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Nextera XT protocol for MiSeq HIV^{PR-RT} sequencing

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HIV GENOTYPIC STUDY BY NGS (ILLUMINA)

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The protocol allows amplification and NGS sequencing of a PR-RT HIV-1 sequence using tagmentation strategy and Illumina MiSeq equipment, followed by analysis of drug-resistance associated mutations by HyDRA

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1 Prepare the sample in BSL2 laboratory. This protocol can be used to amplify and sequence HIV-1 from plasma or culture supernatants, as well as from cell-associated HIV-1. In the latter, skip the step of reverse transcription and go straight to PCR amplification.

2 Perform reverse transcription of HIV-1 and PCR amplification of PR-RT 1168bp fragment using primers and conditions published in Aulicino et al, Antivir Ther 2010;15(4):641-50. PCR amplifications are performed in 25-cycle reactions to avoid introducing artificial mutations.

Aulicino PC, Rocco CA, Mecikovsky D, Bologna R, Mangano A, Sen L (2010). HIV type-1 genotypic resistance profiles in vertically infected patients from Argentina reveal an association between K103N+L100I and L74V mutations.. Antiviral therapy.
https://doi.org/10.3851/IMP1571

3 Check second PCR products with agarose gel electrophoresis (1%). A single 1168bp band should be visible.

4 Perform purification of the PCR product with magnetic beads (Agencourt AMPure XP kit, Beckman Coulter, USA)

5 Perform tagmentation and indexation with Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA), following manufacturer´s instructions.

6 Check electrophoresis tagmentation patterns with QIAxel or another similar equipment. If none is available, a polyacrylamide gel can be used.

7 Quantify DNA using the fluorometric Qubit dsDNA high-sensitivity (HS) assay (Qubit 1X dsDNA HS Assay Kit, ThermoFisher Scientific, USA)

8 Calculate PCR samples concentration and volume to normalize all samples to 2nM, using PCR water.

9 Pool all samples using 5ul of each 2nM dilution of each sample.

10 Prepare PhiX at 12.5 pM. Dilute the library to 8 picomolar in HT1 buffer, and add 20% of PhiX. The purpose of PhiX is to add diversity to the samples.

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11 Run in Illumina MiSeq

MiSeq
Sequencer
illumina SY-410-1003

Controls and Error Rate calculation

12 pNL4-3 HIV-1 was used as control. Using BWA, align fastq reads belonging to the control sample to the reference pNL4-3 HIV-1 genome (GenBank Accession Number AF324493). Calculate the average number of mismatches/ total bases. Error Rate should not exceed 0,5%.

Sequence analysis using HyDRA

13 HyDRA (https://hydra.canada.ca/pages/home?lang=en-CA) is an annotated reference-based bioinformatics pipeline scripted in Perl, which analyses next generation sequencing (NGS) data for genotyping HIV-1 drug resistance (HIVDR) mutations. It utilizes an annotated HXB2 sequence (GenBank Accession number: K03455) for reference mapping by Bowtie2 2 , and stringent data quality assurance and variant calling criteria to identify HIVDR associated mutations based on the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/) and 2009 WHO list for Surveillance of Transmitted HIVDR. All HIVDR mutations found in the pol genes; protease (PR), reverse transcriptase (RT), and integrase (IN), are reported according to classifications outlined in the Stanford Surveillance Drug Resistance Mutation list .

Taylor T, Lee ER, Nykoluk M, Enns E, Liang B, Capina R, Gauthier MK, Domselaar GV, Sandstrom P, Brooks J, Ji H (2019). A MiSeq-HyDRA platform for enhanced HIV drug resistance genotyping and surveillance.. Scientific reports.
https://doi.org/10.1038/s41598-019-45328-3

14 Submit sequences to HyDRA. The following Advanced options for Nextera seqs can be used to inform amino acids in 1% frequency or higher, with a minimum read depth of 1000 x:
Consensus percent: 20
Target coverage: -1
Length cutoff: 100
Score cutoff: 2

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Error rate: include error rate calculated with the control (e.g. 0.005)
Minimum variant quality: 20
Minimum read depth: 1000
Minimum allele count: 7
Minimum amino acid frequency: 0.01

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