UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.
Petitioner,

v.

10X GENOMICS, INC.
Patent Owner

IPR2023-XXXXX
U.S. PATENT NO. 10,155,981

DECLARATION OF GREGORY COOPER, PH.D IN SUPPORT OF PETITIONER FOR *INTER PARTES* REVIEW

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1. Claim 1: 58

a) [1A] A method of analyzing nucleic acids from a plurality of single cells, the method comprising: 58

b) [1B] providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides; 62

c) [1C] generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides, ...66

d) [1D] wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and 71

e) [1E] wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for

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different single cells in the plurality of single cells; and 76

f) [1F] wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: … II. a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell;..... 83

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g) [1G] sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences; 93

h) [1H] using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and 95

i) [1I] using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived. 97

2. Claim 2: The method of claim 1, wherein the method further comprises amplifying the tagged polynucleotides prior to the sequencing step (c). 98

3. Claim 3: The method of claim 1, wherein the sample polynucleotides are selected from DNA and RNA..... 101

4. Claim 4: The method of claim 3, wherein the sample polynucleotides comprise mRNA. 102

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5. Claim 6: The method of claim 1, wherein the tagged polynucleotides are generated through PCR or linear amplification of the plurality of sample polynucleotides using an adapter sequence comprising the MID sequence and an amplification primer. 103

B. Ground 2: Claim 5 is Unpatentable as Obvious over Linnarsson in View of McCloskey, and further in View of the Knowledge of a POSA 108

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I. INTRODUCTION

1. My name is Gregory Cooper. I have been retained by Parse Biosciences, Inc. ("Parse" or "Petitioner") in the above-captioned *inter partes* review ("IPR") to provide my independent evaluation and opinions regarding U.S. Patent No. 10,155,981 (the "'981 patent") (Ex. 1001) and the related prior art.

2. This Declaration sets forth my analyses and opinions based on my technical background, skills, knowledge, training, education and experience in the relevant art, and the materials I considered. As I explain below, it is my opinion that all claims of the '981 patent are rendered obvious by the combination of prior art references discussed herein.

II. QUALIFICATIONS AND COMPENSATIONS

3. I am currently a Faculty Investigator at the HudsonAlpha Institute for Biotechnology ("HudsonAlpha") in Huntsville, Alabama. I have held this position since 2010. HudsonAlpha, established in 2008, is an independent, non-profit institution dedicated to improving human health and welfare through biological research, genomic medicine, education, and economic development. Since coming to HudsonAlpha, I have set up an independent human genomics research lab. My lab works on projects related to the genetics of human disease, with a particular emphasis on research and clinical applications of genome sequencing for diagnosing children with developmental delays and intellectual disabilities. We also study the

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genetics and biochemistry of gene expression regulation, develop new computational approaches for analysis of human genomes and related datasets, and pursue other applications of genomic technologies to understand human biology.

4. I am also concurrently an Associate Professor Adjunct in the Department of Genetics at the University of Alabama at Birmingham ("UAB"). I have held a UAB adjunct faculty position since 2010.

5. I earned a Bachelor of Arts in Microbiology and Bachelor of Science in Mathematics and Statistics, summa cum laude, from Miami University (Oxford, OH) in 2001. As an undergraduate student, I performed molecular biology and genetic research in research laboratories at Miami University, the University of Illinois at Urbana-Champaign, and the University of Cincinnati.

6. I earned a Ph.D. in Genetics from Stanford University in 2006 under the mentorship of Professor Arend Sidow, Ph.D. My thesis work was mainly comprised of computational biology work, with a focus on evolutionary comparisons of mammalian genome assemblies to better annotate functional elements in human genomes and assess whether mutations in those elements might contribute to disease. While there, I worked and/or received training in areas including: theoretical and applied human genetics, molecular evolution, functional genomics, developmental biology, bioinformatics, genomic technologies, and

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analysis of datasets produced by high-throughput experimental platforms, especially DNA sequencers.

7. From 2006 to 2009, I was a Senior Fellow in the Department of Genome Sciences at the University of Washington working with Professors Evan Eichler, Ph.D., and Deborah Nickerson, Ph.D. While there, I conducted my postdoctoral research. I was primarily interested in applying genomic technologies to better understand the genetic basis for human disease. One series of projects centered on analytical and algorithmic developments to identify large deletion and duplication events, also known as "copy-number variants" ("CNVs"), from microarray data generated to perform Single Nucleotide Polymorphism ("SNP") genotyping. This process involved extensive processing, normalization, and analysis of Illumina SNP array data. A second series of projects was aimed at better understanding the genetic basis for a variety of complex traits. These included genetic analyses of transcript levels, for example, and also a genome-wide association study of patient response to the commonly prescribed anticoagulant warfarin. Finally, another series of projects focused on analysis of high-throughput DNA-sequencing data, in particular the locations of genetic variants, from individuals with Mendelian diseases. I completed my postdoctoral studies in 2009.

8. From 2009 to 2010, I was an Acting Assistant Professor in the Department of Genome Sciences at the University of Washington.

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9. I am an author or contributor to more than 100 publications in the areas of human genetics and genomics, including functional and evolutionary annotations of mammalian genomes, DNA sequencing, and DNA analysis, as they relate to human diseases, especially, but not exclusively, intellectual disabilities and other developmental issues. As of March 23, 2023, these publications have been collectively cited more than 42,000 times, according to Google Scholar estimates.

10. Among the awards and honors I have earned are: Harrison Scholar, Miami University (Oxford, OH) (1997-2001), Goldwater Scholar (1999-2001), Howard Hughes Medical Institute Doctoral Fellowship (2001-2006), a University of Washington Genome Training Grant Postdoctoral Fellowship (2007), and a Merck, Jane Coffin Childs Memorial Fund Postdoctoral Fellowship (2007-2010).

11. I am a Principal Investigator ("PI") on two current human genomic research projects funded by the National Institutes of Health ("NIH") and a PI or co-Investigator on several additional projects funded by non-NIH sources, including both governmental and philanthropic sources. I have also been a PI or co-Investigator on multiple additional grants related to human genetics and genomics, from NIH and elsewhere, that have been completed.

12. In general, I have knowledge and experience with both the general scientific contexts relevant to this matter and specific details of the technologies employed. For example, as described here, I have extensive experience with

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molecular techniques such as DNA synthesizing and sequencing, molecular tagging, PCR, gene expression analysis, and single-cell sequencing.

13. I have also previously served as an expert witness in three now concluded patent disputes and one ongoing patent dispute. The three completed cases, in which I worked on behalf of the plaintiffs, are Illumina/Verinata vs Ariosa/Roche (C.A. No. 3:12-5501-SI, N.D. Cal.), Guardant Health vs FMI (C.A. No. 17-1616-LPS-CJB, D. Del.), and Guardant Health vs PGDx (17-1623-LPS-CJB, D. Del.). The ongoing case is Natera vs ArcherDX/Invitae (C.A. No. 20-125-GBW, D. Del.), in which I am working on behalf of the defendants.

14. A copy of my Curriculum Vitae, attached as Exhibit 1020, contains further details on my education, experience, publications, and other qualifications to render an expert opinion in this matter.

15. For this service, I am paid my standard hourly consulting fee of $750/hour, and I am reimbursed for actual expenses. My compensation in no way depends on the outcome of this matter or the substance of this declaration.

III. SUMMARY OF OPINIONS

16. Based on my knowledge, experience, and the materials that I have reviewed, it is my opinion that claims 1-4 and 6 of the ’981 patent are obvious based on International Patent Application Publication No. WO 2010/117620 to Linnarsson (“Linnarsson”) (Ex. 1003) in view of M. McCloskey et al., *Encoding PCR Products*

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with Batch-stamps and Barcodes, Biochemical Genetics, Vol. 45 (Dec. 2007) ("McCloskey") (Ex. 1004). It is further my opinion that claim 5 of the '981 patent is obvious based on Linnarsson in view of McCloskey, and further in view of the knowledge of a person of ordinary skill in the art ("POSA").

IV. LEGAL STANDARDS

17. I am not a lawyer, and I offer no legal opinion in this declaration. I have, however, been advised by counsel as to various legal standards that apply to the technical issues I address in this declaration. I have applied those standards in arriving at my conclusions expressed in this declaration.

A. Written Description Support

18. I have been informed that the claims in a U.S. application are only entitled to the benefit of a foreign priority date or the filing date of a provisional application if the corresponding foreign application or provisional application contains sufficient written description of the invention to enable a POSA to practice the invention claimed in the non-provisional application.

B. Claim Construction

19. I have been informed that the words of the claims of a patent are generally given the ordinary and customary meaning as interpreted by a POSA at the time of the invention. I have been informed that patent claims are construed in light of the claim language, the patent specification (including the drawings), and

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the prosecution history (including the references cited during the prosecution of the patent). For this declaration, I have laid out my opinions on what terms should be construed in Section VIII on Claim Construction below; I have given all other terms their plain and ordinary meaning as would have been understood by a POSA at the time of the invention.

C. Anticipation

20. I understand that a prior art reference "anticipates" an asserted claim and renders that claim invalid if all elements of the claim are disclosed in that prior art reference and arranged in the prior art reference as they are in the claims.

21. I also understand that even if a prior-art reference does not expressly disclose every limitation of a claim, the reference may still anticipate the element if the missing descriptive element is "inherent" in the disclosure. A claim element is inherent if it is necessarily present in, or is the natural result of, subject matter disclosed in the reference. I further understand that inherency may not be established by probabilities or possibilities, and the mere fact that a certain thing might result from a given set of circumstances is insufficient.

D. Obviousness

22. I understand that even if a patent is not anticipated, a claimed invention is "obvious" if the difference between the claimed subject matter and the prior art

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are such that the subject matter as a whole would have been obvious at the time the invention was made to a POSA in the art.

23. I understand that a POSA provides a reference point from which the prior art and claimed invention should be viewed. This reference point prevents a POSA from using one's insight or hindsight in deciding whether a claim is obvious.

24. Also, I understand that a claim can be obvious in light of a combination of multiple prior art references. I understand that such a combination must meet certain requirements. I understand that the prior art must teach or suggest all the requirements of the invention as recited in the claim. I understand that the prior art, coupled with knowledge generally available in the art at the time of invention, must contain some suggestion or incentive that would have motived a POSA to modify a reference or to combine the references. I also understand that this proposed modification must have had a reasonable expectation of success.

25. I understand that only analogous art may be considered in an obviousness analysis. Art is considered analogous if it is in the same field of endeavor as the patent or if it is reasonably pertinent to the particular problem with which the patent is involved.

26. I also understand that in evaluating whether patent claims are obvious, objective indicia of non-obviousness can be considered, such as but not limited to,

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commercial success, long-felt but unsolved needs, praise, unexpected results, industry acceptance, failure of others, and skepticism by experts.

V. THE '981 PATENT

27. The '981 patent is titled "Methods For Analyzing Nucleic Acids From Single Cells." (Ex. 1001). The claims of the '981 patent generally describe methods for analyzing nucleic acids from two or more single cells using polynucleotides comprised of two "tags" – the first of which identifies the single cell from which the polynucleotide is derived and the second of which distinguishes the polynucleotide from other polynucleotides derived from the same single cell. Ex. 1001 at Claims.

28. I note that the specification of the '981 patent describes a method not covered by its claims—a technique called the "reflex method" that relates to the processes for rearranging sequences of interest in a polynucleotide. *See* Ex. 1001 at Summary Of The Invention. As the '981 patent explains in the Background of the Invention section, because the "fragments in the regions of interest of the genome are longer than the lengths that can be sequenced by a particular technology," such fragments are not fully analyzed. Ex. 1001 at 1:34-36. The "reflex method" purports to solve this short-read sequencing problem by introducing multiple copies of "reflex" sequences in functional proximity to one another, thus allowing sequencing of longer fragments in a single run. *See generally* Ex. 1001 at 16:62-19:6. While the specification of the '981 patent focuses heavily on this "reflex method," its

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claims are directed to the routine process of tagging and sequencing polynucleotides, as shown in representative claim 1, reproduced below:

> 1. A method of analyzing nucleic acids from a plurality of single cells, the method comprising:
>
> (a) providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides;
>
> (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides, wherein each tagged polynucleotide comprises:
>
> > (i) a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and
> >
> > (ii) a multiplex identifier (MID) sequence comprising:
> >
> > > I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells; and
> > >
> > > II. a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell;
>
> (c) sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences;
>
> (d) using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and

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(e) using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived.

29. The specification of the '981 patent reflects that the tagging method is not the invention but a prior art technique, explaining that "[w]e have previously described methods that enable tagging each of a population of fragmented genomes and then combining them together to create a 'population library' that can be processed and eventually sequenced as a mixture." Ex. 1001 at 1:26-29.

30. The '981 patent defines "Multiplex Identifier" ("MID") as "a tag or combination of tags associated with a polynucleotide whose identity (e.g., the tag DNA sequence) can be used to differentiate polynucleotides in a sample. In certain embodiments, the MID on a polynucleotide is used to identify the source from which the polynucleotide is derived." Ex. 1001 at 6:33-38.

31. The specification discloses that MIDs used to tag nucleic acids were known in the art, incorporating a patent application and other patents by reference. For example, the specification states that "[a]ny type of MID can be used, including but not limited to those described in copending U.S. patent application Ser. No. 11/656,746, filed on Jan. 22, 2007, and titled 'Nucleic Acid Analysis Using Sequence Tokens', as well as U.S. Pat. No. 7,393,665, issued on Jul. 1, 2008, and titled 'Methods and Compositions for Tagging and Identifying Polynucleotides',

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both of which are incorporated herein by reference in their entirety for their description of nucleic acid tags and their use in identifying polynucleotides." Ex. 1001 at 15:20-29. Additionally, "[e]xemplary nucleic acid tags that find use as MIDs are described in U.S. Pat. No. 7,544,473, issued on Jun. 6, 2009, and titled 'Nucleic Acid Analysis Using Sequence Tokens', as well as U.S. Pat. No. 7,393,665, issued on Jul. 1, 2008, and titled 'Methods and Compositions for Tagging and Identifying Polynucleotides', both of which are incorporated herein by reference in their entirety for their description of nucleic acid tags and their use in identifying polynucleotides." Ex. 1001 at 6:56-64.

32. The specification of the '981 patent explains that "MIDs can be generated in a variety of different ways, e.g., by a combinatorial tagging approach in which one MID is attached by ligation and a second MID is attached by primer extension. Thus, MIDs can be designed and implemented in a variety of different ways to track polynucleotide fragments during processing and analysis." Ex. 1001 at 7:15-22.

33. The '981 patent also states that ligation was known in the art. For example, the '981 patent states that a "variety of template driven ligation reactions are described in the following references, which are incorporated by reference: Whiteley et al, U.S. Pat. No. 4,883,750; Letsinger et al, U.S. Pat. No. 5,476,930; Fung et al, U.S. Pat. No. 5,593,826; Kool, U.S. Pat. No. 5,426,180; Landegren et al,

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U.S. Pat. No. 5,871, 921; Xu and Kool, Nucleic Acids Research, 27: 875-881 30 (1999); Higgins et al, Methods in Enzymology, 68: 50-71 (1979); Engler et al, The Enzymes, 15: 3-29 (1982); and Namsaraev, U.S. patent publication 2004/0110213." Ex. 1001 at 6:23-32.

34. The '981 patent shows that primer extension was known in the art. The specification describes that "'[r]everse transcription PCR,' or 'RT-PCR,' means a PCR that is preceded by a reverse transcription reaction that converts a target RNA to a complementary single stranded DNA, which is then amplified, e.g. Tecott et al, U.S. Pat. No. 5,168,038, which patent is incorporated herein by reference." Ex. 1001 at 8:8-13. A POSA would have understood that reverse transcription requires the use of primer extension. Thus, because the '981 patent discloses that reverse transcription was known in the art, it also discloses that primer extension was known in the art.

35. The specification states that the "practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label." Ex. 1001 at 13:32-39.

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36. The specification of the ’981 patent discloses that there were amplification methods known in the art that could be used with the invention. Specifically, the specification notes that “[a]ny convenient method for performing amplification reactions on a starting nucleic acid sample can be used in practicing the subject invention.” Ex. 1001 at 14:53-56. The ’981 patent indicates that “[o]ne limitation of the overall process stems from limitations of existing DNA sequencing technologies.” Ex. 1001 at 1:32-34. This shows that DNA sequencing existed at the time of the invention.

37. I do not have an opinion on the priority date of the ’981 patent. Based on the face of the patent, I understand that the earliest possible priority date of the ’981 patent is August 20, 2009 based on the filing date of U.S. Provisional Patent Application No. 61/235,595. I have considered August 20, 2009 to be the relevant date for assessing prior art invalidity.

VI. PRIOR LITIGATION AGAINST THE PATENT OWNER

38. I understand that in a prior unrelated case (the “*BD* litigation”)[1] between Patent Owner—10X Genomics, Inc. (“Patent Owner”)—and Becton, Dickinson and Company, Patent Owner challenged the eligibility of seven patents (the “Fodor Patents” – U.S. Patent Nos. 8,835,358; 9,315,857; 9,816,137; 9,290,809;

[1] *Becton, Dickinson and Company et al. v. 10X Genomics, Inc.*, C.A. No. 18-1800-RGA (D. Del.).

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9,290,808; 9,708,659; and 9,845,502), which are directed to tagging DNA molecules for the purpose of tracking and counting individual molecules. Ex. 1016. Similar to the ’981 patent, the Fodor Patents achieve molecular tagging and tracking by attaching nucleic acid label-tags to target nucleic acid molecules, amplifying the tagged nucleic acid molecules, and analyzing them by sequencing. *See* Ex. 1029 at Claims 1, 6, 29, 30 and 75. The Fodor Patents also disclose a two tag system for tracking target nucleic acids utilizing a variety of conventional molecular techniques, including PCR, hybridization, reverse transcription, primer extension, and ligation. *See* Ex. 1029 at Claims 1, 6, 13, 14, 15, 17, 18, 19, 24, 29, 30, 35, 42, 62, 66 and 75.

39. In the *BD* litigation, The Patent Owner analyzed claim 6 of U.S. Patent No. 8,835,358 (“the ’358 patent”) and claim 1 of each of the other six Fodor Patents as representative of the claims in each of the Fodor Patents. Ex. 1016.007, 011. Claim 6 of the ’358 patent is reproduced below:

> 6. A method comprising:
>
> (a) combining a mixture comprising at least two distinct target nucleic acid molecules with a pool of nucleic acid label-tags, wherein the pool of nucleic acid label-tags comprises a plurality of nucleic acid label-tags with different sequences;
>
> (b) attaching at least two nucleic acid label-tags from the pool of nucleic acid label-tags to the at least two distinct target

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nucleic acid molecules to obtain at least two label-tag-target nucleic acid molecules, wherein the distinct target nucleic acid molecules have different sequences from one another;
(c) amplifying at least a portion of the label-tag-target nucleic acid molecules, wherein an amplified portion of the label-tag-target nucleic acid molecules comprises at least a portion of said target nucleic acid molecule; and
(d) detecting an amplified product of step (c).

40. I understand that the Patent Owner stated that the Fodor Patents are "nakedly directed at the abstract idea of labeling different objects (two or more 'nucleic acid molecules', e.g., portions of DNA) with different labels ('a plurality of nucleic acid label-tags with different sequences.')." Ex. 1016.010. The Patent Owner argued that the concept of "labeling different objects with different labels" is "as old as the scientific method and is familiar to every student in any introductory science class" and the Fodor Patents "[m]erely apply[] this 'old solution' of different labels in the particular technological environment of DNA." *Id.*, at .009, .016. Just like the Fodor Patents, the '981 patent is also directed to "tagging polynucleotides."

41. The Patent Owner stated that the other elements of the Fodor Patents including the limitations (c) and (d) of claim 6 above, are "well-known and conventional." *Id*. at .010. The Patent Owner also stated that detecting and sequencing DNA samples are nothing but "generic, routine, well-known laboratory

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techniques." *Id.* at .017. The '981 patent similarly claims "sequencing the plurality of tagged polynucleotides." Ex. 1001 at Claim 1 (c).

42. I also understand that one of the asserted Fodor patents was U.S. Pat. 9,708,659 (the "'659 Patent") (Ex. 1053). Ex. 1016.007. I have reviewed the '659 Patent, and I note that claims 13 and 19 are directed to the idea of dual-tagging. Claims 13 and 19 of the '659 Patent are reproduced below:

> 13. A system for counting n, wherein n is a number of nucleic acid target molecules that are present in a single cell from a sample comprising cells, the system comprising:
>
> a) a diverse set of labels comprising a plurality of oligonucleotides attached to each bead of a plurality of beads, wherein the set of labels comprises m different labels, wherein m>n, and wherein each oligonucleotide of the plurality of oligonucleotides comprises ***a common sequence***, ***a variable target label sequence***, and a target-specific recognition sequence capable of attaching to or hybridizing to the target molecule;
>
> b) a plurality of reaction vessels comprising a single cell from said sample comprising cells and a single bead from said plurality of beads, wherein each reaction vessel is used for attaching a label from the diverse set of labels attached to the bead contained therein to each occurrence of the nucleic acid target molecules from the single cell contained therein to generate a set of labeled nucleic acid target molecules, wherein the individual labeled nucleic acid target molecules

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> comprise all or a portion of the complementary sequence of a nucleic acid target molecule and a label from the diverse set of labels; and
>
> c) processing software for counting n from a number of labeled nucleic acid target molecules detected.
>
> 19. The system of claim 13, wherein the common sequence for the plurality of oligonucleotides attached to a given bead is different from that for the pluralities of oligonucleotides attached to other beads in the plurality of beads.

Ex. 1053 at claims 13, 19 (emphasis added). A POSA would have understood that the recited "common sequence" could be used to tag all molecules in a single-cell with the same cell-tag, and the recited "variable target sequence" could be used to further tag each individual molecule within the single-cell with an additional molecular identifier. For example, I have reviewed BD's claim chart which is consistent with this reading. Ex. 1052.014 (identifying 10X's sample-distinguishing Barcode as the "common sequence" and a 10X's Unique Molecular Identifier as the "variable target label sequence); *id.* at .013 (stating that the Barcode is used to "separately index each cell's transcriptome.").

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VII. PROSECUTION HISTORY OF THE ’981 PATENT

43. On August 15, 2017, Patent Owner filed Nonprovisional Patent Application No. 15/677,957 titled “Methods For Analyzing Nucleic Acids From Single Cells.” Ex. 1005.003-011, 069-071.

44. On December 27, 2017, the Examiner issued a non-final rejection, rejecting all of the pending claims (1-6). Ex. 1005.147-163. The Examiner rejected all of the pending claims as anticipated by International PCT Patent Application Publication No. WO 2010/117620 (“Linnarsson,” Ex. 1003). Ex. 1005.153-156. The Examiner explained that “[t]he cell specific tag (e.g., see figure 3) in the nucleic acid sample from each of the single cell is the first Multiplex Identifier (first MID) sequences associated with a segment of a nucleic acid molecule of (a) uniquely identifies the single isolated cell from which the nucleic acid molecule is provided.” Ex. 1005.155. The Examiner also explained that:

> Linnarsson also teaches that the synthesis of the first strand of cDNA is directed by a cDNA synthesis primer (CDS) that includes an RNA complementary sequence (RCS) that is at least partially complementary to one or more mRNA in an individual mRNA sample (Linnarsson claim 3), wherein a template-switching oligonucleotide (TSO) is added to each individual mRNA sample, wherein said TSO comprises a portion which is at least partially complementary to a portion at the 3' end of the first strand of cDNA (Linnarsson claim 5), wherein the CDS and the TSO each include a different tag (Linnarsson claims 10 and 11).

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> Different tags associated with cDNA segment of Linnarsson meets the limitation of second MID sequences are different from each other.

Ex. 1005.155.

45. In the same December 27, 2017 non-final rejection, the Examiner also rejected all of the pending claims under 35 U.S.C. § 101 as being "directed to an abstract idea of mathematical relationships and a law of nature," "without significantly more." Ex. 1005.150-152. The Examiner determined that "the instant claims are drawn to an abstract idea of analyzing nucleic acids by correlating tag sequences with sequencing data." Ex. 1005.151. The Examiner found that the claims "recit[e] using a first tag to correlate an identified polynucleotide sequence with the single cell from which the polynucleotide sequence is derived and a second tag sequence is used to correlate an identified to correlate an identified polynucleotide sequence with the plurality of sample polynucleotides from which the polynucleotide sequence is derived. These steps involve the mental step of performing a correlation." Ex.1005.150. Further, "[t]he remaining steps in claim 1 were well-understood, routine steps," and "[t]he dependent claims add elements . . . [that] are routine, well-understood and conventional in molecular biology." Ex.1005.151. Finally, the Examiner determined "[g]enerating a plurality of tagged polynucleotides from a plurality of single cells comprising a first tag sequence associated with a single cell and a second tag sequence distinguishing the sample

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polynucleotides from the plurality of other sample polynucleotides and sequencing the plurality of tagged polynucleotides are well-known, routine and conventional in genetics technology." Ex. 1005.152.

46. On March 26, 2018, in response to the Examiner's non-final rejection, Patent Owner asserted that the template switching oligonucleotides "in Linnarsson are essentially a single tag that is associated with a particular well, which may or may not contain a single cell. As such, Linnarsson does not describe a second tag that distinguishes the sample polynucleotides from other pluralities of sample polynucleotides derived from the same cell." Ex. 1005.182-183.

47. On July 10, 2018, the Examiner issued a final rejection, again rejecting all of the pending claims as being anticipated by Linnarsson. Ex. 1005.193-196. The Examiner determined that Linnarsson teaches the "identification of expressed genes and identification of cells from which they are originated, which would necessarily require a means of identifying a particular nucleic acid sequence, as well as a tag for identifying the particular cell from which it came." Ex. 1005.195 (emphasis in original). The Examiner further explained that "Figure 3 of Linnarsson depicts a cell-tag, XXXXX, which is reasonably interpreted to be a different tag for each cell, given that Linnarsson teach identification of genes and further teach identification of the cell from which the gene originated, the cell-tag clearly is present as a separate tag, used to identify a particular cell." Ex. 1005.195. Finally,

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the Examiner determined that "Linnarsson's Claim 14 teaches that each cDNA sample has a distinct tag or combination of tags, which is reasonably interpreted to mean that each cDNA sample comprised 2 distinct tags." Ex. 1005.196 (emphasis in original). For all these reasons, the Examiner found that Patent Owner's arguments were not persuasive. Ex. 1005.195-196. The Examiner withdrew its rejection based on 35 U.S.C. § 101, but stated that "tagging nucleic acid sample with identifier sequence [was] routinely practiced in the art, [which is] exemplified [by] Linnarsson." Ex. 1005.199.

48. On August 6, 2018, Patent Owner responded to the Examiner's final rejection. Ex. 1005.215-225. Patent Owner asserted that "[a]s explained in Linnarsson, the tags in Figure I are used to identify the single cell from which the cDNAs came from, while sequencing of cDNA sequence derived from an expressed gene itself, as opposed to a second tag, are used for identification of the expressed gene. *See, e.g.,* Linnarsson at 22, 11. 26-28." Ex. 1005.219. Patent Owner also asserted that "Figure 3 and page 4, lines 15-16 fail to disclose a second tag sequence." Ex. 1005.221. Further, "Figure 3 and page 4, lines 15-16 simply disclose a 'cell-tag' XXXX. There is no description of the structure or function of such a tag based on this disclosure." Ex. 1005.221. Finally, Patent Owner alleged that "[e]ven assuming *arguendo* that claim 13 discloses a cDNA sample that include two distinct tags, Patent Owner submit that claims 1 and 13 and the remaining claims of

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Linnarsson are silent with respect to multiple tags that are distinct from one another." Ex. 1005.222.

49. On December 18, 2018, the '981 patent issued. Ex. 1005.342. In the Reasons for Allowance, the Examiner stated "[t]he claimed identifier sequence comprises . . . a second tag sequence, which distinguishes a sample polynucleotide from other sample polynucleotides derived from a single cell, which is not taught by Linnarsson." Ex. 1005.238.

50. During the prosecution of the '981 patent, McCloskey (Ex. 1004) was not considered by the Examiner. As discussed below, Section XI.A. *infra*, McCloskey discloses "a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell," as recited by claim 1 of the '981 patent.

VIII. CLAIM CONSTRUCTION

51. I do not believe that any explicit claim construction is required to determine the validity of the '981 patent claims because the prior art teaches and/or discloses the claim limitations under any reasonable construction. My opinion may change if the Patent Owner sets forth its own explicit claim constructions or construes claim limitations contrary to their reasonable construction.

52. I understand that the words of the claims of a patent are generally given the ordinary and customary meaning as interpreted by a POSA in the art at the time

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of invention. I have been informed that patent claims are construed in light of the claim language, the specification (including the drawings), and the prosecution history (including references cited during the prosecution of the patent).

53. Claim 1 of the ’981 patent recites “a multiplex identifier (MID) sequence.” The specification defines “Multiplex Identifier” (“MID”) as “a tag or combination of tags associated with a polynucleotide whose identity (e.g., the tag DNA sequence) can be used to differentiate polynucleotides in a sample. In certain embodiments, the MID on a polynucleotide is used to identify the source from which the polynucleotide is derived.” Ex. 1001 at 6:33-38. I have applied this definition in my analyses.

54. Claim 1 of the ’981 patent recites “providing a sample.” The specification defines a “[s]ample” to mean “a quantity of material from a biological, environmental medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought.” Ex. 1001 at 12:7-10. The ’981 patent’s definition of “sample” is consistent with a POSA’s understanding of this term, and I have applied this definition in my analysis.

IX. PERSON OF ORDINARY SKILL IN THE ART

55. I understand that whether a patent is obvious is analyzed from the perspective of a POSA at the time of the alleged invention. I am knowledgeable

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about the level of ordinary skill in the art of the time of the alleged invention based on my education and experience. *See supra* Section II.

56. In determining the definition of a POSA, I have considered the following factors: (1) the level of education and experience of those in the field; (2) the complexity of the technology; (3) the rate of innovation; (4) the types of problems encountered by those in the art; and (5) prior art solutions to such problems.

57. In my opinion, a POSA would have been someone with a Masters degree and/or Ph.D. in Molecular Biology, Genetics, Chemistry, Engineering, or a related discipline and at least two years of post-Masters, postdoctoral, or industry experience. Alternatively, a POSA would have been someone with a Bachelor of Science degree in Molecular Biology, Genetics, Chemistry, Engineering, or a related discipline and at least five years of industry or academic experience (including any experimental work toward a graduate degree) relating to DNA sequencing technologies, amplification methods, and gene expression analysis..

58. Further, it is my opinion that a POSA would have been familiar with associated tools, methods, and techniques including: (1) polynucleotide tagging, (Section III.C.1); (2) amplification, (Section X.C.2); (3) use of tags to overcome amplification bias, (Section X.C.3); (4) single cell analysis, (Section X.C); (5) ligation and primer extension, (Section X.B); and (6) sequencing, (Section X.C.4).

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X. TECHNICAL BACKGROUND AND STATE OF THE ART

A. DNA and RNA

59. DNA is an informational molecule, containing the information necessary to specify many aspects of development and maintenance of the cells of organisms. This information is stored in the sequence of adjacent bases in the DNA strand, or a "nucleotide sequence."

60. DNA is a double-stranded molecule composed of monomeric units called nucleotides. A polynucleotide is a linear polymer comprised of several nucleotides. An oligonucleotide, or "oligo," is a short polynucleotide. Oligonucleotides are commonly used as primers with various biological techniques such as DNA sequencing, polymerase chain reaction ("PCR"), and molecular cloning.

61. In DNA, each nucleotide comprises a deoxyribose sugar molecule, one of four nitrogenous bases, and one or more phosphate groups. The four nitrogenous bases of DNA are: adenine ("A"), which is complementary to thymine ("T"); and cytosine ("C"), which is complementary to guanine ("G"). The two strands of DNA are connected through complementary base pairing via chemical bonds, also known as "hybridization," as depicted below:

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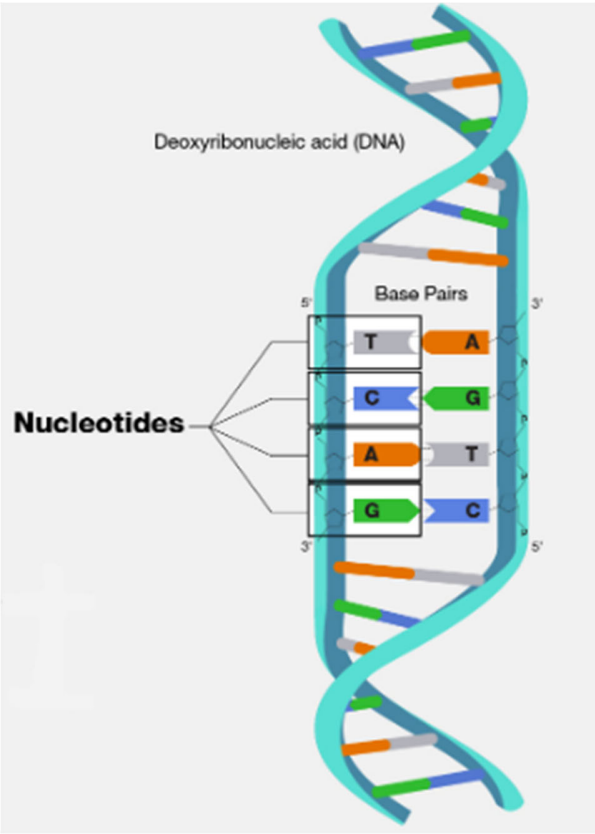

Ex 1049.002.

62. Each strand of the DNA molecule has two ends – one end is referred to as 5' and the other end is referred to as 3'. DNA is synthesized in the 5' to 3' direction.

63. Ribonucleic acid (RNA) is a molecule similar to DNA, both store genetic information and are made up of nucleotides consisting of sugar, nitrogenous bases, and a phosphate backbone. RNA can hybridize to a complementary DNA sequence with the help of specific enzymes. DNA and RNA are molecules that are known in the art as "nucleic acids."

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64. Unlike DNA, RNA is mostly single-stranded. Also, the four nucleotides that make up RNA are adenine, guanine, cytosine, and uracil (U), wherein G hybridizes to C, and A hybridizes to U (as oppose to T in DNA). Despite the structural difference, uracil and thymine are functionally equivalent because they both form base pairs with adenine, and denote the same genetic information. Vértessy at 1 (Ex.1044.001).

65. Genes are sequences of DNA nucleotides and constitute functional units carrying hereditary information. Alleles are variant forms of genes, located at a specific position on a specific chromosome. The differences between alleles of a given gene are reflected by the sequence of the polynucleotide corresponding to that gene. An individual inherits two alleles for each gene, one from each parent. Differences in alleles decide physical traits and influence risks for developing certain diseases, allergies, or reactions to certain medications.

66. Gene expression is the process by which information encoded in a gene is used by a cell by converting the nucleic acid sequence information into a protein. Proteins are made up of amino acids, which serve critical functions of cellular life including repairing and building body's tissues, carrying out metabolic reactions, and coordinating bodily functions.

67. Protein-coding gene expression is achieved by two major steps – transcription and translation. Transcription is the copying of genetic information

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from a DNA sequence into a specific type of RNA: messenger RNA (mRNA), which is made up of nucleotides that are complementary to the DNA sequence. During transcription, an enzyme called RNA polymerase catalyzes the formation of a pre-mRNA molecule using the DNA sequence as a template for complementary base-pairing. The pre-mRNA is then processed to form mature mRNA. Clancy at 1 (Ex. 1030.001). A poly(A) tail, which is a sequence of adenine nucleotides, is added to the 3' end of the mature mRNA (circled in red, below) and makes the mRNA molecule more stable and allows it to be exported from the nucleus to carry out the next step, translation.

Ex. 1042.019 at Figure 2 (annotated).

68. Translation is the decoding of genetic information carried by the mRNA to make proteins. This process takes place within a specialized organelle called the ribosome, which reads the mRNA in a group of three bases, referred to as a codon, which correspond to a particular amino acid. Clancy at 2 (Ex. 1030.002).

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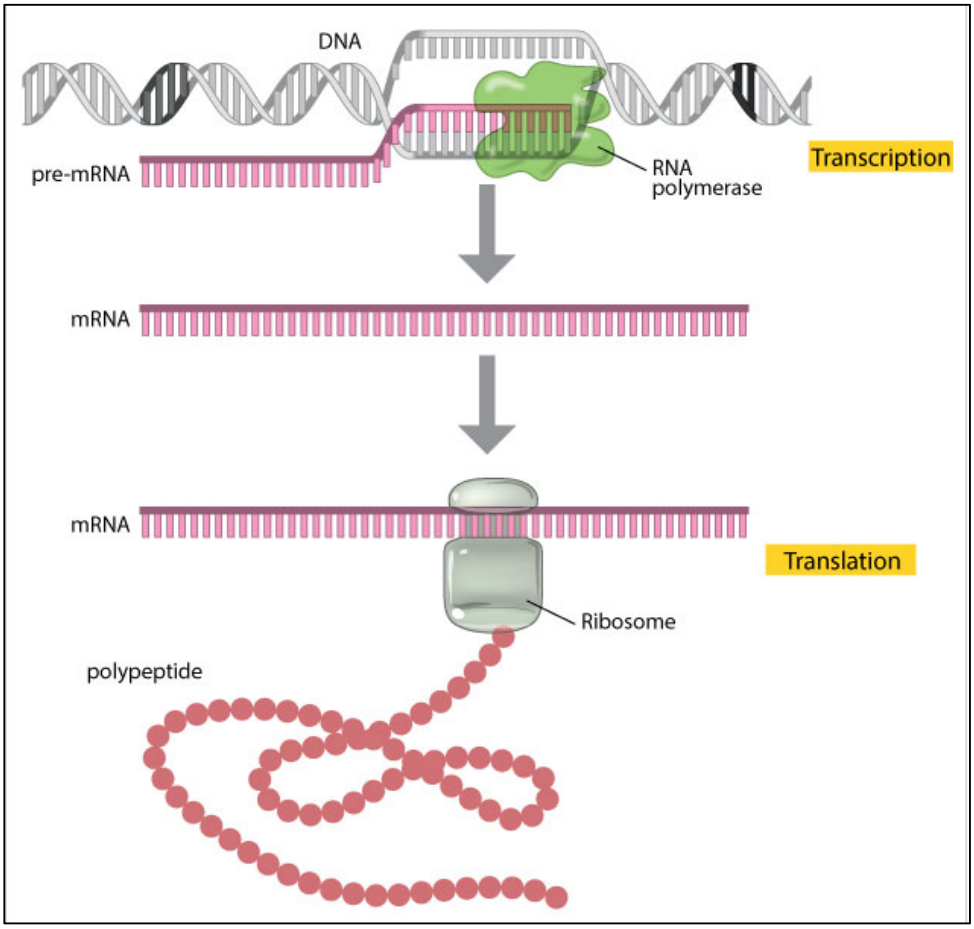

Clancy at 2, Figure 1 (Ex. 1030.002).

69. Genes can be expressed at different levels. In the figure below, gene A is transcribed and translated at a higher level than gene B. As a result, the amount of protein A in the cell is greater than that of protein B. Alberts at 302 (Ex. 1025.005).

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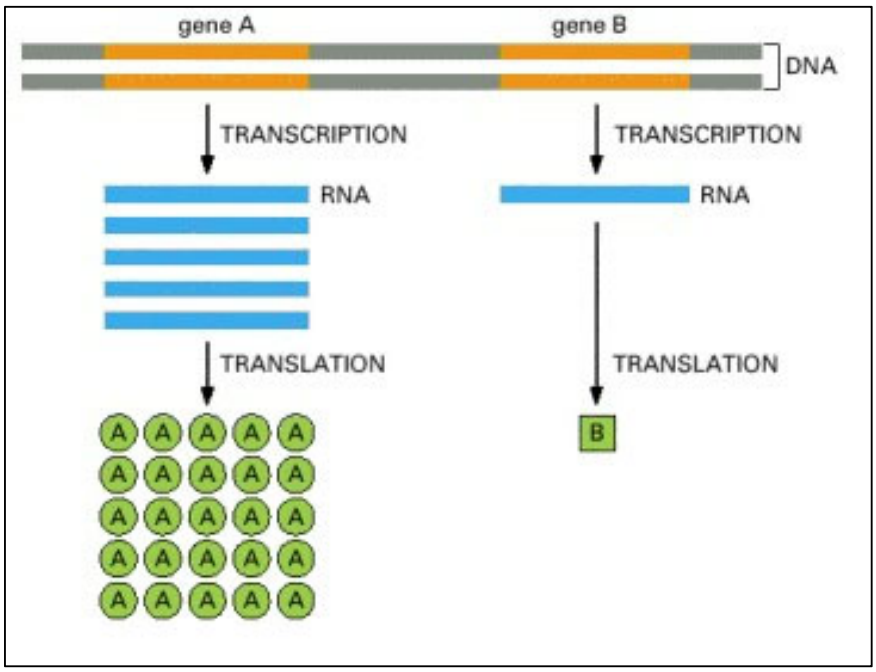

Alberts at Fig. 6-3 (Ex. 1025.005).

70. Gene expression profiling is the determination of the mRNA content in a cell or tissue to understand the pattern of genes expressed. Thus, detecting mRNA molecules is critical to the study of gene expression because they show the portions of DNA copied for the production of proteins. This is useful to understand aberrant gene activity and identify potential drug targets that alter gene expression, among other research and clinical applications.

B. Reverse Transcription

71. Reverse transcription is the process by which complementary DNA (cDNA) is created. Reverse transcription involves two steps: (1) mRNA is isolated from other cellular RNA and (2) isolated mRNA is reverse transcribed into cDNA

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by using an enzyme called reverse transcriptase. The first step—the isolation process—is carried out by adding oligo(dT) primers into a mixture containing all cellular RNA molecules. The oligo(dT) primers are designed to pair with poly(A) tails which are present in most mRNA molecules, but not in other types of cellular RNA molecules. Thus, mRNA are separated from other cellular RNA based on the presence of oligo(dT) primer. The second step—cDNA synthesis—is achieved by adding reverse transcriptase enzymes to the mRNA collection. The reverse transcriptase enzyme recognizes the oligo(dT) primer as a starting point, and utilizes the mRNA strand as a template to synthesize the cDNA by adding nucleotides to the new strand, with each nucleotide complementary to the base on the mRNA strand.

72. Reverse transcription had long been known in the art and serves as an important tool in molecular biology protocols. Okayama at 161 (Ex. 1024.001). For example, reverse transcriptases are helpful in producing cDNA libraries which aid in understanding gene expression. Reverse transcription had also been combined with PCR (as detailed later), allowing detection of RNA at very low levels of gene expression.

C. Single Cell Genomics

73. Single cell genomics is the study of polynucleotides and proteins at a cell-by-cell resolution. A human body has many distinct cell-types, and even different cells of the same cell-type may be distinct from one another. Trapnell at

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1491 (Ex. 1031.001). Traditional techniques rely on bulk analysis to examine nucleic acids obtained from tissue samples, organs, or an entire organism containing millions of cells. These traditional techniques end up giving only the average expression profiles of individual cells and often result in loss of functional information present in single cells. Linnarsson (Ex. 1003 at 1:29-30). Bulk analysis also obscures results caused by cells which vary in pathways that are active, genes that are expressed, and functions performed in a cell at any given point in time. Trapnell at 1491 (Ex. 1031.001).

74. As an improvement over the bulk analysis, single cell genomics allows for the analysis of samples on a single cell basis and preserves unique information of each cell in a sample. Single cell genomics further provides useful information about the heterogeneity of gene expression in tissues, including diseased tissues (e.g., cancer).

75. Gene expression analysis in single cells has been widely practiced since the 1990s. *See, e.g.*, Brady at 17 (Ex. 1032.001), Brail at 45 (Ex. 1006.001), Eberwine at 3010 (Ex. 1033.001). While earlier methods were suitable analyzing small numbers of single cells or cells that are difficult to obtain in large numbers, next-generation sequencing (NGS) technologies have enabled gene expression in tens of thousands of single cells. Linnarsson (Ex. 1003 at 2:28-29).

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76. Single cell sequencing generally requires the following steps: (1) isolation of single cells from a cell population; (2) template preparation and tagging of sequences; (3) amplification of these sequences from each isolated cell; (4) preparation of a sequencing library; and (5) sequencing of the library using a next-generation sequencer. I explain in detail below the steps of tagging, amplification, and sequencing since they are the most relevant to the ’981 patent and prior art references that I have used.

1. DNA Tagging

77. Tagging is a known concept in genetics which involves adding synthetic polynucleotide to a sequence of interest. The ’981 patent notes in its background section that previous methods existed that “enable tagging each of a population of fragmented genomes and then combining them together to create a ‘population library’ that can be processed and eventually sequenced as a mixture.” Ex. 1001 at 1:26-29. The ’981 patent further acknowledges that various methods of tagging existed in the art. Ex. 1001 at 22:38-40 (“Any convenient method for tagging and/or counting individual nucleic acids molecules with unique tags may be employed”).

78. The field has been replete with routine methods of tagging for a variety of uses including acting as a unique “barcode” identifier (Ex. 1034.003, 011); providing primers to facilitate amplification (Ex. 1021.001); counting and tracking

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molecules (Ex. 1007.001); tracing molecules to their sources (Ex. 1003 at 19:9-10); and/or removing amplification bias (Ex. 1013.001).

79. It was a common practice in the art to introduce molecular barcodes to construct nucleic acid libraries before the amplification step. Brenner at 1665 (Ex. 1035.001); Hug at 623 (Ex. 1007.009); Linnarsson (Ex. 1003 at 19:12-15). For instance, in 1996, Bonaldo et al. recommended the use of "library tags" to identify and distinguish between different cDNA libraries. These tags were added to the oligonucleotide used to prime the synthesis of the first template. Bolando at 791, 793, 801 (Ex. 1034.001, 003, 011). Indeed, the '981 patent itself cites Brenner et al., which recommended the use of "unique tags" to each molecule for identification purposes. Brenner at 1665, 1667 (Ex. 1035.001, 003).

80. Molecular tags have also been commonly known in the art to track the origin of nucleic acids. The concept of a "molecular barcode"—a barcode that identifies each individual molecule or a barcode that identifies each sample in a pool of samples—was known in the prior art. This includes the use of nucleic acid sequences to track the origin and efficiency of amplified samples. For example, Linnarsson discloses a method for preparing a cDNA library by tagging cDNA samples originating from different cells with unique tags which act as barcodes to identify which single cell each cDNA sample originated from. Linnarsson (Ex. 1003 at 19:4-11). Binladen further discloses use of a unique tagged primer combination

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applied to specific DNA templates, in order to sort the sequences generated after sequencing into their original template source using the tags. Binladen at 2, 3 (Ex. 1021.002, .003).

81. The concept of using multiple tags or a combination of tags was also known. For example, McCloskey discloses a method of DNA analysis for single-cell samples by utilizing an encoding oligonucleotide with five information regions, two of which are tag sequences – one being a barcode that uniquely identifies and distinguishes one polynucleotide from another and another being a batch-stamp which associates a polynucleotide to "a specific sample on a specific date." McCloskey at 762, 764 (Ex. 1004.002, .004). Linnarsson also discloses tagging a cDNA sample with a combination of tags. Linnarsson (Ex. 1003) at 19:6-7.

82. Thus, tagging was a well-known step in sequencing of polynucleotides. In fact, as mentioned above, the Patent Owner has admitted in the *BD* litigation that the concept of tagging and labelling of two or more nucleic acid molecules was "well-known and conventional" before 2009. Ex. 1016.010.

2. Amplification

83. DNA sequencing requires a minimum amount of DNA or RNA. Before it can be sequenced, a sample with limited amount of DNA or RNA must sometimes be amplified. Amplification refers to the process of creating multiple copies of a specific polynucleotide. Various methods of amplification were known in the art

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before 2009, such as Polymerase Chain Reaction (PCR), Ligase chain reaction (LCR), Nucleic acid based amplification (NASBA), and Transcription-mediated amplification, etc.

84. Linnarsson and McCloskey each describes amplification steps that were "known and widely practiced in the art." Ex. 1003 at 12:19-20; McCloskey at 764-767 (Ex. 1004.004-.007). Indeed, the '981 patent also acknowledges the use of conventional techniques of amplification as a part of its claims and description. I focus on PCR and ligation chain reaction techniques since they are most relevant to the '981 patent and prior art references that I have used.

a) Polymerase Chain Reaction ("PCR")

85. PCR is a technique using repeated cycles of oligonucleotide-directed DNA synthesis to carry out amplification of target nucleic acid sequences. Persing at 1281 (Ex. 1036.001). PCR requires generally involves three steps: (1) denaturation of the double-stranded DNA, (2) annealing of primers, and (3) primer extension. Schochetman at 1154 (Ex. 1009.001).

86. Denaturation is the separation of the complementary strands of DNA by using heat or through other chemical or physical means of dissociation. Schochetman at 1154 (Ex. 1009.001). This results in two complementary single-stranded DNA template strands, which can then be replicated.

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87. Annealing is the attachment of primers or probes which are oligonucleotides complementary to one of the original DNA strands. When the reaction mixture comprising denatured DNA template strands is cooled to a certain temperature, the primers hybridize, that is bind through base pairing interactions to either the left (5') or right (3') side of the sequence of interest. Schochetman at 1154 (Ex. 1009.001). These portions to which the primers bind are called priming sites.

88. During the primer extension step, a thermostable enzyme catalyzes the synthesis of new strands of DNA through primer extension. The enzyme called "DNA polymerase" adds nucleotides complementary to those in the single-stranded DNA template starting at the 3' end of the annealed primer. Schochetman at 1154 (Ex. 1009.001). The result of one cycle of PCR is a double-stranded DNA. Upon completion of one cycle, the DNA strands are subjected to additional cycles, i.e., the same steps of denaturation, primer annealing, and primer extension. This results in an exponential amplification of target DNA sequences, as demonstrated below.

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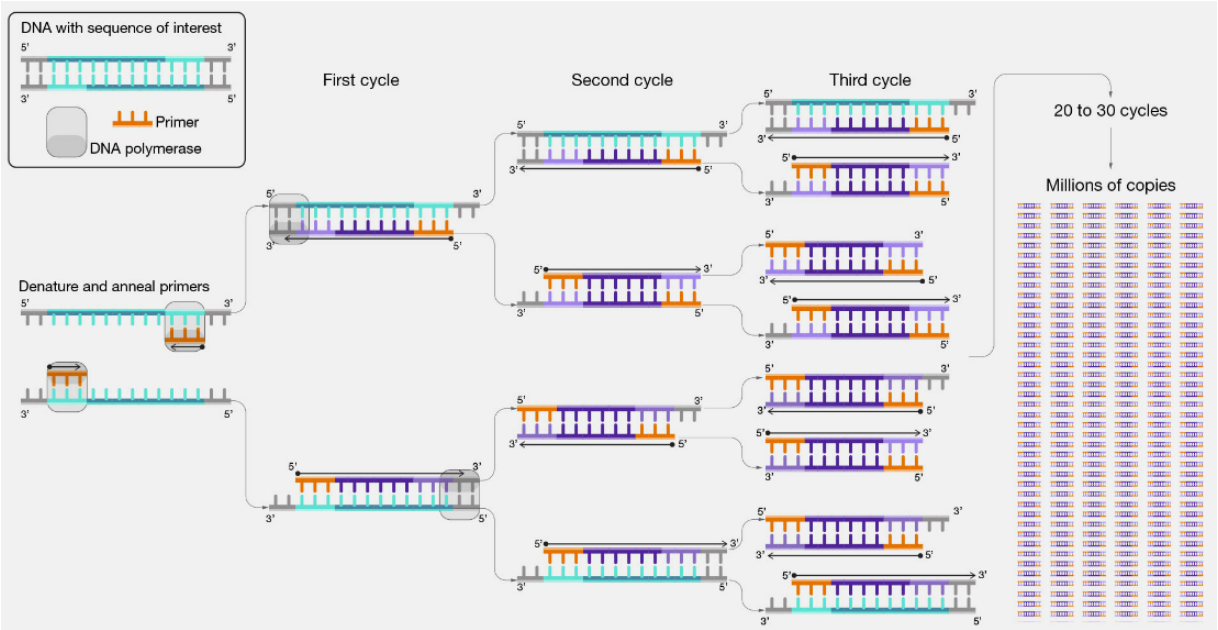

Ex. 1050.001.

89. Methods for applying PCR amplification to a small amount of sample polynucleotides, such as DNA or RNA from single cells, were well established and known in the prior art. *See* Kurimoto (Ex. 1037); Esumi (Ex. 1038).

3. Amplification Bias and Molecular Tagging

90. One of the known issues with nucleic acid amplification is amplification bias. Because the PCR process is stochastic—i.e., randomized—the relative quantity of each sequence in the post-amplification sample likely does not accurately reflect the distribution of such samples in the pre-amplification sample. Degrelle (Ex. 1012); Yang (Ex. 1011). Such problems cloud the ability of researchers or clinicians to understand, for example, how much of a particular gene is being expressed in a particular cell of interest.

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91. The issue of amplification bias can be particularly severe when the amplification starts from a small number of template DNAs, such as in single-cell analyses. Because of the low number of template DNA, more rounds of amplification is necessary, which results in an exponential increase in error rate. Additionally, there is increased risk of amplifying any contaminant DNA in the starting mixture, which results in a skewed end product. Miner at 1 (Ex. 1013.001). Thus, reducing amplification bias is of particular importance in gene expression analysis in single cells.

92. To solve this problem, molecular tags (as discussed above at X.C.1) can be added to target sequences prior to the amplification step. Miner at 1 (Ex. 1013.001), McCloskey at 766 (Ex. 1004.006). Every sample and the polynucleotides within every given sample are first uniquely tagged before being amplified and thereafter sequenced. By uniquely tagging the sample and the polynucleotides, researchers can track the number of polynucleotides and their source by counting the number of unique tags. The number of unique tags correspond to the number of unique polynucleotides in every sample. This results in addressing amplification bias by keeping a track of the polynucleotides in the original sample. EX1013.001; EX1004.006.

93. Tagging has been a known solution to address the problem of amplification bias. For example, McCloskey suggests use of a multiple tag system

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to distinguish sample polynucleotides derived from the same cell. McCloskey at Abstract (Ex. 1004.001) ("We have developed and applied molecular encoding principles to solve this source-uncertainty problem for DNA sequences generated by standard PCR."). Additionally, Miner notes that:

> In response to the challenges of PCR redundancy and contamination associated with PCR amplification of limited amounts of DNA template, we have labeled genomic DNA fragments with molecular sequence barcodes and 'batch-stamps' prior to PCR amplification. This was accomplished by including these molecular labels in the hairpin linker sequence that we use in hairpin-bisulfite PCR (5). This encoded information enables us to track the genomic origin of each sequence obtained from PCR and subsequent bacterial cloning. Each genomic fragment is marked prior to amplification, allowing us to identify contaminant and redundant sequences and to quantify accurately the proportion of cells carrying a particular sequence variant by counting only distinctly tagged sequences. This highly sensitive method offers confirmation of the independent genomic origin of all sequences in final data sets derived from PCR amplification.

Miner at 1 (Ex. 1013.001).

4. Sequencing

94. Nucleic acid sequencing is the process of determining the exact sequence of nucleotides in a nucleic acid molecule. The development of sequencing technologies can be traced to at least 1977 when Frederick Sanger developed DNA

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sequencing technology based on a chain termination method. Hutchinson at 6227, 6299 (Ex. 1043.001, 003).

95. DNA sequencing technologies went through significant improvements in subsequent years. In the early 2000s, NGS technologies emerged which were different from the Sanger method in terms of throughput and cost. Mardis at 388 (Ex. 1045.002). By approximately 2008, techniques and platforms for NGS were commercially available and in widespread use including the Roche/454 FLX, Illumina/Solexa Genome Analyzer and Applied Biosystems SOLiD System. Mardis at 388 (Ex. 1045.002); Shendure at 1136 (Ex. 1046.002).

96. NGS is a type of high-throughput sequencing technology that uses parallel sequencing of many fragments of DNA. Parallel sequencing enables these sequencers to process and detect millions of sequence reads in parallel. Mardis 2 at 133 (Ex. 1047.001). The platforms carrying out NGS are diverse in sequencing and detection biochemistry but generally have a common workflow to produce sequence reads starting with preparing a sequencing library. Shendure at 1136 (Ex. 1046.002).

97. Library preparation is often accomplished by random fragmentation of input DNA and ligation of common adapter sequences to both ends of each DNA fragment. Shendure at 1136 (Ex. 1046.002). Then, the sequences may be subjected to clonal amplification which, depending on the platform, includes approaches such as emulsion PCR or bridge PCR. These are PCR techniques that cause the amplified

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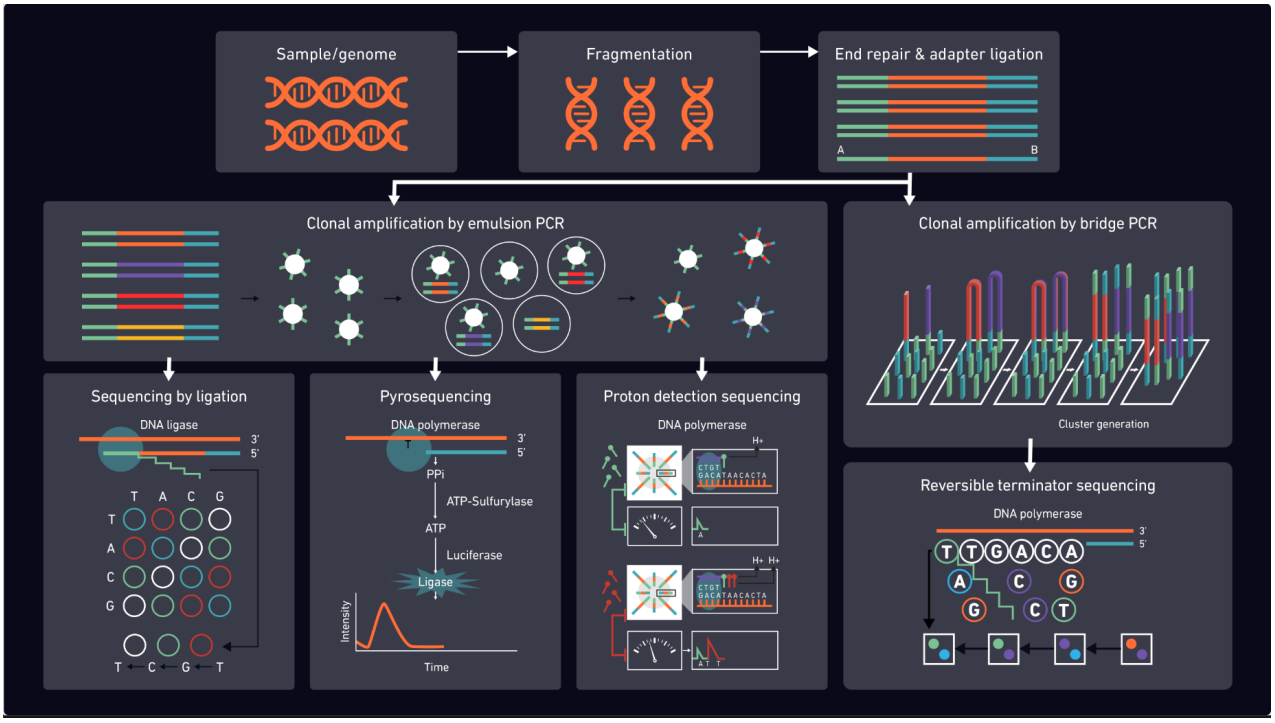
sequences derived from a single library molecule to be spatially clustered either to a single location as in the case of bridge PCR or to the surface of micron-scale beads in case of emulsion PCR, thus facilitating sequencing and detection. Shendure at 1136 (Ex. 1046.002). After the amplification step, again depending on the selected platform, the sequencing step is performed which consists of alternating cycles of enzyme-driven processes and imaging steps as depicted below[2].



Gkazi at Figure 2 (Ex. 1041.005).

98. The Illumina Genome Analyzer, utilizes a sequencing-by-synthesis approach, also called reversible terminator sequencing. Mardis at 391 (Ex.

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1045.005); Shendure at 1139 (Ex. 1046.005). In this sequencing platform, starting with the fragmentation and adapter ligation steps as described above, the sequenced library is added to a "flow cell" which are sample surfaces that have channels coated with oligonucleotides complementary to the sequencing adapters of the library, so that single-stranded adapter-ligated DNA fragments can attach through hybridization. Holt at 839 (Ex. 1048.001). After hybridization, an isothermal polymerase creates a complementary strand of the hybridized template. The double-stranded molecule is then denatured and the single-stranded template is clonally amplified through bridge amplification. Shendure at 1138 (Ex. 1046.004). This results in production of multiple DNA copies or clusters, each representing the single molecule that initiated the cluster amplification. Mardis 2 at 135 (Ex. 1047.003). These DNA clusters are again made single-stranded in order to be used as a template to make copies by the help of "reverse terminators." Shendure at 1139 (Ex. 1046.005). Reverse terminators are nucleotides that carry a base-unique fluorescent label and have their 3'-OH group chemically inactivated such that only that single base is incorporated which corresponds to its respective base (A, G, T, or C) at the position on the single-stranded template. Shendure at 1139 (Ex. 1046.005). After the addition of each nucleotide, an imaging step is performed which excites the clusters by a light source resulting in emission of a characteristic fluorescent signal. The number of cycles determines the length of the read and the emission wavelength

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determines the base call. Mardis at 392 (Ex. 1045.006). For a given cluster, all identical strands are read simultaneously in a computer and millions of clusters are processed in a massively parallel process. Mardis 2 at 135 (Ex. 1047.003), Shendure at 1140 (Ex. 1046.006).

XI. THE PRIOR ART REFERENCES OF THE GROUNDS

A. McCloskey (Ex. 1004)

99. McCloskey *et. al*., "Encoding PCR Products with Batch-Stamps and Barcodes", is an article that was published in Biochemical Genetics on October 23, 2007 and is thus prior art to the '981 patent. McCloskey (Ex. 1004). McCloskey describes a method of DNA analysis for single-cell samples that utilizes an "encoding oligonucleotide" having "five distinct information regions," two of which are tag sequences that distinguish, respectively, polynucleotides and samples. As depicted below, the first tag is a "barcode" (highlighted in yellow), which is a randomly generated sequence intended to uniquely identify and distinguish one polynucleotide from another. McCloskey at 762 (Ex. 1004.002) ("[T]he random barcode distinguishes among sequences arising from different cell or allele copies."), *id.* McCloskey also teaches a second tag sequence, the "batch-stamp" (highlighted in red), that is unique to each experiment and is intended to associate a polynucleotide to "a specific sample run on a specific date." McCloskey at 764 (Ex. 1004.004). The other regions on the encoding oligonucleotide include: "a genomic

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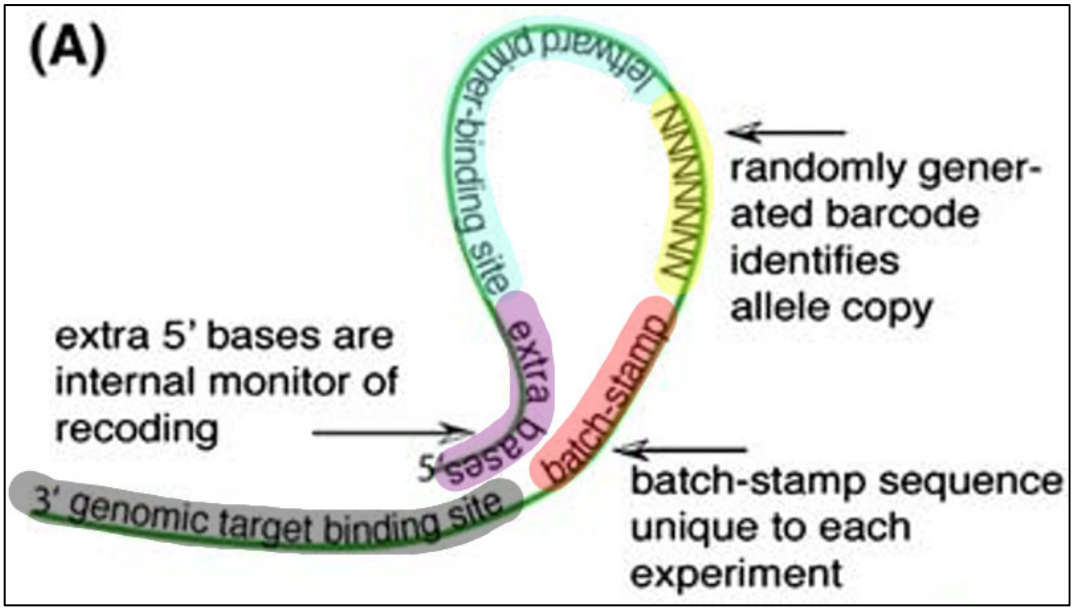
target-binding site" (highlighted in grey), "a primer-binding site for PCR" (highlighted in light blue), and "extra bases that serve as internal sentinels of recoding as well as tethering the oligonucleotide to itself" to minimize non-specific annealing (highlighted in purple, below). McCloskey at 763 (Ex. 1004.003).



Ex. 1004 at Fig. 1.

100. McCloskey's method begins with annealing the 3' end genomic target binding site (highlighted in grey, above) of the encoding oligonucleotide to denatured genomic DNA (*see* step (B) in figure, below). McCloskey at 763-766 (Ex. 1004.003-006). The oligonucleotide-annealed DNA is then extended by Sequenase to build a complementary DNA sequence (*see* step (C) in figure, below). *Id.*

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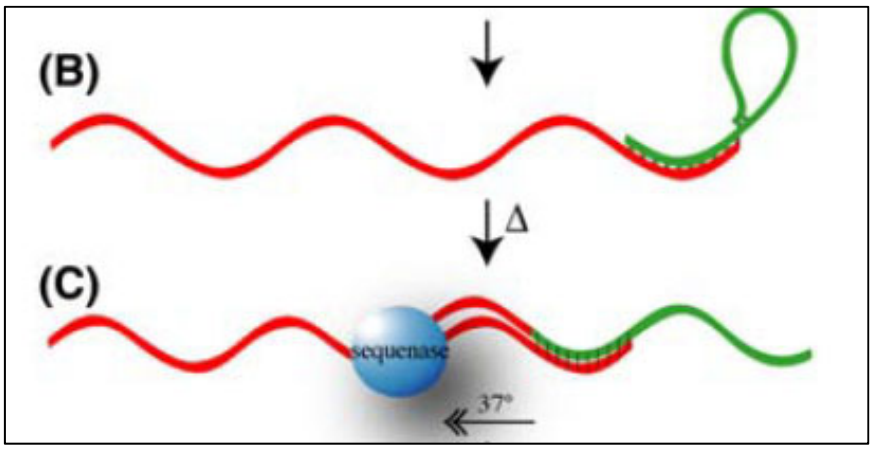
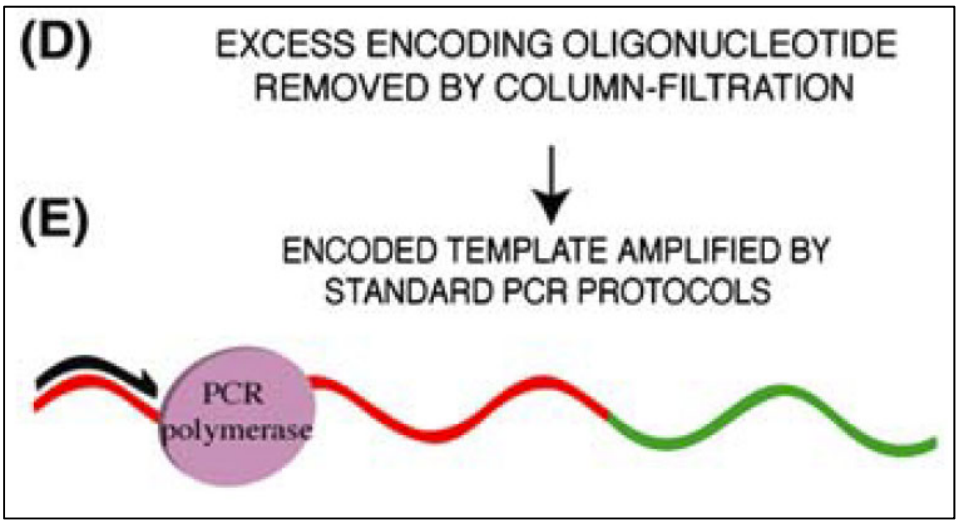


Ex. 1004 at Fig. 1.

101. Subsequent to annealing and extension, the resulting encoded DNA strands are column-purified in order to remove any encoding oligonucleotides that were not annealed (*see* step (D) in figure, below). The sample of encoded DNA strands are then amplified using standard PCR protocols and sequenced for evaluation (*see* step (E) in figure, below). McCloskey at 765, 766 (Ex. 1004.005-006).



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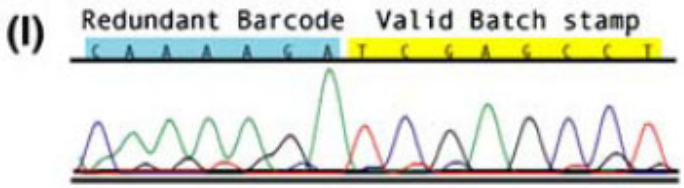
Ex. 1004 at Fig. 1.

102. McCloskey's method addresses the element of uncertainty in sequence information caused by the risks of amplification bias, contamination, and -redundant sequences. McCloskey at 762 (Ex. 1004.002). McCloskey teaches that a benefit of its method is that it distinguishes redundant sequences found in the end product of PCR amplification from redundant sequences existing in the sample before amplification. Researchers can readily identify the redundant sequences from the same PCR reaction since they have identical barcodes and batch-stamp as opposed to any encoded nucleotides with different barcodes which suggests that the redundancy existed in the sample prior to amplification (*see* step (I) in figure, below). *See* McCloskey at 766 (Ex. 1004.006). Thus, by counting sequences with non-redundant barcodes, McCloskey's method is useful in obtaining a quantitative measure of template diversity. *Id.*



Ex. 1004 at Fig. 1.

103. Another benefit that McCloskey's method teaches is the ability to identify valid sequences and distinguish them from contaminant sequences.

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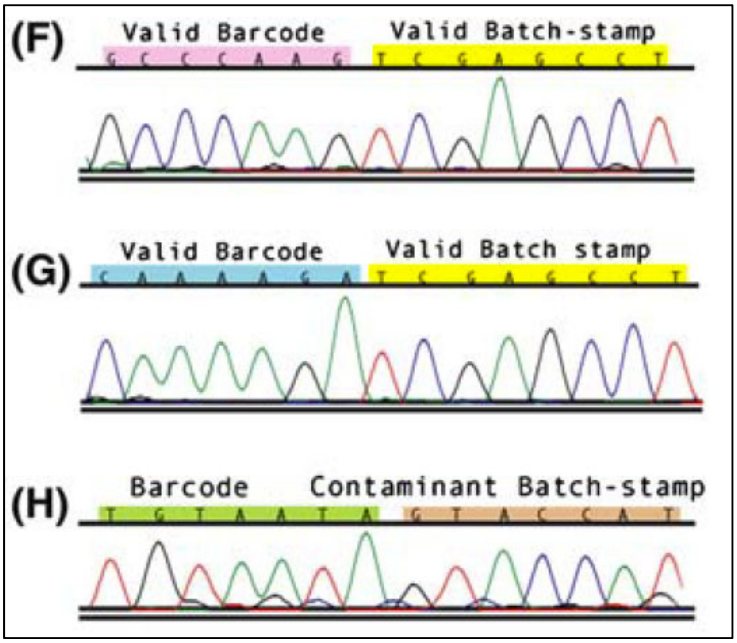
McCloskey at 766 (Ex. 1004.006). Valid sequences of one experiment are identified from the batch stamps specific to each sample and barcodes specific to each cell or allele copy, assigned to that experiment (*see* steps (F) and (G) in figure, below). Contaminant sequences, on the other hand, are identified from inappropriate batch stamps which were assigned to a different experiment (see step (H) in figure, below). McCloskey at 764, 766 (Ex. 1004.004, .006).



Ex. 1004 at Fig. 1.

104. Thus, McCloskey describes its method to increase reliability of PCR-based sequence information by applying molecular encoding principles to distinguish valid from data-corrupting sequences. McCloskey at 761 (Ex. 1004.001) ("Our approach thus enables classification of each PCR-derived sequence as valid, contaminant, or redundant, and provides a measure of sequence diversity."); *see also*

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id. at Figs. 1(F) – 1(I) (showing various barcode and batch-stamp sequences from one experiment), *id.* at .003. McCloskey also recommends the use of batch-stamps and barcodes "when amplifying irreplaceable DNAs and cDNAs for forensic, clinical, single cell, and ancient DNA analyses." McCloskey at 767 (Ex. 1004.007).

B. Linnarsson (Ex. 1003)

1. Linnarsson is § 102(e) Prior Art

105. International PCT patent application publication WO 2010/117620 A2 ("Linnarsson") entitled "Gene Expression Analysis In Single Cells," was published on October 14, 2010. Linnarsson (Ex. 1003). I understand that Linnarsson claims priority to U.S. Provisional Patent Application No. 61/164,759 ("Linnarsson Provisional"), filed on March 30, 2009. Linnarsson Provisional (Ex. 1015) at 1:3-5.

106. I have been asked to opine on whether the Linnarsson Provisional contains adequate written description support for Linnarsson's invention. It is my opinion that the disclosure of the Linnarsson Provisional provides sufficient written description to enable a POSA to practice Linnarsson's invention. Linnarsson and Linnarsson Provisional contain substantially similar specifications and claims, with Linnarsson simply providing additional duplicative exemplary embodiments beyond Linnarsson Provisional. The table below identifies where, for example, written description can be found in Linnarsson Provisional for each and every element of Linnarsson's claim 1:

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Linnarsson	Linnarsson Provisional Application Disclosure
1. A method of preparing a cDNA library from a plurality of single cells, the method comprising the steps of:	"In particular, the invention relates to a method for preparing a cDNA library from a plurality of single cells, and to a cDNA library produced by this method." Ex. 1015 at 1:4-6. "The present invention aims to overcome, or reduce, these problems by providing a method of preparing cDNA libraries which can be used to analyse gene expression in single cells." Ex. 1015 at 2:22-25. "The method of preparing a cDNA library described above may further comprise processing the cDNA library to obtain a library suitable for sequencing." Ex. 1015 at 8:33-35
(i) releasing mRNA from each single cell to provide a plurality of individual mRNA samples, wherein the mRNA in each individual mRNA sample is from a single cell;	"the method comprising: (i) releasing mRNA from each single cell to provide a plurality of individual samples, wherein the mRNA in each individual mRNA sample is from a single cell;" Ex. 1015 at 2:28-32. "mRNA may be released from the cells by lysing the cells. Lysis may be achieved by, for example, heating the cells, or by the use of detergents or other chemical methods, or by a combination of these. However, any suitable lysis method known in the art may be used." Ex. 1015 at 4:11-15. "As each cDNA sample obtained from a single cell is tagged, gene expression can be analysed at the level of a single cell." Ex. 1015 at 3:5-6.
(ii) synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a	"(ii) synthesising a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell;" Ex. 1015 at 2:33-37. "cDNA is typically synthesised from mRNA by reverse transcription. Methods for synthesising cDNA from small amounts of mRNA, including from single cells, have

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Linnarsson	Linnarsson Provisional Application Disclosure
tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell;	previously been described (Kurimoto, Yabuta et al. 2006; Kurimoto, Yabuta et al. 2007; Esumi, Wu et al. 2008)." Ex. 1015 at 5:4-8. "Thus, the synthesis of the first strand of the cDNA in step (ii) may be directed by a cDNA synthesis primer (CDS) that includes an RNA complementary sequence (RCS) that is at least partially complementary to some mRNA in an individual mRNA sample. This allows the primer, which is typically an oligonucleotide, to hybridise to at least some mRNA in an individual mRNA sample to direct cDNA synthesis using the mRNA as a template. The RCS may comprise oligo(dT), or be gene family-specific, or may be composed of a random sequence, such as random hexamers." Ex. 1015 at 5:24-32.
(iii) pooling the tagged cDNA samples; and	"pooling the tagged cDNA samples; and" Ex. 1015 at 3:1. "However, pooling the samples simplifies handling of the samples from each single cell and reduces the time required to analyse gene expression in the single cells, thus allowing high throughput analysis of gene expression. As the samples are pooled before amplification, technical variation between samples is virtually eliminated. In addition, as the cDNA samples are pooled before amplification, less amplification is required to generate sufficient amounts of cDNA for subsequent analysis compared to if cDNA samples from each single cell were amplified and treated separately. This reduces amplification bias, and also means that any bias will be similar across all the cells used to provide pooled cDNA samples." Ex. 1015 at 3:9-20.
(iv) amplifying the pooled cDNA samples to generate a cDNA library comprising	"amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA." Ex. 1015 at 3:2-3. "The tagged cDNA samples are pooled and amplified and then sequenced to produce 100 million reads. This allows

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Linnarsson	Linnarsson Provisional Application Disclosure
double-stranded cDNA,	identification of genes that are expressed in each single cell." Ex. 1015 at 11:14-17. "Full length cDNA amplification. The cDNA was amplified by PCR by adding the reagents shown in Table 3." Ex. 1015 at 14:5-7.

107. Thus, Linnarsson Provisional provides written description support for the invention claimed in Linnarsson. Accordingly, I understand from counsel that Linnarsson is prior art as of Linnarsson Provisional's filing date of August 20, 2009.

2. Linnarsson Overview

108. Linnarsson teaches a method of analyzing gene expression in a plurality of single cells by preparing a tagged cDNA library for sequencing. EX1003 at 1:6-9. The cDNA samples originating from a plurality of cells are appended with unique oligonucleotide sequences, or "tags" which are useful in tracking polynucleotides arising from different single cells in a multi-cell experiment. *Id.* at 37:28-29; 19:9-10 (disclosing that the tag acts as a barcode to "identify which single cell from each cDNA sample originated.").

109. As an initial matter, Linnarsson teaches a method for synthesizing tagged cDNA from mRNA by the process of reverse transcription. With the use of a tagged cDNA synthesis primer ("CDS") and/or a tagged template switching oligonucleotide ("TSO"), a tag is incorporated into the resulting cDNA. Ex. 1003 at

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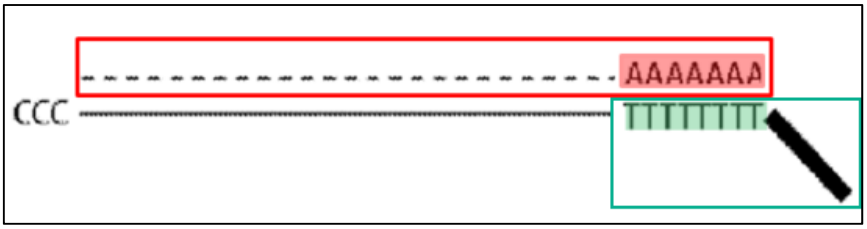
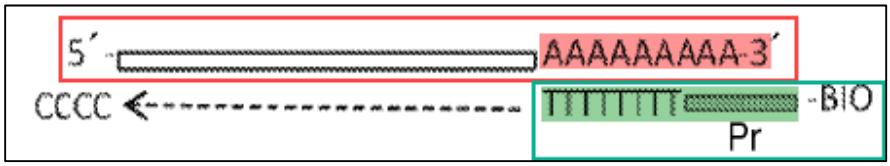
18:31-19:1 ("In some aspects of the invention, a tag can be incorporated into the cDNA during its synthesis. For example, the CDS and/or TSO can include a tag, such as a particular nucleotide sequence"). Further, Linnarsson teaches performing PCR on the tagged cDNA and subsequently analyzing and sequencing the tagged cDNA. Ex. 1003 at 3:29-4:2, 19:16-17, 21:13-20. I will discuss the CDS and TSO in turn.

110. First, Linnarsson's cDNA synthesis primer ("CDS") (boxed in green, below) includes a sequence of thymines (highlighted in green, below) that "directs synthesis of a cDNA strand" by hybridizing with the poly(A) tail (highlighted in red, below), which is a sequence of adenine nucleotides, of an mRNA strand (boxed in red, below)



Ex. 1003 at Fig. 2.



Ex. 1003 at Fig. 11; Ex. 1003 at 5:29-31, 24:13-15.

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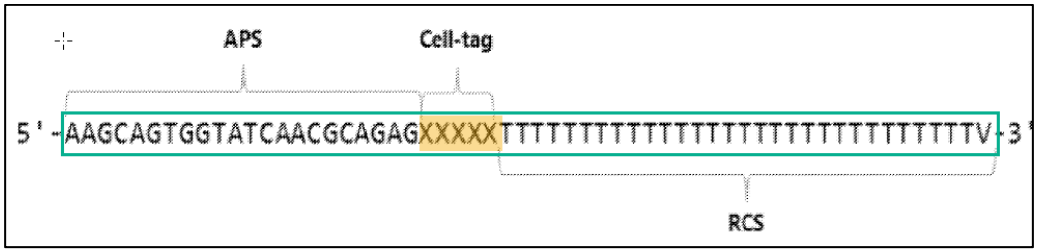
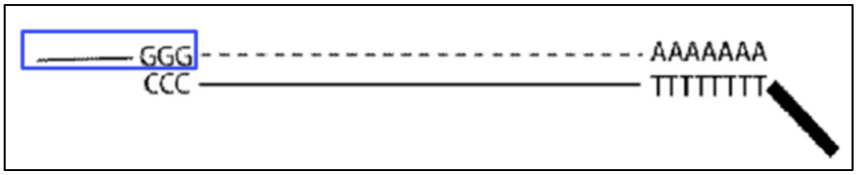
111. The enzyme responsible for synthesizing this strand of cDNA is called a reverse transcriptase, which also naturally causes a series of cytosines to be incorporated at the 3' end of the first strand of cDNA synthesized from mRNA. Ex. 1003 at 16:22-26. Thus, the resulting cDNA molecule also includes at its end a series of cytosine nucleotides. *Id*.

112. Linnarsson discloses that the CDS cell-tag "can be incorporated into the cDNA" and can "be used to identify which single cell from each cDNA sample originated." Ex. 1003 at 18:31, 19:9-10. Figure 4 of Linnarsson depicts an example of a CDS (boxed in green, below) with a cell-tag (highlighted in yellow, below):



Ex. 1003 at Fig. 4.

113. Second, Linnarsson's template switching oligonucleotide ("TSO") (boxed in blue, below) comprises the tag "XXXXX" (highlighted in blue) and "anneals transiently" to the series of cytosine nucleotides at the end of the newly synthesized strand "and cDNA synthesis proceeds using the oligo as a template":



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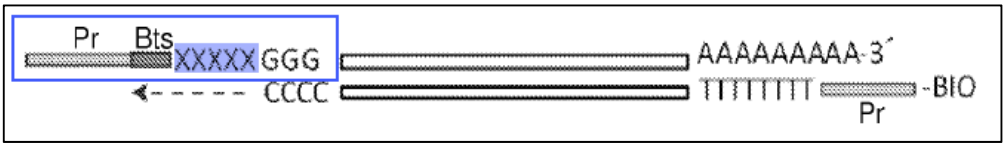
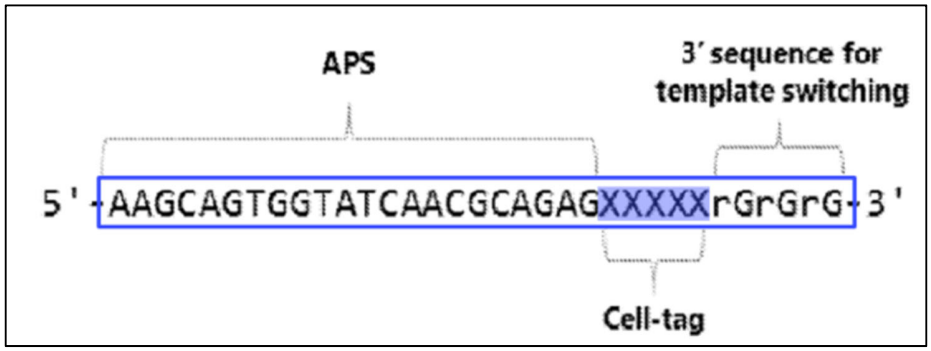
Ex. 1003 at Fig. 2.



Ex. 1003 at Fig. 11; Ex. 1003 at 5:31-33, 24:15-20 (explaining Figure 11).

114. Linnarsson discloses that the TSO cell-tag "can be incorporated into the cDNA" and can "be used to identify which single cell from each cDNA sample originated." Ex. 1003 at 18:31, 19:9-10. Figure 3 depicts an example of a TSO (boxed in blue, below) with a cell-tag (highlighted in blue, below):



Ex. 1003 at Fig. 3.

115. Linnarsson discloses that the tagged CDS and tagged TSO can be used in alone or combination such that each tagged cDNA generated by this method "can have a distinct tag, or a distinct combination of tags," which can be used to "identify which single cell from each cDNA sample originated." Ex. 1003 at 19:7-10; *id*. at 18:31-32 (the "CDS and/or the TSO can include a tag."); *id.* at 19:4-5 ("As the tag

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is present in the CDS and/or TSO it will be incorporated into the cDNA during its synthesis and can therefore act as a "barcode" to identify the cDNA.").

116. Moreover, Linnarsson discloses the processes for amplifying polynucleotides including "PCR [and] ligation amplification (or ligase chain reaction, LCR)," and further explains that these methods are "known and widely practiced in the art." Linnarsson (Ex. 1003 at 12:18-20). Linnarsson also teaches that after generating the tagged cDNA, "sequencing is performed" thereon. Ex. 1003 at 4:8-13, 19:28-32, 21:13-25 ("This cDNA library can be sequenced to provide an analysis of gene expression in single cells or in a plurality of single cells.").

117. Linnarsson further discloses a template switching reverse transcription step where the sample mRNA is denatured from the first synthesized cDNA strand and a new complementary strand of cDNA is formed along the synthesized cDNA strand, which generates a double-stranded cDNA molecule. As a result, one strand of the double-stranded cDNA molecule (boxed in orange, below) has the same sequence as the original mRNA strand (boxed in red, below), including the same sequence of adenines (highlighted in orange, below) that made up the poly(A) tail (highlighted in red, below) of the mRNA:

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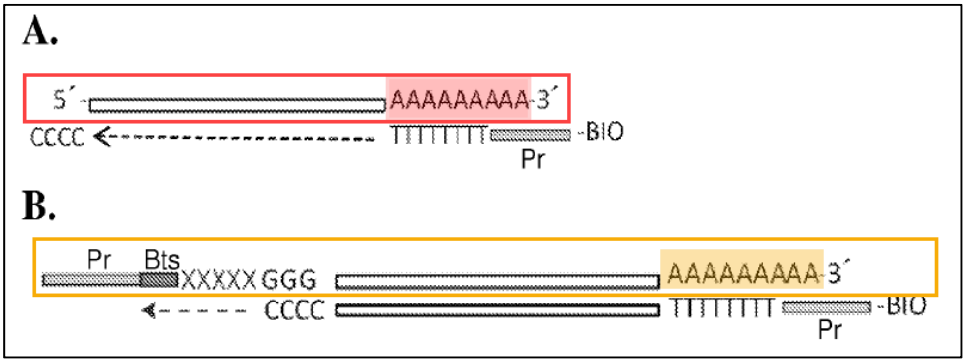
Ex. 1003 at Fig. 2. Figure 11 of Linnarsson discloses the same:



Ex. 1003 at Fig. 11; Ex. 1003 at 6:1-3 (explaining Figure 11).

XII. PRIOR ART ANTICIPATED OR WOULD HAVE RENDERED OBVIOUS THE CLAIMS OF THE '981 PATENT

A. Ground 1: Claims 1-6 Are Unpatentable as Obvious over Linnarsson In View Of McCloskey

1. Claim 1:

a) [1A] A method of analyzing nucleic acids from a plurality of single cells, the method comprising:

118. To the extent the preamble is deemed limiting, it is my opinion that Linnarsson discloses a method of analyzing nucleic acids from a plurality of single cells.

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119. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

120. Linnarsson discloses a method of analyzing nucleic acids from a plurality of single cells. The Linnarsson abstract states "[t]he present invention provides methods and compositions for the analysis of gene expression in single cells or in a plurality of single cells." Linnarsson (Ex. 1003) at Abstract, Linnarsson Provisional (Ex. 1015) at 1:3-8. Linnarsson repeats this disclosure:

> "The present invention provides methods and compositions for the analysis of gene expression in single cells or in a plurality of single cells."

Linnarsson (Ex. 1003) at 9:22-25; Linnarsson Provisional (Ex. 1015) at 1:3-8.

> "Embodiments of the invention provide a method of preparing a cDNA library from a plurality of single cells"

Linnarsson (Ex. 1003) at 10:15-22; Linnarsson Provisional (Ex. 1015) at 2:27-3:3, 9:8-10.

> "An embodiment of the method of the invention is summarized in **Figure 1**. Cells are obtained from a tissue of interest and a single-cell suspension is obtained. A single cell is placed in one well of a 96-wcll plate in Cell Capture Mix. The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA. The tagged cDNA samples

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> are pooled and amplified and then sequenced to produce 100 million reads. This allows identification of genes that are expressed in each single cell."

Linnarsson (Ex. 1003) at 22:22-28 (emphasis in original); Linnarsson Provisional (Ex. 1015) at 11:8-17. As discussed above in Section X.A., gene expression analysis includes analyzing nucleic acids.

121. Moreover, Linnarsson discloses a method for analyzing nucleic acids comprising the following steps: "(A) the tissue of interest is dissected; (B) ***a plurality of single cells are selected***; (C) single cells are placed in separate wells of a 96-well plate and lysed; tagged reverse transcription is performed on each sample to produce cDNA; (D) cDNA samples are pooled and amplified; (E) ***sequencing is performed*** to obtain 100 million reads; and (F) ***identification of expressed genes and identification of cells from which they originated***." Linnarsson (Ex. 1003) at 4:8-13[3], Linnarsson Provisional (Ex. 1015) at 9:35-10:6. This method is illustrated in Figure 1 of Linnarsson:

[3] All emphasis is added unless otherwise stated.

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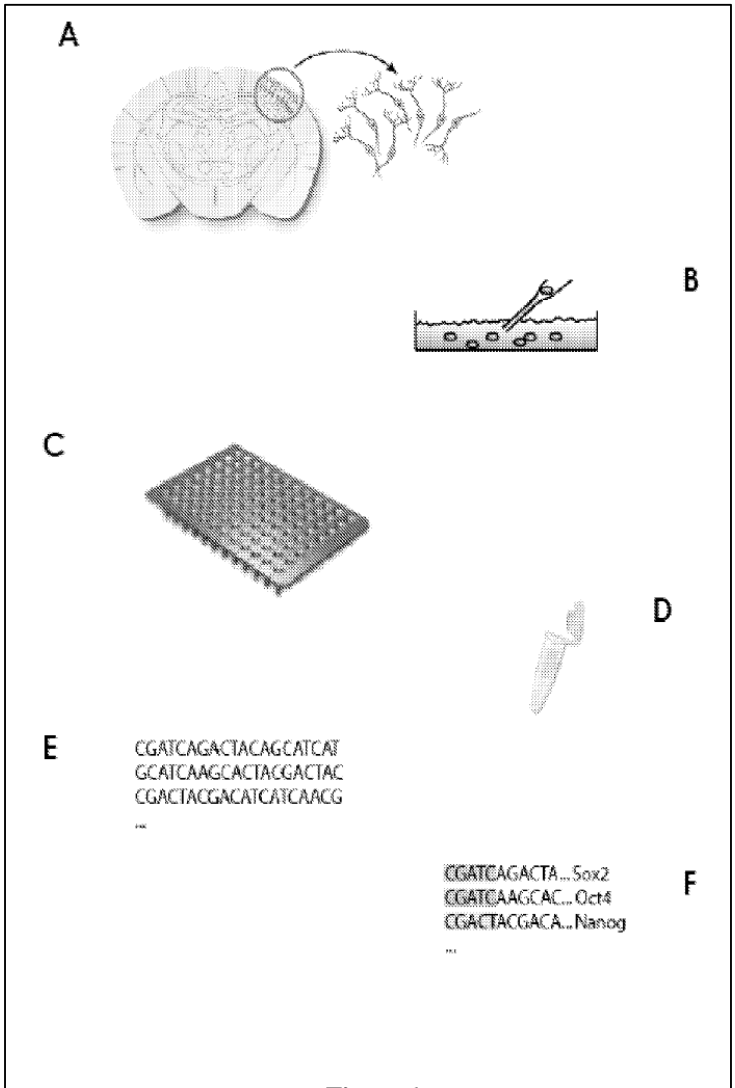

Figure 1

Ex. 1003 at Fig. 1, *see also* Ex. 1015 at Fig. 1.

122. Therefore, because Linnarsson discloses a method for analyzing gene expression in a plurality of single cells, and because a POSA would have understood that analyzing gene expression requires analyzing nucleic acids, it is my opinion that Linnarsson discloses "a method of analyzing nucleic acids from a plurality of single cells," as recited by claim 1 of the '981 patent.

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b) [1B] providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides;

123. It is my opinion that Linnarsson discloses "providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides."

124. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

125. First, Linnarsson discloses the limitation "providing a sample comprising a plurality of single cells." As discussed above in Section VIII, the '981 patent defines a "sample" to mean "a quantity of material from a biological, environmental, medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought." Ex. 1001 at 12:7-10. The '981 patent's definition of "sample" is consistent with a POSA's understanding of this term, and I have applied this definition in my analysis.

126. As I explained above in Section XII.A.1.a., Linnarsson discloses a method for analyzing nucleic acids from a plurality of single cells. Linnarsson further explains that the analysis is performed on "[c]ells [] obtained from a tissue of interest." Linnarsson (Ex. 1003) at 22:22-23; Linnarsson Provisional (Ex. 1015) at 11:9. Such "tissue of interest" constitutes a recited "sample" because it is "a

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quantity of material from a biological . . . source." Ex. 1001 at 12:7-10. Linnarsson also discloses that the plurality of single cells may be obtained from other biological sources, including "from a biopsy, blood sample, or cell culture." Linnarsson (Ex. 1003) at 13:11-12, Linnarsson Provisional (Ex. 1015) at 3:31-34; *see* Ex. 1001 at 12:18-21 (defining "sample" to include "cultures, blood . . . and the like").

127. Linnarsson further discloses that the sample comprises a plurality of single cells. For example, Linnarsson discloses a method for obtaining a plurality of single cells from a tissue (i.e., a biological source), comprising the steps of "(A) the tissue of interest is dissected; (B) ***a plurality of single cells*** are selected." Linnarsson (Ex. 1003) at 4:8-10; Linnarsson Provisional (Ex. 1015) at 9:35-37. A POSA would have understood that step B requires selecting a plurality of single cells ***from the dissected tissue of interest***. Therefore, a POSA would have understood that the tissue of interest comprises a plurality of single cells.

128. As I will explain in Section XII.A.1.c. below, Linnarsson discloses labeling the nucleic acids from the plurality of single-cells. Also, as I will explain in Section XII.A.1.g. below, Linnarsson discloses sequencing the nucleic acids from the plurality of single-cells. Therefore, the sources of single-cells disclosed in Linnarsson—including tissue, biopsy, blood sample, or cell cultures—are "a quantity of material from a biological . . . source in which detection, measurement, or labeling of target nucleic acids is sought," and meets the definition of a "sample."

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129. Second, Linnarsson teaches "wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides," as recited by claim limitation 1B. As shown below, Linnarsson discloses lysing a plurality of sample single cells, thereby releasing polynucleotides—including mRNA—within each single cell.

> "In some aspects of the invention, mRNA can be released from the cells by lysing the cells. Lysis can be achieved by, for example, heating the cells, or by the use of detergents or other chemical methods, or by a combination of these."

Linnarsson (Ex. 1003) at 13:28-30; Linnarsson Provisional (Ex. 1015) at 9:37-10:3.

> "The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA."

Linnarsson (Ex. 1003) at 22:24-26; Linnarsson Provisional (Ex. 1015) at 11:11-14 ; Section X.A., *supra* (explaining that mRNA is a type of polynucleotide).

130. Linnarsson further teaches that each single cell contains a plurality of mRNA molecules. *See, e.g.*, Linnarsson (Ex. 1003) at 3:26-30 ("In one aspect, the method includes the steps of releasing mRNA from each single cell to provide a plurality of individual mRNA samples …."); Linnarsson Provisional (Ex. 1015) at 2:27-3:3.

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131. For the reasons explained above, it is my opinion that Linnarsson discloses this limitation.

132. It is my opinion that McCloskey also discloses providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides.

133. McCloskey discloses providing a sample comprising a plurality of single cells. For example McCloskey recommends "that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for … single cell … DNA analyses." McCloskey at 767 (Ex. 1004.007). Thus, a POSA would understand that McCloskey provides a method recommended to be used on a plurality of single cells.

134. McCloskey further discloses that each single cell of the plurality of single cells comprises a plurality of sample polynucleotides. McCloskey teaches generating tagged polynucleotides (*e.g.*, tagged cDNA) from sample polynucleotides. For example, McCloskey discloses "a method by which molecular codes are added to single-stranded DNA or cDNA templates …." McCloskey at Abstract, 762 (Ex. 1004 at Abstract, 002); McCloskey at 767 (Ex. 1004.007) ("We recommend that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for … single cell … DNA analyses."). As discussed above in Section X.B., a POSA would understand that cDNA refers to DNA molecules that are formed by the reverse transcription of mRNA molecules. Therefore, a person of

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skill in the art would have understood that McCloskey discloses a method of analyses of samples of "single cells" comprising a plurality of sample polynucleotides (mRNA or DNA).

c) [1C] generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides,

135. It is my opinion that Linnarsson discloses "generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides."

136. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution. Further, as discussed above in Section VI., Patent Owner previously challenged claims directed to a method for attaching a tag to a DNA sample was "well-known and conventional before 2009." Ex. 1016.010.

137. As described above, Linnarsson discloses lysing a plurality of sample single cells to release a plurality of polynucleotides—including mRNA molecules—contained within. Sections X.A, XII.A.1.b, *supra*. Linnarsson further discloses performing reverse transcription on the released plurality of mRNA molecules to generate a plurality of cDNA, and tagging the cDNAs during the process. Linnarsson (Ex. 1003) at 22:24-26; Linnarsson Provisional (Ex. 1015) at 11:11-14. ("The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from

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cellular mRNA and incorporation of a tag into the cDNA."); Section X.B., *supra*. Thus, Linnarsson discloses a method of generating a plurality of tagged polynucleotides ***from the sample polynucleotides***, such as sample mRNA.

138. Linnarsson specifically teaches that during cDNA synthesis, tagged oligonucleotides referred to as cDNA synthesis primer (CDS) and/or template switching oligonucleotide (TSO) be used to incorporate a cell-tag into the cDNA. Linnarsson (Ex. 1003) at 19:4-6 ("As the tag is present in the CDS and/or the TSO it will be incorporated into the cDNA during its synthesis and can therefore act as a 'barcode' to identify the cDNA."); Linnarsson Provisional (Ex. 1015) at 7:23-26. The tagged cDNA is the claimed "plurality of tagged polynucleotides." The CDS and TSO are also referred to in Linnarsson as tailed oligo-dT primer and barcoded helper oligo respectively, which a POSA would have understood to refer to the same tagged oligonucleotides.

139. Specifically, Linnarsson first teaches using a tagged CDS during cDNA synthesis to generate tagged cDNA. For example, Figures 2 and 11, shown below, depict an mRNA strand (boxed in red, below) with a poly-A tail (highlighted in red, below), and a CDS or "tailed oligo-dT primer" (boxed in green, below) that "directs synthesis of a cDNA strand":

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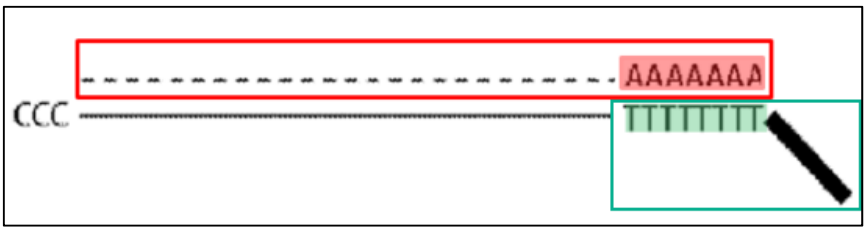
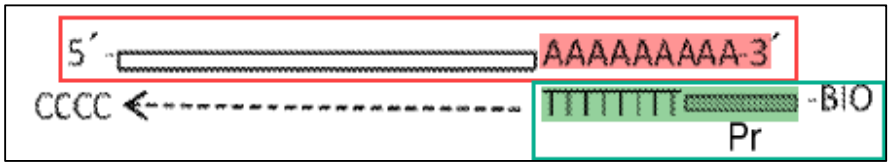


Ex. 1003 at Fig. 2; *see also* Ex. 1015 at Fig. 2.



Ex. 1003 at Fig. 11 ; *see also* Ex. 1015 at Fig. 2; Ex. 1003 at 5:29-31 (explaining the disclosure of Figure 11); Ex. 1015 at 5:24-32, 13:8-14 (same).

140. As demonstrated in Figures 2 and 11 above, the CDS or tailed oligo-dT primer includes a sequence of thymines (highlighted in green, above) that hybridize with the sequence of adenines making up the poly-A tail of the mRNA molecule. This process is further described in Linnarsson's Figure description: "a reverse transcription step. A tailed oligo-dT primer directs synthesis of a cDNA strand. When the end of the template RNA is reached, reverse transcriptase adds 3-4 Cs at 3' of cDNA strand (due to its terminal transferase activity)." Linnarsson (Ex. 1003) at 5:29-31; Linnarsson Provisional (Ex. 1015) at 5:24-32, 13:8-14.

141. For example, Figure 4 depicts an example of a CDS (boxed in green, below) with a cell-tag (highlighted in green, below):

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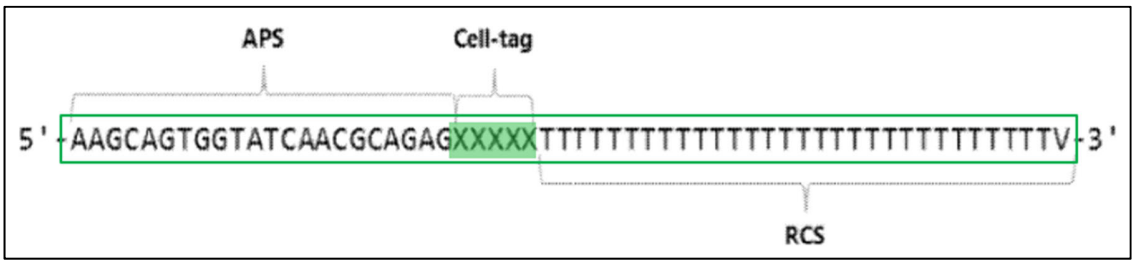

Ex. 1003 at Fig. 4; *see also* Ex. 1015 at Fig.4.

142. Linnarsson discloses that, as part of "synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample . . . incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA …." Linnarsson (Ex. 1003) at 10:18-20, Linnarsson Provisional (Ex. 1015) at 2:33-36; *see also* Linnarsson (Ex. 1003) at 3:25-30 ("The invention provides method for preparing a cDNA library from a plurality of single cells. In one aspect, the method includes the steps of releasing mRNA from each single cell to provide a plurality of individual mRNA samples, synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample and **incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples**. . ."); Linnarsson Provisional (Ex. 1015) at 2:27-3:3.

143. Linnarsson also teaches using a TSO or "[b]arcoded help oligo" (boxed in blue, below), which comprises the tag "XXXXX" (highlighted in blue) and "anneals transiently, and cDNA synthesis proceeds using the oligo as template."

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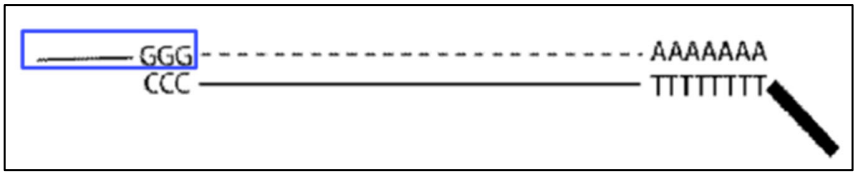
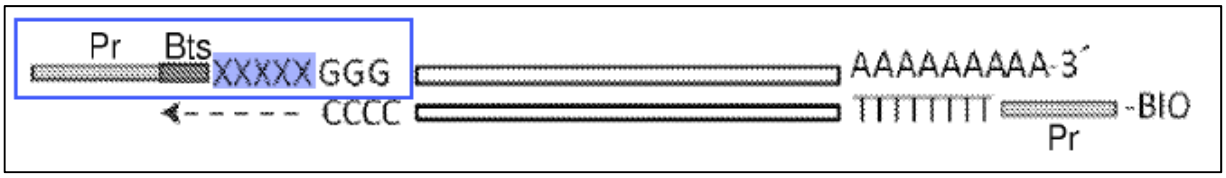
Linnarsson (Ex. 1003) at 5:31-33; Linnarsson Provisional (Ex. 1003) at 13:14-25; *see also* Linnarsson (Ex. 1003) at 16:10-17 (discussing another method of forming the second strand of cDNA); Linnarsson Provisional (Ex. 1015) at 6:1-11. The TSO (boxed in blue) is shown in Figures 2 and 11, reproduced below:

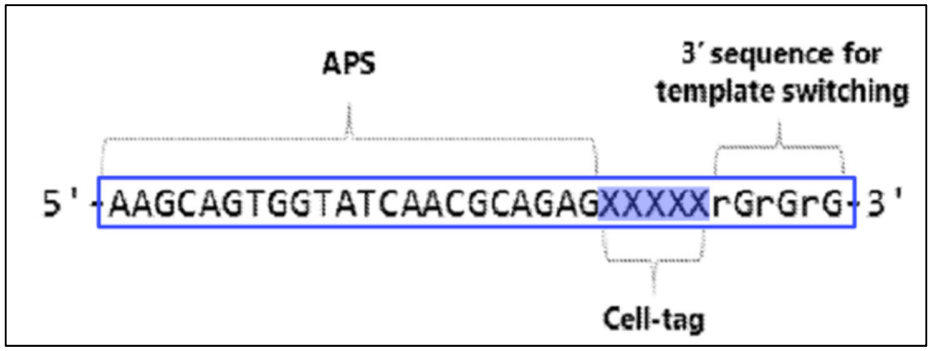


Ex. 1003 at Fig. 2; *see also* Ex. 1015 at Fig. 2.



Ex. 1003 at Fig. 11; Ex. 1015 at Figs. 2, 3.

144. Figure 3 depicts an example of a TSO (boxed in blue, below) with a cell-tag (highlighted in blue, below):



Ex. 1003 at Fig. 3; Ex. 1015 at Fig. 3.

145. Linnarsson also teaches that "[b]oth the CDS and the TSO can include a tag. The CDS and the TSO can each include a different tag, such that the tagged

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cDNA sample comprises a combination of tags." Linnarsson (Ex. 1003) at 19:6-7; Linnarsson Provisional (Ex. 1015) at 7:26-28.

146. After reverse transcription, the resulting tagged cDNA are then amplified to generate copies of the tagged double-stranded cDNA, and these copies of the tagged cDNA are also the claimed plurality of tagged polynucleotides. Linnarsson (Ex. 1003) at 10:20-22 ("… amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA."); Linnarsson Provisional (Ex. 1015) at 3:2-3.

147. For the reasons explained above, it is my opinion that Linnarsson discloses this limitation.

d) [1D] wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and

148. It is my opinion that Linnarsson discloses the limitation "wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides."

149. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

150. As discussed above, Linnarsson discloses that each single cell comprises a plurality of polynucleotides, such as mRNA molecules. *See* Section

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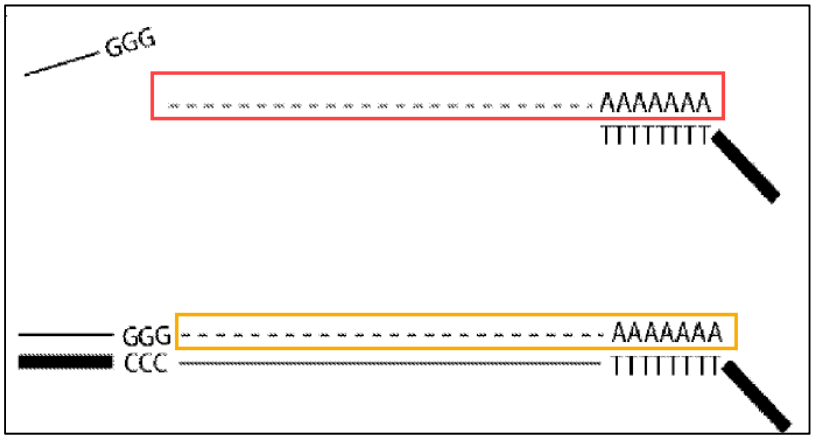
XII.A.1.b. *supra*. A POSA would have understood that the mRNA disclosed in Linnarsson comprise a plurality of nucleotide sequences.

151. It is my opinion that Linnarsson discloses this limitation because it discloses that the tagged cDNA comprises a sequence identical to the sample mRNA sequence. As discussed above in Section XII.A.1.c., Linnarsson teaches generating a plurality of tagged polynucleotides (*i.e.*, tagged double-stranded cDNA and/or amplified copies) from the plurality of sample polynucleotides (*i.e.*, sample mRNA). As shown in Figure 2, Linnarsson discloses that the resulting double-stranded cDNA comprises one strand (boxed in orange, below) which is identical to the original sample mRNA (boxed in red, below):



Ex. 1003 at Fig. 2.

152. The cDNA is synthesized from the sample mRNA using reverse transcription. Thus, a POSA would have understood that one strand of the resulting double-stranded cDNA would be identical to the sequence of the sample mRNA

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because a cDNA synthesized from a sample mRNA comprises the same sample sequence. As discussed above in Section X.A., the sample mRNA comprises the base pair uracil while the cDNA comprises the base pair thymine. Any POSA would have understood that this difference is trivial because uracil and thymine are functionally equivalent. The presence of thymine in the cDNA indicates the presence of uracil in the mRNA, thus preserving the sequence information. Despite the base pair difference, these sequences convey the same genetic information because they code for the same set of proteins such that they are functionally equivalent. Indeed, claim 4 of the '981 patent requires the sample polynucleotide to be mRNA, and a POSA would have understood that the recited "tagged polynucleotide" is thus a cDNA synthesized from the sample mRNA. Ex. 1001 at Claim 4. Thus, it is my opinion that the tagged polynucleotides cDNA comprises a sequence from a sample polynucleotide.

153. Alternatively, it is my opinion that Linnarsson discloses that the tagged cDNA comprises a poly(A) tail sequence, which is a sequence from the sample mRNA. Specifically, Linnarsson discloses that the sample mRNA comprises a poly(A) tail. As explained above in Section X.A., one of the nucleotide sequences making up a mRNA molecule is a poly(A) tail, which is a sequence of adenine nucleotides found at the 3' end of a mRNA molecule. The process of polyadenylation, by which the poly(A) tail is added is naturally occurring and helps

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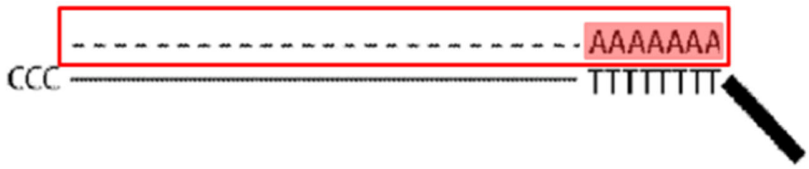
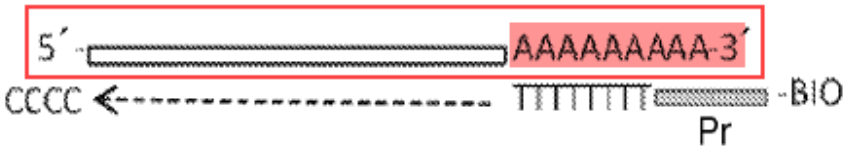
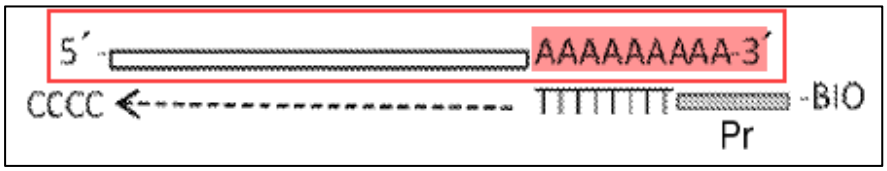
facilitate RNA processing and stability. Linnarsson's Figures 2 and 11, depicted below, show an mRNA molecule with a sequence from the sample polynucleotide (boxed in red) including its poly-A tail (highlighted red):



Ex. 1003 at Fig. 2 Ex. 1015 at Fig. 2.



Ex. 1003 at 4:14 (describing Fig. 2), 24:13-20 (same); Ex. 1015 at 10:8, 13:8-18.



Ex. 1003 at Fig. 11; Ex. 1015 at Fig. 2.

154. As can be seen below in Figures 2 and 11, after reverse transcription, one strand of the double-stranded cDNA molecule (boxed in orange, below) will have the same sequence as the original mRNA strand (boxed in red, below), including the same sequence of adenines (highlighted in orange, below) that made up the mRNA's poly-A tail (highlighted in red, below).

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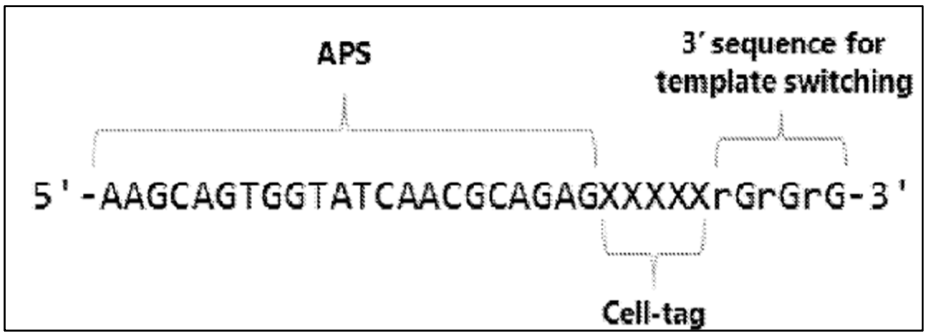
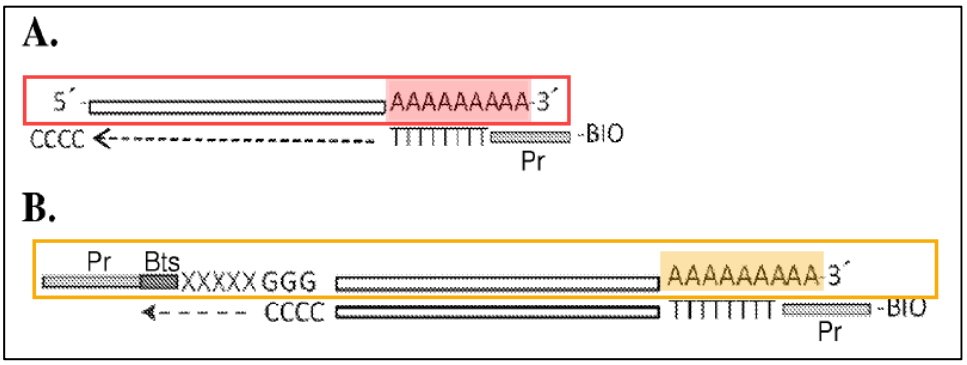
Ex. 1003 at Fig. 2



Ex. 1015 at Fig. 2



Ex. 1003 at Fig. 11 Ex. 1015 at Figs. 2, 3.

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155. Therefore, Linnarsson discloses the limitation "a sequence from a sample polynucleotide of the plurality of sample polynucleotides" because it discloses that the tagged cDNA comprises a sequence identical to the mRNA, or alternatively because it discloses that the tagged cDNA comprises a poly(A) tail sequence from the mRNA.

e) [1E] wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells; and

156. It is my opinion that Linnarsson and McCloskey both disclose a method comprising the step of generating a plurality of tagged polynucleotides, "wherein each tagged polynucleotide comprises a multiplex identifier (MID) sequence comprising a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells."

157. First, it is my opinion that Linnarsson discloses that each tagged polynucleotide comprises a multiplex identifier ("MID").

158. A multiplex identifier is a short polynucleotide sequence barcode that is used as a tag or marker to identify and track polynucleotide samples that are pooled together and sequenced in the same reaction. The ability to combine samples and

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sequence them together increases the efficiency and reduces the cost of processing samples. The ’981 patent provides the following definition for Multiplex Identifier (MID):

> “Multiplex Identifier” (MID) as used herein refers to a tag or combination of tags associated with a polynucleotide whose identity (e.g., the tag DNA sequence) can be used to differentiate polynucleotides in a sample. In certain embodiments, the MID on a polynucleotide is used to identify the source from which the polynucleotide is derived.

Ex. 1001 at 6:33-37; *see also id.* at 6:38-7:22 (further definition an MID).

159. It is my opinion that the definition provided in the ’981 patent is consistent with the understanding of a person of skill in the art. I have applied this definition to my analysis here.

160. I also note that the ’981 patent admits that the use of MIDs for identifying polynucleotides was known in the prior art. *See* Ex. 1001 at 6:56-64 (incorporating by reference prior art patents “for their description of nucleic acid tags and their use in identifying polynucleotides”).

161. Consistent with the ’981 patent’s definition of MID, Linnarsson discloses a tag associated with a polynucleotide, where the tag can be used to differentiate polynucleotides in a sample. For example, Linnarsson discloses “[i]n some aspects of the invention, a tag can be incorporated into the cDNA during its

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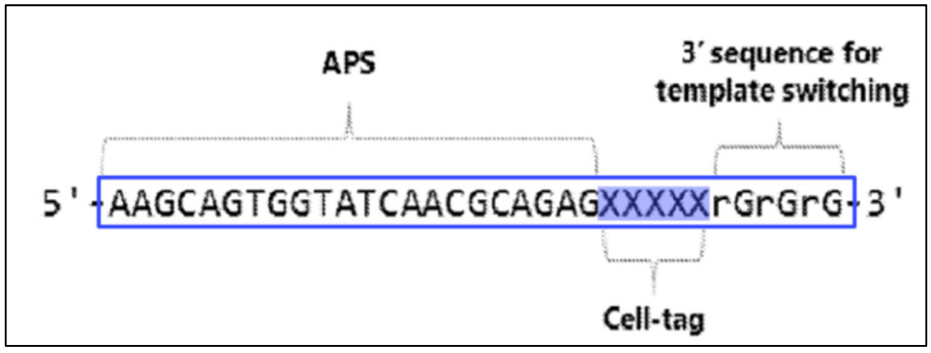
synthesis. . . . As the tag is present in the [cDNA synthesis primer] and/or the [template switching oligonucleotide] it will be incorporated into the cDNA during its synthesis and can therefore act as a 'barcode' to identify the cDNA." Linnarsson (Ex. 1003) at 18:31-19:5; Linnarsson Provisional (Ex. 1015) at 7:17-26.

162. Further, Linnarsson provides that "[e]mbodiments of the invention provide a method of preparing a cDNA library from a plurality of single cells by . . . incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples . . . By utilizing the above method, it is feasible to prepare samples for sequencing from several hundred single cells in a short time and with a minimal amount of work." Linnarsson (Ex. 1003) at 10:15-24; Linnarsson Provisional (Ex. 1015) at 10:10-16.

163. As depicted below in Figure 3, Linnarsson discloses a MID, sequence comprising a first tag sequence. Linnarsson's Figure 3, as shown below, depicts using a TSO comprising a "cell tag" (highlighted in blue below), an amplification primer sequence ("APS"), and a sequence for template switching



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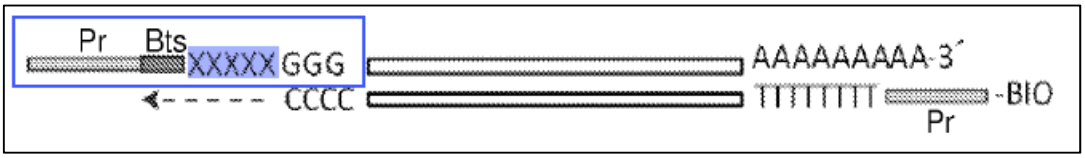
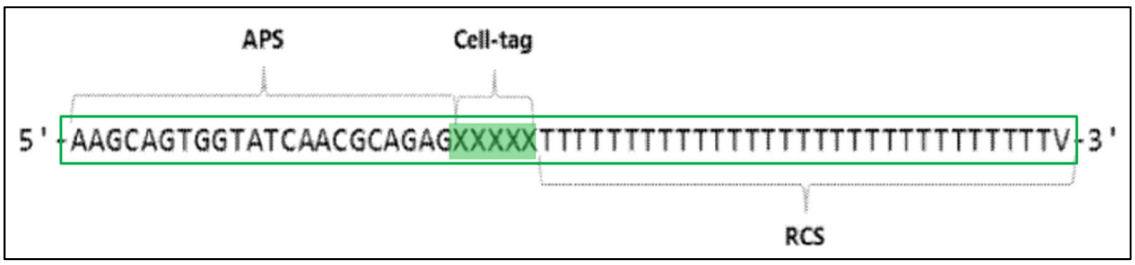
Ex. 1003 at Fig. 3, 4:15-18; *see also* Ex. 1015 at Fig. 3, 2:27-3:3.

164. As shown below in Figure 11, the TSO is added to the cDNA during synthesis:



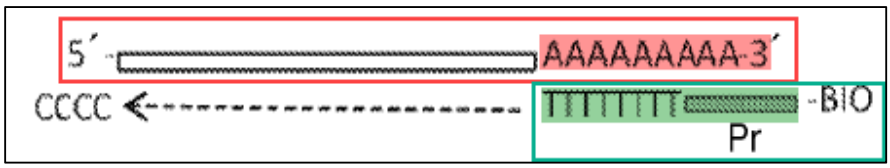
Ex. 1003 at Fig. 11; *see also* Ex. 1015 at Fig. 2, 3, 10:10-13.

165. Alternatively, Linnarsson discloses a CDS as the MID sequence comprising a first tag. Figure 4, as shown below, depicts a CDS comprising a "cell tag" (highlighted in green below), an amplification primer sequence ("APS"), and a RNA complementary sequence ("RCS"):



Ex. 1003 at Fig. 4, 4:17-18; *see also* Ex. 1015 at Fig. 4, 10:14-16.

166. As shown in Figure 11, the cell-tag in the CDS is incorporated into the cDNA during reverse transcription:



Ex. 1003 at Fig. 11, *see also* Ex. 1015 at Fig. 2, 4, 10:14-16.

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167. Thus, it is my opinion that Linnarsson discloses that each tagged polynucleotide comprises a MID, which can be either the TSO or CDS or a combination of both..

168. Second, it is my opinion that Linnarsson discloses that "the MID sequence comprises a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells."

169. Linnarsson discloses that "tagged reverse transcription is performed on each sample to produce cDNA," which allows for "identification of expressed genes and ***identification of cells from which they originated***." Linnarsson (Ex. 1003) at 4:8-13; Linnarsson Provisional (Ex. 1015) at 9:35-10:6. Similarly, Linnarsson explains that the tags present in the CDS and/or the TSO are incorporated into the cDNA to provide a tag or combination of tags, which "can be used to identify which single cell from each cDNA sample originated." Linnarsson (Ex. 1003) at 19:5-11 ("Both the CDS and the TSO can include a tag. The CDS and the TSO can each include a different tag, such that the tagged cDNA sample comprises a combination of tags. ***Each cDNA sample generated by the above method can have a distinct tag, or a distinct combination of tags, such that once the tagged cDNA samples have been pooled, the tag can be used to identify which single cell from each cDNA sample originated.*** Thus, each cDNA sample can be linked to a single cell, even

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after the tagged cDNA samples have been pooled in step (iii) of the above method)."); Linnarsson Provisional (Ex. 1015) at 7:26-34.

170. A POSA would have understood that in order to use MID tags to "identify which single cell from each cDNA sample originated," each cDNA sample must have a tag that is different from those attached to cDNA sample originated from a different single-cell. In other words, each cDNA sample must have "a different [tag] sequence for different single cells in the plurality of single cells," as required by limitation [1E].

171. It is my opinion that McCloskey also discloses a method comprising the step of generating a plurality of tagged polynucleotides, "wherein each tagged polynucleotide comprises a multiplex identifier (MID) sequence comprising a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells."

172. McCloskey teaches the use of an "encoding oligonucleotide" that "specifies the DNA source." McCloskey at 762 (Ex. 1004.002). McCloskey's "encoding oligonucleotide" is depicted in Figure 1, reproduced below:

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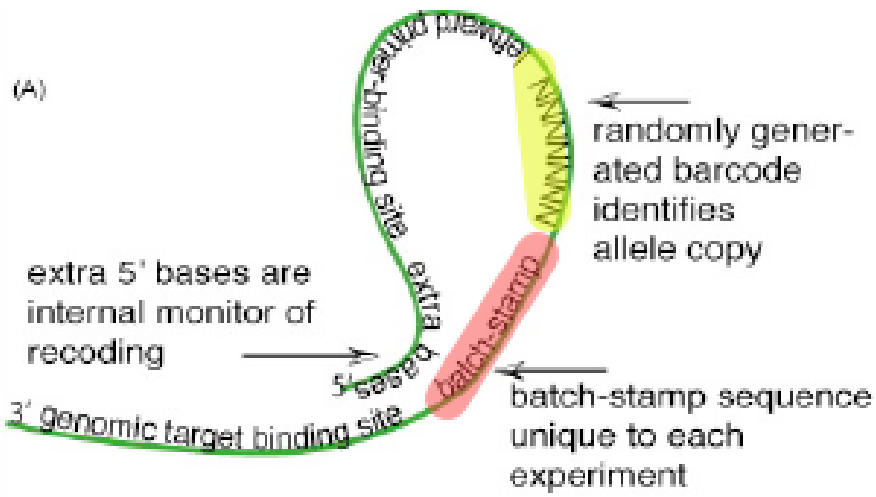

Ex. 1004 at Fig. 1.

173. As described in McCloskey and depicted above, the encoding oligonucleotide comprises two tag sequences: (1) a "batch-stamp" sequence (highlighted in red, above) to identify the experimental batch or genomic source (*e.g.*, a single cell) and (2) a "barcode" (highlighted in yellow, above) to distinguish polynucleotide sequences "arising from different cell or allele copies." McCloskey at 762 (Ex. 1004.002) ("Our method utilizes an encoding oligonucleotide with five distinct informational regions: (1) ***the batch-stamp, unique to each experiment, specifies the DNA source such as the patient or sample identification***, and the date of reaction; (2) ***the random barcode distinguishes among sequences arising from different cell or allele copies***; (3) the primer-binding site facilitates specific PCR amplification; (4) the genomic target-binding site targets the locus to be encoded

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prior to amplification; and (5) the extra 5′ bases serve as an internal sentinel of recoding.”).

174. The batch-stamp is a tag or combination of tags associated with a polynucleotide whose identity can be used to differentiate between polynucleotides in a sample. Thus, the batch-stamp is a MID.

175. A POSA would have understood that the “batch-stamp” sequence disclosed in McCloskey is a MID “associated with the single cell from which the sample polynucleotide is derived,” as recited by claim limitation 1E.

176. The batch-stamp “specifies the DNA source such as the patient or sample identification” and the sample may be a “single-cell sample.” McCloskey at 762 (Ex. 1004.002). Thus, the batch-stamp may be used to specify a single-cell of origin. A POSA would have also understood that McCloskey’s batch-stamp sequence “is a different sequence for different single cells in the plurality of single cells,” as recited by claim limitation 1E.

177. For the reasons above, it is my opinion that Linnarsson and McCloskey both disclose limitation 1E.

f) [1F] wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: … II. a second tag sequence distinguishing the sample

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polynucleotide from other sample polynucleotides derived from the same single cell;

(1) Combination of Linnarsson and McCloskey Teaches This Limitation

178. It is my opinion that the combination of Linnarsson and McCloskey teaches a method comprising the step of generating a plurality of tagged polynucleotides, "wherein each tagged polynucleotide comprises a multiplex identifier (MID) sequence comprising a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell."

179. As explained above in Section XII.A.1.e, Linnarsson discloses that each tagged polynucleotide comprises a multiplex identifier (MID) sequence, which is either a TSO or CDS.

180. In addition, Linnarsson discloses a method of using a combination of two distinct tags. Linnarsson provides:

> Both the CDS and the TSO can include a tag. The CDS and the TSO can each include a different tag. **such that the tagged cDNA sample comprises a combination of tags**. Each cDNA sample generated by the above method can have a distinct tag, or **a distinct combination of tags**, such that once the tagged cDNA samples have been pooled, the tag can be used to identify which single cell from each cDNA sample originated.

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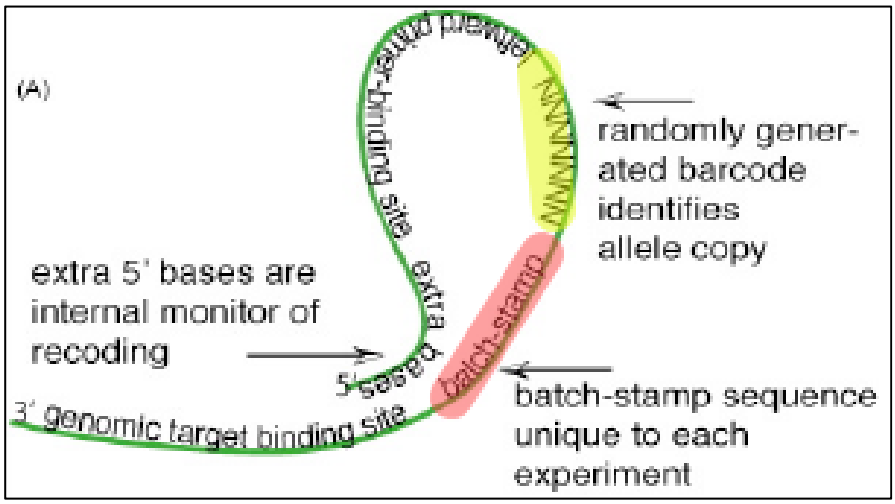
Linnarsson (Ex. 1003) at 19:5-11; Linnarsson Provisional (Ex. 1015) at 7:26-34.

181. I understand that during prosecution of the ’981 patent, the Examiner found that Linnarsson “teaches that each cDNA sample has a distinct tag or combination of tags, which is reasonably interpreted to mean that each cDNA sample comprised 2 distinct tags.” Ex. 1005.196. However, the Examiner’s Allowance found that each claim of the ’981 patent was disclosed by Linnarsson with exception of the recited second tag. Ex. 1005.238.

182. It is my opinion that McCloskey—which the Examiner did not discuss alone or in combination with Linnarsson—teaches an MID comprising a second tag sequence that distinguishes a sample polynucleotide from others.

183. The MID sequence taught by McCloskey is depicted below in Figure 1 as an “encoding oligonucleotide.”



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Ex. 1004 at Fig. 1. As described in McCloskey and depicted above, the disclosed encoding oligonucleotide comprises two tag sequences: (1) a "batch-stamp" sequence (highlighted in red, above) to identify the experimental batch or genomic source (*e.g.*, a single cell as explained in Section XII.A.1.e) and (2) a "barcode" (highlighted in yellow, above) to distinguish polynucleotide sequences "arising from different . . . allele copies":

> Our method utilizes an encoding oligonucleotide with five distinct informational regions: (1) the batch-stamp, unique to each experiment, specifies the DNA source such as the patient or sample identification, and the date of reaction; (2) the random barcode distinguishes among sequences arising from different cell or allele copies; (3) the primer-binding site facilitates specific PCR amplification; (4) the genomic target-binding site targets the locus to be encoded prior to amplification; and (5) the extra 5′ bases serve as an internal sentinel of recoding.

McCloskey at 762 (Ex. 1004.002).

184. The second region disclosed in McCloskey (i.e., the "barcode") constitutes the recited second tag sequence distinguishing the sample polynucleotides from other sample polynucleotides derived from the same single cell. McCloskey discloses that its barcode "distinguishes among sequences arising from different . . . ***allele copies***." McCloskey at 762 (Ex. 1004.002). McCloskey also discloses that its barcode can be used in single cell analyses. McCloskey at 762,

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767 (Ex. 1004.002, 007) ("batch-stamps and barcodes [should] be used when amplifying irreplaceable DNAs and cDNAs for … ***single cell*** … ***analyses***."). Therefore, McCloskey disclose that its barcode can be used to distinguish allele copies in the context of single cell analyses, and a POSA would have understood that such allele copies originate from the same single cell. As explained above in Section X.A., a POSA would have understood that an allele comprises a DNA sequence. *Id.* Therefore, because McCloskey's random barcode distinguishes among "allele copies," it distinguishes sample polynucleotides (i.e., DNA) derived from within the same single cell.

185. Additionally, McCloskey instructs that its method of tagging applies "to single-stranded DNA or cDNA templates," which a person of skill in the art would have understood to mean analysis of mRNA. McCloskey at 762, 767 (Ex. 1004.002, 007). For example, Linnarsson provides "cDNA is typically synthesized from mRNA by reverse transcription." Linnarsson (Ex. 1003) at 14:17; Linnarsson Provisional (Ex. 1015) at 5:4.

(2) It Would Have Been Obvious to a POSA to Combine Linnarsson's and McCloskey's Teachings

186. It is my opinion that it would have been obvious to combine Linnarsson's methods of analyzing tagged nucleic acids with McCloskey's teachings to use an MID (*i.e.*, encoding oligonucleotide) comprising two tag

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sequences, one of which distinguishes a sample polynucleotide from a plurality of sample polynucleotides derived from the same single cell.

187. First, a POSA would have found McCloskey and Linnarsson to be analogous art that focus on significantly the same subject matter. Both references describe methods of genetic analysis of single cells whereby sample polynucleotides are tagged with oligonucleotides (Linnarsson's CDS and TSO; McCloskey's encoding oligonucleotide), after which PCR amplification is performed and the results are sequenced. Linnarsson (Ex. 1003) at 3:25-4:2, Linnarsson Provisional (Ex. 1015) at 2:27-3:3; McCloskey at Fig. 1 (Ex. 1004 at Fig. 1). They are thus in the same field of endeavor.

188. Second, a POSA would have been motivated to combine Linnarsson's method of tagging polynucleotides with McCloskey's MID comprising two tag sequences because McCloskey teaches a solution to a problem discussed in Linnarsson, namely amplification bias. Linnarsson discloses that analyzing gene expression in single cells results in "potential amplification bias." Linnarsson (Ex. 1003) at 2:3-15, 11:6-10; Linnarsson Provisional (Ex. 1015) at 2:6-21, 3:14-20. A POSA would have understood that avoiding amplification bias is particularly important in gene expression analysis in single cells. As explained above, single cell genomics rely on limited amounts of template DNA, thus amplification will be necessary and correcting for bias is particularly important. *Id.* Linnarsson attempts

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to address the issue of amplification bias by using a cell-tag to distinguish single cells, but recognizes its solution is not perfect as it only "reduces," rather than eliminating, amplification bias. *See* Linnarsson (Ex. 1003) at 11:6-9; Linnarsson Provisional (Ex. 1015) at 3:14-18 ("[t]his reduces amplification bias."). McCloskey recognizes the issue of amplification bias, and expressly discloses that its method of including a second tag sequence on the oligonucleotide "solve[s]" this problem. *See* McCloskey at Abstract (Ex. 1004 at Abstract) ("The risks of … biased amplification … are especially high when limited amounts of template DNA are used; *id.* ("We have developed and applied molecular encoding principles to solve this source-uncertainty problem for DNA sequences generated by standard PCR"). Thus, a POSA would have been motivated to combine McCloskey with Linnarsson.

189. Linnarsson discusses the issue of amplification bias associated with single cell analysis. For example, Linnarsson discloses:

> Gene expression in single cells has previously been analyzed using a variety of methods. In particular, single cell gene expression in neural cells has been studied by microarray analysis. However, these methods require that each single cell is analyzed individually and treated separately during the entire procedure, which is time-consuming and expensive. In addition, the preparation and amplification of samples from single cells independently potentially introduces cell-to-cell variation. **Furthermore, as the cDNA of each cell must be amplified to an amount that can be reasonably handled for the subsequent**

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> **analysis, there is potential amplification bias.** For example, a single cell contains about 0.3 pg of mRNA, and at least a 300 ng is commonly needed for subsequent analysis by sequencing. Therefore, an amplification of at least a million-fold is required.

Linnarsson (Ex. 1003) at 2:3-15 (internal citations omitted); Linnarsson Provisional (Ex. 1015) at 2:6-21.

190. However, Linnarsson does not actually solve the problem of amplification bias, instead only "reduces" amplification bias by using a cell-specific tag. Linnarsson (Ex. 1003) at 11:6-10; Linnarsson Provisional (Ex. 1015) at 3:18-22. Linnarsson's tag sequence reduces amplification bias because the tagged samples can be pooled, requiring fewer rounds of amplification and thus less potential amplification bias. Linnarsson (Ex. 1003) at 10:32-11:6; Linnarsson Provisional (Ex. 1015) at 3:5-14. McCloskey, however, provides a more definitive solution. By incorporating a second tag in an oligonucleotide that uniquely identifies each sample polynucleotide prior to amplification, McCloskey's method is able to uniquely account for each individual sample polynucleotide and "solve" amplification bias:

> The risks of contamination, biased amplification, and product redundancy are especially high when limited amounts of template DNA are used. We have developed and applied molecular encoding

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> principles to solve this source-uncertainty problem for DNA sequences generated by standard PCR.

McCloskey at 761 (Ex. 1004.001).

> An additional benefit of our method … is that counting sequences with nonredundant barcodes provides a quantitative measure of template diversity."

McCloskey at 766 (Ex. 1004.006).

191. As mentioned, the risks of amplification bias are "especially high when limited amounts of template DNA are used," such as for single cell genomics or gene expression analysis in single cells. Linnarsson (Ex. 1003) at 2:3-15, 11:6-10, Linnarsson Provisional (Ex. 1015) at 2:6-21, 3:14-20. Because McCloskey provides a direct solution to the problem discussed in Linnarsson, a POSA would have been motivated to incorporate McCloskey's teachings into Linnarsson's methods to remove amplification bias by introducing barcodes to measure true sample complexity/template diversity. By tagging each sample polynucleotide uniquely, true sample complexity can be analyzed because each unique sample sequence can be identified by the tag and amplification bias can be accounted for and corrected.

192. Third, a POSA would have had a reasonable expectation of success to incorporate McCloskey's teachings into Linnarsson's. Linnarsson discloses incorporating oligonucleotides (*e.g.*, CDSs and TSOs) that comprise "a distinct tag,

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or a distinct combination of tags." Linnarsson (Ex. 1003) at 19:5-10; Linnarsson Provisional (Ex. 1015) at 7:26-32. McCloskey similarly discloses annealing an "encoding oligonucleotide." The only difference between Linnarsson and McCloskey is that Linnarsson discloses annealing one or more oligonucleotides (i.e., the CDS and/or TSO) each comprising one tag sequence, whereas McCloskey discloses annealing an oligonucleotide comprising two tag sequences. *See, e.g.*, McCloskey at 762 (Ex. 1004.002) ("Our method utilizes an encoding oligonucleotide with five distinct informational regions [including] the random barcode, [which] distinguishes among sequences arising from different cell or allele copies …."). However, a POSA would have found this difference to be trivial because a POSA could conceptually divide each of Linnarsson's cell-tag sequence into two portions, wherein the first portion performs the function of McCloskey's "batch-stamp,", used to track individual cells, and the second portion performs the function of McCloskey's "barcode", used to differentiate polynucleotides within a cell. For example, Linnarsson disclose that its cell-tag can be "4-20 nucleotides in length." Linnarsson (Ex. 1003) at 19:2-3. McCloskey discloses that its batch-stamp typically comprises 6 to 8 nucleotides, and its barcode comprises 7 nucleotides. McCloskey (Ex. 1004) at 764. Therefore, a POSA could have conceptually designated the first 6-8 nucleotides of Linnarsson's cell-tag sequence to identify the

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single-cell origin of the tagged polynucleotide, and use the next 7 nucleotides to distinguish the tagged polynucleotide from other one from the same single cell.

193. Moreover, a POSA would have had a reasonable expectation combining Linnarsson and McCloksey because Linnarsson discloses using a combination of distinct tags, which is consistent with the two-tag concept disclosed in McCloskey. Linnarsson (Ex. 1003) at 19:6-7; Linnarsson Provisional (Ex. 1015) at 7:27-28 ("the CDS and the TSO can each include a different tag, such that the tagged cDNA sample comprises a combination of tags."). I also note that the Patent Owner previously admitted "using different labels to differentiate and distinguish between different instances of similar objects is commonplace." Ex. 1016.010.

194. Therefore, it is my opinion that it would have been obvious to combine Linnarsson and McCloskey.

g) [1G] sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences;

195. It is my opinion that Linnarsson discloses a method comprising the step of "sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences."

196. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution. Further, as discussed above in Section VI., Patent Owner previously challenged claims directed to

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detecting and sequencing DNA samples as nothing but "generic, routine, well-known laboratory techniques." Ex. 1016.017.

197. Linnarsson teaches that after generating the tagged polynucleotides, "sequencing is performed" thereon. *See* Linnarsson (Ex. 1003) at 4:8-13, Linnarsson Provisional (Ex. 1015) at 8:22-28.

198. Additionally, Linnarsson repeats the disclosure that the method includes a sequencing step:

> The 5' APS can be designed to facilitate downstream processing of the cDNA library. For example, if the cDNA library is to be analyzed by a particular sequencing method, e.g. Applied Biosystems' SOLiD sequencing technology, or Illumina's Genome Analyzer, the 5' A.PS can be designed to be identical to the primers used in these sequencing methods.

Linnarsson (Ex. 1003) at 19:28-32, Linnarsson Provisional (Ex. 1015) at 8:33-9:10.

> The method of preparing a cDNA library described herein can further comprise processing the cDNA library to obtain a library suitable for sequencing. As used herein, a library is suitable for sequencing when the complexity, size, purity or the like of a cDNA library is suitable for the desired screening method. In particular, the cDNA library can be processed to make the sample suitable for any high-throughput screening methods, such as Applied Biosystems' SOLiD sequencing technology, or Illumina's Genome Analyzer. As such, the cDNA library can be processed by fragmenting the cDNA library (e.g. with DNase)

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> to obtain a short-fragment 5' -end library. Adapters can be added to the cDNA, e.g. at one or both ends to facilitate sequencing of the library. The cDNA library can be further amplified, e.g. by PCR, to obtain a sufficient quantity of cDNA for sequencing. Embodiments of the invention provide a cDNA library produced by any of the methods described herein. **This cDNA library can be sequenced to provide an analysis of gene expression in single cells or in a plurality of single cells.**

Linnarsson (Ex. 1003) at 21:13-25; Linnarsson Provisional (Ex. 1015) at 9:35-10:6.

199. A POSA would have understood that the sequencing step of Linnarsson discloses obtaining a plurality of identified polynucleotide sequences.

h) [1H] using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and

200. It is my opinion that Linnarsson discloses a method that comprises the step of using the first tag sequence of a MID to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived.

201. A POSA would have understood that Linnarson's tag "used to identify which single cell from each cDNA sample originated" is the claimed "first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived." As discussed above

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in Section XII.A.1.e., Linnarsson teaches that the tags present in the CDS and the TSO and incorporated into the cDNA can comprise distinct tags, either of which can be used to identify the single cell from which polynucleotide sequence was derived:

> Both the CDS and the TSO can include a tag. The CDS and the TSO can each include a different tag, such that the tagged cDNA sample comprises a combination of tags. Each cDNA sample generated by the above method can have a distinct tag, or a distinct combination of tags, such that once the tagged cDNA samples have been pooled, the tag can be used to identify which single cell from each cDNA sample originated.

Linnarsson (Ex. 1003) at 19:5-11, Linnarsson Provisional (Ex. 1015) at 7:26-34.

202. A POSA would have understood that identifying which cell the cDNA originated from also identifies which cell the identified polynucleotide arose from because the cDNA comprises the identified polynucleotide sequence.

203. It is my opinion the McCloskey also discloses a method that comprises the step of "using the first tag sequence of a MID to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived."

204. As discussed above, Section XII.B.1.e., McCloskey discloses a method utilizing an encoding oligonucleotide with a first tag referred to as a "batch-stamp," which "specifies the DNA source such as the . . . sample identification." McCloskey

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at 762 (Ex. 1004.002). The specific "DNA source" is recommended to include a "single cell." *Id.* at 767 (Ex. 1004.007).

i) [1I] using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived.

205. It is my opinion that the combination of Linnarsson and McCloskey discloses a method that comprises the step of "using the second tag sequence of a MID to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived."

206. As discussed above in Section XII.A.1.f., McCloskey's encoding oligonucleotide comprises two tag sequences: (1) a "batch-stamp" sequence . . . and (2) a "barcode" to distinguish polynucleotide sequences "arising from different cell or allele copies." McCloskey at 763, 764 (Ex. 1004.003, .004). The batch-stamp sequence correlates to the claimed first tag sequence and the barcode correlates to the claimed second tag sequence. The barcode "distinguishes among sequences arising from different cell or allele copies." McCloskey at 762-64 (Ex. 1004.002-004). As explained above, a POSA would have understood McCloskey to disclose that the barcode is useful to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived.

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207. McCloskey teaches that the "batch-stamps and barcodes [should] be used when amplifying irreplaceable DNAs and cDNAs for … ***single cell*** … ***analyses***," and that its method of tagging applies "to single-stranded DNA or cDNA templates," which, as discussed above, a person of skill in the art would have understood to mean analysis of mRNA. McCloskey at 762, 767 (Ex. 1004.002, 007).

208. As explained above, it is my opinion that a POSA would have been motivated to combine Linnarsson and McCloskey, and would have had a reasonable expectation of success in combining them. *See supra* Section XII.A.1.f.(2). Therefore, Linnarsson and McCloskey render obvious limitation [1I].

209. Because Linnarsson and McCloskey render obvious each limitation of claim 1 of the '981 patent, and because a POSA would have been motivated to combine Linnarsson and McCloskey, and would have had a reasonable expectation of success in combining them, it is my opinion that Linnarsson and McCloskey render obvious claim 1 of the '981 patent.

2. Claim 2: The method of claim 1, wherein the method further comprises amplifying the tagged polynucleotides prior to the sequencing step (c).

210. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious "[t]he method of claim 1, wherein the method further comprises amplifying the tagged polynucleotides prior to the sequencing."

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211. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

212. Linnarsson discloses this limitation. For example, Linnarsson discloses that "[t]he cDNA library can be further amplified, e.g. by PCR, to obtain a sufficient quantity of cDNA for sequencing." Linnarsson (Ex. 1003) at 21:21-22; Linnarsson Provisional (Ex. 1015) at 9:4-6.

213. Figure 1 of Linnarsson provides an exemplary walkthrough comprising the steps of collecting a plurality of single cells, tagging and performing reverse transcription to produce tagged cDNA, and pooling and amplifying the tagged cDNA before performing sequencing:

> **Figure 1**, panels A-F, show an overview of a method of analyzing gene expression in a plurality of single cells. (A) the tissue of interest is dissected; (B) a plurality of single cells are selected; (C) single cells are placed in separate wells of a 96-well plate and lysed; tagged reverse transcription is performed on each sample to produce cDNA; (D) **cDNA samples are pooled and amplified**; (E) sequencing is performed to obtain 100 million reads.

Linnarsson (Ex. 1003) at 4:8-12, Linnarsson Provisional (Ex. 1015) at 10:2-3.

214. The following passages from Linnarsson also disclose an amplification step to prepare sufficient sample prior to sequencing:

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> The ends of the cDNA have identical sequences and are amplified using a single PCR primer, which helps suppress short amplicons.

Linnarsson (Ex. 1003) at 6:1-3; Linnarsson Provisional (Ex. 1015) at 10:2-3.

> Embodiments of the invention provide . . . pooling the tagged cDNA samples and amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA. By utilizing the above method, it is feasible to prepare samples for sequencing from several hundred single cells in a short time and with a minimal amount of work.

Linnarsson (Ex. 1003) at 10:15-24; Linnarsson Provisional (Ex. 1015) at 10:2-3.

215. McCloskey also teaches that an amplification step may precede the sequencing step. For example, McCloskey discloses that "PCR products were sequenced and their encoded information was evaluated," thus teaching that PCR was performed to amplify the polynucleotides prior to the sequencing step. McCloskey at 763 (Ex. 1004.003). As I explained above, PCR is a method for amplification. *See* Section X.C.2.a., *supra*. Further, when discussing how sample cDNA is processed prior to sequencing, McCloskey teaches that samples may be "amplified using standard PCR protocols." McCloskey at 762, 764 (Ex. 1004.002, 004).

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3. Claim 3: The method of claim 1, wherein the sample polynucleotides are selected from DNA and RNA.

216. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious t "[t]he method of claim 1, wherein the sample polynucleotides are selected from DNA and RNA."

217. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

218. As described above in Section XII.A.1.b., Linnarsson teaches selecting sample polynucleotides that are mRNA. For example, Linnarsson discloses that "[i]n one aspect, the method includes the steps of releasing mRNA from each single cell to provide a plurality of individual mRNA samples." Linnarsson (Ex. 1003) at 3:26-27; Linnarsson Provisional (Ex. 1015) at 2:27-31.

219. As explained in Section XII.A.1.b., McCloskey discloses providing a sample comprising a plurality of single cells which each comprise a plurality of polynucleotides, which may be DNA or cDNA. As explained in Section XII.A.1.e., McCloskey teaches generating tagged polynucleotides (*e.g.*, tagged cDNA) from sample polynucleotides that are DNA. For example, McCloskey discloses "a method by which molecular codes are added to single-stranded DNA or cDNA templates …." McCloskey at Abstract, 762 (Ex. 1004 at Abstract, 002); *see also* McCloskey at 767 (Ex. 1004.007) ("We recommend that batch-stamps and barcodes

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be used when amplifying irreplaceable DNAs and cDNAs for … single cell … DNA analyses."). Further, as discussed above in Section X.B., a POSA would understand that cDNA refers to DNA molecules that are formed by the reverse transcription of mRNA molecules. Therefore, a person of skill in the art would have understood that McCloskey discloses tagging and sequencing the claimed "sample polynucleotides," which includes either DNA or mRNA.

4. Claim 4: The method of claim 3, wherein the sample polynucleotides comprise mRNA.

220. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 3, wherein the sample polynucleotides comprise mRNA."

221. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

222. As explained in Sections XII.A.1.b. and XII.A.3., Linnarsson discloses sample polynucleotides that are mRNA: "In one aspect, the method includes the steps of releasing mRNA from each single cell to provide a plurality of individual mRNA samples." Linnarsson (Ex. 1003) at 3:26-27; Linnarsson Provisional (Ex. 1015) at 2:27-31.

223. As explained in Section XII.A.3, McCloskey also discloses sample polynucleotides that are mRNA.

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5. Claim 6: The method of claim 1, wherein the tagged polynucleotides are generated through PCR or linear amplification of the plurality of sample polynucleotides using an adapter sequence comprising the MID sequence and an amplification primer.

224. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious "[t]he method of claim 1, wherein the tagged polynucleotides are generated through PCR or linear amplification of the plurality of sample polynucleotides using an adapter sequence comprising the MID sequence and an amplification primer."

225. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

226. First, Linnarsson and McCloskey both teach that tagged polynucleotides are generated through PCR of the plurality of sample polynucleotides. According to Linnarsson, after "synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample" and "incorporating a tag," PCR amplification is performed to generate "a cDNA library comprising double-stranded cDNA"—*i.e.*, tagged polynucleotides. Linnarsson (Ex. 1003) at 10:18-22; Linnarsson Provisional (Ex. 1015) at 2:33-3:3; *see also* Linnarsson at 20:3-6 ("PCR is a reaction in which replicate copies are made of a target polynucleotide …."); Linnarsson Provisional at 5:16-22, 8:7-21. As discussed above, each cDNA copy generated through PCR is a recited "tagged polynucleotide." *See* Section XII.A.1.c,

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supra. McCloskey similarly teaches generating tagged polynucleotides through PCR of the plurality of sample polynucleotides. After annealing the encoding oligonucleotide to the sample polynucleotide and performing extension by Sequenase, the "Sequenase-extension products are … amplified using standard PCR protocols." McCloskey at 762 (Ex. 1004.002); *see also id.* at 003 (describing and depicting the same).

227. Second, it is my opinion that McCloskey and Linnarsson both teach generating the plurality of sample polynucleotides using (1) an adapter sequence comprising the MID sequence and (2) an amplification primer. A POSA would have understood that the recited "amplification primer" is a separate molecule from the recited "adapter sequence" because the specification defines a "primer" to mean "an oligonucleotide," which is in turn defined as a "linear polymer of nucleotide monomers." Ex. 1001 at 9:52, 8:60-62. Further, these definitions are consistent with the understanding of a POSA so I have applied them here.

228. As discussed above, McCloskey teaches an encoding oligonucleotide comprising a MID sequence. *See* XII.A.1.e-f, *supra*. Moreover, it is my opinion that McCloskey's encoding oligonucleotide is an adapter sequences. Specifically, McCloskey discloses:

> Our method utilizes an encoding oligonucleotide with five distinct informational regions (Fig. 1): (1) the batch-stamp, unique to each

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> experiment, specifies the DNA source such as the patient or sample identification, and the date of reaction; (2) the random barcode distinguishes among sequences arising from different cell or allele copies; **(3) the primer-binding site facilitates specific PCR amplification; (4) the genomic target-binding site targets the locus to be encoded prior to amplification**; and (5) the extra 5′ bases serve as an internal sentinel of recoding. This encoding oligonucleotide is annealed to denatured DNA and extended by Sequenase (USB) (Fig. 1).

McCloskey at 762 (Ex. 1004.002).

229. The "primer-binding site" and "genomic target-binding site" are both adaptors, which facilitate the extension and amplification through PCR of the sample polynucleotides. McCloskey at 762-763 (Ex. 1004.002-003); *see also* Section XI.B, *supra*. Thus, the encoding oligonucleotide is an adapter sequence because it contains the primer-binding site and genomic target-binding site.

230. Additionally, it is my opinion that McCloskey's discloses generating the tagged polynucleotides using an amplification primer molecule. Specifically, the "primer-binding site" of McCloskey's encoding oligonucleotide "is the target of one of the **primers used for PCR amplification**." McCloskey at 764 (Ex. 1004.004). These "primers used for PCR" are the claimed "amplification primer." *See* McCloskey at 762 (Ex. 1004.002) (explaining that the encoding oligonucleotide

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comprises a "primer-binding site [that] facilitates specific PCR amplification"); *id.* at 763-765 (Ex. 1004.003-005) (describing the same).

231. It is my opinion that Linnarsson also discloses using an adapter sequence, comprising a MID, to generate the plurality of sample polynucleotides. Specifically, Linnarsson discloses that the TSO and/or CDS can comprise a MID. *See* Section XII.A.1.e, *supra*. It is also my opinion that Linnarsson discloses that the TSO and/or CDS is an adapter sequence. For example, Linnarsson discloses that the TSO and/or CDS comprises an "amplification primer sequence (APS)", the TSO further comprises a "3' sequence for template switching," and the CDS further comprises a "3' RNA complementary sequence (RCS)." Linnarsson (Ex. 1003) at 4:15-18; Linnarsson Provisional (Ex. 1015) at 8. The APS, the 3' sequence for template switching, and the 3' RCS is each an adapter region facilitating amplification. Thus, a POSA would have recognized the TSO and/or the CDS each as an adapter sequence because they contain the above-mentioned adapter regions.

232. Further, Linnarsson teaches generating the plurality of tagged polynucleotides using an amplification primer molecule. Specifically, Linnarsson teaches that each of the CDS and/or TSO comprises an "amplification primer sequence" ("APS"):

> The CDS can comprise a 5′ amplification primer sequence (APS), which subsequently allows the first strand of cDNA to be amplified by

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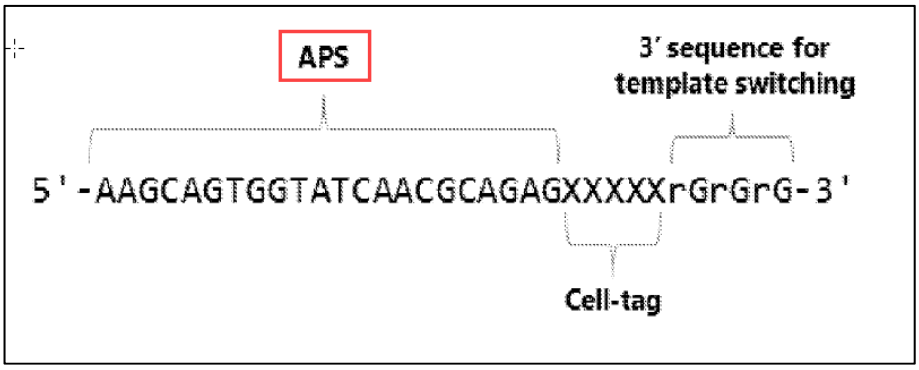
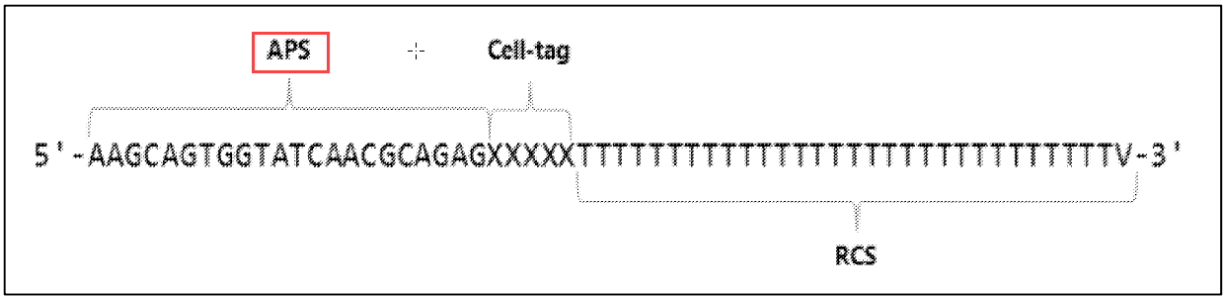
PCR using a primer that is complementary to the 5′APS. The TSO can also comprise a 5′ APS . . .

Ex. 1003 at 19:18-23; Ex. 1015 at 8:9-12. The APS in the TSO is depicted in Linnarsson Figure 3:



Ex. 1003 at Fig. 3. Figure 4 similarly depicts the APS for the CDS:



Ex. 1003 at Fig. 4.

233. As discussed above, Linnarsson discloses "**a primer** that is complementary to the 5' APS." Ex. 1003 at 19:18-23; *see also* Ex. 1015 at 8:9-15. This primer, which binds to the APS, is the claimed "amplification primer."

234. Thus, it is my opinion that Linnarsson and McCloskey render obvious claim 6 of the '981 patent.

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B. Ground 2: Claim 5 is Unpatentable as Obvious over Linnarsson in View of McCloskey, and further in View of the Knowledge of a POSA

235. It is my opinion that the combination of Linnarsson and McCloskey in view of the knowledge of a POSA renders obvious the "method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction."

236. I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

237. As discussed above in Section XII.1.f.ii, a POSA would have been motivated to adopt McCloskey's teachings and modify Linnarsson's design so that the tags include a first region, which could distinguish between different cells, and a second region, which could distinguish tagged polynucleotides originating from the same single-cell. Further, it would have been an obvious and a routine design choice with a reasonable expectation of success to carry out this modification by ligating an oligonucleotide comprising a second tag sequence to Linnarsson's CDS and/or TSO.

238. As of the earliest priority date of the '981 Patent, it was well-known and routine in the art to attach tag sequences using ligation. Indeed, the specification of the '981 Patent states as much:

> A variety of template-driven ligation reactions are described in the following references, which are incorporated by reference: Whiteley et al, U.S. Pat. No. 4,883,750; Letsinger et al, U.S. Pat. No. 5,476,930;

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Fung et al, U.S. Pat. No. 5,593,826; Kool, U.S. Pat. No. 5,426,180; Landegren et al, U.S. Pat. No. 5,871,921; Xu and Kool, Nucleic Acids Research, 27: 875-881 (1999); Higgins et al, Methods in Enzymology, 68: 50-71 (1979); Engler et al, The Enzymes, 15: 3-29 (1982); and Namsaraev, U.S. patent publication 2004/0110213.

Ex. 1001 at 6:23-32.

The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, **ligation**, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Genome Analysis: A Laboratory Manual Series (Vols. I-IV), Using Antibodies: A Laboratory Manual, Cells: A Laboratory Manual, PCR Primer: A Laboratory Manual, and Molecular Cloning: A Laboratory Manual (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) Biochemistry (4th Ed.) Freeman, New York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, A., Principles of Biochemistry 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) Biochemistry,

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5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

Ex. 1001 at 13:32-63.

239. Further, as discussed in Section VI *supra*, Patent Owner previously challenged a claim directed to attaching a tag by ligation, stating that ligation is not "***any*** new, non-conventional attachment technique." Ex. 1017.011-.012 (emphasis in original); *see also* Ex. 1016.020 (admitting that "primer binding [was] known and conventional," citing references "describing known ligation techniques").

240. Given that ligation was routine, conventional, and well-known in the art, it is my opinion that a POSA would also have had a reasonable expectation of success in ligating an additional sequence to Linnarsson's CDS and/or TSO. For example, a POSA would have found it routine and obvious to ligate a tag sequence by using a linker molecule, such as the method described in Hug's 2003 article. Ex. 1007.001 ("Hug").

241. Hug discloses a method whereby a tag sequence is ligated to a polynucleotide with a linker. The linker molecules are designed to hybridize to the target polynucleotide as well as a polynucleotide used as a MID. Hug at 621 (Ex. 1007.007). Once they are aligned through hybridization with the linker, Hug teaches using a T4 DNA Ligase enzyme to ligate the target polynucleotide to the MID, which in the method of Hug are additional linkers used to tag. *Id.*

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242. Hug provides one method by which a POSA would know the routine practice of ligation could be used to attach a tag to the CDS and/or TSO. Such a method was commonplace in the art at the time. *See* Hug at 621 (Ex. 1007.007); *see also* Okayama at 165 (Ex. 1024.005); Shibata at 1250-51, Fig. 1 (Ex. 1039.001-002); Ex. 1040 at Fig. 2, ¶¶ 71-75, ¶¶ 235-239. Specifically, a POSA would have known that a linker could be used to hybridize with the TSO and/or CDS and tag sequences to align them such that they could be ligated together using routine ligation techniques.

243. A POSA would have understood that such an approach, using ligation to add the tag sequences to the CDS and/or TSO, would have been entirely equivalent to the method disclosed by the combination of Linnarsson and McCloskey. The only difference is in the timing: with the combination of Linnarsson and McCloskey discussed above, Section XII.A.1, the tag sequence is part of the CDS or TSO prior to extension whereas by using ligation, the tag sequence is added after extension. Using ligation to add the tag after extension would allow for increased flexibility in selecting tag sequences during an experiment. Thus, a POSA would have understood that using ligation was a routine choice and would have been motivated to use ligation because of the increased flexibility in tag choice.

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244. Thus, it is my opinion that it would have been an obvious design choice to a POSA to ligate the second tag sequence to the TSO and/or CDS to generate the tagged polynucleotide.

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XIII. DECLARATION

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are belief to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

By: _____

Dated: April 19, 2023

Gregory Cooper, Ph.D.

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