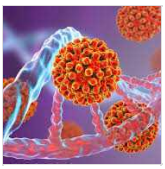

Jun 20, 2021

# Full genome PCR amplification of all African Hepatitis B Virus genotypes

Forked from Full genome PCR amplification of all African Hepatitis B Virus genotypes

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Masters Project

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ABSTRACT

This method is and adaptation of the full genome amplification protocol published by Gunther *et al.*, able to amplify most known genotypes. The primers bind near the fold in the genome where the DNA is partially double-stranded. Since genotype A1 of HBV frequently has two point mutations in the reverse primer binding region, a second primer was designed to circumvent them when amplifying A1 isolates. This protocol was used to amplify specimens from occult infections of HBV with genotypes A, D, and E.

ATTACHMENTS

Full-genome_HBV_PCR_template_UP-MedVir-Le-Clercq.xlsx

DOI

dx.doi.org/10.17504/protocols.io.bvykn7uw

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA737147

PROTOCOL CITATION

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FORK NOTE

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KEYWORDS

HBV, Hepatitis, Full Genome, PCR, Genotypes



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GUIDELINES

- Set up the mixes in a laminar flow cabinet **On ice** .
- Amplicons can be stored at **-20 °C**

MATERIALS TEXT

Reagents:

- Expand™ High Fidelity PCR System **Roche Catalog #11732650001** **High_fildelity_roche.pdf**
  Deoxynucleotide (dNTP) Solution Mix **New England Biolabs Catalog #N0447S** ,
  **25 Milimolar (mM) stock**
- Molecular grade water nuclease-free **Contributed by users**
- Primers, **15 Micromolar (µM) stock**

| A | B | C | D |
|---|---|---|---|
| P1 | 1821--1841 | 5'- CTT TTT CAC CTC TGC CTA ATC A -3' | 52.8 |
| P2 | 1825--1806 | 5'- AAA AAG TTG CAT GGT GCT GG -3' | 54.6 |
| P2_A1 | 1825--1806 | 5'- AAA AAG TTG CAT GAT GAT GG -3' | 49.3 |

Primers used to amplify full genomes of HBV. P1 is the forward primer and P2 or P2_A1 is the reverse primer, depending on the genotype to be amplified. Numbering is based on the EcoR1 site. The sequences and calculated Tm for each primer is indicated.

SeaKem® LE Agarose **Lonza Catalog #50004** Step 3.1

UltraPure™ TBE Buffer, 10X **Thermo Fisher Catalog #15581044** Step 3.1

SYBR SAFE DNA stain **Life Technologies Catalog #S33102** Step 3.1

GeneRuler 1 kb DNA Ladder **Thermo Fisher Scientific Catalog #SM0311** Step 3.2

Equipment:

- Thermal cycler
- Gel Documentation system

SAFETY WARNINGS

- Set up master mixes in a "DNA-free" room and laminar flow cabinet.
- Add DNA to reaction tubes in a "DNA-loading" laminar flow cabinet.
- Always dispose of biohazardous waste appropriately in accordance to lab regulations.
- Always wear gloves and a lab coat.
- Never directly look at the UV lamps.



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BEFORE STARTING

- Thaw reagents/components **On ice** .
- Wipe workspace with **[M]10 % volume** Bleach, followed by **[M]70 % volume** Ethanol, and dd$H_2O$ before (and after).
- UV the relevant laminar flow cabinets.

## Master Mix preparation

1 Prepare the following two mixes **On ice** :

1.1 Master Mix 1:

For one **15 µl** reaction combine the following:

| A | B | C | D |
|---|---|---|---|
| dNTPs | 25 mM | 200 uM | 0.2 uL |
| P1 | 15 uM | 300 nM | 0.5 uL |
| P2 or P2_A1 | 15 uM | 300 nM | 0.5 uL |
| Expand Hi Fi Buffer | 10x | 1x | 2 uL |
| MgCl2 | 15 mM | 1.5 mM | - |
| ddH2O | n.a. | n.a. | 11.8 uL |

Components for Master Mix 1 with their stock and final concentrations and volume needed for one 15 uL reaction.

1.2 Master Mix 2:

For one **5 µl** reaction combine the following:

| A | B | C | D |
|---|---|---|---|
| Expand Hi Fi Buffer | 10x | 1x | 0.5 uL |
| Expand Hi Fi Enzyme | 3.5 U/uL | 2.6 U | 0.75 uL |
| ddH2O | n.a. | n.a. | 3.75 uL |

Components for Master Mix 2 with their stock and final concentrations and volume needed for one 5 uL reaction.

1.3 ⚠

- Add **15 µl Master Mix 1** to **5 µl template DNA** for a **20 µl** reaction in thin walled PCR tubes.
- At the Thermal cycler, add an additional **5 µl Master Mix 2** to each tube after initial denaturation.

## Thermal cycling

2 Perform thermal cycling according to the following conditions:



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- Initial denaturation at **94 °C** for 2 minutes.
- Cooling to **58 °C** before adding Mix 2.
- 40 cycles of:

1. Denaturation at **94 °C** for 40 seconds
2. Annealing at **55 °C** for 90 seconds
3. Elongation at **68 °C** for 180 seconds*

*Add 180 seconds every 10 cycles

- Cooling/hold at **4 °C**

Electrophoresis 50m

3 Perform TBE-gel electrophoresis to confirm success of amplification prior to amplicon clean-up.

3.1 Prepare a **0.7 % (v/v)** gel with Agarose **Lonza Catalog #50004** and UltraPure™ TBE Buffer, 10X **Thermo Fisher Catalog #15581044**, pre-stained with SYBR SAFE DNA stain **Life Technologies Catalog #S33102**.

SYBR SAFE DNA stain **Life Technologies Catalog #S33102** is usually added at a concentration of **1 µl Stock** per **10 mL TBE**.

50m

3.2 Load **5 µl** of PCR product to the gel using **2 µl loading buffer** such as Gel Loading Dye Orange (6X) - 4.0 ml **New England Biolabs Catalog #B7022S** and molecular weight marker, e.g. GeneRuler 1 kb DNA Ladder **Thermo Fisher Scientific Catalog #SM0311**, and run at **100 Volt** for **00:50:00**.

3.3 Visualize and capture gel on an appropriate imager and paired software, e.g. the

Gel Doc XR+ Gel Documentation System
Gel Documentation System
Bio-rad Laboratories 1708195

3.4 Expected result:



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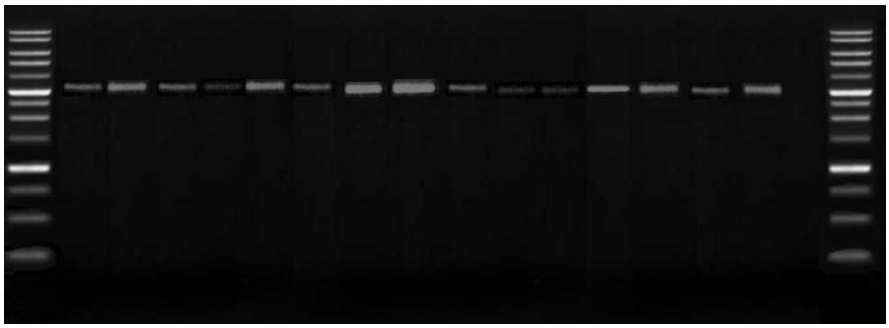

Gel image showing single bands for individually amplified full genome HBV isolates, flanked by a molecular weight marker.



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