Identification of an Unknown Plasmid

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Recent developments in the field of molecular biology and its far-reaching applications have brought biotechnology into the lives and minds of many students. A thorough familiarity with, and understanding of, such topics as recombinant DNA, cloning, and chromosome mapping will be essential for students to adequately tackle the consequent moral and financial concerns brought about by such technology. This laboratory activity is designed to introduce students to some of the strategies and tools employed by molecular biologists using a more problem-solving approach than many other such activities.

Students are presented with an unknown sample of DNA (plasmid, bacteriophage lambda) and are asked to determine its identity. They do this by performing a restriction analysis of the DNA, electrophoretically separating the resultant fragments, staining, and visualizing them. A standard curve is prepared, using lambda H3 marker DNA, which the students then use to determine the fragment sizes of their digested samples. By comparing the restriction profile of their unknown to those of "known" (i.e., determined by the students using actual restriction maps) DNA samples, students can positively determine the identity of their sample.

The restriction maps of two plasmids, pAMP and pKAN, commercially available from Cabisco Technologies (Carolina Biological Supply Company), are shown in Figure 1 (on the next page), along with a stylized representation of their restriction profiles when digested with the endonucleases Bam HI and Hind III. A variety of activities can be prepared through modifications of this approach. I would be interested in hearing about any such modifications.

Bennethum, T. M., J. A. Chiscon, M. O. Chiscon, C. R. Carlin, R. H. Shippee, and J. W. Vanable. 1993. Identification of an unknown plasmid. Pages 129–138, *in* Laboratory manual for *Biology 225-6: The Basic Concepts*. Department of Biological Sciences, Purdue University.

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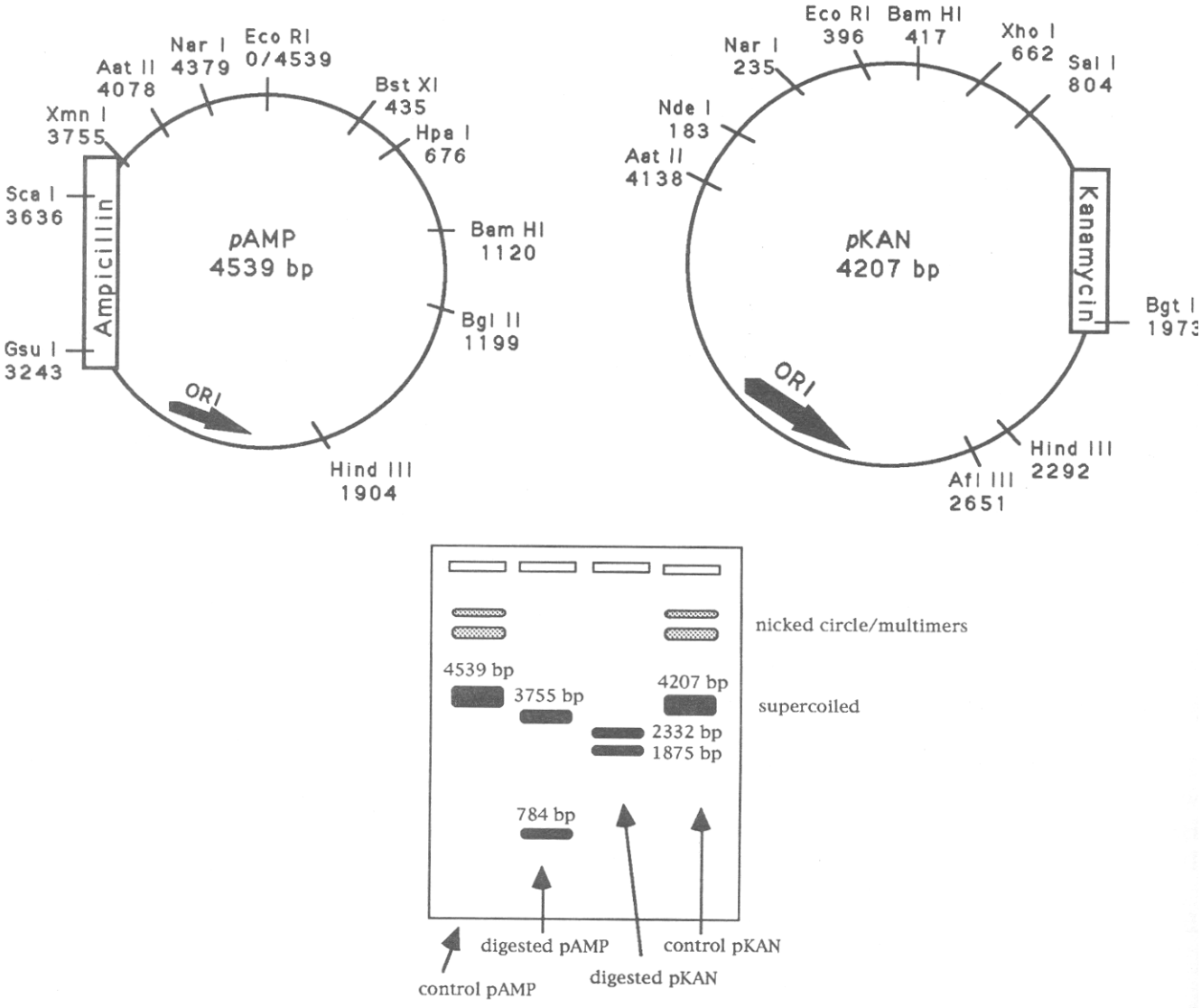

Figure 1. Restriction maps of two plasmids (pAMP and pKAN) and a stylized representation of their restriction profiles when digested with the endonucleases Bam HI and Hind III.