UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.,
Petitioner,

v.

10X GENOMICS, INC.,
Patent Owner.

Case No. IPR2023-00876
U.S. Patent No. 10,155,981

DECLARATION OF GREGORY COOPER, PH.D IN SUPPORT OF PETITIONER'S REPLY TO PATENT OWNER'S RESPONSE

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I. INTRODUCTION

1. I, Gregory Cooper, declare as follows:

2. This declaration responds to Dr. John Quackenbush's opinions regarding the patentability of U.S. Patent No. 10,155,981 (the "'981 patent").

II. RESPONSE TO DR. QUACKENBUSH'S OPINIONS REGARDING THE LEVEL OF ORDINARY SKILL

3. In this declaration, I apply the same definition of a person of ordinary skill in the art ("POSA") as in my original declaration. EX1002 ¶¶ 57-58. I understand that Dr. Quackenbush has provided his own definition of a POSA but has not disputed any aspect of my definition in providing his opinions. EX2013 ¶ 25; *see also id.* ("My opinions would not change if I applied [Dr. Cooper's] definition of a person[] of skill."). Dr. Quackenbush confirmed this in his deposition:

> Q. I'm just asking. There will be a broader question in a minute. But I'm just asking you now, when you say, "My opinions would not change if I applied that definition of a person of skill," you're referring to Dr. Cooper's opinion?
>
> A. Here, specifically, when I say "that definition," ***I'm applying to -- I'm referring to Dr. Cooper's definition***.
>
> Q. Okay. All right. Thank you. And in order to determine that there would be no difference, you performed the obviousness

analysis both under -- from the vantage point of your level of skill in the art and Dr. Cooper's; is that correct?

A. So I initially applied my definition. I also looked at Dr. Cooper's definition. Recognizing that as of 2009, I had at least the skill and experience and training of a person of ordinary skill in the art that would match either my definition or Dr. Cooper's definition and also that I had supervised people as of 2009 who had the relevant opinion of either one. ***In terms of overall knowledge and understanding, I didn't see a great difference. And so essentially at the end of the day, I've applied both definitions. And the opinions I set forth are consistent with those***.

EX1057 at 31:6-32:8.

4. Most important, Dr. Quackenbush provides his opinions with the understanding that a POSA would be familiar with the well-known techniques of ligation, primer extension, and sequencing. EX2013 ¶ 25 (admitting that his "opinions would not change if [he] applied [my] definition of a" POSA who "would be familiar with associated tools, methods, and techniques including: … (5) ligation and primer extension … and (6) sequencing …"). In fact, he admitted to have learned of laboratory techniques prevalent around 1992, including "DNA sequencing; ligation and primer detection," during his deposition. EX1057 at 34:1-8 ("In 1992, I received a five-year fellowship to work on the Human Genome Project

… I went into the lab and threw myself into learning all laboratory techniques, starting with … DNA sequencing; ligation and primer detection …"). *Id*.

5. Again, the key point is that Dr. Quackenbush does not dispute that (1) polynucleotide tagging, (2) amplification, (3) use of tags to overcome amplification bias, (4) single cell analysis, (5) ligation and primer extension, and (6) sequencing all would have been techniques that would have been with the basic competencies of the skilled artisan in August 2009, as set forth in ¶ 58 of my original declaration. As set forth further herein, this is pertinent to several aspects of my opinions. EX1002 ¶ 58.

III. RESPONSE TO DR. QUACKENBUSH'S OPINIONS REGARDING STATE OF THE ART AND '981 PATENT

A. The State Of The Art Of Sequencing

6. My original declaration included a brief overview of DNA sequencing technology. *See id*. ¶¶ 95-98. For the purpose of responding to certain opinions of Dr. Quackenbush regarding how the claims should be interpreted, it is necessary to briefly expand upon that original overview.

7. As I stated in my original declaration, nucleic acid sequencing is the process of determining the sequence of nucleotides in a nucleic acid molecule. *Id.* My original declaration discussed "Next Generation Sequencing" technologies that were the state of the art at the time of the '981 Patent in August 2009. *Id*. ¶¶ 95-98. NGS technologies were able to sequence millions of DNA fragments in

parallel. *See* EX1041.003 ("NGS platform … was … able to amplify millions of copies of a particular DNA fragment in a massively paralleled way in contrast to Sanger sequencing.") These NGS sequencing platforms, however, are "short-read" sequencing platforms, meaning that the platforms could only read relatively short stretches of DNA, typically ranging from a few dozen to a few hundred base pairs. *See* EX1046.004 ("For example, the 454 FLX instrument generates ~400,000 reads per instrument-run at lengths of 200 to 300 bp."); EX1047.001 ("Finally, next-generation sequencers produce shorter read lengths (35-250 bp, depending on the platform) …"). The '981 Patent notes this when discussing "existing sequencing platforms," explaining that such platforms could not read the entire sequence of large DNA fragments in genomic regions of interest:

> One limitation of the overall process stems from limitations of existing DNA sequencing technologies. In particular, if fragments in the regions of interest of the genome are longer than the lengths that can be sequenced by a particular technology, then such fragments will not be fully analyzed (since sequencing proceeds from an end of a fragment inward).

EX1001 at 1:32-38. Dr. Quackenbush likewise notes this aspect of NGS in the 2009 timeframe in his declaration. EX2013 ¶ 98 ("Longer sequences would not be sequenced by many NGS technologies, and longer fragment lengths would therefore be disadvantageous, as persons of skill would have recognized.").

8. As an example, the Roche 454 sequencing platform, which is repeatedly mentioned in the '981 Patent, "produced an average read length of ~250 bp per sample" in 2007. EX1047.002. Similarly, the '981 Patent reported in August 2009 a length for the Roche 454 platform of about 400 bases. EX1001 at 16:65-17:2 ("For example, sequencing a polynucleotide region that is 2 kilobases or more in length using Roche 454 (Branford, Conn.) technology, in which the length of a single sequencing run is about 400 bases."). The Illumina sequencing platforms that were becoming available in the same timeframe would read only dozens to low hundreds of bases. EX1047.002 ("Introduced in 2006, the Illumina Genome Analyzer is based on the concept of 'sequencing by synthesis' (SBS) to produce sequence reads of ~32–40bp from tens of millions of surface-amplified DNA fragments simultaneously.").

B. Response To Dr. Quackenbush's Opinions Regarding The '981 Specification Disclosure

9. Dr. Quackenbush on many occasions has mischaracterized the '981 Patent as allegedly disclosing dual tagging of polynucleotides. *See, e.g.*, EX2013 ¶ 30 (The "specification of the '981 Patent explains that tagging a polynucleotide with the '981 Patent's ***disclosed first and second tag sequences***…"); *id.* ¶ 28 (The "patent describes using a multiplex identifier ("MID") ***with two tag sequences***: 'a first tag sequence associated with the single cell from which the sample

polynucleotide is derived' and 'a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same cell.'").

10. As I described in my original declaration, the specification of the '981 Patent lacks disclosure of dual-tagging of polynucleotides. EX1002 ¶ 28. The specification describes something different—the "reflex method." *Id*. Even to the extent the '981 Patent teaches the use of MIDs, it does not teach a MID to incorporate ***both*** the claimed first and second tag sequence, as characterized by Dr. Quackenbush. *Id.*; *see also id*. ¶ 30.

11. Rather, the '981 patent teaches only the use of a ***single*** tag that can perform different functions. *See, e.g.*, EX1001 at 6:36-38 ("In certain embodiments, the MID on a polynucleotide is used to identify the source from which the polynucleotide is derived…."); *see also id.* at 6:45-47 ("In certain embodiments, MIDs are employed to uniquely tag each individual polynucleotide in a sample."). Nowhere does the '981 Patent disclose the simultaneous use of two tags, wherein a first tag is used to encode the cellular origin of a polynucleotide and a second tag distinguishes among polynucleotides in a cell.

12. I disagree with Dr. Quackenbush that the "innovative methods claimed in '981 Patent [] permitted users to do what had not previously been possible: to determine the quantity of ***each polynucleotide*** present in a cell ***on a cell-by-cell basis*** and to determine the quantity of each type of individual cell." EX2013 ¶ 30. As

detailed in my original declaration, the allegedly "innovative" methods of tagging were well-known and obvious. EX1002 ¶¶ 77-82; 156-194. Indeed, Dr. Quackenbush cites the definition of MID in the '981 Patent in support of this argument, which clearly discloses that MIDs were known in the art. *Id.*; *see also* EX1001 at 6:56-64 ("Exemplary nucleic acid tags that find use as MIDs are described in U.S. Pat. No. 7,544,473, issued on Jun. 6, 2009, and titled 'Nucleic Acid Analysis Using Sequence Tokens', as well as U.S. Pat. No. 7,393,665, issued on Jul. 1, 2008, and titled 'Methods and Compositions for Tagging and Identifying Polynucleotides', both of which are incorporated herein by reference in their entirety for their description of nucleic acid tags and their use in identifying polynucleotides.") Dr. Quackenbush states that he disagrees with my opinion that the "'981 tagging method is not new, but rather a prior art technique," but fails to actually address the substance of my opinion in his declaration. *See* EX2013 ¶ 31.

C. Response To Dr. Quackenbush's Opinions That Depend On Properties Of Mammalian Cells

13. Many of Dr. Quackenbush's opinions are premised on the number of tags that would be required to tag each mRNA molecule in a mammalian cell. Based on the number of molecules in a typical mammalian cell, Dr. Quackenbush opines that one would require "5,000,000 – 30,000,000 unique tags" when discussing whether the barcodes used in the McCloskey reference may be used in context of methods defined by Linnarsson. *Id*. ¶ 28. To the extent Dr.

Quackenbush contends that either the claims or prior art is limited to mammalian cells, it is my opinion that such a contention is erroneous and mischaracterizes my declaration from a related IPR proceeding.[1]

14. First, as to the '981 patent itself, nothing in the claims requires the ability to tag each of the mRNA molecules in a mammalian cell. The claims refer to cells generally, not mammalian cells.

15. Likewise, nothing in the '981 specification would limit the claims to mammalian cells. Just, the opposite, the specification states that the alleged invention can be used with any type of cell, including bacteria, which are simpler than mammalian cells:

> Furthermore, any organism, organic material or nucleic acid-containing substance can be used as a source of nucleic acids to be processed in accordance with the present invention including, but not limited to, plants, animals (e.g., reptiles, mammals, insects, worms, fish, etc.), tissue samples, bacteria, fungi (e.g., yeast), phage, viruses, cadaveric tissue, archaeological/ancient samples, etc.

EX1001 at 14:14-21; *see also id.* at 14:61-63 ("In certain embodiments, the nucleic acid sample being analyzed is derived from a single source (e.g., a single organism, virus, tissue, cell, subject, etc.), …"); *id.* at 12:7-11 ("'Sample' means a quantity of

[1] IPR2023-00958 for U.S. Patent No. 10,697,013

material from a biological, environmental, medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures).").

16. Notably, despite relying upon the notion that the '981 patent claims require uniquely tagging all the mRNA molecules in a mammalian cell, at deposition Dr. Quackenbush did not identify any basis to contend that the claims should be so limited:

> Q. Do you understand that the '981 claims are limited to mammalian sources?
>
> MS. RAYMOND: Objection. Scope.
>
> A. ***I haven't performed such an analysis. I don't believe Dr. Cooper offers any such opinions***, and so it's beyond the scope of what I'm prepared to testify about today.

EX1057 at 130:7-13.

17. While the claims of the '981 Patent are plainly not limited to mammalian cells, neither is the Linnarsson reference I rely upon. Rather, Linnarsson is just like the '981 Patent in that it teaches that its approach may be used on a wide variety of input samples, including simpler cells such as bacteria or yeast. *See* EX1003 at 13:13-16 ("… cells from specific organs, tissues, tumors, neoplasms, or the like can be obtained … Furthermore, in general, cells from any population can be used in the methods, ***such as a population of prokaryotic or eukaryotic single***

celled organisms including bacteria or yeast.”). Indeed, Dr. Quackenbush agrees to this in his deposition:

> Q. In terms of Linnarsson, is it your understanding that Linnarsson can only be used with mammalian sources?
>
> MS. RAYMOND: Objection. Scope.
>
> A. ***So I don't believe Dr. Cooper offers any interpretation of Linnarsson saying that Linnarsson can only be used for mammalian sources. I don't believe I offered any opinions about the limitations of the application of Linnarsson beyond mammalian sources***. However, Linnarsson's CDS, cDNA synthesis primer has an oligo(dT) component. And to the best of my recollection, all eukaryotic mRNAs are polyadenylated. ***So, for example, Linnarsson's application or Linnarsson's method could, sitting here today speculating, could be applied to analysis of single yeast cells or other eukaryotic cells***.

EX1057 at 130:14-131:7.

18. In fact, as detailed below, Linnarsson discloses methods that do not even require tagging of *all* mRNA molecules rather only a subset for which only a small number of tags would be needed. *See* EX1003 at 15:2-6 (“RNA complementary sequence (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 … to direct cDNA synthesis

using the mRNA as a template."); *id.* at 15:6-7 (RSC can "be gene family-specific"); *see also infra* Section VI.A.6.

D. Response To Dr. Quackenbush's Opinions Regarding The State Of The Art Of Single Cell Analysis

19. Dr. Quackenbush asserts that "[u]ntil inventions such as the '981 Patent, most nucleotide sequencing was performed using polynucleotides (e.g., DNA or RNA) from a population of cells in a process called bulk analysis." EX2013 ¶ 29. I disagree.

20. As I describe in my original declaration, in the August 2009 timeframe, single cell analysis had been practiced and "next-generation sequencing (NGS) technologies [] enabled gene expression in tens of thousands of single cells." EX1002 ¶ 74-75; EX1003 at 2:28-29 ("These methods are suitable for the analysis of small numbers of single cells, and in particular may be used to study cells that are difficult to obtain in large numbers …").

21. Dr. Quackenbush further gives the example of detecting overexpression of the HER2 gene in cancer cells as a technique supposedly taught and enabled by the '981 Patent. EX2013 ¶¶ 29-30 ("Applying the inventive methods of the '981 Patent, the medical professional of the example above could identify individual cells with over-expressed HER2 genes and could then diagnose the patient with cancer at an early stage."). In my opinion, however, the '981 Patent teaches nothing of the

sort. In fact, Dr. Quackenbush acknowledged this to be the case during his deposition:

> Q. And in terms of the HER2 gene that you referred to in paragraph 29 and 30, that's just something you used as an example or did you get that from the patent or something else?
>
> A. ***So this is an example that comes from my experience***, commonly referred to the gene as HER2. It's a widely-used expression marker to classify a subtype of breast cancer, and I can tell you about its importance if you'd like.

EX1057 at 67:11-19.

IV. CLAIM CONSTRUCTION

22. I understand that Dr. Quackenbush contends that the '981 Patent requires sequencing of the ***entirety*** of the claimed "tagged polynucleotides." EX2013 ¶¶ 44, 54-60. I disagree with his opinion for several reasons.

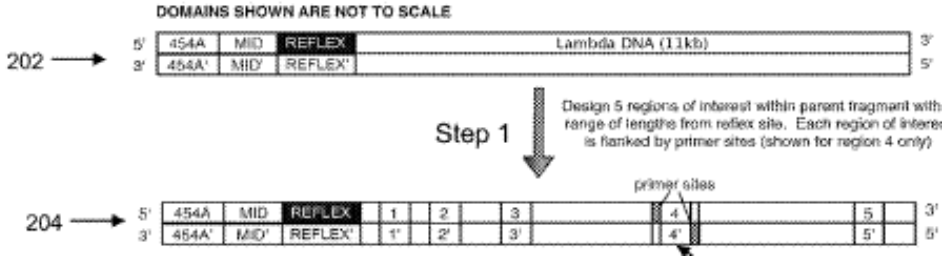
23. First, I do not see anything in the claim language to require sequencing the "entirety" of the "tagged polynucleotide." The claim merely states "sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences" and does not refer to sequencing the "entire" tagged polynucleotide. A POSA in 2009 would understand that "sequencing the plurality of the tagged polynucleotides to obtain a plurality of identified polynucleotide sequences" is simply calling for one to generate sequence information (*i.e.*, the "identified polynucleotide sequences") from the "tagged polynucleotides."

Nothing in this language requires one to sequence the "entirety" of the "tagged polynucleotides."

24. Second, the specification confirms that the claims of the '981 Patent would not be interpreted to require sequencing the entirety of the "tagged polynucleotides." As discussed previously, the specification of the '981 patent is focused on the "reflex method" which involves breaking tagged polynucleotides into smaller fragments suitable for sequencing. The '981 patent specification states, for example, that "***[a]fter tagging each polynucleotide*** in the sample with a sequencing primer site, MID and reflex sequence (as shown in the figures and described above), ***we use the reflex process to break each polynucleotide into lengths appropriate to the sequencing procedure*** being used…." EX1001 at 23:3-9. In other words, the specification teaches that the tagged polynucleotides of interest are not sequenced in their entirety, but are instead broken down into fragments "appropriate to the sequence procedure" and ***then*** sequenced. Notably, this type of fragmentation of tagged polynucleotides prior to sequencing is little different from the fragmentation of tagged polynucleotides prior to sequencing that takes place in Figure 11 of Linnarsson.

25. As another example, consider Figure 3 of the '981 patent. The original tagged polynucleotide appears as item 202 and is an 11 kb fragment of Lambda

DNA. The tagged polynucleotide includes four regions of interest that are flanked by primer sites:



After tagging using the reflex method, what ends up finally being sequenced is not the entire tagged polynucleotide, but rather only small portions thereof that are of interest and that are located between the primer sites (denoted as "1," "2," "3," "4," and "5"):

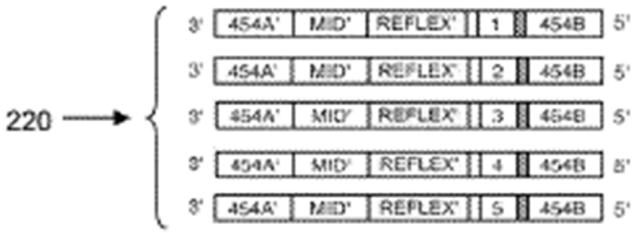


Figure 3 of the '981 patent thus reflects another example of an approach that is inconsistent with the construction that Dr. Quackenbush proposes.

26. Finally, Dr. Quackenbush's interpretation should not be adopted because it is inconsistent with what a skilled artisan would have understood about sequencing technology in the 2009 timeframe. As I explain above, the relevant sequencing platforms in 2009, including the Roche 454 sequencing platform that is discussed repeatedly in the '981 Patent, were short read sequencing platforms. *See*

supra ¶¶ 7-8. These platforms provide only short stretches of sequence information from a particular DNA fragment. As such, it would be odd to limit a patent claim that allegedly is entitled to a 2009 priority date and that requires DNA sequencing to an approach that sequences entire DNA fragments.

V. RESPONSE TO DR. QUACKENBUSH'S CHARACTERIZATION OF THE PRIOR ART

A. Linnarsson

27. At the outset, it is my opinion that Dr. Quackenbush's overview of Linnarsson is deficient. His declaration is exclusively focused on Figure 11, and otherwise ignores many other key teachings, embodiments, and figures disclosed in Linnarsson, for instance, but not limited to, Figures 3 and 4.

28. Dr. Quackenbush states that Linnarsson discloses a "single-cell tagged reverse transcription (STRT) method," aspects of which "are illustrated in Panels A-F of Linnarsson's Figure 11." EX2013 ¶¶ 33, 36. While this is true, it is not what Linnarsson is all about and Figure 11 is ***only one*** out of the numerous figures and "representative examples" of Linnarsson's invention. *See* EX1003 at 4:4-9:21 (Describing 22 figures "intended to illustrate broad concepts of the invention by reference to ***representative examples***" that are "not intended to limit the scope of the invention"). As I discuss in my original declaration, Linnarsson teaches a method of analyzing gene expression in a plurality of single cells by preparing a tagged cDNA library for sequencing. *Id*. at 1:6-9 ("The present invention relates to the

analysis of gene expression in single cells. 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. The cDNA libraries prepared by the method of the invention are suitable for analysis of gene expression by sequencing."); EX1002 ¶ 108.

29. Linnarsson generally teaches this method in Figure 1, comprising the following steps: "(A) the tissue of interest is dissected; (B) a plurality of single cells are selected; (C) single cells are placed in separate wells of a 96-well plate and lysed; tagged reverse transcription is performed on each sample to produce cDNA; (D) cDNA samples are pooled and amplified; (E) sequencing is performed to obtain 100 million reads; and (F) identification of expressed genes and identification of cells from which they originated." EX1002 ¶ 121; EX1003 at 4:8-13.

30. Dr. Quackenbush acknowledges that Linnarsson discloses these general steps. For example, Dr. Quackenbush in ¶ 33 of his declaration states:

> Linnarsson's method involves preparing a cDNA library for sequencing by reverse-transcribing mRNA initially produced by transcription of genes being expressed in the analyzed cells. EX1003, 1 (Abstract), 3:25-4:2; EX1002, ¶109. In a step of this process, "***single cells are placed in separate wells of a 96-well plate and lysed***…." EX1003, 4:8-13. Each ***lysed cell "contain[s] a plurality of mRNA molecules***." EX1003, 3:26-30; EX2012, Abstract; EX2011, ¶256. Following lysis, ***Linnarsson adds cDNA***

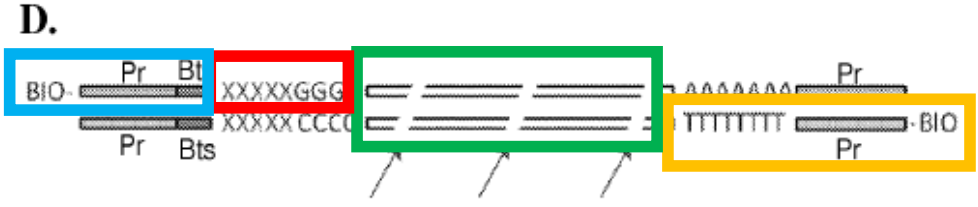
> ***synthesis primers ("CDSs") and/or template switching oligonucleotides ("TSOs"), which are then incorporated into cDNA during reverse transcription from mRNA***. EX1003, 18:32-33. According to Linnarsson, a sequence in the CDSs and/or TSOs can be used to identify the cell from which the tagged polynucleotide originated. EX1003, 19:4-11.

EX2013 ¶ 33; *see also id.* ¶ 35 ("After the tags are added as part of the reverse transcription process, the cDNA from each well is ***pooled***, and ***a cDNA library is created***. EX1003, 18:27-19:11."). Importantly, nothing in this disclosure imposes the requirements for the specific steps that are set forth in Figure 11 and that are used for fragmentation and capture.

31. Despite acknowledging that Linnarsson includes this broad disclosure, Dr. Quackenbush goes on to focus almost exclusively on Figure 11. 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 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. The biotin (in the case of both the CDS and TSO) and restriction enzyme cut site (in the case of the TSO) are part and parcel

of the fragmentation method used in Figure 11. Dr. Quackenbush goes on to characterize Linnarsson as being limited to the specific fragmentation approach set forth in Figure 11.

32. According to Dr. Quackenbush, for instance, Linnarsson's sequencing step is limited to the disclosure in Figure 11. It is his opinion that Linnarsson's method "does not sequence the entirety of the DNA sequences" because of the "fragmentation" and "capturing" steps disclosed in Panel D and E of Figure 11. EX2013 ¶¶ 39-44. Specifically, Dr. Quackenbush alleges that only the sequence corresponding to the DNA fragment tagged with the TSO (boxed in red) is sequenced, after it gets cleaved by a restriction enzyme. *Id*. ¶ 41 ("Only the portion of the 5' fragments that are to the 3' end of the Bts sequence are released from the beads; the 3' fragments are biotinylated, and "remain stuck on the beads" because they do not include Bts sequences."). The "internal fragments," as Dr. Quackenbush terms it (boxed in green) are washed away and the sequences attached to the biotin molecules (boxed in blue and yellow) remain stuck on beads and are thus not sequenced. *Id*. ¶¶ 39-42.



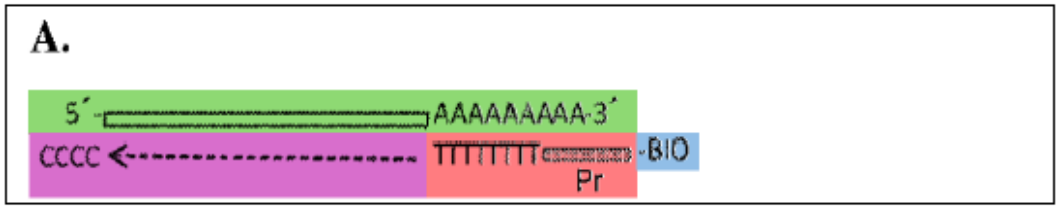
There are several defects with such opinions.

33. To begin, 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." EX1003 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.

34. 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. I explain why this is so immediately below.

35. 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. EX1001 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.”). According to Dr. Quackenbush this “tailed oligo-dT primer” is the CDS (highlighted in red) which “has a poly-T tail at its 3’ end, a primer sequence on the 5’ side of the CDS’s poly-T tail, and is ‘capped’ by a biotin molecule …, in blue.” EX2013 ¶ 37.



This biotin molecule is used in the fragmentation approach to capture the nucleic acid to which it is connected. EX1003 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.").

36. Consider now the CDS in Figure 4 of Linnarsson compared to the 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:

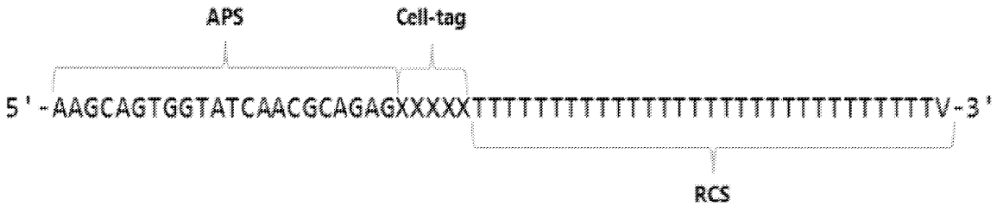


Figure 4

Id. at Fig. 4.

37. 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. EX1064.001; EX1003 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. This further undermines Dr. Quackenbush's reliance on Figure 11. Specifically, Dr. Quackenbush argues that my reliance on the CDS is misplaced because the CDS in Figure 11 is never sequenced. While that may be true of Figure 11, it is not true of the embodiments based on Figure 4. Indeed, it would make no sense whatsoever to include a cell tag within a CDS for tracking the cellular origin of a DNA fragment only to later ignore the cell tag. On the other hand, it makes sense that no such cell tag is part of the CDS in Figure 11 because there is no intention for the CDS in Figure 11 to ever be sequenced.

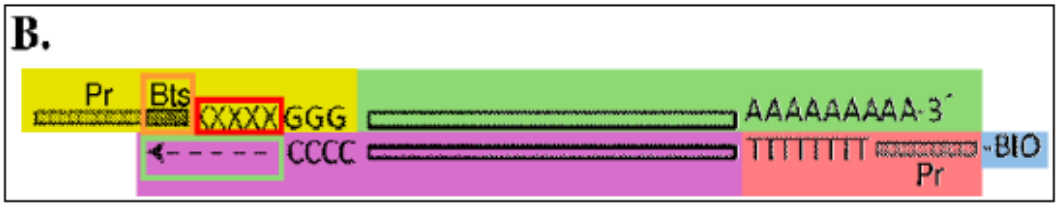
38. Notably, Dr. Quackenbush admits that ***either*** the CDS or TSO in Linnarsson can have a "cell tag" that traces the polynucleotide to its cell of origin.

EX2013 ¶ 33 ("According to Linnarsson, a sequence in the CDSs ***and/or*** TSOs can be used to identify the cell from which the tagged polynucleotide originated. EX1003, 19:4-11."); *id.* ¶ 37 ("A cell tag (according to Dr. Cooper) ***can*** be incorporated between the primer and the poly-T tail [of the CDS]. EX1002, ¶112; EX1003, Fig.4, 4:17-18, 18:31-19:11."). Given that Dr. Quackenbush acknowledges that Linnarsson teaches that a cell tag can be included in the CDS, it is difficult to believe that he would simultaneously contend that Linnarsson never contemplates sequencing the CDS as would be required to use the cell tag for its intended purpose. This is a plainly illogical conclusion and clearly not the way a POSA would have read or understood Linnarsson.

39. 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 capture.

40. Panel B teaches the template switching step with the help of a "[b]arcoded helper oligo," which Dr. Quackenbush calls the TSO (yellow in left column in table below) and 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." EX2013 ¶ 38. The BtsCI type IIS restriction endonuclease sequence is a restriction enzyme that cleaves the

sequences at the Bts sequence cut-site (the sequence of which is GGATG) and releases the captured fragments bound to the streptavidin beads for sequencing. EX1064.001.



41. 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." EX1003 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:

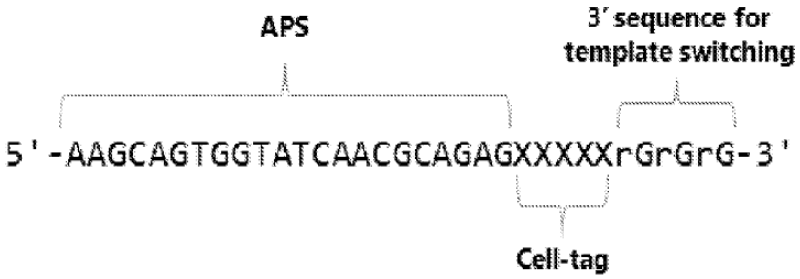


Figure 3

Id. at Figure 3; *see also* EX1064.001.

42. 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.

43. 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." EX1003 at 15:1-10. While Linnarsson states that 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.

44. In short, insofar as Dr. Quackenbush's rebuttals to my opinions are based on the specific aspects of the techniques used in Figure 11, his opinions are irrelevant because (1) Linnarsson is clear that the specific aspects of Figure 11 are optional and (2) there are embodiments in Linnarsson confirming that Linnarsson is not limited to the fragmentation and capture approach of Figure 11. Two prominent examples of such embodiments are reflected in Figures 3 and 4 of Linnarsson, which I cited throughout my original declaration. *See* EX1002 ¶ 112, 114, 141, 144, 163, 165, 232.

45. In addition to ignoring Figures 3 and 4 of Linnarsson and other aspects of Linnarsson describing methodological variations, it is my opinion that Dr. Quackenbush has ignored other disclosures in Linnarsson that are broad and do not limit its application to the steps of "capturing" and "fragmentation" as set forth in Figure 11. For example, as I describe above and in my original declaration, Linnarsson discloses generally that "sequencing is performed," after the steps of tagging and amplification. EX1002 ¶197; *see also* EX1003 at 4:8-13; 3:31-4:2 ("The invention further provides methods for analyzing gene expression in a

plurality of cells by preparing a cDNA library as described herein and ***sequencing*** the library.").

46. Indeed, Linnarsson's disclosures reveal that sequencing is not limited to ***one*** particular method, as misrepresented by Dr. Quackenbush, because Linnarsson's method gives the experimenter the flexibility to choose from different sequencing methods depending on the needs of the experiment. *See* EX1003 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.").

47. 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.

B. McCloskey

48. I understand that Dr. Quackenbush does not dispute McCloskey's publication date of October 23, 2007. EX1057 at 91:21-23. As described in my original declaration, McCloskey discloses a method of DNA analysis for single-cell samples that utilizes an "encoding oligonucleotide" having two tag sequences—a batch stamp and a barcode that distinguishes samples and polynucleotides, respectively. EX1002 ¶¶ 99-104.

49. Dr. Quackenbush argues that McCloskey does not disclose a first-tag sequence that identifies the cell from which the nucleic acid originated. EX2013

¶¶ 48, 63. To be clear, whether McCloskey teaches a tag that can be used to track cell of origin is largely irrelevant because Linnarsson teaches a cell tag, and I rely upon that disclosure in my declaration. EX1002 ¶¶ 156-170. But, in any event, I disagree with Dr. Quackenbush that McCloskey does not disclose a cell tag. McCloskey's batch-stamp 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. *Id*. ¶ 99; EX1004.002, .003, .004. Further, McCloskey discloses that the sample may be a "single-cell sample." EX1004.002. Thus, the 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 sample barcode is the same as the cell tag described by Linnarsson and a POSA would have understood this from reading these references.

50. Dr. Quackenbush further argues that the first tag does not identify the cell of origin because "McCloskey does not disclose using its method for 'single cell analysis.'" EX2013 ¶ 63. I disagree.

51. As noted in my original declaration, McCloskey describes methods of single cell analysis by utilizing molecular tags. EX1002 ¶¶ 104,134,184; *see also* EX1004.001, .007 ("We recommend that batch-stamps and barcodes be used when

amplifying irreplaceable DNAs and cDNAs available for…single cell…analyses."). I disagree with Dr. Quackenbush's argument that McCloskey does not allow single-cell analysis because McCloskey's disclosed example analyzes human genomic DNA that weighs 2.71µg which "is significantly more genomic DNA than that present in a single cell." EX2013 ¶¶ 47-48. It is my opinion that this disclosure is only an exemplary embodiment described in McCloskey and it is clear that McCloskey's teachings are applicable to various analyses including "forensic, clinical, ***single cell***, and ancient DNA analyses." EX1004.001, .007. Indeed, during his deposition, Dr. Quackenbush failed to support his contention that McCloskey fails to teach single-cell analysis. Rather he acknowledged that McCloskey recommends the use of molecular tags for single-cell analysis:

> Q. You'll agree that she recommends the batch-stamps and barcodes that she described for single-cell analysis?
>
> MS. RAYMOND: Objection. Scope.
>
> A. ***So she says she recommends it***, but she doesn't describe any way in which this could be done. And the only method that she presents is one that uses 2.71 micrograms of DNA, which is far more than what you would get for an individual cell.

EX1057 at 106:23-107:7.

52. Further, Dr. Quackenbush's contention is inconsistent with what he states in his declaration, which ultimately confirms that McCloskey teaches single-cell analysis. Dr. Quackenbush acknowledges that "McCloskey discloses the possibility of tagging DNA even when the sample (batch) consists of DNA from only a 'single cell.'" EX2013 ¶ 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. EX1004, 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 POSA 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 POSA 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 barcoding strategy would have been easily understood by a POSA to be relevant to Linnarsson's cell tag strategy given that Linnarsson describes the generation of many single-cell "samples."

53. Additionally, Dr. Quackenbush argues that "there would not be enough oligonucleotide tags in McCloskey to tag each mRNA molecule in a cell." EX2013 ¶ 49. According to Dr. Quackenbush, McCloskey allows creation of only 16,384 unique barcodes which "is far too few to allow unique barcode tags for each mRNA molecule." *Id.* ¶¶ 46, 49. Dr. Quackenbush bases this opinion on the argument that a "person[] of skill would have expected there to be between 50,000 and 300,000 mRNA molecules in the typical mammalian cell, and persons of skill would want to err towards having enough unique tags for the high side of the range." *Id.* I disagree with Dr. Quackenbush. First, McCloskey does not limit its method or provide that it is only capable of generating 16,384 tags. The number is exemplary and, in fact, McCloskey defines a general formula for determining the number of unique sequences that would result from any given barcode length (i.e., "4^n" where n is the number of random bases). *See* EX1004.004 ("We currently use seven

nucleotides for the barcode, giving 16,384 possible barcodes for each encoding oligonucleotide."). Thus, a POSA would understand McCloskey's method to not be limited to generating 16,384 tags but flexible to expand the range depending on the requirements and needs of the experiment. Second, as is also true for both Linnarsson and the '981 patent, McCloskey does not teach that its methods are limited to analysis of polynucleotides in only mammalian cells. Dr. Quackenbush points to no such disclosure. Rather, McCloskey, as stated above, recommends its methods for "forensic, clinical, single cell, and ancient DNA analyses." EX1004.001, .007; *see also id.* at .002 (the batch-stamp, unique to each experiment, specifies the DNA source such as the patient or sample identification.). Thus, McCloskey teaches a general method that is applicable to single cell analysis. Further, as also discussed above, not all of Linnarsson's methods require targeting of all mRNA molecules for cDNA synthesis, tagging, and sequencing. Linnarsson discloses utilizing primers for cDNA synthesis that include sequences "at least partially complementary to one or more mRNA". EX1003 at 15:1-10. Linnarsson further teaches that these sequences may be "gene specific." *Id.* Thus, a POSA would understand that any number of mRNA molecules can be analyzed using Linnarsson and McCloskey's methods depending on the sample type and requirements of the method employed.

VI. RESPONSE TO DR. QUACKENBUSH'S OPINIONS REGARDING OBVIOUSNESS

A. Ground 1: Claims 1-4 And 6 Are Obvious Over Linnarsson In View Of McCloskey

1. Linnarsson Teaches Sequencing the Tagged Polynucleotides

54. As described in my prior declaration, it is my opinion that Linnarsson discloses a method comprising the step of "sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences" as required by claim [1G]. EX1002 ¶¶ 195-199. I understand that Dr. Quackenbush contends that "Linnarsson does not teach 'sequencing the plurality of tagged polynucleotides' as required by [1G]." EX2013 ¶ 54. 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 POSA. First, it is my opinion that the claim does ***not*** impose any requirement to sequence the "entirety" of the tagged polynucleotides. Second, it is my opinion that even if claim [1G] imposes a requirement to sequencing the "entirety," Linnarsson discloses such a method.

55. I have been informed by counsel that, during patent prosecution, the Patent Owner did not dispute that Linnarsson discloses "sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences" as required by claim [1G]. As such, Patent Owner did not raise any argument to

overcome the rejection based on Linnarsson that imposed a limitation of sequencing the "entirety" of the tagged polynucleotides as Dr. Quackenbush now contends.

a. Claim [1G] Does Not Require Sequencing The "Entirety" Of The Tagged Polynucleotides

56. As I summarize above, *see supra* Part VI.A.1.a, it is my opinion that claim [1G] does not require sequencing of the "entirety of the tagged polynucleotides" as Dr. Quackenbush contends in his declaration. EX2013 ¶ 54. Specifically, it is my opinion that the claim language does not require sequencing the "entirety," the '981 patent specification demonstrates that sequencing the "entirety" is not required, and a POSA at the time of the invention would not interpret claim [1G] to impose the requirement Dr. Quackenbush now asserts. Below I provide further detail on these points to further supplement my opinion above that the claims do not require sequencing the "entirety" of the claimed "tagged polynucleotides."

57. 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***."

58. As described in my prior declaration, this is exactly what Linnarsson discloses. EX1002 ¶¶ 195-199. 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 POSA.

59. It is my opinion that the intrinsic record of the '981 patent does not contain any evidence to support Dr. Quackenbush's requirement that the "entirety" of the "tagged polynucleotides" be sequenced. Dr. Quackenbush does not cite any such evidence. EX2013 ¶¶ 54-58. 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.

60. As one example from the '981 patent provides:

> For example, we might want to sequence one thousand viral genomes (or a specific genomic region) or one thousand copies of a gene present in somatic cells. ***After tagging each polynucleotide in the sample*** with a sequencing primer site, MID and reflex sequence (as shown in the figures and described above), ***we use the reflex process to break each polynucleotide into lengths appropriate to the sequencing procedure being used,*** transferring the sequencing

primer site and MID to each fragment (as described above). Obtaining sequence information from all of the reflex-processed samples can be used to determine the sequence of each individual polynucleotide in the starting sample, using the MID sequence to defining linkage relationships between sequences from different regions in the polynucleotide being sequenced. Using a sequencing platform with longer read lengths can minimize the number of primers to be used (and reflex fragments generated).

EX1001 at 23:1-17.

61. 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. Further, I understand that during deposition, Dr. Quackenbush confirmed that he had performed "***no analysis***" on whether the sequencing of claim [1G] requires sequencing the "entirety" of the tagged polynucleotides. For example, Dr. Quackenbush provided the following testimony:

Q. An in terms of Step (c), it says, "sequencing the plurality of tagged polynucleotides . . ."

Does that tagged polynucleotide have to be -- does that permit additional processing of the tagged polynucleotide from Step (b) before Step (c)?

MS. RAYMOND: Objection. Form.

A. Again, I don't think Dr. Cooper offered any opinion about additional processing and so I had nothing to respond to. So I haven't done that analysis, and I'm not prepared to offer an opinion today.

Q. ***Do you have an opinion as to whether the sequencing of plurality of tagged polynucleotides requires sequencing of the entire sequence of the tagged polynucleotide?***

MS. RAYMOND: Objection to form.

Q. Is that a claim construction issue that you evaluated?

MS. RAYMOND: Objection. Form. Scope.

A. So I don't believe Dr. Cooper has offered any opinions, so ***I haven't done that analysis, and I'm not prepared to offer testimony regarding it today.***

EX1057 at 172:20-173:20.

62. It is correct that I did not opine in my original declaration that the claims of the '981 patent require sequencing the entirety of the tagged polynucleotides. I did not then and do not now believe any such requirement exists. Rather, that claim construction opinion originated in Dr. Quackenbush's declaration, and thus it is perplexing that he disavowed it by pointing to the absence of that opinion in my original declaration. In any case, the fact that he was unable or unwilling to articulate a justification for that interpretation is consistent with the conclusion that the '981 patent claims do not require sequencing the entire tagged polynucleotide.

63. Finally, it is my opinion that a POSA at the time of invention of the '981 patent would not understand claim [1G] to require sequencing the "entirety" in the way that Dr. Quackenbush contends in his declaration. As described above, the next generation sequencing technology at the time was limited to short reads to obtain short fragments that the sequencing technology could accommodate. For example, the '981 patent provides as follows with regard to the Roche 454 patent:

> As an exemplary embodiment, suppose we want to sequence a specific polynucleotide region from multiple genomes in a pooled sample where the polynucleotide region is too long to sequence in a single reaction. ***For example, sequencing a polynucleotide region that is 2 kilobases or more in length using Roche 454 (Branford, Conn.) technology, in which the length of a single sequencing run is about 400 bases.*** In this scenario, we can design a set of left hand primers (A_n) and right hand primers (B_n) specific for the polynucleotide region that are positioned in such a way that we can obtain direct sequences of all parts of the insert, as shown in FIG. 1B.

EX1001 at 16:62-17:6. In this embodiment, the '981 patent describes sequencing a long stretch of DNA (2 kilobases) using the existing sequencing Roche 454 technique, which was limited to "about 400 bases." To accomplish this, the '981 patent describes fragmenting the long stretch of DNA and obtaining short stretches that are "within the single-sequencing run read length of the current Roche 454 sequencing platform." *Id*. at 17:28-32. Thus, even the '981 patent acknowledges

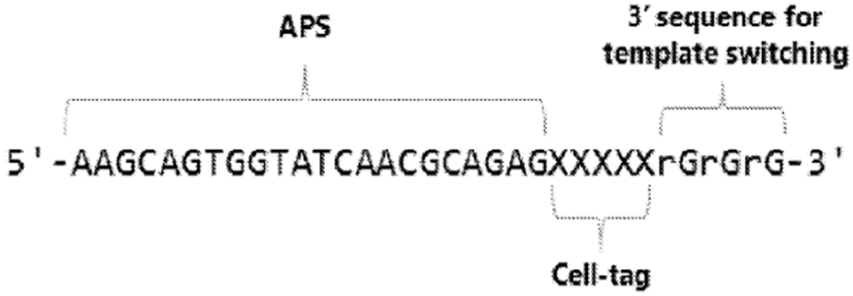
that the sequencing platforms for use with the alleged invention were limited in their ability to read long stretches of DNA. Thus, a POSA would not have interpreted claim [1G] to impose a requirement to sequencing the "entirety" of the tagged polynucleotides as described by Dr. Quackenbush.

b. Dr. Quackenbush Mischaracterizes Linnarsson As Requiring Fragmentation

64. Dr. Quackenbush's arguments that Linnarsson does not disclose sequencing the "entirety" of the fragment is based on the contention that Linnarsson requires the use of fragmentation as set forth in Figure 11. Yet, this argument is erroneous because a skilled artisan would understand that what Linnarsson contemplates is not limited to the fragmentation-based approach in Figure 11.

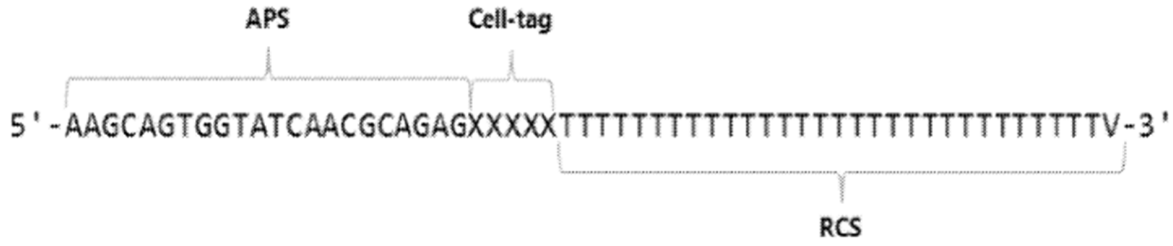
65. As described in my prior declaration, Linnarsson broadly discloses a sequencing step that does not refer to any sort of fragmentation and does not include the steps of Figure 11. EX1002 ¶ 195-199. Dr. Quackenbush's primary contention appears to be that Linnarsson does not disclose sequencing the "entire" tagged polynucleotide because "Linnarsson teaches that the 'amplified library is fragmented'" as set forth in Figure 11, wherein only a terminal end of the DNA molecule is capture for sequencing. EX2013 ¶ 55. Setting aside that, as described above, the '981 patent also teaches fragmenting, it is my opinion that the disclosures of Linnarsson ***do not*** require the specific approach of Figure 11.

66. Figure 11 is simply one exemplary embodiment of Linnarsson. The tagged polynucleotides in Figure 11 do contain a biotin molecule that would be used to capture fragmented polynucleotides for sequencing. EX1003 at Fig. 11; 5:28-6:12. As described above, after washing, these captured fragments would then be released by a restriction enzyme for sequencing. *Id.* Linnarsson, however, contains numerous other exemplary embodiments that ***do not*** include a biotin or any restriction enzyme cut site. For example, as discussed at length in my prior declaration and above, 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. EX1002 ¶¶ 112, 114-117, 144-147, 163, 165. Specifically, 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," and ***no*** biotin or "GGATG" restriction enzyme cut site:



EX1003 at 4:15-16; Fig. 3; *see also* EX1064.001. Similarly, 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)," and ***no*** biotin or "GGATG" restriction enzyme cut site:

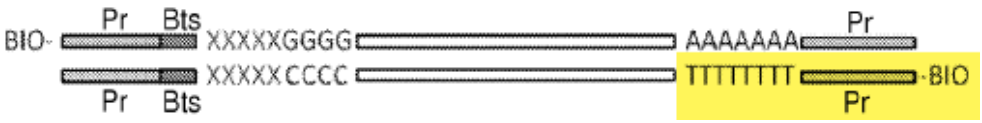


EX1003 at 4:17-18; Fig. 4; *see also* EX1064.001.

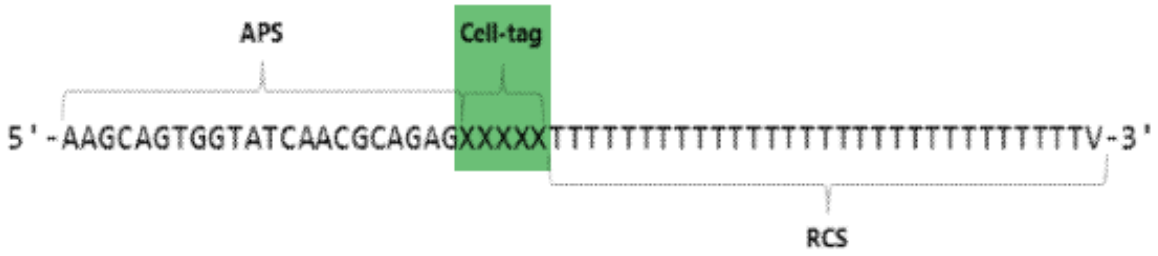
67. As I explain above, where no biotin and no restriction enzyme cut site are present, the embodiment does not involve fragmenting the tagged polynucleotides as disclosed in Figure 11. Thus, as evidenced by Figures 3 and 4, Linnarsson does not require the Figure 11 approach in all of its embodiments.

68. Dr. Quackenbush further points to Figure 11 as evidence that the 3' CDS fragments "do not contain a Bts sequence and, as a result, are not released from the 'beads' to which they are bound." EX2013 ¶ 56. As described above, however, Linnarsson is not limited to the single embodiment described in Figure 11. In fact, Linnarsson provides clear examples of 3' CDS sequences that are ***not*** biotinylated. For example, Figure 4, shown above, provides a CDS sequence with no biotin. Thus, Linnarsson is not limited to the embodiment that Dr. Quackenbush fixates on and in fact the 3' CDS sequences are designed to be sequenced.

69. The fact that the CDS is designed to be sequenced is further evidenced by the inclusion of a "cell-tag" on the CDS. In Figure 11, upon which Dr. Quackenbush relies, the CDS sequence contains no "cell tag":



EX1003 at Fig. 11C (annotated). The Figure 11 CDS sequence, highlighted in yellow, contains no "cell tag" for good reason—it is biotinylated and lacks a restriction enzyme cutting site and will not be released and sequenced. As described at length in my prior declaration and above, the other CDS sequences described by Linnarsson ***do*** contain a "cell tag." EX1002 ¶¶ 112, 138, 141, 163-170. For example, Figure 4 provides an exemplary CDS "comprising a 5' amplification primer sequence (APS), a ***cell tag*** and a 3' RNA complementary sequence (RCS)":



EX1003 at 4:17-18; Fig. 4. The "cell tag," highlighted in green, only functions if the CDS is sequenced. Without sequencing the CDS, including a "cell tag" would serve no purpose. Further, as described above, the CDS in Figure 4 does not

contain biotin. Thus, Linnarsson is here describing a method wherein the CDS is sequenced.

70. Dr. Quackenbush relies on additional exemplary embodiments from Linnarsson, but again these are non-limiting. For example, Dr. Quackenbush cites 21:13-25, which provides that "the cDNA library ***can*** be processed by fragmenting." EX1003 at 21:13-25. Again, Linnarsson is clear that this is describing only an exemplary embodiment that "can" be used, not a limitation that ***must*** be used. As further examples, Dr. Quackenbush cites the methods outlined at 27:6-28:2 and 35:23-36:9. Again, these are only exemplary embodiments as made clear by the fact that they fall under the headings "**EXAMPLE I**" and "**EXAMPLE II**." *Id.* at 23:1; 31:3. In my opinion, all the citations to fragmentation provided by Dr. Quackenbush are merely examples and are not meant to be limiting. As described above, this must be the case given the other embodiments provided that ***do not*** require the fragmentation and capture approach wherein certain DNA fragments are not captured.

71. I understand that Patent Owner further contends that "Linnarsson defines 'cDNA library' as 'a collection of cloned complementary DNA (cNDA) fragments.'" Paper 13 at 23. This quote, however, is taken out of context and fails to support the Patent Owner. The language to which Patent Owner points is not teaching the use of fragmentation of the type that is set forth in Figure 11, which

Dr. Quackenbush relies upon. Rather, this language is merely stating that the cDNA will necessarily be a fragment of DNA since the RNA is itself just a fragment of nucleic acid. With full context, this is clear:

> The term "cDNA library" refers to a collection of cloned complementary DNA (cDNA) fragments, which together constitute some portion of the transcriptome of a single cell or a plurality of single cells. cDNA is produced from fully transcribed mRNA found in a cell and therefore contains only the expressed genes of a single cell. . .

EX1003 at 12:4-8. Thus, this "definition" does not support Patent Owner and, in addition to the reasons described above, demonstrates that Linnarsson does not require fragmentation of the type set forth in Figure 11.

72. As additional confirmation that the methods of Linnarsson cannot ***require*** the specific fragmentation of Figure 11, as Dr. Quackenbush contends, Linnarsson's dependent claims provide a method utilizing fragmenting. Specifically, Linnarsson's claim 27, which depends on claim 1, provides the "method according to claim 26, wherein the processing comprises fragmenting the cDNA library." *Id*. at claim 27. I understand that where a limitation is imposed by a dependent claim, the independent claim is necessarily broader in scope. Thus, by imposing the limitation of fragmenting in dependent claim 27, Linnarsson confirms that fragmenting is ***not*** required by the method taught, but is merely an

optional embodiment. Thus, Linnarsson discloses sequencing the "entirety" of the tagged polynucleotides, even under Dr. Quackenbush's proposed interpretation.

2. McCloskey Discloses A First Tag Sequence

73. Dr. Quackenbush contends that McCloskey does not disclose the "first tag sequence" of claims [1E] and [1H]. EX2013 ¶¶ 61-66. Interestingly, Dr. Quackenbush does not dispute that Linnarsson discloses the "first tag sequence" of claims [1E] and [1H], which is the method primarily relied upon to disclose these claims in my prior declaration. EX1002 ¶¶ 157-170, 200-202. Thus, Dr. Quackenbush appears to admit that these claims are rendered obvious by Linnarsson.

74. Even if Linnarsson did not disclose a "first tag sequence" (which it does), as described in my prior declaration McCloskey also discloses a "first tag sequence." *Id*. ¶¶ 171-177, 203-204. Specifically, McCloskey discloses an encoding oligonucleotide that contains a batch stamp that "specifies the DNA source such as the patient or sample identification." *Id*. ¶ 173; EX1004.002. McCloskey is clear that the samples being identified by the batch stamp may be "single-cell samples." EX1004.002. Thus, as previously described, a POSA would have understood that McCloskey's batch-stamp may be used to identify a specific single-cell of origin—exactly as required by claim [1E]. EX1002 ¶¶ 176-177.

75. Despite these clear disclosures, Dr. Quackenbush's contends that McCloskey's batch stamps do not satisfy claims [1E] and [1H]. To support this

contention, his leading assertion is that "McCloskey does not disclose using its method for 'single cell analysis.'" EX2013 ¶ 63. Dr. Quackenbush's contention, however, is not internally consistent. For example, Dr. Quackenbush acknowledges that "McCloskey discloses the possibility of tagging DNA even when the sample (batch) consists of DNA from only a 'single cell.'" *Id.* Dr. Quackenbush goes on to state that McCloskey's batch stamp allows one "to distinguish between nucleic acids arising from a cell in the sample and contaminant nucleic acids arising from cells from prior samples." *Id*. ¶ 62; *see also id.* ¶ 66. If a sample in McCloskey can be a single cell, and if the McCloskey batch-stamp allows one to distinguish cells from different samples, then McCloskey allows one to distinguish single cells from different samples.

76. For the same reasons, Dr. Quackenbush's confusing contention that McCloskey's batch stamp could be used for a "sample of a single cell" but not "a plurality of cells" is without merit. *Id*. ¶ 64. Dr. Quackenbush acknowledges that McCloskey could be used for "single cell samples" (plural) and that the batch stamp could distinguish between "nucleic acids arising from a cell in the sample and contaminant nucleic acids arising from cells from prior samples." *Id.* ¶¶ 64-66, 78. Thus, a POSA would understand that McCloskey does in fact provide a method for the analysis of single cells and that the batch stamp can be used to distinguish between single cells. EX1002 ¶¶ 171-177. McCloskey confirms this by

specifically recommending "that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for forensic, clinical, ***single cell***, and ancient DNA analyses." EX1004.007.

77. As an additional point, I note that it is generally illogical to assert that a method that is applicable to one thing, i.e., a "single cell", cannot be used for two or more things, i.e., "single cells", when the method can clearly be performed multiple times. As such, Dr. Quackenbush's assertion that a POSA would interpret single-cell analyses described by McCloskey as somehow fundamentally incompatible with single-cell analyses more generally (e.g., paragraph 65), including Linnarsson, is not tenable.

78. To summarize, McCloskey is ***not*** necessary for claim elements [1E] and [1H] because Linnarsson discloses a "first tag sequence" as required by these claims. As explained in my prior declaration, the primary purpose for discussing the first tag sequence of McCloskey was to demonstrate that McCloskey and Linnarsson are analogous art and that McCloskey has conceptual and methodological compatibility with Linnarsson that would motivate a POSA to combine them and expect that combination to succeed. EX1002 ¶ 187. Notably, Dr. Quackenbush does not dispute that McCloskey and Linnarsson are analogous art. Even if McCloskey were necessary, it also discloses a "first tag sequence" as

required by claims [1E] and [1H] because it teaches a "batch stamp" that identifies the DNA source, such as a "single cell." EX1004.002, .007.

3. McCloskey Teaches A Batch-Stamp For Single-Cell Analysis

79. Dr. Quackenbush contends that a POSA would not have been motivated to combine the teachings of McCloskey and Linnarsson because they offer different benefits. Specifically, Dr. Quackenbush contends that McCloskey's batch stamp lacks the benefit of Linnarsson's cell-tag "to identify and eliminate the misattribution of data from a first cell to a second." EX2013 ¶ 67. As explained below, this is not only wrong, but also irrelevant. As a threshold matter, I am not aware of any rule requiring that two references provide the same benefit to establish that a POSA would be motivated to combine. Dr. Quackenbush does not suggest that there is any rule to this effect in his description of the legal standards, nor has counsel apprised me of any such rule.

80. Regardless, Dr. Quackenbush confuses the proposed combination relying on McCloskey's batch stamp for the functionality of the "first tag sequence." *Id*. As explained above, McCloskey's batch stamp does offer the functionality of the first tag sequence, but it is not the primary reference I relied upon for that disclosure. In fact, Linnarsson's cell-tag satisfies the "first tag sequence" requirement of claim 1. EX1002 ¶¶ 157-170, 200-202. Rather than being relied upon for a cell tag, I relied upon McCloskey's disclosure of a barcode for

distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell, as required by claim [1F]. *Id*. ¶¶ 178-185. Thus, Dr. Quackenbush's contentions regarding the different benefits of McCloskey's cell tag and Linnarsson's cell tag are irrelevant to my analysis.

81. Even if a requirement exists that references provide the same benefits, as explained in my prior declaration, McCloskey and Linnarsson do offer the same benefits because both provide methods for tagging polynucleotides from single cells to track the source of the polynucleotides. *Id*. ¶¶ 186-194. As described at length in my prior declaration, both McCloskey and Linnarsson disclose methods for tagging polynucleotides to track the source of the polynucleotide, such as the single cell of origin. *Id*. ¶¶ 99-104, 108-117, 156-177, 186-194.

82. Further, the fact that McCloskey's encoding oligonucleotide does not offer identical benefits for Linnarsson's cell tag does not impact a POSA's motivation to combine their teachings. As explained in my prior declaration, "a POSA would have been motivated to combine Linnarsson's method of tagging polynucleotides with McCloskey's MID comprising two tag sequences because McCloskey teaches a solution to a problem discussed in Linnarsson, namely amplification bias." EX1002 ¶¶ 188-190. McCloskey offers a solution to this problem by tagging each sample polynucleotide uniquely such that they can be distinguished and quantified accurately. EX1002 ¶¶ 188-191; EX1004.001 ("We

have developed and applied molecular encoding principles to solve this source-uncertainty problem."). In fact, Dr. Quackenbush acknowledged exactly this benefit during his deposition:

> Q. Based on your review of McCloskey, what issues do you think she's proposing to address with the barcodes?
>
> MS. RAYMOND: Objection. Scope. Form.
>
> A. So she says at the beginning of the second paragraph in the "Results and Discussions" section of her paper, "In addition to distinguishing valid from contaminant sequence, our methods detect redundant sequences arising from the same cellular DNA template (Fig. 1)."
>
> So in that mix of DNA, each template molecules from a cell, and ***she's saying that her combination of batch stamps and barcodes can identify places where, in the final sequencing reaction, the same original molecule is counted twice from the same cellular DNA template.***

EX1057 at 111:17-112:9. Thus, a POSA would have been motivated to incorporate the teachings of McCloskey into Linnarsson's methods to solve the amplification bias issue described by Linnarsson.

4. McCloskey Teaches A Method Of Generating Enough Unique Tags To Uniquely Tag Each Polynucleotide

83. It is Dr. Quackenbush's argument that "persons of skill would not have thought McCloskey's 7-nucleotide-long barcode would provide enough unique

combinations to tag "each sample polynucleotide" in Linnarsson's method." EX2013 ¶ 72. Dr. Quackenbush contends that McCloskey's 7-nucleotide-long barcode can only generate 16,384 tags which are incapable of tagging every polynucleotide in a cell. *Id.* ¶ 73 ("McCloskey's disclosed barcode is 'seven nucleotides' long and uses one of the four natural deoxyribonucleotides in each position. EX1004, 4; see also EX1002, ¶192. McCloskey teaches that each of the seven positions in its barcode can be composed of one of four deoxyribonucleotides, resulting in 16,384 unique seven nucleotide-long barcodes"). According to Dr. Quackenbush, the number of polynucleotides to be tagged ***are expected*** by a POSA to be between 50,000 and 300,000, as these are the number of mRNA molecules found in a mammalian cell. *Id.* ¶ 74. There are several errors in Dr. Quackenbush's argument.

84. First, McCloskey does not cap its method to the use of ***only*** a 7-nucleotide long barcode. Dr. Quackenbush's opinion is restricting in the face of McCloskey's clear disclosures that no such restriction exists. For example, McCloskey teaches that any number of random bases "n," can be used to create the random barcode. EX1004.004. McCloskey also clearly states that the 7-nucleotide barcode is exemplary and used for a specific experiment.

> The barcode is a random sequence of the four deoxyribonucleotides. The number of distinguishable barcodes in a population of oligonucleotides used in a reaction is

> determined by the number of random bases, ***n***. This enables one to distinguish among 4^n allele copies per reaction. ***We currently use*** seven nucleotides for the barcode, giving 16,384 possible barcodes for each encoding oligonucleotide.

Id.

85. In fact, the McCloskey patent application US2007002640[2], further notes that much longer barcodes, comprising 20-30 random nucleotide bases, can be designed, that would allow distinguishing among 1,099,511,627,776 - 1,152,921,504,606,846,976 polynucleotides.

> The length of the second sequence is sufficient to provide, with high probability, a unique identity to each target nucleic acid molecule in the sample prior to amplification. For example, a second sequence of 7 random nucleotides N selected from A, G, C, and T will provide a maximum of 4^7 or 16,384 unique barcodes. In some embodiments, the length of the second sequence is between 3 and ***30 nucleotides***, such as between 5 and ***25 nucleotides*** or between 7 and 13 nucleotides.

EX2001 ¶ 21. Thus, a POSA would find it obvious from McCloskey's method to expand barcodes to different lengths depending on the size of a sample and needs of

[2] I understand that Patent Owner believes that McCloskey "is simply a less-detailed version of 'US20070020640' to McCloskey *et al.*" *See* Paper 13 at 2.

the experiment. This is something I explained in a related IPR proceeding that I understand Patent Owner has cited in these proceedings. *See* EX2011 ¶¶ 256-57.

86. Second, as I discuss above, Linnarsson does not limit its analysis to mammalian cells:

> As used herein, a "single cell" refers to one cell. Single cells useful in the methods described herein can be obtained from ***a tissue of interest, or from a biopsy, blood sample, or cell culture***. Additionally, cells from specific organs, tissues, tumors, neoplasms, or the like can be obtained and used in the methods described herein. Furthermore, in general, cells from any population can be used in the methods, such as a population of ***prokaryotic or eukaryotic single celled organisms including bacteria or yeast.***

EX1003 at 13:11-16.

87. Just like Linnarsson, the '981 Patent notes that "any organism, organic material or nucleic acid-containing substance can be used as a source of nucleic acids …including, but not limited to, plants, animals (e.g., reptiles, mammals, insects, worms, fish, etc.), tissue samples, ***bacteria***, fungi (e.g., ***yeast***), phage, ***viruses***, cadaveric tissue, archaeological/ancient samples, etc." EX1001 at 14:14-21; *see also id.* at 14:61-63 ("In certain embodiments, the nucleic acid sample being analyzed is derived from a single source (e.g., a single organism, virus, tissue, cell, subject, etc.), …"). For example, bacteria such as E. Coli have a little over 4,000

genes and ~1,380 mRNA molecules in each cell. EX1061.001. Furthermore, there are even simpler bacteria such as *Carsonella ruddii* that have only 182 genes and will thus have even fewer mRNA molecules. *See* EX1062.001. The total number of mRNA per cell in a yeast cell is between 15,000 and 60,000. EX1063.006. Thus, it is my opinion that Dr. Quackenbush is incorrect in opining that a POSA would necessarily expect anywhere from 50,000-300,000 mRNA molecules. This is merely the figure for mammalian cells.

88. Dr. Quackenbush further misrepresents my opinion in my declaration from another IPR petition regarding the number of tags to be used for successful tagging of polynucleotides. Dr. Quackenbush cites to ¶ 256 of that declaration, which contains my opinion regarding a different claim from a related patent,[3] to state that "Dr. Cooper asserted in another declaration that one would want ***'at least 100 times [more] than the number of sample polynucleotides.'***" Dr. Quackenbush is concealing my entire opinion as in ¶ 256, I stated that ***one could ensure*** successful tagging of polynucleotides by using ***e.g,*** 100 times more tags than the polynucleotides. EX2011 ¶ 256. My opinion is that successful tagging of polynucleotides can be "ensured" by employing a significantly higher ratio of tags to the polynucleotides. The use of ***100 times more tags*** than polynucleotides is an

[3] Claim 13 of U.S. Patent No. 10,697,013

illustrative rather than a definitive approach as misunderstood by Dr. Quackenbush, and nothing in the claims requires a method that "ensures" successful tagging of all polynucleotides.

89. Third, Dr. Quackenbush argues that McCloskey does not have enough tags to tag ***each*** polynucleotide in Linnarsson' single cell. Dr. Quackenbush calculates the number of unique tags required to be 30,000,000 in order to be able to tag ***all*** 300,000 mRNA molecules potentially present in a mammalian cell. In parallel district court litigation, however, Patent Owner has asserted that the "'sample polynucleotides' are the polynucleotides in a cell that are being sampled (i.e., the polynucleotides of interest), ***not all polynucleotides of that cell***." EX1058.030.[4] Patent Owner has likewise stated that to "argue that 'sample polynucleotides' means every polynucleotide of the cell would rewrite the claim and replace 'sample' with 'all.'" *Id.* at .031. Similarly, Linnarsson discloses methods

[4] The specific disputed claim language in the district court was from a dependent claim in U.S. Patent No. 10,240,197. But the pertinent claim language from the independent claim in this patent, which appears to be the main evidence on the dispute, is not meaningfully different from the independent claim in the '981 patent. *See* EX1058.031 (relying upon claim 1); *compare* EX1001 at claim 1 *with* EX1060 at claim 1.

that consider only subsets of the mRNA molecules to be targets of interest. For example, Linnarsson states that an "RNA complementary sequence (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 . . . to direct cDNA synthesis using the mRNA as a template." EX1003 at 15:2-6. Linnarsson also states, for example, that the RSC can be "gene family-specific." *Id.* at 15:6-7. These disclosures make clear that Linnarsson's methods allow for tagging and sequencing of subsets of polynucleotides, including potentially very small subsets for which only small numbers of random barcodes would be needed. In my opinion, this is also consistent with the plain language of the '981 patent claims, where the "sample polynucleotides" need not be all the polynucleotides in a cell but merely a subset. EX1001 at 30:24-25 ("generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides") As such, Dr. Quackenbush's arguments about whether McCloskey discloses enough barcodes to tag all the mRNA molecules in a cell are irrelevant because the claims do not require tagging all the molecules in a cell, whether mammalian or otherwise. Overall, it is my opinion that a POSA would understand that McCloskey discloses enough unique tags to tag the polynucleotides in Linnarsson's single cell methods and solve the problem of amplification bias. EX1002 ¶¶ 186-194.

5. McCloskey Describes Single-Cell Analysis

90. Dr. Quackenbush contends that McCloskey does not disclose "single cell analysis." EX2013 ¶ 77. For the reasons described above, McCloskey does describe methods for single cell analysis, even explicitly providing that its methods are recommended to "be used when amplifying irreplaceable DNAs and cDNAs for forensic, clinical, ***single cell***, and ancient DNA analyses." EX1004.007; *see also* EX1002 ¶¶ 99-104, 187.

91. Dr. Quackenbush first contends that "McCloskey's design was not suitable for analyses of very large numbers of nucleic acids such as Linnarsson's." EX2013 ¶ 78. Dr. Quackenbush's contention lacks any citation or supporting evidence and is without merit. It is my opinion that a POSA would have known to utilize McCloskey's method for the analysis of large numbers of nucleic acids. To the extent Dr. Quackenbush's contention is based on the length of McCloskey's barcode, a POSA would have known to use a longer barcode for larger sample sizes. Such methods for generating longer barcodes were routine and conventional at the time as acknowledged by the McCloskey patent application US2007002640.[5]

[5] As also stated above, I understand that Patent Owner believes that McCloskey "is simply a less-detailed version of 'US20070020640' to McCloskey *et al.*" Thus, these are analogous methods and could be similarly modified.

EX2001 ¶ 21 ("The length of the second sequence is sufficient to provide, with high probability, a unique identity to each target nucleic acid molecule in the sample prior to amplification." "In some embodiments, the length of the second sequence is between 3 and 30 nucleotides, such as between 5 and 25 nucleotides or between 7 and 13 nucleotides."). Generating barcodes of different lengths that could accommodate larger samples would have been well known to a POSA at the time. Thus, there is no barrier to using McCloskey's method for even "very large numbers of nucleic acids." This is something I explained in a related IPR proceeding that I understand Patent Owner has cited in these proceedings. *See* EX2011 ¶¶ 256-57.

92. Dr. Quackenbush also contends that "McCloskey lacks a tag that identifies the cell of origin, which is also necessary for single-cell analysis." EX2013 ¶ 78. Again, this assertion is without citation, supporting evidence, or explanation. As explained in detail above and in my prior declaration, McCloskey does teach a tag that identifies the cell of origin. EX1002 ¶¶ 99-104, 171-177. Specifically, McCloskey teaches a "batch stamp" that "specifies the DNA source such as the patient or sample" wherein the sample may be a "single cell." EX1004.002, .007. Thus, McCloskey teaches a tag that identifies the cell of origin.

93. For the foregoing reasons, it is my opinion that a POSA would understand McCloskey to teach a method that could be used for single cell analysis.

6. A POSA Would Have A Reasonable Expectation Of Success In Adding McCloskey's Barcode To Linnarsson

94. Dr. Quackenbush contends that McCloskey "would not have provided enough unique barcodes" to "tag each sample polynucleotide." As described above, Dr. Quackenbush is wrong because he overstates the number of sample polynucleotides and ignores the practical simplicity of modifying McCloskey's barcode to accommodate a larger sample. *See supra* Section III.C. First, the methods of the '981 patent are not limited to mammalian cells and could be utilized for cells containing significantly smaller numbers of mRNA. *Id.* Thus, "at least 30,000,000 unique tags" would not be required to perform the methods of the '981 patent.

95. Second, as described in my other declaration, cited by Dr. Quackenbush, "a POSA would 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." EX2011 ¶ 257. Thus, it would have been a matter of routine optimization to design molecular tags such that an extremely large number of unique tags can be generated in order to ensure sufficient unique tags to identify even extremely large samples of polynucleotides.

B. Ground 2: Claim 5 Is Unpatentable As Obvious Over Linnarsson In View Of McCloskey, And Further In View Of The Knowledge Of A POSA

1. A POSA Would Be Motivated To Modify Linnarsson and McCloskey To Add Tags Via Ligation

96. As described in my original declaration, it is my opinion that the combination of Linnarsson and McCloskey in view of the knowledge of a POSA renders obvious Claim 5. EX1002 ¶¶ 235-244.

97. A skilled artisan, as I clearly spell out in my original declaration, would be motivated to ligate on the "second tag sequence," after the first reverse transcription step in Linnarsson's method that adds the "first tag sequence." A POSA would achieve this by standard and routine methods of ligation, which have been well-known for decades, as also acknowledged by Dr. Quackenbush:

> 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.
>
> MS. RAYMOND: Objection to form.
>
> 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.

EX1057 at 33:18-34:17

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***?

MS. RAYMOND: Objection to form.

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.

Q. Yes

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

MS. RAYMOND: Objection. Form.

Scope.

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

Id. at 59:20-60:18.

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?

MS. RAYMOND: Objection to form.

Q. Without using ligation.

MS. RAYMOND: Objection.

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.

98. As detailed in my original declaration and undisputed by Dr. Quackenbush, Linnarsson's method teaches generation of tagged polynucleotides using a CDS and/or TSO that can incorporate a cell-tag into the cDNA. EX1003 at 22:24-26, 19:4-6. Linnarsson discloses that the cell-tag is incorporated into the cDNA through primer extension. [6] EX1003 at 5:29-31. Further, Dr. Quackenbush does not dispute that this cell tag is the "first tag sequence" that is

[6] Linnarsson discloses that the cDNA synthesis is achieved by reverse transcription. EX1003 at 22:24-26. As I state in my original declaration, a POSA would have understood that reverse transcription requires the use of primer extension. EX1002 ¶ 34.

"used to identify the cell from which the tagged polynucleotide originated." EX2013 ¶¶ 33, 157-170, 200-202; EX1003 at 19:5-11

99. The CDS and/or TSO do not have the "second tag sequence" that differentiates the polynucleotides within a cell. EX1002 ¶ 192. It is my opinion that a POSA would be motivated to combine the teachings of Linnarsson and McCloskey to incorporate this second tag into the sample polynucleotides prior to amplification in Linnarsson's method. *Id*. ¶¶ 178-194. As detailed in my original declaration, a POSA would be motivated to make this modification to address the problem of amplification bias, as expressly taught in McCloskey. *Id.* There are only two ways to include this second tag. A POSA would either include the second tag as part of the CDS or TSO when synthesizing the CDS or TSO such that both the first and second tags are synthesized together in one go from the outset. Alternatively, the first and second tag would be prepared separately, in which case one would attach the second tag to the first by ligation. While it ***might*** be possible to do this attachment through (1) certain complex chemical means without an enzyme or (2) by primer extension, ligation was (and still is) far and away the standard and most convenient approach for directly linking two DNA molecules. There is not any other reasonable way to carry out the task of including the barcode from McCloskey with the tag from Linnarsson.

100. As to the ligation approach, specifically, well before the priority date of the '981 Patent, it was well-known and routine to attach tag sequences using ligation. EX1002 ¶ 238. As I note in my original declaration, both Patent Owner and the '981 Patent acknowledge this. *Id.* And, so did Dr. Quackenbush during his deposition, including adding tags for sequencing applications:

> Q. As of August 2009, were you familiar with the use of ligation for polynucleotide tagging?
>
> MS. RAYMOND: Objection to form.
>
> 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.

EX1057 at 57:12-58:1. Dr. Quackenbush likewise testified about how familiar he was with ligation in 2009 and how widely used it was in connection with sequencing for introduction of barcodes:

> Q. As of August of 2009, were you familiar with techniques in the field for using ligation to add molecular barcodes to polynucleotides?
>
> MS. RAYMOND: Objection. Form. Scope

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

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?

MS. RAYMOND: Objection to form.

Q. Without using ligation.

MS. RAYMOND: Objection.

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 59:21-61:15.

101. Thus, a POSA would be familiar with the well-known technique of ligation and would be able to rely on routine and standard methods of ligation to add the second tag to Linnarsson's CDS and/or TSO. As stated in my original declaration, using linker molecules to ligate as disclosed in Hug would be one obvious and routine option for a POSA to perform ligation. EX1002 ¶¶ 240-241. This approach for ligation was used not just in Hug, but was widely used elsewhere in the art. Specifically, Hug is only one example out of four well known exemplary options that I listed in my prior declaration. *See, e.g.*, *id.* (Okayama at 165 (EX1024.005); Shibata at 1250-51, Fig. 1 (EX1039.001-.002); EX1040 at Fig. 2, ¶¶ 71-75, ¶¶ 235-239.) The foregoing alone establishes why and how a skilled artisan would use ligation to introduce a second tag. In fact, as described above, ligation would be a standard and routine technique known by any skilled artisan.

102. It is further my opinion that a POSA would be motivated to use ligation not just because this would have been a routine design choice given the narrow set of options available, but because, as I explain in my original declaration, this would provide increased flexibility in tag choice. EX1002 ¶ 243. Dr. Quackenbush contends that I did not adequately explain the added flexibility that ligation would

offer, but the flexibility advantage is clear and straightforward. Because the barcodes that track individual molecules are being synthesized separately and then subsequently linked, one has added flexibility in choosing tags during the experiment. For instance, if one needs to tag only a small number of molecules in a given cell, one can use molecular barcodes of shorter overall length and introduce them during the experimental workflow without the need to synthesize a large batch of composite tags (not all of which may be used and would lead to a waste of reagents) in advance. As I explained during my deposition "that's just sort of a – a design choice that you could optimize your particular needs and goals, and it would just be a matter of flexibility for different kinds of – different kinds of setups that allow you maximize efficiency of your overall process." EX2018 at 57:2-12.

103. Dr. Quackenbush makes several assertions in his declaration that are wrong and misrepresent my opinion on Ground 2.

104. First, Dr. Quackenbush contends that "it is unclear what tags are argued to be added via ligation." EX2013 ¶ 81. My declaration is clear that it is the ***second tag*** that is being ligated on. *See* EX1002 ¶ 237 ("…carry out this modification by ligating an oligonucleotide ***comprising a second tag sequence*** to Linnarsson's CDS and/or TSO"); *id.* ¶ 240 ("a POSA would also have had a reasonable expectation of success in ligating an ***additional sequence*** to Linnarsson's CDS and/or TSO"); *id.* ¶ 244 ("[I]t is my opinion that it would have been an obvious

design choice to a POSA to ligate the ***second tag sequence*** to the TSO and/or CDS to generate the tagged polynucleotide.").

105. Second, Dr. Quackenbush confuses my opinion in Ground 2 by asserting that "persons of skill would not have been motivated to add more tags (and more steps) when the CDS or TSO already contains two tags." EX2013 ¶ 82. I am saying no such thing. My opinion is not to add "redundant" tags to Linnarsson's CDS and/or TSO. *Id.* Rather, as I explain above, it is my opinion that a POSA would find it obvious to ligate ***the second tag*** to track individual molecules within a cell, not a third or a fourth tag to Linnarsson's CDS and/or TSO. Even where both the CDS and TSO include a cell tag in Linnarsson's embodiments, these two tags are both used only to track a molecule's cellular origin. As I explain above, Dr. Quackenbush admits that the CDS and/or TSO of Linnarsson only has the cell tag, i.e. the first tag sequence. It would be a routine choice for a POSA to rely on ligation to add the second tag. Dr. Quackenbush further asserts that my theory does not show "how to remove tag(s) from these nucleic acids … to instead add them back by ligation." *Id*. ¶ 83. This is irrelevant and not required. There is no tag to remove. A POSA interested in ***adding*** the second tag sequence to the CDS and/or TSO and would find it obvious to rely on ligation for this purpose. Thus, neither are there any additional tags being added nor any tags being removed, as wrongly contended by Dr. Quackenbush.

106. Third, Dr. Quackenbush, based on the above assertions, contends that "removing the functionality of those tags from the CDS or TSO and then adding tags to the fragments at some point after extension," alters Linnarsson's principle of operation and "persons of skill would not have been motivated to fundamentally alter that process." EX2013 ¶ 84. I disagree. Again, as explained above, my opinion is not to remove the tags and add them back. Persons of skill would be motivated to ligate the second tag to Linnarsson's CDS and/or TSO to overcome amplification bias as detailed in my original declaration. EX1002 ¶¶ 186-194. The issue of amplification bias was well-known at the time and Linnarsson discusses that. EX1003 at 2:3-15 ("Gene expression in single cells has previously been analyzed using a variety of methods … However, these methods require that each single cell is analyzed individually and treated separately during the entire procedure, which is time-consuming and expensive. … as the cDNA of each cell must be amplified to an amount that can be reasonably handled for the subsequent analysis, there is potential amplification bias. … an amplification of at least a million-fold is required."). A POSA would be motivated to add McCloskey's barcode that distinguishes between the polynucleotides of a cell and is recommended to solve the problem of amplification bias, to Linnarsson's CDS and/or TSO. And, it is my opinion that a POSA would find it obvious to use ligation as a method to add this second tag (barcode) because this approach was so routine, accessible,

conventional and well-known. As discussed above, a POSA would ligate after the step of primer extension by any of the standard ligation methods prevalent at the time, such as the one taught in Hug. EX1002 ¶¶ 240-241; EX1007.001, .007.

107. Finally, I disagree with Dr. Quackenbush that a POSA would not be motivated to ligate because an "additional ligation step" would increase complexity, cost or time to practice Linnarsson's method. EX2013 ¶¶ 82, 85. Dr. Quackenbush is basing this argument on his erroneous understanding that the "additional litigation step" involves ***replacing*** tags from the CDS or TSO, ***removing*** these tags and ***adding*** different tags, which as I discuss above is not the case. EX2013 ¶ 85; *supra* ¶¶ 102-105. But there is no replacement or removal of different tags required in my theory. While an additional tag is introduced, I explain above that a POSA would be motivated to do this to address amplification bias, as already proposed by McCloskey. Further, to the extent Dr. Quackenbush argues the step of ligation to be complex over primer extension, he offers no explanation in that regard. I maintain my opinion that a POSA would be familiar and in the practice of routinely using ligation to introduce different tags, primers or barcodes to polynucleotide sequences for experiments in her lab and would not find it complex to ligate the second tag to Linnarsson's CDS and/ or TSO.

2. A POSA Would Have Known How To Successfully Utilize Ligation To Add Tags In Linnarsson

108. Dr. Quackenbush contends that a POSA would not be successful in modifying the combination of Linnarsson and McCloskey because (a) a tag allegedly cannot be ligated to the CDS; (b) tag(s) ligated to the TSO will allegedly be cleaved off prior to sequencing; and (c) Hug's ligation method would be incompatible with Linnarsson and McCloskey. I disagree with Dr. Quackenbush for the reasons below.

a. The Second Tag Can Be Ligated To The CDS

109. I disagree with Dr. Quackenbush that "ligating a tag to the CDS is incompatible with the other steps in Linnarsson's method." EX2013 ¶¶ 88-92. Dr. Quackenbush's argument is that the biotin attached to the 5' end of the CDS "would block ligation of tag(s) to the CDS." EX2013 ¶ 89. As detailed above, however, Dr. Quackenbush is relying on Figure 11, which includes biotin. Dr. Quackenbush ignores other exemplary embodiments of CDS disclosed in Linnarsson including Figure 4, which ***do not*** include a biotin and which would be suitable for ligation. *See supra* Section VI.A.1. Dr. Quackenbush nevertheless seemingly contends that all CDSs in Linnarsson require biotin. EX2013 ¶¶ 89, 90. His reliance on Example I to support this argument is misplaced. Example I notes that the "structure of a ***typical CDS*** is shown in Figure 4." EX1003 at 25:3. As noted, Figure 4 does not include the biotin that would allegedly prevent ligation.

See supra Section VI.A.1. In fact, Linnarsson expressly states that "[a]dditional arbitrary sequences ***can be*** inserted at the 5' end, after the 5' APS, or after the cell-tag." EX1003 at 25:5-6. This confirms that Linnarsson does not view his CDS as being blocked from further introduction of sequences. Moreover, as detailed above, Linnarsson discloses that these examples are only "representative examples" and not limiting. Notably, Dr. Quackenbush acknowledges this. EX2013 ¶ 90 ("…Linnarsson describes other aspects of its method as optional.")

110. Even if a biotin molecule is deemed an essential aspect of the CDS, the claims would still be obvious. I understand that a person of ordinary skill is also a person of ordinary creativity, not an automaton. A skilled artisan seeking to introduce an additional tag to the CDS of Linnarsson by ligation would understand that the biotin would need to be added to the additional tag sequence rather than the CDS to ensure capture of the desired polynucleotide and allow ligation of the second tag. Such modifications would have been routine and obvious to a skilled artisan. Dr. Quackenbush acknowledges in his testimony that a POSA would have known that the biotin would block ligation:

> Q. And would a person of ordinary skill in the art in 2009 know that you couldn't ligate a barcode to the biotin on the five prime end of the CDS?
>
> MS. RAYMOND: Objection. Form. Scope.

A. So a person of ordinary skill in the art would understand that if we look at – the easiest way to understand it is to look at element D of Figure 11, or maybe it's B. Both the five prime and three prime ends of the fragment being analyzed have biotin molecules attached. That biotin is typically attached to the five prime phosphate. And ligation requires the formation of a phosphodiester bond, as I describe in paragraph 89. So if there's already a biotin attached, you wouldn't be able to create that bond, hence, ligation wouldn't occur. And a person in 2009 would have understood this.

EX1057 at 174:12-175:6.

111. Given that a POSA would have known about the effects of biotinylation, it is my opinion that a POSA would have known how to work around it. Specifically, a POSA would have used routine and well-known methods that do not require biotinylation and/or associate the biotin with the second tag to ensure capture of the desired polynucleotide. Dr. Quackenbush's assertions to the contrary require that POSAs lack even basic skill in the art.

112. Dr. Quackenbush further contends that a "Bts sequence is key to Linnarsson's method" and states that without the Bts sequence the CDS is never sequenced. EX2013 ¶ 91. I disagree. As I explain above, the Bts restriction enzyme cut site is an optional feature of Linnarsson's approach, specific to

embodiments that use fragmentation and capture. *See supra* Section VI.A.1. The CDS in Figure 4 includes neither a biotin nor a Bts restriction enzyme cut site but nonetheless includes a cell tag. As I explain above, this is because Figures 3 and 4 of Linnarsson are disclosing embodiments that do not require the fragmentation and capture approach of Figure 11. *See supra* VI.A.1.b. And, even to the extent Dr. Quackenbush is correct that a Bts sequence is required, it can simply be added to the CDS as disclosed in Linnarsson. EX1003 at 25:3-6 ("[a]dditional arbitrary sequences ***can be*** inserted at the 5' end, after the 5' APS, or after the cell-tag").

b. The Second Tag Ligated To The TSO Need Not Be Cleaved Off

113. Dr. Quackenbush complains that my theory is unclear on "at what step in Linnarsson's method the ligation occurs." EX2013 ¶ 80. Dr. Quackenbush is incorrect. As clearly discussed in my original declaration, the second tag is ligated to the sample polynucleotide after the first reverse transcription step in Linnarsson's method. EX1002 ¶ 243 ("by using ligation, the tag sequence is added ***after extension***"). During my cross-examination, I confirmed that I was contemplating ligation after the initial extension step. As I stated, "you can ligate after you've made the ***initial cDNA***…." EX2018 at 53:4-12; *see also id.* at 58:7-8 ("you would ligate it on after the that initial cDNA has been made"). There cannot be any confusion on this point because I proposed to carry out ligation using linker sequences as set forth in prior art such as the Hug reference, which requires a single

stranded region to which the linker sequence may hybridize. *See* EX1007.008 at Fig. 3; EX1002 ¶¶ 240-42. As I explained, a "POSA would have known that a linker could be used to hybridize with the TSO and/or CDS and tag sequences to align them such that they could be ligated together using routine ligation techniques." EX1002 ¶ 242. This is consistent only with an approach where there is single-stranded DNA at the end of the fragment, which happens after Linnarsson's first extension reaction.

114. Further, Dr. Quackenbush, while relying on Figure 11, argues that the second tag cannot be ligated to the TSO after extension because Linnarsson does not disclose a "third extension step." EX2013 ¶ 94. Specifically, he contends that "'after extension' would mean that the ligation occurs after the template switching step," and at that "point in Linnarsson's method, the cDNA has been extended twice, and is complete." *Id.* Thus, as per Dr. Quackenbush, because there is "no disclosed 'third extension' of the cDNA in Linnarsson," the tag "added via ligation would not be included in the cDNA that is subsequently amplified during PCR" and in "the tagged polynucleotide that is sequenced." *Id*. I disagree.

115. As an initial matter, Dr. Quackenbush misses the point that the second tag can be routinely ligated in Linnarsson's method after the first extension of the cDNA and before the second extension. The first extended cDNA is complementary to the RNA sequence and will be amplified and sequenced. And,

as a result, ligating the second tag after the first cDNA extension would ensure its incorporation into the cDNA sequence that will be amplified and sequenced.

116. Further, it is my opinion that a POSA would understand that the second tag ***can*** easily be ligated to Linnarsson's TSO even after the second extension step without any meaningful complication. After the second extension, a skilled artisan would understand that the TSO sequence is part of both strands of the nucleic acid product. As discussed in detail above, Linnarsson's TSO is not limited to one structure and can incorporate tags and additional sequences. Indeed, Linnarsson teaches that the tag of the TSO is incorporated into the cDNA during synthesis. EX1003 at 19:4-5 ("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."). Just as the TSO tag is incorporated into the cDNA, so too is the rest of the TSO. Indeed, Linnarsson further teaches that by using a TSO, the end "of the cDNA can be arbitrarily controlled." *Id.* at 24:19-20. As such, after the second extension, one can simply ligate the barcode of McCloskey to the terminal end of the double-stranded complex wherein the TSO is part of both strands of DNA. This could be done by blunt-end ligation, which Dr. Quackenbush confirmed was a well-known technique as of 2009. EX1057 at 57:12-21, 61:2-15. In fact, Linnarsson itself even discloses the process of ligating sequencing adapters to the cDNA molecules following both extension steps. EX1003.007 ("Panel

shows fragment release and adapter ligation. 5' fragments are released by BtsCI digestion, leaving just the barcode (Be) and insert (white area). 3' fragments remain stuck on the beads. Genome Analyzer paired-end compatible adapters (Pl and P2) are ligated."); EX1003.028 ("The fragments were released by BtsCI digestion, and simultaneously ligated to the FDV and RDV adapters."); EX1003.036 ("5' fragments containing barcodes and cDNA inserts were released from the beads by BtsCI digestion, and adapters were simultaneously ligated to generate a sample suitable for sequencing on the lllumina Genome Analyzer."). A third extension would not be required.

117. Finally, even if there were a need to perform another extension reaction, this is a trivial step that would not create any meaningful level of complication and would not in the slightest dissuade a POSA interested in avoiding amplification bias from attempting to introduce a molecular tag via ligation.

118. I also disagree with Dr. Quackenbush's argument that even if the second tag "were somehow transcribed," it "cannot be ligated to the TSO" because after using a restriction enzyme the second tag will remain stuck to a bead and will hence never be sequenced. Ex. 2013 ¶ 94. Again, Dr. Quackenbush is only focusing on the exemplary Figure 11 in Linnarsson, which is a specific example requiring fragmentation and capture, followed by release using a restriction enzyme. Linnarsson is not limited to this approach. Indeed, the CDS and TSO in Figures 3

and 4 do not include a biotin or a restriction enzyme cut site at all, thus confirming that Linnarsson teaches approaches that do not require them. And, even if it were true that Linnarsson only disclosed Figure 11, a POSA would be successful in ligating the second tag to the TSO ***after the extension*** of the CDS because a skilled artisan would have the basic intelligence to include the restriction enzyme cut site upstream of molecular barcode so that when the restriction enzyme is applied the molecular barcode is released for sequencing. Again, I understand that a person of ordinary skill is also a person of ordinary creativity, not an automaton incapable of making simple and routine modifications.

c. POSA Would Find It Obvious To Ligate The Second Tag Using A Linker Molecule

119. Dr. Quackenbush contends that a POSA would not look to Hug's method or be "motivated to use its linker approach with Linnarsson or McCloskey." EX2013 ¶ 98. As a preliminary matter, Dr. Quackenbush misunderstands my use of Hug. As stated clearly in my prior declaration, Hug is ***not*** a reference used as a basis for the grounds of my opinion. EX1002 ¶¶ 240-42. Rather, Hug is an "example" of a method demonstrating that "a POSA would have found it routine and obvious to ligate a tag sequence by using a linker molecule." *Id*. ¶ 240. Indeed, my prior declaration cited numerous other references providing methods for ligating polynucleotides—Hug is merely one of four exemplary methods for ligation. *Id*. ¶¶ 235-243. In fact, as described above, ligation would be a standard and well

known technique for a skilled artisan. Even the '981 patent describes the method of ligation taught by Hug as an optional technique for accomplishing ligation:

> It is noted here that, while not shown in FIGS. 1A and 1B, any convenient method for adding adapters to a polynucleotide to be processed as described herein may be used in the practice of the reflex process (adaptors containing, e.g., primer sites, polymerase sites, MIDs, restriction enzyme sites, and reflex sequences). **For example, adapters can be added at a particular position by ligation.** For double stranded polynucleotides, an adapter can be configured to be ligated to a particular restriction enzyme cut site. **Where a single stranded polynucleotide is employed, a double stranded adapter construct that possesses an overhang configured to bind to the end of the single-stranded polynucleotide can be used.** For example, in the latter case, the end of a single stranded polynucleotide can be modified to include specific nucleotide bases that are complementary to the overhang in the double stranded adaptor using terminal transferase and specific nucleotides.

EX1001 at 19:7-23.

120. Tellingly, Dr. Quackenbush discusses Hug at length in his declaration despite his repeated admissions that techniques for ligation were routine and conventional. *See supra* ¶¶ VI.B.1; EX2013 ¶¶ 97-105. As previously described, it is my opinion that a POSA would have found it routine and obvious to ligate the second tag and one exemplary method is provided by using a linker molecule such

as described in Hug. EX1002 ¶ 240; *see also* EX1024.005; EX1039.001-.002; EX1040 at Fig. 2, ¶¶ 71-75, ¶¶ 235-239.

121. Dr. Quackenbush disputes that the modification of the combination of Linnarsson and McCloskey in Ground 2 is obvious in view of Hug. EX2013 ¶¶ 97-105. This is not true and as discussed above I rely on Hug as an ***example*** to opine that a POSA would find it obvious and routine to use methods like Hug for ligation, ***not*** as a basis for the grounds. Dr. Quackenbush contends that a POSA would not have looked to Hug because "Hug does not have a MID." *Id*. ¶ 98. Again, my opinion does not rely upon Hug for a MID, but merely an example of a well-known and flexible method for ligating polynucleotides using a linker molecule. EX1002 ¶¶ 240-42.

122. To the extent Dr. Quackenbush argues that Hug's linker molecule cannot be used to ligate McCloskey's unique random barcode to Linnarsson's CDS and/or TSO, I disagree. *See* EX2013 ¶¶ 99-105. Hug's method allows the ligation of a tag sequence to a polynucleotide with a linker. EX1007.007. Hug's linkers hybridize to both the target polynucleotide as well as the oligonucleotide containing the tag sequence. *Id.* Once both are aligned, a T4 DNA Ligase enzyme is used to ligate the target polynucleotide to the oligonucleotide. *Id.*; *see also* EX1002 ¶ 241. Dr. Quackenbush contends that Hug's "linker molecule could not be used with the random barcode of McCloskey," because "there would have to

be a linker molecule that is complementary to each combination of sequences that could be present in McCloskey's barcode." EX2013 ¶¶ 99-101. It is my opinion that a POSA would find it obvious and routine to modify and design Hug's linker molecule to hybridize to a non-random portion of McCloskey's barcode. Similarly, a POSA would find it routine and obvious to modify Hug's linker molecule to hybridize to Linnarsson's CDS and/or TSO. Dr. Quackenbush contends that even if these modifications were made, "Hug does not cure the other technical issues with Ground 2," for example the presence of a biotin. EX2013 ¶105. I disagree with Dr. Quackenbush for the reasons detailed above.

123. Thus, a POSA would be successful in modifying the combination of Linnarsson and McCloskey to generate polynucleotides through at least one ligation reaction.

124. Though I note that Hug is relied upon as exemplary of background art related to ligation, it is my opinion that Hug is analogous art to the '981 patent. Both the '981 patent and Hug relate to methods for the analysis of polynucleotides from single cells. The '981 patent is titled "Methods For Analyzing Nucleic Acids From Single Cells" and its claims provide a "method of analyzing nucleic acids from a plurality of single cells." EX1001 at 1:1-2, 30:18-19. Similarly, Hug provides "a method to calculate the number of molecules of a single mRNA species in a complex mRNA preparation" such as "in a single cell." EX1007.001, .009.

Specifically, Hug distinguishes polynucleotides by ligating "multimeric linkers" or "tags" to sample polynucleotides. *Id.* at .001, .003-.007 ("The tags are used to separate DNA molecules."). Thus, Hug is in the same field of endeavor as the '981 patent, including methods for analyzing and distinguishing polynucleotides in a sample such as a single cell. For these reasons, it is also my opinion that Hug is pertinent to the particular problems addressed by the '981 patent. As discussed, both Hug and the '981 patent disclose methods of tagging nucleic acids from single cells for the purpose of analyzing and distinguishing the tagged nucleic acids. EX1001 at 30:18-48; EX1007.002-.007, .009 ("Again, identical molecules (IMPSMS) are made distinguishable with different tags and the tags are then counted to yield the number of new DMS."). Thus, a POSA would reasonably have consulted Hug and the '981 patent and applied their teachings to problems regarding tagging, distinguishing, and analyzing nucleic acids from single cells.

C. Linnarsson and McCloskey Are Analogous Art To The '981 Patent

125. I understand that Patent Owner contends that Petitioner did not meet its burden in demonstrating that Linnarsson and McCloskey are analogous art to the '981 patent. *See* Paper 13 at 68-69. As described in my prior declaration, "I understand that only analogous art may be considered in an obviousness analysis" and that art "is considered analogous if it is in the same field of endeavor ***as the***

patent or if it is reasonably pertinent to the particular problem with which the patent is involved." EX1002 ¶ 25.

126. With this understanding, I undertook a detailed obviousness analysis of Linnarsson and McCloskey including explanations of how they are analogous and relate to the same field of endeavor as the '981 patent. *Id*. ¶¶ 27-37 (the "claims of the '981 patent generally describe methods for analyzing nucleic acids from two or more single cells using polynucleotides comprised of two 'tags'"); *id.* ¶¶ 99-104 ("McCloskey describes a method of DNA analysis for single-cell samples that utilizes" "tag sequences that distinguish, respectively, polynucleotides and samples"); *id.* ¶¶ 108-117 ("Linnarsson teaches a method of analyzing gene expression in a plurality of single cells by preparing a tagged cDNA library"), *id*. ¶¶ 186-194.

127. It is my opinion that Linnarsson relates to the same field of endeavor as the '981 patent. Both the '981 patent and Linnarsson relate to methods for the analysis of polynucleotides from single cells. The '981 patent is titled "Methods For Analyzing Nucleic Acids From Single Cells" and its claims provide a "method of analyzing nucleic acids from a plurality of single cells." EX1001 at 1:1-2, 30:18-19. Similarly, Linnarsson's Abstract states that it "provides methods and compositions for the analysis of gene expression in single cells or in a plurality of single cells" and its claims are directed to a method of tagging and tracking

polynucleotides "of single cells." EX1003.001, .047-49. Thus, Linnarsson and the '981 patent are directed to the same field of endeavor, including methods of analyzing nucleic acids from single cells through the introduction of tag sequences followed by sequencing. EX1001 at 30:18-62; EX1003.047-.049. For these reasons, it is also my opinion that Linnarsson is pertinent to the particular problems addressed by the '981 patent. As discussed, both Linnarsson and the '981 patent teach methods of tagging nucleic acids from single cells for the purpose of identifying and tracking the tagged nucleic acids. Thus, a POSA would reasonably have consulted Linnarsson and the '981 patent and applied their teachings to problems regarding single cell nucleic acid tagging and analysis.

128. It is my opinion that McCloskey relates to the same field of endeavor as the '981 patent. Exactly akin to the '981 patent and Linnarsson, McCloskey teaches methods of tagging nucleic acids of single cells. For example, McCloskey's Abstract states that its methods for "molecular encoding" can solve the "source-uncertainty problem for DNA sequences generated by standard PCR." EX1004.001. McCloskey describes a method for tagging nucleic acids with an "encoding oligonucleotide" containing multiple tag sequences including a tag that identifies the "DNA source such as the patient or sample identification" and a "random barcode" tag that "distinguishes among sequences arising from different cell or allele copies." *Id.* at .002. McCloskey recommends that the dual tag

approach "be used when amplifying irreplaceable DNAs and cDNAs for forensic, clinical, single cell, and ancient DNA analyses." *Id.* at .007. Thus, McCloskey relates to the same field of endeavor as the '981 patent, "analyzing nucleic acids from single cells using methods that include using tagged polynucleotides containing multiplex identifier sequences." EX1001 at Abstract. For these reasons, it is also my opinion that McCloskey is pertinent to the particular problems addressed by the '981 patent. Specifically, as described at length in my prior declaration, McCloskey's tags can be used to solve common issues with amplifying nucleic acids from single cell samples. EX1002 ¶¶ 90-93, 102-103, 188-193. Thus, a POSA would have consulted McCloskey and applied its teachings to problems regarding single cell nucleic acid tagging, amplification, and analysis such as the '981 patent. EX1001 at 30:18-19, 48-51.

129. In fact, Dr. Quackenbush did not contest that Linnarsson, McCloskey, and the '981 patent are analogous art or that they are directed to the same field of endeavor. *See* EX2013. As further evidence that Linnarsson is analogous art to the '981 patent, I understand that even Patent Owner submitted Linnarsson to the patent office during prosecution of the '981 patent. EX1005.122-.127. In fact, the examiner relied upon Linnarsson as prior art to issue a non-final rejection of the '981 patent. EX1005.147-.163. Similarly, the McCloskey patent application US2007002640 was submitted by Patent Owner to the patent office during

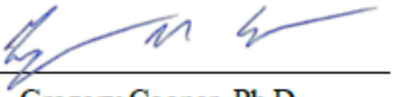
prosecution of the ’981 patent. EX1005.122-.127. Patent Owner has previously characterized McCloskey as “simply a less-detailed version of ‘US20070020640.’” Paper 6 at 2. Thus, through Patent Owner’s own submissions it has admitted that both Linnarsson and McCloskey are analogous to the ’981 patent.

130. Thus, as previously explained, it is my opinion that Linnarsson, McCloskey, and the ’981 patent are analogous art because they are all directed to methods for tagging and tracking polynucleotides from single cells.

VII. DECLARATION

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

Dated: March 29, 2024

By: 
Gregory Cooper, Ph.D.