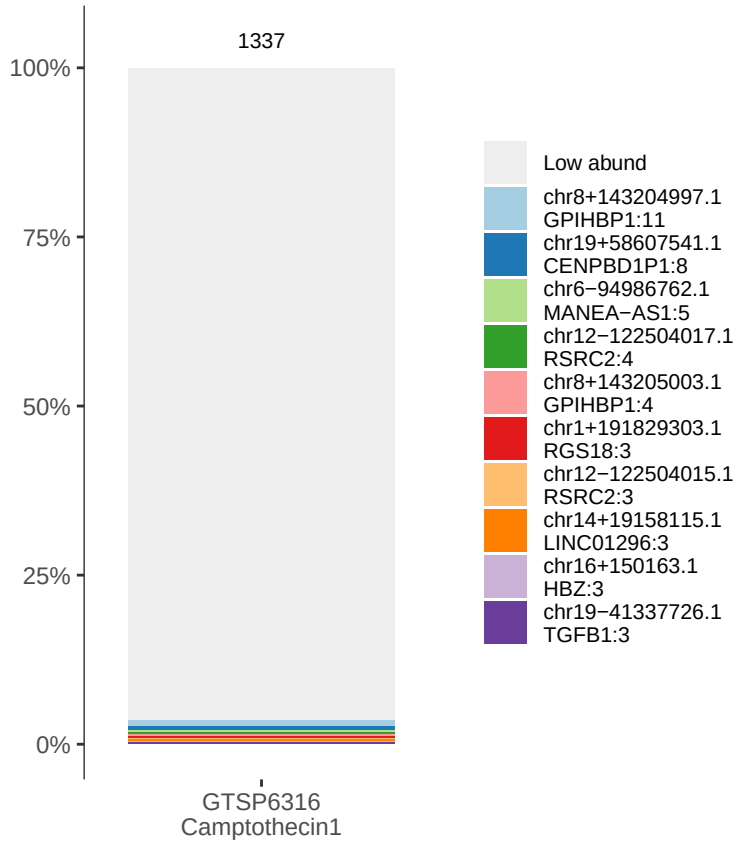
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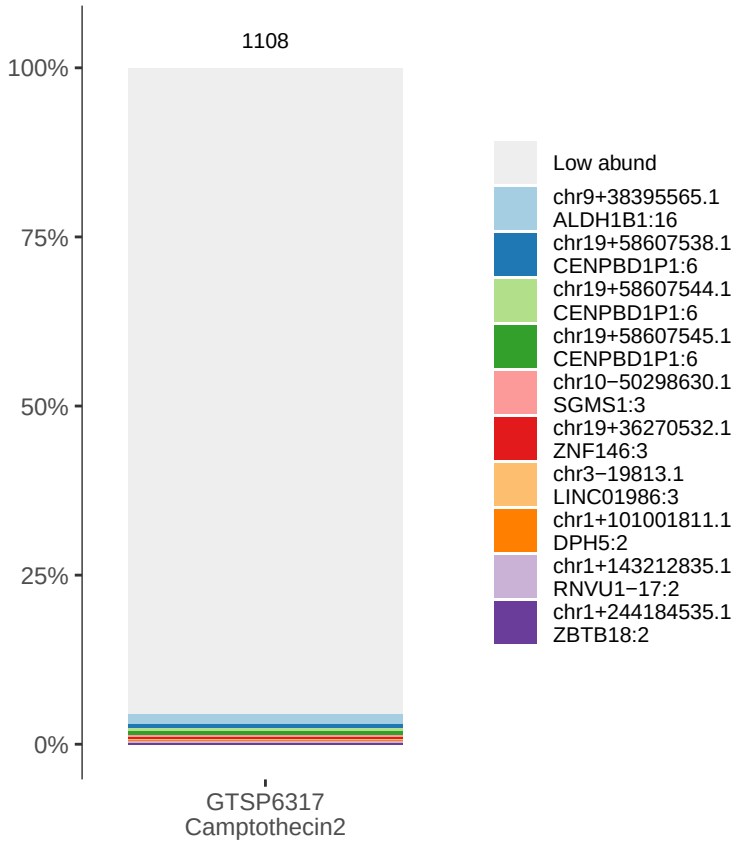
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CENPBD1P1:6
chr10−50298630.1
SGMS1:3
chr19+36270532.1
ZNF146:3
chr3−19813.1
LINC01986:3
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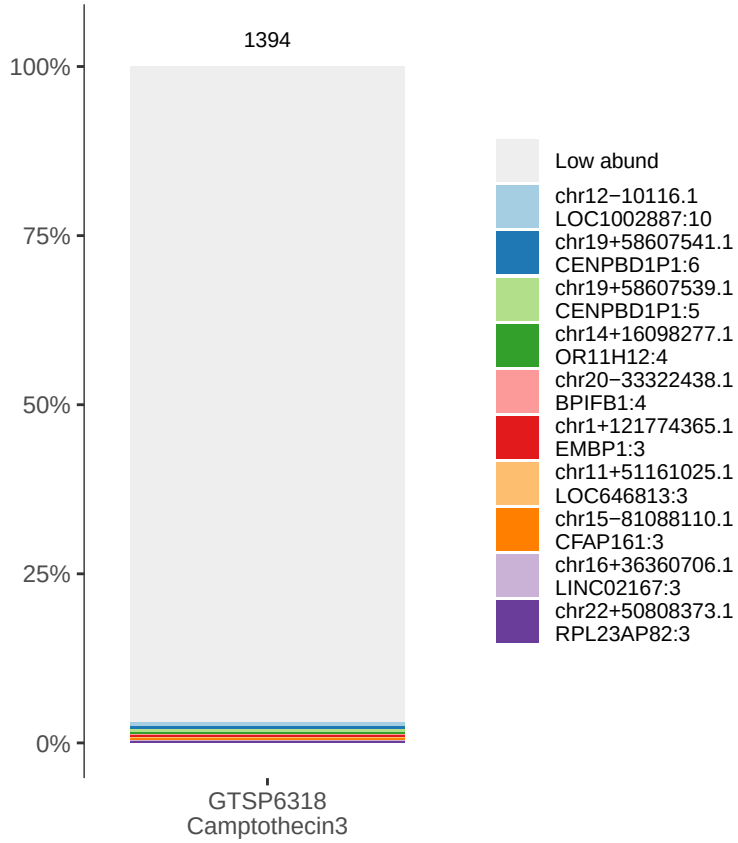
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CENPBD1P1:6
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chr14+16098277.1
OR11H12:4
chr20−33322438.1
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chr1+121774365.1
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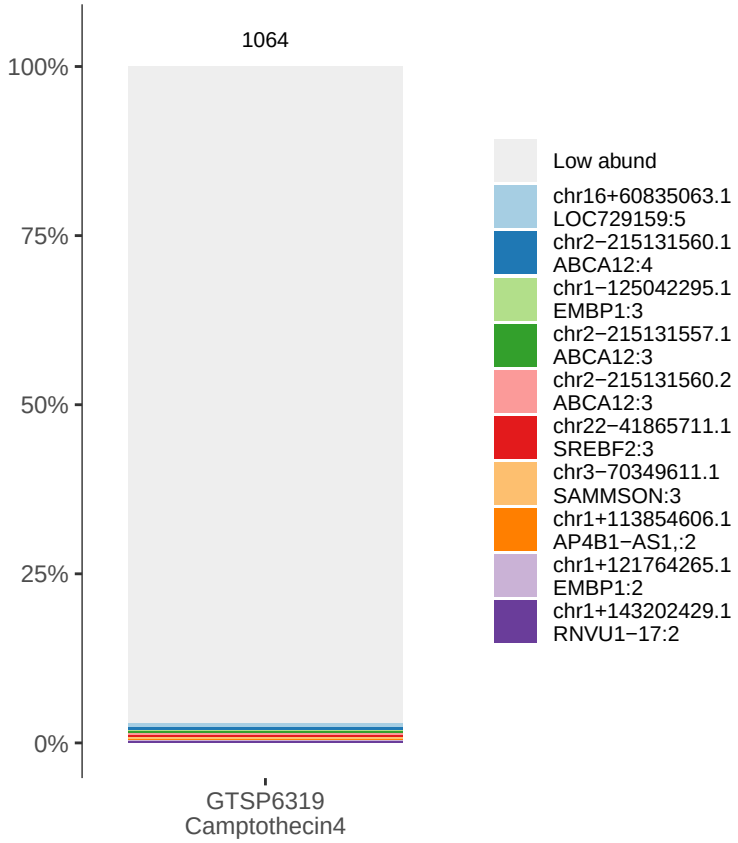
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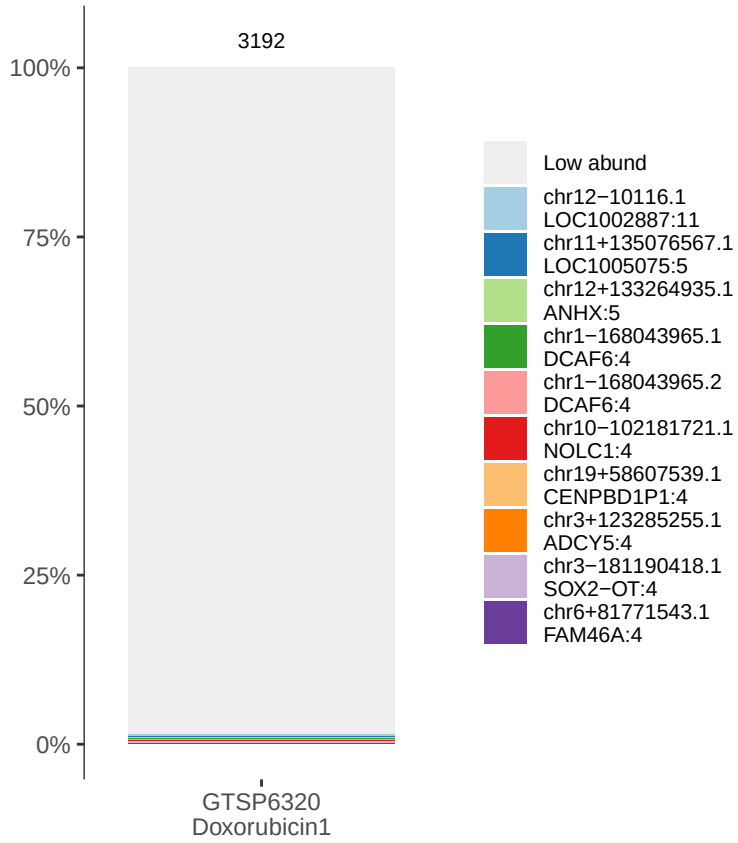
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chr1−168043965.1
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DCAF6:4
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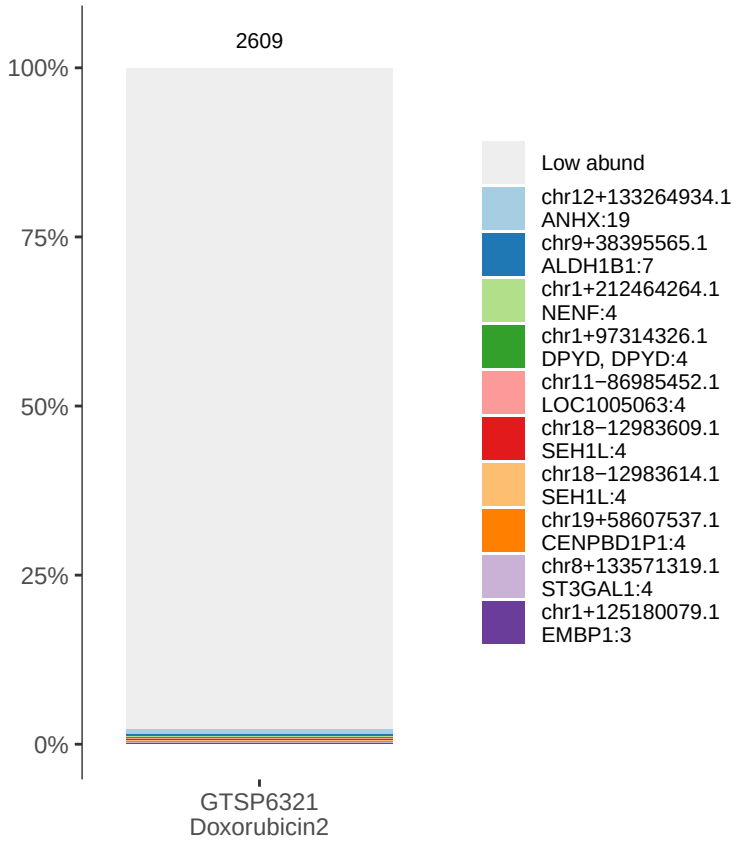
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chr11−86985452.1
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chr18−12983609.1
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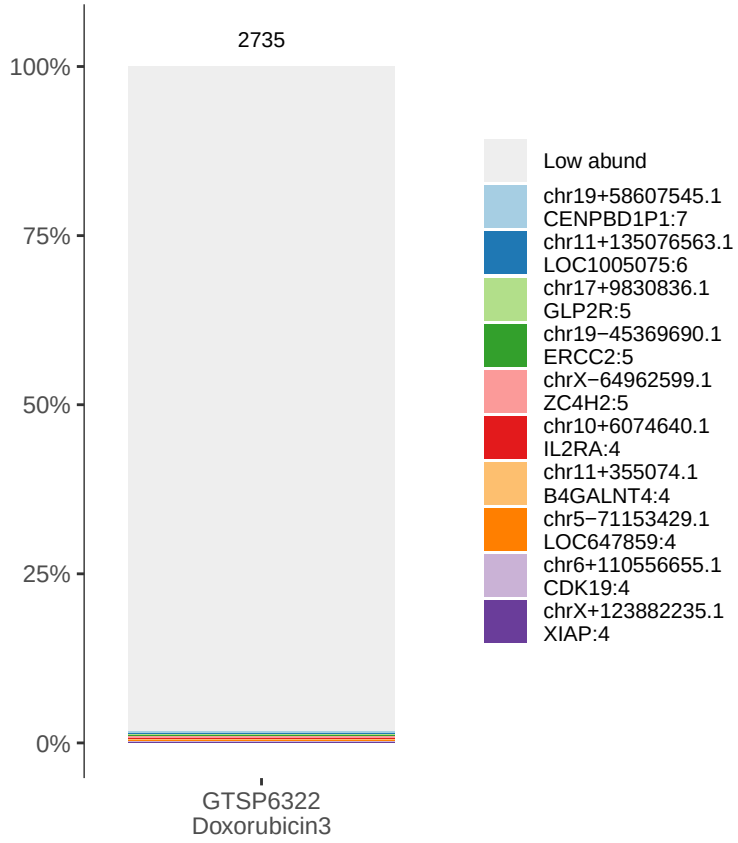
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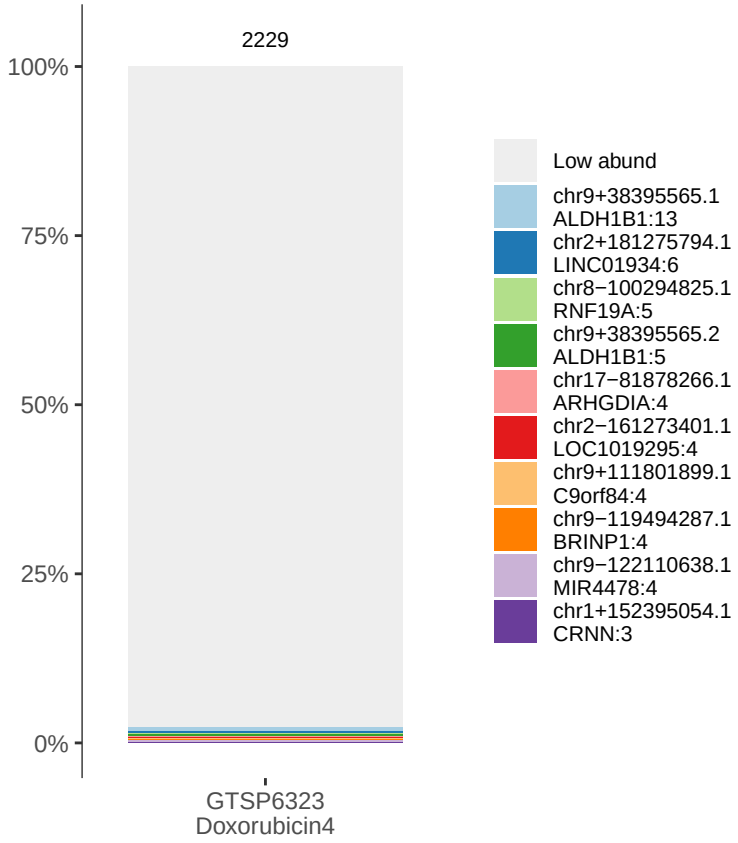
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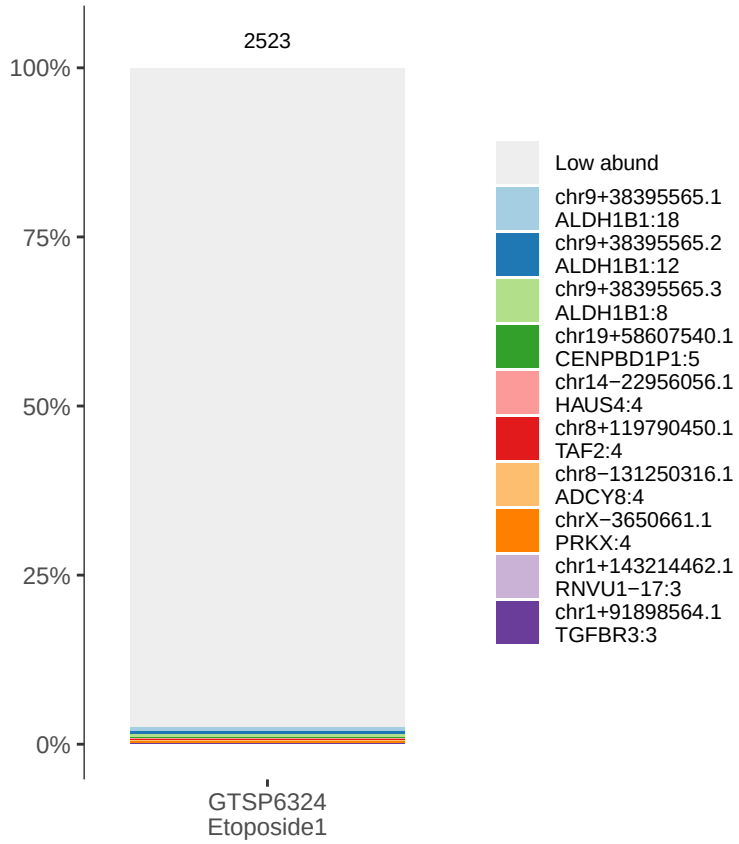
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chr19+58607540.1
CENPBD1P1:5
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HAUS4:4
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TAF2:4
chr8−131250316.1
ADCY8:4
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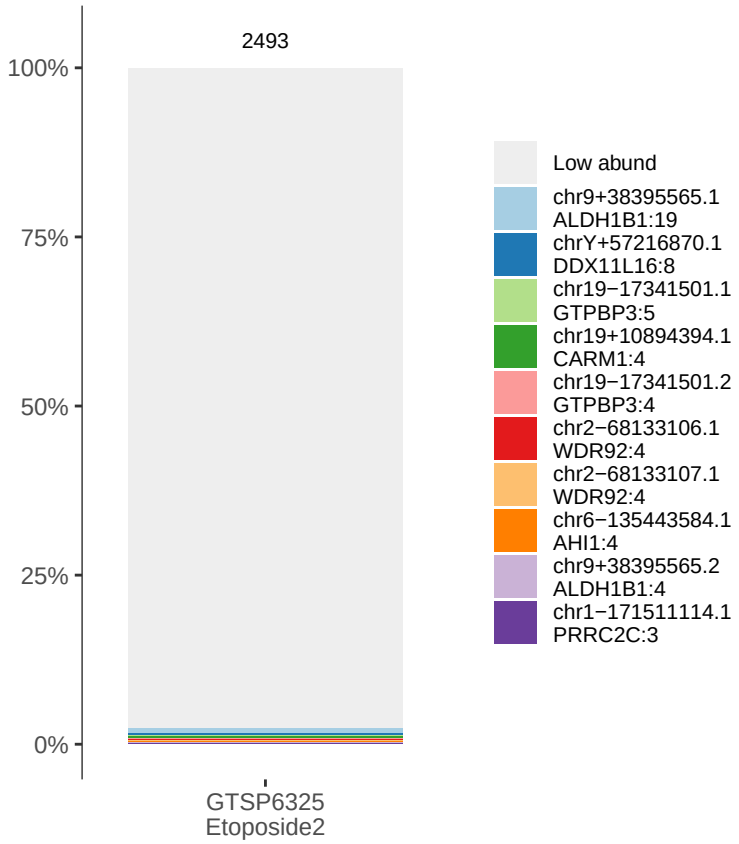
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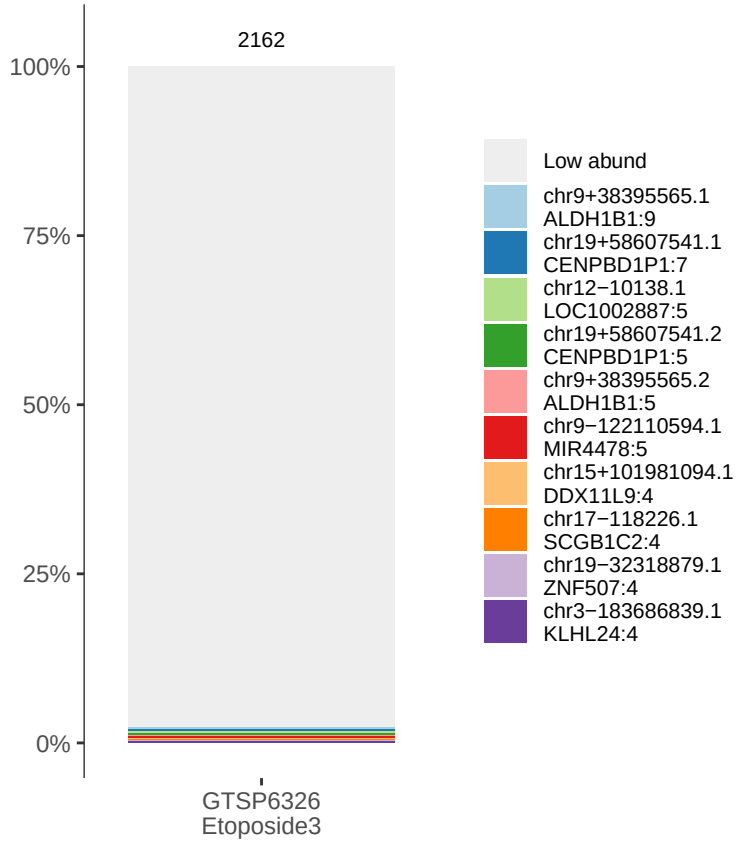
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chr9−122110594.1
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chr15+101981094.1
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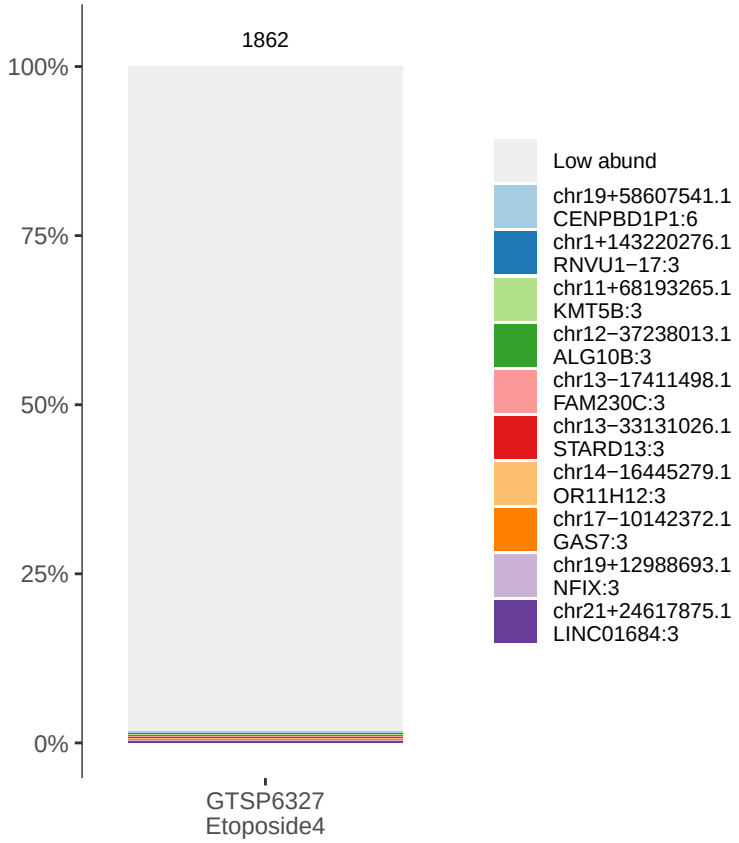
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KMT5B:3
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ALG10B:3
chr13−17411498.1
FAM230C:3
chr13−33131026.1
STARD13:3
chr14−16445279.1
OR11H12:3
chr17−10142372.1
GAS7:3
chr19+12988693.1
NFIX:3
chr21+24617875.1
LINC01684:3

AAV ITR Breakpoint Summary

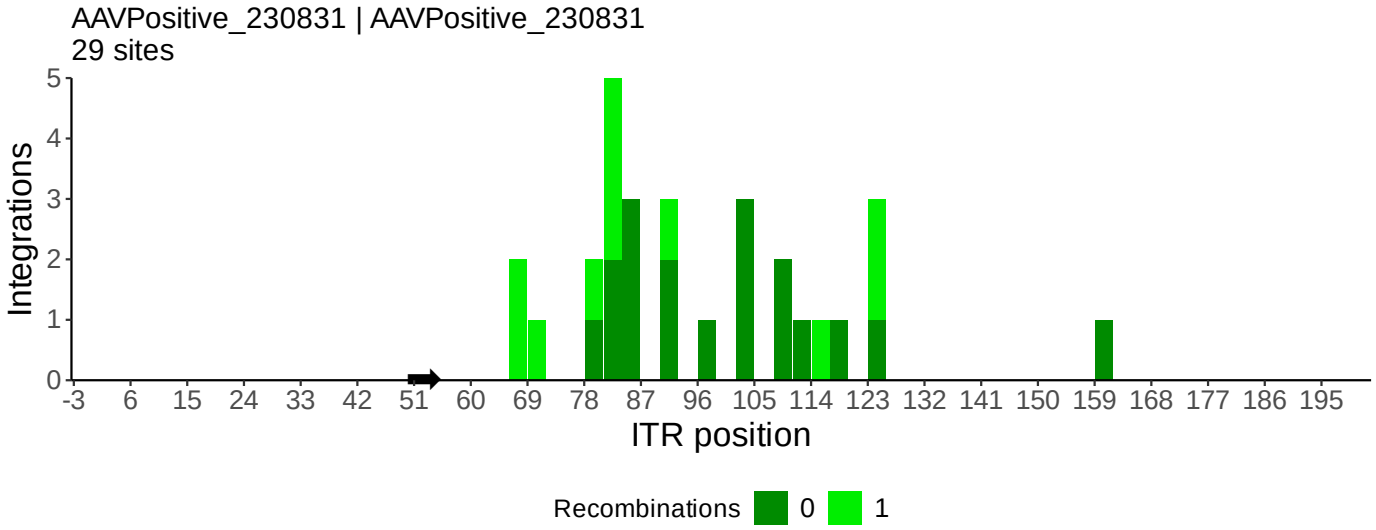
This is a summary table for itr breakpoints.

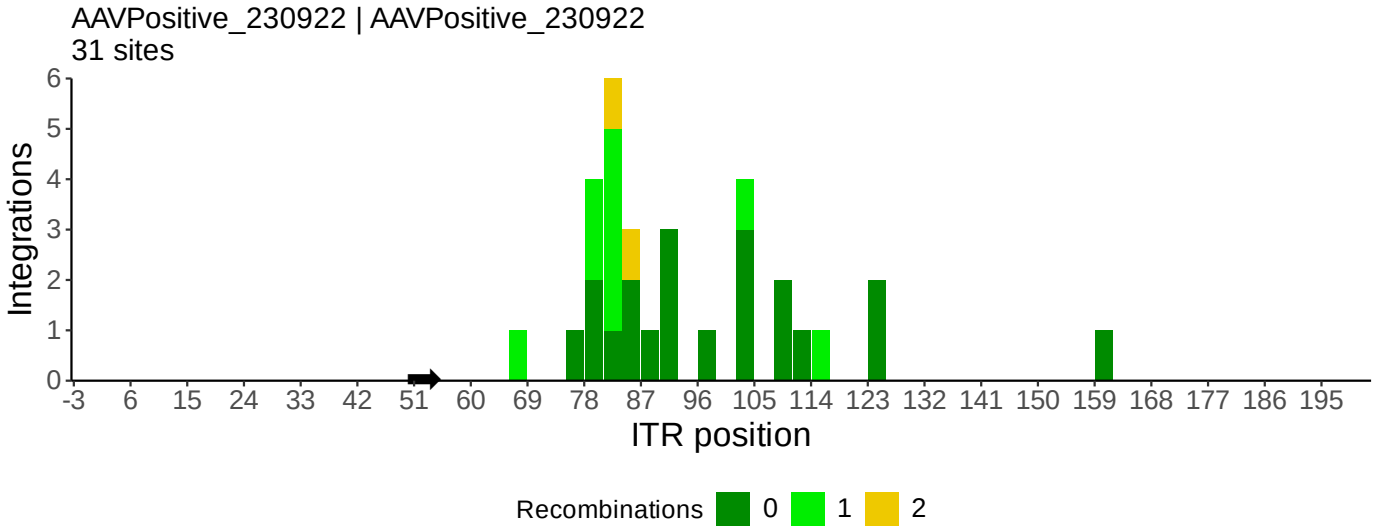
Table 2: Rearrangement Summary Table

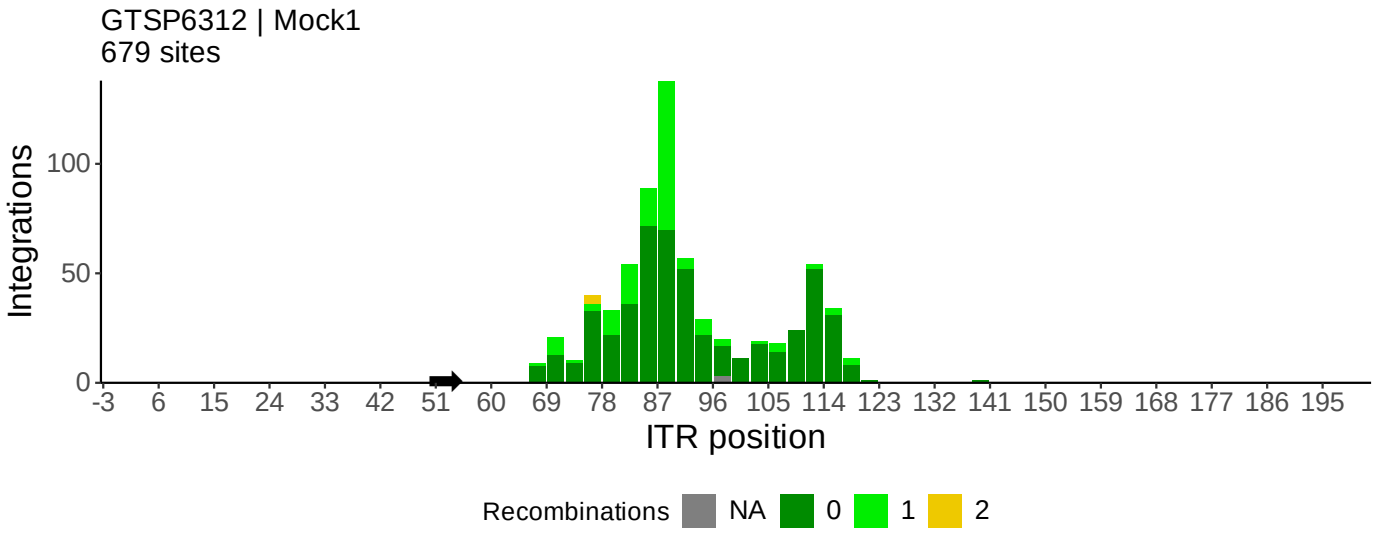
sample	totalBreaks	totalLength	totalBreakBool	break%	breakBool%
AAVPositive_230831	11	591	11	1.86	1.86
AAVPositive_230922	13	611	11	2.13	1.80
GTSP6312	184	15754	162	1.17	1.03
GTSP6313	231	22633	230	1.02	1.02
GTSP6314	208	13278	199	1.57	1.50
GTSP6315	109	6642	101	1.64	1.52
GTSP6316	229	17803	217	1.29	1.22
GTSP6317	366	42222	355	0.87	0.84
GTSP6318	234	16705	225	1.40	1.35
GTSP6319	149	9970	146	1.49	1.46
GTSP6320	565	42792	531	1.32	1.24
GTSP6321	445	39179	429	1.14	1.09
GTSP6322	512	41338	455	1.24	1.10
GTSP6323	485	49780	470	0.97	0.94
GTSP6324	808	73948	744	1.09	1.01
GTSP6325	580	45054	558	1.29	1.24
GTSP6326	575	57544	515	1.00	0.89
GTSP6327	360	29617	341	1.22	1.15

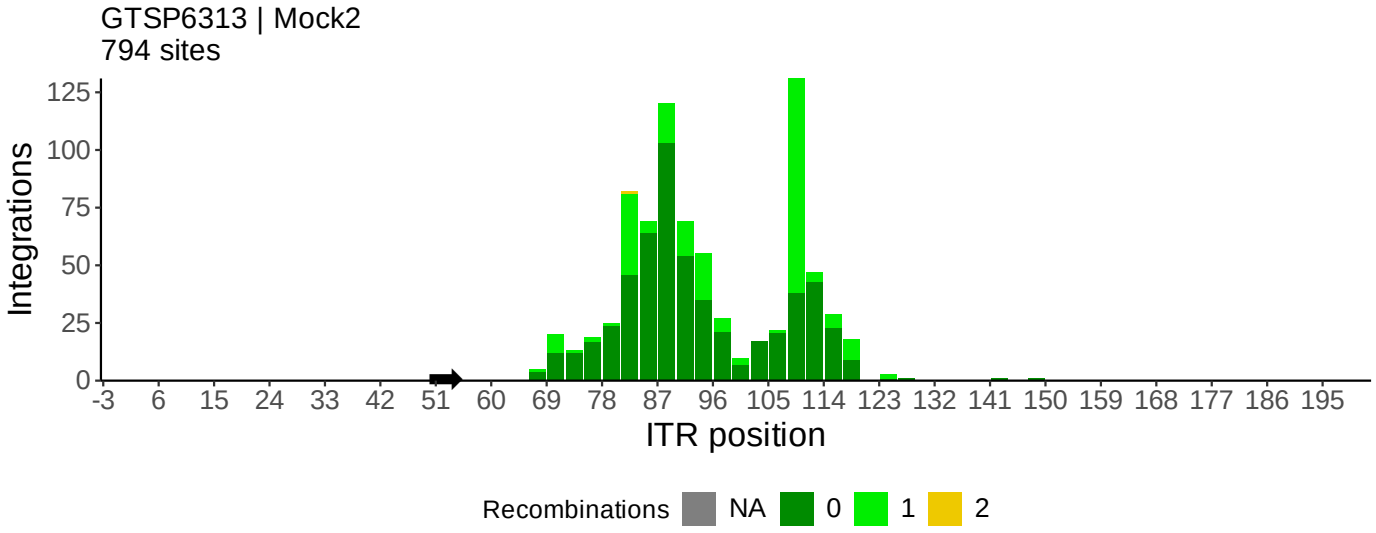
AAV ITR Breakpoint Plots

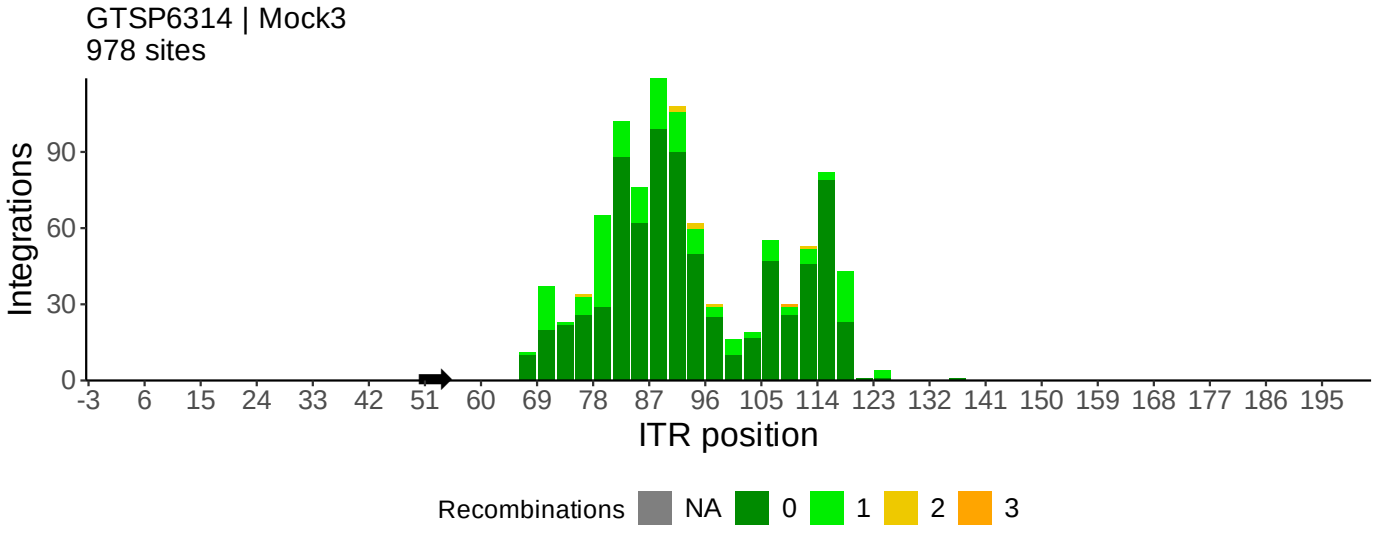
These plots are a visual representation of the ITR breakpoint at the site of genomic integration. The arrow indicates the priming location within the ITR and the x-coordinate of each bar corresponds to a departure from an expected ITR sequence. Each departure from expectation is demarcated as a rearrangement, and bars are colored to reflect the frequency of rearrangement at each integration site.

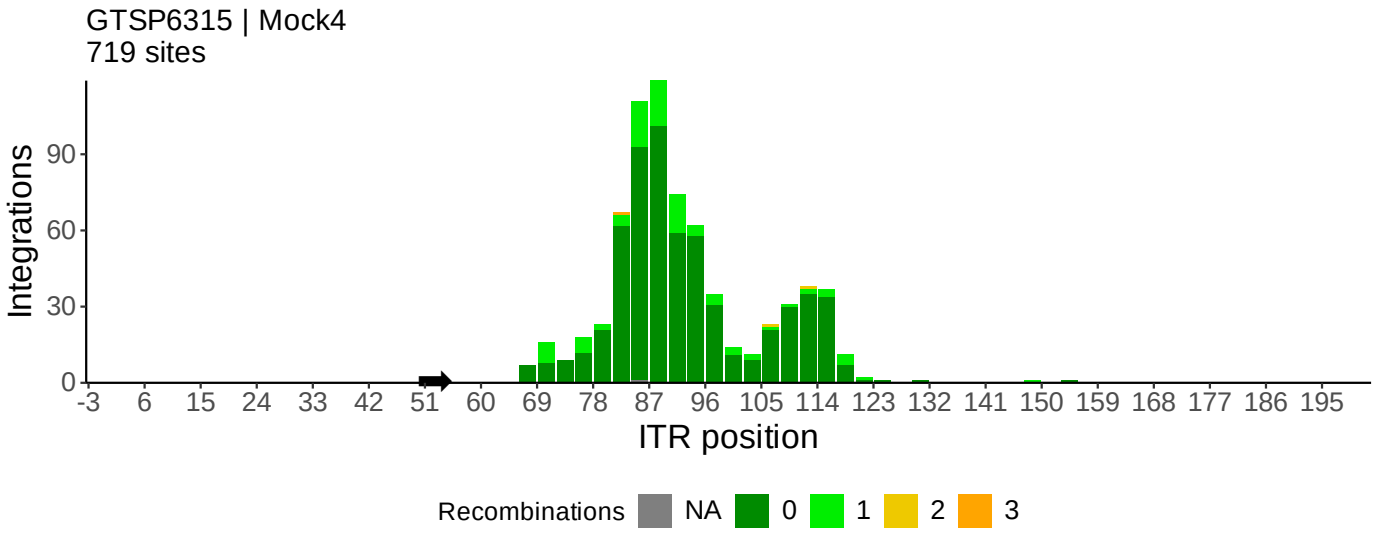


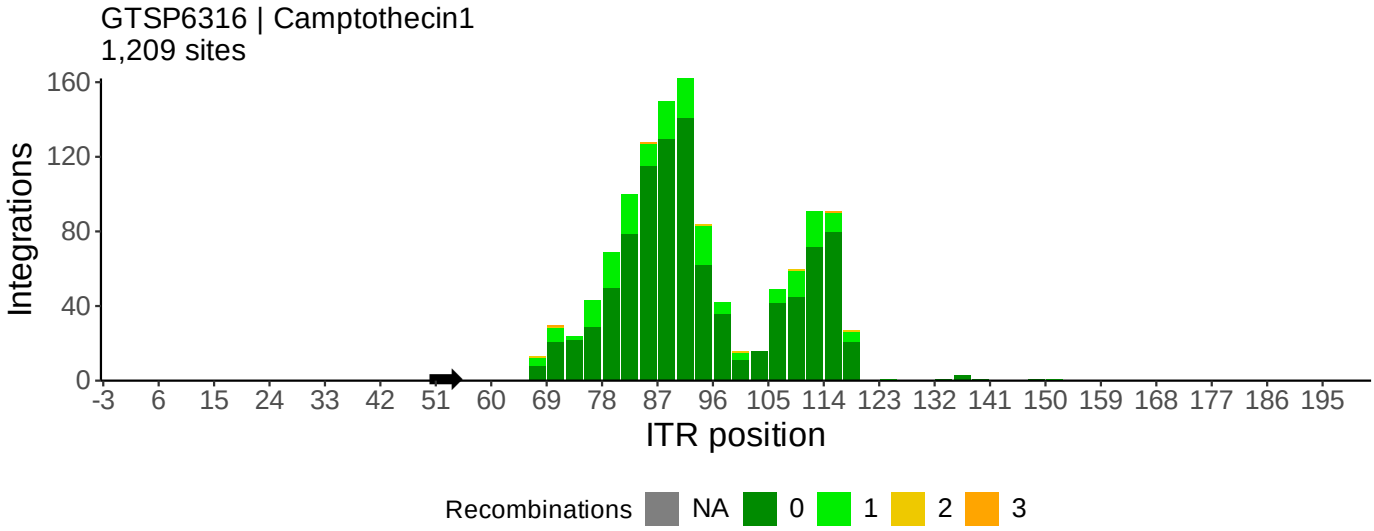


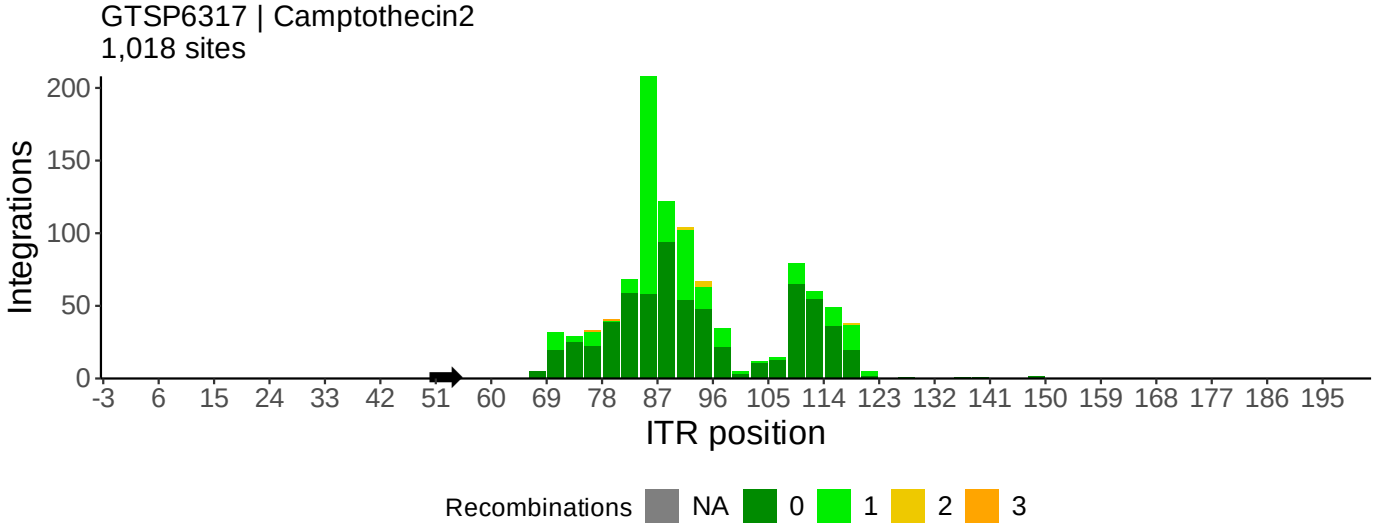

GTSP6312 | Mock1
679 sites
Integrations
0
50
100
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2

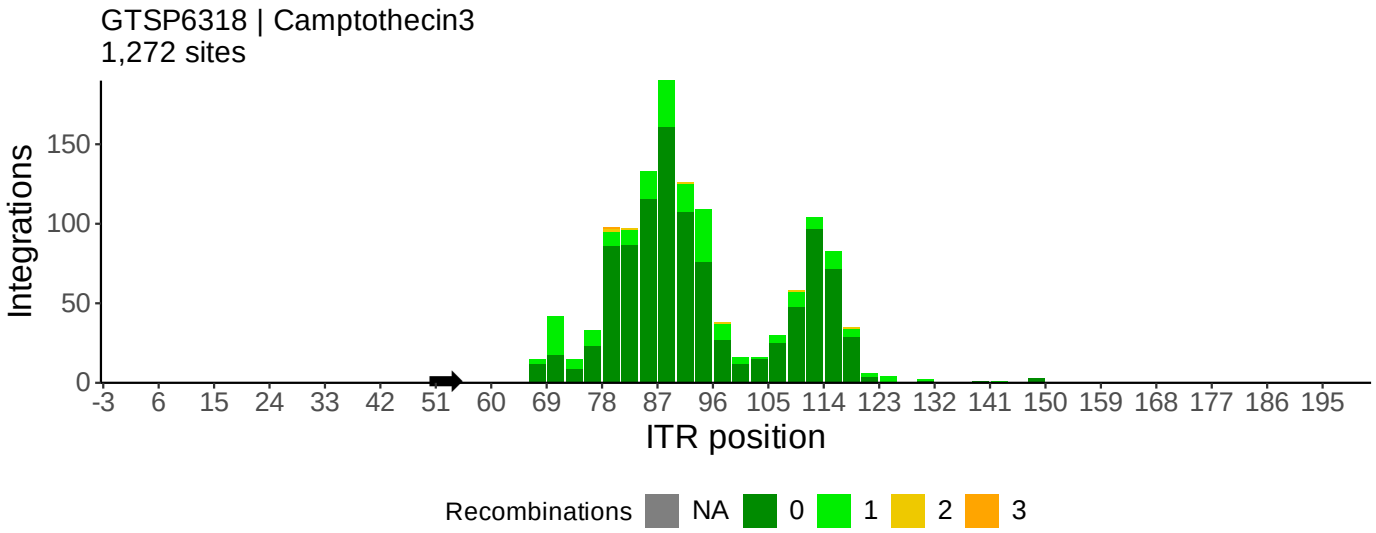

GTSP6313 | Mock2
794 sites
Integrations
0
25
50
75
100
125
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2

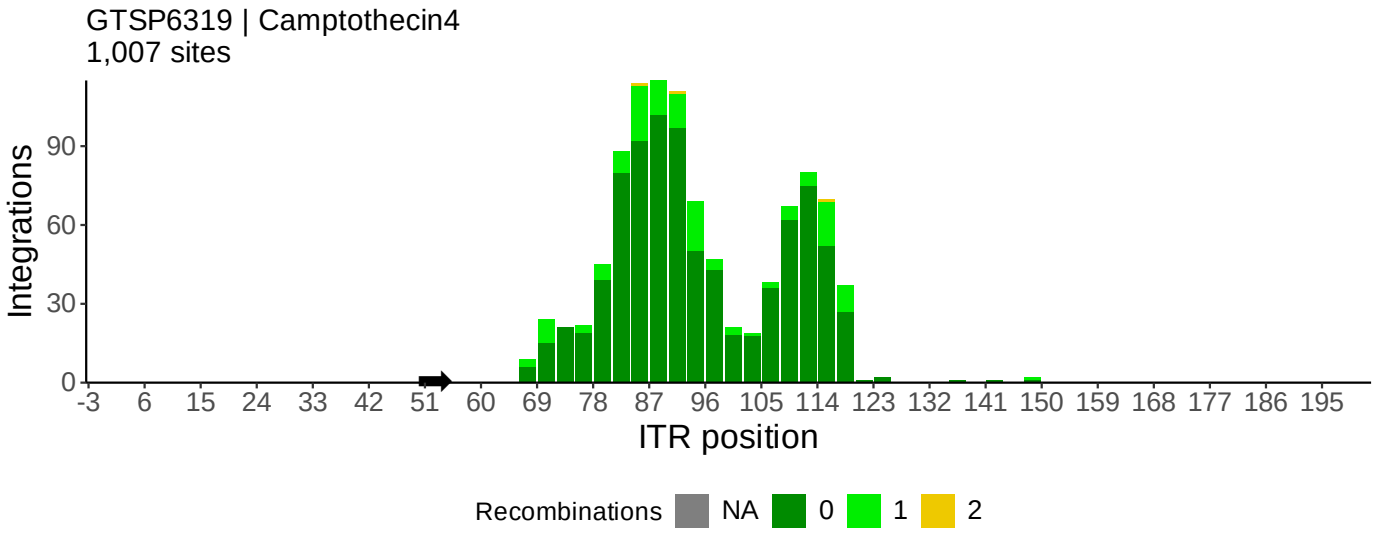

GTSP6314 | Mock3
978 sites
Integrations
0
30
60
90
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

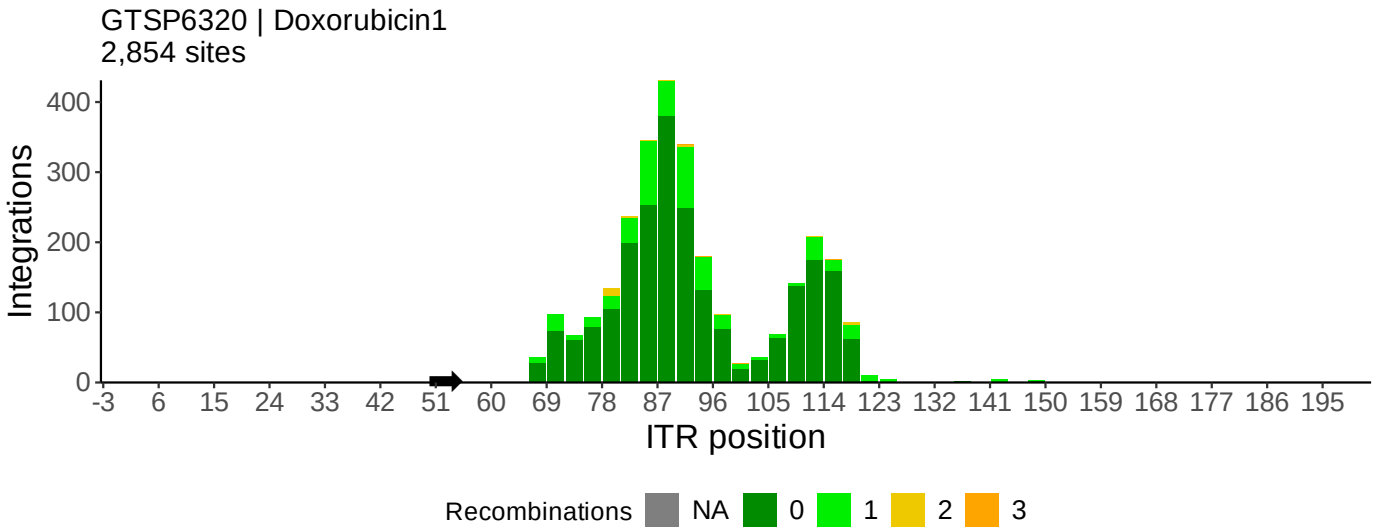

GTSP6315 | Mock4
719 sites
Integrations
0
30
60
90
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

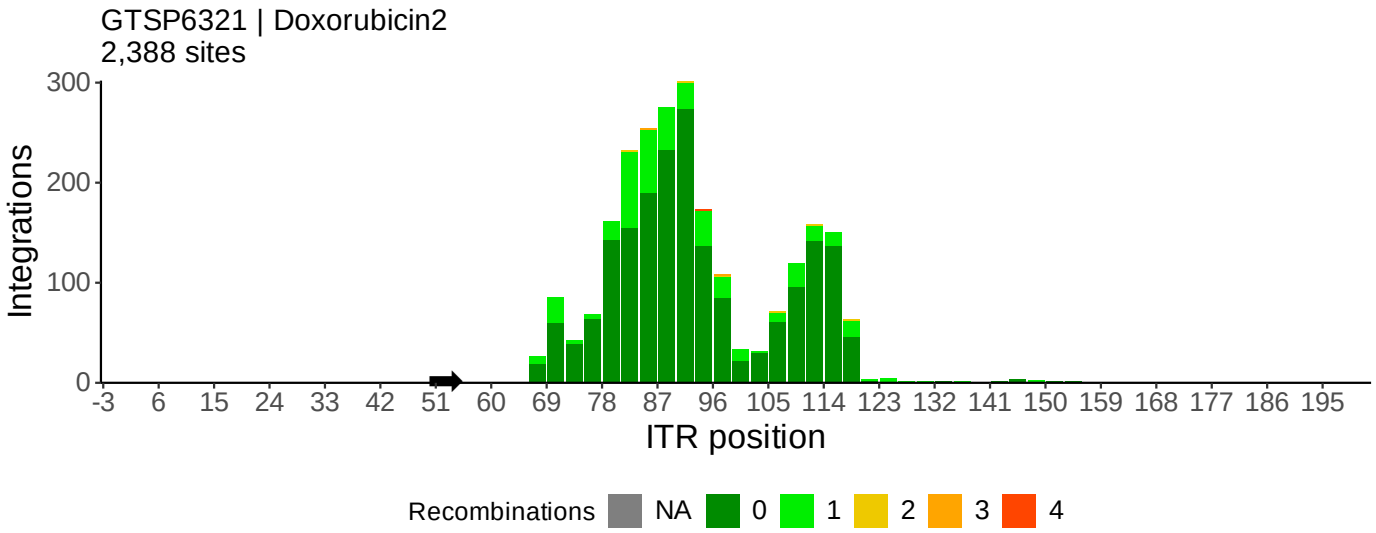

GTSP6316 | Camptothecin1
1,209 sites
Integrations
0
40
80
120
160
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

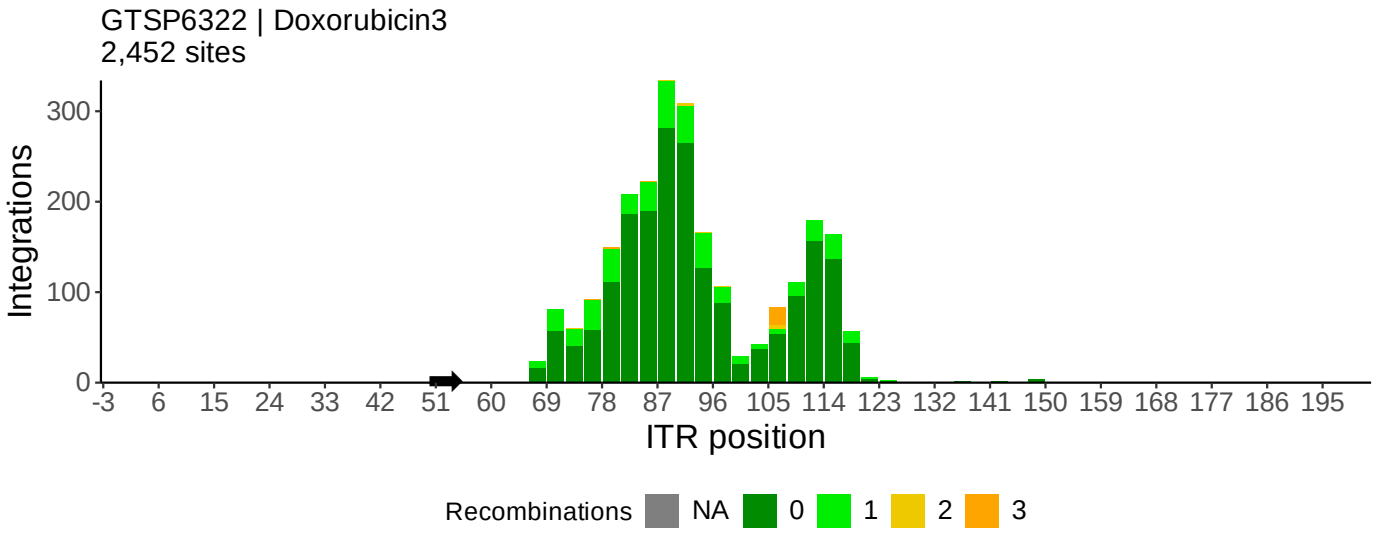

GTSP6317 | Camptothecin2
1,018 sites
Integrations
0
50
100
150
200
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

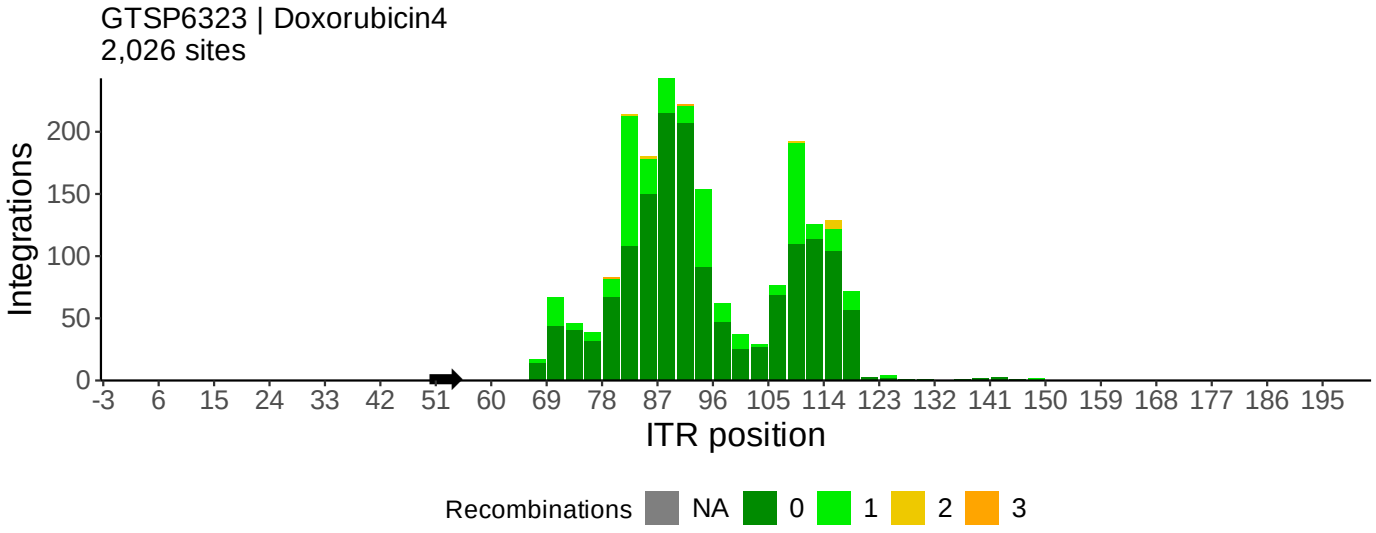

GTSP6318 | Camptothecin3
1,272 sites
Integrations
0
50
100
150
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

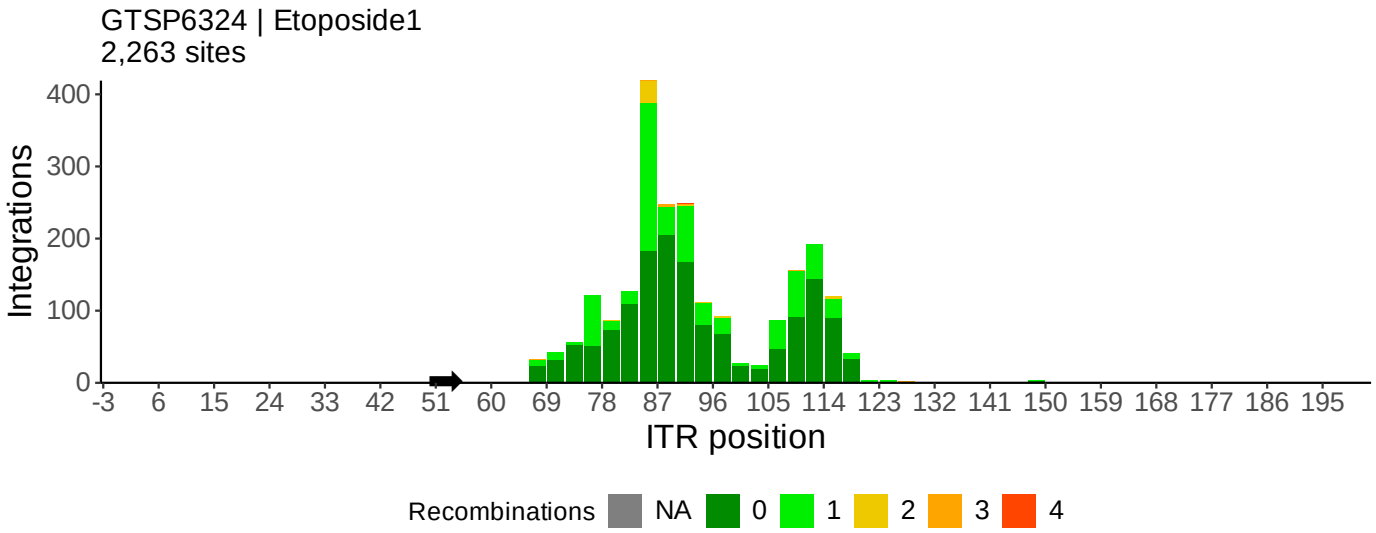

GTSP6319 | Camptothecin4
1,007 sites
Integrations
0
30
60
90
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2

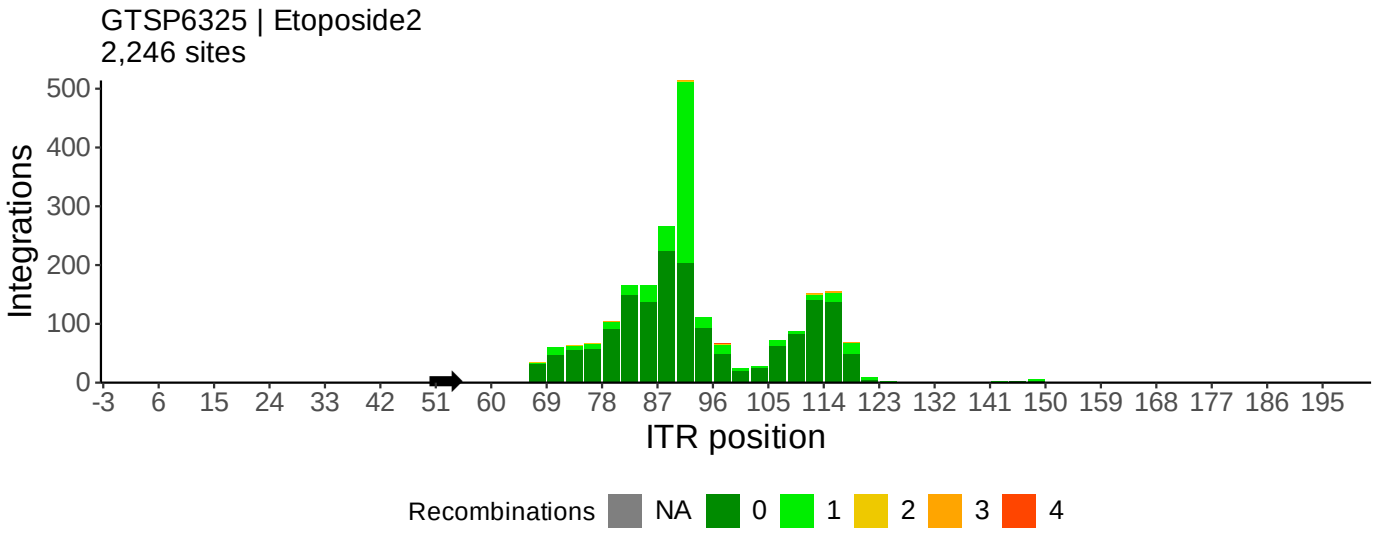

GTSP6320 | Doxorubicin1
2,854 sites
Integrations
0
100
200
300
400
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3

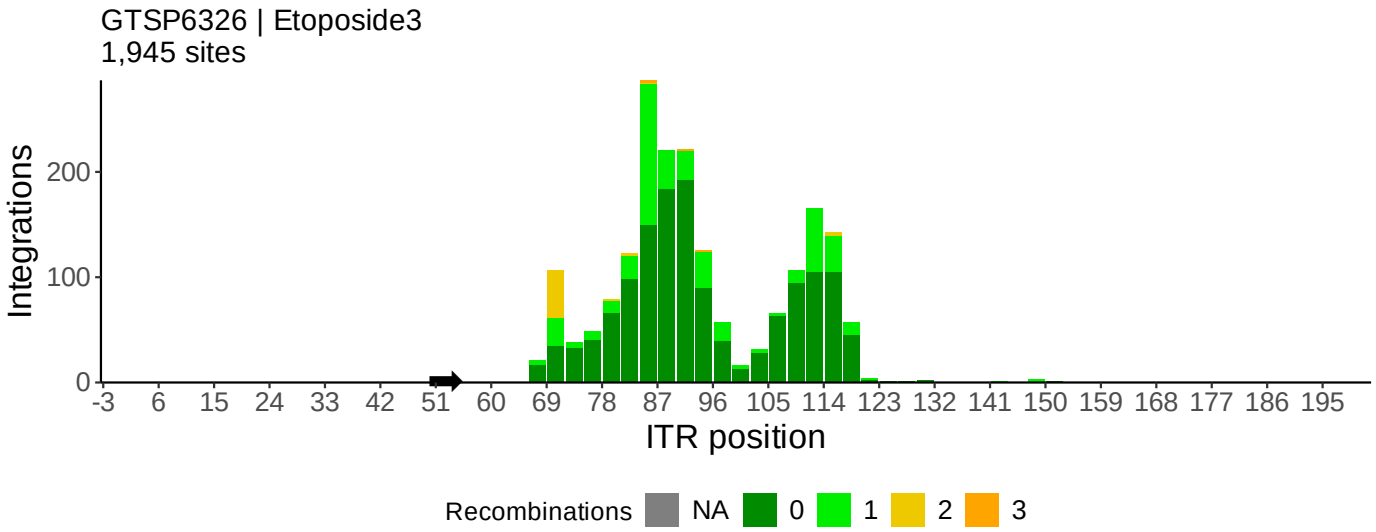

GTSP6321 | Doxorubicin2
2,388 sites
Integrations
300
200
100
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3 4

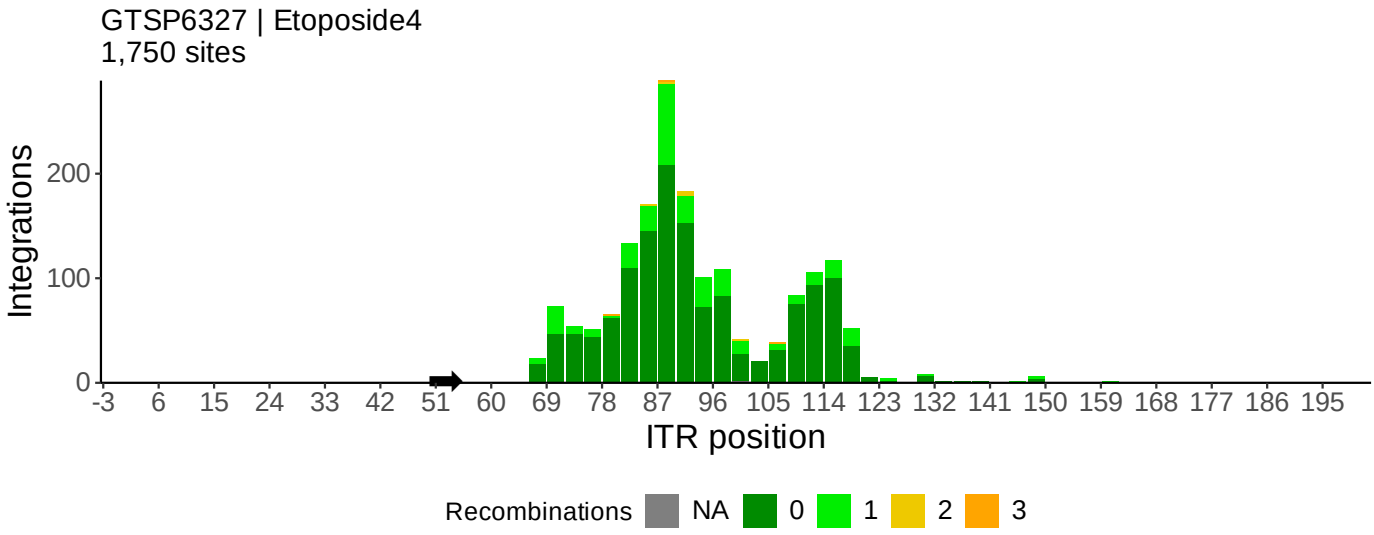

GTSP6322 | Doxorubicin3
2,452 sites
Integrations
300
200
100
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3


GTSP6323 | Doxorubicin4
2,026 sites
Integrations
200
150
100
50
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3


GTSP6324 | Etoposide1
2,263 sites
Integrations
0
100
200
300
400
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3 4


GTSP6325 | Etoposide2
2,246 sites
Integrations
0
100
200
300
400
500
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3 4


GTSP6326 | Etoposide3
1,945 sites
Integrations
0
100
200
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations NA 0 1 2 3


GTSP6327 | Etoposide4
1,750 sites
Integrations
0
100
200
-3
6
15
24
33
42
51
60
69
78
87
96
105
114
123
132
141
150
159
168
177
186
195
ITR position
Recombinations
NA
0
1
2
3

Gene distribution dot plots

The plot below details the % of integration sites within two features, 'in genes' and 'in Exons', summarized per sample. 'in Genes' refers to sites within transcription unit boundaries and 'in Exons' refers to sites within exon boundaries.

Table 3: gene distribution percentage table

sample	info	In Exon%	In Transcription Unit%
AAVPositive_230831	AAVPositive_230831	82.8	82.8
AAVPositive_230922	AAVPositive_230922	80.6	80.6
GTSP6312	Mock1	1.8	34.9
GTSP6313	Mock2	2.8	38.5
GTSP6314	Mock3	2.5	34.3
GTSP6315	Mock4	2.4	39.8
GTSP6316	Camptothecin1	4.4	37.7
GTSP6317	Camptothecin2	4.1	45.4
GTSP6318	Camptothecin3	2.9	39.5
GTSP6319	Camptothecin4	4.8	44.4
GTSP6320	Doxorubicin1	2.2	46.1
GTSP6321	Doxorubicin2	3.3	46.2
GTSP6322	Doxorubicin3	3.2	48.0
GTSP6323	Doxorubicin4	4.2	48.8
GTSP6324	Etoposide1	4.2	42.7
GTSP6325	Etoposide2	4.5	46.7
GTSP6326	Etoposide3	3.6	43.9
GTSP6327	Etoposide4	3.4	43.1

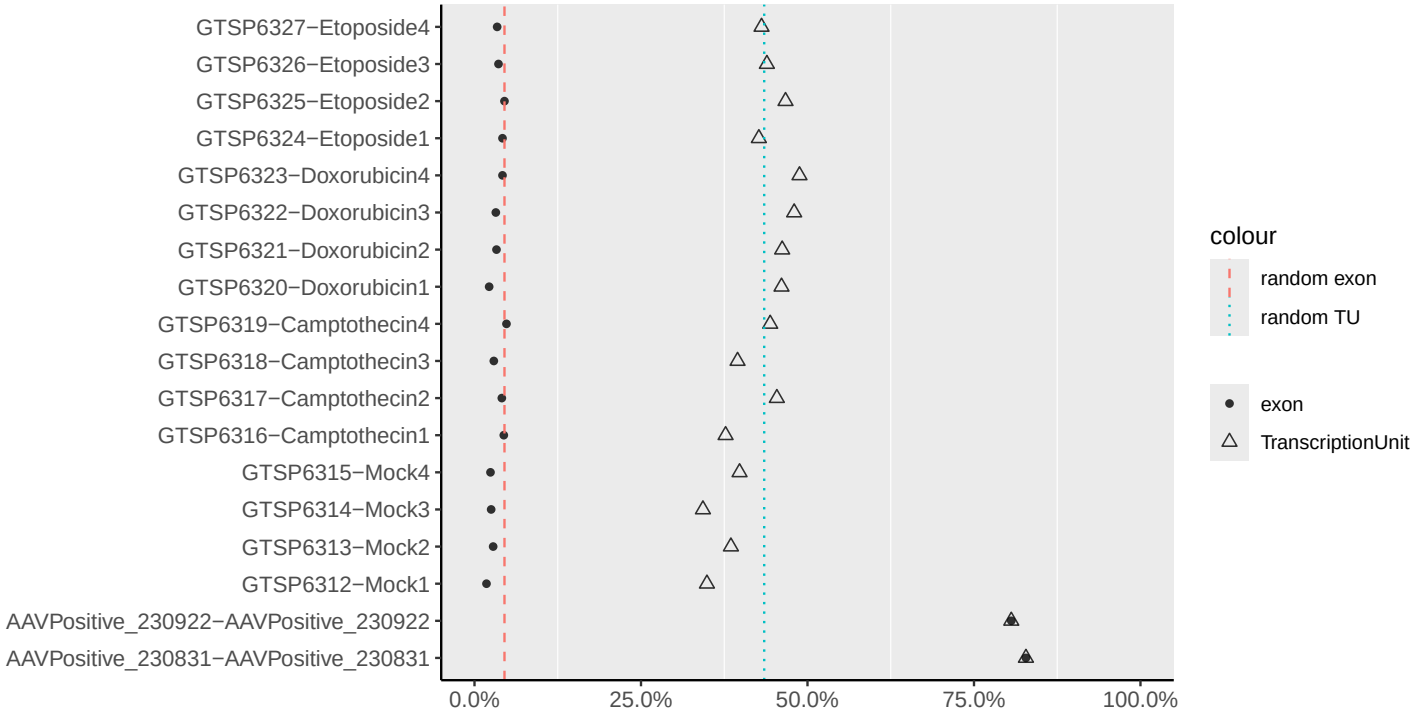


Figure 1: gene distribution scatter plot

Methods

Report Generated on: June 20, 2024
AAVenger Version: 1.1

Modules Called:

- core.R
- mapSiteLeaderSequences.R
- buildAAVremnantPlots.R