bp segment, are recommended for monitoring DNA contamination in enzyme and buffer preparations. The primer sequences are as follows: 515F, GTGCCAGCMGCCGCGG; 1492R, GGTTACCTTGTTACGACTT; 907R, CCGTCAATTCMTTTRAGTTT; where M=A or C, R=A or G.

REFERENCES

1. **Brosius, J., M.L. Palmer, P.J. Kennedy and H.F. Noller.** 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc. Nat. Acad. Sci. USA *75*:4801-4805.
2. **Kitchin, P.A., Z. Szotyori, C. Fromholc and N. Almond.** 1990. Avoidance of false positives. Nature *344*:201.
3. **Kwok, S. and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature *339*:237.
4. **Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science *239*:487-491.
5. **Sarkar, G. and S.S. Sommer.** 1990. Shedding light on PCR contamination. Nature *343*:27.
6. **Williams, J.F.,** 1989. Optimization strategies for the polymerase chain reaction. BioTechniques *7*:762-769.

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Rapid Identification of Recombinant Baculoviruses Using PCR

The baculovirus system is widely used for large-scale production of eukaryotic proteins. Foreign genes are brought under the control of a strong viral promoter, in general the promoter of the polyhedrin gene. Insect cells infected with recombinant virus produce the foreign protein at high levels (3).

Special plasmid vectors for recombination with the wild-type virus have been designed (1). The gene of interest is cloned into these vectors and subsequently co-transfected with the wild-type virus DNA into insect cells. In some of these transfected cells, the viral polyhedrin gene will be replaced by the foreign gene because of homologous recombination, thus rendering the virus unable to form occlusion bodies. The supernatants of such cell cultures are screened for the presence of recombinant viruses by performing a plaque assay. By visual screening for the occlusion-negative phenotype, recombinant plaques are identified and purified by additional rounds of plaque assay (5). Visual screening for the recombinant phenotype is a critical step, and often a high percentage of the plaques picked in the first round turn out not to contain the recombinant DNA.

The development of the AcMNPV-β-gal expression vectors provides a tool to simplify the screening for recombinant virus, but it often leads to a large number of false positives. When using β-gal-expressing virus instead of the normal wild-type virus in a co-transfection experiment, resulting recombinants should form white plaques in contrast to the blue ones. However, the diffusion of the β-galactosidase can obscure other recombinant plaques, while on the other hand the high mutation frequency of the large β-gal gene may lead to false white plaques. Even with this simplified screening method, dot-blot hybridization analyses have been required so far to exclude false positives before doing subsequent cycles of plaque purification.

The PCR technique (4) provides a very sensitive, nonradioactive tool to

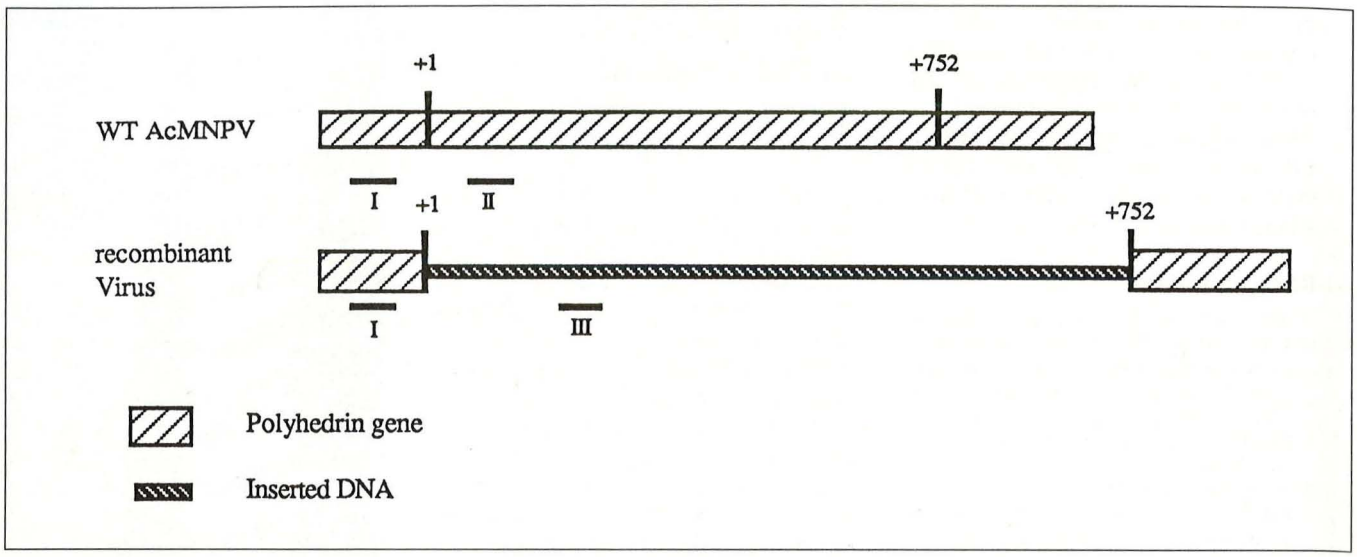

Figure 1. Schematic drawing of the positions of the three primers (I, II, III) used in our experiment.

check quickly a large number of isolates for the presence of the recombinant DNA.

Because the titer of extracellular virus in a primary plaque is too low to be used directly in a PCR, the virus has to be amplified by infection of Sf-9 cells, as for a dot-blot experiment. Multiple-well culture plates are seeded with Sf-9 cells to an appropriate density (9-cm^2 vessels with 6×10^6 cells per well in a total volume of 500 µl). Suspected positive plaques are picked and directly transferred to the wells. After incubation for 48 h, 10 µl of the infectious supernatant are used for a PCR. The extracellular viruses are lysed by adding 90 µl of detergent buffer A (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.1 mg/ml gelatin, 0.45% Nonidet P-40® (NP40), 0.45% Tween 20) containing 6 µg of proteinase K and incubated at 60°C for 1 h. After this incubation, the proteinase K is inactivated at 95°C for 10 min. Twenty-five microliters of this lysate are used in a 50-µl PCR. The reaction volume is scaled up to 50 µl with the nucleotides (0.2 mM each final), the primers (50 pM each final), the *Taq* DNA polymerase (Pharmacia LKB Biotechnology, Freiburg, FRG, 2.5 U) and 2.5 µl detergent buffer B (10× detergent buffer A plus 25 mM $MgCl_2$). Forty reaction cycles are performed in a PCR machine with a Peltier Element as a thermoelectric heat pump (6) (annealing 58°C; elongation 72°C; denaturing 92°C; 1 min each).

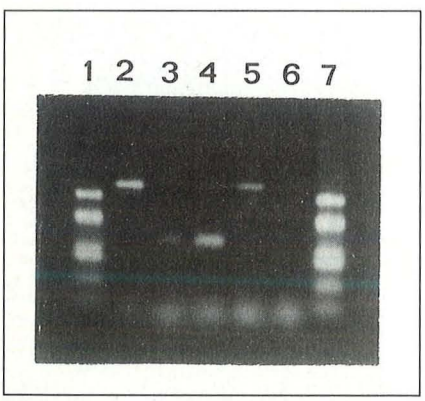

Figure 2. Analysis of the PCR products on a 1.2% agarose gel. Lanes 1 and 7: pUC18/*Hae*III marker fragments; lane 2: control with 20-ng insert-containing vector DNA (pAcYM1mrk); lane 3: control with 20-ng wild-type virus DNA (AcMNPV); lane 4: PCR product after first plaque purification showing wild-type and recombinant band; lane 5: PCR product after second plaque purification showing only recombinant band; lane 6: template-free control reaction.

In our experiments two primers with sequences of the polyhedrin gene (2) were used: (I) $_{-201}$5′TATACTATTGTC TGCGAGCAGTTG3′$_{-177}$; (II) $_{+118}$5′G TTCGGCGAAGTGCTTCTTGC3′$_{+98}$. The third primer was derived from sequences 460 bp downstream of the start codon of the gene to be expressed (Figure 1).

By using this set of three primers amplifying the wild-type virus DNA as well as the recombinant virus DNA, the isolates can be checked for the presence of recombinant virus and for the purity of the isolate in one step (Figure 2).

REFERENCES

1. **Luckow, V. and M.D. Summers.** 1988. Trends in the dvelopment of baculovirus expresion vectors. Bio/Technology *6*:47-55.
2. **Matsuura, J., R.D. Possee, H.A. Overton and D.H.L. Bishop.** 1987. Baculovirus expression vectors: the requirements for high level expression of proteins, including glycoproteins. J. Gen. Virol. *68*:1233-1250.
3. **Miller, L.K.** 1988. Baculoviruses as gene expression vectors. Annu. Rev. Microbiol. *42*: 177-199.
4. **Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Ehrlich and N. Arnheim.** 1985. Enzymatic amplification of β-globin genomic sequnces and restriction site analysis for diagnosis of sickle cell anemia. Science *230*:1350-1354.
5. **Summer, M.D. and G.E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. Texas Agricultural Experiment Station Bull. No. 1555, Texas A&M University.
6. **Wittbrodt, J. and W. Erhardt.** 1989. An inexpensive and versatile computer-controlled PCR machine using a Peltier Element as a thermoelectric heat pump. Trends Genet. *5*: 202-203.

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