pTop User Guide

Version 2.0



pFind.ict.ac.cn

Copyright: ©. The pFind Group, Institute of Computing Technology, CAS, Beijing, China Last modified: 2017-07-03

Table of Contents

1	Inst	allatio	on		3
	1.1		Inst	allation requirements	3
	1.2		Inst	allation steps	3
2	Acti	vatio	n		6
3	Usa	ge			7
	3.1		Star	tup GUI	7
	3.2		Sett	ing common parameters	8
		3.2.3	1	Spectra	8
		3.2.2	2	Search Parameters	9
		3.2.3	3	Quantitation Parameters	13
	3.3		Run	рТор	16
	3.4		Res	ults	
4	Con	tact i	nforr	nation	20

1 Installation

1.1 Installation requirements

Hardware requirements

• GB or higher recommended memory

Software requirements

- Windows 7 or above
- microsoft .NET Framework 4.5 or above
- MSFileReader 2.2 (From Thermo Scientific) or higher version

1.2 Installation steps

The Windows setup package of pTop 2.0 can be downloaded from the website <u>http://pfi</u>nd.ict.ac.cn/download/pTop/pTop2.0 x64.exe.

The pTop setup package includes not only pTop, but also pXtract, pParseTD, pConfig, pLabel and pBuild. pXtract creates MS1 and MS2 input files directly from Thermo Scientific RAW LC-MS/MS data files. pParseTD converts the MS1 and MS2 files to MGF files, in which detecting the relative accurate mono mass of the precursors and deconvoluting and deisotoping the MS/MS. pConfig is a tool that can add or change the basic configurations, such as amino acids, modifications. pLabel is a spectra labeling tool that can visualize the global- and local-view proteoform-spectrum matches, given the results of pTop or any other search engines. pLabel can label both CID and ETD spectra, and implement the manual de novo sequencing. pBuild is a new tool for visualization of proteoform-spectrum match (PrSM).

To install pTop on windows, the following simple steps are needed.

Step 1: Select the installer language (**Figure 1**). Now it only supports English and Chinese (Simplified).

Installer Language									
5	Please select a language.								
	English 💌								
	OK Cancel								

Figure 1. Installer language

Step2: Click Next to start the setup (Figure 2).



Figure 2. Welcome to the setup wizard

Step 3: Choose the install Location (Figure 3). And D drive disk is recommended.

🛐 pTop 2.0 Setup	
Choose Install Location	
Choose the folder in which to install pTop	2.0.
Setup will install pTop 2.0 in the following f and select another folder. Click Install to s	folder. To install in a different folder, click Browse tart the installation.
Destination Folder	
C:\Program Files\pTop	Browse
Space required: 73.9MB	
Space available: 6.6GB	
рТор	
	< Back Install Cancel

Figure 3. Choose install location

Step 4: Just wait a few seconds, the Installation will be finished (Figure 4).

🗾 p	oTop 2.0 Setup	
In	stalling	57
-	riease wait while pilop 2.0 is being installed.	
E	Extract: OxyPlot.dll 100%	
	Extract: MigraDoc.DocumentObjectModel.dll 100%	^
	Extract: MigraDoc.Rendering.dll 100%	
	Extract: MigraDoc.RtfRendering.dll 100%	
	Extract: NL.INI 100%	
	Extract: OxyPlot.Pdf.dll 100%	
	Extract: OxyPlot.WindowsForms.dll 100%	
	Extract: OxyPlot.Wpf.dll 100%	=
	Extract: OxyPlot.Xps.dll 100%	
	Extract: OxyPlot.dll 100%	*
рТор	p	
	< Back	Next > Cancel

Figure 4. Installing

Finally, you can check the box of run pTop and then click Finish to start pTop (Figure 5).



Figure 5. Installation finished

2 Activation

All users are required to go through a software activation process in order to use pTop 2.0. A license wizard will appear to guide users through the activation process the first time pTop 2.0 is launched.

🗾 License Dialog	X						
User Name:							
Institute/Company Name							
Country:	China 🔹						
Email Address:							
Activation Code:	7F0400CEC9C2AE9FDAE822BFE8BE6/						
Send Email	Copy to Clipboard						
The email will be sent to ptop@ict.ac.cn for manual review.							

Figure 6. license wizard

Please carefully fill in the user information required to get the license file on the computer that will be running pTop2.0 (**Figure 6**). Your information will be useful for developers and will be strictly confidential. Thank you.

If you've already installed Microsoft Outlook, and the email address you just filled in is registered in your Outlook, then just click "*Send Email*"; Otherwise, click "*Copy to Clipboard*", then your information is copied to the clipboard, what you need to do is paste the registration information into the body of your email and send it to ptop@ict.ac.cn.



Figure 7. operation tips

Then you will get the license file. Put the license file *pTop.license* in the bin directory of your

installation directory, and you will be able to launch pTop 2.0 successfully.

Important

Once the computer hardware upgraded, the license file also need to be updated.

3 Usage

3.1 Startup GUI

Double click the icon **P**, then pTop will start up. You will see the main dialog window of pTop (**Figure 8**). The first time you start up pTop 2.0, you need to set the thread number as well as the default working directory where the tasks are stored.

🖸 рТор		-				
File Options Help						
MS Data	Identification	Quantitation	Summary			
MS Data Format : MS Instrument : Data File List	Files		Soe		S Settings Lagrand Company	
				Add Delete Clear	Default Date Reportiony Ratio: Dirigit ophilonkipane Basedon Available Space on Drive D : 27.4 S DK Careet	
 Data Extraction 						
Precursors Detection						
Instation Width -		= Marco foreitor				
tablation width :		Minime specia				
Deconvolution						
Maximum Charge	e:	M/Z Tolerance :	ppm			
Maximum Mass :		S/N Ratio :				
Place of Decimal						
M/Z :	-	Intensity :	•			
B Output Save Report						
Ready						

Figure 8. Main dialog window of pTop

Every time you start up pTop 2.0, you are creating a new task, and you need to name the task and

select its storage path (**Figure 9**). You can also open an existing task by click is a specific folder including a "task_name.tsk" file as well as a "param" folder, maybe also some results files (**Figure 10**).

🛐 NewTask	and the second s	
Name	pTopTask20170703071539	
Location	D:\pTopWorkspace\	Browse
	ОК	Cancel

Figure 9. Create a new task

2 打开									
😋 🕞 マ 📕 ▸ 计算机	搜索 testCommandL	索 testCommandLine							
组织 ▼ 新建文件夹							0		
☆ 收藏夹	名称	修改日期	类型	大小					
🚺 下载	2DLC_H4_CIDFT	2017/7/3 2:12	文件夹						
三 桌面	퉬 param	2017/7/3 2:06	文件夹						
📃 最近访问的位置	testCommandLine.tsk	2017/7/3 2:06	TSK 文件	1 KB]				
■ 果叫									
Subversion									
🛃 视频									
■ 图片									
🖹 文档									
👌 音乐									
🚺 рТор									
🖳 计算机									
📬 网络 🔻	-								
文件	培(N): testCommandLine.tsk			-	pTop task files (*.tsk)		•		
					打开(0)	取消			

Figure 10. Open an existing task

3.2 Setting common parameters

The common parameters are listed in the 'MS Data' panel and the 'Identification' panel. How to set the common parameters will be detailed introduced as follows.

3.2.1 Spectra

The important parameters of the input spectra data are 'MS Data Format', 'MS Instrument' and 'Data File List'. (Figure 11)

MS Data Format

Following formats are supported by pTop: RAW and MGF.

MS Instrument

Instrument determines which fragment ion series will be used for scoring. Now HCD, CID, ETD, EThcD, ETciD and UVPD are supported.

Data File List

Click "Add" to put the paths of input files in the list, the path or folder containing the tandem mass spectra.

pT pTop - pTopTask_H4(D	:\pTopWorkspace\pTopTa	sk_H4\)		B 10 10	(5 x	
File Options Help						
MS Data	Identification	Quantitation	Summa	ary		
MS Data Format : MS Instrument : Data File List <u>J:\pTop\Data\HumanF</u>	RAW		Size 261.964MB	Add Delete		
•			Þ			
1 File(s), 262.144 MB						
Data Extraction						
Isolation Width :	15	Mixture Spectra			-	
	15	mixture opectio				
Deconvolution					-	
Maximum Charge :	30	M/Z Tolerance :	20 ppm			
Maximum Mass :	50000	S/N Ratio :	1.5			
Place of Decimal					-	
M/Z :	5 🔹	Intensity :	1 •			
Save Report						
Ready						

Figure 11. MS Data panel

3.2.2 Search Parameters

For the first time you use a database, you should click 'Customize Database...' (Figure 12) to add and open the FASTA file (Figure 13). Then the database you choose will appear in the select box of database, and it will be directed chose in your subsequent search.

🗊 pTop - pTopTask_H4(D:\pTopWorkspace\pTop	Task_H4\)	-	D. D. Barre						
File <u>O</u> ptions Help									
: 🗈 🗁 🔜 🕨 🔳					7				
MS Data Identification	Quantitation	Summary							
Database Search	_								
Database : Customize Database Precursor Tolerance : ± 5.2 Da	 Fragment Tolerance : 	± 15 ppm -							
Max Truncated Mass : 20000 Da	Search Mode :	Tag-Index 🔻	Second Search						
Max PTM Positions : 3 🗢	Max Mod. Mass :	500 Da	Unexpected PTMs:	1 •					
Add Modification									
Fixed	Acetyl[AnyN-tc Acetyl[K] Amidated[Arpu Amidated[Prot Ammonia-loss Biotin[AnyN-te Biotin[K] C+12[AnyN-te Carbamidomet	erm] C-term] (AnyN-termC] erm] try[C]	< H						
Dimethyll(K] Methyl(K] Methyl(R) Trimethyl(K)	Garbamyl[Any] Carbamyl[K] Carboxymethy Cation_Na[Any Cation_Na[D] Cation_Na[D] Cation_VI[K]	N-term] I[C] rC-term]	Ŧ						
	Display Al	II Edit]						
□ Display All Edit ○ Display All Edit ► Result Filter FDR ≤ 1 % Separate Filtering ■ Output									



tabase	es	
Name	Path	
	🔾 Database Info	rmation
	Name:	human_histones
	Path:	E:\workspace\Data\database\human_histones.fasta
		Add contaminant
	Note: Please	input a target-only database. pTop will generate the target-decoy database automatically.
		OK
L		
L		

Figure 13. Add a new database

pTop 2.0 support identification of truncated proteins, thus "Max Truncated Mass" of the N/C terminal of the protein can be configured. pTop 2.0 supports search with fixed/variable modifications as well as one unexpected modifications. Fixed modifications are applied universally, to every instance of the specified residues or terminus. Variable modifications are those which may or may not be present. Unexpected modifications can be set as 0 or 1, and once the unexpected PTMs set as 2, the search may take a much longer time. The left or right arrows mean to add or delete the fixed or variable modifications to the fixed and variable boxes. And you can choose the 'Max Modify Position' to set the maximum variable modifications allowed on each protein in the search. (**Figure 14**)

The modifications on the right side are those common ones. You can check the box of 'display all' to show all the modifications in the modification.ini file. If you still cannot find the modifications you have to add, please click 'Edit...' to add your modifications.

🗊 pTop - pTopTask_H4(D:\pTopWorkspace\pTopTask_H4\)	
File <u>O</u> ptions Help	
	-
MS Data Identification Quantitation Summary	
• Database Search	
Database : 🔹 🔹	
Precursor Tolerance : ± 5.2 Da v Fragment Tolerance : ± 15 ppm v	
Max Truncated Mass : 20000 Da Search Mode : Tag-Index • Second Search	
Max PTM Positions : 3 🗢 Max Mod. Mass : 500 Da Unexpected PTMs:	1
Add Modification	
Fixed Acetyl[AnyN-term] Fixed Acetyl[AnyN-term] Acetyl[ProteinN-term] Amidate[AnyN-term] Biotin[AnyN-term] Biotin[K] Carbampl[AnyN-term] Carbampl[AnyN-term] Dimethyl[K] Carbampl[AnyN-term] Methyl[K] Carbampl[AnyN-term] Carbampl[AnyN-term] Carbampl[AnyN-term] Carbampl[AnyN-term]	
Display All	
FDR ≤ 1 % Ø Separate Filtering	
Output	
Save Report	
Ready	

Figure 14. Select modifications

To add a modification, you have to type in the name, choose its composition and then the mono mass will be calculated automated. You also have to choose the positions that it might occur. And then type in the neutral loss of the modification if it have, and do nothing if not. (错误!未找到引 用源。)

If you choose the 'Common' box, the modification you add will appear in the modification list even if the 'Display All' box is not checked.

51		(6 d		57		
Modifications			Modification I	nformation				
Search			Name:					
Name	Mass	Compo	Composition:			Edit	n	
2-dimethylsuccinyl[C]	144.042	H(8)C(6						
2-monomethylsuccinyl[C]	130.026	H(6)C(Marrie					C
2-nitrobenzyl[Y]	135.032	H(5)C(IVIass:					
2-succinyl[C]	116.010	H(4)C(4						
2HPG[R]	282.052	H(10)C	Position:	Anywhere	-			
3-deoxyglucosone[R]	144.042	H(8)C(
3-phosphoglyceryl[K]	167.982	H(5)C(
3sulfo[AnyN-term]	183.983	H(4)C(Sites:					
4-ONE+Delta_H(-2)O(-1)[C]	136.088	H(12)C						
4-ONE+Delta_H(-2)O(-1)[H]	136.088	H(12)C	Neutral Loss					
4-ONE+Delta_H(-2)O(-1)[K]	136.088	H(12)C	Neutral Loss.					
4-ONE[C]	154.099	H(14)C						
4-ONE[H]	154.099	H(14)C	Is Common:	🗹 Common				
4-ONE[K]	154.099	H(14)C						
4AcAllylGal[C]	372.142	H(24)C						
ADP-Ribosyl[C]	541.061	H(21)C		Apply				
ADP-Ribosyl[D]	541.061	H(21)C						
ADP-Ribosyl[E]	541.061	H(21)C			-	- 0150		
ADP-Ribosyl[K]	541.061	H(21)C(15)N(5)O(13)P(2)	NORMAL	К	False		
ADP-Ribosyl[N]	541.061	H(21)C(15)N(5)O(13)P(2)	NORMAL	N	False		
ADR-Ribosyl(R)	5/11 061	H(21)C(15\N/5\O/12\D/2_I		R	Falca		

Figure 15. Add a custom modification

Parameter	Description
Database	Protein sequence database to be searched, required
Precursor Tolerance	Error tolerance for precursor mass in Dalton. The default value is 5.2 Da.
Fragment Tolerance	Error tolerance for fragment ions in ppm. The default value is 15.
Max Truncated Mass	Max mass allowed to be truncated on the N/C terminus. The default value is 20000.
Search Mode	The two search modes in pTop 2.0 are tag-index mode and ion-index mode. Tag-index mode gets candidate proteins through tag-index, while ion-index mode acquire candidate proteins through ion-index. When ion-index mode is used, the precursor tolerance can be set as the most, e.g. 50 000.
Second Search	Once tag-index mode is selected, a second search switch could be turned on. Second search flow use ion-index to search those spectra missed by tag-index, which may take a little longer time.
Max PTM Positions	The maximum modification sites (including variable and unexpected) allowed on each protein. The default value is 3.
Max Mod. Mass	Maximum absolute value of the mass shift (in Dalton) of an modification. Default value: 500.
Unexpected PTMs	Maximum number of unexpected modifications in a proteoform. Default value: 0.
Fixed Mods.	Fixed modifications which are certain to happen on the proteins.
Variable Mods.	Variable modifications which may happen on some proteins.
FDR	The threshold of false discovery rate (FDR). The default value is 0.01.
Separate Filtering	Whether to calculate FDR and filter the search results for each input file individually. If the switch is turned off, the search results of all the input files will be merged and then estimate FDR and filter out the results above the FDR threshold.

Table 1 Parameters in Identification Tab.

3.2.3 Quantitation Parameters

If the sample data are labeled and can be quantified based on the MS spectra, you can choose "Labeling" to do quantitation analysis (Figure 16). You can set light label and heavy label. If there are three labels, you can select "Multiplicity" as 3, and set "Light Label", "Medium Label", and "Heavy Label" (Figure 17).

To edit labels information, you can click



to open the labels information panel. To add a label, you have to type in the label name, choose the amino acids or modifications it labels, as well as the label element and the element to be replaced (Figure 18).

🗊 pTop - pTopTask_H4(D:\pTopWorkspace\pTopTask_H4\)	
File Options Help	
	-
MS Data Identification Quantitation Summary	
Type : Labeling	
Multiplicity: 2	
Light Label : none Light Label : Name Va Dimethyl_Labeling M:Dimethyl[ProteinN-term][H,2H]M:C SILAC-Arg10Lys8 R:K[N,15N]R:K[C,13C]R:R[N,15N]R:R[C	
Heavy Label : TS: (N.15N)M:D/Samidated(N)(N.15N)M:Clines () () () () () () () () () ()	
Advanced	
NUMBER_SCANS_HALF_CMTG : 200 NUMBER_HOLE_IN_CMTG : 2	
PPM_FOR_CALIBRATION : 0 PPM_HALF_WIN_ACCURACY_PEAK : 15	
TYPE_SAME_START_END_BETWEEN_EVIDENCE : For 1:1 Mixed Samples ELEMENT_ENRICHMENT_CALIBRIATION in one	
Save Report	
Ready	

Figure 16. Quantitation Panel

рТор	ar 1848 18	-	Lation II	-	
File Options	Help				嵌小化
			_		
MS Data	Identification	Quantitation	Summary		
Type : Multiplicity :	Labeling				
Light Label :	none		Name Dimethyl_Labeling SILAC-Arg10Lys8 15N_Labeling	Labels Va M:Dimethyl[ProteinN-term](H,2H)M:D R:K(N,15N)R:K[C,13C)R:R[N,15N]R:R[C R:*(N,15N]M:Deamidated[N][N,15N]N	
Medium Label :		 			
Heavy Label :		 <td><</td><td>Þ</td><td></td>	<	Þ	
 Advanced 					
- 🖬 Output —					
Save Report					
Ready					

Figure 17. Panel with three labels

C pConfig Tool Quantifications	
Name Va None no 15N_Labeling R:" Dimethyl_Labeling M: SILAC-Arg10Lys8 R:1	alue one *{N,15N}M:Deamidated[N]{N,15N}M:Gln->pyro-Glu[AnyN-termQ]{N,15N} :Dimethyl[ProteinN-term]{H,2H}M:Dimethyl[K]{H,2H} K{N,15N}R:K{C,13C}R:R{N,15N}R:R{C,13C}
	Quantification Information Name: Add Label: + AA: Label0: Label1: - AA: Label0: Label1: - AA: Label0: Label1: - AA: Label0: Label1: - AA: Label0: Label1: - Apply
	Add Delete Save

Figure 18. Add Labels

3.3 Run pTop

In the summary panel, you can see all the configuration information. And the red rows stand for those you must fill in but you haven't and the green rows mean you did not fill in while it does not matter. After check all the settings in the summary panel, you can click 'Start' to run pTop, and "Stop" to stop a running task (**Figure 19**). If you don't want to run the task, you can also click "Save" to save the task, mainly its configuration information.

Once you click "Start", you need to once again confirm the task name and its storage path and you still have a chance to change them (**Figure 20**).

🗊 p	lop - pTopTestQuant(D:\pTopWor	<pre>kspace\pTopTestQuant\)</pre>		7 Automatical Automatical	Auto 1		x
File	Options Help						
: 🗅	🗁 🔜 🕨 🔳						
M	S Data Identific	ation Quantitatio	on Summary				
	Decimal Places of M/Z	5					
	Decimal Places of Intensity	1					
() Identification						_
	Property	Value					
	Database	uniprot-proteome-Yeast					_
	Precursor Tolerance	±3.2 Da					
	Fragment Tolerance	±15 ppm					
	Max Truncated Mass	30000					
	Max PTM Positions	5					
	Max Mod. Mass	500					
	Unexpected PTMs	0					
	Fixed Modifications	Dimethyl[K]					
	Variable Modifications	Dimethyl[ProteinN-term] Acetyl[ProteinN-term] Oxidation[M]					
	Search Mode	Tag_Index					
	Second Search	True					
	FDR	1%					
	Separate Filtering	True					
	Quantitation						_
	Property	Value					
	Quantitation	Labeling					Ε
	Multiple Labeling	2					
	Light Label	none;					
	Heavy Label	M:Dimethyl[ProteinN-t					
	NUMBER_SCANS_HALF_CMTG	200					
	PPM_FOR_CALIBRATION	0					
	PPM_HALF_WIN_ACCURACY_PEAK	15					
	NUMBER_HOLE_IN_CMTG	2					
	TYPE_SAME_START_END_BETWEEN	For 1:1 Mixed Samples					
	LL_ELEMENT_ENRICHMENT_CALIBI	none					
				Save	Start	Stop	Ļ
	Output						
	output						
Sa	ve Report						
Read	у						

Figure 19. Summary panel

🗾 SaveTask		
Name	pTopTestQuant	
Location	D:\pTopWorkspace\	Browse
		ОК

Figure 20. Confirm the task name and storage path

When pTop is running, you can see the progress information in the 'Output' box. (Figure 21)

🗾 pl	op - pTopTestQuant2(D:\pTopWor	kspace\pTopTestQuant2\)						-
File	Options Help							
: 🗈	🗁 🔜 🕨 🔲							
M	Data Identifica	ation Ouantita	tion	Summany				
	Identification	uon Quantita	luon	Summary				
	Property	Value						, î
	Database	uniprot-proteome-Yeast						
	Precursor Tolerance	±3.2 Da						
	Fragment Tolerance	±15 ppm						
	Max Truncated Mass	30000						
	Max PTM Positions	5						
	Max Mod. Mass	500						
	Unexpected PTMs	0						
	Fixed Modifications	Dimethyl[K]						
	Variable Modifications	Dimethyl[ProteinN-term] Acetyl[ProteinN-term] Oxidation[M]						
	Search Mode	Tag_Index						
	Second Search	True						
	FDR	1%						
	Separate Filtering	True						
•	Quantitation							
	Property	Value						
	Quantitation	Labeling						
	Multiple Labeling	2						
	Light Label	none;						=
	Heavy Label	M:Dimethyl[ProteinN-t						
	NUMBER_SCANS_HALF_CMTG	200						
	PPM_FOR_CALIBRATION	0						
	PPM_HALF_WIN_ACCURACY_PEAK	15						
	NUMBER_HOLE_IN_CMTG	2						
	TYPE_SAME_START_END_BETWEEN	For 1:1 Mixed Samples						
	LL_ELEMENT_ENRICHMENT_CALIBI	none						J
					Save	Start	Stop	•
- 🔳 (Dutput							
Sa	re Report	0 / 3980						
Tq] [pT [q] Tq] Tq]	op] Read protein information op] Number of proteins: 6749 op] Create tag index op] search op] <search>: 0 / 3980 (0%)</search>	from database.						
[pT	op] <search>: 123 / 3980 (3%</search>)						¥
Runn	ing							

Figure 21. Run pTop

3.4 Results

In the output path (task path), you can see your task folder containing all the results (**Figure 22**). The ".tsk" file is the symbol of a pTop/pBuild task. The "param" folder contains the parameter files of this task. There is a folder for each input data file. In the "summary.txt" file, you can find the overall results about the total MS/MS, the identification rate for each input file. In the "out.log" file, you can find the running log of pTop. The ".cfg" file is also a copy of the search parameters. The "pTop.spectra" file contains all the search results and the "pTop_filtered.spectra" file contains all the identification results above the FDR threshold.

If quantitation analysis is done, there will generate more files. 1.aa/2.aa and 1.mod/2.mod contain the information of amino acids and modifications under different labels. "*pQuant.cfg*" is a copy of pQuant's parameter file. The "pQuant.protein" file and the "pQuant.protein.list" file contain information of quantified proteins, while the "pQuant.spectra" file and the "pQuant.spectra" file contain information of quantified PrSMs.

(D:) ▶ pTopWorkspace ▶ pTopTestQuant1 ▶			
新建文件夹			
名称	修改日期	类型	大小
20160306_YEAST_controlD_HD_A4_1_HCDFT	2017/7/2 20:23	文件夹	
\mu param	2017/7/2 20:08	文件夹	
1.aa	2017/7/2 20:08	AA 文件	1 KB
🔳 1.mod	2017/7/2 20:08	电影剪辑	156 KB
2.aa	2017/7/2 20:08	AA 文件	1 KB
🔳 2.mod	2017/7/2 20:08	电影剪辑	156 KB
📋 out.log	2017/7/2 20:36	文本文档	13 KB
pQuant.cfg	2017/7/2 20:23	CFG 文件	2 KB
pQuant.proteins	2017/7/2 20:36	PROTEINS 文件	893 KB
pQuant.proteins.list	2017/7/2 20:36	LIST 文件	35 KB
pQuant.spectra	2017/7/2 20:36	SPECTRA 文件	78,682 KB
pQuant.spectra.list	2017/7/2 20:36	LIST 文件	2,391 KB
pTop.spectra	2017/7/2 20:23	SPECTRA 文件	4,066 KB
pTop.summary.txt	2017/7/2 20:23	文本文档	1 KB
pTop_filtered.spectra	2017/7/2 20:23	SPECTRA 文件	2,570 KB
pTopTestQuant1.tsk	2017/7/2 20:08	TSK 文件	1 KB
search_task_20170702200816.cfg	2017/7/2 20:08	CFG 文件	1 KB

Figure 22. Output files

In each file folder, there are search results for this input data file (Figure 23). And the finally identification reports are list in the filter.csv file. (Figure 24) And pLabel could open the .plabel file to check the identified proteoform-spectrum-matching (PSM) (Figure 25).

D160306_YEAST_controlD_HD_A4_1_HCDFT.L1.qry.csv	2017/7/2 20:15	Microsoft Excel	3,483 KB
Difference 20160306_YEAST_controlD_HD_A4_1_HCDFT.L1.qry.top10.csv	2017/7/2 20:15	Microsoft Excel	29,343 KB
Difference 20160306_YEAST_controlD_HD_A4_1_HCDFT.L2.qry.csv	2017/7/2 20:23	Microsoft Excel	3,474 KB
Difference 20160306_YEAST_controlD_HD_A4_1_HCDFT.L2.qry.top10.csv	2017/7/2 20:23	Microsoft Excel	31,295 KB
20160306_YEAST_controlD_HD_A4_1_HCDFT.plabel	2017/7/2 20:23	PLABEL 文件	1,306 KB
20160306_YEAST_controlD_HD_A4_1_HCDFT.qry.csv	2017/7/2 20:23	Microsoft Excel	2,714 KB
20160306_YEAST_controlD_HD_A4_1_HCDFT_filtered.csv	2017/7/2 20:23	Microsoft Excel	1,637 KB

Figure 23. Output reports

A	В	с	D	E F	G	Н	I	J	K L	X	N	0	Р	Q	R	S T	U
FileI	Title	Scan	Precursor	Charge StPrecurso	Precurso	aTheoretic	Nass Diff	lass DiffPr	tein AProteir	SPTHs	Matched FNt	era MatCt	ern HatN	term MatC	term MatB	aw ScoreFinal SccL	abel Type
	20160306_YEAST_controlD_HD_A4_1.2318.2318.11.2.dta	2318	2	11 800.3581	8793.866	5 8790, 864	3.002	341.5 sp	POCTO4TEMFIVT	LB (0)Dineth	62	31	31	0.203	0.203	90.73 1.05E-69	1
	20160306_YEAST_controlD_HD_A4_1.2318.2318.11.0.dta	2318	0	11 800.0858	8790, 871	8790.864	0.007	0.8 sp	POCTO4TENFIVT	LB (0)Dincth	62	31	31	0.203	0.203	90.73 1.05E-69	1
	20160306_YEAST_controlD_HD_A4_1.1637.1637.16.0.dta	1637	0	16 752.072	12018.04	4 12018.11	-0.063	-5.2 sp	P22943 SDAGRKG	FC(0)&cety]	68	39	29	0.171	0.23	96.23 8.74B-69	1
(20160306_YEAST_controlD_HD_A4_1.2628.2628.11.0.dta	2628	0	11 967.039	10627.36	5 10626.38	0.978	92.1 sp	Q12349NVIQDL9	LF (0)Dinctl	68	26	42	0.096	0.271	91.89 1.27E-68	1
	20160306_YEAST_controlD_HD_A4_1.2544.2544.7.1.dta	2544	1	7 1225.316	8571.11	8569.161	2.009	234.5 sp	P43582AQSKSNF	PC(0)Dimeti	56	29	27	0.2	0.308	81.67 1.15E-66	1
	20160306_YEAST_controlD_HD_A4_1.2544.2544.7.0.dta	2544	0	7 1225.03	8569.168	8 8569.161	0.007	0.8 sp	P43582 AQSKSNF	PC(0)Dineth	56	29	27	0.2	0.308	81.67 1.15E-66	1
	20160306_YEAST_controlD_HD_A4_1.2374.2374.11.1.dta	2374	1	11 800.3583	8793.869	9 8790, 864	3.005	341.8 sp	POCTO4TENFIVT	LB(0)Dineth	59	30	29	0.203	0.181	85.98 6.04E-66	1
	20160306_YEAST_controlD_HD_A4_1.2374.2374.11.0.dta	2374	0	11 800.0858	8790.871	8790.864	0.007	0.8 sp	POCTO4TENFIVT	LB(0)Dimeth	59	30	29	0.203	0.181	85.98 6.04E-66	1
(20160306_YEAST_controlD_HD_A4_1.2545.2545.7.0.dta	2545	0	7 1225.03	8569.168	8 8569.161	0.007	0.8 sp	P43582 AQSKSNF	PC(0)Dinctl	56	30	25	0.183	0.317	80.68 7.79E-66	1
	20160306_YEAST_controlD_HD_A4_1.2545.2545.7.1.dta	2545	1	7 1225.316	8571.11	8569.161	2.009	234.5 sp	P43582AQSKSNF	PC(0)DinetH	56	30	25	0.183	0.317	80.68 7.79B-66	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.3.dta	1009	3	12 804.3312	9640, 895	5 9637,903	2.992	310.4 sp	P22943 SDAGRKO	FC(0)Acety	64	29	34	0.179	0.27	84.94 1.04E-65	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.0.dta	1009	0	12 803.9978	9636.893	3 9637.903	-1.01	-104.8 sp	P22945 SDAGRKO	FC(0)Acetyl	64	29	34	0.179	0.27	84.94 1.04E-65	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.2.dta	1009	2	12 803.9141	9635.889	9637.903	-2.014	-208.9 sp	P22943 SDAGRKO	FC (0) Acetyl	64	29	34	0.179	0.27	84.94 1.04E-65	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.1.dta	1009	1	12 804.0813	9637.895	5 9637,903	-0.008	-0.8 sp	P22943 SDAGREG	FC(0)&cety)	64	29	34	0.179	0.27	84.94 1.04E-65	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.6.dta	1009	6	12 804.2481	9639, 897	7 9637.903	1.994	206.9 sp	P22943 SDAGREG	FC(0)Acetyl	64	29	34	0.179	0.27	84.94 1.04B-65	1
	20160306_YEAST_controlD_HD_A4_1.1009.1009.12.5.dta	1009	5	12 804.1649	9638, 899	9 9637, 903	0.996	103.3 sp	P22943 SDAGRKG	FC(0)Acety)	64	29	34	0.179	0.27	84.94 1.04E-65	1
	20160306_YEAST_controlD_HD_A4_1.2543.2543.7.0.dta	2543	0	7 1225.03	8569.168	8569.161	0.007	0.8 sp	P43582 AQSKSNF	PC(0)Dineti	54	29	25	0.201	0.287	80.69 1.21E-65	1
(20160306_YEAST_controlD_HD_A4_1. 2543. 2543. 7. 1. dta	2543	1	7 1225.316	8571.11	8569.161	2.009	234.5 sp	P43582 AQSKSNF	PC(0)Dineth	54	29	25	0.201	0.287	80.69 1.21E-65	1
	20160306 YEAST controlD HD A4 1.650.650.13.0.dta	650	0	13 631.2265	8193,851	7 8193, 864	-0.007	-0.9 sp	P50263 AEKLQCN	DE (0)Dimeth	60	30	31	0.134	0.094	89.53 1.69E-65	1
	20160306_YEAST_controlD_HD_A4_1.2629.2629.11.0.dta	2629	0	11 967.039	10627.36	5 10626.38	0.978	92.1 sp	Q12349NVIQDLY	LF (0)Dincth	63	24	39	0.117	0.3	85.53 1.93E-65	1
(20160306_YEAST_controlD_HD_A4_1.870.870.13.0.dta	870	0	13 674.4773	8756.111	8756.122	-0.004	-0.5 sp	P50263NNEFAEB	LC(0)Acetyl	55	26	29	0.36	0.222	78.91 4.16B-65	1
	20160306_YEAST_controlD_HD_A4_1.2320.2320.11.0.dta	2320	0	11 800.0859	8790.872	2 8790, 864	0.008	1 sp	POCTO4TENFIVI	LB (0)Dineth	55	27	29	0.232	0.192	82.62 1.40B-64	1
(20160306_YEAST_controlD_HD_A4_1.2320.2320.11.2.dta	2320	2	11 800.3582	8793, 861	7 8790, 864	3,003	341.6 sp	POCTO4TENFIVT	LB (0)Dineth	55	27	29	0.232	0.192	82.62 1.40E-64	1
	20160306 YEAST controlD HD &4 1.1051.1051.12.5.dta	1051	5	12 758.4418	9090.221	9088.237	1.984	218.3 sp	P50263 SNNNKF	AB (0) Acetyl	53	22	31	0.351	0.248	77.87 1.74E-64	1
	20160306_YEAST_controlD_HD_A4_1.1051.1051.12.8.dta	1051	8	12 758.1101	9086.241	9088, 237	-1.996	-219.6 sp	P50263 SNMNKP	AB (0) Acety	53	22	31	0.351	0.248	77.87 1.74E-64	1
	20160306 YEAST controlD HD A4 1, 1051, 1051, 12, 3, dta	1051	3	12 758, 1932	9087.238	3 9088, 237	-0.999	-109,9 sp	P50263 SNNNNKF	AB(0)Acety)	53	22	31	0.351	0,248	77.87 1.74B-64	1
(20160306 YEAST controlD HD A4 1.1051.1051.12.1.dta	1051	1	12 758, 3589	9089, 226	5 9088, 237	0,989	108,9 sp	P50263 SNNNKF	AS(0)Acety)	53	22	31	0.351	0,248	77.87 1.74B-64	1
(20160306_YEAST_controlD_HD_A4_1.1051.1051.12.0.dta	1051	0	12 758.276	9088.232	2 9088, 237	-0.005	-0.5 sp	P50263 SNMNKF	AB (0) Acety	53	22	31	0.351	0.248	77.87 1.74E-64	1
	20160306 YEAST controlD HD A4 1, 2503, 2503, 11, 0, dta	2503	0	11 906, 1158	9957, 201	9957.208	-0.006	-0.6 sp	OSE754NENDKGG	L5(0)Acetyl	53	22	31	0.06	0.316	77.07 1.82E-64	2
(20160306 YEAST controlD HD A4 1, 2546, 2546, 7, 1, dta	2546	1	7 1225, 316	8571.17	8569, 161	2,009	234.5 pp	P43582 AQSKSNF	PC(0)Dineth	52	28	24	0, 21	0,307	78.64 1.86E-64	1
	20160306 YEAST controlD HD A4 1.2546.2546.7.0.dta	2546	0	7 1225.03	8569.168	8 8569, 161	0.007	0.8 sp	P43582AQSKSNF	PC(0)Dincth	52	28	24	0.21	0.307	78.64 1.86E-64	1
	20160306 YEAST controlD HD A4 1, 875, 875, 13, 0, dta	875	0	13 674, 4773	8756, 117	8756, 122	-0.004	-0, 5 sp	P50263NNEFAER	LC(0)Acetyl	54	27	27	0.364	0.216	77.19 7.03R-64	1
	20160306 YEAST controlD HD A4 1, 2630, 2630, 11, 0, dta	2630	0	11 967.039	10627.36	5 10626.38	0.978	92.1 sp	Q12349NVIODLY	LS (0)Dineth	61	23	38	0,133	0,316	82,84 8,88E-64	1
	20160306 YEAST controlD HD A4 1, 2324, 2324, 12, 2, dta	2324	2	12 733, 6626	8792, 871	8790, 864	2,007	228.3 sp	POCTO4TEMFIVT	LB (0)Dineth	56	28	28	0,152	0,192	84.9 1.21E-63	1
(20160306 YEAST controlD HD A4 1.2324.2324.12.1.dta	2324	1	12 733.413	8789, 876	5 8790, 864	-0.988	-112.4 sp	POCTO4TENFIVT	LS (0) Dinets	56	28	28	0.152	0.192	84.9 1.21E-63	1
	20160306 YEAST controlD HD A4 1, 2324, 2324, 12, 0, dta	2324	0	12 733, 496	8790, 872	2 8790, 864	0.008	0.9 sp	POCTO4TENFIVT	LS (0)Dineth	56	28	28	0.152	0.192	84.9 1.21E-63	1
	20160306 YEAST controlD HD A4 1, 2324, 2324, 12, 4, dta	2324	4	12 733, 5794	8791, 873	3 8790, 864	1.009	114.7 sp	POCTO4TENFIVT	LS (0)Dineth	56	28	28	0,152	0,192	84.9 1.21E-63	1
	20160306 YEAST controlD HD A4 1, 2324, 2324, 12, 6, dta	2324	6	12 733, 3298	8788, 878	8 8790, 864	-1,986	-225, 9 sn	POCTO4TENFIVT	1.8 (0)Dineth	56	28	28	0.152	0.192	84.9 1.21R-63	1
	20160306 YEAST controlD HD A4 1, 2642, 2642, 11, 0, dta	2642	0	11 967.039	10627.36	5 10626.38	0.978	92.1 sp	O12349NVIODLY	LS (0)Dineth	62	24	37	0.135	0.317	82,43 1,51R-63	1
-	20160306 YEAST controlD HD A4 1, 2298, 2298, 11, 0, dta	2298	0	11 800, 0851	8790, 81	8790, 864	0,006	0.7 sp	POCTO4TENFIVI	LB (0)Dineth	57	30	27	0, 229	0,186	81.72 1.81E-63	1
-	20160306 YEAST controlD HD A4 1, 2461, 2461, 12, 1, dta	2461	1	12 830, 6065	9956, 198	8 9957, 208	-1,01	-101.5 sp	Q3E754MENDKCO	Ly(0)Acety	53	13	40	0.012	0.377	76.09 2.53E-63	2
	20160306 YEAST controlD ND 44 1, 1633, 1633, 15, 0, dta	1633	0	15 802.0766	12017.04	12018.11	-1.061	-88.3 m	P22945 SDAGRKO	FC (0)Acety	59	29	30	0.146	0.339	84.9 3.74E-63	1
	00100000 9810F	0600	ő	11 067 090	10007 20	10000 00	0.070	02.1 m	OLOGAC NUTODA S	1 5 (A) Dia a+1	60	22	07	0.140	0.240	70 EC E 000_CO	

Figure 24. Identification list



Figure 25. Matched MS/MS in pLabel

The output results of pTop could also be visualized by **PBuild.exe**. You could open a pTop task (.tsk) with pBuild (**Figure 26**). Then click "Protein" panel to see all the PrSMs both their MS spectra and MS/MS spectra (**Figure 27**).

	There is a start of the start o	
pBuild	Overview	
	pBuild 3.0 is a viewer tool for pFind 3.0. pBuild takes care of every step of result validation	n, visualization and reporting.
Start		
Open	System Requirements	
Exit	pBuild 3.0 is available in both 64 bit and 32 bit version. It supports Windows Operation 3 space to store temporary files and intermediate results. The amount of disk space reequ	ystem (Windows XP, Windows 7 and Windows 8). This program files will only use \sim 40MB disk space. red for this purpose depends on the size of the user's datasets.
	* 在東来 ふ 名作 中収日明	大小 近天
Recent	TE 2017/7/2 22:15	文件夹
pTopTask20 (12 hours ago)	武庫 201/1/12 2201 東京街道的位置 ジョアのアモネルH4.tsk 2017/72 22:13	文件兒 TSK 文件 1 K8
testComman (11 hours ago)	- 43 I	
	10 m	
	Subversion	
	■ 文階 ▶ ec	
	A plop	
	🦉 计算机	
	• 网络 -	
	ZIFA(N)	task hies (*.1sk)
		1171(0) 40.00



so rescummandumeto propivo	respace (restcommand															
l Help																
Spectrum Level 💿 Peptide Level 🔘 🗄	Sequence Level 🔝 Othe	s Filter Copy	Export to TXT	Show Sim	Config	MS2 Config										
Spectrum Charge	Spectrum mass	Sequence		Mod§ites	Specific	MissedGlea	DetiaMass (Da)	DetlaMass (PPM)	Score Targe	Qecoy LabelNar	ne AC					
20LC_H41770177012.0.dta 12	11341.426848	SGRGKGGRGLGRGGAKR	SKVLRDNEQGTEPA	I 16 TrimethylEK](No	or 0	0	-1.00992	8 -89.04	3.95E-046 target	None	\$PE1P1004534	2				
2DUC_H4170917091203ta 12 2DUC_H417441744110.dta 11	11341.419519	SGRGKGGKGLGKGGAKRI	IRKVLRDNIQGITKPAI	I 16.Trimethyl(K)(No I 16.Trimethyl(K)(No	or O	0	-1.01/153	-89.68	5.71E-043 target	None	IPEIPI004534	7				
2DLC_H4.1953.1953.11.0.dta 11	11300.3825	SGRGKGGKGLGKGGAKR	RKVLRDNIQGITKPAL	5,Trimethy@Q(Nor	ne O	0	-0.043709	-3.868	5.96E-042 target	None	IPEIP1004534	7				
2DLC_H4.2052.2052.12.0.dta 12	11299.407823	SGRGKGGKGLGKGGAKR	HRKVLRDNIQGETKPAI	I 23,Methyl(R)(None	e) 0	0	-0.982003	-86.907	4.94E-041 target	None	IPEIPI004534	7				
2DLC H41806180612.0.dta 12	11342.411184	SGROKGOKGLOKGGAKRI	RKVI RDNIOGITKPAI	1 16 Trimethal Kills	er O	0	-0.051979	5 -5.45	1.90F-040 target	None	IPEIPI004534	7				
152 Chromatoscam																
	Kange- DeNovo-															
Title: 1010 H4 1730 1720 17.0 dt	Mode: Dissethet RI 12	Trimertully1:15 Aret	EProteint termi - 0	Label: None Linfo												
Base Peak: 1.52E+005 MS2_Ma	ISS: 11341.426848Da / 946	042241Th MS2_mass - T	heoretical_Mass: 1.00	99260a / -89.048ppm	PSM_Score ((%):56.12										
	ele v le li	IC V CO C A		VID	DINI	6	TVD	A L D D L	A P P C	C V V P	L s c l		E E T	0 6		
	G G A G L	GAGGA	A A H A	JA V L A	DIN	14 6 1	I K F			GVKK	1.201		b52 b53	10 B	A IF IV I	• 1
SGRGK	No. of Concession, Name	e hiz	218	*	1025											
-SGR GR	54 S	e biz 100 V01 V00 V08 V28	127 128 128	¥23 ¥25 ¥28	1024 V18 V18 V17	154 YES 154	913									
		но ула уло уло уло ТГ ГУ ГТ ГЕ ГН	10 VI R K				и к в l	OGRI	YGEG	G						
		ма 100 ул 120 ул 120]Т Ү Т Е Н						Q G R T I		G						
		¥0 ¥0 ¥0 ¥0 ¥0 ¥0 T Y T E H		Y23 Y21 Y20 T V T A Net Net Net		VIA VIT VIA VIV VIV A		Q G R T I	Y G F G	G						
		100 ya	122 Y24 Y24 A K R K bos bos	YD YD YD ∫T V∫T∫A Nat hai ha				Q G∐R T I	Y G F G	G						
		bul JT IY IT E H	X22 Y24 X24 A K RK				JL K R_] ■ 8932	QG_RTI _{baa}	Y G F∐G	G						
			122 Y24 Y24 A K R K bog bog	YD YD YD YD T V T A Net het he			LKR bas	QG∐RTI ^{bee}	Y G F∐G	G						
F_L_E_N V	I R D A V		122 124 124 A K R K bos bos	YD YD YD YD T V_T_A Net bez bez			_L K R.] _L K R.] ₀₀ 6₀2	QG∐RTI bei	YGF∐G	G						
	I R D A V		122 Y24 Y24 A K R_K_ bos bos	YD YD YD YD T V_T_A Net Net Net	bia Yis Yi Yi Yi M D V big big big	YES YES KE A		QG∐RTI	YGF∐G	G						
F LLEENN V	I R D A V						⁹²³ □LKR ■ 00 892	QG∐RTI _{bas}	Y G F_G buo	G						
-5 G K G K ======== F_L_E_N ∨ ==========			10 YOM K R K	YD Y			⁹¹³ ↓LKR ₀₀ bs2	Q G∐R T I	YGF_G ≋uoo	G						
F L [E]N V F L [E]N V	I R D Kas k	লালালা নারী বী পী বী	NATION AND AND AND AND AND AND AND AND AND AN	Y23 Y23 Y29 ∫T V_T_A_ Not Not Not Not		YEA YEA YEA V Y A to ber be	⁹⁽³ ∫LKR ₀ ₀	QG_JR T I	Y G F∬G	G						
F_L_E_N V		TYTE	SEE	Y23 Y23 Y25 T V_ T ▲ Bet Bet Bet Bet		YEA YEA YEA V Y A	¹⁰³ □ L K R 10 10 10 10 10 10 10 10 10 10	QG_RTI _{Nat}	L Y G F_G Nao	G						
		में जे प् में जे प	NU VIA KANA	Y23 Y23 Y26 T V_T_A net her her	bas yas ya MDVV n bas ba	YEA YEA YEA V Y A to her he	100 K R 100 000	QG_RTI	.YGF_G	G						
F L E N V		मा ज ग मा ज ग ग	SEE SEE SEE		hat yis yu yu M D_V a has ha	1916 NOT	100 100 100 100 100 100 100 100	QG_RTI	Y G F_G	G						
		में जो 7 प में जो 7 प -	A K R K	TT VTTA	baa Jos yu yu M D_V_ n kus ka	The form	500 K R 	Q G_R T I	Y G F G	G						
		н а н а т үү	A K R K		baa ms ya m² M D_V_ n ns ne	REAL ROOM	то к к к	Q G_R T I	Y G F G	G			±.2		4	-9
			A K R K		a ma ya no Ma D_V ba ba ba		100 + 100	Q G R T I	Y G F G	G		4.24	2,4 1,000 1,000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000 - 1000			88- 1000-
			**************************************			744+ 260	В К R В 100 100 100 100 100 100 100 10	Q G R T I	Y G F G	G		+ 104 - 105 - 106	-100- 100-	1989 -		-100-
			100 100 100 100 100 100 100 100			1940+ 1960- 1	100 + 10000 + 10000 + 10000 + 1000 + 10000 + 1000 + 1000 + 1000 + 1000 +	Q G R T I	Y G F G biss	G *0/7		+100 +100 +100 +100	1001 1001 - 1001	- 599-	- 100 - 100 - 100	
			100 100 100 100 100 100 100 100	1000 100 100 100 100 100 100 100 100 10		7044+ 500	100 K R 100 +500 +500 +500 +500 +500 +500 +500	Q G R T I	Y G F G	-6 -0	-967 -1967	1011 - 1022 - 1033 -	-104 -104	1989+	-100	- 908- - 1600-
			100 100 100 100 100 100 100 100 100 100			Palette Palette palette palette palette palette palette palette palette palette palette palette	10 K R S12	Q G_R T I	Y G F G	G	<u>ر این این این این این این این این این این</u>		-100 -100	+589	- 100-10-10-1- 10-10-1- 10-10-1-	- 190- - 1000-
			100 100 100 100 100 100 100 100 100 100			100+ 10+ 1	100 1 K R 10 1	Q G R T I	Υ G F G base	G	<u>考読</u> 	+106 +156 +158 +158	*****	- P689-		
			10 10 10 10 10 10 10 10 10 10 10 10 10 1				100 100 100 100 100 100 100 100	Q G R T I	Y G F G base	6	魏 <u>唐</u>		1985 1986 1987		۱۹۹۹ کو ۱۹۹۹ کو	

Figure 27. Matched MS/MS in pBuild

4 Contact information

E-mail: ptop@ict.ac.cn, rxsun@ict.ac.cn

Web: http://pfind.ict.ac.cn

Institute: Institute of Computing Technology, Chinese Academy of Sciences.

Address: No. 6, Kexueyuan South Road, Zhongguancun, Haidian District, Beijing 100190, China