

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~~oligonucleotide, comprising: (a) providing a biological sample obtained from a subject having or suspected of having said target molecule and said anti-sense ~~nucleic acid sequence~~oligonucleotide, wherein said anti-sense oligonucleotide comprises a sequence complementary to a sequence of a messenger ribonucleic acid (mRNA) transcript of said target molecule; (b) using a first detection probe specific for said anti-sense oligonucleotide and a second detection probe specific for said target molecule to detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a first position of said anti-sense ~~nucleic acid sequence~~oligonucleotide in said biological sample, wherein said second set of signals identifies a second position of said target molecule and a change in a level of said target molecule relative to a reference, and wherein said first set of signals or said second set of signals are detected using

fluorescent in situ sequencing (FISSEQ)~~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 first position ~~of each of~~ said anti-sense ~~nucleic acid sequence~~oligonucleotide and said second position of 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

CLAIMS

1. A method for identifying a target molecule and an anti-sense ~~nucleic acid sequence~~oligonucleotide, comprising:
 - (a) providing a biological sample obtained from a subject having or suspected of having said target molecule and said anti-sense ~~oligonucleotide, nucleic acid sequence~~ wherein said anti-sense oligonucleotide comprises a sequence complementary to a sequence of a messenger ribonucleic acid (mRNA) transcript of said target molecule;
 - (b) using a first detection probe specific for said anti-sense oligonucleotide and a second detection probe specific for said target molecule to detecting a first set of signals and a second set of signals from said biological sample, wherein said first set of signals identifies a first position of said anti-sense ~~oligonucleotide, nucleic acid sequence~~ in said biological sample, wherein said second set of signals identifies a second position of said target molecule and a change in a level of said target molecule relative to a reference, and wherein said first set of signals or said second set of signals are detected using fluorescent in situ sequencing (FISSEQ)~~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 first position of ~~each of~~ said anti-sense ~~nucleic acid sequence~~oligonucleotide and said second position of 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 ~~oligonucleotide, nucleic acid sequence~~.
- ~~3. The method of claim 1, wherein said target molecule is a target nucleic acid molecule.~~
- ~~43.~~ The method of claim ~~31~~, wherein (b) comprises identifying a target sequence of said target nucleic acid molecule.
- ~~54.~~ The method of claim 1, wherein said target molecule is a target polypeptide or target protein.
- ~~65.~~ The method of claim 1, wherein said biological sample is a cell, a cell derivative, or a tissue.
- ~~76.~~ The method of claim 1, wherein said output is an image or video.
- ~~87.~~ The method of claim 1, wherein said anti-sense ~~nucleic acid sequence~~oligonucleotide ~~is of a~~ ribonucleic acid, a phosphorothioate deoxyribonucleic acid, a locked nucleic acid, a 2'-O-methoxyethyl ribonucleic acid, a 2'-O-methyl ribonucleic acid, or a 2'-fluoro deoxyribonucleic acid.
- ~~98.~~ The method of claim 1, wherein said ~~anti-sense nucleic acid sequence is of an~~ anti-sense ~~nucleic acid molecule~~oligonucleotide is 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.~~
- ~~119.~~ The method of claim ~~10~~, wherein said ~~one or more~~first detection probes or said second detection probe comprises a nucleic acid.
- ~~1210.~~ The method of claim ~~14~~, wherein (b) further comprises hybridizing ~~a said first~~ detection probe ~~of said one or more detection probes~~ to said anti-sense ~~nucleic acid sequence~~oligonucleotide.
- ~~1311.~~ The method of claim 1, wherein said ~~sequencing FISSEQ~~ 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.~~