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. 10,240,197

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 counting nucleic acids in a sample, the method comprising: 58

b) [1B] (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides; 60

c) [1C] (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags, ... 63

d) [1D] wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (i) a sample sequence from a sample polynucleotide of the plurality of sample polynucleotides; 68

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e) [1E] wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (ii) a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and 73

f) [1F] wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (iii) a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell; 74

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g) [1G] (c) sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence; 84

h) [1H] (d) using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell. 86

2. Claim 2: The method of claim 1, wherein the method further comprises amplifying the plurality of tagged polynucleotides prior to the sequencing step (c). 88

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4. Claim 4: The method of claim 1, wherein the plurality of sample polynucleotides comprises mRNA. 90

5. Claim 6: The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide

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tags comprises said first tag sequence and said second tag sequence. 91

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9. Claim 10: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one reverse transcription reaction. 96

10. Claim 11: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one polymerase chain reaction (PCR). 96

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13. Claim 24: The method of claim 1, wherein step (d) comprises determining the number of different second

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tag sequences associated with said sample sequence, thereby estimating the number of sample polynucleotides having said sample sequence from said cell. 103

14. Claim 25: The method of claim 1, wherein said plurality of sample polynucleotides comprises substantially all mRNA molecules of said cell. 104

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1. Claim 5: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction. 105

2. Claim 12: The method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with the same first tag sequence. 109

3. Claim 21: The method of claim 1, wherein step (c) comprises hybridizing said tagged polynucleotide to a solid support. 112

4. Claim 22: The method of claim 20, wherein said solid support is a bead. 115

5. Claim 26: The method of claim 1, wherein said plurality of sample polynucleotides comprises a subset of polynucleotides of said cell having the same sequence. 116

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6. Claim 18: The method of claim 17, wherein the number of different second tag sequences is at least ten times the number of sample polynucleotides. 122

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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. 10,240,197 (the "'197 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 '197 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

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, 6-11, 20 and 23-25 of the ’197 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 with Batch-stamps and Barcodes*, Biochemical Genetics, Vol. 45 (Dec. 2007) ("McCloskey") (Ex. 1004). It is further my opinion that claims 5, 12, 21, 22 and 26 of the '197 patent are 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"). It is further my opinion that claims 13-19 of the '197 patent are obvious based on Linnarsson in view of U.S. Patent Application No. 11/186636 to McCloskey ("McCloskey II") (Ex. 1055), and further in view of the knowledge of a 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.

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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 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

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

V. THE '197 PATENT

27. The '197 patent is titled "Methods For Analyzing Nucleic Acids From Single Cells." (Ex. 1001). The claims of the '197 patent generally describe methods for counting 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 '197 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 '197 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 '197 patent focuses heavily on this "reflex method," its claims are directed to the routine process of tagging, counting and sequencing polynucleotides, as shown in representative claim 1, reproduced below:

1. A method of counting nucleic acids in a sample, the method comprising:

> (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides;
>
> (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags, wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises:
>
> > (i) a sample sequence from a sample polynucleotide of the plurality of sample polynucleotides;
> >
> > (ii) a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and
> >
> > (iii) a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell;
>
> (c) sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence; and

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(d) using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell.

29. The specification of the '197 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 specification of the '197 patent acknowledges that the methods of counting nucleic acid molecules with unique tags was well-known, incorporating two patents and two patent applications by reference. The specification states that "any convenient method for tagging and/or counting nucleic acid molecules with unique tags may be employed (see, e.g., U.S. Pat. No. 7,537,897, issued on May 26, 2009; U.S. Pat. No. 7,217,522, issued on May 17, 2007; U.S. patent application Ser. No. 12/432,080, filed on Apr 29, 2009; each of which is incorporated by reference herein in their.)" Ex. 1001 at 22:38-45.

31. The '197 patent further discloses that the use of tag sequences to identify, track and count molecules were known in the art. For example, the '197 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.

32. 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', 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.

33. The specification of the '197 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.

34. The '197 patent also states that ligation was known in the art. For example, the '197 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, 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.

35. The '197 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 '197 patent discloses that reverse

transcription was known in the art, it also discloses that primer extension was known in the art.

36. 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.

37. The specification of the '197 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 '197 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.

38. I do not have an opinion on the priority date of the '197 patent. Based on the face of the patent, I understand that the earliest possible priority date of the '197 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

39. 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; 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. 1016.007-008. Similar to the '197 patent, the Fodor Patents achieve molecular tagging, counting 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.

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

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40. 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 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).

41. 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

PARSE EX.1002.023

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 '197 patent is also directed to "tagging polynucleotides."

42. The Patent Owner further stated that this concept of labelling different objects is not "any less abstract when applied, as here, to the context of counting DNA molecules." EX1017.007. Just like the Fodor Patents, the '197 patent is also directed to "counting nucleic acid molecules."

43. Additionally, 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 techniques." *Id.* at .017. The '197 patent similarly claims "sequencing the plurality of tagged polynucleotides." Ex. 1001 at Claim 1 (c).

44. 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***,[2] 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 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.

[2] All emphases added unless otherwise stated.

PARSE EX.1002.025

> 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.").

VII. PROSECUTION HISTORY OF THE '197 PATENT

45. On November 16, 2018, Patent Owner filed Nonprovisional Patent Application No. 10/240,197 titled "Methods For Analyzing Nucleic Acids From Single Cells." Ex. 1005.003-011. On March 26, 2019, the '197 patent was issued. EX1005.167.

46. I have been informed by counsel that during the prosecution of the '197 patent, the Examiner did not issue any non-final rejections, final rejections, or office

actions. I have been further informed by counsel that the Examiner issued the patent after the Patent Owner filed a terminal disclaimer subject to the expiration date of U.S. 10,155,981 (the "'981 Patent"). The '981 patent is being challenged in a co-pending IPR petition and I have submitted a declaration providing my independent evaluation on the validity of the claims of the '981 Patent.

VIII. CLAIM CONSTRUCTION

47. I do not believe that any explicit claim construction is required to determine the validity of the '197 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.

48. 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 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).

49. Claim 1 of the '197 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 '197 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

50. 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 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.

51. 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.

52. 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..

53. 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).

X. TECHNICAL BACKGROUND AND STATE OF THE ART

A. DNA and RNA

54. 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."

55. 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.

56. 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

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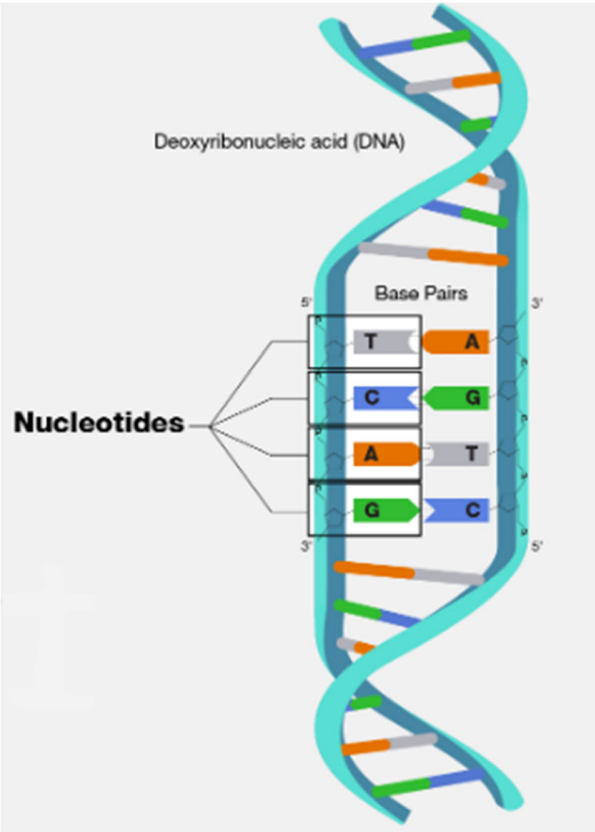
cytosine ("C"), which is complementary to guanine ("G"). The two strands of DNA are connected through complementary base pairing via chemical bonds, also known as "hybridization," as depicted below:



Ex 1049.002.

57. 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.

58. 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."

59. 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).

60. 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.

61. 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

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including repairing and building body's tissues, carrying out metabolic reactions, and coordinating bodily functions.

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

63. Translation is the decoding of genetic information carried by the mRNA to make proteins. This process takes place within a specialized organelle

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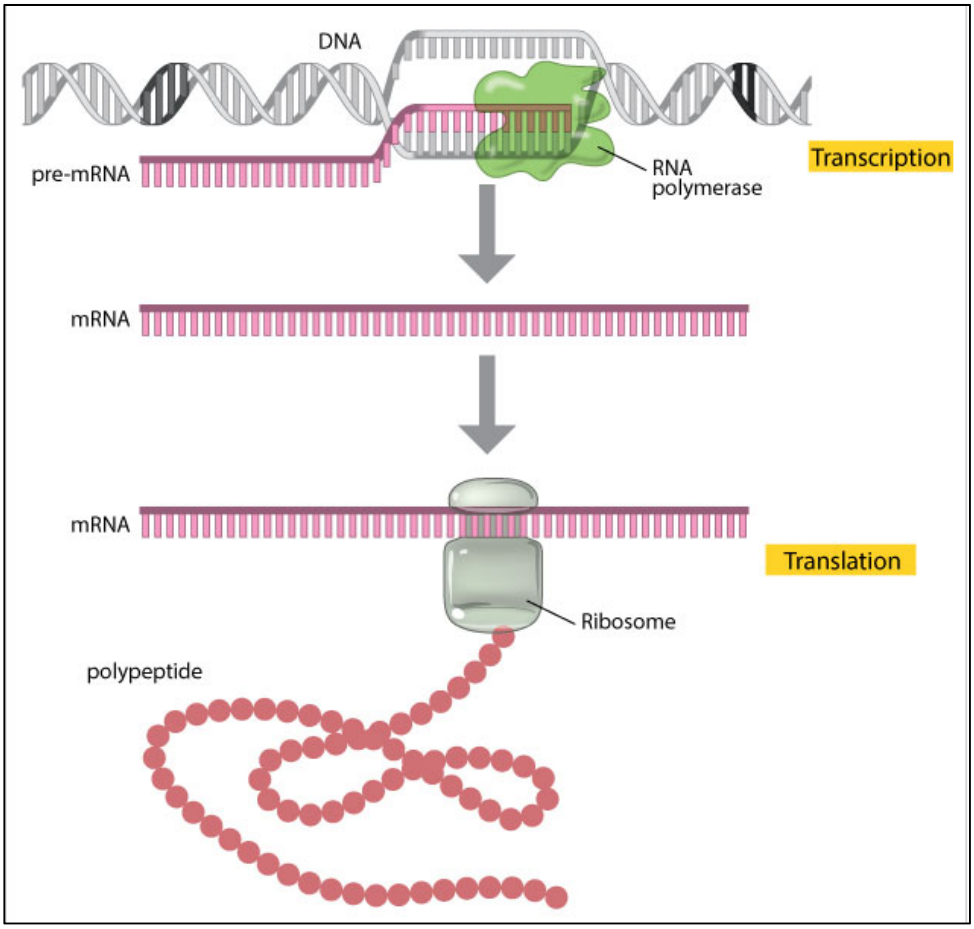
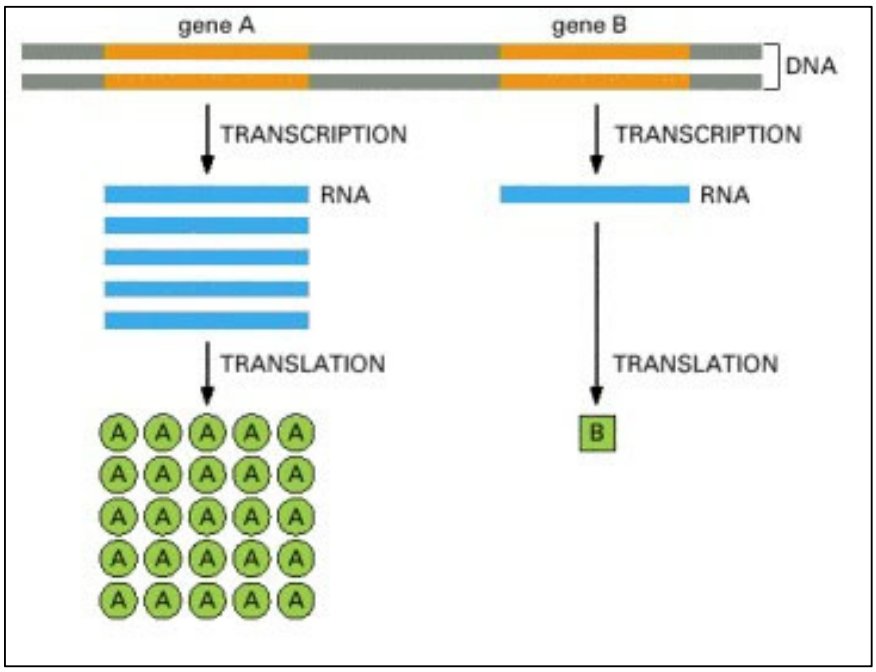
called the ribosome, which reads the mRNA in a group of three bases, referred to as a codon, which correspond to a particular amino acid. Clancy at 2 (Ex. 1030.002).



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

64. 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).



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

65. 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

66. 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

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. The generation of a duplex DNA through the process of reverse transcription is also known to be understood as linear amplification.

67. 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.

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C. Single Cell Genomics

68. 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 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).

69. 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).

70. 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

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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.

71. 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 '197 patent and prior art references that I have used.

1. DNA Tagging

72. Tagging is a known concept in genetics which involves adding synthetic polynucleotide to a sequence of interest. The '197 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 '197 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").

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

74. 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 '197 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).

75. 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 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).

76. 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.

77. It was also known in the art that using a large number of tags can ensure that each polynucleotide in a reaction mixture is uniquely tagged. For example, Hollas discloses that if "the number of different oligonucleotide sequences is large enough (100 times larger than the number of DNA molecules), almost all oligonucleotides that are combined with a DNA molecule will contain a unique sequence." Hollas (Ex. 1056) at 001-002. Thus, this is a typical and routine method

of increasing the chances for every polynucleotide from the original sample to get tagged and be eventually sequenced.

78. 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. Counting Polynucleotides Through Tagging

79. As explained above, unique tagging can also be used for counting individual molecules and these methods have been known in the art. Ex. 1007.001; Hollas (Ex. 1056) at 001. Indeed, as also explained above, the specification of the '197 patent acknowledges that methods for tagging and counting nucleic acid molecules were well-known: "convenient method for tagging and/or counting individual nucleic acid molecules with unique tags may be employed (see, e.g., U.S. Pat. No. 7,537,897, issued on May 26, 2009; U.S. Pat. No. 7,217,522, issued on May 15, 2007; U.S. patent application Ser. No. 11/377,462, filed on Mar. 16, 2006; and U.S. patent application Ser. No. 12/432,080, filed on Apr. 29, 2009; each of which is incorporated by reference herein in their)." Ex. 1001 at 22:38-45. Specifically, U.S. Pat. No. 7,537,897 titled "Molecular Counting" relates to the same concept of counting molecules through unique tagging as claimed in the 197 Patent:

> "The invention provides methods and compositions for counting molecules in a sample, wherein each molecule is labeled with a unique oligonucleotide tag. Such tags are amplified and identified rather than the molecules themselves; that is, the problem of counting molecules is converted into the problem of counting tags."

Ex. 1058 at Abstract.

80. Further, the Patent Owner confirms in the *BD* litigation that the concept of labeling information is not "any less abstract when applied, as here, to the context of counting DNA molecules." EX1017.007.

81. The unique tagging of sample polynucleotides increases the accuracy in counting the number of these sample polynucleotides which further facilitates gene expression analysis. By utilizing significantly more tag sequences than the sample nucleotides, the probability of counting and distinguishing unique sample polynucleotides from a single-cell sample increases. This enables researchers to better analyze the mRNA content in a sample and understand gene activity for clinical or research purposes.

3. Amplification

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

83. 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 '197 patent also acknowledges the use of conventional techniques of amplification as a part of its claims and description. I focus on PCR techniques since they are most relevant to the '197 patent and prior art references that I have used.

a) Polymerase Chain Reaction ("PCR")

84. 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).

85. 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.

86. 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.

87. 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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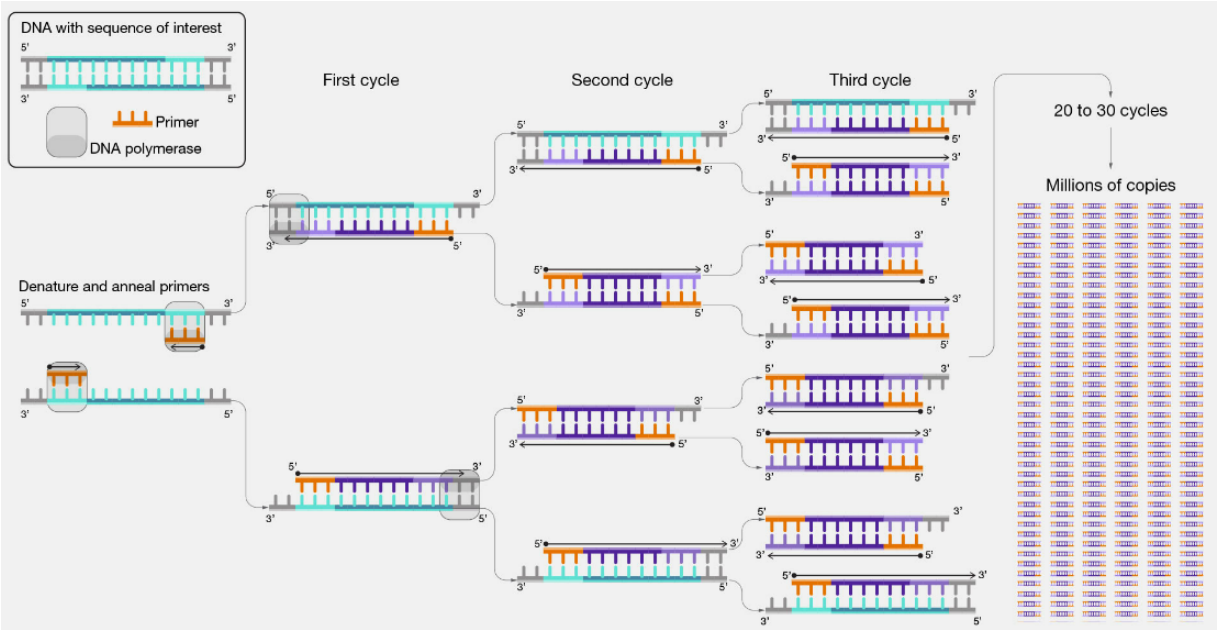

Ex. 1050.001.

88. 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).

4. Amplification Bias and Molecular Tagging

89. One of the known issues with nucleic acid amplification is amplification bias. Because the PCR process is at least partially stochastic—i.e., randomized—the relative quantity of each sequence in the post-amplification sample may 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.

90. 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.

91. To solve this problem, it was well-known and common to add molecular tags (as discussed above at X.C.1) to target sequences prior to the amplification step. *See, e.g.*, Miner at 1 (Ex. 1013.001), McCloskey at 766 (Ex. 1004.006), Stöger at 6 (Ex. 1060.006), Genereux at 2 (Ex. 1061.002), Nicolas at 0371 (Ex. 1062.008). 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.

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

93. Stöger, referring to Miner, notes that "the use of molecular 'batchstamps' and 'barcodes' will improve the quality of DNA sequence data-sets

as they provide a method to validate sequence diversity, and enable researchers to accurately classify valid, contaminant, or redundant sequences." Stöger at 6 (Ex. 1060.006). Similarly, Genereux relies on using molecular batchstamps and barcodes to distinguish valid from contaminant and redundant sequences. Genereux at 2 (Ex. 1061.002).

5. 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 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 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.

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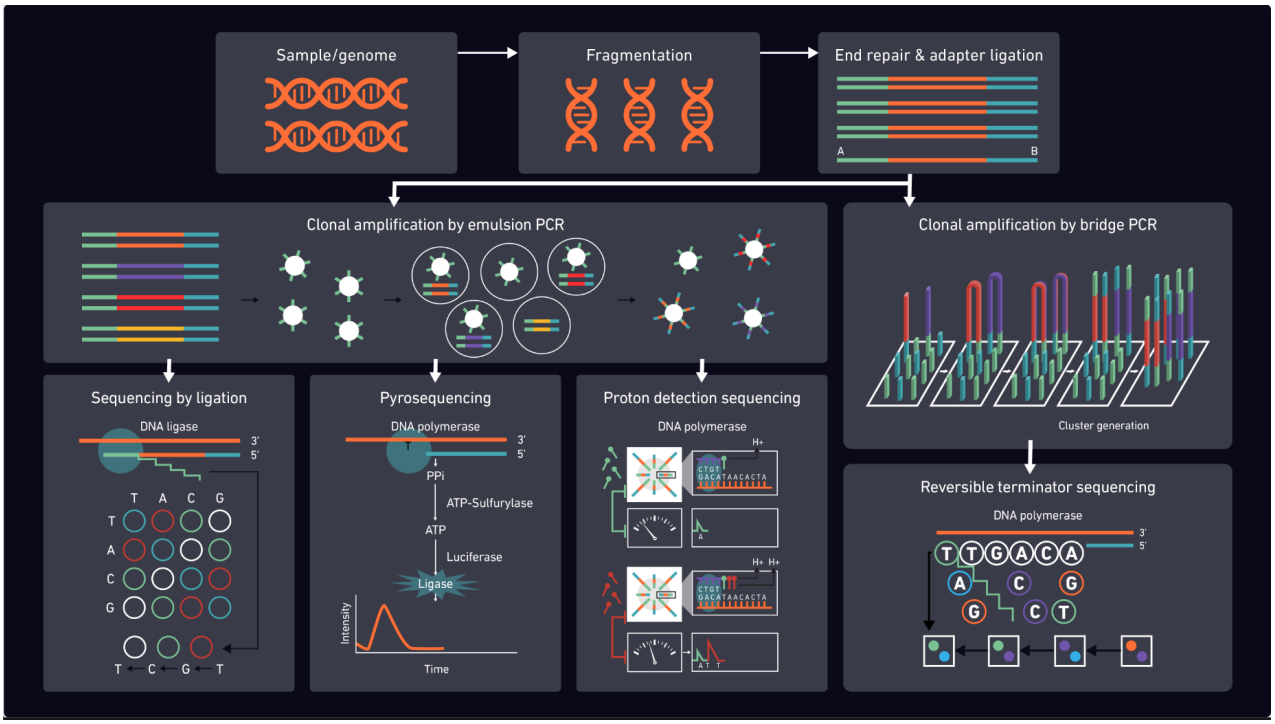


Gkazi at Figure 2 (Ex. 1041.005).

98. One of the first commercially introduced next-generation sequencers, Roche (454) GS FLX, uses a solid support also called a "bead" for immobilization of sample nucleic acids. The platform performs the step of pyrosequencing which utilizes the detection of pyrophosphate generation and light release to determine whether a specific base has been incorporated in a DNA chain. Mardis (Ex. 1045.003). Each incorporation of a nucleotide by DNA polymerase results in the release of pyrophosphate which causes a series of reactions that ultimately produce light by use of an enzyme called luciferase. *Id.* The amount of light produced is proportional to the number of nucleotides incorporated. *Id.*

99. The Illumina Genome Analyzer, on the other hand, utilizes a sequencing-by-synthesis approach, also called reversible terminator sequencing. Mardis at 391 (Ex. 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 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. Linnarsson (Ex. 1003)

1. Linnarsson is § 102(e) Prior Art

100. 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.

101. 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 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:

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	"(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

Linnarsson	Linnarsson Provisional Application Disclosure
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;	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 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.

Linnarsson	Linnarsson Provisional Application Disclosure
(iv) amplifying the pooled cDNA samples to generate a cDNA library comprising double-stranded cDNA,	"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 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.

102. 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.

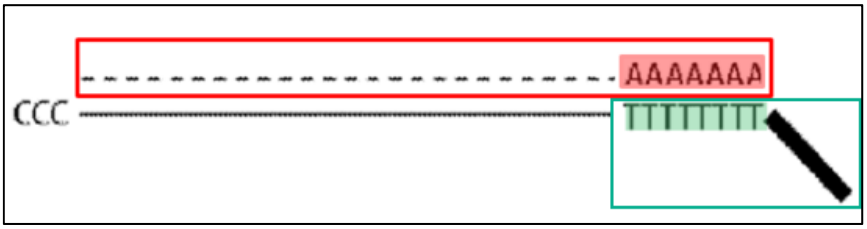
2. Linnarsson Overview

103. 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.").

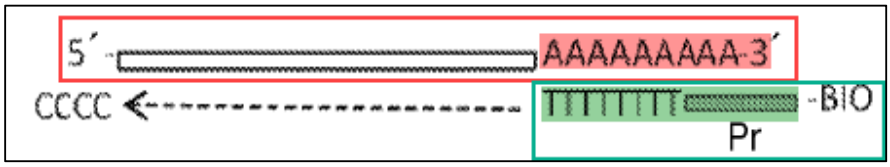
104. As an initial matter, Linnarsson teaches a method for synthesizing tagged cDNA from mRNA by the process of reverse transcription. With the use of

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 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.

105. 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)



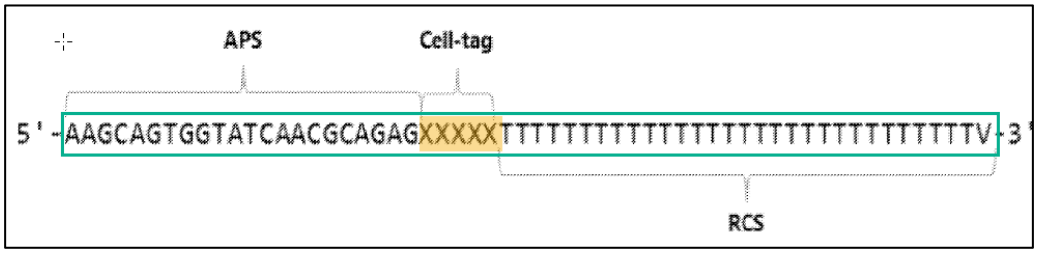
Ex. 1003 at Fig. 2.



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

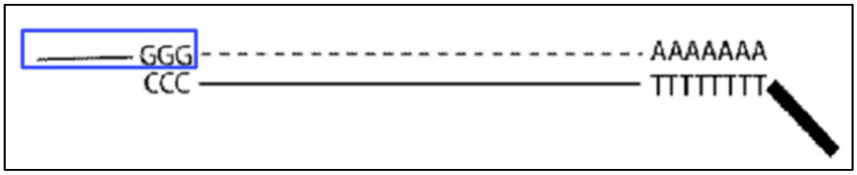
106. The enzyme responsible for synthesizing this strand of cDNA is called a reverse transcriptase, which also naturally causes a series of cytosines to be incorporated at the 3' end of the first strand of cDNA synthesized from mRNA. Ex. 1003 at 16:22-26. Thus, the resulting cDNA molecule also includes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

107. 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):

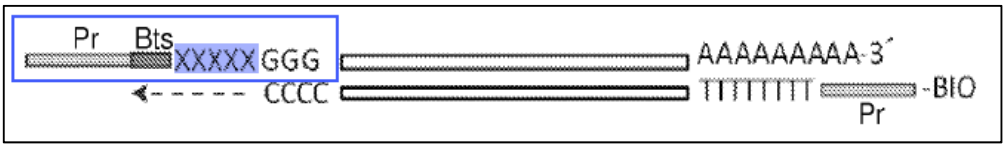


Ex. 1003 at Fig. 4.

108. 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":

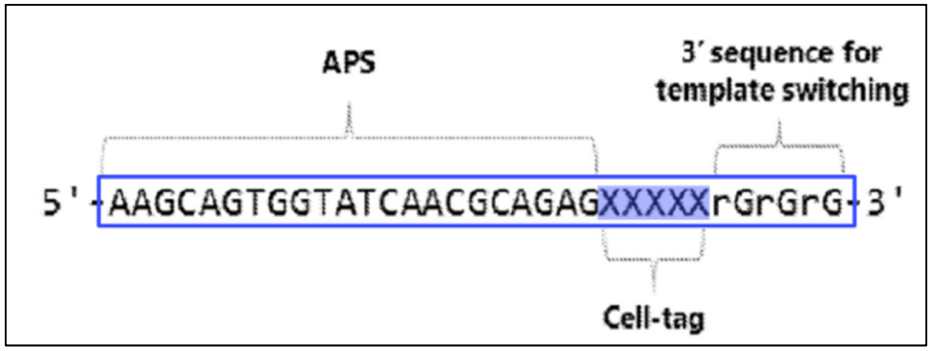


Ex. 1003 at Fig. 2.



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

109. 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.

110. 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 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.").

111. 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.").

112. 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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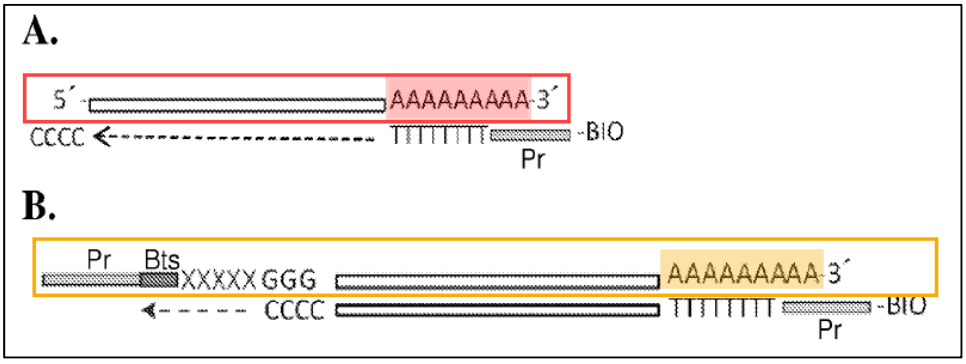
Ex. 1003 at Fig. 2. Figure 11 of Linnarsson discloses the same:

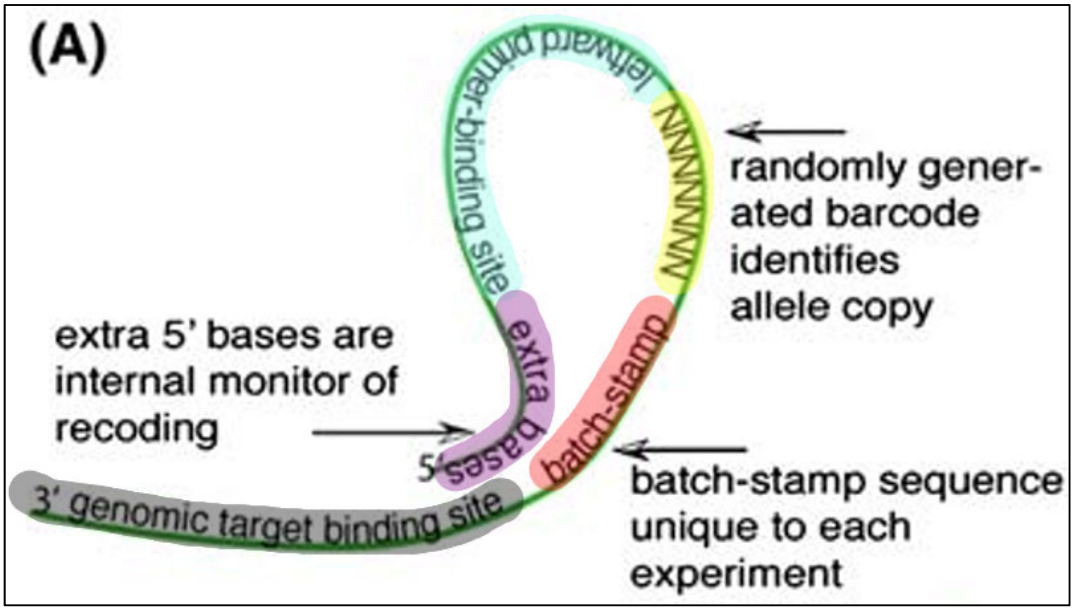


Ex. 1003 at Fig. 11; Ex. 1003 at 6:1-3 (explaining Figure 11).

B. McCloskey (Ex. 1004)

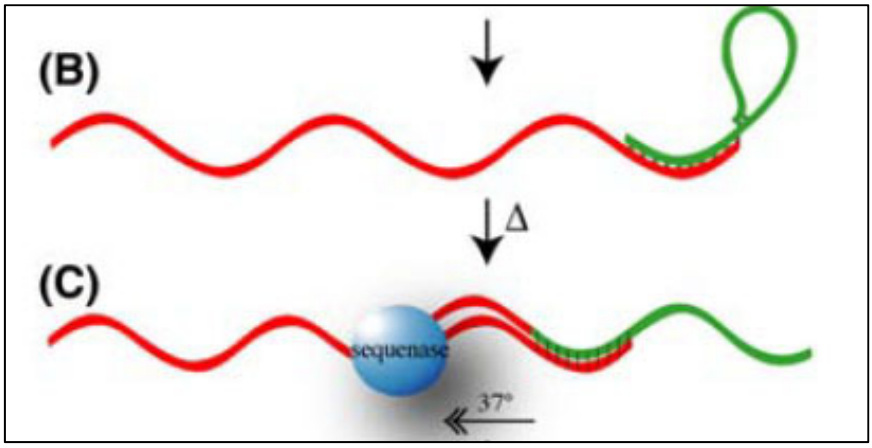
113. McCloskey *et. al*., "Encoding PCR Products with Batch-Stamps and Barcodes", is an article that was published in Biochemical Genetics on October 23, 2007 and is thus prior art to the '197 patent. McCloskey (Ex. 1004). McCloskey describes a method of DNA analysis for single-cell samples that utilizes 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 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.

114. 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.*



Ex. 1004 at Fig. 1.

115. 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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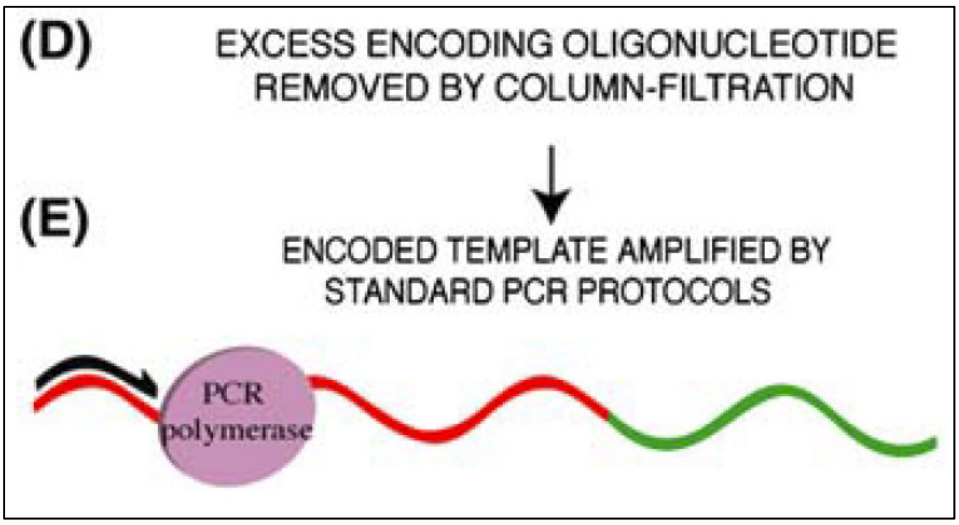
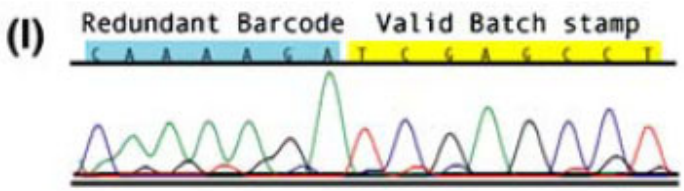
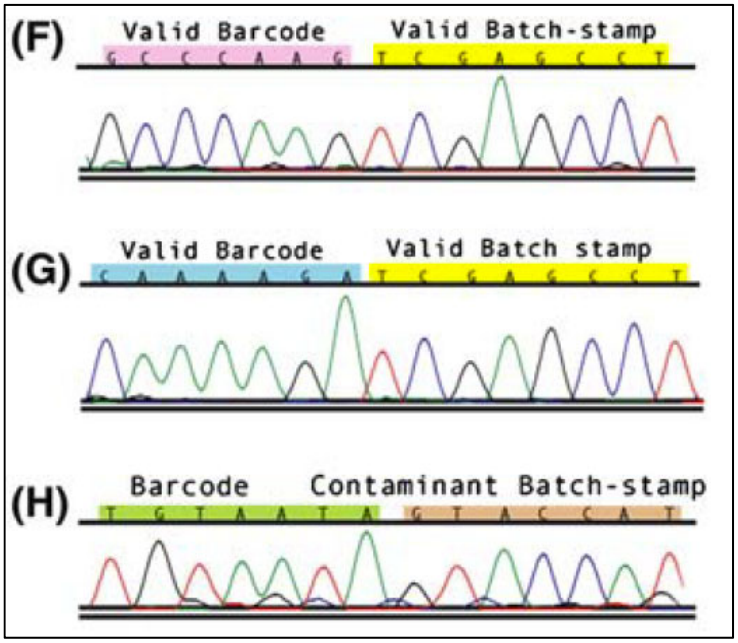


Ex. 1004 at Fig. 1.

116. 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.*



117. Ex. 1004 at Fig. 1.Another benefit that McCloskey's method teaches is the ability to identify valid sequences and distinguish them from contaminant sequences. 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.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 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).

C. McCloskey II (Ex. 1055)

118. U.S. Patent Publication 2007/0020640 ("McCloskey II"), entitled "Molecular Encoding of Nucleic Acid Templates For PCR And Other Forms Of Sequence Analysis," was published on January 25, 2007. McCloskey II (Ex. 1055). I understand from counsel that McCloskey II is prior art under pre-AIA 35 U.S.C. § 102(a) and (b). Further Counsel has explained that during prosecution of the '197 Patent, the Applicant identified McCloskey II as prior art in an IDS. EX1005.106, 147. The Examiner raised no prior art rejection based on McCloskey II.

119. The subject matter of McCloskey II was invented by the authors of McCloskey and teaches substantially the same method with minor additions. Thus, similar to McCloskey, McCloskey II also teaches a method for tagging

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polynucleotides with an encoding oligonucleotide comprising a "molecular barcode and batch-stamp." McCloskey II (Ex. 1055) at Abstract. Similar to McCloskey, the McCloskey II encoding oligonucleotide can be used to identify the origin (cell and molecule) of each tagged oligonucleotide. *Id.* at [0093]. Further, McCloskey II teaches all the same routine processing techniques as McCloskey, such as amplification, sequencing, and ligating and annealing oligonucleotides. *Id.* at [0034], [0035], [0036], [0093], [0098], Claim 5, 8. Beyond the teaching of McCloskey, McCloskey II teaches explicitly that the random barcode sequence may comprise up to 30 random nucleotides. *Id.* at [0021].

XII. PRIOR ART ANTICIPATED OR WOULD HAVE RENDERED OBVIOUS THE CLAIMS OF THE '197 PATENT

A. Ground 1: Claims 1-4, 6-11, 20, and 23-25 Are Unpatentable as Obvious over Linnarsson In View Of McCloskey

1. Claim 1:

a) [1A] A method of counting nucleic acids in a sample, the method comprising:

120. To the extent the preamble is deemed limiting, it is my opinion that Linnarsson and McCloskey each disclose a method of counting nucleic acids from a sample.

121. Specifically, Linnarsson discloses a method of counting nucleic acids in a sample: "Sequence reads are mapped to the genome to reveal sites of transcription, and quantitation is based simply on hit counts, with great sensitivity

and nearly unlimited dynamic range." Linnarsson (Ex. 1003) at 2:21-22; *see also* Linnarsson Provisional (Ex. 1015) at 36:31-33 ("all mapping reads were counted to generate a raw count"). It is my opinion that a POSA would have understood the term "reads" to refer to the results of sequencing nucleic acid sequences, which comprises the recited "nucleic acids."

122. The well-known idea of counting nucleic acids in a sample is also seen in McCloskey, which discloses "counting sequences ***with non-redundant barcodes*** provides a quantitative measure of template diversity." McCloskey at 766 (Ex. 1004.006). As discussed in further detail below, the combination of Linnarsson's method with McCloskey's "non-redundant barcodes" yields a method of counting nucleic acids in a sample. It is my opinion that a POSA would understand the quantitative measure of template diversity refers to a count of the sample polynucleotides based on counting sequences with unique tag sequences.

123. Moreover, methods of counting nucleic acids in a sample, particularly using molecular barcodes or tags, were routine in the art at the time of the '197 patent. Indeed, the '197 patent specification provides that any "convenient method for tagging and/or counting individual nucleic acid molecules with unique tags may be employed (see, e.g., U.S. Pat. No. 7,537,897, issued on May 26, 2009; U.S. Pat. No. 7,217,522, issued on May 15, 2007; U.S. patent application Ser. No. 11/377,462, filed on Mar. 16, 2006; and U.S. patent application Ser. No. 12/432,080, filed on

Apr. 29, 2009; each of which is incorporated by reference herein in their)." Ex. 1001 at 22:38-45.

124. Thus, it is my opinion that, to the extent the preamble is limiting, the combination of Linnarsson and McCloskey teaches and renders obvious this limitation.

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

125. 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," thus Linnarsson teaches and renders obvious this limitation.

126. First, Linnarsson discloses the limitation "providing a sample comprising a plurality of single cells." As discussed above in Section VIII, the '197 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 '197 patent's definition of "sample" is consistent with a POSA's understanding of this term, and I have applied this definition in my analysis.

127. 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 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").

128. 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.

129. 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."

130. 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).

131. 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

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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.

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

c) [1C] (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags,

133. It is my opinion that Linnarsson discloses “generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags.”

134. 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. 1016.011.

135. 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.

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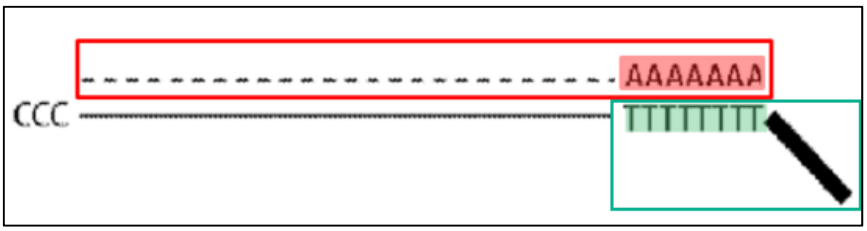
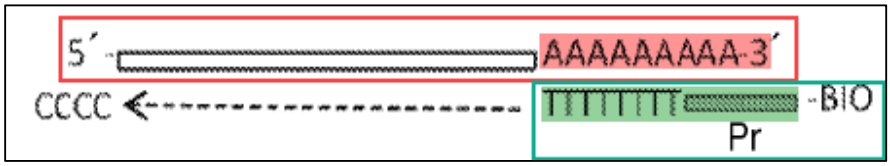
("The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA."); Section X.B., *supra*. Thus, Linnarsson discloses a method of generating a plurality of tagged polynucleotides ***from the sample polynucleotides***, such as sample mRNA.

136. Linnarsson specifically teaches that during cDNA synthesis, oligonucleotide tags 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.

137. 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":



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).

138. 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.

139. 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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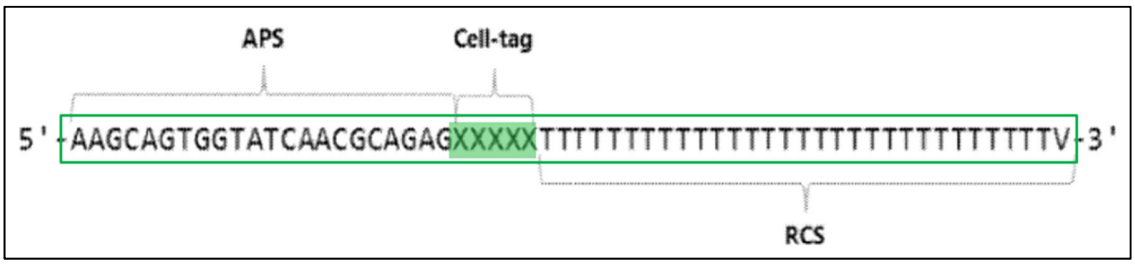
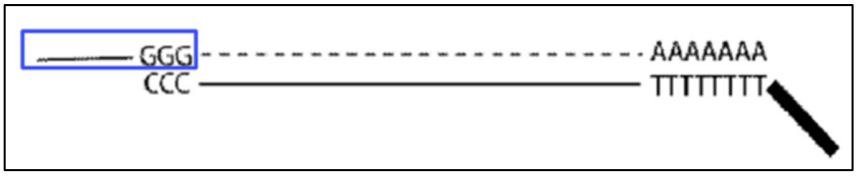


Ex. 1003 at Fig. 4; *see also* Ex. 1015 at Fig.4.

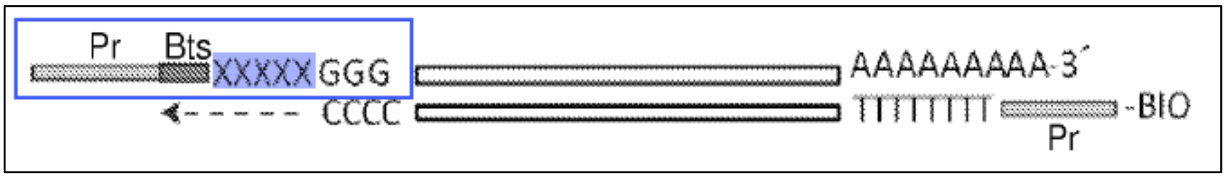
140. Linnarsson discloses that, as part of "synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample . . . incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA …." Linnarsson (Ex. 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.

141. 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."

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:

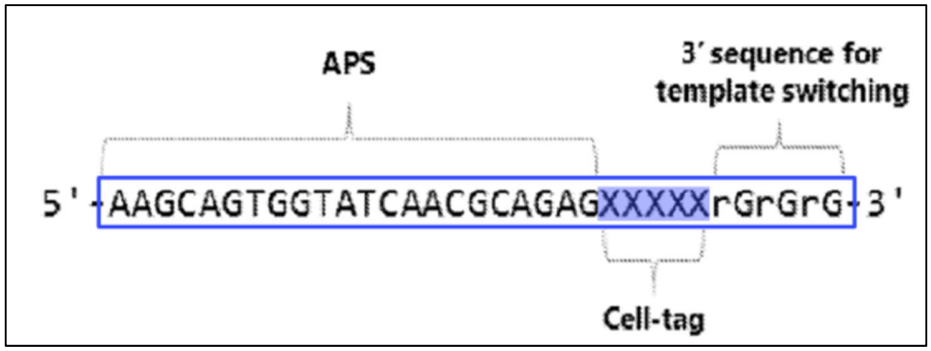


Ex. 1003 at Fig. 2; *see also* Ex. 1015 at Fig. 2.



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

142. 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.

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

144. 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.

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

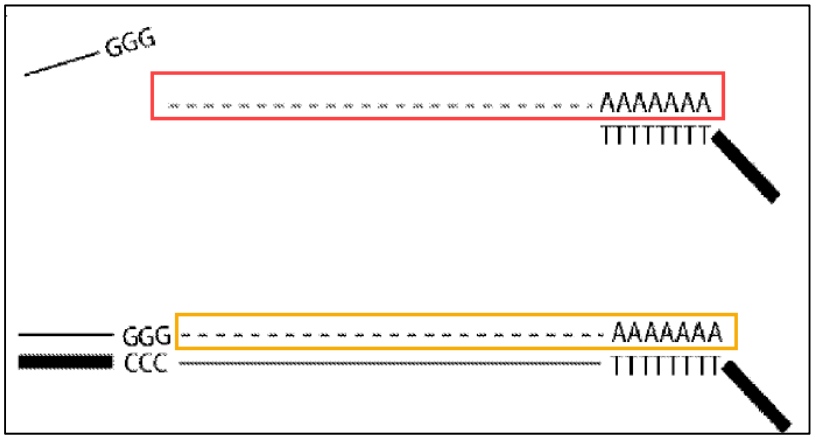
d) [1D] wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (i) a sample sequence from a sample polynucleotide of the plurality of sample polynucleotides;

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

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

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

148. 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):



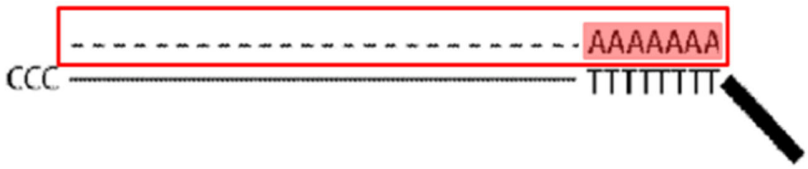
Ex. 1003 at Fig. 2.

149. 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

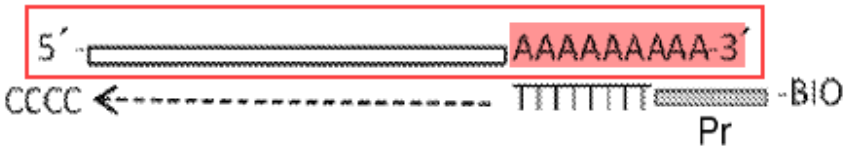
because a cDNA synthesized from a sample mRNA comprises the same sample sequence. As discussed above in Section X.A., the sample mRNA comprises the base pair uracil while the cDNA comprises the base pair thymine. Any POSA would have understood that this difference is trivial because uracil and thymine 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 '197 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.

150. 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

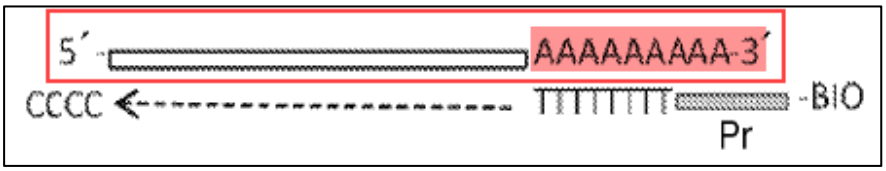
facilitate RNA processing and stability. Linnarsson's Figures 2 and 11, depicted below, show an mRNA molecule with a sequence from the sample polynucleotide (boxed in red) including its poly-A tail (highlighted red):



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.

151. 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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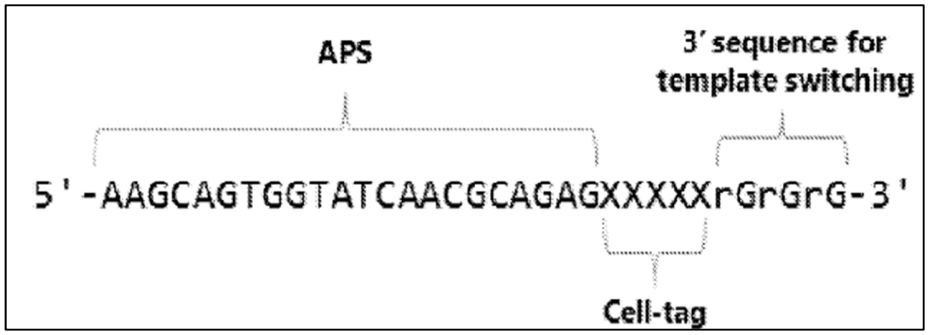
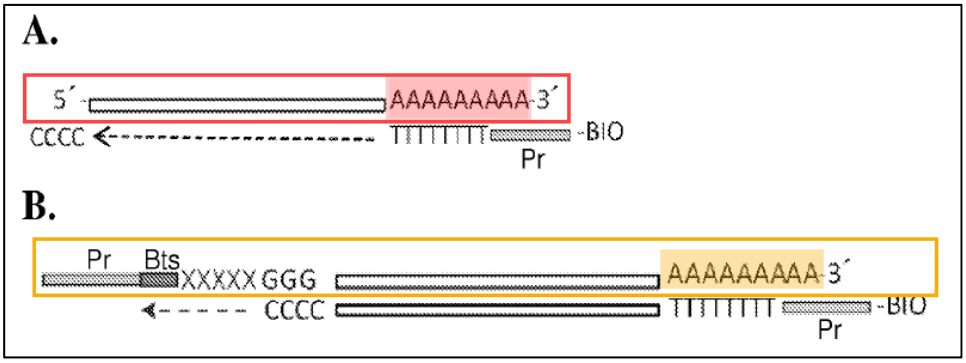
Ex. 1003 at Fig. 2



Ex. 1015 at Fig. 2.



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

152. Therefore, Linnarsson discloses the limitation "a sample 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 a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (ii) a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and

153. It is my opinion that Linnarsson discloses a method comprising the step of generating a plurality of tagged polynucleotides, "wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells."

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

155. A POSA would have understood that in order to use molecular 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].

156. For the reasons above, it is my opinion that Linnarsson discloses limitation 1E.

f) [1F] wherein a tagged polynucleotide of the plurality of tagged polynucleotides comprises: … (iii) a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell;

(1) Combination of Linnarsson and McCloskey Teaches This Limitation

157. It is my opinion that the combination of Linnarsson and McCloskey teaches a method comprising the step of generating a plurality of tagged

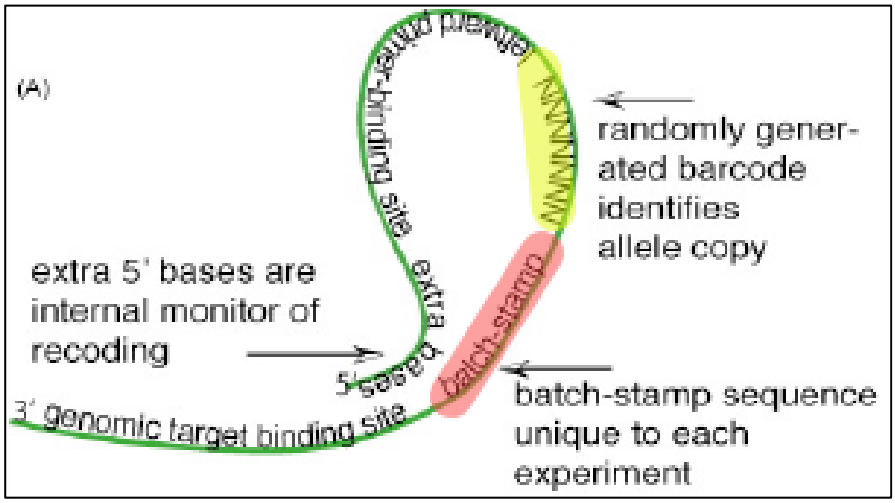
polynucleotides, "wherein each tagged polynucleotide comprises a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell."

158. 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.

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

159. The tag sequence taught by McCloskey is depicted below in Figure 1 as an "encoding oligonucleotide."



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).

160. The second tag 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,

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.

161. 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

162. 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 a molecular tag (*i.e.*, encoding oligonucleotide) comprising two tag

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

163. 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 and teach very similar methods.

164. Second, a POSA would have been motivated to combine Linnarsson's method of tagging polynucleotides with McCloskey's encoding oligonucleotide comprising two tag sequences because McCloskey teaches a solution to a well-known 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 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.

165. 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 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.

166. 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 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).

167. 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.

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

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.

169. Moreover, a POSA would have had a reasonable expectation combining Linnarsson and McCloskey 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. 1016.005.

170. Alternatively, it would be obvious to a POSA that both the CDS tag sequence and the TSO tag sequence could be used in combination, wherein one is used to identify the single-cell of origin of the tagged polynucleotide, and the other tag sequence is used to distinguish tagged polynucleotides originating from the same cell. Linnarsson explicitly contemplates using the CDS and TSO in combination such that tagged polynucleotides would have two associated tag sequences. Linnarsson (Ex. 1003) at 19:6-7 ("The CDS and the TSO can each include a different tag, such that the tagged cDNA sample comprises a combination of tags."). Thus, this alternative design would be identical to the method taught by Linnarsson except

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that either the TSO or CDS would be modified such that it is different for each tagged cDNA.

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

g) [1G] (c) sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence;

172. It is my opinion that Linnarsson discloses a method comprising the step of "sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence."

173. As discussed above in Section VI., Patent Owner previously challenged claims directed to detecting and sequencing DNA samples as nothing but "generic, routine, well-known laboratory techniques." Ex. 1016.012.

174. 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.

175. 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

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> 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) 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.

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176. A POSA would have understood that the sequencing step of Linnarsson discloses determining the sample sequence, the first tag sequence, and the second tag sequence.

h) [1H] (d) using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell.

177. It is my opinion that the combination of Linnarsson and McCloskey discloses this claim element. As discussed above in Sections VIII.A.1.e-f., the combination of Linnarsson and McCloskey discloses and renders obvious using the first tag sequence and the second tag sequence to count a number of sample polynucleotides in said plurality of sample polynucleotides of said cell.

178. I understand that in a prior litigation, Patent Owner alleged that patents directed to counting labeled polynucleotides are abstract. EX1016.012-15, 17-18 (alleging that "the many known techniques for counting molecules" are routine and conventional).

179. As discussed above in Sections XII.A.1.e.-f., the combination of Linnarsson and McCloskey discloses and renders obvious a method of dual molecular tagging using a first tag to identify the single-cell of origin of a sample polynucleotide and using a second tag to uniquely identify each sample polynucleotide arising from the same cell. Further, Linnarsson and McCloskey disclose that this combination of tags allow the precise count of sample

polynucleotides in each single-cell to be determined. For example, Linnarsson provides a method of using tags whereby "mapping reads were counted to generate a raw count." Linnarsson (Ex. 1003) at 38:32. McCloskey provides a method of using the tagged encoding oligonucleotide for "counting sequences with non-redundant barcodes provides a quantitative measure of template diversity." McCloskey (Ex. 1004.006) at 766. Further, I have described exemplary methods whereby the combination of Linnarsson and McCloskey teaches counting sample polynucleotides below with respect to claims 23 and 24. *See* Sections IX.A.20-21.

180. Further, as described above in Section XII.A.1.a., methods of counting polynucleotides in a sample, particularly using molecular tags, were well-known and routine as of the earliest priority date of the '197 Patent. The specification of the '197 Patent provides as much, stating that any "convenient method for tagging and/or counting individual nucleic acid molecules with unique tags may be employed (see, e.g., U.S. Pat. No. 7,537,897, issued on May 26, 2009; U.S. Pat. No. 7,217,522, issued on May 15, 2007; U.S. patent application Ser. No. 11/377,462, filed on Mar. 16, 2006; and U.S. patent application Ser. No. 12/432,080, filed on Apr. 29, 2009; each of which is incorporated by reference herein in their)." Ex. 1001 at 22:38-45.

181. Thus, it is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious using the first and second tag to count a

number of sample polynucleotides in said plurality of sample polynucleotides of said cell.

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

182. 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."

183. 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.

184. 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***

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> ***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.

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

> 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; *see* Linnarsson Provisional (Ex. 1015) at 10:2-3.

186. Thus, as a POSA would understand as standard, Linnarsson teaches amplifying the plurality of tagged polynucleotides prior to sequencing.

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

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

188. 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.

189. I understand from counsel that this claim is written in "Markush form," which means the entire claim is disclosed by the prior art if one alternative in the Markush group is in the prior art. Thus, because Linnarsson discloses a method wherein the plurality of sample polynucleotides are mRNA, Linnarsson discloses this claim.

4. Claim 4: The method of claim 1, wherein the plurality of sample polynucleotides comprises mRNA.

190. 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."

191. 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.

5. Claim 6: The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises said first tag sequence and said second tag sequence.

192. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises said first tag sequence and said second tag sequence."

193. As explained in Section XII.A.1, it is my opinion that a POSA would have been motivated to adopt McCloskey's teachings of using an encoding oligonucleotide comprising two tags sequences and implement it to Linnarsson's method of preparing a tagged cDNA library. It is also my opinion that it would have been obvious to a POSA to conceptually separate the tag sequences in Linnarsson's CDS or TSO into first and second sequences. Thus, the first portion would qualify as the first tag sequence which identifies the cell origin of the tagged polynucleotides from the sample and the second portion would qualify as the second tag sequence which would distinguish the sample polynucleotides originating from the same cell.

6. Claim 7: The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises a sequence that is configured to hybridize to said sample polynucleotide.

194. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein an oligonucleotide

tag of said plurality of oligonucleotide tags comprises a sequence that is configured to hybridize to said sample polynucleotide." Linnarsson's CDS structure as exemplified in Figure 4 and reproduced below, comprises a 3' RNA complementary sequence (RCS) (boxed in red):

Linnarsson (Ex. 1003) at Fig. 4.

195. Linnarsson discloses that the RCS is configured to hybridize to the sample mRNA.

> "In some aspects of the invention, the RCS is at least partially complementary to one or more mRNA in an individual mRNA sample. This allows the primer, which is typically an oligonucleotide, to hybridize to at least some mRNA in an individual mRNA sample to direct cDNA synthesis using the mRNA as a template."

Linnarsson (Ex. 1003) at 15:3-6; Linnarsson Provisional (Ex. 1015) at 5:26-30.

196. Specifically, Figure 11 shows that the RCS has a sequence of thymines (boxed in green) which are able to hybridize with the poly-A tail sequence at the 3' of the mRNA (boxed in red):

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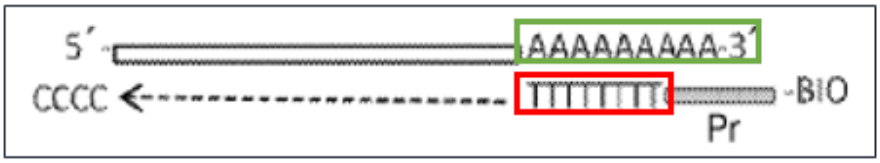

Linnarsson (Ex. 1003) at Fig. 11.

7. Claim 8: The method of claim 1, wherein the plurality of tagged polynucleotides is generated by (i) hybridizing an oligonucleotide tag of said plurality of oligonucleotide tags to said sample polynucleotide and (ii) extending said oligonucleotide tag or said sample polynucleotide or both.

197. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein the plurality of tagged polynucleotides is generated by (i) hybridizing an oligonucleotide tag of said plurality of oligonucleotide tags to said sample polynucleotide and (ii) extending said oligonucleotide tag or said sample polynucleotide or both."

198. As discussed above in Section VI, Patent Owner previously challenged claims directed to tagging polynucleotide samples arguing that hybridizing is a "generic, routine, well-known laboratory technique." Ex 1016.017.

199. As detailed in Section XII A.1.7, Linnarsson teaches the step of hybridization of the oligonucleotide to the sample polynucleotide. Linnarsson discloses that the CDS which is the oligonucleotide tag, includes a sequence of thymines that hybridizes with a sequence of adenines on the ploy-A-tail of the sample mRNA strand, which is the sample polynucleotide. Linnarsson (Ex 1003) at

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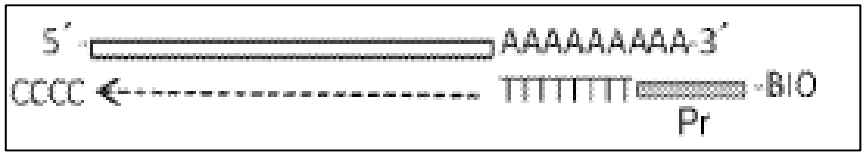
Fig. 2 and 11; Linnarsson Provisional (Ex 1015) at Fig 2 and 11; *see also* Linnarsson (Ex 1003) at 5:29-31; Linnarsson Provisional (Ex 1015) at 5:24-32.

200. Linnarsson further teaches the step of extension of the oligonucleotide tag. As described above in Section XII.A.1.C, Linnarsson discloses that "a tailed oligo-dT primer directs synthesis of a cDNA strand." Linnarsson (Ex. 1003) at 5:29-31; Linnarsson Provisional (Ex. 1015) at 5:24-32, 13:8-14. Specifically, Figure 11 shows the direct synthesis of a cDNA strand by extension of the CDS through the process of reverse transcription:



Linnarsson (Ex. 1003) at Figure 11; Linnarsson Provisional (Ex. 1015) at Fig. 2.

8. Claim 9: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one linear amplification reaction.

201. It is my opinion that Linnarsson discloses and renders obvious the "method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one linear amplification reaction."

202. As described above in Section XII.A.1.C, Linnarsson teaches the step of reverse transcription to generate plurality of tagged polynucleotides. Linear amplification, as discussed above in Section X.B, is the generation of a duplex DNA

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or cDNA through the process of reverse transcription. Thus, a POSA would understand that linear amplification refers to reverse transcription.

203. Linnarsson discloses that "cDNA is typically synthesized from mRNA by reverse transcription." Linnarsson (Ex 1003) at 14:17; Linnarsson Provisional (Ex 1015) at 4:37. Linnarsson describes steps of cDNA synthesis through reverse transcription:

> 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, pooling the tagged cDNA samples and amplifying the pooled cDNA samples to generate a cDNA library having double-stranded cDNA.

Linnarsson (Ex 1003) at 3:25-30; Linnarsson Provisional (Ex 1015) at 2:27-3:3.

204. Linnarsson also discloses that the "cells are lysed and reverse transcription reaction mix is added directly to the lysates….This results in the synthesis of cDNA from cellular mRNA…." Linnarsson (Ex. 1003) at 22:24-26; Linnarsson Provisional (Ex. 1015) at 11:11-14.

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9. Claim 10: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one reverse transcription reaction.

205. It is my opinion that, for the same reason as explained above with respect to claim 9, this claim is rendered obvious by the combination of Linnarsson and McCloskey. *See* Section XII.A.8, *supra*.

10. Claim 11: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one polymerase chain reaction (PCR).

206. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one polymerase chain reaction (PCR)."

207. Linnarsson teaches 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, *supra*.

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11. Claim 20: The method of claim 1, wherein said plurality of sample polynucleotides are randomly associated with said plurality of oligonucleotide tags to generate said plurality of tagged polynucleotides.

208. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein said plurality of sample polynucleotides are randomly associated with said plurality of oligonucleotide tags to generate said plurality of tagged polynucleotides."

209. First, it is my opinion that a POSA would understand molecular tags are routinely added to a plurality of sample polynucleotides in such a way that association between barcodes and sample polynucleotides happens stochastically. Hug at 616 (Ex. 1007.002) ("methods use the fact the hybridization of DNA molecules with identical sequences is competitive and therefore, the tags are selected randomly.") This is the typical method by which barcodes are added—by placing them into a mixture with sample polynucleotides and allowing them to associate randomly.

210. Second, Linnarsson discloses such a method of adding a plurality of oligonucleotide tags to a mixture containing sample polynucleotides to generate tagged polynucleotides. Linnarsson teaches that "the CDS and/or the TSO can be added to each individual mRNA sample during cell lysis, as this will aid hybridization of the oligonucleotides." EX1003 at 18:27-29. Indeed, Linnarsson

teaches that tags hybridize to mRNA in a stochastic non-specific manner because each CDS attaches to mRNA by hybridizing with the poly-A tail, which is identical on all sample mRNA regardless of the unique tag sequence. Thus, a POSA would understand that Linnarsson teaches adding oligonucleotide tags to a mixture containing sample polynucleotides under the appropriate conditions, as disclosed by Linnarsson, such that the tags randomly associate with the sample polynucleotides to generate the plurality of tagged polynucleotides.

12. Claim 23: The method of claim 1, wherein step (d) comprises using said first tag sequence to distinguish (i) the number of sample polynucleotides having said sample sequence from said cell from (ii) the number of sample polynucleotides having said sample sequence from other cells.

211. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein step (d) comprises using said first tag sequence to distinguish (i) the number of sample polynucleotides having said sample sequence from said cell from (ii) the number of sample polynucleotides having said sample sequence from other cells."

212. I understand that in a prior litigation, Patent Owner alleged that patents directed to counting labeled polynucleotides are abstract. EX1016.012-15, 17-18 (alleging that "the many known techniques for counting molecules" are routine and conventional).

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213. First, it is my opinion that the combination of Linnarsson and McCloskey teaches counting the number of sample polynucleotides having the same sample sequence. As explained above in Sections XII.A.1.c. and XII.A.1.f.-h., the combination of Linnarsson and McCloskey teaches a method of attaching unique molecule-tag sequences (*i.e.*, "second tag sequences") to each sample polynucleotide during the process of generating tagged polynucleotides, and counting the number of different molecule-tags in the sequenced cDNA to count the number of sample polynucleotides. Using this method, the second tag, which is molecule (polynucleotide) specific, one can distinguish among tagged polynucleotides which contain the same sample sequence (*i.e.*, sample mRNA sequence). This method can be used to determine the number of sample polynucleotides having a given sample sequence from the same cell. By counting the number of different molecule tags attached to cDNA having the same sequence and the same cell tag, one can estimate the number of identical sample mRNA in the sample single-cell. Further, a POSA would understand that such a technique for determining the number of sample polynucleotides having the same sequence was well-known and routine. Indeed the specification of the '197 patent provides any "convenient method for tagging and/or counting individual nucleic acid molecules with unique tags may be employed." Ex. 1001 at 22:38-40; Hug at 615 (Ex. 1007.001) (providing two exemplary methods "to

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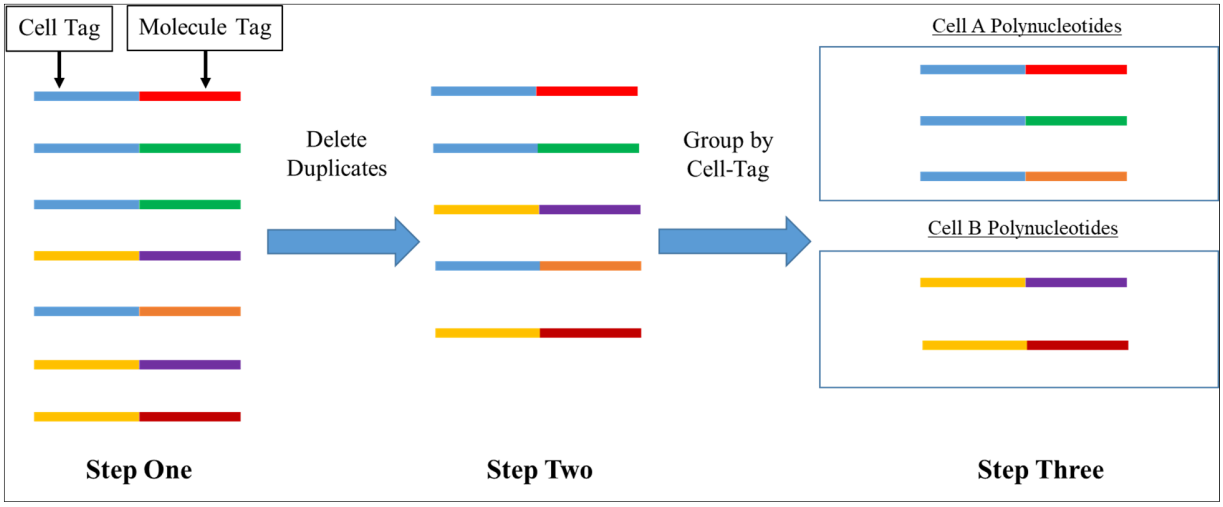
calculate the number of molecules of a single mRNA species in a complex mRNA preparation").

214. For example, the simplified exemplary model below demonstrates how different second tag sequence can be used to estimate the number of sample polynucleotides having said sample sequence. For example, the green molecule tag and the red molecule tag may be associated with the same sample sequence ("sequence 1"). In that event, the duplicate green tag molecule would be removed in step two. Thus, in step three, by counting the total number of molecules with sequence 1 (associated with the red and green tags), one could determine that two copies of sample sequence 1 were present in said cell.



Graphic 1

215. Second, it is my opinion that the combination of Linnarsson and McCloskey teaches "using said first tag sequence to distinguish (i) the number of

sample polynucleotides having said sample sequence from said cell from (ii) the number of sample polynucleotides having said sample sequence from other cells." As explained above in Section XII.A.1.e., the combination of Linnarsson and McCloskey teaches a method of dual tagging polynucleotides using a cell-tag (*i.e.*, the "first tag sequence"), which can be used to identify the single-cell of origin of a sample polynucleotide and a molecule tag (*i.e.*, the "second tag sequence"), which can be used to distinguish sample polynucleotides arising from the same cell. Using this method, tagged cDNA comprising an mRNA sequence from the same single-cell of origin are all associated with the same unique cell tag (*i.e.*, Linnarsson's TSO and/or CDS tag; McCloskey's batch-stamp), which is different and distinguishable from other cell tags for cDNA, which may comprise the same mRNA sequence, but arise from other single-cells.

216. Therefore, this method can be used to determine the number of cDNA comprising a specific mRNA sequence arising from any single-cell by counting the number of specific unique cell tag associated cDNA comprising the same sample sequence and different polynucleotide tags. Additionally, the same procedure could be done for each single-cell in a sample. Thus, by counting the number of other different unique cell tags associated with the sample polynucleotide sequence, one could determine the number of cDNA comprising the same mRNA sequence arising from different cells.

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217. For example, a simplified exemplary model is shown in Graphic 1 above. Step one involves the detection, via sequencing, of the tagged cDNA library generated through the method taught by the combination of Linnarsson and McCloskey. Each of the tag combinations shown would be associated with a sample sequence from a sample polynucleotide such that each sample polynucleotide would be tagged with a different molecule tag, even if they have identical sequences. Once sample sequences and their associated first tag (cell tag) and second tag (molecule tag) have been identified, Step Two involves the removal of duplicate second tags from the count. This is because second tags mark a unique sample molecule and duplicates are the result of amplification, which can skew accurate counts. *See supra* Section X.C.4. (discussing amplification bias). Finally, step three involves the counting of the number of first tags, which allows one to distinguish the number of sample polynucleotides having a given sample sequence from a given cell from the number of sample polynucleotides having said sample sequence from other cells. For example, below the blue tags are associated with "Cell A." After duplicate second tags are removed, there are three remaining molecules associated with a blue tag—thus, the total number of polynucleotide molecules arising from Cell A was three. Similarly, the yellow tag is associated with "Cell B." Thus, there are two molecules arising from Cell B.

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218. Thus, the combination of Linnarsson and McCloskey renders obvious using cell tags to identify the single-cell origins of sample polynucleotides, and therefore renders obvious "using said first tag sequence to distinguish (i) the number of sample polynucleotides having said sample sequence from said cell from (ii) the number of sample polynucleotides having said sample sequence from other cells."

13. Claim 24: The method of claim 1, wherein step (d) comprises determining the number of different second tag sequences associated with said sample sequence, thereby estimating the number of sample polynucleotides having said sample sequence from said cell.

219. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein step (d) comprises determining the number of different second tag sequences associated with said sample sequence, thereby estimating the number of sample polynucleotides having said sample sequence from said cell." As discussed above in Section XII.A.18., it is my opinion that the combination of Linnarsson and McCloskey teaches and renders obvious that by counting the number of different molecule-tags (*i.e.*, "second tag sequences") in the sequenced cDNA comprising the same mRNA sequence, the number of sample polynucleotides having the same sample sequence from the cell can be determined.

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14. Claim 25: The method of claim 1, wherein said plurality of sample polynucleotides comprises substantially all mRNA molecules of said cell.

220. It is my opinion that the combination of Linnarsson and McCloskey discloses and renders obvious the "method of claim 1, wherein said plurality of sample polynucleotides comprises substantially all mRNA molecules of said cell."

221. As explained above in Sections XII.A.1.b and XII.A.3, Linnarsson describes that the plurality of sample polynucleotides may comprise mRNA molecules. It is my opinion that Linnarsson also teaches that said plurality of sample polynucleotides can comprise substantially all mRNA molecules of said cell, as discussed in Section XII.A.13.

222. Linnarsson's method begins with "releasing mRNA from each single cell to provide a plurality of individual mRNA samples." Ex. 1003 at 3:26-28; Ex. 1015 at 2:30-32. A POSA would understand that because the mRNA is indiscriminately released from each single cell, and because Linnarsson does not describe any additional elimination or filtration, the released mRNA sample comprises substantially all mRNA molecules of said cell. As discussed above, Linnarsson's method then tags these sample polynucleotides for the purpose of gene expression analysis. A POSA would understand that the purpose of such a method would be to analyze substantially all mRNA molecules of a cell—therefore, this method would provide associating a tag with substantially every sample mRNA

molecule. Ex. 1003 at 1:11-12, 1:25-26 ("direct sequencing of the ***all***, or parts, ***of the mRNA content*** of a tissue is being increasingly used."); Ex. 1015 at 1:10-11, 1:31-33. Thus, a POSA would understand that Linnarsson describes a method which is designed to be applied to substantially all mRNA molecules of a given cell.

B. Ground 2: Claims 5, 12, 21-22, and 26 are Unpatentable as Obvious Over Linnarsson in View of McCloskey, and Further in View of the Knowledge of a POSA

1. Claim 5: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction.

223. As explained in Section XII.A.1., the combination of Linnarsson and McCloskey teaches and renders obvious the method of claim 1. Further, 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."

224. As discussed above in Section XII.1.f.ii, a POSA would have been motivated to adopt McCloskey's teachings to modify Linnarsson's method so that the tags include a first sequence, which could distinguish between different cells, and a second sequence, which could distinguish sample 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.

225. As of the earliest priority date of the '197 Patent, it was well-known and routine in the art to attach tag sequences using ligation. Indeed, the specification of the '197 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; 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

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> 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, 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.

226. 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").

227. 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

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by using a linker molecule, such as the method described in Hug's 2003 article. Ex. 1007.001 ("Hug").

228. 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 tag. 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 tag sequence, which in the method of Hug are additional linkers used to tag. *Id.*

229. 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.

230. 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.

231. 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.

2. Claim 12: The method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with the same first tag sequence.

232. It is my opinion that the combination of Linnarsson and McCloskey renders obvious the "method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with the same first tag sequence."

233. As described above in Section XII.A.1.E, Linnarsson's cell-tag which is the "first tag sequence", identifies the single-cell origin of sample polynucleotides:

> "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 after the tagged cDNA samples have been pooled in the methods described herein."

Linnarsson (Ex 1003) at 19:5-11

234. First, a POSA would have been motivated to tag substantially every sample polynucleotide with the same first tag sequence (i.e., cell-tag). Linnarsson teaches using a cell-tag to identify the cell-of-origin for each sample polynucleotide. *See supra* Section XII.A.1.h. A POSA would have understood that tagging substantially every sample polynucleotide would maximize the amount of information (i.e., cell origin) one can glean from each cell.[3]

235. Second, a POSA would have had a reasonable expectation of successfully tagging substantially every sample polynucleotide with the same cell-tag. A POSA would have understood that increasing the amount of oligonucleotide tags relative to the number of sample nucleotides would increase the percentage of sample nucleotides being tagged, thus ensuring that substantially every sample polynucleotide is associated with the same first tag. Therefore, it would have been

[3] I understand from counsel that claim 12 depends from claim 1, which recites "***a cell*** of the plurality of cells comprising ***a plurality of sample polynucleotides***." Thus, in my analysis, I have interpreted the recited "every sample polynucleotide of ***said plurality of sample polynucleotides***" in claim 12 to originates from "a cell" (i.e., the same single-cell) as recited in claim 1.

a matter of routine optimization to tag substantially every sample polynucleotide with the same first tag sequence.

236. Indeed, the ’197 Patent specification admits that such techniques were routine, providing:

> “a large collection of sequences is tagged with MID such that each polynucleotide molecule in the sample has a unique MID. In other words, each polynucleotide in the sample (e.g., each individual double stranded or single stranded polynucleotide) is tagged with a MID that is different from every other MID on every other polynucleotide in the sample. In general, to accomplish such molecular tagging the number of distinct MID tags to be used should be many times greater than the actual number of molecules to be analyzed. This will result in the majority of individual nucleic acid molecules being labeled with a unique ID tag (see, e.g., Brenner et al., Proc. Natl. Acad. Sci. USA. 2000 97(4):1665-70).”

Ex. 1001 at 22:48-60.

237. The Brenner 2000 prior art article, cited by the specification of the ’197 Patent, teaches using over 100 times more unique tags than sample polynucleotides. Brenner 2000 then discloses that doing so ensures that each sample polynucleotide is tagged with a unique tag sequence:

> By making the repertoire of tags large relative to the number of polynucleotides in the mixture, samples of the tag-polynucleotide

> conjugates may be selected with virtually every polynucleotide having a unique tag.

Brenner 2000 at 1665 (Ex. 1035.001). A POSA would have understood "virtually every" to mean "substantially every."

238. Similarly, Linnarsson teaches attaching a tag to each individual mRNA sample. *See* 16:18-20 ("A template-switching oligonucleotide (TSO) that includes a portion which is at least partially complementary to a portion of the 3'd end of the first strand of cDNA can be added to ***each*** individual mRNA sample…"), 18:27-30 ("If the lysis is achieved partially by heating, then the CDS and/or the TSO can be added to ***each*** individual mRNA sample during cell lysis…"), 46:22-24 ("wherein a template-switching oligonucleotide (TSO) is added to ***each*** individual mRNA sample ….").

Therefore, the combination of Linnarsson and McCloskey teaches and renders obvious claim 12.

3. Claim 21: The method of claim 1, wherein step (c) comprises hybridizing said tagged polynucleotide to a solid support.

239. It is my opinion that the combination of Linnarsson and McCloskey in view of the knowledge of a POSA discloses and renders obvious the "method of claim 1, wherein step (c) comprises hybridizing said tagged polynucleotides to a solid support"

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240. Specifically, such a method of utilizing a bead as a solid support, was well-known and routine in the art at the time of the earliest priority date of the '197 patent. For example, Linnarsson teaches that sequencing through the commercially available high throughput screening methods including the SOLiD sequencing platform was well-known. Linnarsson (Ex. 1003) at 21:16-18; Linnarsson Provisional (Ex. 1015) at 8:35-9:2 ("the cDNA library can be processed to make the sample suitable for any high-throughput screening methods, such as Applied Biosystems' SOLiD sequencing technology."). *See also* Linnarsson (Ex. 1003) at 22:6-8; Linnarsson Provisional (Ex. 1015) at 9:19-22.

241. The Applied Bio systems SOLiD System was one of the sequencing platforms in widespread commercial use well before 2009. Mardis at 388 (Ex. 1045.002. The platform along with several other common and commercially available sequencing platforms available at the time including Roche 454, rely on a standard method in their protocol of immobilizing sample polynucleotides to a solid support. This solid support is in the form of beads. Mardis notes that the "SOLiD platform uses an adaptor-ligated fragment library similar to those of the other next-generation platforms, and uses an emulsion PCR approach with small magnetic beads to amplify the fragments for sequencing." *Id.* at 393 (Ex. 1045.007). The SOLiD System utilizes a biotin-streptevidin interaction to immobilize the sample

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polynucleotides to the bead. On the other hand, Roche 454 relies on hybridization to achieve the immobilization. *Id.*

242. Linnarsson further discloses that the APS which is incorporated in the oligonucleotide tags i.e. the CDS or TSO, "can be identical to the SOLiD P1 primer, and/or a SOLiD P2 sequence inserted in the CDS, so that the P1 and P2 sequences required for SOLiD sequencing are integral to the amplified library." Linnarsson (Ex. 1003) at 19:31-20:2; Linnarsson Provisional (Ex. 1015) at 8:28-31. Thus, Linnarsson teaches that the tagged polynucleotides comprise of sequences which are integral to sequencing methods.

243. Additionally, since Linnarsson discloses that the sequencing methods include "any high-throughput screening method," it would be obvious for a POSA to use any commercially available sequencing methods around such time including the well-known Roche 454 platform. A POSA would further be motivated to use the Roche 454 system as a standard substitute to the SOLiD System since Roche 454 utilizes hybridization of polynucleotides. As described in detail above in Section X.C.4, Roche sequencing utilizes a method whereby sample oligonucleotides are immobilized by hybridization to a capture bead, which constitutes a solid support. Mardis further describes that in Roche 454:

> "the library fragments are mixed with a population of agarose beads whose surfaces carry oligonucleotides complementary to the 454-

specific adapter sequences on the fragment library, so each bead is associated with a single fragment."

Mardis at 389 (Ex. 1045.003); *see also id.* at Fig 1b.

244. Indeed, the specification of the '197 Patent provides that sequencing may be conducted via the routine and commercially available method of Roche 454 sequencing. Ex. 1001 at 17:7-11 ("For example, sequencing a polynucleotide region that is 2 kilobases or more in length using Roche 454 (Branford, Conn.) technology, in which the length of a single sequencing run is about 400 bases."), 17:26-32, 19:12-15 ("The resulting polynucleotide can be used in subsequent analyses as desired by the user (e.g., Roche 454 sequencing technology)."). The Roche 454 platform, along with several other common and commercially available sequencing platforms available at the time, rely on a standard method in their protocol of immobilizing sample polynucleotides via hybridization to a solid support.

245. Thus, given that the SOLiD system referred to in Linnarsson and other common sequencing platforms at the time relied on a solid support, a POSA would have had a reasonable expectation of successfully performing such sequencing

4. Claim 22: The method of claim 20, wherein said solid support is a bead.

246. It is my opinion that it would have been obvious to a POSA to perform the method disclosed by the combination of Linnarsson and McCloskey by further hybridizing the tagged polynucleotides to a solid support, such as a bead, during the

sequencing step (step (c)), as exemplified by Roche 454 and SOLiD sequencing. *See supra* Section XII.B.2.

5. Claim 26: The method of claim 1, wherein said plurality of sample polynucleotides comprises a subset of polynucleotides of said cell having the same sequence.

247. It is my opinion that the combination of Linnarsson and McCloskey, further in view of the knowledge of a POSA, teaches and renders obvious the method of claim 1, "wherein said plurality of sample polynucleotides comprises a subset of polynucleotides of said cell having the same sequence."

248. It is my opinion that it would have been a simple and obvious design choice to modify the combination of Linnarsson and McCloskey to target a specific sequence such that the plurality of sample polynucleotides comprise a subset of polynucleotides having the same sequence.

249. First, a POSA would have had a reasonable expectation of success targeting a specific sequence or set of sequences. A POSA would understand that many well-known and routine methods were available for the targeted selection of a specific genomic loci (specific nucleotide sequence). Such techniques are a key tool in a variety of modern molecular biology applications. Albert at 903 (Ex. 1057.001) ("The targeted enrichment or specific regions in complex genomes is a fundamental practice of modern molecular biology."). For example, one method that would have been obvious for a POSA to apply is targeted capture, such as a microarray. A

targeted capture approach, which was routine, would have allowed selection of a specific target sequence from a diverse polynucleotide sample—to the exclusion of all other non-targeted polynucleotides. A 2007 article ("Albert") teaches a method where custom microarrays are designed and used to "capture" target polynucleotides by "hybridiz[ing] single-stranded products to the capture arrays." Albert at 904 (Ex. 1057.002). Once target polynucleotides have been "captured" (hybridized to the microarray), this method "removed non-hybridized materials by washing," which leaves only the isolated target polynucleotide sequences. Albert at 904 (Ex. 1057.002). This method allows for isolation, through capture, of a target sequence from a sample, excluding all other non-target polynucleotides.

250. Second, there are numerous reasons why a POSA would have been motivated to target a specific sample sequence. For example, specific genes (genomic loci or target sequences) may be of interest due to their relevance to a disease, common mutation, or to explore variation in a population of research purposes. Albert at 904 (Ex. 1057.002) (explaining that genes may be selected to "determine our ability to discover variation in the human genome.").

Thus, it would have been a simple and obvious design choice for a POSA to have a plurality of sample polynucleotides comprise a subset of polynucleotides having the same sequence.

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C. Ground 3: Claims 13-19 are Unpatentable as Obvious Over Linnarsson, McCloskey, and further in View of McCloskey II

1. Claim 13: The method of claim 1, wherein at least 90 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.

251. It is my opinion that the combination of Linnarsson, McCloskey, and McCloskey II teaches and renders obvious this claim.

252. First, a POSA would have been motivated to ensure that each sample polynucleotide is tagged with a unique second tag sequence. As I explained above, the combination of Linnarsson and McCloskey renders obvious using a second tag sequence for counting and distinguishing sample polynucleotides originating from the same single-cell. *See* Section XII.A.1.f, h, *supra*. A POSA would have understood that the second tag sequence facilitates accurate counting by unique identifying each sample polynucleotide existing in a sample cell prior to amplification. Thus, by counting the number of unique tags in the post-amplification mixture, one can count the number of sample polynucleotides in the pre-amplification mixture. Furthermore, untagged polynucleotides cannot be attributed to sample polynucleotides in the pre-amplification mixture. For example, if the post-amplification mixture contains 10 untagged polynucleotides having the same sequence, it would be impossible to determine the number of polynucleotides having this sequence in the sample cell. Moreover, accuracy in counting number of sample

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polynucleotides in the sample cell is critical for gene expression analyses. *See* Section X.C.2, *supra*. Therefore, a POSA would have been motivated to ensure that each sample polynucleotide is tagged to increase the accuracy of counting.

253. Second, a POSA would have had a reasonable expectation to successfully tag each sample polynucleotide with a unique second tag sequence. As explained above in Section XII.B.2, it would have been obvious to tag ***each*** sample polynucleotide with a tag. For example, a POSA would have understood that one could ensure each sample polynucleotide of said plurality of sample polynucleotides would be tagged by using many times more tags than sample polynucleotides (*e.g.*, at least 100 times more tags than sample polynucleotides). *See also* EX1001 at 22:50-59 (admitting that it was known to ensure that "each polynucleotide in the sample…tagged with a MID…different from every other MID on every other polynucleotide in the sample" by using a "number of distinct MID tags…many times greater than the actual number of molecules to be analyzed.") (citing Brenner 2000 (Ex. 1035)).

254. Furthermore, it is also my opinion that it would have been obvious to tag each sample polynucleotide with a ***unique*** second tag sequence. Indeed, it would have been a matter of routine optimization to design molecular tags such that an extremely large number of unique tags can be generated in order to ensure sufficient unique tags to identify a large sample of polynucleotides. For example, McCloskey

teaches that using a tag sequence containing *n* number of random base pairs "enables one to distinguish 4^n allele copies per reaction." McCloskey at 764 (Ex. 1004.004). This is possible because each base pair can be any of the four nucleotide bases (a, t, g, c/u), as described above in Section X.A. Thus, the longer a tag sequence is, with each nucleotide being a random nucleotide the number of unique tags produced increases exponentially. Thus, a POSA would have understood that to generate *m* number of unique tag sequences, one can design tag sequences to have $\frac{\log(m)}{\log(4)}$ base pairs.

255. For instance, a POSA could have easily designed tag sequences comprising 20-30 random nucleotide bases, which would allow the creation of 4^{20}-4^{30} unique barcodes distinguishing among 1,099,511,627,776-1,152,921,504,606,846,976 polynucleotides. For example, McCloskey II teaches using second tag sequences each comprising up to 30 base pairs. As disclosed in McCloskey II, "the length of the second sequence is between 3 and 30 nucleotides". McCloskey II at ¶ [0021] (Ex. 1055). Linnarsson also discloses tag sequences comprising at least 20 nucleotides. EX1001 at 18:31-19:2. A POSA would have understood that using second tag sequences comprising 20-30 nucleotides would provide significantly more unique tag sequences than would be necessary to ensure that each sample polynucleotide from any single cell receives a unique second tag

sequence. *See* Ex. 1054.001 (estimating between 50,000 and 300,000 mRNA per mammalian cell).

256. Therefore, the combination of Linnarsson, McCloskey and McCloskey II renders obvious claim 13.

2. Claim 14: The method of claim 1, wherein at least 95 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.

It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson, McCloskey and McCloskey II. *See* Section XII.C.1, *supra*.

3. Claim 15: The method of claim 1, wherein at least 99 percent of said plurality of sample polynucleotides is associated with a unique second tag sequence.

257. It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson, McCloskey and McCloskey II. *See* Section XII.C.1, *supra*.

4. Claim 16: The method of claim 1, wherein substantially every sample polynucleotide of said plurality of sample polynucleotides is associated with a unique second tag sequence.

258. It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson, McCloskey and McCloskey II. *See* Section XII.C.1, *supra*.

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5. Claim 17: The method of claim 1, wherein the number of different second tag sequences is larger than the number of sample polynucleotides.

259. It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson, McCloskey and McCloskey II. *See* Section XII.C.1, *supra*. Specifically, it is my opinion that a POSA would have found it obvious to use a number of different second tag sequences larger than the number of sample polynucleotides so that each sample polynucleotide would be tagged with a unique second tag sequence.

6. Claim 18: The method of claim 17, wherein the number of different second tag sequences is at least ten times the number of sample polynucleotides.

260. It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson and McCloskey II. *See* Section XII.C.1, *supra*. Specifically, it is my opinion that a POSA would have found it obvious to use at least 10 times more unique second tag sequences than sample polynucleotides so that each sample polynucleotide would be tagged with a unique second tag sequence.

7. Claim 19: The method of claim 18, wherein the number of different second tag sequences is at least one hundred times the number of sample polynucleotides.

It is my opinion that, for the same reason as explained above with respect to claim 13, this claim is rendered obvious by the combination of Linnarsson and

McCloskey II. *See* Section XII.C.1, *supra*. Specifically, it is my opinion that a POSA would have found it obvious to use at least 100 times more unique second tag sequences than sample polynucleotides so that each sample polynucleotide would be tagged with a unique second tag sequence.

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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: May 24, 2023

Gregory Cooper, Ph.D.

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