# Whole genome sequencing of the Piscine Myocarditis Virus (PMCV) directly from field samples

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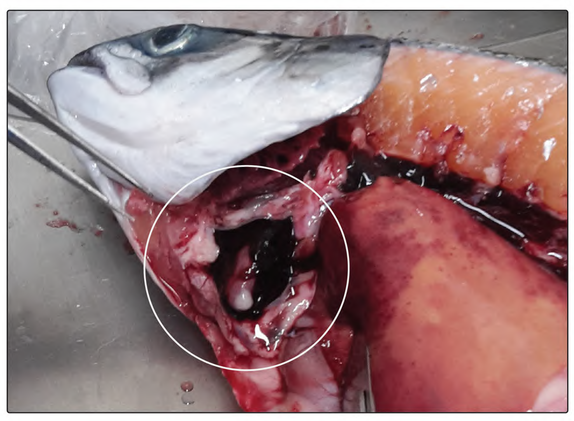
## INTRODUCTION

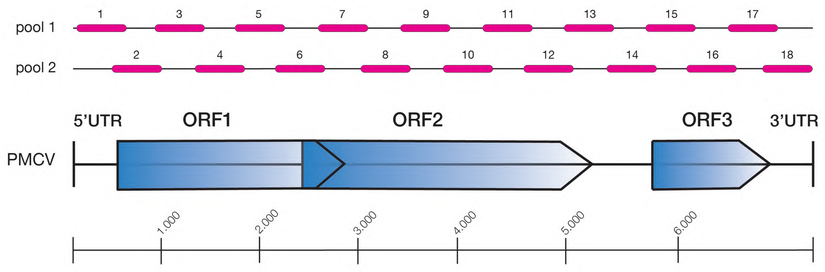
Cardiomyopathy syndrome (CMS) has proven to be of increasing concern to the salmon farming industry. CMS is characterized by severe inflammation and degeneration of the heart, which in acute cases causes it to rupture. The causal virus, Piscine Myocarditis Virus (PMCV), is a dsRNA virus and has as of yet not been successfully cultivated, which has limited the full characterization of it. The main aim of this study was to develop a method for whole genome sequencing of PMCV directly from field samples.



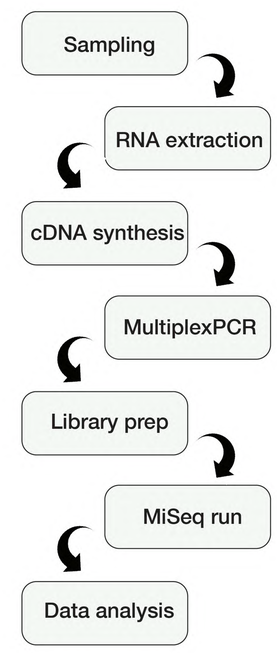
## METHOD

A multiplex PCR with 18 overlapping fragments was designed with fragment sizes varying from 452 bp to 536 bp. All primer pairs were generated with the online primer design tool, Primal Scheme (Quick *et al.,* 2017), using the 6.688 bp sequence from Haugland *et al.,* 2011 as template - which to date is the only published full genome sequence of PMCV. By pooling equimolar amounts of every other primerpair to overlapping fragments in two separate pools, the full genome of a PMCV could be amplified in only two multiplex PCR reactions. These were subsequently sequenced on an Illumina MiSeq system. This method allows for 47 samples per run (+ one negative control).

All samples included in this study have been tested PMCV positive with qPCR, date from 2012 to 2019, and represent all three production compartments as well as wild salmon found in the Faroe Islands.




Schematic presentation of the 18 overlapping fragments covering the PMCV genome.




Overview of the workflow

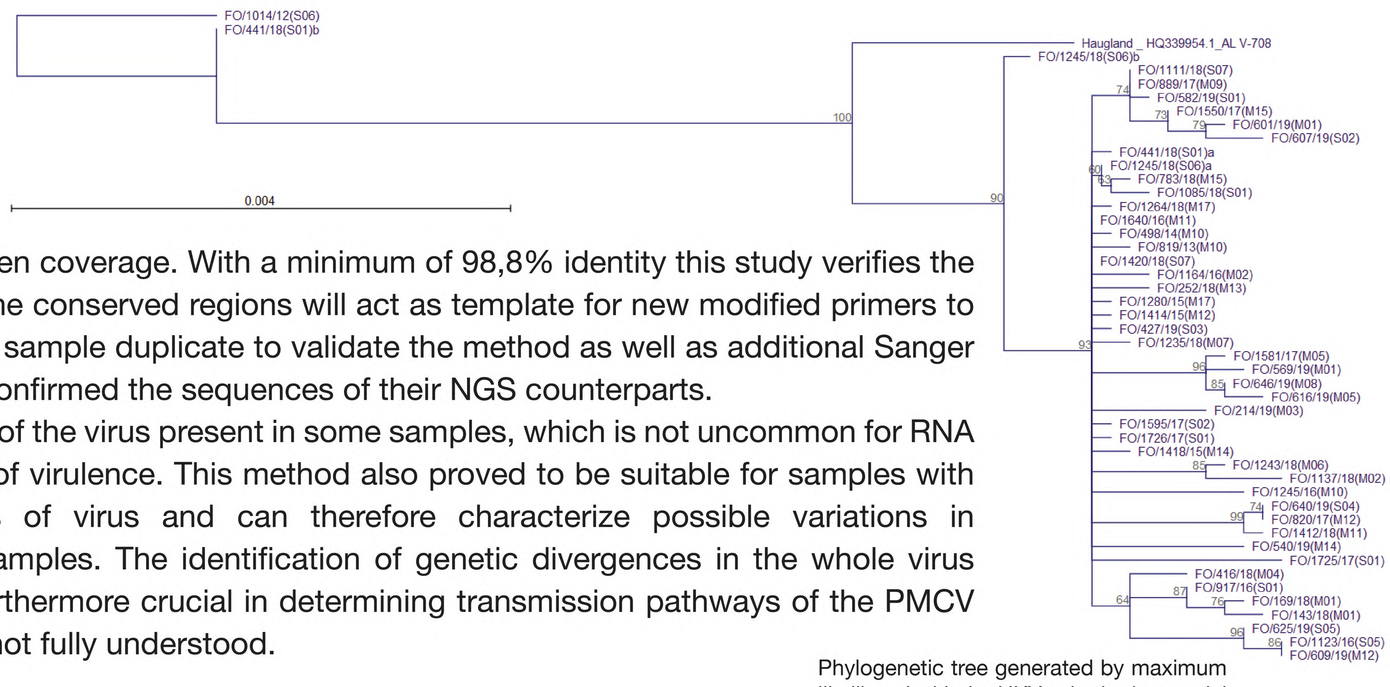
Haugland, O. et al. (2011). Cardiomyopathy syndrome of Atlantic salmon (Salmo salar L.) is caused by a double-stranded RNA virus of the totiviridae family. Journal of Virology, 85, 5275– 5286.

Quick, J. et al. (2017) Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nature Protocols 12, 1261-1276

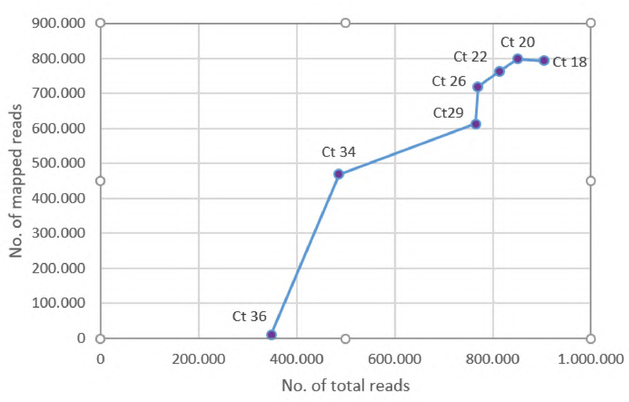
## RESULTS

The MiSeq run yielded 6.585 bp sequences with 100% coverage. Depth of coverage however varied between fragments from a minimum of 600 to a maximum of 131.800 reads. The data analysis detected several SNPs in primer sequences which most likely accounts for some of the uneven coverage. With a minimum of 98,8% identity this study verifies the relatively homogeneous nature of the PMCV genome, and the conserved regions will act as template for new modified primers to as far as possible prevent primer bias. The study included a sample duplicate to validate the method as well as additional Sanger sequencing of samples with Ct values below 28 - these all confirmed the sequences of their NGS counterparts.

The sequencing data further detected more than one variant of the virus present in some samples, which is not uncommon for RNA viruses and can be important for research in e.g. evolution of virulence. This method also proved to be suitable for samples with low amounts of virus and can therefore characterize possible variations in pre-clinical samples. The identification of genetic divergences in the whole virus genome is furthermore crucial in determining transmission pathways of the PMCV which still is not fully understood.




Phylogenetic tree generated by maximum likelihood with the HKY substitution model and bootstrap values from 1000 replicas - threshold is set to 60.




Comparative plot showing a tendency of increasing number of total reads and mapped reads with decreasing Ct values.

Graph of mapped reads illustrating overlapping fragment distribution and relative depth of coverage.

## ACKNOWLEDGMENT

This study is part of an ongoing project in cooperation with P/F Bakkafrost, Mowi ASA, P/F Luna, SpF Aquamed and Andras Strøm and is funded by the Faroese Research Council (Grant No. 0453).