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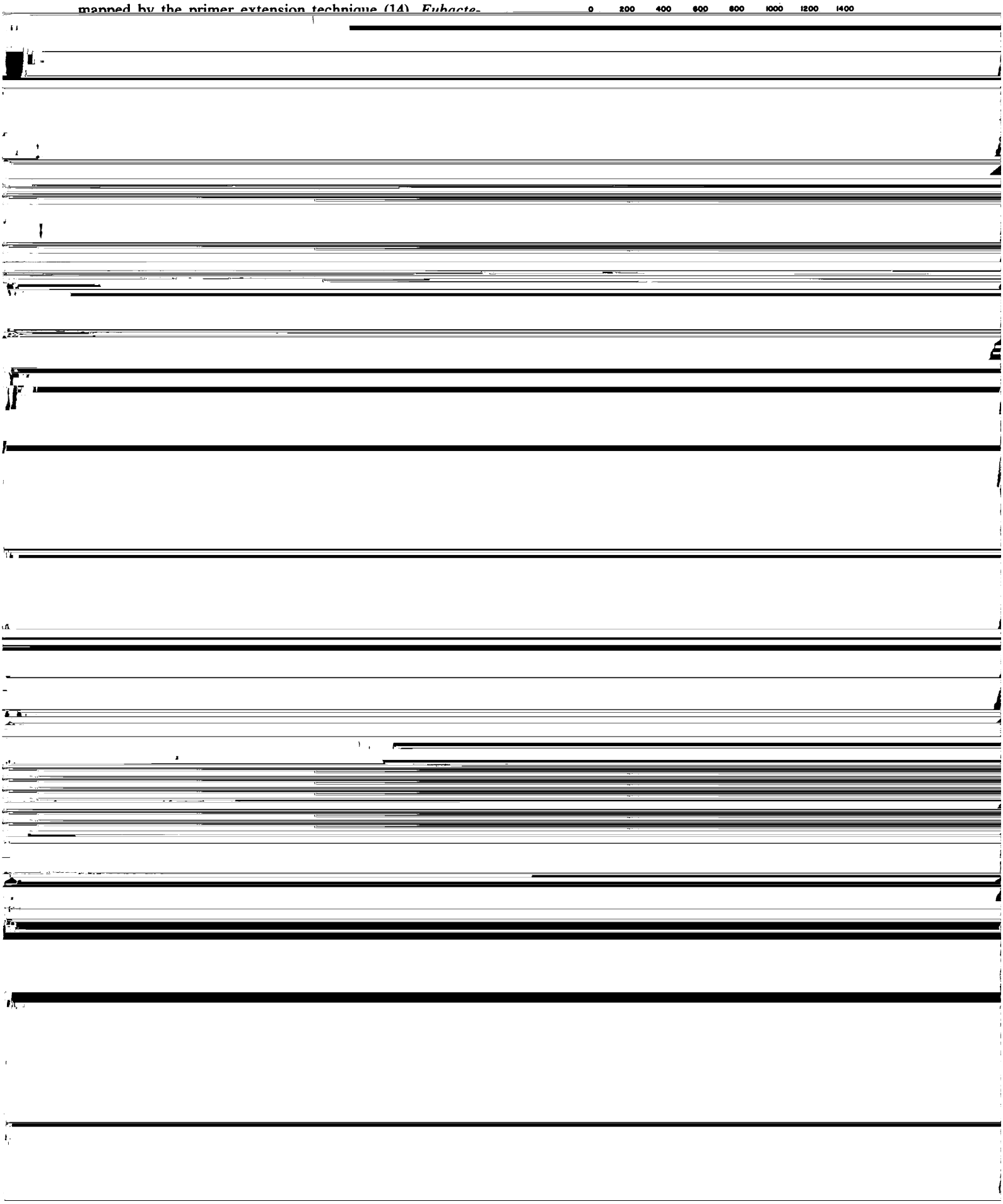
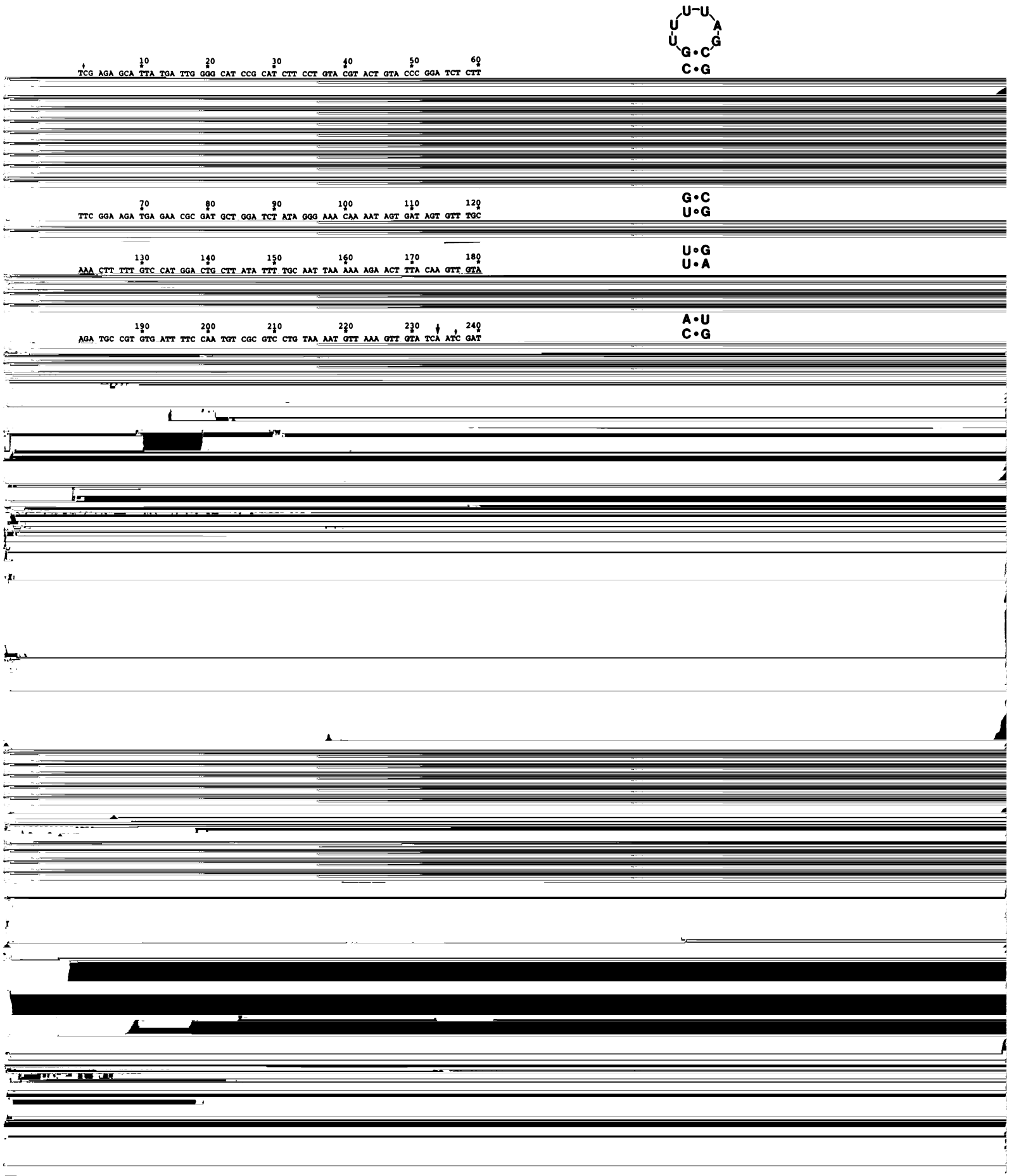
0021-9193/88/052070-08$02.00/0

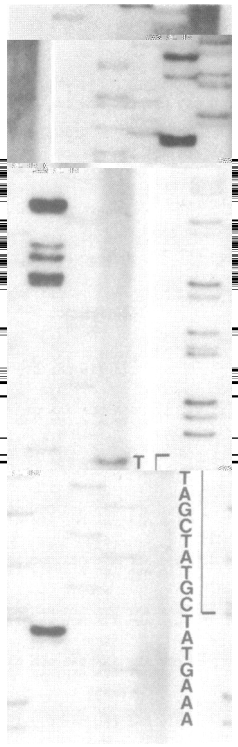
and International Biotechnologies, Inc. S1 nuclease, di-

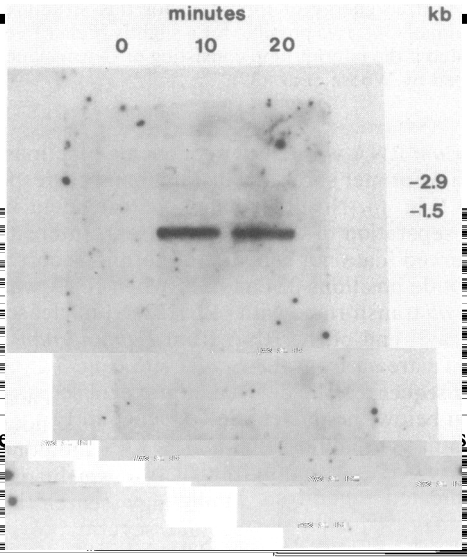


mapped by the primer extension technique (14). *Eubacte-*


U-U
U
A
U
G
G•C
C•G
10 20 30 40 50 60
TCG AGA GCA TTA TGA TTG GGG CAT CCG CAT CTT CCT GTA CGT ACT GTA CCC GGA TCT CTT
70 80 90 100 110 120
TTC GGA AGA TGA GAA CGC GAT GCT GGA TCT ATA GGG AAA CAA AAT AGT GAT AGT GTT TGC
G•C
U•G
130 140 150 160 170 180
AAA CTT TTT GTC CAT GGA CTG CTT ATA TTT TGC AAT TAA AAA AGA ACT TTA CAA GTT GTA
U•G
U•A
190 200 210 220 230 240
AGA TGC CGT GTG ATT TTC CAA TGT CGC GTC CTG TAA AAT GTT AAA GTT GTA TCA ATC GAT
A•U
C•G

*Eco*RI fragment, and the other was an 1,154-bp *Taq*I fragment. The two inserts contained a common 349-bp *Taq*I-*Eco*RI fragment. *E. coli* strains containing the plasmid with the 1,150-bp insert produced a 27,000-molecular-weight polypeptide which was immunologically cross-reactive with P-27 purified from *Eubacterium* sp. strain VPI 12708. In addition, preliminary nucleic acid sequence data analysis





sequence of P-27

(5). Together, the two inserts constitute a continuous 3-kilobase stretch of *Eubacterium* DNA containing the entire coding region for P-27, as well as approximately 2 kilobases of upstream DNA and 329 bp downstream from the P-27 stop

The genus *Eubacterium* has been classified, on the basis of 16S rRNA fingerprint analyses, with the clostridia in the subdivision of "gram-positive eubacteria with low G+C content" of the gram-positive group (40). Because of the

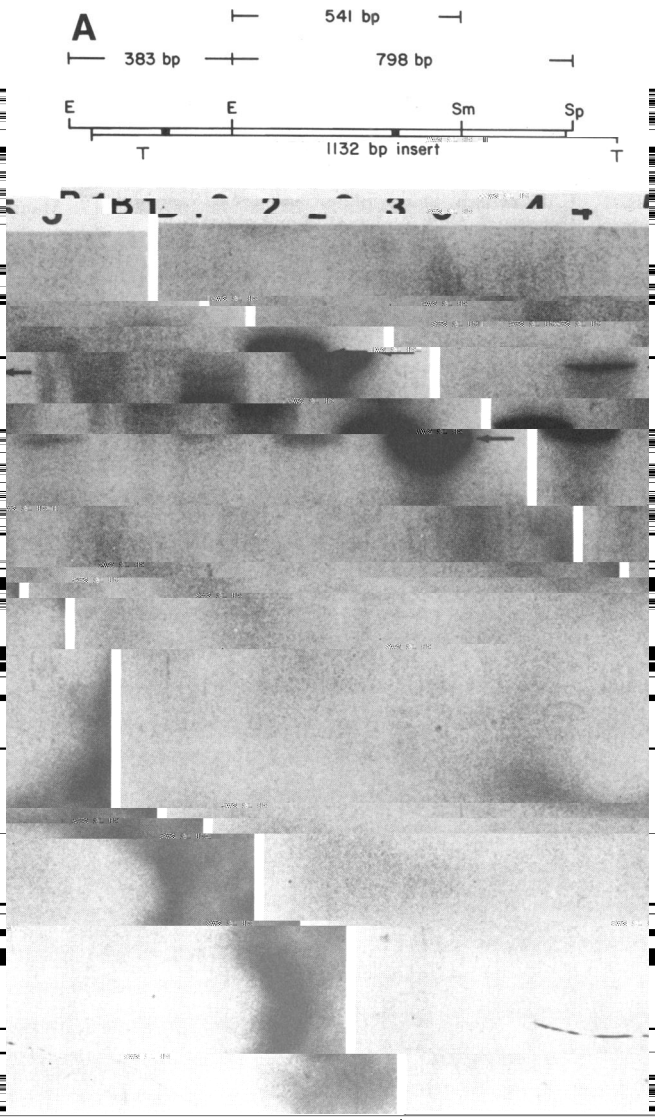


FIG. 7. S1 nuclease mapping of 3′ end of RNA. Total RNA from either cholic acid-induced or uninduced *Eubacterium* sp. strain VPI 12708 was hybridized to a 798-bp *Eco*RI (E)-*Sph*I (Sp) fragment containing 786 bp of the *Eubacterium* sequence plus 12 bp of the pUC19 multiple cloning site (A). The hybrid was digested with S1 nuclease (see Materials and Methods), and the product was run on 798- bp piece and a 383-bp *Eco*RI-*Eco*RI fragment (A). After electrophoresis, the protected species were denatured in dilute NaOH, electroblotted onto a Nylon membrane, and hybridized to 5′-end-labeled ([^{32}P]T4 kinase reaction) synthetic oligonucleotides (dark

mechanism of 7-dehydroxylation (Fig. 1). In the *Eubacte-*

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35. Wallace, R. B., M. J. Johnson, S. V. Suggs, K. Miyoshi, R. E. H. Mosbach, and P. B. Hylemon. 1983. Regulation of bile acid

Bhatt, and K. Itakura. 1981. A set of synthetic oligodeoxyribo- 7-dehydroxylase activity by NAD^+ and NADH in cell extracts