Using ADAR1 chimeric eCLIP to understand the on-target and off-target behaviors of RNA editing oligonucleotides

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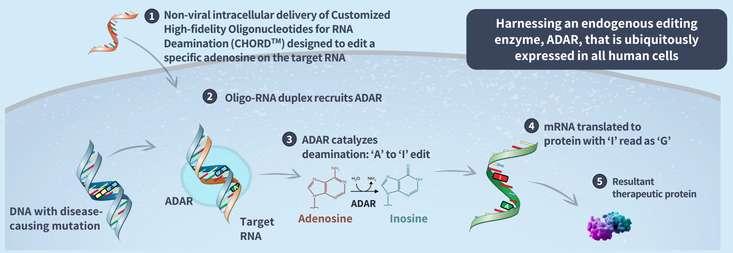
Abstract

Korro's proprietary RNA editing platform (OPERA™) utilizes CHORDs™ (Customized High-fidelity Oligonucleotides for RNA Deamination) and endogenous ADAR to repair disease-causing mutations or to modulate protein functions. Chimeric eCLIP (enhanced UV crosslinking and immunoprecipitation) was originally developed to identify microRNA binding sites[1] but the method can also be used to study intermolecular interactions between RNA molecules bound by specific RNA-binding proteins. We performed ADAR1 chimeric eCLIP to understand the target engagement of a platform toolkit oligo and evaluated if the method could be used to predict off-target editing sites. We identified on-target chimeric reads with both ADAR1 isoforms p110 and p150, and also detected ~500-1500 off-target interactions across the transcriptome. Chimeric reads were enriched for the reverse complement of oligo sequence in transfected samples only, suggesting chimeric eCLIP can successfully identify oligo binding sites. However, RNAseq studies showed that chimeric reads discovered in the eCLIP experiment were not enriched for A to I mutations, which suggests that chimeric eCLIP is not a suitable method for detecting off-target editing sites.

RNA editing platform (OPERA™)



Chimeric eCLIP

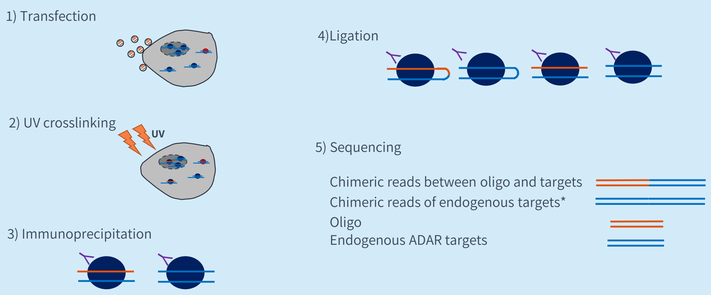


Figure 1: Illustration of the chimeric eCLIP procedure. Chimeric eCLIP for ADAR is adapted from the miR-eCLIP method[1]. Dark blue circles represent ADAR1 protein. Orange lines represent the CHORD™ oligonucleotide (oligo). Blue lines represent endogenous RNAs bound by ADAR1. *Chimeric reads of endogenous targets were not identified in this study due to the analysis method.

Experimental conditions and RNA editing results

A

B

Sample Number	Replicate	Treatment	ADAR1 Isoform for eCLIP
1	1	mock	p110
2	2	mock	p110
3	1	transfected	p110
4	2	transfected	p110
5	1	mock	p150
6	2	mock	p150
7	1	transfected	p150
8	2	transfected	p150

C

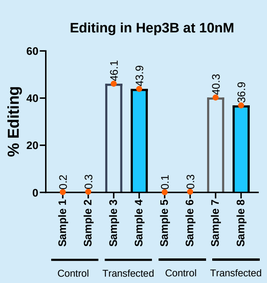


Figure 2: (A) Diagram of the oligo used in this study, a 50mer toolkit oligo targeting the 3'UTR of human ACTB mRNA. **(B)** Details of the samples tested in this study. No interferon was used for any samples. **(C)** ACTB RNA editing results in Hep3B cells 16 hours post-dose (10nM transfection) measured by next generation sequencing (NGS).

Antibodies in chimeric eCLIP pull down different ADAR1 isoforms

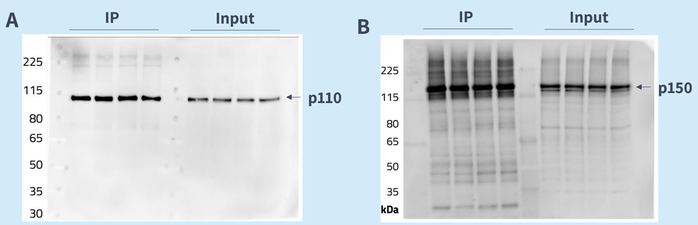


Figure 3: Western blot of the immunoprecipitation during the ADAR1 chimeric eCLIP experiment in Hep3B cells. **(A)** Antibody against both isoforms. There is no significant p150 band present in these samples. The results mostly reflect p110 binding sites. **(B)** Antibody specifically against p150.

Chimeric eCLIP libraries generated

A

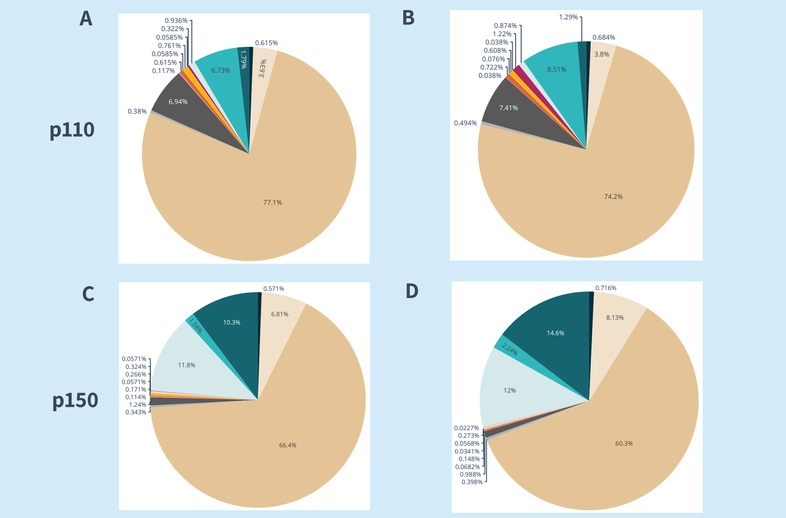
Non-Chimeric Read QC Metrics					
Sample number	Experimental condition	Initial reads	Final reads	Clusters	Peaks
1	Mock_p110	56,065,157	10,753,875	251,837	3,417
2	Mock_p110	59,177,918	10,491,612	252,252	2,631
3	Transfected_p110	52,705,993	10,569,030	240,697	2,083
4	Transfected_p110	55,434,859	11,671,657	258,652	3,085
5	Mock_p150	53,924,888	18,481,047	371,281	5,259
6	Mock_p150	54,609,326	18,072,108	347,946	8,805
7	Transfected_p150	44,164,815	14,461,352	296,179	6,579
8	Transfected_p150	62,147,129	21,949,467	402,934	7,412

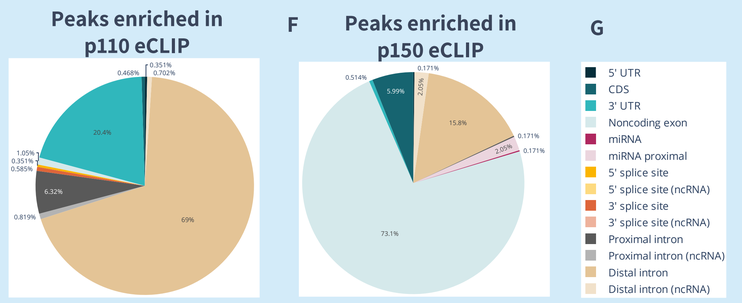
B

Chimeric Read QC Metrics				
Experimental condition	Initial candidate chimeric reads	% of reads containing oligo	Final reads	Clusters
Transfected_p110	19,025,415	0.78%	47,992	383
Transfected_p110	20,725,791	0.79%	53,486	479
Transfected_p150	16,128,794	1.46%	85,913	686
Transfected_p150	22,463,036	1.40%	113,715	1,038

Figure 4: Quality control metrics for **(A)** Non-chimeric reads and **(B)** Chimeric reads. A peak is defined as a cluster with log2 fold enrichment ≥ 3 and p-value ≤ 0.001. A previously published method, CLIPper[2], was used for peak calling. The chimeric portion of the pipeline is run after mapping reads non-chimerically.

Non-chimeric reads show ADAR1 binding sites





H

Top 5 genes enriched in each ADAR1 eCLIP	
p110	p150
IGF2	MALAT1
LSS	KCNQ1OT1
AOPEP	ZNF721
PIAS4	ZNF594
CYP4V2	HNF1A-AS1

Figure 5: Pie charts depict the relative frequency of peaks that map to each feature from non-chimeric reads in mock transfected samples. **(A)** p110 eCLIP replicate 1 (sample 1); **(B)** p110 eCLIP replicate 2 (sample 2); **(C)** p150 eCLIP replicate 1 (sample 5); **(D)** p150 eCLIP replicate 2 (sample 6); **(E)** peaks enriched in p110 eCLIP; **(F)** peaks enriched in p150 eCLIP. **(G)** Legend for **(A)-(F)**. Overall, ADAR1 showed a preference for intronic sequences, which correlates with previous publications[3–4]. **(H)** Top 5 genes with peaks enriched in each ADAR1 eCLIP.

Oligo binding sites in chimeric eCLIP reads

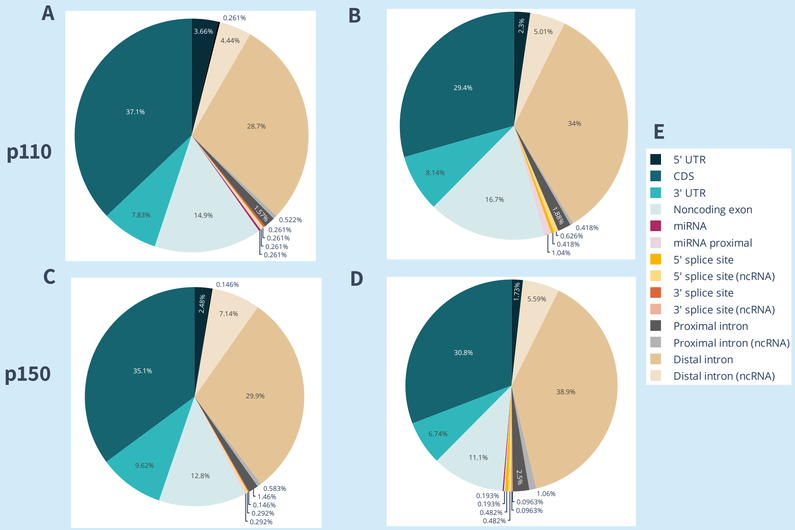


Figure 6: Pie charts depict the relative frequency of peaks that map to each feature from the chimeric reads in oligo transfected samples. **(A)** p110 eCLIP replicate 1 (sample 3); **(B)** p110 eCLIP replicate 2 (sample 4); **(C)** p150 eCLIP replicate 1 (sample 7); **(D)** p150 eCLIP replicate 2 (sample 8). **(E)** Legend for **(A)-(D)**. There are more reads mapped to CDS and fewer reads mapped to introns in the chimeric reads compared to non-chimeric reads **(Figure 5 A-D)**.

Chimeric reads are enriched for oligo complementary sites

A

Oligo sequence (reverse):
3'-GTCCCCTCCACTACCGTAACGAAAGCACATTTAATACATTACGTTTTAAA-5'

B

Experimental condition	Motif	% of Targets	P-value
Transfected_p110		17.91%	1.00E-29
Transfected_p110		11.74%	1.00E-31
Transfected_p150		13.86%	1.00E-50
Transfected_p150		13.25%	1.00E-55

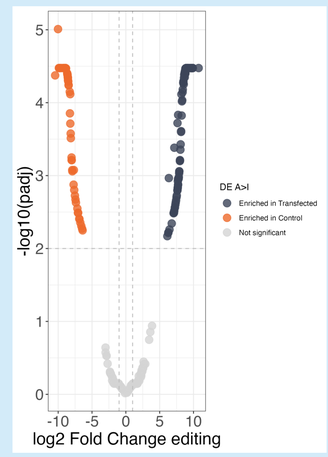
Figure 7: (A) Illustration of the reverse complement motif to the 3'end of the oligo. Red highlights the orphan position and green highlights portion of the oligo sequence reverse complement to the motif. **(B)** Enriched motifs with sequence complementarity to the oligo identified by HOMER[5] in each oligo transfected samples.

Chimeric eCLIP peaks are not enriched for A to I mutations

A

	p110 rep1	p110 rep2	p150 rep1	p150 rep2
NO. of chimeric peaks	530	612	1103	1438
NO. of ACTB peaks	1	1	1	2
NO. of genes with chimeric peaks	218	239	370	517

B



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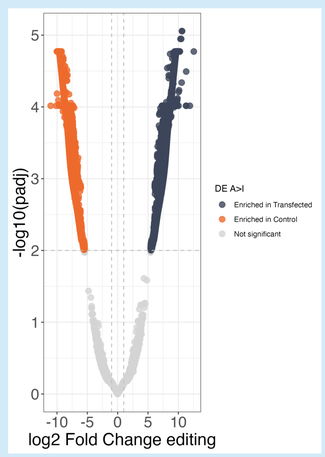

D

	Chimeric peaks	Non-chimeric peaks
Enriched in Transfected	188 (66%)	29,030 (65%)
Enriched in Control	96 (33%)	15,466 (35%)

Figure 8: (A) Number of chimeric peaks identified for each ADAR1 isoform eCLIP sample. Fold change of A to I mutations in **(B)** chimeric peaks and **(C)** non-chimeric peaks comparing transfected or mock (control) samples. A to I mutations were identified by RNAseq and analyzed using SAILOR[6] and the differential mutational rate analysis results were obtained with DESeq2. **(D)** Table of peaks with padj < 0.01 and |log2 fold change| > 2. There is no difference between the chimeric and non-chimeric peaks, which suggests chimeric peaks are not preferentially enriched for A to I mutations.

Conclusions

- Chimeric eCLIP has generated RNA footprints of both ADAR1 isoforms in Hep3B cells upon transfection of a toolkit RNA editing oligonucleotide.
- Both ADAR1 isoforms preferentially bind to introns. Comparing between the two, p110 shows preference for 3'UTRs and there are more peaks mapped to CDS and non-coding RNAs for p150.
- There are more CDS peaks in the chimeric reads. A sequence motif reverse complement to the 3' end of the oligo was recovered in a substantial fraction of the chimeric reads in the transfected samples, which suggests chimeric reads are enriched for oligo binding sites.
- Chimeric reads are not enriched for A to I mutations compared to non-chimeric reads.
- **Overall, chimeric eCLIP method allows the identification of oligo binding sites, but not enriched for off-target editing sites.**

References

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