

TUTORIAL FOR RNA EDITING PLUS

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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).

Search for RNA editing

Search

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)

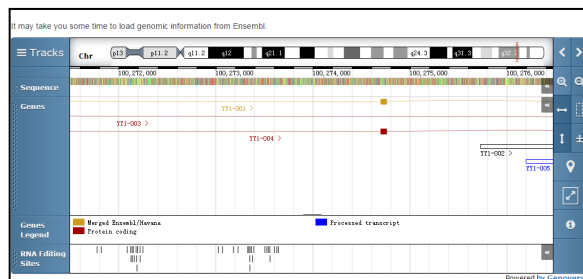
Editing sites Filters (to distinguish editing events by clicking buttons regarding Alu, non-Alu and other location information)

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

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

Editing Hunter

Search results for ATAD3C (60 editing sites found, 22 sites have effects).



Editing sites

Filters

Alu? ☐ All ☒ Non-Repeat element

Annotation: ☒ All ☐ Intronic ☐ 5' UTR ☐ CDS

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 click here to analyse editing sites in your samples.

or the "Analyze" button at the

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

Predict effects by providing a list of editing sites.

File input(list of editing sites) [Demo file](#)

[Choose File](#) | No file chosen

Required columns of the file containing the list of editing sites (LESS THAN 10 Mb):

1. Column 1 (Region): The name of the chromosome or scaffold
2. Column 2 (Position): The starting position of the SNV in the chromosome or scaffold (1-based)
3. Column 3 (Strand): Strand (+/1 or -/0)

2.3 Job submission

Describe 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.

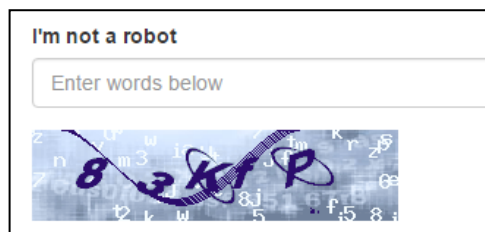
Description for your job

Tissue

adrenal

E-mail address: (Since the whole job might take some time, we will send you the jobs' result to this address when it finished)

Your e-mail address here



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 click here to analyse editing sites in your samples.

or the "Analyze" button at the

top of our home page. You will find NGS frames at the middle of the page.

[Predict effects by providing NGS data.](#)

Sample Type
Paired-end RNA-seq Data

URL of pair-end RNA-seq file [Demo file](#)
Tell REP to download RNA-seq data from web by entering URL in this box

URL of pair-end RNA-seq file [Demo file](#)
Tell REP to download RNA-seq data from web by entering URL in this box

Requirements for RNA-Seq Data:

1. The url must be a direct download link (no redirection, no authorization...);
2. Seq files should be compressed by gzip;
3. Each seq file should be less than 5 Gb.

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)

Job information

| | |
|----------------------------------|-------------------------------------|
| Description: for protocol snap | |
| Create time: 2017-01-08 06:12:08 | |
| Validated editing sites: 14 | Download candidates |
| Sites distribute on: 11 genes | |

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".

Editing effects

| |
|---|
| Caused by 3' UTR editing: 0 |
| Caused by miRNA editing: 1 |
| Caused by Splicing sites or branch sites editing: 8 |
| Caused by coding exon editing: 0 |

Event

3' UTR editing

Gene symbol

Search

3' UTR Editing

miRNA editing

Splicing editing

Coding exon editing

ARHGAP26

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.

Event: miRNA editing

Gene symbol:

Search

3' UTR Editing

miRNA editing

Splicing editing

Coding exon editing

hsa-let-7d-5p

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

Result

Save results as pdf

Download results(SQL)

| Chromosome | Position | Seed region? | New targets | Old targets | Common Targets | Details |
|------------|----------|--------------|-------------|-------------|----------------|---|
| chr9 | 94178843 | ✓ | 3143 | 2294 | 393 | About targets alternation |

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)

miRNA: [hsa-let-7d-5p](#)

Sequence(edited): AGIGGUAGUAGGUUGCAUAGUU

Chromosome: chr9

Position(hg38): 94178843

Strand: +

Event: A>I

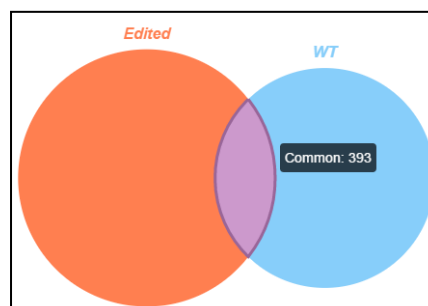
Seed?: ✓

Alu?: [G-](#)

Venn (origin targets and new potential targets)

Common and New targets

Weaken targets (inactive targets of the edited miRNA)



Top 100 targets([Full report](#)):

| | | | | |
|--------|---------|------------|-------|--------|
| AAK1 | ABCG4 | AC174470.1 | ACER3 | ACTR10 |
| ANGPT4 | ANKRD52 | AP5M1 | APH1A | ARAP1 |

Top 100 targets([Full report](#)):

| | | | | | |
|------|------|------|------------|------------|-------|
| A1BG | A1CF | AASS | ABCA6 | ABCB11 | ABCB1 |
| ABI2 | ABL2 | ABT1 | AC005003.1 | AC006486.1 | |

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)

| miRNA Target Show | | | |
|--|--------------|--------------------------|--------------|
| hsa-let-7d-5p - AAGAB(ENST00000261880.5) | | | |
| AUGUUC AUGCUACCAAGUUGUUCAGAAUUGCCAGCUGG | | | |
| UTR Start | 2111 | UTR End | 2117 |
| ΔG duplex | Not evaluate | ΔG binding | Not evaluate |
| ΔG duplex(seed) | Not evaluate | ΔG binding(seed) | Not evaluate |
| View: raw | | | |
| Only Targetscan high confidence. | | | |

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.

RNA Editing 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 | inactivated (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.

[Optional] Providing gene expression data for more accurate prediction:

To get a more accurate prediction for UTR editing's effects, you can provide us a gene expression data via Cufflinks. And this file should not be bigger than 10 MB.

Gene expression data:

No file chosen

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.