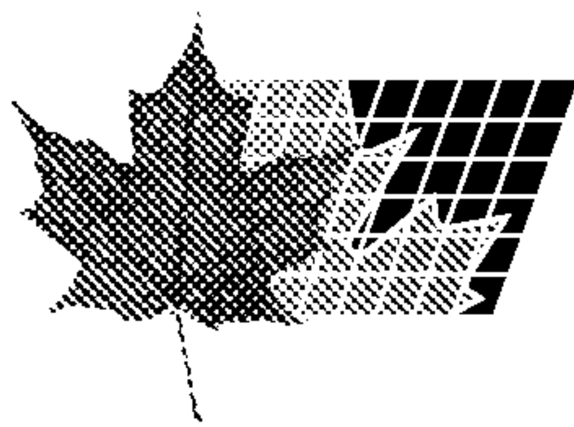

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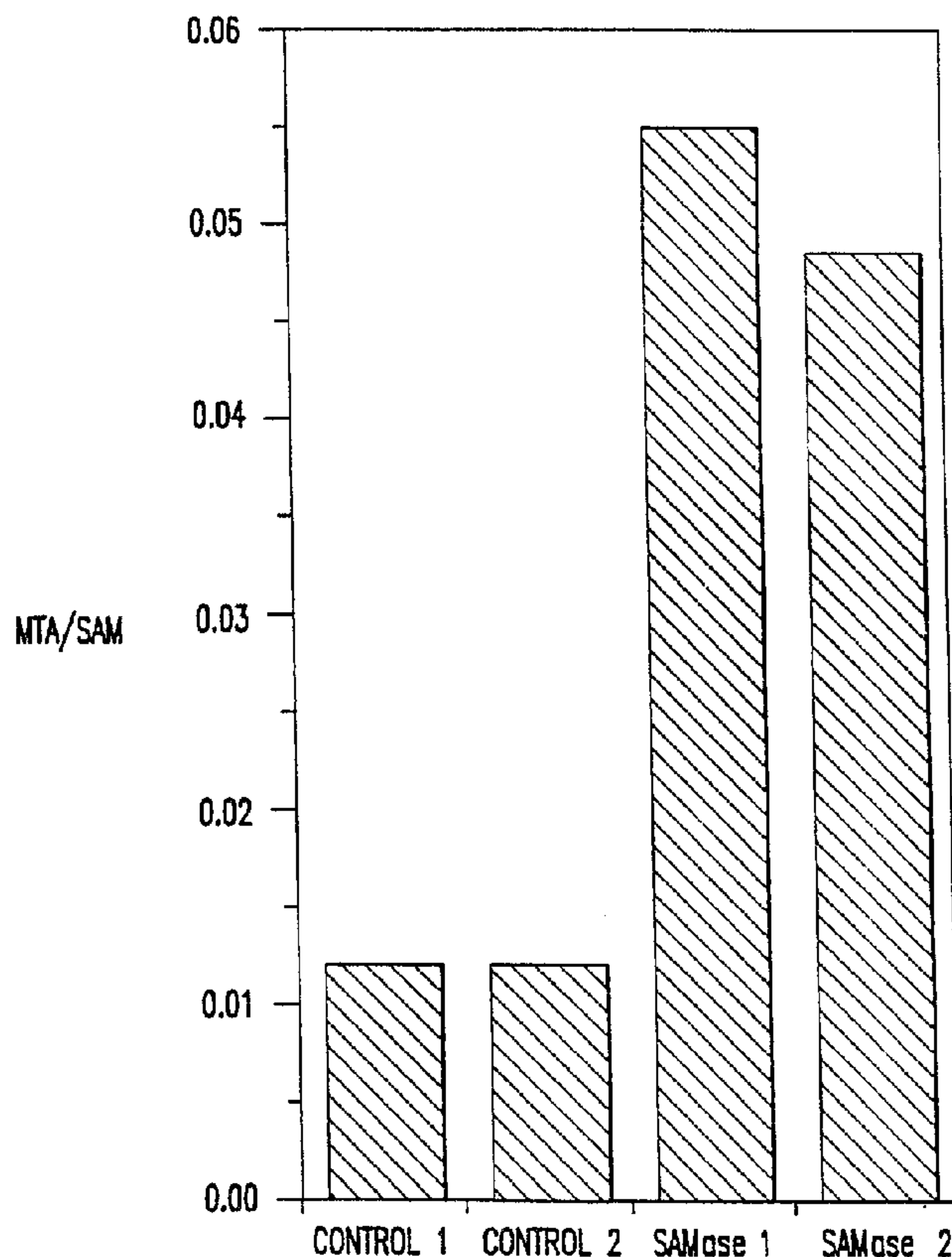
(30) 1989/12/12 (448,095) US

(54) **REGULATION GENETIQUE DE LA BIOSYNTHESE DE L'ETHYLENE DANS LES PLANTES**

(54) **GENETIC CONTROL OF ETHYLENE BIOSYNTHESIS IN PLANTS**



(57) Procédé pour réguler la biosynthèse de l'éthylène chez les plantes, comprenant un vecteur renfermant un gène sélectif soumis à une régulation du promoteur de la plante, une insertion d'ADN comprenant des codons pour un polypeptide hétérologue fonctionnel possédant une activité AdoMétase et doté d'un promoteur de la

(57) A method for control of ethylene biosynthesis in plants comprising a vector containing a selective gene under plant promoter control. and a DNA insert comprising codons for a functional heterologous polypeptide having AdoMetase activity and flanked by a plant promoter on one side and a polyA signal sequence

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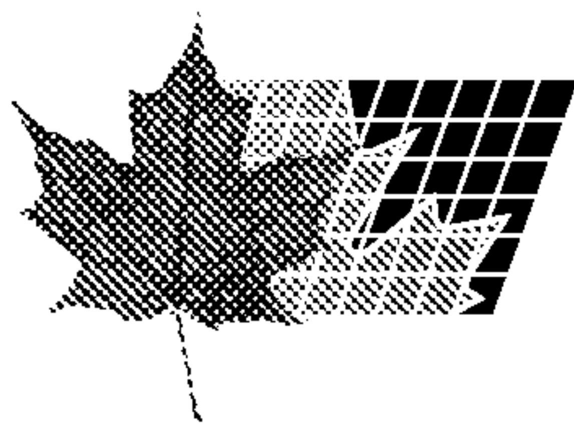

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plante d'un côté et d'une séquence de signal polyA de l'autre côté. On peut transformer une plante hôte avec ledit vecteur, la plante hôte ainsi transformée est alors capable d'exprimer le polypeptide hétérologue ayant une activité AdoMétase sous le contrôle de ladite zone de régulation. La présence et l'expression de l'AdoMétase dans les plantes transgéniques fait baisser les taux d'AdoMet et génère un inhibiteur de synthèse ACC provoquant une baisse correspondante de la biosynthèse de l'éthylène et de la disponibilité du précurseur. La construction actuelle des plantes transgéniques contenant une ou plusieurs copie(s) du gène AdoMétase T3 permet de fabriquer des plantes qui vont effectivement réguler la biosynthèse de l'éthylène pour donner des fruits, des légumes et des fleurs qui ont une durée de consommation prolongée et des qualités de conservation améliorées.

on the other side; and, transforming a plant host with said vector wherein the plant host transformed thereby is capable of expressing the heterologous polypeptide having AdoMetase activity under the control of said control region. The presence and expression of AdoMetase in transgenic plants towers AdoMet levels and generates an inhibitor of ACC synthase causing a corresponding decrease in ethylene biosynthesis and precursor availability. The current construction of transgenic plants containing a copy(s) of the T3 AdoMetase gene allow for construction of plants that will control ethylene biosynthesis resulting in fruits, vegetables, and flowers which have improved shelf life and preservation qualities.

GENETIC CONTROL OF ETHYLENE BIOSYNTHESIS IN PLANTS

Abstract

A method for control of ethylene biosynthesis in plants comprising a vector containing a selective gene under plant promoter control, and a DNA insert comprising codons for a functional heterologous polypeptide having AdoMetase activity and flanked by a plant promoter on one side and a polyA signal sequence on the other side; and, transforming a plant host with said vector wherein the plant host transformed thereby is capable of expressing the heterologous polypeptide having AdoMetase activity under the control of said control region. The presence and expression of AdoMetase in transgenic plants lowers AdoMet levels and generates an inhibitor of ACC synthase causing a corresponding decrease in ethylene biosynthesis and precursor availability. The current construction of transgenic plants containing a copy(s) of the T3 AdoMetase gene allow for construction of plants that will control ethylene biosynthesis resulting in fruits, vegetables, and flowers which have improved shelf life and preservation qualities.

PATENT
PD-1007CIP/SD

GENETIC CONTROL OF ETHYLENE BIOSYNTHESIS IN PLANTS

2068644

BACKGROUND OF THE INVENTION

5 This invention relates to a method and means of genetic control of ethylene biosynthesis in plants.

Ethylene is a plant hormone influencing many aspects of plant growth and development. This simplest of all unsaturated carbon compounds is a powerful regulator of plant metabolism, acting, and interacting with other plant hormones 10 in trace amounts.

Ethylene promotes senescence in plants, both in selected groups of cells and in whole organs such as fruits, leaves, or flowers. Senescence is the natural, genetically controlled degenerative process which usually leads to death in plants. Even at low concentrations (ethylene is a gas under physiological conditions), 15 ethylene has profound hormonal effects on plants. The effects of ethylene, whether produced by the plant itself or applied exogenously, are numerous, dramatic, and of considerable commercial importance. Among the diverse physiological effects are:

a. Stimulation of ripening in fruits and vegetables
20 b. Leaf abscission
c. Fading in flowers
d. Flower wilting
e. Leaf yellowing
f. Leaf epinasty

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Normally, ethylene production from plant tissue is low. Large quantities of ethylene, however, are produced during ripening and senescence processes. A large amount of ethylene is also produced following trauma caused by chemicals, temperature extremes, water stress, ultraviolet light, insect damage, disease, or mechanical wounding. Ethylene produced by plants under such conditions is referred to as "wound ethylene" or "stress ethylene". In fruits and vegetables, the stimulation of ethylene production by cuts or bruises may be very large and bear considerably on storage effectiveness. Ethylene-induced leaf browning is a common basis for loss in many plants, including lettuce and tobacco. In some tissues, exposure to only a small amount of ethylene may cause an avalanche of ethylene production in adjacent plants or plant tissues such as fresh produce. This autocatalytic effect can be very pronounced and lead to loss of fruit quality during transportation and storage.

The mechanism by which ethylene exerts its effects has become apparent only in the last few years. As judged by numerous data, each of the responses to ethylene involves an ethylene receptor site - a metalloenzyme. The reaction of ethylene with its receptors triggers a cascade of physiological events. Marked increases in the amounts of RNA and protein occur in response to ethylene. The levels of several enzymes have also been shown to increase in response to ethylene, such as cellulase, α-amylase, and invertase.

Current technologies that specifically address post-harvest storage life have been in existence for decades and are hampered by such problems as high cost, side effects, and an inability to completely shut off ethylene production. Included in this group are controlled atmosphere (CA) storage, chemical treatment, packaging, and irradiation.

CA facilities slow ethylene biosynthesis through: (1) low temperature, (2) reducing the oxygen level below 3%, and (3) elevating the carbon dioxide level in the storage area to the 3%-5% range. Expensive scrubbers are sometimes added which reduce ethylene already respired to the atmosphere. Drawbacks are that

CA facilities are expensive to construct, have a high utility cost, and are unable to completely eliminate ethylene production and side effects. Also, CA storage techniques can only control external ethylene and not that which resides inside the plant tissue. CA storage can also lead to undesirable side effects. Injury can result from high CO_2 levels, low O_2 levels, or low temperature.

Another approach is to limit ethylene biosynthesis in the plant tissue through chemical treatment. Aminoethoxyvinylglycine (AVG), an analog of the antibiotic rhizobitoxine, is such an inhibitor. Use of the chemical in foods is impossible, however, due to its high toxicity. Silver thiosulfate (STS) is also effective in showing fruit ripening and flower fading but is also toxic and cannot be used on foods. STS works only with certain flowers and often causes black spotting.

The amino acid methionine has been shown to be a precursor of ethylene in plant tissues. Methionine, however,is not the immediate precursor, but first must be converted to the sulfonium compound S-adenosylmethionine (AdoMet) and, subsequently, to 1-aminocyclopropane-1 carboxylic acid (ACC) prior to conversion to ethylene. The following metabolic reactions are now accepted for the synthesis of ethylene from methionine under both normal and stress conditions:

$$\text{Methionine} \rightarrow \text{AdoMet} \rightarrow \text{ACC} \rightarrow \text{Ethylene}$$

The system which converts ACC to ethylene appears to be constitutive in most plant tissues with the notable exception of some preclimacteric fruit tissue. ACC synthase catalyzes the degradation of AdoMet to ACC and 5'-methylthioadenosine (MTA). This enzymatic reaction seems to be the rate-limiting step in ethylene formation. AdoMet is synthesized via a condensation reaction between methionine and Adenosinetriphosphate (ATP). Attempts at regulating the levels of AdoMet by controlling the rate of AdoMet synthesis have failed, mainly because there appear to be at least three different AdoMet synthesizing enzymes coded by three different genes. In addition, the known biochemical inhibitors of

AdoMet synthesis are very toxic to mammalian cells. See S.F. Yang, *et al.*, "Ethylene Biosynthesis and its Regulation in Higher Plants," *Ann.Rev. Plant Physiol,* 35:155-189, 1984; Veen, *et al.*, *SciHortic,* 18:277-286; Sisler, *et al.*, *Plant Physiol,* 63:114-120; and Wang, *et al.*, *Plant Physiol,* 89:434-438.

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Although plant tissues are known to maintain a substantial rate of ethylene production for extended periods, their methionine levels have been shown to be very low. To continue to produce ethylene, the sulfur contained in MTA must be recycled back into methionine so as to provide an adequate supply of methionine

10 for continual ethylene production. This pathway has been recently shown to exist in plant tissue. See also S.F. Yang, *et al.*, "Ethylene Biosynthesis and its Regulation in Higher Plants," *Ann.Rev. Plant Physiol,* 35:155-189, 1984. The degradation of MTA has added significance in light of the finding that MTA is a potent inhibitor of ACC synthase. It should be noted that this pathway merely

15 maintains a methionine supply for ethylene biosynthesis, but does not result in a net increase in methionine synthesis.

An enzyme encoded by the *E. coli* bacteriophage T3 hydrolyzes S-adenosylmethionine (AdoMet) to homoserine and 5'-methylthioadenosine (MTA). This enzyme

20 is known by either its recommended name, AdoMet hydrolase (AdoMetase), or by its other name, S-adenosylmethionine cleaving enzyme (SAMase). See Studier, *et al.*, "SAMase Gene of Bacteriophage T3 is Responsible for Overcoming Host Restriction," *Journal of Virology,* 19:135-145, 1976. Both products of the reaction are recycled to methionine; MTA as previously shown and homoserine via

25 a metabolism pathway known to exist in plant tissues. The AdoMetase gene has been identified, isolated, cloned, and sequenced. J.A. Hughes, *et al.*, "Expression of the Cloned Coliphage T3 S-adenosylmethionine Gene Inhibits DNA Methylation and Poly Amine Biosynthesis in *Escherichia coli*," *J.Bact.*, 169:3625-3632, 1987 and J.A. Hughes, *et al.*, "Nucleotide Sequence And Analysis of the Coliphage T3

30 S-adenosylmethionine Hydrolase Gene and its Surrounding Ribonuclease III Processing Sites," *Nuc. Acids Res.*, 15:717-729, 1987. The gene contains two

inframe reading sequences that specific polypeptides of 17105 and 13978 daltons. Both polypeptides terminate at the same ochre codon. This results in the 14kd polypeptide being identical to 82% of the 17kd polypeptide starting at the carboxyl end of the longer polypeptide. Both polypeptides are present in partially
5 purified preparations of active AdoMetase from T3 bacteriophage infected cells and from *E. coli* expressing the cloned gene. J.A. Hughes, *et al.*, "Nucleotide Sequence And Analysis of the Coliphage T3 S-adenosylmethionine Hydrolase Gene and its Surrounding Ribonuclease III Processing Sites," *Nuc. Acids Res.*, 15:717-729, 1987; and F.W. Studier, *et al.*, "SAMase Gene of Bacteriophage T3 is
10 Responsible for Overcoming Host Restriction," *J. Virol.*, 19:135-145, 1976.

Other bacteriophages that encode the AdoMetase of SAMase genes are coliphage BA14, *Klebsiella* phage K11, and *Serratia* phage IV. See H. Mertens, *et al.*, "Coliphage BA14: a New Relative of Phase T7," *J.Gen.Virol.*, 62:331-341, 1982;
15 R. Hausmann, The Bacteriophages, 1:279-283, 1988, R. Calender (ed.), Plenum Press, NY; and K. H. Korsten, *et al.*, "The Strategy of Infection as a Criterion for Phylogenetic Relationships of Non-Coli Phages Morphologically Similar to Phase T7," *J.Gen.Virol.*, 43:57-73, 1979.

20 SUMMARY OF THE INVENTION

AdoMetase is normally not present in plant tissues. The AdoMetase gene codes for a protein having a very unusual enzymatic activity. Bacteriophage T3, Coliphage BA14, *Klebsiella* phage K11, and *Serratia* phage IV are the only known sources of a gene encoding that activity. The presence of the AdoMetase gene
25 and the expression of AdoMetase in transgenic plants lowers AdoMet levels. Since AdoMet is the sole precursor for ethylene biosynthesis, its reduced availability causes a corresponding decrease in ethylene biosynthesis. Furthermore, the hydrolysis of AdoMet by AdoMetase generates MTA which is an inhibitor of ACC synthase, a principle enzyme in the biosynthesis of ethylene by plants. The net
30 effect is twofold, a reduction in precursor availability and a direct inhibition of ethylene biosynthesis. The current

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construction of transgenic plants containing at least one copy of the T3 AdoMetase gene by use of the *Agrobacterium* transfer systems allow for construction of plants that will control ethylene biosynthesis under restricted conditions. Thus, the present invention combines expertise from two very different fields of study, bacteriophage biochemistry, and plant biochemistry. 5 This invention will result in fruits, vegetables, and flowers which have been modified internally to improve shelf life and preservation qualities.

It is an object of an aspect of the present invention to provide a vector useful for transformation of a plant host, said vector comprises:

10 a first DNA sequence containing a gene useful for genetic selection in plant cells, where said first DNA sequence is flanked by regulatory elements effective to allow expression of the sequence in a plant host, and where said vector further comprises a second DNA sequence which (i) is flanked by regulatory elements effective to allow expression of the sequence in a plant 15 host, and (ii) encodes a S-adenosylmethionine hydrolase enzyme which hydrolyses S-adenosylmethionine to homoserine and 5'-methylthioadenosine.

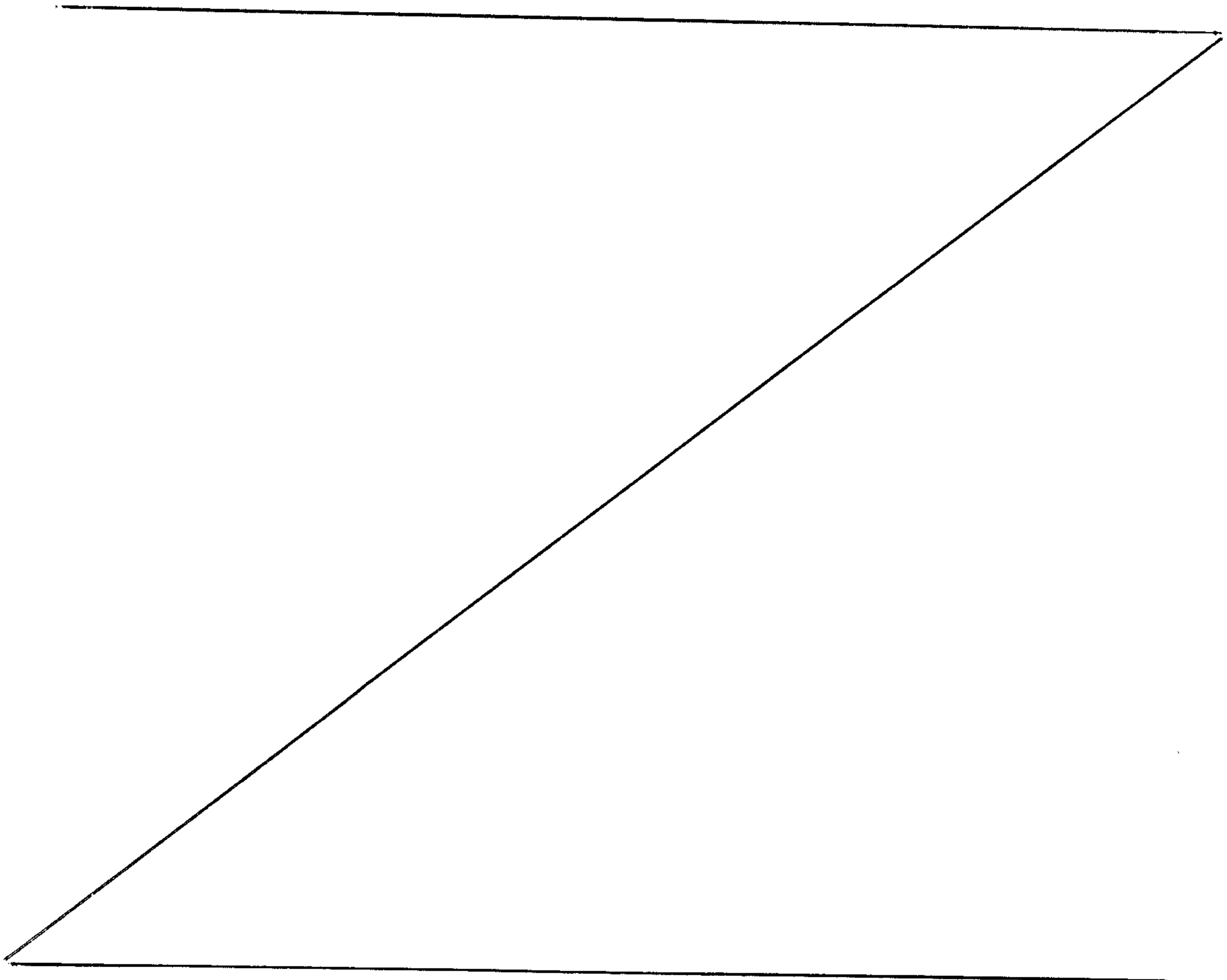
In accordance with another aspect of the invention, there is provided a method for reducing ethylene biosynthesis in plant cells, comprising transforming plant 20 host cells with the above vector wherein the transformed host cells are capable of expressing said enzyme.

According to another aspect of the invention, a transgenic plant cell contains a DNA sequence which encodes and expresses a S-adenosylmethionine hydrolase 25 enzyme, wherein said enzyme can hydrolyze S-adenosylmethionine to homoserine and 5'-methylthioadenosine.

The present further relates to a binary vector system useful for the transformation of a plant host comprising a "T-DNA less" Ti plasmid, and a

broad host-range plasmid containing T-DNA borders, and a selective gene under plant promotor control. The vector also includes a DNA insert comprising codons for a functional heterologous polypeptide having AdoMetase activity, or a heterologous polypeptide having substantially the same biological activity as AdoMetase. The heterologous polypeptide is
5 flanked by a plant promoter on one side and a polyA signal sequence on the other side. The result is the transformed plant host is capable of expressing the heterologous polypetide under the control of the control region.

The present invention further relates to a tripartite vector system useful for
10 transformation of a plant host comprising (a) a "T-DNA less" Ti plasmid, (b) a



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broad host-range P incompatibility group plasmid containing a cloned virG gene, and (c) a broad host-range plasmid containing T-DNA borders, and a selective gene under plant promotor control. The vector also includes a DNA insert comprising codons for a functional heterologous polypeptide having AdoMetase activity, or a heterologous polypeptide having substantially the same biological activity as AdoMetase activity, or functional derivatives thereof. The heterologous polypeptide is flanked by a plant promotor on one side and a polyA signal sequence on the other side. The result is the transformed plant host is capable of expressing the heterologous polypeptide under the control of said control region. See P. Zambryski and J. Schell, "Transfer and Function of T-DNA Genes from Agrobacterium Ti and Ri Plasmids in Plants," *Cell*, 56(2):193-201, 1989. Specifically, it has been constructed using a strain of *Agrobacterium* P 2760 containing a T-DNA-less derivative of pTiA6NC. To this transfer system has been added a plasmid pVK102 containing a plasmid pTiBo542 virG gene insert. This insert enhances the transfer of the DNA containing T-DNA borders that are contained on a third plasmid be it pGA 482-Sam-K or pBI 121-AdoMetase. In summary, the transfer system is provided by three plasmids all contained in the same bacterial strain. The first contains all of the genetic information needed to transfer genes to plants except it lacks the T-DNA borders. The second plasmid contains extra copies of one of the virulence genes which enhances the transfer process. The third plasmid contains the DNA to be delivered to the plant cells engineered between two T-DNA borders. This system differs from those published in that it uses three plasmids and a mixture of virulence gene products (ones from pTiA6NC and pTiBo542) to achieve efficient transfer to a broad variety of plants.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention itself, as well as additional advantages and features thereof, will be more readily and comprehensively understood from the following detailed description of the preferred inventive embodiments, such description making 5 reference to the appended sheets of drawings, wherein:

FIGURE 1a schematically represents the ethylene biosynthetic pathway in plant tissue;

FIGURE 1b schematically represents the ethylene biosynthetic pathway with AdoMetase;

10 FIGURE 1c schematically represents the methionine recycling pathway;

FIGURE 2 schematically represents the construction of pBI121-AdoMetase;

FIGURE 3 schematically represents the genetic engineering of the AdoMetase gene;

FIGURE 4 schematically represents the alternative construction of pGA482-NOS-15 SAM;

FIGURE 5 indicates SAMase activity in transgenic plants; and

FIGURE 6 indicates that part of the nucleotide sequence of pUC19SAM-K that encodes AdoMetase gene with the modified 5' end.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The plant vector system consists of the following. To transfer the AdoMetase gene into plants, a binary *Agrobacterium tumefaciens* system is preferably used. The *Agrobacterium* strain PC2760 containing a "T-DNA less" Ti plasmid and a broad host-range plasmid containing T-DNA borders, a selective kanamycin gene under plant promoter control, and the AdoMetase gene flanked by a plant promoter on one side, and a polyA signal sequence on the other side is constructed as shown in FIGURE 2 and described in the following examples.

It will be appreciated that the AdoMetase gene can be isolated from more than one bacteriophage. Different bacteriophages may be expected to contain AdoMetase genes with variations in their DNA sequences. Furthermore, the amino acid sequence of AdoMetase may be modified by genetic techniques to produce enzymes with altered biological activities. An increase in the biological activity could permit the use of lower amounts of the enzyme to control ethylene biosynthesis in plants. Modifications of the sequence of the AdoMetase gene are within the scope of the present invention.

EXAMPLE 1

The source of the AdoMetase gene is obtained and manipulated as follows. The AdoMetase gene has been identified on an AluI-HaeIII restriction fragment from purified T3 DNA (J.A. Hughes, *et al.*, "Expression of the Cloned Coliphage T3 S-adenosylmethionine Gene Inhibits DNA Methylation and Poly Amine Biosynthesis in *Escherichia Coli*," *J.Bact.*, 169:3625-3632, 1987). Bacteriophage T3 is available under ATCC No. 11303-B3. This DNA fragment was first cloned into the bacteriophage M13 MP8 vector (Pharmacia LKB Biotechnology, Inc.). A Mae 111 to Bam fragment is then subcloned into the pUC19 plasmid vector (Pharmacia) to produce pUC19-AdoMetase (pUC19-SAMase), transformed into *E. Coli* and used as a source of DNA for further construction experiments and for DNA sequence determination. The Mae 111 site is used as the 5' terminus of the

AdoMetase gene fragment since it is only 10 base pairs upstream from the initiation codon for the gene. As shown in FIGURE 2, pUC19-AdoMetase is used as the source of the AdoMetase gene for insertion into an *Agrobacterium tumefaciens* vector as described below.

5 The parent vector, pBI121, is obtained commercially from Clontech Laboratories, Inc. The plant promoter upstream of the AdoMetase gene sequence can be varied to obtain tissue specific expression, temperature dependent expression, low or high level constitutive expression, hormone-induced expression, or light dependent expression in the transgenic plants. In the following example, the 10 promoter is the constitutive Cauliflower Mosaic Virus (CaMV) promoter (Pharmacia).

EXAMPLE 2

The following is an example of the construction and transformation with PC2760/pBI121-AdoMetase. The pUC19-AdoMetase plasmid is digested with Xba 15 I and Sac I to produce a 520bp fragment encoding the entire AdoMetase gene. The DNA fragment is purified by agarose gel electrophoresis followed by electrolution. The vector, pBI121, is also digested with Xba I and Sac I and purified by the same method as described above. The two fragments are ligated together and the resultant plasmid named pBI121-AdoMetase. PBI121-AdoMetase 20 is introduced into *Agrobacterium* using a direct transformation method. *Agrobacterium tumefaciens* PC2760 is deposited with the American Type Culture Collection, Rockville, Maryland, under accession number ATCC 68111. *Agrobacterium tumefaciens* strain PC2760 is grown to mid log phase (OD 600 0.5 to 1.0) in YEP media (10g yeast extract, 10g peptone, and 5g NaCl per liter). 25 After chilling on ice, 50mls of these cells are pelleted, resuspended in 1ml of ice cold 20mM $CaCl_2$ and split into 1ml aliquots. One μg of pBI121-AdoMetase is added to one of the aliquots and incubated on ice for 30 minutes, frozen in liquid nitrogen and thawed at 37°C for 5 minutes. One ml of YEP media is added and incubated at 28°C for 2 hours. The cells are pelleted and resuspended in 50μl

of YEP, then plated on YEP agar plates containing 20μg/ml kanamycin. Kanamycin-resistant transformed colonies appear within 2 days.

A PC2760 clone containing these plasmids is named PC2760/pBI121-AdoMetase and was used to transform leaf discs obtained from Nicotiana tabacum L. cv. Wisconsin by the following direct method. A tobacco leaf is washed once in 95% ethanol for 10 seconds, once in 10% bleach, 0.1% Tween-20 for 20 minutes, four times in water, cut into 5mm discs, and finally placed in a 10ml overnight culture of PC2760/pBI121 for 30 minutes. The leaf discs are then placed on Murishegee and Sckoog callus forming medium for 1 day. The discs are then soaked in 500μg/ml cefatoxamine for 1 hour and placed on Tobacco callus-forming media containing 200μg/ml carbenicillin for 3 days. The discs are then transferred to the same medium containing an additional 100μg/ml kanamycin. Kanamycin-resistant tobacco callus is selected using standard techniques. The regeneration of plants from calli is a known art. Protocols vary with each plant species and specific parameters can be easily determined by one skilled in the art. Plant tissues derived from this callus are shown to contain the AdoMetase gene using DNA-dot blots and Southern blots. Transcription of this gene is demonstrated by extracting RNA from lead tissue and performing Northern blots. Both Southern and Northern blots are probed with a radioactively-labeled AdoMetase gene fragment from pUC19-AdoMetase. The presence of AdoMetase enzyme is confirmed by making crude extracts from lead tissue and performing AdoMetase assays as previously described and as demonstrated in FIGURE 5 where extracts of transgenic plants were analyzed for enzymatic activity based on the ratio of 5'-methylthioadenosine to S-adenosylmethionine. Also demonstrated is the effect of Naphthaleneacetic acid (NAA), a plant hormone which stimulates ethylene production, on control tissues versus transgenic plant tissue in terms of ethylene evolution in tobacco leaf discs after 40 hours of culture. The transgenic tissue is designated Nt-BOB. The transgenic plant shows a marked decrease in ethylene evolution as shown in TABLE 1.

TABLE 1

The Effect of NAA on Ethylene Evolution of AdoMetase Transformed Tobacco Leaf Discs After 40 Hours of Culture

Tissue	NAA (mM)	Ethylene (nmol/g/40 h)
Nt-control	0.00	1.90
	0.01	10.01
	1.00	75.47
Nt-BOB	0.00	0.61
	0.01	5.17
	1.00	13.01

As seen in FIGURE 1, the formation of ACC is a rate limiting step for production of ethylene in plant tissues. S.F. Yang, *et al.*, "Ethylene Biosynthesis and Its Regulation in Higher Plants," *Ann.Rev. Plant Physiol*, 35:155-189, 1984. Various other methods may be employed to elicit transformation of the plant host, such as electroporation, microinjection, and microprojectile bombardment. These methods are well known in the art and detailed in the following representative references. T.M. Klein, *et al.*, "Stable Genetic Transformation of Intact Nicotiana Cells by the Particle Bombardment Process," *Proc.Natl.Acad.Sci. USA*, Washington, D.C.: The Academy, Nov. 1988, vol. 85, Issue 22, pages 8502-8505, ill.; B.L.A. Miki, *et al.*, "Microinjection: An Experimental Tool for Studying and Modifying Plant Cells," *Plant DNA Infectious Agents*, edited by Th. Hohn and J. Schell, Wien: Springer-Verlag, c1987, pages 249-265, ill.; C. Bellini, *et al.*, "Transgenic Plants of Lettuce (Lactuca Sativa) Obtained Through Electroporation of Protoplasts," *Bio/Technol*, New York, NY: Nature Publishing Co., May 1989, Vol. 7, Issue 5, pages 503-508, ill. An analogous PC2760/pBI121-AdoMetase clone containing the virG gene constituting a tripartite vector system is also employed to transform leaf discs by the method described above.

The present method is applicable to all higher plants, and particularly relevant for use with economically significant food crops and ornamentals. The following list of plant species to which the present method may be applied is representative of the wide range of applications, but is by no means limiting thereto.

5 Food crops:

Allium cepa (onion)
Allium sativum (garlic)
Ananas comosus (pineapple)
Ananas sativus (pineapple)
10 Apium graveolens (celery)
Asparagus officinalis (asparagus)
Beta vulgaris (red and sugar beets)
Brassica oleracea (cole crops)
Capsicum annum (peppers)
15 Capsicum frutescens (peppers)
Carica candamarcensis (papaya)
Carica cauliflora (papaya)
Carica papaya (papaya)
Cichorium endivia (endive)
20 Citrullus lanatus (watermelon)
Citrullus sp. (melons)
Citrulius vulgaris (watermelon)
Cucumis melo (cantaloupe)
Cucumis sativus (cucumber)
25 Cynara scolymus (Globe artichoke)
Daucus carota (carrots)
Ficus carica (figs)
Fragaria sp. (strawberry)
Fragaria x ananassa (strawberry)
30 Lactuca sativa (lettuce)
Lycopersicon esculentum (tomato)
Malus pumila (apple)
Malus sylvestris (apple)
Musa acuminata (banana)
35 Musa cavendishii (banana)
Musa sp. (banana)
Olea europaea (olive)
Passiflora edulis (passion fruit)
Persea americana (avocado)
40 Phaseolus vulgaris (bean)
Phoenix dactylifera (date palm)
Pisum sativum (pea)
Prunus avium (cherry)
Prunus domestica (plum)

Prunus institia (plum)
Prunus mariana (prunus rootstock)
Prunus pandora (cherry)
Prunus persica (peach)
5 Prunus sp. (apricot, nectarines)
Punica granatum (pomegranate)
Pyris communis (pear)
Rubus idaeus (raspberry)
Rubus sp. (cane berries)
10 Rubus ursinus (raspberry)
Solanum melongena (eggplant)
Solanum tuberosum (potato)
Spinacla oleracea (spinach)
Vaccinium elliottii (blueberry)
15 Vaccinium macrocarpon (cranberry)
Vaccinium sp. (blueberry)
Vitis labruscana (concord grape)
Vitis rupestris (grape)
Vitis sp. (grapes)
20 Vitis vinifera (wine grapes)
Zea mays (corn)

Ornamentals:

Antirrhinum majus (snapdragon)
Chrysanthemum morifolium
25 Delphinium cardinale
Delphinium elatum
Delphinium nudicaule
Dianthus caryophyllus (carnation)
Euphorbia pulcherrima (poinsettia)
30 Fuchsia hybrida
Gerbera jamesonii (daisy)
Gladiolus grandiflorus
Gladiolus hortulans
Homerocallis sp. (day lilly)
35 Iris hollandica
Iris sp.
Lilium sp. (lily)
Narcissus sp. (daffodil/narcissus)
Pelargonium hortorum (geranium)
40 Pelargonium peltatum (geranium)
Pelargonium sp.
Pelargonium zonale (geranium)
Petunia axillaris
Petunia hybrida
45 Petunia inflata

Petunia parodii
Petunia parviflore
Petunia sp.
Petunia tricuspidata
5 Rhododendron simsii (azalea)
Rhododendron sp.
Rosa canina
Rosa chinensis
Rosa damascena
10 Rosa hybrida
Rosa manetti
Rosa nitida
Rosa multiflora
Rosa sp.
15 Saintpaulia ionantha (african violet)
Tulipa gesneriana (tulip)

Orchids:

Arachnis sp.
Cattleya sp.
20 Cymbidium sp.
Dendrobium sp.
Oncidium sp.
Paphiopedilum sp.
Vanda sp.

25 The AdoMetase gene is genetically engineered further to achieve a preferred sequence. Analysis of the AdoMetase gene sequence indicated a less than optimal DNA sequence surrounding the initiation codon of the gene. According to the studies of M. Kozak, "At least Six Nucleotides Preceding the AUG Initiator Codon Enhance Translation in Mammalian Cells," *J.Mol.Bio.*, 196:947-950, 1987, 30 a consensus initiation sequence for eucaryotic mRNAs exists which allows for efficient translation. The AdoMetase gene is genetically engineered to change the AdoMetase initiation sequence to the consensus Kozak sequence. The changes made to the DNA sequence are shown in FIGURE 3 and carried out as follows.

EXAMPLE 3

The plasmid pUC19-AdoMetase is digested with Xmn I and Bam HI and the 1.9kb and 1.3kb fragments purified by electrolution after agarose gel electrophoresis. A double stranded synthetic oligonucleotide linker having the sequence indicated in FIGURE 3 is ligated to the 1.9kb fragment and this ligated DNA subjected to Xmn I digestion to remove excess linkers. The linkered 1.9kb fragment is then repurified by electrophoresis on low melting temperature agarose and ligated to the 1.3kb fragment to form the plasmid pUC19 SAM-K. The altered gene region is subjected to DNA sequence analysis and shown to contain the expected DNA sequence as shown in FIGURE 6. This gene is named SAM-K and used to construct additional plant expression vectors. A pBI121-SAM-K (PC2760/pBI121-SAM-K) construction is created and transferred into tobacco using the approach described above in EXAMPLE 1. The plasmid DNA can also be used to directly transform the plant host via electroporation, microinjection, or microprojectile bombardment.

EXAMPLE 4

The following Example discloses an alternative construction. FIGURE 4 describes the construction of vector pGA482-NOS-SAM which is analogous to pBI121-AdoMetase or pBI121-SAM-K above. In this construction, a different promoter is employed as well as different parental plasmids. The parental plasmids are pGA482 and pNCN obtained from Pharmacia. The promoter used is the constitutive nopaline synthetase promoter (NOS-pro and NOS-term).

Using standard techniques, the DNA fragments NOS-pro and NOS-term are isolated from pNCN, and the SAMase fragment coding for the altered enzyme is isolated from pUC-SAM-K. The fragments are ligated into pGA482 at the appropriate restriction sites as indicated in the figure with the NOS-pro and NOS-term sequences flanking the SAMase fragment. The plasmid GA482-NOS-

SAM is transferred to *A. tumefaciens* (PC2760/GA482-NOS-SAM) and used to transform plants as above.

While there is shown and described present preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto, 5 but may be otherwise variously embodied and practiced within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A vector useful for transformation of a plant host, said vector comprising:
a first DNA sequence containing a gene useful for genetic selection in plant cells, where said first DNA sequence is flanked by regulatory elements effective to allow expression of the sequence in a plant host, and where said vector further comprises
a second DNA sequence which (i) is flanked by regulatory elements effective to allow expression of the sequence in a plant host, and (ii) encodes a S-adenosylmethionine hydrolase enzyme which hydrolyses S-adenosylmethionine to homoserine and 5'-methylthioadenosine.

2. The vector of claim 1, wherein said regulatory elements flanking the second DNA sequence include a plant promoter and where said plant promoter provides tissue specific, temperature dependent, or light dependent expression of the second DNA sequence.

3. The vector of claim 1 or 2, where said second DNA sequence encodes the protein sequence presented in Figure 6.

4. The vector of claim 1 or 2, where said enzyme is derived from a bacteriophage selected from the group consisting of *Escherichia coli* bacteriophage T3, coliphage BA14, *Klebsiella* phage K11, and *Seratti* phage IV.

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5. The vector of any one of claims 1 to 4, wherein said gene useful for genetic selection in plant cells confers kanamycin resistance.

6. The vector of any one of claims 1 to 5, wherein said regulatory elements flanking the second DNA sequence include a plant promoter and where said plant promoter is a constitutive nopaline synthetase promoter.

7. The vector of any one of claims 1 to 5, wherein said regulatory elements flanking the second DNA sequence include a plant promoter and where said plant promoter is a constitutive expression promoter.

8. The vector of claim 7, wherein said constitutive expression promoter is a Cauliflower Mosaic Virus promoter.

9. A method for reducing ethylene biosynthesis in plant cells, comprising:
transforming plant host cells with a vector of any of claims 1 to 8,
wherein the transformed plant host cells are capable of expressing said enzyme.

10. The method of claim 9, wherein the transformation of a plant host is carried out by a direct transformation methodology selected from the group consisting of *Agrobacterium*-mediated binary vector transformation, electroporation, microinjection, and microprojectile bombardment.

11. The method of claims 9 or 10, wherein said plant host is *Nicotiania tabacum* L. cv. Wisconsin.

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12. The method of claims 9 or 10, wherein said plant host is a tomato.

13. A transgenic plant cell containing a DNA sequence which encodes and expresses a S-adenosylmethionine hydrolase enzyme, where said enzyme can hydrolyse S-adenosylmethionine to homoserine and 5'-methylthioadenosine.

14. A transgenic plant cell obtainable by transformation of a plant cell with a vector of any one of claims 1 to 8.

15. A transgenic plant cell of claim 13 or 14, where said plant cell is obtained from a fruit, vegetable or flower producing plant.

E

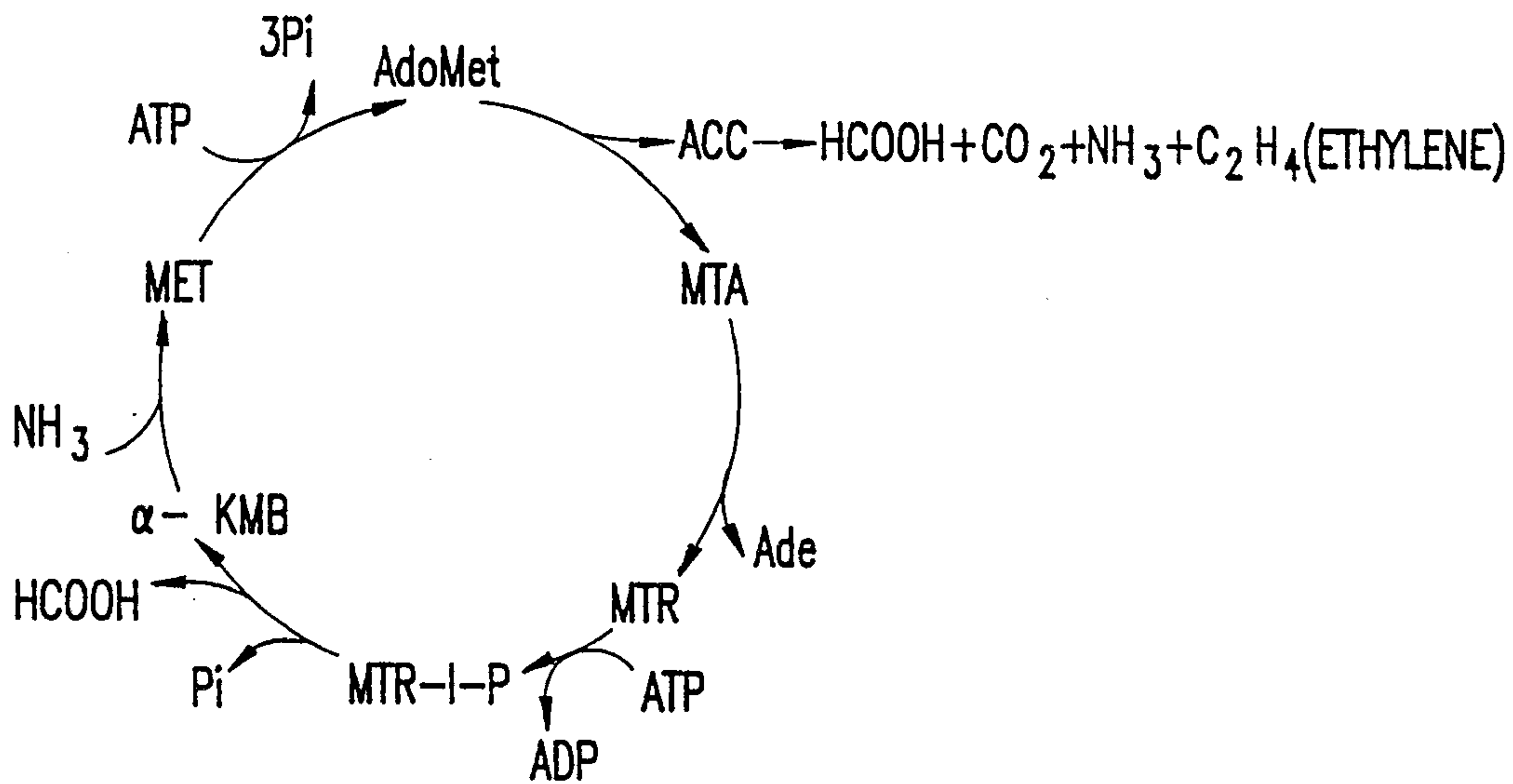

FIG.1a

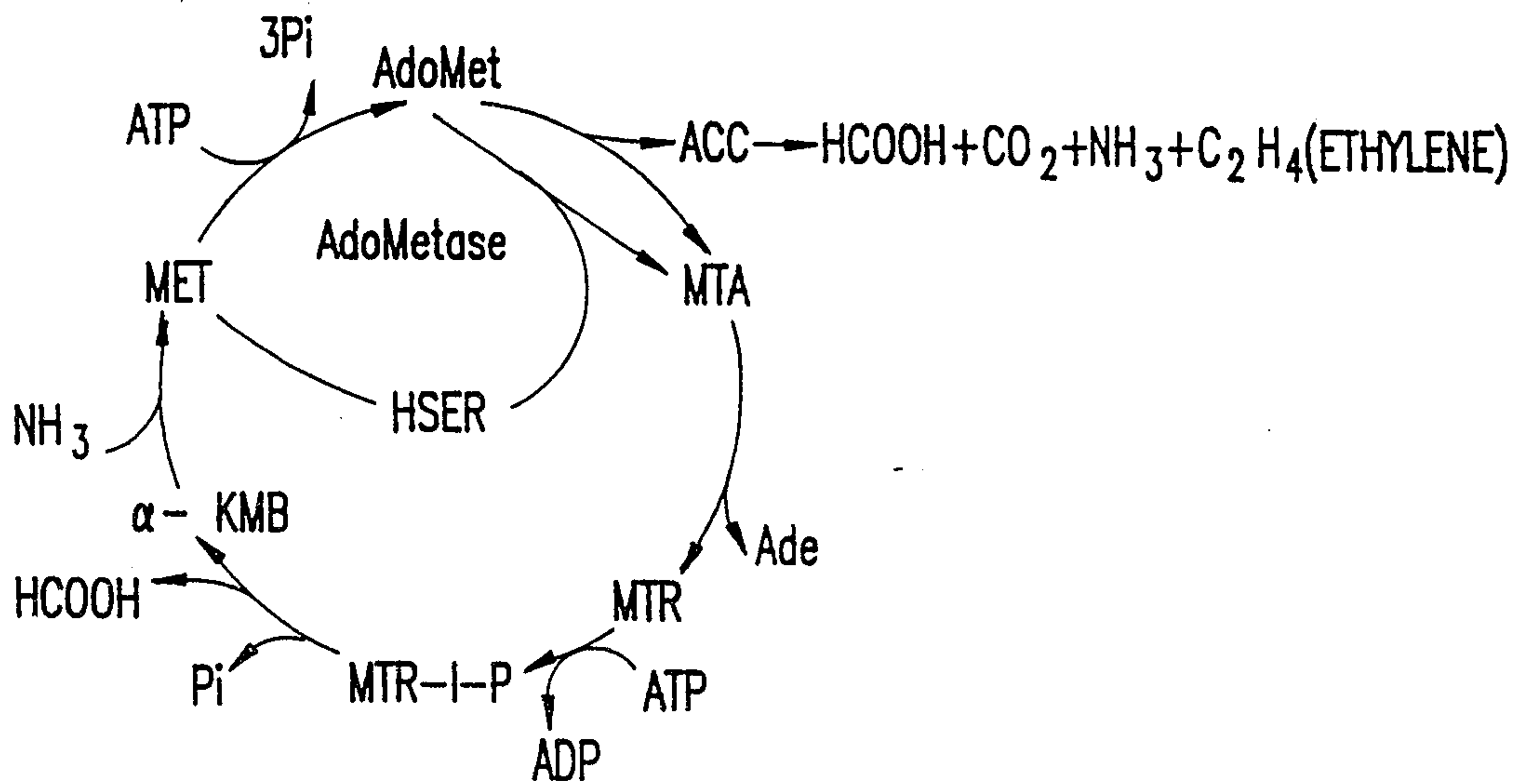

FIG.1b

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METHIONINE RECYCLING PATHWAY

S-ADENOSYLMETHIONINE

ACC → C_2H_4

5'-MEYHYLTHIOADENOSINE

MTA NUCLEOSIDASE

Ade

5-METHYLTHIORIBOSE

ATP

MTR KINASE

5-METHYLTHIORIBOSE-1-PHOSPHATE

1-PHOSPHO-2,3-DIKETO-5-METHYLTHIOPENTANE

Pi

HCOOH

2-KETO-4-METHYLTHIOBUTYRATE

GLUTAMINE

α-KETOGLUTARATE

METHIONINE

ATP

3pi

FIG.1c

SUBSTITUTE SHEET

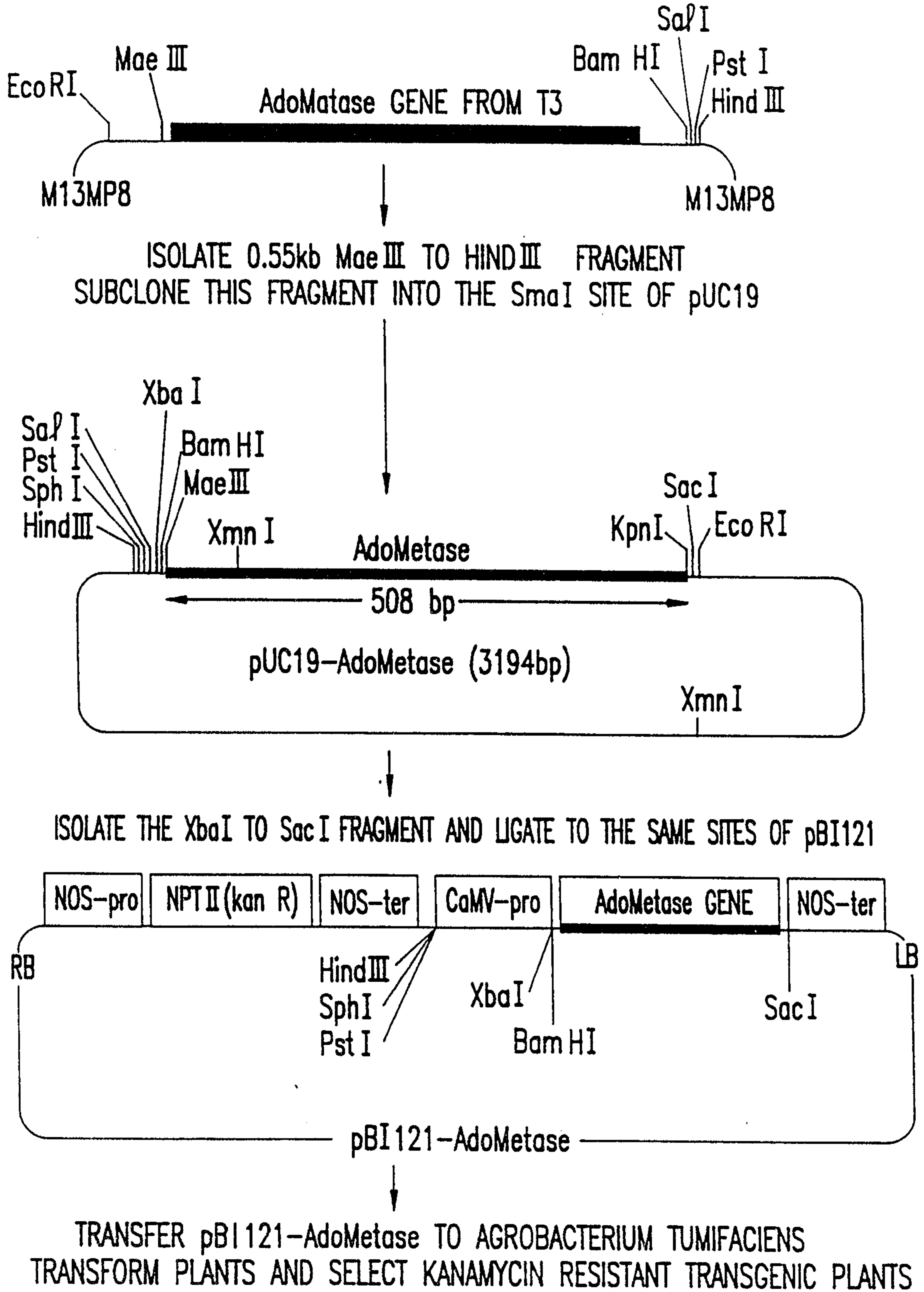


FIG.2

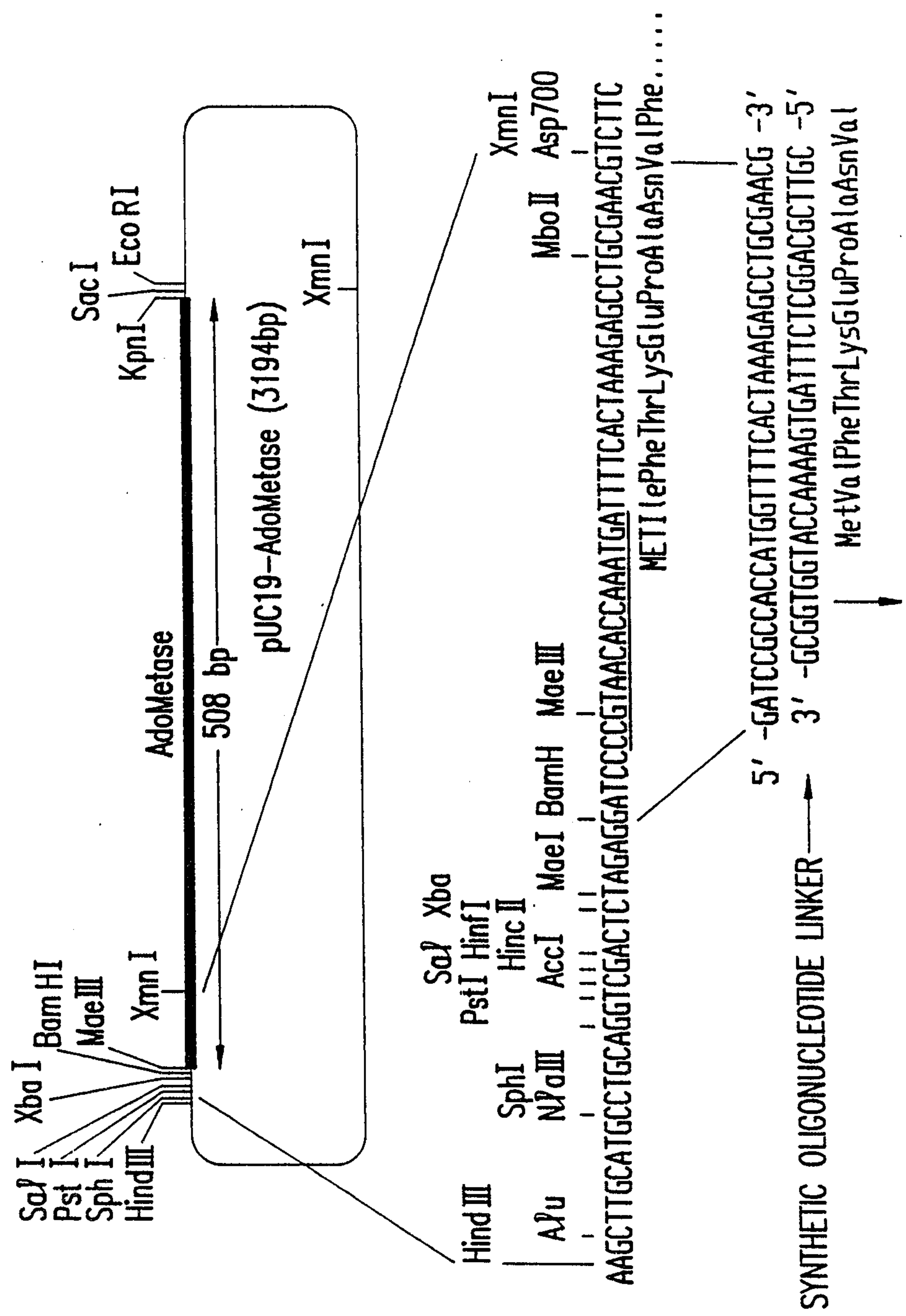


FIG.3

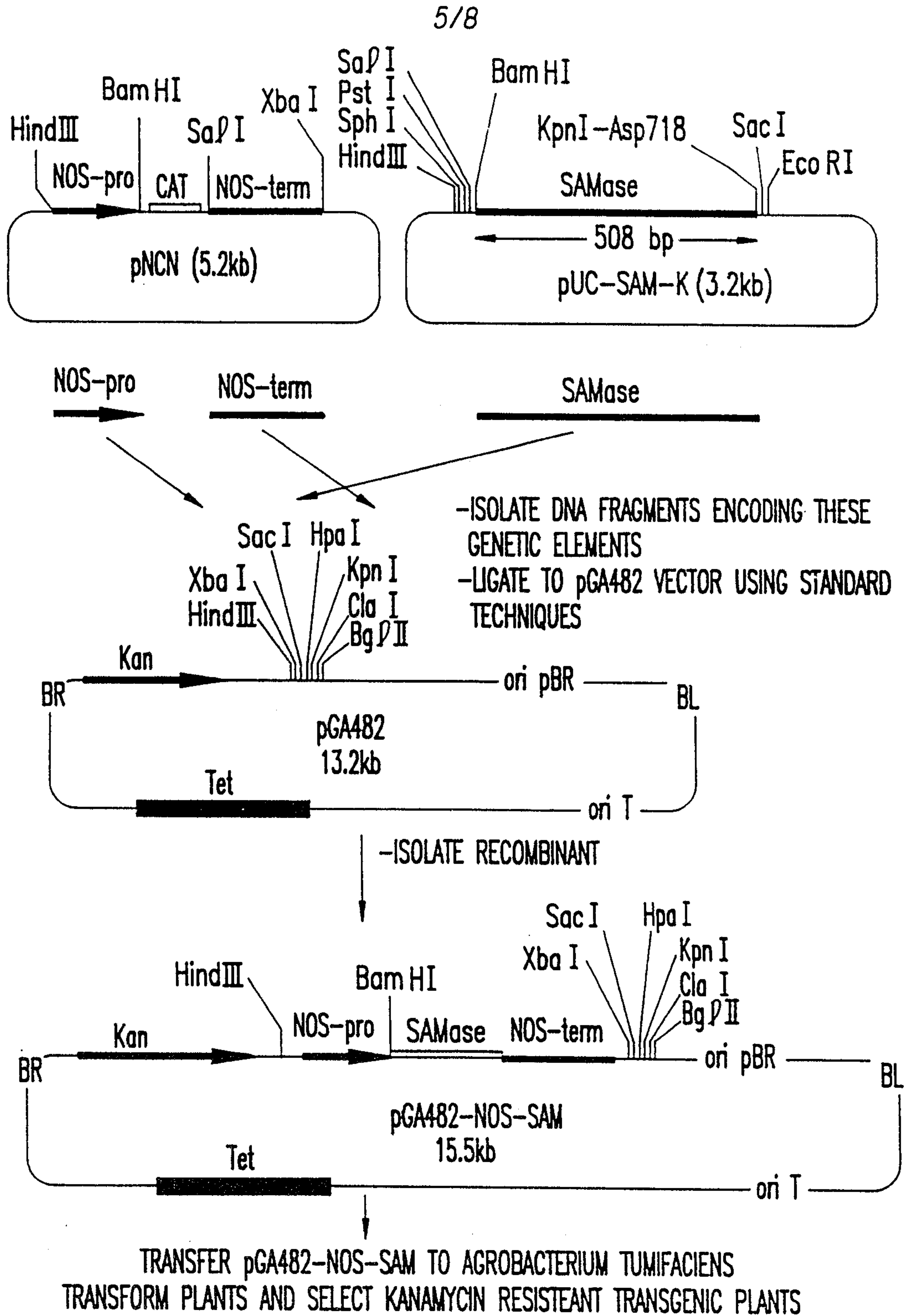


FIG.4

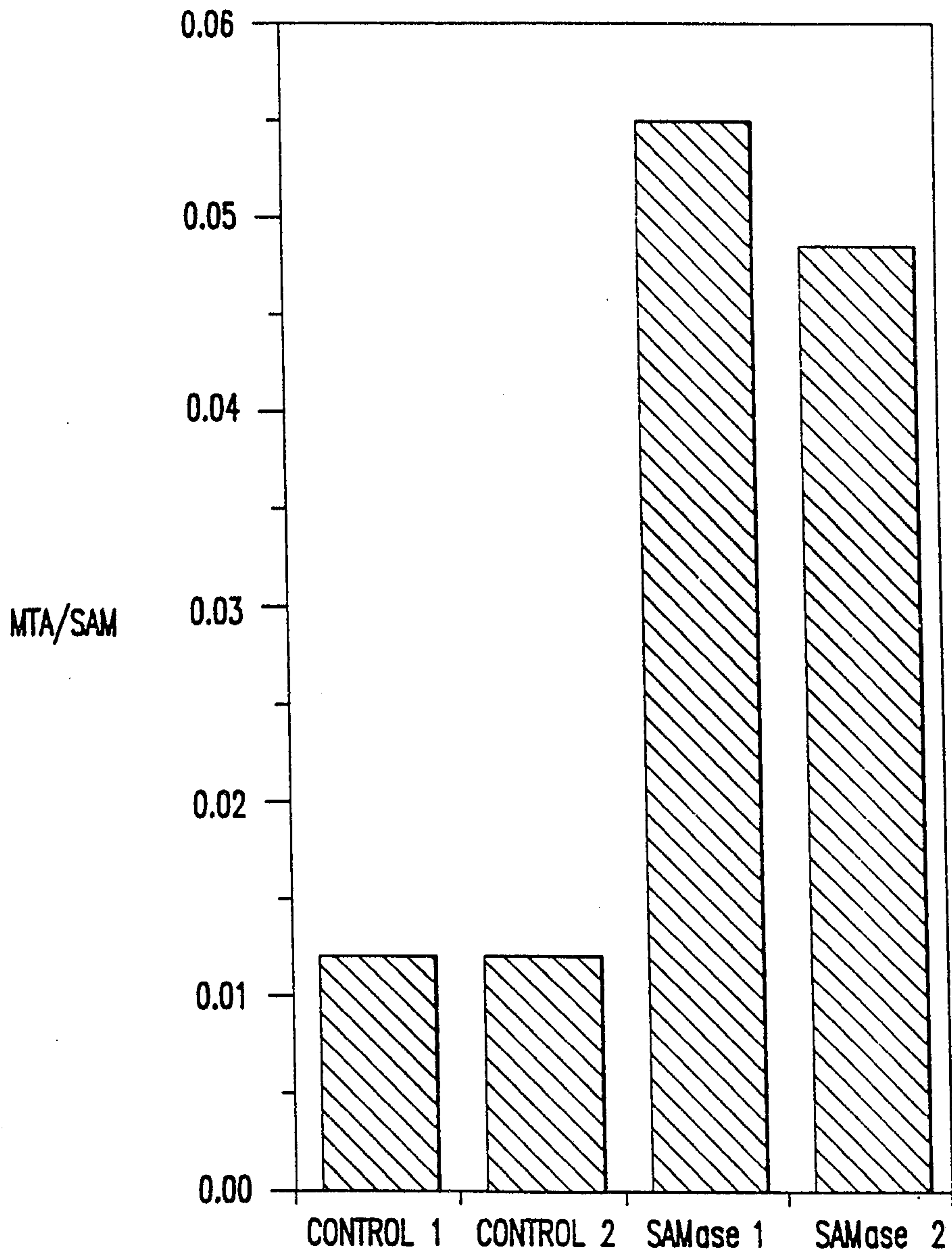


FIG.5

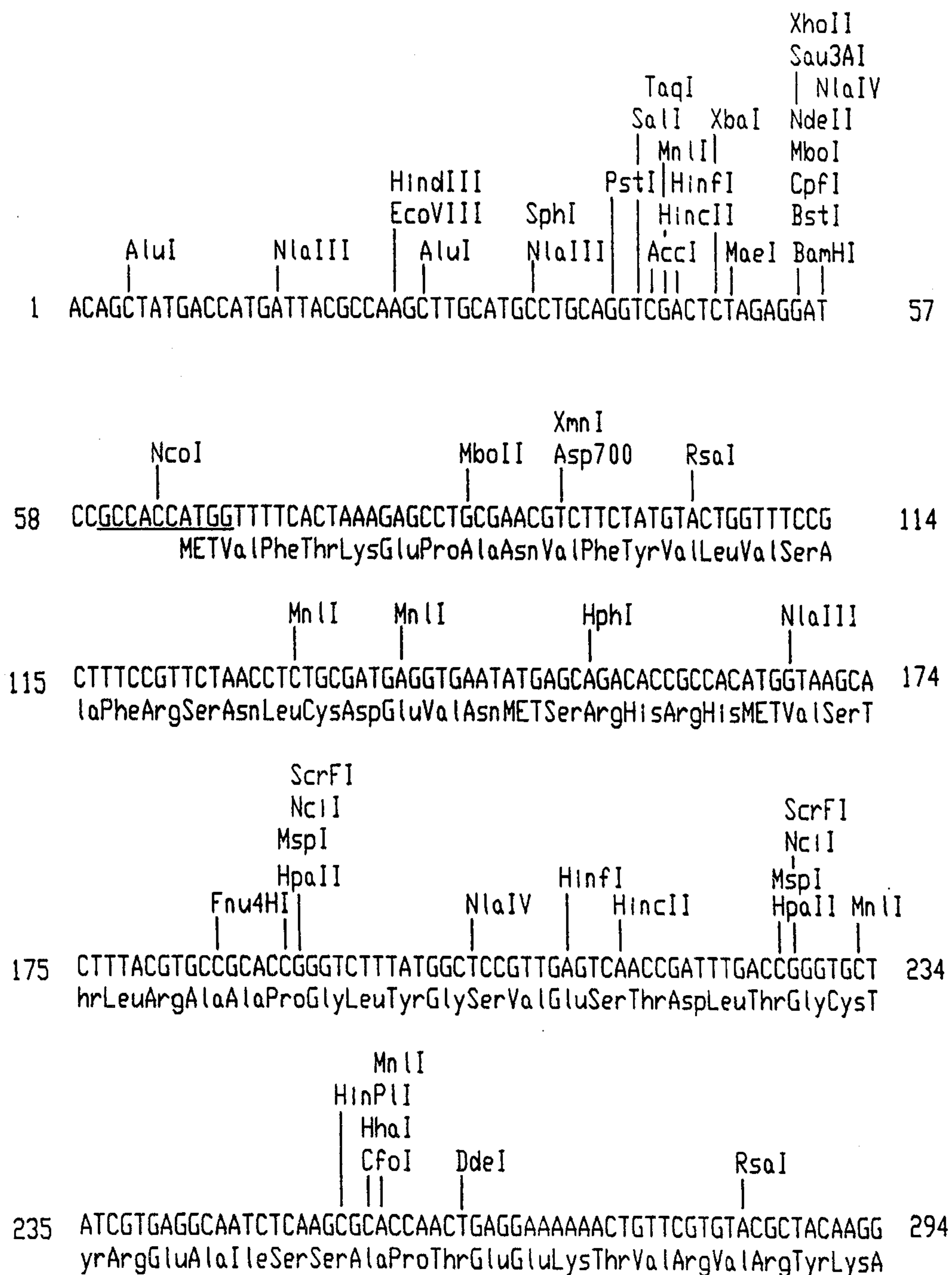


FIG. 6

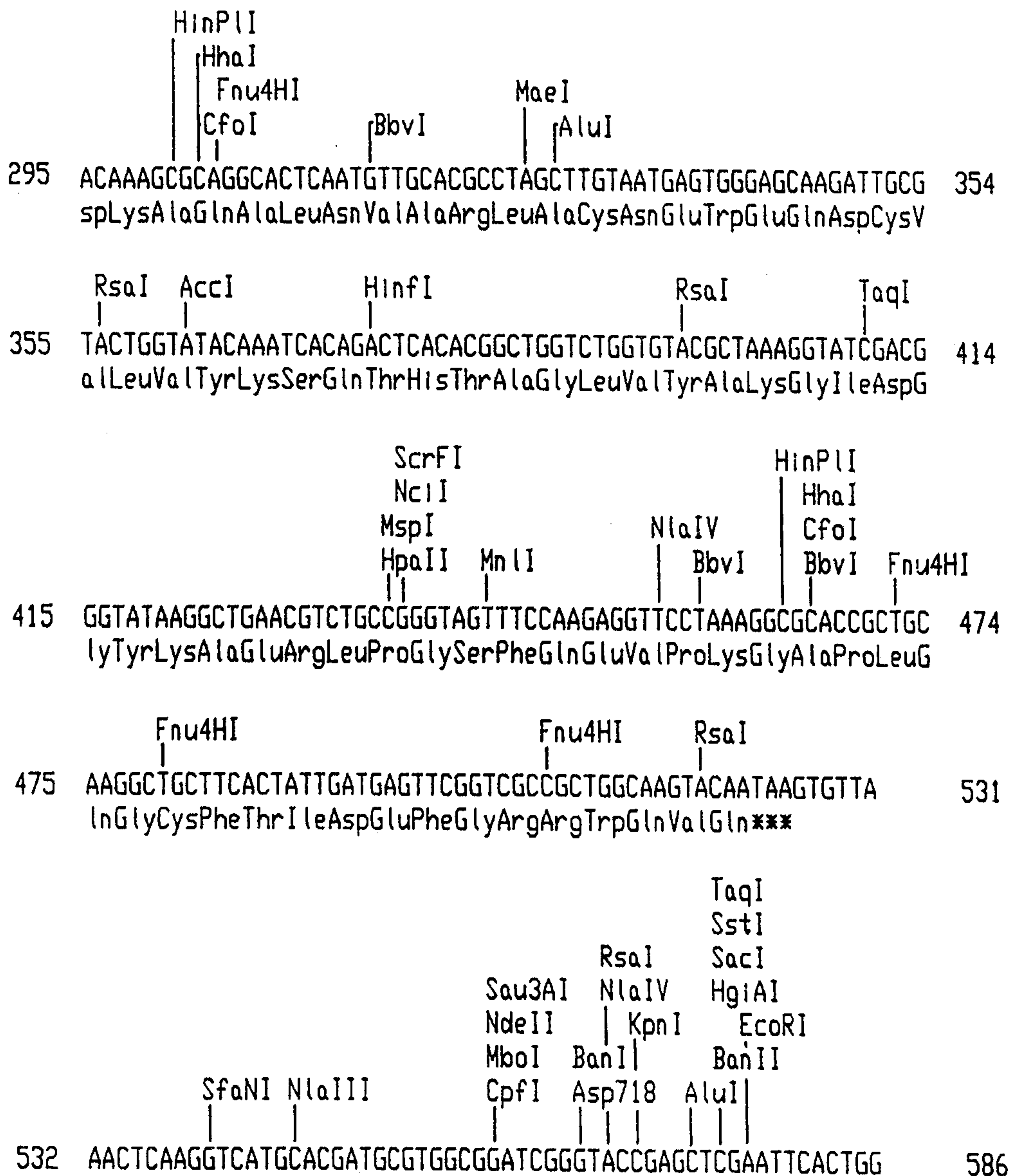
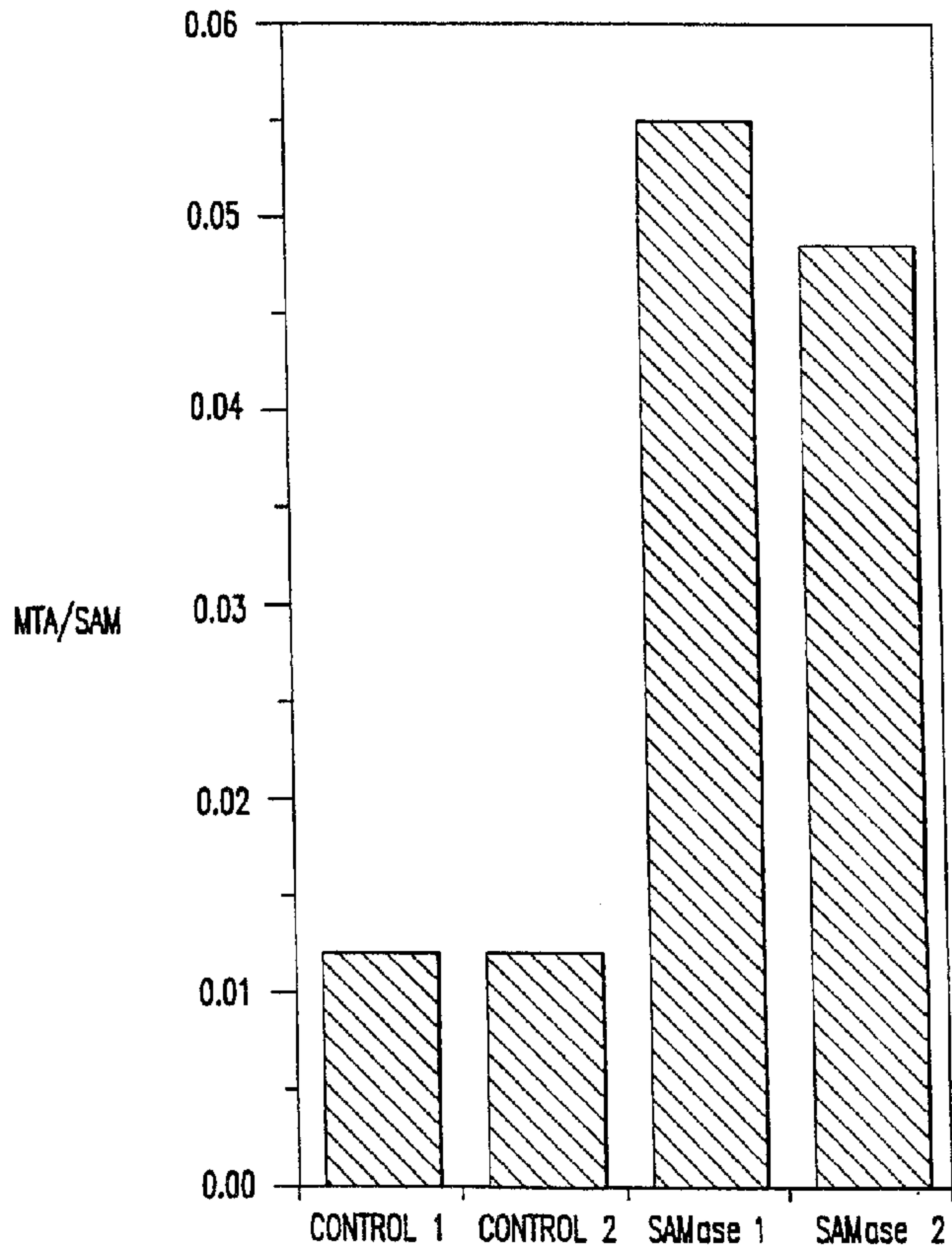


FIG. 6 (cont.)


0.06
0.05
0.04
0.03
0.02
0.01
0.00
MTA/SAM
CONTROL 1
CONTROL 2
SAMase 1
SAMase 2