FluSeq Guide

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

General Description

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

1.0 INSTALLATION

Installation instructions are available for LINUX/UNIX or MacOSX. 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 an updated java.

Pre-requisite

1. Installation of Python 3.4.3 (https://www.python.org/downloads/)
   - Upon 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. Installation of 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 later, 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. Installation of BLAST 2.2.31+ software (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download)

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

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

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

6. Installation of 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 later, 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. Installation of 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 later, 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".

Procedure

1. Download the FluSeq.tar, decompress, and mv the FluSeq Folder to ~.

   ```
   $ tar –xvf  FluSeq.tar
   $ mv FluSeq ~
   ```

2. Change working directory to FluSeq

   ```
   $ cd ~/FluSeq
   ```

3. Execute FluSeq

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

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 alphanumeric characters. It is advisable that a folder can be capitalised at first letter for a better folders and files organization, eg. Folder20151231 (**Figure 1** – Folder in red).

The input folder should contain paired-end reads in fastq.gz format generated by Miseq Reporter or fastq2bcl2, eg. 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>, eg. YYMMDD_machinename_experimentnumber_flowcellnumber.

3.0 Contamination Learning

The Input folder for in-run contamination learning should contain paired-end reads in fastq.gz format generated by Miseq Reporter or fastq2bcl2, eg. 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>, eg. YYMMDD_machinename_experimentnumber_flowcellnumber.

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 Influenza Sequence Database

This section describes the sequence database used for blastn. It contains all influenza sequences available in GenBank, according to the last update.

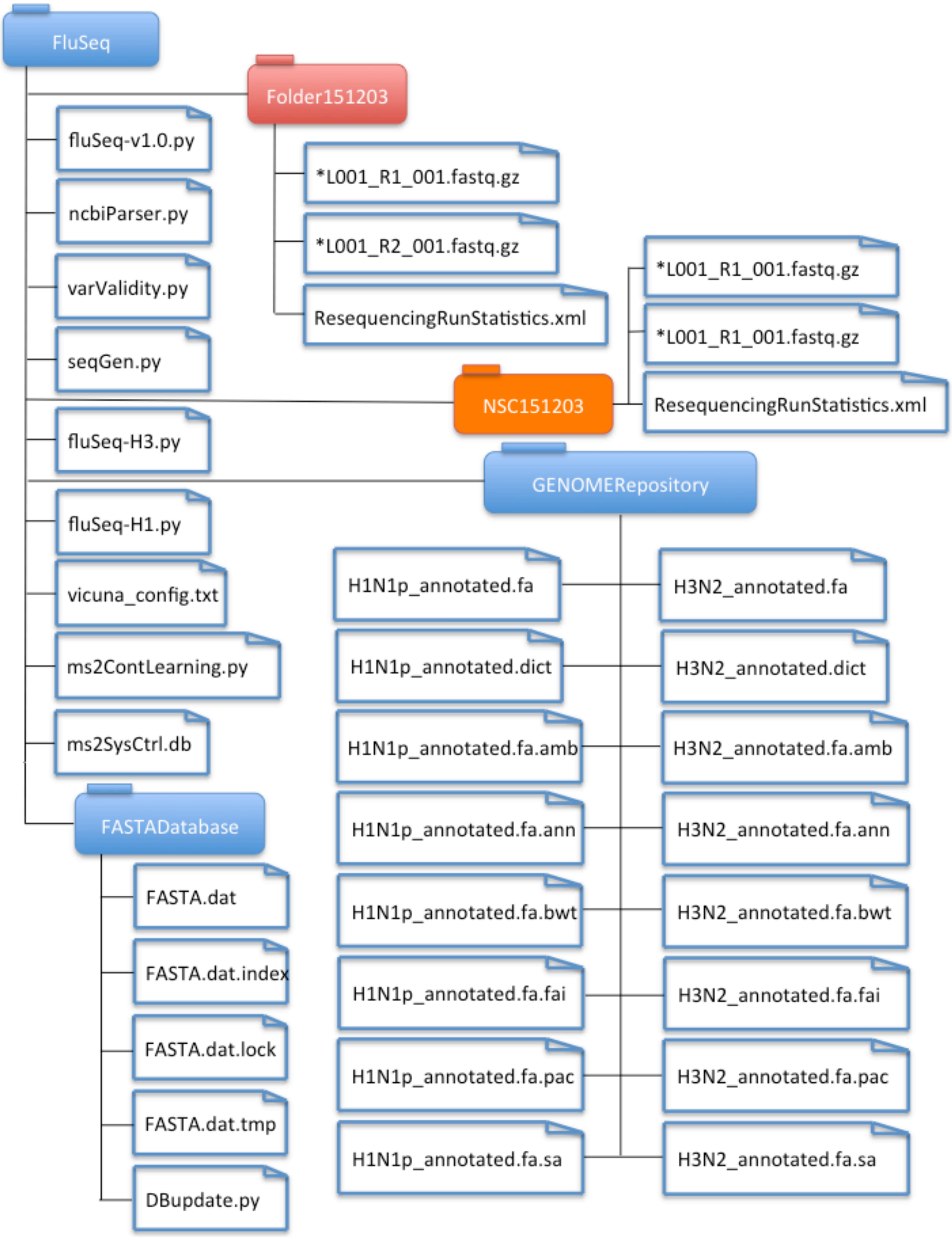
Database Update

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
   ```

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



Reference:

[1] Hong Kai Lee, Chun Kiat Lee, Julian Wei-Tze Tang, Tze Ping Loh, and Evelyn Siew-Chuan Koay. Pairwise comparison of contamination-controlled high-throughput and Sanger sequencing for influenza A genome sequencing. [Article in preparation]