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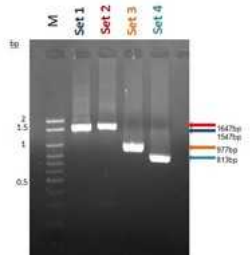
Whole Genome Sequencing of Hepatitis B Virus



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We use this protocol and it's working

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Abstract

This standard operating procedure (SOP) describes the procedure for whole genome sequencing (WGS) of the hepatitis B virus using a next-generation sequencer. Next-generation sequencing (NGS) has been widely used for genome-wide studies in many aspects of infectious as well as non-communicable diseases. However, there is no standardized method for whole genome sequencing of HBV across different genotypes. This SOP was developed using universal primers for HBV genomes across genotypes A to G.

Guidelines

Follow the Good Laboratory Practice in all steps.
Use powder-free gloves to handle the reagents and all libraries.
Use filtered-tips for pipetting.

Materials

1. General
- Micropipettes and tips (10 µL, 200 µL, 1000 µL)
- Disposable powder-free gloves
- 2N NaOH stock
- Microcentrifuge tubes (low bind)
- 15ml tube
- Vortex
- Microcentrifuge
- Centrifuge
- PCR plates with adhesive seals
- Kimwipes
- Lens tissue

2. Targeted amplifications
- GoTaq Long PCR Master Mix (M4021)
- Primers
  - Set 1: 251F (GAC TYG TGG TGG ACT TCT C)
    1797R (CCA ATT TMT GCY TAC AGC CTC)
  - Set 2: 2300F (CCA CMW AAT GCC CCT ATC)
    654R (GSC CCA MBC CCA TAG G)
  - Set 3: 1859F (ACT NTT CAA GCC TCC RAG CTG)
    2835R (GTT CCC AVG WAT AWG GTG AYC C)
  - Set 4: 1584F (ACT TCG MBT CAC CTC TGC ACG T)
    2396R (GTC KGC GAG GYG AGG GAG TT)

3. Library Preparation Kit
- Illumina Microbial Amplicon Prep (iMAP)
- PhiX Control Kit, version 3 (10 µL at 10 nM) (#15017666)
- Illumina DNA/RNA UD Indexes Sets A–D (Catalog nos. 20091654, 20091656, 20091658, and 20091660)
- MiSeq Reagent Kit, version 2 (300 cycles)

4. Other reagents
- Squeeze bottles
- pH sticks
- Molecular grade distilled water
- Tween 20
- Isopropyl Alcohol
- Absolute Ethanol
- Ice bucket with ice
- Water bath

Before start

DNA extraction should be done accordingly using the QIAamp DNA Kit (QIAGEN) in accordance with the manufacturer’s instructions.

General Precaution before the procedure

1 Follow the Good Laboratory Practice in all steps.

2 Use powder-free gloves to handle the reagents and all libraries.

3 Use filtered-pipette tips for pipetting.

4 DNA extraction should be done accordingly using the QIAamp DNA Kit (QIAGEN) in accordance with the manufacturer's instructions.

Amplification

5 PCR preparation

5.1 Thaw the Long PCR mastermix.

5.2 Prepare the PCR reactions as follows: Long PCR mix (2X), DNA template, DDW, Total 20 µL.

6 PCR Amplification

6.1 Prepare the PCR conditions as follows: Initial denaturing 02:00, 01:00, 01:00 35 cycles, 01:15, 05:00, Hold.

7 Gel-electrophoresis

7.1 Confirm the amplification of the samples using 1% agarose gel-electrophoresis.

Library preparation

8 Tagment PCR amplicons

8.1 5 µL each from four PCR amplicons were pooled together in a tube. (Total 20 µL)

8.2 Thaw EBLTS HT (4°C) at RT and vortex thoroughly before use.

8.3 Thaw TB1HT (-20°C) at RT and vortex thoroughly before use.

8.4 Prepare the reagent mix: 12 µL TB1 HT, 4 µL EBLTS HT, 20 µL H2O.

8.5 Use 30 µL of reagent mix to each sample (Add 30 µL of mix to the sample) (Total 50 µL).

8.6 Seal and shake 1600 rpm for 1 min.

8.7 Centrifuge 1000 g for 1 min.

8.8 Run: 55°C for 5 min, 10°C Hold (lid-heated, 50 µL volume).

9 Post Tagmentation Cleanup

9.1 Keep ST2 HT (RT) vortex before use.

9.2 TWB HT (4°C) at RT and vortex before use.

9.3 Centrifuge the plate for 1 min.

9.4 Add 10 µL of ST2 HT.

9.5 Seal and shake.

9.6 Centrifuge.

9.7 Incubate at RT for 5 min.

9.8 Place on magnetic stand until clear (~3 min).

9.9 Remove supernatant.

9.10 Wash the bead by: Remove from magnetic stand, Add 100 µL TWB, Seal and shake (pipette mix), Centrifuge prn, Place on magnetic stand until clear (about 3 min), Remove supernatant.

9.11 Wash again a second time, but leave the supernatant until the next step.

10 Amplify Tagmented Amplicons

10.1 Thaw EPM HT (-20°C) on ice until use (mix by inverting).

10.2 Index 10 bp adapter (-20°C), thaw RT and centrifuge after vortex.

10.3 Prepare the PCR mix by 20 µL of EPM + 20 µL of H2O.

10.4 Vortex to mix the PCR mix.

10.5 Remove the supernatant wash in previous cleanup.

10.6 Use 10 µL tips to remove residual wash solution.

10.7 Remove from magnetic stand.

10.8 Add 40 µL of PCR mix.

10.9 Add 10 µL of index.

10.10 Seal and shake.

10.11 Centrifuge briefly.

10.12 Run inside a thermocycler as follows: 72°C for 3 min, 98°C for 3 min, 7 cycles of 98°C for 20 sec, 60°C for 30 sec, 72°C for 1 min, 72°C for 3 min, Hold at 10°C (lid heated 100°C, vol 50 µL).

11 Pool and cleanup libraries

11.1 Take 7 µL each from libraries and pool to 1.5 ml tube.

11.2 Add 0.9 x of the IPB.

11.3 Shake well and briefly spin down.

11.4 Incubate at RT for 5 min.

11.5 Place on magnetic stand for 3 min.

11.6 Discard supernatant.

11.7 Add 500 µL of freshly prepared 80% Ethanol (while keeping the tube on magnetic stand).

11.8 Remove the supernatants.

11.9 Add a second time 500 µL of 80% Ethanol.

11.10 Remove the supernatant.

11.11 Use 10 µL pipette to remove extra reagents.

11.12 Air dry (about 3 min).

11.13 Add RSB 55 µL to the bead after removing from magnetic stand.

11.14 Incubate 2 min.

11.15 Place to magnetic stand for 2 min.

11.16 Elute 50 µL of pooled libraries.

11.17 Check Qubit and the initial result showed too high. So 5 µL of the library was diluted with 25 µL of the EBT and measured again.

11.18 After diluting to 4 nM, recheck the concentration. (ng/µL that is equal to nM. We proceed accordingly).

12 Denaturing and running in MiSeq

12.1 Prepare the 0.2N NaOH (0.2N NaOH = 100 µL of 1N NaOH + 400 µL of LGW).

12.2 5 µL of lib + 5 µL of 0.2N NaOH.

12.3 Incubate for 5 min at RT.

12.4 Add prechilled HT 990 µL (to become 24 pM, 1000 µL).

12.5 Combine 311 µL of 24 pM + 288 µL of prechilled HT (600 µL of 12.5 pM lib).

12.6 Prepare 12.5 pm (100 µL) of PhiX (by dilute 63 µL of 20 pM + 38 µL of HT).

12.7 Add 5% (30 µL) of PhiX control to load.

Protocol references

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