

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

INTELLIGENT BIO-SYSTEMS,
INC.

Petitioner,

- vs -

ILLUMINA CAMBRIDGE LTD

Patent Owner.

No. IPR2013-00517
U.S. PATENT 7,566,537

VIDEO DEPOSITION OF FLOYD ROMESBERG, Ph.D.

July 8, 2014

Reported by: LYNN PENFIELD, CSR No. 8589, RPR, CRR

Columbia Exhibit 2035
Illumina, Inc. v. The Trustees of Columbia
University in the City of New York
IPR2018-00322

1 I N D E X

2

3 THE WITNESS: EXAMINATION

4 FLOYD ROMESBERG, Ph.D.

5 BY MR. SEGAL 9

6 BY MS. SWAROOP 170

7 QUESTIONS NOT ANSWERED

8 PAGE LINE

9 Did you ask your attorneys for other 159 2
10 data with regard to the cleavage of
11 allyl protecting groups?

12

13 E X H I B I T S

14 DEPOSITION EXHIBIT: PAGE

15 Exhibit 1018 A 2-page copy of a document 111

16 entitled, "A Mild and Rapid
17 Regeneration of Alcohols from
18 their Allylic Ethers by
19 Chlorotrimethylsilane/Sodium
20 Iodide" by Ahmed Kamal and
21 others

22

23

24

25

1 Exhibit 1019 A 14-page copy of a document 112
2 entitled, "Approach to the Total
3 Synthesis of Chlorothricolide:
4 Synthesis of (+-)-19,20-Dihydro-
5 24-O-methylchlorothricolide,
6 Methyl Ester, Ethyl Carbonate"
7 by Robert E. Ireland and
8 Michael D. Varney
9 Exhibit 1020 A 2-page copy of a document 170
10 entitled, "Abstracts,
11 Biophysical Society, Sixth
12 Annual Meeting"
13
14
15
16
17
18
19
20
21
22
23
24
25

1	EXHIBITS REFERRED TO:	PAGE
2	1001	21
3	1002	51
4	1004	112
5	1008	99
6	1106	119
7	2011	13
8	2013	138
9	2014	140
10	2015	141
11	2016	143
12	2018	147
13	2019	81
14	2021	150
15		
16	CONFIDENTIAL SECTION	BEGINS ENDS
17	No. 1	164 168
18		
19		
20		
21		
22		
23		
24		
25		

1 APPEARANCES

2

3 FOR THE PETITIONER:

4 BALLARD SPAHR LLP

5 BY: MARC S. SEGAL, ESQ.

6 1735 Market Street, 51st Floor

7 Philadelphia, Pennsylvania 19103-7599

8 215-665-8500

9 segalm@ballardspahr.com

10 AND

11 BALLARD SPAHR, LLP

12 BY: SCOTT D. MARTY, Ph.D., ESQ.

13 999 Peachtree Street, Suite 1000

14 Atlanta, Georgia 30309-3915

15 678-420-9300

16 martys@ballardspahr.com

17 FOR THE PATENT OWNER ILLUMINA CAMBRIDGE LTD.:

18 KNOBBE MARTENS OLSON & BEAR LLP

19 BY: SHEILA SWAROOP, ESQ.

20 2040 Main Street, 14th Floor

21 Irvine, California 92614

22 949-760-0404; FAX 949-760-9502

23 sheila.swaroop@kmob.com

24 AND

25 ///

1 KNOBBE MARTENS OLSON & BEAR LLP
2 BY: NATHANAEL R. LUMAN, Ph.D., J.D., ESQ.
3 KERRY S. TAYLOR, Ph.D., ESQ.
4 12790 El Camino Real, Suite 100
5 San Diego, California 92130
6 858-707-4000; FAX 858-707-4001
7 Nate.Luman@knobbe.com
8 kerry.taylor@knobbe.com

9 AND

10 ILLUMINA, INC.
11 BY: MARCUS BURCH, ESQ.
12 5200 Illumina Way
13 San Diego, California 92122
14 224-643-7473
15 mburch@illumina.com

16 VIDEO RECORDING PROVIDED BY:

17 JORDAN MEDIA, INC.
18 BY: JON IMEL
19 1228 Madison Avenue
20 San Diego, California 92116
21 619-299-4050
22
23
24
25

1 VIDEO DEPOSITION OF FLOYD ROMESBERG, PH.D.
2 taken by Petitioner at 12790 El Camino Real, Suite 100,
3 San Diego, California 92130, commencing on Tuesday,
4 July 8, 2014, at 9:02 a.m. before Lynn Penfield,
5 Certified Shorthand Reporter, Registered Professional
6 Reporter, Certified Realtime Reporter.

7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

12:35:37 1 San Diego, California July 8, 2014 9:18 a.m.

08:42:13 2 THE VIDEO OPERATOR: Good morning. We are on
09:18:12 3 the record. This is the digital video deposition of
09:18:15 4 Floyd Romesberg, Ph.D., testifying in the matter of
09:18:20 5 Intelligent Bio-Systems, Inc., versus Illumina
09:18:24 6 Cambridge, case number IPR2013-00517.

09:18:31 7 Today we are located at 12790 El Camino Real,
09:18:35 8 San Diego, California. Today's date is July 8th, 2014.
09:18:39 9 The time on the video monitor is 9:18 a.m.

09:18:43 10 My name is Jon Imel, video specialist with
09:18:46 11 Jordan Media, Inc., located at 1228 Madison Avenue,
09:18:51 12 San Diego, California. The certified shorthand reporter
09:18:55 13 today is Lynn Penfield in association with Kramm Court
09:19:00 14 Reporting, located in San Diego, California.

09:19:02 15 Will counsel please introduce yourselves for
09:19:04 16 the record.

09:19:05 17 MR. SEGAL: Mark Segal, Ballard Spahr, for
09:19:09 18 Intelligent Bio-Systems; and with me is Scott Marty,
09:19:14 19 also from Ballard Spahr for Intelligent Bio-Systems.

09:19:18 20 MS. SWAROOP: Sheila Swaroop, Knobbe Martens
09:19:20 21 Olson & Bear, for Illumina. Also with me from Knobbe
09:19:24 22 Marten is Nate Luman, and also attending is a
09:19:28 23 representative from Illumina, Marcus Burch.

09:19:31 24 THE VIDEO OPERATOR: The reporter may swear in
09:19:31 25 the witness.

09:19:33 1 FLOYD ROMESBERG, Ph.D.,
09:19:33 2 having been first duly sworn, testified as follows:

09:19:33 3

09:19:33 4 EXAMINATION

09:19:41 5 BY MR. SEGAL:

09:19:42 6 Q. Good morning, Dr. Romesberg.

09:19:44 7 A. Good morning.

09:19:45 8 Q. Could you please state your full name and
09:19:47 9 address for the record.

09:19:47 10 A. I'm Floyd Romesberg, 8109 Camino del Sur -- del
09:19:53 11 Sol, La Jolla, California.

09:19:55 12 Q. Okay. I know you've been deposed a few times
09:19:59 13 before in these IPR proceedings, but I just want to go
09:20:02 14 through some ground rules for this deposition.

09:20:05 15 Is that okay?

09:20:05 16 A. It is.

09:20:08 17 Q. Do you understand that today you're under oath?

09:20:10 18 A. I do.

09:20:11 19 Q. By that, do you understand that you are sworn
09:20:13 20 to tell the truth?

09:20:15 21 A. I do.

09:20:16 22 Q. Do you also understand that even though we're
09:20:19 23 not in front of a Court or in front of a jury, your
09:20:22 24 answers today have the same force and effect as if we
09:20:27 25 were in a courtroom with a judge and jury?

09:20:29 1 A. I understand.

09:20:30 2 Q. Are you prepared to answer my questions today?

09:20:32 3 A. I am.

09:20:33 4 Q. Is there anything that will prevent you from
09:20:35 5 giving me your full attention today?

09:20:38 6 A. There is not.

09:20:39 7 Q. Are you taking any medications or suffering
09:20:42 8 from any illness that would prevent you from
09:20:45 9 understanding my questions and answering them fully?

09:20:48 10 A. I am currently taking a medication, but it
09:20:50 11 would not prevent me from answering your questions
09:20:53 12 fully.

09:20:55 13 Q. Okay. So throughout the day -- you've gone
09:20:57 14 through this process before -- I'm going to ask you some
09:21:00 15 questions, and if you don't understand my question,
09:21:03 16 please ask me to clarify.

09:21:05 17 Is that okay?

09:21:06 18 A. That is okay.

09:21:08 19 Q. Now, if you don't ask me to clarify the
09:21:11 20 question, can we assume that you've understood the
09:21:14 21 question?

09:21:15 22 A. Yes.

09:21:18 23 Q. Also, throughout the day, your attorney may
09:21:22 24 interpose some objections. Now, unless you are
09:21:26 25 instructed by counsel not to answer, please go ahead and

09:21:29 1 answer the question.

09:21:30 2 Understand?

09:21:31 3 A. I do.

09:21:33 4 Q. As you know, we have a court reporter recording
09:21:35 5 my questions and your answers today, so throughout the
09:21:39 6 day, we want to try to make things easier on her, and so
09:21:43 7 you need to make sure you give oral responses to my
09:21:46 8 questions.

09:21:47 9 Understood?

09:21:48 10 A. Yes.

09:21:48 11 Q. And we should also try not to speak over each
09:21:50 12 other, so please let me finish my question before you
09:21:54 13 start answering, and I will try to let you finish your
09:21:58 14 answer before I ask my next question.

09:22:00 15 Understood?

09:22:01 16 A. Yes.

09:22:05 17 Q. Okay. I'm going to take -- also take regular
09:22:07 18 breaks during the day, but if at any time you need a
09:22:11 19 break for anything, please ask me and we can take a
09:22:14 20 break.

09:22:15 21 Understood?

09:22:16 22 A. Yes.

09:22:19 23 Q. Now, have you been deposed since your last
09:22:22 24 deposition in these IPR proceedings?

09:22:26 25 A. No, I have not.

09:22:28 1 Q. Now, you previously testified that you were
09:22:30 2 serving as an expert for Roche in a case against Enzo.

09:22:35 3 Is that correct?

09:22:36 4 A. I believe it's a case of Enzo against Roche.

09:22:40 5 Q. Okay. But you are serving as an expert in that
09:22:42 6 matter?

09:22:43 7 A. Yeah, but there's -- as I think I mentioned
09:22:46 8 then, there's been zero activity on that and there
09:22:49 9 hasn't been any activity since then.

09:22:51 10 Q. Okay. Just so I understand, there's no
09:22:53 11 activity since your last deposition?

09:22:55 12 A. Correct.

09:22:56 13 Q. Okay. So you haven't filed a declaration in
09:22:59 14 that matter?

09:22:59 15 A. No, I have not.

09:23:00 16 Q. Okay. You haven't been deposed?

09:23:03 17 A. No.

09:23:06 18 Q. Have you been engaged as an expert in any other
09:23:09 19 matter since your last deposition?

09:23:11 20 A. No.

09:23:13 21 Q. Okay. How much time have you spent on this IPR
09:23:17 22 proceeding to date?

09:23:21 23 A. This IPR?

09:23:23 24 Q. Yes.

09:23:29 25 A. I -- again, I -- I always have problems

09:23:33 1 figuring this out. I should have known you'd ask me
09:23:36 2 this, so I should have figured it out.

09:23:39 3 35 hours, 40 hours, I would -- maybe 50 hours
09:23:44 4 including reading, talking to counsel.

09:23:50 5 Q. Okay. Does that include your preparation for
09:23:53 6 the deposition today?

09:23:54 7 A. Yeah.

09:23:56 8 Q. Okay.

09:23:57 9 A. That's a rough estimate.

09:24:05 10 MR. SEGAL: His declaration?

09:24:35 11 I'm going to show you what's been previously
09:24:38 12 marked as Exhibit 2011.

09:24:42 13 (Exhibit 2011 was referred to.)

09:24:47 14 MS. SWAROOP: Counsel, before you start, I just
09:24:49 15 want to note that portions of his declaration have been
09:24:52 16 designated as confidential, so to the extent you get
09:24:55 17 into that portion of the declaration, we'll just need to
09:24:57 18 go on the confidential portion of the record.

09:25:01 19 MR. SEGAL: Okay.

09:25:01 20 BY MR. SEGAL:

09:25:02 21 Q. Do you recognize this document?

09:25:03 22 A. Yes. It would appear to be my declaration.

09:25:05 23 Q. And it's your declaration in this matter, this
09:25:08 24 proceeding?

09:25:10 25 A. Yes, it would appear to be.

09:25:16 1 Q. If you'll turn to the last page, page 43, is
09:25:23 2 that your signature there?

09:25:24 3 A. It is.

09:25:26 4 Q. And you signed it on May 5th of this year?

09:25:30 5 A. Yes.

09:25:34 6 Q. Did you draft this declaration?

09:25:36 7 A. I participated in it. I read carefully all
09:25:41 8 aspects, every part of it, and worked with it, but I did
09:25:49 9 not actually write it. It was written by my lawyers at
09:25:54 10 Knobbe Martens.

09:25:57 11 Q. Did you make any changes after they wrote the
09:26:00 12 declaration?

09:26:00 13 A. Pretty extensively, all through discussion.

09:26:06 14 Q. Was anyone other than your attorneys at Knobbe
09:26:10 15 involved in drafting your declaration?

09:26:13 16 A. No.

09:26:15 17 Q. Are you aware that there is a -- another expert
09:26:19 18 that's testifying on behalf of Illumina in this matter?

09:26:24 19 A. Vaguely.

09:26:25 20 Q. Did you speak with this expert?

09:26:27 21 A. Did I speak with him? No, I did not, verbally.

09:26:32 22 Ironically, I sent him an e-mail. It was
09:26:36 23 unintentional. At the time I didn't know he was an
09:26:38 24 expert and it was on an unrelated thing, and he sent me
09:26:42 25 an e-mail back, and that was the extent of it.

09:26:45 1 Q. All right. Who was this expert?

09:26:49 2 A. Metzker.

09:26:50 3 Q. Metzker?

09:26:51 4 A. Oh, God. This is how informal it was.

09:26:56 5 Q. Is it Dr. Burgess?

09:26:58 6 A. Yes, I'm sorry. No -- yes, it was Dr. Burgess.

09:27:02 7 Q. Okay.

09:27:03 8 A. Thank you.

09:27:03 9 Q. So you didn't speak to him regarding this

09:27:05 10 matter?

09:27:05 11 A. No.

09:27:06 12 Q. Okay. You sent him an e-mail recently?

09:27:10 13 A. Maybe it was three -- two months ago.

09:27:12 14 Q. And that was regarding?

09:27:14 15 A. It was regarding some -- my lab works with the

09:27:21 16 evolution of DNA polymerases and on a -- with a -- to

09:27:27 17 recognize unnatural substrates, and we -- I had a

09:27:31 18 question about an unnatural substrate that would have

09:27:35 19 nothing to do with sequencing by synthesis, and I just

09:27:40 20 wondered if he knew of anyone who had characterized it,

09:27:44 21 and he sent me back an e-mail that said no.

09:27:50 22 Q. Can you turn to page 6 of your declaration.

09:28:04 23 You'll see in paragraph 21 you list some

09:28:08 24 documents that you've considered and reviewed in

09:28:12 25 connection with providing this declaration.

09:28:14 1 Is that right?

09:28:15 2 A. That is right.

09:28:17 3 Q. Are these the only materials you relied on in
09:28:20 4 forming your opinion in your declaration?

09:28:24 5 A. No. There were a couple other papers that I
09:28:28 6 read that I believe are not listed.

09:28:33 7 Q. And what are those papers?

09:28:35 8 A. I don't have them.

09:28:37 9 Q. Can you tell me --

09:28:38 10 A. Oh, what were they?

09:28:42 11 Q. Yes.

09:28:42 12 A. I thought you said where.

09:28:44 13 They were a paper by an author named Reardon
09:28:47 14 and a paper by an author named Handlon, and they were
09:28:52 15 provided to me by my lawyers.

09:29:02 16 Q. Do you know the date of these papers?

09:29:05 17 A. I can estimate. I did look at them. I've read
09:29:08 18 a lot of papers recently on this IPR. I think they
09:29:12 19 were -- I believe the Handlon paper I'm thinking was
09:29:18 20 1988, and I think the Reardon paper was 1992,
09:29:21 21 approximately.

09:29:26 22 Q. Now, why didn't you list those papers in
09:29:28 23 paragraph 21?

09:29:29 24 A. Because I only saw them for the first time last
09:29:38 25 week.

09:29:38 1 So maybe I should clarify my answer. I did not
09:29:41 2 use them for writing this declaration. I used them -- I
09:29:44 3 read them in preparation for this IPR, for this
09:29:47 4 deposition.

09:29:49 5 Q. Okay. So are there any references that aren't
09:29:53 6 listed or material that is not listed in paragraph 21
09:29:56 7 that you relied on in rendering your opinions in the
09:30:00 8 declaration?

09:30:01 9 A. No.

09:30:05 10 I must have misunderstood your question. I'm
09:30:08 11 sorry.

09:30:08 12 Q. Who identified the documents and material
09:30:11 13 listed in paragraph 21?

09:30:13 14 A. Most of them, my lawyers. The -- there were
09:30:17 15 several specifically related to an aspect of -- that's
09:30:21 16 important to the IPR, specifically conditions that
09:30:24 17 denature DNA that I spent a particularly long time
09:30:30 18 looking at, looking for; and I think that none of them
09:30:33 19 wound up being as key as the ones are here, so they're
09:30:36 20 not listed.

09:30:37 21 And so they were not -- they were simply read,
09:30:39 22 but they didn't wind up being as relevant, so I didn't
09:30:43 23 use them and so I think that none of them wound up being
09:30:47 24 here.

09:30:48 25 So I think that in the end, I think all of them

09:30:50 1 here, I believe, were provided by my lawyers.

09:30:58 2 Q. So you mentioned some documents that you found
09:31:00 3 regarding denaturation.

09:31:02 4 Is that right?

09:31:03 5 A. Correct.

09:31:04 6 Q. And those are not listed here?

09:31:06 7 A. They are not.

09:31:09 8 Q. Do any of those documents contradict your
09:31:13 9 opinions in your --

09:31:15 10 A. No.

09:31:15 11 Q. -- declaration?

09:31:17 12 A. No, absolutely not.

09:31:18 13 Q. Do you remember the -- the first named
09:31:21 14 authors --

09:31:22 15 A. No.

09:31:22 16 Q. -- or the titles of these documents?

09:31:24 17 A. No. I apologize. I do not. But they did not
09:31:28 18 contradict in any way. They were the -- the -- the
09:31:33 19 important point was absolutely the same in all of them.

09:31:37 20 Q. Were these references provided to your
09:31:39 21 attorneys?

09:31:42 22 A. I -- I discussed it with them. I don't know --
09:31:45 23 I don't remember if I actually provided them.

09:31:59 24 Q. Now, you mentioned the Reardon and Handlon
09:32:01 25 references that you reviewed in preparation for this

09:32:04 1 deposition.

09:32:05 2 Is that right?

09:32:05 3 A. It is.

09:32:08 4 Q. Did your review of the Reardon and Handlon
09:32:11 5 references in any way change your opinions --

09:32:16 6 A. No.

09:32:16 7 Q. -- that are in your declaration?

09:32:18 8 A. No, it did not.

09:32:29 9 Q. Were there any documents that you located
09:32:31 10 through your own search that you were told not to
09:32:34 11 consider in rendering your opinions?

09:32:36 12 A. No. In fact, my lawyers were very clear --

09:32:44 13 MS. SWAROOP: Dr. Romesberg, I would caution
09:32:45 14 you not to disclose communications with counsel. Those
09:32:48 15 are subject to privilege.

09:32:49 16 So answer that to the extent you can without
09:32:52 17 disclosing privileged communications.

09:32:54 18 THE WITNESS: No. They -- no, there were none
09:32:56 19 that I was told not to use.

09:33:09 20 BY MR. SEGAL:

09:33:09 21 Q. What did you do to prepare for today's
09:33:11 22 deposition?

09:33:12 23 MS. SWAROOP: Again, Dr. Romesberg, exclude
09:33:15 24 from your answer any specific reference to any specific
09:33:16 25 communications with counsel. You can testify generally

09:33:18 1 as to what you did to prepare.

09:33:20 2 THE WITNESS: I met with the lawyers and I read
09:33:23 3 papers and patents, the material described in paragraph
09:33:29 4 21 of my declaration.

09:33:31 5 BY MR. SEGAL:

09:33:31 6 Q. When did you meet with your lawyers?

09:33:37 7 A. Most recently, and the only dates that I can
09:33:40 8 explicitly remember are, I believe, Wednesday and
09:33:47 9 Thursday of last week -- two days last week, I believe
09:33:51 10 it was Wednesday and Thursday -- yesterday -- and
09:33:54 11 yesterday, and also one time earlier, maybe a month ago.

09:34:05 12 I came into the -- to this room and we --
09:34:09 13 that's when we went through the -- the declaration
09:34:12 14 paragraph by paragraph.

09:34:18 15 Q. How long did you meet with your attorney last
09:34:20 16 week? Were they all-day meetings?

09:34:23 17 A. Yes, both days were all-day meetings, I think
09:34:27 18 seven to eight hours.

09:34:29 19 All day doing academic -- "all day" to me can
09:34:33 20 mean longer than that.

09:34:35 21 Q. How about yesterday? Was that an all-day
09:34:38 22 meeting?

09:34:39 23 A. No, it was not. I think we were done at 2:00.

09:34:49 24 Q. Did you discuss your deposition preparation
09:34:52 25 with anyone other than your attorneys?

09:34:54 1 A. No.

09:35:24 2 MR. SEGAL: Dr. Romesberg, I'm going to hand
09:35:26 3 you what's been previously marked Exhibit 1001. It's
09:35:30 4 U.S. patent No. 7,566,537.

09:35:36 5 (Exhibit 1001 was referred to.)

09:35:49 6 BY MR. SEGAL:

09:35:50 7 Q. Have you seen this patent before?

09:35:51 8 A. Yes, I have. It would appear to be the
09:35:53 9 Illumina patent that is the subject of this IPR.

09:35:56 10 Q. Have you reviewed this patent?

09:35:58 11 A. Yes.

09:35:59 12 Q. Have you reviewed the claims of this patent?

09:36:01 13 A. Yes, I have.

09:36:09 14 Q. Have you reviewed the file history for this
09:36:11 15 patent?

09:36:12 16 A. That's a specific document, correct?

09:36:16 17 Q. By "file history," I mean the -- the documents
09:36:20 18 that are exchanged between the patent applicant and the
09:36:23 19 U.S. Patent and Trademark Office to obtain the patent.

09:36:28 20 Did you review that file?

09:36:31 21 A. I -- I'm not sure. I vaguely remember maybe
09:36:36 22 seeing it, but I might be mixing this up with a
09:36:40 23 different IPR that was very long, and I don't think I
09:36:42 24 read it. If it was provided to me, I didn't digest it
09:36:47 25 and retain it.

09:36:51 1 Q. Do you know what claims are at issue in this
09:36:54 2 IPR proceeding?

09:37:01 3 A. I don't understand the question.

09:37:02 4 Q. Do you know what patent claims are at issue in
09:37:06 5 this proceeding?

09:37:08 6 A. So specifically I assumed that all of them were
09:37:12 7 at issue. I guess I'm -- I guess, then, the answer to
09:37:15 8 your question would be no.

09:37:26 9 Q. If you could take a look at claim 1. It's in
09:37:35 10 column 19.

09:37:39 11 Are you there?

09:37:40 12 A. I see it.

09:37:41 13 Q. Claim 1 reads at the very beginning, "A method
09:37:43 14 of labeling a nucleic acid molecule."

09:37:47 15 Did I read that correctly?

09:37:50 16 A. You did.

09:37:50 17 Q. How would a person of ordinary skill in the art
09:37:53 18 as of August 2002 understand a method of labeling in the
09:37:57 19 context of this claim?

09:37:58 20 MS. SWAROOP: Object as outside the scope of
09:38:01 21 his declaration.

09:38:02 22 BY MR. SEGAL:

09:38:03 23 Q. You can answer.

09:38:04 24 A. I assume a person skilled in the art would
09:38:06 25 interpret it exactly as it says: That it was a method

09:38:10 1 of having, producing, or using nucleotides that are
09:38:19 2 modified or labeled in some way that's useful.

09:38:23 3 Q. Now, a method of labeling is different than a
09:38:26 4 method of sequencing, correct?

09:38:28 5 MS. SWAROOP: Again, object as outside the
09:38:29 6 scope.

09:38:32 7 THE WITNESS: A method of labeling is different
09:38:35 8 than a method of its use; for example, for sequencing,
09:38:38 9 yeah.

09:38:40 10 BY MR. SEGAL:

09:38:43 11 Q. The next part of the claim, if you'll read with
09:38:45 12 me, states "the method comprising incorporating into the
09:38:51 13 nucleic acid molecule a nucleotide or nucleoside
09:38:56 14 molecule."

09:38:56 15 Did I read that portion correctly?

09:39:00 16 A. Yes.

09:39:02 17 Q. In the context of this claim, what would
09:39:04 18 "incorporating" mean to a person of ordinary skill in
09:39:08 19 the art as of August 2002?

09:39:11 20 MS. SWAROOP: Okay. I'm going to object as
09:39:13 21 outside the scope of Dr. Romesberg's declaration.

09:39:18 22 THE WITNESS: Are you asking --

09:39:19 23 MS. SWAROOP: The subject of -- the subject of
09:39:20 24 claim construction was not part of his opinions in this
09:39:23 25 case.

09:39:23 1 MR. SEGAL: He's giving an opinion as to the
09:39:25 2 validity of the claims. He has to understand what they
09:39:28 3 mean to a person of ordinary skill in the art.

09:39:30 4 MS. SWAROOP: Well, I object to the extent
09:39:31 5 you're asking him to construe the claims. It's outside
09:39:36 6 the scope of his declaration.

09:39:37 7 MR. SEGAL: Your objection is noted.

09:39:39 8 THE WITNESS: So to clarify, you're asking if
09:39:42 9 one was given that sentence in a vacuum with no other
09:39:45 10 information, how would they interpret it?

09:39:47 11 BY MR. SEGAL:

09:39:48 12 Q. How would a person interpret that phrase that I
09:39:50 13 just read to you in the context of this patent?

09:39:54 14 If they're picking up this patent in August of
09:39:56 15 2002, how would they understand that incorporation
09:40:02 16 phrase --

09:40:02 17 MS. SWAROOP: Same objection.

09:40:03 18 BY MR. SEGAL:

09:40:04 19 Q. -- to mean?

09:40:05 20 MS. SWAROOP: Same objection. Calls for a
09:40:06 21 legal conclusion, outside the scope.

09:40:08 22 THE WITNESS: I should answer?

09:40:10 23 MS. SWAROOP: You can answer to the extent you
09:40:11 24 can.

09:40:13 25 THE WITNESS: I interpreted it as polymerase

09:40:19 1 mediated incorporation. I assume that someone then
09:40:22 2 would have done the same thing.

09:40:26 3 BY MR. SEGAL:

09:40:26 4 Q. Now, this portion of the claim says includes
09:40:32 5 incorporating a nucleoside molecule.

09:40:36 6 How can you incorporate a nucleoside molecule
09:40:41 7 via polymerase?

09:40:44 8 A. That's -- that's straightforward. A
09:40:47 9 nucleoside, as opposed to a nucleotide, is whether -- it
09:40:51 10 depends on whether you're including the phosphate
09:40:54 11 moiety.

09:40:55 12 If you include a nucleoside -- sorry. If you
09:40:59 13 include a nucleoside monophosphate or a nucleotide --
09:41:03 14 that's what you actually include during a reaction;
09:41:05 15 that's what you actually incorporate during a
09:41:08 16 reaction -- that certainly including the incorporation
09:41:11 17 of a nucleoside. It's just the nucleobase part.

09:41:20 18 It would not be traditional to call that a
09:41:23 19 nucleoside. You could call that a nucleoside moiety, I
09:41:27 20 guess, but if you're incorporating it -- the language is
09:41:33 21 a little loose by scientific standards, but I don't
09:41:36 22 think it would have been -- I don't think -- I think it
09:41:38 23 would have been understood.

09:41:39 24 It's a little vernacular, but it's clearly
09:41:44 25 referring to a -- the only reaction that a polymerase

09:41:50 1 performs.

09:41:50 2 Q. So a nucleoside -- the molecule without a
09:41:53 3 triphosphate, that's what I mean by nucleoside.

09:41:57 4 A. Well, a nucleoside just means there's no
09:41:59 5 phosphate.

09:41:59 6 A triphosphate is a nucleoside, but people
09:42:03 7 often refer to that as a -- sorry -- is a nucleotide.

09:42:06 8 People often refer to that as a nucleoside
09:42:10 9 triphosphate, and so they sort of often shorten that to
09:42:13 10 a nucleoside. It is a little sloppy.

09:42:16 11 But a monophosphate is also a nucleotide, or a
09:42:19 12 nucleoside monophosphate. "Nucleotide monophosphate"
09:42:23 13 would be redundant.

09:42:25 14 Q. So a nucleoside without any phosphate groups,
09:42:30 15 is there a way to incorporate that into a nucleic acid
09:42:35 16 molecule?

09:42:38 17 A. By the strict definition of what a nucleoside
09:42:42 18 is, no.

09:42:43 19 Neither by a polymerase nor by chemical
09:42:46 20 synthesis. A polymerase reaction, you would need it to
09:42:50 21 be a nucleoside triphosphate. By -- for a chemical
09:42:54 22 synthesis, you would need it to be what's called a
09:42:56 23 nucleoside phosphoramidite.

09:42:59 24 They're not specifying here, but the claim is
09:43:02 25 clearly referring to a polymerase, so you would

09:43:05 1 immediately have thought of a nucleoside triphosphate
09:43:09 2 being converted to a nucleoside monophosphate during the
09:43:12 3 reaction, and they're simply referring it to a nucleus,
09:43:15 4 as a nucleoside; and that's -- so can you show me
09:43:19 5 exactly where you're referring to, by the way?

09:43:23 6 Q. That phrase in claim 1, again, it says
09:43:25 7 "incorporating into the nucleic acid molecule a
09:43:29 8 nucleotide or nucleoside molecule."

09:43:32 9 A. So they -- sorry. So they do specify
09:43:35 10 nucleotide or nucleoside. Yeah.

09:43:40 11 It's not the way I would have written it,
09:43:42 12 but -- so was my answer sufficiently clear?

09:43:46 13 Q. Yes.

09:43:46 14 A. Okay.

09:43:47 15 Q. So the next part of the claim picks up "wherein
09:43:50 16 the nucleotide or nucleoside molecule has a base that is
09:43:54 17 linked to a detectable label via a cleavable linker and
09:43:58 18 the nucleotide or nucleoside molecule has a ribose or
09:44:02 19 deoxyribose sugar moiety."

09:44:05 20 Do you see that?

09:44:10 21 A. I'm sorry. I'm having trouble finding it.
09:44:14 22 Where does the sentence start?

09:44:16 23 Q. The portion of the claim picks up with the
09:44:17 24 "wherein," the third line of claim 1.

09:44:20 25 A. I have it, yes.

09:44:21 1 Q. Okay. And I just read that portion in.

09:44:24 2 "Wherein the nucleotide or nucleoside molecule
09:44:28 3 has a base that is linked to a detectable label via a
09:44:32 4 cleavable linker and a nucleotide or nucleoside molecule
09:44:36 5 has a ribose or deoxyribose sugar moiety."

09:44:40 6 Do you see that?

09:44:40 7 A. I do.

09:44:41 8 Q. Okay. Did I read that correctly?

09:44:43 9 A. Yes.

09:44:46 10 Q. How would a person of ordinary skill understand
09:44:48 11 that portion of the claim?

09:44:50 12 MS. SWAROOP: And same objection. Outside the
09:44:51 13 scope of his declaration, calls for a legal conclusion.

09:44:56 14 THE WITNESS: I would interpret that as the
09:45:00 15 nucleoside or nucleotide that's being incorporated, it
09:45:04 16 has a nucleic base on it by definition, and they're
09:45:09 17 saying that that -- they're saying two things about it.

09:45:12 18 The nucleic base could be attached to a linker,
09:45:15 19 a cleavable linker, and it's also attached to a sugar,
09:45:20 20 being a ribose or a deoxyribose. They're just
09:45:27 21 specifying the type of nucleoside and a modification.

09:45:31 22 BY MR. SEGAL:

09:45:31 23 Q. What's the difference between a ribose and a
09:45:34 24 deoxyribose?

09:45:37 25 A. Hydroxyl group. The sugar is different.

09:45:41 1 Q. On a certain position?

09:45:43 2 A. Correct, yes. The C2 position.

09:45:49 3 Q. Okay. Just picking up again the claim where I
09:45:52 4 left off, the next part of the claim reads "wherein the
09:45:56 5 ribose or deoxyribose sugar moiety comprises a
09:46:02 6 protecting group attached via the 2' or 3' oxygen atom."

09:46:07 7 Do you see that?

09:46:08 8 A. I do.

09:46:09 9 Q. Did I read that correctly?

09:46:10 10 A. Yes.

09:46:10 11 Q. Again, in the context of this claim and in the
09:46:12 12 context of the patent, what would "protecting group"
09:46:18 13 mean to a person of ordinary skill as of August 2002?

09:46:22 14 MS. SWAROOP: Object as outside the scope,
09:46:24 15 calls for a legal conclusion.

09:46:26 16 THE WITNESS: I read that as a group that could
09:46:27 17 be removed.

09:46:38 18 BY MR. SEGAL:

09:46:38 19 Q. That's all, just that the group can be removed?

09:46:46 20 A. Commonly the term "protecting group" is used in
09:46:49 21 synthetic chemistry when you have a functional group
09:46:53 22 that's reactive under conditions that you want to
09:46:57 23 specifically direct to a different part of the molecule,
09:46:59 24 and its reactivity would prevent specificity.

09:47:05 25 So you put on a protecting group. It prevents

09:47:08 1 reaction, gives you the specificity that you wanted, and
09:47:12 2 it then can be removed.

09:47:21 3 Q. So the same definition would apply here?

09:47:24 4 MS. SWAROOP: Object as outside the scope,
09:47:26 5 calls for a legal conclusion.

09:47:34 6 THE WITNESS: I would read that as "more or
09:47:36 7 less."

09:47:36 8 I mean I hesitate to use the word "exact"
09:47:39 9 because it's a little bit different, but you want to
09:47:42 10 include the nucleoside in a manner that you don't --
09:47:50 11 where the additional reactivity would be continued
09:47:53 12 synthesis, and so your blocking group prevents that so
09:47:56 13 that gives you the specificity for a single
09:47:59 14 incorporation and the removability is similar, I guess.

09:48:09 15 BY MR. SEGAL:

09:48:09 16 Q. Okay. This portion of the claim, it allows for
09:48:12 17 the protecting group to be only attached to the 2'
09:48:16 18 oxygen, correct?

09:48:17 19 MS. SWAROOP: Again, object to the form of the
09:48:18 20 question, object as outside the scope, calls for a legal
09:48:21 21 conclusion.

09:48:21 22 THE WITNESS: No, I think it says -- again, my
09:48:25 23 read of it is it says 2' or 3' oxygen atom.

09:48:30 24 BY MR. SEGAL:

09:48:30 25 Q. Right. So one of the possibilities is that the

09:48:33 1 protecting group could be only attached to the 2'
09:48:34 2 oxygen?

09:48:38 3 MS. SWAROOP: Same objections. Outside the
09:48:40 4 scope, calls for a legal conclusion.

09:48:40 5 THE WITNESS: Yeah, so in this case I'm going
09:48:42 6 to have to agree with my lawyer a little bit in that I
09:48:45 7 don't quite know how to construct legally what that
09:48:48 8 means.

09:48:48 9 I think that me, as a scientist, simply reading
09:48:51 10 this, it would say that protection of the 2' or the 3';
09:48:54 11 and, again, aside from any legal implications of the
09:49:00 12 English language that I'm not familiar with, I assume
09:49:05 13 that would include a case where it's protected at the 2'
09:49:09 14 only.

09:49:14 15 BY MR. SEGAL:

09:49:14 16 Q. Again, back to the claim, the next part of the
09:49:16 17 claim reads, "and said protecting group can be modified
09:49:19 18 or removed to expose a 3' hydroxyl group."

09:49:25 19 Did I read that right?

09:49:29 20 A. Yes.

09:49:33 21 Q. How would a person of ordinary skill in the art
09:49:36 22 remove a protecting group from a 3' oxygen to expose a
09:49:41 23 3' hydroxyl group?

09:49:49 24 A. How would one do that?

09:49:50 25 Q. Yeah.

09:49:51 1 A. Well, by today's standard, I would use an
09:49:55 2 azidomethyl group. I guess that was a joke.

09:49:59 3 There's lots of ways. I guess I don't
09:50:01 4 understand the question.

09:50:03 5 By definition, what a protecting group is, is
09:50:06 6 it allows for chemical removal, exposing what you
09:50:10 7 protected; in this case, the hydroxyl group, so --

09:50:14 8 Q. So the removal of the group on the 3' position,
09:50:23 9 that alone exposes the 3' hydroxyl?

09:50:26 10 A. A group --

09:50:27 11 MS. SWAROOP: Object to the form of the
09:50:27 12 question.

09:50:28 13 THE WITNESS: If I -- if I understood your
09:50:30 14 question, a group attached to the 3' oxygen, its removal
09:50:38 15 would unmask, would leave behind a 3' hydroxyl. It
09:50:46 16 would not, if it were directly attached to the 3'
09:50:50 17 position, which is the carbon.

09:50:52 18 BY MR. SEGAL:

09:50:53 19 Q. Okay. Does the '537 patent give any examples
09:50:59 20 of removing a protecting group from the 3' oxygen to
09:51:02 21 expose a 3' hydroxyl group?

09:51:06 22 MS. SWAROOP: Object to the form of the
09:51:07 23 question. Object as outside the scope.

09:51:09 24 THE WITNESS: I would have to look back through
09:51:11 25 it. I read so many documents in the past week that I

09:51:15 1 would have to look back through and refresh my memory.

09:51:18 2 Would you like me to do so?

09:51:20 3 BY MR. SEGAL:

09:51:20 4 Q. I think we'll do that on a break, so maybe we
09:51:22 5 can hold that for now.

09:51:24 6 A. Okay.

09:51:28 7 Q. How would a person of ordinary skill in the art
09:51:30 8 modify a protecting group that's attached to a 3' oxygen
09:51:35 9 to expose a 3' hydroxyl group?

09:51:38 10 A. My read on that -- and this is a common way
09:51:41 11 protecting groups work -- is that the modification
09:51:43 12 occurs distal -- far away -- from the oxygen, but it
09:51:48 13 triggers a reaction that ultimately releases the
09:51:51 14 hydroxyl group.

09:51:56 15 Again, it's a little bit of an odd language,
09:51:59 16 but that's kind of a common organic synthesis language.
09:52:02 17 And, again, what I read that is an event that produces
09:52:06 18 something, for example, that's unstable, that then
09:52:08 19 rearranges and leaves the hydroxyl group behind.

09:52:11 20 So in that context, what's the protecting group
09:52:14 21 and what's the modification is vague, and people use
09:52:17 22 that loosely.

09:52:23 23 Q. Okay. Is your basis for that understanding of
09:52:25 24 that term, is that supported by the '537 patent?

09:52:29 25 MR. BURCH: Counsel, this in no way has

09:52:33 1 anything to do with the opinions in Dr. Romesberg's
09:52:35 2 declaration. He did not give any opinion with regard to
09:52:38 3 the disclosure of the '537 patent. This is way outside
09:52:42 4 the scope, so I think we need to move on.

09:52:45 5 BY MR. SEGAL:

09:52:46 6 Q. You can answer.

09:52:47 7 MS. SWAROOP: You can answer to the extent you
09:52:49 8 can.

09:52:50 9 THE WITNESS: Could you repeat your question.

09:52:52 10 BY MR. SEGAL:

09:52:52 11 Q. You just gave your understanding of what it
09:52:54 12 means to modify a protecting group at the 3' oxygen to
09:52:58 13 expose a 3' hydroxyl, right?

09:53:01 14 A. Correct.

09:53:01 15 Q. I'm just wondering if there's any support in
09:53:03 16 the '537 patent for your definition.

09:53:06 17 MS. SWAROOP: Counsel, can you point to any
09:53:08 18 portion of Dr. Romesberg's declaration in which he
09:53:11 19 discussed the specification or disclosure of the '537
09:53:14 20 patent?

09:53:15 21 BY MR. SEGAL:

09:53:15 22 Q. You can answer.

09:53:17 23 MS. SWAROOP: No. I want an answer to my
09:53:18 24 question. This is clearly outside the scope --

09:53:21 25 MR. SEGAL: It's not.

09:53:22 1 MS. SWAROOP: -- and this in no way addresses
09:53:23 2 his opinions in his declaration.

09:53:25 3 MR. SEGAL: It's not outside the scope.

09:53:25 4 MS. SWAROOP: We're not going to permit
09:53:27 5 questioning with regard to disclosure of the '537
09:53:30 6 patent. That's not at issue here, and it's not what he
09:53:33 7 opined on.

09:53:34 8 MR. SEGAL: He -- I'm entitled to know what his
09:53:36 9 understanding of how a person with ordinary skill in the
09:53:39 10 art would interpret these claims.

09:53:41 11 He's giving an opinion that these claims are
09:53:44 12 invalid and that requires a comparison of the prior art
09:53:47 13 to the claims as properly construed, so I'm entitled to
09:53:52 14 know what his understanding of those claims are.

09:53:55 15 MS. SWAROOP: You've asked him questions about
09:53:56 16 the claims and asked him questions about claim
09:53:57 17 construction which, again, he never gave an opinion on.
09:53:59 18 Now you're moving on to the disclosure of the '537
09:54:03 19 patent, which has nothing to do --

09:54:04 20 MR. SEGAL: I'm asking if he has a basis.

09:54:06 21 MS. SWAROOP: -- with the issues in this case.

09:54:06 22 We're not going to permit questioning on this
09:54:09 23 line of questioning, so you need to move on. It has
09:54:11 24 nothing to do with his opinions on this case.

25 ///

09:54:13 1 BY MR. SEGAL:

09:54:13 2 Q. Do you have any basis for your understanding of
09:54:15 3 that term "modified" there?

09:54:21 4 MS. SWAROOP: Are you asking about the claims
09:54:22 5 in the patent? Because then I have an objection again
09:54:25 6 as outside the scope, calls for a legal conclusion.

09:54:27 7 MR. SEGAL: You've made your objection.

09:54:29 8 THE WITNESS: I certainly have an opinion based
09:54:31 9 on how that word is used in chemistry.

09:54:36 10 I don't recall how that opinion -- whether that
09:54:40 11 opinion was reinforced explicitly or implicitly in this
09:54:44 12 patent. I would have to go back and look through it.

09:54:47 13 It is certainly not what created that opinion.

09:54:50 14 That opinion on that use of the word "modified" I
09:54:54 15 believe would be common, and how that relates to the
09:54:57 16 patent, I would -- I would have to go back and look.

09:55:04 17 BY MR. SEGAL:

09:55:05 18 Q. Now, how would a person of ordinary skill in
09:55:07 19 the art remove a protecting group attached to the 2'
09:55:12 20 oxygen to expose a 3' hydroxyl group?

09:55:15 21 MS. SWAROOP: Again, object as outside the
09:55:17 22 scope of the declaration.

09:55:18 23 You can answer to the extent you can but,
09:55:20 24 again, we're way outside the scope.

09:55:22 25 THE WITNESS: The 2' and 3' oxygens exchange

09:55:26 1 things quickly. In fact, in a lot of cases you can't
09:55:29 2 make one or the other because they exchange.

09:55:31 3 I would assume it would be possible that if you
09:55:35 4 had something attached at the 2' position -- I'm sorry.
09:55:43 5 Could you repeat the question.

09:55:44 6 Was the question how would removal of the 2'
09:55:47 7 position unmask the 3'?

09:55:50 8 BY MR. SEGAL:

09:55:50 9 Q. Yeah. How would someone with skill in the art,
09:55:53 10 you know, remove a protecting group from the 2' oxygen
09:55:56 11 to expose a 3' hydroxyl?

09:56:00 12 MS. SWAROOP: Again, object as outside the
09:56:02 13 scope.

09:56:03 14 THE WITNESS: How would something -- how would
09:56:05 15 someone remove a protecting group that was attached only
09:56:07 16 to the 2' position?

09:56:10 17 BY MR. SEGAL:

09:56:10 18 Q. Correct.

09:56:11 19 MS. SWAROOP: Counsel, can you point to where
09:56:13 20 in his declaration he addresses this point.

09:56:16 21 THE WITNESS: I would have to think about that.
09:56:17 22 I'm not sure.

09:56:21 23 BY MR. SEGAL:

09:56:21 24 Q. And how about, again, the last possibility of
09:56:23 25 the claim here --

09:56:24 1 A. Can I -- I'm sorry. Can I add something?

09:56:26 2 Q. Sure.

09:56:27 3 A. I'm not trying to obfuscate the point. This is
09:56:31 4 complicated because the 2 and 3' hydroxyls exchange
09:56:33 5 things back and forth. They're right there; they're
09:56:41 6 both nucleophiles --

09:56:43 7 THE REPORTER: Sir, you're going to have to
09:56:43 8 slow down.

09:56:44 9 THE WITNESS: Hydroxyls can exchange some
09:56:46 10 protecting groups, and so -- and they often can -- and
09:56:50 11 the protection of one often involves a covalent bond at
09:56:54 12 the other. That's called an acetal.

09:56:58 13 And so I don't -- I don't really feel
09:57:01 14 comfortable answering the question, because it's
09:57:03 15 complicated and I haven't thought it through.

09:57:06 16 If you'd like me to answer that question, I
09:57:09 17 would like a little time to think about it, and maybe
09:57:13 18 I'm not doing a very good job thinking about it sitting
09:57:16 19 here.

09:57:17 20 BY MR. SEGAL:

09:57:17 21 Q. Okay. Maybe later today we'll revisit that
09:57:20 22 question.

09:57:21 23 MS. SWAROOP: And I'll continue to object as
09:57:22 24 outside the scope.

25 ///

09:57:24 1 BY MR. SEGAL:

09:57:24 2 Q. Now, the last possibility is -- here, how would
09:57:27 3 a person with ordinary skill in the art modify a
09:57:29 4 protecting group that's attached to a 2' oxygen to
09:57:35 5 expose a 3' hydroxyl group?

09:57:37 6 A. That's --

09:57:38 7 MS. SWAROOP: Counsel, again --

09:57:38 8 Let me enter my objections.

09:57:38 9 This is way outside the scope. This is not in
09:57:41 10 Dr. Romesberg's declaration.

09:57:44 11 MR. SEGAL: I think we understand your
09:57:45 12 objection.

09:57:45 13 MS. SWAROOP: Well, then you need to conclude
09:57:47 14 this line of questioning, because this is way outside
09:57:50 15 the scope.

09:57:51 16 MR. SEGAL: I'm almost through this, and then
09:57:52 17 so --

09:57:52 18 BY MR. SEGAL:

09:57:52 19 Q. Can you answer that, please.

09:57:53 20 MS. SWAROOP: You can answer to the extent you
09:57:53 21 can, and if you can't, you don't need to provide an
09:57:57 22 answer.

09:57:57 23 THE WITNESS: That is a -- that is a more
09:57:58 24 complicated version of the former question, which I
09:58:00 25 couldn't answer, so . . .

09:58:06 1 BY MR. SEGAL:

09:58:06 2 Q. Now, does claim 1 require the removal or
09:58:11 3 modification of the protecting group?

09:58:13 4 MS. SWAROOP: Counsel --

09:58:14 5 BY MR. SEGAL:

09:58:14 6 Q. -- just by your understanding of the language?

09:58:16 7 MS. SWAROOP: -- we're not going to permit
09:58:18 8 further questioning with the scope of the claim and
09:58:20 9 asking this witness to give opinions on claim
09:58:23 10 construction.

09:58:24 11 This is not part of his declaration, and it's
09:58:25 12 not part of his opinion, so I'm not going to allow
09:58:28 13 further questioning on this. You need to move on to
09:58:30 14 different topics.

09:58:32 15 Dr. Romesberg, I'm instructing you not to
09:58:34 16 answer these questions.

09:58:36 17 You need to move on.

09:58:38 18 MR. SEGAL: We're going to have to go to the
09:58:39 19 Board, then, because he is giving an opinion, again, to
09:58:41 20 the -- as to the validity of these claims, so I'm
09:58:44 21 entitled to know his understanding.

09:58:46 22 He's also giving an opinion as to secondary
09:58:50 23 considerations, and then certain unexpected results are
09:58:53 24 commensurate with scope of the claim and that they
09:58:55 25 support a finding of non-obviousness. So I don't

09:58:58 1 understand how I can probe his opinions without
09:59:00 2 understanding his understanding and what he believes a
09:59:05 3 person with ordinary skill in the art, how they would
09:59:08 4 interpret such claims.

09:59:09 5 MS. SWAROOP: Well, I disagree. He didn't
09:59:11 6 offer an opinion on claim construction.

09:59:12 7 You can ask him about what the prior art taught
09:59:14 8 and whether -- whether the prior art you're asserting
09:59:17 9 renders the claims obvious, and he can answer those
09:59:20 10 questions.

09:59:20 11 But you're asking him about the scope of the
09:59:22 12 claims, you're asking claim construction, you're asking
09:59:24 13 him to interpret claim terms.

09:59:26 14 None of that is in his declaration, and that's
09:59:28 15 clearly outside the scope, so . . . he's not offering --

09:59:33 16 MR. SEGAL: My understanding of the rules is
09:59:35 17 you have to go to the Board if you want him to not
09:59:38 18 answer a certain question, and we can pull out the rules
09:59:41 19 to that effect, but that's my understanding.

09:59:43 20 Unless you're telling him that it's
09:59:46 21 attorney-client privileged -- that my question is going
09:59:48 22 to impinge on that -- I'm entitled to get answers.

09:59:53 23 If you want to go to the Board, then I'm fine;
09:59:55 24 explain to the Board I'm entitled to understand how he
09:59:59 25 understands the claims that he's giving an opinion on.

10:00:01 1 If you want to go to the Board -- I mean, I
10:00:04 2 only have a few more minutes of questioning. It's up to
10:00:06 3 you.

10:00:06 4 MS. SWAROOP: You're asking him about the
10:00:08 5 requirements of claims. You've already gone through the
10:00:10 6 claims.

10:00:10 7 MR. SEGAL: Right, so let me finish my line of
10:00:12 8 the questioning.

10:00:13 9 MS. SWAROOP: Well, no, now -- you're not
10:00:13 10 asking him the requirements of the claims; you're asking
10:00:14 11 him to opine on the scope of the claims. That's not --
10:00:16 12 that's not --

10:00:16 13 MR. SEGAL: How he understands the claim is
10:00:18 14 what I'm asking him. I have a few more questions in
10:00:20 15 this area.

10:00:21 16 Do you want to go to the Board?

10:00:23 17 MS. SWAROOP: Well, let's -- move on to
10:00:24 18 something else, and we'll see whether we need to revisit
10:00:27 19 it later.

10:00:28 20 MR. SEGAL: No. This is the basis for some of
10:00:30 21 my later questions, so I need to understand how he
10:00:32 22 understands the claims.

10:00:40 23 MS. SWAROOP: May -- why don't we take a break,
10:00:42 24 then, and we can --

10:00:43 25 MR. SEGAL: Can I finish these line of

10:00:45 1 questions first?

10:00:46 2 MS. SWAROOP: I'd prefer to take a break right
10:00:49 3 now, and you can pick up after that.

10:00:51 4 Can we go off the record?

10:00:53 5 THE VIDEO OPERATOR: Okay to go off?

10:00:54 6 MR. SEGAL: Yeah, that's fine.

10:00:56 7 THE VIDEO OPERATOR: We are off the record.

10:00:57 8 The time is 10:00 o'clock.

10:01:00 9 (Recess from 10:01 a.m. to 10:05 a.m.)

10:05:51 10 THE VIDEO OPERATOR: We are back on the record.

10:05:53 11 The time is 10:05.

10:05:59 12 MS. SWAROOP: Counsel, we had a discussion
10:06:00 13 before the break with regard to your line of
10:06:02 14 questioning.

10:06:02 15 As you know, I have an objection as to the
10:06:04 16 extent you're asking this witness about claim
10:06:06 17 construction. We'll permit you to do your limited --
10:06:09 18 the limited questions that you said you had left, and
10:06:12 19 I'll object as needed.

10:06:15 20 BY MR. SEGAL:

10:06:15 21 Q. Dr. Romesberg, now, claim 1, it doesn't require
10:06:21 22 the removal or modification of the protecting group.

10:06:24 23 Do you agree with me there?

10:06:26 24 A. I'm going to read it real quick.

10:06:28 25 Q. Sure.

10:06:40 1 A. It would appear to require the possibility of
10:06:42 2 removing it. My read of it -- and I'm no lawyer -- it
10:06:46 3 would not appear to require removal as part of the
10:06:50 4 claim, but it would appear to require the possibility of
10:06:53 5 removal, I guess is how I would read that.

10:06:56 6 Q. Okay. Now, the final part of the claim reads
10:06:59 7 "and the protecting group comprises an azido group."

10:07:06 8 Do you see that?

10:07:07 9 A. Yes, I do.

10:07:08 10 Q. Does this mean that the protecting group
10:07:12 11 contains an azido group?

10:07:15 12 A. I wish you wouldn't ask me that question,
10:07:16 13 because that word, "comprises," has come up in all three
10:07:20 14 of these IPRs and it appears to be a little bit
10:07:24 15 different from how I would use that word.

10:07:26 16 My understanding is that legally, it means that
10:07:29 17 it includes.

10:07:33 18 Q. So how many possible protecting groups are
10:07:36 19 encompassed by this claim?

10:07:38 20 MS. SWAROOP: Object to the form of the
10:07:39 21 question. Object as outside the scope and also calling
10:07:43 22 for a legal conclusion.

10:07:47 23 You can answer to the extent you can.

10:07:49 24 BY MR. SEGAL:

10:07:49 25 Q. You can answer.

10:07:50 1 A. Would you repeat the -- how many protecting
10:07:53 2 groups does that entail? How many would that include?

10:07:57 3 MS. SWAROOP: Same objections.

10:07:59 4 BY MR. SEGAL:

10:07:59 5 Q. Correct.

10:08:01 6 A. Well, to be realistic, a handful, because
10:08:07 7 you're requiring removal of the azido, triggering -- or
10:08:11 8 modification in some way of the azido group, triggering
10:08:14 9 a deprotection of the 3' hydroxyl or the hydroxyl.

10:08:21 10 So you can imagine a really long chain of
10:08:23 11 carbons with an azido group at the end, and if that were
10:08:26 12 the case, there would be an infinite number of
10:08:29 13 possibilities, but that's clearly not what they're
10:08:30 14 talking about and no one would have thought of that,
10:08:32 15 because that would not allow for removal.

10:08:35 16 So removal means an event involving the azido
10:08:38 17 would have to unmask the hydroxyl. There's really
10:08:40 18 only -- even with the modification approach to doing it
10:08:43 19 where a change at the azido would trigger a reaction
10:08:47 20 that unmasks the hydroxyl, there's only a few carbon
10:08:50 21 atom -- a few bond links that could go through, so it
10:08:53 22 would -- there's really -- given the constraints of it
10:08:56 23 unmasking the hydroxyl, there's a handful.

10:09:00 24 Frankly, the one I would draw is -- is an
10:09:04 25 azidomethyl, but others might think of other things.

10:09:11 1 I would say a few, I guess, given that I'm
10:09:14 2 not -- I mean I guess if you ask me to sit down right
10:09:17 3 now and come up with alternatives, maybe I could come up
10:09:20 4 with a couple. I don't know.

10:09:24 5 Q. Now, you're familiar with the reference --

10:09:28 6 THE REPORTER: I'm sorry?

10:09:29 7 BY MR. SEGAL:

10:09:29 8 Q. Are you familiar with the reference Greene and
10:09:31 9 Wuts?

10:09:33 10 A. Sure. Yes.

10:09:34 11 Q. And your declaration discusses a little bit
10:09:37 12 about Greene and Wuts, correct?

10:09:38 13 A. It does.

10:09:39 14 Q. Now, does Greene and Wuts identify any
10:09:43 15 protecting groups for a hydroxyl group that contain an
10:09:49 16 azido group?

10:09:50 17 MS. SWAROOP: Do you have a copy of the
10:09:51 18 reference for the witness, Counsel?

10:09:53 19 BY MR. SEGAL:

10:09:53 20 Q. Can you answer it without --

10:09:54 21 A. I certainly cannot -- could you repeat the
10:09:57 22 question.

10:09:57 23 Q. Does Greene and Wuts identify any protecting
10:10:00 24 groups for a hydroxyl group that contain an azido group?

10:10:05 25 MS. SWAROOP: Same objection.

10:10:08 1 I would also ask you to provide a copy to the
10:10:11 2 witness.

10:10:15 3 THE WITNESS: I can't speak to how many. It
10:10:18 4 certainly contains a few.

10:10:21 5 For example, an azidomethyl group, I believe,
10:10:24 6 is in Greene and Wuts, because I talk about it a little
10:10:27 7 bit in my declaration, as a protecting group for a
10:10:31 8 phenolic -- for unstable alcohol -- sorry -- for a
10:10:38 9 particularly stable alcohol like a phenol. It does not
10:10:42 10 talk about them for aliphatic alcohols.

10:10:47 11 BY MR. SEGAL:

10:10:47 12 Q. How about -- for aliphatic alcohols, are there
10:10:51 13 any protecting groups listed in Greene and Wuts that
10:10:55 14 contain an azido group?

10:10:57 15 MS. SWAROOP: Object to the form of the
10:10:58 16 question.

10:10:58 17 Again, I would ask that you provide a copy of
10:11:01 18 Greene and Wuts to the witness if you're going to be
10:11:03 19 asking him questions about it.

10:11:05 20 THE WITNESS: I'm not sure. I -- off the top
10:11:08 21 of my head -- and this is evoking my knowledge, more
10:11:11 22 than my knowledge of Greene and Wuts -- I would think --
10:11:17 23 I don't think so, but I'm not sure.

10:11:26 24 BY MR. SEGAL:

10:11:26 25 Q. So, again, in the context of the claim -- we

10:11:29 1 talked about this a little bit earlier -- the azido
10:11:32 2 group -- azido protecting group can be on the -- can be
10:11:36 3 attached to the 2' oxygen; is that right?

10:11:41 4 A. By my read of --

10:11:42 5 MS. SWAROOP: Let me object outside the scope
10:11:45 6 of the declaration. Again, object to the extent you're
10:11:48 7 calling -- asking for a legal conclusion.

10:11:50 8 You can answer.

10:11:52 9 THE WITNESS: By my reading, it would -- as I
10:11:54 10 said earlier, it would appear one of the possibilities
10:11:57 11 would be that it would be a modification of the 2'
10:12:01 12 hydroxyl group.

10:12:02 13 BY MR. SEGAL:

10:12:03 14 Q. Take a look at claim 2. The claim reads, "The
10:12:09 15 method of claim 1, wherein said incorporating is
10:12:12 16 accomplished via a terminal transferase, a polymerase or
10:12:15 17 a reverse transcriptase."

10:12:17 18 Did I read that correctly?

10:12:19 19 A. Yes.

10:12:21 20 Q. What is a terminal transferase?

10:12:25 21 A. It's an enzyme that takes single-stranded DNA
10:12:30 22 and adds nucleotides to it. So all three of what they
10:12:34 23 said, they gave -- this aim explicitly includes the
10:12:39 24 three enzymes that synthesize DNA.

10:12:41 25 They use different mechanisms. They have

10:12:44 1 different substrates. What they all have in common is
10:12:48 2 they -- they'll synthesize oligonucleotides.

10:12:53 3 Q. So the substrate for a terminal transferase is
10:12:56 4 single-stranded DNA; is that correct?

10:13:03 5 A. I'm not pos- -- I mean certainly a substrate is
10:13:07 6 single-stranded DNA. I'm not sure that it's the only
10:13:10 7 substrate. I think it's entirely possible that it would
10:13:13 8 actually act on a primer template. I'm not sure.

10:13:19 9 It's the -- its physiological function is to
10:13:26 10 work on primer templates where one strand is longer than
10:13:33 11 the other and so that it adds nucleotides to that longer
10:13:37 12 strand.

10:13:39 13 Whether it's more efficient -- whether it's
10:13:43 14 slightly less efficient with a fully single-stranded,
10:13:48 15 I'm not sure. So a lot of enzymes have -- don't have
10:13:54 16 one activity that -- they're not so specific, so I would
10:14:00 17 not feel comfortable saying that it's the only
10:14:02 18 substrate.

10:14:05 19 Q. Can terminal transferase be used for
10:14:07 20 sequencing?

10:14:08 21 MS. SWAROOP: Object as outside the scope.

10:14:13 22 THE WITNESS: Not in the context of adding
10:14:15 23 nucleotides to a single strand. But as I just
10:14:18 24 mentioned, it -- I'm not sure how efficient it is in
10:14:22 25 template-directed synthesis, so it certainly would not

10:14:26 1 act for sequencing if it had a single strand if it were
10:14:29 2 not template-directed. Then obviously it would not.
10:14:33 3 That is an activity of terminal transferase.

10:14:38 4 I just don't -- I would -- I would have to see
10:14:42 5 if someone's run that experiment.

10:14:44 6 BY MR. SEGAL:

10:14:44 7 Q. So you don't know --

10:14:46 8 A. I don't know.

10:14:47 9 Q. -- essentially?

10:14:50 10 A. That is correct.

10:14:51 11 Q. What is reverse transcriptase?

10:14:55 12 A. Reverse -- this is another great example of an
10:14:58 13 enzyme that does more than one thing.

10:15:00 14 Reverse -- the physiological function, you
10:15:02 15 know, to the extent that we understand biology, clearly
10:15:06 16 one function of reverse transcriptase is due -- is to
10:15:12 17 take RNA and synthesize, in a template-directed manner,
10:15:16 18 DNA from it. So it's the opposite of an RNA polymerase,
10:15:22 19 and that process is called transcription and that's why
10:15:28 20 the enzyme is called reverse transcriptase, because it
10:15:32 21 does the opposite of transcription.

10:15:35 22 So it typically takes an RNA template and
10:15:39 23 synthesizes DNA from it. In this case, I believe that
10:15:41 24 it also will take a DNA template and synthesize DNA with
10:15:45 25 it.

10:16:07 1 MR. SEGAL: I'm handing you what's been
10:16:09 2 previously marked Exhibit 1002. It's a U.S. patent
10:16:13 3 No. 6,664,079, and the first named inventor is Jingyue
10:16:24 4 Ju.

10:16:26 5 (Exhibit 1002 was referred to.)

10:16:32 6 MS. SWAROOP: Do you have a copy for me?

10:16:34 7 MR. SEGAL: I'm sorry.

10:16:48 8 BY MR. SEGAL:

10:16:49 9 Q. Have you reviewed this reference?

10:16:51 10 A. Yes, I have. This would appear to be what I'll
10:16:54 11 refer to as the Ju reference.

10:17:02 12 Q. I'd like to compare the Ju reference to the
10:17:05 13 elements in claim 1. I believe you sort of went through
10:17:08 14 this process during your first deposition.

10:17:10 15 Do you recall that?

10:17:13 16 A. No.

10:17:17 17 Q. If you'll turn back again to the '537 patent,
10:17:28 18 after that first "comprising" there, it says
10:17:30 19 "Incorporating into the nucleic acid molecule a
10:17:35 20 nucleotide or nucleoside molecule."

10:17:40 21 A. I'm sorry. Can you tell me exactly where
10:17:41 22 you're at? What line are you at?

10:17:44 23 Q. Line 2, right after the "comprising."

10:17:46 24 A. Yes.

10:17:46 25 Q. "Incorporating into the nucleic acid molecule a

10:17:51 1 nucleotide or nucleoside molecule," do you see that?

10:17:54 2 A. I do.

10:17:55 3 Q. Okay. Now, would you agree with me that Ju
10:17:58 4 teaches incorporating nucleotides into nucleic acid
10:18:03 5 molecules as part of his SBS method?

10:18:07 6 A. Yes.

10:18:07 7 Q. Okay. The next part of the claim reads
10:18:09 8 "wherein the nucleotide or nucleoside molecule has a
10:18:13 9 base that is linked to a detectable label via a
10:18:17 10 cleavable and the nucleotide or nucleoside molecule has
10:18:21 11 a ribose or deoxyribose sugar moiety."

10:18:26 12 Do you see that?

10:18:27 13 A. I do.

10:18:28 14 Q. Does Ju's method disclose or teach using such a
10:18:33 15 nucleotide molecule?

10:18:35 16 A. I believe so.

10:18:38 17 Q. Next part of the claim says "wherein the ribose
10:18:45 18 or deoxyribose sugar moiety comprises a protecting group
10:18:50 19 attached via the 2' or 3' oxygen."

10:18:53 20 You would agree with me that Ju discloses using
10:18:55 21 a protecting group attached to the 3' oxygen with its
10:18:57 22 nucleotides for his SBS method?

10:19:00 23 A. I believe so.

10:19:01 24 Q. Next part of the claim says "said protecting
10:19:04 25 group can be modified or removed to expose a 3' hydroxyl

10:19:08 1 group."

10:19:09 2 Would you agree with me that Ju discloses
10:19:12 3 removing the 3' protecting group to expose a 3' hydroxyl
10:19:18 4 group?

10:19:18 5 A. Yeah, Ju, at length, talks about the
10:19:20 6 requirements for that stuff being efficient.

10:19:24 7 Q. So if you look at that claim as a whole, would
10:19:27 8 you agree that the only element in claim 1 that is not
10:19:30 9 specifically taught by Ju is that the protecting group
10:19:33 10 comprises an azido group?

10:19:35 11 MS. SWAROOP: Let me state my objections.

10:19:37 12 I would object as outside the scope. I would
10:19:39 13 also object to the form of the question and object to
10:19:41 14 the extent you're calling -- asking for a legal
10:19:43 15 conclusion.

10:19:46 16 THE WITNESS: I'm not sure if I can do this or
10:19:48 17 not, but you're asking a specific question about
10:19:51 18 claim 1.

10:19:52 19 BY MR. SEGAL:

10:19:52 20 Q. Correct.

10:19:53 21 A. The -- the relationship between the two patents
10:19:57 22 is -- part of the importance -- part of that comparison
10:20:00 23 is in claim 8, and so you don't want me to evoke that?
10:20:05 24 You don't want me to describe how I read that and how
10:20:08 25 that informed my opinion about what each of the

10:20:10 1 claims -- about what each patent claimed?

10:20:14 2 Q. Right now I'm asking you specifically about
10:20:16 3 claim 1.

10:20:18 4 A. So ignoring claim 8?

10:20:20 5 Q. Just about claim 1 --

10:20:22 6 A. Okay. So --

10:20:21 7 Q. -- and you understand what I mean by the
10:20:23 8 elements and limitations of a claim?

10:20:26 9 A. No, I don't.

10:20:27 10 Q. So it's essentially --

10:20:29 11 A. I mean I know what the English -- I apologize
10:20:31 12 for interrupting.

10:20:33 13 I know what the English words that you're
10:20:34 14 saying mean to me.

10:20:35 15 Q. Okay. What do they mean to you?

10:20:37 16 A. What is the claim saying specifically and what
10:20:41 17 is it not saying.

10:20:42 18 Q. Okay. I mean, using that definition, the
10:20:48 19 only -- would you agree that the only element in claim 1
10:20:51 20 that is specifically not taught by the Ju reference is
10:20:55 21 that the protecting group comprises an azido group?

10:20:59 22 MS. SWAROOP: Again, let me object as outside
10:21:01 23 the scope, object to the form of the question, object to
10:21:03 24 the extent you're asking for a legal conclusion from
10:21:06 25 this witness.

10:21:09 1 THE WITNESS: Ignoring claim 8, then I think
10:21:17 2 relative to claim 1 -- because the efficiency is not
10:21:21 3 implied in claim 1; that's implied in claim 8 -- it's
10:21:27 4 removal -- you know, could I -- I believe that's
10:21:31 5 correct.

10:21:33 6 I haven't sat down and compared the claims side
10:21:36 7 by side, but my recollection, I believe -- I believe
10:21:39 8 that's correct, comparing claim 1 to the Ju patent.

10:21:43 9 BY MR. SEGAL:

10:21:43 10 Q. If you look at claim 2, again, it's "The method
10:21:46 11 of claim 1, wherein said incorporating is accomplished
10:21:49 12 via a terminal transferase, a polymerase or a reverse
10:21:53 13 transcriptase."

10:21:54 14 Did I read that right?

10:21:56 15 A. Yes.

10:21:57 16 Q. Ju discloses using, for example, a polymerase
10:22:00 17 in his SBS methods?

10:22:02 18 A. Yes.

10:22:06 19 Q. If you look at claim 3, it says "The method of
10:22:09 20 claim 1, wherein the base is a deazapurine."

10:22:12 21 Did I read that right?

10:22:14 22 A. Yes.

10:22:15 23 Q. Ju teaches using nucleotides that have a
10:22:20 24 deazapurine?

10:22:22 25 A. I believe so.

10:22:25 1 Q. Well, just to confirm that, if you look at
10:22:28 2 the -- the Ju reference, which is Exhibit 1002 -- if you
10:22:33 3 look at column 7, line 53 --
10:22:37 4 A. Column 7.
10:22:47 5 Q. After the figures.
10:22:59 6 A. Yes.
10:23:00 7 Q. So you would agree that Ju teaches using
10:23:05 8 deazapurines?
10:23:06 9 A. Yes.
10:23:09 10 Q. Now, if you look at claim 4 of the '537 patent,
10:23:15 11 it reads "The method of claim 1, wherein the nucleotide
10:23:19 12 is a deoxyribonucleotide triphosphate."
10:23:24 13 Did I read that right?
10:23:25 14 A. Yes.
10:23:26 15 Q. Again, Ju teaches the use of
10:23:28 16 deoxyribonucleotide triphosphates?
10:23:31 17 A. Yes.
10:23:32 18 Q. Take a look at claim 5.
10:23:35 19 It says "The method of claim 1 wherein the
10:23:38 20 label is a fluorophore."
10:23:41 21 Ju teaches using fluorophores attached to its
10:23:46 22 nucleotides for use in his SBS methods?
10:23:50 23 A. Yes.
10:23:51 24 Q. And if you look at claim 8, it says "The method
10:23:56 25 according to claim 1, further comprising detecting the

10:24:00 1 detectable label and cleaving the cleavable linker."

10:24:03 2 Does Ju teach detecting the detectable label on
10:24:10 3 its nucleotides and cleaving the cleavable linker?

10:24:13 4 A. Yes.

10:24:21 5 Q. Turn to column 10 of the -- of the -- in the Ju
10:24:28 6 patent, which is Exhibit 1002. If you look at around
10:24:43 7 line 4 -- I'm going to read this into the record.

10:24:48 8 "Any chemical group could be used as long as
10:24:52 9 the group 1) is stable during the polymerase reaction,
10:24:55 10 2) does not interfere with the recognition of the
10:24:58 11 nucleotide analogue by polymerase as a substrate, and 3)
10:25:02 12 is cleavable."

10:25:04 13 Did I read that right?

10:25:06 14 A. Well, I would have read "is cleavable."

10:25:08 15 Q. "Is cleavable," right. I didn't have the right
10:25:11 16 emphasis.

10:25:12 17 A. No, you read it correctly.

10:25:15 18 Q. Okay. So in that -- in that statement there,
10:25:18 19 Ju's describing the requirements for his 3' hydroxyl
10:25:25 20 protecting group?

10:25:26 21 MS. SWAROOP: Object to the form of the
10:25:27 22 question.

10:25:29 23 THE WITNESS: I would not characterize this as
10:25:31 24 the place that he describes all of the requirements.

10:25:33 25 Later he talks about -- where he refers to

10:25:36 1 sections as the fundamental requirements of the method.
10:25:40 2 I would turn to that for what the actual requirements
10:25:42 3 are.

10:25:43 4 None of them are inconsistent with this. This
10:25:45 5 is sort of a -- this is a good part of them and so it
10:25:48 6 is -- they are -- these are some of the requirements for
10:25:51 7 the method.

10:25:53 8 BY MR. SEGAL:

10:25:53 9 Q. Well --

10:25:54 10 A. Each of them are required.

10:25:55 11 Q. Well, specifically for the 3' hydroxyl
10:25:59 12 protecting group, are these Ju's requirements for the 3'
10:26:04 13 hydroxyl protecting group?

10:26:05 14 MS. SWAROOP: Object to the form of the
10:26:06 15 question. It's been asked and answered.

10:26:08 16 THE WITNESS: It's missing a requirement that's
10:26:11 17 articulated where he talks about the fundamental
10:26:13 18 requirements.

10:26:14 19 BY MR. SEGAL:

10:26:16 20 Q. Let me ask you some questions about the
10:26:17 21 azidomethyl group.

10:26:20 22 So as of August 2002, a person of ordinary
10:26:24 23 skill would know that an azidomethyl group is a chemical
10:26:28 24 group.

10:26:29 25 You would agree with me there, right?

10:26:31 1 A. Yes.

10:26:32 2 Q. Now, as of August 2002, a person of ordinary
10:26:35 3 skill in the art would have thought that azidomethyl
10:26:39 4 protecting group would be stable during the polymerase
10:26:43 5 reaction?

10:26:44 6 MS. SWAROOP: Object to the form of the
10:26:45 7 question. Object as outside the scope.

10:26:47 8 THE WITNESS: No, I don't know that people
10:26:48 9 would have thought that.

10:26:50 10 BY MR. SEGAL:

10:26:50 11 Q. Why?

10:26:52 12 A. Well, in Ju's patent, he talks about the
10:26:56 13 importance of the reversible terminator not being an
10:27:00 14 electrophile, and there is literature that suggests that
10:27:06 15 in some cases an azide could act as an electrophile, and
10:27:10 16 so that would have raised one's concern, because in a
10:27:13 17 paper by Canard, he describes there being a powerful
10:27:16 18 nucleophile in the enzyme -- in the polymerase active
10:27:19 19 site; and he, in fact, expressly calls out azides as
10:27:23 20 being potential electrophiles raising a problem.

10:27:27 21 So combining Ju and Canard, Ju states that you
10:27:30 22 can't have an electrophile; Canard states that an azide
10:27:34 23 is an electrophile. That would have probably raised --
10:27:37 24 that would have raised concerns for using the azide.
10:27:39 25 One of the requirements is that it's stable during

10:27:43 1 incorporation, and obviously for it to act as a
10:27:47 2 reversible terminator, if it -- if it did act as an
10:27:51 3 electrophile the way Canard describes, it would produce
10:27:54 4 an irreversible terminator.

10:27:58 5 Q. I want to ask you some questions about that
10:28:00 6 later.

10:28:01 7 A. Okay.

10:28:01 8 Q. But do you have any other basis to believe that
10:28:06 9 the azidomethyl group -- where someone with ordinary
10:28:11 10 skill in the art would not believe that the azidomethyl
10:28:14 11 protecting group would be stable during the polymerase
10:28:16 12 reaction?

10:28:22 13 A. Specifically are you asking would -- is there
10:28:25 14 any reason that people would have suspected that the
10:28:27 15 azidomethyl moiety would not have been stable, having
10:28:31 16 nothing to do with incorporability, having nothing to do
10:28:34 17 with reversibility; you're asking specifically for
10:28:38 18 stability?

10:28:39 19 Q. For stability. And I understand you gave your
10:28:41 20 answer with respect to a person of ordinary skill
10:28:45 21 suspecting that it would be an electrophile. I
10:28:47 22 understand that point.

10:28:48 23 I just want to know if there's any other basis
10:28:50 24 that I need to explore today per your answer.

10:28:53 25 A. Well, I guess -- and this is related -- Canard

10:28:56 1 actually supports his assertion that there are
10:28:59 2 electrophiles that might be subject to attack in the
10:29:02 3 polymerase active site by noting that -- that AZT, which
10:29:06 4 has an azido group on it, can be recovered as the amine,
10:29:10 5 and -- after being provided to people, injected to
10:29:15 6 people, administered as a drug, and that would be
10:29:17 7 consistent with it acting as an electrophile.

10:29:20 8 Q. Okay. Other than --

10:29:22 9 A. I'll --

10:29:23 10 Q. Other than the electrophile basis, is there
10:29:25 11 anything else --

10:29:27 12 A. Well, I'll say that that's consistent with it
10:29:29 13 becoming an irreversible terminator, and it being an
10:29:32 14 electrophile is one way that could have been. Someone
10:29:34 15 else could have said, Well, I don't believe it was
10:29:36 16 because it was an electrophile; I think it was reduced
10:29:39 17 by one of the many enzymes that reduce azides.

10:29:42 18 And so this simply might have raised to the
10:29:45 19 issue that they were reactive and that they could be
10:29:48 20 reduced to amines.

10:29:50 21 Other than that, I -- I don't see why anyone
10:29:54 22 would have -- I don't see a particular reason people
10:29:59 23 would have thought that the azides would be not stable
10:30:02 24 during polymerase incorporation, other than the two
10:30:05 25 cases that I mentioned.

10:30:14 1 Q. How about as of August 2002, would a person of
10:30:21 2 ordinary skill in the art have thought that a nucleotide
10:30:26 3 with an azidomethyl protecting group would have been
10:30:28 4 incorporated by a polymerase?

10:30:30 5 And I'm just asking about incorporation. We
10:30:33 6 talked about stability a second ago, but incorporated.

10:30:36 7 A. Probably not, is my impression. I mean it's
10:30:39 8 always hard to guess, but it's my opinion that someone
10:30:42 9 skilled in the art -- I mean, look, lots of protecting
10:30:44 10 groups, if -- if that's specifically what we're talking
10:30:48 11 about -- lots had been discussed, and I don't think the
10:30:51 12 azide had been discussed.

10:30:52 13 So I think that the vast majority of the
10:30:56 14 literature was suggesting things like esters, aryl
10:31:02 15 esters or alkyl esters or MOM ethers, that kind of
10:31:08 16 thing; and so that the paucity, the absence of
10:31:11 17 discussion, would lead me to think that it was not in
10:31:14 18 the minds of people talking about it. It was not
10:31:18 19 considered -- I mean I can tell you my opinion is
10:31:20 20 probably -- as to why I think it probably wasn't
10:31:24 21 considered.

10:31:25 22 The azide group, if you wish to know;
10:31:26 23 otherwise -- the answer to the question is I do not
10:31:30 24 think it was -- I don't think it was commonly thought
10:31:32 25 likely to be a polymerase substrate. That's based on

10:31:35 1 the fact that it's never discussed.

10:31:36 2 And I can give you my opinion as to why I think
10:31:39 3 that might have been, but that's just my opinion.

10:31:42 4 Q. Let me ask you, you mentioned AZT earlier.

10:31:44 5 And AZT is incorporated by polymerase, correct?

10:31:48 6 A. AZT is incorporated by a very error-prone
10:31:52 7 polymerase, so a sloppy polymerase, and I think that was
10:31:55 8 a big part of why people were interested in it, because
10:31:58 9 it did not inhibit the high fidelity enzymes that are
10:32:02 10 required for, for example, replication of a genome or
10:32:05 11 sequencing by synthesis in a case where you need high
10:32:09 12 fidelity DNA replication.

10:32:11 13 The very reason, I think, at least a lot of the
10:32:13 14 early ideas as to why AZT was, in fact, a good drug was
10:32:19 15 because it intentionally avoided any polymerase worth a
10:32:23 16 darn and went after only those polymerases that are so
10:32:29 17 sloppy that they don't discriminate. That's the --
10:32:33 18 that's the mechanism of its action.

10:32:36 19 Q. Now, back in 2002, would one with skill in the
10:32:39 20 art been able to modify or engineer polymerases to
10:32:45 21 improve their ability to incorporate nucleotides --

10:32:51 22 A. In 2002?

10:32:51 23 MS. SWAROOP: Let me --

10:32:51 24 BY MR. SEGAL:

10:32:51 25 Q. -- with 3' oxygen protecting groups?

10:32:53 1 MS. SWAROOP: -- object to the form of the
10:32:55 2 question. Object as outside the scope.

10:33:00 3 THE WITNESS: I -- certainly in 2002, people,
10:33:02 4 including myself, were interested in that kind of
10:33:06 5 question.

10:33:08 6 I worked very -- my lab worked very hard, so my
10:33:14 7 opinion is that no one else in the world but me could
10:33:17 8 have done it.

10:33:17 9 That's kind of a joke. Many scientists like to
10:33:20 10 think that way.

10:33:21 11 But that's something that we worked very hard
10:33:23 12 on establishing a system to try to do, not specifically
10:33:28 13 for the case that you mentioned, but in a more broad
10:33:31 14 sense, of all polymerases to be able to take modified
10:33:35 15 substrates, including that type of modification.

10:33:39 16 We spent years getting a system to work, and so
10:33:47 17 what -- I would say that it was definitely state of the
10:33:50 18 art and that there were probably -- we, by far,
10:33:53 19 developed the most sophisticated system to do it. We've
10:33:58 20 developed the most robust system to do it in academics.
10:34:03 21 I can't speak to industry. I don't know what's not
10:34:05 22 published.

10:34:06 23 To this day, it's the most state-of-the-art way
10:34:09 24 to do it. So in 2002 are you asking whether it was
10:34:13 25 common to think that you could do that? Is that what

10:34:15 1 the question is?

10:34:16 2 BY MR. SEGAL:

10:34:16 3 Q. Whether one of ordinary skill in the art would
10:34:19 4 believe that they would be able to do that.

10:34:21 5 A. Judging from the response of my reviews on
10:34:25 6 grants that I submitted, that it was not common, then
10:34:26 7 the answer would be no; people did not believe that you
10:34:29 8 could.

10:34:29 9 Q. They would believe that as of 2002, they
10:34:32 10 wouldn't be able to either choose one of the number of
10:34:35 11 polymerases that were available or modify such
10:34:39 12 polymerases to be able to incorporate an azidomethyl
10:34:45 13 group that was attached to the 3' oxygen?

10:34:48 14 MS. SWAROOP: Object to the form of the
10:34:48 15 question. Object as outside the scope.

10:34:51 16 THE WITNESS: I think it's very likely that
10:34:53 17 people would have been -- people would have not
10:34:54 18 considered that a likely possibility.

10:35:02 19 The whole field of protein evolution, evolving
10:35:08 20 proteins to do things, is an extraordinarily difficult
10:35:12 21 task today. Much progress has been made in the last 10
10:35:15 22 years.

10:35:16 23 So in 2002, were people starting to make
10:35:17 24 progress? A handful of groups, including mine, sure.
10:35:21 25 No one thought it was easy. To this day, it's not easy.

10:35:25 1 There's simply a little bit more success today.

10:35:28 2 2002, I included this in grants that I
10:35:33 3 submitted to the NIH, and they always said, Wow, that
10:35:36 4 would be amazing if you could do that, but good luck.

10:35:41 5 So it was certainly not -- someone would not
10:35:43 6 have looked at that and said, Huh, we could simply
10:35:45 7 evolve a polymerase to do it.

10:35:50 8 BY MR. SEGAL:

10:35:51 9 Q. Are you aware if some researchers were able
10:35:53 10 to -- to get 3' oxygen protecting group nucleotide
10:35:57 11 analogues with 3' oxygen protecting groups, they were
10:36:00 12 able to get incorporation and termination of a -- of a
10:36:06 13 template growing strand using the -- using a particular
10:36:12 14 polymerase?

10:36:13 15 MS. SWAROOP: Object to the form of the
10:36:14 16 question. Object as outside the scope.

10:36:17 17 THE WITNESS: I'm aware that people were
10:36:18 18 looking for that. I'm aware that a lot of groups were
10:36:22 19 tried. I think the O-Allyl was amongst -- was maybe
10:36:28 20 uniquely successful, an O-Allyl protecting group, and
10:36:32 21 even that was limited, meaning a very broad panel of
10:36:35 22 polymerases were looked at and it was the only one that
10:36:39 23 showed any promise, in my opinion; and that, again, just
10:36:42 24 testifies to how difficult I think the problem was.

10:36:45 25 Look, the 3' hydroxyl group mechanistically

10:36:50 1 needs to bind the magnesium ion on the active site. It
10:36:55 2 needs to be activated for attack on the next incoming
10:36:57 3 triphosphate. And part -- sorry. Part of its binding
10:36:59 4 mechanism involves chelating, binding to a metal ion in
10:37:03 5 the active site, if you're going to go putting groups on
10:37:07 6 here.

10:37:07 7 Frankly, my intuition is I'm amazed any of them
10:37:11 8 work, because it's such a mechanistically central part
10:37:14 9 of the enzyme.

10:37:16 10 BY MR. SEGAL:

10:37:16 11 Q. But you would agree that there were some
10:37:17 12 researchers that had some success prior to August 2002?

10:37:21 13 A. Sure. But the question I'm answering is
10:37:23 14 whether it was considered straightforward or whether it
10:37:26 15 was considered -- you know, whether it was considered
10:37:28 16 sort of -- it was certainly not considered easy, and the
10:37:34 17 very few examples -- maybe only the O-Allyl,
10:37:38 18 partially -- and the many, many examples of failure I
10:37:42 19 think support -- that's what -- that's what I'm saying,
10:37:46 20 is that amongst the natural polymerases, these -- this
10:37:49 21 is simply not something that's common.

10:37:54 22 Q. With respect to that last requirement that Ju
10:37:58 23 lists in that -- in column 10 that we just read earlier,
10:38:02 24 as of 2002, a person of ordinary skill would have
10:38:07 25 thought that the azidomethyl could be cleaved from a 3'

10:38:12 1 oxygen.

10:38:13 2 Would you agree with me?

10:38:14 3 MS. SWAROOP: Objection to the form of the
10:38:15 4 question. Object as outside the scope.

10:38:18 5 THE WITNESS: No, I would not agree with you,
10:38:19 6 and that's simply -- it depends on how you read
10:38:22 7 "cleavable." If you read "cleavable" in the absence of
10:38:26 8 anything else in this document, then I might agree with
10:38:29 9 you.

10:38:30 10 BY MR. SEGAL:

10:38:30 11 Q. Let's just take that first question.

10:38:32 12 In the absence of anything else in the
10:38:35 13 document, is it -- is an azidomethyl group attached to a
10:38:41 14 3' oxygen, is it cleavable and would -- and my question
10:38:44 15 actually is, would a person of ordinary skill expect
10:38:48 16 that -- it to be cleavable in 2002?

10:38:51 17 MS. SWAROOP: Object to the form of the
10:38:52 18 question; incomplete question. And object as outside
10:38:57 19 the scope.

10:38:58 20 THE WITNESS: You're sort of asking two
10:38:59 21 questions.

10:39:00 22 One is, is it cleavable in the absence of any
10:39:02 23 other consideration? You don't need to have much skill
10:39:04 24 in any art to know that you could cleave that. You
10:39:07 25 could heat it to a million degrees and you'll get a

10:39:10 1 bunch of atoms and that's cleaved.

10:39:13 2 By saying "someone skilled in the art," I
10:39:16 3 assume you -- as I define it here, it's a
10:39:17 4 sequencing-by-synthesis person, so I'm going to assume
10:39:20 5 that person is constrained by the requirements for
10:39:24 6 sequencing by synthesis.

10:39:25 7 So can I answer your question as to whether or
10:39:27 8 not it's cleavable in that context?

10:39:29 9 BY MR. SEGAL:

10:39:29 10 Q. Sure.

10:39:30 11 A. Then, no, I do not believe that azidomethyl was
10:39:34 12 known to be cleavable.

10:39:36 13 Q. How about just cleavable from a nucleotide,
10:39:39 14 without destroying the other covalent bonds from a
10:39:47 15 nucleotide? Was that known from 2002?

10:39:49 16 MS. SWAROOP: Same objection.

10:39:50 17 THE WITNESS: I think it was known that that
10:39:51 18 was a problem; that, no, 2002 not cleavable.

10:40:07 19 BY MR. SEGAL:

10:40:07 20 Q. If you look at the -- turning back to the Ju
10:40:09 21 reference, you would agree that Ju said that for a 3'
10:40:15 22 hydroxyl capping group, small chemical moieties are
10:40:20 23 preferred?

10:40:20 24 A. I'd have to find where he said that but, yes, I
10:40:23 25 do remember him talking about that.

10:40:25 1 Q. You would agree with me that an azidomethyl is
10:40:29 2 a small chemical moiety?

10:40:31 3 A. "Small" is a relative term. Is it small
10:40:35 4 relative to an O-Methyl? No.

10:40:38 5 Is it small relative to a naphthyl group? Yes.
10:40:42 6 So I guess --

10:40:44 7 Q. How about this: Is it similar in size to an
10:40:47 8 allyl in a MOM group?

10:40:53 9 A. Again, I'm not trying to be difficult. That's
10:40:56 10 a difficult question, because size -- the question of
10:40:58 11 size, shape matters.

10:41:00 12 In a three-dimensional thing -- object, you
10:41:04 13 might have a lot of freedom for size in one dimension,
10:41:08 14 and -- it might be a long narrow tube and so you might
10:41:11 15 have very little freedom in any other dimension; or, you
10:41:16 16 may have a -- a little pocket, in which case you can
10:41:20 17 have something like a phenyl group, that's sort of round
10:41:25 18 like that, that maybe sort of is within, but you can't
10:41:29 19 take something like an azide that's long.

10:41:32 20 So, again, I apologize. I don't mean to be
10:41:33 21 difficult, but it's not a simple question. You know,
10:41:36 22 when I read this, I read people using the word "small"
10:41:39 23 or "large" or "efficient," these are very qualitative
10:41:43 24 terms that you really need to describe the system that
10:41:45 25 you're talking about to appreciate.

10:41:48 1 I would think that with an azide -- an azide is
10:41:53 2 a very long molecule. It's a long, narrow molecule.
10:41:56 3 It's three atoms linear. That means you've got to
10:42:00 4 have -- oh, about, I'm guessing, five angstroms of
10:42:04 5 linear space; half a nanometer.

10:42:08 6 For an enzyme, that's a lot of space, and you
10:42:11 7 have to have the space to accommodate that and it can't
10:42:15 8 wrap around. So it's like a MOM group. A MOM group is
10:42:16 9 only single bond, so it can contort and twist to fit
10:42:20 10 into a site. An azide doesn't have that ability.

10:42:24 11 So is it small? You know, is -- it's smaller
10:42:27 12 than a naphthyl group. Is it as small as a MOM or an
10:42:34 13 O-Allyl? It depends on what you're trying to fit it
10:42:38 14 into.

10:42:38 15 In some cases in an absolute sense, in like a
10:42:41 16 solvent environment where you have a solvent that's able
10:42:44 17 to adapt to whatever it wants, yeah, certainly in that
10:42:49 18 particular case, an azide might be similar to a MOM or
10:42:53 19 an allyl.

10:42:56 20 In an -- an anisotropic -- that's the word that
10:43:00 21 scientists love to use to describe this exact
10:43:02 22 phenomenon. Isotropic means the same in all dimensions.
10:43:07 23 Anisotropic means not the same in all dimensions, so
10:43:11 24 once you're in an anisotropic environment, I would call
10:43:16 25 that unpredictable. I would not say that they're the

10:43:19 1 same.

10:43:20 2 Q. How about with respect to -- just to, like
10:43:22 3 atomic size? Are they similar in that respect?

10:43:26 4 A. In terms of volume? In --

10:43:29 5 Q. Like molecular weight.

10:43:30 6 A. Oh, in terms of molecular weight. Sure,
10:43:36 7 percentagewise.

10:43:40 8 But, again, that's like saying that if you take
10:43:42 9 a linear arrangement of atoms that's very long and
10:43:48 10 compared to a case where you wrap them around themselves
10:43:50 11 that's like a little baseball, I would say those are
10:43:53 12 similar molecular weight.

10:43:55 13 Those might be very -- those might be identical
10:43:57 14 molecular weight but have very different shapes, and so
10:43:59 15 the only thing I'm trying to emphasize is that if you're
10:44:01 16 talking -- because I believe what we're talking about
10:44:03 17 here is expectation of incorporability into the
10:44:07 18 polymerase active site -- volume is not a -- it's
10:44:14 19 certainly not the only requirement.

10:44:17 20 Molecular weight is not a particularly
10:44:22 21 correlated phenomenon with these sorts of things, and
10:44:25 22 what is correlated is physical chemical properties;
10:44:28 23 things like specific dimensions, H-bonding, ionic
10:44:33 24 charge, polarity. Things like that are much more
10:44:38 25 important, and people who think about substrates think

10:44:41 1 about that much more.

10:44:43 2 No one talks about, Oh, this -- might this be a
10:44:46 3 substrate; what's its molecular weight?

10:44:52 4 Q. Now, we've just been talking about the allyl
10:44:55 5 and MOM groups.

10:44:59 6 Ju specifically suggests the allyl and MOM
10:45:02 7 group as possible protecting groups for the 3' hydroxyl.

10:45:08 8 A. He does.

10:45:09 9 Q. Is that right?

10:45:10 10 A. Yes.

10:45:10 11 Q. Now, when the allyl and MOM groups are attached
10:45:13 12 to the 3' oxygen, that's then an ether; am I correct
10:45:17 13 there?

10:45:18 14 A. In both cases, yes.

10:45:19 15 Q. Okay. And when you attach an azidomethyl group
10:45:25 16 to the 3' oxygen, is that also an ether?

10:45:29 17 A. Yes. Pretty much anything that you attach --
10:45:36 18 if you're attaching a carbon to an oxygen -- and I can't
10:45:40 19 imagine you'd be attaching everything else -- everything
10:45:43 20 will be an ether, because that's the definition of an
10:45:46 21 ether.

10:45:48 22 MR. SEGAL: Can we take a break?

10:45:50 23 THE VIDEO OPERATOR: We are going off the
10:45:51 24 record. This is the end of Disk No. 1. The time is
10:45:54 25 10:45.

10:45:56 1 (Recess from 10:45 a.m. to 11:06 a.m.)

11:06:25 2 THE VIDEO OPERATOR: We are going back on the
11:06:27 3 record. This is the beginning of Disk No. 2. The time
11:06:29 4 is 11:06.

11:06:31 5 BY MR. SEGAL:

11:06:32 6 Q. Dr. Romesberg, if you could take out your
11:06:35 7 declaration again -- it's Exhibit 2011. Please turn to
11:06:41 8 paragraph 29.

11:06:56 9 Now, in this paragraph, you have identified
11:06:59 10 four requirements for Ju's method; is that right?

11:07:02 11 A. Yes.

11:07:05 12 Q. Now, is it your testimony that a person of
11:07:08 13 ordinary skill in the art would only choose a protecting
11:07:11 14 group for the 3' hydroxyl that could satisfy these
11:07:15 15 conditions?

11:07:18 16 A. I believe that's what Ju teaches. I don't know
11:07:25 17 that I have an opinion on what anyone else might do.

11:07:31 18 People do all sorts of things for all sorts of
11:07:34 19 crazy ideas. Ju teaches these, so if you are following
11:07:39 20 Ju's teachings, then you would want something that
11:07:42 21 satisfied these.

11:07:44 22 I won't -- I don't care to think about -- I
11:07:48 23 mean, like I said, people do incorrect things, people do
11:07:53 24 things that are unanticipated, and so I'm not commenting
11:07:58 25 on that.

11:08:02 1 Q. Now, in 2002, would you agree that a person of
11:08:07 2 ordinary skill in the art would need to test various
11:08:13 3 protecting groups to determine if such a group would
11:08:16 4 satisfy the requirements that you list in paragraph 29?

11:08:21 5 A. There are components that would require
11:08:23 6 testing. There are components I think that are very
11:08:26 7 obvious that would not require testing, because they're
11:08:29 8 just known in the literature. So some of it would
11:08:32 9 require testing.

11:08:33 10 So if you could ask me about a specific
11:08:35 11 requirement, I could tell you whether I think that would
11:08:38 12 require testing.

11:08:45 13 Q. So look at requirement No. 2, "The four
11:08:51 14 nucleotide analogues need to be efficiently and
11:08:53 15 faithfully incorporated by DNA polymerase as terminators
11:08:56 16 in the polymerase reaction."

11:08:58 17 You would agree with me that in 2002, a person
11:09:01 18 of ordinary skill would have to test a protecting group
11:09:04 19 to determine whether, in fact, it meets that
11:09:07 20 requirement?

11:09:08 21 A. Yes, if it had not been tested in the
11:09:10 22 literature and then -- correct, yes.

11:09:17 23 Q. Now, how about with respect to No. 3 that
11:09:21 24 you've listed in paragraph 29, "the tag and the group
11:09:24 25 capping the 3' hydroxyl need to be removed with high

11:09:28 1 yield to allow the incorporation . . . of the next
11:09:32 2 nucleotide"?

11:09:32 3 A. Yes.

11:09:33 4 Q. You would agree with me that one of skill in
11:09:36 5 the art would need to test to see if that -- you could
11:09:40 6 achieve that requirement?

11:09:42 7 MS. SWAROOP: Object to the form of the
11:09:43 8 question and object as outside the scope.

11:09:45 9 You can answer.

11:09:46 10 THE WITNESS: Yes.

11:10:20 11 BY MR. SEGAL:

11:10:20 12 Q. If you'll turn to paragraph 31 of your
11:10:23 13 declaration, which is Exhibit 2011.

11:10:31 14 Now, you reference Ju and say that "Ju teaches
11:10:34 15 that . . . protecting groups 'with electrophiles such as
11:10:38 16 ketone groups are not suitable for protecting the 3'
11:10:43 17 hydroxyl of the nucleotide in enzymatic reactions due to
11:10:48 18 the existence of strong nucleophiles in the
11:10:53 19 polymerase.'"

11:10:54 20 Is that right? Is that part of your opinion?

11:10:56 21 A. I'm quoting Ju there. That's out of his --
11:10:59 22 that's out of the patent, so that's what Ju's teaching.

11:11:05 23 Q. Do you agree with Ju there?

11:11:11 24 A. Sure. Sure. If it's -- if you have an
11:11:14 25 electrophile and if the polymerase reacted with it in a

11:11:19 1 way that was unintended, that would be a problem.

11:11:26 2 Q. Now, Ju cites to a Canard reference.

11:11:32 3 Is that correct?

11:11:32 4 A. It is.

11:11:34 5 Q. And Ju -- just to be clear, Ju's citing to
11:11:37 6 Canard to support that quote from Ju that I just read
11:11:41 7 into the record?

11:11:42 8 A. I believe so.

11:11:45 9 Q. Now, when Ju says "ketone," isn't Canard
11:11:51 10 actually describing carbonyl groups?

11:11:56 11 A. A carbonyl group -- a ketone contains a
11:12:00 12 carbonyl group, an ester contains a carbonyl group; so
11:12:05 13 if you say -- if one person says a "ketone" and another
11:12:10 14 person says a "carbonyl," there's no inconsistency
11:12:13 15 there.

11:12:17 16 A carbonyl group is a rather -- broader term
11:12:21 17 that includes in addition to ketones; esters, amides.

11:12:30 18 Q. So when Ju is talking about an electrophile
11:12:34 19 such as ketone groups, what atom of the ketone group is
11:12:41 20 the electrophile?

11:12:44 21 A. The carbonyl. It's actually two atoms. It's
11:12:47 22 the carbonyl, so it's the C=O that's the electrophile.

11:12:59 23 Q. Ju talks about strong nucleophiles in the
11:13:03 24 polymerase; is that correct?

11:13:05 25 A. Yes.

11:13:09 1 Q. What strong nucleophiles are present in the
11:13:11 2 active site of polymerases?

11:13:15 3 A. Enzymes -- proteins in general have lots of
11:13:19 4 nucleophiles. Are you asking what -- what are common
11:13:26 5 nucleophiles?

11:13:27 6 So if -- if I was one of skill in the art and I
11:13:30 7 read that polymerase active sites contain a strong
11:13:33 8 nucleophile, are you asking what I would have guessed
11:13:36 9 they would likely be?

11:13:38 10 Q. Yes, and what one of ordinary skill in the art
11:13:43 11 would understand them to be.

11:13:44 12 A. The side chain of a lysine amino acid would be
11:13:48 13 one possibility; the side chain of a serine amino acid,
11:13:52 14 the side chain of a histidine amino acid, the thiol side
11:14:00 15 chain of a cysteine, an activated water molecule. So
11:14:07 16 enzymes use lots of mechanisms to have strong
11:14:12 17 nucleophiles, and those would be some of the common ones
11:14:16 18 that one might have suspected.

11:14:25 19 And also, if I could elaborate I guess a little
11:14:27 20 bit further, in 2002, people certainly knew that there
11:14:30 21 were metals in a polymerase active site, two of them,
11:14:34 22 and I guess one might have thought a likely candidate
11:14:37 23 was an activated water molecule. That would just be
11:14:40 24 sort of standard chemistry.

11:14:47 25 Q. So, again, the list that you provided to me,

11:14:50 1 what is the most reactive nucleophile that would be
11:14:53 2 present and be able to react with the electrophile in a
11:15:02 3 ketone group?

11:15:03 4 A. All of those. Are you asking which one I think
11:15:05 5 is the most common?

11:15:07 6 Q. Well, I'll ask that question.
11:15:09 7 What is the most common?

11:15:12 8 A. They're all common; maybe a side chain amine
11:15:20 9 because they form Schiff bases with the carbonyl.
11:15:25 10 That's the result of the nucleophile attacking the
11:15:28 11 carbonyl and you dehydrate it, and it's a common
11:15:31 12 mechanism.

11:15:34 13 But there are lots of common mechanisms. I
11:15:36 14 don't think -- I don't think anyone -- there are so many
11:15:39 15 candidates as to what that electrophile -- that
11:15:41 16 nucleophile could have been. That's one thing enzymes
11:15:46 17 do, is they have nucleophiles.

11:15:48 18 People study what those nucleophiles are. I
11:15:51 19 mean any time you get a new enzyme, you study it, and
11:15:56 20 get years and years of studying an enzyme to figure out
11:16:00 21 what specific nucleophile it is.

11:16:02 22 Q. So, again, of the list that you gave me of
11:16:06 23 these nucleophiles, which ones are reactive enough to
11:16:11 24 attack the -- the electrophiles of the ketone group?

11:16:16 25 A. Any of them could be. I mean so what enzymes

11:16:23 1 do is they have active sites where they activate
11:16:28 2 nucleophiles. Serine proteases attack a carbonyl group
11:16:32 3 by activating a serine hydroxyl group.

11:16:38 4 As I mentioned, there's an enzyme -- lots of
11:16:41 5 enzymes use side chain amines. Histidine is another
11:16:47 6 common one.

11:16:48 7 Q. What do you think -- well, what is Canard
11:16:54 8 referring to that he believes -- what nucleophile of the
11:16:58 9 polymerase does Canard believe is reacting with the
11:17:02 10 electrophiles of the ketone groups?

11:17:04 11 A. I don't think Canard knows.

11:17:06 12 MS. SWAROOP: Let me object to the form of the
11:17:08 13 question, and I'd also ask to the extent that you're
11:17:11 14 asking the witness about a reference, that you provide
11:17:14 15 him with a copy.

11:17:17 16 THE WITNESS: Again, let me change that
11:17:19 17 question -- not change the question.

11:17:20 18 Let me add something to the former question
11:17:23 19 where you're asking about what people would have
11:17:25 20 suspected was the nucleophile.

11:17:27 21 I think people probably would have suspected it
11:17:30 22 had something to do with those metals.

11:18:05 23 MR. SEGAL: I'm handing you what's been
11:18:07 24 previously marked as Exhibit 2019, a reference with the
11:18:13 25 first author Bruno Canard.

11:18:18 1 (Exhibit 2019 was referred to.)

11:18:25 2 BY MR. SEGAL:

11:18:25 3 Q. Is this the Canard reference that Ju cites to?

11:18:30 4 A. I believe so, yes. It appears to be.

11:18:39 5 Q. If you'll turn to -- I guess it's the last page
11:18:43 6 of the Canard reference, 10863, you cite a sentence from
11:18:55 7 Canard which I believe is at the very bottom of that
11:19:01 8 left-hand column: "Strikingly, the central nitrogen of
11:19:05 9 the 3'-azido group of AZT bears a partial positive
11:19:12 10 charge in a position similar to the carbonyl group of
11:19:15 11 3'-esterified nucleotides, and the 3'-azido group of AZT
11:19:21 12 is metabolized into a 3'-amino group in vivo."

11:19:26 13 Is that the portion of Canard that you're
11:19:29 14 referencing in your declaration?

11:19:32 15 A. It is.

11:19:33 16 Q. Is it your position that the central nitrogen
11:19:36 17 of an azido group is an electrophile?

11:19:45 18 A. Are you asking what my position is today, or
11:19:47 19 what I think the average person of skill in the art
11:19:49 20 would have thought in 2002?

11:19:57 21 Q. Let's take both of those questions.

11:19:59 22 What would the person of ordinary skill in the
11:20:02 23 art thought in 2002?

11:20:05 24 A. I think the person would have read the -- if
11:20:08 25 someone read the Canard paper, I assume they would have

11:20:13 1 assumed that that central nitrogen could act as an
11:20:16 2 electrophile.

11:20:18 3 Q. And what is your understanding today?

11:20:23 4 A. I'd be suspicious of that.

11:20:27 5 Q. What do you base your suspicions on?

11:20:31 6 A. It tends -- in a lot of reactivity of azides,
11:20:36 7 you tend not to get reaction there. So if you just
11:20:41 8 think about the reactivity of azides -- which
11:20:44 9 incidentally is a lot better understood today than it
11:20:47 10 was 10 years ago, 12 years ago -- it just -- it would
11:20:51 11 be -- it would not be the most likely site for
11:20:55 12 reactivity.

11:20:56 13 But what I will say is, again, what they're
11:20:58 14 talking about here is in an enzyme active site, and
11:21:02 15 enzyme active sites have a way of reacting where they
11:21:05 16 want to react; and so most people's opinion about that
11:21:08 17 central nitrogen not being electrophilic today is based
11:21:13 18 on simple reactivity of an azide in solution with
11:21:17 19 something that you're mixing, not an enzyme.

11:21:20 20 Enzymes are structured environments. They
11:21:23 21 position nucleophiles at specific places, so what I
11:21:27 22 think the Canard paper is saying is he's saying there's
11:21:31 23 this nucleophile, and it can attack these carbonyl
11:21:35 24 groups because they are in this position; and, look,
11:21:38 25 this azide -- and I'm going to add a little bit to it.

11:21:42 1 If I were writing this today, what I would say
11:21:44 2 is this position does not typically act as an
11:21:48 3 electrophile, but in the enzyme active site it appears
11:21:51 4 to, because enzyme active sites position -- I mean this
11:21:56 5 is what they do: They position nucleophiles at specific
11:22:00 6 places, or electrophiles, or reactive centers, to do
11:22:03 7 chemistry in a way that they evolved to do.

11:22:05 8 And if there's something -- if there's an
11:22:07 9 electrophile that's -- that's positioned specifically to
11:22:12 10 go after a certain site, then it's not unreasonable to
11:22:17 11 think that it's -- the reactivity of the azide in that
11:22:21 12 context would be different.

11:22:23 13 So I guess that's what I would -- if I were
11:22:26 14 reading this paper today, that's probably what I would
11:22:29 15 think. I would think, Oh, well, that's interesting;
11:22:30 16 usually azides don't react that way, but apparently --
11:22:34 17 enzymes are cool. Enzymes do what they do.

11:22:42 18 Q. Now, in 2002, would agree with me that a person
11:22:45 19 of ordinary skill in the art knew that generally azides
11:22:49 20 were fairly non-reactive?

11:22:54 21 A. "Non-reactive" is a relative term. In certain
11:23:01 22 environments, they are certainly very reactive. In a
11:23:04 23 lot of environments, they're not. So I think that's
11:23:06 24 what you're implying there, that people understood that
11:23:08 25 in a lot of environments, they were not reactive.

11:23:08 1 That's certainly not to say they're not
11:23:11 2 reactive in some. I mean the very essence of the
11:23:13 3 deprotection mechanism that we're talking about today
11:23:16 4 relies on their high reactivity, but certainly in a lot
11:23:19 5 of cases, people understood azides were stable.

11:23:23 6 Q. Do you know whether the carbon of a ketone or
11:23:27 7 carbonyl group is more reactive with nucleophiles of a
11:23:32 8 polymerase than the site central nitrogen of an azido
11:23:36 9 group?

11:23:38 10 A. No, I do not know that.

11:23:39 11 Again, what I know -- and if I can rephrase the
11:23:41 12 question in a way that I can answer -- well, let me give
11:23:44 13 you the answer to a slightly different question.

11:23:46 14 In general, outside of an enzyme, your
11:23:49 15 statement would be correct. Carbonyl groups would tend
11:23:53 16 of better electrophiles than that central nitrogen of an
11:23:56 17 azide; but, again, enzymes are a different game.

11:24:00 18 Enzymes -- the very act of -- the very fact of
11:24:02 19 what enzymes do, enzymes evolve to catalyze reaction.
11:24:06 20 That means they take a reaction that doesn't go
11:24:08 21 otherwise, and they make it happen.

11:24:12 22 So if someone said to me, Would you expect a
11:24:15 23 carbonyl of an ester to be more electrophilic than an
11:24:20 24 azide in general, I would say, Yeah, that's what I would
11:24:24 25 expect.

11:24:25 1 Alternatively, if they said, Would it surprise
11:24:27 2 you that an enzyme went after the central position of an
11:24:31 3 azide with a nucleophile as efficiently as it did an
11:24:34 4 ester, would that surprise me? I would say, Not
11:24:37 5 particularly. This is -- enzymes are -- this is the
11:24:40 6 kind of thing enzymes do.

11:24:44 7 Q. Other than the statement in Canard that you --
11:24:48 8 that you quote in your -- your declaration, as of 2002,
11:25:01 9 do you know of any other evidence that a nucleophile in
11:25:05 10 a polymerase would react with an azido group?

11:25:13 11 A. No, I don't -- I do not know of any report of
11:25:15 12 that.

11:25:16 13 Q. Canard doesn't present any evidence to that,
11:25:19 14 correct?

11:25:20 15 A. No. He suggests it, but he does not present
11:25:22 16 evidence.

11:25:26 17 Well, the evidence that he presents is very
11:25:28 18 tangential, and he -- I think he probably knew that.
11:25:31 19 He's saying that, interestingly, AZT is metabolized into
11:25:36 20 a 3' amino group, and that would suggest -- that does
11:25:40 21 suggest -- that's consistent with his mechanism.

11:25:43 22 Is that the only thing that's consistent with
11:25:45 23 his mechanism? Maybe not, but the reason he's pointing
11:25:50 24 that out is exactly this: He's trying to come up with
11:25:54 25 more data.

11:25:55 1 So other than those two statements, no, I'm
11:25:57 2 unaware of any other examples.

11:26:00 3 Q. You would agree with me that Canard doesn't
11:26:03 4 present any data showing that a nucleophile in a
11:26:06 5 polymerase actually reacts with an azido group?

11:26:09 6 A. I believe that's correct. I would have to read
11:26:11 7 through this to make sure that's correct. I believe
11:26:13 8 that's correct.

11:26:14 9 Q. Now, in that sentence that you quote in your
11:26:18 10 declaration, it cites to two references, 29 and 30.

11:26:28 11 Do you see that in that -- if you look in
11:26:31 12 Canard -- remember that sentence that we were just
11:26:33 13 talking about in Canard?

11:26:35 14 A. Oh, that references 29 and 30 in Canard?

11:26:39 15 Q. Right. Did you review those references, 29 and
11:26:43 16 30 of Canard?

11:26:55 17 A. I don't think so. I looked at a lot of papers.
11:26:59 18 I don't think those were two of them. I don't recognize
11:27:02 19 them.

11:27:29 20 Q. Why don't we turn to -- let's turn to paragraph
11:27:40 21 32 of your declaration. Again, that's Exhibit 2011.

11:27:50 22 Now, in this paragraph, you suggest that Ju's
11:27:52 23 desired read length is more than 30 base pairs.

11:27:57 24 Is that correct?

11:27:58 25 A. That was my reading, yes.

11:28:01 1 Q. Now, the Ju reference itself, or the Ju patent,
11:28:05 2 does not provide any data showing 30 base pair reads,
11:28:10 3 correct?

11:28:11 4 A. No, it does not, so that part is correct.

11:28:17 5 Q. Would you agree with me that a person of
11:28:19 6 ordinary skill would understand any alleged 30 base pair
11:28:26 7 requirement as a goal?

11:28:30 8 A. I think my reading was that Ju had a much
11:28:34 9 longer -- a goal of being much longer than 30
11:28:38 10 nucleotides.

11:28:39 11 Q. Right. But the Ju patent itself doesn't
11:28:41 12 provide any data that actually shows a 30 base pair
11:28:45 13 read, right?

11:28:46 14 A. No. He has no such data, no.

11:28:48 15 Q. Right. So would you agree with me that a
11:28:50 16 person of ordinary skill reading the Ju reference in
11:28:54 17 2002 would understand that as a goal, correct?

11:28:57 18 MS. SWAROOP: Object -- objection. Asked and
11:28:58 19 answered.

11:29:00 20 THE WITNESS: I don't think Ju's goal was to
11:29:02 21 sequence 30 nucleotides; I think Ju's goal was to
11:29:05 22 sequence significantly longer.

11:29:07 23 BY MR. SEGAL:

11:29:08 24 Q. Okay. But they're both goals to the extent
11:29:09 25 they're in the Ju reference?

11:29:12 1 A. I don't read it as 30 nucleotides being a goal
11:29:15 2 of Ju. He actually cites a pyrosequencing method, and
11:29:20 3 he cites the 30 nucleotides as a limitation -- the fact
11:29:24 4 that you can only get a read length of 30 as a
11:29:28 5 limitation. So I think he's -- it's sort of -- it's
11:29:30 6 noted as a limitation, and he then at a later -- at a
11:29:35 7 different spot, talks about much longer reads.

11:29:37 8 So my read, I don't think he actually gives a
11:29:40 9 value of what he says is his goal, but it was
11:29:42 10 clearly long- -- in my read of it, it was clearly longer
11:29:45 11 than 30 because he's disparaging the pyrosequencing
11:29:49 12 method as only providing 30 nucleotide read lengths.

11:29:53 13 Q. Now, a person with ordinary skill in the art to
11:29:57 14 reach a 30 base pair read or longer would understand
11:30:00 15 that you would need to test various protecting groups,
11:30:04 16 correct?

11:30:04 17 A. Sure. Yes.

11:30:06 18 Q. And you'd also have to test the protecting
11:30:08 19 groups that are attached to nucleotides; you'd also have
11:30:12 20 to test polymerases, correct?

11:30:15 21 MS. SWAROOP: Object to the form of the
11:30:16 22 question.

11:30:17 23 THE WITNESS: I think by definition, yes.

11:30:19 24 BY MR. SEGAL:

11:30:19 25 Q. And you would also have to test various

11:30:23 1 cleaving conditions, correct?

11:30:28 2 A. The efficiency, yeah; and the time, sure.

11:30:31 3 Q. And Ju, in fact, teaches that various, for
11:30:35 4 instance, polymerases can be used, correct?

11:30:41 5 A. I -- I'm not sure I understand the question.
11:30:43 6 He teaches -- I think he suggests.

11:30:46 7 Does he demonstrate? Is that what you're
11:30:48 8 asking? Or --

11:30:49 9 Q. Well, I'm asking whether Ju teaches that
11:30:51 10 different polymerases can be used in an SBS method.

11:30:55 11 A. So could you spec- -- could you use a word
11:30:57 12 other than "teaches" in that question?

11:31:00 13 Q. "Disclose" in there, the use of different
11:31:04 14 polymerases --

11:31:05 15 A. I don't believe he has -- there's no data, if
11:31:08 16 that's what you're asking. He doesn't demonstrate.

11:31:11 17 Does he say that they could be? I think so.
11:31:20 18 And the reason I'm qualifying that is I think that
11:31:22 19 "disclose" and "teach" have certain meanings.

11:31:26 20 Q. Okay. How about does Ju suggest the use --

11:31:29 21 A. Yes.

11:31:29 22 Q. -- of different polymerases?

11:31:31 23 A. Yes.

11:31:35 24 Q. And, again, to achieve this 30 base pair read
11:31:39 25 or longer, a person of ordinary skill in the art would

11:31:42 1 understand that you might need to optimize various
11:31:45 2 conditions of Ju's SBS method; is that right?

11:31:52 3 A. To the extent that any parameter doesn't
11:31:55 4 satisfy what he defines as a criteria, if you are going
11:31:59 5 to pursue the method then, by definition, you would have
11:32:02 6 to optimize it.

11:32:18 7 Q. Let's turn to paragraph 33 of your declaration.
11:32:31 8 Is it your position that for Ju's method, a
11:32:37 9 person of ordinary skill in the art would understand
11:32:39 10 that you would need a protecting group that could be
11:32:42 11 removed in nearly quantitative yield?

11:32:48 12 A. Yes.

11:32:53 13 Q. Now, before a person of ordinary skill in the
11:32:56 14 art back in 2002, reading Ju's method -- before such a
11:33:02 15 person would try a protecting group in Ju's method,
11:33:07 16 would they need data showing the removal of the
11:33:11 17 protecting group with nearly quantitative yield?

11:33:15 18 MS. SWAROOP: Object to the form of the
11:33:16 19 question.

11:33:17 20 THE WITNESS: I guess it depends on what level
11:33:20 21 of expectation you're asking.

11:33:22 22 Are you asking if someone expected to be able
11:33:25 23 to use Ju's method with this protecting group? They
11:33:30 24 would -- if you -- I mean it's a confidence question.
11:33:34 25 If you're saying that I have zero confidence and I'm

11:33:37 1 just going to try different things then, sure, you could
11:33:40 2 try anything and you would need no data.

11:33:43 3 If you're going into it with an expectation of
11:33:46 4 success, then -- then I would need data, and I guess the
11:33:51 5 way I would think about it is -- you know, I run a
11:33:53 6 research group with a limited amount of money, so you
11:33:56 7 make choices that have opportunity costs. I -- I can
11:34:01 8 pay students to work on one thing, or I can pay students
11:34:04 9 to work on another.

11:34:05 10 If I were, in 2002, thinking about doing this,
11:34:08 11 I would have needed data that would have convinced me
11:34:12 12 that it wasn't a waste of my time and my students' time
11:34:15 13 and my money.

11:34:16 14 BY MR. SEGAL:

11:34:17 15 Q. So for a reasonable expectation of success,
11:34:19 16 would a person of ordinary skill in the art need data
11:34:24 17 showing that the protecting group can be removed with
11:34:26 18 nearly quantitative yield?

11:34:28 19 A. It depends on what you mean by "reasonable." I
11:34:31 20 mean, again, it's a level of confidence. It depends.

11:34:35 21 If there's data in the literature that suggests
11:34:38 22 they're inefficient, then I would need a lot of
11:34:42 23 convincing to change my mind.

11:34:44 24 If there was no data and it were easy to test,
11:34:48 25 I mean, again, it all comes down to just what level of

11:34:51 1 confidence you want to have, what level of expectation
11:34:54 2 of success you're asking.

11:34:56 3 Again, if you have zero expectation of success,
11:35:00 4 then I don't think you would need any data.

11:35:02 5 Am I being clear?

11:35:06 6 Q. Well, for -- again, for a reasonable
11:35:08 7 expectation of success -- let's ask it a different way.

11:35:12 8 What yield would motivate a person of ordinary
11:35:15 9 skill in the art to try a protecting group with Ju's
11:35:19 10 method?

11:35:22 11 MS. SWAROOP: Object to the form of the
11:35:23 12 question.

11:35:23 13 THE WITNESS: So -- okay. So that's a hard
11:35:25 14 question.

11:35:26 15 Are you asking if I saw a particular reaction,
11:35:29 16 would I -- how prone would I be to think, yeah, that
11:35:34 17 could be used -- the protecting group and its removal by
11:35:38 18 that method could be used in Ju's method? Is that what
11:35:44 19 you're asking?

11:35:45 20 BY MR. SEGAL:

11:35:45 21 Q. Yes.

11:35:46 22 A. So it depends on what that data is. If that
11:35:49 23 data looks like you're getting 10 percent yields and
11:35:52 24 people have worked on it a fair bit, then I'd say, Boy,
11:35:56 25 there's very little chance to optimize it.

11:35:59 1 Then I would be very suspicious. I would have
11:36:02 2 a very low level of confidence.

11:36:04 3 If you were looking at a completely new
11:36:06 4 reaction that had never been examined and my intuition
11:36:09 5 was that, you know, hey, maybe that would work and it
11:36:11 6 wasn't hard, maybe I would test it. So it really
11:36:14 7 depends on what confidence you have of the data that
11:36:17 8 exists and how good that data is, because if there's
11:36:21 9 very little data there and maybe you're hopeful that
11:36:23 10 there could be optimization. If there's a lot of data
11:36:27 11 there from a lot of different groups and they're getting
11:36:30 12 10 percent, then I would have very little data that you
11:36:33 13 could -- I would have very little belief that you could
11:36:35 14 optimize it.

11:36:36 15 Q. How about if the prior art showed yields of
11:36:40 16 80 percent?

11:36:44 17 A. Consistently under different conditions from
11:36:46 18 different groups? So, again, is that -- how solid does
11:36:50 19 that 80 percent look?

11:36:53 20 If it looks solid, then it's a -- there's no
11:36:57 21 pursual there; there's no chance that's going to work if
11:37:01 22 that's the solid -- if that's a solid number.

11:37:07 23 Frankly, if it's a --

11:37:08 24 Q. Again, what do you mean by "solid"?

11:37:10 25 A. It depends on how much optimism that I have

11:37:13 1 that I could improve it, and what that means is what are
11:37:16 2 the conditions that had been examined? Depending on the
11:37:20 3 reaction, how much flexibility is there to optimize it?

11:37:24 4 If you are in a very constrained reaction and
11:37:27 5 there's only one way you can do it, and there's reliable
11:37:31 6 data that shows that gives you an 80 percent yield, I'm
11:37:35 7 looking for another project.

11:37:37 8 Q. But would you look for others -- other research
11:37:40 9 groups that tried to remove that particular protecting
11:37:44 10 group?

11:37:44 11 A. Sure. That would be an important piece of the
11:37:46 12 data, because if there were just one report and they
11:37:49 13 report this -- you know, there's a lot of errors in
11:37:52 14 science, and so I would be suspicious that it could be
11:37:55 15 an error.

11:37:55 16 So if someone reported some quantitative yield
11:37:59 17 of something that looked too good to be true and no one
11:38:04 18 had actually even explored it, I would be pretty
11:38:06 19 suspicious of that.

11:38:07 20 Someone -- in contrast, if three or four or
11:38:10 21 five respectable groups report something and they're all
11:38:14 22 giving 80 percent, 85 percent, then that's the opposite:
11:38:18 23 I'm done.

11:38:19 24 That's what I mean by there being good data, so
11:38:22 25 it all depends on the level of optimization that you

11:38:25 1 think is possible.

11:38:26 2 If there's a lot of groups that tried and can't
11:38:30 3 do it, then you're probably not going to be able to do
11:38:33 4 it.

11:38:34 5 Q. So you would look for other groups that have
11:38:36 6 tried to remove a particular protecting group before
11:38:38 7 ruling it in or ruling it out?

11:38:41 8 A. That would not be the -- you know, that would
11:38:43 9 not be the gating criteria, but I would weigh that into
11:38:46 10 a consideration.

11:38:48 11 But, again, it depends on the specific
11:38:51 12 situation, so you're going to have to tell me a fact
11:38:54 13 pattern, and I'll tell you whether I would consider that
11:38:57 14 promising or not.

11:39:04 15 Q. What conditions could affect the removal
11:39:08 16 efficiency of a 3' protecting group?

11:39:12 17 A. What removal conditions?

11:39:14 18 The reagents that are used, the temperature,
11:39:18 19 the solvent, the efficiency, because if you can -- if
11:39:28 20 you have to take it for a long time, it's likely that
11:39:30 21 competing side reactions might be there.

11:39:35 22 Q. PH?

11:39:36 23 A. Sure.

11:39:39 24 Q. How about the concentration of the reagents?

11:39:42 25 A. The concentration required; sure, that could

11:39:47 1 affect things.

11:40:00 2 Q. Let's turn to paragraph 34 of your declaration,
11:40:04 3 Exhibit 2011.

11:40:13 4 Is it your position that a person of ordinary
11:40:16 5 skill in the art would understand Ju's method requires
11:40:19 6 rapid removal conditions for 3' hydroxyl protecting
11:40:23 7 groups?

11:40:28 8 A. Given the qualitateness of that term "rapid,"
11:40:33 9 yes.

11:40:34 10 Q. Let me ask you about that term, "rapid."

11:40:37 11 Does Ju define what "rapid" is?

11:40:41 12 A. Not explicitly; but implicitly, he does. He
11:40:46 13 describes "efficient," and he's describing a
11:40:49 14 sequencing-by-synthesis method, so I think that anyone
11:40:53 15 thinking about his method would understand that that
11:40:55 16 brings with it certain constraints.

11:41:01 17 Q. So, again, that would be the ultimate goal of a
11:41:04 18 researcher working in sequencing-by-synthesis methods?

11:41:08 19 A. Yes.

11:41:09 20 Q. But the first step is not necessarily you're
11:41:12 21 going to be worrying about extremely rapid process;
11:41:16 22 would you agree with that?

11:41:18 23 MS. SWAROOP: Object to the form of the
11:41:19 24 question.

11:41:21 25 THE WITNESS: Yes and no. I agree with it to

11:41:23 1 the extent that I think it's a valid thing to do to
11:41:27 2 explore one thing and hope to optimize another aspect of
11:41:32 3 it later, but you will also understand that that aspect
11:41:37 4 will eventually have to be optimized, and so you would,
11:41:41 5 at best, ignore it with the optimization -- with the
11:41:48 6 optimism that you could eventually fix it.

11:41:51 7 If you're working with something that will
11:41:53 8 never cleave faster than -- I don't know; I'm just going
11:41:57 9 to make up a number -- five hours, six hours, you're
11:42:00 10 done. I mean, no one will ever sequence anything
11:42:03 11 significant waiting six hours between reads.

11:42:07 12 So if you have zero optimism that you could
11:42:10 13 ever deal with that, then you'd be a fool to try to
11:42:14 14 develop other aspects of it. You're going to get a
11:42:18 15 nice-looking car that can't drive.

11:42:27 16 BY MR. SEGAL:

11:42:28 17 Q. So would you agree that the -- strike that.

11:42:32 18 Would you agree that how rapid the removal of
11:42:34 19 the 3' protecting group is related to the application
11:42:39 20 that you're going to use the SBS method for?

11:42:43 21 A. Could you repeat that question.

11:42:46 22 Q. Does the rapid removal of a 3' protecting group
11:42:52 23 relate to the eventual application of the SBS method?

11:42:57 24 MS. SWAROOP: Object to the form of the
11:42:58 25 question.

11:42:59 1 THE WITNESS: So are you asking if the
11:43:01 2 efficiency of removal depends -- required, depends on
11:43:06 3 the application?

11:43:08 4 BY MR. SEGAL:

11:43:09 5 Q. Sure. That's a better way of asking it. Thank
11:43:11 6 you.

11:43:13 7 A. So that depends -- I mean, so that depends.
11:43:16 8 Are you talking about sequencing-by-synthesis
11:43:19 9 applications?

11:43:20 10 Q. Yes.

11:43:21 11 A. Sure, but they all have to be efficient.
11:43:23 12 There's a minimum that you're going to have to be.

11:43:26 13 You're going to have to get 30 nucleotide read
11:43:29 14 lengths minimum, in a time that's not -- in a time
11:43:33 15 that's feasible.

11:43:37 16 Q. What's a feasible time?

11:43:38 17 A. You know, that's a relative term. I mean, I --
11:43:41 18 it -- I threw out the idea of five hours being not
11:43:47 19 reasonable, and I think that's probably right.

11:43:50 20 What is a reasonable time? I don't know. An
11:43:52 21 hour. That would not be great, I don't think, but that
11:43:55 22 would at least maybe be in the realm of doable.

11:43:59 23 10 hours -- you know, I mean, how long does it
11:44:04 24 take to drive to L.A. and what's acceptable? I mean it
11:44:08 25 depends on how much time you've got and depends on, you

11:44:10 1 know, what -- you know, two hours is better than 20.

11:44:15 2 But what I will say is that there are lots of
11:44:23 3 polymerase reactions, there are lots of read lengths
11:44:30 4 that would simply never be useful for sequencing by
11:44:33 5 synthesis.

11:44:34 6 A read length of one is just an example. You
11:44:37 7 would never be able to do anything with that. So you're
11:44:41 8 going to have to get read lengths of 25 or 30 in -- in
11:44:46 9 a -- with no more than an hour or so between reads to
11:44:52 10 make it -- to -- someone who's reading the Ju material,
11:44:58 11 the Ju patent, and thinking about sequencing by
11:45:01 12 synthesis is going to understand those as restrictions.

11:45:42 13 MR. SEGAL: I'm going to hand you what's been
11:45:45 14 previously marked as Exhibit 1008. It's a PCT published
11:45:50 15 patent application. The first named inventor is Roger
11:45:58 16 Tsien. That's spelled T-s-i-e-n.

11:46:18 17 (Exhibit 1008 was referred to.)

11:46:20 18 BY MR. SEGAL:

11:46:21 19 Q. I'd like to do with you what we did with the Ju
11:46:24 20 reference, and sort of compare what Tsien teaches or
11:46:29 21 suggests in his patent application as compared to the
11:46:32 22 claim language of the claims in the '537 patent, which
11:46:38 23 is Exhibit 1001, okay?

11:46:44 24 A. Yes.

11:46:44 25 Q. So, again, if we look at claim 1 and, again,

11:46:52 1 beginning on line 2 of claim 1 of the '537 patent, after
11:46:56 2 the word "comprising," in the second line it says
11:47:00 3 "incorporating into the nucleic acid molecule a
11:47:07 4 nucleotide or nucleoside molecule."

11:47:10 5 You would agree with me that the Tsien
11:47:12 6 reference discloses that?

11:47:15 7 MS. SWAROOP: Object to the form of the
11:47:16 8 question and object as outside the scope.

11:47:21 9 THE WITNESS: Yes.

11:47:22 10 BY MR. SEGAL:

11:47:24 11 Q. Again, so the next part of the claim reads
11:47:26 12 "wherein the nucleotide or nucleoside molecule has a
11:47:29 13 base that is linked to a detectable label via a
11:47:33 14 cleavable linker," again, the Tsien reference discloses
11:47:38 15 this?

11:47:43 16 A. I think so, yes.

11:47:55 17 Q. Is that a "yes"?

11:47:56 18 A. I believe so. It just --

11:47:56 19 MS. SWAROOP: While he's looking, let me object
11:47:58 20 to the form of the question and also object as outside
11:48:01 21 the scope.

11:48:07 22 THE WITNESS: Could you show me in the Tsien
11:48:09 23 reference where he states this?

11:48:12 24 I believe it is correct, but I'm, again, mixing
11:48:16 25 up so many things that I read that I just want to make

11:48:19 1 sure that I don't make a mistake sitting here.

11:48:25 2 BY MR. SEGAL:

11:48:25 3 Q. Okay. If you'll look on page 27 of the Tsien
11:48:28 4 reference --

11:48:29 5 A. Which numbering system? Bottom or top of the
11:48:32 6 page?

11:48:33 7 Q. The top of the page.

11:48:35 8 A. Okay.

11:48:42 9 Q. If you'll look at the last paragraph -- it
11:48:44 10 begins around line 33 -- and read that on to page 28.

11:49:06 11 A. Yes, it would -- yes.

11:49:07 12 Q. Okay. Now, turning to the next part of claim 1
11:49:14 13 of the '537 patent, the claim reads "the nucleotide or
11:49:21 14 nucleoside molecule has a ribose or deoxyribose sugar
11:49:26 15 moiety wherein the ribose or deoxyribose sugar moiety
11:49:31 16 comprises a protecting group attached via the 2' or 3'
11:49:34 17 oxygen atom."

11:49:37 18 Would you agree with me that Tsien discloses a
11:49:41 19 protecting group attached to the 3' oxygen?

11:49:45 20 MS. SWAROOP: Let me object to the form of the
11:49:46 21 question. Object as outside the scope.

11:49:49 22 THE WITNESS: Yes.

11:49:50 23 BY MR. SEGAL:

11:49:53 24 Q. The next part of claim 1 of the '537 patent
11:49:56 25 reads "said protecting group can be modified or removed

11:50:01 1 to expose a 3' hydroxyl group."

11:50:06 2 Would you agree with me that Tsien discloses
11:50:09 3 removing the 3' protecting group to expose a 3' hydroxyl
11:50:14 4 group?

11:50:14 5 A. Yes.

11:50:20 6 Q. The next part of the claim reads "the
11:50:22 7 protecting group comprises an azido group."

11:50:26 8 Now, would you agree with me that Tsien teaches
11:50:29 9 using an N3 group as a blocking modification to the 3'
11:50:35 10 hydroxyl position of dNTPs?

11:50:42 11 A. No.

11:50:43 12 Q. That's actually a quote from Tsien.

11:50:47 13 If you'll turn the page, using, again, the page
11:50:51 14 numbers at the top, page 21, if you look beginning on
11:51:09 15 line 19, it reads -- the text of Tsien reads "Other
11:51:14 16 blocking modifications to the 3'-OH position of dNTPs
11:51:20 17 include the introduction of groups such as" -- I'm not
11:51:24 18 going to read all the groups in, but the N3 group is
11:51:27 19 there, correct?

11:51:28 20 A. That is what's written there.

11:51:33 21 Q. So you would agree with me that Tsien, at
11:51:36 22 least, suggests that an N3 group, otherwise known as an
11:51:42 23 azido group, would be incorporated by polymerase?

11:51:45 24 MS. SWAROOP: Object to the form of the
11:51:46 25 question.

11:51:47 1 THE WITNESS: As a reversible terminator?

11:51:50 2 So you're mixing two questions here, because
11:51:53 3 the question that you were just talking about was as a
11:51:55 4 blocking -- a removable blocking group, or -- so are you
11:52:01 5 asking me that, or are you asking me if azido group
11:52:05 6 modifications can still be accommodated with --
11:52:08 7 by polymerase? So those are two separate questions.

11:52:12 8 BY MR. SEGAL:

11:52:12 9 Q. Let's start with that last question.

11:52:14 10 A. So Tsien is evoking data that shows that a
11:52:17 11 variety of groups attach to the 3' position; a carbon,
11:52:20 12 and these are not -- these are well-known examples, all
11:52:24 13 of them, and he sites Kraevskii --

11:52:29 14 Q. Now, would you agree with me --

11:52:30 15 A. -- and azido was one of them.

11:52:32 16 Q. Sorry.

11:52:32 17 MS. SWAROOP: Counsel, let him finish his --

11:52:36 18 THE WITNESS: And azido was one of those.

11:52:37 19 BY MR. SEGAL:

11:52:38 20 Q. Okay. Are you finished?

11:52:39 21 A. Yes.

11:52:39 22 Q. Okay. Would you agree with me that Tsien does
11:52:41 23 not teach that the N3 group could not be part of a
11:52:45 24 chemical moiety attached to a 3' oxygen?

11:52:49 25 MS. SWAROOP: Object to the form of the

11:52:50 1 question.

11:52:52 2 THE WITNESS: He doesn't address it. So to the
11:52:58 3 extent that's teaching or not teaching, he doesn't
11:53:01 4 address it.

11:53:03 5 Incidentally, it couldn't be. It's chemical
11:53:07 6 nonsense to attach an azide to a oxygen directly, I
11:53:11 7 believe. I don't think that would be a viable molecule.

11:53:14 8 BY MR. SEGAL:

11:53:14 9 Q. But Tsien doesn't teach that the N3 group
11:53:17 10 couldn't be part of a protecting group and be attached
11:53:20 11 to a 3' oxygen?

11:53:21 12 A. Sure, but he doesn't -- he does not teach that
11:53:23 13 it could not be, but he doesn't teach that it could be.
11:53:26 14 He doesn't breach -- he doesn't address the subject.

11:53:31 15 Q. Turning to claim 2 of the '537 patent, reads
11:53:38 16 "The method of claim 1, wherein said incorporating is
11:53:41 17 accomplished via a terminal transferase, a polymerase or
11:53:46 18 a reverse transcriptase."

11:53:48 19 Do you see that?

11:53:49 20 A. Yes, I do.

11:53:51 21 Q. Tsien discloses using a polymerase to
11:53:53 22 incorporate a nucleotide in his SBS methods?

11:53:57 23 A. Yes.

11:53:58 24 Q. Turning to claim 3 of the '537 patent, "The
11:54:03 25 method of claim 1, wherein the base is a deazapurine,"

11:54:11 1 would you agree that Tsien teaches through the
11:54:15 2 incorporation of the Prober reference using a
11:54:18 3 deazapurine base?

11:54:19 4 MS. SWAROOP: Object to the form of the
11:54:21 5 question and object outside the scope.

11:54:23 6 THE WITNESS: Could you show me that? Because
11:54:24 7 having read the Tsien patent recently, it's mostly about
11:54:29 8 not -- about analogues without that modification.

11:54:32 9 But show me expressly -- I mean I'm aware of
11:54:35 10 the Prober reference that does describe the diaza
11:54:39 11 analogue, so if you could just refresh my memory quickly
11:54:42 12 to make sure that it is the Tsien PCT that's describing
11:54:46 13 that.

11:54:47 14 BY MR. SEGAL:

11:54:47 15 Q. If you look on -- again, using the numbers at
11:54:49 16 the top of Tsien -- page 29 the sentence that begins on
11:55:01 17 line 12, "One quite versatile scheme is based on an
11:55:13 18 approach used by Prober, et al. (1987) to prepare ddNTPs
11:55:19 19 with fluorescent tags."

11:55:22 20 Do you see that sentence?

11:55:23 21 A. I do.

11:55:23 22 Q. So would you agree with me that Tsien teaches,
11:55:26 23 through incorporation of Prober, the use of deazapurine
11:55:30 24 bases?

11:55:31 25 MS. SWAROOP: Object to the form of the

11:55:32 1 question. Object as outside the scope.

11:55:40 2 THE WITNESS: I'm a little confused, because
11:55:41 3 then he then references molecules A through D, and none
11:55:45 4 of them are the diazas as he shows them. They're all
11:55:53 5 attached at the C-8 position, and what he's talking
11:55:55 6 about is the C-8 position.

11:55:58 7 "The . . . approach can be used to prepare
11:56:01 8 the" -- "A number of" --

11:56:16 9 BY MR. SEGAL:

11:56:17 10 Q. Does he suggest -- let me ask a different
11:56:19 11 question.

11:56:20 12 Does Tsien suggest that you could use the
11:56:24 13 attachment mechanism disclosed in Prober for his
11:56:29 14 nucleotides?

11:56:31 15 MS. SWAROOP: Again, I just want to object as
11:56:32 16 outside the scope.

11:56:34 17 THE WITNESS: I would need to think about that,
11:56:35 18 because from my reading right here, what he's saying is
11:56:40 19 that a number of approaches are possible to produce
11:56:43 20 fluorescent derivatives.

11:56:45 21 One versatile element is that taught by Prober.
11:56:48 22 Prober is deazapurines, so that would seem to imply
11:56:54 23 that, yes.

11:57:05 24 BY MR. SEGAL:

11:57:05 25 Q. Turn to claim 4 of the '537 patent. It reads

11:57:09 1 "The method of claim 1, wherein the nucleotide is a
11:57:12 2 deoxyribonucleotide triphosphate."

11:57:15 3 You would agree with me that Tsien discloses
11:57:18 4 such a molecule?

11:57:20 5 A. Yes.

11:57:20 6 Q. And he discloses it for use in his sequencing
11:57:23 7 methods?

11:57:25 8 A. Yes.

11:57:26 9 Q. If you look at claim 5 of the '537 patent,
11:57:31 10 claim 5 reads "The method of claim 1, wherein the label
11:57:35 11 is a fluorophore."

11:57:39 12 Again, would you agree with me that Tsien
11:57:41 13 discloses using fluorophores as a label on its
11:57:46 14 nucleotides for use in his sequencing methods?

11:57:49 15 A. Yes.

11:57:50 16 I'm -- I apologize. Can we revisit the
11:57:53 17 question of the deazapurines?

11:57:57 18 I apologize for not knowing this, but my
11:58:01 19 reading is it's very unclear, because he says that he's
11:58:06 20 talking about the C-8 position of purines, and that is
11:58:11 21 not a deazapurine; that's the other way to do it.

11:58:15 22 And then he starts talking about pyrimidines,
11:58:17 23 and that's C and T. That's not a purine, so that's not
11:58:22 24 a case for a deaza. And it's there that he says --
11:58:25 25 immediately following that, he says you can use the

11:58:27 1 Prober technique to prepare fluorescent tags, so if he's
11:58:31 2 talking about in general everything that he just spoke,
11:58:35 3 purines and pyrimidines, then that would seem to include
11:58:37 4 the deazapurines. But if he's only talking about the
11:58:41 5 pyrimidines, Prober contains the methods for the
11:58:44 6 synthesis of the pyrimidines that don't use, obviously,
11:58:48 7 the deazapurine.

11:58:49 8 So -- and all the compounds that he lists
11:58:51 9 are -- he lists lots of purines, and none of them are
11:58:56 10 deazapurines; they're all regular purines. So I would
11:59:00 11 need a little time to -- and I would need a little time
11:59:02 12 to read this through to make sure that I agree with that
11:59:06 13 statement.

11:59:09 14 Q. Okay. I mean we can move on.

11:59:12 15 Claim 8 reads "The method according to claim 1,
11:59:18 16 further comprising detecting the detectable label and
11:59:21 17 cleaving the cleavable linker."

11:59:24 18 Would you agree with me that Tsien discloses
11:59:27 19 that step in his sequencing methods?

11:59:31 20 MS. SWAROOP: Object to the form of the
11:59:32 21 question. Object as outside the scope.

11:59:36 22 THE WITNESS: Yes.

11:59:59 23 MR. SEGAL: I think I'm about to enter a new
12:00:01 24 line of questioning. I think our lunch just came. I
12:00:03 25 think this would probably be a good time to break.

12:00:07 1 MS. SWAROOP: Fine.

12:00:08 2 THE VIDEO OPERATOR: Okay. We are going off
12:00:09 3 the record. The time is 12:00 o'clock.

12:00:14 4 (Luncheon recess was taken at 12:00 p.m.)

12:00:14 5 * * *

12:00:13 6 San Diego, California July 8, 2014 1:02 p.m.

13:02:00 7 THE VIDEO OPERATOR: We are back on the record.
13:02:01 8 This is the beginning of Disk No. 3. The time is 1:02.

13:02:05 9

13:02:05 10 CONTINUED EXAMINATION

13:02:06 11 BY MR. SEGAL:

13:02:06 12 Q. Now, Dr. Romesberg, if you'll take out your
13:02:09 13 declaration again. It's Exhibit 2011. If you'll turn
13:02:18 14 to paragraph 40.

13:02:31 15 Now, this paragraph is where you begin
13:02:33 16 discussing Zavgorodny; is that correct?

13:02:35 17 A. Yes, it is.

13:02:42 18 Q. Now, is Zavgorodny directed to sequencing by
13:02:46 19 synthesis?

13:02:47 20 A. No, it is not.

13:02:52 21 Q. Is it directed to synthetic organic chemistry?

13:02:57 22 A. Yes.

13:02:57 23 Q. And --

13:03:04 24 A. Again, I've seen so many of these. Yes. Yes.

13:03:09 25 Q. Now, you talk about some other references

13:03:11 1 throughout your declaration, and I believe those also
13:03:15 2 deal with synthetic organic chemistry. I'm just going
13:03:19 3 to ask you about them. If you need to see them, I'm
13:03:22 4 happy to show them to you, but it's just a simple
13:03:24 5 question.

13:03:26 6 Is Greene and Wuts directed to synthetic
13:03:28 7 organic chemistry?

13:03:31 8 A. It is.

13:03:31 9 Q. How about the Loubinoux reference?

13:03:33 10 A. It is.

13:03:34 11 Q. Now, are you familiar that Ju, the Ju
13:03:38 12 reference, cites to a reference, Kamal, that discusses
13:03:45 13 the allyl group? Are you familiar with that reference?

13:03:48 14 A. Yes.

13:03:48 15 Q. Now, is that directed to synthetic organic
13:03:53 16 chemistry?

13:03:54 17 MS. SWAROOP: Object as outside the scope.

13:03:55 18 THE WITNESS: I'd have to see it to -- I would
13:03:56 19 like to see it to remember. Again, I apologize. I've
13:03:59 20 just read so many of these.

13:04:01 21 BY MR. SEGAL:

13:04:01 22 Q. No, that's fine.

13:04:12 23 While we're waiting for that, if you want to
13:04:14 24 look at Ju, column 3, right around line 22 or 23, Ju
13:04:45 25 introduces both the MOM and allyl group and cites to

13:04:49 1 references by Ireland and Kamal.

13:04:53 2 Do you see that?

13:04:54 3 A. I do.

13:05:03 4 Q. I'm going to show you the Kamal reference, and
13:05:05 5 we're going to mark it as Exhibit 1018.

13:05:13 6 MS. SWAROOP: Counsel, as you know, since
13:05:15 7 you're marking it as a new exhibit, it's not a reference
13:05:18 8 that Dr. Romesberg reviewed in forming his opinions in
13:05:21 9 this case, and we would object as outside the scope.

13:05:25 10 (Exhibit 1018 was marked for identification.)

13:05:27 11 BY MR. SEGAL:

13:05:27 12 Q. Again, is this Kamal reference directed to
13:05:30 13 organic synthetic synthesis?

13:05:33 14 MS. SWAROOP: Object as outside the scope.

13:05:34 15 THE WITNESS: So I'm glancing at his table, and
13:05:37 16 I would guess not based on the substrates that he shows.

13:05:55 17 BY MR. SEGAL:

13:05:55 18 Q. Is it directed to a removal method for removing
13:05:58 19 an allyl ether group?

13:06:01 20 MS. SWAROOP: Object as outside as the scope.

13:06:04 21 And, Dr. Romesberg, take as much time as you
13:06:07 22 need to review this.

13:06:14 23 THE WITNESS: From a glance at it, it looks
13:06:16 24 like that's correct, I mean -- and certainly looking at
13:06:19 25 the title suggests that's correct.

13:06:21 1 BY MR. SEGAL:

13:06:22 2 Q. Is Kamal directed to sequencing by synthesis?

13:06:25 3 MS. SWAROOP: Object as outside the scope.

13:06:27 4 THE WITNESS: From my glance as it, as I said a
13:06:29 5 minute ago, I don't think so.

13:06:36 6 MR. SEGAL: I'm going to also show you the
13:06:38 7 Ireland reference that's cited by Ju in that column 3.

13:06:42 8 I'm going to mark the Ireland reference as
13:06:44 9 Exhibit 1019.

13:06:46 10 (Exhibit 1019 was marked for identification.)

13:07:01 11 MS. SWAROOP: Let me object to this exhibit
13:07:03 12 also as outside the scope as it's not a reference that
13:07:06 13 Dr. Romesberg considered in forming his opinions.

13:07:10 14 BY MR. SEGAL:

13:07:10 15 Q. And you can -- I just have one simple question:
13:07:14 16 Is this Ireland reference directed to synthetic organic
13:07:19 17 chemistry?

13:07:19 18 A. It looks to be.

13:07:29 19 MS. SWAROOP: And before you move on, I'd also
13:07:31 20 like to object to both Exhibits 1018 and 1019 on the
13:07:35 21 grounds of relevance.

13:07:59 22 MR. SEGAL: I'm going to hand you a reference
13:08:02 23 with the first named author Sergey Zavgorodny which has
13:08:07 24 been previously marked as Exhibit 1004.

13:08:10 25 (Exhibit 1004 was referred to.)

13:08:19 1 BY MR. SEGAL:

13:08:19 2 Q. Have you seen that reference before?

13:08:20 3 A. It is one of the papers that I considered, yes.

13:08:23 4 Q. And you've reviewed that reference?

13:08:25 5 A. I have.

13:08:28 6 Q. And was Zavgorodny directed to nucleoside
13:08:33 7 chemistry?

13:08:34 8 A. It is, correct, yes.

13:08:46 9 Q. Would you agree with me that Zavgorodny
13:08:48 10 discloses using an azidomethyl to protect the 3'
13:08:56 11 hydroxyl group of a nucleoside?

13:09:01 12 A. Yes.

13:09:05 13 Q. Now, this same position, the 3' hydroxyl
13:09:09 14 position, this is the same position that the nucleotides
13:09:14 15 in Ju's process require for protection?

13:09:17 16 MS. SWAROOP: Let me object to the form of the
13:09:19 17 question.

13:09:22 18 THE WITNESS: They both involve blocking --
13:09:24 19 they both involve a protecting group that's modifying
13:09:28 20 the 3'-O group of a -- of course, it's of a nucleotide
13:09:37 21 in the end in one, and nucleoside in the other; but,
13:09:43 22 yeah, I mean that's -- those obviously are related.

13:09:47 23 And I'd also mention that Zavgorodny's is also
13:09:50 24 one of many -- the azidomethyl is one of many that he
13:09:56 25 suggests.

13:09:56 1 BY MR. SEGAL:

13:09:57 2 Q. Now this 3' hydroxyl position that Zavgorodny
13:10:04 3 is showing the azidomethyl group protecting, is this the
13:10:07 4 same position that the nucleotides in Tsien's process
13:10:11 5 require for protection?

13:10:12 6 MS. SWAROOP: Object to the form of the
13:10:13 7 question.

13:10:15 8 THE WITNESS: It is.

13:10:19 9 BY MR. SEGAL:

13:10:19 10 Q. Now, you would agree with me that a person of
13:10:22 11 ordinary skill back in 2002 that's considering
13:10:28 12 protecting groups for the 3' hydroxyl position of a
13:10:31 13 nucleotide would not disregard a blocking group just
13:10:36 14 because the art only showed it being used with
13:10:40 15 nucleosides?

13:10:44 16 A. No. As I discussed earlier, there would be a
13:10:46 17 lot that would weigh into your decision about how
13:10:49 18 promising a particular protecting group was to explore,
13:10:52 19 and the fact that it only had been looked at with a
13:10:56 20 nucleoside would not have been particularly strong
13:10:58 21 against you -- strong motivation for being against being
13:11:03 22 interested in it.

13:11:04 23 Q. Now, in 2002, would a person of ordinary skill
13:11:08 24 in the art have been able to convert a nucleoside
13:11:11 25 molecule to a nucleotide molecule?

13:11:23 1 A. Yeah. I mean maybe not efficiently, but that's
13:11:26 2 actually a reaction that we made a pretty significant
13:11:29 3 improvement to a couple years ago, so the yields before
13:11:32 4 our improvement were actually pretty lousy.

13:11:35 5 But, yeah, they could have in a very
13:11:37 6 inefficient way. It's actually not a trivial thing to
13:11:41 7 do, to make a nucleoside that way -- sorry, to make a
13:11:44 8 nucleotide triphosphate. But, yeah, they could have
13:11:47 9 accessed the triphosphate.

13:11:50 10 Q. Now, you would agree with me that Zavgorodny
13:11:54 11 teaches that the azidomethyl group can be removed to
13:11:57 12 expose a 3' hydroxyl group?

13:12:01 13 A. I'd have to carefully look at this again, but I
13:12:04 14 don't think he shows data. I think that he describes
13:12:06 15 them as being removable, yes.

13:12:08 16 So, again, it comes to my discomfort on using
13:12:12 17 the word "teaching" in a legal way. He suggests it.

13:12:19 18 Q. Now, as an example, Zavgorodny teaches that
13:12:23 19 azidomethyl can be removed -- or suggests, to use your
13:12:28 20 language -- by using triphenylphosphine and aqueous
13:12:33 21 pyridine.

13:12:34 22 Would you agree with that?

13:12:35 23 A. Yes, I do.

13:12:42 24 Q. Are you familiar with the Staudinger reaction?

13:12:45 25 A. I am.

13:12:50 1 Q. Can you just explain briefly, what the
13:12:53 2 Staudinger reaction is.

13:12:55 3 A. How much detail would you like?

13:12:56 4 Q. Do it at a fairly high level at first.

13:13:00 5 A. So a Staudinger reaction is a reaction that
13:13:02 6 results in the reduction of an azide to an amine.

13:13:07 7 It goes through the intermediate of something
13:13:10 8 called a phospho ylide, so it's -- you have -- you have
13:13:16 9 triphenylphosphine. The triphenylphosphine attacks a
13:13:22 10 nitrogen, a terminal nitrogen.

13:13:24 11 You form a four-membered ring, it's called the
13:13:28 12 ylide, that's y-l-i-d-e; that then expuls- -- the
13:13:32 13 driving force for the decomposition there is expulsion
13:13:36 14 of diatomic nitrogen, N₂, and that leaves behind what's
13:13:40 15 called this ylide, this P-minus attached directly to a
13:13:44 16 nitrogen, and -- sorry, a P-nitrogen ylide, and that
13:13:51 17 then -- then that hydrolyzed -- that cleaves to an
13:13:54 18 amine.

13:13:56 19 Q. You mentioned triphenylphosphine.

13:13:59 20 Is the Staudinger reaction limited to using
13:14:02 21 triphenylphosphine to react with an azide, or can --

13:14:11 22 A. You know, that depends on who you ask. Purists
13:14:15 23 love name reactions, synthetic chemists. And so
13:14:20 24 technically I think it is, but I think in practice, it's
13:14:22 25 not. You can access the same mechanism with different

13:14:25 1 phosphines.

13:14:27 2 Q. Now, back to Zavgorodny, we mentioned that the
13:14:33 3 conditions that Zavgorodny suggests for removing an
13:14:39 4 azidomethyl triphenylphosphine and aqueous pyridine at
13:14:45 5 20 degrees Celsius.

13:14:48 6 Would one of skill in the art recognize these
13:14:51 7 removal conditions as involving the Staudinger reaction?

13:14:59 8 A. Are you asking if the Staudinger reaction
13:15:02 9 requires pyridine as a solvent?

13:15:05 10 Q. No, just would whether one of skill in the art,
13:15:09 11 reading Zavgorodny back in 2002, would they recognize
13:15:14 12 that the mechanism that Zavgorodny suggests,
13:15:18 13 triphenylphosphine -- would they recognize that as
13:15:21 14 involving the Staudinger reaction?

13:15:25 15 A. Yes, that -- you're asking would the -- would
13:15:27 16 someone realize that that deprotection mechanism was via
13:15:31 17 Staudinger reaction?

13:15:33 18 Q. Yes.

13:15:33 19 A. Yeah.

13:15:38 20 Q. In 2002, were water soluble phosphines reagents
13:15:46 21 known?

13:15:46 22 A. Yeah. They were rare, but they were known.

13:15:49 23 Q. In 2002, was TCEP known?

13:15:53 24 A. I believe so, yeah. I think Carolyn
13:15:56 25 Bertozzi -- I don't know if she was the first, but she

13:15:59 1 published a reaction that became, in later years,
13:16:01 2 extraordinarily popular that's related to the Staudinger
13:16:05 3 reaction, so it's called the Staudinger ligation. The
13:16:08 4 differences are, I think from our -- for our issues,
13:16:11 5 trivial.

13:16:12 6 And her method requires water, use an -- a
13:16:19 7 water environment, so she had to modify it to -- for
13:16:22 8 that.

13:16:27 9 Q. Again, looking at conditions that Zavgorodny
13:16:31 10 suggests, the triphenylphosphine and aqueous pyridine at
13:16:34 11 20 degrees Celsius, would a person of ordinary skill in
13:16:40 12 the art understand that you could use the
13:16:43 13 triphenylphosphine with other organic solvents?

13:16:53 14 A. Yes.

13:17:01 15 Q. You mentioned that other water soluble
13:17:04 16 phosphine reagents were known.

13:17:05 17 Do you know what those other --

13:17:07 18 A. No, and I --

13:17:07 19 Q. -- water soluble phosphine reagents are?

13:17:10 20 A. No. Like I said, I think that Bertozzi's
13:17:13 21 reagent was TCEP, but it was a very small segment of
13:17:18 22 the -- it was not used by synthetic chemists at all.

13:17:22 23 It was -- it was the beginning of what became
13:17:25 24 known as bioorthogonal -- the field of bioorthogonal
13:17:29 25 chemistry.

13:17:37 1 Q. If you want to turn to paragraph 44 of your
13:17:39 2 declaration, Exhibit 2011.

13:17:50 3 And in this paragraph you discuss a reference
13:17:52 4 by Loubinoux; is that right?

13:17:55 5 A. Yes.

13:18:01 6 Q. So in 2002, if a person of ordinary skill in
13:18:03 7 the art is considering using an azidomethyl method group
13:18:08 8 to protect the 3' oxygen of a nucleotide, a person of
13:18:12 9 ordinary skill in the art might look to this Loubinoux
13:18:16 10 reference for guidance?

13:18:19 11 MS. SWAROOP: Object to the form of the
13:18:20 12 question.

13:18:22 13 THE WITNESS: Yes.

13:18:23 14 BY MR. SEGAL:

13:18:23 15 Q. Now, I believe it's your opinion that Loubinoux
13:18:27 16 would have discouraged a person of ordinary skill in the
13:18:30 17 art from using an azidomethyl protecting group in the Ju
13:18:34 18 and Tsien methods; is that right?

13:18:37 19 A. It is.

13:19:10 20 MR. SEGAL: I'm going to hand you what's been
13:19:14 21 previously marked as Exhibit 1106. It's a reference
13:19:18 22 with the first named author, Loubinoux.

13:19:22 23 (Exhibit 1106 was referred to.)

13:19:25 24 BY MR. SEGAL:

13:19:25 25 Q. I should also point out for the record that

13:19:28 1 it's an English translation. The original was in
13:19:31 2 French, and we've had it translated and that's what I've
13:19:34 3 handed to you.

13:19:37 4 Are you familiar with this reference?

13:19:39 5 A. I am. It's one that I listed as having --
13:19:42 6 having considered when writing my declaration.

13:19:46 7 Q. And you have reviewed the reference?

13:19:50 8 A. Yes.

13:19:51 9 Q. Would you agree with me that Loubinoux shows
13:19:54 10 that successful use of an azidomethyl group to protect
13:19:58 11 the hydroxyl group of a phenol?

13:20:02 12 A. Yes. Well, "successful" is a relative term.
13:20:09 13 Successful for his applications, sure.

13:20:12 14 Q. Would you agree with me that Loubinoux
13:20:14 15 discloses a number of methods for removing an
13:20:20 16 azidomethyl group?

13:20:22 17 A. I believe he discusses three, so yes.

13:20:25 18 Q. And one of these conditions for removing the
13:20:29 19 azidomethyl group is triphenylphosphine and water in
13:20:33 20 tetrahydrofuran?

13:20:39 21 A. Triphenyl phosphene is aqueous THF, correct,
13:20:43 22 yeah. That is one of the standard conditions.

13:20:47 23 Q. If you'll turn to page 4 -- the number's on the
13:20:56 24 bottom -- you'll see that last sentence there.
13:21:01 25 Loubinoux discloses a removal method for azidomethyl,

13:21:09 1 and he talks about the treatment with triphenylphosphine
13:21:13 2 and then with water, in tetrahydrofuran at 25 degrees
13:21:18 3 Celsius.

13:21:19 4 Do you see that?

13:21:20 5 A. I do.

13:21:20 6 Q. And then you see that there's a reference to 4?

13:21:28 7 A. I do.

13:21:29 8 Q. If you'll turn to the back where the references
13:21:32 9 are, 4 refers to publications by Vaultier's group.

13:21:43 10 Do you see that?

13:21:44 11 A. I do.

13:21:46 12 Q. Did you review the references identified as
13:21:49 13 No. 4 there?

13:21:50 14 A. I did not.

13:22:00 15 Q. Now, if you turn back to page 4, the conditions
13:22:03 16 we just read, triphenylphosphine and then with water in
13:22:07 17 tetrahydrofuran, those conditions are different than the
13:22:11 18 conditions that Zavgorodny suggests; is that right?

13:22:13 19 A. They are.

13:22:21 20 Q. You testified that Loubinoux teaches or
13:22:24 21 discloses three methods for removal of an azidomethyl
13:22:30 22 group?

13:22:31 23 A. I think three. I think he described tin
13:22:32 24 chloride, hydrogenation, and triphenylphosphine.

13:22:39 25 Q. Would you agree with me that that is not an

13:22:42 1 exhaustive list of the methods known to a person of
13:22:45 2 ordinary skill in the art in 2002 for removing an
13:22:49 3 azidomethyl group?

13:22:52 4 MS. SWAROOP: Object to the form of the
13:22:53 5 question and object as outside the scope.

13:22:55 6 THE WITNESS: I haven't thought -- I haven't
13:22:58 7 considered that. I think -- I mean those are certainly
13:23:01 8 the ways I would first consider.

13:23:03 9 Are there other ways? Probably.

13:23:08 10 It would not surprise me if there were other
13:23:10 11 ways. I believe these are certainly the most common, I
13:23:13 12 believe. That's not to say that there's not another
13:23:17 13 reaction that could do it.

13:23:18 14 I mean certainly there are other reactions that
13:23:20 15 can do it. They tend not to be used, to my knowledge,
13:23:24 16 at least not used with the same frequency; in
13:23:31 17 particular, the same frequency as the hydrogenation and
13:23:34 18 the triphenylphosphine.

13:23:39 19 BY MR. SEGAL:

13:23:39 20 Q. If you turn back to paragraph 44 of your
13:23:42 21 declaration, about six lines down, you state that
13:23:51 22 "Loubinoux found no greater than 60 to 80 percent
13:23:54 23 removal of the azidomethyl group from phenol derivatives
13:23:59 24 using triphenylphosphine."

13:24:02 25 Is that right?

13:24:04 1 A. Yes, that -- that is correct.

13:24:09 2 Q. Now, you say in that statement, "60 to
13:24:12 3 80 percent removal."

13:24:15 4 Loubinoux is not actually measuring removal; is
13:24:19 5 that correct?

13:24:37 6 A. I think he is.

13:24:37 7 Q. Let me ask it a different way. The yields
13:24:40 8 given in the Loubinoux reference, and in particular
13:24:44 9 table 2 of the Loubinoux reference on page 7, would you
13:24:52 10 agree that Loubinoux is measuring the yield of the pure
13:24:55 11 products after the removal reaction of the azidomethyl
13:24:59 12 group?

13:25:13 13 A. I don't remember if the yields that he lists in
13:25:16 14 the table are -- so looking at table 1, he captions the
13:25:23 15 yield and says the yield of pure product isolated over
13:25:27 16 two steps; so certainly in table 1, I would agree.

13:25:35 17 And then table 2, he doesn't say how he
13:25:38 18 describes yield, and I'm trying to remember -- in the
13:25:40 19 text, I think he describes -- I thought he described
13:25:45 20 these as measured indirectly and that the yields --

13:25:53 21 Q. Maybe I can help you.

13:25:55 22 If you turn to page 5 of the Loubinoux
13:25:58 23 reference, do you see that, I guess, second paragraph
13:26:04 24 there --

13:26:05 25 A. Yes.

13:26:05 1 Q. -- where it says "Table 2 Reports"?

13:26:06 2 A. Yes.

13:26:07 3 Q. Now, does that confirm to you --

13:26:09 4 A. Yes.

13:26:09 5 Q. -- that Loubinoux --

13:26:10 6 Just for the record, does that confirm to you
13:26:12 7 that Loubinoux's yields are yields of the pure product
13:26:17 8 after a purification?

13:26:18 9 A. Of 60 to 80 percent, yes.

13:26:22 10 Q. Now, you would agree that it's possible that
13:26:25 11 you can lose some of the resulting product during
13:26:29 12 purification process?

13:26:33 13 A. That is possible.

13:26:36 14 Q. And then, in effect, that the actual removal
13:26:39 15 efficiency of the azidomethyl group from the hydroxyl
13:26:47 16 can actually be higher than the actual purification
13:26:50 17 yield?

13:26:52 18 A. One could speculate that but, in general,
13:26:55 19 people want to publish good yields. It's -- certainly
13:26:58 20 the student doing it is under pressure to get the
13:27:01 21 correct yields, and the PI who's publishing the paper
13:27:05 22 wants to make his paper, his method, and his results
13:27:08 23 look good; so, in general, synthetic chemists try to get
13:27:12 24 the highest yields possible.

13:27:15 25 Is it -- there's certainly some amount lost in

13:27:18 1 purification. That's always the case, and you don't
13:27:21 2 know how much that is. What I will say is that
13:27:25 3 reporting purified yields of 60 to 80 percent in no way
13:27:29 4 are going to give someone the expectation that the
13:27:32 5 yields were actually 99 percent or 98 percent and that
13:27:35 6 you're just losing everything up on purification.

13:27:38 7 Q. No?

13:27:39 8 A. No.

13:27:39 9 Q. You're not familiar with any references that
13:27:42 10 actually say that the reaction itself goes quantitative,
13:27:50 11 but when they go to purify the product, they may only
13:27:53 12 get 60 to 80 percent?

13:27:55 13 A. Oh, I'm sure that there are -- I'm sure there
13:27:55 14 are references that you can show that. What I'm talking
13:27:58 15 about is what the average person -- the average expert
13:28:00 16 in the field would have thought, and that is certainly
13:28:03 17 not the typical case.

13:28:04 18 So I'm sure there are cases that you get
13:28:05 19 100 percent conversion and you get 10 percent recovery
13:28:09 20 because of some idiosyncratic aspect of the purification
13:28:14 21 procedure -- it wasn't optimized or there was just some
13:28:17 22 weirdness to it, it was particularly challenging -- but
13:28:20 23 certainly the vast majority of experts in the field
13:28:22 24 would look at 60 to 80 percent and assume that was very
13:28:26 25 close to the theoretical maximum.

13:28:29 1 Q. Now, with respect to pure product yields, a
13:28:34 2 person of ordinary skill in the art could optimize
13:28:38 3 conditions to obtain higher yields.

13:28:43 4 Would you agree with that?

13:28:45 5 A. Up to the maximum conversion, yes.

13:28:49 6 You could -- that is certainly possible, but
13:28:52 7 like I just said, some reactions are tough to purify,
13:28:55 8 some reactions are easy to purify, but usually people
13:29:01 9 consider this to be something that you could get most of
13:29:03 10 your product to be recovered, yes.

13:29:07 11 Q. So if you had a product that was difficult to
13:29:09 12 purify, it's possible that the reaction actually goes
13:29:13 13 quantitative but that because of the difficult
13:29:16 14 purification products, you actually get a low pure
13:29:20 15 product yield?

13:29:21 16 A. That's certainly possible, and I certainly
13:29:24 17 would expect that to be pointed out in the text, because
13:29:28 18 people could say that they measured yields by NMR,
13:29:31 19 people could say that they measured yields by UV and
13:29:35 20 they consistently found the reaction itself yielded
13:29:38 21 this, but then upon purification, they could only
13:29:41 22 recover this.

13:29:41 23 Typically a good scientist then would track
13:29:43 24 down and show where he'd lost the material, was it in
13:29:45 25 some sort of extraction, and then he could show it was

13:29:48 1 partitioning in both layers and they couldn't get it
13:29:52 2 out.

13:29:52 3 So you would track that down. You probab- --
13:29:54 4 certainly would not just leave it at 60 percent or
13:29:57 5 50 percent, or whatever the lower yield was. You would
13:30:00 6 probably point that out, and you would probably do a
13:30:03 7 little science to try to fix it and report it.

13:30:06 8 Q. Now, in organic chemistry, synthetic organic
13:30:09 9 chemistry, isn't -- an 80 percent pure product yield,
13:30:15 10 isn't that considered good?

13:30:16 11 A. It depends who you ask, but generally no.

13:30:21 12 So it is a -- synthetic chemists, how do I
13:30:24 13 describe them? Proud, competitive, yields
13:30:27 14 extraordinarily high are expected, and I mean it's an
13:30:34 15 interesting thing, because in my group, for example --
13:30:36 16 I'm not a synthetic chemist -- and so we do
13:30:40 17 transformations for our own purposes, and I don't allow
13:30:43 18 my students a lot of luxury to optimize things because
13:30:46 19 we're interested in doing something with it.

13:30:49 20 And I'll have fights where I'll say, That's
13:30:52 21 good enough; let's move on to what we really want to do,
13:30:54 22 as opposed to a synthetic chemist, where what they're
13:30:58 23 doing is the art of synthesis. They're producing
13:31:01 24 things, and so it's a constant fight with me and my
13:31:04 25 students to make them get past that. Don't worry that

13:31:07 1 it's in the 70 percent yield, because we want to go on
13:31:11 2 and do something. That just gets buried in the
13:31:14 3 materials and methods. No one cares.

13:31:16 4 In papers that are synthetic papers, this is
13:31:19 5 what distinguishes good ones from bad ones -- good
13:31:23 6 synthetic chemists from poor synthetic chemists: People
13:31:27 7 who achieve 96 percent yields.

13:31:30 8 Q. Are there other ways to measure removal
13:31:32 9 efficiency other than using a pure product yield?

13:31:37 10 A. Sure. As I mentioned earlier, you could
13:31:39 11 measure -- if you were only interested in the reaction
13:31:42 12 and you were trying to quantify that, you could use --
13:31:44 13 you could characterize it in the unpurified state.

13:31:47 14 So if it has a unique NMR signal, a unique UV
13:31:53 15 signal, you could characterize it that way. And that
13:31:56 16 gets a little tricky, and the reason people generally
13:32:00 17 don't like that is because you're going to get a signal,
13:32:00 18 let's say an NMR signal, and you have to then find a way
13:32:02 19 to translate that into a yield.

13:32:04 20 So you have to know what the pure compounds NMR
13:32:06 21 signal looks like and how that NMR signal scales as a
13:32:11 22 function of how much material you have.

13:32:13 23 That means you have to have the pure product.
13:32:15 24 So if you can get the pure product, then you're going to
13:32:20 25 get a yield; and if you can't get the pure product, then

13:32:23 1 you can't really use these techniques, so these
13:32:27 2 techniques are going to be approximate by definition.
13:32:29 3 As a result, they're going to be approximate techniques.

13:32:33 4 Q. If you could use such methods, the NMR method,
13:32:37 5 for example, would they be more accurate as a way to
13:32:40 6 measure the actual removal efficiency than relying on
13:32:43 7 the pure product yield?

13:32:46 8 A. Maybe. I mean, what I just said is they are
13:32:47 9 inherently less accurate because you have to do a
13:32:51 10 translation.

13:32:52 11 There's nothing more accurate than putting
13:32:54 12 something on a scale and weighing it. That's the most
13:32:57 13 accurate way to do it. That's why synthetic chemists
13:33:01 14 always try to do that.

13:33:03 15 Your -- if you're not doing that and you're
13:33:05 16 trying to use a method like NMR, like we're describing,
13:33:09 17 or UV, where you actually measure your product using
13:33:13 18 that as a surrogate signal, you have to then translate
13:33:16 19 that into a quantity that's present, and you need to
13:33:19 20 know -- if it's UV, it's called molar extinction
13:33:22 21 coefficient, and NMR, it's just the -- it's called the
13:33:26 22 gyromagnetic ratio.

13:33:29 23 And so what these are, these are constant
13:33:33 24 proportionalities that relate the intensity of the
13:33:36 25 signal to the compound, and all compounds have different

13:33:39 1 values. So if you can't purify the compound, you don't
13:33:43 2 know what the value is, so what people tend to do is
13:33:46 3 they tend to say, Well, someone else purified this
13:33:49 4 related compound and it has an extinction coefficient of
13:33:54 5 this, so we're going to use that.

13:33:56 6 That is maybe close; maybe it's not. So, by
13:33:59 7 definition it's inherently a less accurate method. It's
13:34:04 8 more accurate from the simple perspective that you're
13:34:07 9 eliminating a step so you're not purifying it, but it's
13:34:11 10 inherently less accurate because you are actually not
13:34:14 11 able to measure what you're trying to measure.

13:34:18 12 Q. Now, you would agree with me that if your
13:34:21 13 purification process is inefficient, then your pure
13:34:26 14 product yield is not a good proxy for your removal
13:34:30 15 efficiency?

13:34:31 16 A. Sure, yes. But, again, if I were -- if I
13:34:37 17 found --

13:34:38 18 Q. There's no question pending.

13:34:43 19 MS. SWAROOP: Dr. Romesberg, finish your
13:34:45 20 response, please.

13:34:46 21 MR. SEGAL: I'm just trying to get through.

13:34:48 22 MS. SWAROOP: Finish your response, please.

13:34:48 23 THE WITNESS: What I would say is that as far
13:34:49 24 as it not being a good proxy, if you can't purify it, I
13:34:52 25 don't think simply reporting -- I think that what the

13:34:56 1 correct prox- -- what the correct response there would
13:34:58 2 be, would be to purify it, show the yield you get,
13:35:02 3 measure the extinction coefficient, use that to go back
13:35:05 4 and measure what the actual yield was by your NMR signal
13:35:10 5 or UV signal or whatever, and describe that.

13:35:13 6 And then if you do that, I would find that
13:35:15 7 convincing and I would say, Okay, it was a purification
13:35:18 8 problem; they couldn't purify it for this reason but
13:35:21 9 that's the purification yield and that's the inherent
13:35:25 10 reaction yield.

13:35:26 11 In --

13:35:27 12 BY MR. SEGAL:

13:35:27 13 Q. I think I understand you.

13:35:29 14 So the best method would be to first purify it,
13:35:32 15 look at the pure product, get the proper NMR -- what did
13:35:37 16 you call that? A signal? NMR --

13:35:39 17 A. Technically, it's a gyromagnetic ratio. It's
13:35:43 18 just the constant proportionality between the signal and
13:35:46 19 the quantity of the material.

13:35:48 20 Q. Use that NMR signal -- for shorthand -- to
13:35:51 21 actually measure in real time the removal efficiency.

13:35:55 22 Would that be the best method?

13:35:57 23 A. That would be a convincing method. I mean --
13:35:59 24 best, that would simply probably give you the most
13:36:03 25 accurate data, yeah.

13:36:06 1 Q. Now, the bottom of paragraph 45, second to last
13:36:17 2 sentence there, you state "One of ordinary skill would
13:36:24 3 view Table 2 of Loubinoux as teaching that azidomethyl
13:36:27 4 cleavage yields can vary widely, even when the same
13:36:31 5 removal conditions are used."

13:36:36 6 Now, isn't it possible that the variation could
13:36:39 7 be due to the fact that some of the products are more
13:36:42 8 easily purified?

13:36:49 9 A. It is technically not impossible for that to be
13:36:51 10 the case. It would be very unlikely.

13:36:53 11 They're related compounds -- and, again, if
13:36:57 12 that was the case -- look, the average expert reading
13:37:00 13 this would not assume that because, in general, again --
13:37:02 14 let me emphasize -- that product isolation tends not to
13:37:07 15 ever limit yields. It's just not very common; and if it
13:37:12 16 does, it's just not much; and if it does, you'd think
13:37:15 17 that the author would go out of his way to explain that.

13:37:18 18 In the absence of such an explanation, I think
13:37:20 19 that people would look at the sort of data that's
13:37:23 20 reported in that table and assume that those were
13:37:27 21 reaction efficiencies.

13:37:29 22 Q. Now, the variation in Table 2 that you refer to
13:37:34 23 in your paragraph 45 --

13:37:36 24 A. This is -- I'm sorry. This is Table 2 in
13:37:38 25 Loubinoux?

13:37:39 1 Q. Loubinoux, right.

13:37:49 2 Could -- that variation in Table 2 of
13:37:52 3 Loubinoux, could that be due to the use of different
13:37:56 4 eluents during the purification process?

13:38:03 5 A. Sure. But, again, what people -- the reason
13:38:06 6 different eluents are used is because they're
13:38:08 7 yielding -- optimizing yield; they're not just randomly
13:38:13 8 selected. And this is common, common practice.

13:38:44 9 Q. If you will turn to paragraph 50 of your
13:38:47 10 declaration, if you're on page 24 there, about three
13:38:57 11 lines up from the bottom, you state that the efficiency
13:39:03 12 of the conversion of an azide to an amine can be
13:39:07 13 evaluated as an upper limit to what could have been
13:39:10 14 expected for the entire azidomethyl group removal
13:39:14 15 reaction.

13:39:15 16 Is that your opinion?

13:39:24 17 A. Yes.

13:39:24 18 Q. What's the other step?

13:39:26 19 A. The other step is a hydrolysis step. You --
13:39:28 20 once you have the amine, it decomposes to -- yeah, I'm
13:39:42 21 sorry. I'm drawing a blank. Hold on one second.

13:39:55 22 It's hydro- -- water mediated hydrolysis to the
13:39:59 23 alcohol.

13:40:02 24 Q. Now, do you have any evidence that this
13:40:05 25 hydrolysis step would not be quantitative?

13:40:10 1 A. In what case?

13:40:14 2 Q. In the case of removing an azidomethyl group.

13:40:17 3 A. It very much depends on what you're removing it
13:40:20 4 from.

13:40:22 5 Would you like me to explain that?

13:40:24 6 Q. How about if you're removing it from a hydroxyl
13:40:28 7 group?

13:40:28 8 A. So a hydroxyl group can be very different. A
13:40:31 9 phenolic hydroxyl group is very different than an
13:40:35 10 aliphatic hydroxyl group.

13:40:37 11 Q. How about a hydroxyl group on a nucleotide
13:40:39 12 molecule?

13:40:40 13 A. That would be aliphatic. That's going to be,
13:40:42 14 in general, much worse than a phenolic oxygen and the
13:40:48 15 reason is the ability to cleave that depends on the
13:40:50 16 stability of the free hydroxyl, in particular the
13:40:55 17 free -- before you protonate it, when you're breaking
13:40:59 18 that bond, there's going to be negative charge building
13:41:03 19 up on that oxygen. This is the mechanism of that step.

13:41:08 20 The stability of that negative charge there
13:41:10 21 will be a large -- will make a very large contribution
13:41:11 22 to how fast and efficient that reaction is.

13:41:14 23 When you have a phenolic oxygen, that electron
13:41:18 24 is very stable because it's delocalized into the
13:41:21 25 phenol -- into the aromatic ring.

13:41:25 1 You don't have such a stabilization option.

13:41:27 2 That's not possible with an aliphatic, so aliphatics are
13:41:32 3 a little bit harder to predict.

13:41:35 4 Let me say that in general, and the reason I
13:41:37 5 tried to be clear about this, was that in general -- in
13:41:41 6 general, this step with a good-leaving group like a
13:41:45 7 phenol, will not limit the yield because it's going to
13:41:48 8 happen fast and instantaneously.

13:41:51 9 I mean if you look at Loubinoux's title of his
13:41:55 10 paper, he talks about unstable phenols, because they're
13:41:58 11 not stable because they're hydrolyzing in this last
13:42:01 12 step.

13:42:01 13 Aliphatics aren't like that, and so the
13:42:06 14 question is, how unstable are they? And there
13:42:10 15 certainly -- there certainly will be examples when they
13:42:14 16 could slow the reaction and actually limit efficiency.
13:42:18 17 That's very unlikely to be the case with phenols and
13:42:21 18 it's just sort of hard to guess as to whether or not
13:42:23 19 with aliphatic compounds. It just depends on how stable
13:42:26 20 those leaving groups are.

13:42:28 21 And so, at best, they can not contribute
13:42:32 22 anything; at worst, they could slow the reaction down
13:42:36 23 and make it less efficient.

13:42:38 24 Q. But do you have any evidence or support that
13:42:44 25 the hydrolysis step would slow it down or make it less

13:42:47 1 efficient?

13:42:48 2 A. No. I -- what I just described were chemical
13:42:50 3 principles.

13:43:02 4 No, let me -- may I modify that question --
13:43:04 5 that answer slightly?

13:43:05 6 Q. Sure.

13:43:06 7 A. For example, we just read here where people
13:43:08 8 talked about the reaction done with triphenylphosphine
13:43:10 9 and THF followed by the addition of water.

13:43:14 10 You don't add that water, you're not going to
13:43:16 11 get that last hydrolysis step. It's going to be
13:43:20 12 infinitely slow. So one could easily imagine an example
13:43:23 13 where you only have a little bit of water in there.
13:43:27 14 That hydrolysis step would become really slow and it
13:43:30 15 certainly would limit the reaction. So if you were
13:43:31 16 trying to run a Staudinger-like reaction in the presence
13:43:33 17 of only a trace amount of water, that step, because it
13:43:36 18 requires the -- it requires the participation of a water
13:43:39 19 molecule, that step will become slow and it will limit
13:43:43 20 the entire reaction, it will limit the entire
13:43:46 21 efficiency, it will limit the overall efficiency. So
13:43:49 22 that's a simple case where it would limit the
13:43:54 23 efficiency.

13:44:12 24 Q. Did you look for any follow-up references that
13:44:16 25 maybe improved upon the pure product yield -- yields of

13:44:20 1 the Loubinoux reference?

13:44:22 2 A. So what I did when I started this work for this
13:44:25 3 IPR, is I just looked in general at Staudinger reactions
13:44:29 4 that had been published.

13:44:30 5 None jumped out to me. I didn't note any of
13:44:35 6 them as being of more significance than what we wound up
13:44:38 7 with here. So I did not see any that had high yields,
13:44:43 8 and that's not to say there aren't any out there. I
13:44:51 9 just didn't find them.

13:44:55 10 Q. If you'll take a look at table 1 in your
13:44:59 11 declaration, starts on page 25.

13:45:05 12 And, again, just so we're clear, you have a
13:45:08 13 column that says "Yield" in that table; is that correct?

13:45:13 14 A. I do, yes.

13:45:14 15 Q. And those yields are, again, pure product
13:45:17 16 yields?

13:45:18 17 A. I believe so.

13:45:20 18 Q. So they're not actually measuring the removal
13:45:22 19 efficiency, or they're -- actually here is the reduction
13:45:27 20 from the azide to the amine?

13:45:30 21 A. Correct. Right. Correct. Again, I'd like to
13:45:34 22 emphasize that you could apply this logic to any
13:45:38 23 reaction, in which case the entire body of our
13:45:41 24 understanding of chemical reactivity would be in
13:45:43 25 question, and there's no one who thinks that.

13:45:46 1 These kind of yields are what the field of
13:45:49 2 synthetic chemistry is based on.

13:46:25 3 MR. SEGAL: I'm going to hand you what's been
13:46:28 4 previously marked as Exhibit 2013, and it's a reference
13:46:32 5 with the first named author, Ranganathan.

13:46:38 6 (Exhibit 2013 was referred to.)

13:46:40 7 BY MR. SEGAL:

13:46:56 8 Q. Is this the reference you're referring to on
13:46:58 9 table 1 on page 25 of your declaration?

13:47:01 10 A. It would appear to be, yes.

13:47:07 11 Q. Again, just to confirm, if you want to look on
13:47:11 12 page 4343, the 50 percent yield in your table 1 is a
13:47:16 13 reference to a pure product yield?

13:47:20 14 A. I believe that is correct.

13:47:25 15 Q. And this Exhibit 2013, the purification process
13:47:34 16 is described as a two-step process; a Dekker column
13:47:39 17 followed by crystallization? Would you agree with me?

13:47:42 18 A. Yes.

13:47:46 19 Q. Would you agree that it's possible that you
13:47:49 20 lose some product when you perform the Dekker column
13:47:53 21 purification step?

13:47:54 22 A. It is.

13:47:54 23 Q. Now, is it also possible that you lose some
13:47:59 24 product when you perform the crystallization step?

13:48:04 25 A. It is.

13:48:05 1 Q. So you would agree with me that the actual
13:48:09 2 efficiency of the reaction, the conversion of the azide
13:48:14 3 to the amine, could actually be much higher than the
13:48:17 4 50 percent yield?

13:48:19 5 A. I would not agree with that, just because you
13:48:21 6 used the word "much."

13:48:23 7 Q. It could be higher?

13:48:24 8 A. Sure.

13:48:34 9 Q. Do you see where we're looking there above the
13:48:37 10 figure 4343, the top figure? In that second full
13:48:41 11 paragraph where Ranganathan discloses his mechanism for
13:48:50 12 reducing the azide to the amine, he says "treatment of
13:48:56 13 compound VIIIf with triphenylphosphine and pyridine."

13:49:02 14 Do you see that?

13:49:03 15 A. I do.

13:49:04 16 Q. He references a reference 11. If you turn to
13:49:10 17 the back, 11 is to a reference by Mungall.

13:49:19 18 Do you know if that's the Mungall reference
13:49:21 19 that you also referred to in your table 1?

13:49:24 20 A. I do not know. I believe it is.

13:49:28 21 Q. Well, if you just compare the citation to --

13:49:31 22 A. It would appear to be, yes.

13:49:51 23 MR. SEGAL: Okay. I'm going to hand to you
13:49:53 24 what has been previously marked as Exhibit 2014. It's a
13:49:58 25 reference with the first named author, Mungall.

13:50:04 1 (Exhibit 2014 was referred to.)

13:50:10 2 BY MR. SEGAL:

13:50:10 3 Q. Is this the Mungall reference that you refer to
13:50:13 4 in your table 1 in your declaration?

13:50:15 5 A. It would appear to be, yes.

13:50:35 6 Q. Now, would you agree that Mungall uses ammonia
13:50:38 7 to hydrolyze the intermediate formed -- I'm going to
13:50:43 8 butcher this -- phosphinimine?

13:50:47 9 A. Yes.

13:50:55 10 Q. You would agree that the yields that you
13:50:57 11 provide in your table are, again, pure product yields --

13:51:01 12 A. Yes.

13:51:01 13 Q. -- is that correct?

13:51:03 14 And each yield in the table represents the
13:51:09 15 yield after a multi-step purification process?

13:51:13 16 A. Yes.

13:51:20 17 Q. And, again, it's possible that the actual
13:51:23 18 conversion efficiency of the azido to the amine could be
13:51:27 19 higher than the pure product yield?

13:51:30 20 A. Unlikely. Possible. It is not impossible.
13:51:37 21 For about the fifth time, it is very unlikely that it
13:51:42 22 would be significantly higher.

13:51:53 23 Q. And is it possible that if you changed some of
13:51:58 24 the reagents to reduce the azido to the amine, that you
13:52:04 25 could end up with higher yields?

13:52:08 1 A. That is possible. Again, it all depends on how
13:52:14 2 good of a job they did in figuring what their conditions
13:52:16 3 are. If you assume they did a poor job . . .

13:52:20 4 Q. Now, did you look for any references by other
13:52:22 5 researchers that followed up on the -- the methods used
13:52:29 6 in Mungall?

13:52:31 7 A. Not explicitly, no.

13:52:43 8 MR. SEGAL: You can put that one aside.

13:52:56 9 I'm going to hand you what's been previously
13:52:59 10 marked as Exhibit 2015. It's U.S. patent 5,959,089.

13:53:11 11 (Exhibit 2015 was referred to.)

13:53:15 12 BY MR. SEGAL:

13:53:22 13 Q. You've seen this document before?

13:53:24 14 A. Yes, I have.

13:53:25 15 Q. Is this the patent that you reference in your
13:53:29 16 table 1 in your declaration?

13:53:30 17 A. It would appear to be.

13:53:35 18 Q. Just looking at your table there, the azido
13:53:44 19 group is not attached to a nucleotide; is that right?

13:53:49 20 A. That is correct.

13:53:49 21 Q. And it's not attached to a nucleoside either;
13:53:53 22 is that correct?

13:53:54 23 A. That is correct.

13:53:57 24 Q. And the hydrolysis step used in Exhibit 2015
13:54:05 25 uses concentrated ammonia; is that right?

13:54:09 1 A. Yes.

13:54:12 2 Q. And, again, that the yield provided in your
13:54:15 3 table and the yield discussed in Exhibit 2015 is the
13:54:20 4 isolated product yield; is that right?

13:54:23 5 A. I believe that is correct, yes.

13:54:27 6 Q. And Exhibit 2015 discusses actually a
13:54:33 7 multi-purification step; is that correct?

13:54:36 8 A. I'm sorry. Would you repeat that.

13:54:38 9 Q. This Exhibit 2015, the patent that we've been
13:54:43 10 discussing for isolating the pure product and for
13:54:48 11 determining the yield, this patent uses a multi-step
13:54:53 12 purification process; is that right?

13:54:55 13 A. Could you point me to that.

13:55:01 14 Q. If you'll look at page 9, column 16, example 9.

13:55:18 15 A. Yes, that's correct.

13:55:25 16 Q. So it's possible during these multiple steps in
13:55:27 17 the purification process, you'll lose some of your
13:55:30 18 product during each one of these steps; is that correct?

13:55:33 19 A. You know, again, every time you ask me this,
13:55:35 20 I'm going to say correct. There's -- it's likely that a
13:55:38 21 small amount could be lost. It is unlikely that that is
13:55:42 22 a significant amount.

13:56:05 23 MR. SEGAL: I'm going to hand to you what's
13:56:07 24 been previously marked as Exhibit 2016. It's a
13:56:11 25 reference with the first named author, Pilard.

13:56:17 1 (Exhibit 2016 was referred to.)

13:56:20 2 BY MR. SEGAL:

13:56:20 3 Q. If you'll look at your table 1 again, is this
13:56:23 4 the reference, Exhibit 2016, that you refer to in
13:56:29 5 table 1 of your declaration?

13:56:31 6 A. It would appear to be, yes.

13:56:34 7 Q. And, again, your table shows that the azido
13:56:38 8 group that's being reduced to the amine is not attached
13:56:41 9 to a nucleotide; is that right?

13:56:44 10 A. That is correct.

13:56:44 11 Q. And it's not attached to a nucleoside; is that
13:56:47 12 correct?

13:56:48 13 A. That is correct.

13:56:49 14 Q. And you would agree with me that the yield
13:56:51 15 shown in your table there is isolated product yield?

13:56:58 16 A. Could you point -- let me look -- let me look
13:57:02 17 at the paper for a second.

13:57:15 18 Yes.

13:57:15 19 Q. I can help you out here.

13:57:17 20 A. Yes.

13:57:22 21 Q. And again, just for completeness, it's possible
13:57:24 22 also that some of the product is lost during the
13:57:27 23 purification process?

13:57:28 24 A. A small amount, unlikely to be significant.

13:57:48 25 I would also point out that in none of the

13:57:50 1 examples that you've shown me, is there any knowledge
13:57:53 2 that these are difficult compounds to obtain. They are
13:57:55 3 completely generic and there is no reason to suspect
13:57:58 4 their isolation would be challenging.

13:58:02 5 Q. Turn to paragraph 55 of your declaration.

13:58:29 6 In paragraph 55, am I correct that your opinion
13:58:32 7 is that you believe that pyridine would denature DNA?

13:58:39 8 A. Yes.

13:58:39 9 Q. And now, is that under any conditions?

13:58:44 10 A. No. It's under conditions where there's a
13:58:48 11 significant amount of pyridine.

13:58:49 12 So a trace amount of pyridine in water would
13:58:53 13 not denature DNA. A significant concentration of
13:58:56 14 pyridine, under any condition, would denature DNA.

13:59:02 15 Q. So regardless of the pH, pyridine would
13:59:06 16 denature DNA?

13:59:10 17 A. Certainly anything physiological. I would have
13:59:13 18 to think about if there was some mechanism -- excuse
13:59:19 19 me -- whereby it would not.

13:59:23 20 I -- I -- I think it's unlikely -- again, with
13:59:31 21 the exception of just simply removing the pyridine, if
13:59:35 22 you want to consider that a condition, and the pH might
13:59:39 23 affect precisely where -- I mean if you're talking 2,
13:59:43 24 3 percent up here, the pH might impact whether it's
13:59:47 25 3 percent or 2 percent that is required to denature.

13:59:51 1 If you're talking 90 percent pyridine or
13:59:53 2 60 percent pyridine -- and, by the way, Staudinger
13:59:56 3 reactions are done in effectively wet pyridine, which
14:00:01 4 means you have only a little amount of water where, in
14:00:03 5 fact, you couldn't even measure a pH because there's not
14:00:07 6 enough water to measure the pH.

14:00:10 7 You can't measure a pH -- pH is not defined in
14:00:11 8 an organic solution, so if you're talking pH, you're
14:00:16 9 effectively talking about so little pyridine that it
14:00:18 10 doesn't matter.

14:00:19 11 So under those conditions, DNA might survive,
14:00:22 12 and the details of how it survives might depend a little
14:00:27 13 on pH, but -- so that's why I tend not to consider --
14:00:31 14 when you're asking these questions, I'm not really
14:00:33 15 considering pH because it's not defined in an organic
14:00:37 16 solution.

14:00:37 17 Q. How about temperature? Can temperature affect
14:00:40 18 whether pyridine will denature DNA?

14:00:42 19 A. Of course. Yes, sure.

14:00:48 20 To be a little more specific about that, the
14:00:51 21 sorts of -- the sorts of DNA that we're talking about
14:00:55 22 denaturation in this discussion are relatively short
14:01:01 23 pieces of DNA, 21 hybrids, that kind of thing. No
14:01:06 24 temperature is going to protect that to pyridine.

14:01:09 25 If you're talking about chromosomal DNA, very

14:01:12 1 large fragments of DNA that are much, much more stable,
14:01:17 2 then temperature's going to play a role because it is
14:01:19 3 going to be so stable, you're going to have to heat it
14:01:22 4 up a little bit.

14:01:23 5 Q. Now, why is denaturation a problem in an SBS
14:01:29 6 reaction?

14:01:29 7 A. You need to maintain the integrity of the
14:01:32 8 primer templates such that each edition you can be sure
14:01:35 9 is reading successive nucleotides in the template.

14:01:45 10 Q. Even if pyridine denatures DNA, if you then
14:01:50 11 change back to aqueous conditions, won't the DNA
14:01:56 12 reanneal?

14:01:57 13 A. Sure, but maybe not in the same way. So
14:02:00 14 especially with the larger you get, the more likely you
14:02:04 15 are to fall into kinetic traps in reannealing. You
14:02:08 16 would have to cool excessively slowly to make sure that
14:02:12 17 you reanneal correctly, and even then there are all
14:02:16 18 sorts of templates that you need to resequence, things
14:02:18 19 like repeats, things like homopolymeric runs where you
14:02:21 20 wouldn't reanneal correctly.

14:02:24 21 Q. If you look at paragraph 55, again, of your
14:02:28 22 declaration, the first sentence you say "Pyridine was
14:02:34 23 known to be an especially effective DNA denaturing agent
14:02:38 24 in aqueous solutions," and you cite to a Kit reference.

14:02:43 25 Is that right?

14:02:44 1 A. Uh-huh, it is.

14:03:03 2 MR. SEGAL: I'm going to show you what's been
14:03:05 3 previously marked as 2018, the reference with the author
14:03:11 4 Saul Kit.

14:03:14 5 (Exhibit 2018 was referred to.)

14:03:17 6 BY MR. SEGAL:

14:03:20 7 Q. Is this the reference that you are citing to in
14:03:23 8 your paragraph 55 of your declaration?

14:03:25 9 A. It would appear to be, yes.

14:03:32 10 Q. Now, Kit's a review article; is that right?

14:03:35 11 A. It is.

14:03:37 12 Q. Now, you quote a sentence from quit -- Kit in
14:03:43 13 your declaration, 55, if you look on line 3 of your
14:03:47 14 declaration.

14:03:49 15 The quotation is "aromatic reagents, such as
14:03:53 16 phenols and pyridine are especially effective" and then
14:03:56 17 you type in "DNA denaturants."

14:03:59 18 Do you see that?

14:04:00 19 A. I do.

14:04:04 20 Q. And the citation is to page 57 and 58.

14:04:09 21 So if you could turn there, and I believe what
14:04:11 22 you're quoting from is at the very bottom of page 57 and
14:04:21 23 goes on to 58.

14:04:22 24 Is that right?

14:04:23 25 A. It is.

14:04:26 1 Q. And Kit cites to a reference identified as 252;
14:04:31 2 is that right?
14:04:32 3 A. Correct.
14:04:32 4 Q. So if we flip back to the back of Kit, and we
14:04:36 5 look for reference 252, which is on page 79 towards the
14:04:43 6 bottom in the right-hand column.
14:04:46 7 Do you see that reference?
14:04:48 8 A. I do.
14:04:50 9 Q. Did you review that reference?
14:04:52 10 A. I did.
14:04:52 11 Q. Okay. Do you have a copy of that reference?
14:04:56 12 A. No, I do not.
14:04:59 13 MR. SEGAL: Okay. Counsel, we'd ask that be
14:05:01 14 provided to us.
14:05:04 15 MS. SWAROOP: The Gordon reference that's cited
14:05:08 16 in Kit?
14:05:09 17 MR. SEGAL: Yes.
14:05:10 18 MS. SWAROOP: Okay. I'll take a look at it and
14:05:12 19 see.
14:05:13 20 THE WITNESS: I'll end the suspense for you.
14:05:17 21 It ironically contains almost precisely -- and the
14:05:17 22 reason I didn't include it is it contains essentially
14:05:20 23 exactly the same sentence.
14:05:31 24 BY MR. SEGAL:
14:05:31 25 Q. You didn't list that in your materials

14:05:33 1 reviewed, did you?

14:05:34 2 A. That is correct, I did not.

14:05:41 3 Q. Was that an abstract that you reviewed?

14:05:44 4 A. Yes, it was, and that's part of why I didn't
14:05:46 5 list it. I considered the review to be a better source.

14:05:49 6 Q. And did you look for any follow-up papers by
14:05:52 7 these authors --

14:05:56 8 A. I didn't.

14:05:56 9 Q. -- that discussed pyridine and the denaturation
14:05:58 10 of DNA?

14:05:59 11 A. Not specifically for them. I actually looked
14:06:01 12 fairly hard myself for rigorous examples of pyridine
14:06:05 13 denaturation. I did not find by these authors. I
14:06:10 14 didn't explicitly look for them.

14:06:14 15 But I was surprisingly able to find a little
14:06:16 16 example that went in more detail other than "organics
14:06:19 17 denature DNA." I mean it's sort of such a well-known
14:06:22 18 effect and the mechanism is well-known as to why they're
14:06:26 19 all going to denature DNA.

14:06:28 20 And I can explain to you why that's the case if
14:06:30 21 you want me to, but I just found this to be a nice
14:06:36 22 summary of all of that.

14:06:44 23 MR. SEGAL: I'm sorry. I think my question
14:06:46 24 maybe got lost in your answer there.

14:06:49 25 THE WITNESS: No problem. I did not

14:06:50 1 specifically look and find additional reviews by those
14:06:53 2 authors, additional sources by those authors.

14:06:55 3 BY MR. SEGAL:

14:06:56 4 Q. Okay. Thank you.

14:07:25 5 Turning back to your declaration again, about
14:07:31 6 five lines down starting on the right-hand side you
14:07:33 7 state --

14:07:34 8 A. I'm sorry. What page?

14:07:35 9 Q. I'm in paragraph 55 of your declaration, five
14:07:43 10 lines down about, the sentence that starts on that line,
14:07:45 11 "In fact, most organic solvents (including
14:07:49 12 tetrahydrofuran 'THF' and methanol) were known to
14:07:54 13 denature DNA."

14:07:55 14 Do you see that?

14:07:56 15 A. I do.

14:07:56 16 Q. And you cite to a Lee reference --

14:07:59 17 A. I do.

14:08:00 18 Q. -- correct?

14:08:19 19 I'm going to hand to you a document that's been
14:08:23 20 previously marked as Exhibit 2021. It's a reference
14:08:28 21 with the first named author, Lee.

14:08:32 22 (Exhibit 2021 was referred to.)

14:08:42 23 BY MR. SEGAL:

14:08:43 24 Q. Is Exhibit 2021 the reference you are citing to
14:08:48 25 in paragraph 55 of your declaration?

14:08:50 1 A. It is.

14:08:57 2 Q. So if you turn back to your declaration where
14:09:01 3 you cite to Lee, you also quote Lee: "'such unwinding
14:09:09 4 of the DNA duplex caused by dehydration is likely to be
14:09:13 5 a common phenomenon in organic solvents.'"

14:09:19 6 Did I read that right?

14:09:20 7 A. You did.

14:09:29 8 Q. Now, am I correct that Lee is dealing with the
14:09:32 9 supercoiling of DNA helixes?

14:09:34 10 A. Yes.

14:09:35 11 Q. So Lee is not dealing with the denaturation of
14:09:39 12 DNA single strands?

14:09:44 13 A. Those are related processes, but technically
14:09:46 14 correct, yes.

14:09:56 15 Q. And the data provided in Lee does not involve
14:10:00 16 the denaturation of DNA, but is again involved to the --
14:10:05 17 involved with the supercoiling of DNA, correct?

14:10:09 18 A. At some point, the linking number will be so
14:10:13 19 small that it will be denatured, and I'm not sure
14:10:16 20 looking at that -- looking at this data, I'm not sure if
14:10:20 21 he reaches that or not. I'd have to read through this
14:10:23 22 more carefully to see.

14:10:24 23 But if you're unwinding DNA, which is what
14:10:26 24 reducing the linking number means, you're going to be
14:10:30 25 denaturing it, because you're going to be pulling those

14:10:33 1 waters out of the minor and major grooves, and that's
14:10:36 2 what leads to the meltdown, because it reduces the
14:10:40 3 stability. So he's following a process where the
14:10:42 4 stability is being lost.

14:10:44 5 Q. But what he's showing here is the unwinding of
14:10:46 6 the DNA helix, not the denaturation of DNA?

14:10:50 7 A. Yeah, but I believe he has very large pieces of
14:10:53 8 DNA.

14:10:54 9 Q. Can you show me where he talks about the
14:10:56 10 denaturation of the DNA --

14:10:58 11 A. No, he doesn't.

14:10:59 12 Q. -- in his results?

14:11:00 13 A. I don't think he does. I mean, I'd have to
14:11:02 14 look more carefully, but what I put this in for was the
14:11:07 15 quote about the dehydration, because that's the
14:11:08 16 mechanism by which you also reduce denaturation.

14:11:14 17 MR. SEGAL: This would be a good time for a
14:11:17 18 break. We can go off for a few minutes.

14:11:21 19 THE VIDEO OPERATOR: Okay. We are going off
14:11:22 20 the record. This is the end of Disk No. 3. The time is
14:11:25 21 2:11.

14:11:26 22 (Recess from 2:11 p.m. to 2:33 p.m.)

14:33:24 23 THE VIDEO OPERATOR: We are going back on the
14:33:25 24 record. This is the beginning of Disk No. 4. The time
14:33:28 25 is 2:33.

14:33:29 1 MS. SWAROOP: Counsel, before you begin your
14:33:31 2 next line of questioning, I just wanted to state one
14:33:34 3 issue for the record, which is you did mark two new
14:33:37 4 exhibits at the deposition today, Exhibit 1018 and
14:33:40 5 Exhibit 1019.

14:33:41 6 I did object to those depositions [verbatim],
14:33:43 7 but I also want to additionally object on the grounds
14:33:45 8 that we believe that it's improper supplemental evidence
14:33:49 9 submitted outside the time that the Board allows for
14:33:52 10 supplemental evidence under Rule 42.123, so I just
14:33:56 11 wanted to make that record with regard to those two
14:34:00 12 exhibits.

14:34:01 13 MR. SEGAL: Well, we disagree with that
14:34:03 14 objection, but it's on the record.

14:34:05 15 MS. SWAROOP: Thank you.

14:34:10 16 BY MR. SEGAL:

14:34:10 17 Q. If you'll turn back, Dr. Romesberg, to your
14:34:13 18 declaration, in particular the section beginning with
14:34:19 19 paragraph 59, page 34.

14:34:29 20 I believe it's your opinion in this section
14:34:33 21 that the '537 patent satisfied a long-felt need in
14:34:37 22 sequencing.

14:34:38 23 Is that right?

14:34:39 24 A. Yes.

14:34:41 25 Q. And I believe if you look at paragraph 60, that

14:34:46 1 the long-felt need that you're describing is a need for
14:34:50 2 a fast and efficient SBS method.

14:34:54 3 Is that right?

14:34:55 4 A. Yes. I guess I --

14:35:02 5 Q. What do you mean --

14:35:02 6 A. Sorry.

14:35:02 7 Q. What do you mean by "fast"?

14:35:05 8 A. That's what I was about to qualify.

14:35:07 9 Q. Okay.

14:35:08 10 A. I would say fast and efficient meaning
14:35:10 11 sufficient to sequence genomes, and I think at the time
14:35:14 12 that this -- this work represented significant progress
14:35:19 13 towards that.

14:35:22 14 Q. And when you say "fast," are you talking about
14:35:26 15 fast incorporation?

14:35:29 16 A. No. I'm talking about the -- overall, the
14:35:31 17 sequencing reaction. So generally I think when I refer
14:35:34 18 to that, I'm referring to the step that limits the
14:35:37 19 overall process, so that would tend to be the
14:35:40 20 deprotection step.

14:35:42 21 Q. So you would need at least, though, the fast
14:35:45 22 incorporation to have a fast and efficient SBS method?

14:35:49 23 A. Sure. I mean it's -- if your incorporation
14:35:52 24 step were -- required more time than your deprotection
14:35:55 25 step, then that would be the step that limited the

14:35:58 1 overall time required.

14:36:04 2 Q. And you've already mentioned that by "fast,"
14:36:07 3 fast also includes fast cleavage of the protecting
14:36:12 4 group; is that right?

14:36:13 5 A. Correct.

14:36:14 6 Q. And the fast and efficient SBS method would
14:36:19 7 also require a fast cleavage of the label; is that
14:36:22 8 correct?

14:36:24 9 A. That is also correct.

14:36:26 10 Q. And for a fast and efficient SBS method, you
14:36:30 11 would also need a fast method for detecting the label;
14:36:34 12 is that right?

14:36:36 13 A. Sure. I mean to the extent that any of those
14:36:40 14 became the slowest step, they will limit the sequencing
14:36:43 15 reaction; and, like I said, the ballpark number that I
14:36:49 16 have in my head is -- things are obviously much better
14:36:53 17 than this today, but I think in 2002 the expectation
14:36:56 18 would have been that you better be getting to something
14:36:58 19 on the order of an hour overall per base read.

14:37:02 20 So if anything limited that, then it would
14:37:05 21 require that -- so obviously that requires every step to
14:37:09 22 fit within that time period.

14:37:11 23 Q. Now, if I could turn to, what do you mean by
14:37:14 24 "efficient," when you say fast and efficient SBS method?

14:37:19 25 A. So there's sort of different but they are

14:37:22 1 certainly related. A reaction -- you could have a
14:37:25 2 reaction that was efficient, meaning it went to very
14:37:28 3 high yield but that it took multiple hours or took a
14:37:31 4 very long time; or you could have something that was
14:37:33 5 very fast, but it was inherently limited to 80 percent
14:37:37 6 or 85 percent or something, so it would be fast but not
14:37:40 7 efficient.

14:37:41 8 So fast and efficient, how -- usually things
14:37:44 9 that are fast are also efficient, but they don't have to
14:37:48 10 be, so being -- saying "fast and efficient" makes sure
14:37:52 11 that you're covering both of those things.

14:37:56 12 Q. Would the efficiency also include the
14:37:59 13 efficiency of incorporation of a nucleotide; is that
14:38:02 14 right?

14:38:03 15 A. To the extent that that step limited things,
14:38:06 16 then sure. The nucleotide has to be efficiently
14:38:09 17 incorporated.

14:38:11 18 Q. And it would also include things like the
14:38:14 19 cleavage efficiency of the protecting group; is that
14:38:16 20 right?

14:38:17 21 A. Correct.

14:38:18 22 Q. And it would include the cleavage efficiency of
14:38:23 23 the label; is that correct?

14:38:25 24 A. The -- so I believe I just answered these exact
14:38:27 25 questions a second ago, but I would add that the errors

14:38:30 1 associated with cleavage of the fluorophore, of the
14:38:35 2 linker, are rather different than the errors associated
14:38:37 3 with cleavage of the protecting group at the sugar.
14:38:41 4 They are rather more stringent at the sugar.

14:38:44 5 Q. If you'll turn to paragraph 66 of your
14:38:58 6 declaration, you'll see the heading right above
14:39:10 7 paragraph 66 says "Unexpected Results Support The
14:39:13 8 Nonobviousness of the Claims."

14:39:15 9 Did I read that right?

14:39:17 10 A. You did.

14:39:18 11 Q. Now, is it your opinion that the azidomethyl
14:39:21 12 group has unexpected properties over prior art
14:39:26 13 protecting groups for a 3' hydroxyl of nucleotides?

14:39:31 14 A. Yes.

14:39:40 15 Q. Now, if you look at paragraph 67, and in
14:39:46 16 particular for the last couple lines of that paragraph,
14:39:50 17 I just want to direct your attention to that and I'm
14:39:52 18 going to ask you a question.

14:39:54 19 The unexpected property with respect to the
14:39:59 20 azidomethyl, is it your opinion that the property is the
14:40:03 21 ability to be cleaved more rapidly and efficiently?

14:40:06 22 A. That is the most obvious unexpected result,
14:40:09 23 yes; the most obvious unexpected property.

14:40:16 24 Q. And that's the property for which you review
14:40:20 25 the experimental data that was provided to you by

14:40:23 1 Illumina; is that right?

14:40:25 2 A. That's correct.

14:40:31 3 Q. Now, in this section, you compare the removal
14:40:34 4 of the azidomethyl protecting group with the removal of
14:40:40 5 an allyl protecting group; is that right?

14:40:42 6 A. That's correct.

14:40:44 7 Q. Did you compare the azidomethyl protecting
14:40:46 8 group removal rate and efficiency to any other
14:40:51 9 protecting groups?

14:40:55 10 A. No.

14:40:57 11 Q. Now, in this section, you discuss and reference
14:41:03 12 notebook pages provided to you by Illumina; is that
14:41:06 13 correct?

14:41:07 14 A. That is correct.

14:41:08 15 Q. Now, did you review the entire notebooks from
14:41:11 16 which these pages come from?

14:41:14 17 A. No, I did not.

14:41:16 18 Q. Did you ask if Illumina had conducted any other
14:41:19 19 experiments regarding the cleavage of an allyl
14:41:23 20 protecting group?

14:41:24 21 A. No, I did not.

14:41:26 22 Q. Did you ask Illumina if it had conducted any
14:41:30 23 other experiments regarding the cleavage of an
14:41:33 24 azidomethyl protecting group?

14:41:35 25 A. I've had no communications with Illumina so,

14:41:37 1 no, I did not ask that.

14:41:41 2 Q. Did you ask your attorneys for other data with
14:41:45 3 regard to the cleavage of allyl protecting groups?

14:41:51 4 MS. SWAROOP: Counsel, you're getting into
14:41:52 5 subject matter of communications with counsel.

14:41:54 6 So, Dr. Romesberg, I instruct you not to answer
14:41:57 7 that.

14:41:58 8 You can ask him generally what he considered,
14:42:00 9 but to the extent you're asking him about specific
14:42:02 10 communications, that's privileged and we're not going to
14:42:05 11 permit testimony on communications.

14:42:06 12 BY MR. SEGAL:

14:42:06 13 Q. Other than the couple experiments that you
14:42:09 14 discuss in this section, did you rely on anything else
14:42:14 15 for your conclusion that the azidomethyl group has
14:42:17 16 unexpected properties?

14:42:29 17 A. Yeah, I'm not sure I know how to answer this
14:42:31 18 question.

14:42:32 19 Kind of yes; and kind of yes, for example, the
14:42:37 20 nature paper that Illumina published, that's the -- the
14:42:40 21 first author's Bentley. I believe it's already in --
14:42:45 22 what do you call it? -- entered into evidence -- it
14:42:50 23 works. It works amazingly. It uses no allyl, and they
14:42:55 24 sequenced a genome. This caught the scientific world
14:43:01 25 totally off guard.

14:43:02 1 Q. Other than the Bentley article --

14:43:04 2 A. And the fact that --

14:43:04 3 Q. -- and the notebook pages that you discussed in
14:43:08 4 your declaration, are you relying on anything else to
14:43:11 5 support your opinion that the azidomethyl group shows
14:43:16 6 unexpected results?

14:43:18 7 A. Just to be clear, this is related to the
14:43:20 8 Bentley paper. The fact that you can sequence what they
14:43:23 9 achieve implies that the cleavage efficiencies are
14:43:29 10 outlandishly high; north of 99 percent, north of
14:43:33 11 99.9 percent or something.

14:43:54 12 Q. Did you search the literature to look for any
14:43:56 13 other data regarding the cleavage of a 3' oxygen allyl
14:44:01 14 protecting group?

14:44:04 15 A. Not explicitly. I know Ju's work. He's -- as
14:44:10 16 I think I've established, I think it's well known he
14:44:12 17 works in a field that's not so distant from what I work
14:44:16 18 in, so that I know his work, and I think reading the
14:44:18 19 literature over the years I'm vaguely familiar with this
14:44:22 20 stuff.

14:44:22 21 And I would have -- I mean I always had sort of
14:44:25 22 an impression as to where the field stood, and so it
14:44:30 23 would be that sort of knowledge in addition to what
14:44:33 24 Illumina provided me that also contributed.

14:44:37 25 And, I'm sorry. I don't have --

14:44:39 1 Q. I don't think that was quite answering my
14:44:41 2 question.

14:44:41 3 Did you look at any data from any other
14:44:44 4 references or literature regarding the cleavage
14:44:48 5 efficiency of a 3' hydroxyl protecting group using the
14:44:53 6 allyl protecting group?

14:44:54 7 A. Yes. What I mean is that Ju has been
14:44:56 8 publishing this for a while, and I -- I assume that I've
14:45:00 9 read most of his papers. Most of that was a long time
14:45:03 10 ago, so it's left a general impression on me and I did
14:45:07 11 not go consult them for this declaration or in
14:45:09 12 preparation for this deposition, but I -- that did
14:45:13 13 inform my opinion.

14:45:34 14 Q. Look at paragraph 75. That first sentence
14:45:42 15 there, I believe you're summarizing the previous
14:45:45 16 discussion of the allyl experiments.

14:45:47 17 And you say "In my opinion, these notebook
14:45:51 18 pages report that even under optimized conditions,
14:45:54 19 long . . . periods are required to achieve 3' oxygen
14:46:00 20 allyl protecting group cleavage efficiencies that are
14:46:02 21 sufficient for SBS."

14:46:04 22 Is that right?

14:46:05 23 A. I do say that, yes.

14:46:07 24 Q. So you used the term "optimized conditions."

14:46:13 25 So what has been optimized through the series

14:46:18 1 of allyl cleavage experiments that you reviewed?

14:46:23 2 A. I believe that different phosphines were used
14:46:26 3 and I think different palladium sources were used. I
14:46:34 4 would have to look through, and it's been a little while
14:46:37 5 since I read this, but I think different additives and
14:46:40 6 maybe different temperatures.

14:46:51 7 Do you want me to come through and come up with
14:46:53 8 a more accurate assessment of what I was writing at
14:46:55 9 the --

14:46:56 10 Q. I don't think that's necessary right at this
14:46:58 11 moment.

14:47:12 12 Why don't we go look at these experiments.

14:47:16 13 Why don't you take your time -- I mean, you did
14:47:19 14 review your declaration before this deposition?

14:47:21 15 A. Yes.

14:47:22 16 Q. Why don't you take your time right now to look
14:47:25 17 through the paragraphs in this section leading up to
14:47:30 18 paragraph 75, and let me know if your answer changes.

14:47:35 19 A. Okay. So the answer to that question, how they
14:47:38 20 optimized?

14:47:40 21 Q. Yes.

14:47:42 22 A. Okay.

14:48:31 23 MS. SWAROOP: Counsel, while Dr. Romesberg is
14:48:33 24 reviewing the portions that you asked him to review, I
14:48:36 25 just want to note that this portion of his declaration

14:48:38 1 was designated as confidential, so to the extent we're
14:48:42 2 going to be discussing the specific notebook pages, I'd
14:48:45 3 like to mark the deposition as confidential.

14:48:48 4 (Confidential section begins.)

14:48:48 5 -o0o-

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

14:58:06 1 MR. SEGAL: I think if we can go on a break, I
14:58:08 2 may be almost finished.

14:58:10 3 MS. SWAROOP: Okay.

14:58:11 4 THE VIDEO OPERATOR: We are going off the
14:58:12 5 record. The time is 2:58.

14:58:14 6 (Recess from 2:58 p.m. to 4:32 p.m.)

16:32:51 7 (Mr. Taylor, Ph.D., Esq., enters the deposition
16:32:51 8 proceedings.)

16:32:54 9 THE VIDEO OPERATOR: We are going back on the
16:32:55 10 record. The time is 4:32.

16:32:58 11 BY MR. SEGAL:

16:32:59 12 Q. Now, Dr. Romesberg, earlier we were talking
16:33:01 13 about the Kit reference, which is Exhibit 2018 and, in
16:33:08 14 particular, the sentence in Kit that you quoted in your
16:33:14 15 declaration that's on page 57 and 58.

16:33:17 16 Do you remember that?

16:33:18 17 A. I do.

16:33:19 18 Q. And Kit cites to reference 252 and, as you
16:33:26 19 recall, the 252 reference is referring to a -- an
16:33:32 20 abstract with the author Gordon.

16:33:35 21 Is that right?

16:33:36 22 A. Uh-huh, uh-huh.

16:33:37 23 Q. And you testified earlier that you had reviewed
16:33:39 24 that reference.

16:33:40 25 Is that right?

16:33:41 1 A. Uh-huh, yes, that's right.

16:33:44 2 MR. SEGAL: I'm just going to mark as

16:33:45 3 Exhibit 1020 an abstract with the first named author

16:33:54 4 Gordon, and it's from the Biophysical Society, Sixth

16:34:03 5 Annual Meeting.

16:34:05 6 (Exhibit 1020 was marked for identification.)

16:34:09 7 MR. SEGAL: I don't have one --

16:34:10 8 MS. SWAROOP: I thought we made -- oh, okay.

16:34:12 9 And it's Exhibit 1020?

16:34:15 10 BY MR. SEGAL:

16:34:15 11 Q. I just want to confirm that this is actually --

16:34:18 12 this is the abstract you were talking about earlier when

16:34:21 13 you said you reviewed the abstract referenced in -- as

16:34:24 14 252 in Kit.

16:34:26 15 A. Yes.

16:34:29 16 MR. SEGAL: Okay. Dr. Romesberg, thank you for

16:34:30 17 your time. I have no further questions.

16:34:34 18

16:34:34 19 EXAMINATION

16:34:34 20 BY MS. SWAROOP:

16:34:35 21 Q. Okay. Dr. Romesberg, I do have a few questions

16:34:37 22 for you.

16:34:45 23 So during cross, counsel had asked you about

16:34:48 24 the Staudinger litigation.

16:34:51 25 Do you recall that?

16:34:52 1 A. I do.

16:34:52 2 Q. Can you explain what a Staudinger ligation is.

16:34:55 3 A. Sure. So just -- this -- unlike the Staudinger
16:35:00 4 reaction, where the leaving group is essentially
16:35:03 5 transferred to water so you get -- the leaving group
16:35:06 6 departs with the hydroxyl group, the Staudinger ligation
16:35:12 7 intercepts that intermediate with a nucleophile that's
16:35:16 8 not water, but instead is attached to a linker; and as a
16:35:20 9 result, that results in your leaving group not being
16:35:23 10 hydrolyzed and free but, in fact, being attached to
16:35:27 11 something else, a linker, and that's why it's called a
16:35:31 12 ligation.

16:35:32 13 Q. Okay. And if you were to use Staudinger
16:35:34 14 ligation on an azido-blocked nucleotide, would the
16:35:39 15 Staudinger ligation result in a free 3' hydroxyl?

16:35:44 16 A. No, it would not.

16:35:45 17 MR. SEGAL: Object. Outside the scope of my
16:35:48 18 cross examination. I did not ask him about Staudinger
16:35:50 19 ligation.

16:35:52 20 MS. SWAROOP: We disagree with that, but I'll
16:35:54 21 move on to the next line of questions.

16:35:58 22 BY MS. SWAROOP:

16:35:58 23 Q. If you can turn to the Tsien reference, which I
16:36:02 24 believe is Exhibit 1008, counsel had asked you some
16:36:21 25 questions on page 21 of the reference.

16:36:31 1 I'm sorry. Exhibit 1008, Tsien.

16:36:36 2 I think you have Ju.

16:36:37 3 A. Oh, I'm sorry.

16:36:38 4 Q. That's okay. I just had it.

16:36:47 5 THE REPORTER: Counsel, may we go off the

16:36:49 6 record?

16:36:51 7 THE VIDEO OPERATOR: Do you want to go off?

16:36:52 8 MS. SWAROOP: Sure.

16:36:53 9 MR. SEGAL: Sure.

16:36:54 10 THE VIDEO OPERATOR: Okay. Off the record.

16:36:54 11 The time is 4:36.

16:37:18 12 (Discussion off the record.)

16:37:24 13 THE VIDEO OPERATOR: We are back on the record.

16:37:25 14 The time is 4:36 -- 7.

16:37:31 15 MS. SWAROOP: Okay. So the court reporter

16:37:32 16 asked us to make clear when we went off the confidential

16:37:36 17 portion of the record.

16:37:36 18 So there was a series of questions to

16:37:39 19 Dr. Romesberg with regard to the Illumina notebook pages

16:37:42 20 and then we broke at that point, so the confidential

16:37:45 21 portion would end at that point, and we're now on the

16:37:47 22 public record.

16:37:48 23 BY MS. SWAROOP:

16:37:48 24 Q. Dr. Romesberg, I was asking about the Tsien

16:37:50 25 reference, Exhibit 1008, and in particular page 21.

16:37:56 1 A. Top number --

16:37:57 2 Q. Top number.

16:37:59 3 A. Got it.

16:38:00 4 Q. And you had testified that the azido group is
16:38:04 5 an irreversible terminator.

16:38:07 6 Do you recall that?

16:38:08 7 A. In the context of what Tsien's describing, yes.

16:38:11 8 Q. And can you explain why?

16:38:13 9 A. Tsien is using them to support the
16:38:15 10 incorporability of modified nucleotides, and he lists a
16:38:19 11 variety including the fluorine and the amino group and
16:38:23 12 an azide, for example, amongst several others, all of
16:38:27 13 which could not be attached to an oxygen of the
16:38:29 14 nucleotide. It would be chemically not possible for
16:38:32 15 them to be.

16:38:33 16 And they're in fact, well-known examples from
16:38:36 17 C3 -- directly attached to C3 nucleotides as reverse --
16:38:40 18 which act as irreversible terminators, and he cites
16:38:45 19 Kraevskii, which explicitly shows them attached directly
16:38:50 20 to the C3 carbon.

16:38:52 21 So they're not reversible terminators; they're
16:38:57 22 irreversible terminators.

16:38:58 23 Q. Okay. And if the azido was reduced to an
16:39:01 24 amine, would it still be an irreversible terminator?

16:39:05 25 A. It would, yes.

16:39:06 1 Q. So would you agree that the azido serves the
16:39:09 2 same function whether or not it gets reduced by the
16:39:13 3 polymerase?

16:39:14 4 A. Yes.

16:39:18 5 Q. Dr. Romesberg, if something were to work well
16:39:21 6 in oligonucleotides manufacturing, does that necessarily
16:39:26 7 mean it would work well for sequencing by synthesis?

16:39:29 8 A. No, it does not.

16:39:31 9 MR. SEGAL: Object to the form of the question.

16:39:38 10 And, again, I'll also object to it's outside
16:39:41 11 the scope of my cross examination.

16:39:43 12 BY MS. SWAROOP:

16:39:44 13 Q. Dr. Romesberg, you were asked during your
16:39:47 14 deposition about, I think, synthetic chemistry papers.

16:39:51 15 Do you recall that?

16:39:52 16 A. I was, yes.

16:39:53 17 Q. Okay.

16:39:54 18 A. I do.

16:39:54 19 Q. And is it reasonable for someone looking to
16:39:56 20 develop an SBS method, to consult synthetic chemistry
16:40:00 21 papers?

16:40:01 22 A. Yes, it is.

16:40:02 23 Q. Does that -- and does it depend upon what's
16:40:05 24 disclosed in those papers?

16:40:07 25 A. Yeah. So I mean if I were looking to develop a

16:40:09 1 sequencing-by-synthesis method, I would certainly
16:40:12 2 consult synthetic chemistry papers that showed things
16:40:17 3 like efficiency of protecting group cleavage, I would
16:40:20 4 look at the conditions that were explored to see if they
16:40:23 5 were compatible with sequencing by synthesis, and I
16:40:26 6 would look at yield to see if they were compatible with
16:40:29 7 the high efficiencies required for sequencing by
16:40:33 8 synthesis.

16:40:34 9 Q. And if the conditions disclosed in a particular
16:40:34 10 reference were incompatible with sequencing by
16:40:37 11 synthesis, would there be any expectation of success?

16:40:41 12 A. That would not provide an expectation of
16:40:43 13 success.

16:40:57 14 Q. I'd like to ask you a few questions about the
16:41:01 15 Zavgorodny reference, if you could pull that exhibit
16:41:04 16 out. It's 1004.

16:41:16 17 Dr. Romesberg, does Zavgorodny disclose any
16:41:20 18 conditions other than triphenylphosphine phosphine in
16:41:24 19 aqueous pyridine at 20 degrees Celsius for removal of
16:41:30 20 its azidomethyl group?

16:41:32 21 MR. SEGAL: Objection. Outside the scope of my
16:41:34 22 cross.

16:41:34 23 THE WITNESS: No. Those are the conditions
16:41:36 24 that he suggests for cleaving these -- the azidomethyl
16:41:42 25 group.

16:41:42 1 BY MS. SWAROOP:

16:41:43 2 Q. Does Zavgorodny suggest any other conditions
16:41:47 3 other than triphenylphosphine in aqueous pyridine at 20
16:41:51 4 degrees Celsius for removal of its azidomethyl group?

16:41:55 5 MR. SEGAL: Same objection.

16:41:55 6 THE WITNESS: No, he does not.

16:41:56 7 BY MS. SWAROOP:

16:41:57 8 Q. Okay. And if you look at the last two lines on
16:42:00 9 page 7595 of Zavgorodny --

16:42:07 10 A. Yes.

16:42:07 11 Q. -- there's a reference to removal under very
16:42:10 12 specific and mild conditions viz. -- v-i-z -- with
16:42:17 13 triphenylphosphine phosphine in aqueous pyridine at 20
16:42:21 14 degrees Celsius.

16:42:23 15 Do you see that?

16:42:24 16 A. I do.

16:42:25 17 Q. What does the word "viz." mean to you?

16:42:27 18 MR. SEGAL: Objection. Outside the scope. I
16:42:29 19 didn't ask him what "viz." means.

16:42:31 20 MS. SWAROOP: You asked whether there was an
16:42:32 21 example in Zavgorodny, and I'm asking him whether there
16:42:36 22 are any other conditions other than the particular
16:42:37 23 condition here, so it's well within the scope of your
16:42:40 24 cross.

16:42:41 25 MR. SEGAL: My objection stands.

16:42:42 1 THE WITNESS: "Viz." means "namely." It does
16:42:45 2 not mean, "for example."

16:42:49 3 BY MS. SWAROOP:

16:42:49 4 Q. All right. Now, Dr. Romesberg, you were asked
16:42:52 5 some questions about the Staudinger reaction.

16:42:54 6 Do you recall that?

16:42:56 7 A. I do.

16:42:56 8 Q. Okay. Does temperature have any effect on the
16:42:59 9 efficiency of the Staudinger reaction?

16:43:01 10 A. Yes. Like all reactions, they'll be more
16:43:04 11 efficient at higher temperature and less efficient at
16:43:07 12 lower temperature.

16:43:13 13 Q. You were also asked some questions about
16:43:16 14 denaturing of DNA.

16:43:19 15 Do you recall that?

16:43:20 16 A. Yes, I do.

16:43:20 17 Q. Would you expect DNA to be denatured at
16:43:24 18 20 degrees Celsius in aqueous pyridine sufficient to
16:43:29 19 solubilize triphenylphosphine?

16:43:31 20 MR. SEGAL: Object. Outside the scope of my
16:43:33 21 cross examination.

16:43:34 22 THE WITNESS: That depends on the DNA. Large
16:43:36 23 pieces of DNA, like chromosomal DNA or maybe even plasma
16:43:40 24 DNA, at 20 degrees in pyridine might actually not,
16:43:45 25 although it might be denatured; but certainly primer

16:43:48 1 templates of the sort used in sequencing by synthesis
16:43:51 2 would be denatured at 20 degrees in pyridine.

16:43:55 3 BY MS. SWAROOP:

16:43:55 4 Q. Can you explain why?

16:43:57 5 A. Well, because they're already not very stable.
16:44:00 6 They're only marginally stable because they're
16:44:03 7 reasonably short.

16:44:05 8 When you have a chromosome, you have millions
16:44:06 9 of base pairs, like in some of the examples we talked
16:44:09 10 about here where they were looking at denaturation, they
16:44:10 11 were using, for example, the genome of T4 --

16:44:16 12 THE REPORTER: Sir --

16:44:16 13 THE WITNESS: In several of the examples here,
16:44:16 14 they were using chromosomal DNA from T4 phage. It's a
16:44:21 15 type of phage. It's millions of base pairs, so you have
16:44:24 16 all that stability that the pyridines have to compete
16:44:27 17 with. The pyridine will certainly denature it, but you
16:44:31 18 might have to go to a slightly higher temperature.

16:44:34 19 You don't sequence by synthesis with millions
16:44:37 20 of base pairs of hybridization. You have primer
16:44:40 21 templates that have 20 nucleotides, maybe 30, something
16:44:43 22 like that at the beginning; maybe you get up to 100 if
16:44:47 23 your read lengths are good, or maybe longer, but you
16:44:50 24 don't get anywhere near the sorts of lengths that would
16:44:53 25 be required to with stand a significant amount of

16:44:58 1 pyridine.

16:44:59 2 Q. Dr. Romesberg, you testified that approximately
16:45:01 3 25 base pairs would be needed for sequencing by
16:45:04 4 synthesis.

16:45:05 5 Do you recall that?

16:45:06 6 A. Yes, I do.

16:45:07 7 Q. Why is that?

16:45:08 8 A. That's the length that you need to uniquely
16:45:12 9 place a sequence within a genome, so you need it to
16:45:15 10 assemble the genome from the sequences that you're going
16:45:18 11 to get by sequencing by synthesis.

16:45:21 12 Q. If you were looking for a single nucleotide
16:45:25 13 polymorphism using a sequencing-by-synthesis method,
16:45:28 14 what minimum read length would you need?

16:45:31 15 A. It's not --

16:45:33 16 MR. SEGAL: Objection. You've got to give me a
16:45:34 17 second to object.

16:45:35 18 Objection. Outside the scope of the cross.

16:45:38 19 BY MS. SWAROOP:

16:45:38 20 Q. You can answer.

16:45:39 21 A. It would not change, because the SNIP
16:45:41 22 identification comes from the assembled genome, so you
16:45:45 23 still need to assemble the genome, you still need to
16:45:48 24 place the sequence within the genome to know whether or
16:45:50 25 not a specific nucleotide is a SNIP. So the SNIP is

16:45:54 1 determined after you assemble the genome, so it doesn't
16:45:57 2 change anything.

16:45:58 3 Q. Okay. And you referred to SNIP in your
16:46:00 4 response.

16:46:01 5 Is that an acronym for single nucleotide
16:46:04 6 polymorphism?

16:46:05 7 A. It is.

16:46:05 8 Q. Okay. Now, counsel asked you some questions
16:46:09 9 about claim 1 of the '537 patent, so if you can pull out
16:46:13 10 that exhibit. I believe it's Exhibit 1002.

16:46:16 11 I'm sorry. 1001. Yes.

16:46:39 12 So counsel asked you about claim 1, and in your
16:46:43 13 response to referred to claim 8.

16:46:45 14 Do you recall that?

16:46:46 15 A. I do.

16:46:46 16 Q. Can you explain why claim 8 is important to
16:46:50 17 you?

16:46:50 18 MR. SEGAL: Objection. Outside the scope. I
16:46:52 19 didn't ask him any questions about claim 8. You can't
16:46:54 20 put on the record new testimony.

16:46:57 21 MS. SWAROOP: I disagree. He referred to
16:46:58 22 claim 8 in his response, so I'm certainly entitled to --

16:47:01 23 MR. SEGAL: So?

16:47:01 24 MS. SWAROOP: I'm certainly entitled to --

16:47:01 25 MR. SEGAL: I didn't ask him questions about

16:47:03 1 claim 8. It's within the scope of my cross examination.

16:47:06 2 BY MS. SWAROOP:

16:47:06 3 Q. Dr. Romesberg, can you explain why claim 8 is

16:47:08 4 important to you.

16:47:10 5 A. Claim 8 is what identifies the method described

16:47:13 6 as the sequencing-by-synthesis method, and implicit in

16:47:17 7 claim 8 is efficient cleavage.

16:47:20 8 Q. What specifically about claim 8 --

16:47:24 9 A. Well, it says "Detecting the detectable label

16:47:27 10 and cleaving the cleavable linker" -- sorry.

16:47:30 11 "Detecting the detectable label and cleaving

16:47:34 12 the cleavable linker."

16:47:43 13 Q. Counsel asked you some questions about your

16:47:45 14 opinions on unexpected results, and particularly your

16:47:48 15 comparison of cleavage efficiency of the azidomethyl

16:47:53 16 group as compared to the allyl group.

16:47:55 17 Do you recall that?

16:47:56 18 A. I do.

16:47:57 19 Q. And why did you compare the azidomethyl group

16:48:00 20 to the allyl group, and not any other protecting groups?

16:48:04 21 A. Well, the O-allyl groups were generally

16:48:06 22 believed to be the most promising candidates for

16:48:09 23 protecting groups for the method, so they represented

16:48:11 24 sort of the state of the art and the most rigorous and

16:48:14 25 relevant comparison.

16:48:20 1 Q. Did any of the prior art references that you
16:48:23 2 reviewed discuss the allyl group?

16:48:29 3 A. Yes. They were -- they were -- yes, and
16:48:31 4 it also would be -- they were also relevant because they
16:48:34 5 were discussed by Ju, Tsien, and Metzker.

16:48:38 6 Q. Okay. Can Ju's method be practiced using
16:48:42 7 nucleosides?

16:48:43 8 A. They can be practiced with a nucleoside
16:48:46 9 triphosphate that gets converted into a nucleoside
16:48:50 10 monophosphate.

16:48:51 11 Q. Can Tsien's method be practiced using
16:48:54 12 nucleosides?

16:48:55 13 A. Same answer.

16:49:08 14 Q. Now, counsel asked you whether you were aware
16:49:10 15 of other methods of cleaving an azido group.

16:49:13 16 Do you recall that?

16:49:14 17 A. I do.

16:49:15 18 Q. And are you aware of any methods of cleaving an
16:49:18 19 azido group, other than what's in your declaration?

16:49:21 20 A. Yes. There's a DTT reported method.

16:49:25 21 Q. Did you review any papers discussing DTT as the
16:49:31 22 cleavage reason?

16:49:32 23 A. I did. I reviewed Reardon.

16:49:34 24 Q. Is that the Reardon paper that you identified
16:49:35 25 in your deposition today?

16:49:36 1 A. It is.

16:49:37 2 Q. And what did Reardon show?

16:49:39 3 A. Reardon showed that the deprotection of a 3
16:49:43 4 azido nucleoside, the deprotection yield efficiency was
16:49:48 5 80 percent; and that with the monophosphate, the
16:49:52 6 efficiency was worse, it was only 19 percent and, in
16:49:55 7 fact, it was one of only seven or eight -- it was one of
16:49:58 8 seven or eight different products that were formed, and
16:50:01 9 it was formed at 19 percent, so it was much worse than
16:50:04 10 the nucleoside.

16:50:09 11 Q. Was Reardon the first to look at DTT cleavage
16:50:13 12 of an azido group?

16:50:15 13 MR. SEGAL: Object as outside the scope.

16:50:16 14 I didn't ask him questions about what Reardon
16:50:19 15 discussed.

16:50:20 16 THE WITNESS: No, it wasn't.

16:50:22 17 BY MS. SWAROOP:

16:50:22 18 Q. Are you aware of others?

16:50:26 19 A. Yes. And I'm drawing a blank.

16:50:35 20 Can I --

16:50:38 21 Q. Well, let me ask you this: Assuming you're
16:50:41 22 drawing a blank and you can't remember the author, but
16:50:43 23 do you remember anything about any other studies with
16:50:46 24 regard to DTT cleavage?

16:50:49 25 A. Yes, there was a study that looked at the

16:50:51 1 nucleoside that reported quantitative cleavage of the
16:50:54 2 same nucleotide that was examined by Reardon.

16:50:57 3 Q. Okay. And did the Reardon paper analyze that
16:51:02 4 previous study?

16:51:04 5 A. Yes, they did, and they showed it was cleaved
16:51:06 6 at 80 percent efficiency, and they also showed -- it
16:51:10 7 was -- so the Reardon study was a much more
16:51:13 8 comprehensive, thorough study. It was published in a
16:51:16 9 much more rigorous journal, and they report extensively
16:51:20 10 the materials and methods, unlike the former paper,
16:51:22 11 which was very -- which essentially had very little
16:51:25 12 detail.

16:51:25 13 And I think what caught their attention was
16:51:27 14 yields that were too high in the original paper, and I
16:51:30 15 just wish I could remember the name of the original
16:51:33 16 paper. I'm drawing a blank.

16:51:34 17 There's no way I can actually get that
16:51:37 18 information, since it is known in this room?

16:51:40 19 MR. SEGAL: Unfortunately, you can't ask
16:51:42 20 questions, so --

16:51:45 21 THE WITNESS: Yeah, it's one of those things
16:51:47 22 where I've got someone else's name in my head and I
16:51:50 23 can't get it out.

16:51:51 24 Anyway, what they reported was, as I said, the
16:51:54 25 3' azide nucleoside. They reported a quantitative

16:51:58 1 yield, and they reported a very strange statement of
16:52:00 2 100 percent recovery, which is unlikely because yields
16:52:04 3 are seldom 100 percent.

16:52:07 4 So this is sort of an example where a yield can
16:52:11 5 actually be too high, and it was probably because they
16:52:15 6 were salts or something that they co-purified it with.

16:52:19 7 But, anyway, it obviously motivated Reardon to
16:52:23 8 go look at this reaction a little bit more and, given
16:52:25 9 the data that Reardon publishes, it was clear that what
16:52:28 10 happened in the original report was that they simply
16:52:31 11 didn't have enough sensitivity on their HPLC detector
16:52:36 12 because it was -- there were multiple peaks in the base
16:52:39 13 line that were just below -- that just weren't detected
16:52:41 14 in the original paper, and when those peaks were taken
16:52:45 15 into consideration it was only an 80 percent yield.

16:52:56 16 MS. SWAROOP: We're going to mark as
16:52:58 17 Exhibit 2139 the Reardon paper.

16:53:04 18 MR. SEGAL: I'm going to object to that. You
16:53:06 19 can't introduce an exhibit during redirect.

16:53:08 20 I had the same discussion -- I think you were
16:53:10 21 there, Nate -- with your partner Bill Zimmerman. So
16:53:13 22 we'll go to the Board on this. This is definitely
16:53:16 23 outside the scope and it's not proper.

16:53:20 24 So I will adjourn the deposition and, like Bill
16:53:25 25 Zimmerman from your firm required us to do, go to the

16:53:29 1 Board, and we'll put it to the Board whether this is
16:53:31 2 proper.

16:53:32 3 MS. SWAROOP: Well, I don't think the Board is
16:53:33 4 open right now, so --

16:53:35 5 MR. SEGAL: We'll have to adjourn the
16:53:36 6 deposition. We can come back tomorrow morning.

16:53:40 7 MS. SWAROOP: Well, I think what I'll do is
16:53:43 8 I'll withdraw this from the record and just read the
16:53:45 9 cite in, and if we need to revisit this with the Board,
16:53:49 10 we can.

16:53:49 11 So I want to make my proffer, so the
16:53:52 12 exhibit that I would have shown to the witness is
16:53:54 13 entitled "Reardon, et al.," and it's "Reduction of
16:54:00 14 3'-azido-3' deoxythymidine (AZT) and AZT Nucleotides by
16:54:09 15 Thiols," Journal of Biological Chemistry, Volume 269,
16:54:15 16 No. 23, issue of June 10th, 1994.

16:54:37 17 And I just want to note for the record that
16:54:39 18 Reardon addresses the Handlon reference, and I'll read
16:54:44 19 that into the record as well.

16:54:46 20 MR. SEGAL: You're testifying now. It's
16:54:48 21 ridiculous. You made your point it refers to the
16:54:51 22 Handlon.

16:54:51 23 MS. SWAROOP: But the witness referred to it,
16:54:52 24 so I just want to make a record.

16:54:54 25 MR. SEGAL: This is completely improper.

16:54:56 1 MS. SWAROOP: He testified that he reviewed it,
16:54:58 2 so I want to make that a record.

16:55:01 3 Handlon and Oppenheimer, 1998, Pharmaceutical
16:55:04 4 Research, Volume V, pages 297 through 299.

16:55:29 5 Without marking it, I'm just going to show
16:55:32 6 Exhibit 1239 to the witness. Again --

16:55:36 7 MR. SEGAL: It's totally improper.

16:55:38 8 MS. SWAROOP: I'm just asking if it refreshes
16:55:40 9 his recollection as to what he's -- what he was talking
16:55:42 10 about, his testimony.

16:55:44 11 MR. SEGAL: This is completely improper. Now,
16:55:45 12 I'll go to the Board on this. This is ridiculous. He
16:55:48 13 didn't cite to these references in his declaration. He
16:55:52 14 can't introduce new testimony during redirect.

16:55:54 15 MS. SWAROOP: During your cross, he did testify
16:55:56 16 that he reviewed these references, and he was talking
16:56:00 17 about a Reardon reference and I just want to --

16:56:01 18 MR. SEGAL: The same issue -- same exact issue
16:56:02 19 came up, and Marcus -- and I don't know if Marcus was
16:56:04 20 there, but Nate was definitely there -- and our witness
16:56:08 21 testified about Zavgorodny during the cross examination,
16:56:11 22 and we tried to ask him some questions about Zavgorodny,
16:56:15 23 and Bill Zimmerman objected and made us go to the Board.

16:56:19 24 So, I mean, as long as we're playing on the
16:56:21 25 same playing field, we'll do the same thing. Come back

16:56:25 1 tomorrow morning if it's necessary, and we'll call the
16:56:27 2 board.

16:56:28 3 BY MS. SWAROOP:

16:56:28 4 Q. Dr. Romesberg, is the Reardon paper that you
16:56:30 5 testified during cross, the same Reardon that we've been
16:56:33 6 discussing in this series of questioning?

16:56:38 7 A. The Reardon paper that I mentioned in -- I
16:56:41 8 guess this is called cross --

16:56:43 9 Q. In the morning session.

16:56:44 10 A. -- in the morning session is the same Reardon
16:56:47 11 paper that I just described; and I also cited -- I hope
16:56:50 12 I can mention this now -- the other paper was Handlon.
16:56:53 13 I've now remembered. I don't know why I was -- Handlon
16:56:57 14 was the original report that Reardon corrected.

16:57:00 15 MR. SEGAL: And again, objection. It's outside
16:57:02 16 the scope. He didn't testify at all with regard to the
16:57:04 17 substance of the Reardon or the Handlon papers during
16:57:08 18 cross examination.

16:57:08 19 MS. SWAROOP: Well, he was asked whether he was
16:57:09 20 aware of other methods of cleaving an azido group, so
16:57:12 21 it's directly related to that portion of the cross.

16:57:24 22 And also, since we have stated our -- if you
16:57:26 23 need to add more, you can, but now I've made my proffer
16:57:29 24 and you've stated your objection on the record, so I
16:57:32 25 don't think there's a need to go to the Board on this.

16:57:34 1 So if there is further record you want to make,
16:57:38 2 please go ahead and do so.

16:58:01 3 MR. SEGAL: I think the record's clear.

16:58:04 4 MS. SWAROOP: Okay. Well, I have no further
16:58:06 5 questions.

16:58:08 6 MR. SEGAL: If we can take a few minutes to
16:58:11 7 determine whether we have any recross questions.

16:58:13 8 MS. SWAROOP: Sure.

16:58:14 9 MR. SEGAL: Thank you.

16:58:15 10 THE VIDEO OPERATOR: We are going off the
16:58:16 11 record. The time is 4:58.

16:58:18 12 (Recess from 4:58 p.m. to 5:20 p.m.)

17:20:53 13 THE VIDEO OPERATOR: We are back on the record.
17:20:54 14 The time is 5:20.

17:20:56 15 MS. SWAROOP: Counsel, before you get started,
17:20:58 16 I just want to note that you did mark during cross
17:21:01 17 Exhibit 1020.

17:21:02 18 I just want -- and asked no questions of the
17:21:05 19 witness with regard to that exhibit. I just want to
17:21:07 20 object on the grounds of relevance and also object as
17:21:10 21 improper supplemental evidence and untimely under
17:21:14 22 Rule 42.123.

17:21:19 23 MR. SEGAL: We obviously don't agree with that
17:21:21 24 objection.

17:21:23 25 Dr. Romesberg, thank you for your time. I have

17:21:27 1 no further questions.

17:21:29 2 I think we're adjourned.

17:21:32 3 THE VIDEO OPERATOR: Okay to go off the record?

17:21:34 4 MS. SWAROOP: Yes.

17:21:35 5 THE VIDEO OPERATOR: Off the record. The time
17:21:36 6 is 5:21.

7 (The Video Deposition concluded at 5:21 p.m.)

8 * * *

9

10

11

12

13

14

15

16

17 _____
FLOYD ROMESBERG, Ph.D.

18

19

20

21

22

23

24

25

1 I, Lynn Penfield, a Certified Shorthand Reporter
2 No. 8589, State of California, RPR, do hereby
3 certify:

4 That the deponent in the foregoing deposition,
5 FLOYD ROMESBERG, Ph.D.,
6 was by me first duly sworn and the foregoing testimony
7 was reported by me and was thereafter transcribed with
8 Computer-Aided Transcription; that the foregoing 190
9 pages is a full, complete and true record of said
10 proceeding.

11 I further certify that I am not of counsel or
12 attorney for either or any of the parties in the
13 foregoing proceeding and caption names or in any way
14 interested in the outcome of the cause in said caption

15 The dismantling, unsealing, or unbinding of the
16 original transcript will render the reporter's
17 certificate null and void.

18 IN WITNESS WHEREOF, I have hereunto set my hand this 9th
19 day of July, 2014.

20 _____ Reading and Signing was requested.

21 _____ Reading and Signing was waived.

22 _____X_____ Reading and Signing was not requested.

23

24

25 _____
Lynn Penfield, CSR No. 8589, RPR, CRR

Errata Sheet

Re: Deposition of Floyd Romesberg

Date: 7-08-2014

Caption: Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd – IPR2013-00517

Page	Line	Correction/Change	Reason
17	19	"ones are here" → "ones here"	error
25	17	"the nucleobase part" → "the nucleobase and sugar part"	accuracy
27	3	"it to a nucleus" → "to it"	accuracy
28	16	"nucleic base" → "nucleobase"	error
28	18	"nucleic base" → "nucleobase"	error
33	25	"MR. BURCH" → "MS. SWAROOP"	error
45	9	"3' hydroxyl or the hydroxyl" → "3' hydroxyl"	error
45	21	"atom" → "atoms"	accuracy
50	12	"Reverse" → "Reverse Transcriptase"	error
50	14	"Reverse" → "Reverse Transcriptase"	error
50	16	"is due" → "is to"	error
53	6	"stuff" → "step"	error
58	1	"sections" → "a section"	error
61	5	"injected to" → "injected into"	error
61	18	"raised to" → "raised"	error
64	14	"of all polymerases" → "for all polymerases"	error
67	1	"ion on" → "ion in"	error
69	1	"cleaved" → "cleavage"	

July 23, 2014

Date


Signature

Errata Sheet

Re: Deposition of Floyd Romesberg

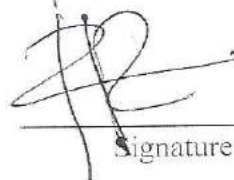
Date: 7-08-2014

Caption: Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd – IPR2013-00517

Page	Line	Correction/Change	Reason
70	8	"in a MDM" → "as a MDM"	error
71	8	"it like a MDM" → "It's not like a MDM"	error
71	8	"A MDM group is only" → "A MDM group has only"	error
72	22	"physical chemical" → "physicochemical"	error
84	16	"of better" → "to be better"	error
84	19	"reaction" → "reactions"	error
95	20	"take" → "react"	accuracy
103	11	"attach" → "attached"	accuracy
105	10	"diza" → "deaza"	error
106	4	"diazas" → "deazas"	error
116	8	"Phospho ylide" → "phosphoylide"	accuracy
118	6	"use an" → "use in"	error
125	6	"everything up on" → "everything upon"	error
129	23	"constant" → "constants of"	accuracy
129	24	"proportionalities" → "proportionality"	accuracy
131	18	"constant" → "constant of"	accuracy
144	24	delete "up here"	accuracy
145	23	"21 hybrids" → "21 base pairs"	accuracy

July 23, 2014

Date


Signature

Errata Sheet

Re: Deposition of Floyd Romesberg

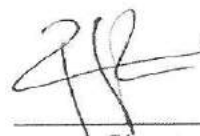
Date: 7-08-2014

Caption: Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd – IPR2013-00517

Page	Line	Correction/Change	Reason
145	25	"to pyridine" → "against pyridine"	error
146	8	"edition" → "addition"	error
147	12	"quit" → "kit"	error
149	15	"a little" → "few"	accuracy
149	16	"example" → "examples"	accuracy
152	2	"melt down" → "melting"	error
152	16	"reduce" → "induce"	error
162	7	"come through" → "comb through"	error
165	15	"went was" → "went with was"	error
165	25	"is ligating" → "is ligated"	accuracy
175	18	"phosphine in" → "in"	error
176	13	"phosphine in" → "in"	error
177	23	"plasma" → "plasmid"	error
178	16	"pyridines have" → "pyridine has"	error
178	25	"With stand" → "Withstand"	error
185	11	"on their HPLC" → "in their HPLC"	error

July 23, 2014

Date



Signature

17:21:27 1 no further questions.

17:21:29 2 I think we're adjourned.

17:21:32 3 THE VIDEO OPERATOR: Okay to go off the record?

17:21:34 4 MS. SWAROOP: Yes.

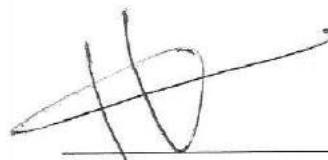
17:21:35 5 THE VIDEO OPERATOR: Off the record. The time

17:21:36 6 is 5:21.

7 (The Video Deposition concluded at 5:21 p.m.)

8 * * *

9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25



FLOYD ROMESBERG, Ph.D.