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

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INTELLIGENT BIO-SYSTEMS, INC.

Petitioner,

v.

ILLUMINA CAMBRIDGE LTD.

Patent Owner.

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Case No. IPR2013-00266 (LMG)

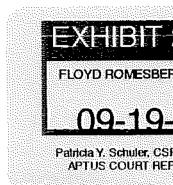
U.S. Patent 8,158,346

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VIDEO DEPOSITION OF FLOYD ROMESBERG, PH.D.

APRIL 10, 2014

Reported by: Margaret A. Smith, CSR #9733, RPR, CRR



Columbia Exhibit 2082  
Illumina, Inc. v. The Trustees  
of Columbia University in the  
City of New York  
IPR2018-00291, 318, 322, 385 & 797

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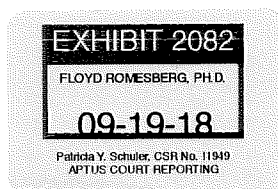
U.S. Patent 8,158,346

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VIDEO DEPOSITION OF FLOYD ROMESBERG, PH.D.

APRIL 10, 2014

Reported by: Margaret A. Smith, CSR #9733, RPR, CRR



Intelligent Bio-Systems, Inc.  
Exhibit 1042  
IBS v. Illumina  
Case IPR2013-00266

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## 1 I N D E X

2

3 EXAMINATION PAGE

4 CROSS BY MR. SEGAL 7

5 REDIRECT BY MR. BABCOCK 64

6 RE-CROSS BY MR. SEGAL 78

7

8 E X H I B I T S

9 (None offered.)

10

11 (Previously marked Exhibits 1028, 1036, 2037, 2053,

12 2054, 2055, and 2056 were referenced.)

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21 DEPOSITION OF FLOYD ROMESBERG, PH.D., taken on

22 behalf of Petitioner, at 12790 El Camino Real,

23 San Diego, California, commencing on Thursday, April 10,

24 2014 at 9:12 a.m., before Margaret A. Smith, Certified

25 Shorthand Reporter, CSR No. 9733, RPR, CRR.

1 San Diego, California; April 10, 2014; 9:12 a.m.

2

3 VIDEOGRAPHER: Good morning. We are on the

09:11:50 4 record. This is the digital video deposition of Floyd

09:11:54 5 Romesberg, Ph.D., testifying in the matter of

09:11:58 6 Intelligent Bio-Systems, Inc., versus Illumina

09:12:02 7 Cambridge, Case No. IPR2013-00266.

09:12:12 8 Today, we are located at 12790 El Camino Real,

09:12:17 9 San Diego, California.

09:12:18 10 Today's date is April 10th, 2014. The time on

09:12:23 11 the video monitor is 9:12 a.m.

09:12:27 12 My name is Jon Imel, video specialist, with

09:12:30 13 Jordan Media, Inc., located at 1228 Madison Avenue,

09:12:37 14 San Diego, California.

09:12:39 15 The certified shorthand reporter today is

09:12:41 16 Maggie Smith in association with Kramm Court Reporting,

09:12:46 17 located in San Diego, California.

09:12:48 18 Will counsel please introduce yourselves for

09:12:50 19 the record.

09:13:23 20 MR. BABCOCK: For the patent owner, this is --

09:13:23 21 Illumina, this is Brent Babcock with Knobbe Martens.

09:13:23 22 Also with me is Counsel David Kujawa.

09:12:51 23 MR. SEGAL: For Petitioner Intelligent

09:12:51 24 Bio-Systems, this is Marc Segal from Ballard Spahr. And

09:12:51 25 with me in Philadelphia -- I'm conducting this via video

09:12:51 1     teleconference -- is Scott Marty. And present at the  
09:12:51 2     deposition site in San Diego is Chrysta Elliott, also  
09:12:51 3     from Ballard Spahr.

09:13:24 4             VIDEOGRAPHER: The reporter may swear in the  
09:13:25 5     witness.

09:13:25 6  
09:13:25 7             FLOYD ROMESBERG, PH.D.,  
09:13:25 8     having been first duly sworn, was examined and testified  
09:13:25 9     as follows:

09:13:25 10

09:13:25 11             CROSS-EXAMINATION

09:13:42 12     BY MR. SEGAL:

09:13:43 13             Q     Good morning.

09:13:45 14             A     Good morning.

09:13:45 15             Q     Can you please -- could you please state your  
09:13:48 16     full name and address for the record.

09:13:50 17             A     Floyd Romesberg. 8109 Camino Del Sol. And  
09:13:57 18     that's in San Diego.

09:14:01 19             Q     I know you were deposed a few months ago in a  
09:14:03 20     related proceeding, but I just want to run through some  
09:14:07 21     ground rules for the deposition.

09:14:12 22             Do you understand that today you are under  
09:14:13 23     oath?

09:14:14 24             A     I do.

09:14:17 25             Q     And by that, you understand that you are sworn

09:14:19 1 to tell the truth?

09:14:21 2 A I do.

09:14:23 3 Q Do you also understand that even though we're  
09:14:25 4 not in a court, your answers today have the same force  
09:14:29 5 and effect as if you were testifying in a court of law?

09:14:34 6 A I do.

09:14:36 7 Q And are you prepared to answer my questions  
09:14:38 8 today?

09:14:39 9 A I am.

09:14:41 10 Q Are you aware of anything that would prevent  
09:14:43 11 you from testifying truthfully today?

09:14:46 12 A No.

09:14:51 13 Q So you're not on any medications or suffering  
09:14:53 14 from any illness that would prevent you from  
09:14:56 15 understanding my questions and testifying accurately and  
09:15:00 16 truthfully today?

09:15:01 17 A No, I am not.

09:15:06 18 Q So, today, I'm going to ask you some questions,  
09:15:09 19 and if any time you don't understand a question, please  
09:15:12 20 ask me to clarify.

09:15:13 21 Do you understand?

09:15:14 22 A I do.

09:15:17 23 Q And if you don't understand one of my questions  
09:15:24 24 or -- excuse me.

09:15:26 25 If I -- if you don't ask a clarifying question

09:15:28 1 of me, I'm going to assume that you understood my  
09:15:31 2 question.

09:15:32 3 Is that okay?

09:15:33 4 A Yes, it is.

09:15:40 5 Q Also, throughout the day, your attorney is  
09:15:42 6 likely to raise objections to some of my questions.

09:15:48 7 Unless you are instructed by counsel not to answer my  
09:15:50 8 questions, please go ahead and just answer the questions  
09:15:54 9 that I've asked you. Okay?

09:15:55 10 A Okay.

09:16:00 11 Q Okay. Today, obviously, we have a court  
09:16:01 12 reporter recording my questions and your answers. To  
09:16:11 13 help that court reporter, we need to make sure we're not  
09:16:13 14 speaking over each other.

09:16:15 15 Do you understand?

09:16:15 16 A Yes.

09:16:16 17 Q And I notice, since we're doing this via video  
09:16:20 18 teleconferencing, there is a little bit of a delay from  
09:16:24 19 when I finish my question until I think that you're  
09:16:30 20 actually receiving it. So I think we need to do an  
09:16:33 21 extra special job of waiting a second or two in between  
09:16:37 22 our questions and answers so we don't end up talking  
09:16:40 23 over each other.

09:16:42 24 Understood?

09:16:42 25 A Yes.

09:16:44 1 Q Okay. And you're doing a great job with the  
09:16:46 2 "yes." Obviously, we need clear, verbal answers. No  
09:16:51 3 "uh-huhs" or no nodding of the heads.

09:16:55 4 Understand?

09:16:56 5 A Yes.

09:16:56 6 Q Okay. And, also, if at any time you need a  
09:17:00 7 break, please let me know, and we can take a break.  
09:17:03 8 Okay?

09:17:04 9 A Okay.

09:17:08 10 Q Okay. Have you been deposed since your last  
09:17:11 11 deposition in the related -- related proceedings in  
09:17:14 12 these matters?

09:17:15 13 A I have not.

09:17:21 14 Q You previously testified that you were serving  
09:17:27 15 as an expert for Roche in a case against Enzo. Is that  
09:17:33 16 correct?

09:17:33 17 A Yes. I think it's -- I think Enzo is -- I  
09:17:37 18 think Roche is the defendant. Yes. It --

09:17:42 19 Q And you are serving as an expert on behalf of  
09:17:44 20 Roche?

09:17:45 21 A Correct.

09:17:50 22 Q Do you know if you filed an expert report in  
09:17:52 23 that matter?

09:17:55 24 A I think I was asked this last time. And I  
09:17:58 25 apologize. I think -- what I said then was I wasn't

09:18:01 1 sure. And I -- that's still the case.

09:18:03 2 As I explained last time, my administrative  
09:18:06 3 assistant handles most of that, and if they asked for  
09:18:10 4 it, I would have given it to her, and she would have  
09:18:13 5 taken care of it. And like -- like most of the  
09:18:15 6 paperwork that goes through my office, she would have  
09:18:17 7 taken care of it. And I would have glanced at it and  
09:18:20 8 made sure everything was correct. And then she would  
09:18:22 9 have sent it to them. But I -- I actually don't recall  
09:18:25 10 that. But it -- it might have happened.

09:18:31 11 Q Do you remember any -- signing any sort of  
09:18:33 12 statements like you've done in these matters, what we  
09:18:36 13 call a declaration? Have you done anything like that?

09:18:38 14 A No. So if I could clarify a little --

09:18:41 15 Q And you --

09:18:42 16 A I'm sorry. If I could clarify a little bit, it  
09:18:45 17 is moving extraordinarily slowly for reasons that I  
09:18:49 18 don't understand. In fact, I haven't spoken with them  
09:18:54 19 in, I think, probably close to a year or eight months or  
09:18:58 20 something. And I think just out of curiosity, I had my  
09:19:02 21 admin contact them a few months ago and asked if -- if  
09:19:07 22 this was still going on. And they said it was but that  
09:19:10 23 it was just being delayed.

09:19:11 24 And so it really is not past them sending me  
09:19:15 25 some material that they asked me to read. And I think

09:19:18 1 maybe two or three phone calls.

09:19:20 2 So it really hasn't -- there's -- not much has  
09:19:25 3 happened.

09:19:29 4 Q Have you been engaged as an expert in any other  
09:19:32 5 matter since your last deposition?

09:19:34 6 A No.

09:19:43 7 Q Do you know how much time, approximately, you  
09:19:44 8 spent on this IPR proceeding?

09:19:51 9 A Um --

09:19:53 10 MR. BABCOCK: And just to be clear, you're  
09:19:55 11 talking about the proceeding for the deposition today,  
09:19:57 12 not -- not the Illumina/IBS group of IPRs?

09:20:04 13 MR. SEGAL: Let me break it down a little bit  
09:20:05 14 if -- if you know the numbers. I don't know.

09:20:07 15 BY MR. SEGAL:

09:20:07 16 Q Do you know the total amount of time you spent  
09:20:10 17 on the group, all the IPRs, between Illumina and IBS?

09:20:14 18 A I'd be happy to estimate that. But I'm really  
09:20:16 19 not sure how accurate that is. I would guess 70 hours,  
09:20:21 20 something, maybe 80 on all of it, ag- -- in aggregate.

09:20:28 21 Q Okay. About how much time on this particular  
09:20:34 22 IPR, the one relating to the '346 patent?

09:20:41 23 A So just to clarify, because I -- I -- I think  
09:20:45 24 of them as the first, second, and third. So I think  
09:20:48 25 this one would be the second. You know --

09:20:51 1 Q That's right.

09:20:52 2 A -- I think the ma- -- my recollection is that  
09:20:54 3 a -- a majority was on the first. And so I think on  
09:20:57 4 this one, maybe 30 hours, maybe 35 or 40. Thirty --  
09:21:04 5 something a little less than half, I think.

09:21:21 6 Q Let me show you what's been marked as  
09:21:22 7 Exhibit 2037.

09:21:28 8 A Thank you.

09:21:29 9 MR. BABCOCK: Thanks.

09:21:40 10 BY MR. SEGAL:

09:21:40 11 Q Do you recognize this document?

09:21:42 12 A I do.

09:21:46 13 Q Is this a copy of your declaration signed in  
09:21:50 14 this proceeding?

09:21:51 15 A Yes, it would appear to be.

09:21:54 16 THE REPORTER: And, Coun- --

09:21:54 17 BY MR. SEGAL:

09:21:55 18 Q And if you'll turn to the last page.

09:22:09 19 THE REPORTER: And, Counsel, this is the  
09:22:09 20 reporter. With the cough, I want to make sure I heard.  
09:22:09 21 The question was "Is this a copy of your declaration  
09:22:09 22 signed in this proceeding"?

09:22:14 23 MR. SEGAL: In this proceeding.

09:22:16 24 THE REPORTER: Thank you.

25

09:22:22 1 BY MR. SEGAL:

09:22:22 2 Q And on the last page, is that your signature?

09:22:27 3 A It is.

09:22:34 4 Q Did you draft this declaration?

09:22:38 5 A I was -- I was responsible for all the

09:22:40 6 scientific content and editing and was provided a first

09:22:46 7 draft after discussion by my -- by -- by the lawyers.

09:22:57 8 Q So the lawyers from Knobbe Martens provided you

09:23:00 9 with a first draft?

09:23:01 10 A After discussion, yes.

09:23:05 11 Q Was there anyone else involved, other than the

09:23:10 12 Knobbe lawyers, in preparing your declaration?

09:23:15 13 A No.

09:23:21 14 Q If you'll turn to page 2 of your declaration.

09:23:31 15 In particular, paragraph 4.

09:23:34 16 Can you please confirm that this is a complete

09:23:36 17 list of the documents you considered in preparing your

09:23:39 18 declaration, in this declaration.

09:23:54 19 A I believe it is, yes.

09:24:05 20 Q And these are the only materials you relied on

09:24:07 21 in forming your opinions in this declaration?

09:24:11 22 A Yes.

09:24:15 23 If I could caveat that slightly, I -- I -- I've

09:24:20 24 been thinking about these sorts of questions, because

09:24:22 25 it's related to my own research, for many, many years,

09:24:27 1 since I started my own independent career in 1999.  
09:24:30 2 So a lot of what went into this declaration is  
09:24:34 3 just the -- the acute -- results from the accumulation  
09:24:39 4 of lots of things. These are the manuscripts that I --  
09:24:45 5 the documents that I read and -- and explicitly  
09:24:48 6 considered for this. But that, of course, is in the  
09:24:51 7 context of years of reading many other things, ranging  
09:24:54 8 from textbooks to literature publications to meetings.  
09:24:58 9 And those would have also informed my opinions in this  
09:25:02 10 as well.

09:25:08 11 Q I notice you didn't list U.S. Patent  
09:25:11 12 No. 8158346 or what we are calling the '346 patent.

09:25:20 13 Does that patent not form the basis of your  
09:25:24 14 opinion?

09:25:25 15 A So I -- I guess I'm not exactly sure I  
09:25:28 16 understand the question. What we're discussing is the  
09:25:30 17 '346 patent. So I guess what I listed here were  
09:25:34 18 documents that informed me about that patent. So, of  
09:25:37 19 course, that patent and the material -- and the material  
09:25:41 20 context of that patent was central to all of this,  
09:25:43 21 because that's why this document is here. I didn't list  
09:25:50 22 it because, I guess, I didn't think that was what was  
09:25:53 23 being listed. These are the -- these are the doc- --  
09:25:55 24 these are the documents that I consulted explicitly  
09:25:57 25 about issues related to that patent. So that I guess

09:26:02 1 that's why the patent is not itself listed.

09:26:07 2 Q How about with respect to Illumina's motion to  
09:26:10 3 amend, does that also inform your opinions in this  
09:26:12 4 declaration?

09:26:18 5 A Do you mean the -- the document -- I'm sorry.  
09:26:20 6 I'm not sure I understand the -- the exact language.  
09:26:22 7 The motion to amend is an actual document?

09:26:27 8 Q Yeah. Are you familiar that Illumina filed a  
09:26:30 9 motion to amend the claims in this proceeding?

09:26:32 10 A Yes.

09:26:36 11 Q Have you -- have you reviewed that document?

09:26:40 12 A I think so. I don't explicitly recall, but I  
09:26:43 13 think I did. But, no, I don't think it informed --  
09:26:47 14 my -- I apologize. But my recollection maybe is that it  
09:26:50 15 was a very long document. And there was nothing in it  
09:26:53 16 that I found that -- that -- that I thought informed  
09:26:56 17 this. When I -- when I was writing this and thinking  
09:26:58 18 about this, I didn't go to that document. So these are  
09:27:01 19 the documents that I -- that I did go to and that I did  
09:27:03 20 think about and that I did sort of cite in this document  
09:27:09 21 or that materially influenced statements in this  
09:27:12 22 document. And none of that document, the motion to  
09:27:16 23 amend document, did I do that with. So -- so it's not  
09:27:21 24 listed.

09:27:28 25 Q Who identified the documents listed in

09:27:30 1 paragraph 4?

09:27:30 2 A It was a combination of the discussions with my  
09:27:36 3 lawyers. Between the two -- between the group of us,  
09:27:38 4 the three of us.

09:27:41 5 And, you know, I don't know that I remember  
09:27:43 6 exactly every one of them. I mean, several of them are  
09:27:48 7 obviously very central to this case. And -- for  
09:27:49 8 example, the JU patent and the Herman patent and the  
09:27:54 9 Ruby patent -- or the Ruby paper. Those obviously would  
09:27:57 10 have been presented to me as background reading  
09:28:00 11 material.

09:28:01 12 In fact, maybe that's the best way to put it.  
09:28:03 13 I would -- I would actually claim that the listing here  
09:28:06 14 is mine. A lot of them, I was provided with. And I  
09:28:10 15 think I would not have been able -- either so central to  
09:28:15 16 the patent that to say that I -- that -- that their  
09:28:19 17 being there was motivated by me alone, it would be kind  
09:28:22 18 of silly, unrealistic.

09:28:25 19 But I think the actual list here was probably  
09:28:29 20 largely mine.

09:28:36 21 Q Did you identify any of these documents that  
09:28:37 22 are on the list? In other words, did you actually  
09:28:39 23 search for and find the documents, any of them on the  
09:28:42 24 list?

09:28:49 25 A Yes, I did. In terms of exactly which ones,

09:28:54 1 I'd -- I'd have to look through the list individually.

09:28:57 2 So, like I said, some of them were presented to me as

09:29:00 3 being so central to the case. Some of them were

09:29:03 4 presented to me as background. And some of them, I

09:29:06 5 found myself.

09:29:11 6 Q Can you tell me which ones you found yourself?

09:29:13 7 A So that might be a little bit difficult. I

09:29:15 8 mean, I will (sic) claim that what I'm about to tell you

09:29:18 9 is an exclusive list of them, because these are just

09:29:21 10 first authors, and there's a lot of them, and I've read

09:29:24 11 a lot of things. And they might have to be shown to me.

09:29:27 12 So if you wanted me to go through every one of them, I'd

09:29:30 13 be happy to do that, if I -- if I had copies of them.

09:29:32 14 But just glancing at the titles, I -- what's

09:29:35 15 written here, I can certainly tell you several of

09:29:36 16 them that -- that I found. But I only want to clarify

09:29:39 17 that I'm not sure that I'll hit all of them.

09:29:41 18 But, for example, the -- the Fersht papers

09:29:45 19 listed as Q&R, for example, I found. And I believe the

09:29:59 20 Bebenek papers. And, again, I'm not -- if you want me

09:30:05 21 to give you an explicit call on -- on any of these, I'd

09:30:07 22 like to see the documents.

09:30:09 23 But I'm -- the first ones, I just happen to

09:30:12 24 remember because I was surprised that Allan Fersht wrote

09:30:16 25 the papers. And I -- and I can tell you what they were

09:30:19 1 about and -- and why they're here. And I believe  
09:30:21 2 several others.

09:30:22 3 My guess is that four or five of them, I found.  
09:30:25 4 And if you want exactly which ones those are, I'd have  
09:30:30 5 to see them. But I believe the -- the two Fersht  
09:30:31 6 papers, certainly, I found.

09:30:33 7 And, like I said, I believe the Bebenek papers  
09:30:36 8 as well.

09:30:37 9 Does that answer your question sufficiently?

09:30:40 10 Q Okay. I mean, just by looking at the list, we  
09:30:42 11 have the Fersht and the Bebenek papers.

09:30:45 12 Just by, again, looking at the list, can you  
09:30:47 13 recall any other ones that -- that you actually located?

09:31:06 14 A No. And I'm not 100-percent sure there aren't  
09:31:09 15 any. But those are the only ones I can recall.

09:31:18 16 Q Were you told not to consider any documents --

09:31:21 17 A No.

09:31:22 18 Q -- in rendering your opinions?

09:31:24 19 A No, I was not.

09:31:31 20 Q Did you do anything to prepare for your  
09:31:33 21 deposition today?

09:31:34 22 A Sure. Yes.

09:31:35 23 Q Can you explain your preparation.

09:31:37 24 A So I -- I -- I met with David and -- and Kerry  
09:31:45 25 and Brent on Monday of this week for a few hours to just

09:31:48 1 discuss things in my dec to make sure that -- you know,  
09:31:52 2 I think it was just to refresh my memory and make sure  
09:31:55 3 that I -- I unfortunately have a lot of other things  
09:31:58 4 that I'm doing and wanted to make sure that I was sort  
09:32:00 5 of -- sort of just touching base just to rethink things.

09:32:04 6 And then I spent an hour and a half on Sunday  
09:32:07 7 just rereading things.

09:32:09 8 And then I spent half an hour this morning over  
09:32:11 9 coffee, trying to wake up, and I glanced at them.

09:32:15 10 So I believe that would be the entirety of  
09:32:17 11 the -- of my getting ready for this meeting/deposition.

09:32:30 12 Q Other than the attorneys at Knobbe, did you  
09:32:32 13 discuss your deposition preparation with anyone else?

09:32:35 14 A No, I did not.

09:32:40 15 Q Did you review any documents to prepare for  
09:32:42 16 today's deposition?

09:32:44 17 MR. BABCOCK: So I'll -- I'll caution the  
09:32:45 18 witness that you can answer "yes" or "no." You're not  
09:32:49 19 permitted to divulge documents that we showed you.  
09:32:54 20 Those are all work product. So you can answer his  
09:32:56 21 question with a "yes" or a "no," but don't identify  
09:32:59 22 anything in particular that you may recall that counsel  
09:33:01 23 showed you.

09:33:03 24 Is that a clear instruction?

09:33:06 25 THE WITNESS: Yeah.

09:33:07 1 I'm sorry. Could you -- I'm sorry. Could you  
09:33:09 2 repeat the question.

09:33:10 3 And then -- and then, Brent, could you -- you  
09:33:13 4 repeat the extra.

09:33:15 5 MR. BABCOCK: Okay.

09:33:16 6 BY MR. SEGAL:

09:33:16 7 Q Let me ask you a slightly different way.

09:33:18 8 Did you review any documents on your own  
09:33:20 9 without input from counsel on which documents to review  
09:33:24 10 to prepare for your deposition today?

09:33:28 11 MR. BABCOCK: Okay. That question, you can  
09:33:29 12 answer fully.

09:33:30 13 THE WITNESS: Yes.

09:33:32 14 BY MR. SEGAL:

09:33:32 15 Q Okay. Which documents?

09:33:34 16 A I don't remember. It is -- I -- I glanced at a  
09:33:39 17 large number of them as I was just rethinking through  
09:33:42 18 all aspects. It's very typical for the way I think  
09:33:45 19 about things. If there's an issue that comes up in my  
09:33:48 20 mind, I'll probably flip to the document, find it in the  
09:33:51 21 document, and so I -- I would say it's actually quite  
09:33:55 22 possible that I glanced at some level at all of them.

09:33:59 23 I probably spent -- if you'd like to know which  
09:34:02 24 ones I spent the most time, I could probably list those,  
09:34:05 25 if you'd like.

09:34:08 1 Q Yes, please.

09:34:09 2 A Okay. Probably the Ruby paper, the Herman  
09:34:15 3 patent, the Chen patent, the Canard paper, the Welch --  
09:34:25 4 the Welch and Metzker papers, and the -- the Letsinger  
09:34:36 5 paper were probably the ones that I spent more -- more  
09:34:38 6 time looking at and would have actually read more --  
09:34:42 7 more of them.

09:34:53 8 Q Okay. Let's take a look at your declaration,  
09:34:55 9 Exhibit 2037. And if you'll turn to page 15, I'm going  
09:35:08 10 to ask you some questions about paragraph 17, which  
09:35:10 11 actually starts on page 14. So if you'd like to review  
09:35:18 12 that paragraph before I ask you questions, or I could  
09:35:20 13 just jump right in.

09:35:22 14 A Sure. I -- I believe I'm pretty familiar with  
09:35:24 15 it. So --

09:35:30 16 Q I want to ask you about a sentence that is on  
09:35:33 17 page 15. It starts about six lines up from the bottom.  
09:35:38 18 And I'm just going to read it into the record.

09:35:42 19 "A person of ordinary skill in the art seeking  
09:35:45 20 to develop sequencing by synthesis methods would  
09:35:53 21 recognize the need to use a nucleotide with a 3'  
09:35:56 22 protecting group that could be incorporated by a  
09:35:59 23 polymerase with high fidelity at moderate to low  
09:36:03 24 concentrations."

09:36:05 25 Do you see that sentence?

09:36:07 1 A I do.

09:36:10 2 Q What do you mean by "sequencing by synthesis  
09:36:13 3 methods" in this sentence?

09:36:17 4 A So I -- I believe -- what I -- what I would --  
09:36:18 5 what I'm trying to articulate there is the suite of  
09:36:24 6 sequencing by synthesis methods, not any one particular  
09:36:28 7 method. There's a variety of them that people are  
09:36:32 8 interested in. And all of them have certain  
09:36:34 9 restrictions that are kind of obvious. And so it isn't  
09:36:40 10 intended to -- to speak to the specifics of one versus  
09:36:43 11 another, but, rather, to the properties that a 3'  
09:36:52 12 protecting group would have to have in order to be even  
09:36:55 13 possible -- for its use to be possible for use in  
09:36:58 14 sequencing by synthesis methods.

09:37:04 15 Q Does the sequencing by synthesis method, the  
09:37:08 16 way you use it in this sentence, does it have to have a  
09:37:10 17 certain number of cycles or read lengths?

09:37:16 18 A Yes.

09:37:18 19 Q And so what are you -- when you say "sequencing  
09:37:21 20 by synthesis," how many cycles or read lengths are you  
09:37:25 21 thinking about?

09:37:25 22 A So let me qualify that, if -- if I may.

09:37:28 23 It wouldn't have to include that, because there  
09:37:30 24 would be -- there would be characteristics of a 3'  
09:37:38 25 protecting group that would even prevent it from being

09:37:41 1 useful for a single round.

09:37:43 2 Now, for any sequencing by synthesis method to  
09:37:47 3 be viable as a commercial product, it's going to  
09:37:51 4 obviously have to have the ability to go through  
09:37:54 5 multiple rounds.

09:37:55 6 But my use of "sequencing by synthesis" here, I  
09:37:58 7 guess I would -- I would not like it to be taken to be a  
09:38:01 8 detailed statement, a -- it's not intended to be  
09:38:05 9 detailed to that extent. It -- it's sort of just  
09:38:10 10 generic for any sequencing by synthesis method.

09:38:12 11 So if -- if -- there could be problems that are  
09:38:15 12 manifest at the single incorporation level, in which  
09:38:18 13 case they could be a problem even if you weren't going  
09:38:21 14 through cycles, or they could be a problem that was  
09:38:22 15 unique for only -- that was only unique to be a pro- --  
09:38:27 16 that was only a problem when you were going through  
09:38:30 17 cycles. But it wouldn't have to be one or the other.  
09:38:33 18 It could be either.

09:38:35 19 Is -- I'm sorry. Is that clear?

09:38:40 20 Q Well, let me ask you a follow-up question.

09:38:42 21 You say "A person of ordinary skill in the art  
09:38:45 22 seeking to develop sequencing by synthesis methods."

09:38:48 23 So in this sentence, what is the person of  
09:38:51 24 ordinary skill in the art seeking to develop? Are  
09:38:54 25 they -- in one -- in -- in a -- what type of sequencing

09:39:00 1 by synthesis methods are they seeking to develop? A  
09:39:03 2 method with, you know, at least 10 read lengths, 20 read  
09:39:06 3 lengths, 50 read lengths?

09:39:09 4 A It could be any of those things. A lot of  
09:39:10 5 times when people do science and they're seeking --  
09:39:14 6 seeking to develop a method, you're not going to exclude  
09:39:16 7 an approach because you don't understand how it might be  
09:39:19 8 used in the end to sequence 100 cycles. And so maybe  
09:39:23 9 you're focused only on that first step. And so in that  
09:39:27 10 case, maybe you would be more tolerant of modifications  
09:39:30 11 that might be only a problem in the cycling step. But  
09:39:34 12 that's okay, because you're focused on the in- -- on the  
09:39:39 13 first step. So it could -- it really could be either.

09:39:42 14 People develop things from a lot of different  
09:39:45 15 perspectives, with a lot of different rigor, and with a  
09:39:48 16 lot of different criteria. And my -- my statement here  
09:39:50 17 is supposed to be about those things that are so broad  
09:39:53 18 and -- and important, that, in my opinion, would be --  
09:39:55 19 would -- would -- would be important for any aspect of  
09:39:58 20 developing sequence -- a sequencing by synthesis method.

09:40:25 21 Q Let me ask you about another term used in that  
09:40:27 22 sentence. You say "with high fidelity."

09:40:37 23 What do you mean by that?

09:40:38 24 A So when a DNA -- so all sequencing by synthesis  
09:40:41 25 methods involve a polymerase incorporating triphosphates

09:40:48 1 against a template. That's how they work. So DNA  
09:40:52 2 synthesis works by having a template strand of DNA and a  
09:40:57 3 polymerase that sequentially incorporates one of the  
09:41:00 4 four nucleotides. That's, in fact, what you're  
09:41:02 5 interested in. That's what gives you the sequence.

09:41:04 6 "Fidelity" is a term that describes -- that you  
09:41:06 7 could think of as error rate. If your sequencing by  
09:41:12 8 synthesis method is high fidelity, that means it has a  
09:41:15 9 low error rate, which means the sequence it produces,  
09:41:18 10 the results that it produces are reliable. If it has a  
09:41:22 11 low error rate or a low -- sorry. If it has a high  
09:41:25 12 error rate or a low fidelity, that would mean that it  
09:41:29 13 produces sequences that are -- that do not reflect what  
09:41:33 14 the template sequence that you're trying to sequence is  
09:41:35 15 and would make them less useful or perhaps even not  
09:41:38 16 useful at all.

09:41:39 17 So "fidelity" simply refers to how accurately  
09:41:43 18 you're reading the piece of DNA that you want to  
09:41:46 19 sequence. And it largely results from the correct  
09:41:48 20 incor- -- it results from the correct incorporation of a  
09:41:52 21 triphosphate, of a nucle- -- of a nucleotide  
09:41:57 22 triphosphate, in this case -- in most cases, a labeled  
09:42:00 23 nucleotide triphosphate, a fluorophore labeled  
09:42:05 24 nucleotide triphosphate.

09:42:08 25 And what the fidelity refers to -- those

09:42:10 1 triphosphates, obviously, are G, C, A, and T, are the --  
09:42:15 2 are the common names that people use for them. They're  
09:42:17 3 just the nucleotides of the -- of DNA.

09:42:19 4 So every time you have a C, fidelity means --  
09:42:23 5 high fidelity means that with a -- you always  
09:42:27 6 incorporate a G. When -- every time you have a G, you  
09:42:30 7 always incorporate a C. Every time you have an A, you  
09:42:33 8 always incorporate a T. And when you have a T, you  
09:42:36 9 always incorporate an A.

09:42:42 10 Q Okay. You mentioned error rate. So low  
09:42:45 11 fidelity would have a higher error rate. So if you have  
09:42:49 12 a high -- just to make sure I understand this, if you  
09:42:52 13 have a high fidelity, then you would have a high correct  
09:42:57 14 incorporation rate.

09:42:57 15 Does that make sense?

09:42:58 16 A Yes.

09:43:03 17 Q Now, is there a certain percentage of -- for --  
09:43:10 18 for the correct incorporation rate that you would want  
09:43:12 19 for a -- a sequencing by synthesis method?

09:43:18 20 A So are you asking what fidelity you would need  
09:43:21 21 for a sequencing by synthesis method?

09:43:25 22 Q Yes.

09:43:26 23 A It really depends on what your application is.  
09:43:31 24 There's a variety of different applications that people  
09:43:34 25 are interested in. For example, one is called de novo

09:43:39 1 sequencing. And what that means is that you have no  
09:43:43 2 information about your sequence that you're trying to  
09:43:44 3 sequence -- the DNA that you're trying to sequence.

09:43:48 4           You need really high fidelity there, because  
09:43:51 5 you don't know -- you have no information. It's maybe a  
09:43:56 6 little bit relaxed, a little -- maybe the fidelity  
09:44:00 7 demands are a little bit lower if you're doing something  
09:44:02 8 called resequencing, in which case you already know the  
09:44:06 9 sequence and you're just looking to confirm that that's  
09:44:09 10 the sequence, for example.

09:44:13 11           If you're looking for mutations, which is, you  
09:44:15 12 know, really what -- something that's emerging as being  
09:44:19 13 what people are interested in using sequencing by  
09:44:21 14 synthesis for, you're back to needing really high  
09:44:24 15 fidelity. Because if you -- you're not just trying to  
09:44:27 16 say, okay, is -- is -- is -- is that particular sequence  
09:44:30 17 present or not. I want to know if it's present and does  
09:44:33 18 it have the correct sequence. So you have -- you have  
09:44:36 19 obviously a higher fidelity there.

09:44:39 20           Now, I can give you my opinion, and this might  
09:44:41 21 not be everyone's opinion, because people who are  
09:44:43 22 interested in developing sequencing by synthesis  
09:44:45 23 methods, maybe their method is not really practical and  
09:44:50 24 they don't really care. They want to get a paper.  
09:44:54 25 Maybe they would claim they would be a little bit more

09:44:57 1 tolerant of lower fidelity.

09:44:58 2 My opinion from a practical perspective, if  
09:45:01 3 you're going to develop something that people are going  
09:45:03 4 to use in an industrial commercial setting, you have to  
09:45:06 5 have as high a fidelity as possible.

09:45:12 6 Q Can you give -- can you give me a number, put a  
09:45:14 7 number on that? Better than a 95 percent?

09:45:20 8 A Certainly. I don't know that I could put a  
09:45:22 9 number on that. I can give you an impression to any  
09:45:25 10 number you give me. That's a hard question to answer,  
09:45:27 11 because it's all relative. I mean, if you have  
09:45:30 12 extraordinarily long read lengths, the ability to read  
09:45:34 13 long, long sequences, and you have a very cheap method,  
09:45:38 14 maybe you'd be a little more tolerant of a slightly  
09:45:42 15 lower fidelity, because you're just going to sequence  
09:45:44 16 over and over again. And you're going to use a  
09:45:47 17 method -- a method we call maximum parsimony. It's a  
09:45:51 18 simple idea. It's majority rules, if you like to think  
09:45:55 19 of it that way. If you sequence something 10 times and  
09:45:57 20 you have a -- a higher error rate, you can just compare  
09:46:00 21 those 10 sequences and say, okay, it got it wrong this  
09:46:03 22 one time. But nine times, it got it right.

09:46:07 23 And so a lower error rate would require a  
09:46:09 24 higher sequencing coverage. You'd have to sequence more  
09:46:13 25 often. And so maybe you have a very cheap method. And

09:46:16 1 so maybe you're going to promote your method by saying,  
09:46:21 2 okay, it's got a little bit lower fidelity, but look how  
09:46:24 3 cheap it is. And we can get really long read lengths.  
09:46:28 4 So that's not going to be a problem.

09:46:30 5 So there's no absolute answer to your question.  
09:46:32 6 It's just that if there's a lower fidelity, it's going  
09:46:35 7 to require more reading. That is going to be more  
09:46:37 8 expensive. That may be more, or less tolerated based on  
09:46:41 9 your method. So it's very much method-dependent. It's  
09:46:43 10 very much application. But the app- -- it's very much  
09:46:46 11 application-dependent. But no one is going to promote a  
09:46:50 12 sequencing approach that has a 90-percent fidelity, for  
09:46:54 13 example. That's just too low. A 95-percent fidelity is  
09:46:59 14 just too low. Now, 99, versus 99.9, versus 99.999,  
09:47:07 15 maybe.

09:47:07 16 Now, remember, to you -- to a lot of people, a  
09:47:10 17 fidelity of 99 percent seems high. DNA polymerases,  
09:47:15 18 themselves, start at fidelities, under the worst cases,  
09:47:20 19 of 99.999999. The prob- -- the reason that you don't  
09:47:26 20 get that sort of fidelity when you're working in a  
09:47:30 21 sequencing by synthesis method is because you had to  
09:47:33 22 change things in order to be able to read them.

09:47:36 23 And so polymerases get angry. Polymerases  
09:47:39 24 don't work as well. And so the fidelity drops to 99.9,  
09:47:45 25 which is four to five orders of magnitude down.

09:47:51 1 And -- and so you're starting from  
09:47:53 2 extraordinarily high fidelities. The method that you  
09:47:56 3 develop, depending on the sort of modifications that you  
09:47:58 4 make to the nucleotides to be able to read them,  
09:48:03 5 strongly impacts that. And so that's what we're talking  
09:48:05 6 about: How much do your analogs reduce fidelity.

09:48:09 7 And so, again, like I said, it's  
09:48:11 8 application-dependent, technique-dependent, and how  
09:48:15 9 willing you are to -- to have to use the method over and  
09:48:21 10 over again to generate reliable data.

09:48:24 11 So there's no absolute answer to your question.  
09:48:27 12 The -- the -- the only real correct answer there is as  
09:48:30 13 high as possible. And -- and -- and things dipping down  
09:48:36 14 into the 97 percent very quickly become untenable.

09:48:45 15 Q How about for methods where you're sequencing,  
09:48:49 16 like, so-called clusters of templates, where you have a  
09:48:52 17 number of identical templates, say, a thousand or so,  
09:48:55 18 would that tolerate a lower fidelity?

09:49:00 19 MR. BABCOCK: Objection. Outside the scope.  
09:49:00 20 Calls for speculation.

09:49:09 21 THE WITNESS: That's a -- a little bit of a  
09:49:10 22 hard question to answer, because I'd have to sort of see  
09:49:13 23 the technique and make sure that -- that I agreed with  
09:49:17 24 it. But it -- it could. It -- because you're  
09:49:24 25 getting -- I mean, I guess that's a little bit similar.

09:49:27 1 And, again, I'd have to see the exact technique to make  
09:49:30 2 sure that -- that this is correct.

09:49:33 3 But, in principal, if you were generating  
09:49:35 4 independent reads within that cluster, then you can use  
09:49:37 5 that maximum parsimony method that I mentioned earlier.  
09:49:43 6 But that isn't necessarily the case, because it depends  
09:49:45 7 on how you're reading what that cluster says, because  
09:49:47 8 you're not going to go into a cluster and read every  
09:49:50 9 individual template within that cluster.

09:49:52 10 So depending on how you were reading it,  
09:49:55 11 there's going to probably be an aggregate signal for  
09:49:58 12 that cluster. And so to answer that question, I would  
09:50:02 13 have to understand how individual fidelities on  
09:50:06 14 individual templates with any given cluster impact the  
09:50:09 15 aggregate signal that you're reading.

09:50:15 16 BY MR. SEGAL:

09:50:44 17 Q So if you'll turn back to your declaration  
09:50:46 18 again. On that same page, at line -- about four lines  
09:50:55 19 down, I'm going to ask you a question about a sentence  
09:51:00 20 in your -- on that page. And I'm just going to read  
09:51:02 21 that into the record.

09:51:03 22 It says "However, polymerases lose the ability  
09:51:06 23 to accurately replicate a DNA strand at high nucleotide  
09:51:12 24 concentrations because the selectivity of polymerases is  
09:51:15 25 hindered at high nucleotide concentrations."

09:51:19 1 Do you see that?

09:51:24 2 A Yes, I do.

09:51:25 3 Q And right after that sentence, you cite to two

09:51:38 4 Fersht papers. Is that correct?

09:51:40 5 A That's correct.

09:51:40 6 Q All right. I'm going to show you what already

09:51:42 7 been marked as Exhibit 2053.

09:51:44 8 A Thank you.

09:51:58 9 Q Is this one of the Fersht papers you were

09:52:01 10 citing to in your declaration?

09:52:03 11 A Yes, it is.

09:52:11 12 Q Okay. In particular, I believe you were citing

09:52:13 13 to page 4947. Correct?

09:52:20 14 A Yes, it is.

09:52:25 15 Q What portion of page 4947 are you relying on to

09:52:29 16 support your statement in your declaration?

09:52:34 17 A I think there's several places. I can glance

09:52:36 18 at it and find them if you'd like. Like, I -- I just

09:52:39 19 glanced right now and saw one statement that would fit

09:52:43 20 that. If -- again, I'm not positive it's all of them.

09:52:47 21 And -- and if you wanted to give me a few minutes, I

09:52:49 22 could read it and come up with all of them. Or I can

09:52:54 23 give you an example.

09:52:56 24 So is an example sufficient, or do you want a

09:52:59 25 more inclusive list?

09:53:01 1 Q An example is fine.

09:53:02 2 A Okay. So just at the -- I -- the first line I  
09:53:04 3 happen to look at was the first line on the paragraph on  
09:53:07 4 the right. So it says "Increasing the concentration of  
09:53:12 5 dGTP relative to the ATP to favor the A-to-G transition,  
09:53:18 6 however, caused a dramatic increase in the number of  
09:53:21 7 revertants."

09:53:27 8 And then it says "At 1 millilimole -- millimolar  
09:53:28 9 dGTP and 10 micromolar ATP, the reversion rate increased  
09:53:34 10 by 2,000 fold."

09:53:36 11 So that would be an example.

09:53:42 12 And another particularly important aspect that  
09:53:45 13 caught my attention -- so this is one of the papers that  
09:53:47 14 I -- that I identified. They talk about the nonlinear  
09:53:52 15 relationship. So this is at the beginning of the next  
09:53:56 16 paragraph, where they say "The nonlinear relationship  
09:53:58 17 between the reversion frequencies and the  
09:54:03 18 concentrations." And then they go on to talk about it.  
09:54:06 19 So the paper observes as you vary the relative  
09:54:08 20 concentrations of the nucleotide triphosphates, for  
09:54:11 21 example, increasing one, you get a nonlinear increase in  
09:54:14 22 the mutation rate, meaning, that you start to get -- if  
09:54:16 23 you double the concentration of that reagent, of that  
09:54:19 24 particular triphosphate, you more than double the  
09:54:22 25 concentration -- the -- the -- the number of mutations

09:54:24 1 that you're producing. So that's what they mean by

09:54:27 2 "nonlinear."

09:54:30 3 Q So Fersht is measuring the misincorporation

09:54:34 4 rate by varying the ratios of the nucleotides. Is that

09:54:37 5 right?

09:54:38 6 A Yes.

09:54:38 7 THE REPORTER: Can the reporter verify, please.

09:54:38 8 The question was -- in the question, was the word

09:54:38 9 "disincorporation," as seen on your realtime screen?

09:54:51 10 MR. SEGAL: Misincorporation.

09:54:57 11 THE REPORTER: Thank you.

09:54:58 12 MR. SEGAL: Misincorporation.

09:55:00 13 THE REPORTER: Thank you.

09:55:01 14 BY MR. SEGAL:

09:55:01 15 Q And -- and some of the experiments described in

09:55:04 16 Fersht, I think, use a ratio of 100 to 1, and that would

09:55:10 17 be the -- the G to the A?

09:55:15 18 A One millimolar to 10 micromolar. So that be

09:55:21 19 correct, 100 to 1.

09:55:25 20 Q And Fersht's results show that where the ratio

09:55:29 21 is greater, the error rate is actually higher?

09:55:36 22 A Could you -- I'm not sure I understand that.

09:55:38 23 They -- as -- as the ratio gets greater -- yes, the

09:55:42 24 mutation rate gets greater. Correct. I believe that's

09:55:45 25 what you said.

09:55:46 1 Q Yes. Thank you.

09:55:48 2 And, conversely, where the ratio is lower, the  
09:55:52 3 error rate is lower?

09:55:54 4 A Yes.

09:56:03 5 Q So if you'll go back to where you -- I think  
09:56:04 6 you were reading earlier in that top of page 4947 in  
09:56:12 7 column 2, I just want to understand what these error  
09:56:14 8 rates are. And I believe -- so what Fersht is reporting  
09:56:20 9 is that at a hundred and one ratio of dGTP to dATP, the  
09:56:27 10 error rate is 1 out of 150, as measured by the progeny  
09:56:33 11 phage release.

09:56:34 12 Would you agree with me?

09:56:35 13 A I believe so, yes.

09:56:41 14 Q So if the error rate is 1 out of 150, as we  
09:56:43 15 discussed earlier, then the correct incorporation rate  
09:56:46 16 would be 149 out of 150.

09:56:56 17 A Correct.

09:56:56 18 Q And if I did the math correctly -- I am a  
09:56:58 19 lawyer. But -- so please check me -- I believe that  
09:57:01 20 equals a 99.3-percent rate.

09:57:06 21 A That sounds about right.

09:57:12 22 Q Okay. Then Fersht also reports results with a  
09:57:18 23 different assay, and I believe right around that same  
09:57:19 24 spot we were looking at, at a hundred and one ratio of  
09:57:22 25 dGTP to dATP, the error rate is 1 out of 300, as

09:57:28 1 measured by the number of infected centers.

09:57:33 2 Would you agree with me?

09:57:37 3 A Yes.

09:57:37 4 Q And, again, if the error rate is 1 out of 300,

09:57:40 5 the correct incorporation rate is 299 out of 300, which

09:57:43 6 works out to about 99.7 percent.

09:57:46 7 Would you agree with me there?

09:57:48 8 A I would.

09:58:00 9 Q And also, the Fersht paper reports that the

09:58:04 10 ineffective center assay is a better way to measure the

09:58:07 11 error rate or is more accurate than the progeny phage

09:58:13 12 assay.

09:58:15 13 Would you agree with me?

09:58:16 14 A I would agree that -- that Fersht states that.

09:58:19 15 I don't know if I agree with Fersht. But --

09:58:31 16 Q So for that statement that we read out of your

09:58:33 17 declaration, that same sentence, you recite to another

09:58:36 18 Fersht paper. Correct?

09:58:37 19 A Yes.

09:58:43 20 Q So I'm going to hand you what's been previously

09:58:45 21 marked as Exhibit 2054 --

09:58:48 22 MR. BABCOCK: Thank you.

09:58:49 23 BY MR. SEGAL:

09:58:49 24 Q -- which is a paper by Fersht, dated 1981.

09:58:59 25 A I -- I'm sorry.

09:59:00 1 Q Is this the other --

09:59:01 2 THE WITNESS: Could I -- could I ask for a

09:59:03 3 60-second break. I apologize. I would like to use the

09:59:09 4 bathroom.

09:59:10 5 MR. SEGAL: Sure.

09:59:11 6 VIDEOGRAPHER: Okay. We are going off the

09:59:12 7 record. The time is 9:59.

10:02:55 8 (Recess from 9:59 a.m. to 10:03 p.m.)

10:03:15 9 VIDEOGRAPHER: We are going back on the record.

10:03:17 10 The time is 10:03.

10:03:25 11 MR. BABCOCK: F-e-r-s-h-t, Fersht, as opposed

10:03:30 12 to First.

10:03:33 13 THE REPORTER: F-e-r- --

10:03:35 14 MR. BABCOCK: -- -s-h-t, Fersht --

10:03:39 15 THE REPORTER: Thank you.

10:03:39 16 MR. BABCOCK: -- for both of them.

10:03:40 17 BY MR. SEGAL:

10:03:41 18 Q Dr. Romesberg, right before the break, you were

10:03:47 19 handed Exhibit 2054.

10:03:50 20 Is this the other Fersht reference that you

10:03:53 21 were citing to in your declaration?

10:03:55 22 A Yes, it is.

10:04:05 23 Q Does exhibit 2054 use the same basic strategy

10:04:09 24 to induce misincorporations as Exhibit 2053, the other

10:04:15 25 Fersht paper, by increasing the concentrations of one

10:04:19 1 nucleotide over the others?

10:04:21 2 A So they use that as a method to increase the  
10:04:23 3 mutation, to -- to -- what the papers are doing is  
10:04:27 4 they're measuring the impact of that altered ratio on  
10:04:29 5 the mutation rate. So the papers are -- are similar,  
10:04:33 6 yes.

10:04:35 7 Q So they're using a higher concentration of one  
10:04:37 8 of the nucleotides and keeping the other three lower.  
10:04:43 9 Is that right?

10:04:44 10 A I seem to recall there are a few experiments  
10:04:47 11 where they vary a couple, but, in general, yes.

10:04:52 12 Q And I believe you cite to pages 4252 to 4253.  
10:05:01 13 Is that right?

10:05:02 14 A Yes, it is.

10:05:10 15 Q I want to ask you about the error rates on  
10:05:13 16 4253.

10:05:17 17 And I believe the highest error rates in that  
10:05:19 18 Figure 1 on 4253 is 80 out of 100,000. And I believe  
10:05:31 19 that's shown in the -- in the bottom right-hand graph on  
10:05:33 20 that page.

10:05:37 21 Do you agree with that?

10:05:40 22 A I would have to take a few moments to say if  
10:05:43 23 that were -- that was the highest. But that looks --  
10:05:47 24 that looks the highest, yes.

10:05:52 25 Q I mean, if you need to look at it for a few

10:05:55 1 more moments, please do.

10:05:56 2 A In this -- in this -- in this figure, Figure 1  
10:05:58 3 of -- of the -- of the -- of the paper, that is the  
10:06:03 4 highest, yes.

10:06:09 5 Q Okay. And just so we both understand that  
10:06:12 6 graph -- and when I mean "that graph," we're both  
10:06:14 7 looking at Figure 1, the bottommost right-hand graph.  
10:06:22 8 And the concentration, I believe, in that experiment is  
10:06:25 9 950 micromolars of dGTP?

10:06:30 10 A Yes.

10:06:31 11 Q Did I get that part right?

10:06:33 12 A You did.

10:06:33 13 Q Okay. And then on the X axis, the  
10:06:36 14 concentration of dATP, as you move from left to right,  
10:06:42 15 actually decreases. Right?

10:06:44 16 A Yeah. Because it's one over dAPT  
10:06:50 17 concentration, yeah.

10:06:53 18 Q So I believe the ratio is approximately  
10:06:55 19 95-to-1, as measuring dGTP as compared to dATP?

10:07:07 20 A I --

10:07:07 21 Q Do you agree with me?

10:07:09 22 A I -- I think so. I'd have to do that math, and  
10:07:20 23 on my feet right now -- that certainly seems correct.

10:07:26 24 Q Okay. And 80 per 100,000 works out to an error  
10:07:30 25 rate of 0.08 percent.

10:07:32 1 Would you agree with me?

10:07:34 2 A That sounds correct.

10:07:39 3 Q And, again, as we talked about earlier, the --

10:07:40 4 the -- the inverse of that is the correct incorporation

10:07:43 5 rate would then be 99.92 percent. Correct?

10:07:50 6 A Yeah. Yeah.

10:07:58 7 Q I think you could put that paper aside.

10:08:04 8 I'm going to go back to that same paragraph

10:08:05 9 we've been looking at in your declaration. So after --

10:08:13 10 after the citations to Fersht, there's a sentence in

10:08:16 11 that paragraph, which reads as follows: "Furthermore,

10:08:21 12 polymerases are prone to insert or delete nucleotide

10:08:25 13 residues during synthesis at high nucleotide

10:08:31 14 concentrations."

10:08:33 15 Do you see that?

10:08:33 16 A I do.

10:08:34 17 Q Did I read that correctly?

10:08:35 18 A Yeah, I think so.

10:08:40 19 Q And in support of that statement, you cite to

10:08:45 20 two papers by Bebenek. Is that correct?

10:08:57 21 A Yes.

10:08:57 22 Q All right. I'm going to show you what's been

10:09:02 23 marked as Exhibit 2055.

10:09:05 24 A But --

10:09:12 25 Q Is this one of the Bebenek papers that you are

10:09:17 1 citing to in your declaration?

10:09:19 2 A Yes, it is.

10:09:26 3 Q Now, if you'll turn to page 4947.

10:09:39 4 Now, one of the things Bebenek is measuring  
10:09:44 5 here is, again, misincorporations. Is that correct?

10:09:53 6 A I believe they're a slightly different sort of  
10:09:57 7 misincorporation. They're leading to -- they're both --  
10:10:00 8 the Fersht papers and these Kunkel -- or the Bebenek  
10:10:06 9 papers are both dealing with mutations. The Fersht  
10:10:10 10 paper is dealing with what is called substitution  
10:10:12 11 mutations. It's where one nucleotide is replaced by  
10:10:17 12 another. The Bebenek papers are dealing with what are  
10:10:20 13 called frame shift mutations. That's where you gain or  
10:10:24 14 lose a nucleotide. So they're rather different.

10:10:31 15 Q Okay.

10:10:31 16 A But they're both a problem for sequencing.

10:10:38 17 Q And Bebenek, like Fersht, is using -- varying  
10:10:46 18 the ratio of the nuc- -- the concentrations of the  
10:10:49 19 nucleotides to so-called force this misincorporation or  
10:10:55 20 frame shift error, whatever you just mentioned.

10:10:57 21 Is that -- is that accurate?

10:11:00 22 A Well, I don't -- I don't know what you mean by  
10:11:01 23 "force." What they -- what they were looking for was  
10:11:04 24 the effect of concentration on frame shift mutation. So  
10:11:07 25 they didn't go in with the intent to force it. They

10:11:10 1 were asking, as you increase the concentration, do  
10:11:13 2 the -- do the frame shift mutations happen.

10:11:19 3 Q Well, can you look on page 4947, under that  
10:11:27 4 first heading on that second column there, it says  
10:11:29 5 "Effect of dNTP substrate imbalances on error  
10:11:30 6 specificity."

10:11:39 7 THE REPORTER: Can the reporter hear that  
10:11:39 8 again, please.

10:11:39 9 BY MR. SEGAL:

10:11:32 10 Q The heading is "Effect of dNTP substrate  
10:11:43 11 imbalances on error specificity." Bebenek states, "To  
10:11:49 12 test the model, we reason that forcing misincorporations  
10:11:53 13 by DNA polymerase lacking a proofreading exonuclease  
10:11:58 14 activity, using an excess of one dNTP" and so forth.

10:12:06 15 Does that -- with that -- reading that, does my  
10:12:08 16 question make more sense that they were using, again, an  
10:12:11 17 imbalance of concentrations of so-called -- what he  
10:12:14 18 says -- force and misincorporation?

10:12:18 19 A Yeah, I don't mean to -- I guess I was just  
10:12:20 20 thinking of it more from a sort of a cause and effect.  
10:12:23 21 If you want to use the word "force," that's fine. I  
10:12:26 22 don't really object to that. It's -- I don't think  
10:12:28 23 it's -- it's just a -- a way of describing it. I --  
10:12:33 24 just scientifically, I think it's more correct to say  
10:12:38 25 that the authors didn't force anything. They simply

10:12:44 1 varied conditions and saw what the system did.

10:12:46 2 So it's just sort of a semantic thing. I

10:12:48 3 don't -- I don't mean to hang us up on that.

10:12:52 4 I -- I wouldn't have used the word "force"

10:12:54 5 for -- in Kunkel's paper either. I would have objected

10:12:59 6 to that.

10:13:00 7 Q Okay. When you say "Kunkel," you mean Fersht?

10:13:04 8 A No. Kunkel is the corresponding PI on Bebenek.

10:13:09 9 Yeah.

10:13:11 10 Q Okay. And in these experiments, Bebenek is

10:13:21 11 using in excess of either the G or the A nucleotide at

10:13:25 12 one millimolar concentration, while keeping the

10:13:29 13 others -- other nucleotides at 50 micromolar

10:13:31 14 concentration. Is that right?

10:13:34 15 A Yes, it is.

10:13:45 16 Q And if you'll look under -- under, I think, the

10:13:48 17 Table 1 on that same page. I believe the highest error

10:13:55 18 rate that Bebenek reports is when there is a 20-fold

10:14:00 19 excess of dATP over the other nucleotides and that error

10:14:06 20 rate is 420 times 10 to the negative 6.

10:14:13 21 Would you agree with me?

10:14:16 22 A Those are the numbers. I don't know, again, if

10:14:18 23 those are the highest that he reports. He doesn't -- he

10:14:20 24 doesn't say that. So I'd have to look through carefully

10:14:23 25 to see that. But at those particular concentrations,

10:14:30 1 those are the mutation rates that he measures.

10:14:32 2 Glancing at the table, I don't see any that are  
10:14:35 3 higher. So I might -- I might agree with you. But I'd  
10:14:40 4 have to look more carefully to know that for sure.

10:14:47 5 Q Okay. At that -- that error rate, 420 out of a  
10:14:51 6 million, it works out to an error rate of .042 percent.

10:14:55 7 Would you agree with me?

10:15:01 8 A I -- I think that you probably can do that  
10:15:03 9 math. So, yes, I would agree with you. It sounds about  
10:15:06 10 right. I don't -- I don't have a calculator. But that  
10:15:08 11 sounds about right.

10:15:13 12 Q So, again, conversely, the -- the correct  
10:15:15 13 incorporation rate, then, would be 99.958 percent.  
10:15:20 14 Correct?

10:15:21 15 A I think so.

10:15:27 16 Q And Bebenek actually measures also the error  
10:15:32 17 rate when they are at equal molar concentrations.

10:15:35 18 If you'll look with me again directly under  
10:15:40 19 Table 1 on that same page, with respect to equal molar  
10:15:45 20 concentrations of 1 millimolar each, the error rate is  
10:15:50 21 360 times 10 to the negative 6. Correct?

10:15:59 22 A I'm sorry. Could you direct me to where  
10:16:01 23 that -- where that sentence is.

10:16:03 24 Q Right underneath the table, Table 1, it says  
10:16:06 25 "Reverant frequencies for the initial template were 360

10:16:11 1 times 10 to the negative 6 with equal dNTP pools."

10:16:16 2 A Yes.

10:16:18 3 Q Do you see that?

10:16:18 4 A Yes.

10:16:18 5 Q Okay. And if you actually look to the left

10:16:23 6 under the heading "DNA Polymerase Reactions," it

10:16:31 7 explains that for the equal molar, they use

10:16:33 8 concentrations of 1 millimolar for all four. Correct?

10:16:45 9 A Yes.

10:16:46 10 Q Okay. So, thus, there is an error rate of 360

10:16:51 11 times 10 to the 6 when all four of the nucleotides are

10:16:56 12 at a concentration of 1 millimolar. Correct?

10:16:59 13 A Yes.

10:17:03 14 Q And, again, just walk -- I'm going to walk you

10:17:06 15 through the math -- this works to a -- a 0.036 error

10:17:11 16 rate. Correct?

10:17:12 17 A I -- I think so. I -- I -- again, that sounds

10:17:17 18 about right.

10:17:18 19 Q It's just moving the decimal place, right, six

10:17:21 20 over. And then to make it a percentage, you move it two

10:17:24 21 back.

10:17:25 22 A Okay.

10:17:25 23 Q Correct?

10:17:30 24 A That --

10:17:31 25 Q So, again, conversely, the correct -- the

10:17:34 1 correct incorporation rate is 99.964 percent. Correct?

10:17:39 2 A I think so, yes.

10:17:47 3 Q Okay. You can put that paper aside.

10:18:16 4 I'm going to show you what's been previously  
10:18:18 5 marked as Exhibit 2056.

10:18:41 6 Is this the other Bebenek paper that you cite  
10:18:45 7 in your declaration?

10:18:45 8 A Yes, it is.

10:18:57 9 Q And, again, you cite to page 3591. Correct?

10:19:02 10 A Yes.

10:19:05 11 Q Okay. If you could turn to that page. Turn  
10:19:19 12 your attention to Table 1. And the heading of Table 1  
10:19:27 13 reads "Effective dNTP biases on one based deletion  
10:19:36 14 errors by HIV-1 reverse transcriptase."

10:19:42 15 Did I read that right?

10:19:44 16 A Yes.

10:19:48 17 Q And HIV-1 reverse transcriptase is a polymerase  
10:19:54 18 that is known to be error prone. Is that right?

10:20:02 19 A Relative to the natural polymerases, the  
10:20:04 20 polymerases that replicate genome in cells? Is that  
10:20:07 21 the -- I mean, error prone relative to what, is what I  
10:20:10 22 would ask?

10:20:13 23 Q Well, let's -- let's use your comparison.

10:20:15 24 A Yes, that is -- that is correct, then. I think  
10:20:19 25 the numbers are something like -- it's something like

10:20:21 1 100-fold more error prone than would be the polymerases  
10:20:25 2 that -- that your and my cells use to replicate our  
10:20:29 3 genomes.

10:20:32 4 Q How about as compared to polymerases that you  
10:20:35 5 would use in a typical sequencing by synthesis method?

10:20:39 6 A It would depend on the polymerase. Different  
10:20:43 7 polymerases can be used.

10:20:51 8 Q Are you aware of any sequencing by synthesis  
10:20:52 9 methods that use HIV-1 reverse transcriptase?

10:20:59 10 A By "methods," do you mean methods that are  
10:21:01 11 commercialized, or have I read papers where people are  
10:21:04 12 developing such methods? So --

10:21:08 13 Q Commercialized.

10:21:10 14 A Commercialized, I'm unaware of anyone that uses  
10:21:12 15 HIV-1 RT. That -- that's, again --

10:21:18 16 Q Turning your attention to --

10:21:20 17 MR. BABCOCK: Look, let him answer.

10:21:21 18 THE WITNESS: I'm sorry. That's a bit of a  
10:21:22 19 difficult question, because there -- I mean, I know what  
10:21:25 20 you're getting at, because the error rate might be a  
10:21:28 21 problem. But there are actually some reasons that you'd  
10:21:30 22 want to use a reverse transcriptase as opposed to a  
10:21:34 23 polymerase for a sequencing method.

10:21:37 24 So a lot of times, people would say, well, I'm  
10:21:40 25 going to be more tolerant of a mutation rate of a chance

10:21:43 1 of a -- of -- of -- of the chance of having more  
10:21:46 2 mutations because it gives me the opportunities to  
10:21:48 3 reverse transcribe RNA. And maybe they're interested in  
10:21:53 4 that. So they -- so -- so lots of people publish papers  
10:21:56 5 using HIV-1 RT potentially as a polymerase for  
10:22:02 6 sequencing.

10:22:10 7 BY MR. SEGAL:

10:22:10 8 Q Turn your attention to Table 1.

10:22:12 9 Would you agree with me that the error rates  
10:22:14 10 are similar when using high equal molar concentrations  
10:22:18 11 of all nucleotides as compared to low equal molar  
10:22:21 12 concentrations of all nucleotides?

10:22:32 13 A They're a little bit higher with high, but  
10:22:35 14 they're very close -- they're reasonably close, yes.

10:22:41 15 Q And the difference between the two are -- are  
10:22:44 16 only 30 additional errors per 100,000? Would you agree  
10:22:49 17 with me there?

10:22:52 18 A Yeah. Percentage-wise, they look like about,  
10:22:55 19 what, 20 percent or something, in terms of the -- of --  
10:23:00 20 of revertant freq- -- oh, no. Those are 10 to the minus  
10:23:03 21 5.

10:23:04 22 Yeah, I think their numbers were right.

10:23:07 23 Q And that 30 per 100,000 works out to about  
10:23:11 24 .03-percent difference in the error rates?

10:23:15 25 A Okay. That sounds about right. I -- I

10:23:19 1 apologize. I don't mean to not agree with you. It's  
10:23:22 2 just that I don't have a calculator to go through all --  
10:23:27 3 all the numbers.

10:23:28 4 Q Is there a calculator there that you can use?

10:23:32 5 MR. BABCOCK: There is.

10:23:36 6 THE WITNESS: Do you really want me to  
10:23:37 7 actually --

10:23:38 8 MR. SEGAL: If you -- so if you -- if you feel  
10:23:41 9 the need. I just don't want --

10:23:42 10 THE WITNESS: No, no.

10:23:42 11 MR. SEGAL: -- you to later on say that --

10:23:44 12 THE WITNESS: No, no. I don't mean to imply  
10:23:45 13 that. Your numbers all sound close. I just want to  
10:23:48 14 explain why I'm -- why I keep saying this sort of  
10:23:51 15 ridiculous "sounds about right." So if you want me to  
10:23:55 16 check something, I -- I will. But -- but, like I said,  
10:23:57 17 your numbers sound right. But the only reason I'm  
10:24:00 18 saying that as opposed to a simple "yes."

10:24:02 19 So if you'd like a "yes," I'll take a few  
10:24:04 20 seconds every time to punch the numbers in myself.

10:24:07 21 BY MR. SEGAL:

10:24:08 22 Q Well, I'll tell what you we'll do. I mean,  
10:24:10 23 after I'm finished, my questions, your counsel will have  
10:24:15 24 an -- have an opportunity to redirect you. And I'm sure  
10:24:18 25 there will be a break in between.

10:24:20 1 So if you feel the need to actually calculate  
10:24:22 2 any of those numbers and you think any of them are  
10:24:26 3 incorrect, then please do so. And then you could -- you  
10:24:29 4 could address the -- whatever miscalculations there  
10:24:32 5 are -- I don't think there are any -- during the  
10:24:35 6 redirect.

10:24:36 7 A Sure.

10:24:36 8 Q Does that sound okay with you?

10:24:39 9 A Yes. Yeah. Absolutely. And I also don't  
10:24:41 10 think there are any -- any errors either. I don't mean  
10:24:43 11 to impinge your mathematics.

10:24:48 12 Q And, again, turning back to -- to Table 1, the  
10:24:54 13 higher error rates are shown when using an excess of one  
10:24:59 14 of the nucleotides. Is that correct?

10:25:01 15 A Yes, it is.

10:25:06 16 Q And when all the nucleotides are at a high  
10:25:09 17 concentration, 1 millimolar, the error rate is 150 per  
10:25:19 18 100,000. Is that correct?

10:25:22 19 A Yes.

10:25:25 20 Q And, again, this -- this works out to an error  
10:25:28 21 rate of .15 percent.

10:25:31 22 Does that sound about right?

10:25:32 23 A It does.

10:25:34 24 Q And then the -- so the correct incorporation  
10:25:37 25 rate would be 99.85 percent. Correct?

10:25:42 1 A Yes.

10:25:46 2 Q Okay. If you'll turn to page 3593. And, in  
10:25:59 3 particular, Table 5. It's going to read in the -- the  
10:26:08 4 title of that table. It reads "Pool bias effects on  
10:26:13 5 minus 1 nucleotide errors with yeast DNA polymerase  
10:26:19 6 alpha."

10:26:21 7 Did I read that correct?

10:26:22 8 A Yep. Yes, you did.

10:26:24 9 Q And the error rate shown in that table when all  
10:26:28 10 are at a high concentration of 1 millimolar, the error  
10:26:33 11 rate is 36 out of 100,000. Is that right?

10:26:40 12 A Yes.

10:26:44 13 Q And, again, that -- that works out to an error  
10:26:46 14 rate of 0.036 percent. Correct?

10:26:54 15 A Yes.

10:26:56 16 Q And then, again, it's a correct incorporation  
10:27:01 17 of 99.964 percent of the time. And, again, you can  
10:27:07 18 check again at -- at a break if you'd like.

10:27:10 19 But does that sound reasonable?

10:27:12 20 A Yeah. I -- I want to be a little bit clear.  
10:27:14 21 Because of what the assay is measuring, it's actually --  
10:27:19 22 the math that you're doing -- the intuitive thing that  
10:27:22 23 you're doing is actually not strictly correct. What it  
10:27:25 24 says is that that's the percent of frame shift mutations  
10:27:30 25 that you get.

10:27:31 1 What -- what you're calling correct isn't  
10:27:34 2 correct. They didn't sequence this. What they know is  
10:27:36 3 that they're simply not a frame shift.

10:27:40 4 Is that -- is that clear?

10:27:42 5 Q Okay.

10:27:42 6 A So all the assays --

10:27:44 7 Q Thank you.

10:27:45 8 A So all that the assay is measuring is the  
10:27:47 9 appearance of a frame shift. They're not actually  
10:27:50 10 characterizing correct versus incorrect sequence. There  
10:27:52 11 are lots of mistakes that a polymerase can make. Frame  
10:27:57 12 shifts are one of them.

10:27:58 13 And the reason I cited these papers was I  
10:28:00 14 wanted to cite literature on the sorts of effects that  
10:28:05 15 led the frame shifts, as opposed to Fersht's papers,  
10:28:10 16 which were the source of effects that led to  
10:28:12 17 substitution mutations.

10:28:15 18 To be clear, neither of the papers actually  
10:28:18 19 sequence anything. Not -- neither of them actually  
10:28:21 20 know, in general -- Fersht, the sequence, just if you --  
10:28:24 21 as a representative examples to show that they were  
10:28:26 22 substitutions. But the assays that they're using to get  
10:28:29 23 the number that you're talking about, they're not based  
10:28:31 24 on going in and sequencing things. They're based on a  
10:28:35 25 cell that's able to grow because it made a substitution

10:28:38 1 or because it made a frame shift. And that way, you can  
10:28:42 2 simply plate out lots and lots of cells and count  
10:28:45 3 colonies, way more than you'd go in and be able to  
10:28:48 4 sequence one by one, especially in an era when  
10:28:54 5 sequencing was expensive and slow.

10:28:56 6 So just to be clear, every time that you say  
10:28:59 7 0.0-percent frame shift, meaning that 100 minus that  
10:29:04 8 number was correct, that's not what these papers are  
10:29:07 9 measuring. The 100 minus that number is simply the  
10:29:12 10 percentage at that site in the chromosome that did not  
10:29:17 11 frame shift.

10:29:21 12 It might have done something else, but it  
10:29:22 13 didn't frame shift. So Fersht papers are mutation,  
10:29:27 14 substitution mutation or not. And the Kunkel or the  
10:29:32 15 Bebenek papers are frame shift or not. So just to be  
10:29:34 16 clear, it's not correct versus incorrect.

10:29:37 17 Is that -- is that clear?

10:29:40 18 Q Right. So the inverse is there's no error  
10:29:43 19 rate. So if there's an error rate of .05 percent, then  
10:29:47 20 there's -- you're not -- you would not have that error  
10:29:50 21 99.9 percent of the time. Correct?

10:29:54 22 A To be clear, when people -- when most people  
10:29:56 23 use the term --

10:30:02 24 Q The error that they're measuring?

10:30:04 25 A Correct. Yeah.

10:30:05 1 THE REPORTER: I didn't -- can the reporter  
10:30:05 2 hear. What was the word before that they're measuring?  
10:30:06 3 MR. BABCOCK: The error.  
10:30:06 4 THE REPORTER: Thank you.  
10:30:07 5 THE WITNESS: Right. So to say it one last  
10:30:08 6 time -- I think -- I think we're in agreement now. When  
10:30:11 7 most people talk about an error rate, they're talking  
10:30:14 8 about a composite of all the types of errors. They're  
10:30:18 9 talking about anything but the correct sequence. The  
10:30:21 10 Fersht and the Kunkel papers are talking about a  
10:30:25 11 specific type of error versus the absence of that  
10:30:28 12 specific type of error.  
10:30:30 13 BY MR. SEGAL:  
10:30:32 14 Q Okay. You could put that paper aside.  
10:30:48 15 If you turn back -- again, we're still on that  
10:30:51 16 same page of your declaration. I want to ask you about  
10:30:55 17 that sentence after the citations to the Bebenek papers.  
10:31:00 18 And I'll just read it again in the record.  
10:31:01 19 "Thus, it is not surprising that Canard taught that  
10:31:07 20 these modified nucleotides are not very efficient chain  
10:31:10 21 terminators. A result awaiting precise determination of  
10:31:15 22 their Km, Vmax, and kcat. (Work in progress.)"  
10:31:36 23 Do you see that?  
10:31:37 24 A I do.  
10:31:37 25 Q I'm going to show you what's been marked as

10:31:39 1 Exhibit 1028.

10:31:40 2 MR. BABCOCK: Thank you.

10:31:40 3 BY MR. SEGAL:

10:31:53 4 Q Is this the Canard paper that you're citing?

10:31:57 5 A It is.

10:32:01 6 Q I believe the sentence you're citing is on

10:32:04 7 page 3, about 10 lines down in the second column. I

10:32:17 8 wanted you to read -- I wanted to ask you questions

10:32:19 9 about this, just so that I can understand a little bit

10:32:23 10 better.

10:32:24 11 I think it would help if you read that, your

10:32:25 12 sentence that you quote, in context.

10:32:30 13 So I believe, to put it in context, you would

10:32:33 14 start maybe three lines from the bottom on column 1 and

10:32:38 15 read through your sentence. But feel free to read

10:32:48 16 whatever you want to read on that page. Okay?

10:32:51 17 A I'm sorry. Are you starting with the sentence

10:32:52 18 that starts "The plateau value"?

10:32:57 19 Q I would suggest starting to read "Although, 3'

10:33:00 20 RTA" --

10:33:02 21 A Oh.

10:33:03 22 Q -- which, again, is three lines up from the

10:33:05 23 bottom.

10:33:06 24 A Okay.

10:33:06 25 Q And then read about 20 lines in the next column

10:33:09 1 until you get to the sentence that you cite.

10:33:11 2 A Okay.

10:33:12 3 Q Understood?

10:33:12 4 A Yes. Okay.

10:34:28 5 Q So that -- again, that sentence that you  
10:34:31 6 quoted, when Canard is talking about chain terminators  
10:34:34 7 in that sentence, again, that you quote in your  
10:34:37 8 declaration, he's referring to, like, a Sanger  
10:34:42 9 sequencing method. Is that right?

10:34:47 10 A Yes. He's -- he's -- he's using -- I'd have to  
10:34:54 11 reread -- rethink now on -- I believe his comparison  
10:35:00 12 with dideoxies is not only based on Sanger sequencing,  
10:35:07 13 but that's clearly part of it. And that's the  
10:35:09 14 comparison that he's drawing here.

10:35:15 15 Q Okay. So when he refers to not very efficient  
10:35:18 16 terminators, he means that the modified nucleotide is  
10:35:21 17 unlikely to sufficiently outcompete dideoxy nucleotides.  
10:35:28 18 Is that right?

10:35:29 19 A Yeah. And I think that he's simply, as an  
10:35:32 20 example, comparing his -- the terminators -- the 3'  
10:35:40 21 block groups that he's talking about in his -- in this  
10:35:43 22 paper, versus dideoxies, which are classical chain  
10:35:43 23 terminators in Sanger sequencing.

10:35:49 24 THE REPORTER: If you can back up, a little  
10:35:49 25 slower for me on that one. "Versus" --

10:35:49 1 THE WITNESS: Dideoxy chain terminators, which  
10:35:57 2 are classical terminators for Sanger sequencing.

10:36:03 3 And saying that his are worse, I think is what  
10:36:06 4 he's saying there. He's saying because that's what he  
10:36:08 5 means by --

10:36:09 6 BY MR. SEGAL:

10:36:10 7 Q Okay. What --

10:36:11 8 A That's what he means by not being able to  
10:36:12 9 outcompete.

10:36:16 10 Q So when Canard says "not very efficient  
10:36:18 11 terminators," he's not talking about the accuracy of  
10:36:22 12 incorporation or the fidelity, like we talked about  
10:36:25 13 earlier?

10:36:27 14 A No.

10:36:37 15 Q He's talking about the competition versus  
10:36:39 16 dideoxy again, not -- you'd agree with me that -- it  
10:36:43 17 might be a little unclear for the record. That's why  
10:36:45 18 I'm asking it again.

10:36:46 19 You'd agree with me that he's not talking about  
10:36:48 20 fidelity when he says "not efficient terminators."  
10:36:51 21 Agree?

10:36:52 22 A I think in this particular spot, yes, I agree,  
10:36:56 23 that he's -- he is simply -- I believe, simply comparing  
10:37:00 24 to dideoxy. So that would be just an efficiency of  
10:37:04 25 termination. And, correct, I think there's no --

10:37:07 1 there's no -- there's no referencing to fidelity here.

10:37:16 2 Q Turn back to your declaration. The next page,

10:37:26 3 paragraph 18. You state in paragraph 18 -- I'm just

10:37:41 4 going to read this into the record -- "Furthermore, it

10:37:43 5 was known in 2002 that polymerases would not incorporate

10:37:50 6 a nucleotide bearing a 2-aminobenzoyl 3'-OH protecting

10:37:59 7 group." You cite to Metzker, Table 2.

10:38:03 8 "The 2-aminobenzoyl protecting group is similar

10:38:09 9 in size and structure to a 2,4-dinitrobenzenesulfenyl

10:38:10 10 protecting group. Therefore, a person of skill in the

10:38:22 11 art would have expected that a nucleotide with a

10:38:23 12 2,4-dinitrobenzenesulfenyl 3'-OH protecting group would

10:38:32 13 not have been incorporated by a polymerase in 2002."

10:38:40 14 Did I read that reasonably correctly?

10:38:42 15 A Absolutely. You did a -- you did a nice job.

10:38:45 16 Q Thank you.

10:38:51 17 So just so I understand your opinion here, so

10:39:00 18 it's your opinion that one of skill in the art as of

10:39:03 19 2002 would not have expected that a nucleotide with a 3'

10:39:06 20 protecting group similar in size and structure to a

10:39:09 21 2-aminobenzoyl protecting group would be incorporated by

10:39:15 22 a polymerase?

10:39:16 23 A Yes, that is my opinion.

10:39:27 24 Q So if you turn to paragraph 22 of your

10:39:33 25 declaration. Again, that's Exhibit 2037. And this is

10:39:44 1 in the -- the section where you're discussing the  
10:39:48 2 Letsinger reference. Correct?

10:39:57 3 A It is. Paragraph 22, yes.

10:39:58 4 Q So I'm going to show you what's been previously  
10:40:00 5 marked as Exhibit 1036. Excuse me. I think that's  
10:40:10 6 Exhibit 1044.

10:40:27 7 MR. BABCOCK: Double-check that, Marc.

10:40:29 8 MR. SEGAL: You know what, give me a second.  
10:40:31 9 I'm just going to go on mute to see if we have the right  
10:40:33 10 exhibit number.

10:40:36 11 MR. BABCOCK: I think -- I think he wanted  
10:40:39 12 Letsinger.

10:40:41 13 THE WITNESS: That's my guess.

10:40:43 14 THE REPORTER: And we're still on the record?

10:40:44 15 MR. BABCOCK: Oh, yeah. We kind of are.

10:40:48 16 MR. SEGAL: Yeah. Correction. That is  
10:40:48 17 Exhibit 1036.

10:40:53 18 THE WITNESS: Thank you. Thanks.

10:41:06 19 BY MR. SEGAL:

10:41:06 20 Q Just so we're on the same page, that's the  
10:41:12 21 Letsinger reference from 1974?

10:41:15 22 A Yes.

10:41:15 23 Q Okay. And this is the -- the reference that  
10:41:18 24 you're -- you discuss in your declaration?

10:41:21 25 A It is.

10:41:30 1 Q You state in your declaration, "A person of  
10:41:36 2 ordinary skill in the art would not expect the three  
10:41:39 3 different types of esters to cleave with the same yield.  
10:41:45 4 It is likely that the ester attached to the 3'-OH group  
10:41:51 5 cleaves with a lower yield than the other two esters."

10:41:57 6 A Yes.

10:41:58 7 Q Is that your opinion?

10:41:59 8 A Yes.

10:41:59 9 Q Now, you say "It's likely that." So you don't  
10:42:01 10 know for sure?

10:42:03 11 A I don't know anything for sure when it comes to  
10:42:05 12 this kind of thing. One can never -- there are always  
10:42:08 13 surprises. I -- I would -- I would bet a paycheck on  
10:42:12 14 the -- and I don't make as much money as you do. So I'm  
10:42:18 15 pretty -- I'm pretty confident that -- that the 3' --  
10:42:21 16 there are certain tenants of -- of chemistry. And --  
10:42:24 17 and you're taught this as a sophomore in organic  
10:42:28 18 chemistry.

10:42:29 19 If you -- if you gave those three esters to a  
10:42:31 20 sophomore chemist on a test, that would be a very  
10:42:35 21 appropriate question. I almost guarantee you, that's  
10:42:39 22 typical of the sort of question they would get. And  
10:42:41 23 they -- and the correct answer would be a clear  
10:42:43 24 ordering. The thymine would -- would -- would cleave  
10:42:47 25 first. Although, it's a little closer. It would

10:42:50 1 cleave -- I'd probably give a lot of -- partial credit  
10:42:53 2 to whether or not they didn't know whether the -- the  
10:42:54 3 ester --

10:42:59 4 THE REPORTER: Yeah. Let me hear that again.  
10:42:59 5 "Whether the ester" --

10:43:00 6 THE WITNESS: On the thymine nucleobase and the  
10:43:04 7 primary ester on the 5' group both cleave similarly --  
10:43:10 8 similarly. But they're going to -- they're going to --  
10:43:14 9 to -- to get any points on the test, they're going to  
10:43:16 10 have to know that the secondary cleaves slower, the  
10:43:19 11 secondary ester at the 3' position cleaves slower.

10:43:36 12 BY MR. SEGAL:

10:43:37 13 Q If you're cleaving -- if you have to remove all  
10:43:39 14 three of those groups and the yield is 80 percent, then  
10:43:43 15 to remove any one of those groups has to be higher than  
10:43:47 16 80 percent. Correct?

10:43:49 17 A Yeah. Yes.

10:43:56 18 Q And later in that same paragraph, as evidence  
10:44:08 19 that the 2,4-dinitrobenzenesulfonyl group attached to  
10:44:14 20 the thymine base is more labile than the group attached  
10:44:17 21 to the 3' hydroxyl, I believe you cite to Letsinger at  
10:44:24 22 2616 showing the conversion of the molecule identified  
10:44:32 23 as Roman numeral VII to the molecule identified as Roman  
10:44:36 24 Numeral IV in Chart 1.

10:44:39 25 Do you see that? Sort of the middle of the

10:44:41 1 page there on page 18?

10:44:54 2 A I do.

10:44:59 3 Q If you'll turn to that Chart 1 of Letsinger.

10:45:07 4 And I believe the agent that's used there to remove that

10:45:12 5 group is acetic acid. Is that right?

10:45:22 6 A Yes, it is.

10:45:23 7 Q And that's not -- that's not a reductive

10:45:25 8 reaction there. Correct?

10:45:26 9 A No, it is not.

10:45:33 10 MR. SEGAL: Can we take a short break, Brent?

10:45:35 11 MR. BABCOCK: Sure, Marc.

10:45:37 12 VIDEOGRAPHER: I need to switch the disks too.

10:45:39 13 MR. SEGAL: Okay.

10:45:39 14 VIDEOGRAPHER: So we're going off the record.

10:45:42 15 MR. SEGAL: Okay.

10:45:42 16 VIDEOGRAPHER: This is the end of Disk No. 1.

10:45:45 17 The time is 10:45.

10:57:14 18 (Recess from 10:45 a.m. to 10:57 a.m.)

10:57:22 19 VIDEOGRAPHER: We are back on the record. This

10:57:23 20 is the beginning of Disk No. 2. The time is 10:57.

10:57:31 21 MR. SEGAL: Well, I promised this would be

10:57:33 22 short. So, thank you, Dr. Romesberg. I have no further

10:57:36 23 questions, subject to -- subject to your -- your

10:57:42 24 counselor's redirect.

10:57:44 25 MR. BABCOCK: Oh. Well, that's --

10:57:46 1 MR. SEGAL: So I'm finished.

10:57:47 2 MR. BABCOCK: Thank you, Marc. We're actually

10:57:49 3 a little bit surprised. We thought you had more.

10:57:52 4 So can you give us maybe 20 minutes for us to

10:57:54 5 regroup offline and -- and we'll come back and let you

10:57:57 6 know --

10:57:58 7 MR. SEGAL: Sure.

10:57:58 8 MR. BABCOCK: -- if we have any questions?

10:58:00 9 VIDEOGRAPHER: Okay to go off?

10:58:02 10 MR. SEGAL: Okay. We'll come back into the

10:58:03 11 room in 20 minutes.

10:58:04 12 MR. BABCOCK: Sounds good. Thank you, guys.

10:58:06 13 We're back in 20 minutes.

10:58:07 14 MR. SEGAL: Okay. Bye-bye.

10:58:09 15 VIDEOGRAPHER: Off the record. The time

10:58:10 16 is 10:58.

12:07:01 17 (Recess from 10:58 a.m. to 12:07 p.m.)

12:07:38 18 VIDEOGRAPHER: We are going back on the record.

12:07:39 19 The time is 12:07.

12:07:43 20

12:07:43 21 REDIRECT EXAMINATION

12:07:44 22 BY MR. BABCOCK:

12:07:45 23 Q Afternoon, Dr. Romesberg.

12:07:47 24 A Afternoon.

12:07:47 25 Q As you know, I represent Illumina, and we have

12:07:50 1 a chance now to ask you a few follow-up questions.

12:07:53 2 Is that okay?

12:07:54 3 A It's okay.

12:07:55 4 Q And we're going to focus on the references

12:07:56 5 that -- that Marc presented to you.

12:07:59 6 Let's first take a look at Canard -- the Canard

12:08:05 7 reference. And that is Exhibit 1028. I believe

12:08:14 8 Mr. Segal pointed you to page 3 on the second column.

12:08:23 9 Do you recall where you -- where you were

12:08:26 10 discussing the sentence towards the top of the second

12:08:28 11 column?

12:08:31 12 A Yes.

12:08:35 13 Q And so the column mentions high concentrations.

12:08:39 14 What -- what is the -- the concentration here?

12:08:44 15 A To achieve --

12:08:46 16 MR. SEGAL: Objection. Outside the scope.

12:08:50 17 THE WITNESS: I should still answer the

12:08:52 18 question?

12:08:52 19 BY MR. BABCOCK:

12:08:52 20 Q Yes, you should.

12:08:54 21 A So to achieve the -- the incorporation

12:08:56 22 efficiencies required for sequencing by synthesis, the

12:09:00 23 concentrations were at least 1 millimolar.

12:09:03 24 Q How -- how do you know that?

12:09:05 25 A If you look at -- take Figure 3 on the

12:09:10 1 following page, he shows the data. I -- I'd point out  
12:09:15 2 that there is a -- appears to be a typographical error  
12:09:18 3 in this manuscript. In the subscript to Figure 3, he --  
12:09:24 4 I think he got the legend wrong. He mislabeled the  
12:09:28 5 legend. I mean, clearly, the lowest incorporations,  
12:09:33 6 which are represented as blocked -- blocked equals  
12:09:35 7 incorporation of the terminator -- the lowest must be  
12:09:37 8 the lowest concentration. The circles -- the closed  
12:09:40 9 circles must be the lower.

12:09:43 10 And -- and then the square -- the blocks must  
12:09:46 11 be the next. And then the triangles and then the  
12:09:49 12 circles. And so there's a -- there's a -- appears to be  
12:09:52 13 a typographical error.

12:09:54 14 But the -- the first -- the one that he shows  
12:09:56 15 sufficient concentrations, there's not an error.  
12:09:59 16 There's the triangles. And that's 1 millimolar.

12:10:03 17 Q Okay. So what's the concentration needed to  
12:10:08 18 reach high incorporation?

12:10:11 19 A Of these substrates would be 1 millimolar.

12:10:13 20 Q Does -- does that same sentence mention whether  
12:10:16 21 or not it is -- it's an efficient chain terminator?

12:10:23 22 A The sentence on -- on page 3?

12:10:26 23 MR. SEGAL: Outside the scope.

12:10:28 24 BY MR. BABCOCK:

12:10:28 25 Q Yeah. We -- yes.

12:10:31 1 A It says that they are not very efficient chain  
12:10:36 2 terminators.

12:10:37 3 Q And what does that mean, to be a "not very  
12:10:39 4 efficient chain terminator"?

12:10:40 5 A That it's not a good substrate.

12:10:42 6 Q And -- and what do you mean by "not a good  
12:10:45 7 substrate"?

12:10:45 8 A It is not recognized well and incorporated by  
12:10:49 9 the polymerase.

12:11:03 10 Q Mr. Segal also had you look at the Fersht  
12:11:10 11 papers, Exhibit 2053. The other one is 2054.

12:11:31 12 Do you recall that discussion?

12:11:33 13 A Yes.

12:11:36 14 Q If you look at particularly 2053, Mr. Segal had  
12:11:42 15 you look at page 4947 on the top of the second column.

12:11:48 16 Do you recall that discussion?

12:11:48 17 A I do.

12:11:51 18 Q And what was the -- what does the paper discuss  
12:11:55 19 about the -- the -- the -- the -- the fold increase with  
12:12:01 20 respect to the missubstitution? What's -- what --  
12:12:05 21 what's the relative fold increase?

12:12:08 22 A When -- when the -- the dGTP concentration is  
12:12:12 23 at 1 millimolar, the fold increases 2,000.

12:12:15 24 Q Okay. Two thousand fold increase.

12:12:17 25 Is that a relative, or absolute fold increase?

12:12:21 1 A It's a -- it -- it's -- when the GTP to ATP,  
12:12:28 2 the concentrations are approximately 1 millimolar and 10  
12:12:31 3 micromolar, the mutation rates increased 2,000-fold.

12:12:37 4 Q Okay. Are those natural nucleotides, or  
12:12:40 5 nonnatural nucleotides?

12:12:42 6 A Those are natural.

12:12:50 7 Q Well, then, with respect to nonnatural  
12:12:52 8 nucleotides, would a person of skill in the art have an  
12:12:56 9 expectation that their error rate would be the same, or  
12:12:59 10 different?

12:13:00 11 MR. SEGAL: Objection. Outside the scope.

12:13:03 12 THE WITNESS: The -- in -- in general, modified  
12:13:08 13 nucleotides have -- show higher mutation rates. In  
12:13:14 14 terms of the concentration change and how that affected  
12:13:17 15 the relative mutation rate, maybe that would be similar.  
12:13:20 16 It certainly wouldn't be better. It might be worse.

12:13:22 17 BY MR. BABCOCK:

12:13:48 18 Q And how does Fersht measure the error rate?

12:13:55 19 THE REPORTER: Can I hear the question again,  
12:13:55 20 please.

12:13:55 21 BY MR. BABCOCK:

12:13:55 22 Q How does Fersht measure the error rate?

12:13:57 23 A Fersht measures the error rate by taking some  
12:14:01 24 DNA and subjecting it to conditions that he's interested  
12:14:03 25 in exploring that involve, in this case, DNA

12:14:06 1 polymerase III, varying triphosphate concentrations, the  
12:14:12 2 other -- whatever other factors he wants to add. And he  
12:14:16 3 performs that reaction in vitro, meaning in a test tube.

12:14:20 4 And then he transfers that DNA, the replicated  
12:14:24 5 DNA, into a cell. And this is related to sort of very  
12:14:30 6 standard assays where the cell then mu- -- where  
12:14:33 7 mutations then cause the cell to do something that you  
12:14:35 8 can visual -- visually see. It might be phage  
12:14:39 9 production. It might be change color. It might be dye.  
12:14:45 10 But the behavior of the cell then reports on what the  
12:14:48 11 sequence was.

12:14:49 12 So, for example, you may -- you may give the  
12:14:52 13 cell an essen- -- the DNA that encodes, an essential  
12:14:55 14 protein, but it has a frame shift. And so only frame  
12:14:58 15 shift mutations that restore that protein would allow  
12:15:01 16 cell growth. And then by gauging the cells that grow  
12:15:04 17 compared to those that die -- that don't grow, you can  
12:15:07 18 gauge what the percentage of that particular frame shift  
12:15:10 19 mutation was.

12:15:11 20 Now, what Fersht is talking about is  
12:15:15 21 substitution mutation. So he might introduce a mutation  
12:15:17 22 that causes a lethal mutation in a cell. And so they  
12:15:21 23 all die, unless when they replicate the DNA, they happen  
12:15:28 24 to have made a mutation, and then they transform that  
12:15:30 25 mutated DNA in, and that would then bestow the cells

12:15:34 1 with the ability to survive.

12:15:36 2 And so, again, by some assay that you can read,  
12:15:40 3 cell color, death, phage production, or something, you  
12:15:43 4 can get very high statistical numbers on -- with good  
12:15:47 5 significance -- statistical significance on what the  
12:15:51 6 mutation rates were.

12:15:52 7 And so, in this case, Fersht is assaying for  
12:15:56 8 substitutions mutations by transforming the DNA into a  
12:16:00 9 cell -- by -- by moving it into a cell and looking for  
12:16:03 10 what the cell does.

12:16:13 11 Q How do the conditions that he measured affect  
12:16:16 12 the error rate that he found?

12:16:21 13 A Could you repeat that question.

12:16:22 14 Q Sure. How do the conditions that he used to --  
12:16:27 15 to measure the error rate -- to measure the substitution  
12:16:33 16 error affect the error rate that he found?

12:16:36 17 A The -- the conditions in vitro, or within the  
12:16:40 18 cell? The fact that he's going into a cell?

12:16:42 19 Q In the cell.

12:16:43 20 A Yeah, obviously, that has a huge impact.  
12:16:45 21 Cells, these are full of DNA repair pathways. So  
12:16:48 22 there's at least four or five independent repair  
12:16:51 23 pathways. These include things like methyl directed  
12:16:55 24 mismatch repair, base excision repair, nucleotide  
12:17:00 25 excision repair, various glycosylase enzymes that

12:17:04 1 recognize damaged DNA, cut the damage out. So while the  
12:17:07 2 cell is reading what the mutations were, it is also  
12:17:09 3 going to be repairing those mutations.

12:17:14 4 Q So with the Fersht papers, how are they  
12:17:17 5 measured in mutations? Is it by -- is it cell death?

12:17:20 6 A It -- it -- in -- in -- in -- in the Fersht  
12:17:23 7 paper, I think it's cell death.

12:17:25 8 Q What about the two Bebenek papers that Mr. --

12:17:29 9 A I believe they're measuring phage mobilization.

12:17:35 10 THE WITNESS: Oh, I'm sorry.

12:17:38 11 THE REPORTER: And I didn't quite get the end  
12:17:38 12 of the question.

12:17:39 13 BY MR. SEGAL:

12:17:40 14 Q With regard to the Bebenek papers, how are they  
12:17:43 15 measuring mutations?

12:17:44 16 A I think in the Bebenek papers, they're  
12:17:46 17 measuring frame shift mutations, and I think they're  
12:17:48 18 measuring frame shift mutations by measuring a frame  
12:17:53 19 shift that makes active phage. And so then the phage  
12:17:56 20 then lyse the cells. So it's again sort of related -- I  
12:18:00 21 believe -- I believe that's the assay. And it's -- it's  
12:18:02 22 again related to a death, in this case, caused by phage.

12:18:05 23 Q Okay. So in both the -- the Bebenek  
12:18:09 24 experiments and the Fersht experiments, are there  
12:18:13 25 opportunities for cell repair?

12:18:15 1 A Ample, because these assays take hours to -- to  
12:18:20 2 read. The cells have to grow up into colonies that are  
12:18:22 3 big enough to see or not -- or -- or to note -- or to  
12:18:27 4 note their absence. So that would be a minimum of eight  
12:18:30 5 hours. So this is through many generations of bacteria  
12:18:33 6 doubling.

12:18:35 7 So the cells would have had, from their  
12:18:37 8 perspective, generations and generations to repair the  
12:18:41 9 DNA.

12:18:42 10 Q And is there a -- an opportunity for cell  
12:18:46 11 repair in sequencing by synthesis?

12:18:49 12 A No, there is not.

12:18:52 13 Q So how would that affect the misincorporation  
12:18:57 14 of errors measured by Fersht and by Bebenek?

12:19:01 15 A Well, they're -- well, the misincorporation  
12:19:03 16 would be only measured by Fersht.

12:19:07 17 Q Okay.

12:19:07 18 A And they're going to dramatically reduce them.  
12:19:10 19 And the absolute values will be much smaller. The  
12:19:15 20 fidelities will be much higher because you're repairing  
12:19:18 21 a lot of the errors.

12:19:19 22 Q What about for Bebenek and the frame shift --

12:19:23 23 A Same thing.

12:19:23 24 Q -- and the frame shift, how would the lack of  
12:19:25 25 opportunity for cell repair affect the efficiencies

12:19:29 1 reported?

12:19:30 2 MR. SEGAL: Objection. Outside the scope. All  
12:19:32 3 these questions.

12:19:33 4 THE WITNESS: All of the mutation rates  
12:19:35 5 reported in these will be much higher in the absence of  
12:19:39 6 repair. So it's not a good idea or useful, even, to use  
12:19:46 7 these numbers to think about errors in sequencing by  
12:19:48 8 synthesis, because they're -- they're going to be much,  
12:19:52 9 much lower when you have the opportunity for repair.

12:19:58 10 BY MR. SEGAL:

12:19:58 11 Q So are the absolute efficiencies reported by  
12:20:00 12 Bebenek or by Fersht, are they accurate estimates for  
12:20:05 13 the enzymes themselves?

12:20:08 14 A No.

12:20:08 15 Q Why not?

12:20:09 16 A Because you're repairing the mutations.

12:20:17 17 Q Does -- does Fersht report absolute, or  
12:20:22 18 relative rates -- substitution error rates?

12:20:27 19 A I believe both. And I believe the -- the -- by  
12:20:34 20 the way, the relative ones are probably more -- more  
12:20:38 21 useful.

12:20:39 22 Q Why is that?

12:20:41 23 A Because when you correct for repair, the  
12:20:45 24 relative increases are probably still accurate.

12:20:58 25 Q Do the absolute values of the misincorporation

12:21:02 1 reported in Fersht, are those reflective of SBS?

12:21:06 2 A No. They -- they -- in SBS, as I said, the  
12:21:09 3 mutation rates will be higher.

12:21:12 4 Q Then the -- the 2,000-fold relative increase in  
12:21:18 5 misincorporation that we talked about at the beginning,  
12:21:21 6 is that relevant to SBS?

12:21:22 7 A It is.

12:21:23 8 Q Why is that, then?

12:21:24 9 A Because they're independent of repair. And so  
12:21:26 10 the fact that sequencing by synthesis doesn't have  
12:21:28 11 repair makes that relative change more relevant. The  
12:21:33 12 relative change more relevant.

12:21:38 13 MR. SEGAL: Objection. Relevance. Objection.  
12:21:39 14 Outside the scope.

12:21:40 15 BY MR. BABCOCK:

12:21:41 16 Q Then back to -- to Canard, would you expect  
12:21:45 17 that his teaching of the 1 millimolar concentration will  
12:21:48 18 result in significant misincorporation?

12:21:52 19 A Yes.

12:21:53 20 Q Why -- why is that?

12:21:57 21 A The -- for a variety of reasons. When -- when  
12:22:02 22 DNA polymerases are reading correct base pairs, they  
12:22:07 23 rely on specific complementarity between the base pairs,  
12:22:12 24 being what's driving incorporation. At high  
12:22:15 25 concentrations, you circumvent this route.

12:22:18 1 For example, there's a large literature where  
12:22:20 2 you can remove the H bond donors and acceptors within  
12:22:24 3 the nucleobase, utterly obviating the possibility for  
12:22:29 4 hydrogen bonding complementarity, and you still -- at  
12:22:33 5 high concentrations, you still incorporate the  
12:22:36 6 nucleotides.

12:22:36 7 So in this case, what's happening is the  
12:22:40 8 nucleotides are simply inserting into the templates by  
12:22:44 9 intercolation between the nucleobases and not by a  
12:22:50 10 mechanism that requires reading the Watson-Crick pair,  
12:22:53 11 the way -- which is the only way that high fidelity is  
12:22:56 12 retained.

12:22:57 13 So at high concentrations, you circumvent that  
12:23:01 14 mechanism, which -- which is why cells evolved to not  
12:23:10 15 allow high concentrations of triphosphates. That's why  
12:23:14 16 cells --

12:23:14 17 THE REPORTER: Can I hear again. "Which is why  
12:23:14 18 cells evolved" --

12:23:14 19 THE WITNESS: To not allow high concentrations  
12:23:14 20 of triphosphates.

12:23:16 21 BY MR. BABCOCK:

12:23:16 22 Q And then focusing on the Bebenek papers, and in  
12:23:21 23 particular to Exhibits 2055, 2056, what is Bebenek  
12:23:31 24 teaching -- what is his teaching regarding the errors?  
12:23:35 25 What is he focusing on?

12:23:37 1 A He is focusing on and only on frame shift  
12:23:39 2 errors.

12:23:40 3 Q Are those -- is that the only type of errors  
12:23:42 4 for SBS that's relevant?

12:23:44 5 A No. Obviously sequencing by synthesis methods  
12:23:51 6 would be -- it would be problematic to have frame shift  
12:23:55 7 mutations or substitution mutations.

12:23:58 8 Q Does -- does Bebenek address substitution  
12:24:03 9 errors?

12:24:04 10 A No. Bebenek's assay itself is blind to  
12:24:06 11 substitution mutations.

12:24:11 12 Q So is this measurement from Bebenek reliable  
12:24:14 13 for indicating absolute error rate in SBS?

12:24:18 14 A It is not.

12:24:19 15 Q And could you explain why not?

12:24:21 16 A Because the mutation rates -- and, in fact, the  
12:24:23 17 ones that you'd really expect to be problematic with  
12:24:26 18 high concentrations with poor substrates would probably  
12:24:30 19 be substitution errors. And Bebenek is simply not  
12:24:32 20 sensitive to substitution errors.

12:24:35 21 I guess, let me -- let me say my point for  
12:24:38 22 having included it in the references was simply to show  
12:24:41 23 that when you get to high concentrations and you get  
12:24:44 24 things like imbalances, that you get frame shifts. And  
12:24:47 25 it was never intended to be a single assay that would

12:24:50 1 show that -- that would be itself relevant to sequencing  
12:24:52 2 by synthesis. I think what the Bebenek references show  
12:24:57 3 is that you can't go to imbalanced concentrations of  
12:25:02 4 triphosphates and not get frame shift mutations. It  
12:25:06 5 does not speak at all to substitution mutations.

12:25:11 6 MR. BABCOCK: All right. Those are the  
12:25:12 7 questions that we have for you, Dr. Romesberg. Thank  
12:25:16 8 you for your time.

12:25:18 9 We'll -- as you know, Mr. Segal has an  
12:25:20 10 opportunity to ask you a few more follow-up questions.  
12:25:23 11 So we'll take a break and then come back when they're  
12:25:26 12 ready. Thank you.

12:25:27 13 VIDEOGRAPHER: Okay to go off the record?

12:25:29 14 MR. BABCOCK: Yeah.

12:25:30 15 MR. SEGAL: Yeah.

12:25:31 16 VIDEOGRAPHER: Okay.

12:25:31 17 MR. SEGAL: Let's go off the record.

12:25:33 18 VIDEOGRAPHER: Off the record, the time is

12:25:35 19 12:25.

12:56:58 20 (Recess from 12:25 p.m. to 12:57 p.m.)

12:57:14 21 VIDEOGRAPHER: We are back on the record. The  
12:57:15 22 time is 12:57.

12:57:17 23

12:57:17 24

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DECLARATION UNDER PENALTY OF PERJURY

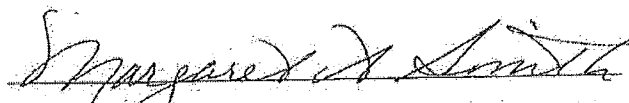
I, FLOYD ROMESBERG, PH.D., the witness herein,  
declare under penalty of perjury that I have read the  
foregoing in its entirety; and that the testimony  
contained herein is a true and accurate transcription of  
my testimony elicited at said time and place.

Executed this \_\_\_\_\_ day of \_\_\_\_\_, 2014.

\_\_\_\_\_  
FLOYD ROMESBERG, PH.D.

1 I, Margaret A. Smith, Certified Shorthand  
2 Reporter licensed in the State of California,  
3 License No. 9733, hereby certify that the foregoing is a  
4 true and accurate transcript of the deposition of said  
5 witness, who was first duly sworn by me on the date,  
6 time and place hereinbefore set forth. I certify that  
7 the list of appearances on the foregoing pages  
8 accurately indicates the individuals that were present  
9 during the deposition of said witness.

10 I further certify that I am neither attorney  
11 nor counsel, nor related to or employed by any of the  
12 parties to the action in which this deposition was  
13 taken, and further that I am not a relative or employee  
14 of any attorney or counsel in this action, nor am I  
15 financially interested in this case. Dated this 11th  
16 day of April, 2014.

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21 MARGARET A. SMITH, CSR NO. 9733, CRR  
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