FIG. 1

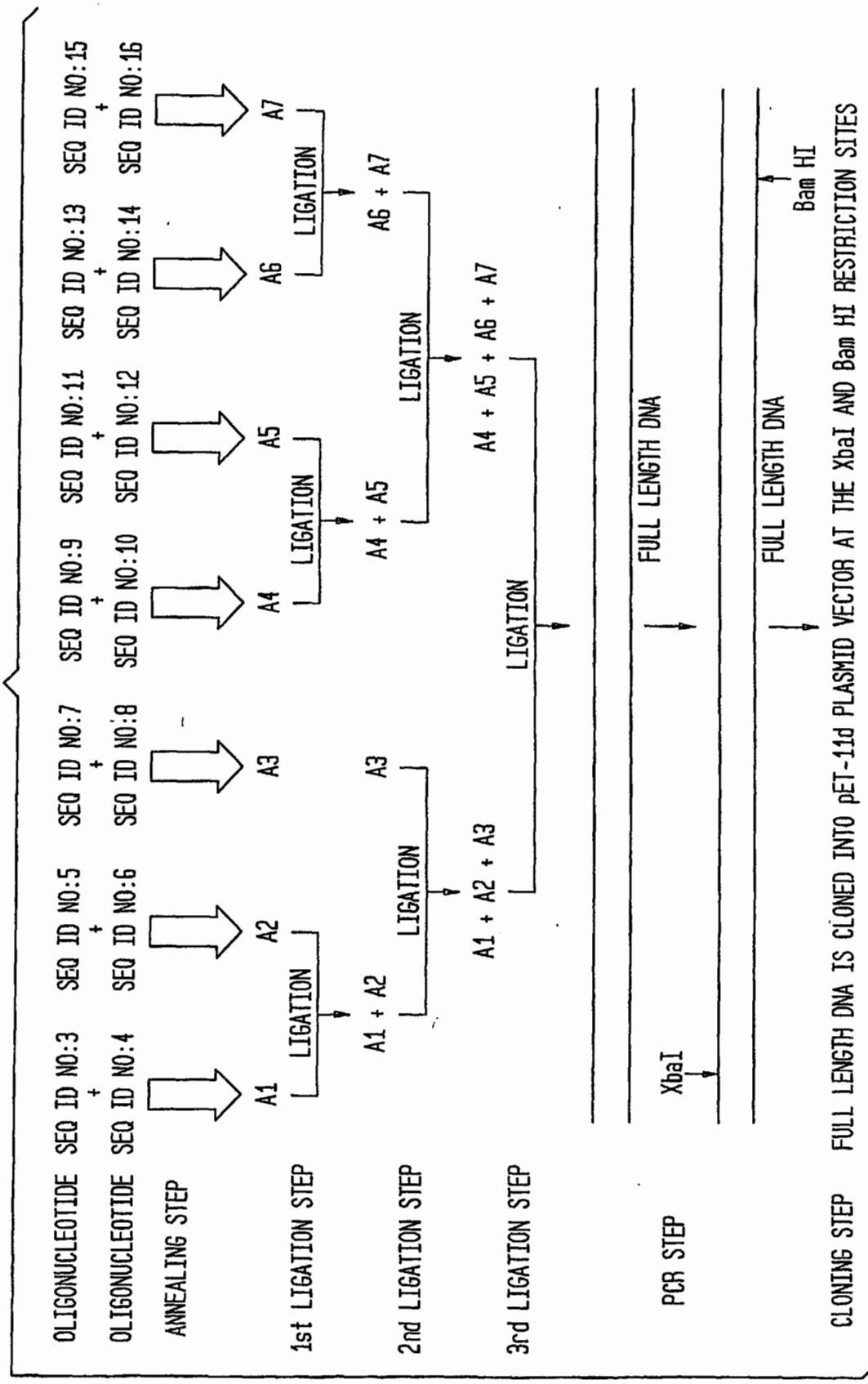

OLIGONUCLEOTIDE SEQ ID NO:3 + SEQ ID NO:5 SEQ ID NO:7 SEQ ID NO:9 SEQ ID NO:11 SEQ ID NO:13 SEQ ID NO:15
OLIGONUCLEOTIDE SEQ ID NO:4 + SEQ ID NO:6 SEQ ID NO:8 SEQ ID NO:10 SEQ ID NO:12 SEQ ID NO:14 SEQ ID NO:16
ANNEALING STEP
A1
A2
A3
A4
A5
A6
A7
1st LIGATION STEP
LIGATION
A1 + A2
A3
A4 + A5
A6 + A7
2nd LIGATION STEP
LIGATION
A1 + A2 + A3
A4 + A5 + A6 + A7
3rd LIGATION STEP
LIGATION
PCR STEP
XbaI
FULL LENGTH DNA
CLONING STEP
FULL LENGTH DNA IS CLONED INTO pET-11d PLASMID VECTOR AT THE XbaI AND Bam HI RESTRICTION SITES
FULL LENGTH DNA
Bam HI

FIG. 2

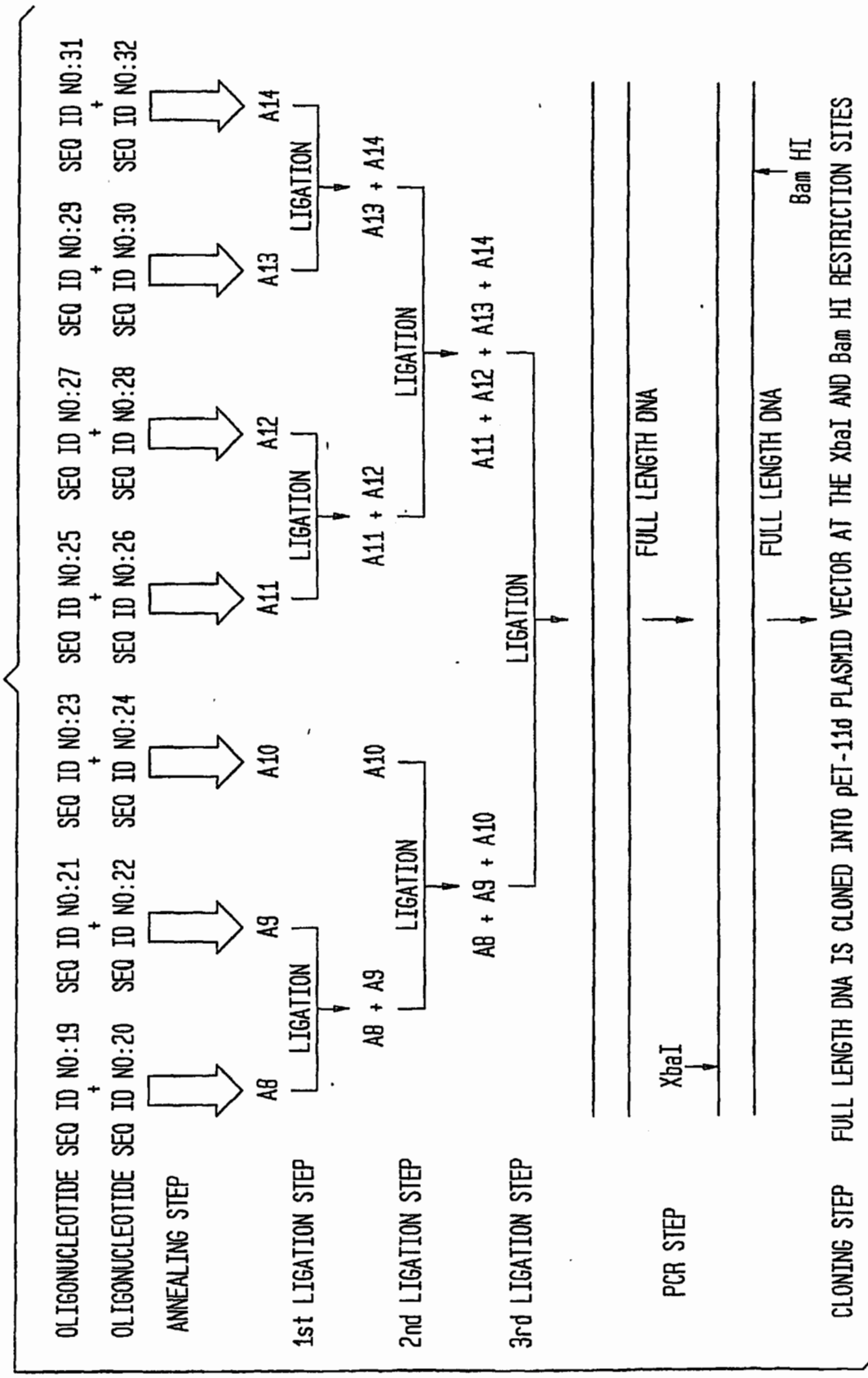

OLIGONUCLEOTIDE SEQ ID NO:19 + SEQ ID NO:21 + SEQ ID NO:23 + SEQ ID NO:25 + SEQ ID NO:27 + SEQ ID NO:29 + SEQ ID NO:31
OLIGONUCLEOTIDE SEQ ID NO:20 + SEQ ID NO:22 + SEQ ID NO:24 + SEQ ID NO:26 + SEQ ID NO:28 + SEQ ID NO:30 + SEQ ID NO:32
ANNEALING STEP
A8
A9
A10
A11
A12
A13
A14
1st LIGATION STEP
LIGATION
LIGATION
LIGATION
A8 + A9
A10
A11 + A12
A13 + A14
2nd LIGATION STEP
LIGATION
LIGATION
A8 + A9 + A10
A11 + A12 + A13 + A14
3rd LIGATION STEP
LIGATION
PCR STEP
XbaI
FULL LENGTH DNA
FULL LENGTH DNA
Bam HI
CLONING STEP FULL LENGTH DNA IS CLONED INTO pET-11d PLASMID VECTOR AT THE XbaI AND Bam HI RESTRICTION SITES

FIG. 3

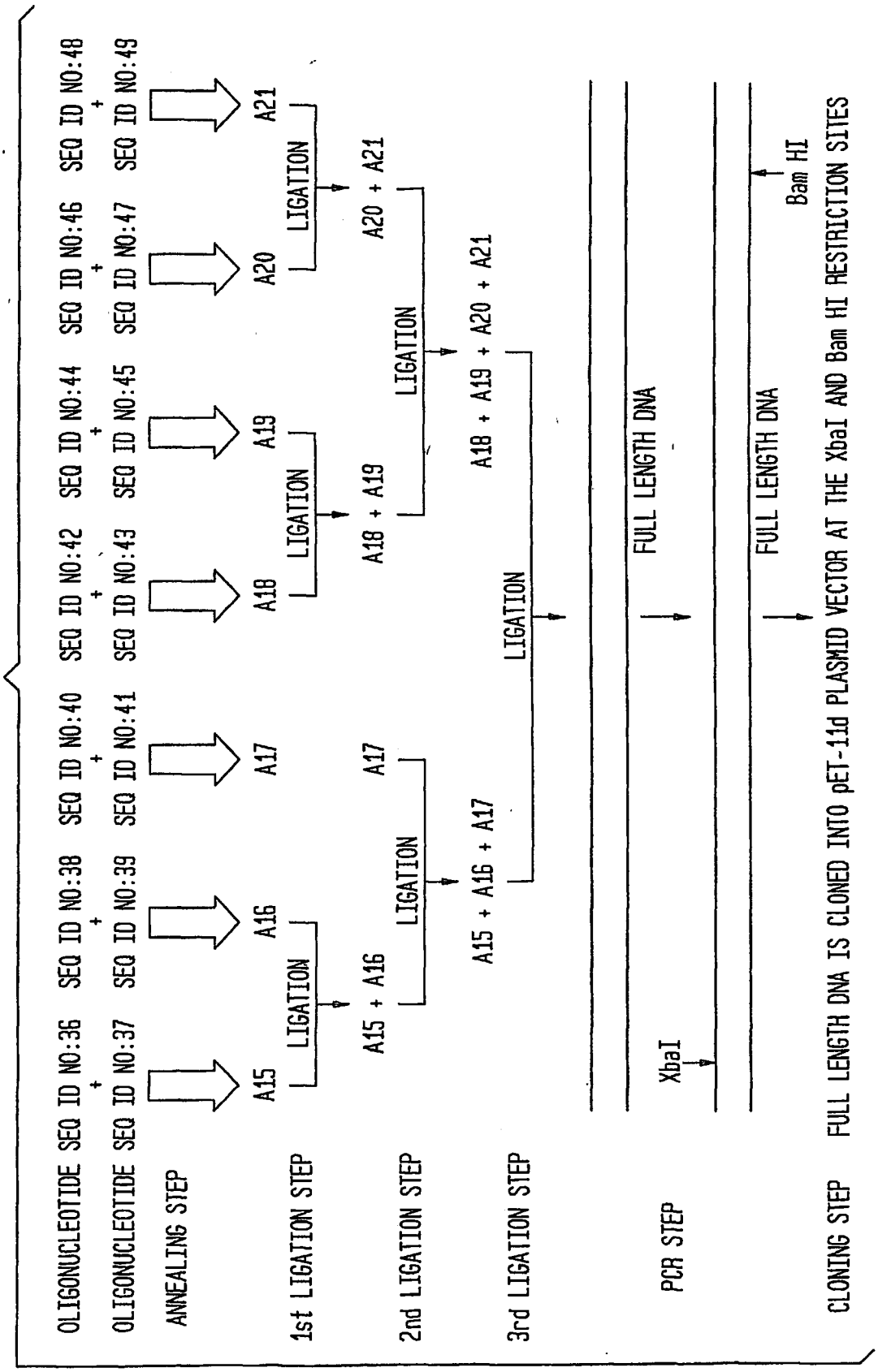

OLIGONUCLEOTIDE SEQ ID NO:36 + SEQ ID NO:38 + SEQ ID NO:40 + SEQ ID NO:42 + SEQ ID NO:44 + SEQ ID NO:46 + SEQ ID NO:48
OLIGONUCLEOTIDE SEQ ID NO:37 + SEQ ID NO:39 + SEQ ID NO:41 + SEQ ID NO:43 + SEQ ID NO:45 + SEQ ID NO:47 + SEQ ID NO:49
ANNEALING STEP
A15
A16
A17
A18
A19
A20
A21
1st LIGATION STEP
LIGATION
A15 + A16
A17
A18 + A19
A20 + A21
2nd LIGATION STEP
LIGATION
A15 + A16 + A17
A18 + A19 + A20 + A21
3rd LIGATION STEP
LIGATION
PCR STEP
XbaI
FULL LENGTH DNA
CLONING STEP
FULL LENGTH DNA
Bam HI
FULL LENGTH DNA IS CLONED INTO pET-11d PLASMID VECTOR AT THE XbaI AND Bam HI RESTRICTION SITES

FIG. 4

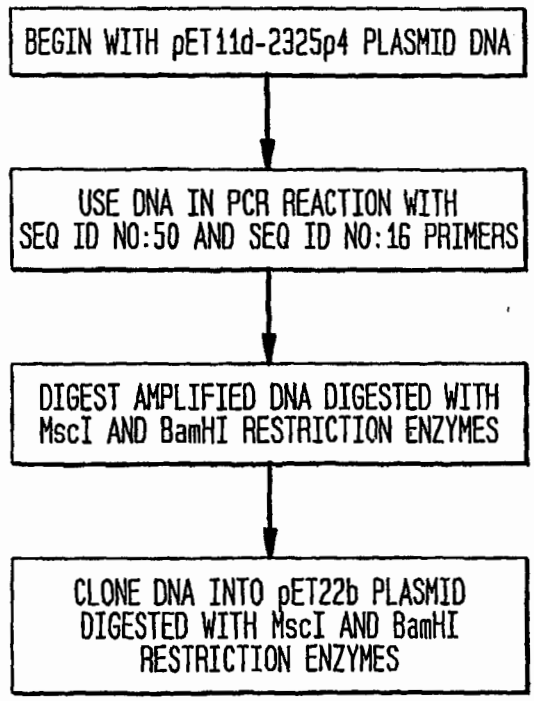

BEGIN WITH pET11d-2325p4 PLASMID DNA
USE DNA IN PCR REACTION WITH SEQ ID NO:50 AND SEQ ID NO:16 PRIMERS
DIGEST AMPLIFIED DNA DIGESTED WITH MscI AND BamHI RESTRICTION ENZYMES
CLONE DNA INTO pET22b PLASMID DIGESTED WITH MscI AND BamHI RESTRICTION ENZYMES

FIG. 5

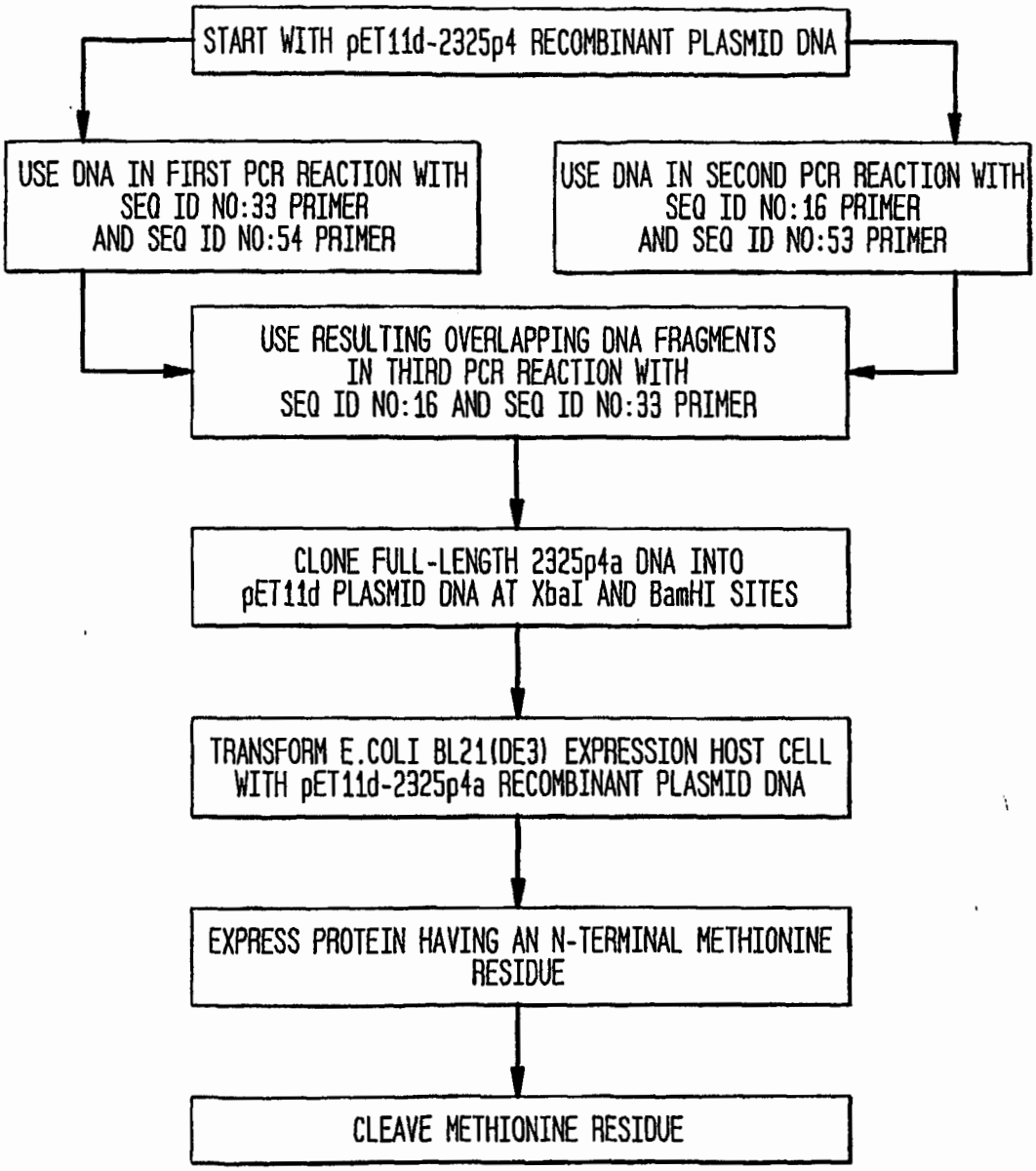

START WITH pET11d-2325p4 RECOMBINANT PLASMID DNA
USE DNA IN FIRST PCR REACTION WITH SEQ ID NO:33 PRIMER AND SEQ ID NO:54 PRIMER
USE DNA IN SECOND PCR REACTION WITH SEQ ID NO:16 PRIMER AND SEQ ID NO:53 PRIMER
USE RESULTING OVERLAPPING DNA FRAGMENTS IN THIRD PCR REACTION WITH SEQ ID NO:16 AND SEQ ID NO:33 PRIMER
CLONE FULL-LENGTH 2325p4a DNA INTO pET11d PLASMID DNA AT XbaI AND BamHI SITES
TRANSFORM E.COLI BL21(DE3) EXPRESSION HOST CELL WITH pET11d-2325p4a RECOMBINANT PLASMID DNA
EXPRESS PROTEIN HAVING AN N-TERMINAL METHIONINE RESIDUE
CLEAVE METHIONINE RESIDUE

FIG. 6

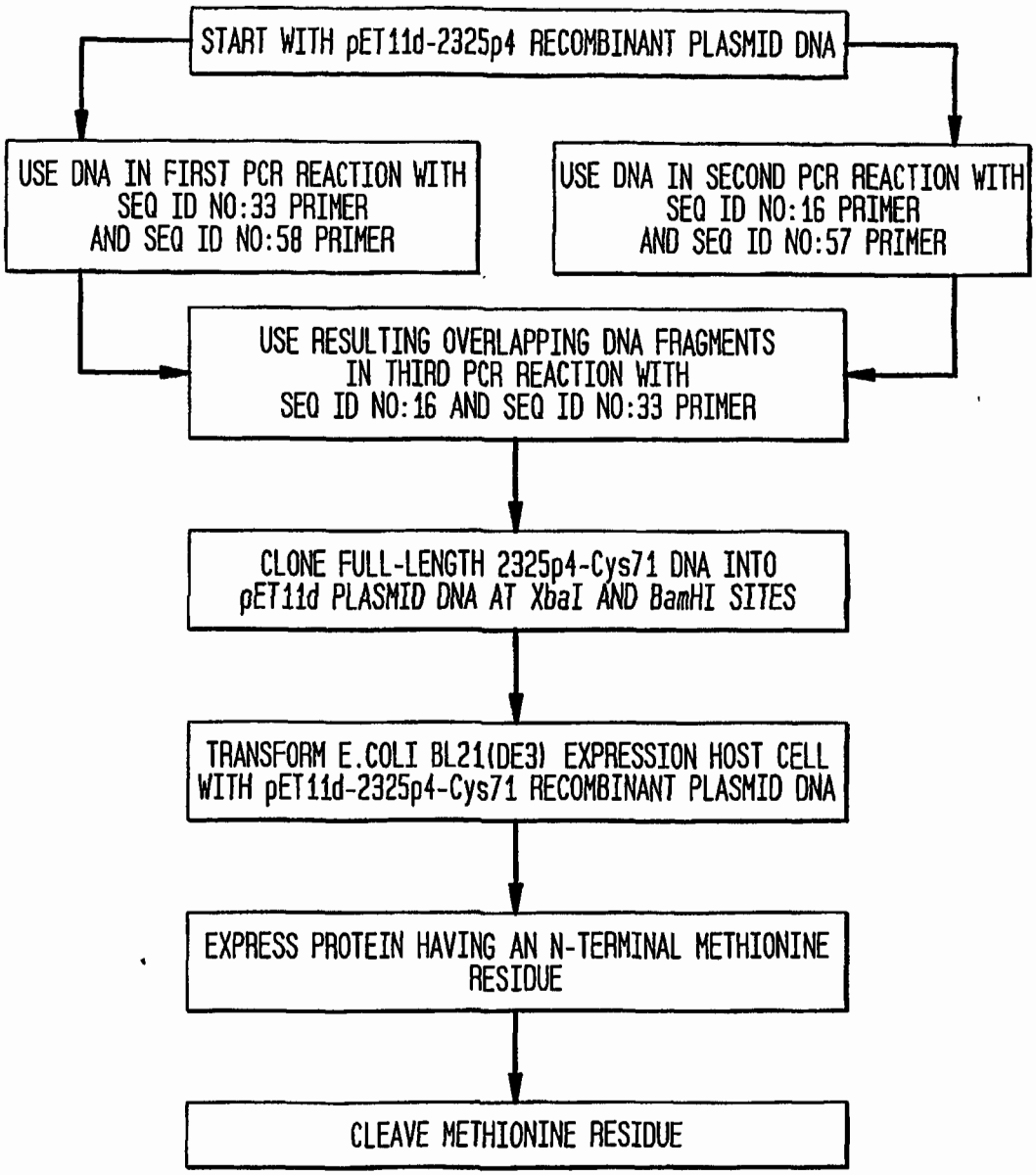
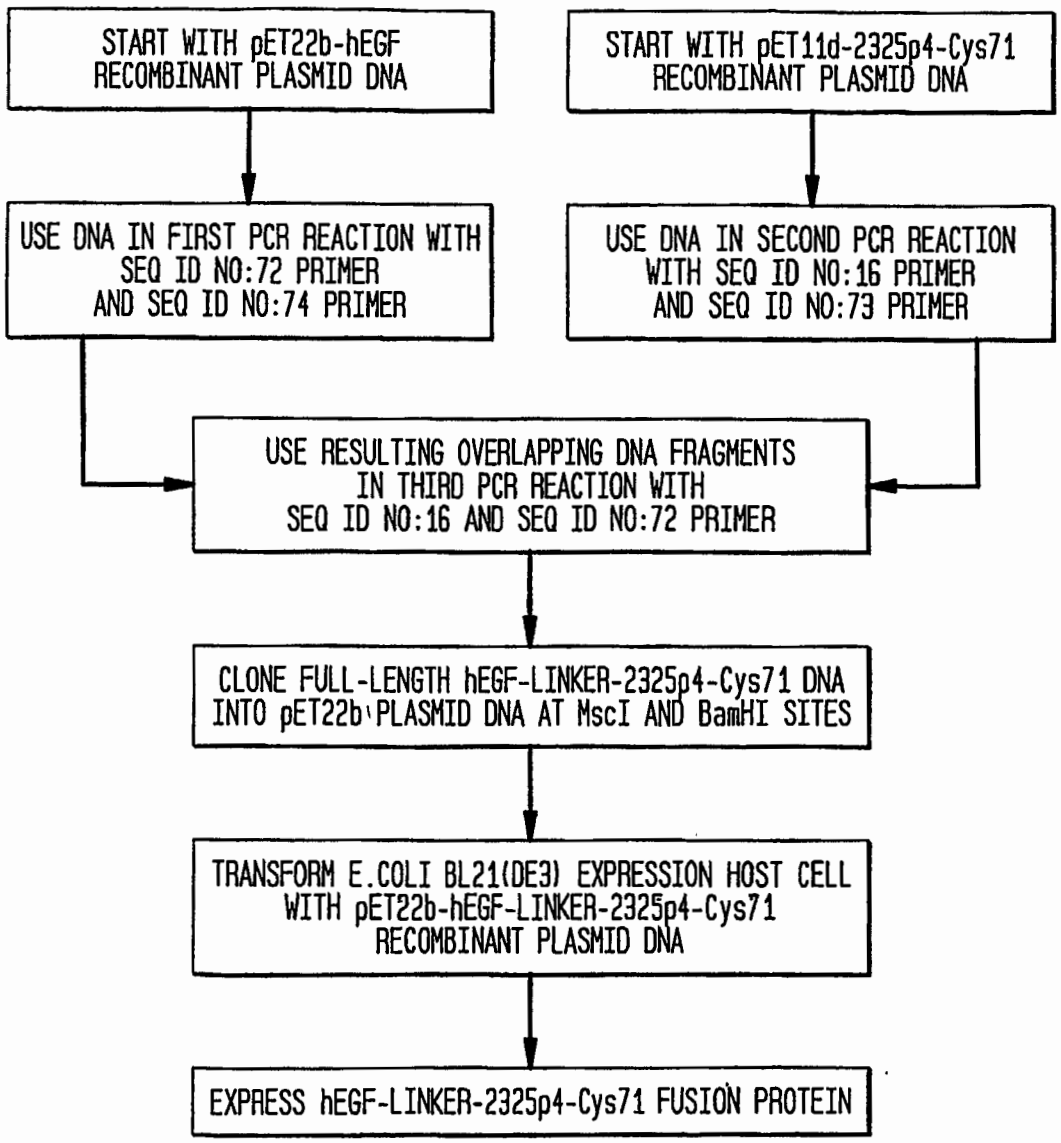

START WITH pET11d-2325p4 RECOMBINANT PLASMID DNA
USE DNA IN FIRST PCR REACTION WITH SEQ ID NO:33 PRIMER AND SEQ ID NO:58 PRIMER
USE DNA IN SECOND PCR REACTION WITH SEQ ID NO:16 PRIMER AND SEQ ID NO:57 PRIMER
USE RESULTING OVERLAPPING DNA FRAGMENTS IN THIRD PCR REACTION WITH SEQ ID NO:16 AND SEQ ID NO:33 PRIMER
CLONE FULL-LENGTH 2325p4-Cys71 DNA INTO pET11d PLASMID DNA AT XbaI AND BamHI SITES
TRANSFORM E.COLI BL21(DE3) EXPRESSION HOST CELL WITH pET11d-2325p4-Cys71 RECOMBINANT PLASMID DNA
EXPRESS PROTEIN HAVING AN N-TERMINAL METHIONINE RESIDUE
CLEAVE METHIONINE RESIDUE

FIG. 7


START WITH pET22b-hEGF RECOMBINANT PLASMID DNA
START WITH pET11d-2325p4-Cys71 RECOMBINANT PLASMID DNA
USE DNA IN FIRST PCR REACTION WITH SEQ ID NO:72 PRIMER AND SEQ ID NO:74 PRIMER
USE DNA IN SECOND PCR REACTION WITH SEQ ID NO:16 PRIMER AND SEQ ID NO:73 PRIMER
USE RESULTING OVERLAPPING DNA FRAGMENTS IN THIRD PCR REACTION WITH SEQ ID NO:16 AND SEQ ID NO:72 PRIMER
CLONE FULL-LENGTH hEGF-LINKER-2325p4-Cys71 DNA INTO pET22b PLASMID DNA AT MscI AND BamHI SITES
TRANSFORM E.COLI BL21(DE3) EXPRESSION HOST CELL WITH pET22b-hEGF-LINKER-2325p4-Cys71 RECOMBINANT PLASMID DNA
EXPRESS hEGF-LINKER-2325p4-Cys71 FUSION PROTEIN