US009127285B2

(12) **United States Patent**
Turkewitz et al.

(10) **Patent No.: US 9,127,285 B2**
(45) **Date of Patent: Sep. 8, 2015**

(54) **GENETICALLY ALTERED CILIATES AND USES THEREOF**

(71) Applicant: **The University of Chicago**, Chicago, IL (US)

(72) Inventors: **Aaron Turkewitz**, Chicago, IL (US); **Joseph Briguglio**, Chicago, IL (US)

(73) Assignee: **The University of Chicago**, Chicago, IL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/773,327**

(22) Filed: **Feb. 21, 2013**

(65) **Prior Publication Data**

US 2013/0224796 A1 Aug. 29, 2013

Related U.S. Application Data

(60) Provisional application No. 61/601,921, filed on Feb. 22, 2012.

(51) **Int. Cl.**

C12P 21/06	(2006.01)
C12N 15/79	(2006.01)
C07K 14/44	(2006.01)
C12P 21/04	(2006.01)
C12P 21/02	(2006.01)
C07H 21/02	(2006.01)
C07K 14/00	(2006.01)
C12N 15/00	(2006.01)

(52) **U.S. Cl.**
CPC ***C12N 15/79*** (2013.01); ***C07K 14/44*** (2013.01); ***C12P 21/02*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,087,124 A	7/2000	Steinbruck et al.	435/69.1
6,846,481 B1	1/2005	Gaertig et al.	424/93.1
2006/0127973 A1	6/2006	Hartmann et al.	435/69.1

FOREIGN PATENT DOCUMENTS

EP	847 444	6/1998
WO	WO 98/01572	1/1998
WO	WO 03/078566	9/2003
WO	WO 2007/006812	1/2007
WO	WO 2010/108182	9/2010

OTHER PUBLICATIONS

Chilcoate et al., "An antisense approach to phenotype-based gene cloning in Tetrahymena" 98(15) Proceedings of the National Academy of Sciences 8709-8713 (2001).*

Yoon et al., "Enhanced Production and Secretion of Heterologous Proteins by the Filamentous Fungus *Aspergillus oryzae* via Disruption of Vacuolar Protein Sorting Receptor Gene Aovps10" 76(17) Applied and Environmental Microbiology 5718-5727 (2010).*

Eisen et al., "Macronuclear Genome Sequence of the Ciliate Tetrahymena thermophila, a Model Eurkaryote" 4(9) PLoS Biology1620-1642 (2006).*

Idiris et al. 85 Applied Microbiology and Biotechnology 667-677 "Enhanced protein secretion from multiprotease-deficient fission yeast by modification of its vacuolar protein sorting pathway" (2010).*

Turkewitz et al., "Functional genomics: the coming of age for *Tetrahymena thermophia*" 18(1) Trends in Genetics 35-40 (2002).*

Zeng et al., "The inactivation of the sortilin gene leads to a partial disruption of prosaposin trafficking to the lysosomes" 315 Experimenatl Cell Research 3112-3124 (2009).*

Becker and Rusing, *J. Eukaryot. Microbiol.*, 50:235-239, 2003.

Blomberg et al., *Mol. Cell Biol.*, 12 :7237-7747, 1997.

Boldrin et al., *Eukaryot Cell*, 2 :422-425, 2006.

Bruns et al., *Proc. Natl. Acad. Sci. USA*, 9 :284-286, 1985.

Brus and Cassidy-Hanley, *Meth. Cell Biol.*, 62:501-512, 2000.

Cassidy-Hanley et al., *Genetics*, 146:135-147, 1997.

Cowan et al., *Mol. Cell. Biol.*, 16 :4046-4060, 2005.

de Coninck et al., *J. Industr. Microbiol. Biotechnol.*, 24 :285, 2000.

Gaertig and Gorovsky, *Proc. Natl. Acad. Sci. USA*, 89:9196-9200, 1992.

Gaertig et al., Surface display of a parasite antigen in the ciliate *Tetrahymena thermophila. Nature Biotech.*, 17:462-465, 1999.

Gaertig et al., *Nucleic Acids Res.*, 22:5391-5398, 1994.

Hellenbroich et al., *Appl. Microbiol. Biotechnol.*, 51 :447, 1999.

Melia et al., Mutational analysis of regulated exocytosis in *Tetrahymena. J. Cell Sci.* 111(Pt1):131-140, 1998.

Orias et al., *Gene*, 2:295-301, 1988.

Shang et al., *Proc. Natl. Acad. Sci USA*, 6 :3734-379, 2002.

Spangler and Blackburn, *J. Biol. Chem.*, 10:6334-6340, 1985.

Taniguchi et al., *j. Biol. Chem.*, 260 :13941-13946, 1985.

Tondravi and Yao, *Proc. Natl. Acad. Sci. USA*, 83:4369-4373, 1986.

Weide et al., *BMC Biotechnol.*, 6 :19, 2006.

Williams et al., *J. Biol. Chem*, 255:296-303, 1980.

Yu and Blackburn, *Proc. Natl. Acad. Sci. USA*, 21:8487-891, 1989.

Frankel, *Meth. Cell Biol.*, 62:27-125, 2000.

Gaertig and Kapler, Transient and stable DNA transformation of *Tetrahymena thermophila* by electroporation. *Meth. Cell Biol.*, 62:486-500, 2000.

Hai et al., Knockout heterokaryons enable facile mutagenic analysis of essential genes in Tetrahymena. *Meth. Cell Biol.*, 62:513-531, 2000.

Lin et al., The use of synthetic genes for the expression of ciliate proteins in heterologous systems. *Gene*, 288(1-2):85-94, 2002.

Pan et al., *Cell*, 28(3):595-604, 1982.

Turkewitz et al., "Regulated Protein Secretion in *Tetrahymena thermophila*." *Meth. Cell Biol.*, 62:347-362, 2000.

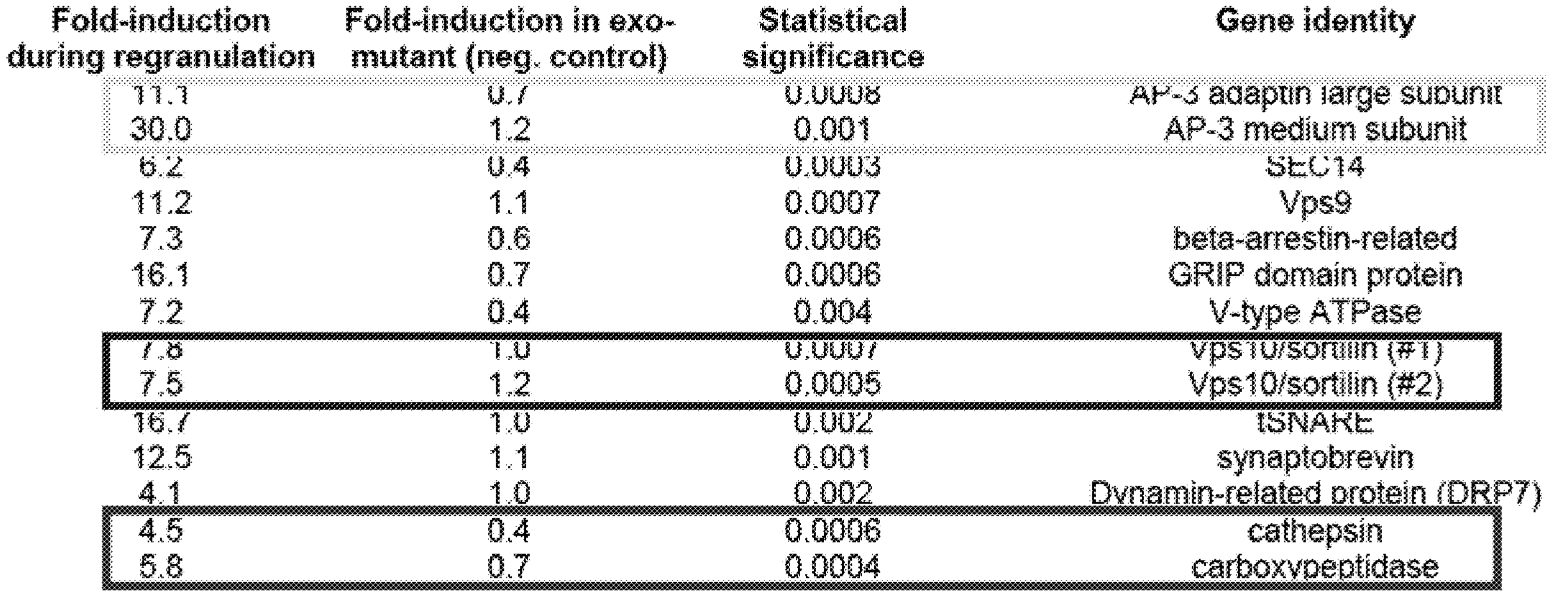
* cited by examiner

Primary Examiner — Anne Gussow
Assistant Examiner — Nancy J Leith
(74) *Attorney, Agent, or Firm* — Norton Rose Fulbright US LLP

(57) **ABSTRACT**

Ciliate organisms are provided that comprise reduced proteolytic processing in granules. For example, ciliates are provided that lack detectable expression of one or more sortilin (SOR) gene product. Methods for producing such genetically altered ciliates and methods for protein production in a these organisms are also provided.

20 Claims, 5 Drawing Sheets



Fold-induction during regranulation	Fold-induction in exo- mutant (neg. control)	Statistical significance	Gene identity
11.1	0.7	0.0008	AP-3 adaptin large subunit
30.0	1.2	0.001	AP-3 medium subunit
6.2	0.4	0.0003	SEC14
11.2	1.1	0.0007	Vps9
7.3	0.6	0.0006	beta-arrestin-related
16.1	0.7	0.0006	GRIP domain protein
7.2	0.4	0.004	V-type ATPase
7.8	1.0	0.0007	Vps10/sortilin (#1)
7.5	1.2	0.0005	Vps10/sortilin (#2)
16.7	1.0	0.002	tSNARE
12.5	1.1	0.001	synaptobrevin
4.1	1.0	0.002	Dynamin-related protein (DRP7)
4.5	0.4	0.0006	cathepsin
5.8	0.7	0.0004	carboxypeptidase

FIG. 1

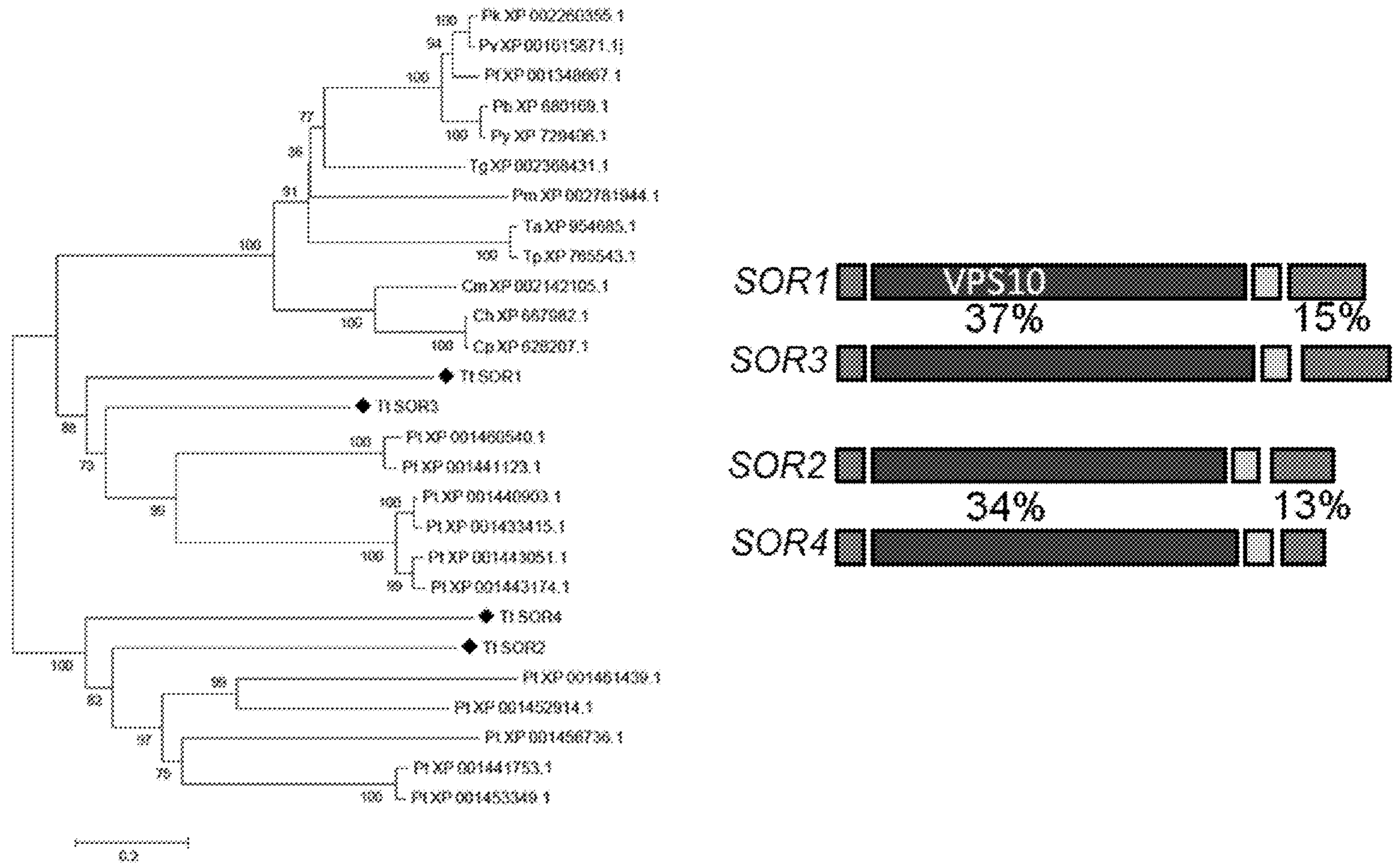

100
Pk XP 002260355.1
94
Pv XP 001615871.1
100
Pf XP 001348667.1
77
Pb XP 680169.1
100
Py XP 729406.1
36
Tg XP 002368431.1
91
Pm XP 002781944.1
100
Ta XP 954685.1
100
Tp XP 765543.1
Cm XP 002142105.1
100
Ch XP 667982.1
100
Cp XP 628207.1
Tt SOR1
88
Tt SOR3
73
100
Pt XP 001460540.1
Pt XP 001441123.1
95
100
Pt XP 001440903.1
Pt XP 001433415.1
100
Pt XP 001443051.1
99
Pt XP 001443174.1
Tt SOR4
Tt SOR2
100
82
98
Pt XP 001461439.1
Pt XP 001452914.1
97
Pt XP 001456736.1
79
Pt XP 001441753.1
100
Pt XP 001453349.1
0.2
SOR1
VPS10
37%
15%
SOR3
SOR2
34%
13%
SOR4

FIG. 2

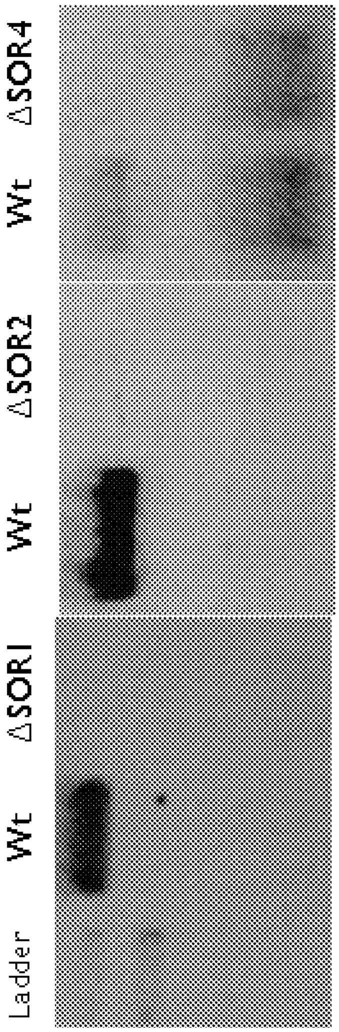


FIG. 3

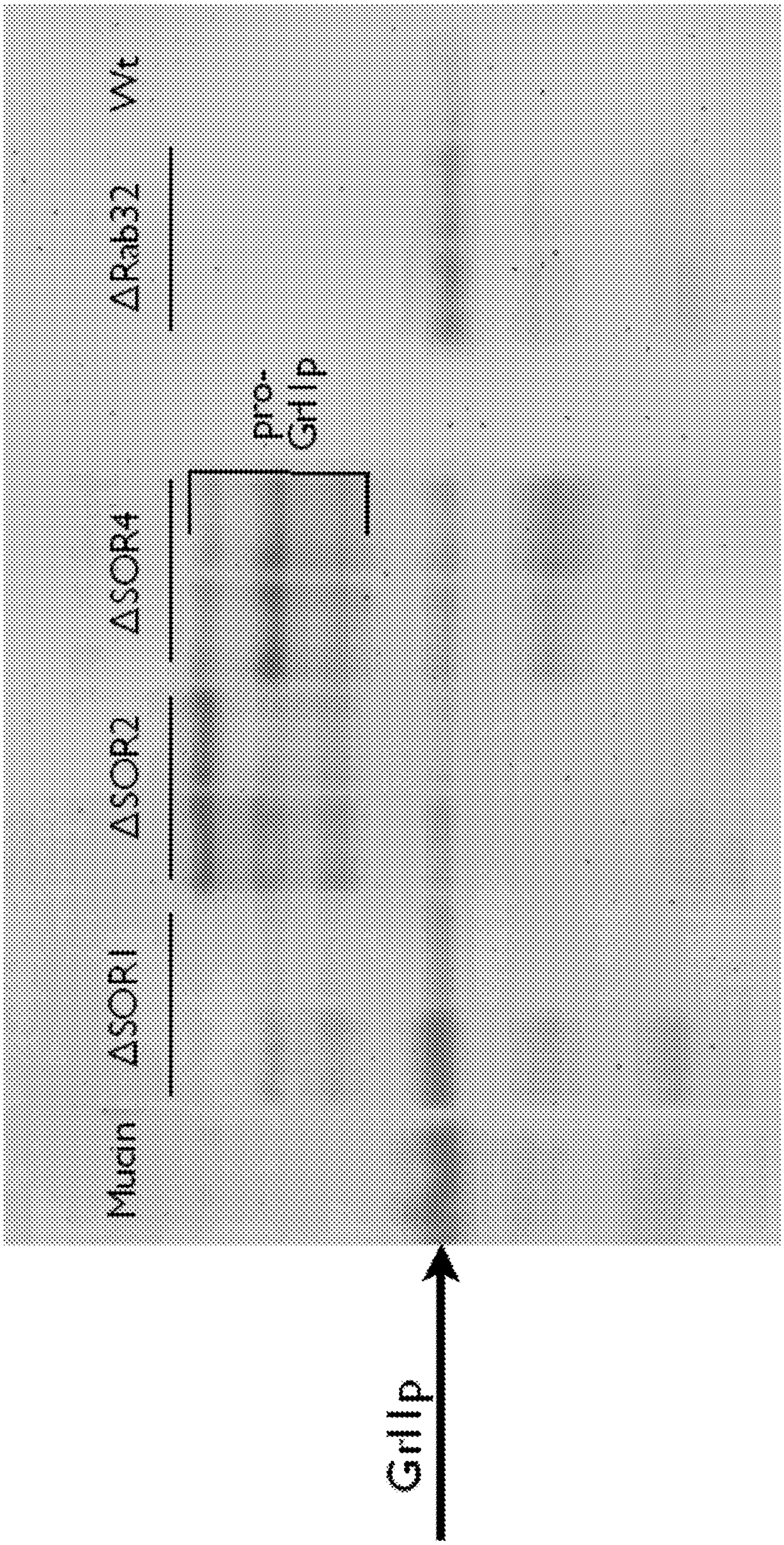


FIG. 4

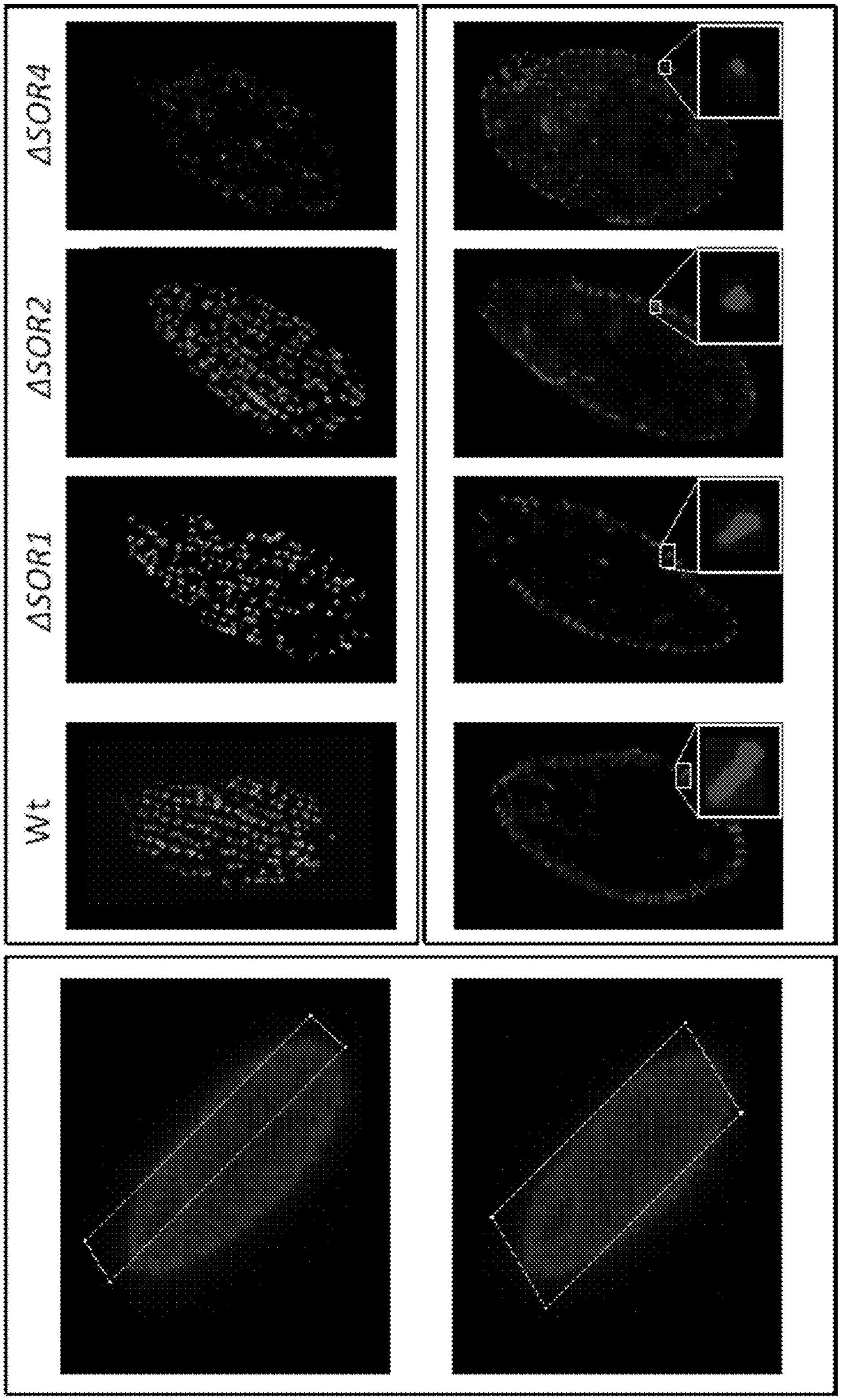


FIG. 5

GENETICALLY ALTERED CILIATES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Ser. No. 61/601,921, filed on Feb. 22, 2012, which is hereby incorporated by reference in its entirety.

The invention was made with government support under Grant No. GM077607 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of genetics and molecular biology. More particularly, it concerns genetically altered ciliate organisms and the use of such organisms in recombinant protein production.

2. Description of Related Art

Recombinant proteins are useful for a wide range of applications including as industrial enzymes and as therapeutics. For example, production of genetically engineered vaccine antigens, therapeutics (including antibodies and antibody fragments), industrial enzymes, biopolymers, and bioremediation agents now constitute a multibillion dollar-per-year industry. There is also a large market for recombinant proteins in the basic research arena (Pavlou and Reichert (2004); Langer (2005)).

Currently available platforms for the production of recombinant proteins are limited to a relatively small number of cell-based systems that include bacteria, fungi, and insect and mammalian tissue culture cells. Although bacteria can offer high yield and low cost alternatives for production of mammalian proteins, cell culture systems based on higher organisms such as insect cells or mammalian cell systems generally provide proteins having greater fidelity to the natural proteins in terms of protein folding and/or post-translational processing (e.g., glycosylation). Whole transgenic plants and animals have also been harnessed for the production of recombinant proteins, but the long development time from gene to final product can be a major drawback with these multicellular organisms, as can their high cost, low yield and many inherent difficulties in purification.

There remains a need in the art for improved methods for rapid, high-fidelity and cost-effective production and purification of recombinant polypeptides.

SUMMARY OF THE INVENTION

In a first embodiment a genetically altered ciliate is provided wherein the ciliate lacks detectable expression (or has reduced expression) of one or more SOR gene product. For example, the SOR gene product may be a product corresponding to SOR1 (SEQ ID NO: 1, 2), SOR2 (SEQ ID NO: 3, 4), SOR3 (SEQ ID NO: 5, 6) and/or SOR4 (SEQ ID NO: 7, 8; indicating the protein and nucleic acid coding sequence respectively). In some aspects, the ciliate may lack detectable expression of a SOR polypeptide or a SOR RNA corresponding to SOR1, SOR2, SOR3 and/or SOR4. In a further aspect, the ciliate lacks detectable expression of 2, 3 or 4 of the SOR1, SOR2, SOR3, or SOR4 genes. For example, a ciliate of the embodiments may lack detectable expression (or have reduced expression) of SOR1 and SOR2; SOR1 and SOR3; SOR1 and SOR4; SOR2 and SOR3; SOR2 and SOR4; SOR3 and SOR4; SOR1, SOR2 and SOR3; SOR1, SOR2 and SOR4; SOR1, SOR3 and SOR4; SOR2, SOR3 and SOR4; or SOR1, SOR2 SOR3 and SOR4.

In certain aspects, a ciliate of the embodiments comprises a genomic alteration, such as an insertion or a deletion in both copies of the germline genome that disrupts expression of one or more SOR gene product. For instance, the ciliate can comprise an insertion or deletion located in the open reading frame of a gene corresponding to SOR1, SOR2, SOR3 and/or SOR4. In some aspects, a genomic insertion comprises a selectable marker, such a drug resistance marker (e.g., a gene for tetracycline or neomycin resistance). Accordingly, in some aspects, a ciliate of the embodiments comprises an insertion or a deletion in all macronuclear copies of a gene corresponding to SOR1, SOR2, SOR3 and/or SOR4.

In further aspects a ciliate of the embodiments expresses a polynucleotide complementary to all or part of an RNA gene product corresponding to SOR1, SOR2, SOR3 and/or SOR4. For example, the ciliate can express an antisense RNA or a double stranded RNA (dsRNA) molecule, such as a small interfering RNA (siRNA), short hairpin RNA (shRNA) or micro RNA (miRNA), complementary to all or part of an RNA gene product corresponding to SOR1, SOR2, SOR3 and/or SOR4.

In still further aspects, a ciliate of the embodiments comprises a transgenic expression cassette, such as an expression cassette encoding a polypeptide. For example, the polypeptide can be a polypeptide for recombinant production in the ciliate. Polypeptides for use in accordance with the embodiments include, but are not limited to, enzymes, immunoglobulin (e.g., immunoglobulin light chains, immunoglobulin heavy chains or single chain antibodies), cytokines, chemokines, and antigens (e.g., bacterial or viral antigens). In some aspects the polypeptide coding sequence can comprise a sequence for cellular trafficking, such as a mucocyst-targeting sequence. For example, the polypeptide can encode a mucocyst-targeting sequence derived from a *Tetrahymena* Grl protein, such as Grl1, Grl2, Grl3, Grl4, Grl5, Grl6, Grl7, Grl8, Grl9 or Grl 10. In still further aspects, the polypeptide encodes a cleavable linker (e.g., between the polypeptide for expression and a mucocyst-targeting sequence).

In some specific aspects, a ciliate of the embodiments is a *Tetrahymena*, such as a *T. thermophila* or *T. pyriformis*.

In yet a further embodiment there is provided a recombinant *Tetrahymena* germline genome (e.g., a recombinant *T. thermophila* or *T. pyriformis* genome) comprising a genomic insertion or deletion in both copies of one or more SOR gene selected from the group consisting of SOR1, SOR2, SOR3, and SOR4. For example, the genomic insertion or deletion can be located in the open reading frame of the gene. In some aspects, a genomic insertion comprises the insertion of a selectable marker, such a drug resistance marker. In still further aspects, a *Tetrahymena* germline genome comprises a genomic insertion or deletion in both copies of 2, 3 or 4 SOR genes corresponding to SOR1, SOR2, SOR3 or SOR4. Thus, the genome can comprise an insertion of deletion in the genes for SOR1 and SOR2; SOR1 and SOR3; SOR1 and SOR4; SOR2 and SOR3; SOR2 and SOR4; SOR3 and SOR4; SOR1, SOR2 and SOR3; SOR1, SOR2 and SOR4; SOR1, SOR3 and SOR4; SOR2, SOR3 and SOR4; or SOR1, SOR2 SOR3 and SOR4.

In a further embodiment there is provided a recombinant *Tetrahymena* germline genome comprising, an expression cassette comprising a sequence encoding a polynucleotide molecule complementary to all or part of an RNA gene product corresponding to SOR1, SOR2, SOR3, or SOR4. For example, genome can comprise sequences encoding an antisense RNA or a dsRNA, such as a siRNA, shRNA or miRNA, complementary to all or part of an RNA gene product corresponding to SOR1, SOR2, SOR3 and/or SOR4.

In still a further aspect of the embodiments a recombinant *Tetrahymena* germline genome can comprise a transgenic expression cassette, such as a cassette encoding a polypeptide, optionally including a mucocyst-targeting sequence.

In still yet a further embodiment there is provided a method of producing a genetically altered ciliate comprising: (a) transforming a ciliate with a polynucleotide comprising a sequence complementary to a SOR gene corresponding to SOR1, SOR2, SOR3 and/or SOR4; and (b) isolating a genetically altered ciliate wherein the ciliate lacks detectable expression of the gene product of said SOR gene. For example, step (b) can comprise isolating a genetically altered ciliate comprising an insertion or deletion in a SOR gene or isolating a genetically altered ciliate expressing a polynucleotide molecule complementary to all or part of an RNA gene product of a SOR gene. In further aspects, step (a) comprises transforming the ciliate with a polynucleotide comprising a sequence complementary to a SOR gene and comprising a selectable marker (e.g., a drug resistance marker). Thus, in some aspects, a genetically altered ciliate is isolated based on expression of a selectable marker (such as by drug selection). Detailed methods for genetic alteration of ciliates are well known in the art and are detailed in PCT Patent Publn. No. WO2010108182, the entirety of which is incorporated herein by reference.

In a further embodiment there is provided a method of producing a genetically altered ciliate of the embodiments comprising obtaining the genetically altered ciliate and vegetatively propagating the ciliate. In further aspects, a genetically altered ciliate can be a produced by sexually propagating a genetically altered ciliate and isolating progeny that comprise the genetic alterations.

In still yet a further embodiment a method of producing a polypeptide is provided comprising: (a) expressing a polynucleotide encoding the polypeptide in a ciliate of the embodiments; and (b) incubating the ciliate in a media under conditions permissible for expression of the polypeptide. In some aspects, the majority (or at least a portion) of the polypeptide is secreted from the ciliate and the method can comprise (c) purifying the expressed polypeptide from the media. In certain aspects, the majority (or at least a portion) of the polypeptide is not secreted by the ciliate and the method can comprise (c) purifying the ciliate from the media and, optionally, (d) purifying the protein from the ciliate. In still further aspects, a method of the embodiments further comprises transforming a ciliate with a polynucleotide encoding a polypeptide. Further methods for polypeptide expression in ciliates are detailed in PCT Patent Publn. No. WO2010108182, the entirety of which is incorporated herein by reference.

In some aspects a expressing a polynucleotide for expression in a ciliate is further defined as an expression cassette encoding a polypeptide. For example, the polypeptide can be a polypeptide of mammalian origin, such as a human polypeptide. In some aspects, the polypeptide comprises sequence encoding an enzyme, an immunoglobulin, a cytokine, a chemokine, or an antigen.

As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. **1**: Two sortilin genes in *Tetrahymena* are dramatically up-regulated during induced granule formation (regranulation) in *Tetrahymena*. First column show fold induction of the genes, significance values are shown in third column.

FIG. **2**: Analysis of the *Tetrahymena* genome indicates that it codes four sortilin/Vps10 genes. Diagram shows the homology between the four sortilin genes (Tt SOR1-4) and structurally related genes.

FIG. **3**: *Tetrahymena* RNA expression was examined in putative SOR knockout lines (SORT, SOR2 and SOR4). In each case wild type (WT) *Tetrahymena* exhibited SOR RNA expression, whereas no expression was observed in the knockout lines.

FIG. **4**: *Tetrahymena* sortilin knockout lines secrete unprocessed precursors of the granule protein Grl1p. Immunoblot media samples show that in the case of each of the SOR knockout lines unprocessed (high molecular mass) Grl1p precursors are released into the media.

FIG. **5**: The sortilin knockout lines each make aberrant secretory granules, which are visualized here by immunefluorescence. Granule-specific immunefluorescence results for the indicated knockout cells (or for wild type "wt") are shown in tangential section (upper panels) or equatorial section (lower panels).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Recombinant protein production has become very important for a variety of applications. For example, many modern therapeutics, such as enzymes and monoclonal antibodies, are proteins that are produced recombinantly. However, adequate and cost-effective protein production systems are lacking Bacterial expression systems, while low cost, often result in proteins that do not incorporate crucial post-translation modifications or are improperly folded. On the other hand, mammalian cell expression systems are very expensive to maintain and produce notoriously low yields of protein products that must be extensively purified. Accordingly, ciliate-based protein production systems could provide an attractive alternative existing systems. However, there remains a need for a ciliate system adapted to provide high quality recombinant protein yield.

Studies detailed herein identify four ciliate gens from *Tetrahymena* that are important regulators of cell trafficking and secretion pathways. In particular, the SOR genes mediate transport of proteases to granules allowing for proteolytic processing of the granule contents. The studies here demonstrate that SOR gene expression can effectively knocked-out (see, FIG. **3**), and that such knockout is not lethal to the organism. Indeed, knockout of SORT, SOR2 and SOR4 all resulted in decreased proteolytic processing of products located in granules (FIG. **4**) and a change in granule structure indicative of reduced proteolytic processing (FIG. **5**).

Accordingly, modified ciliate organisms, such as *Tetrahymena*, are provided that have reduced expression of one or more sortilin gene product. Importantly, these organism exhibit reduced proteolytic processing in granules and are thereby ideal for recombinant protein production. Such organisms can be used to produce a wide range of protein products without aberrant cleavage of the products during expression. Moreover, recombinant proteins can be easily and cost-effectively purified by either isolating the ciliate cells comprising large quantities of highly concentrated (and uncleaved) protein product or by targeting the proteins for secretion and isolating the product from cell media.

I. Ciliates for Use According to the Embodiments

The embodiments may be practiced with a variety of different ciliates which include secretory granules called mucocysts. Heterologous polypeptides can be targeted to these secretory granules by encoding fusion proteins of the desired heterologous polypeptide and an appropriate targeting sequence. After exposing the ciliate to a secretory stimulus that causes the mucocysts to discharge their contents to the extracellular environment, the heterologous polypeptide can be recovered from the resulting matrix and medium.

The free-living ciliate protists are a large and diverse phylum (Ciliata) whose members display a structural and functional complexity comparable to that of higher metazoa (Fankel (2000); Turkewitz et al. (2002)), and include over 7,000 species with 11 major subdivisions. Tetrahymenids and Paramecium belong to the Oligohymenophoreans. Ciliates that include mucocysts useful in the invention include *Tetrahymena* species such as *Tetrahymena thermophila* and *Tetrahymena pyriformis*. Paramecium has dense core granules but does not secrete a proteinaceous gel. Both *Tetrahymena thermophila* and *Tetrahymena pyriformis* produce mucocysts, and both secrete a proteinaceous gel.

Tetrahymena spp. are amenable to genetic manipulation, can be grown on a large scale and have a doubling time of 1.5-3 hrs. Unlike *T. thermophila*, which has an optimal growth temperature of 35° C., the optimal growth temperature for *T. pyriformis* is lower (maximal growth temperature of 34° C.). Cells reach high-density in a short time on a variety of inexpensive media and can be expanded for growth in bioreactors up to several thousand liters in size (Hellenbroich et al. (1999); de Coninck et al. (2000)). Methods for transformation, along with robust, inducible promoters for driving high-level gene expression have recently been described for this system (Bruns and Cassidy-Hanley (2000); Gaertig and Kapler (2000); Shang et al. (2002); Boldrin et al. (2006)).

Tetrahymena spp. devote a large part of their metabolism to membrane protein production due to the hundreds of cilia that extend from its surface (Williams et al. (1980)). Additionally, *Tetrahymena* spp. lack a cell wall and display high-mannose N-glycan protein modifications that lack branched, immunogenic structures (Taniguchi et al. (1985); Becker and Rusing (2003); Weide et al. (2006)). Glycosylation patterns of secreted proteins in *Tetrahymena* spp. are uniform and consist of high-mannose N-glycan structures comprising Man3GlycNac2 core N-glycans similar to those which are produced in the endoplasmic reticulum of mammalian cells.

This glycosylation pattern is unlike the glycosylation pattern produced in other microbial systems. For example, such glycosylation is non-existent in bacteria, and is highly branched and immunogenic in fungi.

II. Genetic Alteration of Ciliates

Methods for genetic alteration of ciliates are well known in the art and may be used in accordance with the instant embodiments. For example, ciliates can be transformed with vectors that express nucleic acid to disrupt expression of a SOR gene (such as siRNAs). In some aspects, the ciliates is transformed with a vector to disrupt an endogenous SOR gene (e.g., by generating an insertion of deletion in a genomic copy of the gene). In still further aspects, a ciliate can be transformed with a vector for the expression of heterologous polypeptides, such as peptides that will be harvested from the cells.

Certain aspects of the embodiments concern ciliates that lack detectable expression (or have reduced expression) of one or more SOR gene product corresponding to SOR1, SOR2, SOR3 or SOR4. In some aspects the SOR gene product is an RNA at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence of SOR1 (SEQ ID NO: 2), SOR2 (SEQ ID NO: 4), SOR3 (SEQ ID NO: 6) or SOR4 (SEQ ID NO: 8). In a further aspect, the SOR gene product is an polypeptide at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SOR1 (SEQ ID NO: 1), SOR2 (SEQ ID NO: 3), SOR3 (SEQ ID NO: 5) or SOR4 (SEQ ID NO: 7). In yet further aspects the SOR gene product is an polypeptide comprising at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890 or 900, contiguous amino acids identical to the amino acid sequence of SOR1 (SEQ ID NO: 1), SOR2 (SEQ ID NO: 3), SOR3 (SEQ ID NO: 5) or SOR4 (SEQ ID NO: 7). In some specific aspects, the gene product corresponding to SOR1, SOR2, SOR3 or SOR4 is one of the gene products listed in the NCBI accession numbers of FIG. **2**, each of which is incorporated herein by reference. Thus, in some aspects, a ciliate of the embodiments comprises an insertion or a deletion in such a gene corresponding to *Tetrahymena* SOR1, SOR2, SOR3 and/or SOR4. In yet further aspects, a ciliate can comprise an expression cassette encoding a polynucleotide (e.g., a dsRNA, siRNA, shRNA or miRNA) complementary to all or part of an RNA corresponding to a *Tetrahymena* SORT, SOR2, SOR3 and/or SOR4 RNA.

Transformation

Genes can be introduced into ciliates using established protocols or any method known to one skilled in the art. Transformation of ciliates can be achieved by microinjection (Tondravi and Yao (1986)), electroporation (Gaertig and Gorovsky (1992)), or biolistically (Cassidy-Hanley et al. (1997)).

Thus, in some embodiments, ciliate cells can be transformed with a chimeric gene by particle bombardment (also known as biolistic transformation) (Cassidy-Hanley et al. (1997)). Particle bombardment transformation can be achieved by several ways. For example, inert or biologically active particles can be propelled at cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the chimeric gene. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Microcarrier bombardment can also be used to transform ciliate cells by means of DNA-loaded gold particles (U.S. Pat. No. 6,087,124; European Pat. EP 847 444; WO 1998/001572). In this approach, microcarrier bombardment with DNA-coated gold is used as a means of introducing foreign genes into ciliates. In one embodiment, microcarrier bombardment can be used to transform ciliates and introduce genes into the (germline) micronucleus

Methods for selection of transformed cells harboring foreign genes are known in the art. For example, the vector can further comprise a selectable cassette marker to permit selection for transformed cells {e.g., a neo 2 cassette) (Gaertig et al. (1994)).

Selection of transformants can be achieved by growing the cultured ciliates in a medium which allows only the transformants to survive. Suitable selection agents include antibiotics which will kill most all non-transformants but allow transformants (which also possess an antibiotic resistance gene) to survive. A number of antibiotic-resistance markers are known in the art. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. For example, selection of the transformants can be performed by means of a resistance marker such as a point mutation in the 17s rDNA, which confers resistance to paromomycin, can allow for selection of rDNA transformants (Spangler and Blackburn (1985); Bruns et al. (1985)). Other methods include the use of a mutant cell line that allows targeting of genes to the beta tubulin-1 locus of *T. thermophila* by homologous recombination, and allows efficient selection of transformed cell lines by growth in the microtubule-stabilizing agent (taxol) (U.S. Pat. No. 6,846,481). Another method for selection of transformed cells harboring foreign genes is to insert full length coding regions into the pD5HA vector (Cowan et al. (2005)). In this method, transcription is driven by the inducible MTT1 promoter. Once cells have been transformed with the pD5HA vector selection of positive transformants is determined by paromomycin resistance (i.e., cell growth in media containing the drug). Presence of the transgene is then verified by PCR and then induced with cadmium chloride to over-express the recombinant gene product.

Many other selectable marker systems are known in the art. Selectable marker genes that confer resistance or tolerance to a normally toxic selection agent allow only successfully transfected cells to survive in the presence of the selection agent, and are referred to as positive selectable markers. Examples of positive selectable marker genes and their corresponding selection agents are: aminoglycoside phosphotransferase (APH) and G418; dihydro folate reductase (DHFR) and methotrexate (Mtx); hygromycin-B-phosphotransferase (HPH) and hygromycin-B; xanthine-guanine phosphoribosyltransferase (XGPRT) and mycophenolic acid; and adenosine deaminase (ADA) and 9-β-D-xylofuranosyl adenine (Xyl-A). In another example of a positive selectable marker system, thymidine kinase (TK) and aminopterin (included, e.g., in hypoxanthine-aminopterin-thymidine (HAT) medium) can be used in cells that are initially thymidine kinase deficient (tk~). The aminopterin will normally kill tk~ cells and, therefore, only successful TK transfectants will survive. Selectable marker genes that confer sensitivity or susceptibility to a normally nontoxic selection agent cause only successfully transfected cells to die in the presence of the selection agent, and are referred to as negative selectable markers. An example of a negative selectable marker system is thymidine kinase (TK) and gancyclovir. Phenotypic selectable marker genes permit selection based upon morphological or biochemical traits rather than cell death or survival. In some cases, the phenotypic marker is detectable only in the presence of an additional selection agent. An example of a phenotypic selectable marker system is β-galactosidase (lacZ) and X-gal.

III. Vectors and Polypeptide Expression

Heterologous nucleic acids can be introduced into the ciliate host on an expression vector that is capable of integrating into the host's genome. For example, expression vectors capable of homologous recombination with a highly expressed gene that is endogenous to the protozoan host, such as a P-tubulin gene are known in the art. Alternatively, a heterologous nucleic acid transformed into a ciliate can be maintained extrachromosomally on an autonomous plasmid.

Expression vectors useful for transforming ciliates in accordance with the methods described herein include but are not limited to replacement vectors, rDNA vectors, and rDNA-based vectors. Replacement vectors accomplish DNA-mediated transformation by replacing or altering endogenous genes using homologous recombination. Integration of the heterologous nucleic acid into the host's genome at the targeted site is accomplished via homologous recombination involving a double crossover event with the vector containing the heterologous nucleic acid. An example of an expression vector useful for genomic incorporation of a heterologous nucleic acid by replacement is one that includes a heterologous coding sequence flanked by portions of the endogenous BTU1 gene of *Tetrahymena thermophile.*

A replacement vector can include a 5' region, followed by a heterologous coding region, followed by a 3' region, wherein at least a portion of each of the 5' and 3' regions is complementary to 5' and 3' regions on an endogenous gene of the host, to allow for genomic integration of the heterologous coding region via homologous recombination. The 5' and 3' regions of the vector can also comprise regulatory elements, such as a promoter and a terminator. The necessary regulatory elements can also be supplied by the endogenous gene into which the heterologous coding region integrates. Suitable regulatory regions include, but are not limited to promoters, termination sequences, signal peptides and proprotein domains involved in the expression and secretion of proteins. For example, such regulatory elements can provide efficient heterologous expression of proteins in *Tetrahymena* spp. under control of promoters and/or terminators which are derived from genes in *Tetrahymena* ssp. Such vectors can comprise naturally occurring promoters and/or terminators from proteins secreted at a high level in *Tetrahymena* ssp. The expression of recombinant polypeptides in *Tetrahymena* spp. can be driven by strong promoters, pre/pro sequences and terminators. In one embodiment, the promoters and/or terminators can be selected from proteins secreted at a high level independent of the cell-cycle in *Tetrahymena* spp. (US Patent Application 2006/0127973; WO2003/078566). Inducible promoters from *Tetrahymena* spp. genes have also been described that allow robust expression of foreign genes. For example, heat-inducible promoters of the heat shock protein family of the ciliate *Tetrahymena* spp. are also suitable for use with the methods described herein. Suitable heat shock promoters from *Tetrahymena* spp. are known in the art (see WO2007/006812).

Methods for creating mitotically stable *Tetrahymena* spp. transformants, for example, by integration of a heterologous gene by homologous DNA recombination, are known in the art. Methods for generating *Tetrahymena* spp. having targeted gene knockouts by homologous DNA recombination are also known in the art (Bruns and Cassidy-Hanley (2000); Hai et al. (2000); Gaertig et al. (1999); Cassidy-Hanley et al. (1997)). The somatic macronucleus or the generative micronucleus can be transformed in alternation. For example, sterile transformants, which may provide improved safety parameters, can be obtained with macronucleus transformation.

Expression vectors can also be maintained extrachromosomally in the ciliates. An expression vector maintained as an extrachromosomal element can be a rDNA-based vector containing an on from *Tetrahymena* spp. rDNA, which is known to support extrachromosomal replication. Such a vector can further comprise a 5' regulatory region from an endogenous *Tetrahymena* spp. gene containing a promoter region operably linked to the heterologous coding region and, optionally, a 3' regulatory region from the same or a different *Tetrahymena* spp. gene. For example, regulatory regions from ciliate genes in such vectors can include, but are not limited to, regulatory regions from genes such as HHFI, rp129, BTU1, BTU2, SerH3, and actin.

There are a number of suitable vectors suitable for transformation of ciliates known in the art. For example, *Tetrahymena* spp. can be transformed with an rDNA vector (Tondravi and Yao (1986); Yu and Blackburn (1989)). The shuttle vector pXS76 allows insertion of transgenes downstream of a cadmium-inducible promoter from the MTT1 metallothionein gene of *T. thermophila* via homologous recombination and selection in paromomycin. Alternatively, inserts can be introduced into high copy number ribosomal DNA vectors (such as pD5H8) under control of the cadmium-inducible MTT1 promoter. The pD5H8 vector takes advantage of a biological feature of *Tetrahymena* spp. in which the ribosomal cistrons become amplified to extraordinarily high copy numbers following conjugation. An rDNA-based vector can be a circular vector that contains a 5' non-translated sequence comprising two or more on sequences from *Tetrahymena* spp. rDNA. A nucleic acid fragment containing a heterologous coding region, for example a selectable marker or transgene, can also be added to the vector. The vector can further comprise a 5' untranslated region of a *Tetrahymena* spp. gene and a 3' untranslated region of a *Tetrahymena* spp. gene, inserted upstream and downstream of the selectable marker and/or the transgene. Methods for transformation, along with robust, inducible promoters for driving high-level gene expression have recently been described for this system (Bruns and Cassidy-Hanley (2000); Gaertig and Kapler (2000); Shang et al. (2002); Boldrin et al. (2006)).

Sequence variations within the origins of replication of rDNA from wild-type B- and C3-strains of *T. thermophila* convey a replicative advantage to the C3-form in B/C3 heterozygotes. Although both B- and C3-forms of rDNA are initially present in the macronucleus in approximately equal amounts, within 30 fissions only the C3 variant remains (Pan et al. (1982); Orias et al. (1988)). pIC19-based shuttle vectors containing the C3 origin of replication have been used as high-copy number vectors for the delivery of foreign DNA to *Tetrahymena* spp. (Yu and Blackburn (1989)) (FIG. **5**).

Although such vectors can become unstable and be lost within about 50 to about 80 generations, micronuclear versions of the C3 rDNA is accurately processed (to form a palindrome) following introduction into *T. thermophila* B cell lines. The micronuclear version is maintained as a stable linear chromosome over many generations (Bruns et al. (1985)). Functional transgenes can be inserted into the 3'-nontranscribed spacer (3'-NTS) of such vectors with no effect on rDNA processing. Within 6-10 generations, recombinant molecules can comprise 50-100% of the total rDNA complement, with as many as 18,000 copies of the transgene per cell (Blomberg et al. (1997)). The use of this approach enables an increase in the number of cloned genes in transformed cell lines by orders of magnitude and leads to increased expression at the protein level. For example, the use of rDNA-based vectors in combination with the MTT1 promoter can be used to drive expression of the endogenous granule lattice protein Grl Ip to approximately 20% of total cell protein (Lin et al. (2002)). Similarly, pD5H8 rDNA-based vectors (Blomberg et al. (1997)) can be used to boost expression of proteins by at least 3-10 fold compared with trans formants in which respective transgenes are integrated at somatic gene loci. Other vectors suitable for use with the methods described here include vectors comprising a ribosomal DNA sequence. Such vectors can replicate at high copy numbers and can be used to deliver a heterologous DNA sequence to *Tetrahymena* spp. for purposes of RNA expression.

Heterologous Polypeptides

Suitable heterologous polypeptides for use with these methods include, but are not limited to, antibodies, antibody fragments, cytokines, growth factors, protein kinases, proteases, protein hormones or any fragment thereof. Similarly, the methods described herein are suitable for the production of specialty proteins. The use of such specialty proteins can include, but is not limited to, prototype vaccines for animal model studies, structural studies, or as therapeutic proteins. For example, quantities of antigens can be produced according to the methods described herein.

Isolation of Desired Polypeptides from the Mucocyst Matrix

In one aspect, the invention provides methods for protein purification from the extracellular matrix formed by the discharge of mucocysts. Because heterologous polypeptides targeted to the mucocyst compartment will be associated within the matrix, the invention provides matrix-based purification strategies. Advantageously, the matrix can be used for rapid purification of recombinant polypeptides associated with it.

Proteins within the gel matrix can be separated from cellular constituents by low-speed centrifugation (See Turkewitz et al. (2000)). Any other method known in the art suitable for separating intact cells, from the discharged material, including, but not limited to filtration harvesting using an appropriately selected mesh, can also be used in conjunction with the methods described herein. After isolation of the matrix, the desired heterologous polypeptide can be liberated from the secreted matrix gel. Methods for liberation of the protein can include chemical methods {e.g., high salt concentrations) and/or enzymatic methods {e.g., site-specific proteases).

Proteins can also be isolated in intact secretory granules. For example, the use of an exocytosis-defective mutant, MN 173, of *T. thermophile* where granules accumulate in the cytoplasm has been described for such purposes (Melia et al. (1998)).

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Sortilin Gene Control Granule Trafficking of Proteases in *Tetrahymena*

Initial studies were undertaken to identify genes that are up-regulated upon regranulation in *Tetrahymena* cells. Results shown in FIG. **1** demonstrate that two sortilin genes are among the genes that are dramatically up-regulated during this process. In view of these studies, the *Tetrahymena* genome was analyzed in comparison with genes from other organisms and four *Tetrahymena* sortilin genes were identified (FIG. **2**). The identified genes were SOR1, SOR2, SOR3, and SOR4, corresponding to NCBI accession nos. XM_001033316.2, XM_001020814.3, XM_001025035.2 and XM_001033494.2, each incorporated herein by reference.

To further determine the function of the SOR genes vectors were constructed to knockout each of the genes *Tetrahymena* (by homologous recombination targeting to the SOR ORFs). The vectors used in the studies are provided as SEQ ID NOs: 9-12, for targeting SOR1, SOR2, SOR3 and SOR4 respectively. Following transformation, knockout cells were successfully isolated for SOR1, SOR2 and SOR4. Studies shown in FIG. **3** confirm that in each case the knockout lines lack detectable expression of the indicated sortilin RNA. Moreover, knockout of the sortilin genes hampered proteolytic processing in the knockout cells. As shown in FIG. **5**, unprocessed forms of Grl1p (pro-Grl1p) were observed in the media of the knockout lines, but not in that of wild type cells or a knockout of the Rab32 gene. Thus the sortilin knockouts result in an inability to effect the normal proteolytic processing of granule proteins.

Immunefluorescence studies were also performed to visualize granules in knockout and wild type cells. Results, shown in FIG. **5**, demonstrate that wild type granules are elongated, a shape that is generated by the proteolytic processing of the content proteins. The granules in the sortilin knockout lines are spherical, consistent with the failure to proteolytically process the contents.

* * *

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Pat. No. 6,846,481

U.S. Pat. No. 6,087,124

U.S. Patent Appln. 2006/0127973

Becker and Rusing, *J. Eukaryot. Microbiol.,* 50:235-239, 2003.

Blomberg et al., *Mol. Cell. Biol.,* 12:7237-747, 1997.

Boldrin et al., *Eukaryot Cell,* 2:422-425, 2006.

Bruns and Cassidy-Hanley, *Meth. Cell Biol.,* 62:501-512, 2000.

Bruns et al., *Proc. Natl. Acad. Sci. USA,* 9:2844-286, 1985.

Cassidy-Hanley et al. *Genetics,* 146:135-147, 1997.

Cowan et al., *Mol. Cell. Biol.,* 16:4046-4060, 2005.

de Coninck et al., *J. Industr. Microbiol. Biotechnol.,* 24:285, 2000.

European Patent EP 847 444

Fankel, *Meth. Cell Biol.,* 62:27-125, 2000.

Gaertig and Gorovsky *Proc. Natl. Acad. Sci. USA,* 89:9196-9200, 1992.

Gaertig and Kapler, *Meth. Cell Biol.,* 62:486-500, 2000.

Gaertig et al. *Nucleic Acids Res.,* 22:5391-5398, 1994.

Gaertig et al., *Nature Biotech.,* 17:462-465, 1999.

Hai et al., *Meth. Cell Biol.,* 62:513-531, 2000.

Hellenbroich et al. *Appl. Microbiol. Biotechnol.,* 51:447, 1999.

Langer, 3^{rd} Annual Rept and Surv. Biopharm. Manufact. Capacity and Prod., BioPlan Assoc., Inc. (2005

Lin et al. *Gene,* 288(1-2):85-94, 2002.

Melia et al., *J. Cell Sd. H,* 1(Pt 1):131-140, 1998.

Orias et al., *Gene,* 2:295-301, 1988.

Pan et al., *Cell,* 3:595-604, 1982.

Pavlou and Reichert, *Nat. Biotechnol.,* 22:1513-1519, 2004.

PCT Publn. WO 1998/001572

PCT Publn. WO 2003/078566

PCT Publn. WO 2007/006812

PCT Publn. WO 2010/108182

Shang et al., *Proc. Natl. Acad. Sci. USA,* 6:3734-379, 2002.

Spangler and Blackburn, *J. Biol. Chem.,* 10:6334-6340, 1985.

Taniguchi et al., *J. Biol. Chem.,* 260:13941-13946, 1985.

Tondravi and Yao, *Proc. Natl. Acad. Sci. USA,* 83:4369-4373, 1986.

Turkewitz et al., *Meth. Cell Biol.,* GI-MI-IGI, 2000.

Weide et al., *BMC Biotechnol.,* 6:19, 2006.

Williams et al., *J. Biol. Chem.,* 255:296-303, 1980.

Yu and Blackburn, *Proc. Natl. Acad. Sci. USA,* 21:8487, 891, 1989.

```
                              SEQUENCE LISTING


<160> NUMBER OF SEQ ID NOS: 12

<210> SEQ ID NO 1
<211> LENGTH: 893
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 1

Met Lys Ala Tyr Lys His Leu Gln Leu Ile Gly Ile Val Leu Leu Ile
1               5                   10                  15

Ser Ala Leu Gln Phe Thr Ala Thr Ala Lys Gln Gln Asp Ile Ser Phe
            20                  25                  30

Ser Lys Asn Phe Leu Asp Ser Glu Ile Val Asp Val Ile Trp Cys Gly
        35                  40                  45

Thr Asp Thr Gln Asn Asp Gln Asn Val Leu Val Gln Thr Asp Ser Gly
    50                  55                  60

Thr Ile Tyr Arg Ser Gln Asn Lys Met Val His Phe Glu Asn Ile Ser
65                  70                  75                  80

Asp Asn Leu Val Asn Ala Gly Ile Lys Tyr Val Ala Asp Asn Ser Gln
                85                  90                  95

Ile Val Glu Ser Glu Val Ile Arg Met Ile Arg Ser Gln Ala Asn Pro
            100                 105                 110

Asn Val Ile Val Leu Gln Gly Lys Asn Glu Val Asn Trp Val Thr Arg
        115                 120                 125

Asp Cys Gly Asn Thr Phe Arg Ala Phe Ser Arg Lys Lys Asp Arg Ile
    130                 135                 140

Asn Thr Phe Lys Leu His Pro Ser Gln Glu Ala Trp Met Leu Ala Ser
145                 150                 155                 160

Thr Asn Asn Val Cys Ala Lys Ser Gln Lys Ala Pro Cys Phe Ser Phe
                165                 170                 175

Ala Ile Leu Trp Leu Ser Lys Asp Leu Gly Asn Ser Trp Glu Lys Leu
            180                 185                 190

Thr Gln Tyr Val Tyr Lys Phe Glu Trp Gly Asn Leu Asn Phe Thr Asn
        195                 200                 205

Ser Gln Val Pro Gln Gln Arg Ile Phe Trp Val Gln Glu Asp Gly Asn
    210                 215                 220

Lys Gln Asn Gln Asn Arg Tyr Gly Leu His Glu Lys Arg Asn Phe Tyr
225                 230                 235                 240

Tyr Ser Asp Asp Phe Leu Ala Ser Lys Lys Leu Leu Met Thr Lys Gly
                245                 250                 255

Asn Val Phe Tyr Ile Asp Tyr Asn Tyr Leu Tyr Val Val Gln Leu Leu
            260                 265                 270

Glu Gln Asn Ser Gln Gln Val Asn Leu Lys Val Ala Asn Pro Gln Asp
        275                 280                 285

Leu Asp Ile Lys Leu Arg Asp Val Gln Leu Gly Glu Lys Leu Gln Asn
    290                 295                 300

His Lys Phe Thr Ile Leu Asp Thr Arg Glu Gly Gln Val Phe Leu Asn
305                 310                 315                 320

Val Asn His Leu Gly Ser Thr Ser Pro Met Gly Thr Leu Tyr Ile Ser
                325                 330                 335

Asp Ser Leu Gly Ala Arg Phe Ser Ser Ser Leu Gln Gly His Leu Arg
            340                 345                 350

Ser Glu Asn Gly Asp Thr Asp Phe Glu Arg Leu His Gly Ile Tyr Gly
        355                 360                 365
```

-continued

```
Ile Tyr Ile Ala Asn Val Tyr Glu Gln Lys Arg Arg Glu Glu Phe Glu
    370                 375                 380

Asn Met Tyr Ala Ser Glu Gln Asn Asp Asp Asp Glu Asn Gln Gly Gln
385                 390                 395                 400

Asp Ser Lys Asn Lys Lys Ser Asn Thr Ser Ile Lys Gln Asp Lys Lys
                405                 410                 415

Ala Val Lys Met Lys Asp Leu Val Thr Gln Lys Ile Gln Thr Met Ile
            420                 425                 430

Thr Phe Asp Lys Gly Gly Met Trp Ser Arg Ile Asn Ala Pro Thr Thr
        435                 440                 445

Asp Gln Glu Asn Lys Glu Ile Lys Cys Gly Asp Asn Cys Phe Leu Asn
    450                 455                 460

Ile His Ser Asn Ser Asn Asp Leu Tyr Asn Ser Phe Tyr Ser Ser Lys
465                 470                 475                 480

Asn Ala Val Gly Leu Val Leu Ala Asn Gly Asn Val Gly Lys Tyr Leu
                485                 490                 495

Ser His Ser Pro Thr Gln Val Asn Thr Tyr Leu Ser Arg Asp Ala Gly
            500                 505                 510

Leu Thr Trp Lys Gln Val Ile Gln Asn Gln Asp Leu Thr Ser Tyr Leu
        515                 520                 525

Phe Ile Leu Ser Met Ile Gln Lys Ile Lys Arg Gly Ala Tyr Val Phe
    530                 535                 540

Glu Ile Gly Asp His Gly Ser Ile Ile Val Met Ala Lys Asp Lys Asp
545                 550                 555                 560

Tyr Gly Thr Thr Lys Phe Ile Glu Tyr Thr Leu Asp Glu Gly Ile Thr
                565                 570                 575

Trp Asn Gln Val Gln Ile Ser Asp Thr Asp Ile Glu Ile Asp Asn Ile
            580                 585                 590

Ile Thr Glu Pro Ser Asn Thr Gly Thr Ser Phe Met Val Leu Ala Lys
        595                 600                 605

Thr Leu Ser Thr Asp Lys Lys Gln Tyr Gly Leu Ala Ile Thr Ile Asp
    610                 615                 620

Phe Ala Asn Gln Phe Asn Arg Asn Cys Ser Gly Ala Thr Ser Pro Asp
625                 630                 635                 640

Asp Pro Asp Ser Asp Tyr Glu Lys Trp Ile Pro His Ser Tyr Lys Ser
                645                 650                 655

Ser Gln Cys Leu Leu Gly Gln Lys Val Thr Tyr Ser Arg Lys Lys Gln
            660                 665                 670

Glu Ser Val Cys Leu Asn Gly Glu Asp Tyr Glu Arg Gln Ile Glu Leu
        675                 680                 685

Gln Ala Cys Val Cys Ser Glu Glu Asp Trp Glu Cys Asp Ile Gly Tyr
    690                 695                 700

Ile Arg Asn Gly Gln Asn Gly Pro Cys Val Lys Asp Gly Thr Leu Ser
705                 710                 715                 720

Asp Glu Glu Tyr Glu Gly Val Ile Pro Glu Ile Cys Thr Asp Tyr Tyr
                725                 730                 735

Gln Val Ser Arg Gly Tyr Arg Lys Ile Pro Tyr Asn Thr Cys Gln Gly
            740                 745                 750

Gly Val Asn Tyr Ser Ala Glu Thr Arg Arg Cys Pro Gly Asn Ser Ile
        755                 760                 765

Phe Ser Phe Asn Thr Leu Lys Asn Leu Ile Leu Leu Ile Leu Ala Ile
    770                 775                 780

Ala Ala Ile Tyr Tyr Gly Ile Gln Tyr Lys Ser Gln Leu Ser Ser Leu
```

```
785                 790                 795                 800

Leu Ile Tyr Leu Ser Ser Leu Ile Pro Leu Ile Tyr Ser His Arg Lys
                805                 810                 815

Asp Tyr Ile Asp Phe Ser Lys Ala Lys Ser Asp His Glu Glu Lys Glu
            820                 825                 830

Asn Lys Phe Met Asn Leu Phe Ser Phe Ser Asn Lys Lys Asn Val Asn
        835                 840                 845

His Tyr Ser Asn Val Asn Glu Ser Glu Asp Tyr Glu Asp Ser Glu Asp
    850                 855                 860

His Gln His Leu Asn Asn Gln Asn Tyr Asn His Leu Asn Gln His Asn
865                 870                 875                 880

Tyr Phe Thr Asp Asn Gln Asp Glu Glu Ser His Tyr Asp
                885                 890


<210> SEQ ID NO 2
<211> LENGTH: 2784
<212> TYPE: DNA
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 2

aaaagtatta atatttagac ttaatgaaag cgtacaagca tttatagctc ataggtattg     60

tattgcttat ttcagctcta cagttcactg caacagctaa atagcaagat atttctttta    120

gcaagaactt tcttgattct gaaatagttg atgtcatttg gtgtggcacc gacacataga    180

atgactaaaa cgttttggtt caaactgata gtggaaccat ttatagatct taaaacaaaa    240

tggtacactt cgaaaatatt agcgataatc ttgttaatgc tggtatcaaa tatgtagctg    300

ataatagtta aatagtagaa agtgaagtta ttagaatgat aagaagtcaa gctaatccta    360

atgttattgt tctttaaggc aaaaatgaag tcaactgggt aacaagagac tgcggtaata    420

catttagagc cttttcaaga aagaaagata gaataaacac atttaagttg catcccagtt    480

aagaagcatg gatgttagca agcactaata acgtttgtgc caaaagctaa aaagctccat    540

gcttttcttt tgctatacta tggttaagta aagatttagg aaatagttgg gagaagctta    600

ctcaatatgt ttacaaattc gaatggggta atttaaactt tactaatagc taagttcctc    660

aacaaagaat attttgggtt taagaagatg gaaataagca aaaccaaaat agatatggat    720

tgcatgaaaa aagaaacttt tattacagcg atgacttttt agcttcaaag aagctactca    780

tgaccaaagg aaatgtcttt tacattgatt ataattacct ttatgttgtt caacttttag    840

aataaaattc atagcaagtt aacctaaaag ttgctaatcc ttaagactta gatattaaat    900

taagggatgt ttagttaggc gagaagctgt aaaaccataa gtttacaatt ttagatactc    960

gtgaaggata ggtttcttta aatgtaaatc atttaggttc aacatctcct atgggtactc   1020

tttatatatc agactcatta ggtgctcgct tttcctcaag cttgtagggt catcttagaa   1080

gtgaaaatgg tgatacagat tttgagcgct tacatggaat ttatggaatt tatatagcaa   1140

atgtttatga ataaaaaaga agagaagagt ttgagaatat gtatgcaagc gaataaaatg   1200

atgatgatga aaattaagga taagactcca aaaataaaaa aagcaataca tcaattaaat   1260

aggataaaaa agcagtaaag atgaaagatt tggtcaccca aaaaatatag acaatgatta   1320

ctttcgataa aggtggtatg tggagtagaa ttaatgctcc aaccacagac taggaaaaca   1380

aagaaattaa atgtggtgac aactgcttct taaatataca ttctaattct aatgacttat   1440

ataattcatt ctactcatca aaaaatgctg taggtttagt tttagcaaat ggaaatgttg   1500

gtaagtatct ttcacatagt ccaactcaag ttaatactta cctttcaaga gatgcaggtt   1560
```

```
taacttggaa ataagtaatt taaaattaag atttaacttc atatttattt attctttcaa   1620

tgatttaaaa gattaaaaga ggagcttatg ttttcgaaat aggtgatcat ggttcaataa   1680

tagttatggc taaagataag gattatggaa ccactaaatt tatcgaatat actttagatg   1740

aaggtattac ttggaaccaa gtttaaatat cagatactga tatcgaaata gataatataa   1800

taacagagcc atcaaatact ggaacctcat tcatggttct tgcaaaaaca ctatcaacag   1860

ataaaaaata atatggatta gctataacaa tagattttgc taatcagttt aatagaaact   1920

gttctggtgc aacaagtcca gatgatcctg attctgatta tgaaaaatgg atacctcata   1980

gctataaatc atctcaatgt ctcttaggtt agaaagtgac ttactcacgt aaaaaacaag   2040

aatctgtttg cttaaatgga gaagattatg aaagacaaat agaactttaa gcatgtgtct   2100

gctctgaaga agactgggag tgtgatatcg gctatattag aaatggataa aatggtccat   2160

gtgtaaagga tggaacactt agtgatgaag aatatgaagg agtgatccca gaaatatgta   2220

ctgattatta ttaagtaagt agaggttata gaaaaattcc ttacaataca tgctaaggag   2280

gtgtaaatta ttcagcggaa actagaagat gccctggaaa ttcaattttt agctttaata   2340

ctttaaaaaa tttgattctt ttgattttag ctattgcagc tatttattat ggaattcagt   2400

ataagagtta actctctagc ttgttaattt acttaagttc tttgatccct ctaatttatt   2460

ctcatcgtaa agattatatt gacttttcca aagcaaagtc agaccatgaa gaaaaggaaa   2520

ataaatttat gaatctattt tcatttagca acaaaaaaaa tgttaatcat tacagcaacg   2580

taaatgaaag tgaagattat gaagatagtg aagatcatta acatcttaat aaccaaaatt   2640

acaatcattt aaattaacat aactatttta ctgataacca agatgaagag agtcattatg   2700

attgaattta attaactaat tgatttttg ttttttcata aatttctttg tagattaatt   2760

taatttaaaa ataattttaa tagt                                          2784


<210> SEQ ID NO 3
<211> LENGTH: 893
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 3

Met Lys Ile Lys Arg Asn Gln Gln Ile Ala Ile Ile Phe Ala Ile Phe
1               5                   10                  15

Ile Leu Thr Ala Ile Gln Ala Ala Asp Asp Val Ala Asp Asp Lys Val
            20                  25                  30

Gln Gln Ala Ile Lys Ser Tyr Gln Lys Gln Val Asp Gly Gly Ile Leu
        35                  40                  45

Glu Phe Glu Trp Cys Gly Thr Asn Glu Ile Tyr Asn Asp Glu Thr Asp
    50                  55                  60

Arg Val Val Val Asp Gln Glu Val Glu Glu Ser Phe Asp Thr Arg Ile
65                  70                  75                  80

Phe Val Leu Thr Asp Glu Gly Gln Val Phe Lys Ser Thr Asn Tyr Gly
                85                  90                  95

Lys Ser Trp Val His Val Thr Lys Ser Phe Tyr Gly Ser Asn Asn Gln
            100                 105                 110

Pro Phe Phe Ser Thr Glu Val Ser Ile Ser Pro Val Asp Gly Lys Thr
        115                 120                 125

Val Tyr Ile Trp Gly His Lys Asp Thr Ser Tyr Val Ser Glu Glu Cys
    130                 135                 140

Gly Lys Thr Trp Lys Lys Leu Asn His Pro Ala Gly Leu Phe Asp Phe
```

-continued

```
145                 150                 155                 160

Arg Phe His Arg Lys Asn Lys Asn Trp Val Leu Ala Phe Thr Asn Ile
                165                 170                 175

Glu Cys Lys Arg Phe Asp Glu Asp Cys Glu Ser Asn Met Arg Asn Leu
            180                 185                 190

Tyr Val Ser Gln Asp Ala Gly Val Thr Phe Thr Phe Leu Ala Thr Lys
        195                 200                 205

Val Leu Glu Ala Ser Trp Asn Arg Met Asn Asn Phe Tyr Asn Val Asp
    210                 215                 220

Ser Pro Gly Ile Leu Met Ala Val Gln Gln Glu Ser Gln Ser Asn Val
225                 230                 235                 240

Val Tyr Thr Glu Asp Phe Gly Lys Thr Met His Thr Val Gln Glu Gly
                245                 250                 255

Gly Asp Asn Phe Phe Gln Ala Glu Tyr Phe Leu Phe Leu Thr Val Lys
            260                 265                 270

Pro Lys Asn Ser Lys Arg Thr Tyr Asp Met Lys Ile Ala Thr Met Phe
        275                 280                 285

Asp Asp Phe Asn Tyr Tyr Val Glu Pro Lys Ser Leu Lys Leu Pro Phe
    290                 295                 300

Glu Asn Thr Asp Gln Leu Ser Phe Thr Ile Leu Lys Ser Asp Gly Ala
305                 310                 315                 320

Met Val Phe Leu Ala Ile His His Glu Thr Gln Asn Met Trp Gln Ser
                325                 330                 335

Asn Ile Tyr Val Ser Asp Trp Arg Gly Tyr Asp Leu Thr Leu Ala Leu
            340                 345                 350

Leu Tyr Asn Val Arg Ala Pro Asn Gly Asp Cys Asp Phe Glu Lys Ile
        355                 360                 365

Glu Ser Asn Glu Gly Val Tyr Ile Ala Asn Thr Tyr Asp Val Glu Lys
    370                 375                 380

Val Glu Lys Leu Arg Asn Glu Val Lys Lys Met Asp Ile Ser Thr Ala
385                 390                 395                 400

Lys Asn Lys Leu Gln Thr Lys Asp Lys Lys Asn Leu His Lys Glu Leu
                405                 410                 415

Thr Asn Tyr Arg Lys Ser Val Ile Ser Phe Asp Ser Gly Ser Ser Trp
            420                 425                 430

His Pro Ile Arg Ala Pro Ser Gln Arg Trp Asn Gly Lys Thr Val Val
        435                 440                 445

Cys Ser Gly Glu Cys Ser Leu His Leu Ala Gly Arg Thr Tyr Tyr Lys
    450                 455                 460

Lys Ser Gln Met Tyr Ser Ser Ser Asn Ala Pro Gly Leu Ile Val Ala
465                 470                 475                 480

Leu Gly Ser Ile Gly Thr His Leu Glu Asn Asn Phe Asn Leu Leu Asn
                485                 490                 495

Thr Tyr Leu Ser Asn Asp Gly Gly His Gln Trp Arg Glu Ile Leu Lys
            500                 505                 510

Gly Pro His Ile Phe Glu Ile Gly Asp His Gly Gly Ile Ile Val Ala
        515                 520                 525

Ala Ser Val Ala Asn Lys Thr Asn Ile Ile Lys Tyr Ser Trp Asp Glu
    530                 535                 540

Gly Lys Thr Trp Ser Glu Tyr Lys Leu Ser Ala Leu Pro Phe Glu Ile
545                 550                 555                 560

Asp Gln Ile Ile Thr Glu Pro Ser Asn Met Glu Gln Arg Phe Val Val
                565                 570                 575
```

```
Tyr Gly Lys Gly Arg Asn Gly Thr Glu Thr Ser Met Ile Val Ser Val
            580                 585                 590

Asp Leu Gln Asp Leu His Ile Arg Gly Cys Val Gly Ala Glu His Pro
        595                 600                 605

Asn Arg Pro Asn Ser Asp Tyr Glu Ile Trp Ile Pro Thr Asn Phe Lys
    610                 615                 620

Gly Glu Gln Cys Ile Phe Gly Arg Lys Val Lys Tyr Val Arg Arg Lys
625                 630                 635                 640

Pro Asp Ala Lys Cys Phe Asn Ser Ile Thr Thr Asp Gln Lys Thr Val
                645                 650                 655

Ile Glu Glu Cys Pro Cys Thr Gln Glu Asp Trp Glu Cys Asp Phe Gly
            660                 665                 670

Phe Tyr Arg Lys Glu Asn Glu Leu Glu Cys Ile Pro Met Asn Glu His
        675                 680                 685

Tyr Ser Pro Asp Asn Leu Ala Lys Pro Pro Ala Asp Cys Ser Trp Ser
    690                 695                 700

Tyr Leu Val Ser Lys Gly Tyr Arg Lys Ile Pro Gly Val Phe Cys Gln
705                 710                 715                 720

Gly Gly Val Asp Leu Ser Pro Glu Tyr Lys Glu Cys Pro Pro Lys Ile
                725                 730                 735

Ser Val Pro Arg Thr Glu Glu Glu Thr Asp Gln Tyr Lys Ser Phe Lys
            740                 745                 750

Glu Ala Gln Lys Glu Ile Ile Ser Gln Tyr Gln Gln Gln Gln Gln Gln
        755                 760                 765

Ser Asn Ser Gln Asn Gly Lys Thr Asp Ser Ser Ser Ser Ile Asn Trp
    770                 775                 780

Gly Val Ile Phe Thr Gln Ile Phe Tyr Ala Gly Leu Ile Leu Thr Ala
785                 790                 795                 800

Leu Ala Leu Ala Phe Ile Phe Arg Glu Asn Ile Lys Gln Val Val Lys
                805                 810                 815

Ser Ile Gly Glu Ile Gly His Asn Lys Glu Arg Lys Gln Tyr Gln Gln
            820                 825                 830

Leu Gln Ser Ser Gln Asn Lys Gln Ser Ser Tyr Thr Gln Gln Lys Asn
        835                 840                 845

Thr Gln Asn Val Arg Ile Gln Glu Thr Glu Glu Arg Asn Tyr Asp Leu
    850                 855                 860

Glu Glu Gln Asp Met His Tyr Pro Glu Asp Glu Lys Pro Val Leu Gln
865                 870                 875                 880

Arg Asp Gln Glu Asp Tyr Tyr Tyr Gln Glu Asp Tyr Asp
                885                 890


<210> SEQ ID NO 4
<211> LENGTH: 2682
<212> TYPE: DNA
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 4

atgaaaataa aaaggaatta gcaaattgca attatatttg ctattttcat cttgactgct      60

atttaggcag cagatgatgt tgcagatgat aaggtttagt aagctataaa aagttattaa     120

aagtaagtag atggaggtat tttagaattc gagtggtgtg gtacaaatga aatttataac     180

gatgaaactg accgtgttgt tgttgattaa gaagttgaag aatcattcga tactcgtata     240

tttgttctta cagatgaagg ttaagttttt aaaagtacaa actatggtaa aagttgggtc     300
```

-continued

```
catgtcacta aatcctttta tggttcaaat aattagccat ttttctctac tgaagtttcc    360

atttctcctg ttgatggtaa aacagtctat atttggggac acaaggatac cagctatgtt    420

tctgaggaat gtggtaagac ttggaaaaag ttaaaccatc ctgctggttt gtttgatttt    480

agatttcacc gtaaaaataa aaattgggta ttagctttca ctaatataga atgtaagaga    540

tttgatgaag attgtgaatc taatatgaga aatctttacg tttcttaaga tgcgggtgtt    600

actttcacat tcttagctac taaagtttta gaagcttcat ggaatagaat gaataacttt    660

tacaacgttg acagtcctgg tattttaatg gccgttcaat aagaatcata aagtaatgta    720

gtttacactg aagacttcgg taaaactatg cacacagttt aagaaggtgg tgataatttc    780

ttttaagcag agtacttcct ctttttaaca gttaagccta aaaacagtaa aagaacctat    840

gatatgaaaa tcgcaactat gtttgacgat tttaattact atgttgaacc caaaagctta    900

aagcttccct ttgaaaacac tgattaactt tcgtttacaa ttctaaagag cgatggtgcc    960

atggttttcc ttgccataca ccacgaaact caaaatatgt ggtaaagcaa tatctatgtt   1020

tctgattgga gaggttatga tttgacttta gctttacttt acaatgttag agctccaaac   1080

ggagattgcg actttgaaaa gatagaaagc aatgaaggtg tttatatagc aaatacatat   1140

gatgttgaaa aagttgaaaa attaagaaac gaagttaaaa aaatggatat cagcactgca   1200

aagaataaat tataaacaaa agataaaaag aatttgcaca aagaactaac taattatagg   1260

aaatcagtca tttcatttga cagcggttct agttggcatc caattagagc tccttcatag   1320

agatggaatg gaaagactgt tgtttgcagt ggagaatgca gtttgcattt agctggtaga   1380

acatattata aaaaatctta gatgtattct tcctctaacg ctcctggttt aattgttgca   1440

ttaggaagca ttggaactca tcttgaaaac aacttcaatc ttcttaacac atatctttca   1500

aacgatggtg gtcactaatg gcgtgaaatt cttaagggtc ctcatatttt tgaaattggt   1560

gatcatggtg gtatcatcgt agctgcttct gttgccaata aaacaaatat catcaaatac   1620

agttgggatg aaggaaaaac atggagcgaa tataaattga gtgctttacc atttgaaata   1680

gattaaataa ttactgagcc tagcaatatg gaacagagat ttgttgttta tggaaaagga   1740

agaaatggaa cagaaacttc tatgattgtt tctgtagatt tataagattt gcacattaga   1800

ggttgtgtag gagctgaaca tcctaataga cctaatagtg attatgaaat ctggattcct   1860

actaatttta aaggtgaaca atgtattttc ggtcgtaaag ttaaatatgt tagaagaaag   1920

cctgatgcaa aatgctttaa ttctatcaca acagattaaa aaacagttat tgaagaatgc   1980

ccatgcacat aagaagattg ggaatgtgac ttcggtttct acagaaaaga aaacgaatta   2040

gaatgtattc caatgaatga gcattattct cctgataatc ttgctaaacc tcctgcagat   2100

tgtagttggt cttacttagt ctcaaaggga tatagaaaaa taccaggagt attttgttaa   2160

ggaggtgttg atttaagtcc agaatataaa gaatgtcctc caaaaatatc agtgcctaga   2220

actgaagaag aaacagatta atataaaagc ttcaaagaag cataaaaaga gattattagc   2280

taatattaat agtaatagta gtaatcaaat agttaaaatg gaaaaactga ttcatcatct   2340

tcaataaact ggggtgttat ttttacataa attttctatg ctggattaat tttaacagct   2400

ttagctttag ctttcatatt tagagagaat atcaaataag tagtaaaaag cattggtgaa   2460

ataggacata ataaagaacg caaataatat taataactct aatcatctta gaataaataa   2520

tcatcataca cttaatagaa aaatactcaa aatgtccgca tttaagaaac tgaagaaaga   2580

aattatgatt tagaagaata agacatgcat tatccagaag atgaaaagcc tgtcttgtaa   2640

agagatcaag aagattacta ttattaagaa gattacgatt ga                      2682
```

```
<210> SEQ ID NO 5
<211> LENGTH: 936
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 5

Met Lys Lys Glu Ile Arg Ile Ala Leu Ile Ala Leu Phe Cys Cys Ile
1               5                   10                  15

Leu Thr Val Asn Cys Arg Asn Glu Tyr Ser Ser Ser Val Ile Gly Asn
            20                  25                  30

Pro Ser Ser Leu Asp Ser Pro Leu Gln Asp Ile Gln Trp Cys Gly Glu
        35                  40                  45

Asn Ser Ser Asn Asp Asn Leu Val Val Leu Leu Thr Gln Lys Gly Ser
    50                  55                  60

Val Tyr Arg Ser Glu Asp Arg Gly Ala Ser Trp Ile Lys Met Val Asp
65                  70                  75                  80

Ser Phe Ala Arg Val Gly Val Asn Val Lys Met Asp Leu Ser Ser Asn
                85                  90                  95

Val Gly Ile Val Thr Gln Met Ile Ala Ser Pro Ile Asp Ser Asn Glu
            100                 105                 110

Ile Val Phe Met Gly Ser Asp Gly Ile Asn Trp Ile Thr Thr Asp Cys
        115                 120                 125

Gly Val Thr Ile Gln Ala Leu Gly Ile Asn Leu Asn Leu Arg Glu Phe
    130                 135                 140

Met Tyr His Pro Thr Glu Lys Asn Trp Met Leu Ala Ser Ser Phe Asn
145                 150                 155                 160

Asn Cys Glu Lys Gln Asn Asn Gln Lys Asp Lys Arg Lys Lys Asp Thr
                165                 170                 175

Glu Cys Phe Lys Thr Lys Asp Leu Phe Phe Ser Glu Asn Lys Gly Lys
            180                 185                 190

Ser Trp Arg Val Leu Leu Lys Tyr Val Val Gln Phe Gly Trp Ala His
        195                 200                 205

Lys Val Asn Ser Lys Leu Thr Asn Val Pro Thr Ser Arg Ile Ile Tyr
    210                 215                 220

Ser Lys Glu Val Gly Ser Asn Ser Phe Phe Phe Asn Glu Ala Ser Gln
225                 230                 235                 240

Gln Thr Asn Ile Ile Ile Lys Asp Ser Gly His Gln Val Met Lys Gly
                245                 250                 255

Trp Ser Met Lys Thr His Leu Phe Tyr Thr Asp Asp Phe Met Lys Asn
            260                 265                 270

Gln Asn Met Ile Val Asn Gln Gly Asn Lys Phe Leu Ile Thr Glu Asn
        275                 280                 285

Tyr Leu Phe Ala Ala Gln Val His Ser Ser Asp Asn Gln Leu Val Lys
    290                 295                 300

Leu Met Val Ser Gln Ser Asn Gln Lys Glu Tyr Ser Phe Thr Tyr Ala
305                 310                 315                 320

Glu Ile Pro Glu Asp Ile His Gln His Ser Phe Thr Ile Leu Asp Thr
                325                 330                 335

Lys Glu Gly Gln Val Phe Leu Asn Ile Asn His Leu Gly Ser Asn Ser
            340                 345                 350

Pro Met Gly Asn Ile Tyr Gln Ser Asp Ser Thr Gly Thr Arg Phe Ser
        355                 360                 365

Leu Ser Leu Glu Asp Asn Val Arg Gly Arg Asp Gly Gln Cys Asp Phe
```

-continued

```
    370                 375                 380

Glu Ser Val Asn Gly Val Glu Gly Ile Phe Ile Ser Asn Ile Phe Ala
385                 390                 395                 400

Pro Ser Lys Lys Leu Lys Gly Ile Lys Gln Met Leu Lys Ser Lys Asn
                405                 410                 415

Pro Asp Thr Ser Asp Glu Asp Ile Pro Thr Glu Asn Thr Arg Lys Lys
            420                 425                 430

Gly Gln Ala Gln Asn Ser Glu Asp Val Leu Lys Glu Ser Leu Lys Ser
        435                 440                 445

Leu Arg Asp Asn Met Val Thr Arg Ile Thr Phe Asp Lys Gly Gly Met
    450                 455                 460

Trp Ser Leu Leu Arg Ala Pro Ala Lys Asp Ser Asn Gly Lys Gln Ile
465                 470                 475                 480

Asn Cys Asp Ile Asn Lys Lys Cys Ser Leu His Leu His Ser Val Ser
                485                 490                 495

Ser Gln Leu Ser Phe Gly Pro Ala Tyr Ser Ser Glu Asn Ser Leu Gly
            500                 505                 510

Leu Ile Ile Ala Thr Gly Asn Thr Gly Gln Phe Leu Ser His Lys Ala
        515                 520                 525

Gly Ser Val Asn Thr Tyr Leu Ser Arg Asp Gly Gly Leu Val Trp Glu
    530                 535                 540

Glu Ile Arg Lys Gly Ser His Ile Tyr Glu Val Ala Asp His Gly Ser
545                 550                 555                 560

Ile Ile Val Met Ala Thr Asp Gln Glu Pro Thr Lys Asn Ile Ile Phe
                565                 570                 575

Ser Trp Asp Glu Gly Arg Thr Trp Asn Thr Lys Gln Ile Ser Asp Thr
            580                 585                 590

Pro Val Met Ile Ser Asn Ile Ile Thr Glu Pro Gly Asn Thr Ser Asp
        595                 600                 605

Lys Phe Leu Val Tyr Gly Ser Ile Glu Gly Glu Ser Asp Ile Ser Gly
    610                 615                 620

Ile Ile Val Leu Leu Asp Phe Ala Ser Leu His Pro Arg Asp Cys Gln
625                 630                 635                 640

Gly Tyr Glu Asn Pro Asp Thr Ser Asp Ser Asp Tyr Glu Tyr Trp Thr
                645                 650                 655

Pro His Asn Pro Ser Glu Phe Cys Leu Leu Gly Arg Glu Ile Lys Tyr
            660                 665                 670

Val Arg Arg Lys Arg Asp Ala Ala Cys Phe Asn Pro Glu Thr Phe Glu
        675                 680                 685

Arg Ser Tyr Val Val Arg Lys Cys Glu Cys Thr Glu Leu Asp Trp Glu
    690                 695                 700

Cys Asp Val Gly Phe Ala Arg Ala Lys Asp Asp Ser Lys Glu Arg Thr
705                 710                 715                 720

Gly Pro Cys Val Pro Leu Lys Asp Phe Lys Val Asp Tyr Asn Pro Pro
                725                 730                 735

Gln Thr Cys Ser Gly Ser Tyr Gln Val Thr Gln Gly Tyr Arg Arg Val
            740                 745                 750

Ala Gly Asn Gln Cys Ile Gly Gly Ile Asp His Ala Pro Ile Gln Tyr
        755                 760                 765

Pro Cys Pro Met Phe Gly Phe Leu Ser Tyr Asn Asn Leu Phe Thr Asn
    770                 775                 780

Val Leu Ile Leu Gly Ala Met Ala Gly Val Phe Tyr Leu Ile Ile Gln
785                 790                 795                 800
```

-continued

```
Asn Lys Glu Val Val Ile Thr Phe Val Ala Thr Ser Asn Leu Asp Ala
                805                 810                 815

Tyr Ile Asn Leu Gly Lys Thr Tyr Leu Lys Lys Gly Tyr Thr Phe Val
            820                 825                 830

Thr Ser Ile Val Leu Pro Gln Ala Ser Asn Gln Gln Gln Gly Tyr Phe
        835                 840                 845

Gln Ala Asn Gln Asp Glu Glu Asn Arg Lys Ser His Ser Leu Lys Asp
    850                 855                 860

Gln His His Gln Phe His Asp Asn Leu Ile Glu Ser His Asp His Asp
865                 870                 875                 880

Asp Glu Glu Glu Gln Ser Asp Ala Val Gln Gln Gln Leu Thr Ser Ser
                885                 890                 895

Gln Val Pro Gln Asn Asn Ser Asn Lys Asn Asn Asn Asn Ser Asn Thr
            900                 905                 910

Pro Asn Gln Ala Gln His Lys Asp Leu Leu Asp Glu His Asp Gly Glu
        915                 920                 925

Glu Asp Pro Phe Asp Pro Arg Asn
    930                 935


<210> SEQ ID NO 6
<211> LENGTH: 3010
<212> TYPE: DNA
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 6

atgaaaaaag aaataagaat agctcttata gctttatttt gctgcatttt gacagtaaat      60

tgtagaaatg aatactcaag cagtgtcatt ggaaacccct caagtttgga ttcacctctt     120

taggacattt aatggtgtgg tgaaaattca tcaaatgata atttggttgt cctcttaact     180

taaaagggta gcgtttacag atcagaagat agaggagcat cttggataaa gatggttgac     240

tcttttgcga gagttggtgt aaatgtaaag atggatctga gctcaaacgt aggtattgtt     300

acttaaatga ttgcaagtcc tattgattct aatgaaatag tctttatggg ctctgatggt     360

attaactgga tcactactga ttgtggtgtt accatttaag cccttggaat caacttaaat     420

ttgagagaat ttatgtatca cccaactgaa aagaattgga tgcttgcttc ttcctttaac     480

aactgtgaaa agcaaaacaa ccaaaaagat aagagaaaaa aggacactga atgctttaag     540

actaaagatt tgtttttctc tgaaaataag ggtaaaagct ggagagtttt acttaaatat     600

gttgtacaat tcggatgggc tcacaaagtt aattctaagc taacaaatgt cccaacttca     660

agaattatat actctaagga agtcggaagt aattcgtttt tctttaatga agcatctcaa     720

taaactaata taataataaa agatagtggt caccaagtga tgaagggttg gagcatgaaa     780

actcatttat tctatactga tgatttcatg aaaaactaga atatgattgt taactaagga     840

aataagtttt tgattactga aaactacttg ttcgctgcat aagttcacag tagtgataat     900

taactagtca agttaatggt ttcttaatct aattaaaaag aatactcttt cacttatgct     960

gaaattcctg aagatataca ctagcactca ttcactattt tagatactaa ggaaggttag    1020

gtattcttaa atattaatca cttgggcagt aactctccta tgggtaatat ttactaatct    1080

gactcaactg gtactcgttt ctctctttct cttgaagata atgtaagagg aagagatggt    1140

taatgcgatt ttgaatcagt taatggtgtt gaaggtattt ttatctcaaa tatattcgct    1200

cctagcaaaa agttaaaggg tatcaagcaa atgttgaaat ccaaaaatcc tgatacaagc    1260

gatgaagata ttccaactga aaacacaaga aagaaaggtc aagcataaaa ttctgaagat    1320
```

-continued

```
gtcttaaaag aatccttaaa aagtcttaga gataacatgg taactcgtat cactttcgac   1380

aagggtggta tgtggagttt gcttagggct cctgctaaag attctaatgg aaaataaatt   1440

aattgtgata ttaataaaaa gtgttctctt caccttcact cagtttcttc ataactaagt   1500

tttggacctg cttactcaag tgaaaattca ttaggtttaa ttattgctac tggtaacaca   1560

ggataattct taagtcataa agcaggtagc gtcaacactt atctttctcg tgatggtggt   1620

cttgtttggg aagaaatccg taaggggtct cacatatatg aagttgctga tcatggctct   1680

atcatagtta tggctactga ttaagaacct actaagaaca ttattttctc ttgggatgaa   1740

ggccgcacat ggaacaccaa gtaaattagc gatactcctg tcatgatttc aaatattatc   1800

actgaacctg gcaatacttc tgacaagttc ttagtttatg gatctattga aggtgaatct   1860

gatatttcag gaataattgt ccttcttgac tttgcttctc ttcatcctcg cgattgctaa   1920

ggttatgaaa accctgacac ttctgattct gattatgaat actggactcc tcataatccc   1980

agtgaattct gtttattagg acgtgaaatt aaatatgtca gaagaaaaag agatgctgct   2040

tgctttaatc ccgaaacttt tgaaagatct tatgttgtta gaaaatgtga atgtactgaa   2100

cttgattggg aatgtgatgt cggatttgct cgtgctaaag acgatagcaa agaaagaact   2160

ggcccttgcg ttcctttaaa agacttcaaa gtggattaca atcctccata aacttgcagt   2220

ggctcttacc aagttacata aggttacaga agagtagctg gtaattaatg tataggcggt   2280

attgatcatg ctccaattta ataccttgt cctatgtttg gcttcttgag ctataacaac    2340

cttttcacca atgttcttat tttaggagct atggctggtg ttttctactt aattatataa   2400

aataaagaag tagtaataac atttgtagct acatcaaatc ttgatgccta cattaactta   2460

ggtaaaactt acctaaagaa gggttatact tttgttacat caattgtcct tccacaagct   2520

tcaaattaat aataaggata tttccaagct aaccaagatg aggaaaatag aaaatctcat   2580

tccttaaagg atcaacatca ttaattccat gataatttaa ttgaaagcca tgatcatgat   2640

gatgaggaag agtaaagtga tgcagtataa taataattaa cttcttctta agtcccttaa   2700

aataatagta acaaaaacaa taataatagt aatacaccaa actaagctca gcacaaagat   2760

cttcttgatg aacatgatgg tgaagaagat ccttttgatc ctagaaattg aaaaataatt   2820

gactgaataa tattgctaat ttattttttt acttaaataa taaataaata aaaataaata   2880

aattaatttt tgtctttcat taatattatt tagaaagttt ttctaagtaa tttaatatag   2940

tgtgtcaagt atctttttct cttaacttat gtattttatc aaatcctttt ttactttatt   3000

attcctagtt                                                          3010


<210> SEQ ID NO 7
<211> LENGTH: 872
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena thermophila

<400> SEQUENCE: 7

Met Lys Lys Gln Asp Leu Thr Val Tyr Val Ala Ala Phe Leu Leu Leu
1               5                   10                  15

Phe Ser Cys Val Ile His Phe Ala Asn Ala Gln Asp Lys Val Ser Glu
            20                  25                  30

Ile Phe Lys Asp Lys Tyr Asp Val Lys Tyr Arg Val Thr Glu Leu Asp
        35                  40                  45

Ser Pro Val Gln Glu Ile Leu Trp Cys Gly Ser Ser Gln Ala Thr Ser
    50                  55                  60
```

-continued

```
Glu Asp Gly Asp Ile Ile Thr Tyr Asp Gln Thr Ala Lys Val Arg Lys
65                  70                  75                  80

Leu Tyr Val Leu Thr Asp Lys Gly Lys Leu Tyr Tyr Ser Glu Asp Tyr
                85                  90                  95

Gly Ile Thr Leu Lys Leu Ile Asn Asp Asp Ile Arg Gln Ser Thr Asn
            100                 105                 110

Ser Lys Gln Thr Gln Val Glu Val Asp Asp Ile Met Ile Ser Pro Val
        115                 120                 125

Lys Asn Arg Lys Val Phe Ile Phe Thr Lys Ser Gly Glu Ser Tyr Tyr
    130                 135                 140

Thr Glu Asn Cys Gly Ala Thr Tyr Thr Ser Phe Lys His Glu Ile Leu
145                 150                 155                 160

Leu Tyr Asp Ile Gln Pro Asn Pro Ser Asp His Lys Ser Leu Ile Gly
                165                 170                 175

Leu Val Pro Val Gln Cys Gln Lys Gly Asp Pro Glu Cys Gln Gly Gly
            180                 185                 190

Asp Ser Asp Leu Tyr Leu Thr Val Asp Ser Gly Met Thr Trp Arg Lys
        195                 200                 205

Ile Val Ser Asn Val Asn Gln Ala Gln Trp Asp Lys Thr Lys Gln Thr
    210                 215                 220

Leu Met Asn Thr Gln Asn Arg Ile Ile Leu Ser His Gln Glu Gln Glu
225                 230                 235                 240

Lys Asn Glu Lys Gly Glu Asn Val Phe Leu Asn Lys Val Ser Tyr Thr
                245                 250                 255

Asp Asn Tyr Gly Lys Asp Leu Lys Val Val Glu Lys Asn Gly Val Arg
            260                 265                 270

Phe Tyr Gln Thr Glu Glu Tyr Ile Phe Val Leu Ile Gln Gly Lys Glu
        275                 280                 285

Phe Gly Lys Tyr Lys Leu Asn Ile Gly Pro Ser Phe Val Thr Gln Ser
    290                 295                 300

Ser Ser Arg Lys Glu Ile Asp Leu Pro Leu Gln Arg Val Lys Asp Glu
305                 310                 315                 320

Ser Phe Thr Val Leu Asp Ile Asp Ala Gly Gln Ile Leu Ile Ala Ile
                325                 330                 335

Asn His Glu Gly Asp Ser Ala Gly Tyr Thr Asn Val Tyr Ile Ser Asn
            340                 345                 350

Ser Gln Gly Glu Gln Phe Thr Leu Ser Leu Gln Tyr Thr Val Gly Asp
        355                 360                 365

Asp Asp Ser Asn Ile Asp Phe Glu Pro Ile Asn Ser Asn Glu Gly Val
    370                 375                 380

Tyr Ile Ala Asn Thr Tyr Thr Ala Ala Ser Ile Ser Lys Tyr Gln Lys
385                 390                 395                 400

Leu Leu Gln Arg Lys Glu Gly Gln Lys Ser Ser Gly Ser Ser Leu Thr
                405                 410                 415

Leu Asp Ser Phe Lys Ile Glu Asn Met Lys Lys Thr Lys Ile Thr Phe
            420                 425                 430

Asn Lys Gly Gly Asp Trp His Ala Ile Lys Ala Pro Glu Phe Asn Tyr
        435                 440                 445

Ala Gly Asn Pro Ile Arg Cys Ser Gly Asp Cys Ser Leu Asn Phe Lys
    450                 455                 460

Gly Arg Thr Glu Ser Gln Gly Thr Pro Val Tyr Ser Thr Asp Asn Ala
465                 470                 475                 480

Pro Gly Ile Ile Leu Ala Thr Gly Asn Val Gly Ser Tyr Leu Thr Asn
```

```
                485                 490                 495

Asn Gln Asp Glu Leu Arg Thr Tyr Leu Ser Ile Asp Gly Gly His Thr
            500                 505                 510

Trp Lys Glu Ile Gln Val Gly Ser His Glu Tyr Glu Ile Gly Asp Gln
        515                 520                 525

Gly Gly Ile Ile Ala Met Ala Arg Asp Asp Lys Leu Thr Asn Glu Val
    530                 535                 540

Ile Tyr Ser Val Asp Glu Gly Glu Thr Trp Arg Lys Leu Asn Phe Lys
545                 550                 555                 560

Asp Glu Asn Lys Phe Lys Val Asp Ser Phe Val Thr Glu Glu Gly Asn
                565                 570                 575

Asp Glu Arg Thr Phe Leu Phe Tyr Gly Thr Lys Thr Gly Ala Asp Gly
            580                 585                 590

Asn Thr Lys Gly Val Ile Gly Ala Ile Asn Phe Ser Asn Leu Phe Lys
        595                 600                 605

Lys Glu Cys Thr Gly Phe Glu Asn Pro Gly Glu Asp Gly Ser Asp Tyr
    610                 615                 620

Glu Arg Trp Val Pro Leu Asn Phe Glu Gly Lys Lys Cys Leu Phe Gly
625                 630                 635                 640

Ser Lys Ile Ser Tyr Ile Arg Lys Lys Thr Asp Ser Ser Cys Phe Asn
                645                 650                 655

Asn Arg Lys Val Gly Asp Leu Arg Met Val Gln Gly Ser Cys Glu Cys
            660                 665                 670

Thr Glu Glu Asp Phe Glu Cys Asp Tyr Gly Phe Thr Lys Asp Leu Ile
        675                 680                 685

Asp Glu Thr Lys Cys Val Pro Ile Asn Ala Lys Phe Ala Lys Lys Arg
    690                 695                 700

Asp Gln Pro Pro Leu Asn Cys Lys Asp Phe Tyr Phe Val Ser Ser Gly
705                 710                 715                 720

Lys Arg Lys Ile Ala Asn Asn Gln Cys Gln Gly Gly Ile Glu Glu Leu
                725                 730                 735

Tyr Thr Lys Lys Lys Val Arg Cys Pro Gly Asn Glu Glu Ala Gln Gln
            740                 745                 750

Thr Gln Gln Gln Thr Gln Asn Thr Gln Ala Asn Thr Ala Gln Asn Asn
        755                 760                 765

Gln Gln Asp Leu Phe Ser Arg Lys Pro Glu Asp Ile Lys Lys Glu Ile
    770                 775                 780

Lys Glu Gln Tyr Gly Asn Gln Thr Asp Gln Thr Ser Gly Ile Ser Phe
785                 790                 795                 800

Leu Gly Val Leu Ala Ala Phe Leu Val Leu Phe Leu Leu Tyr Thr Tyr
                805                 810                 815

Arg Val Glu Ile Leu Ser Lys Ile Lys Glu Tyr Gln Gln Asn Gln Lys
            820                 825                 830

Asn Lys Lys Gly Asp Asn Asn Lys Tyr Gly Tyr Lys Gln Lys Ser Tyr
        835                 840                 845

Gly Asn Asn Ala Glu Gln Tyr Ser Leu Phe Gln Asn Asp Gln Asp Asn
    850                 855                 860

Asp Glu Tyr Asp Ala Asp Met Leu
865                 870


<210> SEQ ID NO 8
<211> LENGTH: 2689
<212> TYPE: DNA
<213> ORGANISM: Tetrahymena thermophila
```

```
<400> SEQUENCE: 8

gaaattacaa aaagcaatct ttttagagta gcatttaaaa taaattataa attaggtatt     60

tgtttagatt atgaaaaaat aagatctgac agtatatgtt gcagctttcc tgcttctctt    120

ttcttgtgtt attcactttg ctaatgctca agataaagtt agtgaaattt ttaaagacaa    180

atatgatgtc aaatatagag taactgaatt agattcacct gtttaggaaa ttctatggtg    240

cggtagttct taagcaacat ctgaagacgg agatattatc acctatgatt aaacagcaaa    300

agttagaaaa ctttatgtct taactgataa aggtaaattg tattactcag aagactatgg    360

cattacattg aagttgatta atgatgatat ccgtcaatca accaattcca aataaactta    420

ggtcgaagtc gatgatatca tgatctcacc tgttaaaaat agaaaagtgt tcatcttcac    480

taaaagcggt gaaagctatt atacagaaaa ctgtggtgcc acttatactt ctttcaagca    540

cgagattctc ctatacgata tctagcccaa tccttctgat cacaagtctt tgataggact    600

tgtacccgtt tagtgctaaa aaggagatcc tgagtgctaa ggtggtgatt ctgatttata    660

cttaacagta gatagcggta tgacttggag aaaaatagtc tctaacgtaa atcaagcata    720

gtgggataag accaaataaa ctctcatgaa cacataaaat agaattattt tgtctcatta    780

agagtaagaa aagaatgaaa aaggagaaaa tgtattcctc aataaagtaa gctacactga    840

taactatggt aaagatttaa aagtggtaga aaagaatgga gttagattct attaaacaga    900

agaatatatt tttgttttaa tctaaggaaa ggaatttggc aaatataaac ttaatattgg    960

accttctttt gttactcaat cttctagcag aaaagagatc gatttacctc tttaaagagt   1020

taaagatgaa tcttttactg tcttggacat agatgcaggc taaattctta tcgctattaa   1080

tcatgaaggt gacagtgctg gatacactaa tgtttacatt tcaaactcct aaggagaata   1140

gttcactctt tcacttcaat atacagtagg tgatgatgat tctaacattg attttgaacc   1200

cattaacagc aacgaaggag tttatattgc aaacacatac actgcagctt caatttcaaa   1260

atatcaaaag cttttgcaaa gaaaagaagg acaaaaatct tctggatctt cactcacttt   1320

ggattcattt aaaattgaaa atatgaaaaa aactaaaatt acatttaaca agggtggtga   1380

ctggcacgca atcaaggctc ccgaattcaa ttatgctgga aatcctattc gttgctctgg   1440

tgactgttct cttaacttta aaggaagaac tgagtctcaa ggtactccag tctattctac   1500

tgataatgct cctggtatta ttttggctac aggtaatgtt ggctcttatc tcactaataa   1560

tcaagatgaa ttaagaactt atctttctat tgatggtgga cacacatgga aagagattca   1620

agttggatct catgaatacg aaattggtga ttaaggcggt atcatcgcta tggctagaga   1680

cgataagctt acaaacgaag ttatttactc tgttgatgaa ggagaaacat ggagaaaatt   1740

gaatttcaag gatgaaaata aatttaaagt agatagtttt gttacagaag aaggcaacga   1800

tgaaagaact ttcttgttct atggaaccaa gactggtgca gatggaaata ctaaaggtgt   1860

aattggtgct atcaactttt caaatttatt caaaaaggaa tgcacaggat ttgaaaaccc   1920

tggcgaagat ggcagtgatt atgagagatg ggtcccatta aactttgaag gaaaaaaatg   1980

cttatttggt tcaaaaattt catacataag aaaaaaaact gattctagtt gctttaacaa   2040

cagaaaagtt ggtgatttaa gaatggtcta aggatcttgt gaatgtacag aagaagattt   2100

cgaatgtgat tatggtttca ctaaagattt aattgatgaa acaaaatgtg ttccaataaa   2160

tgcaaaattt gcaaagaaaa gagactaacc acctttgaac tgcaaagatt tttactttgt   2220

ttcttcagga aaaagaaaaa ttgcaaacaa ctaatgttaa ggcggtattg aagaattata   2280
```

-continued

tacaaagaaa aaagtaagat gcccaggaaa tgaagaagct cagcaaactt agcaataaac 2340

tcaaaatact taagctaata cagcttaaaa taactagtaa gacttattta gcagaaagcc 2400

agaagatata aaaaaagaaa taaaagaata atatggcaat taaacagatt agacatcagg 2460

aatatccttc ctcggtgttt tggcagcttt cttagtatta ttcttattat atacttacag 2520

ggtagaaata cttagcaaga taaaagaata tcaataaaac caaaagaaca aaaagggtga 2580

taacaataaa tatggctata agcaaaaatc ctatggaaat aatgctgaac agtattcact 2640

tttctaaaat gatcaagaca atgatgaata cgatgcagat atgctttga 2689

<210> SEQ ID NO 9
<211> LENGTH: 6277
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA Construct

<400> SEQUENCE: 9

ttgtaagcgt taatattttg ttaaaattcg cgttaaattt ttgttaaatc agctcatttt 60

ttaaccaata ggccgaaatc ggcaaaatcc cttataaatc aaaagaatag accgagatag 120

ggttgagtgt tgttccagtt tggaacaaga gtccactatt aaagaacgtg gactccaacg 180

tcaaagggcg aaaaaccgtc tatcagggcg atggcccact acgtgaacca tcaccctaat 240

caagtttttt ggggtcgagg tgccgtaaag cactaaatcg gaaccctaaa gggagccccc 300

gatttagagc ttgacgggga aagccggcga acgtggcgag aaaggaaggg aagaaagcga 360

aaggagcggg cgctagggcg ctggcaagtg tagcggtcac gctgcgcgta accaccacac 420

ccgccgcgct taatgcgccg ctacagggcg cgtcccattc gccattcagg ctgcgcaact 480

gttgggaagg gcgatcggtg cgggcctctt cgctattacg ccagctggcg aaagggggat 540

gtgctgcaag gcgattaagt tgggtaacgc cagggttttc ccagtcacga cgttgtaaaa 600

cgacggccag tgaattgtaa tacgactcac tatagggcga attgggtacc gggccccccc 660

tcgaggtcga cggtatcgat aagctctgat tgttaaatgt tgaaagagta tttttatgag 720

aagtattttt tgttttgaaa tcagaaattt tattcctctt ttttagtaaa aatacttaat 780

tgttatttat gtaacaaatg taataaaatc gcaaatgaaa tattcttttt aaccaattaa 840

ataaataata ctatttttaa ttaaaatgat gagcatatta attttaaaat ggatcttttt 900

aattaatgtt aaattataat atttaacaat aaaaaatatg ctgttgatat tttaataaat 960

tcgcaatcaa gaaattattg attttattat ttctattaat aatatttatt aattaattat 1020

ttaatgtaga aataaataaa taaattatga aagaaaataa aaatattaga caagatagat 1080

tgatagaaaa caaaaaataa ttagtgaaaa catttttagtt ttaaaacaaa ttaataagac 1140

tgtttattta acaaatattc agtagttagt ttgttagtta gtattgtatt cattttattt 1200

tgtaaaatga ttgattacat taaaattaat aatcattaat taattaattg cttatgctct 1260

caagtaattt tttaatgata agcttgatat cgaattcaga tcccccgggc tgcatttttc 1320

cagtaaaaat ttgaaaattt aatggcaaaa aaaaatatta ttattggatt tgcagacaaa 1380

tttttaagag ctaacatgta tgtgaagagg aatttttttt tttagaaagt taaaaaaaat 1440

aattgacata aaatatatat acaaatgagt tgtaaaataa tgattttagt caatttggaa 1500

taaattatat tttatagtag tatattaaca cgtttttttg gtgctttaat gttaatataa 1560

tacactaaaa attaatttta tataatatat ttattttata tgaaagtttg taaatatata 1620

ttgaattttt aatttaagga tctcagaaga attcgtcaag aagacgatag aaggcgatac 1680

-continued

```
gttgagaatc gggagcggcg ataccgtaaa ggacaaggaa acggtcagcc cattcaccac   1740

caagttcttc agcaatatca cgggtagcta aggcaatatc ttgataacgg tcggcgacac   1800

caagacgacc acagtcgatg aaaccagaaa aacgaccatt ttcaaccatg atattgggta   1860

agcaggcatc accatgggtg acgacaagat cttcaccgtc gggcatacgg gccttaagtc   1920

tggcgaaaag ttcggcaggg gcaagacctt gatgttcttc gtcaagatca tcttgatcga   1980

caagaccggc ttccatacga gtacgagcac gttcgatacg atgtttggct tggtggtcga   2040

aagggcaggt agcgggatca agggtatgaa gacgacgcat agcatcagcc atgatagaaa   2100

ctttttcggc aggagcaagg tgagaagaaa gaagatcttg accggggact tcacctaaaa   2160

gaagccagtc tctaccggct tcagtgacaa cgtcaaggac agcagcgcaa ggaacaccgg   2220

tggtggcaag ccaagaaaga cgggcagctt catcttgaag ttcattaagg gcaccagaaa   2280

ggtcggtctt gacgaaaaga acaggacgac cttgagcaga aagacggaag acggcggcat   2340

cagagcaacc gatggtttgt tgagcccagt cataaccgaa aagtctttcg acccaagcgg   2400

cgggagaacc agcgtgtaaa ccatcttgtt caatcattat tttaagttta gtattattat   2460

ttattttatt agagctttat taaatttttt taattttttt aaattatata aagaataaaa   2520

aagacgaata tatatatata cactatttac attattttat atggatcatt gtataaatcg   2580

tgaatcacgt agctaagaat tatatcagaa atataaaaaa ttactttata ttcaagagag   2640

attcaagaat cacatctata ttttagaata gaagaatttt gaaaattagt taggttgact   2700

catgatttaa atcatgagtc aatcaattta tatttttat cagaaataaa aagatttaca   2760

aataattcat gacacaaaat tcaagaatca caacttaata ttaaaatata atagaaacgg   2820

ataattgaaa ataaaaaata aatgatagcc taaataatga gtaatatttt gaaaattaat   2880

gattcacata ttataattga tgaatgagct atgttttgag cagcttatat atttaataaa   2940

taaaataatt gatatttatc tattttatat ttcatgtttt ctttaaaaaa catgtcatct   3000

tttttatcaa tatatttgaa atttaaagaa aataattgaa taaacgatac aatatatttt   3060

aagatatata aaaaagtttt gctttcaaga tattaaaaat agtgatataa aaaataagta   3120

ctctattatg tttttttcta ttcagtatta tacctttaat cattattatc tttttattta   3180

tttttagtta gttatttttt atttttatga atatttaaag agctaaaaaa aatttaaaaa   3240

tgtgtattta aattaaagga gttattcaaa acccttatta ttttttattt ttaaatattt   3300

tttagaaata aattgtatat cgaattcctg cagctaaaaa gattgctttt tgtaatttct   3360

atgattttaa gagtattttt taatttaata tttttaatat ttaattaatt atgatttttt   3420

tttttttttg atgagaagat acactttatt aaataacata gttcgaaata tcataatcaa   3480

cttcttttaa aaaatttttt ttaagaatca aacaaactct ctaacataca cgcattcgct   3540

catttattaa aatttttacg ttttgcaaat ttaatttgtt ggcacttttg tatcttcact   3600

caattaccaa aattttctct caattttcct tccttttata aaataaccaa tgataatttt   3660

tgatccaata cgttttaaaa tttagtcttt cttttaaaat aataacaaag aaagataaat   3720

acatgagtaa aaataaaaaa agcagatagc tatgcaattt attaattttt ttgaaattta   3780

taaatatttt tggagatatt ttttcattat agtgattaaa attaatttta tttagaaaaa   3840

tcaagtttta tttatgaata aacagttatt tacttaagat tttgttttca ctattagtat   3900

tctgttttaa atctttaagt attttcttag ttaacaatct tacaatcctt attttgattg   3960

ctatttaaaa ttaaaatatt ttaaatagaa catttaacat aacagatatg aaaataaaca   4020
```

-continued

```
gcgttttacg ctagcgcatg ctctagagcg gccgccaccg cggtggagct ccagcttttg   4080

ttccctttag tgagggttaa tttcgagctt ggcgtaatca tggtcatagc tgtttcctgt   4140

gtgaaattgt tatccgctca caattccaca caacatacga gccggaagca taaagtgtaa   4200

agcctggggt gcctaatgag tgagctaact cacattaatt gcgttgcgct cactgcccgc   4260

tttccagtcg ggaaacctgt cgtgccagct gcattaatga atcggccaac gcgcggggag   4320

aggcggtttg cgtattgggc gctcttccgc ttcctcgctc actgactcgc tgcgctcggt   4380

cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg gtaatacggt tatccacaga   4440

atcaggggat aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg   4500

taaaaaggcc gcgttgctgg cgtttttcca taggctccgc ccccctgacg agcatcacaa   4560

aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt   4620

tccccctgga agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct   4680

gtccgccttt ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct   4740

cagttcggtg taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc   4800

cgaccgctgc gccttatccg gtaactatcg tcttgagtcc aacccggtaa gacacgactt   4860

atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc   4920

tacagagttc ttgaagtggt ggcctaacta cggctacact agaaggacag tatttggtat   4980

ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa   5040

acaaaccacc gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa   5100

aaaaggatct caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga   5160

aaactcacgt taagggattt tggtcatgag attatcaaaa aggatcttca cctagatcct   5220

tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga   5280

cagttaccaa tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcatc   5340

catagttgcc tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg   5400

ccccagtgct gcaatgatac cgcgagaccc acgctcaccg gctccagatt tatcagcaat   5460

aaaccagcca gccggaaggg ccgagcgcag aagtggtcct gcaactttat ccgcctccat   5520

ccagtctatt aattgttgcc gggaagctag agtaagtagt tcgccagtta atagtttgcg   5580

caacgttgtt gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc   5640

attcagctcc ggttcccaac gatcaaggcg agttacatga tcccccatgt tgtgcaaaaa   5700

agcggttagc tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc   5760

actcatggtt atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt   5820

ttctgtgact ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag   5880

ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca catagcagaa ctttaaaagt   5940

gctcatcatt ggaaaacgtt cttcggggcg aaaactctca aggatcttac cgctgttgag   6000

atccagttcg atgtaaccca ctcgtgcacc caactgatct tcagcatctt ttactttcac   6060

cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc   6120

gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa gcatttatca   6180

gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg   6240

ggttccgcgc acatttcccc gaaaagtgcc acctaaa                            6277
```

<210> SEQ ID NO 10
<211> LENGTH: 6648

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA Construct

<400> SEQUENCE: 10

cacctaaatt gtaagcgtta atattttgtt aaaattcgcg ttaaattttt gttaaatcag 60

ctcatttttt aaccaatagg ccgaaatcgg caaaatccct tataaatcaa aagaatagac 120

cgagataggg ttgagtgttg ttccagtttg gaacaagagt ccactattaa agaacgtgga 180

ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat ggcccactac gtgaaccatc 240

accctaatca agttttttgg ggtcgaggtg ccgtaaagca ctaaatcgga accctaaagg 300

gagcccccga tttagagctt gacggggaaa gccggcgaac gtggcgagaa aggaagggaa 360

gaaagcgaaa ggagcgggcg ctagggcgct ggcaagtgta gcggtcacgc tgcgcgtaac 420

caccacaccc gccgcgctta atgcgccgct acagggcgcg tcccattcgc cattcaggct 480

gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa 540

agggggatgt gctgcaaggc gattaagttg ggtaacgcca gggttttccc agtcacgacg 600

ttgtaaaacg acggccagtg aattgtaata cgactcacta tagggcgaat tgggtaccgg 660

gccccccctc gaggtcgacg gtatcgataa gcttgattta cgacaaattc aatatgccat 720

ttcaaaagta atctgagttt ctggaagttt aatagaataa aaattacaga attgatattt 780

aaatcaaaat tttcaagcta aattagattg atttttaagt tactcaaata ataaaggtag 840

taaataaaat caaatttctc aaacaataat tatcttctac ataaaactgc tttcttaata 900

cccctaatta aaaaagatat atttttgaaa atttaaacaa aatttggaag aaaaattaat 960

ttcatttgat aaattttatt taaagtaggt tctccataac taacccctcc cctaatcaaa 1020

tatttgtaaa agctttgggt tttttctaa aaaattttca aaatttattt ttttcaaaac 1080

atatttatta tttcaagtta aacattttgt gaatttaatg atttataaaa actcaaaaaa 1140

atagttgttt agaattatta tttagcttta tttgttatat tattaagata tattacattt 1200

tgccttttat aaattaaata cgcatttcac aaaagactgt tcatttataa gattattcat 1260

caatatatta atatattatt ttttgctatt tttttaattt ggttatttaa aattctagaa 1320

tcatgattaa gtatttattt ttaattatct tgattataaa taatctaaat ttttatgtta 1380

actaaaaatc tttggtagta aataaataaa ttattattat tattattaaa aaatcaatac 1440

tattaaaatt atttttaaat taaattaatc tacaaagaaa tttatgaaaa aacaaaaaat 1500

caattagtta attaagcttg atatcgaatt cagatccccc gggctgcatt tttccagtaa 1560

aaatttgaaa atttaatggc aaaaaaaaat attattattg gatttgcaga caaattttta 1620

agagctaaca tgtatgtgaa gaggaatttt tttttttaga aagttaaaaa aaataattga 1680

cataaaatat atatacaaat gagttgtaaa ataatgattt tagtcaattt ggaataaatt 1740

atattttata gtagtatatt aacacgtttt tttggtgctt taatgttaat ataatacact 1800

aaaaattaat tttatataat atatttattt tatatgaaag tttgtaaata tatattgaat 1860

ttttaattta aggatctcag aagaattcgt caagaagacg atagaaggcg atacgttgag 1920

aatcgggagc ggcgataccg taaaggacaa ggaaacggtc agcccattca ccaccaagtt 1980

cttcagcaat atcacgggta gctaaggcaa tatcttgata acggtcggcg acaccaagac 2040

gaccacagtc gatgaaacca gaaaaacgac cattttcaac catgatattg ggtaagcagg 2100

catcaccatg ggtgacgaca agatcttcac cgtcgggcat acgggcctta agtctggcga 2160

-continued

```
aaagttcggc aggggcaaga ccttgatgtt cttcgtcaag atcatcttga tcgacaagac   2220

cggcttccat acgagtacga gcacgttcga tacgatgttt ggcttggtgg tcgaaagggc   2280

aggtagcggg atcaagggta tgaagacgac gcatagcatc agccatgata gaaacttttt   2340

cggcaggagc aaggtgagaa gaaagaagat cttgaccggg gacttcacct aaaagaagcc   2400

agtctctacc ggcttcagtg acaacgtcaa ggacagcagc gcaaggaaca ccggtggtgg   2460

caagccaaga aagacgggca gcttcatctt gaagttcatt aagggcacca gaaaggtcgg   2520

tcttgacgaa aagaacagga cgaccttgag cagaaagacg gaagacggcg gcatcagagc   2580

aaccgatggt ttgttgagcc cagtcataac cgaaaagtct ttcgacccaa gcggcgggag   2640

aaccagcgtg taaaccatct tgttcaatca ttattttaag tttagtatta ttatttattt   2700

tattagagct ttattaaatt tttttaattt ttttaaatta tataaagaat aaaaaagacg   2760

aatatatata tatacactat ttacattatt ttatatggat cattgtataa atcgtgaatc   2820

acgtagctaa gaattatatc agaaatataa aaaattactt tatattcaag agagattcaa   2880

gaatcacatc tatattttag aatagaagaa ttttgaaaat tagttaggtt gactcatgat   2940

ttaaatcatg agtcaatcaa tttatatttt ttatcagaaa taaaaagatt tacaaataat   3000

tcatgacaca aaattcaaga atcacaactt aatattaaaa tataatagaa acggataatt   3060

gaaaataaaa aataaatgat agcctaaata atgagtaata ttttgaaaat taatgattca   3120

catattataa ttgatgaatg agctatgttt tgagcagctt atatatttaa taaataaaat   3180

aattgatatt tatctatttt atatttcatg ttttctttaa aaaacatgtc atcttttta   3240

tcaatatatt tgaaatttaa agaaaataat tgaataaacg atacaatata ttttaagata   3300

tataaaaaag ttttgctttc aagatattaa aaatagtgat ataaaaaata agtactctat   3360

tatgtttttt cttattcagt attatacctt taatcattat tatcttttta tttattttta   3420

gttagttatt ttttattttt atgaatattt aaagagctaa aaaaaattta aaaatgtgta   3480

tttaaattaa aggagttatt caaaaccctt attatttttt atttttaaat atttttttaga   3540

aataaattgt atatcgaatt cctgcagaaa gatatttaat cacttaataa ctaagtctgt   3600

ttctcatgcc aagaaaaatt caactaacaa taagtttatc aaaaattttc tatttagatg   3660

tagaaaagaa aaagaaaaaa caaatctaaa ggttattagc atatttttttc ttcttaaaca   3720

aggattaatt tttacgtttt taaatttcag accaatcaat caatcatgaa tgataataga   3780

tatttttaaa atatagcttt aaaaaaatac aatatttaac gagattataa tattttttttt   3840

taatactaaa attcttcgct ttgctgagca atttgatttg aaaaagctaa tcaactttat   3900

taatttttttt cggattaggt ttttaaaatt ttataaggaa taaacgtttt ttaatgatat   3960

gctatttagg atactgcttt tttaaagtaa ttttttaatt tagatttaag tttactctaa   4020

caataaggat ttaaatataa acaatttaca aataatttta tatagattag aattttaatt   4080

tatttattta tttacttatt aatttaagtt aaattattta atttgattta actaaaattt   4140

attttgaagt tatattacaa aattttcatt ttatgttaaa ctcagttagt ttgatcattg   4200

tttcatacat ctgattaaat attttaatat gatgaggaac caaacttgtg actttaatta   4260

tttgaattaa taaaaaaatt ctgcatatcg ttgctgtctt attttaagtt tagctttaca   4320

ttatataaaa gactatctat tggttggtat tactattatt tattatttaa taatgatgtt   4380

atttactagc tgcctaatcc agactgaggc tagcgcatgc tctagagcgg ccgccaccgc   4440

ggtggagctc cagcttttgt tccctttagt gagggttaat ttcgagcttg gcgtaatcat   4500

ggtcatagct gtttcctgtg tgaaattgtt atccgctcac aattccacac aacatacgag   4560
```

-continued

ccggaagcat aaagtgtaaa gcctggggtg cctaatgagt gagctaactc acattaattg 4620

cgttgcgctc actgcccgct ttccagtcgg gaaacctgtc gtgccagctg cattaatgaa 4680

tcggccaacg cgcggggaga ggcggtttgc gtattgggcg ctcttccgct tcctcgctca 4740

ctgactcgct gcgctcggtc gttcggctgc ggcgagcggt atcagctcac tcaaaggcgg 4800

taatacggtt atccacagaa tcaggggata acgcaggaaa gaacatgtga gcaaaaggcc 4860

agcaaaaggc caggaaccgt aaaaaggccg cgttgctggc gtttttccat aggctccgcc 4920

cccctgacga gcatcacaaa aatcgacgct caagtcagag gtggcgaaac ccgacaggac 4980

tataaagata ccaggcgttt ccccctggaa gctccctcgt gcgctctcct gttccgaccc 5040

tgccgcttac cggatacctg tccgcctttc tcccttcggg aagcgtggcg ctttctcata 5100

gctcacgctg taggtatctc agttcggtgt aggtcgttcg ctccaagctg ggctgtgtgc 5160

acgaaccccc cgttcagccc gaccgctgcg ccttatccgg taactatcgt cttgagtcca 5220

acccggtaag acacgactta tcgccactgg cagcagccac tggtaacagg attagcagag 5280

cgaggtatgt aggcggtgct acagagttct tgaagtggtg gcctaactac ggctacacta 5340

gaaggacagt atttggtatc tgcgctctgc tgaagccagt taccttcgga aaaagagttg 5400

gtagctcttg atccggcaaa caaaccaccg ctggtagcgg tggttttttt gtttgcaagc 5460

agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt tctacggggt 5520

ctgacgctca gtggaacgaa aactcacgtt aagggatttt ggtcatgaga ttatcaaaaa 5580

ggatcttcac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc taaagtatat 5640

atgagtaaac ttggtctgac agttaccaat gcttaatcag tgaggcacct atctcagcga 5700

tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata actacgatac 5760

gggagggctt accatctggc cccagtgctg caatgatacc gcgagaccca cgctcaccgg 5820

ctccagattt atcagcaata aaccagccag ccggaagggc cgagcgcaga agtggtcctg 5880

caactttatc cgcctccatc cagtctatta attgttgccg ggaagctaga gtaagtagtt 5940

cgccagttaa tagtttgcgc aacgttgttg ccattgctac aggcatcgtg gtgtcacgct 6000

cgtcgtttgg tatggcttca ttcagctccg gttcccaacg atcaaggcga gttacatgat 6060

cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc tccgatcgtt gtcagaagta 6120

agttggccgc agtgttatca ctcatggtta tggcagcact gcataattct cttactgtca 6180

tgccatccgt aagatgcttt tctgtgactg gtgagtactc aaccaagtca ttctgagaat 6240

agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat acgggataat accgcgccac 6300

atagcagaac tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga aaactctcaa 6360

ggatcttacc gctgttgaga tccagttcga tgtaacccac tcgtgcaccc aactgatctt 6420

cagcatcttt tactttcacc agcgtttctg ggtgagcaaa aacaggaagg caaaatgccg 6480

caaaaaaggg aataagggcg acacggaaat gttgaatact catactcttc ctttttcaat 6540

attattgaag catttatcag ggttattgtc tcatgagcgg atacatattt gaatgtattt 6600

agaaaaataa acaaataggg gttccgcgca catttccccg aaaagtgc 6648

<210> SEQ ID NO 11
<211> LENGTH: 6681
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA Construct

-continued

```
<400> SEQUENCE: 11

cacctaaatt gtaagcgtta atattttgtt aaaattcgcg ttaaattttt gttaaatcag     60

ctcatttttt aaccaatagg ccgaaatcgg caaaatccct tataaatcaa aagaatagac    120

cgagataggg ttgagtgttg ttccagtttg gaacaagagt ccactattaa agaacgtgga    180

ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat ggcccactac gtgaaccatc    240

accctaatca agttttttgg ggtcgaggtg ccgtaaagca ctaaatcgga accctaaagg    300

gagcccccga tttagagctt gacggggaaa gccggcgaac gtggcgagaa aggaagggaa    360

gaaagcgaaa ggagcgggcg ctagggcgct ggcaagtgta gcggtcacgc tgcgcgtaac    420

caccacaccc gccgcgctta atgcgccgct acagggcgcg tcccattcgc cattcaggct    480

gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa    540

agggggatgt gctgcaaggc gattaagttg ggtaacgcca gggttttccc agtcacgacg    600

ttgtaaaacg acggccagtg aattgtaata cgactcacta tagggcgaat tgggtaccgg    660

gccccccctc gaggtcgacg gtatcgataa gctgaagata aattattgct tcaatcattt    720

gctcagctaa ttatattagc taatttctat caaagcattt gtaggaagac agagaaaaat    780

cacagtcttt aaataaaata acaaaaaatt tattaatttt aaaggcatca tagatttttg    840

atatatataa attatcacat cctctaacga gatcaacata attttaggcc tgttacagct    900

tatcaaagca gtaagagtta ttcccatcat ctattgatat aagatatatt agataagatt    960

cttttctatt attaactgca taatatttcc catatttttc gtttttttatc acctaaaagt   1020

attcattttt attttataaa agctagtcta ttatttaagc tatttatttt tcatttaaaa   1080

tatcatttta tacatttgtt gaaaaccctt aatccatatt tttataattt ttttttttca   1140

aaatttctta tcaaaaattt tttaattaaa acaaattcct ttaaaaacat ttaacttaaa   1200

tttggaagta aattattaga aggtttgtta gaatattttc aaactaggaa taataaagta   1260

aaaaaggatt tgataaaata cataagttaa gagaaaaaga tacttgacac actatattaa   1320

attacttaga aaaactttct aaataatatt aatgaaagac aaaaattaat ttatttattt   1380

ttatttattt attatttaag taaaaaaata aattagcaat attattcagt caattatttt   1440

aagcttgata tcgaattcag atcccccggg ctgcattttt ccagtaaaaa tttgaaaatt   1500

taatggcaaa aaaaaatatt attattggat ttgcagacaa atttttaaga gctaacatgt   1560

atgtgaagag gaattttttt ttttagaaag ttaaaaaaaa taattgacat aaaatatata   1620

tacaaatgag ttgtaaaata atgattttag tcaatttgga ataaattata ttttatagta   1680

gtatattaac acgttttttt ggtgctttaa tgttaatata atacactaaa aattaatttt   1740

atataatata tttattttat atgaaagttt gtaaatatat attgaatttt taatttaagg   1800

atctcagaag aattcgtcaa gaagacgata gaaggcgata cgttgagaat cgggagcggc   1860

gataccgtaa aggacaagga aacggtcagc ccattcacca ccaagttctt cagcaatatc   1920

acgggtagct aaggcaatat cttgataacg gtcggcgaca ccaagacgac cacagtcgat   1980

gaaaccagaa aaacgaccat tttcaaccat gatattgggt aagcaggcat caccatgggt   2040

gacgacaaga tcttcaccgt cgggcatacg ggccttaagt ctggcgaaaa gttcggcagg   2100

ggcaagacct tgatgttctt cgtcaagatc atcttgatcg acaagaccgg cttccatacg   2160

agtacgagca cgttcgatac gatgtttggc ttggtggtcg aaagggcagg tagcgggatc   2220

aagggtatga agacgacgca tagcatcagc catgatagaa actttttcgg caggagcaag   2280

gtgagaagaa agaagatctt gaccggggac ttcacctaaa agaagccagt ctctaccggc   2340
```

-continued

```
ttcagtgaca acgtcaagga cagcagcgca aggaacaccg gtggtggcaa gccaagaaag   2400

acgggcagct tcatcttgaa gttcattaag ggcaccagaa aggtcggtct tgacgaaaag   2460

aacaggacga ccttgagcag aaagacggaa gacggcggca tcagagcaac cgatggtttg   2520

ttgagcccag tcataaccga aaagtctttc gacccaagcg gcgggagaac cagcgtgtaa   2580

accatcttgt tcaatcatta ttttaagttt agtattatta tttattttat tagagcttta   2640

ttaaattttt ttaatttttt taaattatat aaagaataaa aaagacgaat atatatatat   2700

acactattta cattatttta tatggatcat tgtataaatc gtgaatcacg tagctaagaa   2760

ttatatcaga aatataaaaa attactttat attcaagaga gattcaagaa tcacatctat   2820

attttagaat agaagaattt tgaaaattag ttaggttgac tcatgattta aatcatgagt   2880

caatcaattt atatttttta tcagaaataa aaagatttac aaataattca tgacacaaaa   2940

ttcaagaatc acaacttaat attaaaatat aatagaaacg gataattgaa aataaaaaat   3000

aaatgatagc ctaaataatg agtaatattt tgaaaattaa tgattcacat attataattg   3060

atgaatgagc tatgttttga gcagcttata tatttaataa ataaaataat tgatatttat   3120

ctattttata tttcatgttt tctttaaaaa acatgtcatc tttttatca atatatttga   3180

aatttaaaga aaataattga ataaacgata caatatattt taagatatat aaaaaagttt   3240

tgctttcaag atattaaaaa tagtgatata aaaaataagt actctattat gtttttttctt   3300

attcagtatt atacctttaa tcattattat ctttttattt atttttagtt agttattttt   3360

tatttttatg aatatttaaa gagctaaaaa aaatttaaaa atgtgtattt aaattaaagg   3420

agttattcaa aacccttatt atttttttatt tttaaatatt ttttagaaat aaattgtata   3480

tcgaattcct gcagatctat tttttctctc aattattttc ttttcaagga tttgtttttt   3540

ttttgttggt tattctatta attaaggcaa gatgaatgct tctatcaaaa aaaatacgtt   3600

ttttgatttg taatttttttc ctattgattt caattatgtt tttaaaatta agtcatattc   3660

ttgtttatca agtttcatca gtaattcaag ctcattaaaa tctttaaaaa aatctttcta   3720

aatagtttca attatactga agtgattcaa tcatttttta atcaaaaata tatttcagtc   3780

aattataatt tcattcatca aaataaaatg agatatttct aaaactgatt cataatttta   3840

gaaaattctt taatataaaa agatacattt tttaacttaa taatattttg gcattacata   3900

gctaatacaa aaatatgatt aatacaataa tgtaaatcat aagattaata tattagtaaa   3960

acaaaacata aaatcaagta ctgaattgtt ttattaatat attattttag taaaaatact   4020

ttcaaaatat tttttgaact aaagttgtaa ctaattatta ttttaacacc gtaaaaaata   4080

aaaaagttta aaagatttta aatattaaat aaactaacaa accatattca aatatattta   4140

aaaatagtaa aaactaaata ataaatattt cttaaattta tgcttcaaat aaaatttttc   4200

aatcagttaa ctatttttat attcaattta ttagatgtga taaattatat aaattaattc   4260

tttgtttttc atttgttaat tttttatttt gtttcagtaa atgatatctt ttaatttctt   4320

cattcaaatt ccttaaaact atataataag gacaaattaa actcataaat atattctcaa   4380

atagttatta attttatata tcataattct tctatacaat tatccaatca taaaagtgga   4440

agctagcgca tgctctagag cggccgccac cgcggtggag ctccagcttt tgttcccttt   4500

agtgagggtt aatttcgagc ttggcgtaat catggtcata gctgtttcct gtgtgaaatt   4560

gttatccgct cacaattcca cacaacatac gagccggaag cataaagtgt aaagcctggg   4620

gtgcctaatg agtgagctaa ctcacattaa ttgcgttgcg ctcactgccc gctttccagt   4680
```

-continued

cgggaaacct gtcgtgccag ctgcattaat gaatcggcca acgcgcgggg agaggcggtt 4740

tgcgtattgg gcgctcttcc gcttcctcgc tcactgactc gctgcgctcg gtcgttcggc 4800

tgcggcgagc ggtatcagct cactcaaagg cggtaatacg gttatccaca gaatcagggg 4860

ataacgcagg aaagaacatg tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg 4920

ccgcgttgct ggcgtttttc cataggctcc gcccccctga cgagcatcac aaaaatcgac 4980

gctcaagtca gaggtggcga aacccgacag gactataaag ataccaggcg tttccccctg 5040

gaagctccct cgtgcgctct cctgttccga ccctgccgct taccggatac ctgtccgcct 5100

ttctcccttc gggaagcgtg gcgctttctc atagctcacg ctgtaggtat ctcagttcgg 5160

tgtaggtcgt tcgctccaag ctgggctgtg tgcacgaacc ccccgttcag cccgaccgct 5220

gcgccttatc cggtaactat cgtcttgagt ccaacccggt aagacacgac ttatcgccac 5280

tggcagcagc cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt 5340

tcttgaagtg gtggcctaac tacggctaca ctagaaggac agtatttggt atctgcgctc 5400

tgctgaagcc agttaccttc ggaaaaagag ttggtagctc ttgatccggc aaacaaacca 5460

ccgctggtag cggtggtttt tttgtttgca agcagcagat tacgcgcaga aaaaaaggat 5520

ctcaagaaga tcctttgatc ttttctacgg ggtctgacgc tcagtggaac gaaaactcac 5580

gttaagggat tttggtcatg agattatcaa aaaggatctt cacctagatc cttttaaatt 5640

aaaaatgaag ttttaaatca atctaaagta tatatgagta aacttggtct gacagttacc 5700

aatgcttaat cagtgaggca cctatctcag cgatctgtct atttcgttca tccatagttg 5760

cctgactccc cgtcgtgtag ataactacga tacgggaggg cttaccatct ggccccagtg 5820

ctgcaatgat accgcgagac ccacgctcac cggctccaga tttatcagca ataaaccagc 5880

cagccggaag ggccgagcgc agaagtggtc ctgcaacttt atccgcctcc atccagtcta 5940

ttaattgttg ccgggaagct agagtaagta gttcgccagt taatagtttg cgcaacgttg 6000

ttgccattgc tacaggcatc gtggtgtcac gctcgtcgtt tggtatggct tcattcagct 6060

ccggttccca acgatcaagg cgagttacat gatcccccat gttgtgcaaa aaagcggtta 6120

gctccttcgg tcctccgatc gttgtcagaa gtaagttggc cgcagtgtta tcactcatgg 6180

ttatggcagc actgcataat tctcttactg tcatgccatc cgtaagatgc ttttctgtga 6240

ctggtgagta ctcaaccaag tcattctgag aatagtgtat gcggcgaccg agttgctctt 6300

gcccggcgtc aatacgggat aataccgcgc cacatagcag aactttaaaa gtgctcatca 6360

ttggaaaacg ttcttcgggg cgaaaactct caaggatctt accgctgttg agatccagtt 6420

cgatgtaacc cactcgtgca cccaactgat cttcagcatc ttttactttc accagcgttt 6480

ctgggtgagc aaaaacagga aggcaaaatg ccgcaaaaaa gggaataagg gcgacacgga 6540

aatgttgaat actcatactc ttcctttttc aatattattg aagcatttat cagggttatt 6600

gtctcatgag cggatacata tttgaatgta tttagaaaaa taaacaaata ggggttccgc 6660

gcacatttcc ccgaaaagtg c 6681

<210> SEQ ID NO 12
<211> LENGTH: 6564
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA Construct

<400> SEQUENCE: 12

cacctaaatt gtaagcgtta atattttgtt aaaattcgcg ttaaattttt gttaaatcag 60

-continued

```
ctcattttt aaccaatagg ccgaaatcgg caaaatccct tataaatcaa aagaatagac    120
cgagataggg ttgagtgttg ttccagtttg gaacaagagt ccactattaa agaacgtgga    180
ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat ggcccactac gtgaaccatc    240
accctaatca agttttttgg ggtcgaggtg ccgtaaagca ctaaatcgga accctaaagg    300
gagcccccga tttagagctt gacggggaaa gccggcgaac gtggcgagaa aggaagggaa    360
gaaagcgaaa ggagcgggcg ctagggcgct ggcaagtgta gcggtcacgc tgcgcgtaac    420
caccacaccc gccgcgctta atgcgccgct acagggcgcg tcccattcgc cattcaggct    480
gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa    540
agggggatgt gctgcaaggc gattaagttg ggtaacgcca gggttttccc agtcacgacg    600
ttgtaaaacg acggccagtg aattgtaata cgactcacta tagggcgaat tgggtaccgg    660
gccccccctc gaggtcgacg gtatcgataa gctaactaga atattaattg ctaaagtcaa    720
aaatctctaa tataagaaag aaaatattga aatgagaatt taatttaaaa ttaaatgata    780
atgcctatga gttaattaat ttgattaaag aaaggtattt gtttctttga gttcatattt    840
aatcaccagc acttaaaata tgttgatatt tgaattattt aaatactttc ttaaatatta    900
ttaccgtaat agtgtttgaa gtttaagaaa taatgtactt tggttataaa acaatttttt    960
tactattaat agaagggagt gactgtatat ttttcagccg aattattttt tttaaatatt   1020
cgaaattaaa aaataaaaag tttaaaatca taaaaattaa atgacatatc accacctgta   1080
cctacatagt tcgtgatatt ttaattacag gattagcaat atttatacat ataaaaatat   1140
taaatgcttt ttttaagatt ttagttattt ataaaatata cttataagat aaacaatttc   1200
aatttataat aatatattta aattcaatta aaatcctctt ttaaacttta ataattaaca   1260
aaacatttag cataatctgt tttgattgta gattttaaaa tagattaaat taaattatta   1320
agaaattttt tagaaaacta aaaaattaat ttacaaaaaa taaaatatta aacttacaaa   1380
ttaattaaaa tgaagaaatt tgttatttta ataagcttga tatcgaattc agatccccccg   1440
ggctgcattt ttccagtaaa aatttgaaaa tttaatggca aaaaaaaata ttattattgg   1500
atttgcagac aaatttttaa gagctaacat gtatgtgaag aggaattttt tttttttagaa   1560
agttaaaaaa aataattgac ataaaatata tatacaaatg agttgtaaaa taatgatttt   1620
agtcaatttg gaataaatta tattttatag tagtatatta acacgttttt ttggtgcttt   1680
aatgttaata taatacacta aaaattaatt ttatataata tatttatttt atatgaaagt   1740
ttgtaaatat atattgaatt tttaatttaa ggatctcaga agaattcgtc aagaagacga   1800
tagaaggcga tacgttgaga atcgggagcg gcgataccgt aaaggacaag gaaacggtca   1860
gcccattcac caccaagttc ttcagcaata tcacgggtag ctaaggcaat atcttgataa   1920
cggtcggcga caccaagacg accacagtcg atgaaaccag aaaaacgacc attttcaacc   1980
atgatattgg gtaagcaggc atcaccatgg gtgacgacaa gatcttcacc gtcgggcata   2040
cgggccttaa gtctggcgaa aagttcggca ggggcaagac cttgatgttc ttcgtcaaga   2100
tcatcttgat cgacaagacc ggcttccata cgagtacgag cacgttcgat acgatgtttg   2160
gcttggtggt cgaaagggca ggtagcggga tcaagggtat gaagacgacg catagcatca   2220
gccatgatag aaactttttc ggcaggagca aggtgagaag aaagaagatc ttgaccgggg   2280
acttcaccta aaagaagcca gtctctaccg gcttcagtga caacgtcaag gacagcagcg   2340
caaggaacac cggtggtggc aagccaagaa agacgggcag cttcatcttg aagttcatta   2400
```

-continued

```
agggcaccag aaaggtcggt cttgacgaaa agaacaggac gaccttgagc agaaagacgg   2460

aagacggcgg catcagagca accgatggtt tgttgagccc agtcataacc gaaaagtctt   2520

tcgacccaag cggcgggaga accagcgtgt aaaccatctt gttcaatcat tattttaagt   2580

ttagtattat tatttatttt attagagctt tattaaattt ttttaatttt tttaaattat   2640

ataaagaata aaaaagacga atatatatat atacactatt tacattattt tatatggatc   2700

attgtataaa tcgtgaatca cgtagctaag aattatatca gaaatataaa aaattacttt   2760

atattcaaga gagattcaag aatcacatct atattttaga atagaagaat tttgaaaatt   2820

agttaggttg actcatgatt taaatcatga gtcaatcaat ttatattttt tatcagaaat   2880

aaaaagattt acaaataatt catgacacaa aattcaagaa tcacaactta atattaaaat   2940

ataatagaaa cggataattg aaaataaaaa ataaatgata gcctaaataa tgagtaatat   3000

tttgaaaatt aatgattcac atattataat tgatgaatga gctatgtttt gagcagctta   3060

tatatttaat aaataaaata attgatattt atctatttta tatttcatgt tttctttaaa   3120

aaacatgtca tctttttat caatatattt gaaatttaaa gaaaataatt gaataaacga   3180

tacaatatat tttaagatat ataaaaaagt tttgctttca agatattaaa aatagtgata   3240

taaaaaataa gtactctatt atgtttttc ttattcagta ttatacttt aatcattatt   3300

atcttttat ttatttttag ttagttattt tttattttta tgaatattta aagagctaaa   3360

aaaaatttaa aaatgtgtat ttaaattaaa ggagttattc aaaaccctta ttattttta   3420

tttttaaata ttttttagaa ataaattgta tatcgaattc ctgcagcaga cttcctctga   3480

tttcctaaca aagtaattct tactttttg ttaaaacatt taaaaaaaaa caaaaatttt   3540

taataatttc taaaacgtgt tttatttaat ataaattcat gcatatggct aatatcttac   3600

gacttttcta aatatttaat tttataaatc tagattcaac ataaatagcg atcaactttt   3660

ttttatgagt cttaaaaatc tctacattta aaaacgaaaa attataagtt cagtcaactc   3720

caagctatta taagataatc atccatctaa aatcaataca gtcaattttt attttctata   3780

ttttcatagt ataaattttt atattttaaa ttgaaatttt tttaattttt cattttatta   3840

taaaaataga attagccaat ttgtaattta agatattaaa atttaatatt taaaattaaa   3900

tttacttaaa ggaaataaca taaaagtaaa acttacctta aaaattatga ttacctgtcc   3960

agaacatttt ttagcaatat aaaatatata tatccaaaat tttaaataaa attaaaaaaa   4020

actttgaata tattctgata taaaaatagt aaatacacaa tacatataaa aaaaaattca   4080

aatatttggt caaatttgat attatttaag ttcattatta attttaatta aataaataaa   4140

atatttacaa tcaatagtgt aaacttttaa atgataattt tacttttaga tataaatata   4200

aacaaacaaa atagagttat ataaaattta taaatagttt tagaaataat tcattttatt   4260

ttattttatt tgatgaaatt gtgttatgat aaaaaggaat ttacttattc ttcaattaga   4320

atacgctagc gcatgctcta gagcggccgc caccgcggtg gagctccagc ttttgttccc   4380

tttagtgagg gttaatttcg agcttggcgt aatcatggtc atagctgttt cctgtgtgaa   4440

attgttatcc gctcacaatt ccacacaaca tacgagccgg aagcataaag tgtaaagcct   4500

ggggtgccta atgagtgagc taactcacat taattgcgtt gcgctcactg cccgctttcc   4560

agtcgggaaa cctgtcgtgc cagctgcatt aatgaatcgg ccaacgcgcg gggagaggcg   4620

gtttgcgtat tgggcgctct tccgcttcct cgctcactga ctcgctgcgc tcggtcgttc   4680

ggctgcggcg agcggtatca gctcactcaa aggcggtaat acggttatcc acagaatcag   4740

gggataacgc aggaaagaac atgtgagcaa aaggccagca aaaggccagg aaccgtaaaa   4800
```

-continued

```
aggccgcgtt gctggcgttt ttccataggc tccgcccccc tgacgagcat cacaaaaatc   4860

gacgctcaag tcagaggtgg cgaaacccga caggactata aagataccag gcgtttcccc   4920

ctggaagctc cctcgtgcgc tctcctgttc cgaccctgcc gcttaccgga tacctgtccg   4980

cctttctccc ttcgggaagc gtggcgcttt ctcatagctc acgctgtagg tatctcagtt   5040

cggtgtaggt cgttcgctcc aagctgggct gtgtgcacga accccccgtt cagcccgacc   5100

gctgcgcctt atccggtaac tatcgtcttg agtccaaccc ggtaagacac gacttatcgc   5160

cactggcagc agccactggt aacaggatta gcagagcgag gtatgtaggc ggtgctacag   5220

agttcttgaa gtggtggcct aactacggct acactagaag gacagtattt ggtatctgcg   5280

ctctgctgaa gccagttacc ttcggaaaaa gagttggtag ctcttgatcc ggcaaacaaa   5340

ccaccgctgg tagcggtggt ttttttgttt gcaagcagca gattacgcgc agaaaaaaag   5400

gatctcaaga agatcctttg atcttttcta cggggtctga cgctcagtgg aacgaaaact   5460

cacgttaagg gattttggtc atgagattat caaaaaggat cttcacctag atccttttaa   5520

attaaaaatg aagttttaaa tcaatctaaa gtatatatga gtaaacttgg tctgacagtt   5580

accaatgctt aatcagtgag gcacctatct cagcgatctg tctatttcgt tcatccatag   5640

ttgcctgact ccccgtcgtg tagataacta cgatacggga gggcttacca tctggcccca   5700

gtgctgcaat gataccgcga gacccacgct caccggctcc agatttatca gcaataaacc   5760

agccagccgg aagggccgag cgcagaagtg gtcctgcaac tttatccgcc tccatccagt   5820

ctattaattg ttgccgggaa gctagagtaa gtagttcgcc agttaatagt ttgcgcaacg   5880

ttgttgccat tgctacaggc atcgtggtgt cacgctcgtc gtttggtatg gcttcattca   5940

gctccggttc ccaacgatca aggcgagtta catgatcccc catgttgtgc aaaaaagcgg   6000

ttagctcctt cggtcctccg atcgttgtca gaagtaagtt ggccgcagtg ttatcactca   6060

tggttatggc agcactgcat aattctctta ctgtcatgcc atccgtaaga tgcttttctg   6120

tgactggtga gtactcaacc aagtcattct gagaatagtg tatgcggcga ccgagttgct   6180

cttgcccggc gtcaatacgg gataataccg cgccacatag cagaacttta aaagtgctca   6240

tcattggaaa acgttcttcg gggcgaaaac tctcaaggat cttaccgctg ttgagatcca   6300

gttcgatgta acccactcgt gcacccaact gatcttcagc atcttttact ttcaccagcg   6360

tttctgggtg agcaaaaaca ggaaggcaaa atgccgcaaa aaagggaata agggcgacac   6420

ggaaatgttg aatactcata ctcttccttt ttcaatatta ttgaagcatt tatcagggtt   6480

attgtctcat gagcggatac atatttgaat gtatttagaa aaataaacaa ataggggttc   6540

cgcgcacatt tccccgaaaa gtgc                                          6564
```

What is claimed is:

1. A genetically altered ciliate wherein the ciliate lacks detectable expression of one or more sortilin (SOR) gene products selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

2. The ciliate of claim **1**, wherein the one or more sortilin (SOR) gene products has a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7.

3. The ciliate of claim **1**, wherein the ciliate comprises a genomic alteration of one or more of SOR1, SOR2, SOR3, or SOR4.

4. The ciliate of claim **3**, wherein the ciliate comprises a deletion in both copies of the ciliate's germline genome that disrupts expression of a SOR gene product.

5. The ciliate of claim **3**, wherein the ciliate comprises an insertion in both copies of ciliate's germline genome that disrupts expression of a SOR gene product.

6. The ciliate of claim **5**, wherein the insertion is located in the open reading frame of one or more SOR genes selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

7. The ciliate of claim **1**, wherein the ciliate lacks detectable expression of 2, 3, or 4 gene products selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

8. The ciliate of claim **1**, wherein the ciliate expresses a polynucleotide complementary to all or part of an RNA gene product selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

9. The ciliate of claim **8**, wherein the polynucleotide molecule complementary to all or part of an RNA gene product is an antisense RNA or a double stranded RNA (dsRNA).

10. The ciliate of claim **1**, wherein the ciliate is *Tetrahymena.*

11. A recombinant *Tetrahymena* germline genome comprising a genomic insertion or deletion in both copies of one or more SOR genes selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

12. The recombinant genome of claim **11**, wherein the genomic insertion or deletion is located in the open reading frame of the one or more SOR genes.

13. The recombinant genome of claim **11** comprising a genomic insertion or deletion in both copies of 2, 3, or 4 SOR genes selected from the group consisting of SOR1, SOR2, SOR3, and SOR4.

14. A method of producing a genetically altered ciliate comprising:

(a) transforming a ciliate with a polynucleotide comprising a sequence complementary to a SOR gene selected from the group consisting of SOR1, SOR2, SOR3, and SOR4; and

(b) isolating a genetically altered ciliate wherein the ciliate lacks detectable expression of the gene product of said SOR gene.

15. The method of claim **14**, wherein step (b) comprises isolating a genetically altered ciliate comprising an insertion or deletion in a SOR gene.

16. A method of producing a polypeptide comprising: expressing a polynucleotide encoding a polypeptide in the ciliate of claim **1**.

17. The method of claim **16**, further comprising:

(c) purifying the expressed polypeptide from media.

18. The method of claim **16**, wherein the polypeptide is not secreted by the ciliate.

19. The method of claim **18**, further comprising:

(c) purifying the ciliate from media.

20. The method of claim **19**, further comprising:

(d) purifying the polypeptide from the ciliate.

* * * * *