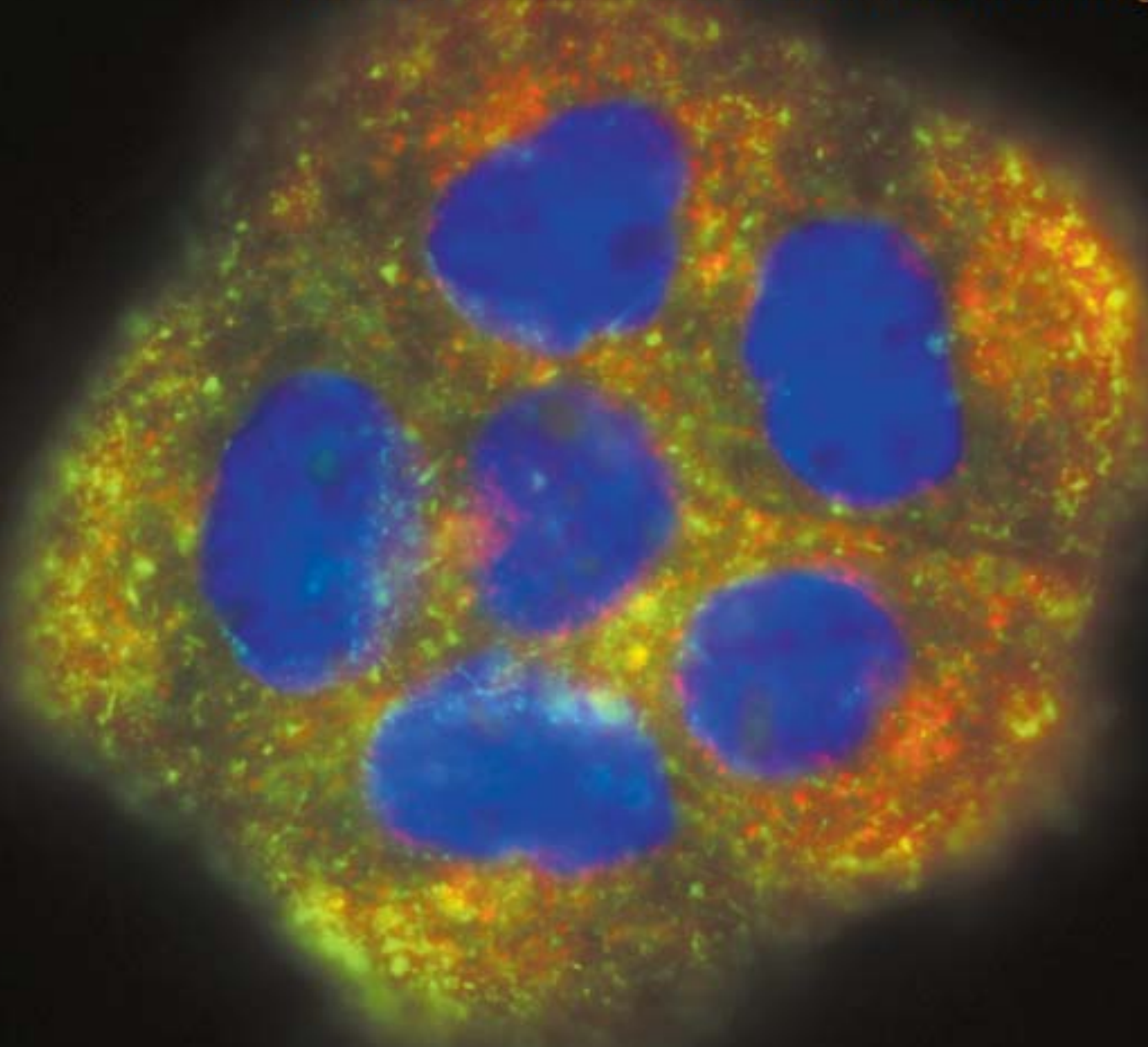


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A Simplified and Rapid Method for Extraction Of DNA from Baculovirus Occlusion Bodies

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Baculoviruses have found many uses in the field of biology, including as a control strategy for major insect pests such as the gypsy moth, the corn earworm, and the velvetbean caterpillar, for high level expression of recombinant proteins in insect cells, and, more recently, as gene delivery vehicles into mammalian cells. Some of these uses entail the need for isolation of baculoviral DNA for molecular biology studies. Currently, there are three ways in which baculoviral DNA can be obtained: 1) from the infection of insect larvae or cell cultures and the recovery of occlusion bodies (OB) containing viral particles, from which the DNA is then extracted; 2) from the recovery of extracellular virus (ECV) or budded virus (BV), usually from infected cell cultures; or 3) from total intracellular DNA harvested from infected cell cultures early in the infection cycle.¹⁻⁷

The advantages of using OB as a source of baculoviral DNA are based on the stability of OB in long-term storage at 4° C or lower, and the ready microscopic ease of quantifying such preparations. Disadvantages consist of the large numbers of OB that must be produced in larvae or cell culture; the lengthy recovery and purification pro-

cedures, especially from larvae; and the alkali liberation of viral particles from OB. We have developed a modified method that uses relatively low numbers of OB produced either *in vitro* or *in vivo*, as well as a simpler modified method that uses a small volume sample for extracting DNA from occlusion-derived virus (ODV) released from OB. These

methods are reported in Table 1.

A plaque-purified isolate from the widely used baculovirus *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV) was used. It was propagated in *Trichoplusia ni* (TN-CL1) cells grown in Ex-Cell 401 (JRH Biosciences, Lenexa, KS) containing 10% inactivated fetal bovine serum (FBS) following inocula-

Table 1. Methods.

1. 2×10^7 OB in 0.2 ml of ultrapure (UP) water (Culligan, Columbia, MO) were placed in a 1.5 ml microcentrifuge tube.
2. The sample was heat treated at 65° C for 15 minutes and cooled to room temperature (RT).
3. 0.2 ml of alkali solution (1 M NaCl + 1 M Na₂CO₃) was added to the contents of the tube and it was placed in a water bath at 37° C for 30 minutes then brought to RT.
4. 75 µl of 0.3 M HCl was added slowly to the tube to lower the pH to approximately 8.0.
5. 158 µl of 2% SDS were added and mixed well.
6. 9.5 µl of proteinase K (Sigma, St. Louis, MO) stock (10 mg/ml) were added to give a final concentration of 200 µg/ml and placed in a water bath at 37° C for 2 h and cooled to RT.
7. 3 µl of RNase A (4 mg/ml, Gentra Systems, Inc., Minneapolis, MN) were added to the sample and it was re-incubated in the water bath for 2 h and then brought to RT.
8. 150 µl of protein precipitation solution (Gentra Systems) were added to the sample and vortexed vigorously for 20 seconds.
9. The sample was placed in a table-top centrifuge (National Labnet Co., Woodbridge, NJ) and centrifuged at 12,000 x g for 3 minutes.
10. The supernatant containing DNA was poured into another 1.5 ml microcentrifuge tube containing 500 µl of isopropanol (Sigma) and the contents mixed by inverting 50 times.
11. The sample was centrifuged at 12,000 x g for 3 minutes and the supernatant carefully poured off, leaving the pelleted DNA.
12. The pellet was washed with 100 µl of 70% ethanol by inverting several times.
13. The DNA sample was centrifuged at 12,000 x g for 3 minutes and the ethanol carefully poured off.
14. The tube was drained on a Kimwipe EX-L (Kimberly-Clark Corp., Neenah, WI), inverted, and allowed to dry for 15 minutes.
15. 100 µl of UP water was added to the pellet and stored overnight at 4° C.
16. The DNA was measured in a spectrophotometer.

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tion at a multiplicity of infection (MOI) of 0.5. Occlusion bodies were released from cells by sonication, followed by centrifugation to pellet OB as previously described. Occlusion bodies from infected TN-CL1 cells and *T. ni* larvae were purified on sucrose gradients and DNA was extracted according to the following modified protocol (Puregene Kit, Gentra Systems, Minneapolis, MN).⁸⁻¹⁰

We were able to recover 59 µg/ml of AcMNPV DNA (ratio 260/280 = 1.7) from 2×10^7 sucrose-gradient purified OB from infected TN-CL1 cells.

Baculovirus DNA was recoverable from AcMNPV OB on a reproducible basis using the protocol outlined here. We were also able to extract DNA (57 µg/ml) from the OB of another baculovirus, namely the single-enveloped nucleopolyhedrovirus of *Helicoverpa zea* (HzSNPV) produced in *H. zea* larvae.¹⁰

TN-CL1 cells (5×10^4) were successfully transfected with 236 ng of the recovered AcMNPV DNA employing the transfection procedure of Clontech (Palo Alto, CA) with minor modifications. The latter consisted of using

serum-free medium (Ex-Cell™ 401) to wash the cells prior to transfection and during transfection, and the use of LT2 (Pan Vera Corp., Madison, WI) as the transfecting reagent. Several days following transfection, OB production in cells was readily observed, and titration of the ECV at seven days post-transfection resulted in a titer of 3×10^4 TCID₅₀/ml from the transfection of 5×10^4 TN-CL1 cells. The results of this transfection study demonstrated that the isolated AcMNPV DNA was biologically active when transfected into TN-CL1 cells.

AcMNPV DNA extracted from OB produced in *T. ni* larvae using this protocol was compared with DNA from AcMNPV OB produced in the TN-CL1 cell line by restriction endonuclease (REN) according to a previously described method. The results are depicted in Figure 1. The profiles following digestion with *Hind*III show no difference between the patterns of DNA extracted from either source, and both are identical to the AcMNPV DNA REN pattern, the DNA of which was prepared by the phenol extraction method. We were able to generate 7×10^7 OB/ml (in 5 ml of UP-water) from one T-75 cm² flask containing 2×10^6 TN-CL1 cells in 10 ml of Ex-Cell 401 containing 10% FBS. This translates into 175 OB per cell, and indicates that TN-CL1 cells are very efficient in their production of OB.¹⁰⁻¹¹

An earlier report describes the use of the chaotropic agent guanidinium isothiocyanate (GIT) for the extraction of DNA from ECV and OB preparations. A direct comparison of efficiency of recovery of DNA between the two methods cannot be made because in the former report, the sample used was based on a weight basis and not the number of OB, as was employed here. In comparison with the GIT procedure, our method is simpler, faster, requires fewer lengthy centrifugation steps, and avoids the use of hazardous materials such as acetone and phenol:chloroform:isoamyl in the preparation and extraction procedures. In addition, low numbers of OB can be used as compared with conventional methods, and DNA extraction can be carried out in a 1.5 ml microcentrifuge tube, resulting in sufficient DNA for

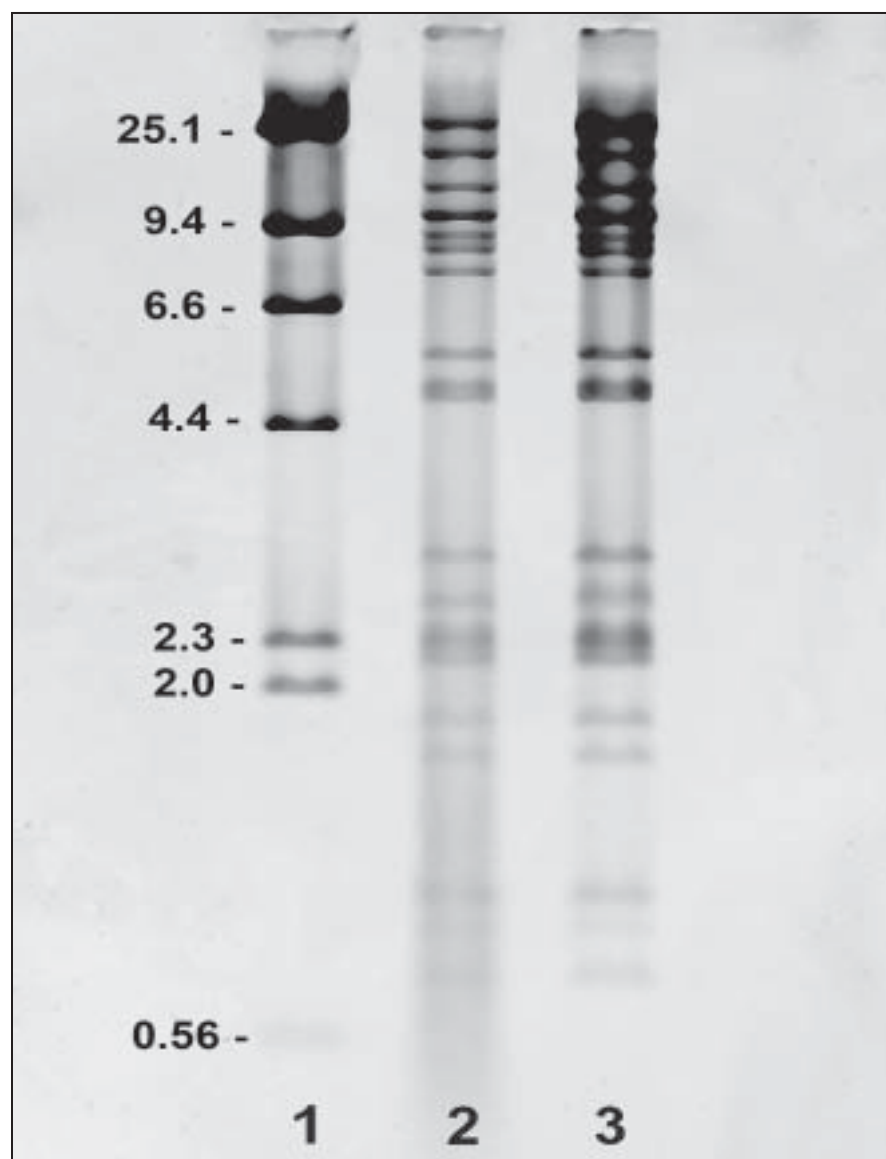


Figure 1. Restriction endonuclease (REN) profiles of AcMNPV DNA, with lambda DNA as a molecular marker, following digestion with *Hind*III. Lane 1. Lambda DNA. Lane 2. AcMNPV DNA profile of DNA from AcMNPV produced in TN-CL1 cells and extracted by the method described in this report. Lane 3. AcMNPV DNA profile of DNA extracted by the same method from OB produced in *T. ni* larvae. Profiles in Lanes 2 and 3 are identical.

REN, transfection, or other molecular biology assays.¹²

Extraction of DNA from ECV using the abbreviated method described here was also successful. ECV was recovered from TN-CL1 infected cells by low-speed centrifugation at 3,000 RPM for 15 minutes in a tabletop centrifuge (Model TJ-6, Beckman Instruments, Palo Alto, CA) to remove any suspended cells. This was followed by ultracentrifugation of the ECV-containing supernatant fluid from the low-speed spin at 20,000 RPM in an SW 28.1 rotor for two hours (Beckman Optima LE-80K). The viral pellets were resuspended in 0.4 ml Tris-EDTA buffer pH 7.6 overnight, and DNA was extracted as described in the protocol.

The conventional methods for releasing ODV from OB and subsequent extraction of viral DNA are rather cumbersome and lengthy, requiring many reagents. The protocol for DNA extraction from OB described here is relatively simple, is rapid (once the OB have been produced), eliminates many purification steps as well as the use of hazardous reagents (such as phenol/chloroform), and produces acceptable yields of relatively pure DNA (260/280 = 1.7). Furthermore, we have found that the sucrose-gradient purification step for OB produced in cell culture can be omitted, thus further reducing the total preparation time. This is probably a result of cleaner *in vitro* system as compared with OB produced in larvae. Another advantage of using cell culture is that ECV can be recovered from virus-infected cells and DNA can be extracted, thus avoiding the alkali treatment of OB for the release of ODV.¹²⁻¹⁵

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