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Whole genome amplification of human parechovirus type 3 (PEV-A3) utilizing tiling-PCR

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

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Abstract

The procol allows whole genome amplification of PEV-A3, prior to next generation library preparation and sequencing. Suitable for Nanopore and Illumina sequencing as well.

DNAse I treatment (to remove contaminating human-derived gDNA) 40m

1 **Reagents**

- TURBO DNase™ (2 U/µL), Invitrogen
- Nuclease-free destillated water

1.1 Prepare the following master mix

	A	B
	TURBO DNase (2 U)	1.0 uL
	TURBO DNase puffer	4.0 uL
	Destillated water	6.0 uL
	RNA	**40.0 uL**

1.2 Incubation in thermocycler 30m

37 °C 00:30:00

2 Post-DNAse I treatment clean-up

Reagents

- AMPure XP Beads, Beckman Coulter
- 70% EtOH (prepared freshly)
- Nuclease-free destillated water

2.1 Add 1.8 µl AMPure XP per 1.0 µl of sample.

2.2 Bind DNA fragments to paramagnetic beads.

2.3 Separation of beads + DNA fragments from contaminants.

2.4 Wash beads + DNA fragments twice with 70% EtOH to remove contaminants.

2.5 Elute purified DNA fragments from beads.

2.6 Transfer to new tubes.

Reverse transcription

1h 20m

3 **Reagents**

- SuperScript™ VILO™ cDNA Synthesis Kit, Invitrogen
- Nuclease-free destillated water

3.1 Prepare the following master mix

	A	B
	5X VILO™ Reaction Mix	4.0 uL
	10X SuperScript™ Enzyme Mix	2.0 uL
	Destillated water	4.0 uL
	RNA	**8.0 uL**

3.2 Incubation in thermocycler

10m

25 °C 00:10:00

3.3 Incubation in thermocycler

42 °C 01:00:00

3.4 Incubation in thermocycler

5m

85 °C 00:05:00

Polymerase chain reaction (tiling-PCR)

2h 30m

4 **Reagents**

- Phusion™ High-Fidelity DNA Polymerase, New England Biolabs
- Nuclease-free destillated water
- dNTP mix

4.1 Prepare the following master mixes

Mix_1

	A	B
	5x Phusion‰ High-Fidelity PCR Master Mix with GC Buffer	4.0 uL
	dNTP mix (10 mM)	0.4 uL
	Phusion‰ High-Fidelity DNA Polymerase	0.2 uL
	Primer pool_2	**3.0 uL**
	Destillated water	7.4 uL
	cDNA template	**5.0 uL**

The mix includes pool_1 primers (see section 4.2)

Mix_2

	A	B
	5x Phusion‰ High-Fidelity PCR Master Mix with GC Buffer	4.0 uL

	A	B
	dNTP mix (10 mM)	0.4 uL
	Phusion‰ High-Fidelity DNA Polymerase	0.2 uL
	Primer pool_2	**3.0 uL**
	Destillated water	7.4 uL
	cDNA template	**5.0 uL**

The mix includes pool_2 primers (see section 4.2)

4.2 Tiling-PCR primers

	A	B	C	D	E
	Primer	Pool	Sequence (5' - 3')	Size	Position (bp)
	hPeV-3_1_LEFT	1	ATACCCCGATTTGCTGAGCTTC	22	41 – 987
	hPeV-3_1_RIGHT	1	GCCATTGAATGAAAGTTATCCACATTTC	28	
	hPeV-3_2_LEFT	2	GATGTAGTGCAAGCTACGACCA	22	899 – 1895
	hPeV-3_2_RIGHT	2	CTACTGTGGCTGCCCAATCAAA	22	
	hPeV-3_3_LEFT	1	TGATCCTAGAACTGCAGGGAGT	22	1750 – 2745
	hPeV-3_3_RIGHT	1	TCTTCAGTGTCATATGTATGAGCCAC	26	
	hPeV-3_4_LEFT	2	AAGAGGGTCATGGCATGTTGTC	22	2625 – 3631

	A	B	C	D	E
	hPeV-3_4_RIGHT	2	TGTAGACAAACAAGCAGTGGTTAGA	25	
	hPeV-3_5_LEFT	1	TCCTCAGCAGCCACAGAAATTC	22	3500 – 4487
	hPeV-3_5_RIGHT	1	TCTCTTCAAGATGTGCCATTGGG	23	
	hPeV-3_6_LEFT	2	GCCAGTGAGTTCATGGATGGTT	22	4340 – 5339
	hPeV-3_6_RIGHT	2	GCTGGTATCCTGCACAATGTGT	22	
	hPeV-3_7_LEFT	1	GCCAAACCAAAGAGTGCTTTCC	22	5192 – 6188
	hPeV-3_7_RIGHT	1	CGGACTTAACAAAGCTGTACCCT	23	
	hPeV-3_8_LEFT	2	GGCAAGGTGTTAAAGCATGTGTC	23	6039 – 7057
	hPeV-3_8_RIGHT	2	GCTGCTTGAATGTGCTGAAGTTT	23	
	hPeV-3_9_LEFT	1	ACCACCATCTTTAACACTTGTCTCA	25	6296 – 7323
	hPeV-3_9_RIGHT	1	TGGTATGTCCAATATTCCAAATTAGTGTTC	30	

4.3 Tiling-PCR thermocycling

6m 50s

1 98 °C 00:00:30

2 98 °C 00:00:10

3 54 °C 00:00:20

4 72 °C 00:00:50

5 Repeat steps 2-3-4 for 40 times.

6 72 °C 00:05:00

7 4 °C hold

4.4 Post PCR-clean-up

Reagents

- Ampure XP Beads, Beckman Coulter
- 70% EtOH (fershly prepared)
- Nuclease-free destillated water

4.5 Add 0.8 µl AMPure XP per 1.0 µl of sample.

4.6 Bind DNA fragments to paramagnetic beads.

4.7 Separation of beads + DNA fragments from contaminants.

4.8 Wash beads + DNA fragments twice with 70% EtOH to remove contaminants.

4.9 Elute purified DNA fragments from beads.

4.10 Transfer to new tubes.

Tiling-amplicon quantification using Qubit Flex fluorometer

10m

5 **Reagents**

- Qubit 1X dsDNA HS Assay Kit

5.1 Add 190 uL of working solution to all wells of two Qubit strips. Label the top of the tubes S1 and S2. Then add 10 uL of the respective HS Standard (1 or 2) to each tube of the two strips.

5.2 For each of your sample tubes, add 198 uL of working solution to labeled tubes. Then add 2 uL of your samples to each tube.

5.3 Vortex all strips and spin down if necessary. Incubate for 3-5 minutes at room temperature.

5.4 On the Qubit, select 1x dsDNA → dsDNA High Sensitivity → Read standards

5.5 Insert strip standard 1 and then select Read standard. Repeat for standard 2.

5.6 Select Run samples and read the respective wells of the strips. Use the 2 uL sample volume. When you are done.