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Measles virus whole genome amplification (D4, B3,D8 genotypes) by in-house tiling-PCR V.1

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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 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

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 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

10m

25 °C 00:10:00

3.3 Incubation in thermocycler

1h

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_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‰ High-Fidelity PCR Master Mix with GC Buffer	4.0 uL
	dNTP mix (10 mM)	0.4 uL
	Phusion‰ 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

	Start	End	Pool nr	Forward/ Reverse	Sequence (5'-3')
	1	29	1	F	CCAAACAAAGTTGGGTAAGGATAGATCA
	1101	1125	1	R	CTACTCCCATGGCATAGCTCCATA
	1066	1091	2	F	TCAGAACAAGTTCAGTGCAGGATCA
	2166	2190	2	R	AGAGTCAGCATCTTGGATTCCCTT
	2094	2118	1	F	TCAAGAAATCTCCAGGCATCAAGC
	3194	3221	1	R	CGACTGGATTTTATAATGGAGCGGATT
	3135	3157	2	F	AAAAAGATGAGCTCAGCCGTCG
	4235	4258	2	R	AGTTGTGCATGGAGAGTCTTGCT
	4200	4224	1	F	GCACCAGTCTTCACATTAGAAGCA
	5300	5322	1	R	GCTGTGGGATGCTGATGTCGTT

	5257	5277	2	F	AACCAGCACCCAAGAGCGAT
	6357	6379	2	R	AGCCTATGTTGTACGAGACCCC
	6318	6341	1	F	CTGTCCGAGATTAAGGGGGTGAT
	7418	7438	1	R	TGCAATGGCTAGCAACCCGA
	7383	7407	2	F	GCTGGCTGTTCTATTCGTCATGTT
	8483	8506	2	R	GACCCCGTATGAAGGAATCCTGT
	8428	8452	1	F	AAGGGTAAAATCCAAGCACTCTGC
	9528	9554	1	R	ACGGATCTTCCTTGTTGACTCTTTGT
	9465	9491	2	F	GTTATCCGACCCACTCTCATATTCCA
	10565	10590	2	R	GCATAAAGCAGCCAAATCTCACTCC
	10533	10554	1	F	AGCAGTGCGTTGACAACTGGA
	11633	11664	1	R	GCCTTTGCCTAAGAATTACAAAATAATCTCT
	11600	11623	2	F	AACCTTAAGAAACGGGAAGCTGC
	12700	12726	2	R	ATTCTAGTACATCAGGGACCTCAAGG
	12658	12680	1	F	CCATATGTGGGCAAGGCTAGCT
	13758	13781	1	R	TGAAGACAACAGCTCACCCATCT
	13717	13748	2	F	GGCATTTGATGTACATTATCATAGACCATCA
	14817	14847	2	R	TGGGGATATTACTGACTATGAAATTGAAGC
	14766	14788	1	F	AGGTGCTCTTTAATGGGAGGCC

	15865	15891	1	R	AGACAAAGCTGGGAATAGAAACTTCG

4.3 Post-PCR clean-up

Reagents

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

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

4.5 Bind DNA fragments to paramagnetic beads.

4.6 Separation of beads + DNA fragments from contaminants.

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

4.8 Elute purified DNA fragments from beads.

4.9 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.