# Lipid annotation with MS2Analyzer

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# Checklist before you start...

You need to have:

- 1.A computer with Java environment and Office(2003 or higher)
- 2.MS/MS spectra in MGF files
- 3.Latest version of MS2Analyzer jar file
- 4.Query text files

For additional validation with lipidblast, download the library and NIST search software in the following link(download the 87 MB full version): <a href="http://fiehnlab.ucdavis.edu/projects/LipidBlast">http://fiehnlab.ucdavis.edu/projects/LipidBlast</a>

# Instruction outline

Step1: Using MS2Analyzer to search the spectra feature
Step2: Using excel filters to annotate lipids
Step3: Confirmation by checking retention time
(optional)

Step 4:Using NIST search for library validation(optional)

1. Double click on MS2Analyzer-ver1. jar to start the program

MS2Analyzer Ver1.0	
Help	
Input MGF/MSP file:	
	Browse
Input query file(txt):	
	Browse
m/z window Intensity threshold	
Output file(.xls):	
	Browse
GO	

2.Click the "Browse" button under the "Input MGF/MSP file" and select the MGF file you want to annotate.

S Open File	
Look In: POS	
Combined_pos_2.mgf	
Combined_pos_3.mgf	
Combined_pos_4.mgf	
pos_1A.mgf	S MS2Analyzer Ver1.0
	Help
File Name:     pos_1A.mgf       Files of Type:     MGF file(*.mgf)	Input MGF/MSP file: d annotation\combined_MGF\POS\pos_1A.mgf Browse
Open Cancel	Input query file(txt): Browse
	m/z window Intensity threshold
	Output file(.xls): Browse

GO

3.Click the "Browse" button under the "Input query file(txt)" and select the query file you want.

Open File      Look In:     pos	
DGDG-pos.txt	
DGTS-pos.txt Lyso-DGTS-pos.txt MGDG-pos.txt	MS2Analyzer Ver1.0
PE-pos.txt         File Name:       MGDG-pos.txt         Files of Type:       Text file(*.txt)         Open       Cancel	Input MGF/MSP file:         d annotation\combined_MGF\POS\pos_1A.mgf         Browse         Input query file(txt):         .ipid annotation\lipids_query\pos\MGDG-pos.txt         Browse
	m/z window Intensity threshold

Output file(.xls):

GO

Browse

4.Type your desired parameters into "m/z window" and "intensity threshold". The first one depends on the mass accuracy of the instrument and the second one depends how noisy the spectra are. Intensity threshold are relative intensity to the base peak, from 0 to 1.0.

🛃 MS2Analyzer Ver1.0	
Help	
Input MGF/MSP file:	
d annotation\combined_MGF\POS\pos_1A.mgf	Browse
Input query file(txt):	
_ipid annotation\lipids_query\pos\MGDG-pos.txt	Browse
m/z window Intensity threshold	
0.005 0.05	
Output file(.xls):	
	Browse
GO	

5.Click the "Browse" button under the "Output files (.xls)" and choose the pathway and name of the output file.

The default name of the output file is same as the name of the MGF files, but you may want to add more information about the lipid class and parameters. Here's an example:

Save File	
Save In: annotation	
	MS2Analyzer Ver1.0
	Help
	Input MGF/MSP file:
	d annotation\combined_MGF\POS\pos_1A.mgf Browse
	Input query file(txt):
File Name: pos_1A_005_05_MGDG xls	.ipid annotation\lipids_query\pos\MGDG-pos.txt Browse
Files of Type: Excel 2003 file(*.xls)	m/z window Intensity threshold
Save Cancel	0.005 0.05
	Output file(.xls):
	of_data\annotation\pos_1A_005_05_MGDG.xls Browse
	GO

6.Click on "GO" button. The program will start searching mass spectra features in the MGF file and generate a Excel 2003 file.

Depending on the speed of the computer, it will take a few minutes or longer. A progress window will show up. When it's done, the progress window will disappear, and you are ready to move to the next Step!

MS2Analyzer Ver1.0	🛃 MS2Analyzer Ver1.0
Help	Help
Inp Progress 🕅	Input MGF/MSP file:
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m/z Cancel	m/z window Intensity threshold
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Output file(.xls):	Output file(.xls):
of_data\annotation\pos_1A_005_05_MGDG.xls Browse	of_data\annotation\pos_1A_005_05_MGDG.xls Browse
GO	GO

#### 1.Open the Excel 2003 files you generated from the last Step.

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19 5h6kMSMSp.d, MS/MS of 371.3161711 0 at 0.110283333333333 mins	0	0	0 0	0	0	0	0		0	0		) 0	0	0			0	0	0 0	0 0	5	0
20 5h6kMSMSp.d, MS/MS of 371.3161711 0 at 0.1132666666666667 mins	0	0	0 0	0	0	0	0	0	0	0	0	) 0	0	0	0	)	0	0	0 0	, 0 0	5	0
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31 5h6kMSMSp.d, MS/MS of 450.3579445 1+ at 0.16468333333333 mins	0	0	0 0	0	0	0	0	0	0	0	(	0 0	0	0	0		0	0	0 0	0	0	0
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39 5h6kMSMSp.d, MS/MS of 436.3415480 1+ at 0.2032 mins	0	0	0 0	0	0	0	0	(	0 0	C	(	) 0	0	0	0		0	0	0 (	) 0	0	0
40 5h6kMSMSp.d, MS/MS of 436.3415480 1+ at 0.2083666666666666 mins	0	0	0 0	0	0	0	0	0	0	C	0	0 0	0	0	0		0	0	0 0	1 0	0	0
41 5h6kMSMSp.d, MS/MS of 416.3737694 1+ at 0.2143666666666666 mins	0	0	0 0	0	0	0	0	0	0	0		0 1	0	0	0		0	0	0 0	0	1	0
42 SHOKMSMSP.U, MS/MS OF 410.3737094 1+ at 0.22035 mins 43 566kMSMSp.d. MS/MS of 237 0834021 1+ at 0.23155 mins	0	0	0 0	0	0	0	0					ט נ ה נ	0	0			0	0			י ר	0
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46 5h6kMSMSp.d, MS/MS of 391.2845829 0 at 0.23901666666666667 mins	0	0	0 0	0	0	0	0	0	0	C	0	) 0	0	0	0	)	0	0	0 0	1 0	0	0
47 5h6kMSMSp.d, MS/MS of 450.3579445 1+ at 0.2418 mins	0	0	0 0	0	0	0	0	0	0 0	C	(	) 0	0	0	0	)	0	0	0 0	) 0	0	0
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3	5h6kMSMSp.d, M	IS/MS of 237.06340	021 0 at 0.04365 mms	3333 mins		0 0	0	0	0		n n	0 0	0 0	0	0			0			0	0	0	0 0		0 0	
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7	5h6kMSMSp.d, M	IS/MS of 450.35794	445 1+ at 0.0567 mins			0 0	0	0	0	0 (	0	0 (	0 0	0	0	0 (	0 0	0 0	(		0	0	0	0 0		0 0	
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11	5h6kMSMSp.d, M	IS/MS of 237.08340	021 1+ at 0.07835 mins			0 0	0	0	0	0 0	D	0 0	0 Ő	Ő	Ő	) Č	0 Č	) Ő	Ċ	1	0	0	0	0 0	)	0 0	
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14	566kMSMSp.d, M	IS/MS OF 391.28438	829 1+ at 0.08611666666 445 1+ at 0.089016666666	666667 mins		0 0	0	0	0		u n		0 0	0	0			J U			0	0	0	0 0		0 0	
16	5h6kMSMSp.d, M	IS/MS of 450.35794	445 1+ at 0.09193333333	333333 mins		0 0	0	0	0	0 0	0	0 0	0 0	0	0		0 0	0 0			0	0	0	0 0		0 0	
17	5h6kMSMSp.d, M	IS/MS of 430.38969	905 1+ at 0.0951 mins			0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
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19	5h6kMSMSp.d, M	IS/MS of 371.3161	711 0 at 0.1102833333333	333 mins		0 (	0	0	0	0 (	0	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
20	5b6kMSMSp.d, M	IS/MS of 371.3161/ IS/MS of 279 16049	711 0 at 0.113266666666 900 1+ at 0 118 mins	667 mins		0 0	n	0	0		J N		0 0	0	0		0 0	) U			0	0	0	0 0		0 0	
22	5h6kMSMSp.d, M	IS/MS of 279.16049	900 1+ at 0.12271666666	66667 mins		0 0	0	0	0	0 0	Ď	0 0	0 0	0	0		0 0	0 0			0	0	0	0 0		0 0	
23	5h6kMSMSp.d, M	IS/MS of 436.34154	480 1+ at 0.12743333333	33333 mins		0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
24	5h6kMSMSp.d, M	IS/MS of 436.34154	480 1+ at 0.13215 mins			0 (	0	0	0	0 (	0	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
25	5h6kMSMSp.d, M	IS/MS of 389.06542	24/ 1+ at 0.13/56666666	66667 mins		0 (	0	0	0	0 0	0	0 0	0 0	0	0			0 0	(		0	0	0	0 0		0 0	
20	5h6kMSMSp.d, M	IS/MS of 237 0834	247 1+ at 0.142505555555 021 1+ at 0.15418333333	3333 mins		0 0	0	0	0	0 0	n	0 0	0 0	0	0		0 0	) 0			0	0	0	0 0		0 0	
28	5h6kMSMSp.d, M	IS/MS of 237.08340	021 1+ at 0.15635 mins			0 (	0	0	0	0 (	0	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0	)	0 0	
29	5h6kMSMSp.d, M	IS/MS of 391.28458	829 1+ at 0.15906666666	66667 mins		0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
30	5h6kMSMSp.d, M	IS/MS of 391.28458	829 1+ at 0.16178333333	33333 mins		0 0	0	0	0	0 0	0	0 0	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
31	5h6kMSMSp.d, M	5/1015 OT 450.35794	440 1+ at 0.16468333333 445 1+ at 0.167566666666	56667 mine		0 0	n	0	0		n	0 0	0 0	0	0			0 0			0	0	0	0 0		0 0	
33	5h6kMSMSp.d. M	IS/MS of 430.38969	905 1+ at 0.170966666666	66667 mins		0 0	0	0	õ	0 0	0	0 0	0 0	0	0		0 0	0 0	(		0	0	0	0 0	)	0 0	
34	5h6kMSMSp.d, M	IS/MS of 430.38969	905 1+ at 0.17436666666	66667 mins		0 0	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0	)	0 0	
35	5h6kMSMSp.d, M	IS/MS of 371.3161	711 1+ at 0.18613333333	33333 mins		0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
36	5h6kMSMSp.d, M	IS/MS of 371.31617	/11 1+ at 0.18885 mins	222 mine		0 0	0	0	0	0 (	U n	0 (	0 0	0	0	) ( ) (	0 0	0 0	0		0	0	0	0 0		0 0	
3/	5b6kMSMSp.d, M	IS/MS of 279.16043	900 0 at 0.193433333333 900 0 at 0 198033333333	333 mine		0 0	n	0	0		u n		0 0	0	0			) U			0	0	0	0 0		0 0	
39	5h6kMSMSp.d, M	IS/MS of 436.34154	480 1+ at 0.2032 mins			0 0	0	0	0	0 0	Ď	0 0	0 0	0	0	0 0	0 0	0 0	(		0	0	Ő	0 0		0 0	
40	5h6kMSMSp.d, M	IS/MS of 436.34154	480 1+ at 0.20836666666	66667 mins		0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
41	5h6kMSMSp.d, M	IS/MS of 416.3737	694 1+ at 0.21436666666	66667 mins		0 (	0	0	0	0 (	0	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
42	5h6kMSMSp.d, M	IS/MIS of 416.37370	694 1+ at 0.22035 mins			0 0	0	0	0	0 0	) N	0 0	0 0	0	0		0 0	0 1			0	0	0	0 0		0 0	
43	5h6kMSMSp.d. M	IS/MS of 237.08340	021 1+ at 0.23371666666	66667 mins		0 0	0	0	0	0 0	0	0 0	0 0	0	0		0 0	, U ) O			0	0	0	0 0	1	0 0	
45	5h6kMSMSp.d, M	IS/MS of 391.28458	829 0 at 0.236366666666	667 mins		0 0	0	0	0	0 0	- D	0 0	0 0	Ő	0	0 0	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
46	5h6kMSMSp.d, M	IS/MS of 391.28458	829 0 at 0.239016666666	667 mins		0 (	0	0	0	0 (	D	0 (	0 0	0	0	) (	0 0	0 0	(	1	0	0	0	0 0	)	0 0	
47	5h6kMSMSp.d, M	IS/MS of 450.35794	445 1+ at 0.2418 mins			0 0	0	0	0	0 (	0	0 (	0 0	0	0	) (	0 0	0 0	(		0	0	0	0 0		0 0	
14	ADDRMSMSn.d.M ADDRMSMSn.d.M Sheet1	2 of 450 35794	445 1+ at 0 7445666666	hbb/ mins		0 (								0					(			u				·	
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3. Filter out the 0 values in the head group(has the same name as lipid class, in this case, MGDG )column, only leave the 1 value there.

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3 5h6kMSMSp.d, MS/MS of 436.3415480 1+	OK Cancel	0	0 0	)	0 0		0 0	0	0 0	0 0	0	0	
4 DIDKM SM SD. 0, M S/M S OF 436.3410480 1+		- U	0 (	)	0 0		0 0	0	0 0	0 0	0	0	
6 5h6kMSMSp.d. MS/MS of 389.0654247 1+ at 0.142983333333333	3 mins	0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	0	
7 5h6kMSMSp.d, MS/MS of 237.0834021 1+ at 0.1541833333333	3 mins	0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	0	
8 5h6kMSMSp.d, MS/MS of 237.0834021 1+ at 0.15635 mins		0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	0	
9 5h6kMSMSp.d, MS/MS of 391.2845829 1+ at 0.159066666666666	7 mins	0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	0	
0 5h6kMSMSp.d, MS/MS of 391.2845829 1+ at 0.16178333333333	3 mins	0 0	0 0	)	0 0		0 0	0	0 0	0 0	0	0	
1 SN6KMSMSD.d, MS/MS of 450.35/9445 1+ at 0.164683333333333 2 556FMSMSD.d, MS/MS of 450.3579445 1+ at 0.46756666666666	3 mins 7 mins	0 0	0 0	)	0 0		0 0	0	0 0	0 0	0	0	
a shokmismispid, mis/mis/or 430.3379443 1+ at 0.167366666666666666666666666666666666666	7 mins	0 0	0 0	)	0 0		0 0	0	0 0	0 0	0	0	
4 5h6kMSMSp.d, MS/MS of 430.3896905 1+ at 0.174366666666666	7 mins	0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	ŏ	
5 5h6kMSMSp.d, MS/MS of 371.3161711 1+ at 0.18613333333333	3 mins	0 0	0 0	)	0 0	)	0 0	0	0 0	0 0	0	0	

4.Click on each filter under the name of lipids(in red), and check if there's 1 value there. If yes, filter out the 0 and show only the 1 value.

•		M1	<b>-</b>	$f_{\infty}$	MGDG34:7[M+NH4]+																
	- 24				А		В	С	D	E	F	G	Н			J	K	L	М	N	C
																			MGDG34	MGDG32	MGD
																			:7[M+NH	:0[M+NH	:1[M+
_	1						MGDG	16:0+179	16:1+179	16:2+179	16:3+179	16:4+179	18:0+179	18:1+179	18:2	+179	18:3+179	18:4+179	4/+	4]+	4]+
	2	Title				T	1/9.0/93 60 T	433.3193	433.3039	431.2002	429.2120	421.2069	463.3008	461.333Z	439.	5195	401.3039	400.2002	052	148.3938	146.0
-	90	5h6kMSMSp (	MS/MS of	279 1546	631 1+ at 0 42085 mins		1	002 0	51 0		0	034	) 040	JJ4		urt Sma	JI Ilect to Large	oro	JJL	412 0	32
	221	5h6kMSMSp.c	I. MS/MS of	504.3187	7866 0 at 0.85913333333	3333 mins	1	0	0	0	0		0 0		* 2 1 c	nt Sine	inest to cargo			0	j –
1	075	5h6kMSMSp.c	I, MS/MS of	762.5173	3035 1+ at 3.6592833333	3333 mins	1	0	0	0	0		î o		1 2	ort Larg	jest to Small	est		0	j l
1	147	5h6kMSMSp.c	d, MS/MS of	764.5323	3079 0 at 3.82168333333	333 mins	1	0	0	0	1		î o	)	S	or <u>t</u> by (	Color			0	)
1	149	5h6kMSMSp.c	I, MS/MS of	765.5352	2173 1+ at 3.8235166666	6667 mins	1	0	0	1	1	(	) ()	•	K 🖸	ear Filt	ter From "762	.5155952"		0	)
_1	150	5h6kMSMSp.o	I, MS/MS of	765.5352	2173 1+ at 3.8244166666	6667 mins	1	0	0	0	1	(	0 0		Fi	lter by	Color			0	J
1	197	5h6kMSMSp.c	I, MS/MS of	764.5323	3079 1+ at 3.942 mins		1	0	0	0	1				N	umbar	Filters			0	<u> </u>
1	237	SHERMSMSD.C	I, MS/MS of	766 5473	3079 1+ at 4.0336666666	6667 mins	1	0	0						14	uniber	Lucers			0	
-	397	566kMSMSp.0	I, MS/MS OF	766 5473	2429 U al 4.12010333333	SSS mins	1	۰ ۱	0		0		ט רייס		S	earch			Q	0	,
1	427	5h6kMSMSp.	I MS/MS of	768 5623	3474 1+ at 4 4836833333	3333 mins	1	۰ ۱	1	1		( 	י ער ו				Select All)			0	ý
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5. Check the 1 values in the acyl chain loss columns(16:0 to 18:4 in this case) and see if the sum of them matches the number in lipids name. If the sum matches, this is a correct hit!

Example 1: 16:4+18:3=34:7 which means this lipid is

MGDG 34:7(16:4/18:3) [M+NH4]+

	A	В	С	D	E	F	G	Н		J	K	L	M	N	0
													MGDG34	MGDG32	MGDG32
													:7[M+NH	:0[M+NH	:1[M+NH
1		MGDG	16:0+179	16:1+179	16:2+179	16:3+179	16:4+179	18:0+179	18:1+179	18:2+179	18:3+179	18:4+179	4]+	4]+	4]+
		179.0793	435.3195	433.3039	431.2882	429.2726	427.2569	463.3508	461.3352	459.3195	457.3039	455.2882	762.5155	748.5938	746.5781
2	Title	× 69	T 862 T	37 💌	878 💌	386 💌	894 💌	846 💌	354 💌	862 💌	37 💌	878 💌	952 🖵	412 💌	92 💌
1075	5h6kMSMSp.d, MS/MS of 762.5173035 1+ at 3.65928333333333 mins		1 (	) (	) (	0	1	0	0	) (	) 1	<b>)</b> (	1	0	0
4970															

Example 2: Sometimes there's only one acyl chain loss found, but maybe that's also a potential hit. In this case, the lipid name is written as MGDG 34:7(16:4/18:3) [M+NH4]+

While 16:4 is found by program, and 18:3 is back calculated: 34:7-16:4=18:3

	A	В	С	D	E	F	G	Н		J	K	L	М	N
													MGDG34	MGDG32
													:7[M+NH	:0[M+NH
1		MGDG	16:0+179	16:1+179	16:2+179	16:3+179	16:4+179	18:0+179	18:1+179	18:2+179	18:3+179	18:4+179	4]+	4]+
	_	179.0793	435.3195	433.3039	431.2882	429.2726	427.2569	463.3508	461.3352	459.3195	457.3039	455.2882	762.5155	748.5938
2	Title	69 🚽	862 💌	37 🔹	878 💌	386 💌	894 💌	846 💌	354 💌	862 🔻	37 💌	878 💌	952 🔻	412 💌
1075	5h6kMSMSp.d, MS/MS of 762.5173035 1+ at 3.659283333333333 mins		r o	) 0	0	0	1	0	0	0		0	1	0
4970														

5. Example 3:Sometimes,there are multiple possibilities...

First spectra:

<u>MGDG 34:5(16:2/18:3) [M+NH4]+</u>

Second spectra:

16:2+18:3=34:5

16:4+18:1=34:5

#### MGDG 34:5(16:4/18:1) [M+NH4]+

	A	В	С	D	E	F	G	Н		J	K	L	М	N
													MGDG34	MGDG34
													:5[M+NH	:7[M+NH
1		MGDG	16:0+179	16:1+179	16:2+179	16:3+179	16:4+179	18:0+179	18:1+179	18:2+179	18:3+179	18:4+179	4]+	4]+
		179.0793	435.3195	433.3039	431.2882	429.2726	427.2569	463.3508	461.3352	459.3195	457.3039	455.2882	766.5468	762.5155
2	Title	- 69 -	r 862 🔹	37 💌	878 💌	386 💌	894 💌	846 💌	354 💌	862 💌	37 💌	878 💌	936 🖵	952 💌
1277	5h6kMSMSp.d, MS/MS of 766.5473429 0 at 4.128183333333333 mins		1 (	) (	) 1	1 1	0	0	C	) (	1	1	1	0
1387	5h6kMSMSp.d, MS/MS of 766.5473429 1+ at 4.38465 mins		1 (	) (	) (	) 0	1	0	1	0	0	0	1	0
4970														
4971														

Both are correct and should be reported separately.

5. (Continue) After you are done with one lipid precursor m/z , clear the filter by checking the 0 value under its filter again, and move to the next precursor m/z.

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1					DGDG	16:0+NI	16-1+NI	16·2+NI	16·3+NI	16·4+NI	18-0+NI	18·1+NI	18·2+NI	18·3+NI	18·4+NI	2[IVI+INF14 ]+	3[IVI+INF14 ]+	4[IVI+INTI4 ]+	0[M+NH4 1 1+ 1-
					341.1321	597.3724	595.3567	593.3411	591.3254	589.3098	625.4037	623.3880	621.3724	619.3567	617.3411	934.6466	932.6310	930.6153	910.6466 9
2	Title			-	9 "Т	072 💌	58 💌	088 -	596	104 💌	056	564 💌	072	58	r 088 🔻	622 💌	13 🐨	638 💌	622 💌 1
1437	5h6kMSMSp.d, M	IS/MS of 932.631347	7 1+ at 4.5070666666	66667 mins	1	1	0	1		0 0	) (	0 1		<mark>≩↓ <u>S</u>ort Sn</mark>	nallest to Larg	est		0	0
1477	5h6kMSMSp.d, M	IS/MS of 932.631347	7 1+ at 4.598 mins		1	1 1	0	(	)	0 0	) (	0 0	1	Z↓ Sort La	rgest to Small	est		0	0
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#### 6. Write the result into a Excel file.

The following is an example of the report:

Report: Title, m/z(with 3 decimals, e.g. 766.547), RT(retention time, with 2 decimals, e.g. 4.13), Name of lipid, and Adduct

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5	5h6kMSMSp.d, M	S/MS of 766.5	473429 1+	at 4.384	65 mins			766.547		4.38	MGDG 34:5(16:	4/18:1)	[M+N	H4]+		- 1
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TIPS:

MGDG, DGDG, DGTS, PE all have head groups and two acyl chains (same as the example).

Lyso-DGTS only has head group and one acyl chain, in this case, the acyl chain loss must be found in order to report.

DG and TG don't have head groups. Therefore,

DG: only look for the two acyl chain loss(both must be found)

TG: only look for the three acyl chain loss(at least two of them must be found)

### Step3: Confirmation by checking retention time

If a RP column is used for the separation, among lipids in each lipid class, the retention times usually follow the following rule:

Retention times increase with the number of carbon, while decrease with the number of double bond.

This can be used as a further confirmation of the annotation.

Step3: Confirmation by checking retention time

For example RT MGDG 34:0< RT MGDG 36:0 RT DGDG 32:2 > RT DGDG 32:3 Quiz: RT PE 34:1 ? RT PE 32:4 RT TG 50:4? RT DG 36:4

Now you have a list of potential lipid with its title and annotation, you can visualize the spectra in NIST search and search against available libraries to see if the annotation results match.

Here we use lipidblast library as an example.

# 1. Extract the LipidBlast-Full.zip file, and double click on the nistms.exe file in the LipidBlast-MSSearch folder.

When you open Nist search for the first time, it will give you a warning message. After clicking OK, a window pops up to ask you to select library directory. Here we just keep it highlighting LipidBlast-MSSearch and click OK, then the software will open.



Browse for Folder	X
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	\mu LipidBlast-Examples
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	OK Cancel

2. Go to the import button, and select the MGF file where you have your lipids in.

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	6	L	ABI-API 4000	QTrap; [M-H]-; GM	12(d18:1/C18:	combin	ed_pos_4.mgf		10/22/2013 3:28 PM	MGF File	98,017 KB		
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Click on "Import All" and in the next window, choose Overwrite or Prepend. It'll take some time to load all the spectra.





Importing spectra	×
5h6kMSMSp.d, MS/MS of 253.0830493 1+ at 11.50043333333	
18%	

3. When the importing is done, go to the Library Search Options button, and set appropriate parameters.

For details, please refer to the user manual of lipidblast.

Library Search Options	Library Search Options
Search MS/MS Libraries Automation Limits Constraints MS/MS Search m/z Tolerance	Search       MS/MS       Libraries       Automation       Limits       Constraints         Available Libs:       Included Libs:       Included Libs:       ★ ★         Custompc+hpos.msp custompc+napos.msp lipidblast.neg       Included Libs:       ★ ★       ↓         Included Libs:       Decormsp       Included Libs:       ★       ↓
Product ions ± 0.4	pc-ac-neg.msp pc-form-neg.msp
	234420 Spectra in 6 Libraries 234420 Spectra in 6 Libraries Spectrum search
OK Cancel Help	OK Cancel Help

# 4. Scroll down in the upper-left window and find your potential lipids by its title(mainly RT, since RT is the increasing order).

Click on its title to show it spectra in the upper-right window.



Double click on the title to search against libraries. Sometimes there's no good match in lipidblast because the specific lipid is not covered in lipidblast.



If the library search result match the MS2Analyzer result, then the annotation is further confirmed. Here's an example of good match.

Title	m/z	RT(min)	Name	Adduct
5h6kMSMSp.d, MS/MS of 586.5369873 1+ at 6.739 mins	586.537	6.74	DG 32:0(16:0/16:0)	[M+NH4]+
名 NIST MS Search 20 - [Peptide, Presearch Default - 17 spectra] D File Search View Tools Qutions Window Help				_ 8 ×
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Marca X         Statutes         Spec List           poldblast oss pc-ac-reg map: pc-fom-reg map: custompc-rhops map: lpidblast-reg: 234221 total spectra         0L           100         500         800         700         600         500         400         300         200         100           1         100         500         800         700         600         500         400         300         200         100         6           1         100         500         800         700         600         500         400         300         200         100         6           1         100         515         158         158         168         160         100         50           2         1         76         144         054         657         105 320; [M-NH44; DG140/16/0/10         50         50         50         50         50         50         50         50         100         50         100         50	16.964530397103 100 520 300 400 500 9.064MSSp.d. MS/MS of 506 5508731 + 45 116 9946530397103 116 9946530397103 40 80 120 160 200 240 2 566/MSMSp.d. MS/MS of 595 53693731 + 26 573 116 9946530397103 116 9946530397103 116 9946530397103 565 152 313 27410 551 52	13 274506757565 812 480265725655 130 min 780 min 13 272450675758 333 296031265086 313 272410 130 320 360 400 440 480 9 min 1 Head to Tail 743	×         III           551 4980085796966         713, 1888 11197751, 812,480265725655           551 52743         551 52743           5520 550 660         640 680 720 760 800 840           MF-319 RMF-485         I/¥         DG 32/0; [M+H4]+           State Desk         I/¥         DG 32/0; [M+H4]+           Nmmax DG 32/0; [M+H4]+         DG 32/0; [M+H4]+         I/¥           State Desk         State J 285 308; [M+H4]+         State J 285 300; [M+H4]+           State Desk         State J 285 300; [M+H4]+         I/¥           State Desk         State J 285 300; [M+H4]+         State J 285 300; [M+H4]+           State Desk         State J 285 300; [M+H4]+         I/¥           State De	, 980 426871641491 880 920 950 1000 DC(16:0/15:0/03) 319 4988 94.8P
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# That's it!

- Thanks for using MS2Analyzer.
- Feel free to email <u>yanma@ucdavis.edu</u> if you have any question and comment.