MAR 26, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.btpqnmw

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.btpqnmw>

MANUSCRIPT CITATION:

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Protocol status: In development
 We are still developing and optimizing this protocol

Created: Mar 26, 2021

Last Modified: Feb 14, 2022

PROTOCOL integer ID:
 48592

HIV-1 Genotyping and Drug Resistance by Next Generation Sequencing

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Jax Sequencing

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

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 sunseting 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

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/>

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

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

3 Reverse-Transcription/Amplification PCR thermocycler settings

38m 45s

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

- 6 Second Amplification PCR thermocycler settings

8m 45s

🧴 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

🧴 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 Preparation

- 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
- 13 Add 5 μL [M] 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

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