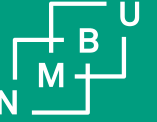


The molecular basis of PMCV infection and the disease, CMS

Norwegian seafood research fund # 901671, Does PMCV primarily infect salmon?

Co-authors: Espen Rimstad, NMBU, Ingvild B. Nyman, NMBU; Torstein Tengs, Nofima, Øystein Wessel, NMBU, Marta Alarcon, Pharmaq Analytiq



The molecular basis of PMCV infection and the disease, CMS

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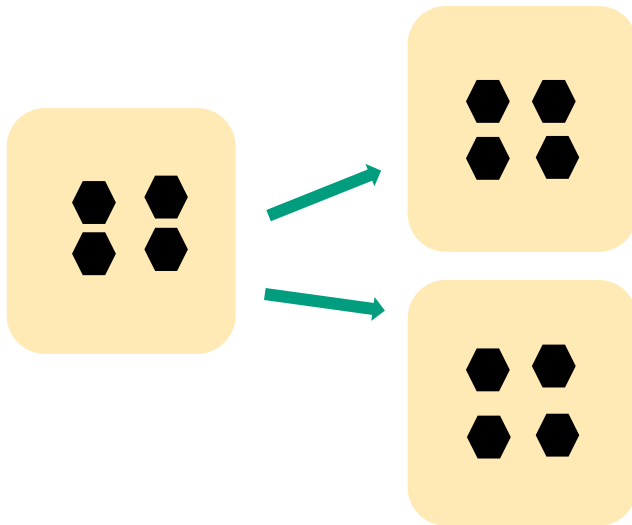
Outline:

- Short about dsRNA virus and Totiviruses
- Spread between fish
- Spread within heart
- Viral RNA genomic dsRNA versus mRNA
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- Does PMCV primarily infect salmon?

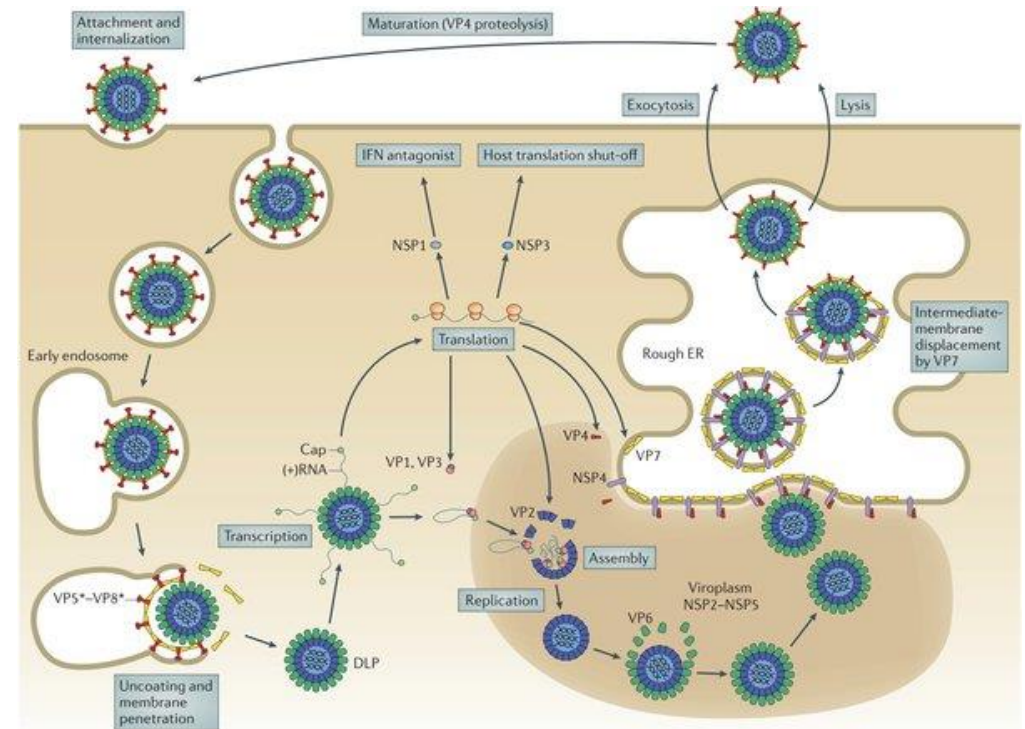
Properties of PMCV versus Totiviruses

PMCV particle

- Classical Totiviruses are associated with latent infections of yeast, protozoa, fungus.
- With a few exceptions, these viruses are not spread outside the cells
- They spread when the unicellular host divides.
- Very little horizontal spread



- This is very different from viruses infecting cells of a vertebrate animal.
- Viruses infecting vertebrates are always spread extracellularly, and between individuals

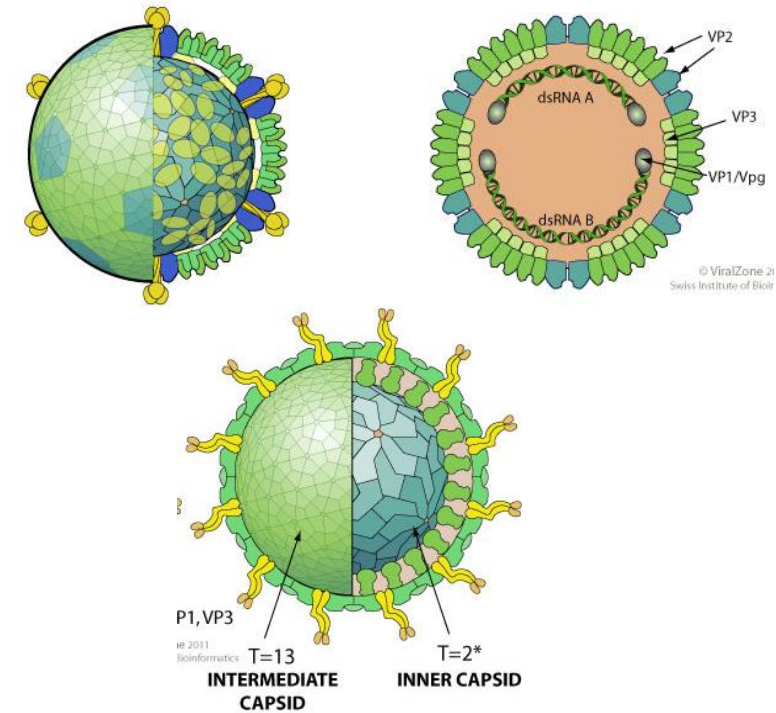
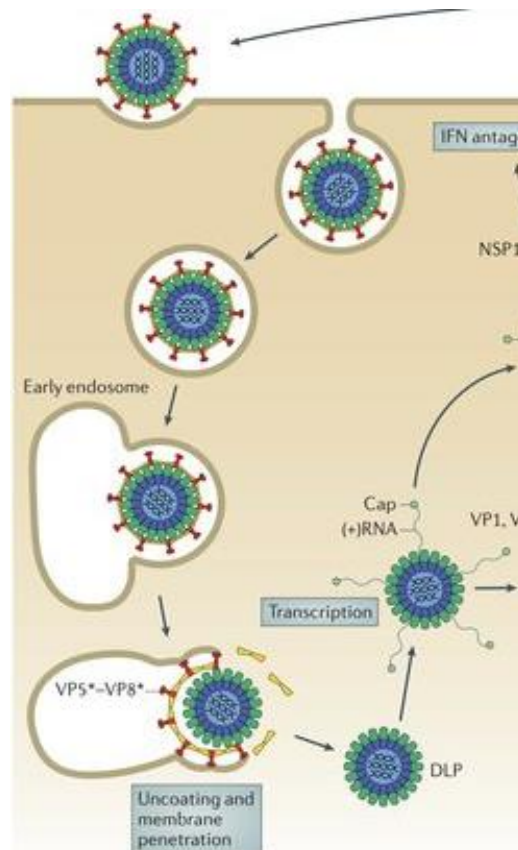
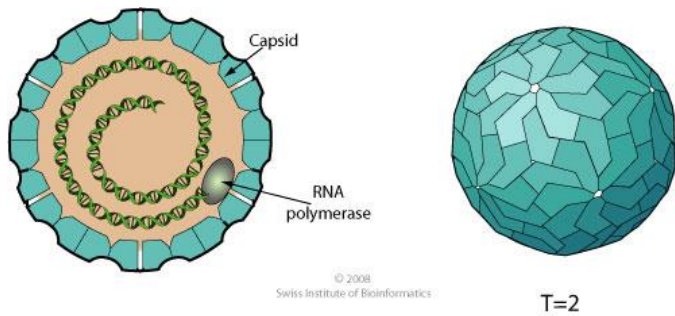


Properties of PMCV versus Totiviruses

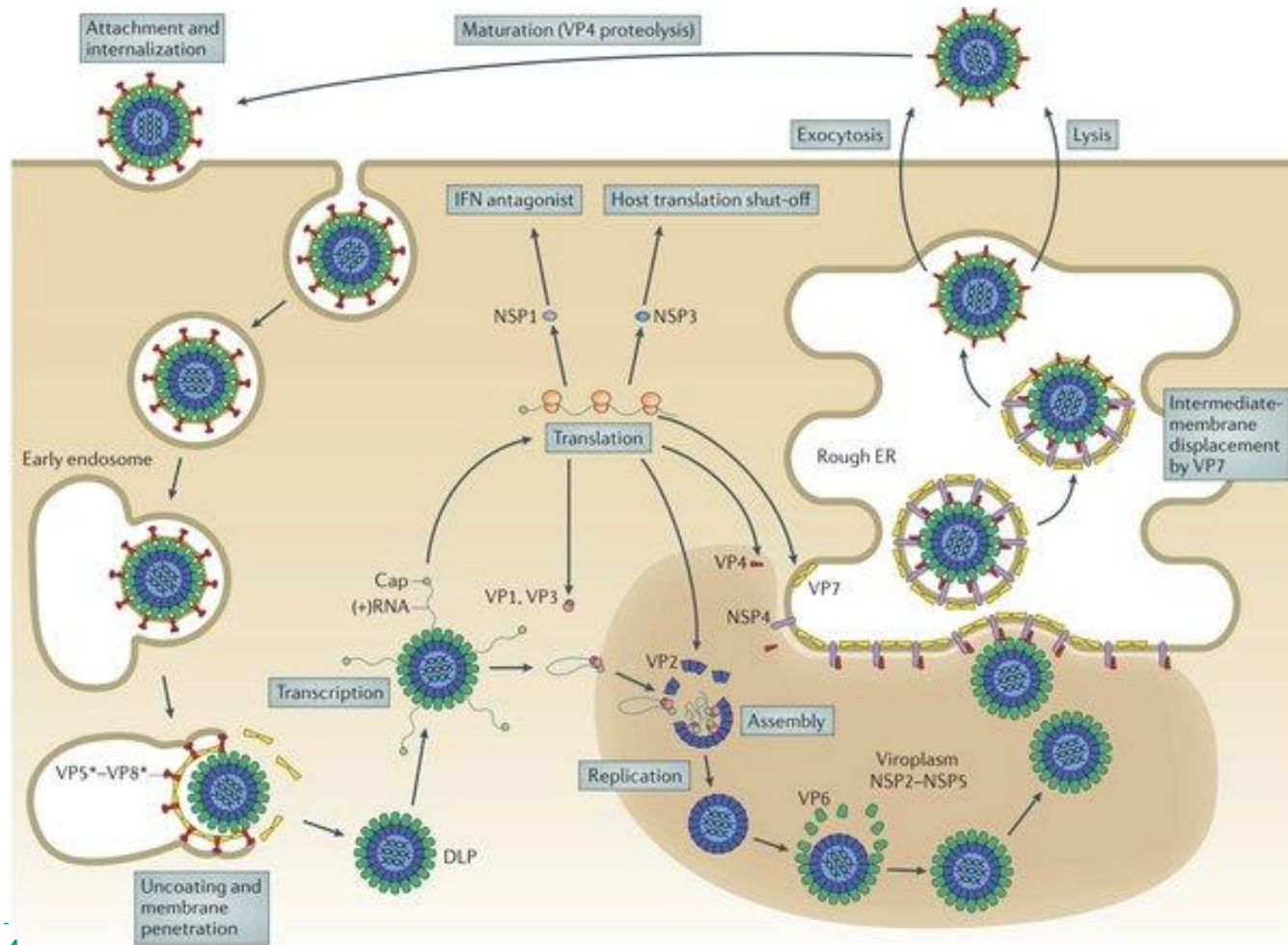
PMCV particle

- **Totiviruses**
- The capsids are single-shelled
- RNA density in the capsid is half of that of viruses infecting vertebrates

- Capsid viruses with dsRNA genome infecting cells of a vertebrate animal have more than one layer in the capsid.
- They need this to enter the cells.

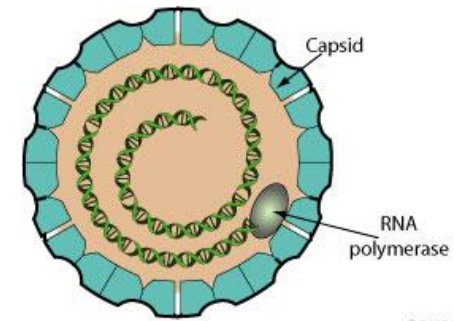
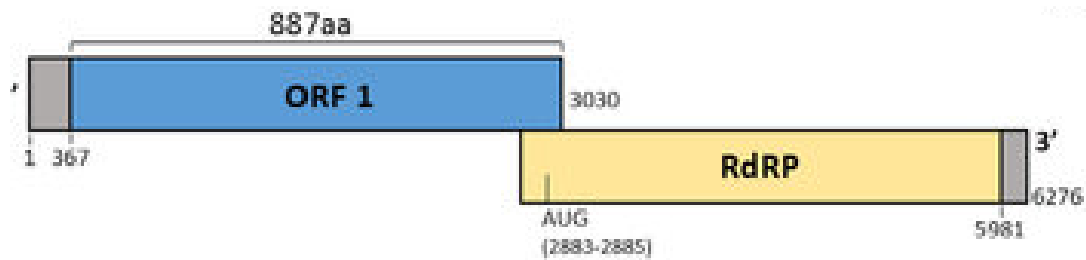


Replication of viral dsRNA in a vertebrate cell

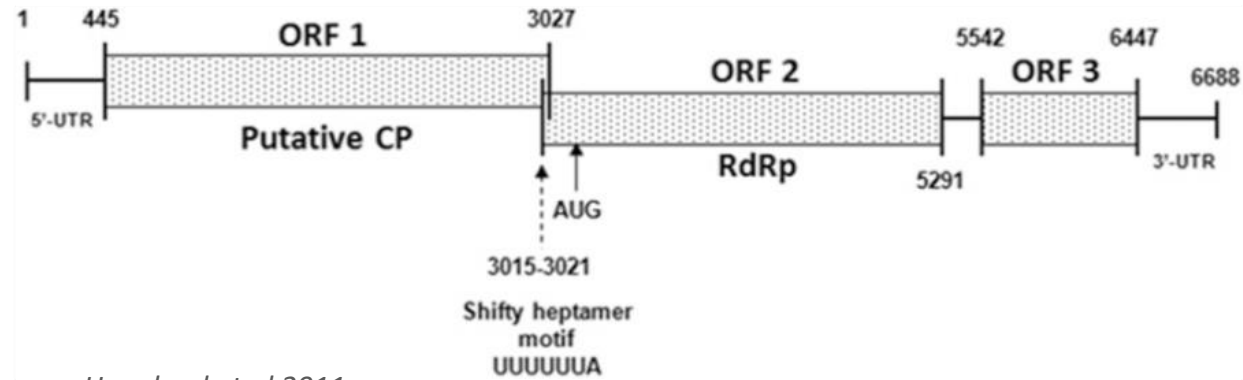


1. The dsRNA genome is never released into the cytoplasm
2. mRNA (identical to the + strand of the dsRNA genome) is emitted from the capsid/core particle.
3. mRNA is translated and packaged before new dsRNA is made.
4. There are thus 2 types of viral RNA in the cell, dsRNA – genomic, +ssRNA = viral mRNA

"PMCV is different from other totiviruses"



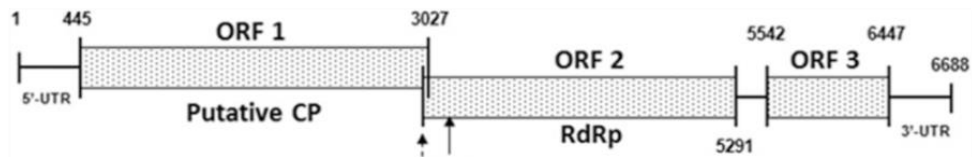
© 2008 Swiss Institute of Bioinformatics



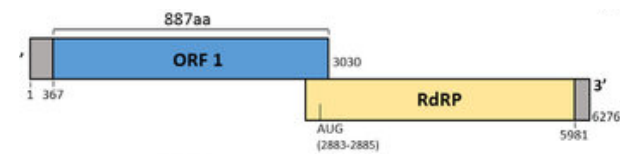
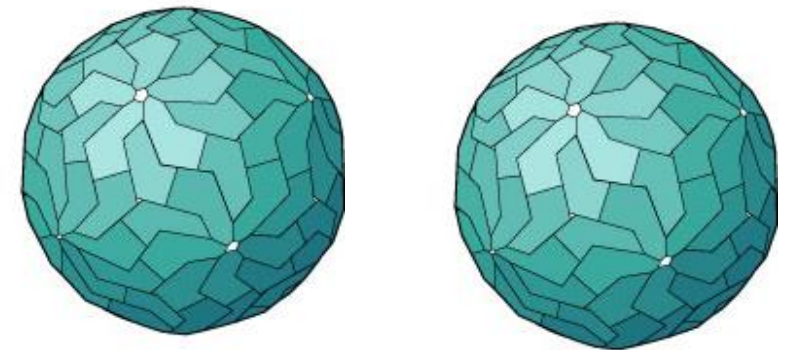
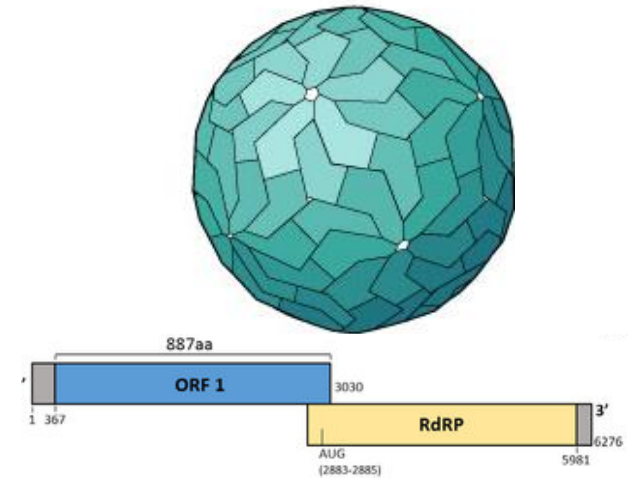
Haugland et.al 2011

The genome of PMCV

- Totiviruses were originally discovered in the 1960-ies because some wine-yeast strains were killing other yeast strains.
- Totivirus isolates (of the yeast *Saccharomyces*) supports the replication of one of several satellite dsRNAs encoding a secreted toxin (killer toxins).
- These additional dsRNAs are also encapsidated separately in capsids encoded by the helper virus genome.



PMCV

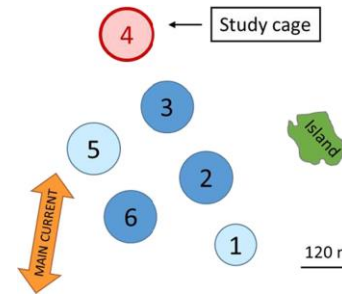


TOX

Spread of PMCV between fish?



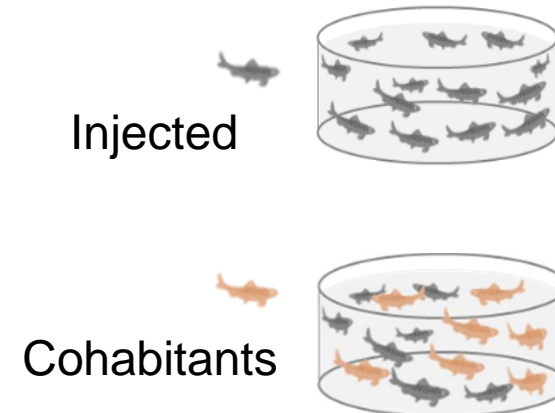
- “...Ct-values below 10 in all CMS-diagnosed fish”
“Our study cage was the only cage at the site with a CMS diagnosis until 49 wpd.” (Fritsvold et al 2021)



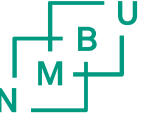
Fritsvold et al. Journal of Fish Diseases, Volume: 44, Issue: 12, Pages: 2067-2082,

1. Field observations; Very little horizontal transmission of the virus!

- “24 weeks after PMCV was injected into the fish, PMCV could be detected by PCR in only 13/24 of the cohabitant fish in the same tank (Su et al. 2021)”



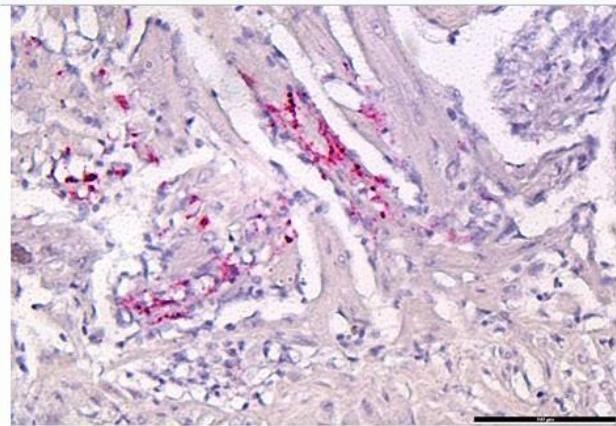
2. Experimental observations; Very little horizontal transmission of the virus!



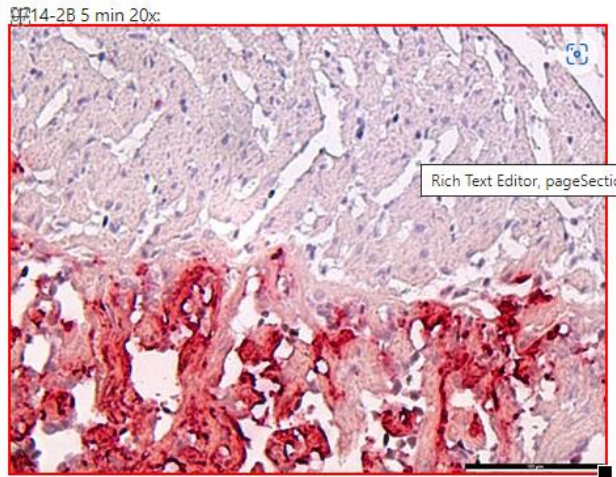
Outline:

- Short about dsRNA virus and Totiviruses *Done*
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- Spread within heart
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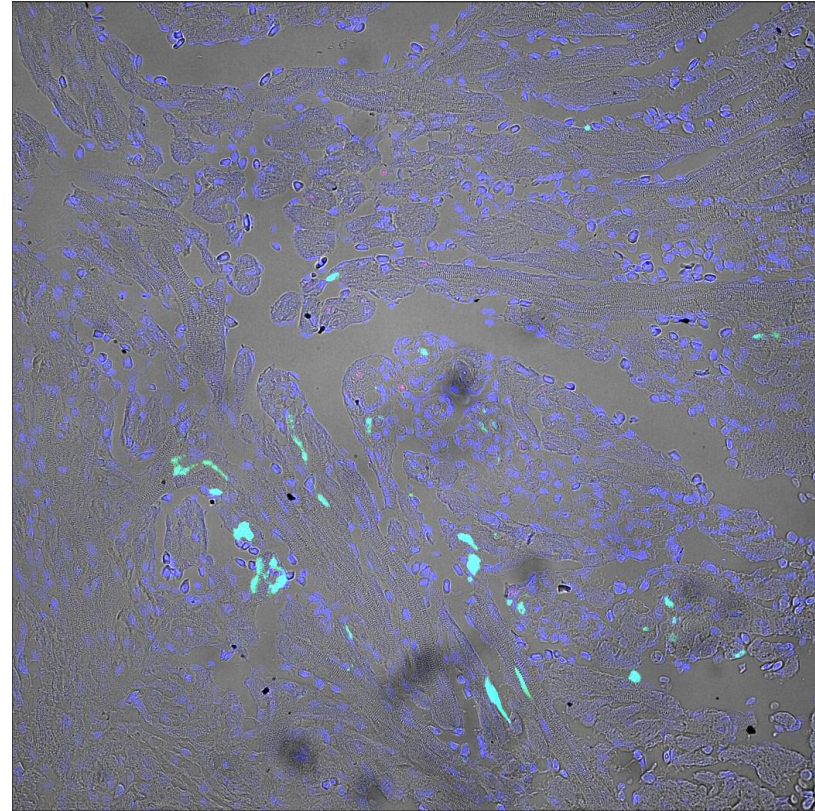
Viral RNA in heart



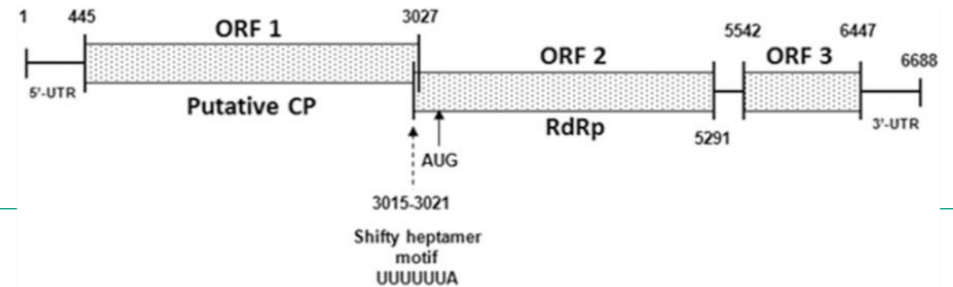
Ct 20

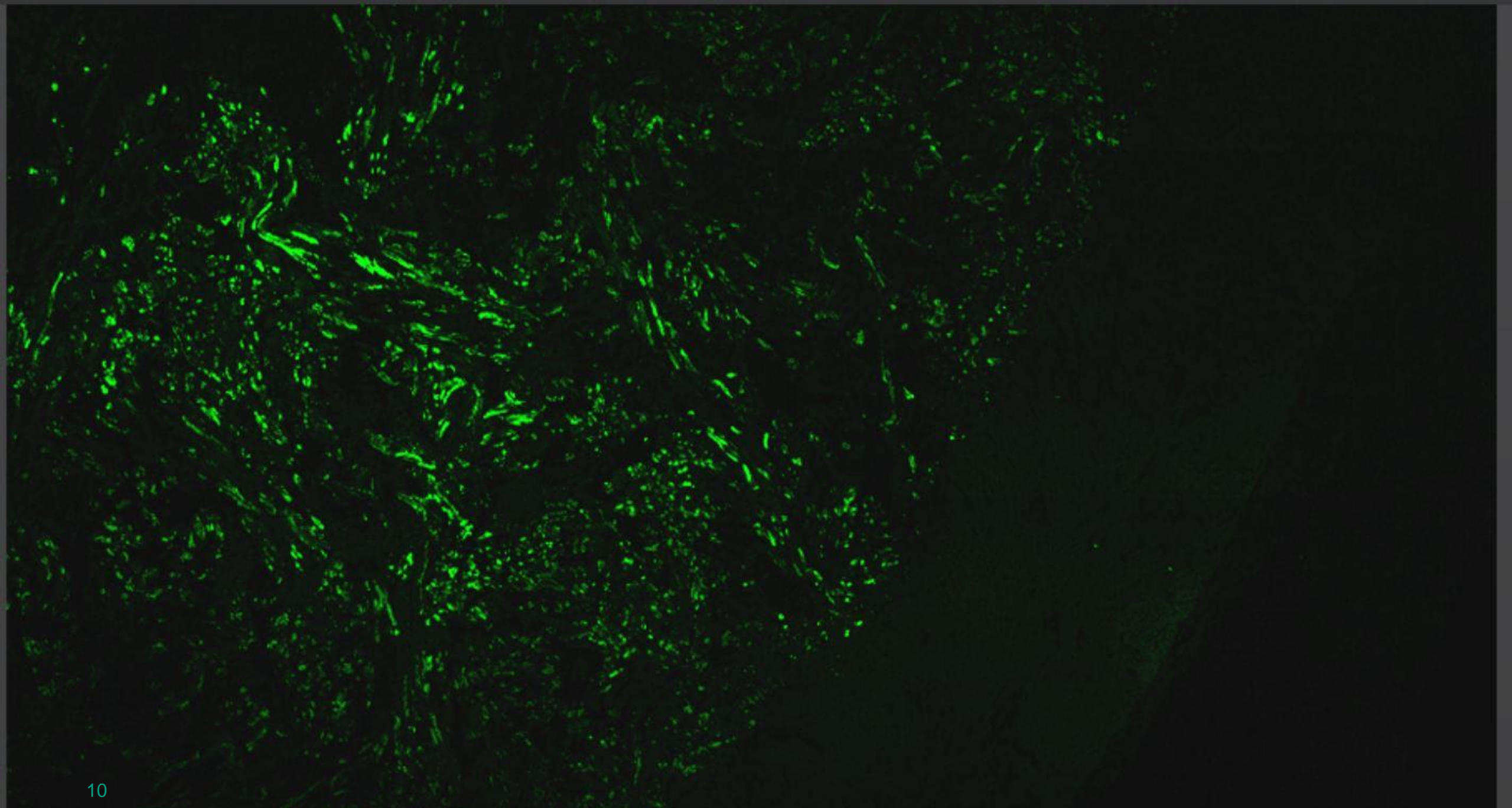


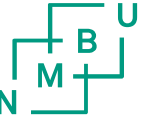
Ct 13



Probe
 V-PMCV-ORF2
 V-Piscine-myocarditis-ORF1
 V-PMCV-ORF2-sense



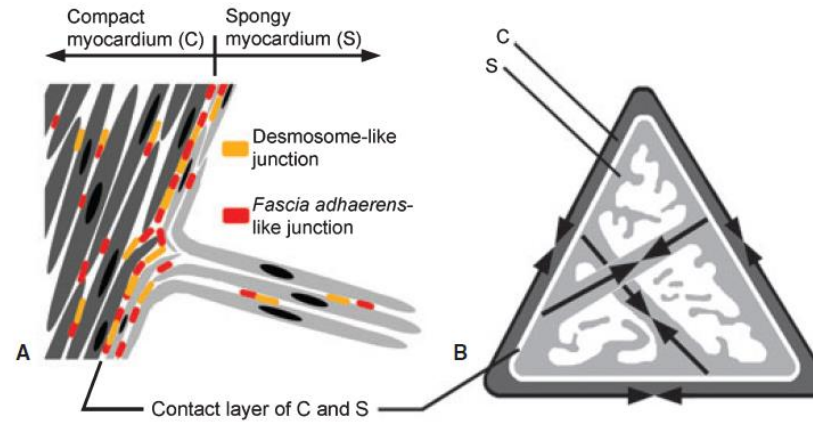
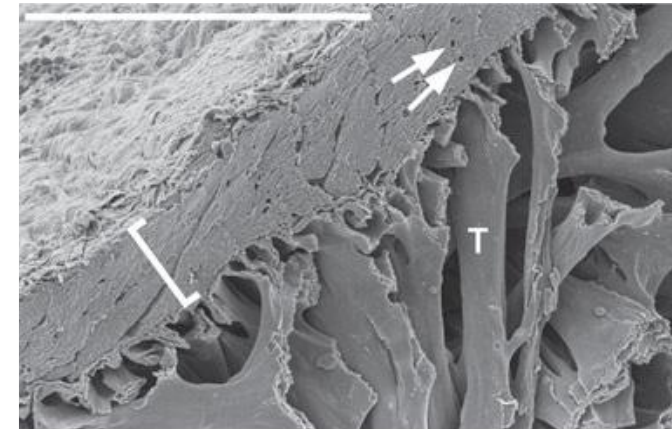
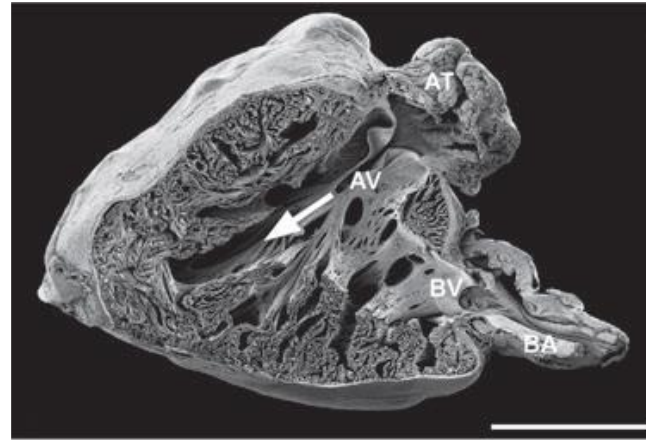
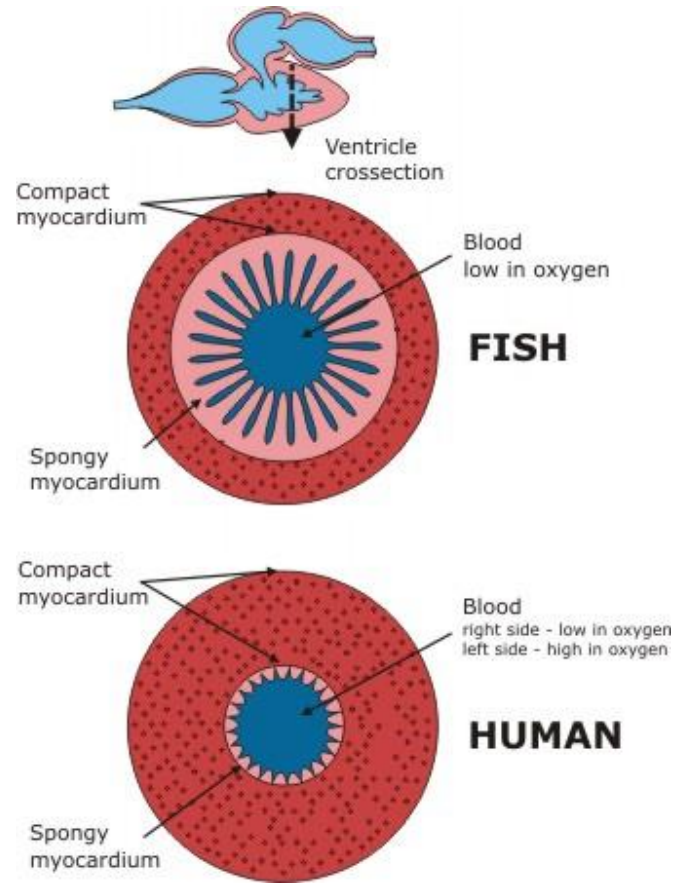




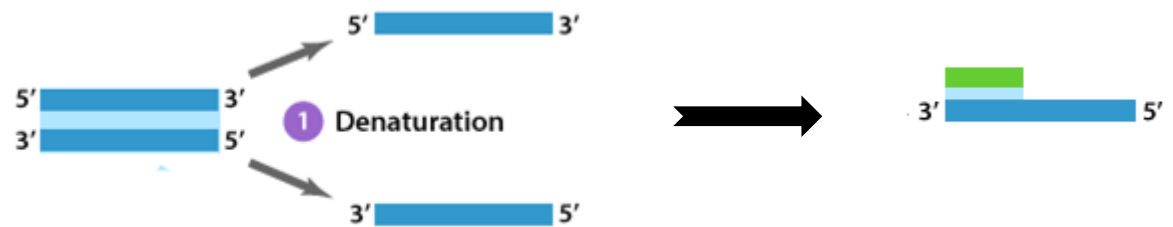
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The spread of PMCV within the fish



Detection of viral dsRNA and mRNA using RT-qPCR




dsRNA must be heated before cDNA-synthesis for detection (dsRNA = genome of the virus)



mRNA do not require denaturation for detection

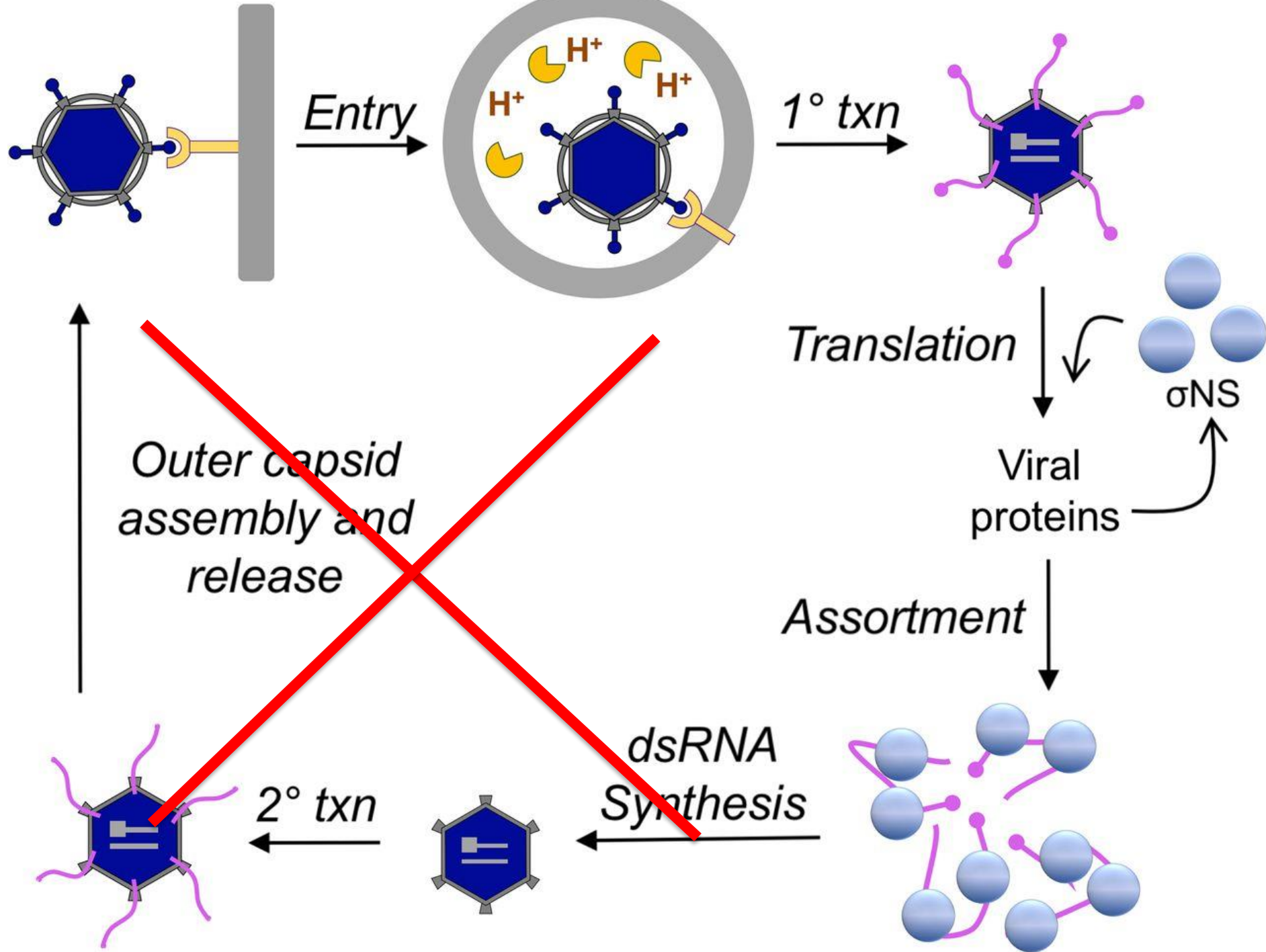
- Extracted RNA heated before cDNA synthesis = detection of **both** dsRNA + mRNA.
- No heating = detection of **only** mRNA

 = primer used in cDNA synthesis

Detection of viral dsRNA and mRNA using RT-qPCR

PMCV RNA heated = ssRNA + dsRNA		PMCV RNA Not heated = ssRNA	Δ Ct dsRNA	Amount of ssRNA RNA
Heart 1	17.05	17.23	-0.18	88%
Heart 2	13.36	13.32	0.04	100%
Heart 3	14.19	14.49	-0.30	81%
Heart 4	12.79	12.78	0.01	100%
Heart 5	17.70	18.65	-0.95	52%
Kidney 1	21.66	25.47	-3,81	7%
Kidney 2	20.20	23.06	-2,28	20%
Kidney 3	20.26	24.24	-3,98	6%
Kidney 4	21.74	25.27	-3,53	9%
Kidney 5	19.00	23.21	-4,21	5%

Only minor production of viral particles in the heart!

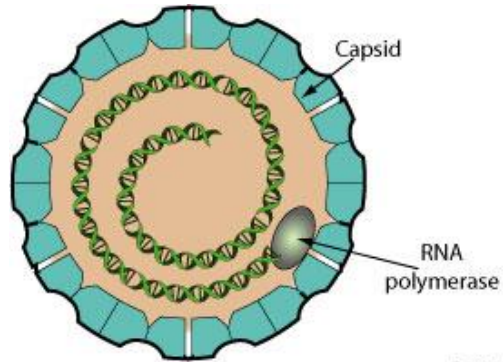




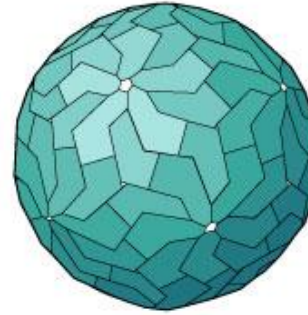
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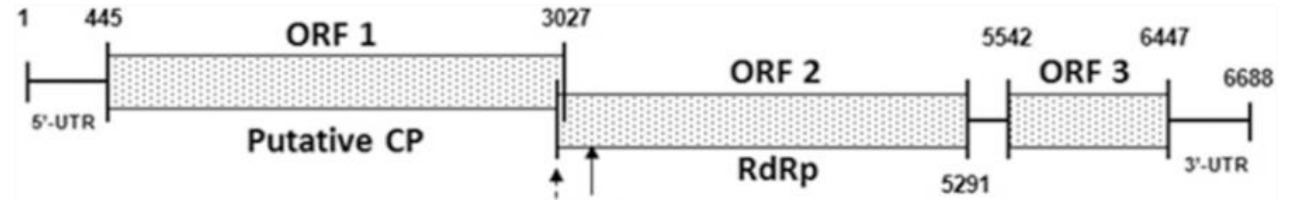
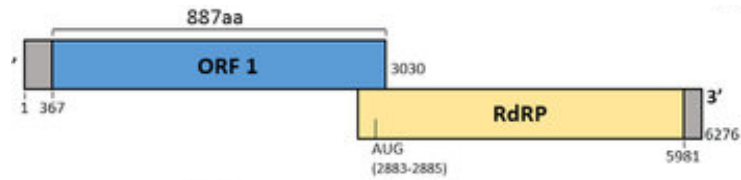
The genome of PMCV



© 2008
Swiss Institute of Bioinformatics



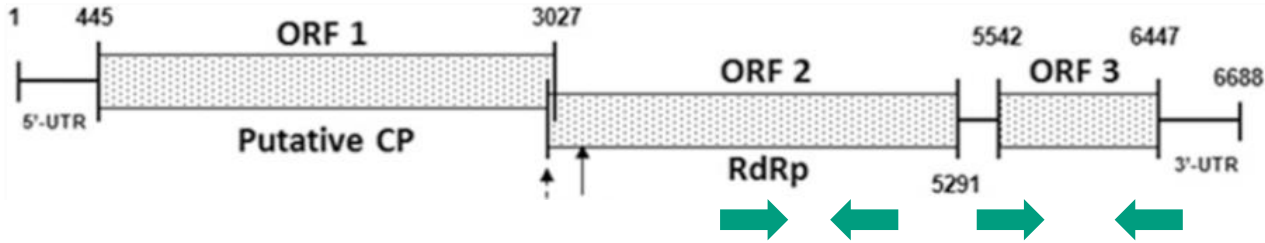
T=2



Classical Totiviruses
Infection of unicellulars
Described also from two fish species
(from metagenomic studies)

“Toti-like” viruses
Extra ORF
Described from four fish species (from metagenomic studies)

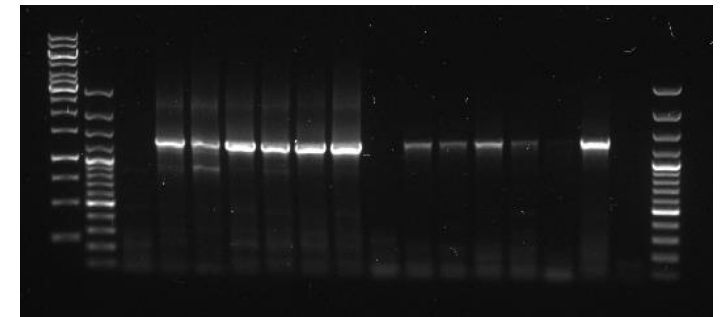
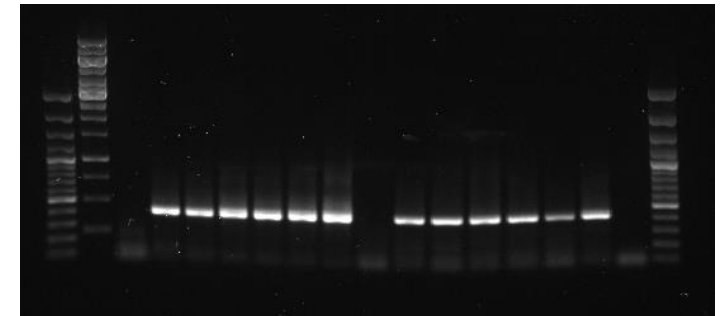
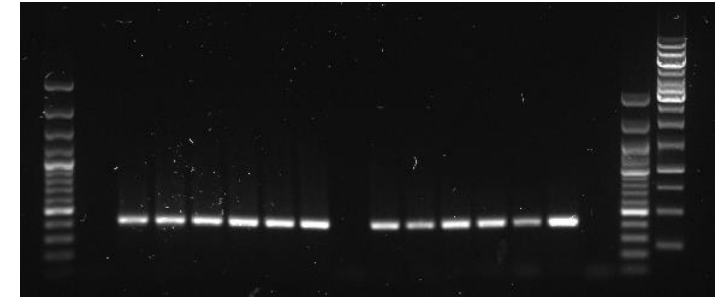
The genome of PMCV



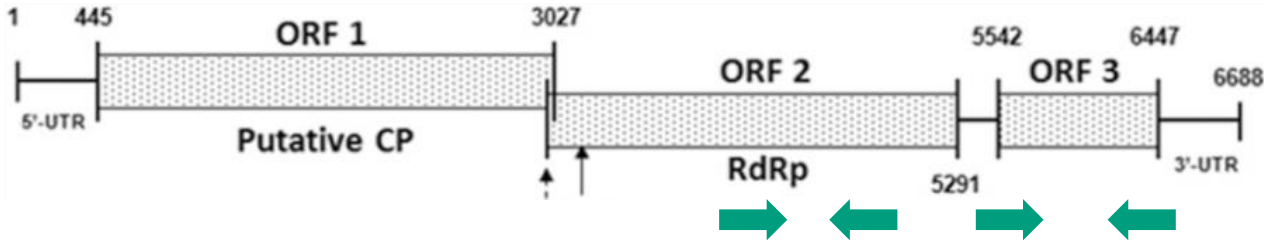
- Starting material:
- Total RNA from RNAlater fixed material
- Primer set 1 in ORF 2
- Primer set 2 in ORF 3
- Combination of primer sets 1 and 2 should show whether there is any break between ORF2 and ORF3.
- Conclusion: The toxic ORF3 protein is encoded by PMCV, on a continuous genome

Heart

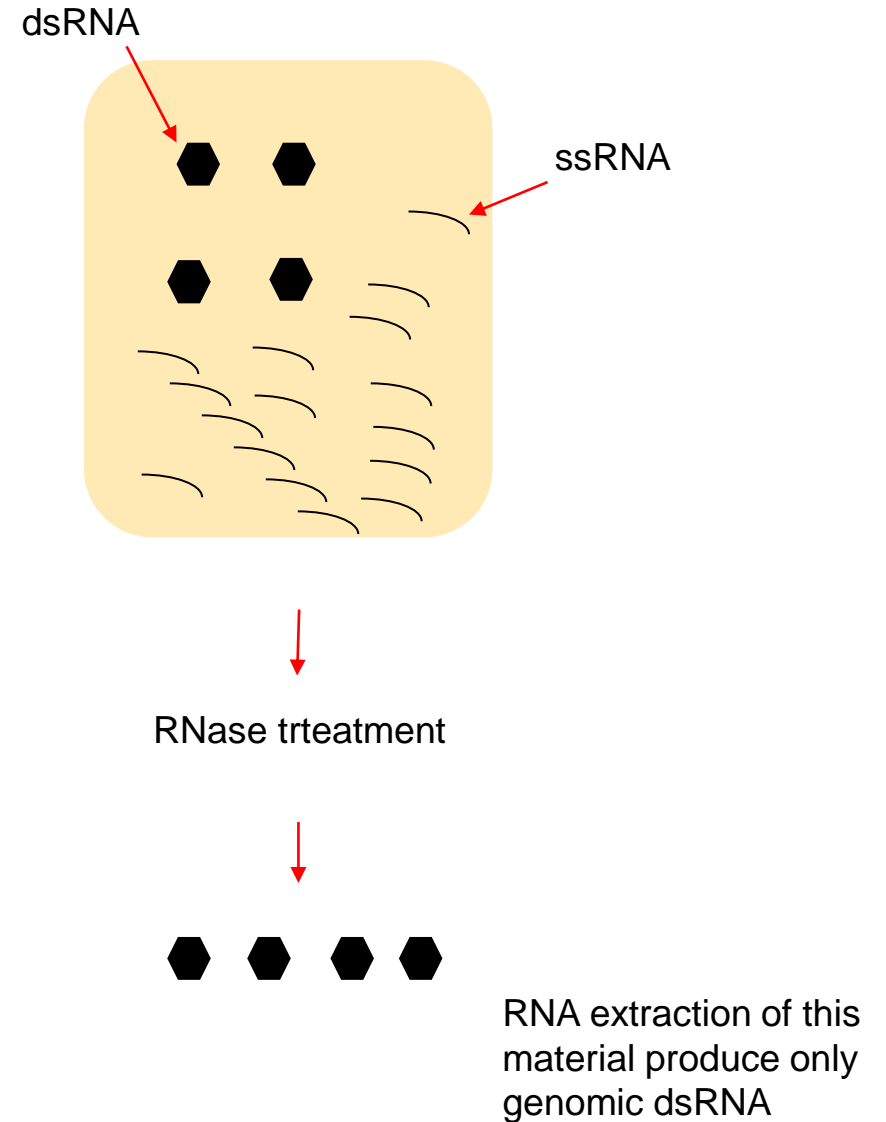
Kidney



The genome of PMCV



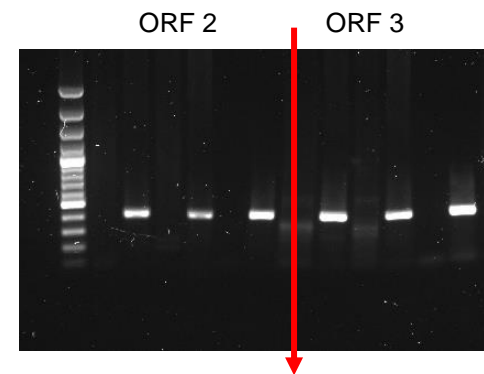
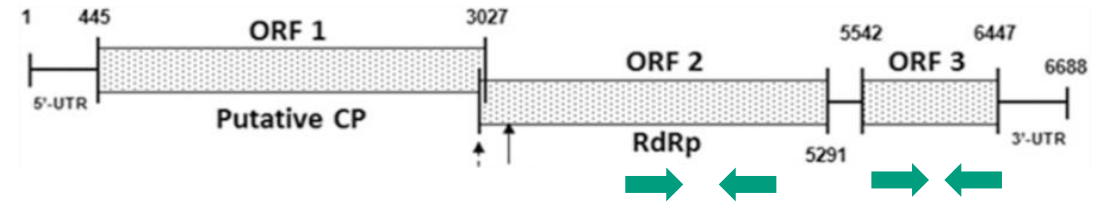
- Samples collected in cell culture media. Treated with RNAase **BEFORE** RNA ekstraktion
- Primerset 1 in ORF 2
- Primerset 2 in ORF3
- Combination of primersets 1 and 2 will indicate eventual break between ORF2 og ORF3.





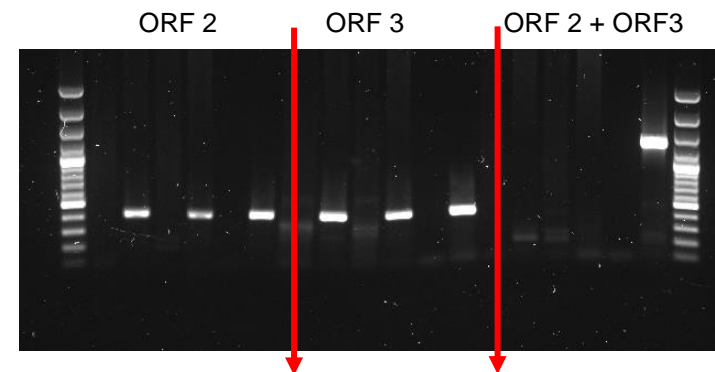
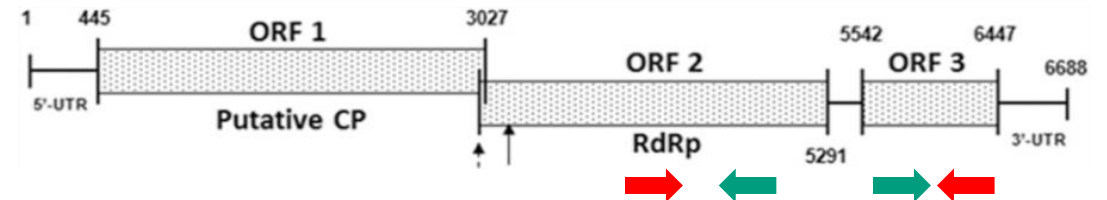
The genome of PMCV

- Starting material: Material stored in cell culture medium, RNase treatment BEFORE RNA extraction
- Primer set 1 in ORF 2
- Primer set 2 in ORF3

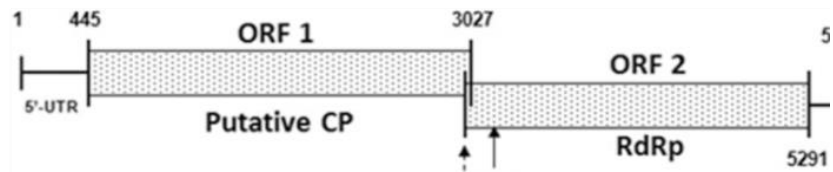
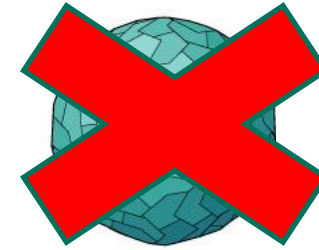
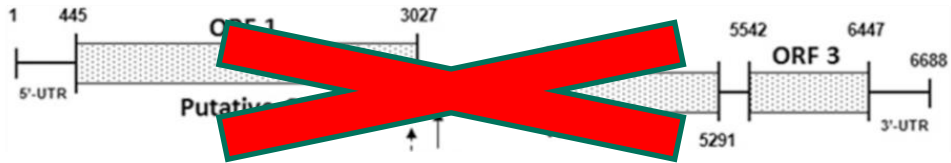


The genome of PMCV

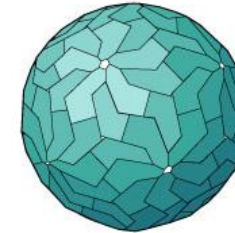
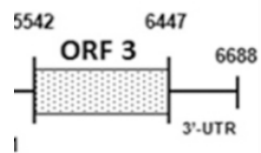
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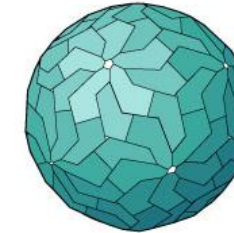
PMCV Genom og partikkel



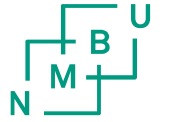
+



ORF 1 + ORF 2



ORF 3



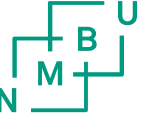
Different expression levels between the parts of the genome?



	ORF 2	ORF 3	ΔCt	Relative amount of ORF3 ssRNA versus ORF2
Heart 2	12.08	15.36	-3.28	10%
Heart 3	12.97	17.94	-4.97	3%
Heart 4	11.34	16.60	-5.26	3%
Heart 5	18.31	21.72	-3.41	9%
Average				6.25%
Kidney 2	22.91	24.23	-1.32	40%
Kidney 3	23.84	25.43	-1.59	33%
Kidney 4	24.68	27.10	-2.42	19%
Kidney 5	23.13	24.71	-1.58	33%
Average				31.25%

Outline:

- Short about dsRNA virus and Totiviruses
- Spread between fish: *PMCV does not spread easily horizontally*
- Spread within heart: *There is no indication of spread from spongiosum to compactum.*
- Viral RNA genomic dsRNA versus mRNA: *There is very little production of viral particles in heart!*
- The genome of PMCV: : *There are indications that the genome is split in two in viral particles*
- Does PMCV primarily infect salmon? *The jury is still out....*



Does PMCV primarily infect salmon?

How to explain a simple fish to fish cycle when:

- *Does not spread easily horizontally*
- *Very little production of viral particles in major target organ*
- *How is it shed? Or transmitted?*
- *Rather chaotic findings of the organization of the replication of genetic elements?*

- *Together this indicates that salmon heart is a blind alley for the virus.*

The end

