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Version 2

Measles virus whole genome amplification (D4, B3,D8 genotypes) by in-house tiling-PCR V.2

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We use this protocol and it's working

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Abstract

The protocols allows whole genome amplification of the Measles virus Genotypes D4, B3 and D8 by utilizing tiling-PCR and in-house designed primer sets in two pools.



DNase I treatment (to remove contaminating human-derived gDNA)

1h 20m

1 Reagents

- TURBO DNaseTM (2 U/ μ L), Invitrogen
- Nuclease-free destillated water

1.1 Prepare the following master mix

	A	B
	TURBO DNase (2 U)	1.0 μ L
	TURBO DNase puffer	4.0 μ L
	Destillated water	6.0 μ L
	RNA	40.0 μL

1.2 Incubation in thermocycler

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 μ L AMPure XP per 1.0 μ L 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 30m

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

🌡️ 25 °C ⌚ 00:10:00

10m

3.3 Incubation in thermocycler

🌡️ 42 °C ⌚ 01:00:00

1h

3.4 Incubation in thermocycler

🌡️ 85 °C ⌚ 00:05:00

5m

Polymerase chain reaction (tiling-PCR)

6m 50s



4 Reagents

- PhusionTM 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 TM High-Fidelity PCR Master Mix with GC Buffer	4.0 uL
	dNTP mix (10 mM)	0.4 uL
	Phusion TM High-Fidelity DNA Polymerase	0.2 uL
	Primer pool_1	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 TM High-Fidelity PCR Master Mix with GC Buffer	4.0 uL
	dNTP mix (10 mM)	0.4 uL
	Phusion TM High-	0.2 uL



	A	B
	Fidelity DNA Polymerase	
	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

	Star t	End	Pool nr	Forward/ Reverse	Sequence (5'-3')
	1	29	1	F	CCAAACAAAGTTGGGTAA GGATAGATCA
	1101	1125	1	R	CTACTCCCATGGCATAGCT CCATA
	106 6	1091	2	F	TCAGAACAAGTTCAGTGCA GGATCA
	216 6	219 0	2	R	AGAGTCAGCATCTTGGATT CCCTT
	209 4	2118	1	F	TCAAGAAATCTCCAGGCAT CAAGC
	319 4	322 1	1	R	CGACTGGATTTTATAATGG AGCGGATT
	313 5	3157	2	F	AAAAAGATGAGCTCAGCC GTCG
	423 5	425 8	2	R	AGTTGTGCATGGAGAGTCT TGCT
	420 0	422 4	1	F	GCACCAGTCTTCACATTAG AAGCA
	530 0	532 2	1	R	GCTGTGGGATGCTGATGTC GTT



	525 7	527 7	2	F	AACCAGCACCCAAGAGCG AT
	635 7	637 9	2	R	AGCCTATGTTGTACGAGAC CCC
	631 8	634 1	1	F	CTGTCCGAGATTAAGGGG GTGAT
	7418	743 8	1	R	TGCAATGGCTAGCAACCC GA
	738 3	740 7	2	F	GCTGGCTGTTCTATTGCTC ATGTT
	848 3	850 6	2	R	GACCCCGTATGAAGGAAT CCTGT
	842 8	845 2	1	F	AAGGGTAAAATCCAAGCA CTCTGC
	952 8	955 4	1	R	ACGGATCTTCCTTGTTGAC TCTTTGT
	946 5	949 1	2	F	GTTATCCGACCCACTCTCA TATTCCA
	105 65	105 90	2	R	GCATAAAGCAGCCAAATCT CACTCC
	105 33	105 54	1	F	AGCAGTGCGTTGACAACT GGA
	1163 3	1166 4	1	R	GCCTTTGCCTAAGAATTAC AAAATAATCTCT
	1160 0	1162 3	2	F	AACCTTAAGAAACGGGAA GCTGC
	1270 0	1272 6	2	R	ATTCTAGTACATCAGGGAC CTCAAGG
	126 58	126 80	1	F	CCATATGTGGGCAAGGCTA GCT
	1375 8	1378 1	1	R	TGAAGACAACAGCTCACC CATCT
	1371 7	1374 8	2	F	GGCATTGTGATGTACATTAT CATAGACCATCA
	1481 7	148 47	2	R	TGGGGATATTACTGACTAT GAAATTGAAGC
	147 66	147 88	1	F	AGGTGCTCTTTAATGGGAG GCC



	158 65	158 91	1	R	AGACAAAGCTGGGAATAG AAACTTCG

4.3 Tiling-PCR thermocycling

6m 50s

- 1 98 °C 00:00:30
- 2 98 °C 00:00:10
- 3 52 °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 10 °C hold

4.4 Post-PCR clean-up

Reagents

- AMPure XP Beads, Beckman Coulter
- 70% EtOH (prepared freshly)
- 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.