

EXHIBIT U

PART 1

Lodish Decl. in Support of Opposition to Roche's Motion for Summary Judgment of Invalidation for Double Patenting Over Claim 10 of the '016 Patent

United States Patent [19]

Goeddel et al.

[11] Patent Number: **4,766,075**

[45] Date of Patent: **Aug. 23, 1988**

[54] HUMAN TISSUE PLASMINOGEN
ACTIVATOR

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[73] Assignee: Genentech, Inc., South San
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[21] Appl. No.: 483,052

[22] Filed: Apr. 7, 1983

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 398,003, Jul. 14, 1982,
and a continuation-in-part of Ser. No. 374,860, May 5,
1982.

[51] Int. Cl.⁴ C12N 5/00; C12N 15/00;
C12N 9/48; C12N 9/72; C12N 1/20; C12N
1/00; C07H 17/00

[52] U.S. Cl. 435/240.2; 435/172.3;
435/212; 435/215; 435/253; 435/320; 536/27;
935/14; 935/29; 935/32; 935/70; 935/73

[58] Field of Search 435/172.3, 253, 317,
435/240, 212, 215, 320, 240.2; 536/27; 935/14,
27, 29, 32, 72, 73, 70

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[57]

ABSTRACT

Human tissue phasminogen activator (t-PA) is pro-
duced in useful quantities using recombinant DNA
techniques. The invention disclosed thus enables the
production of t-PA free of contaminants with which it
is ordinarily associated in its native cellular environ-
ment. Methods, expression vehicles and various host
cells useful in its production are also disclosed.

11 Claims, 14 Drawing Sheets

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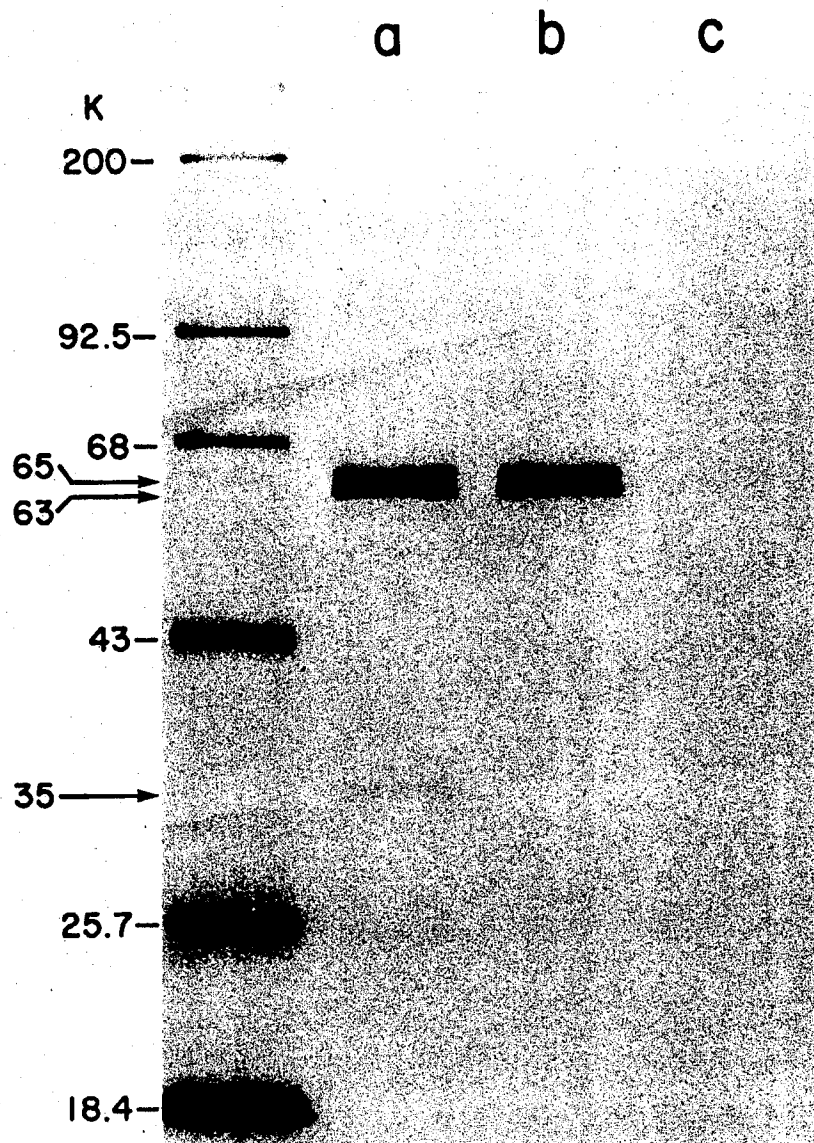


Fig.1.

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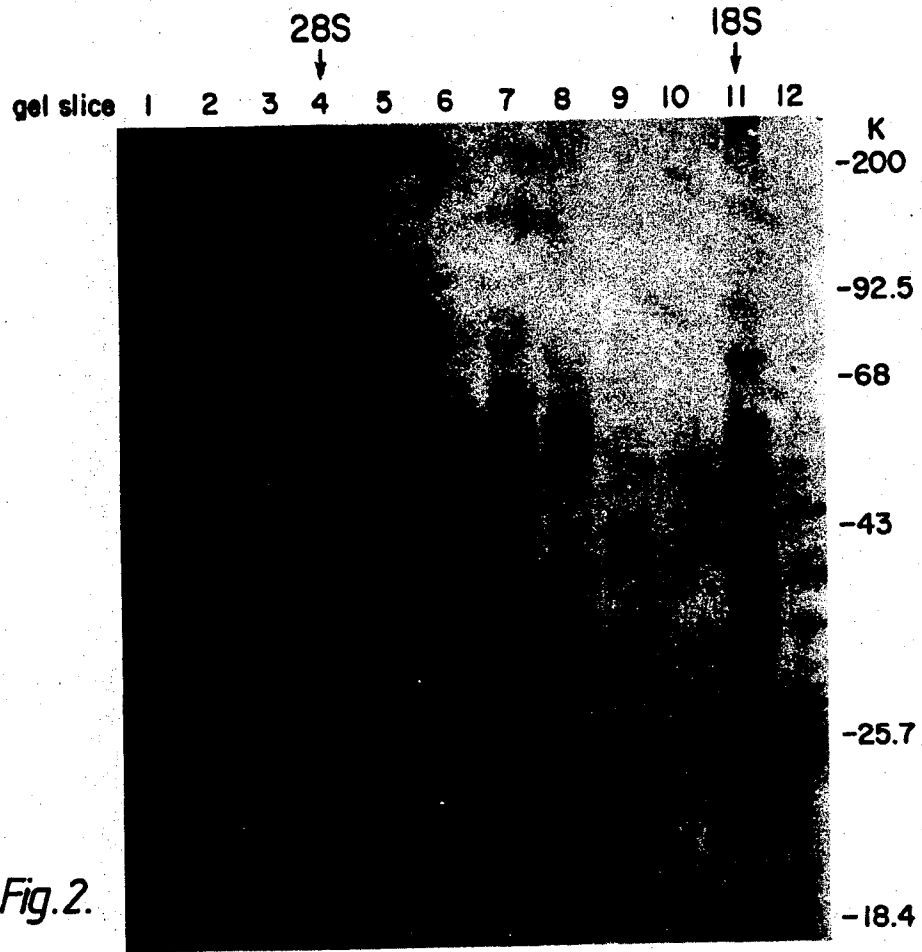


Fig. 2.

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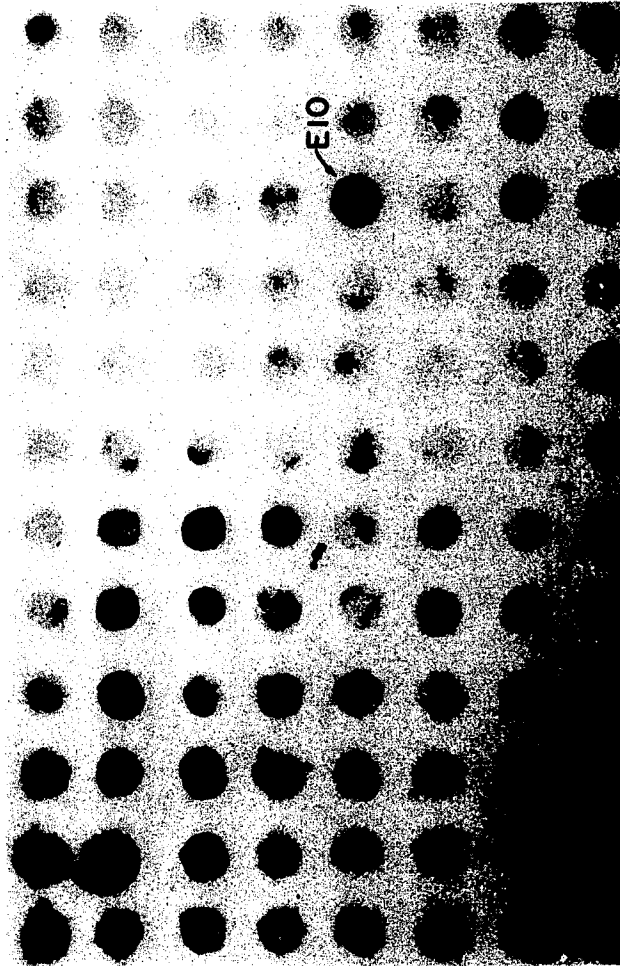
COLONY HYBRIDIZATION

RNA Gel Slice 7 cDNA Clones

vs.

^{32}P -TC(A₆)CA(A₆)TA(C₆)TCCCA Probe

Fig. 3.



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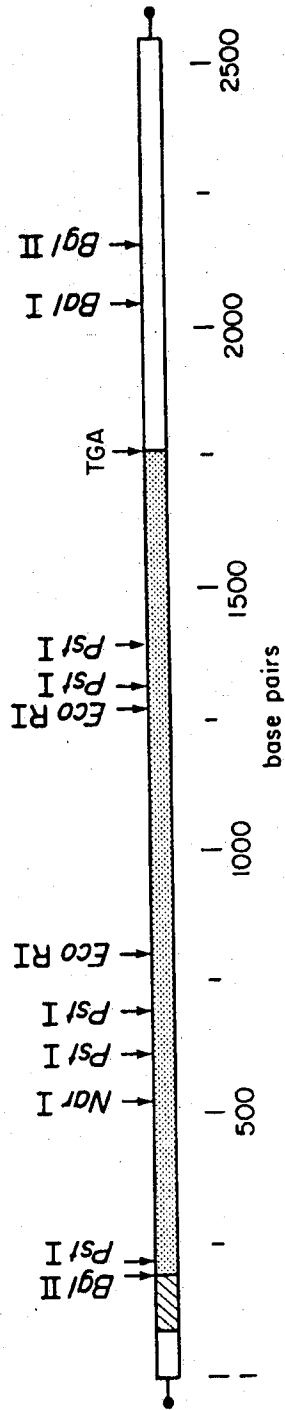


Fig. 4.

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GTTCTGAGCACAGGGCTGGAGAGAAAACCTCTGCGAGGAAAGGGAAGGAGCAAGCCGTGA

-35 -30
 met asp ala met lys arg gly leu
 ATG GAT GCA ATG AAG AGA GGG CTC
 ATTTAAGGGACGCTGTGAAGCAATC

-20
 cys cys val leu leu leu cys gly ala val phe val ser pro ser
 TGC TGT GTG CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC

-10 1
 gln glu ile his ala arg phe arg arg gly ala arg SER TYR GLN
 CAG GAA ATC CAT GCC CGA TTC AGA AGA GGA GCC AGA TCT TAC CAA

10
 VAL ILE CYS ARG ASP GLU LYS THR GLN MET ILE TYR GLN GLN HIS
 GTG ATC TGC AGA GAT GAA AAA ACG CAG ATG ATA TAC CAG CAA CAT

20 30
 GLN SER TRP LEU ARG PRO VAL LEU ARG SER ASN ARG VAL GLU TYR
 CAG TCA TGG CTG CGC CCT GTG CTC AGA AGC AAC CGG GTG GAA TAT

40
 CYS TRP CYS ASN SER GLY ARG ALA GLN CYS HIS SER VAL PRO VAL
 TGC TGG TGC AAC AGT GGC AGG GCA CAG TGC CAC TCA GTG CCT GTC

50 60
 LYS SER CYS SER GLU PRO ARG CYS PHE ASN GLY GLY THR CYS GLN
 AAA AGT TGC AGC GAG CCA AGG TGT TTC AAC GGG GGC ACC TGC CAG

70
 GLN ALA LEU TYR PHE SER ASP PHE VAL CYS GLN CYS PRO GLU GLY
 CAG GCC CTG TAC TTC TCA GAT TTC GTG TGC CAG TGC CCC GAA GGA

80 90
 PHE ALA GLY LYS CYS CYS GLU ILE ASP THR ARG ALA THR CYS TYR
 TTT GCT GGG AAG TGC TGT GAA ATA GAT ACC AGG GCC ACG TGC TAC

100
 GLU ASP GLN GLY ILE SER TYR ARG GLY THR TRP SER THR ALA GLU
 GAG GAC CAG GGC ATC AGC TAC AGG GGC ACG TGG AGC ACA GCG GAG

110 120
 SER GLY ALA GLU CYS THR ASN TRP ASN SER SER ALA LEU ALA GLN
 AGT GGC GCC GAG TGC ACC AAC TGG AAC AGC AGC GCG TTG GCC CAG

130
 LYS PRO TYR SER GLY ARG ARG PRO ASP ALA ILE ARG LEU GLY LEU
 AAG CCC TAC AGC GGG CGG AGG CCA GAC GCC ATC AGG CTG GGC CTG

140 150
 GLY ASN HIS ASN TYR CYS ARG ASN PRO ASP ARG ASP SER LYS PRO
 GGG AAC CAC AAC TAC TGC AGA AAC CCA GAT CGA GAC TCA AAG CCC

160
 TRP CYS TYR VAL PHE LYS ALA GLY LYS TYR SER SER GLU PHE CYS
 TGG TGC TAC GTC TTT AAG GCG GGG AAG TAC AGC TCA GAG TTC TGC

170 180
 SER THR PRO ALA CYS SER GLU GLY ASN SER ASP CYS TYR PHE GLY
 AGC ACC CCT GCC TGC TCT GAG GGA AAC AGT GAC TGC TAC TTT GGG

Fig. 5A.

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190
ASN GLY SER ALA TYR ARG GLY THR HIS SER LEU THR GLU SER GLY
AAT GGG TCA GCC TAC CGT GGC ACG CAC AGC CTC ACC GAG TCG GGT

200
ALA SER CYS LEU PRO TRP ASN SER MET ILE LEU ILE GLY LYS VAL
GCC TCC TGC CTC CCG TGG AAT TCC ATG ATC CTG ATA GGC AAG GTT

210
TYR THR ALA GLN ASN PRO SER ALA GLN ALA LEU GLY LEU GLY LYS
TAC ACA GCA CAG AAC CCC AGT GCC CAG GCA CTG GGC CTG GGC AAA

220
HIS ASN TYR CYS ARG ASN PRO ASP GLY ASP ALA LYS PRO TRP CYS
CAT AAT TAC TGC CGG AAT CCT GAT GGG GAT GCC AAG CCC TGG TGC

230
HIS VAL LEU LYS ASN ARG ARG LEU THR TRP GLU TYR CYS ASP VAL
CAC GTG CTG AAG AAC CGC AGG CTG ACG TGG GAG TAC TGT GAT GTG

240
PRO SER CYS SER THR CYS GLY LEU ARG GLN TYR SER GLN PRO GLN
CCC TCC TGC TCC ACC TGC GGC CTG AGA CAG TAC AGC CAG CCT CAG

250
PHE ARG ILE LYS GLY GLY LEU PHE ALA ASP ILE ALA SER HIS PRO
TTT CGC ATC AAA GGA GGG CTC TTC GCC GAC ATC GCC TCC CAC CCC

260
TRP GLN ALA ALA ILE PHE ALA LYS HIS ARG ARG SER PRO GLY GLU
TGG CAG GCT GCC ATC TTT GCC AAG CAC AGG AGG TCG CCC GGA GAG

270
ARG PHE LEU CYS GLY GLY ILE LEU ILE SER SER CYS TRP ILE LEU
CGG TTC CTG TGC GGG GGC ATA CTC ATC AGC TCC TGC TGG ATT CTC

280
SER ALA ALA HIS CYS PHE GLN GLU ARG PHE PRO PRO HIS HIS LEU
TCT GCC GCC CAC TGC TTC CAG GAG AGG TTT CCG CCC CAC CAC CTG

290
THR VAL ILE LEU GLY ARG THR TYR ARG VAL VAL PRO GLY GLU GLU
ACG GTG ATC TTG GGC AGA ACA TAC CGG GTG GTC CCT GGC GAG GAG

300
GLU GLN LYS PHE GLU VAL GLU LYS TYR ILE VAL HIS LYS GLU PHE
GAG CAG AAA TTT GAA GTC GAA AAA TAC ATT GTC CAT AAG GAA TTC

310
ASP ASP ASP THR TYR ASP ASN ASP ILE ALA LEU LEU GLN LEU LYS
GAT GAT GAC ACT TAC GAC AAT GAC ATT GCG CTG CTG CAG CTG AAA

320
SER ASP SER SER ARG CYS ALA GLN GLU SER SER VAL VAL ARG THR
TCG GAT TCG TCC CGC TGT GCC CAG GAG AGC AGC GTG GTC CGC ACT

330
VAL CYS LEU PRO PRO ALA ASP LEU GLN LEU PRO ASP TRP THR GLU
GTG TGC CTT CCC CCG GCG GAC CTG CAG CTG CCG GAC TGG ACG GAG

340
CYS GLU LEU SER GLY TYR GLY LYS HIS GLU ALA LEU SER PRO PHE
TGT GAG CTC TCC GGC TAC GGC AAG CAT GAG GCC TTG TCT CCT TTC

350
360
370
380
390
400
410
420

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Fig. 5B.

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430
 TYR SER GLU ARG LEU LYS GLU ALA HIS VAL ARG LEU TYR PRO SER
 TAT TCG GAG CGG CTG AAG GAG GCT CAT GTC AGA CTG TAC CCA TCC

 440
 SER ARG CYS THR SER GLN HIS LEU LEU ASN ARG THR VAL THR ASP
 AGC CGC TGC ACA TCA CAA CAT TTA CTT AAC AGA ACA GTC ACC GAC

 460
 ASN MET LEU CYS ALA GLY ASP THR ARG SER GLY GLY PRO GLN ALA
 AAC ATG CTG TGT GCT GGA GAC ACT CGG AGC GGC GGG CCC CAG GCA

 470
 ASN LEU HIS ASP ALA CYS GLN GLY ASP SER GLY GLY PRO LEU VAL
 AAC TTG CAC GAC GCC TGC CAG GGC GAT TCG GGA GGC CCC CTG GTG

 490
 CYS LEU ASN ASP GLY ARG MET THR LEU VAL GLY ILE ILE SER TRP
 TGT CTG AAC GAT GGC CGC ATG ACT TTG GTG GGC ATC ATC AGC TGG

 500
 GLY LEU GLY CYS GLY GLN LYS ASP VAL PRO GLY VAL TYR THR LYS
 GGC CTG GGC TGT GGA CAG AAG GAT GTC CCG GGT GTG TAC ACC AAG

 520
 VAL THR ASN TYR LEU ASP TRP ILE ARG ASP ASN MET ARG PRO OP
 GTT ACC AAC TAC CTA GAC TGG ATT CGT GAC AAC ATG CGA CCG TGA

 CCAGGAACACCCGACTCCTCAAAAAGCAAATGAGATCCCGCCTCTTCTTCTTCAGAAGACA
 CTGCAAAGGCGCAGTGCTTCTCTACAGACTTCTCCAGACCCACCACCCGAGAAAGCGGG
 ACGAGACCCCTACAGGAGAGGGAAGAGTGCATTTTCCCAGATACTTCCCATTTTGGAAGT
 TTTCAGGACTTGGTCTGATTTTCAGGATACTCTGTGATGCGGAAAGACATGAATGCACACT
 AGCCTCTCCAGGAATGCCTCCTCCCTGGGCAGAAAGTGGCCATGCCACCCTGTTTTTCAGCTA
 AAGCCCAACCTCCTGACCTGTCAACCGTGAGCAGCTTTGGAAACAGGACCACAAAAATGAA
 AGCATGTCTCAATAGTAAAAGATAACAAGATCTTTCAGGAAAGACGGATTGCATTAGAA
 ATAGACAGTATATTTATAGTCACAAGAGCCAGCAGGGCCTCAAAGTTGGGGCAGGCTGGC
 TGGCCCGTCATGTTCTCTCAAAGCACCCCTTGACGTCAAGTCTCCTTCCCCTTTCCCCTACT
 CCCTGGCTCTCAGAAGGTATTCCTTTTGTGTACAGTGTGTAAAGTGTAAATCCTTTTTCT
 TTATAAACTTTAGAGTAGCATGAGAGAATTGTATCATTGAACTAGGCTTCAGCATA
 TTTATAGCAATCCATGTTAGTTTTTACTTTCTGTGTCACCAACCCTGTTTTATACTGTA
 CTTAATAAAATTCAGATATATTTTTTCACAGTTTTTCCAAAAAATAAAAAA

Fig. 5C.

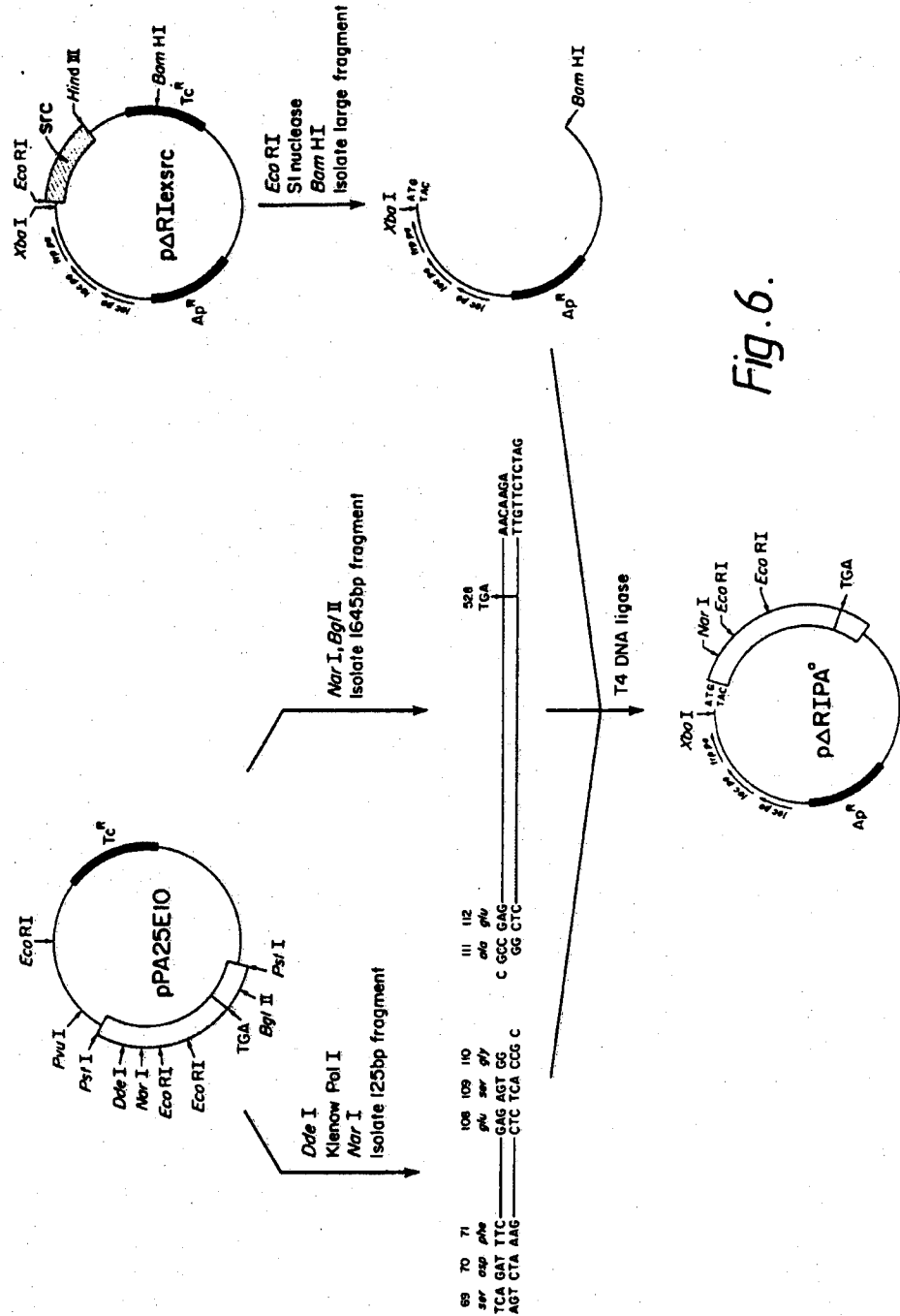


Fig. 6.

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Fig. 7.

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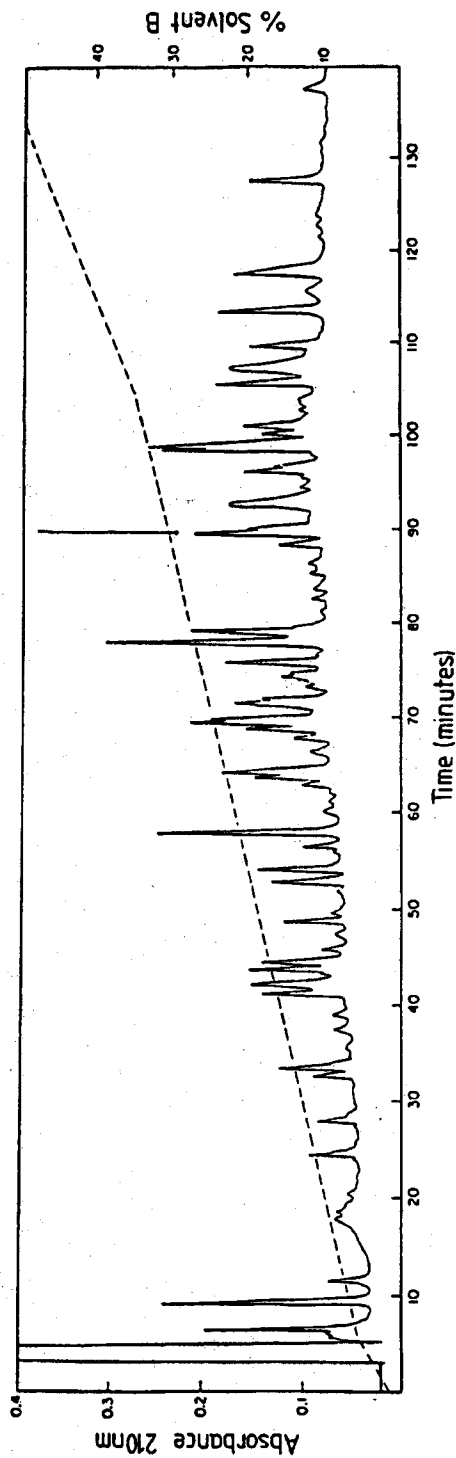


Fig. 8.

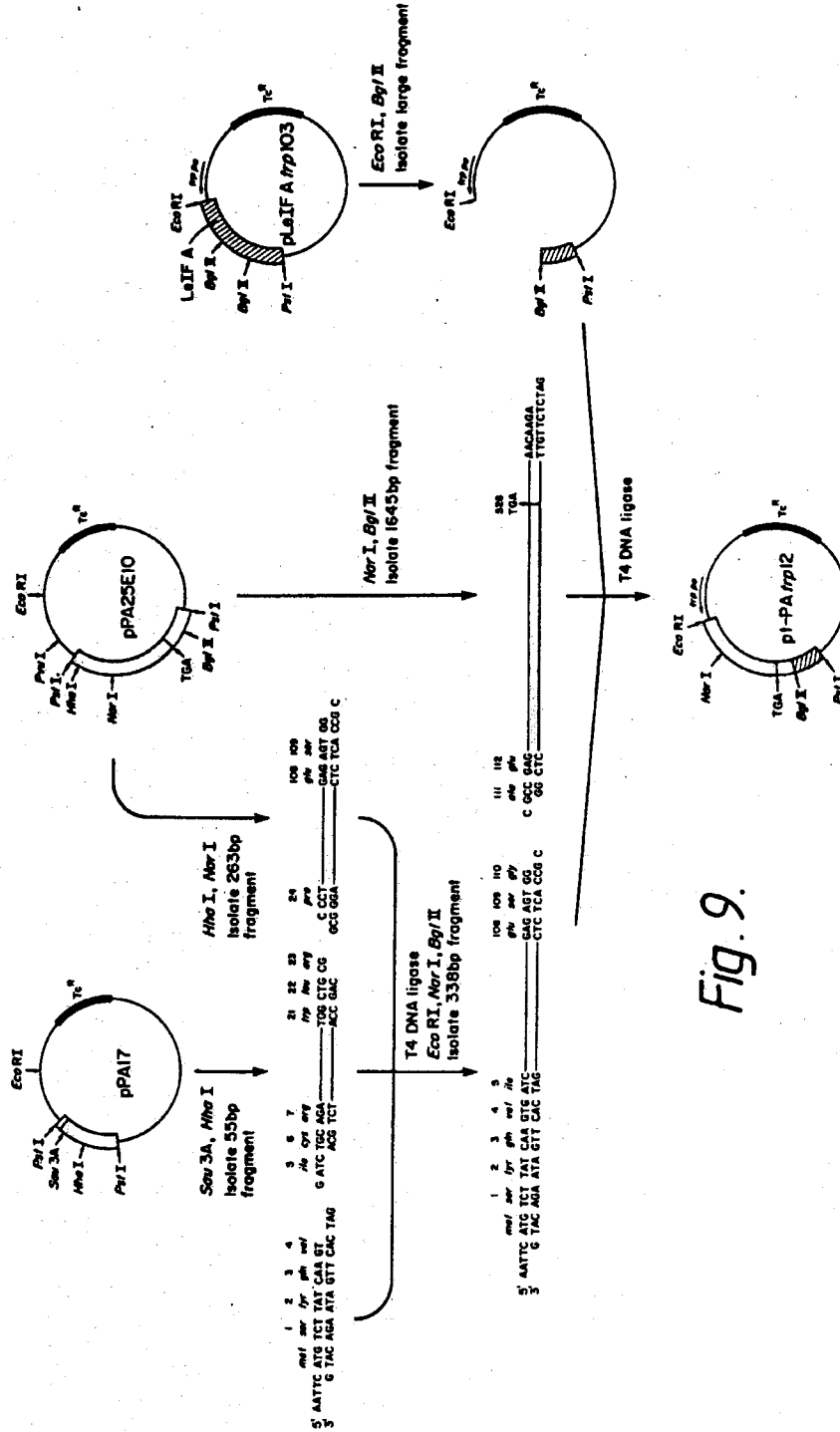


Fig. 9.

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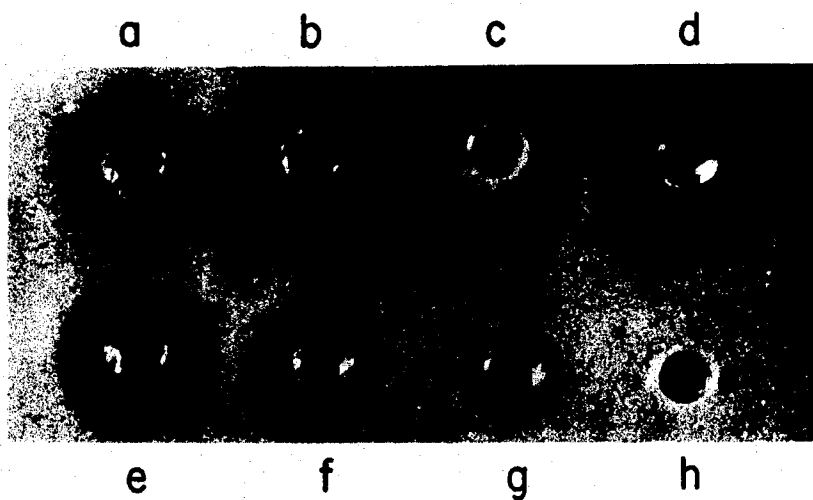


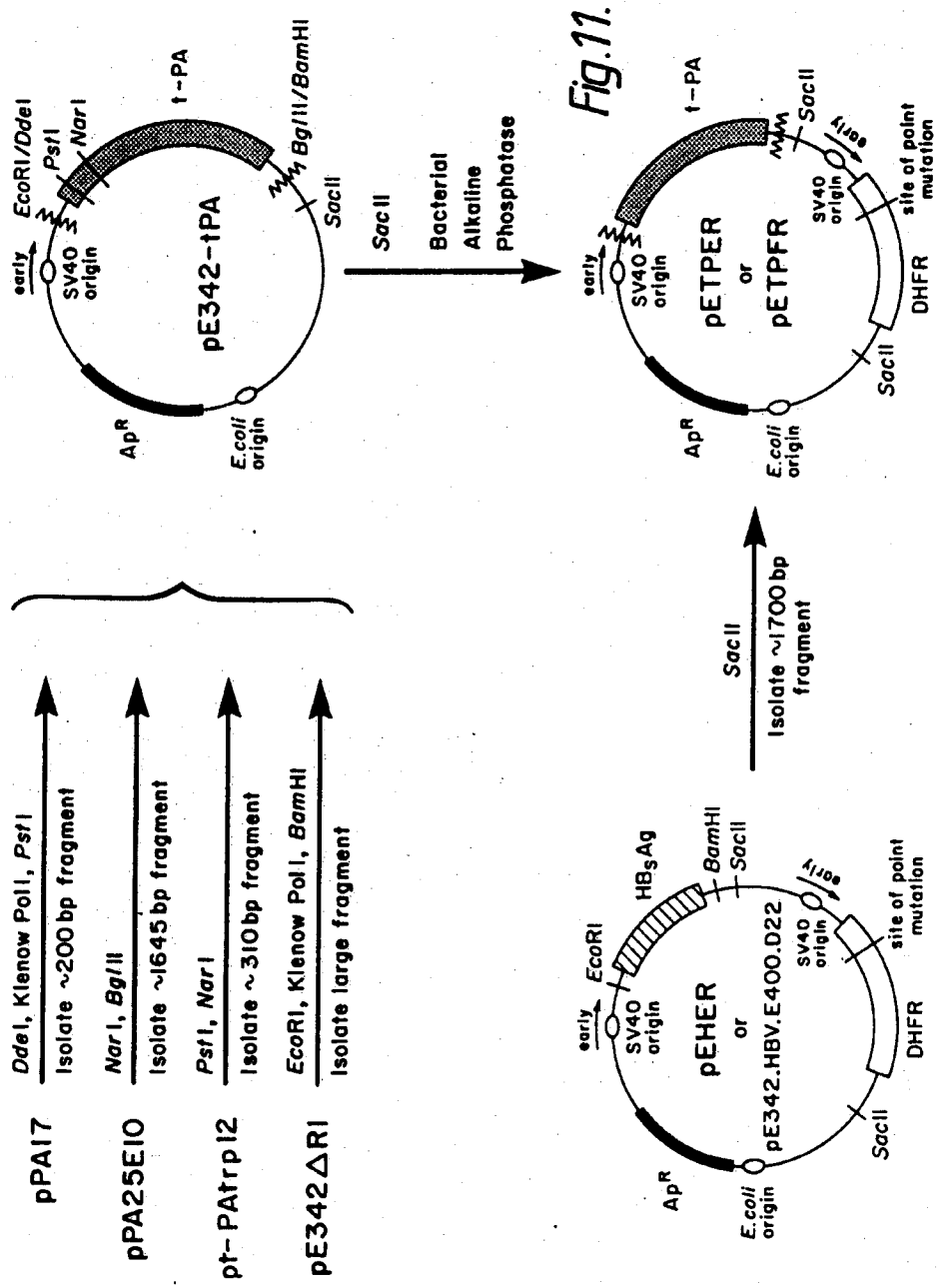
Fig. 10.

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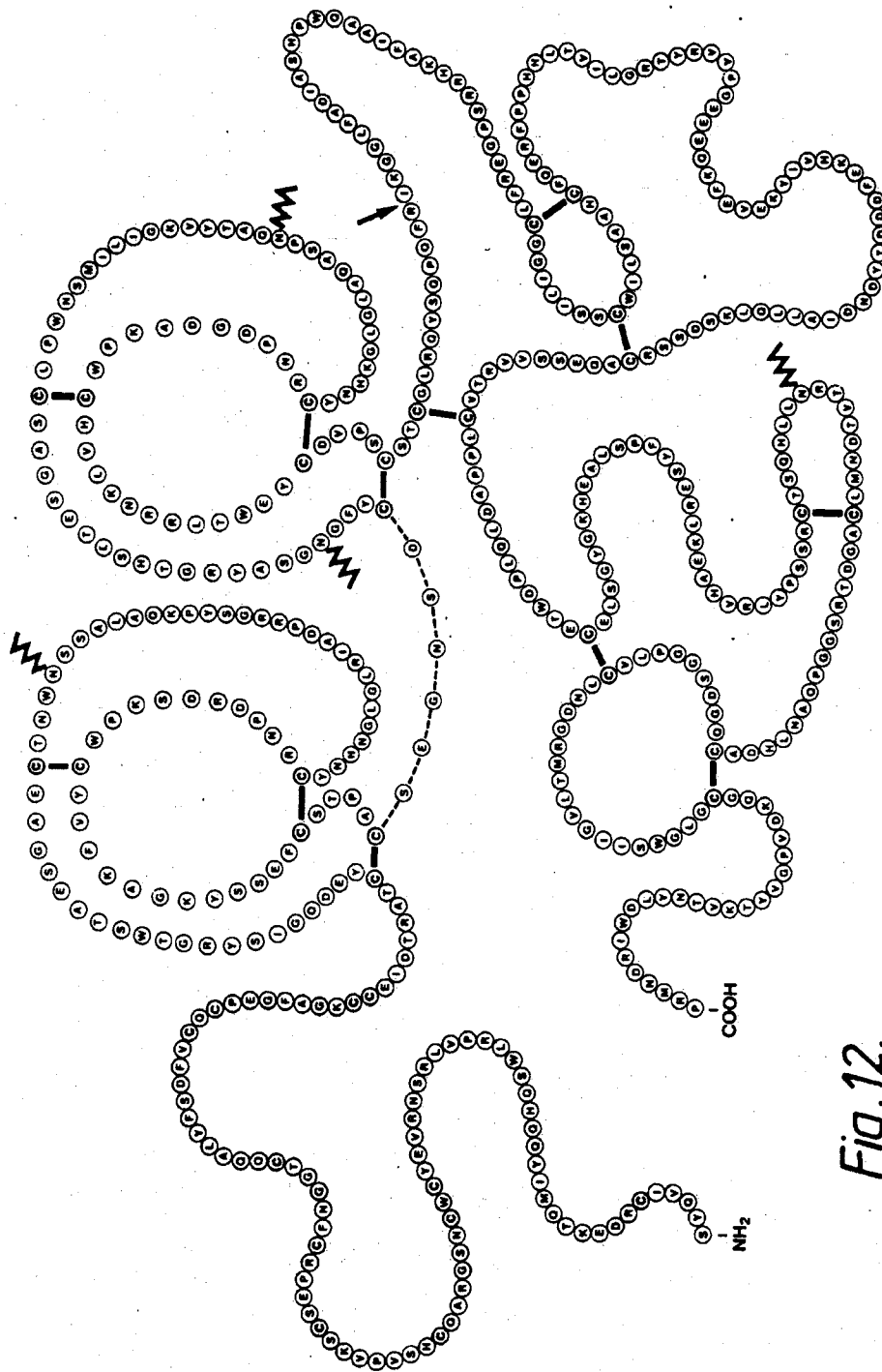


Fig. 12.