

ATTACHMENT 2

Representative Invalidity of U.S. Patent No. 11,098,303 (Claims 1-12) by U.S. Patent No. 10,829,814 Fan and Zhang, “Methods and Compositions for Single Cell Genomics” (“Fan”)

Fan was filed on June 17, 2015, published on December 24, 2015, and claims priority to Provisional Application No. 62/014,538, filed on June 19, 2014. Fan is prior art under 35 U.S.C. § 102. Claims 1-12 of the 303 Patent are anticipated or rendered obvious by Fan alone, or Fan in combination with other prior art, as outlined in the chart below.

10x’s contentions in this document are made under Vizgen and Harvard’s allegations and positions, including Vizgen and Harvard’s apparent interpretation of the asserted claims of the 303 Patent as shown in at least its First Amended Answers, Defense, and Counterclaims, and its Initial Infringement Contentions, for purposes of the invalidity contentions only. The invalidity analysis in this chart is not an adoption or admission by 10x as to the accuracy of Vizgen and Harvard’s allegations, admissions, or positions, including those with respect to claim scope, validity, and interpretation of claim language. Accordingly, contentions in this chart are not necessarily intended to be consistent with other non-infringement or invalidity positions that 10x may take and should not be otherwise construed.

Based on Vizgen’s interpretation, Fan expressly or inherently discloses every limitation of the below-listed asserted claims of the 303 Patent. To the extent that Vizgen contends that Fan does not expressly or inherently disclose every limitation, it would have been obvious to a person of skill in the art reading the Fan to modify it to render the claims invalid under § 103 based on Vizgen’s interpretation.

The following claim chart containing exemplary disclosures is based on the information presently available to 10x. 10x’s investigation of the case continues, and 10x reserves the right to supplement this claim chart as appropriate.

Claim No.	Claim Language	Disclosure
1[preamble]	A method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization, comprising:	To the extent that the preamble is limiting, Fan teaches a method for detecting a plurality of different nucleic acid targets within a sample by in situ hybridization. For example: “Presented herein are methods and compositions for obtaining sequence information from one or more individual cells. The methods are useful for

Claim No.	Claim Language	Disclosure
		obtaining sequence information for a single nucleotide sequence, and for multiplex generation of sequence information from an individual cell. In some embodiments presented herein, the methods comprise first amplifying one or more nucleic acid molecules in situ within a cell, and subsequently hybridizing a barcode probe to the amplified nucleic acid molecules. The identity of the amplified nucleic acid molecules is then determined by decoding of the barcode probes. In other embodiments presented herein, the methods comprise first hybridizing one or more different barcode probes to one or more nucleic acid molecule in situ within a cell, and subsequently amplifying the barcode probe, thereby generating an amplified barcode probe. The identity of the nucleic acid molecules is then determined by decoding of the amplified barcode probes.” Fan at 1:58-2:7 (emphasis added). “A nucleic acid sample that is amplified, sequenced or otherwise manipulated in a method disclosed herein can be, for example, DNA or RNA. Exemplary DNA species include, but are not limited to, genomic DNA (gDNA), mitochondrial DNA, chloroplast DNA, episomal DNA, viral DNA and copy DNA (cDNA). One non-limiting example of a subset of genomic DNA is one particular chromosome or one region of a particular chromosome. Exemplary RNA species include, without limitation, coding RNA such as messenger RNA (mRNA), and non-coding RNA (ncRNA) such as transfer RNA (tRNA), microRNA (miRNA), small nuclear RNA (snRNA) and ribosomal RNA (rRNA). Further species of DNA or RNA include fragments or portions of the species listed above or amplified products derived from these species, fragments thereof or portions thereof. The methods described herein are applicable to the above species encompassing all or part of the complement present in a cell. For example, using methods described herein the sequence of a substantially complete genome can be determined or the sequence of a substantially complete targeted nucleic acid sequences such as mRNA or cDNA complement of a cell can be determined.”

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		Fan at 6:60-7:14. “Cell Handling for In Situ Detection and Decoding The methods and compositions make use of hybridization, amplification, washing and detection within individual cells. These methods are performed in situ on isolated cells or in tissue sections that have been prepared according to methodologies known in the art. Methods for permeabilization and fixation of cells and tissue samples are well-known in the art, as exemplified by Cremer et al., The Nucleus: Volume 1: Nuclei and Subnuclear Components, R. Hancock (ed.) 2008; and Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the content of each of which is incorporated herein by reference in its entirety. Any suitable permeabilization and/or fixation technologies can be used for making cells and the target nucleic acid molecules therein available for the amplification and detection methods provided herein. Detection of Signals The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ. Suitable imaging technologies are known in the art, as exemplified by Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the entire content of which is incorporated by reference herein in its entirety. An example demonstrating in situ imaging and 3D reconstruction of in situ detection is set forth in FIG. 1 and Example 1.” Fan at 10:61-11:22 (emphasis added). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach

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		certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1[a]	(a) producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes,	Fan teaches producing a plurality of first hybridized complexes within a sample containing a plurality of different nucleic acid targets by exposing the sample to a plurality of different encoding nucleic acid probes. For example: “In some embodiments presented herein, the methods comprise first amplifying one or more nucleic acid molecules in situ within a cell, and subsequently hybridizing a barcode probe to the amplified nucleic acid molecules. The identity of the amplified nucleic acid molecules is then determined by decoding of the barcode probes. In other embodiments presented herein, the methods comprise first hybridizing one or more different barcode probes to one or more nucleic acid molecule in situ within a cell, and subsequently amplifying the barcode probe, thereby generating an amplified barcode probe. The identity of the nucleic acid molecules is then determined by decoding of the amplified barcode probes.” Fan at 1:63-2:7 (emphasis added). “FIG. 1 describes one embodiment of padlock capture and hybridization decoding of rolonies. (A) Molecular barcodes are attached to rolonies in a gene specific manner using padlock probes. (B) After padlock capture, each rolony will have a unique barcode in thousands of copies. These barcodes can be accurately decoded by hybridization similar to the decoding of Illumina bead array, using a combinatorial chemistry approach (Gunderson et al. Genome Res. 14(5): 870-877 (2004)).” Fan, Fig. 1 and 3:32-40. “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT

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		primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a ‘DNA ball’ or ‘rolony’. The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.” Fan at 9:1-22. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[a][i]	wherein each of the plurality of encoding nucleic acid probes comprises (i) a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets and	Fan teaches that each of the plurality of encoding nucleic acid probes comprises (i) a target sequence that specifically hybridizes to a different nucleic acid target or a different location of an identical nucleic acid target of the plurality of different nucleic acid targets. For example: “The methods provided herein further comprise annealing in situ a barcode probe to the amplified nucleic acid molecule. As used herein, the term barcode probe refers to a nucleic acid molecule which can hybridize specifically to a nucleic acid of interest, and which comprises a barcode sequence. As used herein, the terms barcode, barcode sequence and like terms refer to a unique identifier sequence on a probe molecule. The unique identifier sequence can be, for example, a nucleic acid sequence which associates the barcode probe with the nucleic acid of interest to which it hybridizes.

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		The barcode probes presented herein can be any probe which specifically hybridizes to a nucleic acid species of interest and which comprises at least a first and a second decoder sequence. In some embodiments presented herein, the barcode probes are used as a template for further amplification, thus generating a clonal object that comprises many repeating copies of the barcode, as described in greater detail in the disclosure of WO 2011/123246, the entire content of which is incorporated herein by reference in its entirety.” Fan at 5:15-35 (emphasis added). “Barcode probes used in the methods presented herein can include padlock probes. Padlock probes are specialized ligation probes and are known in the art, as exemplified generally by Nilsson et al, 1994, Science 265:2085, hereby incorporated by reference. These probes have a first ligation domain that is identical to a first ligation probe, in that it hybridizes to a first target sequence domain, and a second ligation domain, identical to the second ligation probe, that hybridizes to an adjacent target sequence domain. Again, as for OLA, the detection position can be either at the 3' end of the first ligation domain or at the 5' end of the second ligation domain. However, the two ligation domains are connected by a linker, frequently nucleic acid. The configuration of the system is such that upon ligation of the first and second ligation domains of the padlock probe, the probe forms a circular probe, and forms a complex with the target sequence wherein the target sequence is "inserted" into the loop of the circle.” Fan at 7:36-53. “The at least first and second decoding sequences can be any suitable length to ensure specific hybridization. Thus, for example, in some embodiments, each decoding sequence can be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or greater than 20 nucleotides in length.” Fan at 9:44-48.

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		To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1[a][ii]	(ii) one or more read sequences such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations;	Fan teaches one or more read sequences (decoder sequences) such that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations. For example, “The methods and compositions presented herein make use of unique barcode probes having decoder sequences to identify multiple nucleic acid targets in a multiplex fashion. The instant innovations overcome limitations of existing rolon identification methodologies by surprisingly and successfully applying decoding strategies that entail sequential hybridizations of labeled decoder probes to decoder sequences found in immobilized nucleic acids within an individual cell. Such decoding strategies using decoder sequences have until now been used for decoding of randomly assembled arrays of beads. Thus, the decoding strategies presented herein are flexible and scalable and can be used to detect and decode thousands of unique amplified nucleic acid species. In some embodiments, a barcode probe can comprise at least a first decoder sequence and a second decoder sequence. In some embodiments, a barcode probe can comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 decoder sequences. As used herein, the terms decoder sequence, stage, slot and like terms refer to a unique sequence which is part of a plurality of unique sequences are designed to each specifically hybridize to a labeled decoder probe. During decoding, labeled decoder probe oligonucleotides are sequentially hybridized to the decoder sequences to specifically determine the identity of each barcode sequence. Sequential hybridization of decoder probes to decoder sequences is described by Gunderson et al. (Genome Res. (2004) 14:870-877), the content of which is incorporated by reference in its entirety. Additional description of

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		decoding algorithms and the use of sequential hybridization useful in the methods presented herein can be found in U.S. Pat. Nos. 7,060,431; 7,033,754; 7,226,734; and 7,455,971, the disclosures of each of which are hereby incorporated by reference in their entireties. The principles of sequential hybridization to decode bead arrays are surprisingly robust and effective when applied to amplified nucleic acids in situ according to the methods provided herein.” Fan at 8:31-67 (emphasis added). “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a ‘DNA ball’ or ‘rolony’. The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.” Fan at 9:1-22. “An amplicon typically contains multiple, tandem copies of the circularized nucleic acid molecule of the corresponding sample nucleic acid. That is, each amplicon contains multiple, tandem copies of a single sample nucleic acid molecule, which was circularized. The number of copies can be varied by

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		appropriate modification of the amplification reaction including, for example, varying the number of amplification cycles run, using polymerases of varying processivity in the influence amplification yield. Generally, the number of copies of a nucleic acid in an amplicon is at least 100, 200, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10,000 copies, and can be varied depending on the particular application. As disclosed herein, one particular form of an amplicon is as a nucleic acid "ball" having amplification reaction and/or varying the length of time that the amplification reaction is run, as well as modification of other conditions known in the art to desired dimensions. The number of copies of the nucleic acid molecule can therefore provide a desired size of a nucleic acid "ball" or a sufficient number of copies for efficient subsequent analysis of the amplicon, for example, sequencing." Fan at 6:8-29. "Useful methods for clonal amplification from single molecules include, for example, rolling circle amplification (RCA) (Lizardi et al., <i>Nat. Genet.</i> 19:225-232 (1998), which is incorporated herein by reference) and oligonucleotide ligation assay (OLA) (See generally U.S. Pat. Nos. 7,582,420, 5,185,243, 5,679,524 and 5,573,907; EP 0 320 308 B1; EP 0 336 731 B1; EP 0 439 182 B1; WO 90/01069; WO 89/12696; and WO 89/09835, all of which are incorporated by reference) technologies. The enhanced signal-to-noise ratio provided by clonal amplification provides a great advantage over existing detection methodologies. In a particularly useful embodiment, amplicons are generated by rolling circle amplification (RCA), which can be used to generate amplicons having multiple copies of a nucleic acid sequence and which can be used to create nucleic acid 'balls,' as disclosed herein. It will be understood that these 'balls' need not be perfectly spherical and can include other globular or packed conformations. In a particular embodiment, RCA is primed using the at least one universal primer attached to the sample nucleic acid molecule."

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		Fan at 17:15-35. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach that each of the plurality of first hybridized complexes comprises multiple encoding nucleic acid probes of the different encoding nucleic acid probes located in its different locations, such limitations would have been inherent and/or obvious in view Cai and/or Raj as described in 10x's Invalidity Contentions. <i>See</i> Attachments 1 and 4, limitation 1[a][ii]. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1(b)	(b) producing a plurality of readout probe-hybridized complexes by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe, wherein the first readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	Fan teaches producing a plurality of readout probe-hybridized complexes (the decoder probe-detection reagent-analyte conjugate) by exposing each of the plurality of first hybridized complexes within the sample to a first readout nucleic acid probe (decoder probe). The first readout nucleic acid probe (decoder probe) comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences (decoder sequence), and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. For example: “The methods and compositions presented herein make use of unique barcode probes having decoder sequences to identify multiple nucleic acid targets in a multiplex fashion. The instant innovations overcome limitations of existing colony identification methodologies by surprisingly and successfully applying decoding strategies that entail sequential hybridizations of labeled decoder probes to decoder sequences found in immobilized nucleic acids within an individual cell. Such decoding strategies using decoder sequences have until now been used for decoding of randomly assembled arrays of beads. Thus, the decoding strategies presented herein are flexible and scalable and can be used

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		to detect and decode thousands of unique amplified nucleic acid species. In some embodiments, a barcode probe can comprise at least a first decoder sequence and a second decoder sequence. In some embodiments, a barcode probe can comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 decoder sequences. As used herein, the terms decoder sequence, stage, slot and like terms refer to a unique sequence which is part of a plurality of unique sequences are designed to each specifically hybridize to a labeled decoder probe. During decoding, labeled decoder probe oligonucleotides are sequentially hybridized to the decoder sequences to specifically determine the identity of each barcode sequence. Sequential hybridization of decoder probes to decoder sequences is described by Gunderson et al. (Genome Res. (2004) 14:870-877), the content of which is incorporated by reference in its entirety. Additional description of decoding algorithms and the use of sequential hybridization useful in the methods presented herein can be found in U.S. Pat. Nos. 7,060,431; 7,033,754; 7,226,734; and 7,455,971, the disclosures of each of which are hereby incorporated by reference in their entireties. The principles of sequential hybridization to decode bead arrays are surprisingly robust and effective when applied to amplified nucleic acids in situ according to the methods provided herein.” Fan at 8:31-67 (emphasis added). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[c]	(c) detecting a fluorescent signal from each of the plurality of readout probe-hybridized complexes;	Fan teaches detecting a fluorescent signal from each of the plurality of readout probe-hybridized complexes. For example: “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown

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		in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a "DNA ball" or "rolony". The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images." Fan at 9:1-22 (emphasis added). "The decoding strategies presented herein provide several key advantages over existing sequence identification methods. For example, one advantage is the simplicity and robustness of the decoding process, which involves hybridization and washing of oligo pools at room temperature. As such, heating and cooling steps are not necessary, thus minimizing damage to the cellular structure and reducing the potential drift of the specimen throughout the imaging process. As an additional advantage, the signals that are generated are more even and have higher intensity inside a structured tissue than methods that rely on other detection techniques. Further, in many embodiments, only two fluors are needed for decoding, which provides convenient spectral distinction using simple detection devices and leaves enough spectrum for labeling cellular structure using other fluors. Of course more than two colors can be used to expand the number of sequences that are decoded, using modifications of the method set forth in further detail elsewhere herein and in the references that are incorporated by reference above as describing sequential hybridization of decoder probes to decoder sequences."

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		Fan at 9:23-43 (emphasis added). “The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ.” Fan at 11:11-15. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[d]	(d) removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes;	Fan teaches removing the fluorescent signal from each of the plurality of readout probe-hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the plurality of readout probe-hybridized complexes. For example: “In some aspects of the above embodiments, the first labeled decoder probe is removed prior to hybridizing the second labeled decoder probe. In some aspects, the second labeled decoder probe is hybridized prior to the first labeled decoder probe.” Fan at 2:56-60. “The decoding strategies presented herein provide several key advantages over existing sequence identification methods. For example, one advantage is the simplicity and robustness of the decoding process, which involves hybridization and washing of oligo pools at room temperature. As such, heating and cooling steps are not necessary, thus minimizing damage to the cellular structure and reducing the potential drift of the specimen throughout the imaging process.” Fan at 9:23-31.

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		To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1[e]	(e) after step (d), producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprise a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	Fan teaches after removing or inactivating the fluorescent signal, producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe. For example: “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a ‘DNA ball’ or ‘rolony’. The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.” Fan at 9:1-22; <i>see also</i> Fig. 1. Fan teaches that the readout nucleic acid probe (decoder probe) comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a

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		fluorescent signaling entity capable of producing a fluorescent signal. <i>See</i> disclosure for element 1(b). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1[f]	(f) detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes;	Fan teaches detecting a fluorescent signal from each of the different plurality of readout probe hybridized complexes. For example: “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a ‘DNA ball’ or ‘rolony’. The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.” Fan at 9:1-22 (emphasis added). “The decoding strategies presented herein provide several key advantages over existing sequence identification methods. For example, one advantage is the simplicity and robustness of the decoding process, which involves hybridization

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		and washing of oligo pools at room temperature. As such, heating and cooling steps are not necessary, thus minimizing damage to the cellular structure and reducing the potential drift of the specimen throughout the imaging process. As an additional advantage, the signals that are generated are more even and have higher intensity inside a structured tissue than methods that rely on other detection techniques. Further, in many embodiments, only two fluors are needed for decoding, which provides convenient spectral distinction using simple detection devices and leaves enough spectrum for labeling cellular structure using other fluors. Of course more than two colors can be used to expand the number of sequences that are decoded, using modifications of the method set forth in further detail elsewhere herein and in the references that are incorporated by reference above as describing sequential hybridization of decoder probes to decoder sequences. Fan at 9:23-43 (emphasis added). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
1[g]	(g) removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes;	Fan teaches removing the fluorescent signal from each of the different plurality of readout probe hybridized complexes by inactivating the fluorescent signaling entity or removing the fluorescent signaling entity from each of the different plurality of readout probe hybridized complexes. <i>See</i> disclosure for element 1[d]. The inactivation or removal of the fluorescent signaling entity is performed after each detecting step in a readout round. For example: “In some aspects of the above embodiments, the first labeled decoder probe is removed prior to hybridizing the second labeled decoder probe. In some aspects, the second labeled decoder probe is hybridized prior to the first labeled decoder probe.” Fan at 2:56-60.

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		“The decoding strategies presented herein provide several key advantages over existing sequence identification methods. For example, one advantage is the simplicity and robustness of the decoding process, which involves hybridization and washing of oligo pools at room temperature. As such, heating and cooling steps are not necessary, thus minimizing damage to the cellular structure and reducing the potential drift of the specimen throughout the imaging process.” Fan at 9:23-31. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[h]	(h) repeating steps (e) to (g) for one or more times using a different readout nucleic acid probe, wherein the different readout nucleic acid probe comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal;	Fan teaches after removing or inactivating the fluorescent signal, producing a different plurality of readout probe-hybridized complexes by exposing the sample to a different readout nucleic acid probe. For example: “As an example, one or more target nucleic acid species can be amplified, either specifically or non-specifically, to generate an amplified molecule containing many repeats of the amplified nucleic acid in tandem. In the embodiment shown in FIG. 1, cDNA is generated in situ, using either random hexamers or oligo dT primers or both. Then the cDNA molecules are amplified using rolling circle amplification (RCA) to generate a ‘DNA ball’ or ‘rolony’. The amplified nucleic acids can then be hybridized with one or more unique padlock probes with unique molecular barcodes. When a padlock probe specifically hybridizes to a sequence of interest, the probe is ligated to form, for example, a circularized barcoded padlock probe. The amplified nucleic acid molecules and/or the hybridized padlock probes are immobilized within the cell using the crosslinking methods described herein, and decoding of the barcode probe takes place by multiple rounds of sequential hybridization. In the example shown in FIG. 1, decoding takes place via a set of barcode probes having decoder

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		sequences that can be decoded by eight rounds of sequential hybridization with fluorescently labeled oligos and imaging of fluorescent images.” Fan at 9:1-22 (emphasis added). Fan teaches that the readout nucleic acid probe (decoder probe) comprises a first portion and a second portion, the first portion comprising a sequence that specifically hybridizes to one of the read sequences, and the second portion comprising a fluorescent signaling entity capable of producing a fluorescent signal. <i>See</i> disclosure for element 1(b). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[h][i]	(i) producing codewords representing the plurality of different nucleic acid targets at locations within the sample, wherein each of the codeword represents one of the plurality of different nucleic acid targets and comprises multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-	Fan teaches producing codewords (temporal sequence of signal signature) representing the plurality of different nucleic acid targets at locations within the sample, wherein each of the codeword represents one of the plurality of different nucleic acid targets and is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample. For example: “In some aspects, detecting can further comprise evaluating a signal to determine the presence, quantity, frequency, and/or spatial distribution of a disease-causing mutation, allele or common variant within a tissue.” Fan at 3:21-24. “Spatial information is also desirable when interpreting transcriptional heterogeneity of individual cells in a structured tissue. However, RNA sequencing is typically performed on isolated cells, such that spatial information is lost. One existing approach to link transcriptome information from isolated cells to their context is to first acquire the spatial information as well as other ‘phenotypes’ of individual cells within the intact tissue using fluorescent

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	hybridized complexes at a respective location within the sample while a value of 0 is obtained when the fluorescent signal is not detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample;	microscopy, and then extract specific cells (or cellular content) for subsequent transcriptional profiling. Thus, there is a need for methods which provide high-resolution mapping of a large number of transcripts in situ.” Fan at 3:61-4:5. “The methods and compositions set forth herein will be exemplified for detection of nucleic acids in a single cell. However, the methods can be extended to the simultaneous detection of nucleic acids in several individual cells. Parallel detection can be carried out such that several individual cells are resolved with regard to nucleic acid sequences that are present. In some embodiments the spatial location of nucleic acids can be simultaneously determined for several individual cells in a tissue or other cell-containing sample.” Fan at 4:26-34. “Example 1 Design and Optimization of a Set of Padlock Probes and Hybridization Oligos for Targeted Detection and Quantification of a Subset of Genes This example presents an innovative method based on padlock circularization and hybridization-based decoding (FIG. 1). This method is built upon ultra-high degree multiplexed capture (>300,000 per reaction) using padlock probes for useful for genomic, transcriptomic and methylomic analyses, as well as technology for accurate decoding of randomly assembled bead arrays. In some embodiments, colonies for an entire transcriptome can be generated using either random hexamers or oligo-dT primers (FIG. 1A). In some embodiments, colonies for a specific subset of genes of interest can then be tagged with gene-specific barcodes using a library of padlock probes (FIG. 1B). Padlock probes are designed in a similar manner as the full transcriptome probe

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		sets previously developed for digital RNA allelotyping according to the incorporated materials disclosed in Zhang, K. et al. Nat Methods 6, 613-618 (2009) and Noggle, S. et al. Nature 478, 70-75 (2011). A neural network, trained for predicting probe efficiency using experimental data accumulated on over 400,000 probes, is integrated into a probe design algorithm called ppDesigner. The entire probe design, synthesis and production process has been highly optimized. Long oligonucleotides (up to 300 nt in length) acquired from Agilent Technologies contain amplification adaptors to allow production of essentially an unlimited amount of functional padlock probes from a set of seed oligos. The design of the barcoding region incorporates the error-checking scheme described in the incorporated materials of Gunderson et al. (Genome Res. (2004) 14:870-877). For up to 1700 target genes, eight rounds of hybridization decoding are performed. In each round, a colony can have three states: two fluorescent (FAM and Cy3) 'ON' states and one nonfluorescent OFF state. In this three-state, eight-stage decoding scheme, the valid barcode for each gene is required to have exactly two OFF stages and six ON stages for error checking. Each padlock probe only needs six slots for hybridization (corresponding to the six ON stages). For the hybridization sequences used in these six slots, a set of 16 (8 stages x 2 ON states) 20-mers is selected that are orthogonal to the human genome and have uniform annealing parameters. This eight-stage barcoding scheme is sufficient to decode up to 1792 transcripts with a low error rate ($<10^{-4}$)." Fan at Example 1, 14:1-48. "Example 2 Design and Optimization of a Set of Padlock Probes for Targeted Detection and Quantification of BRAF V600E, EGFR T790M and EGFR L858R Mutations In this example, allele-specific (padlock) probes are generated for disease

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		causing mutations. Specifically, point mutations are detected in genomic DNA and the decoding scheme is utilized to measure the frequency and spatial distribution of these mutations within tissue sections at single cell resolution. For each of the specifically targeted mutations described below, a padlock probe is designed for specific hybridization to either the wild-type or mutant version of the gene. Then, the padlock probe serves as a template for RCA as described in greater detail herein. The resulting amplicon is detected and decoded using a barcode sequence integrated into the padlock probe.” Fan at Example 2, 14:49-67. Fan explicitly incorporates Gunderson, et al., Decoding Randomly Ordered DNA Arrays, 14 <i>Genome Res</i>, 870-877 (2004) for its decoding and error-checking methods, which discloses codewords of multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected during a stage of sequential hybridization and a value of 0 is obtained when a fluorescent signal is not detected (or a different signal is detected) in another stage of sequential hybridization. For example, “Our algorithm uses sequential hybridizations of dye-labeled oligonucleotides, or decoders, complementary to bead sequences to create a combinatorial decoding scheme for arrays. It is distinct from sequencing by hybridization (SBH), which has been used successfully to characterize sequences de novo by hybridization to all <i>n</i>-mers or a well-chosen subset, typically in the range of 4- to 10-mers (Drmanac et al. 1996, 1998; Gunderson et al. 1998; Brenner et al. 2000). Our approach uses longer sequences, each designed to hybridize to a defined target with high specificity. It is capable of decoding, with high accuracy, many 1000s of bead types. Each bead type is defined by a unique DNA sequence that is recognized by a complementary decoder.” Gunderson, at 870. “Decoding process. (A) The sequential hybridization process is illustrated for a single bead, of bead type 2. In stage 1, a complementary decoder hybridizes to

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		the oligonucleotide probe that is attached to the bead (for details of the procedure, see Methods). The decoder is labeled with a fluorophore (green in stage 1, red in stage 2, and green in stage 3). The fluorescent signal is read by imaging the entire array. The array is then dehybridized, and the process is repeated for two more stages. (B) A scanning electron micrograph of an array of beads, artificially colored to represent three sequential hybridization stages. The images, taken collectively, reveal a combinatorial code for each bead. Note that the bead circled in yellow has the color signature GRG or code 010. (C) Colors, or states, are assigned to individual decoder sequences in each stage to produce a unique combination across stages. This signature, or code, identifies each bead type. As indicated in the parity code column, an extra decoding stage (data not shown) can be performed to provide an error checking parity bit. After three stages of decoding, all the beads are uniquely identified by their color.” Gunderson, at 872, Figure 2. To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding scheme. Gunderson, at 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three

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		categories: used valid, unused valid, and invalid (table 1).” <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent that Fan and incorporated Gunderson are argued by Vizgen or found by the Court to not explicitly teach codewords comprising multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample while a value of 0 is obtained when the fluorescent signal is not detected from one of the plurality of readout probe-hybridized complexes or one of the different plurality of readout probe-hybridized complexes at the respective location within the sample, such limitations would have been inherent and/or obvious in view of Curry as described in 10x’s Invalidity Contentions. <i>See</i> Attachment 5, limitation 1[h][i]. Moreover, the use of binary codewords representing the plurality of different nucleic acid targets at locations within the sample were well known in the art at the time of the invention and it would have been obvious to combine the teachings of Fan/Gunderson with Curry, Hamady, and/or Chee as described in 10x’s Invalidity Contentions. <i>See</i> Attachments 1 and 5, limitation 1[i]. Hamady (Patent Publication No. US2010/0323348) teaches the use of Hamming code to create an error detection system for nucleic acid barcodes detection to identify nucleic acid targets. <i>See e.g.</i>, Hamady at [0097]-[0100], [0108]-[0110], [0135]-[0145], Figs. 1B and 1C (showing error correction of binary barcodes). Chee (Patent Publication No. US2007/0231824A1) teaches the use of binary code to encode bioactive agents such as nucleic acids. For example: “In a preferred embodiment, each subpopulation of beads comprises a plurality of different IBLs. By using a plurality of different IBLs to encode each bioactive

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		agent, the number of possible unique codes is substantially increased. That is, by using one unique IBL per bioactive agent, the size of the array will be the number of unique IBLs (assuming no ‘reuse’ occurs, as outlined below). However, by using a plurality of different IBLs per bead, n, the size of the array can be increased to 2^n, when the presence or absence of each IBL is used as the indicator. For example, the assignment of 10 IBLs per bead generates a 10 bit binary code, where each bit can be designated as ‘1’ (IBL is present) or ‘0’ (IBL is absent). A 10 bit binary code has 2^{10} possible variants.” Chee at [0102] (emphasis added). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach that the codeword comprises binary values, such limitations would have been obvious in view of Curry as described in 10x’s Invalidity Contentions.
1[j]	(j) matching the codewords with valid codewords in a codebook by comparing the codewords to the valid codewords in the codebook, and if one of the codewords is not matched with one of the valid codewords in the codebook, applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords,	Fan teaches matching the codewords (the temporal order of signal signatures) with valid codewords in a codebook (list of identifiers identifying the probe reagent corresponding to a specific analyte) by comparing the codewords to the valid codewords in the book, wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets. For example: “These barcodes can be accurately decoded by hybridization similar to the decoding of Illumina bead array, using a combinatorial chemistry approach (Gunderson et al. Genome Res. 14(5):870-877 (2004).)” Fan at 3:36-40. “During decoding, labeled decoder probe oligonucleotides are sequentially hybridized to the decoder sequences to specifically determine the identity of each barcode sequence. Sequential hybridization of decoder probes to decoder sequences is described by Gunderson et al. (Genome Res. (2004) 14:870-877), the content of which is incorporated by reference in its entirety. Additional description of decoding algorithms and the use of sequential hybridization

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	wherein the codebook comprises the valid codewords of the plurality of nucleic acid targets; and	useful in the methods presented herein can be found in U.S. Pat. Nos. 7,060,431; 7,033,754; 7,226,734; and 7,455,971, the disclosures of each of which are hereby incorporated by reference in their entireties.” Fan at 8:53-64. “The design of the barcoding region incorporates the error-checking scheme described in the incorporated materials of Gunderson et al. (Genome Res. (2004) 14:870-877). For up to 1700 target genes, eight rounds of hybridization decoding are performed. In each round, a colony can have three states: two fluorescent (FAM and Cy3) "ON" states and one nonfluorescent OFF state. In this three-state, eight-stage decoding scheme, the valid barcode for each gene is required to have exactly two OFF stages and six ON stages for error checking. Each padlock probe only needs six slots for hybridization (corresponding to the six ON stages). For the hybridization sequences used in these six slots, a set of 16 (8 stages x 2 ON states) 20-mers is selected that are orthogonal to the human genome and have uniform annealing parameters. This eight-stage barcoding scheme is sufficient to decode up to 1792 transcripts with a low error rate ($<10^{-4}$).” Fan at Example 1, 14:32-48. To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding scheme. Gunderson, at 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state

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		are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three categories: used valid, unused valid, and invalid (table 1).” <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent Fan/Gunderson is argued by Vizgen or found by the Court not to explicitly teach applying an error detection or correction system, matching the one of the codewords with another of the valid codewords in the codebook, or discarding the one of the codewords, such limitations would have been inherent and/or obvious in view of Curry, Hamady, and/or Chee as described in 10x’s Invalidity Contentions. <i>See</i> Attachment 5, limitation 1[j]. Hamady teaches the use of Hamming code to create an error detection system for nucleic acid barcodes detection to identify nucleic acid targets. <i>See e.g.</i>, Hamady at [0097]-[0100], [0108]-[0110], [0135]-[0145], Figs. 1B and 1C (showing error correction of binary barcodes). Chee teaches the use of parity analysis during decoding to detect errors in binary codes. <i>See e.g.</i>, Chee at [0241]-[0250]. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
1[k]	(k) determining the plurality of nucleic acid targets in the sample based on results from the	Fan teaches determining the plurality of nucleic acid targets in the sample based on results from the matching step. For example: “Presented herein are methods and compositions for obtaining sequence

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	matching step.	information from one or more individual cells. The methods are useful for obtaining sequence information for a single nucleotide sequence, and for multiplex generation of sequence information from an individual cell. In some embodiments presented herein, the methods comprise first amplifying one or more nucleic acid molecules in situ within a cell, and subsequently hybridizing a barcode probe to the amplified nucleic acid molecules. The identity of the amplified nucleic acid molecules is then determined by decoding of the barcode probes. In other embodiments presented herein, the methods comprise first hybridizing one or more different barcode probes to one or more nucleic acid molecule in situ within a cell, and subsequently amplifying the barcode probe, thereby generating an amplified barcode probe. The identity of the nucleic acid molecules is then determined by decoding of the amplified barcode probes.” Fan at 1:58-2:7 (emphasis added). “A nucleic acid sample that is amplified, sequenced or otherwise manipulated in a method disclosed herein can be, for example, DNA or RNA. Exemplary DNA species include, but are not limited to, genomic DNA (gDNA), mitochondrial DNA, chloroplast DNA, episomal DNA, viral DNA and copy DNA (cDNA). One non-limiting example of a subset of genomic DNA is one particular chromosome or one region of a particular chromosome. Exemplary RNA species include, without limitation, coding RNA such as messenger RNA (mRNA), and non-coding RNA (ncRNA) such as transfer RNA (tRNA), microRNA (miRNA), small nuclear RNA (snRNA) and ribosomal RNA (rRNA). Further species of DNA or RNA include fragments or portions of the species listed above or amplified products derived from these species, fragments thereof or portions thereof. The methods described herein are applicable to the above species encompassing all or part of the complement present in a cell. For example, using methods described herein the sequence of a substantially complete genome can be determined or the sequence of a substantially complete targeted nucleic acid sequences such as mRNA or cDNA complement of a cell

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		can be determined.” Fan at 6:60-7:14. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
2	The method of claim 1, wherein the fluorescent signal in step (c) and/or step (f) is detected at a resolution better than 300 nm.	<i>See</i> disclosure for claim 1. Fan teaches that the fluorescent signal in step (c) and/or step (f) is detected at a resolution better than 300 nm. For example: Fan teaches that a suitable imaging technology is one that can detect individual amplicons in situ. A person of ordinary skill in the art at the time of the invention would understand that detecting individual amplicons in situ would be detected at a resolution better than 300 nm. “The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ. Suitable imaging technologies are known in the art, as exemplified by Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the entire content of which is incorporated by reference herein in its entirety.” Fan at 11:11-19. “However, when performing the experiments for detection of sequences with single nucleotide resolution in human and mouse cells (Fig. 1b), we found that Ampligase resulted in a lower proportion of signals from the non-matched probe.” Larsson, supplementary Note 4 at 14.

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		Moreover, equipment for high resolution (better than 300nm) detection of fluorescent signals was readily available prior to the effective filing date of the 303 Patent and one of skill in the art, seeking to detect the fluorescent signals in situ produced by the claimed method would have been motivated to use detection equipment with resolutions greater than 300nm. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
3	The method of claim 1, wherein the sample comprises a cell.	<i>See</i> disclosure for claim 1. Fan teaches that the sample comprises a cell. For example: “Presented herein are methods and compositions for obtaining sequence information from one or more individual cells. The methods are useful for obtaining sequence information for a single nucleotide sequence, and for multiplex generation of sequence information from an individual cell. In some embodiments presented herein, the methods comprise first amplifying one or more nucleic acid molecules in situ within a cell, and subsequently hybridizing a barcode probe to the amplified nucleic acid molecules. The identity of the amplified nucleic acid molecules is then determined by decoding of the barcode probes. In other embodiments presented herein, the methods comprise first hybridizing one or more different barcode probes to one or more nucleic acid molecule in situ within a cell, and subsequently amplifying the barcode probe, thereby generating an amplified barcode probe. The identity of the nucleic acid molecules is then determined by decoding of the amplified barcode probes.” Fan at 1:58-2:7. “Cell Handling for In Situ Detection and Decoding The methods and compositions make use of hybridization, amplification, washing and detection within individual cells. These methods are performed in situ on isolated cells or in tissue sections that have been prepared according to methodologies known in the art. Methods for permeabilization and fixation of

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		cells and tissue samples are well-known in the art, as exemplified by Cremer et al., <i>The Nucleus: Volume 1: Nuclei and Subnuclear Components</i>, R. Hancock (ed.) 2008; and Larsson et al., <i>Nat. Methods</i> (2010) 7:395-397 and associated supplemental materials, the content of each of which is incorporated herein by reference in its entirety. Any suitable permeabilization and/or fixation technologies can be used for making cells and the target nucleic acid molecules therein available for the amplification and detection methods provided herein. Detection of Signals [] The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ. Suitable imaging technologies are known in the art, as exemplified by Larsson et al., <i>Nat. Methods</i> (2010) 7:395-397 and associated supplemental materials, the entire content of which is incorporated by reference herein in its entirety. An example demonstrating in situ imaging and 3D reconstruction of in situ detection is set forth in FIG. 1 and Example 1.” Fan at 10:61-11:22. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
4	The method of claim 3, wherein the cell is fixed on a coverslip.	<i>See</i> disclosure for claim 1. Fan teaches that the cell is fixed on a coverslip under Vizgen’s interpretation. For example: “The methods and compositions make use of hybridization, amplification, washing and detection within individual cells. These methods are performed in situ on isolated cells or in tissue sections that have been prepared according to

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		methodologies known in the art. Methods for permeabilization and fixation of cells and tissue samples are well-known in the art, as exemplified by Cremer et al., <i>The Nucleus: Volume 1: Nuclei and Subnuclear Components</i>, R. Hancock (ed.) 2008; and Larsson et al., <i>Nat. Methods</i> (2010) 7:395-397 and associated supplemental materials, the content of each of which is incorporated herein by reference in its entirety. Any suitable permeabilization and/or fixation technologies can be used for making cells and the target nucleic acid molecules therein available for the amplification and detection methods provided herein.” Fan at 10:62-11:9. Larsson discloses that the fresh frozen sections of mouse embryos were placed on Superfrost Plus Gold slides (Thermo Scientific) and that the breast tissue sections were placed on Starfrost microscope slides (Instrumedics). Larsson, at 398. To the extent it is determined that Fan does not explicitly recite a "coverslip", Fan and the incorporated references teach in situ hybridization methods with fluorescent detection. A person of ordinary skill in the art would understand that tissue samples or cells are conventionally fixed using coverslips (and/or glass slides) for FISH. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
5	The method of claim 1, comprising determining the fluorescent signal entity by imaging at least a portion of the sample using a light microscope.	See disclosure for claim 1. Fan teaches determining the fluorescent signal entity by imaging at least a portion of the sample using a light microscope. For example: “The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ. Suitable imaging technologies are known in the art, as exemplified by Larsson et al., <i>Nat. Methods</i> (2010) 7:395-

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		397 and associated supplemental materials, the entire content of which is incorporated by reference herein in its entirety. An example demonstrating in situ imaging and 3D reconstruction of in situ detection is set forth in FIG. 1 and Example 1.” Fan at 11:11-22. Larsson et al. 2010 discloses: “Image acquisition and analysis. Images of cultured cells were acquired using an AxioplanII epifluorescence microscope (Zeiss) equipped with a 100 W mercury lamp, a CCD camera (C4742-95, Hamamatsu), and a computer-controlled filter wheel with excitation and emission filters for visualization of DAPI, FITC, Cy3, Cy3.5 and Cy5. A ×20 (Plan-Apocromat, Zeiss), ×40 (Plan-Neofluar, Zeiss) or ×63 (Plan-Neofluar, Zeiss) objective was used for capturing the images. Images were collected using the Axiovision software (release 4.3, Zeiss).” Larson, at 10X595-00032300. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
6	The method of claim 1, further comprising determining an abundance and/or spatial distribution of the different nucleic acid targets at locations within the sample.	<i>See</i> disclosure for claim 1. Fan teaches determining an abundance and/or spatial distribution of the different nucleic acid targets at locations within the sample. For example, “In some aspects, detecting can further comprise evaluating a signal to determine the presence, quantity, frequency, and/or spatial distribution of a disease-causing mutation, allele or common variant within a tissue.” Fan at 3:21-24.

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		“Spatial information is also desirable when interpreting transcriptional heterogeneity of individual cells in a structured tissue. However, RNA sequencing is typically performed on isolated cells, such that spatial information is lost. One existing approach to link transcriptome information from isolated cells to their context is to first acquire the spatial information as well as other ‘phenotypes’ of individual cells within the intact tissue using fluorescent microscopy, and then extract specific cells (or cellular content) for subsequent transcriptional profiling. Thus, there is a need for methods which provide high-resolution mapping of a large number of transcripts in situ.” Fan at 3:61-4:5 (emphasis added). “The methods and compositions set forth herein will be exemplified for detection of nucleic acids in a single cell. However, the methods can be extended to the simultaneous detection of nucleic acids in several individual cells. Parallel detection can be carried out such that several individual cells are resolved with regard to nucleic acid sequences that are present. In some embodiments the spatial location of nucleic acids can be simultaneously determined for several individual cells in a tissue or other cell-containing sample.” Fan at 4:26-34. “In some embodiments, an in situ analysis method is performed in a cell that is not viable, but retains the structure and organization of a viable cell. For example, a cell which has been permeabilized and/or fixed is not considered viable, even though subcellular organelles and molecules such as proteins and nucleic acids remain intact and substantially in the same cellular location compared to a viable cell. Thus, analysis methods such as amplification, annealing, hybridizing, decoding and the like which are performed in situ are performed within the structure of a viable or non-viable cell or tissue.” Fan at 4:63-5:4.

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		To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
7	The method of claim 1, further comprising removing the readout nucleic acid probes unhybridized to each of the plurality of first hybridized complex prior to step (c).	<i>See</i> disclosure for claim 1. Fan teaches removing the readout nucleic acid probes unhybridized (e.g., cleaving) to each of the plurality of first hybridized complex prior to step (c). A person of skill in the art would understand that specific hybridization of decoding probes to generate the fluorescent signals involves washing/removal of decoding probes to remove non-specific and unhybridized decoding probes. “(c) hybridizing a first fluorescently labeled decoder probe to said first decoder sequence, wherein the first fluorescently labeled decoder probe that is not hybridized to said first decoder sequence is removed by washing.” Fan at Claim 1. “In some aspects of the above embodiments, the first labeled decoder probe is removed prior to hybridizing the second labeled decoder probe. In some aspects, the second labeled decoder probe is hybridized prior to the first labeled decoder probe.” Fan at 2:56-60. “The decoding strategies presented herein provide several key advantages over existing sequence identification methods. For example, one advantage is the simplicity and robustness of the decoding process, which involves hybridization and washing of oligo pools at room temperature. As such, heating and cooling steps are not necessary, thus minimizing damage to the cellular structure and reducing the potential drift of the specimen throughout the imaging process. As an additional advantage, the signals that are generated are more even and have higher intensity inside a structured tissue than methods that rely on other detection techniques. Further, in many embodiments, only two fluors are needed

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		for decoding, which provides convenient spectral distinction using simple detection devices and leaves enough spectrum for labeling cellular structure using other fluors. Of course more than two colors can be used to expand the number of sequences that are decoded, using modifications of the method set forth in further detail elsewhere herein and in the references that are incorporated by reference above as describing sequential hybridization of decoder probes to decoder sequences.” Fan at 9:23-43. “Cell Handling for In Situ Detection and Decoding The methods and compositions make use of hybridization, amplification, washing and detection within individual cells. These methods are performed in situ on isolated cells or in tissue sections that have been prepared according to methodologies known in the art. Methods for permeabilization and fixation of cells and tissue samples are well-known in the art, as exemplified by Cremer et al., The Nucleus: Volume 1: Nuclei and Subnuclear Components, R. Hancock (ed.) 2008; and Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the content of each of which is incorporated herein by reference in its entirety. Any suitable permeabilization and/or fixation technologies can be used for making cells and the target nucleic acid molecules therein available for the amplification and detection methods provided herein. Detection of Signals The signals generated by fluorescent imaging of one or more probes during decoding, sequencing and other detection methods carried out in situ and can be detected using any suitable imaging and detection technologies which allow for detection of individual amplicons in situ. Suitable imaging technologies are known in the art, as exemplified by Larsson et al., Nat. Methods (2010) 7:395-397 and associated supplemental materials, the entire content of which is

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		incorporated by reference herein in its entirety. An example demonstrating in situ imaging and 3D reconstruction of in situ detection is set forth in FIG. 1 and Example 1.” Fan at 10:61-11:22: To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
8	The method of claim 1, wherein the valid codewords form an error-checking or an error-correcting code space.	<i>See</i> disclosure for claim 1. Fan teaches producing codewords (temporal sequence of signal signature) representing the plurality of different nucleic acid targets at locations within the sample, wherein each of the codeword represents one of the plurality of different nucleic acid targets and is obtained when the fluorescent signal is detected from one of the plurality of readout probe hybridized complexes or one of the different plurality of readout probe-hybridized complexes at a respective location within the sample and wherein the codewords form an error-checking or an error-correcting code space. For example: “In some aspects, detecting can further comprise evaluating a signal to determine the presence, quantity, frequency, and/or spatial distribution of a disease-causing mutation, allele or common variant within a tissue.” Fan at 3:21-24. “Spatial information is also desirable when interpreting transcriptional heterogeneity of individual cells in a structured tissue. However, RNA sequencing is typically performed on isolated cells, such that spatial information is lost. One existing approach to link transcriptome information from isolated cells to their context is to first acquire the spatial information as well as other ‘phenotypes’ of individual cells within the intact tissue using fluorescent microscopy, and then extract specific cells (or cellular content) for subsequent

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		transcriptional profiling. Thus, there is a need for methods which provide high-resolution mapping of a large number of transcripts in situ.” Fan at 3:61-4:5. “The methods and compositions set forth herein will be exemplified for detection of nucleic acids in a single cell. However, the methods can be extended to the simultaneous detection of nucleic acids in several individual cells. Parallel detection can be carried out such that several individual cells are resolved with regard to nucleic acid sequences that are present. In some embodiments the spatial location of nucleic acids can be simultaneously determined for several individual cells in a tissue or other cell-containing sample.” Fan at 4:26-34. “Example 1 Design and Optimization of a Set of Padlock Probes and Hybridization Oligos for Targeted Detection and Quantification of a Subset of Genes This example presents an innovative method based on padlock circularization and hybridization-based decoding (FIG. 1). This method is built upon ultra-high degree multiplexed capture (>300,000 per reaction) using padlock probes for useful for genomic, transcriptomic and methylomic analyses, as well as technology for accurate decoding of randomly assembled bead arrays. In some embodiments, colonies for an entire transcriptome can be generated using either random hexamers or oligo-dT primers (FIG. 1A). In some embodiments, colonies for a specific subset of genes of interest can then be tagged with gene-specific barcodes using a library of padlock probes (FIG. 1B). Padlock probes are designed in a similar manner as the full transcriptome probe sets previously developed for digital RNA allelotyping according to the

Claim No.	Claim Language	Disclosure
		incorporated materials disclosed in Zhang, K. et al. Nat Methods 6, 613-618 (2009) and Noggle, S. et al. Nature 478, 70-75 (2011). A neural network, trained for predicting probe efficiency using experimental data accumulated on over 400,000 probes, is integrated into a probe design algorithm called ppDesigner. The entire probe design, synthesis and production process has been highly optimized. Long oligonucleotides (up to 300 nt in length) acquired from Agilent Technologies contain amplification adaptors to allow production of essentially an unlimited amount of functional padlock probes from a set of seed oligos. The design of the barcoding region incorporates the error-checking scheme described in the incorporated materials of Gunderson et al. (Genome Res. (2004) 14:870-877). For up to 1700 target genes, eight rounds of hybridization decoding are performed. In each round, a colony can have three states: two fluorescent (FAM and Cy3) 'ON' states and one nonfluorescent OFF state. In this three-state, eight-stage decoding scheme, the valid barcode for each gene is required to have exactly two OFF stages and six ON stages for error checking. Each padlock probe only needs six slots for hybridization (corresponding to the six ON stages). For the hybridization sequences used in these six slots, a set of 16 (8 stages x 2 ON states) 20-mers is selected that are orthogonal to the human genome and have uniform annealing parameters. This eight-stage barcoding scheme is sufficient to decode up to 1792 transcripts with a low error rate ($<10^{-4}$). Fan at Example 1, 14:1-48. “Example 2 Design and Optimization of a Set of Padlock Probes for Targeted Detection and Quantification of BRAF V600E, EGFR T790M and EGFR L858R Mutations In this example, allele-specific (padlock) probes are generated for disease causing mutations. Specifically, point mutations are detected in genomic DNA

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		and the decoding scheme is utilized to measure the frequency and spatial distribution of these mutations within tissue sections at single cell resolution. For each of the specifically targeted mutations described below, a padlock probe is designed for specific hybridization to either the wild-type or mutant version of the gene. Then, the padlock probe serves as a template for RCA as described in greater detail herein. The resulting amplicon is detected and decoded using a barcode sequence integrated into the padlock probe.” Fan at Example 2, 14:49-67. Fan explicitly incorporates Gunderson, et al., Decoding Randomly Ordered DNA Arrays, 14 <i>Genome Res</i>, 870-877 (2004) for its decoding and error-checking methods, which discloses codewords of multiple binary values 1 and 0, wherein a value of 1 is obtained when the fluorescent signal is detected during a stage of sequential hybridization and a value of 0 is obtained when a fluorescent signal is not detected (or a different signal is detected) in another stage of sequential hybridization. For example: “Our algorithm uses sequential hybridizations of dye-labeled oligonucleotides, or decoders, complementary to bead sequences to create a combinatorial decoding scheme for arrays. It is distinct from sequencing by hybridization (SBH), which has been used successfully to characterize sequences de novo by hybridization to all <i>n</i>-mers or a well-chosen subset, typically in the range of 4- to 10-mers (Drmanac et al. 1996, 1998; Gunderson et al. 1998; Brenner et al. 2000). Our approach uses longer sequences, each designed to hybridize to a defined target with high specificity. It is capable of decoding, with high accuracy, many 1000s of bead types. Each bead type is defined by a unique DNA sequence that is recognized by a complementary decoder.” Gunderson, at 870. “Decoding process. (A) The sequential hybridization process is illustrated for a single bead, of bead type 2. In stage 1, a complementary decoder hybridizes to the oligonucleotide probe that is attached to the bead (for details of the

Claim No.	Claim Language	Disclosure
		procedure, see Methods). The decoder is labeled with a fluorophore (green in stage 1, red in stage 2, and green in stage 3). The fluorescent signal is read by imaging the entire array. The array is then dehybridized, and the process is repeated for two more stages. (B) A scanning electron micrograph of an array of beads, artificially colored to represent three sequential hybridization stages. The images, taken collectively, reveal a combinatorial code for each bead. Note that the bead circled in yellow has the color signature GRG or code 010. (C) Colors, or states, are assigned to individual decoder sequences in each stage to produce a unique combination across stages. This signature, or code, identifies each bead type. As indicated in the parity code column, an extra decoding stage (data not shown) can be performed to provide an error checking parity bit. After three stages of decoding, all the beads are uniquely identified by their color.” Gunderson, at 872, Figure 2. Moreover, this claim would have been inherent and/or obvious in view of Curry, Hamady, and/or Chee as described in 10x’s Invalidity Contentions. <i>See</i> Attachment 5, claim 8. Hamady teaches that the valid codewords form an error-checking or an error-correcting code space. For example: “Hamming codes may be represented by a subset of the possible codewords that are chosen from the center of multidimensional spheres (i.e., for example, hyperspheres) in a binary subspace. Single bit errors may fall within hyperspheres associated with a specific codeword and can thus be corrected. On the other hand, double bit errors that do not associate with a specific codeword can be detected, but not corrected. Consider a first hypersphere centered at coordinates (0, 0, 0) (i.e., for example, using an x-y-z coordinate system), wherein any single-bit error can be corrected by falling within a radius of 1 from the center coordinates; i.e., for example, single bit errors having the coordinates of (0, 0, 0); (0, 1, 0); (0, 0, 1); (1, 0, 0), or (1, 1, 0). Likewise, a second

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		hypersphere may be constructed wherein single-bit errors can be corrected by falling within a radius of 1 of its center coordinates (1, 1, 1) (i.e., for example, (1,1,1); (1, 0, 1); (0 ,1, 0); or (0, 1, 1). See, FIG. 1A (first hypersphere-blue; second hypersphere-red).” Hamady, at [0098]. Chee teaches that the valid codewords form an error-checking or an error-correcting code space. For example: “In a preferred embodiment the invention includes introducing a ‘redundant stage’ into the decoding system. By ‘redundant stage’ is meant a stage that serves as a parity check. That is, following the decoding stages, an additional stage is included to analyze the parity. This analysis provides an indication of the competence or validity of the decoding. That is, when codes are designed with a pre-determined parity, the redundant stage is used to detect the parity of the signals obtained from the decoding step. That is, the redundant stage detects errors in parity because if there has been an error in decoding, the parity detected following the redundant stage will be different from the parity designed into the codes.” Chee at [0243]; <i>see also</i> Fig. 7; [0241]-[0250]. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
9	The method of claim 1, wherein in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched	<i>See</i> disclosure for claim 1. Fan and its incorporated Gunderson discloses that in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors. For example,

Claim No.	Claim Language	Disclosure
	with one of the valid codewords in the codebook when the one of the codewords has one or more errors.	Gunderson pp. 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three categories: used valid, unused valid, and invalid (table 1).” To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding scheme. Gunderson, at 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three

Claim No.	Claim Language	Disclosure
		categories: used valid, unused valid, and invalid (table 1)." <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent Fan/Gunderson are argued by Vizgen or found by the Court not to explicitly teach that in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors, such limitations would have been inherent and/or obvious in view of Curry, Hamady, and/or Chee as described in 10x's Invalidity Contentions. <i>See</i> Attachments 1 and 5, claim 9. Hamady teaches that in comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors. For example: “Hamming codes may be represented by a subset of the possible codewords that are chosen from the center of multidimensional spheres (i.e., for example, hyperspheres) in a binary subspace. Single bit errors may fall within hyperspheres associated with a specific codeword and can thus be corrected. On the other hand, double bit errors that do not associate with a specific codeword can be detected, but not corrected. Consider a first hypersphere centered at coordinates (0, 0, 0) (i.e., for example, using an x-y-z coordinate system), wherein any single-bit error can be corrected by falling within a radius of 1 from the center coordinates; i.e., for example, single bit errors having the coordinates of (0, 0, 0); (0, 1, 0); (0, 0, 1); (1, 0, 0), or (1, 1, 0). Likewise, a second hypersphere may be constructed wherein single-bit errors can be corrected by falling within a radius of 1 of its center coordinates (1, 1, 1) (i.e., for example, (1,1,1); (1, 0, 1); (0 ,1, 0); or (0, 1, 1). <i>See</i>, FIG. 1A (first hypersphere-blue; second hypersphere-red).” Hamady at [0098].

Claim No.	Claim Language	Disclosure																									
		Chee teaches that one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors. For example: “An example is illustrative. In the following illustration, array elements are decoded in two stages with two colors. This results in the detection of 4 (2^2) codes. The codes are designed to result in odd parity following the analysis with the redundant stage. That is, the code sequences can be made such that each code will contain an odd number of ‘red’ colors (when viewed across all stages, or when viewed across the pre-determined number of stages). In the illustration below, only the stage where the ‘red’ signal is detected is indicated. <table><tr><th>Code</th><th>Stage 1</th><th>Stage 2</th><th>Redundant Stage</th><th>Parity (number of “red” stages)</th></tr><tr><td>1</td><td>0</td><td>0</td><td>red</td><td>1 (odd)</td></tr><tr><td>2</td><td>0</td><td>red</td><td>0</td><td>1 (odd)</td></tr><tr><td>3</td><td>red</td><td>0</td><td>0</td><td>1 (odd)</td></tr><tr><td>4</td><td>red</td><td>red</td><td>red</td><td>3 (odd)</td></tr></table> However, if there is an error during decoding, this will be reflected as detecting a parity that is different from that designed into the codes. In the following illustration the same codes as above are used however, an error is detected. In this case if one detects an even number of ‘red’ stages it can be inferred that there has been a decoding error.”	Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)	1	0	0	red	1 (odd)	2	0	red	0	1 (odd)	3	red	0	0	1 (odd)	4	red	red	red	3 (odd)
Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)																							
1	0	0	red	1 (odd)																							
2	0	red	0	1 (odd)																							
3	red	0	0	1 (odd)																							
4	red	red	red	3 (odd)																							

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		<table><tr><th>Code</th><th>Stage 1</th><th>Stage 2</th><th>Redundant Stage</th><th>Parity (number of “red” stages)</th></tr><tr><td>1</td><td>0</td><td>0</td><td>red</td><td>1 (odd)</td></tr><tr><td>2</td><td>0</td><td>red</td><td>0</td><td>1 (odd)</td></tr><tr><td>3</td><td>red</td><td>0</td><td>0</td><td>1 (odd)</td></tr><tr><td>4</td><td>red</td><td>o (error)</td><td>red</td><td>2 (even)</td></tr></table> Chee at [0244]-[0245]. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.	Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)	1	0	0	red	1 (odd)	2	0	red	0	1 (odd)	3	red	0	0	1 (odd)	4	red	o (error)	red	2 (even)
Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)																							
1	0	0	red	1 (odd)																							
2	0	red	0	1 (odd)																							
3	red	0	0	1 (odd)																							
4	red	o (error)	red	2 (even)																							
10	The method of claim 1, wherein in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one error.	See disclosure for claim 1. Fan and its incorporated Gunderson teaches that in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one error. For example: “The simplest form of error checking we have used has a parity bit and is illustrated in Figure 2C. The assigned four-bit codes all have an even parity bit sum and are termed valid codes. An error in a single decode stage is a one-bit error that creates an odd parity, or invalid code. Although the parity-based approach is effective, we have optimized error checking by designing a more advanced scheme that assigns codes in a way that takes into account inherent biases in error rates and enables estimation of the misclassification rate.” Gunderson, at 871. See also <i>Coding and Information Theory</i>, Richard Hamming. Prentice-Hall, NJ. 1986 (“Hamming 1986”). Chapter 3, pp. 45-46 (“We can express the minimum distance between vertices of a set of message points in terms of the error correctability possible.																									

Claim No.	Claim Language	Disclosure
		The minimum distance must be at least 1 for uniqueness of the code (Table 3.6-1). A minimum distance of 2 gives single-error detectability. A minimum distance of 3 gives single-error correctability; any single error leaves the point closer to where it was than to any other possible message. Of course, this minimum-distance code could be used instead for double-error detection. A minimum distance of 4 will give both single-error correction plus double-error detection. ...”). To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding scheme. Gunderson, at 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three categories: used valid, unused valid, and invalid (table 1).” <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent Fan/Gunderson are argued by Vizgen or found by the Court not to explicitly teach that in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one error, such

Claim No.	Claim Language	Disclosure
		limitations would have been inherent and/or obvious in view of Curry, Hamady, and/or Chee as described in 10x's Invalidity Contentions. <i>See</i> Attachments 1 and 5, claim 10. Hamady teaches that in comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one error. For example: “Hamming codes may be represented by a subset of the possible codewords that are chosen from the center of multidimensional spheres (i.e., for example, hyperspheres) in a binary subspace. Single bit errors may fall within hyperspheres associated with a specific codeword and can thus be corrected. On the other hand, double bit errors that do not associate with a specific codeword can be detected, but not corrected. Consider a first hypersphere centered at coordinates (0, 0, 0) (i.e., for example, using an x-y-z coordinate system), wherein any single-bit error can be corrected by falling within a radius of 1 from the center coordinates; i.e., for example, single bit errors having the coordinates of (0, 0, 0); (0, 1, 0); (0, 0, 1); (1, 0, 0), or (1, 1, 0). Likewise, a second hypersphere may be constructed wherein single-bit errors can be corrected by falling within a radius of 1 of its center coordinates (1, 1, 1) (i.e., for example, (1,1,1); (1, 0, 1); (0 ,1, 0); or (0, 1, 1). See, FIG. 1A (first hypersphere-blue; second hypersphere-red). Codeword regions comprising a length of 16 or more bits may be checked by parity bits at positions 0, 1, 2, and 4, wherein the bits that are checked by each position are marked with 1. See, FIG. 1B. Consequently, a “received” codeword containing a binary value of 3 (0011) (n=7, k=4) may be decoded for possible correction. The first case contains no errors; the second contains a single-bit error at position 6 that is detected and corrected. See, FIG. 3. Note that this is an example of a Hamming error-correcting code: the method claims all error-detecting and error-correcting codes.” Hamady, at [0098]-[0099].

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		“6. Checked whether the barcode is present in the list of valid barcodes: a. If valid, remap to original sample id, assign unique sequence id to the read. b. If not, try to correct barcode using the Hamming decoder software in accordance with Example II. i. If corrected, remap to original sample id, assign unique sequence id to the read, and record the position and type of the error. ii. If not corrected, drop sequence.” Hamady at [0141]-[0145]. Chee teaches that comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has one or more errors. For example: “However, if there is an error during decoding, this will be reflected as detecting a parity that is different from that designed into the codes. In the following illustration the same codes as above are used however, an error is detected. In this case if one detects an even number of ‘red’ stages it can be inferred that there has been a decoding error.” <table><tr><th>Code</th><th>Stage 1</th><th>Stage 2</th><th>Redundant Stage</th><th>Parity (number of “red” stages)</th></tr><tr><td>1</td><td>0</td><td>0</td><td>red</td><td>1 (odd)</td></tr><tr><td>2</td><td>0</td><td>red</td><td>0</td><td>1 (odd)</td></tr><tr><td>3</td><td>red</td><td>0</td><td>0</td><td>1 (odd)</td></tr><tr><td>4</td><td>red</td><td>o (error)</td><td>red</td><td>2 (even)</td></tr></table> Chee at [0245].	Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)	1	0	0	red	1 (odd)	2	0	red	0	1 (odd)	3	red	0	0	1 (odd)	4	red	o (error)	red	2 (even)
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4	red	o (error)	red	2 (even)																							

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		To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x's Invalidity Contentions.
11	The method of claim 1, wherein in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has two errors.	<i>See</i> disclosure for claim 1. Fan and its incorporated Gunderson teaches that in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has two errors. For example: Gunderson, at 871-872: “With this scheme, it is theoretically impossible to misclassify a code through any number of occurrences of the most common error type: a transition from an ON state to an OFF state. The following events can lead to misclassification: a transition from one ON state to the other, or multiple stage errors with at least one ON-to-OFF transition and at least one OFF-to-ON transition. Both events are extremely rare.” <i>See also</i> Hamming 1986, a minimum distance 3 allows for double-error detection: “We can express the minimum distance between vertices of a set of message points in terms of the error correctability possible. The minimum distance must be at least 1 for uniqueness of the code (Table 3.6-1). A minimum distance of 2 gives single-error detectability. A minimum distance of 3 gives single-error correctability; any single error leaves the point closer to where it was than to any other possible message. Of course, this minimum-distance code could be used instead for double-error detection. A minimum distance of 4 will give both single-error correction plus double-error detection. ...” Hamming 1986, Chapter 3, pp. 45-46. To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding

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		scheme. Gunderson pp. 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three categories: used valid, unused valid, and invalid (table 1).” <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent Fan/Gunderson are argued by Vizgen or found by the Court not to explicitly teach in said comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has two errors, such limitations would have been inherent and/or obvious in view of Curry and/or Hamady as described in 10x’s Invalidity Contentions. <i>See</i> Attachments 1 and 5, claim 11. Hamady teaches that in comparing the codewords to the valid codewords in the codebook in step (j), one of the codewords is not matched with one of the valid codewords in the codebook when the one of the codewords has two errors. “Hamming codes may be represented by a subset of the possible codewords that are chosen from the center of multidimensional spheres (i.e., for example, hyperspheres) in a binary subspace. Single bit errors may fall within hyperspheres associated with a specific codeword and can thus be corrected. On

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		the other hand, double bit errors that do not associate with a specific codeword can be detected, but not corrected.” Hamady, at [0098] (emphasis added). To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.
12	The method of claim 1, wherein said comparing the codewords to the valid codewords in the codebook in step (j) comprises comparing the values at each of the positions of the codewords.	<i>See</i> disclosure for claim 1. Fan teaches that comparing the codewords to the valid codewords in the codebook in step (j) comprises comparing the values at each of the positions of the codewords. “During decoding, labeled decoder probe oligonucleotides are sequentially hybridized to the decoder sequences to specifically determine the identity of each barcode sequence. Sequential hybridization of decoder probes to decoder sequences is described by Gunderson et al. (Genome Res. (2004) 14:870-877), the content of which is incorporated by reference in its entirety. Additional description of decoding algorithms and the use of sequential hybridization useful in the methods presented herein can be found in U.S. Pat. Nos. 7,060,431; 7,033,754; 7,226,734; and 7,455,971, the disclosures of each of which are hereby incorporated by reference in their entireties.” Fan at 8:53-64. “The design of the barcoding region incorporates the error-checking scheme described in the incorporated materials of Gunderson et al. (Genome Res. (2004) 14:870-877). For up to 1700 target genes, eight rounds of hybridization decoding are performed. In each round, a colony can have three states: two fluorescent (FAM and Cy3) "ON" states and one nonfluorescent OFF state. In this three-state, eight-stage decoding scheme, the valid barcode for each gene is required to have exactly two OFF stages and six ON stages for error checking. Each padlock probe only needs six slots for hybridization (corresponding to the

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		six ON stages). For the hybridization sequences used in these six slots, a set of 16 (8 stages x 2 ON states) 20-mers is selected that are orthogonal to the human genome and have uniform annealing parameters. This eight-stage barcoding scheme is sufficient to decode up to 1792 transcripts with a low error rate ($<10^{-4}$).” Fan at Example 1, 14:32-48. To the extent that Gunderson is not considered to be an express disclosure of Fan, the motivation to combine Fan with the error correction/detection methods of Gunderson is explicit. Both Fan and Gunderson rely on a three-state, eight-stage decoding scheme. Gunderson, at 871-2, Section "Error Checking": “The error-checking concepts that we have developed, and that underlie the robustness of our hybridization-based approach, are based on algorithms from digital information theory (Shanon 1948a,b; Hamming 1986). ... In our implementation of three-state decoding, the most common errors are transitions from an ON state, one or two, to the OFF state, zero. Less common are transitions from OFF to ON. Transitions from one ON state to the other ON state are extremely rare. This can be explained by the fact that such transitions require two simultaneous classification errors: a mistaken ON in one color channel and a mistaken OFF call in the other color channel. We used these biases in error rates by designing the decode stages so that every valid code has a fixed number of OFF states. ... The space of all possible color codes is divided into three categories: used valid, unused valid, and invalid (table 1).” <i>See</i> Gunderson at 872, Table 1 (summarizing a codebook with respect to the distribution and use of 6561 codes in the three-state, eight-stage system.) To the extent that Fan/Gunderson are argued by Vizgen or found by the Court not to explicitly teach this claim, it would have been inherent and/or obvious in view of Curry, Hamady, and/or Chee as described in 10x’s Invalidity Contentions. <i>See</i> Attachments 1 and 5, Claim 12.

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		Hamady teaches that comparing the codewords to the valid codewords in the codebook in step (j) comprises comparing the values at each of the positions of the codewords. “Hamming codes may be represented by a subset of the possible codewords that are chosen from the center of multidimensional spheres (i.e., for example, hyperspheres) in a binary subspace. Single bit errors may fall within hyperspheres associated with a specific codeword and can thus be corrected. On the other hand, double bit errors that do not associate with a specific codeword can be detected, but not corrected. Consider a first hypersphere centered at coordinates (0, 0, 0) (i.e., for example, using an x-y-z coordinate system), wherein any single-bit error can be corrected by falling within a radius of 1 from the center coordinates; i.e., for example, single bit errors having the coordinates of (0, 0, 0); (0, 1, 0); (0, 0, 1); (1, 0, 0), or (1, 1, 0). Likewise, a second hypersphere may be constructed wherein single-bit errors can be corrected by falling within a radius of 1 of its center coordinates (1, 1, 1) (i.e., for example, (1,1,1); (1, 0, 1); (0 ,1, 0); or (0, 1, 1). See, FIG. 1A (first hypersphere-blue; second hypersphere-red).” Hamady at [0098]. “Of the 3,684 sequences examined with this decoding algorithm, 3,531 (95.8%) had exact bar code matches, 70 (1.9%) had errors in their bar codes that were decodable, and 83 (2.3%) were not decodable.” Hamady at [0086]. “6. Checked whether the barcode is present in the list of valid barcodes: a. If valid, remap to original sample id, assign unique sequence id to the read. b. If not, try to correct barcode using the Hamming decoder software in

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		accordance with Example II. i. If corrected, remap to original sample id, assign unique sequence id to the read, and record the position and type of the error. ii. If not corrected, drop sequence.” Hamady, at [0141]-[0145]. Chee teaches that the comparing the codewords to the valid codewords in the codebook in step (j) comprises comparing the values at each of the positions of the codewords. For example: “An example is illustrative. In the following illustration, array elements are decoded in two stages with two colors. This results in the detection of 4 (22) codes. The codes are designed to result in odd parity following the analysis with the redundant stage. That is, the code sequences can be made such that each code will contain an odd number of ‘red’ colors (when viewed across all stages, or when viewed across the pre-determined number of stages). In the illustration below, only the stage where the ‘red’ signal is detected is indicated. <table><tr><th>Code</th><th>Stage 1</th><th>Stage 2</th><th>Redundant Stage</th><th>Parity (number of “red” stages)</th></tr><tr><td>1</td><td>0</td><td>0</td><td>red</td><td>1 (odd)</td></tr><tr><td>2</td><td>0</td><td>red</td><td>0</td><td>1 (odd)</td></tr><tr><td>3</td><td>red</td><td>0</td><td>0</td><td>1 (odd)</td></tr><tr><td>4</td><td>red</td><td>red</td><td>red</td><td>3 (odd)</td></tr></table> However, if there is an error during decoding, this will be reflected as detecting a parity that is different from that designed into the codes. In the following illustration the same codes as above are used however, an error is detected. In this case if one detects an even number of ‘red’ stages it can be inferred that	Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)	1	0	0	red	1 (odd)	2	0	red	0	1 (odd)	3	red	0	0	1 (odd)	4	red	red	red	3 (odd)
Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)																							
1	0	0	red	1 (odd)																							
2	0	red	0	1 (odd)																							
3	red	0	0	1 (odd)																							
4	red	red	red	3 (odd)																							

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		there has been a decoding error.” <table><tr><th>Code</th><th>Stage 1</th><th>Stage 2</th><th>Redundant Stage</th><th>Parity (number of “red” stages)</th></tr><tr><td>1</td><td>0</td><td>0</td><td>red</td><td>1 (odd)</td></tr><tr><td>2</td><td>0</td><td>red</td><td>0</td><td>1 (odd)</td></tr><tr><td>3</td><td>red</td><td>0</td><td>0</td><td>1 (odd)</td></tr><tr><td>4</td><td>red</td><td>o (error)</td><td>red</td><td>2 (even)</td></tr></table> Chee at [0244]-[0245]; <i>see also</i> Fig. 7. To the extent that Fan is argued by Vizgen or found by the Court not to explicitly teach certain limitations in the asserted claims, such limitations would have been inherent and/or obvious as described in 10x’s Invalidity Contentions.	Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)	1	0	0	red	1 (odd)	2	0	red	0	1 (odd)	3	red	0	0	1 (odd)	4	red	o (error)	red	2 (even)
Code	Stage 1	Stage 2	Redundant Stage	Parity (number of “red” stages)																							
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2	0	red	0	1 (odd)																							
3	red	0	0	1 (odd)																							
4	red	o (error)	red	2 (even)																							