Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Videotaped Deposition of

FLOYD ROMESBERG, PH.D.

September 19, 2018

Volume I

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UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC., Petitioner, v.	Case No. IPR2018-00291	U.S. Patent 9,718,852
	IPR2018-00318	U.S. Patent 9,719,139
THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, Patent Owner.	IPR2018-00322	U.S. Patent 9,708,358
	IPR2018-00385	U.S. Patent 9,725,480

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.
VOLUME I
SAN DIEGO, CALIFORNIA
SEPTEMBER 19, 2018

Reported By:
PATRICIA Y. SCHULER
CSR No. 11949
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Videotaped deposition of Floyd Romesberg, Ph.D., Volume I, taken on behalf of the Patent Owner at 12790 El Camino Real, San Diego, California, beginning at 9:04 a.m. and ending at 3:48 p.m., on September 19, 2018, before PATRICIA Y. SCHULER, Certified Shorthand Reporter No. 11949.

APPEARANCES:

FOR PETITIONER:

KNOBBE MARTENS - SAN DIEGO, CA
BY: NATHANAEL R. LUMAN, ESQ.
12790 El Camino Real
San Diego, California 92130
(858) 707-4000
nate.luman@knobbe.com
- and -
KNOBBE MARTENS - WASHINGTON, DC
BY: WILLIAM R. ZIMMERMAN, ESQ.
1717 Pennyslvania Avenue
Suite 900
Washington, DC 20006
bill.zimmerman@knobbe.com

FOR PATENT OWNER:

FITZPATRICK, CELLA, HARPER & SCINTO
BY: ROBERT S. SCHWARTZ, ESQ.
BY: ZACHARY L. GARRETT, ESQ.
1290 Avenue of the Americas
New York, New York 10104-3800

Also Present: Dr. Steven Menchen
John White, Esq.
John Murnane, Esq.

Videographer:
Daniel Bermudez

I-N-D-E-X

E-X-H-I-B-I-T-S (CONTINUED)

E-X-H-I-B-I-T-S (CONTINUED)

SAN DIEGO, CALIFORNIA; WEDNESDAY, SEPTEMBER 19, 2018
9:04 a.m.

THE VIDEOGRAPHER: Good morning. We are now on the record. Today's date is September 19, 2018, and the time is 9:04 a.m. This is the video deposition of Floyd Romesberg Ph.D. being taken in the matter of Illumina Inc. versus Trustees of Columbia University and the City of New York pending in the United States Patent and Trademark Office before the Patent Trial and Appeal Board, case numbers IPR2018-00291, IPR2018-00318, IPR2018-00322, IPR2018-00385.

We are at Knobbe Martens, San Diego, 12790 El Camino Real. My name is Daniel Bermudez of Aptus Court Reporting located at 600 West Broadway, Suite 300, San Diego, California 92101.

Will counsel please identify yourselves and state whom you represent.

MR. SCHWARTZ: My name is Robert Schwartz. I'm an attorney with Fitzpatrick, Cella, Harper & Scinto in New York. I represent Columbia University, and with me is my colleague Zack Garrett also from Fitzpatrick. And sitting next to him is Dr. Steven Menchen and then sitting next to him is John White from the firm Cooper & Dunham.

And at the end is John Murnane from Fitzpatrick.

MR. ZIMMERMAN: Bill Zimmerman of Knobbe Martens Olson & Bear on behalf of Illumina and the witness. With me is my partner Nate Luman and Marcus Burch of Illumina.

THE VIDEOGRAPHER: The court reporter is Patricia Schuler, and she may now swear in the witness.

FLOYD ROMESBERG, PH.D.,
having been administered an oath, was examined and testified as follows:

EXAMINATION

BY MR. SCHWARTZ:

Q. Good morning, Dr. Romesberg. As I said, my name is Robert Schwartz, I'm usually referred to as Bob Schwartz. I am an attorney representing Columbia University in these IPRs.

Would you please state your full name and a home address for the record.

A. Floyd Romesberg, and my home address is 8109 Camino del Sol, La Jolla, California.

Q. And you have been deposed before, have you not?

Page 9

A. I have.
Q. And the rule that we follow in depositions is that unless your counsel instructs you not to answer a question, you should attempt to answer that question to the best of your ability. You understand that, correct?
A. Yes, I do.
Q. And if at any time you don't understand my question, please let me know. If you do not say otherwise I'll assume that if you answered my question that you understood that question, okay?
A. Yes.
Q. And you've heard this before, but the other rule we try to follow -- it's broken, unfortunately, all the time -- is that we try not to talk over one because the court reporter -- it becomes impossible for the court reporter to take down questions and answers. So we'll try to talk one at a time, okay?
A. I will try.
Q. And the other rule is that we give audible answers, so head shaking or nodding is not acceptable, okay?
Did you meet with your attorneys in preparation for your deposition today?

Page 10

A. Yes.
Q. I am only asking for a yes or no.
A. Yes.
Q. What are the names of the attorneys you met with?
A. Kerry and Nate.
Q. How many times did you meet with those attorneys in preparation for your deposition today?
A. In person?
Q. Let's start with in person.
A. I didn't count, but I believe it would have been, I think, three times, three or four times sounds right.
Q. And then you had other meetings that were I assume by telephone or something?
A. Yes, that's correct.
Q. How many times did you have meetings by telephone?
A. I am going to guess about the same, three or four times.
Q. And when did those meetings take place? Let's start with the in person.
A. Over the past three or so months. One pretty early on, and then one in the middle maybe a month ago, and then a couple this week.

Page 11

Q. And I guess maybe my question was not clear, but I was specifically referring in preparation for the deposition, so when did the meetings take place in preparation for the depo?
A. I'm sorry. These last two -- this week.
Q. And approximately how much time did you spend preparing for the depo?
A. 20 hours, maybe, approximately.
Q. Outside of those meetings, did you meet with your attorneys at any other time -- and I think you probably went into that. You met earlier over the past couple of months; is that correct?
A. Yes.
Q. And did you speak to anybody else other than your attorneys about this case?
A. No, I have not.
Q. And what did you do to prepare for today's deposition?
A. Mostly read literature and documents and refreshed my memory of all of the issues.
Q. And you understand that today's deposition is for four IPRs, which are IPR2018-00291, IPR2018-00318, IPR2018-00322, IPR2018-00385. That is your understanding that the deposition is in relation to those IPRs, correct?

Page 12

A. I believe so. I don't, unfortunately, recognize the numbers, but they are the four nucleotide cases, yes.
Q. And you prepared an expert declaration in each of those IPRs, correct?
A. I did.
Q. I am ready to put the first exhibit out. I'm going to put in front of you what has been marked as Exhibit 1012.
(Exhibit 1012 was previously marked for identification.)
BY MR. SCHWARTZ:
Q. Exhibit 1012. These are the four -- these are the expert declarations that you submitted in these IPRs, correct?
A. That is correct.
Q. And that's the first one. I can provide you with the others if you want them, maybe for the time being, we'll just leave one out on the table if that's okay with you, Counsel, or unless you want them all in front of him?
MR. ZIMMERMAN: That is fine with me.
MR. SCHWARTZ: It is just a lot of paper to put in front of you, so we'll just leave those out, but we have them all here.

Page 13

BY MR. SCHWARTZ:
Q. Just look at the end of that declaration. I just want to confirm that that's your signature.
MR. ZIMMERMAN: Just so we have a clear record, we're looking at Exhibit 1012 in the 291 IPR.
BY MR. SCHWARTZ:
Q. Correct. That is what we're looking at now.
Is that your signature?
A. That is my signature, yes.
Q. Do you recall signing the three expert reports in these cases as well?
A. Yes.
Q. And what did you do to prepare your expert declarations in this case?
A. A lot of reading, discussions with my lawyers, and a lot of writing and editing.
Q. Did you receive any materials such as scientific references or patents from your attorneys that you used to prepare any of your declarations?
A. I did, yes.
Q. And are all of those materials listed in your reports?

Page 14

A. To the best of my knowledge, yes.
Q. And did you do any of your own literature searching independent of the materials that were provided to you by the attorneys?
A. Yes, I did.
Q. And are all of those materials listed in your reports?
A. No, they are not.
Q. How did you decide what to list and what not to list?
A. I chose ones that I thought were most relevant without diluting out into the many articles that I examined.
Q. Did you rely on any materials to form the opinions in any of your reports that are not listed?
A. Yes, in a general way. For several of the issues I looked at many papers, many more than you would have wanted me to list and bring here. And I just wanted to make sure that my recollections of the time period were correct, my impressions were correct. And the ones that I included were the ones that I thought represented that the best.
Q. I am going to request that your counsel

Page 15

provide us any materials that you relied on to form the opinions that are in your report that are not listed. So I'm making that request on the record, okay?
MR. ZIMMERMAN: Okay.
BY MR. SCHWARTZ:
Q. You've been an expert for Illumina in other IPR proceedings, correct?
A. That is correct.
Q. And in some of those proceeding Intelligent Bio-Systems challenged Illumina patents; is that right?
A. Yes.
Q. And you submitted expert declarations on behalf of Illumina in those proceedings?
A. I did.
Q. And you were deposed in those proceedings, correct?
A. I was.
Q. And there was a court reporter at those depositions who created a transcript of those depositions, correct?
A. Correct.
(Exhibit 1013 was previously marked for identification.)

Page 16

MR. SCHWARTZ: I am going to put in front of -- it is marked as Exhibit 1013. It is entitled "DNA Sequencing."
MR. ZIMMERMAN: Just so we're clear, it's 1013 in which IPR?
MR. SCHWARTZ: 1013 in the 00385 IPR, but I believe it's the same in all of them.
MR. ZIMMERMAN: I'm just not sure that they all line up. But I want to make sure we are clear as to which one it is.
BY MR. SCHWARTZ:
Q. And that exhibit I just put in front of you is entitled "DNA Sequencing." And it's a patent application number WO91/06678 by Tsien. And just for the court reporter, the Tsien is spelled T-S-I-E-N.
And, Dr. Romesberg, this is the Tsien reference that you referred to in your declarations in the four IPRs in this case, right?
A. Yes, it is.
(Exhibit 2007 was previously marked for identification.)
BY MR. SCHWARTZ:
Q. I'm going to put in front of you Exhibit 2007, so this happens to be from the 00385

Page 17

case. It's an expert declaration in IPR2013-00517.
Dr. Romesberg, this is one of the expert declarations that you submitted on behalf of Illumina in the IPR challenges made by Intelligent Bio-Systems against Illumina; is that correct?
A. It appears to be, yes.
Q. And the Illumina patents at issue in those IPRs related to SBS, correct?
A. Yes.
Q. Would you please go to Page 14 of your declaration. And on Page 14, towards the bottom of the page there is a header entitled "PCT publication" -- W-zero -- I'm sorry. WO91/00678 to Tsien. Do you see?
A. It's 06678.
Q. 00678? Thank you.
A. One zero.
Q. To Tsien, right?
A. Yes.
Q. That's the same Tsien reference as Exhibit 1013 in the present IPRs, correct?
A. Yes, it is.
Q. Please draw your attention to the last three sentences in paragraph 36. And in paragraph 36 you write -- and this is on Page 15, the last

Page 18

three sentences in paragraph 36: "One of ordinary skill in the art would have understood these teachings of Tsien as stating that Tsien's invention was nucleotide sequenced determination using DNA polymerase, and in particular, sequencing by synthesis."
That is what you wrote, correct?
A. Yes.
Q. And it is your opinion that a person of ordinary skill in the art understood that Tsien's invention was SBS, correct?
MR. ZIMMERMAN: Objection. Vague as to time.
BY MR. SCHWARTZ:
Q. I am referring to a person of ordinary skill as you defined it in your declarations.
MR. ZIMMERMAN: And, again, objection. Vague as to time.
BY MR. SCHWARTZ:
Q. Well, in your declaration you say as of 2000, October 2000. So it's your understanding that a person of ordinary skill -- and again, whenever I use that term I am referring to how you define it in October 2000 -- understood that Tsien's invention was SBS?

Page 19

A. Yes.
Q. And I'll specifically, again, for the record refer to Dr. Romesberg's definition of POSA in paragraph 24 of his declarations in these cases. So whenever I use the word person of ordinary skill or if I abbreviate it as POSA, we'll understand we're using your definition as you have it in your declarations as of October 2000, correct?
A. Okay.
Q. Can you please go back to the Tsien reference, 1013. And again, this is the same Tsien reference that you write about in Exhibit 2007, the declaration that we just looked at, correct?
A. Yes.
Q. Please turn to Page 16 of Tsien and draw your attention to lines 21 to 23.
Are you there?
A. I am.
Q. Tsien writes "One obvious potential shortcoming of the present invention is that it employs a long sequence of serial reactions."
Do you see that?
A. I do.
Q. So a reader of Tsien, a POSA reading Tsien understood that Tsien's SBS method requires

Page 20

multiple serial cycles, correct?
A. Yes.
Q. And earlier I asked you about whether you had been --
A. I would have to think that. I believe it requires serial reactions. I guess I'm not 100 percent positive that simply reading a single nucleotide, a sequence of one -- I would have to check to see if that was covered in this patent or not.
Q. No, I am just saying Tsien writes. When Tsien writes that "One obvious potential shortcoming of the present invention is that it employs a long sequence of serial reactions." A person reading that understood that Tsien was referring to a method that requires multiple serial reactions, correct?
A. That is what he wrote, and that is what a POSA would have understood.
Q. And that's in relationship to his SBS methods?
A. Yes.
Q. And earlier I asked you about whether you had been deposed in the Illumina versus Intelligent Bio-Systems IPRs.

Page 21

Do you remember that?
A. I do.
Q. And you testified you had been deposed, correct?
A. Correct.
Q. And you testified that there were deposition transcripts from those depositions, correct?
A. Correct.
Q. Did you review any of those deposition transcripts in preparation for your deposition today?
A. I looked over them, yes.
Q. I am ready to mark my next exhibit, which is exhibit -- let me get this to the court reporter.
(Exhibit 2035 was marked for identification.)
BY MR. SCHWARTZ:
Q. I am putting in front of Dr. Romesberg Exhibit 2035, which is entitled video deposition of Floyd Romesberg Ph.D. dated July 8, 2014. And please turn to the last page of Exhibit 235, I believe it is page 190. And is that your signature on page 190?

Page 22

A. It is.
Q. Please turn to page 179 of the transcript. And please draw your attention to line 2 on page 179. And starting at line 2 the transcript reads "Question: Dr. Romesberg, you testified that approximately 25 base pairs would be needed for sequencing by synthesis. Do you recall that."
And you answered, "Yes, I do."
That was your testimony, correct?
A. That is what is written, yes.
Q. It is your opinion that SBS requires sequencing about 25 base pairs, correct?
MR. ZIMMERMAN: Objection. Vague as to time.
THE WITNESS: I would have to also --
BY MR. SCHWARTZ:
Q. And I'm referring always to the October 2000 time frame, as I mentioned earlier, whenever I use the word POSA.
A. I would have to look back through this to refresh my memory to see the context of which I was describing this, but it would not surprise me that I would say that. I would imagine I was talking about, as it goes on to explain, the amount of

Page 23

nucleotide reads that he would have to assemble to be able to reassemble a genome and uniquely place that sequence within the genome.
Q. So, again, just so I have a clean record, it is your opinion that SBS requires sequencing of about 25 base pairs. That's what you said, right?
MR. ZIMMERMAN: Same objection.
MR. SCHWARTZ: And, again, vague objections are actually not allowed in patent office proceedings, just so you know. You can just say "objection," but you can't say anything else.
MR. ZIMMERMAN: I'm just trying to give you an opportunity to correct the question so we are clear as to what time frame you are talking about.
MR. SCHWARTZ: I am always talking about the time frame of October 2000, okay, as he defines what a POSA would understand, okay?
THE WITNESS: I think you have to be a little bit careful to define what you mean by sequencing by synthesis, but you can read a sequence, and you can read a sequence of one. I assume that what I was talking about here -- I'd have to take a few seconds to refresh my memory, but I assume what I'm talking about is the ability

Page 24

to, in a practical way, sequence a genome or sequence an unknown piece of DNA, in which case something like 25 would seem like a reasonable answer.
BY MR. SCHWARTZ:
Q. But did you testify just -- your testimony I'm just reading it from your transcript is approximately 25 base pairs would be needed for sequencing by synthesis.
That is what your testimony was, right?
A. That's what is written, yes.
Q. So when Tsien states in this patent application that a long sequence of serial reactions is required for his SBS method, that's consistent with your opinion that SBS requires sequencing about 25 base pairs, correct? It's consistent?
MR. ZIMMERMAN: Object as to form.
MR. SCHWARTZ: You can just object.
THE WITNESS: Given the caveat that I just described, but I believe that that statement is correct.
BY MR. SCHWARTZ:
Q. I'm going to put in front of you my next exhibit. Let me get it to the court reporter.

Page 25

Thank you.
(Exhibit 1015 was marked for identification.)
BY MR. SCHWARTZ
Q. I put in front of you Exhibit 1015, which is patent No. 5547839. And again, this is from one of the IPRs, but I believe they are all the same. This is actually Illumina's exhibit, so it was used in all of your declarations.
The first named inventor is Dower. Exhibit 1015 is the same Dower patent that you rely on in your expert declarations in the present IPRs, correct?
A. It is.
Q. And the Dower patent relates to SBS, correct?
A. That is correct.
Q. And so, like Tsien's SBS method, Dower's SBS method, since it's about SBS, requires sequencing about 25 base pairs. That's your opinion?
A. Again, it is a little bit dependent on what your goal of sequencing by synthesis would be. But if it is to determine the unique -- to uniquely determine the sequence of an unknown piece of DNA

Page 26

that is long and of practical use, the sort of way people would have wanted to use this, then yes.
Q. So practical use of SBS would require that about 25 base pairs be sequenced?
A. As long as that practical use required determining the sequence of a large piece of DNA that you don't know, yes.
Q. And that's consistent with your testimony that about 25 base pairs is required for SBS?
A. Yes. The reason I'm qualifying this is because there was a lot of interest, especially at this time, in determining single nucleotide polymorphisms. And that would be a specific single nucleotide. And so it is entirely possible that someone could have wanted to use these sorts of things for that, but that would not be the de novo sequencing that we're talking about.
Q. But that would still require about 25 base pairs of sequencing to do snips, right?
A. Well, it depends on how you would do it. If you had a small piece of DNA and you just wanted to know what the nucleotide was at position -- at the position immediately following a primer, then no.
Q. Let's go back to your deposition

Page 27

transcript that was Exhibit 2035. And go back to that page 179 we actually looked at before. And we were looking at the top of the page where you testified that approximately 25 base pairs would be needed for sequencing by synthesis. That was your testimony, right?
A. Yes.
Q. And would you look just down to about line 12. And you were asked a question "If you were looking for a single nucleotide polymorphism using a sequencing by synthesis method, what minimum read length would you need?" And then there was -- you started to answer that. There was an objection. And then you were instructed you can answer.
And then you said -- your answer was: "It would not change, because the SNP identification comes from the assembled genome, so you still need to assemble the genome, you still need to place the sequence within the genome to know or not a specific nucleotide is a SNP. So the SNP is determined after you assemble the genome, so it doesn't change anything."
That was your testimony, correct?
A. That is correct.

Page 28

Q. And that is in relation to your testimony above that SBS requires sequencing of 25 base pairs, correct?
A. That is correct.
Q. So even if you are sequencing using SBS for SNPs, your testimony is it still requires about 25 base pairs of sequencing, correct?
A. So again, as I tried to explain a second ago, it would depend on how you were doing it. So if you simply had a genome or a large piece of DNA and you wanted to identify all the differences, the SNPs, then what I said in this testimony is correct. The reason I caveated it a minute ago was because I could also imagine cases where one would have a small piece of DNA and one would need to simply know the identity of a nucleotide at a given position, in which case then you would not need a read length of 25.
So it really depends on the application, but for the sort of practical applications that I think we're going to spend most of our time discussing here, you would need read lengths that were longer than one.
Q. So for a practical application of SBS, whether it would be for SNP or some other reason,

Page 29

it is your opinion that you need to sequence about 25 base pairs?

MR. ZIMMERMAN: Objection. Asked and answered.

MR. SCHWARTZ: You can't make that objection. It's just objection.

BY MR. SCHWARTZ

Q. I'm just looking at your testimony. I just want to make sure that we understand what your testimony is.

A. What I was referring to here was a certain sort of application. I think it's very possible that people would have other applications in mind where they would not need read lengths of 25.

BY MR. SCHWARTZ:

Q. But you did testify that for SBS you need a read length of 25. That is your testimony?

A. The context of what I was talking about at the time, which I hope I made clear, would require read lengths of 25, yes.

Q. And that would be the same if it was for SNPs?

A. In the context of what I was describing here, which I hope I have made clear.

Page 30

Q. And that's a practical application of SBS, is that what you are saying?

A. No. In the particular practical application that I was talking about in this testimony, yes. But as I just said, I can imagine there would be other practical applications -- I don't know all practical applications that people might be interested in -- where you would need a read length of one.

Q. What practical application would you need a read length of one?

A. As I described a minute ago, I'll try to be clear now. You might have a piece of DNA. And you might say I want to know what the nucleotide is at position 28. You could design a primer of 27 length or a primer that terminates immediately before the nucleotide in the template that you're interested in, and you could get a read length of one and determine what that nucleotide was.

Now, that was not the applications that I was describing in the context that we're discussing. But it would seem to be an application that someone might be interested in. I'm just trying to be clear about what people might have read Tsien or Prober and been interested in doing.

Page 31

And lots of people are interested in lots of different things.

Q. But you were asked specifically about SBS, and your testimony was that 25 base pairs would be required.

A. Everything that we just talked about is SBS. SBS in the context of my testimony here was in a specific situation where you would need a read length of 25. I can certainly imagine other applications of SBS where you would not need that.

Q. And the practical applications that you need 25 when you're sequencing for purposes of determining a gene, how it fits into the genome, is that something like that?

A. Yeah. So if you don't know anything about the piece of DNA that you're sequencing, for example, and you need to assemble it, you need certain overlap with other sequences to be able to do that, and so you would need longer read lengths there.

Q. Going back to the Tsien patent application. Tsien doesn't disclose any sequencing data using a 30 allyl nucleotide, correct?

A. He does not have data for a three prime allyl nucleotide, that is correct.

Page 32

Q. I am going to mark another exhibit.

MR. SCHWARTZ: Court reporter, before you hand that over, this is an exhibit that has not yet been marked. Could you please mark this as Exhibit 2080.

(Exhibit 2080 was marked for identification.)

MR. SCHWARTZ: You might want to put the numbers on the bottom in case we have to come back to it. This is going to be 2080.

BY MR. SCHWARTZ:

Q. And this is a document entitled "Video Deposition of Floyd Romesberg Ph.D." dated January 14, 2014.

Do you see that?

A. I do.

Q. And this is the transcript of your deposition in IPR2013-00128, correct?

A. It would seem to be, yes.

Q. Again, the Illumina patents that were being challenged in this IPR relate to SBS, correct?

A. Yes.

Q. Please turn to page 101 of the transcript. Please draw your attention to line 6.

Page 33

And starting at line 6 of the transcript it reads.
"Question: And would you agree with me that in 2002 it was known that you could cleave a linker and a protecting group under identical conditions?
"Answer: No, I would not agree with that.
"Question: So if we can turn to start Exhibit 1012, that is the Tsien reference. And in particular, if can you turn to page 28 of Tsien."
Now I'd like to stop here, because I just want to clarify something about the transcript.
In the transcript, Tsien, the T-S-I-E-N paper is misspelled as C-H-E-N, correct? That is just a misspelling, correct?
A. I believe so, yes.
Q. So what you're actually -- the question and answer in the section relates to the Tsien, T-S-I-E-N, reference that is the same Tsien that we have been looking at in these IPRs, correct?
A. I believe so, yes.
Q. Let's jump to line 25 of the same page, and starting at line 25, the transcript reads, "So you see there that Tsien says the one method involves the use of a tag attached to the base

Page 34

moiety, correct?
"Answer: Yes, correct.
"Question: And that is a fluorescent label attached to the base of the nucleotide, correct?
"Answer: yes.
"Question: Okay. And then the next sentence says the tag may be chemically cleaved, either separately or simultaneously with the deblocking step. Do you see that?
"Answer: I do.
"Question: And the deblocking step that Tsien is referring to there is the three prime OH blocking group, correct?
"Answer: Yes.
"Question: So then would you" -- strike that.
"Question: So then you would agree that the concept that it was known in the art that you could cleave a base labeled, a cleavable linker attaching a label to a base in a three prime protecting group.
"Answer: Just to be clear, I guess what I am -- I think what we are disagreeing here is whether -- with here is whether the suggestion had

Page 35

been made or whether it was known that you could. I see that Tsien is suggesting that, and that was known in, and that had been suggested in the literature built before. I don't believe that Tsien provides any data.
"Question: But the prior art did show, and Tsien is an example of that, that a person of skill in the art could cleave a cleavable linker, attaching a label to the base and a three prime protecting group.
"Answer: I don't.
"Question: Correct?
"Answer: No. I don't know that Tsien provided that. I don't know that anyone -- I don't know that that was known. I'm unaware of that."
That was your testimony, correct?
MR. ZIMMERMAN: Objection.
Mischaracterizes.
BY MR. SCHWARTZ:
Q. That was your testimony, correct?
A. That is what -- you read the transcript correctly. It's a little bit hard for me, because I haven't read and thought about this particular --
Q. I am just asking you about that piece. That was your testimony, correct?

Page 36

A. You read it correctly.
Q. And in your deposition testimony in IPR2013-00128, you specifically pointed out that Tsien does not provide any data for simultaneously cleaving a cleavable linker, attaching a label to a base in a three prime protecting group, correct?
A. That seems to be correct, yes.
Q. And you testified that you were not aware of any other prior art that provided data showing simultaneous cleavage of the cleavable linker in a three prime protecting group, correct?
A. That seems to have been my testimony.
Q. And without having such data it was your testimony that it was not known that a cleavable linker in a three prime protecting group could be cleaved, correct?
A. That seems to have been my testimony.
Q. And going back to Tsien, Tsien doesn't disclose any data showing that a nucleotide with a 3'-O-allyl protecting group could be cleaved under conditions that are compatible with SBS, correct?
A. Tsien shows no data after that, correct.
Q. And sitting here today, are you aware of any prior art -- and again, I'm dealing with your definition as of October 2000 -- that provided any

Page 37

data that a nucleotide with a 30 allyl protecting group could be used successfully for SBS?
A. No. I think there was data that suggested that it could be used.
Q. What data was that?
A. Reaction showing that you could efficiently deprotect the -- you could efficiently remove the deprotecting group.
Q. That wasn't in the context of SBS, was it?
A. No, but you asked about any data.
Q. I asked specifically -- let me ask the question again, okay?
Are you aware of any prior art prior to October 2000 that provided any data that a nucleotide with a 3'-O-allyl protecting group could be used for SBS?
A. I think that the way you asked the question, the way I would interpret that is that any data would include data that a person of ordinary skill in the art would look at a particular reaction, let's say, and draw an impression about the same or similar reaction that could be used in SBS. And I believe there was data for that.

Page 38

Q. I'm not asking you about drawing inferences. I'm asking you for any direct data -- just the way you testified that you weren't aware of any data that showed cleavable linker on three prime protecting group cleavage. Are you aware of any data -- in the context of actually using it for SBS -- in an SBS reaction, are you aware of any data that a 3'-O-allyl protecting group on a nucleotide that was used for SBS?
A. So let me be clear about the answer. My testimony here referred to simultaneously deprotecting a blocking group and a linker on a base. And I testified, and I would have to rethink this, but I believe I understand the context, and I believe that that answer was correct, because no one had ever shown the simultaneous deprotection of two things like that.
This is a rather different question, and I'm going to again explain that if one was simply asking if an O-allyl could be deprotected from a nucleotide, it is common, and any person of ordinary skill in the art would have exercised this exact procedure, you would look for -- he or she would have looked for similar reactions, and then it's more than just an inference. This is the way

Page 39

you draw an impression about how optimistic or pessimistic you are about whether or not something's feasible, whether or not you're interested in it. And there was a lot of data, not on an exact nucleotide that would have been used for sequencing by synthesis. So this is not exactly in a sequencing by synthesis reaction, but there were very similar reactions where people would have drawn an impression.
So there certainly was data, but there was not data that was used within the context of an exact sequencing by synthesis reaction.
Q. So it's your testimony here today that you are not aware of any data prior to October 2000 where an 3'-O-allyl nucleotide was used precisely in a SBS reaction?
A. That is correct.
So can I add one qualifier to that? Metzker did report prior to 2000 a reaction that was getting very, very close. It examined parts of what you would use for sequencing by synthesis reaction, but it was not the complete sequencing by synthesis.
Q. But Metzker didn't show any data using the 3'-O-allyl nucleotide for SBS, he did?

Page 40

A. No, but he did look at steps that would be involved in that.
Q. But he didn't do it for SBS. He never evaluated that nucleotide for SBS, correct?
A. He never used it for sequencing by synthesis, but I just want to be clear that there were examples in literature that was very close that any person of ordinary skill in the art would have relied on to draw an opinion. And more importantly, an opinion was available in 2000 is the point I am trying to make.
Q. There was no data. There was no data using a 3'-O-allyl nucleotide for SBS, because that nucleotide would have required a label attached to it as well, correct?
A. Just to be clear, sequencing by synthesis is a multi-step process, and there were reports of some of those steps, not all of them. So in a complete sequencing by synthesis reaction, there was no data, no one had tried to my knowledge. But there were examples of different part components of this -- different steps of the overall reaction that had been reported.
Q. But not for the complete nucleotide for SBS?

Page 41

A. That's right. Not for the complete scheme for all the steps of the sequencing by synthesis reaction.

Q. And without having any date, the best you could do is speculate at that point?

A. Well, this is the problem I'm having. I disagree with the statement that there would not have been any data, because there are multiple steps. And you have data that shows whether or not the O-allyl -- the 3'-O-allyl variant would have satisfied individual steps of the overall reaction. You did not have any data on the overall reaction, but you did have data on very relevant steps of that overall reaction. And I think that would have been -- I call that data, and I think a person of ordinary skill in the art would have viewed that as data relevant to how he felt -- he or she felt about the O-allyl groups.

Q. But you didn't refer to that kind of steps data when you were testifying about the simultaneous cleavage. There was data. You were asked about data for the individual steps, and you said there was no data for the combined cleavage reaction. That it was not enough to just look at the individual data. You were not aware of any

Page 42

data showing simultaneous cleavage, correct?

MR. ZIMMERMAN: Objection. Compound.

BY MR. SCHWARTZ:

Q. That was your testimony?

MR. ZIMMERMAN: Objection. Compound.

MR. SCHWARTZ: Again, you can object.

MR. ZIMMERMAN: The rules allow me to succinctly state my objection. Objection. Compound.

BY MR. SCHWARTZ:

Q. Okay.

A. What I was referring to in that testimony was a specific step, and no one had ever shown that step.

Q. Okay. And no one has ever shown all of the steps involved in SBS for the 3'-O-allyl nucleotide, correct?

A. That is correct. No one has shown all of the steps for a 3'-O-allyl nucleotide. That is correct.

Q. And without that data, you can only speculate that it would work?

A. I disagree with that.

Q. But let's go to an Exhibit 2007. It's an exhibit that is already on the table. It's your

Page 43

expert declaration in IPR2013-00517. Please turn to paragraph 37, page 15.

In paragraph 37 you write: "According to Tsien, the criteria for the successful use of 3'-O-allyl blocking groups include, one, the ability of a polymerase enzyme to accurately and efficiently incorporate the DNTPs carrying the 3'-O-allyl blocking groups into the CNA chain; two, the availability of mild conditions for rapid and quantitative deblocking; and, three, the ability of the polymerase enzymes to reinitiate the cDNA synthesis subsequent to the deblocking stage. And you cite to Tsien at column 20, lines 28 to column 21, line three.

That was what you wrote, correct?

A. That is correct.

Q. And the Tsien reference that you are referring to in this exhibit is the same Tsien reference that is marked 1013 in these IPRs, correct?

A. That is correct.

Q. So going to the first criteria: "If a nucleotide analog with a 3'-O-allyl blocking group was not accurately and efficiently incorporated into the DNA chain by the polymerase, it would not

Page 44

be expected to be successful in Tsien's SBS method," correct?

A. I think it is important to be careful about what you mean by "accurately and efficiently," because those are absolute statements. And it is certainly true that there is a efficiency and an accuracy requirement for sequencing by synthesis. So I would not want to quantitatively assign any meaning to "accurately and efficiently," but there certainly is a requirement, yes.

Q. I was not asking you at this point to assign any numbers or anything. I'm just saying that you cite to Tsien's three requirements. And I'm just asking you if a nucleotide with three-prime blocking group was not accurately and efficiently incorporated as Tsien requires, then it would not be expected to work for his method?

A. Are you asking me to confirm that that's what Tsien says, or what my opinion is?

Q. I'm asking you -- well, you wrote this. This is what you wrote. You cited this about Tsien's requirements?

A. Right. So according to Tsien, that is correct.

Page 45

Q. So according to Tsien, if any of these criteria are not met, Tsien says it wouldn't be expected to work for his SBS method, correct?
A. Yes.
Q. Let's go to Tsien's second requirement, which is the availability of mild conditions for rapid and quantitative deblocking. We'll talk about that for a little bit, okay?
A. Yes.
Q. Please go back to your declaration, Exhibit 2007, it was your declaration in IPR2013-00517. Do you have that?
A. Yes, I do.
Q. Please go to page 29. Please draw your attention to paragraph 55, and please look down about five lines to a sentence that starts with "in fact most."
Do you see that?
A. I do.
Q. And in paragraph 55 you write: "In fact, most organic solvents, including tetrahydrofuran, THF, and methanol were known to denature DNA." And you cite the Lee paper of 1981, and you say "(such unwinding of the DNA duplex caused by dehydration is likely to be a common phenomenon in organic

Page 46

solvents.)"
That's is what you wrote, correct?
A. That is what I wrote.
Q. So it is your opinion that most organic solvents, including methanol, were known to denature DNA, correct?
A. Under conditions where they dehydrate the DNA, yes.
Q. Your testimony is most organic solvents, including methanol, were known to denature DNA. You cite to the Lee paper, correct?
A. Yes. And I was also trying to be clear that it was due to dehydration.
Q. Let's turn to page 23 of your declaration. And please draw your attention to the second line on the top of the page that starts with "This reaction was performed in methanol."
Do you see that?
A. Yes, I do.
Q. And you write: "This reaction was performed in methanol which was known to denature DNA, required two hours, and resulted in only 75 percent to 91 percent yield." You cite to a paper by Loubinoux. "Further, stannous chloride was known to damage DNA by causing nicks in DNA

Page 47

strands." And you cite to a Dantas paper. Then you write: "Any one of these considerations would have led one of ordinary skill to have expected stannous chloride to be incompatible which ends in Ju's requirements for sequencing by synthesis."
That is what you wrote, correct?
A. That is what I wrote, correct, yes.
Q. And, again, the Tsien reference that you referred to in that statement is the same Tsien reference as Exhibit 2 -- 1013, correct?
A. It is.
Q. And the reaction you are referring to in your declaration is a deprotection reaction of the 3'-O-allyl protecting group, correct?
A. Correct.
Q. It is your opinion that 3'-O-allyl deprotection reactions that denature DNA, such as those that are performed in methanol, are incompatible with Tsien's SBS method.
It's what you wrote?
A. It is what I wrote.
Q. And in fact, you also wrote in your opinion that 3'-O-allyl deprotection reactions that are performed in most organic solvents are compatible with SBS.

Page 48

That's what you wrote, correct?
A. Could you point me to where I wrote that, please?
Q. We just looked at that before, remember?
A. Right. So due to dehydration.
Q. Most organic solvents?
A. Yes. Due to dehydration, yeah. They're not damaging the DNA is what I mean. But they're incompatible if they're denaturing the DNA, yes.
Q. And what we looked at, you don't say anything about dehydration. You just say the reaction was performed in methanol, which is incompatible with Tsien's SBS method. That is what you wrote?
MR. ZIMMERMAN: Objection. Mischaracterizes.
BY MR. SCHWARTZ:
Q. That's what you wrote?
A. That is what I wrote in the section, but I do believe I was trying to be clear in the document that that was due to dehydration.
Q. So you're saying that the mechanism of denaturation by methanol is dehydration?
A. The mechanism of denaturation is dehydration, yes.

Page 49

Q. Let's go back to Tsien, please, Exhibit 2013. And please turn to page 23. And please draw your attention to line 27. There is a section entitled "deblocking methods." Do you see that?

A. I do.

Q. And starting at line 33, Tsien teaches that the deblocking method used in his SBS method must, A, proceed rapidly; B, yield a viable 3'-O-allyl O-H function in high yield; and, C, not interfere with future enzyme function or denature the DNA strand."

Those are Tsien's requirements for deblocking the 3'-O-allyl protecting group, right?

A. He lists those requirements, yes.

Q. And Tsien expressly requires that the deblocking conditions used to remove the 3'-O-allyl protecting group does not denature the DNA, correct?

A. Yes.

Q. Will you please go back to Exhibit 2035, that was your transcript from your deposition in IPR2013-00517.

A. Okay.

Q. You have it?

Page 50

A. Yes.

Q. Can you please turn to page 174. Please draw your attention to line 19, and starting at line 19 the transcript reads:

"Question: And is it reasonable for someone looking to develop an SBS method to consult synthetic chemistry papers?

"Answer: Yes, it is.

"Question: Does that -- and does it depend on what is disclosed in those papers?

"Answer: Yes. So I mean, if I were looking to develop a sequencing by synthesis method, I would certainly consult synthetic chemistry papers that showed things like efficiency of protecting group cleavage. I would look at the conditions that were explored to see if they were compatible with sequencing by synthesis, and I would look at yield to see if they were compatible with the high efficiencies required for sequencing by synthesis.

"Question: And if the conditions disclosed in a particular reference were incompatible with sequencing by synthesis, would there be any expectation of success?

"Answer: That would not provide an

Page 51

expectation of success."

That was your testimony, correct?

A. Yes.

Q. So your opinion is that it is reasonable for someone looking to develop SBS methods to consult synthetic chemistry papers, correct?

A. Yes.

Q. And your opinion is, if the condition disclosed in a synthetic chemistry paper for cleavage of the three-prime protecting group is incompatible with SBS, then there would not be any expectation of success, correct?

A. If a synthetic chemistry paper did not provide conditions that were compatible with DNA sequencing, then that paper itself would not provide an expectation of success.

Q. And that would be true for Tsien's SBS method, correct?

A. Yes.

Q. And it would be true for Dower's SBS method, correct?

A. Yes.

Q. While we have the transcript open, please turn to page 177. And please draw your attention to line 17. And starting at line 17 the transcript

Page 52

reads:

"Question: Would you expect DNA to be denatured at 20 degrees Celsius in aqueous pyrimidine sufficient to solubilize triphenylphosphine?"

There was an objection.

"Answer: That depends on the DNA. Large pieces of DNA like chromosomal DNA or maybe even plasma DNA at 20 degrees in pyrimidine might actually not, although it might be denatured, but certainly primer templates of the sort used in sequencing by synthesis would be denatured at 20 degrees in pyrimidine.

"Question: Can you explain why.

"Answer: Well, because they're already not very stable. They're only marginally stable because they are reasonably short."

That was your testimony, correct?

A. That is correct.

Q. And your opinion is that DNA denaturation is an especially important consideration in SBS, because SBS uses short primer and DNA templates that are only marginally stable due to their short length, correct?

A. I don't know that I would characterize it

Page 53

as an especially critical step, but it is a required step that you do denature -- that you do not dissociate the primer from the template.

Q. And because the primers and the templates are short in length, they are more susceptible to denaturation?

A. Shorter than chromosomal double-stranded DNA, so certainly, yes.

Q. They're already not very stable. That was your testimony.

A. Well, they're certainly less stable, so they're more prone to lose structure when you perturb them by any method; heating or denaturational, whatever.

Q. And so that's why my question was, so they are -- that's a particularly important consideration in SBS because the primers and the templates are short and only marginally stable, they are even more susceptible to denaturing conditions, correct?

A. Yes. I was just a little -- I was commenting on that I did not think that the denaturation was any more criterial than any other requirement. But relative to a chromosome, the conditions that usually would be used for

Page 54

sequencing by synthesis would be more prone to denaturation.

Q. I'm going to ask you to go back to your declaration in IPR2013-00517 that was marked as Exhibit 2007. Okay?

A. I am there.

Q. Please go to page 34. And please draw your attention to paragraph 60. And please look at the third line down in paragraph 60 that starts with "For example, it was known that."

Do you see that?

A. I do.

Q. In paragraph 60 you write: "For example, it was known that stringent requirements for nucleotide analogs with removable 3'-OH terminators presented a formidable obstacle to developing stable and efficiently deprotected 3'-OH protecting groups for SBS." And you cite to Metzker at 4259. That is the Metzker paper that you also use in your declarations, correct?

A. I believe that is correct, yes.

Q. And you continue to say "And it was a major challenge in the field to develop 3'-OH terminators that were removable under aqueous SBS conditions." And you cite to the Welsh paper,

Page 55

correct?

A. Correct.

Q. That's what you wrote?

A. That is what I wrote.

Q. It is your opinion that stringent requirements for nucleotide analogs with removable 3'-OH terminators presented a formidable obstacle to developing stable and efficiently deprotected 3'-OH protecting groups for SBS. That was your opinion, correct?

A. Again, that is what is written there, yes.

Q. And one of those obstacles is that SBS requires aqueous conditions for cleavage of the 3'-OH protecting group, correct?

A. That is correct.

Q. And you agree it was a major challenge in the field to develop 3'-OH terminators for SBS that were removable under aqueous conditions, correct?

A. Well, as I say, they were a stringent requirement for any sequencing by synthesis method of this sort, yes.

Q. And you say though specifically it was a major challenge. I'm just reading from your declaration.

Page 56

A. Yes, I think I was quoting a paper there. If you want me to describe what I'm trying to say, if I can remember the context, any sequencing by synthesis method stringently requires you simply -- of the sequencing by synthesis methods that we're describing, you simply have to remove that protecting group.

Q. Under aqueous conditions?

A. Under conditions where the DNA is not going to denature, so, yeah, it's very likely there is going to have to be water there.

Q. Well, you actually say that there, it was required that they were removable under aqueous conditions?

A. Yes.

Q. That is your testimony that it was required that it be removable under aqueous conditions?

A. Yeah, that's -- just to be clear, that is what I meant to just say.

Q. I am going to put another exhibit in front of you.

MR. ZIMMERMAN: We've been going for a little over an hour. Do you need a break?

THE WITNESS: Yeah, actually.

Page 57

MR. ZIMMERMAN: If now is a good time.
THE WITNESS: It will only take a second.
MR. SCHWARTZ: Fine. And, of course, you've been through this, you've been to this rodeo before. If you ever need a break, you are entitled to have a break.
THE WITNESS: Yeah, I can't understand why I have to go to the bathroom all the time.
THE VIDEOGRAPHER: We are off the record at 10:07 a.m.
(Recess taken.)
THE VIDEOGRAPHER: We are on the record at 10:19 a.m.
BY MR. SCHWARTZ:
Q. Dr. Romesberg, would you please go back to Tsien. That is Exhibit 1013. And please turn to page 23. We may have looked at this before. At the bottom of the page, that's where Tsien lists the deblocking requirements. And we discussed this earlier, one of Tsien's deblocking requirements is rapid deblocking of the 3'-OH protecting group, correct?
A. Proceed rapidly, yes.
Q. Please turn back to Exhibit 2007, your declaration in IPR2013-00517. Please go to

Page 58

page 25. Please draw your attention to the second line on page 25 that starts with "Furthermore, the."
Do you see that?
A. Yes.
Q. And you write: "Furthermore, the amount of time required to achieve those yields, three hours to multiple days, would not have been expected to fulfill the rapid deprotection requirement of Tsien's DNA sequencing method, or provide a fast sequencing system capable of the ultrahigh-throughput in accordance with Ju's DNA sequencing method."
That is what you wrote, correct?
A. That is correct.
Q. It's your opinion that if it takes three hours to achieve high-yield deprotection of the 3' protecting group, it would not have been expected to fulfill Tsien's rapid deprojection requirement?
That's what you wrote?
A. That is what I wrote.
Q. Let's go back to Exhibit 2035, your deposition transcript in this case, in the 00517 case I should say. Please turn to page 97. Please draw your attention to line 22, and starting at

Page 59

line 22, the transcript reads:
"Question: Does the rapid removal of a 3' protecting group relate to the eventual application of the SBS method?"
And there was an objection.
"Answer: So you are asking if the efficiency of removal depends -- required depends on the application?
"Question: Sure. That is a better way of asking it. Thank you.
"Answer: So that depends. I mean, so that depends. Are you talking about sequencing by synthesis applications?
"Question: Yes.
"Answer: Sure, but they all have to be efficient. There is a minimum that you are going to have to be. You are going to have to get 30 nucleotide read lengths minimum in a time that is not -- in a time that's feasible.
"Question: What is a feasible time?
"Answer: You know, that's a relative term. I mean, I threw out the idea of five hours being not reasonable. And I think that is probably right. What is a reasonable time? I don't know. An hour. That would not be great, I don't think,

Page 60

but that would at least maybe be in the realm of doable."
That is your testimony, correct?
A. Yes.
Q. Is it fair to say that your opinion is that SBS requires sequencing somewhere between 25 to 30 bases?
A. I think it's my opinion that I'm not sure doable is a word, but, yes, I think depending on the specific application, and, again, getting back to the discussion that we had earlier, if you are doing the -- if your goal is the application that we are describing here, and that is de novo sequencing of unknown sequences of DNA, then, yeah, you would need read lengths of on that order.
Q. And then the testimony we just read you testified that a reasonable time for deprotection of the 3'-O-allyl protecting group in SBS is an hour or less.
That is what you testified, right?
A. Well, I am hemmed and hawed a little bit because I think that is kind of a tough question. If an hour would have been all that is available, then people probably would have gone with that. But certainly people wanted faster, the fastest

Page 61

possible.
Q. But your testimony was that an hour or less. That was your testimony?
MR. ZIMMERMAN: Objection. Mischaracterizes.
THE WITNESS: Let me look at what I said. "I threw out the idea of five hours being not reasonable, and I think that is probably right. And what time is reasonable? I don't know. An hour, that would not be great I don't think, but that would at least maybe be in the realm of doable."
Yeah, so there's not -- I don't have an answer, an exact answer to this question. People's standards would have adapted to what was required, but clearly faster is better.
BY MR. SCHWARTZ:
Q. I am just asking about your testimony. And your testimony was that an hour might be doable.
A. I certainly believe that an hour might be doable.
Q. But you also said that an hour would not be great.
A. Great is kind of a relative term. I

Page 62

think, you know, today, looking at what we have, I think an hour would not be great.
Q. But in terms of this testimony, that wasn't today. That testimony was in the past. And you were asked about sequencing by synthesis, and your testimony was that an hour might not be great, but it would be doable. So my question is, it was your testimony that in your opinion SBS required that it occur in an hour or less?
MR. ZIMMERMAN: Objection. Compound.
BY MR. SCHWARTZ:
Q. That was your testimony, correct?
MR. ZIMMERMAN: Objection. Compound.
THE WITNESS: I don't believe that was my testimony. If it -- put it this way, if it was, it shouldn't have been. But I don't believe it was. Could you direct me to where I said that it would have to be an hour or less.
BY MR. SCHWARTZ:
Q. "What is a reasonable time? I don't know. An hour, that would not be great, I don't think, but at least that would be in the realm of doable."
A. If you ask me does it have to be an hour or faster to be a great sequencing by synthesis

Page 63

method, I think we could discuss what great means, but I think the point I was making was that an hour is -- you certainly could have hoped faster, but I don't think an hour would have precluded using it. It wouldn't necessarily have been great, but it still -- it certainly would have been doable.
I think probably -- I mean, in reality, 10 hours would have been doable. It would have not been great. It would have started to be pretty bad because you just would not have been able to desequence -- your read lengths wouldn't have been limited by time, but would you still be able to do it? Yes. Would you be able to do it in the high-throughput way that Ju describes? No.
So it really comes down to what does high-throughput mean. And the problem is that scientists use these kinds of terms loosely. So when I used -- when I said "I don't know. An hour, that would not be great, I don't think, but that would at least be in the realm of doable," what I was saying was that an hour, you know, that's doable. It certainly could have been better, but I don't believe I ever intended to define an hour as any sort of cutoff.
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Page 64

BY MR. SCHWARTZ:
Q. But you did testify what is a reasonable time. I don't know, an hour. That would not be great.
That was your testimony?
A. Yes. That is what I wrote, but, again, I don't believe I was -- I don't believe there's anything in that statement that defines an hour as some cutoff. I think it is exactly what it says. I didn't know. An hour. I was speculating that wouldn't be great, but doable.
Q. An hour would not be great?
A. But it would be doable.
Q. So Tsien teaches that his SBS method requires rapid and quantitative deblocking. A POSA -- and, again, a POSA in terms of the way you defined it in October 2000 -- understood that to mean that the 3' protecting group should be cleaved quantitatively in what you consider to be around an hour?
A. I would not be comfortable imposing my speculation of an hour being doable but not great. I guess you could interpret that as saying my reading of Tsien in a doable but not great way would have suggested, I don't know, an hour as a

Page 65

deprotection time. But I don't think I meant to ever state that that was any sort of an absolute requirement in time, you know, 59 minutes okay, 61 minutes not.

Q. Around an hour?

A. Would be good, I guess. It would certainly be doable.

Q. The question was, you asked yourself what is a reasonable time. And your answer to what is a reasonable time was an hour.

A. I agree that one hour would probably be reasonable for a good, but not great sequencing by synthesis implementation of Ju's method.

Q. We are talking about Tsien.

A. I'm sorry. Of Tsien's method.

Q. Please go back to your declaration in IPR2013-00517. That is Exhibit 2007. Please turn to page 27, and please draw your attention to paragraph 52. In paragraph 52 you write: "Tsien and Ju's DNA sequencing methods require high and quantitative efficiency using nucleotides. See example Tsien at column 20, lines 28 to 34, stating that the criteria for the successful use of 3'-O-allyl blocking groups include, one, the ability of polymerase enzyme to accurately and

Page 66

efficiently incorporate the DNTPs, i.e., deoxy nucleotide triphosphates; and, two, the availability of mild conditions for rapid and quantitative deblocking."

That is what you wrote, correct?

A. That is what I wrote.

Q. And Tsien's SBS method requires quantitative deblocking of the 3'-O-allyl protecting group, correct?

A. I'm sorry. I was distracted by an error in my -- please repeat the question.

Q. Tsien's SBS method requires quantitative deblocking of the 3'-O-allyl protecting group, correct?

A. It requires very high efficient deblocking, yes.

Q. You wrote that it "requires high and quantitative efficiency using nucleotides," right?

A. That is what I wrote.

Q. Please go back to Exhibit 2080, which is your deposition transcript in IPR2013-00128. Please turn to page 200, and please draw your attention to line 3 on page 200. And starting at

Page 67

line 3, the transcript reads:

"Question: Okay. And it's greater than the 90 percent that you cite here in paragraph 58 as the disulfide cleavage conditions that would provide an expectation that the disulfide cleavage conditions, looking at cleavage of an OH protecting group with greater than 90 percent efficiency."

There was an objection by counsel. Some back and forth between you and counsel. You answered, "I never suggested that greater than 90 percent was sufficient. It's certainly necessary, but necessary is not necessarily sufficient. And 91 percent is greater than 90, but that would not be sufficient. 95 percent would not be sufficient. Sufficiency is really something approaching 100."

That was your testimony, correct?

A. That is what is written, yes.

Q. So it is your opinion that 95 percent deblocking efficiency of the 3' protecting group is not sufficient for SBS, correct?

MR. ZIMMERMAN: Objection. Mischaracterizes.

THE WITNESS: I would have to read this. If you want me to, I am happy to. I believe what I

Page 68

am talking about is the disulfide cleavage. Do you want me to take a minute to read this to get context?

BY MR. SCHWARTZ:

Q. You can read all you want to get context, but when you worked specifically in the portion that we just read referring to cleavage of a 3'-OH protecting group, when you looked at that, you stated that, that was the question. Cleavage conditions, getting cleavage of a 3'-OH protecting group, and your answer was that "95 percent would not be sufficient. Sufficiency is really something approaching 100."

That was your testimony?

A. Well, I look and I see that what's written is "and it's greater than 90 percent that you cite here in paragraph 58." This is what the question -- as the disulfide cleavage conditions. So I want to make sure I'm talking about not the disulfide cleavage.

Q. You are talking about the cleavage of a 3'-OH protecting group?

A. Can I take a moment?

Q. Okay. But do you see that you say that here in the testimony we just read?

Page 69

A. Can I answer that in a second?
Q. Sure.
A. It seems to me I'm talking about disulfide cleavage.
Q. In relation to cleavage of the 3'-OH protecting group, correct?
A. I think I'm talking about the efficiency of disulfide cleavage.
Q. Of the 3'-OH protecting group.
A. No.
Q. You're talking about cleavage conditions could get cleavage of a 3'-OH protecting group.
A. I'm sorry. I don't follow that. I know one of the issues that we were talking about in this case was disulfide cleavage of a nucleobase.
Q. Okay, but let's look at the transcript again. Starting at page 200, line 3.
"Question."
Are you with me?
A. Yes.
Q. "Okay. And it's greater than 90 percent that the cite here in paragraph 58. It's the disulfide cleavage conditions that would provide an expectation that the disulfide cleavage conditions could get cleavage of a 3'-OH protecting group with

Page 70

greater than 90 percent efficiency."
That was your question. And your answer was: "95 percent would not be sufficient. Sufficiency is really something approaching 100."
A. The problem that I'm having here is that at the time, we were talking about -- and I don't know if that's what we are talking about here, but one of the issues was simultaneous deprotection of a nucleobase linker and the 3' blocking group. So I'm not sure I'm talking about conditions where you get simultaneous cleavage.
Let me answer the question that I think you're getting at is that I think that we would need -- you would need for Tsien's sequencing by synthesis method, I think the answer is applicable to that, that you would need something greater than 95 percent for cleavage of the 3' hydrox group, if that's the question that you're asking.
Q. Yes.
A. Then I would agree with it. I want to caveat that I'm not absolutely sure that that is specifically precisely what I am talking about here, but I am happy to answer that question.
Q. Okay. And, in fact, you said that sufficiency is really something approaching 100?

Page 71

A. The closer you get to 100, the better the method's going to be.
Q. Sufficiency is really something closer to 100, correct?
MR. ZIMMERMAN: Objection. Asked and answered.
BY MR. SCHWARTZ:
Q. That is what you testified, right?
A. I think sufficiency is a relative term. Sufficient for what? But certainly you would have wanted to get as efficient as possible.
Q. So do you agree with your testimony that sufficiency is something approaching 100?
MR. ZIMMERMAN: Objection. Mischaracterize.
THE WITNESS: I wish to characterize that based on what sufficient -- what your application is and what sufficiency you need. It would depend on read length. It would depend on things, all sorts of things. So you would have to give me a set of conditions. I don't know the context that I testified here to know whether I would agree with myself or not. I think it's clear that the closer you get to 100 the better.
///

Page 72

BY MR. SCHWARTZ:
Q. Let's go back to Exhibit 2035, your deposition transcript in the IPR2013-00517 case. Please turn to page 156. Please draw your attention to line 12. And starting at line 12, the transcript reads:
"Question: Would the efficiency also include the efficiency of incorporation of a nucleotide; is that right?
"Answer: To the extent that the step limited things, then sure. The nucleotide has to be efficiently incorporated."
That was your testimony, correct?
A. That was what was written, yes.
Q. And it is your opinion that SBS requires efficient incorporation of the nucleotide by the polymerase, correct?
A. Well, I would like to caveat this in that efficiency is not a quantitative term; it is a relatively qualitative term, but it is certainly true.
Q. And so to successfully practice SBS, there must be both efficient incorporation of the nucleotide and deblocking efficiencies that are efficient?

Page 73

A. Yes.
Q. I'm putting in front of you another document that has not yet been marked. I am putting you in front of you, Dr. Romesberg, a document entitled "Video Deposition of Floyd Romesberg Ph.D. in IPR2013-00266.
MR. SCHWARTZ: Court reporter, could you please mark this as Exhibit 2082?
THE WITNESS: I'm sorry, so this is going to be --
MR. SCHWARTZ: 2082.
THE WITNESS: In the future, this is going to be today, this will be referred to as?
(Exhibit 2082 was marked for identification.)
BY MR. SCHWARTZ:
Q. 2082. Please turn to page 66. And please draw your attention to line 17. And starting at line 17 the transcript reads:
"Question: Okay. So what is the concentration needed to reach high incorporation?
"Answer: Of these substrates would be one millimolar.
"Question: Does that same sentence mention whether or not it is -- it's an efficient

Page 74

chain terminator?
"Answer: The sentence on page 3?"
And there was an objection.
"Question: Yeah, we -- yes.
"Answer: It says that they are not very efficient chain terminators.
"Question: And what does that mean to be a not very efficient chain terminator?
"Answer: That it is not a good substrate.
"Question: And what do you mean by not a good substrate?
"Answer: It is not recognized well and incorporated by the polymerase."
That was your testimony, correct?
A. That is what is written, yes.
Q. And when you are referring to a chain terminator, you are referring to the 3' protecting group, correct?
A. I would have to check. A 3' protected nucleotide is certainly a chain terminator.
Q. So a 3' protecting group that is not efficiently incorporated by a polymerase means that it is not a good substrate for the polymerase, correct?

Page 75

A. Yes.
Q. And the 3' protecting group that is not a good substrate means that it is not recognized well and it is not incorporated well by the polymerase, correct?
A. I think I agree with if it is not incorporated well, it is not a good substrate. I think that's what you mean by incorporation is that it is taken as a substrate so, yes.
MR. SCHWARTZ: And before it gets handed to you, maybe it would be easier, Court Reporter, this is a new exhibit, so could you mark this 2083.
(Exhibit 2083 was marked for identification.)
BY MR. SCHWARTZ:
Q. Dr. Romesberg, I've put in front of you a document entitled "Substitute Declaration of Floyd Romesberg Ph.D. in Support of Patent Owner's Motion to Amend in IPR2013-00128." Please turn to page 37 of that exhibit. And I'm just going to ask you if that is your signature on that page?
A. Yes, it is.
Q. Please go back to page 21. Please draw your attention to the last line on page 21. Do you see there's a sentence that starts with "A person

Page 76

of ordinary skill"?
A. I do.
Q. You write: "A person of ordinary skill in the art would have been well aware that incorporation of each sequential nucleotide and any related deprotection of a 3' protecting group or cleavage of a linker from the nucleotide in a DNA sequencing by synthesis process must be highly efficient to permit accurate sequencing."
That's what you wrote, correct?
A. That is what is written.
Q. So each cycle of that SBS requires highly efficient nucleotide incorporation and highly efficient 3' deprotection, correct?
A. Those words are qualitative but, sure, yes. And as I go on to do, you can simply do the math for anything that is not 100 and see what the ramifications are.
Q. I will. We'll do some of that math later, since you invited it. Thank you.
A. I'll invite triple integrals as well, if you would like to do some of that.
Q. That's above me.
So inefficient incorporation of the 3' protected nucleotide or inefficient deprotection of

Page 77

the 3' protecting group, that would result in an SBS system becoming out of phase, correct?

A. The sequencing read would lose phase, yes.

Q. And when the sequencing -- I'm trying to use your words. When the sequencing system loses phase, it's no longer capable of generating reliable sequencing information, correct?

A. Certainly once it's lost phase, that is correct.

Q. Please go back to Exhibit 2080. That's your deposition transcript in IPR2013-00128. And please turn to page 252. Please draw your attention to line 6. And starting at line 6 the transcript reads:

"Question: If more than half of the strands were out of phase after a given number of cycles, would that be sufficient for SBS?

"Answer. No. No."

That was your testimony, correct?

A. Yes.

Q. It's okay. You can smile.

It is your opinion that if more than half the strands are out of phase after a given number of SBS cycles, then that would not be sufficient

Page 78

for SBS, correct?

A. That was the answer to the question that I was answering.

Q. That's your opinion, right? That if more than half of the strands are out of phase after a given number of cycles, that would not be sufficient for SBS?

A. I think there are ways that you can recover data, and where exactly it fails to be recoverable, I'm not exactly sure. But this sounds about right to me.

Q. So you stand by your testimony?

A. More or less. Certainly losing phase is a problem for sequencing.

Q. And if more than half the strands were out of phase, you testified that would not be sufficient for SBS?

MR. ZIMMERMAN: Objection. Asked and answered.

THE WITNESS: I think there are methods that allow you to still sequence as you are losing phase. I don't know how far currently people can do that. I don't know that the specific software algorithms that allow you to do that as to how far you can read into a lost phase sequence, a losing

Page 79

phase sequence. So I don't know exactly at what point. I think that intuitively I would more or less agree with what I said here, although I don't know the exact value.

BY MR. SCHWARTZ:

Q. I am going to take you up on your suggestion that we do some calculations.

Does anybody have a piece of paper?

A. You're going to make me do calculations?

Q. You bet.

Let's assume that we have 93 percent efficiency of cleavage of the 3' protecting group at each cycle. After 10 cycles there would be .93 to the 10th of the sequence polynucleotide containing the correct sequence, correct?

A. Um-hmm. Sorry, yes.

Q. And so I just want you to do that calculation, .93 --

A. Can I use my calculator?

Q. Absolutely.

A. Actually, I am not sure my calculator has --

Q. Mine doesn't either. You'll have to do it 10 times.

A. Okay.

Page 80

MR. ZIMMERMAN: The answer is the answer. You're going to make him sit here and punch in .93 ten times on a calculator?

MR. SCHWARTZ: Yes, I am going to ask him to confirm that is 48.4 percent.

MR. ZIMMERMAN: Take as much time as you need, Dr. Romesberg.

THE WITNESS: The answer I get is 48 percent.

BY MR. SCHWARTZ:

Q. And that means that 93 percent cleavage efficiency of the 3' protecting group, more than half of the strands are out of phase after 10 cycles, correct?

A. Yes.

Q. Go back to Exhibit 1012, which is your declaration. Take your time.

MR. ZIMMERMAN: Declaration in this case?

BY MR. SCHWARTZ:

Q. Okay, so we're at Exhibit 1012?

A. Yes.

Q. That's your declaration in IPR2018-00291, correct?

A. Yes.

Q. And please turn to page 16. Right above

Page 81

paragraph 40 you have a header which reads: "It was known that the 3' capping group should be small."

Do you see that?

A. I do.

Q. Please keep that open, but I'd like you to go back to Exhibit 2007. That is your declaration in IPR2013-00517. And please go to page 32. Please draw your attention to paragraph 58, and please draw your attention to the second sentence. And you write: "One skilled in the art would have understood that the physical properties underlying, one, nucleotide stability; two, polymerase recognition; and, three, reinitiation of DNA synthesis following deprotection are not necessarily related to; four, size; and, five, the chemical reversibility. A protecting group can have any subset of the aforementioned properties independent of the other properties."

And to save time, I'm not going to read the rest of paragraph 58 into the record, but I would ask that you turn to page 34 where you have a concluding sentence. It starts with "Thus, a person of ordinary skill in the art."

Do you see that?

A. Yes.

Page 82

Q. And you write: "Thus, a person of ordinary skill in the art would not have had an expectation that a small and removal protecting group would also possess all of the listed properties of Ju and Tsien's protecting group."

That is what you wrote, correct?

A. Yes.

Q. And, again, the Tsien reference that you're talking about is the same Tsien reference that we have been talking about in this case?

A. It is.

Q. It is your opinion that a person of ordinary skill in the art would not have had an expectation that a small and removable protecting group would also possess all of the listed properties of the Tsien's protecting group, correct?

A. Sure, yes. I assume that you're going to have me clarify this, but ...

Q. No, I'm just asking you that you heard the question. I could repeat it again. It is your opinion that a person of ordinary skill in the art would not have had an expectation that a small and removable protecting group would also possess all of the listed properties of Tsien's protecting

Page 83

group?

A. Yes.

Q. Go back to Tsien, please. That is Exhibit 1013, back to pages 20 and 21. And, again, Tsien's required properties for successfully practicing his SBS method include; one, the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTP carrying the 3' blocking groups into the CD nature -- my mouth is dry. Let me read that again.

"Tsien's required properties for successfully practicing his SBS method include; one, the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs carrying the 3' blocking groups into the cDNA chain," correct?

A. That is listed as a criteria for the successful use of the 3' blocking group, yes.

Q. And Tsien's required properties include; two, the availability of mild conditions for rapid and quantitative deblocking, correct?

A. That is listed as well, yes.

Q. And Tsien's required properties include; three, the ability of a polymerase enzyme to reinitiate the cDNA synthesis subsequent to the

Page 84

deblocking stage, correct?

A. That is listed as No. 3, yes.

Q. It is your opinion that "a person of ordinary skill in the art" -- and, again, I'm using it in the way you do in October 2000 -- "would not have had an expectation that just because 3' blocking group is small, it would be expected to be accurately and efficiently incorporate the DTNPs carrying 3' blocking groups into the cDNA chain," correct?

A. That is what I testified.

Q. And it is your opinion that a person of ordinary skill in the art "would not have had an expectation that just because the 3' blocking group is small, it would be expected that mild conditions for rapid and quantitative deblocking are available for that blocking group," correct?

A. That is correct.

Q. It is your opinion that a person of ordinary skill in the art would not have had an expectation that just because 3' blocking group is small, it would be expected that the polymerase enzyme is able to reinitiate cDNA synthesis following deprotection, correct?

A. That is correct.

Page 85

(Exhibit 1035 was previously marked for identification.)
BY MR. SCHWARTZ:
Q. I am going to put in front of you a different exhibit.
I am putting in front of you Exhibit 1035 entitled "Cleavage of Allyl Ethers with Palladium Carbon" by Boss and Scheffold. You relied on this paper to form your opinions about deprotection efficiency of allyl groups, correct?
A. Actually, no.
Q. Okay. Why don't we take you back to your declaration that we looked at in IPR2018-00291.
MR. ZIMMERMAN: 10 of 12. Go to page 64.
THE WITNESS: On what page?
BY MR. SCHWARTZ:
Q. Page 32. And do you see where it says "It was also known -- it's paragraph 64 -- "it was also known that allyl groups were generally efficiently removed using palladium including under aqueous conditions," and you cite to Exhibit 1035, which is Boss?
A. Yes.
Q. So you relied on this paper to form your opinions about deprotection efficiencies of allyl

Page 86

protecting groups, correct?
A. Including conditions that -- under which you could get deprotection.
Q. But you relied on this paper to form your opinions about deprotection efficiencies. You say so in your declaration.
A. So I included two specific examples of deprotection. This is a paper that I relied particularly strongly on some aspects that weren't -- there certainly is data in here that lead -- that I included in -- to form my opinion that it was efficient, but this paper also was used for other -- to inform my opinion on other aspects of the reaction as well.
Q. As an additional question, none of the compounds in Boss are nucleotides or nucleosides, correct?
A. They are not.
Q. And when you opined that Boss talked that allyl groups were efficiently removed, you were referring to the first four entries in table one that reported yields of greater than 95 percent, correct?
A. I was.
Q. And that data comes from structures four,

Page 87

five, six and seven, correct?
A. That is correct.
Q. Is it fair to say that yields of 74 percent or 84 percent as shown for the last three compounds in Table 1 would not be understood to exhibit efficient removal of allyl groups?
A. That's correct.
Q. So in forming your opinion that Boss thought that the allyl groups were efficiently removed, you relied only on 8, 9, and 10 to form that opinion, correct?
I'm sorry. You relied only on -- 4, 5, 6, and -- let me ask the question again because I messed it up.
In forming your opinion that Boss thought that the allyl groups were efficiently removed, you did not take into account the yields of compounds 8, 9 or 10, correct?
A. I would not say that I did not take them into account. Because I never said or meant to imply that all groups could be efficiently removed at any level. I was looking through there and saw a wide variety of deprotection efficiencies. And certainly reactions 4 through 7 which show greater than 95 percent yield were the ones that

Page 88

represented the efficient deprotection so, yes.
Q. Let me ask you a few questions about those compounds in Table 1. As an initial question, the allyl protecting group on a nucleotide, 3'-OH. Let me go back.
I'm going to ask you about a few questions about the compounds in Table 1, okay? And as an initial question, the allyl protecting group on a nucleotide, 3'-OH, it's a secondary alcohol, correct?
A. That is correct.
Q. And the only secondary alcohol in Boss is compound 10, correct?
A. That is correct.
Q. Compound 4 is an aromatic alcohol, correct?
A. That is correct.
Q. And compounds five, six, seven, and nine are primary alcohols, correct?
A. That is correct.
Q. And compound 8 is also a primary alcohol, but it would be considered to be a hindered alcohol, correct?
A. Maybe. It is certainly in an environment that might suggest steric hindrance.

Page 89

Q. And it's a primary alcohol?
A. It is a primary.
Q. So a person of ordinary skill in the art understood that the structure that is most relevant in terms of the type of alcohol that is on the protecting group is compound 10 to a nucleotide 3'-OH because they're both secondary alcohols, correct?
A. I think that is hard to say. You have to understand the mechanism of these reactions. They are very complex, and even to understand any one even today, it's a mechanistic study. What you're getting at is whether or not the departure of the hydroxyl, that deprotection step, whether that weight limits the reaction. Sometimes it does; sometimes it doesn't. And so sometimes the efficiency of the stability, what you're getting at is whether or not the stability of that leaving group would impinge -- will affect the reaction. And that is not known. It could, but it might not, and so I think it is a little bit risky to say that that compound in particular represents the sort of yield, the sort of reaction that you would expect with a nucleotide, because that step is often not rate limiting.

Page 90

And if it -- to be clear, if it is not rate limiting that means that in a series of steps, if you have a step that is slower than everyone else, that step is called rate limiting, and that is the step that is going to determine the rate of the reaction. And if you are competing with other things, that determines the efficiency of the deprotection in this case.
These Palladium-catalyzed reactions are multi-step. The palladium has to bind. A nucleophile has to attack. You then have to do a rearrangement that involves displacement of that leaving group. That step may or may not be rate limiting, so the nature of the actual leaving group may or may not identify commonalities between them.
Q. Is it true that in terms of the compounds that we are looking at in the allyl on an aromatic alcohol is generally easier to cleave than an allyl on a secondary alcohol?
A. It might be, but it is not an absolute.
Q. And is it true that an allyl on a primary alcohol is generally easier to cleave than a allyl on a secondary alcohol?
A. If it's the rate limiting step, then, yes; if it is not, then, no. Let me rephrase that.

Page 91

The actual step itself, what you said is correct. But whether you actually can even see that because whether that -- those differences contribute to the rate of the reaction and the efficiency for the reaction that you're looking at, that may make no difference at all. Because they're simply not rate determining.
Q. Suppose in your hypothetical that the deprotection was the rate limiting step?
A. If the -- no, to be clear, all of the steps are the deprotection step. That step is a complex series of steps, and if the specific step that evolves the accumulation of negative charge on the oxygen and its departure and cleavage of that bond, if that specific elementary step is rate limiting for the deprotection, then what you're saying would be -- you would then see those differences in the rates and the efficiencies for the deprotection.
If that particular elementary step were not rate limiting to the overall deprotection step, then you would not see it.
Q. Okay. I think I understand that. But again, we're talking about if we just get down to the simpler level of the primary, secondary, and

Page 92

aromatic alcohols that we're talking about here, generally speaking, the aromatic is easier to cleave than a secondary?
A. It depends if that step is what limits the reaction.
Q. Would you agree that all of Boss's compounds are much simpler in structure than nucleotide, particularly one that has a cleavable label on a base?
A. Simpler?
Q. I asked simpler in the sense that a nucleotide with cleavable label on the base contains additional functional groups, right? For example, amines on the base, amides in the linkers double bonds in the bases, all of those could also react with palladium under the conditions of Boss's method, correct?
MR. ZIMMERMAN: Objection. Compound.
BY MR. SCHWARTZ:
Q. Do you understand my question?
A. Yes, I believe so. I mean, you're asking if any of the functionality in DNA would interfere with the palladium-catalyzed reaction. I don't think so. You know, these reactions -- I see hydroxyl groups here. So first of all, I don't

Page 93

think any of the functionality in DNA is particularly poisoning to a palladium-catalyzed reaction.

I don't see any -- I'm not envisioning any functionality in what I imagine is the standard implementation of a linker, fluora-4, in a sequencing by synthesis methodology that would be incompatible with palladium, I'm not imagining that. I'm not seeing that, if that is what you are asking.

MR. ZIMMERMAN: I think we need to change the tape.

THE VIDEOGRAPHER: This marks the end of media No. 1 of the deposition of Floyd Romesberg Ph.D. We are off the record at 11:12 a.m.

(Recess taken.)

THE VIDEOGRAPHER: We are on the record at 11:18 a.m., and this marks the beginning of media No. 2 of the deposition of Floyd Romesberg Ph.D.

BY MR. SCHWARTZ:

Q. Dr. Romesberg, please go back to Exhibit 2007. It's your declaration in IPR2013-00517. Please turn to page 33, and please draw your attention to the middle of the page where

Page 94

there is a sentence that starts "Furthermore, it was known that a nucleotide."

Do you see that?

A. I do.

Q. And you write: "Furthermore, it was known that a nucleotide having a 3'-O azido ester was not a substrate for polymerases examined by Metzker," and you cite the Metzker '94 paper. And that is the same Metzker paper that you used in your declarations in these cases, correct?

A. That is correct.

Q. And then you write: "Thus, 3'-O azido esters were known to interfere with polymerase recognition and incorporation of the modified nucleotide despite the small size and removability of this protecting group."

That is what you wrote, correct?

A. That is what is written.

Q. So it is your opinion that Metzker 1994 showed that 3'-O azido esters were known to interfere with polymerase recognition and incorporation of the modified nucleotide, despite the small size and removability of this protecting group, correct?

A. That is what is written.

Page 95

Q. Please turn to page 34 of the declaration, and please look at the second sentence and you write: "Thus, a person of ordinary skill in the art would not have had an expectation that 3'-O azido esters would also possess all of the listed properties of Ju and Tsien's protecting group."

That is what you wrote, right?

MR. ZIMMERMAN: Objection. Where are you reading from?

MR. SCHWARTZ: On page 34. Am I in the wrong place? Second sentence.

THE WITNESS: Yeah, you added azido group in particular, and that's not --

MR. ZIMMERMAN: Mischaracterizes the document. What you read is not what is on the page.

MR. SCHWARTZ: Let me get to the right place.

Can I go off the record for a second and try to find it? Because I know -- maybe it is in a different document.

MR. ZIMMERMAN: Okay.

THE VIDEOGRAPHER: We are off the record at 11:22 a.m.

(Recess taken.)

Page 96

THE VIDEOGRAPHER: We are on the record at 11:25 a.m.

BY MR. SCHWARTZ:

Q. We were looking at paragraph 33 where you wrote that -- on page 33, I'm sorry, of the declaration it was known. "Furthermore, it was known that a nucleotide having 3'-O" --

A. I'm sorry. Could you tell me what document we are on?

Q. Exhibit 2007. Page 33.

A. Yes.

Q. And you wrote "Furthermore, it was known that a nucleotide having a 3'-O azido ester was not a substrate for polymerases examined by Metzker." You cite to Metzker 1994. "Thus, 3'-O acetyl esters were known to interfere with polymerase recognition and incorporation of the modified nucleotide, despite the small size and removability of this protecting group."

That's what you wrote?

MR. ZIMMERMAN: Objection. Asked and answered.

BY MR. SCHWARTZ:

Q. You wrote that?

A. That is what is written.

Page 97

Q. So a person with ordinary skill would have had an expectation that 3'-O acetyl esters would be expected to interfere with polymerase recognition and incorporation of the 3'-O acetyl ester nucleotide, correct?

A. With the polymerases examined by Metzker, yes.

Q. Sitting here today are you aware of any other polymerases that had been tested prior to October 2000 with the acetyl ester?

A. I did not research that, so off the top of my head I could not come up with an example. I am not positive that people did not try, but I don't know of one.

Q. Let's go back to your declaration and IPR2013-00517, Exhibit 2007, and please look at paragraph 67 on page 37. In paragraph 67 you write: "I have reviewed several notebook pages provided by Illumina dated" -- blank. And the date is -- and that was redacted.

Do you see that?

A. Yes, I do.

Q. Please look at paragraph 68. In paragraph 68 you write: "Notebook SLB090 describes several experiments conducted in -- it was

Page 98

redacted -- where 3'-O-allyl protecting group was removed from nucleotides under aqueous conditions."

Please look at paragraph 75 on page 40. In paragraph 75 you write: "In my opinion, these notebook pages report that even under optimized conditions long time periods are required to achieve 3'-0 allyl protecting group cleavage efficiencies that are sufficient for SBS. For example, 3'-O-allyl protecting group was at best only -- redacted -- this is insufficient for SBS."

The passages I read into the record is what you wrote, correct?

A. That is what is written, yes.

Q. And in your opinion, the Illumina laboratory notebooks you reviewed show that even under optimized conditions in aqueous media, long time periods are required to achieve 3'-O-allyl protecting group cleavage efficiencies that are sufficient for SBS, correct?

A. Under the optimized conditions that they reported, that is what I'm saying.

Q. And you conclude from looking at those notebooks that the 3'-O-allyl protecting group would not work for SBS?

A. I don't believe I concluded that. I

Page 99

believe I concluded that the conditions -- the precise result they reported under the optimized conditions that they reported would be insufficient for SBS, yes.

Q. And when you came to that conclusion in 2014, you were aware of Metzker 1994 and the data in Metzker 1994 for the 3'-O-allyl nucleotide, correct?

A. I was, yes.

Q. Please go back to Exhibit 2081, that is your second declaration in IPR2013-00266.

MR. ZIMMERMAN: I don't think we have 2081.

THE WITNESS: Yeah, I thought we skipped 2081. 2082?

MR. SCHWARTZ: You don't have this before. I have skipped it before.

(Exhibit 2081 was marked for identification.)

BY MR. SCHWARTZ:

Q. And so are you ready?

A. Um-hmm.

Q. I put in front of you Exhibit 2081, which is your second declaration in IPR2013-00266. Please turn to page 15. Starting at six lines up

Page 100

from the bottom of page 15, there is a sentence that starts with "The person of ordinary skill in the art."

Do you see that?

A. I do.

Q. You write: "A person of ordinary skill in the art seeking to develop sequencing by synthesis methods, would recognize the need to use a nucleotide with a 3' protecting group that could be incorporated by a polymerase with high fidelity at moderate to low concentrations."

That is what you wrote, right?

A. That is what I wrote.

Q. And it is your opinion that a person of ordinary skill in the art seeking to develop an SBS method would recognize the need to use a nucleotide with a 3' protecting group that could be incorporated by a polymerase with high fidelity at moderate to low concentrations, correct?

A. Again, I believe that is what is written.

Q. Please go back to Tsien. To Tsien's paper, Exhibit 1013.

A. I have it.

Q. Please turn to page 38. On page 38 Tsien has a section entitled "Example four:

Page 101

Incorporation of Labeled Nucleotide Analogs Into DNA."

Do you see that?

A. I do.

Q. You can read through this entire paragraph if you need to in order to answer my question, but for now I'll just ask you to look at lines 14 through 19. And Tsien teaches the use of three unlabeled 3' blocked deoxy nucleotide triphosphate dNTP analogs at a concentration of 1.5 micromolar each and one 3' blocked fluorescently labeled dNTP analog at a concentration of 30 micromolar for his SBS method, correct?

A. That would seem to be correct.

Q. And Tsien's teaching of 1.5 to 30 micromolar concentrations -- excuse me -- of nucleotides is consistent with your opinion that a person of ordinary skill in the art seeking to develop sequencing by synthesis methods would recognize the need to use a nucleotide with a 3' protecting group that could be incorporated by a polymerase with high fidelity of moderate to low concentrations, correct?

A. I think that's just the question that you asked earlier, and if that's the case, then, yes,

Page 102

that is what I wrote, so, yes.

Q. A POSA understood that a nucleotide concentration of 200 micromolars is higher than what typically would be expected for SBS, correct?

A. Would a person of ordinary skill in the art thought 200 micromolar was higher than what?

Q. Well, when you were talking about moderate to low concentrations for SBS, would it be higher than the moderate to low concentrations?

A. 200 micromolar, no. That's probably in the moderate range. That is certainly not in the low range.

Q. But Tsien himself uses them at much lower concentrations, correct?

A. That would seem to be the case here, yes.

(Exhibit 1016 was previously marked for identification.)

BY MR. SCHWARTZ:

Q. I am putting in front of you Exhibit 1016, a paper entitled "Termination of DNA Synthesis by Novel 3' Modified Deoxyribonucleoside 5'-triphosphates" by Metzker that was published in 1994.

This is the same Metzker 1994 paper you refer to in your declarations in these current IPRs,

Page 103

correct?

A. That is correct.

Q. Please look at the abstract. In the abstract the authors write "Eight modified-dNTPs were synthesized and tested in two different template assays for incorporation activity. From this" --

MR. ZIMMERMAN: You want to read that sentence again? You did not read it correctly. So I just want to make sure, we left out the three.

BY MR. SCHWARTZ:

Q. "Eight 3'-modified-dNTPs were synthesized and tested in two different template assays for incorporation activity. From this enzymatic screen two 3'-O-methyl-dNTPs were shown to terminate DNA syntheses mediated by a number of polymerases and may be used as alternative terminators in Sanger sequencing."

That is what Metzker wrote, correct?

A. That is correct.

Q. When Metzker refers to the enzymatic screen, he is referring to the tests that are shown in table two on page 4263, correct?

A. I believe that is actually slightly vague, because he reports two different assays of

Page 104

which there's a lot of data, and then table two is sort of a summary of that. And so it is not exactly those assays, it is compiled from the assays.

Q. But if you look on the right side of page 4263 of Tsien, it talks about a --

A. I'm sorry --

Q. I'm sorry. Of Metzker. And he has a section "terminator screen," and he refers to the table two, correct?

A. It summarizes. That's right. And the only reason I point that out is there is an immense amount of little details in this paper that didn't make their way into this table, but I believe all of the things of your interest are going to be in this table.

Q. Again, I am just saying when he refers to enzymatic screen in his abstract, he is referring at least to the information in table two, correct?

A. At least, yes.

Q. You are familiar with the term "screen" as used by scientists, correct?

A. I am.

Q. And is it fair to say that the screen is used by scientists to -- who are involved in the

Page 105

discovery projects to mean a preliminary test that is used to identify compounds that have promising properties?

A. It can be used in that way. That is not the only way it is used.

Q. But that is a way that it is used?

A. It is used in that way. It is also used as a preliminary analysis that is not superficial. A screen itself does not necessarily have to be rough or approximate. It is somehow limited and is expected to then lead to information that would help you move forward.

Q. And is it fair to say that screens are also used to decide which compounds not to move forward with?

A. They can be used in that way.

Q. And that would be because they don't have necessarily the properties you are looking for in your discovery project, right?

A. Yes.

Q. Going back to table two of Metzker. Metzker tested modified nucleotides with seven different 3'-O protecting groups to see how they behaved in Sanger sequencing, correct?

A. Was it exactly Sanger sequencing? I

Page 106

mean, certainly -- he compares to the dideoxy termination pattern, which sort of is Sanger sequencing. So I didn't ask myself were each of his assays precisely Sanger sequencing, but I think essentially that is correct.

Q. Included in that group of seven 3'-O protecting groups is a 3'-O-allyl protecting group, correct?

A. It is compound 3, yes.

Q. And going back to the abstract we just looked at that from those screening tests that are reported in table two, Metzker identified two 3'-O protected nucleotides that Metzker says may be used as alternative terminators in Sanger sequencing, correct?

A. Yes.

Q. And Metzker states in the abstract that "the 3'-O methyl dNTPs were shown to terminate DNA synthesis and may be used as alternative terminators in Sanger sequencing."

That is what he states, correct?

A. That is what is written.

Q. Metzker does not say that the 3'-O-allyl dNTP was shown to terminate DNA synthesis and may be used as an alternative terminator in Sanger

Page 107

sequencing, right?

A. He does not say that.

Q. I would like you to keep Metzker '94 in front of you. I'm going to mark another paper.

MR. SCHWARTZ: This will require a new stamp, Court Reporter, 2053.

(Reporter clarification.)

MR. SCHWARTZ: Some of these are going to be a little bit out of order. I'll be coming back and forth, so instead of changing my whole index, I'm just going to ask you to mark this as 2053. This is actually something already marked in these IPRs, but it wasn't in all four of the early four.

MR. ZIMMERMAN: Is it a record only in the method case or is it a record in the four nucleotide cases?

MR. SCHWARTZ: It's so far only of record in the method case.

MR. ZIMMERMAN: Because we offered the witness today on the method. And you're not taking him on the method, but you're using exhibits from the method case. Just want that to be clear from the record.

MR. SCHWARTZ: We're using an exhibit from the method case.

Page 108

(Exhibit 2053 was marked for identification.)

BY MR. SCHWARTZ:

Q. Dr. Romesberg, I'm putting in front of you a document entitled "Sequencing technologies, the next generation," by Michael Metzker. It was published in 2010. The author of this article is the same Michael Metzker who is an author of the Metzker 1994 paper we were just looking at, correct?

A. That is correct.

Q. Please turn to page 34 of that paper, and please draw your attention to the left column which contains a section header entitled "Cyclic reversible termination."

Do you see that?

A. I do.

Q. And please look at the second paragraph in that section that starts with "The key to the CRT Method."

Do you see that?

A. I do.

Q. CRT means Cyclic Reversible Termination, correct?

A. Correct.

Page 109

Q. And the CRT method is another name for SBS, correct?
A. I would have to read that. It is not a term that I use. In fact, I don't think it is a particularly commonly used term. I'd have to see whether that refers to a specific step of SBS or if it refers to SBS overall, certainly is a step.
Q. Please draw your attention to the section that starts with "The use of Dideoxy Nucleotide." Do you see that?
A. Yes.
Q. Metzker writes "The use of a Dideoxy nucleotide which acts as a chain terminator in Sanger sequencing provided the basis for the initial development of reversible blocking groups attached to the 3' end nucleotides." That is what Metzker wrote, correct?
A. That is what is written, yes.
Q. And Metzker refers to reference 19 and 20 support for that statement, correct?
A. Yes.
Q. And would you please turn to the references section and look at references 19 and 20. And reference 19 is the same Metzker 1994 paper that we just looked at, correct?

Page 110

A. Yes.
Q. So in Metzker's 2010 paper, he states that the work he reported in his 1994 paper on terminators for Sanger sequencing provided the basis for development of 3'-O reversible blocking groups for SBS, correct?
A. That is what it states.
Q. Is it fair to say that a reader of Metzker's 1994 paper understood that the work that it was reported in the 1994 paper on terminators for Sanger sequencing provided the basis for his development of 3'-O reversible blocking groups for SBS?
A. If you're asking me if a person of ordinary skill in the art would have read the 1994 paper and understood that Metzker was looking to develop reversible terminators for what he called -- he didn't use the term sequencing by synthesis, but for that, then, yes, I think a reader -- I think he states that in the 1994 paper.
Q. And is it fair to say the person of ordinary skill in the art -- and, again, whenever I use POSA, I'm referring to POSA that you defined, goes back to October 2000 -- is it fair to say that a person of ordinary skill in the art understand

Page 111

that a cleavable 3'-O protecting group that works for Sanger sequencing would be a good candidate for development of a blocking group for SBS?
A. If it worked for Sanger sequencing, it would satisfy certain requirements that would also be required for sequencing by synthesis, so it would be a piece of data that a person of ordinary skill in the art would look to to form an opinion about sequencing by synthesis, yes.
Q. And by the same token, is it fair to say that a POSA understood that 3' protecting group that inhibits polymerase activity in Sanger sequencing would also likely inhibit polymerase activity in SBS?
A. I don't know what you mean by the word "inhibit."
Q. The way that Metzker uses the word inhibit in his paper.
A. No, I do not believe that that would be a necessary fatal flaw to moving forward with an analog.
Q. Is it fair to say that a person of ordinary skill in the art understood that the 3' protecting group that is an inefficient terminator in Sanger sequencing would also be expected to be

Page 112

an inefficient terminator in SBS?
A. I want to be careful about how I answer this question, because inefficient is not a particularly useful term here. Inefficient relative to what?
Q. Well, you used the word inefficient all the time in what you --
A. And I believe I used it very qualitatively, and if you wish for me to define more specifically what I meant, I'm happy to look at any one of them if it's not clear by context, which I hope it would be.
But in this case it's really not useful to simply categorize something as interference means inefficient. And I'll tell you why. You can imagine a modification of A to C. The natural triphosphate that polymerases use as natural substrates. C is actually recognized less efficiently than A. So would I call that interference? No, I mean, it's a natural substrate. So I think the question that you're asking is at what point does interference preclude its use in sequencing by synthesis. And that's a slightly hard question to answer without qualitative data, but I think it's certainly safe

Page 113

to say that if it's not accepted as a substrate, then that is not going to be a good candidate for sequencing by synthesis.

If it is recognized as well as a natural substrate, that's awesome. People would be really excited, and they would want to move forward in the sequence by synthesis application. But there's a whole range of gray between the two. And at what point it becomes so inefficiently recognized that it could be not be used as a substrate, it's certainly -- there's an area in there where that is going to happen.

Q. I think maybe we're getting a little bit too complicated here. Basically, Metzker in 2010 pointed to the '94 paper and said that the '94 paper provided the basis for his moving forward with protecting groups for SBS, correct?

A. Correct. That would be his moving forward with his opinions of what efficiency was required.

Q. Okay. And in the 1994 paper he lists some nucleotides as being, for example, inhibitors of the polymerase. And I'm just saying would a person understand that he didn't choose those to move forward with in his SBS studies because they

Page 114

were inhibitors in 1994?

A. I think if you looked at the 2010 paper and those compounds that he found inhibited in 1994 weren't there, I think that you would have logically assumed that he got rid of them, he didn't pursue them because they were not promising.

Q. That is the basis for my question to you, the same for inefficient. Those that he found to be inefficient, he didn't move forward with those. He made that clear in the 2010 paper, correct?

A. Are you asking me if this is the only metric that he used to decide to move forward?

Q. No, I am not asking you if it is the only metric.

A. Certainly the efficiency of which it caused termination as he described it in the '94 paper would have been a component of his thought process, of his decision process to decide to what to -- with which -- in his process to decide what he was moving forward with.

Q. And those are reasonable decisions that Metzker made. You said his thought process, but that would be is it reasonable for other people to have that thought process?

A. Everyone is going to weigh things

Page 115

differently. I don't know exactly what he was thinking. I don't know exactly what motivations he had to choose one or the other, but it was certainly a requirement that you have some recognition. He would have never moved forward with something that has no recognition if he had multiple things that had recognition, he may have chosen one over the other for other reasons.

Q. Let's go back to the allyl compound in Metzker, that's compound 3 as you said, right? When I say "Metzker," I should have clarified that Metzker 1994 since there may be several Metzkers on the table.

The 3'-O-allyl dATP had no incorporation with seven of the eight polymerases Metzker used in the screening assay, correct?

A. That is what it is listed.

Q. Based on those results, a POSA understood that the 3'-O-allyl dATP would most likely not work for DNA sequencing with any of those seven polymerases, correct?

A. With those polymerases under the condition employed, a POSA would have not been encouraged -- would not have had an expect -- would not have been provided with an expectation for

Page 116

success, correct.

Q. For DNA sequencing?

A. That is correct.

Q. If I refer to Vent (exo-) DNA polymerase, if I refer to that only as "Vent," will you understand that I am referring to the Vent (exo-) DNA polymerase in Metzker '94?

A. I will now.

Q. Thank you.

Metzker reports that the incorporation of the 3'-O-allyl dATP by the Vent polymerase was incomplete at the final concentration of 250 micromolar, correct?

A. That is what he writes, yes.

Q. Please turn to page 4265. Please draw your attention to the second paragraph on the right side of the page that starts with "Some notable correlations."

Do you see that?

A. I do.

Q. And please look at the second sentence in that paragraph that starts with "First, the protecting groups."

Do you see that?

A. I do.

Page 117

Q. And Metzker writes "First, the protecting groups containing ether linkages at the 3' position, compounds 1, 3, 4, 7 and 8, were incorporated by some of the polymerases."
That is what Metzker wrote, correct?
A. That is what he wrote.
Q. So when Metzker writes that "Compound 3 was incorporated by some of the polymerases," the reader of Metzker understands that the incorporation Metzker refers to is incomplete incorporation with the Vent polymerase at 250 micromolar, correct?
A. Under the conditions that he employed, yes. I'm sorry. Could you repeat that question?
Q. So when Metzker writes that "Compound 3 was incorporated by some of the polymerases," the reader of Metzker understands that the incorporation Metzker refers to is incomplete incorporation with the Vent polymerase at 250 micromolar, correct?
A. Under the conditions that he explored that he is reporting, yes.
Q. And earlier we looked at the Tsien publication, and Tsien suggested using nucleotides in the low micromolar range, 1.5 to 30 micromolar.

Page 118

Do you remember that?
A. I do.
Q. So is it fair to say that a person of ordinary skill would expect that the 3'-O-allyl dATP could show even less incorporation with Vent if it was used at a 10-fold lower concentration that Tsien suggests?
A. That is right. But Tsien is suggesting a different reaction than what Metzker is looking at, and so it's very difficult to compare that.
Q. I am not asking you to compare it, I'm just saying --
A. You just did.
Q. I'm saying that -- no, I said that Tsien suggested using lower concentrations of nucleotides.
A. And then you asked me to extrapolate onto the Metzker data as to what that would have done.
Q. And my question was, at those lower concentrations of nucleotide, would a person of ordinary skill reasonably expect that the incorporation by the allyl nucleotide by Vent could be even less than he reported at 250 micromolars?
A. That is a physical law, yes.
Q. Please draw your attention to the last sentence page -- withdraw the question.
Please turn to page 4263 and please draw your attention to the right side of the page where there is a header "3-O Methyl dATP compound 1 incorporation."
Do you see that?
A. I do.
Q. And please draw your attention to the last sentence in that section that starts with "All RP-HPLC purified."
Do you see that?
A. I do.
Q. And Metzker writes "All RP-HPLC purified 3' modified dATPs, compounds 1 through 7, showed approximately 1 percent dATP contamination, and these trace levels could not be removed by subsequent rp HPLC."
That is what Metzker writes, correct?
A. That is what he writes.
Q. And compound 3, 3'-O-allyl dATP, is one of the compounds included in compounds 1 through 7, correct?
A. That is correct.
Q. So Metzker is teaching that compound 3, the 3'-O-allyl dATP that is listed in table two contains approximately percent of the unprotected nucleotide, correct?
A. Triphosphate, yes.
Q. The unprotected dATP?
A. Yes.
Q. When Metzker reports that compound 3, the 3'-O-allyl dATP, had incomplete termination with Vent at 250 micromolar, it is possible that any incorporation Metzker observed for 3'-O-allyl dATP is actually incorporation of the contaminated dATP, correct?
A. I'm sorry. Could you repeat that, please?
Q. When Metzker reports that compound 3, the 3'-O-allyl dATP, had incomplete termination at 250 micromolar with Vent, it's possible that the incorporation Metzker observed is actually incorporation of the contaminated dATP, correct?
A. I don't think so.
Q. Why not?
A. Because you wouldn't get termination if natural dATP were incorporated. There is no terminating group; there is no blocking group. It would, in fact, lead to reduced termination. In fact, that is what almost certainly Metzker is

Page 119

Page 120

Page 121

seeing. Some level of the reduced termination, the asterisk that he puts there is exactly due to that, because there is natural dATP incorporating, and it is allowing synthesis to continue.

Q. I think maybe we're talking past one another. When he said the termination is incomplete, he's suggesting that there is some?

A. Termination.

Q. There is some termination, but that it is incomplete, meaning that it continues to go on. And I'm saying that is it possible that that is because of the contamination of the unprotected dATP?

A. The incomplete termination is because of the presence of the contaminating dATP. Yes, I think that is certainly correct.

Q. I am going to put in front of you another document.

MR. SCHWARTZ: This will require a marking. 2084.

(Exhibit 2084 was marked for identification.)

BY MR. SCHWARTZ:

Q. I am putting in front of you a document entitled "The NEB Transcript," dated September 19,

Page 122

1992 that I've marked as Exhibit 2084. Please draw your attention to the top far-left side of the page, and under the date of September 1992 it reads "A scientific newsletter from New England Biolabs."

Do you see that?

THE WITNESS: I do.

MR. ZIMMERMAN: Objection. Lacks foundation.

BY MR. SCHWARTZ:

Q. New England Biolabs is a supplier of biochemical products for the scientific community, correct?

MR. ZIMMERMAN: Same objection.

BY MR. SCHWARTZ:

Q. You can answer the question.

A. NEB is a company that sells such reagents, yes.

Q. And including products for DNA sequencing, correct?

A. I think so.

Q. Do you recall reading NEB transcripts in the course of your own work?

A. No, I did not.

Q. On the far right side of the page is an index with page numbers.

Page 123

Do you see that?

A. I do.

MR. ZIMMERMAN: Objection. Foundation.

BY MR. SCHWARTZ

Q. And within the index there is an entry entitled "CircumVent Thermal Cycle DNA Sequencing Kit."

Do you see that?

MR. ZIMMERMAN: Can I have a standing objection to this exhibit on foundation, or do you want me to make the objection every time?

MR. SCHWARTZ: You can have a standing objection, and the foundation will become clear in a minute.

BY MR. SCHWARTZ:

Q. Okay. Do you see where there's an entry "CircumVent Thermal Cycle DNA Sequencing Kit," in the index, page 12?

A. Yes.

Q. Please turn to the next page, which is page 12. And I will represent that Exhibit 284 -- 2084 is not the complete transcript. It's only the portion that refers to the CircumVent Thermal Cycle DNA Sequencing Kit.

Do you see that?

Page 124

A. I do.

Q. And under that header it reads "CircumVent thermal cycle DNA sequencing is a simple and rapid method which generates sequence information from less template than any other protocol. The kit uses the highly thermostable Vent (exo-) DNA polymerase."

That is what it says, right?

A. That is what it says.

Q. Now, please go back to Metzker 1994, which is Exhibit 10,016 [sic], and go back to table two. The Vent polymerase used in Metzker 1994 is the Vent (exo-) DNA polymerase, correct?

A. That is correct.

Q. Now, please turn to Page 4261 of Metzker. And on the right side towards the bottom of page 4161 is a header entitled "Polymerases."

Do you see that?

A. I do.

Q. And please draw your attention to the last sentence in that section. Metzker states that he got the Vent (exo-) DNA polymerase from New England Biolabs, correct?

A. That is written, yes.

Q. So the Vent polymerase in Metzker 1994 is

Page 125

the same Vent polymerase that is being discussed in the NEB September 1992 transcript, correct?
A. I would have to look at this. As I said, I haven't looked at this before. This seems to be a thermal cycle kit. It seems to use Vent. I don't know what else is in the kit.
Q. I'm just asking you specifically the polymerase. It's the same polymerase that Metzker says that he used?
A. It seems to be the same polymerase.
Q. Please go back to that exhibit, the transcript, Exhibit 2084 and turn to the next page, which is page 13.
A. I'm sorry, which? 2084?
Q. Yes, page 13 is the next page in that. Do you see that? And please draw your attention to the middle column. And towards the bottom of the middle column on page 13, there is a header which reads "Can the CircumVent kit be used for sequencing templates to be used on automated fluorescent sequencers."
Do you see that?
A. I do.
Q. The NEB transcript answers that question with a yes, but with a caveat. Now, please draw

Page 126

your attention to the second sentence there which starts with "However, when fluorescent tagged."
Do you see that?
A. I do.
Q. And the transcript reads, "However, when fluorescent-tagged dideoxy dNTPs are used in the sequencing method, the results can be mixed. Inefficient incorporation leads to different intensities and peak heights on the resulting fluorograms, thus, the software has more difficulty calling correct bases."
That is what the transcript states, right?
A. That is what is written, yes.
Q. So the NEB transcript teaches that adding a fluorescent label to a dNTP results in inefficient incorporation by Vent polymerase, correct?
A. They don't describe what -- they don't describe what the modifications are, but they say that inefficient incorporation can lead to different intensities, yes.
Q. When you add a fluorescent tag to the dNTP?
A. Whatever fluorescent tag they are

Page 127

exploring, yes.
Q. And the transcript states that, that inefficient incorporation leads to unreliable sequencing. That's what it says, right?
MR. ZIMMERMAN: Objection. Mischaracterizes.
THE WITNESS: The document says inefficient incorporation leads to different intensities and peak heights and more difficulty in calling correct bases.
BY MR. SCHWARTZ
Q. So would you say that that is what results in unreliable sequencing?
A. You have as much information about it as I do. It says that difficulty in calling correct bases.
Q. So please go back to Metzker 1994, and go back to table two. Metzker reports in table two that the unlabeled 3'-O-allyl nucleotide incompletely terminated the sequencing reaction using Vent (exo-) DNA polymerase at 250 micromolars of the nucleotide, correct?
A. Under the conditions that he is using here, that is what the document says.
Q. And we just looked at the NEB transcript,

Page 128

the September 1992 transcript, and it teaches that fluorescently labeled nucleotides are inefficiently incorporated by the same Vent polymerases Metzker used in those experiments, correct?
A. The polymerase is the same, so given the caveats that we just described, there can be some fluorophore link or modifications that reduce efficiency and complicate base calling, according to NEB.
Q. Given that Metzker reports that the 3'-O-allyl dATP without a label is capable of incomplete termination even at high nucleotide concentrations, a person of ordinary skill in the art would expect that adding a fluorescent label to that nucleotide could result in even less termination activity with a Vent, correct?
A. No, I disagree with that. He has not reported that the 3'-O-allyl is efficiently incorporated.
Q. No, I said incomplete termination.
A. That's inefficient incorporation. So he has not reported that it, on its own, is inefficient at termination. As we just discussed, there is 1 percent dATP in there.
Q. But if you add a fluorescent label to

Page 129

that nucleotide, it could result in even less termination than Metzker reported at the 250 micromolar, correct?

A. That is if -- if the added linker, as was the case apparently with the NEB report, if the added linker reduced the efficiency, then, yes, that would presumably, in an additive way, reduce the termination. But, again, that is termination in the presence of 1 percent dATP.

Q. Yeah, I'm just referring to Metzker's studies.

A. As am I, and his Metzker study has 1 percent dATP in the mixture. No one would sequence that way. No one would include dATP that you're allowing to compete with your modified dATP where you're relying on the modified dAPT terminate.

Q. Right. So the fact that there is -- that there is unlabeled, unmodified dAPT there would be understood to change the way that one would expect that nucleotide to behave if that unmodified dATP wasn't there, correct?

A. A POSA would understand that having that contaminating dAPT would dramatically change this, yes. I guess just to be clear, a POSA would know

Page 130

that the O-allyl modified dAPT at a 100 fold more concentration than unmodified dAPT was competing with the incorporation of that unmodified dAPT, because you are getting some termination.

Q. Please go back to page 63 of Metzker '94. And please look at the header "Terminator Screen" on the right side of the page, and please look at the last sentence in that section that starts with "Compounds 1, 8, 7."

Do you see that?

A. I do.

Q. And Metzker writes "Compounds 1, 8, and 7 showed specific termination and were further evaluated with respect to their concentration-dependent effects."

That is what Metzker writes?

A. Correct.

Q. A person of ordinary skill reading Metzker understood that Metzker was advancing compounds 1, 8, and 7 for further evaluation based on the screening tests that were reported -- at least based on the screening tests that were reported in table two, correct?

A. At least, but that data was available to him when he made the choice to conduct further

Page 131

experiments concentration-dependent effects with 1, 8, and 7.

Q. And a person reading -- a person of ordinary skill reading Metzker 1994 also understood that, based on those screening tests, Metzker did not advance compound 3 for further evaluation, correct?

A. No. If you say based on at least those screening effects, then I might agree with it.

Q. Let me change the question then.

A person of ordinary skill reading Metzker 1994 understood that at least based on the screening test, Metzker did not advance compound 3 for further evaluation?

A. So just to be clear, because I'm not sure I understand that turn of the phrase "at least based on," he had that information available. He certainly had other information available too. And he chose to move forward with 1, 8, and 7, and what you are getting at, obviously, is that he did not move 3 forward, and he had the data from his kinetic data that's reported in table two, and he had a world of other data as well. And I don't know what it was that fed into his choice to not move 3 forward.

Page 132

Q. But he didn't move 3 forward?

A. He did not move 3 forward.

Q. Metzker 1994 did not describe any condition to remove the 3'-O-allyl group, correct?

A. That is correct.

Q. He certainly didn't describe any condition to remove the 3'-O-allyl group that would be compatible with DNA synthesis, right -- or DNA -- let me strike that.

Metzker 1994 did not describe any condition to remove the 3'-O-allyl group that is compatible with DNA, correct?

A. That is correct. And in fact, I think that's probably related to why he did not move 3 forward. Because he had the conditions to easily deprotect 7. He wasn't the chemist. So he probably didn't think about reactions to deprotect 3.

Q. But he didn't describe any conditions?

A. That is right.

Q. Six lines down in the abstract, which is on page -- we read the first part of the abstract, and I'm just going to read the next part of the abstract -- there is a sentence that starts with "3'-O 2-nitrobenzyl dAPT."

Page 133

Do you see that?
A. I do.
Q. And Metzker writes "3'-O- 2-nitrobenzyl dAPT is a UV sensitive nucleotide, and was shown to be incorporated by several thermostable DNA polymerases. Base-specific termination and efficient photolytic removal of the 3' protecting group was demonstrated. Following deprotection, DNA synthesis was reinitiated by the incorporation of natural nucleotides into DNA. The identification of this labile terminator and the demonstration of one-cycle stop/start DNA synthesis are initial steps in the development of a novel sequencing strategy."
That is what Metzker writes, correct?
A. That is correct.
Q. So Metzker advanced a nitrobenzyl nucleotide for further testing and demonstrated it was capable of one complete cycle of SBS, correct?
A. I think that is precisely why he advanced it, because it was easy to show it would be capable of that complete step.
Q. And Metzker didn't advance the 3'-O-allyl cap nucleotide for SBS testing?
A. He did not.

Page 134

MR. SCHWARTZ: Do you want to take a break for lunch at any particular time? I don't know. What are your plans?
MR. ZIMMERMAN: When are you hungry?
THE WITNESS: I generally don't eat big lunches. So whenever anyone else is hungry.
MR. SCHWARTZ: Maybe we should break now.
MR. ZIMMERMAN: If there is a natural stopping point or a place, we are not wedded to a particular time.
THE VIDEOGRAPHER: We are off the record at 12:16 p.m.
(Recess taken.)
THE VIDEOGRAPHER: We are on the record at 1:19 p.m.
BY MR. SCHWARTZ:
Q. Good afternoon, Dr. Romesberg. I am going to put in front of you another exhibit. That doesn't require marking. I'm putting in front of you Exhibit 107. It is entitled "Termination of the DNA Synthesis by N6-Alkylated, Not 3'-O-Alkylated Photocleavable 2'-Deoxyadenosine Triphosphates," by Wu, et al.
You reviewed this paper in forming your opinion in the current IPRs, correct?

Page 135

A. That is correct.
Q. The corresponding author of this paper is Michael Metzker, correct?
A. Correct.
Q. And that's the same Michael Metzker who is the first author of Metzker 1994, correct?
A. That is correct.
Q. Please turn to page 6348 of the paper, and please look at the discussion section on the left side. Please draw your attention to seven lines down in the discussion section where the sentence starts with "This is unlike the situation for."
Do you see that?
A. I do.
Q. And the author writes "This is unlike the situation for 3' modified nucleotides which typically act as poor substrates for DNA polymerases. For example, screening 3'-O-allyl dATP with eight different polymerases revealed limited activity at high micromolar concentrations, with only Vent (exo-) DNA polymerase."
That is what the authors wrote, correct?
MR. ZIMMERMAN: You left out the word DNA.

Page 136

BY MR. SCHWARTZ:
Q. DNA polymerase?
MR. ZIMMERMAN: Yes.
MR. SCHWARTZ: I thought I did say DNA.
THE WITNESS: That is what is written.
BY MR. SCHWARTZ:
Q. And the authors cite to reference 8 as support for those statements, correct?
A. They do.
Q. And can you take a look at the reference list. And reference 8 is the Metzker '94 paper we've been looking at, correct?
A. That is correct.
Q. So the Wu paper which includes Metzker as an author refers back to the screening test reported in Metzker 1994, correct?
A. That is correct.
Q. And the Wu authors which include Metzker characterize the screening test in Metzker '94 as showing that the allyl capped nucleotide had limited activity at high micromolar concentrations with only one out of eight polymerases tested, correct?
A. That is what they state, yes.
Q. And they stated that in 2007, being aware

Page 137

of the fact that there were contaminants in the 1994 studies, correct?
A. That is correct.
Q. So despite the fact that they were aware of contaminants in the 1994 studies, even years later Metzker concluded that the 3'-O-allyl dAPT had limited activity with Vent at high micromolar concentrations.
That is what he says, right?
A. He says that. I just, by way of clarification, I think -- I don't know that I would call that a conclusion. He is using sort of justification for what he is doing. He is -- decided that he's pursuing a different analog, and he is then saying that the -- that all the old ones were not incorporated efficiently. Which, incidentally, I disagreed with, but that is not your question.
Q. And the Wu authors, which include Metzker, characterize the screening test in Metzker '94 as showing that the allyl cap nucleotide had limited activity at high micromolar concentration with only one of the eight polymerases?
MR. ZIMMERMAN: Objection. Asked and answered.

Page 138

BY MR. SCHWARTZ:
Q. That is what they said, correct?
A. They say that, yes.
Q. And a person reading Metzker 1994 in October of 2000 understood that the 3-O-allyl ATP had only limited activity at high micromolar concentrations with only one of the eight polymerases?
A. I would disagree with that.
Q. So you disagree with Metzker's interpretation of his own data?
A. I am.
Q. But you do agree that Metzker himself stated in 2007 that the O-allyl had limited activity high micromolar concentrations?
A. I agreed that he stated what you read.
Q. And the Wu authors also stated that "the 3-O-allyl dAPT is a poor substrate for DNA polymerase." That's what they said?
A. I agree that what you read is out of their paper.
Q. And they said that knowing that in their 1994 paper they had some contaminate in each of their nucleotides, correct?
A. I guess I don't know that the first

Page 139

author knew that, because I don't think he was in the group at the time that that paper was studied, or that paper was written, Metzker certainly was. I certainly think that that first author would have had access to read the paper. Do I know that he thought about that, no, but ...
Q. But the Wu authors do characterize those studies that way, correct?
A. I don't know who wrote the paper. Wu is first author on the paper, Metzker is corresponding author.
Q. Let's just make it simple. That is what the paper says, right?
MR. ZIMMERMAN: Objection. Mischaracterizes the document.
BY MR. SCHWARTZ:
Q. I disagree that I'm mischaracterizing the document. They say that the 3-O-allyl ATP is a poor substrate for DNA polymerase. They say it.
A. I agree with the passages that you read.
Q. Okay.
And that included Metzker on that paper?
A. Metzker is the corresponding author on this paper.
Q. Have you been corresponding author on

Page 140

papers?
A. I have.
Q. Is it your practice to read the papers that you are corresponding author on?
A. I write the papers that I'm corresponding author on.
Q. And is it your practice that, even if there are other authors on the paper where you're a corresponding author, that you would permit information that you did not believe to be accurate to be in that paper?
A. No.
Q. So would you agree that corresponding authors vouch for the veracity of the information in the papers of which they are corresponding authors?
MR. ZIMMERMAN: Objection. Lacks foundation. Calls for speculation.
THE WITNESS: So before I accuse Metzker of falsifying data, which I am not going to do, all the statements that we're talking about are qualitative. He's saying that they aren't great substrates. Well, relative to what? Relative to the natural substrate? Sure.
I'll tell you what. They're good enough

Page 141

that they compete with natural dAPT for incorporation. Metzker seems to characterize that as "not great." I would characterize that as surprisingly good. I guess it's a matter of perspective or taste, I guess. I don't think he was lying or falsifying anything. There is no quantitative data there that's -- you know, it's he simply says -- he uses a qualitative term that's not wrong. You can buy -- again, I don't particularly like the qualitative way he describes it, but what I'll also say is this is life.

People do this. People use qualitative statements to justify where they're at in the program right then. He had chosen, I think, probably because he was seduced into this idea that you could photodeprotect very easily a base protected analog -- sorry, a 3' protected analog. That turned out to be incorrect, as I know you know.

And I think he was enticed into that approach because he thought it was so easy to deprotect with light. And so that is why, in fact, I am almost certain moved compound 8 on in 1994, not because it was particularly better as a substrate than O-allyl. I think he made that

Page 142

choice based on the photodeprotection. So I don't think that he's mischaracterizing things. I just wouldn't characterize -- I just wouldn't use the perspective that he -- I would not take the perspective he is taking.

BY MR. SCHWARTZ:

Q. But you would agree that he did interpret his 1994 data as saying that the 3-O-allyl ATP is a poor substrate for DNA polymerase.

A. I would not agree with that. Those are the words he uses. It is attempting to take words so literally.

Q. I am only asking you if Metzker is saying that.

A. He wrote those words. And that is not how a person of ordinary skill in the art would have written them.

Q. Those are the words in his paper?

MR. ZIMMERMAN: Objection. Asked and answered.

THE WITNESS: Those are the words in his paper.

(Exhibit 2025 was previously marked for identification.)

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Page 143

BY MR. SCHWARTZ:

Q. I am going to put in front of you another document. I'm putting in front of you Exhibit 2025, a document entitled "Improved Nucleotide Selectivity and Termination of 3'-OH Unblocked Reversible Terminators by Molecular Tuning of 2-nitrobenzyl Alkylated HOMedU Triphosphate." And, again, the corresponding author of this paper is Michael Metzker, correct?

A. That is correct.

Q. And this is the same Michael Metzker who is the first author of Metzker '94, correct?

A. It is.

Q. Please turn to Page 10 of the paper. Please draw your attention to the first full paragraph on the left side of the page that starts with "The Use of HOMedU."

Do you see that?

A. Yes, I do.

Q. Will you please look a little over halfway down that paragraph where there is a sentence that starts "We note 3-O-allyl dAPT is."

Do you see that?

A. I'm sorry. I'm having a little bit of trouble.

Page 144

Q. It's about halfway down.

A. Yes, I found it.

Q. You are with me?

A. I am.

Q. And the Litosh authors write "We note that 3'-O-allyl dAPT is not efficiently incorporated by Vent (exo-) polymerase."

That is what the authors wrote, correct?

A. That is what they wrote.

Q. And they refer to reference 46 to support that statement, right?

A. They do.

Q. Reference 46 is the Metzker 1994 paper, isn't it?

A. It is.

Q. So the authors of the Litosh paper, which includes Michael Metzker as the corresponding author, states that the 3'-O-allyl dAPT is not efficiently incorporated by Vent (exo-) polymerase, correct?

A. Those are the words -- your reading of those words is correct.

Q. And they refer to the Metzker 1994 paper as support for that statement, correct?

A. They do.

Page 145

Q. And again, they made that statement in this paper, which was published in 2011, aware of the fact that they had some contamination of the nucleotides that were used in the Metzker 1994 studies, correct?

A. I assume they were still aware of that. I don't know, maybe he wanted to forget it. It's frankly, it's a mistake on his part, and he may have not been proud of that.

Q. So you're putting yourself into Metzker's mind now?

A. No, I'm thinking of how I would feel if I had done that, and I'm just feeling how most people would have. He is drawing conclusions that are really not, in my opinion, founded. And I think they wouldn't have been to the person of ordinary skill in the art as well.

Q. We looked at two papers, post 1994, where Michael Metzker is the corresponding author, and in both of those papers he is interpreting the experiment in the 1994 paper, correct?

A. That is correct.

Q. And he is interpreting those experiments to conclude that the 3'-O-allyl dATP was not suitable for his studies as a DNA terminator,

Page 146

correct?

A. I don't know that he said that. I would have to look to see that he said that, but the --

Q. He says that it is not efficiently incorporated in 2011. And he says in 2007 that it's a poor substrate for the polymerase, correct?

A. So he doesn't refer to sequencing by synthesis as a whole, but, you know, this is why we qualify people's opinions as having to be a person of ordinary skill in the art. A person of ordinary skill in the art, I imagine, would have been confused by these statements.

Q. You don't know that though, do you know?

A. A person of the ordinary skill in the art would have seen 1 percent contamination. A person of ordinary skill in the art would have understood that dAPT would be very efficiently incorporated at its natural rate, and would therefore be contributing to the lack of termination mediated by the protected analog. It is sophomore organic chemistry. I think that anyone practicing in that field would have been interested in that, and then maybe if they then read these papers been confused by Metzker stepping away from it.

And again, I think what they would have

Page 147

concluded is he is interested in these other guys, and he is passing this aside because this is now his direction.

Q. This is all speculation on your part, isn't it?

A. It is. It is.

Q. Do you have any evidence that persons skilled in the art actually believed in what you just said?

A. Yes, because what I just said is sophomore chemistry. It's basic chemistry. And that is why we put a certain burden -- my understanding is that's why we put a certain limit on what we define as a person of ordinary skill in the art. And I think a person of ordinary skill in the art would have fully appreciated the natural substrates for polymerase are very efficiently incorporated.

Q. But Metzker himself who had even extraordinary skill in the art -- would you agree that he had more than a person of ordinary skill in the art? How would you characterize that?

MR. ZIMMERMAN: Objection. Compound. Do you want to pick which one of those questions?

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Page 148

BY MR. SCHWARTZ:

Q. Do you consider Metzker a POSA?

A. Yes.

Q. You consider Metzker a POSA, and Metzker as a POSA wrote that the 3'-O-allyl dAPT is not efficiently incorporated by a Vent polymerase, right?

A. So if you really want to talk about why he said this, I think it's clear. First of all, efficiently is a relative term, and so he's not saying anything wrong, and he is justifying the direction of research that he's going. He's got NIH grants that describe how great his approach is.

Unfortunately, everyone in science has to do this. We have to justify what we are doing relative to other things. You're right. This is all speculation. I don't know why he wrote those words, but it is not speculation that there was 1 percent dAPT in those reactions and that that 1 percent dAPT led to two and a half micromolar of the natural substrate. That's a concentration that is typical of a standard PCR reaction. So it's going in. And so to the extent it goes in, it is going to inhibit the chain termination by the analog. That is not speculation, and that is not

Page 149

something that would not have been obvious to a POSA.

Q. And when Metzker wrote in the other paper, the 2007 paper, that the 3'-O-allyl dAPT is a poor substrate for DNA polymerase, that was also Metzker as a POSA writing that, correct?

A. Yeah. And if you want me to speculate why I think he might have written that, speculate, I'm happy to do so.

Q. I'm not asking you to speculate. I'm only asking you to answer my question.

A. He wrote that. And I would consider him a POSA.

Q. And earlier we looked at Tsien, and you agreed that one of Tsien's specific requirements is efficient incorporation of the 3' block nucleotide by the polymerase, correct?

A. Correct.

Q. You also agree that to be successful for SBS, the nucleotide has to be efficiently incorporated by the polymerase?

A. Are you asking my opinion?

Q. No, you actually said it, I'm just asking if you remember that it was your opinion that to be successful for SBS, the nucleotide has to be

Page 150

efficiently incorporated by the polymerase?

A. Just because I would like to understand the context in which I said that, where are you reading that?

Q. We can go back. We already have testimony on this, but we can go back to your deposition transcript in IPR2013-00517. I forget what exhibit that is.

A. I feel like I can answer the question now without looking further. That would be in the context of a sequencing by synthesis method.

Q. Let's go back to table two of Metzker 1994. That is on page 4263 of Metzker.

A. I am there.

Q. Compound 1 in table two is a 3'-O-allyl dATP, correct?

A. That is correct.

Q. And compound 8 in table two is a 3'-O-Methyl dTTP, correct?

A. That is correct.

Q. The only difference between compound 1 and compound 8 is the base, correct?

A. That is correct.

Q. The base in compound 1 is an adenine. The base is compound 8 is a thymine, correct?

Page 151

A. That is correct.

Q. I want to focus on table two's results for the AMV RT polymerase, okay?

A. Okay.

Q. The AMV RT polymerase was able to incorporate the methyl capped adenine nucleotide, correct?

A. That is correct.

Q. But the same polymerase was unable to incorporate the methyl capped thymine nucleotide, right?

A. That is what is reported.

Q. Again, the only difference between those two Methyl capped nucleotides is that one had an adenine base and the other had a thymine base, correct?

A. That is correct.

Q. I want to focus on table two results for the last four polymerases, the BST DNA polymerase, the AmpliTaq DNA polymerase, the Vent (exo-) polymerase, and the RTH DNA polymerase. We'll focus just on those, okay?

A. Yes.

Q. These four polymerases were capable of incorporating the methyl capped thymine nucleotide,

Page 152

right?

A. That is correct.

Q. But none of those four polymerases was capable of incorporating the methyl capped adenine nucleotide, right?

A. That would seem to be what the table is saying.

Q. You agree none of the polymerases in table two were capable of incorporating both the methyl capped thymine analog and the methyl capped adenine analog, correct?

A. That would seem to be the case.

Q. Please turn to page 4266. Look at the right column. The second full paragraph that starts with "Several unexpected results."

Do you see that?

A. Yes.

Q. And Metzker writes "We have not identified a polymerase that will incorporate both 3'-O-allyl dATP, compound 1, and 3'-O-allyl dTTP, compound 8."

That is what Metzker writes, correct?

A. That is written there, yes.

Q. And you agree that the results we just looked at show that if a polymerase incorporates a

Page 153

nucleotide analog with one particular base, it may or may not incorporate the similar analog containing a different base, correct?

A. That is what is stated there. That is the conclusion -- that is obvious in the conclusion that is stated there.

Q. So is it fair to say that a polymerase's ability to incorporate a nucleotide analog with one base doesn't provide an expectation that that polymerase will also incorporate a similar nucleotide with a different base?

A. I would probably have to disagree with that. It certainly doesn't prove that it is the case, but I think a POSA would have expected it to be, because you are making a modification that is distant from -- you are talking about the modification that change distant from the position you are modifying and most people think that a Watson-Crick base pair is formed. It doesn't particularly matter if it's a GC, AT, TA, CG, so I think most people would have dismissed that and assumed that it was sort of general.

I mean it was very common for people to simply characterize one analog and sort of expect that to transfer, to be transferable to other

Page 154

analogs. I don't think the people would have known that is the case. I think they probably would have expected it, and I think that is probably why he is calling it unexpected. He opens that paragraph by saying "several unexpected results." And so I think that is a bit of a surprise.

I would also point out, again, that all of those O-methyl ATP reactions have dATP in there. So some of those dashes may have been because they were being outcompeted by the natural dAPT. So I think a POSA would have viewed that conclusion cautiously.

Q. That is your speculation?

A. That is my speculation. It is not my speculation that those reactions had natural dAPT in there and those would have officially incorporated. Again, each of those reactions had 1 percent of the natural dAPT in there, and it is not my speculation that a POSA would have recognized that as an efficient substrate. That is my definition of a POSA.

Q. But you will agree that Metzker's own studies demonstrate that just substituting a base with the same polymerase can result in completely different results in terms of incorporation by the

Page 155

polymerase, correct?

A. No, I don't agree with that. I agree that Metzker states that.

Q. Yeah, I am asking you, Metzker says that, correct?

A. Yes, I'm sorry. I thought you were saying I agree with the result.

Q. No. I am saying you agree that Metzker says that?

A. Correct.

Q. And, again, Metzker as a person of ordinary skill, he understood his experiments that there was no polymerase that he found that could incorporate both the 3'-O-allyl dAPT and 3'-O-methyl dTTP. That is what he says.

A. That is what he says.

Q. As a POSA.

So going back to the allyl capped nucleotides, the only allyl capped nucleotide that was tested by Metzker is the adenine, correct?

A. That is correct.

Q. Metzker didn't test the thymine, did he?

A. He does not report that.

Q. So based on Metzker results that we just looked at for the methyl capped adenine nucleotide

Page 156

and the methyl capped thymine nucleotide, a POSA understood it was possible that for the allyl capped nucleotide, changing the base could change the way that it would it be incorporated, if at all, by a polymerase?

A. A POSA would have understood that just as a matter of his education. I don't think a POSA would have interpreted -- I don't think a POSA would have been comfortable interpreting the results in this manuscript as evidence of that. Because I think the POSA would have come into the -- would have approached that question from the beginning saying, yeah, they're probably going to be similar.

And then he would have seen Metzker's conclusion, and he would have said, well, Metzker seems to think otherwise, there is 1 percent dAPT in these. So I think that a POSA would have been -- would have viewed that skeptically.

(Exhibit 1036 was previously marked for identification.)

BY MR. SCHWARTZ:

Q. Let's move on to a different exhibit. I am putting in front of you Exhibit 1036, entitled "Unexpected Enzymatic Fucosylation of the Hindered

Page 157

Tertiary Alcohol of 3-C-Methyl-N-Acetyllactosamine Produces a Novel Analogue of the LeX-Trisaccharide" by Qian that was published in 1998.

This is the same Qian paper that you referred to in these IPRs, correct?

A. That is correct, Qian.

Q. Please turn to Page 2185. Please draw your attention to scheme two. You refer to scheme two to form your opinion that the allyl protecting group is removed quantitatively, correct?

A. Yes, that is correct.

Q. And the compounds in Qian are not nucleotides or nucleosides, correct?

A. That is correct. They are sugars, which is closely related to a -- the sugar of the nucleotide of which the 3'-O-hydroxyl group is part of, meaning that that unit is a sugar. And these are sugars.

Q. These are disaccharides, correct?

A. They're two sugars attached.

Q. Just so that we are understand each other, a nucleotide used for sequencing does not have a disaccharide, does it?

A. No, but a nucleotide used for sequencing has a sugar unit. These have sugar units.

Page 158

Q. Now the scheme that is used to deprotect the allyl in Qian is not performed in aqueous media, correct?

A. It is performed in methanol.

Q. And you agree that protecting groups for use is SBS must be removable under aqueous conditions, correct?

A. That is correct.

Q. And you just said this one, instead of aqueous conditions, uses methanol?

A. That is what's reported.

Q. And you testified earlier that methanol is a DNA denaturance?

A. Neat methanol is a DNA denaturance. As long as -- to the extent that it dehydrates DNA, it is a denaturer. There is no reason these reactions could haven't been performed with added water. The reaction scheme they're showing is absolutely compatible with water.

Q. So you are suggesting that somebody could modify this reaction scheme, is that what you are suggesting?

A. I am suggesting it would have been obvious to a POSA that he would have had the flexibility and had high expectation of success

Page 159

that this would have been equivalent in water or in water/methanol mixtures, if not pure water.

Q. Earlier we looked at your testimony in IPR2013-00517. It was actually your declaration that was Exhibit 2016. Can you go back to that?

A. What is the number?

Q. 2007.

A. I have it.

Q. And if you go back to paragraph 46. You write in paragraph 46: "Furthermore, the conditions used by Loubinoux in table two would not be compatible with Tsien's and Ju's sequencing by synthesis methods. The experiment in table two that achieved 95 percent yield used THF as a solvent, which as with pyrimidine, was a known DNA denaturant," it cites to a Lee paper, "Therefore, one of ordinary skill would not have viewed table two of Loubinoux as teaching that yields vary widely" -- I'm sorry. "Therefore, one of ordinary skill would not only have viewed table two of Loubinoux as teaching that yields vary widely, but would also have considered this method of azido-methyl protecting group removal to be incompatible."

MR. ZIMMERMAN: I want to be clear that

Page 160

he skipped several sentences.

MR. SCHWARTZ: I did.

BY MR. SCHWARTZ:

Q. "But would have also considered this method of azido-methyl protecting group removal to be incompatible with Tsien's and Ju's requirements for sequencing by synthesis."

Now, your position in those IPRs was that the reaction and conditions in the references that IBS's expert was using for the deprotection of the 3' protecting group, the azido-methyl protecting group is not compatible with Tsien's SBS method. You criticized the other expert's use of a method that was not compatible with SBS, correct?

A. I appear to be saying that it would not have been compatible.

Q. You criticized that paper that the other expert was using because you viewed that paper as being incompatible with SBS, correct?

A. Well, I think I was concluding that it was incompatible, but I hope I didn't criticize the expert.

Q. I am not saying you criticized the expert. I'm saying you criticized the expert's use of that paper, because it -- in your opinion, it

Page 161

was not -- did not have compatible 3' protecting group deblocking conditions.

A. If the reaction was done in neat pyridine, then I would have said then and I would say now that that is not compatible with any DNA based method.

Q. So let's go back to the Qian paper. The Qian paper is using conditions that are not compatible with SBS, correct?

A. The difference is that in one case, the transferability to conditions that would be are trivial and well known and commonly practiced. In the other case, they were not.

Q. But you agree that the conditions in the paper that you are referring to in your declaration in these cases those conditions are not compatible with SBS?

MR. ZIMMERMAN: Objection. Asked and answered.

THE WITNESS: A POSA reading the Loubinoux paper would have thought that is unlike -- those conditions are unlike -- there would not have been -- there would not have been high expectation of success of performing a reaction -- of that particular reaction in a way

Page 162

that was compatible with DNA. A palladium chloride mediated deprotection of an allyl group -- of an O-allyl would have been known that it could be done in water, and so when you see it done in methanol, you would have had -- a POSA would have had a high expectation that that would be doable in water.

MR. SCHWARTZ: Can I just take a minute to ask -- can I go off the record for a second, please?

THE VIDEOGRAPHER: This marks the end of media No. 2 of the deposition of Floyd Romesberg Ph.D. We are off the record at 1:57 p.m.

(Recess taken.)

THE VIDEOGRAPHER: On the record at 2:00 p.m. And this marks the beginning media No. 3 of the deposition of Floyd Romesberg Ph.D.

BY MR. SCHWARTZ:

Q. Dr. Romesberg, going back to the reaction, the palladium reaction in Qian. Palladium chloride is only soluble in methanol or an organic solvent, correct? It has to have an organic solvent?

A. It is not highly soluble in water, so to solve that problem, people had worked out additives to get it into water. The most common one is a PEG

Page 163

additive. Trucol is a PEG. Palladium chloride forms polymers, and that precludes it from acting as a catalyst because they precipitate, they become insoluble. What Trucol as a PEG does, is it breaks them up into smaller polymers that are soluble. So it was common knowledge that you could get palladium chloride into water. People wanted to get metallic reactions, metal catalyzed reactions into water to make them green. It was a deal in the '80s. And this was well known. In particular, with palladium chloride, which is part of what drew me to Qian or Qian, I am not sure how to pronounce, but to this reference.

Because it is regarding the mild conditions required for compatibility with DNA sequencing by synthesis this really has everything. It is a sugar, which is very close to a nucleotide. It is the O-allyl, it's quantitative, it's less than two hours to room temperature. It's methanol that people understood that that would not be a limitation, that you could -- people would have had expectation this would have worked in water.

Q. But this reference that you are relying on to say that there was quantitative cleavage of the allyl protecting group uses conditions that are

Page 164

not themselves compatible with SBS, correct?

A. That is correct.

Q. Let's go back to the Boss reference that was Exhibit 1035. Please turn to page 558. On the right side of the page you see there is a section entitled "Experimental"?

A. I do.

Q. And in the experimental section there is an example for compound 7.

Do you see that?

A. I do.

Q. And the reaction -- I'm sorry. Compound 7 is a compound that showed a deprotection yield of greater than 95 percent, correct?

A. That is correct.

Q. And the reaction that is used to deprotect the allyl for compound 7 uses 10 mils of methanol and two mils of water, correct?

A. That is correct.

Q. We can calculate the concentration of methanol in that mixture, can't we?

A. That is possible, correct, yes.

Q. I'm going to walk you through a calculation. So I'm going to ask you, may I have another piece of paper?

Page 165

A. I still have the former one you gave me with very little written on it if that saves you the trouble.

Q. Did you write anything on that paper? We'll keep that paper, because I will mark it at the end. And so we're going to walk through this calculation for the concentration of methanol in that reaction, okay? And I will provide you with some information which I will represent to you is accurate, and your counsel, if they think it is inaccurate, has a chance to take that up with you on your redirect.

So why don't you write down that the molecular weight of methanol is 32 grams per mil. I'm sorry, 32 grams per mole.

And the density of methanol is .79 grams per mil. So 10 mils of methanol weighs 7.9 grams, correct?

MR. ZIMMERMAN: Do you need a calculator, Dr. Romesberg?

THE WITNESS: No. 10 mils would be 7.9 grams, that's correct.

BY MR. SCHWARTZ:

Q. The 7.9 grams, again, the molecular weight of 32 grams per mole is .25 moles, correct?

Page 166

And you can have a calculator.

A. Look, as easy as this is, it is surprisingly tricky to do on your feet in front of when 10 people are watching you. If you tell me that is correct, I'll believe it. It sounds about right. If you really want me to sit here and do that freshman chemistry, I'll do it.

Q. Yeah, let's do it. 7.9 --

A. All right. I will do it.

Q. 7.9 grams, when the molecular weight of 32 grams per mole is .25 moles, correct?

A. Is 7.9 divided by 32.

Q. And it's .25 grams.

A. You want me to check that?

Q. I do.

A. Okay. I will check that. 7.9 divided by 32 is 0.25.

Q. Moles?

A. Moles.

Q. The so the molarity of methanol in the mixture of 10 mils of methanol and two mils of water, that is to say you've got a total of .012 liters of solution, would be .25 moles over .012 liters, correct?

A. That sounds right.

Page 167

Q. Would you do that calculation, .25 over 0.12?

MR. ZIMMERMAN: 0.012.

BY MR. SCHWARTZ

Q. 0.012.

A. 0.012 liters per mole is what you are saying?

Q. Mm-hmm.

A. So it's not divided by.

Q. It's .25 moles over 12 mils, which is 0.012 litters, right?

A. So you want me to calculate the molarity there?

Q. Exactly.

MR. ZIMMERMAN: Take your time.

THE WITNESS: I calculate 21 molar.

BY MR. SCHWARTZ:

Q. 20.8 to be exact?

A. Okay. 20.8.

Q. Do you agree?

A. It's what my calculation says, so probably.

Q. So the concentration of methanol -- what do you mean probably? It is or it isn't.

A. I did the calculation here. I got an A

Page 168

in freshman chemistry in 1984, so I guess I trust my calculation. You got the same result, so I assume it is probably right. But if I were -- if I were you, I would have done this carefully, made sure it is right, and presented me the data.

Q. But your calculation --

A. If you want me to go through it and come up with it, I did it, and that is what I got. So you can believe that for what it is. I hope I didn't make a mistake.

Q. And you got 20.8 molar?

A. I did.

Q. So the concentration of methanol in Boss's allyl deprotection reaction is 20.8 molar, correct?

A. I believe so.

Q. Now, the Boss paper does not provide any details of the deprotection reaction for compounds 4 and 5, which also indicates the same methanol/water mixture. Based on the experimental section we looked at, is it reasonable to believe that the same methanol/water mixture was used for those reactions?

A. I don't know. If he doesn't tell us, then I guess I don't know.

Page 169

Q. It's reasonable though, could he -- he could be using that, correct?
A. Could have.
MR. SCHWARTZ: I'm going to mark another paper. This is going to require a marking. Court Reporter, this will be Exhibit 2085.
(Exhibit 2085 was marked for identification.)
BY MR. SCHWARTZ
Q. I'm putting in front of you a document entitled "The Relationship of Structure to the Effectiveness of Denaturing Agents for Deoxyribonucleic Acid" by Levine, et al. published in 1973.
Please turn to page 171 of the paper. On the top left side you see there is a table two?
A. I see it.
Q. Table two is a list of reagents that were tested for their ability to denature DNA, correct?
A. I see it.
Q. And the DNA being tested here is bacteria 5 DNA, correct?
A. That seems to be the case. I have not read this paper. Actually, I have read this paper, surprisingly, but I haven't read it in years, so

Page 170

that seems to be what it is saying.
Q. You actually relied on this paper in some of your IBS IPRs.
A. That's why I said I have read it, but not in years. That is, I believe, 2014.
Q. You testified earlier that DNA primer templates that were used in SBS are more susceptible to denaturation than large pieces of DNA.
Do you remember that?
A. Yes.
Q. Would you agree that the susceptibility of DNA primary templates in an SBS reaction would be even more susceptible to denaturation for bacteriophage DNA?
A. To the extent that they are smaller, that is correct.
Q. Now, first reagent listed in table two is methyl alcohol.
Do you see that?
A. I do.
Q. And that is the same as methanol, correct?
A. It is.
Q. And table two indicates that 3.5 molar

Page 171

methanol denatures 50 percent of bacteriophage DNA under the conditions of the assay, correct?
A. That is correct.
Q. And the conditions of the assay are given on page 168.
MR. ZIMMERMAN: To the extent you need time to verify that, please do.
BY MR. SCHWARTZ:
Q. The assay conditions for the data reported in table two of Levine. Do you see where it said denaturation of DNA was determined as follows?
A. I do.
Q. And it was 30 minutes under buffered aqueous conditions at close to neutral pH at 73 degrees C.
Do you see that?
A. I do.
Q. And if you go back to table two it indicates that the assay conditions were also done at an ionic strength of 0.043.
Do you see that?
A. I do.
Q. And if you go back to page 169, if you look down toward the bottom of the left column on

Page 172

page 169, about nine lines up from the bottom, there is a sentence that begins with: "At 73 degrees there is essentially."
Do you see that?
A. I do.
Q. And the sentence reads: "At 73 degrees there is essentially no denaturation of DNA in the absence of a denaturing agent."
That is what Levine wrote, correct?
A. That is correct.
Q. So the condition -- the reaction condition used to test the compounds in table two of Levine were not themselves denaturing, correct?
A. No, that is not true at all. They basically took the system to the brink of denaturation by heating it up. 73 degrees. That is the point.
Q. But it says "at 73 degrees there is no denaturation of DNA"?
A. They took it to the brink of denaturation.
Q. But they say that "at 73 degree there is essentially no denaturation in the absence of a denaturing agent."
That is what they say, right?

Page 173

A. None of the strands are dissociating, detectably. But they have -- the whole point of elevating the temperature was to give them a regime where they could see changes.

Q. What temperature do you think is -- in your opinion, what temperature does denature DNA?

A. It depends on the sequence, the sequence length, the sequence context. It's variable. It depends on the conditions. 73 is up there.

Q. Would 80 degrees denature DNA?

A. PCR reactions, which they have much longer strands of DNA, routinely heat up to 90 degrees, 88 degrees, 89 degrees, and assume complete denaturation in no methanol, in just aqueous standard conditions that favor duplex formation. So 73 is a lot closer to 89 or 88 than it is to room temperature of 37. These strands are close to dissociating.

Q. I mean it is your point that 73 degrees, even though Levine reports that there is no denaturation of the DNA in the absence of a denaturing agent, your position is that that is close to the level of where DNA would be denatured, if I understood you correctly?

MR. ZIMMERMAN: Objection.

Page 174

Mischaracterizes the document.

You can answer.

MR. SCHWARTZ: No, I'm asking about his testimony.

MR. ZIMMERMAN: I know, but the question mischaracterizes the document, so I'm going to make the objection.

You can answer.

BY MR. SCHWARTZ:

Q. The sentence reads: "At 73 degrees there is essentially no denaturation of DNA in the absence of a denaturing agent." That is what it says, right? And then you had a position about that that you said I think that that is close to where DNA denatures. You were making a point, right?

A. Yes. The point that I was trying to make -- and it wasn't for this specific strand, because I don't know the temperature at which it would dissociate. 73 degrees -- what -- DNA duplex is susceptible to heating. Heating is a denaturation -- is a denature -- you have a free energy that stabilizes a duplex. When you increase the temperature, the free energy stabilizing the duplex is decreasing, and you can get that to an

Page 175

arbitrarily small value, and it won't dissociate yet. And then a little more denaturation, whether that be provided by further increase in temperature or addition of methanol or whatever sort of denature you are interested in, then makes that free energy increasingly less favorable. And then you will start to see the dissociation of that.

So what they are doing is they are taking their system -- my guess is what they did was they wanted to -- within one piece of DNA, they wanted to be able to add different things and be able to see a range -- they wanted everything to be able to cause denaturation so they could compare them.

I think a chemist or a scientist would call this increasing your dynamic range so you can see the effects of things. If they would have done this at room temperature, I think a bunch of their things probably wouldn't have shown denaturation. And I think the three molar methanol would not have shown denaturation. I think that is probably true.

Q. That is a speculation, correct?

A. Based on this data, probably -- I mean, look, if it were 3 molar that caused denaturation at 78 degrees, then certainly not. It would not have caused denaturation at room temperature. That

Page 176

is not speculation.

Q. But do you remember relying on this very paper and this very table in your IPRs against IBS?

A. I do.

Q. And you relied on this paper and this very table to make conclusions about what agents would be DNA denaturing agents.

Do you remember that?

A. I do.

Q. So you -- at one point you relied on this table to make a conclusion about what agents were DNA denaturing agents, correct?

A. I made no conclusions about quantitatively at this temperature or any temperature what would cause denaturation. I was simply showing -- I believe -- and I have not read that for a little while -- I believe what I was evoking that table for was that pyridine, I think, was a stronger denaturant than a lot of other things. And so it is certainly true that at any given temperature -- and this is, in fact, why they chose to increase the temperature so they could see denaturation by everything, the concentration at which they caused denaturation they could then compare.

Page 177

So it is not disingenuous, and I would still believe this, that if I look at that table, you see pyridine is 0.09 molar. That's much less than methanol. And I believe my point was, and I would have to go look to rethink this if you want me to be sure, but I believe my point was that pyridine is a relatively strong denaturant. And I believe that conclusion is correct.

Q. But you did rely on this paper and on that table to form that conclusion?

A. In the way I just described it, yes. Which is not the same as the way we are looking at it now. Just to be clear.

Q. And in what way is it different?

A. I was comparing pyridine to other things.

Q. I haven't even asked you any questions about this table yet, so -- all I've asked you is what the reaction conditions were.

A. No, you are asking me about methanol being a denaturant, and that is shown there in the concentration.

Q. Yes.

A. And that is in this table.

Q. Yes.

A. The only point that I was making before

Page 178

was that you needed, at any given temperature, less pyridine to cause denature than most other things. And that is true.

Q. And going back to the entry for methanol that you just brought that up again, 3.5 methanol -- 3.5 molar methanol is reported in this paper to cause 50 percent denaturation of the bacteriophage DNA in 30 minutes, correct?

A. At 73 degrees.

Q. Which the paper reports there was essentially no denaturation of DNA at that temperature, the paper reports that, right?

A. I'm not sure if you don't understand what I'm saying or if you just want me to say it again. They took this by heating it up to a temperature where the free energy stabilizing the duplex was almost taken to zero. That allowed them to add different denaturants and get dissociation so everything they added, they would be able to see denaturation. They wanted to do that so they can see how they all compared. That is one conclusion you can draw from this table.

That was not the conclusion that I drew from the table four years ago in that IPR that you evoked. The conclusion I drew at that time was

Page 179

that pyridine under these given conditions is a stronger denaturant than methanol. Not that pyridine would or wouldn't have denatured it at these exact conditions.

Q. Does this paper report that 3.5 molar methanol caused 50 percent denaturation of the bacteriophage DNA in 30 minutes? Does the paper report that?

A. At 73 degrees.

Q. Now, we just did the calculation for the concentration of methanol in the allyl protection reaction in Boss, correct?

A. Yes.

Q. And that was 20.8 molar, correct?

A. Correct.

Q. And that is almost six times the concentration of methanol that caused 50 percent denaturation of bacteriophage DNA in Levine, correct?

A. Are you asking me to calculate that six times?

Q. Sure.

A. So you want me to take 20.8 and divide it by what number?

Q. 3.5.

Page 180

A. 3.5. Let me get my calculator. 20.8 divided by 3.5. I get 5.94.

Q. So that's almost a six fold difference, correct?

A. Yeah, that's almost a six fold.

Q. So a POSA reading this paper, Levine, understood that 20.8 molar would be expected to denature DNA?

MR. ZIMMERMAN: Object as to form.

THE WITNESS: I don't know how to explain this better, because I am not sure if you're simply not listening to me, or if I'm not explaining it well. I will try to explain this again if you want me to, but the conclusion that you just reached is wrong. This reaction was performed at room temperature. The comparison you just made would be at 73 degrees temperature. I'm sure your expert witness will help you -- walk you through this if you don't believe me.

20 molar methanol, this paper certainly suggests you will get full denaturation. It does not tell you what will happen at room temperature.

BY MR. SCHWARTZ:

Q. But you expect 20.8 molar methanol to be compatible with SBS?

Page 181

A. 20 molar methanol at room temperature?
Q. 20.8 molar methanol.
MR. ZIMMERMAN: Objection. Incomplete hypothetical.
THE WITNESS: Are you asking if it would denature DNA or if the entire process would be compatible with that much methanol?
BY MR. SCHWARTZ:
Q. Let's start with the later. Would the process be compatible with that much methanol?
A. Probably. It would probably affect the polymerase.
Q. And at the bottom of table two there is an entry for acetonitrile.
Do you see that?
A. Bottom of table two.
Q. Of that Levine paper.
A. Oh, yes.
Q. And table two reports --
A. I'm sorry, acetonitrile? At the bottom?
Q. At the bottom right.
A. Oh, yeah, sorry. Yes, I found it.
Q. And table two reports that 1.2 molar acetonitrile causes 50 percent denaturation of bacteriophage DNA, correct?

Page 182

A. At 73 degrees.
Q. Which the paper reports there was no denaturation without the denaturant, right?
A. I know you're making --
MR. ZIMMERMAN: Objection. Mischaracterizes the document again.
MR. SCHWARTZ: I disagree with your saying that it mischaracterizes.
MR. ZIMMERMAN: You're leaving out the word "essentially." Every time you characterize you leave out "essentially," so that is a mischaracterization of the document.
MR. SCHWARTZ: The document says that at 73 degrees there is essentially no denaturation in the absence of denaturing agent. That is what the document says, correct?
THE WITNESS: Yes.
MR. SCHWARTZ: Can we take a break? Let's take a break here.
MR. ZIMMERMAN: Sure --
THE VIDEOGRAPHER: We are off the record at 2:26 p.m.
(Recess taken.)
THE VIDEOGRAPHER: We are on the record at 2:46 p.m.

Page 183

BY MR. SCHWARTZ:
Q. Dr. Romesberg, just to step back for a moment. We were referring earlier today about allyl deprotections with palladium chloride in water. Can you point me to any reference prior to October of 2000 showing an allyl deprotection with palladium chloride in water?
A. No. I cannot. What I can show you is a -- I'm sure I could find papers that talk about using palladium chloride in water.
Q. But can you point me to any paper that reports deprotection of an allyl using palladium chloride in water?
A. Not that I know of. In general, palladium chloride deprotections of allyl were most used in sugar chemistry, and those substrates were insoluble in water. People would not have desired to run the reactions in water.
Q. I think I'm ready to go to another exhibit.
MR. SCHWARTZ: Court Reporter, this will be marked as Exhibit 2086.
(Exhibit 2086 was marked for identification.)
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Page 184

BY MR. SCHWARTZ
Q. I am putting in front you, Dr. Romesberg, a document entitled "Deoxyribonucleic Acids" by Kit that was published in 1963. And this is a paper that you relied on in your declarations in the IPR2013-00517 case to form opinions about reagents that are DNA denaturants, correct?
A. I believe that is correct, yes.
Q. And please turn to page 57 of Kit. And please draw your attention to the last paragraph on the page, and five lines up from the bottom of the page there is a sentence that starts with "Thiocyanate."
Do you see that?
A. I do.
Q. And Kit writes "Thiocyanate, trichloroacetate, and perchlorate are more powerful DNA denaturing agents than those prototypical hydrogen bond breakers, urea and clonidinian chloride, and are matched only by certain organic solvents such as formalyde, dimethylformalyde, and dimethylsulfoxide."
That is what Kit writes, correct?
A. That is what is written.
Q. And Kit teaches that perchloric acid is a

Page 185

very powerful DNA denaturant, correct?
A. That is what is written there, yes.
Q. Please go back to the Boss reference that was Exhibit 1035. And please go back to Table 1. And if you look at the legend of Table 1, the legend indicates that deprotection conditions for all of the compounds except compound 8 contained perchloric acid, correct?
A. That is correct.
Q. And the acid is there because it is required for deprotecting an allyl using palladium two, correct?
A. That is not correct.
Q. There is acid there, correct?
A. There is acid, yes.
Q. Okay.
A. So let me be clear about that, in principle, it might have been important. The way people run these reactions if you look at the mechanism, there is a spot where if you do not have a powerful nucleophile, your electrophile has to be made a better leaving group. And in those cases it might be beneficial to add a proton, very common for people if they were not conducting mechanistic studies to simply add some acid because they don't

Page 186

really care if it is needed or not. Because they're sort of generic conditions.
And so I imagine that is what they were doing here.
Q. But clearly it indicates that perchloric acid is present in these deprotection reactions.
MR. ZIMMERMAN: Objection. Asked and answered.
BY MR. SCHWARTZ
Q. And, again, we looked at this before. I know that you don't like doing calculations, so I won't ask you to do it if you don't want to, but I would -- the concentration of perchloric acid that's listed there is either 1 millimolar to 5 millimolar, correct?
A. I think that I remember reading that.
Q. It's actually in the paper.
A. Okay.
Q. Do you see it? Turn it over and look at note a under the table.
A. Yes.
Q. And I want to calculate what the concentration of perchloric acid is when it's 1 millimolar -- one millimole per 12 mils. That's a simple calculation. I got 83 millimolars. Would

Page 187

you like to confirm that it is 83 millimolars?
A. No. I'll take your word for it.
Q. And 5 millimoles of perchloric acid in 12 mils. I calculated it was 417 millimolars.
Do you want to confirm that it is 417 millimolar?
A. If I bought the first, I bought the second.
Q. I am going to ask you now to do a calculation of pHs. And I will provide you with some information so use that table -- or use that piece of paper. The first piece of information I'll provide to you is that the formula for determining pH is pH equals minus log of the proton concentration. So when the hydrogen -- when the proton concentration is .083 molar, pH would be the minus log of .083, correct?
A. That is correct.
Q. And likewise, when the hydrogen -- the proton concentration is .417 molar, the pH will be the minus log of .417, correct?
A. Yes.
Q. We have a calculator that does logs?
I won't ask you to do that. I will tell you that the pH that I got from .083 molar is pH

Page 188

1.1.
A. Sounds about right.
Q. And your counsel will have an opportunity to correct me if I am wrong. And the pH for .417 molar is about .4.
A. Okay.
Q. I mean, would you like to do the calculation to confirm me?
A. No. It's a straightforward calculation.
Q. So maybe you can write that down that the pH of .083 molar is about 1.1, and the pH of .417 molar is about pH of .4.
A. What was the first number?
Q. I'm sorry, 1.1.
A. What was the hydrogen ion concentration?
Q. .083.
A. Zero eight?
Q. .083. I think you may have written that down already. There were two concentrations of perchloric acid given in that figure.
A. So it was 0.417 and 0.083 pH of 1.1 and 0.84?
Q. Yes. So please go back to the Kit reference, Exhibit 2086. Please turn to page 66 of Kit. Eight lines down from the top of page 56,

Page 189

there is a sentence that starts with "DNA is stable over."
Do you see that?
A. Yes.
Q. And Kit writes "DNA is stable over the approximate pH range of 2.7 to 12 --
MR. ZIMMERMAN: That's not the same. You said that is the start of the sentence?
MR. SCHWARTZ: I said about eight lines down there is a sentence that starts with "DNA is stable over."
MR. ZIMMERMAN: There is not a sentence that starts with that.
MR. SCHWARTZ: It's in the middle of the sentence. I apologize.
BY MR. SCHWARTZ
Q. But do you see where I am?
A. I do.
Q. And it says "DNA is stable over the approximate pH range of 2.7 to 12, but denatures at higher or lower pHs."
That is what Kit writes, right?
A. That is what's written.
Q. So pH 1.1, the pH of perchloric acid at 0.83 molars is outside the range of 2.7 to 12 where

Page 190

DNA is stable, correct?
A. That is correct.
Q. And pH of 0.4, the pH of perchloric acid at .417 molar is also outside the range of 2.7 to 12 where DNA is stable, correct?
A. That is correct.
Q. So the concentration of perchloric acid that was used to deprotect the allyl in the Boss deprotection reaction would denature DNA, correct?
A. The Kit reference suggests that it would.
Q. And that deprotection reaction in Boss would not be considered a mild condition that would not denature DNA, correct?
A. That is correct.
Q. Let's switch topics for a minute -- for a few minutes, and let's talk about depurination. If it's been known before 2000 that glycosidic linkages and purines are susceptible to acid hydrolysis, correct?
A. That is correct.
Q. I am going to mark the paper.
(Exhibit 2057 was marked for identification.)
MR. SCHWARTZ: This is, again, this is one of these papers that was present in only one

Page 191

IPR, but not all the IPRs, so we're going to mark it to make sure it is in all the IPRs. And it is 2057.
MR. ZIMMERMAN: So in the case where we're not doing the deposition in that case today, and the case that you refused to do the deposition on that case today, you are marking the exhibits from that case.
MR. SCHWARTZ: Not the exhibits from that case. This is only the second exhibit that has been used in that case. There were many exhibits in that case.
MR. ZIMMERMAN: This exhibit is outside of the scope of the IPRs that we are here to do the deposition for today.
MR. SCHWARTZ: It is not outside the scope, because it deals directly with acid catalyzed denaturation of --
MR. ZIMMERMAN: Was it of record in the four that we are doing?
MR. SCHWARTZ: It is of record now.
MR. ZIMMERMAN: Okay. I am objecting it's outside the scope.
MR. SCHWARTZ: Okay.
MR. ZIMMERMAN: What number did he mark,

Page 192

this one?
MR. SCHWARTZ: This is marked as 2057.
BY MR. SCHWARTZ
Q. So I'm putting in front of you a paper entitled "Instability and Decay of the Primary Structure of DNA" by Lindahl, which was published in 1993. Now, please draw your attention to the first page of Lindahl. It's Page 709. And on the left side of page 709 is a section entitled "DNA Hydrolysis."
Do you see that?
A. I do.
Q. And please draw your attention to the sentence that begins five lines down. It starts with "The instability of DNA."
Do you see that?
A. I do.
Q. Lindahl writes "The instability of DNA glyclosal bonds under physiological solvent conditions was investigated 20 years ago. It was measured by using DNA with carbon 14-labeled purine or pyrimidine residues and determining the rates of release of free bases as a function of temperature, pH, ionic strength, and nucleic acid secondary structure."

Page 193

That is what Lindahl writes, correct?
A. That is what is written.
Q. And Lindahl cites to references five through seven in support for that statement, correct?
A. That is correct.
Q. And please turn to the references list. And reference five is a paper by Lindahl and Nyberg published in 1972, correct?
A. That is correct.
MR. SCHWARTZ: I am going to mark that paper. That is going to be 2087, Court Reporter.
(Exhibit 2087 was marked for identification.)
MR. ZIMMERMAN: Is this already of record in any of the IPRs?
MR. SCHWARTZ: No.
BY MR. SCHWARTZ:
Q. So I am putting in front of you a document entitled "Rate of Depurination of Native Deoxyribonucleic Acid" by Lindahl and Nyberg, published in 1972. This is reference 5 in Lindahl 1993, correct?
A. It appears to be.
Q. Please direct your attention to the

Page 194

abstract. The abstract reads "The rate of depurination of double stranded bacillus subtilis DNA radio actively labeled in the purine residues has been followed as a function of temperature, pH, and ionic strength. In the magnesium containing buffer of physiological ionic strength, the rate constant for depurination of DNA is 4 times 10 to the minus ninth per second at 70 degrees and pH 7.4."
That's what the authors write, correct?
A. That is what is written.
Q. Please turn to page 3616 of the paper, and please draw your attention to figure nine at the top of the page. Figure nine shows the pH dependence of the rate of acid catalyzed depurination of DNA, correct?
A. It does.
Q. And if you compare figure nine to the statement we just read in the abstract, the rate of depurination reported in the abstract of four times 10 to the minus nine per second at pH 7.4 is consistent with where the dotted line in figure nine intersects the X axis, correct?
A. Yeah, I guess it is. It's a little hard to read, but I guess, yes.

Page 195

Q. And figure nine shows that as the pH becomes more the acidic, the rate of acid catalyzed depurination greatly increases, correct?
A. It increases, yes.
Q. And if you drop from pH 7.4 to about pH 4, the rate of acid catalyzed depurination of DNA increases about three logs, correct?
A. That is correct.
Q. That is an increase of about a thousand fold, correct?
A. Three logs is a thousand fold.
Q. And if we were to extrapolate figure nine to pH 1, the rate of acid catalyzed depurination of DNA could increase by about another three logs?
A. If that same linear relationship holds, then that's correct.
Q. And that would be an increase of about a million fold from pH 7.4, right?
A. That would be correct.
Q. So Lindahl shows that DNA is rapidly depurinated in strong acid, correct?
A. Well, it shows it down to pH 4.5.
Q. And he shows that it's rapidly depurinated?
A. Rapidly?

Page 196

Q. We were just talking about the logs. We can do the calculation. I know you don't like to do calculations, but we're talking about four times 10 to the minus nine per second at pH 7.4?
A. So four times 10 to the minus -- it's just that rapidly, again, is a relative term. That is what it is.
Q. And go back to Boss, please, Exhibit 1035. Let's look at Table 1, and we calculated that the pH of the allyl deprotection reactions that use perchloric acid is between 1.1 and 0.4, correct? We put that on that piece of paper?
A. The pHs, yes.
Q. So let me ask the question again so we can get a clean answer.
We calculated that the pH of the allyl deprotection reactions that use perchloric acid in Boss is between 1.1 and 0.4, correct?
A. Yes.
Q. And Lindahl shows that those conditions will lead to rapid DNA depurination all the way down to pHs below 1, correct?
MR. ZIMMERMAN: Objection. Mischaracterizes the reference. Assumes facts not

Page 197

in evidence.
THE WITNESS: Lindahl shows rates, I believe, down to pH 4.5, and the pH -- and it shows pH rates for that that vary from four times 10 to the minus nine, and up by about three orders of magnitude as we just discussed.
BY MR. SCHWARTZ:
Q. And if we extrapolate from that graph, assuming you can extrapolate from the graph.
A. Well, Lindahl does not show that.
Q. I know.
A. If you extrapolate it and it remains linear, then you can multiply that by another thousand.
Q. So it would be a million fold if you did that extrapolation?
A. If it's linear over that range, then would that be correct.
Q. And Lindahl shows that in pHs between 1.4 and 0.4 would be expected to lead to DNA depurination, correct?
A. Lindahl does not -- I would have to look at this carefully, but I do not think Lindahl reports stabilities -- experiments under pH of 4.5.
Q. Right.

Page 198

A. So he doesn't show anything about pH 1, so if you --
Q. He does show that it would be depurinated at pH 4.5?
A. Yes.
Q. And if you dropped it down to pH 1.1, would you also expect depurination at pH 1.1?
A. I would expect that. My only point was that Lindahl does not actually show that. But I would expect that from the logic we just walked through.
Q. And if you dropped it to pH .4, would you expect that there would be depurination?
A. Yes, I would.
Q. And conditions that lead to DNA depurination would not be considered mild conditions that do not denature DNA, correct?
A. That is correct. I don't know why I'm adding this, but just for the record, I wasn't suggesting that they were. And I know you know that, so why am I saying that.
Q. I am trying to understand what you just said. You were not considering that depurination conditions to be mild. Is that what you're saying?
A. No. I'm saying that I was not

Page 199

suggesting, nor would anyone suggest that pH conditions of 1 -- pH 1 would be stable -- would be useful for a DNA -- would be considered mild for a DNA reaction. As I tried to explain, this Boss reference added perchloric acid, and I was simply including this reference to illustrate that water did not poison the plate and catalyze reaction.
The reaction that's much closer to demonstrating mild conditions for DNA is the Qian or Qian, Q-I-A-N, paper which is very -- which is mild. So if you really want to talk about whether or not palladium chloride mediated deprotections of O-allyls are compatible with DNA, we should really be talking -- we shouldn't be calculating pHs for, you know, this Boss paper. If I didn't make this clear, I should have. We should be looking at this -- the Qian paper.
Q. You did put the Boss paper in your declaration, didn't you?
A. I did.
MR. ZIMMERMAN: Objection. Asked and answered.
BY MR. SCHWARTZ:
Q. So you relied on Boss in your declaration?

Page 200

MR. ZIMMERMAN: Objection. Asked and answered.
THE WITNESS: I hope that I clarified what I was relying on it for.
BY MR. SCHWARTZ:
Q. You used it in your declaration to say that allyl deprotection was a mild condition, right?
A. Allyl deprotections can be run under a wide variety of conditions.
Q. But you were relying on that paper?
A. To make that statement, yes. Under a wide variety of conditions, some of which are compatible with -- would be considered mild for DNA, and some of which would not be.
Q. But not under those particular conditions in that particular paper?
A. Correct.
Q. Do you recall earlier when we were walking through Tsien's requirements for SBS, you agree that one of Tsien's requirements is rapid and quantitative deprotection of the 3'-OH protecting group?
A. I do.
Q. Please go back to the Boss reference.

Page 201

Again, please look at Table 1. All of the allyl deprotection conditions in Boss are between three hours and six days, correct?

A. That is correct.

Q. And three hours to six days is not consistent with SBS, is it?

A. Three hours, as we went before, depending on what requirements you insisted on, what efficiency meant to you, what applications you were interested in. Maybe six days, very unlikely.

Q. You don't remember your testimony when you said that three hours was not consistent with SBS?

A. In the context of what I was describing then, that is what I said, and that is what I meant. If you relax one's standards, three hours would not be great. It would take a very long time, but it is not per se inconsistent with running a sequencing by synthesis reaction.

Q. But your position in those other IPRs was that three hours was not compatible with SBS. That was your position there, correct?

A. I believe I was stating something about whether or not it satisfied the requirements of efficient high-throughput sequencing.

Page 202

Q. Of a protecting group?

A. You're not sequencing the protecting group here.

Q. Do you want to go back to your testimony? We can go back to it.

A. Sure. Let's go back to it.

Q. Okay. It was in IPR2013-00517. That was Exhibit 2007, and page 25.

A. If I could take a minute to read this.

Q. Do you know where I'm directing you to?

A. Well, 25 is a pretty short -- very little text on 25, so I assume it's the text.

Q. It is where it says "Furthermore, the amount of time required."

Do you see that?

A. Yes, I do.

Q. And you write: "Furthermore, the amount of time required to achieve those yields, three hours to multiple days, would not have been expected to fulfill the rapid deprotection requirement of Tsien's DNA sequencing method."

You wrote that, right?

A. Yeah, and so just to be very clear with what I just said, and I was correct. I was talking about fulfilling the rapid requirements or the

Page 203

high-throughput, the fast sequence requirements of Tsien.

The question you just asked me was sequencing by synthesis compatible with three hours. And the answer I gave then was, yes. Maybe not very efficient, but I wasn't answering whether it fulfilled a requirement for being fast as I read Tsien.

Q. It doesn't, but it doesn't -- you agree it doesn't fulfill Tsien's requirement for rapid deprotection?

A. That is what I testified here, and, again, it doesn't seem like it -- it does not seem like it would fulfill what Tsien had in mind. But that was not the way -- that was not the question you asked me a moment ago.

Q. So none of Boss's reaction conditions satisfied Tsien's requirement for rapid deprotection, correct?

A. I would agree with that.

MR. SCHWARTZ: I need to ask a question. I'd like to go back to the patents. We have not talked about the patents yet. The patents in this case. Did we circulate those yet?

And also I would like to mark that piece

Page 204

of paper where you have your handwritten calculations and notes on.

(Exhibit 2090 was marked for identification.)

MR. SCHWARTZ: I will start with one patent. I think we all agree that the specifications are the same for the patents. If you would prefer I can put all four patents.

MR. ZIMMERMAN: It's up to you. If you're going to ask him about the claims, he needs all four. If you're asking about the spec, one is probably sufficient.

MR. SCHWARTZ: Well, let's start with one at a time then.

(Exhibit 1001 was marked for identification.)

BY MR. SCHWARTZ:

Q. So I am putting in front of you Exhibit 1001. This happens to be the U.S. patent 9718852 to Ju, et al. This is a patent that is at issue in these IPRs, correct, Dr. Romesberg?

A. That is one of them, that is correct.

Q. It is a patent that you considered in one of your declarations in these IPRs, correct?

A. That is correct.

Page 205

Q. Why don't we turn to the claims.
A. Do you want to point me to a column.
Q. I'm just going to start with this. It is column 34 of the claims.
Do you see after the structure it says wherein RA represents a small chemically cleavable chemical group capping the oxygen at the 3' position of the deoxyribose of the deoxyribonucleotide analog."
Do you see that?
A. I do.
Q. What is your understanding of what is a small group?
A. Small. A small group. I am okay. I accept Dr. Ju's definition, or his use of "small." He doesn't define it. He represents it with examples, which he uses MOM and allyl. Those are both three carbons, so I am okay with that definition. I am okay with accepting his definition of small.
Do you want me to talk about small outside of this? I'm happy to do so.
Q. So you understood from reading this what a small chemical capping group is?
A. I understand that Dr. Ju evokes MOM and

Page 206

allyl as small.
Q. What is your understanding of the chemically cleavable chemical group?
A. That it can be removed by a reaction that allows it to -- by a chemical reaction to produce a free three-prong hydroxyl group that is compatible with continued DNA synthesis.
Q. Do you see in the structure that is drawn for this particular adenine nucleotide that the tag is attached to the base via a structure called Y?
A. A box called Y, yes, I see that.
Q. What is your understanding of the box called y?
A. It would be a cleavable -- part of a cleavable label -- a link or part of a label that would attach what he calls the tag.
Q. So you understand that the tag is not directly connected to the base, correct?
A. Yes. I mean, we'll get a little semantic talking about why the tag and why start and stop, because they're all going to be chemical bonds, but clearly the spirit here is that there is a part that is cleavable that will separate the fluorophore or the tag from the nuclear base.
Q. Is it your understanding that the term

Page 207

small in the other patents -- which I can put in front of you if you want, but I'll represent it uses the same terminology -- would be the same as you just described, your understanding of small? I guess maybe what I'm trying to say is this is not a unique understanding for this particular structure, right?
A. I believe that Dr. Ju uses the same description in all five of the patents.
Q. Again, in terms of understanding what a chemical -- cleavable chemical group is, that is not unique to this particular adenine nucleotide, right?
A. I believe he represents it equivalently in all of the cases we are discussing here.
Q. Let's go back to Tsien. That was Exhibit 1013. Will you please turn to page 24 and please draw your attention to the paragraph that begins at line 24 and Tsien writes "A wide variety of hydroxyl blocking groups are cleaved selectively using chemical procedures other than base hydrolysis. 24 nitrobenzenesulfonyl groups are cleaved rapidly by treatment with nucleophiles such as thylphenyl and phenyl sulfate." And he points to a Letsinger 1964 paper.

Page 208

Allyl ethers are cleaved by treatment with mercury II in acetone water. And he points to Gigg and Warren in 1968.
That is what Tsien writes, right?
A. That is correct.
Q. Please go back to Exhibit 1012, which is your declaration IPR2018-00291.
MR. ZIMMERMAN: Which exhibit?
MR. SCHWARTZ: 1012. It's his declaration in IPR2018-00291.
BY MR. SCHWARTZ:
Q. Please turn to page 55. Please draw your attention to paragraph 98. In paragraph 98 you write: "With respect to 3'-O-allyl groups, Tsien cites to Gigg, Exhibit 1013, Tsien at 24; 29 through 30, allyl ethers are cleaved by treatment with mercury II acetone water. Gigg and Warren 1968."
That is what you wrote, right?
A. That is correct.
Q. Tsien's disclosure at page 24, lines 29 to 30 is of allyl groups, correct? That's what you said?
A. Yes.
Q. And allyl groups is a genus that includes

Page 209

both unsubstituted allyls as well as substitute allyls, correct?

A. No.

Q. It's not a genus that includes those?

A. You can stretch it to that if you want, but it's certainly not common language.

Q. But it is a collection of groups?

A. You could stretch it to include that. It's kind of a slang, it's kind of a vernacular. A lot of chemical names have very long complex names, and a lot of times people don't want to write them out. A lot of times people don't even know how to actually name things. So it's very common for people to use sort of a slang. So that's an allyl group. That's an allyl-type group. That's got an allyl in it, so let's call it an allyl protecting group. It's kind of like that's a red car.

Q. You yourself use the term 3'-O-allyl groups when you refer to Tsien, right?

A. That's right. He wasn't doing single molecule experiments.

Q. And you also refer to Tsien saying allyl ethers, correct?

A. Again, not single molecule experiments. What I meant was that there are many molecules in

Page 210

his reaction. Just like people would describe templates. That's a very common descriptor. It's not that they were all different, it's just you're sequencing templates. Because there are many of them. In a reaction, there is many molecules. So if that caused confusion, I apologize. I never meant to implicate that there were different allyl groups. If people wanted, like I said, to stretch the definition and to sort of use it as a slang, that's certainly not unheard of. But an allyl group is something, and it's a very specific thing. And it would be what anyone would think of.

Q. But you didn't use the term "allyl group," you used the term "allyl groups." That is what you wrote, correct?

A. Yeah, just methyl groups. That's a very common expression. If you want to modify methyl groups, you can use this sort of thing. And there's no derivatives of a methyl group. It's sort of like -- it -- the plural is referring to there are many of them. You're deprotecting -- you're not doing a single molecule reaction. You're not modifying one. You're removing a lot of allyl groups, one for every molecule in there.

Q. You know how to use the word group if you

Page 211

wanted to; you used the word groups. You know how to use the word ether if you wanted to; you used the word in plural, correct? You chose those words?

MR. ZIMMERMAN: Asked and answered.

MR. SCHWARTZ: No, that wasn't asked and answered.

THE WITNESS: I was using them in plural in the sense that there were many of them.

BY MR. SCHWARTZ:

Q. Many of them?

A. I mean, if there's one molecule in a reaction, you still can use the word molecules to describe the reaction. Doesn't that imply --

Q. I don't understand you said there are many of them. You were referring to Tsien, and Tsien is talking about allyl groups. And you said that Tsien refers to 3'-O-allyl groups. What do you mean many of them? Where in Tsien does it talk about many of them?

A. They are not single molecule experiments, so you're not exploring -- you're not doing chemistry on a single molecule. There are many molecules. That doesn't mean there are different molecules. They happen to be many of the same

Page 212

molecule, but still plural. That's all I meant by this.

Q. You had meant that Tsien cites to Gigg and Warren for his disclosure of allyl groups, correct?

A. He does.

Q. Let's look at Gigg and Warren.

MR. SCHWARTZ: Can I ask the court reporter for an approximate amount of time on the record right now? Not the court reporter, I'm sorry. I meant the videographer. You know what I meant.

THE VIDEOGRAPHER: 4:36. Four hours, 36 minutes, around there.

(Exhibit 1046 was previously marked for identification.)

BY MR. SCHWARTZ:

Q. I am putting in front of you Exhibit 1046 entitled "The Allyl Ether as a Protecting Group and Carbohydrate Chemistry, Part II," by Gigg and Warren, published in 1968.

This is the same Gigg and Warren paper cited in Tsien, correct?

A. I believe so.

Q. Please turn to Page 1906 of Gigg and

Page 213

Warren.
MR. SCHWARTZ: You look like you don't agree --
MR. ZIMMERMAN: Not at all. Did you say four hours and 10 minutes. We are almost at five hours.
THE VIDEOGRAPHER: I have to do the math.
MR. ZIMMERMAN: We are 4:49 right now.
BY MR. SCHWARTZ:
Q. All right. So we -- that is the same Gigg and Warren that is cited in Tsien, correct?
A. I believe so, yes.
Q. And please turn to page 1906 of Gigg and Warren, and on the left side towards the bottom of the page are shown compounds XL, IX, L, and L2. Do you see that?
A. I do.
Q. And none of those are three carbon allyl ethers, correct?
A. I think whatever that is, LI is.
Q. I didn't ask about LI. I said XL, IX, L, and L2.
A. Right. That is correct.
MR. ZIMMERMAN: He came up with 4:42.
///

Page 214

BY MR. SCHWARTZ:
Q. So none of those are three carbon allyl groups, correct?
A. That is correct.
Q. And throughout Gigg and Warren there are disclosures of allyl ethers that are both unsubstituted and substituted, correct?
A. Apparently, yes.
Q. And throughout Gigg and Warren there are also disclosures of allyl ethers other than a three carbon allyl ether, correct?
A. That is correct. Obviously, from what you just pointed out. At least.
Q. A reader of Tsien -- referring back to the Gigg and Warren that Tsien refers to for his support of an allyl -- the allyl groups -- understood that Tsien's reference to allyl ether is to a collection of ethers shown by Gigg and Warren, correct?
A. No. People -- as I said, people sometimes use the word allyl, and describing this as an allyl ether, while technically still a little bit weird, is a little less weird than their generalizing a group of things. But still, the only thing in here that in here strictly properly

Page 215

referred to as an allyl group is the single allyl, the three carbon allyl. Other substituted ones technically should have different names, like there should be like, for example, in -- you're going to test my nomenclature. The compound, the first one, which really have be called II methyl allyl, but like I said, people are a little bit loose with those because people don't like to write out things like that.
Q. But do you agree that Tsien refers to Gigg and Warren for his disclosure when he discloses allyl groups, correct?
A. That is correct.
Q. And a POSA reading Gigg and Warren understood that Gigg and Warren teaches other than the three carbon allyl group, correct?
A. Yes, that is correct. There is a small number of -- I mean, frankly, there just aren't that many different sort of allyl groups short of making longer -- there are not that many ways to substitute allyl per se, only three carbons. You can certainly make modifications. They're very seldom used allyl groups are typically the common C3 one, the namesake of the group.
Q. Gigg and Warren discloses allyl groups

Page 216

better than that?
A. As I already said, yes, that is correct --
Q. Let's go back to Exhibit 1001, which was the patent the '852 patent.
A. I'm sorry, where?
Q. The patent. The '852 patent that is Exhibit 1001. Please go to column six, line 23. And in column 23 the patent reads "The MOM."
MR. ZIMMERMAN: Just some confusion, you said go to column six, and now you're saying --
MR. SCHWARTZ: Column 26 -- maybe my --
MR. ZIMMERMAN: We're both sitting here on six.
MR. SCHWARTZ: Column 26.
MR. ZIMMERMAN: Thank you.
MR. SCHWARTZ: Sorry for the confusion.
MR. ZIMMERMAN: I just wanted to make sure we are all at the same place.
BY MR. SCHWARTZ:
Q. And line 23. The patent reads "The MOM" -- and it draws the structure, CH2OCH3, or allyl, and it draws a structure CH2CH-double bond-CH2 -- "is used to cap the 3'-OH group using well established synthetic procedures," and refers

to figure 13.

So the patent specification, Dr. Ju's patent specification, specifically calls the CH2CH double bond CH2 group the allyl group, correct?

A. As most people would, yeah. It's the allyl group, and he parenthetically draws it out.

Q. And that structure is not disclosed in Tsien, correct?

A. It effectively is. Its name is disclosed in Tsien. It's not drawn out in a parenthetical manner like this, but in a way that any POSA would have immediately drawn that conclusion.

Q. I'm just saying that the structure is not shown in Tsien, is it?

A. The structure is not drawn out.

Q. Okay.

MR. SCHWARTZ: I would like to go off the record if I could. I have to mark up some exhibits.

THE VIDEOGRAPHER: Off the record at 3:38 p.m.

(Recess taken.)

THE VIDEOGRAPHER: On the record at 3:48 p.m. This concludes today's deposition of Floyd Romesberg Ph.D. We are off the record at

3:48 p.m.

(The videotaped deposition of FLOYD ROMESBERG, PH.D., VOLUME I, concluded at 3:48 p.m.)

I, the undersigned, a Certified Shorthand Reporter of the State of California, do hereby certify:

That the foregoing proceedings were taken before me at the time and place herein set forth; that any witnesses in the foregoing proceedings, prior to testifying, were duly sworn; that a verbatim record of the proceedings was made by me using machine shorthand which was thereafter transcribed under my direction; that the foregoing transcript is a true record of the testimony given.

Further, that if the foregoing pertains to the original transcript of a deposition in a Federal Case, before completion of the proceedings, review of the transcript [X] was [ ] was not requested.

I further certify I am neither financially interested in the action nor a relative or employee of any attorney of party to this action.

IN WITNESS WHEREOF, I have this date subscribed my name.

Dated: September 21, 2018

PATRICIA Y. SCHULER
CSR NO. 11949

DECLARATION UNDER PENALTY OF PERJURY

Case Name: Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Date of Deposition: 09/19/2018

Job No.: 10047002

I, FLOYD ROMESBERG, PH.D. hereby certify that I have read the transcript of my deposition and that this transcript accurately states the testimony given by me, with the changes or corrections, if any, as noted on an errata sheet or errata sheets.

Executed this _____ day of _____, 2018, at _____.

_____

FLOYD ROMESBERG, PH.D.

NOTARIZATION (If Required)

State of _____

County of _____

Subscribed and sworn to (or affirmed) before me on this _____ day of _____, 20__, by_____, proved to me on the basis of satisfactory evidence to be the person who appeared before me.

Signature: _____ (Seal)

DEPOSITION ERRATA SHEET

Case Name: Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Name of Witness: Floyd Romesberg, Ph.D.

Date of Deposition: 09/19/2018

Job No.: 10047002

Reason Codes: 1. To clarify the record.
2. To conform to the facts.
3. To correct transcription errors.

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DEPOSITION ERRATA SHEET

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_____

FLOYD ROMESBERG, PH.D.

F

Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Videotaped Deposition of

FLOYD ROMESBERG, PH.D.

September 20, 2018

Volume II

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UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC., Petitioner, v.	Case No.	IPR2018-00291 U.S. Patent 9,718,852
		IPR2018-00318
THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, Patent Owner.		U.S. Patent 9,719,139
		IPR2018-00322 U.S. Patent 9,708,358
		IPR2018-00385 U.S. Patent 9,725,480

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.
VOLUME II
SAN DIEGO, CALIFORNIA
SEPTEMBER 20, 2018

Reported By:
PATRICIA Y. SCHULER
CSR No. 11949
Job No. 10047003

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC., Petitioner, v.	Case No.	IPR2018-00291 U.S. Patent 9,718,852
		IPR2018-00318
THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, Patent Owner.		U.S. Patent 9,719,139
		IPR2018-00322 U.S. Patent 9,708,358
		IPR2018-00385 U.S. Patent 9,725,480

Videotaped deposition of Floyd Romesberg, Ph.D., Volume II, taken on behalf of the Patent Owner at 12790 El Camino Real, San Diego, California, beginning at 9:04 a.m. and ending at 10:41 a.m., on September 20, 2018, before PATRICIA Y. SCHULER, Certified Shorthand Reporter No. 11949.

APPEARANCES:

FOR PETITIONER:

KNOBBE MARTENS - SAN DIEGO
BY: NATHANAEL R. LUMAN, ESQ.
12790 El Camino Real
San Diego California 92130
(858) 707-4000
nate.luman@knobbe.com
- and -
KNOBBE MARTENS - WASHINGTON DC
BY: WILLIAM R. ZIMMERMAN, ESQ.
1717 Pennsylvania Avenue, Suite 900
Washington, D.C. 20006
bill.zimmerman@knobbe.com

FOR PATENT OWNER:

FITZPATRICK, CELLA, HARPER & SCINTO
BY: ROBERT S. SCHWARTZ, ESQ.
BY: ZACHARY L. GARRETT, ESQ.
1290 Avenue of the Americas
New York, New York 10104-3800
zgarrett@fchs.com

Also Present: Dr. Steven Menchen
John White, Esq.
John Murnane, Esq.
Marcus Burch, Illumina

Videographer: Daniel Bermudez

I-N-D-E-X

E-X-H-I-B-I-T-S

Page 227

SAN DIEGO, CALIFORNIA; THURSDAY, SEPTEMBER 20, 2018
9:04 a.m.
THE VIDEOGRAPHER: Good morning. We are now on the record. Today's date is Thursday, September 20, 2018, and the time is 9:04 a.m.
This is the video deposition of Floyd Romesberg, Ph.D., Day II, being taken in the matter of Illumina, Inc. versus the Trustees of Columbia University in the City of New York, pending in the United States Patent and Trademark Office before the Patent and Appeal Board, Case Nos. IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385.
We are at Knobbe Martens in San Diego, 12790 El Camino Real. My name is Daniel Bermudez of Aptus Court Reporting located at 600 West Broadway, Suite 300, San Diego, California 92101.
Will counsel please identify yourselves and state whom you represent.
MR. SCHWARTZ: I'm Robert S. Schwartz representing Columbia, from Fitzpatrick, Cella, Harper & Scinto. With me is Zack Garrett, also from Fitzpatrick. Sitting next to him is Dr. Steven Menchen. Sitting next to him is John Light from the Cooper and Dunham firm also

Page 228

representing Columbia. And at the end of the table is John Murnane, also representing Columbia from the Fitzpatrick firm.
MR. ZIMMERMAN: Bill Zimmerman of Knobbe Martens Olson & Bear representing Illumina and the witness, Dr. Romesberg. With me is my partner Nate Luman and Marcus Burch of Illumina.
THE VIDEOGRAPHER: The court reporter today is Patricia Schuler, and she may now swear in the witness.

FLOYD ROMESBERG, PH.D.,
having been administered an oath, was examined and testified as follows:

FURTHER EXAMINATION
BY MR. SCHWARTZ:
Q. Good morning, Dr. Romesberg.
A. Good morning.
Q. Is there any reason you can't give truthful testimony today?
A. No.
Q. Did you consult with your attorneys regarding the substance of your testimony yesterday?

Page 229

A. No, I did not.
Q. And did you consult with your attorneys regarding the substance of what you anticipate discussing today?
A. No, I did not.
Q. I am going to start out by asking you to go back to the Tsien reference. That was Exhibit 1013. Please draw your attention to Page 24, and look at Line 29.
And Tsien writes "Allyl ethers are cleaved by treatment with mercury II in acetone water."
That is what Tsien writes, correct?
A. That is correct.
Q. Please go back to Exhibit 2086, which was the Kitt paper we looked at yesterday, 2086. Got it?
A. I have it.
Q. And please turn to page 59. And please draw your attention to the second full paragraph that starts with "additional evidence."
Do you see that?
A. I do.
Q. And Kitt writes "Additional evidence that partial and reversible denaturation of DNA

Page 230

molecules may occur has been derived from experiments on the reaction of mercury chloride with DNA."
Do you see that Kitt writes that?
A. Yes, I do.
Q. And mercury II is a DNA denaturant, correct?
A. It denatures DNA, yes.
Q. Going back to the allyl ethers in Tsien, the cleavage method for the allyl ethers cited by Tsien is based on the Gigg and Warren reference, correct?
A. That is correct.
Q. And if you wouldn't mind, please, going back to that paper, Exhibit 1046.
Do you have it?
A. I do.
Q. Please draw your attention to Reaction 1 on the left side of Page 1904, in Gigg and Warren.
A. I'm sorry. Where?
Q. Reaction 1 on the left side of 1904.
Got it?
A. Yes.
Q. The first step in that method is for the allyl ethers to be converted to vinyl ethers under

Page 231

strong basic conditions, and then the vinyl ethers are cleaved by mercury II, correct?
A. Yes. I am not reading the first step. If I could take a second to read this?
Q. Sure.
A. That seems to be the case, yes.
Q. The structure of allyl ethers is different from that of vinyl ethers, correct?
A. Yes.
Q. And the conversion --
A. One is an isomer of the other.
Q. They are different structures?
A. You represent -- we think about them differently as different structures. Technically, if you want to know technically the truth, it's a theoretical description of the two.
The connectivities of the atoms are the same. We never know where to draw -- put electrons in reality. And in the two forms, we put the electrons in different places, and they are differen in the two.
Q. And so the conversion of allyl ethers to vinyl ethers requires the use of strong basic conditions. We looked at that where he says "potassium t-butoxide."

Page 232

You saw that, right?
A. Yes.
Q. And that denatures DNA, correct? And that cannot be used for SBS, correct?
A. Certainly, at high concentrations potassium t-butoxide would denature DNA. I don't know exactly at what concentration that would occur, but that is a base, and, you know, I think at sufficiently basic conditions, DNA is damaged.
Q. So a person reading Tsien along with Gigg and Warren would not use Gigg's condition for cleavage of allyl ethers for SBS because there would be no expectation of success using those cleavage conditions, correct?
A. I think that is fair.
(Exhibit 1037 was previously marked for identification.)
BY MR. SCHWARTZ:
Q. I am going to put another exhibit in front of you because I know you don't have enough of them already. I am putting in front of you Exhibit 1037, which is a paper entitled "A Mild and Rapid Regeneration of Alcohols from their Allylic Ethers by Chlorotrimethylsilane/Sodium Iodide" by Kamal et al, published in 1999.

Page 233

Have you seen this paper before, Dr. Romesberg?
A. I have.
Q. Please draw your attention to the last paragraph on the bottom of the first page that starts with "This led us to utilize."
Do you see that?
A. I do.
Q. Kamal writes "This led us to utilize this reagent system for the cleavage of allyl ethers following this procedure. To a solution of allyl ether, one millimole, in acetonitrile, 10 mils, sodium iodide, 1.5 millimoles, was added, and the mixture stirred for two minutes.
To this stirred suspension, a solution of chlorotrimethylsilane, 1.5 millimoles, was added, and the stirring continued for another two minutes. For entry seven, 4.5 equivalents of the reagents were used."
That is what Kamal writes, correct?
A. That is what's written.
Q. The scheme that is used to deprotect the allyl group in Kamal is not performed in aqueous medium, correct?
A. It is performed in acetonitrile.

Page 234

Q. And protecting groups for use in SBS must be removable under aqueous conditions. We went over that yesterday, correct?
A. Yes, that is correct. Under conditions where the DNA is stable and hybridized, yes.
Q. And it has to be done in aqueous conditions?
A. Meaning there has to be water there, yes.
Q. And as you just mentioned, Kamal's deprotection assay uses acetonitrile, correct?
A. That is correct.
Q. And acetonitrile is an organic solvent, correct?
A. It is an organic solvent, correct.
Q. And we talked about this yesterday -- and it is your opinion that deprotection conditions that use organic solvents are not compatible with SBS, correct?
A. I don't believe I said that, and I think that you have to say that there can be an organic solvent there, they just can't be sufficient concentrations to denature or damage the DNA.
Q. But his reaction takes place in acetonitrile with no water present?
A. Yes. And in that case it would denature

Page 235

the DNA. And you would dehydrate the DNA and cause it to denature.

Q. And Kamal's allyl deprotection method uses chlorotrimethylsilane, correct?

A. It does.

Q. And chlorotrimethylsilane is not compatible with water, correct?

A. No, it is not.

(Exhibit 2031 was previously marked for identification.)

BY MR. SCHWARTZ:

Q. I am putting in front of you Exhibit 2031, which is a paper entitled "DNA Polymerase Fluorescent Substrates with Reversible 3'-Tags" by Canard and Sarfati, published in 1994.

Please turn to page 4. You've seen this paper before, haven't you, Dr. Romesberg?

A. I have.

Q. Please draw your attention to the right side of the page, and please look at the bottom where there is a section D.

Do you see that?

A. I do.

Q. Please draw your attention to the third sentence in that section that starts with "We

Page 236

reasoned that."

Do you see that?

A. I do.

Q. Canard writes "We reasoned that ether bonds would be hard to cleave under mild conditions compatible with DNA chemical stability."

That's what Canard writes, correct?

A. That is what is written.

Q. And the Canard paper was published in 1994, correct?

A. That is correct.

Q. And the Tsien application was published in 1991, correct?

A. That is correct.

Q. So even years after Tsien, it was understood that ether bonds are hard to cleave under mild conditions compatible with DNA chemical stability.

That is what Canard says, right?

A. Canard says that they reasoned that -- that ether bonds would be hard to cleave.

Q. But that reasoning was three years after the publication of Tsien?

A. Yes. Canard, in this paper, suggested that they thought that ethers would be difficult to

Page 237

cleave.

Q. And in your declarations that you submitted in the current IPRs, you don't cite to any reference prior to October 2000 that provides data showing that a nucleotide with a 3'-O-allyl protecting group is cleaved under mild conditions compatible with DNA chemical stability, do you?

A. I provided examples of reactions that were very close, that a POSA would have expected, and had high expectation of success that the one difference of methanol versus water would not have been a problem, and that would have provided conditions where a 3'-O-allyl protected nucleoside could have been allyl deprotected. I did not find examples of precisely that reaction in water.

Q. And sitting here today, are you aware of any examples of that precise reaction occurring in water?

A. No, I did not find any last night.

Q. Did you look for them last night?

A. I did not.

MR. SCHWARTZ: I am going to go to another exhibit, please. This one will require a new marking, Court Reporter, 2089.

(Exhibit 2089 was marked for

Page 238

identification.)

BY MR. SCHWARTZ:

Q. I'm putting in front of you a paper entitled "Palladium(II)-Catalyzed Oxidation of Aldehydes and Ketones. 1. Carbonylation of Ketones with Carbon Monoxide Catalyzed by Palladium(II) Chloride in Methanol" by Hamed et al, which was published in 2001.

Please turn to page 182. And please draw your attention to the left side of the page towards the top of the page where there is a reaction scheme entitled "Alkoxy Palladium Root."

Do you see that?

A. I do.

Q. The reaction scheme showing palladium (II)reacting with an olefin plus an alcohol to generate a palladium (II)complex with the olefin with the generation of acid."

That is what is the reaction scheme shows, correct?

A. It does show that.

Q. An allyl protecting group is an example of an olefin, correct?

A. Yes.

Q. So a POSA reading -- I'm sorry, strike

Page 239

that.
A POSA understood that reacting in allyl protecting group with palladium (II) will generate acid, correct?
A. Yes.
MR. ZIMMERMAN: Object to the form of the question. And just so you know my objection, you made clear yesterday that a POSA was in 2002 --
THE WITNESS: 2000.
MR. ZIMMERMAN: 2000. And you are asking him about something dated after that and assuming a POSA would know.
So the timing in your question is nonsensical. So I am objecting as to form.
MR. SCHWARTZ: Okay.
BY MR. SCHWARTZ:
Q. A POSA understood that reacting in allyl protecting group, palladium (II) generates acid, correct?
A. Yes.
Q. So please --
A. Stoichiometric acid, yeah.
Q. Please go back to the first page of Hamed -- strike that.
Yesterday we looked at some papers by

Page 240

Lindahl.
Do you recall that?
There were two papers, Lindahl 1993 and Lindahl and Nyberg 1972, and those papers reported that acid catalyzes depurination of nucleotides.
Do you remember that?
A. Yes.
Q. And conditions that result in depurination of nucleotides are not compatible with SBS, correct?
A. If you're depurinating during sequencing reaction, that would not be compatible with sequencing by synthesis.
Q. And please go back to the Qian reference we looked at yesterday, Exhibit 1036, and go back to Page 2185 where there was Scheme 2. And please look again at the figure legend. The deprotection of the allyl takes place with palladium (II) and methanol, correct?
A. It does.
Q. And that reaction would be expected to produce acid, correct?
A. It would produce stoichiometric amounts, very small amounts of acid compared to a solvent -- compared to the adding solvent at millimolar

Page 241

concentrations of the sort that we talked about yesterday, and of the sort that Lindahl talked about, this would generate the amounts of acid that -- one proton for every time you deprotected an allyl. And that's common, and that would be handled under sequencing by synthesis methods, because I assume that is what we are interested in. And that would be handled by buffering the system. That would be well within the buffer power and standard for any POSA to deal with.
Q. But I'm not talking about a different reaction condition. I'm talking specifically about Qian's reaction condition.
So in Qian's reaction condition that would be expected to produce acid, there is not a buffer there, correct?
A. It would produce stoichiometric acid.
MR. SCHWARTZ: I am going to mark a new exhibit. Court reporter, this will require a new number, 2094.
(Exhibit 2094 was marked for identification.)
BY MR. SCHWARTZ:
Q. I'd like to direct you to a document entitled "Interaction of Palladium (II) with DNA"

Page 242

that was published in 1977 by Pillai and Nandi. Go to the abstract. And the abstract reads -- I'm not going to read the whole thing. I am going to ask you to look at the second sentence that starts with "The results."
Do you see that?
"The results indicate that palladium (II) interacts with both the phosphate and bases of DNA."
That is what the authors write, correct?
A. That is correct.
Q. And the palladium (II) referred to by Pillai is palladium chloride as indicated in the last paragraph of Page 11, correct?
A. That is correct. That seems to be correct. They cite palladium chloride, and in the intro they're talking about palladium II, and the palladium in palladium chloride is palladium II. I haven't read this reference to know if that is all they are talking about, but that would seem to be consistent with that.
Q. And please go to Page 16 of Pillai, and draw your attention to the top of the page. And it reads "The ORD and CD curves of DNA were also altered considerably on addition of palladium

Page 243

chloride, figure 4."
And I'm not going to read the whole thing into the record, but let me just ask you to go to th next paragraph that starts with "The ultraviolet."
Do you see that?
A. I do.
Q. It reads "The ultraviolet spectrum (figure 1), indicates binding of palladium (II) to the bases of DNA. This is expected to modify the electronic state of the bases, and will be reflected in the interaction of their dipoles in the polymer, thus affecting the shape and magnitude of the cotton effects." Cites a reference.
"Unstacking of the bases of polynucleotides is thought to result in diminished magnitudes of cotton effects." And it cites a reference. "Similarly disruption of hydrogen bonds between the base pairs of double-stranded polynucleotides may give rise to a red shift in the position of the maxima." And it cites a reference.
"Figure 4A and 4B show that binding of palladium (II) to DNA results in reduction of ORD and CD bands accompanied by red shifts. These results suggest that stacking interactions are reduced considerably, and the Watson and Crick

Page 244

hydrogen bonded double helical system has been disturbed at least in the regions where binding of palladium (II) takes place."
MR. ZIMMERMAN: Object to norm.
BY MR. SCHWARTZ:
Q. That is what the authors write, correct?
A. That is what is written.
Q. A POSA understood from Pillai that palladium chloride cannot be used in a reaction with DNA, because palladium chloride disturbs the Watson Crick hydrogen bonded double helical system, and the fidelity of the Watson Crick hydrogen bonded double helical system is essential for DNA sequencing, including SBS.
Do you agree?
A. No.
Q. And why don't you agree?
A. There's no evidence that the duplex is disrupted in any manner that would prevent sequencing by synthesis here.
If you want me to explain a little bit abou ORD, ORD stands for optical rotatory dispersion. CD stands for circular dichroism.
Chiral molecules, when you polarize light, interact differently with the two-plane polarized

Page 245

lights, left and right polarized. DNA duplex is chiral. Anything that relaxes that chirality at all leads to changes.
So if you simply relax the duplex slightly, you get differences in the ORD and CD signals.
People don't understand the origins of ORD and CD. It's -- the only way that we use these signals is by model systems. We say, okay, an alpha helix is like this, and a protein and beta sheet's like this. DNA duplex A form is like this; B form is like this.
Little small changes happen all the time, and they are only understandable in the context of a model system. And so if there is no model system to show that, you can't say that it's denatured anything.
Slight relaxations of the DNA is common, it doesn't in any way show that the hydrogen bonds are disrupted. It just shows that the duplex is slightly relaxed. Its chirality is not identical.
There is nothing in that to me -- there is nothing that you read here that to me would suggest that it would no longer be able to be substrate for sequencing by synthesis.
I mean, sodium sort of does the same

Page 246

thing, and sodium is perfectly compatible. Palladium (II)is simply interacting with the nitrogens of the bases in the waters and the phosphates. There's nothing -- it's just -- in some respects it's -- there's no evidence here that palladium (II) is acting as anything other than a counterion in the same way that sodium, which is a cation, would be interacting.
But there is nothing in the change in the CRD and the ORD that suggests to me that the DNA is denatured.
In fact, in all cases, it still has a CD an an ORD signal, suggesting that it is still chiral, suggesting there is still duplex.
Q. The authors themselves, however, interpret their own results by suggesting that the stacking interactions are reduced considerably. That is what they say, correct?
A. If they interpret the CD and ORD specter that way, that's fine, but they're certainly not ablated because -- they're certainly not gone, because there is still ORD and CD signal there.
Q. But they do interpret this data as suggesting that their stacking interactions are reduced considerably. That is what the authors

Page 247

say, correct?
A. I don't see that they relate that to recognition by a polymerase in any manner.
Q. They are simply saying that the presence of palladium results in stacking interactions reduced considerably -- they use the word "considerably" -- and that the Watson Crick hydrogen bonded double helical system is disturbed where the regions of palladium binding are. That is what they say. That is what they teach, right?
A. Could you, again, point me to exactly where they say that?
Q. At the end of the paragraph: "These results suggest that stacking interactions are reduced considerably." Those are their words. "And the Watson and Crick hydrogen bonded double helical system has been disturbed, at least in the regions where binding of palladium takes place."
That was the authors' interpretation of their own data, correct?
MR. ZIMMERMAN: Objection. Mischaracterizes the document. And you read it incorrectly.
MR. SCHWARTZ: Where did I read it incorrectly?

Page 248

MR. ZIMMERMAN: You said palladium not palladium II.
MR. SCHWARTZ: Okay. I will correct it by saying palladium II.
THE WITNESS: That is what they say. And I think a POSA would have understood that those changes that -- number one, that there was no link to any sequencing by synthesis to any polymerase recognition for that matter at all. And a POSA certainly would have still understood that there was still a helical structure there.
So there still had to be pattern. So to whatever extent they're reduced considerably, they are not gone.
Q. But a POSA reading this would understand that the authors interpret this data as showing that the palladium causes stacking interactions to be reduced considerably?
MR. ZIMMERMAN: Objection. Asked and answered.
THE WITNESS: Those are the words there.
BY MR. SCHWARTZ:
Q. What you were talking about before when you gave your long explanation of the ORD and the CD, that was speculation on your part on whether or

Page 249

not these were reduced considerably, correct?
A. I don't understand the question. What part are you asking me if it was speculation?
Q. Well, you gave a very long explanation of ORD and CD, okay, but you didn't find anything -- let's strike that. Let's just let's stick --
A. I'm happy to try to answer.
Q. Let's strike it.
MR. ZIMMERMAN: He doesn't have a question.
BY MR. SCHWARTZ:
Q. Let's just stick with what the paper teaches.
I'm going to ask you to go back to Exhibit 2035.
A. Which is what?
Q. Your deposition transcript in IPR2013-00517. And please turn to Page 63. Please draw your attention to Line 19. And starting at Line 19, the transcript reads:
"Question: Now, back in 2002 would one with skill in the art have been able to modify or engineer polymerases to improve their ability to incorporate nucleotides?
"Answer: In 2002?"

Page 250

And then there is a partial attorney statement, and the question continues with 3'-oxygen protecting groups. The question that was asked of you was, "Back in 2002, would one with skill in the art have been able to modify or engineer polymerases to improve their ability to incorporate nucleotides with 3'-oxygen protecting groups," correct?
A. That's what it says, yes.
Q. And I won't read everything into the record that came after that question, and certainly feel free to review the transcript if you need to to follow along with me, but for now I'm going to ask you to skip ahead to Page 65.
A. I'm there.
Q. And please look at Line 3. And starting at Line 3 the transcript reads:
"Question: Whether one of ordinary skill would believe that they would be able to do that?
"Answer: Judging from the response of my reviews on grants that I submitted that it was not common, then the answer would be, no. People did not believe that you could."
So the portions of your testimony that I read is what you testified, correct?

Page 251

MR. ZIMMERMAN: Objection. Incomplete.
BY MR. SCHWARTZ:
Q. I said the portions that I read were what you testified, correct?
A. Those are the words written, yes.
Q. And you testified that as of 2002, people did not believe that they could modify or engineer polymerases to improve their ability to incorporate nucleotides with 3'-O protecting groups, correct?
MR. ZIMMERMAN: Objection. Mischaracterizes.
THE WITNESS: Could you repeat the question?
BY MR. SCHWARTZ:
Q. You testified that as of 2002 people did not believe that they could modify or engineer polymerases to improve their ability to incorporate nucleotides with 3'-O protecting groups?
MR. ZIMMERMAN: Objection. Mischaracterizes.
BY MR. SCHWARTZ:
Q. That is what you testified, correct?
A. I would like to read this.
Q. Sure.
A. One second please.

Page 252

Q. Take your time.
A. Okay. Could you repeat the question?
Q. Sure. So my question was you testified that as of 2002 people did not believe that they could modify or engineer polymerases to improve their ability to incorporate nucleotides with 3'-O protecting groups?
A. I think that's too general of a statement. That is certainly not what I intended to say in this declaration. What I intended to say was that it would have been viewed by -- in general, as being very difficult.
Q. But you didn't actually say that. You said that people would not believe that you could?
A. No, I did say that. If you read on, I said people would not have considered that a likely possibility. A handful of groups including -- people were starting to make progress. In 2002, people were starting to make progress. A handful of groups, including mine, sure. No one thought it was easy. To this day it is not easy.
So I certainly meant that it was difficult and that people would have viewed it as difficult, n doubt.
Q. And in 2000, it would have been even more

Page 253

difficult, correct?
A. There are no significant differences between 2000 and 2002, in my opinion, in my recollection. I don't know of anything that would have changed my answer for 2000.
Q. But again, in the testimony that we read, your response to the question at that deposition was, no, people did not believe that you could.
A. No, I believe I tried to clarify that and explain that people would have thought it was very difficult. But there were groups working on it.
So if there were groups working on it, people -- some people believed that you could, including my group. So I believed we could.
But what I was trying to say then is that - and this certainly is still the case today, is that it's not easy.
Q. I am going to ask you to turn back to Exhibit 2080, which is your deposition transcript in IPR2013-00128. Please turn to Page 45.
A. I am there.
Q. Please draw your attention to Line 21, and starting at Line 21 the transcript reads:
"Question: So then you've met Dr. Tsien?

Page 254

"Answer: Yes.
"Question: And in the discussions you've had with Dr. Tsien, have you ever discussed modified nucleotides?
"Answer: Surprisingly, no."
I'm not going to read the whole transcript into the record, but -- and of course you can look at any part that you want. I'm going to ask that you skip ahead to Page 46 and draw your attention to Line 8. Starting at Line 8, the transcript reads:
"Answer: And to tell you the truth, I never really knew that he did this work until I read this patent."
And the portions of the transcript I read is what you testified, correct?
A. That is what is written, yes.
Q. And the patent which you were referring to in your testimony is the Tsien patent application, which is marked as Exhibit 1013, correct?
A. It is the Tsien patent. It is marked as 1013.
Q. And you've been doing scientific research on modified nucleotides for over 25 years; is that

Page 255

right?
A. No. I've been doing work on modified nucleotides since 19 -- the end of 1998, so...
Q. About 20 years?
A. About 20 years.
Q. And you've published many research papers on modified nucleotides during that time, right?
A. I have published a number of papers on that topic, yes.
Q. Did you ever cite to Tsien's patent application in any of your papers?
A. No.
Q. Do you recall ever citing to Tsien's patent application in any of your review articles?
A. No.
Q. Do you ever recall citing to Tsien's patent application in any of your grant applications?
A. No, I did not.
Q. Do you ever recall citing to Tsien's patent application in any presentations that you've made?
A. No.
Q. I'm talking about scientific presentations.

Page 256

A. No.
Q. And you testified in 2014 in the transcript that we just looked at that you never even knew that Tsien did any work on modified nucleotides until you became involved as an expert for Illumina in the 2013 time frame, correct?
A. I guess I'm not positive I said that. I certainly was unaware that he had worked on the sequencing aspect of modified nucleotides. I mean, he did lots of work on RNA and DNA, and there were, for example, linkers in that. And I would have to rethink that carefully as to -- I've never cited it, but I am sort of peripherally aware that he did that work, but I believe what would be a factual statement -- and I would have to read this to make sure that this is what I said, but the content of the patent that is under discussion, I was unaware that he had been interested in that.
MR. SCHWARTZ: I'm just looking at the record.
THE WITNESS: While you do that, could I request a bathroom break?
MR. SCHWARTZ: Sure.
THE VIDEOGRAPHER: We're off the record at 9:46 a.m.

Page 257

(Recess taken.)
THE VIDEOGRAPHER: We are on the record at 9:49 a.m.
BY MR. SCHWARTZ:
Q. I asked you this yesterday, Dr. Romesberg, and I think you agree that Tsien doesn't provide any data showing that any 3' block nucleotide works for DNA sequencing, correct?
MR. ZIMMERMAN: Objection. Asked and answered by your own admission.
THE WITNESS: Yes. I believe we had that discussion. And I believe I agreed with that.
BY MR. SCHWARTZ:
Q. And Tsien's application is entirely prophetic, right?
A. On this subject, yes. I'd have to go back and -- I did not have that in mind as I read it, so it is possible that there were spots that had data. I did look at the patent in regards to this particular issue, and there is no data.
Q. And in contrast to Tsien's prophetic patent application, Metzker 1994 does provide data for a number of 3' block nucleotides for use in DNA sequencing, right?
A. Metzker provides data for, I think, eight

Page 258

O-modified nucleotides.
Q. So a person of ordinary skill who is developing nucleotides for use in SBS in the 2000 time frame, it's fair to say that they would give more weight to Metzker's paper that does provide data than Tsien's prophetic application that is completely devoid of data, correct?
A. On certain issues that -- data certainly is important. And I think that there are -- people would have weighted that data differently and certainly more impactfully. They would have -- seeing is believing.
So I just don't want to say in any way that they would have then dismissed a good idea, whether you viewed Tsien's idea as good or not.
The downside of data is that it is a specific question, and you can only get answers to specific questions.
So it is difficult to interpret data that i negative as being -- it is difficult to know how general a negative result is.
Q. I think my question may have been a little bit different, which is, given that Metzker actually provided data for 3'-O protecting groups for use in DNA sequencing, is it fair to say that a

Page 259

skilled artisan would give more weight to a paper with data in terms of understanding how those 3'-O protecting groups would work for DNA sequencing than another application that is entirely prophetic?

A. I mean, I understand what you are saying, and in one perspective I agree with that because, like I said, data speaks louder than suggestions. Seeing is believing. But at the same time, there is always the caveat -- because, as I said, experimental data always is a specific experiment. It answers a specific question.

And you may look at that and say, well, tha is not the question exactly I wanted, or I think tha I could optimize this. There are a variety of responses.

In general, certainly seeing is believing, and you want to see data. That is always good.

So, yeah, what you're saying is certainly sort of true, but at the same time, a good idea is a good idea. And sometimes people want to pursue it more than someone else might.

Q. I think -- again, I'm not sure that you are responding directly to my question, which is,

Page 260

if you have a situation where you have a paper and suppose that you are interested in developing 3'-O protecting groups for DNA sequencing, you have a paper that actually tests 3'-O protecting groups?

A. See, that is the problem. So you can make the suggestion that you could use 3'-O protecting groups, and you cannot address that question in a single paper. You cannot fully address that question.

So you can look at the results from any one paper and you can look at the specific experiments that were run, and you get data there. And if you believe the data, then that is the answer. But a question is often much broader than an experimental result.

So you would not necessarily dismiss an entire idea based on one piece of data, or two piece of data. You might have optimism that you could optimize the experiment or that it could be run differently or in the case that we are talking about you could use different polymerases or whatever, but my point is that on any experimental result where there is a result, you would believe that data more than you would believe a suggestion, obviously.

But to the extent that data answers

Page 261

specific questions and only those specific questions, then you might not want to dismiss an entire idea based on one particular set of data.

Q. Please go back to Exhibit 2035, your deposition transcript in IPR2013-00517.

A. I'm there.

Q. And please turn to Page 99, and starting with the first full sentence, halfway through it states -- the transcript reads -- I'm sorry, the transcript reads "There are lots of read lengths that would simply never be useful for sequencing by synthesis. A read length of one is just an example. You would never be able to do anything with that."

That is what you said, right?

A. That is what is written.

Q. So your testimony is there are lots of read lengths that would simply never be useful for sequencing by synthesis, and a read length of one is an example.

A. For sequencing by synthesis, I believe that is what I was saying, yes.

Q. Please go back to Exhibit 2007. That is your declaration in IPR2013-00517.

A. I'm sorry, what declaration?

Page 262

Q. The IPR2013-00517. It is Exhibit 2007.

A. Yes.

Q. Please turn to Page 23. Please look at the first full sentence. And the sentence reads "This reaction was performed in methanol, which was known to denature DNA. See infer at paragraph 55. Required two hours and resulted in only 75 to 91 percent yield." And you cite to the Loubinoux paper. "Further, stannous chloride, SnCl2, was known to damage DNA by causing nicks in DNA strands." And you cite to a Dontis 1999 paper.

And you write "Any one of these considerations would have led one of ordinary skill to have expected stannous chloride to be incompatible with Tsien's and Ju's requirements for sequencing by synthesis."

That is what you wrote, correct?

A. That is correct. That is what is written.

Q. And it is your opinion that a deprotection reaction that requires two hours is incompatible with Tsien's SBS methods, correct?

A. That is not exactly correct, no.

Q. That is what you wrote.

MR. ZIMMERMAN: Objection.

Page 263

Mischaracterizes.
THE WITNESS: That is not what I intended to write.
BY MR. SCHWARTZ:
Q. Did you write it?
MR. ZIMMERMAN: Objection. Mischaracterizes.
THE WITNESS: I wrote the words that are there. You are interpreting it differently than I intended.
BY MR. SCHWARTZ:
Q. Do you not say that any one of these considerations would have led one of ordinary skill to have expected stannous chloride to be incompatible with Tsien's and Ju's requirements for sequencing by synthesis?
A. That is what I wrote. And that is what I meant.
Q. And any one of those requirements included the two hours?
A. Can I explain it?
Q. Did you write that?
A. I would like to explain it.
Q. Can you answer my question first?
A. You can read it. That is correct.

Page 264

Q. And so you wrote it?
A. It's written. Two hours with 80 percent yield is very different than two hours quantitative.
So the time is inseparable from the yield. Those are related things. So -- because I know wher you are going with this, just be clear that my criteria for two hours, that is influenced by the fact that it is 90 percent yield. So just to be clear.
Q. So if the yield is not satisfactory, two hours is certainly not satisfactory with that yield. Is that what you are saying?
A. I'm sorry. Could you repeat that?
Q. If the deprotection yield is not satisfactory, then certainly two hours with that deprotection yield is not satisfactory?
A. Certainly.
Q. And that would not be compatible with Tsien's method?
A. For any efficiency component of that. As we talked about yesterday, you can still do sequencing by synthesis, just not in any sort of efficient way that I believe Tsien required.
Q. And that's true for SBS methods in

Page 265

general. It's not just for Tsien, correct?
A. For anything to be efficient, if you have a deprotection time that's part of the procedure then, yes, that would add time and make it effectively unrealistic to perform.
Q. I am going to ask you to go back to the Metzker paper. The Metzker 1994 paper, I should say. It is Exhibit 1016.
A. I have it.
Q. And please go back to Table 2 on Page 4263. Yesterday we discussed how Table 2 reports termination asterisk for the 3'-O-allyl dAPT with the Vent polymerase.
Do you remember that?
A. I remember the discussion.
Q. And the asterisk indicates that there was incomplete termination, correct?
A. That is what is written.
Q. And if I understand your position from yesterday, the incomplete termination is the result of the natural dAPT contaminate being incorporated, which allows synthesis to continue.
Is that your position?
A. I think that would have been the likely suspicion of a POSA.

Page 266

Q. And because of the natural dAPT contaminant you would end up with read through; is that correct?
A. It would prevent termination.
Q. And that would result in read through?
A. It would prevent termination. So I think we're using -- I think we're saying the same thing.
Q. So please turn to Page 4263, right column, second full paragraph. Halfway through that paragraph Metzker says in Figure 3B -- do you see that?
A. I do.
Q. Metzker writes in Figure 3B "The O-allyl template gel also shows the incorporation of 3'-O methyl dAPT, compound one, by AMV RT.
In addition to termination, some read through was also observed due to the presence of contaminating dATP."
That is what Metzker writes, correct?
A. That is what is written.
Q. So the read through reported for the 3'-O methyl dATP for AMV indicates that there was incomplete termination, correct?
A. The read through indicates there was incomplete termination. I think those are the same

Page 267

thing.
Q. And so let's look at Table 2 and focus on the 3'-O methyl dAPT with the AMV reverse transcript, please.
Do you see that?
A. Yes.
Q. And Metzker reports that there was complete termination, correct?
A. That is correct.
Q. And Metzker reports that there was complete termination even though there was read through, correct?
A. Some read through he reports.
Q. So the presence of read through caused by the natural dAPT contaminant did not lead Metzker to report incomplete termination for the 3'-O methyl dAPT with that polymerase, correct?
A. That is what is written. And what that would mean is that the O-methyl is even better incorporated.
Q. But despite the presence of the read through, Metzker reports that as complete termination?
A. I don't know how much read through was there, but it might have been such a small amount

Page 268

that you still have -- I believe what termination means here is that you produced a banding pattern the same as the dideoxy.
Now, if there was a little bit of read through but effectively you produced exactly that same banding pattern, I believe that Metzker would have called that complete termination. And that's simply because the contaminating 1 percent dAPT just didn't effectively prevent all those bands from bein formed due to termination.
And I assume that's because the 3'-O methyl is very competitive with the dAPT. And the 3' allyl would have been less competitive with the dAPT.
So I think that's consistent with the O-methyl being more competitive.
MR. SCHWARTZ: I would like to take a break now and discuss whether I have any further questions.
THE VIDEOGRAPHER: We are off the record at 10:04 a.m.
(Recess taken.)
THE VIDEOGRAPHER: We are on the record at 10:25 a.m.
MR. SCHWARTZ: Thank you, Dr. Romesberg. I have no further questions on this

Page 269

cross-examination.
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Page 270

EXAMINATION
BY MR. ZIMMERMAN:
Q. Dr. Romesberg, I appreciate you being here today and yesterday. I have a short series of questions that I would like to run through with you just to make sure we have a clear record.
If you could look at Exhibit 1001.
MR. SCHWARTZ: Would you just give me a chance to get a pad?
MR. ZIMMERMAN: Sure.
THE WITNESS: What is Exhibit 1001?
MR. ZIMMERMAN: Give me just a second.
BY MR. ZIMMERMAN:
Q. So it's the '852 Ju patent.
A. Yes.
Q. Do you recall yesterday you were asked some questions about what small means in the context of Ju?
A. I do.
Q. And you cited to Ju's teaching of MOM and allyl?
A. I did.
Q. I went back and looked at that testimony, and you indicated that both MOM and allyl have three carbons; is that correct?

Page 271

A. Yes.
Q. If you look at Column 8 of Ju at Line 26.
A. Yes.
Q. Do you see there is preference to, quote, "A small chemical moiety such as."
A. I'm sorry. Where are you?
Q. Line 25.
A. Yes.
Q. Column 8. You will see it reads "A small chemical moiety such as CH2OCH3 or CH2CH-double bond-CH2?
A. Yes.
Q. What is the second compound there?
A. That is the allyl.
Q. And what is the first compound?
A. That is the MOM.
Q. And does that show MOM as having three carbons?
A. Did I say three carbons? I apologize. I meant three atoms.
Q. So let me ask you the question again. Do MOM and allyl both have three carbons?
A. No, I'm sorry.
Q. How many carbons does allyl have?
A. Allyl has three carbons.

Page 272

Q. And how many carbons does MOM have?
A. It has two carbons.
Q. Leaving aside hydrogen, how many atoms does MOM have?
A. Three.
Q. And leaving aside hydrogen, how many atoms does allyl have?
A. Three.
Q. If we could turn to the Tsien patent, which is Exhibit 1013.
A. Yes.
Q. Do you have that in front of you?
A. Yes.
Q. Do you recall there was some discussion during your examination of Example 2?
A. Could you direct me to that?
Q. Yes. It is on Page 36 and spans over to Page 37.
A. Yes, I do.
Q. And there was some discussion, if you will recall, about the concentrations in that example, correct?
A. Yes. On Page 35, Example 2?
Q. Yes.
A. Yes, I think I remember.

Page 273

Q. And then there was comparisons of those concentrations to other references?
MR. SCHWARTZ: I just want to make an objection. I don't believe that there was any testimony about Example 2.
MR. ZIMMERMAN: Okay.
BY MR. ZIMMERMAN:
Q. I may have misspoken. If you will turn to Page 38.
A. Yes. Correct.
Q. Do you see Example 4?
A. Yes.
Q. Do you recall some discussion about the concentrations in Example 4?
A. Yes.
Q. Was that the only example of Tsien that you were pointed to with respect to concentrations during your examination?
A. I believe it was.
Q. Does Tsien have other examples?
A. Yes. He has six examples, I believe.
Q. And does Tsien in its teaching have any requirements with respect to concentrations?
A. No.
Q. You can put that aside.

Page 274

Do you recall a number of discussions during your examination about a POSA?
A. Yes, I do.
Q. And you were asked to assume that a POSA was a person -- in October of 2000?
A. That is correct.
Q. Is it safe to say that a person of ordinary skill in the art, if we assume October of 2000, wouldn't have had knowledge of articles that were published after that?
A. That is correct.
Q. Can we agree that the Metzker 2011 article, Exhibit 2025, was published after October 2000?
A. Yes.
Q. And can we agree that the Metzker 2007 article, Exhibit 1017, was published after October 2000?
A. Yes.
Q. And the new one that was marked today, Exhibit 1089, by Hamed, which was published in 2001, can we agree that was after October 2000?
A. Yes.
Q. So would a person of ordinary skill in the art as you've defined them have access to those

Page 275

articles?
A. No.
Q. I would like you to look at the Metzker '94 article, which is Exhibit 1016.
Do you have that?
A. I do.
Q. So between the publication of this paper and October of 2000, did Dr. Metzker publish any retraction or correction of this paper, to your knowledge?
A. Not to my knowledge.
Q. And are you aware of any publication by Metzker or anyone in his group or anyone who reviewed this, that indicated the data in this publication was incorrect between '94 and October 2000?
A. No, I do not believe there were any such publications.
Q. Now, if you look at Table 2 on Page 4263.
A. Yes, I'm there.
Q. Entry No. 3.
A. Yes.
Q. 3'-O-allyl modified dATP?
A. That is correct.
Q. Can you tell me if that is a single

Page 276

compound or multiple compounds?
A. That should be a single compound as he has shown it here. He shows the structure of that compound in Figure 2.
Q. And is that a substituted allyl?
A. No, that is just the canonical allyl group.
Q. Are there any other 3'-O allyls in the Metzker '94 paper?
A. No, there's not.
Q. Does he describe a genus of 3'-O allyls?
A. No, he does not.
Q. Does he describe a substituted 3'-O allyl?
A. Not to my knowledge, no.
Q. So the only 3'-O allyl in Metzker, Table 2, is the one there?
A. Yes, that is correct.
MR. ZIMMERMAN: I have no further questions at this time.
MR. SCHWARTZ: We can go off the record and take a break.
THE VIDEOGRAPHER: Going off the record at 10:35 a.m.
(Recess taken.)

Page 277

THE VIDEOGRAPHER: We're on the record at 10:41 a.m.
MR. SCHWARTZ: Dr. Romesberg, we don't have any recross. Thank you for your time today.
THE WITNESS: You are welcome.
MR. ZIMMERMAN: Thank you.
THE VIDEOGRAPHER: This concludes today's deposition of Floyd Romesberg, Ph.D. We are off the record at 10:41 a.m.
(The videotaped deposition of FLOYD ROMESBERG, PH.D., VOLUME II, concluded at 10:41 a.m.)

Page 278

I, the undersigned, a Certified Shorthand Reporter of the State of California, do hereby certify:
That the foregoing proceedings were taken before me at the time and place herein set forth; that any witnesses in the foregoing proceedings, prior to testifying, were duly sworn; that a verbatim record of the proceedings was made by me using machine shorthand which was thereafter transcribed under my direction; that the foregoing transcript is a true record of the testimony given.
Further, that if the foregoing pertains to the original transcript of a deposition in a Federal Case, before completion of the proceedings, review of the transcript [X] was [ ] was not requested.
I further certify I am neither financially interested in the action nor a relative or employee of any attorney of party to this action.
IN WITNESS WHEREOF, I have this date subscribed my name.
Dated: September 21, 2018

PATRICIA Y. SCHULER
CSR NO. 11949

DECLARATION UNDER PENALTY OF PERJURY

Case Name: Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Date of Deposition: 09/20/2018

Job No.: 10047003

I, FLOYD ROMESBERG, PH.D., hereby certify that I have read the transcript of my deposition and that this transcript accurately states the testimony given by me, with the changes or corrections, if any, as noted on an errata sheet or errata sheets.

Executed this _____ day of _____, 2018, at _____.

_____

FLOYD ROMESBERG, PH.D.

NOTARIZATION (If Required)

State of _____

County of _____

Subscribed and sworn to (or affirmed) before me on this _____ day of _____, 20__, by_____, proved to me on the basis of satisfactory evidence to be the person who appeared before me.

Signature: _____ (Seal)

DEPOSITION ERRATA SHEET

Case Name: Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

Name of Witness: Floyd Romesberg, Ph.D.

Date of Deposition: 09/20/2018

Job No.: 10047003

Reason Codes: 1. To clarify the record.
2. To conform to the facts.
3. To correct transcription errors.

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_____ Subject to the above changes, I certify that the transcript is true and correct

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_____

FLOYD ROMESBERG, PH.D.