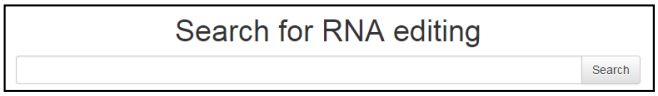
TUTORIAL FOR RNA EDITING PLUS

RNA Editing Plus is an automatically online platform to annotating A-to-I RNA editing sites in all the functional genes, identifying whether these editing occurring in pri-mRNA splicing signals (5'ss, 3'ss or BS), miRNAs (seed or non-seed), or miRNA target regions (3'UTR) from human high-throughput sequencing data and predicting their effects on miRNA targeting, mRNAs alternative splicing, and CDS missense mutation according to bioinformatic analysis.

Researchers are able to investigate RNA editing at different levels by providing RNA-Seq data source or uploading RNA-editing list via *RNA Editing Plus*. Since we have already scanned most previously reported A-to-I editing events, and calculated their potential effects, users may also directly search their interested information according to related gene symbols or chromosome location.

1. Directly search interested previously reported RNA editing events.

1.1 For researchers who want to know whether a previously reported A-to-I editing event happens in specific position. Please type in your interested gene symbols such as YY1, MIR376A1, official symbol name and aliases from NCBI (https://www.ncbi.nlm.nih.gov/) are both accepted. Moreover, you can also search A-to-I editing events by providing the chromosome location information such as chr16:1784776 or chr16:1784776-1785760 (1000bp limits).

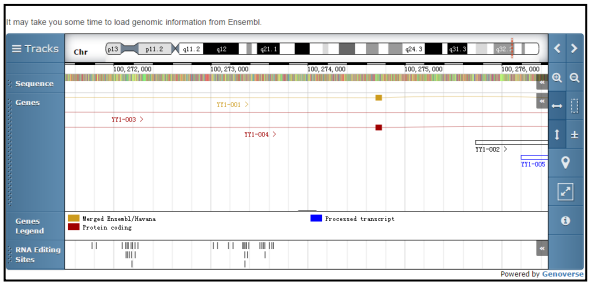


Note: our platform does not support multiple keywords search function, please do not use any spaces when typing in the gene symbols or chromosome location information.

1.2 After clicking the "search" button, the page will automatically redirect to the result part, including:

Editing Hunter (overview of your interested gene)

Map (distribution of the A-to-I editing events and the information of DNA sequences from Ensembl)



Editing sites Filters (to distinguish editing events by clicking buttons regarding Alu, non-Alu and other location information)

Editing sites Results (detailed information of each A-to-I editing event)

Editing effects (detailed information for each effective A-to-I editing event)

Note: please find the description of Results and Editing effects parts *4.2-4.5* from *page 7-10.*

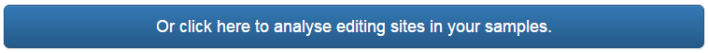
2. Investigate A-to-I RNA editing levels and effects by uploading a RNA editing list.

2.1 For researchers who want to know if certain A-to-I RNA editing events have effects on miRNAs targeting, mRNAs alternative splicing, or CDS missense mutation. You are supposed to upload a prepared editing sites list (template address: https://www.rnaeditplus.org/Public/demo/demo.txt).

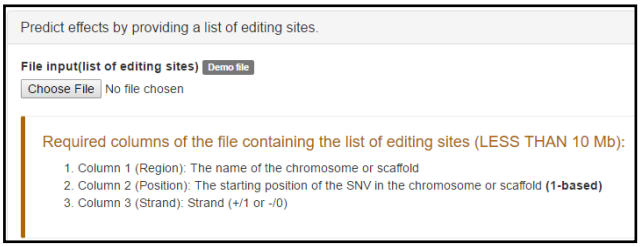
Region	Position	Strand
chr9	94178843	+
chr5	143225441	+
chr4	2970944	+
chr16	29834400	+
chr17	78160763	+
chr16	56940406	+
chr13	30358182	-
chr5	149410136	+
chr21	29581360	-
chr1	45697906	-
chr1	39459245	+

Note: a complete editing list should be written in "txt" format with 10 MB limit, and contain the following information: Region (Chromosome number), Position (detailed location referring to hg38 reference genome, 1-based), Strand (+/1 or -/0).

2.2 After preparation of editing list, click the "blue button" below the "search frame"

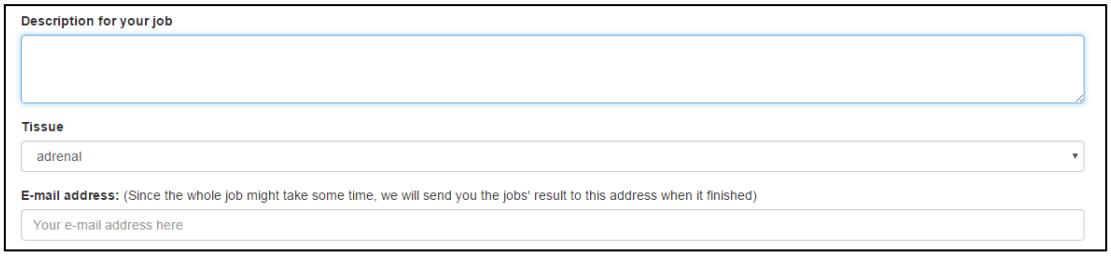


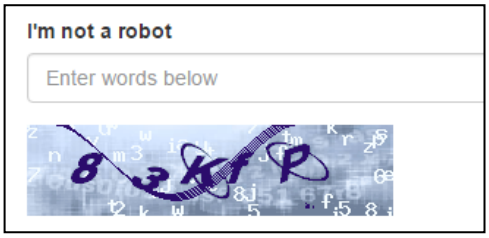
or the "Analyze" button at the top of our home page. You will find a "Choose File" button to upload your editing list.



2.3 Job submission

Discribe the job which can only be seen by yourself (optional), choose a tissue (optional), and input your E-mail address (required) to submit your job.





Note: Click the picture results in verification code change. Once you type a wrong verification code, please return to job submission page and input all related information again.

2.4 Job confirmation

The page will automatically redirect to confirm page: Please confirm your request

Close the page and check your email box, you will receive a confirmation link from no-reply@rnaeditplus.org to continue your job. You can also check the Job status according to the Job ID via "Job status" button at the top of our home page.

2.5 Job Result

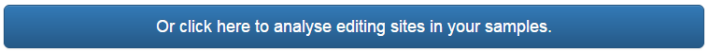
When the Job finished, our system will automatically send you a notification. You can check your job results by click the result link.

Note: please find the detailed description of Results and Editing effects parts *4.1-4.5* from *page 6-10.*

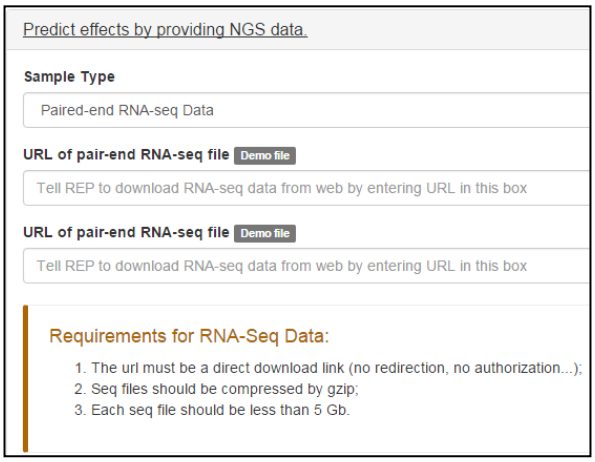
3. Investigate A-to-I RNA editing levels and effects by supporting a RNA-seq data

3.1 The next generation RNA sequencing data (NGS) is accepted by RNA Editing Plus. Besides the RNA editing calling and editing effects prediction, our system will report the expression level of ADAR 1 & 2.

After preparation of your RNA-seq data, click the "blue button" below the "search frame"



or the "Analyze" button at the top of our home page. You will find NGS frames at the middle of the page.



Note: RNA Edit Plus can only process pair-end sequencing data alone. The platform will directly download the paired RNA-Seq data after entering URL (no redirection, no authorization). The RNA-seq data should be provided in "gzip "form, and have a 5GB limit.

3.2 Job submission

3.3 Job confirmation

3.4 Job Result

Note: please find the similar descriptions about Job submission and confirmation in *2.3-2.5* from *page 3-4.* Please find the detailed description of Results and Editing effects parts in *4.1-4.5* from *page 6-10.*

4. Detailed description for the Result and Effects parts RNA Editing on miRNA

4.1 Job result overview includes:

Description (information users typed)

Create time (start time of the job process)

Validated editing sites (detailed location information after annotation)

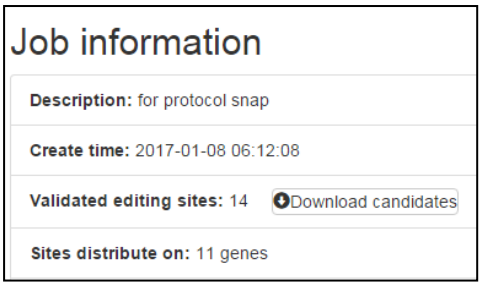
Sites distribute

ADAR 1 & 2 expression

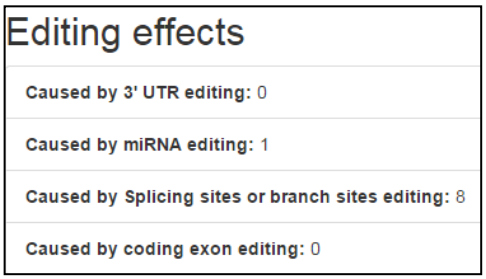
Editing effects (effective editing sites numbers and types)

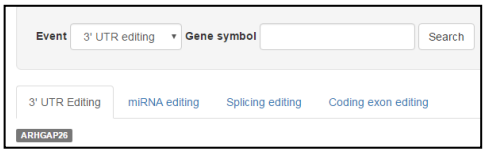
Gene regulation (potentially affected miRNA targets, transcripts information)

Discover (detailed information for each effective editing event, including: 3'UTR editing, miRNA editing, splicing editing, coding exon editing)



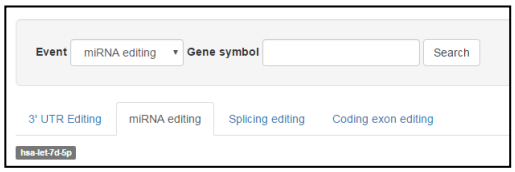
Note: ADAR expression can be only reported from RNA-seq data, and our system will show the normal tissue ADAR expression level as references according to the users pre-selected "tissue".





4.2 RNA Editing effects on miRNA targeting.

Click the "miRNA editing" in "Discover" part all affected candidates are listed below, and if there are too many candidates, users may search the detailed information according to gene symbols.



Click each candidate miRNA, you will see an editing sites map and an overview of RNA editing effects on this gene.

Seed? (√/× means seed or non-seed)

New targets (new potential targets number of the edited miRNA)

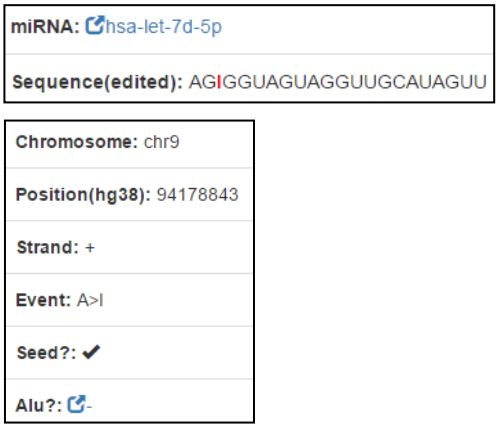
Old targets (origin targets number of the miRNA)

Common targets (common targets number between origin and edited miRNA)

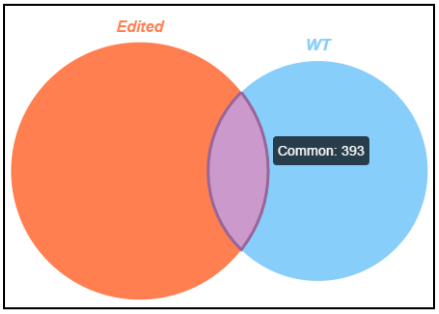
Click the "about targets alternation", our system will display the detailed editing effects:

miRNA Information (miRBase information)

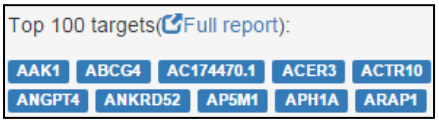
Edit Information (detailed editing information)



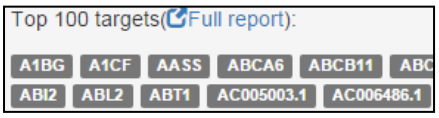
Venn (origin targets and new potential targets)



Common and New targets



Weaken targets (inactive targets of the edited miRNA)



Note: user may save and download the results as "pdf" form.

Click each candidate gene symbol in "Common targets" and "New targets", our system will display the detailed calculated values:

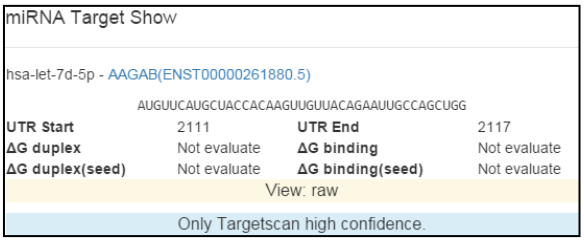
ΔG duplex (miRNA-mRNA duplex minimum free folding energy)

ΔG binding (miRNA-mRNA binding minimum free energy)

ΔG duplex seed (miRNA seed region-mRNA duplex minimum free folding energy)

ΔG binding seed (miRNA seed region-mRNA binding minimum free energy)

UTR start/UTR end (start/end binding site in the target transcript)



Note: Since editing effects has been already identified by other methods, "Not evaluate" means the SVM calculation is skipped. Please find detailed information in *5.2* on *page.11.*

4.3 RNA editing effects on 3'UTR.

Note: please find the similar descriptions in *4.2* from *page 7-8.*

4.4 RNA editing effects on mRNA alternative splicing.

3' UTR Editing | miRNA editing | Splicing editing | Coding exon editing

Gene(Transcript)	Position	Type	Raw score	Edited Score	Variation(%)	#Intron
SEPT7(ENST00000634600)	35832800	inactived (or weakened) 3' splicing site	0.410	0.000	0.000	1

Gene (gene symbol and transcript)

Position (location information according to hg38, 1-based)

Type (editing effects types)

Raw score (origin splicing score)

Edited score (edited splicing score)

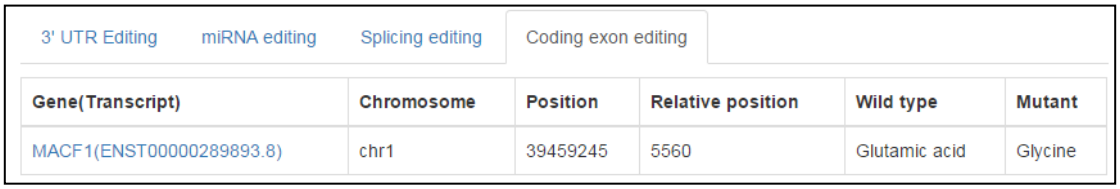
Variation (rate of change from Raw to Edited score)

#intron (intron serial number)

Note: There are 8 types including inactivated (or weakened) 5' or 3' splice site; enhanced 5' or 3' splice site; inactivated branch point; weakened branch point; enhanced branch point; and new branch point.

Note: "1" in #intron means RNA editing occurs in 1st intron.

4.5 RNA editing effects on mRNA coding exon.



3' UTR Editing | miRNA editing | Splicing editing | Coding exon editing

Gene(Transcript)	Chromosome	Position	Relative position	Wild type	Mutant
MACF1(ENST00000289893.8)	chr1	39459245	5560	Glutamic acid	Glycine

Gene (gene symbol and transcript)

Chromosome

Position (location information according to hg38, 1-based)

Relative position (relative position in its transcript, 0-based)

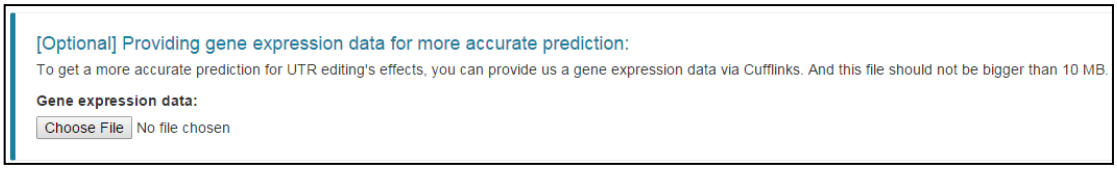
Wild type (wild type amino acids)

Mutant (edited amino acids)

Note: Our platform only shows the missense CDS mutation committed by A-to-I RNA editing.

5. Addition information.

5.1 To get a more accurate prediction for effects of 3'UTR editing, user may provide a gene expression data via Cufflinks. And this file should not bigger than 10 MB.



5.2 Datasets cited in RNA Editing Plus.

GENCODE v24 (comprehensive), hg38, dbSNP146, miRBase 21, RADAR, DARNED, and HERA databases.

5.3 Contact information.

If you have any problem, please do not hesitate to contact us: li_yao@outlook.com, yys@rnaeditplus.org, guangqisong@rnaeditplus.org or zyc@rnaeditplus.org.