US005435730A

United States Patent [19]

Adams et al.

[11] Patent Number: 5,435,730

[45] Date of Patent: * Jul. 25, 1995

[54] GAL OPERON OF STREPTOMYCES

[76] Inventors: Craig W. Adams; Mary E. Brawner; James A. Fornwald; Francis J. Schmidt, all of SmithKline Beecham Corporation, Corporate Patents-U.S. UW2220, P.O. Box 1539, King of Prussia, Pa. 19406-0939

[*] Notice: The portion of the term of this patent subsequent to Sep. 7, 2010 has been disclaimed.

[21] Appl. No.: 99,979

[22] Filed: Jul. 30, 1993

Related U.S. Application Data

[60] Division of Ser. No. 967,949, Oct. 19, 1992, Pat. No. 5,242,809, which is a continuation of Ser. No. 692,769, Apr. 19, 1991, abandoned, which is a continuation-in-part of Ser. No. 9,419, Jan. 30, 1987, abandoned, which is a continuation-in-part of Ser. No. 834,706, Feb. 28, 1986, abandoned.

[51] Int. Cl.[6] C12N 15/63; C12N 15/74; C12N 15/11

[52] U.S. Cl. 435/69.1; 435/172.3; 435/320.1; 435/252.3; 435/252.35; 536/23.2; 536/23.7; 536/24.1; 935/41; 935/75

[58] Field of Search 435/69.1, 172.3, 320.1, 435/252.35, 252.3; 536/23.2, 23.7, 24.1; 935/41, 75

[56] References Cited

PUBLICATIONS

Thompson et al. 1982 Gene 20: 51–62.
Casaban J. Mol. Biol. 1976 104(3) 557–566.

Primary Examiner—Richard A. Schwartz
Assistant Examiner—John LeGuyader

[57] ABSTRACT

A recombinant DNA molecule comprising the Streptomyces gal operon galK gene; galE gene; galT gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region; or the entire Streptomyces gal operon.

45 Claims, 3 Drawing Sheets

FIG. 1

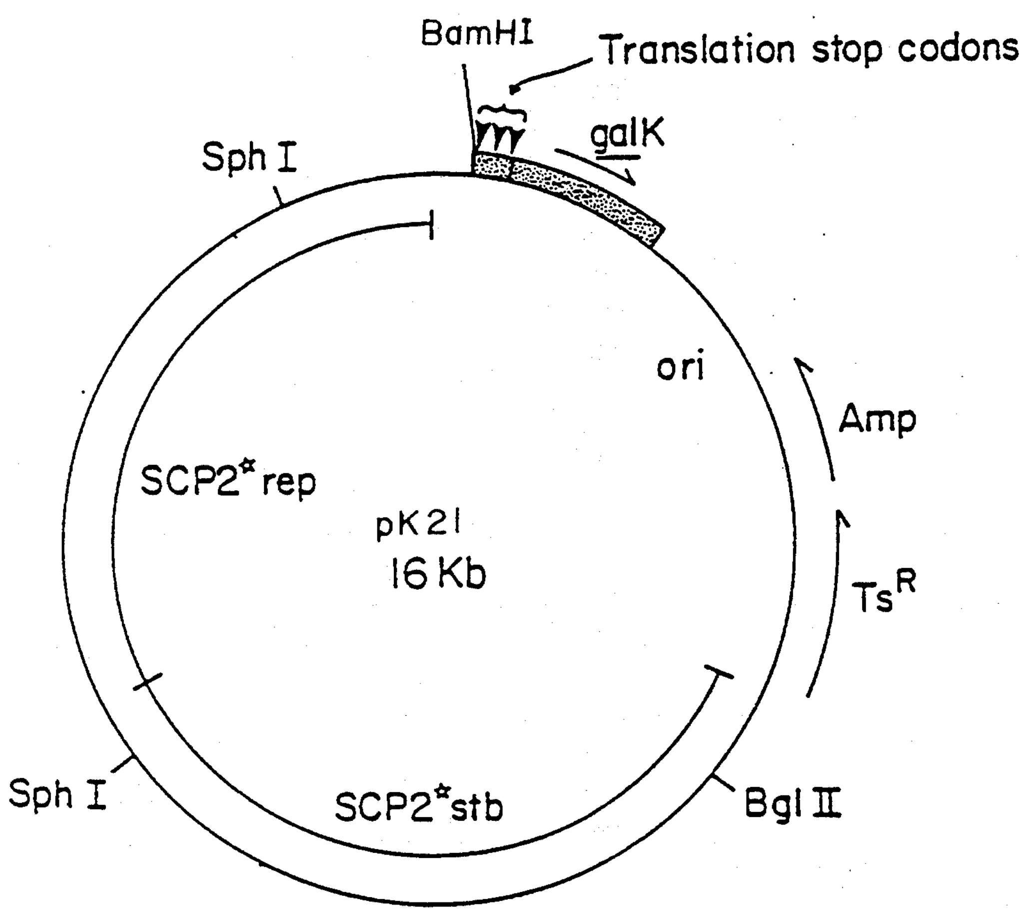
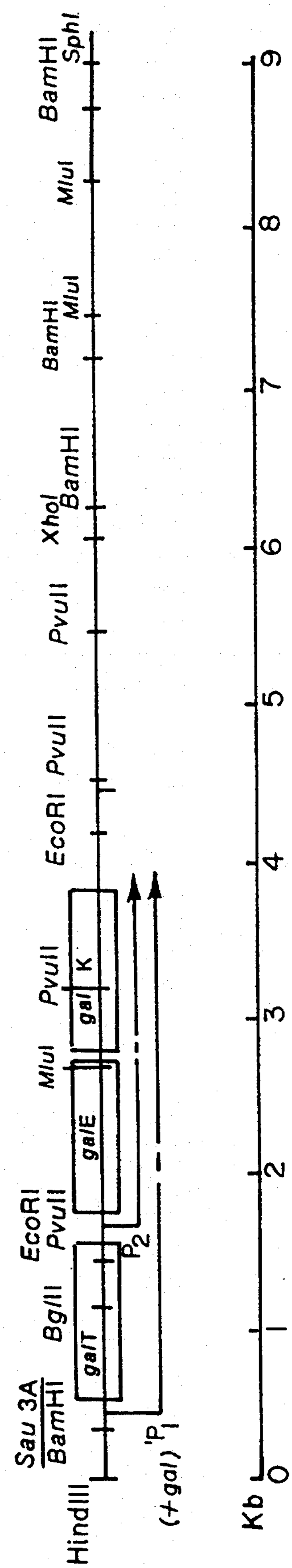

BamHI
Translation stop codons
galK
Sph I
ori
Amp
SCP2* rep
pK21
16 Kb
Ts^R
Sph I
SCP2*stb
Bgl II

FIG. 2


HindIII
Sau 3A/BamHI
BglII
EcoRI
PvuII
MluI
PvuII
EcoRI
PvuII
PvuII
XhoI
BamHI
BamHI
MluI
MluI
BamHI
SphI
galT
galE
gal K
P2
(+gal) 'P1
Kb
0
1
2
3
4
5
6
7
8
9

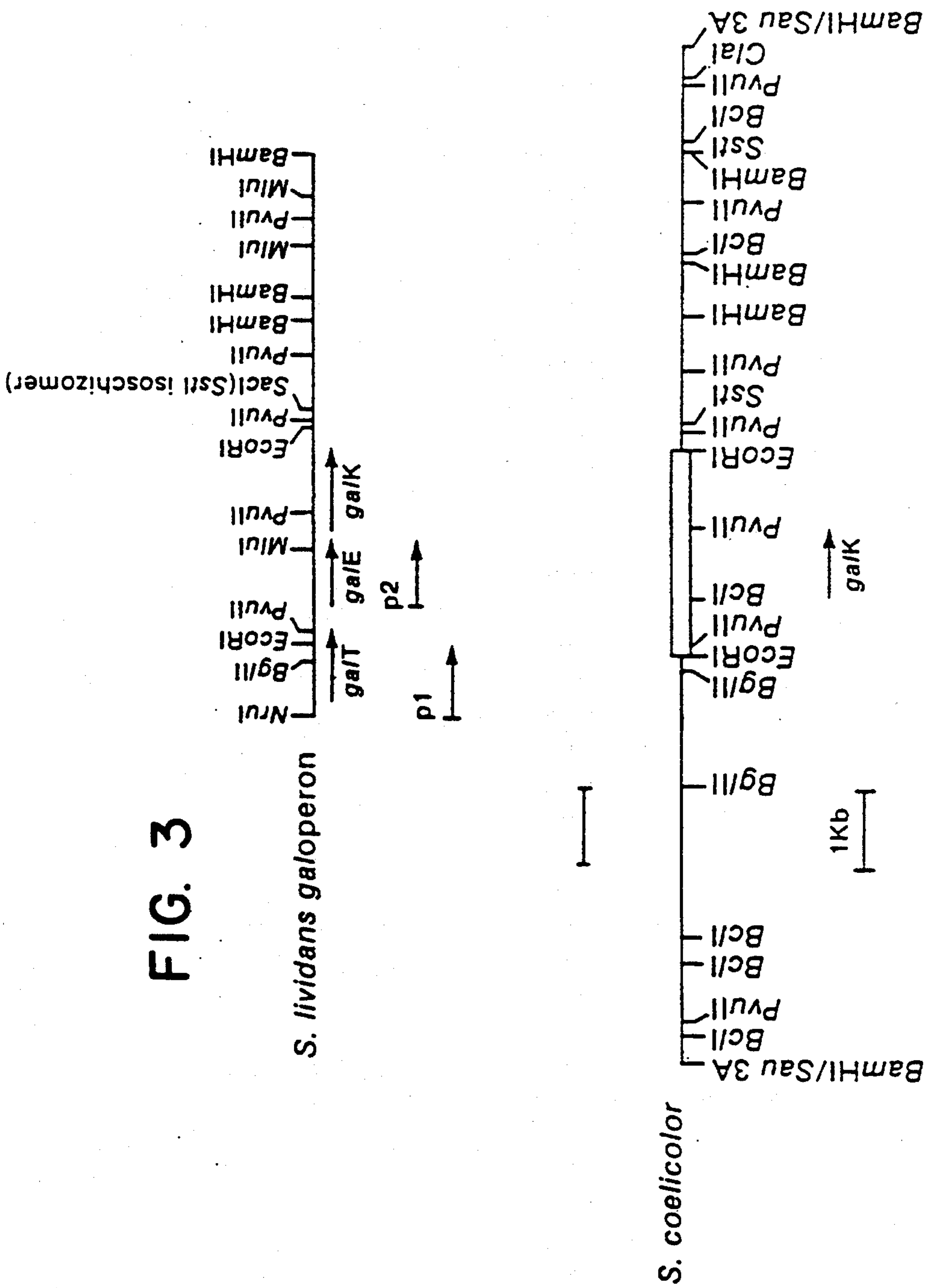

S. lividans galoperon
NruI
BglII
EcoRI
PvuII
MluI
PvuII
EcoRI
PvuII
SacI(SstI isoschizomer)
PvuII
BamHI
BamHI
MluI
PvuII
MluI
BamHI
galT
galE
galK
p1
p2
S. coelicolor
BamHI/Sau 3A
BclI
PvuII
BclI
BclI
BglII
BglII
EcoRI
PvuII
BclI
PvuII
EcoRI
PvuII
SstI
PvuII
BamHI
BamHI
BclI
PvuII
BamHI
SstI
BclI
PvuII
ClaI
BamHI/Sau 3A
galK
1Kb

FIG. 3

GAL OPERON OF STREPTOMYCES

CROSS REFERENCE TO RELATED APPLICATIONS

This is a divisional of application Ser. No. 07/967,949, filed Oct. 27, 1992, now U.S. Pat. No. 5,242,809, which is a continuation of application Ser. No. 07/692,769, filed Apr. 29, 1991, now abandoned, which is a continuation-in-part of application Ser. No. 07/009,419, filed Jan. 30, 1987, now abandoned, which is a continuation-in-part of application Ser. No. 06/834,706, filed Feb. 28, 1986, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a recombinant DNA molecule comprising the Streptomyces gal operon.

Hodgson, *J. Gen. Micro.*, 128, 2417–2430 (1982), report that *Streptomyces coelicolor* A3(2) has a glucose repression system which allows repression at the level of transcription of the arabinose uptake system, one of the glycerol uptake systems, and also repression of the galactose uptake system in wild type strains. There is no report in Hodgson of actual galactose metabolism by *S. coelicolor A*3(2).

Okeda et at. *Mol. Gen. Genet*, 196, 501–507 (1984), report that glucose kinase activity, 2-deoxyglu-cose-sensitivy, glucose utilization and glucose repression were all restored to *S. coelicolor* A3(2) glk (glucose kinase) mutants transformed by a 3.5 kb DNA fragment which contained the glk gene cloned from *S. coelicolor* into a phage vector.

Seno et at., *Mol. Gen. Genet.*, 193, 119–128 (1984), report the glycerol (gyl) operon of *Streptomyces coelicolor*, and state that such operon is substrate-inducible and catabolite-repressible.

Debouck et al., *Nuc. Acids. Res.*, 13(6), 1841–1853 (1985), report that the gal operon of *E. coli* consists of three structurally contiguous genes: which specify the enzymes required for the metabolism of galactose, i.e., galE (uridine diphosphogalactose-4-epimerase), galT (galactose-1-phosphate uridyltransferase) and galK (galactokinase); that such genes are expressed from a polycistronic mRNA in the order E, T, K; that the expression of the promoter distal gene of the operon, galK, is known to be coupled translationally to the galT gene immediately preceding it; that such translational coupling results from a structural overlap between the end of the gait coding sequence and the ribosome binding region of galK; and that the translational coupling of galT and galK ensures the coordinate expression of these genes during the metabolism of galactose.

SUMMARY OF THE INVENTION

This invention relates to a recombinant DNA molecule comprising a Streptomyces gal operon galK gene; galE gene; galT gene; P2 promoter expression unit, or P2 promoter or any functional derivative thereof as well as a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter, P1 promoter regulated region or the entire gal operon or any regulatable and functional derivative thereof.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon or any regulatable and functional derivative thereof; a recombinant DNA molecule comprising a *Streptomyces lividans* or *Streptomyces coelicolor* gal operon containing a wild-type gal operon P1 promoter or any regulatable and functional P1 promoter derivative, a functional DNA molecule operatively linked to such operon; a recombinant DNA vector comprising and such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter expression unit or any functional derivative thereof and a functional DNA molecule operatively linked to such unit; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; and to a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof and a functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter or any regulatable and functional derivative thereof and a foreign functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector; the transformed host prepared by such method; a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed; and to a method of regulating the expression of such functional DNA sequence which comprises cultivating such transformed host under conditions which regulate such expression.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter or any functional derivative thereof and a foreign functional DNA molecule operatively linked to such region; a recombinant DNA vector comprising such DNA molecule, and, optionally, additionally comprising a replicon; a method of preparing a host cell transformed with such vector, the transformed host prepared by such method; and to a method of expressing such functional DNA sequence which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed.

This invention also relates to a method of enabling a non-galactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA molecule comprising a Streptomyces gal operon or any portion of the Streptornyces gal operon, or any functional derivative thereof, which is adequate to enable such transformed host to utilize galactose. This invention also relates to the recombinant DNA vector employed in such method and to the host prepared by such method.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 represents a restriction endonuclease map of the *Streptomyces lividans* 1326 galactose (gal) operon and indicates approximate locations for structural genes and promoters within the operon.

FIG. 2 represents a restriction endonuclease map of plasmid pK21.

FIG. 3 represents a comparison of the restriction endonuclease maps of the *S. lividans* gal operon and a restriction fragment containing the *S. coelicolor* galK gene.

DETAILED DESCRIPTION OF THE INVENTION

It hats now been discovered that the Streptomyces genome contains an operon for the metabolism of galactose (i.e., a gal operon) which comprises three structural genes (galT, galE and galK) and two promoters (P1 and P2). The galT gene product is known as galactose-1-phosphate uridyltransferase (transferase), the gene product is known as uridine diphosphogalactose-4-epimerase (epimerase), and the galK gene product is known as galactose-1-kinase (galactokinase). The function of the gene products of galT, galE and galK in galaclose metabolism in Streptomyces is explained by the following diagram:

1. galactose+ATP galactokinase galactose-1-phosphate+ADP
2. galactose-1-phosphate+UDP-glucose transferase UDP-galactose+glucose-1-phosphate
3. UDP-galactose epimerase UDP-glucose

By the term "promoter" is meant any region upstream of a structural gene which permits binding of RNA polymerase and transcription to occur.

By the term "structural gene" is meant a coding sequence for a polypeptide which serves to be the template for the synthesis of mRNA.

By the term "operon" is meant a group of closely linked genes responsible for the synthesis of one or a group of enzymes which are functionally related as members of one enzyme system. An operon comprises an operator gene, a number of structural genes (equivalent to the number of enzymes in the system) and a regulator gene. By "operator", or "operator gene" is meant a DNA sequence which controls the biosynthesis of the contiguous structural gene(s) within an operon. By "regulator gene" is meant a gene which controls the operator gene in an operon through the production of a repressor which can be either active (enzyme induction) or inactive (enzyme repression). The transcription of the structural gene(s) in an operon is switched on or off by the operator gene which is itself controlled in one or more of three ways: 1) in inducible enzyme systems, the operator is switched off by a repressor produced by the regulator gene and which can be inactivated by some metabolite or signal substance (an inducer) coming from elsewhere in the cell or outside the cell, so that the presence of the inducer results in the operon becoming active; or 2) in repressed enzyme systems, the operator is switched off by a repressor-corepressor complex which is a combination of an inactive repressor produced by the regulator gene with a corepressor from elsewhere, so that the presence of the corepressor renders the operon inactive; or 3) in activated gene systems, the promoter is switched on by an activator produced by a regulator gene which can be activated by some metabolic or signal substance.

The Streptomyces gal operon is naturally present in the Streptomyces genome.

By the term "Streptomyces gal operon" is meant that region of the Streptomyces genome which comprises the P1 promoter, P2 promoter, galT, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes.

By the term "regulatory region" is meant a DNA sequence, such as a promoter or operator, which regulates transcription of a structural gene.

The following model is suggested for gene expression within the Streptomyces gal operon. The P1 promoter is a galactose inducible promoter (i.e., it is induced in the presence of galactose and repressed in the presence of glucose). According to S1 data, the P2 promoter is constitutive, i.e., it is "turned on" regardless of the presence or absence of galaclose or any other carbon source.

A cosmid library was constructed for *Streptomyces lividans* 1326 DNA by using cosmid pJW357 (which encodes the ability to replicate in both Streptomyces and *E. coli*). This library was then transfected into *E. coli* K21 which is a derivative of the *E. coli* strain MM294 which contained a bacteriophage P1 transduced galactokinase (galK) mutation. Transfected cells were plated under media conditions which select for both the presence of the cosmid and the presence of an active galK gene. Weakly positive colonies were isolated and the cosmid DNA derived from these: colonies was transformed into the K21 strain. These transformations yielded two cosmids which consistently produced positive growth with galaclose as the only carbon source. These galK+ cosmids were then transformed into a Streptomyces host (i.e., *Streptomyces lividans* 1326-12K) which had been isolated by the inventors of the subject invention as unable to grow on medium in which galactose was the only carbon source by using 2-deoxygalactose selection [see, Brawner et at., Gene, 40 191 (1985), in press]. Under conditions which differentiate strains able and unable to produce galactoldnase, only one of the cosmids caused the *Streptomyces lividans* 1326-12K host to become galK+. Further studies have demonstrated that this cosmid encodes a gene with galactokinase activity. Additional studies, including DNA sequence analysis and protein studies demonstrate that this Streptomyces gene shares homology with the *E. coli* and yeast galactokinase genes. Regulation studies indicate that the cosmid encoded galactokinase gene regulated in the same manner as the chromosome encoded gene. A. *S. lividans* gal operon was originally isolated from a ca. 9 kilobase (Kb) region of *Streptomyces lividans* 1326. The ca. 9 Kb region of *Streptomyces lividans* 1326 containing the Streptomyces gal operon has been mapped substantially as follows in Table A. By "substantially" is meant (i) that the relative positions of the restriction sites are approximate, (ii) that one or more restriction sites can be lost or gained by mutations not otherwise significantly affecting the operon, and (iii) that additional sites for the indicated enzymes and, especially for enzymes not tested, may exist. The restriction enzymes used herein are commercially available. All are described by Roberts, *Nuc. Acids. Res.*, 10(5):p117 (1982).

TABLE A

Map Position	Restriction Enzyme	Location (kb)
1	HindII	−.40
1a	NruI	0
2	BglII	.75
3	EcoRI	1.05
4	PvuII	1.15
5	MluI	2.30
6	PvuII	2.80
7	EcoRI	4.00
8	PvuII	4.10
8a	SacI	4.25
9	PvuII	5.00
10	Xho	5.50
11	BamHI	5.80
12	BamHI	6.50
13	MluI	6.90
13a	PvuII	7.20
14	MluI	7.80
15	BamHI	8.00
16	SphI	8.30

FIG. **1** represents a restriction endonuclease map of the *Streptomyces lividans* 1326 gal operon and indicates locations for structural genes (galT, galE and galK) and promoters (P1 and P2) comprised within the operon.

Referring to Table A and FIG. **1**, the location of the promoters and structural genes of the *Streptomyces lividans* 1326 gal operon are mapped substantially as follows in Table B:

TABLE B

	Location (Kb)
P1 transcription start site	.10
galT translation initiation codon	.15
P2 transcription start site	1.25
galE translation initiation codon	1.50
galK translation initiation codon	2.40
3' end of galK message	3.60

Microorganisms of the genus Streptomyces have historically been used as a source of antibiotics for the pharmaceutical industry. Consequently, the technical skills necessary to scale-up the production of biological products using Streptomyces as the vehicle for the production of such products are presently available. However, before Streptomyces can be used as a vehicle for the production of bioactive molecules using the new recombinant DNA technologies, there is a need to define regulatory elements in Streptomyces analogous to those which have proved useful in *E. coli*. These regulatory elements include ribosomal binding sites and :regulated transcriptional elements.

The existence of a galE, galT or galT or galK gene or gene product or gall operon in Streptomyces has not been previously reported. The instant invention, i.e., the cloning of the Streptomyces gal operon, enables construction of regulatable expression/cloning vectors in Streptomyces, other actinomycetes, and other host organisms. Furthermore, the instant invention led to the discovery that the Streptomyces gal operon is polycistronic. Perhaps the most important feature of the cloning of the Streptomyces gal operon is the observation that there are sequences essential for regulation of the Streptomyces galK gene. Direct analogy to the initial use of the lac promoter from *E. coli* as an expression system can be made. In fact, Brosius et al., *Proc. Natl. Atari. Sci. USA*, 81, 6929–6933 (1984), utilized the regulatory elements of the *E. coli* lac promoter to regulate the exceptionally strong *E. coli* fibosomal promoters. Because it is likely that the Streptomyces gal operon fibosomal promoters are also exceptionally strong, such promoters enable the construction of regulatable expression vectors which will be very useful in Streptomyces, other actinomycetes, and other host organisms. The instant invention also enabled the unexpected discovery that the 2-deoxygalactose selection which has been used in *E. coli* to select for galK routants also operates in Streptomyces to select for galK mutants [see, Brawner et al., Gene 40, 191 (1985), in press]. This observation, combined with the ability to clone the Streptomyces galK gene and the promoter and regulatory regions required for its transcription and translation on a cosmid, as described herein, allows the direct insertion of any structural gene into the chromosomally located galK gene of Streptomyces by homologous recombination. This manipulation will allow molecular biologists to stably insert DNA fragments of interest into the Streptomyces chromosome. Such an approach will allow researchers to tag or mark a Streptomyces strain of interest or to insert expression cassettes into the organism without the need of maintaining an antibiotic selection such as that presently required by most Streptomyces expression vectors.

This invention relates to a recombinant DNA molecule comprising the Streptomyces gal operon or any regulatable and functional derivative thereof. By "regulatable and functional derivative" is meant any derivative of the Streptomyces gal operon which functions in substantially the same way as the naturally occurring Streptomyces gal operon in terms of regulatable production of the gaT, galE and galK gene products. Such derivatives include partial sequences of the gal operon, as well as derivatives produced by modification of the gal operon coding sequence. Techniques for modifying the gal operon which are known in the an include, for example, treatment with chemical mutagens, irradiation or direct genetic engineering, such as by inserting, deleting or substituting nucleic acids by the use of enzymes or recombination techniques. The naturally occurring Streptomyces gal operon can be isolated from any galactose utilizing Streptomyces strain by employing the techniques described herein. Numerous strains of various Streptomyces species are publicly available from many sources. For example, the American Type Culture Collection, Rockville, Md., U.S.A. has approximately 400 different species of Streptomyces available to the public. The ability of a particular strain of Streptomyces to utilize galactose can be readily determined by conventional techniques, such as by growing such strain on a medium containing galactose as the sole carbon source. The preferred Streptomyces species from which to isolate a gal operon include *S. lividans, S. coelicolor, S. azuraeus* and *S. albus, S. carzinostaticus, S. antibibrinolyticus* and *S. longisporus, S. lividans* is most preferred. The Streptomyces gal operon, and smaller portions thereof, is useful as a nucleic acid probe to obtain homologous sequences from other cells and organisms. The Streptomyces gal operon is also useful as a selection marker in an appropriate host mutant, and for providing regulatory elements. By "appropriate host mutant" is meant a host which does not utilize galactose because it (a) does not contain a gall operon or (b) contains a nonfunctional gal operon, or (c) contains a defect within a homologous structural gene or regulatory region comprised by the Streptomyces gal operon such as a defective P1 promoter, P2 promoter, galT gene, galK gene and/or galE gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable regulatable expression of the foreign functional DNA sequence without the need of maintaining an expensive antibiotic selection. Such operon may therefore also be incorporated on recombinant DNA expression vectors for regulatable expression of a foreign functional DNA sequence operatively linked to such operon in an appropriate host mutant transformed with such vector without the need of maintaining an expensive antibiotic selection. Such operon is also useful for transforming those cells, viruses and microorganisms, such as strains of Streptomyces, other actinomycetes, and other prokaryotic organisms, such as gal *E. coli* strains, which do not utilize galaclose into galactose utilizing strains. Such transformation may have pleiotrophic effects on the transformed host. By the term "functional DNA sequence" is meant any discrete region of DNA derived directly or indirectly form Streptomyces or any other source which functions in a host organism transformed therewith as a gene expression unit, structural gene, promoter or a regulatory region. Preferred functional DNA sequences include those coding for polypeptides of pharmaceutical importance, such as, but not limited to, insulin, growth hormone, tissue plasminogen activator, alpha-1-antitrypsin or antigens used in vaccine production. By the term "foreign functional DNA sequence" is meant a functional DNA sequence not derived from the Streptomyces gal operon coding region.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter expression unit or any functional derivative thereof. By the term "P2 promoter expression unit" is meant that region of the Streptomyces gal operon comprising the Streptomyces gal operon P2 promoter, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes. By "functional derivative" is meant any derivative of the Streptomyces gal operon P2 promoter expression unit which functions in substantially the same way as the naturally occurring region in terms of production of the Streptomyces gal operon galE and galK gene products. Such derivative include partial sequences of the Streptomyces gal operon P2 promoter expression unit coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P2 promoter expression unit can be isolated from the naturally occurring Streptornyces gal operon by conventional techniques. The Streptomyces gal operon P2 expression unit is useful as a selection marker in an appropriate host mutant and for providing regulatory elements. By "appropriate host mutant" is meant a host which does not utilize galactose because it contains a defect within a homologous structural gene or regulatory region comprised by the Streptomyces P2 promoter expression unit such as a defective P2 promoter, gale gene and/or galK gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon P2 promoter expression unit and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable constitutive expression of the foreign functional DNA sequence without the need of maintaining an expensive antibiotic selection. Such expression unit may also be incorporated on recombinant DNA expression vectors for constitutive expression of foreign functional DNA sequences. The Streptomyces gal operon P2 promoter expression unit is also useful for complementation of an appropriate host mutant which can then be used for constitutive expression of a foreign functional DNA sequence operatively linked to such expression unit in an appropriate host mutant transformed with such vector without the need of maintaining an expensive antibiotic selection.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof. By the term "P1 promoter regulated region" is meant that region of the Streptomyces gal operon comprising the Streptomyces gal operon P1 promoter, galT, galE and galK structural genes and any other regulatory regions required for transcription and translation of such structural genes. By "regulatable and functional derivative" is meant any derivative of the Streptomyces gal operon P1 promoter regulated region which functions in substantially the same way as the naturally occurring region in terms of regulatable production of the Streptomyces gal operon galT, galE and galK gene products. Such derivatives include partial sequences of the Streptomyces gal operon P1 promoter regulated region, as well as derivatives produced by modification of the Streptomyces gal operon P1 promoter regulated region coding sequence. Techniques for effecting such modifications am known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P1 promoter regulated region can be isolated from the naturally occurring Streptormyces gal operon by conventional techniques, such as by excising the P2 promoter from the naturally occurring Streptomyces gal operon or inactivating the P2 promoter by a point mutation or by inserting a foreign DNA sequence within the promoter. The Streptornyces gal operon P1 promoter regulated region is useful for the utilities outlined above for the Streptomyces gal operon.

This invention also relates to a recombinant DNA molecule comprising the Streptornyces gal operon P2 promoter or any functional derivative thereof. By "functional derivative" is meant any derivative of the Streptornyces gal operon P2 promoter which functions in substantially the same way as the naturally occurring P2 promoter in terms of enabling the binding of RNA polymerase thereto and transcription of a functional DNA sequence operatively linked to such promoter. Such derivatives include partial sequences of the Streptormyces gal operon P2 promoter, as well as derivatives produced by modification of the gal operon P2 promoter coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P2 promoter can be isolated from the naturally occurring Streptomyces gal operon by conventional techniques. A recombinant DNA molecule (comprising the Streptomyces gal operon P2 promoter and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable constitutive expression of the foreign functional DNA sequence. The Streptomyces gal operon P2 promoter is also useful for incorporation into recombinant DNA expression vectors for constitutive expression of a foreign functional DNA sequence operatively linked thereto in viruses and eukaryotic or prokaryotic cells or organisms, especially in Streptomyces or other actinomycetes, transformed with such vector.

This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P1 promoter or any regulatable and functional derivative thereof. By "regulatable and functional derivative" is meant any derivative of the Streptomyces gal operon P1 promoter which functions in substantially the same way as the naturally occurring P1 promoter in terms of enabling the binding of RNA polymerase thereto and regulating the transcription of a functional DNA sequence operatively linked to such promoter. Such derivatives include partial sequences of the Streptomyces gal operon P1 promoter, as well as derivatives produced by modification of the gal operon P1 promoter coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P1 promoter can be isolated from the naturally occurring Streptomyces gall operon by conventional techniques. A recombinant DNA molecule (comprising the Streptomyces gnl operon P1 promoter and a foreign functional DNA sequence operatively linked thereto), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable regulatable expression of the foreign functional DNA sequence. The Streptomyces gnl operon P1 promoter is also useful for incorporation into recombinant DNA expression vectors for regulatable expression of a foreign functional DNA sequence operatively linked thereto in viruses and eukaroyotic or prokaryotic cells or organisms, especially Streptomyces or other actinomycetes, transformed with such vector.

This invention also related to a recombinant DNA molecule comprising the Streptomyces gal operon galE, galT or galK gene, or any functional derivative thereof. By "functional derivative" is meant any derivative of the Streptomyces gal operon galE, galT or galK gene which functions in substantially the same way as the naturally occurring gene in terms of production of an active galE, galT, or galK type gene product. Such derivatives include partial sequences of the Streptornyces gal operon galE, galT, or galK gene, as well as derivatives produced by modification of the gal operon sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon galE, galT and/or galK gene can be isolated from the naturally occurring Streptornyces gal operon by conventional techniques. The Streptomyces gal operon galE, galT and/or galK gene can be used as a selection marker in an appropriate host mutant. By "appropriate host mutant is meant a host which does not utilize galactose because it contains a defect within a homologous galE, galT and/or galK gene. Thus, a recombinant DNA molecule (comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory region), which can be prepared by conventional techniques, can be transformed into an appropriate host mutant by conventional techniques for incorporation into the host genome by homologous recombination to enable detection of transformants without the need of maintaining an expensive antibiotic selection. Likewise, a recombinant DNA vector comprising the Streptomyces gal operon galE, galT and/or galK gene and a foreign functional DNA sequence, both of which are operatively linked to appropriate regulatory regions, as well as a replicon, can be transformed into an appropriate host mutant by conventional techniques to enable detection of transformants without the need of maintaining an expensive antibiotic selection. The Streptomyces gal operon galE, galK and/or galT gene is also useful for complementation of an appropriate host mutant.

The Streptomyces gal operon galE gene is also useful for providing a ribosome binding; site and initiation codon which can be fused to a foreign functional DNA sequence to enable the expression of such coding sequence when incorporated into an appropriate expression vector and transformed into an appropriate host. If such foreign functional DNA sequence is fused to the galE gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the Streptomyces gal operon P2 promoter expression unit, or the entire gill operon, such DNA sequence will be constitutively expressed when such vector is transformed into an appropriate host organism. If such DNA sequence is fused to the gale gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the Streptomyces gal operon P2 promoter regulated region expression of such DNA sequence can be regulated when such vector is transformed into an appropriate host organism by controlling the presence or absence of galaclose or glucose.

The Streptomyces gal operon galT gene is also useful for providing a ribosome binding site and initiation codon which can be fused to a foreign functional DNA sequence to enable the expression of such coding sequence when incorporated onto an appropriate expression vector and transformed into an appropriate host. If such DNA sequence is fused to the gait gene ribosome binding site and initiation codon in a recombinant DNA expression vector comprising the Streptomyces gal operon P1 promoter regulated region, or the entire gal operon, expression of such coding sequence can be regulated in a host transformed with such vector as outlined above.

This invention also relates to a recombinant DNA vector comprising a replicon, Streptomyces gal operon, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such operon. Such vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally, maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such operon; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed in the method of this invention include viruses, and eukaroyotic and prokaryotic cells or organisms, especially actinomycetes, such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include *Streptomyces lividans, S. coelicolor, S. azuraeus* and *S. albus.* Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chalet et al., Curr. Top. Micro. Imm., 96, 69–95 (1982). This invention also related to a method of expressing the functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed. This invention is also related to a method of regulating the expression of the functional DNA sequence contained by such transformed host which comprises cultivating a transformed host containing such functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the Streptomyces gal operon (and thus the foreign functional DNA sequence) to be regularable. By "regulatable" is meant responsive to the presence of galactose or its metabolites and the presence of glucose or its metabolites in the growth media of the transformed host cell. Such regulation can be carried out by addition or deletion of galactose or glucose to the transformed host's culture medium. The optimal levels of galactose and/or glucose for up or down-regulation of the expression of the foreign functional DNA coding sequence by the transformed host of this invention can be readily determined by one of skill in the art using conventional techniques.

This invention also related to a recombinant DNA vector comprising a replicon, a Streptomyces gal operon P2 promoter expression unit, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such unit. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably, and extrachromosomally, maintain a vector in the host organism which is to be transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon P2 promoter expression unit, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such unit and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. By the term "operatively linked" is meant that a functional DNA sequence is transcriptionally or translationally linked to an expression control sequence (i.e., the Streptomyces gal operon, P2 promoter expression unit, P1 promoter regulated region, P1 promoter or P2 promoter) in such a way so that the expression of the functional DNA sequence can be transcriptionally or translationally linked to the Streptomyces gal operon by inserting such operon within the Streptomyces gal operon P1 or P2 promoter transcript. By the term "replicon" is meant that region of DNA on a plasmid which functions to maintain, extrachromosomally, such plasmid in a host microorganism or cell transformed therewith. It has also been discovered that the Streptomyces gal operon, and smaller portions thereof, is useful as a nucleic acid probe to obtain homologous sequences from other cells and organisms. Appropriate host microorganisms which may be employed in the method of this invention include any virus or eukaroyotic or prokaryotic cell or organism, especially any actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include *Streptomyces lividans, S. coelicolor, S. azuraeus* and *S. albus.* Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., *Curt. Top. Micro. Imm.,* 96, 69–95 (1982). This invention also related to a method of expressing the functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the an using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed.

This invention also related to a recombinant DNA vector comprising a replicon, a Streptomyces gal operon P1 promoter regulated region, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such region. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also related to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, a Streptomyces gal operon P1 promoter regulated region, or a functional and regulatable derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed include any virus or eukaroyotic or prokaryotic cell or organism especially actinomycetes such as those of the microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include *Streptomyces lividans, S. coelicolor, S. azuraeus* and *S. albus*. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69–95 (1982). This invention also related to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed. This invention also related to a method of regulating the expression of the functional DNA sequence contained by such transformed host which comprises cultivating a transformed host containing such functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the Streptomyces gal operon P1 promoter regulated region (and thus the foreign functional DNA sequence) to be regulatable. By "regulatable" is meant responsive to the presence or absence of galaclose or its metabolites and the presence or absence of glucose or its metabolites in the growth media or the transformed host cell. Such regulation can be carried out by addition or deletion of galactose or glucose to the transformed host's culture medium.

This invention also relates to a recombinant DNA vector comprising a replicon, a Streptomyces gal operon P2 promoter, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such promoter. Such a vector can be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, a Streptomyces gal operon P2 promoter, or a functional derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms which may be employed include actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include *Streptomyces lividans, S. coelicolor, S. azuraeus* and *S. albus*. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chater et al., Curr. Top. Micro. Imm., 96, 69–95 (1982). This invention also related to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions; such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the functional DNA sequence to be expressed.

This invention also relates to a recombinant DNA vector comprising a replicon, Streptomyces gal operon P1 promoter, or any regulatable and functional derivative. thereof, and a foreign functional DNA sequence operatively linked to such region. Such a vector cart be prepared by conventional techniques. The replicon employed should be one known for its ability to stably and extrachromosomally maintain a vector in the host organism which is to be the host transformed with the vector.

This invention also relates to a transformed host microorganism comprising a recombinant DNA vector wherein said vector contains a replicon, the Streptomyces gal operon P1 promoter, or any regulatable and functional derivative thereof, and a foreign functional DNA sequence operatively linked to such region; and to the method of preparing such host which comprises transforming an appropriate host microorganism with such vector. Appropriate host microorganisms .which may be employed include viruses or prokaryotic or eukaroyotic cells or organisms, especially actinomycetes such as those of the genus Streptomyces. The most preferred host microorganisms belong to the genus Streptomyces. Preferred species of Streptomyces include *Streptomyces lividans, S. coelicolor, S. azuraeus* and *S. albus*. Transformation of such host microorganism with such vector can be accomplished using conventional techniques such as the method of Chalet et al., Curr. Top. Micro,. Imm, 96, 69–95 (1982). This invention also relates to a method of expressing the foreign functional DNA sequence contained by such transformed host of this invention which comprises cultivating such transformed host under suitable conditions such that the functional DNA sequence is expressed. By "suitable conditions" is meant those conditions which will allow the host to grow and which enable the expression of the functional DNA sequence. Such suitable conditions can be determined by one of skill in the art using conventional techniques and will depend on various factors, such as the host organism employed and the foreign functional DNA sequence to be expressed. This invention also relates to a method of regulating the expression of the functional DNA sequence contained by such transformable host which comprises cultivating a transformed host containing such foreign functional DNA sequence under appropriate conditions such that its expression is regulatable. By "appropriate conditions" is meant those conditions which enable the gal operon P1 promoter (and thus the functional DNA sequence) to be regularable. By "regulatable" is meant responsive to the presence or absence of galactose or its metabolites and the presence of glucose or its metabolites in the growth media of the transformed host cell. Such regulation can be carried out by addition or deletion of galaclose or glucose to the transformed host's culture medium.

EXAMPLES

In the following Examples, specific embodiments of the invention are more fully disclosed. These Examples are intended to be illustrative of the subject invention and should not be construed as limiting its scope. In all Examples, temperature is in degrees Centigrade (°C.).

By utilizing conventional methods, such as those outlined in the following Examples, one of skill in the art can isolate the gal operon from any galactose utilizing; strain of Streptomyces. Furthermore, by utilizing techniques similar to those employed herein to isolate the Streptomyces gal operon, one of skill in the art can attempt to use the Streptomyces gal operon to isolate a gal operon from other galactose utilizing other strains of Streptomyces, especially *S. coelicolor, S. azuraeus, S. albus* and other *S. lividans* strains.

Molecular genetic manipulations and other techniques employed in the following Examples are described in Hopwood et al., *Genetic Manipulation of Streptomyces: A Laboratory Manual*, John Innes Foundation, Norwich, England (1985).

ABBREVIATIONS

In the following Examples, the following abbreviations may be employed:

LB: 10 grams (g) tryptone, 5 g yeast extract, 5 g NaCl

MBSM (modified MBSM): See, Brawner et al., *Gene,* 40, 191 (1985) (in press) MOPS: (3)-N-morpholino-(proprane-sulfonic acid)

YEME+$MgCL_2$+Glycine: [per liter(1)] 3 g yeast extract, 5 g peptone, 3 g malt extract, 10 g glucose, 10 g $MgCL_2$"62H_2O, 340 g sucrose. SL: Mix together $(NH_4)_2SO_4$(1 g/l); L-asparagine (2 g/l); K_2HPO_4 (9 g/l); NaH_2PO_4. (1 g/l) for 0.2% agar and autoclave. Then mix with yeast extract (20 g/l), $MgCl_2$ (5 g/l); $CuCl_2$ (0.1 g/l); Trace elements [20 ml/l—include $ZnCl_2$—40 mg/l; $FeCl_3$"6H_2O (200 mg/l); $CuCl_2$"2H_2O (10 mg/l); NaB_4O_7"10-H_2O (10 mg/l); $(NH_4)_6MO_7O_{24}$"4H_2O (10 mg/l)] filter and sterilize.

YEME (Ym base): (per liter) yeast extract (3 g); peptone (5 g); malt extract (3 g); $MgCl_2$"6H_2O (2 g)

Ymglu: YEME+glucose (10 g)

Ymgal: YEME+galactose (10 g)

BACTERIAL STRAINS

In the following Examples, the following strains of *E. coli* are employed:

CGSC Strain #[a]	Strain Designation	Sex	Chromosomal Markers
4473 (galE^{-})	W3109	F^{-}	galE9,[b]g^{-};IN(rrnD-rrnE)1
4467 (galT^{-})	W3101	F^{-}	galT22[b]g^{-};IN(rrnD-rrnE)1
4498 (galE^{-})	PL-2	Hfr	thi-1, relA1, 921E28, g^{-}, spoT1

[a]CGSC Strain # is the stock number designated for such strain by the *E. coli* Genetic Stock Center of the Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut, 06510, U.S.A.

[b]galE9 is the old Lederberg gal9; galT22 is the old Lederberg gal_1.

S1 ANALYSIS

S1 analysis is used to identify the 5' end of RNAs and the length of a RNA of interest. In the following Examples, S1 analysis refers to S1 experiments carried out according to the method of Weaver et al., *Nucl. Acids Res.,* 7, 1175 (1979) and Berk et at., *Proc. Natl. Acad. Sci. USA,* 75, 1214 (1978).

EXAMPLE I

A. CLONING OF A STREPTOMYCES LIVIDANS GALACTOKINASE GENE

Streptomyces lividans strain 1326 is described by Bibb et al., *Mol. Gen. Genetics,* 184, 230–240 (1981) and was obtained from D. A. Hopwood, John Innes Foundafioni, Norwich, England. *Streptomyces lividans* strain 1326 and *S. lividans* strain 1326 containing the pIJ6 plasmid were deposited in the Agricultural Research Culture Collection, See, Adams et al., Biochem, Biophys, Res. Comm., 89(2), 650–58 (1979)] with 30 mg/ml chloramphenicol. Twenty plates were spread with approximately 200 transformants per plate. After three days incubation at 37° C., no transformants were detected. The minimal plates were then sprayed with nicotinic acid to 5 ug/ml to supplement the nicotinic acid requirement of *E. coli* strain K21, and the incubation was continued for 3 more days at 37° C. and for 2 additional days at room temperature. After such incubation, the surviving colonies were patched to both MacConkey galactose agar (MAC-GAL) [See, Miller et al., cited above] with 30 ug/ml chloramphenicol and to M63 minimal agar [See, Miller et al., cited above] supplemented with .5% galactose, 5 ug/ml nicofinic acid, 5 ug/ml thiamine and 30 ug/ml chloramphenicol. Only two colonies contained cosmid DNA that transformed *E. coli* K21 to a galK+ phenotype. Such cosmids were designated as psLIVGAL-1 and pSLAIVAG-2. Both colonies were light red on MAC-GAL (i.e., they were galK+) and also grew on the M63 medium.

Plasmid pSLIVGAL-1 and PSLIVGAL-2 were isolated from the two galK+ colonies described above and were transformed, according to the method of Chater et at., *Curr. Top. Micro. Imm.,* 96, 69–95 (1982), into *Streptomyces lividans* strain 1326-12K (a galK deficient strain isolated after UV mutagenesis of *S. lividans* strain 1326, See, Brawner et at., *Gene,* 40, 191 (1985), (in press), Plasmid encoded complementation of the *S. lividians* 1326-12K (galK−) host was tested by observing growth of spores plated on MBSM-gal-thiostrepton according to the method of Brawner et at., *Gene,* 40, 191 (1985) (in press). pSLIVGAL-2 showed no detectable complementation of the Streptomyces 1326-12K host.

Cell extracts were prepared from cultures grown in SL medium supplemented with 1% glucose or galactose and 10 ug/ml thiostrepton. The extracts were analyzed forgalactokinase production by immunoblot analysis (see, Brawner et at., *Gene,* 40, 191 (1985), in press) using rabbit antisera prepared against *E. coli* galactokinase. The protein detected by immunoblot analysis was the approximate size of *E. coli* galK. Such protein appeared in galactose supplemented cultures of Streptomyces at levels several fold higher than in glucose cultures.

B.MAPPING OF THE S. LIVIDANS GALK REGION WITHIN A COSMID.

The galK region of the pSLIVGAL1 and pSLIVGAL2 cosmids, prepared as described above, was identified by cloning random fragments from the cosmids into a pUC18 derivative [See, Norrander et at., Gene, 26, 101–106 (1983)] and scoring complementation of *E. coli* strain MM294 (galK−) on MAC-GAL medium. The cosmid clone was partially digested with Sau3AI (using conditions which maximized the yield of 2 to 4 kilobase fragments), and the products of this reaction were ligated into the BglII site of pUC18-TT6, a derivative of pUC18 constructed by insertion of the following synthetic DNA sequence into the BamHI site of pUC18:

5'GATCAGATCTTGATCACTAGCTAGCTAG 3'
3' TCTAGAACTAGTGATCGATCGATCCTAG 5'

Twelve galK+ clones (red on MAC-GAL) were screened for size. One clone, designated as plasmid pSAU10, was the smallest and had an insert size of approximately 1.4 Kb.

In contrast to colonies containing pSLIVGAL1, the pUC clones were very red on MAC-GAL medium, indicating and increased production of galactokinase. The most likely explanation for the increased enzyme level was that the *S. lividans* galK gene was now being transcribed by an *E. coli* promoter which was stronger than the upstream promoter on the cosmid.

The insert of pSAU10 was isolated as an EcoRI to HindIII fragment (these sites flank the insert region of pUC18-TT6) for use as a probe for the *S. lividans* galK gene. The chromosomal DNA used in the cloning was restricted with EcoRI plus MluI and BamHI plus BglII, and then blotted according to the method of Southern, *J. Mol. Biol.*, 98, 503 (1975). The pSAU10 fragment was nick translated and hybridized to the blot. The probe identified a 1.3 kb EcoRI-MluI fragment and a 5 kb BamHI-BglII fragment in the chromosomal digests. When this data was compared to the map of the cosmid insert, the location of the galK gene (between map positions 5 and 7, See Table A) was confirmed.

C. DNA SEQUENCING OF THE S. LIVIDANS GAL OPERON.

The *Streptomyces lividans* gal operon was sequenced by chain termination [(See, Sanger et at., *Proc. Nat'l Acad. Sci., U.S.A.*, 74, 5463 (1977)] and chemical cleavage [See, Maxam and Gilbert, *Methods in Enzymology,* 65, 499 (1980)]. The initial sequences of galK were derived from Sau3AI and SalI fragments of the insert of pSAU6 (a 2.3 Kb sibling of pSAU10) shotgun cloned into the BamHI and SalI sites (respectively) of M13 mp 10 [See, Messing, Methods in Enzymology, 101, 20 (1983)]. Amino acid sequences of the *S. lividans* galT, galE and galK genes were predicted by computer, and further analyzed by comparison with amino acid sequences of the *E. coli* and or *S. cerevisiae* galactokinase, gal-1-phosphate uridyltransferase and UDP-4-epimerase enzymes. The sequences of these proteins were predicted by computer analysis using the total or partial DNA sequence of the genes which encode the gal enzymes [see, Debouck et al., *Nuc Acids. Res.*, 13(6), 1841–1853 (1985), and Citron and Donelson, *J. Bacteriology,* 158,269 (1984)]. Some homology was found between the inferred protein sequence for the *S. lividans* galK, galT, gale gene products and their respective *E. coli* and/or *S. cerevisiae* gene products.

The complete DNA sequence of the *S. lividans* gal operon is shown in Table 1. Included in Table 1 are the transcription start sites for the operon's promoters and the predicted amino acid sequences of the galT, galE and galK products.

TABLE 1

TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS GALACTOSE OPERON

		−120 *			−110 *			−100 *				−90 *			−80 *			−70 *
CTA	CGC	CTC	CGC	GTT	CAG	TAA	TTG	AAC	ACT	TTT	GGT	GAT	GAA	CTT	TGT	TTG	ATT	GTG
			−60 *			−50 *			−40 *				−30 *			−20 *		
ATG	TGA	CAG	GGG	GGT	GGT	GGG	TTG	TGA	TGT	GTT	ATG	TTT	GAT	TGT	GTT	GGA	TGA	TTG
														galP1				
−10 *				1 *			10 *			20 *			30 *				40 *	
ACG	GGC	GTC	CTG	GTG	ACT	CAT	GGG	TGG	GTG	CAG	AGG	AGT	GCG	GCA	GTG	AAG	AAG	ACC
				Met	Thr	His	Gly	Trp	Val	Gln	Arg	Ser	Ala	Ala	Val	Lys	Lys	Thr
				galT														
	50 *			60 *				70 *			80 *			90 *				100 *
TCG	ACC	CGG	CTG	GCC	GAC	GGC	CGT	GAG	CTG	GTC	TAC	TAC	GAC	CTG	CGC	GAC	GAC	ACC
Ser	Thr	Arg	Leu	Ala	Asp	Gly	Arg	Glu	Leu	Val	Tyr	Tyr	Asp	Leu	Arg	Asp	Asp	Thr
		110 *			120 *				130 *			140 *			150 *			
GTC	CGC	GAC	GCC	GTG	GAC	CGC	CGT	CCG	CTG	GAG	CGG	ACC	GTC	ACC	ACG	TCC	GAG	GTG
Val	Arg	Asp	Ala	Val	Asp	Arg	Arg	Pro	Leu	Glu	Arg	Thr	Val	Thr	Thr	Ser	Glu	Val
160 *			170 *			180 *				190 *			200 *			210 *		
CGA	CGC	GAC	CCG	CTG	CTC	GGC	GAC	TCC	GCG	CCG	TCG	CGC	CTC	GCA	CCG	GCA	GGG	GCG
Arg	Arg	Asp	Pro	Leu	Leu	Gly	Asp	Ser	Ala	Pro	Ser	Arg	Leu	Ala	Pro	Ala	Gly	Ala
	220 *			230 *			240 *				250 *			260 *			270 *	
CAC	CTA	CCA	TCC	GCC	GGC	CGA	CCA	GTG	CCC	GCT	GTG	CCc	GTC	GGA	CGG	GGA	ACG	GCT
His	Leu	Pro	Ser	Ala	Gly	Arg	Pro	Val	Pro	Ala	Val	Pro	Val	Gly	Arg	Gly	Thr	Ala
		280 *			290 *			300 *				310 *			320 *			330 *
GAG	CGA	GAT	CCG	GCC	TAT	GAC	GTG	GTG	GTC	TTC	GAG	AAT	CGC	TTT	CCC	TCG	CTG	GCC
Glu	Arg	Asp	Pro	Ala	Tyr	Asp	Val	Val	Val	Phe	Glu	Asn	Arg	Phe	Pro	Ser	Leu	Ala

TABLE 1-continued

TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS GALACTOSE OPERON

```
            340         350         360         370         380
GGT GAC TCC GGG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe

 390         400         410         420         430         440
GCC GAC CTG AGC GAG GAC CAG GCC CGG CTG GTC GTC GAC GCC TGG ACG GAC CGC ACC
Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr

     450         460         470         480         490         500
TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTG TTC TGC TTC GAG AAC CGG GGC
Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly

         510         520         530         540         550
GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe

560         570         580         590         600         610
ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACG
Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr

    620         630         640         650         660         670
GGC GGG GGG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC
Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Gly Arg Val

        680         690         700         710         720
GTC CTG GAG GGT GAG CAC TGG GCG GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
Val Leu Glu Gly Gly His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr

730         740         750         760         770         780
 GAG GTG CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG
 Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala

   790         800         810         820         830         840
GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile

       850         860         870         880         890         900
TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe

           910         920         930         940         950
GGG CAG CTG GAG TTC GAG GGT GTG ACG CGC GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu

 960         970         980         990         1000        1010
TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CGG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser ---             galP2

    1020        1030        1040        1050        1060        1070
TGAACG TGTTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTAGCGAG

 1080        1090        1100        1110        1120        1130
 TTC ATG AGC GGT AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
     Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
     galE

    1140        1150        1160        1170        1180        1190
GCC CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr
```

TABLE 1-continued

TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS GALACTOSE OPERON

```
        1200            1210            1220            1230            1240
GGC TTG CGT GAG GTG TGC CGG CGG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CGC
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg

1250            1260            1270            1280            1290            1300
CGC CAA GTG CGT GGA CGG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser

    1310            1320            1330            1340            1350            1360
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TGG GAC AAC AAC GTC GGT GGC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly

        1370            1380            1390            1400            1410            1420
ACC ATG GCG CTG CTG GAG GCC ATG CGG GGC GCG GGT GTG CGG CGG CTC GTC TTC TCC
Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

            1430            1440            1450            1460            1470
TCC ACG GCC GCC ACG TAC GGC GAG CCC GAG CAG GTT CCC ATG GTC GAG TCC GCG CCG
Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

1480            1490            1500            1510            1520            1530
ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

    1540            1550            1560            1570            1580            1590
GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

        1600            1610            1620            1630            1640
GGC GCG TAC GGG GAG TAC GGC GAG CGC CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

1650            1660            1670            1680            1690            1700
GTC CTT CAA GTG GCG CAG GGC AGG CGG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

    1710            1720            1730            1740            1750            1760
CCG ACG CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCC AGG
Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

        1770            1780            1790            1800            1810
CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GGC
Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

1820            1830            1840            1850            1860            1870
AAC GGC AAC GGG TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CGG CGG GTG ACC GGC
Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

    1880            1890            1900            1910            1920            1930
CAT CCG ATC CCC GAG ATC ATG GCC CCG CGC CGC GGG CGC GAC CCG GCG GTC CTG GTC
His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val

        1940            1950            1960            1970            1980            1990
GCG TCG GCC GGC ACC GCC CGC GAG AAG CTG GGC TGG AAC CCG TCC CGC GCG GAC CTC
Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu

            2000            2010            2020            2030            2040
GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CGG CGC GCG GGC CAG TAG TA
Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---

        2050            2060            2070            2080            2090            2100
ACC GCA GTT ACC GGA AAG GCG AGG GGT CAG GGC ATG GGC GAG GCT GTC GGG GAA CCG
                                            Met Gly Glu Ala Val Gly Glu Pro
                                            galK
```

TABLE 1-continued

TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS GALACTOSE OPERON

2110 2120 2130 2140 2150

TCG GCG AGC GGT TCC GGG AGC TGT ACG GGG CGG AGC CGG AGG GGG TGT GGG CGC CGA

Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg

2160 2170 2180 2190 2200 2210

GCG GGC CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG

Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met

2220 2230 2240 2250 2260 2270

CCT TCG CCC TGC CGC ACC AGG TCG CGG CCG TCT CCC GGC GCG AAC GAC GGC ATC CTG

Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu

2280 2290 2300 2310 2320

CGC CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTG GAG CTG CGC GTC GCC GAC CTG

Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu

2330 2340 2350 2360 2370 2380

GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GGC GTC CTG TGG GCG CGG

Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu

2390 2400 2410 2420 2430 2440

CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCG ACC GTC CCG

Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

2450 2460 2470 2480 2490

TCC GGG GCG GGG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC

Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

2500 2510 2520 2530 2540 2550

GAC CTG TAC GCC CTC GCG CTG CGC GGC TGG CAG CTG GCC CGG CTG TGC CAG CGC GCG

Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

2560 2570 2580 2590 2600 2610

GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC

Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

2620 2630 2640 2650 2660 2670

TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC AGC GGC AGA TCC

Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

2680 2690 2700 2710 2720

CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA AGC

Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

2730 2740 2750 2760 2770 2780

ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CGG GCT GCG AGA AGG GCG CCG

Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

2790 2800 2810 2820 2830 2840

CGC TGC TGG GCG TCG ACG CGC TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG

Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

2850 2860 2870 2880 2890

GAG CGG CTG GGC GAC GAG GAG GAG GTG CGC CGC CTG GTC CGG CAC GTG GTG ACC GAG

Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

2900 2910 2920 2930 2940 2950

GAC GAG CGC GTC GAA CGG GTG GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG

Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser

TABLE 1-continued

TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS GALACTOSE OPERON

	2960			2970				2980			2990			3000				3010
GCG	CCG	TCC	TGG	TCG	AGG	GCC	ACG	CCT	GCT	GCG	CGA	CGA	CTT	CCG	CAT	CTC	CTG	CCC
Ala	Pro	Ser	Trp	Ser	Arg	Ala	Thr	Pro	Ala	Ala	Arg	Arg	Leu	Pro	His	Leu	Leu	Pro
		3020			3030				3040			3050			3060			
CGA	GCT	GGA	CCT	GGT	CGT	CGA	CAC	GGC	CCT	GGC	CTC	CGC	GGC	CCT	CGG	CGC	CGG	ATG
Arg	Ala	Gly	Pro	Gly	Arg	Arg	His	Gly	Pro	Gly	Leu	Arg	Gly	Pro	Arg	Arg	Arg	Met
3070			3080			3090				3100			3110			3120		
ACC	GGC	GGC	GGC	TTC	GGC	GGC	TCG	GCG	ATC	GTC	CTG	GTG	GAG	GCC	GCC	GCG	GTG	GAC
Thr	Gly	Gly	Gly	Phe	Gly	Gly	Ser	Ala	Ile	Val	Leu	Val	Glu	Ala	Ala	Ala	Val	Asp
	3130			3140			3150				3160			3170			3180	
GCC	GTC	ACC	AAG	GCG	GTC	GAG	GAC	GCC	TTC	GCC	GCG	GCG	GGC	CTC	AAG	CGT	CCG	CGG
Ala	Val	Thr	Lys	Ala	Val	Glu	Asp	Ala	Phe	Ala	Ala	Ala	Gly	Leu	Lys	Arg	Pro	Arg
		3190			3200			3210				3220			3230			3240
GTG	TTC	GAG	GCG	GTG	CCT	CGG	CGG	GGC	GCG	GCG	CCT	GGT	CTG	ACG	GTC	AGC	CGA	GCC
Val	Phe	Glu	Ala	Val	Pro	Arg	Arg	Gly	Ala	Ala	Pro	Gly	Leu	Thr	Val	Ser	Arg	Ala
			3250			3260			3270				3280			3290		
GCT	TCA	CCA	GCG	TGT	ACT	CCG	TGA	TCC	CCG	GCG	GGT	AGT	CGG	GGA	TCA	CGC	ACA	TGA
Ala	Ser	Pro	Ala	Cys	Thr	Pro	---											
3300																		
GCT	GCT	AGC	CGC															

EXAMPLE 2

PROMOTERS OF THE *S. LIVIDANS* GAL OPERON

a) P1 promoter

(i) Summary

This promoter is galactose inducible, glucose repressible and is the regulatable promoter for the entire Streptomyces gal operon. S1 data indicates that the *Streptomyces lividans* gal operon encodes a polycistronic transcript of approximately 3.4 kilobases (Kb). The transcript consists of approximately 1 Kb for galT, followed by approximately 1 Kb each for galE and galK. (See, FIG. 1).

Galactose induction of P1 is mediated, at least in part, by an operator sequence whose 5' end is located 31 bp upstream of the transcription start site and a repressor protein which recognizes the operator.

(ii) Experimental: Isolation, Localization, and Characterization of the P1 promoter.

The sequences upstream of the *Streptomyces lividans* galK ATG were screened for promoters using the *E. coli* galK promoter probe system of Brawner, et at., *Gene,* 40, 191, (1985), in press. The HindlII-MluI fragment (See, Table A, map positions 1–5) was restricted with Sau3AI, ligated into the unique BamHI site of pK21 (FIG. 2), and transformed into *E. coli* K21 (galK) according to the method of Example 1. pK21 is a derivative of pSKO3 and is an *E. coli* Streptomyces shuttle vector containing the *E. coli* galK gene (See, FIG. 2). The construction of pSKO3 is described in Rosenberg et at., *Genetic Engineering,* 8, (1986), in press. The clones which expressed galK, i.e., those which had promoter activity, were identified on MacConkey—galaclose plates. Two galK+ clones (designated as pK21 MH1 and 2) were transformed into Streptomyces 1326-12K (galK).

Extracts from transformants were cultured in Ymglu and Ymgal, and were analyzed by western blot analysis using anti-*E. coli* galactokinase antiserum. The blots showed significantly higher levels of galactokinase in the extracts from the galactose induced, cultures.

pK21. MH1 and 2 were shown by restriction analysis to contain a 410 bp Sau3AI insert which is contained within the HindIII and BglII sites (see Table A, map positions 1–2) by Southern blot analysis according to the method of Southern, *J. Mol. Biol.,* 98, 503 (1975). The cloned fragment was analyzed by S1 analysis using RNA isolated from *Streptomyces lividans* 1326-12K and *E. coli* K21 cultures. The fragment yielded a 290 nucleotide protected fragment after S1 digestion (indicating the 5' end of an mRNA 290 bp upstream of the Sau3AI site). Hybridization experiments (using single. stranded M13 clones of this region) have identified the direction of transcription as left to fight as shown in FIG. 2 (i.e., transcription is going toward galK).

Conventional DNA sequence analysis and additional S1 mapping analysis were used to define the 5' end of the mRNA.

The sequences responsible for regulating galactose induction of P1 were localized by removing sequences upstream of the transcription start site by nuclease Bal31. Any change in promoter function or galactose induction by removal of these sequences was assessed using the *E. coli* galK promoter probe plasmid used to identify P1.

(iii) Construction of Gal Promoter Deletions.

Plasmid pHL5 was constructed by cloning a DNA fragment containing 100 bp of sequences downstream from the start of P1 transcription and 216 bp upstream from the start of P1 transcription into plasmid pUC19TT1. Plasmid pUC19TT1 is described in Norrander et at., *Gene*, 26, 101–106 (1983) and has the Unker as pUC18-TT6. See, Example IB. Deletions extending into the upstream sequence preceeding P1 were generated by linearizing pHL5 with HindIII and treating the ends with nuclease Bal31. The uneven ends were subsequently repaired with the Klenow fragment of DNA polymerase I. Bal31-treated pHL5 was then digested with BamHI and run on a 5% acrylamide gel. DNA fragments in the molecular weight range of 100–300 bp were eluted from the gel and subcloned into M13 mp 10 that had been digested with HindII and BamHI. [See, Messing, *Methods in Enzymology*, 101, 20 (1983)]. Individual deletions were then sequenced from the single stranded phage DNA the dideoxy chain termination method of Sanger, et al., cited above.

(iv) Linking the P1 Promoter Deletions to the *E. coli* galK Gene.

The various mp 10 clones were digested with BamHI and HindIII. DNA fragments containing individual deletions were isolated from low-melting point agarose gels and then ligated to pK21 (see, FIG. 2) that had been digested with BamHI and HindIII. After transformation into *E. coli* MM294, plasmid DNA was isolated for each of the deletion derivatives and transformed into Streptornyces Lividans 12K.

(v) Functional Assessment of Bal 31-Generated Deletions in *S. lividans.*

For each individual promoter deletion, a single thiostrepton resistant transformant was; grown to late log in YM base (YEME)+10 ug/ml thiostrepton. Cells were then pelleted, washed once in M56 media and resuspended in M56 media (see Miller, et al., cited above). The washed cells were then used to inoculate YM+0 1M MOPS (pH 7.2)+10 ug/ml thiostrepton supplemented with 1% galactose or 1% glucose. The cells were grown for 16 hours then assayed for galactokinase activity.

Ten individual pK21 derivatives containing either 120, 67, 55, 34, 31, 24, 20, 18, 10 or 8 bp of sequence upstream of the P1 transcription start site were analyzed for galactokinase expression. These results showed that substantially all the information necessary for galactose induction of P1, (i.e., 10–20 fold greater levels of galactolcinase produced in galactose grown cells versus glucose grown cells) is included in the 31 bp of sequence upstream of P1, and that all such information is located in the 67 bp of sequence upstream of P1.

A deletion which leaves 34 bp of sequence upstream of P1 is partially inducible by galactose since galactose induced 6-fold greater amounts of galactokinase. Thus, one end of the operator must be situated within the sequences between the −24 and −31 position. The remaining deletions which leave either 20, 18, 10 or 8 bp of upstream sequence result in a constitutive P1 promoter, that is the levels of galactokinase produced were equivalent when cells were grown in the presence of galactose or glucose. Although the promoter deletions which retained 8 and 10 bp of P1 were constitutive, the amount of galactokinase produced was reduced 10 fold in comparison to the promoter deletions which retained 18 to 120 bp of upstream sequence. This result indicates that sequences between the −10 and −18 positions of −1 are essential for promoter function.

This data supports a model in which galaclose induction of P1 is mediated, at least in part, by an operator sequence. One end of this sequence is within the region 24 to 31 bp upstream of the P1 transcription start site. Removing part or all of the operator results in a promoter which is partially or totally derepressed. The other end of this sequence has now been defined to be contained within the 16 to 21 bp of sequence upstream of the P 1 transcription start site. In addition, we cannot eliminate the possibility that the 3′ end of the operator is also within the 100 bp downstream of the transcription start site since these sequences were contained within the smallest region needed to achieve galactose induction. These data also suggest that the factor which interacts with the operator sequence is a repressor protein. Finally, we do not have any evidence which eliminates the possibility that P1 may be controlled by factors other than a repressor (i.e., positive activator such as lambda phage cII protein) to modulate galactose induction promoter transcript.

(vi) Construction of additional P1 promoter mutations.

Oligonucleotide directed mutagenesis was performed as originally described by Kunkel, et al. 1987 Methods Enzymol. 154:367 using the Mutagene Kit from Biorad (Cat. No. 170–3571) according to the manufacturer's instructions. M13mp18 containing a 196 base pair HindIII-BamH1 fragment that includes the galP1 promoter from −69 to +103 with respect to the apparent transcription start site was used as template. Fragments containing mutations in a single hexamer were constructed by annealing an oligonucleotide (Sanger et al. 1977 Proc. Natl. Acad. Sci USA 7.4:5463–5467) containing the desired base changes to wild type galPl-containing template DNA. Fragments containing mutations in more than one hexamer were constructed by annealing an oligonucleotide (Sanger et al. 1977 Proc. Natl. Acad. Sci USA 74:5463–5467) containing base changes in one hexamer to a template DNA that contained base changes in the other hexamer. The DNA sequence for each construction was confirmed by subjecting the various promoter-containing fragments to the dideoxy sequencing reactions of Sanger et al. 1977 Proc. Natl. Acad. Sci USA 74:5463–5467 using the Sequenase Kit (United States Biochemical Corp., Ohio) and the forward (−40) sequencing primer (P.No. 70736).

(vii)Assay of promoter function using xylE fusions. To assay the effect of various base changes within the galP1 promoter, the 196 bp BamHI-HindlII fragments containing the promoter mutations (prepared as described in Example 2avi, above) were ligated to the larger BamHI-HindlII fragment of pXe4 (Ingram, et. al. 1989, J. Bacteriol. 171:6617–6624) thereby generating transcriptional fusions between galP1 and a promoterless copy of the xylE gene contained in pXE4. In all cases, plasmid DNA was isolated after transformation to verify the presence of the insert. Plasmid DNA was transformed into *S. lividans* 1326 using standard procedures (see Hopwood, et al., Genetic Manipulation of Streptomyces-A Laboratory Manual, F. Crowe & Sons, Ltd., Norwich, England (1985)). Transformants were selected by overlaying the transformation plates with agar (0. 4%) containing 100 mg/ml thiostrepton. Catechol dioxygenase activity was detected on plates and assayed as described by Ingram, 1989, J. Bacteriol., supra, except that the assays were performed at 30 degrees Centigrade. The results are indicated in the following Tables X, Y and Z.

TABLE X

Catechol dioxygenase activity (% fully induced wild type promoter)		
Position/Change	Glucose	Galactose
wild type	7	100
−35, G to C	5	206
−34, G to C	1	12
−33, G to C	7	333
−32, G to C	8	9

TABLE Y

Catechol dioxygenase activity (% fully induced wild type promoter)		
Position/Change	Glucose	Galactose
wild type	9	100
−34, G to A	34	306
−34, G to T	17	850
−32, G to A	65	650
−32, G to T	8	280

TABLE Z

Catechol dioxygenase activity (% fully induced wild type promoter)			
Hexamer/Change	Glucose	Galactose	Glycerol
wild type	10	100	10
(−21 to −16) IV, TCTCAA	76	474	75
(−47 to −42) II, TCTCAA	66	1996	ND
(−53 to −48) I, TATCAA	60	75	55
(−7 to −2) VI, TATCAA	37	91	21

ND = not determined

b) The P2 promoter

(i) Summary

The P2 promoter of the Streptomyces gal operon is upstream of the galE gene and transcribes both galE and galK genes.

P2 promoter expression is constitutive (i.e. not glucose repressed/galactose induced) as shown by S1 analysis

(ii) Experimental: Isolation, Localization and Characterization of the P2 promoter.

The existence of the Streptomyces gal operon P2 promoter became apparent when the BglIII-MluI fragment (see, Table A, map positions 2–5) of *S. lividans* 1326 DNA was inserted into plasmid pK21 (see, FIG. 2) and galactokinase expression was observed in *Streptomyces lividans* 1326-12K transformed therewith.

DNA sequence analysis and S1 analysis were used to identify the 5′ end of the *S. lividans* gal operon P2. The 5′ end of the P2 promoter transcript is within the 100 bp upstream of the predicted galE ATG.

EXAMPLE 3

EVIDENCE OF A POLYCISTRONIC MESSAGE IN THE STREPTOMYCES GAL OPERON

S1 analysis was used to map the transcripts upstream and downstream of the *Streptomyces lividans* gal operon galK gene. In general, overlapping DNA fragments of 1–2 Kb were isolated from subclones, further restricted, and end labelled. The message was followed from the 3′ end of galK to the upstream end at P1.

The 3′ end of the *Streptomyces lividans* gal operon transcript probably occurs within the first hundred bases downstream of galK. Fragments 3′ labelled at sites within the galK sequence were not protected to their full length (S1 analysis) if they extend into this downstream region. One experiment showed a possible protected region that terminated 50–100 bp downstream of the galK translation stop. The existence of a transcription terminator can be confirmed by conventional techniques by using a terminator probe system. The gal operon transcript clearly does not extend to the PvuII site (see, Table A, map position 8) because no full length protection of 5′ labelled PvuaII fragments occurs from that site.

5′ end labelled fragments from two PvuII fragments, fragment I, (map positions 4–6, See, Table A), and fragment II, (map positions 6–8, See Table A), and the insert of pSau10 were used as sources of probes for S1 walking from the 3′ to 5′ end of the message. All fragments through this region are protected, except the fragment containing the P2 promoter which shows partial and full protection. The complete protection from S1 digest indicates a polycistronic message which initiates upstream at P1 and continues to approximately 100 bp downstream of galK.

The above data is indirect evidence of a polycistronic mRNA of the Streptomyces gal operon. S1 analysis using a long contiguous DNA fragment (e.g., the 4.5 kb] HindIII-SacI fragment, see map position 7 of Table A) has been used to confirm the transcript size.

EXAMPLE 4

LOCALIZATION OF *S. LIVIDANS* GAL OPERON GALE AND GALT GENES

(i) Summary

The *S. lividans* gal operon galE gene was localized to 1.5 Kb PvuII fragment (map position, 4–6 of Table A) of pLIVGAL1 (FIG. 1).

The *S. lividans* gal operon galE coding sequences extend through the MluI site (map position 5 of Table A).

The *S. lividans* gal operon galT gene was localized within the 1.15 Kb Nru-PvuII region (see, Table A, map positions 1a-4) of pSLIVGAL1.

The direction of *S. lividans* gal operon galE and galT transcription is the same as galK gene.

(ii) Experimental

It was necessary to identify the other functions contained on pLIVGAL1; specifically, does this plasmid encode for the enzyme galactose epimerase (galE) or the enzyme galactose transferase (galT). The Streptomyces gal operon galK gene was identified by its ability to complement an *E. coli* galK host. Thus, identification of the Streptomyces galT and gale genes was tested for by complementation of *E. coli* galE or galT hosts, respectively. An *E. coli* galT− strain (CGSC strain #4467, W3101) and two galE− strains (CGSC strain #4473; W3109 and CGSC strain #4498; PL-2) were obtained to test for complementation by the pSLIVGAL1 clone.

The ca. 9 Kb HindIII-SphI fragment (see, Table A, map positions 1–16) containing the *Streptornyces lividans* gal operon galK gene was inserted into pUC19. This fragment was situated within pUC19 such that transcription from the Plac promoter of pUC19 is in the same direction as the Streptomyces galK gene. pUC19 is described in Yanisch-Perrou, et al., Gene, 33, 103 (1983). Complementation was assayed by growth on MacConkey-galactose plates. Cells which can utilize galactose [gale+, galT+, galK+] will be red to pink on this medium. *E. coli* strain PL-2 (see, Example 2) containing pUC19 with the HindIII-SphI insert were pink on the indicator plate indicating that the HindIII-SphI fragment contains the *Streptornyces lividans* gale gene.

The gale gene was later mapped to within the 4.5 Kb HindIII-SaCI (the SacI site is near the region around map position 7-8 of Table A) fragment. If the sequences from the MluI site (map position 5 of Table A) to the SacI site were removed galE complementation of *E. coli* PL-2 was not detected. The 5′ end of the galK gene is 70 base pairs (bp) from the MluI site. Therefore it seemed likely that the MluI site was contained within the 5′ or 3′ end of the gale gene. To determine the direction of galE transcription, the HindIII-SacI fragment was inserted into pUC18. In this configuration, the *Streptomyces lividans* galK gene is in the opposite orientation with respect to Plac. The pUC18 HindIII-SphI clone did not complement *E. coli* PL-2 indicating galE is transcribed in the same direction as galK. In addition it was concluded that the MluI site is contained within the 3′ end of the galE gene. DNA sequence analysis of the PvuII-MluI fragment (See, Table A, map position 4-5) has identified an open reading frame which encodes for a polypeptide of predicated molecular weight of 33,000 daltons. The 5′ end of this reading frame is located approximately 176 bp from the PvuII site (See, Table A, map position 4). Therefore, the sequencing results support the conclusion that the 3′ end of galE traverses the MluI site (See, Table A, map position 5).

Similar experiments to localize the galT gene on pSLIVGALI were attempted with the galT hosts.

The region between P1 and the 5′ end of gale was sequenced to identify the galT gone. Translation of the DNA sequence to the amino acid sequence identified a reading frame which encodes a protein showing a region of homology to the yeast transferase.

EXAMPLE 5

GALACTOSE INDUCTION OF *S. LIVIDANS GAL* OPERON (GALK GENE

(i) Summary

Galactokinase expression is induced within one hour after the addition of galactose to culture medium.

Galactokinase expression is 10 times higher in the presence of galactose versus glucose or no additional carbon source within 6 hours after addition of the sugar.

(ii) Experimental

Galactose induction of the *Streptomyces lividans* galK gene was examined by assaying for galactokinase activity at 1, 3, 6 and 24 hours after the addition of galactose. Two liters of YM+0.1M MOPS (pH 7.2) were inoculated with 2×10^7 spores of *Streptomyces lividans* 1326. After 21 hours growth, galactose or glucose were added to a final concentration of 1%. One, three, six and twenty four hours after the addition of sugar, cells were isolated and assayed for galactokinase activity. Total RNA was prepared by procedures described in Hopwood et al., cited above.

An increase in galactokinase synthesis was observed one hour after the addition of galactose. The increase continued over time (1 to 24 hours). S1 analysis or RNA isolated from the induced cultures confirmed that the increase in galK activity was due to increased levels of the P1 promoter transcript.

The S1 data and the induction studies suggest the following model for gene expression within the Streptomyces gal operon. The P1 promoter is the galactose inducible promoter. The P1 transcript includes galT, galE and galK. The P2 promoter is constitutive and its transcript includes galE and galK.

It is interesting to note that the *E. coli* gal operon also has two promoters, P1 anct P2. [See, Nusso et al., Cell, 12, 847 (1977)]. P1 is activated by cAMP-CRP binding whereas P2 is inhibited by cAMP-CRP. Translation of the *E. coli* gal operon galE coding sequence is more efficient when transcription initiates at P2 which serves to supply a constant source of epimerase even in the absence of galactose or the presence of glucose [See, Queen et al., *Cell,* 25, 241 (1981)]. The epimerase functions to convert galactose to glucose 1-phosphate during galactose utilization and convert UDP-glucose to UDP-galactose which is required for *E. coli* cell wall biosynthesis. It is possible that the P2 promoter of the Streptomyces galK operon also serves to supply epimerase and galactokinase in the absence of galactose or during secondary metabolism.

EXAMPLE 6 THE S. COELICOLOR GAL OPERON

(i) Summary

The restriction map of a fragment containing the *S. coelicolor* galK gene is identical to the restriction map of the *S. lividans* gal operon. (See, FIG. 3).

S. coelicolor can grow on minimal media containing galaclose as the sole carbon source.

Galactokinase expression in *S. coelicolor* is induced by the addition of galactose to the growth media.

A promoter analogous and most likely identical to P1 is responsible for galactose induction of the *S. coelicolor* gal operon.

(ii) Experimental

An approximately 14 kb partial Sau3A fragment containing the *S. coelicolor* galK gene was isolated by K. Kendall and J. Cullum at the University of Manchester Institute of Science and Technology, Manchester, UK (unpublished data; personal communication). They were able to localize the *S. coelicolor* galK gene within a 3 kb EcoRI fragment by complementation of a *S. coelicolor* galK mutant. The position of a number of restriction sites within the *S. lividans* gal operon are identical to those found within, upstream and downstream of the EcoRI fragment containing the *S. coelicolor* galK gene (FIG. 3). Thus, it seems likely that the gene organization of the *S. lividans* gal operon is identical to the *S. lividans* gal operon.

Galactose induction of the *S. coelicolor* galK gene was examined by immunoblotting. *S. coelicolor was grown in YM*+1% galactose or 1% glucose (Ymglu or Ymgal) for 20 hours at 28° C. Galactokinase expression was detected using rabbit antisera prepared against purified *E. coli* galactokinase. The protein detected was the approximate site of the *E. coli* and *S. lividans* galK gene product. Galactokinase expression is galactose induced since it was detected only when *S. coelicolor* was grown in Ym+galactose (Ymgal).

S1 nuclease protection studies were performed to determine if galactose induction of the *S. coelicolor* gal operon is directed by a promoter analogous to the *S. lividans* P1 promoter. RNA was isolated from *S. coelicolor* grown in Ym+1% galactose or 1% glucose (Ymgal or Ymglu). The hybridization probe used for S1 analysis of this RNA was 410 pb Sau3A fragment which contains the *S. lividans* P1 promoter. Its transcription start site and the 5′ end of the galT gene. The S1 protected fragment detected by this analysis co-migrated with the protected fragment detected when the probe was hybridized to RNA isolated from *S. lividans* grown in the presence of galactose. Thus, this result shows that galactose induction of the *S. coelicolor* gall operon is directed by a sequence indistinguishable from the *S. lividans* P1 promoter.

It should be noted that the following strains of Streptomyces have been observed to be able to grow on medium containing galactose as the only carbon source:

S. albus J1074 (obtained from Dr. Chater, John Innes Foundation, Norwich, England)
S. carzinostaticus—ATCC accession number 15944
S. carzinostaticus—ATCC accession number 15945
S. antifibrinolyticus—ATCC accession number 21869
S. antifibrinolyticus—ATCC accession number 21870
S. antifibrinolyticus—ATCC accession number 21871
S. longisporus—ATCC accession number 23931

The abbreviation "ATCC" stands for the American Type Culture Collection, Rockville, Md., U.S.A.

While the above descriptions and Examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments. Thus, the invention includes all embodiments coming within the scope of the following claims.

What is claimed is:

1. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon containing a wide-type gal operon P1 promoter or a regulatable and functional P1 promoter deletion derivative.

2. The molecule of claim **1** which further comprises a foreign functional DNA sequence operatively linked to such operon.

3. A transformed host microorganism comprising the molecule of claim **2**.

4. A recombinant DNA vector comprising the molecule of claim **2**, and, optionally, additionally comprising a replicon.

5. A transformed host microorganism or cell comprising the recombinant DNA vector of claim **4**.

6. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism which contains the recombinant DNA vector of claim **4** under appropriate conditions such that expression of the sequence is regulatable.

7. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon P2 promoter expression unit.

8. The molecule of claim **7** which further comprises a foreign functional DNA sequence operatively linked to such expression unit.

9. A transformed host microorganism or cell comprising a recombinant DNA molecule wherein such molecule comprises the molecule of claim **8**.

10. A recombinant DNA vector comprising the molecule of claim **8**, and, optionally, additionally comprising a replicon.

11. A transformed host microorganism comprising the recombinant DNA vector of claim **10**.

12. A recombinant DNA molecule comprising a *Streptotmyces coelicolor* gal operon P1 promoter regulated region or any regularable and functional deletion derivative thereof.

13. The molecule of claim **12** which further comprises a foreign functional DNA sequence operatively linked to such regulated region.

14. A transformed host microorganism comprising the molecule of claim **13**.

15. A recombinant DNA vector comprising the molecule claim **13**, and, optionally, additionally comprising a replicon.

16. A transformed host microorganism comprising a recombinant DNA vector of claim **15**.

17. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism which contains the recombinant DNA vector of claim **15** under appropriate conditions such that expression of the sequence is regulatable.

18. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon P2 promoter.

19. The molecule of claim **18** which further comprises a foreign functional DNA sequence operatively linked to the P2 promoter.

20. A transformed host microorganism comprising the molecule of claim **19**.

21. A recombinant DNA vector comprising the molecule of claim **19** and, optionally, additionally comprising a replicon.

22. A transformed host microorganism comprising the recombinant DNA vector of claim **21**.

23. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon P1 promoter or any regulatable and functional deletion derivative thereof.

24. The molecule of claim **23** which further comprises a foreign functional DNA sequence operatively linked to the P1 promoter.

25. A transformed host microorganism comprising the molecule of claim **24**.

26. A recombinant DNA vector comprising the molecule of claim **24**, and, optionally, additionally comprising a replicon.

27. A transformed host microorganism comprising the recombinant DNA vector of claim **26**.

28. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism which contains the recombinant DNA vector of claim **26** under appropriate conditions such that expression of the sequence is regulatable.

29. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon galE gene.

30. The molecule of claim **29** which further comprises a foreign functional DNA sequence operatively linked to the galE gene.

31. A transformed host microorganism comprising the molecule of claim **30**.

32. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon galT gene.

33. The molecule of claim **32** which further comprises a foreign functional DNA sequence operatively linked to the galT gene.

34. A transformed host microorganism comprising the molecule of claim **33**.

35. A recombinant DNA molecule comprising a *Streptomyces coelicolor* gal operon galK gene.

36. The molecule of claim **35** which further comprises a foreign functional DNA sequence operatively linked to the galK gene.

37. A transformed host microorganism comprising the molecule of claim **36**.

38. A method of enabling a nongalactose utilizing host bacteria cell to utilize galactose which comprises transforming such host with a recombinant DNA vector or molecule comprising a *Streptomyces coelicolor* gal operon, or any portion of the *Streptomyces coelicolor* gal operon which is adequate to enable such transformed host to utilize galactose.

39. A transformed host prepared by the method of claim 38.

40. A recombinant DNA molecule comprising a Streptomyces spp. gal operon P1 promoter or any regulatable and functional delection derivative thereof.

41. The recombinant DNA molecule of claim 40 wherein the Streptomyces spp. is azuraeus, albus, carzinostaticus, antifibrinolyticus, or longisporus.

42. A recombinant DNA molecule comprising a Streptomyces spp. gal operon P1 promoter regulated region or a regulatable and functional deletion derivative thereof.

43. The recombinant DNA molecule of claim 42 wherein the Streptomyces spp. is azuraeua, albus, carzinostaticus, antifibrinolyticus, or longisporus.

44. A recombinant DNA molecule comprising a Streptomyces spp. gal operon containing a wild-type gal operon P1 promoter or a regulatable and functional P1 promoter deletion derivative.

45. The recombinant DNA molecule of claim 44 wherein the Streptomyces spp. is azuraeus, albus, carzinostaticus, antifibrinolyticus, or longisporus.

* * * * *