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Quality Control of Baculoviral Bioinsecticide Production

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1. Introduction

Agriculture is a discipline that has accompanied human beings since the beginning of civilization. The cultivation of different vegetables for centuries has allowed selecting varieties that far exceed the capabilities of many wild type plants originally used as a food source. That situation derived in the manipulation of natural ecosystems, transforming them into spaces where they can only grow and develop the desired species.

In our world, plants are the staple diet of many organisms including invertebrates like Lepidoptera. During the larval stage, these insects can consume a large amount of leaf tissue causing serious damage to the plant. If we think that most vegetables have insect predators, agricultural crops can be transformed into an inviting habitat, allowing the development of these animals. In conclusion, all crops have pests that threaten their productivity. Given this scenario, many pest control strategies have been used by human beings to protect the health of their crops: treatment with chemical insecticides, development of transgenic plants and biological control applications (Christou *et al*, 2006; Gilligan, 2008).

Baculovirus is a large family of insect pathogens that infect and kill different species of Lepidoptera, Hymenoptera and Diptera (Theilmann *et al*, 2005). In particular, many lepidopteron are pests in agriculture transforming these viruses in an important biocontrol tools for their natural hosts (Entwistle, 1998; Moscardi, 1999; Szewczyk *et al*, 2006). Baculoviruses have double-stranded circular DNA genomes of 80,000-180,000 bp, containing between 80 to 180 genes depending on the specie (van Oers & Vlak, 2007; Miele *et al*, 2011). In early stages of virus cycle, this pathogen is produced as Budded Viruses (BVs): the genome contained in a protein capsid (nucleocapsid), which is surrounded by a lipid membrane. In change, in the last phase of multiplication processes appear the Occluded Bodies (OBs): protein crystals (forming polyhedra or granules) containing nucleocapsids wrapped by a lipid membrane with a different composition (ODVs or Occluded Derived Viruses, with single or multiple nucleocapsids depending on the specie) (Rohrman, 2008). These two virus phenotypes have different biological properties; while OBs are specialists (infecting larvae by *per os* route with a narrow host range; responsible of primary infection in midgut cells), the BVs are generalists (infecting a wide range of different insect cells triggering their death; responsible for secondary infection). In the pest control strategies, baculoviruses (OBs) are introduced on the crops to infect and kill larvae through the production of an epizooty.

Genus	Name	Code	Accesion number	Genome (bp)	Total ORFs
Alphabaculovirus – Group I	*Antheraea pernyi* NPV-Z	APN	NC_008035	126629	145
	Antheraea pernyi NPV-L2	AP2	EF207986	126246	144
	Anticarsia gemmatalis MNPV-2D	AGN	NC_008520	132239	152
	Autographa californica MNPV-C6	ACN	NC_001623	133894	154
	Bombyx mori NPV	BMN	NC_001962	128413	137
	Bombyx mandarina NPV	BON	NC_012672	126770	141
	Choristoneura fumiferana DEF MNPV	CDN	NC_005137	131160	149
	Choristoneura fumiferana MNPV	CFN	NC_004778	129593	145
	Epiphyas postvittana NPV	EPN	NC_003083	118584	136
	Hyphantria cunea NPV	HCN	NC_007767	132959	148
	Maruca vitrata MNPV	MVN	NC_008725	111953	126
	Orgyia pseudotsugata MNPV	OPN	NC_001875	131995	152
	Plutella xylostella MNPV	PXN	NC_008349	134417	149
	Rachiplusia ou MNPV	RON	NC_004323	131526	146
Alphabaculovirus – Group II	*Adoxophyes honmai* NPV	AHN	NC_004690	113220	125
	Adoxophyes orana NPV	AON	NC_011423	111724	121
	Agrotis ipsilon NPV	AIN	NC_011345	155122	163
	Agrotis segetum NPV	ASN	NC_007921	147544	153
	Apocheima cinerarium NPV	APO	FJ914221	123876	118
	Chrysodeixis chalcites NPV	CCN	NC_007151	149622	151
	Clanis bilineata NPV	CBN	NC_008293	135454	129
	Ectropis obliqua NPV	EON	NC_008586	131204	126
	Euproctis pseudoconspersa NPV	EUN	NC_012639	141291	139
	Helicoverpa armigera NPV-C1	HA1	NC_003094	130759	135
	Helicoverpa armigera NPV-G4	HA4	NC_002654	131405	135
	Helicoverpa armigera MNPV	HAN	NC_011615	154196	162
	Helicoverpa armigera SNPV-NNg1	HAS	NC_011354	132425	143
	Helicoverpa zea SNPV	HZN	NC_003349	130869	139
	Leucania separata NPV-AH1	LSN	NC_008348	168041	169
	Lymantria dispar MNPV	LDN	NC_001973	161046	163
	Lymantria xylina MNPV	LXN	NC_013953	156344	157
	Mamestra configurata NPV-90-2	MCN	NC_003529	155060	169
	Mamestra configurata NPV-90-4	MC4	AF539999	153656	168
	Mamestra configurata NPV-B	MCB	NC_004117	158482	169
	Orgyia leucostigma NPV	OLN	NC_010276	156179	135
	Spodoptera exigua MNPV	SEN	NC_002169	135611	142
	Spodoptera frugiperda MNPV-3AP2	SF2	NC_009011	131330	143
	Spodoptera frugiperda MNPV-19	SF9	EU258200	132565	141
	Spodoptera litura NPV-II	SLN	NC_011616	148634	147
	Spodoptera litura NPV-G2	SL2	NC_003102	139342	141
	Trichoplusia ni SNPV	TNN	NC_007383	134394	144
Betabaculovirus	*Adoxophyes orana* GV	AOG	NC_005038	99657	119
	Agrotis segetum GV	ASG	NC_005839	131680	132
	Choristoneura occidentalis GV	COG	NC_008168	104710	116
	Cryptophlebia leucotreta GV	CLG	NC_005068	110907	129
	Cydia pomonella GV	CPG	NC_002816	123500	143
	Helicoverpa armigera GV	HAG	NC_010240	169794	179
	Phthorimea operculella GV	POG	NC_004062	119217	130
	Plutella xylostella GV	PXG	NC_002593	100999	120
	Pieris rapae GV	PRG	GQ884143	108592	120
	Pseudaletia unipuncta GV-Hawaiin	PUG	EU678671	176677	183
	Spodoptera litura GV-K1	SLG	NC_009503	124121	136
	Xestia c-nigrum GV	XCG	NC_002331	178733	181
Gamma	*Neodiprion abietis* NPV	NAN	NC_008252	84264	93
	Neodiprion lecontei NPV	NLN	NC_005906	81755	93
	Neodiprion sertifer NPV	NSN	NC_005905	86462	90
Delta	*Culex nigripalpus* NPV	CNN	NC_003084	108252	109

Table 1. Baculovirus complete genomes. Baculoviruses used in this study, sorted by genus (and within them by alphabetical order). MNPV is the abbreviation of multicapsid nucleopolyhedrovirus; NPV is the abbreviation of nucleopolyhedrovirus; SNPV is the abbreviation of single nucleopolyhedrovirus; GV is the abbreviation of granulovirus. The accession numbers are from National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) and correspond to the sequences of complete genomes. Code is an acronym used for practicity

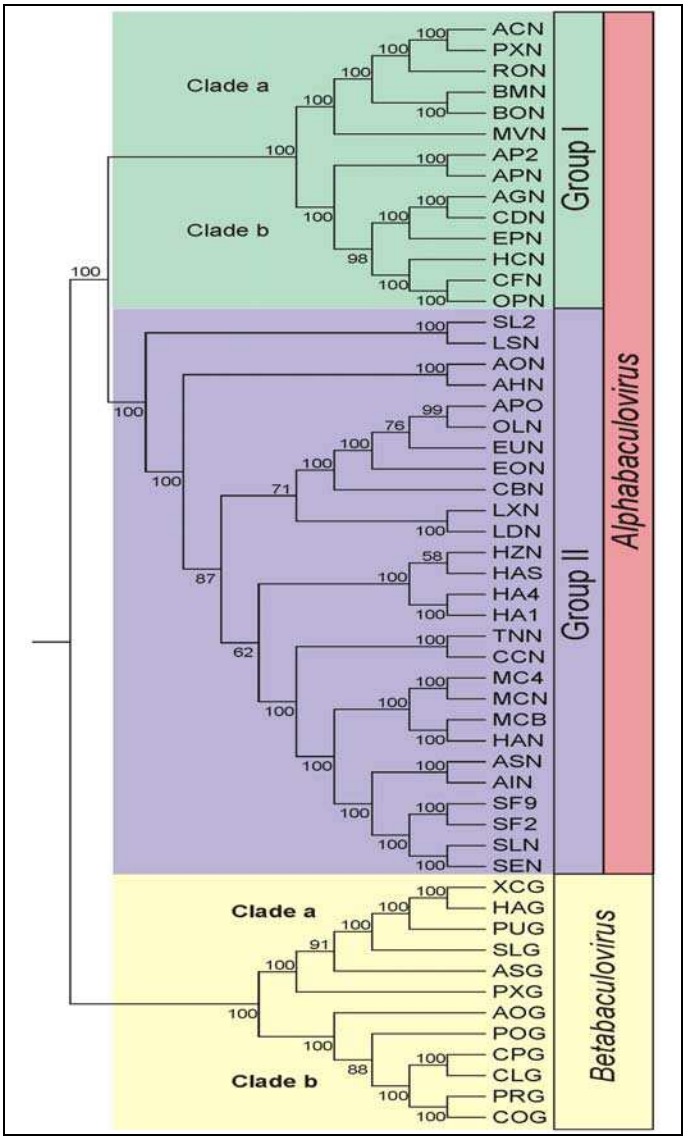


Fig. 1. Lepidopteron Baculovirus genome phylogeny. Cladogram based on amino acid sequence of 31 core genes. Core genes from Lepidopteron Baculoviridae family were independently aligned using MEGA 4 (GOP = 10, GEP = 1 and Dayhoff Matrix. Then, a concatemer was generated and phylogeny inferred using the same software [UPGMA; Bootstrap with 1000 replicates; gap/Missing data = complete deletion; Model = Amino (Dayhoff Matrix); patterns among sites = Same; rates among sites = Different (Gamma Distributed); gamma parameter = 2.25]. Baculoviruses are identified by the acronyms given in Table 1 and distribution in lineages and genera are also indicated. Clades proposed for Betabaculoviruses are shown in bold letters (Miele *et al*, 2011)

Virus code	Host (larvae)	Pest of…
ACN	Alfalfa looper, broad host range	Alfalfa and many other crops
AGN	Velvetbean caterpillar	Soybean crops
AHN	Smaller tea tortrix	Tea plants
AIN	Black cutworm	Vegetables, solanaceous, cucurbitaceous and industrial crops (cotton, essential-oil cultures, maize, tobacco, sunflower)
AOG	Summer fruit tortrix moth	Apples and pears
AON	Tea tree tortrix	Apple, pear, rose, plum, cherry, apricot, sweet cherry, currant, gooseberry, etc.
ASG	Black cutworm	Cotton, essential-oil cultures, maize, tobacco, sunflower, tomatoes, sugar beet and potato and also damage seedlings of tree species
ASN	Turnip moth	Many vegetable and field crops (corn, rape, beet, potatoes, cabbage, cereals, tobacco, vine and many others)
CBN	*Clanis bilineata*	Soybean
CCN	*Chrysodeixis chalcites*	Tomato and sweet pepper.
CDN, CFN	Eastern spruce budworm	Conifeorus trees
CLG	False codling moth, other Tortricid	Citrus, cotton, maize
COG	Western spruce budworm	Coniferous trees
CPG	Codling moth	Apples, pear and quince
EON	The tea looper caterpillar	Tea plants
EPN	Light brown apple moth	Apple, horticultural crops
HA1, HAN, HAS, HAG	Old world bollworm	Cotton, corn, baccy, tomato, maize, chick pea, alfalfa, soybean, pea, pumpkin
HCN	Fall webworm	Trees (cherry, plane, mulberry and persimmon)
LDN	Gypsy moth	Hardwoods

Virus code	Host (larvae)	Pest of…
LSN	Eastern armyworm	Many field crops in China
LXN	Casuarina moth	Casuarina, guava, longan, lychee, acacia
MCN, MC4, MCB	Bertha armyworm	Cruciferous oilseed crops in Canada.
MVN	Maruca pod borer	Leguminous crops (pigeon pea, cowpea, mung bean and soybean)
OLN	White-marked tussock moth	Wide variety of trees, deciduous and coniferous
POG	Potato tuber moth	Solanaceous cultures (potato, eggplant, tomato, pepper, and tobacco).
PRG	Small cabbage white	Cabbage, swede, turnip, radish, horseradish, garden radish, watercress, rape, turnip, and other cruciferous plants
PUG	Armyworm	Turfgrasses, small grains, corn, timothy, millet, and some legumes
PXG, PXN	Diamondback moth	Cruciferous crops
RON	Gray looper moth	Herbaceous plants
SEN	Beet armyworm	Asparagus, beans and peas, sugar and table beets, celery, cole crops, lettuce, potato, tomato, cotton, cereals, oilseeds, tobacco, etc.
SF2, SF9	Fall armyworm	Corn and small grain crops
SLN, SL2, SLG	Oriental leafworm moth	Wide range of plants, like cotton and tobacco.
TNN	Cabbage looper	Wide variety of cultivated plants and weeds (broccoli, cabbage, cauliflower, collards, kale, mustard, radish, rutabaga, turnip, snap bean, spinach, squash, sweet potato, tomato, watermelon, etc.)
XCG	Setaceous hebrew character	Huge variety of plants (tomato, tobacco, carrot, lettuce, alfalfa, potato, grape, maize, apple)

Table 2. Baculovirus and pest control. The table contains some Baculoviruses with their insect hosts, revealing their possible application as bioinsecticide

Actually, baculoviruses are classified in four genera according to their biological properties and gene content: *Alphabaculovirus*, polyhedroviruses that infect Lepidoptera (grouped into two lineages, Group I and Group II, according to their phylogenetic relationships and the identity of the fusogenic membrane protein presents in the BVs); *Betabaculovirus*,

granuloviruses that infect Lepidoptera; *Gammabaculovirus*, polyhedroviruses that infect Hymenoptera; and *Deltabaculovirus*, polyhedroviruses that infect Diptera (Table 1) (Jehle *et al*, 2006a).

Genomic sequence is known more than 50 different baculovirus species, being the recognized prototypes of each genus: AcMNPV, CpGV, NeleNPV and CuniNPV, respectively. Many of them have been used for biological pest control, being excellent biopesticides (Figure 1; Table 2).

However, most baculoviruses cannot efficiently compete with chemical insecticides, especially in the time of death. To overcome this problem, many researchers have been focused to introduce genetic modifications in order to accelerate the lethal effects of bioinsecticide or expand their host range. One strategy that has been explored is the introduction of genes encoding insect toxins, such as different neurotoxins from eukaryotic organism or the bacterial protein Cry (Inceoglu *et al*, 2006; Jinn et al, 2006; De Lima *et al*, 2007). Thus, these genetically modified viruses (GMV) would ensure better performance in biopesticide application.

Baculoviruses are produced by infection processes in susceptible larvae or in *in vitro* cell cultures. First approach is appropriate and inexpensive in small-scale, but big productions prefer the use of cell bioreactors(van Beek & Davis, 2007; Micheloud *et al*, 2009; Mengual Gómez *et al*, 2010). This technology would allow the standardization of production processes and achieve bioinsecticides with reproducible quality.

The main difference among these strategies consists in the starters used, being in one case OBs (in larvae) and BVs in the other (*in vitro* cell cultures); but always with the goal of producing OBs (infective phenotype in nature). Although the trend is moving toward baculovirus production in cell cultures, it is important to note some problems associated with that strategy. One of them is the genome stability. Because only the BVs infect cells growing in laboratory conditions, after successive rounds of infection tend to accumulate defective viral variants with smaller genomes (Lee & Krell, 1992). These quasispecies lose genomic segments encoding late proteins important for generating OBs, because there is no selection pressure associated with oral infection in larvae. Other problems are related to the composition of culture media and the availability of susceptible insect cell lines to each baculovirus. Actually, many researchers are working on the establishment of new cell lines or modifying existing ones to improve their performance, while others have focused on developing proper and cheaper formulations of growth media for cell propagation *in vitro* (Agathos, 2007; Micheloud *et al*, 2009).

2. Quality control assays

The production of baculoviruses for use as bioinsecticides required quality control processes to ensure their proper formulation. In either case above (wild type viruses or GMVs) or regardless of production method applied (larvae or *in vitro* cell cultures), is necessary to carry out a series of phenotypic and genotypic tests against which to assess the quality of each batch produced (Figure 2).

The formulation of one biological entity for some biotechnological application (e.g. baculovirus for agriculture pest control) requires its multiplication under controlled conditions and subsequent procedures for isolation and concentration. In this point, it is important to remember that all biological entities are object of evolution, natural phenomenon that can

influence and alter the biological properties of the product by the accumulation of point mutation or genome rearrangements.

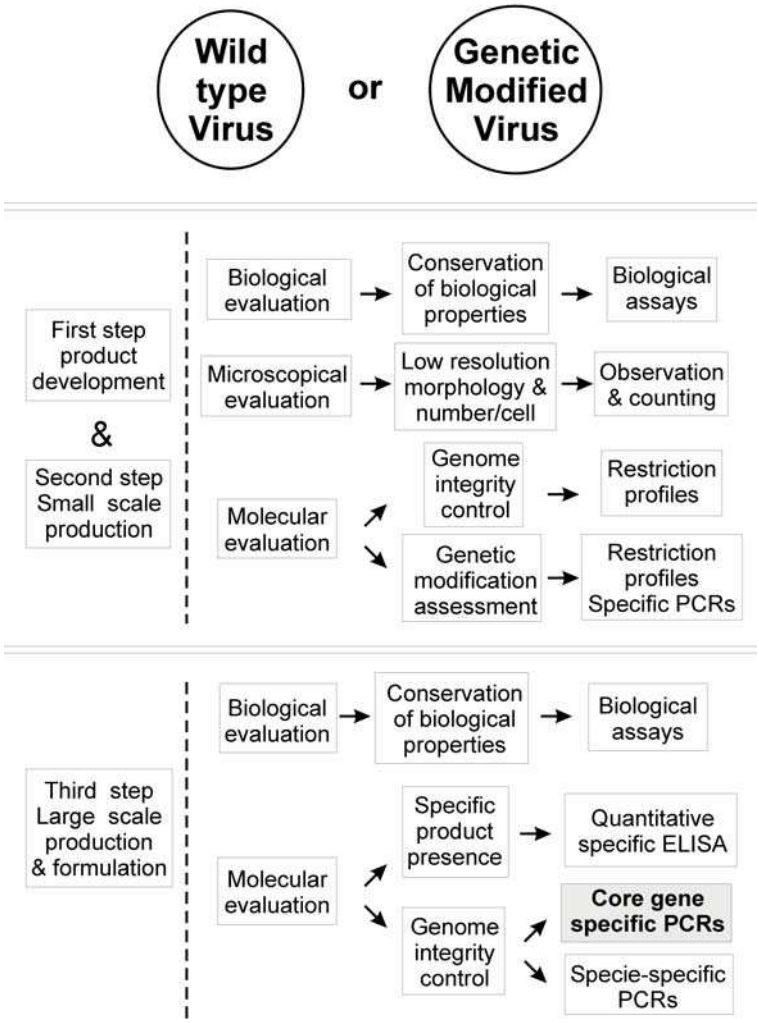


Fig. 2. Quality control scheme. A good quality control strategy is supported in the setting of and in the rigid adhesion to the procedures and protocols. These may include routine examinations of insect/cells stocks, microscopic examinations for infections, routine counting of ODVs, bioassays to assess bioinsecticide potency, restriction profiles of viral DNAs, and so on. First and second steps are developmental phases of the bioinsecticide production, in which the feasibility to obtain high amounts of good quality DNA is not an obstacle. In the third step, is of special importance the availability of sensitive molecular techniques to minimize the interference of formulation components

Thus, quality control assays emerge as central tools for verifying the baculovirus production in each of its stages allowing generating a product that can compete with chemical insecticides, whose production is highly optimized and controlled for years. Also, quality control strategies are useful to standardize the basic studies performed in laboratory scale, necessary for the generation of improved baculovirus.

2.1 Phenotype quality controls

First of all, it is important to have good methods to quantify the number of OBs produced and isolated from larvae or *in vitro* cell cultures. To fulfill this purpose, it is possible to make direct eye count using hemocytometer and optical microscopes. On the other hand, there are methodologies based on immunoassays or carried out by the use of flow cytometers. In the first case, the development of ELISA kits or other similar tests based on the immune detection of OBs (through the use of polyclonal or monoclonal antibodies against polyhedrin or granulin proteins) has standardized the quantitation of baculovirus allowing a more reliable measure (Parola *et al*, 2003). The use of flow cytometers also provides good results, but only so far for the quantification of BVs (Shen *et al*, 2002; Jorio *et al*, 2006).

Once quantified the production of OBs, should determine their biological activity. This involves setting parameters to estimate the ability of baculovirus to kill insect pests and control their population. In view of this, parameters like *median lethal time* (LT 50) and *median lethal dose* (LD 50) work as the best indicators to characterize the baculovirus activity (Li & Bonning, 2007; Lasa *et al*, 2008). These tests consist of exposing susceptible larvae reared in standardized conditions of temperature, light, moisture and food to the virus under evaluation. Then, through the register of deaths and the time in which they occur can be estimated both parameters.

2.2 Genotype quality controls

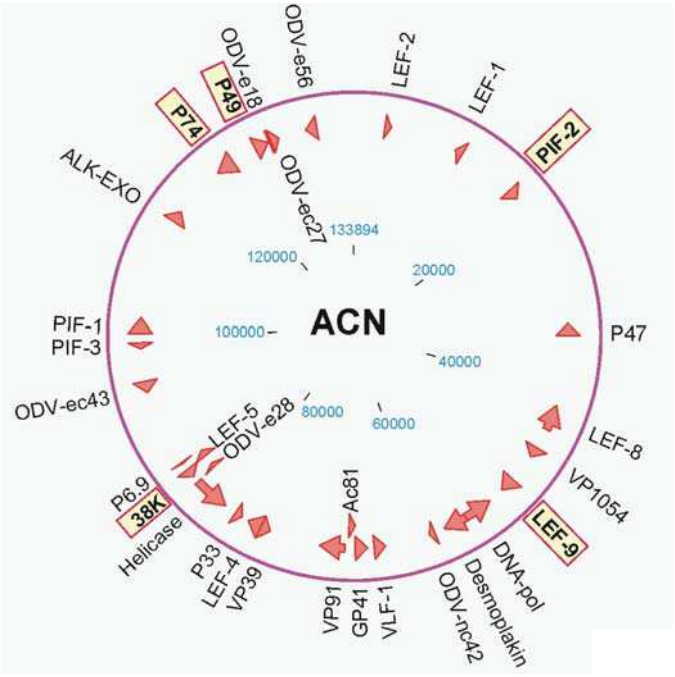
The production of baculoviruses for use as bioinsecticides requires accurate determination of the number of OBs and their biological activity expressed in LT 50 and LD 50 parameters. But it is also important to apply other methodologies that allow considering genotypic evaluations. As mentioned earlier, the processes of baculovirus production in insect cell lines growing in laboratory conditions may derived in problems with the integrity of their genomes. Consequently, the productivity of OBs can be seriously affected both in quantity and activity ruining the entire production. Of course, this is particularly relevant when dealing with GMVs. The stability of putative transgenes should be considered.

Most of baculoviruses applied as bioinsecticides derived from homogenous populations cloned or partially cloned by different procedures (Wang *et al*, 2003; Simón *et al*, 2004). This is a remarkable aspect since it allows establishing genotypic characteristic patterns that can be detected by different approaches. Among them, the visualization of RFLPs (*Restriction Fragment Lenght Polymorphism*) in agarose gel electrophoresis stained by different dyes and UV exposition is usually a good indicator of genome integrity, revealing the gain or loss of DNA (Simón *et al*, 2004; Eberle *et al*, 2009; Rowley *et al*, 2010). In fact, this is a classic approach to characterize genotypic variants of a viral species. The main problem that has this strategy is related to allocate part of baculovirus production to perform the isolation of viral genome, requiring high DNA masses to achieve reliable results. The complementation with hybridization assays solves part of that problem but requires the availability of suitable probes, adding experimental steps and costs of supplies and equipment.

In view of that, methodologies based on PCR (*Polymerase Chain Reaction*) are suitable and reproducible approaches to assess baculovirus productions because this technique can detect desired *locus* with high sensitivity and specificity. These characteristics transform the PCR in the best genotypic evaluation strategy due to its simple, fast and accessible properties for any laboratory production. Since the beginning of studies on the baculovirus genomes, many researchers have designed PCR tests to detect, identify and classify the different species of this virus Family. Thus, PCR assays based on *polyhedrin/granulin*, *p74*, *lef8*, *lef9* or *DNA polymerase* genes, among others, were used to describe new virus isolates which are candidates to bioinsecticide applications (Faktor *et al*, 1996; de Moraes *et al*, 1999; Wang *et al*, 2000; Rosisnki *et al*, 2002; Espinel-Correal *et al*, 2011; Rodríguez *et al*, 2011).
However, there are too many examples of the use of PCR as a technique for quality control in the production of a baculovirus, despite all the advantages mentioned above (Christian *et al*, 2001; Murillo *et al*, 2006).

2.2.1 MP-PCR to control baculovirus production

PCR amplification of several *loci* in the same reaction allows obtaining a profile of products that can be used for genome identification or control test in production processes. MP-PCR (*Multiplex* PCR) assays require the proper design of primers to amplify a set of fragments that are typical for a particular genome. This technique provides results composed of a set of enzymatic amplified fragments that are characteristic for a viral species (when primers were designed completely specific), or for a phylogenetic group (when primers derived from multiple alignments of orthologous sequences). With regard to trials designed to particular viruses, it should be noted the work developed for EpapGV (Manzán *et al*, 2008). Meanwhile, for the detection of groups of related viruses are not many references. Currently, the accepted practice to identify or preliminarily classify a new baculovirus is based on PCR amplification and subsequent sequencing of three genomic fragments corresponding to the *polyhedrin/granulin*, *lef9* and *lef8* genes (Jehle *et al*, 2006b). However, this approach is not itself an MP-PCR. In view of this, we propose an MP-PCR for *alpha* and *betabaculovirus* quality control based on universal primer designs.
Baculoviruses contain 31 core genes conserved by all known members (Miele *et al*, 2011). These orthologous sequences are present in each sequenced baculovirus, but their genomic distribution varies among species. From the analysis of gene distribution in genus prototypes *pif2*, *p49*, *p74*, *lef9*, *38k* genes were selected to primer design targets (Figure 3).
These sequences are properly distributed throughout the entire circular genome. Two genes (*pif2* and *p74*) encode *per os* infectivity factors essentials to the success of primary infection in midgut cells (Song *et al*, 2008; Peng *et al*, 2010). Other two genes (*p49* and *38 K*) encode proteins associated to packaging, assembly, and release of virions (Wu *et al*, 2008; Lin *et al*, 2010). Meanwhile, *lef9* gene encodes a polypeptide involved in virus transcription machinery (Crouch *et al*, 2007). Using multiple alignments derived from sequences corresponding to *P74*, *lef9* and *38k* genes from all *alpha* and *betabaculovirus* members were selected the two better regions of homology to design a set of primers (Figure 4). Thus, these three amplicons certified the presence of lepidopteron baculovirus DNA.
In change, because high divergence of *pif2* and *p49* sequences the primer design was conducted using multiple alignments derived from closest phylogenetic clades (Group I and Group II *alphabaculovirus*, and *betabaculovirus*). According to this, different pairs of primers were designed to generate amplicons from baculovirus genomes (Figure 5).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20000
100000
40000
80000
60000
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LEF-2
LEF-1
PIF-2
P47
LEF-8
VP1054
LEF-9
DNA-pol
Desmoplakin
ODV-nc42
VLF-1
GP41
VP91
P33
LEF-4
VP39
Helicase
38K
P6.9
ODV-ec43
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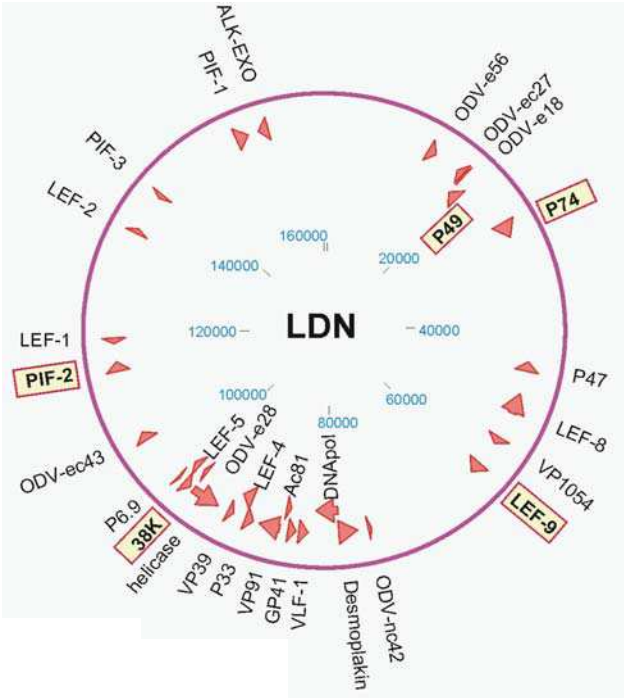
Ac81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38K
helicase
VP39
P33
VP91
GP41
VLF-1
Desmoplakin
ODV-nc42
P47
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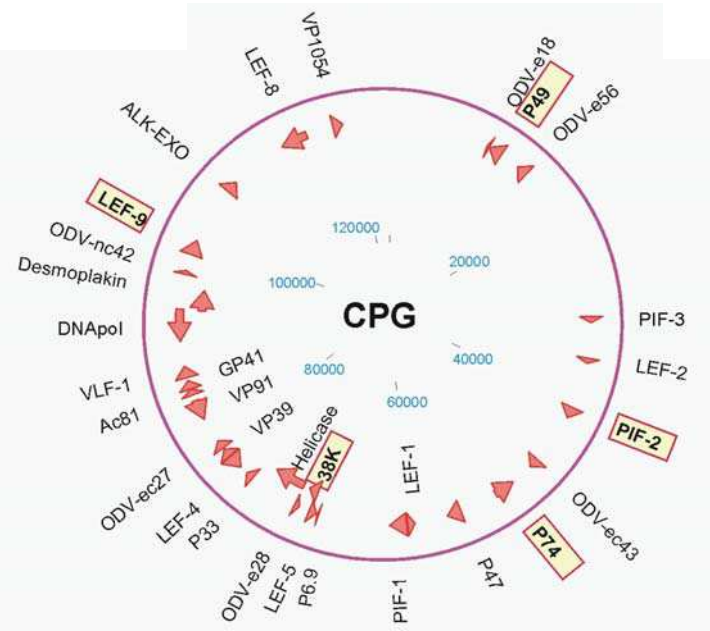
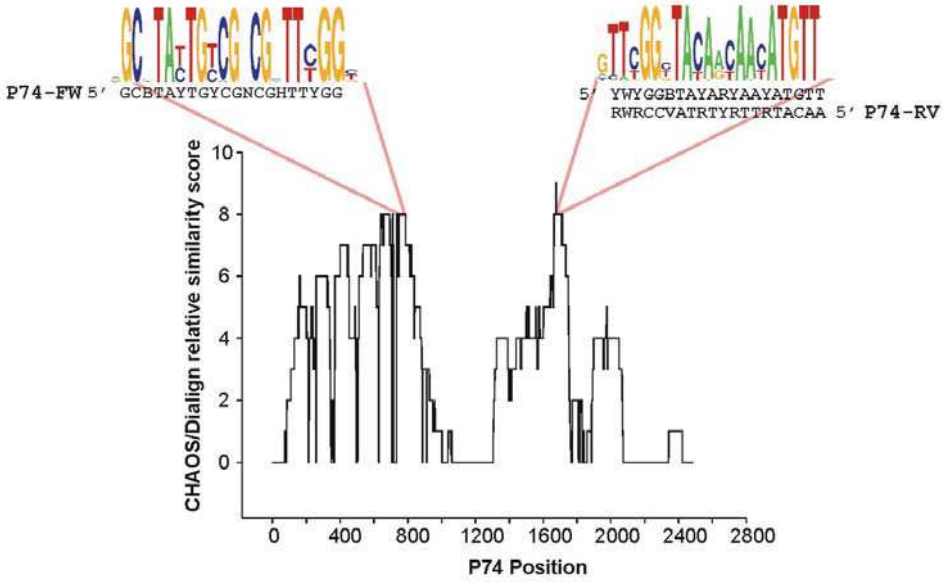


Fig. 3. Physical maps of ACN, LDN and CPG (Arrows shows the physical location of the 31 Core genes. The five selected Core genes for primer designs are highlighted in bold and red boxed.)



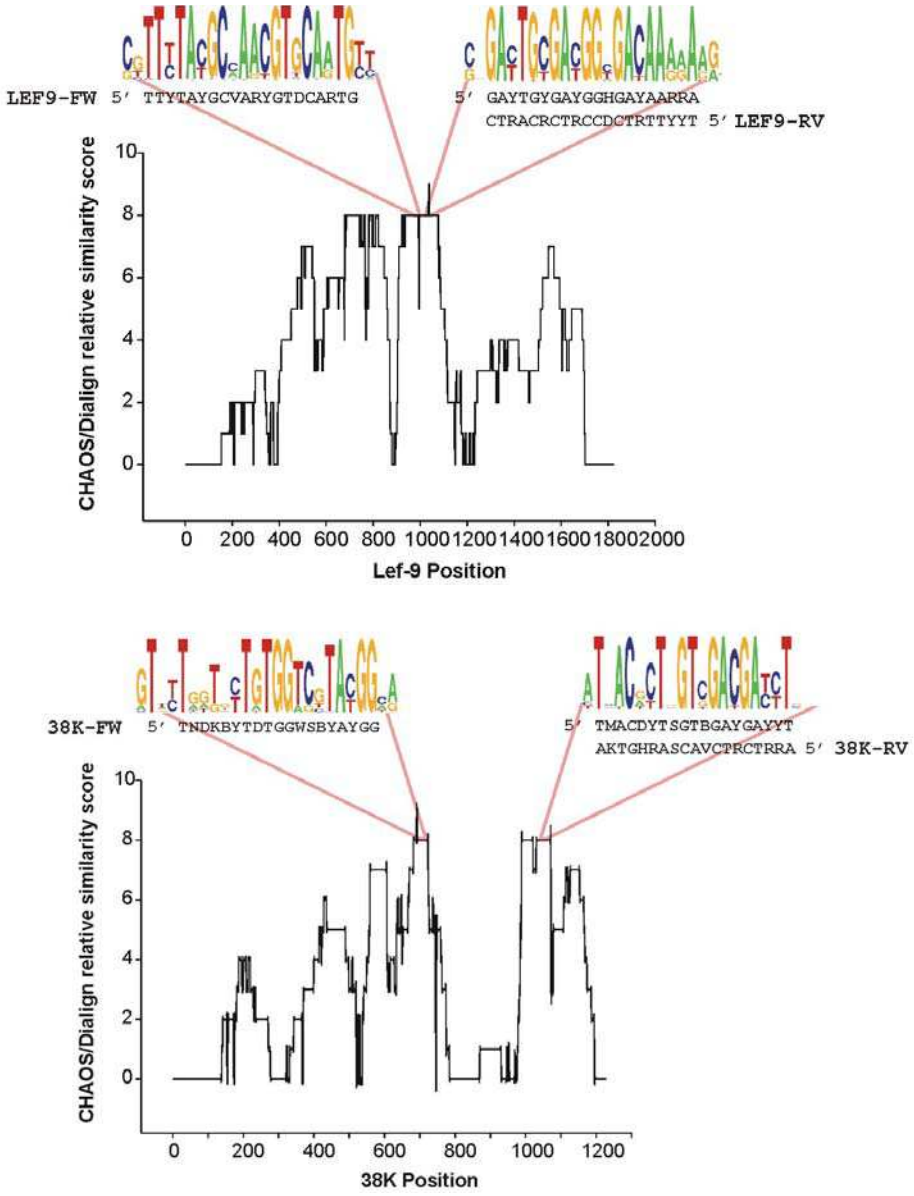


Fig. 4. Primer design for *p74*, *lef-9* and *38K* genes. The orthologous sequences of *p74*, *lef-9* and *38K* genes from Alpha and Betabaculovirus members were aligned by CHAOS/DIALIGN program (Brudno *et al*, 2004). A consensus line in the multiple alignment is a set of numbers (between 0-9) that roughly reflect the degree of local similarity among the sequences. These scores were used to generate plots. The regions with higher relative similarity were selected to design primers. These sequences are showed at the top in Sequence Logos (Crooks *et al*, 2004)

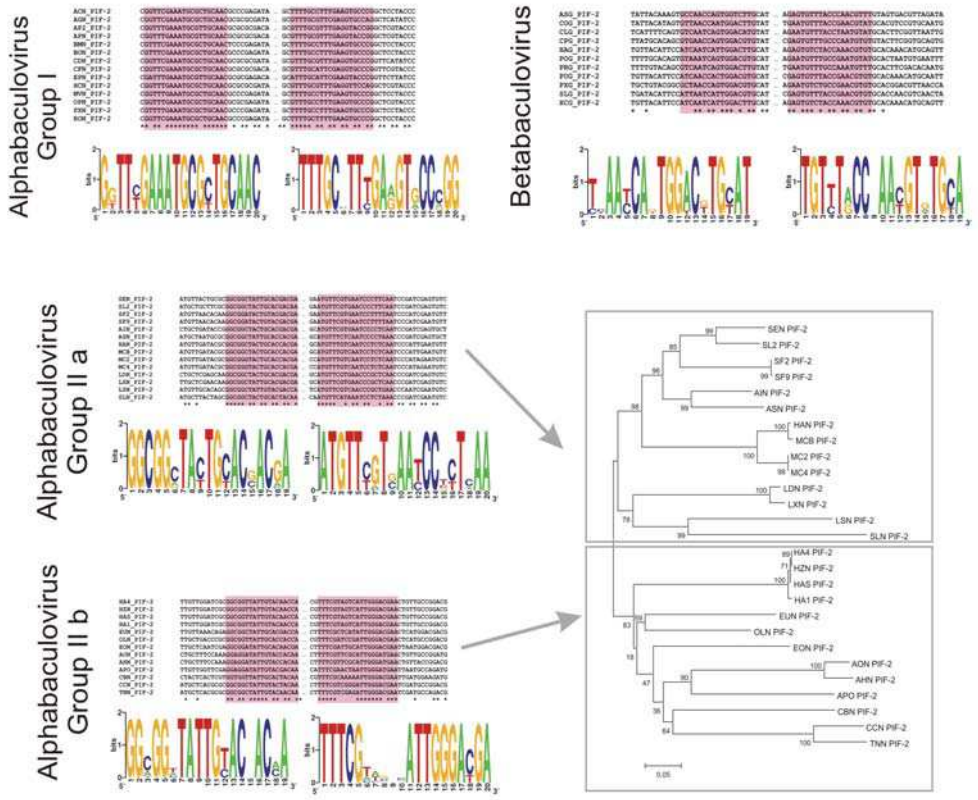


Fig. 5. Primer design for *pif-2* gene. The orthologous sequences of *pif-2* gene from Group I Alphabaculovirus or Group II Alphabaculovirus or Betabaculovirus members were aligned by T-Coffee program (Notredame *et al*, 2000; Poirot *et al*, 2003). The regions with higher similarity were selected to design primers. These sequences are showed at the bottom of each multiple alignment in Sequence Logos (Crooks *et al*, 2004). The cladogram was made with nucleotide sequences of *pif-2* Group II Alphabaculovirus using MEGA 4. It showed a significative grouping in two lineages (Group II a and Group II b), which were considered to design primers. For *p49* sequence analysis a similar approach was conducted (data not shown)

Sets of proposed primers for MP-PCR would allow to detect the proper integrity of genomes in a baculovirus production (Table 3).

Gene	Baculovirus		Primer sequence 5´ to 3´	Product (bp)		
				ACN	LDN	CPG
lef-9	Alpha + Beta	FW	TTYTAYGCVARYGTDCARTG	245	245	247
		REV	TYYTTRTCDCCRTCRCARTC			

Gene	Baculovirus		Primer sequence	Product (bp)		
			5´ to 3´	ACN	LDN	CPG
38K	Alpha + Beta	FW	TNDKBYTDTGGWSBYAYGG	247	260	218
		REV	ARRTCRTCVACSARHGTKA			
pif-2	Alpha Group I	FW	GDTTYGAAATGCGYTGCAAC	382	---	---
		REV	CCBGGHACYTCRAASGCAAA			
	Alpha Group IIa	FW	GGCGGVTAYTGYACBACVA	347	347	---
		REV	TTDARVGGRTTSACRAACAT			
	Alpha Group IIb	FW	GGMGGHTATTGYACNACVA	---	260	---
		REV	TCRTCCCAATBNSDDCGAAA			
	Beta	FW	YYAAYCAKTGGWCDTGYAT	306	306	306
		REV	TRCAHACRTTNGGYARACA			
p74	Alpha + Beta	FW	GCBTAYTGYCGNCGHTTYGG	824	830	935
		REV	AACATRTTRYTRTAVCCRWR			
p49	Alpha GroupI	FW	AGTYTATTTGAYYTRAAARA	1284	---	---
		REV	ACTTTCGTAATCACCTCTTA			
	Alpha GroupII	FW	TAYGCNACNAAYYTKTTYGT	---	970	---
		REV	AATCWCCTCTTATRAWWARAT			
	Beta	FW	CARMGVGAYTAYRTHTWYGA	---	---	596
		REV	AATAARYTYRVWAHVGTRTT			

Table 3. Primer sequences to perform a MP-PCR assay. The table contains all the primer sequences designed by two different approaches and the hypothetical length of amplified fragments using the genome prototypes as reaction template. The specificity of annealing and the size of the amplicons were verified using jPCR (Kalendar *et al*, 2009). FW: forward primer. REV: reverse primer. Ambiguities are indicated in IUPAC code, B=C,G,T; D=A,G,T; H=A,C,T; K=G,T; M=A,C; N=A,C,G,T; R=A,G; S=C,G; V=A,C,G; W=A,T; Y=C,T

3. Conclusion

Integrated control management of agricultural pests requires the combination of different insecticide strategies. Among them, the use of baculovirus is an excellent solution as biological control agent. There are many known members of this viral family, with dozens of sequenced genomes. Some of the limitations that exist in their massive application are given by their time of action and modes for their production. Regarding the latter, quality control methodologies are emerging as essential to ensure proper development and formulation. In view of that, in this work are proposed a series of primers for PCR assays

that would amplify a fragment profile appropriate to certify the genomic integrity and identity of batch production. Furthermore, adding other specific primers (e.g. specific of transgenes) could be confirmed genotypic stability of genetically modified viruses.
Also, the methodology here proposed could be used to characterize new baculoviral isolates, which could be used as bioinsecticides and produced and controlled without the knowledge of their genome sequences.

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5. References

Agathos, S.N. (2007). *Development of serum-free media for lepidopteran insect cell lines*. Methods Mol Biol. Vol 388, pp. 155-186. ISSN: 1064-3745.

Brudno, M.; Steinkamp, R.; Morgenstern, B. (2004). *The CHAOS/DIALIGN WWW server for multiple alignment of genomic sequences.* Nucleic Acids Res., 2004 Jul 1, vol. 32, pp. 41-44. ISSN 0305-1048.

Christian, P.D.; Gibb, N.; Kasprzak, A.B.; Richards, A. (2001). *A rapid method for the identification and differentiation of Helicoverpa nucleopolyhedroviruses (NPV Baculoviridae) isolated from the environment*. J Virol Methods, 2001 Jul, vol 96, pp. 51-65. ISSN: 0166-0934.

Christou, P.; Capell, T.; Kohli, A.; Gatehouse, J.A.; Gatehouse, AM. (2006). *Recent developments and future prospects in insect pest control in transgenic crops.* Trends Plant Sci, 2006 Jun, vol 11, pp. 302-308. ISSN: 1360-1385.

Crouch, E.A.; Cox, L.T.; Morales, K.G.; Passarelli, A.L. (2007). *Inter-subunit interactions of the Autographa californica M nucleopolyhedrovirus RNA polymerase*. Virology, 2007 Oct 25, 367, pp. 265-274. ISSN: 0042-6822.

De Lima, M.E.; Figueiredo, S.G.; Pimenta, A.M.; Santos, D.M.; Borges, M.H.; Cordeiro, M.N.; Richardson, M.; Oliveira, L.C.; Stankiewicz, M.; Pelhate, M. (2007). *Peptides of arachnid venoms with insecticidal activity targeting sodium channels*. Comp Biochem Physiol C Toxicol Pharmacol. 2007 Jul-Aug, vol 146, pp. 264-79. ISSN: 1532-0456.

de Moraes, R.R.; Maruniak, J.E.; Funderburk, J.E. (1999). *Methods for detection of Anticarsia gemmatalis nucleopolyhedrovirus DNA in soil*. Appl Environ Microbiol, 1999 Jun, vol 65, pp. 2307-2311. ISSN: 0099-2240.

Eberle, K.E.; Sayed, S.; Rezapanah, M.; Shojai-Estabragh, S.; Jehle J.A. (2009). *Diversity and evolution of the Cydia pomonella granulovirus*. J Gen Virol, 2009 Mar, vol 90, pp. 662-671. ISSN: 0022-1317.

Entwistle, P.F. (1998). *A world survey of virus control of insect pests*, pp. 189-200. In F. R. Hunter-Fujita, P.F. Entwistle, H.F. Evans, and N. E. Crook (ed.), "Insect Viruses and Pest Management". John Wiley & Sons Ltd., Chichester, England. ISBN0-471-96878-1.

Espinel-Correal, C.; Léry, X.; Villamizar, L.; Gómez, J.; Zeddam, J.L.; Cotes, A.M.; López-Ferber M. (2010). *Genetic and biological analysis of Colombian Phthorimaea operculella granulovirus isolated from Tecia solanivora (Lepidoptera: Gelechiidae)*. Appl Environ Microbiol, 2010 Nov, vol 76, pp. 7617-1625. ISSN: 0099-2240.

Faktor, O. and Raviv, D. (1996). *A polymerase chain reaction for the detection of nucleopolyhedroviruses in infected insects: the fate of the Spodoptera littoralis virus in Locusta migratoria*. J Virol Methods, 1996 Sept, vol 61, pp. 95-101. ISSN: 0166-0934.

Crooks, G.E.; Hon, G.; Chandonia, J.M.; Brenner, S.E. (2004). *WebLogo: a sequence logo generator*. Genome Res, 2004 Jun, vol. 14, pp.1188-1190. ISSN: 1088-9051.

Gilligan, C.A. (2008). *Sustainable agriculture and plant diseases: an epidemiological perspective.* Philos Trans R Soc Lond B Biol Sci. 2008 Feb 27, vol 363, pp. 741-759. ISSN: 1471-2970.

Inceoglu, A.B.; Kamita, S.G.; Hammock, B.D. (2006). *Genetically modified baculoviruses: a historical overview and future outlook*. Adv Virus Res. 2006; vol. 68, pp. 323-360. ISSN: 0065-3527.

Jehle, J.A.; Blissard, G.W.; Bonning, B.C. (2006a). *On the classification and nomenclature of baculoviruses: a proposal for revision*, Archives of Virology, 2006 Jul, vol. 151, pp. 1257–1266. ISSN 0304-8608.

Jehle, J.A.; Lange, M.; Wang, H.; Hu, Z.; Wang, Y.; Hauschild, R. (2006b). *Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera*. Virology, 2006 Mar 1, vol 346, pp. 180-93. ISSN: 0042-6822.

Jinn, T.R.; Tu, W.C.; Lu, C.I.; Tzen, J.T. (2006). *Enhancing insecticidal efficacy of baculovirus by early expressing an insect neurotoxin, LqhIT2, in infected Trichoplusia ni larvae*. Appl Microbiol Biotechnol. 2006 Oct, vol. 72, pp. 1247-1253. ISSN: 0175-7598.

Jorio H.; Tran, R.; Meghrous, J.; Bourget, L.; Kamen, A. (2006). *Analysis of baculovirus aggregates using flow cytometry*. J Virol Methods. 2006 Jun, vol 134, pp. 8-14. ISSN: 0166-0934.

Kalendar, R.; Lee, D.; Schulman, A.H. (2009). *FastPCR Software for PCR Primer and Probe Design and Repeat Search*. Genes, Genomes and Genomics, vol 3, pp. 1-14. ISSN: 1749-0383. [http://primerdigital.com/fastpcr.html]

Lasa, R.; Williams, T.; Caballero, P. (2008). *Insecticidal properties and microbial contaminants in a Spodoptera exigua multiple nucleopolyhedrovirus (Baculoviridae) formulation stored at different temperatures*. J Econ Entomol, 2008 Feb, vol 101, pp. 42-49. ISSN: 0022-0493

Lee, H.Y.; Krell, P.J. (1992). Generation and analysis of defective genomes of Autographa californica nuclear polyhedrosis virus. J Virol. 1992 July, vol. 66, pp. 4339-4347. ISSN: 0022-538X.

Li, H.; Bonning, B.C. (2007). *Evaluation of the insecticidal efficacy of wild-type and recombinant baculoviruses*. Methods Mol Biol, vol 388, pp. 379-404. ISSN: 1064-3745.

Lin, T.; Yu, M.; Wu, W.; Yu, Q.; Weng, Q.; Yang, K.; Yuan, M.; Pang Y. (2010). *Functional analysis of Spodoptera litura nucleopolyhedrovirus p49 gene during Autographa californica nucleopolyhedrovirus infection of SpLi-221 cells*. Virus Genes. 2010 Dec, vol 41, pp. 441-449. ISSN: 0920-8569.

Manzán, M.A.; Aljinovic, E.M.; Biedma, M.E.; Sciocco-Cap, A.; Ghiringhelli, P.D. and Romanowski, V. (2008). *Multiplex PCR and quality control of EpapGV production*. Virus Genes, 2008 Oct, vol 37, pp. 203-211. ISSN: 0920-8569.

Mengual Gómez, D.L.; Belaich, M.N.; Rodríguez, V.A.; Ghiringhelli, P.D. (2010). *Effects of fetal bovine serum deprivation in cell cultures on the production of Anticarsia gemmatalis multinucleopolyhedrovirus*. BMC Biotechnol. 2010 Sep, vol. 15; 10:68. ISSN 1472-6750.

Micheloud, G.A.; Gioria, V.V.; Pérez, G.; Claus J.D. (2009). *Production of occlusion bodies of Anticarsia gemmatalis multiple nucleopolyhedrovirus in serum-free suspension cultures of the saUFL-AG-286 cell line: influence of infection conditions and statistical optimization*. J Virol Methods. 2009 Dec, vol 162, pp. 258-266. ISSN: 0166-0934.

Miele, S.A.B.; Garavaglia, M.J.; Belaich, M.N.; Ghiringhelli, P.D. (2011). *Baculovirus: Molecular Insights on Their Diversity and Conservation*. International Journal of Evolutionary Biology. Vol. 2011, Article ID 379424, 15 pages, 2011 Feb. doi:10.4061/2011/379424. ISSN: 2090052X.

Moscardi, F. (1999). *Assessment of the application of baculoviruses for control of Lepidoptera*. Annu Rev Entomol. Vol 44, pp. 257-489. ISSN: 0066-4170.

Murillo, R.; Muñoz, D.; Williams, T.; Mugeta, N.; Caballero, P. (2006). *Application of the PCR-RFLP method for the rapid differentiation of Spodoptera exigua nucleopolyhedrovirus genotypes*. J Virol Methods, 2006 Jul, vol 135, pp. 1-8. ISSN: 0166-0934.

Notredame, C.; Higgins, D.G.; Heringa, J. (2000). *T-Coffee: A novel method for fast and accurate multiple sequence alignment*. J Mol Biol, 2000 Sep 8, vol 302, pp. 205-17. ISSN: 0022-2836

Parola, A.D.; Sciocco-Cap, A., Glikmann, G., Romanowski, V. (2003). *An immunochemical method for quantitation of Epinotia aporema granulovirus (EpapGV)*. J Virol Methods. 2003 Sep, vol 112, pp. 13-21. ISSN: 0166-0934.

Peng, K.; van Oers, M.M.; Hu, Z.; van Lent, J.W.; Vlak, J.M. (2010). *Baculovirus per os infectivity factors form a complex on the surface of occlusion-derived virus*. J Virol, 2010 Sep, vol 84, pp. 9497-504. ISSN: 0022-538X.

Poirot, O.; O'Toole, E.; Notredame, C. (2003). *Tcoffee@igs: A web server for computing, evaluating and combining multiple sequence alignments*. Nucleic Acids Res, 2003 Jul 1, vol 31, pp. 3503-3506. ISSN 0305-1048.

Rodríguez, V.A.; Belaich, M.N.; Gómez, D.L.; Sciocco-Cap, A; Ghiringhelli, P.D. *Identification of nucleopolyhedrovirus that infect Nymphalid butterflies Agraulis vanillae and Dione juno*. J Invertebr Pathol, 2011 Feb, vol 106, pp. 255-262. ISSN: 0022-2011.

Rohrman, G.F. (2008). *Baculovirus Molecular Biology*, National Library of Medicine (US), NCBI, Bethesda, Md, USA, 2008.

Rosinski, M.; Reid, S.; Nielsen, L.K. (2002). *Kinetics of baculovirus replication and release using real-time quantitative polymerase chain reaction*. Biotechnol Bioeng, 2002 Feb, vol 77, pp. 476-480. ISSN: 0572-6565.

Rowley, D.L.; Farrar, R.R. Jr; Blackburn, M.B.; Harrison, R.L. (2010). *Genetic and biological variation among nucleopolyhedrovirus isolates from the fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae)*. Virus Genes, 2010 Jun, vol 40, pp. 458-468. ISSN: 0920-8569.

Shen, C.F.; Meghrous, J.; Kamen, A. (2002). *Quantitation of baculovirus particles by flow cytometry*. J Virol Methods. 2002 Sep, vol 105, pp. 321-30. ISSN: 0166-0934.

Simón, O.; Williams, T.; López-Ferber, M.; Caballero, P. (2004). *Genetic structure of a Spodoptera frugiperda nucleopolyhedrovirus population: high prevalence of deletion genotypes*. Appl Environ Microbiol, 2004 Sep, vol 70, pp. 5579-5588. ISSN: 0099-2240.

Song, J.; Wang, R.; Deng, F.; Wang, H.; Hu, Z. (2008). *Functional studies of per os infectivity factors of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus*. J Gen Virol, 2008 Sep, vol 89, pp. 2331-2338. ISSN: 0022-1317.

Szewczyk, B.; Hoyos-Carvajal, L.; Paluszek, M.; Skrzecz, I., Lobo de Souza, M. (2006). *Baculoviruses, re-emerging biopesticides*. Biotechnol Adv. 2006 Mar-Apr, vol. 24, pp. 143-60. ISSN: 0734-9750.

Theilmann, D.A.; Blissard, G.W.; Bonning, B.C.; Jehle, J.A.; O'Reilly, D.R.; Rohrmann, G.F.; Thiem, S.M.; and Vlak, J.M. Baculoviridae, In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball. (2005). Virus Taxonomy: (eds.), *Taxonomy of Viruses: VIII Report of the International Committee on Virus Taxonomy*. Elsevier, London, pp. 177-185.

van Beek, N.; Davis, D.C. (2007). *Baculovirus insecticide production in insect larvae*. Methods Mol Biol. 2007, vol 388, pp. 367-378. ISSN: 1064-3745.

van Oers, M. M. and Vlak, J. M. (2007). *Baculovirus genomics*. Current Drug Targets. 2007 Oct vol. 8, pp. 1051–1068. ISSN 1389-4501.

Wang, C.H.; Yang, H.N.; Liu, H.C.; Kou, G.H.; Lo, C.F. (2000). *Nested polymerase chain reaction and in situ hybridization for detection of nucleopolyhedrosis*. J Virol Methods, 2000 Jan, vol 84, pp. 65-75. ISSN: 0166-0934.

Wang, H.; Deng, F.; Pijlman, G.P.; Chen, X.; Sun, X.; Vlak, J.M.; Hu, Z. (2003). *Cloning of biologically active genomes from a Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus isolate by using a bacterial artificial chromosome*. Virus Res, 2003 Nov, vol 97, pp. 57-63. ISSN: 0168-1702.

Wu, W.; Liang, H.; Kan, J.; Liu, C.; Yuan, M.; Liang, C.; Yang, K.; Pang, Y. (2008). *Autographa californica multiple nucleopolyhedrovirus 38K is a novel nucleocapsid protein that interacts with VP1054, VP39, VP80, and itself*. J Virol, 2008 Dec, vol 82, pp. 12356-12364. ISSN: 0022-538X.

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Quality control is a standard which certainly has become a style of living. With the improvement of technology every day, we meet new and complicated devices and methods in different fields. Quality control explains the directed use of testing to measure the achievement of a specific standard. It is the process, procedures and authority used to accept or reject all components, drug product containers, closures, in-process materials, packaging material, labeling and drug products, and the authority to review production records to assure that no errors have occurred.The quality which is supposed to be achieved is not a concept which can be controlled by easy, numerical or other means, but it is the control over the intrinsic quality of a test facility and its studies. The aim of this book is to share useful and practical knowledge about quality control in several fields with the people who want to improve their knowledge.

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