Mar 26, 2021

HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing

Brenna M McGruder Rawson[1], Matthew Schimenti[1], Jason Blanton[1]

[1]Florida Department of Health, Bureau of Public Health Laboratories

In Development dx.doi.org/10.17504/protocols.io.btpqnmmw

Jason Blanton

SUBMIT TO PLOS ONE

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. The amplified *pol* gene regions are used in both genotyping and drug resistance determination. The NGS data generated can also be used in surveillance and outbreak monitoring, giving epidemiologist more information about circulating viral genomes.
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.

DOI

dx.doi.org/10.17504/protocols.io.btpqnmmw

PROTOCOL CITATION

Brenna M McGruder Rawson, Matthew Schimenti, Jason Blanton 2021. HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btpqnmmw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

KEYWORDS

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

CREATED

Mar 26, 2021

LAST MODIFIED

Mar 26, 2021

PROTOCOL INTEGER ID

48592

MATERIALS TEXT

QIAamp DSP Viral RNA Mini Kit DSP or RUO (Qiagen, Germantown, MD)
Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (cat 12574-035)
Primers FL_HIV-1*Ext Nest FW and FL* HIV-1_Ext Nest RV designed in house
Primers HIV2522-F and HIV5073-R designed by Dudley et al.

A	B	C
FL_HIV-1_Ext Nest FW	GAC TTC CCT CAR ATC ACT CTT TGGC	10µM
FL_HIV-1_Ext Nest RV	TGC CAC ACA ATC ATC ACC TGC C	10µM
HIV2252-F	CCC TCA RAT CAC TCT TTG GC	10µM
HIV5073-R	CCA CAC AAT CAT CAC CTG CC	10µM

Primers for HIV-1 Genotyping by Next Generation Sequencing

Single/multichannel pipettes with p20/p200/p1000 tips
Thermocycler
Nuclease-free water
Dimethyl Sulfoxide, biology grade
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/

DISCLAIMER:

RNA Extraction

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

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

1.2 Follow Kit Instructions

Reverse-Transcription and Amplification

Citation: Brenna M McGruder Rawson, Matthew Schimenti, Jason Blanton (03/26/2021). HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing. https://dx.doi.org/10.17504/protocols.io.btpqnmmw

2 Master Mix with Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (cat 12574-035)

25 µl 2x Reaction Mix

1.0 µl HIV2252-F

1.0 µl HIV5073-R

1.0 µl SuperScript III Platinum Taq

2.5 µl DMSO

19.5 µl viral RNA template

38m 45s

3 Reverse-Transcription/Amplification PCR thermocycler settings

105 °C Lid setting

50 °C **00:30:00**

15 cycles

94 °C **00:00:15**

55 °C **00:00:30**

68 °C **00:03:00**

Final extension

78 °C **00:05:00**

4 °C Hold

4 Using a ratio of 0.5 follow the AMPure XP bead protocol for PCR purification

Second Amplification

5 Master Mix with Invitrogen SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity DNA Polymerase (cat 12574-035)

25 µl 2x Reaction Mix

1.0 µl FL_HIV_Ext Nest FW

1.0 µl FL_HIV_Ext Nest RV

1.0 µl SuperScript III Platinum Taq

2.5 µl DMSO

19.5 µl clean cDNA template

8m 45s

6 Second Amplification PCR thermocycler settings

105 °C Lid setting

40 cycles

94 °C **00:00:15**

55 °C **00:00:30**

68 °C **00:03:00**

Final extension

78 °C **00:05:00**

Citation: Brenna M McGruder Rawson, Matthew Schimenti, Jason Blanton (03/26/2021). HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing. https://dx.doi.org/10.17504/protocols.io.btpqnmmw

4 °C Hold

7 Using a ratio of 0.5 follow the AMPure XP bead protocol for PCR purification

8 Visualize samples using Tapestation or agarose gel

8.1 Fragments should be ~2.9 kB

8.2 If the 2.9 kb fragment is isolated then samples should sequence well. If there are additional fragments sequencing is still possible, but samples may require a higher input concentration to achieve desired coverage

Library Preperation

9 Quantitate Samples and dilute as needed to achieve **1.0 ng** input concentration per sample

10 Follow Illumina Protocol for Nextera XT DNA Library Sample Prep

Pooling Samples

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

11.1 Normalize samples prior to pooling by dilution

11.2 Samples should be pooled in an equal volume amount. If desired the pool can be cleaned using an additional AMPure XP bead clean.

11.3 Quantitate the Pooled Library

12 Dilute the Pooled Library to apx. 0.07 ng/µL or a concentration found in the Illumina iSeq manual

Citation: Brenna M McGruder Rawson, Matthew Schimenti, Jason Blanton (03/26/2021). HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing. https://dx.doi.org/10.17504/protocols.io.btpqnmmw

13 Add **5 µl** **1 Nanomolar (nM) PhiX** to the pool

14 Load according to Illumina instructions

Analysis

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

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

References

16 Dudley DM, Bailey AL, Mehta SH, Hughes AL, Kirk GD, Westergaard RP, O'Connor DH. Cross-clade simultaneous HIV drug resistance genotyping for reverse transcriptase, protease, and integrase inhibitor mutations by Illumina MiSeq. Retrovirology. 2014 Dec 23;11:122. doi: 10.1186/s12977-014-0122-8. PMID: 25533166; PMCID: PMC4302432.

Citation: Brenna M McGruder Rawson, Matthew Schimenti, Jason Blanton (03/26/2021). HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing. https://dx.doi.org/10.17504/protocols.io.btpqnmmw