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THE COMMISSIONER OF PATENTS & TRADEMARKS
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Sir:

Transmitted herewith for filing is the patent application of

Inventor: Herrman Bujard, Annie C.Y. Chang, Stanley N. Cohen

For: Cloned High Signal Strength Promoters

Enclosed are:

[X] 1 sheets of drawing.

[X] An assignment of the invention to The Board of Trustees of the Leland Stanford Junior University, a California corporation

[ ] A certified copy of a _____ application.

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CLONED HIGH SIGNAL STRENGTH PROMOTERS

BACKGROUND OF THE INVENTION

Field of the Invention

Having established the feasibility of producing a wide variety of naturally occurring and synthetic polypeptides by means of hybrid DNA technology, there are continuing and extensive efforts to provide for more efficient and economic methods for producing the polypeptides. In developing a process for the commercial production of polypeptides, many factors will be involved in optimizing the economic and efficient production of the polypeptides. Included among these factors are regulatory signals, which are DNA sequences involved with the regulation of replication, transcription and translation.

One area of interest is at the level of transcription. Transcription involves the enzyme RNA polymerase. The RNA polymerase binds to a site called a promoter. It has been observed that promoters vary in their activity, as evidenced by the number of initiations of RNA per unit time or the strength of binding of the enzyme to the promoter site. The promoter may have one or more sequences that bind, which may or may not be contiguous. The more active promoters are referred to as strong promoters.

It was found that when introducing a strong promoter into a vector and employing the resulting plasmid for transformation, one could not select transformants based on expression of markers which allowed for selection. Therefore, cloning of the strong promoters was not feasible. It

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is therefore desirable that methods be provided which would allow for the screening of strong promoters and terminators and their subsequent cloning to be used in conjunction with the replication, transcription and translation of the genes for production of DNA, RNA, and polypeptides.

Description of the Prior Art

Promoters from bacterial and viral sources have been cloned in E. coli, and their signal strength in vitro has been studied using expression from distal promoterless sequences encoding βgalactosidase or other proteins. (Casadaban and Cohen (1980) J. Mol. Biol. 138, ~~179-207~~ 179-207; West and Rodriguez (1980) Gene 9, ~~175-193~~ 175-193). Attempts to clone small DNA fragments carrying the strong promoters of bacteriophage T5 have been unsuccessful. (v. Gabain and Bujard (1979) PNAS USA 76, ~~189-193~~ 189,193), Fragments of T5 DNA having both a strong promoter and a strong termination signal have been cloned. (Breunig (1979) Dissertation (Universitat Heidelberg, Heidelberg, Germany)) Analysis has shown that transcriptional regions of several E. coli plasmids are organized in units where initiation and termination signals are balanced. (Stuber and Bujard (1981) PNAS USA 78: 167-171) P_{25} and P_{26} promoters of the T5 bacteriophage are reported as among the most efficient RNA polymerase binding sequences. (Stuber et al (1978) Mol. gen. Genet. 166 141-149; Niemann (1981) Diplomarbeit (Universitat Heidelberg, Heidelberg, Germany)).

SUMMARY OF THE INVENTION

Methods for cloning, sequencing and using strong promoters and terminators are provided, as well as compositions resulting from the methods. By cleaving T5 phage and selecting fragments specifically binding to RNA polymerase, fragments containing promoters are isolated. A vector is constructed having a strong promoter, followed by a DNA sequence of interest, optionally followed by one or more translational stop codons in one or more reading frames, followed by a balanced terminator, followed by a marker allowing for selection of transformants. Upon introducing the resulting plasmid into a microorganism host, efficient

transcription of the gene is obtained with substantially lesser expression of the marker as compared to the gene. The level of expression of the marker permits selection of transformants having the above described construct. The construct or regulatory portions thereof are used for efficient transcription of RNA or gene expression.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 is a flow chart of the preparation of a plasmid for cloning strong promoters and terminators.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for preparing and cloning strong promoter and terminator regulatory signals and utilization of the strong regulatory sequences in the transcription and expression of genes of interest.

Particularly, T5 phage promoters are isolated, cloned in conjunction with a strong terminator, and appropriate vectors developed for insertion of DNA sequences of interest, usually structual genes, to provide for high and efficient transcription and/or expression of the sequence.

The compositions of this invention are characterized as having in the downstream direction of transcription the following units: a strong T5 phage promoter; optionally a structural gene which may be a marker; a balancing terminator; and optionally a marker allowing for selection of transformants containing the construct, which marker has a relatively low level of expression in comparison with the amount of RNA polymerase initiation at the promoter. In the absence of a promoter in the construct, the construct can be used for the cloning and characterization of promoters of different strengths.

In referring to strong promoters, it is intended that the binding affinity of RNA polymerase is stronger than the commonly employed promoters such as lac and trp and at least comparable to and normally greater than the combination of lac and trp promoters. For the most part, the strongest promoters among prokaryotes are the T5 phage promoters and these will be employed as exemplary of naturally occurring or synthetic strong promoters. It is to be understood, that

other prokaryotic and eukaryotic promoters, either naturally occurring or synthetic, could find application in the subject invention.

The compositions of this invention will include linear segments for insertion of DNA having the strong regulatory signal sequences (i.e., the promoter and terminator) adjacent opposite ends of the linear segment and plasmids formed by introducing a DNA sequence from a source other than the source of the promoter as a bridge between the strong regulatory signal sequences. The termini may be blunt or staggered ended, having the same or different termini to allow for directed positioning of inserted sequences.

The compositions which are employed as already indicated have a promoter, a balanced terminator, and desirably a marker which are in the direction of transcription when the signal sequences are joined. The terminator is balanced, so that a small but useful amount of expression of the marker can occur. Normally, in most situations, a gene will bridge the promoter and terminator to provide circular DNA. The gene may be a non-structural gene or a structural gene providing RNA e.g. ribosomal or messenger, or providing a poly(amino acid).

Desirably, the gene is followed by one or a plurality of translational stop codons e.g. oop or nonsense codons, or preferably a plurality, usually up to about six, more usually from about two to five, where there is at least one stop codon in each reading frame. The stop codons aid in the efficiency of termination, both at the level of transcription and expression.

Next in the direction of transcription is the terminator sequence. The terminator sequence is balanced with the promoter in the sense that the marker is transcribed not more than about ~~onefourth~~ one-fourth of the times that a gene upstream from the terminator is transcribed, where both genes are under the control of the same promoter. Usually, it will be desirable that a sufficient amount of the marker is transcribed to allow for selection of transformants. In view of the fact that the marker is transcribed from a strong pro-

moter, it will usually be sufficient that the marker is transcribed in relation to the transcription of the gene intermediate the promoter and terminator in only about 1 to 20, usually only 5 to 15 number % of the gene. The strength of the promoter is reflected in the level of expression of the marker which is transcribed from the same promoter as the gene whose expression is desired.

In addition to the DNA sequences indicated above, there will normally be other regulatory signals necessary for expression involved with the DNA sequence, such as translational start and stop sites. In addition to the foregoing regulatory signals, other regulatory signals may be included, such as additional promoters, operators, initiators, catabolite activator protein binding sites, etc. Furthermore, the promoter and terminator may be separated by more than one gene, that is, a plurality of genes, including multimers and operons.

The above DNA sequence construct will have a replicating system or be cleaved and be inserted into a vector to provide a plasmid. The vector is distinguished by having one or more DNA sequences which serve to insure stable replication of the plasmid and may also provide opportunities for high copy numbers of the plasmid in the microoganism host. The vectors may be derived from chromosomal or extrachromosomal sources. The sources include plasmids, viruses (phage), chromosomes, or the like. In addition, the vector or the essential portions thereof may be prepared synthetically.

The plasmids may then be used for transformation of an appropriate microorganism host. Methods of introducing DNA into an appropriate host are well known. Illustrative of such methods, but not exhaustive of such methods, are transformation e.g. calcium shock, transfection, and conjugation. Descriptions of these methods may be found in Genetic Engineering, ed. Setlaw and Hollaender, Vol. 1, Plenum Press, New York and London, 1979; Molecular Cloning of Recombinant DNA, ed. Scott and Werner, Vol. 13, Academic Press, Inc. New York, 1973, and references cited therein.

In order to allow for flexibility in preparing the construct and self-replicating sequence or plasmid containing the construct restriction sites should be present to allow for unique insertions and isolation of the various elements. The restriction sites may be naturally present, introduced by linkers, result by partial sequential nucleotide removal from a chain using an exonuclease, or the like. Desirably, the restriction sites will provide for different ends to permit only the proper orientation of the inserted fragment.

A wide variety of structural genes are of interest for production of proteins, including but not limited to proteins of physiological interest, proteins as chemicals, and enzymes which may be of direct interest or of interest in transforming another product, which may be proteinaceous or non-proteinaceous. The proteins may be prepared as a single unit or as individual subunits and then joined together in appropriate ways. Furthermore, as appropriate, the protein products may be modified by glycosylation, acylation with aliphatic acids, e.g. lipid acids, phosphorolation, sulfonation or the like. The different classes of proteins which may be prepared include protamines, histones, albumins globulins, scleroproteins, phosphoproteins, mucoproteins, chromoproteins, lipoproteins, nucleoproteins, and the remaining proteins which are unclassified.

The following is a representative list of proteins of interest.

Insulin; growth hormone; interferon e.g. leukocyte, lumphoblastoid, T-immune and fibroblast; β-lipotropin; β-endorphin; dynorphin; histocompatability proteins; immunoglobulins e.g. IgA, IgD, IgE, IgG and IgM and fragments thereof; hemoglobin, somatomedins; lymphokines; growth factors e.g. epidermal, fibroblast, platelet-derived, multiplication stimulating and nerve; hematoporetic-stimulating factors e.g. erythropoietin, colony-stimulating, erythroid potentating activity or burst-promoting activity and lymphopoietins; albumin and prealbumin;

Prealbumin
Albumin
α_1-Lipoprotein
α_1-Acid glycoprotein
α_1-Antitrypsin
α_1-Glycoprotein
Transcortin
4.6S-Postalbumin
Tryptophan-poor
α_1-glycoprotein
α_1-χ-Glycoprotein
Thyroxin-binding globulin
Inter-α-trypsin-inhibitor
Gc-globulin:
(Gc 1-1),
(Gc 2-1),
(Gc 2-2),
Haptoglobin:
(Hp 1-1),
(Hp 2-1),
(Hp 2-2),
Ceruloplasmin
Cholinesterase
α_2-Lipoprotein(s)
α_2-Macroglobulin
α_2-HS-Glycoprotein
Zn-α_2-glycoprotein
α_2-Neuramino-glycoprotein
Erythropoietin
β-lipoprotein
Transferrin
Hemopexin
Fibrinogen
Plasminogen
β_2-glycoprotein I
β_2-glycoprotein II
Immunoglobulin G
(IgG) or γG-globulin

Mol. formula:

$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$

Immunoglobulin A (IgA)
or γA-globulin

Mol. formula:

$(\alpha_2\kappa_2)^n$ or $(\alpha_2\lambda_2)^n$

Immunoglobulin M
(IgM) or γM-globulin

Mol. formula:

$(\mu_2\kappa_2)^5$ or $(\mu_2\lambda_2)^5$

Immunoglobulin D (IgD)
or γD-Globulin (γD)

Mol. formula:

$(\delta_2\kappa_2)$ or $(\delta_2\lambda_2)$

Immunoglobulin E (IgE)
or γE-Globulin (γE)

Mol. formula:

$(\varepsilon_2\kappa_2)$ or $(\varepsilon_2\lambda_2)$

Free light chains

Complement factors:

C'1

C'1q

C'1r

C'1s

C'2

C'3

β_1A

α_2D

C'4

C'5

C'6

C'7

C'8

C'9.

Importain protein hormones include:

Peptide and Protein Hormones

Parathyroid hormone
(parathormone)

Thyrocalcitonin
Insulin
Glucagon
Relaxin
Erythropoietin
Melanotropin
(Melanocyte-stimulating hormone; intermedin)
Somatotropin
(growth hormone)
Corticotropin
(adrenocorticotropic hormone)
Thyrotropin
Follicle-stimulating hormone
Leuteinizing hormone
(interstitial cell-simulating hormone)
Luteomammotropic hormone
(Luteotropin, prolactin)
Gonadotropin
(chorionic gonadotropin).

Tissue Hormones

Secretin
Gastrin
Angiotensin I and II
Bradykinin
Human placental lactogen

Peptide Hormones from the Neurohypophysis

Oxytocin
Vasopressin
Releasing factors (RF)
CRF, LRF, TRF, Somatotropin-RF, GRF, FSH-RF, PIF, MIF.

In addition to various non-enzymatic proteins of physiological interest, enzymes can also be produced as an end product or for intracellular transformation of a substrate present in the host or substrate introduced extracellularly, or for enzymatic transformation in vitro.

In accordance with the I.U.B. classification, the enzymes fall into varying categories such as 1. oxidoreductases; 2. transferases; 3. hydrolases; 4. lyases; 5. isomerases; 6. ligases. Enzymes of particular interest will be hydrolases and oxidoreductases for use in commercial processing, for example, hydrolases for hydrolysing polysaccharides, lipids and polypeptides; oxidoreductases for oxidation of alcohols and aldehydes, epoxidation, and the like.

The microorganism host may be bacteria, such as Escherichia, Bacillus, Aerobacter, Klebsiella, Proteus, Pseudomonas, Streptococcus, Staphylococcus, Clostridium, Mycobacterium, Streptomyces and Actinomyces; Fungi e.g. Gymnomycota, Dimastygomycota, Eumycota, Zygomycetes, Ascomycetes and Basidomycetes, such as, Candida, Aspergillus, Rhizobus, Microsporum, and Fonsecaea; Protozoa e.g. Mastigophora, Sarcodina, Sporozoa and Celiophora, such as, Trypanosoma, Codosiga, Protospongra and Entameba, and Alga e.g. Dinoflagellates, Euglenoids, and Diatoms.

Higher cells, e.g., mammalian, may also be employed as hosts, where viral, e.g., bovine papilloma virus or other DNA sequence is available which has plasmid-like activity.

Depending upon the nature of the host, a wide variety of vectors may be employed. The vector will have an intact replicon and be capable of replication in the host. In addition, replicons can be developed which may have one or more other regulatory signals. Regulatory signals can include temperature sensitive replicons, runaway-replication sequences, temperature sensitive operators, and the like. Various additional DNA sequences may be present providing for restriction sites, markers, termination sequences, or the like. Desirably, the vector should be of a substantially different size from the construct to allow for excision of the construct and ease of separation of the construct from the vector by molecular weight separation techniques e.g. electrophoresis and density gradient centrifugation.

The subject invention also provides for a technique for screening the strength of promoters and terminators, thus

allowing for the determination of the use of a promoter or terminator in a particular application, where it may be desirable to have promoters or terminators or combinations thereof of varying strength. In screening promoters and/or terminators one employs a promoter or terminator of known activity. A DNA construct is made having the sequence described previously, where appropriate restriction sites are provided for introducing the various elements. The construct provides in the direction of transcription the promoter, a first gene marker, optionally termination codons, such as nonsense codons and oop terminator, the terminator, and a second gene marker. This DNA construct is inserted into an appropriate vector. Where the promoter and terminator are properly balanced, there will be a substantial differentiation between expression of the first gene marker and expression of the second gene marker. The ratio of expression between the first and second marker will provide for a comparative evaluation of the activity of the promoter or terminator, depending upon which is of known value. Thus, one can degrade a DNA sequence such as a chromosome, or an extrachromosomal element, such as a plasmid or double minute, isolate the promoters by selective binding with RNA polymerase and insert the DNA fragments which bind to the RNA polymerase into the previously described construction. By determining the relative proportion of expression of the first and second gene markers, one can determine the strength of promoters in relation to a fixed terminator. Similarly, one can isolate DNA sequences having terminator sequences, insert the sequences into the above described construct at the appropriate site and then measure the relative expression of the two gene markers.

Various markers can be chosen for evaluating the relative activities of promoters and terminators. Conveniently, markers which allow for selection such as resistance to antibiotics, toxins or heavy metals can be used. By varying the concentration of the selective agents in the nutrient medium, one can determine the relative proportions of the enzyme expressed by the genes in relation to the

growth of the host. Alternatively, one can use growth factors e.g. having a gene which complements a mutant gene in an auxotrophic host, where the gene expresses a product necessary for a biosynthetic pathway. A third marker provides virus incompatibility, preventing plaque formation. Other markers which allow for comparison will come readily to mind.

The terminators may be evaluated in the presence and absence of rho, so that one can determine the dependency of the terminator on rho, as well as the effectiveness of the terminator in relation to the concentration of rho.

The methods for preparing the subject compositions will be conventional. The various DNA fragments and sequences can be obtained from a variety of sources by restriction mapping and endonuclease cleavage to provide fragments having the desired intact sequence or gene. The fragments can be further processed employing endo- or exonucleases to remove nucleotides unrelated to desired regulatory sequences or structural genes. By appropriate choice of restriction enzymes, cohesive or blunt ended fragments can be generated. Furthermore, chains can be extended with single nucleotides or oligonucleotides, linkers can be added, or otherwise processing to provide for termini having desired properties.

Desirably, a vector is employed having appropriate restriction sites, a competent replication system for the intended host, and optionally one or more markers which allow for selection. For hybrid DNA technology it would be useful to have a plasmid having a unique restriction site between a T5 promoter and a terminator, desirably having at least one stop codon on the upstream side of the terminator. In this manner, one or more structural genes may be introduced between the promoter and terminator.

As appropriate, downstream from the promoter, but remaining proximal to the promoter, may be an operator, activator, ribosomal start signal sequence, or the like, to allow for controlled expression of the inserted gene(s).

The strategy described above provides a vehicle which can be used with one or more hosts for gene expression,

where the gene after processing in a predetermined way can be directly inserted into the vehicle to provide a competent plasmid for expression of the desired gene(s).

Alternatively, the gene(s) of interest may be ligated to the appropriate regulatory signal sequences before insertion into the vehicle. In this instance, only the promoter and terminator regulatory signals need be present.

To provide for enhanced flexibility, the region between the promoter and terminator may be designed so as to provide for a plurality of restriction cleavage sites, allowing for the introduction and removal of DNA fragments without interruption of the remainder of the vehicle. Thus, by having a plurality of unique restriction sites or restriction sites limited to the region between the promoter and terminator in the downstream direction of transcription, regulatory signals and genes may be readily inserted and removed.

Another strategy is to prepare a construct having all of the desired DNA sequences for transcription and expression in appropriate sequence, with the construct having predetermined termini and inserting the construct into an appropriate vector which has been linearized to provide complementary termini.

In developing the construct, a vector will normally be used in order to clone the various sequences. The construct will allow for the insertion of the different sequences in the correct direction and desirably only in the proper orientation. Therefore, it will usually be desirable to have the sequence and insertion site be asymmetric in having different termini with the termini of the sequence and insertion site being complementary.

The particular restriction enzymes will vary widely with the various sequences, there being a large number of restriction enzymes of known base or sequence specificities commercially available.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Materials and Methods

Restriction endonucleases HindIII, HincII, SalI, BamHI and T4 Ligase were from New England Biolabs (Beverly, Mass., USA), Sau3A was from BRL (Neu-Isenburg, Germany) and EcoRI and HpaII from (Boehringer Mannheim, Federal Republic of Germany). DNA polymerase I, phage fd DNA (replicative form), plasmid AD16/30 containing a 28 bp HindIII/BamHI adapter sequence and, lac represser were supplied by private sources. The isolation of bacteriophage T5 DNA, plasmid DNA E. coli RNA polymerase and E. coli termination factor rho have been described previously, (v. Gabian and Bujard, Mol. gen. Genet. (1977) 157, 301-311; Clewel and Helinski (1969) PNAS USA 62, 1157-1166; and Knopf and Bujard (1975) Eur. J. Biochem. 53, 371-385).

A 780bp DNA fragment carrying the E. coli lac regulatory region (promoter/operator: P/O), an N-terminal portion of the β-galactosidase structural gene sufficient for intracistronic complementation of the M15 deletion, as well as a portion of the i-gene(i) was isolated from a HincII digest of a pACYC 214 plasmid (a plasmid related to pACYC 184 by insertion at a BamHI site of a BamHI restriction cleavage fragment from F'-lac carrying the lac gene) by repressor binding and subsequent adsorption to nitrocellose. This fragment was then employed in the construction of an exemplary plasmid for analyzing strong promoters and strong terminators as depicted in the Figure.

Utilizing the HpaII cleavage site within the β-gal structural genes the fragment was reduced in size and provided with BamHI and SalI cleavage sites by various subcloning. The resulting fragment (left most part of Figure) contains the intact control region of the lac operon and an N-terminal portion of the β-gal structural gene coding for 66 amino acids (α). Introduction of this fragment by blunt end ligation into the HindIII site of pACYC184 (Chang and Cohen (1978) J. Bacteriol. 134, 1141-1156) yielded pBU10, a vector suitable for terminator cloning. The major terminator of the coliphage fd genome was isolated as a 338bp Sau3A fragment

(Beck et al. (1978) Nucl. Acids Res. 5, 4495-4503), ligated with a BamHI/HindIII adaptor sequence and integrated into pBU10 to yield pLBU1.

Cleavage of the lac sequence in pBU10 by HpaII destroys the lac promoter and liberates upon cleavage with HindIII a fragment containing the lac operator and a region coding for a functional α-fragment. Integrating this DNA sequence into pBR322 leads to pBU12a. Finally, replacement of the HindIII/SalI portion of pBU12a by a partial digest of the HindIII/SalI fragment of pLBU1 containing the fd terminator resulted in pLBU3, a vector suitable for integration of efficient promoters at the EcoRI site. The regions encoding chloramphenicol (Cm), ampicillin (Ap) and tetracycline (Tc) resistance are indicated as cat, bla and tet respectively.

The plasmid, pBU10, had the following properties: (i) it contains the α fragment of β-galactosidase (α-protein) and complemented the M15 deletion of the lac operon; (ii) the Tc resistance it specified was under the control of the lac promoter, as shown in M15 I^q strains; (iii) the HindIII site between the lac gene fragment and the tet gene was restored; the stop codon immediately following the HindIII site limited the length of the lacZ gene product to 68 amino acids.

Insertion of the fd terminator upstream to the tet gene resulted in a 90% reduction in the level of Tc resistance, but no detectable change in the levels of β-galactosidase activity in M15 deletion strains. The results obtained as to the properties imparted to various E. coli strains by various plasmids is set forth in the following table:

Seven independent plasmid isolates from clones contained the expected 352bp HindIII-generated fragment containing the fd terminator. Electrophoretic analysis of all seven BamHI-cleaved isolates and DNA sequence analysis of one of these showed that in all instances the fd terminator had been integrated in an orientation opposite the direction of transcription within the fd phage genome. Sequence analysis also revealed a translational stop codon in frame with the α-protein less than 10bp down stream from the

HindIII cleavage forming the conjunction between the lac-derived segment and the fd-derived DNA fragment; a translational stop signal on this position would be expected to result in an α-fragment containing 71 amino acids.

For the cloning of exogenous promoters, the lac promoter on the lac/tet construct had to be removed or destroyed in such a way that a site for the subsequent integration of promoters was retained. To do this, the HpaII cleavage site at position -17 of lac was employed, as described above. Colonies that showed both a reduced level of Tc resistance and the presence of a lac operator sequence, which could be detected on the multicopy plasmid by its ability to bind the lac represser and induce chromosomal β-gal synthesis, were identified. Endonuclease analysis (HindIII/EcoRI double digest) of plasmids recovered from several isolates yielded two types of vectors: one of these represented by pBUl2 harbored the expected 253bp lac fragment; the other represented by pBUl2a yielded a 420bp fragment. DNA sequence analysis showed that in pBUl2a, a 160bp fragment of unknown origin, containing two to three stop codons in each of the possible translational reading frames, had been integrated between the EcoRI site and position -15 of the lac promoter. The presence of the stop codons made the fragment an efficient terminator of any translation that occurred upstream of the translational initiation site for the α-fragment.

When the HindIII/SalI segment of pBUl2a was replaced with an identically generated fragment of pLBUl carrying the fd terminator, the plasmid pLBU3 was obtained, which conferred neither Tc resistance nor β-gal activity to E. coli Ml5 strain. Although this plasmid contained a tet region and a DNA sequence encoding the α-fragment of lac, it conferred neither resistance nor β-gal activity to the E. coli Ml5 strain. It was therefore chosen as the T5 promoter cloning vehicle.

A population of about 200 short fragments of T5 DNA was obtained by double digestion of the 120kb phage genome with HaeIII and AluI endonucleases. These fragments were

ligated with excess synthetic EcoRI links and the resulting molecules were cleaved with EcoRI endonuclease and ligated into the EcoRI cleavage site of pLBU3. Transformation of E. coli C600 and selection for β-gal activity plus high level Tc-resistance yielded 35 colonies resistant to Tc-concentrations between 8 and 70 μg/ml. Plasmids were isolated from 13 colonies resistant to 70 μg/ml, which earlier experiments using multicopy plasmids had suggested was the highest level detectable in E. coli K12 (Cabello et al. (1976) Nature 259, 285-290).

Digestion of the various isolates with EcoRI endonuclease liberated between one and ten fragments of various sizes from each constructed plasmid. Complexing of such fragment mixtures with RNA polymerase, followed by filter binding analysis, identified between one and three fragments of each plasmid that interacted very efficiently with the enzyme; these fragments were isolated from polyacrylamide gels and individually recloned in pLBU3. In each case, they gave rise to colonies resistant to 70 μg/ml Tc. Plasmids isolated from each of these clones carried the expected DNA fragments, as shown by EcoRI cleavage and gel electrophoresis. The promoter library which was obtained contained about 25 different strong promoters of coliphage T5 ~~(Gentz (1981), supra)~~.

A plasmid pGBU207 containing an EcoRI-generated fragment of 212bp was selected for further study. In vitro transcription on the plasmid was mapped by analysis of RNA transcripts made on fragments of the plasmid produced by cleavage with different restriction endonucleases. Cleavage of the plasmid with EcoRI endonuclease yielded principally a single RNA species about 130 nt in length. The size of the transcript increased to 550 nt when a HindIII digest of pGBU207 was used as a template. BamHI digested DNA yielded transcripts of about 740 and 900 nt in length. Correlation of transcript length with the distance of the DNA cleavage site from a fixed point insert indicated that in all of these instances in vitro transcription was initiated at the same promoter and that it progressed toward the tet region of the

plasmid. The experiments also showed the functioning of the termination signal introduced between the lac fragment and the tet gene. Under the high salt conditions used for this in vitro transcription experiment, termination of about 50% of transcription within the fd DNA fragment gave rise to the 740 nt transcript, while a read- through transcript extended to the BamHI cleavage site located 890bp from the promoter. The data strongly suggested that RNA termination occurring in the fd terminator placed in the reverse orientation is rho dependent, in contrast to the rho-independent termination that occurs when the terminator is in its normal orientation.

Since the rate of complex formation between RNA polymerase and promoter signals is a reflection of the strength of the promoter, the relative rate of complex formation of the 212bp fragment of pGBU207 was compared with complex formation involving the previously studied T5 promoters P_{25} and P_{26} (Stuber and Bujard (1981), supra). The results showed that the promoter used to express downstream genetic functions in pGBU207 has a signal strength similar to that of P_{25} and P_{26}, which are among the most efficient RNA polymerase binding sequences identified from any source. (Niemann (1981), supra).

The above results demonstrate that novel DNA sequences can be prepared from the strong T5 promoters, which can then be used for the expression of a wide variety of poly(amino acids). Furthermore, by employing a promoter, optionally a structural gene, a terminator, and a marker, test plasmid structures are provided which allow for screening of the effectiveness of a promoter and/or a terminator, particularly as they interrelate with each other. Therefore, combinations can be prepared which allow for highly efficient transcription of a wide variety of structural genes, with concommitant selection of the transformants by employing an appropriate marker downstream from the balanced terminator.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that

certain changes and modifications may be practiced within the scope of the appended claims.

20 cl. We claim: Claim 1>

IN THE CLAIMS

1. A linear DNA sequence having proximal to one end a strong T5 phage promoter, proximal to the other end a strong terminator balanced with said strong T5 promoter, and having at least one intermediate said promoter and terminator of a marker for selection adjacent to said terminator and distal from said other end, or a replication system foreign to T5.

2. A linear DNA sequence according to Claim 1, having intermediate said other end and said strong terminator at least one stop codon in at least one reading frame.

3. A linear DNA sequence according to Claim 2, having a plurality of stop codons with at least one in each reading frame.

4. A linear DNA sequence according to any of Claims 1, 2 or 3, wherein said marker is a gene imparting biocidal resistance.

5. A linear DNA sequence according to any of Claims 1, 2 or 3, wherein said marker is a DNA sequence having at least one gene in a metabolic synthetic pathway.

6. A linear DNA sequence according to any of Claims 1, 2 or 3, having a marker ~~replication system~~ intermediate said promoter and said terminator.

7. A linear DNA sequence according to Claim 6, wherein said marker provides biocidal resistance.

8. A linear DNA sequence according to Claim 6, wherein said marker has at least one gene for an enzyme in a metabolic synthetic pathway.

9. A linear DNA sequence according to any of Claims 1, 2 or 3, wherein said replication system is for a prokaryote.

10. A linear DNA sequence according to any of Claims 1, 2 or 3, wherein said replication system is for a eukaryote.

11. A circular DNA sequence having in downstream order of transcription a strong T5 phage promoter, a structural gene foreign to T5 phage, a balanced terminator, and a replication system.

12. A circular DNA sequence according to Claim 11, wherein said replication system is recognized by prokaryotes.

13. A circular DNA sequence according to Claim 11, wherein said replication system is recognized by eukaryotes.

14. A circular DNA sequence according to any of Claims 11, 12 or 13, wherein said structural gene expresses a mammalian protein.

15. A circular DNA sequence according to Claim 14, wherein said mammalian protein is an enzyme.

16. A circular DNA sequence according to any of Claims 11, 12 or 13, having intermediate said structural gene and said strong terminator, a plurality of stop codons, having at least one stop codon in each reading frame.

17. A circular DNA sequence according to any of Claims 11, 12 or 13, having a marker for selection downstream from said terminator.

18. A prokaryotic cell having a circular DNA sequence according to Claim 12.

19. A eukaryotic cell having a circular DNA sequence according to Claim 13.

20. A method for preparing a poly(amino acid) which comprises:

growing in a nutrient medium prokaryotic cells according to Claim 18, wherein said structural gene expresses said poly(amino acids).

21. A method for preparing a poly(amino acid) which comprises:

growing said eukaryotic cell according to Claim 19 in a nutrient medium, wherein said structural gene expresses said poly(amino acid).

22. A method according to Claim 21, wherein said eukaryotic cell is yeast.

23. A method for determining the strength of a promoter which comprises:

inserting said promoter into a linear DNA sequence having proximal to one end in the downstream direction for transcription a gene allowing for detection of expression; a terminator of known strength; a marker allowing for determination of expression; and a replication systems recognized by a predetermined host; whereby a circular DNA sequence is obtained;

transforming said host with said circular DNA sequence; and

growing said host in nutrient medium under conditions allowing for determination of the extent of expression of said gene and said marker.

12 11
24. A method according to Claim 23, wherein said host is auxotrophic and said gene provides prototrophy.

13 11 12
25. A method according to any of Claims 23 or 24, wherein said marker provides biocidal resistance.

14 12
26. A method according to Claim 25, wherein intermediate said gene and said terminator are a plurality of stop codons, with at least one stop codon in each reading frame.

Claim 15

Add Bac3

25

CLONED HIGH SIGNAL STRENGTH PROMOTERS

ABSTRACT OF THE DISCLOSURE

Method for preparing high signal strength promoters and terminators and DNA compositions employing such promoters and terminators. T5 phage is cleaved to provide for DNA sequences having intact promoters. These promoters are inserted into vectors separated from a balanced terminator by a gene of interest and the terminator is desirably followed by a marker allowing for selection of transformants. High efficiencies in transcription of DNA can be achieved with the highly active T5 promoters. The promoters and terminators are used in hybrid DNA for efficient expression of structural genes and transcription to provide RNA sequences.

GI P The government has rights in the invention pursuant to Grant Nos. AI 08619 and GM 27241 awarded by the National Institute of Health.

P The research was supported in part by a grant from the Deutsche Forschungsgemeinschaft.

DECLARATION AND POWER OF ATTORNEY

5490-35
TOWNSEND AND TOWNSEND FILE NO.

As a below named inventor, I declare that the information given herein is true, that I believe that I am the original, first and sole inventor if only one name is listed at 201 below, or a joint inventor if plural inventors are named below at 201-203, of the invention entitled: CLONED HIGH SIGNAL STRENGTH PROMOTERS

which is described and claimed in:

☐ the attached specification or ☐ the specification in application Serial No. _____ filed _____

that, as to common subject matter of this application and my earlier United States application(s), if any, described in item 301 below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to such earlier application(s), or in public use or on sale in the United States more than one year prior to such earlier application(s), that the said common subject matter has not been patented or made the subject of an inventor's certificate before the date of such earlier application(s) in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to such earlier application(s) and that no application for patent or inventor's certificate on said common subject matter has been filed by me or my legal representatives or assigns in any country foreign to the United States except those identified in item 101 below, if any; that, as to any subject matter of this application which is not common to any of said earlier application(s) (non-common subject matter), I do not know and do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to the date of this application, or in public use or on sale in the United States more than one year prior to the date of this application, and that said non-common subject matter has not been patented or made the subject of an inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to the date of this application; that no application for patent or inventor's certificate on said non-common subject matter has been filed by me or my legal representatives or assigns in any country foreign to the United States prior to this application except those identified in said item 101; and I acknowledge a duty to disclose information I am aware of which is material to the examination of this application.

	FOREIGN APPLICATION(S) AND INVENTOR'S CERTIFICATE(S), if any, filed within 12 mos. prior to the filing date of this application			
101	COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119
				YES___ NO X
102	ALL FOREIGN APPLICATIONS & INVENTOR'S CERTIFICATES, if any, filed more than 12 mos. prior to the filing date of this application			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Stephen S. Townsend, Reg. No. 15,387; Charles E. Townsend, Jr., Reg. No. 16,218; Anthony B. Diepenbrock, Reg. No. 20,131; Dirks B. Foster, Reg. No. 17,624; Albert J. Hillman, Reg. No. 20,134; Thomas F. Smegal, Jr., Reg. No. 20,732; John L. McGannon, Reg. No. 22,414; Paul W. Vapnek, Reg. No. 24,385; William M. Hynes, Reg. No. 24,168; J. Georg Seka, Reg. No. 24,491; Bertram I. Rowland, Reg. No. 20,015; Roger L. Cook, Reg. No. 24,648; Bruce W. Schwab, Reg. No. 22,321; Ronald S. Laurie, Reg. No. 25,431; Warren P. Kujawa, Reg. No. 25,142; George M. Schwab, Reg. No. 25,748; Robert J. Bennett, Reg. No. 27,533; Kenneth R. Allen, Reg. No. 27,301; Henry C. Bunsow, Reg. No. 27,743; David. N. Slone, Reg. No. 28,572; Sandra S. Schultz, Reg. No. 29,436; Lesley S. Witt, 26,578; James F. Hann, 29,719 and Robert C. Colwell, 27,431.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO: (name, registration number, and telephone number)
TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	Bertram I. Rowland, Reg 20,015 ☐ (415) 543-9600 or ☒ (415) 493-2590

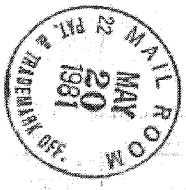
201	FULL NAME OF INVENTOR	Last Name: Bujard	First Name: ~~Hermann~~ HERMANN		Middle Name or Initial: G.
	RESIDENCE & CITIZENSHIP	City: HEIDELBERG	State or Foreign Country: GERMANY BRD		Country of Citizenship: GERMAN
	POST OFFICE ADDRESS	Post Office Address: H.BILLE - STR. 12	City: HEIDELBERG	State or Country: GERMANY	Zip Code: 69
202	FULL NAME OF INVENTOR	Last Name: Chang	First Name: Annie 40200		Middle Name or Initial: C.Y.
	RESIDENCE & CITIZENSHIP	City: Palo Alto	State or Foreign Country: California CA		Country of Citizenship: U.S.A.
	POST OFFICE ADDRESS	Post Office Address: 765 San Antonio Rd. #39	City: Palo Alto	State or Country: California	Zip Code: 94303
203	FULL NAME OF INVENTOR	Last Name: Cohen	First Name: Stanley 40300		Middle Name or Initial: N.
	RESIDENCE & CITIZENSHIP	City: Portola Valley	State or Foreign Country: California CA		Country of Citizenship: U.S.A.
	POST OFFICE ADDRESS	Post Office Address: 271 Gabarda Way	City: Portola Valley	State or Country: California	Zip Code: 94025

301 ☐ Specific reference to my related earlier filed U.S. application(s) of which this application is a ☐Continuation ☐Continuation-in-Part
☐ Division is made on page ____ of the specification for the purpose of receiving benefit of said earlier filing date(s) 35 USC 120.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Signature of Inventor 202	Signature of Inventor 203
Hermann Bujard	ACYC C.Y.	SYC Stanley N. Cohen
Date: 5/19/81	Date: 5/19/81	Date: 5/19/81

FORM T&T 14 REV.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PAT. & TRADEMARK OFF.

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AS ORIGINALLY FILED

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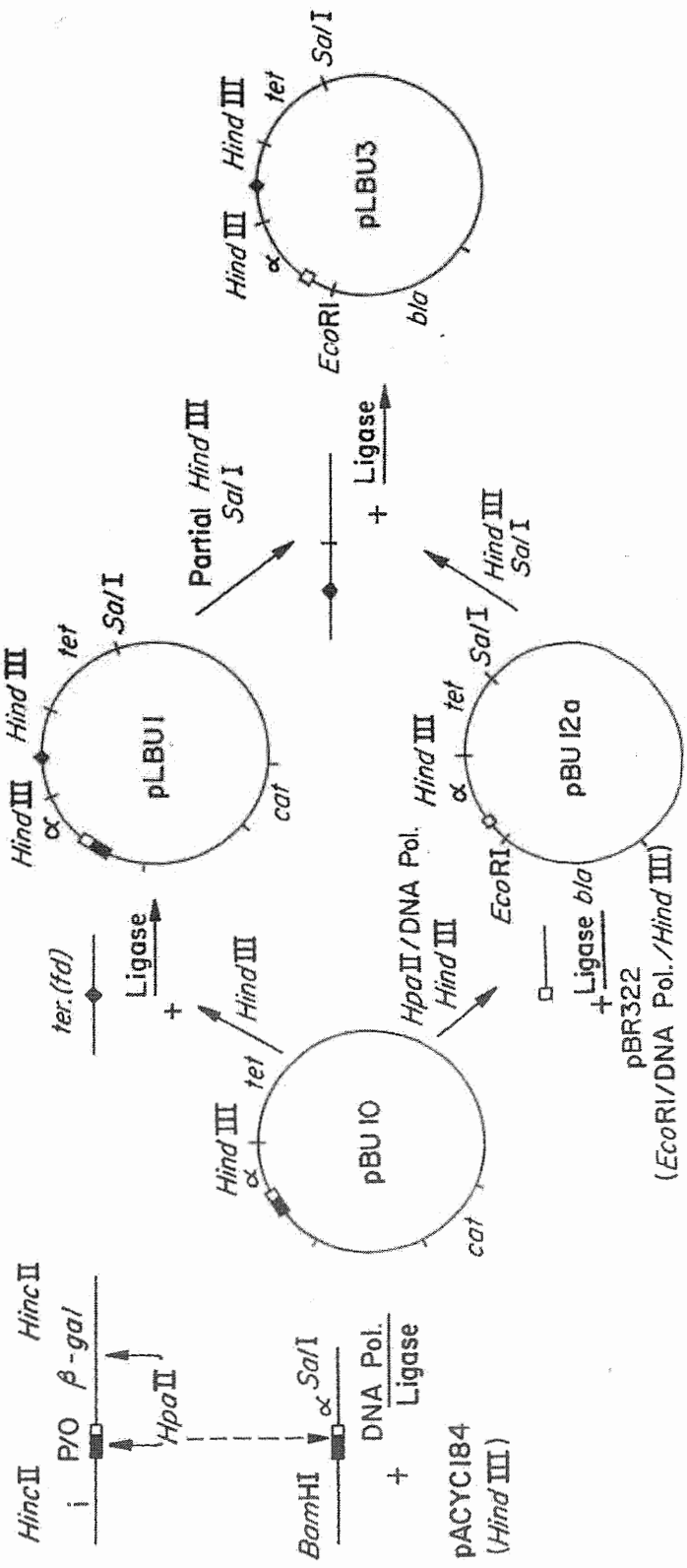

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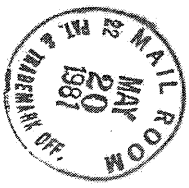
Ck. No. 5490-35
Reimburse Bujard, et al



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
06/265,274	05/20/81	BOLGARD M	5490-35

TOWNSEND & TOWNSEND
STUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

EXAMINER	
KEPPLINGER E	
ART UNIT	PAPER NUMBER
172	2

DATE MAILED: 06/30/82

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined. ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ~~_____ days~~ from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892
2. ☐ Notice of Informal Patent Drawing, PTO-948
3. ☐ Notice of References Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-26 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☑ Claims 1-26 are free of the cited prior art ~~are allowed.~~

4. ☑ Claims 1-26 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ The formal drawings filed on _____ are acceptable.

8. ☐ The drawing correction request filed on _____ has been ☐ approved. ☐ disapproved.

9. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has
   ☐ been received. ☐ not been received. ☐ been filed in parent application, serial no. _____,
   filed on _____.

10. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

11. ☐ Other

PTOL-326 (rev. 7-79)

EXAMINER'S ACTION

The disclosure is objected to as failing to provide an adequate disclosure of the invention as required by the first paragraph of 35 U.S.C. 112. This paragraph of the statute, requires that the specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The journal citations on pages 2 and 15 do not correctly cite the pages and one reference on page 2 is incomplete. Also, on page 17, the Gentz reference is incomplete and no earlier citations thereof can be found.

Claims 1-10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as his or her invention. The claims are indefinite as to what is between the promoter and terminator. It appears that either a marker or foreign replication system are present but the language of the claims does not make that clear.

The disclosure is objected to as failing to provide an adequate disclosure of the claimed invention as required by the first paragraph of 35 U.S.C. 112. This paragraph of the statute, requires that the specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The disclosure requires that the various DNA sequences be in a particular order in order for the invention to function. The claims do not require such order. They do not specify where in the DNA sequence the promoter is inserted which is essential.

Claims 23-26 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the preceding paragraphs.

Claims 23-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as his or her invention. The claims are indefinite in the

recitation of "proximal to one end". Proximal to one end of what?

Applicants are requested to supply copies of the journal articles cited in the specification because they are unavailable to the examiner.

Claims 1-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as his or her invention. The claims are indefinite in the recitation of "a replication system". It is not clear what that encompasses.

703-557-3685/86
Esther M. Kepplinger:ab
6-16-82

Esther M. Kepplinger
ESTHER M. KEPPLINGER
PRIMARY EXAMINER

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED	265276	172	2
	APPLICANT(S) Bujard et al		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
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	F						
	G						
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	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R	Stuber et al, PNAS, 78(1), 167-171 (1981 January). 14
S	
T	
U	

EXAMINER	DATE
E. M. Kepplinger	5/25/82

A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED	265276	172	2
	APPLICANT(S): Beijard et al		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
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FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R	[illegible] (PNAS 78(1), 117-171 (1981 January).
S	
T	
U	

EXAMINER	DATE	
E. M. Kepplinger	5/25/82	

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Herman Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 172
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	AMENDMENT

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

In response to the Office Action of June 30, 1982, please make the following amendments:

IN THE SPECIFICATION:

Page 2, line 10, after "β" insert ~~b~~ - ~~b~~ (a hyphen);

line 11, change "I" to ~~b~~J~~b~~; change "179207" to ~~b~~179-207~~b~~;

line 12, change "175193" to ~~b~~175-193~~b~~;

line 15, change "189193" to ~~b~~189-193~~b~~;

line 21, after "USA" insert ~~b~~78:167-171~~b~~.

Page 14, line 22, change "restruction" to ~~b~~restriction~~b~~.

Page 15, line 1, change "44954503" to ~~b~~4495-4503~~b~~; line 16, after "(α-protein)" insert ~~b~~and~~b~~.

Page 16, line 6, change "om tje" to ~~b~~on the~~b~~.

Page 17, line 24, cancel "(Gentz (1981), supra)".

IN THE CLAIMS:

1. (amended) A linear DNA sequence having proximal to one end a strong T5 phage promoter, proximal to the other end a strong terminator balanced with said strong T5 promoter, and having [at least one intermediate said promoter and terminator of a marker for selection adjacent to said terminator and distal from said other end,] intermediate said promoter and terminator at least one marker for selection adjacent to said terminator or a replication system foreign to T5.

Claim 23, line 8, change "systems" to -system-.

REMARKS

In view of the above amendments and the following remarks, the Examiner is earnestly requested to withdraw the rejections, allow Claims 1-26, and pass this application to issue.

It is noted that the claims are free of the prior art.

Applicants sincerely apologize for the erroneous page numbers. This is an unfortunate result of modern technology where miscoding for a hyphen may result in its absence in the final copy. The appropriate hyphens have now been introduced to indicate the page ranges. The reference to Gentz was in error and has been deleted. A number of minor typographical errors were noted and have now been corrected. The incomplete citation is a reference of record and that citation has now been incorporated into the specification. It is therefore believed, that the minor errors noted by the Examiner have all been corrected.

Turning now to the claims, it is believed some explanation will be helpful in clarifying the situation. Claims 1-10 concern a linear DNA sequence. The terminator appears to be an inverted repeat, so that it has no direction. By contrast, the promoter is directional, allowing for transcription in only one direction. The linear sequence has the promoter near one end and the terminator near the other end. The promoter may provide for transcription in either direction. Thus, the gene of interest, many genes being defined in the claims, may be a portion of the linear sequence or may be provided by insertion between the promoter and the terminator. When the gene is introduced by insertion, the promoter directs transcription toward the end of the linear sequence adjacent the promoter. When the gene of interest is intermediate the promoter and the terminator, the promoter directs transcription along the linear sequence toward the terminator. Claims 1-10 require that the linear DNA sequence either have a replication system (this will be discussed later) or a gene foreign to the T5 phage.

It is hoped that with the change in language of the claim, the claim is now definite and avoids the rejection.

The fact of there being a particular order or direction is not entirely true. As indicated above, the promoter can be pointed in either direction, which then determines whether the gene will be inserted to join the ends of the linear sequence or is present on the linear sequence prior to insertion.

By contrast, Claims 11-19 are concerned with circular DNA where the direction is now important, since downstream defines the direction of transcription. It is believed that Claim 11 clearly defines the proper order.

Claims 20-22 are dependent upon the circular DNA sequence Claims 11-19.

Claim 23 again is concerned with a linear DNA sequence. A linear sequence has two ends. Claim 23 requires that proximal to one end in the downstream direction is a gene. The point of the first step is the insertion of a promoter into the linear DNA sequence to form a circular DNA sequence. Thus, when the promoter is inserted between the ends of the linear DNA sequence, it is followed by the gene and the promoter defines the downstream direction. After the gene is a terminator, which is then followed by another gene, which in turn is followed by a replication system. The purpose of the two genes is to determine the effectiveness of the terminator. If the terminator is ineffective, then the second gene will be expressed. Therefore, Claim 23 provides a basis for evaluating promoters, terminators, and their relative effectiveness.

So far as a replication system is concerned, this is discussed on page 5, lines 18-28. A replication system will include an origin and any other regulatory sequences which are necessary for binding of DNA polymerase to provide for replication of the two DNA strands. It is believed this language has found extensive use, see for example U.S. Patent No. 4,237,224, and should find acceptance.

The articles referred to in the Description of the Prior Art accompany this response, but not the dissertations, which are believed to be of no more relevance than the published articles.

In view of the above amendments and remarks, the application is considered in good and proper form for allowance, and the Examiner is earnestly requested to pass this application to issue.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of this application, the Examiner is invited to call the undersigned attorney, collect, at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 9/30/82

By _____
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

Enclosures
1. Casabadan and Cohen (1980) J. Mol. Biol. 138:179-207
2. West and Rodriguez (1980) Gene 9:175-193
3. Von Gabain and Bujard (1979) PNAS USA 76:189-193
4. Stuber et al. (1978) Molec. gen. Genet. 166:141-149

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 9/30/82
(Date of Deposit)

Date 9/30/82 By TOWNSEND & TOWNSEND _____
Bertram I. Rowland
Reg. No. 20,015

TOWNSEND and TOWNSEND
Steuart Street Tower
One Market Plaza
San Francisco, CA 94105
(415) 543-9600

Docket No. 5490-35

In re application of Herman Bujard et al. Date November 8, 1982

Serial No. 265,276

Filed May 20, 1981

For CLONED HIGH SIGNAL STRENGTH PROMOTERS

THE COMMISSIONER OF PATENTS & TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

☐ No additional fee is enclosed because this application was filed prior to October 25, 1965 (effective date of Public Law 89-83.)

☐ No additional fee is required.

The fee has been calculated as shown below.

CLAIMS AS AMENDED							
	(1)	(2) CLAIMS REMAINING AFTER AMENDMENT	(3)	(4) HIGHEST NO. PREVIOUSLY PAID FOR	(5) PRESENT EXTRA	(6) RATE	(7) ADDITIONAL FEE
	TOTAL CLAIMS	*	MINUS	**	x	x $5	x
	Total CLAIMS	* 29	MINUS	26	x 3	x $10	x 30.00
				TOTAL ADDITIONAL FEE FOR THIS AMENDMENT			$30.00

* If the entry in Column 2 is less than the entry in Column 4, write "0" in Column 5.
**If the "Highest Number Previously Paid For" IN THIS SPACE is less than 10, write "10" in this space.

☐ A check in the amount of $ is attached.

☒ Charge $ 30.00 to Deposit Account No. 20-1430 A duplicate copy of this sheet is enclosed.

☒ Please charge any additional fees or credit overpayment to Deposit Account No. 20-1430 A duplicate copy of this sheet is enclosed.

TOWNSEND and TOWNSEND

Bertram I. Rowland
Attorney of Record
Reg. No. 20,015

BIR/gs
T&T 26 REV.

10/26/82

170

11/22/82

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Herman Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 172
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	SUPPLEMENTAL AMENDMENT

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

Supplemental to the response to the Office Action of June 30, 1982, please add the following claims.

IN THE CLAIMS:

27. A DNA sequence having a replication system recognized by a microorganism, a marker for selection and in downstream order of transcription, a strong promoter, a structural gene foreign to said strong promoter and a balanced terminator, wherein said strong promoter intends a promoter which inhibits expression of said marker in the absence of a balanced terminator.

28. A DNA sequence according to Claim 27, wherein said replication system is recognized by a prokaryote.

29. A DNA sequence according to Claim 27, wherein said replication system is recognized by a eukaryote.

REMARKS

In view of the following remarks, the Examiner is earnestly requested to add Claims 27-29 to the subject application and allow Claims 1-29.

Support for new Claims 27-29 may be found on page 1, lines 26-30, page 2, line 38 and in the original claims.

After responding to the Office Action of June 30, 1982, upon review of the claims, it was found that their scope did not reflect the proper breadth of the subject invention.

The subject claims are believed to be patentable over the prior art for the following reasons. First, it had not be appreciated previously that a strong promoter, such as the T5 phage promoters, could inhibit cloning of a plasmid. Only with the discovery of the subject invention is it shown that one can clone strong promoters and use either the wild type terminator or a different terminator, where the utility of the terminator may be determined by the method disclosed in the subject invention. Therefore, the subject invention now allows for the use of a variety of strong promoters with balanced terminators for the high yield expression of structural genes of interest.

In view of the above remarks, the Examiner is earnestly requested to enter the above amendments and pass this application to issue.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of this application, the Examiner is invited to call the undersigned attorney, collect, at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 11/5/82

By Bertram Rowland

Bertram I. Rowland
Reg. No. 20,015

BIR/gs

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

EXAMINER	
[illegible]	
ART UNIT	PAPER NUMBER
[illegible]	5

DATE MAILED: JAN 03 1983

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined. ☑ Responsive to communication filed on 11/12/82. ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892
2. ☐ Notice of Informal Patent Drawing, PTO-948
3. ☐ Notice of References Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-29 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☑ Claims 1-29 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ The formal drawings filed on _____ are acceptable.
8. ☐ The drawing correction request filed on _____ has been ☐ approved. ☐ disapproved.
9. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has
   ☐ been received. ☐ not been received. ☐ been filed in parent application, serial no. _____,
   filed on _____.
10. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
11. ☐ Other

PTOL-326 (rev. 7-79)

EXAMINER'S ACTION

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide support for the claimed invention. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The recitation that the promoter "intends a promoter which...terminator "is new matter since no basis can be found for such recitation. The areas pointed out by applicants does not provide support.

Claims 27-29 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 27-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are indefinite in the recitation of "intends" since that is not a definite limitation.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide support the claimed invention. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. No basis for having the replication system intermediate the promoter and terminator can be found.

Claims 1-10 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 11-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are indefinite in the recitation of "a balanced terminator". It is not clear in what way or with what it is balanced.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 4-15, 17-22 and 27-29 are rejected under 35 U.S.C. 103 as being unpatentable over Casadaban et al in view of Stüber et al(v). Casadaban et al shows analysis of promoters and terminators by preparing plasmids containing ara promoter, promoters such as T5, structural lac genes, a marker for ampicillin resistance and a terminator. The sequence of the elements appears to be as claimed and the

terminator of the reference appears to be "strong". Stüber et al teach that to prepare stable plasmids the strength of the promoter and terminator should be compatible. Thus, it would be obvious to use balanced promoter and terminator as in Stüber in the plasmids of Casadaban et al for stability. The use of various structural genes and replication systems for either prokaryotes or eukaryotes would be obvious.

EKEPPLINGER:hm

703-557-3685

12/15/82

Esther M. Kepplinger

ESTHER M. KEPPLINGER
PRIMARY EXAMINER

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP–APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED		265276	172	5
		APPLICANT(S) Bujard et al		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

✓	R	Casadaban et al, J. Mol. Biol., 138, 179–207 (1980). 11
✓	S	West et al, Gene, 9, 175–193 (1980). 14
✓	T	Gabain et al, PNAS, 76(1), 189–193 (1979). 14
✓	U	Stüber et al, Molec. gen. Genet., 166, 141–149 (1978). 14
✓	V	Stuber et al, PNAS USA, 78(1), 167–171 (1981). 14

EXAMINER	DATE
E. M. Kepplinger	12/8/82

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

TOWNSEND AND TOWNSEND
Steuart Street Tower
One Market Plaza
San Francisco, CA 94105
(415) 543-9600

Atty. Docket No. 5490-35

Date 3/11/83

In re application of HERMAN J. BUJARD et al.

Serial No. 265,276

Filed 5/20/81

For CLONED HIGH SIGNAL STRENGTH PROMOTERS

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

[ ] Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

[ ] No additional fee is required.

The fee has been calculated as shown below:

	(Col. 1)		(Col. 2)	(Col. 3)	SMALL ENTITY			OTHER THAN A SMALL ENTITY	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDIT. FEE	OR	RATE	ADDIT. FEE
TOTAL	* 30	MINUS	** 29	= 1	x5=	$		x10=	$ 10
INDEP.	*	MINUS	***	=	x15=	$		x30=	$
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$ 10

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found from the equivalent box in Col. 1 of a prior amendment or the number of claims originally filed.

[X] Please charge my Deposit Account No. 20-1430 in the amount of $ 10.00. A duplicate copy of this sheet is attached.

[X] The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 20-1430. A duplicate copy of this sheet is attached.

[X] Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

[ ] Any patent application processing fees under 37 CFR 1.17.

TOWNSEND & TOWNSEND

Reg. No.: 20,015
Attorney of Record Bertram I. Rowland

BIR/gl

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Herman Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 172
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	AMENDMENT

San Francisco, CA. 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

In response to the Office Action dated January 3, 1983, please amend the above-referenced application as follows:

IN THE SPECIFICATION:

Page 4, line 33, cancel "onefourth" and substitute therefor --one-fourth-- .

IN THE CLAIMS:

Claim 1, as amended, at line 7 before "marker" insert --of (1) a -- and at line 8 before "a replication" insert --(2)-- .

Claim 6, line 2, change "replication system" to --marker-- .

Please amend Claim 11 as follows:

11. (amended) A circular DNA sequence having in downstream order of transcription a strong T5 phage promoter, a structural gene foreign to T5 phage under transcriptional control of said promoter, a transcriptional [balanced] terminator which is balanced with said promoter, and a replication system.

5

Please amend claim 27 as follows:

27. (Amended) A DNA sequence having a replication system recognized by a microorganism, a marker for selection and in downstream order of transcription, a strong promoter, a structural gene foreign to said strong promoter and a [balanced] terminator which is balanced with said promoter, wherein said strong promoter is characterized by substantially complete absence of [intends a promoter which inhibits] expression of said marker in the absence of a [balanced] terminator which is balanced with said promoter.

Please add new claim 30 as follows:

30. A DNA sequence according to claim 27, wherein said expression of said marker is at a frequency of less than about one-fourth the frequency at which the structural gene is expressed and said marker is under transcriptional control of said promoter.

REMARKS

Claims 1-29 were examined and rejected. The rejections were based both on 35 U.S.C. 112, first and second paragraphs, and on the prior art. Each of the rejections will be discussed in order below.

Claims 1, 6, and 27 have been amended. New Claim 30 has been added. Re-examination and reconsideration of the Claims, as amended, are requested.

Claims 27-29, added by the amendment dated November 8, 1982, were rejected under 35 U.S.C. 112, first paragraph, as lacking support in the specification. Specifically, the rejection states that the definition of "strong" promoter as one "which inhibits expression of said marker in the absence of a balanced terminator" lacks support. Applicants traverse this rejection. At page 1, lines 26-30, it is clearly stated that "strong" promoters, when inserted into a

vector, prevent expression of markers on the vector. Further support is found at page 2, line 37 through page 3, line 2 where it is disclosed that inclusion of a balanced terminator allows expression of the marker. Based on this disclosure, one skilled in the art could identify "strong" promoters based on their ability to inhibit expression of markers on vectors which lack balanced terminators. Applicants respectfully request that this rejection be withdrawn.

As to the rejection of Claims 27-29 under 35 U.S.C. 112, second paragraph, the definition of "strong" promoter has been amended in Claim 27 to avoid any possible ambiguity in the language.

Claims 1-10 were rejected under 35 U.S.C. 112, first paragraph, as lacking sufficient support in the specification. In particular, the Examiner stated that there was no basis for claiming that the replication system was "intermediate" the promoter and terminator.

Applicants believe that this rejection results from a misunderstanding of the intended coverage of claim 1. Claim 1 is directed specifically at a linear DNA segment, such as that described on page 4, line 4 through page 5, line 28 of the specification. Such a linear segment includes a promoter and a terminator adjacent its opposite ends. The promoter in one orientation (the replication system being present on the linear segment) is oriented to define a direction of transcription away from the terminator so that a structural gene of interest may be joined to each end of the linear segment, whereby the gene will be under the regulatory control of the promoter. Thus, the portion of the segment "intermediate" the promoter and terminator in the linear sequence will not be under the regulatory control of the promoter in the constructed plasmid. This region provides a convenient region for carrying a marker for

selection or a replication system, or both. Such a DNA segment is particularly useful for constructing cloning vehicles for a wide variety of structural genes. Where the marker is present, the promoter may be in either direction, where the structural gene of interest may be introduced to be in tandem with the marker or together with a replication system may serve to join the two ends.

The construct and its use may be formulized as follows:

(1) (a) $-\overrightarrow{P}-M-T-$

(b) $-\overleftarrow{P}-M-T-$

(2) $-\overleftarrow{P}-R-T-$

where P is the promoter and the arrow indicates the direction of transcription;

M is a marker;

T is a terminator; and

R is the replication system. When the ends are joined to form a plasmid, the following constructions can be made; where G is the structural gene of interest:

from (1) (a) $\overrightarrow{P}-G-M-T$

from (1) (b) $-G-\overleftarrow{P}-M-T-$

from (2) $-G-\overleftarrow{P}-R-T-$

Claim 1 has been amended to more clearly define the linear DNA sequence as just described. Claim 6 has been amended to conform with claim 1, as amended. Applicants submit that the rejection of claims 1-10 under 35 U.S.C. 112 has been overcome.

Claims 11-29 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the term "a balanced terminator."

Claims 11 and 27 have been amended to recite "a terminator which is balanced with the promoter" rather than a "balanced terminator." Such terminators are clearly defined in the specification. At page 4, lines 31-34, it is stated that the terminator is balanced when expression of a downstream marker occurs with a frequency not greater than about one-fourth of the expression of the gene upstream from the terminator. Moreover, the method described from page 10, line 37 through page 11, line 31 of the specification is directed specifically at a technique for selecting balanced promoter/terminator pairs. Applicants submit that Claims 11 and 27 now described the nature of the "balanced" terminator with sufficient particularity.

Claims 12-22 and 28-29 dependent on Claims 11 and 27, also avoid the 35 U.S.C. 112 rejections for the reasons just set forth.

Applicants do not understand the rejection of Claims 23-26 based on the recitation of "a balanced terminator." None of said claims 23-26 employ that language. Since no other rejections were stated relative to claims 23-26, applicants believe that claims 23-26 should now be in condition for allowance.

For the above reasons, applicants submit that the formal rejections under 35 U.S.C. 112 have been overcome. The prior art rejections will now be discussed.

Before discussing the prior art rejections and cited references in detail, however, a brief explanation of

the present invention is in order. The present invention provides balanced regulatory systems capable of strong expression of a wide variety of structural and other genes. The regulatory systems are characterized by a strong promoter in conjunction with a strong (balanced) terminator. In particular, the present invention is able to utilize the strong promoters of bacteriophage T5 which have heretofore not been useful in constructing vectors for expression of foreign genes. The present invention successfully uses such promoters by assuring that the strong promoters are matched with a balanced terminator, as defined in the specification. In the absence of a balanced terminator, expression of the genes on the vector is not observed.

The present invention relies on a novel method for selecting balanced terminators to allow for construction of the claimed linear segments and plasmids. The method is best described beginning at the end of page 10 of the specification and continuing on page 11. After selecting a promoter or terminator having a known activity, e.g. a strong promoter such as the T5 promoter, a plasmid can be constructed having a first marker upstream of the terminator and a second marker immediately downstream. By determining the relative proportion of expresssion of the first and second markers, the relative strength of the promoter and terminator can be assessed. Using a known strong promoter, a weak terminator results in diminished expression of both gene markers. A strong terminator, however, provides a much higher relative expression of the gene marker upstream of the terminator compared to that downstream of the terminator.

None of the prior art cited by the Examiner either teaches or suggests such a method for selecting balanced

promoter/terminator pairs or the resulting DNA sequences which utilize such pairs.

Casadaban and Cohen, the primary reference, teaches a method for identifying DNA fragments which include either promoters or terminators, but which does not determine their relative strengths. The prior art method relies on inserting the DNA fragments into a construct comprising the ara promoter and the lac structural gene on a plasmid. The ara promoter is inducible, and when it is turned on, the DNA construct can be used to determine whether the inserted DNA segment acts as a terminator. Expression of the lac gene is greatly reduced if the inserted fragment includes a terminator. When the ara promoter is turned off, the DNA construct can be used to determine if the inserted segment acts as a promoter. Since the ara promoter is inactive, expression of the lac gene will be observed only if the inserted fragment includes a promoter.

The Examiner apparently relies on page 191 of the article where it is disclosed that a HindIII fragment of bacteriophage T5, when inserted into the cloning vehicle just described, provides expression of the lac gene when the ara promoter is turned off. While the particular fragments inserted may have included the strong "early" promoters of the T5 phage (as claimed herein), at least some of the inserted fragments would have included the T5 terminator as well as the promoter, and expression of the lac gene could have resulted from read through past the T5 terminator. HindIII digestion of T5 results in fragments ranging from 2.0kb to about 17.2kb (See von Gabian and Bujard, Table 1), which fragments are sufficiently large to include both the promoter and terminator. The fragments inserted in the

vector pLBU3 in the Experimental section of the present invention, however, had an average length of about 200 base pairs and could not have included both a promoter and matched terminator.

Regardless of the origin of the strong terminator, the article of Casadaban and Cohen nowhere teaches the criticality of providing a terminator which is balanced with the strong promoter, e.g. the T5 promoter. In fact, the observed expression of the lac gene would suggest to one skilled in the art that a balanced terminator is unnecessary since Casadaban and Cohen took no steps to assure its presence. As explained above, such expression based on the above results and their interpretation was most likely the fortuitous result of simultaneously introducting a T5 terminator on the same fragment as the promoter, which allowed read through expression of the lac gene.

The Examiner relies on the article by Stuber and Bujard to teach the inclusion of a balanced terminator in the DNA sequences of Casadaban and Cohen. The basis for this teaching, however, is a single sentence at the end of the article which states that the authors "have strong indications that for plasmid stability the signal strength of the promoters and terminators have to be compatible." There is no indication of what is meant by "compatible," nor is there any suggestion of how such compatibility might be assessed. Applicants submit that such a vague suggestion would hardly teach one skilled in the art how to construct the DNA sequences and plasmids of the present invention where suitable promoters and terminators may be indentified by a specific procedure. The statement is an intuitive prognostication, an invitation to research, not a basis for rejection.

As described above, Claim 1 has been amended to more clearly set forth the linear DNA sequence of the present invention. The sequence comprises a strong T5 promoter at one end and a terminator which balanced with the T5 promoter at the other end. Either an origin of replication or a marker for selection is included intermediate the promoter and terminator. This linear DNA sequence is particularly useful for providing the strong expression of a wide variety of structural genes compatible with the T5 promoter. In the typical case where the sequence includes both a marker and an origin of replication, it is necessary only to insert the structural gene in the proper orientation to obtain the expression vector.

Applicants submit that the structure of Claim 1, as amended, is nowhere taught or suggested by the cited prior art. Casadaban and Cohen do not consider DNA fragments where the T5 promoter is located at one end and there is a marker and/or a replication system on the fragment. More importantly, Casadaban and Cohen do not teach the criticality of providing a balanced terminator. Even when combined with the suggestion of Stuber and Bujard, there is no enabling disclosure as to how one would select such a terminator and provide the DNA structure as claimed, nor is there any reason to prepare the claimed fragment.

Claims 2-10, dependent on Claim 1, are also allowable for the reasons just set forth.

Claim 11 is directed at a circular DNA sequence including in order of transcription, a strong T5 promoter, a structural gene foreign to the T5 phage, a terminator which is balanced with the promoter, and a replication system. Claim 11 has been amended to provide that the structured gene is under transcriptional control of the strong T5 promoter. For the reasons given earlier, the structure taught by Casadaban and Cohen is clearly excluded.

Claims 12-22, dependent on claim 11, are also allowable for the reasons just set forth.

Claim 23 is directed at the method for determining the strength of a promoter as described above. These claims were not rejected based on the art. Moreover, the rejection under 35 U.S.C. 112 appears inapposite. For these reasons, applicants assume that claims 23-26 are now in condition for allowance.

Finally, claim 27 is directed at a DNA sequence including a strong promoter (not limited to the strong T5 promoters) and a balanced terminator. Applicants submit they are entitled to such patent protection based on the advances they have made over the prior art. Although others may have suggested that there might be a need for providing balanced promoter/terminator pairs, applicants herein are the first to provide a method for matching a balanced terminator with any desired strong promoter in order to provide an expression vector capable of enhanced expression of an inserted structural gene, as well as establishing that such need exists.

New claim 30 has been added to provide additional description of the balance between the terminator and the promoter. Support for the amendment is found on page 4, lines 30-35 of the specification.

In view of the above amendments and remarks, the Examiner is earnestly requested to withdraw the rejections and pass this application to issue.

If, in the opinion of the Examiner, a telephone conference would expedite prosecution of this application,

the Examiner is invited to telephone the undersigned attorney at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date: 3/11/83

By: Bertram I. Rowland
Reg. No. 20,015

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 3/11/83
(Date of Deposit)

TOWNSEND & TOWNSEND

Date 3/11/83 By

Name Bertram I. Rowland
Reg. No. 20,015

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
06/265,276	05/20/81	BUJARD	H	5490-35

TOWNSEND & TOWNSEND
STEUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

EXAMINER	
KEPPLINGER, E	
ART UNIT	PAPER NUMBER
174	7

DATE MAILED: 06/20/83

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☑ Responsive to communication filed on 3/14/83 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ~~____~~ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I **THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:**

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449
4. ☐ Notice of informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474
6. ☐ _____

Part II **SUMMARY OF ACTION**

1. ☑ Claims 1 – 30 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☑ Claims 1 – 30 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.

8. ☐ Allowable subject matter having been indicated, formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. These drawings are ☐ acceptable; ☐ not acceptable (see explanation).

10. ☐ The ☐ proposed drawing correction and/or the ☐ proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner, ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved, ☐ disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.

12. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

PTOL-326 (Rev. 7-82) EXAMINER'S ACTION

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide support for the invention as now claimed. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The ~~invention~~ recitation that the "promoter is characterized by....in the absence of a termination which is balanced with said promoter" is not supported by the specification. Applicants point to portions of the specification as basis. Adequate support is not found there, however, since the components of the first vector are not made clear nor is the reason for lack of expression of the marker.

Claims 27-29 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 1-22 and 27-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject

matter which the applicant regards as the invention. The claims are indefinite in the recitation of a terminator which is "balanced with said promoter". It is not clear in what way they are balanced. Recitation that they are balanced to allow a particular amount of marker transcribed would obviate the rejection. For example, the recitation on page 4, lines 31-35 would be sufficient.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide support for the claimed invention. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. There is no basis for having the replication system intermediate the promoter and terminator on a linear piece of DNA. Applicant's arguments have been noted but the issue can best be resolved by applicants pointing out portions of the specification providing the support.

Claims 1-10 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The specification does not disclose what terminators may be utilized to balance the promoter or what terminators of known strength may be used. It would require an undue amount of experimentation for one skilled in the art to determine which terminators would function in the invention. It appears that only the terminator of the coliphage fd genome is disclosed which is not basis for the broad recitation.

Claims 1-30 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 1-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The claims are indefinite in the recitation of "terminator". It is clear what they are a terminator of. It is a transcription terminator?

Claims 23-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are indefinite in that they do not clearly recite where the promoter is inserted into the linear DNA which is essential to the invention.

Claims 23-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are confusing since the preamble recites determination of the strength of a promoter but no positive determination step is recited.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an adequate written description of the invention. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is

most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The specification requires a particular sequence of components for the invention to function and such order must be clearly recited. The claims only limit the position of the gene.

Claims 23-26 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The specification discloses only T5 phage strong promoters and it would require an undue amount of experimentation for one skilled in the art to determine other promoters which could be utilized.

Claims 27-30 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. Although the specification mentions a replication system for eukaryotes, no example or specific disclosures of such are given and the specification is therefore non-enabling for such recitation. It would require an undue amount of experimentation to discover systems and ways of placing such components into a vector for insertion in a eukaryotic system.

Claims 10, 13-17, 19, 21, 22 and 29 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. This paragraph of the statute requires that the specification shall contain a written description

of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. The specification does not adequately show determining the strength of a promoter as claimed. It is not clear that one skilled in the art could perform the invention as claimed. The disclosure does not show measuring unknown promoters of assay their strength.

Claims 23-25 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the

> time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 11-30 are rejected under 35 U.S.C. 103 as being unpatentable over Casabadan et al. Casabadan et al show analysis of promoters and terminators by preparing plasmids containing promoters (ara or T5), structural lac genes, a marker for ampicillin resistance and a terminator in a sequence as claimed. The terminator in a sequence as claimed. The terminator appears to be "balanced" with the promoter since the gene and marker are expressed. The replacement of one promoter for another in Casadaban et al reads on the method of claims 23-26.

Applicants urge that Casadaban et al nowhere teaches the criticality of providing a terminator which is balanced with the strong promoter. The use of such ambiguous language is insufficient to establish a difference over the reference.

Applicants urge that the circular DNA of claim 11 distinguishes over Casadaban et al. Applicants use unclear terms at their point of novelty, however and thus no difference is seen.

Applicant urge that no prior art shows a strong promoter and balanced terminator as in claim 27. The claims do not distinguish over Casadaban et al, however, by reciting such relative and indefinite terms as "strong" and "balanced".

KEPPLINGER:sab
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6-2-83

Esther M. Kepplinger
ESTHER M. KEPPLINGER
PRIMARY EXAMINER

8/D
KT.
9-27-83

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	)	
	)	
Hermann J. Bujard et al.	)	Examiner: E. Kepplinger
	)	
Serial No. 265,276	)	Art Unit: 174
	)	
Filed: May 20, 1981	)	
	)	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	)	AMENDMENT
	)	

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

In response to the Office Action of June 20, 1983, please make the following amendments:

IN THE CLAIMS:

1. (thrice amended) A linear DNA sequence having proximal to one end a strong T5 phage promoter, proximal to the other end a strong transcriptional terminator balanced with said strong T5 promoter, and having intermediate said promoter and terminator at least one of (1) a marker for selection adjacent to said terminator or (2) a replication system foreign to T5, wherein the direction of said promoter is away from said terminator and said marker is expressed at a frequency of less than about one-fourth the frequency of a structural gene under the transcriptional control of said promoter and bridging said linear DNA sequence to provide a circular DNA sequence.

Claim 11, line 4, after "promoter, a" insert ~~a~~transcriptional~~-~~.

H
H
H2

15. (amended) A circular DNA sequence ~~according to any of Claims 11, 12 or 13,~~ having a marker for selection downstream from said terminator, wherein said marker is expressed at a frequency of less than about one-fourth the frequency of which the structural gene is expressed and is under transcriptional control of said promoter.

H1

H3

23. (amended) A method for determining the strength of a promoter which comprises:

inserting said promoter into a linear DNA sequence having in the downstream direction for expression proximal to one end: [in the downstream direction for transcription] a gene allowing for detection of expression; a transcriptional terminator of known strength; a marker allowing for determination of expression; and a replication system recognized by a predetermined host; whereby a circular DNA sequence is obtained;

transforming said host with said circular DNA sequence; [and]

growing said host in nutrient medium under conditions allowing for determination of the extent of expression of said gene and said marker; and

determining the strength of said promoter is determined by the relative degree of transcription of said gene and said marker.

H4

27. (twice amended) A DNA sequence having a replication system recognized by a microorganism, a marker for selection and in downstream order of transcription, a strong promoter, a structural gene foreign to said strong promoter and a transcriptional terminator which is balanced

with said promoter, wherein said strong promoter is characterized by substantially complete absence of expression of said marker in the absence of a terminator, and is at a frequency of less than about one-fourth the frequency at which the structural gene is expressed and said marker is under transcriptional control of said promoter.

Cancel Claim 30.

REMARKS

In view of the above amendments and the following remarks, the Examiner is earnestly requested to withdraw the rejections, allow Claims 1-29, the only claims under consideration, and pass this application to issue.

A sincere effort has been made to avoid the rejections under 35 USC 112. However, it appears that applicants' discussion in the previous response has been inadequate to explain the subject invention. As the Examiner is aware, the promoter is the site for binding of RNA polymerase. Downstream from the promoter sequence is the Pribnow box, which appears to designate a base downstream from the Pribnow box for transcriptional initiation. The theory of strong and weak promoters appears to involve the binding efficiency of RNA polymerase to the promoter site. By contrast, the terminator is usually distant from the promoter, downstream from the structural gene, and involves an inverted repeat, so that a single strand can bind to itself to provide for self-hybridization and looping out. The terminator structure may have additional complexities. Its function is to stop the RNA

polymerase, so that transcription ends at a site upstream from the terminator.

It is not apparent that a promoter acts like a slingshot. Why the nature of the promoter should affect the terminator efficiency is not at all clear. However, it was found that where one had a strong promoter, such as a T5 phage promoter, and used a terminator from a different source, which terminator turned out to be a "weak" terminator, no expression of a marker was observed. Therefore, one could not select for a plasmid in which this construct existed.

For whatever reason, in the absence of a balanced terminator, a transcript or messenger RNA capable of translation was not obtained. This observation was first made by the subject inventors. (This will be discussed subsequently.) Therefore, as a first aspect of patentability, the subject inventors were the first to recognize the existence of the problem. Secondly, because one does not obtain transcription, there would appear to be no obvious reason. Inoperability can be due from a wide variety of sources. It was the subject inventors' insight to realize that the problem might lie with the terminator. Therefore, by changing the 3'-non-coding end of the sequence to include a strong terminator, coupled with a strong promoter, expression of the marker was now achieveable. Based on this observation and the observation that a terminator could be leaky, the subject inventors then conceived of the idea that by placing a marker downstream from the terminator, one could obtain transcription of a structural gene at a high level of transcription and transcription of a marker at a substantially reduced level.

In many situations this is desirable, since the marker and structural gene can be produced concomitantly and the desired product produced at a much higher level than the marker.

Applicants, who are respected academicians at recognized universities, state on page 1, lines 26ff, "It was found that when introducing a strong promoter into a vector and employing the resulting plasmid for transformation, one could not select transformants based on expression of markers which allowed for selection." This is their observation and it is believed that in the absence of reasons for the Examiner to doubt such observation, the Examiner should take the observation at face value. Furthermore, in the Experimental section, that part beginning on page 16, line 27, the manner of using the subject invention to analyze for strong promoters is described. The plasmid pLBU3 was prepared, having a structural gene downstream from a restriction site into which a promoter could be inserted, which structural gene complemented an auxotrophic host, which lacked β-gal activity. The plasmid had the fd terminator and downstream from the fd terminator, a tet gene, which provided tetracycline resistance. The T5 phage was digested with two endonucleases and the various fragments screened for promoters. One plasmid pGBU207 (page 17, line 25) was chosen for further analysis. Finally, it is indicated that the plasmid pGPU207 was shown to have a promoter which appeared to be different from P_{25} and P_{26}, described previously, but had similar signal strength.

It is submitted that Claims 27-29 are clearly supported by the specification. There is no reason to doubt

the statement made by the inventors, nor is it considered necessary that applicants provide a declaration or further evidence of the correctness of their statement based on their observations. For the reasons given above, the Examiner is earnestly requested to withdraw the rejection of Claims 27-29.

Claim 1 has been amended, as well as other claims, to include the limitation which the Examiner suggested, support for which appears on page 4, lines 31-35. The additional amendment to Claim 1 finds support on page 4, lines 4-10 and 18-20.

There is the additional amendment, which indicates that the promoter is directed away from the terminator in the linear DNA. The fact that the promoter is directed away from the terminator and the linear segment follows from the discussion beginning at page 4, line 13. Since the promoter is directed toward the terminator "when the signal sequences are joined" intends that the structural gene which serves to join the promoter and balanced terminator is in the direction of transcription. Since the language is clearly inherent in the language of the specification, it is well established that support for language in the claim may be supplied by its inherency in the specification.

For better understanding of the above discussion, the Examiner's attention is directed to the Figure. Please consider the structural of the plasmid pLBU1. In that plasmid, the promoter, indicated by the black box, is directed toward the terminator, indicated by the black diamond. If one now cleaved the plasmid at the HindIII site intermediate the promoter and the terminator, the promoter

would now be directed away from the terminator on the linear DNA segment.

The rejection of Claims 1-22 and 27-30 under 35 USC 112 has been avoided by amendment as suggested by the Examiner.

The rejection of Claims 1-10 under 35 USC 112 for failing to support having a replication system intermediate the promoter and terminator is respectfully traversed. The Examiner's attention is once again directed to the Figure and the plasmid pLBU1. Again, let us assume that the HindIII site is cleaved. Based on the description of the plasmid, the replication system is now intermediate the promoter and terminator. Therefore, in the linear DNA segment, all that is indicated is that the linear DNA segment provides a convenient portable DNA sequence which has all the necessary elements for controlled expression of a structural gene.

There is clearly language in support of such sequence on page 4, lines 4-10. In that paragraph, the linear DNA sequence is indicated as being useful to be bridged by a structural gene which results in a plasmid. A plasmid is an extrachromosomal element capable of independent replication. On page 5, line 18, it is stated that, "The above DNA sequence construct will have a replicating system. . . ." Furthermore, on page 12, line 24, a vector is described having a competent replication system and a unique restriction site between a T5 promoter and a terminator. Upon cleavage at the unique restriction site, the result would be a linear DNA fragment having a competent replication system intermediate the promoter and terminator. As already discussed, the Figure also supports

this construction. It is therefore submitted, that applicants have ample support for this structure.

The rejection of Claims 1-30 under 35 USC 112 is respectfully traversed. The rejection is based on the allegation that it would require undue experimentation to determine other terminators. First, quite clearly, the terminators of the T5 phage could be used, since it is known that the T5 phage is in fact capable of replication. Secondly, undue experimentation requires a very large class without any direction as to selection. That is not true in the present situation. Strong promoters will be involved with structural genes which are required in relatively high proportions for an organism. Therefore, one would look to those structural genes and their transcriptional regulatory sequences for strong promoters and strong terminators. Furthermore, the subject invention provides for an easy way to screen these regulatory sequences. Under the circumstances, it is submitted that it is not undue experimentation to provide for screening for a promoter, where applicants have shown a number of different strong promoters.

Under the applicable law as stated in Tabuchi et al. v. Nubel et al., 194 USPQ 521 (CCPA 1977), reasonable experimentation is permitted. So long as guidelines are provided, and one can expect success within a reasonable class, this degree of experimentation is permissible. It is submitted that the law recognizes the level of experimentation provided for in the subject application as being acceptable.

The rejection of Claims 1-30 under 35 USC 112 for failing to define the terminator as a transcriptional determinator has been avoided.

The rejection of Claims 23-26 under 35 USC 112 is respectfully traversed. This rejection is based on the failure to indicate where the promoter is inserted. It would appear that the Examiner has overlooked the limitation at the end of the second paragraph which indicates that a circular DNA sequence is obtained. Thus, the insertion acts as a bridge and it therefore inserted between the two ends of the linear DNA. The Examiner is respectfully requested to withdraw this rejection.

The next rejection is of Claims 23-26 under 35 USC 112. This rejection is based on the failure to specify the specific determination step. Claim 23 has been amended to provide the determination step. Support for the new language may be found on page 11, lines 16-19. The rejection having been avoided, the Examiner is earnestly requested to withdraw this rejection.

The next rejection is of Claims 23-26 for failure to indicate the particular sequence of components. The promoter component has already been discussed as to its position. The amendment of Claim 23 has provided the original intention ensuring that the proper order is now provided. Applicants thank the Examiner for bringing the indefiniteness to applicants' attorney's attention.

The rejection of Claims 27-30 under 35 USC 112 because of undue experimentation is respectfully traversed. Applicants are the first to recognize the phenomenon which is provided for in Claim 27, namely the requirement of a balanced terminator with a strong promoter. Furthermore,

applicants now provide for a marker for selection which is downstream from the terminator, which construction is novel and has utility, as has been discussed above. The fact that applicants have used the strongest known prokaryotic promoters does not mean that this invention should be limited to the specific promoters actually exemplified by applicants. As already indicated, strong promoters are known, since they are almost always present in association with products which are expressed in large amounts or high rates by a host. Therefore, there are a large number of promoters which can be used, particularly viral promoters, which are in many cases known to be strong.

The Examiner's rejection of eukaryotic replication systems in Claims 10, 13-17, 19, 21, 22 and 29 is respectfully traversed. The Examiner is well aware that working exemplification is not required. The Examiner has given no reason other than the absence of such working exemplification to reject the subject claims. In the absence of a basis for believing the subject invention is inoperative in eukaryotes, the Examiner is earnestly requested to withdraw this rejection.

The rejection of Claims 23-25 for not adequately showing how to determine strength of the promoter is traversed for the reasons already discussed. Specifically, applicants did exactly that with T5 phage promoters. The Examiner is therefore respectfully requested to withdraw this rejection.

The next rejection appears to be to Claims 11-30, but applicants will treat this rejection as Claims 1-30 based on 35 USC 103, over Casadaban and Cohen. I am sure it does not escape the Examiner's notice, that one of the

coinventors of the subject application is a coauthor of that article. Furthermore, another coinventor Bujard, also appears prominently in the article. If one looks at the discussion on page 194, certain things are evident. On an insertion of T5 phage fragments into the construction described in the article, only one fragment was found to be useful. Of the three hybrid plasmids, the authors state:

> "All three hybrid plasmids contained identical HindIII-generated fragments, one of which was the same size as the vector plasmid. . . and the other was the size of T5 HindIII fragment number 11 or 12, which were not resolved in our gels of HindIII-cleaved T5 DNA. Since no other T5 fragment capable of promoter activity was isolated in three separate experiments, it seems likely that either the other fragments lack a promoter in the appropriate relationship to a HindIII cleavage site to accomplish expression of lac, or that the presence of such a fragment from the virulent T5 phage is lethal to non-infected E. coli."

It would appear that it was this observation that is the basis for the statement on page 1 of the subject application. Namely, where one uses a strong promoter, one does not observe transcription. The strong promoter interferes with transcription of the marker, so that expression is not observed. In contrast, when the promoter and terminator are balanced, transcription is obtained and expression of the lac gene is observed. It should be further noted, that the correct explanation was not suggested in this article.

What conclusions can one come to? First, the promoter which was observed from T5 is unlikely and based on the present invention, cannot be a strong promoter. The Examiner of course realizes that there must be a large number of promoters in T5 phage, since one speaks of promoters 25 and 26. Under the circumstances, since the promoter observed by Casadaban and Cohen was successful in

providing expression, based on the present invention, it cannot be termed a strong promoter. The fact is, that the other promoters did not allow for expression and it is these promoters, such as P_{25} and P_{26}, and the promoter of the subject invention, which prevented transcription. It seems very unlikely in view of the results of the digestion of T5 DNA with HaeIII and AluI, which produced a large number of promoters, that the observation in the article of failure to obtain transcription other than with a single promoter, is based on the fact that HindIII is somehow special and inhibits all the other promoters from being in the right position to provide for transcription.

Based on the above discussion, it is evident that Casadaban and Cohen do not suggest the subject invention, rather they lead away from it. Their observation of an early T5 promoter (see page 194), suggests that the promoter is not a strong one as defined by the subject application and therefore does not suggest the subject invention. Since all the claims in the subject application are concerned with strong promoters and it was only by the observation that a strong promoter must be coupled with a balanced terminator, that the strong promoters were isolated and characterized, it is believed that the rejection over the prior art should be withdrawn.

So far as the unclear language, admittedly the claims are not in the mathematical precision that applicants would like. However, the subject invention does not readily lend itself to such mathematical precision. Rather, it is based on an observation that in certain situations where a promoter is introduced into a vector, which has a terminator not normally associated with the promoter, transcription is

not observed. However, where one provides for a terminator which is balanced with the promoter and the promoter is a strong one, termination is observed. The invention being based on this observation, one must use terminology such as strong and weak, balanced and unbalanced. Based on the application disclosure and the specific description of strong terminators and strong promoters, it is believed that the language is sufficiently clear and definite to allow a person skilled in the art to understand the invention and what is claimed. Furthermore, to the extent that the claims require a marker downstream from the terminator which is under the transcriptional control of the promoter upstream from the terminator, these constructions are novel and provide for a novel result. So far as a means for measuring strength of promoters, the fact that promoters and terminators needed to be balanced was not known until the subject invention. The subject invention provides for a means for measuring balanced terminators and promoters, as well as the strength of the promoter and terminator, and the inventors should be allowed to claim this discovery.

In view of the above amendments and remarks, the application is considered in good and proper form for allowance, and the Examiner is earnestly requested to withdraw the rejections and pass this application to issue.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject

application, the Examiner is invited to call the undersigned attorney at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 9/20/83

By Bertram Rowland
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 9/20/83
(Date of Deposit)

Date 9/20/83 By TOWNSEND & TOWNSEND
Bertram Rowland
Bertram I. Rowland
Reg. No. 20,015

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
[illegible]776	05/20/81	BLANARD H	5390-35

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EXAMINER	
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ART UNIT	PAPER NUMBER
371	9

DATE MAILED: [illegible]

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☑ Responsive to communication filed on 9/23/83 ☑ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-29 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☑ Claims 1-29 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.

8. ☐ Allowable subject matter having been indicated, formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. These drawings are ☐ acceptable; ☐ not acceptable (see explanation).

10. ☐ The ☐ proposed drawing correction and/or the ☐ proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved. ☐ disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.

12. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

PTOL-326 (Rev. 7-82) EXAMINER'S ACTION

Claims 1-10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are indefinite and confusing in the recitation of "wherein the direction of said promoter is away from said terminator", "to provide a circular DNA sequence" and "a structural gene". It is not clear to what the direction refers. The phrase is unclear. The claims are unclear because they recite "a linear DNA sequence" and "to provide a circular DNA sequence. What is being claimed--linear or circular? The reference to a structural gene is unclear since none is positively recited.

Claims 11-16 and 18-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. These claims are indefinite in the recitation of a terminator which is "balanced with said promoter". The suggested amendment was made to other claims but not the recited claims.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. The specification does not

disclose what terminators may be utilized to balance the promoter or what terminators of known strength may be used. It would require an undue amount of experimentation to determine what terminators would function. Applicants urge that the terminators of the T5 phage could be used. Which terminators? Applicants later urge that there are many promoters some of which are not strong. Therefore, there must be many terminators to balance the promoters. It would require an undue amount of experimentation to determine which terminators to utilize. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Applicants urge that some experimentation is permitted and undue experimentation requires a very large class without any direction as to selection since strong promoters are involved with structural genes required in high proportions. Such direction does not appear to be given in the specification but even if it

is, such direction still involves a large class of possibilities which is outside the realm of permitted experimentation.

Claims 1-29 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The specification is objected to under 35 U.S.C.112, first paragraph, as failing to provide an enabling disclosure. The specification is non-enabling for promoters other that T5 phage. This paragraph of the statute requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Applicant urges that other promoters such as viral promoters could be used. Such examples are not given in the specification and it would require an undue amount of experimentation to determine other promoters which could be used.

Claims 27-29 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in Graham v. John Deere Co., 383 U.S. 1,86S.Ct. 684, 15 L.Ed. 2nd 545 (1966) 148 USPQ 459, that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art;
2. Ascertaining the differences between the prior art and the claims at issue; and

3. Resolving the level of ordinary skill in the pertinent art.

Claims 11-16, 18-22 and 27-29 are rejected under 35 U.S.C. 103 as being unpatentable over Casabadan et al for reasons of record. Applicants urge that the promoter of Casabadan et al, based on the present invention, cannot be a strong promoter since successful expression occurred. Such a relative term does not define a difference. Moreover, the fact that expression occurred may be based on the presence of "a balanced terminator". Applicants have not demonstrated a difference in the promoters particularly since the language used to distinguish is "strong" which is a relative term.

Claims 27-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The claims are indefinite in the recitation of "is at a frequency...promoter". It is not clear what is at said frequency.

Applicant's amendment necessitated the new grounds of rejection Accordingly, THIS ACTION IS MADE FINAL. See MPEP 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR

1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

A shortened statutory period for response to this final action is set to expire three months from the date of this action. In the event a first response is filed within two months of the mailing date of this final action and the advisory action is not mailed until after the end of the three-month shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the statutory period for response expire later than six months from the date of this final action.

Any inquiry concerning this communication should be directed to Examiner Kepplinger at telephone number 703-557-2319.

E.Kepplinger/pmj
703-557-2319
12-23-83

Esther M. Kepplinger
ESTHER M. KEPPLINGER
PRIMARY EXAMINER

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
265276	5/20/81	Bujard	5490-35

EXAMINER	
E. Kepplinger	
ART UNIT	PAPER NUMBER
174	10

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Mr. Bertram Rowland (3) _____

(2) _____ (4) _____

Date of interview 3/6/84

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☑ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: all

Identification of prior art discussed: all

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Mr. Rowland will limit the claims to T5 phage promoters and reargue enablement of terminators. No agreement. Will modify claim 1 to clearly indicate that the DNA becomes circular when gene is added. Will cancel claims 27-29 + amend claim 11 concerning a balanced terminator.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate reocrd of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Esther M. Kepplinger
Examiner's Signature

81-3679 PTOL-413 (rev. 1-81)

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5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Hermann Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 174
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	AMENDMENT UNDER RULE 116

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

In response to the FINAL REJECTION of January 10, 1984, please make the following amendments:

IN THE CLAIMS:

1. (fourth amendment) A linear DNA sequence having proximal to one end a strong T5 phage promoter, proximal to the other end a strong transcriptional terminator balanced with said strong T5 promoter, and having intermediate said promoter and terminator at least one of (1) a marker for selection adjacent to said terminator or (2) a replication system foreign to T5, wherein the direction of said promoter is away from said terminator and said marker is expressed at a frequency of less than about one-fourth the frequency of a structural gene, when said structural gene is inserted between said promoter and terminator, so as to be under the transcriptional control of said promoter and [bridging] to bridge said linear DNA sequence to provide a circular DNA sequence.

Cancel Claims 27, 28 and 29.

REMARKS

In view of the above amendments and the following remarks, the Examiner is respectfully requested to withdraw the rejections and allow Claims 1-26, the only claims in this application.

The courteous interview granted applicants' attorney is gratefully acknowledged.

In the interview it was agreed to clarify Claim 1 which appeared to be confusing concerning whether a linear or circular DNA sequence was being claimed. The circular aspect is a limitation on the linear DNA sequence defining a property of the linear DNA sequence when employed for expression. It was explained to the Examiner that the linear DNA sequence could be a product which was sold in the marketplace for use by others as a vector for expression of a gene of interest. Thus, the public would be provided with a readily available DNA sequence which they could use to insert a gene to be expressed, resulting in a circular plasmid which would then be used to transform a compatible host.

The question of the breadth of the terminator was considered. Applicants have in the application a terminator foreign to T5, namely the fd terminator, which is found to be operative with the T5 promoter. The means for selecting appropriate terminators is amply described in the specification, beginning at page 10, line 37. The insight that a terminator can affect the utility of a promoter is in part the basis for the subject invention in providing for the availability of strong T5 promoters for use in DNA constructs. The particular terminator is not crucial to the subject invention, so long as it is operative with the

promoter. Having shown that a terminator from a different phage can be used with the T5 strong promoter it is submitted that the general availability of balanced terminators for the strong promoters has been demonstrated. Furthermore, the T5 terminator would clearly be operable since T5 is viable and able to express its structural genes. Thus, applicants have two species which are useful in the subject invention and are believed to demonstrate the generality of terminators.

It was agreed that the art did not suggest the invention as claimed in Claims 1-26. These claims are limited to T5 phage and it was the insight of applicants to realize that these promoters could only be used with an appropriate terminator. Based on this insight they further established a technique for measuring the strength of promoters and terminators employing two structural genes, one prior to the terminators and one subsequent to the terminator in the direction of transcription. The only reference cited concerning the claims presently under consideration is Casadaban, which is an article co-authored by one of the subject co-inventors. As was pointed out to the Examiner, on page 194 of that article, there is an indication that only a few fragments from the T5 phage provided for expression. In order for a fragment to provide for expression, it would be necessary that the fragment have a promoter at its 3'-end. Thus, when the fragment was inserted in front of the structural gene present in the vector, the gene would be under the transcriptional control of such promoter. However, it was found in many cases that there was no expression and subsequently it was concluded that the terminator which was subsequent to the structural

gene present in the vector was not effective; thus, the strong promoter from the T5 phage was able to inhibit any expression of any structural gene. In effect, the resulting plasmid became a cryptic plasmid. Clearly, the reference does not teach the basis for the observed failures with the various fragments. So far as the few fragments that were operative, it is unlikely that they were strong promoters and it is submitted that the reference does not suggest the subject invention.

The amendments which have been made are not new matter, merely clarify language which was present in the claims, and are therefore believed to be appropriate and do not require citation of specific support in the specification.

In view of the above amendments and remarks, the application is considered in good and proper form for allowance, and the Examiner is respectfully requested to withdraw the rejections and pass this application to issue. Failing this, the Examiner is respectfully requested to enter the above amendments for purposes of further procedure.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 3/12/84

By Bertram Rowland
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 3/12/84
(Date of Deposit)

TOWNSEND & TOWNSEND

4 Date 3/12/84 By

Bertram I. Rowland
Reg. No. 20,015

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
[illegible]	05/20/81	BUJARD	H 5990-35

TOWNSEND & TOWNSEND
STEUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

EXAMINER	
KEPPLINGER, E.	
ART UNIT	PAPER NUMBER
125	12

DATE MAILED: 09 12 83

Below is a communication from the EXAMINER in charge of this application.

COMMISSIONER OF PATENTS, AND TRADEMARKS

ADVISORY ACTION

☑ THE PERIOD FOR RESPONSE ~~IS EXTENDED~~ CONTINUES TO RUN 3 MONTHS FROM THE DATE OF THE FINAL REJECTION. 855 O.G. 1109.

☐ Appellant's Brief is due in accordance with Rule 192 (a).

Applicant's response to the final rejection, filed _____, has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:

   a. ☐ There is no convincing showing under Rule 116(b).
   b. ☐ They raise new issues that would require further consideration and/or search.
   c. ☐ They raise the issue of new matter.
   d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☐ Upon the filing of an appeal, the proposed amendment ☐ will be ☐ will not be, entered and the status of the claims in this application would be as follows:

   a. ☐ Claims _____ would be allowable.

   b. ☐ Claims _____ would not be allowable.

   However:

   (1) ☐ The rejection of claims _____ on references is deemed to be overcome by applicant's response.
   (2) ☐ The rejection of claims _____ on non-reference grounds only is deemed to be overcome by applicant's response.

4. ☐ The affidavit, exhibit or request for reconsideration has been entered but does not overcome the rejection.

5. ☐ The affidavit or exhibit will not be admitted because applicant has not shown good and sufficient reasons why it was not earlier presented.

6. ☐ The application having been examined under the special accelerated examining procedure (M.P.E.P. 708.02), the proposed amendment has not been considered since it does not prima facie place the application in condition for allowance or in better condition for appeal.

PTOL-303 (Rev. 10-80)

THE PERIOD FOR RESPONSE CONTINUES TO RUN 3 MONTHS FROM THE DATE OF THE FINAL REJECTION. Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a) accompanied by the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee.

The amendment filed March 15, 1984 under 37 CFR 1.116 in response to the final rejection will be entered upon the filing of an appeal, but is not deemed to place the application in condition for allowance. Upon the filing of an appeal and entry of the amendment, the status of the claims would be as follows:

Allowed claims: 1-10, 17 and 23 -26

Rejected claims: 11-16 and 18-22

Claims objected to: -

The rejection of claims 11-16 and 18-22 as indefinite in the recitation of "balanced with said promoter" still stands. Also, the rejection of claims 11-16 and 18-22 over Casabadan et al stands. Applicants argue that the plasmids with T5 became cryptic and while a few fragments were operative, it is unlikely that the promoters were strong. On page 194

of Casabadan et al, plasmids containing T5 promoters and lac genes were formed which showed increased lac expression. Thus, the promoter functioned and it appears to be "strong". Since expression occurred, it appears that a terminator was present and was "balanced with the promoter".

Kepplinger:pw
A/C 703
557-3920
4/9/84

Esther M. Kepplinger

ESTHER M. KEPPLINGER
PRIMARY EXAMINER

TOWNSEND AND TOWNSEND
Steuart Street Tower
One Market Plaza
San Francisco, CA 94105
(415) 543-9600

Atty. Docket No. 5490-35

Date April 27, 1984

In re application of Hermann Bujard et al.

Serial No. 265,276

Filed May 20, 1981

For CLONED HIGH SIGNAL STRENGTH PROMOTERS

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 4/27/84
(Date of Deposit)

Date 4/28/84 By TOWNSEND & TOWNSEND
Bertram I. Rowland
Reg. No. 20,015

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

[ ] Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

[X] No additional fee is required.
X Extension Fee (1 month)
The fee has been calculated as shown below:

	(Col. 1)		(Col. 2)	(Col. 3)	SMALL ENTITY			OTHER THAN A SMALL ENTITY	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDIT. FEE	OR	RATE	ADDIT. FEE
TOTAL	*	MINUS	**	=	x5=	$		x10=	$
INDEP.	*	MINUS	***	=	x15=	$		x30=	$
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found from the equivalent box in Col. 1 of a prior amendment or the number of claims originally filed.

[ ] Please charge my Deposit Account No. 20-1430 in the amount of $ -0- . A duplicate copy of this sheet is attached.

[X] The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 20-1430. A duplicate copy of this sheet is attached.

[X] Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

[X] Any patent application processing fees under 37 CFR 1.17.

TOWNSEND & TOWNSEND

Bertram I. Rowland

Reg. No.: 20,015
Attorney of Record

T&T 37

GR 128 5-11-84 A. Thomas

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Hermann Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 128
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	EXTENSION FEE

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

The Commissioner is authorized to charge Deposit Account No. 20-1430 in the amount $50.00 for an extension fee for response within the first month.

The Commissioner is further authorized to charge any additional fees or credit any overpayment to Deposit Account No. 20-1430. This sheet is provided in triplicate for accounting purposes.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 4/27/84

By Bertram Rowland
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

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(Date of Deposit)

TOWNSEND & TOWNSEND

Date 4/27/84 By Bertram Rowland
Bertram I. Rowland
Reg. No. 20,015

TOWNSEND AND TOWNSEND
Steuart Street Tower
One Market Plaza
San Francisco, CA 94105
(415) 543-9600

Atty. Docket No. 5490-35

Date April 27, 1984

In re application of Herrmann, Bujard et al.

Serial No. 265,276

Filed May 20, 1981

For CLONED HIGH SIGNAL STRENGTH PROMOTERS

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

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(Date of Deposit)

Date 4/28/84 By TOWNSEND & TOWNSEND
Bertram I. Rowland
Reg. No. 20,015

Sir:

Transmitted herewith is an amendment in the above-identified application.

[ ] Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

[X] No additional fee is required.

X Extension Fee (1 month)

The fee has been calculated as shown below:

	(Col. 1)		(Col. 2)	(Col. 3)	SMALL ENTITY			OTHER THAN A SMALL ENTITY	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDIT. FEE	OR	RATE	ADDIT. FEE
TOTAL	*	MINUS	**	=	x5=	$		x10=	$
INDEP.	*	MINUS	***	=	x15=	$		x30=	$
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

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*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

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[X] Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

[X] Any patent application processing fees under 37 CFR 1.17.

TOWNSEND & TOWNSEND

Bertram I. Rowland

Reg. No.: 20,015
Attorney of Record

T&T 37

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Hermann Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 128
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	EXTENSION FEE

San Francisco, CA 94105

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

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The Commissioner is further authorized to charge any additional fees or credit any overpayment to Deposit Account No. 20-1430. This sheet is provided in triplicate for accounting purposes.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 4/22/84

By Bertram I. Rowland
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

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(Date of Deposit)

Date 4/22/84

TOWNSEND & TOWNSEND
By Bertram I. Rowland
Bertram I. Rowland
Reg. No. 20,015

TOWNSEND AND TOWNSEND
Steuart Street Tower
One Market Plaza
San Francisco, CA 94105
(415) 543-9600

Atty. Docket No. 5490-35

Date April 27, 1984

In re application of Hermann Bujard et al.

Serial No. 265,276

Filed May 20, 1981

For CLONED HIGH SIGNAL STRENGTH PROMOTERS

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(Date of Deposit)

Date 4/28/84 By TOWNSEND & TOWNSEND
Bertram I. Rowland
Reg. No. 20,015

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

[ ] Small entity status of this application under 37 CFR 1.9 and 1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 1.27 is enclosed.

[X] No additional fee is required.
X Extension Fee (1 month)
The fee has been calculated as shown below:

	(Col. 1) CLAIMS REMAINING AFTER AMENDMENT		(Col. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(Col. 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE	OR	OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
TOTAL	*	MINUS	**	=	x5=	$		x10=	$
INDEP.	*	MINUS	***	=	x15=	$		x30=	$
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

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*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

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[ ] Please charge my Deposit Account No. 20-1430 in the amount of $ -0- . A duplicate copy of this sheet is attached.

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[X] Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

[X] Any patent application processing fees under 37 CFR 1.17.

TOWNSEND & TOWNSEND

Bertram I. Rowland

Reg. No.: 20,015
Attorney of Record

T&T 37

5-12-84
A. Thomas
14/F

5490-35 MISC1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Hermann Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 128
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTERS	Second Amendment Under Rule 116
	San Francisco, CA 94105

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

In response to the Advisory Action of April 12, 1984, please make the following amendments:

Please cancel claims 11-16 and 18-22, without prejudice to renewal.

Please rewrite claim 17 as follows:

--27. A circular DNA sequence having a downstream order of transcription a strong T5 phage promoter, a structural gene foreign to T5 phage, a balance terminator, a marker for selection, and a replication system.--

--28. A circular DNA sequence according to claim 27, wherein said replication system is recognized by prokaryotes.--

--29. A circular DNA sequence according to claim 27, wherein said replication system is recognized by eukaryotes.--

Remarks

In view of the above amendments and the following remarks, the Examiner is earnestly requested to pass this application to issue, since all of the claims are now allowable.

Do not enter Ent 6/29/84

Rejected claims 11-16 and 18-22 have been cancelled. Claim 17 has been written in independent form, since it previously depended upon rejected and now cancelled claims. Since the claim was multiply dependent, it has been divided up into three claims which are identical in scope with claim 17 as originally filed.

In view of the above amendments and remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue.

If for any reason the Examiner feels that a telephone conference would in any way expedite prosecution of the subject application, the Examiner is invited to telephone the undersigned at (415) 493-2590.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 4/27/84

Bertram I. Rowland
Reg. No. 20,015

BIR:svh

(415) 493-2590

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 4/27/84
(Date of Deposit)

Date

By TOWNSEND & TOWNSEND

Bertram I. Rowland
Reg. No. 20,015

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
06/265,276	05/20/81	BUJARD H	5470-35

TOWNSEND & TOWNSEND
STUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

EXAMINER	
HEPPLINGER, E	
ART UNIT	PAPER NUMBER
128	15

DATE MAILED: 05/30/83

Below is a communication from the EXAMINER in charge of this application.

COMMISSIONER OF PATENTS, AND TRADEMARKS

ADVISORY ACTION

☑ THE PERIOD FOR RESPONSE ~~IS EXTENDED~~ CONTINUES TO RUN 4 MONTHS FROM THE DATE OF THE FINAL REJECTION. 855 O.G. 1109.

☐ Appellant's Brief is due in accordance with Rule 192 (a).

Applicant's response to the final rejection, filed _____, has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:
   a. ☐ There is no convincing showing under Rule 116(b).
   b. ☐ They raise new issues that would require further consideration and/or search.
   c. ☐ They raise the issue of new matter.
   d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.
2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.
3. ☐ Upon the filing of an appeal, the proposed amendment ☐ will be ☐ will not be, entered and the status of the claims in this application would be as follows:
   a. ☐ Claims _____ would be allowable.
   b. ☐ Claims _____ would not be allowable.

   However:

   (1) ☐ The rejection of claims _____ on references is deemed to be overcome by applicant's response.
   (2) ☐ The rejection of claims _____ on non-reference grounds only is deemed to be overcome by applicant's response.
4. ☐ The affidavit, exhibit or request for reconsideration has been entered but does not overcome the rejection.
5. ☐ The affidavit or exhibit will not be admitted because applicant has not shown good and sufficient reasons why it was not earlier presented.
6. ☐ The application having been examined under the special accelerated examining procedure (M.P.E.P. 708.02), the proposed amendment has not been considered since it does not prima facie place the application in condition for allowance or in better condition for appeal.

PTOL-303 (Rev. 10-80)

THE PERIOD FOR RESPONSE CONTINUES TO RUN FOUR MONTHS FROM THE DATE OF THE FINAL REJECTION. Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a) accompanied by the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee.

The amendment filed April 30, 1984 under 37 CFR 1.116 in response to the final rejection has been considered but is not deemed to place the application in condition for allowance and will not be entered because:

The proposed amendment raises new issues that would require further consideration and/or search.

The directed claims 27-29 cannot be entered since claims 27-30 were previously in the application and cancelled. Moreover, the claims would be rejected over Casabadan et al as set forth relating to claims 27-29 in the final rejection, Paper No. 9. The proposed cancellation of claims 11-16 and 18-22 would, however, eliminate some rejections if resubmitted.

The proposed amendment is not deemed to place the application in better form for appeal by materially simplifying the issues for appeal.

Applicant is advised that this application and the art to which this application pertains has been transferred to another group. All further papers submitted in this application should carry the following items:

1. Serial Number (checked for accuracy).
2. Group Art Unit 128.
3. Name of Examiner now in charge of this application Esther Kepplinger.
4. Filing date.
5. Title of invention.

Any inquiry concerning this communication should be directed to Examiner Kepplinger at telephone number 703-557-3920.

Kepplinger:rt
A/C 703
557-3920
5/23/84

Esther M. Kepplinger
ESTHER M. KEPPLINGER
PRIMARY EXAMINER
ART UNIT 128

Pg. 128
6-26-84
A. Thomas
#16

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Hermann Bujard et al. Serial No. 265,276 Filed: May 20, 1981 For: CLONED HIGH SIGNAL STRENGTH PROMOTER	Examiner: E. Kepplinger Art Unit: 128 FEE FOR EXTENSION OF TIME

RECEIVED
GROUP 120

San Francisco, CA 94105

Commissioner of Patents of Trademarks

Washington, D.C. 20231

Sir:

The Commissioner is authorized to charge $150.00 to Deposit Account No. 20-1430 for an extension of time to file the enclosed response within the second month.

The Commissioner is further authorized to charge any additional fee or credit any overpayment related to filing the enclosed response to Deposit Account No. 20-1430. This sheet is provided in triplicate for accounting purposes.

Respectfully submitted,

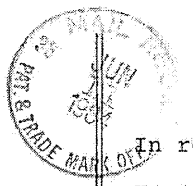
TOWNSEND and TOWNSEND

Date 6/8/84

By _____
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

P2284 06/15/84 265276 20-1430 2 116 150.00CH



5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	)	
	)	
Hermann Bujard et al.	)	Examiner: E. Kepplinger
	)	
Serial No. 265,276	)	Art Unit: 128
	)	
Filed: May 20, 1981	)	
	)	
For: CLONED HIGH SIGNAL STRENGTH PROMOTER	)	FEE FOR EXTENSION OF TIME
	)	

San Francisco, CA 94105

Commissioner of Patents of Trademarks

Washington, D.C. 20231

Sir:

The Commissioner is authorized to charge $150.00 to Deposit Account No. 20-1430 for an extension of time to file the enclosed response within the second month.

The Commissioner is further authorized to charge any additional fee or credit any overpayment related to filing the enclosed response to Deposit Account No. 20-1430. This sheet is provided in triplicate for accounting purposes.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 6/8/84

By _____
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

6-26-84
A. Thomas
17/84

5490-35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Hermann Bujard et al.	Examiner: E. Kepplinger
Serial No. 265,276	Art Unit: 128
Filed: May 20, 1981	
For: CLONED HIGH SIGNAL STRENGTH PROMOTER	THIRD AMENDMENT UNDER RULE 116

San Francisco, CA 94105

Commissioner of Patents of Trademarks

Washington, D.C. 20231

Sir:

OK to enter
Amdt
6/28/84

In response to the Advisory Action of May 30, 1984, please amend the subject application as follows:

IN THE CLAIMS:

Cancel Claims 11-16 and 18-22, without prejudice.

REMARKS

Cancellation of the above claims places the subject application in condition for allowance, Claims 1-10, 17 and 23-26, the only remaining claims in this application, having been allowed. Newly submitted Claims 27-29 (misnumbered) are not to be considered. The Examiner is earnestly requested to pass this application to issue.

Respectfully submitted,

TOWNSEND and TOWNSEND

Date 6/8/84

By Bertram I. Rowland
Bertram I. Rowland
Reg. No. 20,015

BIR/gs

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 6/8/84
(Date of Deposit)

TOWNSEND & TOWNSEND

Date 6/8/84 By Bertram I. Rowland
Bertram I. Rowland
Reg. No. 20,015

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
265276	5/20/81	Bujard et al	5490-35

EXAMINER	
E. Kepplinger	
ART UNIT	PAPER NUMBER
123	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Mr. Bertram Rowland (3) _____

(2) _____ (4) _____

Date of interview August 1, 1984

Type: ☑ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☑ No. If yes, brief description: _____

Agreement ☑ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: 17

Identification of prior art discussed: ———

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: We agreed to changes to make the claim independent. See Examiner's amendment.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1—7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate reocrd of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Esther M. Kepplinger
Examiner's Signature

81-3679 PTOL-413 (rev. 1-81)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
265276			

EXAMINER	
ART UNIT	PAPER NUMBER
	19

DATE MAILED:

This is a communication from the examiner in charge of your application

COMMISSIONER OF PATENTS AND TRADEMARKS

1. [X] THIS IS AN ATTACHMENT TO THE NOTICE OF ALLOWANCE AND BASE ISSUE FEE DUE, PTOL 85.
2. [X] All the claims being allowable, PROSECUTION ON THE MERITS IS CLOSED in this application. If not attached hereto, a Notice of Allowance or other appropriate communication will be sent in due course.

   A. [ ] Note the attached PTO-152, Notice of Informality, which indicates that the declaration (or oath) is deficient and that a substitute is required. The substitute declaration (or oath) MUST BE SUBMITTED WITHIN THE THREE MONTH STATUTORY PERIOD SET FOR PAYMENT OF THE BASE ISSUE FEE IN THE "NOTICE OF ALLOWANCE AND BASE ISSUE FEE DUE" (PTOL-85), preferably with and attached to the base issue fee. Note that the statute does not permit extension of the three month period set for payment of the base issue fee. Failure to timely file the substitute declaration (or oath) will result in ABANDONMENT of the application. The transmittal letter accompanying the declaration (or oath) should indicate the following in the upper right hand corner:
   Issue Batch Number; Date of the Notice of Allowance, and Serial Number.

   B. [ ] Formal drawings are now required and MUST BE SUBMITTED WITHIN THE THREE MONTH STATUTORY PERIOD SET FOR PAYMENT OF THE BASE ISSUE FEE IN THE "NOTICE OF ALLOWANCE AND BASE ISSUE FEE DUE" (PTOL-85). Note that the statute does not permit extension of the three month period set to pay the base issue fee. Failure to timely submit the drawings will result in ABANDONMENT of the application. The drawings should be submitted as a separate paper with a transmittal letter which is addressed to the Official Draftsman and which indicates the following in the upper right hand corner:
   Issue Batch Number; Date of the Notice of Allowance, and Serial Number.

   C. [X] The claims are allowed in view of:
      a. [X] Applicant's communication filed 3/15/84 and 6/11/84.
      b. [X] The interview summarized on the attached EXAMINER INTERVIEW SUMMARY RECORD, PTOL-413.
      c. [X] The attached Examiner's Amendment.
      d. [ ] An Examiner's Amendment which will follow in due course.

   D. [X] The allowed claims are 1-10, 17 and 23-26

3. [ ] Note the attached Examiner's Statement of Reasons for Allowance.
4. [ ] Note attached NOTICE OF REFERENCES CITED, PTO-892, which is part of this communication. The listed references are considered to be pertinent to the claimed invention, but the claims are deemed to be patentable thereover.
5. [ ] Note attached LIST OF ART CITED BY APPLICANT, PTO-1449.
6. [ ] The drawings filed on _____ [ ] are acceptable as filed. [ ] are acceptable subject to correction as indicated on the attached Notice re Drawings, PTO-948. In order to avoid ABANDONMENT of this application, correction is required. Corrections can only be made in accordance with the instructions set forth in the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
7. [ ] The [ ] proposed drawing correction and/or the [ ] proposed additional or substitute sheet(s) of drawings filed on _____ has (have) been approved by the examiner. Applicant is reminded that in order to avoid abandonment of this applicant, execution of the proposed changes or submission of additional or substitute drawings MUST be made in accordance with the instructions set forth in the letter, "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474, attached to Paper No. _____.
8. [ ] The proposed drawing correction, filed _____, has been approved. However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections are required and MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
9. [ ] In order to avoid ABANDONMENT, the drawing informalities noted on the Notice re Drawing, PTO-948, attached to Paper No. _____, must now be corrected. Applicant is reminded that the corrections can only be made in accordance with the instructions set forth in the letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474, attached to the PTO-948.
10. [ ] Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has; [ ] been received. [ ] not been received.

    [ ] been filed in parent application, Serial No. _____ filed on _____.

Esther M. Kepplinger

ESTHER M. KEPPLINGER
PRIMARY EXAMINER
ART UNIT 128

PTOL - 37 (Rev. 8 - 82)

NOTICE OF ALLOWABILITY

In claim 17, line 1, delete "according".

In line 2, deleted "to any of claims 11,12, or 13, "and inserted--having in downstream order of transcription a strong T5 phage promoter, a structural gene foreign to T5 phage under transcriptional control of said promoter, a transcriptional terminator which is balanced with said promoter and a replication system and--.

H 1

The above change was authorized by applicant's attorney, Bertram Rowland, in a telephone interview on August 1, 1984.

Kepplinger:cvm
A/C 703
557-3920
8/2/84

Esther M. Kepplinger

ESTHER M. KEPPLINGER
PRIMARY EXAMINER
ART UNIT 128

PTOL-85 (Rev. 8-82)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

TOWNSEND & TOWNSEND
STEUART STREET TOWER
ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

All communications regarding this application should give the serial number, date of filing, name of applicant, and batch number.

Please direct all communications to the Attention of "OFFICE OF PUBLICATIONS" unless advised to the contrary.

The application identified below has been examined and found allowable for issuance of Letters Patent. PROSECUTION ON THE MERITS IS CLOSED.

	SC/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT		DATE MAILED
	06/345,276	05/20/81	014	KEPPLINGER, E	128	08/09/84

First Named Applicant: BUJARD, HERMANN G.

TITLE OF INVENTION: CLONED HIGH SIGNAL STRENGTH PROMOTERS

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
	5490-35	435-006.000	C12	UTILITY	NO	$500.00	11/09/84

The amount of the issue fee is specified by 37 C.F.R. 1.18 as follows: for an original or reissue patent, except for a design or plant patent, $500; for a design patent, $175; and for a plant patent, $250. If the applicant qualifies for and has filed a verified statement of small entity status in accordance with 37 C.F.R. 1.27, the issue fee is one-half the respective amount aforementioned. The issue fee due printed above reflects applicant's status as of the time of mailing this notice. A verified statement of small entity status may be filed prior to or with payment of the issue fee. However, in accordance with 37 C.F.R. 1.28, failure to establish status as a small entity prior to or with payment of the issue fee precludes payment of the issue fee in the amount so established for small entities and precludes a refund of any portion thereof paid prior to establishing status as a small entity.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE as indicated above. The application shall otherwise be regarded as ABANDONED. The issue fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office. Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of the notice of allowance, the issue fee is charged to the deposit account at the time of mailing of this notice in accordance with 37 C.F.R. 1.311. If the issue fee has been so charged, it is indicated above.

In order to minimize delays in the issuance of a patent based on this application, **this Notice may have been mailed prior to completion of final processing.** The nature and/or extent of the remaining revision or processing requirements may cause slight delays of the patent. In addition, **if prosecution is to be reopened, this Notice of Allowance will be vacated and the appropriate Office action will follow in due course.** If the issue fee has already been paid and prosecution is reopened, the applicant may request a refund or request that the fee be credited to a Deposit Account. However, applicant may wait until the application is either found allowable or held abandoned. If allowed, upon receipt of a new Notice of Allowance, applicant may request that the previously submitted issue fee be applied. If abandoned, applicant may request refund or credit to a Deposit Account.

In the case of each patent issuing without an assignment, the complete post office address of the inventor(s) will be printed in the patent heading and in the Official Gazette. If the inventor's address is now different from the address which appears in the application, please fill in the information in the spaces provided on PTOL-85b enclosed. If there are address changes for more than two inventors, enter the additional addresses on the reverse side of the PTOL-85b.

The appropriate spaces in the ASSIGNMENT DATA section of PTOL-85b must be completed in all cases. If it is desired to have the patent issue to an assignee, an assignment must have been previously submitted to the Patent and Trademark Office or must be submitted not later than the date of payment of the issue fee as required by 37 C.F.R. 1.334. Where there is an assignment, the assignee's name and address must be provided on the PTOL-85b to ensure its inclusion in the printed patent.

Advance orders for 10 or more printed copies of the prospective patent can be made by completing the information in Section 4 of PTOL-85b and submitting payment therewith. If use of a Deposit Account is being authorized for payment, PTOL-85c should also be forwarded. The order must be for at least 10 copies and must accompany the issue fee. The copies ordered will be sent only to the address specified in section 1 or 1A of PTOL-85b.

[X] Note attached communication from Examiner.

[ ] This notice is issued in view of applicant's communication filed _____

PATENT AND TRADEMARK OFFICE COPY

IMPORTANT

ATTENTION IS DIRECTED TO 37 C.F.R. 1.334

THE PATENT WILL ISSUE TO APPLICANT UNLESS AN ASSIGNEE IS SHOWN IN ITEM 3 ON FORM PTOL-85b, ATTACHED

PTOL-85b (Rev. 8-82)

U.S. Department of Commerce
Patent and Trademark Office

B

ISSUE FEE TRANSMITTAL

This form is provided in lieu of a formal transmittal and should be used for transmitting the Issue Fee. Sections 1A through 4 must be completed as appropriate.

INVENTOR(S) ADDRESS CHANGE | SC/SERIAL NO.

INVENTOR'S NAME

Street Address

City, State and Zip Code

CO-INVENTOR'S NAME

Street Address

City, State and Zip Code

☐ Check if additional changes are on reverse side.

MAIL ROOM OCT 26 1984 PAT. & TRADEMARK OFF.

MAILING INSTRUCTIONS

All further correspondence including the Issue Fee Receipt, the Patent, and advanced orders will be mailed to the addressee entered in section 1 on PTOL-85c, unless you direct otherwise by specifying the appropriate name and address in 1A below.

2A. The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified below.

TOWNSEND and TOWNSEND

(Signature of party in interest of record) — Bertram I. Rowland, Reg. 20,015 — (Date) 10/24/84

Note: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

	SC/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT		DATE MAILED
	06/265,276	05/20/81	014	KEPPLINGER, E	128	08/09/84
First Named Applicant	BUJARD,		HERMANN G.			

TITLE OF INVENTION: CLONED HIGH SIGNAL STRENGTH PROMOTERS

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
	5420-35	435-006.000	C12	UTILITY	Yes	~~$340.00~~ $250.-	11/09/84

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 10/24/84 (Date of Deposit)

TOWNSEND & TOWNSEND

Date 10/24/84 By _____

Bertram I. Rowland
Reg. No. 20,015

2B. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR, alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed.

1 Bertram I. Rowland
2
3

USE THIS SPACE

3. ASSIGNMENT DATA (print or type)

50422 10/30/84 265276

A. (1) ☐ This application is NOT assigned.
(2) ☒ Assignment previously submitted to the Patent and Trademark Office.
(3) ☐ Assignment submitted herewith.

B. For Printing On The Patent: (Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data below is only appropriate when an assignment has been previously submitted to the PTO or is submitted herewith. Completion of this form is NOT a substitute for filing of an assignment as required by 37 C.F.R. 1.334).

(1) NAME OF ASSIGNEE: The Board of Trustees of the Leland Stanford Jr. University

(2) ADDRESS: (City & State or Country)
Stanford, California

(3) STATE OF INCORPORATION, IF ASSIGNEE IS A CORPORATION: California

4.

The following fees are enclose:

☐ Issue fee 01630 1 ☐ Advanced order 500 ☐ Assignment recording
20-1430 1 501 10.00CH

The following fees should be charged to deposit acc. no. 20-1430
(PTOL-85c or additional copy of PTOL-85b must be enclosed)

☒ Issue fee
☒ Advanced order
☐ Assignment recording

Number of advanced order copies requested. 10
(must be for 10 or more copies)

TRANSMIT THIS FORM WITH FEE

5490-35 #040

Applic· Patentee: HERMANN . BUJARD ET AL Docket No. S81-17
Serial or Patent No.: 265,276
Filed or Issued: May 20, 1981
For: "CLONED HIGH-SIGNAL STRENGTH PROMOTERS"

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS [37 CFR 1.9(f) and 1.27(d)] — NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: STANFORD UNIVERSITY
ADDRESS OF ORGANIZATION: 105 Encina Hall, Stanford, CA 94305

TYPE OF ORGANIZATION
[X] UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
[ ] TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(a) and 501(c) (3)]
[ ] NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OR THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
[ ] WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(a) and 501(c) (3)] IF LOCATED IN THE UNITED STATES OF AMERICA
[ ] WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention entitled Cloned High-Signal Strength Promoters

by inventor(s) Hermann G. Bujard, Annie Chang, and Stanley Cohen

described in
[ ] the specification filed herewith
[XX] application serial no. 265,276, filed May 20, 1981
[ ] Patent no. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC §1001, and may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Katharine Ku
TITLE IN ORGANIZATION Associate Director, Technology Licensing
ADDRESS OF PERSON SIGNING 105 Encina Hall, Stanford, CA 94305

SIGNATURE Katharine Ku DATE 10/17/84

T&T 36

FORM PTO-875
(REV. 11-77)

U.S. DE[...]MENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

PATENT APPLICATION FEE DETERMINATION RECORD

SERIAL NUMBER	FILING DATE
265276	5-20-81

APPLICANT (*First Named Only*)

Bujard et al

CLAIMS AS FILED - PART I

(1) FOR	(2) NUMBER FILED		(3) NUMBER FILED		(4) RATE	(5) BASIC FEE $65.00
TOTAL CLAIMS	44	-10=	34	=	x $2.00	68
INDEPENDENT CLAIMS	3	- 1=	2	=	x $10.00	20
					TOTAL FILING FEE →	153

CLAIMS AS AMENDED - OART II

	(1)	(2) CLAIMS REMAINING AFTER AMENDMENT	(3)	(4) HIGHEST NO. PERVIOUSLY PAID FOR	(5) PRESENT EXTRA	(6) RATE	(7) ADDITIONAL FEE
AMENDMENT	TOTAL	* 14	MINUS	**	=	x $2	=
	INDEP.	* 1	MINUS		=	x $10	=
					TOTAL ADDITIONAL FEE FOR THIS AMENDMENT →		
AMENDMENT	TOTAL	*	MINUS	**	=	x $2	=
	INDEP.	*	MINUS		=	x $10	=
					TOTAL ADDITIONAL FEE FOR THIS AMENDMENT →		
AMENDMENT	TOTAL	*	MINUS	**	=	x $2	=
	INDEP.	*	MINUS		=	x $10	=
					TOTAL ADDITIONAL FEE FOR THIS AMENDMENT →		
AMENDMENT	TOTAL	*	MINUS	**	=	x $2	=
	INDEP.	*	MINUS	**	=	x $10	=
					TOTAL ADDITIONAL FEE FOR THIS AMENDMENT →		

* If the entry in Column 2 is less than the entry in Column 4, write "0" in Column 5.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 10, write "10" in this space.

The "Highest Number Previously Paid For" ("Total" or "Indep.") is the highest number found in the appropriate box in Column 2.

USCOMM-DC 3987-P79

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

(FOR USE WITH FORM PTO-875)

SERIAL NO. 265276 | FILING DATE 5-20-81

APPLICANT(S). Bujard et al

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT			*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.		IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1						51						
2		1					52						
3		1					53						
4		3					54						
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~~12~~		~~1~~					62						
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~~18~~		~~1~~					68						
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46							96						
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48							98						
49							99						
50							100						
TOTAL IND.	3						TOTAL IND.						
TOTAL DEP.		41					TOTAL DEP.						
TOTAL CLAIMS	44						TOTAL CLAIMS						

PTO-1360 (3-78)

*MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS

U.S. DEPARTMENT of COMMERCE
Patent and Trademark Office

PTO-1130 (rev. 9-79)

PALM III APPLICATION FILE DATA CODING SHEET

U.S. DEPARTMENT of COMMERCE-Pat. & TM Off.

PREPARED BY 417 DATE 7-14-81

FORMAT NO. 2 / SERIAL NO.	TYPE APPL	FILING DATE Month	Day	Year	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	SECURITY CASE?
06 265276	1	05	20	81		172	435	1	22	3	153	N

FORMAT NO. 3	ATTORNEY DOCKET NUMBER (12 spaces)	Atty's. Reg. Nos.	FORMAT NO. 4 - Applicant's Name & Address	FORMAT NO. 5- Title of Invention	FORMAT NO. 6 & 7 Correspondence Address
	54 90 -35	☐ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 8

		CONTINUITY CODE	PARENT APPLICATION SERIAL NUMBER	PARENT FILING DATE Month Day Year	STATUS CODE	PARENT PATENT NUMBER
RECORD	801		0			
RECORD	802		0			
RECORD	803		0			
RECORD	804		0			
RECORD	805		0			
RECORD	806		0			
RECORD	807		0			
RECORD	808		0			
RECORD	809		0			
☐ MORE ON SUPPLEMENTAL CODING SHEET RECORD	810		0			

FORMAT NO. 9

		COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FILING DATE Month Day Year
FOREIGN PRIORITY CLAIMED? ☐ YES ☐ NO RECORD	901			
RECORD	902			
RECORD	903			
RECORD	904			
RECORD	905			
RECORD	906			
☐ APPLICATION PAPERS RECORD	907			
RECORD	908			
RECORD	909			
☐ MORE ON SUPPLEMENTAL CODING SHEET RECORD	910			

FORMAT NO. 3 ATTORNEY'S REGISTRATION NO.(S)

RECORD 301

No.1 No.2 No.3 No.4 No.5 No.6 No.7

RECORD 302

No.1 No.2 No.3 No.4 No.5 No.6 No.7

RECORD 303

No.1 No.2 No.3 No.4 No.5 No.6 No.7

☐ MORE ON SUPPLEMENTAL CODING SHEET

FORMAT NO. 4 APPLICANT(S) NAME AND ADDRESS

RECORD 4 0 1 AUTHORITY FAMILY NAME *(25 spaces)*

GIVEN NAMES *(25 spaces)* NAME–SUFFIX

CITY *(15 spaces)* STATE/COUNTRY

RECORD 4 0 2 AUTHORITY FAMILY NAME *(25 spaces)*

GIVEN NAMES *(25 spaces)* NAME–SUFFIX

CITY *(15 spaces)* STATE/COUNTRY

☐ MORE ON SUPPLEMENTAL CODING SHEET

FORMAT NO. 5 TITLE OF INVENTION *(70 spaces)*

RECORD 501

RECORD 502

RECORD 503

RECORD 504

FORMAT NO. 6 CORRESPONDENCE ADDRESS *(40 spaces)*

RECORD 601

RECORD 602

FORMAT NO. 7

RECORD 701

RECORD 702

RECORD 703

In vivo site-specific genetic recombination promoted by the EcoRI restriction endonuclease.
Chang, S. ; Cohen, S.N.
(Dep. Med. and Genet., Stanford Univ. Sch. Med., Stanford, CA 94305, USA)
Proc. Natl. Acad. Sci. USA 74(11), 4811-4815, 1977

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$8.06 Estimated Total Cost

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ESCHERICHIA COLI PLASMID PHL 24
BEIERSTORF K; VON GABAIN A; BUJARD H
HOPPE-SEYLER'S Z PHYSIOL CHEM 357 (3). 1976 308 Coden: HSZPA

? t23/3/1-7

23/3/1
22001837
CLONED DNA ENCODING THE STRUCTURE FOR THE BOVINE ACTH BETA LIPOTROPIN PRECURSOR PROTEIN
COHEN S N; CHANG A C Y; SHIGETADA; NAKANISHI; INOUE A; KITA T; NAKAMURA M ; NUMA S
DEP. GENET., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
ZIMMERMAN, M., R. A. MUMFORD AND D. F. STEINER (ED.). ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, VOL. 343. PRECURSOR PROCESSING IN THE BIOSYNTHESIS OF PROTEINS; SYMPOSIUM, NEW YORK, N.Y., USA, MAY 2-4, 1979. IX+449P. NEW YORK ACADEMY OF SCIENCES: NEW YORK, N.Y., USA. ILLUS. PAPER. ISBN 0-89766-073-0(PAPER); ISBN 0-89766-072-2(CLOTH). 0 (0). 1980. P415-424. Coden: ANYAA

23/3/2
72066269
CLONING AND EXPRESSION IN STREPTOMYCES-LIVIDANS OF ANTIBIOTIC RESISTANCE GENES DERIVED FROM ESCHERICHIA-COLI
SCHOTTEL J L; BIBB M J; COHEN S N
DEP. OF GENETICS, STANFORD UNIV. SCH. OF MED., STANFORD, CALIF. 94305.
J BACTERIOL 146 (1). 1981. 360-368. Coden: JOBAA

23/3/3
71009999
STRUCTURAL ORGANIZATION OF HUMAN GENOMIC DNA ENCODING THE PRO OPIOMELANOCORTIN PEPTIDE
CHANG A C Y; COCHET M; COHEN S N
DEP. GENET. MED., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
PROC NATL ACAD SCI U S A 77 (8). 1980. 4890-4894. Coden: PNASA

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20014592
THE DEVELOPMENT OF A CLONING SYSTEM FOR STREPTOMYCES
BIBB M J; SCHOTTEL J L; COHEN S N
DEP. OF GENETICS, STANFORD UNIV. SCH. OF MED., STANFORD, CA 94305.
BIOL ACTINOMYCETES RELAT ORG 15 (1). 1980. 35-46. Coden: BAORD

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70078074
IN-VITRO GENE FUSIONS THAT JOIN AN ENZYMATICALLY ACTIVE BETA GALACTOSIDASE SEGMENT TO AMINO TERMINAL FRAGMENTS OF EXOGENOUS PROTEINS ESCHERICHIA-COLI PLASMID VECTORS FOR THE DETECTION AND CLONING OF TRANSLATIONAL INITIATION SIGNALS
CASADABAN M J; CHOU J; COHEN S N
DEP BIOPHYS. THEOR. BIOL., UNIV. CHIC., CHICAGO, ILL. 60637, USA.
J BACTERIOL 143 (2). 1980. 971-980. Coden: JOBAA

23/3/6
70064246
A DNA CLONING SYSTEM FOR INTERSPECIES GENE TRANSFER IN ANTIBIOTIC PRODUCING STREPTOMYCES-SPP
BIBB M; SCHOTTEL J L; COHEN S N
DEP. GENET., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
NATURE (LOND) 284 (5756). 1980. 526-531. Coden: NATUA

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70023276
ANALYSIS OF GENE CONTROL SIGNALS BY DNA FUSION AND CLONING IN ESCHERICHIA-COLI
CASADABAN M J; COHEN S N

DEP. BIOPHYS. THEOR. BIOL., UNIV. CHIC., CHICAGO, ILL. 60637, USA.
J MOL BIOL 138 (2). 1980. 179-208. Coden: JMOBA

? t23/3/8-14

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19045494
EXPERIMENTAL TECHNIQUES AND STRATEGIES FOR DNA CLONING
COHEN S N
DEP. GENET., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
MORGAN, J. AND W. J. WHELAN (ED.). RECOMBINANT DNA AND GENETIC EXPERIMENTATION; PROCEEDINGS OF A CONFERENCE, KENT, ENGLAND, APR. 1-4, 1979. XXIV+309P. PERGAMON PRESS, INC.: NEW YORK, N.Y., USA; OXFORD, ENGLAND. ILLUS. ISBN 0-08-024427-0. 0 (0). 1979 (RECD. 1980). P49-52. Coden: 08752

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18003738
CLONING AND ENDO NUCLEASE MAPPING OF THE HEPATITIS B VIRAL GENOME
SNINSKY J J; SIDDIQUI A; ROBINSON W S; COHEN S N
DEP. MED., STANFORD UNIV., SCH. MED., STANFORD, CALIF. 94305, USA.
NATURE (LOND) 279 (5711). 1979. 346-348. Coden: NATUA

23/3/10
68047599
NUCLEOTIDE SEQUENCE OF CLONED COMPLEMENTARY DNA FOR BOVINE CORTICOTROPIN BETA LIPOTROPIN PRECURSOR
NAKANISHI S; INOUE A; KITA T; NAKAMURA M; CHANG A C Y; COHEN S N; NUMA S
DEP. MED. CHEM., FAC. MED., KYOTO UNIV., KYOTO 606, JPN.
NATURE (LOND) 278 (5703). 1979. 423-427. Coden: NATUA

23/3/11
67073251
INSTABILITY OF PLASMID DNA SEQUENCES MACRO AND MICRO EVOLUTION OF THE ANTIBIOTIC RESISTANCE PLASMID R-6-5
TIMMIS K N; CABELLO F; ANDRES I; NORDHEIM A; BURKHARDT H J; COHEN S N
MAX-PLANCK-INST. MOL. GENET., IHNESTR. 63-73, D-1000 BERLIN 33, W. GER.
MOL GEN GENET 167 (1). 1978. 11-20. Coden: MGGEA

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67066667
STRUCTURAL AND FUNCTIONAL ANALYSIS OF CLONED DNA SEGMENTS CONTAINING THE REPLICATION AND INCOMPATIBILITY REGIONS OF A MINI PLASMID DERIVED FROM A COPY NUMBER MUTANT OF NR-1
TAYLOR D P; COHEN S N
DEP. GENET., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
J BACTERIOL 137 (1). 1979. 92-104. Coden: JOBAA

23/3/13
67054054
CONSTRUCTION OF BACTERIAL PLASMIDS THAT CONTAIN THE NUCLEOTIDE SEQUENCE FOR BOVINE CORTICOTROPIN BETA LIPOTROPIN PRECURSOR
NAKANISHI S; INOUE A; KITA T; NUMA S; CHANG A C Y; COHEN S N; NUNBERG J; SCHIMKE R T
DEP. MED. CHEM., KYOTO UNIV. FAC. MED., YOSHIDA, SAKYO, KYOTO 606, JPN.
PROC NATL ACAD SCI U S A 75 (12). 1978 (RECD. 1979). 6021-6025. Coden: PNASA

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67002630
CLONING AND CHARACTERIZATION OF ECO-R-I AND HIN-D-III RESTRICTION ENDO NUCLEASE GENERATED FRAGMENTS OF ANTIBIOTIC RESISTANCE PLASMIDS R-6-5 AND R-6
TIMMIS K N; CABELLO F; COHEN S N
MAX-PLANCK-INST. MOL. GENET., IHNESTR. 63-73, D-1000 BERLIN 33, W. GER.

MAX-PLANCK-INST. MOL. GENET.; INVESTR. 63-73; D-1000 BERLIN 33; W. GER.
MOL GEN GENET 162 (2). 1978 121-138. Coden: MGGEA

? t23/3/15-22

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16057919
DNA CLONING AND THE ANALYSIS OF PLASMID STRUCTURE AND FUNCTION
TIMMIS K N; COHEN S N; CABELLO F C
HAHN, F. E. ET AL. (ED.). PROGRESS IN MOLECULAR AND SUB CELLULAR BIOLOGY, VOL. 6. XI+354P. ILLUS. SPRINGER-VERLAG: BERLIN, WEST GERMANY; NEW YORK, N.Y., USA. ISBN 3-540-08588-2; ISBN 0-387-08588-2. 1978 1-58 Coden: 07001

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16010722
CLONING OF HIN-D-III AND ECO-R-I RESTRICTION ENDO NUCLEASE FRAGMENTS OF THE R-6-5 PLASMID BY INSERTIONAL INACTIVATION
CABELLO F; TIMMIS K; COHEN S N
SCHLESSINGER, DAVID (ED.). MICROBIOLOGY (WASHINGTON, D.C.)-1978. 2ND ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE ON EXTRACHROMOSOMAL ELEMENTS, JACKSON, WYO., FEB. 20-25, 1977; ASM CONFERENCE ON NITRIFICATION AND NITROGEN OXIDES ATHENS, GA., SEPT. 7-10, 1976; ANNUAL MEETING OF THE

ASM, NEW ORLEANS, LA., MAY 11, 1977. X+449P. ILLUS. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ISBN 0-914826-15-8. 1978 42-44 Coden: 06674

23/3/17
66039533
A SENSITIVE RADIO IMMUNOASSAY FOR DETECTING PRODUCTS TRANSLATED FROM CLONED DNA FRAGMENTS
ERLICH H A; COHEN S N; MCDEVITT H O
DIV. IMMUNOL., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
CELL 13 (4). 1978 681-690. Coden: CELLB

23/3/18
66039386
CONSTRUCTION AND CHARACTERIZATION OF AMPLIFIABLE MULTI COPY DNA CLONING VEHICLES DERIVED FROM THE P-15A CRYPTIC MINI PLASMID
CHANG A C Y; COHEN S N
DEP. MED., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305, USA.
J BACTERIOL 134 (3). 1978 1141-1156. Coden: JOBAA

23/3/19
15020305
RECENT ADVANCES IN THE MOLECULAR BIOLOGY OF DNA CLONING
KRETSCHMER P J; COHEN S N
PROC AUST BIOCHEM SOC 10. 1977 Q1 Coden: ABIPB

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15001933
A SENSITIVE RADIO IMMUNOASSAY FOR DETECTING TRANSLATION PRODUCTS OF FOREIGN DNA FRAGMENTS CLONED INTO PLASMID OR PHAGE VECTORS
ERLICH H A; COHEN S N
FED PROC 37 (6). 1978 1376 Coden: FEPRA

23/3/21
78055117
DNA CLONING AS A TOOL FOR THE STUDY OF PLASMID BIOLOGY
COHEN S N; CABELLO F; CHANG A C Y; TIMMIS K
BEERS, ROLAND F. AND EDWARD G. BASSETT (ED.). MILES INTERNATIONAL SYMPOSIUM SERIES, NO. 10. RECOMBINANT MOLECULES. IMPACT ON SCIENCE AND SOCIETY. CAMBRIDGE, MASS., USA. XVI+540P. ILLUS. RAVEN PRESS: NEW YORK, N.Y., USA. ISBN 0-89004-131-8. 1977 91-105 Coden: 06313

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2573722
63008014
  REPLICATION REGION FRAGMENTS CLONED FROM F-LAC-PLUS ARE IDENTICAL TO
ECO-R-I FRAGMENT F-5 OF PLASMID F
  SKURRAY R A; GUYER M S; TIMMIS K; CABELLO F; COHEN S N; DAVIDSON N; CLARK
A J
  J BACTERIOL  127 (3). 1976 1571-1575.  Coden: JOBAA

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  $7.43  Estimated Total Cost


File55:BIOSIS Previews - 1969 thru 1976
(Corr. Biological Abstracts Inc.)
        Set Items Description
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             35379 DNA
                 0 DEOXYRIBONUCLEIC
          1 35379 DNA OR DEOXYRIBONUCLEIC

          2  4429 VECTOR? ?
              974 PLASMID? ?
                0 PARAGENE? ?
          3   974 PLASMID? ? OR PARAGENE? ?
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          4   254 T5 OR T(1W)5
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               22 COLIPHAGE? ?
          5 11931 PHAGE? ? OR COLIPHAGE? ?
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             1036 STRONG
             2203 STRENGTH
          7  3223 STRONG OR STRENGTH
          8  4210 CLON?
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         13     0 12*6
         14     0 6*8
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            23569 AMINO(W)ACID? ?
         15103905 PROTEIN? ? OR AMINO(W)ACID? ?
         16     0 14*15
? t12/3

12/3/1
52002070
  HOST CONTROLLED MODIFICATION AND RESTRICTION OF FOREIGN CHROMOSOMAL AND
PLASMID DNA IN SHIGELLA-FLEXNERI STRAINS
  KETYI J; ORSKOV F
  ACTA PATHOL MICROBIOL SCAND SECT B: MICROBIOL IMMUNOL  78 (1).  1970
51-58.  Coden: APMIB

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? sau=cohen s n

         18    77 AU=COHEN S N
? c17*18

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          20     35 (17+18)*(2+3+5+6)
? c20*8

          21      1 20*8
? t21/3;c20-(21+12)

21/3/1
60049046
  CLONING  ISOLATION AND CHARACTERIZATION OF REPLICATION REGIONS OF COMPLEX
PLASMID GENOMES
  TIMMIS K; CABELLO F; COHEN S N
  PROC NATL ACAD SCI U S A   72 (6). 1975 2242-2246.   Coden: PNASA

          22     34 20-(21+12)
? t22/3/1-7

22/3/1
62013967
  REPLICATION  CONTROL  IN  A  COMPOSITE  PLASMID  CONSTRUCTED  BY IN-VITRO
LINKAGE OF 2 DISTINCT REPLICONS

  CABELLO F; TIMMIS K; COHEN S N
  NATURE (LOND)   259 (5541). 1976 285-290.   Coden: NATUA

22/3/2
62008033
  PHYSICAL  MAPPING  OF  THE  HIND-III  ECO-RI SAL AND SMA RESTRICTION ENDO
NUCLEASE CLEAVAGE FRAGMENTS FROM BACTERIO PHAGE T-5 DNA
  VON GABAIN A; HAYWARD G S; BUJARD H
  MOL & GEN GENET   143 (3). 1976 279-290.   Coden: MGGEA

22/3/3
61037184
  STUDIES  OF  MOUSE  MITOCHONDRIAL  DNA  IN ESCHERICHIA-COLI STRUCTURE AND
FUNCTION OF THE EUKARYOTIC PROKARYOTIC CHIMERIC PLASMIDS
  CHANG A C Y; LANSMAN R A; CLAYTON D A; COHEN S N
  CELL   6 (2). 1975 231-244.   Coden: CELLB

22/3/4
61013939
  INDIRECT  SELECTION OF BACTERIAL PLASMIDS LACKING IDENTIFIABLE PHENOTYPIC
PROPERTIES
  KRETSCHMER P J; CHANG A C Y; COHEN S N
  J BACTERIOL   124 (1). 1975 225-231.   Coden: JOBAA

 22/3/5
76099125
  UNIFORM NOMENCLATURE FOR BACTERIAL PLASMIDS A PROPOSAL/
  NOVICK R P; CLOWES R C; COHEN S N; CURTISS R III; DATTA N; FALKOW S
  BACTERIOL REV   40 (1). 1976 168-189   Coden: BAREA

22/3/6
60066345
  ALPHA BETA SEQUENCE OF F IS INSERTION SEQUENCE 3
  HU S; PTASHNE K; COHEN S N; DAVIDSON N
  J BACTERIOL   123 (2). 1975 687-692.   Coden: JOBAA

22/3/7
60031281
  STRUCTURE  AND  FUNCTION  OF  THE  GENOME OF COLI PHAGE T-5 TRANSCRIPTION
IN-VITRO OF THE NICKED AND NICK-FREE T-5-PLUS DNA
  KNOPF K-W; BUJARD H
  EUR J BIOCHEM   53 (2). 1975 371-386.   Coden: EJBCA
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?t22/3/8-20

22/3/8
60025274
OCCURRENCE OF INSERTION SEQUENCE REGIONS ON PLASMID DNA AS DIRECT AND INVERTED NUCLEOTIDE SEQUENCE DUPLICATIONS
PTASHNE K; COHEN S N
J BACTERIOL 122 (2). 1975 776-781. Coden: JOBAA

22/3/9
60025251
SITE SPECIFIC RECA INDEPENDENT RECOMBINATION BETWEEN BACTERIAL PLASMIDS INVOLVEMENT OF PALINDROMES AT THE RECOMBINATIONAL LOCI
KOPECKO D J; COHEN S N
PROC NATL ACAD SCI U S A 72 (4). 1975 1373-1377. Coden: PNASA

22/3/10
59036492
UTILIZATION OF 2 DISTINCT MODES OF REPLICATION BY A HYBRID PLASMID CONSTRUCTED IN-VITRO FROM SEPARATE REPLICONS
TIMMIS K; CABELLO F; COHEN S N
PROC NATL ACAD SCI U S A 71 (11). 1974 4556-4560. Coden: PNASA

22/3/11
59002019
TRANSFORMATION OF SALMONELLA-TYPHIMURIUM BY PLASMID DNA
LEDERBERG E M; COHEN S N
J BACTERIOL 119 (3). 1974 1072-1074. Coden: JOBAA

22/3/12
75093748
MAPPING OF FRAGMENTS PRODUCED FROM BACTERIO PHAGE T-5 DNA BY RESTRICTION ENZYMES HIN-III ECO R-1 AND SAL-1
BUJARD H; GABAIN A V; HAYWARD G S
HOPPE-SEYLER'S Z PHYSIOL CHEM 356 (3). 1975 224 Coden: HSZPA

22/3/13
75093741
ELECTRON MICROSCOPIC TITRATION OF ESCHERICHIA-COLI POLYMERASE BINDING SITES IN THE BACTERIO PHAGE T-5 GENOME
BLIN N; BUJARD H
HOPPE-SEYLER'S Z PHYSIOL CHEM 356 (3). 1975 221 Coden: HSZPA

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75067669
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$9.51 0.144 Hrs File55 10 Descriptors

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  $0.98  Tymnet
 $10.49  Estimated Total Cost


File76:Life Sciences Collection - 78-81/Dec
(Copr. Cambridge Scientific ABS.)
         Set Items Description
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                 679 DEOXYRIBONUCLEIC
             1 22872 DNA OR DEOXYRIBONUCLEIC
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                   0 PARAGENE? ?
             3  3864 PLASMID? ? OR PARAGENE? ?
                 142 T5
                 204 T(1W)5
             4   346 T5 OR T(1W)5
                5102 PHAGE? ?
                 181 COLIPHAGE? ?
             5  5117 PHAGE? ? OR COLIPHAGE? ?
             6   107 PROMOTOR? ?

                5389 STRONG
                2920 STRENGTH
             7  8177 STRONG OR STRENGTH
             8  5830 CLON?
             9  2579 1*2+1*3
            10    93 4*5
            11     3 6-8/*
            12     6 9*10
            13     0 12*6
            14    16 6*8
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                12754 AMINO(W)ACID? ?
            15 51428 PROTEIN? ? OR AMINO(W)ACID? ?
            16     4 14*15
? c11+16

            17     6 11+16
? t17/3/1-6;t12/3/1-6

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19 14 AU=COHEN, S.N.
?

20 34 AU=COHEN,S.N.
? c(19+20)-18

21 48 (19+20)-18
? c21*(2+3+5+6)

22 33 21*(2+3+5+6)
? c22*8

23 18 22*8
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Timmis, K. ; Cabello, F. ; Cohen, S.N.
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? sstrong or strength;sclon?

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               7756 STRENGTH
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           8 16153 CLON?
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? c9*10

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? c12*6
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11/7/1
70057410
  NUCLEOTIDE SEQUENCE OF MOLONEY LEUKEMIA VIRUS 3' END REVEALS DETAILS OF
REPLICATION ANALOGY TO BACTERIAL TRANSPOSONS AND AN UNEXPECTED GENE
  SUTCLIFFE J G; SHINNICK T M; VERMA I M; LERNER R A
  DEP. CELL. DEV. BIOL., SCRIPPS CLIN. RES. FOUND., LA JOLLA, CALIF. 92037,
USA.
  PROC NATL ACAD SCI U S A   77 (6). 1980.  3302-3306.   Coden: PNASA
  Language: ENGLISH
  The sequence of a cloned DNA fragment 1108 base pairs long which
corresponds to the 3' end of the Moloney murine leukemia provirus was
determined. The clone was obtained as the primary product of reverse
transcription and begins with the Moloney strong stop sequence, then
extends towards the 5' end of the provirus. The sequence: proves that
reverse transcriptase switches templates during minus strand synthesis;
defines the limits of the 515-base-pair repeats which occupy both ends of
the integrated provirus; shows that the structure of the proviral repeats
has strong analogy to bacterial insertion sequences, indicating that the
Moloney provirus is a transposon; identifies the putative promotor for

genomic transcription within these repeats; shows that the presumed origin
of 2nd strand synthesis, which lies just outside the 3' repeat, has
tertiary structure analogous to single-stranded bacteriophage origins of
replication; solves the amino acid sequence of most of p15E, the
carboxy-terminal product of the env gene; allows detailed mapping of the
mink cell focus-forming virus substitution locus in a central location
within the gp70 region of the env gene; and identifies a long open
translation frame to the right of the env gene (R gene) which could be
involved in leukemogenesis.

? t12/3/1-5

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73078508
  MOLECULAR WEIGHT DETERMINATION OF TRANSPOSON TN-233.
  CHEN J; TAIKIN W; WANG Z; HONG M
  LAB. OF MICROBIOLOGY, SHANGHAI INST. OF PLANT PHYSIOLOGY, ACADEMIA SINICA

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70023276
  ANALYSIS OF GENE CONTROL SIGNALS BY DNA FUSION AND CLONING IN
ESCHERICHIA-COLI
  CASADABAN M J; COHEN S N
  DEP. BIOPHYS. THEOR. BIOL., UNIV. CHIC., CHICAGO, ILL. 60637, USA.
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  A NEW SYSTEM FOR STUDYING MOLECULAR MECHANISMS OF MUTATION BY CARCINOGENS
  BHANOT O S; KHAN S A; CHAMBERS R W
  DEP. BIOCHEM., N.Y. UNIV. SCH. MED., NEW YORK, N.Y. 10016, USA.
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  A NEW HOST VECTOR SYSTEM ALLOWING SELECTION FOR FOREIGN DNA INSERTS IN
BACTERIO PHAGE LAMBDA-GT
  DAVISON J; BRUNEL F; MERCHEZ M
  UNIT MOL. BIOL., INT. INST. CELL. PATHOL., AVE. HIPPOCRAT 75, B 1200
BRUSSELS, BELG.
  GENE (AMST.)   2 (1). 1979 (RECD. 1980).  49-59   Coden: GENED
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69057559
CLONING OF BACTERIO PHAGE T-5 DNA FRAGMENTS IN PLASMID PBR-322 AND BACTERIO PHAGE LAMBDA-GT-WES
BRUNEL F; DAVISON J; MERCHEZ M
UNIT MOL. BIOL., INT. INST. CELL. PATHOL., AVE. HIPPOCRATE 75, B 1200 BRUSSELS, BELG.
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FLUORESCENCE CHANGES OF A MEMBRANE BOUND DYE DURING BACTERIO PHAGE T-5 INFECTION OF ESCHERICHIA-COLI
GLENN J; DUCKWORTH D H
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DEP. BIO
1E UNIV., NEW HAVEN, CONN. 06520, USA.
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INTERACTION OF ESCHERICHIA-COLI RNA POLYMERASE EC-2.7.7.6 WITH PROMOTERS OF SEVERAL COLI PHAGE AND PLASMID DNA
VON GABAIN A; BUJARD H
MOL. GENET. UNIV., IM NEUENHEIMER FELD 230, 6900 HEIDELBERG, W. GER.
PROC NATL ACAD SCI U S A 76 (1). 1979. 189-193. Coden: PNASA
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65034396
ELECTRON MICROSCOPY OF DNA DETERMINATION OF ABSOLUTE MOLECULAR WEIGHTS AND LINEAR DENSITY
STUEBER D; BUJARD H
MOL. GENET., UNIV. HEIDELB., IM NEUENHEIMER FELD 230, D-6900 HEIDELBERG, W. GER.
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77068741
INCORPORATION OF DNA FRAGMENTS OF BACTERIO PHAGE T-5 INTO THE ESCHERICHIA-COLI PLASMID PML-21
BEIERSTORF K; VON GABAIN A; BUJARD H
HOPPE-SEYLER'S Z PHYSIOL CHEM 357 (3). 1976 308 Coden: HSZPA

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72080433
  THE ARAI-C MUTATION IN ESCHERICHIA-COLI B-R
  CASS L G; HORWITZ A H; WILCOX G
  DEP. MICROBIOL., UNIV. CALIFORNIA, LOS ANGELES, CALIFORNIA 90024.
  J BACTERIOL   146 (3). 1981.  1098-1105.   Coden: JOBAA

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  NUCLEOTIDE SEQUENCE OF MOLONEY LEUKEMIA VIRUS 3' END REVEALS DETAILS OF
REPLICATION ANALOGY TO BACTERIAL TRANSPOSONS AND AN UNEXPECTED GENE
  SUTCLIFFE J G; SHINNICK T M; VERMA I M; LERNER R A
  DEP. CELL. DEV. BIOL., SCRIPPS CLIN. RES. FOUND., LA JOLLA, CALIF. 92037,
USA.
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69023057
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RIBOSOMAL PROTEIN GENES CLONED ON COMPOSITE COL-E-1 PLASMIDS IN THE
BACTERIUM ESCHERICHIA-COLI
  DENNIS P P; FIIL N P

  DEP. BIOCHEM., UNIV. B.C., VANCOUVER, B.C. V6R 1W5, CAN.
  J BIOL CHEM   254 (16). 1979.  7540-7547.   Coden: JBCHA

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? c19*8

          20     23 19*8
? c18*17

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73053357
  CLONING AND ANALYSIS OF STRONG PROMOTERS IS MADE POSSIBLE BY THE
DOWNSTREAM PLACEMENT OF A RNA TERMINATION SIGNAL.
  GENTZ R; LANGNER A; CHANG A C Y; COHEN S N; BUJARD H
  MOLEKULARE GENETIK DER UNIV., 6900 HEIDELBERG, FRG.
  PROC NATL ACAD SCI U S A   78 (8). 1981.  4936-4940.   Coden: PNASA

          22      9 17-21
          23     22 20-21
? t22/3/1-9

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72080449
  PROMOTER SITES IN THE GENOME OF BACILLUS-SUBTILIS PHAGE SPP-1
  STUEBER D; MORELLI G; BUJARD H; MONTENEGRO M A; TRAUTNER T A
  MAX-PLANCK-INST. MOL. GENETIK, ABT. TRAUTNER , IHNESTRASSE 63/73,  D-1000
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72030851
ORGANIZATION OF TRANSCRIPTIONAL SIGNALS IN PLASMIDS PBR-322 AND PACYC-184
STUEBER D; BUJARD H
MOLEKULARE GENETIK, UNIV. HEIDELBERG, NEUENHEIMER FELD 230, 6900 HEIDELBERG, FED. REP. GERMANY.
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67066746
INTERACTION OF ESCHERICHIA-COLI RNA POLYMERASE EC-2.7.7.6 WITH PROMOTERS OF SEVERAL COLI PHAGE AND PLASMID DNA
VON GABAIN A; BUJARD H
MOL. GENET. UNIV., IM NEUENHEIMER FELD 230, 6900 HEIDELBERG, W. GER.
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ELECTRON MICROSCOPIC ANALYSIS OF IN-VITRO TRANSCRIPTIONAL COMPLEXES MAPPING OF PROMOTERS OF THE COLI PHAGE T-5 GENOME
STUEBER D; DELIUS H; BUJARD H
MOL. GENET., UNIV. HEIDELB., IM NEUENHEIMER FELD 230, D-6900 HEIDELBERG, W. GER.
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HAYWARD G S; BUJARD H; GUNTHER M
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INTERACTION OF ESCHERICHIA-COLI RNA POLYMERASE WITH PROMOTERS OF COLI PHAGE T-5 THE RATES OF COMPLEX FORMATION AND DECAY AND THEIR CORRELATION WITH IN-VITRO AND IN-VIVO TRANSCRIPTIONAL ACTIVITY/
VON GABAIN A; BUJARD H
MOL. GENET., UNIV. HEIDELB., IM NEUENHEIMER FELD 230, D-6900 HEIDELBERG, W. GER.
MOL GEN GENET 157 (3). 1977 (RECD 1978) 301-312. Coden: MGGEA

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ELECTRON MICROSCOPY OF DNA DETERMINATION OF ABSOLUTE MOLECULAR WEIGHTS AND LINEAR DENSITY
STUEBER D; BUJARD H
MOL. GENET., UNIV. HEIDELB., IM NEUENHEIMER FELD 230, D-6900 HEIDELBERG, W. GER.
MOL GEN GENET 154 (3). 1977 299-304. Coden: MGGEA

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77068749
THE CHROMOSOME OF BACTERIO PHAGE T-5 PHYSICAL STRUCTURE AND ORGANIZATION OF THE GENETIC TEXT
BUJARD H
HOPPE-SEYLER'S Z PHYSIOL CHEM 357 (3). 1976 311 Coden: HSZPA

22/3/9
77068741
INCORPORATION OF DNA FRAGMENTS OF BACTERIO PHAGE T-5 INTO THE ESCHERICHIA-COLI PLASMID PML-21

United States Patent [19]

Bujard et al.

[11] **Patent Number: 4,495,280**

[45] **Date of Patent: Jan. 22, 1985**

[54] **CLONED HIGH SIGNAL STRENGTH PROMOTERS**

[75] Inventors: **Hermann G. Bujard,** Heidelberg, Fed. Rep. of Germany; **Annie C. Y. Chang,** Palo Alto; **Stanley N. Cohen,** Portola Valley, both of Calif.

[73] Assignee: **The Board of Trustees of the Leland Stanford Jr. University,** Stanford, Calif.

[21] Appl. No.: **265,276**

[22] Filed: **May 20, 1981**

[51] **Int. Cl.**[3] **C12Q 1/68;** C12Q 1/02; C12P 21/00; C12P 19/34; C12N 15/00

[52] **U.S. Cl.** **435/6;** 435/29; 435/68; 435/172.3; 435/91; 536/27; 935/6; 935/36; 935/37; 935/41; 935/76

[58] **Field of Search** 435/29, 68, 6, 172, 435/240, 253, 254, 257, 258, 317, 91; 536/27, 28, 29

[56] **References Cited**

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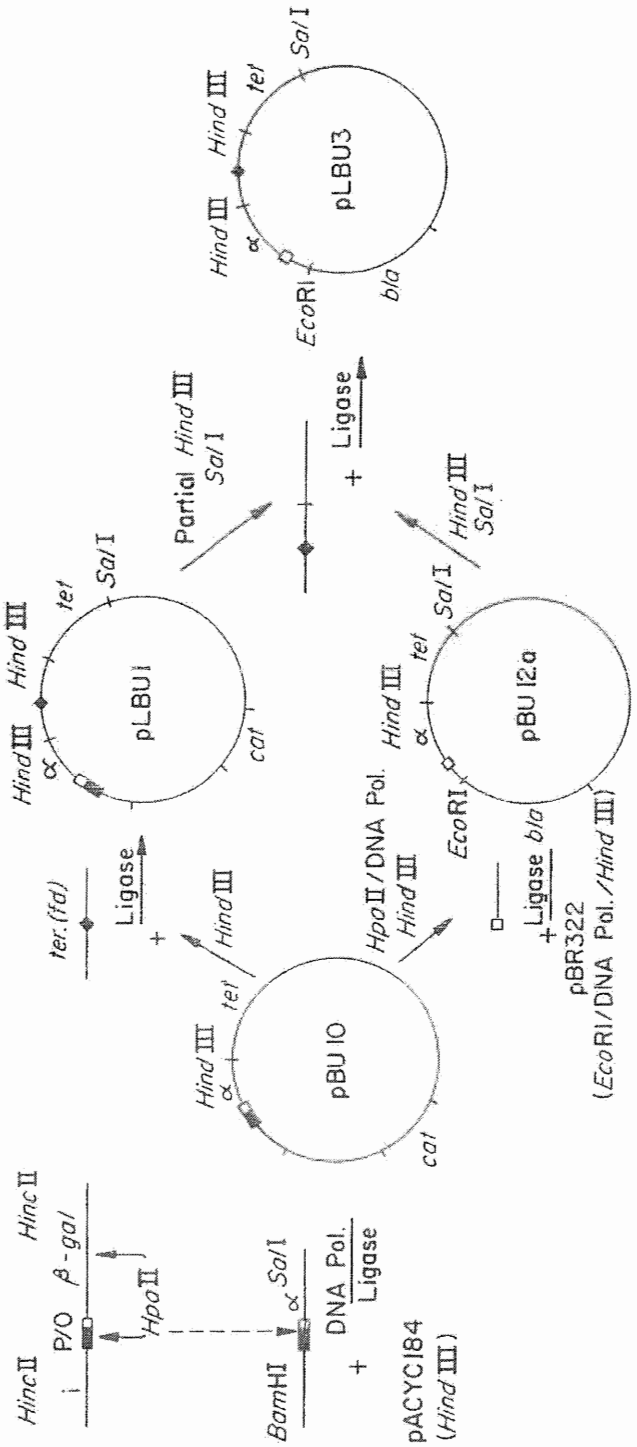
Primary Examiner—Esther M. Kepplinger

Attorney, Agent, or Firm—Bertram I. Rowland

[57] **ABSTRACT**

Method for preparing high signal strength promoters and terminators and DNA compositions employing such promoters and terminators. T5 phage is cleaved to provide for DNA sequences having intact promoters. These promoters are inserted into vectors separated from a balanced terminator by a gene of interest and the terminator is desirably followed by a marker allowing for selection of transformants. High efficiencies in transcription of DNA can be achieved with the highly active T5 promoters. The promoters and terminators are used in hybrid DNA for efficient expression of structural genes and transcription to provide RNA sequences.

15 Claims, 1 Drawing Figure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α
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Sal I
cat
ter. (fd)
Ligase
+
Hind III
pBU 10
Hind III
tet
α
cat
HpaII / DNA Pol.
Hind III
Hind III
Sal I
pBU 12.a
Hind III
tet
Sal I
α
EcoRI
bla
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HincII
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β-gal
HpaII
BamHI
α
Sal I
+ DNA Pol. / Ligase
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CLONED HIGH SIGNAL STRENGTH PROMOTERS

The government has rights in the invention pursuant to Grant Nos. AI 08619 and GM 27241 awarded by the National Institute of Health.

The research was supported in part by a grant from the Deutsche Forschungsgemeinschaft.

BACKGROUND OF THE INVENTION

1. Field of the Invention

Having established the feasibility of producing a wide variety of naturally occurring and synthetic polypeptides by means of hybrid DNA technology, there are continuing and extensive efforts to provide for more efficient and economic methods for producing the polypeptides. In developing a process for the commercial production of polypeptides, many factors will be involved in optimizing the economic and efficient production of the polypeptides. Included among these factors are regulatory signals, which are DNA sequences involved with the regulation of replicaton, transcription and translation.

One area of interest is at the level of transcription. Transcription involves the enzyme RNA polymerase. The RNA polymerase binds to a site called a promoter. It has been observed that promoters vary in their activity, as evidenced by the number of initiations of RNA per unit time or the strength of binding of the enzyme to the promoter site. The promoter may have one or more sequences that bind, which may or may not be contiguous. The more active promoters are referred to as strong promoters.

It was found that when introducing a strong promoter into a vector and employing the resulting plasmid for transformation, one could not select transformants based on expression of markers which allowed for selection. Therefore, cloning of the strong promoters was not feasible. It is therefore desirable that methods be provided which would allow for the screening of strong promoters and terminators and their subsequent cloning to be used in conjunction with the replication, transcription and translation of the genes for production of DNA, RNA, and polypeptides.

2. Description of the Prior Art

Promoters from bacterial and viral sources have been cloned in *E. coli*, and their signal strength in vitro has been studied using expression from distal promoterless sequences encoding β-galactosidase or other proteins (Casadaban and Cohen (1980) J. Mol. Biol 138, 179–207; West and Rodriguez (1980) Gene 9, 175–193). Attempts to clone small DNA fragments carrying the strong promoters of bacteriophage T5 have been unsuccessful (v. Gabain and Bujard (1979) PNAS USA 76, 189, 193), Fragments of T5 DNA having both a strong promoter and a strong termination signal have been cloned. (Breunig (1979) Dissertation (Universitat Heidelberg, Heidelberg, Germany)) Analysis has shown that transcriptional regions of several *E. coli* plasmids are organized in units where initiation and termination signals, are balanced. (Stuber and Bujard (1981) PNAS USA 78: 167–171) P_{25} and P_{26} promoters of the T5 bacteriophage are reported as among the most efficient RNA polymerase binding sequences. (Stuber et al (1978) Mol. gen. Genet. 166 141–149; Niemann (1981) Diplomarbeit (Universitat Heidelberg, Heidelberg, Germany)).

SUMMARY OF THE INVENTION

Methods for cloning, sequencing and using strong promoters and terminators are provided, as well as compositions resulting from the methods. By cleaving T5 phage and selecting fragments specifically binding to RNA polymerase, fragments containing promoters are isolated. A vector is constructed having a strong promoter, followed by a DNA sequence of interest, optionally followed by one or more translational stop codons in one or more reading frames, followed by a balanced terminator, followed by a marker allowing for selection of transformants. Upon introducing the resulting plasmid into a microorganism host, efficient transcription of the gene is obtained with substantially lesser expression of the marker as compared to the gene. The level of expression of the marker permits selection of transformants having the above described construct. The construct or regulatory portions thereof are used for efficient transcription of RNA or gene expression.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a flow chart of the preparation of a plasmid for cloning strong promoters and terminators.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for preparing and cloning strong promoter and terminator regulatory signals and utilization of the strong regulatory sequences in the transcription and expression of genes of interest.

Particularly, T5 phage promoters are isolated, cloned in conjunction with a strong terminator, and appropriate vectors developed for insertion of DNA sequences of interest, usually structual genes, to provide for high and efficient transcription and/or expression of the sequence.

The compositions of this invention are characterized as having in the downstream direction of transcription the following units: a strong T5 phage promoter; optionally a structural gene which may be a marker; a balancing terminator; and optionally a marker allowing for selection of transformants containing the construct, which marker has a relatively low level of expression in comparison with the amount of RNA polymerase initiation at the promoter. In the absence of a promoter in the construct, the construct can be used for the cloning and characterization of promoters of different strengths.

In referring to strong promoters, it is intended that the binding affinity of RNA polymerase is stronger than the commonly employed promoters such as lac and trp and at least comparable to and normally greater than the combination of lac and trp promoters. For the most part, the strongest promoters among prokaryotes are the T5 phage promoters and these will be employed as exemplary of naturally occurring or synthetic strong promoters. It is to be understood, that other prokaryotic and eukaryotic promoters, either naturally occurring or synthetic, could find application in the subject invention.

The compositions of this invention will include linear segments for insertion of DNA having the strong regulatory signal sequences (i.e., the promoter and terminator) adjacent opposite ends of the linear segment and plasmids formed by introducing a DNA sequence from a source other than the source of the promoter as a bridge between the strong regulatory signal sequences.

The termini may be blunt or staggered ended, having the same or different termini to allow for directed positioning of inserted sequences.

The compositions which are employed as already indicated have a promoter, a balanced terminator, and desirably a marker which are in the direction of transcription when the signal sequences are joined. The terminator is balanced, so that a small but useful amount of expression of the marker can occur. Normally, in most situations, a gene will bridge the promoter and terminator to provide circular DNA. The gene may be a non-structural gene or a structural gene providing RNA e.g. ribosomal or messenger, or providing a poly(amino acid).

Desirably, the gene is followed by one or a plurality of translational stop codons e.g. oop or nonsense codons, or preferably a plurality, usually up to about six, more usually from about two to five, where there is at least one stop codon in each reading frame. The stop codons aid in the efficiency of termination, both at the level of transcription and expression.

Next in the direction of transcription is the terminator sequence. The terminator sequence is balanced with the promoter in the sense that the marker is transcribed not more than about one-fourth of the times that a gene upstream from the terminator is transcribed, where both genes are under the control of the same promoter. Usually, it will be desirable that a sufficient amount of the marker is transcribed to allow for selection of transformants. In view of the fact that the marker is transcribed from a strong promoter, it will usually be sufficient that the marker is transcribed in relation to the transcription of the gene intermediate the promoter and terminator in only about 1 to 20, usually only 5 to 15 number % of the gene. The strength of the promoter is reflected in the level of expression of the marker which is transcribed from the same promoter as the gene whose expression is desired.

In addition to the DNA sequences indicated above, there will normally be other regulatory signals necessary for expression involved with the DNA sequence, such as translational start and stop sites. In addition to the foregoing regulatory signals, other regulatory signals may be included, such as additional promoters, operators, initiators, catabolite activator protein binding sites, etc. Furthermore, the promoter and terminator may be separated by more than one gene, that is, a plurality of genes, including multimers and operons.

The above DNA sequence construct will have a replicating system or be cleaved and be inserted into a vector to provide a plasmid. The vector is distinguished by having one or more DNA sequences which serve to insure stable replication of the plasmid and may also provide opportunities for high copy numbers of the plasmid in the microorganism host. The vectors may be derived from chromosomal or extrachromosomal sources. The sources include plasmids, viruses (phage), chromosomes, or the like. In addition, the vector or the essential portions thereof may be prepared synthetically.

The plasmids may then be used for transformation of an appropriate microorganism host. Methods of introducing DNA into an appropriate host are well known. Illustrative of such methods, but not exhaustive of such methods, are transformation e.g. calcium shock, transfection, and conjugation. Descriptions of these methods may be found in Genetic Engineering, ed. Setlaw and Hollaender, Vol. 1, Plenum Press, New York and London, 1979; Molecular Cloning of Recombinant DNA, ed. Scott and Werner, Vol. 13, Academic Press, Inc. New York, 1973, and references cited therein.

In order to allow for flexibility in preparing the construct and self-replicating sequence or plasmid containing the construct restriction sites should be present to allow for unique insertions and isolation of the various elements. The restriction sites may be naturally present, introduced by linkers, result by partial sequential nucleotide removal from a chain using an exonuclease, or the like. Desirably, the restriction sites will provide for different ends to permit only the proper orientation of the inserted fragment.

A wide variety of structural genes are of interest for production of proteins, including but not limited to proteins of physiological interest, proteins as chemicals, and enzymes which may be of direct interest or of interest in transforming another product, which may be proteinaceous or non-proteinaceous. The proteins may be prepared as a single unit or as individual subunits and then joined together in appropriate ways. Furthermore, as appropriate, the protein products may be modified by glycosylation, acylation with aliphatic acids, e.g. lipid acids, phosphorolation, sulfonation or the like. The different classes of proteins which may be prepared include protamines, histones, albumins globulins, scleroproteins, phosphoproteins, mucoproteins, chromoproteins, lipoproteins, nucleoproteins, and the remaining proteins which are unclassified.

The following is a representative list of proteins of interest.

Insulin; growth hormone; interferon e.g. leukocyte, lumphoblastoid, T-immune and fibroblast; β-lipotropin; β-endorphin; dynorphin; histocompatability proteins; immunoglobulins e.g. IgA, IgD, IgE, IgG and IgM and fragments thereof; hemoglobin, somatomedins; lymphokines; growth factors e.g. epidermal, fibroblast, platelet-derived, multiplication stimulating and nerve; hematoporetic-stimulating factors e.g. erythropoietin, colony-stimulating, erythroid potentating activity or burst-promoting activity and lymphopoietins; albumin and prealbumin;

Prealbumin
Albumin
α_1-Lipoprotein
α_1-Acid glycoprotein
α_1-Antitrypsin
α_1-Glycoprotein
Transcortin
4.6S-Postalbumin
Tryptophan-poor
α_1-glycoprotein
α_1-χ-Glycoprotein
Thyroxin-binding globulin
Inter-α-trypsin-inhibitor
Gc-globulin:
(Gc 1-1),
(Gc 2-1),
(Gc 2-2),
Haptoglobin:
(Hp 1-1),
(Hp 2-1),
(Hp 2-2),
Ceruloplasmin
Cholinesterase
α_2-Lipoprotein(s)
α_2-Macroglobulin
α_2-HS-Glycoprotein

Zn-α_2-glycoprotein
α_2-Neuramino-glycoprotein
Erythropoietin
β-lipoprotein
Transferrin
Hemopexin
Fibrinogen
Plasminogen
α_2-glycoprotein I
α_2-glycoprotein II
Immunoglobulin G
(IgG) or γG-globulin
Mol. formula:
$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$
Immunoglobulin A (IgA) or γA-globulin
Mol. formula:
$(\alpha_2\kappa_2)^n$ or $(\alpha_2\lambda_2)^n$
Immunoglobulin M (IgM) or γM-globulin
Mol. formula:
$(\mu_2\kappa_2)^5$ or $(\mu_2\lambda_2)^5$
Immunoglobulin D (IgD) or γD-Globulin (γD)
Mol. formula:
$(\delta_2\kappa_2)$ or $(\delta_2\lambda_2)$
Immunoglobulin E (IgE) or γE-Globulin (γE)
Mol. formula:
$(\epsilon_2\kappa_2)$ or $(\epsilon_2\lambda_2)$
Free light chains
Complement factors:
C'1
C'1q
C'1r
C'1s
C'2
C'3
β_1A
α_2D
C'4
C'5
C'6
C'7
C'8
C'9.
Important protein hormones include:

Peptide and Protein Hormones

Parathyroid hormone: (parathormone)
Thyrocalcitonin
Insulin
Glucagon
Relaxin
Erythropoietin
Melanotropin: (Melanocyte-stimulating hormone; intermedin)
Somatotropin: (growth hormone)
Corticotropin: (adrenocorticotropic hormone)
Thyrotropin
Follicle-stimulating hormone
Leuteinizing hormone: (interstitial cell-simulating hormone)
Luteomammotropic hormone: (Luteotropin, prolactin)
Gonadotropin: (chorionic gonadotropin).

Tissue Hormones

Secretin
Gastrin
Angiotensin I and II
Bradykinin
Human placental lactogen

Peptide Hormones from the Neurohypophysis

Oxytocin
Vasopressin
Releasing factors (RF): CRF, LRF, TRF, Somatotropin-RF, GRF, FSHRF, PIF, MIF.

In addition to various non-enzymatic proteins of physiological interest, enzymes can also be produced as an end product or for intracellular transformation of a substrate present in the host or substrate introduced extracellularly, or for enzymatic transformation in vitro.

In accordance with the I.U.B. classification, the enzymes fall into varying categories such as 1. oxidoreductases; 2. transferases; 3. hydrolases; 4. lyases; 5. isomerases; 6. ligases. Enzymes of particular interest will be hydrolases and oxidoreductases for use in commercial processing, for example, hydrolases for hydrolysing polysaccharides, lipids and polypeptides; oxidoreductases for oxidation of alcohols and aldehydes, epoxidation, and the like.

The microorganism host may be bacteria, such as Escherichia, Bacillus, Aerobacter, Klebsiella, Proteus, Pseudomonas, Streptococcus, Staphylococcus, Clostridium, Mycobacterium, Streptomyces and Actinomyces; Fungi e.g. Gymnomycota, Dimastygomycota, Eumycota, Zygomycetes, Ascomycetes and Basidomycetes, such as, Candida, Aspergillus, Rhizobus, Microsporum, and Fonsecaea; Protozoa e.g. Mastigophora, Sarcodina, Sporozoa and Celiophora, such as, Trypanosoma, Codosiga, Protospongra and Entameba, and Alga e.g. Dinoflagellates, Euglenoids, and Diatoms.

Higher cells, e.g., mammalian, may also be employed as hosts, where viral, e.g., bovine papilloma virus or other DNA sequence is available which has plasmid-like activity.

Depending upon the nature of the host, a wide variety of vectors may be employed. The vector will have an intact replicon and be capable of replication in the host. In addition, replicons can be developed which may have one or more other regulatory signals. Regulatory signals can include temperature sensitive replicons, runaway-replication sequences, temperature sensitive operators, and the like. Various additional DNA sequences may be present providing for restriction sites, markers, termination sequences, or the like. Desirably, the vector should be of a substantially different size from the construct to allow for excision of the construct and ease of separation of the construct from the vector by molecular weight separation techniques e.g. electrophoresis and density gradient centrifugation.

The subject invention also provides for a technique for screening the strength of promoters and terminators, thus allowing for the determination of the use of a promoter or terminator in a particular application, where it may be desirable to have promoters or terminators or combinations thereof of varying strength. In screening promoters and/or terminators one employs a promoter or terminator of known activity. A DNA construct is made having the sequence described previously, where appropriate restriction sites are provided for introducing the various elements. The construct provides in the direction of transcription the promoter, a first gene marker, optionally termination codons, such as nonsense codons and oop terminator, the terminator, and a second gene marker. This DNA construct is inserted into an appropriate vector. Where the promoter and

terminator are properly balanced, there will be a substantial differentiation between expression of the first gene marker and expression of the second gene marker. The ratio of expression between the first and second marker will provide for a comparative evaluation of the activity of the promoter or terminator, depending upon which is of known value. Thus, one can degrade a DNA sequence such as a chromosome, or an extrachromosomal element, such as a plasmid or double minute, isolate the promoters by selective binding with RNA polymerase and insert the DNA fragments which bind to the RNA polymerase into the previously described construction. By determining the relative proportion of expression of the first and second gene markers, one can determine the strength of promoters in relation to a fixed terminator. Similarly, one can isolate DNA sequences having terminator sequences, insert the sequences into the above described construct at the appropriate site and then measure the relative expression of the two gene markers.

Various markers can be chosen for evaluating the relative activities of promoters and terminators. Conveniently, markers which allow for selection such as resistance to antibiotics, toxins or heavy metals can be used. By varying the concentration of the selective agents in the nutrient medium, one can determine the relative proportions of the enzyme expressed by the genes in relation to the growth of the host. Alternatively, one can use growth factors e.g. having a gene which complements a mutant gene in an auxotrophic host, where the gene expresses a product necessary for a biosynthetic pathway. A third marker provides virus incompatibility, preventing plaque formation. Other markers which allow for comparison will come readily to mind.

The terminators may be evaluated in the presence and absence of rho, so that one can determine the dependency of the terminator on rho, as well as the effectiveness of the terminator in relation to the concentration of rho.

The methods for preparing the subject compositions will be conventional. The various DNA fragments and sequences can be obtained from a variety of sources by restriction mapping and endonuclease cleavage to provide fragments having the desired intact sequence or gene. The fragments can be further processed employing endo- or exonucleases to remove nucleotides unrelated to desired regulatory sequences or structural genes. By appropriate choice of restriction enzymes, cohesive or blunt ended fragments can be generated. Furthermore, chains can be extended with single nucleotides or oligonucleotides, linkers can be added, or otherwise processing to provide for termini having desired properties.

Desirably, a vector is employed having appropriate restriction sites, a competent replication system for the intended host, and optionally one or more markers which allow for selection. For hybrid DNA technology it would be useful to have a plasmid having a unique restriction site between a T5 promoter and a terminator, desirably having at least one stop codon on the upstream side of the terminator. In this manner, one or more structural genes may be introduced between the promoter and terminator.

As appropriate, downstream from the promoter, but remaining proximal to the promoter, may be an operator, activator, ribosomal start signal sequence, or the like, to allow for controlled expression of the inserted gene(s).

The strategy described above provides a vehicle which can be used with one or more hosts for gene expression, where the gene after processing in a predetermined way can be directly inserted into the vehicle to provide a competent plasmid for expression of the desired gene(s).

Alternatively, the gene(s) of interest may be ligated to the appropriate regulatory signal sequences before insertion into the vehicle. In this instance, only the promoter and terminator regulatory signals need be present.

To provide for enhanced flexibility, the region between the promoter and terminator may be designed so as to provide for a plurality of restriction cleavage sites, allowing for the introduction and removal of DNA fragments without interruption of the remainder of the vehicle. Thus, by having a plurality of unique restriction sites or restriction sites limited to the region between the promoter and terminator in the downstream direction of transcription, regulatory signals and genes may be readily inserted and removed.

Another strategy is to prepare a construct having all of the desired DNA sequences for transcription and expression in appropriate sequence, with the construct having predetermined termini and inserting the construct into an appropriate vector which has been linearized to provide complementary termini.

In developing the construct, a vector will normally be used in order to clone the various sequences. The construct will allow for the insertion of the different sequences in the correct direction and desirably only in the proper orientation. Therefore, it will usually be desirable to have the sequence and insertion site be asymmetric in having different termini with the termini of the sequence and insertion site being complementary.

The particular restriction enzymes will vary widely with the various sequences, there being a large number of restriction enzymes of known base or sequence specificities commercially available.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Materials and Methods

Restriction endonucleases HindIII, HincII, SalI, BamHI and T4 Ligase were from New England Biolabs (Beverly, Mass., USA), Sau3A was from BRL (Neu-Isenburg, Germany) and EcoRI and HpaII from (Boehringer Mannheim, Federal Republic of Germany). DNA polymerase I, phage fd DNA (replicative form), plasmid AD16/30 containing a 28 bp HindIII/BamHI adapter sequence and, lac represser were supplied by private sources. The isolation of bacteriophage T5 DNA, plasmid DNA *E. coli* RNA polymerase and *E. coli* termination factor rho have been described previously, (v. Gabian and Bujard, Mol. gen. Genet. (1977) 157, 301–311; Clewel and Helinski (1969) PNAS USA 62, 1157–1166; and Knopf and Bujard (1975) Eur. J. Biochem. 53, 371–385).

A 780bp DNA fragment carrying the *E. coli* lac regulatory region (promoter/operator: P/O), an N-terminal portion of the β-galactosidase structural gene sufficient for intracistronic complementation of the M15 deletion, as well as a portion of the i-gene(i) was isolated from a HincII digest of a pACYC 214 plasmid (a plasmid related to pACYC 184 by insertion at a BamHI site of a BamHI restriction cleavage fragment from F'-lac carrying the lac gene) by repressor binding and subsequent

adsorption to nitrocellose. This fragment was then employed in the construction of an exemplary plasmid for analyzing strong promoters and strong terminators as depicted in FIG. 1.

Utilizing the HpaII cleavage site within the β-gal structural genes the fragment was reduced in size and provided with BamHI and SalI cleavage sites by various subcloning. The resulting fragment (left most part of Figure) contains the intact control region of the lac operon and an N-terminal portion of the β-gal structural gene coding for 66 amino acids (α). Introduction of this fragment by blunt end ligation into the HindIII site of pACYC184 (Chang and Cohen (1978) J. Bacteriol. 134, 1141–1156) yielded pBU1O, a vector suitable for terminator cloning. The major terminator of the coliphage fd genome was isolated as a 338bp Sau3A fragment (Beck et al. (1978) Nucl. Acids Res. 5, 4495–4503), ligated with a BamHI/HindIII adaptor sequence and integrated into pBU10 to yield pLBU1.

Cleavage of the lac sequence in pBU10 by HpaII destroys the lac promoter and liberates upon cleavage with HindIII a fragment containing the lac operator and a region coding for a functional α-fragment. Integrating this DNA sequence into pBR322 leads to pBU12a. Finally, replacement of the HindIII/SalI portion of pBU12a by a partial digest of the HindIII/SalI fragment of pLBU1 containing the fd terminator resulted in pLBU3, a vector suitable for integration of efficient promoters at the EcoRI site. The regions encoding chloramphenicol (Cm), ampicillin (Ap) and tetracycline (Tc) resistance are indicated as cat, bla and tet respectively.

The plasmid, pBU10, had the following properties: (i) it contains the α fragment of β-galactosidase (α-protein) and complemented the M15 deletion of the lac operon; (ii) the Tc resistance it specified was under the control of the lac promoter, as shown in M15 I^q strains; (iii) the HindIII site between the lac gene fragment and the tet gene was restored; the stop codon immediately following the HindIII site limited the length of the lacZ gene product to 68 amino acids.

Insertion of the fd terminator upstream to the tet gene resulted in a 90% reduction in the level of Tc resistance, but no detectable change in the levels of β-galactosidase activity in M15 deletion strains. The results obtained as to the properties imparted to various *E. coli* strains by various plasmids is set forth in the following table:

Seven independent plasmid isolates from clones contained the expected 352bp HindIII-generated fragment containing the fd terminator. Electrophoretic analysis of all seven BamHI-cleaved isolates and DNA sequence analysis of one of these showed that in all instances the fd terminator had been integrated in an orientation opposite the direction of transcription within the fd phage genome. Sequence analysis also revealed a translational stop codon in frame with the α-protein less than 10bp down stream from the HindIII cleavage forming the conjunction between the lac-derived segment and the fd-derived DNA fragment; a translational stop signal on this position would be expected to result in an α-fragment containing 71 amino acids.

For the cloning of exogenous promoters, the lac promoter on the lac/tet construct had to be removed or destroyed in such a way that a site for the subsequent integration of promoters was retained. To do this, the HpaII cleavage site at position −17 of lac was employed, as described above. Colonies that showed both a reduced level of Tc resistance and the presence of a lac operator sequence, which could be detected on the multicopy plasmid by its ability to bind the lac represser and induce chromosomal β-gal synthesis, were identified. Endonuclease analysis (HindIII/EcoRI double digest) of plasmids recovered from several isolates yielded two types of vectors: one of these represented by pBU12 harbored the expected 253bp lac fragment; the other represented by pBU12a yielded a 420bp fragment. DNA sequence analysis showed that in pBU12a, a 160bp fragment of unknown origin, containing two to three stop codons in each of the possible translational reading frames, had been integrated between the EcoRI site and position −15 of the lac promoter. The presence of the stop codons made the fragment an efficient terminator of any translation that occurred upstream of the translational initiation site for the α-fragment.

When the HindIII/SalI segment of pBU12a was replaced with an identically generated fragment of pLBU1 carrying the fd terminator, the plasmid pLBU3 was obtained, which conferred neither Tc resistance nor β-gal activity to *E. coli* M15 strain. Although this plasmid contained a tet region and a DNA sequence encoding the α-fragment of lac, it conferred neither resistance nor β-gal activity to the *E. coli* M15 strain. It was therefore chosen as the T5 promoter cloning vehicle.

A population of about 200 short fragments of T5 DNA was obtained by double digestion of the 120kb phage genome with HaeIII and AluI endonucleases. These fragments were ligated with excess synthetic EcoRI links and the resulting molecules were cleaved with EcoRI endonuclease and ligated into the EcoRI cleavage site of pLBU3. Transformation of *E. coli* C600 and selection for β-gal activity plus high level Tc-resistance yielded 35 colonies resistant to Tc-concentrations between 8 and 70 μg/ml. Plasmids were isolated from 13 colonies resistant to 70 μg/ml, which earlier experiments using multicopy plasmids had suggested was the highest level detectable in *E. coli* K12 (Cabello et al. (1976) Nature 259, 285–290).

Digestion of the various isolates with EcoRI endonuclease liberated between one and ten fragments of various sizes from each constructed plasmid. Complexing of such fragment mixtures with RNA polymerase, followed by filter binding analysis, identified between one and three fragments of each plasmid that interacted very efficiently with the enzyme; these fragments were isolated from polyacrylamide gels and individually recloned in pLBU3. In each case, they gave rise to colonies resistant to 70 μg/ml Tc. Plasmids isolated from each of these clones carried the expected DNA fragments, as shown by EcoRI cleavage and gel electrophoresis. The promoter library which was obtained contained about 25 different strong promoters of coliphage T5.

A plasmid pGBU207 containing an EcoRI-generated fragment of 212bp was selected for further study. In vitro transcription on the plasmid was mapped by analysis of RNA transcripts made on fragments of the plasmid produced by cleavage with different restriction endonucleases. Cleavage of the plasmid with EcoRI endonuclease yielded principally a single RNA species about 130 nt in length. The size of the transcript increased to 550 nt when a HindIII digest of pGBU207 was used as a template. BamHI digested DNA yielded transcripts of about 740 and 900 nt in length. Correlation of transcript length with the distance of the DNA cleavage site from a fixed point insert indicated that in

all of these instances in vitro transcription was initiated at the same promoter and that it progressed toward the tet region of the plasmid. The experiments also showed the functioning of the termination signal introduced between the lac fragment and the tet gene. Under the high salt conditions used for this in vitro transcription experiment, termination of about 50% of transcription within the fd DNA fragment gave rise to the 740 nt transcript, while a read- through transcript extended to the BamHI cleavage site located 890bp from the promoter. The data strongly suggested that RNA termination occurring in the fd terminator placed in the reverse orientation is rho dependent, in contrast to the rho-independent termination that occurs when the terminator is in its normal orientation.

Since the rate of complex formation between RNA polymerase and promoter signals is a reflection of the strength of the promoter, the relative rate of complex formation of the 212bp fragment of pGBU207 was compared with complex formation involving the previously studied T5 promoters P_{25} and P_{26} (Stuber and Bujard (1981), supra). The results showed that the promoter used to express downstream genetic functions in pGBU207 has a signal strength similar to that of P_{25} and P_{26}, which are among the most efficient RNA polymerase binding sequences identified from any source. (Niemann (1981), supra).

The above results demonstrate that novel DNA sequences can be prepared from the strong T5 promoters, which can then be used for the expression of a wide variety of poly(amino acids). Furthermore, by employing a promoter, optionally a structural gene, a terminator, and a marker, test plasmid structures are provided which allow for screening of the effectiveness of a promoter and/or a terminator, particularly as they interrelate with each other. Therefore, combinations can be prepared which allow for highly efficient transcription of a wide variety of structural genes, with concommitant selection of the transformants by employing an appropriate marker downstream from the balanced terminator.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

We claim:

1. A linear DNA sequence having proximal to one end a strong T5 phage promoter, proximal to the other end a strong transcriptional terminator balanced with said strong T5 promoter, and having intermediate said promoter and terminator at least one of (1) a marker for selection adjacent to said terminator or (2) a replication system foreign to T5, wherein the direction of said promoter is away from said terminator and said marker is expressed at a frequency of less than about one-fourth the frequency of a structural gene, when said structural gene is inserted between said promoter and terminator, so as to be under the transcriptional control of said promoter and to bridge said linear DNA sequence to provide a circular DNA sequence.

2. A linear DNA sequence according to claim 1, having intermediate said other end and said strong terminator at least one stop codon in at least one reading frame.

3. A linear DNA sequence according to claim 2, having a plurality of stop codons with at least one in each reading frame.

4. A linear DNA sequence according to any of claims 1, 2 or 3, wherein said marker is a gene imparting biocidal resistance.

5. A linear DNA sequence according to any of claims 1, 2 or 3, wherein said marker is a DNA sequence having at least one gene in a metabolic synthetic pathway.

6. A linear DNA sequence according to any of claims 1, 2 or 3, having a marker intermediate said promoter and said terminator.

7. A linear DNA sequence according to claim 6, wherein said marker provides biocidal resistance.

8. A linear DNA sequence according to claim 6, wherein said marker has at least one gene for an enzyme in a metabolic synthetic pathway.

9. A linear DNA sequence according to any of claims 1, 2 or 3, wherein said replication system is for a prokaryote.

10. A linear DNA sequence according to any of claims 1, 2 or 3, wherein said replication system is for a eukaryote.

11. A method for determining the strength of a promoter which comprises:

inserting said promoter into a linear DNA sequence having in the downstream direction for expression proximal to one end; a gene allowing for detection of expression; a transcriptional terminator of known strength; a marker allowing for determination of expression; and a replication system recognized by a predetermined host; whereby a circular DNA sequence is obtained;

transforming said host with said circular DNA sequence;

growing said host in nutrient medium under conditions allowing for determination of the extent of expression of said gene and said marker; and

determining the strength of said promoter is determined by the relative degree of transcription of said gene and said marker.

12. A method according to claim 11, wherein said host is auxotrophic and said gene provides prototrophy.

13. A method according to any of claims 11 or 12, wherein said marker provides biocidal resistance.

14. A method according to claim 13, wherein intermediate said gene and said terminator are a plurality of stop codons, with at least one stop codon in each reading frame.

15. A circular DNA sequence having in downstream order of transcription a strong T5 phage promoter, a structural gene foreign to T5 phage under transcriptional control of said promoter, a transcriptional terminator which is balanced with said promoter and a replication system and having a marker for selection downstream from said terminator, wherein said marker is expressed at a frequency of less than about one-fourth the frequency of which the structural gene is expressed and is under transcriptional control of said promoter.

* * * * *

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INITIALS

REQ10831109 WNR-01-04-040-1-016-01-002

Transfer#: W241-97-0252 Box: CC:P4 59

ARR1-408678920 Asset#: AAC1-22872716 Whole Container: N

C/F: 4495280 Created: 8/30/2011

General Reference Temporary Loan of Records

Standard Customer Pick-up N/A

To: PRADIEU MASSILLON
600 DULANEY ST. ,
ALEXANDRIA, VA, 22314
P : (703) 924-9382 F : (703) 924-2674

Box #59

PATENT NUMBER

APPLICATION SERIAL NUMBER
265276

APPLICANT'S NAME (PLEASE PRINT)
BUJARD ET AL

IF REISSUE, ORIGINAL PATENT NUMBER

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
435	6

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
435	29	68	172.3	91
536	27			
935	6	36	37	41
935	76			

INTERNATIONAL CLASSIFICATION (INT. CL.[3])

C	12	Q		1/68
C	12	Q		1/02
C	12	P		21/00
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ISSUE CLASSIFICATION SLIP

SEARCH NOTES

	Date	Ex'r
Computer Search	5/25/82	Emk
Genetic Engineering books in Group 170	5/25/82	Emk

SEARCHED

Class	Sub	Date	Ex'r
435	29, 68, 6 172, 240 253, 254 257, 258 317, 91	5/24/82	Emk
536	27, 28, 29	5/25/82	Emk
435 536	update "	12/9/82 "	Emk "
435 536	update "	5/24/83 "	Emk "
435 536	update "	12/9/83 "	Emk "
435 536	update "	6/28/84 "	Emk "

INTERFERENCE SEARCHED

Class	Sub	Date	Ex'r
435	6, 29, 68 91, 172.3	6/28/84	Emk
536	27	"	"

PRINT CLAIM(S):

1, 11

INDEX OF CLAIMS

Claim Final	Claim Original	Date 5/25/82	Date 12/10/82	Date 5/25/83	Date 12/9/83	Date 6/28/84
1	(1)	✓	✓	✓	✓	=
2	2	✓				
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Claim Final	Claim Original	Date 5/25/82	Date 12/10/82	Date 5/25/83	Date 12/9/83	Date 6/28/84
14	26	✓	✓	✓	✓	=
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	~~28~~			✓	✓	
	~~29~~			✓	✓	
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SYMBOLS — STATUS

- ✓ ..... Rejected
- = ... Allowed
- –(Through numeral) Canceled
- + ... Restriction requirement
- N ..... Nonelected invention or species
- I ..... Interference
- A ..... Appeal
- O ..... Objected

265276

[redacted]

Entered

CONTENTS

1. Applications ____ papers.
2. Rej (3) June 30, 1982 5/26
3. Cont. 9/30 Amdt A Aug. Oct. 4, 1982 C
4. Suppl. Amdt B Nov. 12, 1982
5. Rej (3) Jan. 3, 1983 12
6. Amdt C Mar. 14, 1983 C
7. Rej (3) June 20, 1983 5/31
8. Amdt D Sept 23, 1983 12/12
9. FINAL Rejection (3) JAN. 10, 1984
10. Personal Interview March 6, 1984
11. Amdt E (N.E.) Mar. 15, 1984
12. Advisory Action 3 mos. April 12, 1984
13. Ext Time grant (1) April 30, 1984
14. Amdt F NE April 30, 1984
15. Advisory (4) May 30, 1984 5/21
16. Ext time grant (2) mo. June 11, 1984
17. Amdt G NE June 11, 1984
18. Examiner interview Aug 1, 1984 7/2
19. Exam Amdt Aug 9, 1984
20. ~~Small Entity~~ Oct 26, 1984
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