SHRIMP ENDOGENOUS VIRAL ELEMENTS (EVE) OF INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV) AND THEIR IMPLICATIONS FOR SHRIMP DIAGNOSIS

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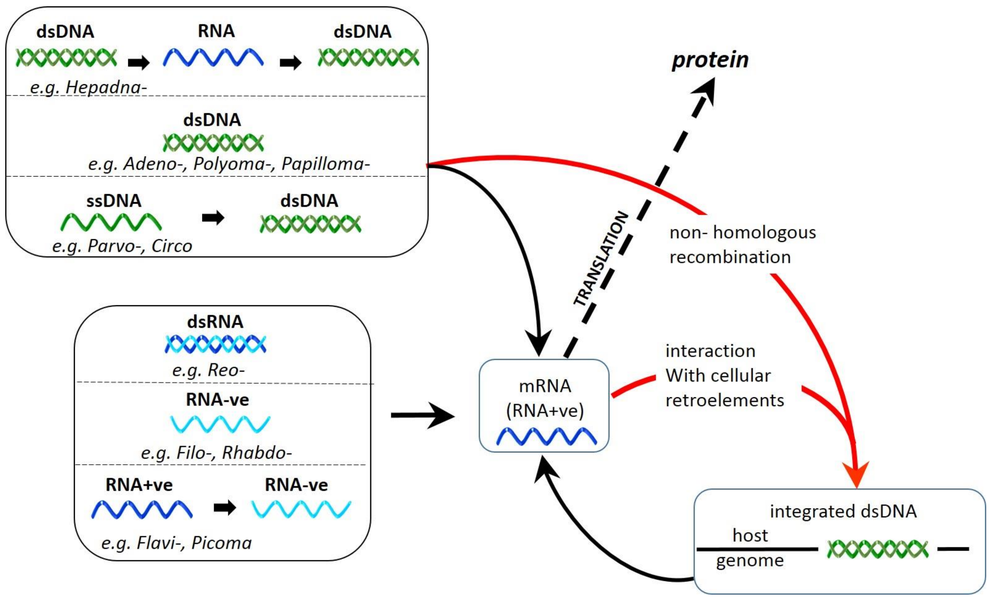
Abstract

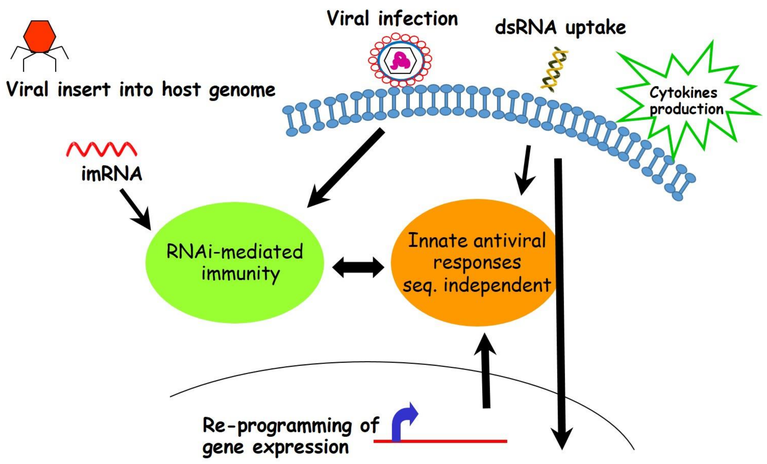
Integration of non-retroviral fragments into animal genomes has been found for a few decades as a result of infection and completion of their life cycles. To date, "endogenous viral elements" (EVE) of infectious hypodermal and hematopoietic necrosis virus (IHHNV) and white spot syndrome virus (WSSV) have been found in penaeid shrimp. For practical diagnostic approach, it necessitated a change in the routine method to distinguish between infected shrimp and EVE containing samples. In the case of IHHNV, infected shrimp species have been developed resistance to the virus, thus it is a challenge to separate shrimp carrying both infectious form of virus and EVE from those that are infected only (i.e. the former and latter would be recognized by conventional PCR testing). Discard of domesticated shrimp breeding stocks based on such false positive results might have negative consequences, if such inserts are related to shrimp viral disease tolerance according to "viral accommodation hypothesis." It is thus necessary to improve accuracy in diagnosis of IHHNV infection. For example, multiplex PCR analysis is developed to amplify the entire IHHNV genome, ensuring the accurate diagnosis, and the technique must be convenient for practical application. More recently, isothermal nucleic acid amplification techniques such as loop-mediated isothermal amplification (LAMP) comprises primers targeting OIE-recommended region and the 3'end of the viral genome that has been reported to be less occurrence of EVEs in the shrimp genome.

OCCURRENCE OF ENDOGENOUS VIRAL ELEMENTS (EVE)



(Adapted from Katzourakis and Gifford (2010))

Shrimp specific response mechanism for antiviral defense based on EVEs

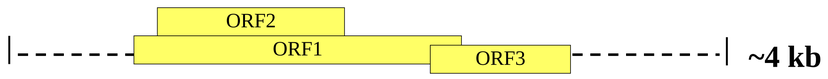


Adapted from (Flegel (2009); Robalino et al. (2007))

Abbreviations: dsDNA (double stranded Deoxyribonucleic acid), RNA (Ribonucleic acid), ssDNA (single stranded Deoxyribonucleic acid), dsRNA (double stranded Ribonucleic acid), mRNA (messenger Ribonucleic acid), RNA+ve (positive sense, single stranded Ribonucleic acid), RNA–ve (negative sense, single stranded Ribonucleic acid

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) VS IHHN-EVE

- Synonyms: *Penaeus stylirostris* densovirus (PstDNV) (assigned by Int'l committee on the Taxonomy)
- The smallest of the known penaeid shrimp viruses
- Genome is a linear ssDNA of ~4 kb that encodes 3 ORFs, ORF1 (non-structural protein), ORF2 (unknown) and ORF3 (structural protein)



- Regarding evidence of common occurrence of random and variable IHHNV genome inserts (Saksmerprome et al.,2011), it would be more appropriate to assign the infectious form of the virus as "IHHNV" and homologous sequence to virus embedded in shrimp genome as "IHHN-EVE."

IHHNV infection


Diseased


Non-diseased


IHHN-EVE

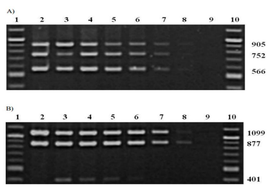
http://www.vinnbio.com/blog/entry/infectious-hypodermal-and-haematopoietic-necrosis-virus-ihhnv-part-2

Currently available detection methods of infectious IHHNV

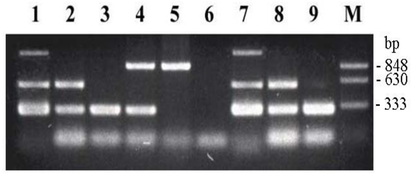
Polymerase chain reaction (PCR)

Primer	Product	Sequence	GenBank
389F	389 bp	5'-CGG-AAC-ACA-ACC-CGA-CTT-TA-3'	AF218266
389R		5'-GGC-CAA-GAC-CAA-AAT-ACG-AA-3'	
77012F	356 bp	5'-ATC-GGT-GCA-CTA-CTC-GGA-3'	AF218266
77353R		5'-TCG-TAC-TGG-CTG-TTC-ATC-3	
392F 3	92 bp	5'-GGG-CGA-ACC-AGA-ATC-ACT-TA-3'	AF218266
392R		5'-ATC-CGG-AGG-AAT-CTG-ATG-TG-3'	
309F	309 bp	5'-TCC-AAC-ACT-TAG-TCA-AAA-CCA-A-3'	AF218266
309R		5'-TGT-CTG-CTA-CGA-TGA-TTA-TCC-A-3'	
MG831F	831 bp	5'-TTG-GGG-ATG-CAG-CAA-TAT-CT-3'	DQ228358
MG831R		5'-GTC-CAT-CCA-CTG-ATC-GGA-CT-3'	

OIE

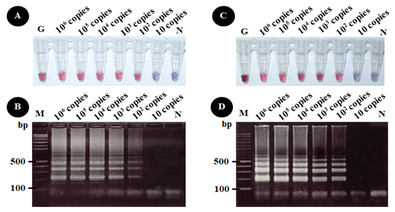


Multiplex PCR

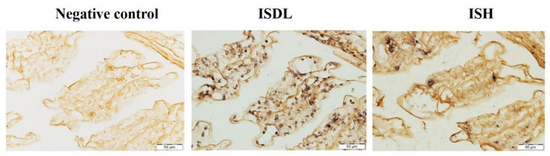


IQ 2000

Loop-Mediated Isothermal Amplification (LAMP)



In situ DIG-labeling LAMP *(ISDL)*



Recommendations for development of international standard detection targets for specific pathogen free (SPF) stocks of shrimp used in commerce

- Highly accurate and practical detection methods to validate EVE-containing stocks, and calls for scientific agreement on healthy shrimp should be prioritized to reduce impact of EVE
- If beneficial inserts could be identified, it would be possible to preserve them by monitoring breeding stocks using PCR methods based on chimeric shrimp-viral primer pairs that would be designed for specificity to desired beneficial inserts
- Monitored IHHNV inserts would be the ability to avoid of IHHNV positive test results in the offspring from IHHNV-negative parental stocks.

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