

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2016/081619 A1

(43) International Publication Date

26 May 2016 (26.05.2016)

WIPO | PCT

(51) International Patent Classification:

C12Q 1/02 (2006.01) G01N 33/566 (2006.01)  
C12N 15/09 (2006.01)

(21) International Application Number:

PCT/US2015/061373

(22) International Filing Date:

18 November 2015 (18.11.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/081,441 18 November 2014 (18.11.2014) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

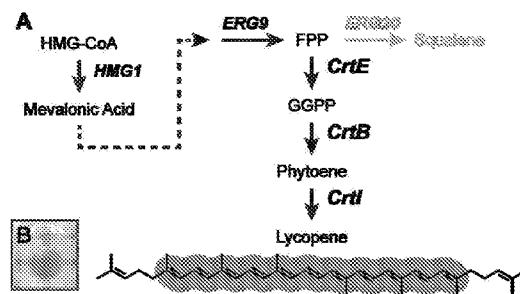
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: DETECTION OF ANALYTES USING LIVE CELLS

FIGURE 1



(57) Abstract: The present invention provides sensor cells comprising a receptor that binds to an analyte indicative of the presence of an agent, where binding of the analyte to the receptor triggers a detection event that is indicative of the presence of the agent. In certain embodiments, the detection event is appearance of a reporter detectable by the naked eye. The present invention also provides uses of such sensor cells for detecting the presence of an agent in a sample.

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## DETECTION OF ANALYTES USING LIVE CELLS

### CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No. 62/081,441, filed November 18, 2014, the content of which is incorporated by reference in its entirety, and to which priority is claimed.

### GRANT INFORMATION

10 This invention was made with government support under Grant Nos. 1R01AI110794-01A1 from National Institutes of Health, and HR0011-15-2-0032 from Defense Advanced Research Projects Agency, and Graduate Research Fellowship number DGE-11-44155 from the National Science Foundation. The government has certain rights in the invention.

15 1. INTRODUCTION

The present invention relates to methods and compositions for detecting the presence of an agent in a test sample using a whole cell reporter. In certain embodiments, detection can be performed without the aid of instrumentation, for example outside of a laboratory setting, permitting home and field tests for 20 interrogating the status of biological systems. The present invention may be used, for example, to identify pathogens and thereby limit the dissemination of disease.

2. BACKGROUND

2.1. WHOLE-CELL BIOSENSORS

Microbial whole-cell bio-reporters present unique advantages for 25 environmental sensing, such as the probing of complex biochemical processes, compatibility with aqueous media, self-renewal by replication, portability by freeze-drying, availability of numerous natural sensing pathways, and ease of engineering

new functions (e.g., by directed evolution).<sup>1,2</sup> Bacterial whole cell sensors have previously been demonstrated for detection of DNA damage,<sup>3</sup> heat shock,<sup>4</sup> oxidative stress,<sup>5</sup> heavy metals,<sup>6-8</sup> viruses,<sup>9</sup> and light.<sup>10</sup> Yeast and mammalian whole cell sensors have also been reported. For yeast whole cell sensors, *see* Hollis (2000) and Radhika (2007). For mammalian whole cell sensors, *see* Rider, (2003).

## 2.2. PEPTIDES AS ANALYTES

While natural receptors can be utilized for detection of a broad range of analytes, proteins and their peptide epitopes present a ubiquitous pool of natural biomarkers which are highly characteristic of the organisms that produce them.

10 Peptides can thus be used as targets for detection of pathogenic organisms, food born toxins, immunogens and bioterrorism agents. For example, see the recent development of mass spectrometry of proteolized samples as a diagnostic tool for various diseases.<sup>11,12</sup>

## 2.3. USING GPCRs FOR DETECTION

15 G-protein coupled receptors (GPCR) constitute a large family of seven-transmembrane receptors for hormones, neurotransmitters, chemokines, calcium, odorants, taste molecules and even light.<sup>19</sup> GPCR signaling pathways are highly conserved among diverse species. Furthermore, GPCR-activation of the Mitogen-activated protein kinase (MAPK) phosphorylation cascade is conserved from yeast to

20 mammals,<sup>19</sup> with different MAPK families activated by multiple different GPCRs.

It was shown that yeast pheromone receptors can be functionally replaced by expressing mammalian GPCRs that couple to the endogenous MAPK signaling pathway, so that the corresponding mammalian agonist activates the yeast pheromone response using different reporter genes<sup>21-23</sup> beta-galactosidase<sup>24-26</sup> or auxotrophic markers.<sup>27-29</sup>

G-protein coupled receptors (GPCRs) have previously been implemented in yeast to develop high-throughput drug discovery assays based around mammalian receptors by using a growth based reporter.<sup>13,14</sup> Additionally, yeast has also been used to functionally express native fungal receptors to study the biology of the respective fungi.<sup>15-18</sup> These previous studies coupled the GPCRs to the endogenous pheromone response pathway by using laboratory assays requiring instrumentation.

### 3. SUMMARY OF THE INVENTION

The present invention relates to methods and compositions for detecting the presence of an agent, for example, but not limited to, a human disease agent (e.g., a pathogenic agent), an agricultural agent, an industrial and model organism agent, a bioterrorism agent, or a heavy metal contaminant, by detecting the presence of an analyte indicative of the presence of the agent in a test sample. In certain embodiments, the analyte is the agent itself, a portion of the agent (e.g., a portion generated by proteolysis), or a product of the agent. The methods utilize a sensor cell bearing a receptor that is specific for the analyte, where binding of the receptor to the analyte triggers a detection event that is indicative of the presence of the agent. The reporter can be coupled to the receptor. In certain embodiments, the sensor cell is a microbe that is easy and quick to propagate, for example a yeast cell, and the reporter gene product is detectable to the naked eye, for example a pigmented compound such as (red) lycopene. In certain non-limiting embodiments, the present disclosure provides an engineered baker's yeast that uses G-protein coupled receptors (GPCRs) to detect a range of peptide ligands associated with specific target agents and uses the red plant pigment lycopene as a fast, non-technical, visual readout. In certain non-limiting embodiments, the present disclosure provides methods of engineering peptide-activated GPCRs to detect non-cognate agent-specific peptides and to

improve performance (*e.g.*, sensitivity and/or specificity) against peptide ligands, using directed evolution.

The present invention provides methods of detecting the presence of an agent of interest in a sample. In certain embodiments, the method comprises: contacting the sample with a sensor cell comprising a non-native G-protein coupled receptor (GPCR) that binds to an analyte indicative of the presence of the agent, wherein binding of the analyte to the receptor triggers appearance of a reporter detectable by the naked eye, wherein the increased expression is indicative of the presence of the agent. The agent can be selected from the group consisting of human disease agents, agricultural agents, industrial and model organism agents, bioterrorism agents, and heavy metal contaminants. In certain embodiments, the non-native GPCR receptor is engineered to bind to the analyte. In certain embodiments, the non-native GPCR receptor is engineered by directed evolution. In certain embodiments, the non-native GPCR receptor is a fungal pheromone GPCR. In certain embodiments, the non-native GPCR receptor is selected from the group consisting of the GPCRs listed in Tables 2 and 6.

In certain embodiments, the sensor cell is a microbe. In certain embodiments, the sensor cell is a fungal cell. In certain embodiments, the sensor cell is a yeast cell. In certain embodiments, the sensor cell is *S. cerevisiae*. In certain embodiments, the sensor cell comprises a nucleic acid encoding the receptor. In certain embodiments, the nucleic acid is linked to a promoter.

In certain embodiments, the analyte is a cognate ligand for the non-native GPCR receptor. In certain embodiments, the analyte is a non-cognate ligand for the non-native GPCR receptor.

In certain embodiments, the analyte is a peptide. In certain embodiments, the peptide is a fungal mating pheromone. The fungal mating pheromone can be selected from the group consisting of human fungal mating pheromones (meaning mating pheromones of fungi that can colonize or infect humans), non-human animal fungal 5 mating pheromones (meaning mating pheromones of fungi that colonize or infect a non-human animal), plant fungal mating pheromones (meaning mating pheromones of fungi that colonize or infect a plant), food fungal mating pheromones (meaning mating pheromones of fungi that colonize or infect human or non-human animal food items), and industrial/model fungal mating pheromone. In non-limiting examples, the 10 human fungal mating pheromone can be selected form the group consisting of the mating pheromones of *C. albicans*, *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, *S. scheckii*, and *Candida krusei*. An example of a non-human animal fungal mating pheromone is the mating pheromone of *P. destructans*. In non-limiting examples, the plant fungal 15 mating pheromone can be selected from the group consisting of the mating pheromones of *F. graminearum*, *M. oryzae*, *B. cinerea*, *G. candidum*, and *C. purpurea*. In non-limiting examples, the food fungal mating pheromone can be selected from the group consisting of the mating pheromones of *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, and *N. fischeri*. In non-limiting examples, the 20 industrial/model fungal mating pheromone can be selected from the group consisting of the mating pheromones of *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (*receptor 1*), *V. polyspora* (*receptor 2*), *S. stipitis*, *S. japonicas*, *S. castellii*, and *S. octosporus*, *A. oryzae*, *T. melanosporum*, *D. haptotyla*, *C. tenuis*, *Y. lipolytica*, *T. delbrueckii*, *B. bassiana*, *K. pastoris*, *A. nidulans*, *N. crassa*, and *H. jecorina*.

In non-limiting examples, the peptide can be selected from the group consisting of the peptides listed in Table 5. In certain embodiments, the peptide has a length of about 5-25 residues. In certain embodiments, the peptide has a length of about 9-23 residues.

5       In certain embodiments, the peptide is associated with a bacterial infection. In certain embodiments, the peptide is associated with *Vibrio cholera*. In non-limiting examples, the peptide associated with *Vibrio cholerae* can be selected from the group consisting of a peptide having an amino acid sequence set forth in VEVPGSQHIDSQKKA (SEQ ID NO: 26), a peptide having an amino acid sequence 10 that is at least 80%, at least 90% or at least 95% about homologous to SEQ ID NO: 26, a peptide having an amino acid sequence set forth in VPGSQHIDS (SEQ ID NO: 27), and a peptide having an amino acid sequence that is at least about 80%, at least 90% or at least 95% homologous to SEQ ID NO: 27. In certain embodiments, the peptide is derived from cholera toxin. The peptide derived from cholera toxin can be 15 selected from the group consisting of the peptides listed in Table 7.

In certain embodiments, the non-native GPCR receptor is coupled to the reporter. In certain embodiments, the method further comprises culturing the sensor cell for an effective period of time; and determining expression of the reporter gene . In certain embodiments, determining expression of the reporter gene does not 20 comprise instrumentation. In certain embodiments, the reporter is a biosynthesized visible-light pigment. In certain embodiments, the reporter is lycopene. In certain embodiments, the sensor cell is engineered to express the receptor.

In certain embodiments, the sample is selected from the group consisting of water samples and body fluid samples. The water sample can be selected from the 25 group consisting of fresh water, sea water, and sewage samples. The body fluid

sample can be selected from the group consisting of intestinal fluids, diarrhea, mucus, blood, cerebrospinal fluid, lymph, pus, saliva, vomit, urine, bile, and sweat.

Additionally, the present invention provides a sensor cell comprising a non-GPCR receptor that binds to an analyte indicative of the presence of the agent, 5 wherein binding of the analyte to the receptor triggers appearance of a reporter detectable by the naked eye, wherein the increased expression is indicative of the presence of the agent.

Furthermore, the present invention provides a kit for detecting the presence of an agent of interest, comprising a sensor cell as described above. In certain 10 embodiments, the kit further comprises a negative control. In certain embodiments, the kit further comprises a substrate that comprising the sensor cell. In certain embodiments, the kit further comprises a nutrient source.

#### 4. BRIEF DESCRIPTION OF THE FIGURES

FIGURES 1A and 1B depict biosynthesis of lycopene. (A) Introduction of *E. 15 herbicola* carotenoid enzymes (CrtEBI) result in biosynthesis of lycopene from endogenous yeast farnesyl pyrophosphate. (B) A lycopene-producing yeast strain becomes visibly colored.

FIGURE 2 depicts eukaryotic biosensor design. Binding of one or more agent-specific analyte (e.g., a peptide) to a receptor triggers a signal transduction 20 cascade, resulting in induction of CrtI (or other Crt) gene responsible for a reporter (e.g., lycopene) biosynthesis or other reporter genes. The G-protein coupled receptor operates via the mating signaling pathway in yeast.

FIGURE 3 depicts one embodiment of cell-based detection of cholera pathogen in drinking water. Engineered sensor is added to cholera-contaminated 25 water or a clinical sample. Binding of the cholera pathogen-specific peptide induces a

signal cascade in the sensor cell, resulting in amplification of a color reporter gene colorimetric signal.

FIGURES 4A and 4B depict experimental results with yeast strains that produced lycopene in response to activation of the endogenous GPCR Ste2. Figure 5 4A shows induction of lycopene biosynthesis by the natural yeast peptide,  $\alpha$ -factor. Figure 4B shows improvement of lycopene readout speed with modification of the yeast strain, in laboratory conditions.

FIGURE 5 depicts viability of yeast after freeze-drying.  $10^8$  cells were freeze dried and resuspended in YPD. Cell was then plated to quantify survival after 0, 1 or 10 4 hours in YPD media.

FIGURE 6 depicts functional and specific response of fungal GPCRs measured by fluorescence. “Xx.a” denotes peptide pheromones derived from species Xx. Species abbreviations: Sc, *S. cerevisiae*; Ca, *C. albicans*; Pb, *P. brasiliensis*; Fg, *F. graminearum*; Mo, *M. oryzae*; Bc, *B. cinerea*.

15 FIGURE 7 depicts a peptide-centric directed evolution (DE) approach. The peptide-centric DE approach permitted direct use of hybrid peptides that march from  $\alpha$ F to the target peptide analytes. After rounds of DE, mutant engineered receptors gained activity to an intermediate peptide and then further increased EC50.

FIGURE 8 depicts one embodiment of cell-based detection of an agent of 20 interest. A yeast-based biosensor constructed around engineered baker’s yeast is extremely cheap to produce, portable as a freeze-dried product, and simple to use. A non-technical user simply adds a sample and waits for a color change signaling the presence of the agent.

FIGURES 9A-9C depicts specific detection of fungal peptides. (A) Mining of 25 fungal receptor-pheromone pairs. Fungal receptor gene was cloned into *S. cerevisiae*

sensor strain, and tested using a synthetic fungal peptide pheromone, using a fluorescent readout. (B) Orthogonality matrices of fungal receptors, measured in biosensor strain using fungal GPCR-peptide pairs. (C) EC50 values for fungal receptors.

5 FIGURE 10 depicts functional characterization of fungal GPCR-peptide pairs. GPCR was engineered into *S. cerevisiae* sensor cell, and induced using its native fungal peptide (synthetic peptide). Induction of fluorescent marker was monitored in culture.

FIGURES 11A-11C depict common topology of fungal GPCRs. (A)  
10 Topological model of the *S. cerevisiae* Ste receptor was predicted by TMHMM v2.0. All the GPCRs characterized have similar topological profile which includes three key regions of higher homology to *S. cerevisiae* Ste2 (gray boxes). Region I corresponds to the third intracellular loop and shows two positively charged residues with high conservation at positions 233 and 234 relative to the *S. cerevisiae* Ste2.  
15 Region II corresponds to the sixth transmembrane helix and contains an essential proline that is conserved across all the receptors at position 258 relative to the *S. cerevisiae* Ste2. Region III shows the highest level of conservation and also includes an essential proline conserved across all the receptors at position 290 relative to the *S. cerevisiae* Ste2. (B) Sequence logo results after alignment of the 23 characterized  
20 receptors. These three key regions have higher density of conserved residues with some residues conserved across all receptors. (C) Percent homology of different regions the 23 receptors when compared to the corresponding region of the *S. cerevisiae* Ste2.

FIGURES 12A and 12B depict characteristics of peptide ligands. (A)  
25 Functional domains within *S. cerevisiae* alpha factor. Residues in blue were shown to

have a strong impact on binding when changed to alanine, while residues in purple were shown to be involved in signaling. [Naider et al. (2004)]. These findings led to the simplified designation of the N-terminus of alpha factor as the signaling domain and the C-terminus as the binding domain, with internal residues L<sub>6</sub> and G<sub>9</sub> strongly contributing to peptide binding. (B) Functional peptide ligands were aligned and clustered according to [Andreatta et al. (2013)]. Positive and negative charges (red and green, respectively) were indicated in colored bolt. Sequences within each of the clusters were shown along with the resulting sequence logos. Logos only highlight the identified 13-residue motifs.

FIGURES 13A-13D depict enhancement of lycopene output. (A) Detailed lycopene pathway w/ co-factors and improved yield lycopene yield & time of visible detection. (B and C) Lycopene yield (B) and response time (C) were optimized using the natural *S. cerevisiae* alpha factor response. Overexpression of genes tHMG1, CrtI and Fad1 showed gradual increase in lycopene yield allowing faster visible response. (D) Characterization of lycopene output in response to alpha factor peptide of pathogenic fungi *C. Albicans*.

FIGURES 14A-14C depict detection of pheromone-producing *C. albicans* strain via biosensor strain. (A) Design of “Yeast Block” product and functional demonstration of integrated biosensor. (B) Dose-response curve of lycopene-producing biosensor using synthetic *C. Albians* alpha pheromone. (C) Biosensor response to different pheromone-producing *C. albicans* strains, as measured using fluorescence output. Each of the *C. albicans* were grown first on Phloxine B stained agar and opaque colonies were selected. These opaques colonies were cultured and their supernatants were assayed.

FIGURE 15 depicts a process from biomarker identification to a novel biosensor. Workflow starts with identification of potential peptide biomarkers by mass spectrometry, leading to identification of parent GPCR used for directed evolution. The resulting GPCR which binds the selected biomarker is incorporated 5 into the biosensor cell.

FIGURE 16 depicts best matching fungal library member/peptidome member pair. The sample peptide HFGVLDEQLHR (SEQ ID NO:132) is similar in length and sequence (36% identity) to the natural mating pheromone activating the mating GPCR of *Zygosaccharomyces rouxii*.

## 10 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and compositions for detecting the presence of an agent of interest in a test sample.

For clarity and not by way of limitation, the detailed description is divided into the following subsections:

- 15 (i) Agents of Interest  
(ii) Sensor cells;  
(iii) Receptors and coupling systems;  
(iv) Detection events;  
(v) Analytes;  
20 (vi) Methods of use; and  
(vii) Kits

### 5.1. AGENTS OF INTEREST

Presently disclosed sensor calls can be used to detect the presence of a variety of agents. Non-limiting examples of suitable agents include human disease agents

(human pathogenic agents), agricultural agents, industrial and model organism agents, bioterrorism agents, and heavy metal contaminants.

Human disease agents include, but are not limited to infectious disease agents, oncological disease agents, neurodegenerative disease agents, kidney disease agents, 5 cardiovascular disease agents, clinical chemistry assay agents, and allergen and toxin agents.

Infectious disease agents include, but are not limited to, fungal pathogens, bacterial pathogens, viral pathogens, and protozoan pathogens, as well as toxins produced by same. Non-limiting examples of fungal pathogens include *C. albicans*, 10 *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, *S. scheckii*, and *Candida krusei*.

Non-limiting examples of bacterial pathogens include *Vibrio cholerae*, *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* (MRSA) strains, *Bacillus subtilis*, *Streptococcus pneumonia*, Group B *Streptococcus*, 15 *Salmonella* sp., *Listeria monocytogenes*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Clostridium difficile*, *Yersinia enterocolitica*, *Legionella* sp., *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Neisseria meningitis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, botulinum toxin of *Clostridium botulinum*, 20 *Shigella*/ Enteroinvasive E.coli, Shiga toxin from the Shiga toxin-producing *Escherichia coli* (STEC), and Verotoxin derived from *Shigella dysenteriae*. Analytes that are indicative of the presence of bacterial pathogens include, but are not limited to, quorum sensing small molecules such as the *Vibrio Cholera* CAI-1,<sup>69</sup> inter-species bacterial quorum sensing AL-2,<sup>70</sup> or components of the bacterial LPS.

Non-limiting examples of viral pathogens include Ebola virus, HPV, HIV, influenza, Hepatitis C Virus, Hepatitis B Virus. Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Respiratory syncytial virus (RSV), Norovirus, Sapovirus, and measles virus. Analytes that are indicative of the presence of viral pathogens include,  
5 but are not limited to, capsid protein or peptides, and other viral particles.

Non-limiting examples of protozoan pathogens include *Trichomonas vaginalis*, *Cryptosporidium*, *Cyclospora cayetanensis*, *Giardia lamblia*, and biomarkers for Amoebiasis derived from *Entamoeba histolytica* such as *E. histolytica* ADP-forming acetyl-CoA synthetase (EhACS) or related peptides [Huat (2014)],  
10 Leishmaniasis biomarkers such as the amastin signature peptide [Rafati (2006)].

Oncological disease agents include, but are not limited to, lung, breast, colorectum, prostate, stomach, liver, kidney or cervix cancer, leukemia, Kaposi sarcoma, Testis, Ovary, thyroid, and other cancer peptide biomarkers unique for certain cancer types, which can be identified by mass spectrometry.<sup>60-63</sup>

15 Neurodegenerative disease agents include, but are not limited to, peptide biomarkers indicated in Alzheimer's,<sup>64</sup> [notably fungal biomarker for Alzheimer's were recently suggested in Pisa (2015)], the protein DJ-1 or peptides thereof as biomarkers for Parkinson disease,<sup>65</sup> and biomarkers for prion disease such as proteins or peptides of the 14-3-3 family in cerebrospinal fluid for detection of Creutzfeldt-  
20 Jakob disease [Van Everbroeck (2005) and Huzarewich (2010)].

Clinical chemistry assay (for general health diagnostics) agents include, but are not limited to, peptide hormones. Peptide hormones include, but are not limited to, neurohypophyseal hormones (e.g., oxytocin and vasopressin) and pancreatic hormones (e.g., glucagon, insulin and somatostatin).

Allergen and toxin agents include, but are not limited to, peptide derived from immunogenic wheat peptide (e.g., gluten), and carcinogen aflatoxin B1 derived from the fungi *A. flavus*.

Kidney disease agents include, but are not limited to, proteins and peptides 5 identified as urinary biomarkers for kidney disease, such as  $\beta$ 2-microglobulin, and differential patterns of peptides in type 2 diabetes<sup>66</sup>.

Cardiovascular disease agents include, but are not limited to, proteins and peptides indicative for atherothrombosis or risk markers for stroke. Markers for primary cardiovascular events include peptides derived from C-reactive protein, 10 fibrinogen, cholesterol, apolipoprotein B, high density lipoprotein, and small molecules like vitamin D. Markers for secondary cardiovascular events include peptides derived from cardiac troponins I and T, C-reactive protein, serum creatinine, and cystatin C. Risk markers for primary stroke, include peptides derived from fibrinogen and serum uric acid [Van Holten et al. (2013)]

15 Agricultural agents include, but are not limited to, fungal pathogens of animals and plants, and fungal agents causing food spoilage. Fungal pathogens of animals and plants include, but are not limited, to animal fungal pathogens and plant fungal pathogens. Animal fungal pathogens include, but is not limited to, *P. destructans*. Non-limiting examples of plant fungal pathogens include *F. graminearum*, *M. oryzae*, 20 *B. cinerea*, *G. candidum*, and *C. purpurea*. Non-limiting examples of fungal agents causing food spoilage include *Z. bailii*, *Z. rouxii*, and *N. fischeri*.

Industrial and model organism agents include, but are not limited to, fungal agents used for genetic studies and industrial applications such as food production, pharmaceutical production, fine chemical production, bioremediation, including, but

not limited to, *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (receptor 1), *V. polyspora* (receptor 2), *S. stipitis*, *S. japonicus*, *S. castellii*, and *S. octosporus*.

Bioterrorism agents include, but are not limited to, peptide biomarkers for *Bacillus anthracis* (causative agent of anthrax - e.g., one of three polypeptides that 5 comprise the anthrax toxin secreted by the pathogen: protective antigen (PA), lethal factor (LF) and edema factor (EF)),<sup>67</sup> *Clostridium botulinum* (causative agent of botulism- e.g., Botulinum neurotoxin peptides such as the cyclic peptide C11-019),<sup>68</sup> viral agents such as smallpox (Variola virus) and Viral encephalitis, Ebola virus.

Heavy metal contaminant include, but are not limited to, cadmium, mercury, 10 lead or arsenic, as bound to biological receptors.

In certain embodiments, the agent is the same as the analyte, as disclosed herein. In certain embodiments, the agent is different from the analyte.

## 5.2. SENSOR CELLS

The sensor cell can be engineered to comprise one or more component of the 15 assay system disclosed herein. As used herein, the term “engineered” means that one or more component is introduced into a sensor cell or its parent cell by a method selected from the group consisting of recombinant DNA techniques (e.g., Reiterative Recombination and CRISPR), natural genetic events, conjugation, and a combination thereof. Sensor cells can be prokaryotic cells or eukaryotic cells. In certain 20 embodiments, a presently disclosed sensor cell is a microbe, including, but not limited to, bacteria, fungi, and slime molds. In certain embodiments, the sensor cell is a fungal cell. In certain embodiments, the fungal cell is a yeast cell. Non-limiting examples of yeast cells include *Saccharomyces cerevisiae*, *Pichia pastoris* and *Schizosaccharomyces pombe*. In one non-limiting embodiment, the sensor cell is 25 *Saccharomyces cerevisiae*. Additional non-limiting examples of fungal cells include

*Candida albicans*, *Paracoccidioides brasiliensis*, *Fusarium graminearum*, *Magnaporthe oryzae*, and *Botrytis cinerea*. In certain embodiments, the sensor cell is a bacterial cell. Non-limiting examples of bacterial cells include *Escherichia coli*, *Bacillus subtilis*, and *Lactobacillus acidophilus*.

### 5 5.3 RECEPTORS AND COUPLING SYSTEMS

The present invention provides for receptors and coupling systems wherein a sensor cell comprises (e.g., bears) a receptor that binds to an analyte, where binding of the analyte triggers a detection event that is indicative of the presence of the agent (e.g., expression of a detectable reporter gene, including increased or decreased 10 expression), release of a therapeutic molecule that directly remediates the agent, production of a redox active molecule, or a change in the membrane potential of the sensor cell). In certain embodiments, the sensor cell is engineered to bind to the analyte.

As used herein, the term “receptor” means a molecule (e.g., a ligand) that 15 binds to a presently disclosed analyte that is indicative of the presence of an agent of interest. A presently disclosed receptor is positioned, either inherently or by association with a membrane protein, at the cell surface exposed to the extracellular environment. In certain embodiments, the receptor is a protein. In certain embodiment, the receptor is a naturally occurring (native) protein or a portion thereof. 20 In certain embodiments, the receptor is a portion of a naturally occurring protein comprised in a fusion protein with one or more heterologous proteins. In certain embodiments, the receptor is a mutated version of a naturally occurring protein. In certain embodiments, the receptor is a synthetic protein. In certain embodiments, the receptor is a partly-synthetic protein. In certain embodiments, the receptor comprises 25 one or more non-protein element.

In certain embodiments, the receptor is a non-protein molecule. In one non-limiting embodiment, the receptor is an aptamer or a riboswitch. The receptor may be comprised of a single element or may be comprised of a plurality of elements/subunits.

5        In certain non-limiting embodiments, the sensor cell comprises a receptor that binds to an analyte, wherein the receptor is coupled to a detectable reporter gene such that when the analyte binds to the receptor, expression of the reporter gene is increased or induced. In certain embodiments, the receptor is coupled to a detectable reporter gene such that when an analyte binds to the receptor, expression of the 10 reporter gene is inhibited (for example, by binding of a transcriptional repressor). In certain embodiments, the analyte is a peptide, *e.g.*, an agent-specific peptide.

As used herein, the term “coupled to” means that binding of an analyte to a receptor is causally linked, directly or indirectly, to and triggers a detection event that is indicative of the presence of the agent (*e.g.*, expression of a detectable reporter gene 15 (induced or inhibited expression), release of a therapeutic molecule that directly remediates the agent, production of a redox active molecule, or a change in the membrane potential of the sensor cell). In certain embodiments, the detection event is expression of a detectable reporter gene. In certain embodiments, the detection event is induced expression of a detectable reporter gene. The receptor may be linked to 20 expression level of the reporter gene through, for example, a pathway of interacting molecules. This pathway may be host-endogenous or engineered.

In certain embodiments, the sensor cell is engineered to express the receptor, for example, by the introduction of a nucleic acid encoding the receptor. In certain embodiments, the nucleic acid is operably linked to a promoter element. In certain 25 embodiments, the promoter element is constitutively active. In certain embodiments,

the promoter element is inducibly active. In certain embodiments, the receptor is expressed on the surface of the sensor cell. In certain embodiments, the receptor is expressed on internal membranes of the sensor cell. In certain embodiments, the receptor is expressed in the cytoplasm of the sensor cell.

5        In certain embodiments, the analyte is a natural (cognate) ligand of the receptor; the coupled analyte-receptor system utilizes a receptor and its natural (cognate) ligand as the analyte. In certain embodiments, the coupled analyte-receptor system is a receptor engineered to bind a different non-cognate ligand as analyte, by way of directed evolution detailed below.

10      In certain non-limiting embodiments, the sensor cell expresses a single species of analyte receptor. In certain non-limiting embodiments, the sensor cell expresses a plurality of species of analyte receptor.

In certain non-limiting embodiments, the sensor cell comprises an analyte-specific receptor which is coupled to a detectable reporter gene by a G-protein 15 signaling pathway. Hence, in certain embodiments, the receptor is a G-protein coupled receptor (GPCR) polypeptide or protein. In certain embodiments, the receptor is a non-native GPCR receptor.

In certain non-limiting embodiments, a yeast pheromone sensing system is used for analyte detection. The yeast pheromone signaling pathway is well studied 20 structurally and is functionally similar to hormone and neurotransmitter signaling pathways in mammals.<sup>20</sup> In certain non-limiting embodiments, the receptor is a variant of the yeast Ste2 receptor or Ste3 receptor, wherein the receptor is modified so that it binds to the analyte rather than yeast pheromone. In certain embodiments, the receptor or portion thereof is a polypeptide that is at least about 40%, at least about 25 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at

least about 95%, at least about 98% homologous, or at least about 99% homologous to the native yeast Ste2 or yeast Ste3 receptor. “Homologous” or “homology” can mean sequence (nucleotide sequence or amino acid sequence) homology or structural homology. In certain embodiments, “homology” or “homologous” refers to sequence (nucleotide sequence or amino acid sequence) homology. The sequence homology can be determined by standard software such as BLAST or FASTA. The receptor binds specifically to the analyte (e.g., agent-specific peptide) under assay conditions or under natural conditions (for example, but not limited to, at room temperature (e.g., 5-20-25°C, at or around body temperature (e.g., 30-40°C), field temperature (e.g., 5-40°C ) or between about 20-40°C). In certain non-limiting embodiments, the receptor is a chimeric protein comprising one or more fragment originating from other receptor proteins, or evolved from non-homologous receptor protein to bind to the analyte (e.g., agent-specific peptide) and interface with a signaling pathway. In certain non-limiting embodiments the receptor is a yeast GPCR polypeptide other than a pheromone binding receptor, such as Gpr1 putative sugar binding receptor and the cognate G $\alpha$  protein Gpa2.

The present invention also provides a nucleic acid encoding the receptor and a host cell comprising said nucleic acid. The nucleic acid can be used to produce a presently disclosed sensor cell. The nucleic acid can be introduced into the host cell such that it is operably linked to an inducible or constitutively active promoter element. In certain embodiments, the sensor cell is a yeast cell, and a nucleic acid encoding a receptor is introduced into the yeast cell either as a construct or a plasmid in which it is operably linked to a promoter active in the yeast cell or such that it is inserted into the yeast cell genome at a location where it is operably linked to a suitable promoter. Non-limiting examples of suitable yeast promoters include, but are

not limited to, constitutive promoters pTef1, pPgk1, pCyc1, pAdh1, pKex1, pTdh3, pTpi1, pPyk1, and pHxt7 and inducible promoters pGal1, pCup1, pMet15, and pFus1.

In certain non-limiting embodiments, receptor activation induces reporter gene expression under a FUS1 promoter, which allows for a convenient screen using 5 reporter gene activation. In one non-limiting example, a GPCR polypeptide is expressed in a yeast cell and is coupled to the yeast pheromone mating system such that GPCR binding activates the yeast Fus1 promoter to express a downstream reporter gene.<sup>27</sup> The GPCR DNA sequence can then be varied, and this library of altered receptors may be screened for binding of an analyte (e.g., an agent-specific 10 peptide) using production of reporter gene as an indicator of binding.<sup>13,26</sup>

In certain non-limiting embodiments, where the pathway includes the yeast pheromone sensing pathway, a nucleic acid encoding the reporter is operably linked to at least a transcription controlling portion of the Fus1 promoter, for example, but not limited to, an activating sequence located in the region (-300) to (+400) of the 15 Fus1 gene (Gene ID: 850330). In certain non-limiting embodiments, where the pathway includes the yeast pheromone sensing pathway, a nucleic acid encoding the reporter is operably linked to a Ste12-binding element [(A/T)GAAACA], such that binding of Ste12 acts as a transactivator of the expression of the reporter. In certain non-limiting embodiments, where the pathway includes the yeast pheromone sensing 20 pathway, a nucleic acid encoding the reporter is alternatively linked to one or more inducible promoter other than pFus1, e.g., pFus2, pFig2, and/or pAgal. In certain embodiments, receptor-activation is linked to an engineered pheromone-responsive transcription factor, which binds a synthetic transcription controlling element distinct from the Ste12-binding element. The transcription factor Ste12 is composed of a 25 DNA-binding domain, a pheromone responsive domain and an activation domain.

The feasibility of engineering Ste12 to bind to non-natural control elements but remain to activate transcription in a pheromone-responsive manner has been shown [Pi et al (1997)].

In certain embodiments, a GPCR is engineered by directed evolution (DE) to alter its stability, specificity, and/or sensitivity. Hence, a receptor that is activated by a desired analyte can be generated by mutagenesis and selection in the laboratory. Several research groups have established DE in yeast as tool for changing mammalian GPCR ligand specificity.<sup>13,14,30-32</sup> Non-limiting examples of such engineered GPCRs include mammalian tachykinin receptors, secretin receptors, opioid receptors, and calcitonin receptors. Non-limiting examples of DE to develop a stable reporter strain are provided in the Examples section.

In certain embodiments, the GPCR is a fungal GPCR. In certain embodiments, the GPCR is a fungal pheromone GPCR. In certain non-limiting embodiments, a fungal Ste2-type or Ste3-type GPCR derived from one or more fungus is engineered into *S. cerevisiae* or other yeast cells to serve as a receptor for detecting an agent of interest. While any peptide-sensing GPCR can be repurposed as a detection element in a yeast cell, fungal pheromone GPCRs have several key advantages for biosensor engineering. First, this type of GPCRs (GPCRs homologous to the *S. cerevisiae* Ste2) couple robustly to the host/native pheromone pathway (see Figures 9 and 10), and several have been expressly validated in *S. cerevisiae* with little to no further modifications.<sup>15-18</sup> Second, fungal pheromone GPCRs from related fungi recognize different peptides based on the natural evolution of this class of GPCR.<sup>33</sup> For example, as shown in Figure 12 and Table 1, these fungal GPCRs recognize a diverse set of peptide ligands. Third, fungal pheromone GPCRs are highly specific for their respective peptides (see Figure 9), since they must mediate

the species-specific mating reaction while preventing interspecies breeding.<sup>34</sup> Furthermore, though there is no crystal structure of these GPCRs, extensive biochemical characterization and mutagenesis data indicates that the *S. cerevisiae* GPCR has a large binding interface across the seven transmembrane helices and the 5 extracellular loops modulating ligand binding.<sup>35-40</sup>

Based on these characteristics, fungal pheromone GPCRs offer a highly viable platform for DE towards binding of novel peptide ligands (e.g., non-cognate peptide ligands) through mutagenesis of specific portions of the receptor, the peptide or both.

In certain embodiments, the receptors are identified by searching protein and 10 genomic databases (e.g., NCBI, UniProt) for proteins and/or genes with homology (structural or sequence homology) to *S. cerevisiae* Ste2 receptor. In certain embodiments, the receptor has an average amino acid sequence homology of 33% to *S. cerevisiae* Ste2, ranging from 66% to 15% as calculated with Clustal Omega 15 [Sievers (2014)].

In certain embodiments, the receptors have seven transmembrane helices, an extracellular N-terminus, an intracellular C-terminus, three extracellular loops and three intracellular loops when analyzed by TMHMM v2.0 [Krogh et al. (2001)]. As shown in Figure 11, there are three key regions that have higher density of conserved residues with some residues conserved across all receptors: Region I, Region II, and 20 Region III. Region I corresponds to the third intracellular loop and shows two positively charged residues with high conservation at positions 233 and 234 relative to the *S. cerevisiae* Ste2. Region II corresponds to the sixth transmembrane helix and contains an essential proline that is conserved across all the receptors at position 258 relative to the *S. cerevisiae* Ste2. Region III shows the highest level of conservation 25 and also includes an essential proline conserved across all the receptors at position

290 relative to the *S. cerevisiae* Ste2. Based on previous mutational studies of the *S. cerevisiae* Ste2 receptor, these three regions are important in mediating signal transduction and interactions with the downstream G-protein. [Ćelić et al. (2003); Martin et al. (2002)]. In certain embodiments, the receptor has at least about >30%, at 5 least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or least about 100% homologous to Region 1 and/or Region 2 and/or Region 3. The receptor functions in a *S. cerevisiae* biosensor.

In certain embodiments, when coupled to a lycopene reporter system, as 10 described below, a fungal-derived GPCR, optionally further modified by directed evolution, generates lycopene in the sensor cell in response to the peptide pheromones produced by an agent of interest. Pheromone GPCRs from related fungi can naturally recognize different peptide pheromones based on the highly specific characteristics of this class of GPCRs, which mediate the species-specific mating reaction while 15 preventing interspecies breeding. As described in the Example section, putative GPCRs can be cloned and screened against their putative cognate peptide pheromones using a detector gene, e.g., a fluorescent reporter gene.

The present invention provides a sensor cell (e.g., a yeast cell) comprising a receptor, which is a fungal receptor modified to bind to a bacterial pathogen-specific 20 analyte, such as one from *V. cholerae*. In certain embodiments, this modification is achieved via directed evolution. The natural yeast pheromone mating receptors Ste2 or Ste3, evolved to bind to a peptide pheromone ligand, are not necessarily likely to adjust to bacterial pathogen-specific analyte and therefore can be deleted from the strain to prevent false activation of reporter gene. A mammalian or hybrid G-protein 25 can be used to enhance GPCR signal transduction in a yeast cell. The remaining

genes in the pathway may be endogenous to the yeast sensor cell, or may be engineered for improved performance.

One or more rounds of DE can be performed to generate a GPCR responsive to the natural cholera analytes and peptides. In certain embodiments, cholera-specific peptides can be generated by adding sequence-specific proteases (e.g., trypsin, chymotrypsin, LysN, or GluC) to a given sample. Also, using available computational methods, a peptide database of *in-silico* proteolized proteomes from bacterial pathogens (e.g., *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumonia*, *Salmonella* sp., *Listeria monocytogenes*), fungal pathogens (e.g., *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Histoplasma capsulatum*, *Pneumocystis jirovecii* and *Stachybotrys*) viral pathogens (e.g., Ebola virus, HPV, HIV, influenza viruses), or proteolysis pattern of any single protein of interest e.g. produced during an industrial process, can be generated. This peptide database can be searched using peptide motifs derived from analysis of the natural diversity of fungal pheromones.

A computational approach can also be used to discover target peptide analytes that are amenable to detection by an engineered fungal GPCR. This computational method generates a pool of high priority targets that can be highly amenable to a DE approach. Engineered receptors such as 15C11 and 31E4, that show increased ligand promiscuity as starting points to generate engineered GPCRs, can be used to detect these new target peptide ligands from a diverse set of bacterial pathogens. Additionally, some of the natural peptide pheromones produced by bacterial pathogens can be targeted.

DE can be implemented to optimize any engineered GPCR for improved signal levels, enhanced EC50 and/or signal transduction kinetics. Of the six GPCR

families, the secretin and fungal pheromone receptor families naturally sense peptides. Moreover, the rhodopsin receptor family also contains members with peptide ligands. Representative members of each of these families have been heterologously expressed in yeast and functionally coupled to the pheromone response pathway: neurotensin 5 NT1 (rhodopsin-like), growth-hormone-releasing-hormone receptor (secretin-like), *Sordaria macrospora* pheromone receptor (fungal pheromone-like). These GPCRs can be engineered into a yeast cell as a method for detecting their cognate peptide ligands, *e.g.*, growth hormone or neurotensin, for monitoring or quantification.

Fungal Ste2-type or Ste3-type GPCRs as well as other peptide-specific GPCRs 10 mentioned above can be used as a platform for developing engineered peptide-activated GPCRs to generically detect agent-specific analytes. In certain embodiments, the present disclosure provides a step-wise Directed Evolution (DE) strategy based on intermediate hybrid peptides to change the ligand specificity of the parent GPCRs to bind the target peptides.

15 In certain embodiments, the engineered GPCR is an engineered receptor for the detection of *Vibrio cholerae*. The receptor can detect a peptide derived from the Cholera toxin (CTx). Additionally, there is a reservoir of biochemical and mutational data of the yeast Ste2 and Ste3 receptor in the literature.<sup>35-37,39,40,43</sup> The same strategy can be used for detection of other fungal, viral or bacterial analytes described below.

20 GPCRs constitute a large class of cell-surface receptors that can be activated by a variety of other ligands, *e.g.*, full proteins, small molecules (*e.g.*, nucleotides and lipids), or light. A variety of these non-peptide sensing receptors have been functionally expressed in yeast.<sup>44</sup> These receptors can be employed and engineered into the biosensor to sense analytes other than peptides, *e.g.*, small molecules, proteins 25 or heavy metals.

Non-limiting examples of DNA encoding certain GPCRs of the invention are set forth in Tables 2 and 6 below; the invention further provides for proteins encoded by said DNA sequences.

#### 5.4. DETECTION EVENTS

5            Being of the analyte to the receptor triggers a detection event that is indicative of the presence of the agent. The detection events include, but are not limited to, appearance of a reporter (including expression (increased or decreased expression) of a detectable reporter gene), release of a therapeutic molecule that directly remediates the agent, production of a redox active molecule, and a change in the membrane  
10          potential of the sensor cell.

In certain embodiments, the detection event is appearance of a reporter. The reporter can be a result of expression of a reporter gene. A reporter can include an enzyme that can produce chromogenic product on a substrate. In certain embodiments, the detection event is increased expression of a reporter gene.

15          In certain embodiments, the reporter is a laboratory reporter. A “laboratory reporter” means a reporter that cannot be detected by the naked eye (e.g., the change or appearance of the color cannot be detected by the naked eye), and/or a reporter whose detection requires instrumentation. Suitable laboratory reporters include, but are not limited to, bioluminescent, fluorescent, and certain chromogenic reporters.  
20          Bioluminescent reporters include, but are not limited to, luciferase. Fluorescent reporters include, but are not limited to, various fluorescent proteins (e.g., a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, a blue fluorescent protein). Non-laboratory chromogenic reporters include, but are not limited to, beta-galactosidase, beta-glucuronidase, and horse-radish peroxidase. In  
25          certain embodiments, the reporter is a fluorescent protein.

In certain embodiments, the reporter does not comprise a laboratory reporter. In certain embodiments, the reporter is a non-laboratory reporter. A “non-laboratory reporter” means a reporter that can be detected by the naked eye (e.g., the change or appearance of the color can be detected by the naked eye), and/or whose detection 5 does not require instrumentation (e.g., reporters that are not conventionally used as research tools). Non-laboratory reporters include, but are not limited to, enzymes in the biosynthetic pathways of pigments (biosynthesized pigments that absorb in the visible light spectrum, also referred to as “biosynthesized visible-light pigments”), electrochemical, and reporters which constitute release of one or more therapeutic 10 molecule. Certain chromogenic reporters are non-laboratory reporters, e.g., lycopene.

Biosynthesized visible-light pigments include, but are not limited to, terpenoids, carotenoids, lycopene, violacein and its precursors, melanin, and indigo. In certain embodiments, the reporter is a terpenoid. In certain embodiments, the reporter is a carotenoid. In certain embodiments, the reporter is lycopene. In certain 15 embodiments, the receptor does not comprise a fluorescent protein.

Binding of analyte can induce or alternatively repress reporter gene expression. In the absence of an analyte, there may be essentially no reporter gene expression, reporter gene expression may occur at an undetectable level (e.g., undetectable by the naked eye), or reporter gene expression may occur at a baseline 20 level that detectably increases upon analyte binding.

Violacein and deoxyviolacein are blue pigments isolated from several bacteria. [Sánchez (2006)]. Heterologous expression of the involved genes vioABCDE and optimization of production yields has been shown in *E. coli* and *S. cerevisiae*. [Lee (2013)].

Melanin is a black diffusible macromolecule whose overproduction has been achieved from L-tyrosine as precursor by heterologous co-expression of a tyrosinase in *E. coli* [Santos (2008)].

Production of the blue pigment bio-indigo from tryptophan as a precursor 5 using a bacterial flavin-containing monooxygenase from the methylotrophic bacteria *Methylophaga aminisulfidivorans* has been achieved and optimized in *E. coli* [Hwan Han (2008)].

Carotenoids are a class of terpenoids composed of 8 isoprene units totaling 40 carbon atoms. Lycopene is a specific naturally produced carotenoid pigment whose 10 heterologous expression in *E. coli* using the genes CrtE, CrtB and CrtI has been extensively studied.<sup>45</sup> If lycopene is used as a reporter, a presently disclosed sensor cell can be engineered to contain the genes required for synthesis and at least one of said genes can be the detectable reporter gene coupled to activation by peptide receptor binding (e.g., at least a portion of the Fus1 promoter). As a non-limiting 15 example, the gene coupled may be CrtI, CrtE or CrtB.

Lycopene can be visualized by the naked eye, is widely validated in yeast metabolic engineering, and is non-toxic. Lycopene is the first intermediate in carotenoid biosynthesis that has a sufficiently conjugated  $\pi$ -system to absorb in the visible region.<sup>46</sup> Thus, unlike standard laboratory reporters like *lacZ* that require 20 exogenously added caged dyes (X-gal) or fluorescent proteins that require specialized equipment (fluorimeter), lycopene can be directly observed by a non-technical person. Additionally, the biosynthesis of lycopene from endogenous yeast farnesyl pyrophosphate is well established in yeast, requiring only three heterologous genes (Figure 1).<sup>47</sup>

Use of a biosynthesized visible-light pigment as a simple visual readout has a number of advantages. Use of a biosynthesized visible-light pigment readout requires no complex equipment since it can be seen by the naked eye and requires no expensive externally added reagent, since it can be biosynthesized from endogenous substrates. In contrast, most whole-cell biosensors reported in the literature use laboratory readouts such as fluorescent proteins, lacZ, or luciferase, which require the use of expensive equipment, externally added chromogenic reagents or both.<sup>48-51</sup>

In certain embodiments, lycopene is modified to achieve better response times, signal-to-noise and robustness. For example, in certain embodiments, one or more alternate pheromone-responsive promoter is used.<sup>52</sup> In certain embodiments, one or 10 more synthetic Fus1-like promoter is used.<sup>53</sup> In certain embodiments, one or more variant of the transcription factor Ste12 is used.<sup>54</sup> In certain embodiments, one or more enhancement to the pheromone response pathway is made.<sup>55-58</sup> In certain embodiments, one or more variant of the *Crt* genes including homologues is used.<sup>59</sup>

15 In certain embodiments, one or more codon optimized version and engineered version with enhanced activity or activation modality is used.

Additional biosynthesized visible-light pigments include mutants of CrtI disclosed in Schmidt-Dannert, C., Umeno, D. & Arnold, F. H. Molecular breeding of carotenoid biosynthetic pathways. Nat Biotech 18, 750–753 (2000), biosynthetic 20 enzymes that generate alternate carotenoid pigments disclosed in Umeno, D. & Arnold, F. H. Evolution of a Pathway to Novel Long-Chain Carotenoids. J. Bacteriol. 186, 1531–1536 (2004), and lycopene enzymes from alternate organism disclosed in Verwaal, R. et al. High-Level Production of Beta-Carotene in *Saccharomyces cerevisiae* by Successive Transformation with Carotenogenic Genes from 25 *Xanthophyllomyces dendrorhous*. Appl. Environ. Microbiol. 73, 4342–4350 (2007).

A presently disclosed sensor cell may also report in a non-measurable, non-visible way by releasing a therapeutic molecule that directly remediates the detected agent. In general, microbial cells have been used to produce therapeutic molecules such as peptides, proteins and other bioactive small-molecules. [Bourbonnais (1988); 5 Miyajima (1985); Ro (2006)]. Similar to the generation of lycopene, a presently disclosed sensor cell can be coupled to the biosynthesis and secretion of such therapeutic molecule.

In certain embodiments, the detection event is release of a therapeutically relevant molecule, which can be reported through an electronic device. Interfacing to 10 an electronic device can allow reporting to occur much more rapidly and produce a quantitative result. Additionally or alternatively, the release of a therapeutic molecule can be used to directly remediate the agent detected by a presently disclosed sensor cell.

In certain embodiments, the detection event is production of a redox active 15 molecule. Others have in general coupled whole cells electrochemically to electrodes. This is usually done by mixing the cells with a redox-active molecule (a mediator) that couples a redox-active enzymatic process within the cell to a redox reaction on the electrode surface. [Su (2011); Eilam (1982); Garjonyte (2009)].

In certain embodiments, the production or release of a redox active molecule 20 is detected by a redox reaction on an electrode. The redox active molecule can be biosynthesized in an analogous way as lycopene, e.g., by introducing the relevant biosynthetic enzymes into a presently disclosed sensor cell. Similarly, the production of this redox active molecule can be triggered by coupling one of the relevant biosynthetic enzymes to the pheromone signaling pathway. In certain embodiments, 25 the redox active molecule is phenazine. The relevant biosynthetic enzymes are

known [Mavrodi (2001)], and their secretion from a bacteria has been measured through the use of an electronic device [Bellin (2014)].

In certain embodiments, the detection event is a change in the membrane potential of the sensor cell. Electronic device that can measure changes in the 5 membrane potential of cells are very common in neuroscience (e.g., multi electrode arrays). [Spira (2013)]. Such a device can be used to measure changes in membrane potential in our biosensor. In certain embodiments, the, a change in the membrane potential of the sensor cell is expression of a cAMP-activated ion channel in the sensor cell (e.g., a yeast cell). This type of channel has been shown to be functional 10 in yeast. [Ali (2006)]

*Signal amplification:* In order to improve the robustness of the reporter signal, quorum sensing signal amplification strategy can be used. Specifically, binding of analyte not only induces expression of visible reporter gene but also induces the expression of enzymes responsible for synthesis of quorum sensing molecules in 15 yeast, or alternative GPCR ligands such as a-factor or alpha-factor. Thus, enhanced sensitivity can be achieved by signal amplification using a positive feedback loop. Signal amplification in this form naturally exists in *S. cerevisiae* and other fungi using the same GPCRs described below such as Ste2

### 5.5. ANALYTES

20 Suitable analytes can be any ligand which is capable of binding to a receptor, where such binding triggers a detection event that is indicative of the presence of the agent, including triggering a cellular response by the sensor receptor. Suitable analytes include, but are not limited to, proteins, polypeptides (including amino acid polymers), and peptides. “Protein” generally refers to molecules having a particular 25 defined 3-dimensional (3D) structure, whereas “polypeptide” refers to any polymers

of amino acids, regardless of length, sequence, structure, and function. “Peptide” is generally reserved for a short oligomer that often but not necessarily lacks a stable conformation. [Creighton Proteins: Structures and Molecular Properties 2<sup>nd</sup> Edition, ISBN-10: 071677030X]. Proteins can be longer than 50 amino acid residues and peptides can be between 3 and 50 amino acid residues or longer.

In certain embodiments, an analyte is a peptide epitope. As used herein, the term a “peptide epitope” refers to a sub-region of amino acids within a larger polypeptide or protein. A peptide epitope can be composed of about 3-50 residues that are either continuous within the larger polypeptide or protein, or can also be a group of 3-50 residues that are discontinuous in the primary sequence of the larger polypeptide or protein but that are spatially near in three-dimensional space. The recognized peptide epitope can stretch over the complete length of the polypeptide or protein, the peptide epitope can be part of a peptide, the peptide epitope can be part of a full protein and can be released from that protein by proteolytic treatment or can remain part of the protein molecule.

Some sensor cells (e.g., yeast cells, e.g. *S. cerevisiae* or *Candida albicans*) are surrounded by a thick cell wall, which can cause a permeability barrier to large molecules. The permeability of the *S. cerevisiae* cell wall was shown to be strongly growth phase-dependent, being most porous and plastic during exponential phase. [Nobel et al. (1991)]. The cell wall was shown to be permeable to molecules of a hydrodynamic radius of 5.8nm, corresponding to a globular protein of 400 kDa. [Nobel (1990)]. Similar sized proteins are functionally secreted from yeast cells like *S. cerevisiae*, *C. albicans*, *C. glabrata* by passing the cell wall [Nobel (1991)]. Therefore, polypeptides or proteins of up to at least 400 kDa may be accessible to the cell surface receptor as analytes. However, proteins or polypeptides beyond this

range can also be detected. In certain embodiments, proteolysis are used to fragment the polypeptide or protein to release smaller polypeptides that can serve as the analyte and be accessible to the cell surface receptors.

The analytes can be natural, engineered or synthetic analytes. Virtually any 5 peptide and modified peptide can be assayed using the composition and methods of this invention, including secreted peptides or fragments of proteins which may be released from the protein by a protease. Proteolysis can be induced by one or more host-specific proteases and/or by addition to a given sample of sequence-specific proteases such as trypsin, chymotrypsin, Gluc, and LysN. Modifications of peptides 10 include but are not limited to post-translational farnesylation, glycosylation, deamination, and proteolytic processing.

In certain embodiments, the peptide is a fungal mating pheromone, e.g., a peptide specific to a fungal pathogen. Non-limiting examples of fungal mating pheromones include human fungal mating pheromones (meaning mating pheromones 15 of fungi that can colonize or infect humans), non-human fungal mating pheromones (meaning mating pheromones of fungi that colonize or infect a non-human animal), plant fungal mating pheromones (meaning mating pheromones of fungi that colonize or infect a plant), food fungal mating pheromones (e.g., food safety/spoilage) (meaning mating pheromones of fungi that colonize or infect human or non-human 20 animal food items), and industrial/model fungal mating pheromones. In certain embodiments, the industrial/model fungal mating pheromones are fungi species that are used for making food (e.g., fermentation of alcohol). In certain embodiments, the industrial/model fungal mating pheromones are fungi species that are used for industrial microbiology, e.g., production of drugs, or pesticides in agriculture. In

certain embodiments, the industrial/model fungal mating pheromones are fungi species that are used for academic research.

Non-limiting examples of human fungal mating pheromones include the mating pheromones of *C. albicans*, *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, *S. scheckii*, and *Candida krusei*.

Non-limiting examples of non-human animal fungal mating pheromones include the mating pheromone of *P. destructans*.

Non-limiting examples of plant fungal mating pheromones include the mating pheromones of *F. graminearum*, *M. oryzae*, *B. cinerea*, *G. candidum*, and *C. purpurea*.

Non-limiting examples of food fungal mating pheromones include the mating pheromones of *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, and *N. fischeri*.

Non-limiting examples of industrial/model fungal mating pheromones include the mating pheromones of *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (receptor 1), *V. polyspora* (receptor 2), *S. stipitis*, *S. japonicas*, *S. castellii*, and *S. octosporus*, *A. oryzae*, *T. melanosporum*, *D. haptotyla*, *C. tenuis*, *Y. lipolytica*, *T. delbrueckii*, *B. bassiana*, *K. pastoris*, *A. nidulans*, *N. crassa*, and *H. jecorina*.

In certain embodiments, the peptide is a peptide disclosed in Table 5.

In certain embodiments, the physicochemical properties, e.g., peptide length, overall charge, charge distribution and hydrophobicity/hydrophilicity, of a peptide are determined by using the program ProtParam on the Expasy server [Walker (2005) ISBN 978-1-59259-890-8]. In certain embodiments, the peptide has a length of 3 residues or more, a length of 4 residues or more, a length of 5 residues or more, 6 residues or more, 7, residues or more, 8 residues or more, 9 residues or more, 10

residues or more, 11 residues or more, 12 residues or more, 13 residues or more, 14 residues or more, 15 residues or more, 16 residues or more, 17 residues or more, 18 residues or more, 19 residues or more, 20 residues or more, 21 residues or more, 22 residues or more, 23 residues or more, 24 residues or more, 25 residues or more, 26 residues or more, 27 residues or more, 28 residues or more, 29 residues or more, 30 residues or more, 31 residues or more, 32 residues or more, 33 residues or more, 34 residues or more, 35 residues or more, 36 residues or more, 37 residues or more, 38 residues or more, 39 residues or more, 40 residues or more, 41 residues or more, 42 residues or more, 43 residues or more, 44 residues or more, 45 residues or more, 46 residues or more, 47 residues or more, 48 residues or more, 49 residues or more, or 50 residues or more. In certain embodiments, the peptide has a length of 3-50 residues, 5-50 residues, 3-45 residues, 5-45 residues, 3-40 residues, 5-40 residues, 3-35 residues, 5-35 residues, 3-30 residues, 5-30 residues, 3-25 residues, 5-25 residues, 3-20 residues, 5-20 residues, 3-15 residues, 5-15 residues, 3-10 residues, 3-10 residues, 5-10 residues, 10-15 residues, 15-20 residues, 20-25 residues, 25-30 residues, 30-35 residues, 35-40 residues, 40-45 residues, or 45-50 residues. In certain embodiments, the peptide has a length of 9-25 residues. In certain embodiments, the peptide has a length of 9-23 residues. In one non-limiting embodiments, the peptide has a length of 9 residues. In one non-limiting embodiments, the peptide has a length of 10 residues.

In one non-limiting embodiments, the peptide has a length of 11 residues. In one non-limiting embodiments, the peptide has a length of 12 residues. In one non-limiting embodiments, the peptide has a length of 13 residues. In one non-limiting embodiments, the peptide has a length of 14 residues. In one non-limiting embodiments, the peptide has a length of 15 residues. In one non-limiting embodiments, the peptide has a length of 16 residues.

embodiments, the peptide has a length of 17 residues. In one non-limiting embodiments, the peptide has a length of 18 residues. In one non-limiting embodiments, the peptide has a length of 19 residues. In one non-limiting embodiments, the peptide has a length of 20 residues. In one non-limiting 5 embodiments, the peptide has a length of 21 residues. In one non-limiting embodiments, the peptide has a length of 22 residues. In one non-limiting embodiments, the peptide has a length of 23 residues.

In certain embodiments, the peptide is hydrophobic. In certain embodiments, the peptide is mildly hydrophilic.

10 In certain embodiments, the peptide is a *S. cerevisiae* pheromone alpha-factor. The C-terminus of the *S. cerevisiae* pheromone alpha-factor is involved in binding to the receptor. The N-terminus of the *S. cerevisiae* pheromone alpha-factor contributes to signaling due to receptor activation.

15 Non-limiting examples of classes of peptide analytes include the following.  
5.5.1. PEPTIDES AS ANALYTES IN DISEASES  
5.5.1.1. Peptides in fungal infections

Suitable analyte peptides associated with fungal infections include, but are not limited to, a peptide from *Aspergillus* (e.g., *Aspergillus niger*), *Candida* (e.g., *C. albicans* or *C. glabrata*), *Cryptococcus* (e.g., *Cryptococcus neoformans* or 20 *Cryptococcus gattii*), *Histoplasma* (e.g., *Histoplasma capsulatum*), *Pneumocystis* (e.g., *Pneumocystis jirovecii*), or *Stachybotrys* (e.g., *Stachybotrys chartarum*).

In certain embodiments, the agent-specific peptide is a peptide pheromone produced by a pathogenic fungus or a proteolytic product from a pathogenic fungus.

### 5.5.1.2. Peptides in bacterial infections

Suitable analyte peptides associated with bacterial infections include, but are not limited to, a peptide from *V. cholera* (e.g., Cholera toxin), *Staphylococcus aureus* (e.g., staphylococcal auto-inducing peptide or portion of beta toxin), and *Salmonella spec.* (e.g., Salmonella Exotoxins). In certain embodiments, an agent-specific analyte is a peptide derived from the cholera toxin or a proteolytic product from cholera. The proteolytic product from cholera can be generated by a host-specific protease and/or by an exogenous protease. In certain embodiments, an agent-specific analyte is a small molecule secreted or derived from *Vibrio cholera*. In certain embodiments, an agent-specific peptide is *Vibrio cholerae* specific or at least specific to a small group of bacteria including *Vibrio cholerae* (for example a group of up to 10 known species or up to 5 known species).

In certain embodiments, the peptide derived from the cholera toxin is selected from the group consisting of the peptides disclosed in Table 7.

In certain embodiments, the peptide associated with *V. cholera* is selected from the group consisting of a peptide having an amino acid sequence set forth in VEVPGSQHIDSQKKA (SEQ ID NO: 26), a peptide having an amino acid sequence that is at least about 80% (e.g., at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%) homologous to SEQ ID NO: 26, a peptide having an amino acid sequence set forth in VPGSQHIDS (SEQ ID NO: 27), and a peptide having an amino acid sequence that is at least 80% (e.g., at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%) homologous to SEQ ID NO: 27.

#### 5.5.1.3. Peptides in viral infections

Suitable analyte peptides associated with viral infections include, but are not limited to, a peptide from Ebola virus (*e.g.*, secreted glycoprotein), Influenza virus (*e.g.*, Hemagglutinin), or HIV (*e.g.*, HIV glycoprotein)

#### 5 5.5.1.4. Peptides in non-infectious disease

Patterns of peptide biomarkers unique for certain cancer types have been identified by mass spectrometry.<sup>60-63</sup> Suitable analyte peptides associated with cancer include, but are not limited to, protein portions released from human endogenous proteins by tumor-specific exopeptidases or antibody-derived peptide biomarkers for 10 well characterized disease states.

Peptide or protein biomarkers have been identified in other diseases, *e.g.*, Alzheimers,<sup>64</sup> Parkinson,<sup>65</sup> or different kidney diseases.<sup>66</sup> Such peptides and proteins may also function as analytes.

### 5.5.2. PEPTIDES AS ANALYTES IN FOOD SAFETY

#### 15 5.5.2.1. Toxins

Suitable analyte peptides associated with food toxins include, but are not limited to, a peptide from *Clostridium botulinum* (*e.g.*, Botulinum toxin), Shiga toxin-producing *Escherichia coli* (STEC) (*e.g.*, Shiga toxin), and *Shigella dysenteriae* (*e.g.*, Verotoxin).

#### 20 5.5.2.2. Immunogens and allergens

Suitable analyte peptides associated with food immunogens and allergens include, but are not limited to, immunogenic wheat peptide (*e.g.*, gluten).

### 5.5.3. PEPTIDES IN PLANT & CROP INFECTIONS

Suitable analyte peptides associated with plant and crop infections include, but 25 are not limited to, a peptide of *Fusarium graminearum*, *Botrytis cinerea*, *Magnaporthe oryzae*, and *Geotrichum candidum*.

#### 5.5.4. PEPTIDES IN BIOTERRORISM

Suitable analyte peptides associated with bioterrorism include, but are not limited to, peptides of *Bacillus anthracis* (anthrax), e.g., one of three polypeptides that comprise the anthrax toxin secreted by the pathogen: protective antigen (PA), lethal factor (LF) and edema factor (EF),<sup>67</sup> or *Clostridium botulinum* (botulism), e.g., Botulinum neurotoxin peptides such as the cyclic peptide C11-019.<sup>68</sup>

#### 5.5.5. OTHER ANALYTES

Non-peptide analytes can include, but are not limited to, quorum sensing small molecules such as the Vibrio Cholera CAI-1,<sup>69</sup> inter-species bacterial quorum sensing 10 AL-2,<sup>70</sup> aflatoxin B1 produced by Aspergillus flavus, components of the bacterial LPS, or heavy metals contaminants such as cadmium, mercury, lead or arsenic.

### 5.6. METHODS OF USE

The present invention provides for a method of detecting the presence of an agent of interest in a sample using the sensor cell disclosed herein. In certain 15 embodiments, the method comprises contacting the sample with a sensor cell (e.g., a yeast sensor cell) comprising (e.g., bearing) a receptor (e.g., a non-native GPCR receptor) that binds to an analyte indicative of the presence of the agent, wherein binding of the analyte to the receptor triggers a detection event that is indicative of the presence of the agent (e.g., increased expression of a reporter gene).

20 In certain embodiments, the receptor is coupled to the reporter gene. The method further comprises culturing the sensor cell for an effective period of time; and determining expression of the reporter gene. In certain embodiments, determining whether expression of the reporter gene comprises detecting the expression of the reporter gene by the naked eye and does not require instrumentation. In certain non-25 limiting embodiments, the reporter is lycopene.

In certain embodiments, the detection event is release of a therapeutic molecule that directly remediates the agent.

In certain embodiments, the detection event is production of a redox active molecule. The method further comprises measuring the production of the redox 5 active molecule. In certain embodiments, measuring the production of the redox active molecule comprises an electronic device. The redox active molecule can be phenazine.

In certain embodiments, the detection event is a change in the membrane potential of the sensor cell. The change in the membrane potential of the sensor cell 10 comprises expression of a cAMP-activated ion channel in the sensor cell.

The particulars of the receptor, coupling, and reporter gene are described in the sections above.

The method for determining whether the reporter gene is or has been expressed depends upon the particular reporting gene used. If the reporter gene 15 produces a visibly detectable product, such as lycopene, it can be detected with the naked eye or colorimetrically. Means of detection of reporter genes known in the art can be used.

In certain non-limiting embodiments, the receptor is a G-protein coupled receptor (GPCR) engineered to bind to the analyte.

20 By way of non-limiting example, a method of detecting the presence of *Vibrio cholerae* in a water sample can include detecting the presence of a peptide associated with *Vibrio cholerae* in the water sample by a method comprising:

contacting the water sample with a sensor yeast cell bearing a GPCR polypeptide that binds to the analyte coupled to a CrtI gene such that when the peptide 25 binds to the receptor, expression of the CrtI gene is induced and lycopene is produced;

culturing the sensor yeast cell for an effective period of time; and  
determining whether lycopene has been produced.

The analyte associated with *Vibrio cholerae* can be a peptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 5 at least about 99% homologous to VEVPGSQHIDSQKKA (SEQ ID NO: 26) or VPGSQHIDS (SEQ ID NO: 27). The effective period of time can be hours (e.g., about 24 hours, about 18 hours, about 12 hours, about 8 hours, about 6 hours, about 4 hours, about 3 hours, or about 2 hours) or minutes (e.g., about 90 minutes, about 60 minutes, about 45 minutes, about 30 minutes, about 20 minutes, about 15 minutes, 10 about 10 minutes, about 5 minutes, about 3 minutes, about 2 minutes, or about 1 minute).

In certain non-limiting embodiments, the present invention provides for a method of detecting the presence of a fungus or a fungal pathogen, comprising detecting the presence of an analyte associated with said fungus or a fungal pathogen 15 in a sample by a method comprising:

contacting the sample with a sensor cell comprising (e.g., bearing) a receptor that binds to the analyte coupled to a reporter gene such that when the analyte binds to the receptor, expression of a detectable reporter gene is induced;

20 culturing the sensor cell for an effective period of time; and  
determining whether the reporter gene is expressed. In certain non-limiting embodiments, the receptor is a G-protein coupled yeast receptor engineered to bind to the analyte. In certain non-limiting embodiments, the reporter gene expression is detected by the naked eye and does not require instrumentation. In certain non-limiting embodiments, the reporter gene product is lycopene.

In certain embodiments, the sensor cell is a freeze-dried or other dried cell, *e.g.*, a freeze-dried yeast cell. The cell can be activated for use by addition of a food source, *e.g.*, sugar or agar.

Non-limiting examples of samples can include a water sample and a sample of body fluid. Non-limiting examples of water samples include fresh water, sea water, and sewage samples. Non-limiting examples of body fluid samples include intestinal fluids, diarrhea or other feces, mucus (*e.g.*, sputum), blood, cerebrospinal fluid, lymph, pus, saliva, vomit, urine, bile, and sweat. In certain embodiments, the agent to be detected is a plant fungal pathogen. A plant can be shaken in water to provide a water sample containing the fungal pathogen, or a soil sample can be mixed with water and tested for the fungal pathogen, or a portion of plant material (*e.g.*, a fluid obtained from the plant) can be used as a sample.

### 5.7. KITS

The present invention provides kits for detecting the presence of an agent of interest, for example but not limited to a chemical or a pathogen, as described above. Kits can include one or more sensor cells, as described above, and can be used to perform methods of detecting the presence of an agent, as described above. Kits can further include one or more controls. Kits can include both a positive and a negative control. Kits can include a substrate that comprises the sensor cells and on which or in which detection can occur, *e.g.*, a dish, cup, bowl, plate, paper, chip, gel, bag, stick, syringe, jar, or bottle. Kits can include a food or nutrient source, *e.g.*, sugar or agar. Kits can include components to improve cell viability, including one or more carbon sources, one or more nitrogen sources, one or more trace nutrient sources, and one or more additional nutrient sources to improve response speed. Kits can include additional assay components, including proteases to release target peptides, dyes,

filters, and/or cryo-protectants. Kits can be produced by combining all required assay components (e.g., nutrients, sensor cells, and proteases) and freeze-drying, air-drying, or binding this component mix to a substrate. In certain embodiments, the kit comprises a protease (e.g., a protease from prokaryote sources or a protease from 5 eukaryote sources) for digestion of the agent into smaller detectable peptides.

Figure 14A represents a kit (“Yeast Block”) in accordance with one non-limiting embodiments. As shown in Figure 14A, the kit comprises a yeast cell, a piece of paper, a negative control, and a nutrient source.

## 6. EXAMPLES

10 6.1. EXAMPLE 1: Yeast Strains That Produce Lycopene In Response To Activation Of The Endogenous GPCR Ste2

A yeast strain producing lycopene in response to the activation of the endogenous GPCR, Ste2 was generated by the natural *S. cerevisiae* peptide pheromone,  $\alpha$ -Factor ( $\alpha$ F). A parental reporter strain was made by deleting the 15 cyclin-dependent kinase inhibitor Far1 to prevent cell-cycle arrest and deleted the G-protein activating protein Sst2 to prevent signal attenuation. For general procedures, see Pausch, M. H. G-protein-coupled receptors in *Saccharomyces cerevisiae*: high-throughput screening assays for drug discovery. Trends Biotechnol. 15, 487–494 (1997). Then, the carotenoid genes derived from *E. herbicola*, CrtE, and CrtB were 20 placed under the control of the constitutive promoters pTef1 and pPgk1, respectively. The final biosynthetic gene CrtI was placed under control of the Fus1 promoter, a downstream target of the pheromone response pathway. See Bardwell, L. A walk-through of the yeast mating pheromone response pathway. Peptides 26, 339–350 (2005). This lycopene reporter cassette was introduced into the parental reporter 25 strain through Reiterative Recombination. See Wingler, L. M. & Cornish, V. W. Reiterative Recombination for the *in vivo* assembly of libraries of multigene

pathways. Proc Natl Acad Sci U S A 108, 15135–15140 (2011). This v1.0 reporter strain became visibly orange 36 hours after exposure to αF, as shown in Figure 4A.

Through modification of the v1.0 strain, a lycopene response time of 2 hours under optimal culture conditions and less than 6 hours in a stringent product prototype assay was observed. To do so, the CrtI amount was increased with an additional chromosomal copy of the pFus1-CrtI construct. This led to a 9.8-fold improvement in response time. The catalytic activity of CrtI was improved by increasing FAD content in the cell through the overexpression of the FAD synthetase FAD1. See Schaub, P. et al. On the Structure and Function of the Phytoene Desaturase CRTI from *Pantoea ananatis*, a Membrane-Peripheral and FAD-Dependent Oxidase/Isomerase. PLoS ONE 7, e39550 (2012); Wu, M., Repetto, B., Glerum, D. M. & Tzagoloff, A. Cloning and characterization of FAD1, the structural gene for flavin adenine dinucleotide synthetase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15, 264–271 (1995). This modification independently led to a 10.3-fold improvement in the response time, and to a 21.1-fold improvement when combined with the increased CrtI copy number. These results are shown in Figure 4B.

Table 1. Key genes and sequences.

Key Genes	Nucleotide Sequence
<i>E. herbicola</i> CrtI	ATGAAGAAAACCGTAGTGATTGGTGCAGGTTTGGTGGTTAGC TTGGCTATACGTCTACAAGCTGCAGGTATTCCTACAGTGCTATT GGAGCAAAGAGACAAACCAGGAGGAAGAGCTTATGTTGGCAC GATCAAGGCTTACCTTGATGCTGGCCTACAGTCATCACTGAT CCTACTGCATTGGAAGCTTGTTCACCTAGCTGGTAGAAGAAC GGAAGATTATGTCCGTCTATTGCCTGTCAAGCGTTTACAGATT GTGTTGGAAATCTGGTAAACCCCTAGATTACGCCATGACAGTG CTGAACCTAGAACGCTCAGATTACGCAGTTAACCCCAGAGATGTC GAAGGTTACAGGAGATTCCCTGCCTATTCCAAGCTGTTTCCA AGAGGGTTATCTCGTTGGGTTCAAGTCCATTCCCTGCCTTAG GGATATGCTTAGAGCAGGTCCCTCAGTTGTGAAGCTACAAGCAT GGCAAAGTGTGTATCAGTCTGTTGAGATTATCGAGGATGAA CATCTGAGACAAGCATTCTCATTCCACAGTCTTAGTTGGAGG TAATCCCTTACACATCGAGCATATACGTTGATTACCGCTTT GGAAAGAGAATGGGGAGTTGGTTCCCTGAAGGTGGAACAGGT

	GCTTGTTAATGGTATGGTAAGCTATTACCGGATTGGGTGG AGAAATAGAGCTGAATGCAAGAGTGGAAAGAACTTGTAGCA GACAACAGAGTCTCACAAAGTAGACTGCTGATGGTAGGATCTT CGATACAGATGCTGTAGCTCAAACGCAGATGTAGTAAACACTT ATAAAAAGTTGTGGGACATCATCCTGTTGGACAAAAGAGAGC AGCTGCTTGGAGAGGAAATCTATGAGCAACTCGTTGTCC TTTACTTGGGCTGAATCAACCACACTCACAACTAGCTCATCAC ACAATCTGCTTGGCCTAGATAACAGAGAGCTGATAGATGAAAT TTTCACTGGATCTGCTTAGCAGACGATTTCCCTGTACTGCA TTCACCATGTGTTACTGATCCCTTTAGCACCACCTGGTTGTGC TAGCTCTATGTTACTGACACCTGTACCACTTGGTAATGCTCC ATTAGATTGGGCACAAGAAGGACCGAAATTGAGGGATAGGATC TTCGACTATTGGAAGAACGTTACATGCCAGGTTGAGATCTCA GTTGGTACACAGAGGATTACACACCAGCTGATTTCATGATA CTCTAGATGCGCATTAGGTAGCGCTTTCCATTGAGCCACTTT TGACGCAAAGTGCTTGGTTAGACCACACAACAGAGATTCTGAC ATTGCCAATCTGTACCTAGTAGGTGCAGGAACATCCAGGAGC TGGTATTCCCTGGAGTTGTAGCTCTGCTAAAGCTACTGCTAGTCT GATGATCGAGGATTGCAGTAA ( <b>SEQ ID NO: 1</b> )
<i>E.</i> <i>herbicola</i> CrtE	ATGGTTCTGGTCGAAAGCAGGGATCACCTCATAGGGAAAT CGAAGTCATGAGACAGTCCATTGATGACCACCTAGCAGGATTGT TGCCAGAACAGATTCCCAGGATATCGTTAGCCTGCTATGAGA GAAGGTTTATGCCACCTGTTAACGTATCAGACCTTGCTGAT GTTACTTGCTGCAAGAGACCTGAGATATCAGGGTTCTATGCCTA CACTACTGGATCTAGCTTGCTGTTGAACTGACACATACTGCTT CCTTGATGCTGGATGACATGCCTGTTGACAATGCGGAACCT AGAAGAGGTCAACCAACAACCCACAAGAAATTGGAGAACTG TTGCCATTGGCTCTGTAAGGTCTGTCGAAAGCTTTGGCT TGATTGCTGCACTGGTATCTCCAGGTGAAAGGAGAGCACAA GCTGTAACCGAGCTATCTACTGCAAGTGGTCTCAAGGTCTAGT CTTAGGACAGTTCAGAGATTGAATGACCGAGCTTGGACAGAA CTCCTGATGCTATCCTGTACGAACCATCTGAAGACTGGCATCT TGTTCTCAGCTATGTTGCAAATCGTAGCCATTGCTCTGCTTCTT CACCATCTACTAGGGAAACGTTACACGCATTGCGATTGGACTTT GGTCAAGCCTTCAACTGCTAGACGATTGAGGGATGATCATCC AGAGACAGGTAAGACCGTAACAAAGACGCTGGAAAAGCACT CTAGTCAACAGATTGGGTGCTGATGCACTAGACAGAAACTGA GAGAGCACATTGACTCTGCTGACAAACACCTGACATTGCATGT CCACAAGGAGGTGCTATAAGGCAGTTATGCACCTATGGTTGG ACACCATCTGCTGATTGGCTCCAGTGTGATGAAGATCGCCTAA ( <b>SEQ ID NO: 2</b> )
<i>E.</i> <i>herbicola</i> CrtB	ATGAGTCACCAACACCTTGGATCATGCTACTCAAACGATGGC TAATGGTTCCAAGTCCTTGCTACAGCAGCTAAACTGTTGACCC AGCTACTAGAAGATCAGTGCTTATGCTGTACACTGGTGTAGAC ACTGTGATGACGTTAGATGACAGACACATGGTTGCGCATCT GAAGCTGCTGCAGAAGAAGAGGCTACTCAGAGATTGGCTAGAT TGAGAACGCTTACACTGCAAGCTTGAAGGTGCTGAGATGCAA GATCCTGCTTGTGCTGCATTCCAAGAAGTTGCACTAACACACGG TATTACGCCAAGAACGGACTTGATCACTGGATGGTTGCAA TGGATGTTGCTCAAACCTCGTTACGTGACCTTGAAGACACCTG

	AGATACTGCTACCATGTTGCTGGAGTAGTTGGTTGATGATGGC AAGAGTAATGGGTGTAAGAGACGAAAGGGTTTGGACAGAGCT TGTGATCTAGGTTGGCTTTCAGCTGACAAACATCGCGAGAGA TATTATCGACGATGCAGCTATTGACAGATGCTATCACCTGCTG AATGGTTGCAAGATGCTGGCTAACCTCCTGAGAATTACGCTGCA AGAGAGAACAGAGCTGCATTAGCAAGAGTTGCTGAAAGGCTGA TAGACGCTGCTGAACCCTATTACATCTCAAGTCAAGCTGGATTG CATGATCTACCACCTAGATGTGCTTGGCTATAGCTACTGCAAG ATCTGTCTACAGAGAGATTGGCATCAAGGAAAAGCTGCAGGTG GTTCTGCTTGGATAGACGTCAACACACTAGCAAAGGAGAGAA GATTGCGATGCTTATGGCTGCACCAGGACAAGTCATTGTC AAACAACCAAGAGTTACACCAAGACCTGCTGGTTATGGCAAAG ACCTGTCTAA (SEQ ID NO: 3)
<i>S. cerevisiae</i> Fad1	ATGCAGTTGAGCAAGGCTGCTGAGATGTGTTATGAGATAACAAA CTCTTACTTACACATAGACCAGAAATCTCAGATAATAGCAAGTA CACAGAACGATAACGGTTGACAAGAAAATCTACTAAGTGA AATTTTGACGTTGGAGTCCACTGAATGGGGAAATATCATTCT CGTACAACGGAGGAAAAGATTGCCAGGTATTACTACTGTTATAT CTGAGTTGCTTATGGGAATATTCTTCATTAAGGCTAAAATTCC CAATTGATTGAGTTCAAAGCTTCCCCATGCAAAGACTTCC AACTGTTTCATTGATCAAGAAGAAACTTCCCTACATTAGAGA ATTTGACTGGAAACCTCAGAGCGATTGCCTTCCTTACG AATCACAAAGGCAATCTGGTCATCGTCAATATGGCAGACGC ATTTAGAGATTTATAAAGATATACCCTGAGACCGAAGCTATAG TGATAGGTATTAGACACACAGACCCATTGGTGAAGCATTAAAG CCTATTCAAAGAACAGATTCTAACTGGCCTGATTGAGTT GCAACCTCTCTTACACTGGGACTTAACCAATATGGAGTTCTT ACTGTATTCTAATGAGCCAATTGACTATATGGTAAAGGTT TCACATCAATCGGCGGAATTAAACAACCTGCATTGCCTAACCCACAC TTGAGAAAGGACTCCAATAATCCAGCCTGCATTGAAATGGGA AATCATTGATGCATTGGCAAGGACGCAGAAGGCGAACGTAGTT CCGCTATAAACACGTCACCTATTCCGTGGGATAAGGAAAGA TTCAGCAAATACCATGACAATTACTATCCTGGCTGGTATTGGTT GATGACACTTTAGAGAGAGCAGGCAGGATCAAGAATTAA (SEQ ID NO: 4)

## 6.2. EXAMPLE 2: Cloning And Screening Of Putative GPCRs Against Putative Cognate Fungal Peptide Hormones.

Several putative GPCRs were screened against their putative cognate peptide pheromones using a fluorescent reporter gene.<sup>33</sup> Recognition of pheromones from the following pathogenic fungi was shown in *S. cerevisiae*:

### Human pathogens:

-*Candida albicans* (functional expression in yeast previously shown)<sup>17</sup>

-*Paracoccidioides brasiliensis* (functional expression in yeast previously shown)<sup>16</sup>

-*Candida glabrata*  
Plant Pathogens:

5 -*Fusarium graminearum* (grain disease)

-*Magnaporthe oryzae* (Rice blast)

-*Botrytis cinerea* (Grey mould)

As shown in Figure 6, these receptors were orthogonal to the endogenous *S. cerevisiae* pheromone receptor and demonstrated a high level of specificity. Their  
10 EC50 values were as follows: *C.albicans*, 51 nM; *P. brasiliensis*, 9 nM; *F. graminearum*, 230nM; *M. oryzae*, 5 uM; *B. cinerea*, <1 nM. Additionally, the GPCR from *B. cinerea* showed activity against the putative pheromone from *Aspergillus flavus* and therefore may provide a useful diagnostic against this human pathogen.  
The results also demonstrated that these receptors successfully generate lycopene in  
15 the disclosed reporter strain.

Table 2. Pathogens and associated sequences

Pathogen	Amino acid sequence of peptide analyte used	Amino acid sequence of GPCRs used	DNA coding sequence of corresponding GPCRs that sense peptide analyte
<i>Candida albicans</i>	GFRLTNFG YFEPG (SEQ ID NO: 5)	MNINSTFIPDKPGDII ISYSIPGLDQPIQIPFH SLDSFQTDQAKIALV MGITIGSCSMTLIFLI SIMYKTNKLTNLKL KLKLKYILQWINQKI FTKKRNDNKQQQQ QQQQQIESSSYNNTT TTLGGYKLFFLFYLN LILLIGIIRSGCYLNY NLGPLNSLSFVFTG WYDGSSFISSDVTN GFKCILYALVEISLG FQVYVMFKTSNLKI WGIMASLLSIGLGLI VVAFQINLTLISHRF SRAISTNRSEEEESSSS LSSDSVGYVINSIWM	ATGAATATCAATTCAACTTTCATACCTGAT AAACCAGCGATATAATTATTAGTTATTCA ATTCCAGGATTAGATCAACCAATTCAAATT CCTTCACATTCAATTAGATTCAATTCAAACC GATCAAGCTAAATAGCTTAGTCATGGG GATAACTATTGGGAGTTGTCAATGACATT AATTTTTTGATTCTATAATGTATAAAACT AATAAAATTAACAAATTAAAATTAAAATT AAATTAATATATCTTGCATGGATAAAT CAAAAAATCTTACCAACAAAAAGGAATGA CAACAAACAACAACAACAACAACAAC AACAAATTGAATCATCATCATATAACAATA CTACTACTACGCTGGGGGGTTATAAATTAT TTTATTATCTTAATTCAATTGATTITATT AATTGGTATTATTCACTCAGGTTGTATT AAATTATAATTAGGTCCATTAAATTCACT TAGTTTGTAATTACTGGTTGGTATGATGG ATCATCATTATCATCCGATGTAACCAA

		DLPTILFSISINIMTIL LIGKLIIAIRTRRYLG LKQFDSFHILLIGFSQ TLIIPSILVVHYFYLS QNKSLLQQISLLLII LMLPLSSLWAQTAN NTHNINSSPSLSFISR HHLDSDSSRGGSNTI VSNGGSNGGGGG GNFPVSGIDAQLPPD IEKILHEDNNYKLLN SNNEVNDGDIIND EGMITKQITIKRV <b>(SEQ ID NO: 6)</b>	TGGATTAAATGTATTATGCTTAGT GGAAATTTCATTAGTTCCAAGTTATGT GATGTTCAAAACTCAAATTAAAAATTG GGGGATAATGGCATCATTATTATCAATTGG TTAGGATTGATTGTTGCCTTCAAAATC AATTAAACAATTATCTCATATTGATTT CCCAGGCTATATCAACTAACAGAAGTGAA GAAGAACATCATCATCATATTATCATCTGAT TCGGTTGGGTATGTGATTAATTCAATATGG ATGGATTACCAACAATTATTATTCATT AGTATTAAATAATGACAATTATTGATT GGTAAACTATAATTGCTATTAGAACAGA CGTTATTAGGATTGAAACAATTGATAGT TTCCATATTATTAATTGTTCACTGATTT CATTAATTATCCCTCAATTATTTGGTGGT TCATTATTTATTATCACA AAAATAAGA TTCTTATTACAACAAATTAGTCTTTATTG ATTATTAAATGTTACCATTAAGTTCTTAT GGGCTCAAACGTCAATAACTCATAATA TTAATTCTCATCTCCAAGTTATCATTATC TCGTCATCATCTGTCTGATAGTAGTCGTAG TGGTGGTCCAATACAATTGTTAGTAATGG TGGTAGTAATGGTGGTGGTGGTGGTGGT GGAATTCCCTGTTCAAGGTATTGATGCAC AATTACCACTGATATTGAAAAAAATCTTAC ATGAAGATAATAATTATAAATTACTTAATA GTAATAATGAAAGTGTAAATGATGGAGAT ATTATCATTAAATGATGAAGGTATGATTACT AAACAAATCACCATCAAAAGAGTGTAG <b>(SEQ ID NO: 7)</b>
<i>Candida glabrata</i>	WHWVRL RKGQGLF <b>(SEQ ID NO: 8)</b>	MEMGYDPRMYNPR NEYLNFTSVYDVND TIRFSTLDAIVKGLL RIAIVHGVRLGAIFM TLIIMFISSNTWKPI FIINMVSLMLVMIHS ALSFHYLLSNYSSIS YILTGFQLITSNNK RIQDAASIVQVLLVA AIEASLVFQIHVMFT IENIKLIREIVLSISIA MGLATVATYLAACI KLIRGLHDEVMPQT HLIFNLSIILLASSINF MTFLVIKLFPAIRSR RYLGLRFDAFHILL IMFCQSLLIPSVLYII VYAVDSRSNQDYLI PIANLFVVLSPLSSI WANTSNNSSRSPKY WKNSQTNKSNGSFV SSISVNSDSQNPPLYK KIVRFTSKGDTTRSI VSDSTLAEVGKYSM QDVSNNSFECRDLD FEKVKHTCENGRIS ETYSELSTLDTALN ETRLFWKQQSQCDK <b>(SEQ ID NO: 9)</b>	ATGGAGATGGGCTACGATCCAAGAATGTA TAATCCAAGAAATGAATAACTTGAATTTCAC GTCGGTATATGATGTAATGACACAATCA GATTTCGACTCTGGACGCCATTGTAAG GATTGCTAGAATTGCCATTGTTCATGGAG TTAGATTGGGAGCAATATTCTATGACGTTAA TAATAATGTTATCTCATCAAATACATGGA AAAAACCCATATTATAATTAAACATGGTGT CGTTGATGTTAGTTATGATTCTTCGGCAC TTAGCTTCCATTACCTTATCGAATTATTC TTCAATTCTTATATAACTGACAGGGTTCCCT CAGTTGATTACAAGCAATAATAACGAAT TCAAGATGCAGCGAGTATAGTCCAAGTTT ATTGGTTGCTGCGATAGAACATCATTGGT ATTCAGATTCTATGTTATGTTACGATTGA AAACATTAAGCTTATTAGAGAAATAGTACT CTCTATATCGATAGCAATGGGATTGGCAAC AGTGGCTACATATCTGCTGCAGCAATAAA GCTGATAAGAGGACTGCATGATGAGGTAA TGCCACAAACACATCTTATTTCATTATT CTATAATATTGCTGCATCCTCCATAAAATT TTATGACATTTATATTGGTCATTAAACTTT CTTCGCTATTAGATCTAGAAGATATCTGG TCTTCGTCATTCTGATGCTTTCATATTGAT TTAATCATGTTCTGCCAGTCATTATTGATA CCCTCAGTATTATATTAGTTACGCG GTTGATAGCAGATCTAATCAGGATTATCTG ATTCCAATTGCCAATTATTGTTGTTTAT CTTGCCATTATCCTCATCTGGCTAACAC CATCAAATAACTCATCCAGATCTCCAAAAT

			ATTGGAAAAACTCTCAAACGAATAAGAGC AATGGGTCTTGTCTCTCAATATCTGTCA ATAGTGACTCACAAAACCCTTGACAAAAA AGATTGTACGTTTACATCAAAGGCGACA CTACCCGTAGTATTGTAAGTGATTCAACAT TAGCAGAGGTGGAAAATACTCTATGCAA GACGTTAGCAATTCAAACCTTGAAATGTCGA GACCTTGATTGAGAAGGTAAAACATACT TGCAGAAAATTGGCAGAAATATCTGAAAC ATATAGTGAGTTAAGTACTTAGATACCAC TGCCCTCAATGAGACTCGGTTGTTGGAA ACAACAAAGTCAGTGTGACAAATAG ( <b>SEQ ID NO: 10</b> )
<i>Paracoccidioides brasiliensis</i>	WCTRPGQ GC ( <b>SEQ ID NO: 11</b> )	MAPSFDPFNQSVVF HKADGTPFNVSIHEL DDFVQYNTKVCINY SSQLGASVIAGLML AMLTHSEKRRLPVF FLNTFALAMNFarL LCMTIYFTGFKNSY AYFGQDYSQVPGSA YAASVLGVVFTLL VISMEMSLLIQTRVV CTTLPDIQRYLLMA VSSAISLMAIGFRLG LMVENCIAIVQASN APFIWLQSASNITITI STCFSAVFVTKLAY ALVTRIRLGLTRFGA MQVMFIMSCQTMVI PAIFSLQYPLPKYE MNSNLFTLVAIFLPL SSLWASVATRSSFET SSSGRHQYLWPSEQ SNNVTNSEIKYQVSF SQNHHTLRSGGSVA TTLSPDRLDPVYCEV EAGTKA ( <b>SEQ ID NO: 12</b> )	ATGGCACCCCTCATTCGACCCCTCAACCAA AGCGTGGTCTTCCACAAGGCCGACGGAAC TCCATTCAACGTCCTCAATCCATGAACCTAGA CGACTTCGTGCAGTACAACACCAAAGTCTG CATCAACTACTCTTCCCAGCTCGGAGCATC TGTCAATTGCAGGACTCATGCTTGCCATGCT GACACACTCAGAAAAGCGTCGTGCCAG TTTCTCCTAAACACATTGCACGGCCA TGAACATTGCCGCTGCTCTGCATGACCA TCTACTTCACCACCGGCTCAACAAAGTCCT ATGCCTACTTGGTCAGGATTACTCCAGG TGCCTGGAGCGCCTACGCAGCCTCTGTCT TGGCGTTGTCTCACCACCTCCTGGTAA TCAGCATGGAATGTCCCTCCTGATCCAAA CAAGGGTTGTCACGACCCCTCCGGATA TCCAACGTTATCTACTCATGGCAGTTCT CCCGCATTTCCCTGATGGCCATCGGGTTCC GCCTGGCTTAATGGITGAGAACTGCATTG CCATTGTGCAGCGTCGAATTGCCCCCTT TTATCTGGCTTCAAAGCGCCTCGAACATCA CCATTACGATCAGCACATGTTCTTCAGTG CCGTTGGTACGAAATTGGCATATGCAC TCGTCACTCGTATACGACTAGGCTTGACGA GGTTTGGTGCTATGCAGGTTATGTTCATCA TGTCTGCCAGACTATGGTATTCCAGCCA TCTTCTCAATTCTCAATACCCACTCCCCA AGTACGAAATGAACCTCAACCTCTTACGC TGGTGGCCATTTCCTCCCTTTCCTCGCT ATGGGCTTCAGTTGCTACGAGATCCAGTT CGAGACGTCTCTCCGGCCATCAGTA TCTTGCCAAGCGAACAGAGCAATAACG TCACCAATTGGAAATTAAAGTATCAGGTCA GCTTCTCTCAGAACACACTACGTTGCGGT CTGGAGGGTCTGTGGCCACGACACTCTCCC CGGACCGGCTGACCCGGTTATTGTGAAG TTGAAGCTGGCACAAAGGCCTAG ( <b>SEQ ID NO: 13</b> )
<i>Fusarium graminearum</i>	WCWWKG QPCW ( <b>SEQ ID NO: 14</b> )	MSKEVFDPFTQNVT FFAPDGKTEISIPVA AIDQVRMMVNNTI NYATQLGACLIMLV VLLVMVPKEKFRP FMILQITSLVISCCR MLLSIFHSSQFLDF YVFWGDDHSRIPRS AYAPSVAGNTMSLC LVisVETMLMSQAW	ATGTCTAAGGAAGTTTCGACCCATTCACT CAAAACGTTACTTCTCGCTCCAGACGGT AAGACTGAAATCTATCCCAGTTGCTGCT ATCGACCAAGTTAGAAGAATGATGTTAA CACTACTATCAACTACGCTACTCAATTGGG TGCTTGTGATCATGTTGGTTGTTGTTG GTTATGGTCCAAAGGAAAAGTCAGAAG ACCATTATGATCTGCAAATCACTTCTT GGTTATCTCTGTTGAGAATGTTGTTGTTG TCTATCTTCACTCTCAATTCTGGACT

		TMVRLWPNVWKYII AGVSLIVSIMAISVR LAYTIIQNNAVLKLE PAFHMFWLIKWTVI MNVASISWWCAIFN IKLVWHLISNRGILP SYKTFTPMEVLIMT NGILMIIPVIFASLEW AHFVNFEASLTLS VAVILPLGLAAQR ASSAPSSANSTGASS GIRYGVSGPSSFTGF KAPSFTGTTDRPHV SIYARCEAGTSSREH INPQGVELAKLDPET DHHRVVDRAFLQRE ERIRAPL (SEQ ID NO: 15)	TCTACGTTTCTGGGTGACGACCACTCTA GAATCCAAGATCTGCTTACGCTCCATCTG TTGCTGTAACACTATGTCCTTGTGTTGGT TATCTCTGTTGAAACTATGTTGATGTCTCA AGCTTGACTATGGTAGATTGTGCCAAA CGTTGGAAGTACATCATCGCTGGTGTTC TTTGTGCTTCTATCATGGCTATCTCTGTT AGATTGGCTTACACTATCATCCAAAACAAC GCTGTTGAAGTTGGAACCAGCTTCCAC ATGTTCTGGTTGATCAAGTGGACTGTTATC ATGAACGTTGCTTCTATCTCTGGTGGTGT GCTATCTCAACATCAAGTTGGTTGGCAC TTGATCTCTAACAGAGGTATCTGCCATCT TACAAGACTTCACTCCAATGGAAGTTTG ATCATGACTAACGGTATCTTGATGATCATC CCAGTTATCTCGCTTCTTGGATGGGCT CACTTCGTTAACCTCGAATCTGCTTCTTGA CTTGACTCTGTTGCTGTATCTGCCATT GGTACTTGGCTGCTCAAAGAACGCTTC CCATCTTCTACTGGTACTACTGACAGA CCACACGTTCTATCTACGCTAGATGTGAA GCTGGTACTCTCTAGAGAACACATCAAC CCACAAGGTGTTGAATTGGCTAAGTGGAC CCAGAAACTGACCACCACGTTAGAGTTGA CAGAGCTTCTGCAAAGAGAACAGAA TCAGAGCTCCATTGTAG (SEQ ID NO: 16)
<i>Magnapor the oryzae</i>	QWCPRRG QPCW (SEQ ID NO: 17)	MDQTLSATGTATSP PGPALTVDPRFQTIT MLTPALMGQGFEEV QTPPAEINDVYFLAF NTAIYGYSTQIGACFI MLLVLLTMTAKARF ARIPTIINTAALVVSI RCTLLVFFSTMMME FYTISSDDFSVHPN DIRRSAVATVFAPLQ LALVEAALMVQAW AMVELWPRAWKVS GIAFSLILATVTVAF KCASAATVKSALLE PLDPRPYLWIRQTDL AFTTAMVTWFCLF NVRLIMHMWQNRSI LPTVKGLSPMEVLV MANGLLMVFPVLFA GLYYGNFGQFESAS LTITSVVLVPLGLT VAQRALAVNNTVAGS SANTDMDDKLAFLG NATTVTSSAAGFAG SSASATRSRLASPRQ NSQLSTSVSAGKPR ADPIDLELQRIDDED DDFSRSGSAGGVRV ERSIERREERL (SEQ ID NO: 18)	ATGGACCAAACCTTGTCTGCTACTGGTACT GCTACTTCTCACCAGGTCCAGCTTGA TTGACCCAAGATTCCAACACTATCACTATG GAAGAAAGTTCAAACACTACTCCAGCTGAAAT CAACGACGTTACTCTCTGGCTTCAACAC TGCTATCGGTTACTCTACTCAAATCGGTGC TTGTTTCATCATGTTGTTGTTGTTGACT ATGACTGCTAAGGCTAGATTGCTAGAATC CCAACATATCATCAACACTGCTGCTTGGTT GTTTCTATCATCAGATGTACTTGTGTTGTT TCTTCTCACTCTACTATGATGGAATTCTA CACTATCTCTGACGACTCTCTTCTGTT CACCCAAACGACATCAGAAGATCTGTTGCT GCTACTGTTTCGCTCCATTGCAATTGGCTT TGGTTGAAGCTGTTGATGGTTCAAGCTT GGGCTATGGTTGAATTGTGGCCAAGAGCTT GGAAGGTTCTGGTATCGCTTCTCTTGA TCTTGGCTACTGTTACTGTTGCTTCAAGTG TGCTTCTGCTGCTGTTACTGTTAAGTCTGCT TTGGAACCATTGGACCAAGACCATACTTG TGGATCAGACAAACTGACTTGGCTTCACT ACTGCTATGGTTACTTGGTTCTGTTCTTGT TCAACGTTAGATTGATCATGCACATGTGGC AAAACAGATCTATCTGCCAAGTGTAAAGG GTTTGTCTCCAATGGAAGTTGGTTATGG CTAACGGTTGTTGATGGTTCTTCCCAGTTT GTTGCGCTGGTTGTTACTACGGTAACCTCGG TCAATTGCAATCTGCTTCTTGA TCTGTTGTTGGTTCTTGCATTGGTACTT TGGTTGCTCAAAGATTGGCTGTTAACACAA

			CTGTTGCTGGTTCTTCTGCTAACACTGACA TGGACGACAAGTGGCTTCTGGGTAACG CTACTACTGTTACTTCTGCTGCTGGTT CGCTGGTTCTCTGCTCTGCTACTAGATCT AGATTGGCTTCTCCAAGACAAAACCTCTAA TTGTCTACTTCTGTTCTGCTGGTAAGCCA AGAGCTGACCCAAATCGACTTGGAAATTGCA AAGAATCGACGACGAAGACGACGACTTCT CTAGATCTGGTTCTGCTGGTGGTAGAG TTGAAAGATCTATCGAAAGAAGAGAAAGAA AGATTGTAG ( <b>SEQ ID NO: 19</b> )
<i>Botrytis</i> <i>cinerea</i>	WCGRPGQ PC ( <b>SEQ ID NO: 20</b> )	MASNSSNFDPLTQSI TILMADGITVSVFTP LDIDFFYYYNVACCI NYGAQAGACLLMFF VVVVLTKAVKRKTL LFVLNVLSLIFGFLR AMLYAIYFLQGFND FYAAFTFDFSRVPRS SYASSVAGSVIPLCM TITVNMSLYLQAYT VCKNLDDIKRIILTT LSAIVALLAIGFRFA ATVVNSVAILATSAS SVPMQWLVKGTLV TETISIWFFSLIFTGK LVWTLYNRRNGW RQWSAVRILAAMG GCTMVIPSIFAILEYV TPVSFPEAGSIALTS VALLPISSLWAGM VTDEETSADVSNLT GSRTMLGSQSGNFS RKTHASDITAQSSH DFSSRKGSNATMMR KGSNAMDQVTTIDC VVEDNQANRGLRDS TEMDLEAMGVRVN KSYGVQKA ( <b>SEQ ID NO: 21</b> )	ATGGCTCTAACACTCTAACCTTCGACCCA TTGACTCAATCTACACTATCTGATGGCT GACGGTATCACTACTGTTCTTCACTCCA TTGGACATCGACTTCTACTACTACAAAC GTTGCTTGTATCAACTACGGTGCCTAA GCTGGTGCTTGTGTTGTTGATGTTCTCGTTG TTGTTGTTTGACTAAGGCTGTTAAGAGAA AGACATTGTTGTCGTTGAACGTTTGTC TTTGATCTCGGTTCTGAGAGCTATGTTG TACGCTATCTACTTCTGCAAGGTTCAAC GACTTCTACGCTGCTTCACTTCGACTTCT CTAGAGTTCCAAGATCTCTTACGCTTCTT CTGTTGCTGGTTCTGTTATCCCATTGTTGAT GACTATCACTGTTAACATGTCTTGTACTT GCAAGCTTACACTGTTGTAAGAACTTGG CGACATCAAGAGAATCATCTTGACTACTT GTCTGCTATCGTTGCTTGTGCTATCGGT TTCAGATTGCTGCTACTGTTGTTAACTCT GTTGCTATCTGGCTACTCTGCTTCTCTG TTCCAATGCAATGGTTGGITAAGGGTACTT TGGTTACTGAAACTATCTCTATCTGTTCTT CTCTTGATCTTCACTGGTAAGTTGTTTG GACTTTGTACAACAGAAGAAGAAACGGTT GGAGACAATGGTCTGCTGTTAGAATCTTGG CTGCTATGGGTGGTTGTTACTATGGTTATCC CATCTATCTTCGCTATCTGGAATACGTTA CTCCAGTTCTTCCCAGAAGCTGGTTCTA TCGCTTGAATTCTGTTGCTTGTGTTGCC AATCTCTCTTGTGGCTGGTATGGTTAC TGACGAAGAAACTCTGCTATCGACGTTTC TAACCTGACTGGTTCTAGAACTATGTTGGG TTCTCAATCTGGTAACTCTCTAGAAAGAC TCACGCTTCTGACATCACTGCTCAATCTC TCACCTGGACTTCTCTAGAAAGGGTTTC TAACGCTACTATGATGAGAAAGGGTTCTA ACGCTATGGACCAAGTTACTACTATCGACT GTGTTGTTGAAGACAACCAAGCTAACAGA GGTTGAGAGACTCTACTGAAATGGACTTG GAAGCTATGGGTGTTAGAGTTAACAGTCT TACGGTGTCAAAAGGCTTAG ( <b>SEQ ID NO: 22</b> )

### 6.3. EXAMPLE 3: Reduction To Practice Of Directed Evolution

#### 6.3.1. DIRECTED EVOLUTION OF REPORTER STRAIN

A stable reporter strain to perform DE on plasmid-borne receptor variants based on previous methods for DE of GPCRs in yeast was established. This strain 5 was analogous to the lycopene reporter with the lycopene biosynthetic genes replaced by the reporters: pFus1-mCherry (fluorescence), pFus1-His3 (growth advantage), pFus2-Ura3 (negative selection). The chromosomal copy of Ste2 was deleted.

#### 6.3.2. LIBRARY GENERATION AND SELECTION SCHEME

The endogenous *S. cerevisiae* Ste2 pheromone receptor was mutated by error-prone PCR and selected for active mutants by fluorescence-activated cell sorting (FACS). The enriched libraries were screened in microtiter plates using a growth 10 based assay using pFus1-His3 as previously reported.<sup>30</sup>

#### 6.3.3. PEPTIDE LIGAND DESIGN FOR STEP-WISE DE

A stepwise selection framework that has been used to change substrate 15 specificity of proteins and enzymes was used.<sup>72</sup> Peptide targets that allow generation of a wide range of intermediate hybrid ligands that march from the native peptide ligand (*e.g.* native yeast α-Factor) to the desired target ligand (*e.g.* peptides derived from Cholera Toxin) were used for directed evolution.

#### 6.3.4. SUCCESSFUL DEMONSTRATION OF DE STRATEGY

This DE strategy was applied to CTx and two intermediate peptides (as shown 20 in Figure 7) were designed. An engineered receptor binding a hybrid peptide that is 71% identical to a peptide derived from the Cholera toxin (intermediate-2, “int-2”) was successfully generated. Int-2 had the sequence WHWLELPGSQHIDS (**SEQ ID NO: 23**). The initial mutant receptor, 15C11, shows an EC<sub>50</sub> of 31uM to 25 intermediate-2. Through further rounds of DE, a mutant receptor, 31E4, was generated with an enhanced EC<sub>50</sub> of 11uM for intermediate-2 (see Figure 7).

Table 3. Peptides used in directed evolution and associated sequences

Name of peptides used in DE	Amino acid sequence
<i>α-Factor, wild type S. cereviseae</i>	WHWLQLKPGQPMY (SEQ ID NO: 24)
intermediate-1 (int-1)	WHWLEVPGSQPMY (SEQ ID NO: 25)
intermediate-2 (int-2)	WHWLEVPGSQHIDS (SEQ ID NO: 26)
cholera toxin epitope long (CTxL)	VEVPGSQHIDSQKKA (SEQ ID NO: 27)
cholera toxin epitope short (CTxS)	VPGSQHIDS (SEQ ID NO: 28)

Table 4. GPCRs and associated sequences

Name of hit GPCRs	Amino acid sequence of GPCR	Corresponding DNA coding sequence
<i>Ste2, wild type S. cereviseae</i>	MSDAAPSLSNL FYDPTYNPGQS TINYTSIYGNGS TITFDELQGLV NSTVTQAIMFG VRCGAAALTII VMWMTSRSRK TPIFINQVSLFL IIIHSALYFKYL LSNYSSVTYAL TGFPQFISRGDV HVYGATNIIQV LLVASIETSLVF QIKVIFTGDNFK RIGLMLTSISFT LGIATVTMYFV SAVKGMIVTYN DVSATQDKYF NASTILLASSIN FMSFVLVVKLI LAIRSRRFLGLK QFDSPFHILLIMS CQSLLVPSIIFIL AYSLKPNQGTD VLTTVATLLAV LSPLLSSMWAT AANNASKNTI TSDFTTSTDRF YPGTLSSFQTD SINNDAKSSLRS RLYDLYPRRKE TTSDKHSERTF VSETADDIEKN QFYQLPTPTSS KNTRIGPFADA SYKEGEVEPVD MYTPDTAADE	ATGTCTGATGCGGCTCCTTCATTGAGCAATCTATTATG ATCCAACGTATAATCCTGGTCAAAGCACCATTAACTACAC TTCCATATATGGGAATGGATCTACCATCACTTTCGATGAG TTGCAAGGTTAGTTAACAGTACTGTTACTCAGGCCATT TGTTGGTGTAGATGTGGTGAGCTGCTTGACTTGAT TGTCAATGTGGATGACATCGAGAACAGCAGAAAAACGCCGAT TTTCATTATCAACCAAGTTTCAATTGTTTAATCATTG ATTCTGCACTCTATTAAATATTACTGTCTAATTACTCT TCAGTGACTTACGCTCTCACCGGATTTCCTCAGTTCA GTAGAGGTGACGTTCATGTTATGGTGCTACAAATATAAT TCAAGTCCCTTGTGGCTTCTATTGAGACTTCACTGGTGT TTCAGATAAAAGTTATTTCACAGGGCACAACCTCAAAA GGATAGGTTGATGCTGACGTCGATATCTTCACTTAGG GATTGCTACAGTTACCATGTATTGTAAGCGCTGTTAAA GGTATGATTGTGACTTATAATGATGTTAGTGCACCCAAG ATAAAACTTCATGCAATGCCACAATTACTGCACTC AATAAAACTTATGTCAATTGTCCTGGTAGTTAAATTGATT TTAGCTATTAGATCAAGAAGATTCCCTGGTCTCAAGCA TCGATAGTTCCATATTACTCATAATGTCATGTCATCT TTGTTGGTTCATCGATAATATTCACTCCTCGCATACAGTT GAAACCAAAACAGGGAACAGATGTCTGACTACTGTTGC AACATTACTGCTGTATTGCTTACATTATCATCAATGT GGGCCACGGCTGCTAATAATGCATCCAAAACAAACACAA TTACTTCAGACTTACAACATCCACAGATAGGTTTATCC AGGCACGCTGTCTAGCTTCAAACAGATGATAGTACAAAC GATGCTAAAAGCAGTCTCAGAAGTAGATTATGACCTA TATCCTAGAAGGAAGGAAACACATCGGATAAACATTG GAAAGAACTTTGTTCTGAGACTGCAGATGATATAGAG AAAAATCAGTTTATCAGITGCCACACCTACGAGTTCAA AAAATACTAGGATAGGACCGTTGCTGATGCAAGTTACA AAGAGGGAGAAGTTGAACCCGTCGACATGTACACTCCCG ATACGGCAGCTGATGAGGAAGCCAGAAAGTTCTGGACTG AAGATAATAATAATTAA (SEQ ID NO: 30)

	EARKFWTEDNNL (SEQ ID NO: 29)	
MClone: 15C11	same as Ste2 with mutation: V276A	ATGTCTGATCGGGCTCCTTCATTGAGCAATCTATTATG ATCCAACGTATAATCCTGGTCAAAGCACCATTAACACTACAC TTCCATATATGGGAATGGATCTACCACACTTCGATGAG TTGCAAGGTTAGTTAACAGTACTGTTACTCAGGCCATTA TGTTGGTGTAGATGTGGTGAGCTGCTTGAATTGAT TGTCAATGTGGATGACATCGAGAAGCAGAAAAACGCCGAT TTTCATTATCAACCAAGTTCAATTGTTTAATCATTTGC ATTCTGCACTCTATTAAATATTACTGTCTAATTACTCT TCAGTGACTTACGCTCACCAGGATTTCCTCAGTTCA GTAGAGGTGACGTTCATGTTATGGTGCTACAAATATAAT TCAAGTCCTCTGTGGCTTCTATTGAGACTTCACGGTGT TTCAGATAAAAGTTATTTCACAGGCCGACAACCTCAAAA GGATAGGTTGATGCTGACGTGATATCTTCACTTCA GATTGCTACAGTTACCATGTATTGTAAGCGCTGTTAAA GGTATGATTGTGACTTATAATGATGTTAGTGCCACCCAAG ATAAAACTTCAATGCATCCACAATTACTGCATCCTC AATAAAACTTATGTCAATTGCTGGTAGTTAAATTGATT TTAGCTATTAGATCAAGAAGATTCCCTGGTCTAACAGT TCGATAGTTCCATATTAACTCATAATGTCAATGTCATCT TTGTTGGTCCATCGATAATATTCACTCCTCGCATAAGTT GAAACCAACCAGGGAACAGATGCCTGACTACTGTTGC AACATTACTGCTGTATTGCTTACATTATCATCAATGT GGGCCACGGCTGCTAATAATGCATCCAAAACACAA TTACTCAGACTTACAACATCCACAGATAGGTTTATCC AGGCACGCTGTCTAGCTTCAAACGTGATAGTATCAACAAAC GATGCTAAAAGCAGTCTCAGAAGTAGATTATATGACCTA TATCCTAGAAGGAAGGAAACACATCGGATAAACATTG GAAAGAACTTTGTTCTGAGACTGCAGATGATATAGAG AAAAACTAGTTTATCAGTTGCCACACCTACGAGTTCAA AAAATACTAGGATAGGACCCTGCTGATGCAAGTTACA AAGAGGGAGAAGTTGAACCGCTGACATGTACACTCCCG ATACGGCAGCTGATGAGGAAGCCAGAAAGTTCTGGACTG AAGATAATAATAATTAA (SEQ ID NO: 31)
MClone: 31E4	same as Ste2 with mutation: V276A and Y193C	ATGTCTGATCGGGCTCCTTCATTGAGCAATCTATTATG ATCCAACGTATAATCCTGGTCAAAGCACCATTAACACTACAC TTCCATATATGGGAATGGATCTACCACACTTCGATGAG TTGCAAGGTTAGTTAACAGTACTGTTACTCAGGCCATTA TGTTGGTGTAGATGTGGTGAGCTGCTTGAATTGAT TGTCAATGTGGATGACATCGAGAAGCAGAAAAACGCCGAT TTTCATTATCAACCAAGTTCAATTGTTTAATCATTTGC ATTCTGCACTCTATTAAATATTACTGTCTAATTACTCT TCAGTGACTTACGCTCACCAGGATTTCCTCAGTTCA GTAGAGGTGACGTTCATGTTATGGTGCTACAAATATAAT TCAAGTCCTCTGTGGCTTCTATTGAGACTTCACGGTGT TTCAGATAAAAGTTATTTCACAGGCCGACAACCTCAAAA GGATAGGTTGATGCTGACGTGATATCTTCACTTCA GATTGCTACAGTTACCATGTATTGTAAGCGCTGTTAAA GGTATGATTGTGACTTATAATGATGTTAGTGCCACCCAAG ATAAAACTTCAATGCATCCACAATTCTACTGCATCCTC AATAAAACTTATGTCAATTGCTGGTAGTTAAATTGATT TTAGCTATTAGATCAAGAAGATTCCCTGGTCTAACAGT TCGATAGTTCCATATTAACTCATAATGTCAATGTCATCT TTGTTGGTCCATCGATAATATTCACTCCTCGCATAAGTT GAAACCAACCAGGGAACAGATGCCTGACTACTGTTGC AACATTACTGCTGTATTGCTTACATTATCATCAATGT GGGCCACGGCTGCTAATAATGCATCCAAAACACAA TTACTCAGACTTACAACATCCACAGATAGGTTTATCC

		AGGCACGCTGTCTAGCTTCAAACGTAGATAGTATCAACAAAC GATGCTAAAAGCAGTCTCAGAAGTAGATTATGACCTA TATCCTAGAAGGAAGGAAACACATCGGATAAACATTG GAAAGAACTTTGTTCTGAGACTGCAGATGATATAGAG AAAAATCAGTTTATCAGITGCCACACCTACGAGTTCAA AAAATACTAGGATAGGACCCTTGCTGATGCAAGTTACA AAGAGGGAGAAGTTGAACCCGTCGACATGTACACTCCCG ATACGGCAGCTGATGAGGAAGCCAGAAAGTTCTGGACTG AAGATAATAATAATTAA (SEQ ID NO: 32)
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### 6.3.5. DEMONSTRATION OF PROTEASES TO RELEASE TARGET LIGANDS

A simple proteolytic degradation of commercially purified CTx was performed. CTx was specifically degraded with either Trypsin or a combination of 5 LysN and GluC. The expected target peptide was successfully detected by mass spectrometry showing it to be released from the full protein. The experiment resulted in a list of released peptides of different length and physicochemical properties which can be used as additional target analytes. Analogous degradation of CTx in the gut or the environment may make target peptides available in field samples. Additionally 10 and alternatively, these extremely robust and cheap proteases may be incorporated into a product formulation.

### 6.4. EXAMPLE 4- Yeast Cholera Biosensor

The strain is engineered to respond to a cholera specific peptide by generating a color output.

15 To develop a cholera peptide binding receptor, the GPCR is subjected to mutagenesis and the resulting library is expressed in the same yeast host. All variants are screened against the peptide, which is synthetically synthesized or originates from bacterial cultures, and strains that show reporter gene expression are further investigated and optimized. Enhanced binding may be achieved by more stringent 20 screening conditions such as lower concentration of target molecule or less copies of the receptor expressed on the cell surface. In certain embodiments, color change is rapid - for example 10 grams, 1 gram, 100 mg, 10 mg, or even 1 mg of freeze dried

yeast may result in sufficient red color to be readily apparent to the naked eye, and the assay is desirably sensitive enough to detect low levels of peptide. Non-engineered yeast may be used as controls to test biosensor specificity and false-positive rate. Native alpha factor/Ste2 receptor activation can also be used as a control.

5     6.5. EXAMPLE 5- Expressing GPCRs In Yeast

GPCRs were cloned into yeast using the Reiterative Recombination DNA assembly system. The desensitization of the receptor, where prolonged stimulation leads to an attenuated response, was eliminated by deletion of SST2, allowing cells to respond to doses of pheromone that are roughly two orders of magnitude lower than 10 those detected by normal cells and prevent recovery from pheromone-induced growth arrest, even if the ligand was removed.<sup>20</sup> Deletion of Far1 also prevented pheromone-induced cell cycle arrest. The endogenous pheromone receptor Ste2 was deleted to avoid cross talk with yeast mating signal.

6.6. EXAMPLE 6 - Freeze-Drying Yeast

15         Viability of *S. cerevisiae* was determined after different freeze-drying treatments.<sup>73</sup> The results are shown in FIGURE 5. Cell viability of ~1-2% was observed, in agreement with previously published literature.

6.7. EXAMPLE 7: Detection of Pathogenic Fungi Pheromones Using An Integrated Lycopene Biosensor

20         The engineering of *S. cerevisiae* as a specific and sensitive biosensor for the presence of pathogenic fungi that may be easily used outside the laboratory. The sensor may be used by non-experts, and thus consists of non-technical mixing and color change output that is visible to the naked eye.

Baker's yeast, a safe organism broadly used in the food industry for centuries 25 and easily grown in a robust manner was reprogrammed to express the tomato red

pigment lycopene in response to binding of natural pathogen-specific peptides by expressing natural fungal binding receptors. This user-friendly and equipment-free signal is compatible with household use at local communities at-risk for fungi infections.

5 Fungal pathogens have recently been identified as increasing cause of human disease as well as a cause of population decline in animals and crops. The annual number of cases of sepsis caused by fungal organisms in the U.S. increased by 207% between 1979 and 2000 [Pfaller, Diekema, (2007)]. Several factors contribute to the increase in fungal infections, among which are the increasing number of  
10 immunocompromised HIV, cancer and transplantation patients, aging population, and increased global mobility which expands the habitats of endemic opportunistic fungal strains [Pfaller, Diekema, (2007)].

Candida fungal species are the major cause of opportunistic mycoses worldwide with 72.8 million annual candida species infections cases worldwide and a  
15 33.9% case/fatality ratio [Pfaller, Diekema, (2007)]. Candida infections are associated with a high crude mortality of 46% to 75% and a long hospital stay which causes tremendous health care burden. Two fungal species, *C. albicans* and *C. Glabrata*, were shown to be the causative agents of 62% and 12% of *Candidasis*, respectively. [Ramirez-Zavaleta (2010)]. *Candida albicans* is a fungi naturally found  
20 in human gastrointestinal, genitourinary tracts and skin, but under compromised immunity it could result in kidney, heart or brain infection [Berman, Sudbery (2002)].

It is difficult to diagnose and distinguish fungal infections. While several anti-fungal therapeutics are available, mortality rates of invasive fungal diseases remain extremely high, often exceeding 50%. This is due to a major clinical bottleneck in  
25 early treatment, rooted in significant lack of rapid diagnosis [Brown et al. (2012)].

For example, although several methods are currently available for detection of pathogenic fungi in the laboratory, the current gold standard for confirming candida infection in patients remains slow methods such as cultures or cost prohibitive methods such as coagulation assays which are often unavailable in high risk areas for 5 fungal infections. In this Example, a non-technical biosensor that could be used outside of the laboratory for detection of pathogenic fungi was developed.

In order to detect fungal pathogens, fungal receptors that are naturally binding the fungal peptide mating pheromone were generated. *Candida albicans* cells are diploid (a/alpha) and both homothallic and heterothallic mating have been observed in 10 clinical samples, making mating peptide a relevant biomarker for fungal detection. *C. albicans* must switch its phenotype from white to opaque before secretion of pheromones can occur to induce mating, a transition triggered by different environmental signals. The opaque “mating” phenotype was found to be stabilized by the presence of CO<sub>2</sub> and GlcNAc and observed during passage through mouse 15 intestines, suggesting persistence of mating-compatible, pheromone producing *C. albicans* cells in the host [Ramirez-Zavala (2008); Huang (2010)]. Mating was also observed in systemic infections and colonization of the skin and intestines. [Hull et al. (2000), Lachke et al (2003), Dumitru (2007)]. *C. glabrata* population is mostly 20 clonal, and while distinct mating types have been identified, pheromone genes are not expressed in most isolates and neither mating types responds to pheromone.

#### 6.7.1. *Fungal GPCRs as the detection element*

Natural fungal GPCRs were cloned and tested for functionality with their respective natural ligands in *S. cerevisiae* biosensor strain. The results for GPCR activation experiments in biosensor strain are presented in Figures 9 and 10.

Sequence analysis of receptor and peptides are presented in Figures 11 and 12 and further discussed in Example 6.8 below.

As shown in Figure 9, fungal receptors were found to be highly specific for their respective peptide pheromones, with very little crosstalk between receptors.

5 This is due to the critical role of pheromone recognition in fungal mating and conservation of species integrity. For example, species cohabitating a common host, *C. Glabrata* and *C. albicans* did not respond to the other species pheromone. However, *S. cerevisiae* native Ste2 receptor responded to *C. glabrata*, but not to *C. albicans* pheromone, reflecting the difference in phylogenetic distance between the

10 three strains. Interestingly, the *P. brasiliensis* receptor seemed more promiscuous, showing moderate activity when induced with *A. fumigatus* or pheromone.

Most receptor-pheromone pairs were found to be highly sensitive to their ligand peptide, with EC50 values of 4 nM, 51 nM and 34 nM for *C. albicans*, *L. elongisporous*, and *P. brasiliensis*, respectively, notably higher than the natural activation of the *S. cerevisiae* GPCR–pheromone pair (EC50=190 nM). *C. glabrata* was less active EC50 =3.6 μM) in biosensor settings (see Figure 9).

#### 6.7.2. Lycopene as a simple, low-cost readout

Having established fungal GPCRs as the detection element, the inventors then implemented and optimized a lycopene biosynthetic pathway as a direct, low-cost readout for the biosensor (see Figure 13). By overexpressing key pathway genes (CrtI, tHMG1, Fad1), there was significant improvement in the maximal yield of lycopene produced after induction with α-factor. These changes also greatly reduced the time required to reach half maximal biosynthesis of lycopene after induction by α-factor (see Figure 13C).

25 6.7.3. An integrated biosensor

A product profile that satisfies the unique requirements of a live yeast cell sensor as diagnostic device was developed. Specifically, a core product component, the “Yeast Reporter Tab”, maintaining viable, functional yeast cells while enhancing color contrast and ease of use (see Figure 14) was developed. Importantly, this kit 5 design incorporates a nutrient gel, a white paper to enhance signal contrast, a concentrated yeast spot to enhance apparent color intensity of the produced lycopene and a control yeast spot to eliminate false positives. The design was viable and functioned.

The integrated biosensor properly responded to a synthetic peptide derived 10 from the human pathogen *C. albicans*. Importantly, the biosensor retained a high level of sensitivity and speed while producing a signal visible to the naked eye (see Figure 14B).

Furthermore, Figure 14C shows observed dose-response of the biosensor strain (using fluorescent readout) when exposed to culture supernatants from the 15 homozygous *C. albicans* strains P37005, GC75 or a mixture of the two pathogen strain.

#### 6.8. EXAMPLE 8: Peptide-Activated Receptors And Peptide Ligands

EXAMPLE 8 is an updated study of EXAMPLE 2. Whole-cell diagnostic device enables the use of integral membrane receptors to mediate highly specific and 20 sensitive detection of biologically relevant ligands. Notably, membrane proteins such as GPCRs have not been amenable for *in vitro* diagnostics as they are notoriously difficult to express outside of their natural membrane environment. A whole-cell provides access to the untapped repertoire of molecular recognition of GPCRs in much the same way ELISAs allowed access to antibody recognition [Lequin (2005)].

The inventors focused on implementing the highly specific fungal peptide-activated GPCRs, such as Ste2 from *S. cerevisiae*, for detection of fungal peptides.

Fungal GPCRs have several key advantages for biosensor engineering. First, GPCRs homologous to the *S. cerevisiae* Ste2 robustly coupled to the host pheromone pathway. (see Figure 9 and 10). Second, these fungal GPCRs recognized a diverse set of peptide ligands (see Figure 12, Table 5). Third, fungal GPCRs showed very highly specificity for their respective peptides (see Figure 9). Furthermore, these fungal GPCRs offered a highly viable platform for directed evolution towards binding of novel peptide ligands through mutagenesis of either receptor or peptide.

10 **Table 5. Physicochemical properties of functionally verified peptide ligands, ordered by peptide length**

Sequence	Length	MW	IP	Charge (-/+)	GRAVY <sup>a</sup>
WCGRPGQPC (SEQ ID NO:20)	9	1	8.07	0/1	-0.878
WCTRPGQGC (SEQ ID NO:11)	9	1.007	8.07	0/1	-0.778
WCGHIGQGC (SEQ ID NO:33)	9	0.960	6.72	0/0	0.078
WCWWKGQPCW (SEQ ID NO:14)	10	1.379	8.06	0/1	-0.800
QWCPRRGQPCW (SEQ ID NO:17)	11	1.416	9.02	0/2	-1.491
WMWTRYGRFSPV (SEQ ID NO:34)	12	1.585	10.84	0/2	-0.558
HLVRLSPGAAMF (SEQ ID NO:35)	12	1.298	9.76	0/1	0.800
HFIELDPGQPMF (SEQ ID NO:36)	12	1.430	4.35	2/0	-0.125
WHWTSYGVFEPG (SEQ ID NO:37)	12	1.465	5.24	1/0	-0.558
WHWLQLKPGQPMY (SEQ ID NO:38)	13	1.670	8.6	0/1	-0.869
GFRLTNFGYFEPG (SEQ ID NO:5)	13	1.500	6	1/2	-0.315
WHWVRLRKGQGLF (SEQ ID NO:8)	13	1.682	12.1	0/3	-0.585
WSWITLRPGQPIF (SEQ ID NO395)	13	1.600	9.75	0/1	0.054

WHWLELDNGQPIY (SEQ ID NO:40)	13	1.670	4.35	2/0	0.785
WHWLRLRYGEPIY (SEQ ID NO:41)	13	1.789	8.6	1/2	-0.769
KPHWTTYGYYEPQ (SEQ ID NO:42)	13	1.669	6.75	1/1	-1.838
NWHWLRLDPGQPLY (SEQ ID NO:43)	14	1.795	6.74	1/1	-0.964
KFKFRLTRYGWFSPN (SEQ ID NO:44)	15	1.947	11.1	0/4	-0.92
KKNSRFLTYWFFQPIM (SEQ ID NO:45)	16	2.106	10.29	0/3	-0.375
GDWGFWYWVPRPGDPAM (SEQ ID NO:46)	17	2.037	4.21	2/1	-0.635
TYADFLRAYQSWNTFVNPDRLNL (SEQ ID NO:47)	23	2.789	5.63	2/2	-0.778
VSDRVKQMLSHWWNFRNPDTANL (SEQ ID NO:48)	23	2.815	8.72	2/3	-0.883
TYEDFLRVYKNWWSFQNPDRPDL (SEQ ID NO:49)	23	2.990	4.68	4/3	-1.265

<sup>a</sup>The GRAVY value is the average hydropathy of the given sequence. Positive values indicate overall hydrophilicity of the sequence and negative values relative hydrophobicity. Index range is -4.5 to 4.5

#### 6.8.1. Key characteristics of fungal GPCRs

5 Candidate receptors for biosensor engineering were identified by searching protein and genomic databases (NCBI, UniProt) for proteins and/or genes with homology to *S. cerevisiae* Ste2 receptor. Functionally characterized receptors (described below) had an average amino acid sequence homology of 33% to *S. cerevisiae* Ste2, ranging from 66% to 15% as calculated with Clustal Omega [Sievers  
10 (2014)].

Additionally, all receptors were predicted to have seven transmembrane helices, an extracellular N-terminus, an intracellular C-terminus, three extracellular loops and three intracellular loops when analyzed by TMHMM v2.0 [Krogh et al. (2001)]. Notably, while large portions of the extracellular loops and transmembrane

helices had low conservation across receptors, three key regions with increased homology (see Figure 11) were observed. Based on previous mutational studies of the *S. cerevisiae* Ste2 receptor, these three regions have been shown to be important in mediating signal transduction and interactions with the downstream G-protein. [Ćelić 5 et al. (2003); Martin et al. (2002)]. Thus, cell surface receptors with homology to these key regions have a high likelihood of functioning in a *S. cerevisiae* biosensor.

#### 6.8.2. List of functionally characterized receptors

Twenty three receptor-peptide pairs were cloned and functionally characterized in sensor strain, as shown in Figures 9 and 10 (see Table 6 for 10 sequences).

- Human pathogen: *C. albicans*, *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, *C. parapsilosis*,
- Plant pathogen: *F. graminearum*, *M. oryzae*, *B. cinerea*, *G. candidum*.
- Food Safety/Spoilage: *Z. bailii*, *Z. rouxii*
- Industrial/Model fungi: *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (receptor 1), *V. polyspora* (receptor 2), *S. stipitis*, *S. japonicas*, *S. castellii*, *S. octosporus*.

#### 6.8.3. List of additional cloned receptors (see Table 6 for sequences)

A. nidulans, A. oryzae, B. bassiana, C. lusitaniae, C. tenuis, N. fischeri, N. crassa, P. destructans, H. jecorina, T. melanosporum, D. haptotyla, S. scheckii, Y. 25 lipolytica, T. delbrueckii, K. pastoris

**Table 6. Sequences of Fungal GPCRs and Peptide Ligands**

Fungi	sequence of peptide analyte used	sequence of GPCRs used (all sequences are wild type)	DNA coding sequence of corresponding GPCRs that senses peptide analyte (WT or codon-optimized noted)
Saccharomyces cerevisiae	WHWLQL KPGQPM Y	MSDAAPSLSNLFY DPTYNPGQSTINY TSIYGNNGSTIFDDE LQGLVNSTVTQAI	(wild type)  ATGTCTGATGCGGCTCCTTCATTGAGC AATCTATTTATGATCCAACGTATAAT

		MFGVRCGAAALT LIVMWMTSRSRKT PIFIINQVSLFLIILH SALYFKYLLSNYS SVTYALTGFQPQFIS RGDVHVYGVATNII QVLLVASIETSLVF QIKVIFTGDNFKRI GLMLTSISFTLGIA TVTMYFVSAVKG MIVTYNDVSATQD KYFNASTILLASSI NFMSFVLVVKLIL AIRSRRFLGLKQFD SFHILLIMSCQSLL VPSIIFILAYSLKPN QGTDVLTIVATLL AVLSPLSSMWAT AANNASKNTITS DFTTSTDRFYPGTL SSFQTDSINNDAKS SLRSRLYDLYPRR KETTSDKHSERTF VSETADDIEKNQF YQLPTPTSSKNTRI GPFADASYKEGEV EPVDMYTPDTAA DEEARKFWTEDN NNL (SEQ ID NO:50)	CCTGGTCAAAGCACCATTAACTACAC TTCCATATATGGGAATGGATCTACCAT CACTTCGATGAGTTGCAAGGTTAGT TAACAGTACTGTTACTCAGGCCATTAT GTTTGGTGTCAAGATGTGGTGCAGCTGC TTTGACTTTGATTGTCATGTGGATGAC ATCGAGAAGCAGAAAAACGCCGATTT TCATTATCAACCAAGTTTCATTGTTTT TAATCATTTCGATTCTGCACTCTATT TTAAATATTTACTGTCTAATTACTCTT CAGTGACTTACGCTCTCACCGGATTC CTCAGTTCATCAGTAGAGGTGACGTT ATGTTATGGTGCACAAATATAATTC AAGTCCTTCTTGTGGCTCTATTGAGA CTTCACTGGTGTTCAGATAAAAGTTA TTTCACAGGCGACAACCTCAAAAGG ATAGGTTGATGCTGACGTCGATATCT TTCACTTTAGGGATTGCTACAGTTACC ATGTATTTGTAAGCGCTGTTAAAGGT ATGATTGTGACTTATAATGATGTTAGT GCCACCCAAGATAAAACTTCATGC ATCCACAATTACTGCATCCTCAAT AAACTTATGTCATTGCTCTGGTAGT TAAATTGATTTAGCTATTAGATCAAG AAGATTCTTGGTCTCAAGCAGTTCGA TAGTTCCATATTACTCATAATGTC ATGTCAATCTTGTGGTCCATCGAT AATATTCATCCTCGCATAACAGTTGAA ACCAAAACCAGGAACAGATGTCTTGA CTACTGTTGCAACATTACTTGCTGTAT TGTCTTACCATATTATCATCAATGTGGG CCACGGCTGCTAATAATGCATCCAAA ACAAACACAATTACTCAGACTTTAC AACATCCACAGATAGGTTTATCCAG GCACGCTGTCTAGTTCAAACATGATA GTATCAACAACGATGCTAAAGCAGT CTCAGAAGTAGATTATGACCTATAT CCTAGAAGGAAGGAAACAAACATCGGA TAAACATTGGAAAGAACATTGTTTC TGAGACTGCAGATGATATAGAGAAAA ATCAGTTTATCAGTTGCCACACCTA CGAGTTCAAAAATACTAGGATAGGA CCGTTGCTGATGCAAGTTACAAAGA GGGAGAAGTTGAACCCGTCGACATGT ACACTCCGATACGGCAGCTGATGAG GAAGCCAGAAAGTTCTGGACTGAAGA TAATAATAATTATAG (SEQ ID NO:51)
Candida albicans	GFRLTNF GYFEPG	MNINSTFIPDKPGD IIISYSIPGLDQPIQI PFHSLDSFQTDQA	(wild type)  ATGAATATCAATTCAACTTTCATACCT

		<p>KIALVMGITIGSCS MTLIFLISIMYKTN KLTNLKLKLKY ILQWINQKIFTKRR NDNKQQQQQQQQ QIESSSYNNNTTTL GGYKLFLYLNSLI LLIGIIRSGCYLNY NLGPLNSLSFVFTG WYDGSSFISSDVT NGFKCILYALVEIS LGFQVYVMFKTSN LKIWGIMASLLSIG LGLIVVAFQINLT LSHIRFSRAISTNRS EEESSSSLSSDSVG YVINSIWMDLPTIL FSISINIMTILLIGKL IIAIRTRRYLGLKQ FDSFHILLIGFSQTL IIPSIILVVHYFYLS QNKDSLLQQISLLL IILMLPLSSLWAQT ANNTHNINSSPSLS FISRHHLSDSSRSRG GSNTIVSNGGSNG GGGGGGNFPVSGI DAQLPPDIEKILHE DNNYKLLNSNNES VNDGDIINDEGMI TKQITIKRV</p> <p>GATAAACCCAGGCGATATAATTATTAG TTATTCAATTCCAGGATTAGATCAACC AATTCAAATTCCCTTCACATTAGA TTCATTCAAAACCGATCAAGCTAAAAT AGCTTAGTCATGGGATAACTATTG GGAGTTGTTCAATGACATTAATTTTT TGATTCTATAATGTATAAAACTAATA AATTAACAAATTAAAATTAAAATTA AAATTAAAATATCTTGCAATGGAT AAATCAAAAAATCTTCACCAAAAAAA GGAATGACAACAAACAACAACAACA ACAACAACAACAACAATTGAATCAT CATCATATAACAATACTACTACTACGC TGGGGGGTTATAAATTATTTTATT ATCTTAATTCTATTGATTTATTAAATTG GTATTATTGATCAGGTTGTTATTAA ATTATAATTAGGTCCATTAAATTCA TTAGTTTGTTACTGTTGGTATG ATGGATCATCATTATATCATCCGATG TAACTAATGGATTAAATGTATT ATGCTTAGTGGAAATTCAATTAGGTT TCCAAGTTATGTGATGTTCAAAACTT CAAATTAAAAATTGGGGGATAATG GCATCATTATTATCAATTGGTTAGGA TTGATTGTTGTTGCCTTCAAATCAAT TTAACAAATTATCTCATATTGATT TCCCAGGCTATATCAACTAACAGAAG TGAAGAAGAACATCATCATCATATT CATCTGATTGGTTGGTATGTGATTA ATTCAATATGGATGGATTACCAACA ATATTATTTCATTAGTATTAAATATA ATGACAATATTGATTGGTAAACTT ATAATTGCTATTAGAACAAAGACGTTA TTAGGATTGAAACAATTGATAGTT CCATATTATTAATTGGTTCA AACATTAAATTATCCTCAATT GGTGGTCATTATTTATTACACA AAATAAGATTCTTATTACAACAAA TTAGTCATTGATTATTTAATGTT ACCATTAAAGTTCTTATGGGCTAAAC TGCTAATAACTCATAATATTAAATTC ATCTCCAAGTTATCATTCAATCTCG TCATCATCTGTCTGATAGTAGTCGTAG TGGTGGTCCAATACAATTGTTAGTAA TGGTGGTAGTAATGGTGGTGGTGGT GTGGTGGAAATTCCCTGTTCA TTGATGCACAATTACCAACCTGATATTG AAAAAATCTTACATGAAGATAATAAT TATAAATTACTTAATAGTAATAATGA AAGTGTAAATGATGGAGATATTATCA TTAATGATGAAGGTATGATTACTAAA</p>
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			CAAATCACCATAAAAGAGTGTAG
Candida glabrata	WHWVRL RKGQGLF	MEMGYDPRMYNP RNEYLNFTSVYDV NDTIRFSTLDAIVK GLLRIAIVHGVR GAIFMTLIIMFISSN TWKKPIFIINMVSL MLVMIHSALSFHY LLSNYSSISYILTGF PQLITSNNKRIQDA ASIVQVLLVAAIEA SLVFQIHVMFTIEN IKLIREIVLSISIAM GLATVATYLAAAI KLIRGLHDEVMPQ THLIFNLSSIILLASSI NFMTFILVIKLFFAI RSRRYLGRLQFDA FHILLIMFCQSLLIP SVLYIIVYAVDSRS NQDYLIPIANLFVV LSPLSSIWANTSN NSSRSPKYWKNSQ TNKSNGSFVSSISV NSDSQNPLYKKIV RFTSKGDTTRSIVS DSTLAEVGKYSM QDVSNSNFECRDL DFEKVKHTCENFG RISETYSELSTLDT TALNETRLFWKQQ SQCDK	(wild type)  ATGGAGATGGGCTACGATCCAAGAAT GTATAATCCAAGAAATGAATACTTGA ATTTCACGTCGGTATATGATGTAAATG ACACAATCAGATTTCGACTCTGGAC GCCATTGTAAAAGGATTGCTTAGAAT TGCCATTGTCATGGAGTTAGATTGGG AGCAATATTGACGTTAATAATAA TGTTTATCTCATCAAATACATGGAAAA AACCCATATTATAATTAAACATGGGT CGTTGATGTTAGTTATGATTCAATTCCG CACTTAGCTCCATTACCTTTATCGA ATTATTCTCAATTCTTATATACTGA CAGGGTTTCAGTTGATTACAAGCA ATAATAAACGAATTCAAGATGCAGCG AGTATAGTCCAAGTTTATTGGGTGCT GCGATAGAAGCATCATTGGTATTCA GATTGATGTTATGTTACGATTGAAAAA CATTAAGCTTATTAGAGAAAATAGTAC TCTCTATATCGATAGCAATGGGATTGG CAACAGTGGCTACATATCTGCTGCA GCAATAAAGCTGATAAGAGGACTGCA TGATGAGGTAATGCCACAAACACATC TTATTTCATTTATCTATAATATTGCT TGCATCCTCCATAAATTTATGACATT TATATTGGTCATTAACCTTTCTTCGC TATTAGATCTAGAAGATATCTCGGTCT TCGTCAATTGATGCTTTCATATT ATTAATCATGTTCTGCCAGTCATT GATACCCCTCAGTATTATATTATAGT TTACGCGGTTGATAGCAGATCTAAC AGGATTATCTGATTCCAATTGCCAATT TATTGTTGTTTATCTTGCCTTATC CTCTATCTGGGCTAACACATCAAATA ACTCATCCAGATCTCCAAAATATTGG AAAAACTCTAAACGAATAAGAGCAA TGGGTCTTGTCTCTCAATATCTGT CAATAGTGAECTCACAAAACCCCTTGT ACAAAAAAGATTGTACGTTTACATCA AAAGGCGACACTACCCGTAGTATTGT AAGTGATTCAACATTAGCAGAGGTGG GAAAATACTCTATGCAAGACGTTAGC AATTCAAACCTTGAATGTCGAGACCTT GATTTGAGAAGGTAAAACATACTTG CGAAAATTGGCAGAATATCTGAAA CATATAGTGAAGTAAAGTACTTTAGATA CCACTGCCCTCAATGAGACTCGGTTGT TTTGGAAACAACAAAGTCAGTGTGAC

			AAATAG
Paracoccidioides brasiliensis	WCTRPG QGC	MAPSFDPFNQSVV FHKADGTPFNVI HELDDFVQYNTK VCINYSQLGASVI AGLMLAMLTHSE KRRLPVFFLNTFA LAMNFARLLCMTI YFTTGFNKSAYF GQDYSQVPGSAY AASVLGVVFVTL VISMEMSLLIQTRV VCTTLPDIQRYLL MAVSSAISLMAIG FRGLGMVENCIAI VQASNFAFPFIWLQ SASNITITISTCFFS AVFVTKLAYALVT RIRLGTRFGAMQ VMFIMSCQTMVIP AIFSILQYPLPKYE MNSNLFTLVAIFLP LSSLWASVATRSS FETSSSGRHQYLW PSEQSNNVTNSEIK YQVSFSQNHTTLR SGGSVATTSPDR LDPVYCEVEAGTK A	(wild type)  ATGGCACCCCTCATTGACCCCTTCAC CAAAGCGTGGTCTTCACAAGGCCGA CGGAACCTCCATTCAACGTCTCAATCCA TGAACTAGACGACTTCGTGCAGTACA ACACCAAAGTCTGCATCAACTACTCTT CCCAGCTCGGAGCATCTGTCATTGCA GGACTCATGCTGCCATGCTGACACA CTCAGAAAAGCGTCGTCTGCCAGTTT CTTCCTAAACACATTGCACGGCCAT GAACCTTGCCCCCCTGCTCTGCATGAC CATCTACTTCACCACGGGCTCAACAA GTCCTATGCCTACTTGGTCAGGATTA CTCCCAGGTGCCTGGGAGCGCCTACG CAGCCTCTGTCTGGCGTTGTCTTC CCACTCTCCTGGTAATCAGCATGGAA ATGTCCCTCCTGATCCAACAAAGGGTT GTCTGCACGACCCCTCCGGATATCCAA CGTTATCTACTCATGGCAGTTCCCTCC GCGATTTCCTGATGCCATGGGTTTC CGCCTTGGCTTAATGGTTGAGAACTGC ATTGCCATTGTGCAGGCGTCGAATTTC GCCCTTTATCTGGCTTCAAAGGCC TCGAACATCACCATTACGATCAGCAC ATGTTCTTCAGTGCCGTCTTGTTAC GAAATTGGCATATGCACTCGTCACTC GTATACGACTAGGCTTGACGAGGTTT GGTGCTATGCAGGTTATGTTCATCATG TCCTGCCAGACTATGGTGATTCCAGCC ATCTTCTCAATTCTCCAATACCCACTC CCCAAGTACGAAATGAACCTCCAACCT CTTTACGCTGGTGGCCATTTCCTCCC TCTTCCTCGCTATGGGCTTCAGTGC TACGAGATCCAGTTCGAGACGTCTTC TTCCGGCCGCCATCAGTATCTTGCC AAGCGAACAGAGCAATAACGTACCA ATTCGAAATTAAAGTATCAGGTAGC TTCTCTCAGAACCAACTACGTTGCC TCTGGAGGGTCTGTGGCCACGACACT CTCCCCGGACCGGCTCGACCCGGTTA TTGTGAAGTTGAAGCTGGCACAAAGG CCTAG
Fusarium graminearum	WCWWK GQPCW	MSKEVFDPTQNV TFFAPDGKTEISIP VAAIDQVRRMMV NTTINYATQLGAC	(codon optimized)  ATGTCTAAGGAAGTTTCGACCCATT ACTCAAAACGTTACTTCTCGCTCCA GACGGTAAGACTGAAATCTCTATCCC

		LIMLVVLLVMVPK EKFRRPFMILQITS LVISCCRMLLISIF HSSQFLDFYVFWG DDHSRIPRSAYAPS VAGNTMSLCLVIS VETMLMSQAWTM VRLWPNVWKYIIA GVSLIVSIMAISVR LAYTIIQNNAVLK LEPAFHMFWLKW TVIMNVASISWWC AIFNIKLVWHLISN RGILPSYKTFPMEM VLIIMTNGILMIIPVI FASLEWAHFVNFE SASLTLSVAVILP LGLTLAAQRIASSAP SSANSTGASSGIRY GVSGPSSFTGFKAP SFSTGTTDRPHVSI YARCEAGTSSREH INPQGVELAKLDP ETDHHRVVDRAFL QREERIRAPL	AGTTGCTGCTATCGACCAAGTTAGAA GAATGATGGTTAACACTACTATCAAC TACGCTACTCAATTGGGTGCTGTTTG ATCATGTTGGTTGTTTGTGGTTATG GTTCCAAAGGAAAAGTTCAGAAGACC ATTCATGATCTGCAAATCACTCTTT GGTTATCTCTTGTAGAATGTTGTT GTTGTCTATCTCCACTCTCTCAATT TTGGACTCTACGTTCTGGGTGAC GACCACTCTAGAATCCCAAGATCTGC TTACGCTCCATCTGTTGCTGGTAACAC TATGTCCTTGTGTTGGTTATCTCTGTT GAAACTATGTTGATGTCTCAAGCTGG ACTATGGTTAGATTGTGGCCAACGTT TGGAAGTACATCATCGCTGGTGTCT TTGATCGTTCTATCATGGCTATCTCT GTTAGATTGGCTTACACTATCATCAA AACAAACGCTGTTTGAAGTTGGAACC AGCTTCCACATGTTCTGGTGTGATCAA GTGGACTGTTATCATGAACGTTGCTTC TATCTCTGGTGGTGTGCTATCTCAA CATCAAGTTGGTTGGCACITGATCTC TAACAGAGGTATCTGCCATCTTACAA GACTTCACTCCAATGGAAGTTTGAT CATGACTAACGGTATCTGATGATCAT CCCAGTTATCTCGCTTCTTGGAAATG GGCTCACTTCGTTAACITCGAATCTGC TTCTTGACTTTGACTTCTGTTGCTGTT ATCTTGCCATTGGTACTTGGCTGCT CAAAGAAATCGCTCTCTGCTCCATCT TCTGCTAATCTACTGGTGTCTCTTCT GGTATCAGATAACGGTGTCTGGTCCA TCTTCTTCACTGGTTCAAGGCTCCA TCTTCTACTGGTACTACTGACAGA CCACACGTTCTATCTACGCTAGATGT GAAGCTGGTACTTCTTAGAGAAC CATCAACCCACAAGGTGTGAATTGG CTAAGTTGGACCCAGAAACTGACCAC CACGTTAGAGTTGACAGAGCTTCTTG CAAAGAGAAGAAAGAATCAGAGCTCC ATTGTAG
Magnaport he oryzae	QWCPRR GQPCW	MDQTLSATGTATS PPGPALTVDPRFQ TITMLTPALMGQG FEEVQTTPAEINDV YFLAFNTAIGYST QIGACFIMLLVLLT MTAKARFARIPTII NTAALVVSIIRCTL LVIFFTSTMMEFYT	(codon optimized)  ATGGACCAAACCTTGTCTGCTACTGGT ACTGCTACTTCTCCACCAAGGTCCAGCT TTGACTGTTGACCCAAGATTCAAAC ATCACTATGTTGACTCCAGCTTGATG GGTCAAGGTTCGAAGAAGTTCAAAC TACTCCAGCTGAAATCAACGACGTTT ACTTCTTGGCTTCAACACTGCTATCG

		IFSDDFSFVHPNDI RRSVAATVFAPLQ LALVEAALMVQA WAMVELWPRAW KVSGIAFSLLATV TVAFKCASAATV KSALEPLDPRPYL WIRQTDLAFTTAM VTWFCLFNVRLI MHMWQNRSILPT VKGLSPMEVLVM ANGLLMVFPVLFA GLYYGNFGQFESA SLTITSVVLVLPLG TLVAQRALVNNT VAGSSANTDMDD KLAFLGNATTVTS SAAGFAGSSASAT RSRLASPRQNSQL STSVSAGKPRADPI DLELQRIDDEDDD FSRSGSAGGVRRVE RSIERREERL	GTTACTCTACTCAAATCGGTGCTTGTT TCATCATGTTGGTTGGTTGACTA TGACTGCTAAGGCTAGATTGCTAGA ATCCCAACTATCATCAACACTGCTGCT TTGGTTGTTCTATCATCAGATGTACT TTGTTGGTTATCTCTCACTTCTACTA TGATGGAATTCTACACTATCTCTCTG ACGACTTCTCTTCGTTCACCCAAACG ACATCAGAAGATCTGTTGCTGCTACTG TTTCGCTCCATTGCAATTGGCTTGG TTGAAGCTGCTTGTGATGGTTCAAGCTT GGGCTATGGTTGAATTGTGGCCAAGA GCTTGBAAGGTTCTGGTATCGCTTTC TCTTGATCTTGCTACTGTTACTGTT GCTTCAAGTGTGCTCTGCTGCTGTT ACTGTTAAGTCTGCTTGGAACCAATTG GACCCAAGACCATACTGTGGATCAG ACAAACTGACTTGGCTTCACTACTGC TATGGTTACTTGGTTCTGTTCTGTT AACGTTAGATTGATCATGCACATGTG GCAAAACAGATCTATCTGCCAAGTG TTAAGGGTTGTCTCCAATGGAAGTTT TGGTTATGGCTAACGGTTGTTGATGG TTTCCCAGTTTGTGCTGGTTGTA CTACGGTAACCTCGGTCAATTGAATC TGCTCTTGACTATCACTCTGTTGTT TTGGTTTGGCATTGGGTACTTGGTT GCTCAAAGATTGGCTGTTAACACAC TGTTGCTGGTTCTGCTAACACTGA CATGGACGACAAGTGGCTTCTGG GTAACGCTACTACTGTTACTCTCTG CTGCTGGTTCGCTGGTTCTGCT CTGCTACTAGATCTAGATTGGCTCTC CAAGACAAAACCTCAATTGTCTACTT CTGTTCTGCTGGTAAGCCAAGAGCTG ACCCAATCGACTTGGAAATTGCAAAGA ATCGACGACGAAGACGACGACTTCTC TAGATCTGGTTCTGCTGGTGGTTGTT AGTTGAAAGATCTATCGAAAGAAGAG AAGAAAGATTGTAG
Botrytis cinerea	WCGRPG QPC	MASNSSNFDPKTQ SITILMADGITVS FTPPLDIDFFYYYYNV ACCINYGAQAGAC LLMFFVVVVLTKA VKRKTLLFVLNVL SLIFGFLRAMLYAI YFLQGFNDFYAAF TFDFSRVPRSSYAS SVAGSVIPLCMTIT	(codon optimized)  ATGGCTTCTAACTCTCTAACTTCGAC CCATTGACTCAATCTATCACTATCTG ATGGCTGACGGTATCACTACTGTTCT TTCACCTCCATTGGACATCGACTTCTC TACTACTACAACGTTGCTGTTGTT AACTACGGTGCTCAAGCTGGTGTGTT TTGTTGATGTTCTGCTGTTGTTGTT TGACTAAGGCTGTTAAGAGAAAGACT

		VNMSLYLQAYTV CKNLDDIKRIILTT LSAIVALLAIGFRF AATVVNSVAILAT SASSVPMQWLVK GTLVTETISIWFFS LIFTGKLWVTLYN RRRNGWRQWSAV RILAAMGGCTMVI PSIFAILEYVTPVSF PEAGSIALTSVALL LPISSLWAGMVTD EETSайдVSNLTGS RTMLGSQSGNFSR KTHASDITAQSSH LDFSSRKGSNATM MRKGSNAMDQVT TIDCVVEDNQANR GLRDSTEMDLEA MGVRVNKSYGVQ KA	TTGTTGTTCGTTTGAAACGTTTGCTCT TGATCTCGGTTCTGAGAGCTATGT TGTACGCTATCTACTCTTGCAAGGTT TCAACGACTCTACGCTGCTTCACTT TCGACTTCTAGAGTTCCAAGATCTT CTTACGCTTCTCTGTTGCTGGTTCTGT TATCCCATTGTGTATGACTATCACTGT TAACATGTCTTGTACTTGCAAGCTTA CACTGTTGTAAGAACCTGGACGACA TCAAGAGAACATCTGACTACTTGT CTGCTATCGTTGCTTGTGGCTATCG GTTTCAGATTGCTGCTACTGTTGTA ACTCTGTTGCTATCTGGCTACTCTG CTTCTCTGTTCCAATGCAATGGTTGG TTAAGGGTACTTGGTTACTGAAACTA TCTCTATCTGGTTCTCTCTTGTATCTT CACTGGTAAGTTGGTTGGACTTTGTA CAACAGAAGAACGGITGGAGA CAATGGTCTGCTGTTAGAATCTTGGCT GCTATGGGTGGTTGACTATGGTTATC CCATCTATCTCGCTATCTTGAATAC GTTACTCCAGTTCTTCCCAGAACGCT GGTTCTATCGCTTGTACTCTGTTGCT TTGTTGTTGCCAATCTCTTGTGG GCTGGTATGGTTACTGACGAAGAAC TTCTGCTATCGACGTTCTAACTGAC TGGTTCTAGAACTATGTTGGTTCTCA ATCTGGTAACTCTCTAGAAAGACTCA CGCTTCTGACATCACTGCTCAATCTC TCACTTGGACTCTCTCTAGAAAGGG TTCTAACGCTACTATGATGAGAAAGG GTTCTAACGCTATGGACCAAGTTACTA CTATCGACTGTGTTGTTGAAGACAACC AAGCTAACAGAGGTTGAGAGACTCT ACTGAAATGGACTTGGAAAGCTATGGG TGTTAGAGTTAACAAAGTCTACGGTGT TCAAAAGGCTTAG
Lodderomyces elongisporous	WMWTRY GRFSPV	MDEAINANLVSGD IIVSFNIPGLPEPVQ VPFSEFDSDFHKDQ LIGVIIILGVTIGACS LLLILLLGMLYKS REKYWKSLLFML NVCILAATILRSGC FLDYYLSDLASISY TFTGVYNGTSFAS SDAANVFKTIMFA LIETSLTFQVYVMF QGTTWKNWGHA VTALSGLLSVASV	(wild type)  ATGGACGAAGCAATCAATGCAAACCT TGTTCCTGGAGATATTATAGTCTCTT TAACATTCTGGTTGCCAGAACCGGT ACAAGTGCCTTCAGCGAATTGATTTC GTTTCATAAAGACCAAGCTCATGGAG TCATCATTCTGGAGTCACTATTGGAG CATGCTCGCTTGTGATATTGCTAC TTGGAATGTTACAAGAGCCGTGAA AAGTATTGAAATCACTATTATATG CTCAATGTATGCATCTGGCTGCCACA ATCTTAAGGAGCGGTTGCTTCTAGAC

		AFQIYTTILSHNNF NATISGTGTLTSGV WMDLPTLLFAASI NFMTILLFLKLM AIRQRRLGLKQF DGFHILFIMFTQTL FIPSILLVIHYFYQA MSGPFIINMALFLV VAFLPLSSLWAQT ANTTKKIESSPSMS FITRRKSEDESPLA ANDEDRLRKFTTT LDLSGNKNNTNN NNNSNNNNMSN INYPSTGLGEDDK SFIFEMEPSRERA IEEIDLGARIDTGL PRDLEKFLVDGFD DSDDGEGMIREV TMLKK (SEQ ID NO:52)	TATTATCTAAGTGATTGGCCAGTATC AGTTATACATTTACTGGAGTATAACAAT GGTACCAAGCTTGCTAGCTCTGACCGC GCAAATGTGTTCAAGACTATTATGTT GCCTTGATTGAAACTTCGTTAACCTTT CAAGTGTATGTCATGTTCAAGGGAC CACTTGAAAAATTGGGCCATGCTG TCACTGCATTATCGGGCTCTGTCTG TTGCCTCAGTGGCGTTCCAGATCTACA CCACGATTTATCCCACAATAATTCA ATGCTACAATCTCGGGAACCGGTACA TTAACCTCAGGTGTTGGATGGACTTA CCAACACTCTGTTGCCAAGTATC AATTTATGACCATTGTTGTTATT AAGTTGGAATGCCATTAGACAAAG AAGGTATTTAGGTTAAAACAGTTG ATGGGTTCCATATCTTATTCACTCATGT TTACCCAAACATTGTCATACCCTCGA TTTGCTTGTGATCCACTACTTTACC AGGCAATGTCTGGACCATTCAATC AACATGGCGTTGTTCTTGGTGGCA TTCTGCCATTGAGTCATTATGGCA CAAAC TGCAAACACTACTAAAAAGAT TGAATCTCGCCAAGTATGAGCTTAT TACTAGACGAAAATCAGAGGATGAGT CACCACTGGCTGCTAACGACGAGGAT AGGTTACGAAAATTCAACCACAAC GGATTGTCGGCAACAAGAACAAATA CAACAAACAATAATAACAATAGCAAC AACATTAACAACAATATGAGCAACAT CAACTACCCCTCTACAGGACTGGGAG AAGACGATAAACCTTTATATTGAG ATGGAACCCAGTCGGAAAGAGCTGC AATAGAAAGAGATTGATCTGGAGCAA GGATCGATACCGGTTGCCCAGAGAT TTAGAGAAATTCTAGTTGATGGGTT GACGATAGTGTGACGGAGAAGGAAT GATAGCCAGAGAAGTGAATATGTTGA AAAAATAG (SEQ ID NO:53)
Penicilliu m rubens	WCGHIG QGC	MATSSPIQPFDPFT QNVTFRLQDGTEF PVSVKALDVFVM YNVRVCINYGCQF GASFVLLVILVLLT QSDKRRSAVFILN GLALFLNSSRLLFQ VIHFSTAFEQVYPY VSGDYSSVPWSAY AISIVAVVLTLVV VCIEASLVIQVHV	(codon optimized)  ATGGCTACCTCTTCCCCAATCCAACCA TTTGACCCATTCAACCAAAACGTTACC TTCCGTTGCAAGACGGTACCGAATTC CCAGTTCTGTCAAGGCTTGGACGTC TTCGTCATGTACAACGTTAGAGTCTGT ATTAACACTACGGTTGTCAATTGGTGCC TCCTTCGTCCTGTTAGTCATTAGTCT TGTAACTCAATCCGACAAGAGAAGA TCTGCTGTCTTCATTITGAACGGTTG

		VCSTLRRRYRHPL LAISILVALVPIGFR CAWMVANCKAI KLTYTNDVWWIES ATNICVTISICFFCV IFVTKLGFAIKQRR RLGVREFGPMKV FVMGCQTMVVPAI FSITQYYVVVPEFS SNVVTLVVISLPLS SIWAGAVLENARR TGSQDRQRRLNL WRALVGGAESLLS PTKDSPTSLSAMT AAQTLCYSDHTMS KGSPTSRDTDAFY GISVEHDISINRVQ RNNSIV (SEQ ID NO:54)	GCTTTGTTCTTGAACCTCTTAGATTG TTGTTCAAGTTATTCACTCTCCACT GCCTCGAACAGTCTACCCATACGTC TCTGGTGACTACTCCTCTGTCCCCTGG TCCGCTTACGCTATCTCCATTGTCGCT GTTGTTTGACTACCTGGTCGTTGTT TGTATCGAAGCTTCTTGTTATTCAA GTTCACGTTGTCTGCCACCTGAGA CGTAGATAACAGACACCCATTATTAGC TATTCTATTGGTCGCTTGGTTCCA ATCGGTTAGATGTGCTGGATGGTC GCTAACTGTAAGGCTATTATTAAATTG ACCTACACCAACGACGTTGGTGGAT CGAATCTGCTACTAACATCTGTGTAC TATCTCATCTGTTCTCTGTGTTATC TTCGTTACCAAGTTGGGTTTCGCCATC AAGCAAAGAAGAAGATTGGGTGTTAG AGAATTGGTCCAATGAAGGTTATT CGTCATGGGTTGTCAAACATGGTTGT TCCAGCTATTCTCCATCACCAATA CTACGTCGTCGCTCCAGAATTCTCCTC TAACGTCGTTACTTGGTTGTCATTTC TTTACCATTATCTCCATTGGGCCGG TGCTGTCGGAAAACGCTAGAAGAA CCGGTCCCAAGATAGACAAAGAAGA CGTAACCTGTGGAGAGCTTGGTTGGT GGTGCAGATCCCTGTTATCCCCAACT AAGGACTCTCCAACCTCTTGTCGCT ATGACTGCTGCTCAAACCTATGTTAC TCTGATCACACCAGTCCAAGGGTTCT CCAACCTCCAGAGACACCGATGTTTC TACGGTATCTCCGTTGAACACGACATC TCCATTAAACAGAGTTCAACGTAACAA CTCCATCGTCTAG (SEQ ID NO:55)
Candida guillermo ndii	KKNSRFL TYWFFQP IM	MKSCSIGFGIPFINE PNFETVSILMDVS FIDADVNPDNILLN FTIPGYQNGFSVP MVVINELQKSQM KYAIVYGCVGAS LILLFVVWILCSRK TPLFIMNNIPLVLY VISSSLNLAYITGP LSSVSVFLTGILTS HDAINVYVASNAL QMLLIFSIQSTMAY HVYVMFKSPQIKY LRYMLVGFLGCLQ IVTTCLYINYNVLY SRRMHKLYETGQT	(codon optimized)  ATGAAGTCCTGCTCCATCGGTTTCGGT ATCCCATTCAATTAAATGAACCAAACCTTC GAAACTGTTCTATTGACCATGGAC GTTTCTTCATTGACGCTGACGTCAAT CCTGACAATATCTGTTGAACCTCACC ATTCCCTGGTTACCAAAACGGTTCTCT GTTCCAATGGTTGTTATTACGAATTG CAAAGTCTCAAATGAAATACGCTAT TGTTTACGGTTGTGGTGTGGTGCCTC CTTGATTTGTTGTTGTCGTCGGATT TTGTGTTCTAGAAAGACTCCATTGTT ATCATGAACAAACATTCCATTAGTTTG TACGTCATCTCCTCTTGAACACTG GCTTACATTACCGGTCCATTGTCCTCT

		YQDGTVMTFVPFI LFQCSVNFSSIFLV LKLIMAIRRRYL GLRQFGGFHILMI VSLQTMLVPSILVL VNYAAHKAVPSN LLSVSMMIIVSL PASSMWAAAANA SSAPSSAASSLFRY TTSDDSDRTLETKS DHFIMKHESHNSS PNSSPLTLVQKRIS DATLELPKELEDLI DSTSI (SEQ ID NO:56)	GTTTCCGTCTTCTTGACCGGTATCTTG ACTTCTCACGATGCCATTAAACGTCGTT TACGCTTCCAACGCTTGCAAATGTTG TTGATCTTCTATCCAATCTACCATG GCCTACCACGTTACGTTATGTTCAAA TCTCCACAAATTAAACTTGAGATAC ATGTTAGTCGGTTCTGGGTTGTTA CAAATTGTCACCACCTGTTATACATC AACTACAATGTTTGTACTCTCGTAGA ATGCACAAATTGTACGAAACTGGTCA AACCTACCAAGATGGTACCGTTATGA CTTTCGTTCATTCACTTGTCCAAT GTTCTGTCAACTCTCTTCTATTITCTT GGTTTGAAGTTGATTATGCCATTAG AACCAGACGTTACTTGGGTTGCGTCA ATTGGTGGTTTCATATTTGATGAT CGTTCTTACAAACTATGTTGGTCCC ATCTATTTGGTTTGGTTAACTACGC CGCTCATAAGGCTGTTCCCTCCAACCT GTTATCTCCGTTCTATGATGATCAT TGTTTGTCTTACAGCTCTTCTATG TGGGCCGCTGCTGCTAACGCCCTTCT GCCCTCCTCCCGCTGCTTCCTCCTG TTCAGATACACCACTCTGATTCCGAT AGAACTTGGAAACTAAATCTGACCA CTTCATCATGAAGCATGAGTCCCACA ACTCTCTCCAAATTCTCCCCATTGA CTTTGGITCAAAGAGAATTCTGATG CCACCTAGAATTACCAAAAGAGTTA GAAGACTTGATCGACTCCACCTCCATC TAG (SEQ ID NO:57)
Candida tropicalis	KFKFRLTRYGWFSPN	MDINNTIQSSGDIII TYTIPGIEEPFELPF EVLNHFQSEQSKN CLVMGVMIGSCSV LLIFLVGILFKTNK FSTIGSKNLSKNF LFYLNCLITFIGIIR AACFSNYLLGPLN SASFAFTGWYNGE SYASSEAANGFRV ILFALIETSMVFQV FVMFRGAGMKKL AYSVTILCTALAL VVVGFQINSAVLS HRRFVNTVNEIGD TGLSSIWLDPIL FSVSVNLMCSVLLI GKLIMAIKRRYL GLKQFDHFVLLIC	(codon optimized) ATGGACATCAACAACACCATCCAATC TTCCGGTGACATCATCATTACCTACAC CATCCCAGGTATCGAAGAAACCATTG AATTGCCATTGGAAGTTTGAACCAACT TCCAATCTGAACAATCCAAGAACTGT TTGGTCATGGGTGTTATGATCGTTCT TGTTCGTTTGTGATCTCTTGGTCG GTATTTGTTCAAACCAACAAATTCT CTACTATTGGTAAGTCTAAGAACTTGT CTAAGAACITCTGTTACTTGAAC GTTTGATCACCTTCATCGGTATCATTC GTGCTGCCTGTTTCTAACTACTTGT TGGGTCCATTGAACCTGCTTCTTCG CTTCACTGGITGGTACAACGGTGAAT CTTACGCTTCTCCGAAGCTGCTAACG GTTTCAGAGTCATCTGTTGCTTGA TTGAAACTCTATGGTCTTCCAAGTT

		STQTLVPSLILFV HYFLFFRNANVML INISILLVMLPFS SLWAQTANTTQYI NSSPSFSFISREPSA NSTLHSSSGHYSE KSYGINKLNTQGS SPATLKDDHNSVI LEATNPMSGFDAQ LPPDIARFLQDDIRI EPSSTQDFVSTEVT YKKV (SEQ ID NO:58)	TCGTTATGTTCAGAGGTGCTGGTATGA AAAAGTTGGCTTACTCCGTTACCATTT TGTGTACCGCTTGGCTTGGTCGTG TTGGTTCAAATTAACCTCCGCTGTCT TATCTCACAGAACAGATTGGTCAACACC GTTAACGAAATTGGTGATACTGGTTG TCCTCCATTGGTGGACTGCCAAC ATCTTGTCTCCGTCTGTCAACTTA ATGTCTGTTTGTGATCGGTAAATTG ATCATGGCTATTAAGACTAGAACATA CTTGGGTTGAAACAATTGATTCCCT CCACGTTTGTAAATTGTTCCACTCA AACTTGTGGTCCCACATTTAATCTT GTTCGTTCACTACTTCTTGTCTTAG AAACGCCAACGTTATGTTGATTAACA TTTCCATCTGTTGATCGTCTTGATGTT GCCATTCTCTCCTGTGGGCTCAAAC CGCCAACACCACCCAAATACATCAACT CTTCCCCATCCTCTCTTCATCTCTAG AGAACCATCTGCTAACTCTACTTGC CTCCTCTCCGGTCACTACTCTGAAAA GTCCTACGGTATTAACAAATTGAACA CCCAAGGTTCTCCCCAGCCACCTAA AGGATGATCACAACCTCCGTATCTG GAAGCTACCAACCCAAATGCTGTTTC GACGCCAATTGCCACCAGACATTGC TAGATTCTGCAAGATGACATCAGAA TTGAACCATCTTCTACCCAAAGATITCG TTTCCACTGAAGTCACCTACAAGAAC GTCTAG (SEQ ID NO:59)
Candida parapsilosiss	KPHWTT YGYYEPQ	MNKIVSKLSSSDVI VTVTIPNEEDGTY EVPFYAIDNYHYS RMENAVVLGATIG ACSMLLIMLIGILF KNFQRLRKSLLFNI NFAILLMLILRSAC YINYLMNNLSSISF FFTGFDDESFMSS DAANAFKVILVAL IEVSLTYQIYVMFK TPMLKSWGIFASV LAGVLGLLATLATQ IYTTVMSHVNFVN GTTGSPSQVTSAW MDMPTILFSVSINV LSMFLVCKLGLAI RTRRYLGLKQFDA FHILFIMSTQTMIIIP SIILFVHYFDQNDS	(codon optimized)  ATGAACAAAGATTGTCCTCCAAGTTGTCT TCTTCTGACGTACCGTTACCGTCACC ATCCCAAACGAAGAACGATGGTACTTA CGAAGTCCCATTCTACGCTATTGACAA CTACCAACTCTCCGTATGGAAAACG CTGTTGTTTGTGCTACCATGGTG CTTGTCTATGTTGATCATGTTGA TTGGTATTTGTTCAAGAACATTCCAAA GATTGAGAAAGTCTTGTGTTCAACA TCAACTCGCTATCTTATTGATGTTGA TTTGAGATCCGCTTGTACATCAACT ACTTGATGAACAACTTGCTTCCATT CTTTCTCTCACCAGGTATTCGATG ATGAATCTTCATGTCTCCGACGCTG CCAACGCCCTCAAGGTTATCTGGTTG CCTTGATTGAAGTTCTGACCTACC AAATTACGTTATGTTCAAGACCCAA TGTTGAAGTCCTGGGGTATTCGCT

		QTTLVNISLLLVI SLPLSSLWAQTN NVRRIDTSPSMSFI SREASNRSGNETL HSGATISKYNTSN TVNTTPGTSKDDS LFILDRSIPEQRIVD TGLPKDLEKFINN DFYEDDDGGMIARE VTMLKTAHNQNQ (SEQ ID NO:60)	CTGTCTTGGCCGGTGTGTTGGGTTGG CTACTTTGGCTACCCAAATCTACACTA CCGTTATGTCTCACGTTAACCTCGTCA ACGGTACCACCGGTTCTCCATCTCAAG TTACTTCCGCTTGGATGGACATGCCAA CTATCTTATTCTCCGTTCTATTAAACGT TTTGTCTATGTCTTGGTTGTAAGTT GGGTTTGGCCATCAGAACCAGACGTT ACTTGGGTTAAAGCAATTGACGCTT TCCACATTTATTCAATTGTCCACTC AAACCATGATCATTCCATCCATCATCT TGTTGTTCACTACTTCGATCAAACAG ACTCTCAAACACACCTGGTCAACATCT CTTTGTTATTGGTCGTCTTCCTTGCC ATTGTCTTCTTGTCGGCTCAAACACTG TAACAACGTTAGAAGAATTGACACTT CTCCATCCATGTCCTTCATCTCTAGAG AAGCTTCCAACAGATCTGGTAACGAA ACCTTGCACTCTGGTGCTACTATCTCT AAGTACAACACCTCCAACACCGTTAA CACTACCCCAGGTACTTCTAAGGATG ACTCTTGTTCACTTGGACAGATCCA TTCCAGAACAAAGAATTGTCGACACT GGTTGCCAACAGGACTTGGAAAAGTT CATTAACAAACGATTTCACGAAGACG ATGGTGGTATGATTGCCAGAGAAGTC ACCATGTTGAAGACCGCTCACAAACAA CCAATAG (SEQ ID NO:61)
Geotrichum candidum	GDWGF WYVPRP GDPAM	MAEDSIFPNNSTSP LTNPIVVETIKGTA YIPLHYLDDLQYE KMLLASLFSVRIA TSFVVIWYFVAV NKAKRSKFLYIVN QVSLLIVFIQSILSL IYVFSNFSKMSTIL TGDYTGITKRDIN VSCVASVFQFLFIA CIELALFIQATVVF QKSVRWLKFSVSL IQGSVALTTALY MAIIVQSIYATLNP YAGNLIKGRFGYL LASLGKIFFSISVTS CMCIFVGKLVFAI HQRRRTLGIKFQFDG LQLIVIMSTQSMIIP TIIVLMSFLRRNAG SVYTMATLLVALS LPLSSLWAEAKTT	(codon optimized)  ATGGCCGAAGACTCCATCTCCAAA CAACTCCACCTCTCCATTGACCAACCC AATTGTTGTTGAAACCATTAAAGGGTA CCGCTTACATTCCATTACACTACTTGG ATGATTGCAATACGAAAAGATGTTG TTGGCTCCTTGTCTCCGTTAGAATT GCTACTTCCCTCGTTGTTATTATTTGGT ACTTCGTCGCTGTCAACAAGGCTAAG AGATCTAAGTTTTGTACATTGTCAAC CAAGTTCTTGTTGATCGTTTATCC AATCCATTGTCTTGTATTACGTCTT CTCCAACCTCTCCAAGATGTCTACCAT TTTGACCGGTGATTACACCGGTATCAC TAAGAGAGACATTAACGTCTTGTGT TGCCTCCGTTTCCAATTCTGTTCATC GCTTGTATCGAATTGGCTTGTTCATC CAAGCTACTGTCGTTTCCAAGAAATCT GTTAGATGGTTGAAGTTTCCGTTCT TTGATCCAAGGTTCCGTCGCTTGACT ACTACCGCCTGTACATGCCATTATT

		RDSASYTAYRPSG SPNNRSLFAIFSDR LACGSGRNNRHD DDSRGNGSVNAR KADVESTIEMSSC YTDSPTYSKFEAG LDARGIVFYNEHG LPVVSGEVGGSSS NGTKLGSGHKYE VNTTVVLSDVDSP SPTDVTRK (SEQ ID NO:62)	GTCCAATCCATCTACGCTACTTTGAAC CCATACGCTGGTAACITGATTAAAGG TCGTTTCGGTTACTTATTAGCTTCTTG GGTAAGATTTCTCTCTATTCTGTGTT ACTTCTTGTATGTATCTCGTTGGT AAGTTGGTCTTGCTATTACCAAAAGA AGAACCTTGGGTATTAAGCAATTGCA CGGTTGCAAATTGGTCATTATGTC TACTCAATCCATGATCATCCAACTAT TATCGTCTTGATGTCTTTGAGACG TAACGCTGGTTCTGTTACACCATGGC TACCTTGTGGTCGCTTGTCCCTGCC ATTGTCCCTGTTGGGCTGAAGCAA GAECTACCAGAGACTCTGCTTCTACAC CGCTTACAGACCATCTGGTCTCCAAA CAACCGTTCTTGTTCGCCATCTCTC TGATAGATTGGCTGTGGTCTGGTAG AAACAACAGACACGATGATGATTCTA GAGGTAACGGTCTGTTAACGCCAGA AAGGCTGACGTCGAATCTACTATCGA AATGTCCTCTGTTACACTGATTCCCC AACCTACTCCAAGTCGAAGCTGGTT GGACGCTAGAGGTATCGTCTTCTACA ACGAACACGGTTGCCAGTTGTCTCCG GTGAAGTTGGTGGTCTCCTCCAACG GTACTAAGTTGGGTCTGGTCATAAGT ACGAAGTCAACACTACTGTTGTTTG CTGATGTTGACTCTCCATCTCCAACCG ACGTCACCCGTAAGTAG (SEQ ID NO:63)
Zygosacch aromyces bailii	HLVRLSP GAAMF	MSGLANNTSYNPL ESFIIFTSVYGGDT MVKFEDLQLVFTK RITEGILFGVKVGA ASLTIMVWMISR RRTSPIFIMNQLSL VFTILHASFYFKYL LDGFGSIVYTLTF PQLITSSDLHVFAT ANVVEVLLVSSIE ASLVFQVNVMFA GSNHRKFAWLLV GFSLGLALATVAL YFVTAVKMIASAY ASQPPTNPIYFNVS LFLLAASVFLMTL MLTVKLILAIRSRR FLGLKQFDSFHILL IMSCQTLIAPSPLY ILGFILDHRKGND	(codon optimized)  ATGTCTGGTTGGCTAACAAACACCTCT TACAACCCATTGGAATCTTCATTATT TTCACCTCTGTTACGGTGGTGTAC ATGGTTAAGTCGAAGACTTGCAATT AGTCTTCACCAAGCGTATTACTGAAG GTATTTGTTGGTGTCAAGGTTGGTG CCGCTTCTTGACTATGATTGTTATGT GGATGATTCCAGAAGAAGAACCTCC CCAATCTTCATCATGAACCAATTGTCT TTGGTTTCACCATCTGCACGCTTCT TTTACTTTAAGTACTTATTGGACGGT TTCGGTTCTATTGTCTACACTTTGACC TTGTTCCCACAATTAAATTACTTCCTCT GACTTGCACGTTTCGCTACTGCTAAC GTTGTTGAAGTCTTATTGGTTCTCC ATCGAAGCCTCTTGGTTCTAACAC AACGTCATGTTCGCTGGTTCTAACAC AGAAAGTTCGCTTGGTTGTTGGTCGGT

		YLITVAQLLVLS LPLSSMWATTAND ASSGTSMSKESV YGSDSLYSKSKCS QFRTFMNRFSTK PTKNDEISDAFVA VDSLEKNAPQGIS EHVCEFPQSLS QATSISSRKKEAV VYASTVDEDKGFS SSDINGYVTNMP LASAASANCENSP CHVPRPYEENEG VETRKIILKKNVK W (SEQ ID NO:64)	TTCTCTTGGGTTGGCTTGGCCACT GTCGCTTGACTTCGTTACTGCTGTC AAGATGATCGCTCCGCTTACGCTCT CAACCACCAACTAACCCAATCTACTTC AACGTTCTTGTCTTGTGGCTGCC TCCGTTTCTTGATGACTTTAATGTTG ACCGTCAAGTTGATCTGGCTATCAGA TCCAGAAGATTCTGGGTTGAAGCA ATTGACTCTTCCACATTGTTGAT TATGCTTGTCAAACTTGATCGCTCC ATCTGTTTGTACATCTGGGTTTATT TTGGATCACAGAAAGGGTAACGACTA CTTGATTACCGTCGCTCAATTGTTGGT CGTTTGTCTTGCCTATTGCTCCAT GTGGGCCACTACTGCTAACGATGCTTC CTCCGGTACTTCTATGTCTTCCAAGGA ATCCGTCTACGGTTCTGATTCTTATA CTCTAAAGTCTAAAGTGTCCCAATTAC CAGAACCTTCATGAACAGATTCTCTAC TAAGCCAACTAAGAACGACGAAATT CTGATTCCGCTTCGTCGCTGTTGATT CCTTGGAAAAGAACGCTCCACAAGGT ATCTCTGAACACGTTGTGAATTCCA CAATCTGACTTATCTGATCAAGCTACT TCCATCTCCTCCAGAAAAAGGAAGC TGTGTTTACGCTCCACTGTTGATGA AGATAAGGGTTCTTCTCCTCTGACAT CAACGGTTACACTGTTACCAACATGC CATTGGCTTCCGCTGCTTCTGCTAACT GTGAAAACCCCCATGTCACGTTCCA AGACCATACGAAGAAAACGAAGGTGT CGTCGAAACCAAGAAAAATTATTTGA AGAAGAACGTCAAATGGTAG (SEQ ID NO:65)
Zygosacch aromyces rouxii	HFIELDP GQPMF	MSEINNSTYNPMN AYVTFTSIYGDDT MVRFKDVELVN KRVTEAIMFGVKV GAASLTLIIMWMIS KKRTTPIFIINQSSL VFTIIHASLYFGYL LSGFGSIVYNMTSF PQLISSNDVRVYA ATNIFEVLLVASIEI SLVFQVKVMFAN NNGRRWTWCLM VVSIGMALATVGL YFATAVELIRAAY SNDTVSRHVFYNV SLILLASSVNLMTL	(wild type)  ATGAGTGAGATTAACAATTCTACCTA CAATCCAATGAATGCATATGTAACGT TTACATCAATATATGGTGATGATACTA TGGTACGTTCAAAGATGTGGAATTG GTAGTTAACAAAAGGGTACAGAAC CATTATGTTCCGGCGTCAAAGTTGGTGC AGCTCGTTGACACTCATCATCATGTG GATGATCTCTAACGAAAAGAACAC CGATATTATCATAATCAGTCTTCGC TTGTATTACCATATAACATGCTTCGC TTTATTGTTGGGTACCTTTGTCAGGAT TTGGTAGTATAGTTACAATATGACAT CGTTCCCGCAGTTAATAAGCTCCAATG ACGTTCGTGTGTACGCAGCTACAAAT

		MLVVKLVLAIRSR RFLGLKQFDASFHIL LIMSCQTLIAPSILF ILGWTLDPHTGNE VLITVGQLLIVSL PLSSMWATTANNT SSSSSVSCNDSSF GNDNLCSKSSQFR RTFMNRFRPKSVN GDGNSENTFVTID DLEKSVFQELSTP VSGESKIDHDHAS SISCQKTCNVHA STVNSDKGSWSSD GSCGSSPLRKTSV NSEDLPPHILSAYD DDRGIKESKKIILK KL (SEQ ID NO:66)	ATTTTGAGGTCTGTTGGTAGCATCT ATCGAAATCTCTCTGGTTTCAGGTC AAAGTTATGTTGCCAACATAATGG TCGAAGATGGACTTGGTGTGATGGT AGTTCCATAGGGATGGCACTAGCTA CTGTAGGACTTATTTGCCACTGCCG TTGAGTTGATCAGAGCTGCTTACAGC AATGATACTGTTAGCCGCCATGTTTT TACAATGTTCTGATCTACTAGCG TCATCTGTCATCTAATGACACTAATG CTAGTGGTAAAATTAGTATTAGCGAT CAGATCAAGAAGATTTGGGTTAA AACAGTTGACAGTTCCACATATTAC TTATAATGTCCTGCCAGACTCTAATAG CACCTCCATTCTATTCAATTGGGTT GGACCTTAGACCCTCATACTGGTAAT GAGGTTTAATTACAGTTGGTCAATTG CTAATAGTACTGTCATTACCGCTGTCA TCTATGGGCTACAACCGCTAACAA TACCAAGTTCATCTAGTAGTTGGTGTGTC CTGTAATGACAGCTTTGGTAATGA CAATCTCTGTTCCAAGAGAGTCGCAATT TAGAAGAACTTTATGAATAGATTCC GTCCCAAGTCGGTTAATGGTACGGT AATTCTGAAAATACCTTGTACAATT GATGATTGGAAAAAGCGTTTCA AGAATTATCAACACCTGTTAGCGGAG AATCAAAGATAAGATCATGATCATGCA AGTAGTATTTCATGTCAAAAGACATG TAATCATGTTCATGCTTCGACAGTGAA TTCAGATAAGGGATCTGGTCCTCTGA TGGTAGTTGTGGCAGTTCTCCGTTAAG AAAGACTTCCACCGTTAATTCTGAAG ATTACCTCCACATATATTGAGCGCCT ACGATGACGATCGAGGTATAGTAGAA AGTAAAAAAATTATCCTAAAGAAATT ATAG (SEQ ID NO:67)
Kluyveromyces lactis	WSWITLR PGQPIF	MSEEIPSLNPLFYNT ETYNPLQSVLTYSI SIYGDGTEITFQQL QLVHENITQAIIF GTRIGAAGLALIIM WMVSKNRKTPIFII NQSSLVLTIVQSAL YLSYLLSNFGGV FALTLFPQMIGDR DKHYGAVTLIQC LLVACIEVSLVFQ VRVIFKADRYRKI GIILTGVVSASFGAA	(wild type)  ATGTCAGAAGAGATAACCCAGTTGAA CCCATTGTTCTACAATGAGAGACATATAA TCCATTGCACTCCGTCCTAACATACAG TTCAATTACGGAGATGGGACTGAAA TAACATTCAACAGCTACAAAATCTTG TCCATGAAAACATCACCCAAGCAATT ATTGGAAACAAGGATCGGCGCTGC TGGATTAGCGTTGATTATAATGTGGAT GGTCTCTAAGAATAGAAAGACGCCGA TATTCTATAATAATCAGAGTTCTTGG TTCITACAATTGTTCAATCTGCTTAT

		TVAMWMITA KSII VVYDSPLNKVD TY YYNIAVILLAC SIN FITLLSVKLFLAF RARRHLGLKQFDS FHILLIMSTQTLIG P SVLYILAYALNNK GVKSLSIATLLVV LSLPLTSIWAAA NDAPSASTFYRQF NPYSAQNRRDSSS YSYGKA FSDKYSF SNSPQTSDGCSSKE LELSTQLEMDLES GESFMDRAKRSDF VSSPGSTDATVIK Q LKASNIYTSETDA DEEARAFWVN AIH ENKDDGLMQSKT VFKE LR (SEQ ID NO:68)	ATCTATCATATTGTTGAGCAATTG GAGGAGTTCCCTTGCTCTAACATTG TCCCACAGATGATAGCGACC GTGAC AAACATCTTACGGTGCCGTGACTCTA ATTCAATGTCTATTGGTGCGTGATT GAGGTCTCGTTAGTC TT CAGGTAAGA GTCATTTC AAGCAGATAGATATA GAAGATAGGAATCATT TGACTGGCG TCTCCGCTAGTTGGTGCTGCAACTG TAGCCATGTGGATGATT ACTGCAATA AAATCTATTATTG TAGTGTATGATAGT CCATTGAACAAAGTTGACACATATTA TTACAACATAGCAGTTATT TACTTGC ATGTTCAATAAATT CATCACTCTTCT TCTATCAGTGAAACTTTCCCTGGCTT CAGAGCTAGGAGACATTAGGTTGA AACAA TT GACTCATTCACATTCTAC TCATCATGTCTACTCAGACATTAATAG GTCCATCGGTTTGATATTCTCGCCT ACCGCCTGAACAATAAAGGAGTTAAG TCGTTGACTTCTATTGCTACATTGCTT GTAGTTCTTCCCTACCTTGACATCT ATCTGGGCTGCTGCTGCAAATGATGC ACCAAGTGCCAGTACTTCTATCGCCA ATTCAACCC TTACTCTGCACAAATCG TGATGATT CATCATCCTACTCTTATGG TAAAGCCTTACTGACAAATACTCTT CAGTAACTCACCACAAACTCGGATG GTTGTAGTTCAAAGGAACTGAACTA TCTACACAGTTGGAGATGGATTAGA GTCTGGCGAATCTTATGGATAGAGC AAAAAGGTCCGATTTGTTCTTCTCC AGGATCAACAGATGCAACAGT GATTA AACAA TTGAAAGCTTCCAACATCTAT ACCTCAGAAACAGATGCTGATGAAGA GGCAAGGGCATT TGGGTGAATGCAA TTCATGAAAACAAAGATGACGGTTA ATGCAATCGAAAACCGTATTCAAAGA ATTAAGATAG (SEQ ID NO:69)
Schizosac charomyce s pombe	TYADFLR AYQSWN TFVNPDR PNL	MRQPWWKDFTIP DASAIHQNITIVSI VGEIEVPVSTIDAY ERDRLLTGMTLSA QLALGVLTILMVC LLSSSEKRKH PVF VFNSASIVAMCLR AILNIVTICNSNSYI LVNYGFILNMVH MYVHFVNILLLL APVIIFTAEMSMMI	(wild type)  ATGAGACAACC ATGGTGAAAGACTT TACTATTCCCGATGCATCCGCAATTAT TCACCAAAATATTACCAT TGTCTCTAT TGTAGGAGAGATTGAAGTGCCAGTT CAACAATTGATGCATATGAAAGAGAT AGACTTTA ACTGGAATGACTTGTCT GCCCAACTTGCTT TAGGAGTCCTTACC ATTTGATGGTTGTCTATTGT CATCA TCCGAAAACGAAAACACCCAGTTT

		<p>QVRIICAHDRKTQ RIMTVISACLTVLV LAFWITNMCQQIQ YLLWLTPLSSKTIV GYSWPYFIAKILFA FSIFHSGVFSYKLF RAILIRKKIGQFPF GPMQCILVISQCQL IVPATFTIIDSFIHT YDGFSSMTQCLLII SLPLSSLWASSTAL KLQSMKTSSAQGE TTEVSIRVDRTFDI KHTPSDDYSISDES ETKKWT (SEQ ID NO:70)</p>	<p>TGTTTTAATTCCGGCAAGTATTGTTGC AATGTGTCTCGGGCCATTGAATAT AGTGACCATATGCAGCAATAGCTACA GTATCCTGGTTAATTACGGGTTATCT TAAACATGGTTCATATGTATGTCCATG TGTTTAATATTAAATTGTTGCTTGC ACCGGTACATCATTACTGCTGAGAT GAGCATGATGATTCAAGITCGTATAA TTTGTGCACATGATAGAAAGACACAA AGGATAATGACTGTTATTAGTGCCTGC TTAACCTGTTGGTCTCGCATTGG ATTACTAACATGTGTCAACAGATTCA GTATCTGTTATGGTTAATTCCACTTAG CAGCAAGACCATTGTTGGATACTCTTG GCCCTACTTTATTGCTAAAATACTTT TGCTTTAGCATTATTTCACAGTGG TGTTTTTCATACAAACTCTTCGTGC CATATTAATACGGAAAAAAATTGGGC AATTCCATTGGTCCGATGCACTGTA TTTGTAGTTATTAGCTGCCAATGTCTTA TTGTTCCAGCTACCTTACTATAATAG ATAGTTTATCCATACGTATGATGGCT TTAGCTCTATGACTCAATGTCTGCTAA TCATTCTCTTCCCTCTTCGAGTTATG GGCGTCTAGTACAGCTCTGAAATTGC AAAGCATGAAACTTCATCTGCGCAA GGAGAAACCACCGAGGTTGATTAG AGTTGATAGAACGTTGATATCAAAC ATACTCCCAGTGACGATTATTCGATT CTGATGAATCTGAAACTAAAAAGTGG ACGTAG (SEQ ID NO:71)</p>
Vanderwal tozyma polyspora (receptor 1)	WHWLEL DNGQPIY	<p>MSSQSHPPLIDL F DSSYDPGESLIYY T SIYGNNTYITFDEL QTIVNKKVTQGILF GVRCGAFLMLV AMWLISKNRSRI FITNQCCLVFMIM HSGLYFRYLLSRY GSVTFILTGFQQLL TRNDIHIYGATDFI QVALVACIELSLIF QIKVIFAGTNYGK LANYFITLGSL LGL ATFGMYMLTAING TIKLYNNEYDPNQ RKYFNISTILLASSI NMILTLILKLVAA IRTRRYLGLKQFD SFHILLIMSTQTLII</p>	<p>(wild type)</p> <p>ATGAGTTCCAATCACACCCACCGCT AATCGATTATTACGATTCCAGTTA TGACCCGGTCAAAGTTAATTATTA CACATCCATCTATGGTAATAATACATA CATAACTTTGATGAACCTCCAGACGAT AGTGAACAAGAAGGTACACAAAGGT TCTTATTGGTGTCAAGATGTGGTGT CTTCCCTGATGTTGGTAGCAATGTGGT TGATTTCAAAAATAAAAGATCTAGA ATTTTCATTACCAACCAATGTTGT GTCTTCATGATAATGCATTCTGGTCTT TATTTAGGTACCTGCTTCAAGGTAC GGTCAGTTACTTCATTCTAACAGGG TTCCAACAACTGCTTACAAGAAATGA CATTCAATTATGGAGCTACTGATT TATCCAAGTAGCTTGGTAGCTTGCAT AGAATTATCTCTTATTTCACAAATAAA</p>

		PSILFILSYSLRED MHTDQLIIIGNLIV VLSPLSSMWASS LNNSSKPTSLNTDF SGPKSSEEGLTAISL LSQNMEPSIVTKY TRRSPGLYPVSVG TPIEKEASYTLFEA TDIDFESSSNDITR TS (SEQ ID NO:72)	AGTGATATTCGCTGGTACAAACTATG GTAAGITGGCTAATTATTTCATCACTC TAGGTTCATATTGGGTTAGCCACCT TTGGTATGTACATGCTTACTGCTATT ACGGTACAATAAAATTATACAATAAC GAATATGACCCAAACCAAAGGAAATA CTTTAACATTCTACAATATTGCTTGC ATCATCAATTAAATATGCTAACGCTGAT ACTTATATTGAAGCTGGTGGCAGCAA TTAGAACAAAGACGTTACTTAGGTTG AAGCAATTGATAGTTTCACATCCTA TTAACATGTCGACTCAAACATTAATA ATTCCCTCTATCTTATTATTCTATCAT ACAGTTGAGAGAGGATATGCATACT GATCAATTAAATAATCATCGGAAATCT GATCGTGGTATTGTCATTACCATTGTC CTCAATGTGGGCTCGTCTAAACAA TTCAAGTAAACCTACATCTTGAATAC TGATTCTCAGGGCCAAAATCAAGTG AAGAAGGGACAGCAATAAGTTGCTA TCACAAAACATGGAACCACATCAATAGT CACTAAATATACAAGAAGATCACCTG GGTTATACCCAGTAAGCGTGGGTACA CCAATTGAAAAAGAAGCATCATAACAC TCTTTGAAGCTACTGACATTGATT TGAAAGCAGTAGTAACGATATCACAA GGACTTCATAG (SEQ ID NO:73)
Vanderwal tozyma polyspora (receptor 2)	WHWLRL RYGEPIY	MSGIDDMGDKPDI LGLFYDANYDPGQ GILTFISMYGNNTI TFDELQLEVNSLIT SGIMFGVRCGAAC LTLLIMWMISKNK KTPIFIINQCSLILII MHSGLYFKNILSN LNSLSYILTGTQN ITKNNIHVFGAANI IQVLLVATIELSLV FQIRVMFKGDSFR KAGYGLLSIASGL GIATVVVMYFYSAI TNMIAVYNQTYNS TAKLFNVANILLST SINFMTVVLIVKLF LAVRSRRYLGKQ FDSFHILLIMSCQT LIVPSILFILSYALS TKLYTDHLVVIAT LLVVLSPPLSSMW ASAANNSPKPSSFT	(wild type)  ATGTCAGGAATTGATGATATGGGTGA TAAACCAGATATTAGGTTATTITA TGATGCTAACTATGATCCAGGTCAAG GTATACTCACATTATTCATGTACG GGAATACTACTATAACTTTGATGAGT TACAGTTAGAGGTCAATAGTTAATTA CAAGTGGTATTATGTTCGCGTCAAGAT GTGGTGCTGCTTGTGACATTGTTAA TAATGTGGATGATTCTAAGAATAAG AAGACTCCAATTATTATTAATCAA TGCTCGCTAATCCTATTATTATGCAT TCAGGTTATATTAAAGAATATTCTA TCAAATTGAATTCTTATCATATATC TTAACTGGGTTACTCAAATATCACT AAAAATAATATACATGTCCTGGTGC GCTAATATTATTCAAGTTTATTAGTA GCAACCATTGAACTGTCGTTAGTGT CAAATTGAGTCATGTTAAAGGTGA CAGTTTAGAAAAGCTGGTACGGTT GTTGTCATTGCGTCTGGTTGGGTAT AGCTACTGTCGTATGTATTACTC

		TDYSNKNPSDTPS FYSQSISSSMKSKF PSKFIPFNFKSKDN SSDTRSENTYIGNY DMEKNGSPNHSYS SKDQSEVYTIGVSS MHTDIKSQKNISG QHLYTPSTEIDEA RDFWAGRavnns VPNDYQPSELPAI LEELNSLDENNEG FLETKRITFRKQ (SEQ ID NO:74)	TGCCATTACAAATATGATTGCTGTTA TAATCAAACCTACAACACTCCACTGCTAA ATTATTAAACGTTGCAAACATTCTTCT GTCTACATCGATAAAATTATGACGGT AGTATTAATTGTTAAATTATTTGGC TGTTAGATCAAGAAGATATTGGGTT AAAGCAGTTGATAGTTCCATATTT ATTGATTATGTCATGTCAAACATTGAT TGTACCATCAATTCTTTATCTTAC ATACGCTTAAGTACTAACGCTGTACAC TGATCATTAGTTGTCATTGCAAAC ATTAGTCGTTCTATCTTACATTATC TTCGATGTGGGCAAGCGCTGCAAATA ATTCTCCTAAACCAAGCTCGTTACAA CCGATTATTCAAACAAGAATCCTAGT GACACACCAAGCTCTACAGTCAAAG TATTAGTCCTCGATGAAAAGCAAATT CCCAAGCAAATTCAACCCCTCAATT CAAGTCTAAAGACAATTCTTCTGACA CTAGATCAGAAAATACATATATTGGC AATTATGACATGGAAAAGAATGGATC ACCAAATCACTCTTATTCTCAAAGA TCAAAGTGAAGTTACACTATAGGTG TAAGCTCTATGCACACAGATATAAAG TCACAAAAGAATATCAGTGGACAGCA TTTATATACCCCAAGTACAGAGATTG ATGAAGAAGCTAGAGACTCTGGCG GGCAGAGCTGTTAATAATTCAAGTCC AAATGACTATCAACCCTGAGTTAC CAGCATCGATTCTGAAGAATTGAATT CACTGGATGAAAATAATGAAGGTTTC TTGGAGACAAAAAGAATAACATTAG AAAACAATAG (SEQ ID NO:75)
Scheffersomyces stipitis	WHWTSY GVFEPG	MDTSINTLNPNII VNYTLPNDPRVIS VPGAFDEYVNQS MQKAIHGVSIGSC TIMLLIILIFNVKRK KSPAFYLNSTVLT AMIIIRSALNLAYLL GPLAGLSFTFSGLV TPETNFSVSEATN AFQVIVVALIEAS MTFQVFVVFQSPE VKKLGIALTSISAF TGAAAVGFTINSTI QQSRIYHSVNGT PTPTVATWSWVR DVPTILFSTSvnIM SFILILKLGFIAIKTR	(wild type)  ATGGATACTAGTATCAATACTCTCAAC CCTGCGAATATCATTGTCAACTACACC TTGCCAAATGATCCTAGAGTAATTAGT GTCCCATTGGAGCTTGTACGAATAT GTTAACCAATCTATGCAAAGGCCAT TATCCATGGAGTTCCATTGGTTCATG CACCATATACTGCTTTAATTATTTGAT CTTCAATGTCAAACGCAAGAAGTCGC CAGCTTCTATCTTAATTGGTTACGT TGACTGCAATGATTATTCGGTCTGCTC TTAATTGGCATATTGCTAGGTCCTT TGGCTGGATTAAGTTACGTTCTCCG GCTTGGTAACCTCAGAAACCAATTCT CTGTCTCTGAAGCCACCAATGCTTCC AGGTTATTGTTGCTCTATCGAGG

		RYLGLRQFGSLHIL LMMATQTLLAPSI LILVHYGYGTSLN SQLILISYLLVVLS LPVSSIWAATANN SPQLPSSATLSFMN KTTSHFSES (SEQ ID NO:76)	CGTCCCATGACATTTCAGGTGTTCGTCG TCTTCCAATCACCAAGAAGTGAAGAAG TTGGGTATAGCTCTTACCTCCATATCT GCATTACGGGTGCTGCTGCTGTAGG ATTTACTATCAATAGTACAATCCAACA ATCGAGAATTATCATTCAAGTGTCAA TGGAACCTACGCCAACGGTCGCTA CCTGGTCTGGGTTAGAGATGTGCCTA CGATACTTTTCTACTTCGGTTAAC TAATGTCTTCATCTGATTCTCAAGT TAGGGTTGCCATAAAGACAAGAAGA TACCTTGGCCTTCGGCAATTGGCAGT TTGCACATCTTATTGATGATGGCTACT CAAACATTATTGGCCCCATCTATTCTC ATTCTGTACATTACGGATATGGCACA TCTCTGAATAGCCAGCTCATTCTTATA AGTTACTTGCTTGTGTTGTCITTAC CAGTATCCTCTATCTGGCAGCAACA GCCAACAAATTCTCCTCAACTCCATCT TCCGCAACTCTTCATTATGAACAAA ACGACCTCTCACTTTCTGAAAGCTAG (SEQ ID NO:77)
Schizosaccharomyces japonicus	VSDRVK QMLSHW WNFRNP DTANL	MYSWDEFRSPPKQ AEVLNQTVTLETI VSTIQLPISEIDSME RNRLLTGMTVAV QVGLGSFILVLMCI FSSSEKRKKPVFIF NFAGNLVMTLRAI FEVIVLASNNYSIA VQYGFAGFAAVRQ YVHAFNIIILLGPF ILFIAEMSLMLQVR IICSQHRPTMITTT VISCIFTVVTLAFW ITDMSQEIAYQLFL KNYNMKQIVGYS WLYFIAKITFAASII FHSSVFSFKLMRAI YIRRKIGQFPFGPM QCIFIVSCQCLIVP AIFFLIDSFTHTYD GFSSMTQCLLIISL PLSSLWATHTAQK LQTMKDNTNPPSG TQLTIRVDRTFDM KFVSDSSDGFSFE KTEETLP (SEQ ID NO:78)	(codon optimized)  ATGTACTCCTGGGACGAATTCA CCC AAACCGTTACCTTGGAAACTATTGTT CCACCATTCAATTGCCAATCTCTGAAA TTGACTCCATGGAAAGAACAGATTG TTGACCGGTATGACTGTCGCTGTTCAA GTTGGTTAGGTTCCCTCATTTAGTT TGATGTGTATTCTCTTCTCTGAAA AGAGAAAGAACGCCAGTCTTCATCTTC AACTCGCTGGTAACTTGGTTATGACT TTGAGAGCTATTTCGAAGTTATCGTT TTGGCTCTAACAAACTACTCTATCGCT GTTCAATACGGTTTCGCTTTGCTGCC GTCAGACAATACGTTCACGCCCTCAA CATTATCATCTTGTGTTGGGTCCATT CATCTTGTTCATCGCTGAAATGTCTTT GATGTTGCAAGTTAGAATCATTGTT CCA CCACTGTTATCTCTGTTATTCAC TGTTACCTTGGCCCTCTGATC CATGTC GTTCTGAAAAACTACAACATGAAGC AAATTGTTGGTTACTCCTGGTTGACT TTATCGCTAAGATCACCTCGCTGCTT CCATTATCTCCATTCCCGTCTTC CTTCAAATTGATGCGTGCTATTACAT

			TCGTAGAAAGATCGGTCAATTCCCATT CGGTCCAATGCAATGTATCTTCATTGT TTCCTGTCAATGTTGATCGTCCAGC TATTTCACTTGATCGATTCTTCACC CACACTACGATGGTTCTCCTCCATG ACTCAATGTTGTTGATCATCTCCTTA CCATTGTCTCCTGTGGGCCACCCAC ACCGCTAAAAGTTGCAAACCATGAA GGATAACACTAACCCACCATCTGGTA CCCAATTAAACCATCAGAGTTGATCGT ACTTCGACATGAAGTCGTTCCGAC TCCTCTGACGGTTCTTCACTGAAAAG ACCGAAGAAACTTGCCA (SEQ ID NO:79)
Saccharomyces castellii	NWHWLR LDPGQPLY	MSDAPPPLSELFY NSSYNPGLSIISYTS IYNGNGTEVTFNEL QSIVNKKITEAIMF GVRCGAAILTHIVM WMISKKKTPIFII NQVSLFLILLHSAF NFRYLLSNYSSVT FALTGFPQFIFHRND VHVYAAASIFQVL LVASIEISLMFQIR VIFKGDNFKRIGTI LTALSSLGLATV AMYFVTAIKGIIAT YKDVNNDTQQKYF NVATILLASSINFM TLILVIKLILAIRSR RFLGLKQFDHFIL LIMSFQSLLAPSILF ILAYSLDPNQGTD VLVTVATLLVVLS LPLSSMWATAAN NASRPSSVGSDWT PSNSDYYNSGPSS VKTESVKSDEKVS LRSRIYNLYPKSKS EFEQSSEHTYVDK VDLENNFYELSTPI TERSPSSIKKKGKQ GISTRETVKKLDL DDIYTPNTAADEE ARKFWSEDVSNEL DSLQKIEETETSDEL SPEMLQLMIGQEE EDDNLLATKKITV KKQ (SEQ ID	(codon optimized)  ATGTCTGACGCTCCACCACCATGTCC GAATTGTTCTACAACCTCCTCTACAAAC CCAGGTTGTCTATCATTTCTTACACT TCCATTACGGTAACGGTACTGAAGTT ACCTTTAACGAATTACAATCTATCGTC AACAAAGAAGATTACTGAAGCTATCAT GTTCGGTGTCAGATGTGGTGCCTGCTAT TTTGACTATCATTGTCATGTGGATGAT TTCTAAGAAGAAAAGACCCCCAATT TCATCATCAACCAAGTTCTTTATTCT TGATTTTGTTGCACTCCGCTTCAACT TCAGATACTTGTGTCTAACTACTCTT CCGTCACTTTCGCCCTGACCGGTTTCC ACAATTCACTCACAGAAACGACGTC CACGTCTACGCTGCTGCTCTATCTC CAAGTCTTGTGGTCGCTCTATTGAA ATTTCTTAATGTTCAAATCAGAGTC ATTTCAAGGGTGATAACTCAAGAG AATTGGTACTATCTTGACCGCTTTGTC CTCTTCTTTGGGTTTAGCTACTGTGTC TATGTACTTTGTCAACCGCTATTAAAGGG TATTATTGCTACCTACAAGGATGTTAA CGATACTCAACAAAAGTACTCAACG TTGCTACTATCTGTTGGCTTCTCTAT CAACTTTATGACCTTGATCTGGTTAT CAAGTTGATCTGGCTATCAGATCCAG AAGATTCTGGGTTGAACAAATTG ACTCTTCCATATCTTGTGATCATGT CTTTCAATCTTGTGGCCCCATCCA TTTGTTCATTTGGCTTACTCTTGG CCCAAACCAAGGTACCGACGTCTGG TTACTGTCGCTACTTGTGGTCGTCT TATCTTGCATGTCCTCCATGTGGG CTACTGCTGCTAACAAACGCCCTCCAGA

		NO:80)	CCATCCTCTGTTGGTCCGACTGGACT CCATCTAACTCCGACTACTACTCTAAC GGTCATCTTGTCAAGACCGAATCT GTCAAATCTGATGAAAAGGTCTCCCT GAGATCCAGAATTACAACCTGTAC CAAAGTCTAAGTCTGAATTGAACAA TCCTCCGAACACACTTACGTGACAA GGTCGACTTGAAAACAACCTCTACG AATTGTCCACCCAAATCACCGAAAGA TCTCCATCTCTATCATTAAGAAGGGT AAGCAAGGTATTCTACTAGAGAAC CGTCAAAAAGTTGGACTCCTGGATG ACATTACACTCCAAACACTGCTGCTG ATGAAGAAGCCAGAAAGTTCTGGTCT GAAGATGTTCTAACGAATTGGATTCC TTACAAAAATCGAAACTGAAACTTC CGATGAATTATCCCCAGAAATGTTAC AATTGATGATTGGTCAAGAAGAAGAA GACGATAACTTATTGGCTACCAAGAA GATCACCGTCAAGAAGCAA (SEQ ID NO:81)
Schizosac charomyce s octosporus	TYEDFLR VYKNWW SFQNPDR PDL	MREPWWKNYYT MNGTQVQNQSIPI LSTQGYIQVPLSTI DKAERNRILTGMT VSAQLALGVLIMV MSILLSSPEKRKTP VFIVNSASIISMCIR AILMIVNLCSSESY LAVMYGFVFELV GQYVHVFDILVMII GTIIIITAEVSMLLQ VRIICAHDRKTQRI VTCISSLGLSLIVVA FWFTDMCQEIKYL LWLTPYNNHQISG YYWVYFVGKILFA VSIMFHSAVFSYK LFHAIQIRKKIGQF PFGPMQCILIISCI CLFVPAIFTIIDSFI HTYDGFSSMTQCL LIVSLPLSSLWASS TALKLQSLKSTTSP GDTTQVSIRVDRT YDIKRIPEELSSV DETEIKKWP (SEQ ID NO:82)	(codon optimized)  ATGCGTGAACCATGGTCCAAGAACTA CTACACCCTGAACGGTACCCAAAGTCC AAAACCAATCCATCCAAATTGTCCA CCCAAGGTTACATTCAAGTTCCATTGT CCACCATCGATAAGGCTGAAAGAAAC AGAATTGTACTGGTATGACCGTTCT GCTCAATTGGCCTGGGTCTTGTATC ATGGTCATGTCTATTGTGTCTTGT CCAGAAAAGAGAAAGACCCAGTTTT CATCGTCAACTCTGCCTCTATCATTT CATGTGTATTAGAGCTATCTTGTATG TGTCAACTTGTGTCTGAATCCTACTC TTTGGCTGTTATGTACGGTTCTGCTT CGAATTGGTGGTCAATACGTTCACGT TTTGACATTGGTATGATTATTGG TACCATCATCATTATTACCGCTGAAGT TTCCATGTTGTGCAAGTCAGAAATTAT TTGTGCTCACGACAGAAAGACTCAA GAATTGTTACCTGTATCTCTTGT TATCCTTGATCGTCGTTGCCCTCTGGT TCACTGATATGTGTCAAGAAATTAAAG TACTTGTGTTGGTGAACCCATACAAC AACCAACAAATCTCTGGTTACTACTGG GTTTACTTCGTCGGTAAGATCTTGTTC GCCGTTCCATTATGTTCCACTCTGCC GTCTTCTCCTACAAGTTGTTCCACGCT ATCCAAATTAGAAAGAAGATTGGTCA

			ATTCCCATTGGTCCAATGCAATGTAT TTTAATTATTCCTGTCAATGTTGTC GTTCCAGCTATTTCACATCATCGAC TCTTTCATCCACACTTACCGACGGTTT TCCTCCATGACCCAATGTTGTTGATC GTCTCTTGCCATTGTCCTCCTGTGG GCCTCTTCACTGCTTAAAGTTGCAA TCTTGAAAGTCTACCACCTCCAGGT GACACTACTCAAGTTCCATTAGAGTC GACAGAACCTACGACATCAAGAGAAT CCCAACTGAAGAATTGCTTCTGTTGA CGAAACCGAAATCAAGAAGTGGCCA (SEQ ID NO:83)
Aspergillus nidulans	WCRFRGQVCG	MATHNQISDQCQ WSYPEVFTTQAVE EPTAEPASYHLHS TLTIMASNFPWN QTITFRLEDGTPFD ISVDYLDGILQYSI RACVNYAAQLGA SVILFVILVLLTRA EKRASCLFWLNSL ALLLNFARLLCDV LFFTGNFVRIYTLI SADESRTVTASDLA TSIVGAIMTALLLT TIEISLVLQVQVVC SNLRRYYRALLC VSAVVATATIAIR YSLLAVNIRALEF SDPTTYNWLESLA TVALTISICYFCVIF VTKLGFAIRLRRK LGLSELGPMKVVF IMGCQTLVIPGKR TLSSLIPPVIVSITH YVSDVPELQTNVL TIVALSLPLSSIWA GTTIDKPVTHSNV RNWLQILSFSGYR PKQSTYIATTTAT TNAKQCTHCVSES RLLTEKESGRNNND TSSKSSSQYGIAVE HDISVRSARRESFD V (SEQ ID NO:84)	(codon optimized)  ATGGCTACCCACAACCAAATCTCTGA TCAATGTCAATGGTCTTACCCAGAAGT CTTCACCACTCAAGCTGTCGAAGAAC CAACCGCCGAACCAGCTTCTTACCACT TGCACCTACCTTGACTATTATGGCTT CTAACCTCGACCCATGGAACCAAACC ATTACCTTCAGATTGGAAGACGGTAC TCCATTGACATTCTGTCGACTACTT GGACGGTATCTGCAATACTCTATCAG AGCTTGTGTCAACTACGCTGCTCAATT GGGTGTTCTGTCATTTGTTGTTAT CTTGGTCTTGTGACTAGAGCCAAA AAAGAGCTTCTGTTGTTCTGGTTAA ACTCCTTAGCTTGTGTTGAACCTCG CCAGATTGTTGTGACGTCTGTTCT TCACCGGTAACCTCGTCAGAATTAC CTTGATCTCCGCTGACGAATCTAGAG TTACTGCTTCCGACTTGGCTACTTCCA TCGTCGGTGCTATCATGACCGCTTGT TGTGACCACTATTGAAATTCTTGG TTTGCAAGTCCAAGTCGTTGTTCTA ACTTGAGAAGAATCTACAGAAGAGCC TTGTTGTGTGTTCCGCCGCGTTGCC ACTGCTACCATTGCTATTAGATACTCC TTGTTGGCTGTCAACATTAGAGCTATT TTGGAATTCTCCGACCCAACTACTTAC AACTGGTTGGAATCTTAGCTACCGTC GCCTGACCATCTCCATCTGTTACTTC TGTGTCATCTCGTCACCAAGTTAGGT TTCGCTATTAGATTGAGAAGAAAGTT GGGTTATCTGAATTGGGTCCAATGA AGGTGCTTCTCATCATGGGTGTCAAA CCITGGTCATCCAGGTAAAAGAAC TTGTCTTCTTGATTCCACCAAGTCATT GTITCTATTACTCACTACGTCTCCGAC

			GTCCCAGAATTGCAAACACTAACGTTTG ACTATCGTCGCCCTGTCCTGCCATTG TCCTCTATTGGGCTGGTACCACCATC GACAAGCCAGTCACTCACTCTAACGT TAGAAACTTGTGGCAAATCTTGTCTT CTCTGGTTACAGACCAAAGCAATCTA CCTACATTGCTACCACTACTACCGCTA CTACCAACGCTAACGAATGTACCCAC TGTTACTCTGAATCTAGATTGTTGACT GAAAAGGAATCTGGCGTAACAAACGA CACTCTTCTAACGTCTCCCTCCAATA CGGTATCGCTGTCGAACACGATATTTC CGTTAGATCTGCTCGTCGTGAATCTT TGACGTCTAG (SEQ ID NO:85)
Aspergillus oryzae	WCALPG QGC	MDSKFDPYSQNLT FHAADGTPFPQVPV MTLNDFYQYCIQI CINYGAQFGASVII FIILLLTRPDKRA SSVFFLNGGALLL NMGRLLCHMIYFT TDFVKAYQYFSSD YSRAPTSAYANSIL GVVLTTLLLVCIET SLVLQVQVVCANL RRRYRTVLLCVSIL VALIPVGLRLGYM VENCKTIVQTDTP LSLVWLESATNIVI TISICFFCSIFIUKLG FAIHQRRLGVRD FGPMKVIFVMGCQ TLTVPA LLQYA VSVPELNSNIMTL VTISLPLSSIWAGV SLTRSSSTENSPSR GALWNRLTDSTGT RSNQTSSTDAVA MTYPSNKSSTVCY ADQSSVKRQYDPE QGHGISVEHDVSV HSCQRL (SEQ ID NO:86)	(codon optimized)  ATGGACTCTAACGTTGACCCATACTCT CAAAACTTGA CTTCCACGCTGCTGAC GGTACCCCATTCAAGITCCAGTCATG ACCTTGAACGACTTTACCAACTACTGT ATTCAAATTGTATCAACTACGGTGCT CAATTGGTGCTTCCGTACATTTC ATTATCTTGTGTTATTGACTAGACCA GACAAAAGAGCTTCTCTGTTTCTTC TTAACCGGTGGTGCCCTGTTGAAC ATGGTAGATTGTTGTCACATGATT TACTCACTACTGACTTCGTCAAGGCT TACCAAACTTCTCTCTGATTACTCT AGAGCCCCAACCTCTGCCTACGCTAA CTCCATTGGGTGCGTCTGACCAC CTTGGTTTACAAGTCCAAGTCGTCTG TGCTAACTTGAGACGTAGATACAGAA CCGTCTTATTGTGTTCTATCTGGT CGCCTTGATCCCAGTCGGTTGAGATT GGGTTACATGGITGAAAAGTGAAGA CTATTGTTCAAACACTGATAACCCATTGT CTTGTTGGTTGGAATCTGCTACTA ACATCGTCATTACCATCTCCATCTGTT TCTTCTGTTCTATCTTCATCATCAAGTT GGGTTCGCCATTCAACAAAGAAGAA GATTGGGTGTCAGAGATT CGGTCCA ATGAAGGTCA TTGTCATGGGTGT CAAACATTGACTGTTCCAGCTTGTG TCTATTGCAATACGCTGCTCTGTC CCAGAATTGAAC TCAACATTATGACT TTGGTTACTATCTCTTGCCTATTGTC CCATTGGGCTGGTGTCTTGTGACCC GTTCTCCTCCACCGAAAAC TCTCCAT CCAGAGGTGCTTGTGGAACCGTTG

			ACCGACTCTACCGGTACCAAGATCTAA CCAAACCTCTTCCACCGACACCGGCCGT CGCTATGACCTACCCATCTAACAAAGTC TTCTACTGTCTGTTACGCCGATCAATC TTCTGTCAAGAGACAATACGATCCAG AACAAAGGTACCGGTATCTCTGTTGAA CACGATGTTCTGTCCACTCCTGTCAA AGATTGTAG (SEQ ID NO:87)
Beauvaria bassiana	WCMRPG QPCW	MDGSSAPSSPTPDP TFDRFAGNVTFFL ADHITTSVPMPV LNAYYDESLCTTM NYGAQLGACLVM LVVVVALTPAAKL ARRPASALHLVGL LLCAVRSGLLFAY FVSPISHFYQVWA GDFSAVSRRYWD ASLAANTLAFPLV VVVEAALINQAW TMVAFWPRAAKA AACACSAVILLTI GTRLAYTIVQNHA IVTAVPPEHFLWAI QWSAVMGAWSIF WFCAVFNVKLVC HLVANRGILPSISV VNPMEVLVMTNG TLMIIIPSIFAGLEW AKFTNFESGSLLTLT SVIIILPLGTLAAQR ISGQGSQGYQAGH LFHEQQQQQARTR SGAFGSASQQSHP TNKVPSSITLSTSG TPITPQISAGSRPEL PLVDRSERLDPIDL ELGRIDAFRGSSDF SPSTARPKRMQRD NFA (SEQ ID NO:88)	(codon optimized)  ATGGATGGTTCTTCTGCTCCATCTTCT CCAACCTCCAGATCCAACCTTCGACAG ATTGCCGGTAACGTCACTTTCTTCTT GGCTGACCACATCACCACCTACCTCCGT TCCAATGCCAGTCTGAACGCCTACTA CGACGAATCCTTGTGACTACCATGA ACTACGGTGCTCAATTAGGTGCTTGT TAGTTATGTTGGTTGTCGTTGTTGCTT TGACCCCAGCTGCTAAGTTGGCTAGA AGACCAGCTTCTGCTTGCTATTGGTT GGTTTGTGTTGCTTACTTCGTCTCCC CAATCTCTCACTTTACCAAGTTGGG CTGGTGACTTCTGCCGTTCCAGAA GATACTGGGACGCCCTTGGCTGCCA ACACTTCTGCTTCCCATTGGTTGTC TCGTTGAAGCTGCTTGATCAACCAAG CTTGGACCATGGTTGCTTCTGGCCAA GAGCCGCTAAGGCCGCTGCCGTGCT TGTCTGCTGTCATTGTTGACT ATTGGTACTAGATTGGCCTACACTATC GTCCAAAACCACGCTATTGTTACTGCC GTCCCACCAGAACACTCTTGTGGGCT ATTCAATGGTCCGCTGTTATGGGTGCT GTTTCCATCTTCTGGTTGTGCCGTT TCAACGTCAAGTTGGCTGTCACTTAG TCGCTAACAGAGGTATCTTGCCATCTA TCTCTGTTGTTAACCCAATGGAAGTCT TGGTTATGACTAACGGTACCTTGATGA TTATCCCCTATCTTCGCTGGTTGG AATGGGCTAACGTTACCAACTTCGAA TCCGGTCTTGACTTTGACTTCCGTT ATTATTATCTTGCCTATTGGGTACTTTG GCTGCCAACGTATTCTGGTCAAGGT TCCCAAGGTTACCAAGCTGGTCACTTA TTCCACGAACAAACAACAACAAGC TCGTACCCGTTCCGGTGCCTCGGTT CGCTTCTCAACAATCCCACCAACTAA CAAGGTTCCATCCTCTATTACCTTGTC TACCTCTGGTACTCCAATTACTCCACA

			AATCTCTGCCGGTTCCCGTCCAGAATT ACCATTGGTTGATAGATCCGAACGTTT GGACCCAATTGACTTCCAATTGGGTA GAATCGATGCTTCAGAGGTTCTCCG ACTTCTCTCCATCCACCGCTAGACCAA AGCGTATGCAACGTGATAACTTCGCC TAG (SEQ ID NO:89)
Candida lustaniae	KWKWI FRNTDVI G	MNPADINIEYTLG DTAFSSTFADFEA WKTRNTQFAIVNG VALACGILMVVS WIIVNKRAPIFAM NQMLVIMVIKSA MYLKHMGPLNSL TFRFTGLMEEWSA PYNVYVTINVHLV LLVAAVESSLVFQI HVVFKSSRARVAG RAIVSAMSTLALLI VSLYLYSTVRHAQ TLRAELSHGDTTT VEPWVDNVPLILF SASLNVLCLLLAL KLVFAVRRRHLG LRQFDSFHILIIMA TQTFVIPSSLVIAN YRYASSPLLSSISII VAVCNLPLCSLWA CSNNNSSYPTSSQ NTILSRYETETSQA TDASSTTCAGIAE KGFDKSPDSPTFG DQDSVSISHLDSDL EKDVEGVTHRRLT (SEQ ID NO:90)	(codon optimized)  ATGAACCCAGCTGACATCAACATCGA ATACACCTTGGGTGATACTGCTTCTC TTCCACTTCGCTGATTCGAAGCTTG GAAAACTAGAAACACTCAATTGCGTA TTGTCAACGGTGTGCTTGGCTTGTG GTATTATCTGATGGCGTTCTTGG TTATTATTGTTAACAGAGAGCTCCAA TCTTCGCTATGAACCAAACATATGTTGG TTATCATGGTTATAAGTCCGCTATGT ACTTGAAGCATATCATGGGTCCATTG AACTCCTGACCTTCCGTTTACCGGT TTAATGGAAGAACCTGGGCTCCATA CAACGTTACGTCACTATTACGTCTT GCATGTTTGTGGTCGCTGCTGTCGA ATCCTTTGGCTTCCAAATCCATGT TGTTTCAAGTCTCTAGAGCCAGAGT TGCTGGTAGAGCCATTGTTCTGCTAT GTCCACTTGGCCTTGTGATCGTTTC TTTGTACTTGTACTCTACTGTTAGACA TGCTCAAACTTGCGTGTGAATTATC TCATGGTGACACTACCACTGTTGAACC ATGGGTCGATAACGTTCCATTGATTIT GTTTCCGCTTCTTGAACGTTTGTGT TTGTTGTTGGCCTTGAATTGGTTITC GCTGTCAGAACAGAACATTTAGG TTTAAGACAATTGACTCTTCCACAT CTTGATTATTATGCCACTCAAACATT CGTTATCCCATTCCCTTGGTCATCGC TAACTACAGATAACGCTTCTCCCCATT GTTGCTTCCATTCCATCATCGTCGC CGTCTGTAACCTGCCATTGTGTTCCCT GTGGGCTTCTAACAAACAACACTTTC CTACCCAACCTCTCTCAAAACACTAT TTTGTCCAGATAACGAAACTGAAACCT CTCAAGCTACTGACGCTTCCCTACCA CCTGTGCCGGTATTGCTGAAAAGGGT TTCGACAAGTCTCCAGACTCTCCAAC TTCGGTGACCAAGACTCCGTCTATC TCCCATATCTTGGACTCTTGGAAAAG GATGTTGAAGGTGTCACCACCCATAG

			ATTGACTTAG (SEQ ID NO:91)
Candida tenuis	FSWNYRL KWQPIS	MDSYLLNHPGDIS LNFAFLPLSDEVYTI TFNDLDSQSSFSIQ YLVHSCAITVCLT LLVLLNLFIRNKKT PVFVLNQVILFFAI VRSSLFIGFMKSPL STITASFTGIHSDDQ KHFYKVSVAAANA ALIILVMLIQVSFT YQIYIIFRSPEVRKF GVFMTSALGVLM AVTGFYVNSAVA STKQYQHIFYSTD YIMDSWVTGLPPI LYSASVIAMSLVL VLKLVAAVRTRR YLGKQFSSYHILL IMFTQTLFVPTILTI LAYAFYGYNDILI HISTTIVVLLPFTS IWASIANNSSRLM SAASLYFSGSNSSL SELSSPSPSDNDTL NENVFAFPDKLQ KMNSSEAVSAVD KVVVHDHFDTISQ KSIPHDIILEILQGN EGGQMKEHISVYS DDFSKTTTPPIVGG NLLITNTDIGMK (SEQ ID NO:92)	(codon optimized)  ATGGACTCCTACTTGTGAACCACCCA GGTGACATCTCTTGAACCTCGCCTG CCATTGTCCGATGAAGTCTACACTATT ACCTTCAACGACTTAGACTCTCAATCT TCTTTTCCATTCAATACTTGGTCATC CACTCTGTGCCATTACCGTCTGTTG ACCTTGTGGTTTGTGAACCTGTT ATCAGAAACAAGAAGACTCCAGTCTT CGTTTGAACCAAGTCATCTTGTCTT CGCTATCGTCAGATCTTCTTGTTCAT CGGTTTATGAAGTCTCCATTGTCCAC CATCACCGCCTCTTCACCGGTATCAT TTCTGATGACCAAAAACACTTCTACA AGGTCTCCGTCGCTGCTAACGCCGCTT TGATCATTGGTCATGTTGATTCAAG TTTCAGATCCCCAGAAGTTAGAAAG TTCGGTGTCTTCATGACCTCCGCCTG GGTGTCTTGATGGCTGTTACCTTCGGT TTTACGTTAACCAAACTACATTA TTTCAGATCCCCAGAAGTTAGAAAG TTCGGTGTCTTCATGACCTCCGCCTG GGTGTCTTGATGGCTGTTACCTTCGGT TTTACGTTAACCAAACTACATTA CTCTACCGACCCATACATCATGGACTC TTGGGTCACTGGTTGCCACCAATCTT GTACTCTGCTTCCGTCATCGCTATGTC TTTGGTCTTGGTTTGAAGTTGGTCGC TGCTGTCAGAACAGAACAGAACATCT ACCAAGCAATACCAACACATCTTCTA CTCTACCGACCCATACATCATGGACTC TTGGGTCACTGGTTGCCACCAATCTT GTACTCTGCTTCCGTCATCGCTATGTC TTTGGTCTTGGTTTGAAGTTGGTCGC TGCTGTCAGAACAGAACAGAACATCT GTTTGAAGCAATTCTCCTCCTACCACA TCTTGTGATTATGTTACCCAAACCT TGTTGTCATGACCATCTTGTGACCATCT TAGCTTACGCTTCTACGGTTACAACG ATATCTTGATCCATATTCTACCA TCACCGTTGTCTTGTGCCATTACCA CCATTGGGCTTCTATGCCAACAACT CTAGATCCTTGATGTCTGCCGCTTCT TGTACTTCTCCGGTTCCAACCTCCTCT TGTCTGAATTGTCTTCTCCATCTCCAT CTGATAACGACACTTTGAACGAAAAC GTCTTCGCTTCTTCCAGACAGAACAGTTG CAAAAGATGAACCTCTGTAAGCCGT TTCTGCTGTCGACAAGGTCGTTGTCA CGACCACTTGATACCATCTCCAAAAA GTCTATCCCACACGACATCTGGAAAT TTTGCAAGGTAACGAAGGTGGTCAA TGAAGGAACACATCTCTGTACTCTG ATGACTCTTCTCCAAGACTACTCCAC CAATTGTCGGTGGTAACCTGTTGATCA CCAACACCGACATCGGTATGAAG

			(SEQ ID NO:93)
Neosartor ya fischeri	WCHLPG QGC	MNSTFDPWTQNIT LTQSDGTTVISSLA LADDYLHYMIRLG INYGAQLGACAVL LLVLLLLTRPEKR VSSVFVLNVALL ANIIRLGQLSYFS TGFARMYALLAG DFSRVSRGAYAGQ VMASVFFTIVFICV EASLVLQVQVVCS NLRRQYRILLGA STLAALVPIGVRLT YSVLNCMVIMHA GTMDHLDWLESA TNIVTTVSICFFCA VFVVKLGLAIKMR KRLGVKQFGPMR VIFIMGCQTMPTPA IFAICQYFSRIPEFS HNVLTLVIISLPLSS IWAGFALVQANST ARSTESRHHLWNI LSSDGATRDKPSQ CVSSPMTSPTTTC YSEQSTSXPQQDP ENGFGISVAHIDI HSFRKDAHGDI (SEQ ID NO:94)	(codon optimized)  ATGAACTCCACCTTCGACCCATGGAC CCAAAACATTACTTGTACTCAATCCGA CGGTACCACTGTCATCTCCTCTTGCG TTTGGCCGATGACTACTTGCACCAT GATTAGATTGGGTATCAACTACGGTG CCCAATTGGGTGCTTGTGCTGTTTGT TGTGGTTTGTATTGTTGACTAGAC CAGAAAAGAGAGTTCTTGTCTCG TTTGAACGTCGCTGCTTGTGGCTA ACATCATCAGATTGGTTGTCAATTGT CCTACTTCTCTACCGGTTTCGCTAGAA TGTACGCCCTGTTGGCCGGTACTTCT CCAGAGTCTCTCGTGGTGCTACGCCG GTCAAGTTATGCCCTCCGTCTTCTCA CCATTGTCTTCAATTGTGTTGAAGCTT CTTGGTTTGTCAAGTTCAAGTCGTCT GTTCTAACTTGAGAAAGACAATACAGA ATCTTGTTATTGGGTGCTTCAACTTGT GCTGCCCTGGTCCAATTGGTGTCTG TTGACTTACTCCGTTAAACTGTATG GTTATTATGCACGCTGGTACTATGGAC CACTGGATTGGTGGAAATCTGCTACC AACATCGTTACTACCGTTCTATTGT TTCTCTGTGCTGTTCGTTGTCAAAT TAGGTTGGCTATCAAGATGAGAAAG CGTTGGGTGTCAAACAATTGGTCCA ATGAGAGTTATCTCATCATGGTTGT CAAACCATGACCATCCCAGCTATTTC GCTATTGTCAATACTTCTCTAGAATT CCAGAATTCTCATAACGTTTGACT TTGGTTATCATCTTGTGCCATTGTCTT CTATCTGGGCCGGTTTGCTTGGTCC AAGCCAACCTACCGCCAGATCTACC GAATCTAGACATCATTTGTGGAACATT TTGTCTCCGATGGTGCTACAGAGAC AAGCCATCCAAATGTGTTCTTCTCCA ATGACCTCTCCAACCAACTACCTGTAC TCCGAACAATCCACCTCTAACGCCACA ACAAGACCCAGAAAACGGTTTGGTA TTTCTGTTGCCACGATATTCCATCC ACTCTTCAGAAAGGACGCCACGGT GATATTAG (SEQ ID NO:95)
Neurospor a crassa	QWCRIHG QSCW	MASSSSPPADIFSG ITQSLNSTHATLTL PIPPADRDHLENQ	(codon optimized)  ATGGCGTCCTCTCCTCACCCACCTGCA GACATTTCAGGGATCACGCAATC

		<p>VLFLEFDNHGQLLN VTITTYIDAFNNML VSTTINYATQIGAT FIMLAIMLLMTPR RRFKRLPTIISLLAL CINLIRVVLLALFF PSHWTDFYVLYSG DWQFVPPGDMQIS VAATVLSIPVTALL LSALMVQAWSMM QLWTPLWRALVV LVSGLLSLVTVAM SFANCIFQAKNILY ADPLPSYWVRKLY LALTTGSISWFTFL FMIRLVMHMWTN RSILPSMKGLKAM DVLIIITNSILMLIPV LFAGLEFLDSASGF ESGSLTQTSVVIVL PLGTLVAQRRIATR GYMPDSLEASSGP NGSLPLSNLSFAG GGGGGSGGHKDK ENGGGIIPPTTNNT AATNFSSSIACSGI SCLPKVKRMTASS ASSQRPLLTMTN STIASNDSSGFPSP GIHNTTTTTQYQ YSMGMNMPNFPP VPFPGYQSRTTGV TSHIVSDGRHHQG MNRHPSVDHFDR LARIDDEDDDGYP FASSEKAVMHGD DDDDVERGRRRA LPPSLGGVRVERTI ETRSEERMPSPDPL GVTKPRSFE (SEQ ID NO:96)</p> <p>ACTAAATAGTACACACGGCAGCGCTTA CACTACCGATTCCGCCAGCGGACAGG GATCATCTGGAAAATCAAGTATTATT TTGTTTGACAATCACGGTCAGTTACTT AATGTAACTACAACATTACATTGACGCT TTAACAAATATGCTGGTCTCTACTACT ATAAAACTATGCAACGCAAATTGGAGC TACTTTATAATGCTAGCCATTATGTT ATTAATGACTCCCAGAAGGAGGTTCA AACGTTACCAACAATTATTAGCTTGT TAGCCTTATGTATTAATTGATCAGGG TGGTTTGCTGGCCCTGTTTTCTCTC TCACTGGACAGACTCTACGTGTTGTA TTCCGGTGAUTGGCAGTTGTACCTCC AGGGGATATGCAAATATCTGTTGCTG CTACGGTTTGCTATCCCAGTGACGG CATTATTATTGAGCGCATTGATGGTTC AAGCCTGGTCAATGATGCAATTATGG ACACCACTGTGGAGGGCACTAGTGGT ACTAGTGTCCGGGCTATTGTCACTGGT AACTGTGGCAATGAGTTCGCGAATT GCATTTCCAAGCGAAAAATATTGTT ATGCCGACCCTTACCCCTCCTACTGGG TCAGAAAATTGTACTTAGCATTAAACG ACTGGGTCTATAAGTTGGTTACATTC CTTTTATGATAAGATTGGTTATGCAT ATGTGGACAAACAGATCTATATTACC AAGCATGAAGGGTTGAAGGCTATGG ATGTATTGATTATTACGAATTCTATAT TGATGTTAATCCCAGTGTGTTGCAG GCTTGBAATTCTGGATAGTCCTCTG GATTGAGTCCGGGCTTTGACTCAA CCTCTGTAGTGATTGTCCTGCCTTGG GTACTTTAGTAGCACAAAGAATAGCT ACGAGGGTTACATGCCGATAGTCT GGAGGCTCTAGCGGACCAAATGGTT CATTGCCATTCTAATTAAAGTTG CTGGAGGGGGCGGTGGTGGTTCTGGG GGACATAAAAGATAAAGAAAACGGTG GCGGTATTATACCGCCTACTACGAAC AATACTGCTGCTACTAATTCTTCA TCAATCGCGTGTCTGGTATATCTTGT TTACCAAAAGTCAAAAGAATGACCGC GAGTTCAAGTCAGTACGCCAGAGAC CGTTGTTGACAATGACTAACTCAACC ATAGCGAGTAATGACAGTTCAAGGTT CCCTTCTCCTGGCATAACATAATAC TACTACGACAACACAATACCAATT CCATGGGAATGAACATGCCGAAC CCTCCAGTCCCGTTCCCAAGGTTAC TCACGTACTACCGGTGTTACTCCC CAT</p>
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			ATTGTGTCCGACGGTAGACATCACCA GGGTATGAACAGGCACCCATCTGTTG ACCATTGATAGGGAACTTGCTAGG ATTGATGATGAAGATGACGATGGTA CCCTTCGCATCAAGTAAAAGGCCG TTATGCACGGAGACGATGACGACGAT GTGGAAAGGGACGTCGTAGAGCTCT ACCACCATCCTAGGTGGAGTTAGAG TTGAAAGGACGATCGAGACCAGGAGC GAGGAACGTATGCCATCTCCGGACCC ATTGGGTGTTACGAAGCCTAGATCATT CGAGTAG (SEQ ID NO:97)
Pseudogymnoascus destructans	FCWRPG QPCG	MSTANVHPADFD PTRQNITIYTPDGT PVVATLPMINLFN RQNNEICVVYGCQ LGASLIMFLVVLL TTRVSKRKSPIFVL NVLSLIISCLRSLL QILYYIGPWTEIYR YLSFDYSTVPASA YANSVAATLLTF LLITIEASLVLQTN VVCKSMSHIRWP VTALSMVVSLAI SFRGLTIRNIEGIL GATVKSDSLMFG ASLISETASIWFFC TIFVIKLGWTLYQ RKKMGLKQWGP MQIITIMAGCTMLI PSLFTVLEFFPEET FYEAGTLAICLVAI LLPLSSVWAAAII DGDEPVRPHGSTP KFASFNMGSODYKS SSAHLPRSIRKAVS PAEHSRTSEEELG DDGTLNRGGAYG MDRMSGISPRGV RIERTYEVHTAGR GGSIEREDIF (SEQ ID NO:98)	(codon optimized)  ATGTCCACTGCCAACGTTCATTTACCA GCTGATTTCGATCCAACTAGACAAAA CATCACTATCTATACCCCAGACGGTAC CCCAGTTGTTGCTACCTGCCAATGAT CAATTGTTAACAGACAAAACAACG AAATCTGTGTTGTTACGGTTGTCAAT TGGGTGCCTCTTAATTATGTTCTTGG TTGTTTGTGACCACCAAGAGTTCCA AGAGAAAATCTCCAATCTCGTCTGA ACGTTTGTCCTTGATTATTCTTGT AAGATCCTTGTGCAAATTTATACTA TATTGGTCCATGGACCGAGATCTACA GATACTTGTCTTCGATTACTCTACTG TCCCAGCTTCCGCTTACGCTAATTCTG TTGCTGCCACTTTATTAAACCTTATTCTT ATTGATTACCATTAAGCTTCTTAGT TTTACAAACTAACGTTGCTGCAAGTC TATGTCCTCTCACATTGTTGGCCAGT TACTGCTTGTCCATGGTTGTCTCTT ATTGGCTATTCTTTAGATTGGTTT GACCATCCGTAACATCGAAGGTATCT TAGGTGCTACTGTCAAATCCGACTCCT TAATGTTCTCTGGTGCCTTGT CTGAAACTGCTTCTATCTGGTTCTTCT GCACTATTTCGTTATTAAATTGGGTT GGACCTTGTACCAAAGAAAGAAGATG GGTTGAAGCAATGGGGTCCAATGCA AATTATCACTATCATGGCTGGTTGCAC CATGTTGATCCCACATGGCTGGTTGCA CTACGAGGCCGGTACTTGGCTATCTG TTTGGTTGCTATTGTTGCCATTATCT TCCGTCTGGCTGCCGCTGCTATTGAT GGTGATGAACCAAGTCCGTCACATGG TTCTACCCAAAATTGCTTCTTCAA CATGGGTCCGACTACAAATCTTCTTC

			TGCTCACTGCCAAGATCTATTAGAAA GGCCTCCGTCCCAGCTGAACATTTATC TAGAACTTCTGAAGAAGAGAGTTAGGTG ACGACGGTACTTTGAACAGAGGTGGT GCCTACGGTATGGACAGAAATGTCCGG TTCTATCTCCCCTAGAGGTGTCAGAAT TGAAAGAACTTACGAAGTTCATACCG CTGGTAGAGGTGGTTCTATCGAGAGA GAGGACATCTCTAG (SEQ ID NO:99)
Hypocrea jecorina	WCYRIGE PCW	MSSFDPYTQNITIL VSPSSPPISIPIPVID AFNDETAISIITNYA AQLGAALAMLLV LLAATPTARLLRA DGPSLLHALALLV CVVRTVLLIYFFLT PFSHFYQVWTGDF SQVPAWNYRASIA GTVLSTLLTVVTD AALVNQAWTMVS LFAPRTKRAVCVL SLLITLLAISFRVA YTVIQCEGIAELAA PRQYAWLIRATLIF NICSIAWFCAFNS KLV AHL VTN RGV LPSRRAMSPMEVL IMANGILMIVPVVF AILEWHHFINFEA GSLTPTSIAILPLSS LAAQRIANTSSS (SEQ ID NO:100)	(codon optimized)  ATGTCTTCCTCGACCCATACACTCAA AACATTACTATTTGGTTCTCCATCC TCTCCACCAATTCCATTCCAATCCA GTTATCGACGCTTCAACGACGAAAC CGCTTCTATCATTACTAACTACGCCGC TCAATTAGGTGCTGCTTGGCCATGTT ATTAGTTTGTGGCCGCTACTCCAAC CGCTAGATTGTTAAGAGCTGATGGTC CATCCTGTTGCACGCTTGGCCTTGT TAGTCTGTGTCGTCAGAACTGTCTTAT TGATCTACTTCTCTTGACCCCCATTCT CTCACTTCTACCAAGTCTGGACC GG TG ACTTCTCTCAAGTCCAGCTTGGAAACT ACAGAGCTTCTATTGCTGGTACCGTTT TGTCTACTTGTGACCGTTGTTACCG ACGCTGCTTGGITAACCAAGCTTGG CTATGGTTCTTATTGCTCCAAGAA CTAAGAGAGCCGTTGTGTTGCTTGT TGTTAATCACCTGTTGGCCATTCTT TCAGAGTCGCTTACACCGTCATTCAAT GTGAAGGTATCGCTGAATTGGCTGCT CCAAGACAATACGCTTGGTTGATCAG AGCCACTTGATCTTAACATCTGTT CATTGCTGGTTCTGTGCTTGTCAA CTCTAAGTTGGTTGCTCACTGGTTAC CAACAGAGGTGCTTGGCCATTCCGTA GAGCCATGTCCCCAATGGAAGTTTG ATTATGGCCAACGGTATCTTGATGATT GTTCCAGTTGTTTCGCTATCTGGAA TGGCACCACTTCATTAACCTCGAAGCT GGTTCTTAACCCAACCTCCATGCC ATTATCTTGCCATTGCTCTTGGCC GCCCAAAGAACATGCCAACACTTCTC CTCTTAG (SEQ ID NO:101)
Tuber melanospo rum	WTPRPGR GAY	MEQIPVYERPGFN PHKQNITLFKHDG STVTVGLHELDAM	(codon optimized)  ATGGAGCAAATCCCAGTCTACGAGCG TCCAGGTTCAACCCACACAAAGCAAA

		FTHSIRVAVVFAS QIGACALLSVIVA MVTKREKRALFF LHISLLLVVRSV LQILYFVGWPWAET YNYVAYYYEDIPL SDKLISIWAGIIQLI LNICILLSLILQVRV VYATSPKLNTIMT LVSCVIASISVGFF FTVIVQISEAILNG VGYDGWVYKVHR GVFAGAIAFFSFIFI FKLAFAIRRKAL GLQRFGPLQVIFIM GCQTMIVPAIFATL ENGVGFEQMSSLT ATLAVISLPLSSM WAAAQTDGPSPQS TPRDGYRRFSTRR SALNRSDPSGGRS VDMNTLDSTGND SLAHVDKTFTVE SSPSSQSQAGPHKE RGFEFA (SEQ ID NO:102)	ACATTACCTTGTCAAGCATGATGGTT CTACTGTTACTGTCGGTTGCATGAGT TGGACGCCATGTTCACTCATTCCATCA GAGTTGCTGTCGTCTCGCCTCTCAA TTGGTGCTTGTGCTTGTGTTAT CGTTGCTATGGTCACCAAGAGAGAAA AGAGACGTGCTTGTCTTGCACA TTATTCCTGTTGTTGGTCGTTGTCG TTCCGTCTGCAAATCTGTACTTCGT CGGTCCATGGGCTGAAACTATAATT ACGTCGCCACTACTATGAAGACATT CTTTGTCTGACAAATTGATTCCATT GGGCTGGTATTATCCAATTGATTGAT ATATCTGTATTTGTTATCTTGATCTT GCAAGTTCTGTCGTTACGCCACCTC TCCAAAATTGAACACTATTATGACTTT AGTCTCTGTGTTATCGCTCTATTCT GTCGGTTCTTCTTACTGTCATCGTTC AAATTCTGAGGCTATTAAACGGTG TTGGTTACGACGGTTGGGTTACAAA GTCCATAGAGGTGCTTCGCTGGTGCT ATCGCCTTCTCTTCTTACATCTTACAT TTAAGTTGGCCTCGCTATCAGAAGA AGAAAGGCTTGGGTTGCAAAGATT CGGTCCATTGCAAGTTATCTTACAT GGGTTGTCAAACATATGATTGTTCCAGC TATCTTGCTACTTGAAAACGGTGT TGGTTTCGAAGGTATGTCCTCTTGAC TGCTACCTTGGCTGTCATTCTTAC ATTGTCTTCTATGTGGGCCGCCGCTCA AACCGACGGTCCATCTCCACAATCCA CTCCAAGAGACGGTTATAGAAGATT TCTACTCGTAGATCTGCCTTGAACAGA TCTGACCCATCTGGGGTAGATCTGTT GACATGAACACCTGGACTCTACCGG TAACGATTCTTAGCTTGCACGTTGA TAAGACTTTACTGTTGAATCTCCCC ATCCTCCAATCTCAAGCTGGTCCACA CAAGGAAAGAGGGTTCGAATTGCC AG (SEQ ID NO:103)
Dactylellina haptotyla	WCVYNS CP	MDHNTQHFNRPE YIEIPVPPSKGFNP HTNPAFFIYPDGSN MTFWFGQIDDFRR DQLFTNTIFSQIGA ALVILCVMFCVTH ADKRKTIVYLLNV SNLFVVIIRGVFFF HYFMGGLARTYTYT TFTWDTSVDVQQSE	(codon optimized)  ATGGACCACAAACACCCAACACTTCAA CAGACCTGAATACATTGAAATCCCAG TTCCACCATCTAAGGGTTCAACCCAC ACACCAACCCCTGCTTCTTCATCTACC CAGACGGTTCTAATATGACCTTTGGT TCGGTCAAATCGACGATTTCAGACGT GACCAATTATTCACTAACACCATCTT TCCATTCAAATTGGTGCCGCTTGGTC

		<p>KATSIVSSICSLILM IGTQISLLLQVRIC YALNPRSKTAILV TCGSISGIATTAYL LLGAYTIQLREKPP DMKFMWKAKPV VNALVALSIVSFSG IFSWRMFQSVRNR RRMGFTGIGSLESL LASGFQCLVFPGL VTALTAVAGSTW YIAVNLTTPSDLTA IYNCSAFFAYAFSI PLLKERAQVEKTIS VVIAIAGVLVVAY GDGADDGSTSNGE KARLGGNVLIGIG SVLYGLYEVLYKK LLCPPSGASPGRSV VFSNTVCACIGAF TLLFLWIPLPLLH WSGWEIFELPTGK TAKLLGISIAANAT FSGSFLILISLTGPV LSSVAALLTIFLVA ITDRILFGRELTSA AILGGLLIIAAFAL LSWATWKEMIEE NEKDTIDSISDVGD HDD (SEQ ID NO:104)</p> <p>ATCTTATGTGTCACTGTTTGTGTTACC CACGCTGATAAGCGTAAAACCATTGT CTACTTGTAAACGTTCCAACTTGTT CGTTGTTATCATTAGAGGTGTTTCTT TGTTCATTAACATGATGGTCAACAACTGAGA AGAACCTATACCACTTCACCTGGG ATACTTCTGATGTTCAACAATCTGAGA AGGCTACTTCCATTGTCTCCTCTATT GTTCTTGATTGTGATGATCGGTACTC AAATCTCCTTATTGTTGCAAGTCAGAA TCTGTTACGCTTGAACCCAAGATCCA AGACCGCTATCTGGTACTTGTGGTT CTATTICGGTATTGCTACCACTGCTT ATTTATTGTTGGGTGCTTACACTATT AATTGAGAGAAAAGCCACCAAGACATG AAGTTCATGAAGTGGGCTAAGCCAGT TGTTAACGCTTGGTGCCTGTCCAT TGTCTCCTTCTGGTATTCTCTGTGG AGAATGTTCAATCTGTCAGAAACAG AAGAAGAATGGGTTCACTGGTATCG GTTCTTGGAAATCTTGTGGCTTCTG GTTTCCAATGTTAGTCTCCCTGGTT TGGTTACTACCGCTTGACCGTCGCCG GTTCCACTGGTATATCGCTGTTAACT TAACTACTCCATCTGACTTGACCGCTA TTTACAACGTGCTCCGCTTTTCGCTTA TGCTTCTCCATTCCATTGTTAAAGGA AAGAGCTCAAGTGGAAAGACCATT CTGTTGTCATTGCTATCGCTGGTGTCT TAGTCGTTGCTTACGGTGACGGTGTG ACGACGGTCCACCTCTAACGGTGAA AAGGCTAGATTGGGTGGTAACGTCTT GATCGGTATCGGTTCTGCTTGTATGG TTTATACGAAGTCTTGTATAAGAAGTT ATTATGTCACCACATCTGGTGCTTCCCC AGGTAGATCTGTTGTTCTCTAATAC CGTTTGTGCTTGCATCGGTGCTTCAC TTTGTATTCTGTGGATCCCATTGCC ATTGTTGCACTGGTCCGGTTGGGAAAT TTTGAATTGCCAACCGGTAAAGACTGC TAAGTTATTGGGTATTCCATTGCCGC TAACGCCACCTCTCTGGTTCTTCTT GATCTTAATTCTTGTACTGGTCCAGT TTTGTCTCTGTTGCCGCTTGTGAC CATTTCTTGTGGTGTCTTACTGACAG AATTTATTGGTAGAGAATTGACTTC TGCTGCCATTGGGTGGTTGTGAT CATCGCTGCCTCGCTTGTATCTG GGCTACTGGAGAGAATGATTGAAG AGAACGGAGAAGGATACTATCGATTCC ATCTCTGACGTTGGTGACCAACGATGA</p>
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			CTAG (SEQ ID NO:105)
Sporothrix scheckii	YCPLKGQ SCW	MKPAAGPASSPFD PFNQTFYLTGPDN TTVPVSVPQVDYI WHYIIGTSINYGSQ IGACLLMLLVMLT LTSKSRSRAATLI NVASLLIGVIRCVL LAVYFTSSLTELY ALFVGDYSQVRSS DLCVSAVATFFSL PQLVLIEAALFLQA YSMIKMWPSSLWR AVVLAMSVVVAV CAIGFKFASVVMR MRSTLTLDLDSLDF WLVEVDLAFTATT IFWFCFIYIIRLVIH MWEYRSILPPMGS VSAMEVLVMTNG ALMLVPVIFAAIEI NGLSSFESGLSVHT SVIVLLPLGLSLIAQ AMTRPDGYVQRT NTSGASGASGAHP GRNGSGHGGHGG AYSRAMTNLNTL DTLDTVDSKTSIM HHHHHHHRNHSN GMSKTKANGTW SHASDANSTNAMI SGGIATQVRIQAN QSTLGNMSGGS GAPNSHTRNNSLA AMEPVEKQLHDID ATPLSASDCRVWV DREVEVRDMV (SEQ ID NO:106)	(codon optimized)  ATGAAACCCGCCGCTGGACCTGCATC TAGTCATTGACCCATTAAACCAAAC GTTTACCTGACCGGTCCAGATAATAC CACTGTACCAGTCTCAGTCCCACAAGT TGACTATATCTGGCATTATATTATTGG AACATCCATCAACTATGGITCTCAGAT CGGAGCCTGTTACTTATGCTTCTGT GATGTTGACATTGACTTCAAAGTCAA GATTTCTCGTGCAGGCCACTCTGATTA ACGTAGCAAGCTTATTGATTGGAGTA ATTCTGTTGTTCTTTAGCTGTCTACT TTACTTCTCTCTAACTGAATTGTATG CTCTGTTGTTGGCGATTACAGCCAGG TCCGTAGGTCTGATCTTGTGTCTCTG CTGTGGCAACCTCTTAGTCTACAC AATTAGTTCTAAATAGAAGCTGCTTGT TTCTACAGGCTTATAGTATGATCAAAA TGTGGCCATCCCTGTGGAGAGCAGTG GTTTCTAGCTATGTCAGTGGTGGTGGCT GTGTGTGCAATCGTTTAAGTTCGCG TCCGTGTTATGCGTATGAGGTCAACA TTAACATTGGACGATTCTTGGATTTC TGGCTAGTGGAAAGTCGATCTGGCTTT ACAGCAACTACTATTTTGGTTTGT TTCATCTACATTATAAGGTTGGTTATT CATATGTGGAAATATAGAAGCATT ACCAACCAATGGGGTCTGTTCTGCTAT GGAGGTTCTGTTATGACCAATGGAG CGTTGATGTTAGTCCAGTGATTTCG CCGCAATAGAAATCAATGGTTATCA AGCTTGAATCAGGGTCACTGGTTCAT ACATCAGTGATTGTATTATTACCTTTA GGTAGCTTGATAGCGCAAGCAATGAC ACGTCCAGATGGGTATGTCCAAGAA CGAATACATCTGGAGCATCAGGCGCA AGTGGTGACATCCTGGTAGAAATGG ATCCGGACACGGTGGTCATGGTGGTG CGTACTCAAGAGCCATGACTAATACC CTAAATACATTGGATACATTGGATAC CGTAGACAGTAAGACATCCATAATGC ATCATCATCATCACCATCATAGAAAC CACTCAAATGGCATGAGTAAGACGAA GGCAAATAGTGGAACATGGAGCCATG CGTCAGATGCTAACTCCACCAATGCT ATGATCAGCGGTGGTATCGCAACTCA AGTTAGGATTCAAGCTAATCAGTC CCTTAGGAAATACGGGGATGTCCGGG

			GGCTCTGGAGCCCCATAATTCTCATACT CGTAATAACTCATTGGCTGCTATGGA ACCAGTGGAGAACGCACTGCATGATA TCGATGCCACACCTTAAGCGCATCTG ATTGCAGGGCTGGGTTGATCGTGAG GTCGAGGTCAAGAGGACATGGTCTA G (SEQ ID NO:107)
Yarrowia lipolytica	WRWFWL PGYGEPN W	MQLPPRPDFDIATL VASITVPETELVLG QMPLGALEQLYQ NRLRLAILFGVRV GAAVLTLIAMHLI SKKNRTKILFLAN QMSLIMLIIHAALY FRFLLGPFAASMLM MVAYIVDPRSNVS NDISVSVATNVFM MLMIMSVQLSLAV QTRSVFHAWLKS IYVTVGLILLSLVV FVFVTTHTIVSCIV LTHPTRDLPSMGW TRLASDVSFACSI FASLVLLAKLVTAI RVRKTLGKKPLGY TKVLVIMSTQSLV VPSILIIVNYYALPEK NSWILSGVAYLMV VLSLPLSSIWATAV HDDEMQSNYLLS ALKDGHVQPSESK LKTVFLNRLRPFST TTNRDDESSVDSP AMPSPEDVTFLN TGFECDENKM (SEQ ID NO:108)	(codon optimized)  ATGCAATTGCCACCACGCCAGACTTC GACATTGCCACTTGGTTGCCTCTATC ACTGTTCCAGAAACTGAATTGGTCTTG GGTCAAATGCCATTGGGTGCTTAGA ACAATTGTACCAAAACAGATTGCGIT TGGCTATTTGTTCGGTGTCAGAGTCG GTGCTGCTGTTTGACCTTGATTGCTA TGCACITAATCTCCAAGAAGAACAGA ACCAAGATCTGTTCTGGCTAACCAA ATGTCTTGATCATGTTGATCATCCAT GCTGCTTGACTTCAGATTCTGTTG GGTCACATCGCCCTCATGTTGATGATG GTTGCTTACATCGTTGATCCAAGATCT AACGTCTCTAACGATATCTCTGTTCT GTTGCCACCAACGTTTCATGATGTTG ATGATTATGTCCTGCTCAATTGTCCTG GCTGTTCAAACCCGTTCTGTTCCAC GCTTGGTTGAAGTCTCGTATTTACGTT ACCGTTGGTTAACCTGTTGTCCTG GTCGCTTCGTTCTGGACCACCCAC ACTATCGTTCTGTATCGTTAAC CATCCAACTAGAGAGACTGCCATCTATG GGTTGGACTAGATTAGCTCTGACGTT TCCTTCGTTGTTCTATCTCTTCGCTT CTTTGGCTTGTGGCTAAGTTGGTCA CCGCCATCAGAGTTAGAAAGACCTTG GGTAAGAAGCCATTGGGTACACCAA GGTTTGGTCATCATGTCACATCAATC TTTAGTCGTTCCATCTATCTGATTAT CGTTAACTACGCTTGGCAGAAAAAAA ACTCTTGGATCTGTCTGGTGTGCGCTT ACTTGATGGTTGTTTGTCCCTTACCAT TGTCCCTCCATTGGGCTACCGCCGTCC ATGACGACGAAATGCAATCCAACATAC TTGTTGTCCTGCCTTGAAAGATGGTCAC GTTCAACCACCGAATCTAAGTTGAA GAUTGTTTCTGAAACAGATTGAGACC ATTCTCTACTACCAACTAACAGAGACG ATGAATCCTCTGTTGATTCCCCAGCCA TGCCATCTCCAGAATCTGATGTTACCT TCTTGAACACTGGTTCGAATGTGACG

			AAAAGATGTAG (SEQ ID NO:109)
Torulaspor a delbruecki i	GWMRLR LGQPL	MSDSAQNLSDLAF NSSYNPLDSFITFT SIYGDNTAVKFSV LQDMVDVNTNEAI VYGTRCGASVLTQ IIMWMISKNR RTP VFIINQVSLLILIH SALYFKYLLSGFG SVVYGLTAFPQLI KPGDLRAFAAANI VMVLLVASIEASLI FQVKVIFTGDNMK RVGLILITIICMG LATVTMYFITAVK SIVSLYRDMMSGST VLYNVSLIMLASSI HFMALILVVKFL AVRSRRFLGLKQF DSFHILLIISCQTL VPSLLFIAYSFSS KNIESLKAIAVLT VLSPLSSMWATA ANNFTNSSSGSDS APTNGGFYGRGSS NLYPEKTDNRSPK GARNALYELRSKN NAEGQADIYTVTD IENDIFNDLSKPVE QNFSDVQIDSHS LHKACSKEDPVMT LYTPNTAIEGEERK LWTSDCSCSTNGS TPVKKKSTGEYAN LPPHLLRYDENYD EEAGGRRKASLK W (SEQ ID NO:110)	(codon optimized)  ATGTCTGACTCCGCCAAAACCTTGTC GATTGGCCCTCAACTCTCTTATAAC CCATTGGACTCCTTATTACCTTTACC TCTATCTACGGTGATAACACTGCTGTT AAGTTCTCCGTTTACAAGACATGGTT GACGTTAATACTAATGAAGCCATCGT TTACGGTACCCGTTGTGGTGCCTCTGT CTTGACCCAATTATCATGTGGATGAT TTCTAAAAACAGAAGAACCCCAGTCT TTATTATTAACCAAGTTCTTGACTT TGATTTAAATTCACTCTGCCTTGTACT TCAAGTACTTGTGTCTGGTTGGTT CCGTTGTCTACGGTTGACTGCTTCC CACAAATTGATTAAGCCAGGTGATTG AGAGCTTTCGCTGCTGCTAACATCGTT ATGGCTTGTGGTCGCTCTATTGAA GCTTCCTTAATCTCCAAGTCAGTT ATCTTCACCGGTGATAACATGAAGAG AGTCGGTTAACATTGACTATTATTG TACTTGTATGGGTTAGCTACTGTTAC CATGTACTTTATTACTGCCGTCAAGTC TATTGCTCTTTGTACCGTGACATGTC TGGTTCCCTCACCGTTTATATAACGT TTCTTAATTATGTTGGCTCCTCCATC CACTTATGGCTTGATCTGGTTGTC AAATTGTTCTGGCTGTTAGATCTAGA AGATTCTGGGTTGAAACAATTGAT TCTTCCACATTGATCATCTCTT GTCAAACATTGTTGGTCCATCTTAT TATTCAATTGCTTACTCTTCCATC TTCTAACATTGAATCTTGAAGGC TATCGCTTTGACCGTCGTTTGTC TTTGCCATTGTCCTATGTGGGCTAC TGCTGCTAATAACTCACTAATCTTC CTCCTCCGGTTCCGACTCCGCTCCAAC CAATGGTGGTTCTACGGTAGAGGTT TTCCAACCTGTATCCTGAAAAGACTGA TAACAGATCCCCAAAGGGTGCCAGAA ACGCTTATACGAATTAAAGATCTAAG AACAAATGCTGAGGGTCAAGCTGATAT TTACACCGTTACCGATATTGAAAACG ATATTTCACGATTGTCCAAGCCAG TTGAGCAAAACATTCTCTGATGTT AAATTATTGATTCTCATTCTTGCATA AGGCTTGTCTAAAGAAGACCCAGTC ATGACTTGTACACTCCAAACACTGCT ATTGAAGGTGAGGAGAGAAAATTGTG

			GACTTCTGACTGTTCCCTGTTCCACTAA CGGTTCCACCCCAGTTAAGAAGAAGT CCACCCGGTAATACGCCAATTACCA CCACACTTATAAGATATGATGAAAA CTACGATGAAGAAGCTGGTAGAC GTAAGGCCTCCTGAAATGGTAG (SEQ ID NO:111)
Komagata ella pastoris	FRWRNN EKNQPPFG	MEEYSDSFDPSSQ LLNFTSLYGETDA TFAELDDYHFYVV KYAIIVYGARIGVG MFCTLMLFVVSKS WKTPIFVLNQSSLI LLIHSGFYIHLYT NQFSSLTYMFTRIP NETHAGVDLRINV VTNTLYALLILSIEI SLIYQVFVIFKGVY ENSLRWIVTIFTAL FAAAVVAINFYVT TLQSVSMYNSNVD FPRWASNVPILFA SSVNWACLLSLK LFFAIKVRRSLGLR QFDTFHILAIMFSQ TLIIPSILIVLGYTG TRDRDSLASLGFL LIVVSLPSSMWA ATANNSNIPTSTGS FAWKNRYSKPSTYS DDTTAVSKSFTIM TAKDECFTTDTEG SPRFIKGDRTSEDL HF (SEQ ID NO:112)	(codon optimized)  ATGGAAGAATACTCCGACTCCITCGA CCCATCCCAACAATTGTTGAACITTCAC TTCCCTATACGGTGAACCGATGCTAC TTTCGCTGAATTGGACGACTACCACCT CTACGTCGTTAAGTACGCCATCGTTA CGGTGCCAGAATTGGTGTGGTATGTT TTGTACTTTGATGTTGTTGTTCC AAGTCTTGGAAAGACTCCAATCTTCGTC TTGAACCAATCTTCTTGATTITGTTG ATTATTCACTCCGGTTCTACATCCAC TACTTGACCAACCAATTCTCTTCCTG ACCTACATGTTCACTAGAACCCAAA CGAAACCCATGCTGGTGTGATTG GTATTAAACGTCGTTACCAACACCTGT ACGCTTGTGATCTTATCTATTGAAA TTTCCTTAATTACCAAGTCTTCGTTA TCTTCAAAGGTGTCTACGAAAACCTTT TAAGATGGATTGTTACTATTTCACCG CTTTATTGCCGCCGCCGTGTTGCTA TTAACCTCTACGTCACTACTTGC CTGTCTCTATGTACAACCTAACGTTG ACTTTCCAAGATGGGCTTCTAACGTC CATTGATCTTGTGCTTCTCTGTCA ACTGGGCTTGTGTTGTTGCTGCTTGA AGTTGTTCTCGCTATCAAGGTTAGAA GATCTTGGGTTGAGACAATTGACA CTTTTCACATCTGGCCATCATGTTCT CTCAAACCTTGATTATCCCATCCATT TGATTGTCTGGGTTACACTGGTACCA GAGACAGAGACTCCTGGCTTCTTGG GTTTCTTGTGATCGTTGTTCTTGCC ATTTTCCCTCATGTGGGCTGCCACTGC TAACAACTCCAACATCCCAACCTCTAC CGGTTCTTCGCTGGAAAGAACAGAT ACTCCCCATCTACTTACTCCGACGATA CCACTGCTGTTCCAAGTCCCTCACTA TTATGACCGCTAAGGATGAATGTTCA CCACTGATACCGAAGGTTCTCCAAGA TTCATCAAGGGTGACAGAACCTCCGA AGATTGCACTCTAG (SEQ ID NO:112)

			NO:113
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#### 6.8.4. Key characteristics of peptide Ligands

Twenty three natural fungal peptides were synthesized and tested for activation of their corresponding receptor in the biosensor strain. Physico-chemical properties, e.g., peptide length, overall charge, charge distribution and hydrophobicity/hydrophilicity were determined for all 23 functionally verified peptide ligands using the program ProtParam on the Expsy server [Walker (2005) ISBN 978-1-59259-890-8]. Sequence variability and conserved sequence motifs within the set of peptide ligands were determined using an alignment and clustering method described in [Andreatta et al. (2013)].

##### A. Physicochemical characteristics of peptide ligands

Natural mating peptide ligands featured diversity in length (9-23 residues), overall charge and number of charged residues as well as hydrophobicity (GRAVY, Grand average of hydropathy [Kyte and Doolittle (1982)] ranging from hydrophobic to mildly hydrophilic (see Table 9).

##### B. Sequence-function relationship and sequence diversity

*Functional domains within alpha-factor:* previously reported Alanine scanning mutagenesis revealed defined functional domains within the *S. cerevisiae* mating pheromone alpha-factor [Naider et al. (2004)]. Residues at the C-terminus were found to be mainly involved in binding to the receptor, while residues at the N-terminus were shown to contribute to signaling due to receptor activation. NMR studies also showed that alpha factor adopts a bended secondary structure due to the tendency of the internal residue stretch to form a loop [Higashijima et al. (1983)].

*Sequence motifs of peptide ligands:* A motif search for the peptides listed below was performed using a 13-residue motif length as an input parameter, because this is the length of the well characterized alpha factor. The peptides were clustered into 3 groups by conservation of residues (*see* Figure 12B): all three clusters showed 5 conservation of internal prolines and Cluster 1 and cluster 3 sequence motif featured the conservation of the aromatic N-terminal “activation domain” also found in *S. cerevisiae* alpha factor.

*Correlation between sequence motifs and physicochemical properties:* The peptide alignments within the clusters showed that sequences within the same cluster 10 varied in length, overall charge, distribution of charged residues and hydrophobicity/hydrophilicity (*see* Figure 12). Cluster 1 featured high variability in overall charge (from negative to positive) and charge distribution across the sequence as well as hydrophobic and hydrophilic members. Cluster 1 and 2 featured variability in the length of group members showing a variation of up to 3 additional residues.

15 6.9. Example 9: Identification Of Biomarkers Specific For A Disease Sample

The design of *S. cerevisiae* biosensor allowed for simple plug-and-play engineering of new receptor-ligand pairs into the existing biosensor strain. The first step in developing yeast biosensors for additional targets using this platform was the identification of specific peptide biomarkers, for which specific receptors can be 20 adapted via receptor engineering and directed evolution. As shown in Figure 15, a pipeline for identification of viable peptide biomarkers was developed.

First, mass spectrometric analysis is used to identify the peptidome of a given sample. A sample can be anything from a blood sample to a nasal swab or water sample. The peptidome of a sample includes peptides a priori present in the sample or

otherwise released after proteolytic treatment (e.g. treatment with trypsin or chemotrypsin).

The resulting peptides are then compared against our existing fungal ligand library to identify the highest homology match. The inventors' fungal ligand library 5 is a list of fungal peptide pheromones - unmodified peptides between 9-15 residues in length - which are predicted or have been validated to activate their cognate fungal mating GPCR. The GPCR corresponding to homologous library peptide is then used as parent for biosensor engineering and provides an advantageous starting point for directed evolution experiments towards the peptide target.

10 6.10. EXAMPLE 10: Trypsination Of Cholera Toxin To Release Target Ligands

Cholera toxin (CTx) is a heteromeric protein complex secreted by the bacterium *Vibrio cholerae*. It is responsible for the massive, watery diarrhea characteristic of cholera infection and it was shown to be an abundant protein in stool samples of cholera-infected patients. [LaRocque et al. (2008)]. CTx is composed of 2 15 subunits, CtxA (27 kDa) and CtxB (11.6kDa), where CtxB assembles in a pentameric ring around a single CtxA subunit.

Trypsin digestion of un-denatured, completely folded Ctx (the protein form expected in an untreated stool sample) was performed and the resulting peptidome was determined by mass spectrometry (*see* peptide list in Table 7). Then, a similarity 20 search of the resulting Ctx peptidome was performed with the inventors' existing library of functional peptides tested in their sensor strain. A peptide HFGVLDEQLHR (SEQ ID NO: 132) with 36% identity to a functional member of the inventors' fungal peptide library, the fungi *Zygosaccharomyces rouxii* (*see* Figure 16) was detected.

The conservation of N-termini of these peptides is encouraging since the N-terminal end of mating pheromones was shown to be significant for receptor activation. [Naider et al. (2004)]. In addition, while tryptic release of some peptides may be less efficient than others because several predicted trypsin cleavage sites 5 might not be solvent exposed and accessible, the high peptide count of the identified peptide (Table 7) indicates its high abundance in the analyzed sample. Importantly, the same peptide identified in this work was previously reported in tryptic digests of clinical stool samples from cholera infected patients. [LaRocque et al. (2008)]. Directed evolution experiments towards GPCR binding of the identified Ctx peptide 10 is performed.

**Table 7. Peptidome of Cholera Toxin after trypsin treatment**

Peptide released by trypsin digest	Peptide count
<b>Cholera toxin subunit A</b>	
ADGYGLAGFPPEHR (SEQ ID NO: 114)	7
ADSRPPDE (SEQ ID NO: 115)	2
ADSRPPDEIK (SEQ ID NO: 116)	4
ADSRPPDEIKQS (SEQ ID NO: 117)	1
ADSRPPDEIKQSGGLMPR (SEQ ID NO: 118)	9
AGFPPEHR (SEQ ID NO: 119)	2
ALGGIPYSQIYGWYR (SEQ ID NO: 120)	1
APAADGYGLAGFPPEHR (SEQ ID NO: 121)	5
ATAPNMFNNDVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 122)	4
AYSHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 123)	1
DIAPAADGYGLAGFPPEHR (SEQ ID NO: 124)	1
DRYYSNLDIAPAADGYGLAGFPPEHR (SEQ ID NO: 125)	34
DSRPPDEIK (SEQ ID NO: 126)	3

DVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 127)	1
FGVLDEQLHR (SEQ ID NO: 128)	8
FLDEYQSKVKRQIFSGYQSDIDTHNR (SEQ ID NO: 129)	2
FLDEYQSKVKRQIFSGYQSDIDTHNRIKDEL (SEQ ID NO: 130)	5
FNVNDVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 131)	1
GAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 132)	2
GGIPYSQIYGWYR (SEQ ID NO: 133)	2
GQSEYFDR (SEQ ID NO: 134)	4
GQSEYFDRGTQMNINLYDHAR (SEQ ID NO: 135)	6
GTQMNINLYDHAR (SEQ ID NO: 136)	45
GTQTGFVR (SEQ ID NO: 137)	15
GTQTGFVRHDDGYVSTSISLR (SEQ ID NO: 138)	3
GYQSDIDTHNR (SEQ ID NO: 139)	1
GYRDRYYSNLIDIAPAADGYGLAGFPPEHR (SEQ ID NO: 140)	3
HDDGYVSTS (SEQ ID NO: 141)	1
HDDGYVSTSISLR (SEQ ID NO: 142)	38
HFGVLDEQLHR (SEQ ID NO: 143)	76
KQSGGLMPR (SEQ ID NO: 144)	5
LDIAPAADGYGLAGFPPEHR (SEQ ID NO: 145)	2
NVNDVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 146)	11
QEVSALGGIPYSQIYGWYR (SEQ ID NO: 147)	1
QIFSGYQSDIDTH (SEQ ID NO: 148)	1
QIFSGYQSDIDTHN (SEQ ID NO: 149)	1
QIFSGYQSDIDTHNR (SEQ ID NO: 150)	41
QSDIDTHNR (SEQ ID NO: 151)	2
QSGGLMPR (SEQ ID NO: 152)	6
RHDDGYVSTSISLR (SEQ ID NO: 153)	21
RQIFSGYQSDIDTHNR (SEQ ID NO: 154)	7
SAHLVGQTILSGH (SEQ ID NO: 155)	1

SAHLVGQTILSGHSTY (SEQ ID NO: 156)	1
SAHLVGQTILSGHSTYY (SEQ ID NO: 157)	5
SAHLVGQTILSGHSTYYIYVIATAPNMF (SEQ ID NO: 158)	5
SDIDTHNR (SEQ ID NO: 159)	98
SGYQSDIDTHNR (SEQ ID NO: 160)	6
SNLDIAPIADGYGLAGFPPEHR (SEQ ID NO: 161)	14
SQIYGWYR (SEQ ID NO: 162)	4
SRPPDEIKQSGGLMPR (SEQ ID NO: 163)	1
TAPNMFNVNDVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 164)	2
VIATAPNMFNVNDVLGAYSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 165)	1
VKRQIFSGYQSDIDTHNRIKDEL (SEQ ID NO: 166)	2
VLDEQLHR (SEQ ID NO: 167)	1
YQSDIDTHNR (SEQ ID NO: 168)	2
YSNLDDIAPIADGYGLAGFPPEHR (SEQ ID NO: 169)	17
YSPHPDEQEVSALGGIPYSQIYGWYR (SEQ ID NO: 170)	1
YSQIYGWYR (SEQ ID NO: 171)	1
YYSNLDIAPIADGYGLA (SEQ ID NO: 172)	1
YYSNLDIAPIADGYGLAGFPPEHR (SEQ ID NO: 173)	29
<b>Cholera subunit B</b>	
AIAAISMAN (SEQ ID NO: 174)	1
EMAIITFK (SEQ ID NO: 175)	1
FSYTESLAGK (SEQ ID NO: 176)	1
IFSYTESLAGK (SEQ ID NO: 177)	2
NDKIFSITESLAGK (SEQ ID NO: 178)	2
NGATFQVEVPGSQH (SEQ ID NO: 179)	1
NGATFQVEVPGSQHIDSQK (SEQ ID NO: 180)	10
NGATFQVEVPGSQHIDSQKK (SEQ ID NO: 181)	18
SYTESLAGKR (SEQ ID NO: 182)	5

TPHAI AAIISMAN (SEQ ID NO: 183)	2
YTESLAGK (SEQ ID NO: 184)	1

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Various references are cited herein, the contents of which are hereby  
incorporated by reference in their entireties.

**What is claimed is:**

1. A method of detecting the presence of an agent of interest in a sample, comprising contacting the sample with a sensor cell comprising a non-native G-protein coupled receptor (GPCR) that binds to an analyte indicative of the presence of the agent, wherein binding of the analyte to the receptor triggers appearance of a reporter detectable by the naked eye, wherein said increased expression is indicative of the presence of the agent.
2. The method of claim 1, wherein the agent is selected from the group consisting of human pathogenic agents, agricultural agents, industrial and model organism agents, bioterrorism agents, and heavy metal contaminants.
3. The method of claim 1 or 2, wherein the non-native GPCR receptor is engineered to bind to the analyte.
4. The method of claim 3, wherein the non-native GPCR receptor is engineered by directed evolution.
5. The method of any one of claims 1-4, wherein the non-native GPCR receptor is a fungal pheromone GPCR.
6. The method of any one of claims 1-5, wherein the non-native GPCR receptor is one selected from the group consisting of the GPCRs listed in Tables 2 and 6.
7. The method of any one of claims 1-6, wherein the sensor cell is a microbe.
8. The method of claim 7, wherein the sensor cell is a fungal cell.
9. The method of claim 8, wherein the sensor cell is a yeast cell.
10. The method of claim 9, wherein the sensor cell is *S. cerevisiae*.
11. The method of any one of claims 1-10, wherein the sensor cell is engineered to express the non-native GPCR receptor.

12. The method of any one of claims 1-11, wherein the sensor cell comprises a nucleic acid encoding the non-native GPCR receptor.
13. The method of claim 12, wherein the nucleic acid is linked to a promoter.
14. The method of any one of claims 1-13, wherein the analyte is a cognate ligand for the non-native GPCR receptor.
15. The method of any one of claims 1-13, wherein the analyte is a non-cognate ligand for the non-native GPCR receptor.
16. The method of any one of claims 1-15, wherein the analyte is a peptide.
17. The method of claim 16, wherein the peptide is a fungal mating pheromone.
18. The method of claim 17, wherein the fungal mating pheromone is selected from the group consisting of human fungal mating pheromones, non-human animal fungal mating pheromones, plant fungal mating pheromones, food fungal mating pheromones, and industrial/model fungal mating pheromones.
19. The method of claim 18, wherein the human fungal mating pheromone is selected from the group consisting of the mating pheromones of *C. albicans*, *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, *S. schechii*, and *Candida krusei*.
20. The method of claim 18, wherein the non-human animal fungal mating pheromone is the mating pheromone of *P. destructans*.
21. The method of claim 18, wherein the plant fungal mating pheromone is selected from the group consisting of the mating pheromones of *F. graminearum*, *M. oryzae*, *B. cinerea*, and *G. candidum*, and *C. purpurea*.
22. The method of claim 18, wherein the food fungal mating pheromone is selected from the group consisting of the mating pheromones of *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, and *N. fischeri*.

23. The method of claim 18, wherein the industrial/model fungal mating pheromone is selected from the group consisting of the mating pheromones of *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (*receptor 1*), *V. polyspora* (*receptor 2*), *S. stipitis*, *S. japonicas*, *S. castellii*, *S. octosporus*, *A. oryzae*, *T. melanosporum*, *D. haptotyla*, *C. tenuis*, *Y. lipolytica*, *T. delbrueckii*, *B. bassiana*, *K. pastoris*, *A. nidulans*, *N. crassa*, and *H. jecorina*.

24. The method of any one of claims 16-23, wherein the peptide is selected from the group consisting of the peptides listed in Table 5.

25. The method of any one of claims 16-24, wherein the peptide has a length of about 3-30 residues.

26. The method of claim 25, wherein the peptide has a length of about 9-23 residues.

27. The method of claim 16, wherein the peptide is associated with a bacterial infection.

28. The method of claim 27, wherein the peptide is associated with *Vibrio cholera*.

29. The method of claim 28, wherein the peptide associated with *Vibrio cholerae* is selected from the group consisting of a peptide having an amino acid sequence set forth in VEVPGSQHIDSQKKA (SEQ ID NO: 26), a peptide having an amino acid sequence that is at least about 80%, at least about 90%, or at least about 95% homologous to SEQ ID NO: 26, a peptide having an amino acid sequence set forth in VPGSQHIDS (SEQ ID NO: 27), and a peptide having an amino acid sequence that is at least about 80%, at least about 90%, or at least about 95% homologous to SEQ ID NO: 27.

30. The method of any one of claims 27-29, wherein the peptide is derived from cholera toxin.
31. The method of claim 30, wherein the peptide derived from cholera toxin is selected from the group consisting of the peptides listed in Table 7.
32. The method of any one of claims 1-31, wherein the non-native GPCR receptor is coupled to the reporter gene.
33. The method of any one of claims 1-32, further comprising culturing the sensor cell for an effective period of time; and determining expression of the reporter.
34. The method of claim 33, wherein determining expression of the reporter gene not comprise instrumentation.
35. The method of any one of claims 1-34, wherein the reporter is a biosynthesized visible-light pigment.
36. The method of claim 35, wherein the reporter is lycopene.
37. The method of any one of claims 1-36, wherein the sample is selected from the group consisting of water samples and body fluid samples.
38. The method of claim 37, wherein the water sample is selected from the group consisting of fresh water, sea water, and sewage samples.
39. The method of claim 37, wherein the body fluid sample is selected from the group consisting of intestinal fluids, diarrhea, mucus, blood, cerebrospinal fluid, lymph, pus, saliva, vomit, urine, bile, and sweat.
40. A sensor cell comprising a non-native GPCR receptor that binds to an analyte indicative of the presence of the agent, wherein binding of the analyte to the receptor triggers appearance of a reporter detectable by the naked eye, wherein said increased expression that is indicative of the presence of the agent.

41. The sensor cell of claim 40, wherein the agent is selected from the group consisting of human pathogenic agents, agricultural agents, industrial and model organism agents, bioterrorism agents, and heavy metal contaminants.
42. The sensor cell of claim 40 or 41, wherein the non-native GPCR receptor is engineered to bind to the analyte.
43. The sensor cell of claim 42, wherein the non-native GPCR receptor is engineered by directed evolution.
44. The sensor cell of any one of claims 40-43, wherein the non-native GPCR receptor is a fungal pheromone GPCR.
45. The sensor cell of any one of claims 40-44, wherein the non-native GPCR receptor is one selected from the group consisting of the GPCRs listed in Tables 2 and 6.
46. The sensor cell of any one of claims 40-45, wherein the sensor cell is a microbe.
47. The sensor cell of claim 46, wherein the sensor cell is a fungal cell.
48. The sensor cell of claim 47, wherein the sensor cell is a yeast cell.
49. The sensor cell of claim 48, wherein the sensor cell is *S. cerevisiae*.
50. The sensor cell of any one of claims 40-49, wherein the sensor cell is engineered to express the receptor.
51. The sensor cell of any one of claims 40-50, wherein the sensor cell comprises a nucleic acid encoding the receptor.
52. The sensor cell of claim 51, wherein the nucleic acid is linked to a promoter.
53. The sensor cell of any one of claims 40-52, wherein the analyte is a cognate ligand for the non-native GPCR receptor.

54. The sensor cell of any one of claims 40-52, wherein the analyte is a non-cognate ligand for the non-native GPCR receptor.
55. The sensor cell of any one of claims 40-54, wherein the analyte is a peptide.
56. The sensor cell of claim 55, wherein the peptide is a fungal mating pheromone.
57. The sensor cell of claim 56, wherein the fungal mating pheromone is selected from the group consisting of human fungal mating pheromones, non-human animal fungal mating pheromones, plant fungal mating pheromones, food fungal mating pheromones, and industrial/model fungal mating pheromones.
58. The sensor cell of claim 57, wherein the human fungal mating pheromone is selected from the group consisting of the mating pheromones of *C. albicans*, *C. glabrata*, *P. brasiliensis*, *L. elongisporous*, *P. rubens*, *C. guillermondi*, *C. tropicalis*, and *C. parapsilosis*, *C. lusitaniae*, *S. scheckii*, and *Candida krusei*.
59. The sensor cell of claim 57, wherein the non-human animal fungal mating pheromone is the mating pheromone of *P. destructans*.
60. The sensor cell of claim 57, wherein the plant fungal mating pheromone is selected from the group consisting of the mating pheromones of *F. graminearum*, *M. oryzae*, *B. cinerea*, *G. candidum*, and *C. purpurea*.
61. The sensor cell of claim 57, wherein the food fungal mating pheromone is selected from the group consisting of the mating pheromones of *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, and *N. fischeri*.
62. The sensor cell of claim 57, wherein the industrial/model fungal mating pheromone is selected from the group consisting of the mating pheromones of *S. cerevisiae*, *K. lactis*, *S. pombe*, *V. polyspora* (receptor 1), *V. polyspora* (receptor 2), *S. stipitis*, *S. japonicas*, *S. castellii*, *S. octosporus*, *A. oryzae*, *T. melanosporum*, *D.*

*haptotyla, C. tenuis, Y. lipolytica, T. delbrueckii, B. bassiana, K. pastoris, A. nidulans, N. crassa, and H. jecorina.*

63. The sensor cell of any one of claims 55-62, wherein the peptide is selected from the group consisting of the peptides listed in Table 5.

64. The sensor cell of any one of claims 55-63, wherein the peptide has a length of about 3-50 residues.

65. The sensor cell of claim 64, wherein the peptide has a length of about 9-23 residues.

66. The sensor cell of claim 55, wherein the peptide is associated with a bacterial infection.

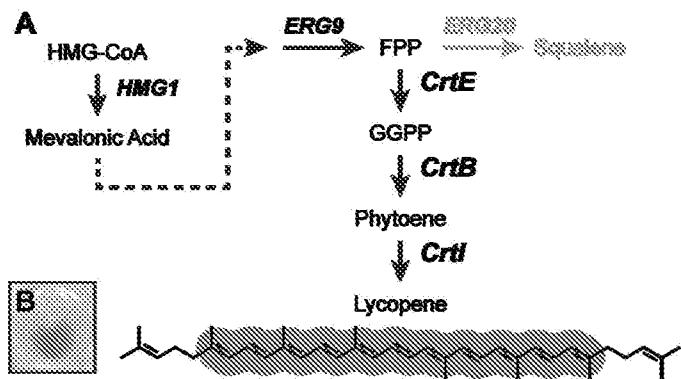
67. The sensor cell of claim 66, wherein the peptide is associated with *Vibrio cholera*.

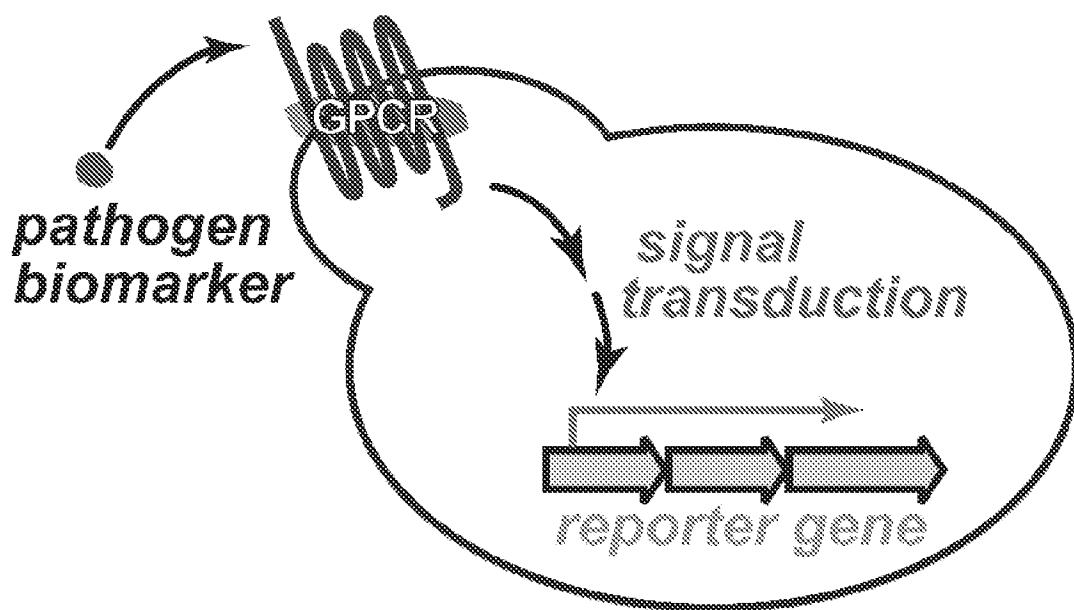
68. The sensor cell of claim 67, wherein the peptide associated with *Vibrio cholerae* is selected from the group consisting of a peptide having an amino acid sequence set forth in VEVPGSQHIDSQKKA (SEQ ID NO: 26), a peptide having an amino acid sequence that is at least about 80%, at least about 90%, or at least about 95% homologous to SEQ ID NO: 26, a peptide having an amino acid sequence set forth in VPGSQHIDS (SEQ ID NO: 27), and a peptide having an amino acid sequence that is at least about 80%, at least about 90%, or at least about 95% homologous to SEQ ID NO: 27.

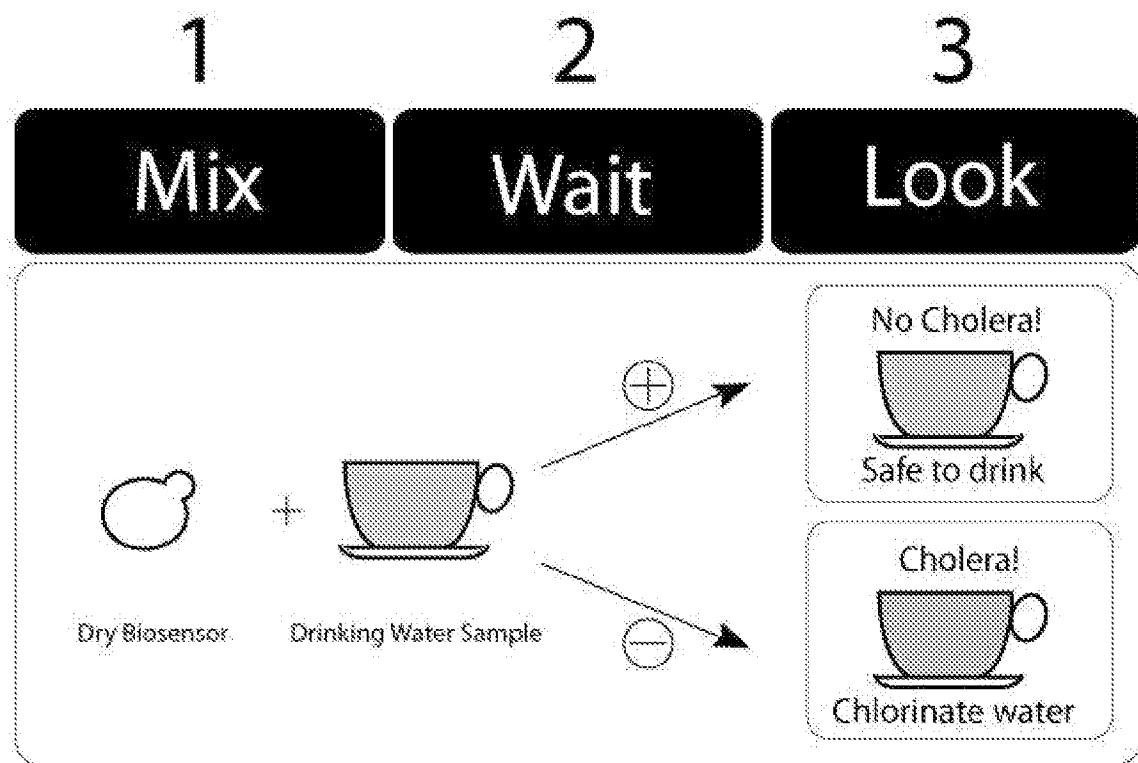
69. The sensor cell of any one of claims 66-68, wherein the peptide is derived from cholera toxin.

70. The sensor cell of claim 69, wherein the peptide derived from cholera toxin is selected from the group consisting of the peptides listed in Table 7.

71. The sensor cell of any one of claims 40-70, wherein the non-native GPCR receptor is coupled to the reporter.
72. The sensor cell of any one of claims 40-71, wherein the reporter is a biosynthesized visible-light pigment.
73. The sensor cell of claim 72, wherein the reporter is lycopene.
74. A kit for detecting the presence of an agent of interest, comprising a sensor cell of any one of claims 40-73.
75. The kit of claim 74, further comprising a negative control.
76. The kit of claim 74 or 75, further comprising a substrate that comprising the sensor cell.
77. The kit of any one of claims 74-76, further comprising a nutrient source.

**FIGURE 1**

**FIGURE 2**

**FIGURE 3**

**FIGURE 4**

Figure 4A. Induction of lycopene biosynthesis by the natural yeast peptide,  $\alpha$ -factor.

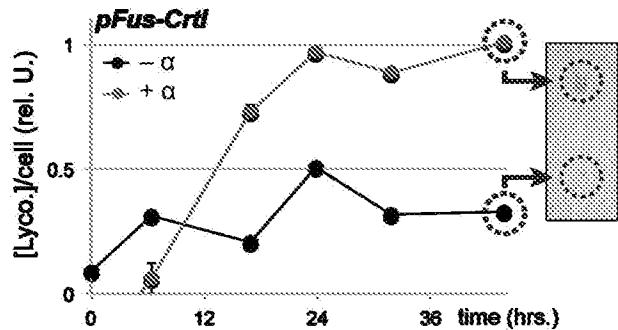
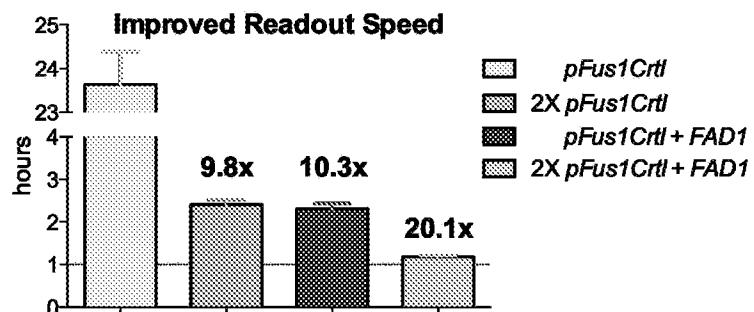
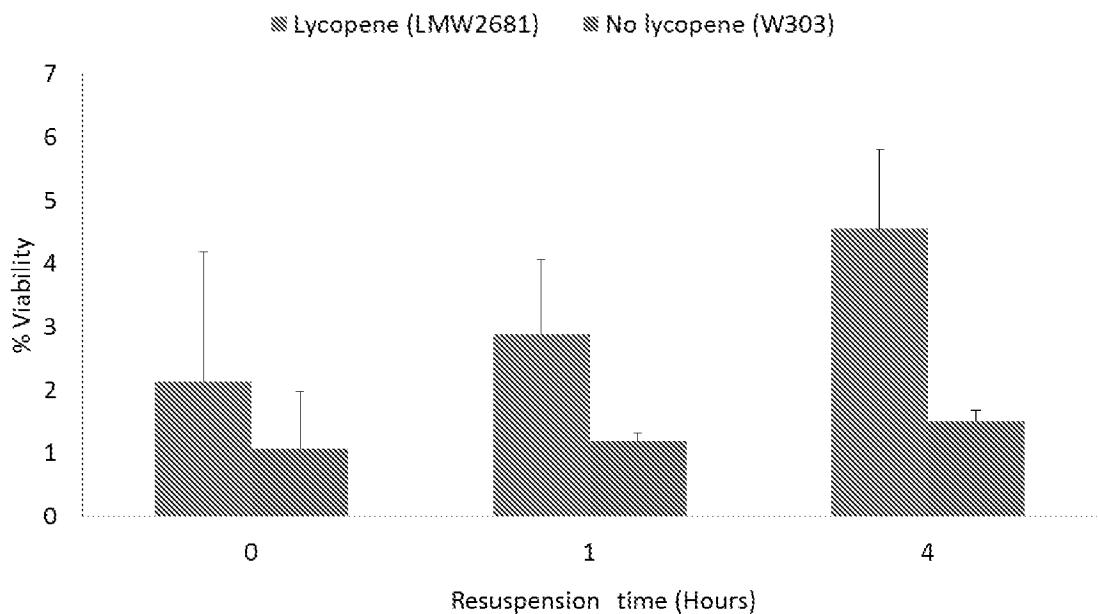
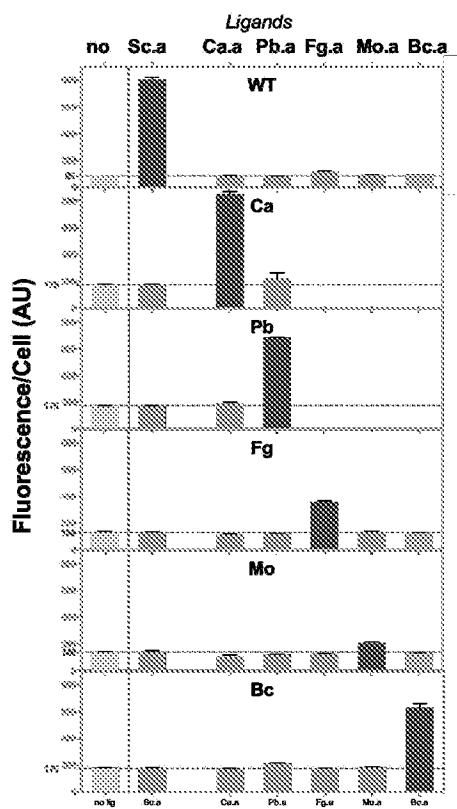
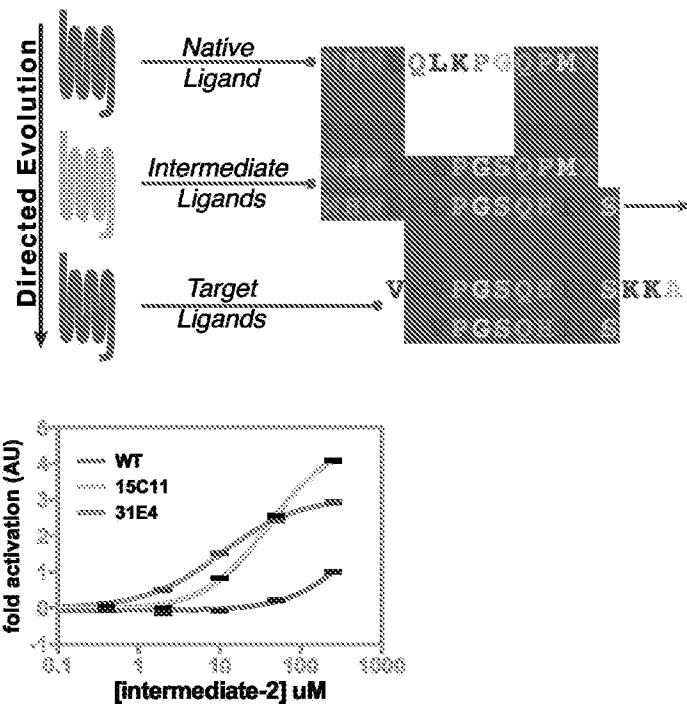


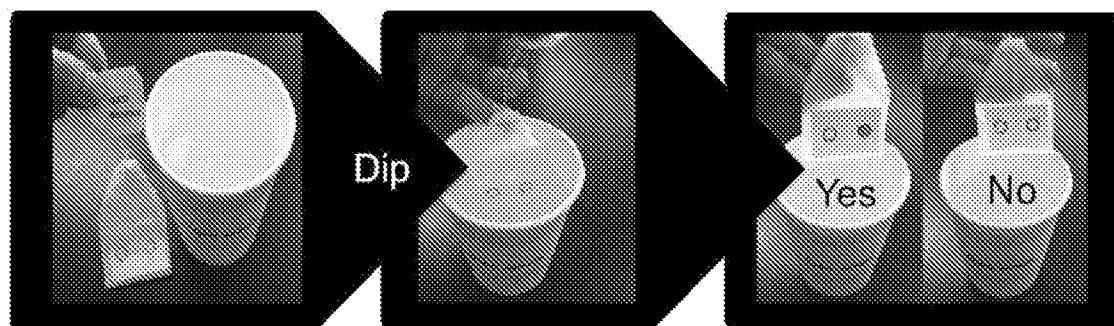
Figure 4B. Improvement of lycopene readout speed in optimal laboratory conditions.

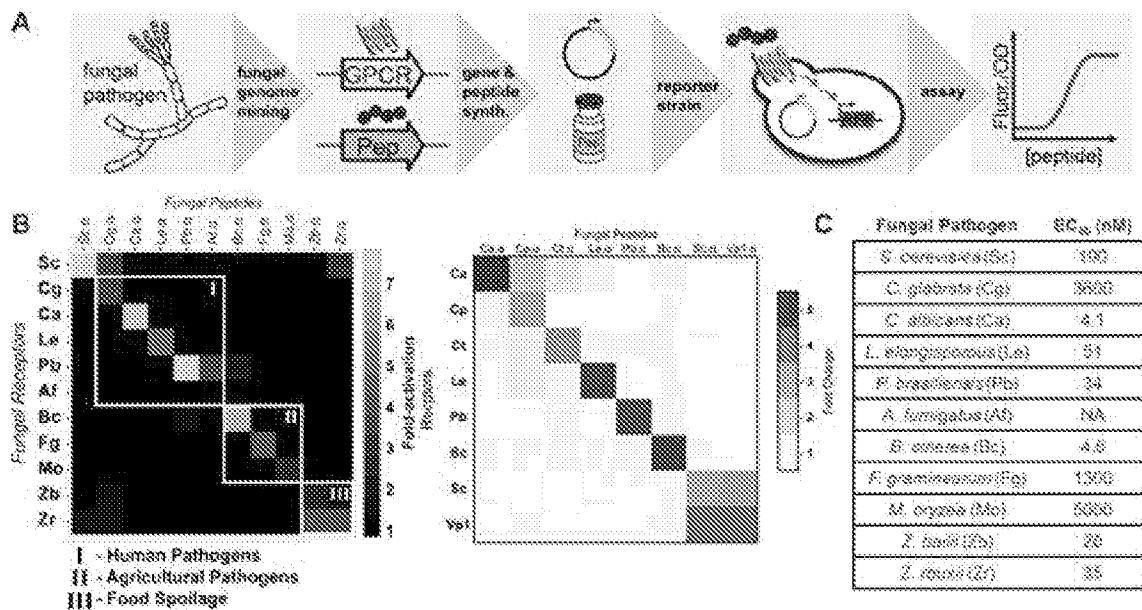


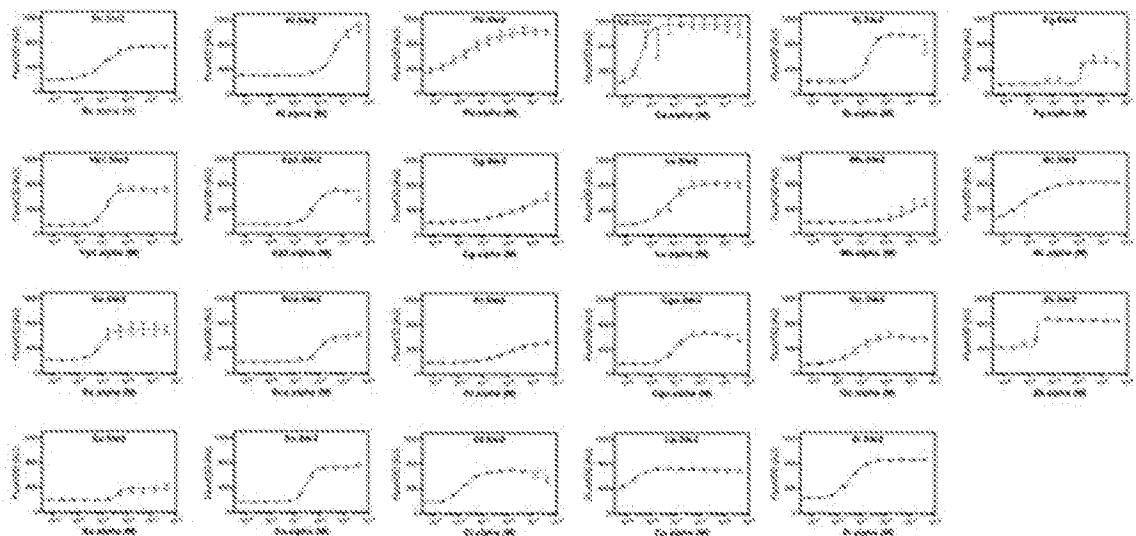
**FIGURE 5**

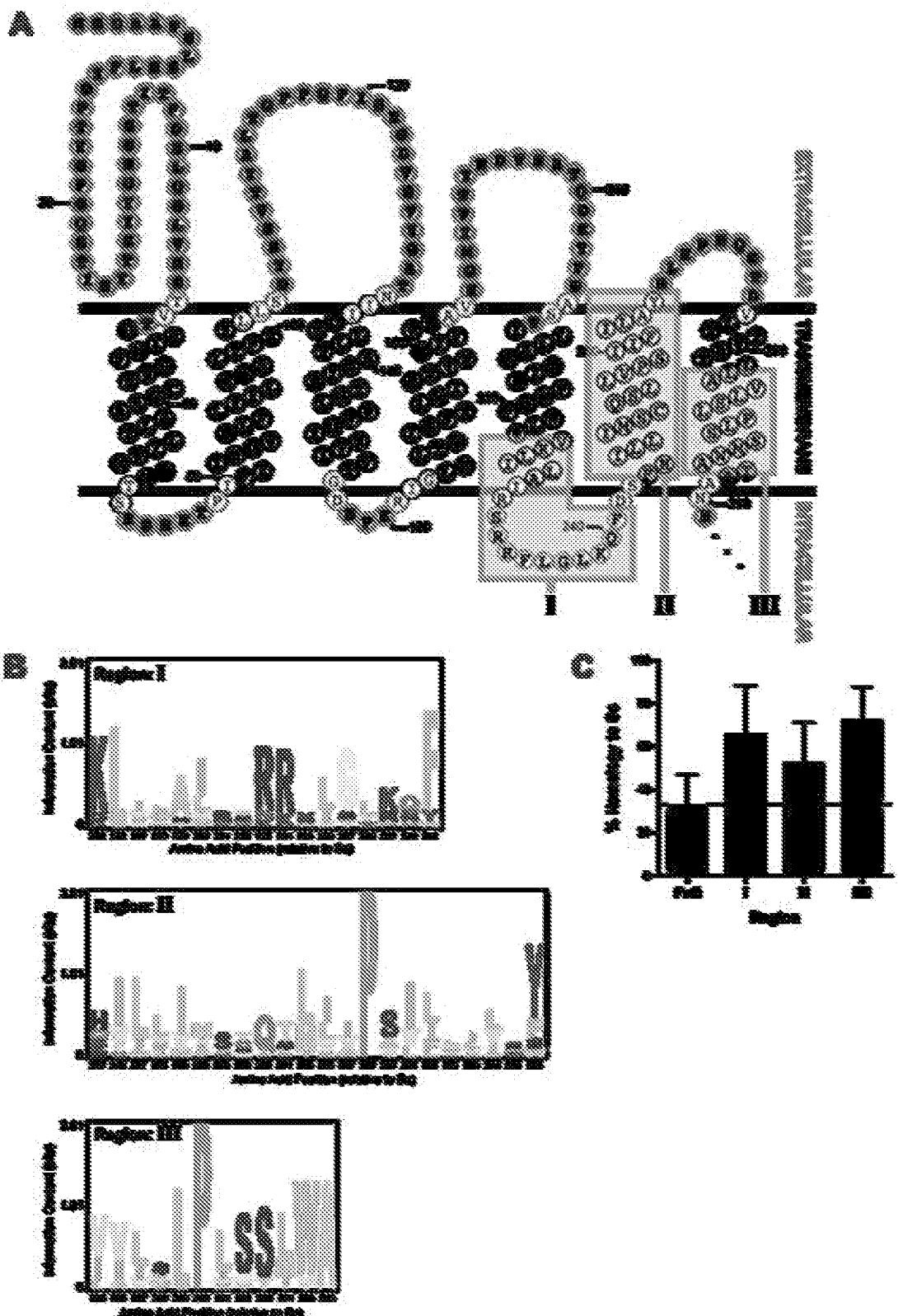
**FIGURE 6**

**FIGURE 7**

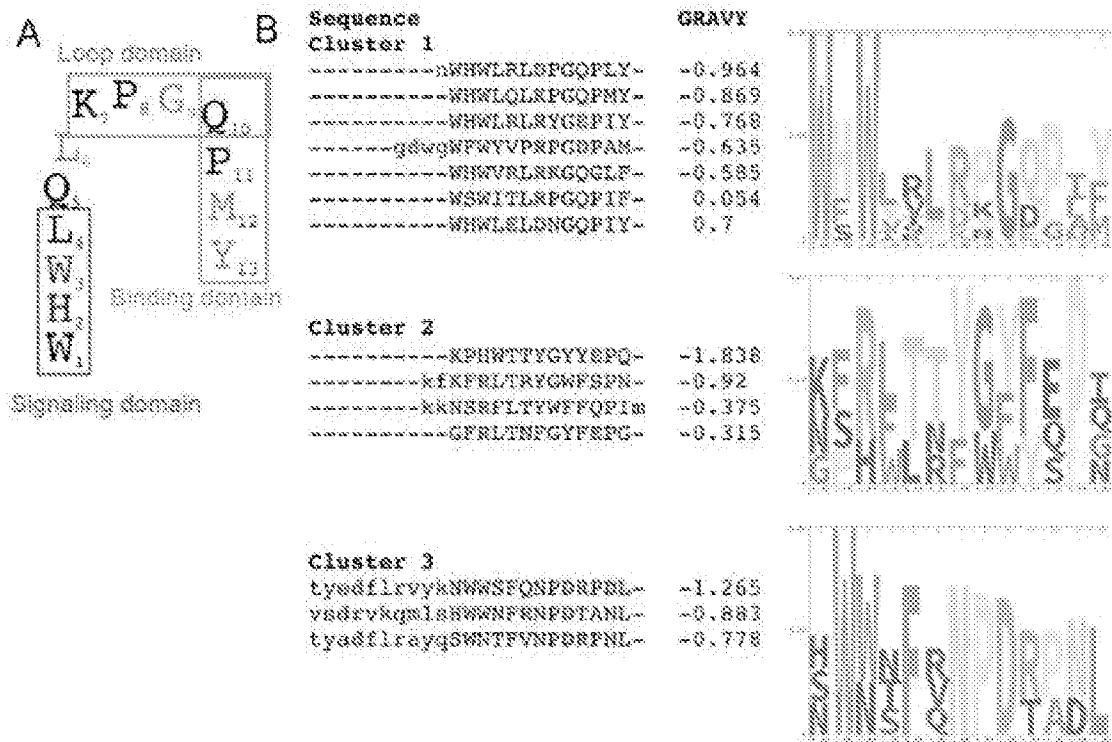
**FIGURE 8**

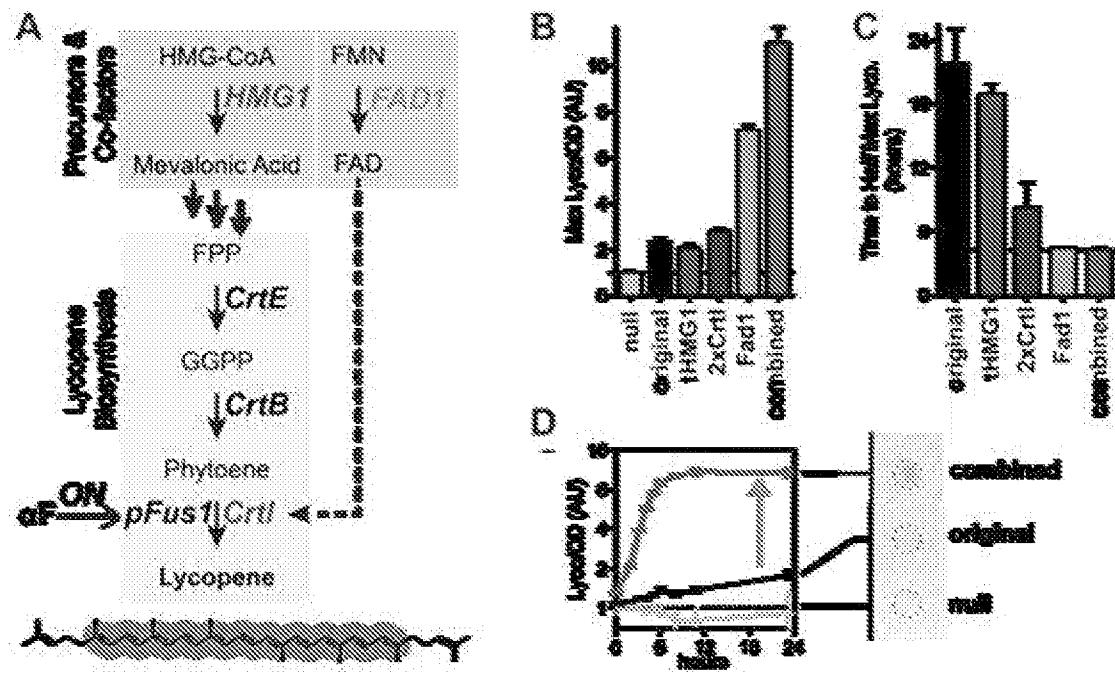
**FIGURES 9A-9C**

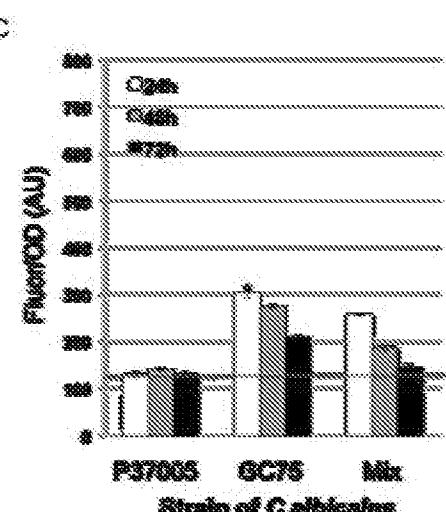
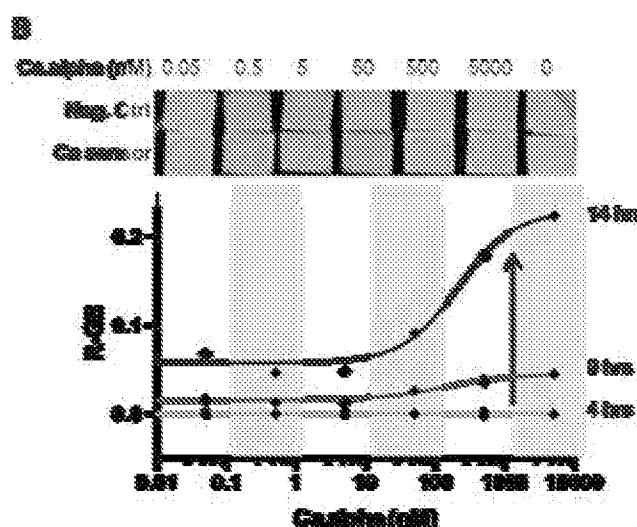
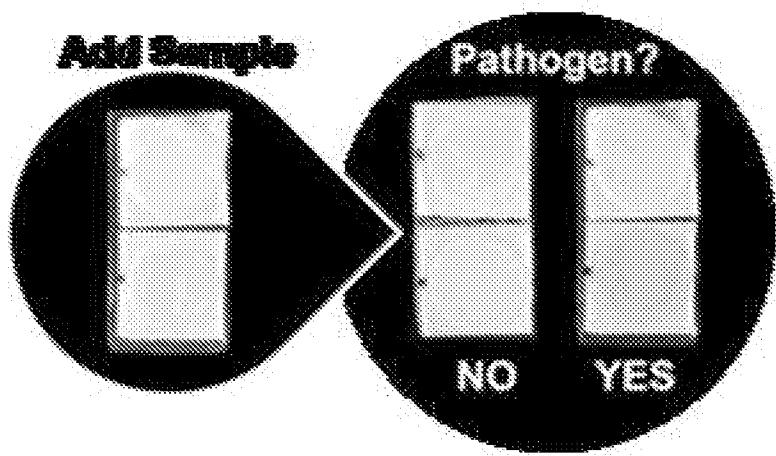
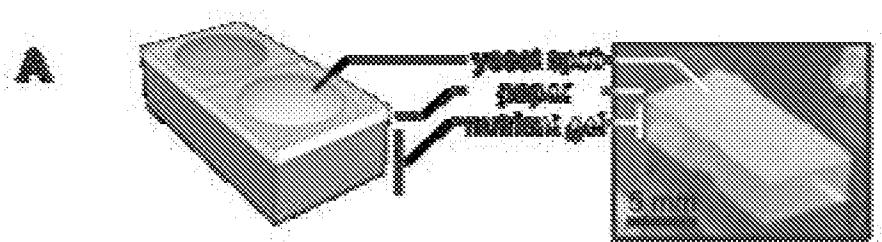
**FIGURE 10**

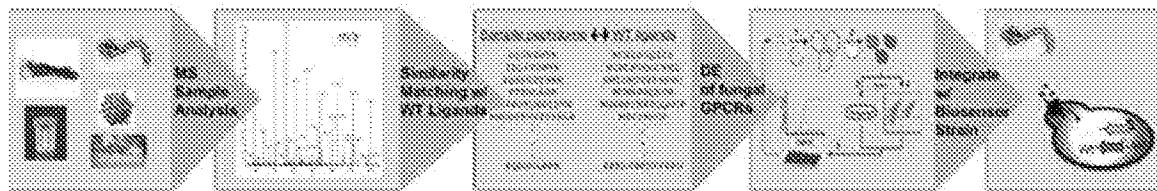
**FIGURES 11A-11C**

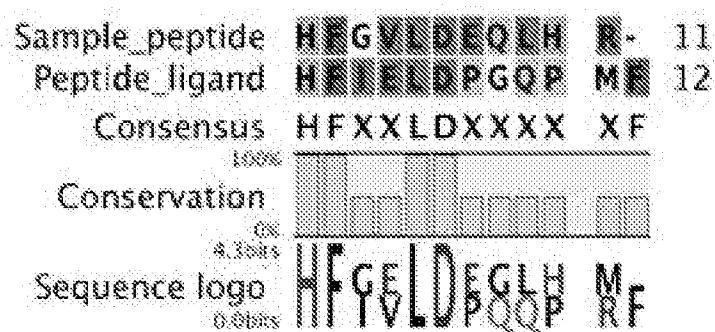
## FIGURES 12A and 12B



**FIGURES 13A-13D**

**FIGURES 14A-14C**

**FIGURE 15**

**FIGURE 16**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/61373

A. CLASSIFICATION OF SUBJECT MATTER  
IPC(8) - C12Q 1/02, C12N 15/09, G01N 33/566 (2016.01)

CPC - G01N 33/566, C12N 15/1034

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC(8): C12Q 1/02, C12N 15/09, G01N 33/566 (2016.01)  
CPC: G01N 33/566, C12N 15/1034Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPC: 435/7.21, 435/7.31, 435/287.3Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
PatBase, Google Patents, Google Scholar, Google Web, search terms: agent of interest, sensor cell, non-native Gprotein coupled receptor, GPCR, analyte, binding, human pathogenic agents, agricultural agents, industrial and model organism agents, bioterrorism agents, heavy metal contaminants, reporter, detectable, directed evolution, fungal pheromone

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,692,696 B1 (Alberte) 17 February 2004 (17.02.2004) col 3, ln 31-33, 4, ln 62-67, col 9, ln 3-11, col 16, ln 15-47, col 21, ln 33-46, col 22, ln 29-34, col 23, ln 44-67 - col 24, ln 1-18, col 28, ln 60-66	1-3, 40-42 ----- 4, 43
Y	US 2003/0008331 A1 (Lerner) 9 January 2003 (09.01.2003) para [0058]	4, 43

 Further documents are listed in the continuation of Box C. 

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
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Date of the actual completion of the international search

5 March 2016 (05.03.2016)

Date of mailing of the international search report

01 APR 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 15/61373

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a.  forming part of the international application as filed:
    - in the form of an Annex C/ST.25 text file.
    - on paper or in the form of an image file.
  - b.  furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c.  furnished subsequent to the international filing date for the purposes of international search only:
    - in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
    - on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 15/61373

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 5-39, 44-77 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.