

- [54] NOVEL CLONING VEHICLES FOR POLYPEPTIDE EXPRESSION IN MICROBIAL HOSTS
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[\*] Notice: The portion of the term of this patent subsequent to Feb. 17, 2004 has been disclaimed.

Primary Examiner—James Martinell  
 Attorney, Agent, or Firm—Gottlieb, Rackman & Reisman

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- [22] Filed: May 14, 1982
- [51] Int. Cl.<sup>4</sup> ..... C12P 21/00; C12N 1/20; C12N 15/00
- [52] U.S. Cl. .... 435/68; 435/252.3; 435/252.33; 435/320; 435/172.3; 935/38; 935/39; 935/43; 935/44; 935/66; 935/72; 935/73
- [58] Field of Search ..... 435/68, 70, 91, 172, 435/253, 317, 172.3, 172.1, 320, 252.3, 252.31-252.35, 66, 72, 73; 935/38, 39, 41, 43

[57] ABSTRACT

Methods and compositions are provided for regulated expression of polypeptides in transformed bacterial hosts. A novel class of plasmid cloning vehicles includes a DNA sequence coding for the desired polypeptide (or an insertion site therefor) linked for transcriptional expression in reading phase with one or more functional fragments derived from an outer membrane protein gene of a Gram-negative bacterium. The plasmids also include an inducible promoter sequence positioned in the proper orientation for transcriptional expression of the desired polypeptide, as well as a separate DNA sequence coding for a repressor molecule which can interact with the inducible promoter to prevent transcription therefrom. Expression of the desired polypeptide is under the control of both the constitutive promoter and the inducible promoter, although transcription from either promoter is normally blocked by the repressor molecule. However, the repressor can be selectively inactivated by means of an inducer molecule to permit transcriptional expression of the desired polypeptide from both promoters. The methods utilize such plasmids to introduce genetic capability into microorganisms for the production of proteins, such as medically or commercially useful hormones, enzymes, immunogenic proteins, or intermediates therefor, but only in the presence of an appropriate inducer.

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40 Claims, 30 Drawing Sheets

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FIG. 1A

TGGCTCTGCAGAGCA  
ACGGAGGCTCTCGT

-350 . . . . .  
 -300 . . . . .  
 ATCTGGCACACAAAGGCTGAGTTATGGTTCTGTGTCACCTGGTACCGAGCGGCACTACTAACACCGCATCTGTTACAGTCTGTGTAATATTGCTT  
 TAGACCGTGTGTTCCCACTGCAACATCAATACCAAGACCACCGTGACCATGGCTGCGCCGTGATGATTTGGCGTAGACAAGTGCAGGACATTATAACGAA

-250 . . . . .  
 -200 . . . . .  
 TTGTGAATTAATTTGTATATCGGGCTTTTTTTTAAATCGATACCAAGCAATAAAAAATCAATCGGATTTCACTATATAATCTCAGCTTTATCTA  
 AACACTTAATTAACATATAGCCGGAAAAAATAAATAGCTATTGGTCTTCGTTATTTTTAGTTAGCCTAAAGTGATATATTAGAGTGAATAGAT

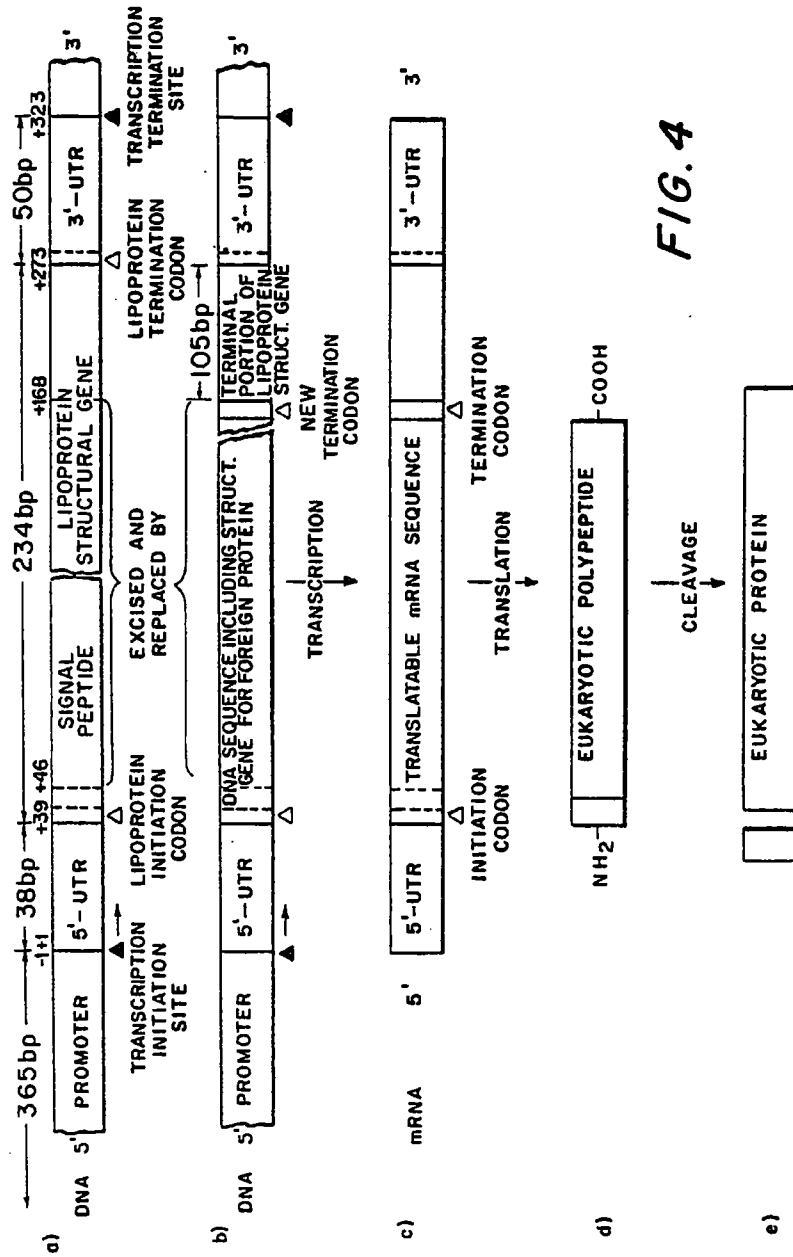
-150 . . . . .  
 -100 . . . . .  
 AGATGAATCCGATGGAGCATCCTGTTTTCTCTCAATTTTTTAAACCCAGCGTTCGATGCTTTTGGCGAAGCATCAAAAAAATAGTGCCTTC  
 TCTACTTAGGCTACCTTCGTAGBACAAAAGAGAGTTAAAAAATAGATTTTGGGTCCGAAGCTACGAAGAACTGGCTTCTAGTTTTTATTCACGGGAA

-50 . . . . .  
 -1+1 . . . . .  
 +50 . . . . .  
 CCATCAAAAAAATTTCTCAACATAAAAAACTTTGTGTAATCTGTAAAGCTACATGGAGATTAACTCAATCTAGAGGGTATTAATGAAGTACT  
 GGTAGTTTTTTAAGAGTTGTATTTTTGAACACATTATGAACATTCGGATGTACCTCTCTAATTTGAGTTAGATCTCCCAATTTACTTTCGATGA  
 mRNA Start  
 MetLysAlaThr









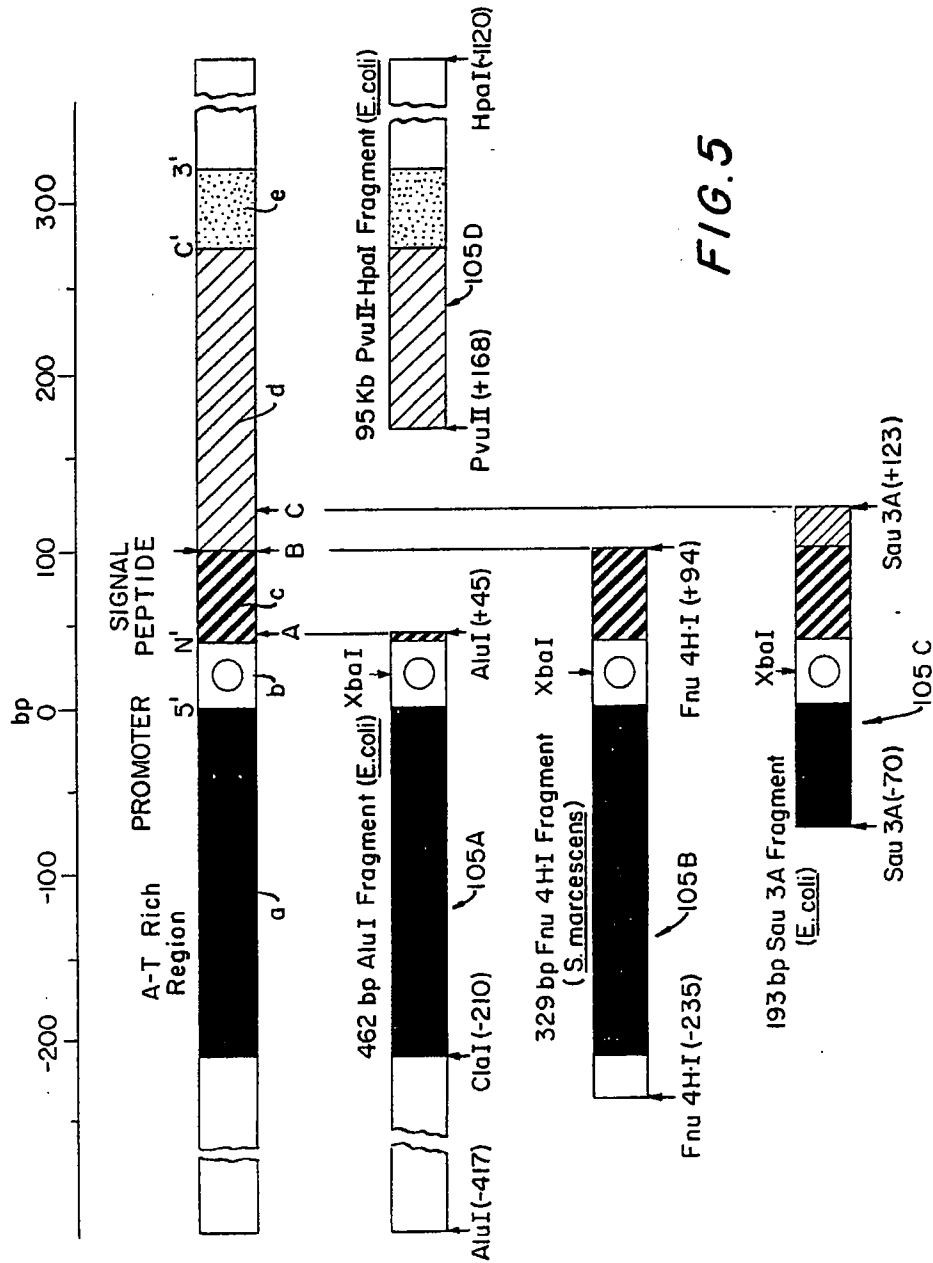


FIG. 5

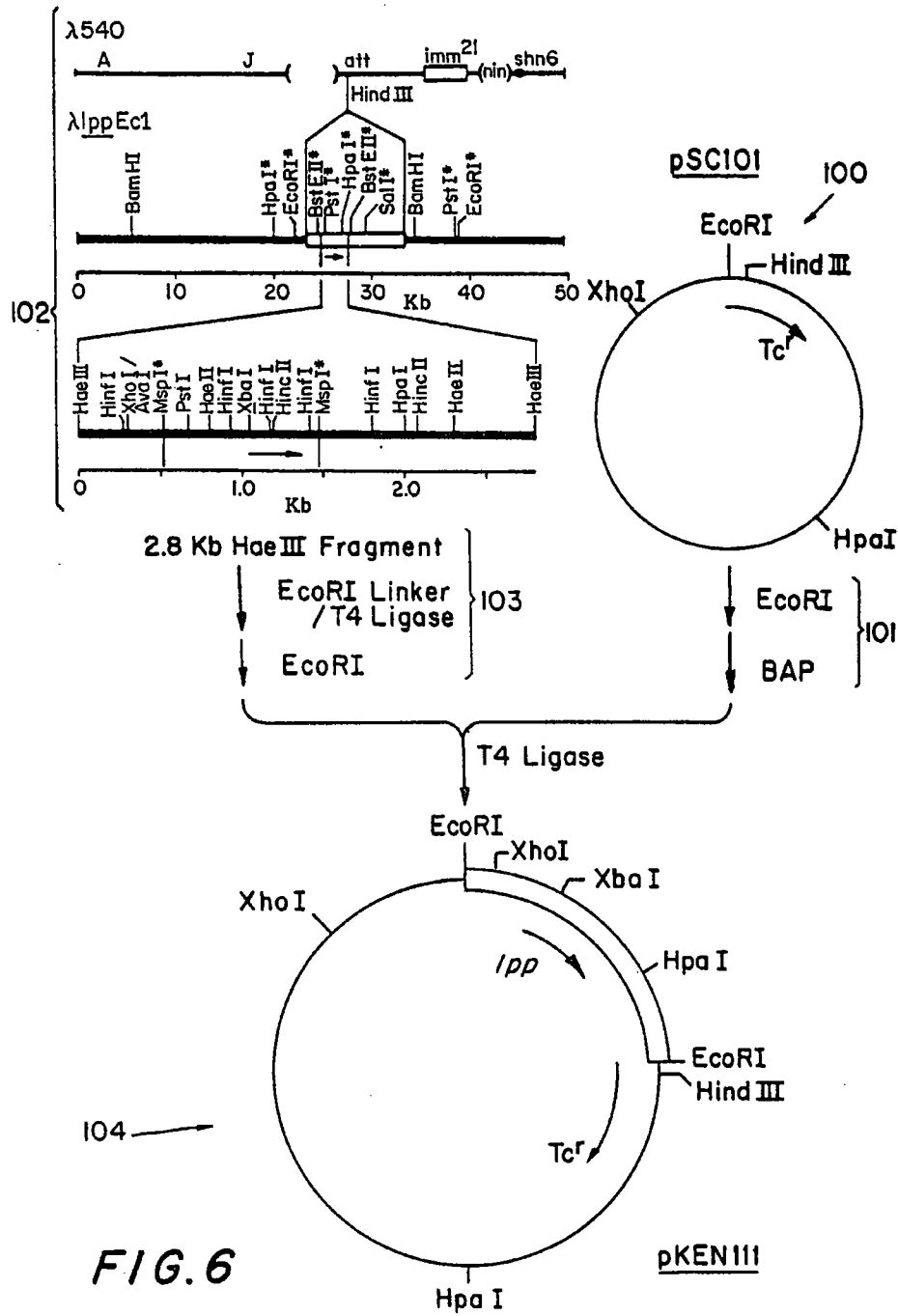
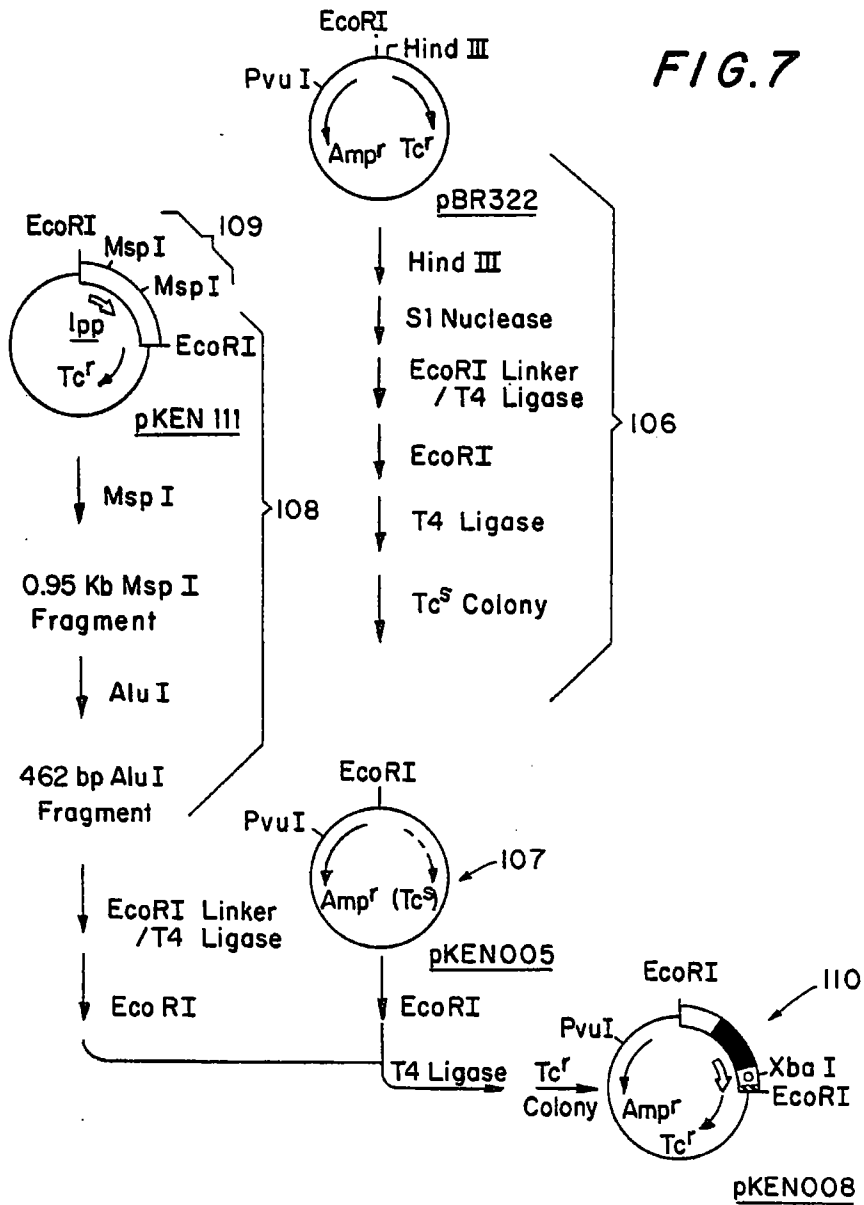


FIG. 6

FIG. 7



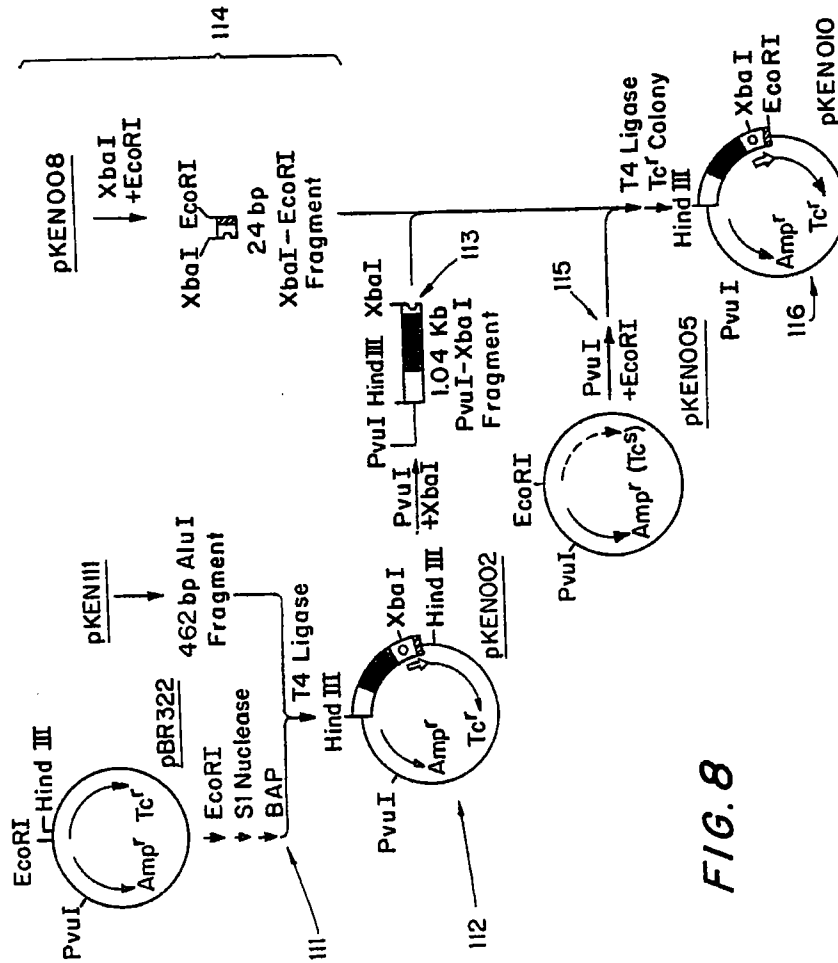
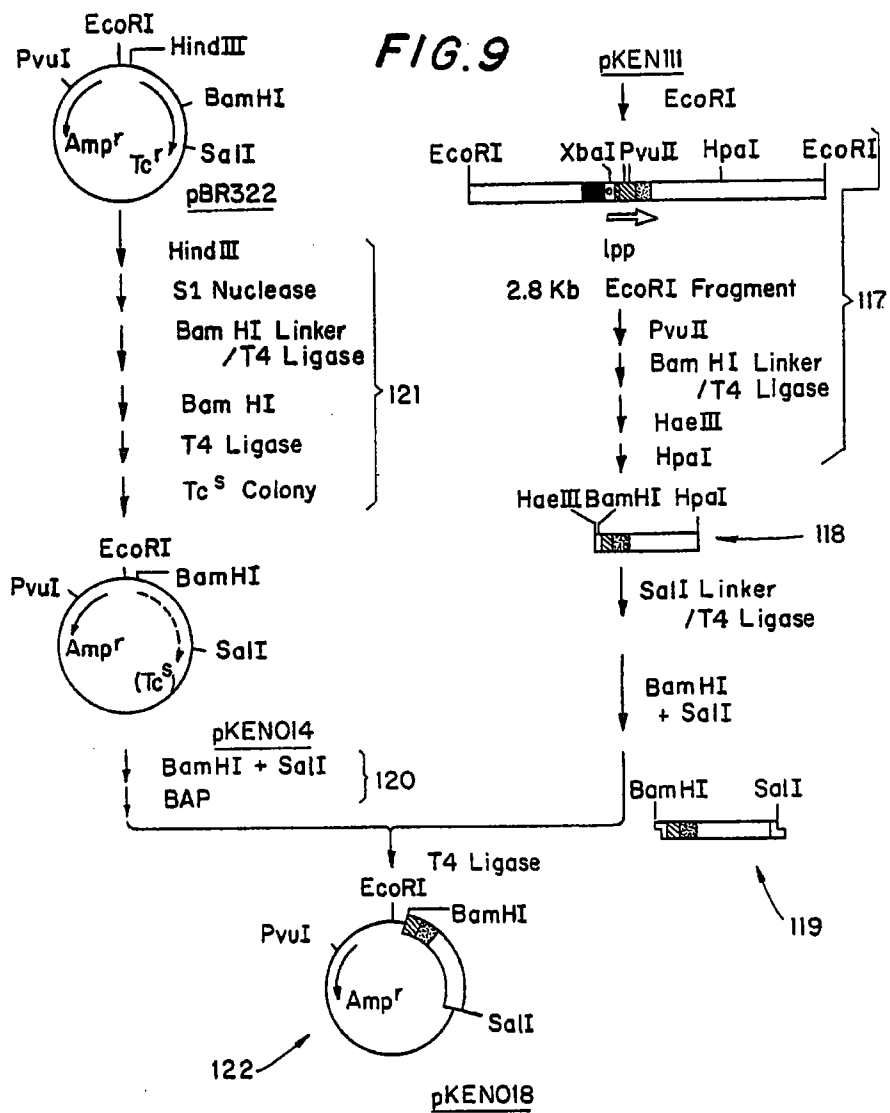


FIG. 8



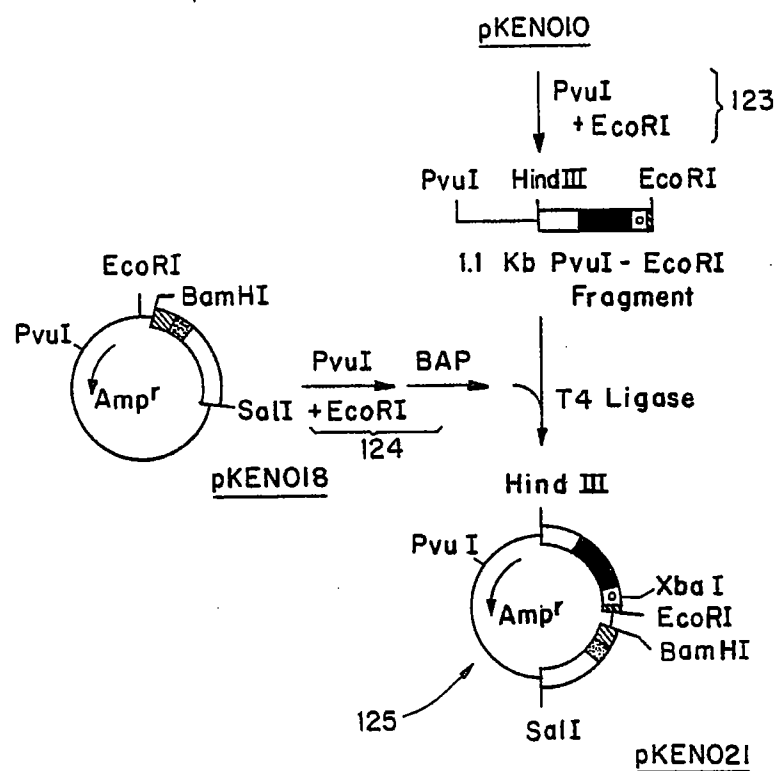
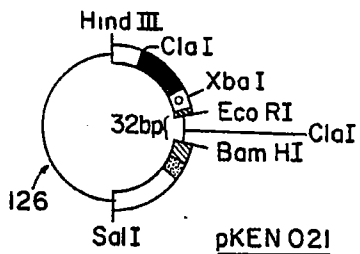
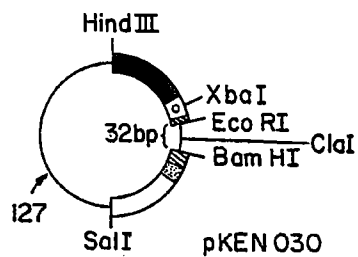


FIG. 10

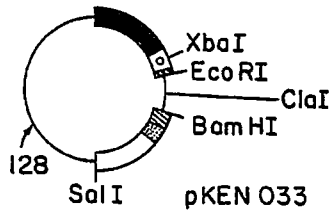
FIG. II



- ↓ ClaI (partial)
- ↓ S1 Nuclease
- ↓ Hind III Linker / T4 Ligase
- ↓ Hind III
- ↓ T4 Ligase



- ↓ Hind III
- ↓ S1 Nuclease
- ↓ T4 Ligase



- ↓ EcoRI + Bam HI
  - ↓ T4 DNA Polymerase (4 dNTPs)
  - ↓ Hind III Linker (p[CCAAGCTTGG]) / T4 Ligase
  - ↓ Hind III
  - ↓ T4 Ligase
- } 129

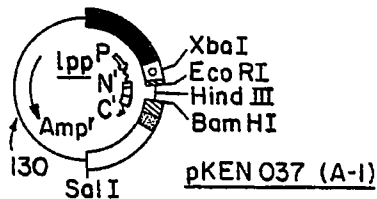
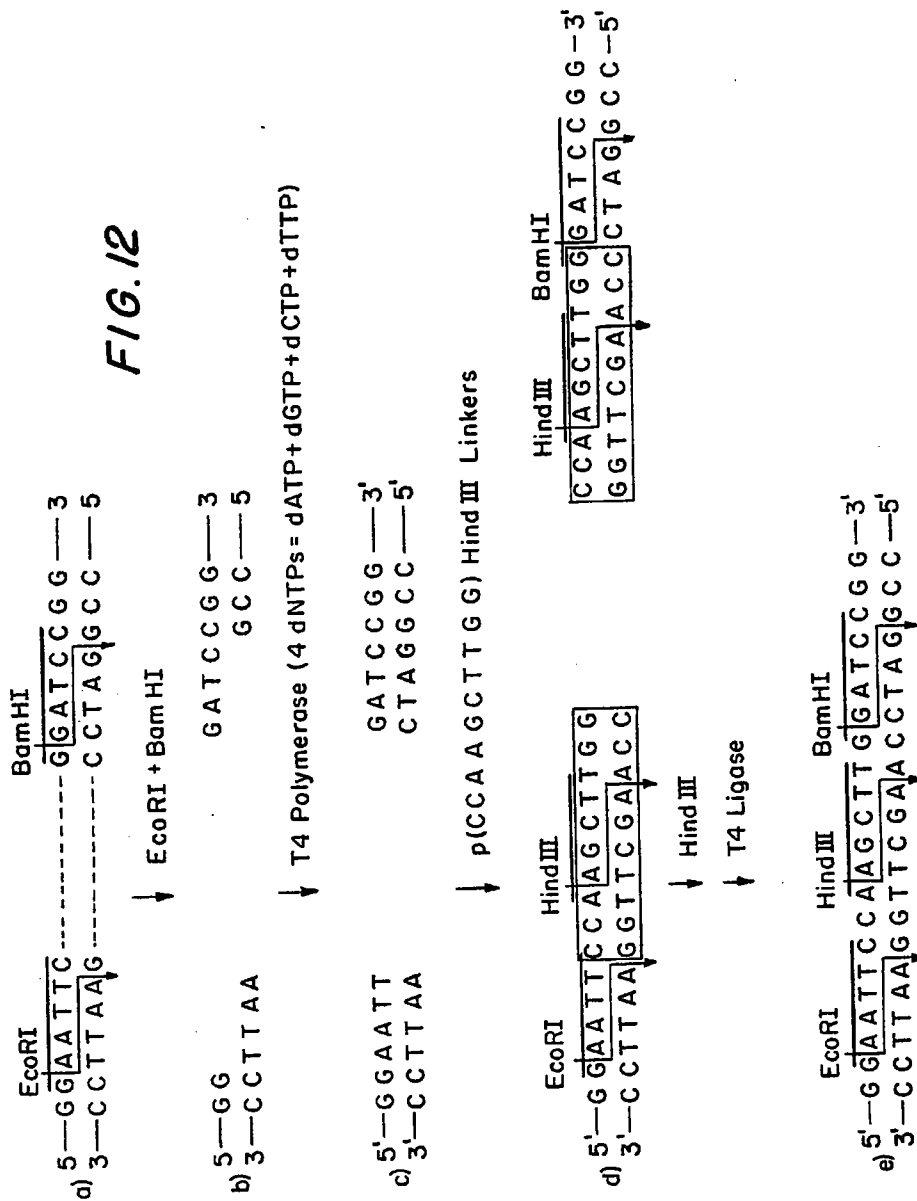


FIG. 12



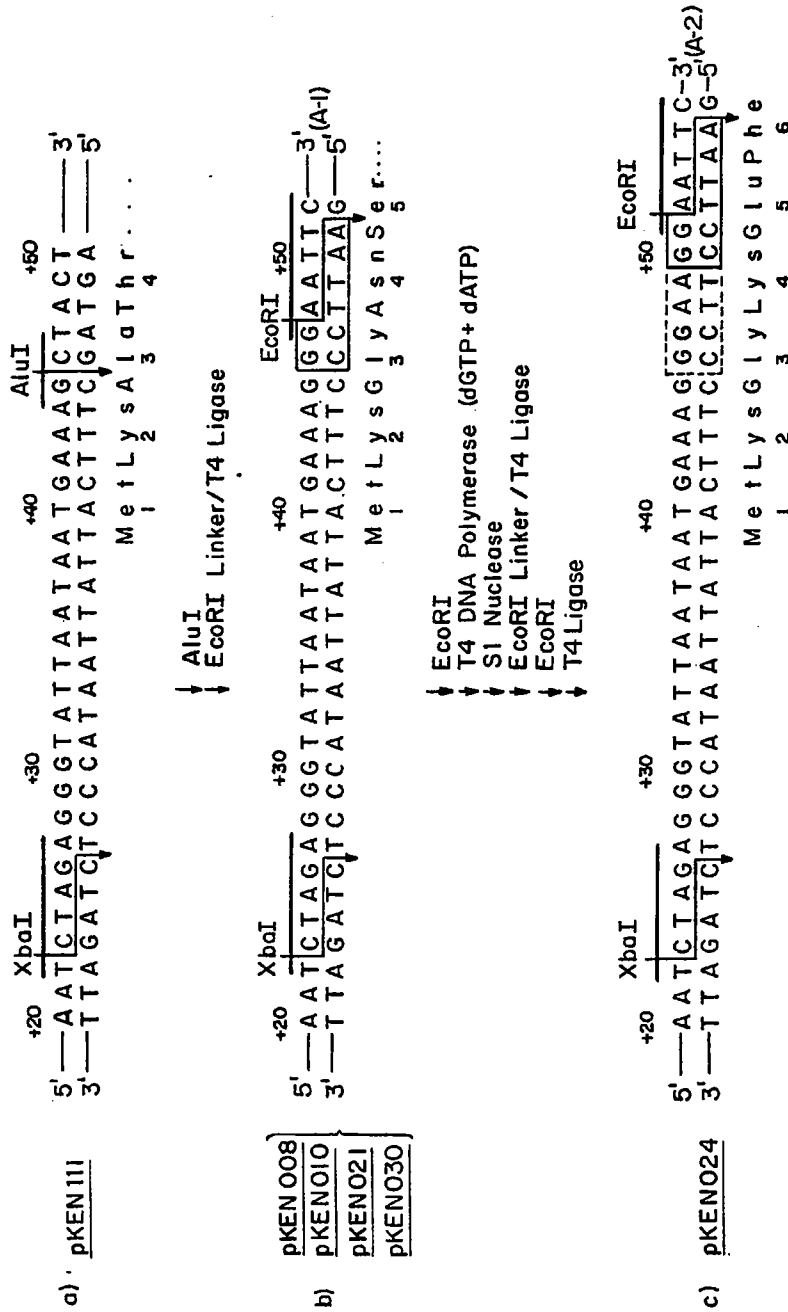


FIG. 13

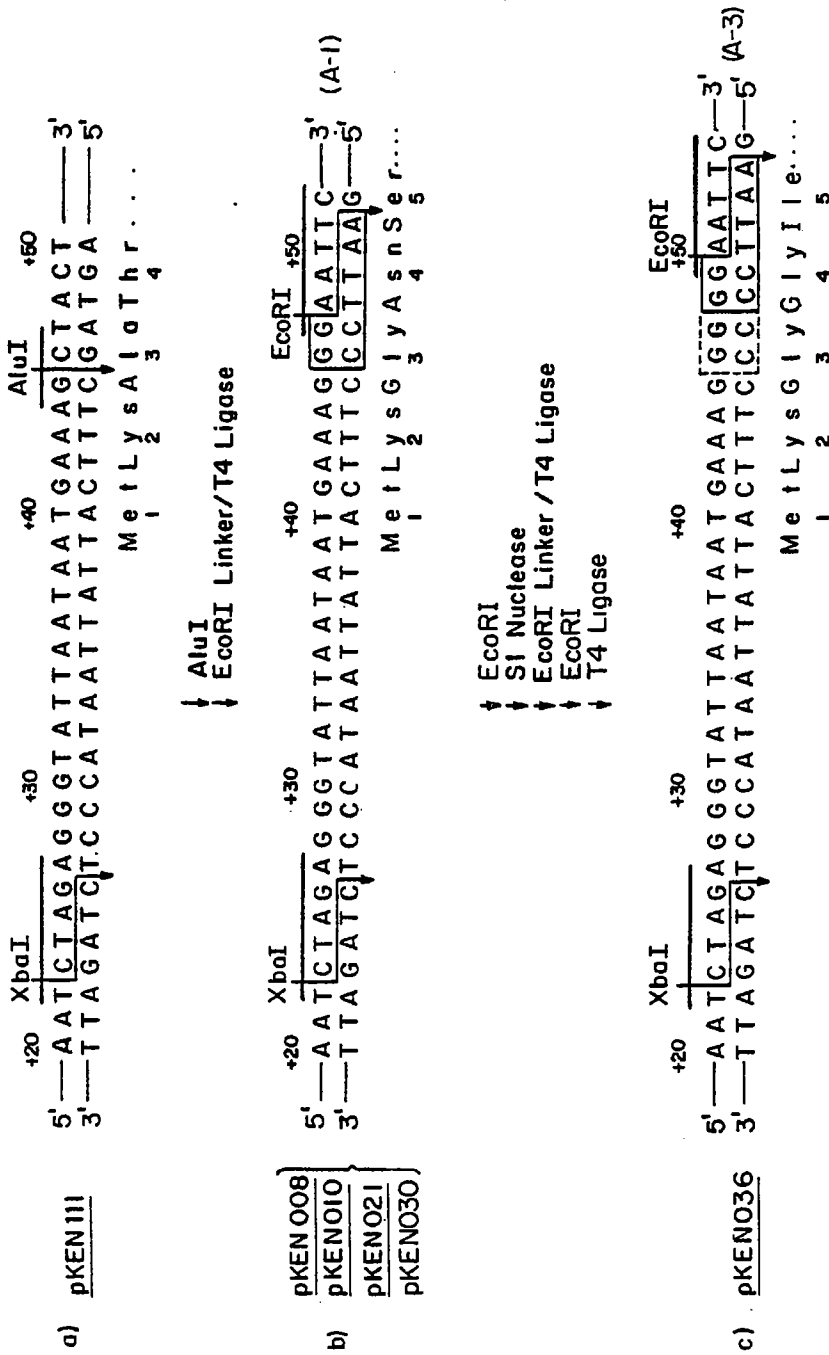
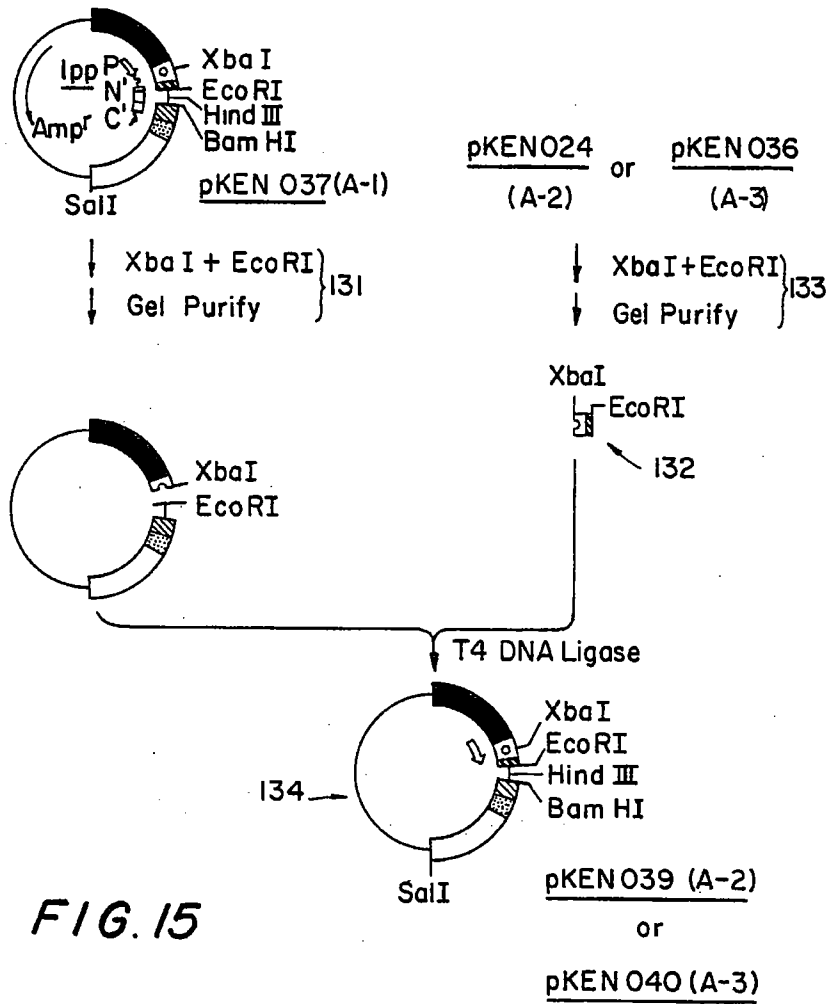


FIG. 14



**FIG. 15**

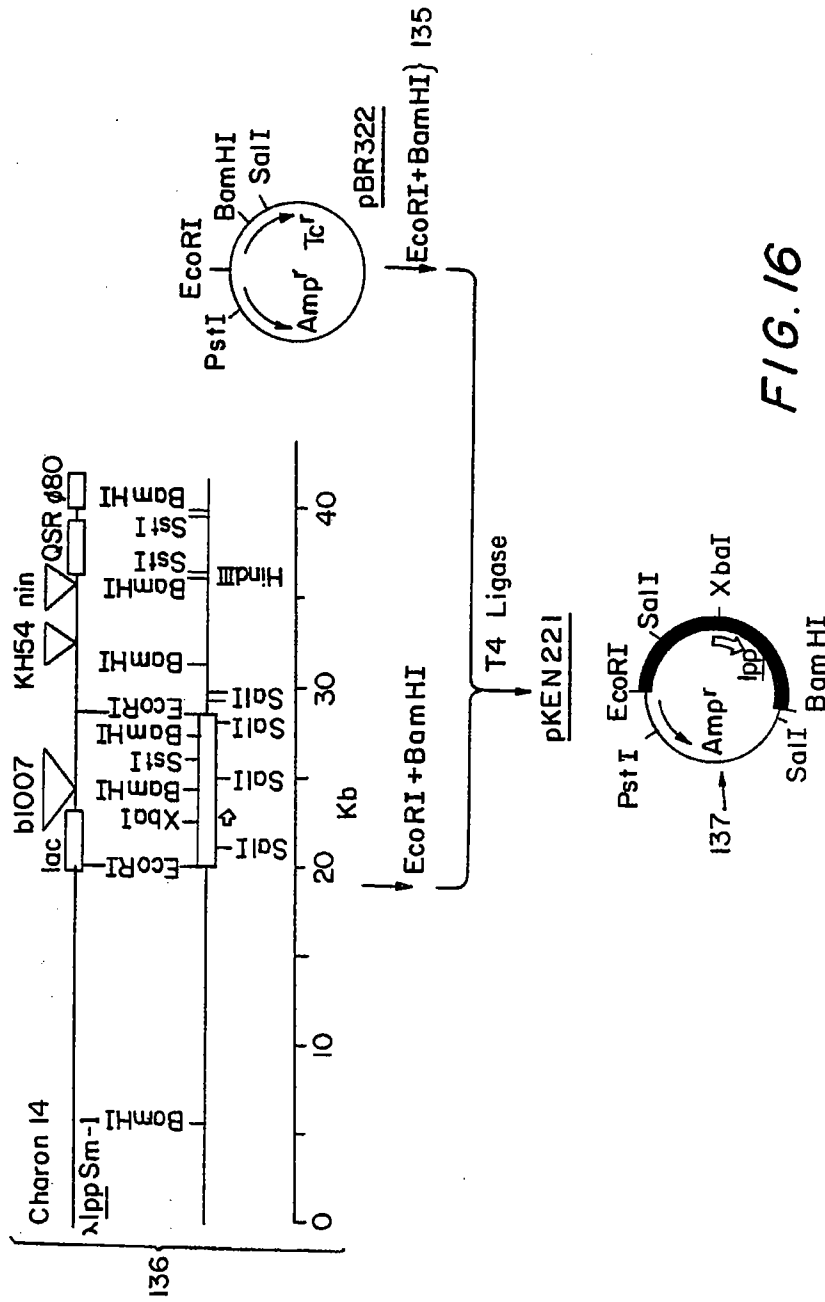


FIG. 16

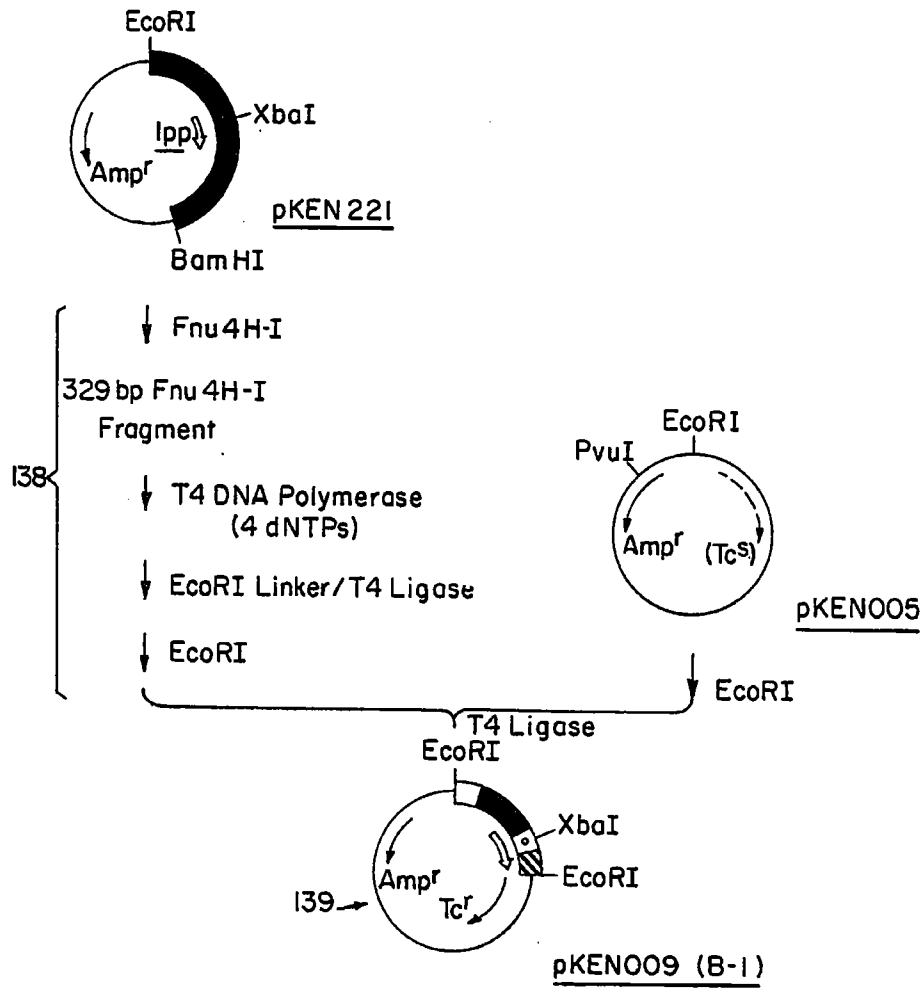


FIG. 17

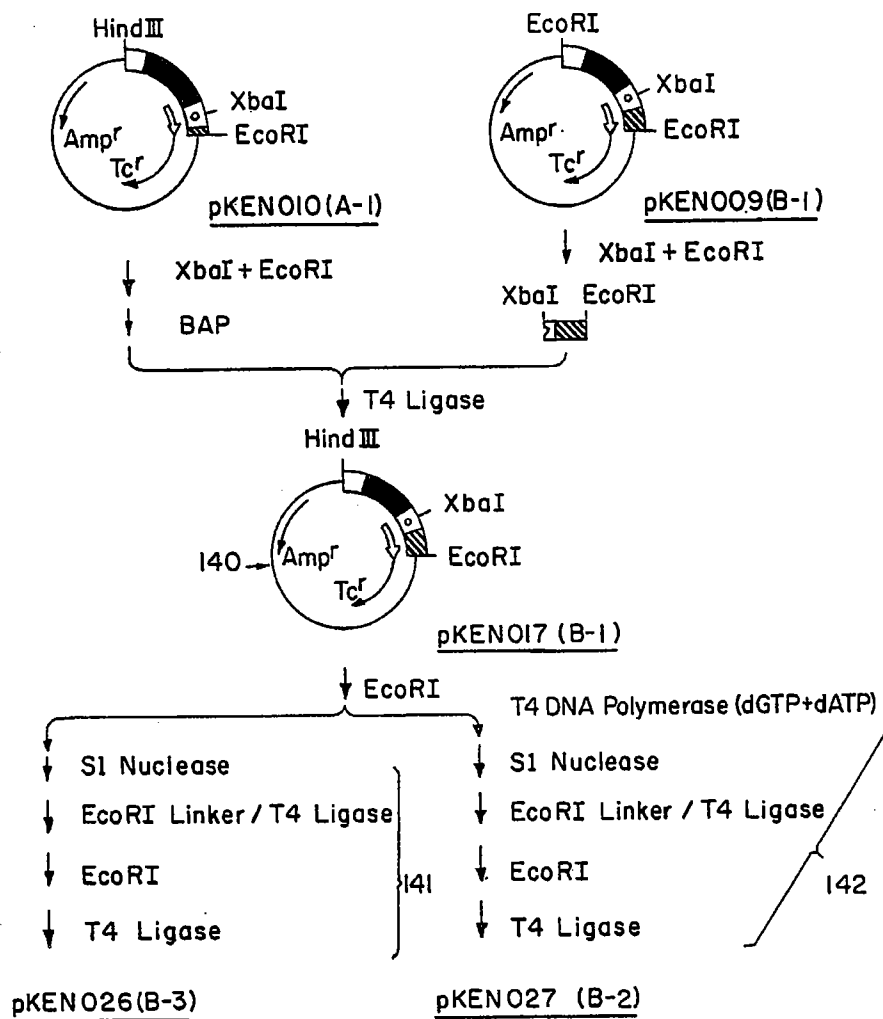


FIG. 18

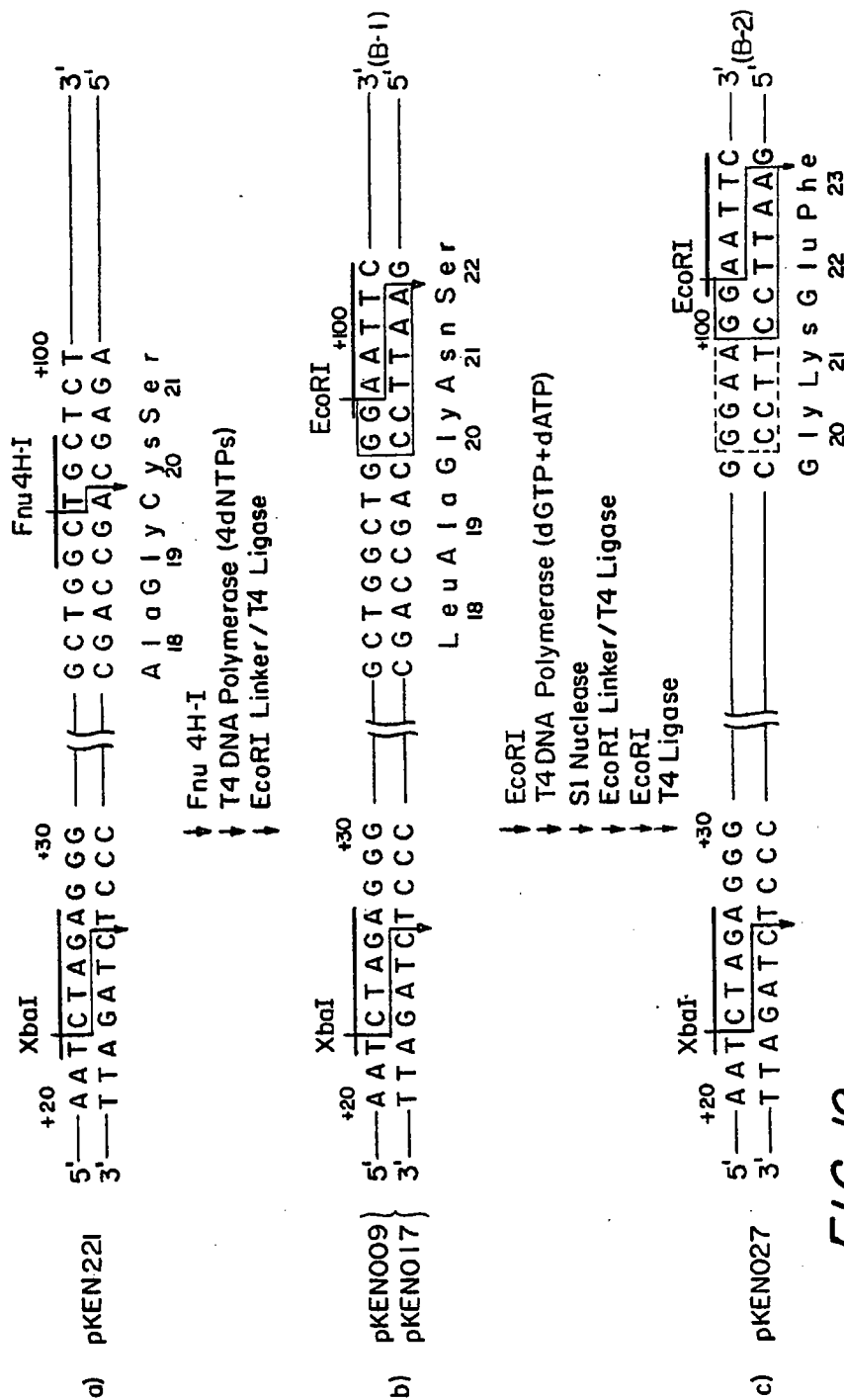


FIG. 19



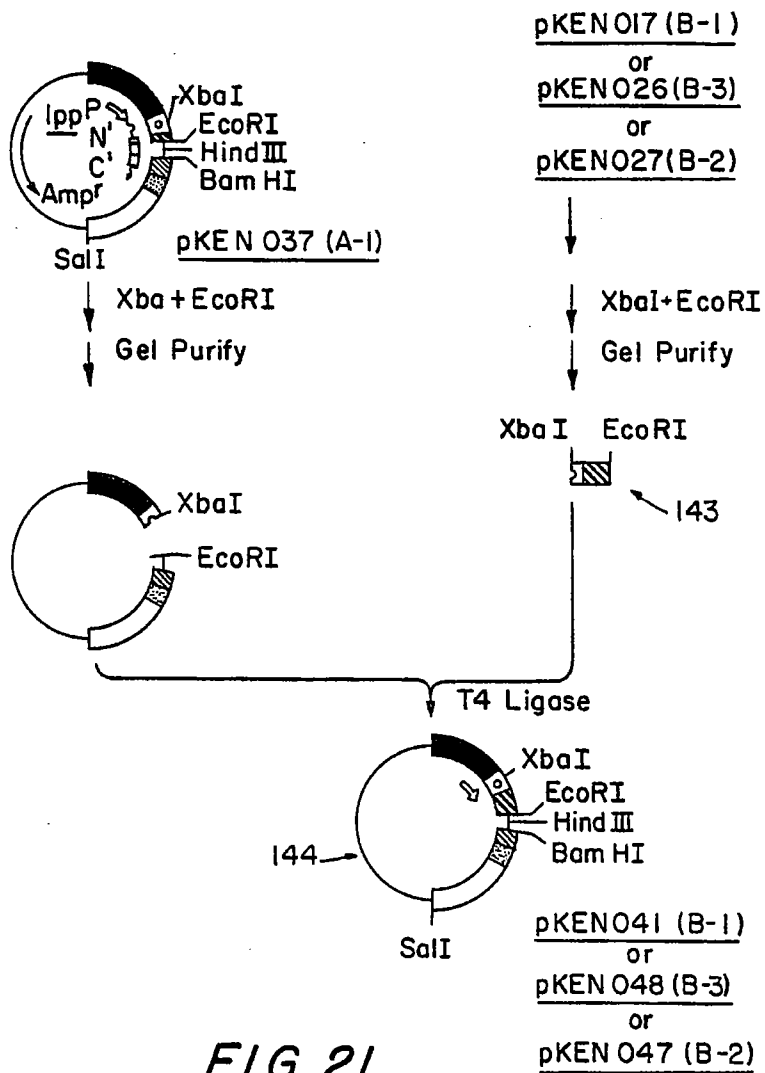
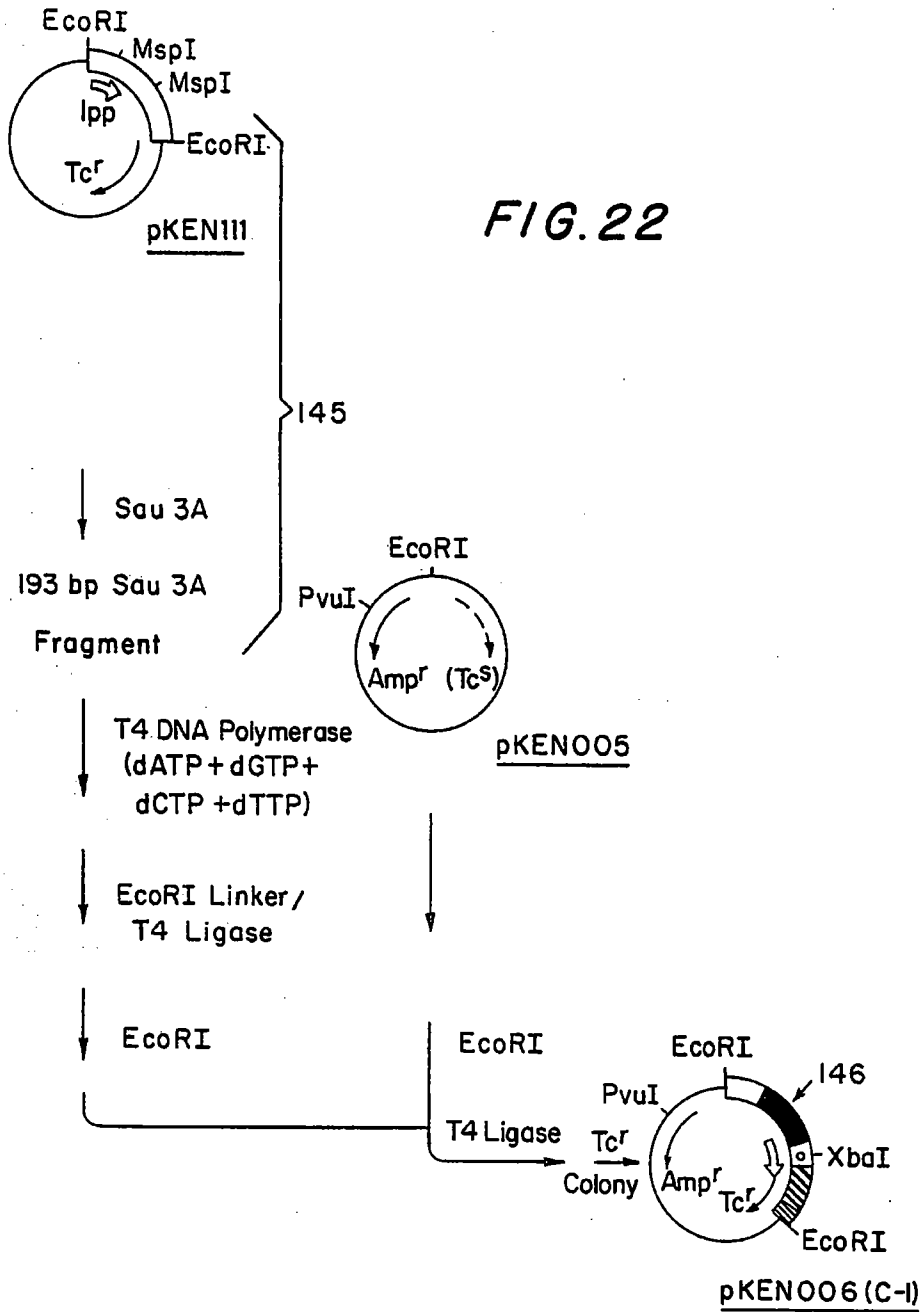


FIG. 21

FIG. 22



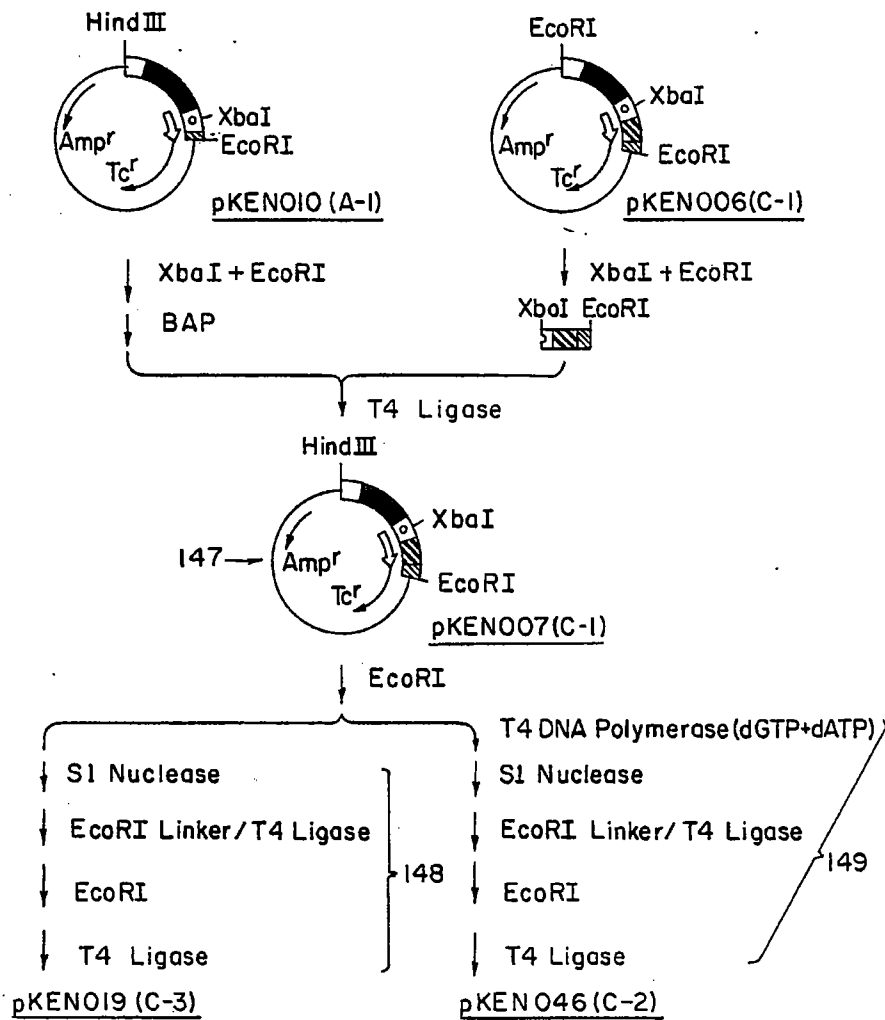


FIG. 23

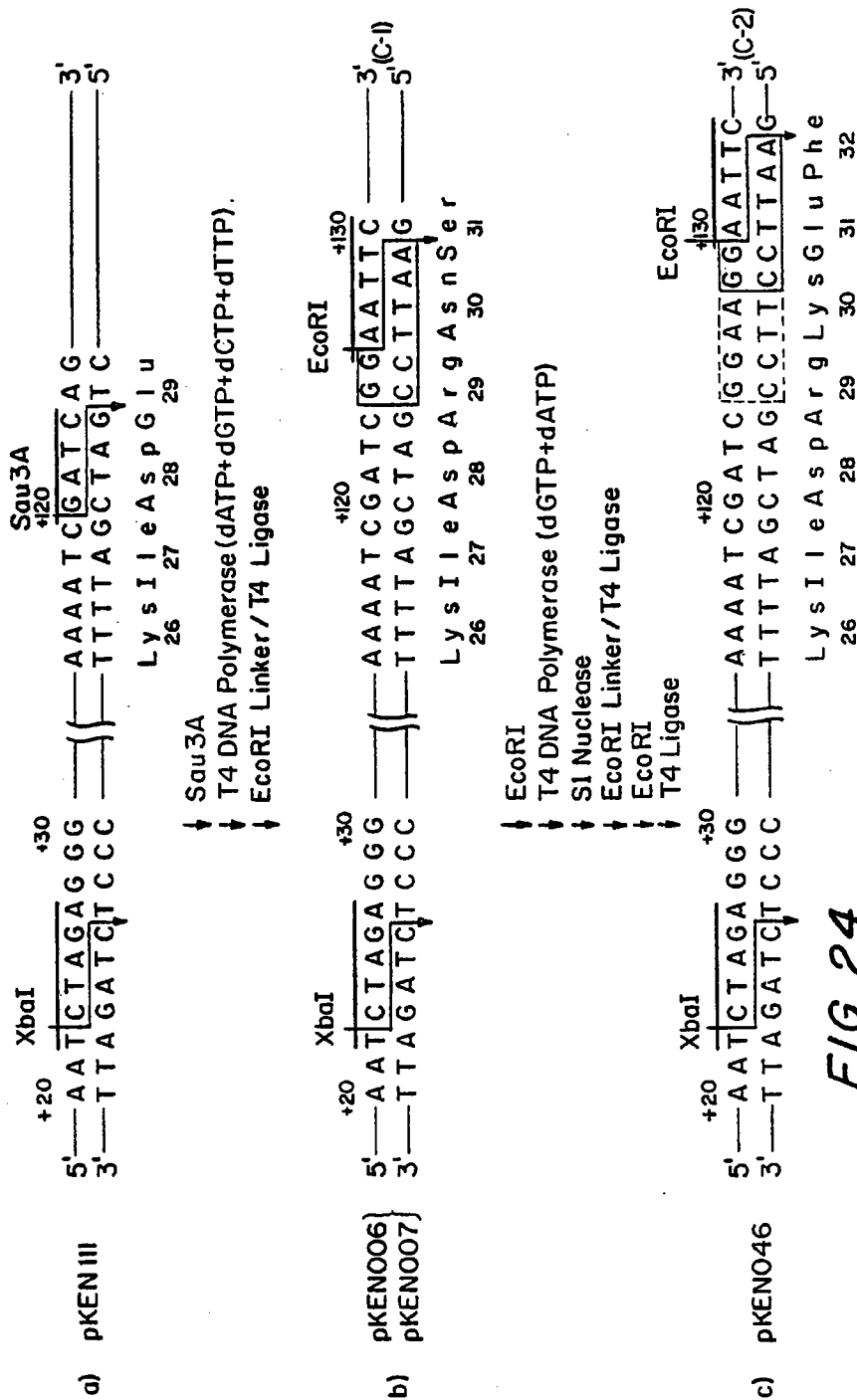


FIG. 24

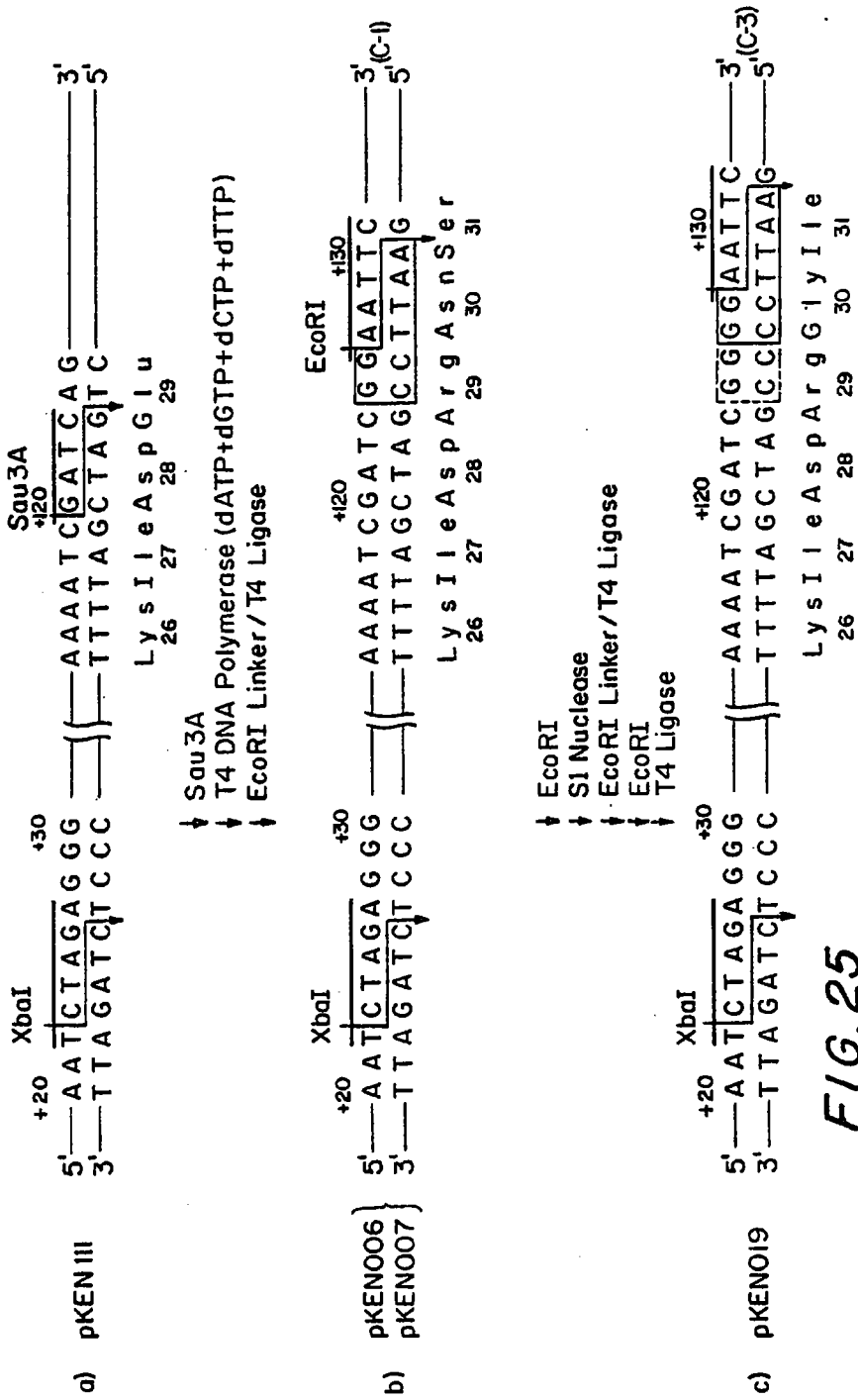


FIG. 25

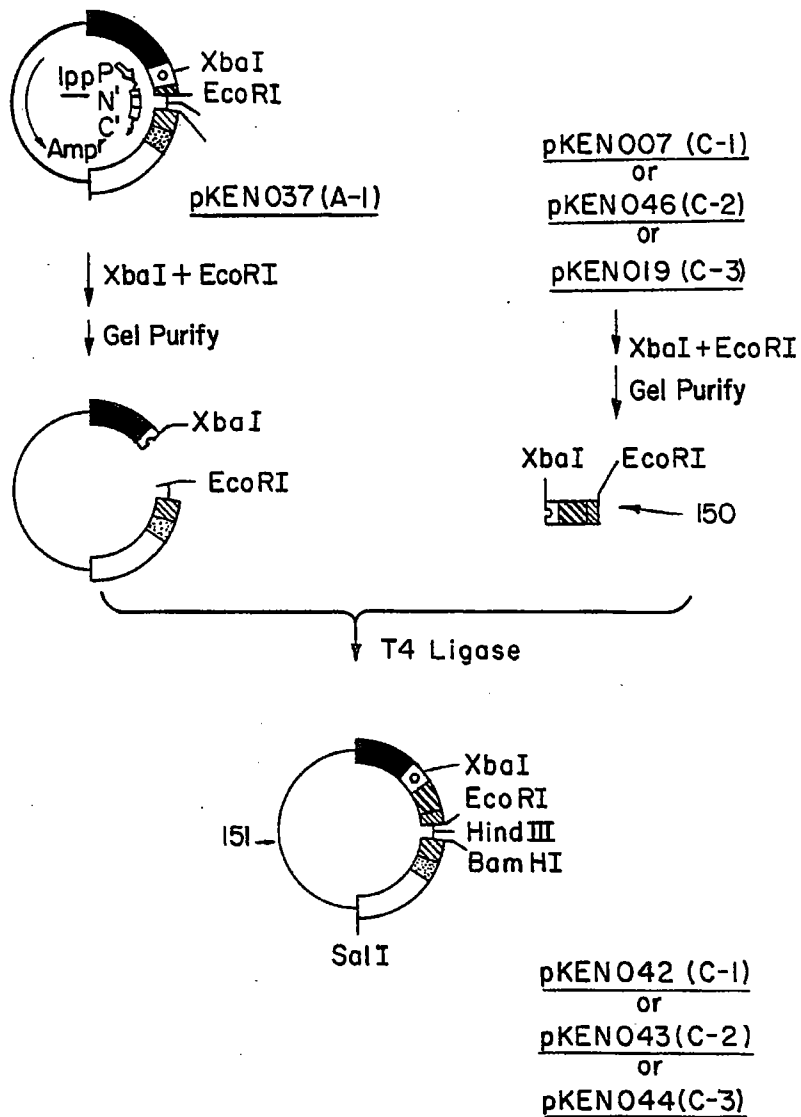


FIG. 26

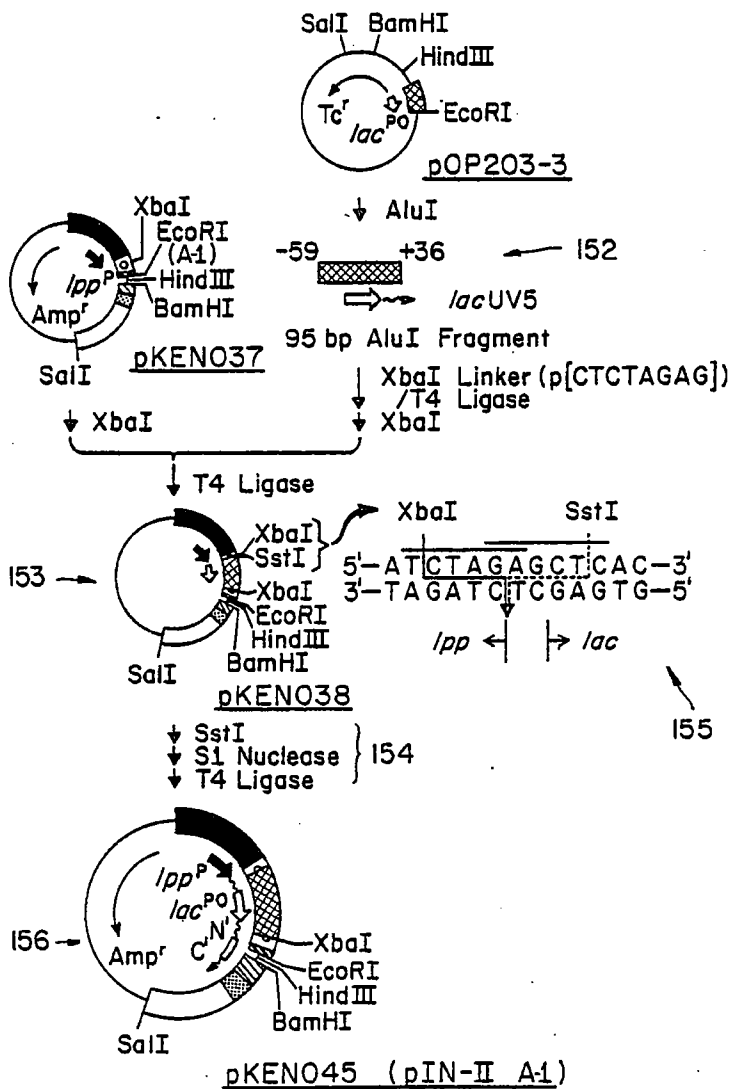


FIG. 27

