



(51) International Patent Classification:

A61K 31/7088 (2006.01) *A61K 39/395* (2006.01)
A61K 38/00 (2006.01) *A61K 45/00* (2006.01)
A61K 39/00 (2006.01) *A61K 48/00* (2006.01)

(21) International Application Number:

PCT/US2017/040853

(22) International Filing Date:

06 July 2017 (06.07.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/358,878 06 July 2016 (06.07.2016) US

(71) Applicant: **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).

(72) Inventors: **KAAS, Christian, Schroeder**; 19 Wiget Street, Apt. 202, Boston, MA 02115 (US). **CHAVEZ, Alejandro**; 108 Sewall Avenue, Unit 4, Brookline, MA 02446 (US).

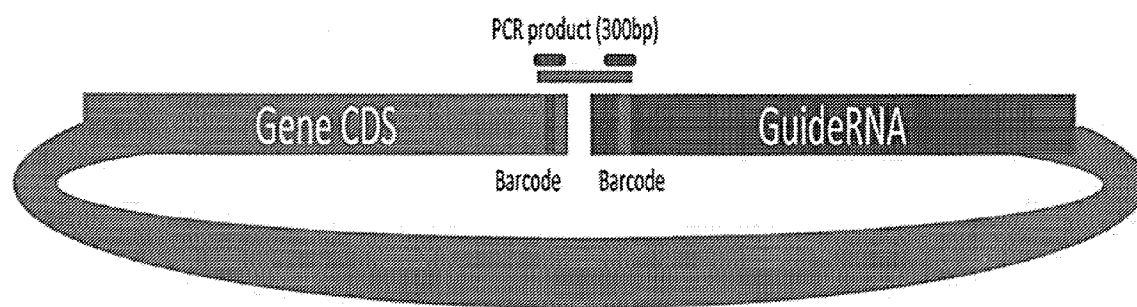
(74) Agent: **IWANICKI, John, P.**; Banner & Witcoff, Ltd., 28 State Street, Suite 1800, Boston, MA 02109 (US).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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,

(54) Title: METHODS OF SCREENING USING BARCODED LIBRARIES

FIG. 6



(57) Abstract: A method of screening is provided including providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode within its genome that is different from other proliferating cell types of the plurality and wherein each proliferating cell type includes an exogenous gene that when expressed produces an associated phenotype to the proliferating cell type which alters proliferation of the cell type, introducing a perturbation to one or more of the plurality of proliferating cell types, inducing expression of one or more of the exogenous genes, determining the relative number of unique associated barcodes after a period of proliferation, and comparing the relative number of unique associated barcodes to a control relative number of unique associated barcodes to indicate the effect of the perturbation on the one or more of the plurality of proliferating cell types.



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))*

METHODS OF SCREENING USING BARCODED LIBRARIES

RELATED APPLICATION DATA

This application claims priority to U.S. Provisional Application No. 62/358,878 filed on July 6, 2016, which is hereby incorporated herein by reference in its entirety for all purposes.

STATEMENT OF GOVERNMENT INTERESTS

This invention was made with government support under HG008525 awarded by National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Methods of screening using model organisms are known. However, existing genetic and small molecule screening methods are costly, labor intensive and non-scalable.

SUMMARY

The present disclosure provides for the use of cell proliferation and differing growth rates between different cell lines or strains to screen for candidate compounds or treatment modalities. If cells under a given set of conditions are exhibiting growth rates below the maximum possible growth rate, for example, because of the presence of a toxic protein associated with a disease state, and a given cell line or variant is able to exhibit a higher growth rate in response to a given permutation, then the cell line or variant will dominate the cell pool over time and the permutation will be identified as a candidate for treatment of the disease associated with the toxic protein.

A proliferating cell type is genetically modified to include an exogenous gene to produce an exogenous protein resulting in an altered cell type. The altered cell type is analyzed to determine a phenotype resulting from the exogenous protein. The proliferating

normal or healthy cell type (unaltered cell type) or the altered cell type may also include a nucleic acid barcode unique to the cell type. The nucleic acid barcode may be sequenced and counted to determine the number of cells representing the proliferating cell type or the altered cell type. According to one aspect a plurality of altered cell types may be provided with each altered cell type being genetically modified to include a different exogenous gene to produce an exogenous protein and a nucleic acid barcode unique to each altered cell type. According to one aspect, an altered cell type exhibits a phenotype produced by the exogenous protein. In this manner, the plurality of altered cell types (having associated phenotypes) may be sequenced and the nucleic acid barcodes counted to determine the relative number of cells in each altered cell type.

The exogenous protein is responsible for a phenotype in the cell type, such as reduced growth. The exogenous gene and its associated protein may be linked to a particular disease or condition. Accordingly, the proliferating cell type that is genetically modified to express an exogenous gene to produce an exogenous protein provides a model system to study the exogenous gene and its associated exogenous protein to identify compounds or treatment modalities that reverse or alter the phenotype. According to one aspect, the exogenous protein is toxic to the cell type such that it results in reduced or slowed or inhibited growth of the cell type. In this manner, the exogenous protein is said to be toxic to the cell type. The unaltered or normal cell type exhibits a much faster growth rate compared to the altered cell type where growth or proliferation is impaired. When the unaltered or normal cell type is mixed or placed with the altered cell type, the unaltered cell type overtakes the altered cell type after a proliferation period. However, the altered cell type may be contacted with a candidate compound or otherwise subjected to a permutation event, such as modulation or editing of a target nucleic acid, and the altered cell type growth may be determined. If the candidate compound or other permutation event counters the toxic effect of the exogenous

protein, then the altered cell type grows at an increased rate compared to the altered cell type without the candidate compound or other permutation event, thereby identifying a possible treatment for the disease state associated with the exogenous protein. In this manner, a plurality of altered cell types each with its own different exogenous gene and its own unique barcode may be provided in a combination or mixture which may then be subjected to a candidate compound or other permutation event. After a suitable growth period after the permutation, the combination or mixture may be sequenced and the plurality of barcodes counted and the number of cells of each altered cell type determined. In this manner, cell types that have increased in number identify the candidate compound or other permutation event as a possible treatment modality since the toxic effect of the exogenous protein was rescued or altered. Accordingly, a method is provided for screening factors that enable the rescue of growth defects induced by various toxic proteins to identify drugs or genes that influence toxicity of the exogenous protein.

According to the present disclosure, a method of screening is provided that includes providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode within its genome that is different from other proliferating cell types of the plurality and wherein each proliferating cell type includes an exogenous gene that when expressed produces an associated phenotype to the proliferating cell type which alters proliferation of the cell type, introducing a perturbation to one or more of the plurality of proliferating cell types, inducing expression of one or more of the exogenous genes, determining the relative number of unique associated barcodes after a period of proliferation, and comparing the relative number of unique associated barcodes to a control relative number of unique associated barcodes to indicate the effect of the perturbation on the one or more of the plurality of proliferating cell types. According to one aspect, wherein expression of the exogenous gene produces an associated phenotype to the

proliferating cell type which reduces proliferation of the cell type. According to one aspect, the endogenous gene is a toxicity gene that when expressed causes toxicity to the proliferating cell type thereby reducing proliferation of the proliferating cell type. According to one aspect, the perturbation is a chemical perturbation. According to one aspect, the perturbation is a genetic perturbation. According to one aspect, the control relative number of unique associated barcodes is determined by providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode that is different from other proliferating cell types of the plurality and wherein each proliferating cell type has an exogenous gene that when expressed produces an associated phenotype to the proliferating cell type which alters proliferation of the proliferating cell type, inducing expression of one or more of the exogenous genes, and determining the control relative number of unique associated barcodes after a period of proliferation. According to one aspect, the perturbation is accomplished by introduction of a candidate compound to the combination of the plurality of proliferating cell types. According to one aspect, the the perturbation is accomplished by introduction of a candidate drug compound to the combination of the plurality of proliferating cell types, wherein the candidate drug compound inhibits the associated phenotype. According to one aspect, the perturbation is accomplished by inducing expression of a target gene within the combination of the plurality of proliferating cell types. According to one aspect, the perturbation is accomplished by reducing expression of a target gene within the combination of the plurality of proliferating cell types. According to one aspect, the relative number of unique associated barcodes is determined by sequencing of the genome of the plurality of proliferating cell types. According to one aspect, the proliferating cell type is yeast or bacteria.

The disclosure provides a method of screening including providing a yeast strain transformed with a plurality of vectors, with each vector in the plurality comprising a first

nucleic acid sequence encoding a toxicity gene that when overexpressed causes toxicity to the yeast strain, a guide RNA having a spacer sequence complementary to a target nucleic acid sequence in the genome of the yeast strain, and a unique barcode associated with the toxicity gene and the guide RNA, and introducing an RNA guided DNA binding protein to the yeast strain, inducing expression of the first nucleic acid sequence of the plurality of vectors such that the toxicity gene is expressed and the guide RNA is produced within the yeast strain and colocalizes with the RNA guided DNA binding protein at the target nucleic acid sequence in the genome of the yeast strain and wherein the target nucleic acid is modulated, determining the relative number of unique barcodes associated with the toxicity gene and the guide RNA after a period of proliferation, and comparing the relative number of unique barcodes associated with the toxicity gene and the guide RNA to a control relative number of unique barcodes to indicate the effect of the modulation of the target nucleic acid on the yeast strain. According to one aspect, the RNA guided DNA binding protein is a Cas protein.

Further features and advantages of certain embodiments of the present invention will become more fully apparent in the following description of embodiments and drawings thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. The foregoing and other features and advantages of the present embodiments will be more fully understood from the following detailed description of illustrative embodiments taken in conjunction with the accompanying drawings in which:

Fig. 1A is a graph depicting barcode number for various yeast strains. Fig. 1B is a graph depicting barcode number for various yeast strains. Fig. 1C is a graph depicting barcode number for various yeast strains.

Fig. 2A is a graph demonstrating activation of the hnRNPA1 gene in yeast. Fig. 2B is a graph demonstrating activation of the FUS gene in yeast.

Fig. 3 is an illustration showing rescue of a yeast strain from the toxic effect of an endogenous protein by a drug or a genetic change.

Fig. 4A is a graph showing diversity of barcodes in a series of barcoded yeast strains. Fig. 4B is a graph showing average representation of the various genes within the library (as determined by barcode sequencing) plotted for two independent biological replicates with the cells being grown under non-inducing conditions. Fig. 4C is a graph showing average representation of the various genes within the library (as determined by barcode sequencing) plotted for two independent biological replicates with the cells being grown under inducing conditions.

Fig. 5A is a graph showing diversity of barcodes in a series of barcoded yeast in the presence of Lovastatin. Fig. 5B is a graph showing diversity of barcodes in a series of barcoded yeast in the presence of Celestrol.

Fig. 6 is an illustration of a plasmid including a nucleic acid encoding a disease causing gene, a barcode associated with the disease causing gene and a guide RNA associated with a target nucleic acid.

Fig. 7 is a schematic of a dual barcode detection system.

Fig. 8 is a graph of data directed to results of a library experiment using barcode sequencing to quantify the abundance of each member before and after overnight growth in inducing conditions. In addition, library members were grown either in the presence of an

empty vector or vector expressing the molecular chaperone HSP104. Error bars ± 1 s.d., $n=3$, $*p<0.01$.

DETAILED DESCRIPTION

The present disclosure provides screening methods based on alteration of cell growth or proliferation as an indication of modulating the phenotypic effect of an exogenous protein on a cell. For example, when an exogenous protein that causes cellular toxicity is expressed, one phenotype to quantify is a decrease in growth rate. Perturbations, such as chemical or small molecule or genetic editing or regulation, that ameliorate toxicity are discerned by observing a restoration of cell division. Perturbations that enhance toxicity are typified by further reductions in cell growth as compared to control conditions. The exogenous protein can be inhibited or activated, such as by introduction of one or more candidate compounds or by altering one or more genes within the cell, such as gene activation or inhibition. The growth rate (or absolute number of cells) for the cell type is determined. This may be referred to as a normal control cell growth rate. The cell type is altered to include an exogenous protein, such as by providing the cell with an exogenous gene encoding the exogenous protein, thereby producing a genetically altered cell type or strain. The cell expresses the exogenous gene and the phenotypic effect of the exogenous protein of the exogenous gene is determined. In one aspect, cell growth rate is lowered as a result of the exogenous protein. Accordingly, one phenotypic effect of an exogenous protein is a lowering or slowing of cell growth rate compared to the cell type without the exogenous protein. The growth rate of the cell type with the exogenous protein is determined. This may be referred to as an exogenous protein control cell growth rate. The cell type with the exogenous protein is then subjected to a perturbation event. Exemplary perturbation events include introduction of one or more candidate compounds, such as chemicals or small molecules or drug or biologics. Exemplary perturbation events include altering expression of one or more genes within the cell, such as

by gene activation or inhibition or gene cutting or gene nicking. The growth rate of the cell type with the exogenous protein and subjected to the perturbation event is determined. This may be referred to as a perturbation event cell growth rate. According to one aspect, the perturbation event cell growth rate is compared to the exogenous protein control cell growth rate and/or the normal control cell growth rate to determine whether the perturbation inhibits the exogenous protein, activates the exogenous protein or has no effect on the exogenous protein, as determined by cell growth rate after a proliferation or growth period. It is to be understood that the number of cells can also be determined and compared for the normal cell type, the cell type including the exogenous protein and the cell type including the exogenous protein and subjected to the perturbation. Whether the perturbation inhibits the exogenous protein, activates the exogenous protein or has no effect on the exogenous protein is based on the increase or decrease in the number of cells after a perturbation period.

The disclosure provides methods of using a combination or mixture of a plurality of proliferating cell types, such as different strains of yeast or bacteria, with each cell type (i.e., each strain of yeast or bacteria) having a particular exogenous protein. The term "cell type" may be used interchangeably with "strain." According to one aspect, the methods employ greater than 5 cell types or strains, greater than 10 cell types or strains, greater than 20 cell types or strains, greater than 30 cell types or strains, greater than 40 cell types or strains, greater than 50 cell types or strains, greater than 60 cell types or strains, greater than 70 cell types or strains, greater than 80 cell types or strains, greater than 90 cell types or strains, greater than 100 cell types or strains, greater than 150 cell types or strains, greater than 200 cell types or strains, greater than 300 cell types or strains, greater than 400 cell types or strains, greater than 500 cell types or strains, greater than 600 cell types or strains, greater than 700 cell types or strains, greater than 800 cell types or strains, greater than 900 cell types or strains, greater than 1000 cell types or strains, greater than 2000 cell types or strains,

greater than 3000 cell types or strains, and the like. Each cell type or strain including a particular exogenous protein includes a nucleic acid barcode unique to the cell type or strain. Each cell type or strain including a particular exogenous protein exhibits an altered rate of cell growth or proliferation, i.e. a lowered or slowed rate of cell growth or proliferation, compared to the cell type or strain without the exogenous protein. Determining the number of barcodes within a population of cells, such as by DNA sequencing, is representative of the number of cells in a cell type or strain in the population. In this manner, the number of barcodes is representative of the number of cells in a cell type or strain and an increase or decrease in the number of barcodes is an indication of whether a particular perturbation has inhibited or has activated or has had no effect on the exogenous protein. According to this aspect, a plurality of cell types or strains in a mixture, each having a different exogenous protein and associated growth rate, may be subjected to a particular perturbation and then the growth rates or amount of cells within a cell type or strain may be determined by sequencing the population of cells within the mixture to determine the number of barcodes. The number of barcodes indicates the number of cells of a cell type or strain present after a perturbation period and, therefore, indicates whether the growth rate of a cell type with a particular exogenous protein increased, or decreased or stayed the same in response to the particular perturbation. In this manner, many different cell types or strains with exogenous proteins may be assayed in response to a particular perturbation event.

According to one aspect, the exogenous protein is a toxicity protein, i.e., a protein that has a toxic effect on the cell type into which it is introduced or otherwise contacted. The phenotype of the toxicity protein is a lowered or slowed or declining growth rate in the altered cell type compared to the normal or healthy cell type. The toxicity protein kills the cells or otherwise inhibits proliferation. The cells with the toxic protein have a slower growth rate or lower absolute number of cells (i.e., toxicity control cell growth rate) after a

proliferation period compared to the cell type without the toxic protein. The cells including the toxic protein are subjected to a perturbation and cell growth is determined (i.e., perturbation cell growth rate) and compared to the normal control cell growth rate and/or the toxicity control cell growth rate to identify whether the perturbation has decreased toxicity, increased toxicity or has no effect on toxicity, as determined by cell growth rate or absolute number of cells determined after a proliferation period. According to one aspect, the cell type including a particular toxic protein includes a nucleic acid barcode unique to the cell type. Determining the number of barcodes within a population of cells, such as by DNA sequencing, is representative of the number of cells in the population. In this manner, the number of barcodes is representative of the number of cells and an increase or decrease in the number of barcodes is an indication of whether a particular perturbation has decreased or increased toxicity. A plurality of cell types with each cell type having an exogenous protein and a bar code unique to that cell type and different from other cell types may be assayed or screened for whether a particular perturbation rescues the cell type from the toxic effect of the exogenous protein. After the perturbation is introduced to a mixture of the plurality of cell types, the mixture is sequenced after a perturbation period and the number of unique barcodes is counted to determine whether growth of any particular cell type increased as a result of the perturbation, thereby serving as an indication that the perturbation countered the toxic effect of the exogenous protein.

According to one aspect, the disclosure provides a method of screening for an effect of a candidate compound or other permutation event on genetically altered strains of proliferating cells such as yeast strains or bacterial strains. With yeast strains being exemplary, a set of yeast strains are provided with each yeast strain being represented by a unique nucleic acid or DNA barcode. The growth or proliferation rate for a particular or normal yeast strain is determined or known such that the number of cells of a yeast strain

after a growth or proliferation period is known or determinable. Each yeast strain is genetically modified to include a particular gene that when expressed or overexpressed produces a protein that may cause toxicity to the yeast strain and thereby may reduce growth or proliferation of the yeast strain compared to the unaltered or normal yeast strain. Exemplary genes are those which are associated with human neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia, spinocerebellar ataxia and the like. The yeast strains are mixed together in a pool and the yeast strains produce the proteins associated with the particular genes and, after a proliferation or growth period, the relative number of each unique barcode within the pool of cells is analyzed, for example using amplification and sequencing methods known to those of skill in the art. The number of each unique barcode serves as a proxy for the number of cells in each yeast strain. Barcodes associated with toxic proteins will show a decrease in abundance within the population or pool of cells. The pool of altered yeast strains with each having a particular growth rate is contacted with a candidate compound or otherwise subjected to a permutation event and the relative number of each unique barcode within the pool of cells is analyzed, for example using amplification and sequencing methods known to those of skill in the art. Candidate compounds or other permutation events include chemical compounds, small molecules, drugs, biologics, gene overexpression, gene underexpression, gene knockouts, single nucleotide polymorphisms and the like. A candidate compound or other permutation event that rescues or inhibits or prevents or reduces the toxicity of a given protein to a yeast strain will prevent a decrease in the relative representation of the barcoded strain within the pool, i.e. the growth or proliferation of the altered yeast strain will increase in the presence of the candidate compound or other permutation event relative to the growth or proliferation of the altered yeast strain in the absence of the candidate compound or other permutation event at a greater rate. The change in different barcodes are determined or

examined for a plurality of candidate compounds or other permutation events to identify candidate compounds or other permutation events that influence or affect toxicity of the protein to the altered yeast strain.

EXEMPLARY PROLIFERATING CELL TYPES

Proliferating cell types or organisms useful in the methods described herein are those which proliferate at a rate sufficient to carry out experiments in a desirable period of time. The proliferating cell types or organisms are also capable of expressing a phenotype associated with an exogenous protein, such as a toxic protein. According to one aspect, exemplary proliferating cell types are capable of exhibiting a lowering of cell growth in response to the presence of an endogenous protein that is toxic to the cell type. The proliferating cell type or organism may be genetically altered to include the toxicity gene which is expressed by the cell or otherwise induced to be expressed to produce the toxic protein.

Exemplary proliferating cell types include eukaryotic cells or prokaryotic cells.

Exemplary eukaryotic cells include yeast strains or fungus strains.

Exemplary yeast strains include *Saccharomyces cerevisia* (and subtypes such as S288C, CEN.PK etc), genus *Saccharomyces* (e.g., *S. cerevisiae*, *S. bayanus*, *S. boulardii*, *S. pastorianus*, *S. rouxii* and *S. uvarum*), *Schizosaccharomyces* (e.g., *S. pombe*), *Kluveromyces* (e.g., *K. lactis* and *K. fragilis*), genus *Candida* (*C. albicans*, *C. krusei* and *C. tropicalis*) and *Pichia pastoris* and the like.

Exemplary fungus strains include *Aspergillus nidulans*, *A. oryza*, *A. niger*, *A. sojae* and the like.

Exemplary eukaryotic cells include mammalian cells.

Exemplary mammalian cells include HEK 293, Chinese Hamster Ovary cells, HEK 293F, HEK 293H, HEK 293A, HEK 293FT, HEK293T, CHO DG44, CHO-S, CHO-DXB11, Expi293F, ExpiCHO-S, T-Rex, HeLa, MCF7, COS7, NIH 3T3, U2OS, A375, A549, N2A, PGP1 iPS, BHK, Hap1, Jurkat, N0 and the like.

Exemplary prokaryotic cells include bacteria strains. Exemplary bacterial strains include *E. coli*, *B. subtilis*, *S. aureus*, *S. typhi*, *M. genitalium*, *V. cholera*, *P. putida* and the like.

EXEMPLARY EXOGENOUS GENES

Exemplary toxicity genes are those responsible for or otherwise involved in a particular disease or disease state. Exemplary toxicity genes include Abeta, Androgen receptor (AR), a-syn A30P, a-syn A53T, a-syn WT, ataxin1, Ataxin1 [Q84], ataxin3, ATXN7, C9orf72 GA100, C9orf72 GA200, C9orf72 GA50, C9orf72 GR100, C9orf72 GR50, C9orf72 PR50, CHOPS M8, CHOPS Wt, EWSR1, EWSR1c1655t, EWSR1g1532c, EWSR1g1750a, FUS WT, FUS-P525L, hnRNPA1 WT, hnRNPA1D262V, hnRNPA2B1 D290V, hnRNPA2B1WT, htt72Q, htt103Q, Htt46Q, PABPN1, SOD1 A4V, SOD1 G85R, SOD1 G93A, SOD1 WT, TAF15, TAF15c1222t, TAF15g1172a, Tau, TDP43, TDP-43 G294A, TDP-43 M337V, TDP-43 Q331K, UBQLN2, CHMP2B, PABPN1, ARX, SOX3, RUNX2, ZIC2, PHOX2B, HOXD13, HOXA13, FOXL2, ATXN2, CACNA1A, PrP, and TBP.

EXEMPLARY METHODS OF MODIFYING A CELL TYPE WITH AN EXOGENOUS NUCLEIC ACID

Cell types according to the present disclosure may be modified to include one or more exogenous nucleic acids which are expressed by the cell to produce an exogenous protein. As an example, yeast cells may be genetically modified using methods known to those of

skill in the art including by LiAc, Electroporation, Biolistic transformation as described in Kawai S, Hashimoto W, Murata K. Transformation of *Saccharomyces cerevisiae* and other fungi: Methods and possible underlying mechanism. *Bioengineered Bugs*. 2010;1(6):395-403 hereby incorporated by reference in its entirety.

AMPLIFICATION METHODS

Nucleic acids within cells of a pool of proliferating cells, such as yeast or bacteria or mammalian cells, may be amplified using methods known to those of skill in the art. Exemplary amplification methods include contacting a nucleic acid with one or more primers that specifically hybridize to the nucleic acid under conditions that facilitate hybridization and chain extension. Exemplary methods for amplifying nucleic acids include the polymerase chain reaction (PCR) (see, e.g., Mullis et al. (1986) *Cold Spring Harb. Symp. Quant. Biol.* 51 Pt 1:263 and Cleary et al. (2004) *Nature Methods* 1:241; and U.S. Patent Nos. 4,683,195 and 4,683,202), anchor PCR, RACE PCR, ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:360-364), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:1874), transcriptional amplification system (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86:1173), Q-Beta Replicase (Lizardi et al. (1988) *BioTechnology* 6:1197), recursive PCR (Jaffe et al. (2000) *J. Biol. Chem.* 275:2619; and Williams et al. (2002) *J. Biol. Chem.* 277:7790), the amplification methods described in U.S. Patent Nos. 6,391,544, 6,365,375, 6,294,323, 6,261,797, 6,124,090 and 5,612,199, isothermal amplification (e.g., rolling circle amplification (RCA), hyperbranched rolling circle amplification (HRCA), strand displacement amplification (SDA), helicase-dependent amplification (HDA), PWGA) or any other nucleic acid amplification method using techniques well known to those of skill in the art.

SEQUENCING METHODS

Nucleic acids within cells of a pool of proliferating cells, such as yeast or bacteria or mammalian cells, may be sequenced using methods known to those of skill in the art such as high throughput disclosed in Mitra (1999) *Nucleic Acids Res.* 27(24):e34; pp.1-6. Sequencing methods useful in the present disclosure include Shendure et al., Accurate multiplex polony sequencing of an evolved bacterial genome, *Science*, vol. 309, p. 1728-32. 2005; Drmanac et al., Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays, *Science*, vol. 327, p. 78-81. 2009; McKernan et al., Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding, *Genome Res.*, vol. 19, p. 1527-41. 2009; Rodrigue et al., Unlocking short read sequencing for metagenomics, *PLoS One*, vol. 28, e11840. 2010; Rothberg et al., An integrated semiconductor device enabling non-optical genome sequencing, *Nature*, vol. 475, p. 348-352. 2011; Margulies et al., Genome sequencing in microfabricated high-density picolitre reactors, *Nature*, vol. 437, p. 376-380. 2005; Rasko et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany, *N. Engl. J. Med.*, Epub. 2011; Hutter et al., Labeled nucleoside triphosphates with reversibly terminating aminoalkoxyl groups, *Nucleos. Nucleot. Nucl.*, vol. 92, p. 879-895. 2010; Seo et al., Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides, *Proc. Natl. Acad. Sci. USA.*, Vol. 102, P. 5926-5931 (2005); Olejnik et al.; Photocleavable biotin derivatives: a versatile approach for the isolation of biomolecules, *Proc. Natl. Acad. Sci. U.S.A.*, vol. 92, p. 7590-7594. 1995; US 2009/0062129 and US 2009/0191553.

Exemplary next generating sequencing methods known to those of skill in the art include Massively parallel signature sequencing (MPSS), Polony sequencing, pyrosequencing

(454), Illumina (Solexa) sequencing by synthesis, SOLiD sequencing by ligation, Ion semiconductor sequencing (Ion Torrent sequencing), DNA nanoball sequencing, chain termination sequencing (Sanger sequencing), Heliscope single molecule sequencing, Single molecule real time (SMRT) sequencing (Pacific Biosciences) and nanopore sequencing such as is described at world wide website nanoporetech.com.

EXAMPLE I

The Number of Cells of Different Yeast Strains May Be Identified by Unique Barcodes

The present disclosure provides a screening method that uses a plurality of DNA barcoded cell lines, such as uniquely barcoded yeast strains. Each yeast strain contains a unique DNA barcode that allows determination of the number of cells of a particular yeast strain within a pool or larger population or mixture of a plurality of different barcoded yeast strains. All barcodes within the population are sequenced and counted and the relative number of cells of each yeast strain is determined. To determine if there exists a strong correlation between a given strain prevalence within a population and the prevalence of its specific barcode upon sequencing, a series of mixed barcoded populations were generated. Three yeast mixtures were generated each containing 80+ uniquely barcoded strains manually mixed at an equal ratio. To this population, an additional uniquely barcoded strain was added that was delivered at a level equal to the other strains already present within the population, 10 times higher than the other strains already present within the population, or 10 times lower than the other strains already present within the population. DNA was extracted from the population of cells and barcodes were amplified out and sequenced.

As shown in Fig. 1A, barcode representation correlated well with population structure. In particular, 83 different barcoded yeast strains were mixed together at equal ratios. Into this pool, an additional barcoded strain (noted by red line) was added at a similar ratio. The pool of cells was then lysed and barcodes were amplified from the bulk population

and subject to next generation sequencing. In the case where all barcodes (including the additional strain) were mixed at equal ratios, all barcodes within the population were present at a similar ratio to each other. As shown in Fig. 1B, the additional strain (noted by the red line) added at 10 times higher than the other strains already present within the population is shown to be enriched as indicated by its barcode. As shown in Fig. 1C, the additional strain (noted by the red line) added at 10 times lower than the other strains already present within the population is shown to be depleted as indicated by its barcode.

In a second experiment, 40 mixtures were made of 96 different healthy yeast cell strains with each strain having a unique barcode. After one day of growth, the barcodes were amplified and sequenced by a next generation sequencing method. A complex mixture of 94 unique cell lines was able to be detected in a single sample.

EXAMPLE II

Expressing Toxic Proteins Lowers Yeast Cell Growth or Proliferation

The present disclosure provides methods of genetically altering yeast strains to include an exogenous gene encoding a protein toxic to the yeast strain and a barcode associated with the toxic protein and measuring its effect on proliferation.

Aspects of the present disclosure are directed to proteins associated with disease conditions and the use of proliferating cells expressing such proteins as models for screening of candidate compounds or permutation events for their effect on such proteins. According to one aspect, a yeast strain is genetically modified to include a target gene to express a target protein and the altered cells are analyzed to determine whether any particular phenotype of the altered cell is associated with the target protein. In one aspect, the altered strain is analyzed to determine whether the protein has affected the ability of the altered strain to grow

or proliferate compared to the unaltered strain. A barcode is used to count the number of cells as a measure of determining whether the protein has affected the ability of the altered strain to grow or proliferate compared to the unaltered strain.

According to one aspect, the target protein is an exogenous protein associated with a neurodegenerative disease. There are several human neurodegenerative diseases that are caused by the accumulation of toxic aggregation prone proteins within neurons. These protein aggregates when present cause cellular dysfunction and ultimately neuronal loss. The toxicity induced by these aggregation prone proteins can be modeled within yeast by overexpressing the given toxic gene and observing its effects on growth rate.

A multiplexed screening method is provided based on the depletion of barcodes associated with toxic proteins within a pool of altered yeast strains. Three uniquely barcoded yeast strains were genetically modified to include either a control vector that expresses a benign control gene (eYFP) or one of two aggregation prone proteins hnRNPA1 or FUS associated with human neurodegenerative diseases that upon expression causes growth arrest in yeast. (see Kim, H. J. *et al.* Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* **495**, 467–473 (2013); Kwiatkowski, T. J. *et al.* Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323**, 1205–1208 (2009) and Vance, C. *et al.* Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323**, 1208–1211 (2009).) All genes were placed under galactose inducible control and therefore are not expressed when cells are grown in glucose containing media. The genes are induced upon growth in the presence of galactose. The eYFP, hnRNPA1 and FUS containing strains were mixed at 1:1, 1:50 or 1:500 ratios and grown up overnight in either non-inducing (glucose) or inducing (galactose) containing media. The relative ratios of each barcode under non-inducing (glucose) and inducing (galactose) conditions were examined.

Fig. 2A depicts results from a mixture of barcoded cells containing either eYFP or hnRNPA1 at various initial seeding ratios under glucose or galactose outgrowth. Fig. 2B depicts results from a mixture of barcoded cells containing either eYFP or FUS at various initial seeding ratios under glucose or galactose outgrowth. As shown in Figs. 2A and 2B, when placed in non-inducing conditions the relative ratio of each barcode was as expected based on the initial seeding. In contrast, when cells were grown under inducing conditions, despite the eYFP containing strain in some instances being seeded at a 500x lower starting ratio by the end of the outgrowth it was present at nearly an equal ratio or greater to the toxic gene expressing strain. Accordingly, a method is provided for examining barcodes to track growth rates of cells carrying various genetic cargo.

EXAMPLE III

Multiplexing Analysis of a Candidate Compound for Its Effect on Toxicity

The present disclosure provides methods of screening a candidate compound for its ability to inhibit the toxic effect of a protein on an altered yeast strain within a plurality of altered yeast strains. Depicted in general in Fig. 3, a library of DNA barcoded yeast strains are employed with each uniquely barcoded yeast strain being depicted by a particular color. Each of the barcodes is associated with a different gene of interest some of which cause a growth defect in yeast when expressed. In the control situation, upon expression of the various toxic genes all yeast grow poorly and so no particular strain is enriched upon induction (an equal ratio of each colored yeast strain exists at the beginning as at the end of the screen). When an exogenous drug or a genetic perturbation is introduced into the barcoded yeast strains such that it is able to rescue the slow growth phenotype of one of the strain expressing a toxic gene, the strain enriches over time (depicted by the yellow yeast that enriches over time).

To demonstrate the concept of using DNA barcodes to track growth rate, a library of DNA barcoded yeasts were associated with either control expression plasmids, expression plasmids that carry an aggregation prone protein some of which are associated with neurodegenerative diseases or a gene that causes toxicity in yeast independent of protein aggregation and is not involved in neurodegeneration. Several experiments were conducted in which all members of the library were mixed at equal amounts and the library was grown under non-inducing conditions (glucose) or in the presence of galactose (inducing condition) with or without a small molecule of interest. After overnight outgrowth, genomic DNA was extracted from each of the experimental conditions and barcodes were quantified.

With respect to Fig. 4A, a series of barcoded yeast strains each containing a different expression plasmid (denoted on x-axis) were mixed and grown overnight in glucose containing media. The diversity of barcodes within the population was then determined through next-generation sequencing and the average representation for 2-3 replicates of each gene of interest was plotted. Under glucose conditions, the library showed an equal amount of diversity among all of its members with biological replicates showing a high degree of correlation to each other. Fig. 4B depicts the average representation of the various genes within the library (as determined by barcode sequencing) plotted for two independent biological replicates where cells were grown under non-inducing conditions. Fig. 4C depicts the average representation of the various genes within the library (as determined by barcode sequencing) plotted for two independent biological replicates where cells were grown under inducing conditions. "416" and "426" refers to the plasmid background used to express each of the genes tested.

A library of barcoded yeast and their associated genes of interest were subjected to growth under inducing conditions in the presence or absence of small molecules. In Fig. 5A, Lovastatin at 2ug/ml was added to the outgrowth media. In Fig. 5B, Celestrol at 1ug/ml was

added to the outgrowth media. Growth in the presence of drug was normalized to the without drug condition and plotted relative to the no drug condition. Bars highlighted in red represent samples that showed a difference >3 standard deviations as compared to the control no drug condition. "416" and "426" refers to the plasmid background used to express each of the genes tested.

Both Lovastatin and Celestrol showed an ability to rescue the growth of some of the toxic aggregation prone proteins that were examined. For both Lovastatin and Celestrol at the concentrations that were tested, none of the proteins that cause toxicity in yeast through alternative methods outside of protein aggregation showed significant rescue nor did control strains expressing benign proteins such as eYFP and mCherry exhibit a significant change in their growth, demonstrating that the rescue associated with Lovastatin and Celestrol is specific. According to one aspect, barcoding methods as described herein are used to interrogate the effects of various genetic perturbations on the library en masse. For example, overexpression of HSP104 is able to rescue particular mutants within the population (See Fig. 8).

EXAMPLE IV

Materials and Methods

The present disclosure utilizes the following material and methods in one or more of the Examples described herein.

Yeast Strains and Plasmids

The BY4741/4742 strain background was employed for all experiments. Gateway-compatible pAG CEN/ARS (416) or 2um (426) destination vectors with galactose inducible promoters were utilized for over-expression experiments. See Alberti, S., Gitler, A. D. & Lindquist, S. A suite of Gateway® cloning vectors for high-throughput genetic analysis in

Saccharomyces cerevisiae. *Yeast* **24**, 913–919 (2007). Yeast were transformed using the Li-Ac method as previously described. See Gietz, R. D. & Schiestl, R. H. High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat. Protocols* **2**, 31–34 (2007). All strains were grown at 30°C in YDP or minimal media with appropriate auxotrophic supplementation. The library of DNA barcoded yeast strains described herein have been previously described. See Ho, C. H. *et al.* A molecular barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds. *Nat. Biotechnol.* **27**, 369–377 (2009).

Barcoded Mixed Pool Experiment

For growth competition experiments, yeast strains each with a unique DNA barcode along with containing a plasmid encoding a control or toxic protein of interest were first grown overnight to saturation in SC-URA+glucose. The various barcoded lines were then mixed in defined ratios and passaged at a 1:1000 dilution into fresh SC-URA+glucose or SC-URA+galactose media. For experiments involving small molecules, yeast were diluted into SC-URA+galactose media containing a given small molecule of interest and allowed to grow for 36 hours before genomic DNA was extracted from the population of cells.

Yeast Lysis

Yeast lysis was performed as previously described. See Lõoke, M., Kristjuhan, K. & Kristjuhan, A. Extraction of genomic DNA from yeasts for PCR-based applications. *BioTechniques* **50**, 325–328 (2011). Briefly, 300-900ul of overnight culture was spun down and resuspended in 300ul of 200mM LiAc, 1% SDS solution. Cells were then vortexed several times and incubated at 70°C for 10-15 minutes. 900ul of >95% ethanol was then added to the samples and they were inverted several times along with vortexing to mix the solution well. The samples were then spun at 15,000g for 3 minutes and the supernatant was discarded. Pellets were washed 1x with 70% ethanol, spun at 15,000g for 3 minutes and the

supernatant was again removed. The pellet was then allowed to air dry for 5-10 minutes before adding in 100ul of 10mM Tris pH 8.5 and pipetting up and down to break up the pellet. Samples were then placed in a heating block set to 37°C for 10 minutes, spun again at 15,000g for 3 minutes and then the supernatant was collected and used in downstream DNA analysis.

Barcode Quantification Using Next Generation Sequencing

Genomic DNA was extracted and barcoded using one of four internally barcoded forward primers, CTTTCCCTACACGACGCTCTTCCGATCT NNNN CACAG tttaaactaatatacacattttacgg;

CTTTCCCTACACGACGCTCTTCCGATCT NNNN GTGTT tttaaactaatatacacattttacgg;

CTTTCCCTACACGACGCTCTTCCGATCT NNNN TCTTG tttaaactaatatacacattttacgg;

CTTTCCCTACACGACGCTCTTCCGATCT NNNN AGCTC tttaaactaatatacacattttacgg; in

combination with the reverse primer,

GGAGTTCAGACGTGTGCTCTTCCGATCTaagcgccatccagtttaaagcag. Following PCR,

the amplicons were purified and normalized using SequalPrep Normalization Plate (96) kit

(Thermo Fisher). A second round of PCR was next performed using the Illumina Truseq

adapters in order to allow individual samples to be identified during subsequent data analysis.

The reads were aligned to a list of the 96 possible barcodes (see Ho, C. H. *et al.* A molecular

barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds. *Nat.*

Biotechnol. **27**, 369–377 (2009) using BWA. The depth of coverage for each barcode was

calculated for each of the samples using a custom script and normalized to the total number

of reads per sample.

Plasmid Synthesis

A landing platform allowing insertion of a disease causing gene was made by digesting pRS413-gal1:TAP-NAT with SpeI/MluI in buffer 2.1 and purifying the backbone from gel. gBlock PAC8404 containing 50bp overhangs with the plasmid was inserted into the backbone using Gibson assembly (Cat E5510S, New England Biolabs), following manufacturer's instructions to generate pC01. The alpha-synuclein (Genbank NM_000345.3) with a G157A mutation was amplified from plasmid provided by A. Gilter, Stanford University using fw: AAAAACCCCGGATTCTAGAAAAATGGATGTATTCATGAAAGGACTT, rv: AAGAAATTCGCATCGATTTTTCGCACCAGTTCGGCACATGACCATTATTAGGCTT CAGGTTCGTAGTCTTGA. The PCR product was inserted into plasmid pC01 (linearized using SpeI/MluI) using Gibson assembly (Cat E5510S, New England Biolabs), following manufacturer's instructions to generate pC08.

Yeast Transformation and Plasmid Detection

BY4741 cells were transformed with the pC08 plasmid and gDNA was purified. The barcode segment was amplified using fw: TGGTCATGTGCCGAACTGG and rv: CGAAACTTCTCCGCAGTGAAAG. The unpurified PCR product was sent for sequencing using primer TGGTCATGTGCCGAACTGG (Genewiz).

Supplemental Sequences:

gBlock PAC8404 containing the landing platform:

CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCTAGAACTAGTGTAG
GATCGATGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAA
AAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTCTT
AAGACATAAAAAACAAAAAAGCACCACCGACTCGGTGCCACTTTTTCAAGTTG

ATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCGAGACGCA
TTAATCGTCTCGGATCATTTATCTTTCACTGCGGAGAAGTTTCGAACGCCGAAAC
ATGCGCACCAACTTTCACTTCTACAGCGTTTGACCAAAATCTTTTGAACATAACA
TTGTAGGGTGTGAAAAAATGCGCACCTTTACCGCTAGCCCAAGAGGGCACTACA
AAATCTAGAGTTGTACTTCAAACGTACATGTAATCCCTTGTATATACTCGAAAGA
AAACATCAAGTTTCTGTATAAATATGAGTGAAAGCATAATCATACATTATCTTTT
CAAAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTT
TGG

Alpha-synuclein (Genbank NM_000345.3) G157A (mutation marked in BOLD and Italics)

ATGGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAGGGAGTTGTGGCTGCT
GCTGAGAAAACCAAACAGGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGG
TGTTCTCTATGTAGGCTCCAAAACCAAGGAGGGAGTGGTGCATGGTGTGACAAC
AGTGGCTGAGAAGACCAAAGAGCAAGTGACAAATGTTGGAGGAGCAGTGGTGA
CGGGTGTGACAGCAGTAGCCCAGAAGACAGTGGAGGGAGCAGGGAGCATTGCA
GCAGCCACTGGCTTTGTCAAAAAGGACCAGTTGGGCAAGAATGAAGAAGGAGCC
CCACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGACAATGAGGCTTAT
GAAATGCCTTCTGAGGAAGGGTATCAAGACTACGAACCTGAAGCCTAATAA

pC01-gall:alpha-synuclein:barcode:ADHterminator:guideRNA_expression_cassette

GACGCTCGAAGGCTTTAATTTGCGGCCGGTACCCAATTGCGCCTATAGTGAGTCG
TATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTG
GCGTTACCCAACCTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAA
TAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGG
CGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGT

TACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCT
TTCTTCCCTTCCTTTCTCGCCACGTTGCGCCGGCTTTCCCCGTCAAGCTCTAAATCG
GGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCCAAAAA
CTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC
GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGG
AACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGA
TTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTT
TAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGCATCT
GTGCGGTATTTACACCGCATAGATCCGTCGAGTTCAAGAGAAAAAAAAGAAA
AAGCAAAAAGAAAAAAGGAAAGCGCGCCTCGTTCAGAATGACACGTATAGAAT
GATGCATTACCTTGTCATCTTCAGTATCATACTGTTTCGTATACATACTTACTGACA
TTCATAGGTATACATATATACACATGTATATATATCGTATGCTGCAGCTTTAAAT
AATCGGTGTCACTACATAAGAACACCTTTGGTGGAGGGAACATCGTTGGTACCAT
TGGGCGAGGTGGCTTCTCTTATGGCAACCGCAAGAGCCTTGAACGCACTCTCACT
ACGGTGATGATCATTCTTGCCTCGCAGACAATCAACGTGGAGGGTAATTCTGCTA
GCCTCTGCAAAGCTTTCAAGAAAATGCGGGATCATCTCGCAAGAGAGATCTCCT
ACTTTCTCCCTTTGCAAACCAAGTTGACAACCTGCGTACGGCCTGTTCGAAAGAT
CTACCACCGCTCTGGAAAGTGCCTCATCCAAAGGCGCAAATCCTGATCCAAACCT
TTTTACTCCACGCGCCAGTAGGGCCTCTTTAAAAGCTTGACCGAGAGCAATCCCG
CAGTCTTCAGTGGTGTGATGGTCGTCTATGTGTAAGTCACCAATGCACTCAACGA
TTAGCGACCAGCCGGAATGCTTGGCCAGAGCATGTATCATATGGTCCAGAAACC
CTATACCTGTGTGGACGTTAATCACTTGCGATTGTGTGGCCTGTTCTGCTACTGCT
TCTGCCTCTTTTTCTGGGAAGATCGAGTGCTCTATCGCTAGGGGACCACCTTTA
AAGAGATCGCAATCTGAATCTTGGTTTCATTTGTAATACGCTTTACTAGGGCTTTC
TGCTCTGTCATCTTTGCCTTCGTTTATCTTGCCTGCTCATTTTTTAGTATATTCTTC

GAAGAAATCACATTACTTTATATAATGTATAATTCATTATGTGATAATGCCAATC
GCTAAGAAAAAAAAAAGAGTCATCCGCTAGGGGAAAAAAAAAATGAAAATCAT
TACCGAGGCATAAAAAAATATAGAGTGTACTAGAGGAGGCCAAGAGTAATAGA
AAAAGAAAATTGCGGGAAAGGACTGTGTTATGACTTCCCTGACTAATGCCGTGTT
CAAACGATACCTGGCAGTGACTCCTAGCGCTCACCAAGCTCTTAAACGGGAAT
TTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCC
GACACCCGCCAACACGCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATC
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGTCAGAGGTTTTCA
CCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTAT
AGGTAAATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACCT
ACACGCGCCTCGTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTA
TTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGA
GTTACGGAATGAAGAAAAAAAAAATAACAAAGGTTTAAAAAATTTCAACAAAA
AGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATAGATATAC
ATTCGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCTTC
TACACAGACAAGATGAAACAATTCGGCATTAAATACCTGAGAGCAGGAAGAGCAA
GATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAA
ACAAAACTATTTTTTCTTTAATTTCTTTTTTTACTTTCTATTTTTTAATTTATATATT
TATATTAAAAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCC
AGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAAT
AATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCC
CTTTTTTGCGGCATTTTGCTTCCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAG
TAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATC
TCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATGAT

GAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG
CAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCA
GTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGAT
CGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAAC
TCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG
TGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAAGTGG
CGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT
AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTG
ATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGC
CAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAA
CTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGC
ATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTT
CATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCA
AAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGAT
CAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAA
AAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCT
TTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACC
TCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT
TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACT
GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAA
GGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGG
AGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCT

CTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAA
AACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA
CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTG
AGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGA
GCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGC
CGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTG
AGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACA
CTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTTACA
CAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAA
GGGAACAAAAGCTGGAGCTCTAGTACGGATTAGAAGCCGCCGAGCGGGCGACA
GCCCTCCGACGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCGTTCT
GAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAAT
ACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAA
CCTTCAAATTAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTT
TTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAAC
AGATATATAAATGGAAAAGCTGCATAACCACTTTAACTAATACTTTCAACATTTT
CAGTTTGTATTACTTCTTATTCAAATGTCATAAAAGTATCAACAAAAAATTGTTA
ATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCTAGAACTAGT
GTAGGATCGATGCGAATTTCTTATGATTTTATGATTTTTATTATTAAATAAGTTATA
AAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAACGAAAAT
TCTTAAGACATAAAAAACAAAAAAGCACCACCGACTCGGTGCCACTTTTTCAA
GTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCGAGA
CGCATTAATCGTCTCGGATCATTTATCTTTCACTGCGGAGAAGTTTCGAACGCCG
AAACATGCGCACCAACTTTCACTTCTACAGCGTTTGACCAAAATCTTTTGAACAT
AACATTGTAGGGTGTGAAAAAATGCGCACCTTTACCGCTAGCCCAAGAGGGCAC

TACAAAATCTAGAGTTGTACTTCAAACGTACATGTAATCCCTTGTATATACTCGA
AAGAAAACATCAAGTTTCTGTATAAATATGAGTGAAAGCATAATCATAACATTATC
TTTTCAAAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAG
GTTTTGG

pC10 gal1:alpha-synuclein:barcode:ADHterminator:guideRNA_expression_cassette

GACGCTCGAAGGCTTTAATTTGCGGCCGGTACCCAATTTCGCCCTATAGTGAGTCG
TATTACGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCGTG
GCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAA
TAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGG
CGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGT
TACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCT
TTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCG
GGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAA
CTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTC
GCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGG
AACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGA
TTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTT
TAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGCATCT
GTGCGGTATTTACACCGCATAGATCCGTCGAGTTCAAGAGAAAAAAAAAAGAAA
AAGCAAAAAGAAAAAAGGAAAGCGCGCCTCGTTCAGAATGACACGTATAGAAT
GATGCATTACCTTGTCATCTTCAGTATCATACTGTTTCGTATACATACTTACTGACA
TTCATAGGTATACATATATACACATGTATATATATCGTATGCTGCAGCTTTAAAT
AATCGGTGTCACTACATAAGAACACCTTTGGTGGAGGGAACATCGTTGGTACCAT
TGGGCGAGGTGGCTTCTCTTATGGCAACCGCAAGAGCCTTGAACGCACCTCTCACT

ACGGTGATGATCATTCTTGCCTCGCAGACAATCAACGTGGAGGGTAATTCTGCTA
GCCTCTGCAAAGCTTTCAAGAAAATGCGGGATCATCTCGCAAGAGAGATCTCCT
ACTTTCTCCCTTTGCAAACCAAGTTCGACAACCTGCGTACGGCCTGTTGCAAAGAT
CTACCACCGCTCTGGAAAGTGCCTCATCCAAAGGCGCAAATCCTGATCCAAACCT
TTTTACTCCACGCGCCAGTAGGGCCTCTTTAAAAGCTTGACCGAGAGCAATCCCG
CAGTCTTCAGTGGTGTGATGGTCGTCTATGTGTAAGTCACCAATGCACTCAACGA
TTAGCGACCAGCCGGAATGCTTGGCCAGAGCATGTATCATATGGTCCAGAAACC
CTATACCTGTGTGGACGTTAATCACTTGCGATTGTGTGGCCTGTTCTGCTACTGCT
TCTGCCTCTTTTTCTGGGAAGATCGAGTGCTCTATCGCTAGGGGACCACCCTTTA
AAGAGATCGCAATCTGAATCTTGGTTTCATTTGTAATACGCTTTACTAGGGCTTTC
TGCTCTGTCTATCTTTGCCTTCGTTTATCTTGCCTGCTCATTTTTTAGTATATTCTTC
GAAGAAATCACATTACTTTATATAATGTATAATTCATTATGTGATAATGCCAATC
GCTAAGAAAAAAAAAAGAGTCATCCGCTAGGGGAAAAAAAAAAATGAAAATCAT
TACCGAGGCATAAAAAAATATAGAGTGTACTAGAGGAGGCCAAGAGTAATAGA
AAAAGAAAATTGCGGGAAAGGACTGTGTTATGACTTCCCTGACTAATGCCGTGTT
CAAACGATACCTGGCAGTGACTCCTAGCGCTCACCAAGCTCTTAAAACGGGAAT
TTATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCC
GACACCCGCCAACACGCGCTGACGCGCCCTGACGGGCTTGCTGCTCCCGGCATC
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCA
CCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTAT
AGGTTAATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACCTT
ACACGCGCCTCGTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTA
TTATTTTTTATGTTTTGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGA
GTTACGGAATGAAGAAAAAAAAAATAACAAAGGTTTAAAAAATTTCAACAAAA
AGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATAGATATAC

ATTCGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCTTC
TACACAGACAAGATGAAACAATTCGGCATTAAATACCTGAGAGCAGGAAGAGCAA
GATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAA
ACAAAACTATTTTTTCTTTAATTTCTTTTTTTACTTTCTATTTTTAATTTATATATT
TATATTAAAAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCC
AGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAAT
AATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCC
CTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAG
TAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATC
TCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGAT
GAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG
CAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCA
GTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGAT
CGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC
TCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG
TGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAAGTGG
CGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGAT
AAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTG
ATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGC
CAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAA
CTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGC
ATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTT
CATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCA

AAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGAT
CAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAA
AAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCT
TTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTA
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACC
TCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCT
TACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACT
GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAA
GGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGG
AGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCT
CTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAA
AACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA
CATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTG
AGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGA
GCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGC
CGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTG
AGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACA
CTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGATAACAATTCACA
CAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAA
GGGAACAAAAGCTGGAGCTCTAGTACGGATTAGAAGCCGCCGAGCGGGCGACA
GCCCTCCGACGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCGTTCT
GAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAAT
ACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAA
CCTTCAAATTAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTT

TTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAAC
AGATATATAAATGGAAAAGCTGCATAACCACTTTAACTAATACTTTCAACATTTT
CAGTTTGTATTACTTCTTATTCAAATGTCATAAAAGTATCAACAAAAAATTGTTA
ATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCTAGAAAAAT
GGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAGGGAGTTGTGGCTGCTGC
TGAGAAAACCAAACAGGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGGTG
TTCTCTATGTAGGCTCCAAAACCAAGGAGGGAGTGGTGCATGGTGTGACAACAG
TGGCTGAGAAGACCAAAGAGCAAGTGACAAATGTTGGAGGAGCAGTGGTGACG
GGTGTGACAGCAGTAGCCCAGAAGACAGTGGAGGGAGCAGGGAGCATTGCAGC
AGCCACTGGCTTTGTCAAAAAGGACCAGTTGGGCAAGAATGAAGAAGGAGCCCC
ACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGACAATGAGGCTTATGA
AATGCCTTCTGAGGAAGGGTATCAAGACTACGAACCTGAAGCCTAATAATGGTC
ATGTGCCGAACCTGGTGCGAAAAATCGATGCGAATTTCTTATGATTTATGATTTTT
ATTATTAAATAAGTTATAAAAAAATAAGTGTATACAAATTTTAAAGTGA CTCTT
AGGTTTTTAAAACGAAAATTCTTAAGACATAAAAAACAAAAAAGCACCACCGAC
TCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTT
TAGCTCTAAAACCGAGACGCATTAATCGTCTCGGATCATTTATCTTTCACTGCGG
AGAAGTTTCGAACGCCGAAACATGCGCACCAACTTTCACTTCTACAGCGTTTGAC
CAAAATCTTTTGAACATAACATTGTAGGGTGTGAAAAAATGCGCACCTTTACCGC
TAGCCCAAGAGGGCACTACAAAATCTAGAGTTGTACTTCAAACGTACATGTAAT
CCCTTGTATATACTCGAAAGAAAACATCAAGTTTCTGTATAAATATGAGTGAAAG
CATAATCATAATTATCTTTTCAAAGACGCGTGTACGCATGTAACATTATACTGA
AAACCTTGCTTGAGAAGGTTTTGG

EXAMPLE V

Cas/Guide RNA Genome Editing as a Perturbation of an Altered Yeast Strain

The present disclosure provides methods of screening genetic perturbations for their ability to inhibit the toxic effect of a protein on an altered yeast strain within a plurality of altered yeast strains. The genetic perturbation is carried out using a RNA-guided DNA binding protein, such as a Cas/guide RNA genome editing approach as is known in the art. A library of plasmids are created with each including an endogenous or exogenous gene which encodes an endogenous or exogenous protein, i.e. a toxic protein, a guide RNA having a spacer sequence complementary to a target nucleic acid sequence, and a barcode associated with the endogenous/exogenous protein and the guide RNA. An endogenous gene or protein is one that is native to the cell, i.e., is naturally occurring within the cell. An exogenous gene or protein is one that is not native to the cell, i.e. it is not naturally occurring within the cell, for example by it being a foreign gene that is introduced into the cell to produce a foreign protein. Yeast is transformed with the library of plasmids, the endogenous gene is expressed and the phenotype of slowed growth or proliferation is observed. The guide RNA and an RNA-guided DNA binding protein, such as a Cas enzyme for example, form a colocalization complex at a target nucleic acid, and the target nucleic acid is modulated or cut or nicked to provide the genetic permutation. After a proliferation period, the relative abundance of the barcodes is analyzed, such as by next generation sequencing, and correlated with the endogenous gene, the guide RNA and the target nucleic acid which has been modulated, cut or nicked. Instances are detected where a given barcode associated with a toxic protein and a guide RNA changing the expression of a target endogenous gene within yeast are represented more or less than the average. For those barcodes with an increased abundance or which are more highly represented, the genetic permutation caused by the modulation, nicking or cutting of the target nucleic acid is deemed to have inhibited the toxic effect of the endogenous protein, i.e. has increased tolerance towards the toxic gene being analyzed. For those barcodes with a decreased abundance or which are less highly represented, the genetic

permutation caused by the modulation, nicking or cutting of the target nucleic acid is deemed to have improved the toxic effect of the endogenous protein, i.e. has increased sensitivity towards the toxic gene being analyzed. In this manner, plasmids can be created which include guide RNA to target any nucleic acid in the yeast cell thereby providing a method of multiplexing the screening of genetic permutations for the ability to rescue a cell from the toxic effect of an exogenous protein or several or a plurality of exogenous proteins within the pool of cells.

Fig. 6 illustrates a plasmid including a nucleic acid sequence encoding an exogenous disease causing gene, a barcode unique to the exogenous disease causing gene and a guide RNA. A PCR product including the barcode and the guide RNA is produced and sequenced to identify the barcode, and thus the gene of interest, as well as the guide RNA (which may also serve as a barcode), and thus the target nucleic acid.

Fig. 7 illustrates a nucleic acid sequence encoding an exogenous disease causing gene, a barcode unique to the exogenous disease causing gene and a guide RNA. In particular, nucleic acid sequence encodes a disease causing gene (alpha-synuclein) under the control of an inducible promoter. The gene is flanked downstream by a primer binding site followed by a gene specific barcode, a minimal gene terminator and then a guideRNA expression cassette in the minus orientation. By amplifying the sequence between the two primers (283bp) both the gene barcode and the guideRNA can be sequenced allowing the user to couple the disease causing gene and the guideRNA that ameliorates the disease allowing the cell to grow. Accordingly, the genetic construct allows one to amplify out a single contiguous piece of DNA and from that amplicon determine what exogenous gene is being expressed and what genetic perturbation is being performed by the Cas enzyme to alter the effect of expressing the exogenous gene of interest.

According to certain aspects, the RNA guided DNA binding protein can be provided to a cell by genetically modifying the cell to include a nucleic acid encoding the RNA guided DNA binding protein or otherwise providing a vector or plasmid encoding the RNA guided DNA binding protein wherein the nucleic acid is expressed to produce the RNA guided DNA binding protein. The RNA guided DNA binding protein may also be provided to the cell as a native protein, i.e. not as a product of expression of a nucleic acid sequence. Methods of providing an RNA guided DNA binding protein to a cell are known in the art.

Exemplary RNA-Guided DNA Binding Proteins

The RNA-guided DNA binding protein includes an RNA-guided DNA binding protein nuclease, a thermophilic RNA-guided DNA binding protein nuclease, an RNA-guided DNA binding protein nickase, or a nuclease null RNA-guided DNA binding protein. According to one aspect, the RNA-guided DNA binding protein includes a Cas nuclease, a Cas nickase or a nuclease null Cas protein. A Cas nickase or a nuclease-null Cas protein is provided where one or more amino acids in Cas, such as Cas9, are altered or otherwise removed to provide a Cas nickase or a nuclease null Cas protein. According to one aspect, the amino acids include D10 and H840 of Cas9. See Jinek et al., *Science* 337, 816-821 (2012). RNA guided DNA binding proteins are readily known to those of skill in the art to bind to DNA for various purposes. Such DNA binding proteins may be naturally occurring. DNA binding proteins having nuclease activity are known to those of skill in the art, and include naturally occurring DNA binding proteins having nuclease activity, such as Cas9 proteins present, for example, in Type II CRISPR systems. Such Cas9 proteins and Type II CRISPR systems are well documented in the art. See Makarova et al., *Nature Reviews, Microbiology*, Vol. 9, June 2011, pp. 467-477 including all supplementary information hereby incorporated by reference in its entirety.

A Cas as described herein may be any Cas known to those of skill in the art that may be directed to a target nucleic acid using an RNA as known to those of skill in the art. The Cas may be wild type or a homolog or ortholog thereof, such as Cpf1 (*See, Zetsche, Bernd et al., Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System, Cell, Volume 163, Issue 3, pgs 759 – 771, hereby incorporated by reference in its entirety*). The Cas may be nonnaturally occurring, such as an engineered Cas as disclosed in Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., Yan, W.X. and Zhang, F., 2016. Rationally engineered Cas9 nucleases with improved specificity. *Science*, 351(6268), pp.84-88 hereby incorporated by reference in its entirety. The Cas may have one or more nucleolytic domains altered to prevent nucleolytic activity, such as with a Cas nickase or nuclease null or “dead” Cas. Aspects of the present disclosure utilize nicking to effect cutting of one strand of the target nucleic acid. A nuclease null or “dead” Cas may have a nuclease attached thereto to effect cutting, cleaving or nicking of the target nucleic acid. Such nucleases are known to those of skill in the art.

According to one aspect, the RNA-guided DNA binding protein includes a Cas9 nuclease, a Cas9 nickase or a nuclease null Cas9 protein. According to one aspect, the RNA-guided DNA binding protein includes a spCas9 nuclease, a spCas9 nickase or a nuclease null spCas9 protein. According to one aspect, the RNA-guided DNA binding proteins includes *S. pyogenes* Cas9, *S. thermophilis* Cas9, *N. meningitides* Cas9, *T. denticola* Cas9, or *S. aureus* Cas9. According to one aspect, the RNA-guided DNA binding protein includes a Cpf1 nuclease, a Cpf1 nickase or a nuclease null Cpf1 protein.

According to one aspect, the Cas9 protein, Cas9 protein nickase or nuclease null Cas9 includes homologs and orthologs thereof which retain the ability of the protein to bind to the DNA and be guided by the RNA. According to one aspect, the Cas9 protein includes the sequence as set forth for naturally occurring Cas9 from *S. thermophiles* or *S. pyogenes* or *N.*

meningitides or *T. denticola* or *S. aureus* or Cpf1 or NgAgo or C2C2 or protein sequences having at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% homology thereto and being a DNA binding protein, such as an RNA guided DNA binding protein.

An exemplary CRISPR system includes the *S. thermophiles* Cas9 nuclease (ST1 Cas9) (see Esvelt KM, et al., Orthogonal Cas9 proteins for RNA-guided gene regulation and editing, *Nature Methods*, (2013) hereby incorporated by reference in its entirety). An exemplary CRISPR system includes the *S. pyogenes* Cas9 nuclease (*Sp.* Cas9), an extremely high-affinity (see Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C. & Doudna, J.A. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature* **507**, 62-67 (2014) hereby incorporated by reference in its entirety), programmable DNA-binding protein isolated from a type II CRISPR-associated system (see Garneau, J.E. et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **468**, 67-71 (2010) and Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816-821 (2012) each of which are hereby incorporated by reference in its entirety). According to certain aspects, a nuclease null or nuclease deficient Cas 9 can be used in the methods described herein. Such nuclease null or nuclease deficient Cas9 proteins are described in Gilbert, L.A. et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* **154**, 442-451 (2013); Mali, P. et al. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature biotechnology* **31**, 833-838 (2013); Maeder, M.L. et al. CRISPR RNA-guided activation of endogenous human genes. *Nature methods* **10**, 977-979 (2013); and Perez-Pinera, P. et al. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nature methods* **10**, 973-976 (2013) each of which are hereby incorporated by reference in its entirety. The DNA locus targeted by Cas9 (and by its nuclease-deficient mutant, "dCas9" precedes a three nucleotide (nt) 5'-NGG-3' "PAM" sequence, and matches a

15–22-nt guide or spacer sequence within a Cas9-bound RNA cofactor, referred to herein and in the art as a guide RNA. Altering this guide RNA is sufficient to target Cas9 or a nuclease deficient Cas9 to a target nucleic acid. In a multitude of CRISPR-based biotechnology applications (see Mali, P., Esvelt, K.M. & Church, G.M. Cas9 as a versatile tool for engineering biology. *Nature methods* **10**, 957-963 (2013); Hsu, P.D., Lander, E.S. & Zhang, F. Development and Applications of CRISPR-Cas9 for Genome Engineering. *Cell* **157**, 1262-1278 (2014); Chen, B. et al. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* **155**, 1479-1491 (2013); Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84-87 (2014); Wang, T., Wei, J.J., Sabatini, D.M. & Lander, E.S. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* **343**, 80-84 (2014); Nissim, L., Perli, S.D., Fridkin, A., Perez-Pinera, P. & Lu, T.K. Multiplexed and Programmable Regulation of Gene Networks with an Integrated RNA and CRISPR/Cas Toolkit in Human Cells. *Molecular cell* **54**, 698-710 (2014); Ryan, O.W. et al. Selection of chromosomal DNA libraries using a multiplex CRISPR system. *eLife* **3** (2014); Gilbert, L.A. et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell* (2014); and Citorik, R.J., Mimee, M. & Lu, T.K. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nature biotechnology* (2014) each of which are hereby incorporated by reference in its entirety), the guide is often presented in a so-called sgRNA (single guide RNA), wherein the two natural Cas9 RNA cofactors (gRNA and tracrRNA) are fused via an engineered loop or linker.

Cas9 proteins are known to exist in many Type II CRISPR systems including the following as identified in the supplementary information to Makarova et al., *Nature Reviews, Microbiology*, Vol. 9, June 2011, pp. 467-477: *Methanococcus maripaludis* C7; *Corynebacterium diphtheriae*; *Corynebacterium efficiens* YS-314; *Corynebacterium glutamicum* ATCC 13032 Kitasato; *Corynebacterium glutamicum* ATCC 13032 Bielefeld;

Corynebacterium glutamicum R; *Corynebacterium kroppenstedtii* DSM 44385;
Mycobacterium abscessus ATCC 19977; *Nocardia farcinica* IFM10152; *Rhodococcus erythropolis* PR4; *Rhodococcus jostii* RHA1; *Rhodococcus opacus* B4 uid36573;
Acidothermus cellulolyticus 11B; *Arthrobacter chlorophenolicus* A6; *Kribbella flavida* DSM 17836 uid43465; *Thermomonospora curvata* DSM 43183; *Bifidobacterium dentium* Bd1;
Bifidobacterium longum DJO10A; *Slackia heliotrinireducens* DSM 20476; *Persephonella marina* EX H1; *Bacteroides fragilis* NCTC 9434; *Capnocytophaga ochracea* DSM 7271;
Flavobacterium psychrophilum JIP02 86; *Akkermansia muciniphila* ATCC BAA 835;
Roseiflexus castenholzii DSM 13941; *Roseiflexus* RS1; *Synechocystis* PCC6803;
Elusimicrobium minutum Pei191; uncultured Termite group 1 bacterium phylotype Rs D17;
Fibrobacter succinogenes S85; *Bacillus cereus* ATCC 10987; *Listeria innocua*; *Lactobacillus casei*;
Lactobacillus rhamnosus GG; *Lactobacillus salivarius* UCC118; *Streptococcus agalactiae* A909;
Streptococcus agalactiae NEM316; *Streptococcus agalactiae* 2603; *Streptococcus dysgalactiae* equisimilis GGS 124;
Streptococcus equi zooepidemicus MGCS10565; *Streptococcus gallolyticus* UCN34 uid46061; *Streptococcus gordonii* Challis subst CH1;
Streptococcus mutans NN2025 uid46353; *Streptococcus mutans*; *Streptococcus pyogenes* M1 GAS;
Streptococcus pyogenes MGAS5005; *Streptococcus pyogenes* MGAS2096; *Streptococcus pyogenes* MGAS9429;
Streptococcus pyogenes MGAS10270; *Streptococcus pyogenes* MGAS6180; *Streptococcus pyogenes* MGAS315;
Streptococcus pyogenes SSI-1; *Streptococcus pyogenes* MGAS10750; *Streptococcus pyogenes* NZ131;
Streptococcus thermophiles CNRZ1066; *Streptococcus thermophiles* LMD-9; *Streptococcus thermophiles* LMG 18311;
Clostridium botulinum A3 Loch Maree; *Clostridium botulinum* B Eklund 17B; *Clostridium botulinum* Ba4 657; *Clostridium botulinum* F Langeland;
Clostridium cellulolyticum H10; *Fingoldia magna* ATCC 29328; *Eubacterium rectale* ATCC 33656; *Mycoplasma gallisepticum*; *Mycoplasma mobile* 163K; *Mycoplasma*

penetrans; *Mycoplasma synoviae* 53; *Streptobacillus moniliformis* DSM 12112; *Bradyrhizobium BTAi1*; *Nitrobacter hamburgensis* X14; *Rhodopseudomonas palustris* BisB18; *Rhodopseudomonas palustris* BisB5; *Parvibaculum lavamentivorans* DS-1; *Dinoroseobacter shibae* DFL 12; *Gluconacetobacter diazotrophicus* Pal 5 FAPERJ; *Gluconacetobacter diazotrophicus* Pal 5 JGI; *Azospirillum B510* uid46085; *Rhodospirillum rubrum* ATCC 11170; *Diaphorobacter TPSY* uid29975; *Verminephrobacter eiseniae* EF01-2; *Neisseria meningitidis* 053442; *Neisseria meningitidis* alpha14; *Neisseria meningitidis* Z2491; *Desulfovibrio salexigens* DSM 2638; *Campylobacter jejuni* doylei 269 97; *Campylobacter jejuni* 81116; *Campylobacter jejuni*; *Campylobacter lari* RM2100; *Helicobacter hepaticus*; *Wolinella succinogenes*; *Tolumonas auensis* DSM 9187; *Pseudoalteromonas atlantica* T6c; *Shewanella pealeana* ATCC 700345; *Legionella pneumophila* Paris; *Actinobacillus succinogenes* 130Z; *Pasteurella multocida*; *Francisella tularensis novicida* U112; *Francisella tularensis holarctica*; *Francisella tularensis* FSC 198; *Francisella tularensis tularensis*; *Francisella tularensis* WY96-3418; and *Treponema denticola* ATCC 35405. The Cas9 protein may be referred by one of skill in the art in the literature as Csn1. An exemplary *S. pyogenes* Cas9 protein sequence is provided in Deltcheva et al., *Nature* 471, 602-607 (2011) hereby incorporated by reference in its entirety.

Modification to the Cas protein is a representative embodiment of the present disclosure. CRISPR systems useful in the present disclosure are described in R. Barrangou, P. Horvath, CRISPR: new horizons in phage resistance and strain identification. *Annual review of food science and technology* 3, 143 (2012) and B. Wiedenheft, S. H. Sternberg, J. A. Doudna, RNA-guided genetic silencing systems in bacteria and archaea. *Nature* 482, 331 (Feb 16, 2012) each of which are hereby incorporated by reference in their entireties.

According to one aspect, the RNA-guided DNA binding protein includes an effector moiety or group attached thereto to affect or alter or modulate a target nucleic acid. The

RNA-guided DNA binding protein may be a nuclease null RNA-guided DNA binding protein including an effector moiety or group attached thereto. An effector moiety or group includes a modulator moiety or group. Modulating may refer to the function of the effector group or moiety attached to the RNA-guided DNA binding protein or guide RNA. A target nucleic acid may be modulated by being cut or nicked by the RNA-guided DNA binding protein. A target nucleic acid may be modulated by being bound by the RNA-guided DNA binding protein. A target nucleic acid may be modulated by the function of the effector group or moiety attached to the RNA-guided DNA binding protein or the guide RNA. A target nucleic acid may be modulated by being bound by the RNA-guided DNA binding protein and the function of the effector group or moiety attached to the RNA-guided DNA binding protein or the guide RNA.

Additional exemplary RNA-guided DNA binding proteins includes Cas9 proteins include Cas9 proteins attached to, bound to or fused or connected or tethered with a functional protein or effector group or modulator such as transcriptional regulators, such as transcriptional activators or repressors, a Fok-domain, such as Fok 1, an aptamer, a binding protein, PP7, MS2 and the like. The nuclease null Cas9 protein and the guide RNA colocalize to the target nucleic acid or the nucleic acid encoding the guide RNA resulting in binding but not cleaving of the target nucleic acid. The activity or transcription of the target nucleic acid is regulated by such binding. The Cas9 protein can further comprise a transcriptional regulator or DNA modifying protein attached thereto. According to one aspect, the transcriptional regulator protein or domain is a transcriptional activator. According to one aspect, the transcriptional regulator protein or domain upregulates expression of the target nucleic acid. According to one aspect, the transcriptional regulator protein or domain is a transcriptional repressor. According to one aspect, the transcriptional regulator protein or domain downregulates expression of the target nucleic acid. Transcriptional activators and

transcriptional repressors can be readily identified by one of skill in the art based on the present disclosure. Exemplary transcriptional regulators are known to a skilled in the art and include VPR, VP16, VP64, P65 and RTA. See Zhang et al., *Nature Biotechnology* 29, 149-153 (2011) hereby incorporated by reference in its entirety. The transcriptional regulatory domains correspond to targeted loci. Accordingly, aspects of the present disclosure include methods and materials for localizing transcriptional regulatory domains to targeted loci of target nucleic acids by fusing, connecting or joining such domains to an RNA-guided DNA binding protein such as Cas or a guide RNA.

Exemplary effector groups or moieties include a detectable moiety, a transcriptional regulator, a protein domain, a nuclease, a phosphatase, deaminase, kinase, polynucleotide kinase, Uracil-DNA glycosylase, nuclease, endonuclease, exonuclease, site-specific nuclease, ligase, polymerase, recombinase, methyl-transferase, fluorescent protein, beta-galactosidase, antibody, scFv single-chain variable fragment of an antibody, nanobody, transcriptional activator, transcriptional repressor, biotin, streptavidin, aptamer, nanoparticle, gold nanoparticle, quantum dot, magnetic bead, paramagnetic particle, or oligonucleotide. Exemplary DNA-modifying enzymes are known to a skilled in the art and include Cytidine deaminases, APOBECs, FokI, endonucleases and DNases.

Exemplary Guide RNA

Embodiments of the present disclosure are directed to the use of a RNA-guided DNA binding protein/guide RNA system, such as a CRISPR/Cas system and, in particular, a guide RNA which may include one or more of a spacer sequence, a tracr mate sequence and a tracr sequence. The term spacer sequence is understood by those of skill in the art and may include any polynucleotide having sufficient complementarity with a target nucleic acid sequence to hybridize with the target nucleic acid sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. According to certain aspects, an

exemplary spacer sequence is between 10 and 30 nucleotides in length. According to certain aspects, an exemplary spacer sequence is between 15 and 25 nucleotides in length. An exemplary spacer sequence is between 18 and 22 nucleotides in length. An exemplary spacer sequence is 20 nucleotides in length.

The guide RNA may be formed from a spacer sequence covalently connected to a tracr mate sequence (which may be referred to as a crRNA) and a separate tracr sequence, wherein the tracr mate sequence is hybridized to a portion of the tracr sequence. According to certain aspects, the tracr mate sequence and the tracr sequence are connected or linked such as by covalent bonds by a linker sequence, which construct may be referred to as a fusion of the tracr mate sequence and the tracr sequence. The linker sequence referred to herein is a sequence of nucleotides, referred to herein as a nucleic acid sequence, which connect the tracr mate sequence and the tracr sequence. Accordingly, a guide RNA may be a two component species (i.e., separate crRNA and tracr RNA which hybridize together) or a unimolecular species (i.e., a crRNA-tracr RNA fusion, often termed an sgRNA).

Tracr mate sequences and tracr sequences are known to those of skill in the art, such as those described in US 2014/0356958 and as shown in Fig. 2. An exemplary tracr mate sequence and tracr sequence is N20 to N8-gttttagagctagaaatagcaagttaaaataaggctagtcggttatcaacttgaaaaagtggcaccgagtcggtgctttttt with N20-8 being the number of nucleotides complementary to a target locus of interest. According to certain aspects, the tracr mate sequence is between about 17 and about 27 nucleotides in length. According to certain aspects, the tracr sequence is between about 65 and about 75 nucleotides in length. According to certain aspects, the linker nucleic acid sequence is between about 4 and about 6. According to certain methods, two or more or a plurality of guide RNAs may be used in the practice of certain embodiments. According to certain aspects, the guide RNA is between about 10 to about 500 nucleotides. According to

one aspect, the guide RNA is between about 20 to about 100 nucleotides. According to certain aspects, the spacer sequence is between about 10 and about 500 nucleotides in length and particularly between about 14 and about 22 nucleotides in length. According to certain aspects, the tracr mate sequence is between about 10 and about 500 nucleotides in length. According to certain aspects, the tracr sequence is between about 10 and about 100 nucleotides in length. According to certain aspects, the linker nucleic acid sequence is between about 4 and about 100 nucleotides in length, and particularly between about 4 and about 6 nucleotides in length.

According to one aspect, the guide RNA includes an effector moiety or group attached thereto. An effector moiety or group includes a modulator moiety or group. Exemplary effector groups or moieties include a detectable moiety, a transcriptional regulator, a protein domain, a nuclease, a phosphatase, deaminase, kinase, polynucleotide kinase, Uracil-DNA glycosylase, nuclease, endonuclease, exonuclease, site-specific nuclease, ligase, polymerase, recombinase, methyl-transferase, fluorescent protein, beta-galactosidase, antibody, scFv single-chain variable fragment of an antibody, nanobody, transcriptional activator, transcriptional repressor, biotin, streptavidin, aptamer, nanoparticle, gold nanoparticle, quantum dot, magnetic bead, paramagnetic particle, or oligonucleotide.

Exemplary Target Nucleic Acid

Target nucleic acids as described herein include any nucleic acid sequence to which a co-localization complex as described herein can be useful to either cut, nick or regulate or modulate. Target nucleic acids include nucleic acid sequences, such as genomic nucleic acids, such as genes, capable of being expressed into proteins. For purposes of the present disclosure, a co-localization complex can bind to or otherwise co-localize with the target nucleic acid at or adjacent or near the target nucleic acid and in a manner in which the co-localization complex may have a desired effect on the target nucleic acid. One of skill based

on the present disclosure will readily be able to identify or design guide RNAs and Cas9 proteins which co-localize to a target nucleic acid. One of skill will further be able to identify effector groups or modulators or transcriptional regulator proteins or domains which likewise co-localize to a target nucleic acid.

Claims:

1. A method of screening comprising
providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode within its genome that is different from other proliferating cell types of the plurality and wherein each proliferating cell type includes an exogenous gene that when expressed produces an associated phenotype to the proliferating cell type which alters proliferation of the cell type,
introducing a perturbation to one or more of the plurality of proliferating cell types,
inducing expression of one or more of the exogenous genes,
determining the relative number of unique associated barcodes after a period of proliferation, and
comparing the relative number of unique associated barcodes to a control relative number of unique associated barcodes to indicate the effect of the perturbation on the one or more of the plurality of proliferating cell types.
2. The method of claim 1 wherein expression of the exogenous gene produces an associated phenotype to the proliferating cell type which reduces proliferation of the cell type.
3. The method of claim 1 wherein the endogenous gene is a toxicity gene that when expressed causes toxicity to the proliferating cell type thereby reducing proliferation of the proliferating cell type.
4. The method of claim 1 wherein the perturbation is a chemical perturbation.

5. The method of claim 1 wherein the perturbation is a genetic perturbation.
6. The method of claim 1 wherein the control relative number of unique associated barcodes is determined by providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode that is different from other proliferating cell types of the plurality and wherein each proliferating cell type has an exogenous gene that when expressed produces an associated phenotype to the proliferating cell type which alters proliferation of the proliferating cell type,
inducing expression of one or more of the exogenous genes, and
determining the control relative number of unique associated barcodes after a period of proliferation.
7. The method of claim 1 wherein the perturbation is accomplished by introduction of a candidate compound to the combination of the plurality of proliferating cell types.
8. The method of claim 1 wherein the perturbation is accomplished by introduction of a candidate drug compound to the combination of the plurality of proliferating cell types, wherein the candidate drug compound inhibits the associated phenotype.
9. The method of claim 1 wherein the perturbation is accomplished by inducing expression of a target gene within the combination of the plurality of proliferating cell types.

10. The method of claim 1 wherein the perturbation is accomplished by reducing expression of a target gene within the combination of the plurality of proliferating cell types.

11. The method of claim 1 wherein the relative number of unique associated barcodes is determined by sequencing of the genome of the plurality of proliferating cell types.

12. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of yeast strains.

13. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of bacterial strains.

14. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of mammalian cell lines.

15. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of human cell lines.

16. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of neuronal cell lines.

17. The method of claim 1 wherein the plurality of proliferating cell types is a plurality of human neuronal cell lines.

18. The method of claim 1 wherein the proliferating cell type is a quickly proliferating organism or cell line.

19. The method of claim 1 wherein the proliferating cell type is a member selected from the group consisting of HEK 293, Chinese Hamster Ovary cells, HEK 293F, HEK 293H, HEK 293A, HEK 293FT, HEK293T, CHO DG44, CHO-S, CHO-DXB11, Expi293F, ExpiCHO-S, T-Rex, HeLa, MCF7, COS7, NIH 3T3, U2OS, A375, A549, N2A, PGP1 iPS, BHK, Hap1, Jurkat, and N0.

20. The method of claim 1 wherein the exogenous gene is a member selected from the group consisting of Abeta, Androgen receptor (AR), a-syn A30P, a-syn A53T, a-syn WT, ataxin1, Ataxin1 [Q84], ataxin3, ATXN7, C9orf72 GA100, C9orf72 GA200, C9orf72 GA50, C9orf72 GR100, C9orf72 GR50, C9orf72 PR50, CHOPS M8, CHOPS Wt, EWSR1, EWSR1c1655t, EWSR1g1532c, EWSR1g1750a, FUS WT, FUS-P525L, hnRNPA1 WT, hnRNPA1D262V, hnRNPA2B1 D290V, hnRNPA2B1WT, htt72Q, htt103Q, Htt46Q, PABPN1, SOD1 A4V, SOD1 G85R, SOD1 G93A, SOD1 WT, TAF15, TAF15c1222t, TAF15g1172a, Tau, TDP43, TDP-43 G294A, TDP-43 M337V, TDP-43 Q331K, UBQLN2, CHMP2B, PABPN1, ARX, SOX3, RUNX2, ZIC2, PHOX2B, HOXD13, HOXA13, FOXL2, ATXN2, CACNA1A, PrP, and TBP.

21. A method of screening comprising
providing a combination of a plurality of proliferating cell types wherein each proliferating cell type has a unique associated barcode within its genome that is different from other proliferating cell types of the plurality and wherein each proliferating cell type includes a toxicity gene that when overexpressed causes toxicity to the proliferating cell type,

introducing a perturbation to one or more of the plurality of proliferating cell types,
inducing expression of one or more of the toxicity genes,
determining the relative number of unique associated barcodes after a period of proliferation, and

comparing the relative number of unique associated barcodes to a control relative number of unique associated barcodes to indicate the effect of the perturbation on the one or more of the plurality of proliferating cell types.

22. A method of screening comprising

providing a yeast strain transformed with a plurality of vectors, with each vector in the plurality comprising a first nucleic acid sequence encoding a toxicity gene that when overexpressed causes toxicity to the yeast strain, a guide RNA having a spacer sequence complementary to a target nucleic acid sequence in the genome of the yeast strain, and a unique barcode associated with the toxicity gene and the guide RNA, and

introducing an RNA guided DNA binding protein to the yeast strain,

inducing expression of the first nucleic acid sequence of the plurality of vectors such that the toxicity gene is expressed and the guide RNA is produced within the yeast strain and colocalizes with the RNA guided DNA binding protein at the target nucleic acid sequence in the genome of the yeast strain and wherein the target nucleic acid is modulated,

determining the relative number of unique barcodes associated with the toxicity gene and the guide RNA after a period of proliferation, and

comparing the relative number of unique barcodes associated with the toxicity gene and the guide RNA to a control relative number of unique barcodes to indicate the effect of the modulation of the target nucleic acid on the yeast strain.

23. The method of claim 22 wherein the RNA guided DNA binding protein is a Cas protein.

24. The method of claim 22 wherein the RNA guided DNA binding protein is a Cas9 protein.

25. The method of claim 22 wherein the RNA guided DNA binding protein is a Cas9 enzyme, a Cas9 nickase or a nuclease null Cas9.

26. The method of claim 22 wherein the RNA guided DNA binding protein is *S. thermophiles* Cas9, *S. pyogenes* Cas9, *N. meningitides* Cas9, *T. denticola* Cas9, *S. aureus* Cas9 or Cpf1, NgAgo, or C2C2.

27. The method of claim 22 wherein the toxicity gene is a member selected from the group consisting of Abeta, Androgen receptor (AR), a-syn A30P, a-syn A53T, a-syn WT, ataxin1, Ataxin1 [Q84], ataxin3, ATXN7, C9orf72 GA100, C9orf72 GA200, C9orf72 GA50, C9orf72 GR100, C9orf72 GR50, C9orf72 PR50, CHOPS M8, CHOPS Wt, EWSR1, EWSR1c1655t, EWSR1g1532c, EWSR1g1750a, FUS WT, FUS-P525L, hnRNPA1 WT, hnRNPA1D262V, hnRNPA2B1 D290V, hnRNPA2B1WT, htt 72Q, htt103Q, Htt46Q, PABPN1, SOD1 A4V, SOD1 G85R, SOD1 G93A, SOD1 WT, TAF15, TAF15c1222t, TAF15g1172a, Tau, TDP43, TDP-43 G294A, TDP-43 M337V, TDP-43 Q331K, UBQLN2, CHMP2B, PABPN1, ARX, SOX3, RUNX2, ZIC2, PHOX2B, HOXD13, HOXA13, FOXL2, ATXN2, CACNA1A, PrP, and TBP.

28. The method of claim 22 wherein the modulation of the target nucleic acid results from cutting or nicking of the target nucleic acid.

29. The method of claim 22 wherein the modulation of the target nucleic acid results from activating or repressing the target nucleic acid.

30. The method of claim 22 wherein the modulation of the target nucleic acid results from binding of the co-localization complex to the target nucleic acid.

31. The method of the claim 22 wherein the RNA guided DNA binding protein includes an effector group or modulator group bound thereto.

32. The method of the claim 22 wherein the guide RNA includes an effector group or modulator group bound thereto.

33. The method of claim 22 wherein the proliferating cell type is a member of the group consisting of genus *Saccharomyces*, genus *Schizosaccharomyces*), genus *Kluveromyces*, genus *Candida* and *Pichia pastoris*.

34. The method of claim 22 wherein the proliferating cell type is a member of the group consisting of *Aspergillus nidulans*, *A. oryza*, *A. niger*, and *A. sojae*.

35. The method of claim 22 wherein the proliferating cell type is a member of the group consisting of *E. coli*, *B. subtilis*, *S. aureus*, *S. typhi*, *M. genitalium*, *V. cholera*, and *P. putida*.

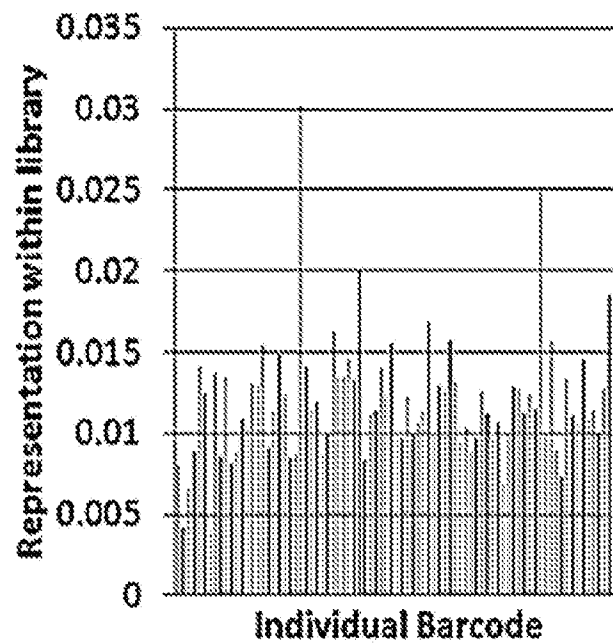
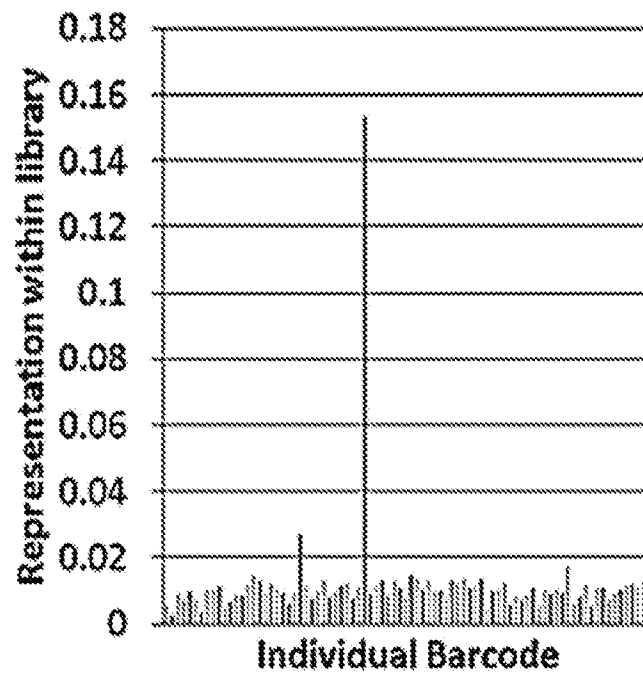
FIG. 1A**FIG. 1B**

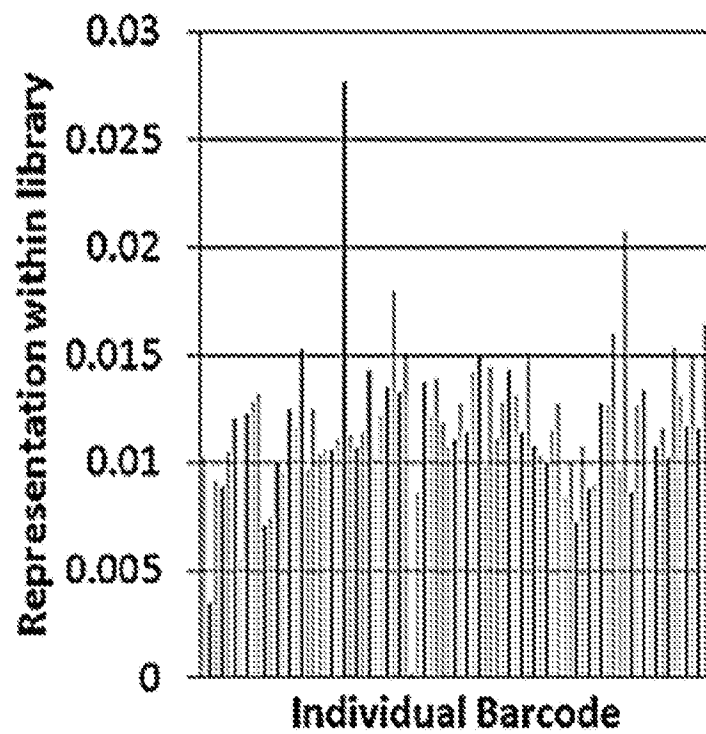
FIG. 1C

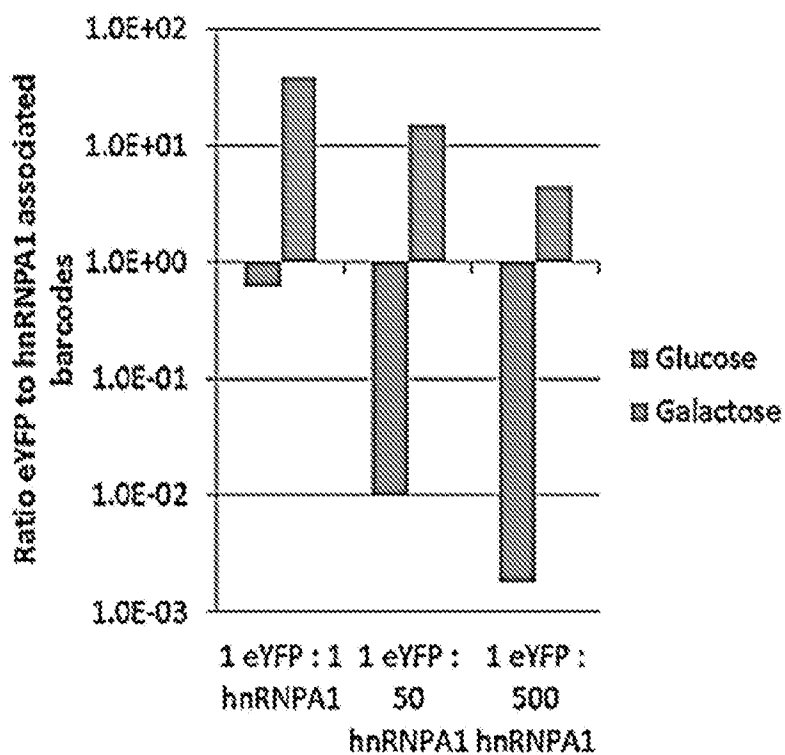
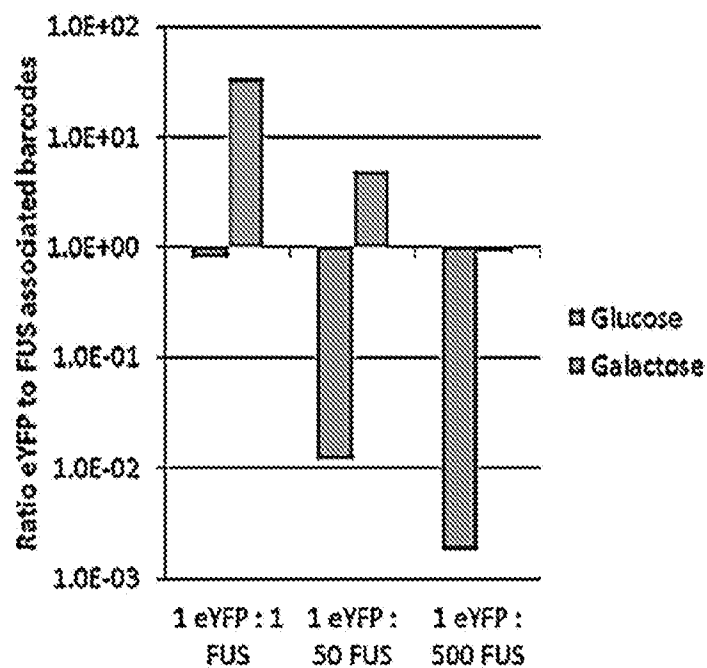
FIG. 2A**FIG. 2B**

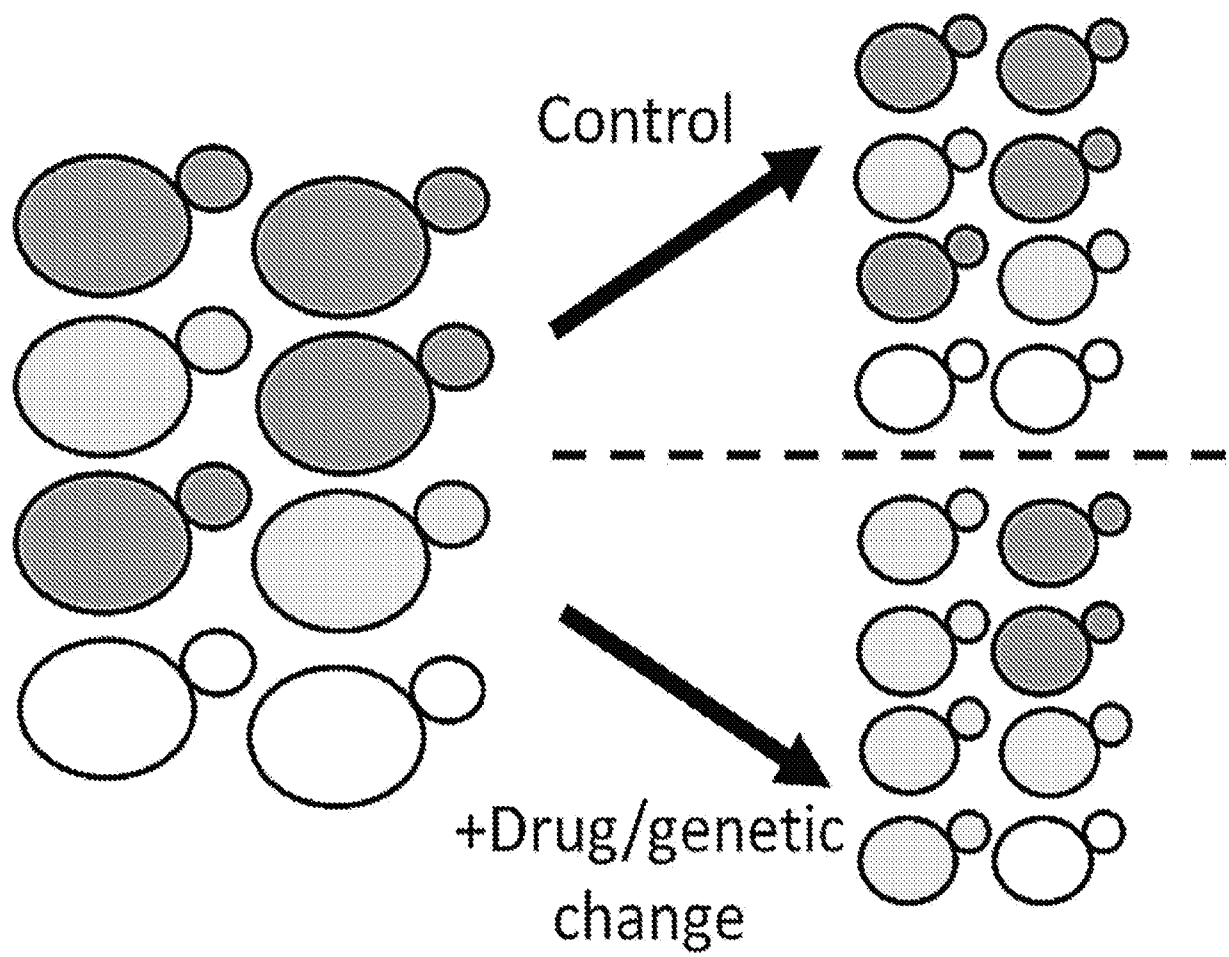
FIG. 3

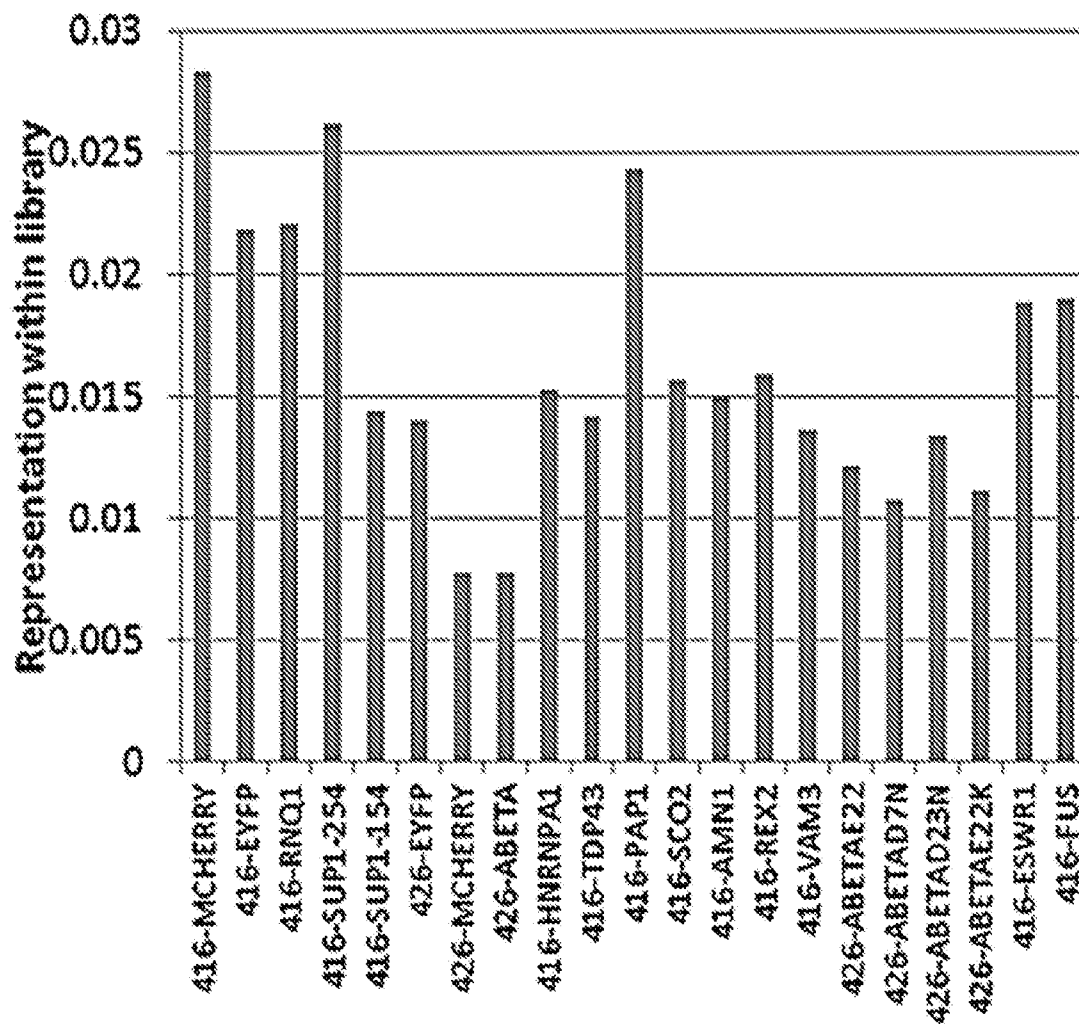
FIG. 4A

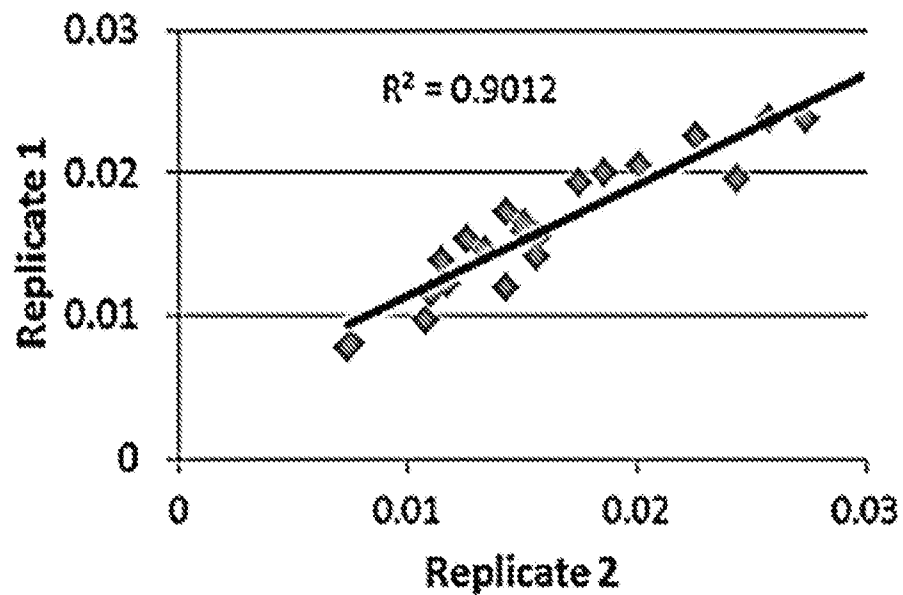
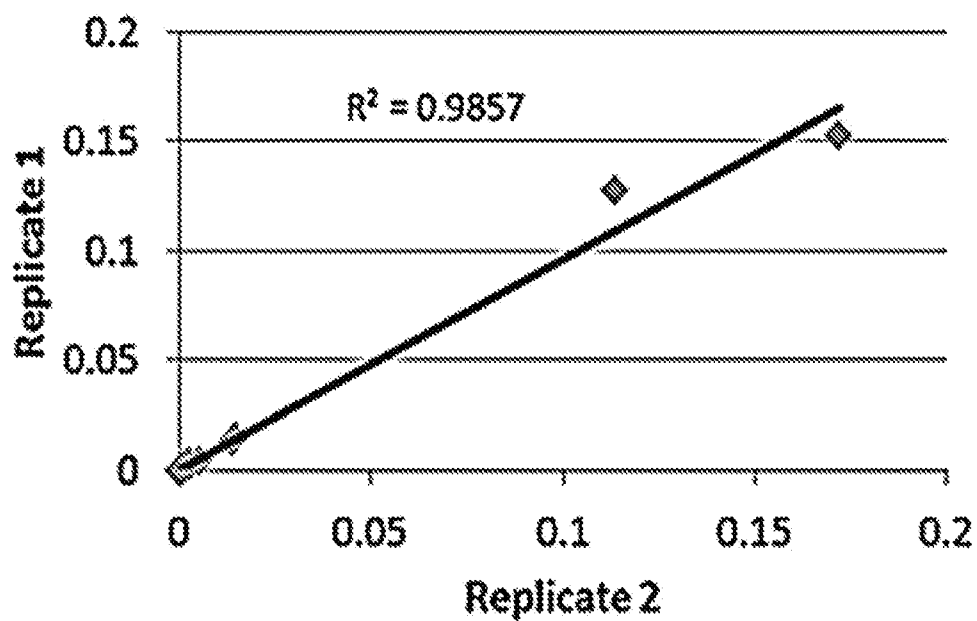
FIG. 4B**FIG. 4C**

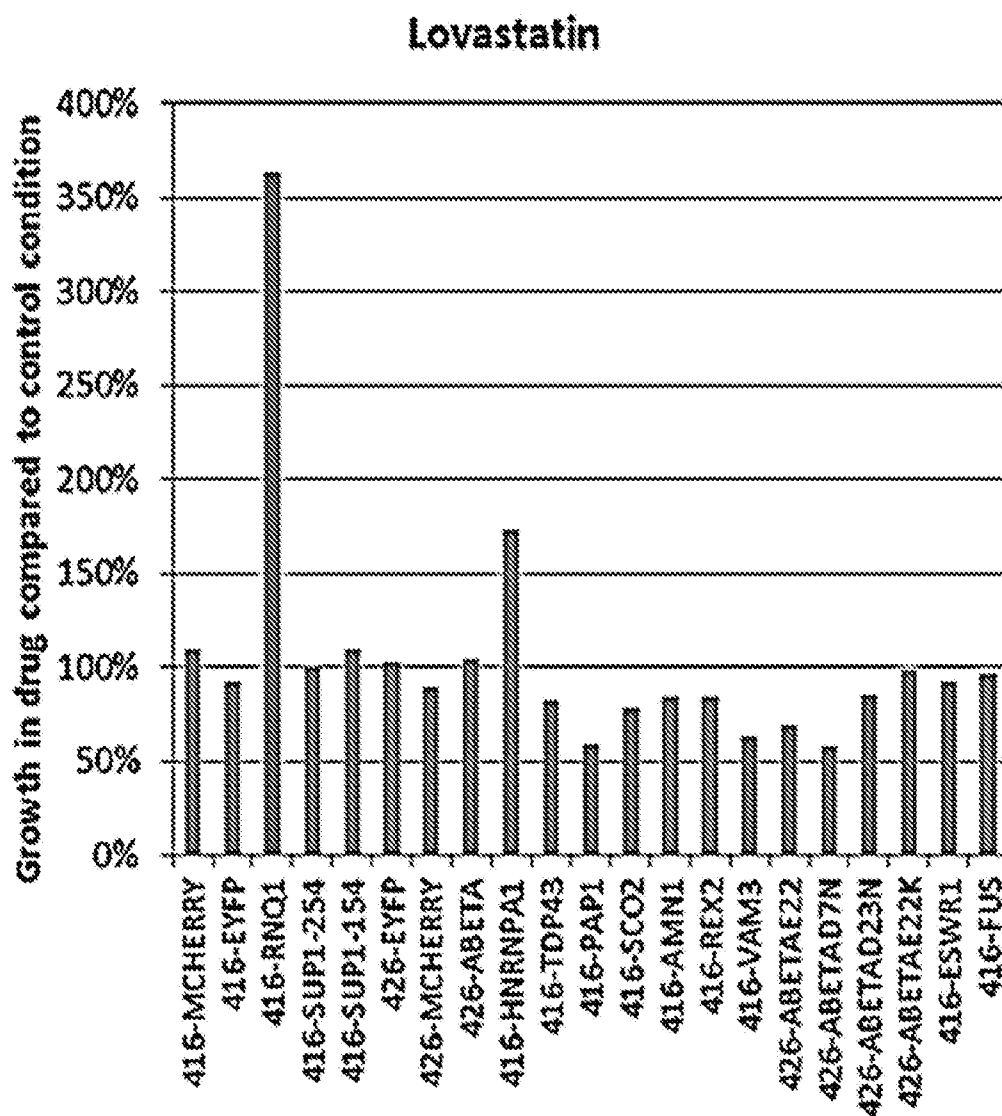
FIG. 5A

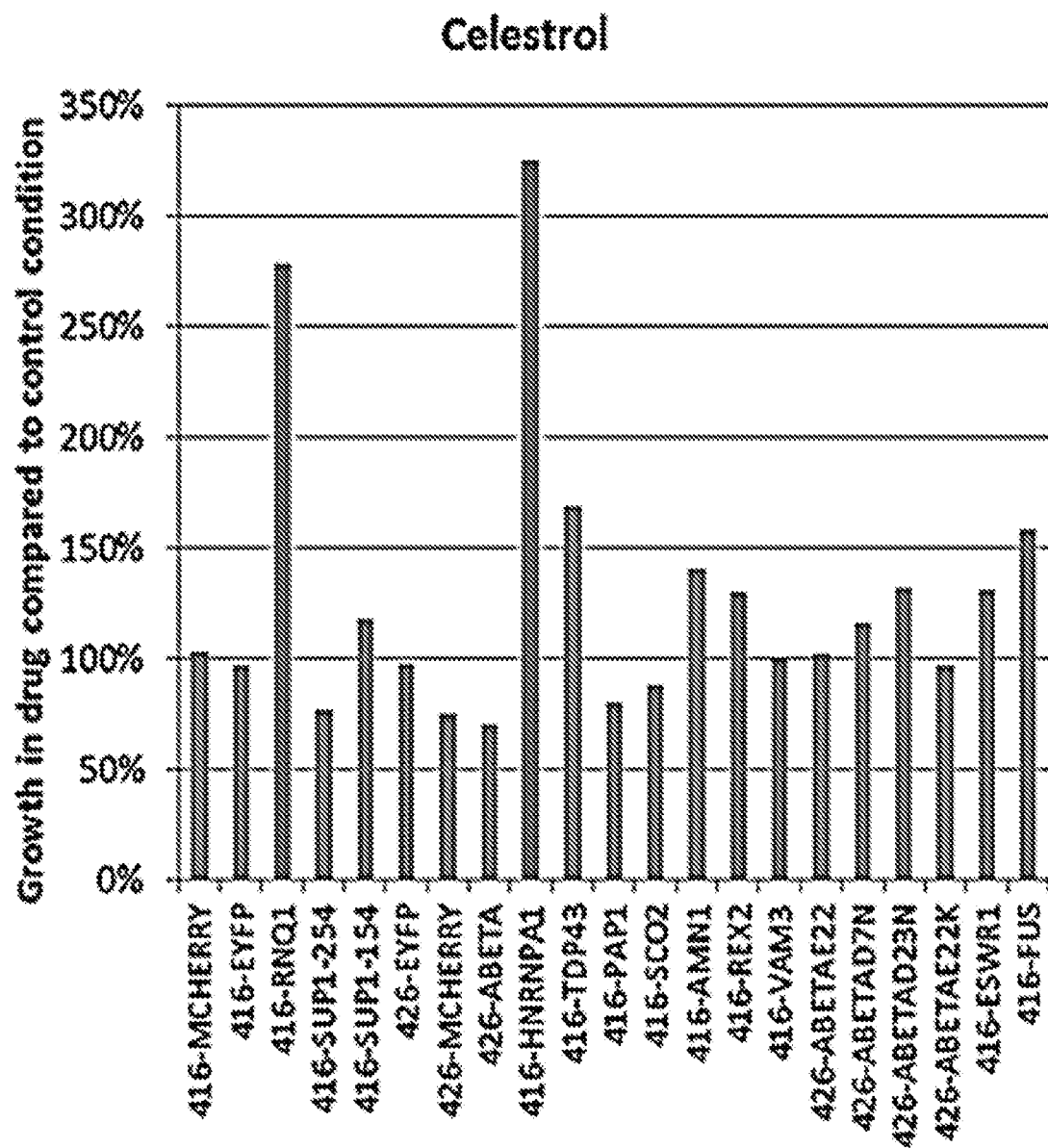
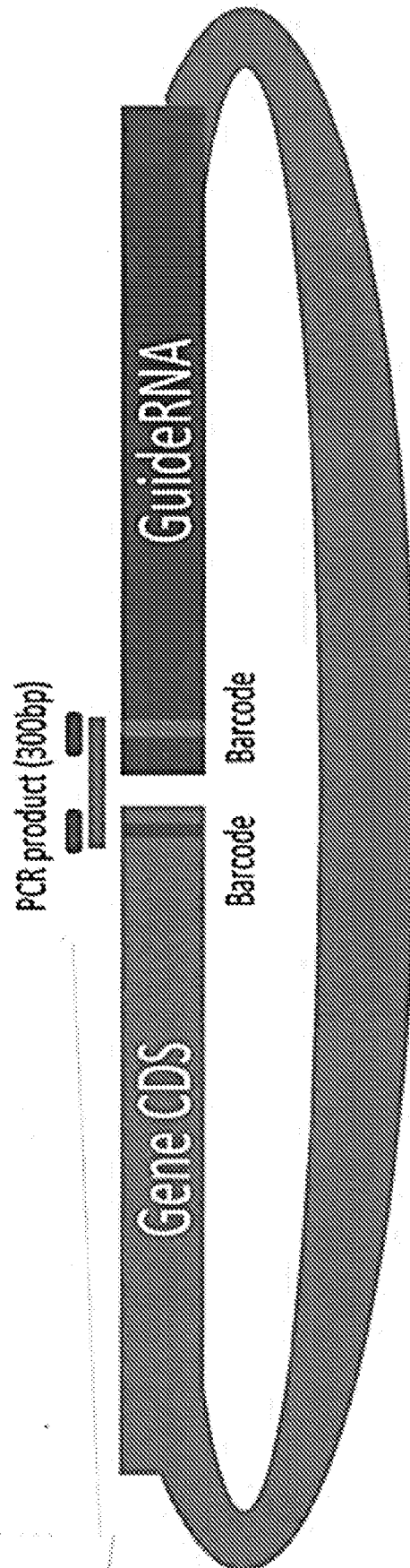
FIG. 5B

FIG. 6



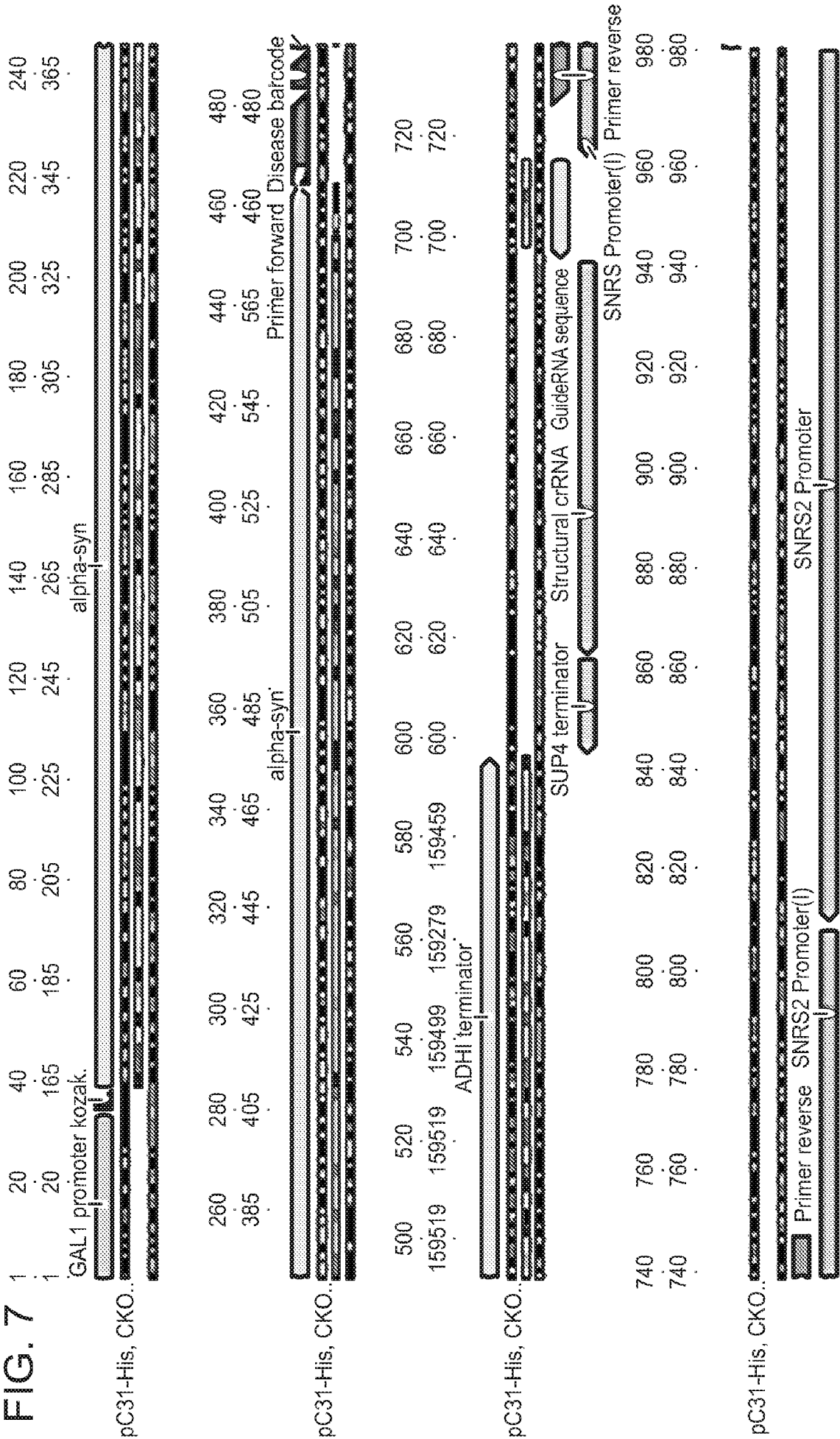


FIG. 8

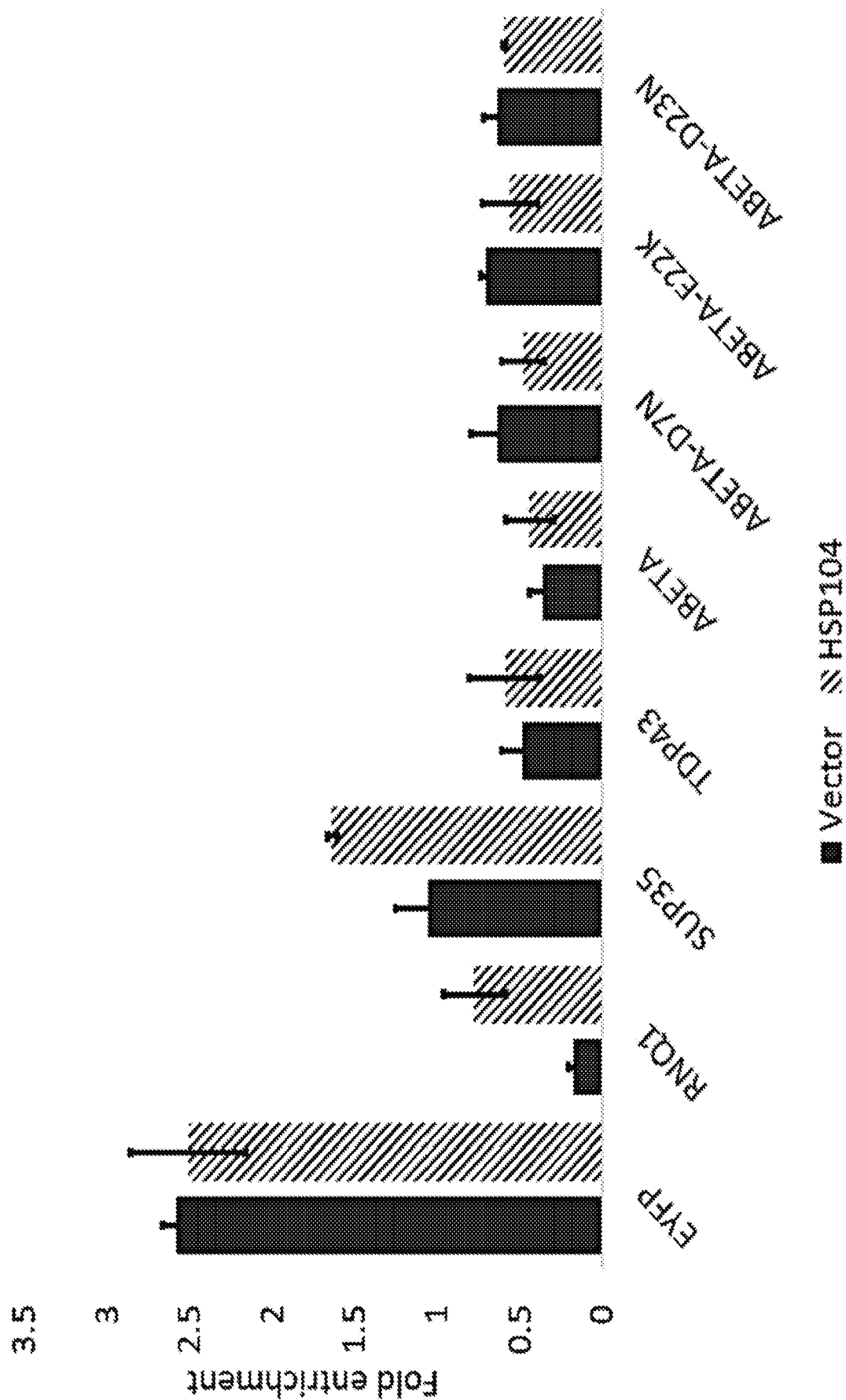


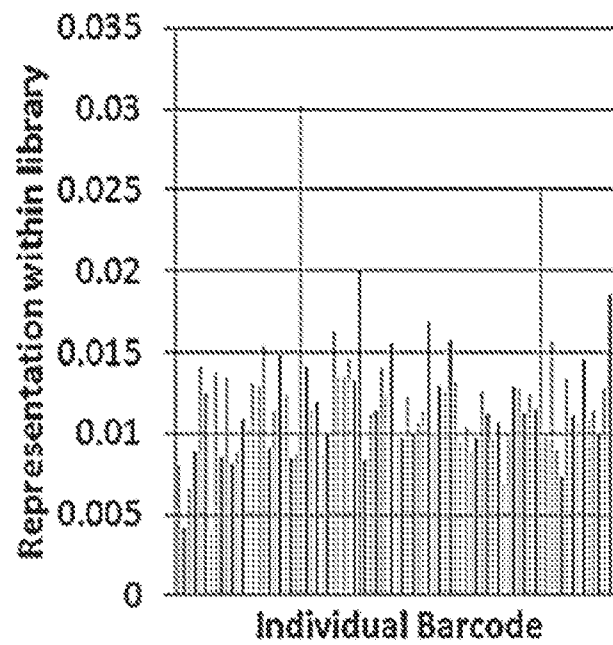
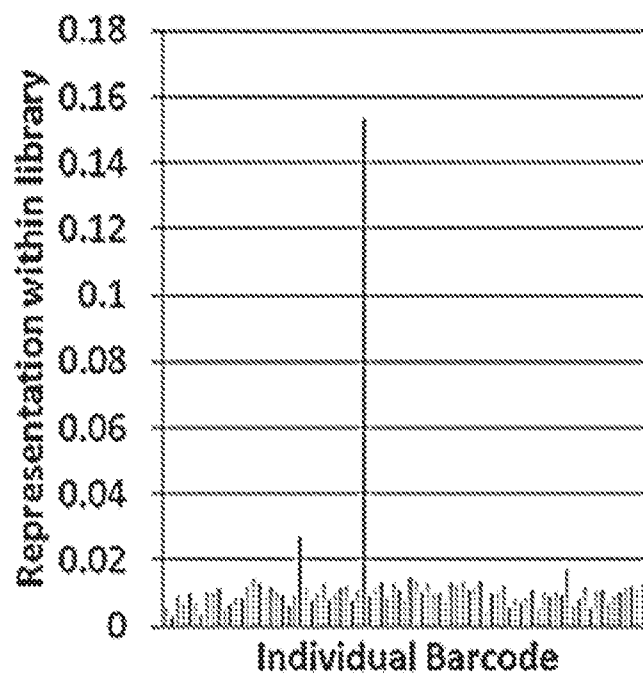
FIG. 1A**FIG. 1B**

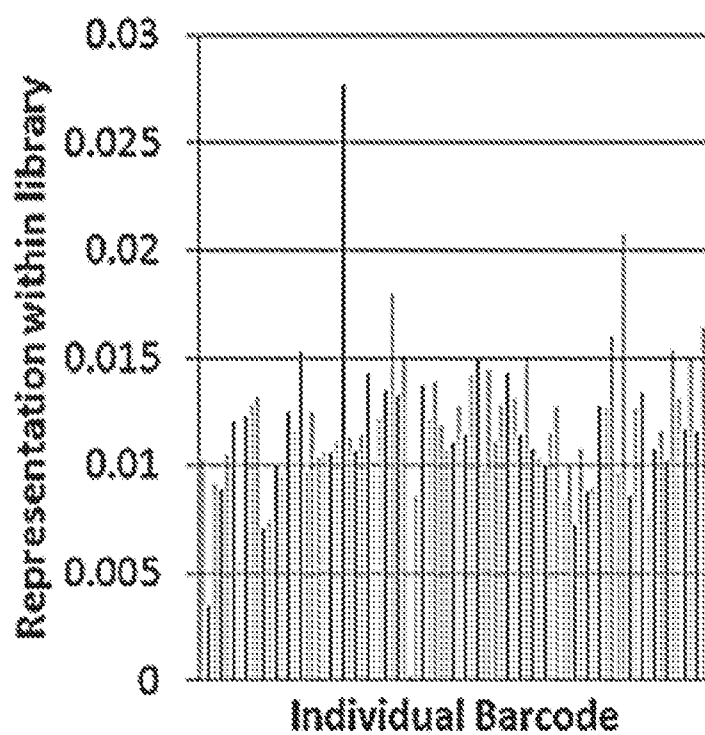
FIG. 1C

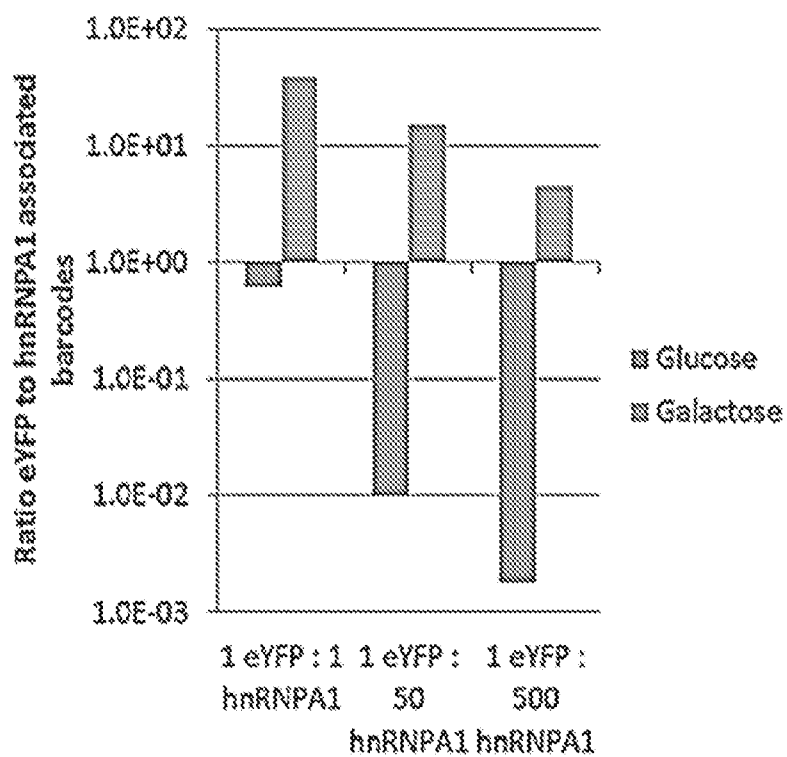
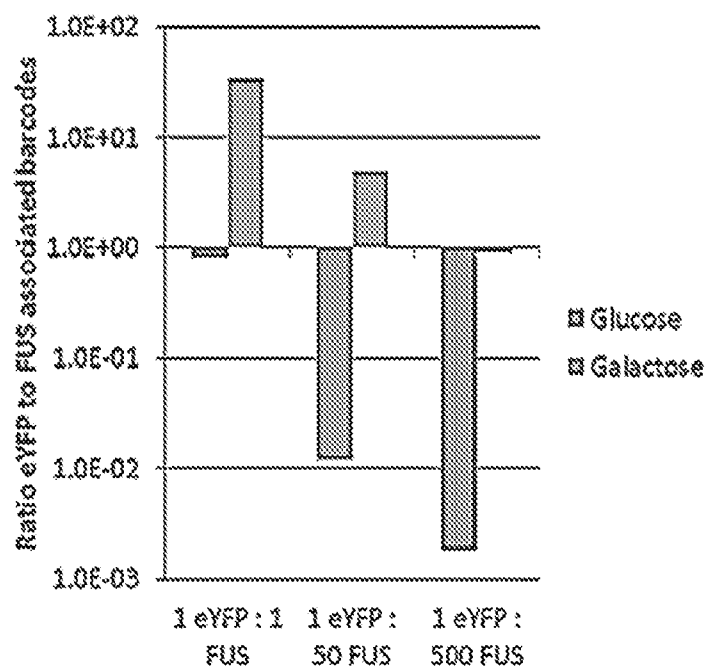
FIG. 2A**FIG. 2B**

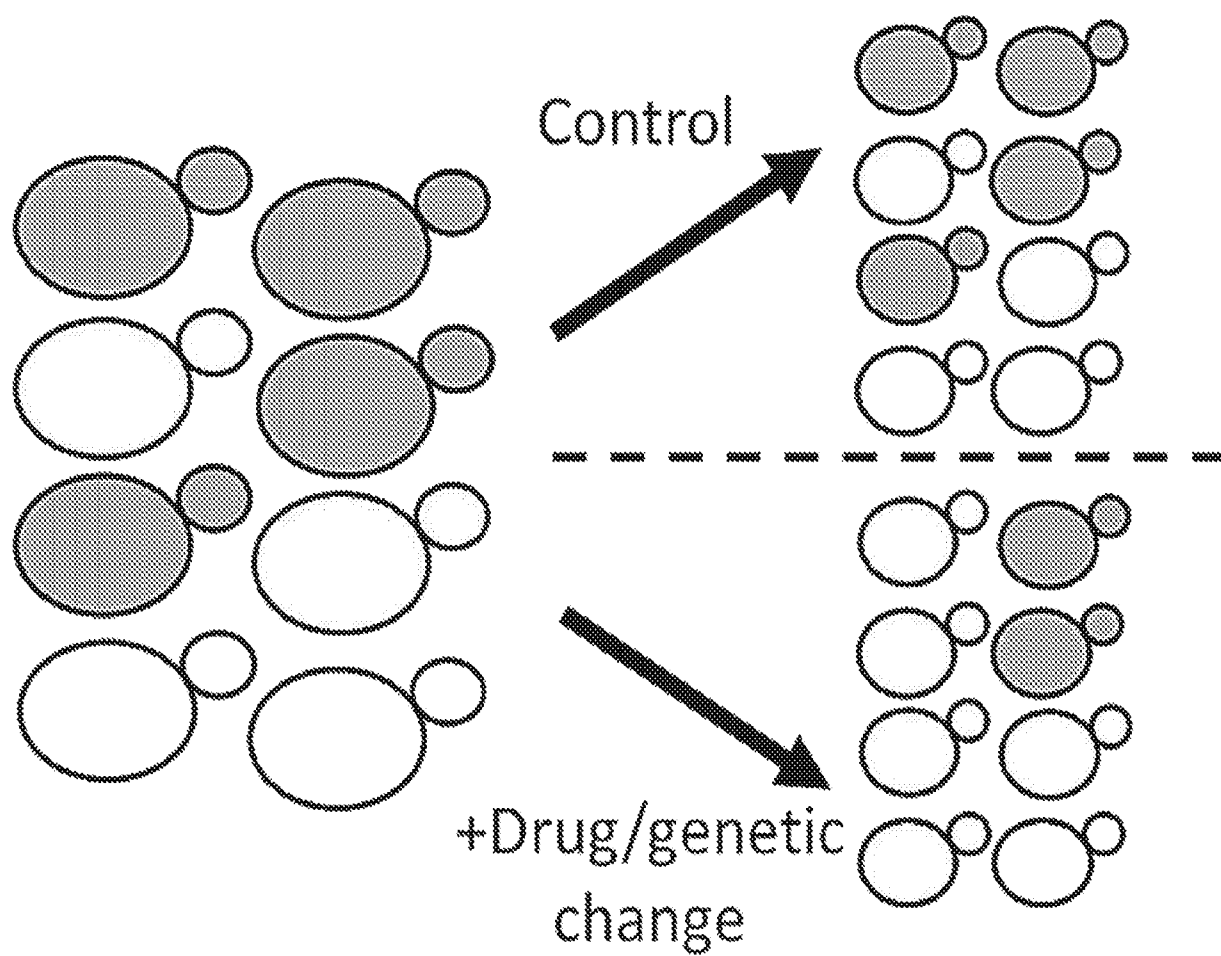
FIG. 3

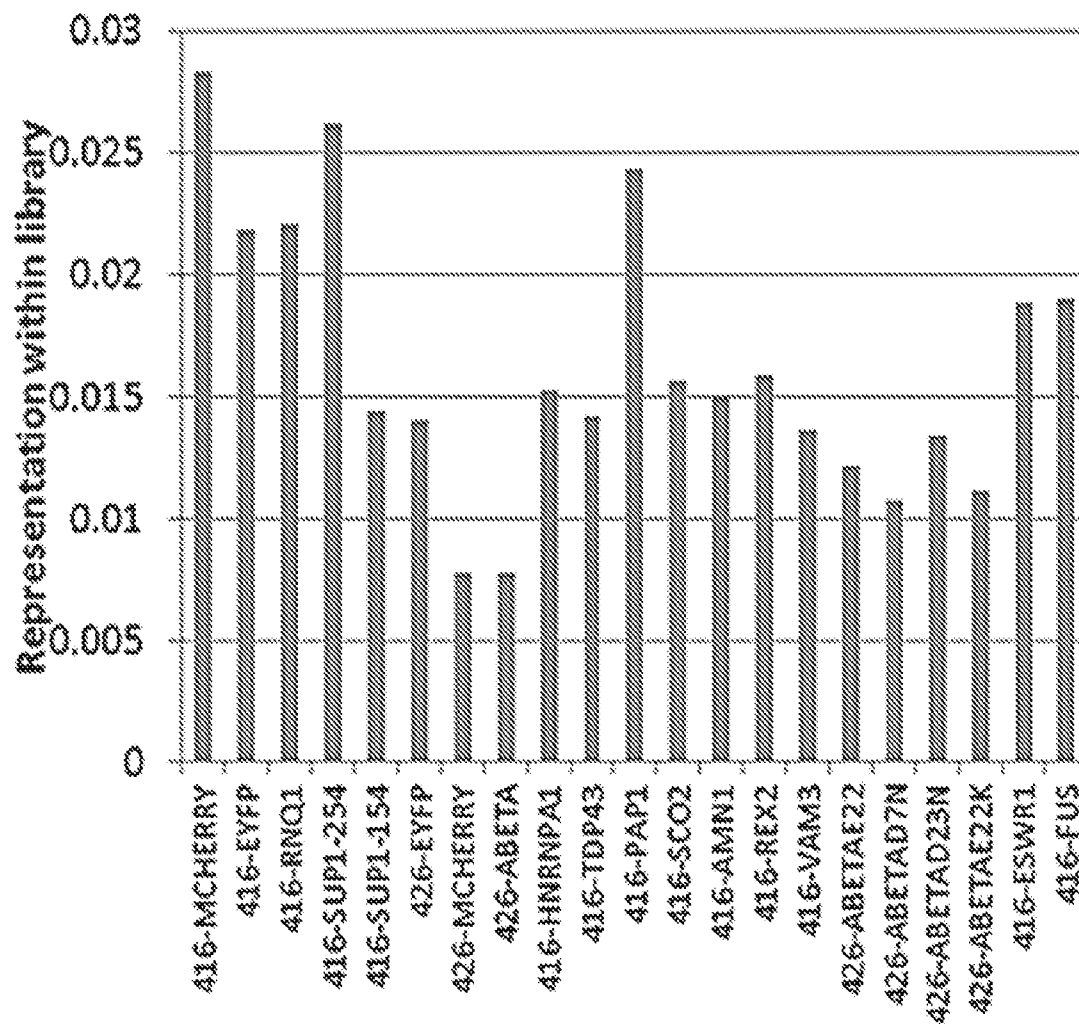
FIG. 4A

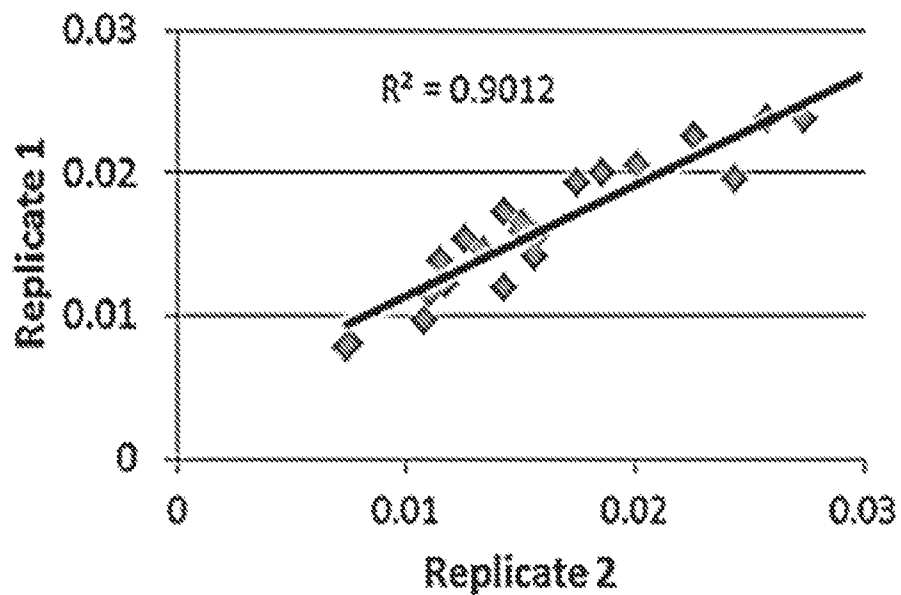
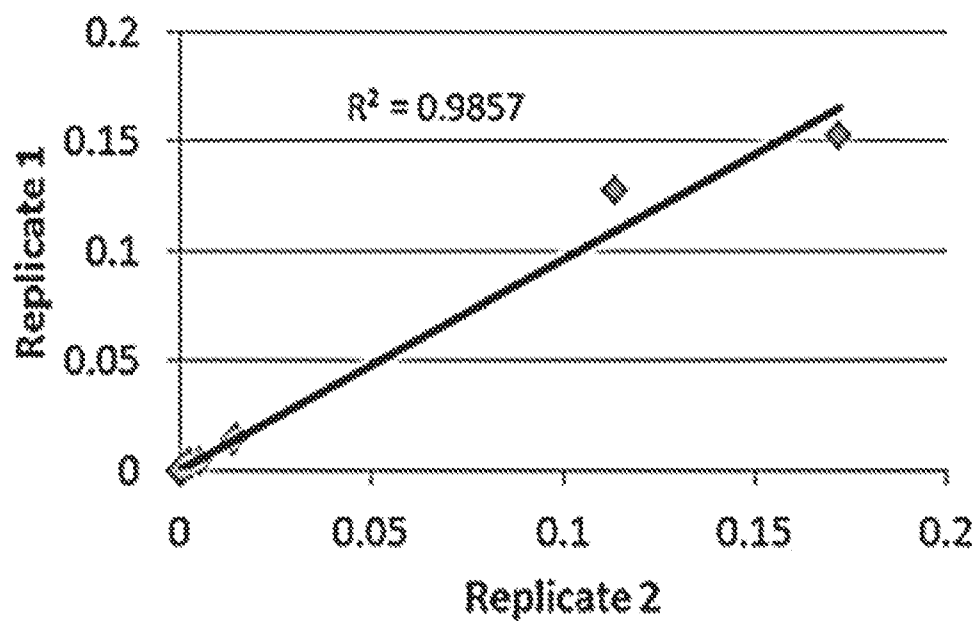
FIG. 4B**FIG. 4C**

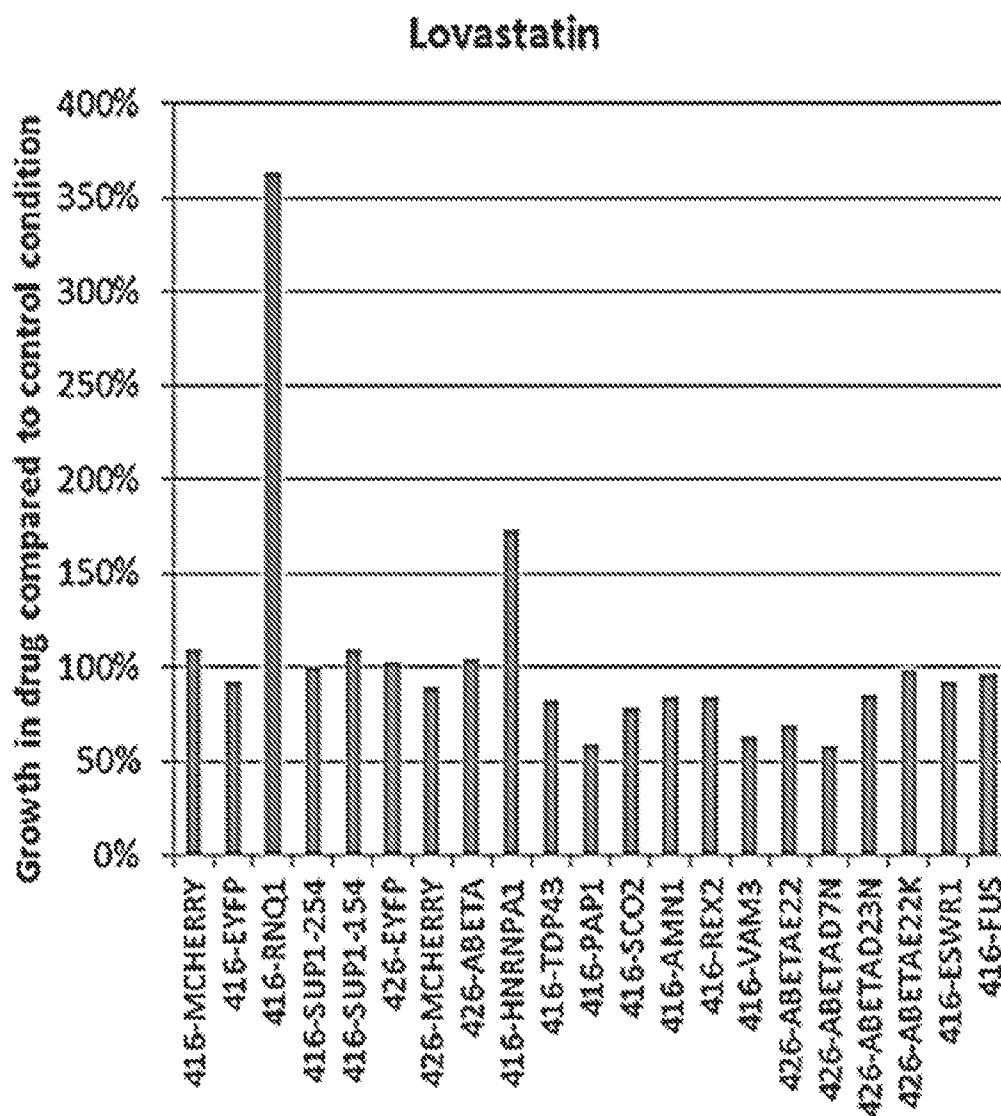
FIG. 5A

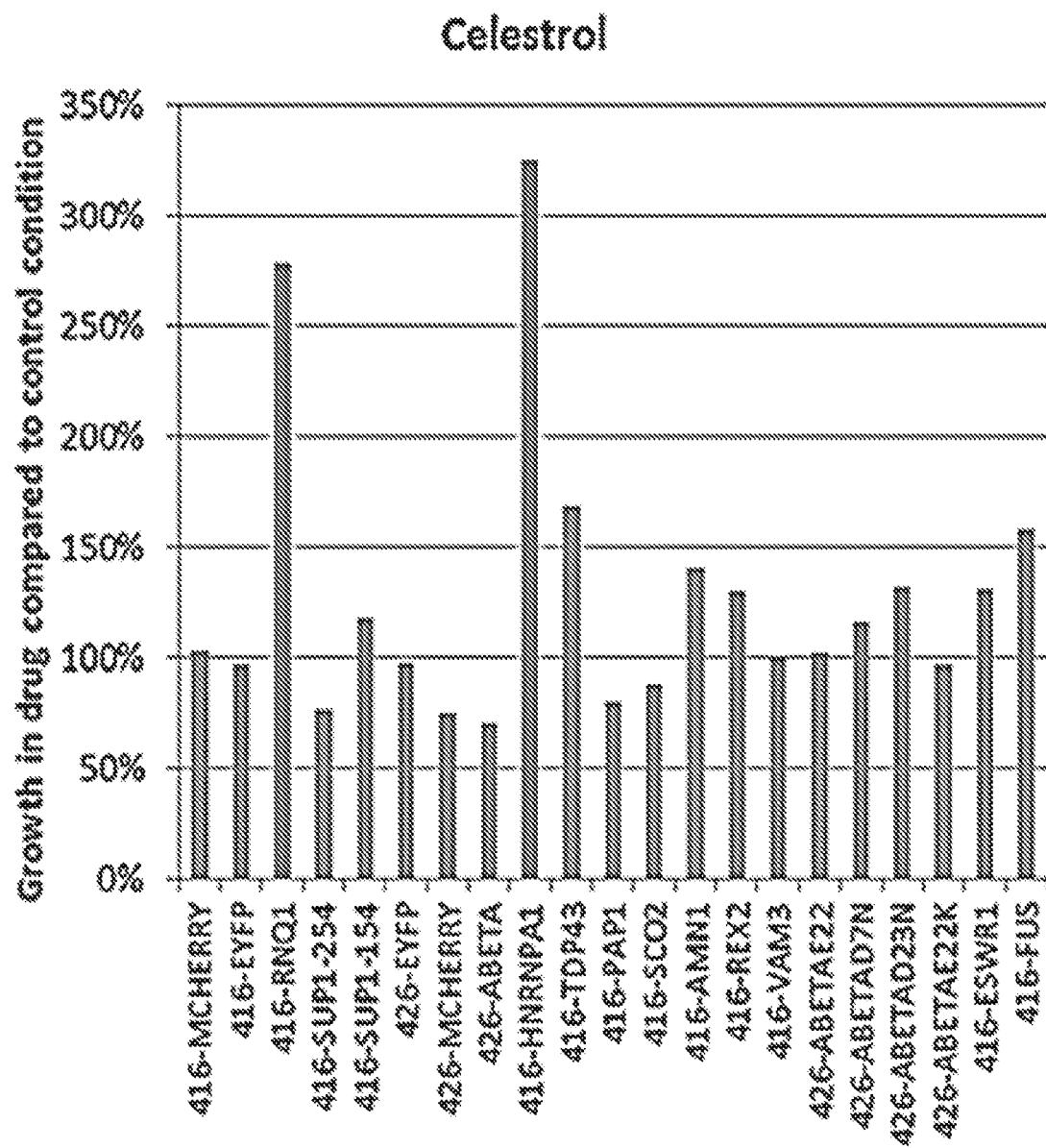
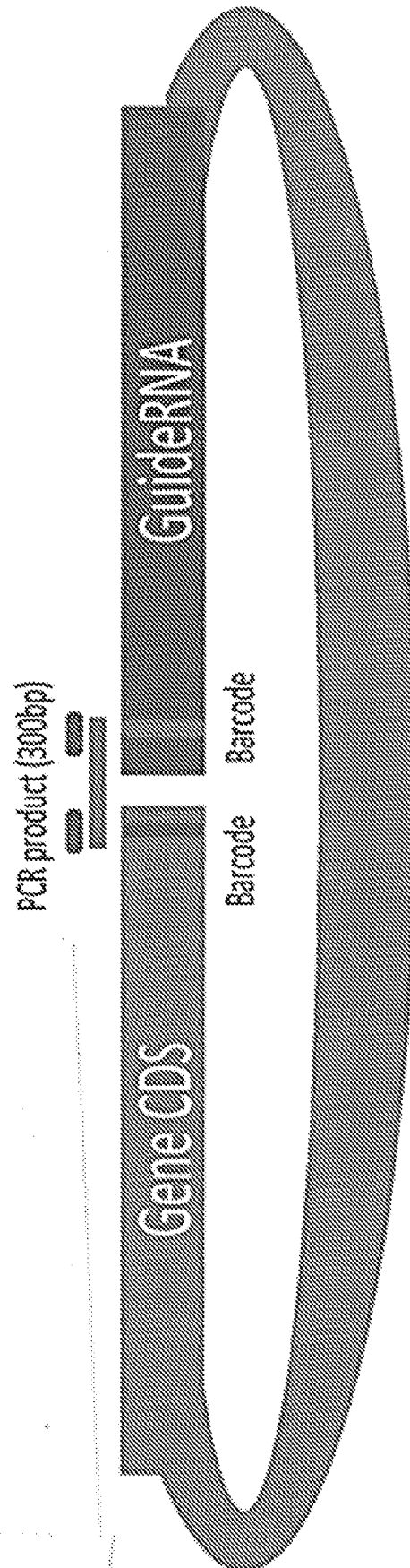
FIG. 5B

FIG. 6



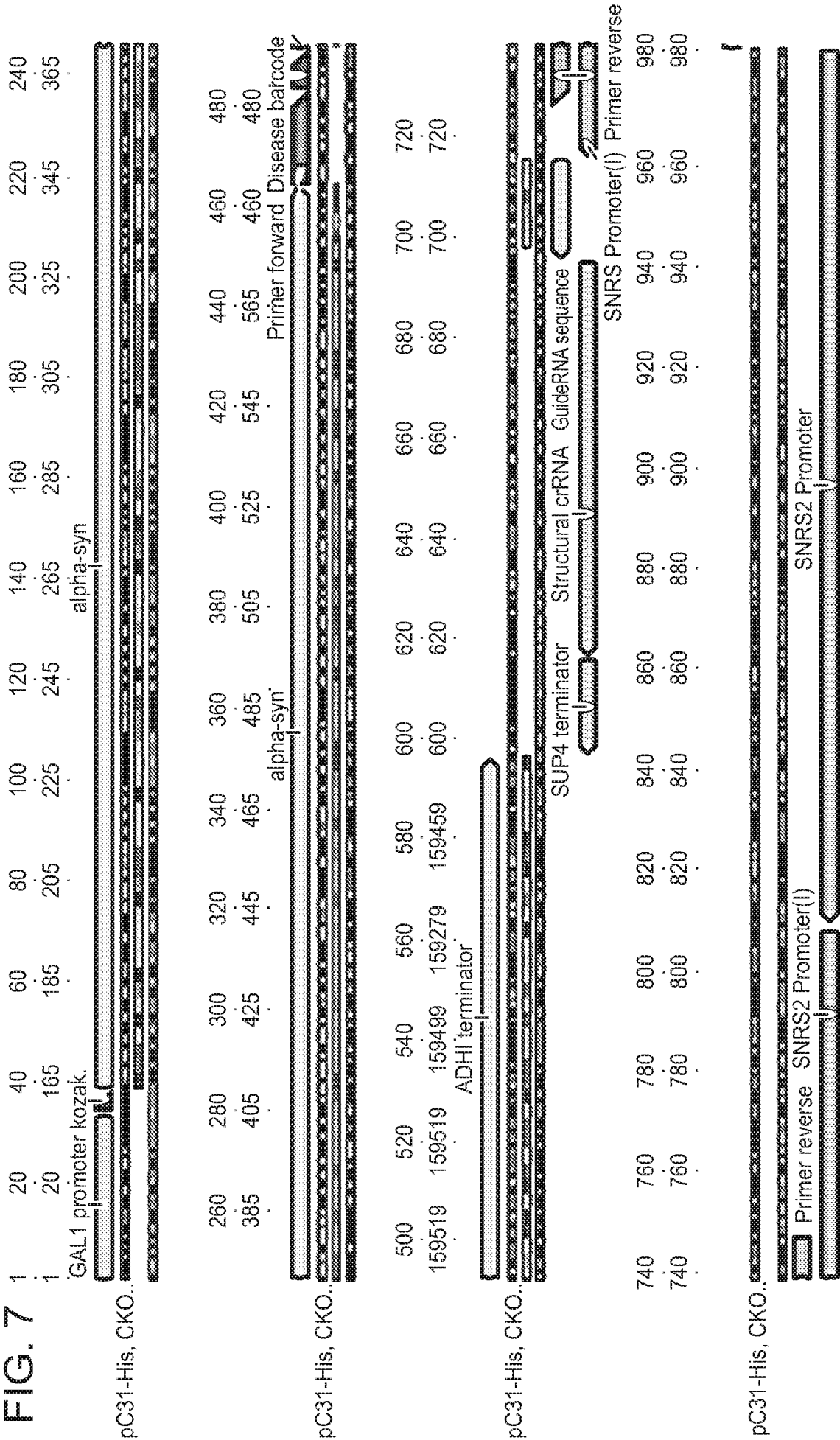
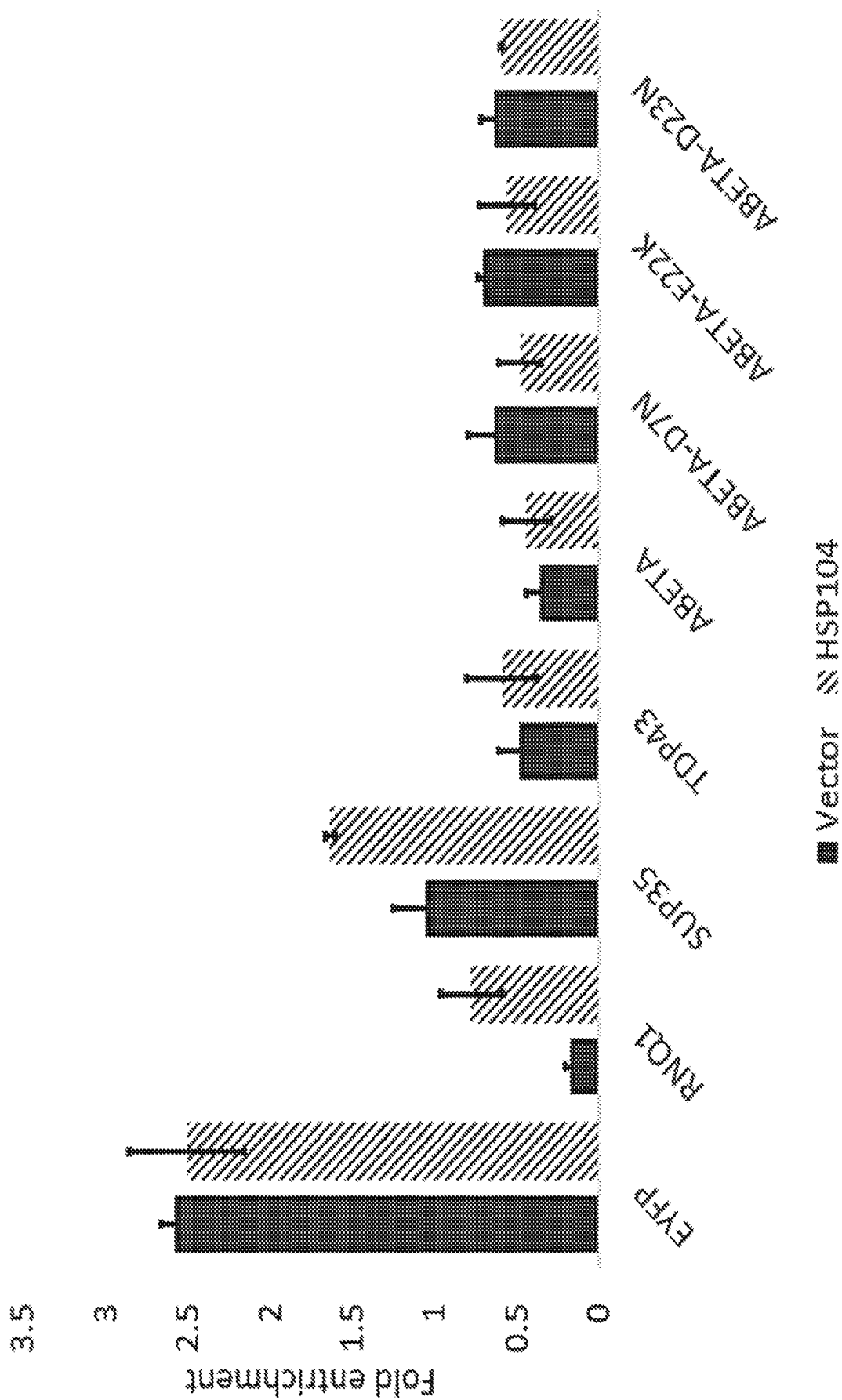


FIG. 8



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/040853

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7088; A61K 38/00; A61K 39/00; A61K 39/395; A61K 45/00; A61K 48/00 (2017.01)
CPC - C07K 14/37; C07K 14/40; C07K 2319/00; C07K 14/4702; C12N 15/65; C12N 15/1034; C12N 15/1082; C12N 15/1086; C12Q 2600/112; C12Q 1/686; C12Q 1/6886; C12Q 1/6897; C12R 1/645; C12R 1/725 (2017.08)

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 435/6.11; 435/44; 435/483; 702/19 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0079619 A1 (ROEMER et al) 14 April 2005 (14.04.2005) entire document	1, 4-7, 9-14, 18-20
Y		2, 3, 8, 15-17, 21, 34
Y	KOSZELA, J. "Novel Screening Methods for Inhibitors of the Human Ubiquitin-Conjugating Enzymes," Thesis, The University of Edinburgh, 28 June 2014 (28.06.2014), Pgs. 1-182 (only Pgs 1-103 provided). Retrieved from the Internet: < https://www.era.lib.ed.ac.uk/bitstream/handle/1842/17893/Koszela2014.pdf?sequence=1&isAllowed=y > on 20 September 2017 (20.09.2017). entire document	2, 8
Y	SMITH et al. "A Survey of Yeast Genomic Assays for Drug and Target Discovery," Pharmacology & Therapeutics, 28 May 2010 (28.05.2010), Vol. 127, Iss. 2, Pgs. 156-164. entire document	3, 21-35
Y	WO 2016/049258 A2 (THE BROAD INSTITUTE INC. et al) 31 March 2016 (31.03.2016) entire document	15-17, 22-35
A	US 6,324,479 B1 (FRIEND et al) 27 November 2001 (27.11.2001) entire document	1-35
A	LIU et al. "Construction of a Gal1-Regulated Yeast Cdna Expression Library and Its Application to the Identification of Genes Whose Overexpression Causes Lethality in Yeast," Genetics, 01 November 1992 (01.11.1992), Vol. 132, No. 3, Pgs. 665-673. entire document	1-35
A	US 2007/0161031 A1 (TRINKLEIN et al) 12 July 2007 (12.07.2007) entire document	1-35

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search

20 September 2017

Date of mailing of the international search report

23 OCT 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

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