Anna Maurer HDR inhibition study

October 20, 2023

Contents

Introduction

There has been interest in the relationship between second-strand synthesis of AAV genomes and the DNA repair pathways non-homologous end joining (NHEJ) and homology-directed repair (HDR). These pathways may also play a role in the integrity of ITR-genome junctions in integrated vectors.

This preliminary study aims to understand the relationship between HDR and AAV integration integrity. U2OS cells were treated with DMSO or HDR inhibitor B02 (RAD51) prior to AAV transduction. The contribution of purification of the AAV preparations was also explored.

Samples are classified as follows:
DMSOCrude: Cells treated with DMSO and transduced with a crude AAV prep
DMSOPure: Cells treated with DMSO and transduced with a pure AAV prep
HDRiCrude: Cells treated with B02 and transduced with a crude AAV prep
HDRiPure: Cells treated with B02 and transduced with a pure AAV prep
Negative: Negative Control

Samples were also run with a positive control containing 6 synthetic yeast integration sites, each with breakpoints at the various regions of the ITR dumbbell (DA, DAB, DABB', DABB'C', DABB'C'C, DABB'C'CA)

Sequencing and Integration Summary Table

Presented below are summary details of each sample. The Unique Molecular Identifiers (UMIs) estimate the number of unique molecules queried. Unique Sites refers to the number of unique integration sites detected. Chao1 estimates the total population sizes of integration sites in the sample.

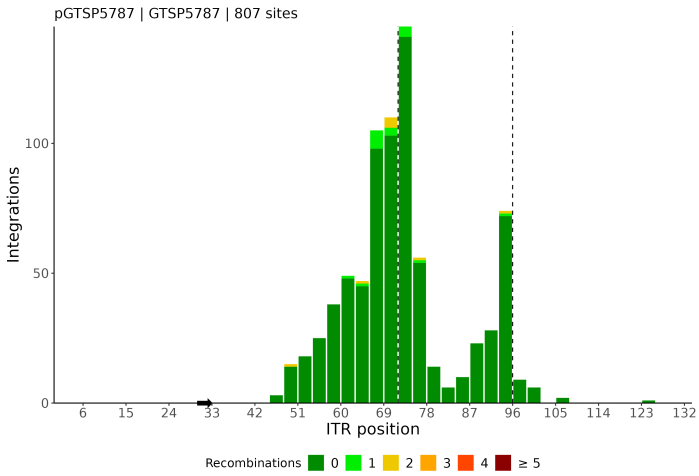
Prelimary work with negative controls support the use of a filtering parameter in such that sites only supported by one read are considered artifactual and removed.

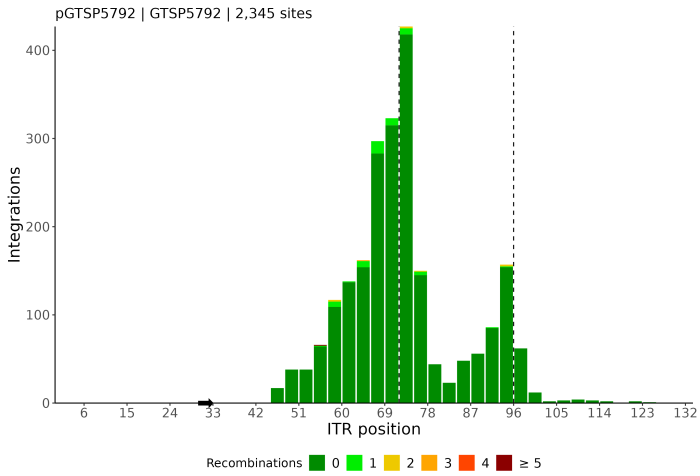
subject	Drug	UMIs	Total Reads	Unique Sites	Chao1	Inferred Cells
pControl	Positive Control	2344	257133	24	26.500	1776
pGTSP5786	HDRiCrude	10374	25771	4757	21366.097	7339
pGTSP5787	DMSOCrude	2155	12877	807	2387.302	1473
pGTSP5788	Negative	2	2	2	3.000	2
pGTSP5789	HDRiPure	17278	51104	5353	16968.643	10660
pGTSP5790	DMSOPure	2204	7953	1017	3773.110	1660
pGTSP5791	HDRiCrude	15158	37892	9996	85261.335	12579
pGTSP5792	DMSOCrude	4207	10200	2345	16388.490	3284
pGTSP5793	Negative	11	10266	5	5.500	11
pGTSP5794	HDRiPure	19700	37800	11612	82895.918	15237
pGTSP5795	DMSOPure	5414	11523	2450	11833.096	3828

AAV ITR Breakpoint Plots

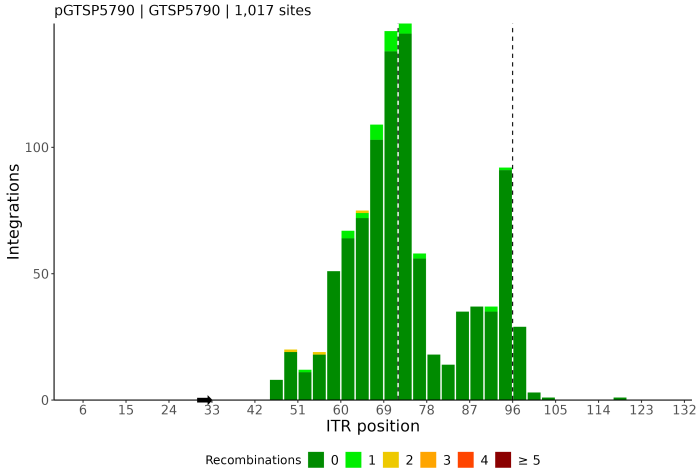
These plots are a visual representation of the ITR integration breakpoint. The x-axis indicates the point of departure from the expected ITR, where zero refers to the annotated start of the D-region. The arrow indicates the location within the ITR of the sequencing primer and the dotted lines indicate the tips of the B and C dumbells. Colors indicate the number of rearrangements in the ITR remnant.

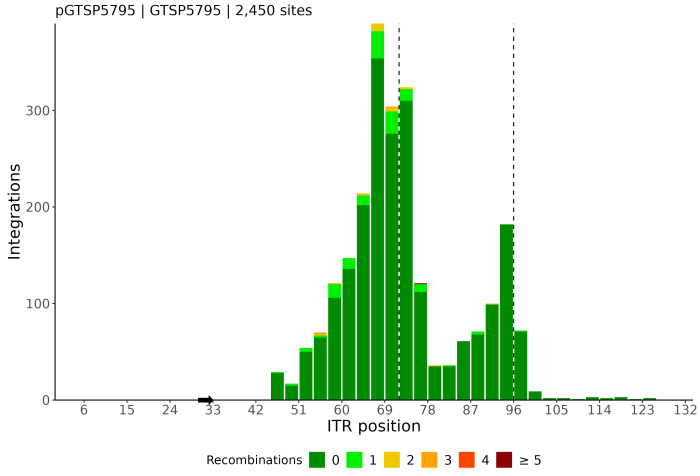
DMSOCrude



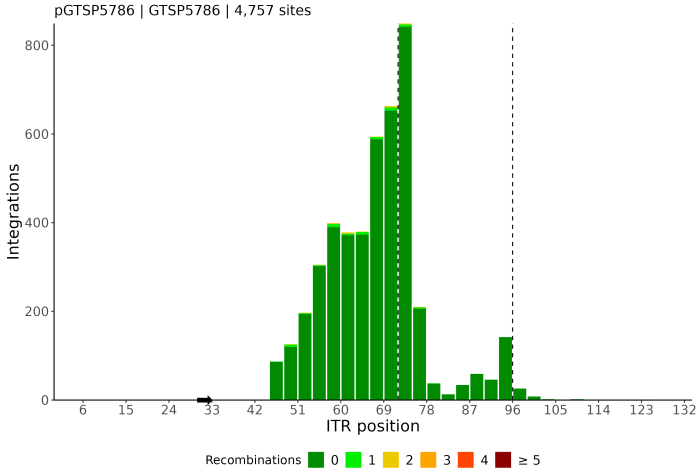


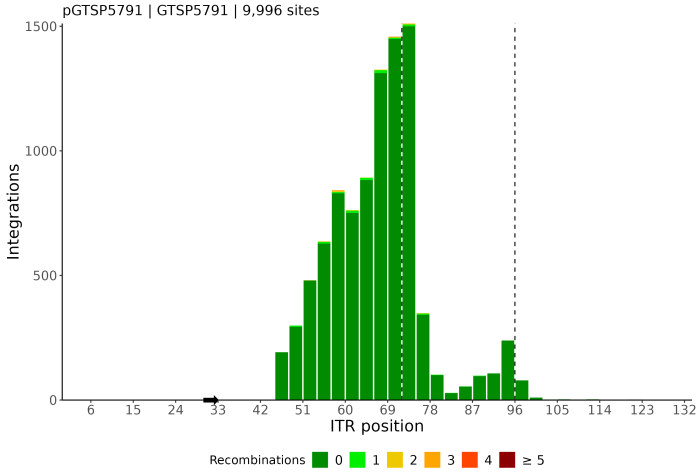
DMSOPure



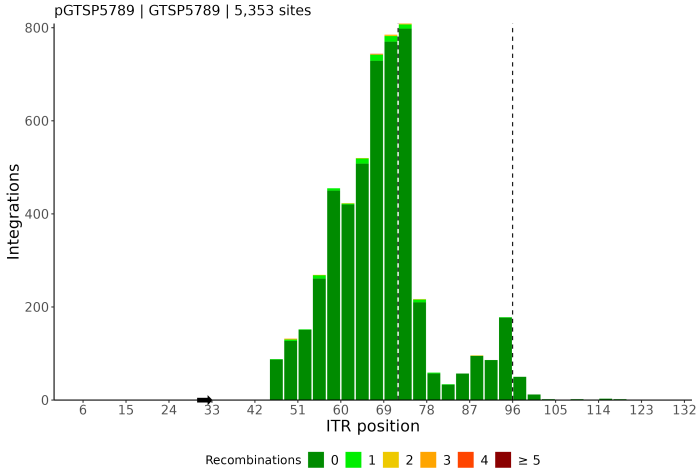


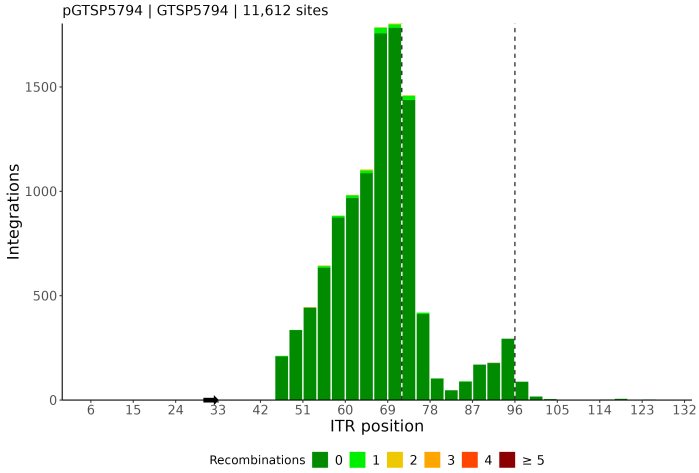
HDRiCrude



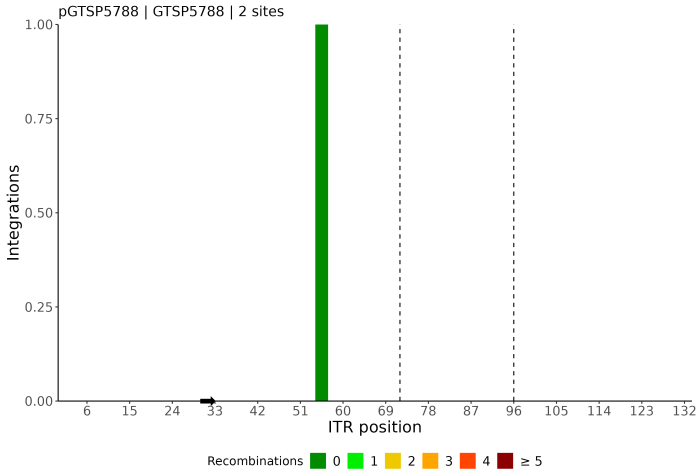


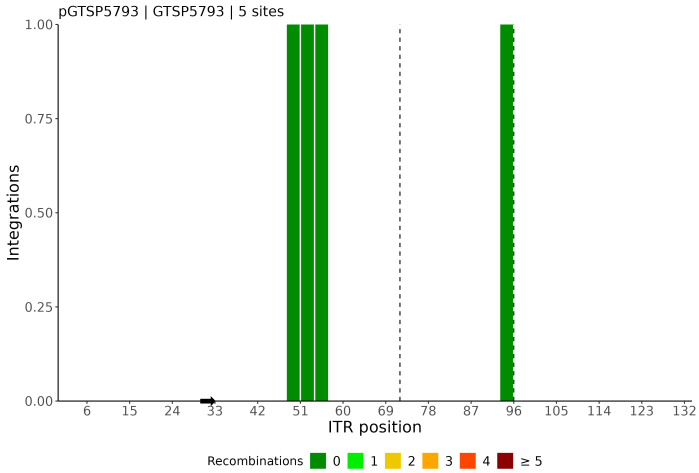
HDRiPure





Negative

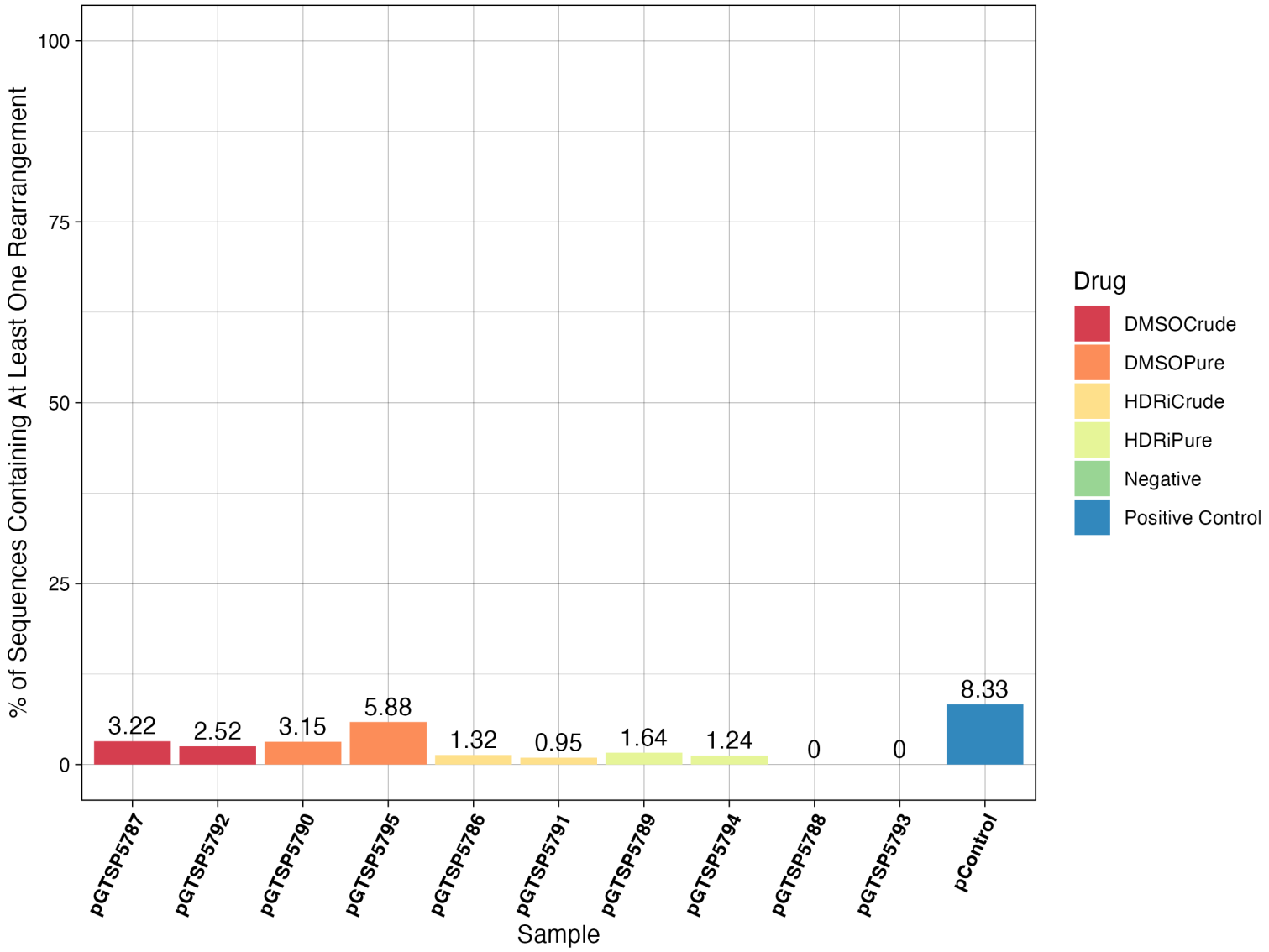




Rearrangements in AAV integration sites

This output takes all the sequences of integration sites and evaluates the region between the ITR primer and the host genome junction for alignment to the vector. Any deviation from the expected ITR sequence is considered a rearrangement.

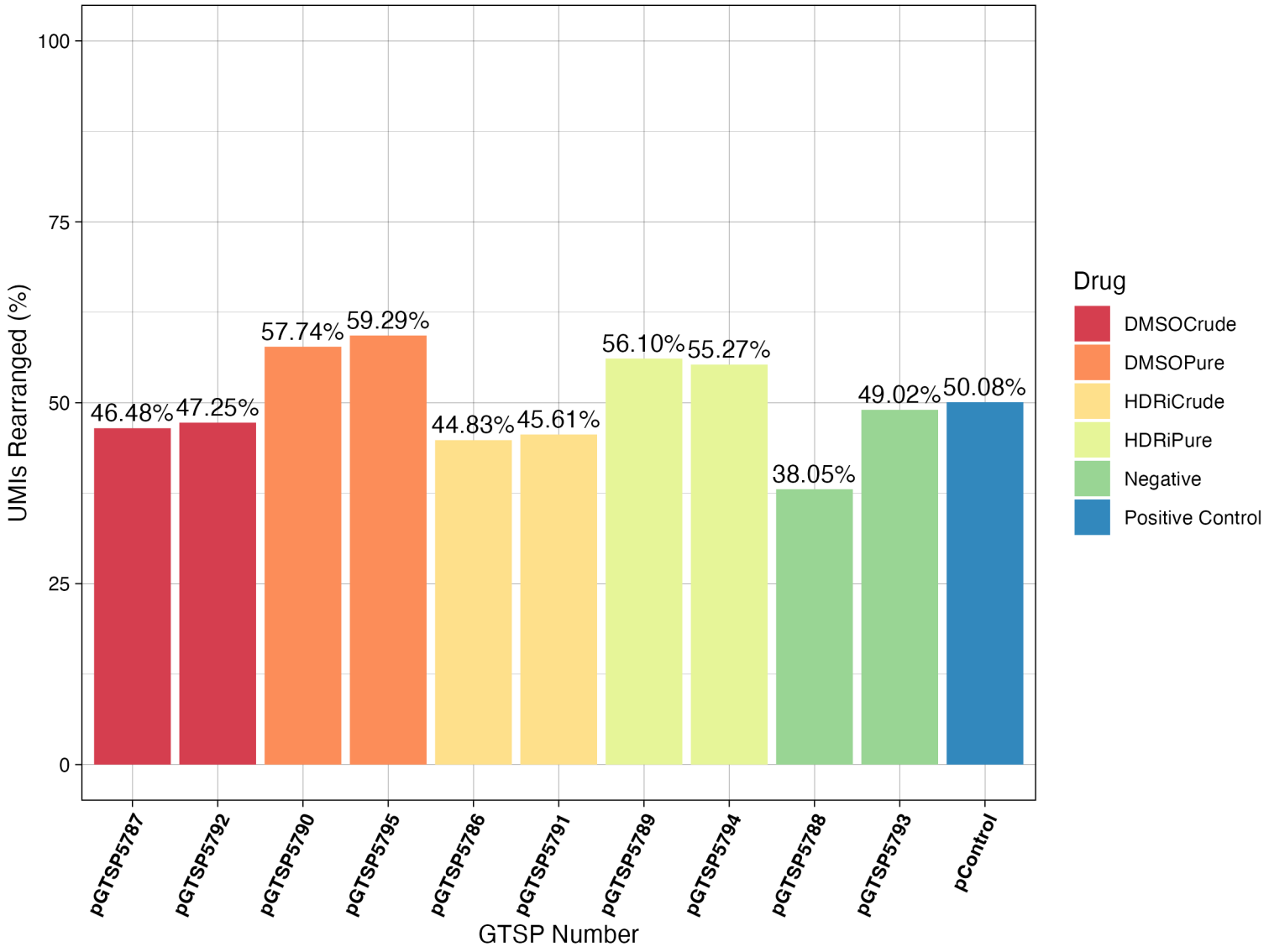
The plot below depicts the percentage of integration sites with an ITR remnant containing at least one rearrangement.



Rearrangements in all ITR containing sequences

This output takes all the ITR containing reads and evaluates them for alignment to the vector. Any deviation from the expected ITR sequence is considered a rearrangement.

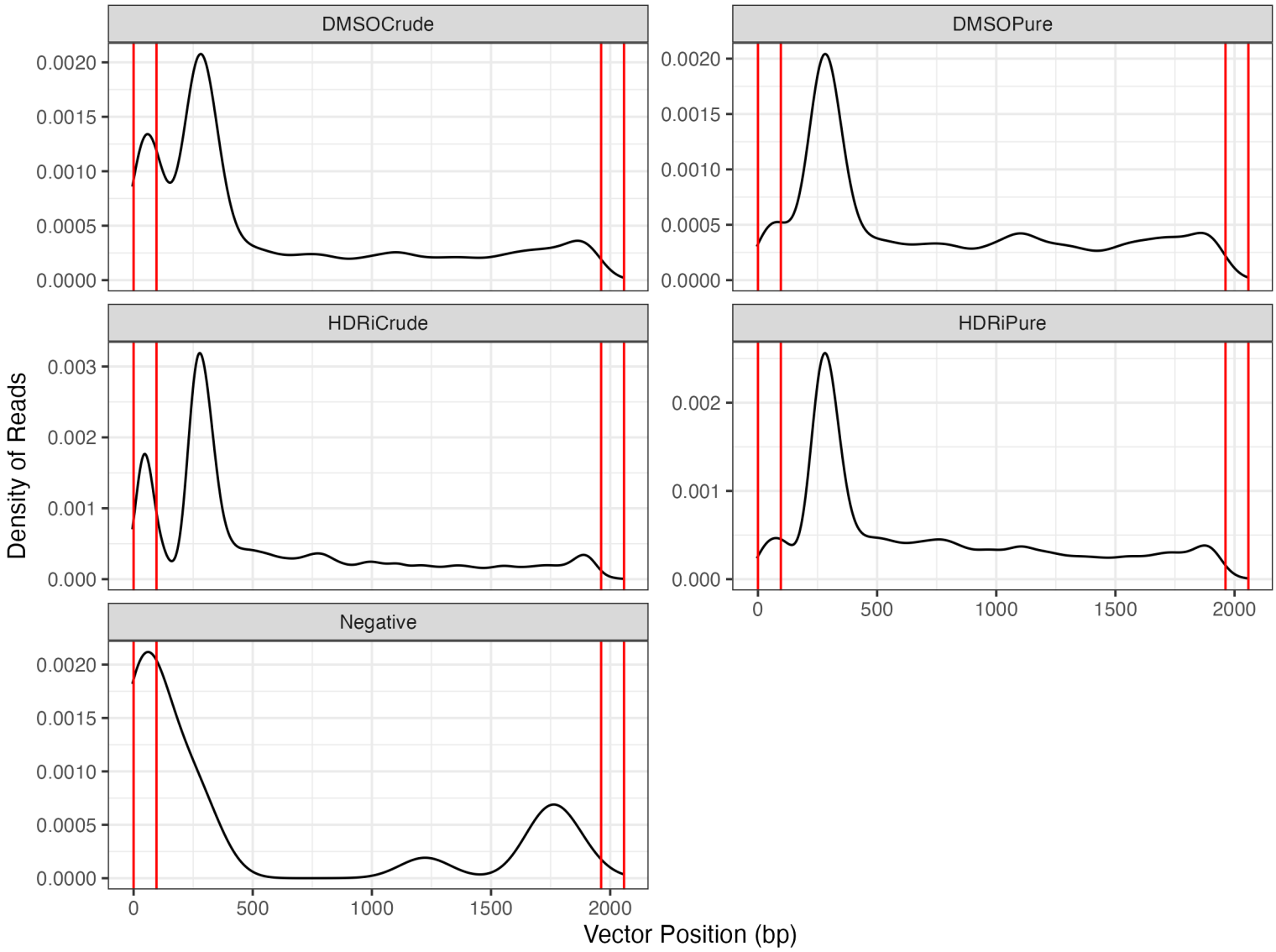
Duplicate reads with the same unique molecular identifiers are collapsed into one molecule for this analysis. The plot below depicts the percentage of unique molecules with an ITR remnant containing at least one rearrangement.



ITR into ITR vs. ITR into Vector

Evaluating anchor reads that break into vector instead of human genome shows a distribution of of break-points into ITR again or into the vector genome. The red lines demarcate the ITR boundaries.

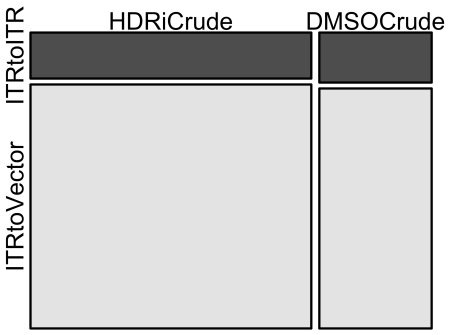
Position 300 on the vector corresponds to the collapsed lacO and mScarlet repeats.



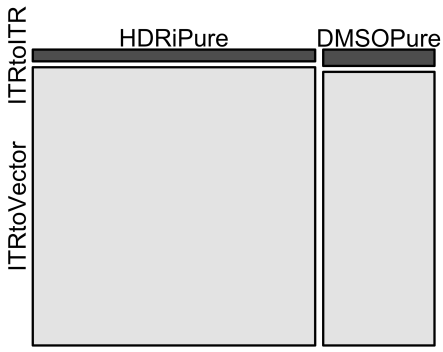
The relationship between the drug conditions and the ratio of ITR into ITR vs ITR into Vector junctions is evaluated below.

The relationship is significant if the p-value is less than 0.05 and the strength of the relationship is weak at Cramer's V greater than 0.10 and strong at Cramer's V greater than 0.50.

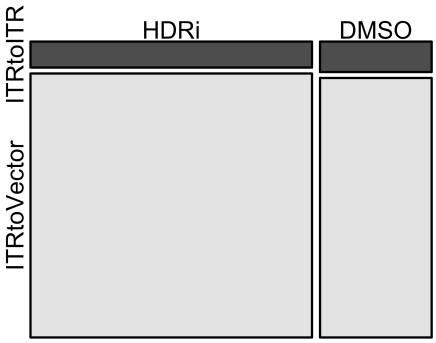
Mosaic Plot of Crude Samples



Mosaic Plot of Pure Samples



Mosaic Plot by Drug Condition



	P-value by chisquared	CramersV
Crude Prep Comparison	0	0.01687
Pure Prep Comparison	0	0.03444
Total Drug Comaprison	0	0.02382

Methods

Report Generated on: October 20, 2023
AAVenger Version: 1.1

Modules Called:

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