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Protocol status: In development
We are still developing and optimizing this protocol

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bHEV genotpying RT-PCR

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ABSTRACT

bHEV genotpying RT-PCR

PROTOCOL REFERENCES

https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-022-04781-0#Sec11

MATERIALS

- Superscript IV Reverse Transcriptase
- 2X Plus High Fidelity Fast PCR Master Mix
- Genotyping Primers
- Nuclease-free water
- Template RNA
- TBE Buffer
- Agarose
- 1 Kb ladder/Loading Dye

Reverse Transcription

1 Pre-warm the 5x SSIV buffer to RT

2 Make up the primer annealing master mix in a 2ml Eppendorf. Per sample volumes:

Component	Volume
dNTPs 10mM	1 µL
Reverse Primer 2mM	1 µL
Nuclease-free water	Up to 13 µL

3 Divide 13 µL of master mix between reaction tubes.

4 Add up to 5 µg RNA template to reaction tubes.

5 Mix and briefly centrifuge.

6 Heat the RNA-primer mix at 65°C for 5 minutes.

7 Incubate on ice for at least 1 minute.

8 Vortex and briefly centrifuge the 5x SSIV Buffer.

9 Make up the RT master mix in a 2ml Eppendorf. Per reaction volumes:

Component	Volume
5x SSIV Buffer	4 µL
DTT 100 µM	1 µL
RNase Inhibitor	1 µL
Reverse Transcriptase (200 U/µL)	1 µL

Note

Mastermix should be made up and aliquoted into PCR tubes in a mastermix cabinet. Cabinet and tubes should be cleaned with decontamination wipes/70% ethanol and UV sterilised before and after use.

10 Mix and briefly centrifuge RT master mix

11 Add 7 µL of RT master mix to annealed RNA

Note

This should be done in the pre-PCR template addition room.

12 Incubate the combined reaction mixture at 50°C for 10 minutes

13 Inactivate the reaction by incubating it at 80°C for 10 minutes. During this incubation, PCR mastermix can be setup (next step).

Polyermase Chain Reaction

18m

14 Setup mastermix in a 1.5 mL eppendorf tube. The following amounts are for a single reaction

Component	Volume
2x Reaction Mix	25 µL
Forward Primer 10 µM	2 µL
Reverse Primer 10 µM	2 µL
Nuclease-free water	16 µL
Total	50 µL

Note

Mastermix should be made up and aliquoted into PCR tubes in a mastermix cabinet. Cabinet and tubes should be cleaned with decontamination wipes/70% ethanol and UV sterilised before and after use.

15 Add 5 µL template cDNA to the PCR tubes.

Note

This should be done in the pre-PCR template addition room.

16 Gently mix contents of each tube by pipetting and spin down.

17 Program the following PCR cycles into the thermal cycler.

Step	Temperature	Time	Cycles
Reverse transcription	50 °C	00:10:00	1

Inactivation	98 °C	00:02:00	1
Denaturation	98 °C	00:00:10	10
Annealing	50 °C	00:00:10	10
Extension	72 °C	00:00:30	10
Denaturation	98 °C	00:00:10	30
Annealing	60 °C	00:00:10	30
Extension	72 °C	00:00:30	30
Final extension	72 °C	00:05:00	1