Ariadne
MS/MS Search
User’s Manual

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Ariadne Development Team

0. Introduction

This manual introduces how to characterize RNA MS/MS data by using the Ariadne server via the Internet (https://ariadne.riken.jp/). How do you start searching sequence database using MS/MS data will be explained in Sections 1 and 2. In Section 3, how to browse your search result interactively is described. Manuals on defining your own parts (Nucleotide Parts Editor) and calculating mass values of oligonucleotides (Mass Calculator) are also available at the Ariadne site. If you have any questions and/or comments, please feel free to contact us via email (ariadne_dev_team@riken.jp).

Ariadne is a web service that assists researchers to identify RNAs in a sample and to characterize their post-transcriptional modifications by searching sequence database using MS/MS data. To identify RNAs in the sample, the software conducts a two-step searching algorithm, MS/MS ion search and Nucleotide mapping as shown in Figure 1.

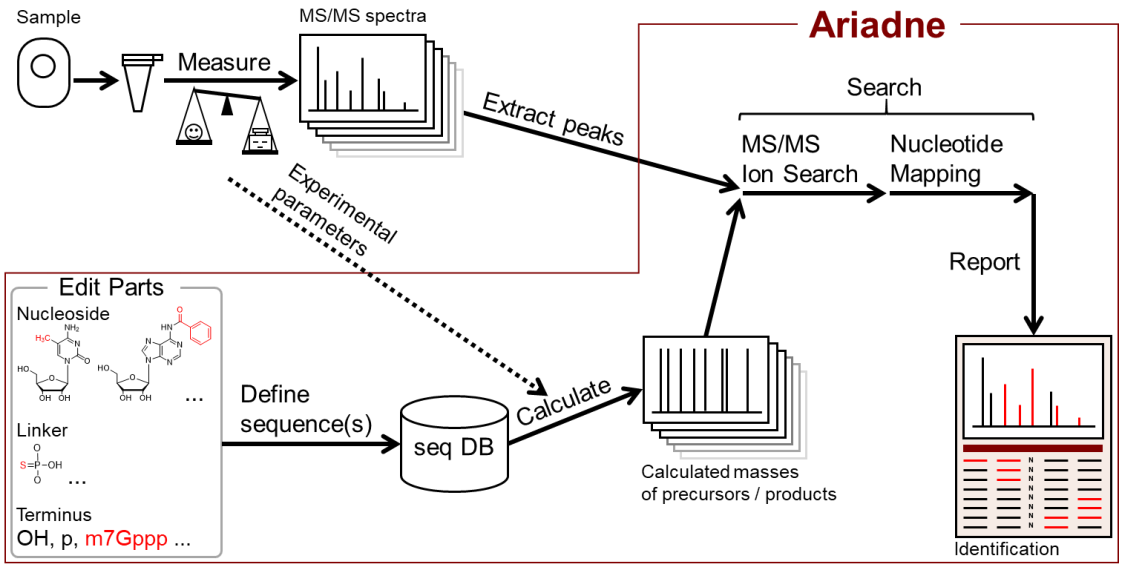


Figure 1. Schematic of Ariadne MS/MS Search

The Ariadne server is publicly available at https://ariadne.riken.jp/. The screen shot of the top page is shown in Figure 2.

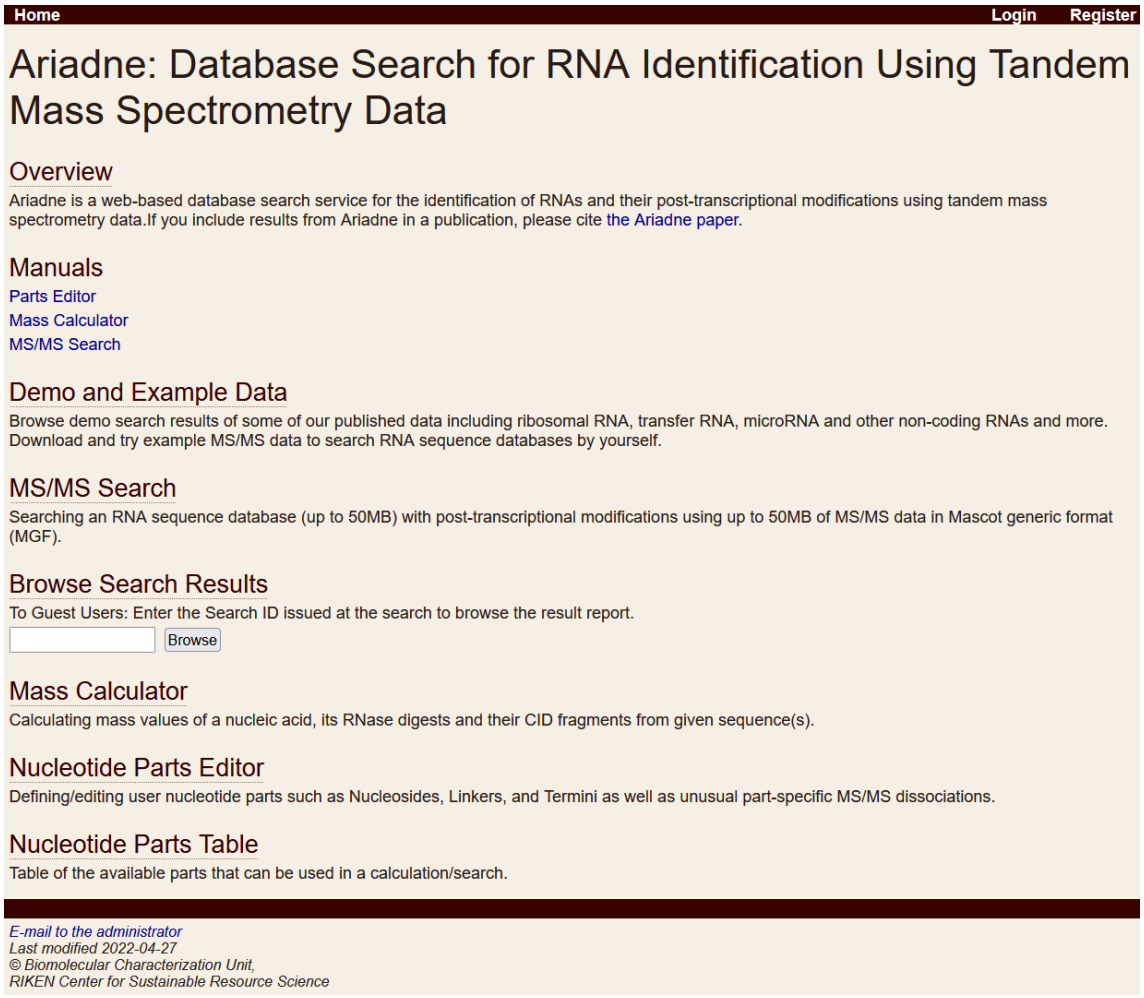


Figure 2. The top page of Ariadne service at https://ariadne.riken.jp/

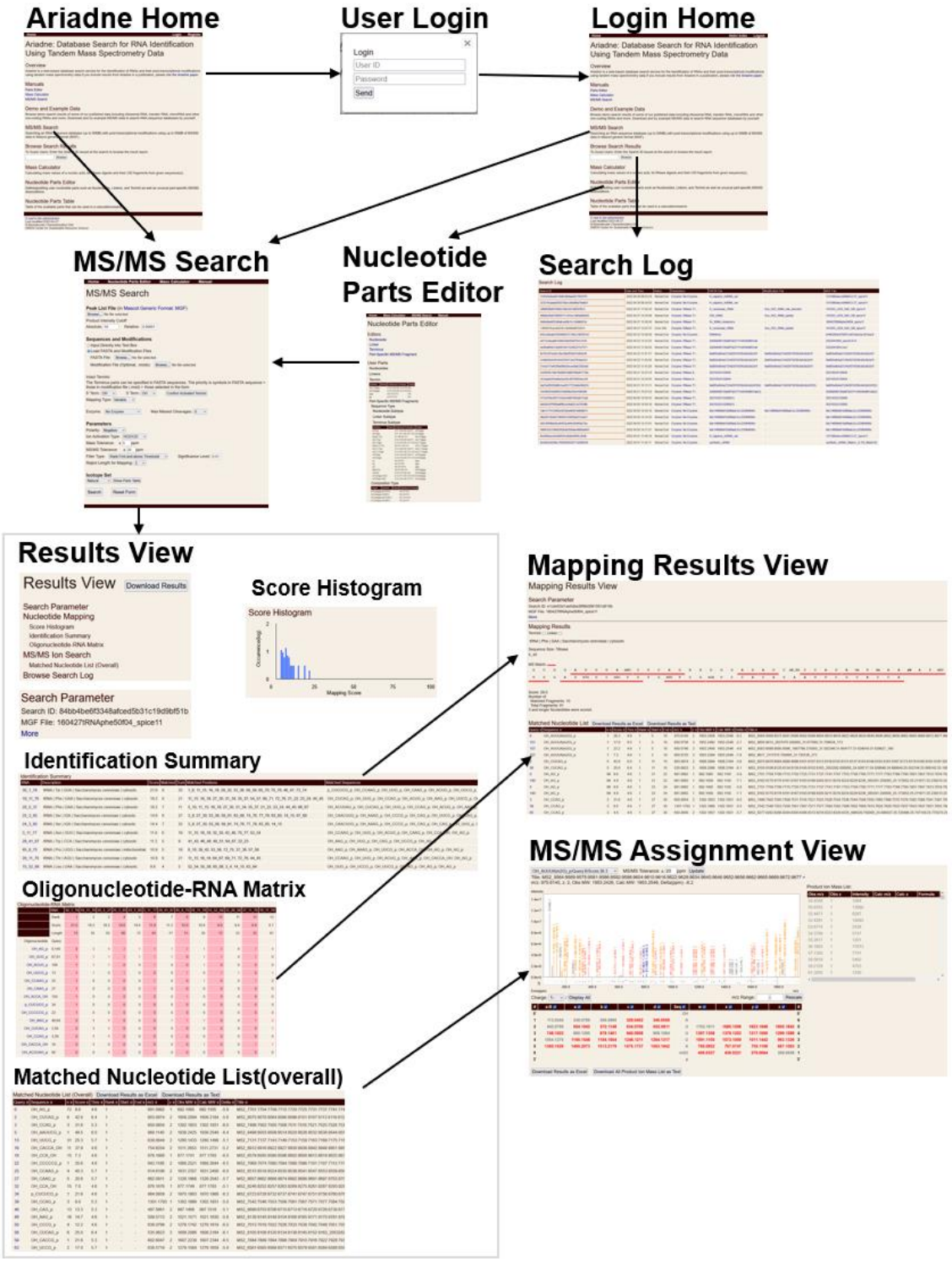


Figure 3. The transition of html pages of Ariadne

Since user interface to Ariadne is a web browser, the search is defined using a web form for an interactive search. In the form, a user can specify MS/MS data of the sample's

RNA(s), sequence database to be searched, which contains RNA sequence(s) and possible post-transcriptional modifications, and other search parameters. The data and parameters inputted in the form is uploaded to the Ariadne server in which they are processed to identify/characterize RNAs in the sample. Just after transferring the query, the server issues the Search ID, which can be specified the search afterward. On completion of the search, the server will return the search results to the user's web browser, which shows an html report containing summary and detailed views of the results as shown in Figure 3.

1. Preparation of data for use in searches

1.1. Requirements

System and data formatting requirements are described in this section. Since user interface to the Ariadne server is a web browser, the search is defined using a web form for an interactive search. A search requires at least a peak-list file containing MS/MS data and a nucleotide sequence as database to be searched.

1.1.1. System requirements

The software has the following system requirements:

Computer: A personal computer with a working Internet connection and one of the following web browsers.

Web browser: Although all the main calculations for the search are done on the Ariadne server, a web browser is necessary for interactive communication with the server. This software uses the JavaScript language that enables it to provide a better user experience within the web browser. Most modern browsers run Ariadne without any issues; we recommend Mozilla Firefox (73.0a1 or later), although we have confirmed that the program also works well with Google Chrome 79.0.3945.117 or later, Microsoft Edge 79.0.309.58 or later, and Brave 1.2.41 or later on a Windows 10 platform. When using an individual user's specific function, *e.g.* the Nucleotide Parts Editor page, you should accept the creation of a Cookie / use your own account (See Section 1.2 and the Ariadne Nucleotide Parts Editor User's Manual).

Microsoft Excel: The results of Ariadne's searches can be downloaded as a Microsoft Excel Workbook (.xlsx) file. Excel version 2007 or later is required to open the .xlsx file.

1.1.2. MS/MS peak list

The Ariadne search required at least a peak list. Currently the program supports peak-list files containing MS/MS data in Mascot™ generic format (MGF). An MGF file is a text file containing at least one MS/MS query unit, each of which begins with a line

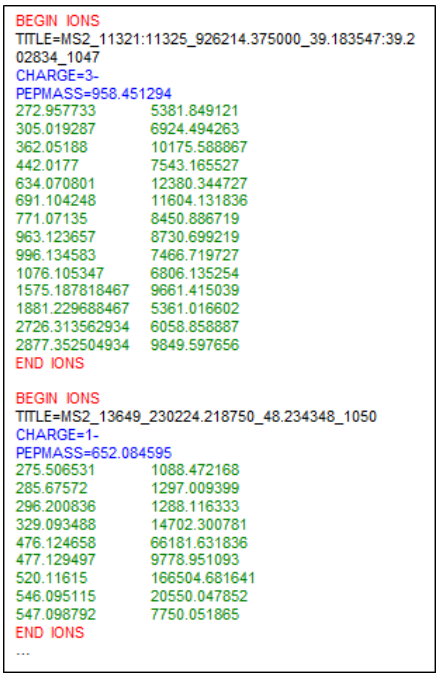
having only BEGIN IONS statement and ends with another line having only END IONS. In between both the lines are the information on a precursor ion and that on the corresponding product ions which are pairs of mass value and its intensity with tab- or space-delimited format. See Data file format page of Mascot (www.matrixscience.com/help/data_file_help.html) for more information on MGF. Most MS vender's software tools support to export MS/MS data as MGF. Consult each vender's manual for details. To view or modify an MGF peak list, the use of a text editor is recommended. Windows has a simple text editor called Notepad (notepad.exe); however, better editors can be downloaded through the web.

1.1.3. Sequence

The search requires that the user input at least one nucleic acid sequence into the web form. The web form can accept three forms of input for sequence(s): (1) either direct inputting and/or pasting to the text box, (2) uploading a file that contain the sequence(s) in FASTA format, or (3) selecting one of preinstalled genome database. Other parameters are optional unless data from a sample which was hydrolyzed with an RNase is searched; in that case, the parameter Enzyme should be specified as the enzyme used in the experiment. If a parameter is not specified by the user, the parameter for the default setting will be used. Steps to prepare sequences and how to define parameters are described in Sections 1.3 and 1.4, respectively. To view or modify a sequence, the use of a text editor is recommended.

1.2. MS/MS Peak List

The peak list should be a text file containing the information on precursor ion's mass and charge as well as product ion's mass values and the corresponding intensities. Ariadne supports only MGF at present. An MGF file contains at least one MS/MS query unit. As shown in Figure 4 each unit begins with a line having only BEGIN IONS statement and ends with another line having only END IONS. The content between BEGIN IONS and END IONS includes the information on a precursor ion like CHARGE and PEPMASS (blue letters in Figure 4) and that on the corresponding product ions which are pairs of mass value and its intensity with tab- or space-delimited format (green letters in Figure 4). The charge value of a product ion can be optionally specified as a third column. See Data file format page of Mascot (www.matrixscience.com/help/data_file_help.html) for more information on MGF. Most MS vender's software tools support to export MS/MS data as MGF. Consult each vender's manual for details.



```
BEGIN IONS
TITLE=MS2_11321:11325_926214.375000_39.183547:39.2
02834_1047
CHARGE=3-
PEPMASS=958.451294
272.957733          5381.849121
305.019287          6924.494263
362.05188           10175.588867
442.0177            7543.165527
634.070801          12380.344727
691.104248          11604.131836
771.07135           8450.886719
963.123657          8730.699219
996.134583          7466.719727
1076.105347         6806.135254
1575.187818467      9661.415039
1881.229688467      5361.016602
2726.313562934      6058.858887
2877.352504934      9849.597656
END IONS

BEGIN IONS
TITLE=MS2_13649_230224.218750_48.234348_1050
CHARGE=1-
PEPMASS=652.084595
275.506531          1088.472168
285.67572           1297.009399
296.200836          1288.116333
329.093488          14702.300781
476.124658          66181.631836
477.129497          9778.951093
520.11615           166504.681641
546.095115          20550.047852
547.098792          7750.051865
END IONS
...
```

Figure 4. An example of MGF file. Lines between BEGIN IONS and END IONS represent a peak list for a single MS/MS measurement or for accumulated multiple MS/MS spectra with the same precursor (dependent on the peak extraction software used).

1.3. User account (optional)

All of the calculator's functions are accessible and can be used without a user account, with the exception of the Nucleotide Parts Editor function and Browse Search Result. A user account is required for this function so that your data can be saved privately and securely (password protected) on the server. Once you have defined and saved the edited parts, this information can be used and accessed from anywhere. You can sign up for a user account on the top right of the page (https://ariadne.riken.jp/).

To create a new account, click on the Register button at the right top of the top page (Figure 2).

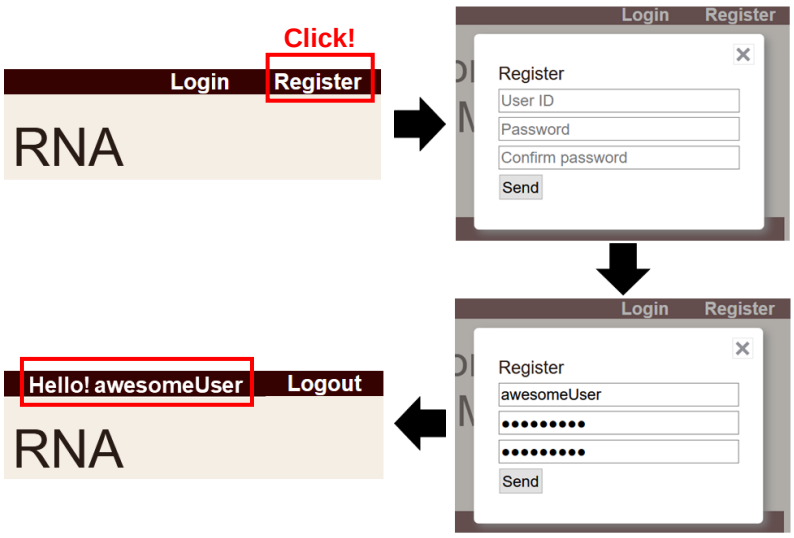


Figure 5. Registration of a new user account

If another user is already logged in, click on the Logout button to log out before registering your user account. Click the Register button and a dialog box will pop up. Input your User ID and Password, and click the Send button. If your User ID and Password were successfully registered on the server, the user status will be changed to Login, and your User ID will be shown on the top right of the page.

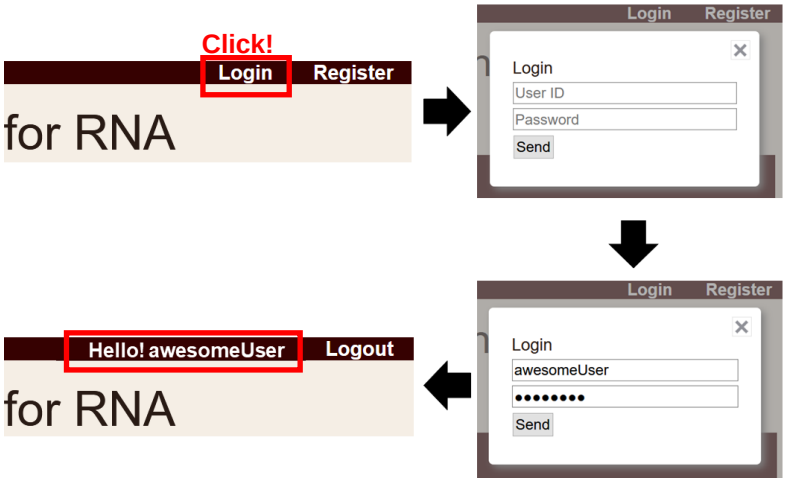


Figure 6. Login procedure

To log in to your account, click on the Login button. A dialog box will appear. Input your User ID and Password, and click the Send button. If you have successfully logged in, the user status will have changed to login and your User ID will be displayed at the top right of the page (See Figure 6). Users are automatically logged out every 24 hours.

1.4. Parts

Typical (and canonical) nucleic acids consist of 5′- and 3′-termini, nucleosides and linker(s) as shown in Figure 7.

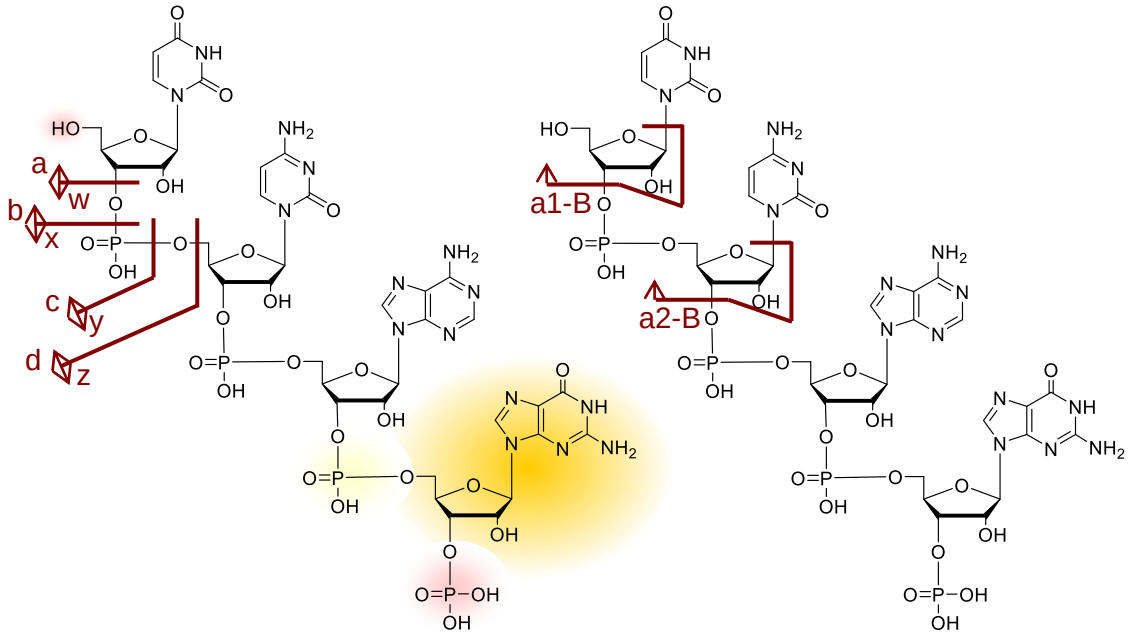


Figure 7. The structure of nucleic acids and their MS/MS dissociation sites. The backbone-cleavage sites and their nomenclature are indicated on the left; an ion generated by simultaneous backbone 3′ C-O bond cleavage and loss of base (termed as a-B) is represented on the right. The 3 components, namely, a nucleoside, a linker, and the termini, are shown in the left in orange, yellow and pink, respectively.

To emulate this structure, Ariadne allows the definition of these three parts to compose a sequence. In the program, a nucleoside is further divided into base and sugar sub-components to calculate the mass values of ions that have lost a base, such as M-B [base loss(es) from molecular ion] and a-B [loss of the base closest to the 3′end from an a-type ion]. These parts are defined by their elemental composition as the software calculates mass values for nucleic acids based on their elemental composition and atomic mass values from the NIST Physical Measurement Laboratory (http://physics.nist.gov/cgi-bin/Compositions/stand_alone.pl?ele=&ascii=html&isotype=some). In addition to these three types of parts, their known MS2 fragments can also be defined. A table that lists available parts and known MS2 fragments can be browsed from the Nucleotide Parts Table link on the top page (See Figure 2). Most nucleoside symbols listed on the table are cited from the Short Name field in MODOMICS, a

publicly available database that compiles post-transcriptional modified nucleosides (https://iimcb.genesilico.pl/modomics/modifications). If you would like to use parts other than those included in the default parts table, you can define your own with the Nucleotide Parts Editor page. Using the editor requires a user account, as described above. Further information on how to define parts is described in the Nucleotide Parts Editor manual.

1.5. Nucleotide sequences with or without site-directed modification(s)

At present, the Genome MS/MS Search service is suspended for preparing a new version. During the downtime, if you would like to search such a large database, contact us via email (ariadne_dev_team@riken.jp).

The MS/MS Search program accepts nucleotide sequences submitted in the FASTA format (http://blast.ncbi.nlm.nih.gov/blastcgihelp.shtml). Briefly, entry of a sequence in that format contains a header line beginning with “>” and either single or multiple lines of a nucleotide sequence. This format can be used to submit multiple entries. To input a nucleotide sequence, you may use any of the parts in the Nucleotide Parts Table page (http://ariadne.riken.jp/html/parts_table.html). If you would like to use your own parts, you can define these parts using the Nucleotide Parts Editor function. After defining these parts, they will be added to the user’s Nucleotide Parts Table page and can be included in a sequence. To create various types of nucleic acids, place the symbols for nucleoside and linker parts in the order of the sequence. A linker must be inserted between nucleosides and must not be placed at either end of the sequence. For example, an RNA with base sequence 5′- A C U G -3′ is represented as ApCpUpG, where p denotes a phosphodiester linker. The sequence can also be written as ACUG because inclusion of the default p linker is optional.
A nucleoside part symbolized by more than two characters (this is the case with most modified nucleosides) must be enclosed within parentheses in a sequence. A linker must not be written with parentheses, even if it has more than two characters. For example, (Ad)C(m5U)GYsG, where Ad, m5U, Y, and s denote deoxyadenosine, 5-methyluridine, pseudouridine, and a phosphorothioate linker, respectively.
Nucleosides that are symbolized by a single character, for example I (Inosine) and Y (Pseudouridine), may be used as is or enclosed in parentheses. Thus, both AYUG and A(Y)UG are acceptable and will be recognized as the same sequence. A typical FASTA for modified RNA is shown below:

>tRNA | Ala | AGC | Saccharomyces cerevisiae | cytosolic
GGGCGUGU(m1G)GCGUAG(D)CGG(D)AGCGC(m2,2G)CUCCCUU(I)GC(m1I)(Y)GG
GAGAGG(D)CUCCGG(m5U)(Y)CGAUUCCGGACUCGUCCACCA

>tRNA | Arg | UCU | Saccharomyces cerevisiae | cytosolic
GCUCGCGU(m1G)(m2G)CGUAA(D)GGCAACGC(m2,2G)(Y)CUGACU(mcm5U)CU(t6
A)A(Y)CAGAAGA(D)UAUGGG(m5U)(Y)CG(m1A)CCCCCAUCGUGAGUGCCA

To input a sequence in FASTA format, you can denote the 5′- and 3′-termini in the sequence as well. If you do not denote each terminus, it can be specified separately in a selectable box within the web form (See Section 2.1). Both 5′- and 3′-termini can be marked using the Intact 5′ Term and Intact 3′ Term boxes, respectively. As the default, OH and p (phosphate) are available as options for the 5′-end, and OH, cp (2′, 3′-cyclic phosphate) and p are available for the 3′-end. After you define a terminal using the Nucleotide Parts Editor function, the defined terminus will be added to the part list and, if you will activate the terminus, it appears in the corresponding selectable box for the calculation.
You can also specify modification(s) using a different file (that has the extension .mods) from the FASTA file. This is a modification file that contains the “>”-starting header line(s) and the next line(s) that specify the site and type of modification(s). A pair of a FASTA file that contains unmodified sequences and the corresponding modification file is shown below:

.fasta

>tRNA | Ala | AGC | Saccharomyces cerevisiae | cytosolic
GGGCGUGUGGCGUAGUCGGUAGCGCGCUCCCUUAGCAUGGGAGAGGUCUCCG
GUUCGAUUCCGGACUCGUCCACCA

>tRNA | Arg | UCU | Saccharomyces cerevisiae | cytosolic
GCUCGCGUGGCGUAAUGGCAACGCGUCUGACUUCUAAUCAGAAGAUUAUGGGU
UCGACCCCCAUCGUGAGUGCCA

.mods

>tRNA | Ala | AGC | Saccharomyces cerevisiae | cytosolic

9 m1G

16 D

20 D

26 m2,2G

34 I

37 m1I

38 Y

47 D

54 m5U

55 Y

>tRNA | Arg | UCU | Saccharomyces cerevisiae | cytosolic

9 m1G

10 m2G

16 D

25 m2,2G

26 Y

33 mcm5U

36 t6A

38 Y

46 D

53 m5U

54 Y

57 m1A

If the modification file has the same header line as the FASTA file, the sequence will be modified with the modification(s). The lines that follow, *e.g.* “9 m1G” indicate modification sites with the symbol of the modification represented in space- or tab-delimited text. In addition to modification for nucleosides, linker modifications can also be defined in the file. The unmodified parts for each modification (Origin field of the Nucleotide Parts Table page) in the modification file must match that in the corresponding site in the sequence file.

In addition to the single site-directed modification at a site, the software can also consider plural modifications at a site. You can write two or more modification lines for a single position in a .mods file.

.mods

>tRNA | Ala | AGC | Saccharomyces cerevisiae | cytosolic
9 m1G
9 m2G
16 D
...

With this setup, the software can generate two sequences having m1G or m2G at the 9th position. Likewise, the software can define plurally modified linkers. The modification timing is just after *in silico* digestion with an Enzyme (See Section 1.5).

The option to plurally modify 5′ - or 3′ -termini is also available. If you choose the "-" symbol on the web form, the software will calculate the sequences with all possible combinations of activated 5′ - and 3′ -termini. For example, with "-" selected for 5′ Term and "OH" for 3′ Term, the software will derive from the nucleotide sequence "ACUG" to the sequences OH_ACUG_OH and p_AUCG_OH, where the activated 5' Terms are: p and OH.

Note that you can define partially modified nucleosides and linkers as below. For example, if you want to consider m1G modification at the 9th is partially modified, define two nucleosides: m1G and G (the Origin of m1G), at the 9th position in .mods file. Then, the software generates two sequences having m1G or G at the 9th position. Therefore, the software calculates both modified and unmodified sequences.

.mods

>tRNA | Ala | AGC | Saccharomyces cerevisiae | cytosolic
9 m1G
9 G

16 D
...

1.6. Variable modifications

A search can incorporate another type of modifications: variable modifications. If you have information on modified specie(s) and position(s), use the site-directed modification (See Section 1.5). Otherwise use the variable modification. Both types can be used in a single search. Specifying variable modifications will generate a combination of all the possible modified/unmodified sequences to be searched.

As in searches with site-directed modifications, any parts in the Nucleotide Parts Table can be used in those with variable modifications. To show the list of available nucleoside, linker, and terminus parts, click the Nucleotide Parts Table link on the top page or the Show Parts Table button of the Isotope Set section on the web form (See Section 1.2).

Variable modifications can be written in a separate text file with extension .mods.
A .mods file must consist of the site-directed and variable modification sections. The site-directed modification section should start with a line of the same description as the corresponding sequence in the accompanying FASTA file for a search (See Section 1.5).
In contrast, the variable modification section should specify modifications at nucleoside, linker, and terminus separately. Each section should contain a header line and following modification lines.

The nucleoside section should start with a header line containing only the max_v_mod_nucleoside label and its value delimited with a tab or white space. The value represents a maximum number of modified nucleosides in an (enzyme-cleaved) oligonucleotide. The recommended value is 1 or 2 although the maximum is 4. The header line is followed by nucleoside symbol lines. Each line should have only a single symbol as shown below. The linker section should start with max_v_mod_linker and its value, and contains modified linker symbols (one at each line). The terminus section does not have header line. Otherwise, each terminus line should start with fp (5′ terminus) or tp (3′ terminus) followed by modified terminus symbol.

An example of variable modification part of a .mods file

```
-----
max_v_mod_nucleoside  2
mA
mC
mG
mU

max_v_mod_linker      2
ceps
ps

fp      m2,2,7Gppp
tp      p
-----
```

Note that nucleoside symbols with more than two characters should NOT be enclosed in parentheses in a modification file. The file accepts “#” starting lines as comments and blank lines for readability.

1.7. Other data and parameters

Additionally, the software can also account for parameters that simulate experimental conditions (Table 1). The software also considers the information on expected structures of the RNAs including 5′- and 3′-termini of intact molecules, and their isotopic distribution. Search queries that relate with the experiment are also to be specified: the polarity, mass tolerances for MS1 and MS2, and precision of the result mass expressions. If the user does not specify some of the parameters listed, default settings will be used to perform the search (See Section 1.1.3 for default queries).

Table 1. Definable parameters and default values used

Parameter Name	Modifiable Condition	Default Setting
5′ Term	5′ functional group of intact nucleic acids. Default available values: hydroxy (OH) or phosphate (p). After you define your own terminus and activate it, you can choose it.	OH

3′ Term	3′ functional group of intact nucleic acids. Default available values: hydroxy (OH), 2′,3′-cyclic phosphate (cp), or phosphate (p). After you define your own terminus and activate it, you can choose it.	OH
Mapping Type	The length of mapping regions in FASTA. Available options are Fixed or Variable. Fixed: mapping region is fixed to the entire length of each FASTA entry; Variable: looking for mapping region of the highest score within each FASTA entry.	Variable
Max Number of Variable Terminal Truncations 5′	Maximum number of the truncation from 5′ Term. The 5′ of newly generated nucleotides are same as the intact 5′ terminus.	0
Max Number of Variable Terminal Truncations 3′	Maximum number of the truncation from 3’ Term. The 3′ of newly generated nucleotides are same as the intact 3′ terminus.	0
Product Intensity Cutoff	Absolute	10
Product Intensity Cutoff	Relative	0.00001
Enzyme	Specification of an endonuclease used in the experiment. Available options: ribonuclease (RNase) T1, RNase T1 + Bacterial Alkaline Phosphatase (BAP), RNase T2, Colicin E5, MazF, RNase A, RNase U2, or No Enzyme (no cutting by nuclease). The sequence specificity for each enzyme is listed in Table 2.	No Enzyme
Max Missed Cleavages	Max number of missed cleavages to be considered. Usually 0 or 1 is sufficient for simulating RNase digestion. When No Enzyme is selected, this parameter will not be applied.	0
Polarity	Positive or Negative. This program does not support data that has switched polarity during	Negative

	measurement.	
Ion Activation Type	The dissociation method used to generate product ions. Options include conventional collision-based methods such as higher-energy collision dissociation (HCD) or collision-induced dissociation (CID). Radical-generating methods such as ultraviolet photodissociation (UVPD) can also be selected.	HCD/CID
Mass Tolerance	Precursor ion's mass tolerance in parts per million (ppm).	5
MS2 Tolerance	Product ion's mass tolerance in ppm. Within this tolerance, all matches between observed and calculated masses are equally scored.	20
Filter Type	Rank First Only, above Threshold, or Rank First and above Threshold.	Rank First and above Threshold
Significance Level	Determining the threshold for MS/MS Ion Search.	0.01
Reject Length for Mapping	Oligonucleotides with length more than this value are used in the Nucleotide Mapping. If there are many short nucleotides in a sample (*e. g.* RNase A digest), smaller number should be specified.	2
Number of Decimals	This parameter specifies decimals used in the html report of calculation / search. The setting will also use in the downloaded files.	4
Isotope Set	Ariadne calculates the mass values of RNA based on specified parts in the mass table. This parameter allows selection of the set of isotopes used for each part and is especially useful for the characterization of site-specific stable isotope labeled RNAs or other nucleic acids. The contents of this table will appear when the Show Parts Table button is clicked. At present, the options of: Natural	Natural

(non-labeled), 13C10_G for SILNAS* with RNase T1, and 13C10_A, 13C9_C, 13C9_U, 13C9_CU, 5D_CU, 56D2_CU or 15N5_G for pseudouridine identification are available. If you would like to use a different mass table for a specific isotope labeling, please contact us *via* email (ariadne_dev_team@riken.jp).

*: Taoka M et al. A mass spectrometry-based method for comprehensive quantitative determination of post-transcriptional RNA modifications: the complete chemical structure of *Schizosaccharomyces pombe* ribosomal RNAs. Nucleic Acids Res. 2015 Oct 15;43(18): e115. doi: 10.1093/nar/gkv560. Epub 2015 May 26.

Among the definable parameters is the sequence-specific nuclease used (Enzyme), which generates a mixture of shorter oligonucleotides from the input nucleic acid sequence(s). The upper limit number of missed cleavages for the enzyme can also be specified (Max Missed Cleavages). Enzymes available for selection are listed in Table 2.

Table 2. The Enzyme parameters available for selection

Enzyme Name	Specificity	5′ Terminus	3′ Terminus
No Enzyme	-	-	-
RNase T1	[G|m1G|m2G|I]* ^ N	OH	cp or p
RNase T2	C ^ [A|G|U]*	OH	cp or p
Colicin E5	G ^ U	OH	cp or p
MazF	N ^ AC	OH	cp or p
RNase A	[C|U|m5C|Y|m1Y|m3Y]* ^ N	OH	cp or p
RNase U2	[A|G]* ^ N	OH	cp or p

^: cleavage site

*: [A|B] represents A or B

cp: 2′,3′-cyclic phosphate

p: phosphate

The default setting is No Enzyme, which instructs Ariadne to calculate mass values of intact sequences without cleavage at any site. You should use No Enzyme to calculate mass values of intact nucleic acids. Besides Enzyme parameters, the Max Number of

Variable Terminal Truncations 5′ and 3′ consider truncations from 5′ and 3′, respectively. These parameters are useful for the analysis of variations in RNA termini and for the identification of metabolites of therapeutic oligonucleotides. The termini of truncated nucleotides are combination of the activated termini in 5′ Term and 3′ Term. Another useful parameter is Isotope Set, which replaces the mass table used for the calculation and enables the program to simulate nucleic acids that are metabolically labeled with stable-isotope-containing monomers like nucleobases and/or nucleosides. Furthermore, the user can specify other parameters that have a relationship with mass measurement. These parameters include polarity, the way that ions are generated, and mass precision. Those parameters are dependent on the instrument and methods used. The parameter named Ion Activation Type defines what types of product ions will be included in the calculation. Currently, 2 options are available for this calculation: either collision-induced dissociation (CID) / higher-energy collisional dissociation (HCD), or radical-mediated dissociations such as ultraviolet photodissociation (UVPD). The types of product ions included in the calculation for both Ion Activation Type options are listed in Table 3.

Table 3. Product ions that are considered for each ion activation type. The nomenclature of product ions is shown in Figure 4.

Ion Activation Type	Product Ions
HCD/CID	a, a-B, b, c, d, w, x, y, z
UVPD	a, a-B, b, c, d, w, x, y, z, a•, a•-B, b•, c•, d•, w•, x•, y•, z•

For Nucleotide Mapping, there are three parameters: Mapping Type, Filter Type, and Reject Length for Mapping. The Mapping Type determines the type of Nucleotide Mapping. The option “Fixed” instructs the software to fix the mapping regions to the entire length of FASTA entries; the option “Variable” instructs it to look for the region having the highest score within FASTA entries. The Filter Type parameter teaches the software what type(s) of MS/MS-Ion-Search identified nucleotides will use for Nucleotide Mapping. The Reject Length for Mapping limits the lower nucleotide length.

2. Set up for MS/MS Search

As described in Section 1, an Ariadne search requires at least a peak-list file containing MS/MS data in Mascot™ generic format (MGF) and a nucleotide sequence database. In

this section, how to input peak lists, sequences, modifications and search parameters in the web form will be illustrated.

At present, the Genome MS/MS Search service is suspended for preparing a new version. During the downtime, we are glad to search either human or mouse genome database using your MGF and parameters with our local machine, and send you the search result. if you would like to search such a large database, contact us via email (ariadne_dev_team@riken.jp).

2.1. Filling in the MS/MS Search web form

Click the MS/MS Search link on the top page (https://ariadne.riken.jp/), and the web form will appear as shown in Figure 8. We offer example data and parameter sets for evaluating the program. Those can be downloaded from Examples: MGF File, FASTA, and Modification Files in Demo and Example Data on the top page .

The sequence database to be searched can be inputted as a text in the text box field, or a FASTA file in the file upload field. If RNAs in the sample are expected to have post-transcriptional modifications, the search can also include the information on the modifications. Available modifications are listed on the Nucleotide Parts Table page (https://ariadne.riken.jp/html/parts_table.html). To identify RNA which was hydrolysed with an RNase, the parameter Enzyme must be specified as the enzyme used in the experiment.

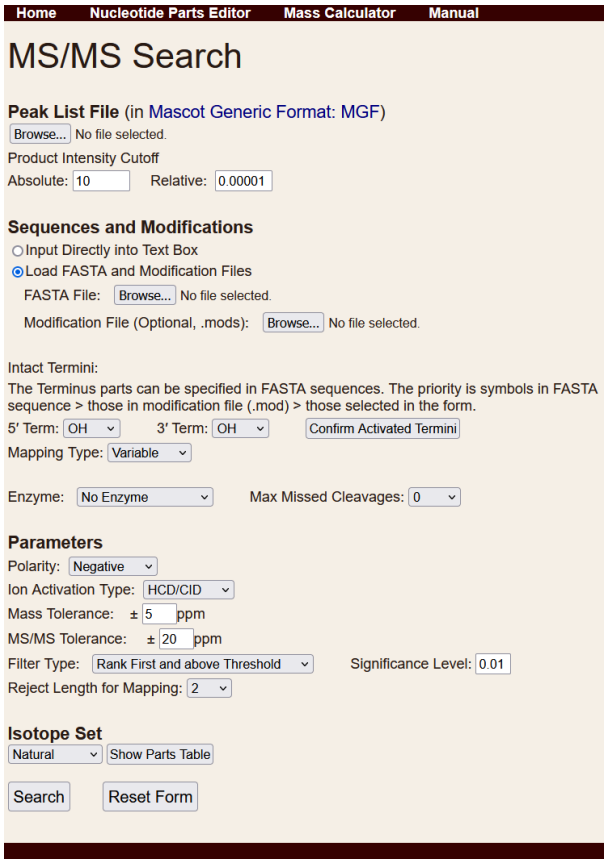


Figure 8. Web form of MS/MS Search

2.2. Input of MS/MS data, sequences, modifications, and other parameters

Ariadne offers two search programs: upload search and genome search. Read Section 1.1.2 to select an appropriate one.

Several sample data and parameters are available at the Ariadne server.

Click the Browse... button of the Peak List File section (Choose File… for Chrome and Chromium-based browser). Choose an MGF file you would like to search and then press the OK button in the dialog box. Then, the name of the selected file will appear at the right of the Browse… button (Figure 9).

Figure 9. Selecting Peak List File. The selected MGF file is shown after browsing/selecting files.

Press the Browse... button of FASTA file of the Sequences and Modifications section of the form. Choose a FASTA file you would like to search and press OK button. Then, the name of the selected file will appear at the right of the Browse… button. Then, if necessary, the Browse... button of Modification File (Optional) of the same section (Figure 10). Choose a .mods file you would like to use and press OK button. Then, the name of the selected file will appear at the right of the Browse… button.
If you would like to input sequences directly or paste them from another application, select the Input Directly into Text Box radio button. Symbols having more than two letters should be enclosed in parenthesis (Figure 11). In this mode, since modifications have to be placed in the FASTA format, the variable modification is not available.

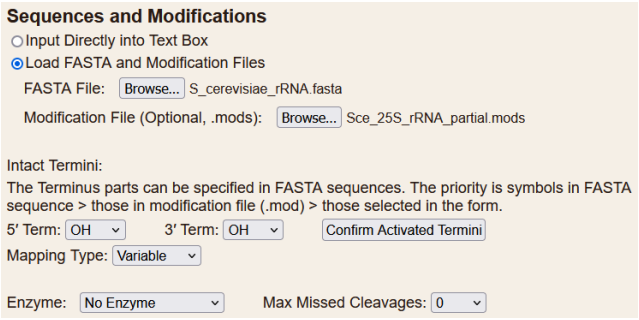


Figure 10. Loading sequence and modification files

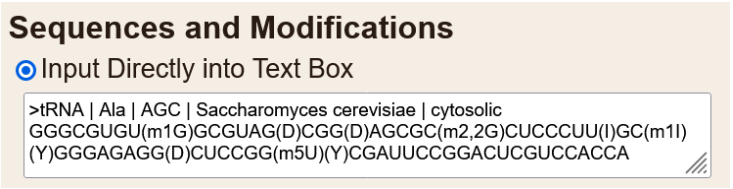


Figure 11. Input sequence with site-directed modifications through text box of the form

Choose appropriate search parameters according to your experimental conditions. The detailed description and the default value of each parameter are shown in Table 1. For selecting the Enzyme parameter, consult Table 2.

2.3. Starting the search

After all data and parameters above are set, a search can be started by pressing the Search button at the bottom of the web form (Figure 8). Searching a large sequence database with a lot of modifications will take some amount of time. Ariadne thus issues a Search ID when the search is accepted to the server. You can browse the search result afterward using the Search ID. If you have your account, you can browse a

search log after logging-in the account. On completion of the search, the browser window is updated to show an html search report. See Section 3 to browse/interpret the results.

3. Browsing the search results

3.1. Showing a search result pages

You can see a specific search result by inputting its Search ID into the Browse Search Result section of the top page (See Figure 2). A Search ID is issued when the search is correctly accepted to the server. Please write down the Search ID if you do not have a user account. If you have user account, the list view of the search results is available after logging-in to the account. Click the Browse Search Results link on the top page (https://ariadne.riken.jp/). Click one of the Search ID fields on the appeared list, and the new window or browser tab will be opened to show Results View of the search.

3.2. Browsing the Results View page

As shown in Figure 12, the Results View page has three sections: Search Parameter, Nucleotide Mapping, and MS/MS Ion Search. The topmost Search Parameter section represents main search parameters used for the search. All parameters can be seen by clicking the More link (Figure 13). The second section represents the Nucleotide Mapping result consisting of the Score Histogram (Figure 14), Identification Summary (Figure 15), and Oligonucleotide-RNA Matrix (Figure 16) subsections. The third section exhibits a list of identified oligonucleotides by MS/MS Ion Search (Figure 17).

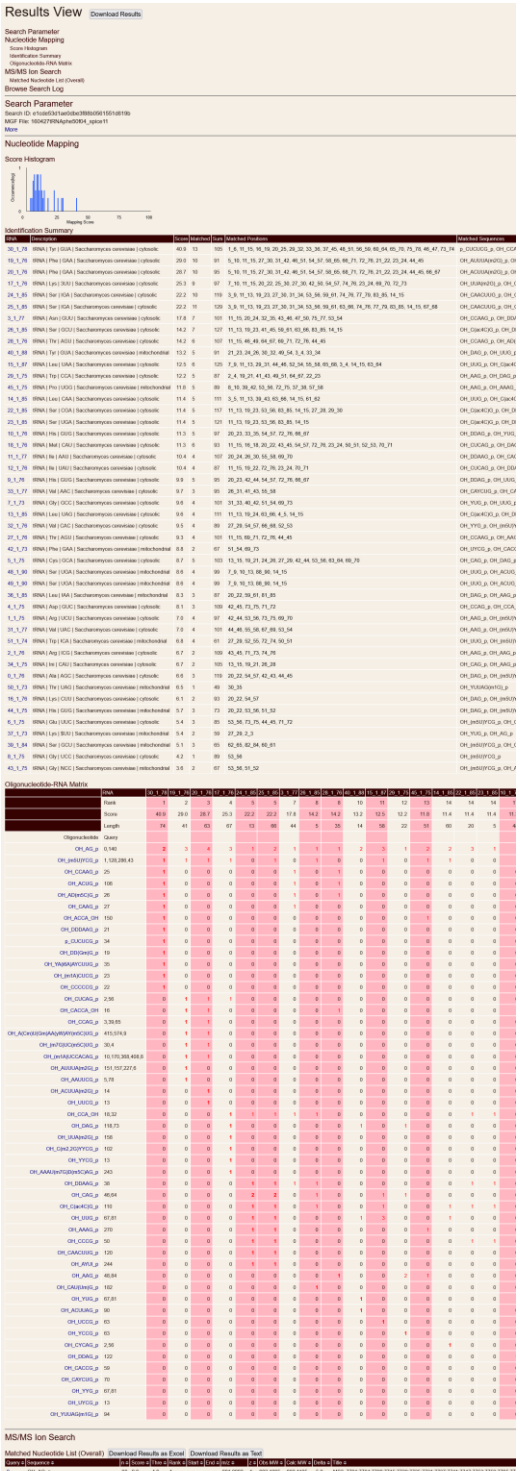


Figure 12. An example of the Results View page

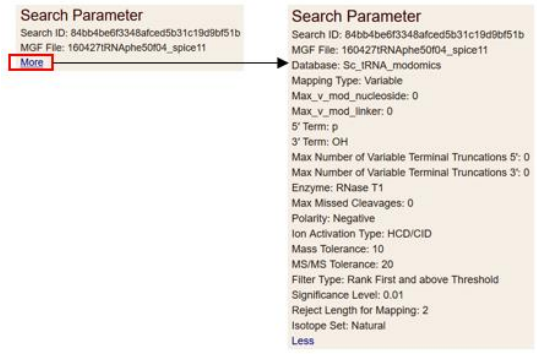


Figure 13. Confirming all the Search Parameters. In the initial view (left) only Search ID and MGF File are shown. Clicking the More link will show the detailed parameters as in the right panel. Clicking the Less link will fold the parameters.

3.2.1. Nucleotide Mapping

3.2.1.1. Score Histogram

The histogram represents the statistics of nucleotide mapping scores for the search. The x-axis is the score for Nucleotide Mapping, and the y-axis is logarithm of numbers of identified RNA regions having the nominal score (Figure 14); Actually the y-value is log(n + 1), where n is the number in a bin (The size of bin is 1.).

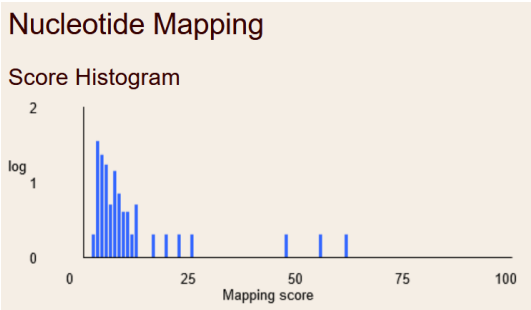
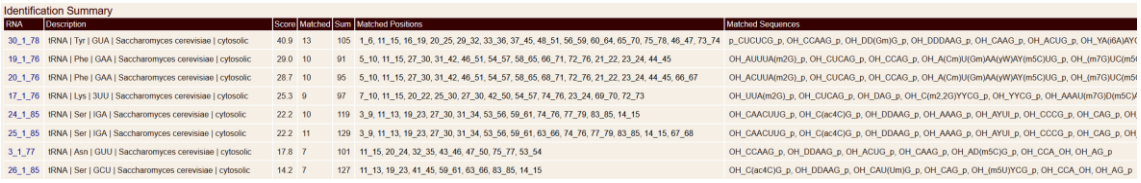


Figure 14. An example of Score Histogram for the Nucleotide Mapping section

3.2.1.2. Identification Summary

The Identification Summary table represents the number for the identified sequence region of the RNA, its description, its nucleotide mapping score, matched oligonucleotide number by MS/MS ion search, total oligonucleotide number which is obtained by *in silico* cleavage of the specified RNase, the position list of the matched oligonucleotides, and the sequence list of the matched oligonucleotides in Figure 15. The results are sorted by the descending order of the nucleotide mapping score. When

clicking a region number of RNA on the left most RNA column, the Mapping Results View page will appear which shows detailed results of nucleotide mapping of the corresponding RNA.



Identification Summary

RNA	Description	Score	Matched	Sum	Matched Positions	Matched Sequences
30_1_78	tRNA | Tyr | GUA | Saccharomyces cerevisiae | cytosolic	40.9	13	105	1_6, 11_15, 16_19, 20_25, 29_32, 33_36, 37_45, 48_51, 56_59, 60_64, 65_70, 75_78, 46_47, 73_74	p_CUCUCG_p, OH_CCAAG_p, OH_DD(Gm)G_p, OH_DDDAAG_p, OH_CAAG_p, OH_ACUG_p, OH_YA(i6A)AY(
19_1_76	tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic	29.0	10	91	5_10, 11_15, 27_30, 31_42, 46_51, 54_57, 58_65, 66_71, 72_76, 21_22, 23_24, 44_45	OH_AUUUA(m2G)_p, OH_CUCAG_p, OH_CCAG_p, OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p, OH_(m7G)UC(m5
20_1_76	tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic	28.7	10	95	5_10, 11_15, 27_30, 31_42, 46_51, 54_57, 58_65, 68_71, 72_76, 21_22, 23_24, 44_45, 66_67	OH_ACUUA(m2G)_p, OH_CUCAG_p, OH_CCAG_p, OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p, OH_(m7G)UC(m5
17_1_76	tRNA | Lys | 3UU | Saccharomyces cerevisiae | cytosolic	25.3	9	97	7_10, 11_15, 20_22, 25_30, 27_30, 42_50, 54_57, 74_76, 23_24, 69_70, 72_73	OH_UUA(m2G)_p, OH_CUCAG_p, OH_DAG_p, OH_C(m2,2G)YYCG_p, OH_YYCG_p, OH_AAAU(m7G)D(m5C)A
24_1_85	tRNA | Ser | IGA | Saccharomyces cerevisiae | cytosolic	22.2	10	119	3_9, 11_13, 19_23, 27_30, 31_34, 53_56, 59_61, 74_76, 77_79, 83_85, 14_15	OH_CAACUUG_p, OH_C(ac4C)G_p, OH_DDAAG_p, OH_AAAG_p, OH_AYUI_p, OH_CCCG_p, OH_CAG_p, OH
25_1_85	tRNA | Ser | IGA | Saccharomyces cerevisiae | cytosolic	22.2	11	129	3_9, 11_13, 19_23, 27_30, 31_34, 53_56, 59_61, 63_66, 74_76, 77_79, 83_85, 14_15, 67_68	OH_CAACUUG_p, OH_C(ac4C)G_p, OH_DDAAG_p, OH_AAAG_p, OH_AYUI_p, OH_CCCG_p, OH_CAG_p, OH
3_1_77	tRNA | Asn | GUU | Saccharomyces cerevisiae | cytosolic	17.8	7	101	11_15, 20_24, 32_35, 43_46, 47_50, 75_77, 53_54	OH_CCAAG_p, OH_DDAAG_p, OH_ACUG_p, OH_CAAG_p, OH_AD(m5C)G_p, OH_CCA_OH, OH_AG_p
26_1_85	tRNA | Ser | GCU | Saccharomyces cerevisiae | cytosolic	14.2	7	127	11_13, 19_23, 41_45, 59_61, 63_66, 83_85, 14_15	OH_C(ac4C)G_p, OH_DDAAG_p, OH_CAU(Um)G_p, OH_CAG_p, OH_(m5U)YCG_p, OH_CCA_OH, OH_AG_p

Figure 15. An example of the Identification Summary table for the Nucleotide Mapping section

3.2.1.3. Oligonucleotide-RNA Matrix

This matrix shows relationship between the oligonucleotides identified by the MS/MS ion search and the RNAs identified by the nucleotide mapping. A typical matrix is shown in Figure 16. Each row represents statistics of how many times the identified oligonucleotide appears in RNA. Otherwise, each column represents which oligonucleotides are mapped onto the corresponding RNA.

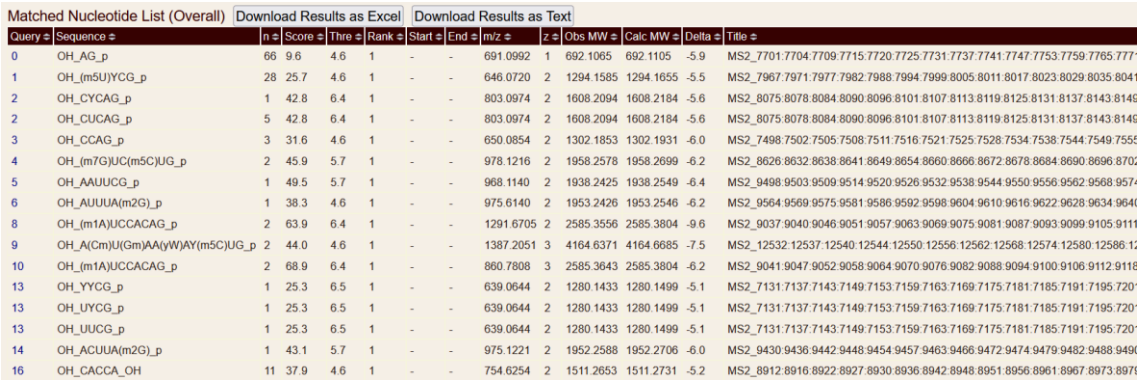
Oligonucleotide-RNA Matrix

	RNA	30_1_78	19_1_76	20_1_76	17_1_76	24_1_85	25_1_85	3_1_77	26_1_85	28_1_76	40_1_88	15_1_87	29_1_75	45_1_75
	Rank	1	2	3	4	5	5	7	8	8	10	11	12	13
	Score	40.9	29.0	28.7	25.3	22.2	22.2	17.8	14.2	14.2	13.2	12.5	12.2	11.8
	Length	74	41	63	67	13	66	44	5	35	14	58	22	51
Oligonucleotide	Query													
OH_AG_p	0,140	2	3	4	3	1	2	1	1	1	2	3	1	2
OH_(m5U)YCG_p	1,128,286,43	1	1	1	1	0	1	0	1	0	0	1	0	1
OH_CCAAG_p	25	1	0	0	0	0	0	1	0	1	0	0	0	0
OH_ACUG_p	106	1	0	0	0	0	0	1	0	1	0	0	0	0
OH_AD(m5C)G_p	26	1	0	0	0	0	0	1	0	1	0	0	0	0
OH_CAAG_p	27	1	0	0	0	0	0	1	0	0	0	0	0	0
OH_ACCA_OH	150	1	0	0	0	0	0	0	0	0	0	0	0	1
OH_DDDAAG_p	21	1	0	0	0	0	0	0	0	0	0	0	0	0
p_CUCUCG_p	34	1	0	0	0	0	0	0	0	0	0	0	0	0
OH_DD(Gm)G_p	19	1	0	0	0	0	0	0	0	0	0	0	0	0
OH_YA(i6A)AYCUUG_p	35	1	0	0	0	0	0	0	0	0	0	0	0	0
OH_(m1A)CUCG_p	23	1	0	0	0	0	0	0	0	0	0	0	0	0
OH_CCCCCG_p	22	1	0	0	0	0	0	0	0	0	0	0	0	0
OH_CUCAG_p	2,56	0	1	1	1	0	0	0	0	0	0	0	0	0
OH_CACCA_OH	16	0	1	1	0	0	0	0	0	1	0	0	0	0
OH_CCAG_p	3,39,65	0	1	1	0	0	0	0	0	0	0	0	0	0
OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p	415,574,9	0	1	1	0	0	0	0	0	0	0	0	0	0
OH_(m7G)UC(m5C)UG_p	30,4	0	1	1	0	0	0	0	0	0	0	0	0	0

Figure 16. An example of the Oligonucleotide-RNA Matrix table

3.2.2. MS/MS Ion Search

Clicking the Change Filter (beta) button after selecting Filter Type and inputting Significant Level will filter the search result with the new filter type and threshold.



Matched Nucleotide List (Overall) Download Results as Excel Download Results as Text

Query	Sequence	n	Score	Thre	Rank	Start	End	m/z	z	Obs MW	Calc MW	Delta	Title
0	OH_AG_p	66	9.6	4.6	1	-	-	691.0992	1	692.1065	692.1105	-5.9	MS2_7701:7704:7709:7715:7720:7725:7731:7737:7741:7747:7753:7759:7765:777
1	OH_(m5U)YCG_p	28	25.7	4.6	1	-	-	646.0720	2	1294.1585	1294.1655	-5.5	MS2_7967:7971:7977:7982:7988:7994:7999:8005:8011:8017:8023:8029:8035:804
2	OH_CYCAG_p	1	42.8	6.4	1	-	-	803.0974	2	1608.2094	1608.2184	-5.6	MS2_8075:8078:8084:8090:8096:8101:8107:8113:8119:8125:8131:8137:8143:814
2	OH_CUCAG_p	5	42.8	6.4	1	-	-	803.0974	2	1608.2094	1608.2184	-5.6	MS2_8075:8078:8084:8090:8096:8101:8107:8113:8119:8125:8131:8137:8143:814
3	OH_CCAG_p	3	31.6	4.6	1	-	-	650.0854	2	1302.1853	1302.1931	-6.0	MS2_7498:7502:7505:7508:7511:7516:7521:7525:7528:7534:7538:7544:7549:755
4	OH_(m7G)UC(m5C)UG_p	2	45.9	5.7	1	-	-	978.1216	2	1958.2578	1958.2699	-6.2	MS2_8626:8632:8638:8641:8649:8654:8660:8666:8672:8678:8684:8690:8696:870
5	OH_AAUUCG_p	1	49.5	5.7	1	-	-	968.1140	2	1938.2425	1938.2549	-6.4	MS2_9498:9503:9509:9514:9520:9526:9532:9538:9544:9550:9556:9562:9568:957
6	OH_AUUUA(m2G)_p	1	38.3	4.6	1	-	-	975.6140	2	1953.2426	1953.2546	-6.2	MS2_9564:9569:9575:9581:9586:9592:9598:9604:9610:9616:9622:9628:9634:964
8	OH_(m1A)UCCACAG_p	2	63.9	6.4	1	-	-	1291.6705	2	2585.3556	2585.3804	-9.6	MS2_9037:9040:9046:9051:9057:9063:9069:9075:9081:9087:9093:9099:9105:911
9	OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p	2	44.0	4.6	1	-	-	1387.2051	3	4164.6371	4164.6685	-7.5	MS2_12532:12537:12540:12544:12550:12556:12562:12568:12574:12580:12586:12
10	OH_(m1A)UCCACAG_p	2	68.9	6.4	1	-	-	860.7808	3	2585.3643	2585.3804	-6.2	MS2_9041:9047:9052:9058:9064:9070:9076:9082:9088:9094:9100:9106:9112:911
13	OH_YYCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131:7137:7143:7149:7153:7159:7163:7169:7175:7181:7185:7191:7195:720
13	OH_UYCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131:7137:7143:7149:7153:7159:7163:7169:7175:7181:7185:7191:7195:720
13	OH_UUCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131:7137:7143:7149:7153:7159:7163:7169:7175:7181:7185:7191:7195:720
14	OH_ACUUA(m2G)_p	1	43.1	5.7	1	-	-	975.1221	2	1952.2588	1952.2706	-6.0	MS2_9430:9436:9442:9448:9454:9457:9463:9466:9472:9474:9479:9482:9488:949
16	OH_CACCA_OH	11	37.9	4.6	1	-	-	754.6254	2	1511.2653	1511.2731	-5.2	MS2_8912:8916:8922:8927:8930:8936:8942:8948:8951:8956:8961:8967:8973:897

Figure 17. An example of Matched Nucleotide List (Overall) in the MS/MS Ion Search section

3.2.2.1. Matched Oligonucleotide List (Overall)

Since the list shows a summary of MS/MS ion search results for all of MS/MS queries, it may be sometimes a very large list representing the information on the identified sequence, its mass values, and title field of the MGF for each MS/MS peak list (Figure 17). The meanings of each column are explained as shown in the Table 4 below. The Query contains a hyperlink to MS/MS Assignment View of the corresponding MS/MS spectrum and its assignment table.

Table 4. Entities in Matched Oligonucleotide List (Overall)

Name	Explanation
Query	sequential number unique to each MS/MS
Sequence	nucleotide sequence including modifications
Score	probability-based score of MS/MS ion search
Thre	statistical threshold for the score. The significant level is 0.05.
Rank	order of the scores for each query
m/z	observed mass to charge ratio of the precursor ion
z	charge of the precursor ion
Obs MW	MW calculated from the m/z and z
Calc MW	MW calculated from the modseq
Delta	relative mass difference between obs_MW and calc_MW in part-per-million
Q_title	'title' field of the MGF file

Clicking the Download Results as Excel button will download the Search Parameter and Matched Nucleotide List sections as Excel (.xlsx) file in Figure 18.

Clicking the Download Results as Text button will download the same data as tab-delimited text file (tab-separated values; TSV) in Figure 19.

The file name will be YYYYMMDDHHMMSS_result.txt, where YYYY, MM, DD, HH, MM, SS are year, month, day, hour, minute, second at the download, respectively.

The Excel file consists of two worksheets: Search Parameter and Matched Nucleotide List worksheets. The Search Parameter sheet represents the parameters used for the search. The Matched Nucleotide List sheet contains the contents of the Matched Nucleotide List (Overall) table in the web page in Figure 17. In the downloaded text file, the same data are contained as TSV format.

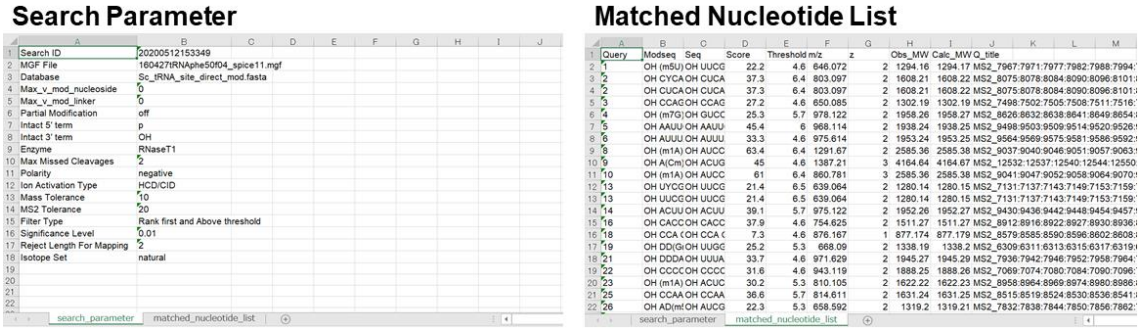


Figure 18. Downloaded Search Parameter and Matched Nucleotide List worksheets in a downloaded Excel file

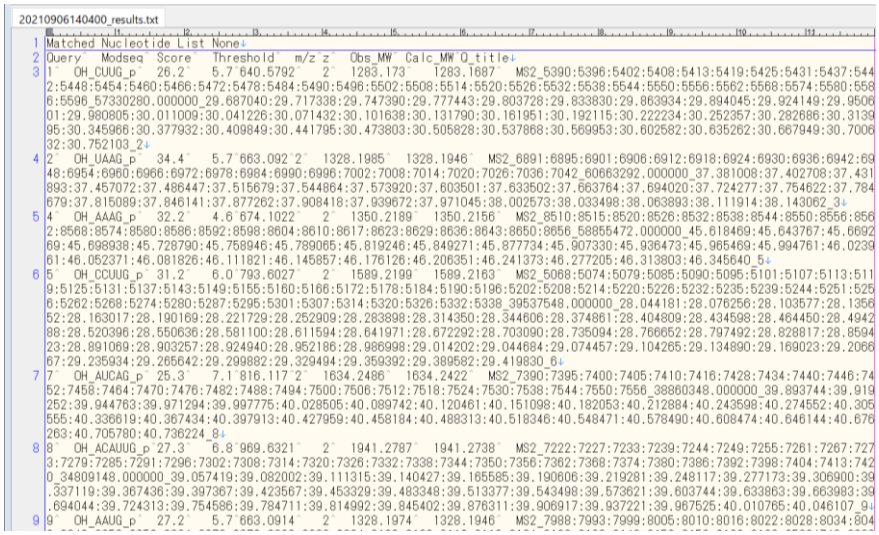


Figure 19. Downloaded Matched Nucleotide List as text file

3.3. Browsing the Mapping Results View page

The Mapping Results View page shows Nucleotide Mapping and Matched Nucleotide List for the identified region (subset) of RNA after a brief header section representing the description of the corresponding database query and positions of the sequence in the query.

3.3.1. Mapping Results

The map in Figure 20 represents the identified sequence region of the database query. The region is shown in boldface black letter, and neighboring sequence region is also shown in gray letter. The identified oligonucleotides in the region are highlighted with red underlines. Each underline contains a hyperlink to MS/MS Assignment View page of the corresponding MS/MS spectrum and its assignment table (Figure 21). Clicking the BLAST Search button will appear a new NCBI blastn search page, the search form of which contains the sequence identified by Ariadne.

Shown just below the map is a summary of the nucleotide mapping.

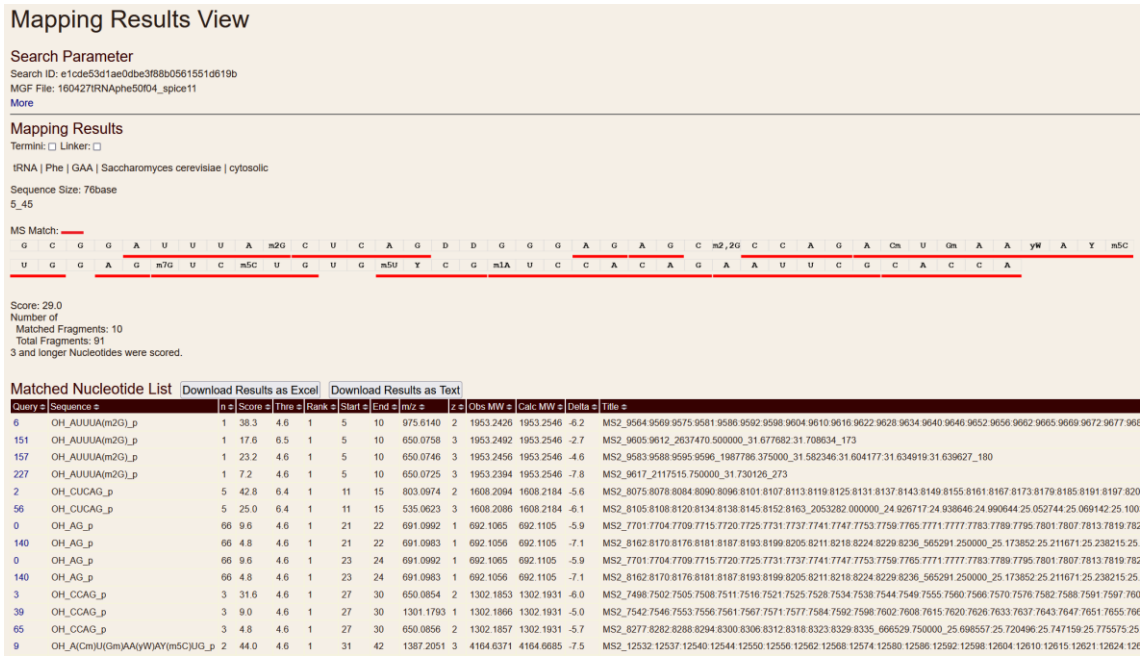


Figure 20. An example of the Mapping Results View page for a single RNA

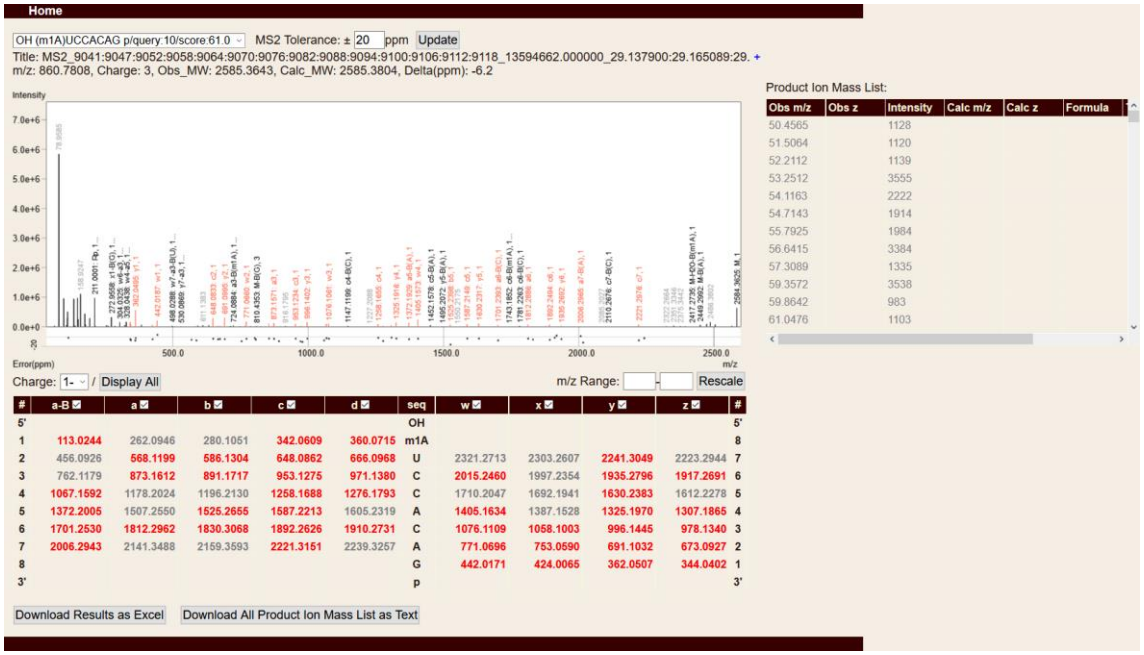


Figure 21. An example of the MS/MS Assignment View page that shows the spectrum for the selected sequence and the corresponding assignment table.

3.3.2. Matched Nucleotide List

The list shows a summary of MS/MS ion search results, representing the information on the identified oligonucleotide sequence, its mass values, and title field of the MGF for

each MS/MS peak list. The explanations of each column are as shown in Table 4. The Query contains a hyperlink to MS/MS Assignment View page of the corresponding MS/MS spectrum and its assignment table.

3.4. Browsing the MS/MS Assignment View page

Clicking each red underline in the sequence of the Mapping Results page or the value of Query column in the Matched Nucleotide List table opens a new tab representing the MS/MS Assignment View page (Figure 21). This page shows the MS/MS information of the selected query. At the top of the page describes the summary of the identification of the query. Below the description is the MS/MS spectrum with peak assignments and the corresponding assignment table. In addition, at the right top of the page is Product Ion Mass List, which shows all the observed product ions (Obs) in the query compared with the calculated chemical formulas, mass values and assignments. Matched peaks (within the tolerance of the search) in the spectrum are shown in red. If the *m/z* is matched but charge (*z*) is different (it might mean incorrect peak detection), the peak is indicated in gray. The spectrum can be enlarged by mouse drag & drop from left-top to right-down (drag at the start *m/z* and drop at the end *m/z*; See Figure 22). The Y-axis is automatically normalized to the most intense peak in the range. Undoing the last enlargement can be done by drag & drop in left-up (opposite) direction. The *m/z* range can also be rescaled by inputting start and end values in text boxes right below of the spectrum.

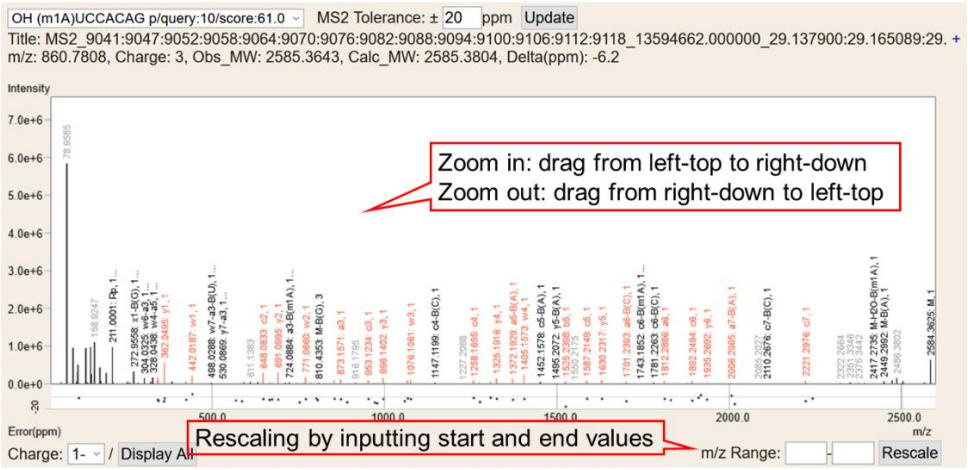
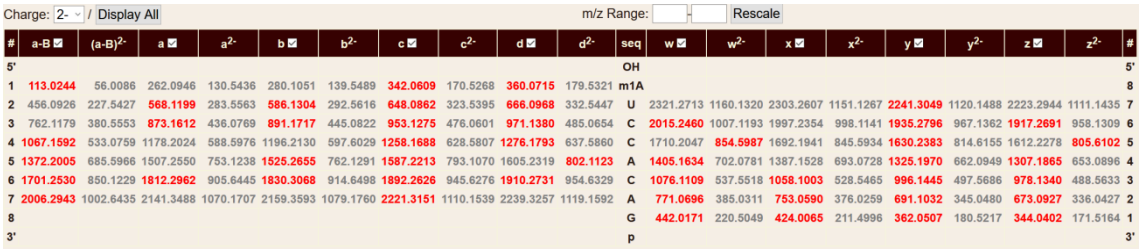


Figure 22. Zooming in and out of assigned MS/MS spectrum

The assignment table shows the calculated mass values of product ions expected from the identified sequence. Although only singly charged mass values are shown by default,

selecting value from the Charge select box rewrite *m/z* values of the table up to the selected value of charge (Figure 23). The colors of mass values in the table correspond to those of peaks in the spectrum.


Charge: 2- / Display All m/z Range: Rescale

#	a-B	(a-B)$^{2-}$	a	a^{2-}	b	b^{2-}	c	c^{2-}	d	d^{2-}	seq	w	w^{2-}	x	x^{2-}	y	y^{2-}	z	z^{2-}	#
5'											OH									5'
1	113.0244	56.0086	262.0946	130.5436	280.1051	139.5489	342.0609	170.5268	360.0715	179.5321	m1A									8
2	456.0926	227.5427	568.1199	283.5563	586.1304	292.5616	648.0862	323.5395	666.0968	332.5447	U	2321.2713	1160.1320	2303.2607	1151.1267	2241.3049	1120.1488	2223.2944	1111.1435	7
3	762.1179	380.5553	873.1612	436.0769	891.1717	445.0822	953.1275	476.0601	971.1380	485.0654	C	2015.2460	1007.1193	1997.2354	998.1141	1935.2796	967.1362	1917.2691	958.1309	6
4	1067.1592	533.0759	1178.2024	588.5976	1196.2130	597.6029	1258.1688	628.5807	1276.1793	637.5860	C	1710.2047	854.5987	1692.1941	845.5934	1630.2383	814.6155	1612.2278	805.6102	5
5	1372.2005	685.5966	1507.2550	753.1238	1525.2655	762.1291	1587.2213	793.1070	1605.2319	802.1123	A	1405.1634	702.0781	1387.1528	693.0728	1325.1970	662.0949	1307.1865	653.0896	4
6	1701.2530	850.1229	1812.2962	905.6445	1830.3068	914.6498	1892.2626	945.6276	1910.2731	954.6329	C	1076.1109	537.5518	1058.1003	528.5465	996.1445	497.5686	978.1340	488.5633	3
7	2006.2943	1002.6435	2141.3488	1070.1707	2159.3593	1079.1760	2221.3151	1110.1539	2239.3257	1119.1592	A	771.0696	385.0311	753.0590	376.0259	691.1032	345.0480	673.0927	336.0427	2
8											G	442.0171	220.5049	424.0065	211.4996	362.0507	180.5217	344.0402	171.5164	1
3'											p									3'

Figure 23. Showing multiple charged ions in assignment table

Reassignment of the spectrum with altered MS2 tolerance can be done by entering a desired value into the text box above the spectrum and pressing the Update button (Figure 24).

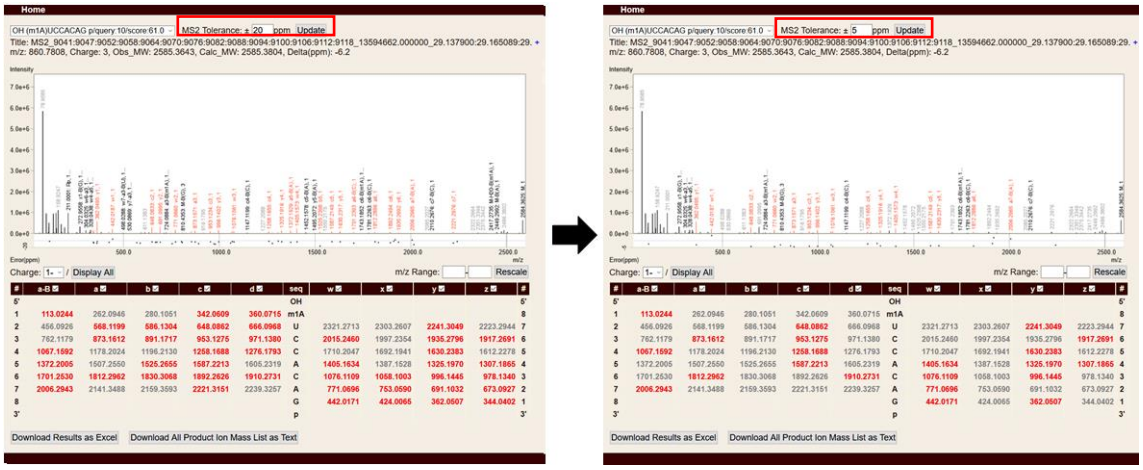
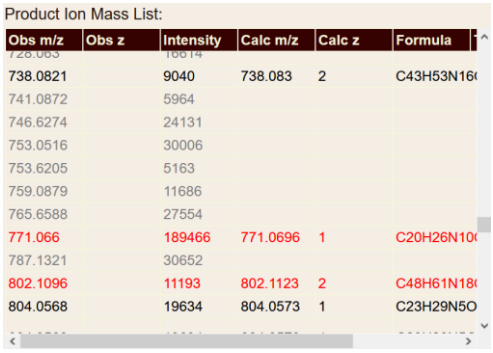


Figure 24. Re-assignment of MS/MS spectrum with different MS2 tolerance. 20 ppm (left), 5 ppm (right).

The Product Ion Mass List table (Figure 25) represents all observed product ions in the MGF query. Some of them, which were assigned to calculated mass values by the search, also exhibit calculated *m/z* (Calc m/z), charge (z), chemical formulas (Formula), and assignments. The assignments are corresponded to the MS/MS spectrum.

Clicking the Download All Product Ion Mass List as Text button will download the Product Ion Mass List with all the calculated product ions, *i.e.* the unassigned calculated product ions. The downloaded file in tab-delimited text (TSV format) will be named as YYYYMMDDHHMMSS.txt, where YYYY, MM, DD, HH, MM, SS are year, month, day,

hour, minute, second at the download, respectively.


Product Ion Mass List:

Obs m/z	Obs z	Intensity	Calc m/z	Calc z	Formula
738.0821		9040	738.083	2	C43H53N16(
741.0872		5964			
746.6274		24131			
753.0516		30006			
753.6205		5163			
759.0879		11686			
765.6588		27554			
771.066		189466	771.0696	1	C20H26N10(
787.1321		30652			
802.1096		11193	802.1123	2	C48H61N18(
804.0568		19634	804.0573	1	C23H29N5O

Figure 25. Product Ion Mass List

Clicking the Download Results as Excel button at the bottom of the MS/MS Assignment View page (See Figure 21) will download an Excel file containing 6 worksheets: Summary, Precursor Mass, Sequence Ladder Ion (Mass), Sequence Ladder Neutral, All Product Ions, Atomic Weights as shown in Figure 26. The file name will be YYYYMMDDHHMMSS.xlsx, where YYYY, MM, DD, HH, MM, SS are year, month, day, hour, minute, second at the download, respectively.

The Summary worksheet contains the selected modified sequence (Sequence) and with its position indicated on the full sequence with parameters that were used for the calculation.

The Precursor Mass worksheet contains the MW of the selected nucleotide and expected *m/z* values of its multiply charged ions.

The Sequence Ladder Ions (Mass) worksheet contains expected *m/z* values for multiply charged product ions (where the upper limit of the charge is the length - 1) that are generated by fragmentation at a single site.

The Sequence Ladder Neutral worksheet contains expected unionized chemical formulas for products and mass values in a table format. The All Product Ions worksheet contains all expected *m/z* values for calculated ions, *i.e.* sequence-ladder ions, internal fragment ions, base losses from the molecular ion, and known MS2 fragments, which are compared with the observed *m/z* values from the search query as in the Product Ion Mass List (Figure 25).

The Atomic Weights worksheet contains the relative atomic weights used for the calculation/search. The values are cited from NIST.

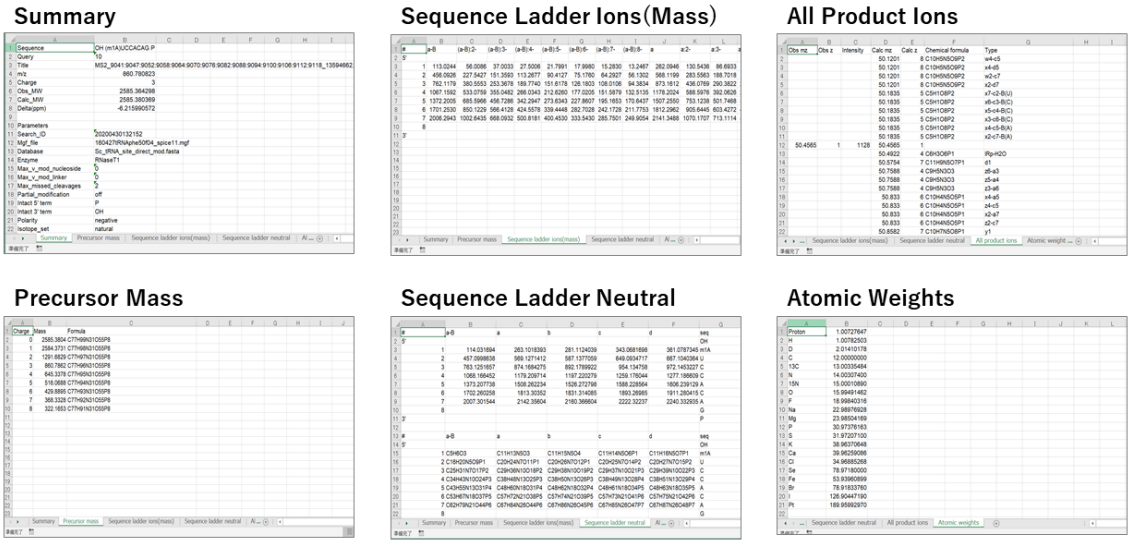


Figure 26. Worksheets in a downloaded Excel file

Clicking the Download All Product Ion Mass List button will download the same contents of the All Product Ions worksheet as text file (Figure 27).

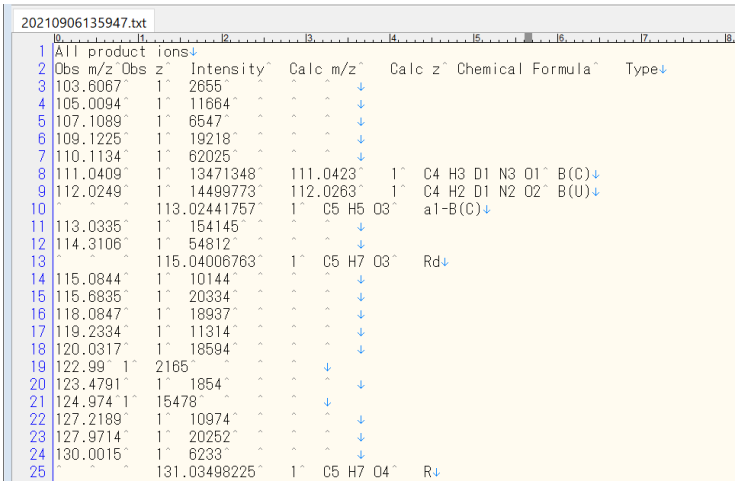


Figure 27. Downloaded All Product Ions as text file