User manual of JUMPp Batch

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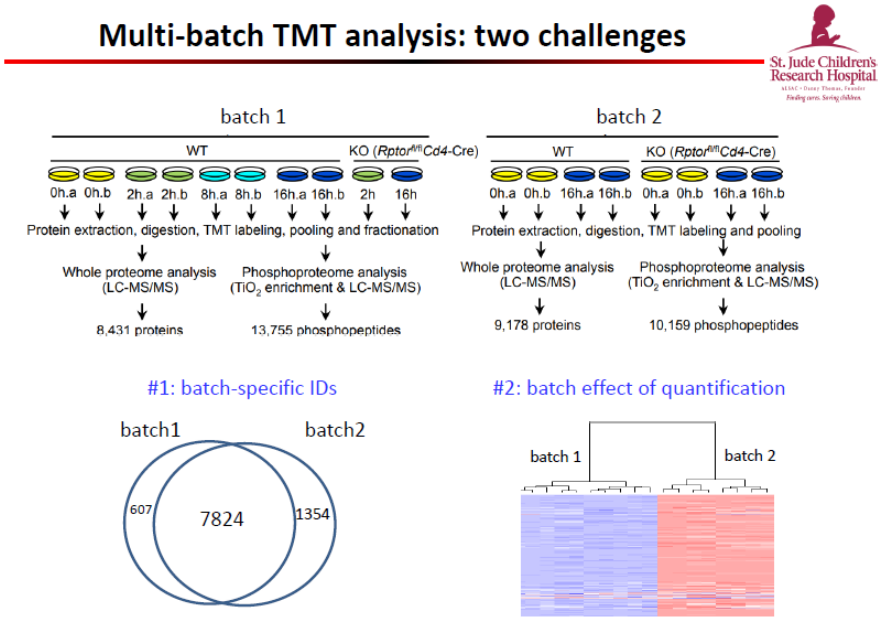
Contents

1. Introduction

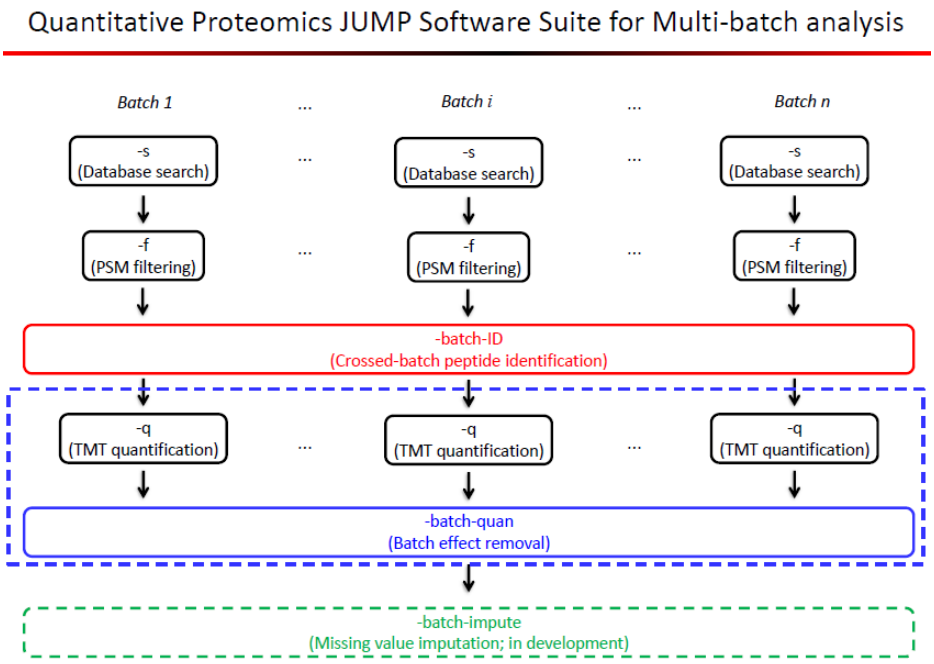
There are two kinds of challenges in multi-batch TMT analysis as below.



For challenge #1, we developed JUMPp -batch-ID by using peptides identified from other batches to rescue peptides in the current batch.

For challenge #2, we developed JUMPp -batch-quan by using internal standard or linear model fitting to normalize crossed-batch signals.

Here is the overall workflow for the multi-batch TMT analysis.



2. How to setup and run JUMPp Batch

2.1 Run JUMPp Batch

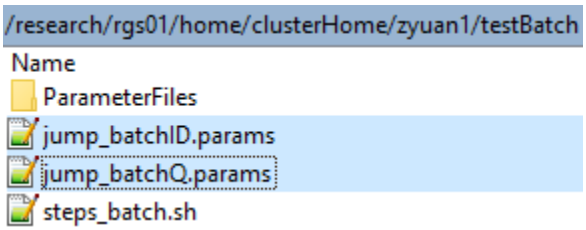
2.1.1 JUMPp Batch setup

Login HPC (PuTTY and WinSCP) and go to a work path (e.g. /home/zyuan1/testBatch).

a. In the work path, load batch params in PuTTY.
   module load jump/1.13.003
   jump -batch-params

```
[zyuan1@splprhpc05 testBatch]$ module load jump/1.13.003
[zyuan1@splprhpc05 testBatch]$ jump -batch-params
```

b. Edit batch params (i.e. jump_batchID.params, jump_batchQ.params) in WinSCP. Skip this step if you want to run the example data.



c. Run batch-ID in PuTTY (by default the results are in folder 'batch_id').
   jump -batch-id jump_batchID.params

```
[zyuan1@splprhpc06 testBatch]$ jump -batch-id jump_batchID.params
```

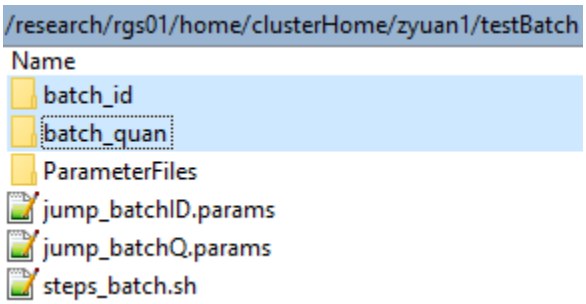
d. Run batch-quan in PuTTY (by default the results are in folder 'batch_quan').
   jump -batch-q jump_batchQ.params

```
[zyuan1@splprhpc06 testBatch]$ jump -batch-q jump_batchQ.params
```

Alternatively, step c & d can be run in one command: bash steps_batch.sh

```
[zyuan1@splprhpc06 testBatch]$ bash steps_batch.sh
```

Here show the results folders.



2.1.2 Edit batch params

jump_batchID.params

```
# Inputs: absolute path of publication tables from JUMP -f results (IDwDecoy.txt)
input_path_batch1 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/batch_test/b1/sum_HH_tmt/IDwDecoy.txt
input_path_batch2 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/batch_test/b2/sum_HH_tmt/IDwDecoy.txt

# thresholds:
min_Jscore = 10          # minimum Jscore cutoff to be considered (10 for JUMP; 1 for Comet)
multiHit_max_dJn = 0.1       # for considering non-top hits for a PSM: PSMs within such dJn range will be considered in a re

enable_group_specific_Jscore = 1     # enable group specific Jscore filtering. each group defined by charge states and pepti
score_cutoff_quantile = 0    # Jscore threshold for each group, defined as the lower quantile (default 5%) of Jscores in eac

# modifications
mods = 0             # Display modified peptides and their unmodified (0:Off, K:Lys, STY: Phosphorylation, ...); same as -f
#mods = STY          # Display modified peptides and their unmodified (0:Off, K:Lys, STY: Phosphorylation, ...); same as -f

# Output:
output_folder = batch_id         # output folder name

# other parameters:
jump_f_path = /research/rgs01/applications/hpcf/authorized_apps/proteomics_apps/pipeline/release/version1.13.003/JUMP/bin/b
# (in JUMP, pit_file = 0; in Comet, set pit_file according to search)
# pit_file = /hpcf/authorized_apps/proteomics_apps/database/20150201/human_ft_mc2_c57_TMT_K229.pit
pit_file = 0
database = 0

# HPC parameters
dispatch = localhost
```

jump_batchID.params: pay attention to the below params, and change others if you need.

(1) input_path_batch1, …, input_path_batchn: absolute path of publication tables from JUMP -f results (IDwDecoy.txt)

(2) min_Jscore: minimum Jscore cutoff to be considered (10 for JUMP; 1 for Comet)

(3) output_folder: output folder name (the default is batch_id). If it is changed here, remember to change it in ‘path_batch_id’ of ‘jump_batchQ.params’.

(4) pit_file: in JUMP, pit_file = 0; in Comet, set pit_file according to search

jump_batchQ.params

```
# JUMP batch correction parameter file
input_mode = 1                  # 1: proteins (for whole proteome); 2: pho site; 3: peptides (from either phosphor- or whole

# output path of -batch-id results
path_batch_id = /research/rgs01/home/clusterHome/zyuan1/testBatch/batch_id

# ATTENTION: jump -q will run automatically for all batches using DEFAULT parameters
# Otherwise, User can edit filtering options within ParameterFiles/TMThh/jump_qj_HH_tmt10_human.params to customize jump -q r

# Specify TMT-plex for each batch (that match to jump -batch-id results)
input_n_batch1 = 10
input_n_batch2 = 10

# Outputs:
output_folder = quan        # output folder suffix name; prefix always 'batch'

# Parameters:
normalization_method = 1            # 0: None (i.e., just combine publication tables); 1: using internal standard; 2: using l
isoform_rescue = 1              # 0: turn off; 1: turn on function. Suppose for a gene, there are two isoforms (say a and b)

# Internal standards for each batch. The most comprehensive batch should be put first
internal_standard_batch1 = sig126           # internal standard for each batch
internal_standard_batch2 = sig126           # internal standard for each batch

# -i parameters
jump_i_path = /research/rgs01/applications/hpcf/authorized_apps/proteomics_apps/pipeline/release/version1.13.003/JUMP/bin/bat

# jump -q parameters (the values here will overwrite the default values copied from the ParameterFiles/ folder above)
ppi_filter = 50                             # precursor peak intensity percentage threshold
impurity_correction = 1                         # 1 = Yes; 0 = No; if only a part of reporters are used, it should be set to
loading_bias_correction = 1                     # 1 = Yes; 0 = No;
interference_removal = 0                        # 1 = Yes; 0 = No;
```

jump_batchQ.params: pay attention to the below params, and change others if you need.

(1) path_batch_id: output path of -batch-id results, the same folder name as 'output_folder' of 'jump_batchID.params'.

(2) jump -q will run automatically for all batches using DEFAULT parameters. Users can edit filtering options within ParameterFiles/TMThh/jump_qj_HH_tmt10_human.params to customize jump -q runs.

(3) input_n_batch1, …, input_n_batchn: specify TMT-plex for each batch (that match to jump -batch-id results).

(4) internal_standard_batch1, …, internal_standard_batchn: internal standards for each batch if normalization_method = 1 (using internal standard).

(5) jump -q parameters (the values here will overwrite the default values copied from the ParameterFiles/ folder above)

Here we show 4 cases to edit parameters.

Case1: phos data, input_mode = 1

jump_batchID.params:

input_path_batch1 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b1/sum_HH_tmt/IDwDecoy.txt

input_path_batch2 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b2/sum_HH_tmt/IDwDecoy.txt

mods = 0

It can be edited: /ParameterFiles/TMThh/jump_qj_HH_tmt10_human.params

jump_batchQ.params:

input_mode = 1 # 1: proteins (for whole proteome)

input_n_batch1 = 11

input_n_batch2 = 11

Case2: phos data, input_mode = 2

jump_batchID.params:

input_path_batch1 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b1/sum_HH_tmt_mod/IDwDecoy_mod.txt

input_path_batch2 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b2/sum_HH_tmt_mod/IDwDecoy_mod.txt

mods = STY

It can be edited: /ParameterFiles/jump_l.params

It can be edited: /ParameterFiles/TMThhpho/jump_qj_HH_pho_tmt10_human.params

jump_batchQ.params:

input_mode = 2 # 2: pho site

input_n_batch1 = 11

input_n_batch2 = 11

Case3: phos data, input_mode = 3

jump_batchID.params:

input_path_batch1 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b1/sum_HH_tmt_mod/IDwDecoy_mod.txt

input_path_batch2 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/phos_test/b2/sum_HH_tmt_mod/IDwDecoy_mod.txt

mods = STY

It can be edited: /ParameterFiles/TMThhpho/jump_qj_HH_pho_tmt10_human.params

jump_batchQ.params:

input_mode = 3 # 3: peptides (from either phosphor- or whole proteome)

input_n_batch1 = 11

input_n_batch2 = 11

Case4: comet data, input_mode = 1

jump_batchID.params:

input_path_batch1 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/comet_test/b1/sum_HH_tmt/IDwDecoy.txt

input_path_batch2 = /hpcf/authorized_apps/proteomics_apps/pipeline/release/SampleData/comet_test/b2/sum_HH_tmt/IDwDecoy.txt

min_Jscore = 1

mods = 0

pit_file = /home/yli4/database/MS_proteomics/2017Feb_human_comprehensiveDB/humanComprehensive_v1_ft_mc2_c57_TMT_K229.pit

It can be edited: /ParameterFiles/TMThh/jump_qj_HH_tmt10_human.params

jump_batchQ.params:

input_mode = 1 # 1: proteins (for whole proteome)

2.2 Troubleshoot

(1) If you cannot use jump as below, run 'module load jump/1.13.003' first.

```
[zyuan1@splprhpc05 testBatch]$ jump -batch-id jump_batchID.params
-bash: jump: command not found
```

(2) If batch numbers of 'input_path_batch' in 'jump_batchID.params', 'input_n_batch' in 'jump_batchQ.params', and 'internal_standard_batch' in 'jump_batchQ.params' are not the same, the workflow will stop and ask you to make them consistent.

(3) If 'path_batch_id' of 'jump_batchQ.params' is not consistent with 'output_folder' of 'jump_batchID.params', it will stop and ask you to make them consistent.

(4) If you make changes in jump_batchID.params and jump_batchQ.params in the work path, jump_qj_HH_tmt10_human.params in /ParameterFiles/TMThh, the changes will not be replaced if you rerun 'jump -batch-params'.

(5) Please be patient when applying a compute node with required RAM as sometimes there is long waiting time.