

Evaluation and Comparison of Aligners for De Novo Sequencing

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Abstract

In high-throughput proteomics research of tandem mass spectrometry, de novo sequencing provides a novel method to interpret MS/MS data without any help of sequence database and discover new organisms. In this paper, we have systematically evaluated and compared the capability of mainstream de novo sequencing software via testing data sets which have been correctly identified by Mascot and Sequest, so we can intuitively find out the optimal de novo sequencing software for protein identification.

Introduction

In recent years, tandem mass spectrometry has got a great development in the field of proteomics. In a typical LC-MS/MS experiment[1, 2, 4], thousands of MS/MS spectra can be generated, how to interpret LC-MS/MS data rapidly and efficiently is still a challenge, although many peptide identification algorithms have been proposed[3, 5, 9, 10].

Database search and de novo sequencing are parallel methods for protein identification, the former is the most popular approach, which basic thought is to score the mass spectra against a database of all candidate peptides to detect significant matches[4, 6, 9, 15], such as Mascot, Sequest, SQUID, OMMSA, ProVerB and pFind. However, the basic idea of the latter is to reconstruct the original peptide sequence without knowledge of genomic sequences or organism, including Lutefisk, PepNovo, pNovo, SeqMS and Peaks[3, 5, 6, 12]. Database search assumes that the genome are accurately sequenced and annotated which are hardly satisfied. De Novo sequencing provides a novel way to identify the MS/MS data only according to spectra itself[14].

De novo sequencing contains four mainly procedures: construct the graph of MS/MS spectra, confirm the ions type, establish sequencing algorithm and scoring model[3-6]. How to construct spectrum graph and determine ions type are the fundamental of de novo algorithms, sequencing algorithm provides searching methods for spectrum graph, scoring function is the heart of de novo algorithms, varies protein identification algorithms have different score function, there are often determine the advantages or disadvantages of the algorithms[4, 8, 14].

Here, we have systematically recommend the mainstream methods of sequencing algorithms and the categories of scoring model. Meantime, in order to evaluate and compare the sensitivity of widely used de novo sequencing software, we utilize the testing dataset which correctly identified by

commercial database search software Mascot and Sequest[7, 11, 15]. We hope our research can provide a reference for researchers.

1 De Novo Sequencing Algorithms

1.1 Constructing The Spectrum Graph

An alternative approach of de novo sequencing is to transform the spectra data as a directed a cyclic graph, where a node corresponds to a mass peak and an edge is connected by two nodes which differ by the total mass of an amino acid. For a spectrum $S = \{s_1, s_2, \dots, s_m\}$, the corresponding mass is $M = \{w_1, w_2, \dots, w_m\}$, the parent mass of S is W , the spectrum graph can be constructed follows:

- Generated two vertexes, denoted as v_0 and v_m , which mass are 0 and $W - 18$, respectively.
- The type of fragment s_i is N -terminal or C -terminal can not be determined, so it is necessary to create complementary \bar{s}_j , which mass are $w_j - 1$ and $W - w_j + 2$, respectively.
- Assume the differ mass by two fragments denoted as : $|w_i - w_j| \leq \Delta m$, meantime, the mass of amino acid $AA = \{aa_1, aa_2, \dots, aa_{20}\}$, δ denoted as the allowable error of instrument. If $\Delta m \leq \delta$, connecting the two fragments (i.e. two nodes) by an edge.

1.2 Confirming The Ions Type

How to confirm the ions type is crucial for de novo sequencing, in a general way, there is not enough information to confirm the ions type. Hence, we need to find out immonium ions in the region of the lower mass, then confirm the other ions type. SHERENGA utilize the offset frequency function to confirm the ions type. PRIME use graph theory to distinguish the ions type.

1.3 Sequencing Algorithm and Scoring

The de novo sequencing problem is to reconstruct the

peptide sequence from a given tandem mass spectral data, given a spectrum graph, our aim is to find a maximum score path for s_0 to s_0 . A number of de novo sequencing algorithms have been reported for deduction of protein sequences from MS/MS spectra data. Although several instrument manufacturers have developed, they are not satisfy for proteomics research. Most of software packages, including Lutefisk, PRIME, SHERENGA, and SeqMS use dynamic program to search spectrum graph.

Scoring function is the core of the de novo sequencing algorithms, varies algorithms have different scoring model. In order remedy the disadvantage of missing peaks, Peaks provides a new mathematics model to compute the reward/penalty that a y (or b) ion has mass m , the formula as follows:

$$f\left(\frac{h_1}{h}\right) \times f\left(\frac{h_2}{h}\right) \times f\left(\frac{h_3}{h}\right) \times \exp\left(-\left(\frac{m' - m}{\delta}\right)^2\right) \times \log h$$

Where:

m' = the mass of a y-ion

m = the mass of the observed peak for that y-ion

δ = the mass error tolerance of the spectrometer

h_1, h_2, h_3, h_4 = the relative abundances of the observed y-ion peak and the corresponding x, y-H₂O, y-NH₃ peaks

The approach of Peaks is different from the spectrum graph model used by previous algorithms, which consideration is attempt to give a reward/penalty score for improving the accuracy of the de novo sequencing results. PepNovo proposed a probabilistic network model which attempted to interpret the MS/MS data, the algorithms can score any number of peptides, the heart consideration is to utilize the likelihood ration hypothesis test to determine whether the peaks observed in the mass spectrum are more likely to have been produced under its fragmentation model than under a model that treats peaks as random events. The mainly scoring function as follows:

$$Score(m, S) = \log \frac{P_{CID}(\vec{I} | m, S)}{P_{RAND}(\vec{I} | m, S)}$$

The details of the parameters reported by ref 4, if the $Score(m, s) > 0$, the denotes generated by actual peaks, otherwise, generated by random peaks[4].

on the whole, all of de novo sequencing algorithms are according to the quality of parent ions, enumerate parts of possible candidate peptides, then compare the candidate peptides by scoring function, finally, find out the best matching of candidate peptides.

2 Searching and Comparison

In order to validate the accuracy and effectiveness of mainstream de novo sequencing software, including Lutefisk, Peaks, PepNovo, pNovo+. We utilize 18 MS/MS spectra from Micromass/Waters QTOF Ultima and LTQ-Orbitrap instruments, which correctly identified by Mascot and Sequest. The parameters set as follows:

- Micromass/Waters QTOF Ultima
 - Parent ions tolerance error : 0.2 Da
 - Fragment ions tolerance error: 0.5 Da
- LTQ-Orbitrap
 - Parent ions tolerance error : 10 ppm
 - Fragment ions tolerance error: 0.5 Da

The specific identifying results as the following table.

Table1 The results of de novo sequencing search

Spectra	Sequenc e	Peaks	pNovo	PepNov o	Lutefisk
QT20060328_Den18mix_06.2112.2112.2.dta	DGPLT GTYR	MHNT YSVK	YMSTP PQR	None	[382.14] T[WY] K
QT20060328_Den18mix_03.2565.2565.2.dta	AWMS AAAIA K	FDPLA DWR	MMDF HNPk	None	[262.10] PLAD[R W]
QT20060328_Den18mix_02.2326.2326.2.dta	VKVNS VLM*K	QVVG DC(+57.02)TR	QVVG DAFAK	None	VQVG D[261.11]R
QT20060328_Den18mix_02.3025.3025.2.dta	ASGG AAVSIS IK	GTGW NLPMG K	TGWG NNGLG GQ	None	[158.07] GWNLP CR
QT20060328_Den18mix_04.3013.3013.2.dta	SAGTN TVASL R	ASGSY YFGPK	TGGAP CNTGN GK	None	DQGT[227.17]F PGK
QT20060328_Den18mix_01.2517.2517.2.dta	VAAAF PGDVD R	VAAAF LLAAD R	VAAAE TDVW R	None	[170.11] AAF[198.10]AG ANK

QT20060328 _Den18mix_ 10.2121.212 1.2.dta	SRGRG GGGGG FR	SSKLTS LAYK	GVEHY EGSDK	None	[SS][335 .11]PNV N[AP]
QT20060328 _Den18mix_ 06.2070.207 0.2.dta	LALGA STGLG LR	LAANE SKGPN K	LATQT NMPPR	None	LAANE Y[EY]R
SXS_D39_0 90104_0.435 1.4351.1.dta	NVALA VK	NVALA VK	KVGLL GAG	VYPVA LAAR	NVALA VK
SXS_D39_0 90104_0.223 5.2235.2.dta	VNAEA GLK	VNAR WR	SSGLV PNK	VNDAN LR	VNATK LR
SXS_D39_0 90104_0.432 6.4326.2.dta	NLMPN PK	NLMPP NK	ARARD PGA	LNNLV LK	VQ[227. 12]VLK
SXS_D39_0 90104_20.45 94.4594.2.dt a	VGING FGR	VGLNG FGR	PASGK GFR	VGLNG FGR	VGLNG FGR
SXS_D39_0 90104_20.19 94.1994.2.dt a	YGGLG TQK	YGGLG TQK	GTFGK GEK	YGGLG DNK	YG[212. 15]LMK
SXS_D39_0 90104_0.157 4.1574.2.dta	QATPG GAVK	FFHLH K	KPPNS SAK	YETND GK	[170.11] R[NS]G PK
SXS_D39_0 90104_20.62 90.6290.2.dt a	EITGLG LK	ELTGL GLK	GKMA KAPK	ELTGL GLK	NKT[17 0.11]GL K
SXS_D39_0 90104_0.389 1.3891.2.dta	LDVEA SAK	LDVEA SAK	LGASD LEK	LDVEA SAK	LDVEA SAK
SXS_D39_0 90104_0.300 0.3000.2.dta	YDTTQ GR	YDTGA SAR	YDAM KGGV	DYTG WAK	[278.09] TG[229. 11]R
SXS_D39_0 90104_0.556 1.5561.2.dta	LSGGV AVIK	LSGTK PLK	SLGGK LLVG	GNLAV EK	LSR[NP]TR

3 Discussion

In this paper, we can see that PEAKS and PepNovo are performed more accuracy and robust than other software below LTQ-Orbitrap instrument. However, we also can find almost all spectra generated by Micromass/Waters QTOF Ultima instrument has lower quality and the searching results showing multifarious. Although de novo sequencing algorithms still beset with difficulties, the technology of instruments have been develop rapidly, and the research of

protein identification algorithms become more thorough, we have hope of de novo sequencing algorithms.

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