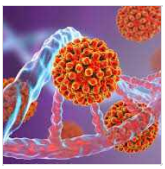

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Full genome PCR amplification of all African Hepatitis B Virus genotypes

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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.* in 2005, able to amplify most known genotypes.

DOI

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EXTERNAL LINK

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

PROTOCOL CITATION

Louis S IV Le Clercq 2021. Full genome PCR amplification of all African Hepatitis B Virus genotypes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvx2n7qe

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Le Clercq, L.S., 2014. Molecular characterization of full genome hepatitis b virus sequences from an urban hospital cohort in Pretoria, South Africa (Masters dissertation, University of Pretoria). DOI: http://dx.doi.org/10.13140/RG.2.2.33619.71204

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.

Equipment:

- Thermal cycler

BEFORE STARTING

Thaw reagents/components **On ice** .

1 Prepare the following two mixes on ice:

1.1 Master Mix 1:

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

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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.

2 Perform thermal cycling according to the following conditions:

- 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**

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

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