FluSeq Guide

Version 1.0 for Illumina Miseq system.
For Research Use only. Not for use in diagnostic procedures.

FluSeq is a bioinformatics program developed and tested for an influenza A genome-wide amplicon-based high-throughput sequencing (HTS) method, using Illumina Miseq system [1]. It includes contigs assembly, blastn, filters for in-run contamination reads, and consensus sequence calling. Users are advised to refer the end-to-end laboratory protocol described by Lee, et al. 2016 [1]. Users are encouraged to modify the FluSeq program written in python scripts for other clinical virus amplicon-based HTS methods.

INSTALLATION

Installation instructions are available for LINUX/UNIX or MacOSX only. The entire analytic workflow was implemented and tested by the author on CentOS-6.6/RedHat Linux and MacOSX, but not on other operating systems. The operating system should contain the latest version of java.

1. Install Python 3.4.3 (https://www.python.org/downloads/)
   - Upon completion of installation, log in as root from Terminal (Linux and MacOSX):

```
# pip install pandas
# pip install numpy
# pip install ZODB
# pip install lxml
# pip install xlrd
# pip install xlwt
# pip install beautifulsoup4
# pip install scipy
# pip install transaction
```

2. Install VICUNA-v1.3
   (http://www.broadinstitute.org/scientific-community/science/projects/viral-genomics/vicuna)

   - The vicuna_config.txt used by this analytic workflow is stored in FluSeq Folder. Compilation of vicunAnalysis is not required during the installation.

   - Path to the compiled vicuna executive file needs to be changed accordingly in the FluSeq-v1.0.py, i.e. Line 100: First argument of the subprocess.Popen, to “YourPathInstalled/VICUNA_v1.3/bin/vicuna-omp-v1.0”

   - Optional: Line 104 - logging.info: Change program path to “YourPathInstalled/VICUNA_v1.3/bin/vicuna-omp-v1.0”.

3. Install BLAST 2.2.31+ software
   (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download)

4. Install BWA-0.6.2 software
   (http://sourceforge.net/projects/bio-bwa/files/)

   - Users are advised to install BWA-0.6.2 software but not any version after this, as bwa-aln/sampe in this version was found to be more stable in reads alignment for this study.

5. Install samtools-1.2
   (http://sourceforge.net/projects/samtools/files/samtools/1.2/)

6. Install GATK-3.4-46 software
   (https://www.broadinstitute.org/gatk/download/)

   - Path to the GenomeAnalysisTK.jar executive file needs to be changed accordingly in the FluSeq-v1.0.py, i.e. Lines 233, 259, and 269: Third or Fourth argument of the subprocess.Popen, to "YourPathInstalled/GenomeAnalysisTK.jar".

   - Optional: Lines 243, 265, and 278: logging.info: Change program path to "YourPathInstalled/GenomeAnalysisTK.jar".

7. Install picard-tools-1.138
   (https://github.com/broadinstitute/picard/releases/tag/1.138)

   - Path to the GenomeAnalysisTK.jar executive file needs to be changed accordingly in the FluSeq-v1.0.py, i.e. Line 146: Third argument of the subprocess.Popen, to "YourPathInstalled/picard-tools-1.138/picard.jar".

   - Optional: Line 152: logging.info: Change program path to "YourPathInstalled/picard-tools-1.138/picard.jar".

8. Install FluSeq

   a. Download the FluSeq.tar, decompress, and mv the FluSeq Folder to ~.
      ```
      $ tar –xvf FluSeq.tar
      $ mv FluSeq ~
      ```

   b. Change working directory to FluSeq
      ```
      $ cd ~/FluSeq
      ```

USAGE

1.0 Influenza Sequence Database

This section describes the sequence database used for blastn. It contains all influenza sequences available in GenBank, updated to the date accessed by the user.

Creating/Updating Sequence Database

1. Download all influenza sequences available from NCBI Influenza Virus Resource with a customized FASTA defline as ">{accession}|{strain}|{segment}|{serotype}" and save the fasta file as FASTA.fa in FASTADatabase directory

2. Change working directory to FASTADatabase
   $ cd ~/FluSeq/FASTADatabase

3. Execute DBupdate
   $ ./ DBupdate.py FASTA.fa
   or
   $ python3 DBupdate.py FASTA.fa
   or
   $ python3.4 DBupdate.py FASTA.fa

2.0 FluSeq INPUT FILES and FOLDER

This section describes the input FluSeq-v1.0 requires.

Folder and File Naming

The top-level input folder can be named in any combination of alphanumeric characters. It is advisable that the first letter of the name of a folder is in upper case for a better folder and file organization, e.g. Folder20151231 (**Figure 1** – Folder in red).

The input folder should contain paired-end reads in fastq.gz format generated by Miseq Reporter or fastq2bcl2, e.g. SampleID_L001_R1_001.fastq.gz and SampleID_L001_R2_001.fastq.gz (**Figure 1**). Also, it should contain the ResequencingRunStatistics.xml that can be copied from the top-level run folder named according to Miseq <ExperimentName>, e.g. YYMMDD_machinename_experimentnumber_flowcellnumber.

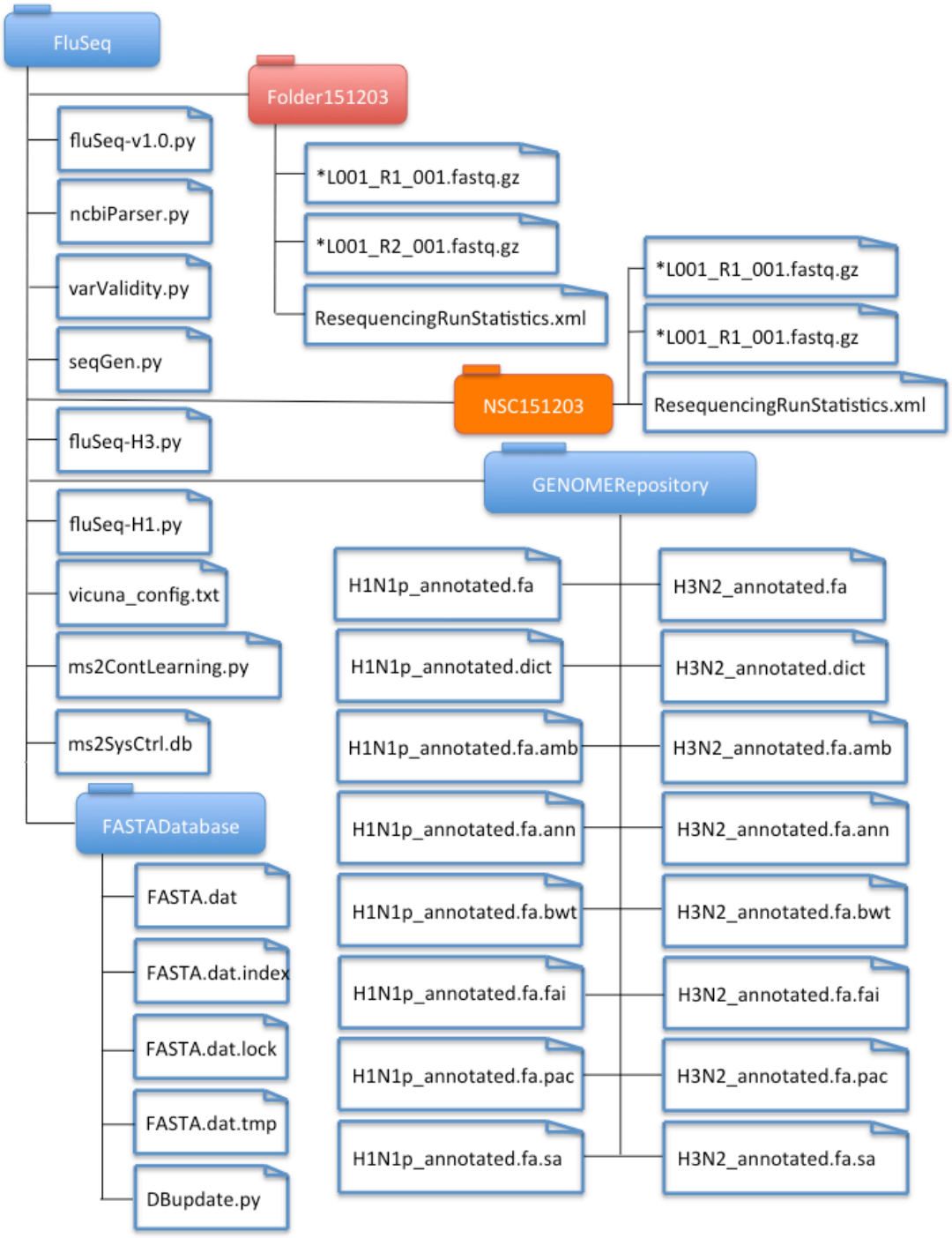

Figure 1. Directory of FluSeq and input folder. Input folders for run analysis and contamination learning are colored in red and orange, respectively.

3.0 Contamination Learning (Optional)

The current FluSeq software provides the database file for MS2 contamination statistics (i.e. ms2SysCtrl.db). However, it is recommeded that individual laboratories generate a laboratory-specific database file to allow more accurate statistics.

The input folder for in-run contamination learning should contain paired-end reads for an in-run negative system control (NSC) in fastq.gz format generated by Miseq Reporter or fastq2bcl2, e.g. SampleID_L001_R1_001.fastq.gz and SampleID_L001_R2_001.fastq.gz (**Figure 1 –** Folder in orange). Also, it should contain the ResequencingRunStatistics.xml that can be copied from the top-level run folder named according to Miseq <ExperimentName>, e.g. YYMMDD_machinename_experimentnumber_flowcellnumber.

The database will be updated according for each execution. A new database file will be generated if the ms2SysCtrl.db file is not found in the FluSeq folder.

Procedure

1. Change working directory to FluSeq
   $ cd ~/FluSeq

2. Execute FluSeq
   $ ./ms2ContLearning.py InputFolder 151206
   or
   $ python3 ms2ContLearning.py InputFolder 151206
   or
   $ python3.4 ms2ContLearning.py InputFolder 151206

4.0 Execution of FluSeq analysis

a. Change working directory to FluSeq
   $ cd ~/FluSeq

b. Execute FluSeq
   $ ./FluSeq-v1.0.py InputFolder
   or
   $ python3 FluSeq-v1.0.py InputFolder
   or
   $ python3.4 FluSeq-v1.0.py InputFolder

REFERENCE:

[1] Hong Kai Lee, Chun Kiat Lee, Julian Wei-Tze Tang, Tze Ping Loh, and Evelyn Siew-Chuan Koay. Contamination-controlled high-throughput whole genome sequencing for influenza A viruses using the MiSeq sequencer. [Article in preparation]