

Ariadne MS/MS Search User's Manual

Rev 1.4

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Ariadne Development Team

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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](#).

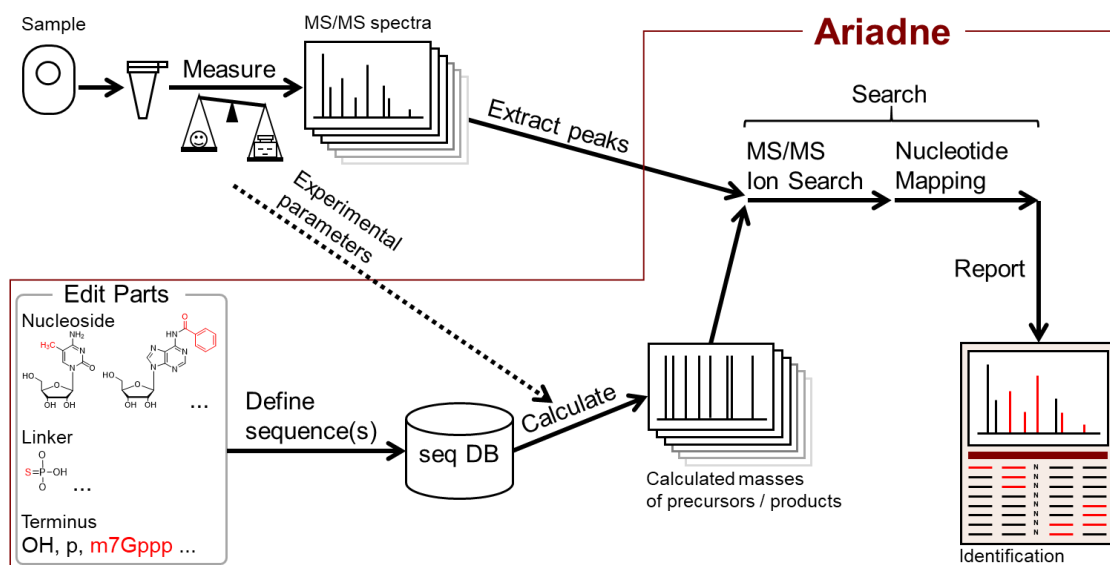


Figure 1. Schematic of Ariadne MS/MS Search

The Ariadne server is publicly available at <https://ariadne.riken.jp/>. The screen shot of the top page is shown in [Figure 2](#).

[Home](#)[Login](#)[Register](#)

Ariadne: Database Search for RNA Identification Using Tandem Mass Spectrometry Data

Overview

Ariadne is a web-based database search service for the identification of RNAs and their post-transcriptional modifications using tandem mass spectrometry data. If you include results from Ariadne in a publication, please cite [the Ariadne paper](#).

Manuals

[Parts Editor](#)
[Mass Calculator](#)
[MS/MS Search](#)

Demo and Example Data

Browse demo search results of some of our published data including ribosomal RNA, transfer RNA, microRNA and other non-coding RNAs and more. Download and try example MS/MS data to search RNA sequence databases by yourself.

MS/MS Search

Searching an RNA sequence database (up to 50MB) with post-transcriptional modifications using up to 50MB of MS/MS data in Mascot generic format (MGF).

Browse Search Results

To Guest Users: Enter the Search ID issued at the search to browse the result report.

Mass Calculator

Calculating mass values of a nucleic acid, its RNase digests and their CID fragments from given sequence(s).

Nucleotide Parts Editor

Defining/editing user nucleotide parts such as Nucleosides, Linkers, and Termini as well as unusual part-specific MS/MS dissociations.

Nucleotide Parts Table

Table of the available parts that can be used in a calculation/search.

[E-mail to the administrator](#)
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Figure 2. The top page of Ariadne service at <https://ariadne.riken.jp/>

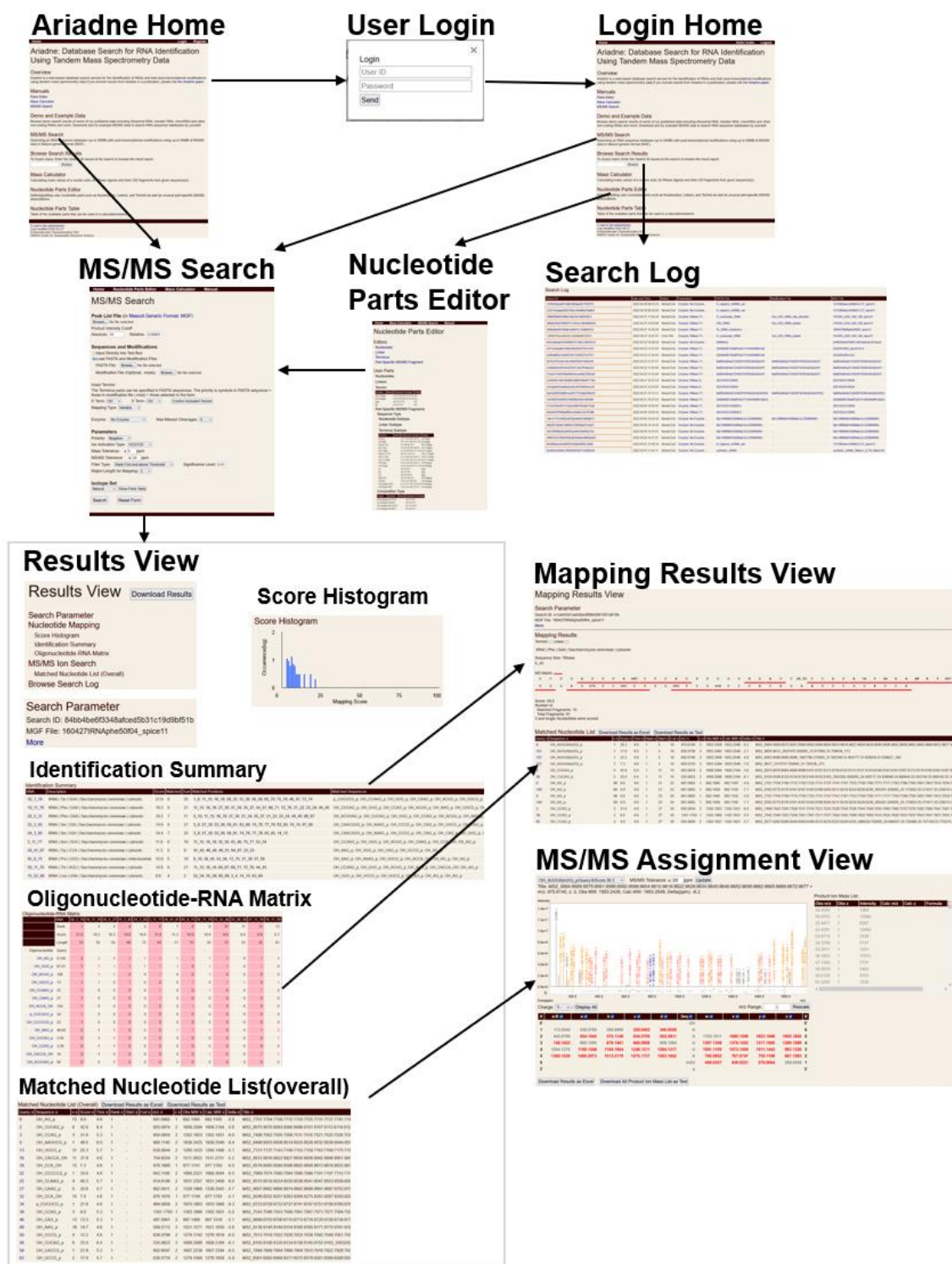


Figure 3. The transition of html pages of Ariadne

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).

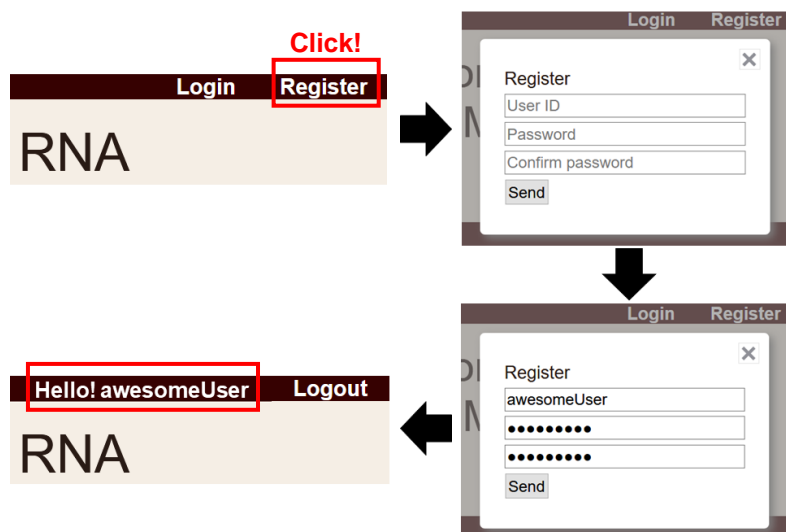


Figure 5. Registration of a new user account

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.

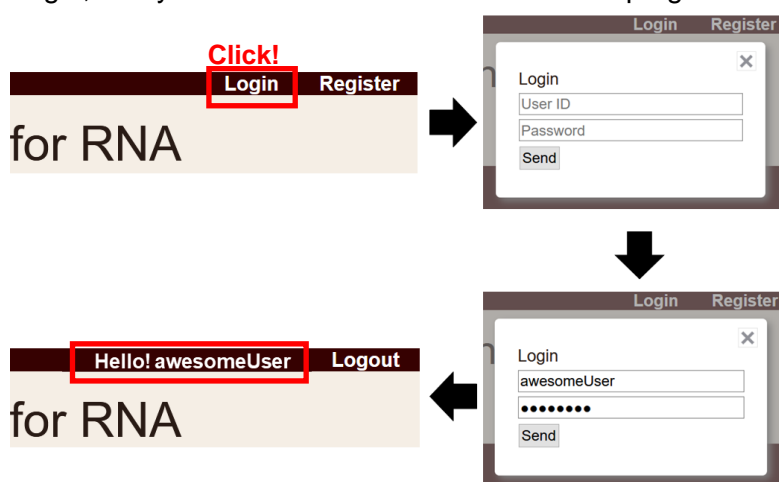


Figure 6. Login procedure

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.

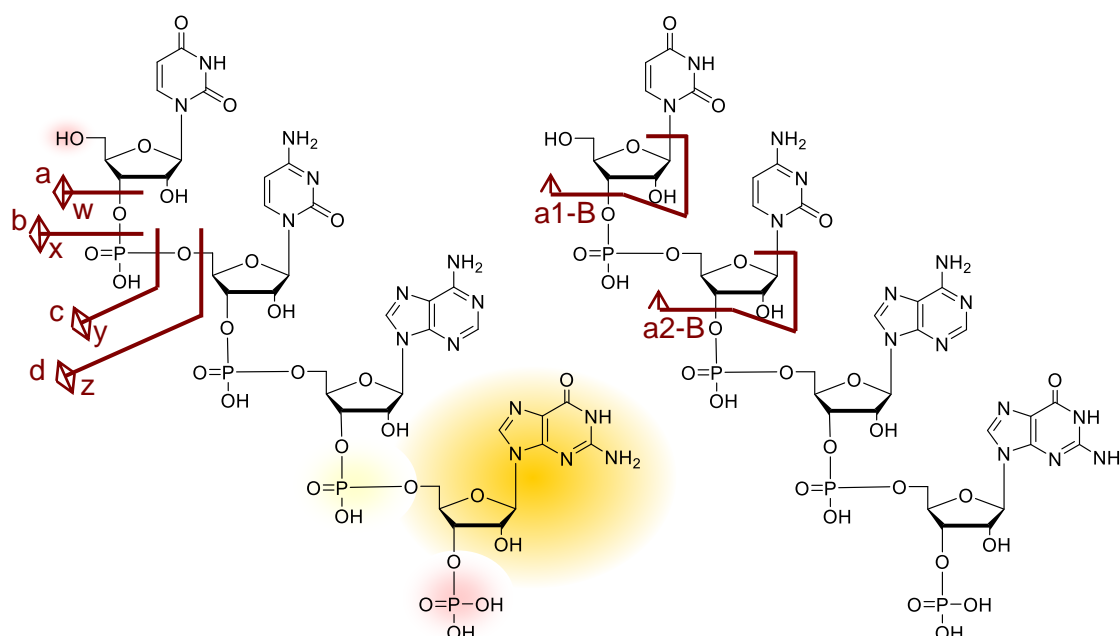


Figure 7. The structure of nucleic acids and their MS/MS dissociation sites. The backbone-cleavage sites and their nomenclature are indicated on the left; an ion generated by simultaneous backbone 3' C-O bond cleavage and loss of base (termed as a-B) is represented on the right. The 3 components, namely, 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/blastcqihelp.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(l)GC(m1l)(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	0.0000 1
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.

Home Nucleotide Parts Editor Mass Calculator Manual

MS/MS Search

Peak List File (in Mascot Generic Format: MGF)
 Browse... No file selected.

Product Intensity Cutoff
 Absolute: 10 Relative: 0.00001

Sequences and Modifications
☐ Input Directly into Text Box
☒ Load FASTA and Modification Files
 FASTA File: Browse... No file selected.
 Modification File (Optional, .mods): Browse... No file selected.

Intact Termini:
 The Terminus parts can be specified in FASTA sequences. The priority is symbols in FASTA sequence > those in modification file (.mod) > those selected in the form.
 5' Term: OH 3' Term: OH Confirm Activated Termini
 Mapping Type: Variable

Enzyme: No Enzyme Max Missed Cleavages: 0

Parameters
 Polarity: Negative
 Ion Activation Type: HCD/CID
 Mass Tolerance: ± 5 ppm
 MS/MS Tolerance: ± 20 ppm
 Filter Type: Rank First and above Threshold Significance Level: 0.01
 Reject Length for Mapping: 2

Isotope Set
 Natural Show Parts Table

Search Reset Form

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](#)).

Peak List File (in Mascot Generic Format: MGF)
 Browse... 160427tRNAphe50f04_spice11.mgf

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](#)).

Results View

Search Parameter

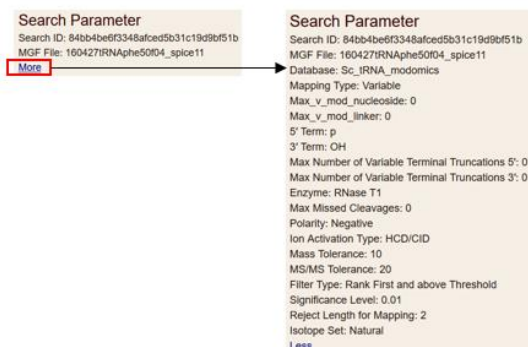


Figure 13. Confirming all the Search Parameters. In the initial view (left) only Search ID and MGF File are shown. Clicking the More link will show the detailed parameters as in the right panel. Clicking the Less link will fold the parameters.

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.).

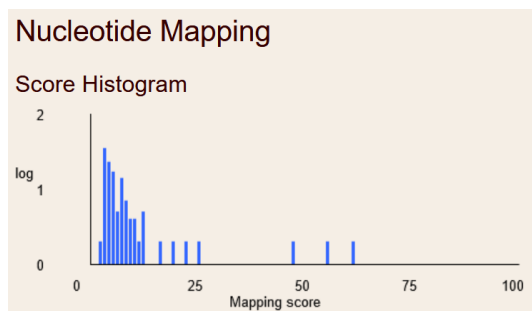


Figure 14. An example of Score Histogram for the Nucleotide Mapping section

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	IRNA Tyr GUA Saccharomyces cerevisiae cytosolic	40.9	13	105	1,6,11,15,16,19,20,25,29,32,33,36,37,45,48,51,56,59,60,64,65,70,75,78,48,47,73,74	p_CUCUCG_p, OH_CCAAG_p, OH_DD(Gm)G_p, OH_DDAAG_p, OH_CAAG_p, OH_ACUG_p, OH_YA(i6A)AY	
19_1_76	IRNA 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_AUUA(m2G)_p, OH_CUCAG_p, OH_CCAAG_p, OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p, OH_m7GUC(m5C)	
20_1_76	IRNA Phe GAA Saccharomyces cerevisiae cytosolic	28.7	10	95	5,10,11,15,27,30,31,42,46,51,54,57,58,65,66,71,72,76,21,22,23,24,44,45,66,67	OH_ACUUA(m2G)_p, OH_CUCAG_p, OH_CCAAG_p, OH_A(Cm)U(Gm)AA(yW)AY(m5C)UG_p, OH_m7GUC(m5C)	
17_1_76	IRNA Lys GUU 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(m2G)YCG_p, OH_YYCG_p, OH_AAAU(m7G)(m5C)G	
24_1_85	IRNA Ser UGA 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_CAAACUG_p, OH_C(m2G)G_p, OH_DDAAG_p, OH_AAAG_p, OH_AYU(p), OH_CCCG_p, OH_CAG_p, OH	
25_1_85	IRNA Ser UGA 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_CAAACUG_p, OH_C(m2G)G_p, OH_DDAAG_p, OH_AAAG_p, OH_AYU(p), OH_CCCG_p, OH_CAG_p, OH	
3_1_77	IRNA 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_CCAAG_p, OH_AD(m5C)G_p, OH_CCA, OH_CAG_p	
26_1_85	IRNA 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(m2G)G_p, OH_DDAAG_p, OH_CAU(m5C)_p, OH_CAG_p, OH_m5UYCG_p, OH_CCA, OH_CAG_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_CCCCG_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	-3.9	MS2_7701.7704.7709.7715.7720.7725.7731.7737.7741.7747.7753.7759.7765.7771
1	OH_(m5U)YCG_p	28	25.7	4.6	1	-	-	646.0720	2	1294.1585	1294.1655	-5.5	MS2_7967.7971.7977.7982.7988.7994.7999.8005.8011.8017.8023.8029.8035.8041
2	OH_CYCAG_p	1	42.8	6.4	1	-	-	803.0974	2	1608.2094	1608.2184	-5.6	MS2_8075.8078.8084.8090.8096.8101.8107.8113.8119.8125.8131.8137.8143.8149
2	OH_CUCAG_p	5	42.8	6.4	1	-	-	803.0974	2	1608.2094	1608.2184	-5.6	MS2_8075.8078.8084.8090.8096.8101.8107.8113.8119.8125.8131.8137.8143.8149
3	OH_CACAG_p	3	31.6	4.6	1	-	-	650.0854	2	1302.1853	1302.1931	-6.0	MS2_7498.7502.7505.7508.7511.7516.7521.7525.7528.7534.7538.7544.7549.7555
4	OH_(m7G)UC(m5C)UG_p	2	45.9	5.7	1	-	-	978.1216	2	1958.2578	1958.2699	-6.2	MS2_8626.8632.8638.8641.8649.8654.8660.8666.8672.8678.8684.8690.8696.8702
5	OH_AAUUCG_p	1	49.5	5.7	1	-	-	968.1140	2	1938.2425	1938.2549	-6.4	MS2_9498.9503.9509.9514.9520.9526.9532.9538.9544.9550.9556.9562.9568.9574
6	OH_AUUUA(m2G)_p	1	38.3	4.6	1	-	-	975.6140	2	1953.2426	1953.2546	-6.2	MS2_9564.9569.9575.9581.9586.9592.9598.9604.9610.9616.9622.9628.9634.9640
8	OH_(m1A)UCCACAG_p	2	63.9	6.4	1	-	-	1291.6705	2	2585.3556	2585.3804	-9.6	MS2_9037.9040.9046.9051.9057.9063.9069.9075.9081.9087.9093.9099.9105.9111
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_12532.12537.12540.12544.12550.12556.12562.12568.12574.12580.12586.12592
10	OH_(m1A)UCCACAG_p	2	68.9	6.4	1	-	-	860.7808	3	2585.3843	2585.3804	-6.2	MS2_9041.9047.9052.9058.9064.9070.9076.9082.9088.9094.9100.9106.9112.9118
13	OH_YYCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131.7137.7143.7149.7153.7159.7163.7169.7175.7181.7185.7191.7195.7201
13	OH_UYCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131.7137.7143.7149.7153.7159.7163.7169.7175.7181.7185.7191.7195.7201
13	OH_UUCG_p	1	25.3	6.5	1	-	-	639.0644	2	1280.1433	1280.1499	-5.1	MS2_7131.7137.7143.7149.7153.7159.7163.7169.7175.7181.7185.7191.7195.7201
14	OH_ACUUA(m2G)_p	1	43.1	5.7	1	-	-	975.1221	2	1952.2588	1952.2706	-6.0	MS2_9430.9436.9442.9448.9454.9457.9463.9466.9472.9474.9479.9482.9488.9494
16	OH_CACCA_OH	11	37.9	4.6	1	-	-	754.6254	2	1511.2653	1511.2731	-5.2	MS2_8912.8916.8922.8927.8930.8936.8942.8948.8951.8956.8961.8967.8973.8979

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.

Search Parameter										
1	Search ID	2020051215349								
2	MGF File	1604271RNAPhe50I04_spice11.mgf								
3	Database	Sc_IRNA_site_direct_mod.fasta								
4	Max_v_mod_nucleoside	0								
5	Max_v_mod_inser	0								
6	Partial Modification	off								
7	Intact 5' term	p								
8	Intact 3' term	OH								
9	Enzyme	RNaseT1								
10	Max Missed Cleavages	2								
11	Polarity	negative								
12	Ion Activation Type	HCD/CID								
13	Mass Tolerance	10								
14	MS2 Tolerance	20								
15	Filter Type	Rank first and Above threshold								
16	Significance Level	0.01								
17	Reject Length For Mapping	2								
18	Isotope Set	natural								
19										
20										
21										
22										
search_parameter matched_nucleotide_list										

Matched Nucleotide List												
1	Query	Modseq	Seq	Score	Threshold	m/z	z	Obs_MW	Calc_MW	Q_title		
2	1	OH (mS)	OH UUCG	22.2	4.6	646.072	2	1294.16	1294.17	MS2_7967:7971:7977:7982:7988:7994:		
3	2	OH CYCA	OH CUCA	37.3	6.4	803.097	2	1608.21	1608.22	MS2_8075:8078:8084:8090:8096:8101:		
4	2	OH CUCA	OH CUCA	37.3	6.4	803.097	2	1608.21	1608.22	MS2_8075:8078:8084:8090:8096:8101:		
5	3	OH CCA	OH CCA	27.2	4.6	650.065	2	1302.19	1302.19	MS2_7486:7502:7505:7508:7511:7516:		
6	4	OH (mT)	OH GUCC	25.3	5.7	978.122	2	1958.26	1958.27	MS2_8626:8632:8638:8641:8649:8654:		
7	5	OH AAU	OH AAU	45.4	6	968.114	2	1938.24	1938.25	MS2_9498:9503:9509:9514:9520:9526:		
8	6	OH AAU	OH AAU	33.3	4.6	975.614	2	1953.24	1953.25	MS2_9564:9569:9575:9581:9586:9592:		
9	6	OH (mT)	OH AUCC	63.4	6.4	1291.67	2	2585.36	2585.38	MS2_9040:9046:9051:9057:9063:		
10	9	OH A(Cm)	OH ACUG	45	4.6	1387.21	3	4164.64	4164.67	MS2_12532:12537:12540:12544:12550:		
11	10	OH (mT)	OH AUCC	61	6.4	860.781	3	2585.36	2585.38	MS2_9041:9047:9052:9058:9064:9070:		
12	13	OH UYCG	OH UUCG	21.4	6.5	639.064	2	1280.14	1280.15	MS2_7131:7137:7143:7149:7153:7159:		
13	13	OH UUCG	OH UUCG	21.4	6.5	639.064	2	1280.14	1280.15	MS2_7131:7137:7143:7149:7153:7159:		
14	14	OH ACU	OH ACU	39.1	5.7	975.122	2	1952.26	1952.27	MS2_9430:9436:9442:9448:9454:9457:		
15	16	OH CACC	OH CACC	37.9	4.6	754.625	2	1511.27	1511.27	MS2_8912:8916:8922:8927:8930:8936:		
16	16	OH CCA	OH CCA	7.3	4.6	876.167	1	877.174	877.179	MS2_8579:8585:8590:8596:8602:8608:		
17	19	OH DDV	OH UUCG	25.2	5.3	668.09	2	1338.19	1338.2	MS2_6309:6311:6313:6316:6317:6319:		
18	21	OH DDA	OH UUA	33.7	4.6	971.629	2	1945.27	1945.29	MS2_7936:7942:7946:7952:7958:7964:		
19	22	OH CCCC	OH CCCC	31.6	4.6	943.119	2	1888.25	1888.26	MS2_7069:7074:7080:7084:7090:7096:		
20	23	OH (mT)	OH ACUC	30.2	5.3	810.105	2	1622.22	1622.23	MS2_8958:8964:8969:8974:8980:8986:		
21	25	OH CCA	OH CCA	36.6	5.7	814.611	2	1631.24	1631.25	MS2_8515:8519:8524:8530:8536:8541:		
22	26	OH AD	OH AUCC	22.3	5.3	658.592	2	1319.2	1319.21	MS2_7832:7838:7844:7850:7856:7862:		
search_parameter matched_nucleotide_list												

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

20210906140400_results.txt

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	12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Mapping Results View

Search Parameter

Search ID: e1cde53d1ae0db3f88b0561551d619b
MGF File: 160427IRNAPhe50104_spice11
[More](#)

Mapping Results

Termini: ☐ Linker: ☐

tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic

Sequence Size: 76base
5_45

MS Match: _____

G C G G A U U U A n2G C U C A G D D G G A G A G C n2,2G C C A G A Cn U Qn U Qn A A Y n5C
U G G A G m7G U C n5C U G U G n5U Y C G n1A U C C A C A G A A U U C G C A C C A

Score: 29.0

Number of
Matched Fragments: 10
Total Fragments: 91
3 and longer Nucleotides were scored.

Matched Nucleotide List

[Download Results as Excel](#)

[Download Results as Text](#)

Query	Sequence	n	Score	Time	Rank	Start	End	m/z	z	Obs MW	Calc MW	Delta	Title
6	OH_AUUUA(m2G)_p	1	38.3	4.6	1	5	10	975.6140	2	1953.2426	1953.2546	-6.2	MS2_9564.9569.9575.9581.9586.9592.9598.9604.9610.9616.9622.9628.9634.9640.9646.9652.9656.9662.9665.9669.9672.9677.9681
151	OH_AUUUA(m2G)_p	1	17.6	6.5	1	5	10	650.0758	3	1953.2492	1953.2546	-2.7	MS2_9605.9612.2637470.500000_31.677682.31.708634_173
157	OH_AUUUA(m2G)_p	1	23.2	4.6	1	5	10	650.0746	3	1953.2456	1953.2546	-4.6	MS2_9583.9588.9595.9596.1987786.375000_31.582346.31.604177.31.634919.31.639627_180
227	OH_AUUUA(m2G)_p	1	7.2	4.6	1	5	10	650.0725	3	1953.2394	1953.2546	-7.8	MS2_9617.2117515.750000_31.730126_273
2	OH_CUACAG_p	5	42.8	6.4	1	11	15	803.0974	2	1608.2094	1608.2184	-5.6	MS2_8075.8078.8084.8090.8096.8101.8107.8113.8119.8125.8131.8137.8143.8149.8155.8161.8167.8173.8179.8185.8191.8197.820
56	OH_CUACAG_p	5	25.0	6.4	1	11	15	535.0623	3	1608.2086	1608.2184	-6.1	MS2_8105.8108.8120.8134.8138.8145.8152.8163_2053282.000000_24.936646.24.990644.25.052744.25.069142.25.100
0	OH_AG_p	66	9.6	4.6	1	21	22	691.0992	1	692.1065	692.1105	-5.9	MS2_7701.7704.7709.7715.7720.7725.7731.7737.7741.7747.7753.7759.7765.7771.7777.7783.7789.7795.7801.7807.7813.7819.78
140	OH_AG_p	66	4.8	4.6	1	21	22	691.0983	1	692.1056	692.1105	-7.1	MS2_8162.8170.8176.8181.8187.8193.8199.8205.8211.8218.8224.8229.8236_565291.250000_25.173852.25.211671.25.238215.25
0	OH_AG_p	66	9.6	4.6	1	23	24	691.0992	1	692.1065	692.1105	-5.9	MS2_7701.7704.7709.7715.7720.7725.7731.7737.7741.7747.7753.7759.7765.7771.7777.7783.7789.7795.7801.7807.7813.7819.78
140	OH_AG_p	66	4.8	4.6	1	23	24	691.0983	1	692.1056	692.1105	-7.1	MS2_8162.8170.8176.8181.8187.8193.8199.8205.8211.8218.8224.8229.8236_565291.250000_25.173852.25.211671.25.238215.25
3	OH_CGAG_p	3	31.6	4.6	1	27	30	650.0854	2	1302.1853	1302.1931	-6.0	MS2_7498.7502.7505.7508.7511.7516.7521.7525.7528.7534.7538.7544.7549.7555.7560.7566.7570.7576.7582.7588.7591.7597.760
39	OH_CGAG_p	3	9.0	4.6	1	27	30	1301.1793	1	1302.1866	1302.1931	-5.0	MS2_7542.7548.7553.7556.7561.7567.7571.7577.7584.7592.7598.7602.7608.7615.7620.7626.7633.7637.7643.7647.7651.7655.766
65	OH_CGAG_p	3	4.8	4.6	1	27	30	650.0856	2	1302.1857	1302.1931	-5.7	MS2_8277.8282.8288.8294.8300.8306.8312.8318.8323.8329.8335_666529.750000_25.698557.25.720496.25.747159.25.775575.25
9	OH_A(Cm)U(Gm)AA(W)AY(m5C)UG_p_2	2	44.0	4.6	1	31	42	1387.2051	3	4164.6371	4164.6685	-7.5	MS2_12532.12537.12540.12544.12549.12550.12556.12562.12568.12574.12580.12586.12592.12598.12604.12610.12615.12621.12624.126

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

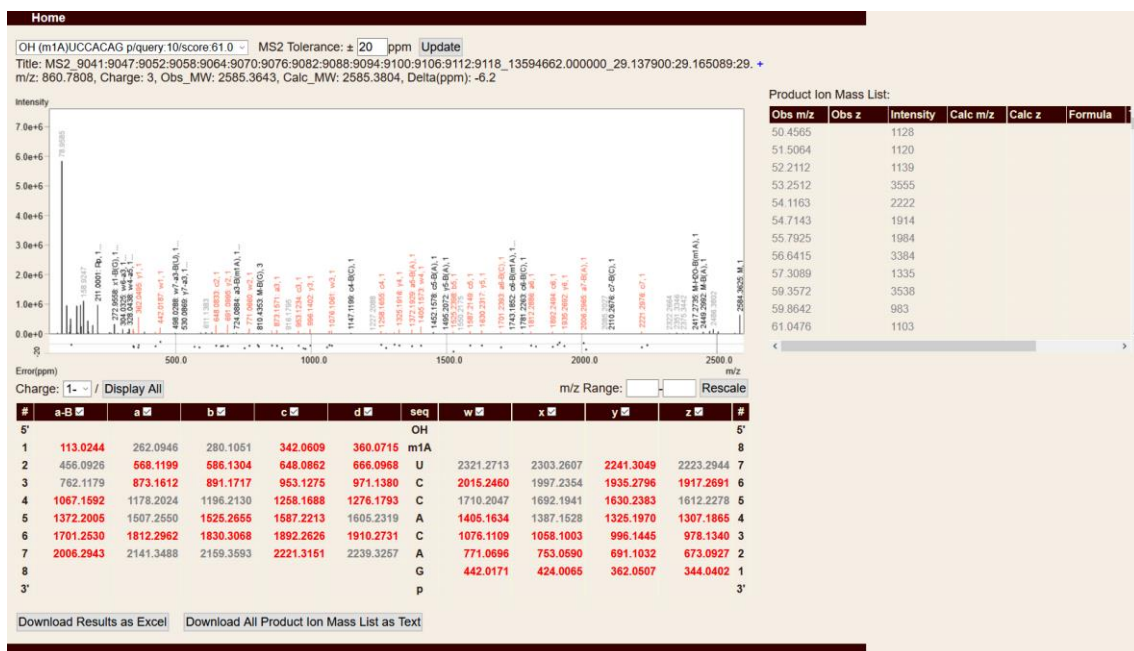


Figure 21. An example of the MS/MS Assignment View page that shows the spectrum for the selected sequence and the corresponding assignment table.

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.

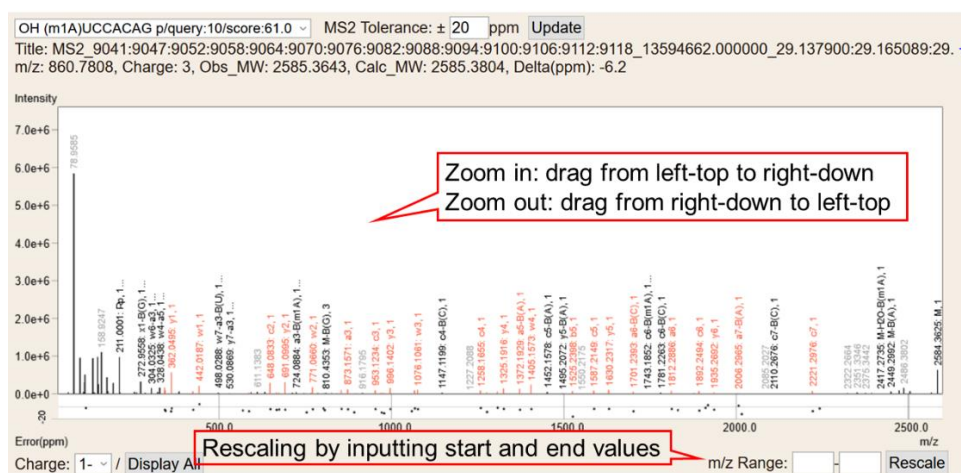


Figure 22. Zooming in and out of assigned MS/MS spectrum

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).

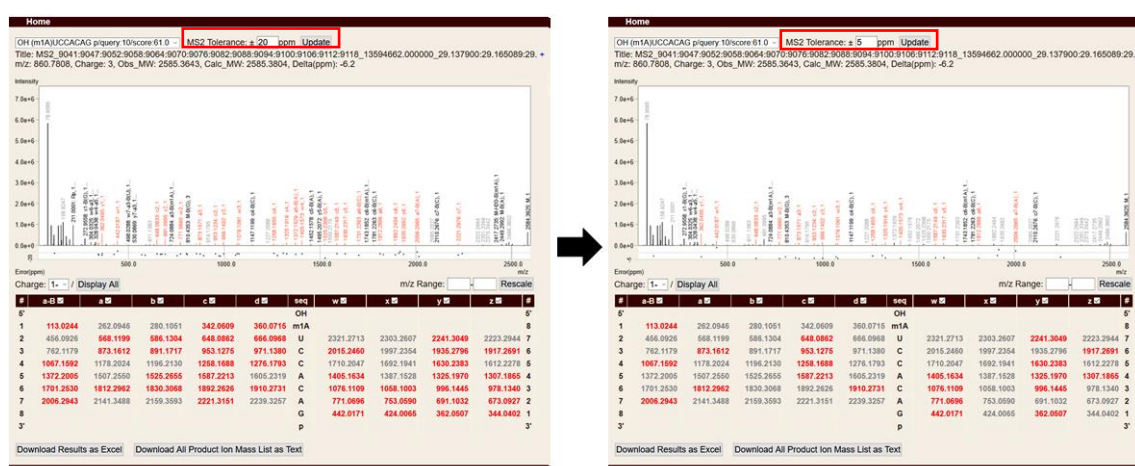


Figure 24. Re-assignment of MS/MS spectrum with different MS2 tolerance. 20 ppm (left), 5 ppm (right).

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	C43H53N16O
741.0872		5964			
746.6274		24131			
753.0516		30006			
753.6205		5163			
759.0879		11686			
765.6588		27554			
771.066		189466	771.0696	1	C20H26N10O
787.1321		30652			
802.1096		11193	802.1123	2	C48H61N18O
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.

Summary

Sequence	Ch (H1A)UCCAG P
1	Chrg
2	Time
3	Chrg
4	Chrg
5	Chrg
6	Chrg
7	Chrg
8	Chrg
9	Chrg
10	Chrg
11	Chrg
12	Chrg
13	Chrg
14	Chrg
15	Chrg
16	Chrg
17	Chrg
18	Chrg
19	Chrg
20	Chrg
21	Chrg
22	Chrg
23	Chrg
24	Chrg
25	Chrg

Sequence Ladder Ions(Mass)

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

All Product Ions

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

Precursor Mass

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

Sequence Ladder Neutral

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

Atomic Weights

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

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).

Obs m/z	Intensity	Calc m/z	Chemical Formula	Type
1	100	100	[M+H] ⁺	100
2	100	100	[M+H] ⁺	100
3	100	100	[M+H] ⁺	100
4	100	100	[M+H] ⁺	100
5	100	100	[M+H] ⁺	100
6	100	100	[M+H] ⁺	100
7	100	100	[M+H] ⁺	100
8	100	100	[M+H] ⁺	100
9	100	100	[M+H] ⁺	100
10	100	100	[M+H] ⁺	100
11	100	100	[M+H] ⁺	100
12	100	100	[M+H] ⁺	100
13	100	100	[M+H] ⁺	100
14	100	100	[M+H] ⁺	100
15	100	100	[M+H] ⁺	100
16	100	100	[M+H] ⁺	100
17	100	100	[M+H] ⁺	100
18	100	100	[M+H] ⁺	100
19	100	100	[M+H] ⁺	100
20	100	100	[M+H] ⁺	100
21	100	100	[M+H] ⁺	100
22	100	100	[M+H] ⁺	100
23	100	100	[M+H] ⁺	100
24	100	100	[M+H] ⁺	100
25	100	100	[M+H] ⁺	100

Figure 27. Downloaded All Product Ions as text file