

# topoDefault

June 28, 2024

## Contents

Introduction	2
PI Summary	2
Sequencing and Integration Summary Table	3
Abundance Frequency Plots	4
AAV ITR Breakpoint Summary	9
AAV ITR Breakpoint Plots	10
Gene distribution dot plots	17
Methods	19

# 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.

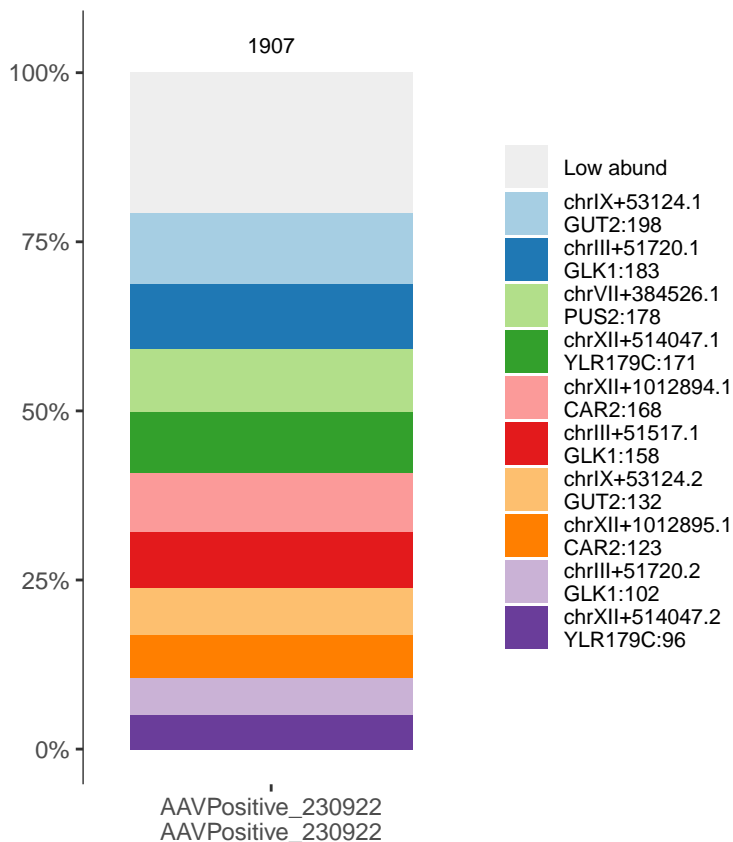
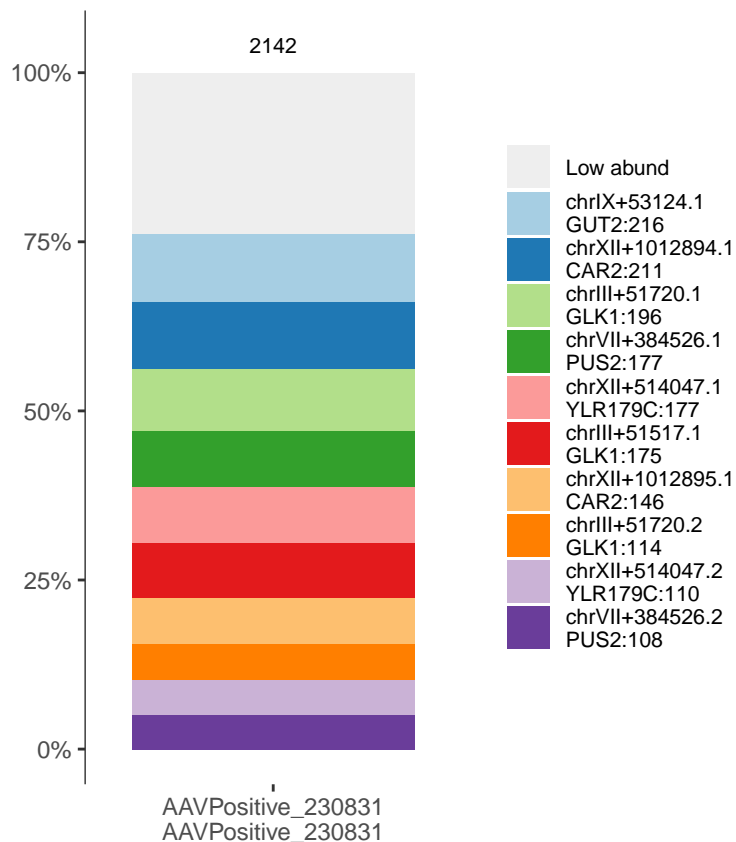
Under most circumstances, only a subset of sites will be sampled. We thus include an estimate of sample size based on the frequency of isolation information from the SonicLength method (Berry, 2012). The ‘S.chao1’ column denotes the estimated lower bound for population size derived using Chao estimate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

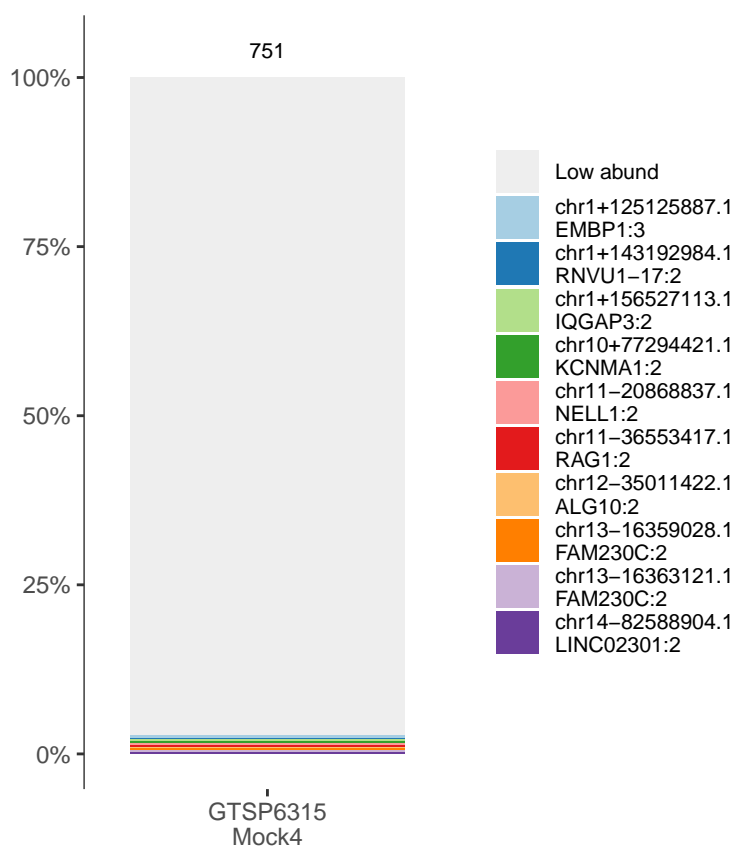
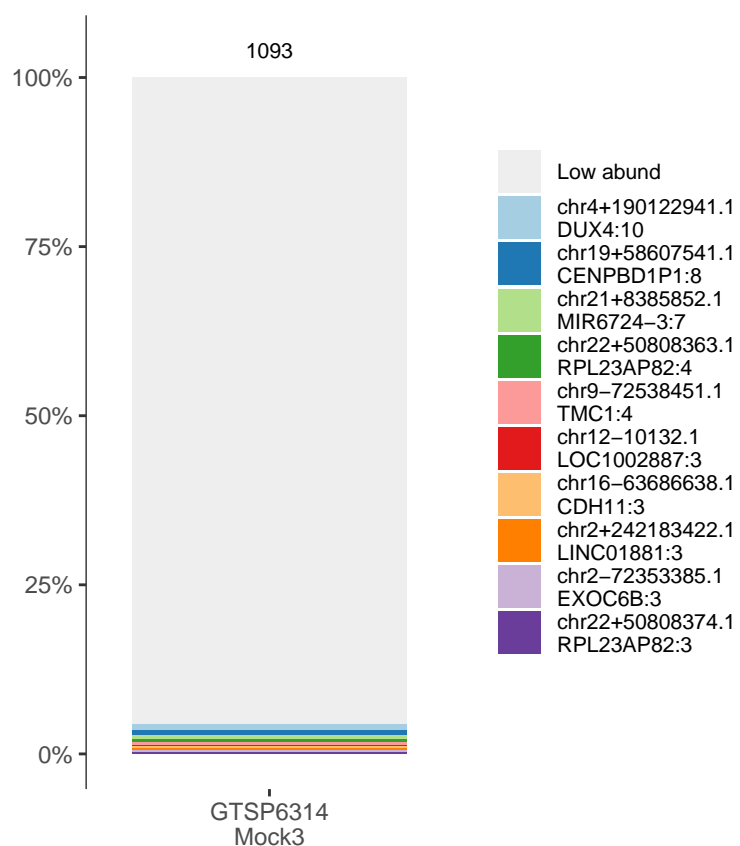
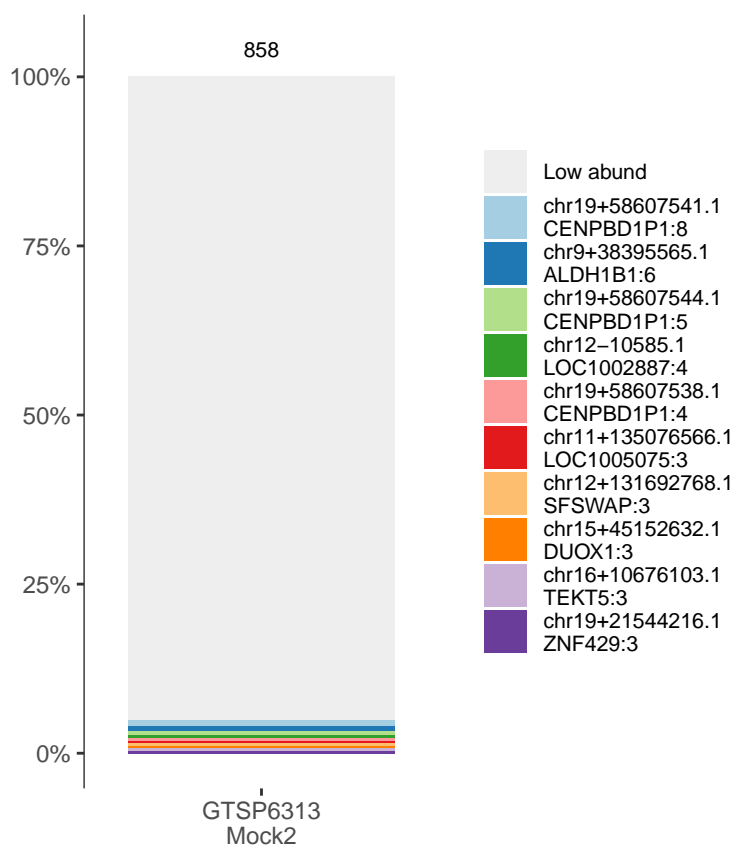
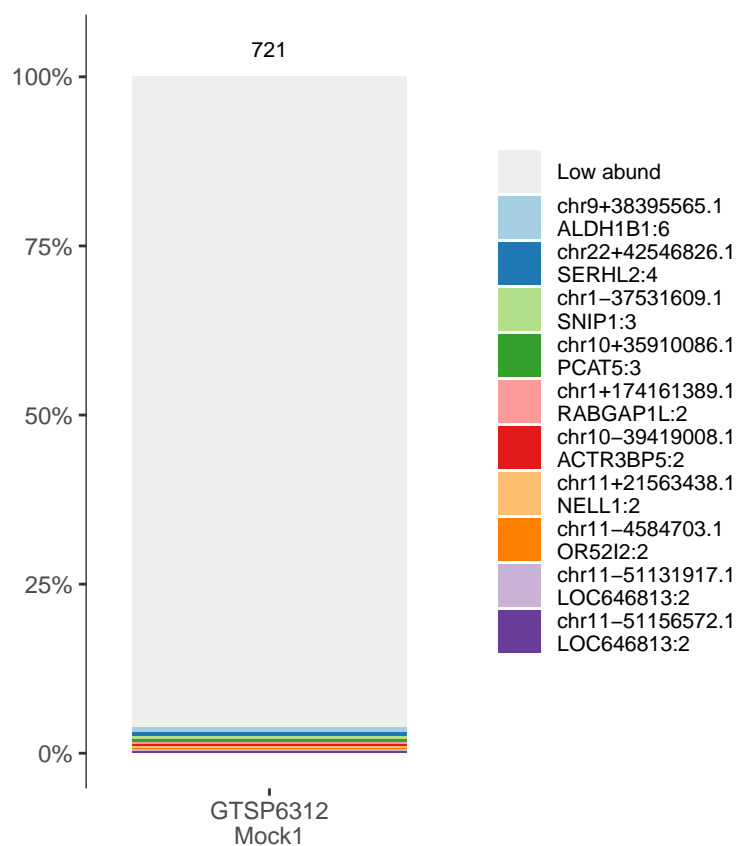
Table 1: integration summary table

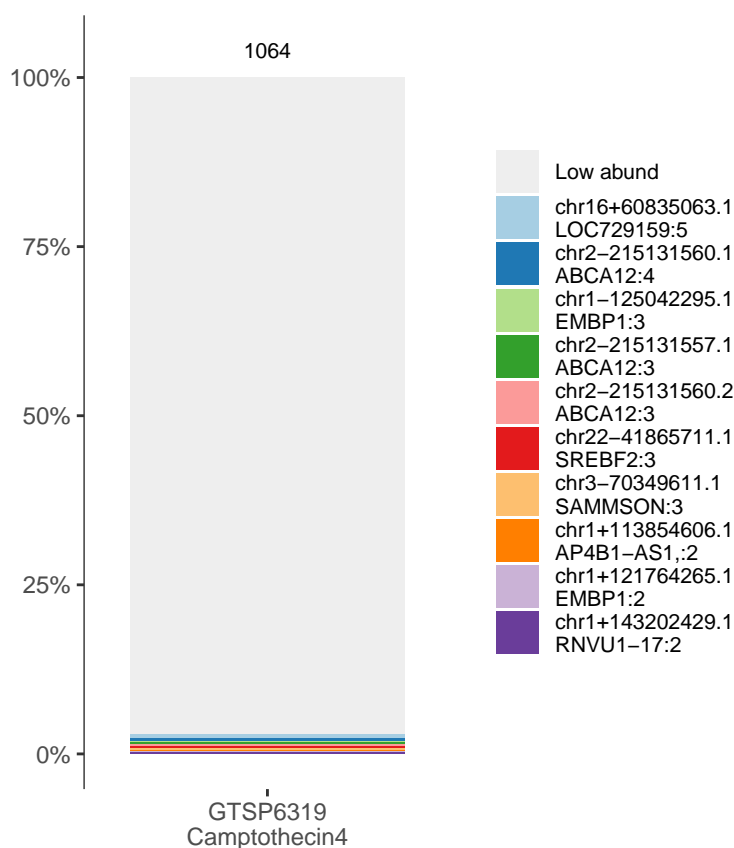
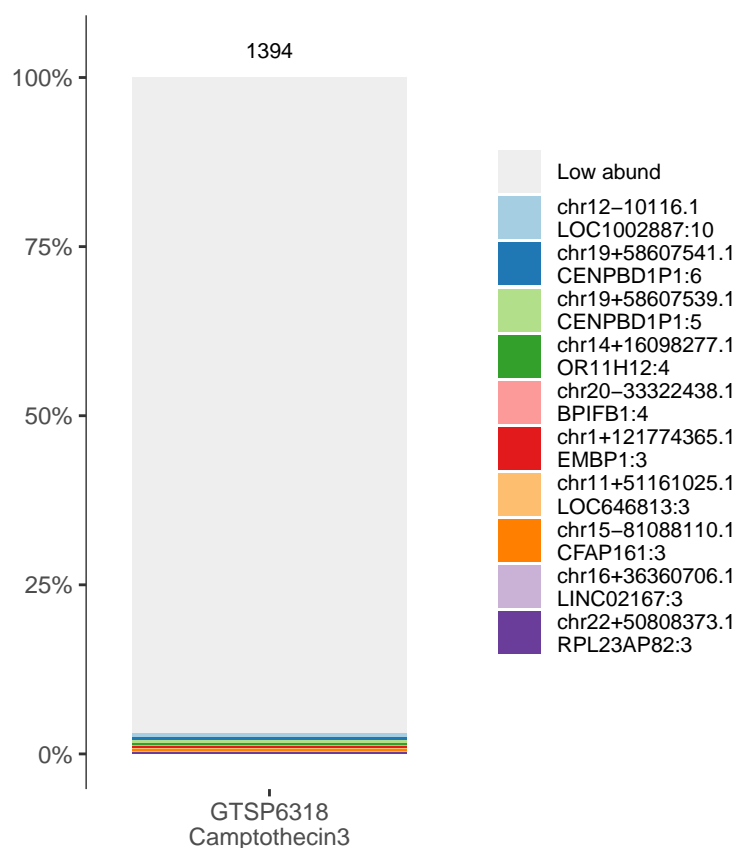
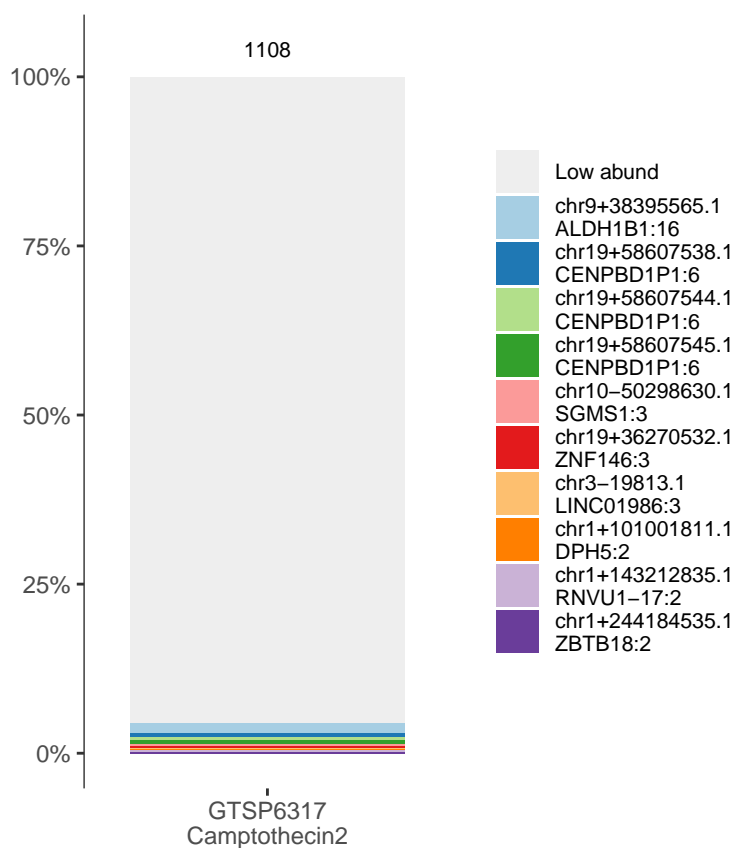
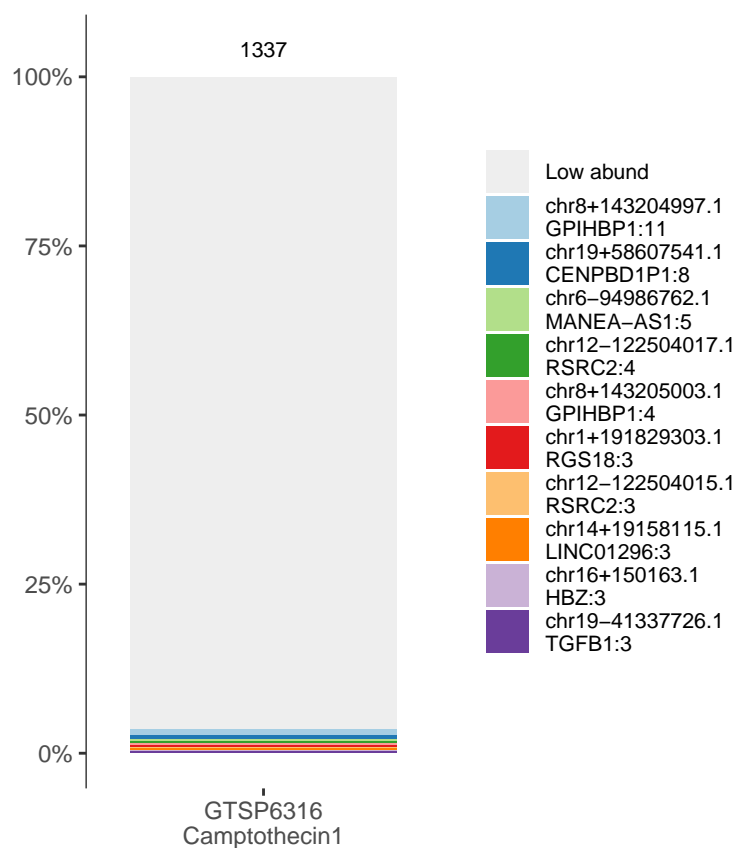
sample	patientID	info	Total Reads	Unique Sites	Inferred Cells	Chao1
AAVPositive_230831	AAVPositive_230831	AAVPositive_230831	319719	29	2142	31.500
AAVPositive_230922	AAVPositive_230922	AAVPositive_230922	208373	31	1907	32.000
GTSP6312	Mock1	Mock1	79003	679	721	7378.677
GTSP6313	Mock2	Mock2	138471	794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

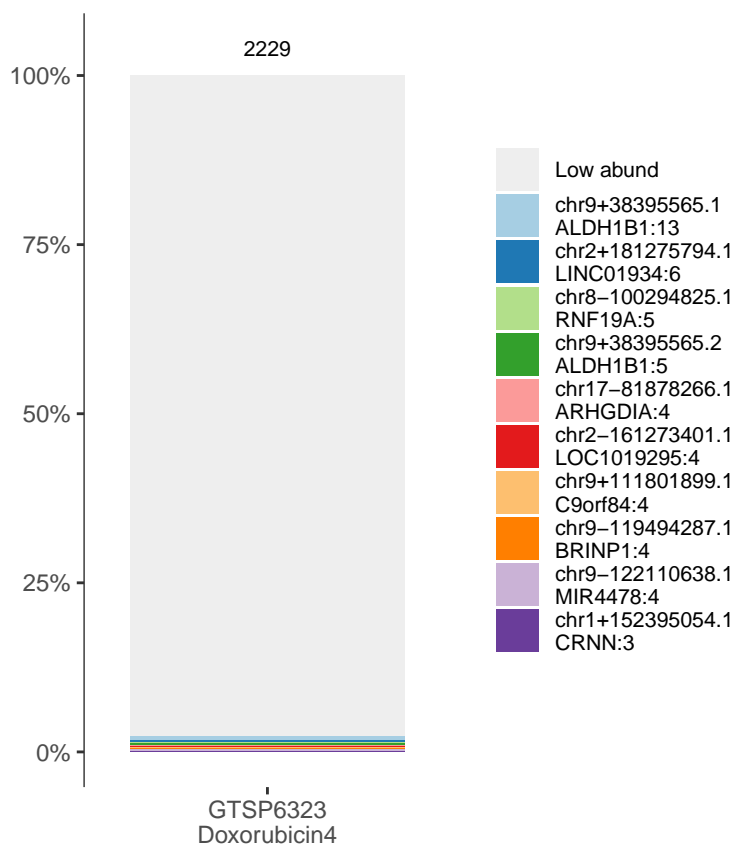
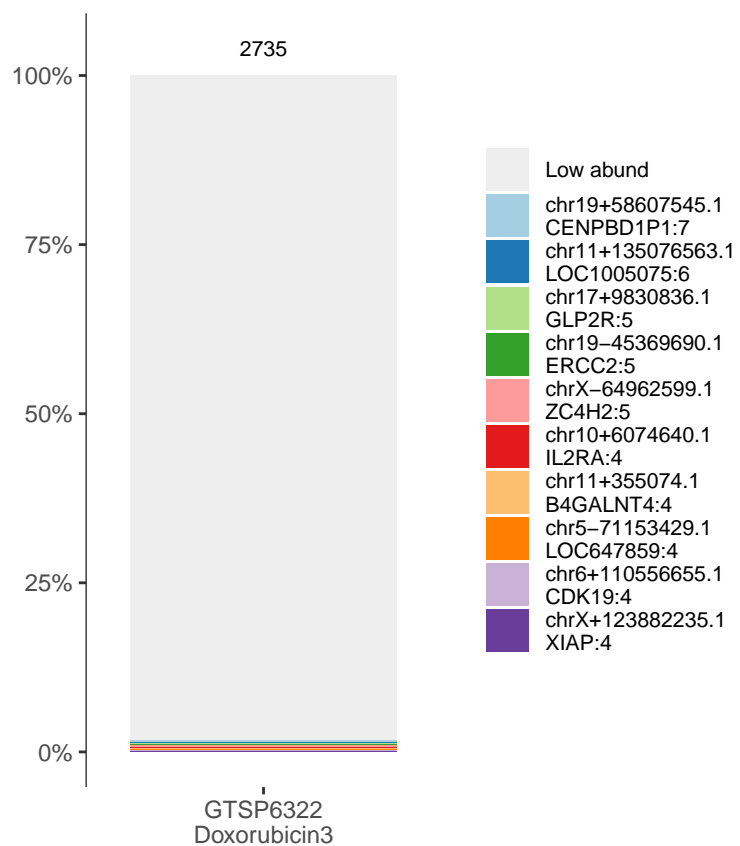
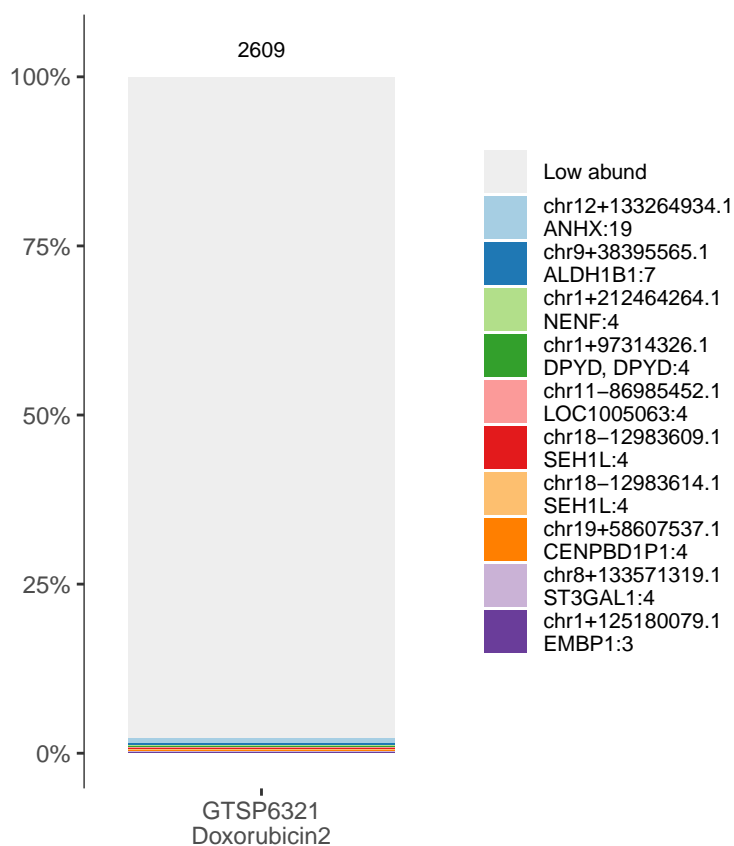
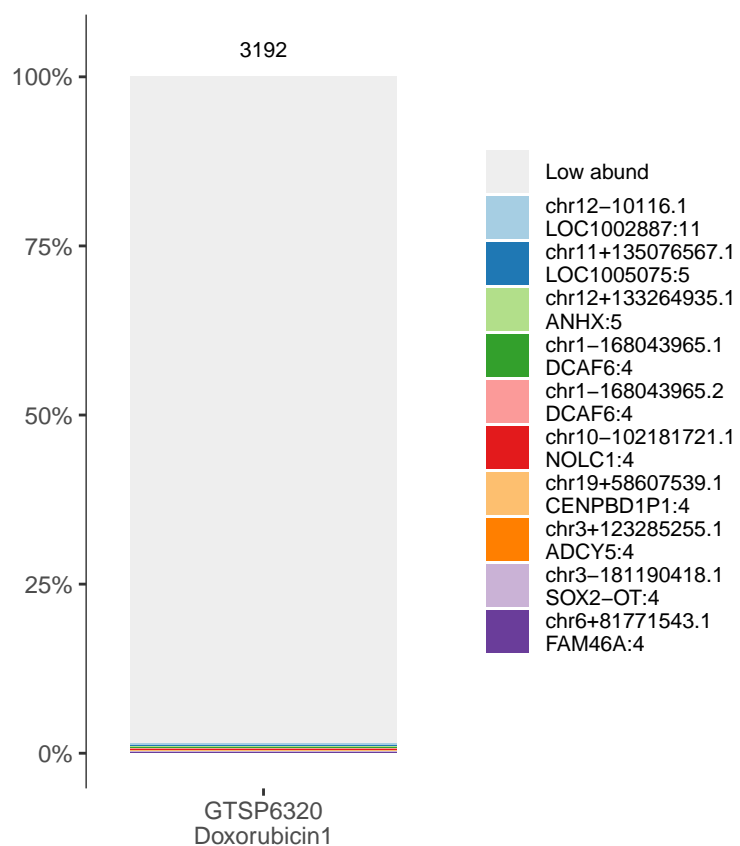
# Abundance Frequency Plots

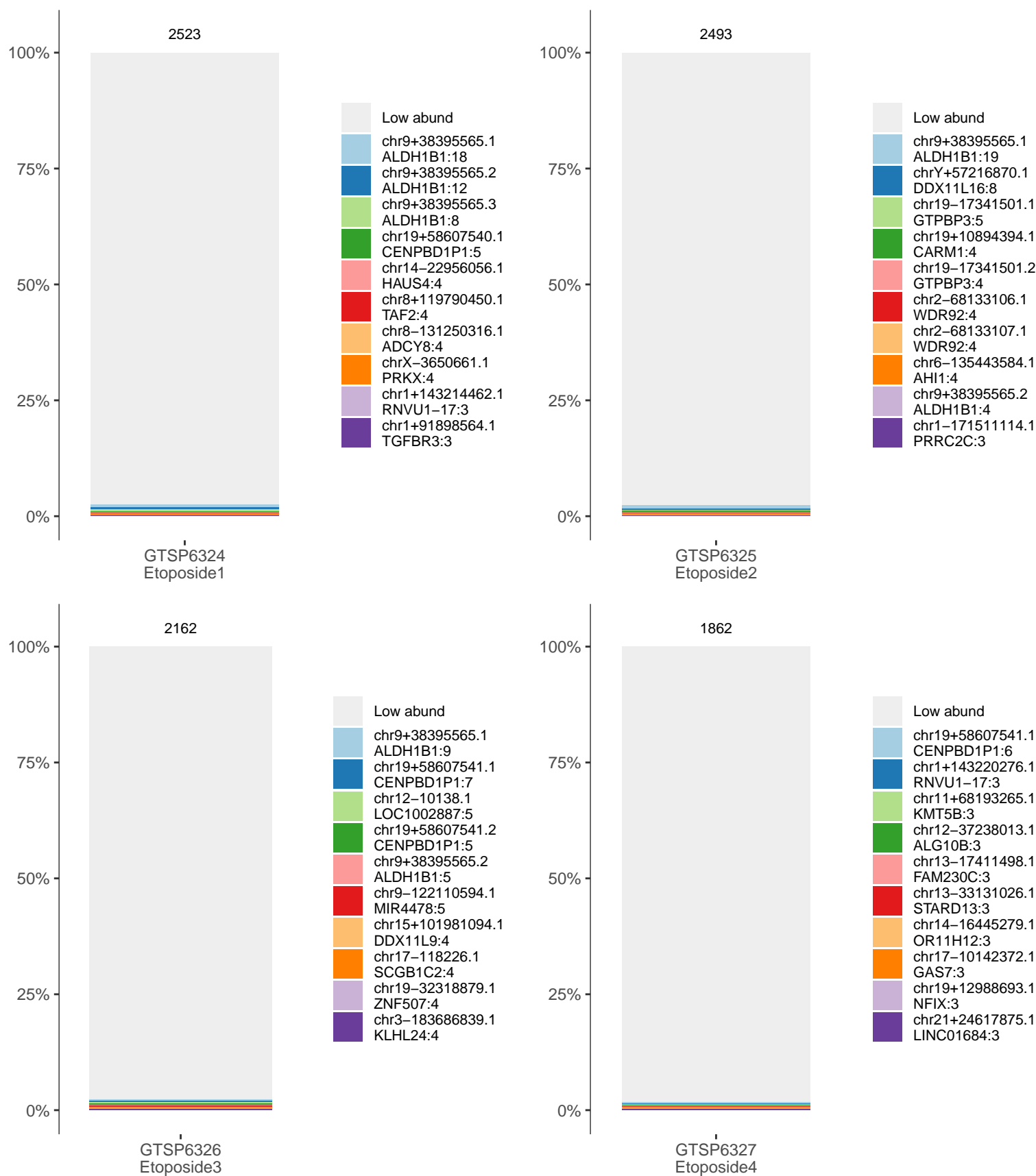
Stacked bar plots indicate clonal abundance frequency. Only the top 10 clonal types are being plotted, and the rest will be plotted in grey as “low abundance”. The number above the stacked bar plots indicates the total clonal types. The legend in each plot correspond to the clonal type integration sites.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













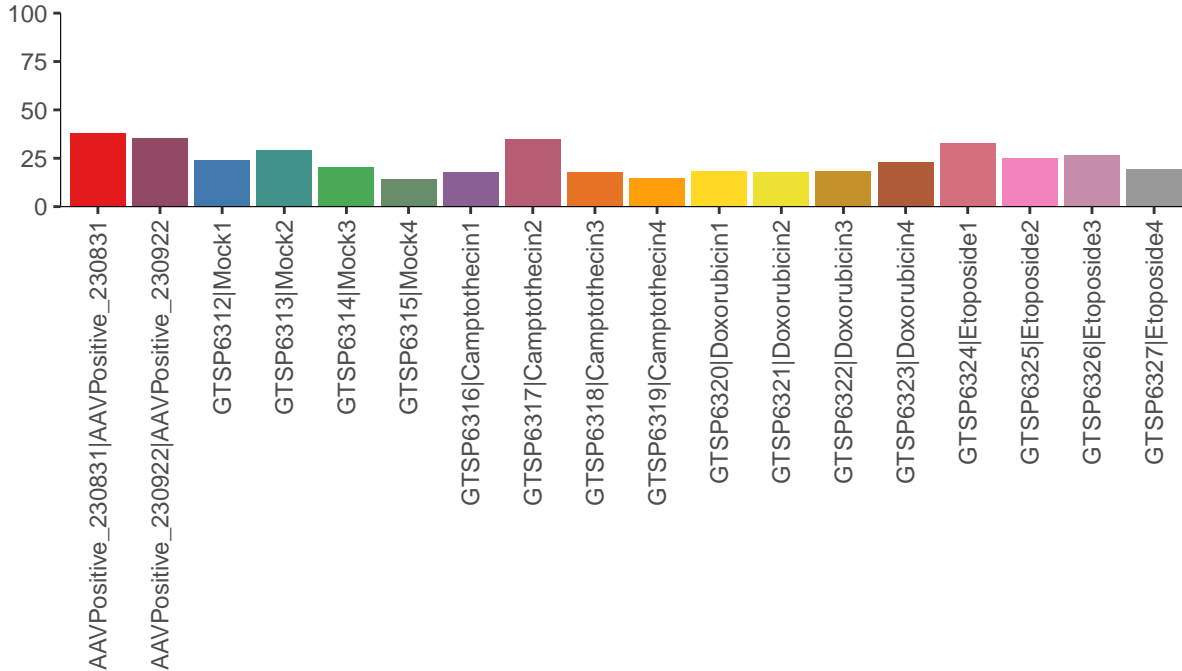
# AAV ITR Breakpoint Summary

This is a summary table for itr breakpoints.

Table 2: Rearrangement Summary Table

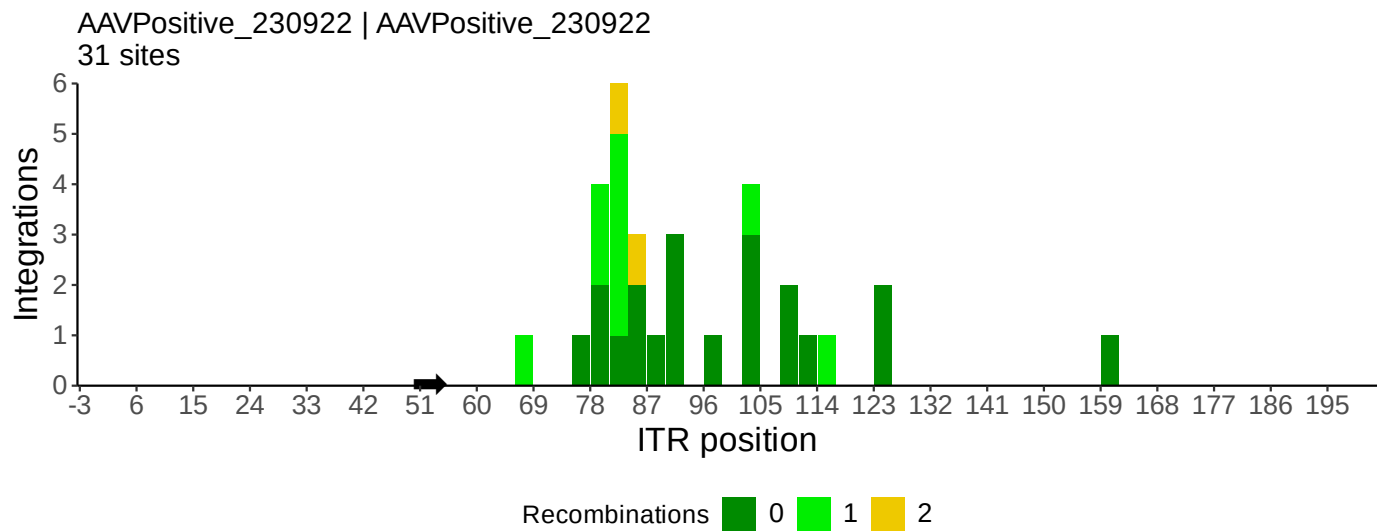
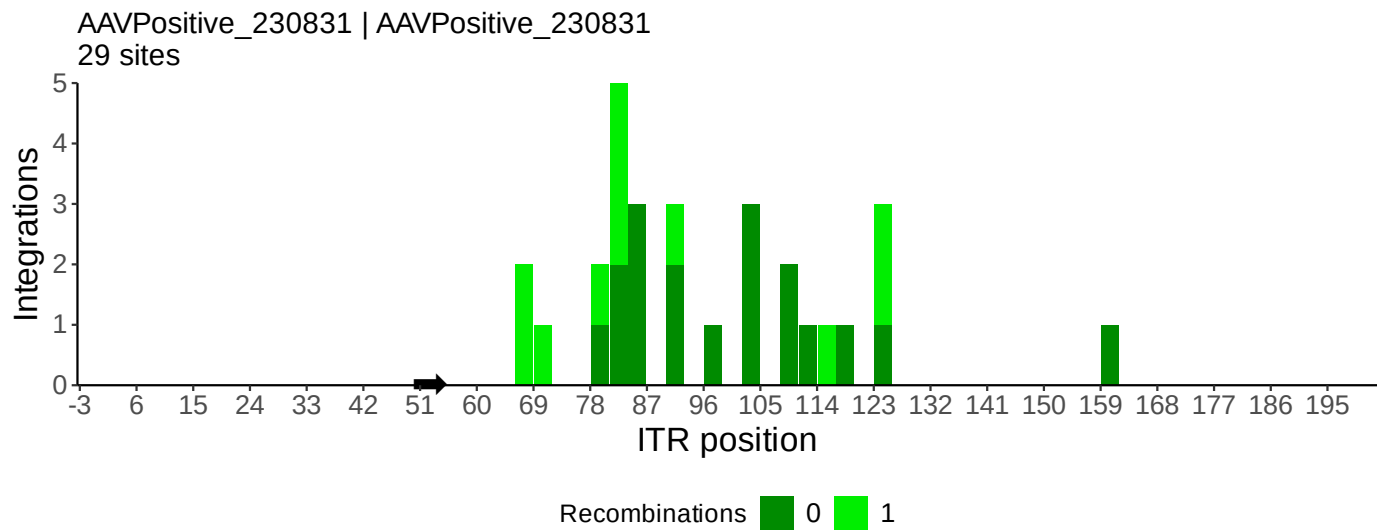
sample	info	break%/length	breakBool%/length	break%/count	breakBool%/count
AAVPositive_230831	AAVPositive_230831	7.54	7.54	37.93	37.93
AAVPositive_230922	AAVPositive_230922	8.44	7.14	41.94	35.48
GTSP6312	Mock1	5.10	4.49	27.10	23.86
GTSP6313	Mock2	5.15	5.13	29.09	28.97
GTSP6314	Mock3	4.65	4.45	21.27	20.35
GTSP6315	Mock4	3.53	3.27	15.16	14.05
GTSP6316	Camptothecin1	3.96	3.75	18.94	17.95
GTSP6317	Camptothecin2	5.31	5.15	35.95	34.87
GTSP6318	Camptothecin3	3.99	3.84	18.40	17.69
GTSP6319	Camptothecin4	3.26	3.20	14.80	14.50
GTSP6320	Doxorubicin1	4.20	3.95	19.80	18.61
GTSP6321	Doxorubicin2	3.80	3.67	18.63	17.96
GTSP6322	Doxorubicin3	4.22	3.75	20.88	18.56
GTSP6323	Doxorubicin4	4.27	4.14	23.94	23.20
GTSP6324	Etoposide1	6.00	5.53	35.70	32.88
GTSP6325	Etoposide2	5.14	4.94	25.82	24.84
GTSP6326	Etoposide3	4.97	4.45	29.56	26.48
GTSP6327	Etoposide4	4.19	3.97	20.57	19.49

Percentage of Boolean Breaks over Sites per Sample

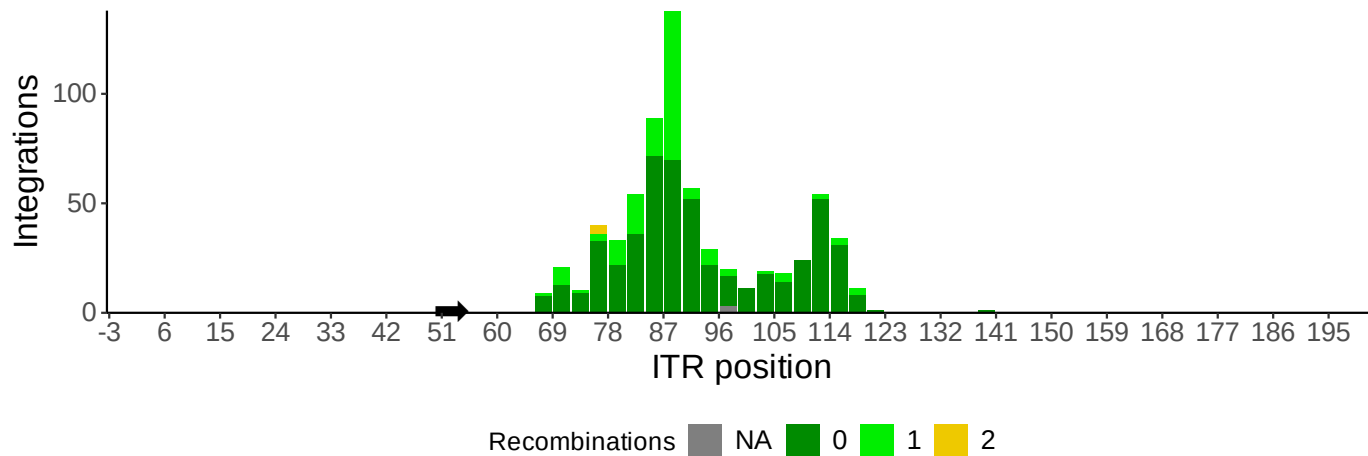


# AAV ITR Breakpoint Plots

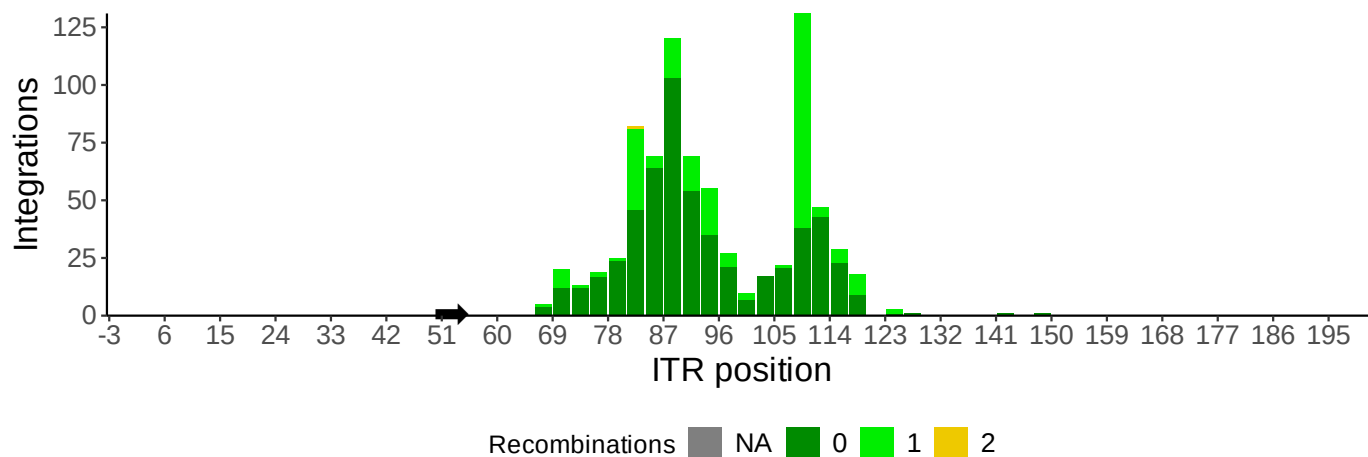
These plots are a visual representation of the ITR breakpoint at the site of genomic integration. The arrow indicates the priming location within the ITR and the x-coordinate of each bar corresponds to a departure from an expected ITR sequence. Each departure from expectation is demarcated as a rearrangement,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



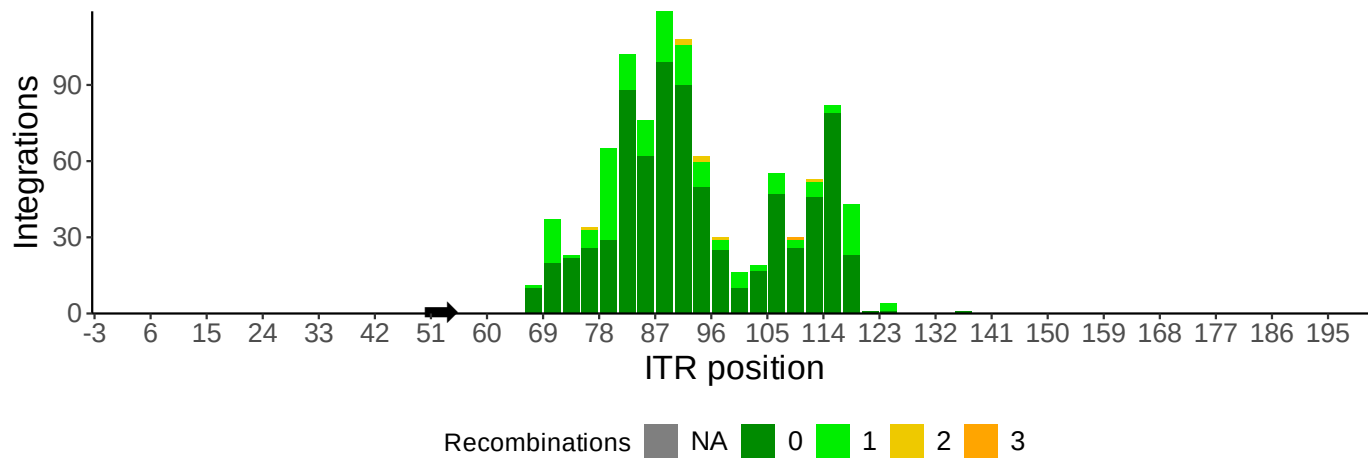
GTSP6312 | Mock1  
679 sites



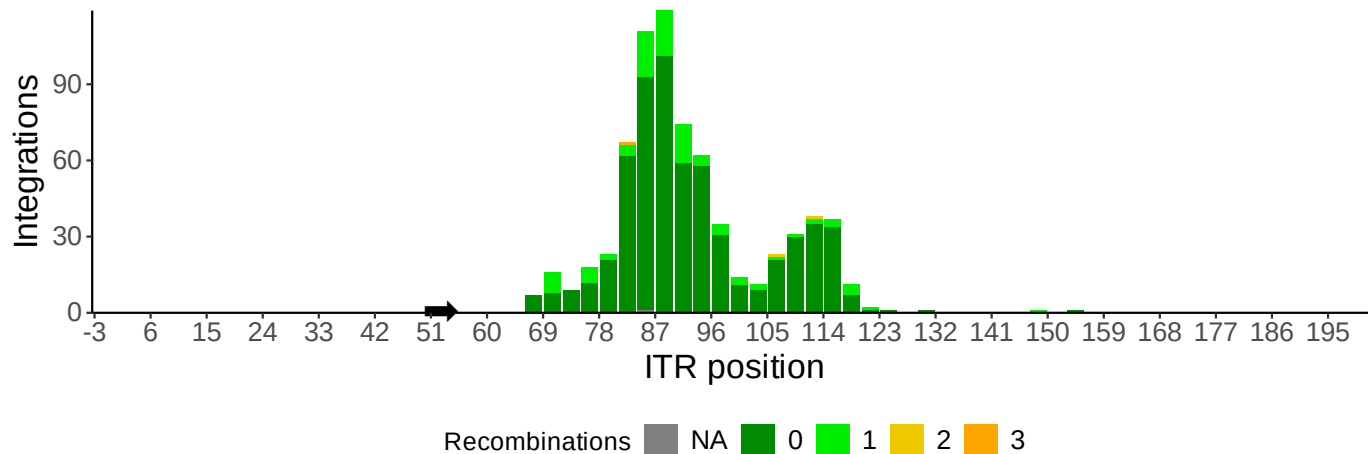
GTSP6313 | Mock2  
794 sites



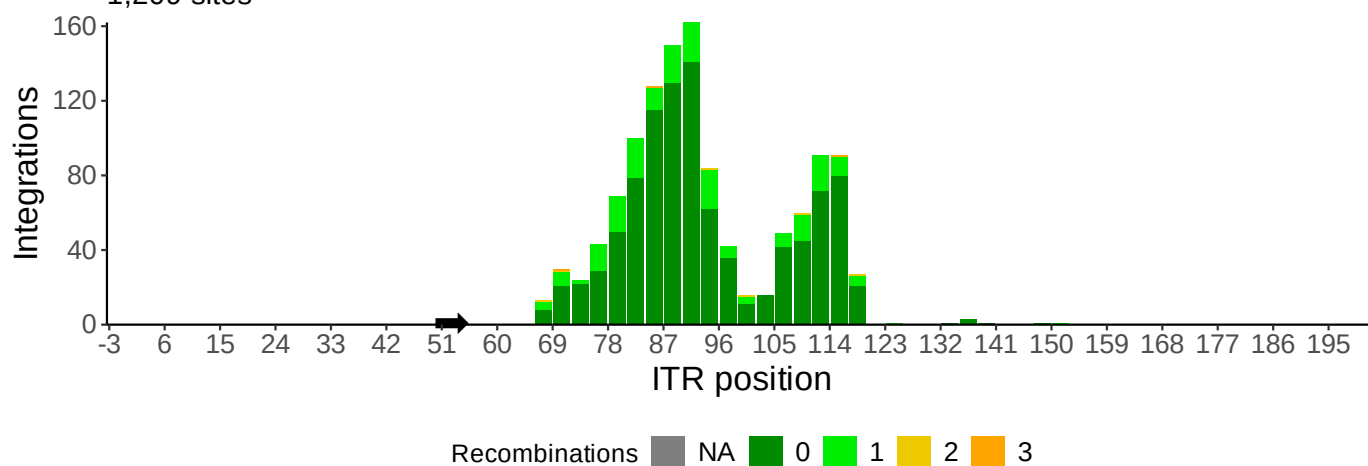
GTSP6314 | Mock3  
978 sites



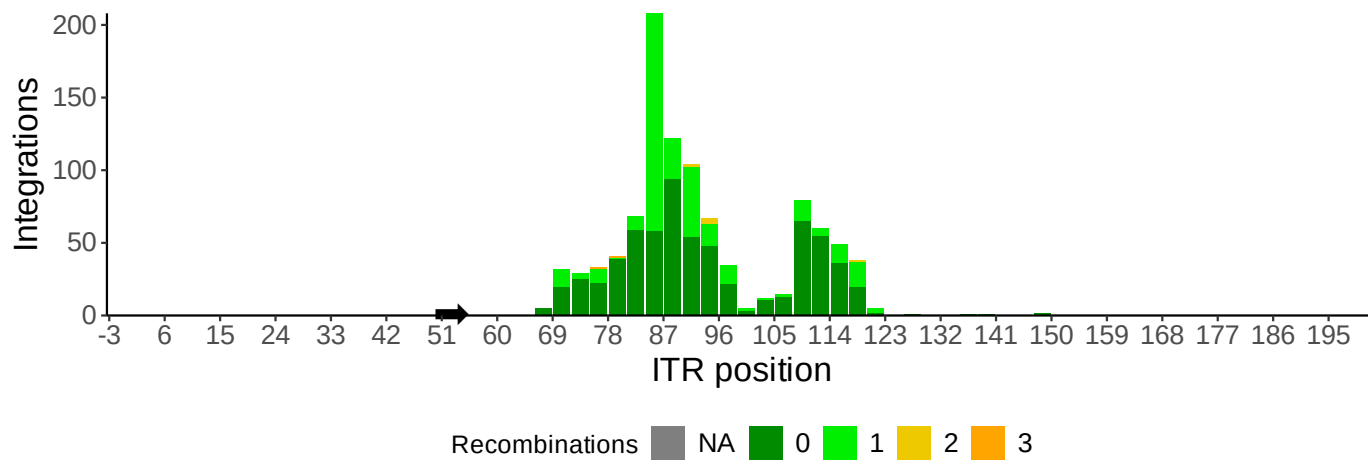
GTSP6315 | Mock4  
719 sites



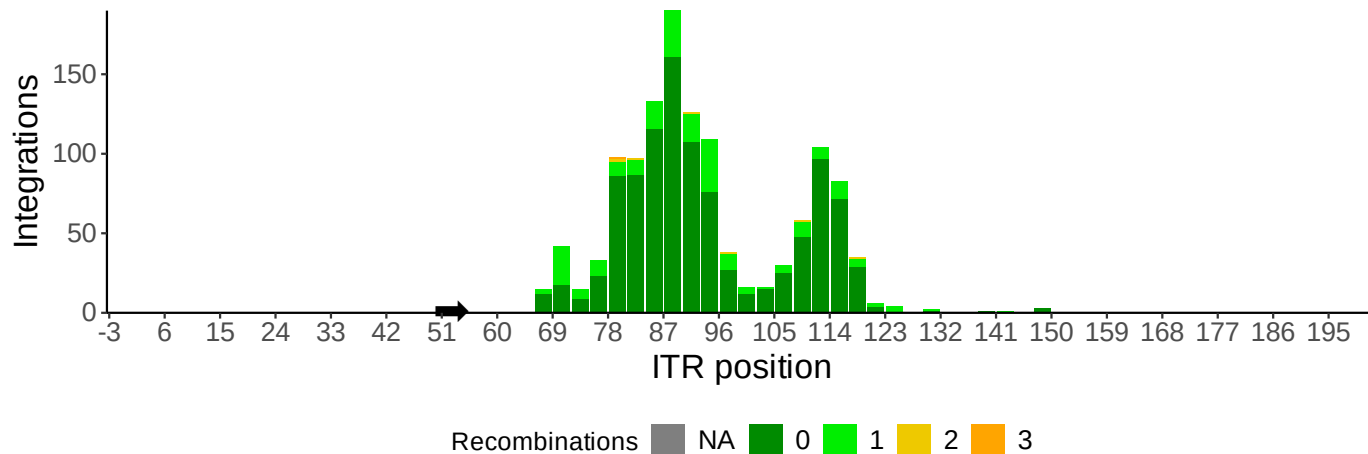
GTSP6316 | Camptothecin1  
1,209 sites



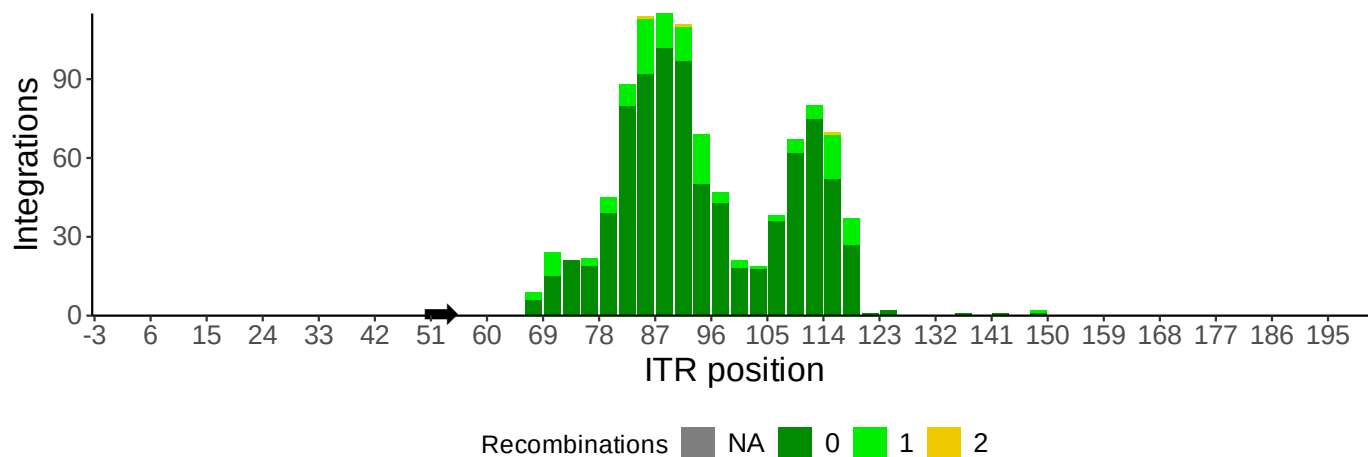
GTSP6317 | Camptothecin2  
1,018 sites



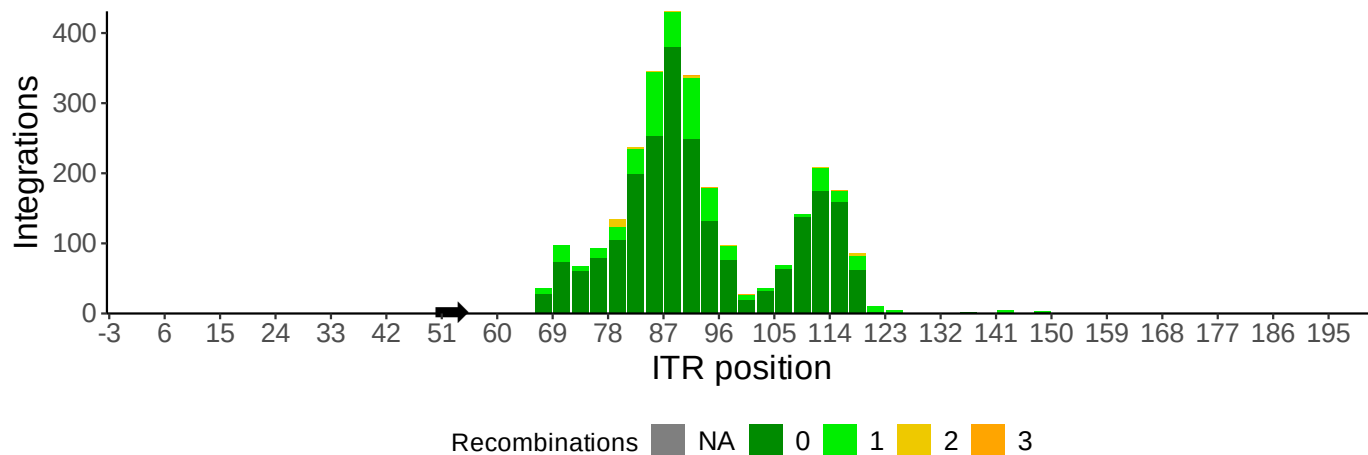
GTSP6318 | Camptothecin3  
1,272 sites

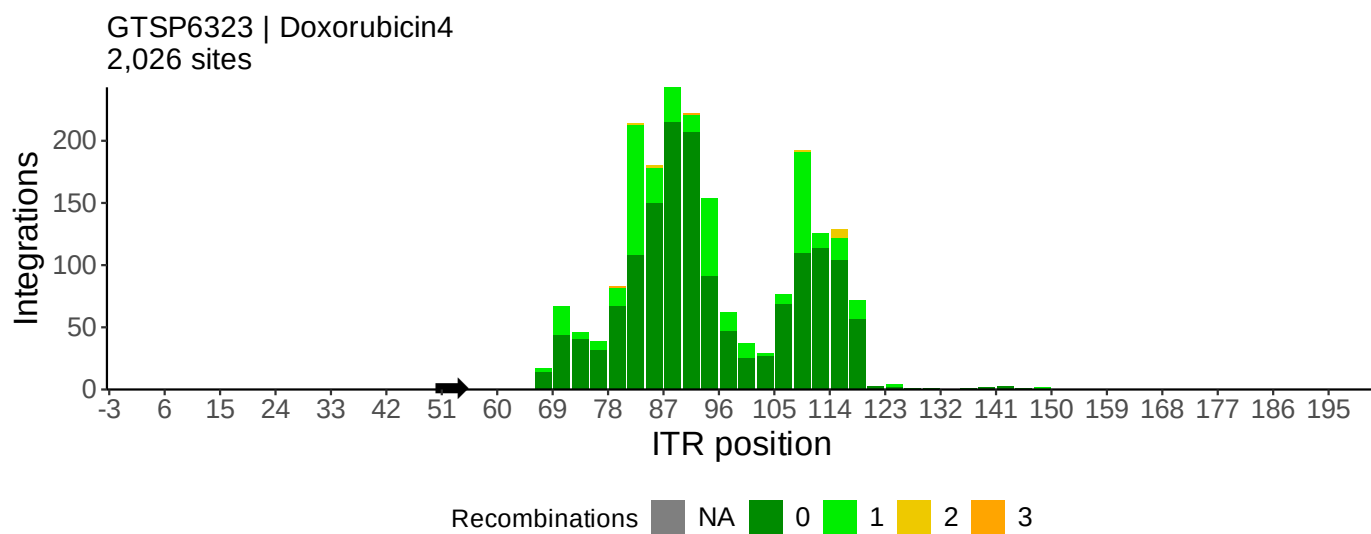
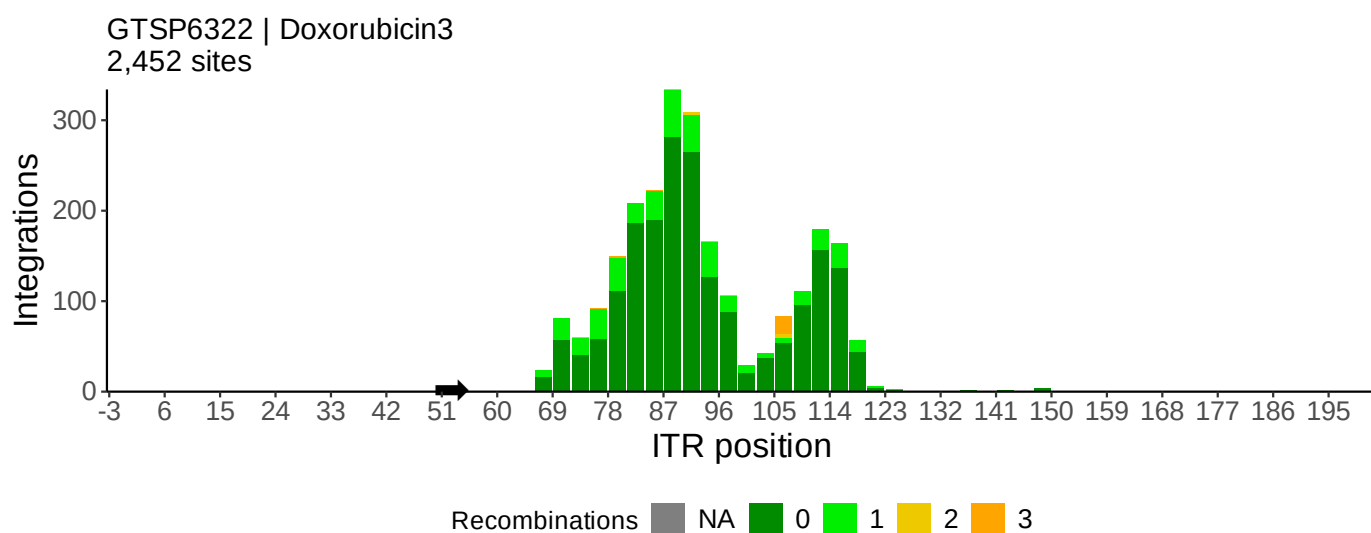
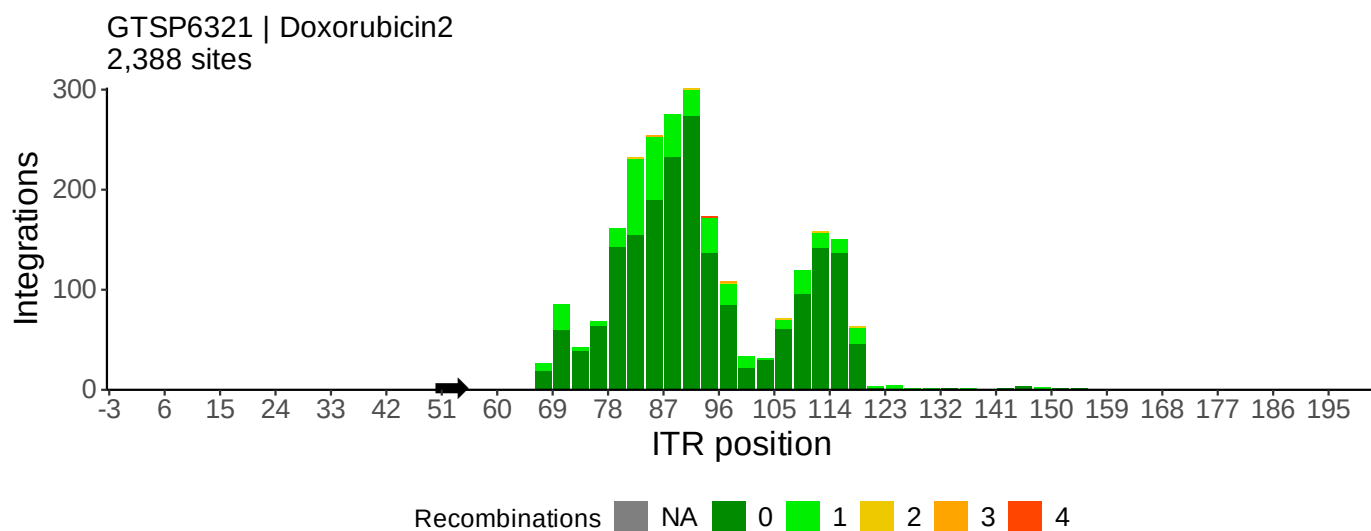


GTSP6319 | Camptothecin4  
1,007 sites

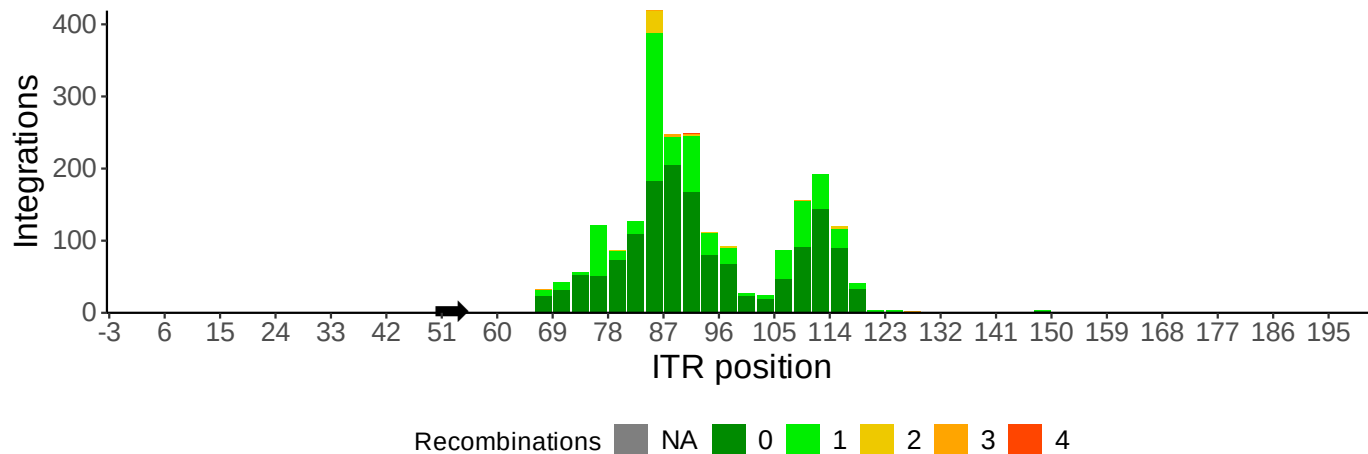


GTSP6320 | Doxorubicin1  
2,854 sites

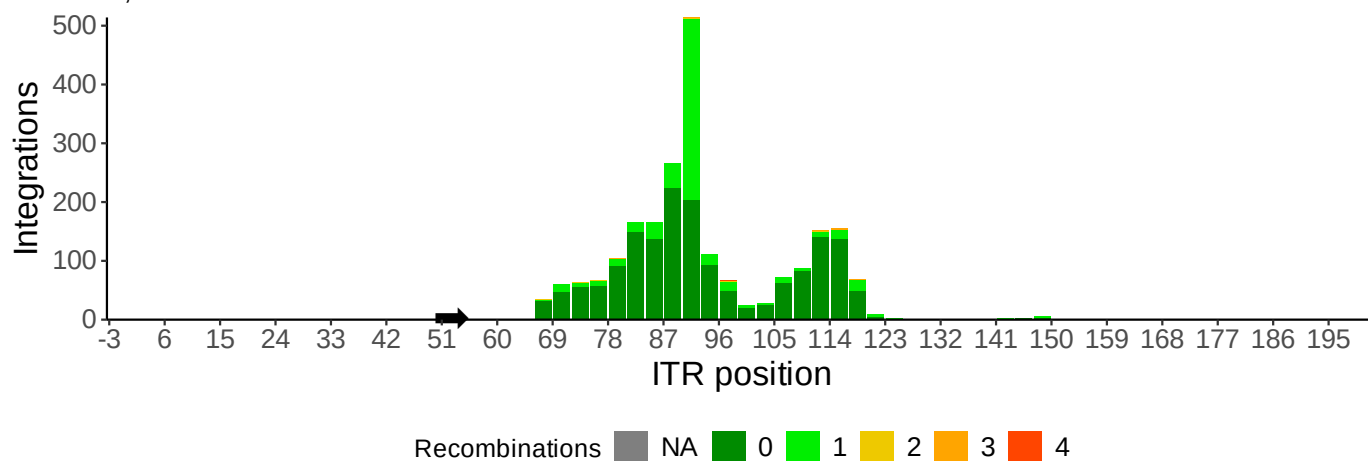




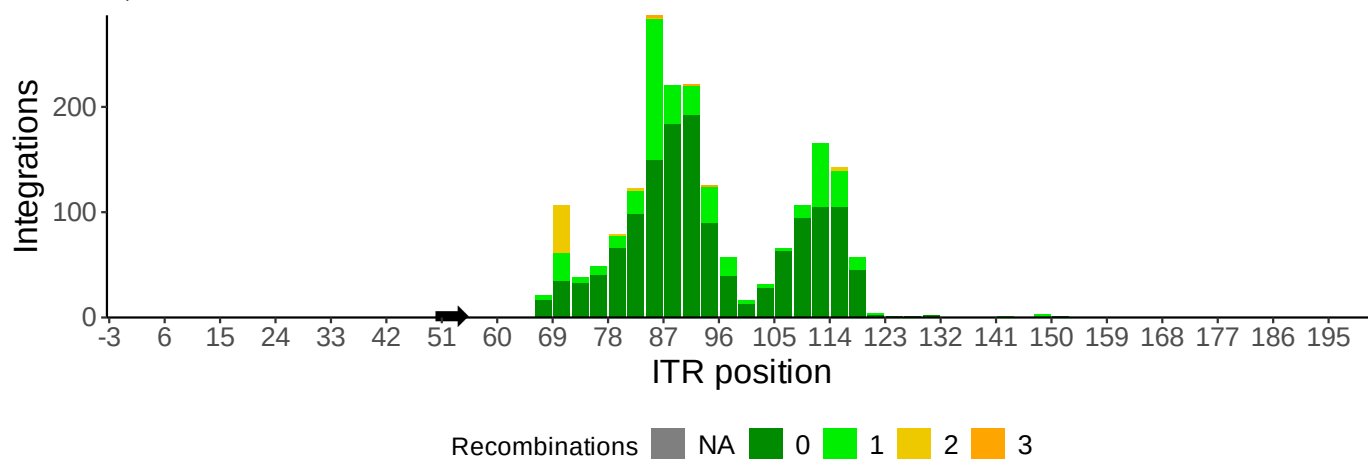
GTSP6324 | Etoposide1  
2,263 sites



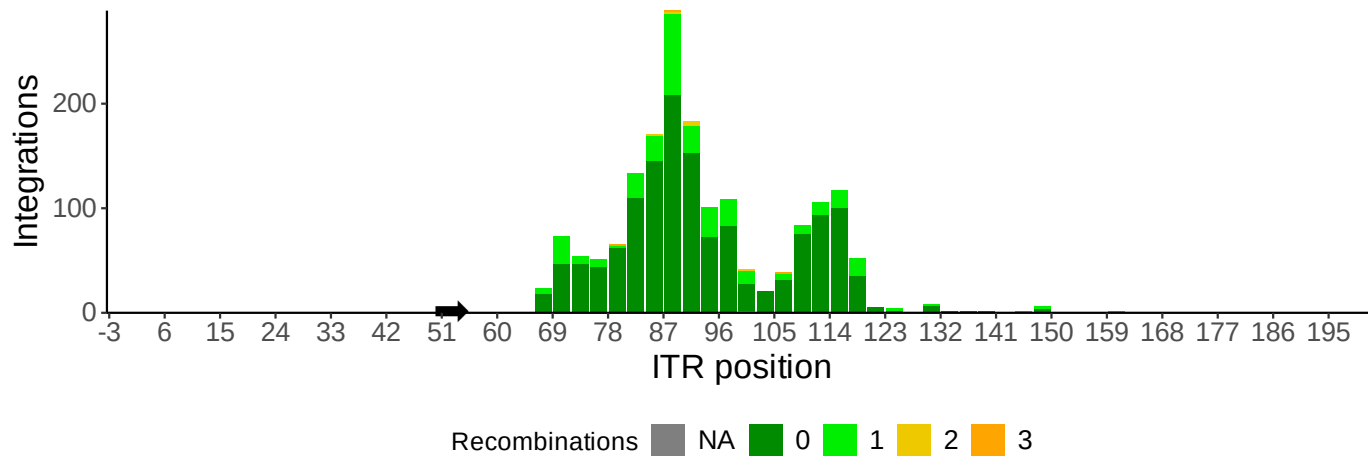
GTSP6325 | Etoposide2  
2,246 sites



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	82.76	82.76
AAVPositive_230922	AAVPositive_230922	80.65	80.65
GTSP6312	Mock1	1.77	34.90
GTSP6313	Mock2	2.77	38.54
GTSP6314	Mock3	2.45	34.25
GTSP6315	Mock4	2.36	39.78
GTSP6316	Camptothecin1	4.38	37.72
GTSP6317	Camptothecin2	4.13	45.38
GTSP6318	Camptothecin3	2.91	39.54
GTSP6319	Camptothecin4	4.77	44.39
GTSP6320	Doxorubicin1	2.17	46.08
GTSP6321	Doxorubicin2	3.31	46.23
GTSP6322	Doxorubicin3	3.22	47.96
GTSP6323	Doxorubicin4	4.24	48.77
GTSP6324	Etoposide1	4.24	42.73
GTSP6325	Etoposide2	4.50	46.75
GTSP6326	Etoposide3	3.60	43.91
GTSP6327	Etoposide4	3.37	43.09

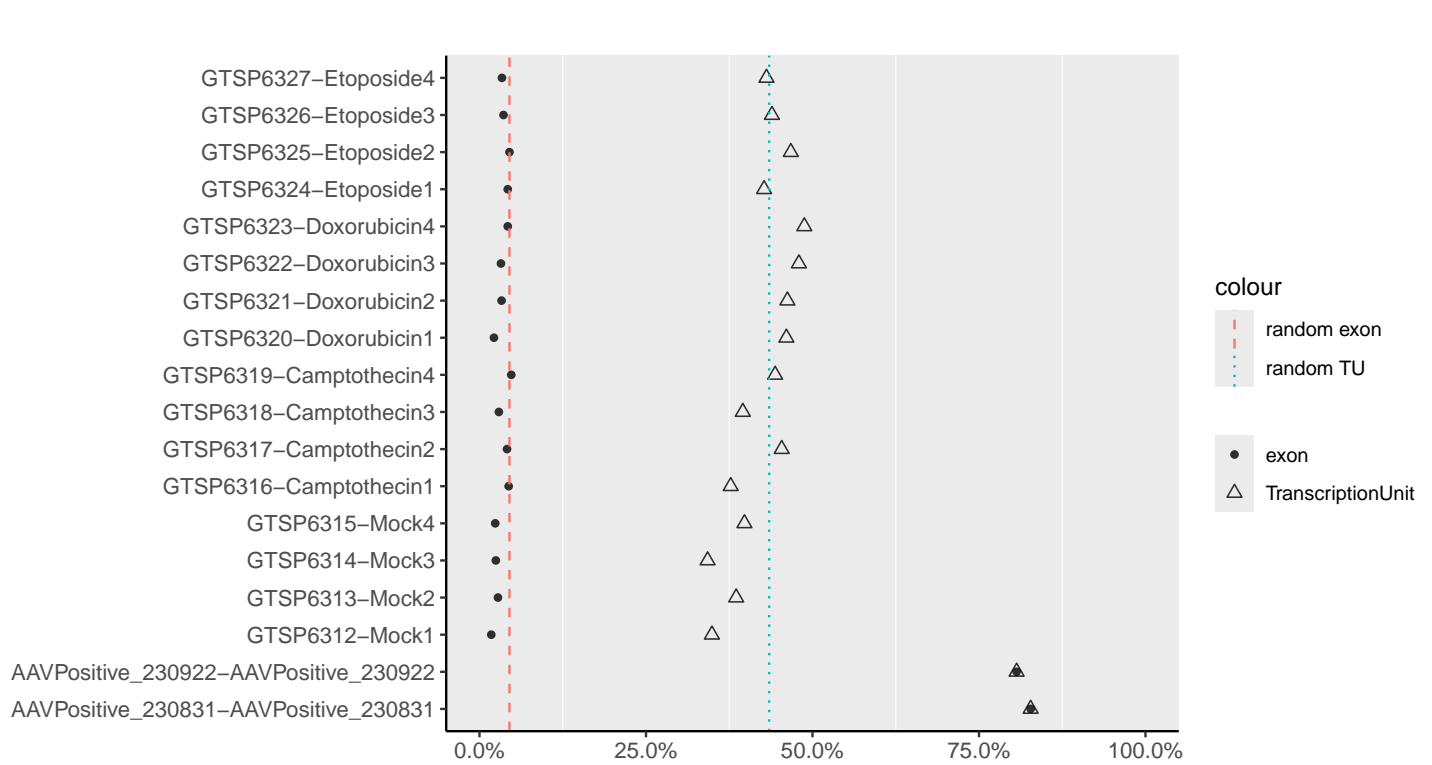


Figure 1: gene distribution scatter plot

# Methods

Report Generated on: June 28, 2024  
AAVenger Version: 1.1

Modules Called:

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