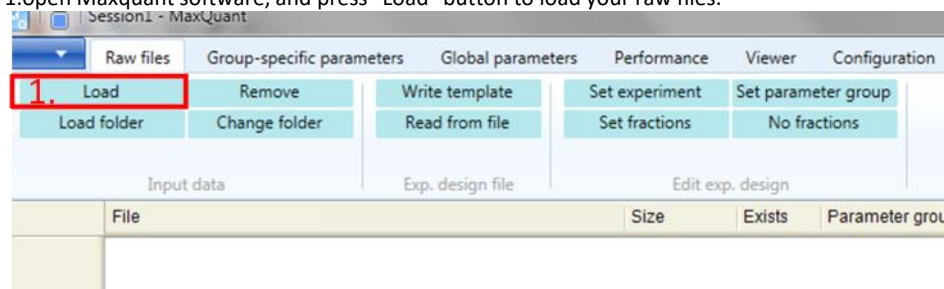


# MaxQuant Search

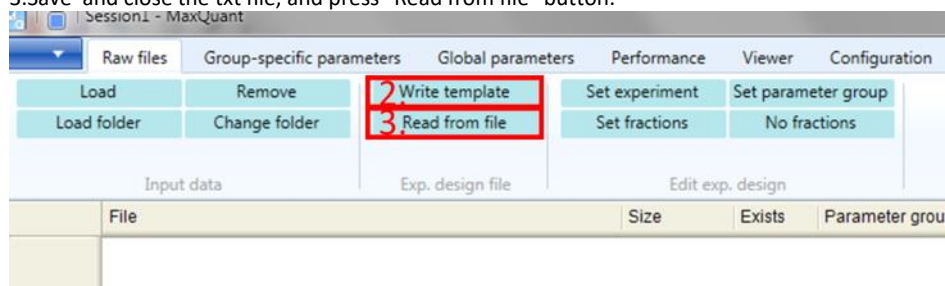
2016年3月31日 上午 11:33

1. open Maxquant software, and press "Load" button to load your raw files.

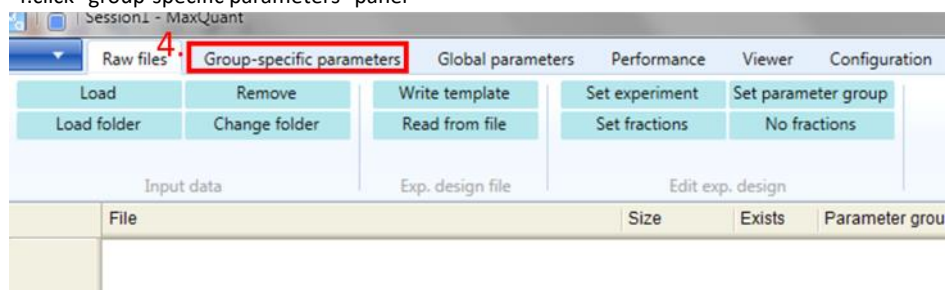


2. press "Write template" button, and open "designtemplate.txt" file to set sample name and number of fractions.

3. Save and close the txt file, and press "Read from file" button.



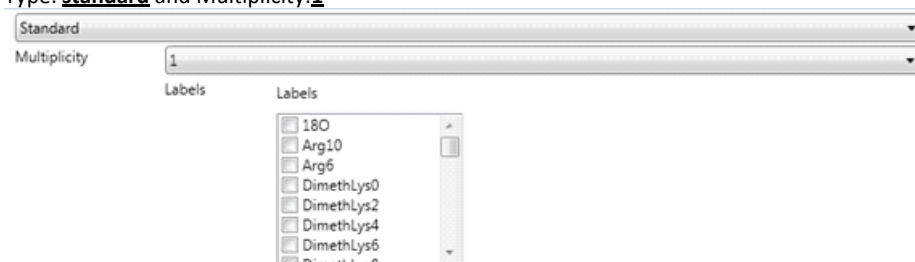
4. click "group-specific parameters" panel



4. select the type of data analysis:

a) identification only and label-free quantification (LFQ)

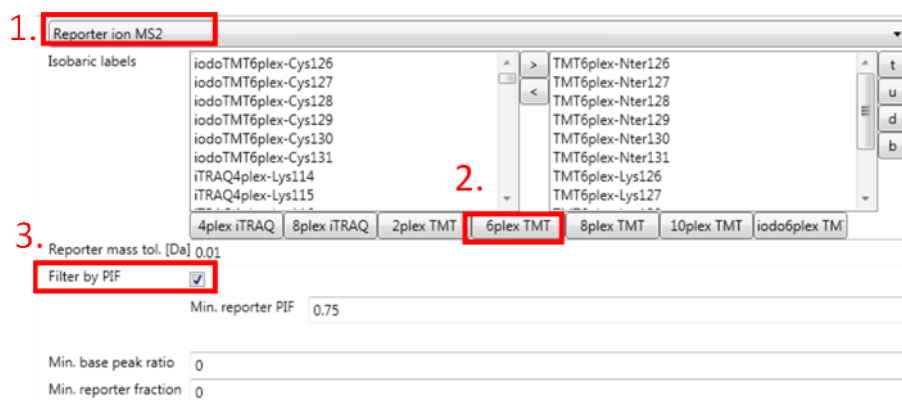
Type: standard and Multiplicity: 1



b) 6-plex TMT

Type: Reporter ion MS2 and click 6plex TMT

enable "filter by PIF"



c) 2plex SILAC and dimethyl labeling

Type: standard and Multiplicity: 2  
 Select the light and heavy isotope labels you used

5. click "Digestion" tab, and select the digestion enzyme you used (usually Trypsin/P or Trypsin)

6. click "modifications" tab, and select the variable modifications you would like to search to the right panel

e.g. add phospho(STY) for phosphoproteomic data

7. If you would like to perform LFQ and the number of samples more than 10, click "label-free quantification" tab, and (1) enable LFQ, and (2) LFQ ratio count: 1

If the number of your samples less than 10, disable "fast LFQ".

8. click "Instrument" tab, (1) main search tolerance: 6 ppm (2) if you use CID fragmentation, then enable "use MS1 and MS2 centroids"

Orbitrap

First search peptide tolerance **1.** 20

Main search peptide tolerance **6**

Peptide tolerance unit ppm

Individual peptide mass tolerance ☒

Isotope match tolerance 2

Isotope match tolerance unit ppm

Centroid match tolerance 8

Centroid match tolerance unit ppm

Centroid half width 35

Centroid half width unit ppm

Time valley factor 1.4

Isotope time correlation 0.6

Theoretical isotope correlation 0.6

Recalibration unit **2.** ppm

Use MS1 centroids ☒

Use MS2 centroids ☒

Intensity dependent calibration ☐

Min. peak length 2

Max. charge 7

Min score for recalibration 70

Cut peaks ☒

Gap scans 1

Advanced peak splitting ☐

Intensity threshold 500

Intensity determination Value at maximum

9. click "Global parameters" panel, add the database fasta file

**2.** Sequences

**1.** Global parameters

Parameter section

Fasta files

**3.** Add file Remove file

C:\Purdue\Lab\Tao\TAIR10\_pep\_20101214.fasta

Include contaminants ☒

Fixed modifications

Acetyl (K)  
Acetyl (N-term)  
Acetyl (Protein N-term)  
Amidated (C-term)  
Amidated (Protein C-term)  
Carbamidomethyl (C)  
Carbamyl (N-term)  
Cation/Na (DE)  
Cys-Cys  
Deamidation (N)

Carbamidomethyl (C)

Min. peptide length 7

Max. peptide mass [Da] 4600

Min. peptide length for unspecific search 8

Max. peptide length for unspecific search 25

10. If you perform LFI, (1) click "adv. Identification", (2) enable 'match between runs', and (3) match time window: **1.0** min

**1.** Adv. identification

Parameter section

Second peptides

Match between runs **2.** ☒

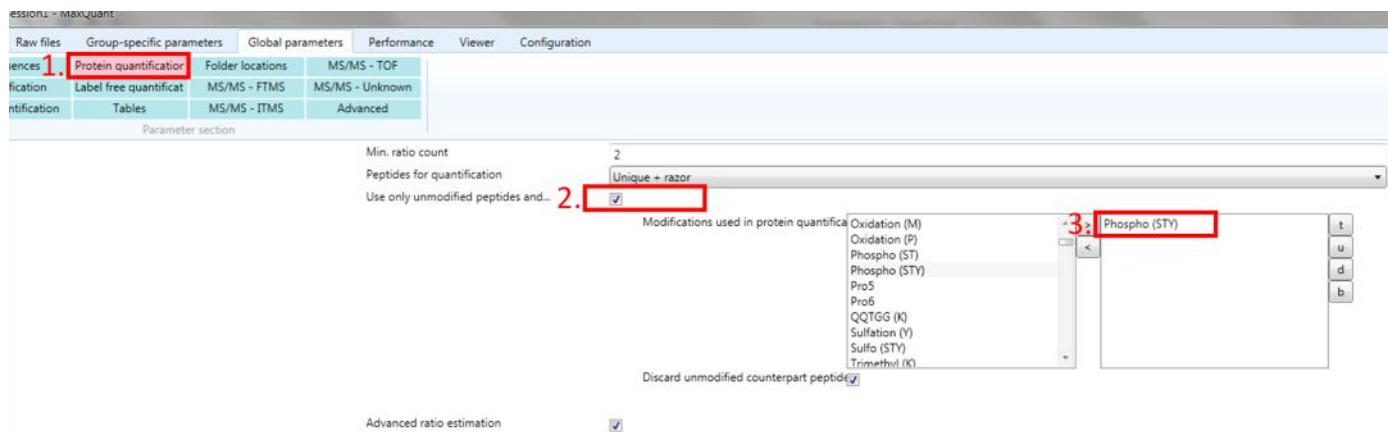
**3.** Match time window [min] 1.0

Alignment time window [m] 20

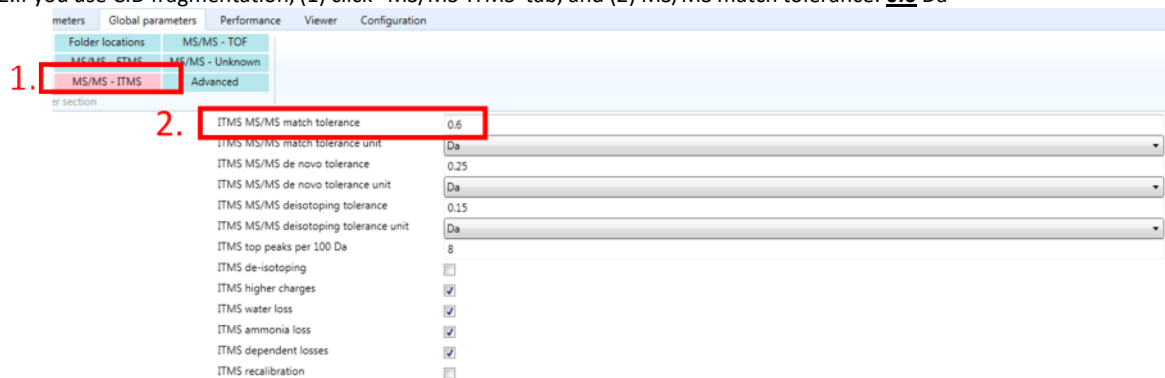
Match unidentified features ☐

Dependent peptides ☐

11. if you would like to quantify the amount of protein based on the specific modifications, (1) click "protein quantification" tab, and (2) enable use "unmodified and modified peptides" (3) select the modification you want



12. if you use CID fragmentation, (1) click "MS/MS-ITMS" tab, and (2) MS/MS match tolerance: 0.6 Da



13. select the number of threads you want to use (usually 4-8 threads), and click "start" tab to perform Maxquant analysis

