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

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

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HIV

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We use this protocol and it's working

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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.

Materials

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)

RNA Extraction

1 Extract RNA using the Thermofisher MagMAX Viral and Pathogen(MVP) Nucleic Acid Isolation Kit I or MVPII using the Kingfisher Flex
- The difference is that the MVPI is Reasearch Use Only (RUO) and the MVPII is In Vitro Diagnostic (IVD) approved
-Both kits are the same, however the MVPI kit is able to be purchased in a smaller format, whereas the MVPII kit is only available in 1,000 or 2,000 reactions.

1.1 Prepare the following 4 plates for the appropriate number of sample/controls (Following the Procedures for viral nucleic acid isolation- 200uL sample volume):
1. Wash Solution 1 plate- 500uL of MVP Wash Solution to each well
2. Wash Solution 2 plate- 1000uL of freshly prepared 80% Ethanol
3. Elution plate- 50uL of MVP Elution Solution
4. Sample Plate
   a. Add 5.0uL Proteinase K to each sample/control well
   b. Add 200uL of sample to designated well
   c. Add 275uL of Binding Bead solution to each sample/control well
      -Binding Bead mix is 265uL Binding Solution + 10uL Magnetic Beads per sample (ie 100uL beads+
      2.65mL Binding solution)
      - Do NOT vortex the Binding Bead solution- only mix well by inversion

1.2 Run the program on the KingFisher Flex (200uL)

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

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.

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