

CLAIMS

In the claims:

1. A method, comprising:
 - (a) providing a plurality of cells, wherein the plurality of cells comprise at least one cell comprising at least one genetic perturbation, wherein said at least one cell comprising the at least one genetic perturbation comprises a barcode sequence associated with the genetic perturbation, optionally wherein at least one cell comprises a CRISPR system;
 - (b) performing at least one round of fluorescence *in situ* hybridization (FISH);
 - (c) performing pooled optical screening in human cells (POSH), comprising amplifying the barcode sequence in the at least one cell and sequencing the barcode sequence *in situ*.
2. The method of claim 1, further comprising analyzing the phenotype of the at least one cell;
wherein analyzing the phenotype comprises at least one assay selected from the group consisting of label-free imaging, high content imaging, calcium imaging, immunohistochemistry, cell morphology imaging, protein aggregation imaging, cell-cell interaction imaging, live cell imaging, and any other imaging-based assay modality.
3. The method of claim 1 or claim 2, wherein the plurality of cells:
 - (i) are selected from the group consisting of immortalized cancer cell lines, primary cells, primary tissue biopsies, and patient-derived cancer cells; or
 - (ii) are derived from induced pluripotent stem cells (iPSCs), optionally further comprising differentiating the iPSCs to derive the plurality of cells and/or optionally wherein the iPSCs are differentiated into hepatic stellate cells.
4. The method of any one of claims 1-3, wherein the at least one cell has been contacted with a gRNA to generate the at least one perturbation; and optionally wherein:
 - (i) the gRNA is or contains the barcode sequence; or
 - (ii) the barcode sequence comprises the gRNA sequence or a portion of the gRNA sequence.

5. The method of any one of claims 1-4, the method further comprising contacting the plurality of cells with a gRNA to generate the at least one genetic perturbation.
6. The method of any one of claims 1-5, further comprising fixing the plurality of cells on a surface prior to step (b), optionally wherein fixing the plurality of cells comprises at least one of paraformaldehyde treatment and methanol treatment.
7. The method of any one of claims 1-6, further comprising permeabilizing the plurality of cells.
8. The method of any one of claims 1-7, wherein the at least one round of FISH comprises at least one round of RNA FISH, wherein each round of RNA FISH is uniquely associated with at least one mRNA transcript from the at least one cell.
9. The method of claim 8, wherein:
 - (A) the at least one round of RNA FISH comprises:
 - (i) contacting a plurality of mRNA transcripts comprising the at least one mRNA transcript with a plurality of 3' loop probes, wherein each 3' loop probe comprises a first target hybridization sequence complementary to a first portion of an mRNA transcript of the plurality of mRNA transcripts;
 - (ii) contacting the plurality of mRNA transcripts comprising the at least one mRNA transcript with a plurality of 5' probes, wherein each 5' probe comprises a second target hybridization sequence complementary to a second portion of the mRNA transcript of the plurality of mRNA transcripts, wherein each 5' probe is capable of specifically hybridizing with a 3' loop probe, wherein hybridization of a 5' probe with a corresponding 3' loop probe forms a loop in the 3' loop probe;
 - (iii) connecting the ends of the loop in each 3' loop probe to form a plurality of circular probes;
 - (iv) amplifying a plurality of target sequences using the circular probes as templates to form a plurality of DNA amplicons, optionally wherein the DNA amplicon is formed using rolling circle amplification (RCA); and
 - (v) detecting the DNA amplicons by the at least one round of RNA FISH;
 - and/or

(B) wherein the at least one round of RNA FISH comprises:

- (i) contacting a plurality of mRNA transcripts comprising the at least one mRNA transcript with a plurality of 5' loop probes, wherein each 5' loop probe comprises a first target hybridization sequence complementary to a first portion of an mRNA transcript of the plurality of mRNA transcripts;
- (ii) contacting the plurality of mRNA transcripts comprising the at least one mRNA transcript with a plurality of 3' probes, wherein each 3' probe comprises a second target hybridization sequence complementary to a second portion of the mRNA transcript of the plurality of mRNA transcripts, wherein each 3' probe is capable of specifically hybridizing with a 5' loop probe, wherein hybridization of a 3' probe with a corresponding 5' loop probe forms a loop in the 5' loop probe;
- (iii) connecting the ends of the loop in each 5' loop probe to form a plurality of circular probes;
- (iv) amplifying a plurality of target sequences using the circular probes as templates to form a plurality of DNA amplicons, optionally wherein the DNA amplicon is formed using rolling circle amplification (RCA); and
- (v) detecting the DNA amplicons by the at least one round of RNA FISH.

- 10. The method of claim 9, wherein detecting the DNA amplicons comprises:
 - (a) labeling the DNA amplicons with a fluorophore, an isotope, a mass tag, or a combination thereof; and/or
 - (b) hybridizing an adapter oligonucleotide to the DNA amplicon; and/or
 - (c) hybridizing a detection probe to the adapter oligonucleotide; and/or
 - (d) imaging the DNA amplicons.
- 11. The method of any one of claims 9-10, further comprising fixing the DNA amplicons to a surface, optionally wherein the fixing comprises treatment with bis-N-succinimidyl-(pentaethylene glycol) (bis-MHS-PEG).
- 12. The method of any one of claims 1-11, wherein the at least one round of FISH comprises at least one round of RNA foci FISH comprising:

- (i) contacting RNA foci with a fluorescent oligonucleotide probe, wherein each fluorescent oligonucleotide probe comprises a target hybridization sequence complementary to a portion of a sequence in the RNA foci; and
 - (ii) detecting the fluorescent oligonucleotide probe by imaging.
13. The method of any one of claims 1-12, wherein POSH comprises:
- (A) reverse transcribing the barcode sequence to form a reverse transcribed barcode sequence;
 - (B) hybridizing at least one padlock probe to the reverse transcribed barcode sequence, wherein:
 - the at least one padlock probe comprises a first barcode hybridization sequence and a second barcode hybridization sequence,
 - the reverse transcribed barcode sequence comprises a first padlock probe hybridization sequence and a second padlock probe hybridization sequence flanking a target sequence, and
 - the first barcode hybridization sequence hybridizes with first padlock probe hybridization sequence and the second barcode hybridization sequence hybridizes with second padlock probe hybridization sequence; and
 - (C) connecting the ends of the at least one padlock probe to form a circular probe; the method optionally further comprising (D) forming a barcode amplicon using the circular probe as a template, wherein the barcode amplicon comprises a plurality of copies of the barcode sequence.
14. A non-transitory computer-readable storage medium storing one or more programs for analyzing known phenotypes or identifying new phenotypes of a plurality of cells, the one or more programs comprising instructions, which when executed by one or more processors of an electronic device having a display, cause the electronic device to perform the operations of:
- receiving a first image depicting the plurality of cells, wherein the first image indicates each cell of the plurality of cells by a boundary and associates each cell of the plurality of cells with a corresponding cell identifier;

receiving a second image depicting locations of a plurality of barcode sequences, wherein the plurality of barcode sequences are associated with the plurality of cells after one or more *in situ* sequencing cycles are performed;

aligning the first image and the second image;

based on the alignment of the first image and the second image, identifying an association between each cell of the plurality of cells and a corresponding barcode sequence of the plurality of barcode sequences;

receiving a FISH image of the plurality of cells after a FISH cycle is performed on the plurality of cells;

aligning the first image and the FISH image;

based on the alignment between the first image and the FISH image, resizing the FISH image;

associating each cell of the plurality of cells with a portion of the resized FISH image, the corresponding barcode sequence, and the corresponding cell identifier; and

analyzing known phenotypes or identifying new phenotypes of the plurality of cells.

15. A system for analyzing known phenotypes or identifying new phenotypes of a plurality of cells, comprising:

one or more processors;

a memory; and

one or more programs, wherein the one or more programs are stored in the memory and configured to be executed by the one or more processors, the one or more programs including instructions for:

receiving a first image depicting the plurality of cells, wherein the first image indicates each cell of the plurality of cells by a boundary and associates each cell of the plurality of cells with a corresponding cell identifier;

receiving a second image depicting locations of a plurality of barcode sequences, wherein the plurality of barcode sequences are associated with the plurality of cells after one or more *in situ* sequencing cycles are performed;

aligning the first image and the second image;

based on the alignment of the first image and the second image, identifying an association between each cell of the plurality of cells and a corresponding barcode sequence of the plurality of barcode sequences;

receiving a FISH image of the plurality of cells after a FISH cycle is performed on the plurality of cells;

aligning the first image and the FISH image;

based on the alignment between the first image and the FISH image, resizing the FISH image;

associating each cell of the plurality of cells with a portion of the resized FISH image, the corresponding barcode sequence, and the corresponding cell identifier; and

analyzing known phenotypes or identifying new phenotypes of the plurality of cells.