a selective pressure of interest, such as but not limited to drug treatment, genomic engineering, and engraftment into a host.

[05] In an example, a method for screening cells for a trait is provided. The method may include: (a) obtaining a plurality of barcoded cells, wherein each barcoded cell includes a single, unique barcode, or barcode combinations; (b) performing a first sequencing of RNA and/or DNA on a subset of the plurality of barcoded cells; (c) culturing the plurality of barcoded cells in the presence of a selection pressure for a first period of time, thereby forming a first plurality of cells; (d) performing a second sequencing of RNA and/or DNA on a subset of the first plurality of cells; (e) culturing the first plurality of cells in the presence of the selection pressure for a second period of time, thereby forming a second plurality of cells; and (f) performing a third sequencing of RNA and/or DNA on at least a subset of the second plurality of cells. The method may also include: (g) determining the relative abundance of a barcode sequenced in the first sequencing, second sequencing, and/or third sequencing. In one aspect, the present disclosure refers to a method for screening cells for a trait, the method comprising:

(a) obtaining a plurality of barcoded cells, wherein each barcoded cell comprises a single, unique barcode;

(b) performing a first sequencing of RNA and/or DNA on a subset of the plurality of barcoded cells;

(c) culturing the plurality of barcoded cells in the presence of a selection pressure for a first period of time, thereby forming a first plurality of cells;

(d) performing a second sequencing of RNA and/or DNA on a subset of the first plurality of cells;

(e) culturing the first plurality of cells in the presence of the selection pressure for a second period of time, thereby forming a second plurality of cells;

(f) performing a third sequencing of RNA and/or DNA on at least a subset of the second plurality of cells; and

(g) determining a level of a barcode sequenced in the first sequencing, second sequencing, and/or third sequencing

wherein steps (e) and (f) are repeated for one or more iterations thereby forming one or more subsequent pluralities of cells and thereby performing one or more subsequent sequencing on a subset of said pluralities of cells.

[06] In another example, a method for screening cells for a response trait to a therapeutic agent is provided. The method may include: (a) obtaining a plurality of barcoded cells, wherein each barcoded cell comprises a single, unique barcode; (b) performing a first sequencing of RNA and/or DNA on a subset of the plurality of barcoded cells; (c) culturing the plurality of barcoded cells in the presence of the therapeutic agent for a first period of time, thereby forming a first plurality of cells; (d) performing a second sequencing of RNA and/or DNA on a subset of the first plurality of cells; (e) culturing the first plurality of cells with the therapeutic agent for a second period of time, thereby forming a second plurality of cells; and (f) performing a third sequencing of RNA and/or DNA on a subset of the second plurality of cells. The method may also include: (g) determining the relative abundance of a barcode sequenced in the first sequencing, second sequencing, and/or third sequencing. In one aspect, the present disclosure refers to a method for screening cells for a response trait to a therapeutic agent, the method comprising:

(a) obtaining a plurality of barcoded cells, wherein each barcoded cell comprises a single, unique barcode;
(b) performing a first sequencing of RNA and/or DNA on a subset of the plurality of barcoded cells;
(c) culturing the plurality of barcoded cells in the presence of the therapeutic agent for a first period of time, thereby forming a first plurality of cells;
(d) performing a second sequencing of RNA and/or DNA on a subset of the first plurality of cells;
(e) culturing the first plurality of cells with the therapeutic agent for a second period of time, thereby forming a second plurality of cells;
(f) performing a third sequencing of RNA and/or DNA on a subset of the second plurality of cells; and
(g) determining a level of a barcode sequenced in the first sequencing, second sequencing, and/or third sequencing

wherein steps (e) and (f) are repeated for one or more iterations thereby forming one or more subsequent pluralities of cells and thereby performing one or more subsequent sequencing on a subset of said pluralities of cells.

[07] In another example is provided a method for comparing responses to selective pressures. The method may include: (a) obtaining a first plurality of barcoded cells, wherein each barcoded cell comprises a single, unique barcode; (b) obtaining a second plurality of barcoded cells that is substantially similar to the first plurality of barcoded cells; (c) performing a first sequencing of RNA and/or DNA from the first plurality of barcoded cells and/or the second plurality of barcoded cells; (d) culturing the first plurality of barcoded cells in the presence of a first selection pressure, thereby forming a first plurality of cells; (e) culturing the second plurality of barcoded cells in the presence of a second selection pressure,

thereby forming a second plurality of cells; (f) performing a second sequencing of RNA and/or DNA from the first plurality of cells and/or the second plurality of cells; (g) culturing the first plurality of cells in the presence of the first selection pressure, thereby forming a third plurality of cells; (h) culturing the second plurality of cells in the presence of the second selection pressure, thereby forming a fourth plurality of cells; and (i) performing a third sequencing of RNA and/or DNA from the third plurality of cells and/or the fourth plurality of cells. The method may also include: (j) determining the relative abundance of one or more barcodes sequenced in the first sequencing, second sequencing, and/or third sequencing. In one aspect, the present disclosure refers to a method for comparing responses to selective pressures, the method comprising:

(a) obtaining a first plurality of barcoded cells, wherein each barcoded cell comprises a single, unique barcode;

(b) obtaining a second plurality of barcoded cells that is substantially similar to the first plurality of barcoded cells;

(c) performing a first sequencing of RNA and/or DNA from the first plurality of barcoded cells and/or the second plurality of barcoded cells;

(d) culturing the first plurality of barcoded cells in the presence of a first selection pressure, thereby forming a first plurality of cells;

(e) culturing the second plurality of barcoded cells in the presence of a second selection pressure, thereby forming a second plurality of cells;

(f) performing a second sequencing of RNA and/or DNA from the first plurality of cells and/or the second plurality of cells;

(g) culturing the first plurality of cells in the presence of the first selection pressure, thereby forming a third plurality of cells;

(h) culturing the second plurality of cells in the presence of the second selection pressure, thereby forming a fourth plurality of cells;

(i) performing a third sequencing of RNA and/or DNA from the third plurality of cells and/or the fourth plurality of cells; and

(j) determining a level of one or more barcodes sequenced in the first sequencing, second sequencing, and/or third sequencing

wherein steps (g) to (i) are repeated for one or more iterations, thereby forming one or more subsequent pluralities of cells and one or more subsequent sequencings.

[08] In an example is provided method of screening cells for a trait in a cell. The method may include: (a) providing a mixture of cells comprising multiple clonal populations wherein each clonal population comprises an identifier that is unique to the respective clonal populations, and wherein initial genetic, transcriptomic, and/or proteomic information of at least one representative member of each clonal population is known; (b) culturing the mixture of cells in the presence of a first selective pressure for a first period of time, and at the end of the first period of time, obtaining second genetic, transcriptomic, and/or proteomic information for at least one member of a surviving clonal population from within the mixture of cells; (c) subjecting the mixture of cells that were subjected to the first selective pressure to a second selective pressure for a second period of time, and at the end of the second period of time, obtaining third genetic, transcriptomic, and/or proteomic information of at least one member of a surviving clonal population from within the mixture of cells; and (d) determining the relative abundance of each clonal population present in the final mixture of cells based upon the unique identifier for the clonal population. The method may also include (e) identifying an adaptive trait, wherein the adaptive trait is a genetic and/or proteomic trait present in or absent from a clonal population in the final mixture of cells. The method may also include (f) comparing information from the initial genetic, transcriptomic, and/or proteomic information, second genetic, transcriptomic, and/or proteomic information, and/or third genetic, transcriptomic, and/or proteomic information, and/or one or more subsequent genetic, transcriptomic, and/or proteomic information. The method may also include (g) comparing the initial genetic, transcriptomic, and/or proteomic information, second genetic, transcriptomic, and/or proteomic information, and/or third genetic, transcriptomic, and/or proteomic information, and/or one or more subsequent genetic, transcriptomic, and/or proteomic information to genetic, transcriptomic and/or proteomic

information of a different clonal population of cells having a different unique barcode that was subjected to the selective pressure. In one aspect, the present disclosure refers to a method of screening cells for a trait in a cell, comprising:

(a) providing a mixture of cells comprising multiple clonal populations wherein each clonal population comprises an identifier that is unique to the respective clonal populations, and wherein initial genetic, transcriptomic, and/or proteomic information of at least one representative member of each clonal population is known;

(b) culturing the mixture of cells in the presence of a first selective pressure for a first period of time, and at the end of the first period of time, obtaining second genetic, transcriptomic, and/or proteomic information for at least one member of a surviving clonal population from within the mixture of cells;

(c) subjecting the mixture of cells that were subjected to the first selective pressure to a second selective pressure for a second period of time, and at the end of the second period of time, obtaining third genetic, transcriptomic, and/or proteomic information of at least one member of a surviving clonal population from within the mixture of cells; and

(d) determining the level of a clonal population present in the final mixture of cells based upon the unique identifier for the clonal population.

[09] In another example, a method of identifying a cellular program that facilitates adaptation to a pressure is provided. The method may include: (a) transducing cells with a plurality of barcodes such that each cell contains a single, unique barcode, or barcode combinations; (b) expanding the cells in culture to create a starting cell pool of clones of cells containing each barcode; (c) obtaining first genetic, transcriptomic, and/or proteomic information from a first subset of the starting cell pool; (d) culturing a second subset of the starting cell pool in the presence of a selective pressure to expand the starting cell pool and form an intermediate cell pool; (e) obtaining second genetic, transcriptomic, and/or proteomic information from a first subset of the intermediate cell pool; (f) continuing to culture a second subset of the intermediate cell pool in the presence of the selective pressure to expand the intermediate cell pool and form a final cell pool; (g) obtaining third genetic, transcriptomic, and/or proteomic information from at least a subset of the final cell pool; and (h) quantifying a level of each barcode in the final cell pool, intermediate cell pool, and/or starting cell pool. The method may also include: (i) assigning cells with barcodes enriched in the final cell pool as winning clones and/or assigning cells with barcodes depleted in the final cell pool as losing clones. The method may also include: (j) determining a genetic mutation, transcription program, and/or protein expression associated with at least one winning clone and/or at least one losing clone. In one aspect, the present disclosure refers to a method of identifying a cellular program that facilitates adaptation to a pressure, comprising:

a. transducing cells with a plurality of barcodes such that each cell contains a single, unique barcode;
b. expanding the cells in culture to create a starting cell pool of clones of cells containing each barcode;
c. obtaining first genetic, transcriptomic, and/or proteomic information from a first subset of the starting cell pool;
d. culturing a second subset of the starting cell pool in the presence of a selective pressure to expand the starting cell pool and form an intermediate cell pool;
e. obtaining second genetic, transcriptomic, and/or proteomic information from a first subset of the intermediate cell pool;
f. continuing to culture a second subset of the intermediate cell pool in the presence of the selective pressure to expand the intermediate cell pool and form a final cell pool;
g. obtaining third genetic, transcriptomic, and/or proteomic information from at least a subset of the final cell pool;
h. quantifying a level of each barcode in the final cell pool, intermediate cell pool, and/or starting cell pool;
i. assigning cells with barcodes enriched in the final cell pool as winning clones and/or assigning cells with barcodes depleted in the final cell pool as losing clones; and
j. determining a genetic mutation, transcription program, and/or protein expression associated with at least one winning clone and/or at least one losing clone

wherein approximately equal numbers of clones of cells containing each barcode are used to create the starting cell pool.

BRIEF DESCRIPTION OF THE DRAWINGS

[010] FIG. 1 is a schematic showing an example overview of longitudinal transcriptomics of barcoded cells (bottom), which combines traditional endpoint studies (top left) with clonal analysis (top right) to allow analysis of response and adaptation of individual cells/clones over time in response to a selective pressure.

[011] FIG. 2 is a schematic showing an example of expression-based high complexity barcoding coupled to single cell analysis for analysis of sensitivity and resistance to a drug treatment (or other selective pressure) over time.

[012] FIG. 3 illustrates an example scRNA-seq compatible barcoding construct with the barcode insertion site, T2A (self-cleaving peptide), and EGFP and puromycin selection genes