

Phenyx Search Guide

Introduction :

Phenyx uses a scoring scheme based on signal detection theory and pattern recognition to calculate a likelihood ratio that distinguishes true from false peptide identifications (Colinge *et al* 2003). Phenyx generally performs searches in one or two sequential rounds. The first round is a search with conservative parameters on a full database. Valid peptide identifications that pass the first round are accepted and processed in the second round. The second round is a more liberal search that may result in increase in sequence coverage, by searching for more modifications or other spectral features. In case of a spectrum being assigned more than one valid peptide, the algorithm attempts to resolve conflicts by using other spectral features.

Getting Started :

The image shows a screenshot of the Phenyx Submission Page (iTRAQ - Do you ?) with four numbered callouts pointing to specific form fields:

- 1**: Select the database that you want to search the dataset against. (Points to the Database(s) dropdown menu)
- 2**: Select appropriate title for your search. (Points to the Title text input field)
- 3**: Select Instrument type and scoring model according to your experimental setup. (Points to the Instrument Type and Scoring Model dropdown menus)
- 4**: Select appropriate charge. (Points to the Default Parent Charge and Trust Parent Charge dropdown menus)

The form fields visible in the screenshot include:

- Profiles: iTRAQ - Do you ? (prack)
- Title: [Empty text input field]
- Database(s): [Dropdown menu with options: ipi.HUMAN, ipi.MOUSE, ipi.RAT, uniprotKB_sptr, uniprot_sprot]
- Taxonomy: -root
- AC list: [Empty text input field]
- Search Engine: MS/MS
- Instrument Type: MALDI TOF-TOF
- Scoring Model: TOF-TOF iTRAQ
- Round # 1
- Default Parent Charge: 1
- Trust Parent Charge: medium

1. Information regarding accessing Phenyx can be found on the following website :
<http://www.phenyx-ms.com/index.html>

Phenyx typically performs its search in two rounds. The first round is generally run with stringent parameters against a broader database.

FIRST ROUND :

The screenshot shows the 'Search Engine MS/MS' configuration window. Callout 5 points to the 'AA Modif. (def.)' list, which includes ITRAQ_K (+144Da), ITRAQ_Nterm (+144Da), METH_core (+14Da), METH_cterm (+14Da), METH_nterm (+14Da), and MMTS (+46Da). Callout 6 points to the 'AA Modif. Details' table. Callout 7 points to the 'Error Tol' and 'Coverage' fields. Callout 8 points to the 'Acceptance Parameters' section. Callout 9 points to the 'Parent Error Tolerance' field.

Name	Type	Tolerance
ITRAQ_K (+144Da)	fixed	all
ITRAQ_Nterm (+144Da)	fixed	all
MMTS (+46Da)	fixed	all

Acceptance Parameters	
Min. Peptide Length	6
Min. Peptide z-Score	5.0
Max. Peptide p-Value	1.0E-4
AC Score	6.0

8. Minimum peptide length: Peptides shorter than set value (6 aas in above case) are reported in results but do not contribute to protein score. **Minimum peptide Z-score:** It measures the calculated peptide score deviation from mean of distribution. It is a conversion of score to standard deviation and a value of 6.0 is accepted as a reasonable minimum. **AC score:** is the sum of best scores for valid peptide identifications. Protein matches scoring below 6.0 (in above case) are not selected as candidate proteins.

SECOND ROUND :

The second round is performed with looser parameters on a more restricted protein database of identifiable proteins that is generated from the first round. Non-specific cleavages, post-translational modifications, amino acid substitutions can be explored in the second round. Moreover, it is recommended that conflict resolution is used in second round and not in the first round.

Select fixed and variable modifications according to the experimental set up. 10

Select Conflict Resolution in the second round to resolve valid peptide identifications for a spectrum. 11

Thresholds for peptide lengths, scores can be adjusted by the user. 12

Input peaklists can be in formats such as dta, pkl, mgf, mzData, mzXML, etc. 13

Browse and select peak list for search. 14

Click on "Submit". 15

Conflict Resolution: none

round # 2

AA Modif. (def.): ITRAQ_K (+144Da), ITRAQ_Nterm (+144Da), METH_core (+14Da), METH_cterm (+14Da), METH_nterm (+14Da), MMTS (+46Da)

Enzyme (def.): Trypsin_(KR_noP)

Missed Cleavage: 2

Cleavage Mode: normal

Turbo:

Conflict Resolution: yes

AA Modif. Details:

DEAM_N (+1Da)	variable	<=1
DEAM_Q (+1Da)	variable	<=1
iTRAQ_K (+144Da)	fixed	all
iTRAQ_Nterm (+144Da)	fixed	all
MMTS (+46Da)	fixed	all

Parent Error Tolerance: 0.7 Da

Acceptance Parameters:

Min. Peptide Length	6
Min. Peptide z-Score	5.0
Max. Peptide p-Value	0.01
AC Score	6.0

Peak Lists

File Format: mgf (zip and tar.gz accepted)

Peak List(s): [Browse...]

Submit

Server Log

For a more detailed discussion on parameters used please visit: <http://phenyx.vital-it.ch/docs/pwi/SubmissionEffects.html>

ID	User	Status	Date	Title	Comment
<input type="checkbox"/> 47870	pratik	Completed	2008-02-20	A549 SECRETOME ASMS 2008	605 matches (9917 peptides): IPI:IPI00854510.1 TRE...
<input type="checkbox"/> 47436	pratik	Completed	2008-02-06	Secretome ASMS 2008	578 matches (9934 peptides): IPI:IPI00854510.1 TRE...

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The job is submitted to the queue for search and colour changes according to job status.

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Once job is completed "Comment" gives an overview of proteins and peptides identified.

GENEBIO
PHENYX

Logged as : 'pratik' (USER) Log Out

Job N: 47870

- Resubmit
- Parameters
- Proteins Overview
- Compounds Overview
- EXPORT EXCEL**
- Export XML
- Export Text
- User Permissions

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Check the box next to your ID search to open various export options.

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Click on EXPORT EXCEL to download Protein and Peptide Report that can be used for further analysis (FPR calculation, quantification, etc.)