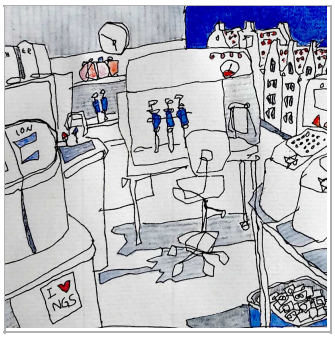

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We use this protocol and it's working

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Measles whole-genome sequencing

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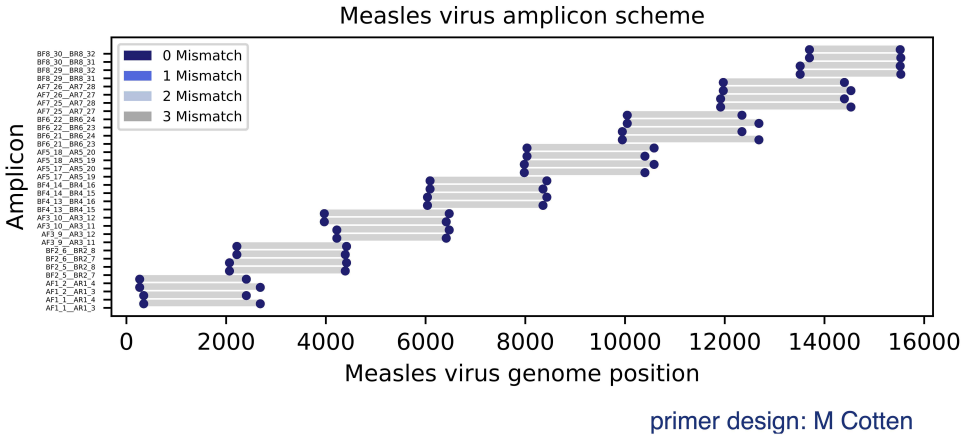
ABSTRACT

This is an optimised protocol for PCR amplification of the Measles virus (MV) genome using specific primers designed for B3 genotype. The method generates cDNA amplicons suitable for whole genome sequencing using the MinION platform. The method covers the entire ca. 16,000 nt MV genome in 8 overlapping amplicons of about 2200 bp (see Figure below). Both the reverse transcription and PCR steps are performed in two separate reactions to avoid short overlapping PCR products.

The method has worked successfully with MV directly from oral fluid samples and with tissue culture passaged material. Primer sequences can be found at the following GitHub site: https://github.com/mlcotten13/Measles_primers



Amplicon layout: Grey bars indicate individual amplicons, blue circles indicate primer location, color coded by number of mismatchs to test genomes, in this instance all primers had 100% match to the genome seqeunces. Each amplicon was prepared with two forward and two reverse primers for security agains virus evolution.

PCR amplification of measles virus genome

1 cDNA synthesis

> **Note**
>
> Please use fresh RNA extracts, and always include 1 positive control and 1 negative control (water). Prepare the mastermix in a clean mastermix working cabinet, separated from template adding cabinet.

1.1 6m

For each sample, prepare 2 separate reactions called "FPM_A" and "FPM_B". For each reaction, add the following:

- Primers: 2 µL FPM_A or FPM_B primer
- Template: 10 µL RNA extract

Then incubate the reactions using the following conditions:

- 65 °C for 00:05:00
- On ice for 00:01:00

1.2 1h

For each FPM reaction, add the following:

- 4 µL 5X First strand buffer
- 1 µL DTT
- 1 µL dNTPs of 10mM each
- 1 µL RNAse OUT Inhibitor (40 U/µL)
- 1 µL SuperScript III polymerase (200 U/µL)

Then incubate the reactions using the following conditions:

- 42 °C for 00:50:00
- 70 °C for 00:10:00

2 PCR amplifications 14m 30s

> **Note**
>
> Prepare the mastermix in a clean mastermix working cabinet, separated from template adding cabinet. For each sample, the reaction PPM_A is for amplification of cDNA from FPM_A reaction. The reaction PPM_B is for amplification of cDNA from FPM_B reaction. Do not mix up the A and B reactions.

For each of the FPM_A and FPM_B reactions, prepare the mastermix for PPM_A and PPM_B, respectively. The mastermix for each PPM reaction include:

- 7 µL Nuclease-free Water
- 5 µL 5X HF Buffer
- 0.5 µL dNTPs of 10mM each
- 2 µL Primer mixes of PPM_A or PPM_B
- 0.5 µL Phusion DNA polymerase

Add 10 µL cDNA from reactions **FPM_A** or **FPM_B** to each of the corresponding tube for **PPM_A** or **PPM_B**, respectively.

Set up the PCR cycling conditions:

- 98 °C heat inactivation for 00:00:30
- Cycles **35-40 times** using the following:

1. Denaturation at 98 °C for 00:00:15
2. Anneal at 55 °C for 00:00:45
3. Extension at 72 °C for 00:03:00

- Polish at 72 °C for 00:10:00
- Then hold at 10 °C for indefinite

Note

Thermocycler instruments have varied temperature profiles. For initial experiments, you should check results using annealing temperatures from 55 to 58 degrees and decide the temperature that work best for your instrument.

3 Running the PCR amplification products on Agilent Tapestation or agarose gel electrophoresis to check for products at approximately 2 - 2.5 kb.
For samples with amplifications for both PPM_A and PPM_B reactions, pool PCR products of PPM_A and PPM_B. This pooled PCR product can be used directly for library preparation for sequencing on MinION using LSK109 kits. For Illumina sequencing, the pooled PCR products will need to be sheared for appropriate size. For Ion Torrent sequencing, please refer to the manufacturer's instructions for library preparation.