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 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]. The users are encouraged to modify the FluSeq program written in python scripts for other clinical virus amplicon-based HTS methods.

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.

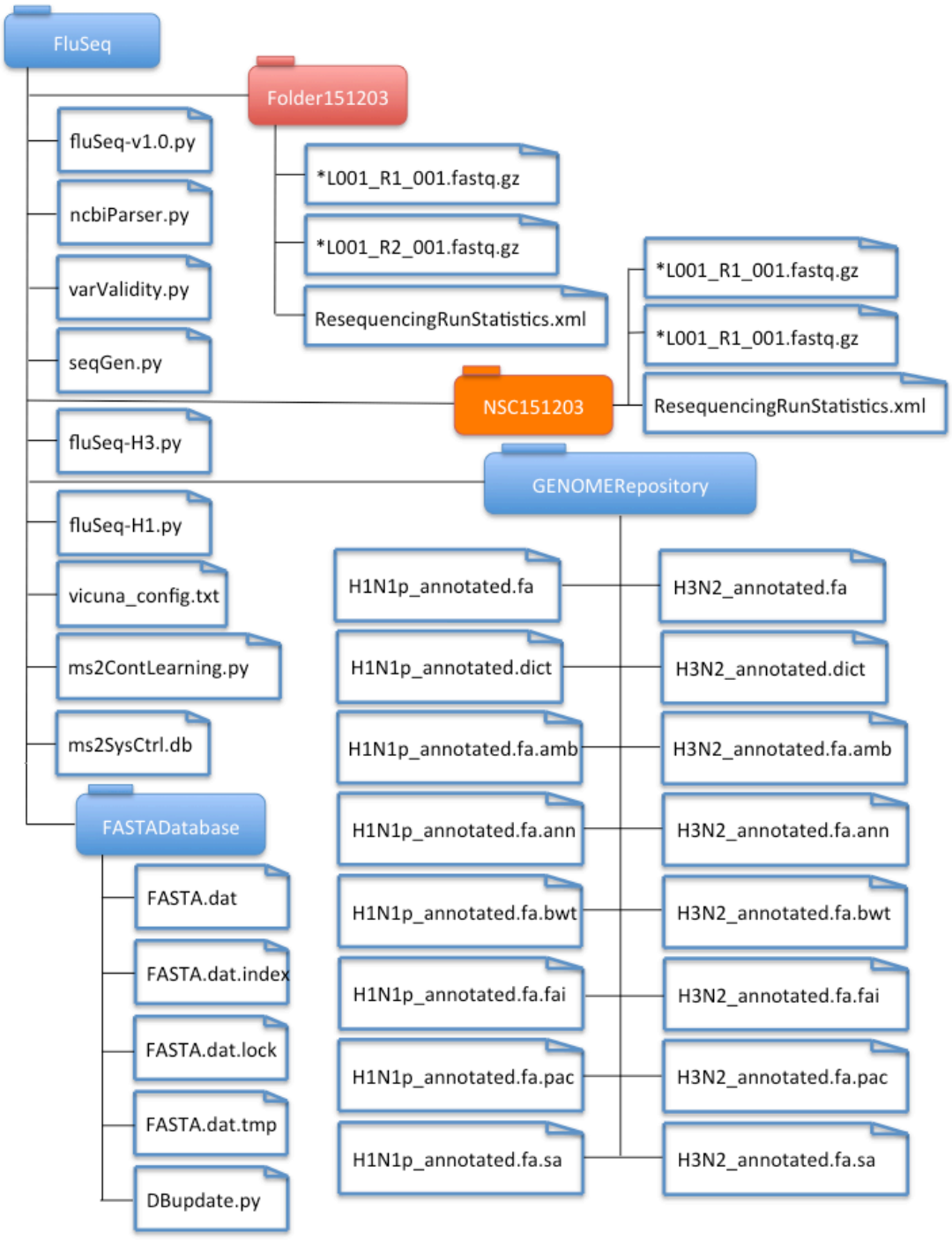
Creating/Updating Sequence Database

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

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. Contamination-controlled high-throughput whole genome sequencing for influenza A viruses using the MiSeq sequencer. [Article in preparation]