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https://protocols.io/view/bhev-genotpying-rt-pcr-dabx2apn

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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

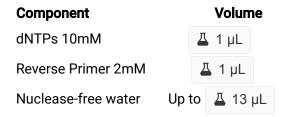
https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-022-04781-0#Sec11

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

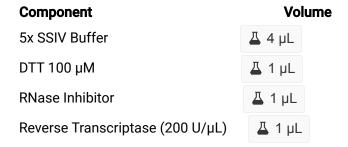
Reverse Transcription

- 1 Pre-warm the 5x SSIV buffer to RT
- 2 Make up the primer annealing master mix in a 2ml Eppendorf. Per sample volumes:



- 3 Divide \perp 13 μ L of master mix between reaction tubes.
- 4 Add up to \triangle 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:



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.

- Mix and briefly centrifuge RT master mix
- 11 Add $\underline{\underline{A}}$ 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

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



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 \perp 5 μ L 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.

StepTemperatureTimeCyclesReverse transcription\$ 50 °C\$ 00:10:001

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Inactivation	₽ 98 °C	© 00:02:00	1
Denaturation	\$ 98 °C	© 00:00:10	10
Annealing	8° 50 °C	© 00:00:10	10
Extension	₽ 72 °C	© 00:00:30	10
Denaturation	₿ 98 °C	(5) 00:00:10	30
Annealing	8° 60 °C	© 00:00:10	30
Extension	₽ 72 °C	© 00:00:30	30
Final extension	3° 72 °C	© 00:05:00	1