Apr 04, 2025 Version 1

HBV Whole-Genome Sequencing using Oxford Nanopore MinION V.1

Journal of Medical Virology

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldrow9g5b/v1

Joakim Bedner Stenbäck[1], Johan Ringlander[1]

[1]University of Gothenburg

Joakim Bedner Stenbäck
University of Gothenburg

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ldrow9g5b/v1

External link: https://doi.org/10.1002/jmv.70630

Protocol Citation: Joakim Bedner Stenbäck, Johan Ringlander 2025. HBV Whole-Genome Sequencing using Oxford Nanopore MinION. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldrow9g5b/v1

Manuscript citation:
Stenbäck JB, Schmidt D, Noborg U, Gustafsson J, Norberg P, Andersson ME, Fu MX, Harvala H, Ringlander J (2025) Accurate and Cost-Efficient Whole Genome Sequencing of Hepatitis B Virus Using Nanopore. Journal of Medical Virology 97(10). doi: 10.1002/jmv.70630

Protocol status: Working
We use this protocol and it's working

Created: March 10, 2025

Last Modified: April 04, 2025

Protocol Integer ID: 124083

Keywords: HBV, Sequencing, Nanopore, Whole Genome, Genotyping, using oxford nanopore minion, oxford nanopore minion this protocol, oxford nanopore minion with flongle flowcell, hbv whole, hbv, sequencing kit, genome sequencing, genome, whole genome, sequencing

Abstract

This protocol outlines the steps for preparing and sequencing the hepatitis B virus (HBV) whole genome using Oxford Nanopore MinION with Flongle flowcells. It details primer-based amplification, quality control using Agilent TapeStation, and library preparation using the Oxford Nanopore RBK114.24 or RBK114.96 barcoding sequencing kit.

Materials

Reagents
- 2X Platinum Polymerase
- Primers (10 µM):
  - Forwards: 56F, 1689F
  - Reverses: 1827R, 262R
- Nested (if required): 251F, 1801 R
- Nuclease-free water (SuperQ water)
- DNA Sample
- Agilent TapeStation D5000 Screentape Kit (or equivalent such as Gel Electrophoresis)
- Oxford Nanopore RBK114.24 or RBK114.96 Barcoding Sequencing Kit

Equipment:
- PCR Thermal Cycler
- Agilent TapeStation
- Oxford Nanopore MinION with Flongle Flowcells

Step 1: Requirements and Master Mix Preparation

30m

1 **Reagent Requirements:**

- ThermoFisher Platinum Hot Start PCR Master Mix (2X) (**Cat.** 13000014)
- System 1 Forward Primer 56F (**CCTGCTGGTGGCTCCAGT**)
- System 1 Reverse Primer 1827R (**GAAAAAGTTGCATGGTGCTGGT**)
- System 2 Forward Primer 1689F (**ACCGACCTTGAGGCCTACTTCA**)
- System 2 Reverse Primer 262R (**CCACCACGAGTCTAGACTCT**)
- Nesting System 1 Forward Primer 251F (**GTGGTGGACTTCTCTCAATTTTC**)
- Nesting System 1 Reverse Primer 1801R (**CAGACCAATTTATGCCTACAGCCT**)

Hardware Requirements:

- Thermocycler
- Agilent Tapestation System *(optional)*
- Agilent D5000 Screentape *(**Cat.** 5067-5589.) (optional)*

2 Prepare two separate master mixes for the two primer systems: 30m

- ThermoFisher Platinum Hot Start PCR Master Mix (2X) 15 µL
- Forward Primer (56F or 1689F) 1 µL
- Reverse Primer (1827R or 262R) 1 µL
- Nuclease-free water 8 µL
- DNA sample 5 µL

- Total Volume: 30 µL

Step 2: PCR Amplification

3h

3 Run both master mixes in single PCR reactions under the following cycling conditions:

	Step	Cycles	Temperature (°C)	Time
	Initial Denaturation	1	95°C	2:00
	Denaturation	40	95°C	0:45
	Annealing	40	58°C	1:00
	Extension	40	72°C	3:00

	Step	Cycles	Temperature (°C)	Time
	Final Extension	1	72°C	3:00
	Storage	1	4°C	∞

Nesting PCR settings if required as listed in Step 3:
Utilize primers 251F and 1801R fitted for system 1.

	Step	Cycles	Temperature (°C)	Time
	Initial Denaturation	1	95°C	2:00
	Denaturation	20	95°C	0:45
	Annealing	20	58°C	1:00
	Extension	20	72°C	3:00
	Final Extension	1	72°C	3:00
	Storage	1	4°C	∞

Step 3: Quality Control

1h

4 Run amplified products on Agilent TapeStation using the D5000 Screentape kit according to the manufacturer's instructions.
The expected product size for system 1 and 2 is ~1800bp.
If bands are detected, proceed to Step 4, otherwise proceed to Step 3.1.

4.1
1. If no band is detected for system 1, perform a nested PCR using primers 251F and 1801R.
2. Repeat TapeStation analysis to confirm amplification.

Step 4: Library Preparation and Sequencing

2h

5
1. If bands are visible, pool the systems for each sample and proceed with library preparation using the Oxford Nanopore RBK114.24 or RBK114.96 kit according to the manufacturer's protocol. Samples that drastically differ in concentration are normalised to ensure even distribution across the flow cell array.
2. Load the prepared library onto the Oxford Nanopore MinION with Flongle flowcells alternatively the Nanpore MinION with standard flow cells and initiate sequencing with the desired run settings.

Step 5: Bioinformatic analysis

6 After sequencing, an array of varied analyses are possible.
For genotyping and resistance mutation identification it is recommended to establish a consensus sequence from the data by utilising the Oxford Nanopore Technologies tool Medaka. This pipeline will polish the reads and further reduce any present error rate. Prior to Medaka, mapping can be done with software like Minimap2 to ensure high quality mapping of long reads, Samtools for file conversion followed by the Medaka consensus function.

The approach used in our study for clinical application is available on GitHub: https://github.com/ClinicalGenomicsGBG/hbv_nano/tree/hbv_nano_daniel