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Hepatitis E and Enterovirus Multiplex Nested RT-PCR

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Enterovirus primers: designed by Dr Alex Shaw, unpublished.

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

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Abstract

For multiplex, nested amplification of Hepatitis E (HEV) and Enterovirus (EV) samples.

Primer sets and target amplicon size

Outer HEV: HEV_38_f and HEV_39_r (538 bps)
HEV_38_f: GARGCYATGGTBGAGAARG
HEV_39_r: GCCATRTTCCARACRGTRTTCC

Inner HEV: HEV_37_f and HEV_27_r (306 bps)
HEV_37_f: GGTTYCGYGCYATTGARAARG
HEV_27_r: TCRCCRGARTGYTTCTTCC

Outer EV: For1_5NCR and Rev7_5NCR (535 bps)
For1_5NCR: CGGTACCYTTGTRCGCCTG
Rev7_5NCR: ATTGTCACCATAAGCAGCC

Inner EV: For2_5NCR and Rev6_5NCR (388 bps)
For2_5NCR: CAAGCACTTCTGTTWCCC
Rev6_5NCR: CCAAAGTAGTCGGTTCCGC

Materials

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072

SuperScript III One-Step RT-PCR System with Platinum Taq **Invitrogen - Thermo Fisher Catalog #**12457-026

Approximate total cost per sample: £8.01
8 primers required (cost excluded from estimate as primers do not need to be ordered each time)
Superscript cost per sample: ~£7.56
Dreamtaq cost per sample: ~£0.45 per sample

Extra equipment required:
Vortex, mini centrifuge, thermocycler

Protocol materials

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072 Materials, Step 1.3

SuperScript III One-Step RT-PCR System with Platinum Taq **Invitrogen - Thermo Fisher Catalog #**12457-026

Materials, Step 1.1

Hepatitis E and Enterovirus Multiplex Nested PCR

1 First round PCR for combined enterovirus and hepatitis E samples

1.1

SuperScript III One-Step RT-PCR System with Platinum Taq **Invitrogen - Thermo Fisher Catalog #**12457-026

Prepare master mix on ice per number of samples/controls + 10%. Flick master mix gently to mix, and spin down. Aliquot 20 ul into each well, then add 5 ul of template.

	A	B
	Reagent	**Volume for 1 rxn (ul)**
	2x reaction mix	12.5
	HEV_38_f primer	0.5
	HEV_39_r primer	0.5
	For1_5NCR primer	0.5
	Rev7_5NCR primer	0.5
	Nuclease free water	4.5
	SSIII taq	1
	Template	5 ul
	Total volume	25 ul

1.2 Cycling conditions for first round PCR

Flick samples gently to mix, spin down tubes. Amplify samples using the following conditions:

	A	B	C
	Number of cycles	**Temperature (°C)**	**Time**
	1	50	30 minutes
	1	94	5 minutes
	40	94	30 seconds
		52	30 seconds
		68	45 seconds
	1	68	5 minutes
		10	Hold

1.3 Second round PCR

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072

Prepare on ice per number of samples/controls + 10%. Vortex briefly, and spin down. Aliquot 24 ul of master mix into each well, then add 1 ul of template.

	A	B
	Reagent	**Volume per 1 reaction (ul)**
	Dreamtaq	12.5
	HEV_37_f	0.5
	HEV_27_r	0.5
	For2_5NCR	0.5
	Rev6_5NCR	0.5
	Nuclease free water	9.5
	Template (amplicon from first round PCR)	1
	Total	25 ul

1.4 **Cycling conditions for second round PCR**

Vortex samples briefly, spin down, and amplify using the following conditions:

	A	B	C
	Number of cycles	**Temperature (oC)**	**Time**
	1	95	10 minutes
	40	95	30 seconds
		52	30 seconds
		72	45 seconds
	1	72	5 minutes
		10	Hold

1.5 Check all samples and controls on an agarose gel or a tapestation.

Protocol references

Wang B, Harms D, Papp CP, Niendorf S, Jacobsen S, Lütgehetmann M, Pischke S, Wedermeyer H, Hofmann J, Bock CT. Comprehensive Molecular Approach for Characterization of Hepatitis E Virus Genotype 3 Variants. J Clin Microbiol. 2018 Apr 25;56(5):e01686-17. doi: 10.1128/JCM.01686-17. PMID: 29514938; PMCID: PMC5925713.