UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.,
Petitioner

v.

10X GENOMICS, INC.,
Patent Owner

Case IPR2023-00958
Patent 10,697,013

EXPERT DECLARATION OF JOHN QUACKENBUSH, PH.D

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I, Dr. John Quackenbush, hereby declare under penalty of perjury:

I. INTRODUCTION

1. I have personal knowledge of the facts and opinions set forth in this declaration, and if called upon to do so, I would testify competently thereto.

2. I have been retained by Groombridge, Wu, Baughman & Stone LLP, on behalf of Patent Owner 10x Genomics, Inc. ("Patent Owner" or "10x") to offer opinions regarding certain issues relating to U.S. Patent No. 10,697,013 (the "'013 Patent"), assigned to 10x, as well as other documents presented to me by counsel for 10x, in connection with *Inter Partes* Review of U.S. Patent No. 10,697,013 under 35 U.S.C. §§ 311-319 and 37 C.F.R. § 42 ("Petition").

3. I am being compensated at a rate of $750/hour for time testifying and $750/hour for my other time spent on this proceeding and reimbursed for expenses incurred as a result of my role as an expert. My compensation is not in any way contingent upon my performance, the result of this proceeding, or any of the issues involved therein. I have no financial interest in the outcome of this case.

II. BACKGROUND AND QUALIFICATIONS

4. I am the Henry Pickering Walcott Professor of Computational Biology and Bioinformatics and Chair of the Department of Biostatistics at the Harvard T.H. Chan School of Public Health. I am also Founding Director and co-Director of the MS Program in Computational Biology and Quantitative Genetics and Director of

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the Big Data to Knowledge Training Program, co-Director of the Cancer Genomics Training Program, and the Faculty Director for the Harvard Quantitative Biomedical Research Center. In addition, I hold appointments as Professor of Biostatistics and Computational Biology at the Dana-Farber Cancer Institute and at the Channing Division of Network Medicine of Brigham and Women's Hospital.

5. In addition to my academic appointments, I co-founded Genospace LLC in 2012. Genospace produces software solutions to operationalize precision medicine through the integration of clinical and genomic data and its presentation to clinical and research users in an intuitive format. Genospace was purchased by Hospital Corporation of America in January of 2017.

6. In addition to my academic and research responsibilities, over the years I have taken significant regional and national research leadership responsibilities. I have served on the Scientific Advisory Board of the St. Jude's Children's Research Hospital and the Board of Scientific Counselors of the Environmental Protection Agency. I have also served on numerous scientific review panels for the National Institutes of Health, the National Science Foundation, the Department of Energy, the National Aeronautics and Space Administration, the Wellcome Trust, Genome British Columbia, Genome Canada, the National Sciences and Engineering Council of Canada, and the European Union. And I have served on numerous study panels

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for the National Research Council, the National Academy of Sciences, and the Institute of Medicine.

7. I have presented more than 350 talks, courses, and workshops since joining the Harvard School of Public Health and Dana-Farber in 2005. I have also taught numerous courses and workshops on the analysis of genomic data and served as an organizer of the EMBO course on DNA microarray analysis for more than 5 years.

8. Prior to joining the faculty at Dana-Farber and Harvard, I was a Professor of Chemical Engineering at the University of Maryland from 2003 – 2005; an Investigator with The Institute for Genomic Research from 2002 – 2006; a Professor in the Department of Biochemistry at The George Washington University from 2000 – 2005; a Lecturer in The Department of Biostatistics at The Johns Hopkins University from 1998 – 2005; an Associate Investigator at The Institute for Genomic Research from 1997 – 2001; and an Assistant Investigator at The Institute for Genomic Research in 1997.

9. I received my Ph.D. in Theoretical Particle Physics at the University of California, Los Angeles in 1990. I also hold a Master of Science in Physics from University of California, Los Angeles and a Bachelor of Science in Physics from the California Institute of Technology.

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10. I have studied, taught, practiced, and conducted research involving the use of genomic technologies, including DNA sequencing and DNA microarrays, and involving computational analysis of data related to genomic technologies. I have expertise in genomic technologies, computational analysis, and modeling of a plant, animal, and microbial systems with an emphasis on the study of human diseases, such as cancer.

11. In describing my work, I often use terms to refer to techniques and technologies, as well as specific application areas. During the course of my career, I have worked in genomics, functional genomics, epigenomics, bioinformatics, biostatistics, computational biology, systems biology, network biology, and network medicine. Although the boundaries between these various fields are often not well defined, one possible distinction is that some are directed toward data generation while others are directed toward developing and applying methods for data analysis and interpretation. For example, genomics, functional genomics, and epigenomics are generally associated with the design and conduct of experiments for generation of large-scale data on hundreds, thousands, or tens of thousands of genes, RNA transcripts, or epigenetic sites within the genome (or other alterations such as identifying regulatory interactions or structural changes in the genome). Fields including bioinformatics, biostatistics, computational biology, systems biology, network biology, and network medicine generally involve the development of new

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methods for data analysis and application of those methods to the biological and biomedical data sets, including those generated using techniques such as genomics, functional genomics, and epigenomics. The beauty of all of these techniques is that their development and use is, largely although not entirely, independent of their application area. So methods developed in one area are often used in other areas.

12. I have worked in the analysis of genes in hundreds of animal, plant, unicellular, and parasitic species, including human, mouse, rat, cattle, sheep, brown bear, salmon, corn, tomato, rice, yeast, the causative agent of malaria, and countless bacteria and viruses. Despite this diversity of applications, my primary research emphasis has been in the study of human diseases, with the greatest body of my recent work in the analysis of human cancers and pulmonary diseases. However, I have also explored a wide range of other issues in health and disease, including Alzheimer's disease, Parkinson's disease, Huntington's disease, neonatal sepsis, and Down syndrome. My work has involved analysis of data generated from bulk tissue samples, small numbers of cells, single cells, and cell-free DNA.

13. I have also done work relevant to reproductive health, an area that became personally important to me as my wife and I, then in our forties, became enmeshed in the use of assisted reproductive technology. This gave me first-hand exposure to a wide range of techniques, including pre-implantation prenatal screening and the use and limitations of technologies directed toward prenatal

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detection of genetic abnormalities including aneuploidies. Following the birth of my son in 2006 at Brigham and Women's Hospital in Boston, I also worked with, and mentored, physician scientists at Harvard, published a genomic analysis of diminished ovarian reserve, and presented the President's plenary lecture at the 63rd American Society for Reproductive Medicine Annual Meeting in 2007 in which I described the use of genomic technology in reproductive, prenatal, and neonatal health.

14. I have received numerous awards and honors including being recognized as a White House Open Science Champion of Change, an Australia Fellow, and a Distinguished International Advisor for the International Conference of BioInformatics and BioEngineering sponsored by the Institute of Electrical and Electronics Engineers. In 2022 I was elected to the National Academy of Medicine, one of three academies that make up the National Academies of Sciences, Engineering, and Medicine (the National Academies) in the United States.

15. I have published more than 320 peer reviewed scientific papers that have been cited more than 94,000 times. I have had continuous grant support for my research from the National Institutes of Health and other organizations for nearly thirty years. In 2018, I received an NCI Outstanding Investigator Career Award, a seven-year research grant recognizing a lifetime record of substantial contributions to the field.

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16. Attached hereto as **Appendix A** is a true and correct copy of my Curriculum Vitae describing my background and experience and includes a list of my publications, presentations, and expert testimony experience.

III. MATERIALS RELIED UPON

17. In preparing this Declaration, I have relied upon the following: the '013 Patent (EX1001) and its File History (EX1005), the Declaration of Gregory Cooper, Ph.D. (EX1002), and EX1003, EX1004, EX1006, EX1007, EX1009, EX1013, EX1035, EX1055, EX2003, EX2007, EX2012, EX2014, EX2015, EX2016, EX2017, EX2018, EX2020, EX2022, and all of the materials cited in this Declaration.

IV. LEGAL STANDARDS

A. Obviousness

18. I understand that a claim is obvious pursuant to 35 U.S.C. § 103 if the differences between the claimed invention and the prior art are such that the claimed invention, as a whole, would have been obvious to persons having ordinary skill in the art ("persons of skill" or "POSITA") to which the claimed invention pertains—before the earliest filing date of the claimed invention. I understand that a claim is valid unless the differences between the claimed invention and the prior art are such that the subject matter as a whole would have been obvious at the time of the

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invention to persons of skill. Here, I have been asked to assume that the earliest filing date of the '013 patent is August 20, 2009.

19. I understand that the following factors are used to make an obviousness determination: (i) the scope and content of the prior art; (ii) the level of ordinary skill in the art; (iii) the differences between the claimed invention and the prior art; and (iv) other "objective indicia" (sometimes called "secondary considerations") of non-obviousness.

20. I also understand that for a claim to be found obvious, each element must be disclosed in the prior art. I understand, however, that when support for obviousness is argued based on a combination of prior art references, an invention is not proved obvious merely by demonstrating that each of the elements in the claim was independently known in the prior art.

21. Rather, I understand that for obviousness, there must be a reason or motivation that would have prompted persons of skill in the relevant field to combine or modify the elements in the way the claimed invention does. I understand that using the claimed invention as a blueprint to piece together various elements in the prior art amounts to hindsight reasoning and is prohibited.

22. I also understand that, for a patent to be obvious, persons of skill must have had, in addition to a motivation to combine, a reasonable expectation of success in practicing the claimed invention based on the prior art.

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23. I further understand that, in making a determination as to whether or not the claimed invention would have been obvious to persons of skill, the Board must consider certain objective "indicia" or factors, such as commercial success (e.g., the profitability of the claimed method or device practicing the claims), long-felt but unsolved need, teaching away (e.g., literature telling persons of skill not to do something that would be required by the claims), unexpected results, copying, and praise by others in the field. The presence of such factors is evidence of nonobviousness. I also understand that a connection, or nexus, must exist between the objective factor and the claimed invention.

24. I understand that, among other things, the Board has instituted an inter partes review of claims 1-28 of the '013 Patent. I understand that the instituted grounds are as follows: Ground 1 challenges claims 1-12 and 19-28 of the '013 Patent as unpatentable as obvious over the combination of Linnarsson (EX1003) in view of McCloskey (EX1004), and further in view of a knowledge of a POSA, and Ground 2 challenges claims 13-18 of the '013 Patent as unpatentable as obvious over the combination of Linnarsson (EX1003) in view of McCloskey (EX1004), McCloskey II (EX1055) and further in view of the knowledge of a POSA. I understand that for *inter partes* review, invalidity must be shown under a preponderance of the evidence standard.

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B. One Of Ordinary Skill

25. I have been asked to assume that persons of skill of the ’013 Patent would have had Ph.D. in molecular biology, molecular genetics, biochemistry, physics, mechanical engineering, or a related discipline, with two years of academic, research, or industry experience in the field of genomic or transcriptome sequencing. I understand Dr. Cooper based his opinions on a different definition of a persons of skill, which is an individual with “a Masters degree and/or Ph.D. in Molecular Biology, Genetics, Chemistry, Engineering, or a related discipline and at least two years of post-Masters, postdoctoral, or industry experience. Alternatively, a POSA would have been someone with a Bachelor of Science degree in Molecular Biology, Genetics, Chemistry, Engineering, or a related discipline and at least five years of industry or academic experience (including any experimental work toward a graduate degree) relating to DNA sequencing technologies, amplification methods, and gene expression analysis” and “would have been familiar with associated tools, methods, and techniques including: (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.” EX1002 (Cooper Declaration), ¶¶52. My opinions would not change if I applied that definition of a persons of skill.

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26. Given my education and professional experience described above and in my curriculum vitae, I would have qualified as a person of skill as of August 20, 2009 (under both my definition and Dr. Cooper's), and I have applied the knowledge of persons of skill in my analysis below. Well before August 20, 2009, my level of skill in the art was at least that of a person of skill. I am qualified to provide opinions concerning what persons of skill would have known and understood at that time, and my analysis and conclusions herein are from the perspective of persons of skill as of August 20, 2009. I understand that a person of skill is presumed to have knowledge of all relevant prior art, and therefore would have been familiar with each of the references cited herein and the full range of teachings they contain.

V. BACKGROUND ON THE STATE OF THE ART AND THE CHALLENGED CLAIMS

A. The '013 Patent's Invention

27. The '013 Patent is titled "Methods For Analyzing Nucleic Acids From Single Cells." EX1001, 1. I understand that the '013 Patent claims priority to an August 20, 2009 provisional, and I have used that date for assessing the prior art and Dr. Cooper's opinions. EX1001, 1; EX1002, ¶37. The '013 Patent's Challenged Claims relate to methods of counting polynucleotides from a plurality of single cells that allow the user not only to differentiate among the different polynucleotides in an overall sample (including *e.g.*, mRNA), but also to track from which particular

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cell each polynucleotide originated. EX1001, 6:41-7:30, 15:16-58, claim 1 (30:38-31:9).

28. The patent describes using 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." *Id.* Put differently, the claimed method employs both (1) cell-specific tags and (2) polynucleotide-specific tags. *Id.* Cells contain many types of polynucleotides, including DNA such as nuclear DNA and mitochondrial DNA (mtDNA), and RNA such as transfer RNA (tRNA), messenger RNA (mRNA), ribosomal RNA (rRNA), micro RNA (miRNA), and small nucleolar RNA (snoRNA), each of which can be analyzed using the claimed invention. EX1001, 14:16-22. According to Dr. Cooper, a single mammalian cell has between 50,000 and 300,000 mRNA molecules alone. EX1002, ¶257; EX1054, Abstract. As the '013 patent notes, "In general, to accomplish such molecular tagging the number of distinct MID tags to be used should be many times greater than the number of molecules to be analyzed." EX1001, 22:63-66. According to Dr. Cooper, tagging each molecule would require "at least 100 times more tags than sample polynucleotides." EX1002, ¶256. In other words, applying Dr. Cooper's tag ratio, tagging each mRNA molecule in a mammalian cell requires ***5,000,000 – 30,000,000 unique tags***. *Id.*; EX1054, Abstract. In my opinion, persons of skill would choose

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to err towards the high end of that range, since it is impossible to know in advance whether the cell of interest contains mRNA molecules at the low end of the expected range (50,000 by Dr. Cooper's analysis) or at the high end (300,000).

29. Until inventions such as the '013 Patent, most nucleotide sequencing was performed using polynucleotides (e.g., DNA or RNA) from a population of cells in a process called bulk analysis. EX1003, 2; EX1006, 2. Bulk analysis provided users an understanding of the polynucleotides found in the overall population of cells in the sample, but was unable to capture key differences among *individual* cells. EX1002, ¶67. For example, overexpression of the HER2 gene is associated with an aggressive form of breast cancer, but in the early stages of cancer a biopsy sample might contain only a small number of cancerous cells among many stromal and other cells. Bulk RNA sequencing analysis might well detect the expression of the HER2 gene, but would almost certainly not detect an overexpression of HER2 in the cancer cells, as the signal would be diluted as the detected bulk signal would be "averaged" *across the entire population of cells* in a sample. Thus, analyzing samples through a bulk analysis would not provide a critical piece of information. In contrast, single-cell RNA sequencing as enabled by the '013 patent would allow not only overexpression of HER2 in individual cells within a multi-cell sample to be identified, but also would provide a quantitation of the number of tumor cells in the sample. *See* EX1002, ¶¶71-72.

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30. The specification of the ’013 Patent explains that tagging a polynucleotide with the ’013 Patent’s disclosed first and second tag sequences before amplification, identifying the source cell and the individual polynucleotide respectively, provides a “correlation between a polynucleotide and its source” so that researchers can determine, for example, how many individual polynucleotides are in each single cell within a sample. EX1001, 6:46-59. The innovative methods claimed in the ’013 Patent thus 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. EX1001, 6:41-7:30, claim 1 (30:38-31:9). Applying the inventive methods of the ’013 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.

31. I disagree with certain of Dr. Cooper’s characterizations of the specification of the ’013 Patent. For example, Dr. Cooper states that the ’013 tagging method is not new, but rather a prior art technique. EX1002, ¶29 (citing EX1001, 1:38-41). I disagree. The portion of the specification of the ’013 Patent Dr. Cooper cites does not describe dual tagging in the manner claimed. EX1002, ¶29 (citing EX1001, 1:38-41). Indeed, that portion, excerpted below, does not describe what tags are used, let alone “reflect[] that the tagging method is not the invention but a

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prior art technique." EX1002, ¶29. Instead, the passage in the '013 patent that Dr. Cooper cites clearly relates to a technique known as "multiplex" sequencing in which individual DNA samples (from distinct bulk sources) are labeled with sample-specific molecular barcodes (or "sample bar codes") during library construction, multiple sample libraries are pooled and sequenced (referred to as "multiplex sequencing") and the resulting data "demultiplexed" to group sample-specific sequences based on their sample bar codes.

> We have previously described methods that enable tagging each of a population of fragmented genomes and then combining them together to create a 'population library' that can be processed and eventually sequenced as a mixture.

32. The Challenged Claims, as broken out by Dr. Cooper, are listed below:

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

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

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

[1D] wherein a tagged polynucleotide of the plurality of tagged polynucleotides is generated by;

[1E] ligating a second oligonucleotide tag to said extension product, and

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

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

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

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

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

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

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

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

Claim 4. The method of claim 3, wherein step (B) comprises (i) ligating said second oligonucleotide tag to said cDNA.

Claim 5. The method of claim 3, wherein said hybridizing comprises hybridizing a poly-dT sequence of said first oligonucleotide tag to a poly-dA sequence of said mRNA.

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

Claim 7. The method of claim 1, wherein said first oligonucleotide tag comprises a sequence that is configured to hybridize to said sample polynucleotides.

Claim 8. The method of claim 1, wherein step (A) comprises (i) hybridizing said first oligonucleotide tag to said sample polynucleotide and (ii) extending said first oligonucleotide tag or said sample polynucleotide or both to provide said extension product.

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

Claim 10. The method of claim 1, wherein said second tag sequences of said plurality of oligonucleotide tags are random sequences.

Claim 11. The method of claim 1, wherein step (c) comprises generating said plurality of amplified polynucleotides using polymerase chain reaction (PCR).

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

Claim 13. The method of claim 1, wherein at least 90 percent of said plurality of tagged polynucleotides have a second tag sequence that is different from second tag sequences of the other tagged polynucleotides.

Claim 14. The method of claim 1, wherein substantially every tagged polynucleotide of said plurality of tagged polynucleotides has a second tag sequence that is different from second tag sequences of the other tagged polynucleotides.

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

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Claim 16. The method of claim 15, wherein said plurality of oligonucleotide tags comprises a number of different second tag sequences that is at least ten times the number of sample polynucleotides.

Claim 17. The method of claim 16, wherein said plurality of oligonucleotide tags comprises a number of different second tag sequences that is at least one hundred times the number of sample polynucleotides.

Claim 18. The method of claim 1, wherein said plurality of oligonucleotide tags comprises at least 200,000 different second tag sequences.

Claim 19. The method of claim 1, wherein an oligonucleotide tag of said plurality of oligonucleotide tags comprises a sequencing adaptor.

Claim 20. The method of claim 19, wherein step (d) comprises hybridizing said amplified polynucleotides or derivatives thereof to a solid support via said sequencing adaptor or derivative thereof.

Claim 21. The method of claim 20, wherein said solid support is a bead.

Claim 22. The method of claim 1, wherein step (e) comprises

(i) using second tag sequences of said plurality of amplified polynucleotides to determine that said plurality of amplified polynucleotides are amplified from said tagged polynucleotide and/or

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(ii) using sample sequences, first tag sequences, and second tag sequences of said plurality of amplified polynucleotides to determine that said plurality of amplified polynucleotides are amplified from said tagged polynucleotide.

Claim 23. The method of claim 1, wherein step (e) comprises

(i) determining the number of different second tags sequences associated with said sample sequence, thereby estimating the number of sample polynucleotides having said sample sequence from said single cell and/or (ii) using second tag sequences of said plurality of amplified polynucleotides to provide a digital count of said sample polynucleotides.

Claim 24. The method of claim 1, wherein said plurality of sample polynucleotides are pooled with sample polynucleotides from other single cells of said plurality of cells prior to the generating of step (b).

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

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

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Claim 27. The method of claim 1, wherein step (e) comprises using first tag sequences of said plurality of amplified polynucleotides to correlate the sample sequences of said plurality of amplified polynucleotides with the single cell from which the sample sequences are derived based on amplified polynucleotides from the same cell having the same first tag sequence.

Claim 28. The method of claim 1, wherein step (e) comprises using second tag sequences of said plurality of amplified polynucleotides to correlate the sample sequences of said plurality of amplified polynucleotides with the sample polynucleotide from which the sample sequences are derived based on amplified polynucleotides from the same sample polynucleotide having the same second tag sequence.

B. Overview Of Prior Art

1. Linnarsson (EX1003)

33. EX1003 ("Linnarsson") discloses a "single-cell tagged reverse transcription (STRT) method." EX1003, 5:10-11, 23:1-4 (Example I disclosing STRT method), 31:3-5 (Example II disclosing STRT method); 37:31-32; *see generally* Pet.24-29. Linnarsson's method involves preparing a cDNA library for sequencing by reverse-transcribing mRNA initially produced by transcription of

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genes being expressed in the analyzed cells. EX1003, 1 (Abstract), 3:25-4:2, 12:4-8 (defining cDNA library as "a collection of cloned complementary DNA (cDNA) fragments")[1]; EX1002, ¶104. 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; EX1054, Abstract; EX1002, ¶125. 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.

[1] Persons of skill would have understood this to refer to fragments of the reverse-transcribed cDNA. This is supported by Linnarsson as a whole, which relies on sizing the reverse-transcribed polynucleotides such that they are suitable for sequencing. *Infra*, ¶¶45-47.

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34. The CDSs are designed to anneal[2] to mRNA molecules. According to Dr. Cooper, "Linnarsson's cDNA synthesis primer… includes a sequence of thymines…." EX1002, ¶101. Because virtually every eukaryotic mRNA molecule has a poly-A tail, the CDSs with a sequence of thymines, also referred to as an oligo-dT, can hybridize or anneal to virtually every mRNA molecule in a well. EX1003, 18:27-30; *infra* n.18.

35. The TSOs are designed to anneal to newly synthesized single-stranded cDNA from reverse transcribed mRNA. EX1003, 16:18-17:2; 18:19-26. Linnarsson's TSOs include a poly-guanosine sequence. EX1003, 18:19-21. In the process of reverse-transcribing mRNA into cDNA, two to five cytosines are typically added to the 3' end of the single-stranded cDNA by the reverse transcriptase. EX1003, 16:18-31. The poly-guanosine sequence in TSOs "'anneals transiently' to the series of cytosine nucleotides at the end of [a] newly-synthesized strand." EX1003, 18:19-23. The TSO annealing to cDNA causes "the reverse transcriptase to switch template and continue to synthesis [sic] a strand

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[2] Annealing is the joining of two complementary strands of nucleic acids through hydrogen bonding. EX1002, ¶¶55, 82; EX1003, 13:3-10; EX1009, 2 ("Denaturation separates the complementary strands of DNA held together in the duplex by hydrogen bonds.").

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complementary to the TSO." EX1003, 18:23-24. 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, and the "5' fragment[s]" are sequenced. EX1003, 18:27-19:11.

36. Linnarsson provides two examples of the STRT method—Examples I and II. Example I employs a biotinylated CDS and a TSO with a Bts cut site on the 5' end of the cell-tag. EX1003, 23:9-19. Figures 2, 3, and 4 depict aspects of the Example I STRT method. EX1003, 23:1-25:4. Figure 2 is a generic overview demonstrating how the CDS anneals to mRNA with a poly-A tail, and TSO anneals to a poly-C sequence of reverse transcribed DNA. Figures 3 and 4 show a "typical" TSO and CDS sequence used in Figure 2. Figure 2 includes no explanation or depiction of purification, sequencing, or any of the other steps persons of skill would recognize as necessary to prepare and subsequently analyze a cDNA library. EX1003, 4:14, 51. Moreover, persons of skill would have understood that the black lines extending from the CDS and TSO in Figure 2 represent STRT method features, including the disclosed TSO and CDS used in Example I, which include the amplification primer sequence ("APS")[3] and Bts cut site for the TSO, and at least the APS, and biotin for the CDS. EX1003, Figs.2-4; EX2015, 1. Further, persons

[3] The APS allows the first strand of cDNA to be amplified by PCR. EX1003, 19:18-23.

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of skill would have understood that the slanted black line on the CDS indicated a tail, and that the tail included a sequence or molecule that is not complementary to the sequence to which it binds. EX1003, Fig.2. In the context of Example I, persons of skill would have understood this tail in Figure 2 refers to at least the universal primer and the biotin present in Linnarsson's disclosures.

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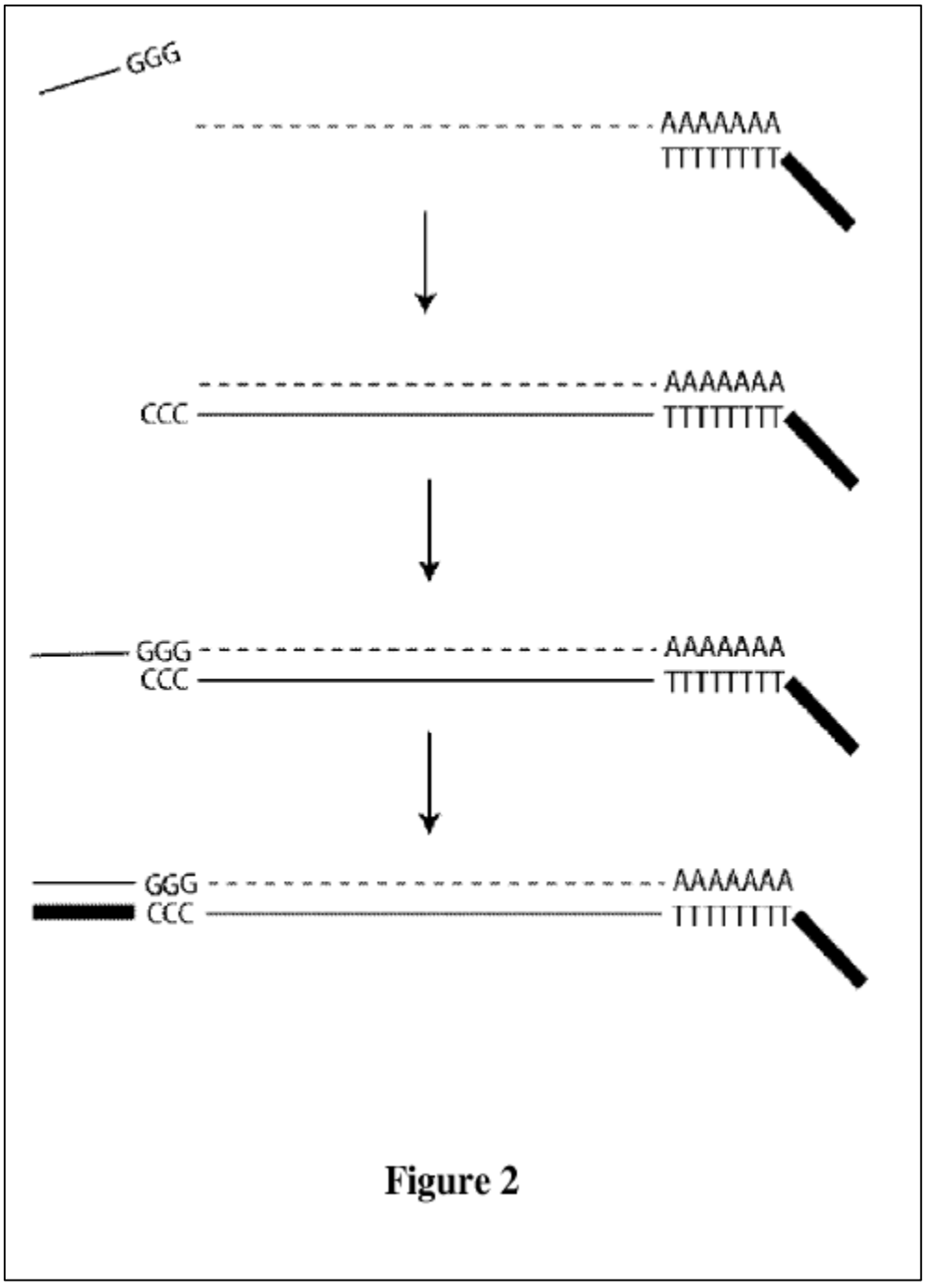


Figure 2

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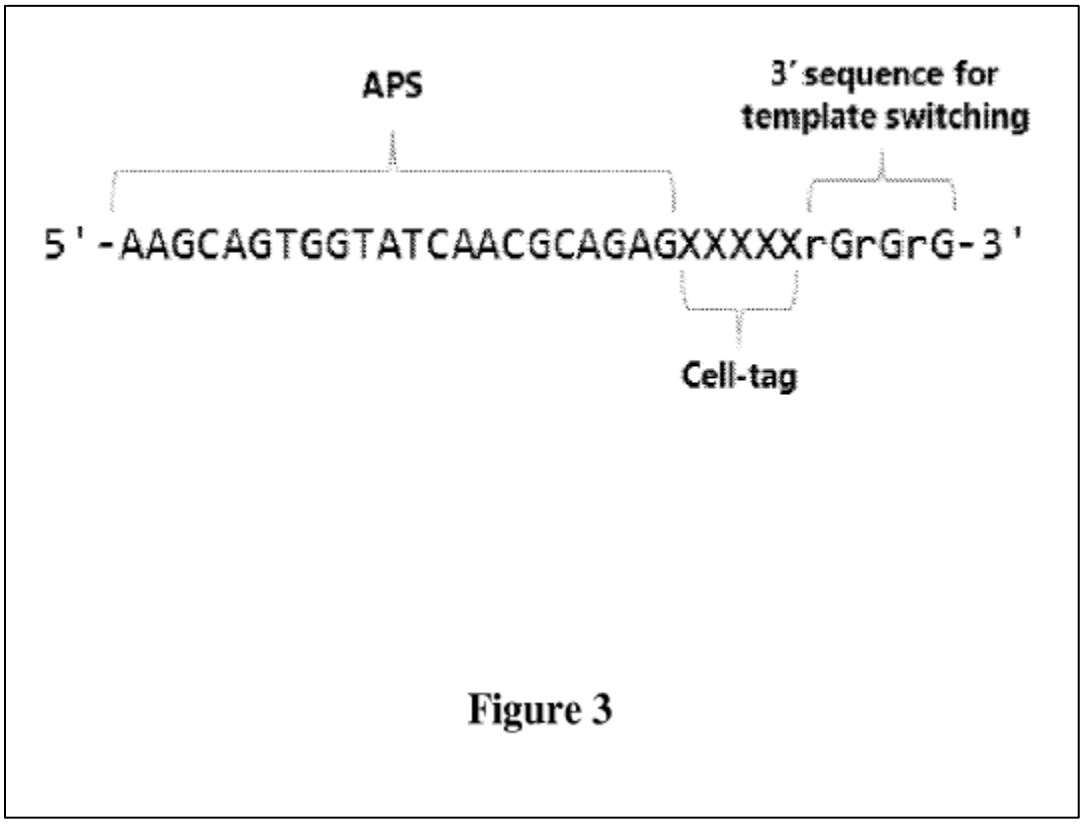


Figure 3

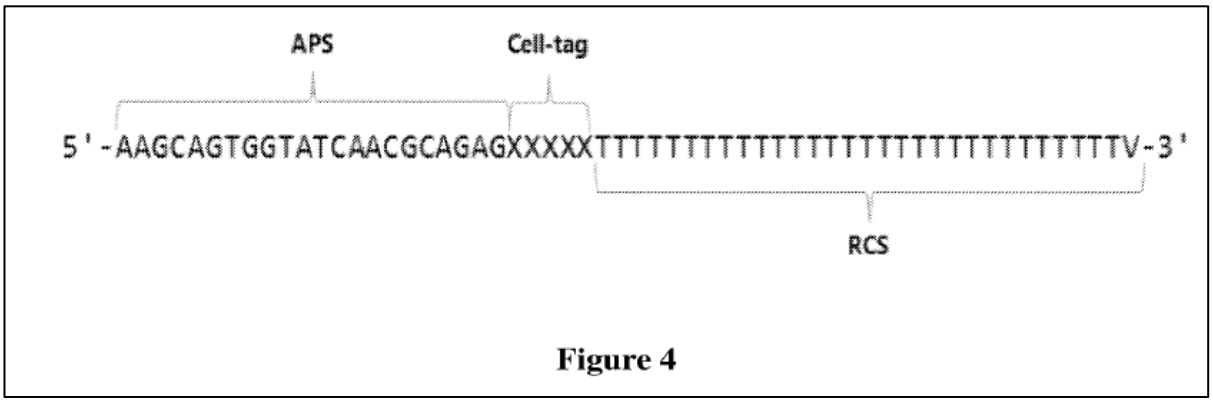


Figure 4

37. Example II describes another example of the STRT method. EX1003, 31:3-32:10. Example II, like Example I, also employs a biotinylated CDS and a Bts cut site on the 5' of the reverse transcribed DNA. EX1003, 32:10-27. Figure 11 is discussed with and depicts steps of a method used in Example II (which are the same

methods used in Example I), and includes details of the process in Panels A-F, which are summarized here. EX1003, 37:31-32, 5:28-6:12;[4] *see also infra*, ¶¶64-65.

38. **Panel A**: The CDS, in red, has a poly-T tail at its 3' end,[5] a primer sequence on the 5' side of the CDS's poly-T tail, and is "capped" by a biotin

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[4] The Figure 11 disclosures are also consistent with the STRT method disclosures in Example I. As in Example I, Figure 11 uses a biotinylated CDS and a TSO with a cell-tag and a Bts cut site to generate a 5'-end fragment cDNA library to analyze gene expression of a cell. EX1003, 5:28-6:12, Fig.11. The Figure 11 disclosures are also consistent with Figures 2-4. As described above, Figures 2-4 are simplified depictions of aspects of the CDS and TSO used in Example I (and the same as those used in Example II) and depicted in Figure 11, and would have been understood to include the features of the CDS and TSO disclosed in Examples I and II and Figure 11, including for the CDS the (1) primer (2) Bts cut site (3) cell-tag and (4) biotin and for the TSO the (1) primer (2) Bts cut site and (3) cell-tag. Thus, my opinions on Figure 11 apply equally to Examples I and II and Figures 2-4.

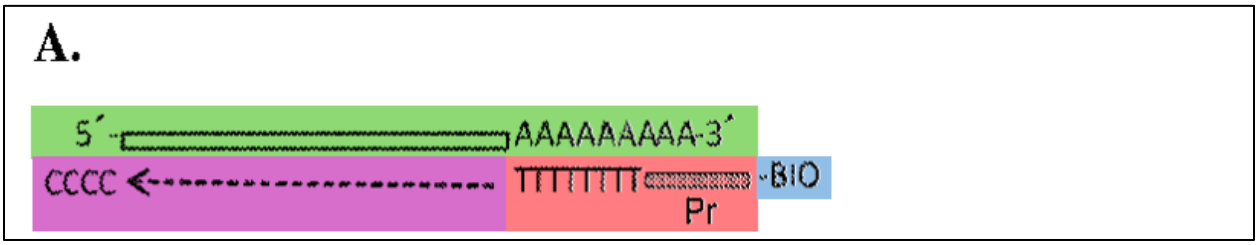
[5] Each single-stranded nucleic acid has a 5' and 3' end. EX1002, ¶56. These are identified by a phosphate group on the 5' end, and a hydroxyl group on the 3' end.

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molecule (attached to the 5' deoxyribose phosphate group, preventing the further addition of nucleotides), in blue. EX1003, 5:28-31, Fig.11 (Panel A), 23:13-19; EX2018, 23:21-25:21. A cell-tag (according to Dr. Cooper) can be incorporated between the primer and the poly-T tail. EX1002, ¶103; EX1003, Fig.4, 4:17-18, 18:31-19:11. The CDS's 3' end poly-T tail is hybridized to the mRNA molecule's (green) poly-A tail because Adenine and Thymine are complementary nucleic acids. EX1003, 5:28-31; EX2018, 23:21-25:21. Once the CDS anneals to the mRNA molecule, a reverse transcriptase enzyme recognizes the annealed structure and reverse transcribes the mRNA to create cDNA (purple). *See* EX1003, 5:28-31, Fig.11 (Panel A). The resulting cDNA (purple) includes an added poly-C tail at its 3' end ("CCCC") due to the terminal transferase activity of reverse transcriptase. EX1003, 5:30-32, Fig.11 (Panel A); EX2018, 25:22-26:6.

Linnarsson refers to the double-stranded ends of the *double*-stranded DNA generated through its process as 5' and 3' "fragments" or "ends." EX1003, 6:5-8, 26:22. The "5' fragment" is the end of the double-stranded DNA corresponding to the region with the TSO sequences, and the "3' fragment" is the end corresponding to the region with the CDS sequences. EX1003, 6:5-8.

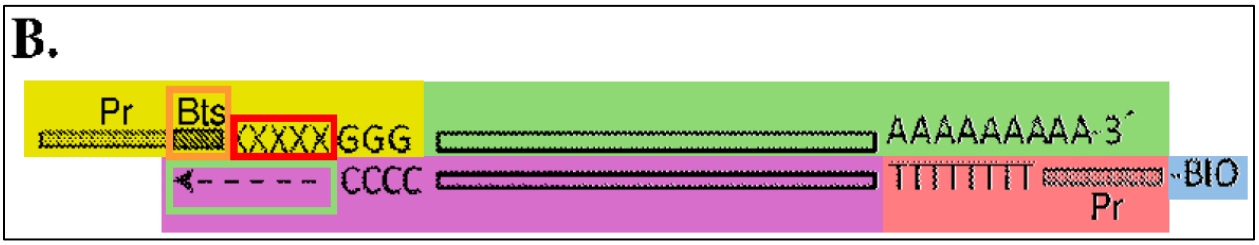
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39. **Panel B**: To extend the synthesis of the cDNA (purple) from Panel A, Linnarsson uses a TSO (yellow). EX1003, 5:31-6:1, Fig.11. The TSO's poly-G tail hybridizes to the poly-C tail of the cDNA because Cytosine and Guanine are complementary to each other. EX1003, 5:31-33, Fig.11 (Panel B); EX2018, 25:22-26:12. The TSO 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. EX1003, 5:31-6:1, 23:13-19, Fig.11 (Panel B); EX2018, 27:14-28:15. A reverse transcriptase enzyme recognizes the annealed structure, and then second-strand cDNA synthesis proceeds using the TSO as a template (depicted by the dotted arrow boxed in green), which adds the cell-tag and Bts to the 3' end of the cDNA (boxed in green) in a second extension step. EX1003, 5:31-33, Fig.11 (Panel B).[6]

[6] The mRNA molecule is optionally digested by an RNAse enzyme. EX1003, 16:10-17. Alternatively, the top strand may be separated from the cDNA during

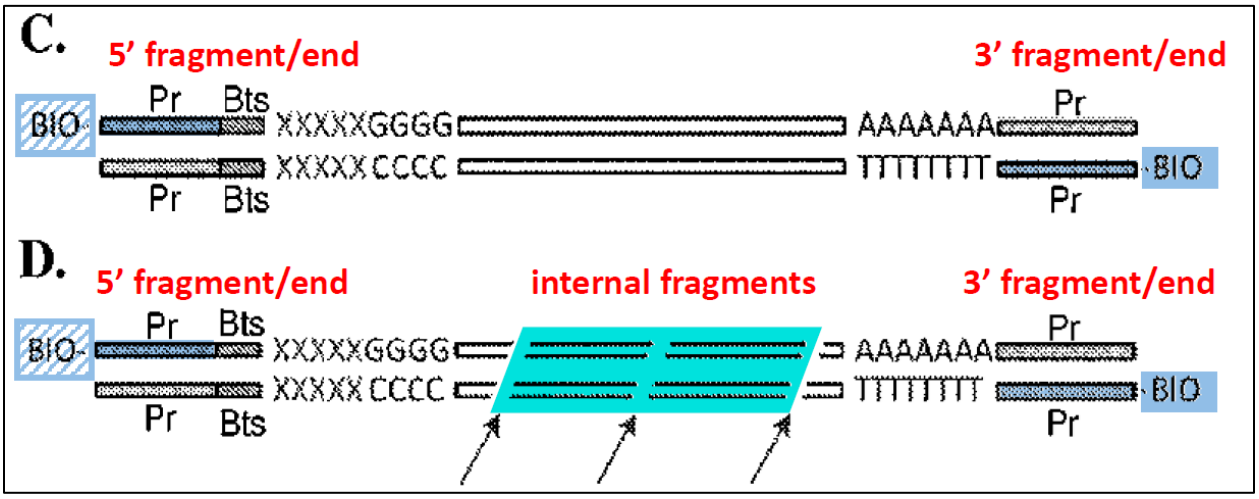
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40. **Panels C and D**: The cDNA sequences generated through the steps shown in Panels A and B are then amplified via PCR, during which a biotin (shaded blue) is added to all cDNA fragments on their 5' ends. EX1003, 25:17-20, Fig.11 (Panel C); EX2018, 28:19-29:7. At this point, the 5' end of each DNA strand is capped by a biotin molecule. EX1003, 25:17-20, Fig.11 (Panel C). Following PCR, the sequences are subjected to a "fragmentation step" with a DNase enzyme, which randomly cleaves the double stranded DNA. EX1003, 6:3-4 (Panel D), 12:4-8; EX2018, 29:8-13. The internal fragments (teal) from Panel D are washed away in a later step (Panel E) (and are therefore never sequenced). EX1003, 6:3-4 (Panel D), 26:26-26.

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the PCR process, which includes a step where the double-stranded DNA is separated into single-stranded DNA. EX1002, ¶¶80-81.



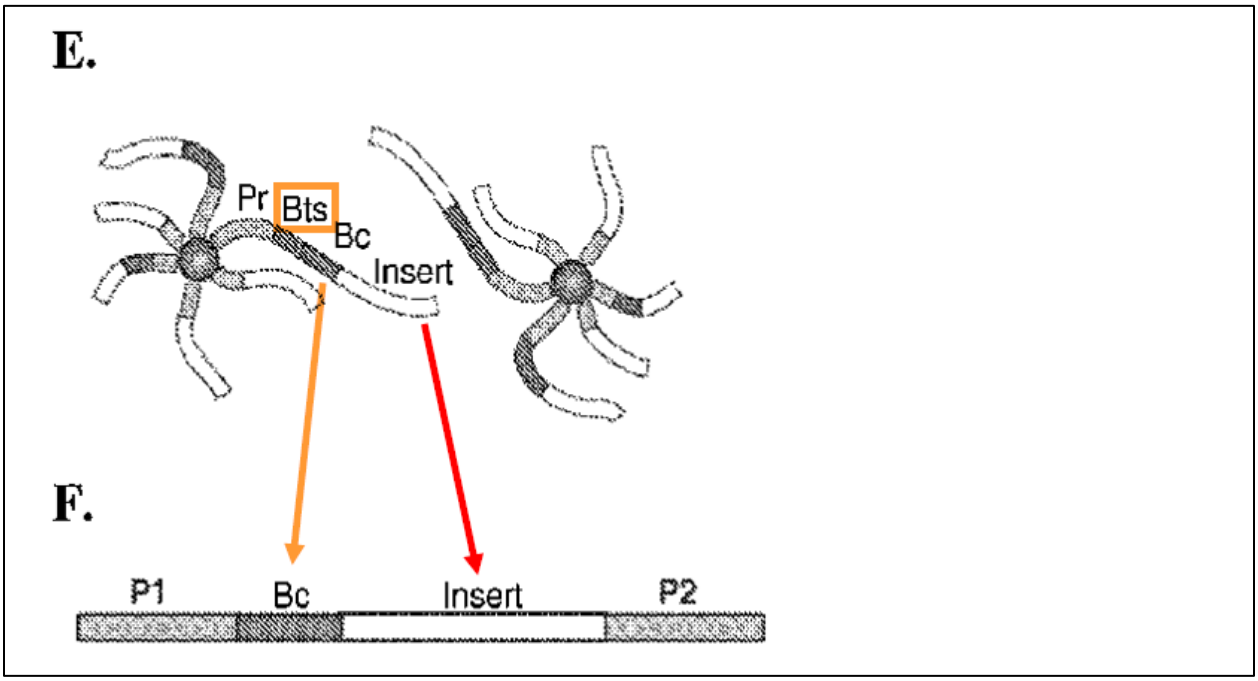
41. **Panels E and F**: The remaining biotinylated and fragmented DNA segments—the 5' fragments with "barcode (Br) and BtsCI site (Bts)" and the 3' fragments (which do not have a Bts site) from Panel D—are then bound to streptavidin beads. EX1003, 6:5-6, 26:21-26, Fig.11 (Panels D-E), 23:13-16, 32:18-27; EX2018, 31:12-22; *see also* EX1003, 21:13-25, 27:6-28:2, 35:23-36:9; EX1002, ¶186; EX2018, 43:21-44:22. It is at this point that the internal fragments from Panel D, which are not biotinylated, and therefore are not bound to beads, are washed away. EX1003, 6:3-4 (Panel D), 26:26-26; EX2018, 43:21-44:22. As I discuss in Paragraph 45, fragmentation may be required in preparation for NGS sequencing. As discussed above, Linnarsson describes no alternative to this step.

42. Following immobilization to the beads for the biotinylated 3' and 5' fragments of the DNA, another enzyme, BtsCI is added, which cleaves the bound fragments at the Bts (orange outline) sequence. EX1003, 6:6-8, Fig.11 (Panel E), 27:6-28:2, 35:23-36:9. 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. EX1003, 6:6-8, Fig.11 (Panel E); *see also* EX1003, 21:13-25, 27:6-28:2, 35:23-36:9; EX1002, ¶186; EX2018, 43:21-44:22. This is confirmed in Panel E, which depicts two different fragments: the first set of fragments has four sequences, and the second set has two sequences. EX1003, Fig.11 (Panel E); *see also* EX1003, EX1003, 21:13-25, 27:6-28:2, 35:23-36:9; EX1002, ¶186; EX2018, 43:21-44:22. The set of fragments with four sequences is, as identified in Panel E, made up of the 5' fragments, and correspond to the side of the DNA tagged with the TSO. EX1003, Fig.11 (Panel E). The set of fragments with two sequences does not contain a Bts sequence and corresponds to the end of the DNA tagged with the CDS. EX1003, Fig.11 (Panel E).

43. Two adapter sequences, P1 and P2, are then ligated to the ends of those 5' fragments released by BtsCI digestion to allow the next-generation sequencer to sequence the fragments (Panel F). EX1003, 6:9-12, 27:6-28:2, 35:28-36:9. If the library is sequenced from the P1 primer, the "reads begin with the 5bp barcode, followed by 3-4 Gs, followed by the cDNA insert." EX1003, 6:9-12. If the P2 sequenced is used, the "reads would yield only the cDNA sequence." EX1003, 6:9-12. The 3' fragments are not released, do not have P1 or P2 sequences added, and are not sequenced. EX1003, 6:6-8, 35:28-36:9.

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44. The method is depicted below.

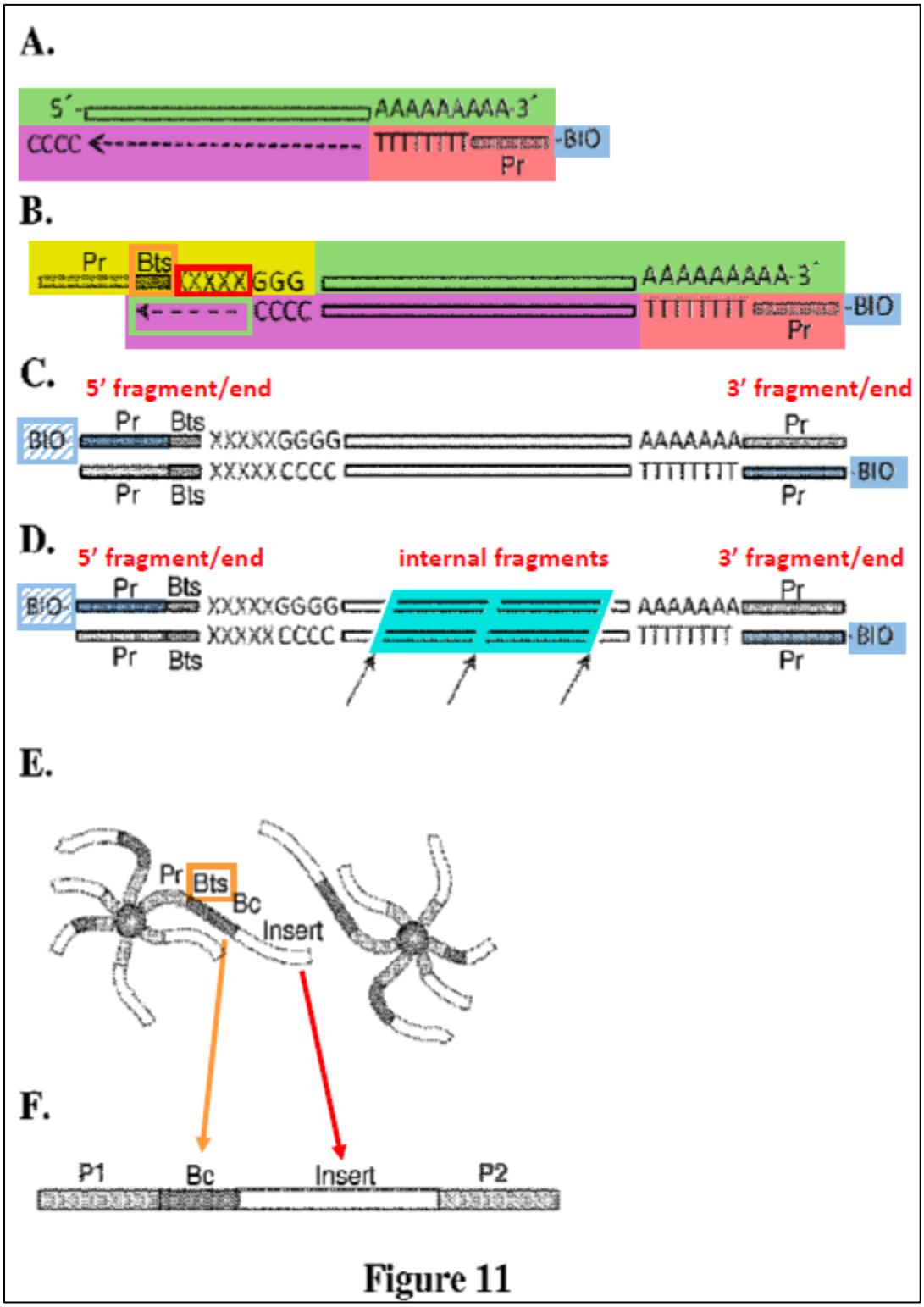


Figure 11

45. Persons of skill would have understood that after the cDNA library is generated, as shown in Examples I and II and Figure 11, further processing steps

(after, *e.g.*, Figure 11 Panel F) are contemplated to prepare it for sequencing, including in NGS. *E.g.,* EX1003, 21:13-25, 48:9-10 (Claim 27). For example, Linnarsson discloses that the cDNA library can be further fragmented, such as for use in shotgun sequencing, which, unlike the process disclosed in Figure 11, would result in each fragment being sequenced (in this case no fragments are "washed away"). EX1003, 22:5-21. Persons of skill would have understood that this further processing would be useful or even necessary depending on the downstream sequencing process being employed. EX1003, 21:13-25, 22:5-21. As another example, further fragmentation might be used to prepare the cDNA fragments such that they are an optimal size for sequencing depending on the sequencing method. EX2020, 57:4-20.

46. Linnarsson's method results in tagging the mRNA molecules extracted from a single cell with the same barcode through the process of reverse transcribing it into cDNA. EX1003, 3:25-4:2, Fig.11. Thus, after amplification and sequencing of the cDNA, Linnarsson's method does not allow persons of skill to distinguish, among the multiple copies of polynucleotides, those that are the result of amplification from those that were created endogenously (*e.g.*, multiple copies of mRNA molecules resulting from overexpression of a particular gene), and thus does not determine how many instances of that particular polynucleotide existed *in a particular cell* before amplification. *See* EX1003, 22:22-28. Linnarsson's method

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also does not sequence the entirety of the DNA sequences. Not only are the internal fragments washed away, but the 3' ends are not released from the beads and are not sequenced. EX1003, 6:5-8.

47. As is clear from the consistent, repeated disclosures of Linnarsson, the features disclosed in Examples I (including Figures 2-4) and II (including Figure 11)—Linnarsson's only provided examples—both affirmatively use CDS and/or TSO, biotin, a restriction site (*e.g.*, the Bts cut site), and fragmentation, which are essential features of Linnarsson's methods. EX1003, Figs. 2-4, 11, Examples I-II; EX2015, 1. Figure 4, for example, depicts a polynucleotide sequence with an APS, a cell-tag, a poly-dT RCS, and a Bts cut site. EX1003, Fig.4; EX2015, 1. Persons of skill would understand Figure 4 as illustrating additional sequence details of a typical CDS used in Figure 2, and not as attempting to illustrate other molecules (like biotin), which the text confirms are part of the Example I process. The CDS is designed to target the mRNA molecules in the cell, and triggers reverse transcription to generate the strand of cDNA with the poly-C tail. EX1003, 5:30-31, 15:1-11, 25:2-22, Figs.2, 11 (Panel A). The TSO then binds and triggers the generation of the rest of the strand of cDNA. EX1003, 16:18-17:2; 18:19-26, Figs.2, 11 (Panel B). Together, these two reverse transcription steps ensure the tags and PCR primers are added. EX1003, Figs.2, 11 (Panel C). And the biotin ensures the tagged fragments are bound to the beads, and not washed away with the internal fragments

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and untagged polynucleotides the researcher is not interested in sequencing (e.g., DNA in Linnarsson's method). EX1003, 21:13-25, 35:23-36:9. This is how Linnarsson discloses purifying its sequences that will become the cDNA library. The Bts cut site located on the 5' fragments is then recognized by the Bts enzyme, and when cut releases the 5' fragments for clean-up and sequencing following purification. *See* EX1003, 12:4-8 (defining "cDNA library" as "DNA (cDNA) fragments."). This is further confirmed by the absence of any alternative approaches in Linnarsson for performing the STRT method.

2. McCloskey (EX1004)

48. EX1004 is an article by Megan L. McCloskey, Reinhard Stöger, R. Scott Hansen, and Charles D. Laird, titled "Encoding PCR Products with Batch-stamps and Barcodes." EX1004 ("McCloskey"), 1. McCloskey is focused on addressing laboratory molecular contamination issues for irreplaceable samples. EX1004, 1.

49. As part of the DNA amplification process, McCloskey adds an "encoding oligonucleotide" with a "batch-stamp" and barcode, to copies of alleles for a particular targeted gene. EX1004, 2-4. McCloskey's encoding oligonucleotide includes, *inter alia*, (1) a batch-stamp; and (2) a random barcode that distinguishes alleles of the same gene with (3) a genomic target-binding site that targets the

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specific gene on the chromosomal DNA to be barcoded prior to amplification. EX1004, 2. The batch-stamp identifies the batch sample from which the polynucleotides originated in order to identify contamination (*i.e.*, to identify sequences originating from a different patient or sample left over from a prior analysis) and the barcode distinguishes between copies of an allele for a particular gene in the sample (without identifying the particular cell of origin). EX1004, 2-4.[7] The only specific length taught in McCloskey for its random barcodes is seven nucleotides, and that barcode is comprised of the four natural deoxyribonucleotides.[8] EX1004, 4. This allows for the creation of up to 16,384 (4^7) unique barcodes and provides enough tag sequences to tag different alleles for the same gene differently in McCloskey's collection of cells. EX1004, 4.[9]

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[7] Alleles are different versions of the same gene. EX2003, 3; EX1002, ¶59.

[8] "Deoxyribonucleotides" are nucleotides that have a deoxyribose as their sugar component. EX1002, ¶¶54-55; EX2014, 31; EX1001, 7:31-56. The four natural deoxyribonucleotides are adenine (A), guanine (G), cytosine (C), and thymine (T). EX1002, ¶¶54-55; EX2014, 31.

[9] I agree with Dr. Cooper that there are, at most, two alleles per "normal" cell. EX1002, ¶59.

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50. McCloskey's method involves lysing the cells and combining the contents (including the DNA) of those cells before tagging the copies of a particular gene within this combined material to determine the presence of alleles. EX1004, 2, 5. Among other things, this is confirmed by the amount of DNA used in the described experiment. EX1004, 5. The 2.71μg used is significantly more genomic DNA than that present in a single cell. EX2016 ("6.46 pg" mean weight in a diploid cell); EX2018, 34:19-35:3. 2.71μg of DNA corresponds to approximately 420,000 cells'-worth of DNA (2.71 μg ÷ 6.46 pg = 419,504.64). EX2016. In my opinion, this amount of DNA in McCloskey indicates many cells were lysed, the genetic material combined, and then isolated.

51. After this material is combined, copies of a particular gene (including any alleles or mutations) are tagged by annealing the genomic target-binding site of the oligonucleotide to the complementary DNA sequence found on one end of the target gene. EX1004, 5. McCloskey discloses that the presence of an encoding oligonucleotide in the DNA for the particular gene of interest allows researchers to identify and remove contaminating sequences (from different batch samples) and redundant sequences (amplified copies containing the same random barcode) after the gene of interest is amplified. EX1004, 3 (Fig. 1), 7. However, McCloskey's method does not allow a user to determine whether or which copy of a particular allele (or mutation) is present in a given cell because *no copies of the target allele*

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are tagged with a sequence that tracks an allele to its cell of origin. EX1004, 3. Single-cell analysis requires separate tagging of polynucleotides for each cell *prior* to pooling to ensure that the same source identifying tag is introduced only to nucleic acids from the same cell. EX1002, ¶70 ("(1) isolation of single cells from a cell population; (2) template preparation and tagging of sequences…"). But McCloskey pools the nucleic acids from all of the cells in the sample *prior to tagging*, making it impossible to track a nucleic acid to its cell of origin. EX1004, 2, 5. Thus, unlike the Challenged Claims, McCloskey's approach to removing redundant copies simply permits a user to determine whether the target gene was present, including whether there were any alleles or mutations present, *in the sample when it was tagged*. EX1004, 2, 5.

52. McCloskey's disclosures are focused on tagging specific genes, the sequence of which is already known; they are not focused on tagging all mRNA. In my opinion, there would not be enough oligonucleotide tags in McCloskey to tag each mRNA molecule in a cell. EX1003, 1; EX1004, 4. As discussed above, persons 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 that range. EX1054, Abstract. McCloskey's barcode, with just 16,348 unique combinations, is far too few to allow unique barcode tags for each mRNA molecule. There would not have

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been enough unique tags for even the 50,000 mRNA molecules (the low end of the range of expected mRNA molecules) to be tagged, even under ideal conditions (where each unique tag is present and anneals to a mRNA molecule), let alone the many multiples of tags that would have been required to tag those 50,000 molecules. *Supra*, ¶28.

3. McCloskey II (EX1055)

53. EX1055 is U.S. Patent Publication 2007/0020640, titled "Molecular Encoding Of Nucleic Acid Templates for PCR And Other Forms Of Sequence Analysis." EX1055 ("McCloskey II").

54. McCloskey II focuses on addressing laboratory molecular contamination issues for irreplaceable samples by tagging specific genes with known sequences. EX1055, ¶¶3-5, ¶9.

C. Claim Construction

55. I understand that, for purposes of *inter partes* review, claim terms are construed in accordance with their ordinary and customary meaning. I understand claim construction begins with the claim language itself. Claim terms are presumed to have their ordinary and customary meaning in light of the patent's specification as understood by persons of skill at the time of invention, unless (1) the patentee sets out a definition and acts as his own lexicographer, or (2) the patentee disavows the

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full scope of a claim term either in the specification or during prosecution. I understand that the "ordinary and customary" meaning of a claim term is the meaning that the term would have to persons of skill at the time of the invention, and that in addition to the claim language, the terms are also understood in the context of the patent's specification and file history.

56. I have assumed the following construction in my analysis, which is found in the specification of the '013 Patent: "sample" means "a quantity of material from a biological, environmental, medical, or patient source in which detection, measurement, or labeling of target nucleic acids is sought" (EX1001 ('013 Patent), 12:15-18).

57. For all other terms, I have been asked to assume that they have their plain and ordinary meaning as they would have been understood by persons of skill in light of the specification.

VI. THE CHALLENGED CLAIMS ARE VALID

A. Ground 1: Linnarsson In View of McCloskey and Further in View of the Knowledge of POSA Does Not Render Claims 1-12, or 19-28 Obvious.

1. Dr. Cooper's Motivation To Modify Dr. Cooper's Ground 1 Fails

58. Dr. Cooper's Ground 1 theory fails because there is no motivation to modify Linnarsson to add a tag via ligation.

59. In my opinion Persons of skill also would not have been motivated to change and complicate the Linnarsson method only to yield what Dr. Cooper asserts is an "equivalent" process. Dr. Cooper's Ground 1 theory would require persons of skill to, at minimum, buy or create another set of tags as well as any other materials necessary to implement the ligation (such as Hug's linker molecules and enzymes). This would also require adding at least one additional ligation step at some point in the process. In my opinion, persons of skill would not have added steps simply to ligate tags rather than *adding* a "second tag sequence" to the tags Dr. Cooper says already existed in the TSO or CDS. Certainly, persons of skill would not have added such steps merely to achieve an "entirely equivalent" method. EX1002, ¶149.

60. Dr. Cooper attempts to justify modifying Linnarsson by asserting "it would allow for increased flexibility in selecting tag sequences during an experiment." EX1002, ¶149. First, Dr. Cooper's assertion about ligation providing "extra flexibility" is contradicted by Dr. Cooper's assertion about the method being "entirely equivalent." EX1002, ¶149. Second, Dr. Cooper's assertion is entirely conclusory, and, in my opinion, is wrong. EX1002 ¶149. Dr. Cooper has not identified any "flexibility" that is gained when *ligating* a tag rather than, *e.g.*, adding it via primer extension (which Dr. Cooper asserts would have been obvious in other proceedings) *before* further modifying its combination with ligation. Assuming one

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could add a tag via ligation in Linnarsson's method (*infra,* §VI.A.2), those same tag sequences could be added via primer extension with the CDS and/or TSO.

61. The lack of benefit is confirmed by Dr. Cooper's theories. Dr. Cooper does not identify any tags that could be added by ligation that would not have been included in the CDS or TSO under his own Ground 1 theory. EX1002, ¶149.

2. Dr. Cooper Has Not Shown A Tag Can Be Successfully Added Via Ligation In Linnarsson While Still Practicing the Claimed Method

a) Dr. Cooper Did Not Show That A Tag Can Be Ligated To The CDS

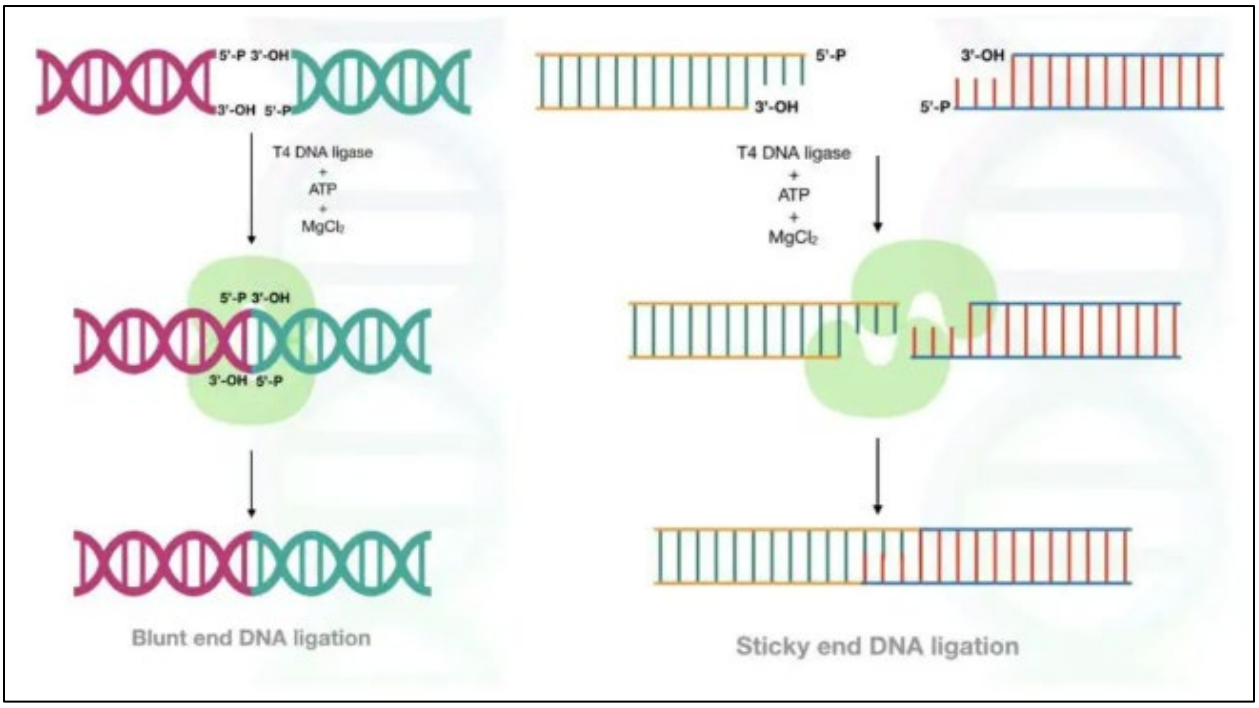
62. In my opinion, as the combination is presented by Dr. Cooper,[10] ***ligating*** a tag to the CDS is incompatible with the other steps in Linnarsson's method, and would not result in ***a method that practices claims 1-12 or 19-28***.

63. First, Linnarsson teaches that a biotin is attached to the 5' end of the CDS. *Supra*, §VI.A.1. The biotin on the 5' end of the CDS would block ligation of a tag to the CDS. EX1003, Fig.11, 5:28-6:8, 23:13-14. Ligation "means to form a covalent bond or linkage between ***the termini of two or more nucleic acids.***" EX1001, 6:15-32. This occurs when the ends of DNA fragments are joined by the

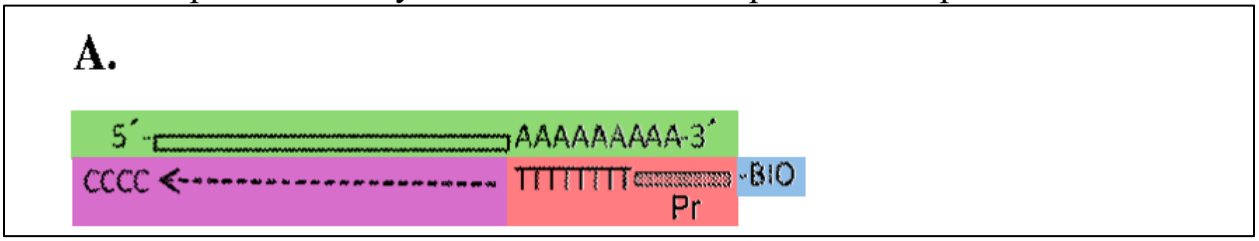
[10] Dr. Cooper does not mention, let alone explain, any other modifications to its combination that would make it possible to ligate any tag to the CDS.

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formation of phosphodiester bonds between the 3'-hydroxyl of one DNA terminus with the 5'-phosphoryl of another. EX1001, 6:23-40. Both of the two forms of ligation, depicted in the picture below, result in formation of phosphodiester bonds between adjacent nucleotides. The attached biotin would prevent ligation because the biotin would be attached to the 5' phosphate and thus would block the formation of a phosphodiester bond required for ligation.



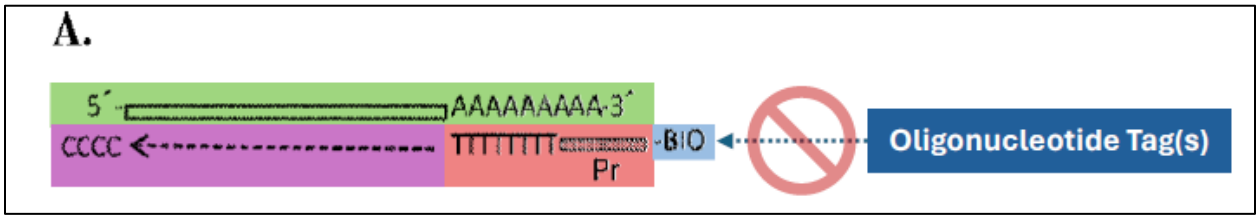
EX2017. An example of a biotinylated nucleic acid is depicted in the picture below.



EX1003, Fig.11. Linnarsson's biotin molecule is bound to the 5'-phosphoryl group in the cDNA strand. EX1003, Fig.11, 23:13-14, 25:17-18, 32:18-25. This is both depicted in Figure 11 and is disclosed in the CDSs used in Examples I and II, which persons of skill would understand to mean that the biotin is bound to the 5' end of the nucleic acid, which has a phosphoryl group on the end of the last nucleotide. *Id.* This means that a nucleic acid tag cannot be covalently bound to the 5' phosphoryl group as that bond is occupied by the biotin. *Id*. As shown in Figure 11 and described in Examples I and II, the CDS is capped by a "BIO" (biotin) molecule at its 5' end. EX1003, Fig. 11; 23:13-15 (The sequence of STRT-**BIO** (which is a CDS) is: 5'-**BIO**-AAGCAGTGGTATCAACGCAGAGT$_{30}$VN-3'"); 32:19-20 ("STRT-T30-**BIO** (5'-**biotin**-AAGCAGTGGTATCAACGCAGAGT$_{30}$VN-3')"), 32:18-27 (disclosing the same CDSs and TSOs for Example II); EX2018, 23:15-25:21, 29:14-30:2. Thus, the tag cannot be ligated to the "CDS" because the biotin blocks the necessary covalent bond from being formed with its 5' end. For this reason, it is my opinion that Dr. Cooper's CDS theory fails. Dr. Cooper offers no evidence that ligation could occur to a molecule with a 5' biotin, and one is left only with his unsupported (and incorrect) assertion, which ignores the existence of the biotin in Linnarsson's approach, as the only "evidence" that one would have any expectation of success. Given the basic physical configuration of the molecules, it is hard to

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imagine how one might carry out Cooper's proposed reaction.



64. I disagree with Dr. Cooper's deposition testimony implying that the use of biotin is optional in Linnarsson's method. EX2018, 25:10-21, 31:1-22; EX2020, 10:20-11:2; *supra*, §VI.A.1. Dr. Cooper expressly relies on Figure 11 (disclosed in the context of Example II), which persons of skill would have understood requires biotin. EX1002, ¶¶101, 104, 108, 112, 135, 136, 139, 155, 156, 199, 200; *supra*, 83. Although Linnarsson describes other aspects of its method as optional, Linnarsson never describes biotin as optional, and the only examples of Linnarsson's method (Examples I and II) include the use of biotinylated CDSs and biotinylating the "5' end" during PCR. *See* EX1003, Fig.11, Example I, Example II. And Dr. Cooper (whose report never suggests eliminating biotin in any case) does not address how Linnarsson's method would work *without* biotinylated cDNA. For example, Linnarsson discloses that the biotin captures the tagged fragments and enables their purification. EX1003, 5:28-6:12, Fig.11. The fragments *without* biotin, described as the "internal fragments," are "washed away." Without biotin, all the fragments would be "washed away." *Id.* Moreover, in Linnarsson the biotin allows these sequences to be attached to the streptavidin beads, which allows these sequences to

be isolated such that adapter sequences may be added, and then sequenced. EX1003, Fig.11.

65. Second, these CDS sequences used in Examples I-II and Figure 11 ***do not include a Bts*** sequence. EX1003, Examples I-II, Fig.11 (Panel A). The Bts sequence is key to Linnarsson's method, as it is the only disclosed approach for releasing the 5' fragments of cDNA from the streptavidin beads and proceed to the sequencing step. EX1003, 5:28-6:12, 27:6-28:2, 35:18-36:9, Fig.11; *Infra,* ¶68. Therefore, these CDS sequences, corresponding to the 3' fragments, would remain bound to the beads in Panel E and would not be sequenced since only sequences with the Bts sequence are released by BtsCl digestion, and Dr. Cooper identifies no teaching in Linnarsson of releasing the CDS sequence from the beads. EX1003, 6:4-8 ("3' fragments remain stuck on the beads."), 27:6-8, Fig.11; EX2018, 43:21-44:22. Thus, any tag allegedly ligated to the CDS would not practice the method of claim 1 because these sequences would not be sequenced. EX1001, Cl.1.

66. And, finally, even if the tag were ligated to the biotin (such that it were cDNA-biotin-tag), and some portion were somehow released from the beads, it is unclear how the tag would be released with the 3' fragment. The biotin group would somehow be ***between*** the cDNA sequence and the added tag, and thus at least one sequence (the second tag or the 3' fragment) would not be released by Bts digestion. Further, if both fragments were somehow released (if, for example, a Bts sequence

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was added to both sides of the biotin), the fragments would no longer be connected, and the second tag could not be used to identify the 3' fragment, as required by claim 1. EX1001, Cl.1.

67. For all these reasons, it is my opinion that Dr. Cooper's theory of ligating a tag to the CDS fails.

b) Linnarsson Teaches That Any Tag Ligated To The TSO Will Be Cleaved Off Prior To Sequencing

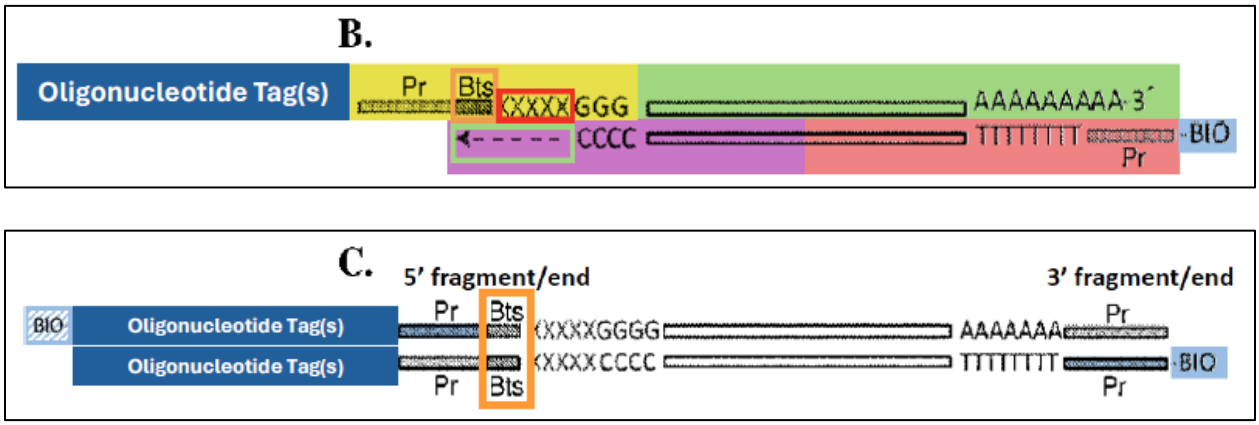
68. It is additionally my opinion that the tag also cannot be ligated to the TSO to arrive at a method that practices claim 1 under Dr. Cooper's theory. Dr. Cooper asserts the tag would be ligated to the end of the TSO "after extension." EX1002, ¶149. Dr. Cooper's theory fails for at least two reasons. First, ligating the "second tag" to the TSO "after extension" would mean that the ligation occurs after the template switching step in Panel B (the next extension step following the addition of the TSO) is completed. EX1003, Fig.11. At this point in Linnarsson's method, the cDNA has been extended twice, and is complete. There is no third "extension" step to reverse transcribe this newly ligated sequence to the resulting cDNA. EX1003, Fig.3. In Panel A, the cDNA is extended a first time to generate a complementary sequence to the mRNA. EX1003, Fig.11 (Panel A). In Panel B, the cDNA is extended a second time to include a sequence complementary to the TSO. EX1003, Fig.11 (Panel B). There is no disclosed "third extension" of the cDNA in

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Linnarsson that would result in any sequence complementary to the ligated tag if the tag were ligated "after extension." Thus, this theory fails because the tag added via ligation would not be included in the cDNA that is subsequently amplified during PCR in Panel C, and therefore would not be part of the tagged polynucleotide that is sequenced in Panel F. EX1003, Fig.11.

69. Second, even if the tag were somehow reverse transcribed, Dr. Cooper's TSO theory fails for another reason. The ligated tag would be located on the 5' end of the Bts sequence, shown in the annotated Panels B and C below. EX1003, Fig.3, Fig.11; *see also id.* 27:6-7, 35:28-36:9. But according to Linnarsson, any sequence on the 5' side of the Bts sequence will be ***removed***—*i.e.*, "released by BtsCI digestion, ***leaving just the barcode (Bc) and insert***" that are released from the streptavidin bead for sequencing. EX1003, 5:28-6:12, Fig.11 (Panel F); *see also id.* 27:6-7, 35:28-36:9. Thus, even if the tag were ligated to the end of Linnarsson's TSO, Dr. Cooper ignores that the tag would be ***cleaved off*** from the sample polynucleotide in Panel E. EX1003, 5:28-6:12, Fig.11. Once cleaved, there would be no "second tag sequence" (as required by claim element [1F]) on the released polynucleotide, and thus the "tagged polynucleotide" would not be sequenced (as required by claim element [1G], nor would it be possible to distinguish among sample polynucleotides as required by claim element [1I]. EX1001, cl.1.

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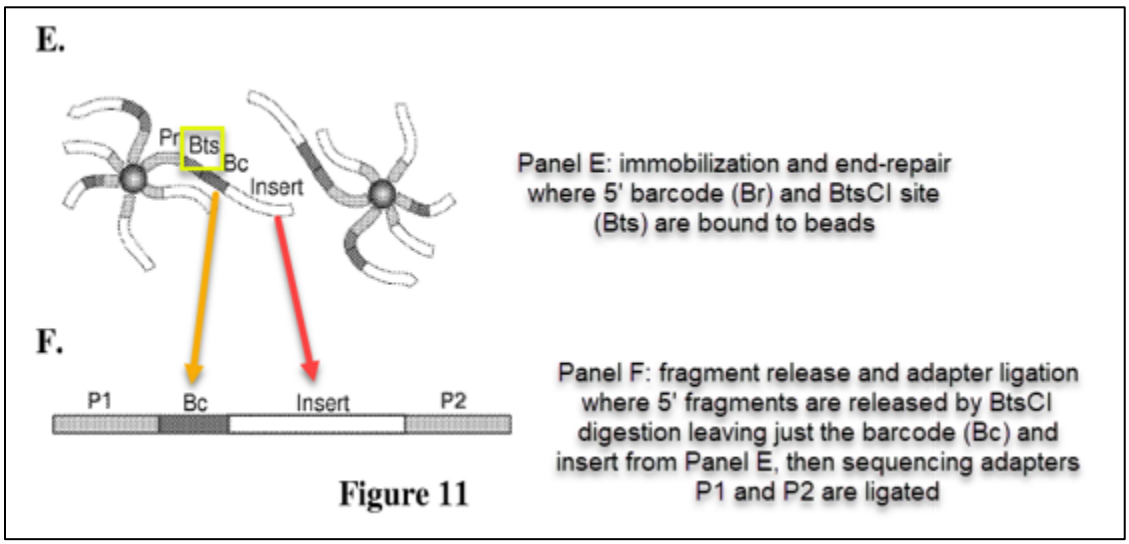


Figure 11

3. Dr. Cooper's Other Ligation Assertions Fail

70. In my opinion, neither Linnarsson nor McCloskey teaches ligation of a "second oligonucleotide tag sequence" to generate the tagged polynucleotides. Dr. Cooper also does not assert that ligation of a tag to a polynucleotide is taught by either reference. EX1002, ¶¶143-144.

71. Dr. Cooper's Ground 1 is "Linnarsson in View of McCloskey, and further in View of the Knowledge of a POSA." EX1002, §XII.A. However I note

that Dr. Cooper relies on EX1007, titled "Measurement of the Number of Molecules of a Single mRNA species in a Complex mRNA Preparation, by Hubert Hug and Rainer Schuler" ("Hug"), when analyzing Ground 1 and arguing the obviousness of the ligation limitation of claim 1. Even to the extent Hug is considered in analyzing Ground 1, I disagree that claim 1 is obvious.

72. First, it is my opinion that persons of skill would not have looked to Hug's fundamentally different (and theoretical) approach or been motivated to use its linker approach with Linnarsson or McCloskey, let alone in a Linnarsson-McCloskey combination. For example, Hug does not sequence the polynucleotides to which it attaches linkers. Hug uses linkers that hybridize to each other to randomly add a number of identical linker units to a molecule that had been cleaved by a BamH1 enzyme so that, rather than DNA-sequence based barcodes, Hug's barcdoes are based on sequence-length (or number of repeated linker elements). EX1007 (Hug), 7-8, Fig.3 ("addition of linkers with a defined ration of oligonucleotide terminators to create DMS of different lengths"). These linkers are then ligated to each other through the use of an enzyme, T4 DNA Ligase. EX1007 (Hug), 7-8, Fig.3 ("ligation with T4 DNA ligase"). Hug then analyzes the DNA fragments by length in order to distinguish between individual molecules and to then

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estimate the number of molecules in a sample.[11] EX1007 (Hug), 7-8, Fig.3 ("determination by PAGE") ("DNA length analysis by PAGE"); EX2018, 49:8-50:9. This is a fundamentally different approach to analyzing nucleic acids from those used in Linnarsson or McCloskey, which both rely on sequencing the nucleic acids they have tagged. EX1003 (Linnarsson), 6:9-12, Fig.11; EX1004 (McCloskey), 6-7. It is also an approach that persons of skill would have been motivated to avoid using in a method where the nucleic acids were sequenced because it would add a significant number of nucleic acid bases as linker-based bar codes, which would then need to be sequenced. This would be a significant disadvantage when using sequencing methods in which only a small number of base pairs can be sequenced—each "wasted" base added by Hug would represent one fewer base that could be read in the mRNA sequences, and thus possibly mean one misses meaningful sequence variants or fails to distinguish transcript isoforms. The short sequence reads characteristic of "Next Generation Sequencing" (NGS) in use at the time are why Linnarsson teaches fragmenting the tagged polynucleotides with DNAse to "200 – 300 bp." EX1003, 6:4-6, 22:5-21. Longer sequences would not

[11] Hug does not have a MID. These linkers are not "unique," cannot be used to identify source, and are not sequenced so as to permit differentiation among nucleic acids. EX1001, 6:41-7:30.

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be sequenced by many NGS technologies, and longer fragment lengths would therefore be disadvantageous, as persons of skill would have recognized.

73. Second, it is my opinion that Hug's linker molecule could not be used with the random barcode of McCloskey. Dr. Cooper incorrectly asserts that Hug's "linker" would have been "*hybridize[d] with the TSO and/or CDS and tag sequences to align them such that they could be ligated together using routine ligation techniques*." EX1002, ¶148. This is incorrect because Hug's disclosed linkers are not designed to link a series of random nucleotides, like those in McCloskey's barcode, with another sequence, like Linnarsson's CDS or TSO, and Dr. Cooper offers no modifications to any of the molecules to allow such linking.

74. Hug describes adding a BamH1 recognition site to the generated double-stranded DNA such that when it is cut with BamH1 (a particular endonuclease), the resulting double-stranded DNA has a 5' sticky end with a particular overhanging sequence. EX1007, 7-8, Fig.3. Two different sets of short linkers are subsequently added. EX1007, 7-8, Fig.3. The first set of linkers consists of the recognition site of the BamH1 restriction enzyme and an end with a short polynucleotide sequence that hybridizes to the other linker.[12] EX1007, 7-8, Fig.3.

[12] Unlike tags that distinguish polynucleotides (EX1001, cl.1), Hug's linkers are not unique.

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These linkers can form a chain of a random length because they can continuously hybridize to each other. *Id*. The second set of linkers are "Terminator Sequences," which terminate the chain because no linkers have the complementary sequence necessary to hybridize to the terminator sequence to continue the chain of linkers. EX1007, 7-8, Fig.3. This is the process by which Hug proposes to differentiate between the sample mRNA molecules. EX1007, 7-8, Fig.3. Hug's theory is that, using the randomly generated linker chains of various *lengths*, one can differentiate between mRNA molecules originating in the sample and estimate the number of each that were endogenous to the sample. EX1007, 7-8, Fig.3. And Hug's theory is premised on the first set of linkers being able to hybridize to each sample polynucleotide that was cut with BamH1. EX1007, 7-8, Fig.3. There is no "selectivity" in the linkers, and the method requires that the same BamH1 site be present. EX1007, 7-8, Fig.3.

75. Dr. Cooper ignores these details of Hug's ligation method and ignores the fact that Hug's linkers are not designed to accommodate oligonucleotide barcodes with *unique sequences*, such as McCloskey's barcodes, or designed to hybridize to Linnarsson's CDSs or TSOs, neither of which contain a BamH1 recognition site. EX1007, 7-8, Fig.3; EX1003, Fig.11; EX1004, 2-4. For a linker to work with McCloskey's barcode, there would have to be a linker molecule that is complementary to each combination of sequences that could be present in

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McCloskey's barcode, and there must be enough of each to ensure the linkers hybridize with the barcode.

76. Moreover, the other end of the linker would need to have a sequence complementary to Linnarsson's CDS or TSO such that it actually "links" the TSO and/or CDS with McCloskey's barcode. In other words, there must be a linker for each of the possible barcode sequences. And for each of these unique linkers, there must be enough of each to ensure that the linker "finds" the barcode and hybridizes to it, and then hybridizes to a CDS and/or TSO. Again, none of this was addressed by Dr. Cooper.

77. And, there would be further issues with this approach because if a linker binds to the CDS or TSO, and another linker binds to the barcode, those two linkers would not then be able to hybridize to each other, and therefore there would be potentially many sample polynucleotides that are never tagged, or insufficiently tagged (*e.g.*, only tagged with a linker, which is unable to act as the second tag). Dr. Cooper does not explain how this would be achieved, particularly in view of the issues below, or why persons of skill would have been motivated to make this modification, which introduces numerous issues, as outlined above.

78. Alternatively, if Hug's linkers were not modified, McCloskey's barcodes and Linnarsson's CDS and/or TSO would have to be modified to include a BamH1 recognition sequence. Dr. Cooper does not propose this modification, or

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explain how it would be achieved or why persons of skill would have been motivated to make this modification.

79. Further, even if persons of skill were to have looked at Hug and made whatever modifications were necessary to allow Hug's linkers to work with McCloskey's barcodes and Linnarsson's CDSs and/or TSOs, it is my opinion that Hug does not cure the other technical issues with Ground 2. Hug, for example, does not address ligation to the end of a polynucleotide that is capped with a biotin. Regardless of whether any modifications to the Linnarsson's CDS, Hug's linker, or McCloskey's barcode were made that would permit Hug's linker to hybridize to both, the CDS includes a biotin at its 5' end. *Supra*, §VI.A.2.a. As explained above, no molecule could be ligated at the end of a biotinylated nucleic acid. *Supra*, §VI.A.1. Similarly, Hug does not change where the Bts sequence is located on the TSO or add one to the CDS. *See supra*, §§VI.A.1, VI.A.2.a-b. Thus, even if Hug's linkers ligated McCloskey's barcode to the CDS or TSO, (1) the barcode would not be released from the bead for sequencing if ligated to the CDS because there is no Bts sequence, or, (2) if ligated to the TSO, the barcode would still be cleaved off from the portion of the polynucleotide that was sequenced because it is on the 5' end of the Bts sequence. *Supra*, §VI.A.2.a-b.

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4. Dr. Cooper Fails To Show Linnarsson Teaches "Sequencing The Tagged Polynucleotide"

80. In my opinion, Linnarsson does not teach sequencing "the tagged polynucleotide" as required by [1J]. Dr. Cooper asserts that the "tagged polynucleotide" in Linnarsson is the cDNA generated during reverse transcription, and that, in his opinion, these tagged polynucleotides are sequenced. EX1002, ¶130 ("The tagged cDNA is the claimed 'plurality of tagged polynucleotides.'"), ¶¶179-182. In my opinion, Dr. Cooper has not shown this limitation is disclosed by Linnarsson. Dr. Cooper ignores, and therefore does not address, how Linnarsson's sequencing of only *a portion* of each tagged polynucleotide, as opposed to the entirety of the tagged polynucleotides, could satisfy this limitation in his proposed combination. *Supra*, ¶34.

81. Linnarsson teaches that the "amplified library is fragmented to 200 – 300 bp using controlled DNase digestion," which creates 5', 3', and internal fragments. EX1003, 6:3-6, 21:13-25, 27:6-28:2, 35:23-36:9; EX1002, ¶186; EX2018, 43:21-44:22; *supra*, §VI.A.1. The internal fragments are portions of the sample polynucleotide. EX1003, Fig.11. Linnarsson discloses that the "***internal fragments are washed away***" before sequencing. EX1003, 6:3-6; *id.* at Figs.11 (Panels C-E); *supra*, § VI.A.1. As explained above, this is because the internal fragments are not biotinylated, and, as a result, are not bound to streptavidin beads.

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Supra, §VI.A.1. Thus, these portions of the sample polynucleotides are not sequenced.

82. After the internal fragments are washed away, the 5' and 3' fragments remain. The 5' fragments correspond to the portions of the cDNA molecules with the TSO sequences, and the 3' fragments correspond to the portions of the cDNA molecules with the CDS sequences. Both ends of the "tagged" cDNA molecules are biotinylated (at the 5' end of each strand of cDNA), and therefore both end-fragments of double-stranded DNA "are bound to beads" after fragmentation. EX1003, 6:3-6. As explained above, the 3' fragments do not contain a Bts sequence and, as a result, ***are not released from the "beads"*** to which they are bound. EX1003, 6:3-8; *id.* at Figs.11 (Panels C-E); EX2018, 43:21-44:22. Thus, the 3' fragments of the "tagged polynucleotide" also cannot be sequenced in Linnarsson's method because they remain bound to the beads. EX2018, 43:21-44:22. Only the 5' fragment portions of the "tagged polynucleotide" are sequenced. *Supra*, §VI.A.1. Dr. Cooper does not address this aspect of Linnarsson.

83. My paragraphs 81 and 82, immediately above, reflect how a person of skill would understand Linnarsson's STRT method—the method Dr. Cooper relies on. EX1002, ¶¶101, 104, 108, 135, 136, 139, 155, 156, 199, 200 (same); EX2018, 23:15-25:21, 29:14-30:2; EX1003, 5:10-11, 23:1-4, 37:31-32, Fig.11. Linnarsson's STRT method discloses reverse transcribing mRNA to prepare a cDNA library,

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which can then be sequenced using NGS, and Linnarsson does not disclose sequencing without fragmentation (or fragmenting the tagged polynucleotide and then sequencing each resulting fragment). EX1003, 6:3-12 ("fragmentation step"), 12:4-8 ("**The term 'cDNA library' refers to a collection of cloned complementary DNA (cDNA) fragments**…"), 21:13-22 ("the cDNA library can be processed by fragmenting the cDNA library (e.g., with DNase) to obtain a short-fragment 5'end library"), 23:15-20, 26:9-20, 35:16-36:9, Fig.11. Thus, it is my opinion that Dr. Cooper failed to show that Linnarsson discloses "sequencing" the "tagged polynucleotides" in his proposed combination.

84. In Dr. Cooper's deposition, he asserted that Figure 11 is described as "optional steps that can be used within STRT" and that "fragmentation is one optional step" in that process. EX2018, 23:4-14, 30:3-12. I acknowledge the description of Figure 11 as a whole says "optional," but disagree that the *fragmentation* shown in Figure 11 is optional, including because Linnarsson discloses no other way of preparing the cDNA library for sequencing, nor does Dr. Cooper identify any. *Supra*, ¶47. And, to the extent Dr. Cooper is arguing that he showed in his report that Linnarsson teaches sequencing of the entirety of each tagged polynucleotide, I disagree with that as well. First, persons of skill would understand that Linnarsson's consistent disclosure of fragmenting then sequencing indicates that only fragments are sequenced in Linnarsson. (Linnarsson's repeated

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reference to sequencing the 5' fragment, without any disclosure of sequencing any other fragment, further confirms this understanding.) Second, Dr. Cooper's three citations regarding sequencing do not support an argument that Linnarsson teaches sequencing the full length of tagged polynucleotides. EX1002, ¶¶185-186. Linnarsson at 4:8-13 is a description of Figure 1, which is a high-level overview of Linnarson's STRT method.[13] EX1003, 4:8-13. That introductory description does not indicate what steps are taken to sequence *any* cDNA, let alone whether all or some of the tagged polynucleotides are sequenced. EX1003, 4:8-13. Persons of skill would not make assumptions about what is sequenced without reading the *rest* of Linnarsson's description of its STRT method, which, in context, indicates that the sequencing is of just the 5' fragment. Further, Linnarsson at 19:28-32, also cited by Dr. Cooper, explains that the "5' APS can be designed to facilitate downstream processing of the cDNA library" and that it "can be designed to be identical to the primers used in [certain] sequencing methods." EX1003, 19:28-32. But this statement, too, does not indicate that the entire tagged polynucleotide is sequenced or that biotin is optional. Dr. Cooper's final citation in connection with his assertion that Linnarsson discloses sequencing the tagged polynucleotide directly supports my

[13] It also refers to "the tissue of interest." which persons of skill would have understood refers to a eukaryotic multi-cellular organism.

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opinion that the entirety of the tagged polynucleotide is *not* sequenced in Linnarsson or that fragmenting is optional. EX1002, ¶186 (Citing EX1003, 21:13-25. The cited portion of Linnarsson states that the "cDNA library can be processed *by fragmenting the cDNA library* (e.g. with DNase) *to obtain a short-fragment 5'-end library*." EX1002, ¶186 (citing EX1003, 21:13-25). This citation explains that the cDNA library (created after fragmentation, as shown in, *e.g.*, Figure 11) may be further processed (including by additional fragmentation) to make it suitable for high-throughput sequencing. This disclosure similarly fails to indicate the "entire tagged polynucleotide" is sequenced or that fragmentation is optional. Indeed, Linnarsson does not disclose any other approach to processing the cDNA library for sequencing, and its only descriptions include fragmentation. EX1003, Fig.11, 26:9-20 (Example I's "DNase treatment"), 35:17-27 (Example II "was fragmented by DNase 1"); EX2018, 21:21-22:12.

85. Relatedly, Dr. Cooper's theory of ligating McCloskey's barcode to Linnarsson's CDS fail for the additional reason that the CDS is part of the 3' fragment, and Dr. Cooper identifies no teaching in Linnarsson showing the 3' fragment is sequenced. EX1003, Fig.11 (Panel E); EX1003, 6:8; EX2018, 43:21-44:22. Although the 3' fragment (the end of the cDNA with the CDS) is not part of the internal portions that are washed away, it is nevertheless not sequenced. *See* EX1003, Fig.11 (Panel E); EX1003, 6:8 ("3' fragments remain stuck on the beads.").

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And nowhere does Linnarsson describe releasing any 3' fragment from the bead or sequencing it. EX2018, 43:21-44:22. And under Dr. Cooper's "alternativ[e]" theory, Dr. Cooper identifies no way to associate the 3' fragment with the corresponding 5' fragment even if both were released. EX1002, ¶¶136, 170. In my opinion, it is not clear how this would be accomplished based on the approach of Linnarsson. Thus, Dr. Cooper's theory relying on the CDS of the Linnarsson-McCloskey combination fails because any tags in the CDS would be on the 3' fragment, and would not be sequenced.

86. Thus, Dr. Cooper's Ground 1 fails because Dr. Cooper failed to show that Linnarsson (including Linnarsson as modified by McCloskey) teaches sequencing the "tagged polynucleotide" identified by Dr. Cooper.

5. There Are Not Enough Unique Tags In McCloskey To Tag "Each Polynucleotide" In Linnarsson

87. To the extent Dr. Cooper's theory is that the barcode that would be ligated to Linnarsson's CDS or TSO would be 7-nucleotides long, persons of skill would not be motivated to modify Linnarsson to incorporate McCloskey's teachings. EX1002, ¶173. Dr. Cooper's assertion motivation to combine Linnarsson with McCloskey is because, according to Dr. Cooper, McCloskey "provides a more definitive solution" and "solve[s]" Linnarsson's alleged problem of amplification bias. EX1002, ¶173. According to Dr. Cooper, this amplification bias is resolved

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in his theory "[b]y incorporating a second tag in an oligonucleotide that uniquely identifies ***each sample polynucleotide*** prior to amplification." EX1002, ¶173 ("By incorporating a second tag in an oligonucleotide that uniquely identifies each sample polynucleotide prior to amplification, McCloskey's method is able to uniquely account for each individual sample polynucleotide and 'solve' amplification bias"), ¶87 ("***To solve this problem [of amplification bias]… Every sample and the polynucleotides within every given sample are first uniquely tagged before being amplified***…").

88. Dr. Cooper also asserts that "a POSA would have understood that [Linnarsson's] method was designed to be applied to substantially all mRNA molecules of a given cell." EX2022, ¶222.[14] I agree with Dr. Cooper. Persons of skill would have understood that the purpose of the disclosures of Linnarsson on which Dr. Cooper relies, including the design of Linnarsson's tags, and the purpose of Linnarsson's method, is to tag substantially all mRNA molecules of a given cell. For example, Dr. Cooper relies on Linnarsson's disclosures of a CDS with a poly-dT sequence, which would indiscriminately tag the eukaryotic mRNA present in the

[14] While this opinion is included in Dr. Cooper's claim 25 analysis in that proceeding, claim 25 depends from claim 1, and his interpretation of Linnarsson is relevant to the analysis of both claims.

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cell. *See* EX1002, ¶¶104, 105, 107, 108, 110, 112, 136-139, 141, 150, 151, 154, 158, 196, 199-200; EX2002, 222.

89. For these reasons, I disagree that Dr. Cooper's proposed combination would be able to solve this alleged amplification bias problem. In my opinion, persons of skill would not have thought McCloskey's 7-nucleotide-long barcode would provide enough unique combinations to tag "each sample polynucleotide"—all mRNA of a mammalian cell—in Linnarsson's method to eliminate amplification bias as Dr. Cooper argues. EX1002, ¶¶174-175. Thus, persons of skill would not have been motivated to make the modification proposed by Dr. Cooper.

90. In my opinion, McCloskey's 7-nucleotide barcode does not disclose enough unique tags to tag "each sample polynucleotide."[15] McCloskey's disclosed

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[15] Dr. Cooper opined that "Linnarsson's method then tags these sample polynucleotides for the purpose of gene expression analysis. ***A POSA would understand that the purpose of such a method would be to analyze substantially all mRNA molecules of a cell—therefore, this method would provide associating a tag with substantially every sample mRNA molecule. . . . Thus, a POSA would understand that Linnarsson describes a method which is designed to be applied to substantially all mRNA molecules of a given cell***." EX2022, 222. Therefore, I interpret

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barcode is "seven nucleotides" long and uses one of the four natural deoxyribonucleotides in each position. EX1004, 4; *see also* EX1002, ¶176. 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.[16] EX1004, 4 (4^7 = 16,384). For Linnarsson's purposes, a barcode of that length is insufficient to distinguish among each of the vast number of mRNA molecules present in a mammalian cell. "In general, to accomplish such molecular tagging the number of distinct MID tags to be used should be many times greater than the actual number of molecules to be analyzed." EX1001, 22:54-60. Dr. Cooper asserted that one would want "***at least 100 times [more] than the number of sample polynucleotides.***" EX1002, ¶256. For purposes of my opinion, I have assumed this is true (but this does not alter my opinion: even if fewer unique tags

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the "sample polynucleotides" of claim 1 to include "substantially all mRNA molecules of a given cell."

[16] These barcodes are typically generated randomly. *E.g.*, EX1035, 1, 3-4. Persons of skill would understand that this is a theoretical maximum, unlikely to be achieved in practice, and therefore the actual number of unique tags in McCloskey is less than 16,348.

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were needed per polynucleotide, McCloskey's barcode would still not provide enough). *See also*, ¶76.

91. In contrast to McCloskey's analysis of alleles, Linnarsson is focused on analyzing gene expression by measuring and identifying the mRNA present in a cell. EX1004, Abstract. Persons of skill would expect anywhere from 50,000 – 300,000 mRNA molecules to be present in the typical mammalian cell addressed by Dr. Cooper. EX1054, Abstract; EX1002, ¶257. McCloskey's barcodes, which are intended to distinguish between a small set of *alleles* (one to two per cell), would be insufficient to distinguish among each of the vast number of *mRNA* molecules present in such a mammalian (or other eukaryotic) cell. EX1054, Abstract; EX1002, ¶256. If Dr. Cooper's assertion that 100 unique tags would be needed for every sample polynucleotide to be tagged is assumed, for example, then at least 30,000,000 unique tags would be required to have enough to tag the expected mRNA. EX1002, ¶257. Persons of skill would not have known how many mRNA molecules would be present in a cell before the sample is analyzed, and would therefore have used enough unique tags to assure that each mRNA molecule could be tagged even if the

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number of mRNA molecules in the cell were at the high end of the expected range.[17] EX1002, ¶256; EX1054, Abstract.

92. It is also my opinion that tagging the mRNA molecules in Linnarsson's gene expression analysis method using a seven nucleotide barcode as taught in McCloskey would not resolve amplification bias. At most, only a subset of the mRNA molecules could be tagged using the no more than 16,348 barcodes taught by McCloskey. Dr. Cooper argues that resolving amplification bias requires tagging "[e]very sample and the polynucleotides within every sample." EX1002, ¶91. But tagging only a subset of the mRNA molecules with unique tags would not be tagging "each" or "every" polynucleotide. EX1002, ¶¶87, 173. It also would not allow persons of skill to determine the number of molecules in the sample because persons of skill would not have been able to determine which of the *untagged* molecules were original to the sample or were a result of amplification bias.

93. Thus, Dr. Cooper has not shown that persons of skill would have been motivated to modify Linnarsson's method for analyzing *gene expression* to include a barcode where that barcode is deficient for that purpose and there would not be

[17] Based on Dr. Cooper's assumptions, even for the low end of the range, persons of skill would need at least 5,000,000 unique tags, which is far greater than the 16,348 McCloskey's tag may generate.

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enough barcodes to "uniquely identif[y] each sample polynucleotide" as Dr. Cooper claims is the motivation for Petitioner's proposed combination. EX1002, ¶173.

6. Dr. Cooper's Claim 1 Reasonable Expectation of Success Fails

94. In my opinion, to the extent Dr. Cooper's theory is that one would use McCloskey's seven nucleotide barcode, persons of skill also would not have had a reasonable expectation of success in "tagging each polynucleotide" with Dr. Cooper's proposed combination of Linnarsson and McCloskey. EX1002, ¶¶174, 176; EX2022, 222. As explained above, persons of skill would not have had reasonably expected to be able to tag each sample polynucleotide with a different barcode by incorporating McCloskey's barcode teachings into Linnarsson because McCloskey would not have provided enough unique barcodes to do so. *Supra*, §VI.A.5. As discussed above, McCloskey's 16,384 tags are an insufficient number of unique tag sequences to tag the 50,000 – 300,000 mRNA molecules in a cell even under an ideal scenario where each tag hybridizes to a sample polynucleotide. And it is far less than the 5,000,000 – 30,000,000 unique tags that would be required, based on Dr. Cooper's assumptions, to tag each of those mRNA molecules in a real-world application. *Supra*, §VI.A.5.

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B. Ground 2: Linnarsson In View Of McCloskey In View Of McCloskey II Does Not Render Claims 13-18 Obvious

95. Dr. Cooper does not address claim 1 in his Ground 2 theory, and it is thus unclear how the elements of claim 1 are satisfied in the dependent claims of Ground 2. I have been asked to assume that Dr. Cooper's Ground 2 arguments build on his Ground 1 claim 1 analysis. For the reasons I stated in Ground 1, I disagree that Ground 2 renders any claim obvious.

96. Moreover, I note that the motivation Dr. Cooper identifies in his Ground 2 analysis—"to ensure that each sample polynucleotide is tagged with a unique tag sequence"—is the same motivation Dr. Cooper articulated in Ground 1—to "remove amplification bias by introducing barcodes to measure true sample complexity/template diversity. By tagging each sample polynucleotide uniquely, true sample complexity can be analyzed because each unique sample sequence can be identified by the tag and amplification bias can be accounted for rand corrected." EX1002, ¶174. However, Dr. Cooper does not explain why Ground 1 would be further modified or improved.

VII. CONCLUSION

97. In summary, for the reasons described above, I have concluded that each of the Challenged Claims is not invalid as obvious under 35 U.S.C. § 103.

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98. I reserve the right to supplement by opinions in the future and to respond to any arguments that Petitioner or its expert may raise and to take into account any new information as it becomes available to me.

99. I have been warned that willful false statements and the like are punishable by fine or imprisonment, or both. I make this declaration of my own personal knowledge, and all statements made of my own knowledge are true. All statements made on information and belief are believed to be true. If called to testify as to the truth of the matters stated herein, I could and would testify competently.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on May 3, 2024 at Dover, Massachusetts.



John Quackenbush, Ph.D.

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APPENDIX A

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CURRICULUM VITAE

Date: October 31, 2023

Name: John Quackenbush, Ph.D.

Office Address: Department of Biostatistics, 655 Huntington Avenue, Building II, Room 421, Boston, MA 02115

Home Address: 86 Walpole Street, Dover, MA 02030, USA

Work e-mail: johnq@hsph.harvard.edu

Work Phone: +1.617.432.9028

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Mobile Phone: +1.301.526.1740

Date & Place of Birth: January 4, 1962 Kingston, PA, USA

Education:

1983	Physics	B.S.	California Institute of Technology, Pasadena, CA
1984	Physics	M.S.	University of California, Los Angeles, CA
1990	Theoretical Particle Physics	Ph.D.	University of California, Los Angeles, CA

Postdoctoral Training:

1990-1992 Postdoctoral Fellow, Experimental Particle Physics, University of California, Los Angeles, CA

1992-1994 Staff Scientist, Molecular Genetics and Genomics, The Salk Institute for Biological Studies, La Jolla, CA

1994-1996 Research Associate, Genomics and Bioinformatics, Stanford Human Genome Center, Palo Alto, CA

Academic Appointments:

2018- present Henry Pickering Walcott Professor of Computational Biology and Bioinformatics; Chair, Department of Biostatistics; Director, Harvard Health Data Science Center, Harvard TH Chan School of Public Health
Research Activities: Development of methods in network medicine and systems biology aimed at understanding the multifactorial drivers of human health and disease, identifying disease subtypes and new therapeutic interventions, and exploring differences between the sexes in

disease etiology and response to therapy; development of open-source software tools supporting research and clinical applications.
Activities: Founding Director/Co-Director, SM Program in Computational Biology and Quantitative Genetics (CBQG); Director, BD2K T32 Training Grant; Director T32 Cancer Training Grant; Founding Co-Director and Executive Committee, Joint Biostatistics and Bioinformatics T32 Training Grant; Director, Harvard Quantitative Biomedical Research Center.

2005- present Professor of Biostatistics and Computational Biology**,**
Department of Data Science, Division of Computational Biology,
Dana-Farber Cancer Institute;
Professor of Cancer Biology, Department of Cancer Biology,
Dana-Farber Cancer Institute
Research Activities: Application of genomic technologies and integrative computational analysis to model cellular systems with an emphasis on understanding of human cancers and other diseases; development of open-source software tools supporting research and clinical applications.

2005- 2018 Professor of Computational Biology and Bioinformatics**,**
Department of Biostatistics, Harvard TH Chan School of Public Health
Research Activities: Application of genomic technologies and integrative computational analysis to model cellular systems with an emphasis on understanding of human cancers and other diseases; development of open-source software tools supporting research and clinical applications.
Activities: Founding Director, SM Program in Computational Biology and Quantitative Genetics (CBQG); Director, BD2K T32 Training Grant; Founding Co-Director and Executive Committee, Joint Biostatistics and Bioinformatics T32 Training Grant.

2008- 2018 Director, Canter for Cancer Computational Biology,
Dana-Farber Cancer Institute
Research Activities: Integrative approaches to data analysis focused on clinical and translational applications and linking clinical and laboratory data in support of basic research; creation of software tools and systems to support research; overall analytical support for investigative research activities for all Dana-Farber Investigators.

2007 – present Adjunct Professor of Bioinformatics, Boston University
Activities: Mentoring PhD student thesis research.

2003- 2005 Professor, Chemical Engineering, University of Maryland
Activities: Mentoring PhD student thesis research.

2002 - 2006 Investigator, The Institute for Genomic Research
Research Activities: Development and implementation of technology and strategies, including computational methods and software tools,

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necessary for functional analysis of the human and other genomes. Analysis of human gene expression in colon tumors using microarrays, rodent models of heart, lung, blood and sleep disorders and gene expression in Arabidopsis. Director of the TIGR Gene Index Project, providing freely available databases and software to estimate gene content in more than 100 organisms.

2000- 2005 Professor, Department of Biochemistry,
The George Washington University
Activities: Instructor in various courses, curriculum committee for Genomics.

1998 – 2005 Lecturer, The Department of Biostatistics,
The Johns Hopkins University
Activities: Instructor in the Masters In Biotechnology Program

1997 - 2001 Associate Investigator, The Institute for Genomic Research
Research Activities: Development and implementation of technology and strategies, including computational methods and software tools, necessary for functional analysis of the human and other genomes. Analysis of human gene expression in colon tumors using microarrays, rodent models of heart, lung, blood and sleep disorders and gene expression in Arabidopsis. Director of the TIGR Gene Index Project, providing freely available databases and software to estimate gene content in more than 50 organisms.

1997 Assistant Investigator, The Institute for Genomic Research
Research Activities: Development and implementation of technology and strategies, including analytical methods and software tools, necessary for functional analysis of the human and other genomes.

1994 - 1997 Research Associate, Stanford Human Genome Center
Stanford University
Research Activities: Project leader for development and implementation of a transposon-mediated strategy for large-scale genomic DNA sequencing including development and implementation of laboratory protocols, analytical methods, computer software, and instrumentation. Mapping, sequencing, and annotating regions of human chromosomes 4 and 21.

1992 - 1994 Staff Scientist, The Salk Institute for Biological Studies
Research Activities: Development of improved methods for DNA sequencing, combinatoric strategies and devices for screening large libraries, simulation and optimization of single pass sequencing strategies including genome sequence sampling. Project director for the STS content mapping of chromosome 11. Development of methods and software tools to support genome mapping.

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1990 - 1992 Postdoctoral Fellow, Department of Physics
University of California, Los Angeles
Research Activities: Research in particle physics, field theory and phenomenology. Founding member of the Antiproton Experiment (APEX) collaboration that set the world's best experimental limit on the lifetime of the antiproton.

1987 - 1992 Visiting Lecturer, Department of Physics
University of California, Los Angeles

1984 - 1996 Physics Instructor, Southern California Science Institute
New College of California

1983 – 1990 Teaching Fellow, Department of Physics
University of California, Los Angeles

1983 - 1990 Research Fellow, Department of Physics
University of California, Los Angeles
Thesis Supervisor: E.T. Tomboulis
Research Activities: Research in elementary particle physics, mathematical physics, field theory focusing on the development of two-dimensional gauge field theory models and the construction of associated string theory models.

Nonacademic Employment

2011-2014 Co-Founder, CEO and Board Chairman, Genospace LLC
Activities: Genospace provides software solutions to operationalize precision medicine through the integration of clinical and genomic data and its presentation to clinical and research users in an intuitive format.

2014-2017 Co-Founder and Board Chairman, Genospace LLC
Activities: Genospace provides software solutions to operationalize precision medicine through the integration of clinical and genomic data and its presentation to clinical and research users in an intuitive format. In January 2017, Genospace was sold to the Hospital Corporation of America to support research and to acquire our world-leading solution for clinical trials matching.

Hospital or Affiliated Institution Appointments:

2003 – 2005 Adjunct Professor, Department of Biostatistics
Bloomberg School of Public Health, The Johns Hopkins University

2005- present Professor, Department of Biostatistics and Computational Biology
Dana-Farber Cancer Institute

2014- present Professor, Channing Division of Network Medicine, Brigham and Women's Hospital

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Hospital and Health Care Organization Service Responsibilities:

2005- present Primary research areas are computational and system biology focused on the integration of diverse data types to provide insight into biological processes that drive human disease phenotypes.
Department of Data Science, Division of Computational Biology, Dana-Farber Cancer Institute

2014- present Research at the interface of systems biology and pulmonary disease, exploration of how sex and gender affect disease risk, development of methods to better handle sex differences in systems approaches, and advising faculty in their research and the Director in setting directions for future work.
Channing Division of Network Medicine, Brigham and Women's Hospital

Major Committee Assignments:

1994	National Laboratory Genome Project Visit Review Panel, DOE
1995	Caltech/BAC Library Construction Site Visit Review Panel, DOE
1996-1998	Bioinformatics Grant Review Panel, DOE
1997-2001	Grant Review Panel, DOE
1997	Hollander Fellowship Review Panel, DOE
1997	Five-Year Program Advisory Committee, NCRR
1997	Cancer Chromosome Anatomy Project program Steering Committee, NCI
1998	National Laboratory Functional Genomic Review Panel, DOE
1998	Full-Length cDNA Library Construction and Sequencing Advisory, Committee, NCI
1998	Functional Genomics Panel, Welcome Trust
1998	Genomics Grant Review Panel, NSF SBIR
1998	Special Program in Tropical Disease Research Review, UNDP/World Bank/WHO
1999-2000	Low-Dosage radiation Grant Review Panel, DOE
1999	Bioinformatics Review Panel, NSF
1999	Special Emphasis Review Panel, NHLBI
1999	Microarray Working Group Advisory Panel, NIDCR
1999	Special Emphasis Review Panel, NIMH
1999	Plant Biology Review Panel, NSF
1999-2007	Board of Directors, MGED
2000	Grant Review Panel, NIDA SBIR
2000	Plant Biology Review Panel, USDA
2000	Plant Biology Review Panel, NSF
2000	Exceptional Chromosome Regions Working Group, DOE
2000	Working Group on US Scientific Interactions, NSF
2000-2006	Genome/GCAT study section, NIH
2000-2003	PGA Coordinating Committee, HHLBI
2000-2003	PGA Bioinformatics Committee, NHLBI

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2000-2003	PGA Microarray Committee, NHLBI
2000	Working Group on Australia Scientific Interactions, NSF
2001	Review Meeting RFP ES-01-01 National Center for Toxicogenomics (NCT) Microarray Resource, HIEHS
2001-2003	Plant Genome Scientific Advisory Board, University of Georgia
2001-2003	Innovative Technology Review Panel, NCI
2001-2002	Genome Study Section, NIH
2002-2006	GCAT Study Section, NIH
2001	Bioinformatics in Neuroscience and Sleep Research Workshop, NIDDK
2001	Plant Genome Site Visit Panel, NSF
2001-2003	BISTI Review Panel, NIH
2001-2005	Grant Review Panel, NCI
2002	Microarray Supplement Review Panel, NHLBI
2002-2006	Panel on Emerging Issues in Toxicogenomics, NRC
2004-2006	Panel on Applications of Toxicogenomics, NRC
2003-2010	Genome British Columbia Scientific Advisory Board
2005-2014	EPA Board of Scientific Counselors
2005-2011	St. Jude's Children's Research Hospital Scientific Advisory Board
2006-2007	Harvard School of Public Health, Lefkopoulou Award Committee Member
2006	Dana Farber Cancer Institute, Computational Biology Search Chair
2005	EU FP6 Grant Review Panel
2006-2007	Harvard School of Public Health, Seminar Committee Member
2006-2019	Harvard School of Public Health, Curriculum Committee Member
2006-2009	Harvard School of Public Health, Computational Biology Search Chair
2007-present	EU FP7 Genomics Grant Review Panel
2008-present	Lovelace Respiratory Research Institute, Scientific Advisory Board
2008-2010	NAS Panel on Collecting, Storing, and Distributing Biodata linked to Social Science Surveys
2009	RC1 Grant Review "Editorial Review" Panel
2009	RC2 Genomic Science Grant Review
2009	Epigenomics Roadmap Grant Review Panel
2009-2014	Epigenomics Roadmap Advisory Board
2010	NCI EUREKA Grant Review Panel
2010	Chair, NCRR S10 Grant Review Panel
2016	NASA ISS Genomic Applications Study Section
2019	NASA Human Research Program Long-Duration Flight Advisory Panel
2016	21st Century Cures Act Biomarker Advisory Group
2012-2019	Research Oversight Committed, Genome British Columbia
2014-present	Shriner's Hospital standing review panel
2005-present	Various NIH grant review panels, either as an ad hoc member or as an ad hoc reviewer. I typically serve on at least six per year and can provide a more detailed list on request.
2005-present	Various EU and Canadian grant review panels, either as an ad hoc member or as an ad hoc reviewer. I can assemble a detailed list on request.
2017-present	Research Foundation Flanders (FWO) standing review panel.
2018-present	Chair, Department of Biostatistics, Harvard T.H. Chan School of Public Health

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Nonacademic Board and Advisory Board Positions (active or past five years):

2005-2016	Nabsys Inc., Scientific Advisory Board
2010-2017	Lovelace Respiratory Research Institute, Scientific Advisory Board
2012-2016	SynapDx, Scientific Advisory Board
2012-2017	Genospace LLC, Founder, CEO, Board of Directors; Chairman of the Board
2012-2020	Blueprint Genomics, Scientific Advisory Board
2013-2017	Thomson-Reuters, Genomics Advisory Board
2013-present	Caris Life Sciences, Scientific Advisory Board
2020-present	Caris Life Sciences, Data Science Advisory Board
2014-present	Shriner's Hospitals, Scientific Advisory Board
2014-present	Olema Pharmaceuticals, Scientific Advisory Board
2015-present	Haymakers for Hope, Board of Directors
2015-present	Lacriscience, Scientific Advisory Board
2017-present	Merck KGaA, Precision Medicine Advisory Board
2018-present	Renalytix AI, Advisory Board
2018-present	Dover, MA, Capital Budget Committee
2019-present	StitchBio, Scientific Advisory Board
2020-present	Charles River School Health and Safety Committee (COVID Advisory Board)
2020-present	Boston University Academy Health and Safety Committee (COVID Advisory Board)
2021-present	Dover, MA, Board of Health Special Advisor
2021-present	Congreso Futuro International Advisory Board, Chile

Professional Societies:

1988	American Physical Society	Member
1988	APS Biophysics Division	Member
1988	APS International Physics Group	Member
1988	APS Division of Particles and Fields	Member
1988	World Federation of Scientists	Member
1990	Microarray Gene Expression Data Society (MGED)/ Functional Genomics Data Society (FGED)	Member
		Board Member
	(2011-2014)	President
1995	The Human Genome Organization (HUGO)	Member
1996	Association of Science Professionals (ASP)	Member
1998	American Association for the Advancement of Science (AAAS)	Member
1999	Center for the Study of the Evolution of Life (CSEOL)	Member
2005	American Association for Cancer Research	Member

Community Service Related to Professional Work:

I have presented more than 350 talks, courses, and workshops since joining Dana-Farber and the Harvard T.H. Chan School of Public Health in 2005; detailed lists will be

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provided upon request. I have also taught numerous courses and workshops on the analysis of genomic data and have served as an organizer of the EMBO course on DNA microarray analysis for more than five years.

In addition, I have served on the Scientific Advisory Board of the St. Jude's Children's Research Hospital, the Scientific Advisory Board for the Lovelace Respiratory Research Institute, Research Oversight Committees for various Genome Canada programs, and the Board of Scientific Counselors of the Environmental Protection Agency. I have also been a member of advisory boards for a number of NIH initiatives, including the Roadmap Epigenome Project, the Extracellular RNA Communication Consortium, the Atopic Dermatitis Research Network (ADRN), and the Trans-Omics for Precision Medicine (TOPMed) program.

Since the onset of the COVID19 pandemic, I have served numerous local school and community groups by advising these on policy and practice. I am particularly proud that the two schools I advised instituted a series of policies addressing air quality, masking, distancing, hygiene, and vaccination that allowed them to remain open without any documented cases of in-school transmission during the 2020-2021 and 2021-2022 academic years. I have also become a special advisor to the Dover, MA Board of Health and provide advice to the Dover-Sherborn Regional School District on COVID and other heath-related matters.

(Peer Review for Scientific Journals)

1994-2016	*Genomics*
1994-2009	*BioTechniques*
1996-2010	*Genome Research*
2000-2010	*Genome Biology*
1997-1998	*Analytical Biochemistry*
1997-present	*Nature*
1997-2010	*Nucleic Acids Research*
1999-2010	*Nature Genetics*
1999-2009	*EMBO Reports*
1999-2017	*Nature Biotechnology*
1999-present	*Science*
2000-2016	*Bioinformatics*
2000-2009	*Journal of Theoretical Biology*
2000-2009	*Mammalian Genomics*
2000-2006	*Gene*
2000-2016	*BMC Genomics, BMC Bioinformatics*
2001-2009	*Structural and Functional Genomics*
2001-2010	*Nature Reviews Genetics*
2001-2006	*American Journal of Applied Statistics*
2009-present	*PLoS Computational Biology*
2015-present	*Proceedings of the National Academy of Sciences*
2017-present	Molecular Systems Biology

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Editorial Boards:

2000-2009	Editorial Board	*BioTechniques*
2000-2012	Associate Editor	*Bioinformatics*
2000-present	Editorial Board	*BMC Genomics*
2000-2006	Editorial Board	*Genomics*
2006-2017	Editor-in-Chief	*Genomics*
2018-present	Editorial Board	*Cancer Research*
2022-present	Editorial Board	*GEN Biotechnology*

Awards and Honors:

1985 - 1991	**Outstanding Teaching Award (7)**, UCLA Physics Department
1986	**Jun John Sakurai Scholarship**, 24th International School of Subnuclear Physics, Ettore Majorana Centre for Scientific Culture, Erice, Italy
1987	**UCLA Distinguished Teaching Assistant Award**, UCLA Academic Senate
1987	**Prize for Best Student** and **Prize for Best Scientific Secretary**, 25th International School of Subnuclear Physics, Ettore Majorana Centre for Scientific Culture, Erice, Italy
1988	**Graduate Distinguished Scholar Award**, UCLA Alumni Association
1992 - 1997	**SERCA Fellow**, National Human Genome Research Institute
2006	**George D. Wilbanks Lectureship in Gynecological Oncology**, University of South Florida College of Medicine
2007	**President's Distinguished Lectureship**, American Society of Reproductive Medicine
2007	**Leopold G. Koss Lectureship**, Universität Bern, Switzerland
2007	**Distinguished International Advisors** at IEE BIBE, the original and the oldest IEEE flagship international conference in Bioinformatics and Bioengineering.
2008	**Distinguished Lecture in Computer Science**, Wayne State University
2010	**IPM Distinguished Lecture**, Mt. Sinai Medical School, New York
2010	**The Ian Lawson Van Toch Memorial Seminar Series in Computational Biology**, Ontario Cancer Institute
2010	**Harvard-Australia Foundation Fellowship**
2010	**Bancroft Fellow-in-Residence**, Queensland Institute for Medical Research, Queensland, Australia
2010	**Jackson Memorial Fellowship**, Griffith University, Queensland, Australia
2011	**Australia Fellow**, National Health and Medical Research Council, Australia
2013	**White House Open Science Champion of Change**, Office of the President of the United States of America
2016	**Mentor of the Year Award**, Postdoc and Graduate Student Organization, Dana-Farber Cancer Institute
2018	**National Cancer Institute Outstanding Investigator Award** (R35)

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2022 **Elected Fellow, National Academy of Medicine**

Part II: Research, Teaching, and Clinical Contributions

A. Narrative report (*700 words or less*) of Research, Teaching, and Clinical Contributions. Please focus primarily on the areas in which most of your time and effort is spent.

My current research involves development of new analytical methods for the integrated analysis of large-scale biomedical data, including genomic data, to better understand the nature of human diseases, including cancer and pulmonary diseases, with the goal of developing better methods for diagnosis and treatment. Two defining characteristics of my work are a commitment to reproducible research through development of open-source software implementations of analytical methods, and an emphasis on studying sexual dimorphism in health and disease.

A longstanding component of my work is methods development for robust biomarkers. We have applied this to identifying reproducible predictive biomarkers in cancer and have explored reproducibility in large, published data sets, demonstrating that many are inconsistent. In collaboration with Hugo Aerts, I have been working to extend our methods to the analysis of quantitative feature data extracted from radiographic images (an emerging field called "radiomics"), to use these to identify predictive biomarkers, and to link these features to genetic mutations in cancer. I have also come to recognize that while individual "features" such as the expression of genes or specific mutations may not be sufficient on their own to identify drivers of biological states or to predict outcome or response to therapy in disease.

I have also been developing methods for inferring gene regulatory network models using gene expression and other sources of data. We published a method, PANDA, that builds on "message passing" (an idea developed communications theory) to optimize information flow through a gene regulatory network model, allowing us to infer condition-specific networks and to compare these between phenotypes. We have developed many extensions of PANDA that incorporate other data types and have applied these to the study of diseases, including ovarian cancer, colorectal cancer, chronic obstructive pulmonary disease, asthma, and Alzheimer's disease, as well as to the investigation of sexual dimorphism. Consistent with our commitment to open and reproducible science, we have collected these methods (all named for animals) with standardized source code in R, C, Matlab, and Python, into the "Network Zoo" (https://netzoo.github.io/). To support the widespread use of these methods, we developed "netBooks" (http://netbooks.networkmedicine.org/), a collection of online tutorials that use Jupyter notebooks to introduce the use of our methods and, increasingly, to allow all of our published analyses to be rerun by those interested in the analyses.

We have also been exploring the connection between genotype and phenotype, using gene expression as a surrogate for phenotype. We have developed a new approach, CONDOR, to the analysis of expression quantitative trait loci that uses a bi-partite graph to represent associations between gene expression levels and both local (*cis-acting*) and

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distant (*trans-acting*) significant genetic variants. We find that the overall structure of the graph provides insight not only into how genetic variants work together to influence phenotype, but also into evolutionary constraints on the genetic variants themselves. This has given us new insight into how many genetic variants of small effect-size can collectively exert strong influence on phenotype.

More recently, we have been asking whether how one can model regulatory networks in the context of genetic background, recognizing that an individual's genotype will manifest its effects through alterations of patterns of gene expression that ultimately will influence the functional processes active in individuals. That method, EGRET, represents an important step forward in the analysis of the link between genotype and phenotype and is the first method for linking genetic cause and phenotypic effect through the action of individual regulatory processes.

Our group also builds and maintains a variety of software tools to support genomic analysis. the TM4 suite of open-source software tools for the analysis of DNA microarray data. The most widely used of these, MeV, is a powerful data mining software tool, that places sophisticated analytical tools behind an intuitive graphical user interface. Developed to be accessible to laboratory biologists, MeV has more than 260,000 users worldwide. We recently transitioned MeV to a cloud-based platform, WebMeV (http://mev.tm4.org) to take advantage of cloud scalability to better handle large sequence-based data. WebMeV now has *5000* registered users and provides more than 200 analysis sessions per day.

I am also very involved in teaching and mentoring. I developed, launched, and direct the Computational Biology and Quantitative Genetics (CBQG) master's degree program, I was co-PI and co-director of the first joint Biostatistics and Bioinformatics training grant in the US and recently stepped down to develop and direct a training grant program as part of the NIH's Big Data to Knowledge (BD2K) program. I also developed and teach an introductory genomics tools and methods course and have co-taught an online course on reproducible research through HarvardX (https://www.edx.org/course/principles-statistical-computational-harvardx-ph527x).

I have trained more than 80 postdoctoral fellows and graduate students (including MS and PhD students whom I have mentored for their thesis projects) and have mentored a number of junior faculty members since joining the Harvard TH Chan School of Public Health and Dana-Farber Cancer Institute in 2005. I have emphasized diversity in hiring postdocs; more than half of my trainees have been female and four of the five postdocs from my group who have moved onto faculty positions in the past year have been female. I also maintain an extensive network of former trainees (and their current trainees) which meets weekly as we continue to work collaboratively in the development and application of network methods.

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B. Funding Information

I have had more than twenty-five years of continuous grant support from the NIH and other organizations. My current support includes:

Active Research Support

5R01CA205406-03 (Ng, K.) **3/1/2017 – 2/28/2022**

NIH/NCI

Novel Randomized Controlled Trials of Vitamin D Supplementation in Patients with Colorectal Cancer: Impact on Survival and Biology

This project is exploring the biology underlying the effects of vitamin D on survival of colorectal patients.

Role: Co-Investigator

1R35CA220523 (Quackenbush, J.) **9/1/2018 – 8/31/2025**

NIH/NCI

Unraveling the Complexities of Risk and Mechanism in Cancer

This NCI Career Award supports Dr. Quackenbush's ongoing work to model genetic processes in cancer and to elucidate the link between genotype and phenotype so as to better understand the drivers of cancer and to identify potential targets for therapeutic intervention, with a particular emphasis on understanding differences between men and women. Role: Principal Investigator

1R01HG011393 (DeMeo, D / Quackenbush, J.) **9/1/2018 – 06/30/2025**

NIH/NCI

Network Tools to Understand Sex- and Gender-Specific Drivers of Disease

This project will develop robust methods for handling male and female multi-omic data, with an emphasis on applying these to the inference of gene regulatory network models and the use of such models for gaining insight into the regulatory processes that drive clinical differences in human health and disease. Role: co-Principal Investigator

1U24CA231846-01A1 (Quackenbush, J.) **9/1/2019 – 8/31/2024**

NIH/NCI

MeV: A Robust Platform for Intuitive Genomic Data Analysis

As part of the Informatics Technology for Cancer Research (ITCR) program, we will develop and support WebMeV, a robust, scalable data analysis software tool that uses intuitive visual interfaces to provide users with access to advanced data analysis methods for large-scale genomic data in a system that helps ensure their research is reproducible.

Role: Principal Investigator

2P50CA127003-11A1 (Bass, A.) **9/1/2019 – 5/31/2024**

NIH (Subcontract: Dana Farber Cancer Institute

Dana Farber/ Harvard Cancer Center SPORE in Gastrointestinal Cancer

The GI SPORE supports state-of-the-art investigator-initiated translational research that will contribute to improved prevention, early detection, diagnosis, and treatment of gastrointestinal cancers. Dr. Quackenbush will serve as Co-Director of the Biostatistics and Bioinformatics Core, which will support the quantitative needs of the investigators in the design of experiments and the analysis of the resulting data.

Role: Co-Investigator/Core Co-Director

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C. Report of Current Research Activities other than those mentioned above (bench research, clinical trials, outcome studies, efficacy studies as applicable)

None to report.

D. Report of Teaching (use only those categories that are applicable)

1983 - 1990	Teaching Fellow, Department of Physics, University of California, Los Angeles
1984 - 1996	Physics Instructor, Southern California Science Institute, New College of California
1987 - 1992	Visiting Lecturer, Department of Physics, University of California, Los Angeles
1998 - 2005	Lecturer, The Johns Hopkins University Instructor in the Masters in Biotechnology Program
2000 - 2005	Professor, Department of Biochemistry, The George Washington University
2003 - 2005	Adjunct Professor, Department of Biostatistics, Bloomberg School of Public Health, The Johns Hopkins University Developed program in genomics and bioinformatics; instructor in various courses.
2005-present	Professor, Department of Biostatistics, Harvard TH Chan School of Public Health
2013-present	Director, MS in Computational Biology and Quantitative Genetics

E. Report of Clinical Activities

1. Description of clinical practice (field, areas of major focus, site(s) of practice [private office, HMO, teaching hospital etc.])

None

2. Patient load (indicate complexity of cases, as appropriate)

None

3. Clinical contributions (e.g., introduction of new methods of clinical diagnosis, prevention, treatment, care delivery)

None

4. Other relevant information about clinical role (receipt of clinical awards, locally or nationally, invitation to participate in clinical activities at other sites, special recognition by peers or professional organizations as a leader in a clinical field)

None

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Part III: Bibliography (as of October 31, 2023)

Total citations: 93,116

h-index: 110

i10-index: 308

Original Articles (325)

Complete List of Published Works in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/john.quackenbush.1/bibliography/public/
(Note that this list includes publications by students funded on training grants on which I am a PI.)

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Educational Material: I have taught numerous courses and workshops and make the materials freely available. I developed and co-taught a HarvardX course on reproducible research (https://www.edx.org/course/principles-statistical-computational-harvardx-ph527x).

Thesis: Gauge Field Theory in Two Space-Time Dimensions: Anomalies and Applications to String Models.

Nonprint Materials, i.e., film strips, films, videotapes and computer-based materials relevant to appointee's academic field. *Include description of item, who commissioned, purpose, users, penetration in field.*

None

Patents

None

Abstracts (*only those containing data not yet published in complete form.)*

None

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Cases in which I have testified at deposition or trial.

To the best of my knowledge, this is a complete list. The parties with whom I was engaged as an expert are shown in bold.

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33. ***HYSEQ, Inc****. v. Affymetrix, Inc.*, Case No. 5:00-cv-20050-JF (N.D. Cal.)

Cases in the past five years for which I have submitted sworn declarations in the past five years, but not testified at deposition or trial.

To the best of my recollection, this is a complete list. The parties with whom I was engaged as an expert are shown in bold.

1. ***Foresight Diagnostics Inc.*** *v. Personalis Inc.,* Petitions for Post-Grant Review Case No. IPR2023-00545
2. ***Foresight Diagnostics Inc.*** *v. Personalis Inc.,* Petitions for Post-Grant Review Case No. IPR2023-00546
3. *Illumina, Inc., et al. v.* ***BGI Genomics Co****., Ltd., et al*., Case Nos. 3:19-cv-03770-WHO and 3:20-cv-01465-WHO (N.D. Cal.)
4. ***10x Genomics*** - Inter partes review of U.S. Patent No. 10,190,115, Docket No.: IPR2021-00133