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# Reduced expression of *Autographa californica* nucleopolyhedrovirus ORF34, an essential gene, enhances heterologous gene expression

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#### ABSTRACT

*Autographa californica* multiple nucleopolyhedrovirus ORF34 is part of a transcriptional unit that includes ORF32, encoding a viral fibroblast growth factor (FGF) and ORF33. We identified ORF34 as a candidate for deletion to improve protein expression in the baculovirus expression system based on enhanced reporter gene expression in an RNAi screen of virus genes. However, ORF34 was shown to be an essential gene. To explore ORF34 function, deletion (KO34) and rescue bacmids were constructed and characterized. Infection did not spread from primary KO34 transfected cells and supernatants from KO34 transfected cells could not infect fresh Sf21 cells whereas the supernatant from the rescue bacmids transfected cells by electron microscopy, nor were viral proteins detected from the transfection supernatants by western blots. These demonstrate that ORF34 is an essential gene with a possible role in infectious virus production.

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#### Introduction

Baculoviruses are large rod shaped DNA viruses that infect insects. They have practical applications as biological insecticides and protein expression vectors. Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) is the type species of the Alphabaculovirus genus of the family Baculoviridae (International Committee on Taxonomy of Viruses, 2009). Two clades, group I and group II, have been identified in Alphabaculovirus (Zanotto et al., 1993), which differ in the envelope fusion protein of the budded virus (BV) (Ijkel et al., 2000; Pearson et al., 2000). AcMNPV is member of group I and has a 134 Kb genome encoding approximately 150 proteins (Rohrmann, 2011). The functions of approximately 50% of these are known (Cohen et al., 2009). AcMNPV is a lytic virus and its life cycle involves the production of two morphological forms of the virus and a regulated cascade of gene expression (for reviews see (Miller, 1997; Rohrmann, 2011). The first form of the virus produced during infection is BV,

which is formed when nucleocapsids are transported from their site of replication and assembly in the nucleus to the plasma membrane and acquire an envelope as they bud from the infected cells. The second form, occlusion derived virus (ODV), is produced late in infection when nucleocapsids retained in the nucleus acquire an envelope, most likely derived from the inner nuclear membrane (Braunagel and Summers, 2007), and are occluded in large protein crystals called occlusion bodies. The primary component of the occlusion bodies is a single virus protein called polyhedrin (polh). AcMNPV gene expression comprises four gene classes. Immediate early genes, which do not require viral gene products, and delayed early genes are transcribed by the host RNA pol II and encode proteins required for virus replication. Late and very late genes are transcribed by a virus RNA polymerase from virus-specific promoters and encode primarily structural proteins. The very late or occlusion-specific genes, which encode polh and another small protein, p10, are expressed to very high levels at the end of the replication cycle. At this stage in the infection cycle polh makes up approximately 50% of the newly synthesized protein in the infected cells. The high-level of expression from the *polh* promoter is achieved through the use of the strong virus RNA polymerase. In addition, the production of large amounts of virus DNA that accumulates in the nucleus effectively amplifies



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#### Table 1

AcMNPV ORFs silenced by RNAi.

ORF(s) targeted by dsRNA	Annotation	dsRNA size bp	dsRNA template positions in AcMNPV genome (bp)
ORF1	ntnase	451	524_974
ORF3	Ctv	150	224-374
ORF21	arif_1	190	16038-16454
ORF46	Odv-e66	403	37898-38394
ORF61	Fn25k	374	49086-48713
ORF94	(Odv-e25) p25	370	79974-80343
ORF105	He65	496	93011-92516
ORF148	Odv-e56	405	130128-129724
ORF15	egt	681	11852-12513
ORF22	pif-2	665	17702-18366
ORF23	•	692	19020-19711
ORF27	iap1	684	22609-23292
ORF32	fgf	534	27581-27048
ORF34+ORF35 (v-ubi)		535	28655-29189
ORF49+ORF48+ORF47 (PCNA)		540	39713-39174
ORF63+ORF64 (gp37)		554	51969–51416
ORF71	iap2	633	61118-61750
ORF109		631	95518–94888
ORF115	pif3	503	99703-99202
ORF119	pif1	616	101140-101755
ORF131	pe/pp34	604	110989–111592
ORF136 (p26) + ORF137 (p10) + ORF138 (p74)		1172	118536-119707
ORF134	94K	989	115972-114984
ORF106+ORF107		601	93750-94334
ORF112+ORF113		669	96584-97253
ORF12	Date	480	9108-9480
ORF2 OPF11	BLO	640	1/83-1120
OPE12		416	0065 0490
OPE12		410	10270 0880
ORF13		563	15110-14557
ORF18		310	22214_22522
ORF29		200	24259-24060
ORF30		507	25531-25025
ORF31	Sod	425	25835-26259
ORF33		474	28235-27762
ORF42	gta	504	34829-35332
ORF43	0	201	35569-35769
ORF44		354	35768-36121
ORF45		501	36155-36655
ORF51		565	43335-43899
ORF52		340	44708-44369
ORF55		191	46418-46608
ORF56		251	46634-46884
ORF57		461	47073–47533
ORF58		150	47733-47584
ORF60		259	48363-48105
ORF63		403	50820-51222
ORFO		517	58740-59256
ORF09 ORF72		251	59515-59910 62202 62052
OPE74		516	62044 62520
ORF78		299	65276-64978
ORF79		303	65602-65300
ORF82	tlp	472	67918-67447
ORF83	p95	605	68012-68616
ORF84	I · ·	401	71247-71647
ORF86	pnk/pnl	602	73269-72668
ORF87	p15	301	74397-74697
ORF88	cg30	604	75505-74902
ORF91		502	78490–77989
ORF93		423	79523–79945
ORF96		455	84406-84860
ORF108		220	94696-94477
ORF110+ORF111		313	96321-95961
ORF114		750	98868-98119
ORF116		150	99958-99809
ORF117		200	99939-100138
		411	100001-100251
		201	102321-102321
ORF122 ORF123	nk2	131	102697-102747 103561_103031
ORF123	рка	524	10301-10301 103854_104487
ORF129	n24	413	109900-110312
ORF130	ר∠ <del>י</del> מח16	306	110524–110829
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