

topoDefault

June 18, 2024

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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.

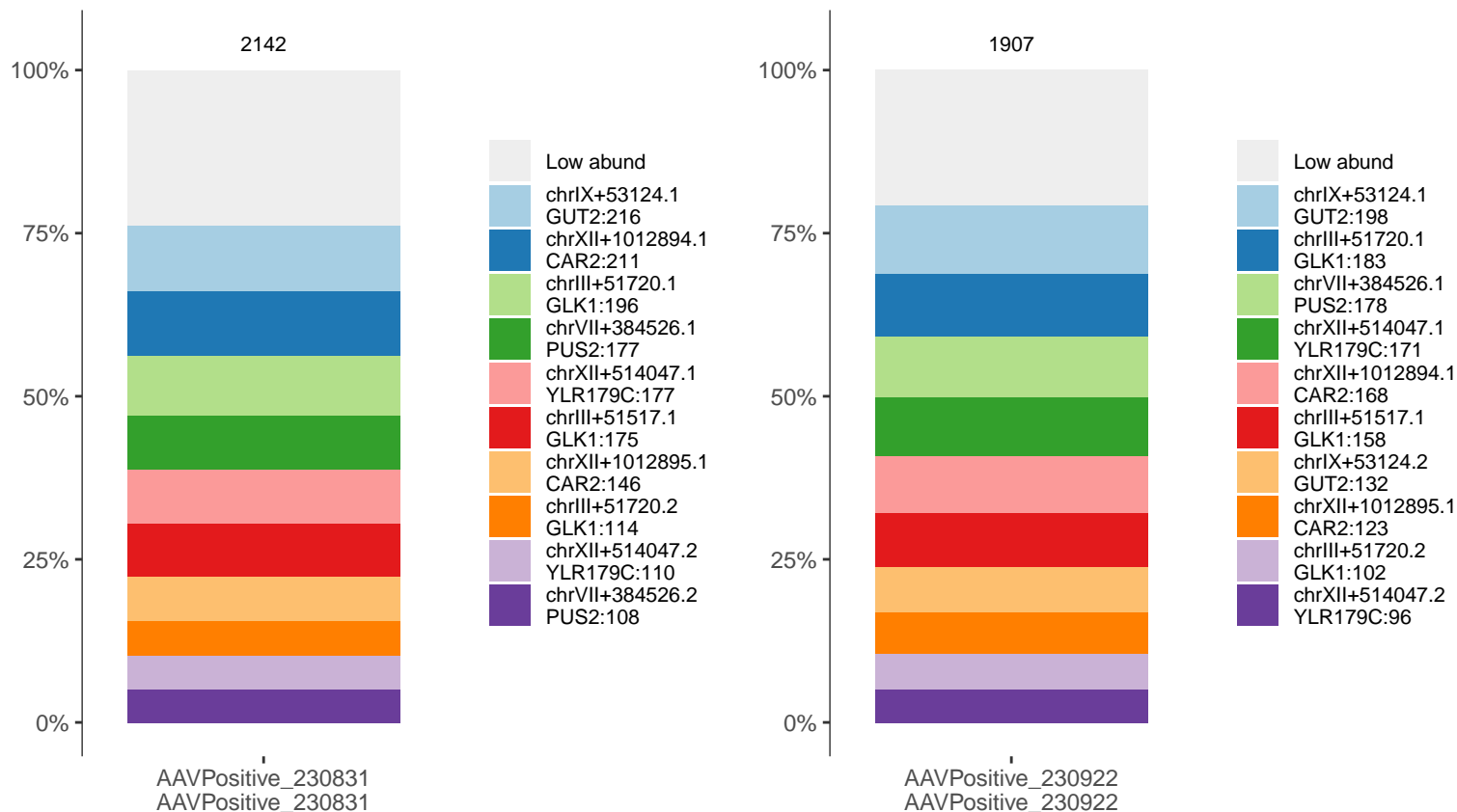
Under most circumstances, only a subset of sites will be sampled. We thus include an estimate of sample size based on the frequency of isolation information from the SonicLength method (Berry, 2012). The ‘S.chao1’ column denotes the estimated lower bound for population size derived using Chao estimate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

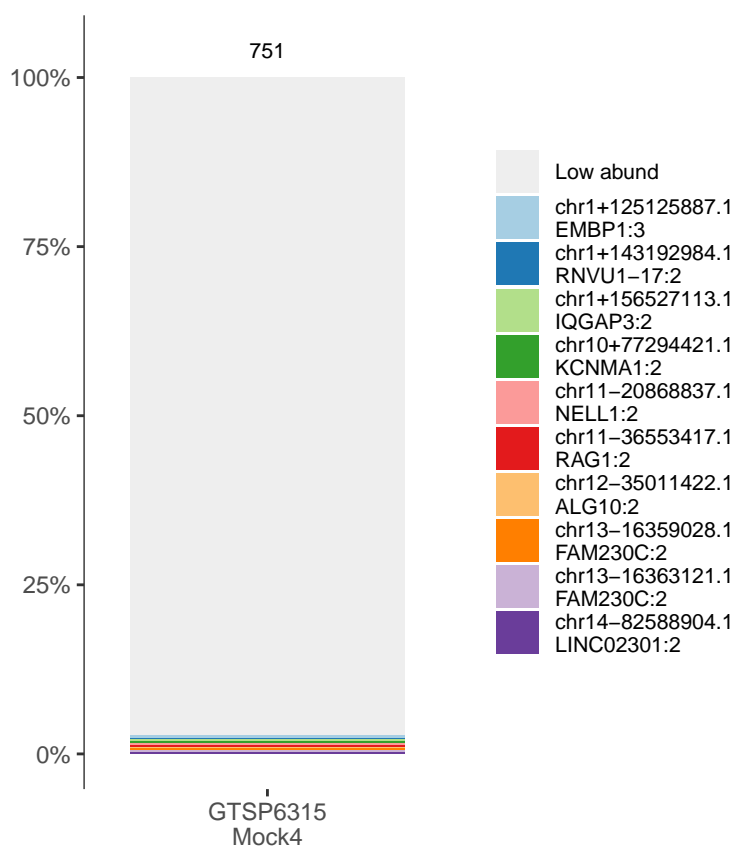
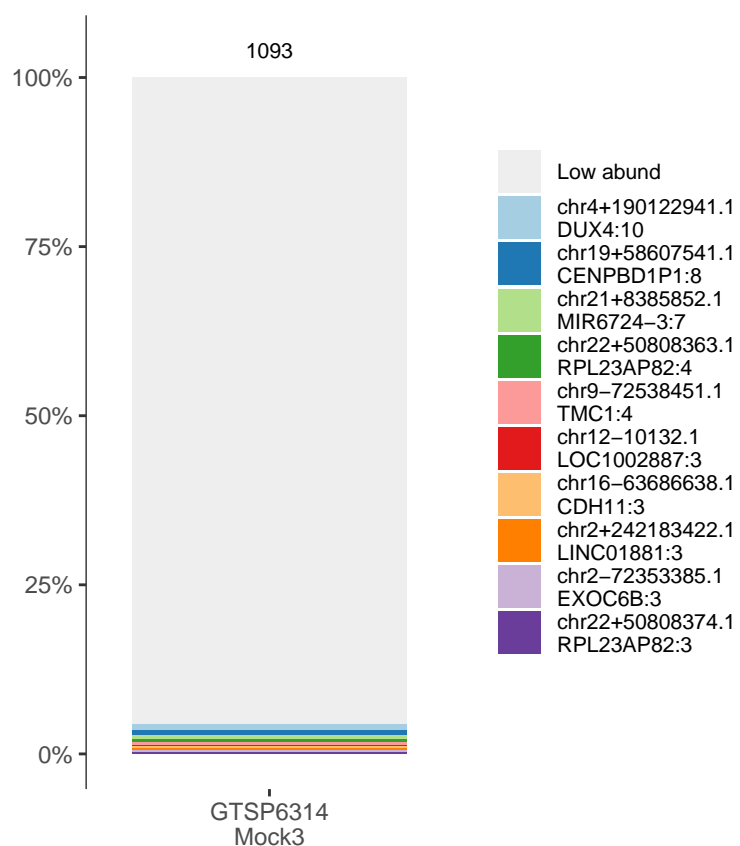
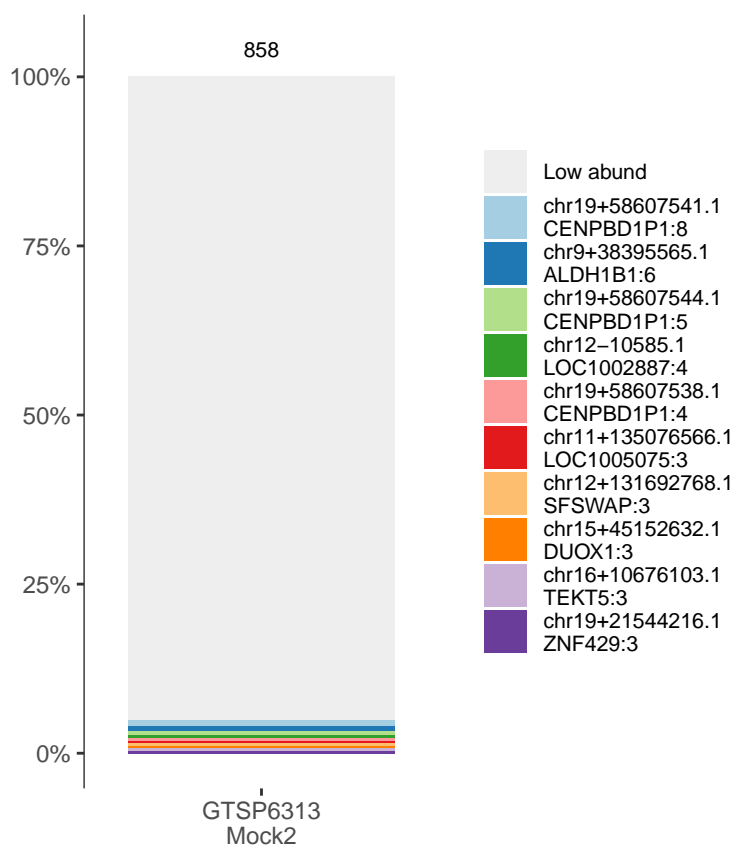
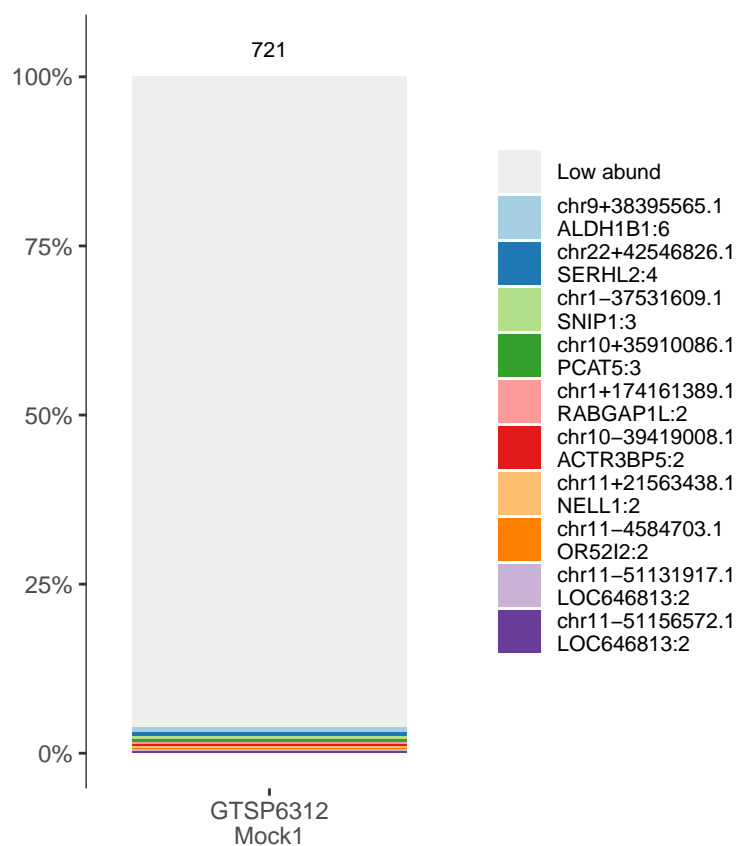
Table 1: integration summary table

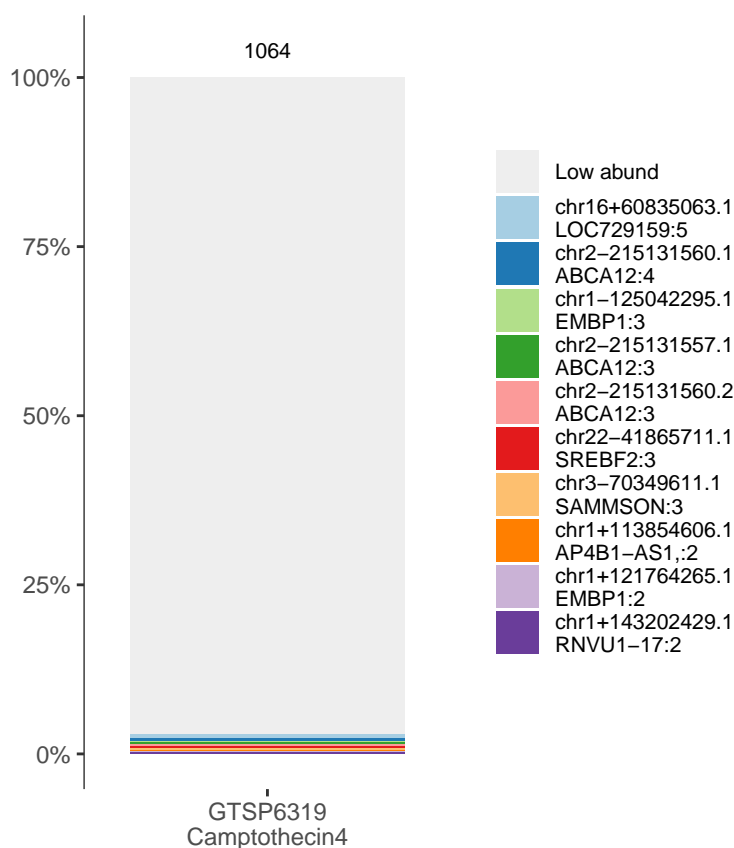
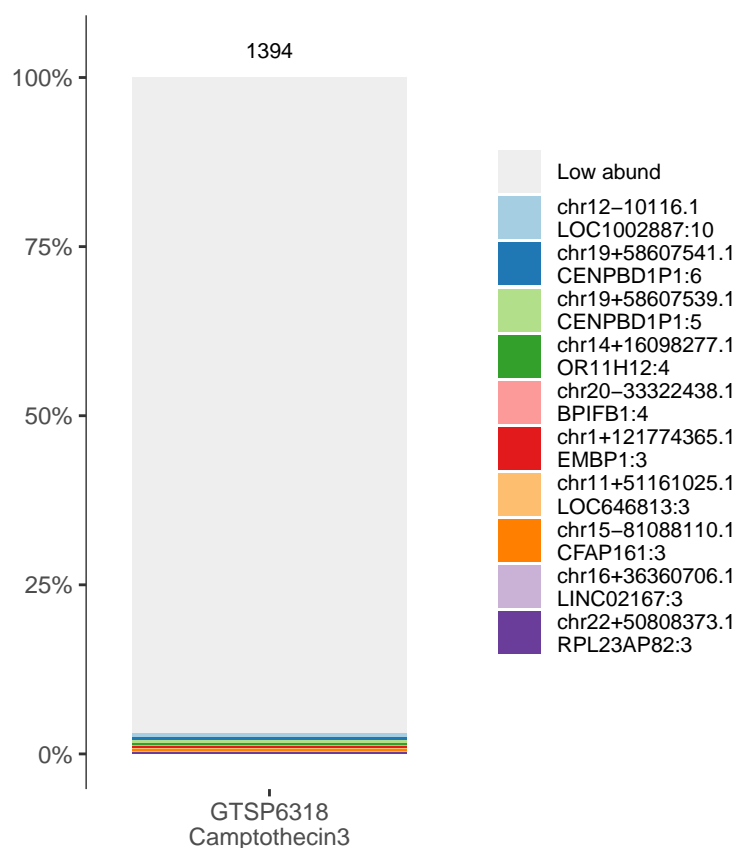
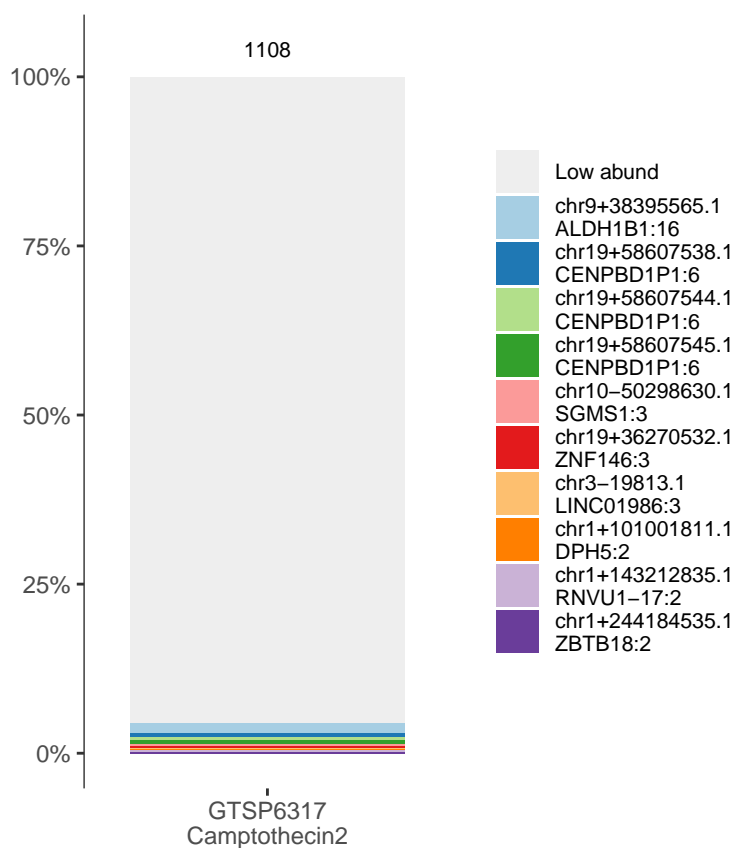
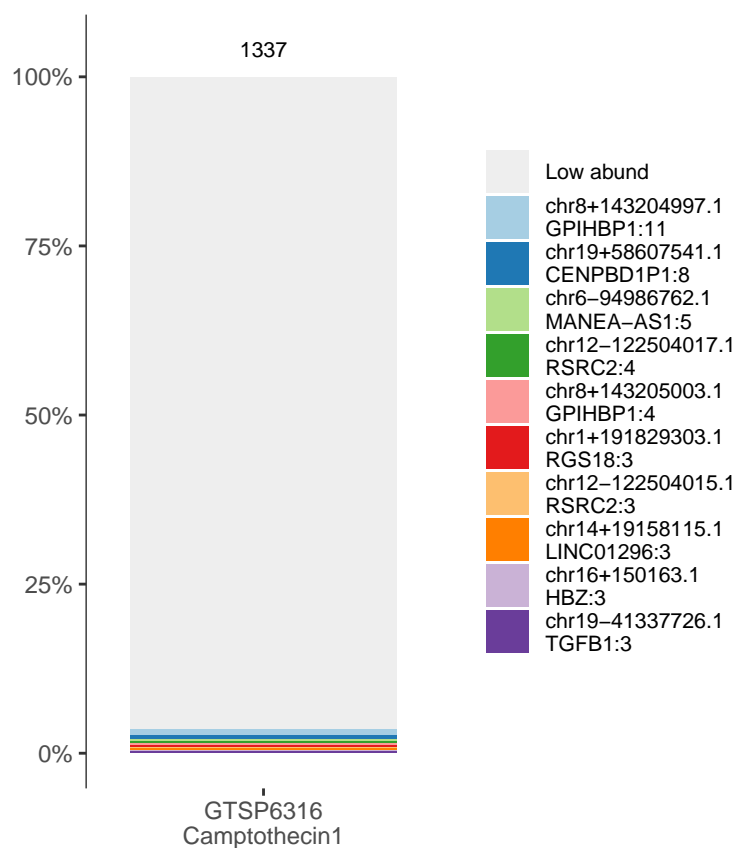
sample	patientID	total reads	Unique Sites	inferred cell	Chao1	info
AAVPositive_230831	AAVPositive_230831	319719	29	2142	31.500	AAVPositive_230831
AAVPositive_230922	AAVPositive_230922	208373	31	1907	32.000	AAVPositive_230922
GTSP6312	Mock1	79003	679	721	7378.677	Mock1
GTSP6313	Mock2	138471	794	858	10583.000	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

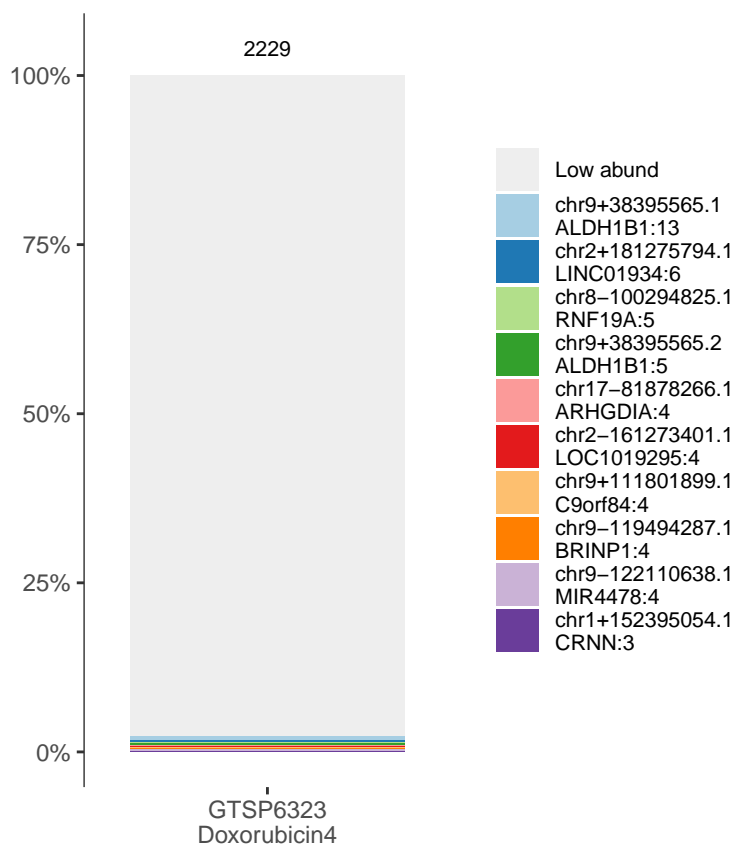
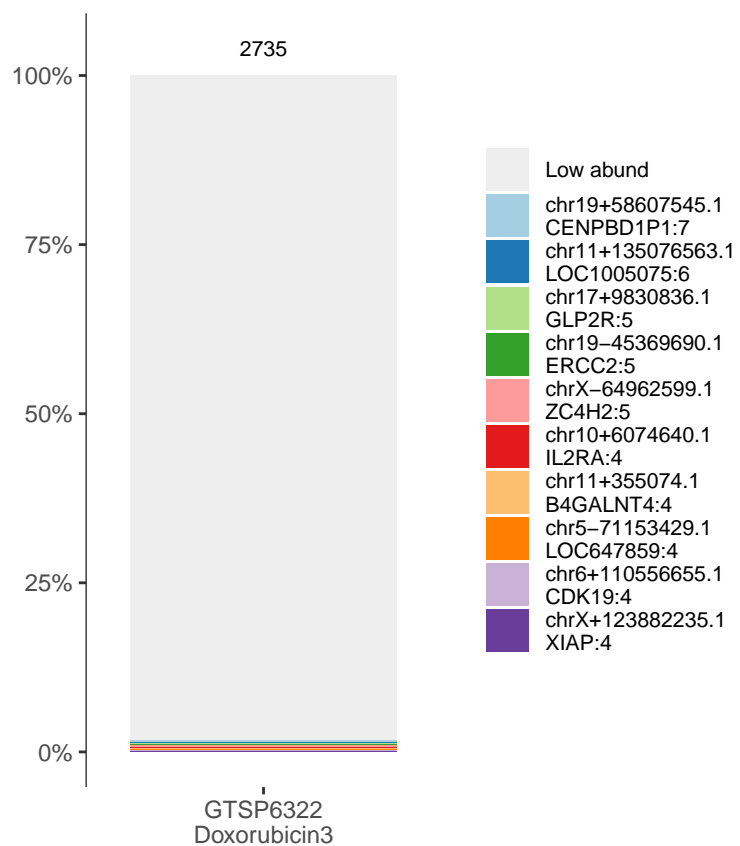
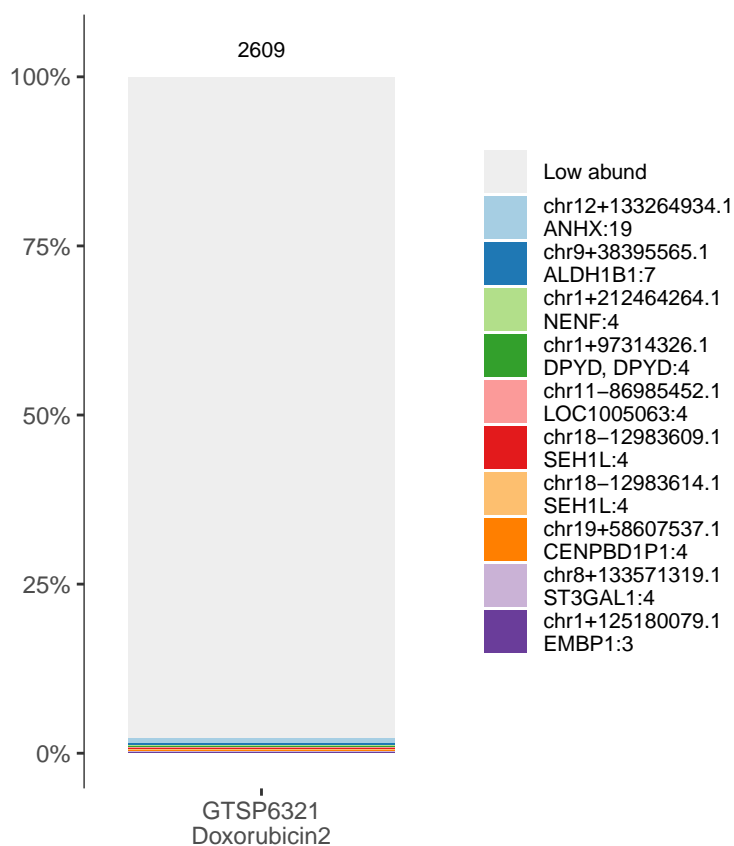
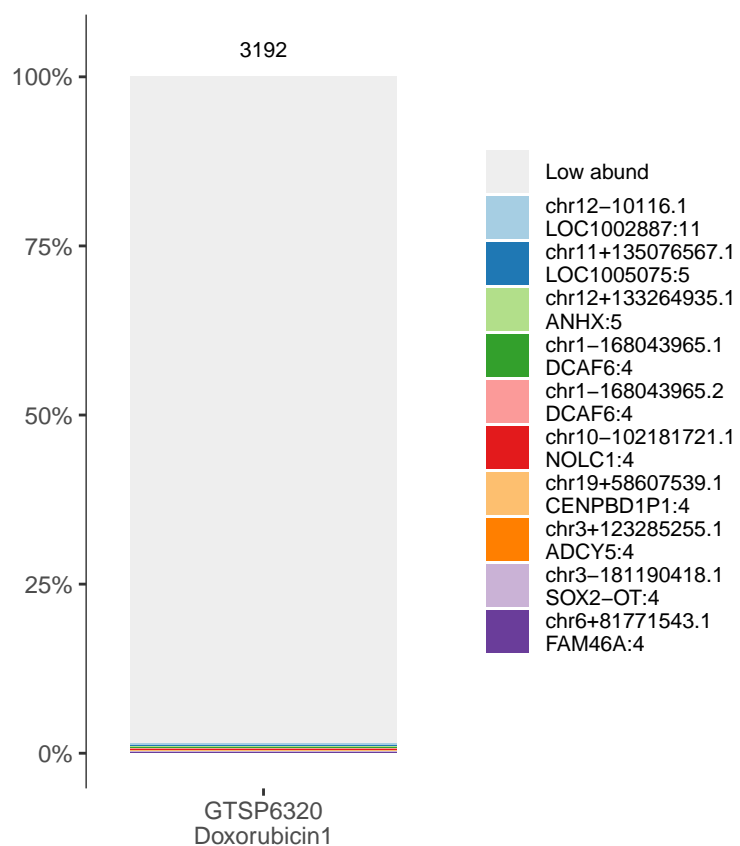
Abundance Frequency Plots

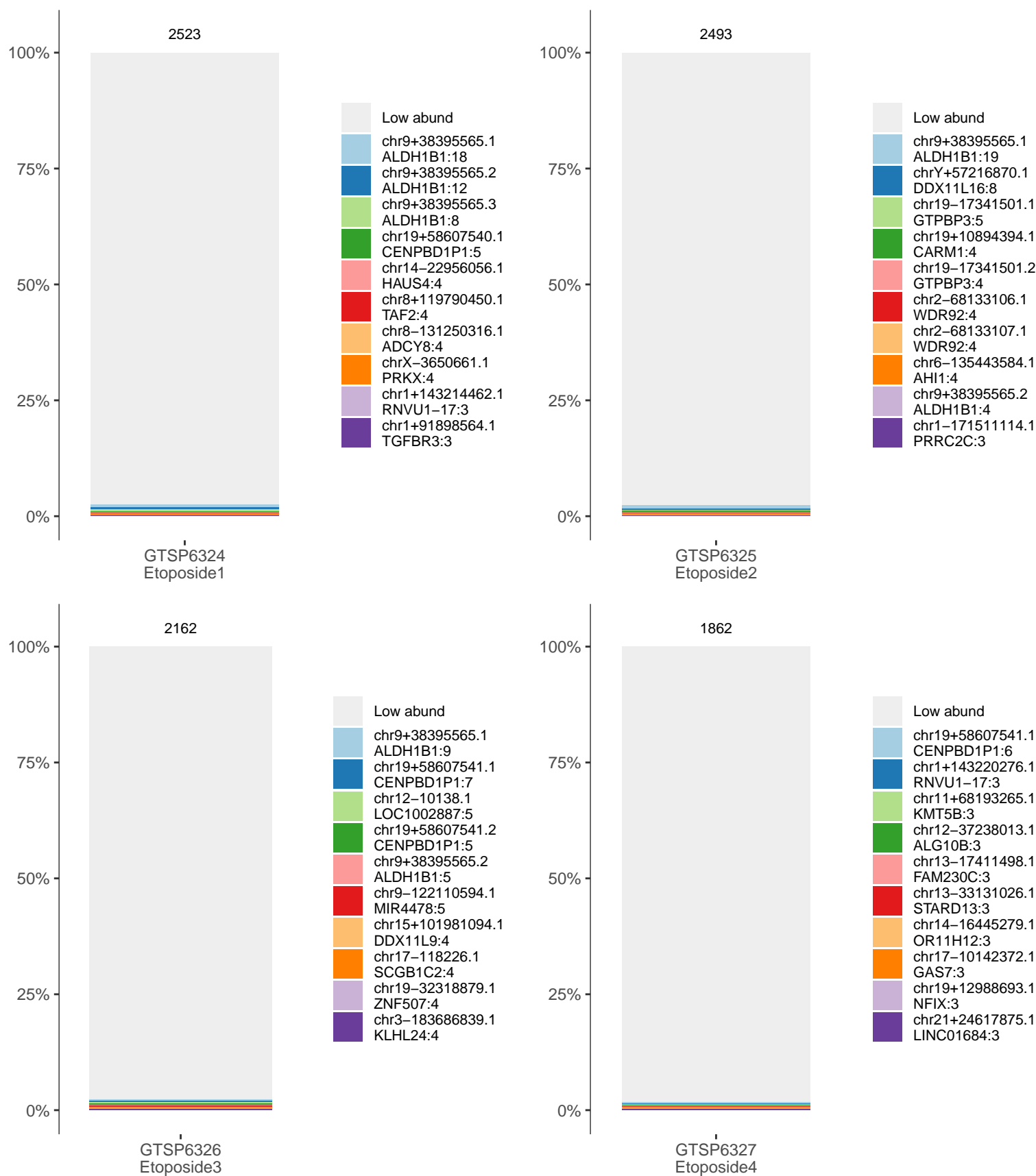
Stacked bar plots indicate clonal abundance frequency. Only the top 10 clonal types are being plotted, and the rest will be plotted in grey as “low abundance”. The number above the stacked bar plots indicates the total clonal types. The legend in each plot correspond to the clonal type integration sites.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











AAV ITR Breakpoint Summary

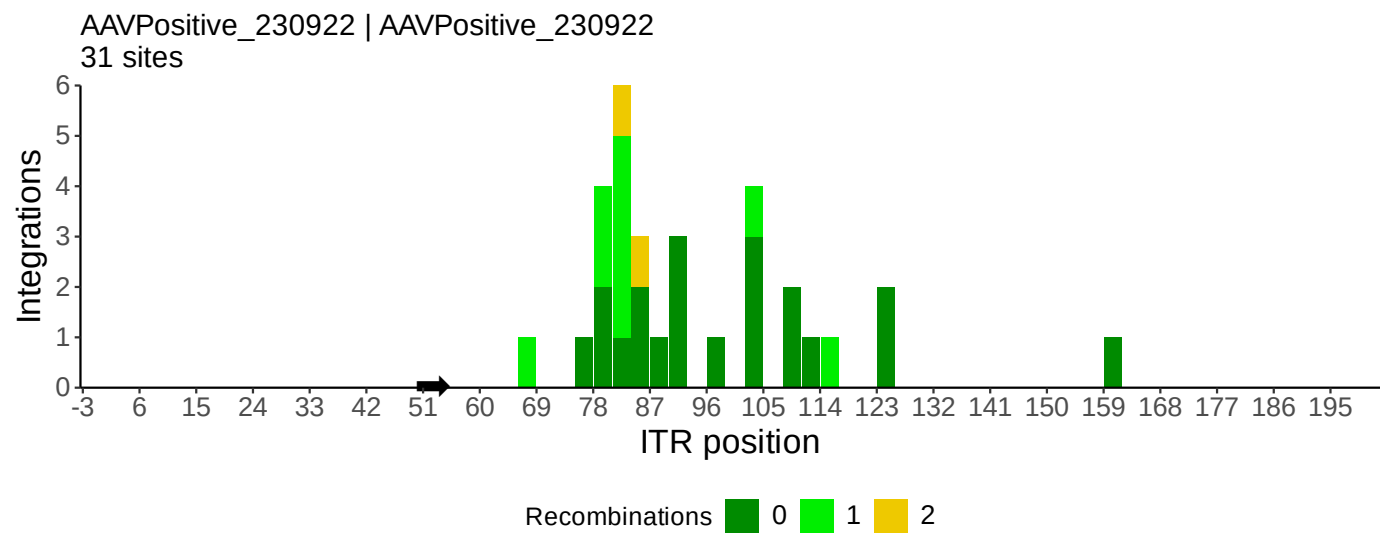
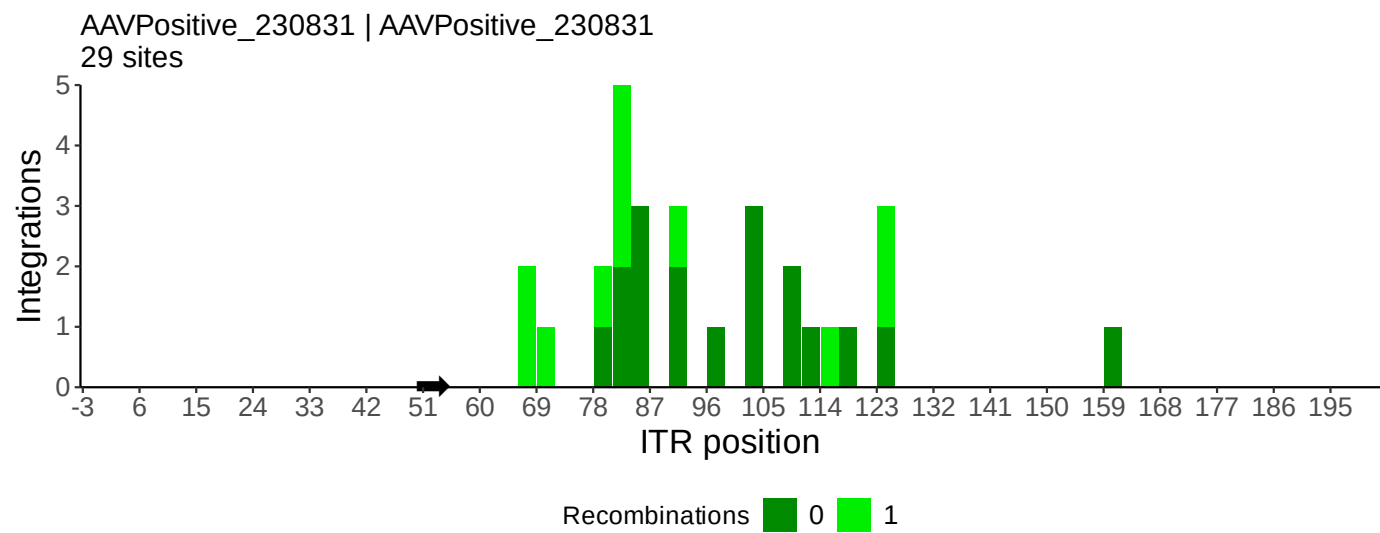
This is a summary table for itr breakpoints.

Table 2: Rearrangement Summary Table

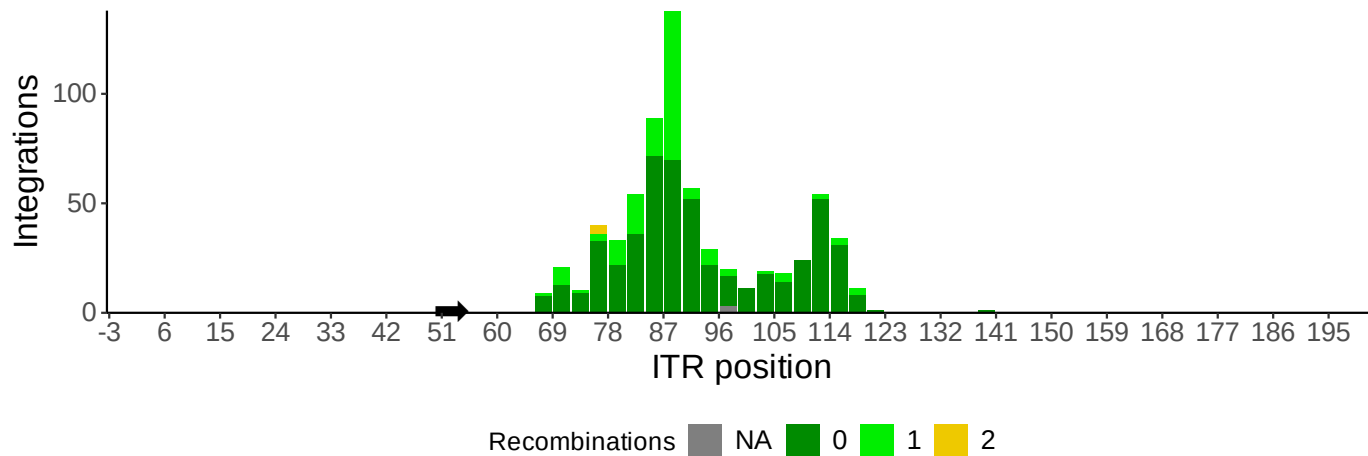
sample	subject	info	break%	breakBool%
AAVPositive_230831	AAVPositive_230831	AAVPositive_230831	37.9	38
AAVPositive_230922	AAVPositive_230922	AAVPositive_230922	41.9	35
GTSP6312	Mock1	Mock1	27.1	24
GTSP6313	Mock2	Mock2	29.1	29
GTSP6314	Mock3	Mock3	21.3	20
GTSP6315	Mock4	Mock4	15.2	14
GTSP6316	Camptothecin1	Camptothecin1	18.9	18
GTSP6317	Camptothecin2	Camptothecin2	36.0	35
GTSP6318	Camptothecin3	Camptothecin3	18.4	18
GTSP6319	Camptothecin4	Camptothecin4	14.8	14
GTSP6320	Doxorubicin1	Doxorubicin1	19.8	19
GTSP6321	Doxorubicin2	Doxorubicin2	18.6	18
GTSP6322	Doxorubicin3	Doxorubicin3	20.9	19
GTSP6323	Doxorubicin4	Doxorubicin4	23.9	23
GTSP6324	Etoposide1	Etoposide1	35.7	33
GTSP6325	Etoposide2	Etoposide2	25.8	25
GTSP6326	Etoposide3	Etoposide3	29.6	26
GTSP6327	Etoposide4	Etoposide4	20.6	19

AAV ITR Breakpoint Plots

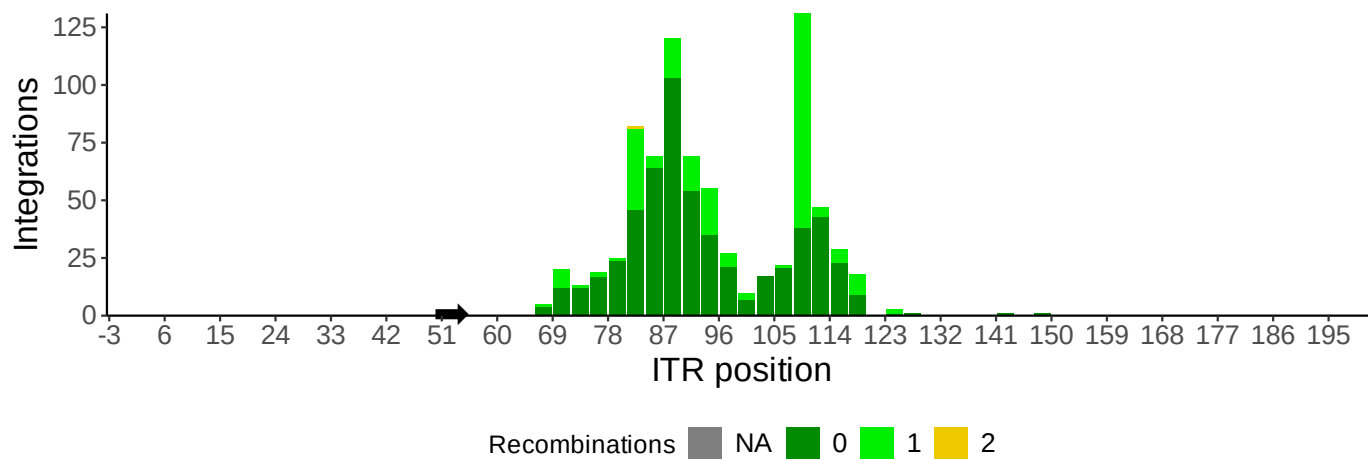
These plots are a visual representation of the ITR breakpoint at the site of genomic integration. The arrow indicates the priming location within the ITR and the x-coordinate of each bar corresponds to a departure from an expected ITR sequence. Each departure from expectation is demarcated as a rearrangement,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



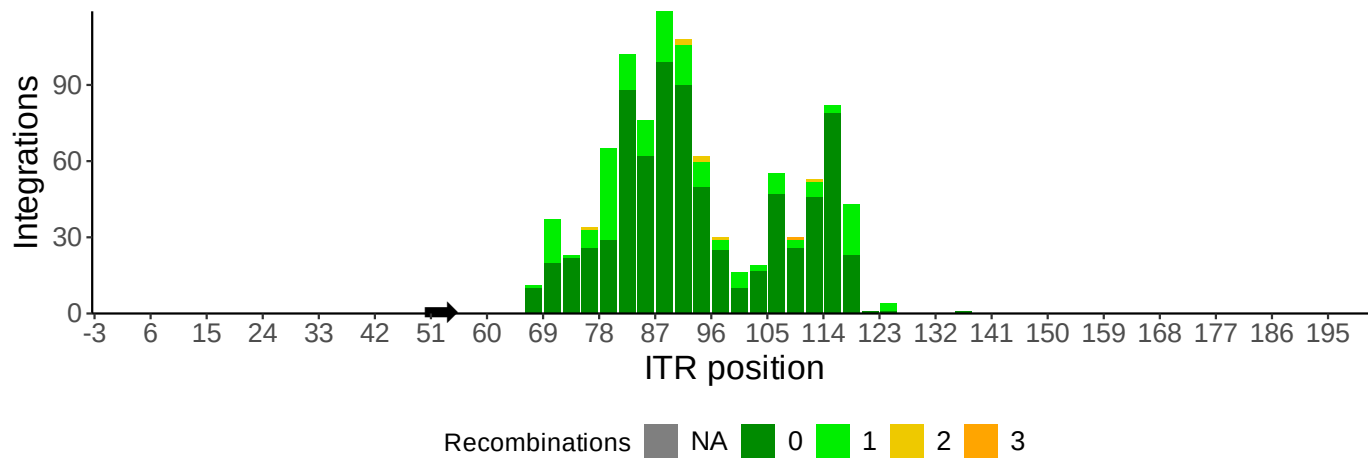
GTSP6312 | Mock1
679 sites



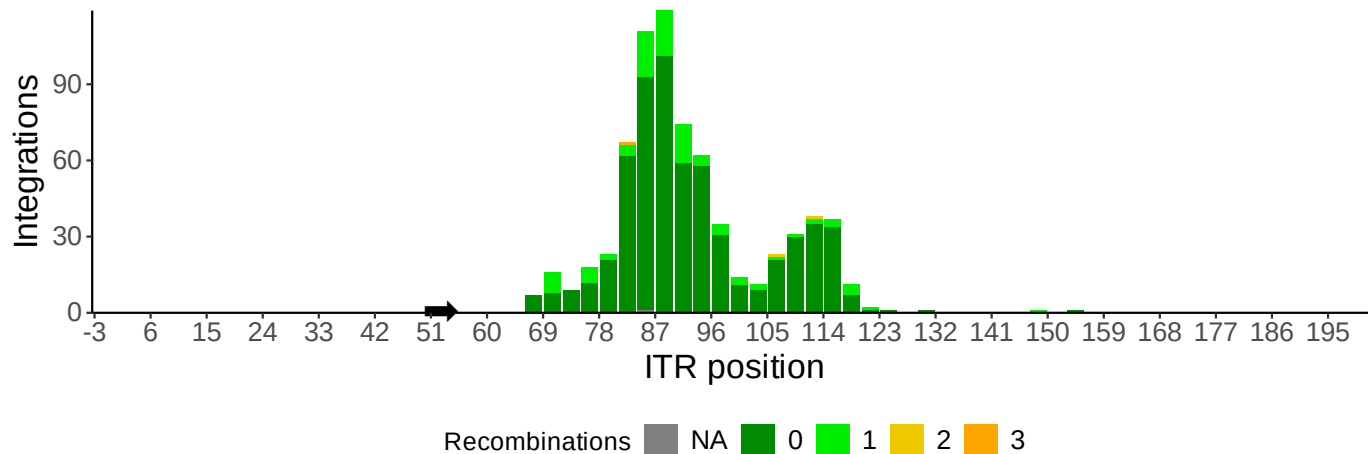
GTSP6313 | Mock2
794 sites



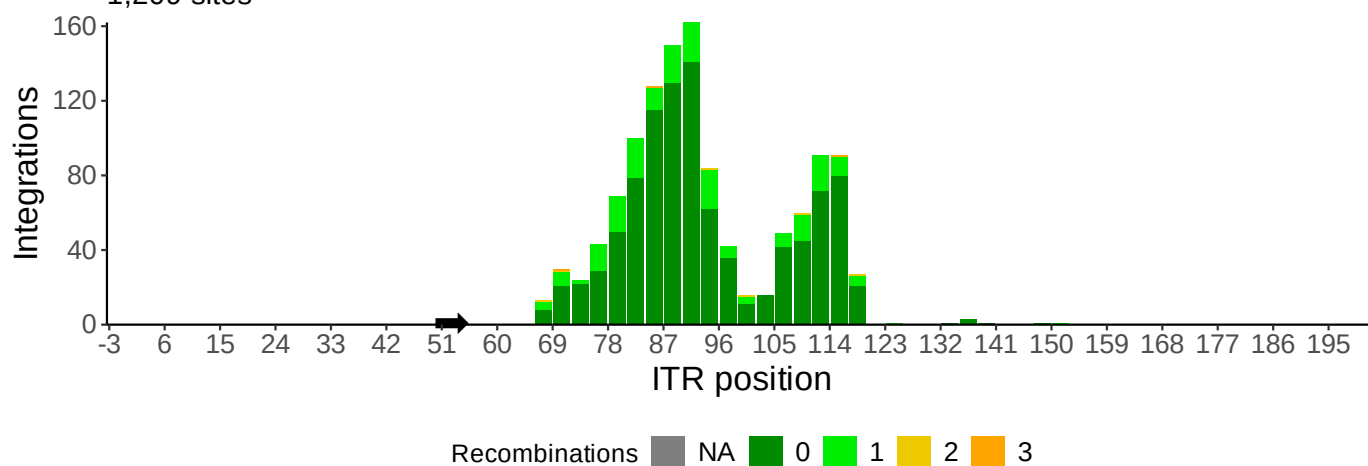
GTSP6314 | Mock3
978 sites



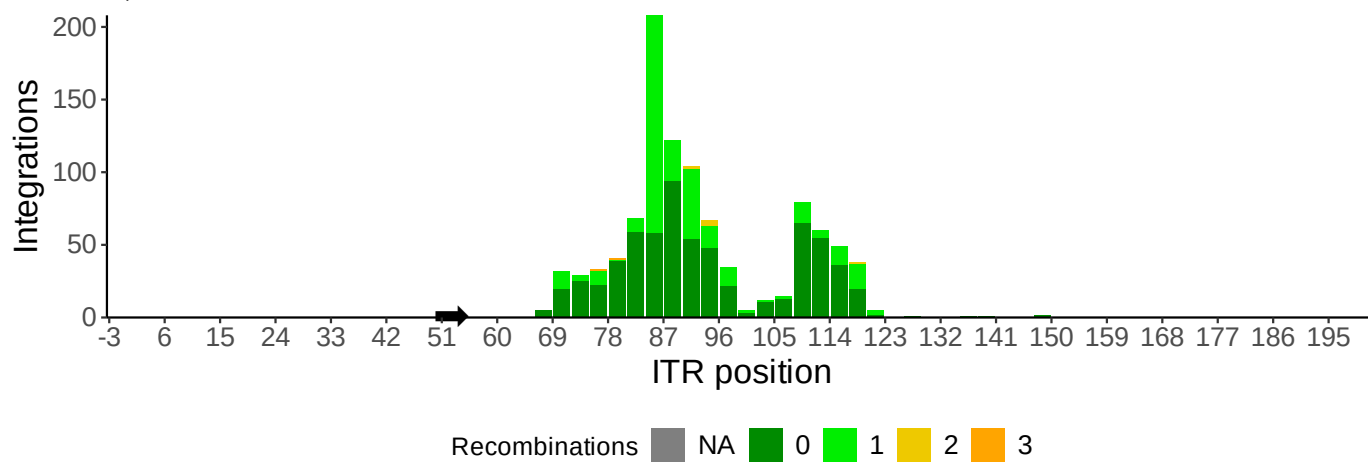
GTSP6315 | Mock4
719 sites



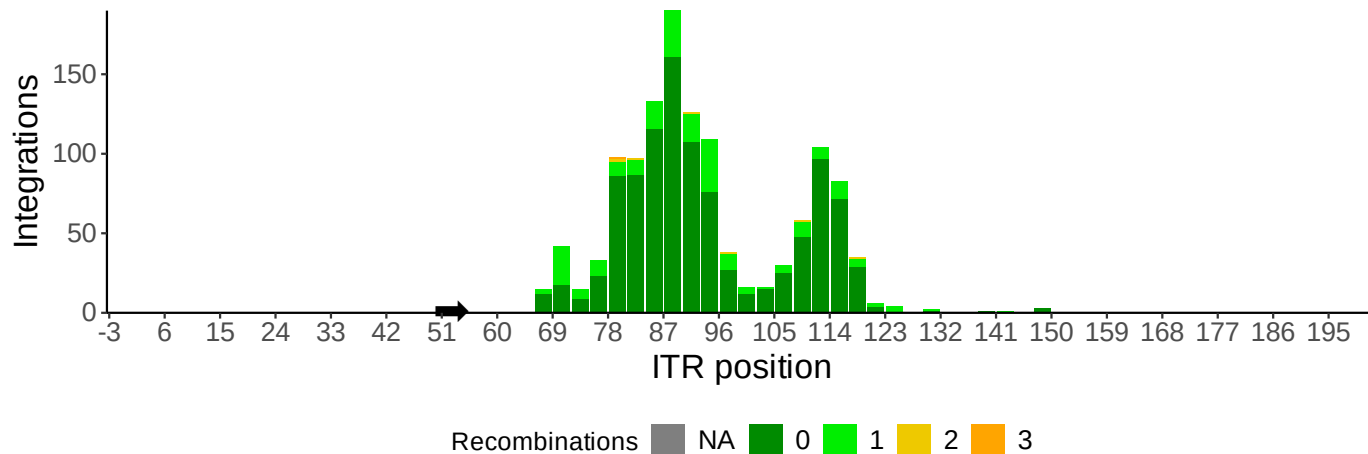
GTSP6316 | Camptothecin1
1,209 sites



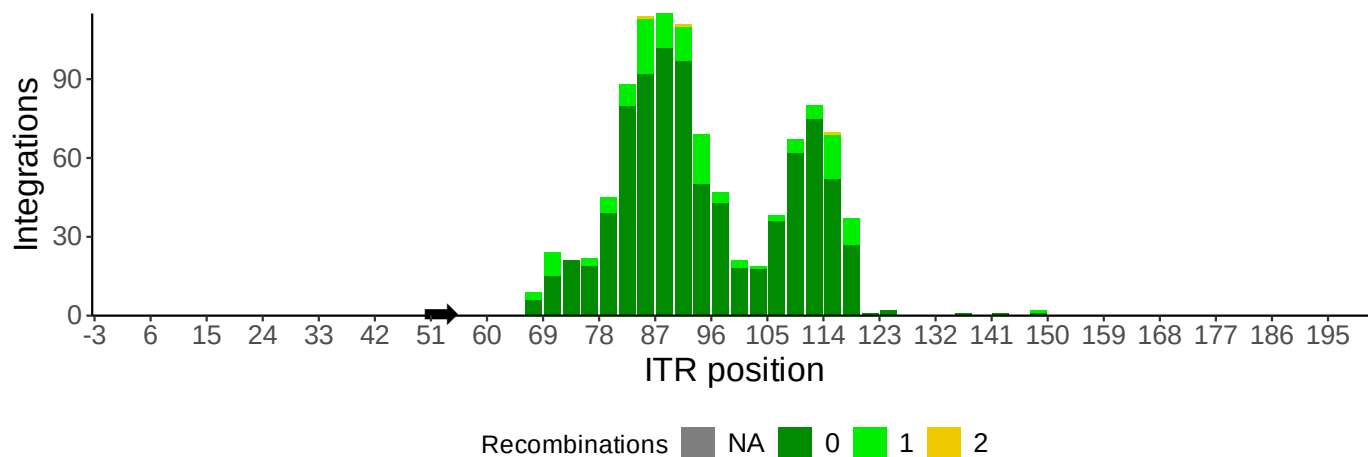
GTSP6317 | Camptothecin2
1,018 sites



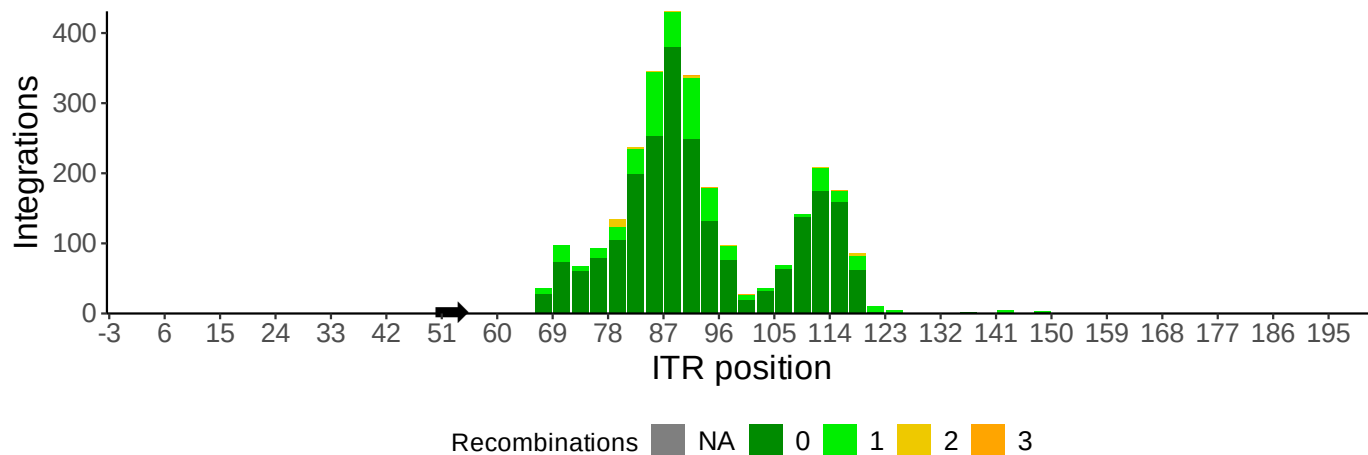
GTSP6318 | Camptothecin3
1,272 sites

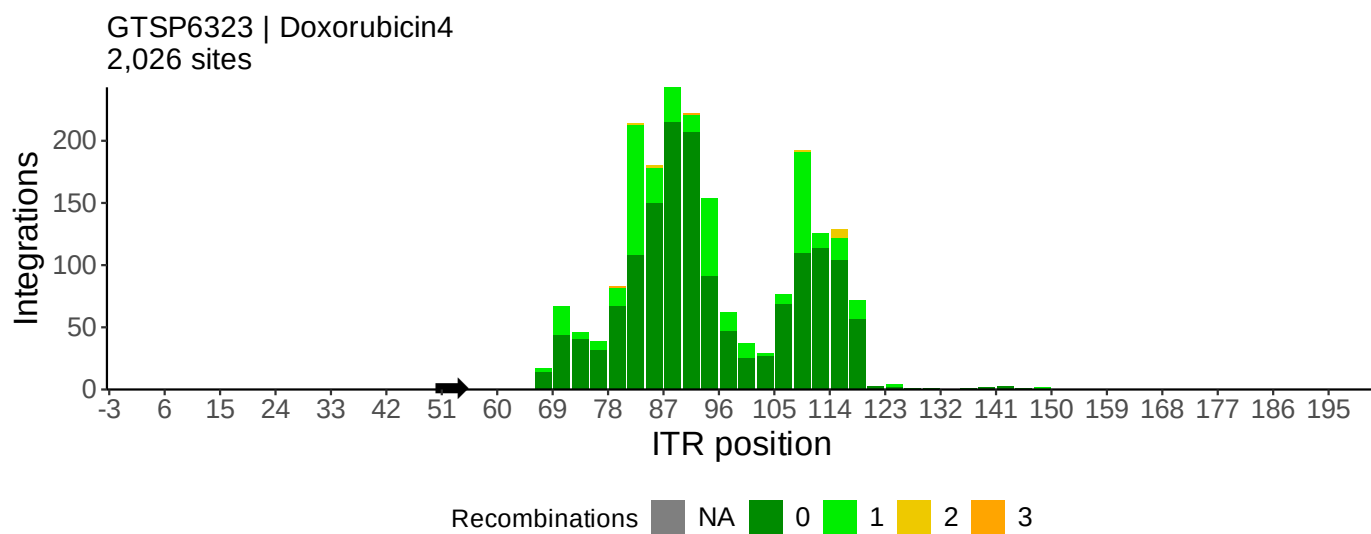
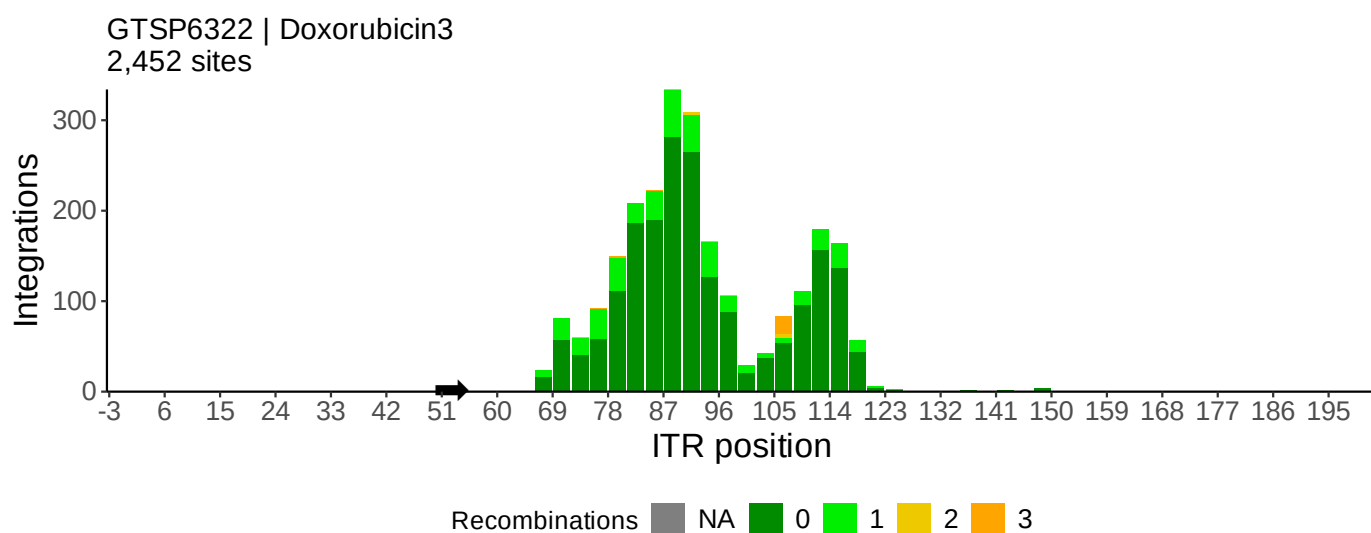
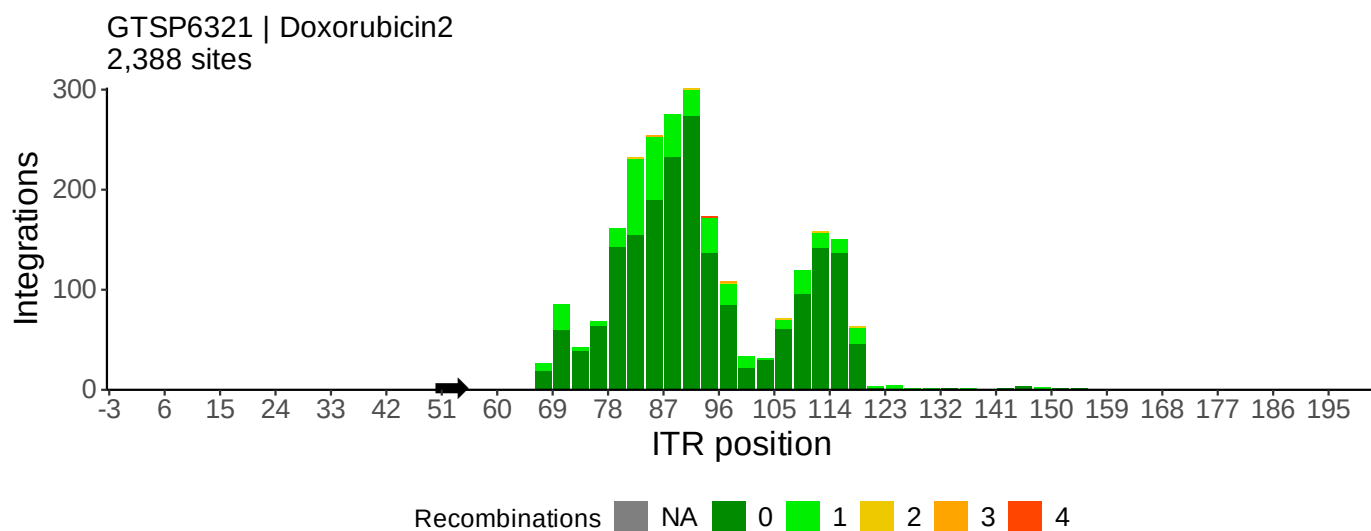


GTSP6319 | Camptothecin4
1,007 sites

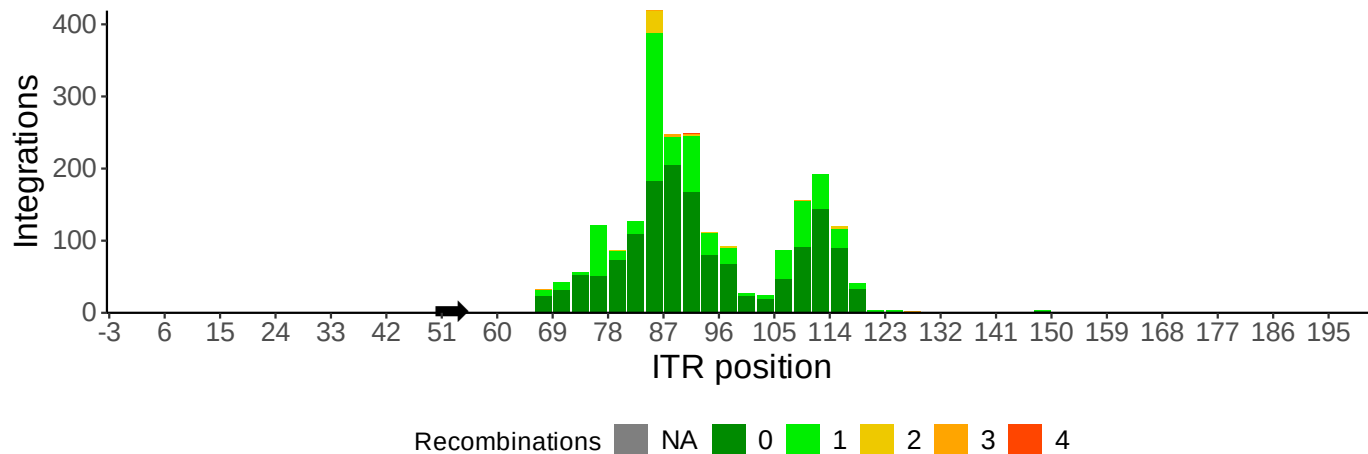


GTSP6320 | Doxorubicin1
2,854 sites

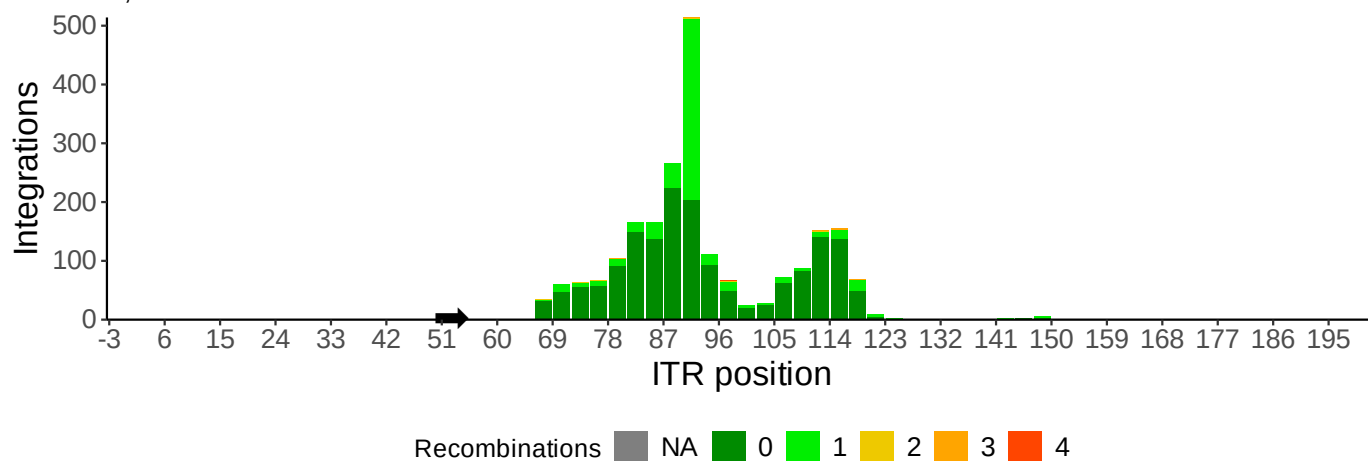




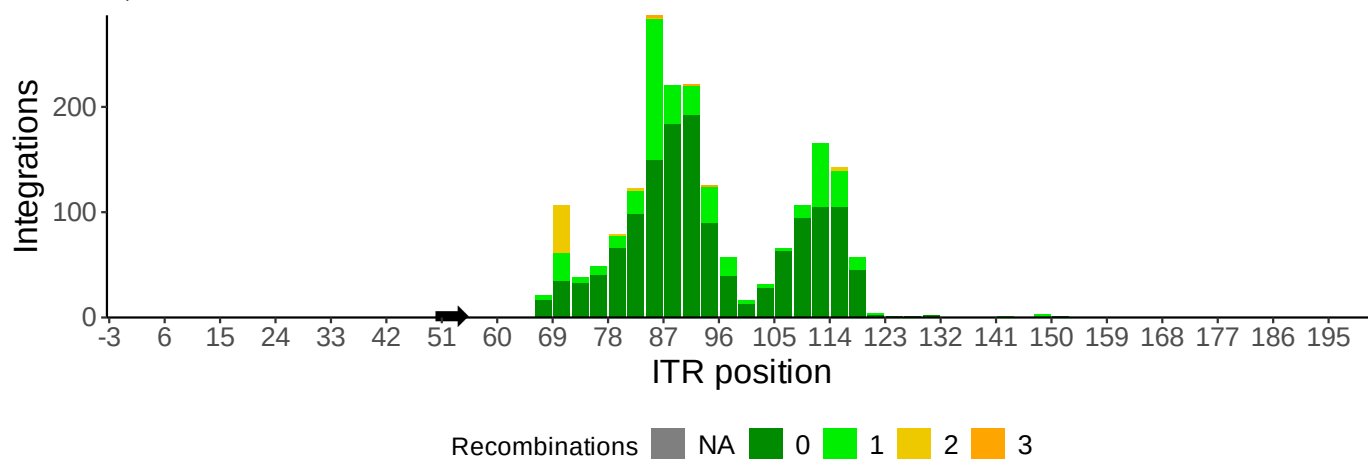
GTSP6324 | Etoposide1
2,263 sites



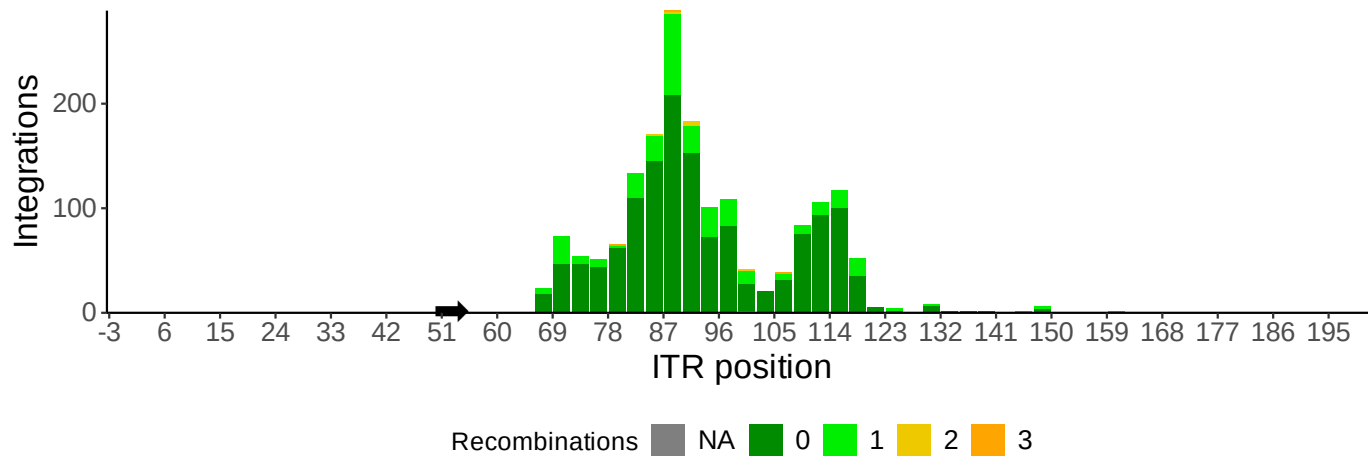
GTSP6325 | Etoposide2
2,246 sites



GTSP6326 | Etoposide3
1,945 sites



GTSP6327 | Etoposide4
1,750 sites



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

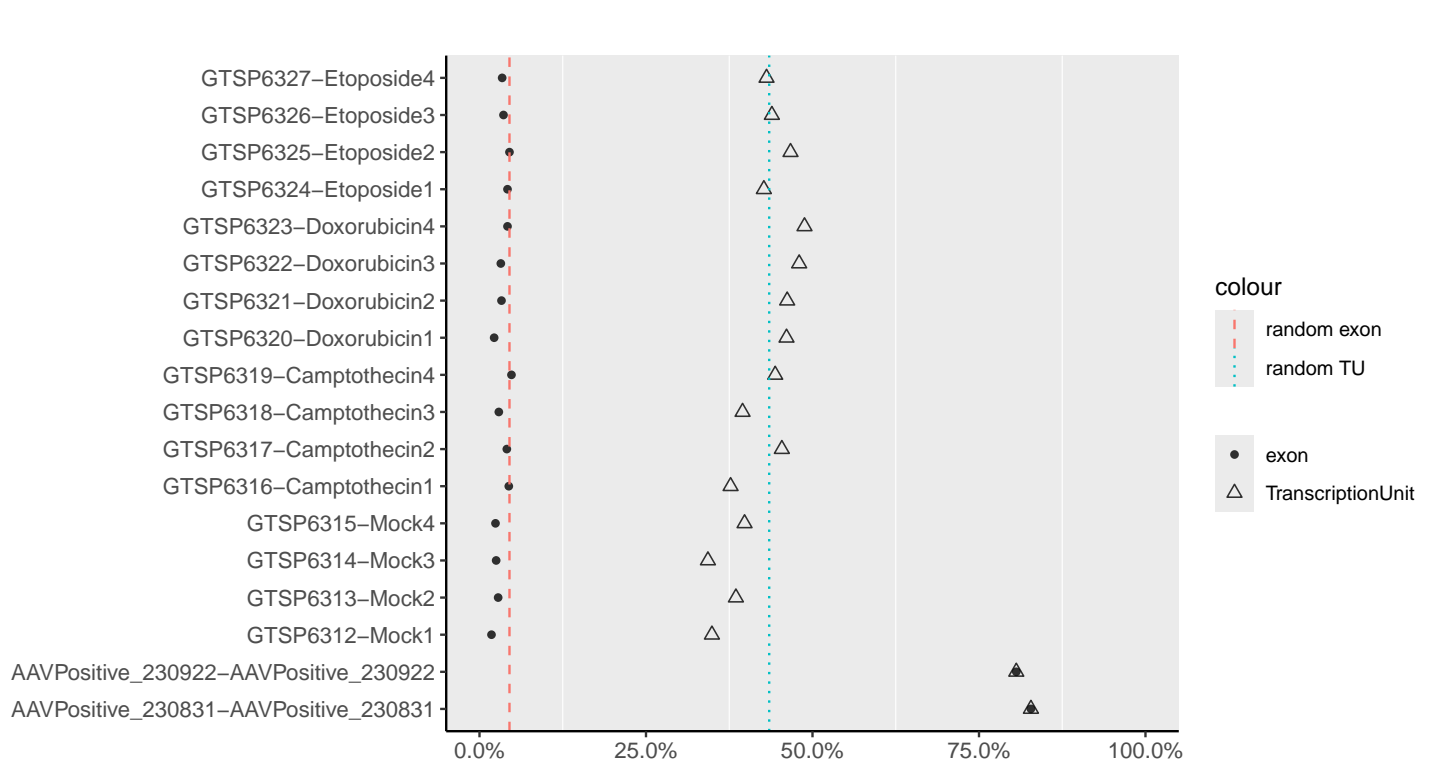


Figure 1: gene distribution scatter plot

Methods

Report Generated on: June 18, 2024
AAVenger Version: 1.1

Modules Called:

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