Ariadne
Mass Calculator
User’s Manual

Rev 1.5
2022-05-09
Ariadne Development Team

The Mass Calculator software calculates expected molecular weight, and *m/z* values of multiply charged molecular (precursor) and product ions from provided nucleic acid sequences, and under defined experimental conditions. This program calculates accurate mass values for a given nucleic acid, based on relative atomic masses of its composition elements. The software is a web-based service that can be accessed through the following link: https://ariadne.riken.jp/. Figure 1 shows an image of the Ariadne top page, with a link to the calculator highlighted with a red rectangle.

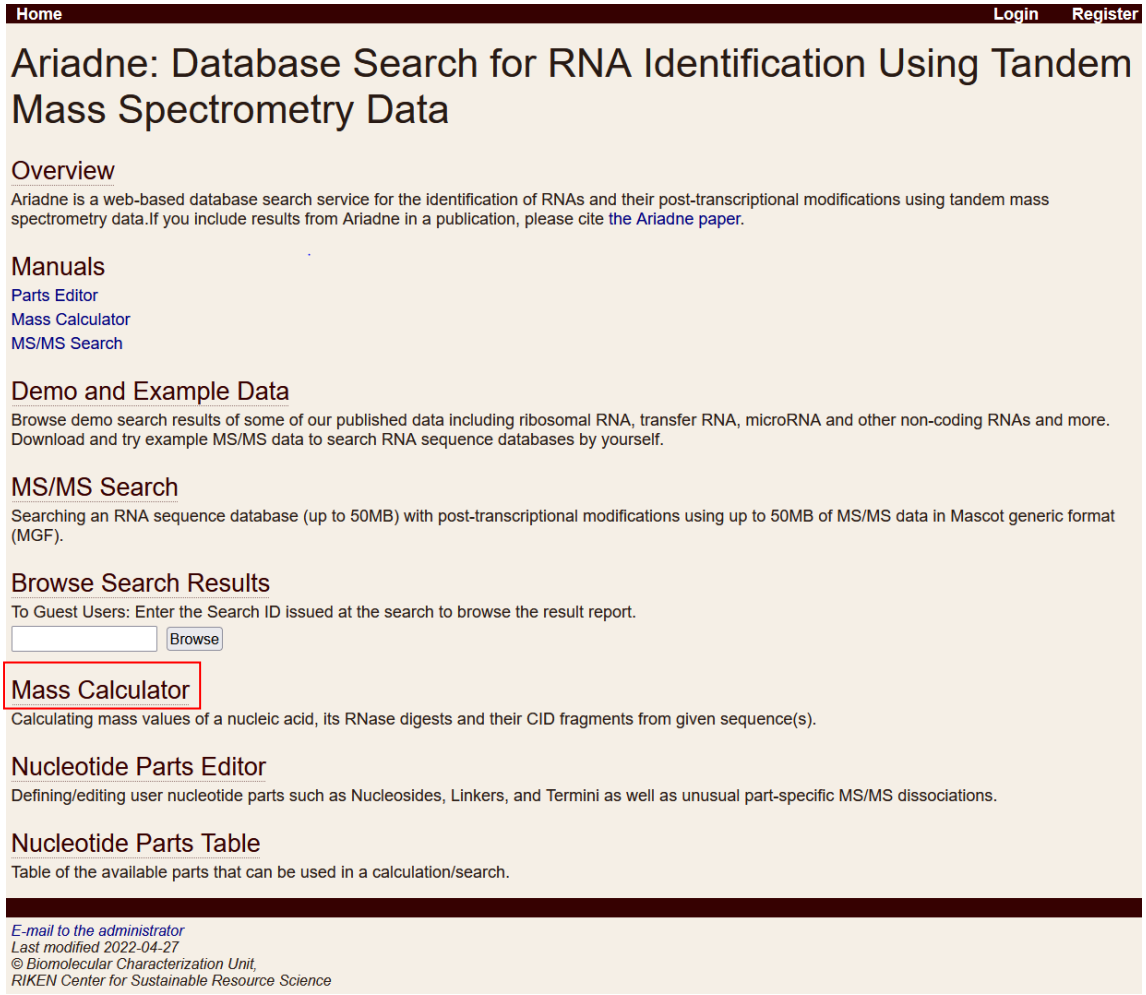


Figure 1. The Ariadne top page

1. Preparation of data for use in calculations

1.1. Requirements

System and data formatting requirements are described in this section.

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 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 calculations 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. Sequence

The calculation requires that the user input at least one nucleic acid sequence into the web form. The web form can accept two forms of input for sequence(s): (1) either direct inputting and/or pasting to the text box, or (2) uploading a file that contain the sequence(s) in FASTA format. Other parameters are optional. 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. Windows has a simple text editor called Notepad (notepad.exe); however, better editors can be downloaded through the web.

1.2. User account (optional)

All 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).

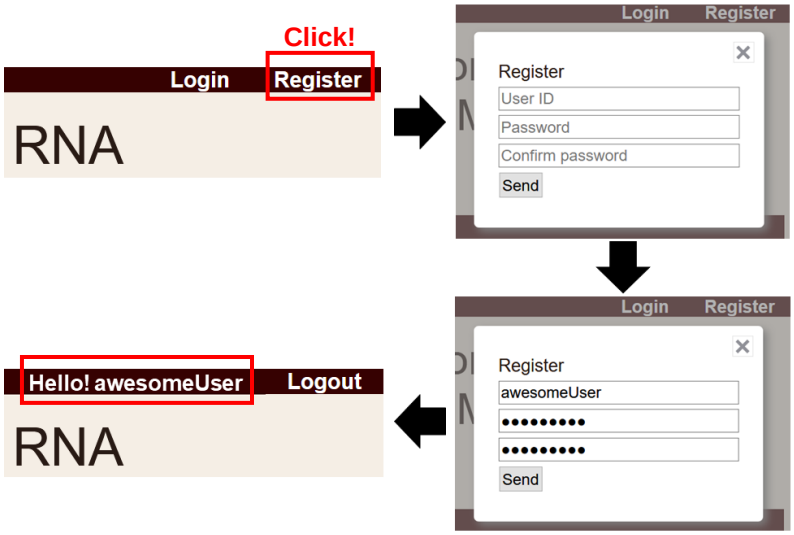


Figure 2. Registration of a new user account

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.

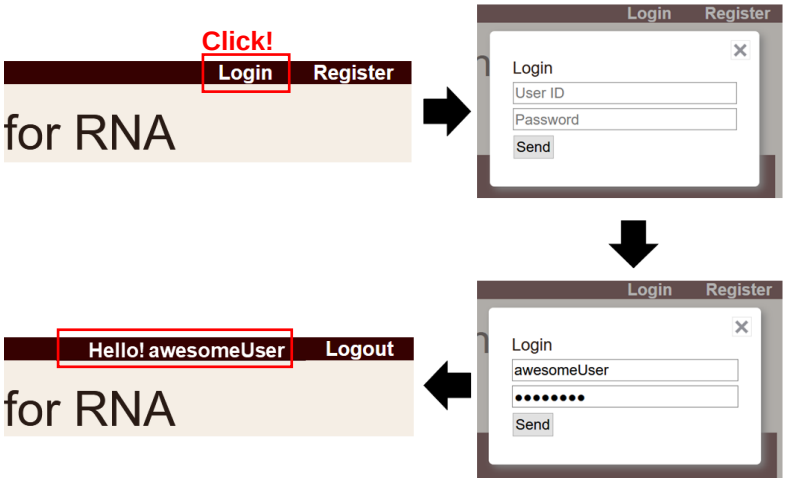


Figure 3. Login procedure

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 3). Users are automatically logged out every 24 hours.

1.3. Parts

Typical (and canonical) nucleic acids consist of 5′- and 3′-termini, nucleosides and linker(s) as shown in Figure 4.

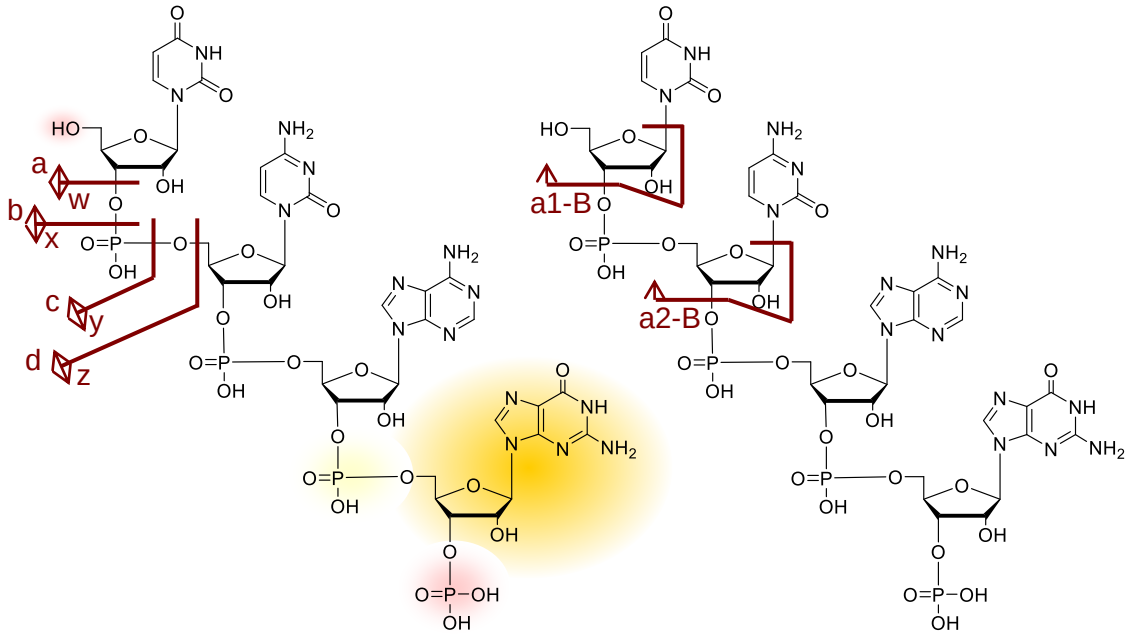


Figure 4. The structure of nucleic acids and their MS/MS dissociation sites. The backbone-cleavage sites and their nomenclature are indicated on the left; an ion generated by simultaneous backbone 3′ C-O bond cleavage and loss of base (termed as a-B) is represented on the right. The 3 components, namely, 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 1). 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 Ariadne Nucleotide Parts Editor User's Manual.

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

The mass calculator 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)GGGA
GAGG(D)CUCCGG(m5U)(Y)CGAUUCCGGACUCGUCCACCA

>tRNA | Arg | UCU | Saccharomyces cerevisiae | cytosolic
GCUCGCGU(m1G)(m2G)CGUAA(D)GGCAACGC(m2,2G)(Y)CUGACU(mcm5U)CU(t6A)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. Each terminus should be connected to the sequence using an underscore character (_). For example, OH_AAA_p, OH_AAA or AAA_p. If you do not denote a terminus, the program refers to the selected terminus in a selectable box in 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 terminus 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
GGGCGUGUGGCGUAGUCGGUAGCGCGCUCCCUUAGCAUGGGAGAGGUCUCCGGU
UCGAUUCCGGACUCGUCCACCA

>tRNA | Arg | UCU | Saccharomyces cerevisiae | cytosolic
GCUCGCGUGGCGUAAUGGCAACGCGUCUGACUUCUAAUCAGAAGAUUAUGGGUUC
GACCCCCAUCGUGAGUGCCA

.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

...

1.5. Other parameters

Additionally, the software can also account for parameters that simulate experimental conditions (Table 1). If the user does not specify some of the parameters listed, default settings will be used to perform the calculation.

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
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
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 measurement.	Negative
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
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 (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).	Natural

*: 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|m2,2G|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	[ac4C|C|ho5C|m5C|m5U|mo5U|T|U|m1Y|m3Y|Y]* ^ N	OH	cp or p
RNase U2	[A|G]* ^ N	OH	cp or p
RNaseT1+BAP	[G|m1G|m2,2G|m2G|I] * ^ N	OH	OH

^: 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•

2. Set-up for the calculation

In this section, we will explain in detail how to input and upload data and specify parameters.

2.1. Filling in the calculator web form

Click on the Mass Calculator button on the top page (See Figure 1), and the web form will appear as shown in Figure 5. This form contains Sequence and Modifications, Parameters and Isotope Set sections and Calculate and Reset Form buttons.

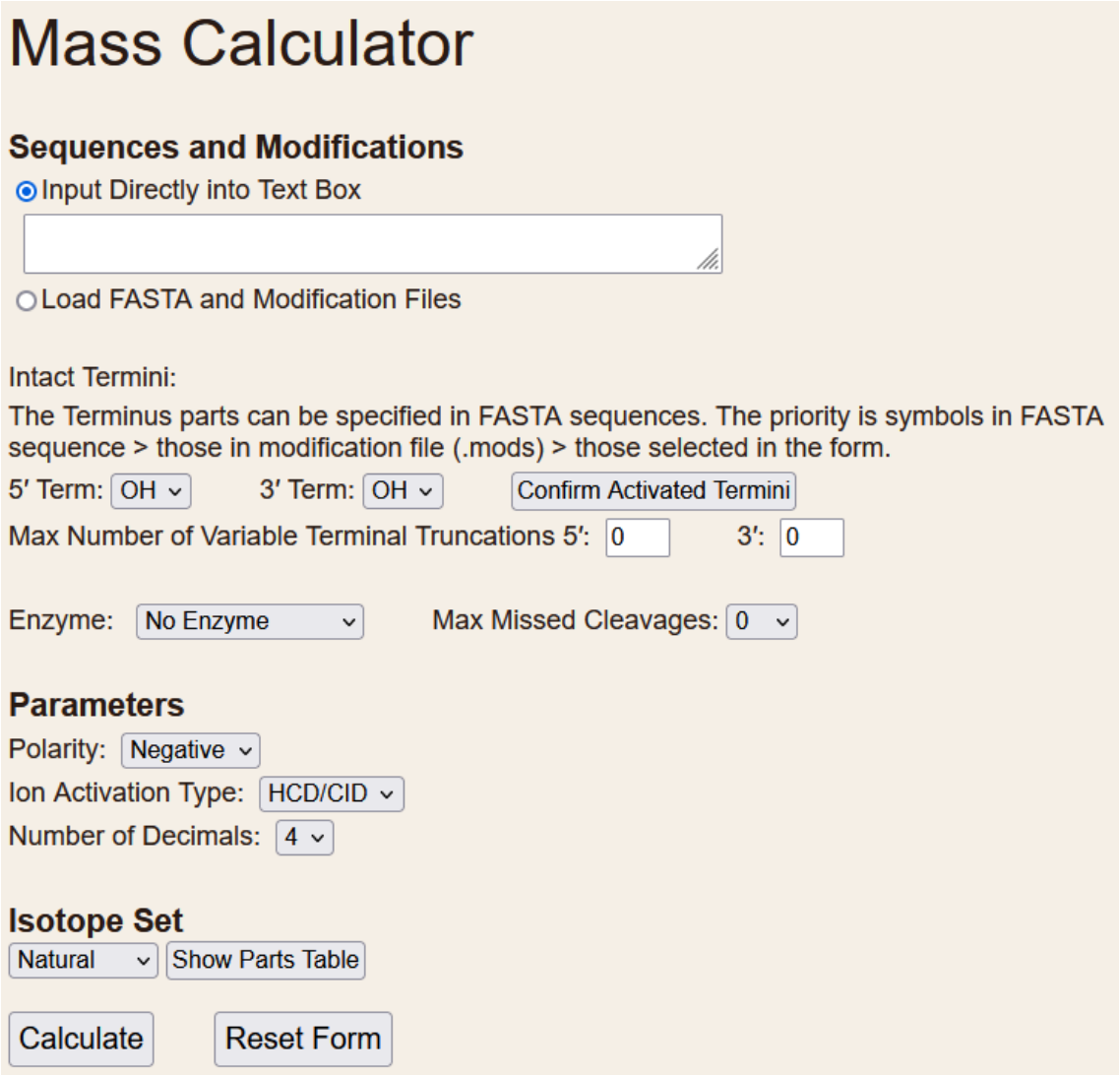


Figure 5. Mass Calculator web form

2.2. Input of sequence(s) and parameters

The nucleic acid sequence can either be directly input or uploaded in the Sequences and Modifications section of the web form. To directly input or paste sequence(s), check the Input Directly into Text Box radio button (default), shown in Figure 6. Note that, in FASTA

format, plural sequences can be inputted directly into the text box, while single sequence without description line (>xxxx) is also acceptable in the box.

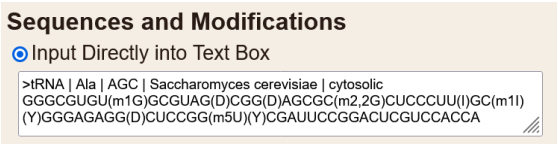


Figure 6. Direct input of a sequence into the text box

The rules for composing a sequence are in Section 1.4. For calculations involving entry of a single sequence, only line(s) of nucleic acid sequence, without the header line, can be accepted in the text box. Otherwise, to upload a FASTA file (and a modification file), select Load FASTA and Modification File button (Figure 7). Then click the Browse… button (In the case of Firefox; for Chrome and Edge, click Choose file... button) for FASTA file.

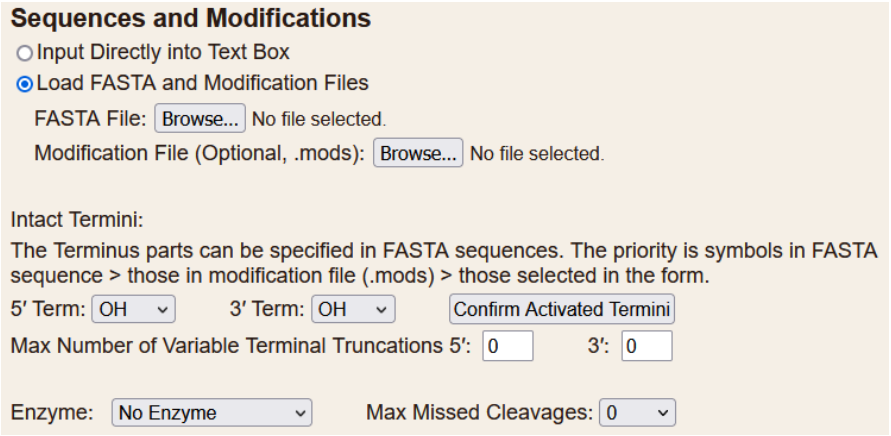


Figure 7. Loading FASTA and modification files

Select the appropriate FASTA file to be marked for uploading and calculation, and press the OK button. Then, the name of the selected file will appear at the right of the Browse… button. (Figure 7). If necessary, click on the Browse... button to load the Modification File (Optional) in the same section (Figure 7). Select a .mods file you would like to upload, and press the OK button. Then, the name of the selected file will appear at the right of the Browse… button. (Figure 7).

Input/Select appropriate parameters according to the sample information and experimental conditions used. Detailed descriptions and the default values of each parameter are shown in Table 1 (See Section 1.6).

2.3. Starting the calculator

After all data and parameters described above are defined, the calculation is started by clicking the Calculate button at the bottom of the web form (Figure 5). On completion of the calculation, the browser window is updated to show an html calculation report. See Section 3 to browse/interpret the results.

3. Browsing the calculation results

3.1. Browsing the Calculation Results page

After the calculation is complete, the Calculation Results page will appear. The page is the homepage of browsing the result report as shown in Figure 8. The reports are divided into *m/z* values for intact/digested nucleotides and their MS/MS in a separate web page. The reports can be downloaded as Excel or tab-delimited text format.

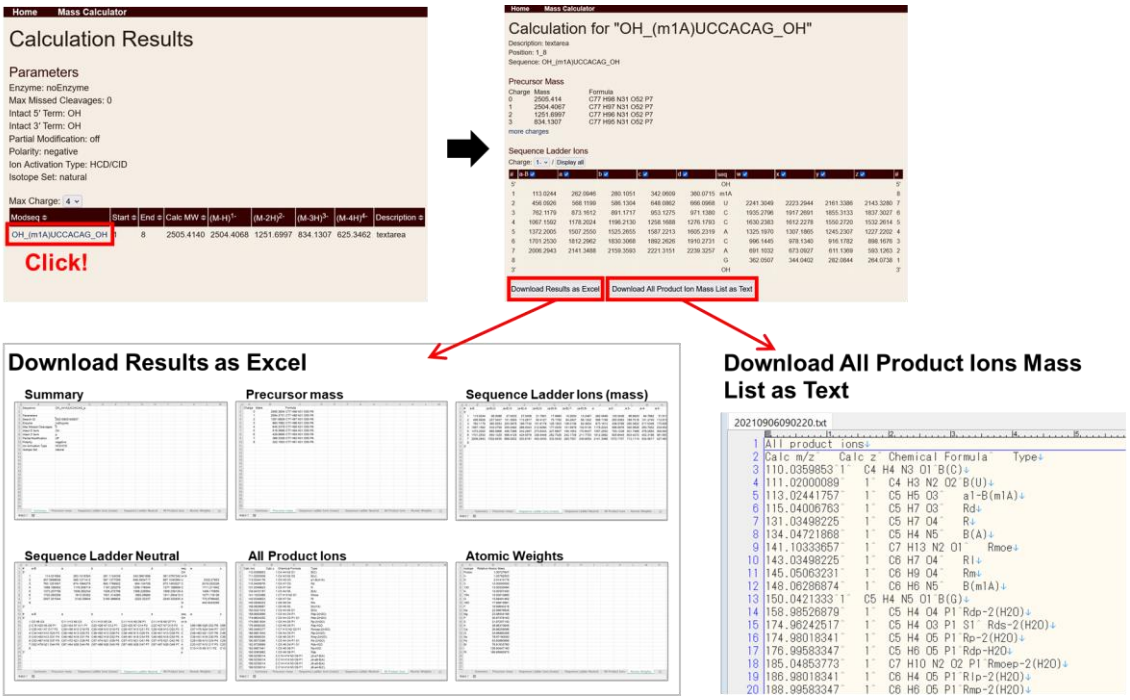
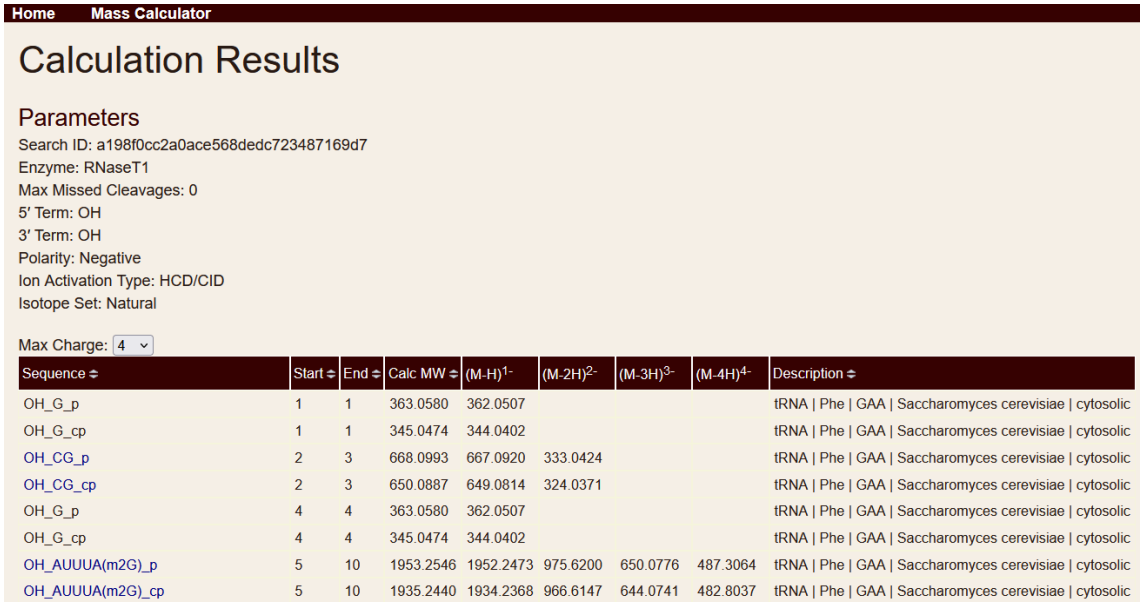


Figure 8. Navigation of the Calculation Results page

The Calculation Results page (Figure 9) contains a table representing molecular weight (MW) and multiply charged *m/z* values for enzymatically cleaved nucleotides generated from the input sequence(s). The parameters (Enzyme, Max Missed Cleavages, 5′ Term, 3′ Term, Polarity, Ion Activation Type, and Isotope Set) that were used in the calculation are also shown at the top of the page.



Sequence	Start	End	Calc MW	$(M-H)^{1-}$	$(M-2H)^{2-}$	$(M-3H)^{3-}$	$(M-4H)^{4-}$	Description
OH_G_p	1	1	363.0580	362.0507				tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_G_cp	1	1	345.0474	344.0402				tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_CG_p	2	3	668.0993	667.0920	333.0424			tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_CG_cp	2	3	650.0887	649.0814	324.0371			tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_G_p	4	4	363.0580	362.0507				tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_G_cp	4	4	345.0474	344.0402				tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_AUUUA(m2G)_p	5	10	1953.2546	1952.2473	975.6200	650.0776	487.3064	tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic
OH_AUUUA(m2G)_cp	5	10	1935.2440	1934.2368	966.6147	644.0741	482.8037	tRNA | Phe | GAA | Saccharomyces cerevisiae | cytosolic

Figure 9. View of the Calculation results page

3.1.1. Precursor mass table

The sequence and mass values of products from enzymatic digests are shown in the table (Figure 9). An explanation for each column entity is shown in Table 4. Clicking the arrow head icon on the header for each column will sort the table by alphabetical order (Sequence or Description) or numerical order (Start, End, or Calc MW). The Max Charge selectable box alters the maximum charge number for which oligonucleotides will be displayed in the table. Clicking a sequence in the Sequence (modified sequence) column will generate a new tab that represents calculation results on product ions for the selected Sequence (Figure 10).

Table 4. Column entities for the oligonucleotide table

Name	Explanation
Sequence	nucleotide sequence including site-directed modifications
Start	the start position of the oligonucleotide in the sequence region
End	the end position of the oligonucleotide in the sequence region
Calc MW	MW calculated from the Sequence
m/z [(M-H)1-, (M-2H)2-, etc]	*m/z* value for the specified charge state
Description	The content of ">"-starting line for the Modseq. The value TextBox is shown unless specified.

3.2. Browsing the Calculation for (specified sequence) page

As described above, clicking on an oligonucleotide sequence in the Sequence field will open another browser tab (or window), in which mass values for corresponding product ions are represented. The page will be titled Calculation for "*XX*", where *XX* is the specified sequence in the selected Sequence field. The upper half of the page displays information on the Sequence and the Precursor mass section, and the lower part of the page displays a Sequence Ladder Ions table, which shows product ions that are generated by fragmentation at a single site (Figure 10). There are also Download Results as Excel and Download All Product Ions Mass List as Text buttons located at the bottom of the page.

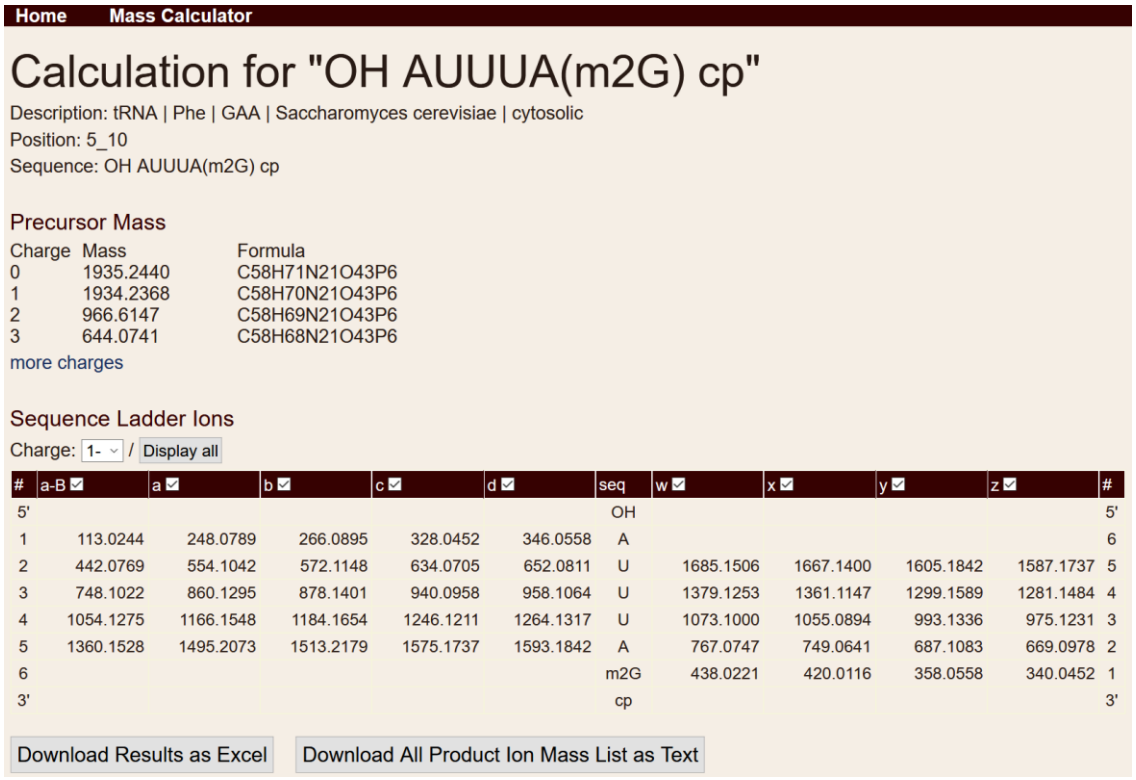


Figure 10. View of the Calculation for (specified sequence) page. The selected modified sequence (Sequence) is displayed in the title of the page.

3.2.1. Precursor mass

This section shows calculated molecular weight (MW) and *m/z* values for multiply charged precursor ions (default setting: up to triply charged ions). To view values of more highly charged ions for Sequences, click more charge link, shown in Figure 11. This will display values up to (*N-1)*-charged ions, where *N* is the length of the nucleotide. Selecting Less Charges link restores the initial view.

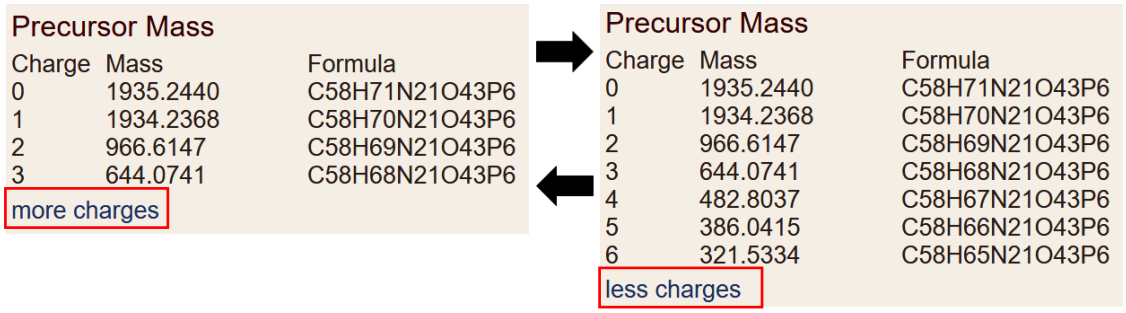


Figure 11. Changing charge upper limit of molecular ions for the display of *m/z* values

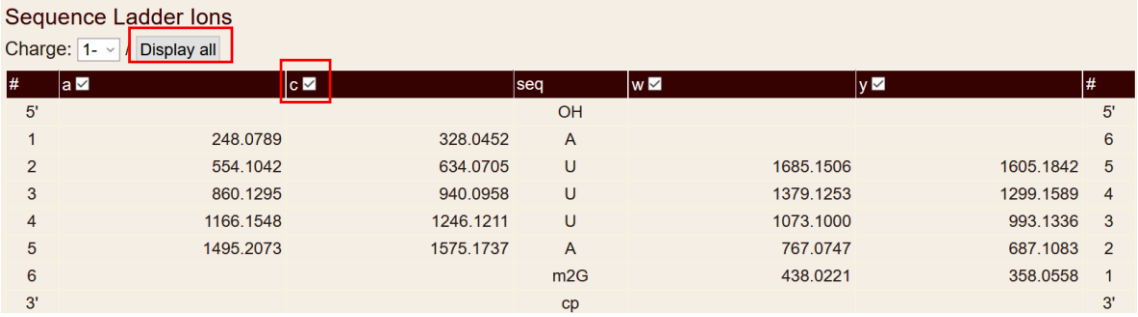
3.2.2. Sequence-ladder ions

Typical single-site backbone dissociations in the gas phase generate what we call sequence-ladder ions in this software. The types of sequence-ladder ions are defined for each Ion Activation Type parameter. At present, 2 ion activation types (See Table 3) are provided as default and these can be specified within the web form (See Section 2.2). The cleavage site for each type of sequence-ladder ions for RNA is shown in Figure 4.

The calculator displays a table for sequence-ladder ions as shown in Figure 10. The initial view only displays singly charged ions for each ion series. To view *m/z* values of multiply charged ions, the upper limit for charge on the ion can be changed to *n* (See Figure 12), and the *m/z* values of ions up to *n*th-charged ions will be displayed in the table.

Figure 12. Changing the upper charge limit to display *m/z* values of multiply charged product ions

If you wish to hide a particular ion series, remove the check mark next to the type symbol (a, b, c and so on) in the table header (See Figure 13) and the column(s) for the corresponding ion series will be hidden. Clicking the Display All button restores the default view.



#	a	c	seq	w	y	#
5'			OH			5'
1	248.0789	328.0452	A			6
2	554.1042	634.0705	U	1685.1506	1605.1842	5
3	860.1295	940.0958	U	1379.1253	1299.1589	4
4	1166.1548	1246.1211	U	1073.1000	993.1336	3
5	1495.2073	1575.1737	A	767.0747	687.1083	2
6			m2G	438.0221	358.0558	1
3'			cp			3'

Figure 13. Selective hiding of a specific ion series

3.3. Downloading of calculated results

The calculation result for a specific sequence shown in Figure 10 can be downloaded in either a Microsoft Excel file or text file.

3.3.1. Results as Excel file

The calculation result for the selected sequence can be saved to your local disk as an Excel format (.xlsx) containing 6 different worksheets as shown in Figure 14.

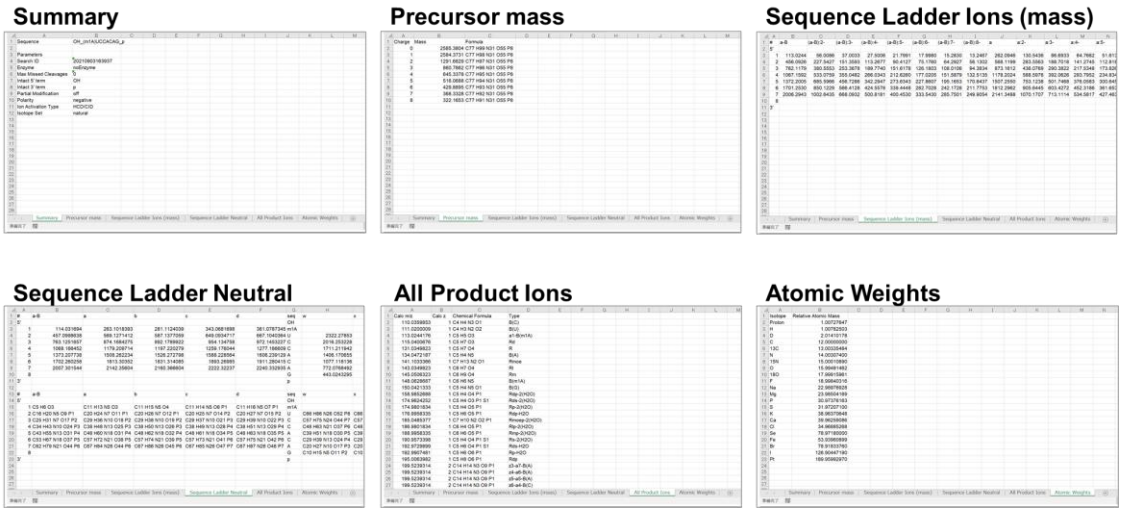


Figure 14. The 6 worksheets of a downloaded Excel file

In Figure 14, 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. The Atomic Weights worksheet contains the relative atomic weights used for the calculation/search. The values are cited from NIST.

To download them, press Download Results as Excel button. A dialog box will appear (Figure 15).

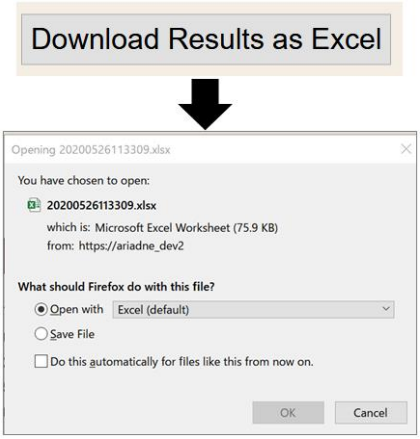


Figure 15. Dialog appeared when clicking the Download Results as Excel format.

If you click on the Download Results as Excel button, all the results shown on the page will be downloaded. In the case of a relatively long sequence (that will generate a relatively large list), the download may take some time.

3.3.2. All product ions mass list as text file

If you would like to download the calculated product ion mass values, you can also download it as a simple text file (.txt). As shown in Figure 16, the file contains calculated *m/z*, *z*, chemical formula, and annotation of every product ion that has calculated by the software, *i. e.* not only sequence-ladder ions but also other frequently detected ions such as internal ions, neutral losses from the molecular ion, and known MS2 fragments.

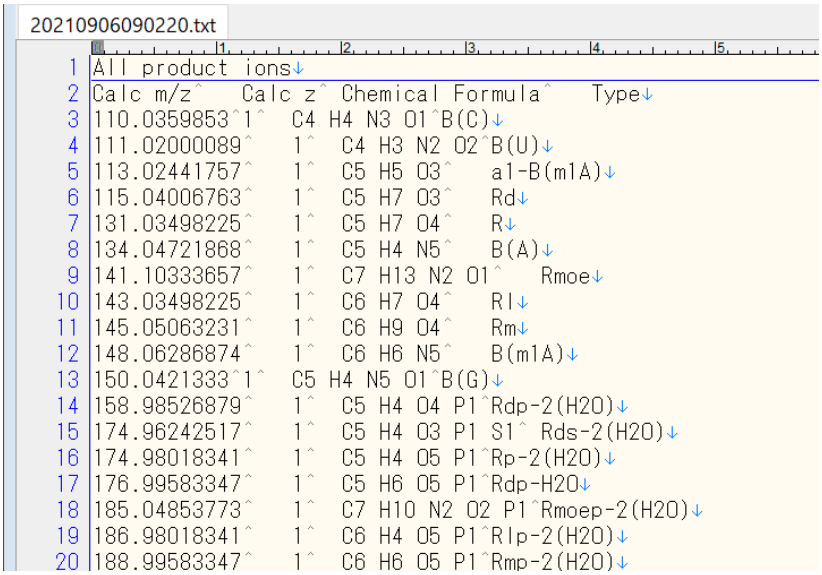


Figure 16. An example of All Product Ions Mass List, which is viewed in a text editor. The "^" marks in the file indicate tabs.

Clicking the Download All Product Ions Mass List as Text in Figure 17, the calculation result of all the product ions can be saved. As there would be a large number of possible product ions to be downloaded in the list, it may take a long time to calculate and be downloaded if you download the result of a long nucleotide.

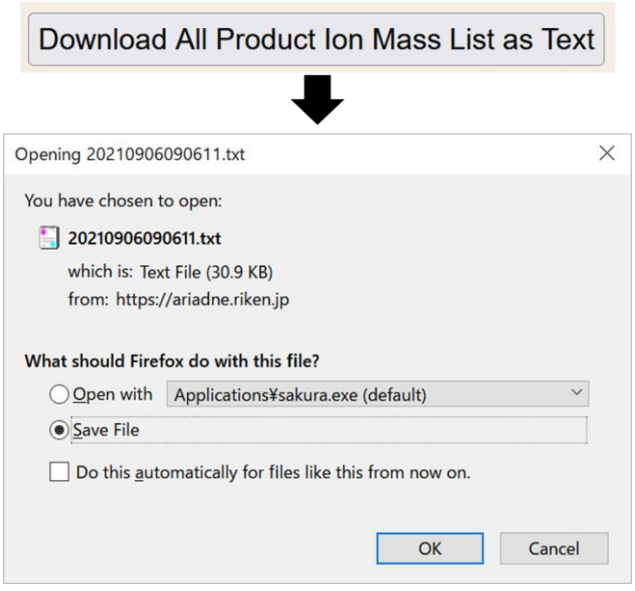


Figure 17. Dialog appeared when clicking the Download All Product Ions Mass List as Text file format