# Investigation of the Role of Frog Virus 3 Gene ORF60R in First Stage Viral DNA Replication

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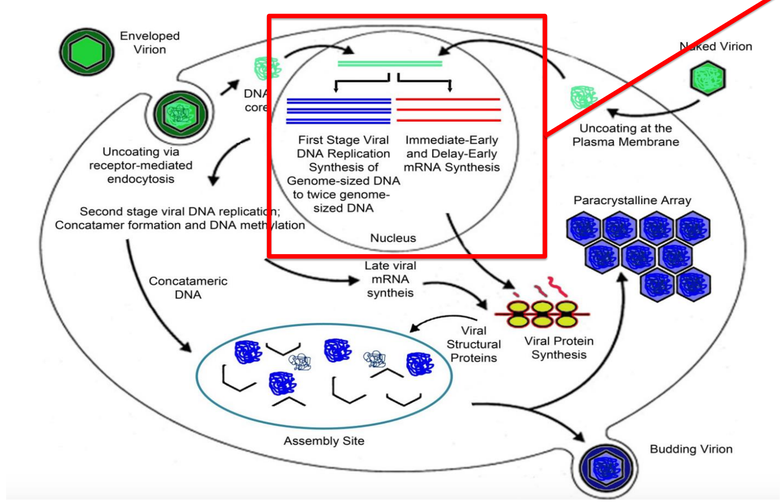
## Ranavirus Introduction

Ranavirus is a genus of DNA viruses that infects cold-blooded vertebrates, such as reptiles and amphibians. The Ranavirus Frog Virus 3 (FV3) has been linked to die-offs of amphibians across the world, yet not much is known about its molecular biology. Symptoms of ranaviral infection include swelling, redness of the skin, and pinpoint hemorraghing. All of these symptoms can be seen in the above image of infected American bullfrog tadpoles.


Ranavirus infected tadpoles
Miller et al Viruses 2011, 3, 2351-2373;

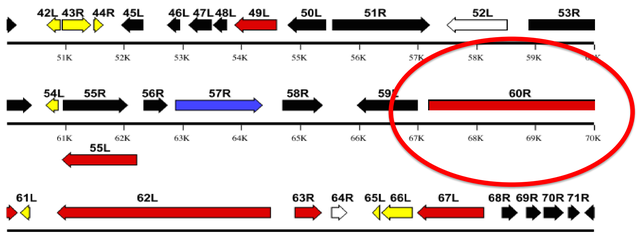
## Frog Virus 3 DNA Replication

This virus has a double-stranded DNA genome and replicates via a unique two-stage strategy. In the first stage of replication, the viral genome is replicated in the nucleus then sent to the cytoplasm where these genomic monomers are then concatemerized and packaged into newly formed virus particles. The goal of this project was to identify genes of FV3 that have function pertaining to this unique method of DNA replication.




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

## ORF60R

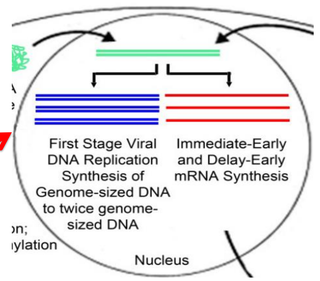



ORF60R highlighted (in the red circle) within context of the full Frog Virus 3 genome. W.G.H. Tan et al. Virology 323 (2004) 70–84

ORF60R is an open reading frame in the FV3 genome that bears sequence similarity to the B DNA polymerase family. B DNA polymerases replicate DNA during cell division.

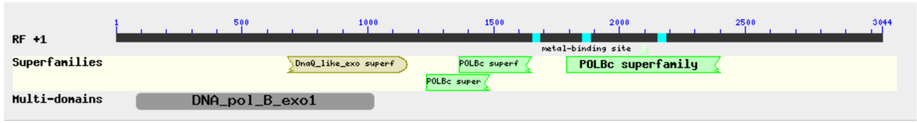
**We hypothesize the gene product of ORF60R is active in the first stage of FV3 DNA replication when viral DNA is synthesized.**

## ORF60R In First Stage Replication




In the first stage of replication, the viral genome is copied several times. This process requires a DNA polymerase. We hypothesize that ORF60R is the polymerase for this DNA synthesis. Future experiments will test this by using qPCR to quantify the amount of viral DNA in infected cells compared to the amount of viral DNA in infected cells in which ORF60R is overexpressed.

## Sequence Alignment Results



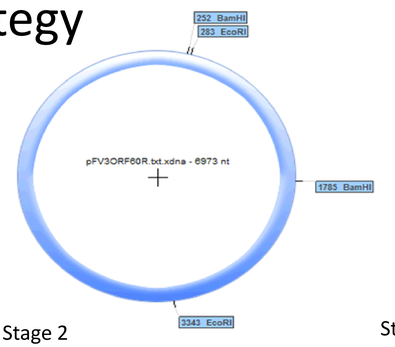

NCBI BLAST showing protein domain alignment to the transcribed ORF60R sequence. All of these domains are found in family B DNA polymerases.

| blastx Sequence Match | Max Score | Total Score | Query Cover | E-Value | Identity | Accession # |
|---|---|---|---|---|---|---|
| DNA Polymerase Delta Catalytic Subunit [Lucilla cuprina] | 221 | 221 | 80% | 5e-56 | 28% | KNC34945.1 |
| DNA Polymerase Delta Catalytic Subunit [Sphaeroforma arctica JP610] | 185 | 294 | 71% | 2e-44 | 27% | XP_014159835.11 |
| DNA Polymerase Delta Catalytic Subunit [Grifola frondosa] | 176 | 176 | 80% | 2e-41 | 25% | OBZ73506.1 |

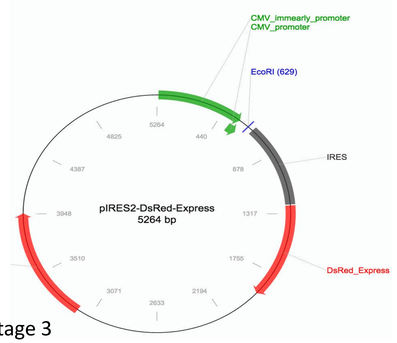
- The above table shows a blastx alignment of the ORF60R nucleotide sequence
- ORF60R shows a relationship to DNA polymerases from a variety of different organisms
  - Lucilla cuprina is an Australian Sheep Blowfly
  - Sphaeroforma arctica is a protozoan
  - Grifola frondosa is a fungus
- The identity percentage of these matches indicates that these matches are not random

## Cloning Strategy


Stage 1
- ORF60R sequence was amplified by PCR using custom primers




Stage 2
- Sequence ligated into cloning vector pCR2.1
- Presence of ORF60R was verified by PCR and restriction screening




Stage 3
- ORF60R sequence excised from cloning vector using EcoRI digest and gel purification
- Plan to ligate into pIRES2-DsRED expression vector (above)