

# Viral metagenomics of mussels from Slovenian coast

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## Introduction

Shellfish are commonly involved in foodborne outbreaks, especially when consumed raw. Noroviruses and hepatitis A virus are implicated in a number of outbreaks. Viruses are usually present in shellfish in low numbers, but sufficient to pose a health risk. Shellfish are filtering organisms that concentrate pollutants present in the water, thus are ideal for monitoring pollution of the environment. In Slovenia, noroviruses are frequently detected in mussels using RT-qPCR method but with very low copy numbers (Ct values > 35), while hepatitis A virus was detected only once. In this study, we used 5 samples of mussels harvested from Slovenian coastal areas in which noroviruses (4 samples) or hepatitis A (1 sample) were detected to investigate the utility of next generation sequencing (NGS) technology for virus detection with metagenomics approach.

## Materials and Methods

### Sample Preparation

Sample: Suspension of Mussels Digestive Glands → RNA/DNA Extraction → cDNA Synthesis → Fragmentation (COVARIS M220) → Library Preparation → Quantification (Qubit qPCR) → Sequencing (Ion PGM)

### Bioinformatic Analysis

Sequencing Reads → Quality Trimming (Geneious: BBDuk) → BlastX (DIAMOND) → Taxonomic Assignment (MEGAN6 using LCA Algorithm)

## Results and Discussion

A total of 3,196,693 (mean length of 250 nt) clean reads were obtained from the 5 samples. The BlastX search identified 785,240 reads in the samples, of which 131,359 belonged to viruses. Other reads belonged to cellular organisms (454,608 reads), unclassified and other sequences (1,259 reads) and unassigned sequences (189,332 reads). Most of the virus sequences were assigned to viruses originating from environmental samples (87,488 reads). Other virus sequences belonged to 22 virus families and also to unclassified viruses, including dsDNA, dsRNA, ssDNA and ssRNA viruses of various origin (bacterial, plant, insect, invertebrate, fish, mammalian, algae).

Although, using NGS technology with metagenomic approach, noroviruses or hepatitis A virus were not detected in mussel samples, this technology was able to detect other viruses infecting mammals, namely picornaviruses, parvoviruses and circoviruses.

Group	Order	Family/Subfamily (Genus)	Number of reads	Host
dsDNA viruses	Caudovirales	<i>Myoviridae</i>	2749	bacteria
		<i>Podoviridae</i>	3027	bacteria
		<i>Siphoviridae</i>	1595	bacteria
		unclassified <i>Caudovirales</i>	56	bacteria
	/	<i>Iridoviridae</i>	38	amphibia, fish, invertebrates, insects
	/	<i>Marseilleviridae</i>	8	amoeba
	/	<i>Mimiviridae</i>	103	amoeba
	/	<i>Phycodnaviridae</i>	648	algae
	/	unclassified dsDNA phages	591	/
	/	unclassified dsDNA viruses	63	/
dsRNA viruses	/	<i>Partitiviridae</i>	7	plant, fungi
	/	<i>Picobirnaviridae</i>	3	mammals
	/	<i>Circoviridae</i>	398	mammals, birds
	/	<i>Geminiviridae</i>	7	plant
ssDNA viruses	/	<i>Microviridae</i>	3992	bacteria
	/	<i>Parvoviridae</i> / <i>Densovirinae</i>	60	invertebrates
	/	<i>Parvoviridae</i> / <i>Parvovirinae</i>	3	vertebrates
	/	unclassified ssDNA viruses	94	/
	/	<i>Dicistroviridae</i>	8	invertebrates
ssRNA viruses	Picornavirales	environmental samples	332	/
		<i>Picornaviridae</i> ( <i>Cardiovirus</i> )	21	human, vertebrates
		unassigned <i>Picornavirales</i> ( <i>Bacillarnavirus</i> )	1003	algae
	/	<i>Tombusviridae</i>	57	plant
Ortervirales	<i>Tymovirales</i>	<i>Tymoviridae</i>	3	plant
	/	<i>Virgaviridae</i>	6	plant
	/	<i>Nodaviridae</i>	153	fish, insects, mammals
	/	unclassified ssRNA viruses	6102	/
	<i>Retroviridae</i> ( <i>Alpharetrovirus</i> )	26	birds	
	/	environmental samples viruses	87488	/
	/	unclassified phages	2182	/
/	unclassified virophages	83	/	
/	unclassified viruses	332	/	