

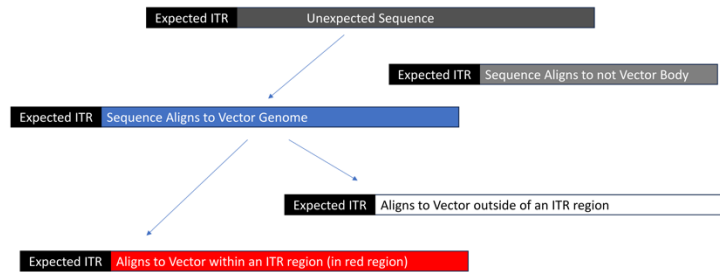
November 14th Update on Berkley Work

By: Aradhana Kasimsetty

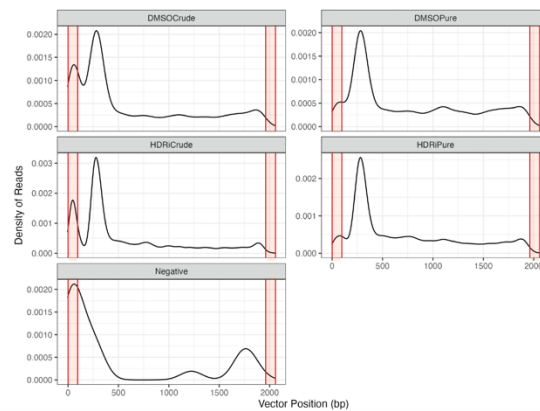
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Initial Method for Selecting Reads of Interest after Demultiplex

How The Data Was Selected

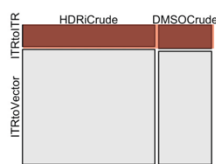


Density Plots based on those Reads (sent to Berkley)



Mosaic Plots, Chi-Squared and CramersV (sent to Berkley)

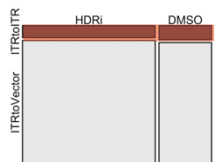
Mosaic Plot of Crude Samples



Mosaic Plot of Pure Samples



Mosaic Plot by Drug Condition



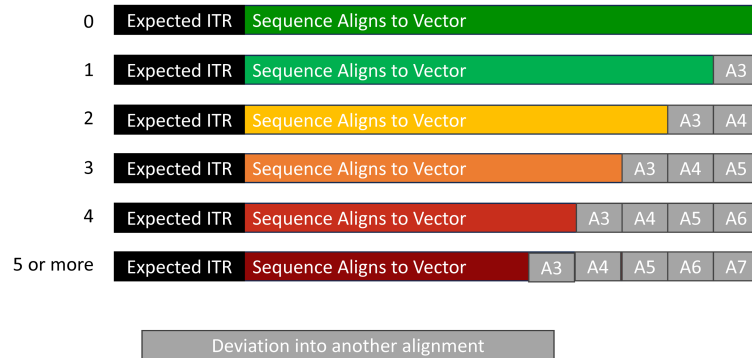
| | P-value by chisquared | CramersV |
|-----------------------|-----------------------|----------|
| Crude Prep Comparison | 0 | 0.01687 |
| Pure Prep Comparison | 0 | 0.03444 |
| Total Drug Comparison | 0 | 0.02382 |

CramersV: Strength of Association

0.1 – 0.5: Weak
0.5 – 0.8 : Moderate
0.8 – 1.0: Strong

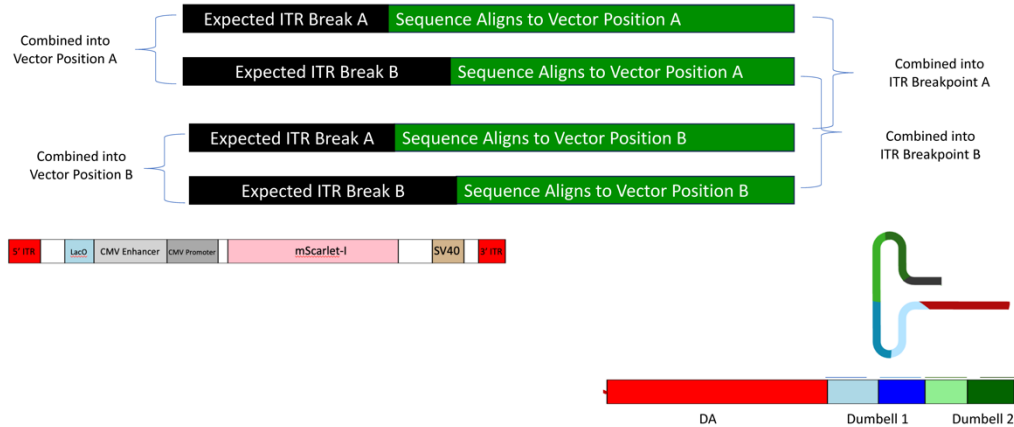
Methodology for Breakpoint Plot Data (not yet sent to Berkley)

Recombinations Post Jump:



First Plot: Vector Breakpoint

Second Plot: ITR Breakpoint

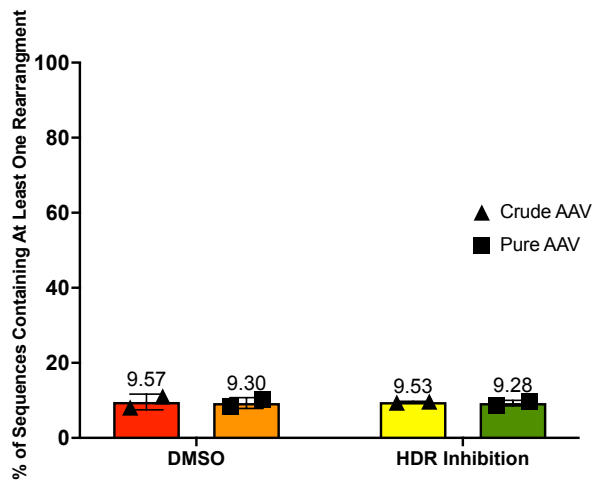


*For ITR breakpoint plots, Flip and Flop are combined and we consider the first Dumbbell vs second Dumbbell

$$\frac{\text{Total Number of Reads with at least one deviation}}{\text{Total Number of Reads with vector alignment}} \times 100$$

Expected ITR Vector Sequence Deviation

Expected ITR Vector Sequence

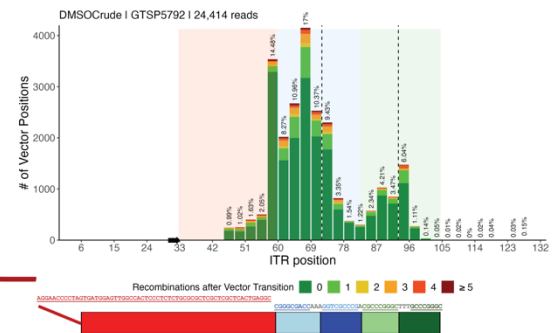
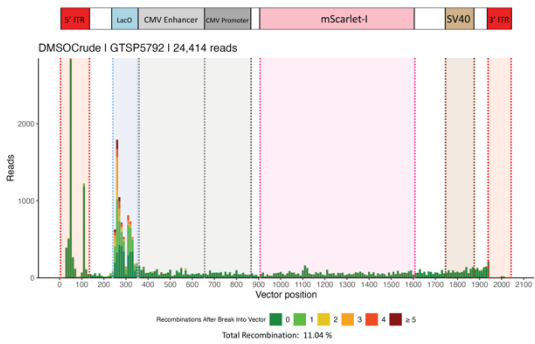
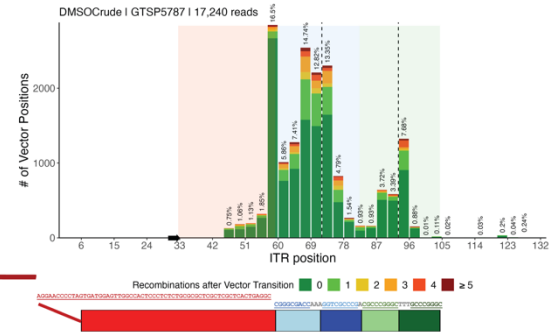
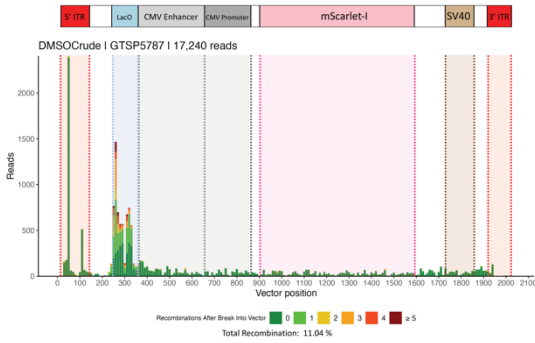


Breakpoint Plots

Vector Breakpoint

ITR Breakpoint

Crude : DMSO

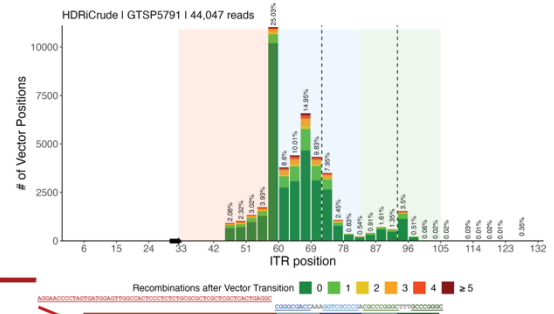
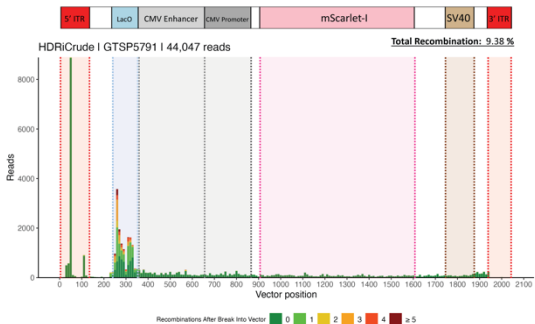
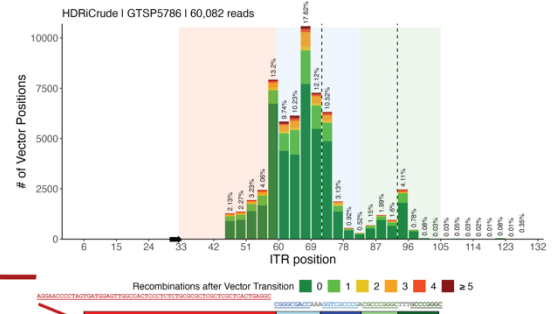
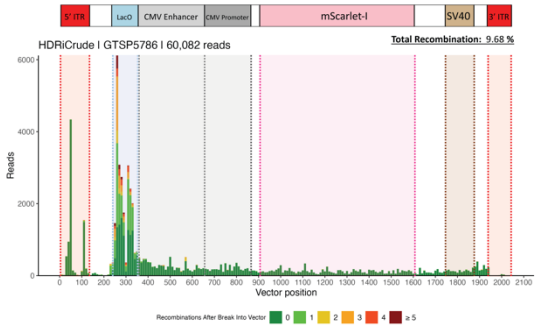


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Vector Breakpoint

ITR Breakpoint

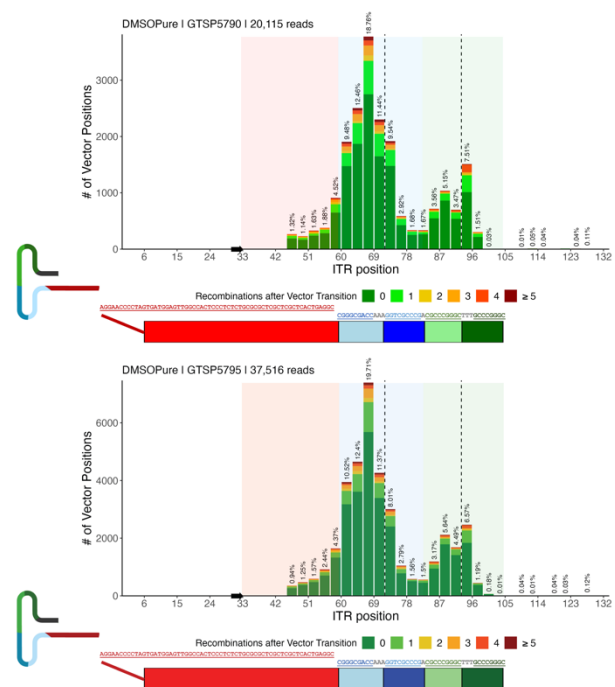
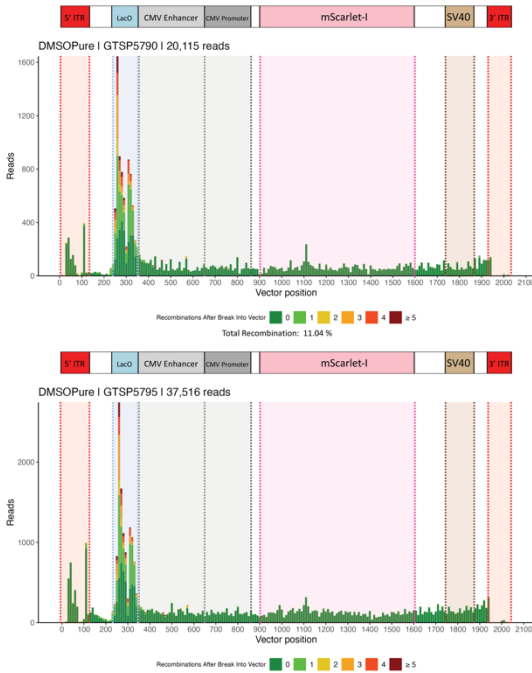
Crude : HDRI



Vector Breakpoint

ITR Breakpoint

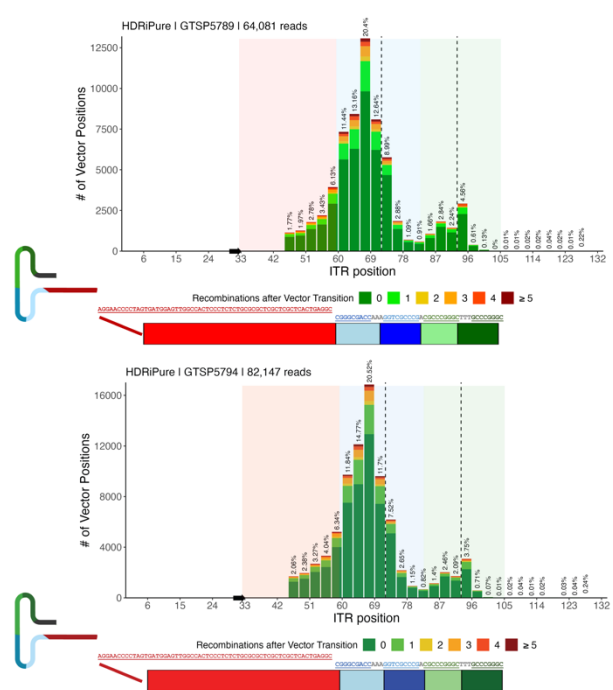
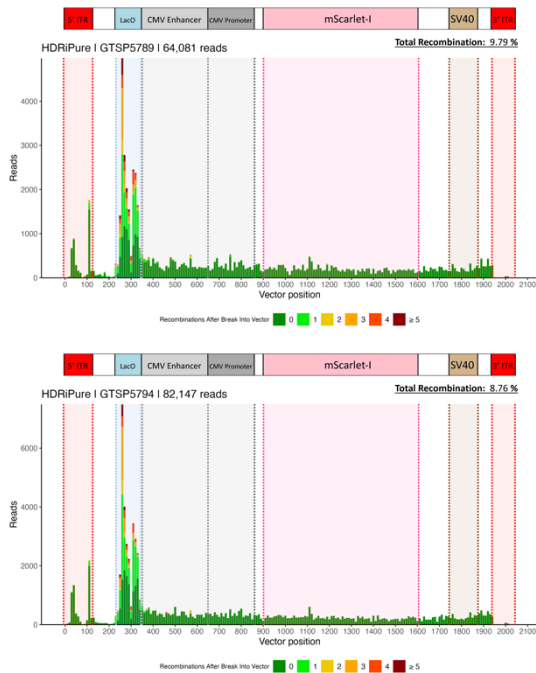
Pure : DMSO



Vector Breakpoint

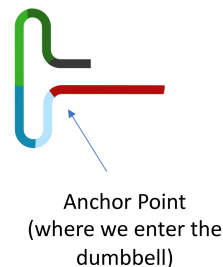
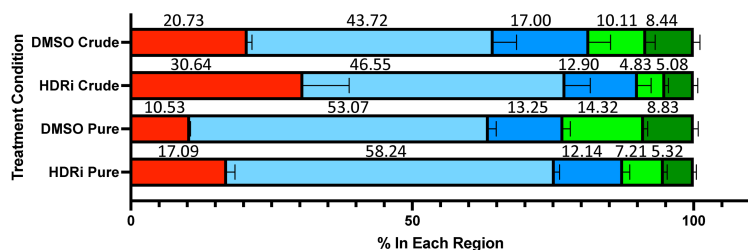
ITR Breakpoint

Pure : HDRI



Numerically Representing the Percentages of Breaks in Various ITR regions Each bar represents the average of n=2 biological replicates

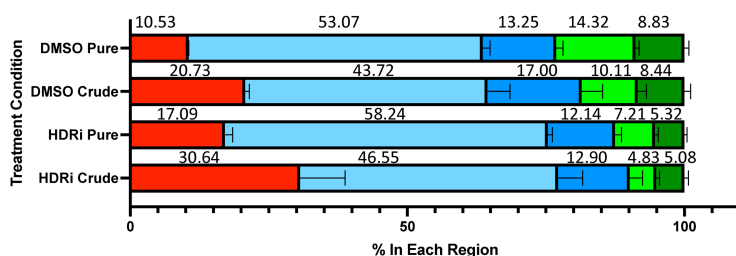
Crude AAV vs Pure AAV



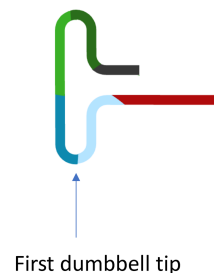
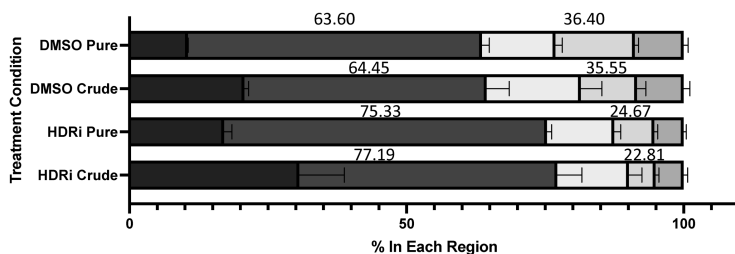
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DMSO vs HDR Inhibition

All Regions of the ITR Dumbbell



Regions of the ITR Before or After the First Dumbbell Tip



Initial Patterns:

- Looking at Crude vs Pure AAV Preps
 - Vector Breakpoint: It looks like the crude prep samples are showing more ITR into ITR hits in the vector breakpoint graphs
 - ITR Breakpoint: It looks like the crude prep samples are breaking off before we hit the anchor point (transition from DA to first dumbbell)
 - Hypothesis: Is it possible that these are not actually breaks, but rather indication of mutated ITRs that are being aligned as two different ITR alignments
- Looking at DMSO vs HDRi
 - Vector Breakpoint: No striking pattern is clear
 - ITR Breakpoint: Looking at the breakpoint graphs, it appears that there are fewer reads breaking off in the second dumbbell region.
 - ITR Breakpoint % graphs: To try and account for the contribution of AAV prep to the DA arm vs first dumbbell, the graphs were also shaded as before dumbbell tip and after dumbbell tip
 - It looks like the HDRi treated cells have more breakpoints prior to the first dumbbell tip

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****Note that this update is for internal Bushman lab planning use only. Code listed below is still in rough draft form and needs to be vetted before data can be shared externally. ****

Details for internal record-keeping

- AAVenger Version 1.1
- AAVenger run by John October 3rd 2023 (ThirdPass folder)
 - Code for filtering anchorReads: inITRoutITR.R
 - Mosaic and Density Plots: inITRoutITR_stats.R
 - ITR Breakpoint plots: ITRintoVector_ITRBreakpoints.R
 - Vector Breakpoint plots: ITRintoVectorBreakpoints.R
- ITR Breakpoint % of Region: SummarizingPercents_ITRBreakpoint.R

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