

METHODS OF SCREENING AND RELATED SYSTEMS

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Technical Field

10 **[0002]** This disclosure relates generally to methods of screening and related systems, and in particular the disclosure relates to methods of screening involving gain of function mutations and related systems.

Summary

[0002a] In one aspect, the present disclosure refers to method of high-
15 throughput screening test agents for treating a disease, the method comprising cultivating pathogenic transduced cells overexpressing a gene of interest associated with a disease to exhibit a disease phenotype in cultivated pathogenic transduced cells; cultivating non-pathogenic transduced cells not overexpressing the gene of interest to exhibit a control phenotype in cultivated non-pathogenic
20 transduced cells; morphologically profiling the cultivated pathogenic transduced cells to develop a disease phenotype morphological profile; morphologically profiling the cultivated non-pathogenic transduced cells to develop a control phenotype morphological profile; adding one or more of a plurality of test agents, or combinations thereof, to the cultivated pathogenic transduced cells to form
25 treated cultivated pathogenic transduced cells; morphologically profiling the treated cultivated pathogenic transduced cells; and identifying any of the plurality of the test agents, or combinations thereof, that modulate the disease phenotype morphological profile of the treated cultivated pathogenic transduced cells to a treated morphological profile that is similar to that of the control phenotype
30 morphological profile. In another aspect, the present disclosure refers to a method of high-throughput screening diseases, the method comprising providing a first disease phenotype morphological profile associated with a first disease; comparing the first disease phenotype morphological profile with a library of a plurality of

phenotype morphological profiles, wherein each of the plurality of phenotype morphological profiles were generated from cultivated pathogenic transduced cells that overexpressed one or more genes, underexpressed one or more genes, or combinations thereof; and identifying any of the plurality of the phenotype

5 morphological profiles that are similar to the first disease phenotype morphological profile. In another aspect, the present disclosure refers to a high-throughput screening system, the system comprising: a memory to store a plurality of phenotype morphological profiles; and processor circuitry to receive a first set of image data for cultivated pathogenic transduced cells overexpressing a gene of

10 interest associated with a disease; receive a second set of image data from cultivated non-pathogenic transduced cells not overexpressing the gene of interest; receive a third set of image data from treated cultivated pathogenic transduced cells treated with one or more of a plurality of test agents, or combinations thereof; generate a disease phenotype morphological profile from

15 the first set of image data; generate a control phenotype morphological profile from the second set of image data; generate a treated phenotype morphological profile from the third set of image data for each of the one or more of the plurality of test agents, or combinations thereof; and assign a similarity value to each of the treated phenotype morphological profiles relative to the control phenotype

20 morphological profile. In yet another aspect, the present disclosure refers to a high-throughput screening system, the system comprising an interface to access a library of a plurality of phenotype morphological profiles; and processor circuitry to access, via the interface, a library of a plurality of phenotype morphological profiles, wherein each of the plurality of phenotype morphological profiles were

25 generated from cultivated pathogenic transduced cells that overexpressed one or more genes, underexpressed one or more genes, or combinations thereof; compare a first disease phenotype morphological profile with; and identify any of the plurality of the phenotype morphological profiles that are similar to the first disease phenotype morphological profile. In another aspect, the present disclosure

30 refers to a method of high-throughput screening test agents for treating a disease, the method comprising cultivating pathogenic transduced cells overexpressing a gene of interest associated with a disease to exhibit a disease phenotype in cultivated pathogenic transduced cells; cultivating non-pathogenic transduced cells not overexpressing the gene of interest to exhibit a control phenotype in cultivated

non-pathogenic transduced cells; profiling the cultivated pathogenic transduced cells to develop a disease phenotype profile; profiling the cultivated non-pathogenic transduced cells to develop a control phenotype profile; adding one or more of a plurality of test agents, or combinations thereof, to the cultivated
5 pathogenic transduced cells to form treated cultivated pathogenic transduced cells; profiling the treated cultivated pathogenic transduced cells; and identifying any of the plurality of the test agents, or combinations thereof, that modulate the disease phenotype profile of the treated cultivated pathogenic transduced cells to a treated profile that is similar to that of the control phenotype profile.

10 Brief Description of the Drawings

[0003] The embodiments disclosed herein will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. The drawings depict primarily generalized embodiments, which embodiments will be described with additional specificity and detail in
15 connection with the drawings in which:

[0004] FIG. 1 is a fluorescent microscopy image of BRAF V600E transduced cells (left panel) and BRAF V600E nonsense transduced cells (right panel) from Example 1.

[0005] FIG. 2 is a heatmap of cosine similarity between pairs of vectors of
20 image-based features extracted from images of cells treated by the reagents in the BacMam titration experiment of Example 1.

[0006] FIG. 3 is a zoom-in image of FIG. 2.

[0007] FIG. 4A is a fluorescent microscopy image of INS-1E cells (left panel) and INS-1E cells overexpressing wildtype MFN1 (right panel) from Park et al.
25 Korean J Physiol Pharmacol. 2012 Feb; 16(1): 71–77.

[0008] FIG. 4B is a fluorescent microscopy image of U2OS cells (left panels) and U2OS cells overexpressing wildtype MFN1 (right panels) from Example 2.

[0009] FIG. 5 are microscopy images of KB cells from Bretscher et al. Current Biology Volume 8, Issue 12, 4 June 1998, Pages 721-724, S1-S4 and U2OS cells
30 transduced to overexpress EGFR FLAG from Example 2. Unstimulated KB cells