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May 30, 2024

The Honorable Joel H. Slomsky
13614 U.S. Courthouse
601 Market Street
Philadelphia, PA 19106

VIA ELECTRONIC FILING

Re: *10x Genomics, Inc. and The Board of Trustees of the Leland Stanford Junior University v. Parse Biosciences, Inc.*, Case No. 22-1117 (JHS)

Dear Judge Slomsky:

Plaintiffs 10x Genomics Inc. and The Board of Trustees of Leland Stanford Junior University (collectively, "10x") respectfully move to strike from Parse Biosciences Inc.'s ("Parse") opening expert report of Dr. Cooper the invalidity defense Parse is asserting in parallel proceedings with the Patent Trial and Appeal Board ("PTAB"). Specifically, Plaintiffs move to strike the portions of Dr. Cooper's invalidity report in which Parse asserts invalidity of the Brenner Patents based on the prior art combination of Linnarsson in view of McCloskey (the "Linnarsson / McCloskey prior art defense"). Ex. 1 ((Redacted Cooper Expert Report) (excerpt)) at §§ IX.B.1–2, IX.C.1, IX.D.1, IX.E.1, IX.F.1.

Parse's assertion of the Linnarsson / McCloskey prior art defense means that it wishes to litigate in this action the same issues raised in the *inter partes* review ("IPR") proceedings before the PTAB, and creates the potential for inconsistent outcomes between the PTAB and district court. This is improper because Parse already stipulated to the PTAB that it would not raise here the same grounds that it relies on in its IPR petitions: "If instituted, Petitioner will stipulate to not pursuing, in the district court litigation, invalidity arguments based on any instituted IPR ground." Ex. 2 (IPR2023-00876 '981 Patent Petition (excerpt)) at 75; Ex. 3 (IPR2023-00955 '197 Patent Petition (excerpt)) at 75–76; Ex. 4 (IPR2023-00958 '013 Patent Petition (excerpt)) at 70.

Parse does not deny that it made this representation to the PTAB. There is also no dispute that Parse's IPR petitions rely on the Linnarsson / McCloskey prior art defense, also relied on in Dr. Cooper's expert report in this action. *See* D.I. 88, Ex. 1, at 5 (IPR2023-00876 '981 Patent Institution Decision); D.I. 153, Ex. 1, at 5 (IPR2023-00955 '197 Patent Institution Decision); D.I. 153, Ex. 2, at 6 (IPR2023-00958 '013 Patent Institution Decision). Accordingly, Parse is

precluded from arguing in this action that the Brenner Patents are obvious over Linnarsson in view of McCloskey.[1]

That Parse made its representation to the PTAB is not an accident. This type of representation is called a *Sand Revolution* stipulation, and it is commonly made by patent defendants as part of a petition to the PTAB to avoid the PTAB using its discretion to deny institution on the basis that the same issue will be decided by a district court. *See Sand Revolution II, LLC v. Cont'l Intermodal Grp.-Trucking LLC*, No. IPR2019-01393, 2020 WL 3273334, at *5 (P.T.A.B. June 16, 2020) (*informative*). As U.S. Patent & Trademark Office guidance has explained, discretionary denials are a way to "minimize potential conflict between the Patent Trial and Appeal Board (PTAB) and district court proceedings" given the "potential for inefficiency and gamesmanship in AIA proceedings." Ex. 5 (K. Vidal, "Interim Procedure for Discretionary Denials in AIA Post-Grant Proceedings with Parallel District Court Litigation" (June 21, 2022)) at 1. Where the IPR petition includes the same or substantially the same claims, grounds, arguments, and evidence as presented in the parallel district court proceeding, this fact has favored denial by the PTAB of the IPR petition. *Apple Inc. v. Fintiv, Inc.*, No. IPR2020-00019, 2020 WL 2126495, at *5–6 (P.T.A.B. Mar. 20, 2020) (*precedential*). Conversely, if the petition includes materially different grounds, arguments, and/or evidence than those presented in the district court, this fact has tended to weigh against exercising discretion to deny institution. *Id*. Where a party stipulates not to pursue in a parallel district court proceeding the same grounds as in the petition, it mitigates concerns of potentially conflicting decisions and duplicative efforts between the district court and the PTAB. *See Sand Revolution II, LLC*, 2020 WL 3273334, at *5 (concluding petitioner's stipulation that "if the IPR is instituted, Petitioner will not pursue the same grounds in the district court litigation" mitigated concerns of "duplicative efforts" and weighed against discretionary denial). Accordingly, the PTAB is less likely to discretionarily deny institution of an IPR in view of parallel litigation where, as here, Parse has made a *Sand Revolution* stipulation.

After Parse made its *Sand Revolution* stipulation, the PTAB instituted the relevant IPR petitions on October 13, 2023 and February 5, 2024. *See* D.I. 88, Ex. 1, at 1 (IPR2023-00876 '981 Patent Institution Decision); D.I. 153, Ex. 1, at 1 (IPR2023-00955 '197 Patent Institution Decision); D.I. 153, Ex. 2, at 1 (IPR2023-00958 '013 Patent Institution Decision). Thus, Parse cannot rely on the Linnarsson / McCloskey prior art defense in this action. Nevertheless, Parse attempts to renege on its representation to the PTAB in an apparent attempt to get two bites at the apple: litigate invalidity on the same grounds in this action and before the PTAB. This is improper. District courts regularly enforce such PTAB stipulations. *See, e.g.*, *Fujifilm Sonosite, Inc. v.*

[1] 10x's motion to strike applies to all of the Brenner Patents. For the '981 Patent, the PTAB will issue an IPR final written decision before the district court trial, and Parse will be estopped by statute from raising at the district court trial the same invalidity grounds that were presented in Parse's IPR petition. *See* 35 U.S.C. § 315(e)(2). For the '197 and '013 Patents, the PTAB will issue an IPR final written decision for each patent after the district court trial, but Parse represented that it would not raise in the district court trial the same prior art grounds that it has raised in its IPR petitions for those patents.

Butterfly Network, Inc., C.A. No. 22-309-JPM, D.I. 159, at 6 (D. Del. Sept. 18, 2023) (attached as Ex. 6); *InVue Sec. Prods., Inc. v. Vanguard Prods. Grp., Inc.*, No. 8:18-cv-2548-T-33SPF, 2020 WL 2425721, at *2 (M.D. Fla. May 12, 2020) (district court enforcing defendants' stipulation not to use patents or publications in district court if IPR not successful).

For example, in *Fujifilm*, the defendant's petitions each included a representation that it would not "not pursue in district court litigation [. . .] any ground raised or that could have been reasonably raised before the PTAB in this IPR if this Petition is instituted." Ex. 6 at 5–6. After the PTAB instituted each petition, the defendant tried to incorporate the same invalidity grounds that it raised in its IPR petitions into its district court counterclaims. Ex. 6 at 6. The Delaware district court struck the invalidity defenses that the defendant had represented to the PTAB it would not pursue. *Id.* The court reasoned that the defendant "is not allowed to take a second bite at the apple by re-litigating its IPR invalidity contentions . . . in this Court." *Id.* The same result is appropriate here.

Parse now argues its PTAB stipulation should no longer apply because of "substantial unforeseeable and changed circumstances" in that two of the three IPRs would not reach final written decisions prior to trial. Ex. 7 (May 28, 2024 email from D. Walter to J. Reich). This fails. The timing of trial in parallel district court proceedings cannot change the enforceability of Parse's representation. Otherwise, *Sand Revolution* stipulations to the PTAB would have no meaning because their enforceability would be subject to schedule changes by the PTAB or district court. Further, by statute, if a final written decision issues prior to a district court trial, then Parse would be estopped from presenting its IPR invalidity grounds in the district court action. *See* 35 U.S.C. § 315(e)(2). Thus, the only purpose of *Sand Revolution* stipulations is to prevent the PTAB from instituting IPRs based on invalidity grounds that will be presented in a district court before a PTAB final written decision. That is exactly this situation, and Parse cannot repudiate the stipulation now because it is operating as intended.

Parse's "unforeseeability" argument also is belied by the record. Over one year ago, on March 7, 2023, the Court set trial in this case for December 2024. Despite knowing the trial date, Parse continued pursuing the Linnarsson / McCloskey prior art defense in its IPR petitions in its January 2024 rehearing request. IPR2023-00955, Pap.8 (Jan. 2, 2024); IPR2023-00958, Pap.8 (Jan. 5, 2024). Specifically, Parse asked the PTAB to institute IPR proceedings as to those Brenner Patents even though it knew that the final written decisions for those IPRs (if instituted as they were) would be set for after the December 2024 trial in this action. *See* 35 U.S.C. § 316(11). The PTAB then instituted those petitions by considering, among other things, the *Sand Revolution* stipulations.

To the extent Parse argues that the Court should stay the trial in this action until after the PTAB issues final written decisions for all three of the Brenner Patents, this fails. This Court has already twice denied Parse's attempt to stay this case pending decision on the IPRs. D.I. 121, 164. The fact that Parse reneged on its stipulation does not justify a stay of trial. Nor is 10x's motion untimely. After the PTAB instituted the Brenner Patent IPR petitions, Parse served invalidity

contentions on April 16, 2024 but those contentions did not disclose specific combinations of prior art references. All that was disclosed in those contentions were combinations of categories of art, which included at least 561 combinations. Based on Parse's *Sand Revolution* stipulation, 10x reasonably believed that Parse would not assert the specific Linnarsson / McCloskey prior art combination that Parse had promised not to assert. It was only when Dr. Cooper's report was served on May 17, 2024 that 10x had notice of Parse's intent to disavow its representation to the PTAB. For Parse to claim now that 10x should have known that Parse should not be held to its PTAB representation would flip Rule 11 and its administrative equivalents on their heads. *See* 37 C.F.R. § 11.18(b)(1).

For these reasons, 10x requests a teleconference on its motion to strike Dr. Cooper's arguments relying on the Linnarsson / McCloskey prior art defense. 10x requests expedited treatment of this letter motion because the parties' rebuttal expert reports are due on June 14, 2024 and reply expert reports are due on July 12, 2024. Counsel are available at Your Honor's convenience should the Court have any questions.

Respectfully,

/s/ Karen Jacobs

Karen Jacobs (#2881)

KJ/jmm
Attachments

cc: All Counsel of Record (via electronic mail; w/attachments)

EXHIBIT 1

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

10X GENOMICS, INC., and THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY, Plaintiffs, v. PARSE BIOSCIENCES, INC., Defendant,	C.A. No. 22-cv-1117-JHS **JURY TRIAL DEMANDED**

EXPERT REPORT OF DR. GREGORY COOPER REGARDING INVALIDITY

HIGHLY CONFIDENTIAL - ATTORNEYS' EYES ONLY

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1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey 44

(a) Claim [1A]: A method of analyzing nucleic acids from a plurality of single cells, the method comprising: 45

(b) Claim [1B]: providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides; 48

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(c) Claim [1C]: generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides, ...49

(d) Claim [1D]: wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and 52

(e) Claim [1E]: wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells; and 53

(f) Claim [1F]: wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: … II. a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell 56

(g) Claim [1G]: sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences; 59

(h) Claim [1H]: using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and....61

(i) Claim [1I]: using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived. 61

(j) Claim 3: The method of claim 1, wherein the sample polynucleotides are selected from DNA and RNA.....62

(k) Claim 5: The method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction.....62

2. The Asserted Claims Are Obvious Over Hug and Miner 66

(a) Claim [1A]: A method of analyzing nucleic acids from a plurality of single cells, the method comprising:.....66

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(b) Claim [1B]: providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides; 67

(c) Claim [1C]: generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides, ...68

(d) Claim [1D]: wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and 69

(e) Claim [1E]: wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells; and 70

(f) Claim [1F]: wherein each tagged polynucleotide comprises: … a multiplex identifier (MID) sequence comprising: … II. a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell 73

(g) Claim [1G]: sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences; 74

(h) Claim [1H]: using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and.....75

(i) Claim [1I]: using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived. 76

(j) Claim 3: The method of claim 1, wherein the sample polynucleotides are selected from DNA and RNA. 76

(k) Claim 5: The method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction. 77

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1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey 78

(a) Claim [1A]: A method of counting nucleic acids in a sample, the method comprising: 79

(b) Claim [1B]: (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides; 79

(c) Claim [1C]: (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags, ...79

(d) Claim [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; 79

(e) Claim [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 80

(f) Claim [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; 80

(g) Claim [1G]: (c) sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence; 80

(h) Claim [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. 80

(i) Claim 5: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction. 81

(j) Claim 17: The method of claim 1, wherein the number of different second tag sequences is larger than the number of sample polynucleotides. 81

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(k) 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. 82

(l) 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. 83

2. The Asserted Claims Are Obvious Over Hug and Miner 83

(a) Claim [1A]: A method of counting nucleic acids in a sample, the method comprising: 84

(b) Claim [1B]: (a) providing a sample comprising a plurality of cells, wherein a cell of the plurality of cells comprises a plurality of sample polynucleotides; 86

(c) Claim [1C]: (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides of said cell and a plurality of oligonucleotide tags, ...86

(d) Claim [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; 86

(e) Claim [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 86

(f) Claim [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; 86

(g) Claim [1G]: (c) sequencing the tagged polynucleotide to determine the sample sequence, the first tag sequence, and the second tag sequence; 86

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(h) Claim [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. 87

(i) Claim 5: The method of claim 1, wherein the plurality of tagged polynucleotides is generated through at least one ligation reaction. 87

(j) Claim 17: The method of claim 1, wherein the number of different second tag sequences is larger than the number of sample polynucleotides. 87

(k) 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. 89

(l) 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. 89

F. Obviousness Grounds (’013 Patent) 90

1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey 90

(a) Claim [1A]: A method for multiplexed analysis of nucleic acids from single cells, the method comprising: 90

(b) Claim [1B]: providing a sample comprising a plurality of cells, wherein a single cell of the plurality of cells comprises a plurality of sample polynucleotides; 90

(c) Claim [1C]: performing combinatorial tagging to generate a plurality of tagged polynucleotides from said plurality of sample polynucleotides and a plurality of oligonucleotide tags, ...90

(d) Claim [1D]: wherein a tagged polynucleotide of the plurality of tagged polynucleotides is generated by providing an extension product by primer extension using a first oligonucleotide tag and a sample polynucleotide of said plurality of sample polynucleotides, and: 91

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(e) Claim [1E]: ligating a second oligonucleotide tag to said extension product, and 93

(f) Claim [1F]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: a sample sequence corresponding to said sample polynucleotide of the plurality of sample polynucleotides; 93

(g) Claim [1G]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and 94

(h) Claim [1H]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell; 94

(i) Claim [1I]: amplifying said tagged polynucleotide, thereby generating a plurality of amplified polynucleotides corresponding to the tagged polynucleotide; and 94

(j) Claim [1J]: sequencing said plurality of amplified polynucleotides to determine sequences of the amplified polynucleotides corresponding to the sample sequence, the first tag sequence, and the second tag sequence of the tagged polynucleotide; and 95

(k) Claim [1K]: using the sequences determined in step (d) to count sample polynucleotides for multiple different sample polynucleotides of multiple different single cells of said plurality of cells. 95

(l) Claim 2: The method of claim 1, wherein said plurality of sample polynucleotides comprise messenger ribonucleic acid (mRNA). 95

(m) Claim 3: The method of claim 2, wherein step (A) comprises (i) hybridizing said first oligonucleotide tag to said mRNA and (ii) extending said first oligonucleotide tag using said mRNA as a template to generate complementary DNA (cDNA). 95

(n) Claim 6: The method of claim 1, wherein said plurality of cells is provided in a plurality of wells. 97

(o) Claim 9: The method of claim 1, wherein step (b) comprises randomly associating said plurality of sample

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polynucleotides with said plurality of oligonucleotide tags. 97

(p) Claim 15: The method of claim 1, wherein said plurality of oligonucleotide tags comprises a number of different second tag sequences that is larger than the number of sample polynucleotides. 98

(q) Claim 25: The method of claim 1, wherein said plurality of tagged polynucleotides are pooled with tagged polynucleotides from other single cells of said plurality of cells prior to the amplifying of step (c).....98

(r) Claim 26: The method of claim 1, wherein said plurality of amplified polynucleotides are pooled with amplified polynucleotides from other single cells of said plurality of cells prior to the sequencing of step (d). 98

2. The Asserted Claims Are Obvious Over Hug and Miner 99

(a) Claim [1A]: A method for multiplexed analysis of nucleic acids from single cells, the method comprising:.....99

(b) Claim [1B]: providing a sample comprising a plurality of cells, wherein a single cell of the plurality of cells comprises a plurality of sample polynucleotides; 99

(c) Claim [1C]: performing combinatorial tagging to generate a plurality of tagged polynucleotides from said plurality of sample polynucleotides and a plurality of oligonucleotide tags, ...99

(d) Claim [1D]: wherein a tagged polynucleotide of the plurality of tagged polynucleotides is generated by providing an extension product by primer extension using a first oligonucleotide tag and a sample polynucleotide of said plurality of sample polynucleotides, and:.....100

(e) Claim [1E]: ligating a second oligonucleotide tag to said extension product, and 101

(f) Claim [1F]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: a sample sequence corresponding to said sample polynucleotide of the plurality of sample polynucleotides; 101

(g) Claim [1G]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a first

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tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and 101

(h) Claim [1H]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell; 101

(i) Claim [1I]: amplifying said tagged polynucleotide, thereby generating a plurality of amplified polynucleotides corresponding to the tagged polynucleotide; and 101

(j) Claim [1J]: sequencing said plurality of amplified polynucleotides to determine sequences of the amplified polynucleotides corresponding to the sample sequence, the first tag sequence, and the second tag sequence of the tagged polynucleotide; and 103

(k) Claim [1K]: using the sequences determined in step (d) to count sample polynucleotides for multiple different sample polynucleotides of multiple different single cells of said plurality of cells. 103

(l) Claim 2: The method of claim 1, wherein said plurality of sample polynucleotides comprise messenger ribonucleic acid (mRNA).....103

(m) Claim 3: The method of claim 2, wherein step (A) comprises (i) hybridizing said first oligonucleotide tag to said mRNA and (ii) extending said first oligonucleotide tag using said mRNA as a template to generate complementary DNA (cDNA).....103

(n) Claim 6: The method of claim 1, wherein said plurality of cells is provided in a plurality of wells. 104

(o) Claim 9: The method of claim 1, wherein step (b) comprises randomly associating said plurality of sample polynucleotides with said plurality of oligonucleotide tags. 104

(p) Claim 15: The method of claim 1, wherein said plurality of oligonucleotide tags comprises a number of different second tag sequences that is larger than the number of sample polynucleotides. 105

(q) Claim 25: The method of claim 1, wherein said plurality of tagged polynucleotides are pooled with tagged

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(r) Claim 26: The method of claim 1, wherein said plurality of amplified polynucleotides are pooled with amplified polynucleotides from other single cells of said plurality of cells prior to the sequencing of step (d). 105

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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. For a given cluster, all identical strands are read simultaneously and millions of clusters are processed in a massively parallel process. Mardis 2 at 135, Shendure at 1140.

IX. OBVIOUSNESS

A. Overview

123. It is my opinion that the '981, '013, and '197 Patents are invalid as obvious under at least the following combinations. As a preliminary note, the Asserted Brenner Patents are also invalid for lack of enablement, written description, or indefiniteness. As I explain below in connection with enablement and written description, the specifications of the patents-in-suit do not allow one to practice the full scope of the claimed inventions without undue experimentation. As I explain below, the evidence I have reviewed shows that the alleged inventions of the Asserted Brenner Patents pertain to a technique called the "reflex method" that relates to the processes for rearranging sequences of interest in a polynucleotide. Thus, to the extent that this Court or any other proceeding determines that the claims including anything novel, to practice the full scope of the claimed invention, one would need to engineer de novo new techniques for generating and using dual molecular tags wherein one tag distinguishes sample polynucleotides from sample polynucleotides from other cells and a second tag distinguishes sample polynucleotides from sample polynucleotides from the same cell. The gap in the disclosure regarding how to perform such techniques is considerable, and I do not believe that what was known in the art could fill this gap in the disclosure of the Asserted Brenner Patents. Nothing in my opinions on anticipation or obviousness that I present below should be construed as a suggestion to the contrary.

124. With that caveat, it is my opinion that, to the extent the Asserted Brenner Patents are not deemed invalid under for lack of enablement, written description, or indefiniteness, they are necessarily obvious. Specifically, it is my opinion that the '981, '013, and '197 Patents are necessarily invalid under the following combinations, which would have been obvious to a POSITA at the time of patent submission, if they are not deemed invalid for lack of enablement or written description. I have been asked to assume that the references I cite to in Section VI qualify as prior art, and this assumption is consistent with my understanding of priority outlined in Section IV.B, above.

B. Prior Art

1. Linnarsson

(a) Linnarsson is § 102(e) Prior Art

125. International PCT patent application publication WO 2010/117620 A2 ("Linnarsson") entitled "Gene Expression Analysis In Single Cells," was published on October 14, 2010. *See* PARSE10X0883861 ("Linnarsson"). I understand that Linnarsson claims priority to U.S. Provisional Patent Application No. 61/164,759 ("Linnarsson Provisional"), filed on March 30, 2009. PARSE10X0883827 at 1:3-5 ("Linnarsson Provisional").

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126. I have been asked to opine as to 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 POSITA to practice Linnarsson's invention. Linnarsson and Linnarsson Provisional contain substantially similar specifications and claims, with Linnarsson simply providing additional duplicative exemplary embodiments beyond Linnarsson Provisional. The table below identifies where, for example, written description can be found in Linnarsson Provisional for each and every element of Linnarsson's claim 1:

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." Linnarsson Provisional 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." Linnarsson Provisional 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." Linnarsson Provisional 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;" Linnarsson Provisional 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." Linnarsson Provisional 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." Linnarsson Provisional at 3:5-6.
(ii) synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a	"(ii) synthesising a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell;" Linnarsson Provisional 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.

Linnarsson	Linnarsson Provisional Application Disclosure
tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA from a single cell;	2006; Kurimoto, Yabuta et al. 2007; Esumi, Wu et al. 2008)." Linnarsson Provisional 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." Linnarsson Provisional at 5:24-32.
(iii) pooling the tagged cDNA samples; and	"pooling the tagged cDNA samples; and" Linnarsson Provisional 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." Linnarsson Provisional at 3:9-20.
(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." Linnarsson Provisional 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." Linnarsson Provisional at 11:14-17. "Full length cDNA amplification. The cDNA was amplified by PCR by adding the reagents shown in Table 3." Linnarsson Provisional at 14:5-7.

127. 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.

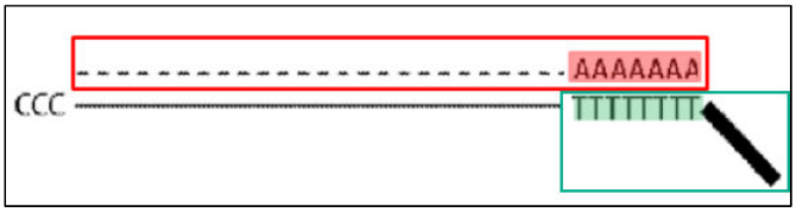
(b) Linnarsson Overview

128. Linnarsson teaches a method of analyzing gene expression in a plurality of single

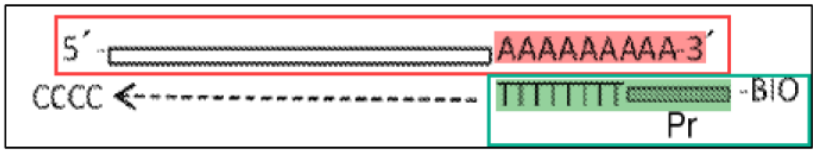
cells by preparing a tagged cDNA library for sequencing. Linnarsson at 1:6-9. The cDNA samples originating from a plurality of single cells are appended with unique oligonucleotide sequences, or "tags" which are useful in tracking polynucleotides arising from different single cells in a multi-cell experiment. *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.").

129. As an initial matter, Linnarsson teaches a method for synthesizing tagged cDNA from mRNA by a 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. Linnarsson at 18:31-19:1 ("In some aspects of the invention, a tag can be incorporated into the cDNA during its synthesis. For example, a 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. Linnarsson at 3:29-4:2, 19:16-17, 21:13-20. I will discuss the CDS and TSO in turn.

130. First, Linnarsson discloses use of a cDNA synthesis primer ("CDS") (boxed in green, below) which includes an "RNA complementary sequence (RCS)." Linnarsson further states that the "RCS is at least partially complementary to one or more mRNA in an individual mRNA sample." An example CDS is shown in Figure 2 and 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). An additional example is shown in the "optionally used" method provided by Figure 11. Linnarsson at 5:28-6:12, Fig. 11.



Linnarsson at Fig. 2.

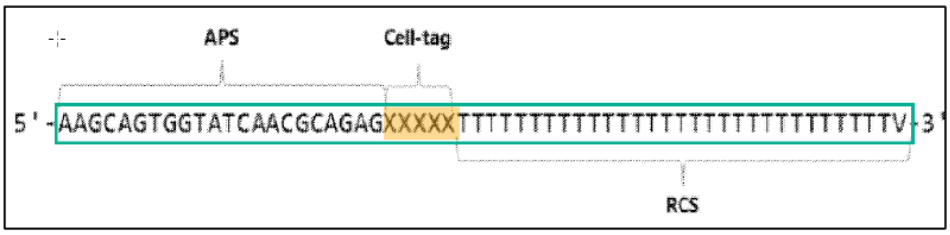


Linnarsson at Fig. 11, 5:29-31, 24:13-15.

131. 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. Linnarsson at 16:22-26. Thus, the resulting cDNA molecule also includes at its end a series of cytosine nucleotides. *Id.*

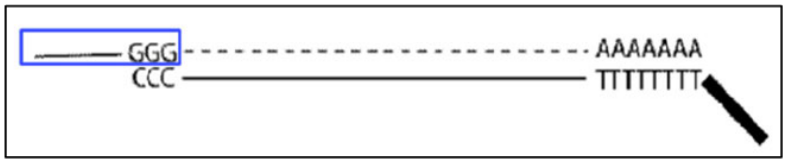
132. Linnarsson discloses that a CDS cell-tag "can be incorporated into the cDNA"

and can "be used to identify which single cell from each cDNA sample originated." Linnarsson 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):

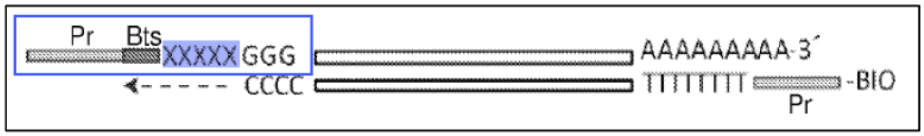


Linnarsson at Fig. 4.

133. Second, Linnarsson discloses a template switching oligonucleotide ("TSO") (boxed in blue, below). Example TSOs are shown in Figure 2 and Figure 11 (below). These TSOs comprise 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":

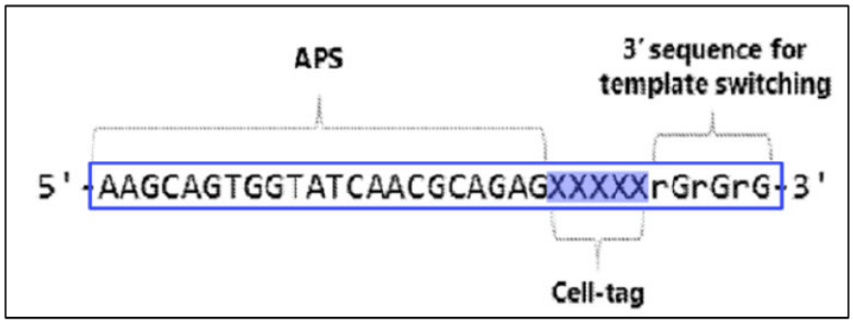


Linnarsson at Fig. 2.



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

134. 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." Linnarsson 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):

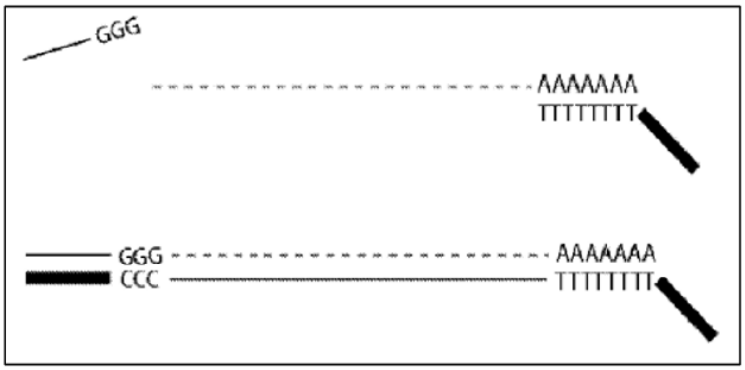


Linnarsson at Fig. 3.

135. Linnarsson discloses that the tagged CDS and tagged TSO can be used individually or in 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." Linnarsson 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.").

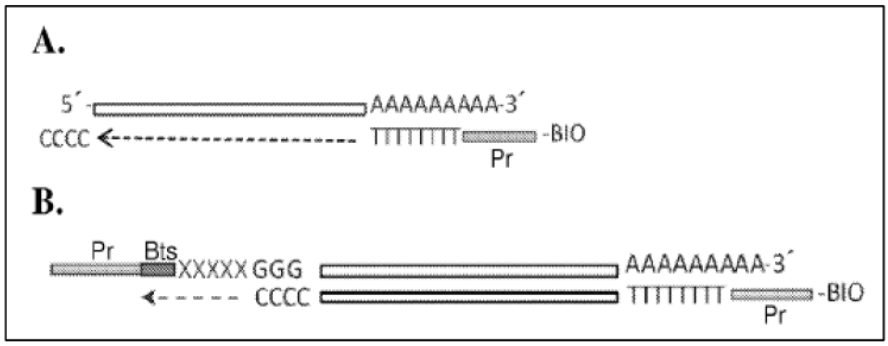
136. Moreover, Linnarsson discloses processes for amplifying polynucleotides including "PCR [and] ligation amplification (or ligase chain reaction, LCR)," and further explains that these methods are "known and widely practiced in the art." Linnarsson at 12:18-20. Linnarsson also teaches that after generating the tagged cDNA, "sequencing is performed." Linnarsson 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.").

137. 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 generated has the same sequence as the original mRNA strand. Figures 2 and 11, portions of which are shown below, provide examples of this process.



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



Linnarsson at Fig. 11, 6:1-3 (explaining Figure 11).

138. I note that Linnarsson's Figure 11 discloses an approach that relies upon fragmentation followed by the use of streptavidin beads to capture the specific DNA fragments containing the cell tag (DNA fragments without a biotin are not captured and not sequenced), and finally the release of these streptavidin beads through the use of a restriction enzyme. As such, the exemplary CDS and TSO in Figure 11 include a biotin molecule for use with the streptavidin capture. The TSO further includes a restriction enzyme cut site so that the capture DNA fragment can be released. Critically, Figure 11 is only a representative example. As Linnarsson states, the "figures are intended to illustrate broad concepts of the invention by reference to representative examples for ease of discussion. They are not intended to limit the scope of the invention by showing one out of several alternate embodiments or by ***showing or omitting optional features*** of the invention." Linnarsson at 4:4-7. Indeed, the description on Figure 11 notes that "panels A-F, show graphical representation of steps ***optionally*** used in the STRT method." *Id.* at 5:28-29.

139. Furthermore, besides expressly stating that the examples are not limiting and that the steps in Figure 11 may be "optionally" used, Linnarsson discloses embodiments that are distinct from the approach of Figure 11. For example, in Figures 3 and 4, Linnarsson discloses a TSO and CDS that are incompatible with the "fragmentation" and "capturing" approach set forth in Figure 11. *Id.* at 4:15-18; *see also id.* at Figs. 3, 4. These embodiments establish that Linnarsson's disclosure cannot possibly be limited to the fragmentation and capture approach of Figure 11.

140. Figure 11's Panel A teaches a reverse transcription step directed by a "tailed oligo-dT primer" that includes a biotin molecule. *Id.* at 5:29-31 ("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)."). Biotin is a molecule that can be linked to nucleic acid and that can be used to immobilize the nucleic acid through binding to beads coated with streptavidin protein, which binds to biotin extremely tightly. '981 Patent at 17:67-18:5 ("Binding moieties and their corresponding binding partners are sometimes referred to herein as binding partner pairs. Any convenient binding partner pairs may be used, including but not limited to biotin/avidin (or streptavidin), antigen/antibody pairs, etc."). This capture approach is useful for purification and detection purposes. *Id.* at 18:25-29

("The biotin moiety (i.e., the binding partner of streptavidin) on the extended strands will bind to the solid-phase streptavidin. Denaturation and washing is then performed to remove all non-biotinylated polynucleotide strands.").

141. This biotin molecule is used in the fragmentation approach to capture the nucleic acid to which it is connected. Linnarsson at 26:21-26 ("The fragments were next bound to beads to capture 5'and 3' ends, and then treated with TaqExpress to repair frayed ends and nicks. 30µL Dynabeads MyOne C1 Streptavidin were washed twice in 2x B&W (Dynal), then added to the Dnase-treated sample, incubated for 10 minutes, and then washed 3x in 1x B&W. About 10% of the sample was bound to the beads (i.e. about 30-60 ng), since internal fragments were not biotinylated."); *id*. at 35:23-25 ("3' and 5' fragments were immobilized on 30 µL streptavidin-coated paramagnetic beads (Dynabeads MyOne C1, Invitrogen), then resuspended in 30 µL TaqExpress buffer (Genetix, UK)."); *id*. at 6:4-6 ("Panel E shows an immobilization and end-repair step. Both 5' (with barcode (Br) and BtsCI site (Bts)) and 3' fragments are bound to beads, while internal fragments are washed away.").

142. Consider now the exemplary CDS in Figure 4 of Linnarsson compared to the exemplary CDS in Figure 11. Linnarsson's Figure 4 "shows an example of a cDNA synthesis primer (CDS) comprising a 5' amplification primer sequence (APS), a cell tag and a 3' RNA complementary sequence (RCS)." *Id*. at 4:17-18. Critically, Figure 4 of Linnarsson ***does not*** contain any biotin and rather has a cell tag that is not present in Figure 11's CDS:



Figure 4

Id. at Fig. 4.

143. The key point is that the CDS in Figure 4, unlike the CDS in Figure 11, does ***not*** include a biotin molecule. As such, the embodiments of Linnarsson based on the use of Figure 4 ***do not*** involve fragmentation and fragment capture set forth in Figure 11. This is because the fragmentation and capture approach of Figure 11 depends on the biotin being present, and there is no biotin in Figure 4. Additionally, the BtsCI restriction enzyme cut site, comprising the sequence "GGATG," is ***not*** present in Figure 4. Linnarsson at Fig. 4. Again, this demonstrates that the CDS in Figure 4 is not being used with the fragmentation and capture approach of Figure 11. Further, whereas the CDS in Figure 11 does not include a cell-tag, the CDS in Figure 4 does indeed include a cell tag.

144. Now let us consider the TSO in Figure 11, which is set forth in Panel B of Figure 11. In my opinion, this provides an even more compelling illustration of how Linnarsson is not limited to the specific approach set forth in Figure 11 based on fragmentation and streptavidin

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capture.

145. Panel B teaches the template switching step with the help of a "[b]arcoded helper oligo," that includes "a cell tag (red box), a Bts Sequence (orange box; recognized by the BtsCI type IIS restriction endonuclease), and a primer sequence (Pr) on the 5' side of the poly-G tail." Consider how this disclosure compares to the TSO in Figure 3 of Linnarsson. Linnarsson's TSO in Figure 3 "shows an example of a template switching oligonucleotide comprising a 5' amplification primer sequence (APS), a cell tag and a 3' sequence for template switching." Linnarsson at 4:15-16. The TSO as exemplified in Figure 3 ***does not*** contain the "GGATG" restriction enzyme cut site sequence, nor does it include a biotin molecule:

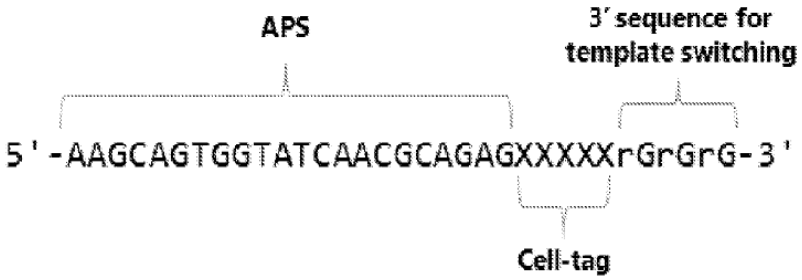


Figure 3

Linnarsson at Fig. 3.

146. The lack of a restriction enzyme cut site and biotin in Figure 3 is critical because it shows that Linnarsson is not limited to the fragment and capture approach of Figure 11. Indeed, without the biotin, one cannot perform the fragment capture as set forth in Figure 11. Further, it cannot be the case that Linnarsson is contemplating the fragmentation and capture of Figure 11 in all its embodiments because the restriction enzyme cut site that is needed to release the fragments following capture is also absent from Figure 3.

147. As yet additional evidence that Figure 11 cannot be considered to limit the scope of Linnarsson, consider the disclosure in Linnarsson about the nature of the mRNAs to be targeted to cDNA synthesis, tagging, and sequencing. In Figure 11, as well as Figures 3 and 4, a series of "T" nucleotides are shown, referred to as "oligo (dt)"; this is a common approach for capturing mRNAs as these Ts hybridize to the stretches of "A" nucleotides, often called a "polyA tail", that are at the end of many mRNA molecules. Linnarsson, however, makes clear that other types of primers may be used to initiate cDNA synthesis. For example, Linnarsson describes various options for CDS design, including use of sequences that are "at least partially complementary to one or more mRNA." Linnarsson at 15:1-10. While Linnarsson states that

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these sequences "can comprise oligo (dt)", it also states that they may be "gene family-specific." *Id.* As such, despite the fact that Figure 11 includes oligo (dt) as an exemplary sequence to target mRNAs, it is clear that oligo (dt) primers are not necessary for Linnarsson's methods to function. Again, this makes clear that Figure 11 is merely one possible set of method options and not the only set of method options disclosed by Linnarsson.

148. Indeed, Linnarsson's disclosures also reveal that sequencing is not limited to ***one*** particular method, because Linnarsson's method gives the experimenter the flexibility to choose from different sequencing methods depending on the needs of the experiment. *See* Linnarsson at 22:5-7 ("The cDNA library ***can be sequenced by any suitable screening method***. In particular, the cDNA library can be sequenced using a high-throughput screening method, such as Applied Biosystems' SOLiD sequencing technology, or lllumina's Genome Analyzer"); *see also id.* at 21:14-18 ("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-throughout screening methods, such as Applied Biosystems' SOLiD sequencing technology, or Illumina's Genome Analyzer."); *id*. at 19:28-32 ("The 5' APS can be designed to facilitate downstream processing of the cDNA library. For example, if the cDNA library is to be analyzed by a particular sequencing method, e.g. Applied Biosystems' SOLiD sequencing technology, or Illumina's Genome Analyzer, the 5' A.PS can be designed to be identical to the primers used in these sequencing methods.")

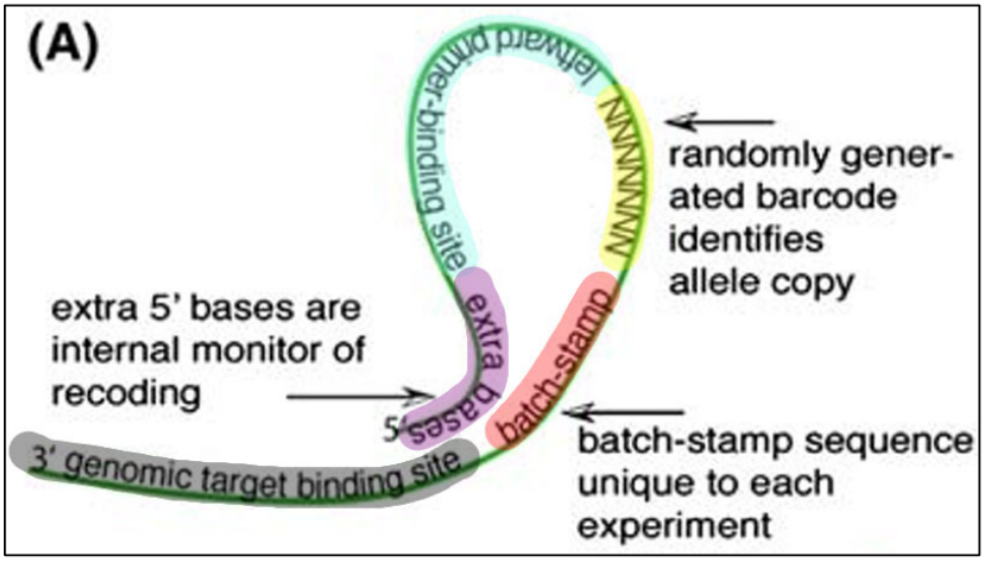
149. Finally, the fact that Linnarsson is not limited to an approach based on fragmentation and capture as set forth in Figure 11 is further evident from the dependent claims of Linnarsson. Claim 26 of Linnarsson recites the "method according to claim 1, wherein the method further comprises processing the cDNA library to obtain a library suitable for ***sequencing***." *Id*. at claim 26. Claim 27 which is a dependent claim of Claim 1 and Claim 26, further recites the "method according to claim 26, wherein the processing ***comprises fragmenting the cDNA library***." *Id*. at claim 27. It is my opinion that since fragmenting is only required in a dependent claim, Linnarsson's method is not limited to the fragmentation approach set forth in Figure 11. If fragmentation were required in Linnarsson, it would not be listed as an optional feature as part of a dependent claim.

2. McCloskey

150. 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 Asserted Brenner Patents. PARSE10X0884005 ("McCloskey"). McCloskey describes a method of nucleic acid analysis 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 ("[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. The other regions on the encoding oligonucleotide also comprise: "a

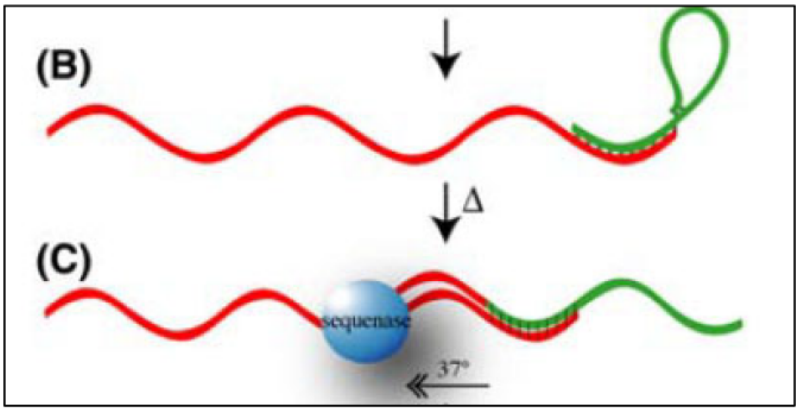
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genomic target-binding site" (highlighted in grey) and "a primer-binding site for PCR" (highlighted in light blue). McCloskey at 763. McCloskey teaches that its methods are suitable for a variety of applications, including for gene expression and for single-cell samples. McCloskey at Abstract, 763, 767.



McCloskey at Fig. 1.

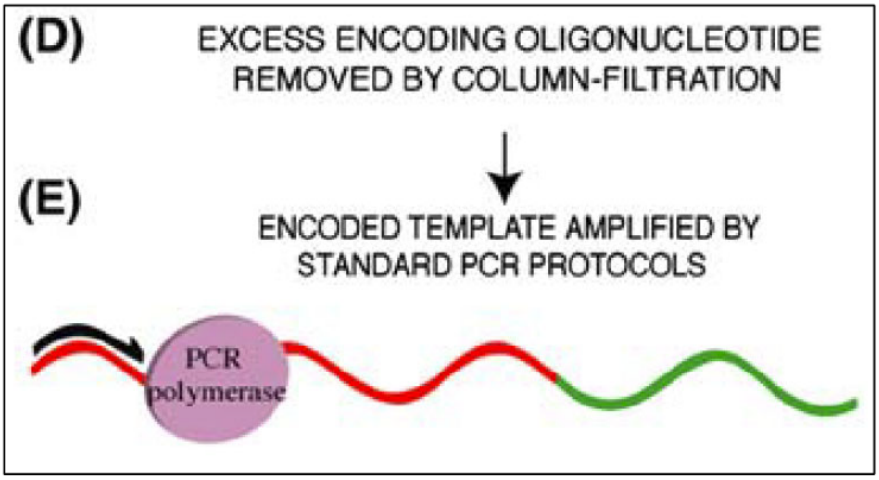
151. McCloskey's method begins with annealing the 3' end genomic target binding site (highlighted in grey, above) of the encoding oligonucleotide to a single-stranded nucleic acid (*see* step (B) in figure, below). McCloskey at 763-766. The oligonucleotide-annealed nucleic acid is then extended by Sequenase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



McCloskey at Fig. 1.

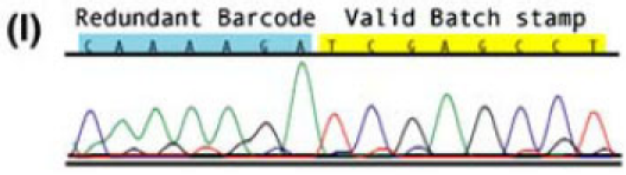
152. Subsequent to annealing and extension, the resulting encoded nucleic acids are

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.



McCloskey at Fig. 1.

153. 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. McCloskey teaches that a benefit of its method is that it distinguishes redundant sequences produced in the process of PCR amplification from redundant sequences that existed 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 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. Thus, by counting sequences with non-redundant barcodes, McCloskey's method is useful in obtaining a quantitative measure of template diversity and correcting for amplification bias. *Id.*



McCloskey at Fig. 1.

154.

5. Miner

158. Miner *et. al*., "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR", is an article that was published in Nucleic Acids Research on September 30, 2004 and is thus prior art to the Asserted Brenner Patents. PARSE10X0884024 ("Miner"). Further, Counsel has explained that during prosecution of the Brenner Patents, the Applicant identified Miner as prior art in an IDS. '981 Patent File History at 10X_PARSE_000000404; '197 Patent File History at 10X_PARSE_000000795. The Examiner raised no prior art rejection based on Miner. Miner teaches a method of tagging polynucleotides with two tags—(a) batchstamps; and (b) barcodes. Miner at 1. The molecular batch-stamps "label each genomic template with a sample ID and analysis date." Miner at Abstract. The molecular barcodes "label each genomic DNA template with an individual sequence." *Id.* The barcodes and batch-stamps are included in a hairpin linker sequence before amplification. *Id.* Miner's method enables tracking "the genomic origin of each sequence obtained from PCR." Miner at PARSE10X0884024. It further allows identification of "contaminant and redundant sequences and" accurate qualification of "the proportion of cells carrying a particular sequence variant by counting only distinctly tagged sequences." *Id.*

C. Motivation to Combine and Reasonable Expectation of Success

1. Linnarsson and McCloskey

159. Both McCloskey and Linnarsson teach methods of genetic analysis of single cells whereby sample polynucleotides are tagged with oligonucleotides after which PCR amplification is performed and the results are sequenced. Linnarsson at 3:25-4:2, Linnarsson Provisional (Linnarsson Provisional) at 2:27-3:3; McCloskey at Fig. 1. They are thus in the same field of endeavor and analogous art as well as analogous art to the Asserted Brenner Patents.

160. As one example, a POSITA would have been motivated to combine Linnarsson's method of tagging polynucleotides with McCloskey's MID because McCloskey teaches a solution to a problem discussed in Linnarsson, namely amplification bias. Linnarsson discloses that analyzing gene expression in single cells results in "potential amplification bias." Linnarsson at 2:3-15, 11:6-10; Linnarsson Provisional at 2:6-21, 3:14-20. A POSITA 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 at 11:6-9; Linnarsson Provisional 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 ("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 POSITA would have been motivated to combine McCloskey with Linnarsson.

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161. Additionally, a POSITA would have had a reasonable expectation of success to incorporate McCloskey's teachings into Linnarsson's. Linnarsson discloses incorporating oligonucleotides (*e.g.*, CDSs and TSOs) that comprise "a distinct tag, or a distinct combination of tags." Linnarsson at 19:5-10; Linnarsson Provisional at 7:26-32. McCloskey similarly discloses annealing an "encoding oligonucleotide." Moreover, a POSITA 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 at 19:6-7; Linnarsson Provisional 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."

162. Both McCloskey and McCloskey II teach substantially the same method with minor additions. Similar to McCloskey, McCloskey II also teaches a method for tagging polynucleotides with an encoding oligonucleotide. Thus, for the reasons explained above, a POSITA would be motivated to combine the teachings of Linnarsson, McCloskey, and McCloskey II.

2. Hug and Miner

163. Both Hug and Miner teach methods of nucleic acid analysis of single cells whereby sample polynucleotides are tagged with oligonucleotides after which PCR amplification is performed and the results are sequenced. Hug at 615-617; Fig. 1; Miner at 1-3; Fig 1. They are thus in the same field of endeavor and analogous art as well as analogous art to the Asserted Brenner Patents.

164. As one example, a POSITA would have been motivated to combine Hug's method of tagging polynucleotides with Miner's MID because Miner teaches a solution to a problem discussed in Hug, i.e. normalization of data from different experiments. Hug discusses that:

> Growth and differentiation of a cell in a tissue is dependent on the expression levels of the genes. While the number and type of genes is usually identical in all cells of an organism, ***the mRNAs expressed and mRNA expression levels are specific for the cell type*** and state of differentiation.
>
> RNA is not very stable and therefore, it is transcribed into complementary DNA (cDNA) for analysis. The cDNA concentrations of a large number of genes can be measured in parallel by using an addressable DNA micro–array or DNA chip (chips, 2002). Thereby ***the normalization of data obtained from different experiments is a difficult problem***.

Hug at 615.

165. Hug attempts to address this issue by attaching a unique tag to *each* individual mRNA to distinguish each mRNA sequence from a complex mRNA pool, but its solution is not

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perfect as it lacks a method to distinguish among mRNA sequences arising from different samples or experiments. In other words, in Hug's mRNA concentration, each mRNA strand has a unique tag and thus it is not possible to preserve information on which of the mRNA sequences came from the same experiment or sample. Miner provides a solution to this problem and expressly discloses that its method of including a "batch-stamp" that encodes "information that would designate the sample analyzed and the date of analysis." As such, Hug's attempt to solve the "difficult problem" of "normalization of data obtained from ***different experiments***" is effectively addressed by utilizing a batch-stamp that is unique to each experiment/sample. Thus, a POSITA would have been motivated to combine Miner with Hug.

166. Hug further recognizes the problem of amplification bias. Hug at 615-616. ("Each round of a PCR amplification roughly doubles the number of DNA molecules making a following quantitative analysis difficult."). A POSITA 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. A POSITA would be particularly motivated to utilize Miner's two tag method because it is the relevant art around the time to address this problem of redundancy and contamination in PCR.

> PCR amplification of limited amounts of DNA template carries an increased risk of product redundancy and contamination. We use molecular barcoding to label each genomic DNA template with an individual sequence tag prior to PCR amplification. In addition, we include molecular 'batch-stamps' that effectively label each genomic template with a sample ID and analysis date. This highly sensitive method identifies redundant and contaminant sequences and serves as a reliable method for positive identification of desired sequences; we can therefore capture accurately the genomic template diversity in the sample analyzed.

Miner at Abstract.

167. Additionally, a POSITA would have had a reasonable expectation of success incorporating Miner's teachings into Hug's. Hug discloses incorporating oligonucleotides that consist of various sequences including "the reverse complementary sequence of a part of the mRNA sequence followed by a random tag sequence, a restriction enzyme recognition site (R) and an unique primer binding site." Hug at 618. Miner similarly discloses ligating a "hairpin linker" that consists of the two tags – barcodes and batchstamps. Moreover, a POSITA would have had a reasonable expectation combining Hug and Miner because Hug allows for using a combination of distinct sequences, which would allow the two-tag concept disclosed in Miner to be utilized in Hug's oligonucleotide.

D. Obviousness Grounds ('981 patent)

1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey

168. It is my opinion that the Asserted Claims of the '981 Patent are obvious in view

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of Linnarsson and McCloskey. As I explain in Section IX.C, a POSITA would be motivated to combine Linnarsson and McCloskey and reasonably expect the combination to succeed. In particular, someone with skill in the would be motivated to combine the first tag of Linnarsson which distinguishes sample polynucleotides from sample polynucleotides arising from different cells and the second tag of McCloskey which distinguishes sample polynucleotides from sample polynucleotides from the same single cell.

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

169. To the extent the preamble is limiting, it is disclosed by both Linnarsson and McCloskey.

170. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution. Linnarsson discloses a method of analyzing nucleic acids from a plurality of single cells. The Linnarsson abstract states "[t]he present invention provides methods and compositions for the analysis of gene expression in single cells or in a plurality of single cells." Linnarsson at Abstract, Linnarsson Provisional at 1:3-8. Linnarsson repeats this disclosure:

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

Linnarsson at 9:22-25; Linnarsson Provision at 1:3-8.

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

Linnarsson at 10:15-22; Linnarsson Provisional at 2:27-3:3, 9:8-10.

> An embodiment of the method of the invention is summarized in **Figure 1**. Cells are obtained from a tissue of interest and a single-cell suspension is obtained. A single cell is placed in one well of a 96-wcll plate in Cell Capture Mix. The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA. The tagged cDNA samples are pooled and amplified and then sequenced to produce 100 million reads. This allows identification of genes that are expressed in each single cell.

Linnarsson at 22:22-28; Linnarsson Provisional at 11:8-17. As discussed above, gene expression analysis includes analyzing nucleic acids.

171. Moreover, Linnarsson discloses a method for analyzing nucleic acids comprising the following steps: "(A) the tissue of interest is dissected; (B) ***a plurality of single cells are selected***; (C) single cells are placed in separate wells of a 96-well plate and lysed; tagged reverse

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transcription is performed on each sample to produce cDNA; (D) cDNA samples are pooled and amplified; (E) ***sequencing is performed*** to obtain 100 million reads; and (F) ***identification of expressed genes and identification of cells from which they originated***." Linnarsson at 4:8-13[3], Linnarsson Provisional at 9:35-10:6. This method is illustrated in Figure 1 of Linnarsson:

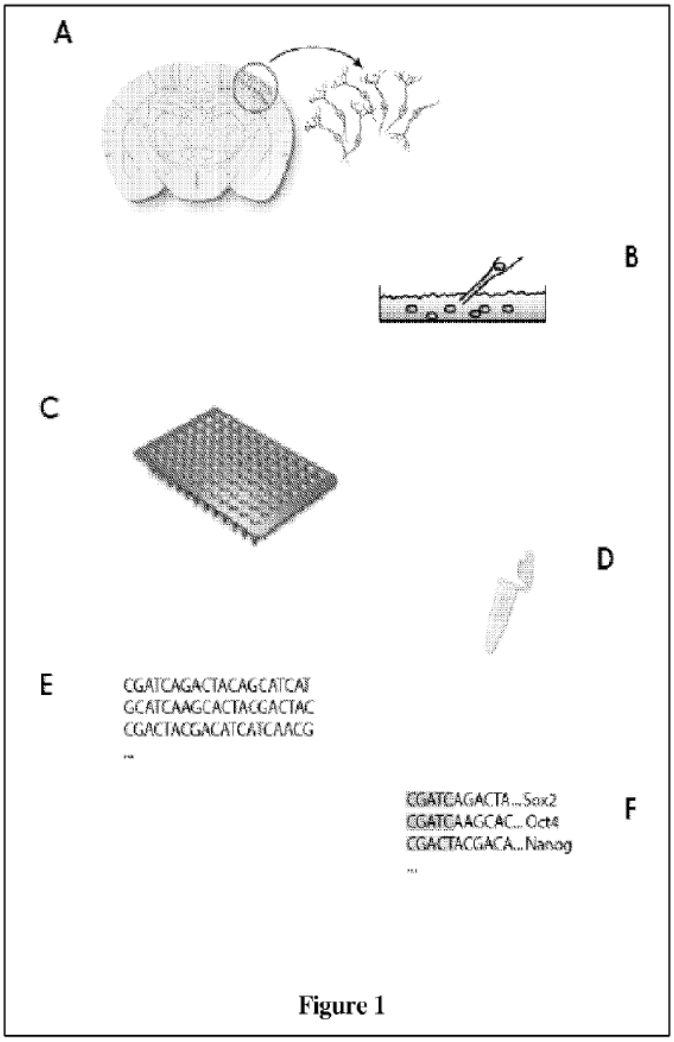


Figure 1

Linnarsson at Fig. 1.

172. Therefore, because Linnarsson discloses a method for analyzing gene expression in a plurality of single cells, and because a POSITA would have understood that analyzing gene expression requires analyzing nucleic acids, it is my opinion that Linnarsson discloses "a method

[3] All emphasis is added unless otherwise stated.

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of analyzing nucleic acids from a plurality of single cells," as recited by claim 1 of the '981 Patent.

173. Similarly, McCloskey teaches a method of analyzing nucleic acids from a plurality of single cells.

174. McCloskey teaches methods of single cell analysis by utilizing molecular tags. McCloskey at 1, 7 ("We recommend that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs available for … single cell … analyses.").

175. McCloskey teaches an encoding oligonucleotide that contains a batch-stamp that is "unique to each experiment" and "specifies the DNA source such as the patient or sample identification" by distinguishing it from polynucleotides from other samples. McCloskey at 72-64. McCloskey discloses that the "sample" may be a "single-cell sample." McCloskey at *Id*. To the extent 10x argues that McCloskey does not disclose a single cell, it is their own position that "a cell" is "a single cell" and as such, McCloskey discloses methods of analysis in "single cells." 10x's VP of Intellectual Property confirmed during deposition that "a cell is a single cell." *See* Wu Depo at 47:15-16; *see also id.* at 52:22-25 ("I don't think it matters whether you call it 'a cell' or 'a single cell.'"). Thus, a POSITA would understand that McCloskey's batch-stamp may be used to identify and distinguish a single cell of origin. Indeed, the methods described by Linnarsson provide for generating many single-cell "samples", and McCloskey defines a process for barcoding each "sample" in a series of samples. As such, the McCloskey batch-stamp serves the same function as the cell tag described by Linnarsson and a POSITA would have understood this from reading these references.

176. Further, in a parallel *inter partes* review proceeding, Plaintiffs' expert, Dr. Quackenbush, has acknowledged that "McCloskey discloses the possibility of tagging DNA even when the sample (batch) consists of DNA from only a 'single cell.'" Quackenbush 981 Decl. at ¶ 63. Dr. Quackenbush also does not dispute the distinguishing function of the batch-stamp. *Id*. ¶ 62 ("McCloskey teaches that its batch-stamp allows one to distinguish between nucleic acids arising from all the cells *in the sample*, on one hand, and the contaminant nucleic acids arising from cells *from different, prior samples*, on the other hand. [McCloskey at] 3-4."); *see also id*. ¶ 66 ("McCloskey's encoding oligonucleotide's batchstamp allows one, instead, to distinguish between nucleic acids arising from a cell *in the sample* and contaminant nucleic acids arising from cells *from prior samples*.") If, as Dr. Quackenbush acknowledges, McCloskey teaches that a sample may consist of only a single cell, i.e., "all the cells in the sample" includes only one cell, and, as Dr. Quackenbush likewise acknowledges, the batch-stamp would allow one to distinguish between different samples, then, McCloskey necessarily teaches that its batch stamp may be used to track single cells. Thus, a POSITA would understand the batch-stamp in McCloskey to be capable of identifying the cell of origin of a polynucleotide. I note further, that this is clear for a POSITA evaluating McCloskey by itself, as McCloskey states explicitly that the methods function on "single-cell" samples. It is even clearer, however, when viewing Linnarsson in combination with McCloskey. For example, claim 1, part (i) of Linnarsson states "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." Thus, the McCloskey sample batch-stamp strategy would have been easily understood by a POSITA to be relevant to Linnarsson's cell tag strategy given that Linnarsson describes the generation of many

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single-cell "samples."

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

177. 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 was well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

178. Linnarsson discloses "providing a sample comprising a plurality of single cells." As discussed above, the definition of "sample" that the parties agreed upon provided by the Brenner patents is "a quantity of material from a biological, environmental, medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought." '981 Patent at 12:7-10.

179. 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 at 22:22-23; Linnarsson Provisional at 11:9. Such "tissue of interest" constitutes a recited "sample" because it is "a quantity of material from a biological . . . source." '981 Patent 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 at 13:11-12, Linnarsson Provisional at 3:31-34; see '981 Patent at 12:18-21 (defining "sample" to include "cultures, blood . . . and the like").

180. 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 at 4:8-10; Linnarsson Provisional at 9:35-37. A POSITA would have understood that step B requires selecting a plurality of single cells from the dissected tissue of interest. Therefore, a POSITA would have understood that the tissue of interest comprises a plurality of single cells.

181. Linnarsson teaches "wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides." 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 at 13:28-30; Linnarsson Provisional 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

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> in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA."

Linnarsson at 22:24-26; Linnarsson Provisional at 11:11-14.

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

183. McCloskey further discloses that each single cell of the plurality of single cells comprises a plurality of sample polynucleotides. McCloskey teaches generating tagged polynucleotides (*e.g.*, tagged cDNA) from sample polynucleotides. For example, McCloskey discloses "a method by which molecular codes are added to single-stranded DNA or cDNA templates …." McCloskey at Abstract, 762; McCloskey at 767 ("We recommend that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for … single cell … DNA analyses."). A POSITA would understand that cDNA refers to DNA molecules that are formed by the reverse transcription of RNA molecules. Therefore, a person of skill in the art would have understood that McCloskey discloses a method of analyses of samples of "single cells" comprising a plurality of sample polynucleotides (RNA or DNA).

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

184. Generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides was well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

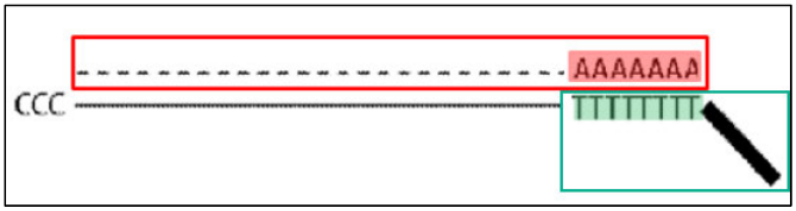
185. As described above, Linnarsson discloses lysing a plurality of single cells to release a plurality of polynucleotides—including mRNA molecules—contained within. Linnarsson further discloses performing reverse transcription on the released plurality of mRNA molecules to generate a plurality of cDNA, and tagging the cDNAs during the process. Linnarsson at 22:24-26; Linnarsson Provisional at 11:11-14. ("The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA."). Thus, Linnarsson discloses a method of generating a plurality of tagged polynucleotides from the sample polynucleotides, such as sample mRNA.

186. Linnarsson specifically teaches that during cDNA synthesis, tagged oligonucleotides referred to as cDNA synthesis primer (CDS) and/or template switching oligonucleotide (TSO) be used to incorporate a cell-tag into the cDNA. Linnarsson 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 (Linnarsson Provisional) at 7:23-26. The tagged cDNA is the claimed "plurality of tagged polynucleotides." Exemplary CDSs and TSOs are also referred to in Linnarsson as tailed oligo-

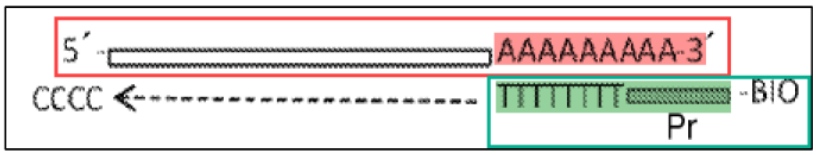
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dT primer and barcoded helper oligo respectively, which a POSITA would have understood to refer to the same exemplary tagged oligonucleotides.

187. Linnarsson 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, defined in the context of these figures as a "tailed oligo-dT primer" (boxed in green, below) that "directs synthesis of a cDNA strand":



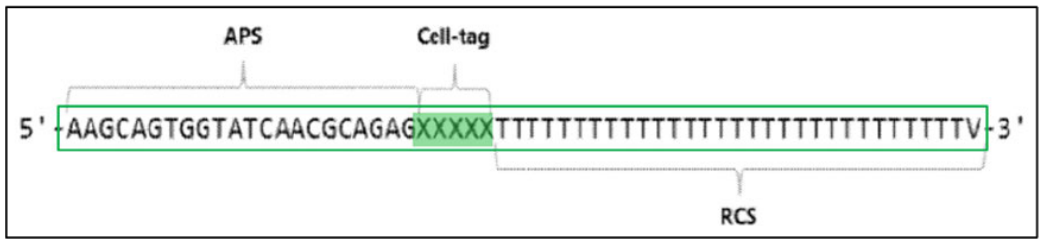
Linnarsson at Fig. 2; *see also* Linnarsson Provisional at Fig. 2.



Linnarsson at Fig. 11; *see also id.* at 5:29-31.

188. 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 at 5:29-31; Linnarsson Provisional at 5:24-32, 13:8-14.

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



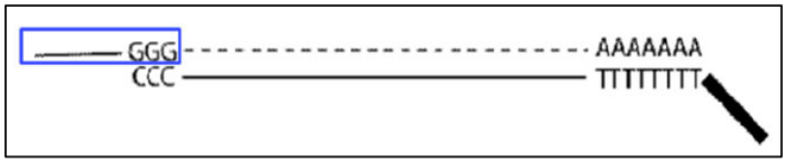
Linnarsson at Fig. 4; *see also* Linnarsson Provisional at Fig. 4.

190. Linnarsson discloses that, as part of "synthesizing a first strand of cDNA from

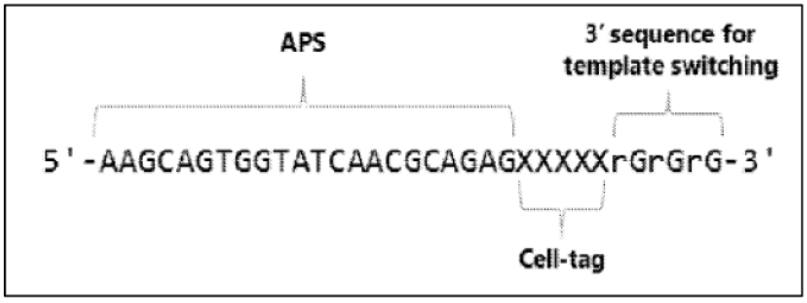
the mRNA in each individual mRNA sample . . . incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, wherein the cDNA in each tagged cDNA sample is complementary to mRNA ….” Linnarsson at 10:18-20, Linnarsson Provisional at 2:33-36; *see also* Linnarsson at 3:25-30 (“The invention 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 at 2:27-3:3.

191. I note that, as discussed above, while the above examples include an oligo-dT primer sequence within an exemplary CDS, Linnarsson discloses other options for the CDS wherein a CDS includes an “RNA complementary sequence (RCS)” that is complementary to, and can therefore hybridize to, “one or more mRNA.” Linnarsson also discloses, for example, that the RCS can be “gene family-specific” or may be composed of “random sequence, such as random hexamers.” Combined with the disclosure of oligo-dT as a possible RCS, a POSITA would understand that a CDS can be used to target a range of mRNA sequences ranging from just one or a few to substantially all mRNAs in a sample.

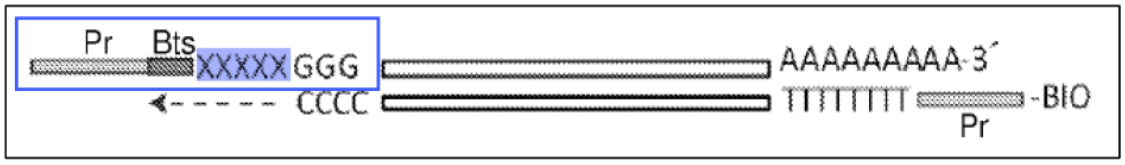
192. Linnarsson also teaches using a TSO or “[b]arcoded help oligo” (boxed in blue, below), which comprises the tag “XXXXX” (highlighted in blue) and “anneals transiently, and cDNA synthesis proceeds using the oligo as template.” Linnarsson at 5:31-33; Linnarsson Provisional at 13:14-25; *see also* Linnarsson at 16:10-17 (discussing another method of forming the second strand of cDNA); Linnarsson Provisional at 6:1-11. Example TSOs (boxed in blue) are shown in Figures 2 and 11, reproduced below:



Linnarsson at Fig. 2; *see also* Linnarsson Provisional at Fig. 2.



Linnarsson at Fig. 3; *see also* Linnarsson Provision at Fig. 3.



Linnarsson at Fig. 11; Linnarsson Provisional at Figs. 2, 3.

193. 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 at 19:6-7; Linnarsson Provisional at 7:26-28.

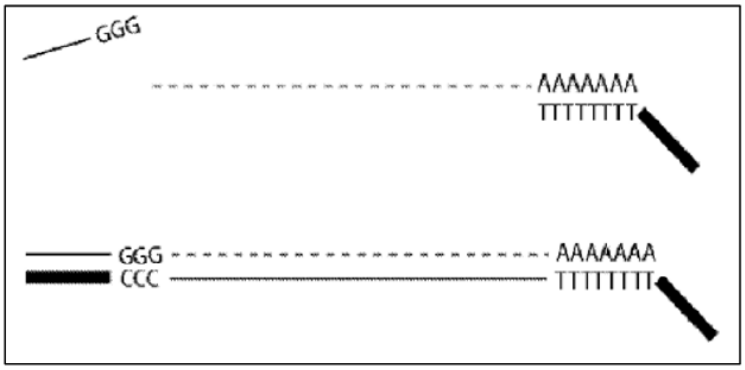
194. McCloskey teaches generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides. For example, McCloskey teaches "an encoding oligonucleotide with five distinct informational regions (Fig. 1): (1) the batch-stamp, unique to each experiment, specifies the DNA source 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 50 bases serve as an internal sentinel of recoding. This encoding oligonucleotide is annealed to denatured DNA and extended by Sequenase (USB) (Fig. 1)." McCloskey at 762.

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

195. Generating tagged polynucleotides wherein each tagged polynucleotide comprises: a sequence from a sample polynucleotide of the plurality of sample polynucleotides was well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

196. Linnarsson discloses that the tagged cDNA comprises a sequence identical to the sample mRNA sequence. As discussed above, 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 one strand of the double-stranded cDNA molecule generated has the same sequence as the original mRNA strand. Figures 2, portions of which are shown below, provides an example of this process:

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Linnarsson at Fig. 2.

197. The cDNA is synthesized from sample mRNA using reverse transcription. Thus, a POSITA 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, the sample mRNA comprises the base pair uracil while the cDNA comprises the base pair thymine. Any POSITA 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. Indeed, claim 4 of the '981 Patent requires the sample polynucleotide to be mRNA, and a POSITA would have understood that the recited "tagged polynucleotide" is thus a cDNA synthesized from the sample mRNA. '981 Patent at Claim 4. Thus, it is my opinion that the tagged polynucleotides cDNA comprises a sequence from a sample polynucleotide.

198. Similarly, McCloskey teaches "a method by which molecular codes are added to single-stranded DNA or cDNA templates." McCloskey at 762. McCloskey's "encoding oligonucleotides are annealed to denatured genomic DNA for 15 min at 37°C." McCloskey at 763. Thus, it is my opinion that McCloskey discloses that the tagged oligonucleotides comprise a sequence identical to the sample oligonucleotide.

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

199. Linnarsson and McCloskey both teach methods for generating tagged polynucleotides wherein each tagged polynucleotide comprises a multiplex identifier (MID) sequence comprising: I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells.

200. Linnarsson discloses tagged polynucleotides that comprise a MID. A multiplex

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identifier is a polynucleotide sequence barcode that is used as a tag or marker to identify and track polynucleotides that are pooled together and sequenced in the same reaction. The ability to combine samples and sequence them together increases the efficiency and reduces the cost of processing samples. The Brenner patents provide the following definition for Multiplex Identifier (MID):

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

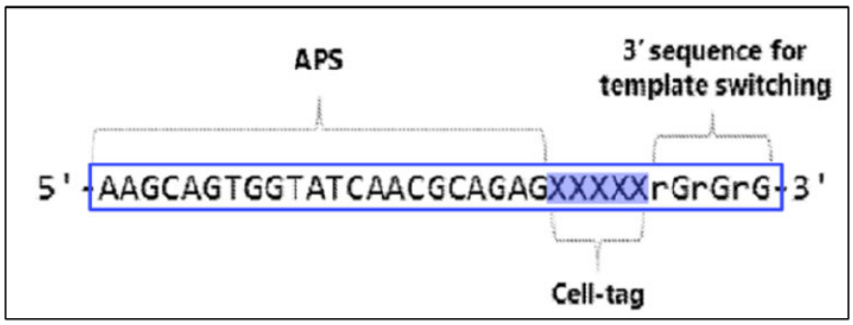
'981 Patent at 6:33-38; *see also id.* at 6:38-7:22 (further definition an MID).

201. I also note that the Brenner patents admit that the use of MIDs for identifying polynucleotides was known in the prior art. *See* '981 Patent at 6:56-64 (incorporating by reference prior art patents "for their description of nucleic acid tags and their use in identifying polynucleotides").

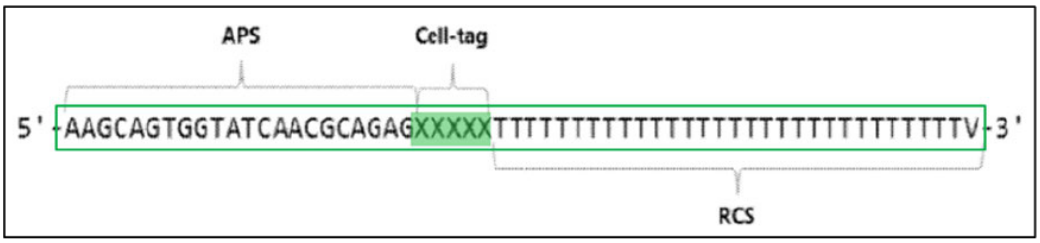
202. Linnarsson discloses a tag associated with a polynucleotide, where the tag can be used to differentiate polynucleotides in a sample. For example, Linnarsson discloses "[i]n some aspects of the invention, a tag can be incorporated into the cDNA during its synthesis. . . . As the tag is present in the [cDNA synthesis primer] and/or the [template switching oligonucleotide] it will be incorporated into the cDNA during its synthesis and can therefore act as a 'barcode' to identify the cDNA." Linnarsson at 18:31-19:5; Linnarsson Provisional at 7:17-26.

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

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



Linnarsson at Fig. 3. Additionally, Linnarsson discloses that a CDS can have a MID sequence. Figure 4, as shown below, depicts an example CDS comprising a "cell tag" (highlighted in green below), an amplification primer sequence ("APS"), and a RNA complementary sequence ("RCS"):



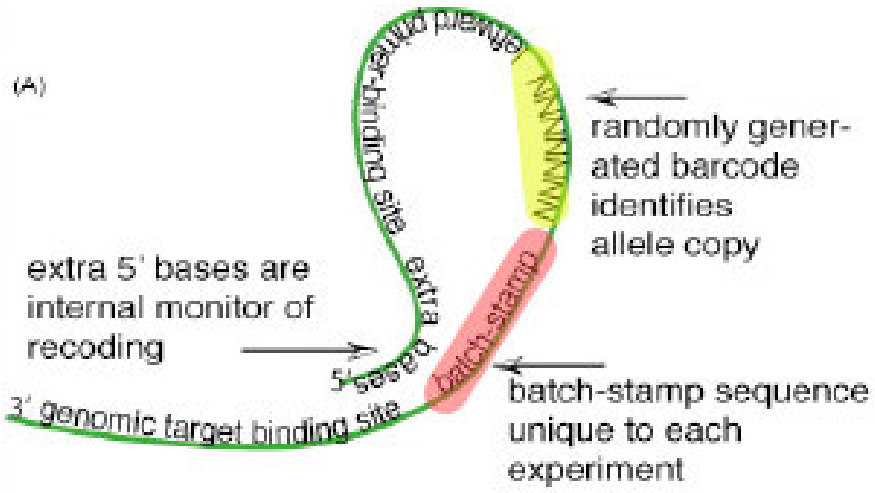
Linnarsson at Fig. 4.

205. Linnarsson discloses a MID that comprises a first tag sequence associated with the single cell from which the sample polynucleotide is derived. 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 at 4:8-13; Linnarsson Provisional at 9:35-10:6. Similarly, Linnarsson explains that the tags present in an exemplary CDS and/or 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 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)."); *see also* Linnarsson Provisional at 7:26-34.

206. A POSITA would have understood that in order to use MID tags to "identify which single cell from each cDNA sample originated," each cDNA sample must have a tag (or combination of tags) that is different from those attached to a 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."

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

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



McCloskey at Fig. 1.

209. As described in McCloskey and depicted above, the encoding oligonucleotide comprises two tag sequences: (1) a "batch-stamp" sequence (highlighted in red, above) to identify the experimental batch or genomic source (*e.g.*, a single cell) and (2) a "barcode" (highlighted in yellow, above) to distinguish polynucleotide sequences "arising from different cell or allele copies." McCloskey at 762 ("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.").

210. The batch-stamp is a tag or combination of tags associated with a polynucleotide whose identity can be used to differentiate between polynucleotides in a sample. Thus, the batch-stamp is a MID. A POSITA would have understood that the "batch-stamp" sequence disclosed in McCloskey is a MID "associated with the single cell from which the sample polynucleotide is derived." The batch-stamp "specifies the DNA source such as the patient or sample identification" and the sample may be a "single-cell sample." McCloskey at 762. Thus, the batch-stamp may be used to specify a single-cell of origin.

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

211. The combination of Linnarsson and McCloskey teaches a method wherein a second tag sequence distinguishes sample polynucleotides from other sample polynucleotides derived from the same single cell.

212. As explained above in Section XII.A.1.e, Linnarsson discloses that each tagged

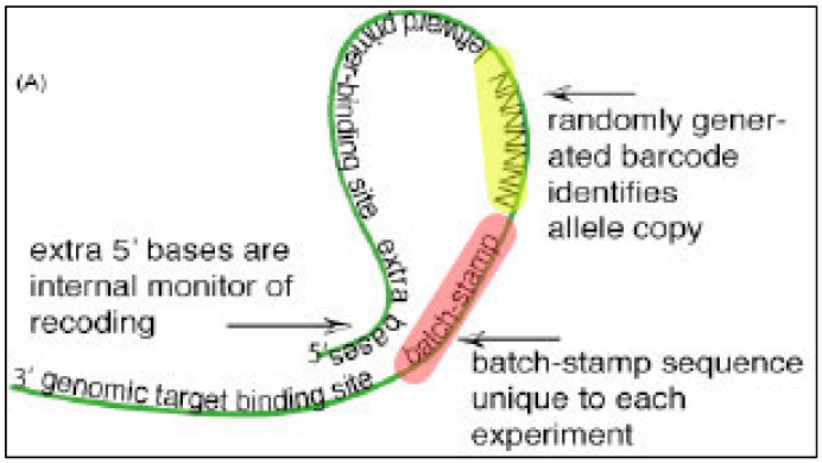
polynucleotide comprises a multiplex identifier (MID) sequence, which is from a TSO and/or CDS. In addition, Linnarsson discloses a method of using a combination of two distinct tags. Linnarsson provides:

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

Linnarsson at 19:5-11; Linnarsson Provisional at 7:26-34.

213. To the extent Linnarsson does not disclose this limitation, it would have been obvious to a POSITA. Specifically, for the reasons discussed above, it would have been obvious to a POSITA to modify the second tag taught by Linnarsson with routine and conventional methods to distinguish between sample polynucleotides arising from the same single cell.

214. McCloskey teaches an MID comprising a second tag sequence that distinguishes a sample polynucleotide from others. The MID sequence taught by McCloskey is depicted below in Figure 1 as an "encoding oligonucleotide.



McCloskey at Fig. 1.

215. 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.

216. The second region disclosed in McCloskey (i.e., the "barcode") constitutes the recited second tag sequence distinguishing the sample polynucleotides from other sample polynucleotides derived from the same single cell. McCloskey discloses that its barcode "distinguishes among sequences arising from different . . . ***allele copies***." McCloskey at 762. McCloskey also discloses that its barcode can be used in single cell analyses. McCloskey at 762, 767 ("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 POSITA would have understood that such allele copies originate from the same single cell. As explained above, a POSITA 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.

217. McCloskey further discloses that the random barcode can be variable in size so as to yield a variable number of unique tags. For example, McCloskey states that the barcode is "currently" 7 nucleotides yielding 16,384 possible sequences, and further discloses that the number of unique sequences that arise from a barcode of length "n" is "4^n". McCloskey at 764. A POSITA would thus understand that the number of unique barcodes could be optimized to their experimental needs by varying the length of the random sequence. This is further consistent with disclosures in Linnarsson as it pertains to combining McCloskey with Linnarsson. For example. Linnarsson discloses that the "tag can be a nucleotide of 4-20 nucleotides in length". Linnarsson at 19:1-3. Further, Linnarsson discloses that both an exemplary CDS and TSO can have a tag, providing additional options for combining the first cell tag from Linnarsson and the second, polynucleotide, tag of McCloskey.

218. Related to the previous point, Linnarsson discloses that while human cells may be tested, the methods may be used for "prokaryotic or eukaryotic single celled organisms including bacteria or yeast." Linnarsson at 13:14-16. Cells from different species harbor very different levels of complexity in terms of the numbers of distinct polynucleotides. For example, some bacteria may include just a few hundred mRNAs, while human cells are estimated to include 50,000 to 300,000. Furthermore, within these cell-types, Linnarsson discloses CDS choices that would isolate very few mRNAs to very many RNAs from among the mRNAs present in a given cell. *See e.g. id*.

219. Thus, the McCloskey strategy of a random sequence barcode to differentiate polynucleotides could easily be combined with the Linnarsson cell tag based approach.

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220. 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. For the reasons explained above, a POSITA would have had a motivation to combine Linnarsson and McCloskey's teaches and would have had a reasonable expectation of success in doing so.

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

221. Sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences was well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

222. Linnarsson teaches that after generating the tagged polynucleotides, "sequencing is performed" thereon. *See* Linnarsson at 4:8-13, Linnarsson Provisional (Linnarsson Provisional) at 8:22-28. Linnarsson repeats the disclosure that the method includes a sequencing step:

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

Linnarsson at 19:28-32, Linnarsson Provisional at 8:22-28.

> 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 at 21:13-25; Linnarsson Provisional at 9:35-10:10.

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223. I understand that in the parallel IPR proceedings, Dr. Quackenbush contends that "Linnarsson does not teach 'sequencing the plurality of tagged polynucleotides' as required by [1G]." In my opinion, Dr. Quackenbush's analysis inappropriately restricts the claim language without any evidence in the '981 Patent and in a way that is inconsistent with the knowledge of a POSITA. First, it is my opinion that the claim does ***not*** impose any requirement to sequence the "entirety" of the tagged polynucleotides as Dr. Quackenbush contends. Second, it is my opinion that even if claim [1G] imposes a requirement to sequencing the "entirety," Linnarsson discloses such a method.

224. It is my opinion that claim [1G] does not include any requirement that the "entirety" of the "tagged polynucleotide" be sequenced. The full language of claim [1G] is "sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences." The term "entirety" does not appear anywhere in the claims of the '981 patent. In fact, the language of claim [1G] requires only "sequencing the plurality of tagged polynucleotides ***to obtain a plurality of identified polynucleotide sequences***." This is exactly what Linnarsson discloses. In my opinion, "sequencing" simply requires generating sequence information from the polynucleotide in order to obtain "identified polynucleotide sequences." There is no explicit or implicit requirement that the "entirety" of the tagged polynucleotide be sequenced. This is consistent with both the '981 patent and the understanding of a POSITA.

225. While I note that it is my opinion that the specification of the '981 patent, which describes the "reflex method", is largely irrelevant to the claims of the '981 patent, which describe barcoding and sequencing of single-cell polynucleotides, there are examples and embodiments in the '981 patent where only portions of the tagged polynucleotides are sequenced. *See e.g.*, '981 at 23:1-17. In this embodiment, the '981 Patent is clear that the "entirety" of tagged polynucleotides do not need to be sequenced. Rather, "after tagging," portions are sequenced after the tagged polynucleotides are broken into smaller fragments.

226. Even if claim [1G] imposes a requirement to sequencing the "entirety," Linnarsson discloses such a method because as described above, Linnarsson broadly discloses a sequencing step that does not require any sort of fragmentation. For example, as discussed at length above in Section IX.B.1, Linnarsson's Figure 3 and Figure 4 show exemplary TSO and CDS that do not contain a biotin and do not contain any restriction enzyme cut site and thus do not require fragmentation.

227. McCloskey teaches sequencing the plurality of tagged polynucleotides. McCloskey provides that "[s]equencing reactions were carried out with fluorescent dideoxynucleotides." McCloskey at 766.

228. To the extent Linnarsson and McCloskey do not disclose this limitation, it would have been obvious to a POSITA because the purpose of tagged polynucleotides for identifying and tracking sample polynucleotides would have been accomplished via routine and conventional sequencing technology whereby the sequence of the sample polynucleotide and MID would be determined.

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

229. Linnarsson teaches a tag "used to identify which single cell from each cDNA sample originated." As discussed above, Linnarsson teaches that the tags present in an exemplary CDS and TSO and incorporated into the cDNA can comprise distinct tags, either or both of which can be used to identify the single cell from which polynucleotide sequence was derived:

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

Linnarsson at 19:5-10, Linnarsson Provisional at 7:26-24.

230. McCloskey also discloses a method that comprises the step of "using the first tag sequence of a MID to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived." As discussed above, McCloskey discloses a method utilizing an encoding oligonucleotide with a first tag referred to as a "batch-stamp," which "specifies the DNA source such as the . . . sample identification." McCloskey at 762. The specific "DNA source" is recommended to include a "single cell." *Id.* at 767

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

231. Linnarsson and McCloskey disclose a method that comprises the step of "using the second tag sequence of a MID to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived."

232. As discussed above, Linnarsson discloses a combination of tags, one of which "can be used to identify which single cell from each cDNA sample originated." Linnarsson at 19:5-11. It would have been obvious to a POSITA to modify the second of the combination of tags using routine and conventional methods to correlate the identified polynucleotide sequence with the sample polynucleotide form which the identified polynucleotide sequence is derived.

233. As discussed above, McCloskey's encoding oligonucleotide comprises two tag sequences: (1) a "batch-stamp" sequence . . . and (2) a "barcode" to distinguish polynucleotide sequences "arising from different cell or allele copies." McCloskey at 763, 764. The batch-stamp sequence correlates to the claimed first tag sequence and the barcode correlates to the claimed second tag sequence. The barcode "distinguishes among sequences arising from different cell or allele copies." McCloskey at 762-64. As explained above, a POSITA would

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have understood McCloskey to disclose that the barcode is useful to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived. The POSITA would furthermore have benefitted from disclosures in both McCloskey and Linnarsson about the ranges of options for tags (e.g., tag at either or both ends of the cDNA, each of which may be variable in length) and combine them to track individual sample polynucleotides from within single-cells.

234. McCloskey further teaches that the "batch-stamps and barcodes [should] be used when amplifying irreplaceable DNAs and cDNAs for … ***single cell*** … ***analyses***," and that its method of tagging applies "to single-stranded DNA or cDNA templates," which, as discussed above, a person of skill in the art would have understood to mean analysis of mRNA. McCloskey at 762, 767. As explained above, it is my opinion that a POSITA would have been motivated to combine Linnarsson and McCloskey, and would have had a reasonable expectation of success in combining them.

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

235. Methods wherein sample polynucleotides are DNA and RNA were well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

236. As described above, 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 at 3:26-27; Linnarsson Provisional at 2:27-31.

237. As explained above, McCloskey discloses providing a sample comprising a plurality of single cells which each comprise a plurality of polynucleotides, which may be DNA or cDNA. McCloskey teaches generating tagged polynucleotides (*e.g.*, tagged cDNA) from sample polynucleotides that are DNA. For example, McCloskey discloses "a method by which molecular codes are added to single-stranded DNA or cDNA templates …." McCloskey at Abstract, 762; *see also* McCloskey at 767 ("We recommend that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for … single cell … DNA analyses."). Further a POSITA would understand that cDNA refers to DNA molecules that are formed by the reverse transcription of RNA molecules. Therefore, a person of skill in the art would have understood that McCloskey discloses tagging and sequencing the claimed "sample polynucleotides," which includes either DNA or RNA.

(k) Claim 5: The method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction.

238. Methods wherein tagged polynucleotides are generated through at least one ligation reaction were well-known in the art, as demonstrated by both Linnarsson and McCloskey. I understand that Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution.

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239. As described above, methods for ligation were well-known in the art. It would have been an obvious and routine design choice with a reasonable expectation of success to ligate an oligonucleotide comprising the second tag sequence.

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

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

'981 Patent at 6:23-32.

> The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Genome Analysis: A Laboratory Manual Series (Vols. I-IV), Using Antibodies: A Laboratory Manual, Cells: A Laboratory Manual, PCR Primer: A Laboratory Manual, and Molecular Cloning: A Laboratory Manual (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) Biochemistry (4th Ed.) Freeman, New York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, A., Principles of Biochemistry 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) Biochemistry, 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

'981 Patent at 13:32-55.

241. I understand that Patent Owner previously challenged a claim directed to attaching a tag by ligation, stating that ligation is not "***any*** new, non-conventional attachment technique." I understand that Plaintiffs' expert Dr. Quackenbush acknowledges that methods of

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ligation have been well-known for decades:

Q. If you could just describe what you knew about polynucleotide tagging as a person skilled in the art as of August 2009 time period.

A. My Ph.D. was in theoretical physics. I worked for two years as a postdoctoral fellow in physics. In 1992, I received a five-year fellowship to work on the Human Genome Project. I began my career at the Salk Institute in San Diego. At the Salk, I went into the lab and threw myself into learning all the laboratory techniques, starting with things like PCR, so that's an amplification technology; DNA sequencing; ***ligation*** and primer detection, the use of tags. And there are a variety of different types of tags that one can use to overcome amplification bias that would include things like universal primers or single primers, ways of tagging different polynucleotides, and we can discuss about radioactive tags, fluorescent tags, other tags like biotin, mass tags that people have used. I'm not providing an exhaustive list.

Quackenbush Tr. (IPR2023-00876) at 33:18-34:17(objection omitted).

Q. ***As of August 2009, you were familiar with ligation*** and primer extension as techniques; is that correct?

A. Broadly speaking, ***yes***.

Q. ***As of August 2009, were you familiar with the use of ligation for polynucleotide tagging***?

A. ***So as of 2009, I personally performed a number of ligation experiments***, both what we refer to as sticky-ended and blunt-ended ligation. ***A number of those introduced a variety of different tags***, including sequencing tags such as sequencing primers. I was also familiar with ligation in the context of sequencing instruments like the Applied Biosystems SOLiD, which performed sequencing by ligation, as well as other applications.

Id. at 57:8-58:1(objection omitted).

Q. ***As of August of 2009, were you familiar with techniques in the field for using ligation to add molecular barcodes to polynucleotides?***

A. So I don't believe I rendered any opinion about that in response to Dr. Cooper's arguments. If you can point me to places where I rendered opinions about that, I'd be happy to refresh my memory. ***As of 2009, I was familiar with ligation.*** I was familiar with Illumina sequencing. There may or may not have been applications of and other sequencing platforms. Earlier we discussed multiplex sequencing using sample barcodes to deconvolute the source of different sequences, and ***I believe that some of the techniques that were in use at the time involved use of ligation to***

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> ***introduce a variety of different tags on universal primers, potentially multiplex barcodes and potentially others.***

Id. at 59:20-60:18 (objection omitted).

> Q. When you say that as of August of 2009, you were aware of techniques to introduce a variety of different tags to polynucleotides, you say potentially multiplex barcodes, were you aware of that or not as of August 2009?
>
> Q. Without using ligation.
>
> A. So sitting here today, I don't recall discussing that because I don't think it was necessary to respond to Dr. Cooper's arguments. ***I was familiar with ligation as a technique dating back to the 19- -- my personal experience with it dating back to the 1990s, both blunt end and sticky ligation***. And I believe that as of 2009, there were protocols for high throughput sequencing, such as that used by Illumina and Applied Biosystems, Helicos Biosystems, 454, other companies for doing multiplex sequencing that used ligation as a way of introducing sample-specific barcodes for multiplex sequencing and demultiplex.

Id. at 60:19-61:15 (objection omitted).

242. Given that ligation was routine, conventional, and well-known in the art, it is my opinion that a POSITA would also have had a reasonable expectation of success in ligating an additional sequence to Linnarsson's CDS and/or TSO. For example, a POSITA would have found it routine and obvious to ligate a tag sequence by using a linker molecule, such as the method described in Hug's 2003 article. *See* Hug at 618-621.

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

244. Hug provides one example method by which a POSITA would know the routine practice of ligation could be used to attach a tag to a CDS and/or TSO. Such a method was commonplace in the art at the time. *See* Hug at 621; *see also* Okayama at 165; Shibata at 1250-51, Fig. 1. Specifically, a POSITA 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.

245. A POSITA would have understood that such an approach, using ligation to add the tag sequences to a 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, the tag sequence is part of a 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

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in selecting tag sequences during an experiment. For example, one might wish to synthesize one set of oligonucleotides for use in the cDNA synthesis and cell-tagging steps separately from those used to provide the second, polynucleotide barcoding oligonucleotides. This separation and modularity would allow a POSITA to combine different sets of tags optimized for different tasks. For example, one set of first (or second) tags might be designed to be usable across many experimental designs while the second (or first) are customized to particular experiments (e.g., differing organisms, cell-types, sample preparations, target mRNAs, etc); such a "mix-and-match" scheme might reduce overall costs (e.g., by producing one set of oligonucleotides in large batches) whilst retaining the ability to customize (by tailoring one set of tags to a given experiment).

246. Further, I note that for a POSITA motivated to add a second tag, as taught by McCloskey, so as to reduce amplification bias (as I discuss above), there are only two common methods available. One is to include the second tag in one or both of the primers used to generate the initial cDNA (a CDS and/or TSO), while the other is to ligate them to the ends of the cDNA. The obviousness of this dependent claim is thus clear given how routine and conventional it was to add tags to polynucleotides via ligation and the fact that ligation of tags was one of a very limited number of options to add tags to polynucleotides prior to amplification.

2. The Asserted Claims Are Obvious Over Hug and Miner

247. It is my opinion that the Asserted Claims of the '981 Patent are obvious in view of Hug and Miner. As I explain in Section IX.C, a POSITA would be motivated to combine Linnarsson and McCloskey and reasonably expect the combination to succeed. In particular, someone with skill would be motivated to combine the first tag of Miner which distinguishes sample polynucleotides from sample polynucleotides arising from different cells and the second tag of Hug which distinguishes sample polynucleotides from sample polynucleotides from the same single cell.

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

248. To the extent the preamble is limiting, it is disclosed by both Hug and Miner.

249. Hug discloses a method of analyzing nucleic acids from a plurality of single cells. Hug describes "a method of calculate the number of molecules of a single mRNA species in a complex mRNA preparation." Hug at Abstract, 615. Hug further describes that its "method could be suitable to detect changes in mRNA levels in total RNA or mRNA preparations which are derived of only a ***few cells***." *Id.* at 623. Furthermore, Hug discloses that a further application of its method "is to determine the number of a certain mRNA … species ***in a single cell*** when the number of cells is counted before RNA isolation." *Id.* As discussed above, determination and detection of mRNA content in a cell is called gene expression and gene expression analysis includes analyzing nucleic acids.

250. Therefore, because Hug discloses a method of determination of mRNA content or gene expression analysis in a plurality of single cells, and because a POSITA would have understood that analyzing gene expression requires analyzing nucleic acids, it is my opinion that

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using ligation to add the oligonucleotides containing the tag sequence to the sample polynucleotide, would be entirely equivalent to the method disclosed in Hug's first approach.

296. Miner also teaches generation of tagged polynucleotides through a ligation reaction. As discussed above, Miner teaches the use of a hairpin linker containing the two tags which is ligated to the sample polynucleotide before amplification. *See* Miner at 1 (tagging "genomic DNA fragments with molecular sequence barcodes and 'batch-stamps' prior to PCR amplification.") This hairpin linker is then ligated to the genomic DNA:

> Conditions for hairpin-bisulfite PCR of human genomic *FMR1* sequences (5) were as follows: 5 µg of genomic DNA was cleaved by 10 U each of restriction endonucleases DraIII and AluI for 1 h at 37˚C, followed by enzyme inactivation at 65˚C for 20 min. The use of a second restriction endonuclease, in this case AluI, removed the CG-rich sequence distal to the region analyzed. ***Ligation of the hairpin linker*** (5′P-AGCGATGCDDDDDDDGCATCGCT-TGA, with variations in the non-random nucleotides for batch-stamps) to DraIII-cleaved genomic DNA was for 15 min at 20˚C, using 400 U of T4 ligase in 20 µl with 1x ***ligation buffer*** (New England Biolabs), followed by enzyme inactivation at 65˚C for 20 min.

Miner at 1.

> Hairpin-bisulfite PCR involves the ***ligation of a synthetic hairpin linker to the ends of a double-stranded genomic DNA fragment prior to bisulfite conversion and PCR amplification***.

Miner at 2.

E. Obviousness Grounds ('197 Patent)

1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey

297. It is my opinion that the Asserted Claims of the '197 Patent are obvious in view of Linnarsson and McCloskey. As I explain in Section IX.C, a POSITA would be motivated to combine Linnarsson and McCloskey and reasonably expect the combination to succeed. In particular, as described above regarding the '981 Patent in Section X.D.1, someone with skill in the would be motivated to combine the first tag of Linnarsson which distinguishes sample polynucleotides from sample polynucleotides arising from different cells and the second tag of McCloskey which distinguishes sample polynucleotides from sample polynucleotides from the same single cell. A POSITA would understand this method to further allow for counting nucleic acids in a sample. In fact, the key motivation for a POSITA to combine McCloskey and Linnarsson is to use the benefits of McCloskey's second, molecule barcode to reduce "amplification bias" and thereby come up with more accurate counts of polynucleotides originally present in a sample

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

298. To the extent the preamble is limiting, it is my opinion that Linnarsson and McCloskey disclose the preamble. *See supra* Section IX.D.1.a.

299. 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 at 2:21-22; *see also id.* at 36:31-33 ("all mapping reads were counted to generate a raw count"). It is my opinion that a POSITA would have understood the term "reads" to refer to the results of sequencing nucleic acid sequences, which comprises the recited "nucleic acids."

300. 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. 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 POSITA would understand the quantitative measure of template diversity refers to a count of the sample polynucleotides based on counting sequences with unique tag sequences.

301. 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)." '197 Patent at 22:38-45.

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

302. It is my opinion that Linnarsson and McCloskey disclose Claim [1B], for the reasons as stated in connection with '981 Patent Claim [1B]. *See supra* Section IX.D.1.b.

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

303. It is my opinion that Linnarsson and McCloskey disclose Claim [1C], for the reasons as stated in connection with '981 Patent Claim [1CB]. *See supra* Section IX.D.1.c.

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

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a sample polynucleotide of the plurality of sample polynucleotides;

304. It is my opinion that Linnarsson and McCloskey disclose Claim [1D], for the reasons as stated in connection with '981 Patent Claim [1D]. *See supra* Section IX.D.1.d.

(e) Claim [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

305. It is my opinion that Linnarsson and McCloskey disclose Claim [1E], for the reasons as stated in connection with '981 Patent Claim [1E]. *See supra* Section IX.D.1.e.

(f) Claim [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;

306. It is my opinion that Linnarsson and McCloskey disclose Claim [1F], for the reasons as stated in connection with '981 Patent Claim [1F]. *See supra* Section IX.D.1.f.

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

307. It is my opinion that Linnarsson and McCloskey disclose Claim [1G], for the reasons as stated in connection with '981 Patent Claim [1G]. *See supra* Section IX.D.1.g.

(h) Claim [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.

308. As discussed above, 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. For example, as explained above, Linnarsson and McCloskey disclose using a first tag to distinguish between cells and using a second tag to distinguish between polynucleotides. *See supra* Sections IX.E.1.e-f. 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 at 38:32. McCloskey provides a method of using the tagged encoding oligonucleotide as "counting sequences with non-redundant barcodes provides a quantitative measure of template diversity." McCloskey at 766.

309. Further, methods of counting polynucleotides in a sample, particularly using molecular tags, were well-known and routine as of the earliest priority date of the Brenner

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Patents. 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)." '197 Patent at 22:38-45.

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

310. It is my opinion that Linnarsson and McCloskey disclose Claim 5, for the reasons as stated in connection with '981 Patent Claim 5. *See supra* Section IX.D.1.k.

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

311. Methods for generating a different number of second tag sequences larger than the number of sample polynucleotides were well-known in the art. For example, providing sufficient second tag sequences to uniquely tag substantially every sample polynucleotide were well-known in the art, as demonstrated by both Linnarsson and McCloskey. As described above, Linnarsson and McCloskey render obvious using a second tag sequence for counting and distinguishing sample polynucleotides originating from the same single-cell. A POSITA would have understood that the second tag sequence facilitates accurate counting by uniquely 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 polynucleotides in the sample cell is critical for gene expression analyses. Therefore, a POSITA would have been motivated to utilize well-known methods to ensure that each sample polynucleotide is tagged uniquely to increase the accuracy of counting.

312. A POSITA would have had a reasonable expectation of successfully tagging each sample polynucleotide with a unique second tag sequence using well-known methods. As explained above, it would have been obvious to tag each sample polynucleotide with a tag. For example, a POSITA would have understood that one could ensure each sample polynucleotide of said plurality of sample polynucleotides would be tagged by using more tags than sample polynucleotides (e.g., 100 times more tags than sample polynucleotides). *See e.g.*, '197 Patent 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.").

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313. 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 a sufficiently large number of unique tags can be generated in order to ensure sufficient unique tags to identify the sample of polynucleotides. For example, and as discussed above, McCloskey teaches that using a tag sequence containing n number of random base pairs "enables one to distinguish 4n allele copies per reaction." McCloskey at 764. This is possible because each base pair can be any of the four nucleotide bases (a, t, g, c/u), as described above in the technical background section. *See supra* Section IX.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 POSITA would have understood that to generate m number of unique tag sequences, one can design tag sequences to have log ⟦(m)⟧ /(log (4)) base pairs.

314. For instance, a POSITA 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]. Linnarsson also discloses tag sequences comprising at least 20 nucleotides. Linnarsson at 18:31-19:2. A POSITA 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. Furthermore, Linnarsson discloses using a combination of two tags, one each in a CDS and TSO, providing yet additional avenues to increase tag diversity, as two tags that each include a random sequence of length n produce 4^(2n) distinct possibilities (e.g., two 7-nucleotide tags produce 4^14 or 268,435,456 distinct possible tags when used in combination).

315. As an additional point, I note that the '197 Patent claims, including claim 1, cite to counting of merely a "plurality" of sample polynucleotides. Thus, they encompass methods in which as few as 2 sample polynucleotides are tagged and counted. Similarly, Linnarsson also discloses methods for use on as few as 2 sample polynucleotides; for example, Linnarsson discloses that the RCS component of a CDS may be "gene family-specific" and may hybridize to "one or more" mRNA. Linnarsson at 15:2-11. In light of the fact that both the '197 Patent claims and Linnarsson contemplate counting small numbers of small polynucleotides, even relatively short random barcodes, such as the 7-nucleotide barcode described as "currently" in use by McCloskey, would be sufficient to achieve a high degree of uniqueness for the second tags when used within at least some embodiments that are both disclosed by Linnarsson and within the scope of claims of the '197 Patent.

(k) 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

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cell from (ii) the number of sample polynucleotides having said sample sequence from other cells.

316. As described above, Linnarsson and McCloskey teach counting the number of sample polynucleotides having the same sample sequence. Linnarsson and McCloskey teach a method of attaching a first, "cell tag" and second, unique molecule-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 first tag can be used to determine the cell from which the polynucleotide was derived (i.e., distinguishing the polynucleotides of one cell from another cell) and the second tag, which is molecule (polynucleotide) specific, can distinguish among tagged polynucleotides within a given cell 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 within 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 and distinguish them from sample polynucleotides that have the same sequence but were derived from other cells. Further, a POSITA 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." '197 Patent at 22:38-40; *see also* Hug at 615 (providing two exemplary methods "to calculate the number of molecules of a single mRNA species in a complex mRNA preparation").

317. Therefore, a POSITA would understand that 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.

(l) 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.

318. It is my opinion that Linnarsson and McCloskey disclose Claim 24, for the reasons as stated in connection with Claim 23. *See supra* Section IX.E.1.k.

2. The Asserted Claims Are Obvious Over Hug and Miner

319. It is my opinion that the Asserted Claims of the '197 Patent are obvious in view of Hug and Miner. As I explain in Section IX.C, a POSITA would be motivated to combine Hug and Miner and reasonably expect the combination to succeed. In particular, as described above regarding the '981 Patent in Section X.D.2, someone with skill in the would be motivated to

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number of sample polynucleotides having said sample sequence from said cell.

341. It is my opinion that Hug and Miner disclose Claim 24, for the reasons as stated in connection with Claim 23. *See supra* Section IX.E.2.k.

F. Obviousness Grounds ('013 Patent)

1. The Asserted Claims Are Obvious Over Linnarsson and McCloskey

342. It is my opinion that the Asserted Claims of the '013 Patent are obvious in view of Linnarsson and McCloskey. As I explain in Section IX.C, a POSITA would be motivated to combine Linnarsson and McCloskey and reasonably expect the combination to succeed. In particular, as described above regarding the '981 Patent in Section X.D.1, someone with skill in the would be motivated to combine the first tag of Linnarsson which distinguishes sample polynucleotides from sample polynucleotides arising from different cells and the second tag of McCloskey which distinguishes sample polynucleotides from sample polynucleotides from the same single cell.

(a) Claim [1A]: A method for multiplexed analysis of nucleic acids from single cells, the method comprising:

343. To the extent the preamble is limiting, it is my opinion that Linnarsson and McCloskey disclose the preamble. *See supra* Section IX.D.1.a.

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

344. It is my opinion that Linnarsson and McCloskey disclose Claim [1C], for the reasons as stated in connection with '981 Patent Claim [1CB]. *See supra* Section IX.D.1.c.

(c) Claim [1C]: performing combinatorial tagging to generate a plurality of tagged polynucleotides from said plurality of sample polynucleotides and a plurality of oligonucleotide tags,

345. As described above Section IV.A.1, I understand that the construction of the term "combinatorial tagging," offered by the Court is an "approach in which one MID is attached by ligation and a second MID is attached by primer extension." It is my opinion that Linnarsson and McCloskey render this claim limitation obvious when adopting this construction.

346. As described above, Linnarsson discloses lysing a plurality of sample single cells to release a plurality of polynucleotides—including mRNA molecules—contained within. 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 at 22:24-26; Linnarsson Provisional at 11:11-14. ("The cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the

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cDNA."). Thus, Linnarsson discloses a method of generating a plurality of tagged polynucleotides from the said plurality of sample polynucleotides, such as sample mRNA.

347. Linnarsson specifically teaches that during cDNA synthesis, tagged oligonucleotides referred to as cDNA synthesis primer (CDS) and/or template switching oligonucleotide (TSO) be used to incorporate a cell-tag into the cDNA. Linnarsson 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 at 7:23-26. The tagged cDNA is the claimed "plurality of tagged polynucleotides." Linnarsson further 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 at 19:6-7; Linnarsson Provisional at 7:26-28. Thus, Linnarsson alone in fact discloses the use of multiple tags in generating tagged polynucleotides, while the combination of Linnarsson and McCloskey discloses the addition of a first tag for tracking cells and a second tag for tracking sample polynucleotides. I discuss the concept of combinatorial tagging in *infra* Sections IX.F.1.d-e, below. Specifically, Linnarsson and McCloskey disclose the step of attaching a tag by primer extension (*see infra* Section IX.F.1.d) and the step of adding a tag by ligation (*see infra* Section IX.F.1.d; *see also supra* Section IX.D.1.k).

(d) Claim [1D]: wherein a tagged polynucleotide of the plurality of tagged polynucleotides is generated by providing an extension product by primer extension using a first oligonucleotide tag and a sample polynucleotide of said plurality of sample polynucleotides, and:

348. Linnarsson teaches the step of primer extension by performing reverse transcription on released plurality of mRNA molecules to generate tagged cDNA molecules. Linnarsson discloses that "cDNA is typically synthesized from mRNA by reverse transcription." Linnarsson at 14:17; Linnarsson Provisional at 4:37. Linnarsson describes steps of cDNA synthesis through reverse transcription:

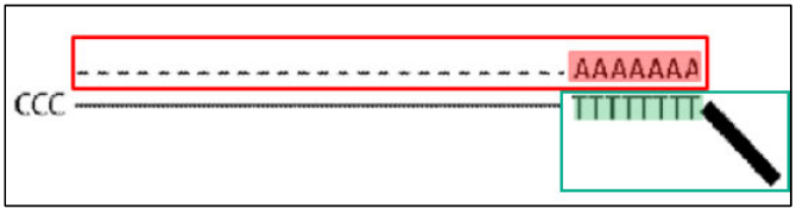
> In one aspect, the method includes the steps of releasing mRNA from each single cell to provide a plurality of individual mRNA samples, synthesizing a first strand of cDNA from the mRNA in each individual mRNA sample and incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples, pooling the tagged cDNA samples and amplifying the pooled cDNA samples to generate a cDNA library having double-stranded cDNA.

Linnarsson at 3:25-30; Linnarsson Provisional at 2:27-3:3.

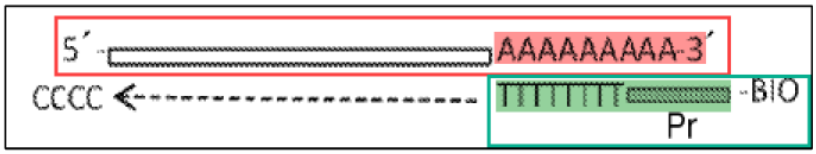
349. Linnarsson also discloses that the "cells are lysed and reverse transcription reaction mix is added directly to the lysates without additional purification. This results in the synthesis of cDNA from cellular mRNA and incorporation of a tag into the cDNA." Linnarsson at 22:24-26; Linnarsson Provisional at 11:11-14. Further, Linnarsson teaches tagging the cDNA with oligonucleotides (*i.e.*, CDS and/or TSO) during primer extension. The tagged cDNA is the claimed "plurality of tagged polynucleotides." Specifically, Linnarsson first teaches using a

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tagged CDS during primer extension 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, in this case comprising a "tailed oligo-dT primer" (boxed in green, below) that "directs synthesis of a cDNA strand":

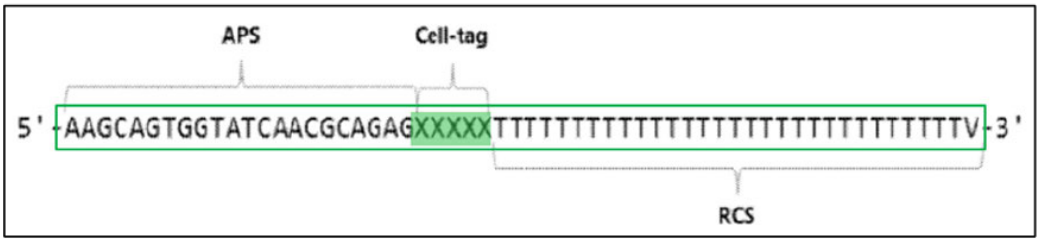


Linnarsson at Fig. 2.



Linnarsson at Fig. 11; *see also id.* at 5:29-31 (explaining the disclosure of Figure 11).

350. As demonstrated in Figures 2 and 11 above, this examplary CDS includes a sequence of thymines (highlighted in green, above) that hybridize with the sequence of adenines making up the poly-A tail of an 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 at 5:29-31; Linnarsson Provisional at 5:24-32, 13:8-14. For example, Figure 4 depicts another example of a CDS (boxed in green, below) with a cell-tag (highlighted in green, below):

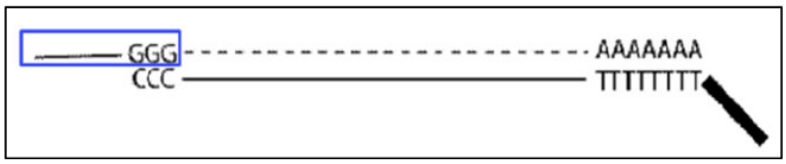


Linnarsson at Fig. 4.

351. 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 at 10:18-20, Linnarsson Provisional at 2:33-36; *see also* Linnarsson 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

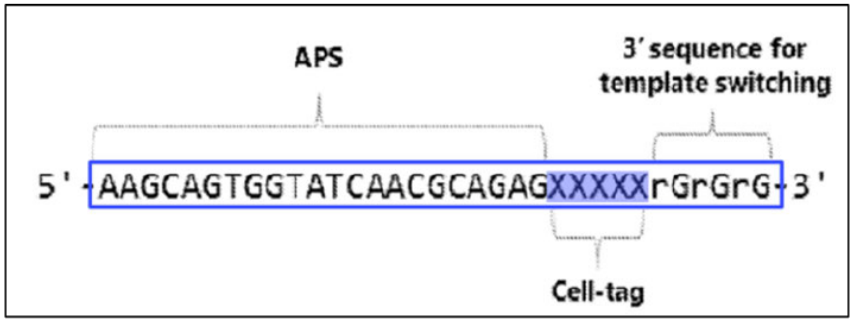
of cDNA from the mRNA in each individual mRNA sample and ***incorporating a tag into the cDNA to provide a plurality of tagged cDNA samples***. . ."); Linnarsson Provisional at 2:27-3:3.

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



Linnarsson at Fig. 2.

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



Linnarsson at Fig. 3.

354. 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 at 19:5-7; Linnarsson Provisional at 7:26-28.

(e) Claim [1E]: ligating a second oligonucleotide tag to said extension product, and

355. It is my opinion that Linnarsson and McCloskey disclose Claim [1E], for the reasons as stated in connection with '981 Patent Claim 5. *See supra* Section IX.D.1.k.

(f) Claim [1F]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: a sample sequence

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corresponding to said sample polynucleotide of the plurality of sample polynucleotides;

356. It is my opinion that Linnarsson and McCloskey disclose Claim [1F], for the reasons as stated in connection with '981 Patent Claim [1D]. *See supra* Section IX.D.1.d.

(g) Claim [1G]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a first tag sequence distinguishing said sample polynucleotide from sample polynucleotides from other cells; and

357. It is my opinion that Linnarsson and McCloskey disclose Claim [1G], for the reasons as stated in connection with '981 Patent Claim [1E]. *See supra* Section IX.D.1.e.

(h) Claim [1H]: wherein said tagged polynucleotide of the plurality of tagged polynucleotides comprises: . . . a second tag sequence distinguishing said sample polynucleotide from other sample polynucleotides from said cell;

358. It is my opinion that Linnarsson and McCloskey disclose Claim [1H], for the reasons as stated in connection with '981 Patent Claim [1F]. *See supra* Section IX.D.1.f.

(i) Claim [1I]: amplifying said tagged polynucleotide, thereby generating a plurality of amplified polynucleotides corresponding to the tagged polynucleotide; and

359. Methods for amplifying tagged polynucleotides were well-known in the art, as demonstrated by both Linnarsson and McCloskey. 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 at 21:21-22; Linnarsson Provisional at 9:4-6. Figure 1 of Linnarsson provides an exemplary walkthrough comprising the steps of collecting a plurality of single cells, tagging and performing reverse transcription to produce tagged cDNA, and pooling and amplifying the tagged cDNA before performing sequencing:

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

Linnarsson at 4:8-12, Linnarsson Provisional at 10:2-3; *see also id.* at 6:1-3, 10:15-24.

360. McCloskey also teaches an amplification step. For example, McCloskey discloses that "PCR products were sequenced and their encoded information was evaluated," thus teaching that PCR was performed to amplify the polynucleotides prior to the sequencing step. McCloskey at 763. As I explained above, PCR was a well-known method for amplification.

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Further, when discussing how sample cDNA is processed prior to sequencing, McCloskey teaches that samples may be "amplified using standard PCR protocols." McCloskey at 762, 763.

361. Finally, I note above that the problem of "amplification bias" would have motivated a POSITA to combine Linnarsson and McCloskey (given that Linnarsson discusses the problem and that McCloskey provides methods to solve the problem). This problem intrinsically arises from "amplification", further underscoring the obviousness of this step.

(j) Claim [1J]: sequencing said plurality of amplified polynucleotides to determine sequences of the amplified polynucleotides corresponding to the sample sequence, the first tag sequence, and the second tag sequence of the tagged polynucleotide; and

362. It is my opinion that Linnarsson and McCloskey disclose Claim [1J], for the reasons as stated in connection with '981 Patent Claim [1G]. *See supra* Section IX.D.1.g.

(k) Claim [1K]: using the sequences determined in step (d) to count sample polynucleotides for multiple different sample polynucleotides of multiple different single cells of said plurality of cells.

363. It is my opinion that Linnarsson and McCloskey disclose Claim [1H], for the reasons as stated in connection with '197 Patent Claim [1H]. *See supra* Section IX.E.1.h.

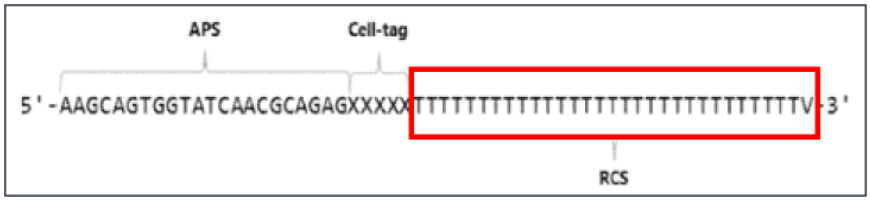
(l) Claim 2: The method of claim 1, wherein said plurality of sample polynucleotides comprise messenger ribonucleic acid (mRNA).

364. It is my opinion that Linnarsson and McCloskey disclose Claim 2, for the reasons as stated in connection with '981 Patent Claim 3. *See supra* Section IX.D.1.j.

(m) Claim 3: The method of claim 2, wherein step (A) comprises (i) hybridizing said first oligonucleotide tag to said mRNA and (ii) extending said first oligonucleotide tag using said mRNA as a template to generate complementary DNA (cDNA).

365. The combination of Linnarsson and McCloskey renders obvious the "method of claim 2, wherein step (A) comprises (i) hybridizing said first oligonucleotide tag to said mRNA and (ii) extending said first oligonucleotide tag using said mRNA as a template to generate complementary DNA (cDNA)." Linnarsson discloses that a CDS can comprise an oligonucleotide tag and an RNA complementary sequence (RCS) which is complementary to "one or more mRNA" which allows it to hybridize to "at least some mRNA" in a sample and is used in cDNA synthesis. For example, in Figure 4, the example CDS includes a "cell-tag" and a series of thymines (boxed in red) which hybridize to a polyA tail present in mRNAs for the purpose of cDNA synthesis:

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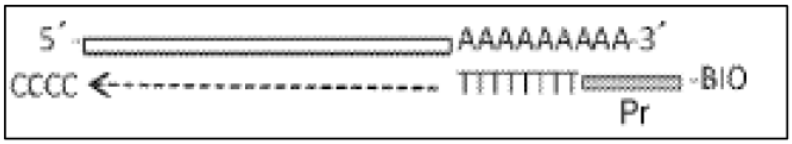
Linnarsson at Fig. 4.

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

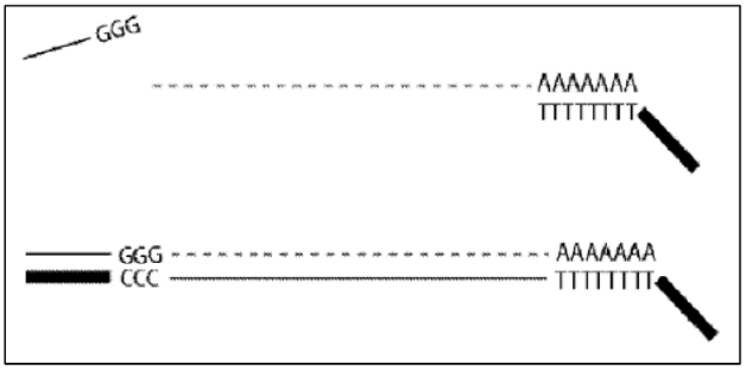
> In some aspects of the invention, the RCS is at least partially complementary to one or more mRNA in an individual mRNA sample. This allows the primer, which is typically an oligonucleotide, to hybridize to at least some mRNA in an individual mRNA sample to direct cDNA synthesis using the mRNA as a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

Linnarsson at 15:2-6; Linnarsson Provisional at 5:26-30.

367. Linnarsson further teaches the step of extension of the oligonucleotide tag using the said mRNA as a template to generate cDNA. As described above, for example, Linnarsson discloses that "a tailed oligo-dT primer directs synthesis of a cDNA strand." Linnarsson at 5:29-31; Linnarsson Provisional at 5:24-32, 13:8-14. As examples, Figure 2 and Figure 11 shows the direct synthesis of a cDNA strand by extension of a CDS through the process of reverse transcription:



Linnarsson at Fig. 11.



Linnarsson at Fig. 2.

(n) Claim 6: The method of claim 1, wherein said plurality of cells is provided in a plurality of wells.

368. Providing a sample comprising a plurality of single cells in a plurality of wells was well-known in the art, as demonstrated by both Linnarsson and McCloskey.

369. For example, the following passages from Linnarsson disclose that each single cell is provided in separate wells:

> (A) the tissue of interest is dissected; (B) a plurality of single cells are selected; (C) single cells are placed in separate wells of a 96-well plate and lysed; tagged reverse transcription is performed on each sample to produce cDNA; (D) cDNA samples are pooled and amplified; (E) sequencing is performed to obtain 100 million reads; and (F) identification of cells from which they originated.

Linnarsson at 4:9-13; Linnarsson Provisional at 9:35-10:6.

> Single cells can be placed in any suitable reaction vessel in which single cells can be treated individually. For example, a 96-well plate, such that each single cell is placed in a single well.

Linnarsson at 13:20-22; Linnarsson Provisional (Linnarsson Provisional) at 3:36-4:2.

370. To the extent Linnarsson and McCloskey do not disclose Claim 6, such a method would have been obvious given knowledge and common sense available to a POSITA.

(o) Claim 9: The method of claim 1, wherein step (b) comprises randomly associating said plurality of sample polynucleotides with said plurality of oligonucleotide tags.

371. Methods for randomly associating oligonucleotide tags with tagged polynucleotides were well-known in the art, as demonstrated by both Linnarsson and McCloskey. A POSITA 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. *See e.g.*, Hug at 616 ("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.

372. Linnarsson discloses 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." Linnarsson at 18:27-29. Linnarsson teaches that tags hybridize to mRNA in a stochastic, non-specific manner. For

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example, in some embodiments Linnarsson discloses use of a CDS that attaches to mRNA by hybridizing with the poly-A tail, which is shared on many sample mRNAs regardless and thus will lead to tags being randomly distributed across mRNAs. Further, Linnarsson also discloses that a CDS may include random sequences, such as "random hexamers", to serve as RNA complementary sequences and thus achieve a random assignment of tags to sample RNAs. Thus, a POSITA would understand that Linnarsson teaches adding oligonucleotide tags in a way such that the tags randomly associate with the sample polynucleotides to generate the plurality of tagged polynucleotides.

(p) Claim 15: The method of claim 1, wherein said plurality of oligonucleotide tags comprises a number of different second tag sequences that is larger than the number of sample polynucleotides.

373. It is my opinion that Linnarsson and McCloskey disclose Claim 15, for the reasons as stated in connection with '197 Patent Claim 17. *See supra* Section IX.E.1.j.

(q) Claim 25: The method of claim 1, wherein said plurality of tagged polynucleotides are pooled with tagged polynucleotides from other single cells of said plurality of cells prior to the amplifying of step (c).

374. A POSITA would know that pooling would be performed prior to step of amplification. It is also obvious, for example, from McCloskey's method that polynucleotides from different cells of a patient may be pooled before amplificaiton, as it utilizes its batch-stamp for various purposes including to identify "DNA source such as the patient or sample" (which encompasses tagging polynucleotides from multiple cells pooled together) in addition to labelling and tracking single cells. McCloskey at 762.

375. Additionally, Linnarsson teaches that pooling is performed prior to amplification. *See* Linnarsson at 3:29-30; 10:20-22 ("… pooling the tagged cDNA samples ***and amplifying the pooled cDNA*** samples to generate a cDNA library having double-stranded cDNA."); Linnarsson Provisional at 3:2-3.

(r) Claim 26: The method of claim 1, wherein said plurality of amplified polynucleotides are pooled with amplified polynucleotides from other single cells of said plurality of cells prior to the sequencing of step (d).

376. It is my opinion that Linnarsson and McCloskey disclose Claim 26, for at least the reasons as stated in connection with Claim 25. See supra Section IX.F.1.q.

377. For example, the pooling step taught by Linnarsson could naturally occur before the step of sequencing which follows after amplification. In fact, this is acknowledged at the very face of the '013 Patent which cites to a 2009 technical bulletin titled "Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry – Basic MID Set":

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> When different libraries are prepared with different MIDs, they can be amplified by emPCR and sequenced together, in a multiplex fashion; the sequencing reads can be deconvoluted by the data analysis software after the sequencing Run, such that the reads from each of the pooled libraries are identified by their MID tag and correctly assigned.

See PARSE10X0144963.

378. A POSITA would have understood that amplified polynucleotides can be pooled before sequencing because, as described, samples have been associated with unique cell and molecule tags, which allow one to identify each samples origin after pooling samples for processing (either amplification or sequencing). Additionally, a POSITA would be motivated to pool samples because it increases efficiency and speed and decreases cost.

2. The Asserted Claims Are Obvious Over Hug and Miner

379. It is my opinion that the Asserted Claims of the '013 Patent are obvious in view of Hug and Miner. As I explain in Section IX.C, a POSITA would be motivated to combine Hug and Miner and reasonably expect the combination to succeed. In particular, as described above regarding the '981 Patent in Section IX.D.2, someone with skill in the would be motivated to combine the first tag of Miner which distinguishes sample polynucleotides from sample polynucleotides arising from different cells and the second tag of Hug which distinguishes sample polynucleotides from sample polynucleotides from the same single cell.

(a) Claim [1A]: A method for multiplexed analysis of nucleic acids from single cells, the method comprising:

380. To the extent the preamble is limiting, it is my opinion that Hug and Miner disclose the preamble. *See supra* Section IX.D.2.a.

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

381. It is my opinion that Hug and Miner disclose Claim [1C], for the reasons as stated in connection with '981 Patent Claim [1B]. *See supra* Section IX.D.2.b.

(c) Claim [1C]: performing combinatorial tagging to generate a plurality of tagged polynucleotides from said plurality of sample polynucleotides and a plurality of oligonucleotide tags,

382. As described above Section IV.A.1, I understand that the construction of the term "combinatorial tagging," offered by the Court is an "approach in which one MID is attached by ligation and a second MID is attached by primer extension." It is my opinion that Hug and Miner render this claim limitation obvious when adopting this construction.

383. As described above, Hug discloses using oligonucleotides "consisting of the

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573. Further, claim 5 of the '013 Patent and claim 10 of the '917 patent claim on oligo dT sequence for use in tagging. 10x, has, again, characterized that this was "known and conventional." *Id*. ("Claim 1 of the 809 patent claims, apart from the abstract labeling idea, 'an oligo dT sequence' and 'a sequencing primer binding site' but again the Fodor patents admit the use of oligo dT sequences and primer binding were known and conventional."). Claim 9 of the '013 Patent and claim 20 of the '197 Patent of the Brenner patents recite the "random" attachment of tags to the polynucleotides. I understand that 10x acknowledged this concept to be no more than a "mathematical abstract idea" that would not contribute to patent eligibility. *See id.* at 16 ("The dependent claims requiring attaching of the labels to be "stochastic" fail Mayo step 2 for the same reason—the specification admits that stochastic attachment is itself a mathematical abstract idea that was well known for more than 40 years."). *Id.*

574. Furthermore, claims 3-4 of the '981 Patent, claims 2 and 6 of the '013 Patent, and claims 3-4 and 25-26 of the '197 Patent recite the use of various naturally occurring and routinely used sample types, such as DNA, RNA or mRNA. I note that the use of these standard sample forms add nothing to the abstract idea to confirm patent eligibility. Claims 24-26 of the '013 Patent deal with pooling samples, which is also a concept that the patents admit to be prior art. *See* '981 Patent at 1:26-29. Indeed, it is my opinion that pooling samples is undisputedly routine, well-known, and conventional. Claim 23 of the '013 Patent and claims 23-24 of the '197 Patent recite counting molecules, which is also an abstract idea. 10x admits that. *See* D.I. 13-5 at 12-13 ("in addition to the ***many known techniques for counting molecules***..."). Claim 21 of the '013 Patent and claim 22 of the '197 Patent pertain to the use of solid supports, such as a "microscope slide," which is just a routine prior art technique. *See* '981 Patent at 11:25-36.

575. Finally, claims 12-18 of the '013 Patent and claims 12-19 of the '197 Patent recite adjusting the number of tags relative to the DNA sequences. Such techniques, as admitted by 10x, were nothing more than "an ineligible abstract idea that cannot save" the claims. D.I. 13-5 at 15-16 ("However, the specification confirms that ratio selection is just performing math, and is thus itself an ineligible abstract idea that cannot save these claims.").

576. Therefore, it is my opinion that because the Asserted Claims of the Brenner Patents are directed to an abstract idea and do not recite any further inventive step or application, the claims are not eligible for patentability.

Dated: May 17, 2024

By: _____
Gregory Cooper, Ph.D.

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