

METHODS AND SYSTEMS FOR THERAPEUTIC AGENT ANALYSIS

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/727,295, filed September 5, 2018, which is entirely incorporated herein by reference.

BACKGROUND

[0002] Therapeutic agents such as small interfering RNAs (siRNA) and microRNAs (miRNA) are transforming the therapeutic landscape from cancer to neurologic disorders. However, few molecules have reached FDA approval. Challenges in delivery verification and off target knockdowns represent a significant barrier to clinical utility. Bulk RNA sequencing can determine global transcriptional changes; however, small effects are often lost in the noise. In addition, due to homogenizing the samples, bulk RNA sequencing cannot localize effects to specific tissues, cell-types, or individual cells. Fluorescent in situ hybridization targeting ribonucleic acid molecules (RNA-FISH) can localize transcriptional changes to specific cells, however, its low multiplexity limits the number of targets observed. Finally, RNA-FISH may require long probes (250-1,500 bases), thus it cannot directly detect and localize these small molecules, 20-50 bases.

SUMMARY

[0003] The present disclosure provides methods and systems for therapeutic agent detection in a biological sample having a three-dimensional matrix.

[0003a] In one aspect, provided herein is a method for identifying a target molecule and an anti-sense nucleic acid sequence, comprising: (a) providing a biological sample having or suspected of having said target molecule and said anti-sense nucleic acid sequence; (b) detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a position of said anti-sense nucleic acid sequence in said biological sample, wherein said second set of signals identifies a change in a level of said target molecule relative to a reference, and wherein said first set of signals is detected with the aid of sequencing; and (c) using said first set of signals and said second set of signals to provide an output indicative of said position of each of said anti-sense nucleic acid sequence and said target molecule in said biological sample.

[0003b] In one aspect, provided herein is a method for identifying a target ribonucleic acid (RNA) molecule and an anti-sense RNA sequence, comprising: (a) providing a biological

sample having or suspected of having said target RNA molecule and said anti-sense RNA sequence, wherein said anti-sense RNA sequence is anti-sense to a sequence of said target RNA molecule; (b) detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a position of said anti-sense RNA sequence in said biological sample, and wherein said second set of signals identifies a change in a level of said target RNA molecule relative to a reference, wherein said first set of signals or said second set of signals are detected with the aid of sequencing; and (c) using said first set of signals and said second set of signals to provide an output indicative of said position of said anti-sense RNA and said target RNA molecule in said biological sample.

[0004] According to an example, provided herein is a method for identifying an anti-sense nucleic acid molecule and a target molecule, comprising: (a) providing a biological sample having or suspected of having the target molecule and the anti-sense nucleic acid molecule, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) using detection probes separate from the anti-sense nucleic acid molecule and the target molecule to detect a first set of signals and a second set of signals from the biological sample, wherein the first set of signals identifies a relative position of the anti-sense nucleic acid molecule in the biological sample, and wherein the second set of signals identifies an increase or a decrease in a level of the target molecule relative to a reference; and (c) using the first set of signals and the second set of signals to provide an output indicative of a position of each of the anti-sense nucleic acid molecule and the target molecule in the biological sample.

[0005] In some examples, the method further comprises, prior to (a), providing the biological sample, contacting the biological sample with a solution having the anti-sense nucleic acid molecule, and generating the 3D matrix subsequent to contacting the biological sample with the solution. In some examples, the target molecule is a target nucleic acid molecule.

[0006] In some examples, (b) comprises using the detection probes to identify a target sequence of the target nucleic acid molecule, thereby providing the second set of signals. In some examples, (b) comprises using the detection probes to sequence the target nucleic acid molecule, thereby identifying the target sequence. In some examples, (b) comprises using the detection probes to identify an anti-sense sequence of the anti-sense nucleic acid molecule, thereby providing the first set of signals. In some examples, (b) comprises using the detection probes to sequence the anti-sense nucleic acid molecule, thereby identifying the anti-sense sequence.

[0007] In some examples, the target molecule is a target polypeptide or protein. In some examples, the biological sample is a cell, a cell derivative, or a tissue. In some examples, the

anti-sense nucleic acid molecule is a therapeutic agent. In some examples, the output is an image or video. In some examples, the anti-sense nucleic acid molecule is a ribonucleic acid, a phosphorothioate deoxyribonucleic acid, a locked nucleic acid, 2'-O-methoxy-ethyl ribonucleic acid, 2'-O-methyl ribonucleic acid, or a 2'-fluoro deoxyribonucleic acid. In some examples, the anti-sense nucleic acid molecule is from 15 to 60 nucleotides in length.

[0008] According to another example, provided herein is a method for identifying an anti-sense ribonucleic (RNA) molecule co-localized with a target nucleic RNA molecule, comprising: (a) providing a biological sample having or suspected of having the target RNA molecule and the anti-sense RNA molecule of the target RNA molecule, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) detecting a first set of signals and a second set of signals from the biological sample, wherein the first set of signals identifies a relative position of the anti-sense RNA molecule in the biological sample, and wherein the second set of signals identifies an increase or a decrease in a level of the target RNA molecule relative to a reference; and (c) using the first set of signals and the second set of signals to provide an output indicative of the anti-sense RNA molecule co-localized with the target RNA molecule in the biological sample.

[0009] In some examples, the method further comprises, prior to (a), providing the biological sample, contacting the biological sample with a solution having the anti-sense RNA molecule, and generating the 3D matrix subsequent to contacting the biological sample with the solution. In some examples, the biological sample is a cell, a cell derivative, or a tissue. In some examples, (b) comprises using detection probes to identify an anti-sense sequence of the anti-sense RNA molecule, thereby providing the first set of signals. In some examples, (b) comprises using the detection probes to sequence the anti-sense RNA molecule, thereby identifying the anti-sense sequence. In some examples, (b) comprises using detection probes to identify a target sequence of the target RNA molecule, thereby providing the second set of signals. In some examples, (b) comprises using the detection probes to sequence the target RNA molecule, thereby identifying the target sequence. In some examples, the anti-sense nucleic acid molecule is a therapeutic agent. In some examples, the output is an image or video. In some examples, the anti-sense RNA molecule is a modified anti-sense nucleic acid molecule. In some examples, the modified anti-sense nucleic acid molecule is a ribonucleic acid, a phosphorothioate deoxyribonucleic acid, a locked nucleic acid, 2'-O-methoxy-ethyl ribonucleic acid, 2'-O-methyl ribonucleic acid, or a 2'-fluoro deoxyribonucleic acid. In some examples, the anti-sense RNA molecule is from 15 to 60 nucleotides in length.

[0010] According to another example, provided herein is a method for identifying two or more genetic aberrations of a target nucleic acid, comprising: (a) providing a biological sample having or suspected of having at least a first genetic aberration and a second genetic aberration of the target nucleic acid, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) using detection probes to detect a first set of signals and a second set of signals from the biological sample, wherein the first set of signals identifies a first relative position of the first genetic aberration in the biological sample, and wherein the second set of signals identifies a second relative position of the second genetic aberration in the biological sample; and (c) using the first set of signals and the second set of signals to provide an output indicative of a position of each of the first genetic aberration and the second genetic aberration in the biological sample.

[0011] In some examples, the detection probes are targeted to a same sequence of the first genetic aberration and the second genetic aberration. In some examples, the first genetic aberration and the second genetic aberration have at least a single nucleotide difference. In some examples, the first genetic aberration comprises an additional sequence than the second genetic aberration. In some examples, the biological sample is a cell, a cell derivative, or a tissue.

[0012] According to another example, provided herein is a method for identifying two or more locations of an anti-sense nucleic acid molecule, comprising: (a) providing a biological sample having the anti-sense nucleic acid molecule, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) using a detection probe to detect a set of signals of the anti-sense nucleic acid molecule within the biological sample; (c) detecting a first set of signals and a second set of signals from at least a first location and a second location within the biological sample, wherein the first location and the second location are within different sub-cellular compartments; and (d) using the set of signals of the anti-sense nucleic acid molecule and the first and the second set of signals of the first location and the second location to provide an output indicative of the anti-sense nucleic acid molecule co-localized with the first location and the second location.

[0013] In some examples, the anti-sense nucleic acid molecule is from 15 to 60 nucleotides in length. In some examples, the sub-cellular compartments comprise mitochondria, endoplasmic reticulum, P-bodies, Golgi apparatus, vesicle, endosome, lysosome, nucleus, microtubule, or a combination thereof. In some examples, the anti-sense nucleic acid molecule is a therapeutic agent. In some examples, the biological sample is a cell, a cell derivative, or a tissue.

[0014] According to another example, provided herein is a method for identifying co-localized molecules in a biological sample, comprising: (a) providing the biological sample having or suspected of having a target molecule and an agent capable of binding or interacting with the target molecule, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) using detection probes separate from the target molecule and the agent to detect a first set of signals and a second set of signals from the biological sample, wherein the first set of signals identifies a relative position of the target molecule in the biological sample, and wherein the second set of signals identifies a relative position of the agent in the biological sample; and (c) using the first set of signals and the second set of signals to provide an output indicative of the target molecule co-localized with the agent in the biological sample.

[0015] In some examples, the agent is a therapeutic agent. In some examples, the therapeutic agent is a polynucleotide, a polypeptide, or a small molecule.

[0016] According to another example, provided herein is a method for identifying an agent and a metabolite of the agent in a biological sample, comprising: (a) providing the biological sample having or suspected of having the agent and the metabolite of the agent, wherein the biological sample comprises a three-dimensional (3D) matrix; (b) using detection probes separate from the target molecule and the agent to detect a first set of signals and a second set of signals from the biological sample, wherein the first set of signals identifies a relative position of the agent in the biological sample, and wherein the second set of signals identifies a relative position of the metabolite in the biological sample; and (c) using the first set of signals and the second set of signals to provide an output indicative of a position of each of the agent and the metabolite.

[0017] In some examples, the biological sample is a cell, a cell derivative, or a tissue. In some examples, the agent is a therapeutic agent. In some examples, the therapeutic agent is a polynucleotide, a polypeptide, or a small molecule.

[0018] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0019] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0020] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0021] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be

CLAIMS

1. A method for identifying a target molecule and an anti-sense nucleic acid sequence, comprising:
 - (a) providing a biological sample having or suspected of having said target molecule and said anti-sense nucleic acid sequence;
 - (b) detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a position of said anti-sense nucleic acid sequence in said biological sample, wherein said second set of signals identifies a change in a level of said target molecule relative to a reference, and wherein said first set of signals is detected with the aid of sequencing; and
 - (c) using said first set of signals and said second set of signals to provide an output indicative of said position of each of said anti-sense nucleic acid sequence and said target molecule in said biological sample.
2. The method of claim 1, further comprising, prior to (a), contacting said biological sample with a solution having said anti-sense nucleic acid sequence.
3. The method of claim 1, wherein said target molecule is a target nucleic acid molecule.
4. The method of claim 3, wherein (b) comprises identifying a target sequence of said target nucleic acid molecule.
5. The method of claim 1, wherein said target molecule is a target polypeptide or target protein.
6. The method of claim 1, wherein said biological sample is a cell, a cell derivative, or a tissue.
7. The method of claim 1, wherein said output is an image or video.
8. The method of claim 1, wherein said anti-sense nucleic acid sequence is of a ribonucleic acid, a phosphorothioate deoxyribonucleic acid, a locked nucleic acid, a 2'-O-methoxy-ethyl ribonucleic acid, a 2'-O-methyl ribonucleic acid, or a 2'-fluoro deoxyribonucleic acid.
9. The method of claim 1, wherein said anti-sense nucleic acid sequence is of an anti-sense nucleic acid molecule from 15 to 60 nucleotides in length.
10. The method of claim 1, wherein, in (b), said detecting is performed with one or more detection probes.
11. The method of claim 10, wherein said one or more detection probes comprise a nucleic acid.
12. The method of claim 11, wherein (b) further comprises hybridizing a detection probe of said one or more detection probes to said anti-sense nucleic acid sequence.
13. The method of claim 1, wherein said sequencing comprises sequencing-by-synthesis, sequencing-by-ligation or sequencing by hybridization.
14. A method for identifying a target ribonucleic acid (RNA) molecule and an anti-sense RNA sequence, comprising:
 - (a) providing a biological sample having or suspected of having said target RNA molecule and said anti-sense RNA sequence, wherein said anti-sense RNA sequence is anti-sense to a sequence of said target RNA molecule;
 - (b) detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a position of said anti-sense RNA sequence in said biological sample, and wherein said second set of signals identifies a change in a level of said target RNA molecule

relative to a reference, wherein said first set of signals or said second set of signals are detected with the aid of sequencing; and

(c) using said first set of signals and said second set of signals to provide an output indicative of said position of said anti-sense RNA and said target RNA molecule in said biological sample.

15. The method of claim 14, further comprising, prior to (a), contacting said biological sample with a solution having said anti-sense RNA sequence.

16. The method of claim 14, wherein said biological sample is a cell, a cell derivative, or a tissue.

17. The method of claim 14, wherein, in (b), said detecting is performed with one or more detection probes.

18. The method of claim 17, wherein (b) further comprises hybridizing a detection probe of said one or more detection probes to said anti-sense nucleic acid sequence.

19. The method of claim 17, wherein (b) comprises hybridizing a detection probe of said one or more detection probes to a target sequence of said target RNA molecule.

20. The method of claim 14, wherein said sequencing comprises sequencing-by-synthesis, sequencing-by-ligation or sequencing-by-hybridization.