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FL-BEEHIV HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing

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ABSTRACT

The Florida Department of Health's Bureau of Public Health Laboratories in Jacksonville is developing a protocol for the Next Generation Sequencing (NGS) of HIV, primarily for the purpose of drug-resistant mutation identification. This HIV-1 protocol uses amplicon based sequencing based on primers designed by the BEEHIVE Consortium (https://www.beehive.ox.ac.uk/). The amplified pol gene regions are used in both genotyping and drug resistance determination. Our protocol utilizes newer enzymes with higher fidelity for sequencing and Illumina sequencing technology. The NGS data generated can also be used in surveillance and outbreak monitoring, giving epidemiologist more information about circulating viral genomes. There is also the potential that this protocol can be expanded to whole genome sequencing for HIV-1.
The imminent sunsetting of ViroSeq (Abbott Molecular) has required many labs to look for new methods to continue identifying HIV-1 drug resistance strains for both clinical management and epidemiological study. NGS was chosen as it is more cost effective than investing in a single pathogen platform. NGS allows for one sample to produce results and data that can aid not just a patient but an entire population.

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PROTOCOL CITATION

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KEYWORDS

null, HIV-1, HIV-1 genotyping, HIV-1 drug resistance, Next Generation sequencing, drug resistance mutations

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MATERIALS TEXT

RNA Extraction by Qiagen Viral RNA Mini Kit (DSP or Regular)
SSIV VILO Master Mix (Thermofisher, Cat 11756050)
Q5 Master Mix (NEB, Cat M0492S)
Primers (Gall A, et al. Journal of Clinical Microbiology. 2012; 50:12)

Set and primer	Sequence (5'–3')	Positions[a]	Product size[a]
2			
Pan-HIV-1_2F	GGG AAG TGA YAT AGC WGG AAC	1031–1051	3,574 bp
Pan-HIV-1_2R	CTG CCA TCT GTT TTC CAT ART C	4604–4583	
3			
Pan-HIV-1_3F	TTA AAA GAA AAG GGG GGA TTG GG	4329–4351	3,066 bp
Pan-HIV-1_3R	TGG CYT GTA CCG TCA GCG	7394–7377	

[a]According to HIV-1 reference strain HXB2 (GenBank accession number NC001802).

Single/multichannel pipettes with p20/p200/p1000 tips
Thermocycler
Nuclease-free water
AMPure XP Beads (Beckman Coulter)
Magnetic stand
Tapestation or Agarose gel
Qubit or other quantitation method
Illumina Nextera XT DNA Library Prep Kit
Illumina Nextera v2 Index Kits
Illumina iSeq
Illumina iSeq 100 i1 v2 cartridge
https://www.smartgene.com/
Pipeline: HIV1-PR+RT+IN (2.2.0HIV1 V1.6)

DISCLAIMER:

RNA Extraction

1 Extract RNA using the Qiagen Viral RNA Mini Kit (DSP or RUO)

1.1 Add **5.6 µl carrier RNA** per sample

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1.2 Follow Kit instructions for extraction

cDNA Synthesis 25m

2 Master Mix

4.0 µl SuperScript IV VILO Master Mix

6.0 µl Nuclease Free Water

10.0 µl RNA template

3 Run the following protocol on a thermocyler 25m

25 °C **00:10:00**

50 °C **00:10:00**

85 °C **00:05:00**

Amplicon PCR 6m

4 Each fragment will need to be amplified in an individual PCR reaction

Set 1

Pan-HIV-1_2F GGG AAG TGA YAT AGC WGG AAC

Pan-HIV-1_2R CTG CCA TCT GTT TTC CAT ART C

Set 2

Pan-HIV-1_3F TTA AAA GAA AAG GGG GGA TTG GG

Pan-HIV-1_3R TGG CYT GTA CCG TCA GCG

4.1 Master Mix

12.5 µl 2x Q5 Master Mix

0.5 µl Forward Primer **[M]20 Micromolar (µM)**

0.5 µl Reverse Primer **[M]20 Micromolar (µM)**

6.5 µl Nuclease Free Water

5.0 µl cDNA template

5 Run the following protocol on a thermocycler 6m

105 °C Lid

50 °C **00:00:30**

98 °C **00:00:30**

35 cycles

72 °C **00:05:00**

4 °C Hold

6 Bead clean up using a ratio of 0.5- follow the AMPure XP bead protocol for PCR purification

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7 Check fragment on Tapestation or gel

7.1 Band size should be
Amplicon 1- 3.5 kB
Amplicon 2- 3.0 kB

Fragment Normalizing and Pooling

8 Fragments can be pooled in eqimolar amounts or in equal concentrations.

8.1 Pool fragments

8.2 Dilute as needed to achieve **1.0 ng** input concentration for library preparation

Library Prep

9 Follow Illumina Protocol for Nextera XT DNA Library Sample Prep

Pooling Samples

10 Amplicon quality can effect how many samples can be pooled onto one run. Use caution in deciding how many samples to pool.

Analysis

11 We currently use SmartGene HIV-1 pipeline (https://www.smartgene.com/) for analysis
Pipeline Name: HIV-1 PR+RT+IN
Version 2.2.0HIV1 V1.6
Noise Filter [%] 0.5
Interpretation cut off [%] 5.0

Minimum read depth and additional criteria should be determined by your institution

References

12 Gall A, Ferns B, Morris C, Watson S, Cotten M, Robinson M, Berry N, Pillay D, Kellan P. Universal Amplification, Next-Generation Sequencing, and Assembly of HIV-1 Genomes. Journal of Clinical Microbiology. 2012; 50:12. doi: 10.1128/JCM.01516-12

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