test1

April 05, 2024

Contents

Introduction **2**

PI Summary **2**

Sequencing and Integration Summary Table **2**

Abundance Frequency Plots **3**

AAV ITR Breakpoint Plots **6**

Gene distribution dot plots **10**

Methods **11**

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

Notes from PI.

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.

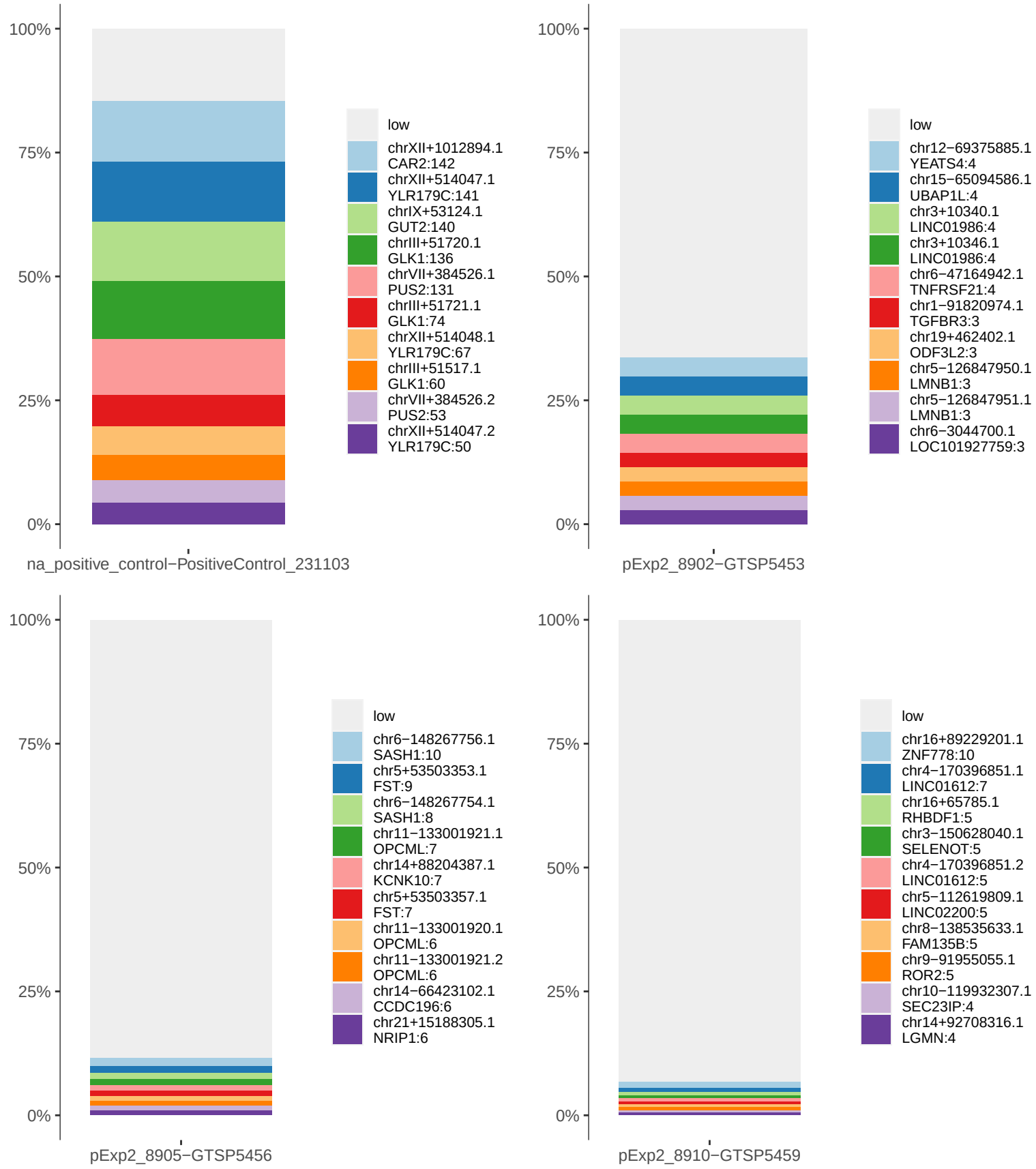
Under most circumstances, only a subset of sites will be sampled. We thus include an estimate of sample size based on the frequency of isolation information from the SonicLength method (Berry, 2012). The 'S.chao1' column denotes the estimated lower bound for population size derived using Chao estimate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

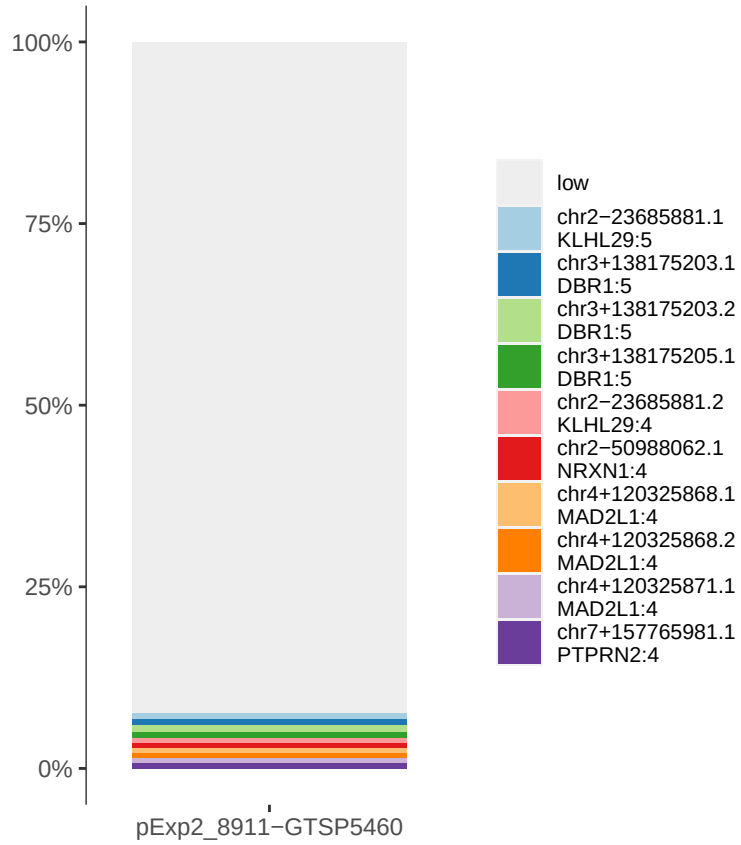
Table 1: integration summary table

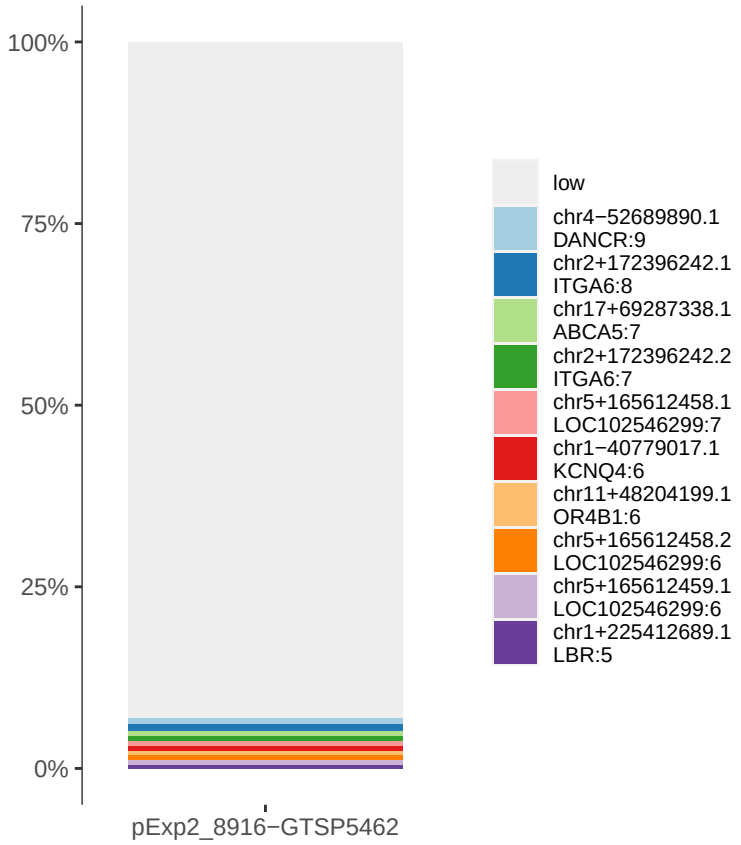
sample	patientID	total reads	Unique Sites	inferred cell	Chao1
GTSP5453	pExp2_8902	7627	68	104	180.8000
GTSP5456	pExp2_8905	444847	345	623	917.5000
GTSP5459	pExp2_8910	404014	513	818	1110.9677
GTSP5460	pExp2_8911	450612	429	579	1126.6800
GTSP5462	pExp2_8916	184739	597	970	1295.3036
GTSP5463	pExp2_8918	167473	599	851	1326.6080
GTSP5465	pExp4_9889	139845	257	696	567.1622
GTSP5467	pExp4_9891	236374	344	1118	644.5849
GTSP5468	pExp4_9883	248734	301	623	485.3636
GTSP5470	pExp4_9888	335405	276	576	927.0370
PositiveControl_231103	na_positive_control	80191	27	1165	27.3750

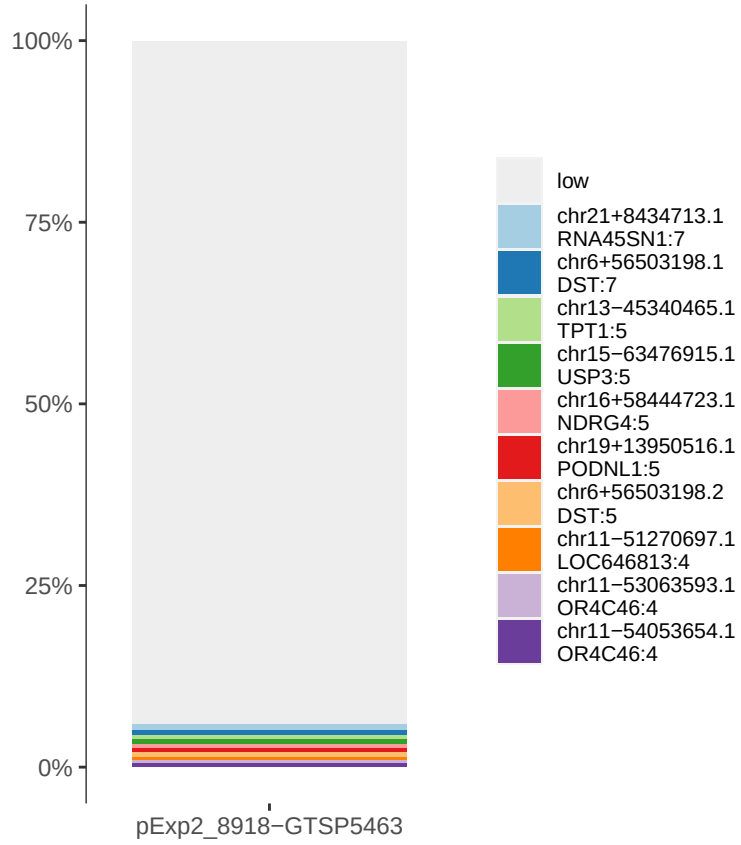
Abundance Frequency Plots

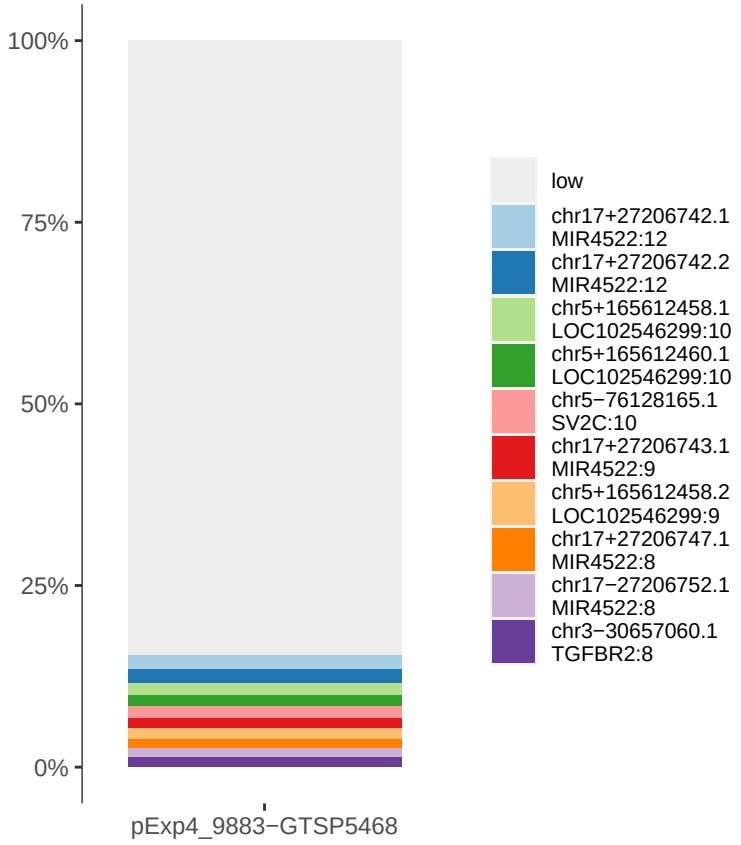
Abundance

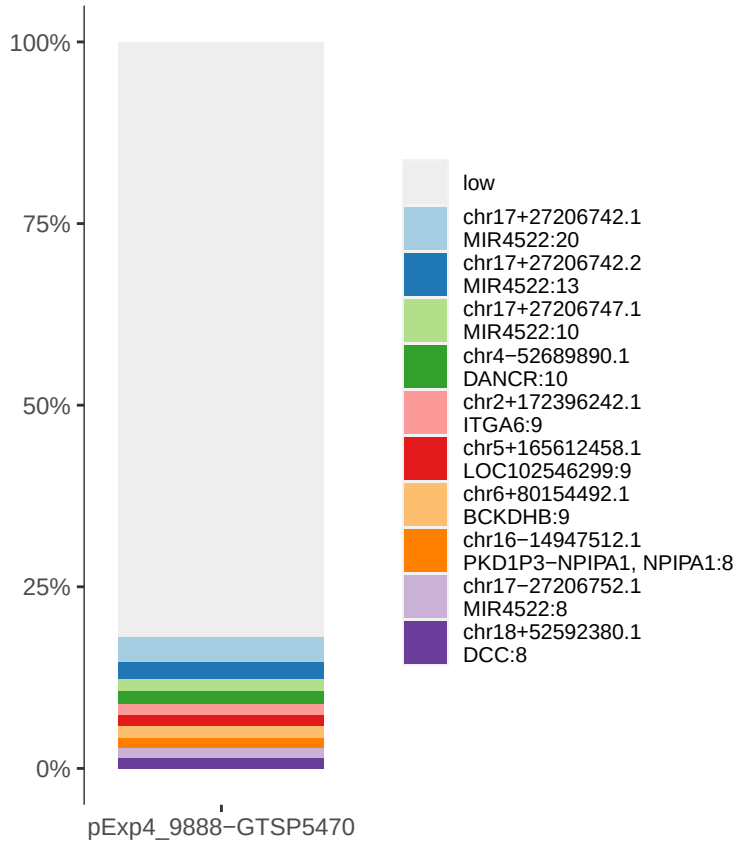


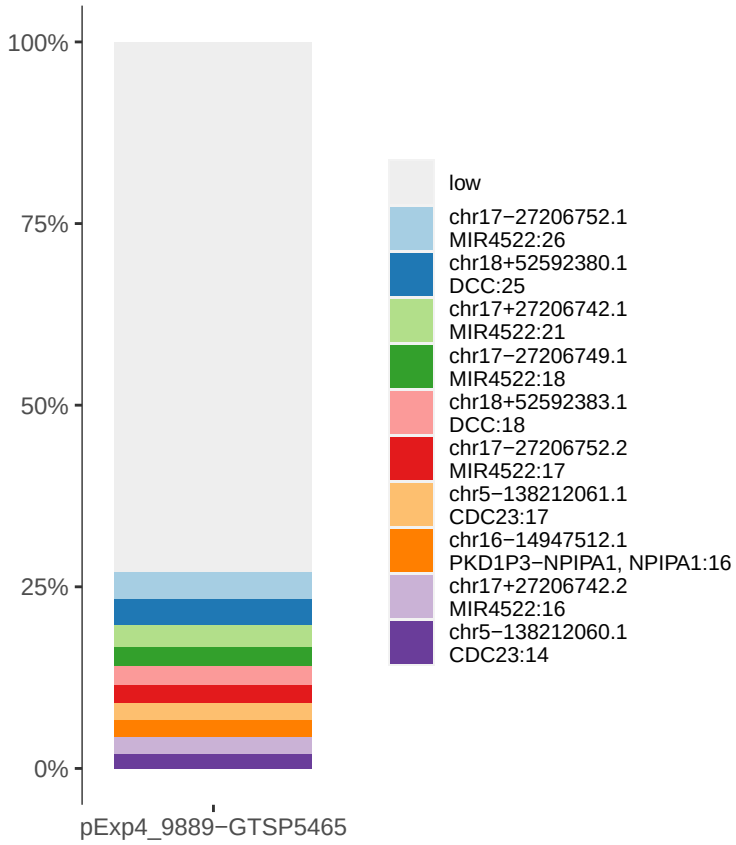

100%
75%
50%
25%
0%
pExp2_8911−GTSP5460
low
chr2−23685881.1
KLHL29:5
chr3+138175203.1
DBR1:5
chr3+138175203.2
DBR1:5
chr3+138175205.1
DBR1:5
chr2−23685881.2
KLHL29:4
chr2−50988062.1
NRXN1:4
chr4+120325868.1
MAD2L1:4
chr4+120325868.2
MAD2L1:4
chr4+120325871.1
MAD2L1:4
chr7+157765981.1
PTPRN2:4

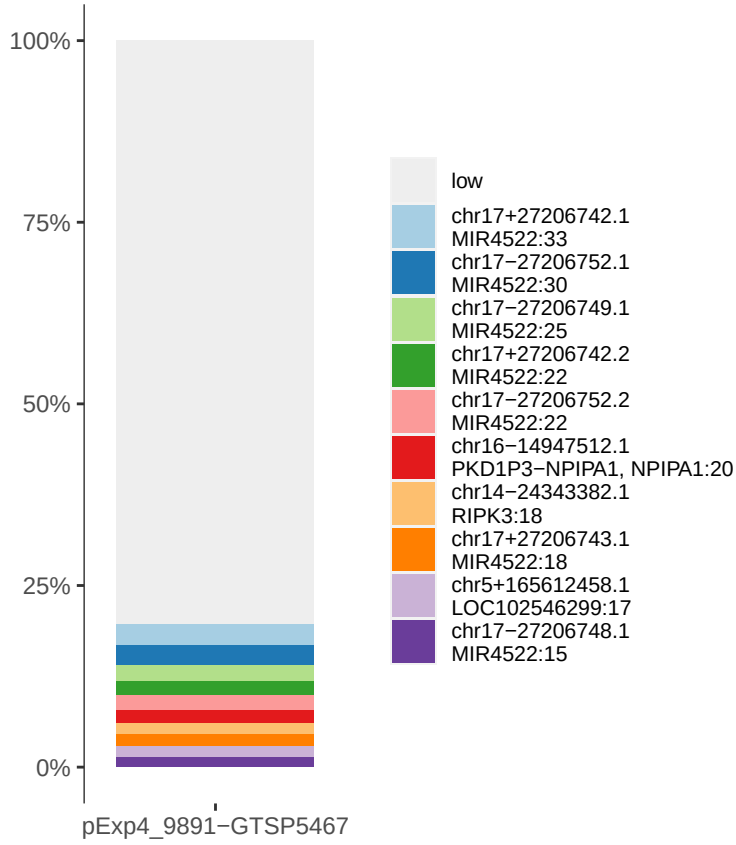

100%
75%
50%
25%
0%
pExp2_8916−GTSP5462
low
chr4−52689890.1
DANCR:9
chr2+172396242.1
ITGA6:8
chr17+69287338.1
ABCA5:7
chr2+172396242.2
ITGA6:7
chr5+165612458.1
LOC102546299:7
chr1−40779017.1
KCNQ4:6
chr11+48204199.1
OR4B1:6
chr5+165612458.2
LOC102546299:6
chr5+165612459.1
LOC102546299:6
chr1+225412689.1
LBR:5


100%
75%
50%
25%
0%
pExp2_8918−GTSP5463
low
chr21+8434713.1
RNA45SN1:7
chr6+56503198.1
DST:7
chr13−45340465.1
TPT1:5
chr15−63476915.1
USP3:5
chr16+58444723.1
NDRG4:5
chr19+13950516.1
PODNL1:5
chr6+56503198.2
DST:5
chr11−51270697.1
LOC646813:4
chr11−53063593.1
OR4C46:4
chr11−54053654.1
OR4C46:4


100%
75%
50%
25%
0%
pExp4_9883−GTSP5468
low
chr17+27206742.1
MIR4522:12
chr17+27206742.2
MIR4522:12
chr5+165612458.1
LOC102546299:10
chr5+165612460.1
LOC102546299:10
chr5−76128165.1
SV2C:10
chr17+27206743.1
MIR4522:9
chr5+165612458.2
LOC102546299:9
chr17+27206747.1
MIR4522:8
chr17−27206752.1
MIR4522:8
chr3−30657060.1
TGFBR2:8

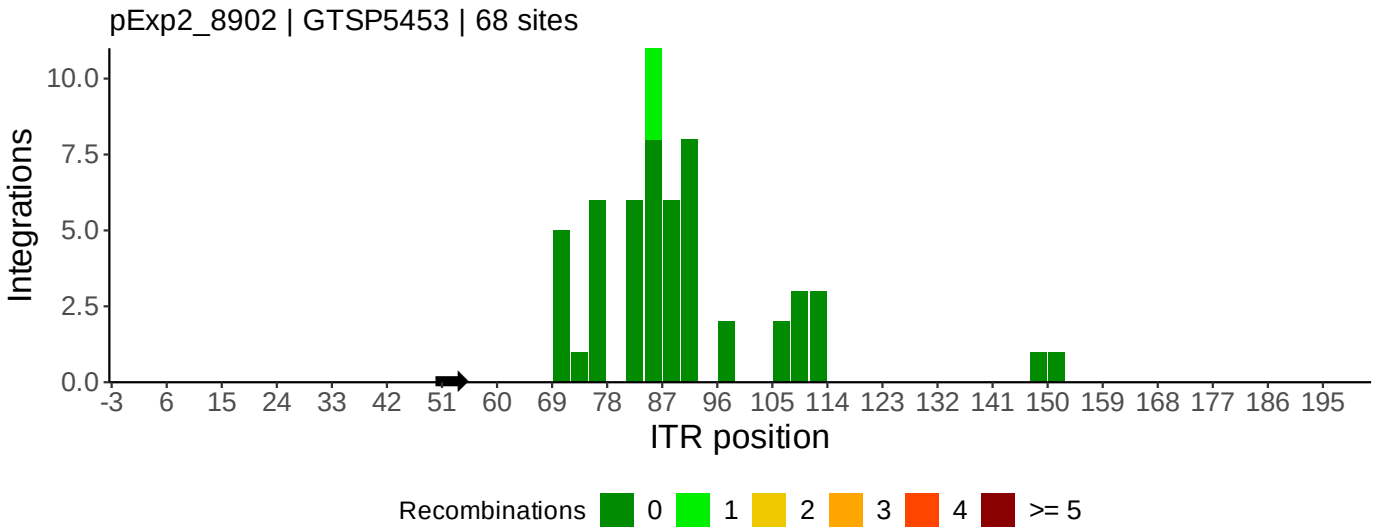

100%
75%
50%
25%
0%
pExp4_9888−GTSP5470
low
chr17+27206742.1
MIR4522:20
chr17+27206742.2
MIR4522:13
chr17+27206747.1
MIR4522:10
chr4−52689890.1
DANCR:10
chr2+172396242.1
ITGA6:9
chr5+165612458.1
LOC102546299:9
chr6+80154492.1
BCKDHB:9
chr16−14947512.1
PKD1P3−NPIPA1, NPIPA1:8
chr17−27206752.1
MIR4522:8
chr18+52592380.1
DCC:8

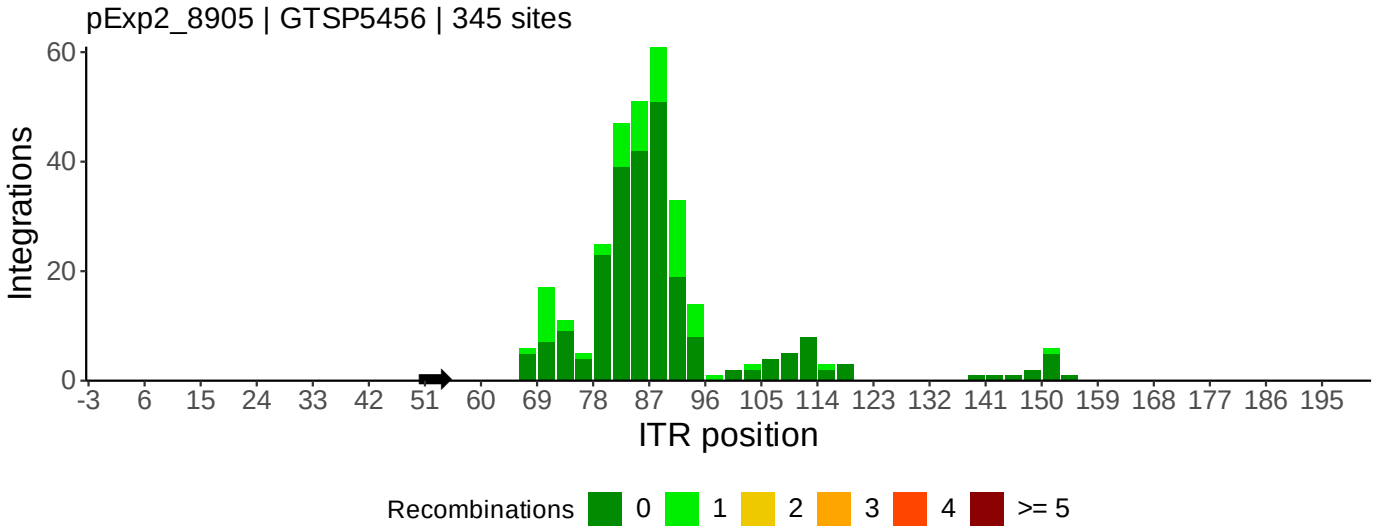

100%
75%
50%
25%
0%
pExp4_9889−GTSP5465
low
chr17−27206752.1
MIR4522:26
chr18+52592380.1
DCC:25
chr17+27206742.1
MIR4522:21
chr17−27206749.1
MIR4522:18
chr18+52592383.1
DCC:18
chr17−27206752.2
MIR4522:17
chr5−138212061.1
CDC23:17
chr16−14947512.1
PKD1P3−NPIPA1, NPIPA1:16
chr17+27206742.2
MIR4522:16
chr5−138212060.1
CDC23:14

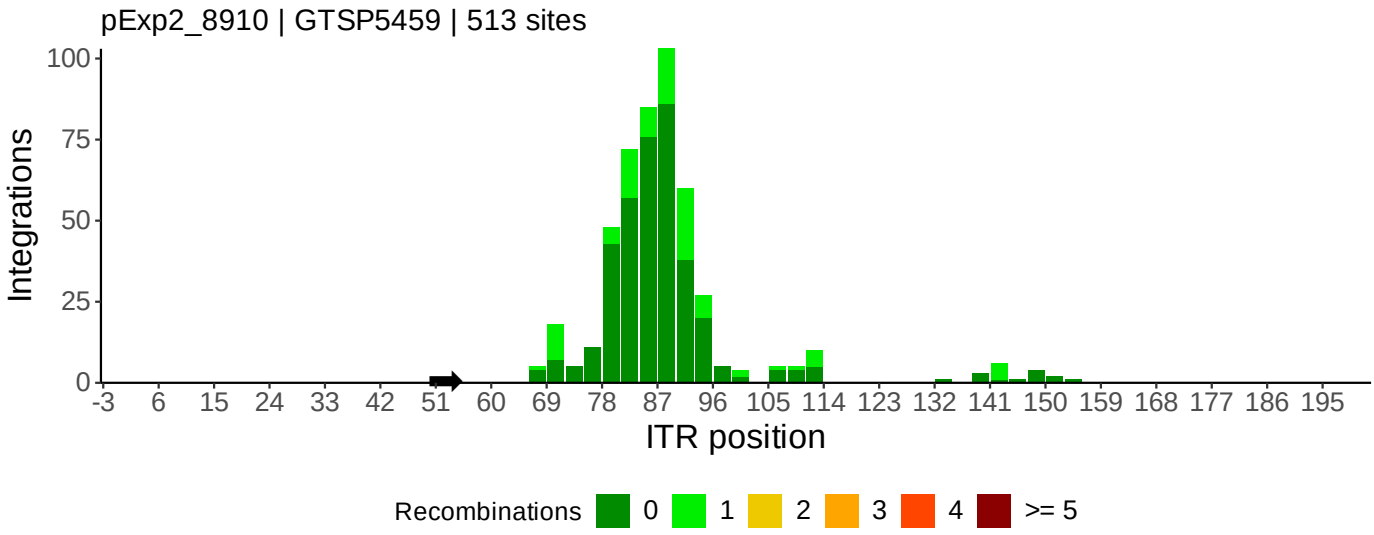

100%
75%
50%
25%
0%
pExp4_9891−GTSP5467
low
chr17+27206742.1
MIR4522:33
chr17−27206752.1
MIR4522:30
chr17−27206749.1
MIR4522:25
chr17+27206742.2
MIR4522:22
chr17−27206752.2
MIR4522:22
chr16−14947512.1
PKD1P3−NPIPA1, NPIPA1:20
chr14−24343382.1
RIPK3:18
chr17+27206743.1
MIR4522:18
chr5+165612458.1
LOC102546299:17
chr17−27206748.1
MIR4522:15

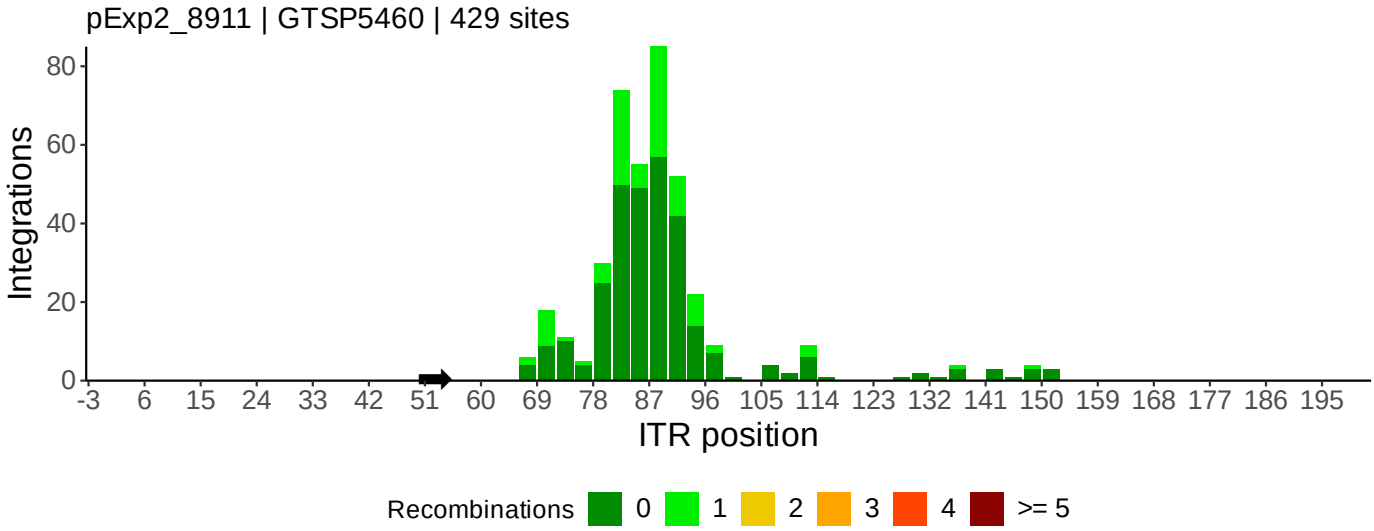
AAV ITR Breakpoint Plots

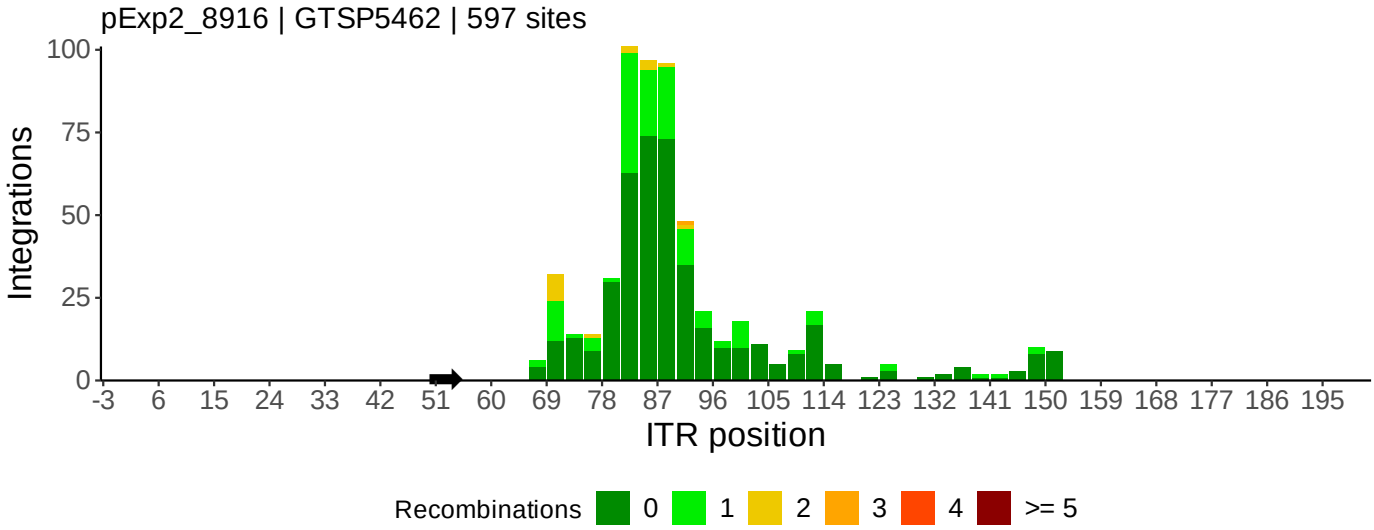
These plots are a visual representation of the ITR breakpoint at the site of genomic integration. The arrow indicates the priming location within the ITR and the x-coordinate of each bar corresponds to a departure from an expected ITR sequence. Each departure from expectation is demarcated as a rearrangement,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

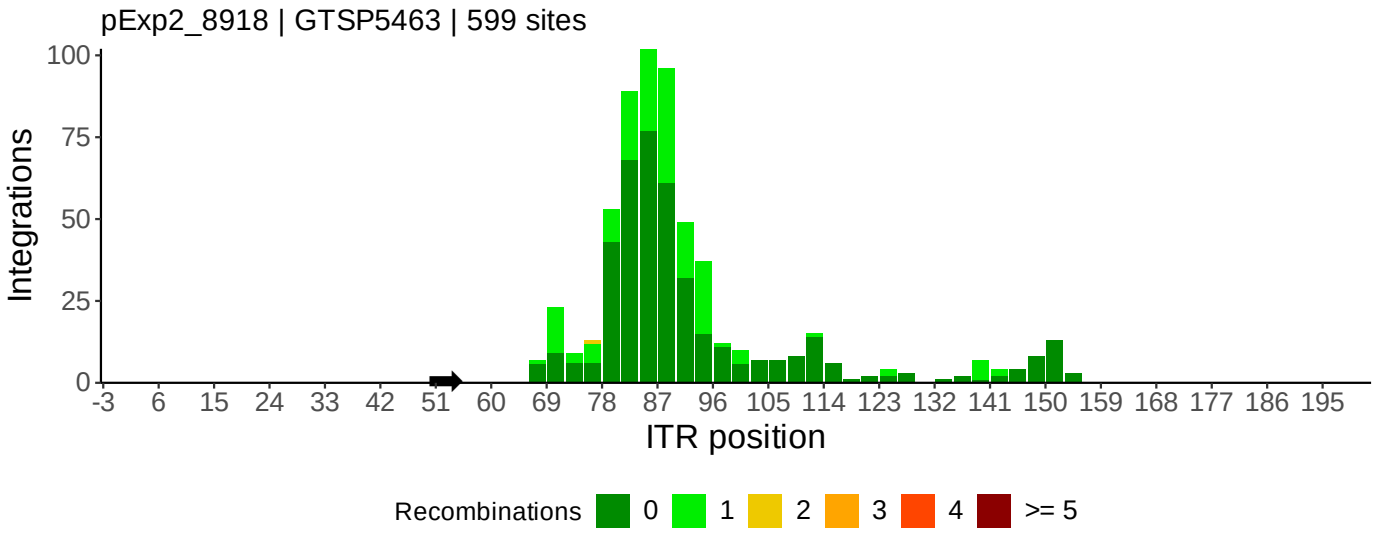


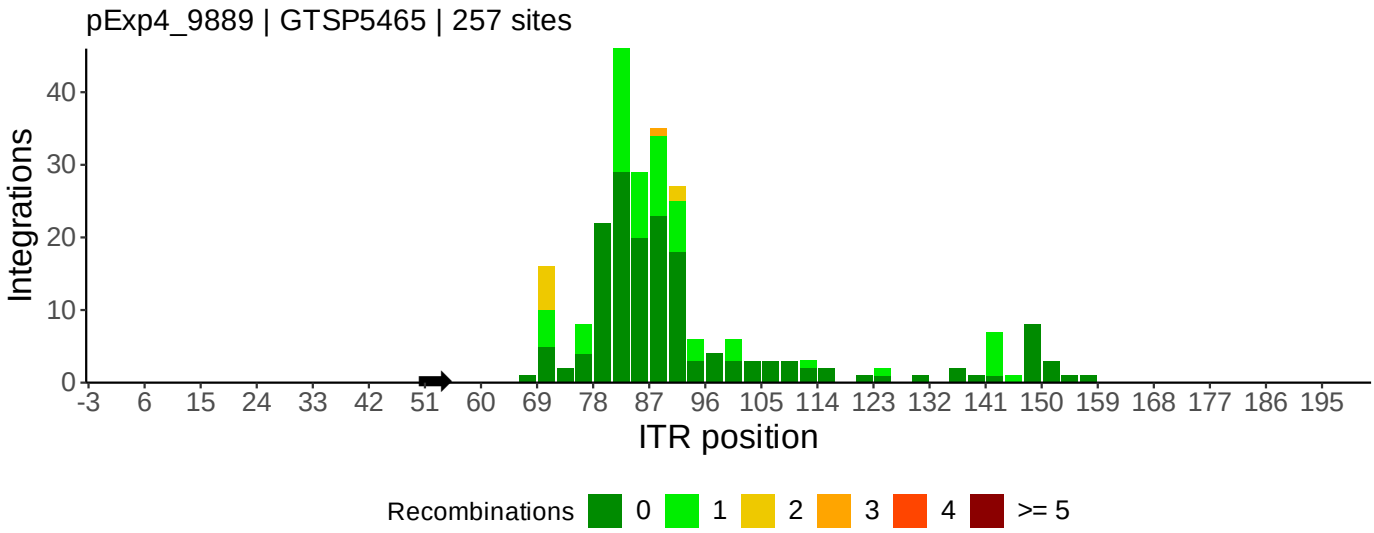


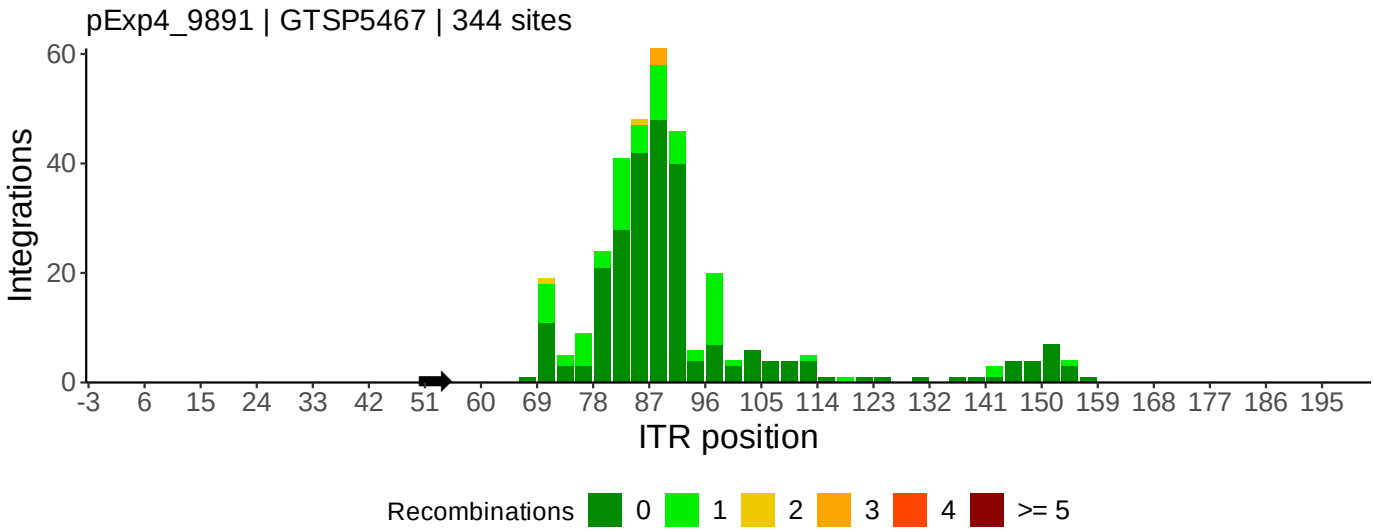

pExp2_8910 | GTSP5459 | 513 sites
Integrations
100
75
50
25
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations 0 1 2 3 4 >= 5

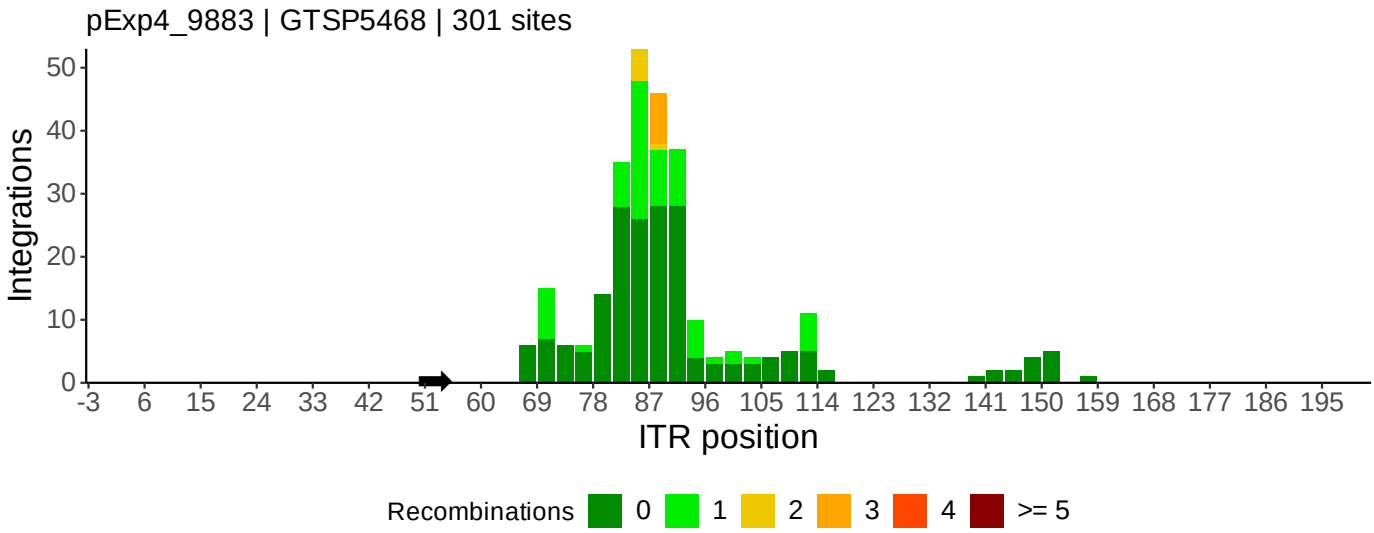

pExp2_8911 | GTSP5460 | 429 sites
Integrations
80
60
40
20
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations 0 1 2 3 4 >= 5

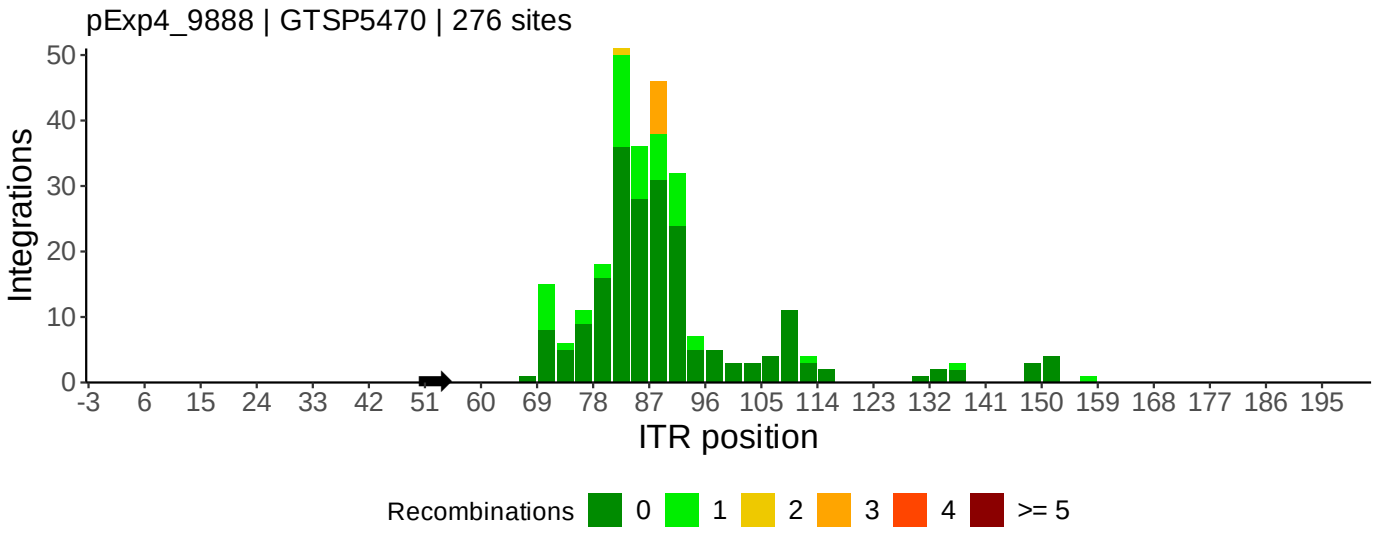

pExp2_8916 | GTSP5462 | 597 sites
Integrations
100
75
50
25
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations 0 1 2 3 4 >= 5

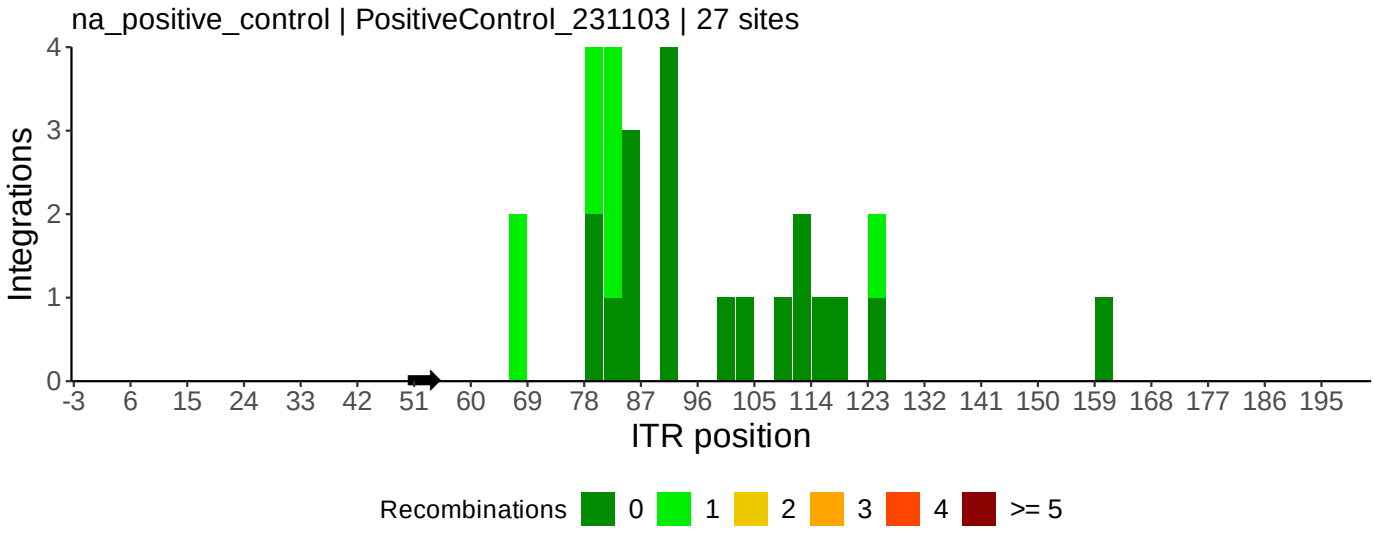

pExp2_8918 | GTSP5463 | 599 sites
Integrations
100
75
50
25
0
-3 6 15 24 33 42 51 60 69 78 87 96 105 114 123 132 141 150 159 168 177 186 195
ITR position
Recombinations 0 1 2 3 4 >= 5


pExp4_9889 | GTSP5465 | 257 sites
Integrations
40
30
20
10
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 0 1 2 3 4 >= 5


pExp4_9891 | GTSP5467 | 344 sites
Integrations
60
40
20
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 0 1 2 3 4 >= 5


pExp4_9883 | GTSP5468 | 301 sites
Integrations
0
10
20
30
40
50
-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 0 1 2 3 4 >= 5


pExp4_9888 | GTSP5470 | 276 sites
Integrations
0
10
20
30
40
50
-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 0 1 2 3 4 >= 5


na_positive_control | PositiveControl_231103 | 27 sites
Integrations
0
1
2
3
4
-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 0 1 2 3 4 >= 5

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 2: gene distribution percentage table

sample	In Exon%	In Transcription Unit%
PositiveControl_231103	74.1	74.1
GTSP5467	2.6	46.5
GTSP5465	3.1	58.8
GTSP5470	0.7	43.5
GTSP5468	1.7	43.5
GTSP5456	2.3	40.6
GTSP5459	7.0	46.8
GTSP5462	4.2	47.6
GTSP5463	4.5	50.4
GTSP5460	5.1	48.7
GTSP5453	4.4	57.4

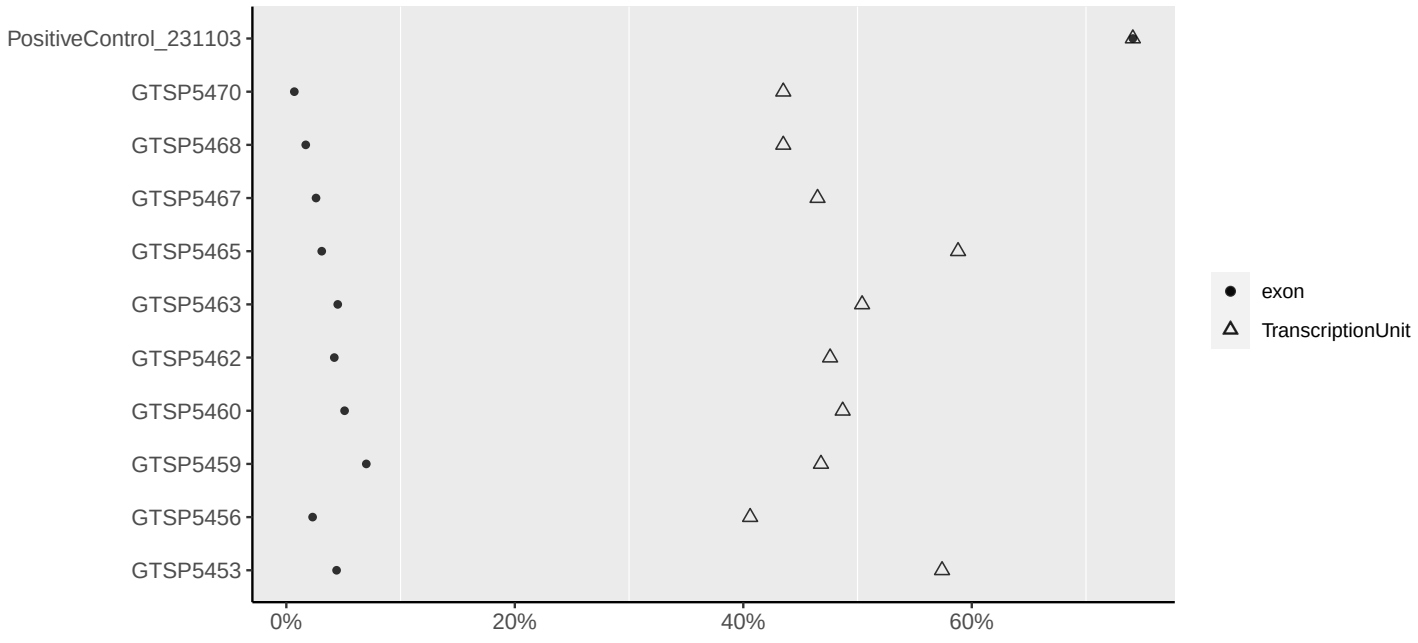


Figure 1: gene distribution scatter plot

Methods

Report Generated on: April 05, 2024
AAVenger Version: 1.1

Modules Called:

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