In the Matter Of:

Parse Biosciences, Inc. v

10X Genomics, Inc.

JOHN QUACKENBUSH, PH.D.

February 28, 2024



UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC., Petitioner,	)	
v.	)	Case No. IPR2023-00876
10X GENOMICS, INC., Patent Owner.	)	

- - -

Wednesday, February 28, 2024

- - -

Videotaped deposition of JOHN QUACKENBUSH, Ph.D. was taken before Elizabeth M. Kondor, Certified Court Reporter and Notary Public, on the above date, commencing at 9:05 a.m.

A P P E A R A N C E S:

WEIL GOTHAL & MANGES, LLP
BY: EDWARD R. REINES, ESQ.
201 Redwood Shores Parkway
Suite 400
Redwood City, California 94065
edward.reines@weil.com
(650) 802-3000
Appearing on behalf of the Petitioner,
Parse Biosciences Inc.

GROOMBRIDGE WU BAUGHMAN & STONE, LLP
BY: MEGAN F. RAYMOND, ESQ.
JOSHUA REICH, ESQ. (NY)
801 17th Street NW
Suite 1065
Washington, DC 20006
megan.raymond@groombridgewu.com
joshua.reich@groombridgewu.com
(202) 505-5878
Appearing on behalf of the Patent Owner,
10x Genomics, Inc.

ALSO PRESENT:
JONATHAN PERRY, Videographer

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VIDEOGRAPHER: We are now on the record. My name is Jonathan Perry. I'm a videographer retained by Lexitas.

This is a video deposition for the United States Patent and Trademark Office, before the Patent Trial and Appeal Board, IPR No. 202300876.

Today's date is February 28, 2024. The time is approximately 9:05 a.m.

We are at the offices of Weil Gotshal & Manges, 2001 M Street Northwest in Washington, D. C.

This is being taken in the matter of Parse Biosciences, Incorporated versus 10x Genomics, Incorporated.

The name of the witness is John Quackenbush, Ph.D.

And would counsel present please introduce themselves and state whom they represent.

MR. REINES: Ed Reines, Weil, Gotshal, Parse.

MS. RAYMOND: Megan Raymond from Groombridge Wu Baughman & Stone for 10x. With me is Josh Reich.

VIDEOGRAPHER: And the court reporter is Elizabeth Kondor, also with Lexitas.

Would you please swear in the witness.

J O H N Q U A C K E N B U S H, P h. D., having been first duly sworn, testified as follows;

EXAMINATION BY MR. REINES:

Q. Please state your name for the record.

A. My name is John Quackenbush, Q-U-A-C-K-E-N-B-U-S-H.

Q. You understood that today's deposition is to address your opinions given in this case?

A. Yes, I do.

Q. And you did your best to prepare for the deposition; is that correct?

A. Yes, I did.

Q. What did you do?

A. I reviewed the document cited in my report, the expert report. I had a meeting yesterday with the attorneys representing 10x, and had, I believe, one short phone or Zoom conference with Josh on Friday and then a

half-hour Zoom conference with him on Monday.
Q. How much time have you invested in being prepared for the deposition?
A. So I was here most of the day yesterday. And then spent some time over the weekend and time in the week leading up. I don't know exactly how long.
Q. More or less than 30 hours?
A. Probably less, but I'd have to look.
Q. More or less than 20 hours?
A. Probably more, but, again, I'd have to take a look.
Q. So your best estimate currently sitting here now is that you spent about 20, 30 hours preparing for the deposition; is that correct?
A. That's a reasonable guess, but the exact tally, I don't know.
VIDEOGRAPHER: Off the record at 9:10.
(Off the record.)
VIDEOGRAPHER: On the record at 9:11.
BY MR. REINES:
Q. I'd like to show you Exhibit 2013, which is your declaration.

How much time did you expend preparing the opinions in this declaration and the declaration itself?
A. Sitting here today, I couldn't tell you.
Q. What's your best estimate?
A. I don't know. Probably more than 40 hours, but I couldn't tell you. I'd have to look back at my records. A lot of this came together at a very busy time of year.
Q. When did you first receive the assignment to work on this declaration?
A. I don't recall the exact date.
Q. What's your best estimate?
A. I couldn't give you an estimate. I really don't know.
Q. Was it in 2023?
A. It was almost certainly at some point in 2023.
Q. Was it before or after the summer of 2023?
A. So I was contacted by the attorneys in Groombridge and asked if I would be willing to work on this. I really started working on this report, though, after Dr. Cooper's --

sometime after Dr. Cooper's report, I believe after his deposition, although I don't know exactly when, because this report is a response to Dr. Cooper's report in this PTAB matter.
Q. When you started working on your opinions in this case set forth in your declaration, did you have the Cooper deposition transcript?
A. I don't recall exactly when I started working. I don't recall when Dr. Cooper was deposed.
In putting this together, I did have access to Dr. Cooper's deposition transcript, but I don't know at what point I received it in the process.
Q. If you turn to page 7 of your declaration, please.
And do you see the section on "Obviousness" setting forth the legal standard?
A. Yes, I do.
Q. Where did you receive this information?
A. So I'm not an attorney or a patent agent, and so I was instructed as to the meaning of obviousness by the attorneys representing

10x.
Q. When did that happen?
A. I don't recall the exact date. It happened early in our discussions.
Q. Was this after you received the Cooper report?
A. I don't recall exactly when. I've worked as an expert witness a number of times and I'm familiar with the concept of obviousness, in anticipation. But this particular set of instructions reflect the instructions and information I was provided by the attorneys from Groombridge.
Q. What was the assignment that you received in this case?
A. So Dr. Cooper had filed a motion before the PTAB, and I was asked to respond.
So now thinking back, I may have submitted earlier declarations. I don't recall -- I don't recall the earlier work I did, but the assignment for this report in particular was a response to Dr. Cooper's report that was filed before the PTAB.
Q. And in terms of how you responded in terms of what portions of Dr. Cooper's report

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you contested and the basis for contesting it, was that from you or was that from the attorneys?

A. So I had the opportunity to read through Dr. Cooper's report to try to understand it to the best of my ability, given what he was presenting; to read over the patents since he was stating that elements of the patent were rendered obvious; to look at the references that Dr. Cooper cited. And then I had a series of discussions with the attorneys about the different theories that Dr. Cooper had presented about the obviousness of the patent given the references that he cited.

Q. In terms of how you responded to Dr. Cooper's report regarding what you contested and the basis for contesting it, did that come from you or did that come from the attorneys?

MS. RAYMOND: Objection. I just caution the witness not to reveal the substance of conversations with attorneys.

A. It arose during discussions with the attorneys about what was represented in Dr. Cooper's report. We spent a lot of time trying to figure out together what Dr. Cooper

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meant when he advanced certain ideas. There are places in his report that we struggled to try to make sense of.

Q. So you didn't understand Dr. Cooper's theories in total, correct?

MS. RAYMOND: Objection to form.

A. I wouldn't characterize it that way. To the best of my ability, I analyzed what Dr. Cooper presented. There are places where Dr. Cooper seems to leave things out, and so, to the best of my ability, I tried to respond to that.

And in preparing my responses, I actually looked at a variety of different alternatives and presented those in different subsections of round 1 as I really tried to go through and understand the potential arguments that Dr. Cooper was making.

Again, there are places where Dr. Cooper I think has incomplete representations of how certain references including Linnarsson and McCloskey might be combined.

Q. Was there any constraint put on you in how you responded to Dr. Cooper's report by

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the attorneys?

A. I'm not sure I understand the question.

Q. Were there any limits? Is there anything that you wanted to include or paths you wanted to follow that you were told not to?

A. No.

Q. And who decided which part of Dr. Cooper's report you would be contesting and which you wouldn't be contesting?

MS. RAYMOND: Objection.

I just caution the witness not to reveal the substance of communications with the attorneys in preparing this report.

A. So I'm not sure -- I'm not sure what you're asking.

I don't contest Dr. Cooper's current employment or where he's working. It was really looking at the substantive arguments he made that I tried my best to respond to.

Q. Who decided whether you contested particular substantive arguments made by Dr. Cooper or not?

A. I believe we responded to all the substantive arguments that Dr. Cooper advanced.

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Q. Okay.

Dr. Cooper's report includes a number of opinions, correct?

A. You might characterize it that way.

Q. There's -- Dr. Cooper's report has opinions with bases and grounds in it, correct?

A. That is correct, yes.

Q. Okay.

And some of them you contest and some of them you don't contest, right?

A. I don't recall grounds that we didn't -- or arguments that Dr. Cooper made that I didn't contest. If you'd like to point me to those in Dr. Cooper's report, I'd be happy to refresh my memory.

Q. Okay.

So you think it may be that you contested every point that Dr. Cooper made in his report?

A. Again, I don't have Dr. Cooper's report in front of me. I think I responded to the substantive arguments that Dr. Cooper made.

Q. My question is, do you believe you contested every point, substantive point made by Dr. Cooper?

PARSE EX. 1057.005

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MS. RAYMOND: Objection to form.
A. Sitting here today, I don't know what you would characterize as a substantive point that I didn't respond to. And I believe I reserved my right to respond to -- to amend my report if there was anything that I missed.
Q. Is there any aspect of Dr. Cooper's substantive analysis with which you agreed?
A. If you can point me to different places in my report where I stated that, I'd be happy to discuss it.
There are -- you know, I don't know what you would consider substantive and places where you believe I didn't contest Dr. Cooper's opinions.
Q. Okay.
So you're not comfortable saying that there's any point of substance that you agree with Dr. Cooper on without going through your report?
MS. RAYMOND: Objection to form.
A. I don't understand what you consider to be of substance, and so I really can't provide you with an informed opinion.
I believe I responded to Dr. Cooper's

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substantive arguments and provided grounds for my analysis.
Q. And the reason you can't answer my question is because you don't understand what I mean by "substance"; is that correct?
A. You could have -- you could consider some things substantive. I don't know -- I don't know what you're considering.
Q. All right.
If you turn to paragraph 19 of your report.
Do you have that in front of you?
A. I do, yes.
Q. You understand that these are the four factors that are the part of the obviousness determination?
A. Yes, that's my understanding.
Q. And in terms of the fourth factor, objective indicia, what did you do to investigate whether there was objective indicia supporting your position?
A. So to the best of my recollection, I didn't offer any opinions on objective indicia. I don't believe Dr. Cooper provided any arguments regarding that.

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I respond to Dr. Cooper's arguments about obviousness, which were largely combinations of two references, a Linnarsson patent and an article by McCloskey. And then with regard to claim 5, those two references and an article by Hug.
Q. Please try to answer my question.
What did you do to investigate whether there were objective indicia supporting your position?
MS. RAYMOND: Objection. Asked and answered.
A. So I believe I answered that question to the best of my ability -- or to the best of my recollection.
I didn't provide any opinions here about objective indicia. I don't believe Dr. Cooper offered any opinions regarding objective indicia.
Q. Did you do anything to investigate whether there were objective indicia supporting 10x?
A. I don't believe -- I think I answered the question twice. I don't believe I provided any opinions in my report regarding objective

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indicia. My report was a response to Dr. Cooper, and I don't believe Dr. Cooper himself offered any opinions regarding that.
Q. The reason why, as the patent owner, you didn't include objective indicia opinions in your report is because Dr. Cooper didn't; is that what you're saying?
A. I was responding -- as I said earlier, I was responding to Dr. Cooper's arguments about the obviousness and validity of the '918 [sic] patent. And I don't believe Dr. Cooper provided any arguments that I needed to respond to.
Q. Is that the reason why you didn't -- well, let me ask you this. Try to answer as directly as you can.
Did you investigate whether there was objective indicia of nonobviousness supporting your position since you listed that as one of the four factors used to make an obviousness determination?
A. So my assignment in preparing this report was to respond to Dr. Cooper's opinions that he had provided in his report. I don't believe Dr. Cooper provided any objective

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indicia arguments. And so in not responding -- in not having to respond to those, I simply didn't include those in my report and didn't spend time investigating them.

Q. If you turn to paragraph 23, do you see where you state, "...the Board must consider objective 'indicia' or factors..."

A. Yes.

Q. And do you see where you say, "The presence of such factors is evidence of nonobviousness"?

A. Yes.

Q. And given that you stated that the board must consider objective indicia of nonobviousness, how come you didn't even investigate that?

A. So I believe the attorneys representing 10x may have presented that in their own briefs.

Again, my assignment was not to provide a comprehensive analysis but really to provide a comprehensive response to Dr. Cooper's allegations of obviousness. So given the fact that Dr. Cooper didn't present any arguments about objective indicia, I had nothing to

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respond to.

Q. In other cases where you were responding to an obviousness analysis, you've presented opinions regarding objective indicia, correct?

A. I may have. It would depend on the case. I've rendered opinions about obviousness both in district court and before the PTAB and I don't recall -- I don't recall all the circumstances in which I provided those opinions.

Q. My question wasn't about all the circumstances.

In fact, you have given objective indicia testimony in response to an obviousness report, correct?

A. I almost certainly have. It would depend on the circumstances whether or not I believe it was necessary.

Q. And did you reach a conclusion here it was unnecessary to consider objective indicia?

A. I wouldn't say it's unnecessary. Again, the PTAB is likely to consider that in evaluating the '918 [sic] patent. I don't know

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all the motions that have been filed and the briefs that have been filed before the PTAB.

I can tell you that my assignment was to respond to Dr. Cooper, and I don't believe Dr. Cooper provided any opinions regarding the objective indicia.

I'd be happy to look at his report if you provide it to me and see if he does provide those opinions.

Q. In terms of who has the burden of going forward on objective indicia, you understand that that's the party that is the patent owner?

A. I don't know who carries what burdens in what circumstances.

Q. Do you understand that if the patent owner wants to rely on objective indicia of nonobviousness, that it needs to present evidence of that? Do you have at least that understanding?

A. I would assume that if a party wanted to rely on some criteria, they would have to provide that. But, again, I'm not a patent attorney or patent agent.

Q. Are the claimed inventions of the

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'981 patent commercially successful?

MS. RAYMOND: Objection. Scope.

A. So I haven't done an analysis of the '918 [sic] patent. I don't know if 10x claims they're practicing the invention of the '918 [sic] patent. I didn't provide an opinion about that, and I'm not prepared to offer an opinion today.

Q. Was there a long-felt need for the claimed inventions of the patent-in-suit?

MS. RAYMOND: Objection. Scope.

A. I didn't provide any opinions about that, and I'm not prepared to offer an opinion today.

Q. Do the claimed inventions of the patents-in-suit -- can you identify any unexpected results offered by the claimed inventions of the patent --

MS. RAYMOND: Objection. Scope.

Q. -- of the '981 patent?

A. I haven't performed that analysis, and I'm not prepared to offer an opinion today. I haven't formed that analysis in the context of this matter.

Q. Are you aware of any -- can you

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identify any copying of the claimed inventions of the patents-in-suit?
MS. RAYMOND: Objection. Scope.
A. Again, I have not done that analysis in the context of the matter we're discussing today, and I'm not prepared to provide an opinion.
Q. Can you identify any praise by those in the field for the claimed inventions?
MS. RAYMOND: Objection. Scope.
A. So I don't believe Dr. Cooper offered any opinions for me to respond to. I didn't do that analysis in the context of the matter we're discussing today, and I'm not prepared to offer any opinions now.
Q. Have you ever seen a patent challenger include objective indicia analysis in their opening report?
A. To the best of my recollection sitting here today, I don't know.
Q. Can you identify any instance where you've ever seen that?
A. I haven't looked back at other matters that I've been involved in, and so I couldn't give you an informed answer.

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Q. Did you investigate whether there was a long-felt need for the claimed inventions?
A. I believe I answered that question. My assignment here was to respond to Dr. Cooper's arguments. I don't believe I did that analysis, and it doesn't appear in my report, so I'm not prepared to offer an opinion today.
Q. Do you believe that objective indicia of nonobviousness, if they existed, would be an appropriate response to the opinions provided by Dr. Cooper?
MS. RAYMOND: Objection. Scope.
A. So you're asking me to render what I believe is a legal opinion. I'm not prepared to do that.
I provided a pretty comprehensive analysis of Dr. Cooper's report, and I'm happy to discuss those opinions I rendered with you today.
Q. Who made the decision that you weren't including objective indicia and an objective indicia analysis in your report when you state, "The Board must consider certain objective 'indicia'..."?

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MS. RAYMOND: Objection to the form.
A. My understanding is that the board -- that this is part of what the board is required to do.
Again, I'm not an attorney. I don't know what other arguments have been made in this case about the objective indicia. So I was asked to respond to Dr. Cooper's arguments, and those are the responses that are contained in my report and that I'm happy to discuss today.
Q. Please focus on my question.
Who made the decision that you weren't to include objective indicia supporting the nonobviousness of the patent in your report?
MS. RAYMOND: Objection. Form.
A. So I worked with the attorneys from Groombridge, who are representing 10x, in preparing my report. And in doing that, my assignment was to respond to Dr. Cooper's arguments, which I believe I provided a fulsome response in the report, and I'm happy to discuss those.
Q. And as to whether or not objective indicia of nonobviousness were part of the response to Dr. Cooper's opinions included in

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your report, whose decision was that?
A. I don't know if there were responses or not. I'm not a party to all of the documents that have been filed in this case. I was asked to respond to Dr. Cooper's theories about obviousness and those are what I provided in my report that I'm happy to discuss.
Q. Who made the determination that including objective indicia of nonobviousness was not a part of the response to Dr. Cooper's opinions --
MS. RAYMOND: Objection to form.
Q. -- for your declaration that you prepared?
MS. RAYMOND: Objection. Form.
And I caution the witness not to reveal privileged conversations.
A. So in preparing my report, I worked with the attorneys from Groombridge. We carefully analyzed Dr. Cooper's report, the patents and a number of other documents that are cited in my report and arrived at the opinions that I've set out here.
Q. Was it outside the assignment that you were given in this case to consider

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objective indicia of nonobviousness?
A. I believe that my response and my assignment was to respond to Dr. Cooper, and I believe my response is very thorough and complete and demonstrates that Dr. Cooper's opinions about obviousness are incorrect. And I'd be happy to discuss those today.
Q. Was it inside or outside your assignment in this case to consider objective indicia of nonobviousness?
MS. RAYMOND: Objection. Form.
A. I met with the attorneys from Groombridge. My assignment was to respond to Dr. Cooper. And I believe I provided a fulsome response, and I'd be happy to discuss the response that's included in my report.
Q. Can you state one way or the other, answering as specifically as you can, whether considering objective indicia of nonobviousness was inside or outside your assignment in this case?
MS. RAYMOND: Objection. Form.
A. I was asked to respond to Dr. Cooper's theories about obviousness. I believe I provided a complete and thorough

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analysis of Dr. Cooper's theories. And I believe what I have here is a fulsome response. And I'm happy to discuss what I included in my report.
Q. Can you answer my question directly?
A. I believe I've answered your question multiple times.
I was asked to respond to Dr. Cooper's assertions about the '918 [sic] patent and I believe -- or, sorry, '981, I may have said '918 previously too -- the '918 [sic] patent and I believe I provided a fulsome response.
Q. Why did you include in your report that objective indicia was part of the obviousness analysis and that the board must consider it if you didn't include any such analysis in your report?
A. In the section on obviousness, I was trying to include as complete an understanding as necessary -- as complete an understanding as possible, given the fact that I'm not an attorney or a licensed patent agent. And so I believe this is a reasonable summary of what the criteria are.

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Again, I don't know all the arguments that have been presented in this case and don't know whether this has been addressed in other areas.
I provided a fulsome response to Dr. Cooper's theories, and I'm happy to discuss those with you today.
Q. Can you identify any teaching away from the claimed invention in the suit from the literature or prior art?
MS. RAYMOND: Objection. Form.
A. I'm not sure what specifically you're referring to. If you can point me to places in my report where I talk about teaching away, I'd be happy to refresh my memory.
Q. Okay.
If you turn to paragraph 23.
A. Sure.
Q. And you list as one of the objective indicia of nonobviousness that the board must consider is whether there's teaching away. And then you wrote, "(...e.g., literature telling persons of skill not to do something that would be required by the claims),..."
Do you see that?

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A. Yes, I do.
Q. Can you identify any teaching away?
A. I don't recall. If you can point me to places in my report where I discuss teaching away, I'm happy to refresh my memory.
Q. Do you have an opinion here one way or the other whether there is teaching away as an objective indicia of nonobviousness?
MS. RAYMOND: Objection. Form.
A. I don't recall rendering such an opinion. If you can point me to places in my report where I offered such an opinion, I'd be happy to refresh my memory.
Q. Did you investigate whether there was a teaching away from the claimed inventions?
MS. RAYMOND: Objection. Form.
A. I was asked to respond to Dr. Cooper's assertions about obviousness. To the extent that Dr. Cooper rendered or offered any opinions, I responded to those regarding teaching away. And if it does appear in my report, if you'd point me to them, I'd be happy to discuss them with you.
Q. Do you remember investigating whether there was the objective indicia of teaching away

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in the prior art from the claimed inventions?
MS. RAYMOND: Objection.
Q. Did you investigate that from your -- based on your memory?
MS. RAYMOND: Objection to form.
A. Sitting here today, what I was asked to do was to respond to Dr. Cooper's arguments. And so I don't recall offering such opinions in my report. If you'd like to point me to those, I'd be happy to refresh my memory.
Q. If you turn to paragraph 25, please.
A. All right.
Q. In terms of the skill level in the art, you addressed that in paragraph 25; is that correct?
A. Twenty-five and 26.
Q. And you formed your analysis, basically, under the skill level in the art identified by Dr. Cooper and the skill level in the art identified by you; is that correct?
Well, let me make it easier. Do you see at the end, you say, "My opinions would not change if I applied that definition of a person of skill"?
Do you see that?

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A. Yes, I do.
Q. Okay.
And there, you're referring to Dr. Cooper's definition; is that right?
A. So --
Q. I'm just asking. There will be a broader question in a minute. But I'm just asking you now, when you say, "My opinions would not change if I applied that definition of a person of skill," you're referring to Dr. Cooper's opinion?
A. Here, specifically, when I say "that definition," I'm applying to -- I'm referring to Dr. Cooper's definition.
Q. Okay. All right. Thank you.
And in order to determine that there would be no difference, you performed the obviousness analysis both under -- from the vantage point of your level of skill in the art and Dr. Cooper's; is that correct?
A. So I initially applied my definition. I also looked at Dr. Cooper's definition. Recognizing that as of 2009, I had at least the skill and experience and training of a person of ordinary skill in the art that would match

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either my definition or Dr. Cooper's definition and also that I had supervised people as of 2009 who had the relevant opinion of either one.
In terms of overall knowledge and understanding, I didn't see a great difference. And so essentially at the end of the day, I've applied both definitions. And the opinions I set forth are consistent with those.
Q. You believe that you qualify as a person of skill in the art in 2009 under Dr. Cooper's definition; is that correct?
A. So I took the opportunity to look back at Dr. Cooper's multiple definitions. So he lists people with both a master's degree or Ph.D., as well as people with -- individuals with a bachelor's degree, periods of experience, familiarity with a variety of different techniques. And then also I looked back at my definition, and as I state in paragraph 6, well before August 2009, my level of the skill in the art was at least that of a person of ordinary skill in the art. So, yes, I do.
Q. Where the answer is just "yes," you don't -- you know, please try to be as concise as you can. You don't need to give long, you

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know, just verbiage if the answer is "yes."
In paragraph 26, you state that you would have qualified as a person of skill in the art under Dr. Cooper's definition in 2009; is that right?
A. Yes. I would have qualified under both my and Dr. Cooper's definition.
Q. And before August 2009, were you familiar with the tools, method and techniques listed as 1 through 6 in your report on page 10?
A. So what's listed as 1 through 6 are a part of Dr. Cooper's criteria, and I was familiar with those as of August 2009.
Q. And what was your knowledge of polynucleotide tagging as of before August 2009?
A. Would you like a comprehensive review of my work prior to 2009.
Q. If you could just describe what you knew about polynucleotide tagging as a person skilled in the art as of August 2009 time period.
MS. RAYMOND: Objection to form.
A. My Ph.D. was in theoretical physics. I worked for two years as a postdoctoral fellow in physics.

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

And then in the early 2000s, people were very interested in analyzing, while I was at the Dana-Farber Cancer Institute, circulating tumor cells and began to become familiar with techniques that --

(Reporter clarification.)

A. At the Dana-Farber Cancer Institute, people were interested in analyzing circulating

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tumor cells. And so I became familiar with methods that had been developed in the early 2000s for doing that.

So my background with all of this really extends back to the start of my career in the biological sciences in 1992. And I can probably even come up with examples before that.

Q. Did I have any experience with molecular barcoding as a means for polynucleotide tagging prior to August 2009, you personally?

MS. RAYMOND: Objection to form.

A. So one would have to look at how one would describe molecular barcoding. When I was at the Stanford Human Genome Center in the late 1990s, Ron Davis and some of his post-docs and students had been developing what they called, I believe, a zip code array in which they were tagging yeast cells to track those yeast cells. And those tags represented what would call a molecular barcode.

I believe there's literature that exists describing that and so -- and describing other approaches. So I'm familiar with molecular barcoding as a type of tagging.

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Q. When you refer to this zip coding, was the molecular barcoding at Stanford in the 1990s, was that polynucleotide barcoding?

MS. RAYMOND: Objection to form.

A. It would depend on how you describe polynucleotide barcoding or polynucleotide tagging. The tags were polynucleotides.

Q. The tags themselves or they were part of polynucleotides?

MS. RAYMOND: Objection to form.

A. It's going to depend on how you define polynucleotide tagging. They were polynucleotides that were used as tags.

Q. Did you have any awareness of polynucleotide tagging in which random barcodes were added to polynucleotides prior to August of 2009?

MS. RAYMOND: Objection to form.

A. So would you repeat the question, please?

Q. Yes.

Were you aware of polynucleotide tagging using random barcodes added to polynucleotides prior to August 2009?

MS. RAYMOND: Objection to form.

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A. So in paragraph 31 of my report, I actually respond to an argument Dr. Cooper makes about claim 1 of the '918 [sic] -- or '981. I keep on saying '918 [sic], and I apologize -- '981 patent that I've called out in a box on page 15. And that passage in the patent that Dr. Cooper cites relates to, and I'm quoting my report, "...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, multiplex sample libraries are pooled and sequenced (referred to as 'multiplex sequencing') and the resulting data 'demultiplexed' to group sample-specific sequences based on their bar codes."

So there are barcoding techniques that have been used for multiplex sequencing, some of those may have involved I believe you referred to as random barcodes, but the use of barcodes from multiplex sequencing was well known in the art before 2009.

Q. And in terms of what you knew, how could you accomplish polynucleotide tagging with

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barcodes prior to August 2009, what were the methods --

A. There were a variety --

Q. -- that you knew of?

A. There were a variety of different methods that people used. This type of multiplex tagging that I'm referring to here in paragraph 31 was largely accomplished in the library preparation steps from Illumina since a lot of the sequencing that was done at the time was on Illumina sequencers. But each of the technology companies, at least the major players, Illumina Applied Biosystems, who had the solid sequencer. I believe the 454 was still providing sequencing technology. To the best of my recollection, they all had multiplex sequencing tech capabilities that relied on sample barcodes. And there were a variety of different ways that those could be introduced.

They could include -- and it would depend on the application and how they were used. They could be introduced by PCR. They could be introduced in a variety of different ways.

Q. Other than PCR, what other ways were

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there that you were aware of prior to August 2009 to add barcodes to polynucleotides?

MS. RAYMOND: Objection to form.

A. I would have to look back and refresh my memory as to the breadth and scope of those. I don't believe that I needed to really look back at all the possible ways of introducing polynucleotides because Dr. Cooper doesn't provide an extensive list of different ways polynucleotide tags would be introduced.

Q. My question was in terms of what you knew yourself personally in 2009 regarding polynucleotide tagging. I understand you're saying you were familiar with that at that time.

My question is, were you familiar with ways other than PCR, you personally?

MS. RAYMOND: Objection to form.

A. So the '918 [sic] patent describes -- sorry, the Linnarsson patent, which we discussed, provides at least one way of introducing those barcodes, a type of molecular barcode through their template switching oligos. I mean, there's a laundry list of different methods, and I couldn't provide a comprehensive list sitting here today. I'm happy to discuss

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those methods that are included in my report.

Q. Other than PCR, can you, sitting here now, identify any other known technique as of August 2009 for polynucleotide barcoding?

MS. RAYMOND: Objection to form and scope.

A. I mentioned earlier the work that Ron Davis and his colleagues had done in tagging yeast, and they used molecular recombination yeast.

So there were a variety of different ways that people could perform this. It would depend on the application and the particular goals. Those that are relevant to responding to Dr. Cooper's arguments are presented in my report.

Q. The second technique that you say you were familiar with as of August of 2009 was amplification.

What did you know about amplification as a tool for nucleic acid analysis in August 2009?

MS. RAYMOND: Objection to form and scope.

A. By August of 2009, I personally had

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probably done tens of thousands, if not hundreds of thousands of PCR amplifications. So I'm familiar with many different PCR techniques and variations; isothermal amplification, amplification -- a variety of different amplification techniques like MDA. I can't tell you exactly when all of these were invented or presented. There are a large number of ways, rolling circle amplification, padlock probes, all of which can be used for different aspects of DNA analysis and in different circumstances and different situations.

I amplified many things for DNA sequencing. I amplified them for analysis on agarose gels. You know, there are polyacrylamide gels. You know, there are many, many, many different techniques that have been developed for DNA amplification.

Q. Did you personally have experience as of August 2009 with amplification of RNA?

A. RNA typically is not directly amplified. RNA is first converted to something called cDNA. It uses an enzyme from retroviruses called reverse transcriptase, which copies the RNA, typically messenger RNA, into

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what's called cDNA, complementary DNA, and that's typically what's amplified.

Q. Did you personally perform techniques or have them performed under your supervision of converting mRNA to cDNA and then amplifying cDNA?

A. Yes. I launched a whole program in gene expression analysis when I was at the Institute for Genomic Research. I used to teach workshops every summer at the Jackson Laboratory in Maine where I teach people how to do that. And I oversaw a large gene expression program at TIGR. And then when I moved to Dana-Farber, in March of 2005, transitioned the DNA array-based analysis of gene expression to RNA sequencing. So, yes, I mean, my experience by that time was fairly extensive.

Q. As of August 2009, were you familiar with molecular biology techniques such as those described in Linnarsson --

MS. RAYMOND: Objection to form.

Q. -- where there's mRNA and then you convert that to cDNA?

A. By 2009, I was very familiar with techniques for analyzing gene expression,

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including the creation of cDNAs for a variety of different applications.

At TIGR, The Institute for Genomic Research, I oversaw a project before arrays that included sequencing, what we called ESTs, or expressed sequence tags, that involved converting RNA to DNA and then sequencing them.

I worked with a large consortium centered -- really centered at the Riken Laboratory in Japan doing full-length sequencing in mouse. So I've had extensive experience with analyzing -- full-length RNA sequencing. So I've had extensive experience with reverse transcription and cDNA preparation and analysis.

Q. One of the techniques you say you were familiar with as of August of 2009 was the use of tags to overcome amplification bias.

Please describe what experience you've had with that before August of 2009.

A. So there are many different ways of using -- potentially using tags to overcome amplification bias. One of the simplest is the use -- the introduction and use of what one could call universal primers for PCR. And that's something since -- this is relevant to my

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discussion of the Linnarsson patent.

In Linnarsson, one of the figures that Dr. Cooper presents in his report and that I discussed fairly extensively is Figure 11 in the Linnarsson patent.

Linnarsson introduces in Step C of his process two regions -- or in Step B they're introduced and then tracked through -- that are called PR. And these represent universal primers. And he uses a single primer extension to do his analysis.

So I was at least familiar with these types of techniques, as well as others.

Q. And please describe those others.

A. It would be a fairly extensive catalogue, and, yeah, I'm not prepared to give you the entire list today.

Q. Other than the one that Dr. Cooper brought to your attention, what other use of tags to overcome amplification bias were you familiar with as the standard techniques prior to August 2009?

MS. RAYMOND: Objection. Scope.

A. So I don't believe I discussed any of those in my report, and so I couldn't provide

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you with a comprehensive catalogue sitting here today.

Q. Yeah, I'm not asking for a comprehensive catalogue. Just examples is fine.

MS. RAYMOND: Objection. Scope.

A. I'd have to think back to the relevant time frame as of 2009.

I'm not prepared to do that today.

Q. So other than what Dr. Cooper brought to your attention in his report, you're not -- you, as of August 2009, you can't say you're aware of any other tags used to overcome amplification bias with polynucleotides?

MS. RAYMOND: Objection. Form. Scope.

A. So I'm sorry, I'm looking for -- there's a place that I recall in my report quoting Linnarsson, where Linnarsson talks about the use of primers to reduce bias in PCR. And I forget the adjective that Linnarsson uses to describe his primers. It may have been single primers, single universal primers, I don't recall exactly, as a way of reducing bias in PCR.

Depending on the application, there

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are other ways to reduce bias in PCR in amplification. And I don't provide a comprehensive list of those here. Sitting here today, I'm not prepared to provide a list since that list wasn't required for me to respond to Dr. Cooper's arguments about the obviousness of the '981 patent.

Q. In terms of your opinion that, as of August 2009, you were familiar with the use of tags to overcome amplification bias, you supported that by stating that you were familiar with the use of universal primers for that.

Is there any other support that you have for that opinion?

A. I wouldn't say that's an opinion. I would say that's a fact. And I talk about the use of -- again, there's some place in this report, and I'm happy to read through it and find it if you'd like. If you can point me to the place where I refer to that or provide me with a searchable copy, I'm happy to find it.

Q. Sure. Turn to paragraph 26, please.

A. Sure.

Q. You state that you are qualified as a person of skill in the art as of August 20,

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2009, under Dr. Cooper's definition.

Do you see that?

A. Yes, I do.

Q. Okay.

And do you recall earlier in the deposition, you said that, in that regard, you would have been familiar with tools, methods and techniques identified by Dr. Cooper listed as 1 through 6 as of August of 2009?

A. Yes, I do.

Q. And one of the things that you're saying now that you would have been familiar with as of August 2009 was the use of universal primers to address amplification bias, right? It's Item 3.

A. He says use of tags to overcome amplification bias, one type of tag could be a primer. And so that's at least one technique that I can point you to. And I believe that one is relevant to the discussion provided in my report.

Q. As of August 2009, how did you understand primers could be used as a tag to overcome amplification bias?

A. Some people refer to primers as a

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tag. And the use of universal primers is one way, and it's discussed in Linnarsson. I'm happy to read through my report in Linnarsson, if you'd like, to find where Linnarsson discusses -- or I discuss Linnarsson's presentation of producing amplification bias.

Q. When you say that you were familiar in August of 2009 of the use of tags to overcome amplification bias, do you consider primers to be tags?

MS. RAYMOND: Objection. Form.

A. It would depend on the context in which it's used.

Q. In that way, where Dr. Cooper says the use of tags to overcome amplification bias was a known technique for people of ordinary skill in the art in August 2009 and you say you had that competency, my question to you is, in that context, is a primer a tag?

MS. RAYMOND: Objection to form.

A. I don't -- I can't tell you what Dr. Cooper was thinking about when he wrote that. If you provide me with his report or show me where he provides more detail as to what he describes as tags, I would be happy to refresh

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my memory of what Dr. Cooper is arguing.

I will tell you that primers are one type of tag that is sometimes referred to in the literature. And so that may or may not have been what Dr. Cooper is referring to.

I don't believe Dr. Cooper presents more detail than what's presented here. So as one example that I've provided, the use of tags as a way of reducing bias in amplification is something that's discussed in Linnarsson. And so sitting here today, that's what I recall. I haven't done a comprehensive analysis to list all the different methods because it's not relevant to the opinions offered in my report.

Q. In August 2009, were you familiar with the techniques described in Linnarsson to use tags to overcome amplification bias, you personally?

A. So Linnarsson's -- I don't know if Linnarsson refers directly to the -- I don't know if -- I'm not sure exactly how Linnarsson describes different types of tags. Linnarsson describes a barcode, which is one type of tag, which can be used to reduce bias.

Linnarsson also describes the use

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of -- I think he refers to them as universal. I think he actually talks about single primer PCR, which would be a type of universal PCR, as a way of reducing bias.

So at least those two are methods that are discussed in Linnarsson that I'm familiar with.

Q. Would you have been familiar with them -- are you saying you were familiar with them in August of 2009?

(Clarification by the reporter.)

Q. Let me ask the full question.

As of August 2009, were you familiar with the two techniques you just described in Linnarsson for the use of tags to overcome amplification bias, you personally?

A. So you're asking a complex question because Linnarsson describes a multistep multiparameter process. So to the extent that your question is limited to single primer PCR or universal primer PCR, and Linnarsson, I believe -- I believe he refers to them as barcodes, sample barcodes. To the extent that Linnarsson is using both of those and we limit our discussion to those two elements, I was very

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familiar with those as of 2009.

Q. Where did you gain that knowledge?

A. I've been working in the field since -- directly in the field since I began my work in molecular biology at the Salk Institute in September of 1992. So at the Salk, at Stanford, at the Institute for Genomic Research, and by 2009, at the Dana-Farber Cancer Institute.

MS. RAYMOND: I think we've been going for more than an hour at this point. I don't know if the witness needs a break.

THE WITNESS: We should give the court reporter a break. I'm happy to sit and go for a long time.

MR. REINES: If we can go to the bottom of the hour, if that works?

THE WITNESS: Is that all right with you? I'm more concerned about you than me right now.

BY MR. REINES:

Q. Are you saying August of 2009, you were familiar with single-cell analysis, you, Dr. Quackenbush?

A. So people use the phrase single-cell analysis to refer to a variety of different

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approaches and different techniques.

Q. What single-cell analysis were you familiar with as of August of 2009?

A. I'd have to think back and look at all the different methods that had been proposed and had been used. I don't recall all of them. I do recall being involved in projects before 2009 that involved the analysis of circulating tumor cells. I also was involved in a process -- in a project that looked at -- it was one started by one of my graduate students in which we looked at the distribution of RNA molecules in individual cells to assert that they followed the Poisson distribution across populations. And we did a series of directed PCR experiments doing limited -- limiting dilutions of individual -- I think they were human cells, they may have been yeast cells, I don't recall -- looking at a subset of specific molecules and their expression levels.

So at least, yeah, in a variety of different applications, I was familiar with the analysis of single cells. Whether or not that would qualify as single-cell analysis would depend on the context.

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Q. And in terms of your statement that, as of August 2009, you were familiar with single-cell analysis like a person of ordinary skill in the art as set forth here, do you believe in that sense that you were familiar with single-cell analysis?

A. So I'm not sure what Dr. Cooper specifically is referring to here. Again, he doesn't provide a fulsome definition of what he means by single-cell analysis.

There are a variety of different ways of examining this, and we'd have to look at them in context. If we limit it to the analysis of a single cell -- well, we'd have to look at it in context.

But by August of 2009, I was involved in analysis of single cells, and my familiarity of that in the literature was sufficient to be able to read Linnarsson, to read Dr. Cooper's arguments about Linnarsson and to render the opinions set forth in my report.

Q. As of August 2009, at Dana-Farber, were you -- was your lab interested in pursuing single-cell analysis as a research area?

MS. RAYMOND: Objection to form.

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A. So I'm not sure exactly what you mean by "single-cell analysis" since it's used in different ways in different contexts.

We were very interested in the analysis of particular mutations that occurred in circulating tumor cells and also in the analysis of gene expression in circulating tumor cells. But the analysis, the approaches and the techniques depended on the particular experimental question they were trying to address.

I was certainly familiar enough with the methods in the literature to render the opinions set forth in my report.

Q. Prior to August 2009, was your lab at Dana-Farber interested in being able to trace mRNA expression to particular cells within a sample? Was that something that was on your radar?

A. So, again, I'm not sure I fully understand your question in what context you're asking it. I can tell you, that as of 2009, we were very interested in the analysis of circulating tumor cells, and some of that may have involved trying to analyze the expression

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in those individual tumor cells.

Q. When you say "may have," was it or you don't know?

A. I don't recall the exact work we were doing with circulating tumor cells. That's not something that I looked back to refresh my memory in preparing this report.

Q. What was the state of the art of single-cell analysis in August of 2009?

MS. RAYMOND: Objection to form.

A. So that's a rather broad question. It would depend on the context in which you were going to describe or discuss single-cell analysis. I didn't review all of the existing technologies and techniques in preparing this report. It wasn't necessary to respond to Dr. Cooper's arguments.

Q. In terms of your lab at Dana-Farber, what was your interest in single-cell analysis at that time?

MS. RAYMOND: Objection to form.

A. So that was 15 years ago, and I don't recall exactly all the work that we were doing at that time. I do recall that I was working with some groups that were very interested in

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the analysis of circulating tumor cells. I do recall that in 2009, my group received funding to get the first Illumina sequencer at Dana-Farber, and so there was a great deal of interest in a wide variety of different applications. But reviewing my work at the time and the extent of it was not necessary to respond to Dr. Cooper's arguments and so I can't provide you with detailed answer sitting here today.

Q. Who were the groups that you were working with that were very interested in the analysis of a circulating tumor cells at the single-cell level?

MS. RAYMOND: Objection. Scope.

A. I am not very good at remembering names, unfortunately. And I recall a number of different individuals I worked with at the Dana-Farber Cancer Institute, Brigham and Women's Hospital and Massachusetts General Hospital at a minimum, but I couldn't tell you the individuals sitting here today. It's not something I reviewed in preparing for my testimony.

Q. So you can't identify any individual

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or group?

MS. RAYMOND: Objection to form. Scope.

A. Sitting here today, I can't tell you. That's not something I reviewed in preparation for in either preparing my report or in preparation for my testimony today.

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

A. Broadly speaking, yes.

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

MS. RAYMOND: Objection to form.

A. So as of 2009, I personally performed a number of ligation experiments, both what we refer to as sticky-ended and blunt-ended ligation. A number of those introduced a variety of different tags, including sequencing tags such as sequencing primers.

I was also familiar with ligation in the context of sequencing instruments like the Applied Biosystems SOLiD, which performed sequencing by ligation, as well as other

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applications.
Q. As of August 2009, were you familiar with the use of ligation for added tags to overcome amplification bias?
MS. RAYMOND: Objection to form.
A. So I'm not sure what particular approaches you're referring to with regard to this combination of techniques that Dr. Cooper lists. I don't believe Dr. Cooper provides explicit examples of those combinations.
To the extent that at a very minimum one could introduce sequencing primers and one could on call a sequencing primer a particular tag, I was familiar with those approaches. I was also familiar with the introduction of amplification primers through ligation. And the use of universal primers is one way of overcoming amplification bias.
"Overcoming" is a challenging term to use. Of addressing amplification bias would with probably be a better way of describing what Dr. Cooper is referring to.
Q. I just need to ask that again because I'm not sure I tracked your response, if you had a specific one.

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What did you personally know of as techniques for using ligation to introduce barcodes for addressing amplification bias?
MS. RAYMOND: Objection to form.
A. So Dr. Cooper doesn't refer to tags -- to barcodes. He says use of tags. One of the types of tags, sitting here today, that I can tell you that we used was universal primers and that we introduced universal primers in a variety of different ways, including through ligation, and that the use of universal primers is one way of reducing amplification bias.
Q. In August of 2009, you were not aware of any way to use ligation to add barcodes to polynucleotides; is that correct?
MS. RAYMOND: Objection to form.
A. I don't believe I said that.
Q. Is it true?
A. Sorry, can you repeat the question?
Q. Yes.
As of August of 2009, were you familiar with techniques in the field for using ligation to add molecular barcodes to polynucleotides?
MS. RAYMOND: Objection. Form.

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Scope.
A. So I don't believe I rendered any opinion about that in response to Dr. Cooper's arguments. If you can point me to places where I rendered opinions about that, I'd be happy to refresh my memory.
As of 2009, I was familiar with ligation. I was familiar with Illumina sequencing. There may or may not have been applications of and other sequencing platforms.
Earlier we discussed multiplex sequencing using sample barcodes to deconvolute the source of different sequences, and I believe that some of the techniques that were in use at the time involved use of ligation to introduce a variety of different tags on universal primers, potentially multiplex barcodes and potentially others.
Q. When you say that as of August of 2009, you were aware of techniques to introduce a variety of different tags to polynucleotides, you say potentially multiplex barcodes, were you aware of that or not as of August 2009?
MS. RAYMOND: Objection to form.
Q. Without using ligation.

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MS. RAYMOND: Objection.
A. So sitting here today, I don't recall discussing that because I don't think it was necessary to respond to Dr. Cooper's arguments. I was familiar with ligation as a technique dating back to the 19- -- my personal experience with it dating back to the 1990s, both blunt end and sticky ligation. And I believe that as of 2009, there were protocols for high throughput sequencing, such as that used by Illumina and Applied Biosystems, Helicos Biosystems, 454, other companies for doing multiplex sequencing that used ligation as a way of introducing sample-specific barcodes for multiplex sequencing and demultiplex.
MR. REINES: Why don't we take that break.
VIDEOGRAPHER: We're off the record at 10:34. This ends is Media Unit No. 1.
(Recess.)
VIDEOGRAPHER: We're on the record at 10:49. This begins Media Unit 2 in the deposition of John Quackenbush, Ph.D.
BY MR. REINES:
Q. Going back to your skilled in the art

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opinion on paragraph 25.
Do you have that in front of you?
A. Yes, I do.
Q. In the first sentence, you say, "I've been asked to assume that persons skilled in the art..." and then you go on to have a definition.
Do you see that?
A. Yes.
Q. And when you say "assume," is that an opinion that you reach based on your analysis or is that just an assumption that you've been asked to make by counsel?
A. So as I mentioned earlier, I prepared this report collaborating with the attorneys from Groombridge representing 10x. And in our discussions about the skill level of a person of ordinary skill in the art, this was what, in our discussions, we believed to be appropriate. And so I was asked to make the assumptions that that's what background a person of ordinary skill in the art would have.
Q. Do you have an affirmative opinion that this is, in fact, the right definition or are you just assuming it for purposes of this proceeding?

A. I believe this is an appropriate definition. But as I wrote, I could either assume my opinion or Dr. Cooper's opinion about what a person of ordinary skill in the art would have in their background, and my opinions overall about the validity of the '981 patent would not change.
Q. How come you didn't include an opinion contesting Dr. Cooper's definition of the skill level in the art?
A. It wasn't necessary because as I stated here in the bottom, my opinions would not change if I applied either Dr. Cooper's definition or my definition.
Q. If you turn to paragraph 28.
A. All right.
Q. You refer in paragraph 28, about five lines down, to a cell-specific tag.
Do you see that?
A. Yes, I do.
Q. And do you know what a cell-specific tag is?
A. So in the context of the '918 [sic] patent -- sorry, I keep saying '918 -- '981. In the context of the '981 patent, the patent

describes the use of a multiplex identifier. And I don't have the patent in front of me, but in paragraph 32, I break out claim 1 of the patent as broken down by Dr. Cooper. And the patent describes the use of what are called multiplex identifiers or MIDs. And they have two tag sequences. The first tag sequence is associated with the single cell from which the sample polynucleotide is derived. And the second sequence -- tag sequence distinguishing the sample polynucleotides from other sample polynucleotides derive from the same cell.
So in this context, the cell-specific tag is the part of that MID that's used to identify the source of molecules and trace them back to individual cells.
Q. And the second part of that is the polynucleotide-specific tags that you read into the record?
A. Yes. The second tag sequence distinguishing the sample polynucleotide from others.
Q. Down further in paragraph 28, you state, "...'to accomplish'..." -- you quote, excuse me, the '981 patent. And I'm happy to

give you that since you mentioned that.
A. Thank you.
(Exhibit 1001 was produced.)
Q. Let me show you Exhibit 1001, which is the '981 patent.
A. Thank you.
Q. But you cite this patent to state, "'...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.'"
Do you see that?
A. Yes, I do.
Q. Did you know, as of August of 2009, that for cell-specific and polynucleotide-specific tagging, you would need tag -- the number of distinct tags would have to be many times greater than the number of molecules to be analyzed?
A. So I'm not sure I understand your question, but it's a fairly-simple analysis that one has to do. So when we're talking about the number of distinct tags --
Q. Right.
A. -- being greater than the number of

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molecules to be analyzed, I can look back at column 22 in the patent, but I believe this is referring to the number of polynucleotides or number of molecules in each individual cell.

If each molecule is to be uniquely tagged within each individual cell, then that second part of the tag -- so every cell is going to have a first sequence associated with that cell, which is going to be the same, and then the second part is going to be variable. And that's going to be useful for identifying the individual molecules.

And it's a simple statistical analysis that one has to do to realize that if you want each molecule to be uniquely tagged, the number of tags has to be greater than the number of molecules.

It's, basically, the same statistical problem as being in a party and trying to identify unique birthdays. If you have more than about 30 people, there's a greater than 50 percent chance that two individuals will have the same birthday, even though there are 365 days and there's only 30 people.

Q. In paragraphs 29 and 30, you refer to this HER2 gene.

Do you see that?

A. Yes, I do.

Q. Do you know whether the claimed methods of the '981 patent have ever been used clinically?

A. That's not an analysis that I performed, and so sitting here today, I can't tell you whether or not they have been used clinically.

Q. And in terms of the HER2 gene that you referred to in paragraph 29 and 30, that's just something you used as an example or did you get that from the patent or something else?

A. So this is an example that comes from my experience, commonly referred to the gene as HER2. It's a widely-used expression marker to classify a subtype of breast cancer, and I can tell you about its importance if you'd like.

Q. Were you familiar with the HER2 gene at Dana-Farber prior to August of 2009?

A. I knew about HER2 for a very long period of time. HER2 positive breast cancer is a subtype of breast cancer that affects about 15 percent of individuals with breast cancer. It's also now become -- HER2 has become an important marker in colon cancer. There's a drug called Herceptin that specifically targets HER2 amplification in breast cancer.

And as of 2005, I published a number of papers, at least one that I can recall immediately, that provided a molecular characterization of breast cancer subtypes using HER2 expression explicitly.

Q. In terms of HER2 expression analysis, did you at Dana-Farber use bulk analysis or did you use single-cell analysis?

A. Most of the analysis that we've done used bulk tissue analysis because those were primarily the clinical assays being performed at the time.

Q. Prior to August of 2009, were you aware of the benefits of performing a single cell analysis for the HER2 gene as compared to the bulk analysis that you seem to be using?

MS. RAYMOND: Objection. Scope.

A. So I don't recall performing that analysis. If you asked me about the potential value of being able to do this with an individual cell or with a collection of cells, as of 2009, I probably could have outlined why that might be useful.

Q. Was there a desire in the field in the 2009 time period to engage in single-cell analysis for gene expression as compared to bulk analysis?

MS. RAYMOND: Objection. Form. Scope.

A. So the question you're asking would depend on the context. I've outlined here at least one example where analysis of HER2 expression could have been valuable. How widespread that would have been in the field as of 2009, I'm not prepared to offer an opinion on today.

Q. What was the level of interest in performing single-cell analysis as compared to bulk analysis for gene expression in 2009?

MS. RAYMOND: Objection. Scope.

A. So I haven't done that analysis. I'm not prepared to offer an opinion today. If we talk about breast cancer, I can tell you why HER2 is important. It's a cell surface receptor that promotes cell growth. And as I said earlier, it's clinically relevant.

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Q. In terms of just your memory of your time working on the gene expression prior to August of 2009, were you aware of interest in the community in single-cell analysis as compared to bulk analysis?

MS. RAYMOND: Objection. Scope.

A. It would depend on the type of analysis one wanted to do. As I mentioned earlier, by 2009, I was working with some groups doing analysis of circulating tumor cells. So there was definitely some interest in being able to understand what was happening with individual cells.

Q. Did you personally pursue any effort to perform single cell analysis as compared to bulk analysis prior to August 2009, you or your lab?

A. So it would depend on how you define single-cell analysis. I don't recall as of 2009 all the techniques that we were using. It's not something I prepared or reviewed in preparation for my deposition today or in preparing the declaration I filed. So sitting here today, I don't recall.

Q. If you turn to page 15 and 16 of your

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report.

A. All right.

Q. And you referred me to the claims as broken up by Dr. Cooper.

Do you see that?

A. Yes, I do.

Q. Okay.

In terms of the polynucleotide of Step 1D, do you have that in front of you?

A. Yes, I do.

Q. Okay.

It states there that it comprises a sequence from a sample polynucleotide of the plurality of sample polynucleotides.

You see that, right?

A. Yes, Step 1D.

Q. Right.

And in terms of the sequence from the sample polynucleotide that is included in the tag polynucleotide, does that have to be the entire sequence from the sample polynucleotide?

MS. RAYMOND: Objection. Scope.

A. Would you repeat your question, please?

Q. Can the sequence from the sample

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polynucleotide that's included in the tag polynucleotide be the entire sequence of the sample polynucleotide?

MS. RAYMOND: Objection. Scope.

A. So a polynucleotide is typically either RNA or DNA in its native state in a molecule and there are different flavors and varieties of DNA that we can discuss. But as a series of nucleotides, hence a polynucleotide, that series of polynucleotides for any individual molecule is referred to as its sequence.

So the polynucleotide now has a sequence and that is the sequence of the polynucleotide.

In this context, that sequence I believe refers to the entire sequence of the polynucleotide and from a sample polynucleotide of the plurality. So each individual polynucleotide is going to have its own sequence. And the tag polynucleotides also comprise a multiplex identifier sequence. And that multiplex identifier sequence comprises a first tag sequence associated with the cell from which it was derived and a second tag sequence

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in Step 1F distinguishing the sample polynucleotide from other sample polynucleotides. So the polynucleotide comprises its sequence so that it includes at least that plus these additional tags.

Q. When the tag nucleotides are being generated in Step 1C, can that be done using PCR?

MS. RAYMOND: Objection. Scope.

A. So I haven't -- in the context of preparing this report, I haven't done a full analysis of the '981 patent and what's disclosed in the specification. I was responding to Dr. Cooper's arguments about obviousness. And so I'm not prepared today to render an opinion about all the different ways that sample polynucleotide -- that tagged polynucleotides can be prepared.

Q. Do you know whether PCR can be used to generate tag polynucleotides?

MS. RAYMOND: Objection. Scope.

A. In an abstract -- in the abstract, one could potentially use PCR to generate tagged polynucleotides. We talked about the introduction of universal primers as one way of

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doing that earlier -- or one way of generating tag polynucleotides. And there are a variety of different ways to do that.

However, in the context of the patent and what the patent is referring to here, I haven't performed that analysis in the context of preparing this report and am not prepared to render an opinion today.

Q. Okay.

So you're saying you're not prepared to state an opinion as to whether, in terms of claim 1, generating a plurality of tag polynucleotides, whether that can be accomplished using PCR?

MS. RAYMOND: Objection. Scope.

Counsel, if you want to bring him to a part of his declaration where he disputes this or addresses this, we can talk about it.

But in the context of a PTAB deposition, the PTAB depositions are limited to the scope of his declaration.

MR. REINES: No, it's fine if he has no -- I mean, I don't understand what the issue is.

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Q. If you don't have an opinion, just state that. That would be perfect.

A. I haven't performed an analysis of the scope of the claims of the '981 patent for the -- within the context of my declaration and deposition today, and so I'm not prepared to render an opinion.

Q. Turning to the '981 patent, can you show me where the generating step is performed in the figures?

MS. RAYMOND: Objection. Scope.

A. I'm sorry. I'm not sure where you're referring to the generating -- oh, Step 1C.

Q. Yes.

A. So I'm not prepared to offer an opinion as to that today. I didn't do that analysis in the context of preparing my report and responding to Dr. Cooper's argument. I'm more than happy to spend the next few hours reading over the patent and performing that analysis, but it's really outside the scope of what I'm prepared to testify about today.

Q. So in order to show where the generating tag polynucleotide step is in the -- is amplified in the '981 patent, that would take

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you hours of review that you'd have to do now because you haven't formulated any opinion on it, right?

A. I don't believe there's an opinion about where the generating step appears in the '981 patent, excuse me, presented in my report. If you can point me to that, I'd be happy to discuss it. But otherwise, it's not something that I've prepared to testify about in the deposition today.

Q. If PCR is used to take sample polynucleotides and tag them, would that require the use of the entire sequence from the sample polynucleotide?

MS. RAYMOND: Objection. Scope.

A. So I'm not prepared to render an opinion about a hypothetical approach to practicing the '918 -- sorry, '981 patent. It's not something I prepared to testify about today. And I have not reviewed that, and so I can't render a valid opinion.

Q. And in terms of, in Step 1D, the sequence from the sample polynucleotide, do you know whether that has to be the entire sequence from the original sample polynucleotide of

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Step 1B or whether it can be some portion of that if you're using PCR?

MS. RAYMOND: Objection. Scope.

A. So you're offering a compound hypothetical. The tagged polynucleotide comprises a sequence, right. So the sequence is part of that tagged polynucleotide. The tagged polynucleotide also comprises the multiplex identifier sequence, which comprises a first tag sequence associated with the cell and a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides.

So the polynucleotide has its own sequence and these multiplex identifiers at a minimum.

Q. And my question to you is, is it just focusing on 1D? You don't have to read into the record 1E. In terms of 1D where it says that the tag polynucleotide comprises a sequence from a sample polynucleotide, do you have an opinion one way or the other whether that has to be the entire sequence of the sample polynucleotide from Step 1B?

MS. RAYMOND: Objection. Scope.

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Q. If you're using PCR?
A. The tagged polynucleotide is -- the tagged polynucleotide has its own sequence. So I believe you may be confused about the fact that sequence refers to the actual physical structure of the molecule. It might also refer to sequence determined from that molecule.
The answer to your question would depend on the context in which it's used. Beyond reading the literal language here, I'm not prepared to render an opinion as to the scope of what that comprising term means in the context of claim 1 of the '981 patent and the deposition we're conducting today.
Q. Answering as directly as you can, is the sequence in the tagged polynucleotide in Step 1D, does that have to be the entire sequence of the sample polynucleotide from 1B?
MS. RAYMOND: Objection. Scope.
Asked and answered.
A. So I'm not sure about the context of your use of the word "sequence." I haven't performed that analysis, and I'm not prepared to offer an opinion today.
Q. Do you have an opinion one way or the

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other as to whether or not the generating plurality of tagged polynucleotides from the plurality of sample polynucleotides can include targeted PCR?
MS. RAYMOND: Objection. Scope.
A. So I believe you answered -- asked me the question about PCR earlier in the context of claim element 1C as broken out by Dr. Cooper. I haven't performed that analysis of the '981 patent, and I'm not prepared to offer an opinion today.
Q. Are the sample polynucleotides of Step 1B genomic DNA or amplified polynucleotides?
MS. RAYMOND: Objection to form.
A. You're referring, I'm sorry, to Step 1D?
Q. No, no, I'm sorry. Step 1B, as in "boy."
So turning to Step 1B, do you have that in front of you?
A. Yes, I do.
Q. Is the plurality of sample polynucleotides, are those polynucleotides genomic material directly from the single cells

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or are those copies from an amplification process?
MS. RAYMOND: Objection. Scope.
A. So performing such an analysis wasn't necessary for me to respond to Dr. Cooper's arguments, so I haven't performed that analysis and I'm not prepared to offer an opinion today.
Q. In the process of going from the sample polynucleotides of Step 1B to the sequencing of the polynucleotides in Step 1G, can you lose some of the sequence from the original sample polynucleotides in Step 1B?
MS. RAYMOND: Objection. Scope.
Form.
A. So I don't believe I've offered an opinion to that in the declaration we're here to discuss. If you can point me to that, I'd be happy to refresh my memory. Otherwise, I'm not prepared to offer an opinion sitting here today.
Q. If you turn to paragraph 23 -- excuse me, page 23, paragraph 40.
A. Certainly.
Q. And there's a reference to, in Linnarsson, the barcode identified as Br.
Do you see that?

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A. Yes, I do.
Q. Okay.
And is that shown in Panel E and F?
A. So I don't have the Linnarsson reference in front of me right now. There is a figure caption that appears earlier in the Linnarsson reference. And Linnarsson and the figure caption for Figure 11, I believe, refers to the series of Xs that appear here as representing that barcode. But the barcode Br, that label doesn't appear in the figure panel itself. It does appear later in Figure 1E as Bc, instead of Br. That may have been a typo when -- or an oversight when Linnarsson prepared the figure caption.
Q. Do you know what barcode Br, what that's referring to in Panels E and F as you set this forth in your report?
MS. RAYMOND: Counsel, would it be possible to give the witness a copy of the reference?
MR. REINES: Sure.
(Exhibit 1003 was produced.)
Q. Here is Exhibit 1003, which is the Linnarsson reference.

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A. Thank you.
Q. My question is really focused on what you've written in your report, but if reading Linnarsson somehow helps you with that, go ahead.
A. Well, you're asking me explicitly about the barcode and where it's labeled. If we look at Linnarsson on the bottom of page 6, sorry, page 5 of -- well, the Bates number has a 6, the page number is 5, it's the last line. And so I may or may not have pulled the entirety of this out into the report, but I definitely refer to the figure caption for Figure 11. And it says, "As a consequence, the 3' end of the cDNA will carry the barcode (XXXX)."
So there are four Xs and that's the barcode that's referred to. Later on where I quote here, it talks about the Br, the next page, line 5. So it doesn't explicitly tell us that barcode is the Xs, and it seems to leave Br out of the figure.
So those are the places I referred to earlier, the Xs and the Br.
Q. Is the barcode in Linnarsson the same thing as the cell tag, what you refer to as cell

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tag on page 21 in a couple of spots?
A. The cell tag in Figure B where I've called it out and highlighted it is part of the template switching oligo TSO, and this is in paragraph 38 of my report. And I put a box around that cell tag.
Linnarsson refers to -- this is a barcode in the figure. I believe elsewhere Linnarsson refers to this as a cell tag. It's used to identify the individual cell that's being labeled and analyzed in this particular step.
Q. So when you refer to cell tag with respect to Panel B, that's the same thing as barcode that you referred to in paragraph 40?
A. So I've used "cell tag" and highlighted it in a red box, the red box is around the five Xs that appear here. Again, it's interesting Linnarsson uses four Xs in the figure caption, five Xs here. So there's some small inconsistencies. But the cell tag that I referred to here in the red box are the series of Xs and that's what Linnarsson in the figure caption calls the barcode.
And, in fact, Linnarsson on the

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bottom of page 24 says, referring to Figure 3, the cell tag is shown as now a series of five Xs.
Q. Prior to August of 2009, were you aware of cell tags such as in this Illumina publication based on your ownership of an Illumina system?
MS. RAYMOND: Objection. Scope.
Q. The filing date is March 2009.
A. So I hadn't seen this Illumina patent application as of March of 2009 or, I guess, issued patent as of March of 2009. I don't recall what products Illumina might have had on the market at the time. I don't recall if this had been discussed at some of the conferences that I had attended.
So for this particular application, I can't say as to whether or not I'm familiar with what Linnarsson was referring to here.
Q. Were you generally familiar with cell tags as a nucleic acid technique as of August of 2009, you personally?
MS. RAYMOND: Objection. Form.
A. I'm familiar enough with the concept of multiplex tagging to understand what

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Linnarsson is describing, to understand Dr. Cooper's arguments and to respond to those.
Q. Yeah, no, my question is just whether you're familiar with cell tags as of August of 2009, very simple question, you personally, Dr. Quackenbush?
MS. RAYMOND: Objection to form.
A. So it would depend on the use. Linnarsson has a particular use here, and I don't recall when I first heard of Linnarsson's particular application of the term.
Q. Let me ask the question again, and try to answer as directly as you can.
Were you aware in August 2009 of the use of cell tags in nucleic acid analysis.
MS. RAYMOND: Objection. Form.
Q. You personally.
A. So that's a rather broad question. I mentioned that in the 1990s, when I was at Stanford, Ron Davis and his group were tagging individual yeast cells with what they refer to as zip codes or what are the equivalent of molecular barcodes. They would do experiments in which they knocked out individual genes and inserted those zip codes so that each strain of

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yeast had its own; therefore, each cell had its own zip code or barcode. They would grow those yeast strains together in a competitive growth experiment to identify whether particular gene knockouts had a growth effect. And then they analyzed the data by performing a molecular analysis and identifying the barcodes.

So in that context, definitely. People had also used a variety of different tags, cell tags in techniques like fluorescence-activated cell sorting to sort individual cell types into individual tubes and then perform molecular analyses on those.

So for molecular analysis, there are all sorts of cell tags that people had used. This particular application, I can't tell you when I first came to know about it.

Q. Okay.

So just to be clear, in terms of the particular cell tag as illustrated in Linnarsson, whether you were aware of that in August of 2009 or not, that's not something that you remember one way or the other; is that fair?

A. I don't recall one way or another when I first learned of Linnarsson's specific

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application of cell tags. But I understand the concept of multiplex tagging of individual samples well enough to understand what Linnarsson was presenting. I would have understood the patent reading it in 2009. And I understand it well enough to analyze Dr. Cooper's opinions and identify the flaws in Dr. Cooper's arguments.

Q. Were you aware of the use of cell tags as described in Linnarsson before this matter, in other words, before you were given the Linnarsson application as part of this proceeding?

A. Before 2023, the concept of cell tags was something I was definitely familiar with from a variety of different applications.

Q. Including the Illumina applications?

A. I've seen the Linnarsson patent before, before 2023, so I was definitely familiar with what Linnarsson presents.

Q. What was the context that you saw Linnarsson's publication first?

A. I'm not someone who, in the course of my day, typically, reads patents. But as you know, I've served as an expert in other matters,

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and I believe the Linnarsson patent appeared in some of those.

Q. Do you remember which matter you first saw Linnarsson?

A. I don't recall.

Q. If you turn to paragraph 44, please.

A. All right.

Q. Do you have that in front of you?

A. Yes, I do.

Q. It states here, "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."

Do you see that?

A. Yes, I do.

Q. And the mRNA molecules extracted from a single cell, are you referring there to the actual RNA molecule that was expressed or is this a copy?

A. So Linnarsson's method, if I recall it in its entirety, begins with putting individual cells into individual wells in what's referred to as a 96-well microtiter plate. In each well, reagents are added and some of those

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reagents include the template switching oligos, so they're the CDSs, and I would have to look at what CDS is. I always default to something called coding sequence, which is not what it refers to. But it is the sequence which is used to prime first strand -- here it is -- cDNA synthesis primers or CDSs. So a CDS is added. A template switching oligo is added, other reagents are added to each well.

The original messenger RNA has a poly-A tail, right, and the original messenger RNA is shown in green, it has a poly-A tail. The CDS in Figure 11 that I've highlighted in red has an oligo (dT) portion and a primer sequence. That primes actually first strand cDNA synthesis. We can talk about some of the details later.

So the molecules that are being bound by the CDS are the original molecules that arise -- that appear in the individual cells.

I hope I'm answering your question.

But what's shown in here in green are the original molecules derived from each individual cell in an individual well in the 96-well plate.

PARSE EX. 1057.024

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Q. When you say derived, do you mean --
A. Those are the molecules that were in that cell.
Q. Okay.
A. The cells lysed or ruptured to release the mRNAs.
Q. Turning to page 28. And this is your discussion of McCloskey?
A. Yes.
Q. You state, "McCloskey distinguishes alleles of the same gene."
Do you see that?
A. Yes, I do.
MS. RAYMOND: Counsel, if we're turning to Dr. McCloskey, I'd like Dr. Quackenbush to have a copy of that.
(Exhibit 1004 was produced.)
Q. I'd like to give you Exhibit 1004, which is the McCloskey reference.
A. Thank you.
Q. Do you see a reference to the random barcode distinguishing alleles in the same gene in McCloskey?
A. I see that, yes.
Q. And just while you have it, were you

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familiar with the McCloskey reference when it came out in 2007?
A. This is something I can tell you that I definitely didn't see in 2007. It referred -- it's in a pretty obscured journal, and it's discussing a solution to a cross-contamination problem, which is not one of the ones that -- not one of the problems that I was working on directly at the time.
Q. I just want to follow up.
So you're saying you definitely didn't see this at the time?
A. I'm pretty sure I didn't see this in 2007.
Q. Is that based on the journal?
A. It's based on the journal. It's also based on the subject matter.
Q. You don't doubt this was published in 2007?
MS. RAYMOND: Objection. Scope.
A. Oh, I have no doubt. I mean, if you look at the date up at the top, it says it was published in 2007.
Q. Was it part of your assignment to determine whether McCloskey was analogous prior

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art?
A. I'm not sure what you mean by "analogous prior art."
Dr. Cooper cites to McCloskey, and I respond to Dr. Cooper's arguments.
Q. Have you ever given opinions on whether prior art is analogous in your many prior experting efforts?
A. I don't -- I don't recall hearing the term "analogous" in the past, so I'm not sure what that means, so I can't -- I can't provide an opinion. I don't recall hearing the term "analogous prior art."
Q. While we're on McCloskey, in terms of the what McCloskey was directed to, if you could turn to page 2 of the exhibit.
A. Sure.
Q. And at the top, McCloskey identifies three issues, contamination, biased amplification and product redundancy.
Do you see that?
A. The first paragraph, second paragraph?
Q. Sorry, the first sentence. Top of the page. Let me ask the question.

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McCloskey identifies issues of contamination, biased amplification and product redundancy, correct?
A. So in that sentence, McCloskey says, talking about DNA sequencing in the prior sentence in the "Introduction," and this sentence states that, "Sequence information obtained by PCR...carries an element of uncertainty...," and it lists the three risks that she asserts; contamination, biased amplification and product redundancy.
And then down below immediately describes the potential clinical impact of this being a PRC analysis and sequencing analysis of whooping cough, where cross-contamination was actually the problem that had occurred.
So she spends -- she mentions these things, but the one she describes is the problem of cross-contamination.
Q. And one of the other issues that the McCloskey paper addresses is redundant sequences from the same cell?
MS. RAYMOND: Objection to form.
Q. You can turn to page 6 if that helps. You can probably remember.

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A. So I'm sorry, where do you see this?
Q. The second paragraph, the "Results and Discussion" is focused on it.
A. So I don't believe your characterization of that was correct. She says redundant sequences recovered from the same PCR reaction are readily identified because they're identical in both barcode and batch-stamp.
Q. And you'll agree that redundant sequences is an issue that the tagging method of McCloskey is also directed in addressing beyond contamination?
MS. RAYMOND: Objection. Form.
Q. If there's something complicated about that, let me know.
A. So McCloskey doesn't specifically say that she's addressing the quote/unquote problem of redundancy. McCloskey in her paper describes an analysis of a single gene, a gene called FMR1, and a bulk tissue sample. And the reason I know it's a bulk tissue sample, she describes the analysis of 2.71 micrograms of DNA. The amount of DNA in a single cell is about 6 picograms, so substantially thousands of times less. And what she's referring to here is her

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ability in that DNA, looking at a single gene, to be able to identify both alleles -- and so alleles are different forms of the same gene. Since we inherit DNA from both our mothers and our fathers, we tend to carry two copies. Sometimes the variants can be distinguished. But the individual copies of those molecules she's able to identify using what we talked about earlier, the randomly-generated barcode to identify allele copies.
So she describes she was able to do that. She doesn't describe that as a problem that has been solved.
Q. Do you see where it says, "In addition to distinguishing valid from contaminant sequences, our methods detect redundant sequences arising from the same cellular DNA template"?
A. Yes, I see that.
Q. And are you denying that the tagging of McCloskey is being identified as a way of removing redundant sequences from the datasets?
MS. RAYMOND: Objection. Form.
A. So McCloskey doesn't describe removing DNA sequences. She describes detecting

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redundant sequences. But remember we talked earlier about McCloskey identifying this problem or working on trying to solve this problem of contamination. And in the previous paragraph, she says "Contaminant sequences were identified by batch-stamps assigned to separate reactions, (Fig. 1). The appearance of sequences bearing inappropriate batch-stamps enable us to trace contaminated sequences...," blah, blah, blah.
So this is consistent with my previous statement that McCloskey's -- the problem she was really trying to solve was this problem with cross-contamination between PCR. She talks in the introduction about this problem of whooping cough being detected in individuals with -- who didn't have it because of PCR-based contamination.
And so that seems to be the problem she's addressed and solved. She also says that by using the randomly-generated barcode, she can distinguish between the different alleles, but she doesn't necessarily talk about removing them or what she's doing.
Q. Do you see in that same paragraph that you're looking at now where it says,

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"Identifying redundant sequences and removing them from data sets is especially important..."?
A. I'm sorry, where?
Q. The same place, the second full paragraph under "Results and Discussion," the same one.
A. Okay.
So she says, "Identifying redundant sequences and removing them from the data sets is especially important when analyzing mosaic samples, which occur in both genetic and epigenetic diseases."
So a mosaic occurs --
Q. I'm just asking if you saw it.
A. I see it, yes.
Q. Okay.
And in terms of -- are you denying that McCloskey is saying that one of the benefits of the tagging approach is that you can identify redundant sequences and remove them from the analysis?
A. So she's talking about specific examples like mosaic samples. The easiest example is going to be a tumor sample where you have a mix of mutated DNA appearing in tumor

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cells and normal DNA in infiltrating lymphocytes. And if you wanted to count how many tumor cells, then being able to identify and count the mutant alleles is something she asserts could be done in those mosaic samples.

So in doing this bulk analysis, which she does and describes, that kind of molecular counting could be important for applications like tumor analysis.

Q. Okay.

But just like a simpler question, in addition to addressing contamination, McCloskey also states that her tagging approach can detect redundant sequences such that they can be removed from the analysis, at least under particular -- and gives an example, right?

A. In bulk samples, that's something she asserts her method can do.

Q. Okay.

MR. REINES: By the way, it's -- we can go off the record for a second.

VIDEOGRAPHER: We're going off the record at 11:52.

(Luncheon recess.)

VIDEOGRAPHER: Back on the record at

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12:38. This begins Media Unit 3 in the deposition of John Quackenbush, Ph.D.

BY MR. REINES:

Q. If you could open up the '981 patent.

A. All right.

I have it.

Q. First of all, did you review and understand the '981 patent in formulating your opinions in this case?

A. Yes, I did.

Q. And with respect to Figure 3, in Step 7, the original -- there's sequence from the original sample polynucleotide.

That's the Illumina, correct?

A. So I don't believe Dr. Cooper offered any opinions about Figure 3. Reading column 2 of the patent, it says, "FIG. 3 is a schematic diagram illustrating an exemplary embodiment for moving a primer site and a MID...," a molecular identifier "...to a specific location in a nucleic acid of interest."

So I haven't performed an analysis of exactly what's happening in each step of this seven-step figure in the context of the patent. And so at this moment, I'm not prepared to offer

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an opinion.

Q. Do you know whether the reflex method as described in the '981 patent embodies the claims in the '981 patent?

MS. RAYMOND: Objection. Form. Scope.

A. So I don't believe Dr. Cooper offers opinions about whether or not the reflex method is embodied in the claims of the '981 patent. The figure legend says that this is, I believe, an exemplary embodiment, but I haven't performed the analysis, and I'm not prepared to offer an opinion.

Q. Embodiment of -- it doesn't say an embodiment of the claims, does it?

Or is that how you read it? Do you read this where it references that Figure 3 is an embodiment, to be an embodiment of the claims that issued on the '981 patent?

MS. RAYMOND: Objection to form.

A. I haven't performed the analysis, and I'm not prepared to offer an opinion.

Q. Do you know whether the nucleic acid sequence in Step 202 is the same as the nucleic acid sequence in Step 7?

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A. So I'm not sure what you're referring to by "sequence." Is it the sequence or all of the sequence? I haven't performed that analysis, and I'm not prepared to offer an opinion sitting here right now.

Q. Is there any version of the reflex method in the '981 patent that embodies the claimed inventions of the '981 patent?

A. So I don't believe Dr. Cooper rendered an opinion about what you're referring to as the reflex method, so I haven't performed that analysis and can't offer an opinion sitting here today.

Q. Does the '981 patent have any embodiment of the claimed inventions of the '981 patent?

A. I'm assuming it does, but I haven't performed that analysis and can't point you to those embodiments.

Q. Back to McCloskey, if you would.

A. Sure.

Q. In the second sentence of the "Results and Discussion," it refers to "...unique cell or allele copy-specific barcodes."

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Do you see that?
A. Yes, I do.
Q. And do you understand that to be two different types of barcodes?
MS. RAYMOND: Objection. Form.
A. So in the sentence you're referring to in McCloskey, she points to Figure 1. In Figure 1, the only thing that she labels is a randomly-generated barcode identified as the allele copy. And in the caption for Figure 1, she simply refers to a barcode. So I'm assuming that she's using the "or" here to have two specific adjectives to refer to the same barcode that she might refer to as a cell or allele-specific barcode.
In the example that she provides in her paper, she actually doesn't have the capability of tracking things back to individual cells. She can only point to specific alleles.
Q. Do you understand what's referenced here with the unique cell-specific barcode?
A. So she doesn't provide any examples of that in her paper. In the experiment she describes doing here with the sequencing of the FMR1 gene from a bulk DNA sample where cells

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from many DNAs were mixed -- excuse me -- in which DNA from many cells is mixed, therefore, I'm not sure specifically what she's referring to here.
Q. Do you have any idea at all?
A. She refers to Figure 1. And in Figure 1, she only describes allele-specific barcodes. She doesn't provide any examples of cell-specific barcodes. And given the experiment she describes, there's no way to identify and trace any sequence back to an individual cell. And I actually refer to that in paragraph 48 of my declaration on page 30.
I write, "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."
Q. And what are you relying on in McCloskey for the statement that the pooling -- the tagging must take place after the pooling?
A. I'm sorry, would you repeat the question?
Q. What are you relying on in McCloskey for your statement that the pooling has to take

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place before the tagging in McCloskey, if that's what you're saying?
A. So McCloskey describes a single experiment. And this experiment is an analysis of the gene called FMR1. And she talks about doing an analysis in which, on page 5 of the document, document page 765, in the section entitled "Sequenase v2.0 (USB) Extension," Human genomic DNA (2.71 micrograms) was cleaved with HinfI from (Boehringer Mannheim GmbH) and another enzyme, AluI, A-l-u-I, and then describes that further analysis of the aliquot, 10 percent of that digested genomic DNA.
So the fact that she's doing this analysis on 2.71 micrograms of DNA is an indication that she's not using individual cells. Cells contain about 6 picograms of DNA, so about a thousand times less. And so her analysis has got to be on DNA from multiple cells. Therefore, there's no way to know, based on her tagging, which individual cell any molecule came from.
Q. Would a person of ordinary skill in the art have known that tagging needs to take place before pooling in order to use barcodes as

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cell-specific barcodes?
A. So I'm not sure about the context of your question, but if you had a pool of DNA from a large number of cells, each cell has two copies of the FMR1 gene. And so any individual molecule I look at in that pool of hundreds, if not thousands, of cells, or DNA from that many cells, couldn't be traced back to an individual cell. So the only way to do that, and Dr. Cooper talks about the Linnarsson reference - not the only way - but Linnarsson describes a way of tagging individual cells. Linnarsson describes cell tags, which I believe he refers to as either cell tags or barcodes. And those are introduced in individual cells of 96-well microtiter plate.
Q. All right.
If you look at the last sentence of McCloskey's results, is that in front of you?
A. Yes, it is.
Q. And that states, "We recommend that batch-stamps and barcodes be used when amplifying irreplaceable DNAs and cDNAs for forensic, clinical, single cell, and ancient DNA analyses."

Do you see that?
A. Yes, I do.
Q. And is that consistent with your reading of McCloskey as to what they're recommending?
A. So McCloskey actually repeats this sentence twice. First, at the last sentence in the Abstract and then also the last sentence in the Results.
So she's making this recommendation and talking about irreplaceable DNAs and cDNAs, and she talks about forensic analysis where, you know, she's clearly, I would guess, addressing the question of cross-contamination, but I don't know. That seems to be the major thrust of her work. Clinical data, single-cell analysis, ancient DNA analysis. It's not exactly clear to me what she's talking about here except these are rare samples in which you would want to avoid cross-contamination. She clearly doesn't describe the use of her method for any kind of single-cell analysis.
Q. You'll agree that she recommends the batch-stamps and barcodes that she described for single-cell analysis?

MS. RAYMOND: Objection to form.
A. So she says she recommends it, but she doesn't describe any way in which this could be done. And the only method that she presents is one that uses 2.71 micrograms of DNA, which is far more than what you would get for an individual cell.
All the processing steps involving cleavage and other analyses to analyze the single gene in a single cell, even that would be incredibly difficult. A single gene, excuse me, even that would be very difficult to do in a single cell given the small quantity of DNA and all the losses that would happen with every step that she includes.
Q. And in terms of the contamination, that is addressed by the batch-stamp, is that correct, as compared to the barcode?
A. So she tells you the batch-stamp is unique to each experiment. And she also tells us in her discussion of the results on page -- document page 6, page -- I don't know what the final page is, but that we talked about earlier that the contaminant sequences were identified by batch-stamps assigned to separate reactions.

And so the batch-stamp is what she's using to identify the contaminants.
Q. As you understand the McCloskey reference, what is the purpose of the barcode if you have the batch-stamp to address contamination issues?
A. So she says on the second page of her paper, "The random barcode distinguishes among sequences arising from different cell or allele copies." And there's another place where she refers to the barcode as distinguishing between copies of an allele for a particular gene in the sample -- oh, sorry, that's what I described. But sequences arising from different cell or allele copies.
So remember earlier we talked about the fact that we have two copies of our genome, so we potentially have two different alleles with variants, and the batch-stamp can be used to identify those.
She also talked about mosaic samples, right, tumor and normal cells mixed with each other, where you would expect the tumor to carry certain mutations that the normal cells wouldn't. And so in the kind of bulk sample

analysis that could be done, the barcodes can help distinguish between those two types of cells.
Q. Why does McCloskey propose doing anything more than just using batch-stamps for what you identify as the contamination issue?
MS. RAYMOND: Objection. Form.
A. Sorry. Could you repeat the question, please?
Q. Okay.
Let me try it this way: In McCloskey, does the cell-specific barcodes, do you understand them to be addressing contamination?
MS. RAYMOND: Objection. Form.
A. So I don't think McCloskey refers to cell-specific barcodes. Well, so the sentence you read earlier talks about unique cell or allele copy-specific barcodes. It refers to Figure 1. In Figure 1, she talks about the randomly-generated barcodes to identify allele copies.
You know, what all her motivations are, I can't speak to, but --
Q. Well, let me ask -- go ahead.

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A. You know, I just want to say that given the experiment she describes with 2.71 micrograms of DNA, you couldn't trace any of the alleles or any of the molecules back to any particular cell.

Q. In terms of the barcodes, do you contend that the barcodes in McCloskey are directed to the contamination issue?

MS. RAYMOND: Objection. Form.

Q. I just want to be clear on that.

A. So I haven't thought through all the ways in which one might be able to use the random barcodes to potentially identify contamination. I don't believe that McCloskey discusses that. So she refers to the batch-stamp as being used to identify contamination. And the portion that she refers to as a barcode, the 7-base oligonucleotide to distinguish between alleles in these bulk samples.

So I think most of her cross-contamination, at least the way she describes it, points to the batch-stamp as being the primary way; but, again, I haven't thought through all the possibilities.

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Q. Will you agree that McCloskey describes the use of tags to address issues other than contamination?

A. So I don't know if it's addressing issues. But she does talk about the use of these barcodes, right, as distinct from the batch-stamps to -- well, she says Valid sequences were identified by their date and sample-specific batch-stamp and their unique cell or allele-specific barcodes.

So I'm not sure how she's defining validity here, but there may be other issues she's trying to address with the barcodes and the batch-stamps together since she includes them both as being key to identifying valid sequences.

Q. Based on your review of McCloskey, what issues do you think she's proposing to address with the barcodes?

MS. RAYMOND: Objection. Scope. Form.

A. So she says at the beginning of the second paragraph in the "Results and Discussions" section of her paper, "In addition to distinguishing valid from contaminant sequences, our methods detect redundant sequences arising from the same cellular DNA template (Fig. 1)."

So in that mix of DNA, each template molecules from a cell, and she's saying that her combination of batch-stamps and barcodes can identify places where, in the final sequencing reaction, that same original molecule is counted twice from the same cellular DNA template.

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Q. And in terms of the clinical use of cDNA, how would that -- how would barcodes help in that context?

MS. RAYMOND: Objection. Form.

A. So McCloskey doesn't define -- doesn't describe the analysis of cDNA. She describes the analysis of a single gene FMR1 and a sample of genomic DNA coming from what I expect is a bulk tissue sample, although she doesn't really tell us. It could be from a cell culture. I don't know where she gets her DNA. But she doesn't describe applications to cDNA, and she doesn't -- she doesn't talk about this -- the use of these other than a way of measuring template diversity or analyzing mosaic samples.

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Q. Would a skilled artisan in 2009, such as yourself, know how to use the McCloskey tagging with cDNA, as she recommends?

MS. RAYMOND: Objection to form.

A. So McCloskey's ability to identify, right, she says, "An additional benefit of our method...is that counting sequences with nonredundant barcodes provides a quantitative measure of template diversity."

So if you want to see how many individual templates are there, then given her method, right, and she describes the use of this with genomic DNA, her analysis, to the best of my read, labels the genomic DNA molecules that she eventually sequences with individual barcodes. And I'd have to go back and review all the details of her method.

To be able to cut DNA with an enzyme as she describes, you'd actually have to create double-stranded cDNA. You would be somewhat removed from the individual molecules.

So I haven't thought through all the ways in which one could potentially adapt this, but as it's described here, it would be -- it would be -- it would take -- I haven't thought

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of different ways in which to describe the use of this method she discloses to analyze cDNA, and I don't believe Dr. Cooper proposes a particular adaptation of McCloskey's method as described here in detail for the analysis of cDNA.

Q. Would you in 2009 have been able to apply the barcodes of McCloskey to cDNA?

MS. RAYMOND: Objection to form. Scope.

A. So I haven't thought through the way in which one might do that. As I mentioned, as laid out here, this method would be difficult as written, as described in this paper, to describe -- to directly to apply to cDNA.

I could attach barcodes to lots of different molecules. Whether it would achieve the goal she lays out here, I'd have to think in more detail about how one would actually go about doing that.

Q. Do you have any explanation for why McCloskey and her team recommended using her barcodes for single-cell analysis?

MS. RAYMOND: Objection to form. Scope.

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A. So single-cell analysis can mean a variety of different things. And one of the reads that one could place here in that sentence that we referred to earlier, the last sentence in the paper, also the last sentence in the abstract, which is the only place she mentions or uses the phrase "single cell," she doesn't make it clear what she's proposing here; how one would go about doing that, given the method that's outlined. She could be talking simply about the analysis of a single cell as opposed to other methods, like Linnarsson, which Linnarsson describes, I believe, as single-cell analysis. Or I believe the '918 [sic] patent also uses the phrase "single-cell analysis," although I don't recall all of the details.

So this could simply be a reference to analyzing single cell. But, again, the method she describes here would be difficult to implement for a single cell.

Q. Was there literature on the use of barcoding in the context of cDNA for single cells as of 2009?

MS. RAYMOND: Objection. Scope.

A. So I don't know if -- I don't believe

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Dr. Cooper pointed to any. There certainly was literature about multiplex analysis of cDNA samples, but I don't know if there's any analysis of single-cell cDNA samples. I would assume that Dr. Cooper would have pointed those out if he had been able to find them. I did no search for them.

Q. If you turn to page 4 of the exhibit, under the heading of "Barcode."

A. All right.

Q. And here, McCloskey states, "The number of distinguishable barcodes in a population of oligonucleotides used in a reaction is determined by the number of random bases, n. This enables one to distinguish among 4n allele copies per reaction."

Do you see that?

A. Yes, I do.

Q. And do you understand "n" there to be a variable?

A. So the way it's presented in the two sentences you read and is a variable, she then states, "We currently use seven nucleotides for the barcode, giving 16,384 possible barcodes for each encoding oligonucleotide."

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And I believe Dr. Cooper in his analysis suggests the use of McCloskey's barcodes which she describes as seven nucleotides.

Q. And did you understand from McCloskey that the number of possible barcodes could be increased or decreased by changing the number of random bases used in the barcode?

MS. RAYMOND: Objection. Scope.

Q. Is that what it's teaching?

A. So McCloskey doesn't describe that. A read of this is that one could choose the number of random bases n. But the number of bases one chooses is going to depend on a variety of different experimental constraints and goals.

And in the context of what we're discussing today, Dr. Cooper's analyses, I don't believe Dr. Cooper provides any suggestion that the number of bases be chosen to be anything other than n when he suggests adapting McCloskey's batch-stamp and barcode to the analysis of single cells as laid out in Linnarsson.

Q. When McCloskey says "We currently use

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seven nucleotides for the barcode...," do you understand McCloskey to be saying that that wouldn't be changeable if the experiment warranted it?

MS. RAYMOND: Objection. Form. Scope.

A. So there are a variety of different considerations that would go into this. McCloskey's barcodes have a very particular -- or the oligonucleotides that contain the barcodes have a very particular structure. So they have a target-specific region that would bind to a particular genomic target locus or genomic target sequence, potentially a gene sequence or some other element. They have the batch-stamp. And I don't recall immediately how long that batch-stamp is. There's her seven randomly-generated barcode. There's some additional sequences to give her little hairpin structure. It's a three-dimensional structure and then some additional bases that bind to, I believe, the genomic region.

So she's got a very particular structure that she's using in her analysis. And I don't know the entirety. I haven't really

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done an analysis for what the impact would be in expanding this barcode or in what conditions one might be able to do it and for what particular application.

So, yeah, there are a lot of things you could potentially vary, but she doesn't provide any guidance on how to do that or why one would want to do it.

Q. A person skilled in the art reading McCloskey would understand that you would want to increase the number of random bases in the barcode if you were addressing a situation where you would have more different molecules than you would barcodes, right?

MS. RAYMOND: Objection. Form. Scope.

A. So would you repeat the question, please?

Q. A person skilled in the art reading McCloskey would understand that you would want to increase the number of random bases in the barcode if you were addressing a situation where you would have more different molecules than you would have barcodes if you were using the seven nucleotide barcode?

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MS. RAYMOND: Objection. Form. Scope.

A. So there are a wide variety of considerations one would have to look at in adjusting that length, and a lot of it would have to do with the experimental conditions. So one of the things I point out in my report is that Dr. Cooper states, and I agree, that one would need many more copies of a molecular tag that - and I'm quoting Dr. Cooper here - I really am quoting the patent in column 22, lines 54 to 57. "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 analyzed."

All right. So that's one consideration one would have to look at.

And then Dr. Cooper, in his report on page -- paragraph 256, tells us that tagging each molecule would require at least a hundred times more tags than sample polynucleotides.

So if I look at the 7 mers that McCloskey uses, she has 16,000 possible barcodes. That tells me that, according to Dr. Cooper's logic, she should be able to tag

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about 160 different molecules.

One might envision you can make that longer, but, again, it would depend on your application.

So on page 6 of McCloskey, she talks about sequence recovery and talks about performing DNA sequencing which was carried out using fluorescent dideoxynucleotides in applied -- using BigDye terminator chemistry from Applied Biosystems, and one skilled in the art would understand that this is an implementation of Sanger sequencing.

At the time, in 2009, the reads were over a thousand bases long, so adjusting that size of a barcode might be -- might not have an impact on what you're actually able to do with the rest of the sequence.

I believe at the time, the sequencing technologies, the Illumina sequencer read 35 bases, maybe 50, in 2009. And so adjusting that number could have a significant impact on other aspects of the experiment.

So, you know, you'd really have to think through how to do it. It's not just simply, Oh, I'm going to make this longer

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without having any potential impact on the rest of it. And Dr. Cooper doesn't propose a way to make this longer without negatively affecting other aspects of the incorporation of these kind of barcodes in Linnarsson.

Q. If you turn to page 32, please.

A. Of my report?

Q. Your report.

A. Sure.

All right. I'm there.

Q. You state in the middle of paragraph 51, "In certain embodiments, the MID on a polynucleotide is used to identify the source from which polynucleotide is derived."

Do you see that?

A. Yes, I see that.

Q. And what did you mean by that, derived?

A. So you're referring me to a section in my report in which I discuss claim construction and how to interpret different terms.

In paragraph 51, I say, I have assumed the following constructions in my analysis, both of which are found in the

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specification of the '981 Patent: Multiplex Identifier or (MID), it means a tag or combination of tags associated with a polynucleotide whose identity - right - the tag DNA sequence can be used to differentiate polynucleotides in the sample, which is what you referred me to.

If I look at that, and I'm interpreting the claims of the patent, the MID, this multiplex identifier, comprises a first tag sequence associated with a single cell from which a sample polynucleotide is derived, and a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same cell.

So if I'm reading this in the context of the line you quoted, which comes from column 6, lines 33 to 38, and it goes further, "In certain embodiments, the MID on polynucleotide is used to identify the source from which that polynucleotide is derived," if I read that in the context of the '981 patent, that MID is going to have a component, an element which allows me to trace each polynucleotide, each molecule back to an

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individual cell and then to trace it back to a specific polynucleotide, a specific molecule that came from that same single cell.

Q. So when you say trace it back to a polynucleotide, is that referring to the original biological material or a copy?

MS. RAYMOND: Objection. Scope. Form.

A. So I'm not sure how you're defining "copy," and I'm not sure the context in which you're looking at defining a copy.

Now we're talking about the language of the patent. And the MID, as we see in column 6, can be used to identify the source from which the polynucleotide is derived. The multiplex sequence comprises a first cell tag associated with the single cell from which the sample polynucleotide is derived and a second tag sequence distinguishing the polynucleotide from other sample polynucleotides derived from the same cell.

I don't believe Dr. Cooper contests this, and so I haven't done a fulsome analysis to really look at the breadth and scope of what that might be referring to. And I'm not

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prepared to offer an opinion beyond what is presented in my report. I don't believe Dr. Cooper contests that.

Q. In paragraph 51, you say, and this is in your Claim Construction section, "...'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.'"

Do you see that?

A. I see that, yes.

Q. And there, you're -- that's what you understand is the construction of the term "sample" as used in the claims; is that correct?

Let me ask the question again.

A. All right.

Q. In paragraph 51, you state, "I have assumed the following constructions in my analysis."

Do you see that?

A. Yes, I do.

Q. And then you have the terms from the claims that you're construing are in quotes: multiplex identifier, MID, and sample; is that right?

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A. So I'm not offering an extensive claim construction analysis here. I'm providing definitions which I don't believe Dr. Cooper contests. These definitions are derived from the patent itself. "Sample," I quoted part of the first sentence of the very long paragraph that describes samples. And the claim itself tells us the sample comprises a plurality of single cells and then goes on to discuss that further.

So, you know, the sample I'm taking here to mean in the context of the report I filed today or that we're discussing today in which Dr. Cooper, in response to Dr. Cooper, a sample is a quantity of material from a biological, environmental, medical, patient source or patient source in which detection measurement or labeling of target nucleic acids is sought.

Q. You stated you provided two constructions from the specifications of the '981 patent in paragraph 51.

What are the two? Can you just identify the terms construed?

A. So I'm not offering a claim

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construction for the entirety of the '981 patent. That would be outside the scope of what I'm providing.

I'm responding to Dr. Cooper's analyses. And I believe that the two terms, "molecular identifier MID" and "sample" as described in paragraph 51, quoting two portions in the '981 patent, are consistent with Dr. Cooper's analysis.

Q. Okay.

And did you, in fact, assume the construction of sample to be a quantity of material from a biological, environmental, medical or patient source in which detection, measurement or labeling of target nucleic acids is sought?

A. That's the assumption of the definition of that term I made in the context of responding to Dr. Cooper and his arguments. And I believe that's consistent with Dr. Cooper's arguments regarding obviousness of the '981 patent claims and in his analysis of Linnarsson, McCloskey.

Q. In terms of the term "sample," you understand sample to mean the actual biological

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material that's at the source rather than a copy of it; is that right?

A. I'm not sure how one would copy a chunk of tissue.

Q. You understand the term "sample" is used also to describe the polynucleotides in claim 1, correct?

MS. RAYMOND: Objection. Form.

A. So I don't believe I provided an analysis of that in my report. It wasn't necessary to respond to Dr. Cooper. I'd have to think about that analysis in greater detail, and I'm not prepared to offer an opinion today.

Q. Do you know what sample polynucleotide means in the claims in the '981 patent?

A. In the context of responding to Dr. Cooper's arguments, I believe that my use of the term "sample polynucleotide" is consistent with the way in which he uses it. And so I'm not responding or I'm not offering claim construction opinions with regard to the '981 patent. I'm providing definitions only to the extent that I'm able to respond to Dr. Cooper's arguments.

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Q. Did you disagree with the way Dr. Cooper applied any of the claim terms for the '981 patent?

MS. RAYMOND: Objection. Form.

A. So that's a rather broad question. I don't have Dr. Cooper's report available in front of me to look through it.

Dr. Cooper, I think, misapplies a lot of terms. Dr. Cooper offers a lot of vague suggestions without really describing in detail what he means in many places.

I have many pages in which I talk about some of the shortcomings in Dr. Cooper's analyses. I'd be willing to walk through those if you want to talk about specific examples.

Q. With respect to plain and ordinary meaning, you applied -- excuse me.

With respect to sample polynucleotide, you applied the plain and ordinary meaning, correct? Paragraph 52.

A. In responding to Dr. Cooper's arguments, wherever possible, I applied the plain and ordinary meaning as they would have been understood in light of the specification. I don't believe I offer any particular

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construction or interpretation of sample polynucleotide beyond the plain and ordinary meaning.

I always, to the best of my ability, try to respond to the opinions as presented by Dr. Cooper.

Q. Do you understand that the '981 claims are limited to mammalian sources?

MS. RAYMOND: Objection. Scope.

A. I haven't performed such an analysis. I don't believe Dr. Cooper offers any such opinions, and so it's beyond the scope of what I'm prepared to testify about today.

Q. In terms of Linnarsson, is it your understanding that Linnarsson can only be used with mammalian sources?

MS. RAYMOND: Objection. Scope.

A. So I don't believe Dr. Cooper offers any interpretation of Linnarsson saying that Linnarsson can only be used for mammalian sources. I don't believe I offered any opinions about the limitations of the application of Linnarsson beyond mammalian sources. However, Linnarsson's CDS, cDNA synthesis primer has an oligo(dT) component. And to the best of my

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recollection, all eukaryotic mRNAs are polyadenylated.

So, for example, Linnarsson's application or Linnarsson's method could, sitting here today speculating, could be applied to analysis of single yeast cells or other eukaryotic cells.

Q. Would you have known in 2009 how to adapt the Linnarsson method to single yeast cells?

MS. RAYMOND: Objection. Scope.

A. So I haven't fully done that analysis, but if yeast cells, if -- and I believe they are; I believe the mRNA is polyadenylated. I could imagine using it for yeast cells. But, again, I haven't fully thought through all the implications, and so I haven't thought about what one would want to do in 2009, other than analyzing the methods presented here in Linnarsson in the context of Dr. Cooper's arguments.

MS. RAYMOND: If we could take a break in the next couple of minutes, that would be great.

MR. REINES: Sure.

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BY MR. REINES:

Q. Can you identify any reason why the Linnarsson approach wouldn't be applicable in the eyes of a skilled artisan to yeast cells?

MS. RAYMOND: Objection. Scope. Form.

A. So I don't believe Dr. Cooper makes any arguments about the limitations of the application of Linnarsson. And so I'm not prepared to offer an opinion as to what the limitations might be sitting here today.

MR. REINES: Okay. Why don't we take a break. Thanks.

VIDEOGRAPHER: Off the record at 1:40. This ends Media Unit No. 3.

(Recess.)

VIDEOGRAPHER: On the record at 1:53. This begins Media Unit 4 in the deposition of John Quackenbush, Ph.D.

BY MR. REINES:

Q. Based on your analysis of Dr. Cooper's declaration, have you identified any terms that warrant claim construction?

MS. RAYMOND: Objection. Form. Scope.

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THE WITNESS: Do you want to repeat that for him?

MS. RAYMOND: Objection. Form. Scope.

A. So I didn't perform a thorough analysis with regard to claim construction. My opinions are laid out in the section of my report we referred to earlier, which I believe appears on claim 31. Beyond that, I don't have any further opinions to offer.

Q. Did you say claim 31?

A. Page 31.

Q. Oh, okay.

But I'm saying if you had seen any area where, you know, based on Dr. Cooper's report, where there was a need for claim construction, you would have identified that; is that right?

MS. RAYMOND: Objection. Form. Scope.

A. I didn't perform that analysis, other than what's offered in Section C on page 31 and 32 of my report. I was simply trying to respond to Dr. Cooper's arguments as best I could.

Q. Did you perform any analysis to see

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if there was any claim construction that was warranted in view of Dr. Cooper's report?

A. I didn't perform an analysis to see whether or not claim construction was warranted. I responded to Dr. Cooper's arguments in the context of the claim construction I presented here.

Q. One of the terms you offered for claim construction was the term "sample."

Does the definition that you identify in paragraph 51 apply to the term "sample" as used in the phrase "sample polynucleotide" in the claims?

MS. RAYMOND: Objection. Form.

A. I haven't fully analyzed the term "sample polynucleotide." If you can point me to places in Dr. Cooper's report where he offers a construction, I'd be happy to review it and comment on it.

Q. No. My question was different.

You give a definition for the term "sample" that you used in your analysis.

Did you use that definition of "sample" as it relates to the phrase "sample polynucleotide" in the claims?

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MS. RAYMOND: Objection to form.

A. So I didn't perform an analysis of the term "sample polynucleotide" as laid out in the claims of the '981 patent because I don't think -- I didn't believe it was necessary to respond to Dr. Cooper's arguments.

Q. You only included the constructions of the terms in your report where you thought it was necessary in view of Dr. Cooper's report; is that correct?

A. My exercise in carrying out this work was to respond to Dr. Cooper's analysis presented in his report. I don't have his report sitting in front of me right now, and I don't recall if he offered any independent or contradictory constructions. But the constructions I used are laid out in paragraphs 51 through 52 of my report.

Q. And how did you select which terms to include construction of in paragraph 51?

A. Again, I don't have Dr. Cooper's report sitting in front of me. I don't recall why these were selected.

Particularly, MID I believe it was important because the MID laid out in the claims of the '981 patent have a very particular structure, particularly in the claims.

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And the terms "barcode" are often used loosely in a variety of different ways in the '981 patent.

Dr. Cooper specifically talks about different types of barcodes, so the barcode that appears in Linnarsson, the batch-stamp plus barcode that appears in McCloskey, another type of barcode that appears in Hug. And so this is just to be very clear about what the MIDs were in claim 1 since that was at the heart of Dr. Cooper's arguments.

I don't recall exactly why I included "sample" here. I'd have to look back at Dr. Cooper's report.

Q. If you turn to Linnarsson and specifically Figure 3.

A. All right.

Q. And with respect to the molecule shown in Figure 3, that includes a cell tag; is that right?

A. That's what the figure says, yes.

Q. And what is the purpose of the cell tag in the molecule of Figure 3?

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A. So Figure 3 is only showing the construction of -- if you look at the description of the drawing that occurs on page 5 of Linnarsson, it's an example of a template switching oligonucleotide that has a five prime amplification primer sequence or (APS), a cell tag and a three prime sequence for template switching.

So, I mean, here, it's just labeling the components of the TSO. So in the figure itself, it's not ascribing any particular use to the cell tag, but I believe the cell tag is described in greater detail in Figure 11.

But, in general, the cell tag is used to distinguish each of the 96 wells -- a unique cell tag is assigned to each of the 96 wells in Linnarsson's 96 well plate and is used to identify the cell which is placed into that plate and the molecules derived from it.

Q. And if you turn to Figure 4 --

A. All right.

Q. -- do you have opinions regarding Figure 4 in this matter?

MS. RAYMOND: Objection. Form.

A. So -- go ahead.

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Q. I didn't see any reference to Figure 4 in your report, and I just want to know, do you have a reason for that?
A. So I didn't call Figure 4 explicitly. The caption for Figure 4 says it "...shows an example of cDNA synthesis primer, (CDS) comprising a 5' amplification primer sequence (APS), a cell tag and a 3' RNA complementary sequence (RCS)." And that's showing the different segments here.
This is, I believe, something that Linnarsson refers to as an alternate form of his CDS. It's not the form of the CDS that appears in either Figure 11 which provides an outline of the method that Linnarsson is describing in his patent. And this method in Figure 11 is also consistent with the two examples, Example 1 and 2 that appear in the patent.
Dr. Cooper notes, and I believe this is stated in Linnarsson, that a cell tag could be used in either the TSO or the CDS. So to the extent that Dr. Cooper makes arguments about the cell tag appearing in the CDS, I discussed that in my response without necessarily explicitly calling out Figure 4.

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Q. Do you know what the sequence is of the cut site used by Linnarsson?
A. So by "the cut site," I believe you're referring to BST CL1. [sic] Off the top of my head, I don't know the recognition sequence for that restriction enzyme.
Q. Did you look it up?
A. At some point, I probably did, but I don't recall what it is.
Q. And why did you want to know what the sequence is of the cut site?
A. There are different types of restriction enzymes and I believe I looked just to make sure it was a Type 1 restriction enzyme.
Q. And how did you look it up?
A. NEB is the biggest supplier of restriction enzymes, New England Biolabs. I think I probably looked it up on their website; I don't recall. The sequence of the BST CL1 [sic] recognition site wasn't important for the analysis I performed, and I don't believe Dr. Cooper made any particular arguments about it.
Q. And in terms of Figure 3, how come there's no cut site in Figure 3?

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A. You would have to ask Dr. Linnarsson why he didn't include it there and why he included the cell tag in Figure 4. He doesn't offer an explanation and I can't speculate.
Q. Do you know why there's no cut site in Figure 4?
A. Again, you would have to ask Dr. Linnarsson why he chose not to do that.
Q. Do you have any understanding why there's no identification of biotin in Figures 3 and 4?
A. Again, I can't comment on why Dr. Linnarsson made certain choices in preparing his figures. I have no way of knowing.
Q. Using the Illumina Genome Analyzer that you had at Dana-Farber, were you able to create cDNA libraries without fragmentation?
MS. RAYMOND: Objection. Scope.
A. I don't recall all the details of the cDNA library sequencing that I did at Dana-Farber. I don't believe it's germane to the arguments that Dr. Cooper presents in his report and that I responded to.
Q. Right, but my question wasn't any of that. It was just when you -- or maybe you

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don't remember, but when you used cDNA libraries with the Illumina Genome Analyzer, did you use fragmentation?
MS. RAYMOND: Objection. Scope.
A. So I don't recall all of the details. We may or may not have fragmented molecules. It would depend on the particular application we were interested in.
Q. And which applications would you tend to have wanted to fragmented cDNA libraries?
MS. RAYMOND: Objection to form. Scope.
A. Again, there are a lot of reasons why one might or might not do particular things in a particular laboratory setting. Thinking back to what I was doing 15 years ago, yeah, I haven't thought back and done that analysis and I'm not prepared to do it right now.
Q. Yes, it wasn't an analysis. It's a memory.
Let me ask it this way: When you were creating cDNA libraries, would you be more inclined to use fragmentation if the sequences were longer?
MS. RAYMOND: Objection to form.

A. In creating cDNA libraries, you get typically cDNA -- you get all-comers, so long and small cDNAs. Again, it would depend on the particular application and what one wanted to do.

There are a whole host of different experiments. You could do surveys of all the RNAs. You can do analyses of a subset. You can do mutational analysis. It would depend on the particular goal of the experiment.

Q. For the experiment example that Linnarsson includes, do you have an understanding why he would use fragmentation for that cDNA library, based on your review of Linnarsson?

A. So there are a variety of different reasons why Linnarsson may have fragmented the molecules. Probably the simplest to understand is that Linnarsson eventually uses the biotin to bind the ends of the molecule to streptavidin beads, and then uses BTS CL1 to release the five prime fragment ends from the beads and then does further processing in preparation of doing sequencing.

So part of it is to release the five

prime end as part of the overall strategy to release the five prime ends. There are other reasons why one might choose to do that. Again, it would depend on a particular downstream goals and applications.

Q. In the example in Linnarsson where he uses biotin, do you have an understanding as to why he uses biotin?

MS. RAYMOND: Objection. Scope.

Q. Let me ask the question again.

Based on your understanding of the Figure 11 example in Linnarsson, why -- what is your understanding is the purpose of using biotin and then the cutting site and attaching it to the bead and doing the wash and then cutting it?

MS. RAYMOND: Objection. Form.

A. So I think you got the order wrong and I lay out it doesn't bind and cut, it cuts and then binds. And I lay this out starting with the detailed description of Figure 11 that begins on page 19.

On page 22, I talk about Panels C and D, and I've sort of grouped them together.

So Panel C -- and, again, some of

this is reflected in Linnarsson's description of Figure 11. So Panel C is a step that involves PCR using single primers. And in that reaction, the single primers are biotinylated. The fragments are then cut, in Figure D, with I believe two enzymes. I believe that's what he -- or, actually, I don't recall. I think maybe one enzyme. I'm thinking of McCloskey where there are two enzymes. And I'd have to look and see -- oh, I'm sorry, not with restriction of enzyme, but with DNAs. So it's a general enzyme that digests DNA. And he refers to this as a fragmentation step that randomly cleaves the double-stranded DNA. And he then describes binding the two ends to streptavidin beads. Streptavidin beads are typically magnetic beads coated with streptavidin.

So you can bind both ends of these fragments and then wash away the fragments that aren't bound. So the internal fragments that were cut with DNAs now are washed away.

And what Linnarsson then describes in Panels E and F is the binding of these fragments in Step E using BTS CL1 to cut the BTS site and release the barcode, and then doing some

additional steps to put on P1 and P2, which, if I recall correctly, are the sequencing primers that are used.

So there are a variety of different reasons why one would do this. One of them is simply to be able to release the five prime ends, which if both the five prime and three prime ends were bound, if one were to cut with BTS CL1, the molecule of the cDNAs, the double-stranded cDNAs would still be attached to the streptavidin beads, so you couldn't carry out the Steps E and F.

Q. My question is maybe simpler, which is why use biotin at all and perform a wash?

A. There are a variety of different reasons, including the fact that he was very interested in purifying these segments of the five prime end, those segments containing the cell tag. These internal segments wouldn't contain the cell tag. They would interfere with -- they would participate in the end repair and ligation of the primers for sequencing. And they -- I mean, there could be a variety of other reasons why Linnarsson chose to do it, but the simplest reason for including biotin is

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because he was interested in doing a purification to isolate the fragments that included the cell tag.

Q. Let me ask you this question: If you were creating a cDNA library without fragmentation, would there be any reason in Linnarsson to use biotin and the wash?

MS. RAYMOND: Objection. Form. Scope.

A. Linnarsson describes the use of biotin, and so I'm not going to speculate on other things that Linnarsson could have done. This is what Linnarsson did. And so in the context of Dr. Cooper's arguments that Linnarsson plus McCloskey is invalidating prior art, I relied on what Linnarsson described as his overall approach.

Q. Let me ask it this way: In Linnarsson, is there any purpose for the biotin and the BTS sequence other than to support the fragmentation approach?

MS. RAYMOND: Objection. Form. Scope.

A. Biotin with streptavidin beads is commonly used as a way of purifying molecules.

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Biotin and the use of BTS CL1 is used to cleave and then further analyze the five prime fragments. So it's what Linnarsson describes as doing.

I haven't done an analysis to think about other approaches that Linnarsson could have used, and Dr. Cooper doesn't provide any speculation about that that I was in a position to respond.

Q. Do you have any -- have you performed any analysis as to what the purpose might be for biotin in the BTS site and the streptavidin beads in Linnarsson beyond supporting fragmentation? Have you done an analysis of that?

MS. RAYMOND: Objection to form. Scope.

A. So I don't believe Dr. Cooper offers any theories as to the use of biotin and BTS CL1 that would differentiate it from what Linnarsson actually describes and proposes.

As I mentioned, the use of biotin and streptavidin beads is a common approach for purifying the DNA samples.

Q. Do you have an opinion as to whether

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that's why Linnarsson uses biotin and BTS sequence in his experiment is for purification or do you have an opinion one way or the other on that?

A. So Linnarsson doesn't describe in detail why he does it. I haven't performed a detailed analysis of all the reasons why. But if we read beyond Figure D, if we go to Figures E and F, if one didn't purify the samples and then in going from E to F did end repair and ligation of different molecules, different primers, all of those molecules would be labeled. All of the molecules would be sequenced.

There are many more molecules potentially that represent these internal fragments and the other fragments. Those internal fragments, the way Linnarsson describes his approach, would not contain the cell tags. They don't have cell tags.

And so there's -- you know, Linnarsson is what Linnarsson is. And I'm not in a -- I wasn't required to speculate about other things Linnarsson could have done or why Linnarsson did different things, because

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Dr. Cooper didn't offer any suggestions as to alternative implementations of Linnarsson.

Q. If you turn to page 46 of Linnarsson.

A. All right.

Q. In the method of claim 1, the cDNA is synthesized from mRNA -- well, let me start again.

Do you have claim 1 in front of you?

A. Yes, I do.

Q. In Linnarsson, the cDNA library is generated according to the method of claim 1 without any fragmentation or use of biotin, correct?

MS. RAYMOND: Objection. Form.

A. So I don't believe Dr. Cooper provides an analysis of claim 1 in his report. I haven't included an analysis -- consequently, I have not included an analysis of each step of claim 1 or each step of the claimed invention in my report. And so I haven't performed a sufficient analysis to be able to tell you exactly what is encompassed in the scope of claim 1 as presented in Linnarsson.

Q. So did you review claim 1 when you reviewed Linnarsson?

PARSE EX. 1057.039

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A. I reviewed it, but I didn't review it in great enough detail to answer your question, because Dr. Cooper didn't provide any opinions that I was required -- that required a response.
Q. Okay.
But you can see in claim 1, there's no reference to fragmentation, correct; there's no fragmenting step?
A. I haven't read all of claim 1, and I haven't read it in the context of the written description to fully understand what Linnarsson is claiming. And I don't believe Dr. Cooper offered any analysis and so I'm not prepared to offer an opinion today as to what it encompasses.
Q. Okay.
So you're saying you can't answer the question whether there's a fragmentation step in claim 1 of Linnarsson?
A. I haven't analyzed claim 1 to the extent where I would feel confident offering an informed opinion sitting here today.
Q. All right.
And you understand that the claims of the Linnarsson patent are part of the disclosure

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of the patent application?
A. I believe that's correct, yes.
Q. And then in terms of -- can you see that there's no attachment using biotin in Claim 1?
A. Again, I haven't done that analysis and I believe if Dr. Cooper felt it was important to single out claim 1, he would have done that in his declaration. And I didn't see anything in his declaration making that argument, and so I'm not in a position to render an opinion because I have nothing to respond to.
Q. And with respect to claim 10, do you see where it says that both the CDS and the TSO include a tag?
A. Yes, I do.
Q. Okay.
So why would the CDS be tagged if it was never -- if it was attached to a streptavidin and it never cut for sequencing?
A. So I haven't done that analysis. Dr. Cooper makes statements about either the CDS or TSO including a tag, but Dr. Cooper doesn't provide an analysis as to how the TSO tag would be read given his description of Linnarsson.

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And his assertion that Linnarsson, as he describes it, represents invalidating prior art.
Q. If you could turn to Figure 11, if you could, please.
A. Sure.
All right.
Q. In terms of Figure 11, did you -- it's the other page?
A. It's right here (indicating). I'm looking at the caption.
Q. Okay.
For Figure 11, did you believe that the CDS is released for sequencing according to this method?
A. So the CDS is the three prime fragment end. The way it's depicted in this figure and in both Example 1 and Example 2, which corresponds to this figure, there is no potential release of the CDS as a double-stranded molecule to then be processed through Steps E and F.
Q. And why would there be a cell tag if it was never going to be sequenced?
A. So Dr. Cooper doesn't provide any arguments as to how one would modify what's

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presented in Linnarsson to sequence that. I can speculate about ways in which it might be done. But to the extent that Dr. Cooper doesn't provide such an argument, I don't have anything to respond to.
Q. For your opinion that -- do you believe Linnarsson requires fragmentation to create cDNA libraries, according to his method?
A. So if you use the term "cDNA library," this is a term that Dr. Linnarsson actually defines in his patent on page 12, starting at line 4. The term "cDNA library" refers to a collection of cloned complementary DNA, cDNA fragments, which together constitute some portion of the transcriptome of a single cell or a plurality of single cells.
So Dr. Linnarsson's description of the cDNA library, and the library is what is eventually sequenced, consists of cDNA fragments. So it at least suggests that the cDNA library has undergone this fragmentation step.
Q. But do you have an opinion one way or the other?
A. As to?

PARSE EX. 1057.040

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Q. Do you have an opinion one way or the other as to whether Linnarsson requires fragmentation for the creation of cDNA libraries using his tagging methods?
A. So I haven't done that analysis. If I look at Figure 11 and Examples 1 and 2, which are the examples presented, Linnarsson's method uses this fragmentation step using DNAs and this is consistent with his definition of the term "cDNA libraries" which appears on page 12.
Q. If you go to claim 27, please.
A. Of?
Q. I'm sorry. Of Linnarsson.
A. All right.
I'm there.
Q. And you see that there's a claim step added. It says, "Wherein the processing comprises fragmenting the cDNA library"?
A. I see that in claim 27.
Q. Okay.
And you understand that's a dependent claim?
A. Yes, that's my understanding.
Q. And you understand that the normal claim construction rules are that a dependent

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claim is presumed to add something that is not otherwise required by the independent method claim?
MS. RAYMOND: Objection. Form. Scope.
A. So my general understanding of dependent claims is that they add some additional requirements, but I haven't done a full analysis of the claims, the independent and dependent claims appearing in Linnarsson.
Q. When you reviewed claim 1 of Linnarsson to understand that this patent disclosure for purposes of your obviousness analysis, did you recognize that there was no fragmenting step in claim 1 in terms of the generating the cDNA library?
MS. RAYMOND: Objection. Form.
A. So I don't believe Dr. Cooper presented a claim-by-claim analysis of the Linnarsson patent. My report was responding to the theories presented by Dr. Cooper. And so to the extent that Dr. Cooper didn't perform such an analysis, I didn't feel it was incumbent upon me to perform such an analysis. I can only respond to what Dr. Cooper offered as his

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theories for obviousness.
Q. And you understand that part of Dr. Cooper's opinion was based on Figure 4 of Linnarsson?
A. I don't have Dr. Cooper's --
(Exhibit 1002 was produced.)
Q. I'd like to show you Exhibit 1002.
A. Thank you.
All right.
Q. You understand that Dr. Cooper for his opinion relied on the Linnarsson disclosure at least in part based on Figure 4?
A. So where does Dr. Cooper refer to Figure 4?
Q. Here is one example, page 79 at paragraph 165.
Do you see the reference to Figure 4?
A. Yes, I do.
Q. And then I think in 166 and so forth.
Did you evaluate Linnarsson as a whole as to whether the Linnarsson method claim 1 without fragmentation was something that was taught by this reference, including Figure 4?
MS. RAYMOND: Objection. Form.
MR. REINES: Well, that seems like a

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valid objection. Let me ask the question again.
Q. Did you evaluate whether Linnarsson, Figure 4 -- let me start again.
How come you never addressed Figure 4?
MS. RAYMOND: Objection. Form.
A. So I don't explicitly call out Figure 4 in my analysis, but I do talk about some of Dr. Cooper's suggestions that Linnarsson's method as presented in Figure 11 in the examples could be modified.
One of the suggestions that --
Q. I just asked about your reference to Figure 4.
It doesn't refer to Figure 4 there, does it?
A. It refers to the CDS, and the CDS is represented in Figure 4.
Q. Okay.
A. And I can describe in detail why Cooper's suggestions about modifying the CDS fall flat.
Q. Understood.
Now, with respect to claim 1 and

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whether -- did you ever evaluate whether the claim 1 method, which doesn't include a fragmentation step, whether that covers the embodiment of Figure 4?

A. So that would be Dr. Cooper's burden. Dr. Cooper doesn't provide a claim-by-claim analysis of Linnarsson. Dr. Cooper makes arguments largely about what's presented in Figure 11. And so in responding to Dr. Cooper, I responded to his assertions about Figure 11 and how Figure 11 might be modified.

If I look at Figure 4, Figure 4 presents the CDS without a BTS sequence and how that would be released in sequence, how it would be joined to -- Dr. Cooper makes some suggestions that you could put a batch or some kind of McCloskey's barcode on the TSO and something else on the CDS and the TSO, but doesn't really explain how those pieces would be put together.

So, you know, Dr. Cooper doesn't perform that analysis and so I didn't do it because I didn't have to respond to this analysis.

Q. You understood that Dr. Cooper stated

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that Figure 4 approach of Linnarsson was an alternative to the approach of Figure 11?

A. Could you point me to that, please?

Q. Yes. Page 79. Hopefully, it's still open --

A. Yes.

Q. -- to that.

A. I'm sorry, where on page 79?

Q. I'm going to ask the question again.

A. All right.

Q. Maybe you can follow me.

When Dr. Cooper -- when you reviewed Dr. Cooper's report in preparing your own, you understood that he was stating that Figure 4 was an alternative approach to Figure 11, correct? This is at paragraph 165 where he says "alternatively"?

A. So I don't believe he's proposing it as an alternative to Figure 11. I believe he's proposing it as an alternative to putting the cell tag on the TSO. But Dr. Cooper doesn't suggest that -- or doesn't provide a mechanism by which the cell tag -- by which the CDS presented in Figure 4 would actually be substituted into Figure 11 in a way that would

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facilitate the downstream analysis.

Q. So when you reviewed Dr. Cooper's report, you didn't understand that he was stating that there was an alternative disclosure in Figure 4 that was different from what was in Figure 11; is that correct?

MS. RAYMOND: Objection to form.

Q. Can you answer "yes" or "no" whether you understood that?

A. He's not presenting it as an alternative to Figure 11. He's presenting the CDS modification as an alternative to the TSO modification, but what he shows doesn't actually correspond --

Q. Yeah, I'm not asking --

A. I'm sorry --

Q. If you want to argue it --

A. -- I'm not --

Q. -- instead of answering my question.

A. I'm answering your question, all right?

You're -- I think you're misrepresenting what Dr. Cooper is presenting.

Q. All right.

Well, it's here and everyone can read

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it.

A. Yes.

Q. Okay.

A. And he's presenting a modification to the CDS is a modification to the alternative of the TSO. All Figure 3 or Figure 4 shows is a sequence that has an oligo (dT), a cell tag and the universal primer sequence. It doesn't include all the steps in Figure 11. So if you're telling me this is an alternative to Figure 11, that doesn't make any sense.

Q. And in terms of why you never referenced Figure 4 in your report, it's because of that, because you didn't view it as an alternative disclosure to Figure 11; is that right?

MS. RAYMOND: Objection to form. Foundation.

A. So I think you're mischaracterizing all of my prior testimony and what's represented in my report.

Q. Okay.

We have your report so we're able to see it.

A. Yes. And I discussed Dr. Cooper's

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theory that one could modify the CDS to include a cell tag and present why that is inconsistent with what's disclosed in Figure 11.

Q. In Figure 4, a CDS has a cell tag, correct?

A. It has a cell tag, but it doesn't include --

Q. I'm just asking you -- you need to just answer my question at some point.

In Figure 4, the CDS has a cell tag? It's a simple question. Let's test how responsive you can be.

Will you agree with that?

MS. RAYMOND: Counsel, please. Treat my witness with respect.

A. I agree that it includes a cell tag, but it lacks other key features that would be required to substitute it into Figure 11. And I'd be happy to describe those in detail for you.

Q. Okay.

And you understood that Figure 4 was just a substitution that you would make to Figure 11?

MS. RAYMOND: Objection. Form.

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A. Dr. Cooper asserts that without any explanation. Dr. Cooper fails to address the deficiencies that appear in Figure 4, including the lack of a biotin and the lack of the BTS sequence that would be required if one were to even imagine trying to do this. And Dr. Cooper provides no analysis of -- or no description of how this would be substituted in or how it would work in the overall workflow to render the claims of the '981 patent obvious.

Q. And do you have an explanation for why there's no biotin or fragmentation in the disclosure of Figure 4?

A. Figure 4 is not describing a workflow. Figure 4 is describing an oligonucleotide. So this is Dr. Cooper's burden and Dr. Cooper failed to provide any description of how this would function.

Dr. Cooper suggests putting part of the tag or taking McCloskey's tag and splitting it between the TSO and the CDS, but doesn't describe how that would work, how it would all be put together.

So Dr. Cooper throws out a lot of little pieces, but he's basically got a bowl of

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fruit without a bowl to hold it together.

Q. And that's the best you can answer that question, correct?

A. It wasn't my burden to describe how Dr. Cooper should have presented any kind of argument. Dr. Cooper fails to present a cogent argument that I can respond to.

Q. Would you agree that Figure 4 is a tag polynucleotide?

A. It's a polynucleotide that contains a cell tag, but it fails -- it's a representation of a polynucleotide that carries a cell tag, but Dr. Cooper fails to disclose how this would work in the workflow of Figure 11.

Q. How does -- what does claim 26 -- excuse me, claim 27, which states -- for Linnarsson claim 27 where it adds fragmenting the cDNA library.

Do you see that?

A. Yes, I do.

Q. What does that add to the claim 1?

A. Dr. Cooper didn't present an analysis of the claims of Linnarsson in the context of representing invalidating prior art, and so I haven't done that analysis because I didn't have

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to do it to respond to Dr. Cooper.

Q. And did you -- when you reviewed Linnarsson, did you have any reaction at all to the fact that he described his method for generating a cDNA library not to include fragmenting or the use of biotin?

A. I didn't do that kind of detailed analysis because it wasn't required to respond to Dr. Cooper's arguments about obviousness.

Q. And you consider that a detailed analysis; is that correct?

A. I performed an analysis to the extent that I understood the patent well enough to respond to Dr. Cooper's arguments.

If Dr. Cooper had presented arguments about claim 27 - and I don't believe he does - then I would have responded to them in kind.

Q. Did you attempt to respond as best you could to Dr. Cooper's point that the Linnarsson disclosure describes a broader approach that doesn't require fragmentation?

MS. RAYMOND: Objection. Form.

A. So if you can point me to where that occurs in Dr. Cooper's declaration, I'd be happy to comment on it.

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If we look at Figure 11, if the molecules in Figure 11 are not fragmented, then if I go from Step D to Step E, if I cut with BTS CL1, all it's going to do is release one end of the molecule not the other, and so the double-stranded cDNA molecule would still be bound to the streptavidin beads and the rest of the workflow wouldn't be possible.

Q. But you understand that Figure 11 of Linnarsson is just one embodiment of the inventions -- of that application?

A. Dr. Cooper doesn't provide an explanation for how this would work without fragmentation, and so I have nothing else to respond to but Dr. Cooper's arguments.

Q. Right.

I'm just asking, did you understand that Figure 11 is just one embodiment of the inventions of the Linnarsson patent document?

A. Dr. Cooper doesn't discuss any other embodiments and so I had nothing else to respond to.

Q. But you do understand that it's one embodiment?

A. I haven't done an analysis of all of

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the patent to the extent that I've looked at all the possible embodiments. Linnarsson may or may not describe it that way.

Q. In reviewing Linnarsson for formulating your opinions, did you consider any embodiment of Linnarsson other than Figure 11 embodiment?

A. I considered what Dr. Cooper presented. Dr. Cooper suggests adding, for example, a cell tag to the CDS, and so I considered that. That doesn't appear directly in Figure 11.

Dr. Cooper doesn't describe how that would work, and so I responded to Dr. Cooper's argument to the best of my ability, given what Dr. Cooper presented. I can't respond -- I was not in a position to respond to what Dr. Cooper might have said or might not have said -- or might have said. I can only respond to what he did say.

Q. Did you consider any embodiment of Linnarsson other than the Figure 11 embodiment in performing your analysis?

A. Again, Dr. Cooper suggested some modifications to what's in Figure 11 and I

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responded to those.

To the extent that Dr. Cooper presented arguments about other potential embodiments, I responded to those. I didn't respond to things Dr. Cooper didn't present.

Q. I understand that.

Did Dr. Cooper rely on other embodiments other than Figure 11?

A. So I'm not sure exactly what you're referring to. Dr. Cooper suggested combining Linnarsson with McCloskey, so I don't know if you would consider that another embodiment.

Dr. Cooper suggested including a cell tag in the CDS rather than the TSO or in both the CDS and TSO. To the extent that he proposed that, I responded to it.

Q. Do you know whether Dr. Cooper relied on Figure 4 for his opinions in this case?

A. Dr. Cooper presents Figure 4. And to the extent that he presents it, you may consider that, meaning that Dr. Cooper relied on Figure 4, but Dr. Cooper doesn't address the deficiencies that appear in Figure 4.

Q. And in terms of any explanation you have for not directly addressing Figure 4, why

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don't you provide those now if you have them?

Why didn't you cite Figure 4 in your report?

MS. RAYMOND: Objection. Foundation.

A. So, again, I believe you're mischaracterizing what's presented in my report. I'm happy to read through the entire report to find all the places where I discuss the limitations that appear in Figure 4. I didn't directly call them out, but responded to Dr. Cooper's arguments and felt that there was no reason to call out Figure 4 explicitly. But I will point you to paragraph 59 in my report, beginning on page 37.

Q. You are mischaracterizing --

A. Repeatedly, Dr. Cooper's theory of modifying Linnarsson's CDS using McCloskey's teaching of a barcode fails for the same reason. All right. So the CDS with now some kind of oligonucleotide.

"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. As discussed above, only the TSO has a BTS sequence. The CDS does

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not." And the CDS is what you're referring to in Figure 4.

"The 3' fragment (the CDS end) remains bound to the beads in Panel E." Right? And I cite to the patent, the "'(3' fragments remain stuck on the beads.)'"

"Each of the 3' fragments bound to the beads in Panel E is not released for sequencing in Panel F. And nowhere does Linnarsson describe any releasing any 3' fragment from the bead or sequencing it. Thus, Dr. Cooper's theory relying on the Linnarsson-McCloskey combination fails because any tagging in the CDS would be on the 3' fragment, and it would not be sequenced. [as read]

All right. So you are absolutely correct, I didn't cite to Figure 4, but I'm explicitly describing Dr. Cooper's modifications or proposed modifications of the CDS appearing in Figure 11 to include some type of tag and that's what Dr. Cooper points to in Figure 4.

MS. RAYMOND: I think we've been going for a chunk more than an hour now. If we can take a break.

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Q. You mischaracterized my question. My question was just why you didn't cite Figure 4. So I move to strike all your argument.

MR. REINES: If you want to take a break now, that's fine.

VIDEOGRAPHER: Off the record at 3:04. This ends Media Unit No. 4.

(Recess.)

VIDEOGRAPHER: On the record at 3:18. This begins Media Unit 5 in the deposition of John Quackenbush, Ph.D.

BY MR. REINES:

Q. If you turn to Figure 11 of Linnarsson.

A. All right. I'm there.

Q. The polynucleotide at the bottom at F, do you see that?

A. I see the representation of it, yes.

Q. That's tagged, correct?

MS. RAYMOND: Objection to form.

A. So it has a type of tag. It has Linnarsson's barcode, which I think he refers to as a cell tag.

Q. Now, turning to the claims of the '981 patent, claim 1. If you look at (b), little (i), do you have that in front of you?

A. Yes, I do.

Q. Okay.

It says, "a sequence from a sample polynucleotide of the plurality of sample polynucleotides..."

Do you see that?

A. Yes, I do.

Q. Does that require, in terms of your understanding of the claim, that the entire sequence of the sample polynucleotide is included in the tagged polynucleotide?

MS. RAYMOND: Objection. Form. Scope.

A. So I haven't done that analysis. I don't believe Dr. Cooper offered any opinions regarding that that I was required to respond to, and so I'm not prepared to offer an opinion about that sitting here today.

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

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

MS. RAYMOND: Objection. Form.

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

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

MS. RAYMOND: Objection to form.

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

MS. RAYMOND: Objection. Form. Scope.

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

Q. Okay.

Would a user of the Linnarsson process benefit from the advantages promoted by McCloskey of the barcodes that are disclosed in McCloskey of identifying redundant sequences?

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MS. RAYMOND: Objection to the form.
A. So I'm not sure what you're asking me. I'm not sure that Dr. Cooper presented an analysis of advantages and so I haven't performed that analysis. I'm not prepared to offer an opinion today.
Q. I want to ask you -- well, now one of your opinions is that "The biotin on the 5' end in the CDS would block ligation of tag(s) to the CDS." You state this in paragraph 89?
A. Yes, correct.
Q. And would a person of ordinary skill in the art in 2009 know that you couldn't ligate a barcode to the biotin on the five prime end of the CDS?
MS. RAYMOND: Objection. Form. Scope.
A. So a person of ordinary skill in the art would understand that if we look at -- the easiest way to understand it is to look at element D of Figure 11, or maybe it's B. Both the five prime and three prime ends of the fragment being analyzed have biotin molecules attached. That biotin is typically attached to the five prime phosphate. And ligation requires

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the formation of a phosphodiester bond, as I describe in paragraph 89. So if there's already a biotin attached, you wouldn't be able to create that bond, hence, ligation wouldn't occur. And a person in 2009 would have understood this.
Q. Would a person of skill in the art with the knowledge of the techniques described in your report in paragraph 25 know how to ligate a tag to the CDS before placing biotin on the five prime end?
MS. RAYMOND: Objection. Scope.
A. So I don't think Dr. Cooper presents any analysis of such an approach. Dr. Cooper -- ligation is a reaction that occurs with double-stranded DNA molecules. And sitting here today, I can't conceive of some way in which that would happen.
The biotin appears early in the process in this -- on the CDS side, in the construction of the CDS; and on the TSO side, in the PCR step; and in both ends, in the PCR step.
Dr. Cooper doesn't provide any suggestion for how that would be handled, and, therefore, I'm not in a position to speculate on

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how that might have been done.
Q. Do you have an opinion whether or not a person skilled in the art would be able to do that?
MS. RAYMOND: Objection. Scope.
Q. And by "that," I mean ligate a tag to the CDS before placing biotin on the five prime end?
MS. RAYMOND: Objection. Scope and form.
A. So biotin isn't placed on the five prime end. Biotin is incorporated into the five prime end in the PCR step between Step B and Step C in Figure 11.
Dr. Cooper doesn't offer any alternative which would allow such ligation. And so while I can speculate about different ways in which it could possibly be carried out, I wouldn't know all the ramifications unless I thought about that more deeply, and so I'm not prepared to speculate today.
Q. Do you have an opinion one way or the other as to whether a person of ordinary skill in the art in 2009 would be able to ligate a tag to the CDS before putting biotin on? Just that

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question; not how it would be done, but whether it would be within the skill in the art?
MS. RAYMOND: Objection. Scope and form.
A. So I don't understand what you mean by "before putting biotin on." The biotin is on the primer itself, and so in going from Step B to Step D when those double-stranded molecules are made, the biotin is incorporated.
Q. We'll address that case as well. But right now I'm just asking you, would a person of skill in the art know how to adjust the process of Linnarsson, such that the tag could be ligated to the CDS before biotin is introduced to the molecule at the five prime end?
MS. RAYMOND: Objection. Form. Scope.
Q. Just whether it's within the scope of the skilled level in the art, whether you have an opinion on that.
A. So it's a complex question that doesn't have a "yes" or "no" answer. Dr. Cooper doesn't provide an explanation for how that would be done.
While one could speculate about

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different ways that it could be done, potentially be done, one would have to think about whether or not it would actually be compatible with the rest of what's disclosed in Linnarsson or actually prevent Linnarsson from carrying out the method that's proposed here.

So I haven't done that analysis because Dr. Cooper hasn't presented a scenario in which it would have.

Q. Is that as responsive as you can be?

MS. RAYMOND: Objection. Form.

A. If you ask the question again, I'll try to respond more clearly.

Q. Okay.

Leaving aside how it would be done, do you have an opinion, sitting here now, as to whether it would be within the scope of the competency of a skilled artisan in 2009 to modify the Linnarsson process so that the tag is ligated before biotin is added?

MS. RAYMOND: Objection. Form and scope.

A. You're asking a question which doesn't have a simple answer and I wish I could give you a simple answer, right. You're saying

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biotin is added. It's not physically added to a double-stranded molecule. It's a part of the synthesis of the double-stranded molecule the way Linnarsson describes it. So that's the first place where I'm having a trouble giving a simple "yes" or "no" answer.

Second, while you can imagine ways of removing -- potentially removing the biotin through chemical reactions, I haven't thought through whether or not that would destroy will the rest of the molecule in some way that you couldn't analyze it.

So, you know, it would really depend on the application and what you wanted to do. And I don't have a simple answer sitting here today.

Q. Do you have any reasonable answer?

MS. RAYMOND: Objection to form.

A. I think I gave you a very reasonable answer. You presented an incomplete hypothetical and it's impossible for me to respond to.

Q. Do you have an opinion whether it's within the scope of the -- let me start again.

Do you have an opinion as to whether

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it's within the level of skill in the art to modify the Linnarsson process to remove the biotin, ligate the tag to the CDS and then put the biotin back on the five prime end?

MS. RAYMOND: Objection. Scope.

A. So this is outside the scope of what I was asked to do here today. Dr. Cooper presented an obviousness argument based on the combination of Linnarsson and McCloskey. And in my report, I've done a detailed analysis of Dr. Cooper's suggestions.

Now you're asking me to invent a completely new process different from Linnarsson, and I haven't sat down to do the analysis of how one might reinvent Linnarsson because that's outside the scope of understanding whether or not Linnarsson, as presented, is invalidating prior art.

Q. Would a person of skill in the art have the competency to do it? Do you have an opinion on that?

MS. RAYMOND: Objection. Scope.

A. It would depend on a lot of factors, and I'm happy to reiterate those as I did previously.

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I haven't done an analysis to understand whether or not it would actually completely disrupt the process in Linnarsson such that it would not carry out its stated goals.

Q. Can you be any more direct than that?

MS. RAYMOND: Objection to form.

A. I think I've been as direct as possible. You could do lots of different things, right. You could ask me, you know, is there a way to modify the wheels on my car and I could describe a way of modifying the wheels such that my car wouldn't run any longer, right. And, you know, you're asking me the same sort of questions. Can I do something here or could someone have done it? And the goal would really be to understand if one carried out a whole host of biochemical processes, whether or not those biochemical processes would have other unexpected consequences and whether or not it would actually disrupt the entire rest of the workflow.

Q. Based on your review of the '981 process -- excuse me.

Based on your review of the '981

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patent, do you have an understanding of how the reflex process works?
A. Generally, yes.
Q. Okay.
And can you identify anywhere in the '981 patent where it states that using two tags is inventive?
MS. RAYMOND: Objection. Scope.
A. So I don't believe Dr. Cooper presents arguments about the inventiveness of the '981 patent. Dr. Cooper presents arguments about obviousness, and I respond to Dr. Cooper's arguments. I didn't provide the analysis of the '981 patent to the extent that I can easily answer your question.
Q. Well, leaving aside what's easier, can you provide any other answer to my question beyond that?
MS. RAYMOND: Same objections.
Q. Given that, I'm going to withdraw the question given the time it's taking.
If you could turn to column 13, please.
A. Of?
Q. Of the '981 patent.

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A. Sure.
All right. I'm there.
Q. All right.
Do you see where it says, "The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology...cell biology, biochemistry and immunology, which are within the skill of the art."
Do you see that?
A. Yes, I do.
Q. Okay.
And do you understand that the use of tagging the MID, that the cell tag is within the skill of the art as a conventional technique?
Let me ask the question this way. Let me step back.
Did you analyze whether the '981 patent describes particular techniques as otherwise indicated? In other words, did you go through and determine what's otherwise indicated as not being conventional and what's just conventional?

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MS. RAYMOND: Objection to form. Scope.
Q. Let me ask the question again because I'm just asking you what you did.
Did you go through the '981 patent to determine where there was an indication that something was nonconventional?
MS. RAYMOND: Objection. Form. Scope.
A. So as we've discussed numerous times today, my goal in preparing this report and providing this deposition today was not to provide a full analysis of the '981 patent, but to respond to Dr. Cooper's arguments regarding obviousness.
So Dr. Cooper cites to three different references in presenting his arguments, but primarily Linnarsson and McCloskey. And so I've done a detailed point-by-point analysis of Dr. Cooper's assertions.
I haven't done the type of analysis that you're suggesting. I provide a summary on page 11, 12, 13, 14, 15, 16 and 17 of the '918 [sic] patent, and I'd be happy to walk through

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that to see if any of what I present here address the question you're asking.
Q. All right.
Now, with respect to ligation, if you could turn to column 6, please.
Do you see where ligation is described from line 15 to 32?
A. Yes.
Q. And you'll agree that ligation is described in column 6 of the '981 patent is described as a conventional prior art technique?
A. So I don't think they use the term -- I don't see the term "conventional" here. But ligation was a method that was well known in the art before the priority date of the '981 patent.
Q. Is there anywhere in the '981 patent where it indicates that ligating -- that ligation in terms of generating the tag polynucleotide is not conventional?
MS. RAYMOND: Objection. Form.
A. So I haven't performed a detailed analysis of the '981 patent with regard to what individual steps may or may not be conventional. I looked at Dr. Cooper's arguments about the '981 patent. Dr. Cooper offers arguments

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regarding the obviousness of claim 5 of the '981 patent. And claim 5 is the claim, you may recall, that says, "The method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction."

So ligation is used. Ligation is a method that was known in the art just like PCR was known in the art. But it's a way of carrying out claim 1.

Dr. Cooper doesn't present any arguments about ligation being nonconventional. But Dr. Cooper makes assertions about how one might modify Linnarsson through ligation. And Dr. Cooper's arguments about how that would occur are actually deficient, and I'd be happy to step you through those. I believe they begin on page 51 of my report and run through page 69.

Q. Please identify where in the '981 patent there's an indication that the use of ligation as specified in dependent claim 5 is not conventional.

MS. RAYMOND: Objection.

Q. If it's in here.

MS. RAYMOND: Objection. Scope.

A. So I'm not sure I understand your

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question. I haven't done an analysis of the '981 patent with regard to the conventionality of ligation. You'll recall that ligation is used in the dependent claim to carry out -- as one way of carrying out the invention described in claim 1. I'd be happy to look back at the invention in claim 1. But Dr. Cooper doesn't present any arguments about ligation being nonconventional. And so I haven't performed the analysis you're asking me to do now, and I'm not prepared to offer testimony today.

Q. If you turn to Cooper report at 239.

A. All right. I'm there.

Q. Actually, let's just go to 238; it would be easier.

A. Okay.

Q. Contrary to what you were saying, Dr. Cooper does state that it was well known and routine in the art to attach tag sequences using ligation.

Do you see that?

MS. RAYMOND: Objection. Foundation.

A. So I'm not contesting that ligation was a well-known method of attaching oligonucleotides. What I do contest in my

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report is Dr. Cooper's assertion that the tags could be ligated on to the method proposed in Linnarsson and still represent an effective analysis.

So the first reason we talked about that not being feasible is the presence of biotin.

The second reason that would not be feasible is that Linnarsson describes the use of BTS CL as an enzyme to cut the sequences and release them from the streptavidin beads, and anything that was ligated on outside of the BTS sequence would not have been then present in downstream sequencing.

So we can discuss Dr. Cooper's deficiencies and Dr. Cooper's arguments. Whether or not ligation -- I told you earlier ligation was a conventional technique, but, you know, you're asking me different questions about the inventiveness of the patents and Dr. Cooper's arguments.

So I wouldn't contest it's well known, but it just wouldn't work with Dr. Cooper's presentation of ligation as a way to represent invalidating prior art in the

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context of modifying Linnarsson and McCloskey.

Q. Was it well known and routine in the art to attach tag sequences using ligation in 2009?

A. It could be but not in the context of modifying Linnarsson in a way that would be functional given Linnarsson's approach.

Q. Okay.

Leaving aside your arguments, can you say something more than it could be? Was it, in fact -- well, let me see.

Dr. Cooper does, in fact, state that it was well known and routine in the art to attach tag sequences using ligation.

Do you contest that? It's in paragraph 238 as of the earliest priority date of the '981 patent.

A. I don't contest that, but not in the context of modifying Linnarsson.

Q. All right.

Now, with respect to the dependent claim 5, do you contend that dependent claim 5 of the '981 patent, which adds using ligation to generate the tag polynucleotides, do you contend that that's inventive?

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MS. RAYMOND: Objection. Scope. Form.

A. So I haven't done a thorough analysis of the inventiveness of the '981 patent. My assumption is because the patent issued, that the patent examiner found it to be inventive.

If you look at Claim 5, Claim 5 is more than just ligation; it's claim 1 with an additional limitation that ligation is the way that tags are attached. But I am not really in a position today to offer an opinion about a thorough analysis of the inventiveness of the '918 [sic] patent because my assignment and task here in presenting my report and providing my deposition today is to analyze Dr. Cooper's arguments. And Dr. Cooper's arguments about ligating fail for a variety of reasons, which I would be happy to step through with you.

Q. Is the addition of the ligation step of claim 5, is that inventive compared to the method of claim 1?

MS. RAYMOND: Objection. Scope. Form.

Q. I'm just talking about the addition of that ligation step, do you consider that to

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be inventive?

A. I haven't done a thorough analysis of claim 5 of the '981 patent. I'm happy to discuss the deficiencies in Dr. Cooper's ligation theories with regard to claim 5, but I'm not in a position today to render an opinion about individual inventiveness of individual steps in the patent.

Q. Did you consider the file history in preparing your opinions?

A. I did, to the extent that it was required for me to respond to Dr. Cooper's arguments about obviousness. And to the best of my recollection, I didn't have to rely on that in presenting the arguments that I'm here to testify about today.

Q. Did you review the file history?

A. I believe I did, but not in great detail because it wasn't required for me to respond to Dr. Cooper.

Q. Dr. Cooper stated that the applicant, when they were trying to identify inventive features of the claims compared to Linnarsson, didn't rely on the ligation step of claim 5.

Do you have any reason to dispute

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that?

A. I'm sorry, can you point me to where in Dr. Cooper's report?

Q. Yes. It's where we just were. Paragraph 236.

Do you deny that the applicant didn't identify ligation as an inventive feature when it was arguing for the claims over Linnarsson?

MS. RAYMOND: Objection. Scope.

A. Dr. Cooper himself does not perform a detailed analysis of the patent prosecution history. He simply says, "I have been informed by counsel that the Patent Owner did not dispute that Linnarsson discloses this limitation during prosecution."

So it's hard for me to respond to that without knowing what Dr. Cooper is referring to, other than someone working with him had informed him of that. He doesn't provide any documentary evidence to that.

Dr. Cooper doesn't appear to rely on that in presenting his arguments about claim 5. And I'd be happy to step through the places where Dr. Cooper's arguments about ligation are deficient, which are laid out between page 51

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and page 59 in my report.

Q. When you reviewed the prosecution history in view of Dr. Cooper's statement about how claim 5 was treated during prosecution, did you see anything where the patent owner argued that the ligation of claim 5 was inventive relative to Linnarsson?

A. That wasn't the analysis that I performed. It wasn't required to examine Dr. Cooper's assertions that ligation could occur after Step C in Linnarsson's Figure 11.

I pointed out reasons why that argument is deficient, including the presence of biotin on both the three prime and five prime ends of the cDMA molecule, and also the fact that if the tag were attached to the five prime fragment end, they wouldn't be sequenced because BTS sequence would leave those behind attached to the streptavidin bead and also because anything attached to the three prime fragment end would also be left behind and attached to the streptavidin bead and therefore not sequenced.

So ligation would be a pointless exercise because it wouldn't provide any

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information in sequencing.
So I didn't have to review the patent prosecution history because Dr. Cooper's arguments are flawed based on the way they're presented in his declaration.
MR. REINES: Move to strike everything after "that wasn't the analysis that I performed."
Q. And I remind the witness to attempt to be responsive and not to put in nonresponsive argument, because you're now doing that systematically.
A. You're asking me about Dr. Cooper's report and so I'm trying to provide context.
MR. REINES: The reader can just go up and read the question and answer and evaluate responsiveness for themselves.
Q. If you turn to paragraph 40 of the Cooper report.
A. All right.
(Exhibit 1016 was produced.)
Q. And I'll give you Exhibit 1016, which is the supporting documentation.
A. All right.
Q. And did you review this Exhibit 1016

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in preparing your opinions in this case?
That should be an easy one. Or explain to me why it's complicated. Let me ask the question again.
Did you review Exhibit 1016 in formulating your opinions in this case?
A. I don't believe I did. On page 7 of my declaration, I cited the materials I relied upon. I don't list Exhibit 1016, and so I don't recall reviewing it. I may have seen it, but I don't recall it.
Q. You referred to what within your declaration?
A. So page 7, paragraph 17. There also should have been an Appendix B that was mistakenly left out, but it would have listed the documents. So these are the documents that I cite. I don't believe I cited to Exhibit 1016. So if this list is incomplete and I cited to Exhibit 1016 in my declaration, please point me to where I did.
Q. And how come you didn't review this document?
A. So I don't recall Dr. Cooper, in presenting any of his arguments about the

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obviousness of the '918 [sic] patent in the context of Linnarsson, McCloskey and Hug, presenting any arguments regarding Exhibit 1016.
Dr. Cooper only cites, to the best of my recollection, Exhibit 1016 in discussing prior litigation against the patent owner, 10x Genomics. So given the fact that he didn't offer this as an obviousness argument, I didn't feel that it was necessary for me to review it in detail and cite to it. If I'm mistaken and I did cite to it in my report, I'd ask you to point me to where I did.
Q. If you turn to paragraph 40, please, of the Cooper report.
A. Okay.
I am there.
Q. Do you see where it says, "The Patent Owner argued the concept of 'labeling different objects with different labels' is 'as old as the scientific method and is familiar to every student in any introductory science class'..."
Do you see that?
A. Yes, I see that.
Q. Do you have any reason to dispute that?

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MS. RAYMOND: Objection. Scope.
A. So I don't know what context this quote is taken from. I'd be happy to review this document. I'd probably also want to look at the patents that are being discussed to understand the scope and scale of what Dr. Cooper is presenting here.
In the context of the analysis I performed, I don't believe Dr. Cooper cited to these in his obviousness arguments; and, therefore, I don't have any context in which to render an opinion about the meaning of this statement.
Q. Do you have any basis to dispute Dr. Cooper's analysis in paragraph 42?
MS. RAYMOND: Objection. Scope.
A. So I don't know what context Dr. Cooper is relying on to make these statements. Again, I haven't reviewed the document you labeled -- or provided labeled Exhibit 1016. I don't believe Dr. Cooper cites to that in his analysis that begins on page 58 of his declaration where he states that prior art anticipated or would have rendered obvious the claims of the '981 patent.

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So this is out of context for me. I don't believe that he cited to it in those sections, and so I didn't respond to it. If I'm mistaken, you can point me to where I cited to that in my declaration, I'd be happy to refresh my memory.

MR. REINES: Why don't we take a break.

VIDEOGRAPHER: Off the record at 4:09.

(Recess.)

VIDEOGRAPHER: On the record at 4:25.

BY MR. REINES:

Q. I'd like to show you the institution decision in this matter.

Turn to page 17, please.

A. All right. I'm there.

Q. And do you see where the decision states, "Patent Owner successfully distinguished Linnarsson during prosecution exclusively on the basis that it does not describe the second tag sequence element which is recited in limitations 1F and 1I."

A. Yes, I see that.

Q. Do you have any reason to disagree with that?

A. I mean, it says that. I mean, I don't know. This is the first time I believe I'm seeing this document.

If this is the analysis from the PTAB, I'm assuming the PTAB is correct in its analysis, but I have no basis to render an opinion.

Q. And just so if I understand you correctly, you're saying in preparing your response to the petition, you didn't review the PTAB's institution decision regarding that petition?

A. Does this have an exhibit number?

Q. No.

A. No, I don't recall -- I don't recall citing to it. I was responding to Dr. Cooper's arguments, and I don't believe he cited to it either.

Q. Do you normally -- let me just ask you again.

So you're saying that you didn't review the patent office institution decision when you prepared your response to the IPR petition to understand how the board was looking at the petition?

A. I may have. I don't recall.

In response to your earlier question, the PTAB actually does cite to the prosecution history.

Q. Could you turn to page 50 of your declaration, please.

A. I'm there.

Q. And is this the section of your report where you identify why you don't believe there's a reasonable expectation of success in the Linnarsson and McCloskey combination?

A. So it's a brief summary. My arguments about Linnarsson and McCloskey in light of claims 1 through 4 and claim 6 being -- or Linnarsson and McCloskey rendering claims 1 through 4 and claim 6 obvious are described starting on page 32 and running through page 50.

So this is just a brief summary of one of the reasons why -- or some of the reasons why there was no reasonable expectation of success based on Dr. Cooper's arguments.

Q. And when you say the one reason that you identify in the reasonable expectation of success section is the issue regarding the number of unique tags; is that correct?

A. So Dr. Cooper argues that you should -- you need about 100 times more tags than the number of molecules. Dr. Cooper also states in his report that there are 50- to 300,000 mRNA molecules per cell, which is the number consistent with what I know is present, which would argue there should be, I think, between 5 million and 30 million independent tags, and McCloskey discloses 16,038 tags, which would be insufficient. So that's one reason why there would be no reasonable expectation of success.

Previously I discussed problems with -- I guess later on I discuss, with respect to claim 5, problems with ligation. But I point out other arguments, too, about why these methods wouldn't work as stated.

Q. And how did you select the number of unique tag argument as being the only thing to include in the Reasonable Expectation of Success section? How did you pick that one to do it? And try to be as responsive as you can.

MS. RAYMOND: I just caution the witness not to reveal substantive

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communications with the attorneys.
A. So it's the easiest one to understand, right, if, as Dr. Cooper asserts, you need 100 time more tags than molecules, if you have between 50,000 and 300,000 RNA molecules you want to tag, that means you need between 5 million and 30 million tags to be successful.
So even if we buy all of the other arguments that Dr. Cooper makes, and we can discuss all the deficiencies in those arguments, even if you buy all of those, you wouldn't be able to uniquely tag each one of the mRNA molecules present in the cell based on Dr. Cooper's own assertions.
And so, again, just from a numerical perspective, it's simply the easiest to understand.
Q. What is inventive about claim 1 of the '981 patent?
MS. RAYMOND: Objection. Scope.
A. So to the extent that I'm responding to Dr. Cooper's arguments, claim 1 is not rendered obvious based on the combination of Linnarsson and McCloskey, and, therefore, is at

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least inventive relative to Linnarsson and McCloskey. Beyond that, I haven't done a detailed analysis of the inventiveness of claim 1 because it was outside of the scope of what was necessary to respond to Dr. Cooper.
Q. Are you able to describe, based on all of your work on this matter, what you think is inventive about any of the claims in the '981 patent?
MS. RAYMOND: Objection. Scope.
A. I haven't done that analysis, other than to respond to Dr. Cooper's arguments that claim 1 is rendered obvious by the combination of Linnarsson and McCloskey. And I'm happy to describe the reasons why claim 1 is not obvious.
Q. Can you answer my question beyond that?
MS. RAYMOND: Same objections.
A. Would you repeat the question, please?
Q. Are you able to describe, based on all your work on this matter, what you think is inventive about any of the claims in the '981 patent?
MS. RAYMOND: Same objections.

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Q. Let's say someone wanted to know and asked you.
A. I haven't performed that analysis, so I would be hesitant to render an opinion today since it's outside the scope of my report.
MR. REINES: All right.
I may be done. So why don't we take a break and check that out.
VIDEOGRAPHER: Off the record at 4:36.
(Recess.)
VIDEOGRAPHER: On the record at 4:46.
BY MR. REINES:
Q. Earlier you referenced the 454 sequencer.
Do you recall that, just generally?
A. Yes, I do.
Q. And you're aware of the use of MIDs with the 454 sequencer?
MS. RAYMOND: Objection. Form.
A. So I'm bad with acronyms. I forget exactly how a MID is defined.
Q. Multiplex identifier.
A. Multiplex identifier.
I believe, I don't recall exactly,

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but I believe that there were applications on 454 that allowed multiplex sequencing, but I don't recall fully. I am much more familiar with the applications on the Illumina sequencer allowing for multiplex sequencing.
Q. You're not saying that the '981 patent invented MIDs?
MS. RAYMOND: Objection. Scope.
Q. Well, you were using the term "MID" earlier today.
A. So I don't recall the exact context of my comment and so I may have misspoken.
A multiplex identifier as defined by the '918 [sic] patent has two specific components. What I was talking about earlier, I believe, when I was talking about the '918 [sic] patent -- or, sorry, when I was talking about the 454 sequencer was the use of multiplex tags so that each individual sample and all the molecules in that sample had a sample-specific tag. So, for example, if I wanted to sequence a mixture of your DNA and my DNA, I could use a tag that represented your DNA and a tag that represented my DNA and we could load them on the same lane in the sequencer and then demultiplex

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them.
So in the context of multiplex sequencing, at least the Illumina platform, I'm pretty sure the Applied Biosystems platform and I think the 454 platform allowed sample multiplexing, but none of them used a MID as claimed in the '918 [sic] patent that had two separate components.
Q. If you could go to the front page of the patent '981.
A. All right.
Q. Do you have that in front of you?
A. Yes, I do.
Q. Do you see under "OTHER PUBLICATIONS," it says, "'Using Multiplex Identifier Adaptors for the GS FLX..."
Do you see that?
A. Yes, I do.
Q. Okay.
And then you see the URL is a 454 publication so-called GS FLX --
A. Yes.
Q. -- Technology?
Okay.
In terms of just the acronym "MID" or

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the use of the term "Multiplex Identifier (MID)" that existed before the '981 patent application was filed in 2009, right?
MS. RAYMOND: Objection. Form.
A. So I don't know how this document uses the term "MID." That document -- I believe that use of MID would probably be limited to just the first element of the MID claimed in the '981 patent, but I'd have to review that document to really understand what type of MID they were referring to.
The idea of multiplex sequencing is something that was known, but the MID here has two separate components. And to the best of my knowledge, this second element of the MID, the second tag sequence, wasn't something that was well known.
Q. The acronym M-I-D for multiplex identifier, described as MID, you heard that before 2009 in your work in connection with sequencing; is that right?
MS. RAYMOND: Objection to form.
A. It's not an acronym that I was widely familiar with. People use lots of acronyms. Sometimes different acronyms for the same thing.

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And so I may have heard it referred to as a MID. I'm not really sure.
Q. So are you saying that you believe that the acronym MID in the '981 patent was coined by the applicants for the '981 patent?
MS. RAYMOND: Objection to form.
A. I don't know who coined the term. I don't know if the MID referred to in the 454 document that you pointed out on the front page of the '918 [sic] patent included all of the elements of the MID that occurred and that are claimed in claim 1 of the '981 patent. I simply don't know.
Q. Let me address it this way: Without you agreeing that the term "MID" as used for multiplex identifier to relate to sequencing, whether that's the same MID performing the same functions as the claimed MID, had you heard of the term "MID" used prior to 2009 by people to refer to multiplex identifiers generally?
A. I don't recall hearing that term, but whether or not I heard it, it would have been different from what's claimed in the '981 patents.
Q. Do you know one way or the other

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whether you heard it before then?
A. I don't recall. I know I've heard about many multiplex sequencing tags. I don't know if they were referred to as MIDs.
Q. All right. Thank you.
MS. RAYMOND: We may need a minute.
VIDEOGRAPHER: Off the record at 4:53.
(Off the record.)
VIDEOGRAPHER: On the record at 4:57.
EXAMINATION BY MS. RAYMOND:
Q. Dr. Quackenbush, do you recall being asked a question about whether you opined on whether Linnarsson would benefit from McCloskey's barcodes?
A. Yes, I do.
Q. Okay.
And could you open your report and go to paragraph 72 to 76.
A. All right. I'm there.
Q. Here, do you address Dr. Cooper's motivation to combine arguments?
A. Yes, I do.
Q. And do you address Dr. Cooper's amplification bias argument in these paragraphs?

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A. Yes, I do.
Q. And you also address -- you also address Dr. Cooper's opinions that persons of skill would have been motivated to modify Linnarsson's cell tag to incorporate the functionality of McCloskey's batch-stamp and barcode also in paragraph of 67 to 71 of your report?
A. Yes, I address Dr. Cooper's arguments there about modifying the Linnarsson cell tag to include the batch-stamp and the McCloskey barcode.
Q. Thank you.
Could you please turn to paragraph 53 of your report.
A. All right.
I'm there.
Q. And could you read the second sentence.
A. So it says, "I reviewed the Institution Decision in connection with this Element and understand the Board interpreted Dr. Cooper's combination as conceptually dividing the nucleotides of Linnarsson's cell-tag into two parts, with one part (six to

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eight of the nucleotides) being used for the functionality of McCloskey's 'batch-stamp,' which Dr. Cooper asserts is the 'first tag sequence,' and the second part (seven of the nucleotides), being used for the functionality of McCloskey's barcode, relying on Dr. Cooper's Paragraph 192."
And then I cite to the institution decision.
Q. Do you recall one way or another reviewing that institution decision now?
A. I do recall it now, yes. And I cited to it here.
Q. Then towards the end of this, after today's exercise, you were asked about a citation to a publication on the face of the '981 patent referring to it, I think, as the 454.
Do you recall that?
A. Yes, I do.
Q. Okay.
And I believe you said that -- did you review that for the purpose of your opinions here?
A. No. I didn't review that or any of

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the other -- any of the other publications cited in the '918 [sic] patent.
Q. And I believe you said earlier that everything from one person is tagged with one tag and everything from another person is tagged with a different tag.
Did I interpret that correctly?
A. So I was discussing multiplex sequencing. And in the common use of multiplex sequencing, one would like to run on the same sequence in the same run samples of different origins. And so multiplex sequencing typically used what we would refer to as multiplex tags so that individual samples from different sources would be tagged with sample-specific barcodes. All right.
So that was a practice that was well known in the art. I believe as I stated earlier, I haven't reviewed this document and don't know the scope of the multiplex identifiers that were described in the 454 documentation.
Q. So if I understand you correctly, the 454, to the best of your recollection, tags all the cells from one origin or sample the same way

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and then it tags cells from a different -- an entirely different sample a different way?
MR. REINES: Objection. Leading. Lack of foundation.
A. So I'm not sure what this document describes, all right. In common parlance, multiplex sequencing refers to running different -- samples of different origin on the same lane of a sequencer.
To accomplish that, one used what one would often -- what is commonly referred to as a set of multiplex tags. So we could use a tag for tumor and normal from the same individual and run them -- label all of the molecules from the collection of cells in the tumor and the collection of cells in the normal with one tag in the tumor and a separate tag for the normal, and then run those together, then demultiplex them by detecting the tags.
Similarly, I could run a sequence derived from you and a sequence derived from me. All the cells from you would be tagged with one multiplex tag; all of the cells from me would be tagged with another multiplex tags. Those would be run and we could identify the source of

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origin either being Megan or John based on the individual multiplex tags.

MS. RAYMOND: Thank you.

I have no further questions.

FURTHER EXAMINATION BY MR. REINES:

Q. Just a few follow-ups.

In terms of MIDs being used to tag polynucleotides to identify their origin, was there anything specific in the prior art as to, you know, limits on what origins could be designated with the tag?

MS. RAYMOND: Objection. It's outside the scope of the redirect.

A. So I haven't done that analysis about where particular MIDs might be used. I'm not sure about the use of MID in the context of this document we referred to on the cover of the '918 [sic] patent, so I can't really provide an opinion today.

Q. When you were responding on cross-examination from 10x regarding MIDs, your testimony was that MIDs could be used to multiplex polynucleotides from different sources, correct?

MS. RAYMOND: Objection. Foundation.

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A. So I think you're mischaracterizing --

Q. I don't know if I'm mischaracterizing. Maybe I misunderstood.

A. I didn't use the term "MID." I used the term "multiplex tags." I'm not sure how MIDs are being used here. But I described how sample-specific tags could be used in a multiplex sequencing application. And that's what I was referring to in my testimony earlier today about the different sequencing platforms.

Q. Okay.

Let me try to address that then.

In terms of the sample-specific tags for polynucleotides being sequenced, in terms of the origins, they could be designated by the different tag, is there any limits on what could be identified? In other words, you gave the example of two different people; you gave the example of tumor versus non-tumor. Is there any limit in the prior art on what sources could be designated?

MS. RAYMOND: Objection. Form.

A. So I'm not sure what you're referring to as different sources. It would depend on the

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particular application. Obviously, one couldn't run tens of thousands of samples -- well, there would be some technical barriers to doing that.

I don't recall reviewing any documentation that would allow me to provide examples from the prior art on limitations and so I can't do that sitting here today.

Q. And then in terms of the other publications, this GS FLX product from Roche for the 454 was a kit of an MID set. You were familiar with the 454.

Were you aware that they were selling a product of adaptors that could be used to apply MIDs?

MS. RAYMOND: Objection. Outside the scope of the redirect.

A. So I don't believe they're actually talking about selling kits. So the GS FLX was, I believe, their sequencing instrument. The titanium chemistry was the chemistry used in that instrument. I don't know what these molecular identifiers were. I don't know if they're being sold. This may be -- this is a technical bulletin that may describe some kind of use of multiplex sequencing. I don't know.

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I don't have the document to review. And so I can't tell you sitting here today what that document referred to.

Q. Do you know what Roche's basic MID set was for its 454 sequencer?

MS. RAYMOND: Objection. Outside the scope of redirect.

A. I don't have that documentation and so I can't tell you what they were selling 15 years ago.

MR. REINES: All right. That's it. Thank you.

MS. RAYMOND: Thank you. I don't have anything more.

VIDEOGRAPHER: Off the record at 5:09. This ends today's deposition.

MS. RAYMOND: I'll take a rough.

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WITNESS'S CERTIFICATION

_____

JOHN QUACKENBUSH, Ph.D.

On _____, 2024, the foregoing deposition was submitted to JOHN QUACKENBUSH, Ph.D., the witness, taken on Wednesday, February 28, 2024, for his examination.

At which time the deposition was read by the witness and any proposed changes desired were subsequently entered upon the attached errata sheet.

Thereafter, the deposition was duly witnessed and signed by:

_____

Notary Public in and for the

County of _____

State of _____

_____

My Commission Expires

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E R R A T A S H E E T

WITNESS'S NAME _____

DATE OF DEPOSITION_____

CASE NAME _____

PAGE	LINE	CORRECTION

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C E R T I F I C A T E

I, ELIZABETH M. KONDOR, a Certified Court Reporter, License ##30XI00117200, and Notary Public, do hereby certify that prior to the commencement of the examination, JOHN QUACKENBUSH, Ph.D. was duly sworn by me to testify the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a true and accurate transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor counsel of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not financially interested in the action.

_____

Notary Public

Dated: Monday, March 11, 2024

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