AMENDED 1

WE CLAIM :

1. A vector comprising each of the following elements arranged sequentially to form a module open reading frame, said vector comprising

a) a first restriction site that codes for a first group of amino acids, wherein the first group of amino acids comprises at least two amino acids;

b) a first open reading frame coding for a polypeptide of interest, wherein the coding sequence of the polypeptide of interest is in frame with the coding sequence of at least two amino acids of the first restriction site;

c) a second restriction site that codes for a second group of amino acids, wherein the second group of amino acids comprises at least two amino acids, wherein the coding sequence of the second group of amino acids of the second restriction site is in frame with the coding sequence of the polypeptide of interest of the first open reading frame, and wherein the polynucleotide sequence of the second restriction site is not the same as the polynucleotide sequence of the first restriction site;

d) a spacer polynucleotide sequence, wherein the spacer polynucleotide sequence codes for at least three spacer amino acids, and wherein the coding sequence for the spacer sequence is in frame with the polynucleotide sequence of the second restriction site; and

e) a third restriction site that codes for a third group of amino acids, wherein the third group of amino acids comprises at least two amino acids, wherein the coding sequence of the third group of amino acids of the third restriction site are in frame with the coding sequence of the at least three spacer amino acids and wherein the polynucleotide sequence of the third restriction site is not the same as the polynucleotide sequence of the first restriction site and the third restriction site is not the same as the polynucleotide sequence of the second restriction site,

wherein elements (a) through (e) are in-frame linked to one another to form the module open reading frame,

wherein the first restriction site is susceptible to a restriction enzyme that would produce a first single-stranded overhang comprising at least 2 nucleotides, wherein the second restriction site is susceptible to a restriction enzyme that would produce a second single-stranded overhang comprising at least 2 nucleotides, and

wherein said first and second overhangs are complementary to one another,

whereupon the annealing of the first and second overhangs, the first restriction site and the second restriction site are destroyed.

2. The vector as claimed in claim 1, wherein the polypeptide of interest is a full length protein or polypeptide, a protein functional domain, a protein structural domain, a protein enzymatic domain, a protein inhibition domain, a protein binding domain, a protein localization domain, or an epitope.

3. The vector as claimed in any one of claim 1 or claim 2, wherein said first restriction site comprises a polynucleotide sequence recognized by the NgoM IV restriction enzyme.

4. The vector as claimed in any one of claims 1-3, wherein the second restriction site comprises a polynucleotide sequence recognized by the Xma I restriction enzyme.

5. The vector as claimed in any one of claims 1-4, wherein the third restriction site comprises a polynucleotide sequence recognized by the Cla I restriction enzyme.

6. The vector as claimed in any one of claims 1-5, wherein the spacer polynucleotide sequence codes for three glycine amino acids.

7. The vector as claimed in any one of claims 1-6, wherein at least one additional open reading frame is inserted between the first open reading frame and the second restriction site, wherein each of the at least one additional open reading frames is in frame with both the first open reading frame and the second restriction group, and wherein each

of the additional open reading frames codes for at least one additional polypeptide of interest.

8. The vector as claimed in claim 7, wherein there are no restriction sites in any of the open reading frames between the first and second restriction groups that occur in either the first or second restriction sites.

9. A method of making a fusion polypeptide comprising

a) digesting the vector as claimed in any one of claims 3 to 8 with restriction enzymes that recognize the second and third restriction sites to create a linear vector with a first and second singlestranded overhang, wherein the first and second overhangs are incompatible with one another;

b) providing a first insert, wherein the first insert comprises at least one additional open reading frame, wherein the at least one additional open reading frame comprises 5' and 3' overhangs that are compatible with the first and second overhangs, wherein each of the at least one additional open reading frames codes for at least one additional polypeptide of interest;

c) ligating the first insert into the linear vector to form a ligated expression vector, wherein the ligation of the 5' overhang of the at least one open reading frame anneals with the first singlestranded overhang of the linear vector, wherein the ligation of the 3' overhang of the at least one open reading frame anneals with the second singlestranded overhang of the linear vector, and wherein the ligation of the 3' overhang recreates the third restriction site,

wherein the first open reading frame and the at least one additional open reading frames are ligated in frame with one another.

10. A method of making fusion protein DNA constructs comprising,

a) providing first and second polynucleotide reagents, wherein each reagent contains an open reading frame of interest, and wherein each open reading frame of interest is flanked by at least 3 predetermined restriction endonuclease sites, wherein the

first restriction site is susceptible to a restriction enzyme that would produce a first single-stranded overhang comprising at least 2 nucleotides, wherein the second restriction site is susceptible to a restriction enzyme that would produce a second single-stranded overhang comprising at least 2 nucleotides, and wherein said first and second overhangs are complementary to one another, whereupon the annealing of the first and second overhangs, the first restriction site and the second restriction site are destroyed.

b) digesting the first polynucleotide reagent with 2 different restriction endonucleases that cleave at 2 of the predetermined restriction endonuclease sites, generating a polynucleotide with 2 different overhangs,

c) digesting the second polynucleotide reagent with 2 different restriction endonucleases that cleave at 2 of the predetermined restriction endonuclease sites, wherein a polynucleotide containing an open reading frame of interest is released, wherein the released polynucleotide has 2 different overhangs, and wherein one of the overhangs is compatible with an overhang of the polynucleotide digested in step b),

d) mixing together, annealing, and ligating the polynucleotide generated in step b) and the released polynucleotide containing an open reading frame of interest of step c), wherein a third polynucleotide containing a fusion of open reading frames is generated, wherein a junction between the open reading frames of interest is no longer susceptible to digestion with any of the endonucleases that cut at the 3 predetermined restriction sites, and wherein sequences flanking the fusion of open reading frames of interest contain the 3 predetermined restriction sites.

11. A method of making fusion protein DNA constructs comprising,

a) providing first and second polynucleotide reagents, wherein each reagent contains an open reading frame of interest, and wherein each open reading frame of interest is flanked by at least 3 predetermined restriction endonuclease sites, wherein the first restriction site is susceptible to a restriction enzyme that would produce a first single-stranded overhang comprising at least 2 nucleotides, wherein the second restriction site is susceptible to a restriction enzyme that would produce a second single-stranded overhang

comprising at least 2 nucleotides, and wherein said first and second overhangs are complementary to one another, whereupon the annealing of the first and second overhangs, the first restriction site and the second restriction site are destroyed,

b) digesting the first polynucleotide reagent with 2 different restriction endonucleases that cleave at 2 of the predetermined restriction endonuclease sites, generating a polynucleotide with 2 incompatible overhangs,

c) digesting the second polynucleotide reagent with 2 different restriction endonucleases that cleave at 2 of the predetermined restriction endonuclease sites, wherein 1 of the restriction endonucleases is identical to a restriction endonuclease of step b), and wherein a polynucleotide containing an open reading frame of interest is released, and wherein the released polynucleotide has 2 incompatible overhangs,

d) mixing together, annealing, and ligating the polynucleotide generated in step b) and the released polynucleotide containing an open reading frame of interest of step c), wherein a third polynucleotide containing a fusion of open reading frames is generated, wherein the fusion of open reading frames of interest is flanked by said predetermined restriction sites, and wherein a junction between the open reading frames of interest lacks said 3 predetermined restriction endonuclease sites.

12. The method as claimed in claims 10 or 11, wherein the predetermined restriction sites are sequences cleavable by NgoM IV, Xma I, and Cla I.

Dated 5th May, 2008.

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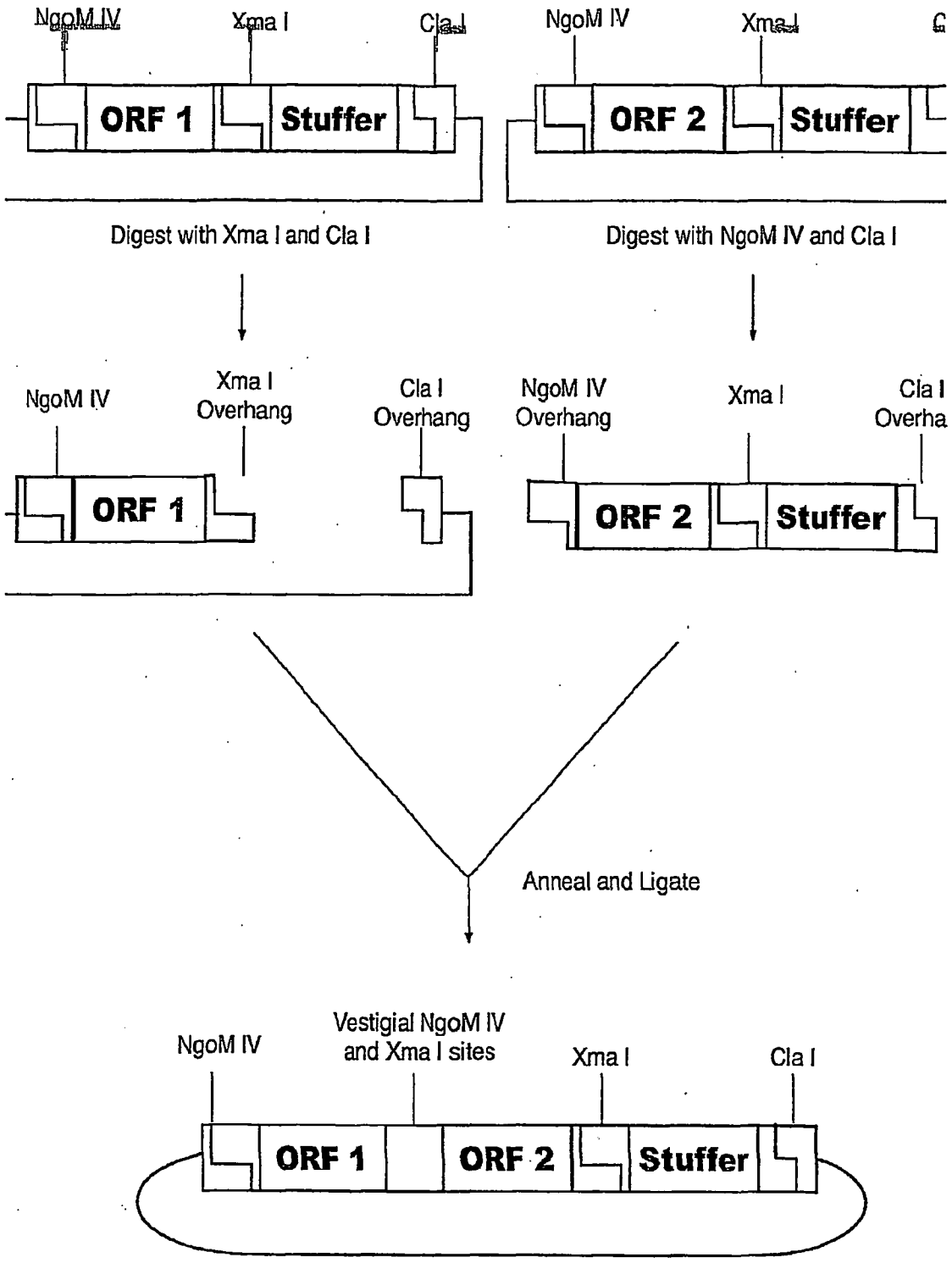

FIGURE 1

S. D. AHUJA
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APPLICANT'S AGENT

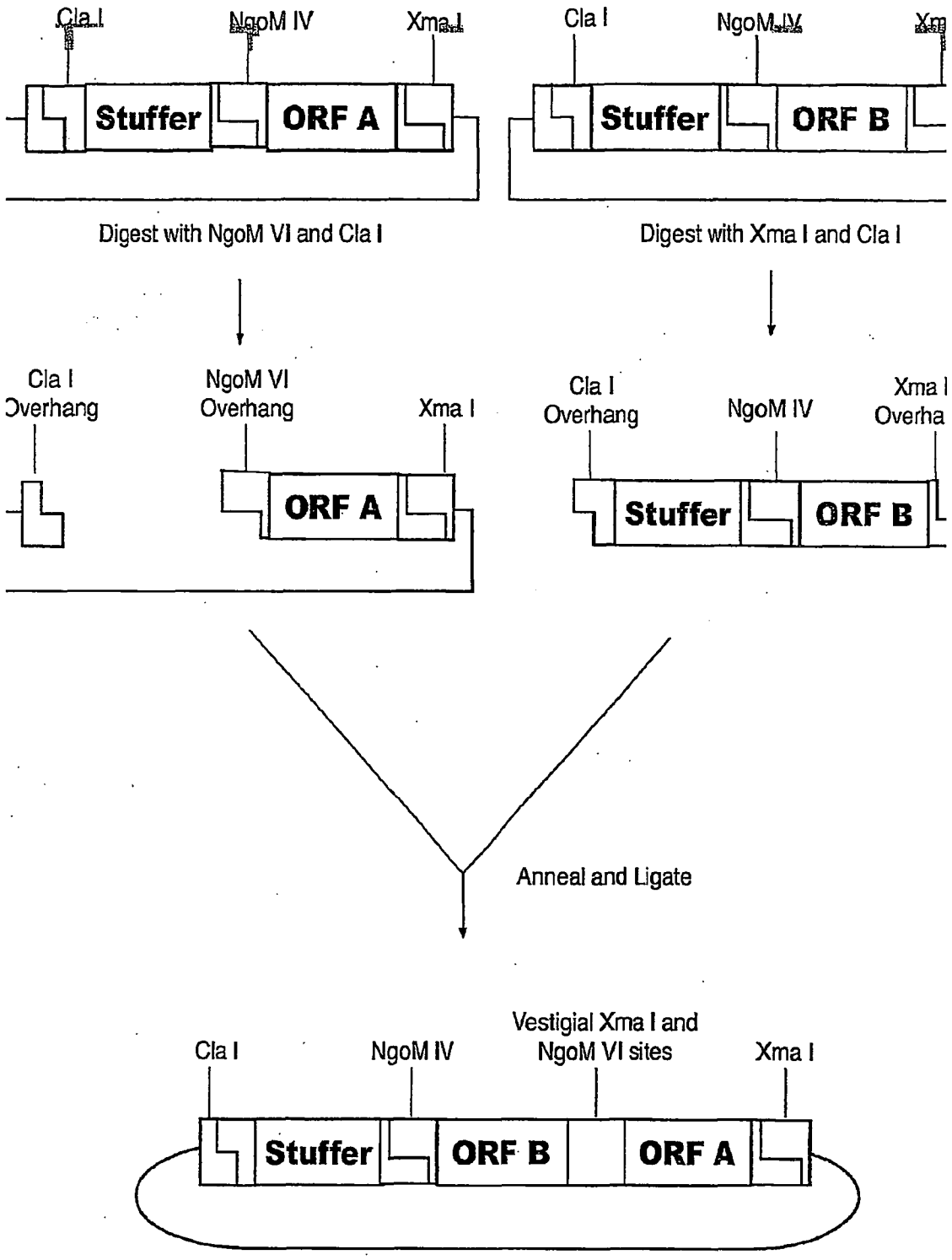

FIGURE 2

S. D. AHUJA
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APPLICANT'S AGENT

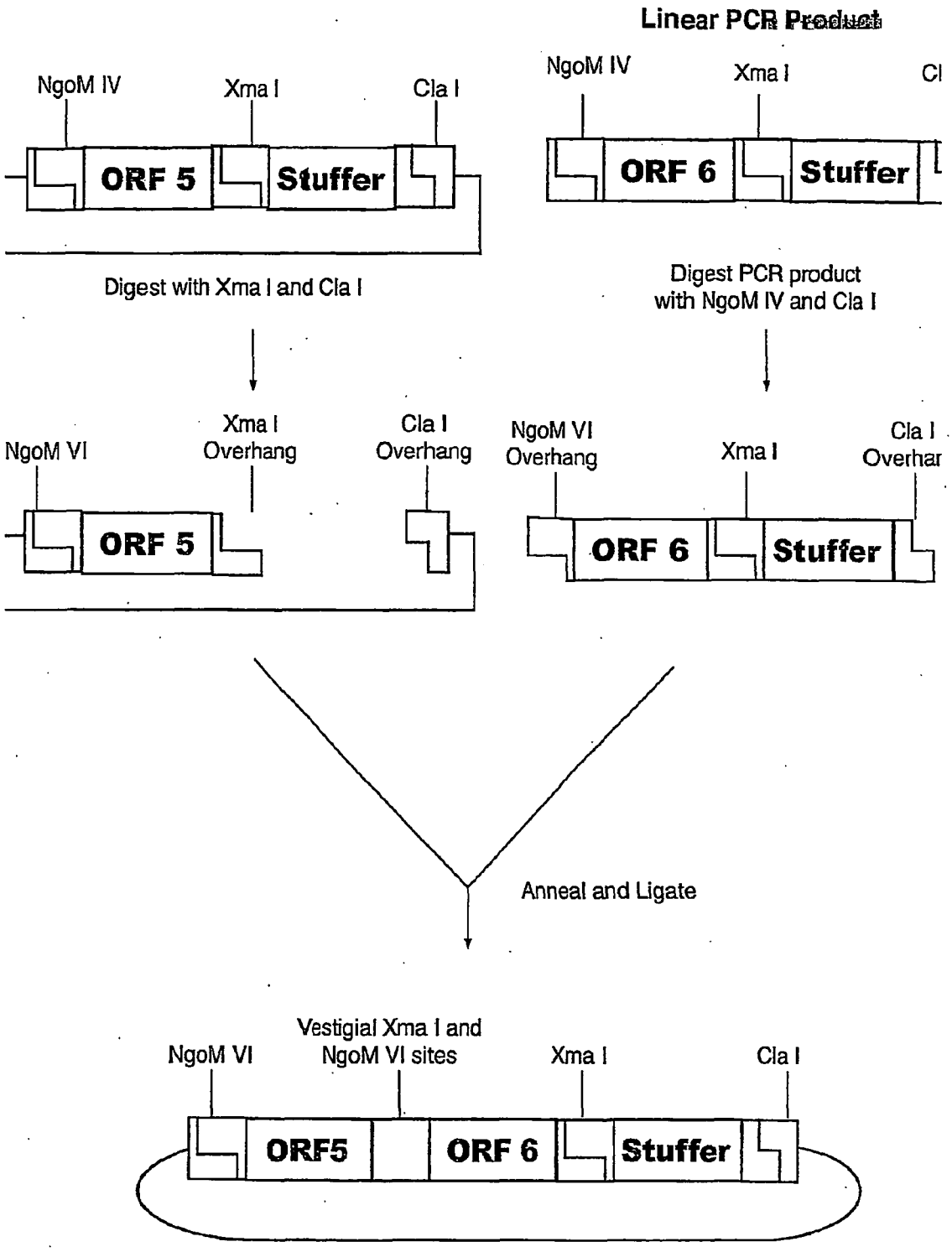

FIGURE 3

S. D. AHUJA
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APPLICANT'S AGENT

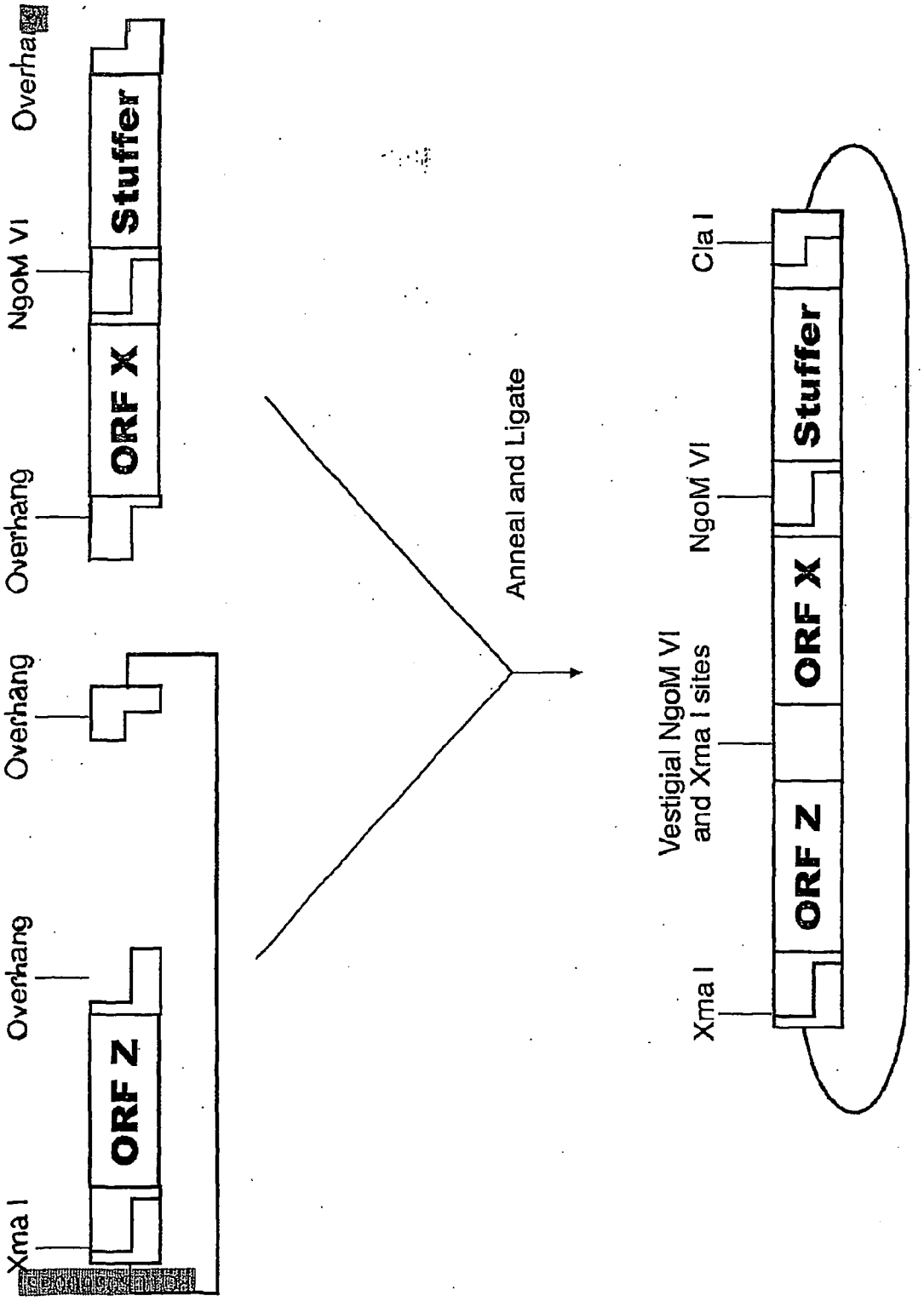

FIGURE 4

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

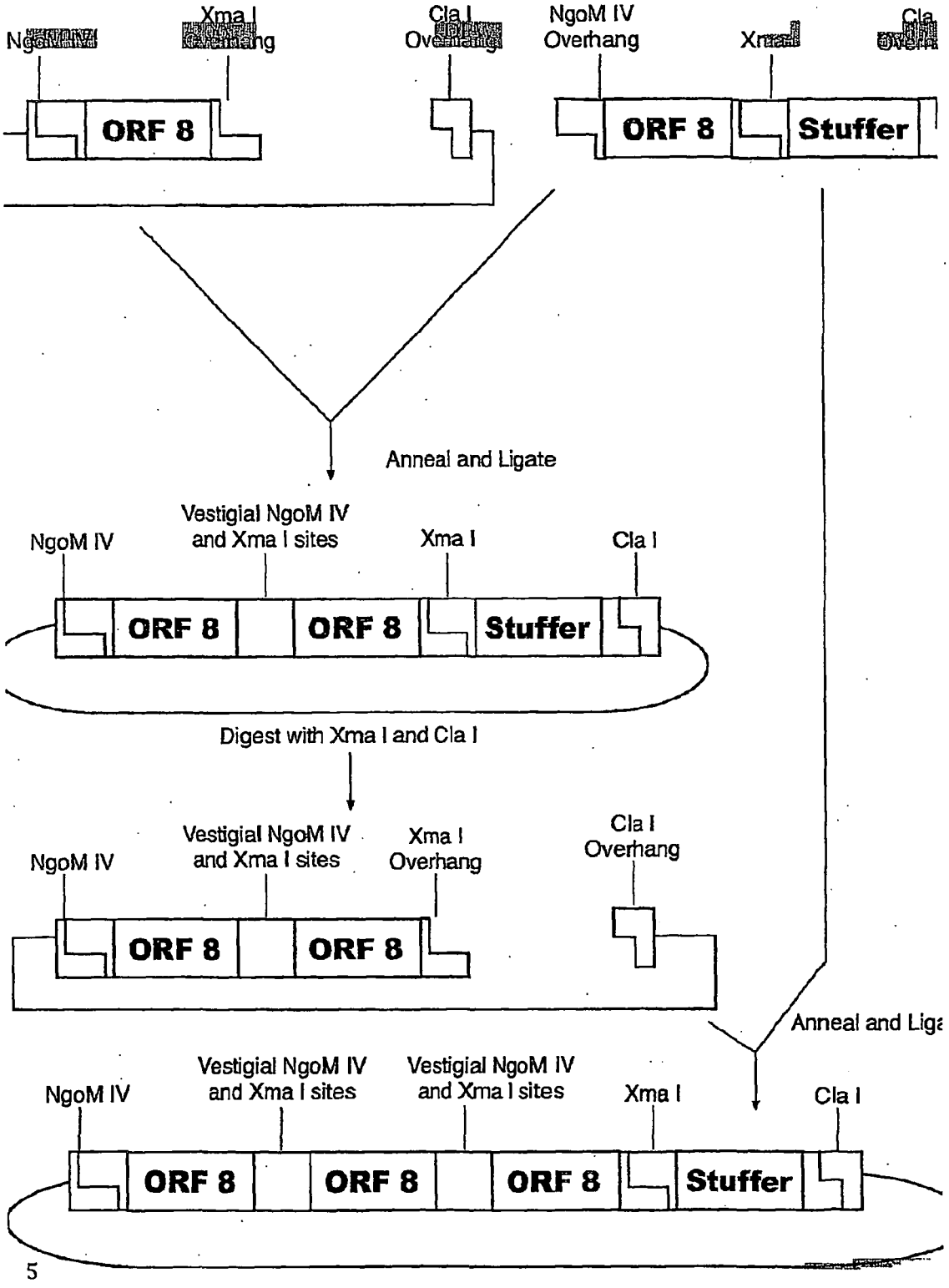

FIGURE 5

S. D. AHUJA
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APPLICANT'S AGENT

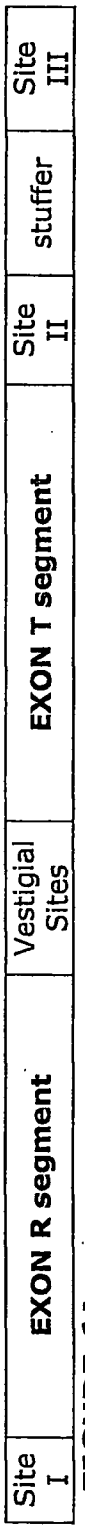

FIGURE 6A

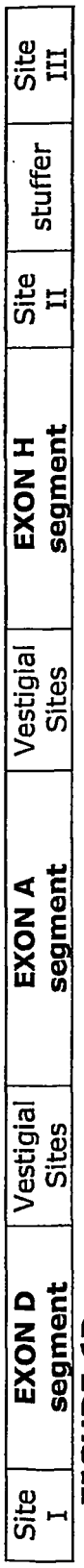

FIGURE 6B

FIGURE 6C

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Site I	**ORF Y**	Vestigial Sites	**ORF Z**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7A

Site I	**LOCALIZATION SIGNAL**	Vestigial Sites	**PEPTIDE 3**	Vestigial Sites	**PEPTIDE 4**	Vestigial Sites	**PEPTIDE 5**	Site II	stuffer	Site III

FIGURE 7B

Site I	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7C

S. D. AHUJA
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APPLICANT'S AGENT

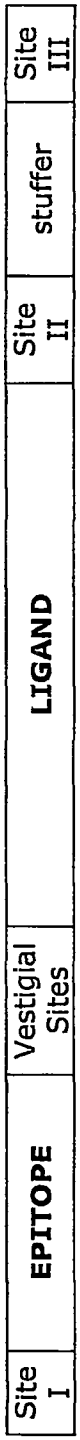

FIGURE 8A

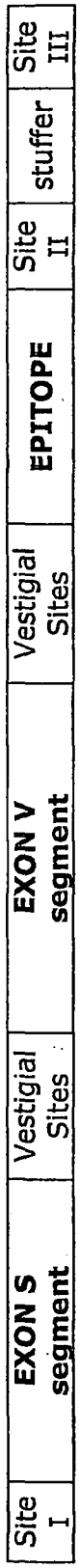

FIGURE 8B

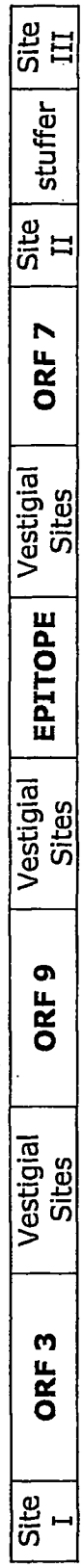

FIGURE 8C

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

FIGURE 9A

FIGURE 9B

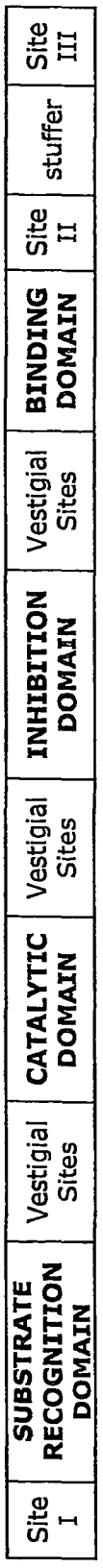

FIGURE 9C

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FIGURE 10

S. D. AHUJA
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APPLICANT'S AGENT

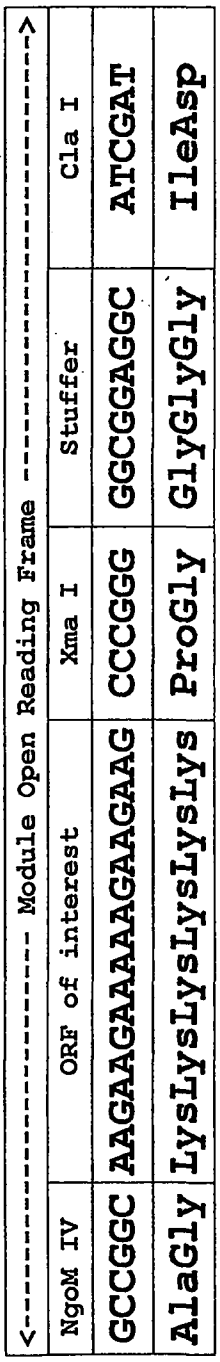

FIGURE 11

S. D. AHUJA
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Site I	Phosphorylation Domain	Site II	Stuffer	Site III
Site I	Zinc Finger Region	Site II	Stuffer	Site III
Site I	Peptide Inhibitor	Site II	Stuffer	Site III
Site I	Hormone Binding Domain	Site II	Stuffer	Site III
Site I	Epitope	Site II	Stuffer	Site III
Site I	Dimerization Domain	Site II	Stuffer	Site III
Site I	Lipid Binding Domain	Site II	Stuffer	Site III
Site I	Glycosylation Domain	Site II	Stuffer	Site III
Site I	Peptide Hormone	Site II	Stuffer	Site III
Site I	Ion Pore Region	Site II	Stuffer	Site III
Site I	Reporter Peptide	Site II	Stuffer	Site III

Site I	Catalytic Domain	Site II	Stuffer	Site III
Site I	DNA Binding Domain	Site II	Stuffer	Site III
Site I	Leucine Zipper Region	Site II	Stuffer	Site III
Site I	Transmembrane Domain	Site II	Stuffer	Site III
Site I	Activation Domain	Site II	Stuffer	Site III
Site I	Inhbition Domain	Site II	Stuffer	Site III
Site I	G Protein Binding Region	Site II	Stuffer	Site III
Site I	Highly Acidic Region	Site II	Stuffer	Site III
Site I	Ligand	Site II	Stuffer	Site III
Site I	Protein Subunit	Site II	Stuffer	Site III
Site I	Localization Signal	Site II	Stuffer	Site III

FIGURE 12

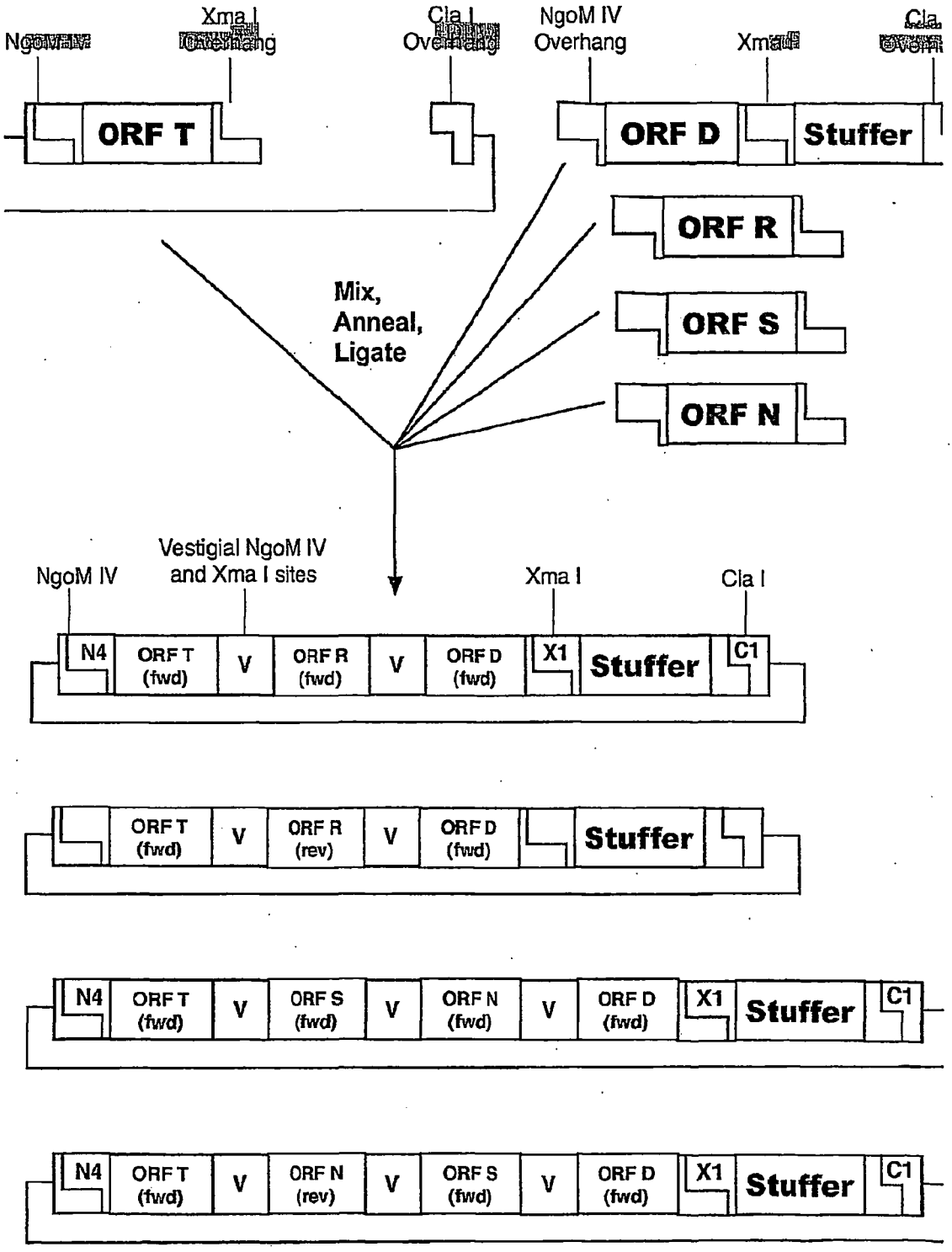

FIGURE 13

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APPLICANT'S AGENT

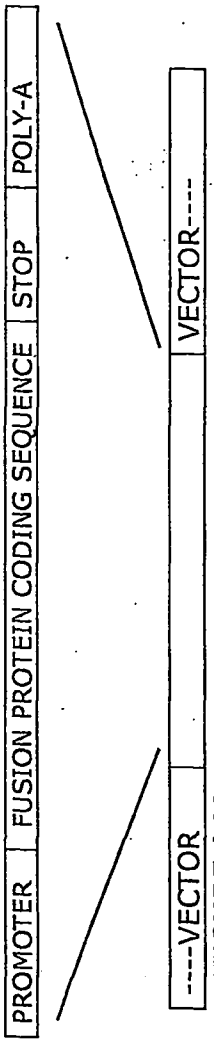

FIGURE 14A

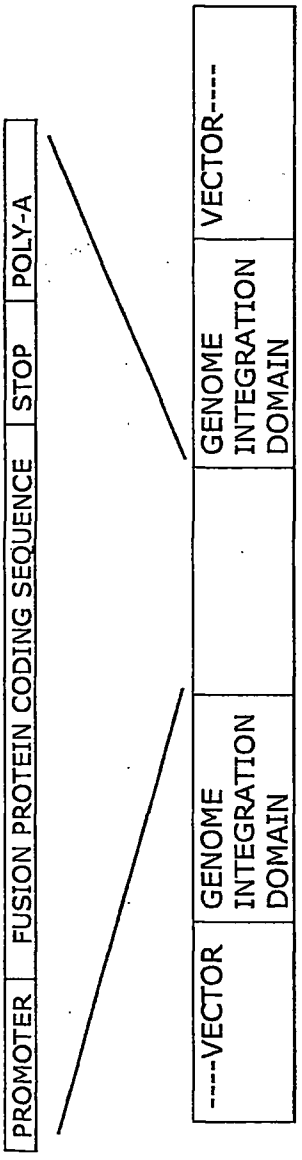

FIGURE 14B

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APPLICANT'S AGENT

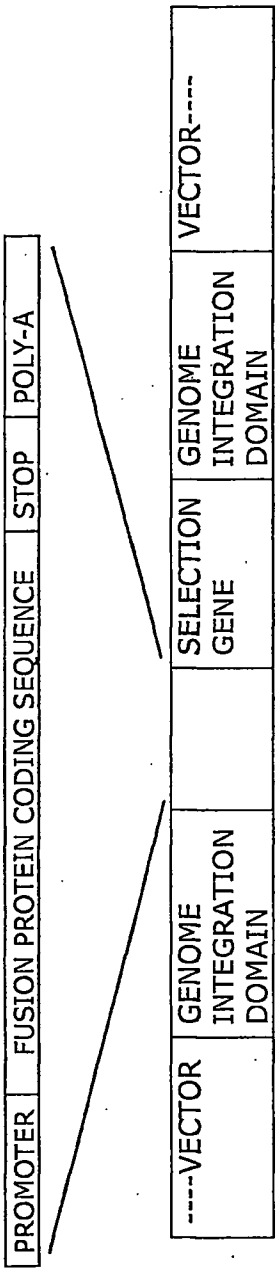


FIGURE 14C

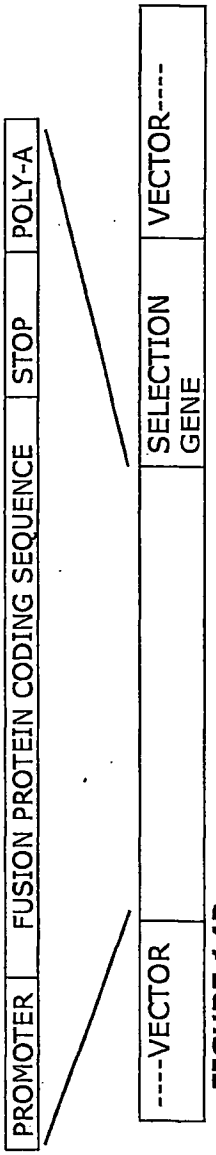


FIGURE 14D

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

AMENDED 1

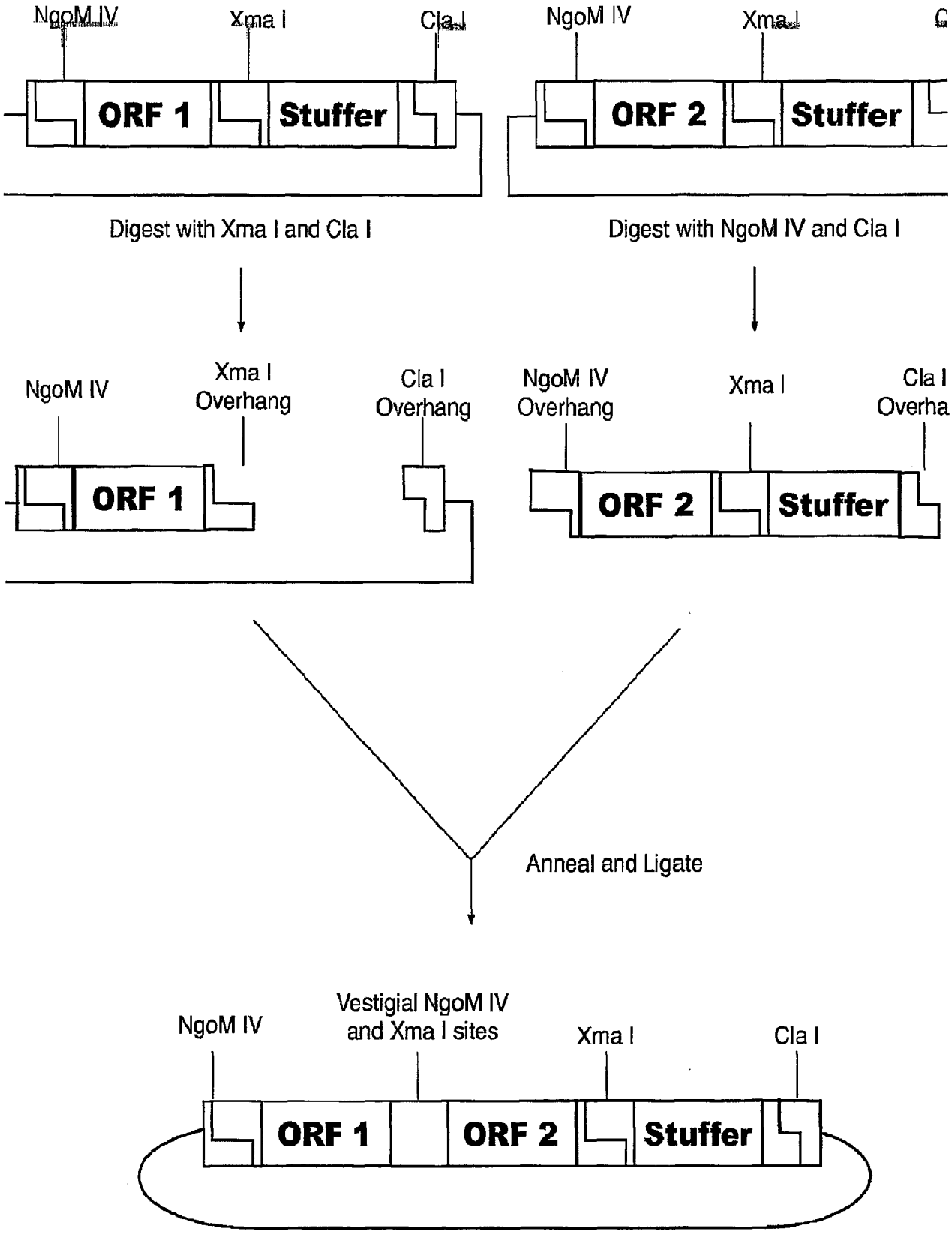
PATENT APPLICATION NO.: 1806/KOLNP/2008

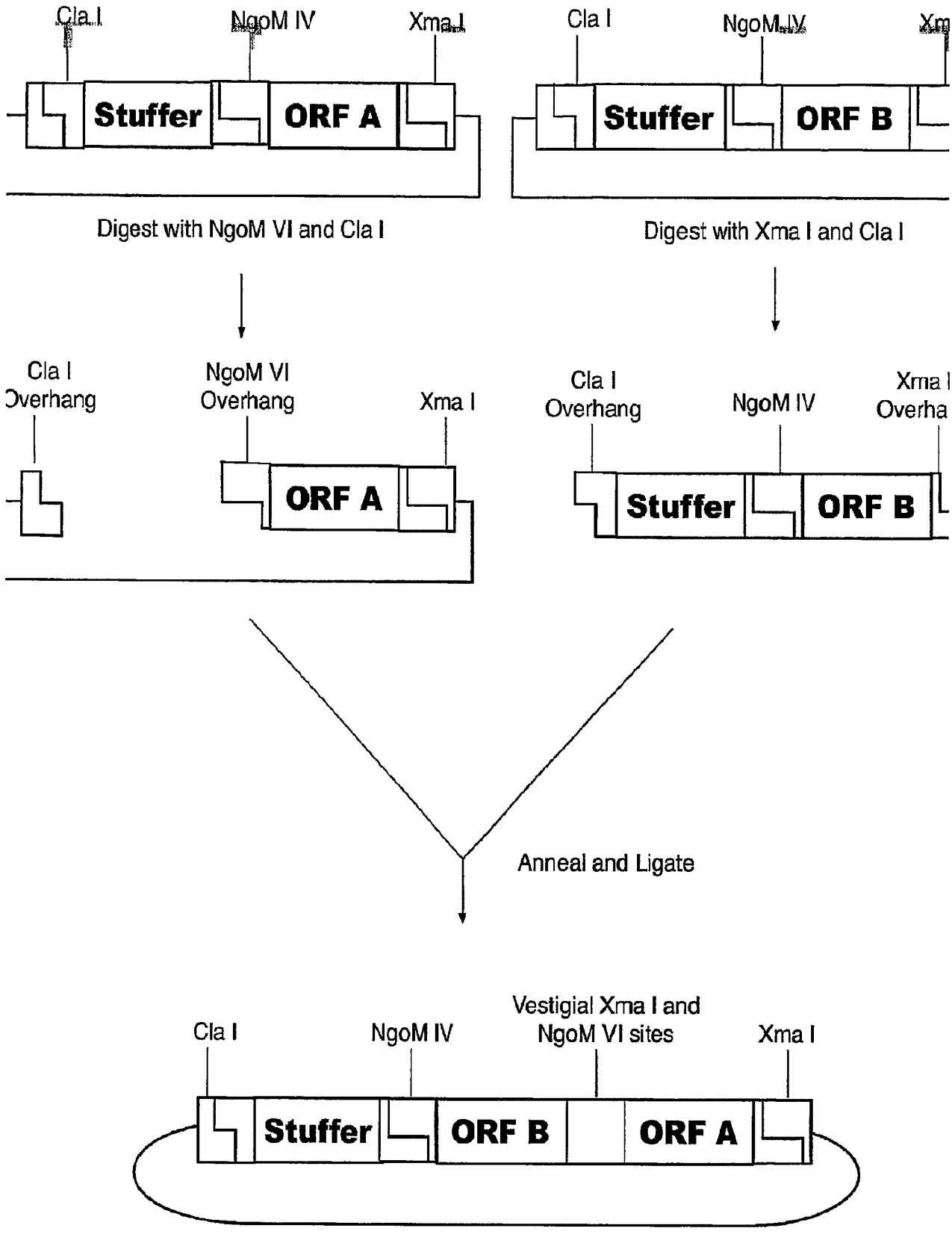
ABSTRACT

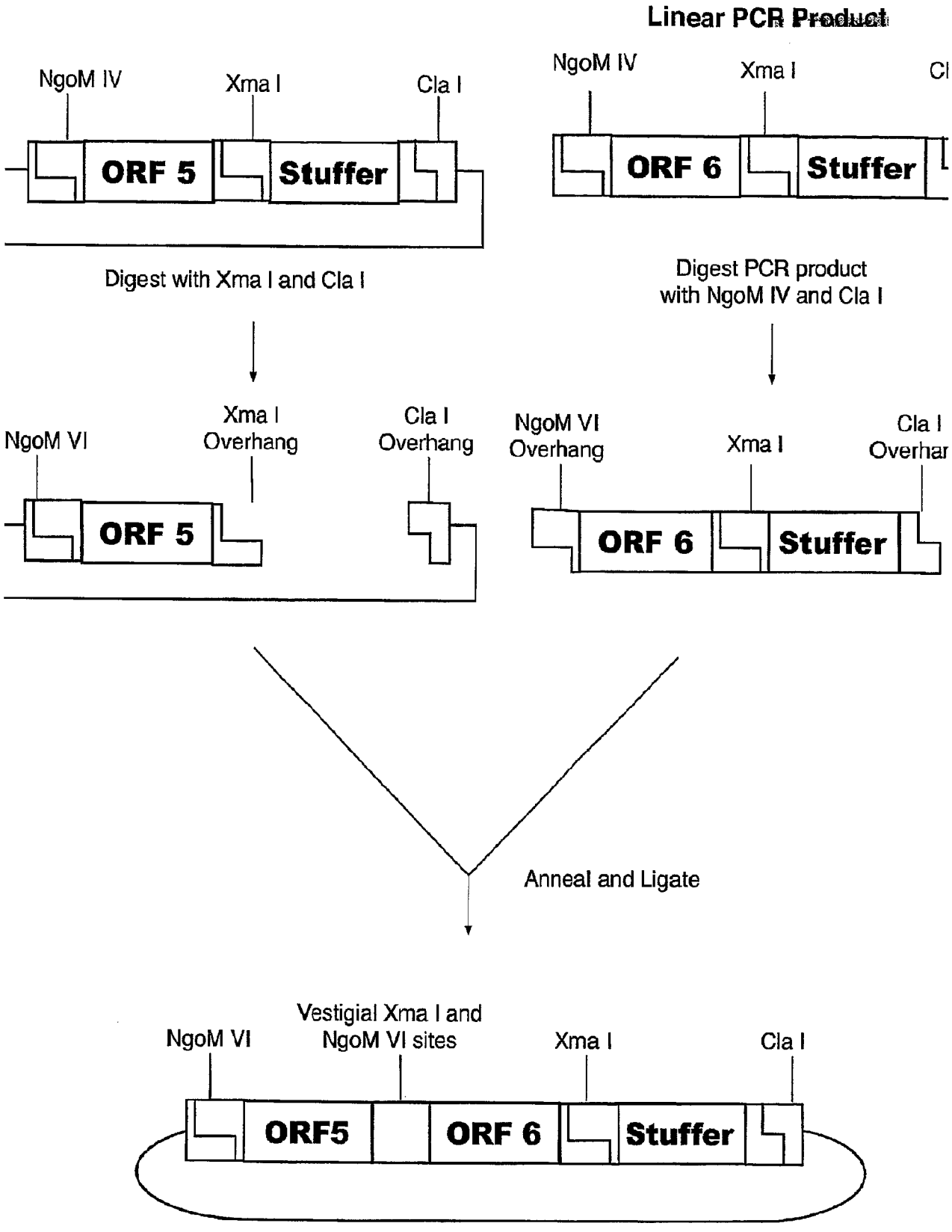
TITLE: METHODS OF MAKING MODULAR FUSION PROTEIN EXPRESSION PRODUCTS

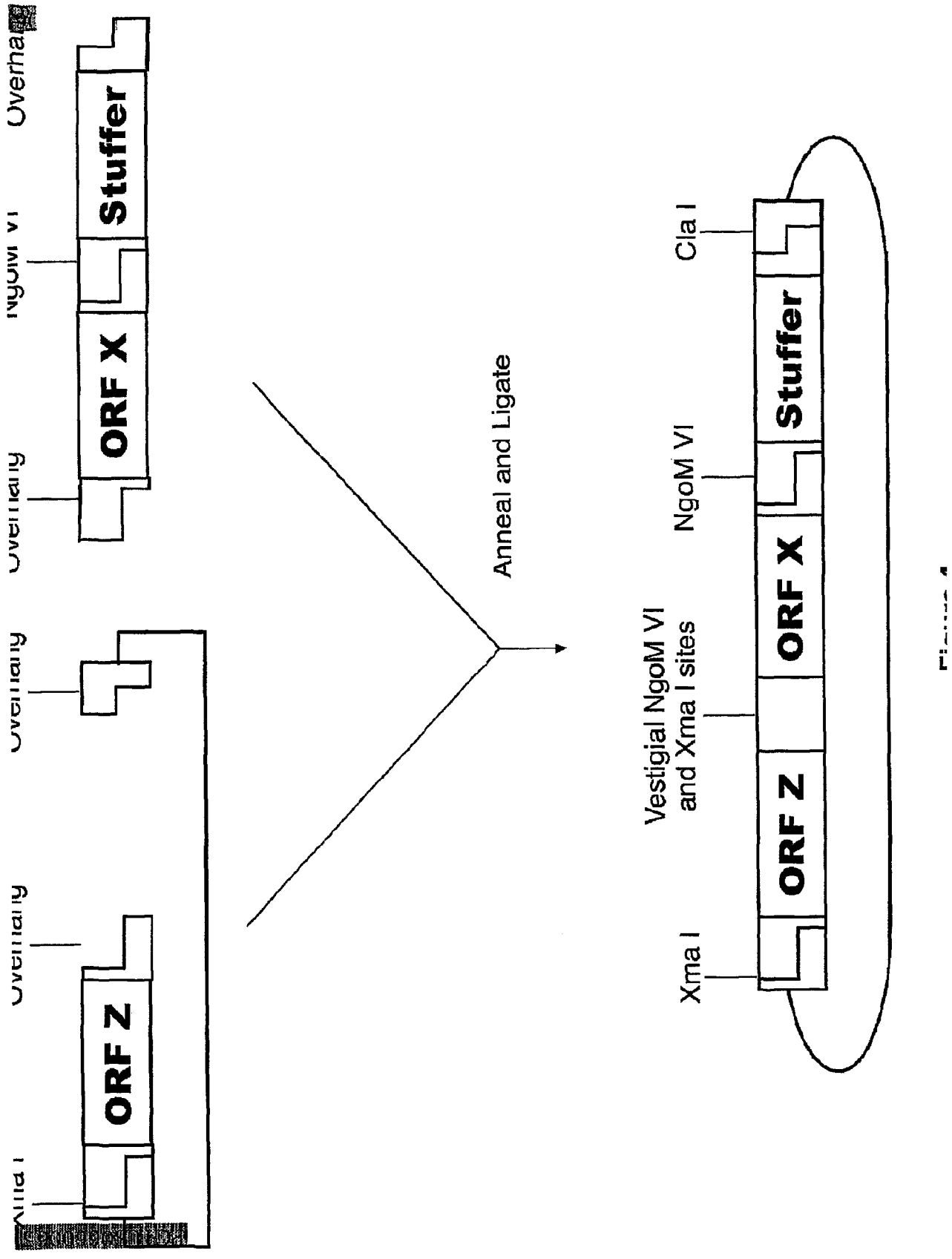
A vector comprising each of the following elements arranged sequentially to form a module open reading frame, said vector comprising a) a first restriction site, b) a first open reading frame coding for a polypeptide of interest, c) a second restriction site that codes for a second group of amino acids, d) a spacer polynucleotide sequence, and e) a third restriction site that codes for a third group of amino acids, wherein elements (a) through (e) are in-frame linked to one another to form the module open reading frame, wherein the first restriction site is susceptible to a restriction enzyme that would produce a first single-stranded overhang comprising at least 2 nucleotides, wherein the second restriction site is susceptible to a restriction enzyme that would produce a second single-stranded overhang comprising at least 2 nucleotides, and wherein said first and second overhangs are complementary to one another, whereupon the annealing of the first and second overhangs, the first restriction site and the second restriction site are destroyed. The present invention also relates to a method of making a fusion polypeptide.

Fig. 1


NgoM IV
Xma I
Cla I
ORF 1
Stuffer
NgoM IV
Xma I
ORF 2
Stuffer
Digest with Xma I and Cla I
Digest with NgoM IV and Cla I
NgoM IV
Xma I Overhang
Cla I Overhang
ORF 1
NgoM IV Overhang
Xma I
Cla I Overha
ORF 2
Stuffer
Anneal and Ligate
NgoM IV
Vestigial NgoM IV and Xma I sites
Xma I
Cla I
ORF 1
ORF 2
Stuffer


Cla I
NgoM IV
Xma I
Stuffer
ORF A
Digest with NgoM VI and Cla I
Cla I
Overhang
NgoM VI
Overhang
Xma I
ORF A
Cla I
NgoM IV
Stuffer
ORF B
Digest with Xma I and Cla I
Cla I
Overhang
NgoM IV
Xma I
Overha
Stuffer
ORF B
Anneal and Ligate
Cla I
NgoM IV
Vestigial Xma I and
NgoM VI sites
Xma I
Stuffer
ORF B
ORF A


Linear PCR Product
NgoM IV
Xma I
Cla I
ORF 5
Stuffer
NgoM IV
Xma I
Cl
ORF 6
Stuffer
Digest with Xma I and Cla I
Digest PCR product
with NgoM IV and Cla I
NgoM VI
Xma I
Overhang
Cla I
Overhang
ORF 5
NgoM VI
Overhang
Xma I
Cla I
Overhar
ORF 6
Stuffer
Anneal and Ligate
NgoM VI
Vestigial Xma I and
NgoM VI sites
Xma I
Cla I
ORF5
ORF 6
Stuffer


ORF Z
ORF X
Stuffer
Anneal and Ligate
Xma I
Vestigial NgoM VI and Xma I sites
NgoM VI
Cla I

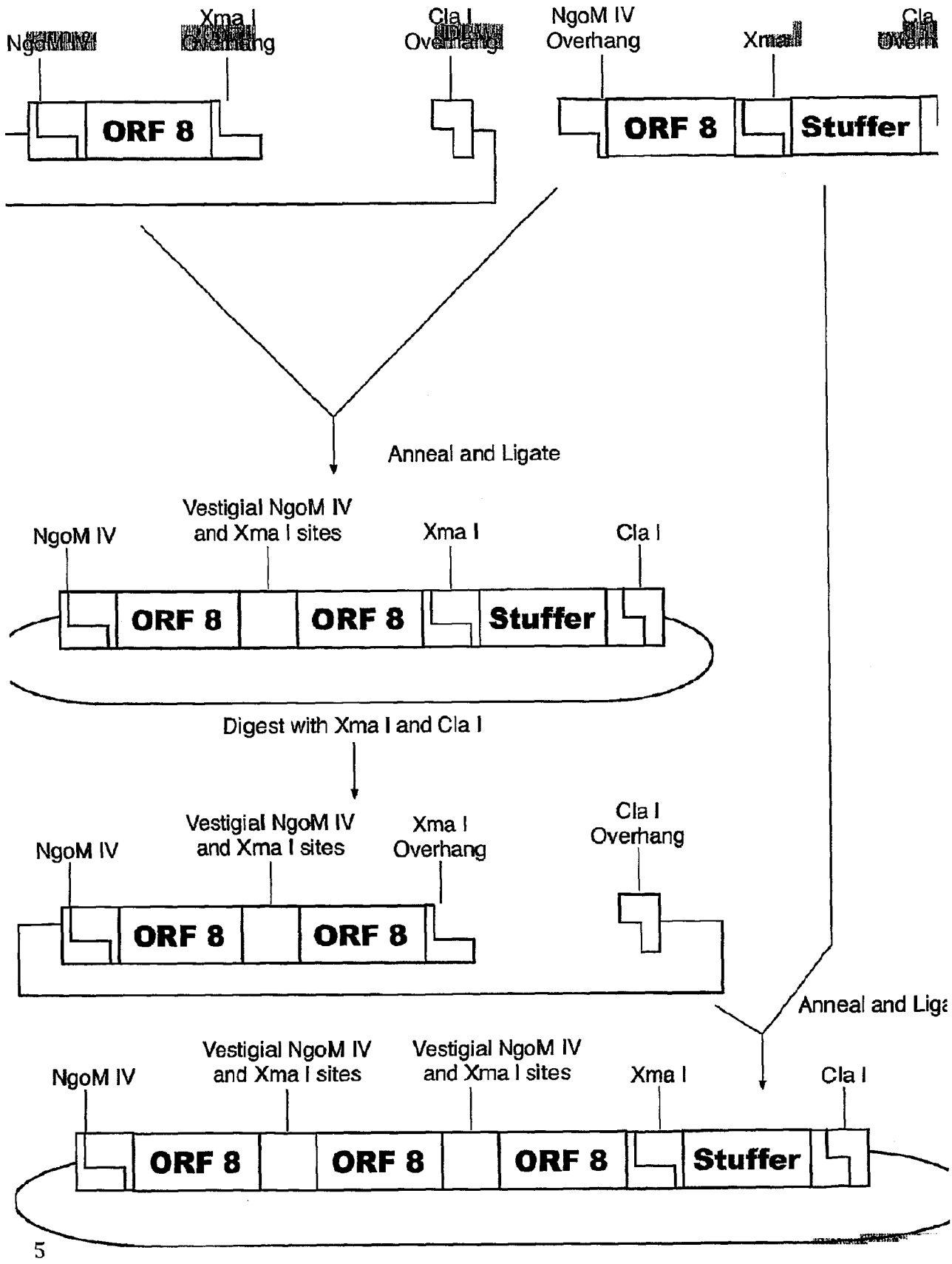

NgoM IV
Xma I
Cla I
NgoM IV Overhang
Xma I
ORF 8
ORF 8
Stuffer
Anneal and Ligate
Vestigial NgoM IV and Xma I sites
NgoM IV
Xma I
Cla I
ORF 8
ORF 8
Stuffer
Digest with Xma I and Cla I
Vestigial NgoM IV and Xma I sites
NgoM IV
Xma I Overhang
Cla I Overhang
ORF 8
ORF 8
Anneal and Liga
Vestigial NgoM IV and Xma I sites
Vestigial NgoM IV and Xma I sites
NgoM IV
Xma I
Cla I
ORF 8
ORF 8
ORF 8
Stuffer

FIGURE 6A

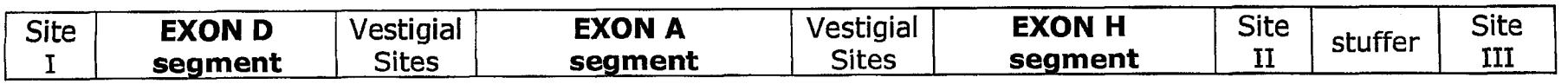


FIGURE 6B

FIGURE 6C

Site I	**ORF Y**	Vestigial Sites	**ORF Z**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7A

Site I	**LOCALIZATION SIGNAL**	Vestigial Sites	**PEPTIDE 3**	Vestigial Sites	**PEPTIDE 4**	Vestigial Sites	**PEPTIDE 5**	Site II	stuffer	Site III

FIGURE 7B

Site I	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7C

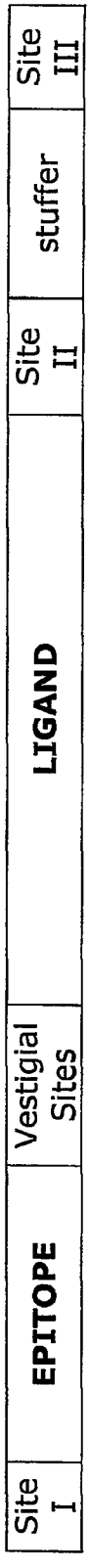


FIGURE 8A

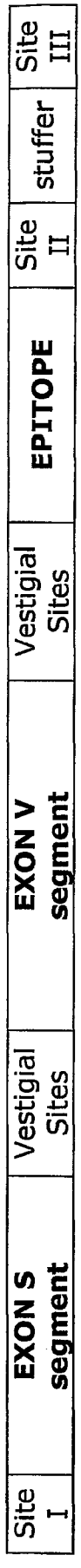


FIGURE 8B

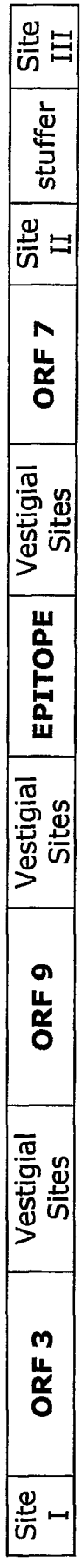


FIGURE 8C

Site I	**CYTOPLASMIC DOMAIN**	Vestigial Sites	**TRANSMEMBRANE DOMAIN**	Vestigial Sites	**EXTRACELLULAR DOMAIN**	Site II	stuffer	Site III

FIGURE 9A

Site I	**DNA BINDING DOMAIN**	Vestigial Sites	**HORMONE BINDING DOMAIN**	Vestigial Sites	**DIMERIZATION DOMAIN**	Vestigial Sites	**ACTIVATION DOMAIN**	Site II	stuffer	Site III

FIGURE 9B

Site I	**SUBSTRATE RECOGNITION DOMAIN**	Vestigial Sites	**CATALYTIC DOMAIN**	Vestigial Sites	**INHIBITION DOMAIN**	Vestigial Sites	**BINDING DOMAIN**	Site II	stuffer	Site III

FIGURE 9C

FIGURE 10

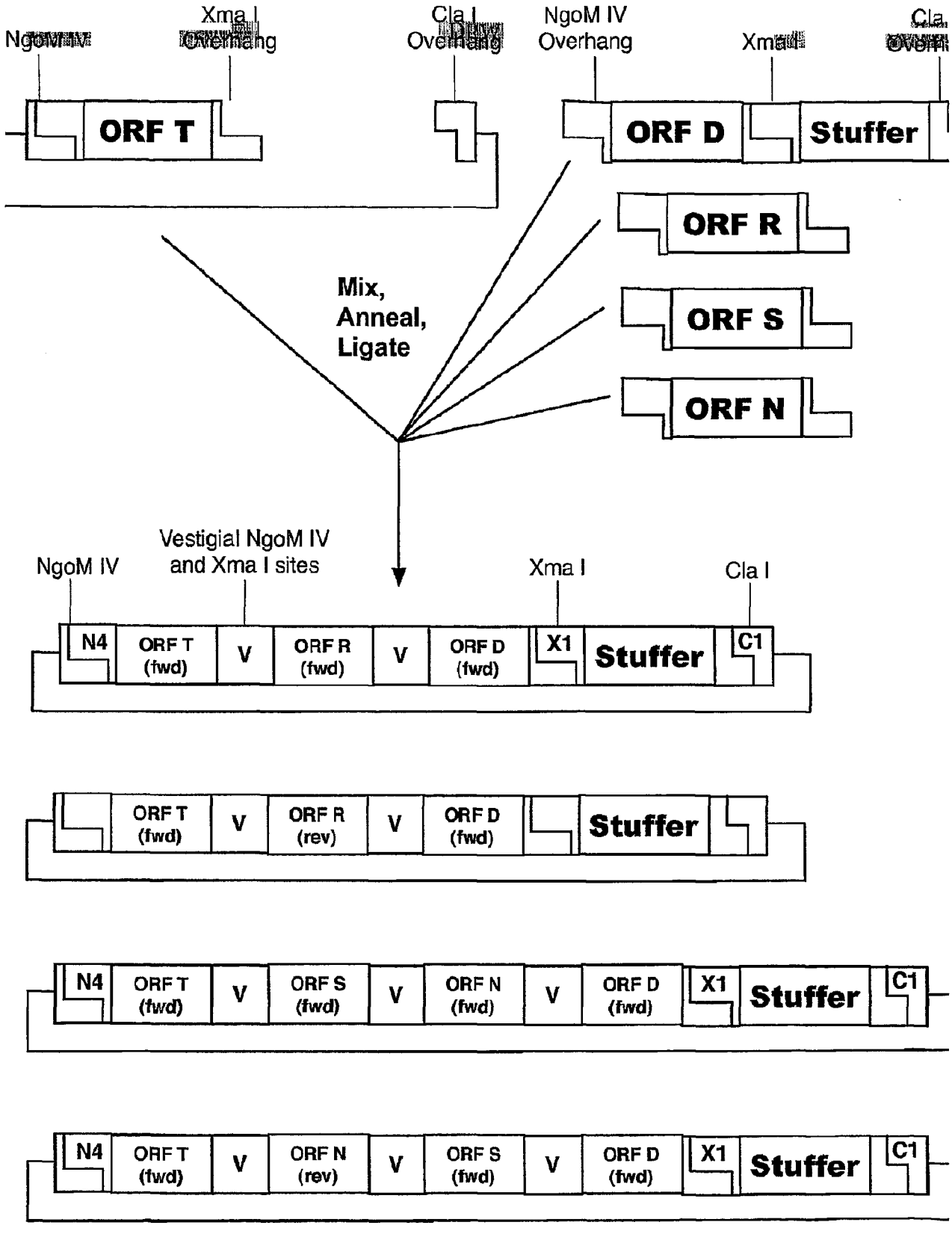
<---------------------- Module Open Reading Frame ------------------------->				
NgoM IV	ORF of interest	Xma I	Stuffer	Cla I
GCCGGC	AAGAAGAAAAAGAAGAAG	CCCGGG	GGCGGAGGC	ATCGAT
AlaGly	LysLysLysLysLysLys	ProGly	GlyGlyGly	IleAsp

FIGURE 11

Site I	Phosphorylation Domain	Site II	Stuffer	Site III
Site I	Zinc Finger Region	Site II	Stuffer	Site III
Site I	Peptide Inhibitor	Site II	Stuffer	Site III
Site I	Hormone Binding Domain	Site II	Stuffer	Site III
Site I	Epitope	Site II	Stuffer	Site III
Site I	Dimerization Domain	Site II	Stuffer	Site III
Site I	Lipid Binding Domain	Site II	Stuffer	Site III
Site I	Glycosylation Domain	Site II	Stuffer	Site III
Site I	Peptide Hormone	Site II	Stuffer	Site III
Site I	Ion Pore Region	Site II	Stuffer	Site III
Site I	Reporter Peptide	Site II	Stuffer	Site III

Site I	Catalytic Domain	Site II	Stuffer	Site III
Site I	DNA Binding Domain	Site II	Stuffer	Site III
Site I	Leucine Zipper Region	Site II	Stuffer	Site III
Site I	Transmembrane Domain	Site II	Stuffer	Site III
Site I	Activation Domain	Site II	Stuffer	Site III
Site I	Inhbition Domain	Site II	Stuffer	Site III
Site I	G Protein Binding Region	Site II	Stuffer	Site III
Site I	Highly Acidic Region	Site II	Stuffer	Site III
Site I	Ligand	Site II	Stuffer	Site III
Site I	Protein Subunit	Site II	Stuffer	Site III
Site I	Localization Signal	Site II	Stuffer	Site III

FIGURE 12


NgoM IV
Xma I Overhang
Cla I Overhang
NgoM IV Overhang
Xma I
Cla I Overhang
ORF T
ORF D
Stuffer
ORF R
ORF S
ORF N
Mix, Anneal, Ligate
NgoM IV
Vestigial NgoM IV and Xma I sites
Xma I
Cla I
N4
ORF T (fwd)
V
ORF R (fwd)
V
ORF D (fwd)
X1
Stuffer
C1
ORF T (fwd)
V
ORF R (rev)
V
ORF D (fwd)
Stuffer
N4
ORF T (fwd)
V
ORF S (fwd)
V
ORF N (fwd)
V
ORF D (fwd)
X1
Stuffer
C1
N4
ORF T (fwd)
V
ORF N (rev)
V
ORF S (fwd)
V
ORF D (fwd)
X1
Stuffer
C1

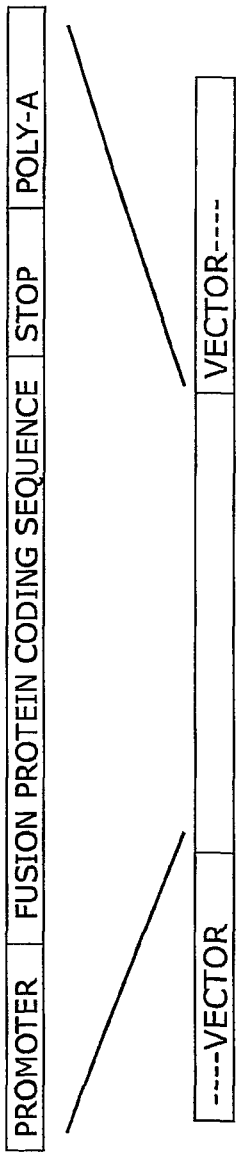


FIGURE 14A

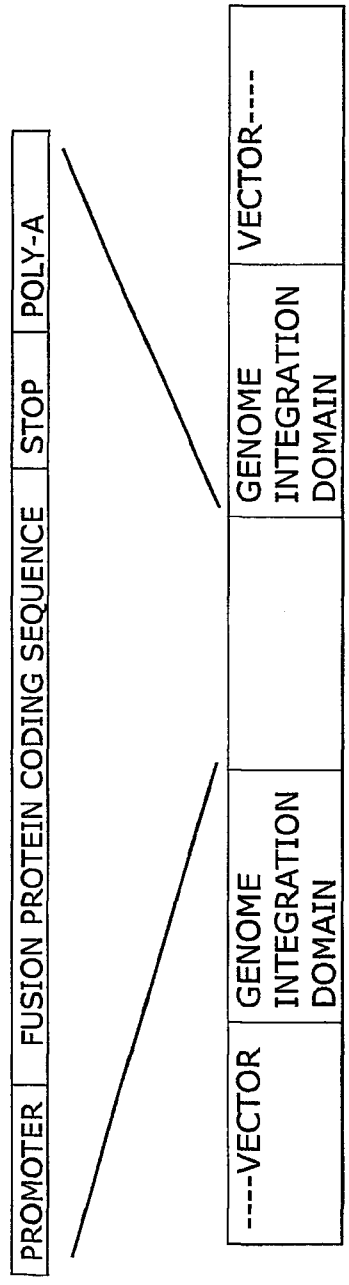


FIGURE 14B

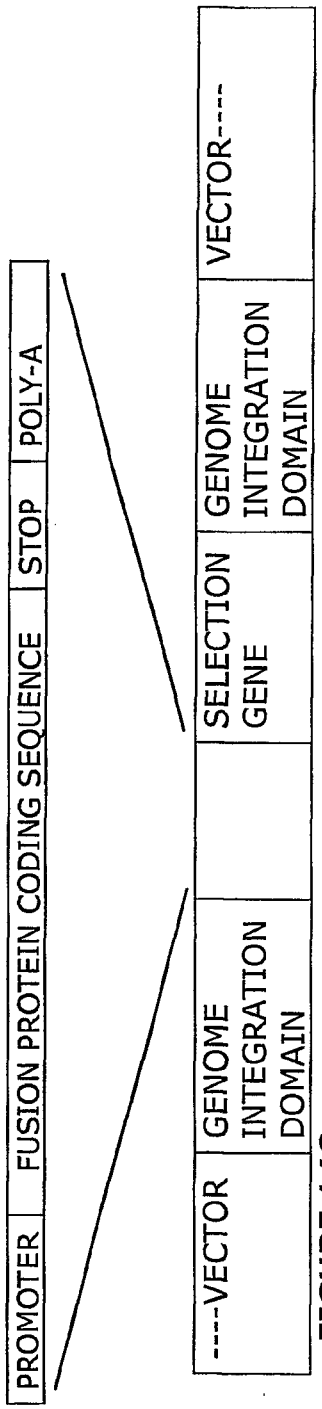


FIGURE 14C

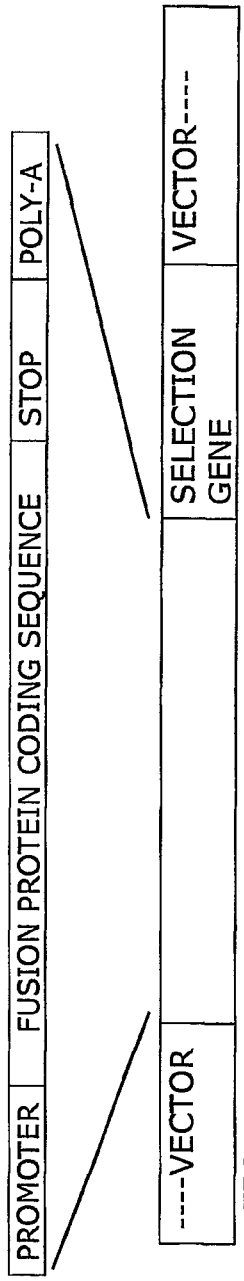


FIGURE 14D

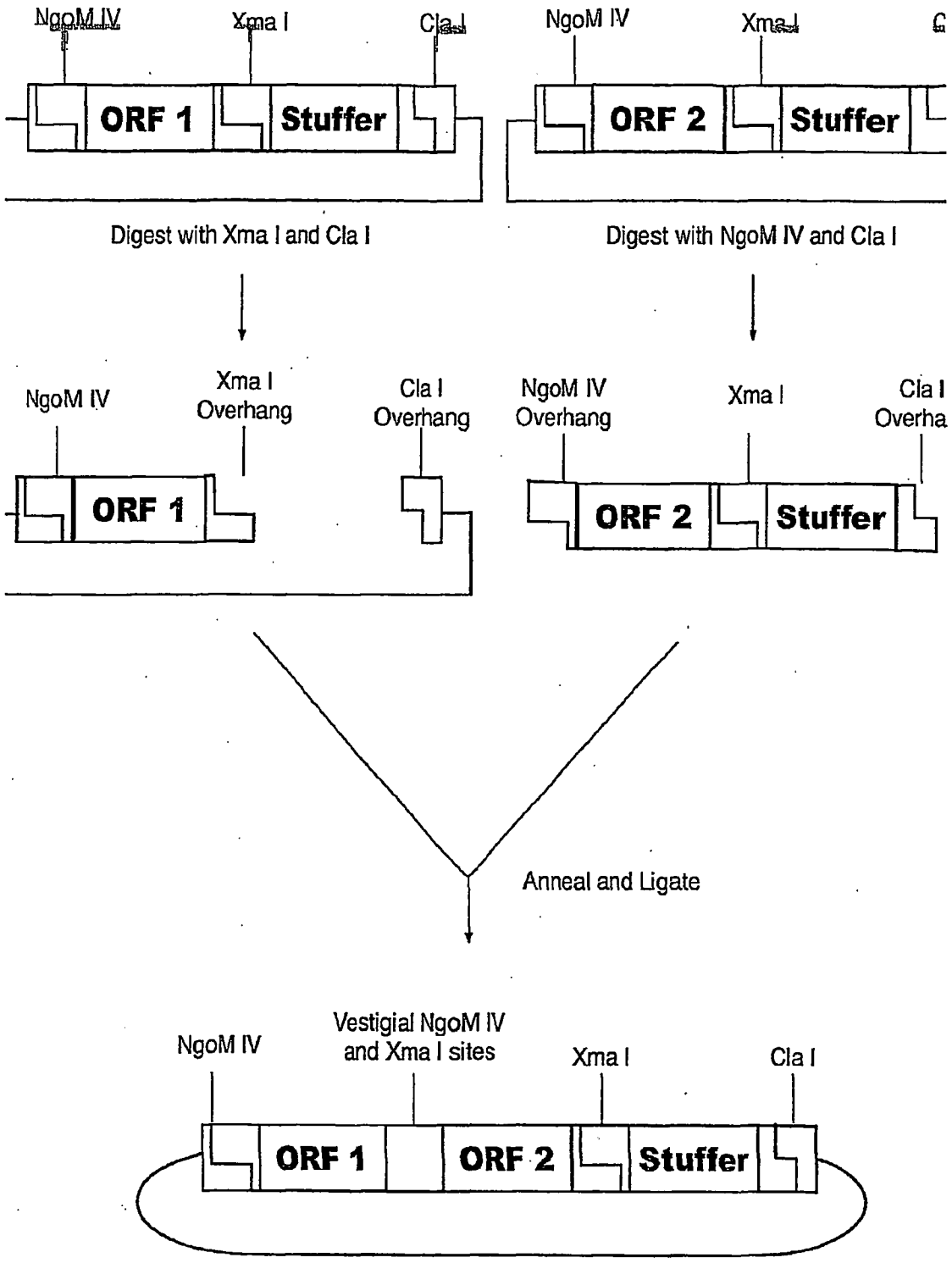


FIGURE 1

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

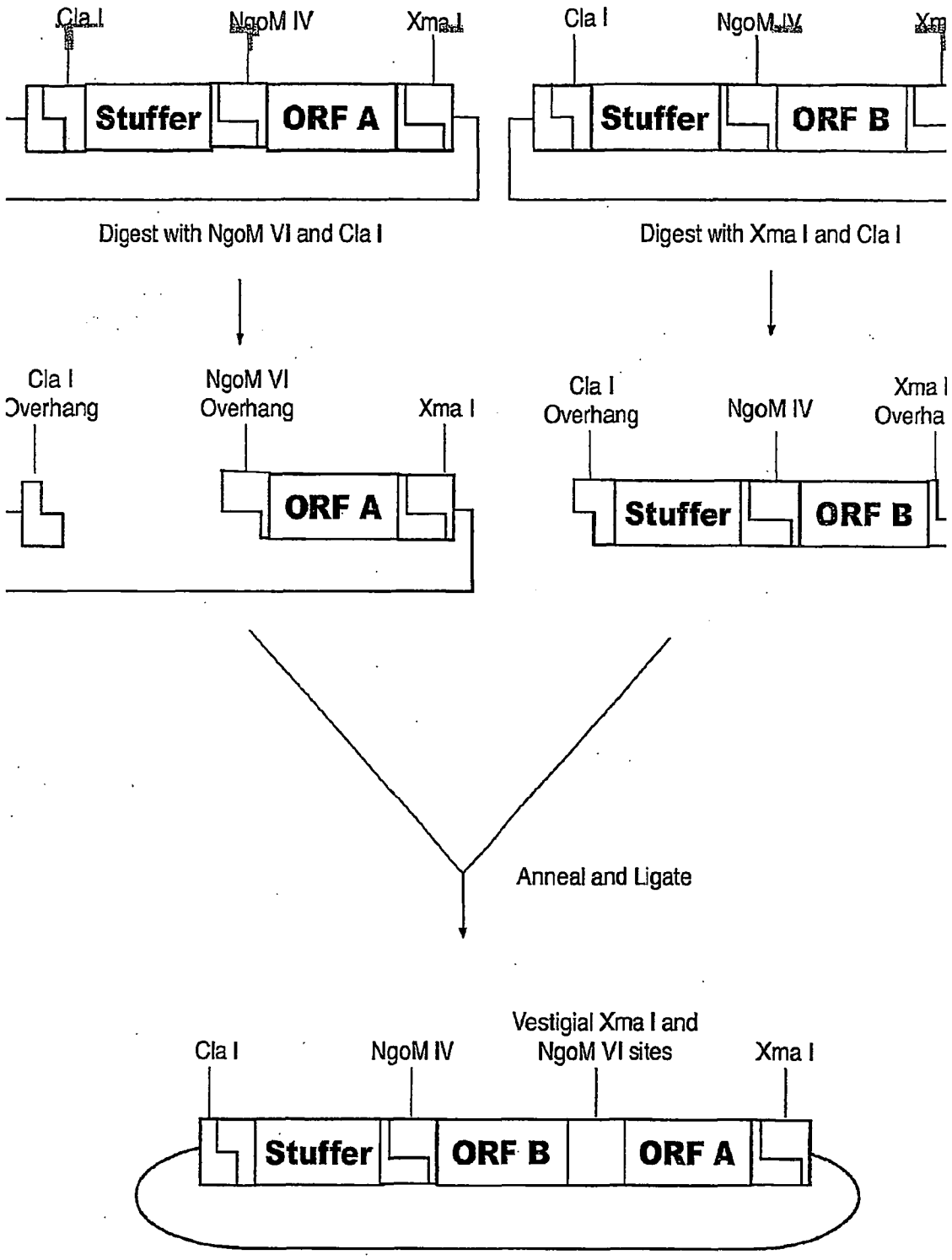


FIGURE 2

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

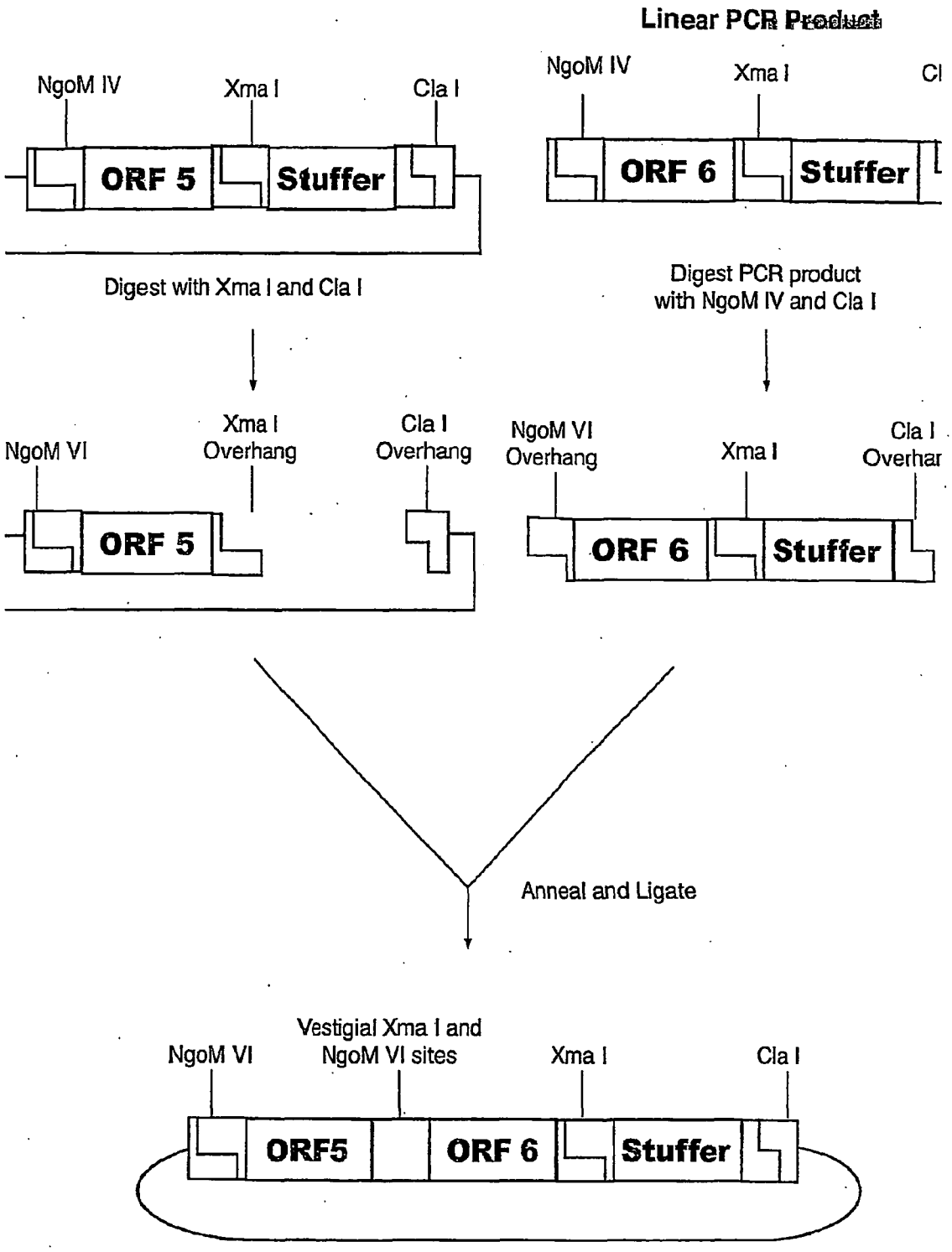


FIGURE 3

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

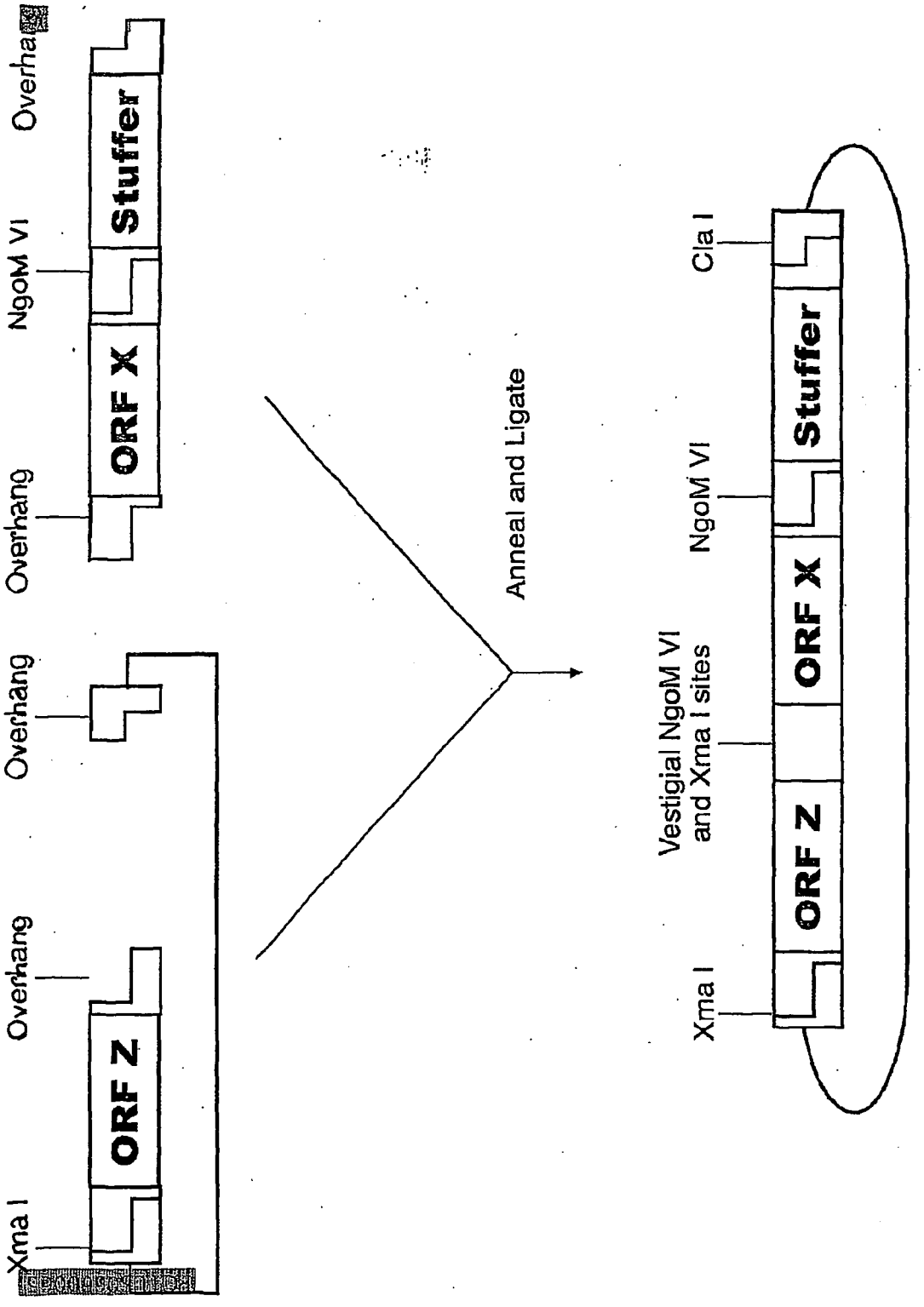


FIGURE 4

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

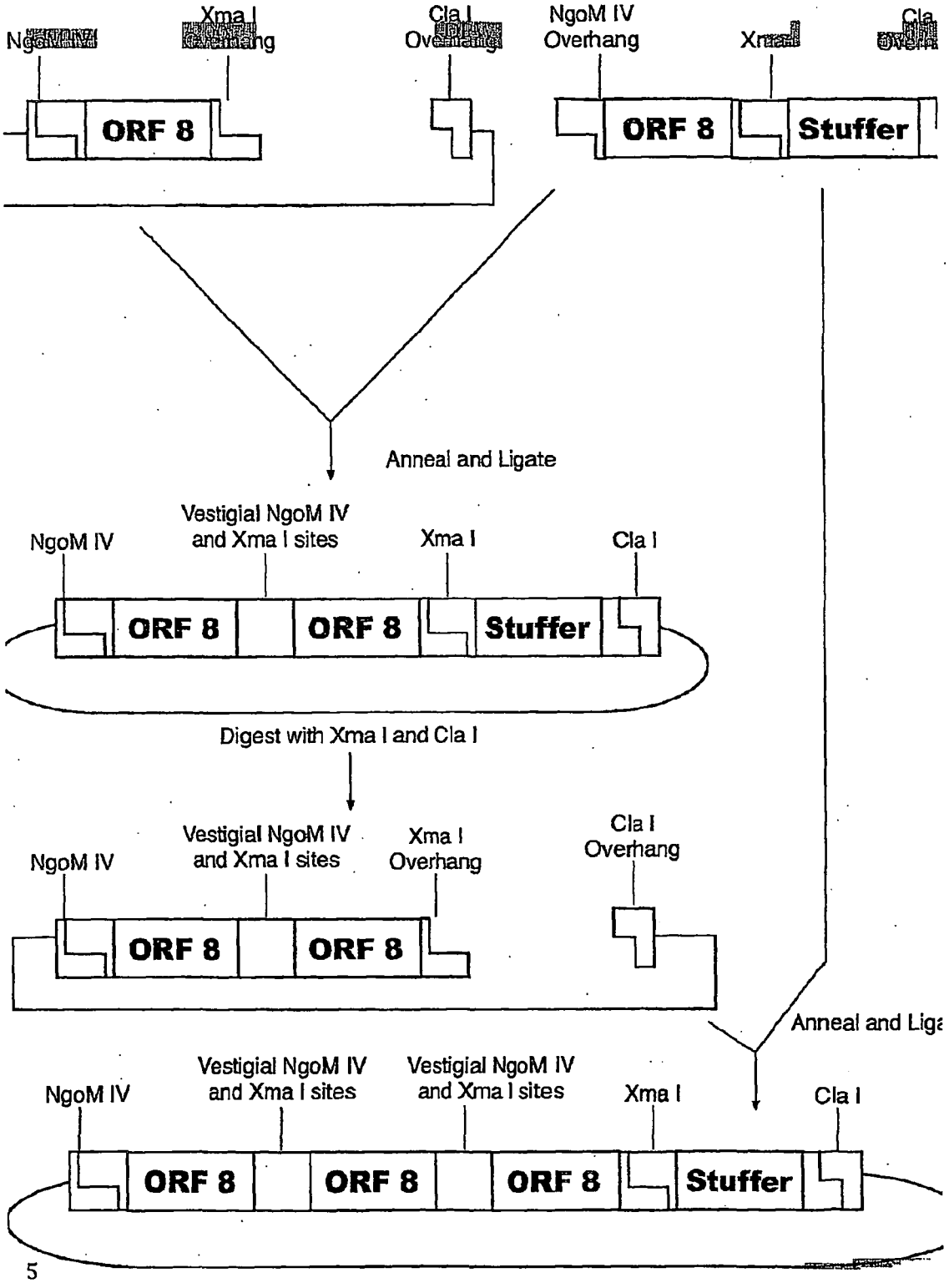


FIGURE 5

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

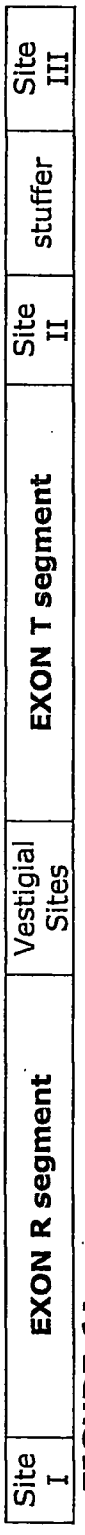


FIGURE 6A

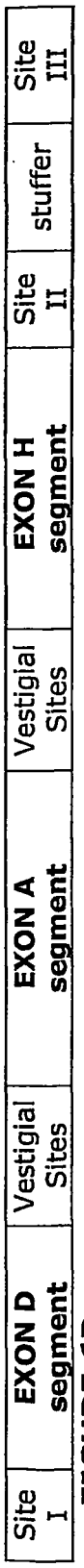


FIGURE 6B

FIGURE 6C

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

Site I	**ORF Y**	Vestigial Sites	**ORF Z**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7A

Site I	**LOCALIZATION SIGNAL**	Vestigial Sites	**PEPTIDE 3**	Vestigial Sites	**PEPTIDE 4**	Vestigial Sites	**PEPTIDE 5**	Site II	stuffer	Site III

FIGURE 7B

Site I	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LIGAND X**	Vestigial Sites	**LOCALIZATION SIGNAL**	Site II	stuffer	Site III

FIGURE 7C

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

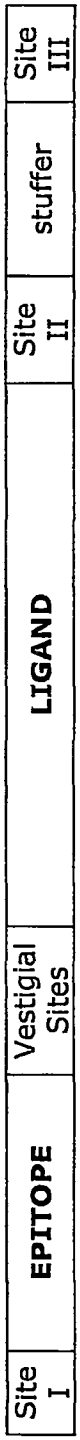


FIGURE 8A

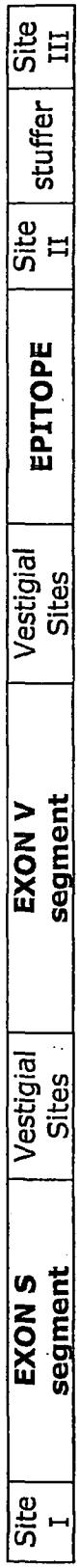


FIGURE 8B

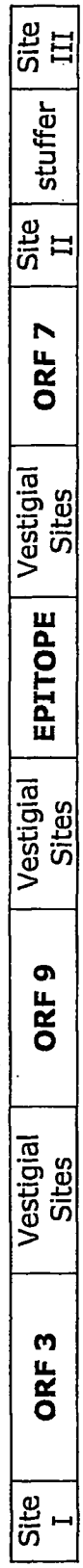


FIGURE 8C

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

FIGURE 9A

FIGURE 9B

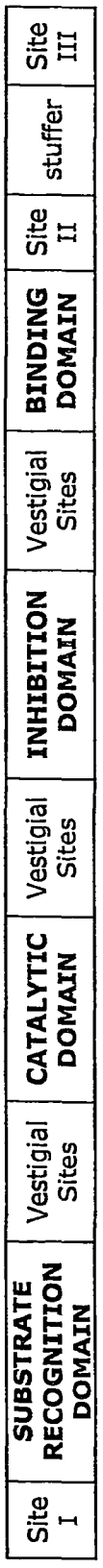


FIGURE 9C

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

FIGURE 10

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

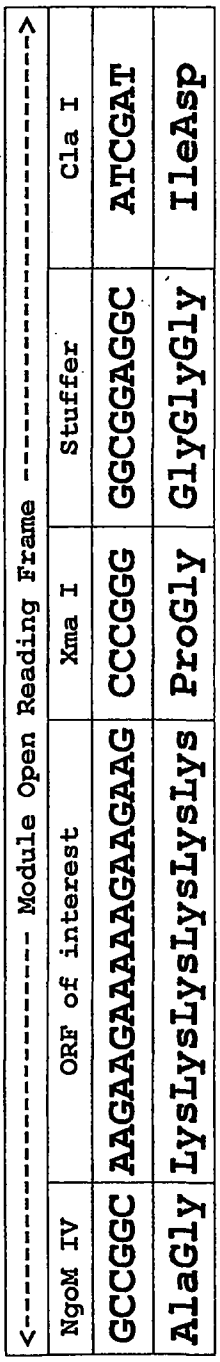


FIGURE 11

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

Site I	Phosphorylation Domain	Site II	Stuffer	Site III
Site I	Zinc Finger Region	Site II	Stuffer	Site III
Site I	Peptide Inhibitor	Site II	Stuffer	Site III
Site I	Hormone Binding Domain	Site II	Stuffer	Site III
Site I	Epitope	Site II	Stuffer	Site III
Site I	Dimerization Domain	Site II	Stuffer	Site III
Site I	Lipid Binding Domain	Site II	Stuffer	Site III
Site I	Glycosylation Domain	Site II	Stuffer	Site III
Site I	Peptide Hormone	Site II	Stuffer	Site III
Site I	Ion Pore Region	Site II	Stuffer	Site III
Site I	Reporter Peptide	Site II	Stuffer	Site III
Site I	Catalytic Domain	Site II	Stuffer	Site III
Site I	DNA Binding Domain	Site II	Stuffer	Site III
Site I	Leucine Zipper Region	Site II	Stuffer	Site III
Site I	Transmembrane Domain	Site II	Stuffer	Site III
Site I	Activation Domain	Site II	Stuffer	Site III
Site I	Inhbition Domain	Site II	Stuffer	Site III
Site I	G Protein Binding Region	Site II	Stuffer	Site III
Site I	Highly Acidic Region	Site II	Stuffer	Site III
Site I	Ligand	Site II	Stuffer	Site III
Site I	Protein Subunit	Site II	Stuffer	Site III
Site I	Localization Signal	Site II	Stuffer	Site III

FIGURE 12

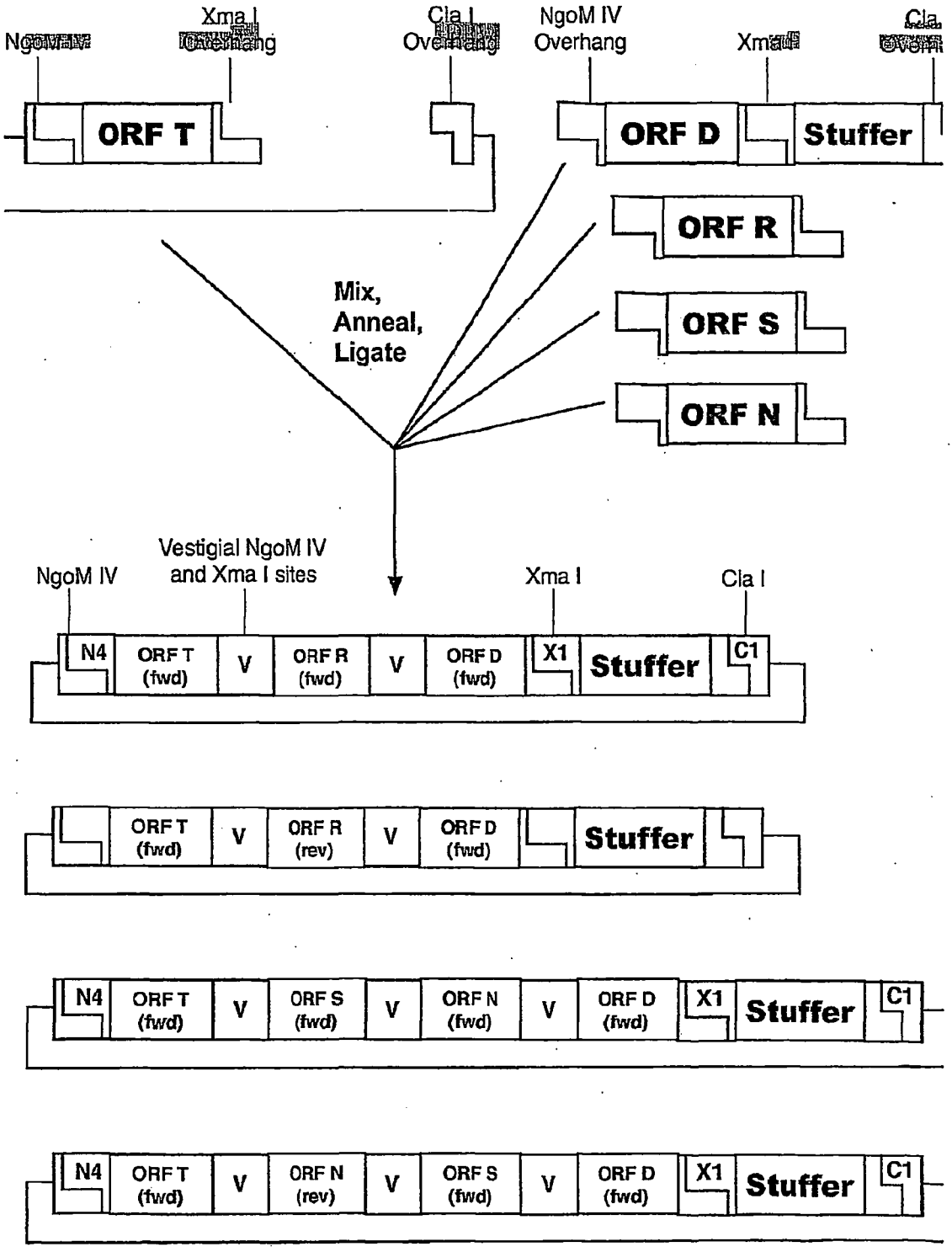


FIGURE 13

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

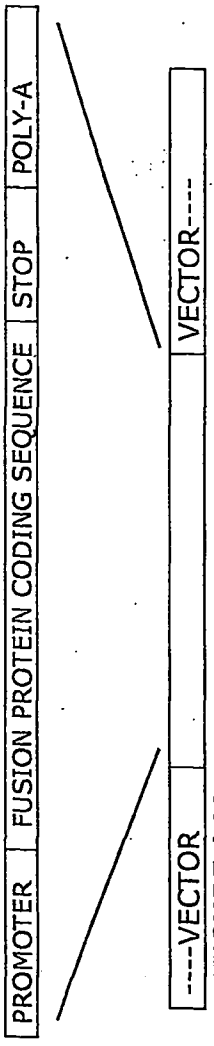


FIGURE 14A

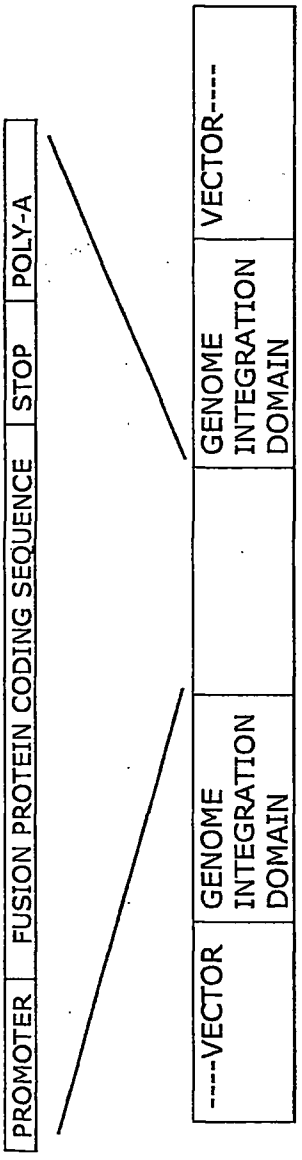


FIGURE 14B

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

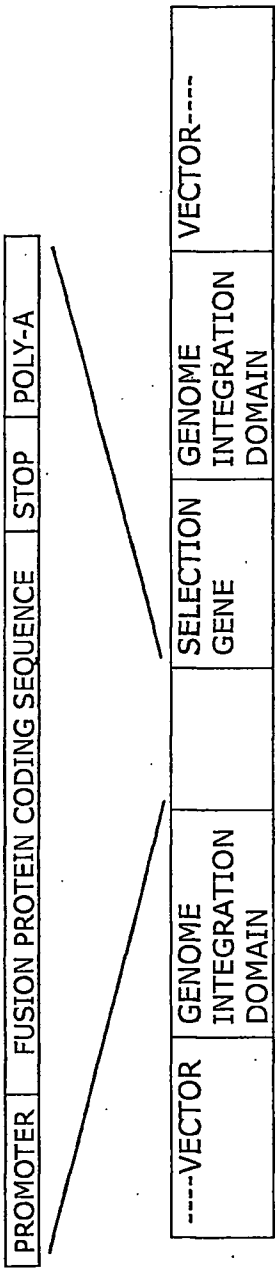


FIGURE 14C

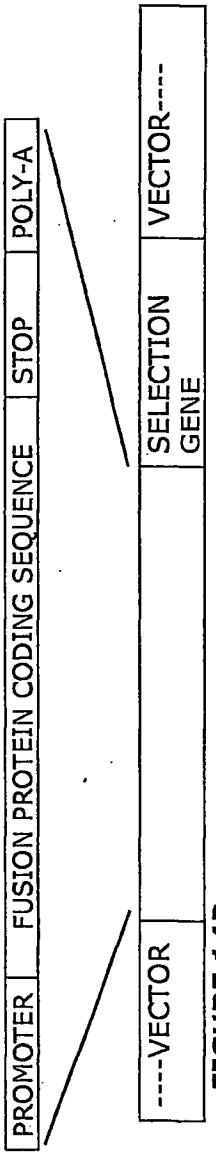


FIGURE 14D

S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT