Ranavirus Replication

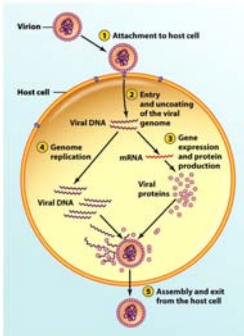
James K. Jancovich
California State University San Marcos
Department of Biological Sciences
jjancovich@csusm.edu

Ranavirus Replication – Lecture Outline

- Background
  - viral replication basics
- Quantify viral replication
- Replication of ranaviurses
- Ranavirus genomes
- Understanding ranavirus gene function

Student Learning Outcomes

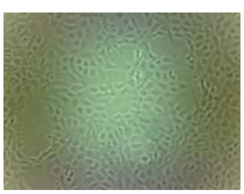
- Understand the basics of viral replication.
- Be able to quantify viral growth by plaque assay.
- Understand the steps of ranavirus replication.
- Understand how ranavirus mutants are constructed in order to characterize gene function.

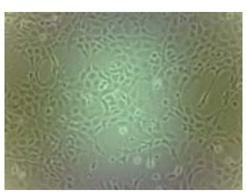


Definitions:

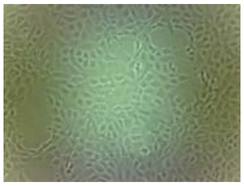
- A <u>susceptible</u> cell has a functional receptor for a given virus the cell may or may not be able to support viral replication.
- A <u>resistant</u> cell has no receptor – it may or may not be competent to support viral replication.
- A <u>permissive</u> cell has the capacity to replicate virus; however, it may or may not be susceptible.
- A <u>susceptible</u> AND <u>permissive</u> cell is the only cell that can take up a virus particle and replicate it

cytopathic effects (CPE)





cytopathic effects (CPE)



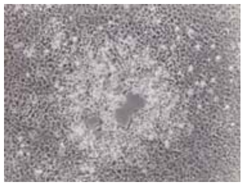


Table 2.1 Some examples of cytopathic effects of viral infection of animal cells

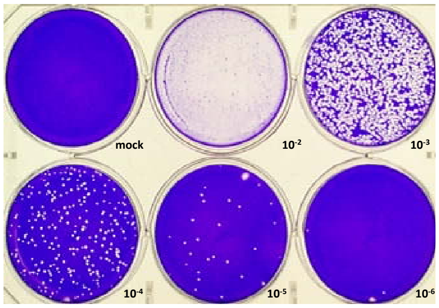
Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Papovaviruses
Syncytia (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of tissue culture cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses
Inclusion bodies	
Virions in nucleus	Adenoviruses
Virions in the cytoplasm (Negri bodies)	Rabies virus
"Factories" in the cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesviruses

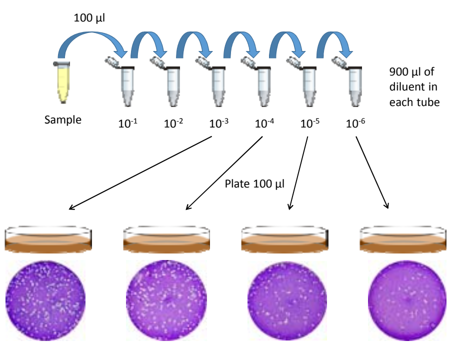
Flint *et al.*, 2009

How can we determine the number of ranavirus particles in a solution?

- Direct count
- End-point assay
- Plaque assay
- PCR assays
- Immuno-assays

Plaque Assay





Plaque Assay

$$\#pfu/ml = \frac{\#\ pfu}{\text{plating factor (ml)}} \times DF$$

DF = 1/dilution

want between 20 – 200 pfu

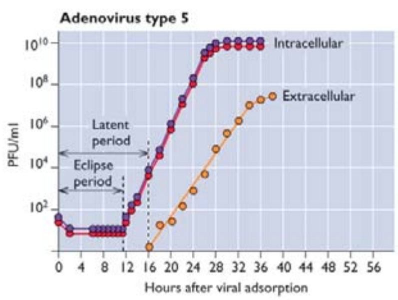
Plaque Assay

plate 100 µl
10^{-5} dilution

$$\#pfu/ml = \frac{\#\ pfu}{\text{plating factor (ml)}} \times DF$$

DF = 1/dilution

want between 20 – 200 pfu



Flint *et al.*, 2009

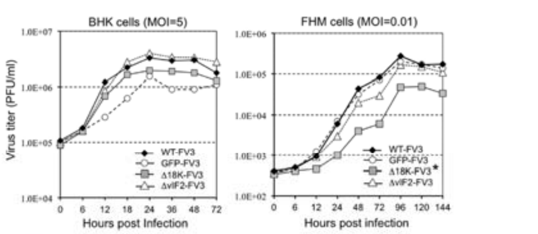
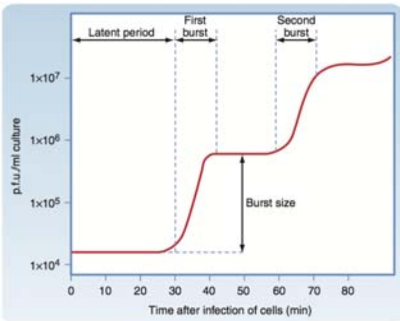


FIG. 5. Comparison of WT and recombinant FV3 replication in cell culture. (A) One-step growth curve assay in BHK-21 cells. (B) Multiple-step growth curve assay in FHM cells. Equivalent numbers of cells were infected with either WT or recombinant virus at an MOI of 5 (BHK-21) or 0.01 (FHM). Viruses were harvested at the indicated time points and quantified by plaque assay. *, $P < 0.0027$ for FV3-Δ18K relative to WT or GFP-FV3 using the Kruskal-Wallis statistical test (Graphpad Prism software 5.0). Other curves in either panel A or panel B did not show statistical significance.

Chen et al., 2011



Multiplicity of Infection (MOI)

- Number of virus particles per cell
  [MOI (pfu/cells) = # pfu/# cells]
- Example:
  - Infect 10^6 cells with 10^7 virions
  - MOI is 10
  - However, not all cells receive 10 virions!

MOI

- Infection depends on random interaction between virus and cell.
- Therefore, some cells are infected with 1, 2, 3 or more virions….while others not infected.
- We can explain this by the **Poisson distribution**

$P(k) = e^{-m}m^k/k!$

P(k): fraction of cells infected by k virus particles

m: multiplicity of infection (moi)

uninfected cells: $P(0) = e^{-m}$
cells receiving 1 particle: $P(1) = me^{-m}$
cells multiply infected: $P(>1) = 1-e^{-m}(m+1)$

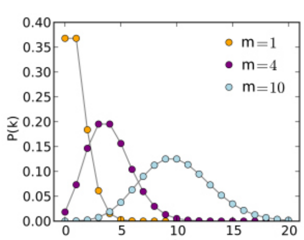
[obtained by subtracting from 1 {the sum of all probabilities for any value of k} the probabilities *P(0)* and *P(1)*]

Examples:

If 10^6 cells are infected at moi of 10:
45 cells are uninfected
450 cells receive 1 particle
the rest receive >1 particle

If 10^6 cells are infected at moi of 1:
37% of the cells are uninfected
37% of the cells receive 1 particle
26% receive >1 particle

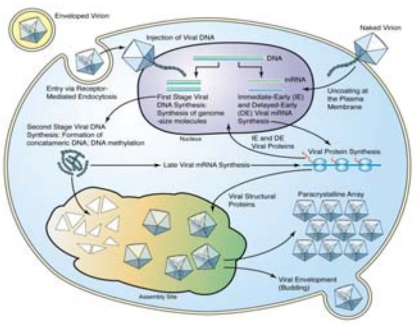
If 10^6 cells are infected at moi of .001:
99.9% of the cells are uninfected
00.099% of the cells receive 1 particle (990)
00.0001% receive >1 particle



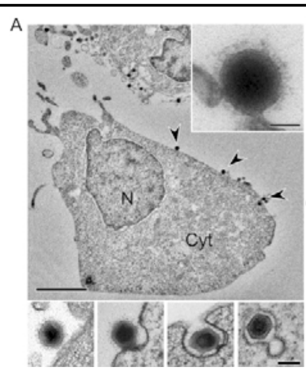
You have a stock of virus with a titer of 6.8×10^8 pfu/ml. What volume of this virus you would need to infect 1×10^6 cells with the following multiplicity of infection (MOI): (Note: You cannot measure volumes less than 0.5 µl.)

A. 0.001

B. 5

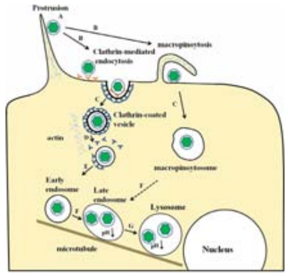


Jancovich et al., 2015



Liu et al., 2016

Model entry route of SGIV into GS cells.



Shaowen Wang et al. J. Virol. 2014;88:13047-13063

JOURNAL OF VIROLOGY, Aug. 1982, p. 519–528 Vol. 43, No. 2
0022-538X/82/080519-10$02.00/0

Frog Virus 3 DNA Replication Occurs in Two Stages

R. GOORHA
Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

Received 28 December 1981/Accepted 19 April 1982

Viral DNA synthesis in frog virus 3 (FV3)-infected cells occurs both in the nucleus and in the cytoplasm (Goorha et al., Virology **84**:32–51, 1978). Relationships between viral DNA molecules synthesized in these two compartments and their role in the virus replication were examined. The data presented here suggest that (i) FV3 DNA replicated in two stages and (ii) nucleus and cytoplasm were the sites of stages 1 and 2 of DNA replication, respectively. Stages 1 and 2 were further distinguished by their temporal appearance during infection and by the sizes of the replicating DNA as determined by sedimentation in neutral sucrose gradients. In stage 1, replicating molecules, between the size of unit and twice the unit length, were produced early in infection (2 h postinfection). In contrast, stage 2 of DNA replication occurred only after 3 h postinfection, and replicating molecules were large concatemers. Results of pulse-chase experiments showed that the concatemeric DNA served as the precursor for the production of mature FV3 DNA. Denaturation of concatemeric DNA with alkali or digestion with S1 nuclease reduced it to less than genome size molecules, indicating the presence of extensive single-stranded regions. Analysis of replicating DNA by equilibrium centrifugation in CsCl gradients after a pulse-chase suggested that these single-stranded regions were subsequently repaired. Based on these and previous data, a scheme of FV3 replication is presented. According to this scheme, FV3 utilizes the nucleus for early transcription and stage 1 of DNA replication. The viral DNA is then transported to the cytoplasm, where it participates in stage 2 DNA replication to form a concatemeric replication complex. The processing of concatemers to produce mature viral DNA and virus assembly also occurs in the cytoplasm. This mode of replication is strikingly different from any other known DNA virus.

Circularly Permutated, Terminally Redundant

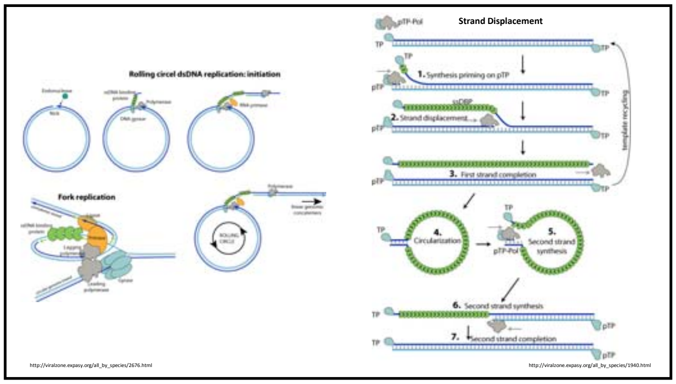
Unit length genome: ABCDEFGHIJKLMNOPQRSTUVWXYZ

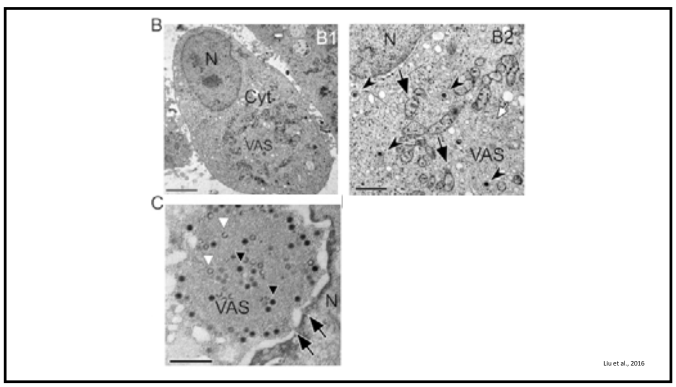
ABCDEFGHIJKLMNOPQRSTUVWXYZABCDEFGHIJKLMNOPQRSTUVWXYZABCDEFGHIJKLMNOPQRSTUVWXYZABCDEFGHIJKL…

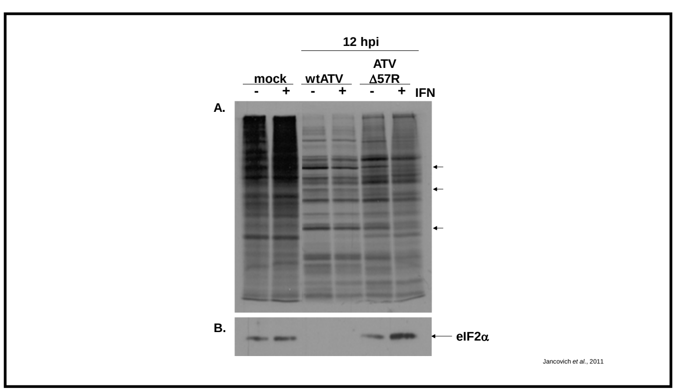
ABCDEFGHIJKLMNOPQRSTUVWXYZABCDEFGHIJ

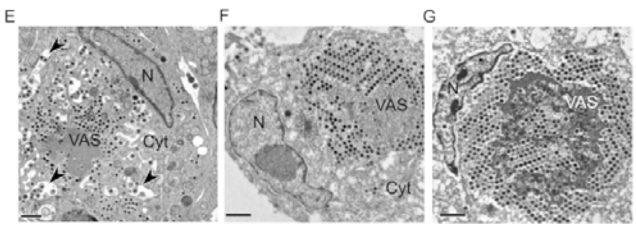
KLMNOPQRSTUVWXYZABCDEFGHIJKLMNOPQRST

UVWXYZABCDEFGHIJKL….

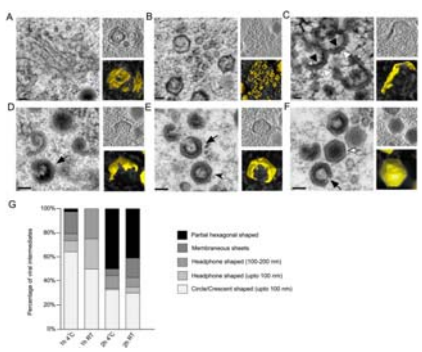

Rolling circel dsDNA replication: initiation
Fork replication
pTP-Pol
Strand Displacement
TP
1. Synthesis priming on pTP
pTP
2. Strand displacement
3. First strand completion
template recycling
4. Circularization
5. Second strand synthesis
pTP-Pol
6. Second strand synthesis
7. Second strand completion
http://viralzone.expasy.org/all_by_species/2676.html
http://viralzone.expasy.org/all_by_species/1940.html


B
B1
B2
N
Cyt
VAS
C
Liu et al., 2016

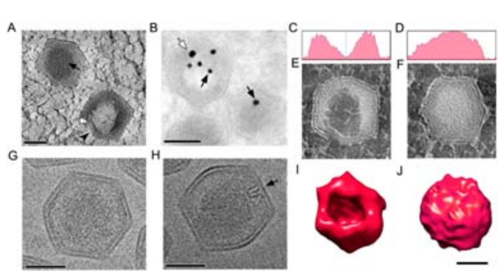

12 hpi
mock
wtATV
ATV Δ57R
- + - + - + IFN
A.
B.
eIF2α
Jancovich et al., 2011



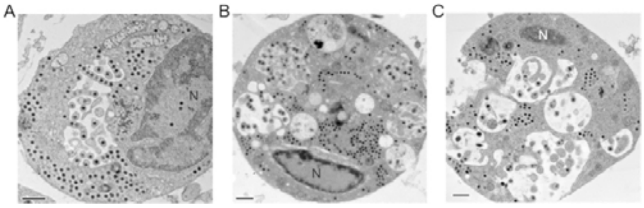
Liu et al., 2016



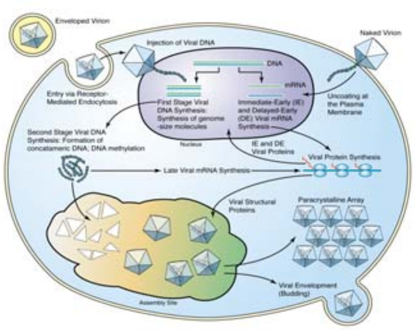
Liu et al., 2016



Liu et al., 2016



Liu et al., 2016



Jancovich et al., 2015

TABLE 1: Sequenced genomes from members of the genus *Ranavirus*.

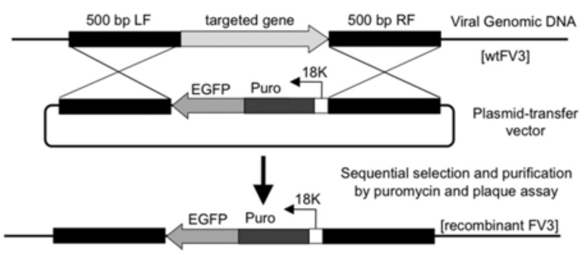
Virus	Host	Genome size (kb)	GC (%)	Predicted ORFs	Accession #	Reference
ADRV	Chinese giant salamander	106,719 106,734	55	101	KF033124 KC865735	Wang *et al.*, 2014 Chen *et al.*, 2013
ATV	Salamander	106,332	54	96	AY150217	Jancovich *et al.*, 2003
CMTV	Frog	106,878	55	104	JQ231222	Mavian *et al.*, 2012
EHNV	Fish	127,011	54	100	FJ433873	Jancovich *et al.*, 2010
ESV	Fish	127,732	54	136	JQ724856	Mavian *et al.*, 2012
FV3	Frog	105,903	55	98	AY548484	Tan *et al.*, 2004
GIV	Fish	139,793	49	139	AY666015	Tsai *et al.*, 2004
RGV	Frog	105,791	55	106	JQ654586	Lei *et al.*, 2012
SGIV	Fish	140,131	48	162	AY521625	Song *et al.*, 2005
STIV	Turtle	105,890	55	105	EU627010	Huang *et al.*, 2009
TFV	Frog	105,057	55	105	AF389451	He *et al.*, 2002

Abbreviations: ADRV, Andrias davidianus ranavirus (aka, Chinese giant salamander ranavirus); CMTV, Common midwife toad ranavirus ; FV3, *Frog virus* 3; RGV, Rana grylio virus; STIV, soft-shelled turtle iridovirus; TFV, tiger frog virus; ATV Ambystoma tigrinum virus; EHNV, *Epizootic hematopoietic necrosis virus*; ESV, European sheatfish ranavirus ; SGIV, Singapore grouper iridovirus; GIV, grouper iridovirus.

Jancovich et al., 2015

How to understand ranavirus gene function?

- Ectopic expression
- Knock-down
- Knock-out
- Induced expression



Chen et al., 2011

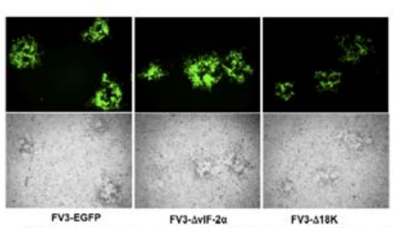


FIG. 2. Selection of recombinant FV3. Fluorescence (top) and phase-contrast (bottom) microscopy of FHM cells infected with recombinant viruses after six consecutive rounds of selection. All the plaques produced by these recombinant viruses are EGFP positive, indicating that they are not contaminated with WT virus.

Chen et al., 2011

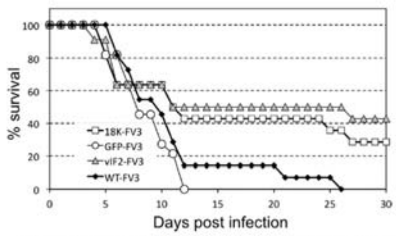
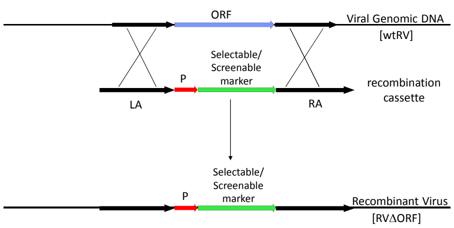


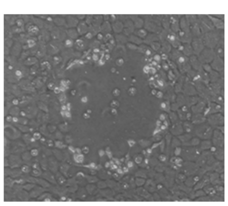
FIG. 6. Susceptibility of *X. laevis* larvae to WT and recombinant FV3. Groups (11 individuals per group) of 2-week-old susceptible larvae (stage 49 to 50) were infected by bath immersion for 1 h in 2 ml of water containing 5×10^6 PFU. Death was recorded daily for 30 days, and FV3 infection was confirmed by PCR.

Chen et al., 2011

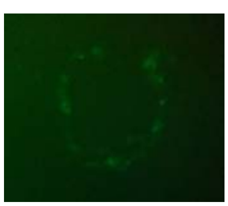
A.



Aron et al., 2016

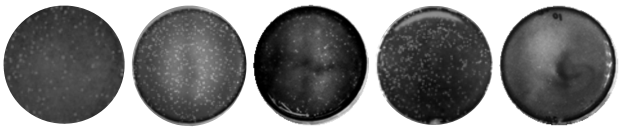


phase contrast

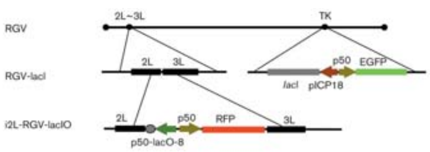


fluorescence

Aron et al., 2016



Aron et al., 2016



Jancovich et al., 2011

Table of Recombinant Ranaviruses

Virus	ORF	Predicted Function	Mutant Phenotype	Reporter Marker	Reference
FV3					
	26R	eIF2α homologue	antagonist of PKR; increased apoptosis; reduced pathogenesis	EGFP-puromycin resistance	Chen et al., 2011
	82R	ICP-18	increased apoptosis; reduced pathogenesis	EGFP-puromycin resistance	"
	52L	β-hydroxysteroid dehydrogenase homolog	tbd; reduced pathogenesis	EGFP-puromycin resistance	Andino et al., 2015
	64R	caspase activation and recruitment domain-containing (CARD) protein	IFN^{s}; increased apoptosis; reduced pathogenesis	EGFP-puromycin resistance	"
ATV					
	57R	eIF2α homologue	antagonist of PKZ; ; reduced pathogenesis	neomycin resistance	Jancovich and Jacobs 2011
	11R	unknown	essential gene	GFP-neomycin resistance	Aron et al., accepted
	25R	RNase III	degrades RNA	GFP-neomycin resistance	"
	40L	CARD-containing gene	tbd; see FV3 above	GFP-neomycin resistance	"
	53R	unknown	essential gene	GFP-neomycin resistance	"
	54R	unknown	tbd	GFP-neomycin resistance	"
RGV					
	53R	viral envelope protein	green virus	EGFP	He et al., 2012
	92R	thymidine kinase (TK)	non-essential	EGFP	"
	53R	viral envelope protein	required for viral production; reduced growth when not expressed	IPTG inducible; EGFP	He et al., 2013
	2L	viral envelope protein	required for viral production; reduced growth when not expressed	IPTG inducible; EGFP	He et al., 2014
	92R/67R	TK and deoxyuridine triphosphatase (dUTPase, DUT)		EGFP/RFP	Huang et al., 2016
ESV					
	114L	dihydrofolate reductase (DHFR)	non-essential	EGFP-neomycin resistance	Martin et al., 2015
STIV					
	VP55	viral envelope protein	green virus	EGFP-VP55 fusion	Huang et al., 2011

Robert and Jancovich, submitted

Additional Readings

- Andino et al., 2015. Characterization of Frog Virus 3 knockout mutants lacking putative virulence genes. *Virology*, 485: 162-170.
- Liu et al., 2016. Visualization of Assembly Intermediates and Budding Vacuoles of Singapore Grouper Iridovirus in Grouper Embryonic Cells. *Scientific Reports*, 6: 18696