DeepDIA Demo: Spectral Library Generation From Peptide Lists

Using deep learning to generate in silico spectral libraries from peptide lists for data-independent acquisition (DIA) analysis.

1. System Requirements

In this demo, spectral library generation has been tested on a workstation with Intel Xeon E5-2690 v3 CPU, 16 GB RAM, and Microsoft Windows Server 2016 Version 1607 (OS Build 14393.2430) operating system with the following softwares:

- Anaconda 4.2.0 (Python 3.5.2).
- Keras 2.2.4 and TensorFlow 1.11.
- Microsoft R Open 3.5.1.
- RStudio 1.1.447.
- R pakages `rjson` and `readr`.

DIA data analysis is performed on a workstation with Intel Core i9-7960X CPU, 128 GB RAM, and Microsoft Windows 10 Version 1809 (OS Build 17763.503) 64-bit operating system with the following softwares:

- Spectronaut 12.0.20491.

2. Demo Data

LC-MS/MS data of 3 DIA technical replicates of 2 h gradient of HeLa cells on Q Exactive HF are available at ProteomeXchange (http://proteomecentral.proteomexchange.org/) with the data set `PXD005573`. (Bruderer, R. et al. Mol. Cell. Proteomics 2017, 16, 2296-2309.)

- Fig1_MP-DIA-120min120kMS1-22W30k-8dppp_MHRM_R01.raw
- Fig1_MP-DIA-120min120kMS1-22W30k-8dppp_MHRM_R02.raw
- Fig1_MP-DIA-120min120kMS1-22W30k-8dppp_MHRM_R03.raw

SwissProt *Homo sapiens* database (FASTA) can be downloaded from UniProt (https://www.uniprot.org/). The FASTA file (2018-04 version, 20,301 entries)
has been deposited to ProteomeXchange via the iProX partner repository with the data set identifier `PXD014108/IPX0001628000`.

- swissprot_human_201804_validated.fasta

Pre-trained models for Q Exactive HF trained with HeLa data from `PXD005573` are available at ProteomeXchange/iProX with identifier `PXD014108/IPX0001628000`.

- HeLa.model.zip

They are also provided in `data/models` folder.

Peptide lists (CSV) collected from the Pan Human Library (Rosenberger, G. et al. Sci. Data 2014, 1, 140031) are provided in `data/peptides` folder.

- Pan_human.peptide.csv
- Pan_human_charge2.peptide.csv
- Pan_human_charge3.peptide.csv

Generated spectral libraries and the saved projects from Spectronaut are also available at ProteomeXchange/iProX with identifier `PXD014108/IPX0001628000`.

- Pan_human_prediction.library.zip
- Pan_human_prediction.kit
- HeLa_Pan_prediction.sne.zip
- HeLa_Pan_prediction.csv.zip

3. Spectral Library Generation

3.1. Prepare Peptide Lists

A peptide list is stored in a comma-separated values (CSV) file including a column named `sequence`. DeepDIA only supports peptide sequences with standard amino acids (ACDEFGHIKLMNPQRSTVWY) and length <= 50.

In this demo, the peptide list files are provided in `data/peptides` folder.

3.2. Predict MS/MS Spectra

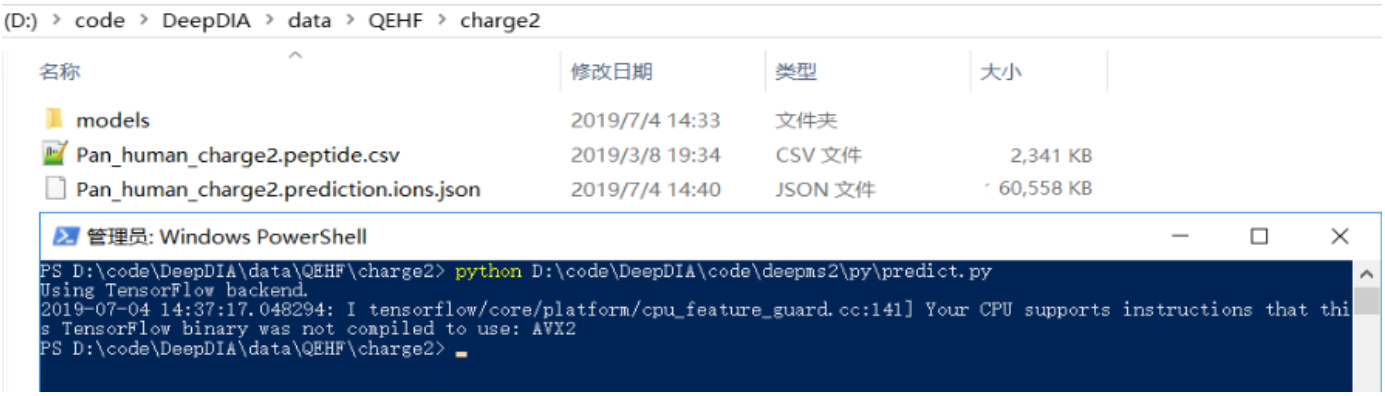
In this demo, pre-trained models `data/model/charge2` and `data/model/charge3` are used for MS/MS prediction.

Copy the peptide list file `data/peptides/Pan_human_charge2.peptide.csv` to the model directory.

Run `deepms2/py/predict.py` in the directory.

```
cd {PATH_TO_MODEL}
python {PATH_TO_CODE}/deepms2/py/predict.py
```

Expected run time depends on the number of peptides and the performance of the computer. In this demo, this command may take about 5 min.



Predict MS/MS spectra for charge 3+ following the same steps.

3.3. Predict iRT

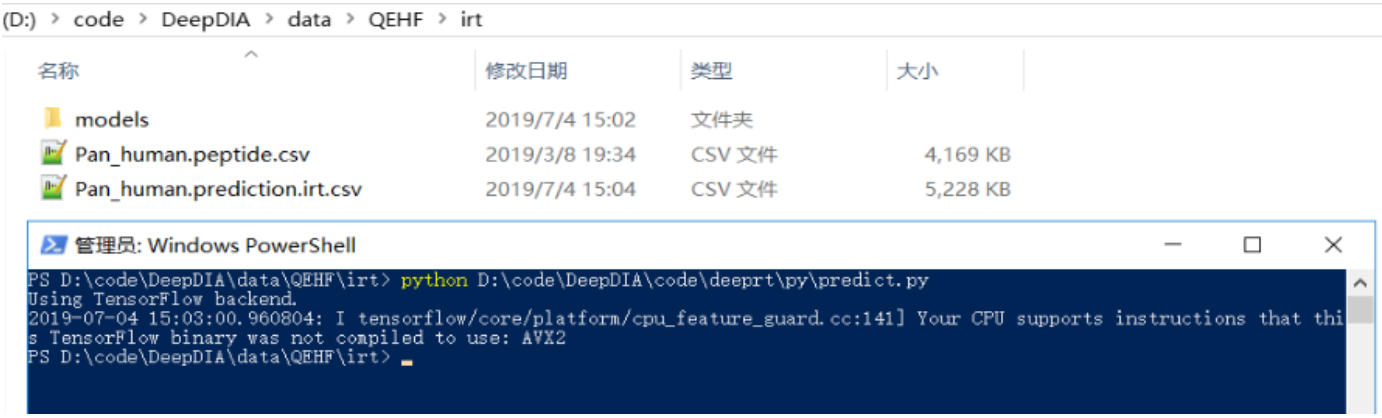
In this demo, a pre-trained model `data/model/irt` is used for iRT prediction.

Copy the peptide list file `data/peptides/Pan_human.peptide.csv` to the model directory.

Run `deeprt/py/predict.py` in the directory.

```
cd {PATH_TO_MODEL}
python {PATH_TO_CODE}/deeprt/py/predict.py
```

Expected run time depends on the number of peptides and the performance of the computer. In this demo, this command may take about 2 min.



3.4. Generate Spectral Library

Move the predicted MS/MS and iRT files to the same directory with the peptide list.

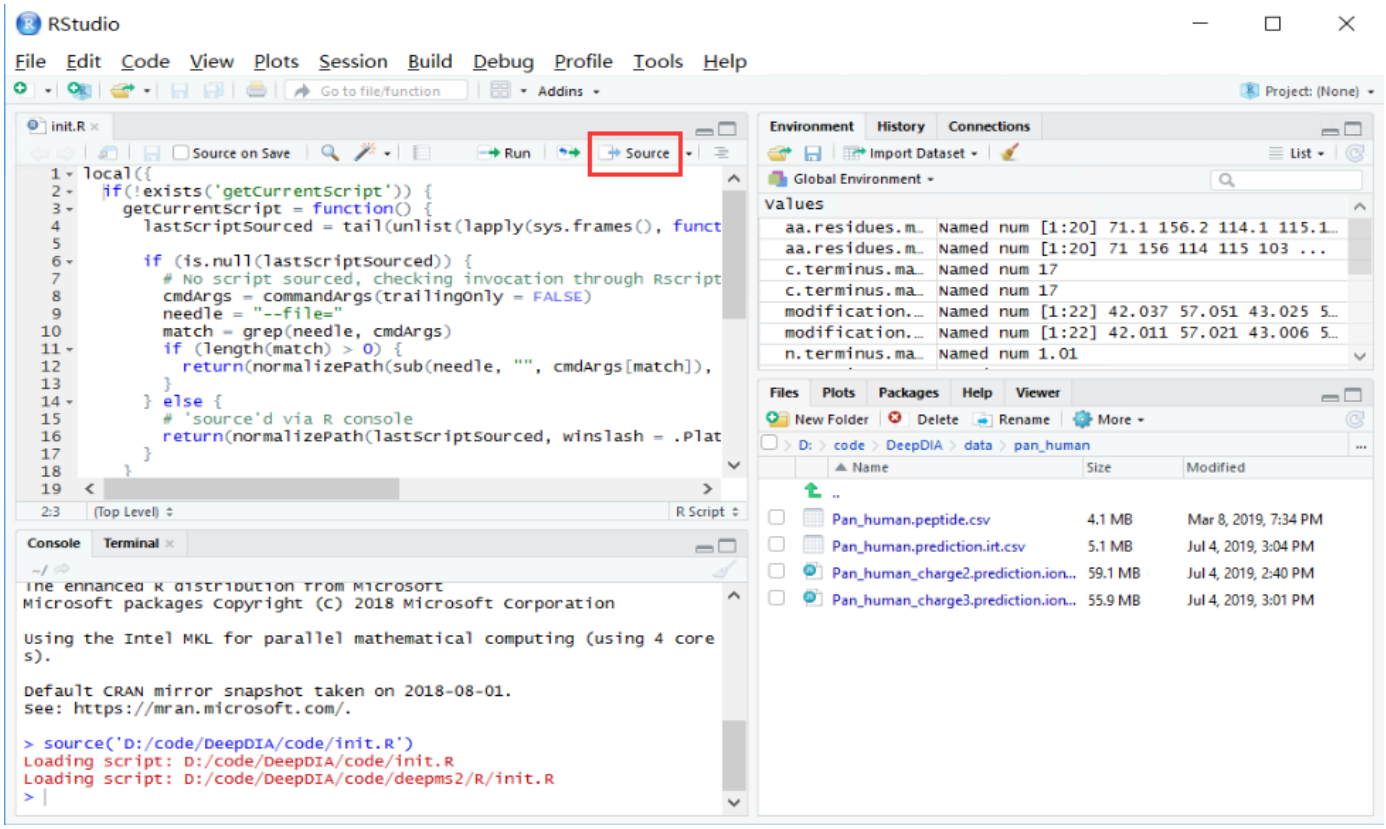
- Pan_human.peptide.csv
- Pan_human_charge2.prediction.ions.json
- Pan_human_charge3.prediction.ions.json
- Pan_human.prediction.irt.csv

Start RStudio, ensure packages `readr` and `rjson` have been installed.

```
install.packages("readr")
install.packages("rjson")
```

Open `init.R` and run the script by clicking `Source`.

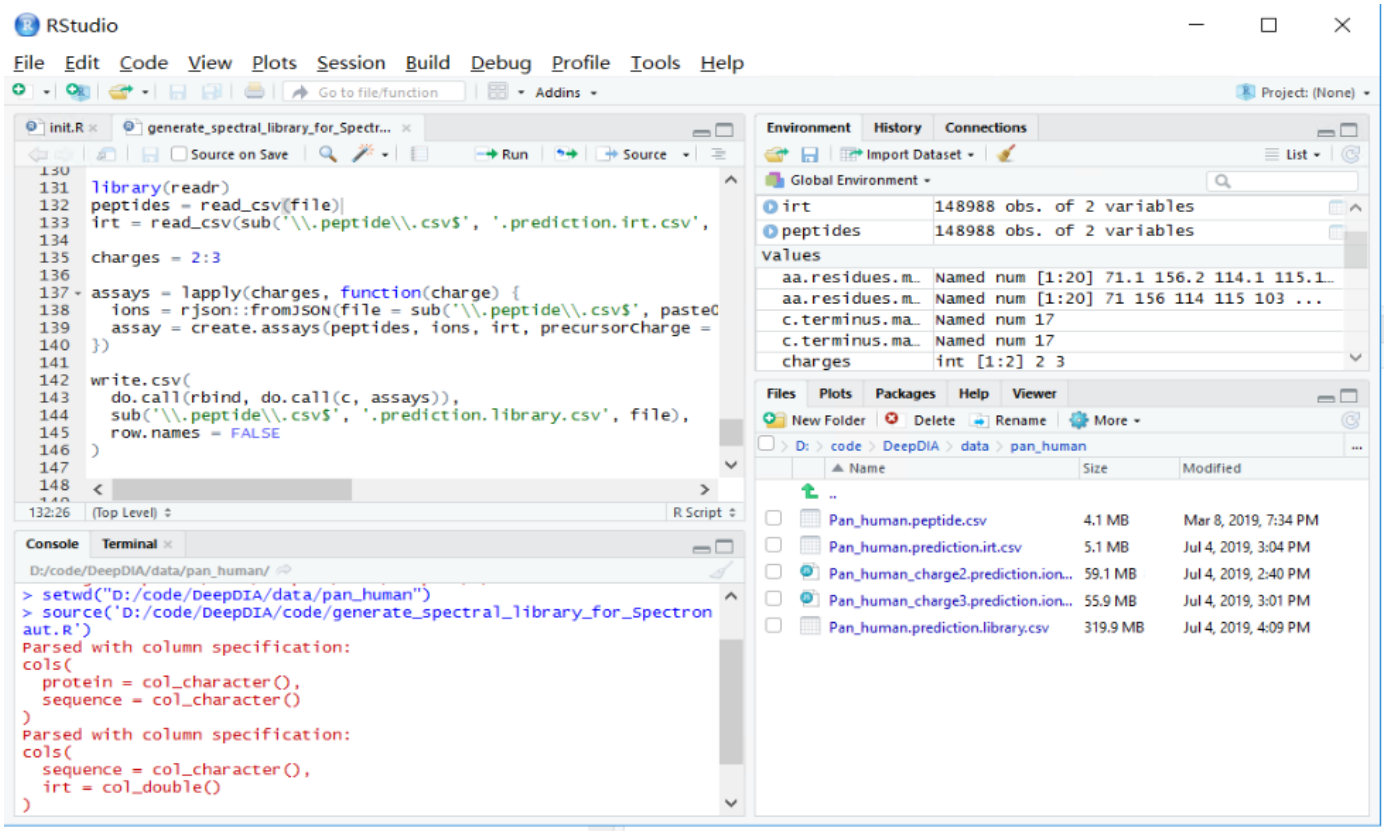
```
source("{PATH_TO_CODE}/init.R")
```



Set the peptide list directory as working directory and run `generate_spectral_library_for_Spectronaut.R`.

```
setwd("{PATH_TO_DATA}")
source("{PATH_TO_CODE}/generate_spectral_library_for_Spectronaut.R")
```

Expected run time depends on the number of peptides and the performance of the computer. In this demo, this command may take up to 1 h.



The output library file can be imported into Spectronaut.

- Pan_human.prediction.library.csv

4. DIA Data Analysis

4.1. Import the Spectral Library

Open Spectronaut, import library `Pan_human.prediction.library.csv` with FASTA file `swissprot_human_201804_validated.fasta` with the default parameters.

4.2. Perform DIA Analysis

Set a DIA experiment, load the raw data and the spectral library with the default parameters.

```
Settings Used: BGS Factory Settings
    ├── Data Extraction
    |   ├── MS1 Mass Tolerance Strategy:   Dynamic
    |   |   └── Correction Factor:  1
    |   ├── MS2 Mass Tolerance Strategy:   Dynamic
    |   |   └── Correction Factor:  1
```

```
|   ├── Intensity Extraction MS1:  Maximum Intensity
|   └── Intensity Extraction MS2:  Maximum Intensity
├── XIC Extraction
|   └── XIC RT Extraction Window:  Dynamic
|       └── Correction Factor:  1
├── Calibration
|   ├── Calibration Mode:  Automatic
|   ├── Precision iRT: True
|   |   ├── iRT <-> RT Regression Type: Local (Non-Linear) Regression
|   |   └── Exclude Deamidated Peptides:    True
|   ├── MZ Extraction Strategy:    Maximum Intensity
|   ├── Allow source specific iRT Calibration: True
|   ├── Used Biognosys’ iRT Kit:   True
|   └── Calibration Carry-Over:    False
├── Identification
|   ├── Generate Decoys:   True
|   |   ├── Decoy Limit Strategy:   Dynamic
|   |   |   └── Library Size Fraction:   0.1
|   |   └── Decoy Method:   Mutated
|   ├── Machine Learning:  Per Run
|   ├── Exclude Duplicate Assays:  True
|   ├── Protein Qvalue Cutoff: 0.01
|   ├── Exclude Single Hit Proteins:   False
|   ├── PTM Localization:  True
|   |   └── Probability Cutoff: 0.75
|   ├── Pvalue Estimator:  Kernel Density Estimator
|   ├── Precursor Qvalue Cutoff:   0.01
|   └── Single Hit Definition: By Stripped Sequence
├── Quantification
|   ├── Interference Correction:   True
|   |   ├── MS1 Min:    2
|   |   ├── MS2 Min:    3
|   |   └── Exclude All Multi-Channel Interferences:    True
|   ├── Major (Protein) Grouping:  by Protein Group Id
|   ├── Minor (Peptide) Grouping:  by Stripped Sequence
|   ├── Minor Group Top N: True
|   |   ├── Min:    1
```

```
    |   |   └── Max:    3
    |   ├── Minor Group Quantity:  Sum precursor quantity
    |   ├── Major Group Top N: True
    |   |   ├── Min:    1
    |   |   └── Max:    3
    |   ├── Major Group Quantity:  Sum peptide quantity
    |   ├── Quantity MS-Level: MS2
    |   ├── Quantity Type: Area
    |   ├── Proteotypicity Filter: None
    |   ├── Data Filtering:    Qvalue
    |   └── Cross Run Normalization:   True
    |       ├── Row Selection:  Automatic
    |       └── Normalization Strategy: Local Normalization
    ├── Workflow
    |   ├── Profiling Strategy:    None
    |   ├── Unify Peptide Peaks Strategy:  None
    |   └── Multi-Channel Workflow Definition: From Library Annotation
    |       └── Fallback Option:    Labeled
    ├── Protein Inference
    |   └── Protein Inference Workflow:    Automatic
    ├── Post Analysis
    |   ├── Calculate Sample Correlation Matrix:   False
    |   ├── Calculate Explained TIC:   None
    |   ├── Gene Ontology: C:\Users\admin\AppData\Roaming\Spectronaut\geneOntology/Ontologies\bgs_default_go-basic.obo
    |   ├── Differential Abundance Grouping:   Major Group (Quantification Settings)
    |   |   └── Smallest Quantitative Unit: Precursor Ion (Quantification Settings)
    |   └── Differential Abundance Testing:    Student's t-test
    |       ├── Run Clustering: True
    |       |   ├── Distance Metric: Manhattan Distance
    |       |   ├── Linkage Strategy:    Ward's Method
    |       |   └── Z-score transformation:  False
    |       └── Group-Wise Testing Correction:  False
    └── Pipeline Mode
        ├── Post Analysis Reports:
        |   ├── Scoring Histograms: False
        |   ├── Data Completeness Bar Chart:    False
```

```
|   ├── Run Identifications Bar Chart:  False
|   ├── CV Density Line Chart:  False
|   └── CVs Below X Bar Chart:  False
├── Generate SNE File: False
├── Report Schema: BGS Factory Report (Normal)
└── Reporting Unit:    Across Experiment
```

Spectronaut Manual is available at https://www.biognosys.com/shop/spectronaut.

For benchmarking purpose, the saved projects using a sample-specific spectral library generated by data-dependent acquisition (DDA) experiments and the Pan Human library are also available at ProteomeXchange/iProX with identifier `PXD014108/IPX0001628000`.

- HeLa_DDA.kit
- HeLa_prediction.kit
- HeLa_DDALib.sne.zip
- HeLa_DDALib.csv.zip
- HeLa_Pan.sne.zip
- HeLa_Pan.csv.zip