1 Общая информация о пайплайне

Пайплайн для xQuest/xProphet написан на языке snakemake, являющимся расширением языка Python. Snakemake отслеживает зависимость между этапами пайплайна через граф. Если какой-либо из используемых файлов был изменен (например, конфиг), то все последующие этапы пайплайна, зависящие от этого файла, переделываются.

2 Установка

1. Клонируем репозиторий https://github.com/noel-noel/xquest-pipeline:

```
git clone https://github.com/noel-noel/xquest-pipeline
```

2. В директории xquest-pipeline из терминала запускаем

```
chmod +x *.sh
./install.sh
```

3 Анализ

1. XQuest использует конфигурационные файлы для задания параметров эксперимента и анализа. Создадим в произвольной директории шаблонные конфиги, вызвав из терминала

```
/usr/local/share/xquest/deffiles/create_configs.sh
```

- 2. В любом текстовом редакторе изменяем конфиги под себя. На сервере можно воспользоваться nano или vim. В разделе 4 приведены наиболее важные параметры.
- 3. В любом текстовом редакторе открываем Snakefile. Необходимо задать **все** параметры из секции CONFIGURATION:
 - proj_name название проекта.
 Например, proj_name = "ph-analysis-sample-1".
 - xquest_def_path абсолютный путь файла xquest.def (см. п.1.). Haпример, xquest_def_path = "/home/noel/xquest.def".
 - xmm_def_path абсолютный путь файла xmm.def (см. п.1.). Например, xmm_def_path = "/home/noel/xmm.def".
 - xprophet_def_path абсолютный путь файла xproph.def (см. п.1.). Haпример, xprophet_def_path = "/home/noel/xproph.def".
 - raw_path абсолютный путь директории, в которой хранятся сырые файлы (.raw). Например, raw_path = "/home/noel/Documents/RAW/".

Комментарий 1: запрещено использовать системные директории в качестве raw_path. Можно: "/home/user/", "/home/user/dir/", "/home/user/dir1/.../dirn/". Нельзя: "/usr/lib/", "/mnt/data/", "/home/".

Kомментарий 2: Все файлы .raw, содержащиеся в директории raw_path будут использоваться для анализа.

• Запускаем пайплайн анализа, вызвав:

```
snakemake all
```

Комментарий 1: можно посмотреть количество запланированных snakemake задач:

```
snakemake -n all
```

Комментарий 2: можно распараллелить пайплайн, указав число потоков (e.g. 10):

```
snakemake -j 10 all
```

4 Конфигурационные файлы

$4.1 \quad xmm.def$

TABLE 1 | Important parameters of xmm.def when used with isotopically labeled cross-linkers or unlabeled cross-linkers.

Parameter	Value type	Example value	Description	
Parameters for isotopically labeled cross-linkers				
Isopair_Mr_tolerance_measure	String	p.p.m.	a.m.u. p.p.m., tolerance measure a.m.u. corresponds to atomic mass units (Da)	
Isopair_Mr_tolerance	Number in unit	15	Allowed tolerance for scan pairs in tolerance measure units	
Isopair_Tr_tolerance	Number in unit	3.0	Maximum relative retention time tolerance of light and heavy scans (in minutes)	
Isotopeshift	Mass	12.075321	Isotope shift or the cross-linker (always in Da)	
Printisotopicscanpairs	Integer	1	Use this to print the isotopic scan pairs, which are then used by the subsequent programs	
Parameters that need to be adjusted if an unlabeled cross-linker is used (light-only search)				
Isopair_Mr_tolerance	Mass	0		
Isopair_Tr_tolerance	Number in unit	0.02		
Isotopeshift	Mass	0		
Printisotopicscanpairs	Integer	0		
Printlightonlypairs	Integer	1	Prints only the light scans	

4.2 xquest.def

TABLE 2 | Important xQuest parameters defined in xquest.def.

Parameter	Value type	Example value	Description
Spectrum comparison parame	eters (compare_peaks	3.pl)	
cp_threshold	Integer	1	Minimum peak intensity
cp_tolerancemeasure	String	Da	(Da p.p.m.) Tolerance measure in Da or p.p.m.
cp_tolerance	Mass	0.2	Allowed tolerance in Da or p.p.m. for peak matching of common ions
cp_tolerancexl	Mass	0.3	Allowed tolerance in Da or p.p.m. for peak matching of cross-linker ions
cp_peakratio	Ratio	0.3	Required peak-intensity ratio lower peak/higher peak to be considered the same peak
cp_isotopediff	Mass	12.075321	Isotope difference of light and heavy cross-linker in Da
cp_nhighest	Integer	100	Use only n highest peaks after spectrum comparison
cp_minpeaknumber	Integer	25	Minimum number of common ions to be considered as a spectrum for the search
xQuest search modes and dat	tabase parameters		
Iontagmode	Integer	1	1 0: If 1, then the ion-tag mode is used
Enumerate	Integer	0	1 0: If 1, then the enumeration mode is used
RuntimeDecoys	Integer	1	$1 \vert 0 \colon \text{If 1, search in target-decoy mode used in combination with the ion-tag mode}$

Database Path /path/ Path to the target database (please do not use whitespaces in the paths) database_dc Path /path/ Path to the decoy database don't changet Generol x Quest search parameters enzyme_num Integer 1 Enzyme to be used for the in silico digest of the database. See enzyme reference at the end of the definition file missed_cleavages Integer 2 Maximum number of allowed missed cleavages. (recommended minimum 2) Mindigestlength Integer 5 Maximal digest length for a peptide to be considered for the search Maxdigestlength Integer 50 Maximal digest length for a peptide to be considered for the search Nocutatxlink Integer 1 1 1/0: If 1, then positions that correspond to the enzyme cleavage site are excluded as a potential cross-linking site Ionseries Sequence 010010 1/0 corresponding to the sequence abcxyz. Ion series that should be considered for the search. e.g., 010010 # abcxyz, considers be and y-ions ioncharge_common Integers 1,2,3 Charge states to be considered for common fragment ions. e.g., 1,2,3 ioncharge_stink Integers 2,3,4,5 Charge states to be considered for cross-linker containing fragment ions. e.g., 2,3,4,5 Cross-linker-specific search parameters Averquired Amino acid K Amino acid separated by comma, e.g., K,5 Xiinkermw Mass 138.0680796 Net mass shift for cross-linked, For more than one cross-linker in Da. Multiple masses can be separated by comma Avouest search tolerances Tolerancemasure—String Da (Da p.p.m.) MS1 tolerance measure in Da or p.p.m. ms1tolerance Number in unit 10 Tolerance for parent mass matching MS1 tolerance for parent mass matching for cross-linker containing fragment ions (Da p.p.m.) MS2 tolerance measure in Da or p.p.m. ms2tolerance Number in unit 0.2 Tolerance for parent mass matching for cross-linker containing fragment ions LID—match2ndisotope Integer Number in unit 0.3 Tolerance for parent mass matching for cross-linker containing fragment ions LID—match2ndisotope Integer Number in unit 0.3 Maximum ion size in MS2 mode to be considered in	xQuest database definitions			
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·	CID_match2ndisotope	Integer	1	1 0, 1 allows matching of 2nd isotope
Maximum ion size in MS2 mode to be considered in m/z	Minionsize	m/z	200	Minimum ion size in MS2 mode to be considered in $\ensuremath{m/z}$
	Maxionsize	m/z	2000	Maximum ion size in MS2 mode to be considered in $\it m/\it z$
xQuest modification settings				
variable_mod Amino acid, M,15.99491 0 mod: Defines a variable modification for a particular amino acid, separated by comma	variable_mod		M,15.99491	·
nvariable_mod Integer 1 Maximum number of variable modifications per peptide	nvariable_mod	Integer	1	Maximum number of variable modifications per peptide
modifications fixed Amino acid, C, 57.02146 Defines fixed modification for a particular amino acid, separated by tab	modifications fixed		C, 57.02146	
Parameters that need to be adjusted if an unlabeled cross-linker is used (light-only search)				
cp_isotopediff Mass 0	cp_isotopediff	Mass	0	

4.3 xproph.def

TABLE 3 | Important xProphet parameters defined in xproph.def.

Parameter	Value type	Example value	Description
Minborder	Number in unit	-10	Filter for minimum precursor mass error in precursor mass tolerance unit used for the xQuest search (usually p.p.m.) $\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$
Maxborder	Number in unit	10	Filter for maximum precursor mass error in precursor mass tolerance unit used for the xQuest search (usually p.p.m.) $\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$
Mindeltas	Number in unit	0.95	O for not using this filter; filter for delta score, minimum delta score required, hits are filtered if larger or equal than the given value
Uniquexl	Integer	1	1 0: 1 calculate statistics based on unique IDs
Qtransform	Integer	1	1 0: 1 transform simple FDR to q-FDR values as described in ref. 48
Nidx	Integer	1	1 0: 1 reparse xml file