Elucidating Function of ORF95R in FV3 DNA Replication

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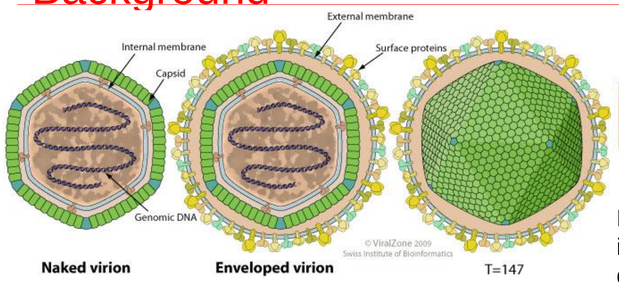
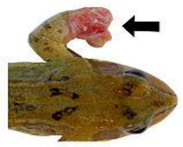
Abstract

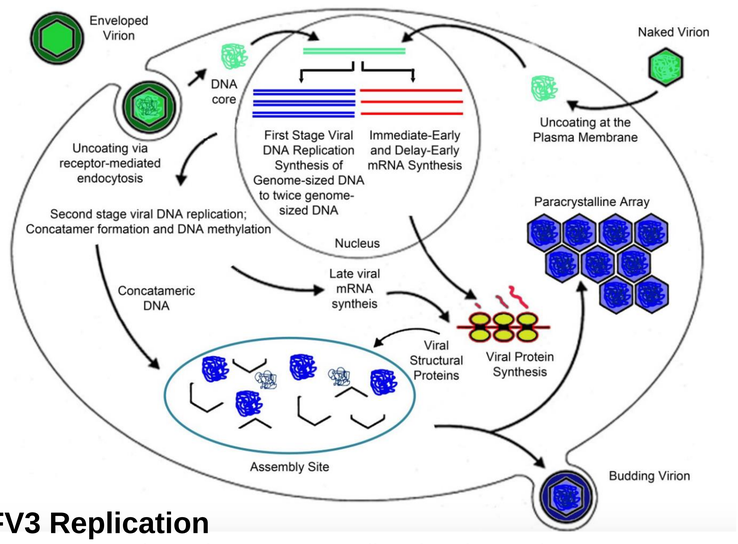
Ranavirus is a genus of double-stranded DNA viruses that infect cold-blooded vertebrates. These viruses use a unique method of DNA replication involving concatamerization of genomic monomers in the cytoplasm. Frog Virus 3 (FV3) is the prototypical member of the species *Ranavirus*. This virus contains many open reading frames, however it has not been determined which of these ORFs represent genes and contribute functional proteins. We are interested in ORFs predicted to play a role in FV3 DNA replication. Using nucleotide alignment and comparison to known sequences, ORF95R is predicted to be a RAD2 DNA repair homolog. We believe that the gene product of ORF95R is active in the concatamerization stage of DNA replication. To determine function, we have cloned of this gene from an isolate of the FV3 genome. This clone was sequenced and compared to reference ORF95R sequences. Discrepancies were identified between the two sequences and analyzed for potential impact on functionality. Further experiments will involve inserting ORF95R into an expression vector and transfecting into cells to determine the effect on cell viability and its role in viral DNA replication.

Background





FV3 is an icosahedral double-stranded DNA virus.



FV3 Replication

1. Enters host via receptor-mediated endocytosis
2. Viral particles shuffled into cell nucleus
3. Viral DNA replication begins
4. Replicated Viral DNA moves into cytoplasm
5. Replicated DNA monomers are concatamerized
6. Concatamers are packaged into viral heads
7. Once full, the DNA is cut at a non-specific location
8. New virions form at cell membrane and release by budding

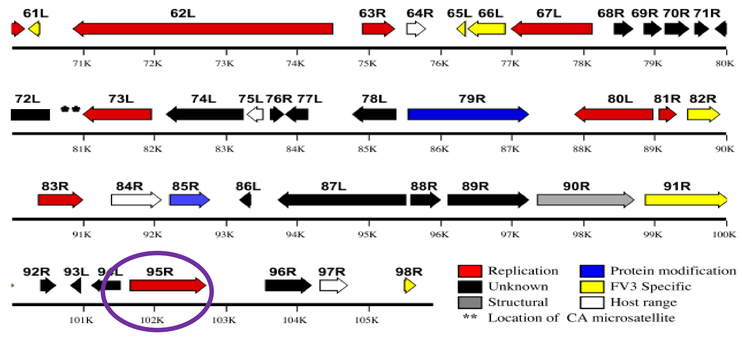
Protein Sequence Result	Max Score	Query Cover	E-Value	Identity	Accession #
Rad27p [Rhizophagus irregularis DAOM 197198w]	75.9	28%	9e-16	42%	EXX77562.1
Rad27p [Saccharomyces cerevisiae YJM193)	65.5	28%	2e-12	38%	AJS30426.1
flap endonuclease-1 (FEN1, RAD2) [uncultured marine thaumarchaeote KM3_87_F05]	70.1	41%	4e-14	29%	AIF19757.1

ORF95R is suspected to function in the 2nd DNA replication stage. Comparing against reported sequences found homology with sequences that encode RAD2 endonucleases in fungi and bacteria.

Methods

The sequence of ORF95R was amplified from an isolated FV3 genome via PCR using primers designed to flank the sequence with no extra nucleotides outside of the open reading frame.

Forward Primer: AAAGAATTCTGATGGGCATAAAAGGACTGAAACCCC
Reverse Primer: AAAGGATCCTCACTTGCGCTTGCACTTCTCAAAGG



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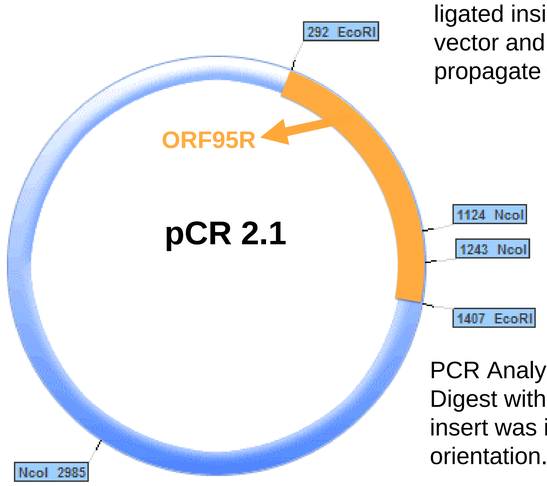
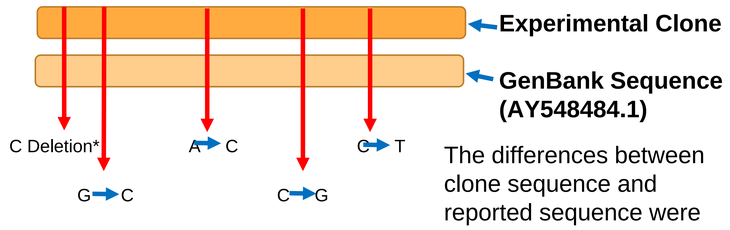


The amplified insert was then ligated inside a pCR 2.1 cloning vector and was used to further propagate ORF95R.

PCR Analysis and Restriction Digest with NcoI confirmed the insert was in the correct orientation.

The ligated pCR2.1 was transformed into E. Coli cells, and the DNA from the resulting colonies was sequenced.

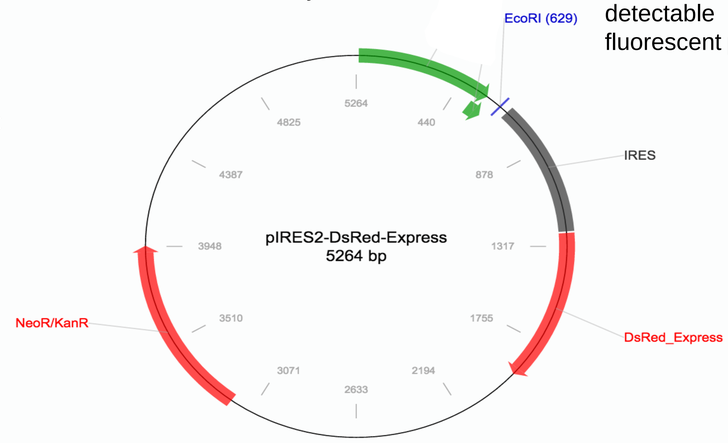


The differences between clone sequence and reported sequence were analyzed. (*only 1 clone)

Future Experiments

EcoRI is used to splice out ORF95R from pCR2.1 after sequence and orientation are confirmed. This extracted DNA is then ligated into pIRES-DsRED, an expression vector with an inserted IRES sequence, or internal ribosome entry site.

This site allows for ribosomes to bind to our past the ORF95R stop codon, producing a detectable fluorescent protein.



Cells will be transfected with pIRES and effect of ORF95R expression will be analyzed.

Cells infected with a temperature sensitive mutant will also be transfected with pIRES, to see if it has an effect on the Stage 2 DNA replication the mutant virus is defective in.

Aknowledge/References

-Chinchar, V.G.; Robert, J.; Storfer, A.T. Ecology of viruses infecting ectothermic vertebrates – the impact of ranavirus infections on amphibians. In Studies in Viral Ecology; Hurst, C.J., Ed.; Wiley-Blackwell: Hoboken, New Jersey, USA, 2011; Volume 2, pp. 231-260