UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,

Petitioner,

v.

FOUNDATION MEDICINE, INC.,

Patent Owner.

REPORTER CERTIFIED TRANSCRIPT

Nos. IPR2017-01170
IPR2017-01447
IPR2017-01448

VIDEOTAPE DEPOSITION OF

JOHN QUACKENBUSH, PH.D.

SEATTLE, WASHINGTON

WEDNESDAY, JANUARY 31, 2018

FOUNDATION EXHIBIT 2029
GUARDANT V. FOUNDATION
IPR 2017-01447

REPORTED BY:
RONALD L. COOK
CA CSR NO. 13928
WA CCR NO. 2523
CCR, CRR, RDR, CCRR, FAPR
FILE NO.: 18-24426

Deposition of JOHN QUACKENBUSH, PH.D., taken on behalf of Patent Owner at 701 Fifth Avenue, Suite 5100, Seattle, Washington, commencing at 9:15 a.m. on Wednesday, January 31, 2018, before Ronald L. Cook, California Certified Shorthand Reporter No. 13928, Washington Certified Court Reporter No. 2523, CRR, RDR, FAPR, California Certificate in Realtime Reporting.

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Personalis EX2009.003

I N D E X

EXAMINATION	PAGE
BY MS. WANG	6

EXHIBITS

PATENT OWNER'S

MARKED	DESCRIPTION	PAGE
Exhibit 1	Document entitled "Declaration of Professor John Quackenbush, Ph.D.," dated February 27, 2017.	33
Exhibit 2	Document entitled "Polymerase Chain Reaction (PCR) Fact Sheet - National Human Genome Research Institute (NHGRI)," dated January 23, 2018.	51
Exhibit 3	Document entitled "Bioinformatic tools and guideline for PCR primer design," by Kamel A. Abd-Elsalam.	56
Exhibit 4	Document entitled "Expert Declaration of John Quackenbush, Ph.D.," dated March 6, 2017.	143

WEDNESDAY, JANUARY 31, 2018; SEATTLE, WASHINGTON

9:15 A.M.

- - -

THE VIDEOGRAPHER: Good morning. We are going on the record. The time on the screen is 9:15 a.m., and today's date is January 31, 2018. We are located at 701 5th Avenue, Suite 5100, in Seattle, Washington.

This marks the beginning of Media Unit No. 1 of the video deposition of Dr. John Quackenbush, in the matter of Guardant Health vs. Foundation Medicine, venued in the US Patent and Trademark Office. Case number is IPR2017-01170.

My name is Michael Hehenkamp. I am a legal video specialist, here on behalf of Chase Litigation Services. The court reporter today is Ronald Cook, also on behalf of Chase Litigation Services.

Counsel, would you please voice identify yourselves and state whom you represent.

MS. WANG: Sophie Wang, from Choate Hall & Stewart, for patent owner Foundation Medicine.

MS. SCHONEWALD: Dr. Stephanie Schonewald, from Choate Hall & Stewart, on behalf of

Personalis EX2009.005

Foundation Medicine.

MS. WANG: And joining us via telephone is Dr. Stacey Gabriel, also for Foundation Medicine.

MR. ROSATO: Mike Rosato, for Guardant Health.

MS. GERRARD: Sonja Gerrard, for Guardant Health.

MR. SMITH: And Bill Smith, from Guardant Health.

THE VIDEOGRAPHER: And the court reporter may now swear in the witness.

JOHN QUACKENBUSH, PH.D.,
having first declared under penalty of perjury to tell the truth, was examined and testified as follows:

EXAMINATION

BY MS. WANG:

Q. Good morning, Dr. Quackenbush.

A. Good morning.

Q. You heard just now that this is IPR2017-1170, but you understand you're also here to testify regarding your opinions in IPR2017-01447 and -01448?

A. Yes.

Personalis EX2009.006

Q. Okay.
A. I trust you the numbers are right.
Q. I will put on the record that I believe those numbers are correct.
You've been deposed before; is that correct?
A. That's correct.
Q. And, in fact, you've been deposed in the related District Court litigation concerning the same patent that's at issue in these IPRs; is that correct?
A. Yes.
Q. And that's Patent No. 934,830; is that correct?
A. Yes.
Q. And your deposition in the related litigation was March of last year; is that correct?
A. I believe that's right. It was sometime last year.
Q. Between that deposition and today have you been deposed in any other matters?
A. I believe I did one additional deposition last year --
Q. And what --
A. -- between those two.
Q. What was that case in?

Personalis EX2009.007

A. I have to think about the timing, but I believe it was a case involving 10x Genomics against Raindance Technologies.

Q. And you were testifying as an expert in that case; is that correct?

A. Yes, I was.

Q. So you've been deposed before, but before we begin, I'll go over some basic ground rules just to refresh your memory.

As you know, there is a court reporter, who is taking down everything we're saying, so I would ask that we not talk over each other and that you answer my questions affirmatively, with a yes or no, or at least with some vocalization other than ums or nods or things like that. Okay?

A. I tend speak clearly, so I'll do my best.

Q. Excellent.

If you don't understand my question, feel free to ask me to clarify, but if you answer my question, I'm going to expect that you understood it when you gave your answer; is that correct?

A. Yes.

Q. We can take breaks if you would like. I am happy to accommodate any breaks that you feel that you need. The only thing that I would state is that

Personalis EX2009.008

if there's a question pending, I would ask that you answer the question before we take the break. Do you understand that?

A. Yes.

Q. And you understand your counsel here is here to object on behalf of Guardant, which he will do, but unless he instructs you not to answer a question, I will expect you to answer my questions. Do you understand?

A. Yes.

Q. So you are currently retained as an expert for Guardant in the related District Court litigation; is that correct?

A. Yes.

Q. That case is still pending, correct?

A. To my understanding, it is.

Q. And you've offered opinions in that case regarding this particular patent, correct?

A. Yes.

Q. There's no reason to think that your opinions with respect to this patent in that litigation should differ from your opinions regarding the same patent in these IPRs; is that correct?

A. I believe the matters are different, that this is really looking at the obviousness of the

Personalis EX2009.009

patent rather than infringement, so today I'm prepared to talk about obviousness.

Q. But when we're talking about this patent, we're talking about the same patent, the same claims, the same specification, correct?

A. Yes, we are.

Q. And so your interpretation of what the specification says, what the claims say, is the same in this case as it would be in the District Court litigation; is that correct?

A. I believe the standards are different in the two, but to the best of my ability, I'm going to provide as much information as I can about what the claims are, what they mean.

Q. The claim language is going to be the same with respect to this case and also the District Court litigation, right?

A. The claim language is the same, but my understanding is that in District Court litigation the judge provides guidance as to how to interpret those claims, and that that guidance doesn't necessarily exist in the context of IPR litigation.

Q. Do you expect that your opinions with respect to what those claims mean in the IPRs will be different than what's in the litigations?

Personalis EX2009.010

A. I don't necessarily expect that they will, but, again, we'd have to look at each individual claim term and try to understand what they mean.

I don't recall what the claim construction was in the context of the District Court case.

Q. But under your interpretation, you are not obligated to apply the claim constructions from the District Court litigation; is that right?

A. No. Not here in this -- this current matter.

Q. Is it your view that what you've said in the District Court litigation would not apply in this context?

A. Again, the -- I don't think whether it applies or not is the appropriate question. The question really is: If the District Court told us we were to interpret claim language a certain way, that claim language may not necessarily -- that interpretation may not necessarily apply to what we're discussing here today.

Q. I understand that, Dr. Quackenbush. I'm asking about what you have said previously regarding your opinions about the patent, regardless of what the District Court has said.

Personalis EX2009.011

Do your opinions regarding what this patent covers apply equally in this particular proceeding?

A. Again, I think that's a difficult question to answer. In the District Court case, we are instructed as how to interpret certain claim language. I don't recall what those interpretations are or were, and so I'm not sure that they necessarily are applicable to the discussion we're going to have today about obviousness.

Q. Do you recall when the District Court gave you those instructions in the litigation?

A. No, I don't.

Q. Do you recall making statements before the District Court actually gave those instructions to you?

A. I don't recall the timing, no.

Q. But assuming that the District Court gave you instructions after you had made statements, those statements you'd made previously would not apply the District Court's instruction, correct?

A. I think we'd have to look at the individual statements to understand what we're talking about.

Q. Okay.

Personalis EX2009.012

Can you take a look at your binder, please.

A. Sure.

Q. And we're going to look at Exhibit 1002, which I believe is the same exhibit number in each of the three IPRs, which is your declaration; is that correct?

A. So I don't have Exhibit 1002, and the declaration actually comes in three individual pieces.

Q. Okay.

I'm sorry. Your declaration in the IPR-11 -- 01170.

A. Okay. Yes. It wasn't marked 1002.

Q. Sorry.

A. But, yes, I have that.

MR. ROSATO: Counsel, we brought copies for Dr. Quackenbush, but if you have copies that you're --

MS. WANG: Sure.

MR. ROSATO: -- referring to, I'd like to get a copy of that.

Thank you.

MS. WANG: And I'll just note that on the copy that I'm handing you for IPR-01170, it is marked as Guardant Exhibit 1002 on the bottom.

Personalis EX2009.013

THE WITNESS: This is the 1170?

Q. BY MS. WANG: Yeah.

A. Okay.

And mine's marked 1002.

Q. Thank you.

And, Dr. Quackenbush, just to make sure we're talking about the same thing, the document you're looking at, it says "Declaration of Dr. John Quackenbush (Claims 65, 66, 72, 73, 75-77, 80-85)"; is that correct?

A. That's correct.

Q. Okay.

And this is your declaration that you submitted with respect to IPR-01170, correct?

A. Yes.

Q. And you signed this declaration?

A. Yes, I believe I did.

Signed it electronically, so . . .

Q. But there's no reason to think that that electronic signature is not yours, correct?

A. No, that's mine.

Q. And it was correct and truthful when you signed this document?

A. To the best of my ability, yes.

Q. Who drafted your declaration?

Personalis EX2009.014

A. I worked with the attorneys here to draft this declaration.

Q. Do you recall approximately how much time you spent drafting this declaration?

A. There was a substantial amount of time. 20 or 30 hours, at least. But some of that was leveraging work that I had done in the District Court case.

Q. Suffice it to say that you've spent a significant amount of time putting forward the opinions in this declaration, correct?

A. Yes, I have.

Q. And then I would like to have you look at the declaration for 00 -- sorry -- 01447, which I believe is also, unfortunately, marked as Exhibit 1002 as well.

MR. ROSATO: Thank you.

THE WITNESS: I think it's 01147.

Q. BY MS. WANG: Should be 1447. Is that -- that must be a typo.

A. All right.

I think it's a typo. You're right.

Q. Okay.

And do you have that in front of you right now?

Personalis EX2009.015

A. Yes, I do.
Q. Okay.
And do you recognize this also as a declaration that you submitted in IPR-01447?
A. Yes, I do.
Q. And you signed this one as well; is that correct?
A. Yes, I believe it is the same basic -- digital signature at the end.
Q. And it was true and correct when you signed it, correct?
A. Yes.
Q. And how much time did you spend drafting this declaration?
A. So they were drafted as a collection, and then I believe separated later on. So with the entire collection, again, probably about 20 or 30 hours specifically on these, but that built on the work that I had done at the District -- for the District Court case.
Q. Okay.
And then I'll have you just turn to the third declaration, which is 01448, which is also marked as Exhibit 1002.
A. Yes.

Personalis EX2009.016

Q. And do you recognize this as the declaration you submitted in IPR-01448?

A. Yes.

Q. And you also signed this declaration, correct?

A. That is correct.

Q. And it was true and correct when you signed it?

A. To the best of my ability, yes.

Q. And you spent, as you have stated previously, the same substantial amount of time preparing this and your opinions that are in this declaration, correct?

A. That's correct.

Q. Dr. Quackenbush, when did you first read the '830 patent?

A. I don't recall the exact date. It was, I believe, sometime in 2016.

Q. And was that in connection with the Foundation litigation?

A. It was in connection with the District Court case, yes.

Q. Okay.

How many times have you read it, would you say?

Personalis EX2009.017

A. In whole, probably three or four. In part, many times.

Q. And have you reviewed the litigation pleadings in the related litigation?

A. So the --

MR. ROSATO: Objection. Form.

THE WITNESS: The materials I reviewed are provided -- for this -- for these documents are provided in the appendix, and these are largely published works in the literature, and those are things I reviewed in preparing these. In addition, I reviewed the patent and depositions from Dr. Gabriel.

Q. BY MS. WANG: Sure. I'm not asking you what you have listed in your declarations. I'm asking have you generally reviewed litigation pleadings from the District Court litigation?

A. I don't recall all the things I -- I reviewed in preparing for the District Court case, but in preparing these documents, these are the individual references and materials I reviewed.

Q. So the list of references that's contained in your declaration, as you've been pointing out, is the exclusive list of documents that you reviewed in preparation for these declarations?

A. In putting together these declarations, I

Personalis EX2009.018

did a lot of research, I looked at a lot of things. These are the documents that are cited here.

Q. Let me try that again. You mentioned that these are the documents that you reviewed in preparation for these declarations, correct?

A. These are documents that are cited in these declarations. Right?

When you're looking for references describing things, you often do PubMed searches, and in doing a PubMed search, you'll find lots of different references, you review the abstract of those references, and sometimes those papers look good. You look at the papers to see what they say, what they describe, and you try to identify papers that actually capture the essence of what you're hoping to communicate.

So I wouldn't say the things I've looked at are limited to this, but these are the references which are cited supporting the arguments put forth in these declarations.

Q. With respect to the other things that you may have reviewed, through PubMed or through any of your other searches, suffice it to say that none of them are included in here, correct?

A. As -- you know, one skilled in the art,

Personalis EX2009.019

one brings one's background and one's knowledge to preparing any kind of document like this, so things I've learned over the course of my career are reflected in some of what's written here, but the citations included in these declarations are the ones listed in the appendix.

Q. Sitting here today, can you think of any other publications specifically that you reviewed that are not included in this appendix?

A. Off the top of my head, no.

Q. Okay.

Dr. Quackenbush, what did you do to prepare for your deposition today?

A. I had a call with Ms. Gerrard last week, and then began looking over the declarations again. They were written six, nine months ago, a distant time in the past. Began looking at those, began looking at the documents listed in the appendix, and then yesterday spent some time meeting with Ms. Gerrard and others to go over the declarations and the materials.

Q. Who else did you meet with other than Ms. Gerrard?

A. There was another attorney present. I'm terrible with names. I don't recall his name. His first name is Derek.

Personalis EX2009.020

Q. Anybody else other than Derek and Ms. Gerrard?

A. I forget Mike's last name.

MR. ROSATO: Mr. Rosato.

THE WITNESS: Mr. Rosato.

I met with him yesterday briefly too.

Q. BY MS. WANG: Anybody else other than Mr. Rosato, Derek, and Ms. Gerrard?

A. I believe those are the only three people who were with me at any point in time yesterday.

Q. And did you meet or speak with anybody else outside of yesterday other than Ms. Gerrard?

A. Not that I recall, no.

Q. Dr. Quackenbush, I'm going to look at the declaration from 01170 as the primary declaration, and then if I'm directing -- so if I'm directing you to a particular paragraph, it's in that one, and I'll tell you specifically if we move on. Okay?

A. All right.

Q. So can you turn to Paragraph 6 of your declaration, please.

A. Sure.

Q. It's on Page 2.

And do you see there that it says that you received your PhD in theoretical particle physics,

Personalis EX2009.021

a Master of Science in physics, as well as a Bachelor of Science in physics. Do you see that?

A. Yes.

Q. And you don't have any other degrees in any other subjects, do you?

A. I have a master's degree in biostatistics, I believe, from Harvard University, but that was granted to me in recognition of being hired as a professor of computational biology in bioinformatics, in the department of biostatistics at the Harvard T.H. Chan School of Public Health.

Q. So there was no associated coursework that you completed or lab work that you did in order to obtain that master's; is that correct?

A. Well, I think the master's reflects the entirety of all the training and work I've done since receiving my PhD in 1992, in particular the expertise that I've gained in the fields of computational biology, bioinformatics, and its application in a wide range of disciplines, including, most notably, the work that I've done in studying cancer and applications in genomics in cancer.

Q. I appreciate that, but did you do any specific work that earned you that particular master's, or was it in recognition, as you say, of

Personalis EX2009.022

your broader experience?

A. I think --

MR. ROSATO: Objection. Form.

THE WITNESS: I think the master's was in recognition of my broader experience.

I have done a number of courses, and, in fact, I teach many courses and subjects relevant to the material here.

Q. BY MS. WANG: But no other degrees in any particular subjects; is that correct?

A. No.

Q. Dr. Quackenbush, you do not have any clinical experience; is that correct?

A. I'm not a practicing physician, but I've worked extensively with clinical samples, worked extensively with clinicians on studying a wide range of diseases and understanding what's relevant to those diseases.

I began -- in 2012 -- 2011, 2012, I launched with a partner a company called Genospace, and one of Genospace's -- it's a precision medicine software company that was purchased by the Hospital Corporation of America, and one of our primary products was actually clinical reporting tools that were used by physicians to communicate the outcome of

Personalis EX2009.023

genomic profiling experiments. So -- or genomic profiling assays, clinical assays, and in that context worked exclusively with -- extensively with clinicians to understand what their needs are in terms of clinical reporting for a wide variety of different assays.

I've worked on -- my very first NCI grant came I believe in 1998, to study breast -- colon cancer, was involved in the NCI Directors Challenge project, looking at colorectal cancer and understanding genomic signatures in colorectal cancer.

I published extensively in breast cancer, ovarian cancer, head and neck -- somewhat head and neck cancers -- chronic pulmonary disease, asthma.

So I've been involved in a lot of studies analyzing clinical data and understanding the clinically relevant questions associated with analyzing genomic data.

Q. Have you, in fact, conducted any diagnosis of patients in a clinical setting?

A. So I don't know what you mean by "diagnosis." In the context of working at Genospace in particular, we worked extensively in understanding and preparing clinical reports. And what --

Excuse me.

Personalis EX2009.024

What that involved was really looking at mutational profiles that came from conducting assays, integrating that with clinical records and clinical information, tying that to guidance that came from the NCCN, the National Cooperative Cancer Network, FDA guidance, information about clinical trials, and other data, to synthesize and produce reports that were then provided to physicians to help them make clinically relevant decisions.

So it was really analyzing clinical data and genomic data -- excuse me -- in the context of understanding what physicians and their patients needed to know.

Q. But you don't have any experience in actual clinical practice; is that correct?

MR. ROSATO: Objection. Form, asked.

THE WITNESS: I have a PhD, not an MD. It would not be appropriate, really -- appropriate for me to treat patients.

Q. BY MS. WANG: So the answer is, no, you have no experience in a clinical practice, correct?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: I'm not a clinician; I do not treat patients. I work with physicians who treat

Personalis EX2009.025

patients. I've worked with genomic data, again, in a wide variety of situations that are relevant to patients.

I've worked with Dana-Farber in establishing their profile assay, and understand there what information was being collected, synthesis of genomic data and clinical data, and the preparation and communication of that information to physicians.

I've worked with data from clinical trials, where we've looked at -- we've looked at information that's been collected in the context of treating patients and understanding their response.

So am I a practicing clinician? Absolutely not. Do I see patients? Absolutely not. Have I worked extensively with clinical data and with applications that are relevant to treating patients? I absolutely have.

Q. BY MS. WANG: Do you have any experience with FDA approval of products used to diagnose patients?

A. I have not been involved in any FDA submissions, no.

Q. Do you have any experience with commercialization of products that are used to diagnose patients?

Personalis EX2009.026

A. Medical devices I haven't been involved with, but, again, in the context of Genospace, that was a commercial operation. I've worked with companies that are offering assays and commercialize them. And, in fact, we commercialized our software. So -- to that extent, yes.

Q. Sure.

Based on that experience, do you have any experience in terms of valuations of the products that you're commercializing?

A. I can talk about our software, but in terms of diagnostic assays, no.

Q. Now, Dr. Quackenbush, are you familiar with the Guardant360 and the FoundationOne CDx products?

MR. ROSATO: Objection. Form.

THE WITNESS: I'm vaguely -- you know, I'm familiar with them. I know the assays, but I couldn't recite the content of individual assays.

Q. BY MS. WANG: Have you used them?

A. Personally, no.

Q. Let's turn to your declaration.

A. I should say, some of our clients at Genospace in the past have used those assays, and so one of the other things that we have done is created a

Personalis EX2009.027

set of research tools that import that kind of data.

But, again, personally I haven't used them.

Q. Okay. Thank you. I will appreciate you've answered my question, so until I ask another question, I'd appreciate, unless you think that there's something wrong with your previous answer, please -- please let me actually ask a question.

MR. ROSATO: Objection. Form. He's completing his answer.

THE WITNESS: You've asked me a question. I just wanted to complete it.

MS. WANG: You answered my question. Thank you.

Q. So can we turn to your declaration, please.

A. Yes.

Q. And can you go to Paragraph 29 for me, please.

A. Sure.

Q. It's on Page 11.

A. Yes.

Q. Can you just read that briefly to yourself.

A. To myself or out loud?

Personalis EX2009.028

Q. To yourself, just to make sure you know what is said in there.

A. Okay.

All right.

Q. And that's an accurate statement; is that correct?

A. Yes.

Q. So let's talk a little bit about targeted enrichment methods --

A. Sure.

Q. -- which you discuss in Paragraph 29.

And you state that they include PCR, molecular inversion probes, microarray, and solution-phase hybridization; is that correct?

A. That's correct.

Q. These are a number of different methods. Are there other different methods in addition to these?

MR. ROSATO: Objection. Form.

THE WITNESS: There are probably many other methods that people have used to do targeted enrichment. You know, I wouldn't claim that this is exhaustive, but -- in fact, this is actually referencing one of the exhibits, Exhibit 1030, which I believe was a review paper discussing widely used

Personalis EX2009.029

methods. So I wouldn't say it's exclusive, but -- or that it's exclusive, but it indicates widely used methods.

Q. BY MS. WANG: And you would agree that solution-phase hybridization, another way of describing that is bait-based hybrid capture; is that correct?

A. One could include bait-based hybridization as an example of solution-phase hybridization. There may be others that people might describe or other terms that people might use.

Q. Okay.

Let's start with PCR, which is the first one that you listed in your Paragraph 29.

A. Sure.

Q. PCR stands for polymerase chain reaction; is that correct?

A. Polymerase chain reaction.

Q. Polymerase chain reaction. Thank you.

And it's been used in preparing samples for about 20 years or more; is that correct?

A. I don't know about preparing samples and what you mean by that. I believe PCR was invented and patented in the 1970s, and has been used widely in molecular biology assays since the 1970s.

Personalis EX2009.030

Q. And since the 1970s it's largely -- the principles are largely the same for PCR; is that correct?

A. I'm not sure exactly what you mean by saying the principles are the same, but PCR is a well-established technology. There are many variations on PCR that people have used. So we'd have to talk about individual assays.

Q. Sure.

You would agree with me that PCR has three essential steps, though; is that correct?

A. Well, you might want to tell me what those steps are, and then we can discuss whether or not I agree with the statement.

Q. Sure.

You would agree with me that the three essential steps are denaturation, annealing, and extension?

MR. ROSATO: Objection. Form.

THE WITNESS: PCR involves more than that, so -- PCR often begins with double-stranded molecules, although not always. It often begins with nucleic acids. Typically I guess it begins always with some type of nucleic acid. Typically RNA or DNA is an initial target, although not necessarily

Personalis EX2009.031

exclusively. You can have modifications to that.

And then what one typically does is design oligonucleotide primers to synthetic pieces of DNA that target specific regions, and then allows those to anneal or bind to target sequences in the DNA. One then typically performs other operations, including extension with a polymerase, and then repetitive cycles involving multiple rounds.

But PCR has many variations. People have done many different things with PCR.

I think some of the key elements are identifying targets and synthesizing oligonucleotide probes to those targets.

Q. BY MS. WANG: So you -- I'm sorry. I'm not sure I understand. So you agree or disagree with me that there are three basic steps in the PCR process?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, PCR takes on many forms and can be used in many different ways, and I think I've characterized what I think the essential -- or what typical components are of PCR. But hesitant -- I'd be hesitant to say that there are three essential steps.

MS. WANG: Okay.

Can you mark this for me, please.

(Deposition Exhibit 1 was marked for identification and sealed separately.)

THE REPORTER: Exhibit 1.

Q. BY MS. WANG: Dr. Quackenbush, you've been handed what's been marked as Exhibit 1 to your deposition.

A. Yes.

Q. Do you recognize this document?

A. Yes, I do.

Q. And this is a declaration that you submitted in that 10x Genomics case that you mentioned earlier, correct?

A. That's correct.

Q. Where you were offering your opinions as an expert witness?

A. That is correct.

Q. Can you go to Page 2 of this declaration, please.

A. Sure.

Q. And do you see Paragraph 6?

A. Sorry. Yes.

Q. And do you see in Paragraph 6 where you state that, "While there are numerous ways to amplify nucleic acid sequences, PCR is a particular three-step

Personalis EX2009.033

method that amplifies specific nucleic acid sequences."

Do you see that?

A. Yes, I do.

Q. And then if you go to the next page, Page 3, Paragraph 7.

A. Yes.

Q. Are you there?

Okay.

And do you see in Paragraph 7 where you state, "As discussed above, Plaintiffs' construction" -- not relevant to this case -- "does not reflect that PCR is a process for amplifying specific nucleic acid sequences," and then later you say, "Plaintiffs' construction also fails to recite that PCR consists of three basic steps (as described in paragraph 5 above)."

Do you see that?

A. I see where it says that, yes.

Q. Okay.

And is it your understanding that those three basic steps are denaturation, annealing, and extension?

A. So this -- the statement, the way you're phrasing or the way you're presenting it, is taken out

Personalis EX2009.034

of context, in the sense that in making the statement, we're trying to distinguish what was referred to as traditional PCR versus a variety of different modifications on PCR.

And so in the context of the statement, I was talking about traditional PCR as laid out by Kary Mullis.

In the literature, and in this reference that you referred to earlier, which is Exhibit 1030, the call-out to PCR in that reference didn't limit itself to traditional PCR.

So when you ask me about the question about PCR consisting of three individual steps, I was answering in the context of this reference, which you had asked me about. If you had asked me about how Kary Mullis defined PCR and what we referred to in this matter as traditional PCR, I would have said, yes, traditional PCR does consist of those three individual critical steps.

But even in this paragraph -- right? -- if you look at language which comes from the '430 patent, which is a different patent, which was in suit here, and is referenced in Paragraph 7, they list a variety of different types of PCR. So nested polymerase chain reaction, polymerase chain

Personalis EX2009.035

reaction-single strand conformation polymorphisms, ligase chain reaction, ligase detection reaction, strand displacement amplification and restriction fragment, polymorph -- polymorphism assays, transcription-based amplification systems, nucleic acid sequence-based amplifications, et cetera.

So in discussing PCR here and describing it as three steps, that was to really characterize it as traditional PCR. So in the context that's absolutely correct. In the broader context of how PCR is used in this article I referred to, I wouldn't say that's correct.

Q. Traditional PCR is still being used today; is that correct?

A. It's still widely used today.

Q. Now, you were talking about the review article, which we'll get to in a little bit, and how you disagree that, as the review article refers to PCR, it has these three basic steps; is that correct?

A. I didn't say that. I said that if we look at PCR, it can encompass many different things. And so if we want to talk about traditional PCR, we can talk about those steps.

I wouldn't say I disagree with the article. I'd have to look at the article to

Personalis EX2009.036

understand exactly what they're talking about. But in the common parlance, PCR can refer to a lot of different assays that involve amplification.

Q. Nontraditional PCR.

MR. ROSATO: Objection. Form.

THE WITNESS: I don't know if I would call it nontraditional PCR. I'd call it variations on PCR.

Q. BY MS. WANG: Do these variations on PCR lack one of the three basic steps?

A. I'd have to look at the individual types. I can think of lots of different things that people have done to do amplification that are characterized as PCR.

Q. To the best of your knowledge sitting here today, are there any examples of PCR that do not involve denaturation, annealing, or extension?

MR. ROSATO: Objection. Form, asked.

THE WITNESS: As a first step?

Many PCRs -- PCR can start with single-stranded targets, so they may not involve denaturation as a first step.

Q. BY MS. WANG: Okay.

Let's look at the article that you're referencing, which I believe in your binder is

Personalis EX2009.037

Exhibit 1030.

A. Yes.

MR. ROSATO: Copy, Counsel?

MS. WANG: Sorry about that.

MR. ROSATO: Thank you.

MS. WANG: Sure.

Q. And this is the Mamanova article that you were referencing; is that right?

A. Yes.

Q. And this describes the different targeted enrichment methods that you mentioned: PCR, molecular inversion probes, solution-phase hybridization, and I believe --

Yes, those three; is that correct?

A. So, yes, the article mentions PCR, molecular inversion probes, and solid-phase and in-solution hybridization assays.

Q. And PCR is referred to and I believe you refer to it as an amplification technique; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: PCR is widely used as an amplification technique, yes.

Q. BY MS. WANG: And to amplify something, that means to increase the amount of that particular

Personalis EX2009.038

thing; is that correct?

A. I don't know -- in some contexts, yes. Typically when we talk about amplification we're making additional copies of some -- some segment of DNA. However, it's broadly used.

So, for example, in random prime PCR, one can copy an entire collection of DNA from an organism.

So typically amplification is used to make additional copies.

Q. And that's how PCR works to enrich a target, correct? By amplifying the particular targeted segment, as you described?

A. So the way PCR is used, yes, one typically uses it to amplify specific targets.

Q. And that is to make multiple copies of that particular target, correct?

A. Yes, one can use it to make copies of the target.

Q. I do want to talk a little bit about traditional PCR, as we've characterized, and what denaturation is. Can you tell me what denaturation is?

A. So DNA is a double-stranded molecule. It consists of a sugar phosphate background -- so DNA is deoxyribonucleic acid. Deoxyribose is a long-chain

Personalis EX2009.039

polymer sugar, and it's the backbone for DNA.

DNA then consists of nucleic acid bases, adenine, cytosine, guanine and thymine, or A, G, C and T, and as a double-stranded molecule, DNA is actually directed, so each deoxyribose strand has a direction -- we refer to them as the 5' and 3' ends -- and those molecules actually have the sugar phosphate backbone and base pairs, and the -- they're bases, the nucleic acid bases, and they form base pairs.

So we have rules in which -- which are made by DNA where we form complementary pairs, so A pairs with T and C pairs with G in those complementary strands.

Denaturation is typically referred to as the process -- is -- typically refers to the process in which those strands are separated, and when the strands separate, that is typically achieved by applying gentle heating, although there are other ways of separating the strands. So denaturation is the separation of those strands of DNA.

Q. So in traditional PCR, as we've talked about, the first step when you have your double-stranded DNA is to denature or to separate those strands from one another; is that correct?

A. So typically, yes, one will separate the

Personalis EX2009.040

strands, because the natural state of DNA is as a double-stranded molecule.

Q. Now, in the second step of traditional PCR is what we've referred to as annealing; is that correct?

A. That's correct, yes.

Q. And annealing refers to when you, in fact, bind the primers that you discussed earlier to each of those strands; is that correct?

A. So before starting a PCR assay, what one does is one designs oligonucleotide probes, so synthetic pieces DNA, that are complementary to a region or target sequence that one wishes to capture and then ultimately amplify.

So one designs the synthetic primers, the synthetic oligonucleotides, and then adds them in significant excess to a reaction, allows them to come in and bind, and that binding step is referred to as annealing.

Q. And the primers bind to each end of the double-stranded target sequence; is that correct?

A. That's not correct. The primers bind to target sequences which they wish to capture -- which one wishes to capture. So if I had an entire genome and I wanted to try to amplify or make many copies of

Personalis EX2009.041

a particular gene or a particular region, what I could do in traditional PCR is design two primers, one which would be complementary to each strand. They would bind in the region of that gene.

Now, they might bind in the middle of a long DNA strand, they might bind at the end of a DNA fragment if the DNA was fragmented somehow in preparing it. So they bind to specific target sequences that one wishes to capture and amplify.

Q. Where do they bind with respect to the particular target sequence?

A. At the places to which they are complementary.

Q. So let's say you have a target sequence of approximately 30 nucleotides. Do you agree with just -- agreeing with my premise here that we have target sequence.

A. One would typically not amplify a target sequence 30 nucleotides in length. If one wanted to, that's -- we can imagine we have a target sequence which is 30 nucleotides.

Q. Just follow with my hypothetical here.

A. All right.

Q. So if you have a target sequence of 30 nucleotides in length, and that particular sequence,

as we've discussed, comprises of the double strands, correct?

A. So in its natural state -- right? If it were in your genome -- right? -- it would probably be in a long double-stranded complex.

Q. Okay.

After denaturation the double strands are broken apart, correct?

A. They're not broken apart. They separate. So they're held together with weak hydrogen bonds, and they reversibly separate. So they can reanneal. They can come back together again.

Q. So after denaturation the double strands are separated; is that correct?

A. They're typically separated into single-stranded molecules.

Q. And each of those separated strands, they are complementary to each other; is that correct?

A. Yes, they're complementary to each other.

Q. And in annealing, you would apply a primer to each of those separated strands; is that correct?

A. I would typically design primers -- there are cases where people design single primers, but in traditional PCR, I would identify a target region and

Personalis EX2009.043

I would design two primers to amplify that target region.

Q. And where would you apply those two primers to amplify that target region of the 30 nucleotides?

MR. ROSATO: Objection. Form.

THE WITNESS: So, again, it would depend on my assay and what I wanted to do. Right? I might try to bind those primers outside of the target region in genomic DNA; I might try to bind them within that target region. I might try to bind them -- I might design multiple primers. People sometimes use nested PCR.

So in traditional PCR I would design two primers that would in some way bracket the target region that I wanted to try to amplify.

Q. BY MS. WANG: Okay.

Now, after you've attached those primers bracketing the target region that you would like to try to amplify, the last step in the PCR cycle is extension, correct?

A. The last step in the first cycle would be extension, so one would add a DNA polymerase, and then allow that polymerase to extend the molecule. Extend from the site of hybridization.

So if I have one single-stranded molecule, the primers would come and bind, and extend, and create a longer piece. So that piece can be relatively short, it can be extremely long. It depends on how long that single-stranded fragment of DNA is.

Q. But the DNA polymerase would create a new strand, essentially, that would extend out; is that correct?

A. I don't know what you mean by a new strand. It would copy DNA. So it creates a complementary strand to the original strand.

Q. But it's a copy, correct?

A. It -- one could refer to it as a copy, yes.

Q. Well, you just said it was a copy of the original strand, correct?

A. You create a -- it's not a copy. It's complementary to the original strand.

There's an important difference. So for every A in this strand, I'd have a T in the -- it's almost like a negative to a photograph. So for every A I'd have a T; for every G I'd have a C.

Q. Okay.

If you start with a double-stranded

Personalis EX2009.045

target sequence, that we've talked about, you separate through this process and you have two strands, correct?

A. So my initial DNA is double-stranded. I apply gentle heating and I have two single-stranded molecules.

Q. Then you apply two primers to those two strands; is that correct?

A. In traditional PCR, where one is targeting a small region, one would use two primers, because in subsequent steps one would want to specifically amplify that target region.

Q. So you would use two primers in traditional PCR to those two separated strands, correct?

A. One would use primers complementary to those two strands.

Q. But two of them; is that correct?

A. In traditional PCR, yes. Again, there are many variations on PCR that are used.

Q. Understood.

In traditional PCR you would use two primers to those two strands, and then you would use DNA polymerase to extend and create a complementary copy of those two strands; is that correct?

Personalis EX2009.046

A. That's typically how PCR -- traditional PCR functions, yes.

Q. So at the end of your first cycle of traditional PCR, instead of one double-stranded target segment, you now have two double-stranded target segments; is that correct?

A. Not completely. You would have --

Again, imagine this is a portion of your chromosome and imagine we have a large fragment of that chromosome, which is millions of bases in length. If we had only one copy -- and, again, for extracting DNA from even any one of your cells, they start with two copies of your genome. Typically extract DNA from multiple copies, but if we focus on one of those, we start with the double-stranded molecule that is effectively a million base pairs in length. We would allow those primers to bind, and we would create copies that are, again, typically less -- complementary copies that are typically less than a million bases in length, depending on where those primers bind and how much additional sequence there is.

Q. But if you start -- I'm only talking about one cycle in traditional PCR. If you start with one double-stranded sequence, what you get at the end

Personalis EX2009.047

of that first cycle are two copies, essentially, of that same double-stranded sequence, correct?

A. Not full copies. Right?

So, again, if I have a long molecule and I bind here and I start to copy this way, this piece to the -- to the right -- I guess to your left -- to the left of where the primer bound on the first strand would still be single-stranded.

You can refer to the entire hybrid -- the hybrid complex as a double-stranded molecule. Again, it depends on the context and how you want to discuss it. But it would be partially single-stranded, partially double-stranded.

Q. So I think I understand where we're talking past each other. With respect to the traditional PCR in the first cycle --

A. Mm-hmm.

Q. -- where you start with a double-stranded target sequence -- and I'm not talking about a full chain but I'm talking about the targeted sequence of our 30 nucleotides as we used in our example. What you end up -- what you end up with at the end of that first cycle are two copies of that 30-nucleotide sequence; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: That's not correct. Right? If you look at how Kary Mullis designed PCR, it was to amplify targeted regions from complete genomic DNA. Right?

So it's not a 30-nucleotide synthetic piece that you're copying. You're trying to capture a piece of a larger genomic sequence.

So you don't make just a targeted copy of that tiny little piece. Right? What you do is you copy a target region from the entire genome.

Q. BY MS. WANG: At the end of the first cycle of a traditional PCR, do you end up with two copies of that target region from the entire genome?

MR. ROSATO: Objection. Form.

THE WITNESS: You don't end up with two copies of that target region. Right?

Again, if I have -- and let's -- let's say your genome is only a million bases long. Okay? Instead of the over 3 billion copies. And let's imagine that you only had one copy of your genome. So let's imagine this is a bacterial genome or a viral genome. Right?

So in that bacterial genome, I may want to run a diagnostic assay, I may want to look for a drug-resistance gene. And so if I have a

Personalis EX2009.049

drug-resistance gene in the middle of a million base-pair-long genome, if I allow the primer to bind and allowed it to extend -- all right? So I design a primer that would target my region of interest, the gene I want to try to amplify. I'd allow it to bind, and I would create a complementary DNA strand that would begin at that primer but capture the target sequence I'm looking for as well as some of the genomic context.

So it's not just the target that's copied, it's the target and additional DNA in the region.

If I wanted to target multiple genes, I'd design multiple primers that would align in those -- that would fall into those genes.

If I wanted to sequence that gene, I may design multiple sets of primers that fall within and are nested within. Right?

So, you know, when you say you just get two copies of that target, that's not entirely correct in the first round of PCR.

Q. BY MS. WANG: Have you personally designed primers?

A. I have designed millions of PCR primers.

Q. So you're familiar -- well familiar with

Personalis EX2009.050

the process of designing primers?

A. I'm well familiar with the process of designing primers and all sorts of oligonucleotide probes for a wide range of applications.

MS. WANG: Can you mark this for me? Thank you.

(Deposition Exhibit 2 was marked for identification and sealed separately.)

THE REPORTER: Exhibit 2.

Q. BY MS. WANG: Dr. Quackenbush, you've been handed what's been marked as Exhibit 2 to your deposition, and do you see that this is a printout from a web page entitled "Polymerase Chain Reaction"?

MR. ROSATO: I'm just going to note an objection. This is not a document currently of record, correct?

MS. WANG: That is correct.

MR. ROSATO: Okay. So I'll just note for preservation objection, any evidentiary objections in scope as well.

Q. BY MS. WANG: Dr. Quackenbush, my question was: You note this is a printout of a web page that's entitled "Polymerase Chain Reaction (PCR)"; is that correct?

A. That's the title, and I can see the URL

Personalis EX2009.051

at the bottom.

Q. And it appears to be from the National Human Genome Research Institute?

A. Yes.

Q. Okay.

And you'll note that in Paragraph 2 of this document it mentions Kary Mullis, who you've been referring to as the inventor of PCR; is that correct?

A. Can I have a minute to read this?

Q. Sure.

A. Okay.

Q. Are you done with reading the page?

A. Yes, I have finished reading it.

Q. Okay.

Can you look to the paragraph that says "How does PCR work?" Do you see that?

A. Sure.

Q. And do you see that it says, "To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA."

You would agree with that, right?

A. That's what it says, yes.

Q. And, "Next, an enzyme called 'Taq polymerase' synthesizes - builds - two new strands of

Personalis EX2009.052

DNA, using the original strands as templates"; is that correct?

A. It says that, but it leaves out the hybridization of primers, which is an essential step.

Q. Correct. Agreed.

But then it next says that, "This process results in the duplication of the original DNA, with each of the new molecules containing one old strand and one new strand of DNA."

Do you see that?

A. I see where it says that, yes.

Q. And you agree that that is what happens at the end of the cycle?

A. So --

MR. ROSATO: Objection. Form.

THE WITNESS: That is what happens during the first cycle, and if you look at the figure, which is really hard to see, this shows exactly what I talked about earlier, where you have a DNA -- a polymer -- sorry -- an oligonucleotide primer bind and copy the target region plus additional DNA.

So one can characterize that as -- the way this is described, as one new and one old strand of DNA.

Q. BY MS. WANG: Okay.

Personalis EX2009.053

And then you would agree that, as it says here, "each of these strands can be used to create two new copies, and so on, and so on"; is that correct?

A. So -- yes. In subsequent steps. Right?

The process is cyclic. That's why it's called a chain reaction. In subsequent steps you create multiple copies of your target.

Q. And so you would go through multiple cycles to get multiple copies; is that correct?

A. Typically if you want to amplify something, you want more than just two copies, and the way Kary Mullis designed PCR was to isolate that target sequence from the larger genomic sequence.

Q. Now, you mentioned earlier that sometimes in PCR, not traditional PCR, you start with a single strand; is that correct?

A. One can, yes.

Q. And if you start with a single strand for the first cycle, there is no denaturation, correct?

A. You wouldn't have to denature the DNA, no.

Q. But if you were to continue and have a second cycle and a third cycle and a fourth and so on and so forth, you would have to include a denaturation step; is that correct?

A. Subsequent cycles of PCR include denaturation, yes.

Q. Now, Dr. Quackenbush, you've said you've had experience designing primers; is that right?

A. Yes, I have.

Q. And are you aware of a typical length of primers?

MR. ROSATO: Objection. Form.

THE WITNESS: The length of primer depends on the application.

Q. BY MS. WANG: In your experience designing primers, what have been some typical lengths that you have used?

A. So primer design, we've used -- for amplifying specific -- so the very first project I worked on when I began my career in molecular biology in 1992 was a large-scale library screening project. We were amplifying small fragments of DNA, about 200 base pairs in length, and the primer sequences we used were typically 22 to 27 bases in length. They were designed with a set of stringent design criteria that included the reaction condition, salt concentrations, and the desired annealing temperature for subsequent PCR reactions.

So for a given set of conditions, yes, I

Personalis EX2009.055

can talk about -- in the reactions we did, I can talk about the length of the primers.

Sometimes primers are modified to contain additional sequences so they're longer. Sometimes one wants specificity so one can adjust the annealing temperatures. It all depends on the context of the reaction.

So I'd hesitate to say there's a typical set of conditions or lengths.

MR. ROSATO: We're coming up on about an hour, so whenever you find a good time for a break would be appreciated.

MS. WANG: Got it. We just have a couple more here.

Will you mark this for me, please.

(Deposition Exhibit 3 was marked for identification and sealed separately.)

THE REPORTER: Exhibit 3.

Q. BY MS. WANG: Dr. Quackenbush, you've been handed a document marked as Exhibit 3 to your deposition, and it's entitled "Bioinformatic tools and guideline for PCR primer design," and it's by Kamel A. Abd-Elsalam, I think. Do you see that?

A. Yes, I do.

MR. ROSATO: Can I take a moment to look

Personalis EX2009.056

at this, Counsel?

MS. WANG: Sure.

MR. ROSATO: It's a new exhibit?

MS. WANG: This is a new exhibit.

MR. ROSATO: Okay. So I'm going to note and preserve any evidentiary objections as well as objections to scope.

You can go ahead.

Q. BY MS. WANG: Dr. Quackenbush, if you can turn to Page 94 as it's noted on the top of this document.

A. Sure.

Q. You'll see a paragraph there about primer length, and take your time and read that paragraph, please.

A. Okay, I see -- I've read the paragraph.

Q. And you see that it says that -- I think as you testified, "both specificity and the temperature and time of annealing are at least partially dependent on the primer length"; is that correct?

A. Well, I added also reaction conditions and salt concentrations, but the first sentence does say that specificity and the temperature and time of annealing are dependent on primer length.

Q. And then it states that "this parameter," primer length, "is critical for successful PCR." Do you see that?

A. That's what it says, yes, and it references a 1991 paper by Wu, which I don't have available.

Q. But would you agree that primer length is, in fact, critical for a successful PCR?

A. Primer length is an important component of successful PCR.

So, again, I haven't read this paper, I haven't had a chance to review it. I don't know exactly what the author is discussing here, but primer length, yeah, is -- it's one of the things we consider in designing a PCR assay.

Q. And the next sentence states, "For broad-spectrum studies, primers of typically 18-30 nucleotides in length are the best." Do you see that?

A. I see where it says that, yes.

Q. Do you agree with that?

A. So this is a review paper that was written in 19 -- sorry -- in 2003. It was published in the African Journal of Biotechnology. All right? So I don't know much about the journal and the criteria they use to review this.

Personalis EX2009.058

This gentleman works in a Plant Pathology Research Institute and the Agriculture Research Center in Orman, Giza, Egypt. So I don't know what he's actually talking about. He may be talking about particular plant assays, since this seems to be, you know, where he works. Again, I haven't had a chance to review this document.

So in the context of the applications he may be discussing here, in the context of this paragraph, his conclusion is that that length of nucleotides are the best. But for different assays you might have different length primers. Again, it really depends on the context and what you want to do. Right?

These primers may have significant secondary structure that interfere with hybridization. There are all sorts of things that play into this.

So I can see where he says this, but it's taken out of context in what he's actually trying to do.

Q. In your experience, have you ever designed primers that are greater than 50 nucleotides in length?

A. I'd have to think very carefully. I've designed some very long primers for specific

Personalis EX2009.059

applications.

Q. Do you recall if you've ever designed primers that are greater than 50 nucleotides in length?

MR. ROSATO: Objection. Form. Asked and answered.

THE WITNESS: I don't recall how long they are, no.

MS. WANG: All right, we can take a break here.

THE VIDEOGRAPHER: We are going off the record. The time is 10:17 a.m.

(Short recess.)

THE VIDEOGRAPHER: We are back on the record. The time is 10:35 a.m.

MS. WANG: Thank you.

Q. Dr. Quackenbush, just to ask, during the break did you speak with your counsel at all regarding your answers to this deposition?

A. No, I did not.

Q. Okay. Thank you.

Now, before the break we were talking about primer length.

A. Yes.

Q. And you had mentioned that you have

Personalis EX2009.060

designed, I think you said, millions of primers; is that correct?

A. Probably, yes.

Q. Okay.

And over the course of your career?

A. I designed the largest number when I was just getting started, but, yes, we've designed primers over the course of my career for a variety of different applications.

Q. And you mentioned that there would be reasons as to why you would want to design longer primers. Do you recall saying that?

A. Yes.

Q. What do you mean by "longer primers"?

A. Well, you were asking me about a range from 18 to 30 bases, and there might be times when you design primers that are longer than that.

Q. How much longer?

A. Again, it's going to depend on your application.

Q. But I think you testified previously that, despite the millions of primers that you've designed, you don't recall if you've actually designed ones that are greater than this range of 18 to 30; is that correct?

A. I know I've --

MR. ROSATO: Objection. Form.

THE WITNESS: I know I've designed primers longer than 30 bases in length. You were asking me about 50 bases, and I'm not sure.

Q. BY MS. WANG: Longer than 40 bases in length?

A. Again, I -- I'd be -- I don't recall the length of all the primers I've designed, so I'd be hesitant to set a size limit.

Q. Sitting here today, of all the millions of primers you've designed, can you think of a single primer that you've designed that was over a hundred bases in length?

MR. ROSATO: Objection. Form.

THE WITNESS: I've been working in molecular biology since 1992, so, you know -- I've designed oligonucleotides that are longer than a hundred base pairs in length. Whether they were specifically used for PCR, I can't say.

Q. BY MS. WANG: And the same with respect to whether you recall designing any primers that are more than a hundred fifty nucleotides in length.

A. I can say I probably haven't ever designed a PCR primer that's a hundred fifty base

Personalis EX2009.062

pairs in length.

Q. Now, you also mentioned that one of the factors -- and I know you mentioned several, but one of the factors that's related to primer length is temperature; is that correct?

A. The annealing temperature of the primer, yes.

Q. And I think you mentioned earlier that during the denaturation step as well you used gentle heat. That was the term that you used. Do you recall saying that?

A. It's typically referred to as gentle heating, yes.

Q. What is the temperature range for gentle heating?

A. So to denature double-stranded genomic DNA, and, in fact, in most PCR -- most PCR protocols using traditional PCR -- so I'm not going to comment on all the variations of PCR and what someone might do, but in traditional PCR as designed by Kary Mullis, heating typically stops at 95 degrees Celsius.

Q. And why is that the case?

A. You want to assure that the double-stranded DNA is fully denatured.

Q. Is there a risk of having a higher

Personalis EX2009.063

temperature beyond 95 degrees?

A. A hundred degrees water boils, so it wouldn't be a good idea to boil the DNA.

Q. Because that would destroy the DNA that you're working with?

A. Yeah.

Q. Okay.

And with respect to annealing temperature, is there also a typical temperature that's used for annealing?

A. It depends on your length of primers and the assay that you want to run. It depends on the salt conditions, it depends on a wide variety of different elements in the protocol and what you're hoping to achieve.

Q. Just like with denaturation temperatures, is there a point at which an annealing temperature is not ideal?

A. Again, it depends on your application. If you try to anneal primers at 95 degrees C, again, for short primers they probably wouldn't work. If you were trying to anneal them at zero degrees C -- right? -- on a block of ice, they wouldn't work. At room temperature you may get some nonspecific binding, but PCR protocols often vary the temperature over the

Personalis EX2009.064

course of a reaction, and can even vary the annealing temperatures depending on the objective of the assay and what you want to achieve.

Q. So for the primers that we were talking about before, between 18 or 30 nucleotides in length, what would be a typical annealing temperature for those?

A. I'd have to know the GC content, the length of the primer, the salt concentrations, and other factors in the assay.

Q. So you can't tell me what a typical temperature would be used for those primers of that particular length?

MR. ROSATO: Objection. Form.

THE WITNESS: So this -- you know, you're referring back to this paper from the African Journal of Biotechnology --

Q. BY MS. WANG: I'm actually not referring to that paper. I'm actually asking you generally in your experience, if you had primers between 18 and 30 nucleotides in length, if there's a typical annealing temperature that you would use to -- in those reactions.

A. So you said as we discussed earlier, which was in this paper, so I don't know what assay

Personalis EX2009.065

this gentleman is using, what the conditions are. Again, it would depend on application. I'd be hesitant to offer a temperature because there may be circumstances in which that temperature wouldn't be appropriate. It depends on the assay itself.

Q. In the context of any primers that you yourself have designed, can you point to any specific temperatures that you've used for annealing?

A. When we were running -- when I was running PCR at the Salk Institute, we would typically design our primers so that they would hybridize in the range of 62 to 65 degrees Celsius. At least that's my recollection.

Q. And when you say "so that they would hybridize," is it that temperature affects whether or not they would actually, in fact, hybridize to the complementary strand?

A. So hybridization is a stochastic process. Hybridization occurs at many different temperatures. Right? At any temperature. As long as you don't have solid ice or boiling water. Right? DNA molecules can find each other and form bonds across the complementary strands. Okay?

So when we design an assay for a hybridization temperature -- particular hybridization

Personalis EX2009.066

temperature, we're trying to balance sensitivity and specificity. Right?

If I wanted to get complete hybridization with every primer, I'd do it at a low temperature but I might hybridize to sequences which aren't a perfect match. If I do it at too high a temperature, I'm still going to get some hybridization, but it may not be -- may not give me sufficient sensitivity to be able to fully amplify the targets that I'm trying to amplify.

So, you know, there -- in any kind of design -- in any kind of experiment, you're always trying to strike a balance between different elements. Right?

Molecular biology is a science of optimization. Right? And pretty routine optimization in the way we approach lots and lots of different assays.

PCR represents that. It depends on the application and what you're trying to gain from the assay itself.

Q. So just going back, when you say specificity, you're referring to whether you're, in fact, hybridizing to the target that you're looking for versus everything else in the pool; is that

Personalis EX2009.067

correct?

A. So specificity can refer to lots of different things. In the context I'm using it right now, specificity that I was referring to is an exact match. All right?

But, again, we even run assays at times where you want to bind but you may want to have a single base pair mismatch.

So specificity is sort of context dependent, but in the context I'm using it, what you want to do is match the appropriate sequence which is complementary to your primer.

Q. And the other -- you also talked about sensitivity, correct?

A. Yes.

Q. And in that context you were talking about whether it's sufficiently sensitive to actually find the particular sequence that you're looking for; is that correct?

A. Not to find it, necessarily.

Q. To hybridize to it?

A. Not necessarily to hybridize. Right? If I design PCR primers to hybridize at 58 degrees Celsius but I set my annealing temperature at 70 degrees Celsius, I would still get some

Personalis EX2009.068

hybridization. Right?

If I carried out the additional steps of PCR, I would still get amplification. But I may not amplify a sufficient quantity for me to carry on the next steps of the assay. Right?

So, again, this is, you know, a process of optimization, where you try to balance a variety of different factors, including sensitivity and specificity.

Q. Let's switch gears a little bit.

You said earlier that you have a lot of experience in dealing with samples from cancer cells; is that correct?

A. Well, cancer cells, cancer cell lines, tumor samples, yes.

Q. And I think you've testified previously in this case and in the litigation about the difference between germline mutations and somatic mutations, correct?

A. That's correct.

Q. And that somatic mutations are really what we're talking about in the context of the '830 patent and in this case; is that correct?

A. I don't recall what I said previously. I can discuss the differences between somatic and

Personalis EX2009.069

germline mutations. Typically, although not always, one is interested in cancer diagnosis and treatment and identifying those somatic mutations, because in the context of precision medicine, those somatic mutations are often the ones that are therapeutically valuable, therapeutically informative.

But if, for example, you carried a germline BRCA1 or BRCA2 mutation --

Q. Hopefully not.

A. Hopefully not, right?

But if you did, that would be a germline mutation which still would be informative for treating cancer.

So typically when we're looking at tumors and tumor mutations, the emphasis is on somatic mutations, but not always.

Q. And somatic mutations are those that actually exist in the tumor cells; is that correct?

A. In the context of cancer, we talk about them as arising after your body grows and develops, during the process of growth and development and aging. Some mutations exist in precan -- somatic mutations exist in a precancerous state.

So, again, you, over the course of your lifetime, accumulate mutations. Not all of those

Personalis EX2009.070

mutations -- those mutations we refer to as somatic because they're not inherited or germline. Those mutations may or may not lead to cancer.

When we talk about the development and progression of cancer, we talk about those somatic mutations which facilitate the uncontrolled growth and expansion of cells, and that growth and expansion is what we refer to as a tumor.

Q. Is it true that in a tumor there would be cells that do not have somatic mutations?

A. There are often infiltrating cells -- right? -- like lymphocytes, that may not carry those mutations. There's often normal tissue mixed in.

So if we look at the bulk tumor, there are cells which may not carry somatic mutations. They still may. We have margin effects in tumors, where the surrounding tissue may, in fact, carry some precursor mutations. With the addition of additional mutations, may lead to cancer development and progression.

A lot of the models of cancer are multihit models, that you have to accumulate a series of somatic mutations to initiate full progression to what we think of as cancer.

If we look at that bulk tumor sample,

Personalis EX2009.071

there may be infiltrating tumor tissue. Often, especially in laboratory investigations, we separate the tumor cells from the somatic cells.

So tumor cells themselves may -- even there, the separation may be incomplete. So there may be cells that may not carry the particular somatic mutations that are relevant for therapy.

Again, there are lots of different classes of mutations. We talk about driver mutations and passenger mutations, and I'd be happy to discuss those as well.

Q. We do not need to discuss those.

A. That's what I figured.

Q. Are you familiar with the term somatic mutation frequency?

A. Yes, I am.

Q. And what do you understand that term to mean?

A. So it's often used in different situations. You can talk about somatic mutation frequency in populations, and there are lots of databases and places to characterize those. There are also somatic mutation frequencies within individuals. So not all the cells in your tumor may have exactly the same set of mutations.

Personalis EX2009.072

And, in fact, what we know is a tumor is a heterogeneous mass, with cells that carry different mutations, and in particular some of those mutations may induce metastases, and those metastases then may contain -- may develop additional mutations.

So when we talk about the frequency, we're talking about how often we see it in a particular background. And that can be a population level, it could be in the context of an individual patient's disease.

Q. And one of the databases that you mentioned that discuss population somatic mutation frequency is the COSMIC database; is that correct?

A. COSMIC -- the COSMIC database is one of those which does characterize population-level frequencies.

Q. Now, you mentioned metastasis.

A. Yes.

Q. Can you describe for me what that is?

A. Sure. If we think about a tumor -- right? -- a tumor is a mass that grows when a set of cells begin to accumulate mutations such as they grow out of control. In many of the models we have of cancer, one of the key driving mutations is the mutation in either DNA repair or in checkpoint

Personalis EX2009.073

processes which detect DNA damage. So the tumor begins to start to grow in its own local environment. Right?

For example, my father-in-law had colorectal cancer, so in a portion of his intestine he had a primary tumor. Some of the cells in that tumor picked up additional -- the tumor grew in the wall of the mucosa -- that's the inner lining of the colon -- it then grew into the local musculature, and at some point some of those cells in the tumor were shed and traveled to his liver, where they ended up growing in a new site, and those cells growing, forming new tumor masses in a new site, are referred to as metastases.

Q. And they traveled to his liver through the circulation; is that correct?

A. In his case I don't know if it was through blood circulation or through the lymphatic system.

Q. Tumor cells that travel through circulation are referred to as circulating tumor cells; is that right?

A. Typically people refer to circulating tumor cells as tumor cells that are carried in the blood, but you can also look at them in lymphatic fluid or other bodily fluids.

Personalis EX2009.074

Q. What are some examples of circulating tumor cells?

A. There are lots of different examples. Solid tumors will shed cells into circulation.

Leukemias carry circulating tumor cells. So if we look at a patient with leukemia, the vast majority of the white blood cells in an early stage may be normal white blood cells, but then circulating with those normal white blood cells would be tumor cells.

Q. The leukemias don't originate with the tumor, that then sheds tumor cells to then metastasize in a different location in the body, correct?

A. Some tumor cells -- some lymphomas can metastasize and form secondary tumors. They're really cells that originate in the bone marrow. So they begin in the bone marrow and make their way into circulation.

Q. So we were talking about population frequency, and I think we talked about the COSMIC database.

A. Mm-hmm.

Q. Are you aware of any other databases that talk about population somatic mutation frequency?

A. There are probably lots of custom

Personalis EX2009.075

databases. I'm sure there are databases at the NCBI that characterize tumor mutation frequencies.

The primary resource we often use when looking at mutation frequencies is COSMIC. They do a very good job of curating other resources and bringing them in.

Q. So you can't think of a specific other database that deals with population mutation frequencies?

A. I'd be happy to do a search and find some, but COSMIC is a -- is probably the most widely used resource so it's the one I'm most familiar with.

Q. Okay.

What are SNPs?

A. So SNP is an acronym. It refers to single-nucleotide polymorphism.

Q. And is it true that they are changes in a DNA base pair?

A. I don't know what you mean by "changes."

Q. What do you understand "changes" to mean?

A. I don't know how you're using it in context.

Q. Are SNPs considered a change in one DNA base pair?

A. So if you're talking about change, you

Personalis EX2009.076

have to talk about change relative to something. Right?

So if we look at our genomic DNA -- right? You and I are clearly not identical. You're female, I'm male. That means you carry two X chromosomes, probably, and I carry an X and Y chromosome, probably.

If we set aside the sex chromosomes, we have 44 other pairs of chromosomes. Those are referred to as autosomes. They're numbered from 1 to 22. They're pairs. They're about 3 billion bases of DNA length. The fact that I have light eyes and you have dark eyes and I'm taller and you're shorter is reflected in variations in our DNA.

If we look across our genomes, though -- right? You and I breathe the same air, can eat the same food. I have O negative blood; I can give you a blood transfusion. Right?

Even though we carry out the same basic metabolic processes, there are subtle differences. Those differences are reflected in our DNA. Single base pair differences characterized across the population are referred to as single-nucleotide polymorphisms. Those single-nucleotide polymorphisms typically refer to variations at fixed positions in

Personalis EX2009.077

DNA that are common across populations.

Although we're increasingly looking at rare variants, so a variant you may carry that no one else or very few other people do, but typically when we talk about SNPs, we're talking about variants that are cataloged and seen at a population frequency of greater than five percent. But, you know, if they're a hundred percent, then the other variant doesn't exist. So somewhere between five and 95 percent.

And we often distinguish those from SNVs, single-nucleotide variants, which one could call a SNP, a difference in DNA, but one that somatically occurs. So it occurs in your, say, tumor cell or in a surrounding tissue, but wasn't inherited from your parents.

Q. Are there SNPs that are in your tumor cell, for example, that really have no impact on the cancer itself?

A. Absolutely. Your tumor cells carry lots of SNPs that don't -- don't have any implication in the development and progression of the disease.

And there are also lots of single-nucleotide variants that exist in your tumor cells that don't have any implication for the disease. Right?

Personalis EX2009.078

And I say that in the context of treating the disease. I should also say but, for example, if you did carry a mutation and BRCA1 -- right? -- that would be a SNP that would potentially have implications for your disease. But there are many others that don't.

Q. It would be possible, though, that I would have a SNP for BRCA1, for example, that somebody else who also carries the BRCA1 gene would also have the same SNP; is that correct?

A. It's sort of the definition of SNP that it has to occur in a certain frequency of population.

Again, we now talk about rare variants. Some people still refer to those as SNPs. A SNP is basically a single base pair change relative to the consensus DNA sequence. The most common DNA sequence.

Q. Let's go to -- back to the article that we were looking at earlier, which is Exhibit 1030 of your binder.

A. Okay.

Q. It's the Mamanova article from earlier.

A. Yes.

Q. And we talked about this earlier, where it describes -- it's a review article that describes these different targeted enrichment methods, and we've

Personalis EX2009.079

talked about PCR, so I'd like to turn next to hybrid capture, which I think is on Page 115 of the article. Do you see that?

A. Yes.

Q. Okay.

And you've talked about hybrid capture in your declaration, correct?

A. Yes, I have.

Q. And we talked about how baits-based hybrid capture is, you said, I think, one type of solution-phase hybridization; is that correct?

A. I may have said that. It's -- I may have said that, so I'll take your word for it.

Q. Okay.

And it's true that baits-based hybrid capture was first reported in a publication called Gnirke in 2009; is that correct?

A. I don't know where it was first reported. I actually cited to a Gnirke paper that describes it, but bait-based hybridization is, in fact, a variation on methods that have been used for a very long period of time. So, you know, it really grows out of Southern blotting from the -- I believe the 1970s. Right? All these hybridization-based assays are variations on each other.

Personalis EX2009.080

So, you know, I don't know exactly when bait-based hybridization was first reported.

Q. Can you turn to the very back page of the Mamanova article that you're looking at.

A. Sure.

Q. If you go to the column on the right, on the bottom, No. 40.

A. Okay.

Q. And do you see that that's a citation to Gnirke A., et al., "Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing." That is in Nature Biotechnology from 2009. Do you see that?

A. Yes, I do.

Q. And then do you see the note below it that says, "This paper was the first description of the method, now commercialized by Agilent, for solution-phase hybridization-based capture using complex libraries of RNA 'bait' to capture from a shotgun DNA 'pond' library"?

A. I see where it says that, yes.

Q. So the term "bait," according to the Mamanova review article, originated from the Gnirke paper; is that correct?

MR. ROSATO: Objection. Form,

Personalis EX2009.081

foundation.

THE WITNESS: So if you read this -- right? -- it says, "This paper was the first description of the method, now commercialized by Agilent, for solution-phase hybridization-based capture" -- right? -- et cetera.

So this is the first description of what is now the Agilent -- or what became the Agilent SureSelect product. So it doesn't say that the word "bait" was, in fact, first used in this Gnirke paper, and, again, I don't know where "bait" first was potentially used.

"Bait" has been used in the context of solid-phase hybridization, and I'm sure it was used for other liquid-phase hybridizations as well.

So what you're referring me to here is really the first description of what's Agilent SureSelect, a commercial project.

Q. BY MS. WANG: So you said, "I'm sure it was used for other liquid-phase hybridizations as well." Are you aware of any other disclosures of solution-phase hybridizations using the term "bait" before Gnirke 2009?

A. Off the top of my head, no. Again, I'd be happy to do a PubMed search and see what I can

Personalis EX2009.082

find.

Q. Sitting here today, you don't recall a paper before 2009 that mentions bait in the context of solution --

MR. ROSATO: Objection. Form.

Q. BY MS. WANG: -- phase hybridization?

THE WITNESS: I don't recall a specific paper. As I said, the term "bait" has long been used as a term in the art for designing -- for an oligonucleotide that's designed to capture a particular DNA fragment. Right?

PCR primers you can think of as baits, because they're designed to be specific to a target DNA that you want to capture.

So, you know, when people first used the term "bait," I'm not sure, but I can tell you it's a term that's long been used in the art. And I can tell you that I heard the term "bait" long before the Gnirke paper in 2009 or 2008. 2009.

Q. BY MS. WANG: Are you aware of any other paper before Gnirke 2009 that referred to the term "bait" in the context of solution-phase hybridization?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, off the top of my head, I can't point to a specific paper. I'd be happy

Personalis EX2009.083

to search for one, but, you know, the term "bait," again, has been widely used in context of hybridization assays.

And one, you know, working in the field would understand immediately what the bait would be without having to read this paper.

Q. BY MS. WANG: Can you point to any other paper or any publication or any disclosure in the art before 2009 that refers to solution-phase hybridization using the term "bait"?

MR. ROSATO: Objection. Form. Asked and answered a couple times now.

THE WITNESS: Any particular paper which talked about solution-phase hybridization, and there are papers that go back to probably the 1970s talking about solution-phase hybridization, may have described the term -- or may have used the term "bait." I do not recall a specific paper.

Again, I'd be happy to search and try to find one. I'm sure they exist.

Q. BY MS. WANG: And you didn't cite any of those papers going back to 1970 that may or may not have used the term "bait" relating to solution-phase hybridization in your declaration, correct?

A. In my declaration I tried to cite papers

Personalis EX2009.084

that were published in the 2009-2010 time frame.

But, again, molecular biology is a process of constant optimization and building on previous technologies, to perfect our assays and make them better.

Q. So you didn't cite to any paper before 2009 referring to "bait" -- specifically the word "bait" in the context of solution-phase hybridization; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I cannot recall whether or not any of the other papers used the term "bait" that were included in the appendix to my report, which were papers that I cited to. Some of the references in those papers may have used the word "bait."

Again, I'm not clear -- I don't recall at this moment whether or not any of those papers might have referred to baits. But "bait" is a term that was commonly used in the art to talk about an oligonucleotide which was designed to a specific target sequence.

Q. BY MS. WANG: When you say "commonly used in the art," are you referring to before 2009?

A. It's a -- it's a term that someone in 2008, 2009, probably 2006, 2005 -- I can't tell you

Personalis EX2009.085

how far back someone would know what that term meant immediately. But I can tell you in 2009 it was a common -- commonly used term.

Q. Let's turn to -- let's turn to Exhibit 1023, which is in your binder.

A. Sure.

Q. And that is, I believe, the Gnirke paper; is that correct?

A. It is a paper by Gnirke, and it's one of those that I cited in my report.

Q. Had you read this paper in 2009, when it was published?

A. I don't recall. I probably read it, but I wouldn't say with certainty that I read it in 2009.

Q. And if you look at the abstract of this paper, which is on the first page, in bold, the second sentence reads, "We developed a capture method that used biotinylated RNA 'baits' to fish targets out of a 'pond' of DNA fragments."

Do you see that?

A. I see where it says that, yes.

Q. Now, this Gnirke paper, when they're talking about fishing targets out of a pond, do you understand that pond to refer to a library?

A. I'd have to read this paper. That pond

Personalis EX2009.086

may be a library. That pond may be fragmented genomic DNA. Again, "library" is a term which is often used in many different contexts, so --

I'd have to actually read more of the paper in context to refresh my memory of what they were referring to as a pond of DNA fragments.

Q. Okay.

Let's go -- let's take a step back first. What is your understanding of solution-phase hybridization?

A. Again, there are many different applications and instantiations of solution-phase hybridization. A typical use of solution-phase hybridization is -- involves the design of oligonucleotides that are targeted to one or more genomic regions, sometimes independent genomic regions, sometimes overlapping genomic regions, and typically what one does is one designs an oligonucleotide primer, which can be DNA or RNA or PNA or LNA, anything which has nucleotides which can be complementary to a DNA or RNA fragment that one wishes to capture. One adds that typically in solution.

Those primers are typically modified with some kind of group, that can be then used to fish out the hybridized complexes from solution.

Personalis EX2009.087

So in a standard application, like the commercial Agilent SureSelect technology, primers have -- or oligonucleotides have a biotinylated modification, so it's a particular molecule biotin added to one end, and that biotin can then bind to magnetic beads, which are coded with another molecule called streptavidin. Right?

So in that context what one does is one allows a hybridization to occur and then pulls the captured targets out of solution. Right?

And, again, there are many different variations on this, but that's one typical example of solution-phase hybridization.

Q. Now, in your opinion, as somebody who's designed millions of primers, as you talked about previously, have you ever designed primers that are attached to streptavidin beads?

A. I have designed primers that have biotin modifications that are eventually captured by a streptavidin substrate.

Q. And in your opinion, as an expert, the term "primer" refers to any oligonucleotide that can be used to hybridize to a particular sequence; is that correct?

MR. ROSATO: Objection. Form,

Personalis EX2009.088

foundation.

THE WITNESS: So you just asked me if I've designed primers that are designed to hybridize. Right?

Primers are a form of oligonucleotide, and the design of oligonucleotides is something I'm very familiar with, and I've done many assays involving the design of oligonucleotides, including primers, and I've designed and ordered and used primers that have -- primers and other oligonucleotides that have a variety of modifications, including biotinylation.

Q. BY MS. WANG: What are other oligonucleotides that are not primers?

A. Primers typically refer to oligonucleotides that are used in PCR, since they initiate the reaction, hence the term primers. They prime the reaction. But if I looked at a DNA fragment, I would not know ahead of time, if I simply had a native strand of DNA, whether it was designed to be used as a primer or for any other type of assay. It could be used for a simple hybridization assay. Right?

So oligonucleotides are the generic term, and primer is a context-specific term, where

Personalis EX2009.089

generally -- again, molecular biology -- biologists are really imprecise in how they use language, but generally used as an initiation oligonucleotide for polymerase chain reaction.

Q. The oligonucleotides that you used in PCR are used in combination with DNA polymerase, correct?

A. Typically, yes.

Q. In the context of solution-phase hybridization, there is no DNA polymerase used; is that correct?

A. Generally at the stage of capture, no, but, again, it depends on the application. Right?

For example, in the paper described -- the paper from Gnirke and his colleagues, I believe that they use the DNAs that they capture in sequencing, and those are amplified through a process called bridge amplification in the Illumina flow cell, which one might characterize as a generalization of PCR, because it does involve a DNA polymerase. Right?

So, again, you know, is solution-phase hybridization in capture -- up to the capture does it typically use polymerase? Not generally.

But, again, there are lots of different variations on lots of different assays.

Q. You could use PCR and polymerase before

Personalis EX2009.090

solution capture, correct?

A. Again, if you want to give me a specific example, we can talk about that. In general, one can do many things before one does solution-phase hybridization.

Q. But you could use PCR to amplify your pool that you then are applying your baits to, for example; is that correct?

A. Again, there are lots of different variations on one -- on what one might do. I could design an assay where I did PCR and use biotinylated probes and then capture those from the resulting solution. I could design an assay where I did PCR and then designed additional oligonucleotides that were biotinylated that I could use to fish things out of solution.

So, you know, this idea of fishing things out of solution is widely used, and, again, I wouldn't begin to comment on how many possible iterations and permutations of techniques and technologies people have dreamed up.

Q. But it's also possible -- you -- I think you said it's possible to use PCR before you do the actual hybridization capture. It's also possible to do it after, to amplify your results; is that correct?

A. Again, in molecular biology there are lots of different ways to combine and optimize techniques, depending on your goals.

Q. But that is one way that you could do it; is that correct?

A. It's one of many ways and many different things that one could possibly do.

MS. WANG: Let's take a quick break.

THE VIDEOGRAPHER: Here marks the end of Media Unit No. 1 in the deposition of John Quackenbush. We are going off the record, and the time is 11:12 a.m.

(Short recess.)

THE VIDEOGRAPHER: Here marks the beginning of Media Unit No. 2 in the deposition of John Quackenbush. We are back on the record, and the time is 11:30 a.m.

Q. BY MS. WANG: Dr. Quackenbush, if you could turn back to your declaration.

A. Sure.

Q. And please turn to Page 12, Paragraph 30.

A. All right.

Q. Are you there?

A. Yes.

Q. Great.

Personalis EX2009.092

A. Opened right there.

Q. Excellent.

Do you see that you have a figure that's in your declaration in Paragraph 30?

A. Yes.

Q. And in the paragraph you state, "Figure 1 (shown here) is of the Gnirke published application." Do you see that?

A. Yes.

Q. Let's look at Figure 1.

A. Okay.

Q. Now, does this describe the solution-phase hybridization that we were talking about earlier?

A. Well, if you don't mind, I'd like to look at the publication to understand exactly where this came from.

Q. I think before we get there, I just want to understand sort of the general principles here a little bit. And, again, this Figure 1 that's included in your declaration, I'm asking is it generally describing what you talked about earlier with respect to solution-phase hybridization?

A. Again, I'd like to look at the document to make sure I don't mischaracterize what's there.

Personalis EX2009.093

Q. The document being your declaration Figure 1 or --

A. No. If you recall, this figure as noted here was taken from the Gnirke published patent application, which is Exhibit 1024.

Q. Okay. So you're turning to Exhibit 1024; is that correct?

A. Yes.

Q. Okay.

Counsel, for you.

MR. ROSATO: Thank you.

THE WITNESS: And, in fact, I'm looking at Exhibit 1024, Column 5, the first paragraph. I think it's Paragraph -- or first column -- Page 5, first column. I think it's Paragraph 68. And there's a figure caption.

Q. BY MS. WANG: Correct. And that figure caption states that, "Figure 1 schematically shows an exemplary selection process of an embodiment of the invention," correct?

A. That's what the first sentence says, yes.

Q. And then it follows that, "As shown, bait sequences are hybridized in solution with a group of nucleic acids (the 'pond)," correct?

A. Yes.

Personalis EX2009.094

Q. And then it says that, "The hybridized sequences are then captured using a moiety linked to or incorporated in the bait sequences."

Do you see that?

A. Yes.

Q. And then it follows that, "The hybrid-selected targets represent a subgroup of the starting group of sequences ('pond'), and referred to here as the 'catch'."

Do you see that?

A. Yes.

Q. Okay.

And then it says that, "This subgroup of sequences can then be subjected to sequencing."

Is that correct?

A. Yes.

Q. Okay.

Let's turn back to that figure now.

A. Okay.

Q. Now, according to this figure, the first section has two boxes, one of which says "Bait" in quotes and the other one which says "Pond" in quotes. Do you see that?

A. Yes.

Q. And the "Bait" in quotes states that it's

Personalis EX2009.095

made from a pool of synthetic oligonucleotides, correct?

A. Correct.

Q. And the "Pond" says that it's genomic DNA fragments with universal adapters; is that correct?

A. Yes.

Q. Now, the second, I guess, column of pictures is labeled "Hybridization in solution." Do you see that?

A. Yes.

Q. And what is being depicted here?

A. So the caption doesn't provide all the details and information so I can speculate on what's happening here. So it appears that a library was constructed that has universal adapters, and if we look at the right-hand column, this is eventually going to sequencing, so my assumption is that these universal adapters are, in fact, the Illumina sequencing primers. So these are synthetic pieces of DNA.

So, again, I'm assuming that what's happened here is that genomic DNA was purified from some sort of sample, that it was fragmented and prepared for Illumina sequencing.

So somehow a sequencing library was

Personalis EX2009.096

prepared, and then it appears, since it looks like these double-stranded -- what I would guess are double-stranded molecules in the pond -- it would appear that these were gently heated to denature the DNA, and then probably cooled, to allow what looks like these bait molecules to bind. And so this second column appears to show an example of a type of hybridization in solution in which the bait molecules are binding to single-stranded elements of the pond of sequencing library fragments.

Q. And do you see that on these bait -- the depiction of the bait molecules, that there's a little dot that's attached to them? Do you see that?

A. Yes.

Q. And is that the biotinylated, that you were speaking of, portion of that bait?

A. So I don't know -- again, I'd have to read this patent application in context.

Biotin is an example of one of the -- if we refer back to the figure legend, one of the moieties that one could use. One could also use, for example, a protein that was conjugated to an enzyme. You could use a variety of different things.

So biotin is an example of a moiety linked to or incorporated in the bait sequences.

Personalis EX2009.097

So the dot appears to indicate that this moiety, whatever it is, potentially biotin, is somewhere in the middle of these baits. And so I would assume that the -- the dots represent those moieties, one of which could be biotin.

Q. And in the third column of this figure it's titled "Hybrid capture." Do you see that?

A. Yes, I do.

Q. And it appears to depict a large circle, which has prongs sticking out of it, and two of these bait and also pond DNA kind of hybrids attached to that circle. Do you see that?

A. I see that figure, yes.

Q. And what does that depict?

A. So, again, this is just my interpretation based on a quick read, but this would appear to be some kind of solid support, and my guess would be that is a streptavidin-coated bead, but it has some kind of moiety which will capture the moieties on the -- the baits.

So what this is depicting is a molecule which -- sorry -- a solid which has some kind of capture elements attached to it that can bind to these moieties and pull them out of solution. And so it would appear that this bead is capturing both a number

Personalis EX2009.098

of these baits, the oligonucleotides with the additional moieties, and some hybrid complexes, where the baits have hybridized to part of this pond, the library.

Q. And the way that they're capturing, if it's streptavidin coated, these are magnetic beads; is that correct?

A. So the beads are magnetic, but it's not magnetism that's capturing it. Right? It's an interaction between biotin and streptavidin.

Again, I'm making assumptions, but one example which would work would be to include biotin and streptavidin, and then to use a magnetic bead.

The reason you use the magnetic bead is that you'd want to fish these beads out of solution -- carrying on the pond and bait language, you want to fish these out of solution so you apply a magnet, you'd pour off the solution, you'd wash it, trusting that the bio -- the streptavidin, for example, on the beads would strongly bind the biotin and allow you to pull these attached fragments out of solution.

Q. So the principle is essentially once you have this capture, you wash away everything else and you trust that the relationship between the streptavidin and the biotin would hold onto your bait

Personalis EX2009.099

DNA hybrid molecule; is that correct?

A. Well, so -- you know, I'm not sure how "trust" is being used, but the reason why you would run this assay or use these magnetic beads is so you could pull what is ever hybridized to the baits, specifically or nonspecifically. Right?

And, again, all of that is going to depend on conditions.

Q. Sure.

A. But to pull whatever has been attached to these baits out of solution.

Q. While washing away everything else; is that correct?

A. One would typically wash away everything else. That's why you'd use a magnetic bead.

Q. So the end result of this particular process is that you have in this last column what is in quotes here a catch and enriched library; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So the figure says that what comes out of this -- again, I'm assuming after washing is that you have the catch, which is an enriched library, which would contain anything which had either bound to the bait oligonucleotides or had

Personalis EX2009.100

nonspecifically been attached to other things and pulled out of solution.

Q. BY MS. WANG: This process doesn't make copies of any of the genomic DNA that was originally in the pool, does it?

A. At this step, no. There's no copying which is shown in this figure.

Again, I don't know exactly what they're going to describe in entirety in referring to this figure, but the figure itself does not appear to have any copies of the later steps. There may have been copies made before. Right?

And the creation of the pond of genomic DNA fragments, those universal adapters could have easily been applied -- or been attached using PCR.

Q. But the actual streptavidin beads that are pulling down the biotinylated baits with their targets -- their capture targets, there's no copies being made during that process; is that correct?

A. The binding of the baits in the DNA hybrid complexes does not at that stage involve any copying, no.

Q. Now, once you -- I know it's not depicted here, but once you have a catch, there is, at the bottom corner of this figure, just a block that says

Personalis EX2009.101

"Sequence." Do you see that?

A. Yes.

Q. And is that referring to, under your knowledge of this process, when you would actually sequence what you have obtained in your catch?

MR. ROSATO: Objection. Form.

THE WITNESS: So if we look at this, there's "Catch" and there's an arrow that says it goes to "Sequence." So there's not a description of what that arrow actually represents, but what I would assume is that there are a variety of different purification and other processes that are carried out to then prepare this and move it into DNA sequencing, which itself is a multistep enzymatic process.

Q. BY MS. WANG: Okay.

So you're familiar with next-generation sequencing, correct?

A. Yes, we have had multiple sequencing -- sequencers and sequencing technologies in our lab.

Q. And Illumina is one of them; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: We have had Illumina sequencers, we've had Ion Torrent sequencers, I've worked with Applied Biosystems sequencers over the

Personalis EX2009.102

years. I'm familiar with many different sequencing technologies.

Q. BY MS. WANG: And you've mentioned that next-generation sequencing also involves a series of steps; is that correct?

A. Any process of molecular biology involves a large series of steps.

Q. Including next-generation sequencing?

A. Including sequencing.

Q. Okay.

Can you describe for me at a broad level what the steps of next-generation sequencing are?

A. Sure. So, again, next-generation sequencing incorporates or encompasses many different technologies. If you'd like, I can limit that to Illumina sequencing, since --

Q. That would be preferable yes. Thank you.

A. Good.

So Illumina uses a process referred to as sequencing by synthesis. Okay?

It was a technology first developed by a company called Solexa, which Illumina purchased.

But what one does is one first prepares a library -- and there are many different steps involved in preparing that library -- right? -- which can

Personalis EX2009.103

include DNA fragmentation, PCR, a whole bunch of different assays that one can use to attach priming sequences for DNA, these -- what people often call universal adapters, to DNA fragments.

Q. And Figure 1 that we were just looking at is a depiction of potentially one way of acquiring a library for sequencing; is that correct?

A. Figure 1 is one way that you could use to create a sequencing library.

Q. Okay.

A. Yes.

One -- even if we practiced Figure 1, if we took this through and knew all the steps involved, we may do additional things between when we capture the DNA fragments and when we put it on the sequencer.

And, again, "library" is often used in a kind of sloppy way. For the context of what I'm going to describe right now, the library is what I'm going to physically add to a device called the flow cell, which is then going to be put on the sequencer. Right?

So the library is typically added in a relatively low concentration. So, again, low is a relative term and it's something you have to optimize, but it's added in a concentration where adapting --

Personalis EX2009.104

adapter sites can flow through the flow cell and bind to synthetic oligonucleotides. Right? Some might refer to it as probes or baits, whatever, that capture elements of that library.

And the reason you use a low concentration is you want them spatially distributed. You don't want them immediately adjacent to each other. And the reason is if you ultimately want to detect DNA -- right? -- you want to determine the DNA sequence, you want these individual fragments spatially separated so that they can be resolved. Because the Illumina sequencer uses a confocal microscope, essentially, and fluorescence to detect the addition of individual bases. Right?

So we flow the DNA in at relatively low concentrations.

There are synthetic oligonucleotides which bind these DNA molecules in the library because they're complementary to the adapter sequences.

One then runs a process known as bridge amplification. So it is similar to PCR. I don't believe it uses a thermostable polymerase, which is most of what PCR does, so DNA molecules are copied, they actually have two oligonucleotide adapters, and the process is designed so that they can bend over and

Personalis EX2009.105

attach at both ends, be copied, heated, stand up, as they denature, and then the process repeats many times. So it's called cluster generation.

And now, instead of distributed molecules on the surface, we create little clusters of molecules that are similar to each other. All right?

At that step one then typically takes it to DNA sequencing. So reagents are added -- that's a DNA polymerase -- and oligonucleotides that are fluorescently labeled. The fluorescent labels have -- are cleavable, though, so they can later be cut off.

So we'll flow in DNA polymerase and, for example, first add the A nucleotide. The A nucleotide is complementary to the next base adjacent to the adapter that will be added. If not, hopefully it won't be added. But the -- if there's a T, an A base will be added. Light will be shown on that to excite the fluorescence, and an A will be detected at a certain site.

One then adds the next base. Let's say it's T. The T will flow in and will add to sites where there's an A on the complementary strand, and the process will repeat through each base for one cycle, and then through this set of four bases will be repeated many, many cycles.

Personalis EX2009.106

When Illumina sequencing was first introduced, it was that kind of single base -- we refer to it as a single-color assay, and typical read lengths were 30 to 50 nucleotides. Through process of continuous optimization, the read lengths are now 100, 200 -- 150, 200 bases in length. And I've heard reports of people getting even longer fragments.

But it's a process of adding nucleotides, allowing a polymerase to extend and bind them, and then detection of that, and then all of the data flows into a computer, which, again, does multiple levels of processing.

Modern Illumina instruments use a two-color detection scheme, but the overall process is essentially the same.

Q. And you would agree that the preparation of the library that you're actually using for sequencing is fairly important in terms of what types of reads you'll get at the end; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Everything you do in the entire process is important. Right?

So, you know -- bad reagents in, bad data out; bad processes in the lab, bad data out.

Q. BY MS. WANG: Does it matter what you put

Personalis EX2009.107

into your library?

A. I'm not sure what you're asking, but obviously, yes. You know, if I want to sequence fish DNA, I'm going to put fish DNA in. If I want to sequence malarial parasite DNA, I'm going to put in malarial parasite DNA. If I want to -- if I want to sequence the RNA population, I'm going to put in probably complementary DNA. So . . .

Q. Let's go to your declaration again.

A. Sure.

Q. Let's go to Paragraph 36.

A. All right.

Q. Now -- are you there?

A. Yes.

Q. Okay.

So in Paragraph 36, that's actually under the subheading B, which in your declaration you state is "Bait Design." Do you see that?

A. Yes.

Q. And in Paragraph 36 you state that "those of ordinary skill in the art knew that certain bait parameters influenced hybridization and sequence coverage."

Do you see that?

A. Yes.

Q. And then further down you state, "Thus, those of ordinary skill in the art knew, and the prior art described, how baits could be designed in advance to account for target abundance, hybridization and the desired depth of sequence coverage."

Do you see that?

A. Yes.

Q. Okay.

Now, sequence coverage, as you described earlier, what does that refer to?

MR. ROSATO: Objection. Form.

THE WITNESS: So I don't think I talked about sequence coverage earlier.

Q. BY MS. WANG: Sorry. I meant in Paragraph 36.

A. Yeah.

So here sequence coverage is the number of sequence reads that one would like to get on average for individual bases. Right? Or for individual regions of DNA.

Q. After sequencing, for example, via the Illumina process; is that correct?

A. The sequence coverage would be what was eventually reported after the DNA sequencing process. So the raw data comes out of the sequencer, it's

Personalis EX2009.109

processed. Again, there are a lot of steps involved in processing that, but the simplest way to understand sequence coverage is that for a particular base, which has been mapped to a region in the genome, the coverage is the number of times that base is read.

Q. And when you say at the end of this paragraph "the desired depth of sequence coverage," that's referring to the average number of sequencing reads for that particular sequence; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: At the -- you mean in the middle of the paragraph?

Q. BY MS. WANG: It's at the bottom of Page 16, into the top of Page 17.

A. Okay.

Q. "Desired depth of sequence coverage."

A. All right. So "those of ordinary skill in the art knew . . . described, how baits could be designed" --

Sorry.

I'll repeat that. "Those of ordinary skill in the art knew, and the prior art described, how baits could be designed in advance to account for target abundance, hybridization and desired depth of sequence coverage," yes.

Personalis EX2009.110

Q. So my question is: "Desired depth of sequence coverage" refers to the desired number of sequence reads generated after sequencing of that particular target; is that correct?

A. Right. The approximate number of sequence reads that you would like to obtain.

Remember, sequencing, like any reaction, any measurement, is a stochastic process, so there's always some variability, but typically one designs an assay with a desired sequence coverage on average in mind.

Q. And just so I have the terminology right, a greater depth of sequence coverage means you have more reads; is that correct?

A. Greater depth would mean more sequence reads.

Q. Okay.

Now, you also say "to account for target abundance." What do you mean by that?

A. Well, so we talk about target abundance. Right? Some targets may be very common, some targets may be very rare, and so, generally speaking, target abundance is the relative representation of a target in the input to your DNA sequencing reaction.

Q. So it's just the amount -- relative

amount of that particular target in your sample; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Generally speaking, yes. Again, the relative amount can vary depending on how you define those targets.

Q. BY MS. WANG: Are there particular sequence coverages that when you're designing baits you would target?

A. It would depend on the assay and the goal of the experiment.

Q. Have you yourself ever designed baits?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So I don't know exactly what you mean by have I designed them. I've used software tools that are available online to design what you could refer to as baits, for targeted resequencing of DNA.

Q. BY MS. WANG: Have you personally designed baits for use in solution-phase hybridization?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: I have designed

Personalis EX2009.112

oligonucleotides that have been used in solution-phase hybridization.

Again, we'd have to precisely define what we mean by "baits" to understand what that means, but, yes, I've designed oligonucleotides that have been used in a wide variety of hybridization assays.

Q. BY MS. WANG: So I just want to make sure I understand this, because in Paragraph 36 you say, "those of ordinary skill in the art knew that certain bait parameters influenced hybridization and sequence coverage." Do you see that?

A. Yes.

Q. And so there, where it's talking about bait parameters, do you understand that to refer to baits that are used in solution-phase hybridization?

A. So --

I just want to read this.

All right. So, yes, that if one were to use the term "bait" to refer to solution hybridization, there are a variety of parameters that influence hybridization, efficiency and sequence coverage.

Q. And have you personally designed baits with reference to solution hybridization in contemplating these parameters?

Personalis EX2009.113

A. There are no parameters listed here, but, again, depending on the application, there are a wide variety of different parameters one would include, and, yes, I've designed baits -- what one could refer to as baits reflecting a desire to obtain a certain level of sequence coverage.

The -- again, you have to think about these not in isolation but in the context of the entire experiment you want to do.

So one could increase sequence depth simply by running additional lanes in the sequencer which have the same sample. Right?

So you have to think about these in context; you can't just talk about them in isolation.

So given the design of the experiment, what one is hoping to achieve, then, yes, I've designed bait -- what could be referred to as baits, taking into account a wide variety of different parameters.

Q. With respect to the figure that we were looking at earlier from Gnirke, Figure 1, which you included in Paragraph 30 of your declaration --

A. Mm-hmm.

Q. -- and the process that we discussed going through with respect to streptavidin-coated

Personalis EX2009.114

magnetic beads and washing away of other things in the library, have you yourself personally designed baits for use in a process like that?

MR. ROSATO: Objection. Form.

THE WITNESS: So I don't know what exactly you mean by "a process like that." Right? The figure's a little bit vague.

I have designed all the nucleotides that were used --

I have to think.

I think -- I recall designing oligonucleotides -- I don't know if I -- I don't know if I can say that it was used exactly in this process, but in processes which are close enough to this that I feel comfortable talking about the general process being used.

Q. BY MS. WANG: So have you personally designed baits that were used to hybridize to genomic DNA in a pool in solution?

A. So this figure doesn't refer to genomic DNA, it refers to something else. Right? It's hybridizing to a library.

But have I designed what one could refer to as baits to hybridize in solution? Yes.

Q. So I just want to make sure. Figure 1

Personalis EX2009.115

states that the pond is made of genomic DNA fragments. Do you see that?

A. Actually, it says, "Genomic DNA fragments with universal adapters." So it's not just raw DNA that's come out of a cell. Right? This has been manipulated through a variety of different steps.

The figure doesn't -- the figure caption doesn't actually tell us what they did to arrive at this. Right?

It appears that these were derived from genomic DNA, but, again, molecular biologists are very loose in the way they use their language. This could have been the result of PCR. I don't know how they arrived at this pool, but it's clearly not native genomic DNA.

Q. Have you personally designed baits to be applied to a pool of manipulated genomic DNA fragments like this?

A. I can't recall all the different ways of design what you could refer to as baits.

Q. So the answer is you don't recall whether you've ever designed baits that have been applied to a pool of genomic DNA fragments like shown in Figure 1?

MR. ROSATO: Objection. Form.

THE WITNESS: To be honest, you know,

I've designed lots of oligonucleotides for lots of different situations that hybridize to lots of different molecules. Can I say I've designed them exactly the way that this figure allegedly depicts it? No.

And I'd be hesitant to say that because I can't recall all the different ways we've designed oligonucleotides.

Processes like this, sure. This exact process, I don't know.

Q. BY MS. WANG: Have you ever run a solution-phase hybridization as shown in Figure 1?

A. I have not -- I don't know exactly what Figure 1 is depicting, so no.

Q. You included Figure 1 in your declaration, correct, Dr. Quackenbush?

A. Yes.

Q. And you included Figure 1 in your declaration to show in the paragraph describing solution-phase hybridization, correct?

A. Yes.

Q. But you don't know exactly what Figure 1 is depicting?

A. Figure 1 is showing a schematic with a general overview of how Gnirke describes

Personalis EX2009.117

solution-phase hybridization in the patent application. So I understand the basic principles, I understand how this assay operates.

Can I tell you everything that he's done? No.

And so I wouldn't be honest if I said, "Oh, I understand every aspect of this."

Can I discuss the general principles? Absolutely.

Q. Have you yourself personally designed baits to be used in a process like this applying those general principles?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: So I don't know what you mean by "like this."

Have I run what's depicted in this assay? I would say not. But I understand the principles well enough to discuss them with you.

Q. BY MS. WANG: Okay.

A. The figure itself does not provide a great deal of clarity about all of the steps that were run and everything that's involved in this process. So the question, in fact, is not one that I think anyone could answer except maybe Dr. Gnirke.

Q. You've read the Gnirke patent application?
A. I read it a long time ago, yes.
Q. And you've read the Gnirke paper?
A. I've read that a while ago too.
Q. Okay.
Let's go back to the Gnirke paper.
A. The paper?
Q. Yes.
A. Or the patent application?
Q. We're going to go to the paper first, please.
A. Okay. So --
Q. And that is Exhibit 1023.
A. All right.
Q. And going back to the -- let's go to Page 183 of Gnirke.
A. Sorry. 183?
Q. Yup.
A. Okay.
Q. Okay.
And do you see there's a Figure 1 on Page 183 as well?
A. Yes.
Q. And Figure 1 -- the caption of Figure 1

Personalis EX2009.119

states, "Overview of hybrid selection method. Illustrated are steps involved in the preparation of a complex pool of biotinylated RNA capture probes (bait; top left), whole-genome fragment input library (pond; top right) and hybrid-selected enriched output library (catch; bottom)."

Do you see that?

A. Yes.

Q. Okay.

And so this is Gnirke describing a pool of biotinylated RNA capture probes. Do you see that?

A. Sorry. Where is that?

Q. The language says "complex pool of biotinylated RNA capture probes (bait)." Do you see that?

A. Yes.

Q. Have you yourself created or designed biotinylated RNA capture probes?

A. No, I haven't.

Q. You see the next part says it's a "whole-genome fragment input library (pond; top right)." Do you see that?

A. Yes.

Q. Have you yourself created a whole-genome fragment input library pond?

A. So have I exactly carried out Gnirke's protocol? Right? Which involves shearing, adapter ligation, and PCR (optional)?

I'd have to read the methods section, and I'm not sure it's included -- a lot of the methods can be supplementary.

So have I done exactly what Gnirke describes here? I'd say it is -- it may be unlikely that I did that. I don't know what his genomic DNA input is, I don't know what all the parameters are.

Have I designed -- have I prepared libraries for sequencing? Yes.

Q. And do you see in the picture it takes the arrows down to the center, which says "Solution hybridization." Do you see that?

A. Yes.

Q. And have you designed baits that were used in solution hybridization as depicted in this figure?

A. So if we read the figure caption, the baits are biotinylated RNA capture probes, and I can honestly say that I have not designed RNA capture probes.

Q. And then if you go down to "Bead capture," and there's a depiction of -- similar to the

Personalis EX2009.121

other Figure 1 we saw, of what I believe is a streptavidin bead. Do you see that?

A. I see the figure, yes.

Q. And do you see that the figure demonstrates capture using some form of moiety -- I realize it's not depicted, but some form of moiety that is linked to the bait molecule in the figure?

MR. ROSATO: Objection. Form.

THE WITNESS: So, again, the figure caption tells us that they're biotin moieties attached to -- well, they simply say biotinylated; they don't use the word "moiety." And they show what appears to be a solid support capturing the biotin, so I'm assuming it's a streptavidin-coated bead capturing the results of this solution hybridization.

Q. BY MS. WANG: Okay.

And have you ever performed an experiment that uses a streptavidin bead or something similar like that to capture a biotinylated capture probe?

A. I've used -- I've used streptavidin beads to capture things that are the result of solution-phase reactions, so in that general sense, yes.

Have I done exactly what Gnirke describes here? No, I haven't.

Personalis EX2009.122

Q. Okay.

If you go to Page 185 of Gnirke.

A. Sure.

Q. In the left-hand column the heading says "Evenness of coverage." Do you see that?

A. Yes.

Q. And the sentence right underneath says, "Uniformity of capture, along with specificity, is the main determinant for the efficiency and practical utility of any bulk enrichment method for targeted sequencing."

Do you see that?

A. I see that, yes.

Q. What is meant by "evenness of coverage"?

A. So in this assay, what Gnirke is describe -- in fact, in this paper what Gnirke is describing is a process that they developed to assume -- or to generate relatively uniform coverage. Right?

What that represents -- or what that requires is that one optimize the reaction -- right? -- every step in this reaction, to try to do even capture and uniform sequencing of a wide variety of fragments.

So I don't recall the exact applications

Personalis EX2009.123

that Gnirke's looking at, but if you think about the optimization problem -- right? If I were to try to capture elements -- if I just had one gene and I want to sequence that gene, and I wanted to capture elements, what I would do is I'd start off by starting my assay and I'd look at the relative sequence coverage. And I discovered that there were some elements that were covered at a lower depth, and I'd want to raise those, or I discovered elements were covered at a higher depth, and I'd want to lower those.

So molecular biology is a process of ongoing optimization. Right?

You turn some knobs. And some of those knobs are knobs which are involved in optimizing things like hybridization, optimizing the -- the reaction conditions. Right?

So if we look at the paragraph -- or the column adjacent to the one that you talk about, they give one example, which is the effects of base composition.

In fact, I could go to something like the Agilent SureSelect website and design a set of baits -- right? -- to use the language we're using here, that would capture a region of DNA that I'd want

Personalis EX2009.124

to sequence, and I could adjust the parameters so that I could either make the coverage very uniform or make the coverage not uniform. Right? Because we're dialing up and down different regions.

And so in the application that Gnirke has here, his goal apparently is to get uniform coverage. That may not be the case in other assays.

Q. Why, according to Gnirke, is uniform coverage important?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: Again, I don't know exactly what Gnirke was trying to do here. In discovery, one might want to have uniform coverage. Right?

Kind of clinical assays we talked about, one might not want to have uniform coverage. One might want to estimate -- or one might want to emphasize particular regions.

And so it was well known in the art how to turn those dials in molecular biology to increase or decrease coverage, and you could do that by changing GC composition, you could do it by changing the length of the oligos. If you had oligos which were tiling over a particular region, you could change how much they overlap. You could change even the

Personalis EX2009.125

relative concentrations of a large number of, to use the term baits, oligonucleotides in solution -- right? -- to either dial up or dial down the coverage.

Gnirke's motivation for getting universal coverage is something I'm not sure of, but his goal was obviously uniform coverage, and what he describes here, if I recall correctly, is an approach to try to even out the coverage over genomic regions.

Q. BY MS. WANG: You mentioned that one reason why you would want uniform coverage is discovery. What did you mean by that?

A. So let's imagine that I had a child with a rare disease, and I know that that rare disease, by looking at his family and looking at patterns of inheritance, has a genetic component to it. Right? So I'd want to try to understand which genes might be mutated. Okay?

So if I were to do that, with no preconceived idea of what might be important and what might not, I could conceive of designing an assay where I'd want to uniformly cover all of the genes, to make sure I didn't miss something. Right?

So there are lots of other examples, but that's one very nice example, where you want the coverage to be uniform because you don't have any

preconceived idea of where that mutation might want to be, so you want to look evenly across the gene.

Q. And I think you mentioned two factors that could be used to create uniform coverage. One I think you said was GC content and the other one was tiling. How does tiling create uniform coverage?

A. So they're two of many factors. Right?

Q. Sure.

A. They include GC coverage, tiling, concentrations, length, melting temperature, secondary structure. But if we are attempting to capture a large region of DNA that, for example, contains a gene that, for example, may contain mutations, splice mutations, lots of different variants -- right? -- that we might want to look for, one could design capture probes that are very far apart.

So if I only design two, to capture each end of the genomic region, I could imagine that the fragments break rapidly, so I'd miss some of the stuff in the middle. Right?

I could design a very dense coverage. I might want to adjust the density of coverage to be higher at the ends and lower in the middle. Right?

It's an empirical process often to try to tune the parameters to capture lots of different

Personalis EX2009.127

fragments of DNA, many of which might be incomplete fragments of the region that I'm trying to analyze.

Q. But in terms of achieving a uniform coverage, how does tiling help?

A. Again, if I designed two baits that didn't overlap, that sat near the end of a region I wanted to target, the coverage in those areas that I captured would generally be higher than areas in the middle where I don't have baits. Right?

And I can adjust the capture by adjusting how those baits overlap, because each -- you know, DNA fragments break in different places.

Q. So to ensure that you have uniform coverage if you're looking at that large of a fragment, you would want to tile closely together, in order to make sure you didn't lose any of those fragments; is that right?

A. Again, it depends on the exact assay you want to run. One potential way to change coverage, either to increase or decrease coverage, would be to change the tiling frequency.

In fact, I'd probably want to adjust the frequency so I tiled more deeply at the ends than in the middle, but I'd want to run an assay first to see whether or not, for my particular DNA fragment that I

want to understand, I'm making the appropriate changes. So I'd probably run a series of assays, different concentrations, different tilings, to try to optimize it. Right?

Microbiology is all about optimization.

Q. But in your experience and based on what you just said, if you were trying to achieve uniform coverage across your entire sequence, you would expect to want to tile pretty closely together as opposed to on the ends; is that correct?

A. It would depend on the application.

Q. So you don't have an answer as to whether you would expect to tile closely or farther apart?

MR. ROSATO: Objection. Form.

THE WITNESS: It would depend on the application. It would depend on exactly what I wanted to do, it would depend on the parameters, it would depend on the GC contract. Right?

There are a whole host of parameters that go into this.

I might simply change it by changing the concentration of oligonucleotides. Right?

It's -- it's an optimization process that one goes through, and there's no single parameter that is always the right answer in every -- in every

Personalis EX2009.129

potential situation.

Q. BY MS. WANG: So you have no opinion as to whether tiling can, in fact, create uniform coverage; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: As I stated earlier, tiling is one of the parameters that can be used to either increase or decrease coverage, and changing the pattern of tiling can lead to uniform or nonuniform coverage, depending on the goals of your experiment and depending on other conditions.

Q. BY MS. WANG: The pattern of tiling can lead to uniform coverage how?

MR. ROSATO: Objection. Form.

THE WITNESS: Depends on the exact assay. One way one might change coverage to make it either more uniform or less uniform is to increase the density of tiling oligonucleotides and their overlaps in particular regions or to decrease it.

Again, there are lots of dials that we can turn, and exactly how we turn those dials is going to depend -- which dials we turn and how we turn them is going to depend on the goal of our experiment.

Gnirke talks about optimizing an assay to achieve uniform coverage. Anyone will read this and

Personalis EX2009.130

understand that you could optimize the assay to get nonuniform coverage. Right?

There are dials we turn, there are parameters we can change. It's the root of molecular biology. It's optimization.

Q. BY MS. WANG: So I, again, want to go back. You just said that one could increase the uniformity of coverage or decrease the uniformity of coverage by increasing the density of tiling oligonucleotides and their overlaps or to decrease it. So increasing the density of tiling oligonucleotides leads to more uniformity and decreasing the density of tiling oligonucleotides leads to less uniformity; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: That's not what I said at all.

Q. BY MS. WANG: Okay.

A. Depend -- so I'm going to run an experiment and I am going to start off, and probably the natural way I would start, if I knew nothing -- right? If I had a big region I wanted to sequence, I might, for example, start with uniform tiling across a region. Right?

What do I mean by uniform? Well, in some

Personalis EX2009.131

ways it's going to be even. Is it more -- is there greater overlap or lesser overlap?

I don't know. It depends on how I start, what I want to do, the parameters of the experiment I want and what I want to get out of it.

So I'm going to start with some initial tiling. Right? And then what I could potentially do if I wanted to increase a region, a natural assumption would be is I could -- if I had low coverage in a region, a natural assumption would be that I would increase the tiling density. Right?

If we look at the extreme example I gave you earlier. If I had only two oligos across a 10,000 base pair window I wanted to sequence, the coverage in the middle would probably be close to zero. So I'd want to increase the density in the middle to increase the coverage. Right?

On the other hand, if I had a uniform region and -- if I had uniform tiling in the region which were covered too highly, if I wanted to decrease the coverage, one potential solution out of many others, given all the parameters that go into this reaction, would be to decrease the tiling density. Right?

Or I could decrease the concentration, or

Personalis EX2009.132

I could alter the probe sequences, or I could change reaction conditions, or I could add adjuncts to the reaction that might influence the formation of -- and stability of hybridization complexes. Or I could consult a database of variation -- right? -- to see if there's particular variants in that region that I might have to compensate for. Or I could look at the repeat structure of the genome and design parameters to compensate for that repeat structure. Or I could look at copy number variations and polymorphisms that exist in DNA structure, or look at secondary structure. Right?

There are a whole host of things you can do to adjust coverage. Changing tiling is one of those, and one can change tiling to increase uniformity or to decrease uniformity, increase coverage or to decrease coverage, depending on what you want to do.

Q. Is it, in your view, when you were designing baits for use in solution-phase hybridization and next-generation sequencing after you obtain your library -- is there a particular coverage that is more preferable for people with skill in the art?

MR. ROSATO: Objection. Form,

Personalis EX2009.133

foundation.

THE WITNESS: Again, I think the coverage and the density of the baits is going to depend on your application. I don't think there's an established standard coverage, in large part because there's so many other parameters that one can use to try to get the desired level of coverage you have for any particular region.

Q. BY MS. WANG: If the goal of your particular assay was to understand the relative expression of particular targets, is there a particular type of coverage that you would be looking for?

A. Again, it would depend on lots of other assays. Right?

One of the papers that I cited in my report was a paper by Levin. Levin was sequencing cDNAs. And Levin actually states that if one wanted to get rare targets, one could change some of the parameters in the design of the baits that allow you to pull out those rare targets. Right?

It depends on what you want to do and it depends on the particular assay and its goals.

Q. Sure. But my question is: If you wanted to understand the relative expression of targets

Personalis EX2009.134

within your sample, would you want uniform coverage or would you not want uniform coverage?

A. Each transcript is different, so I can't answer that question with an absolute yes or no. It's going to depend on the sequences I'm looking at and the parameters of those sequences.

Q. Based on your experience, you don't have a more likely or less likely answer?

A. No.

MR. ROSATO: Objection. Form.

Q. BY MS. WANG: So you have no opinion as to whether or not you'd want uniform coverage or not uniform coverage if you wanted to preserve the relative expression of your transcripts?

MR. ROSATO: Objection. Form.

THE WITNESS: It's going to depend on the absolute goals. It's going to depend on the sequences you're trying to assay. It's going to depend on a whole host of other parameters.

And, again, I could adjust -- I could adjust coverage, I could adjust tiling, I could adjust lots of different things. Right?

I may be interested in simply determining the approximate level of gene expression. I may be actually looking for splice variants, or I may be

Personalis EX2009.135

looking for single-nucleotide polymorphisms, or I may be looking -- or single-nucleotide variants and mutations. I may be looking for fusion transcripts. Right?

So there's no generally accepted set of parameters that work in all those situations, and the choice I make would be dictated by my experimental goals and the conditions of the particular sample and particular assay I wanted to conduct.

Q. BY MS. WANG: So you have no opinion -- if your experimental goal was to preserve the relative expression of transcripts, you have no opinion as to whether you'd want uniform coverage or not uniform coverage; is that correct?

MR. ROSATO: Objection. Form, mischaracterizes. Asked and answered.

MS. WANG: Mike, we know that speaking objections are not appropriate, so --

MR. ROSATO: You're asking the same question over and over, so --

MS. WANG: And I'm entitled to do that --

MR. ROSATO: And you're --

MS. WANG: -- so --

MR. ROSATO: And you're restating his opinion in incorrect ways.

Personalis EX2009.136

MS. WANG: I'm entitled to ask my question. Your objection is an objection that's been preserved. You don't need to coach the witness.

MR. ROSATO: I'm not coaching. You're engaging me in this conversation.

If you want him to leave the room, you can ask him to do that. You're the one engaging me in this conversation.

MS. WANG: Right now I am, but your previous objection, as you well know, was not appropriate.

Q. So I will ask one more time. If the goal of your experiment is to preserve the relative expression of transcripts, is it true that you have no opinion as to whether you desire uniform coverage or not uniform coverage?

A. So I've --

MR. ROSATO: Same objection.

THE WITNESS: That I've had no opinion. I've given my opinion, and I've told you that I'd need to know a lot more about all the other parameters in the assay and the goals of the assay. There are times when I would like to have uniform coverage; there are times when I wouldn't want to have uniform coverage. Right?

Personalis EX2009.137

Levin talks about this, and it's one of the reasons I cited his paper.

Q. BY MS. WANG: I'm not asking about Levin, Dr. Quackenbush. I'm asking you, again -- you have no opinion if you're -- the desire of your assay is to preserve the relative expression of your transcripts, you're telling me you have no opinion, absent other parameters, as to whether you'd want uniform coverage or not uniform coverage; is that correct?

MR. ROSATO: Same objection.

THE WITNESS: There are lots of different ways to measure expression. Right? You'd measure expression by just targeting small fragments of a gene. All right? Of an RNA. You can target entire fragments. If you're looking at splice variants, and you want to know the relative expression. Knowing the entire fragment is going to give you a different set of design parameters than in another type of experiment.

So I've told you that my opinion is not that I have no opinion, but my opinion is that the choice of experimental parameters is going to depend on your exact goals and it's going to depend on a variety of other factors that simply aren't contained in the question you asked.

Personalis EX2009.138

Q. BY MS. WANG: If your exact goal is to preserve the relative expression of the transcripts, would you want uniform coverage or not uniform coverage?

A. What's the GC content of the different targets? What is the secondary structure of the RNA? What is my goal when I say I want to assay the relative expression levels? Does this include splice variants or not? Are there single-nucleotide polymorphisms or not.

There are a whole host of other parameters which go into answering that question, and I'm not prepared to give you a general answer.

Q. So you can't answer my question without knowing those other parameters; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I would want to understand what these parameters were if I were to give you an answer, because in molecular biology there are very rarely uniform answers that apply universally in all situations.

So, you know, I don't have enough information to be able to tell you exactly what kind of coverage one might want to achieve, and one can change that coverage, depending on one's goals, using

Personalis EX2009.139

a wide range of well-known parameters, that have been known for a long period of time, that one can use to adjust coverage.

Q. BY MS. WANG: So you cannot answer my question, correct? Unless I give you each of those other parameters. Is that what you're telling me?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: I believe I have answered your question.

Q. BY MS. WANG: You have not, respectfully.

You are telling me you cannot answer my question unless I give you all of those other parameters; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I wouldn't necessarily need to know them all. I'd want to know the situation and I'd want to design an experiment that is appropriate for answering the question that I am posing, and for potentially answering other questions.

When we're doing sequencing, a lot of times there are multiple questions we're trying to address, and we adjust parameters again to give us the most informative data for the questions we're trying to answer given the characteristics and given the

Personalis EX2009.140

constraints of the assays and the system we're trying to study. Right?

If I were looking at two genes and wanted to know the relative expression, that might -- I might have a very different answer than if I were looking at an entire genome. I wouldn't even necessarily have to use RNA sequencing; I could use other techniques.

So your question is too vague for me to give you an absolute answer.

Q. BY MS. WANG: So absent the parameters that you have at length described, you cannot answer my question as to whether you need uniform coverage or not uniform coverage in order to preserve the expression of transcripts, true?

MR. ROSATO: Objection. Form.

THE WITNESS: The coverage that you choose would depend on the experimental goals, which are more complex than have been captured in the simple question you asked.

MS. WANG: I think this is probably a good place to break for lunch.

THE VIDEOGRAPHER: We are going off the record. The time is 12:27 a.m. -- p.m. Sorry.

(A luncheon recess was taken at 12:27 p.m.)

Personalis EX2009.141

SEATTLE, WASHINGTON; WEDNESDAY, JANUARY 31, 2018

1:18 P.M.

--o0o--

THE VIDEOGRAPHER: We are back on the record. The time is 1:18 p.m.

EXAMINATION RESUMED

BY MS. WANG:

Q. Welcome back, Dr. Quackenbush.

Just to confirm, during the break did you speak with counsel or anyone else about your answers with respect to this deposition?

A. No.

Q. Okay.

So, Dr. Quackenbush, let's talk about the '830 patent for a little bit, which I think you've had experience in studying, as you've mentioned.

One of the terms that's mentioned in the '830 patent is the term "bait set"; is that correct?

A. Yes, "bait set" is used.

Q. And generally speaking, the '830 patent claims describe five different categories of bait sets that are tailored to capture different categories of mutations or tumor members; is that correct?

Personalis EX2009.142

MR. ROSATO: Objection. Form.

THE WITNESS: Generally speaking, some of the claims -- for example, Claim 1, I believe Claim 65, refers to different bait categories.

Q. BY MS. WANG: So taking, for example, Claim 1, it refers to five different categories of bait sets that are tailored to capture different categories of tumor members; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So I'm not sure exactly how "tumor member" is being used. The claim language in Claim 1 -- let me find that. Sorry -- talks about the use of -- it talks about analyzing a tumor sample "wherein the at least two bait sets of step (b) are chosen from two of the following bait sets," and describes five different types of bait sets, which in the context of the claim are generally applied to analyzing tumors.

Q. BY MS. WANG: And each of those five different types of bait sets are tailored to select different types of targets; is that correct?

A. I don't know what you mean by "different types of targets." They're all selecting DNA.

MS. WANG: Can you mark this, please.

(Deposition Exhibit 4 was marked for

Personalis EX2009.143

identification and sealed separately.)

MR. ROSATO: Counsel, real quick, is there a protective order in the litigation?

MS. WANG: There is, but this document was filed publicly.

MR. ROSATO: It was. Okay.

We'll want to verify that, obviously. I'll take your word for it now, but we'll maintain any -- any objections that might be appropriate.

MS. WANG: We can definitely address that after.

MR. ROSATO: Sure.

Q. BY MS. WANG: Dr. Quackenbush, you've been handed what's been marked, I believe, as Exhibit 4 --

A. Yes.

Q. -- to your deposition. Do you recognize this document?

A. Yes, I do.

Q. And this is an expert declaration submitted by you in the Foundation vs. Guardant District Court litigation; is that correct?

A. I believe that's correct.

Q. And if you look at the very back of this document, it appears that you signed it on March 6th

Personalis EX2009.144

of 2017; is that correct?

A. Yes.

Q. Under penalty of perjury, according to the signature block?

A. Yes.

Q. Can you turn to Page 21 for me, please, of your declaration.

MR. ROSATO: Counsel, we'll -- I don't want to interrupt your questioning, but I'll just maintain any scope objections and obviously see where your questioning goes.

MS. WANG: Sure.

Q. Can you turn to Page 21, please.

A. Yes.

Q. And look at Paragraph 58.

A. Yes.

Q. Paragraph 58 states, "The '830 patent claims describe using five different categories of 'bait sets' that are tailored to capture different categories of tumor members based on their frequencies in the tumor sample or their physical locations in the tumor genes."

And it cites Claim 1 and Claim 65. Do you see that?

A. Yes, I do.

Q. And then the next sentence reads, "Each 'bait set' is distinguished by a 'unique preselected efficiency for selection.'"

Do you see that?

A. I see where it says that, yes.

Q. Okay.

So you agree that the '830 patent is describing five different categories of bait sets that are tailored to capture different categories of tumor members, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So can you repeat the question, please?

Q. BY MS. WANG: Sure.

You have stated in this declaration, and you agree, that the '830 patent claims describe using five different categories of bait sets that are tailored to capture different categories of tumor members, correct?

A. So that's what the declaration says, yes.

Q. Do you agree with your statement from this prior declaration?

A. So I probably want to read it in context, and I believe in context it is correct.

Q. And you said in your declaration as well

that "Each 'bait set' is distinguished by 'a unique preselected efficiency for selection.'"

Do you see that?

A. I believe the quotation marks refer to the fact that that is how the language of Claims 1 and Claim 65 distinguish the different bait sets.

Q. And that is how the claim distinguishes the bait sets from each other; is that correct?

A. So the wherein clause at the end of Claim 1, and the same clause is at the end of Claim 65, says, "wherein each bait set of said plurality has a unique preselected efficiency for selection for its target as compared with the other bait sets in the plurality."

So in the set of two or more, those two would be distinguished.

Q. From each other, correct?

A. From each other.

Q. And I believe you've testified about this previously, but "preselected" means selected in advance of performing the method; is that correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So if you point me to that, I -- it seems like a reasonable assumption, but I

Personalis EX2009.147

don't know exactly what I said in this declaration.

Q. BY MS. WANG: Sure.

A. As you pointed out, it was filed 10 months ago.

Q. Sure. If you go to Page 26.

A. Sure.

All right.

Q. And Paragraph 66.

Do you see the first sentence reads, "A POSITA," which is a person of ordinary skill in the art, "would understand that because the 'efficiency of selection' as recited in the '830 patent claims must be 'preselected,' one would have to determine the desired sequencing depth for each target mutation in advance of performing the method."

Do you see that?

A. Yes, I do.

Q. So do you agree that "preselected" means in advance of performing the method?

A. Yes, "preselected" would mean that one would have to determine the desired sequencing depth to preselect for each target mutation in advance of performing the method.

Q. Dr. Quackenbush, are you familiar with normalization techniques for cDNA libraries?

A. Yes, I am.

MR. ROSATO: Objection. Form, foundation.

Q. BY MS. WANG: And what are, generally speaking, normalization techniques?

MR. ROSATO: Same objection.

THE WITNESS: So normalization techniques refer to a broad class of techniques that were used to adjust the relative concentration of different sequences in, for example, cDNAs, in a library of cDNAs that would then be profiled to analyze gene expression.

Q. BY MS. WANG: Why would persons of skill in the art want to normalize a particular library of cDNAs?

A. Again, there are lots of different reasons why one might do that. One example might be to increase efficiency of detecting rare transcripts.

In the early days of cDNA sequencing, cDNA sequencing was used for gene discovery. We didn't know the gene content in humans or other organisms. Common transcripts like actin were easy to find. Rare transcripts were more difficult to find.

So there are situations in which you want to increase the representation of different elements

Personalis EX2009.149

in that -- the sequencing library, and normalization refers to that broad class of methods.

Q. So in the example that you just gave, for example, where rare transcripts are more difficult to find in a particular library, does normalization involve increasing the abundance of those particular transcripts as opposed to all other transcripts in that library?

A. There are techniques which involve increasing concentration; there are also techniques which involve decreasing conversation. So one of the common techniques that was used in the early days of cDNA sequencing was a subtraction technique, where you would actually sequence libraries, and you would actually go through cycles of pulling out the common transcripts to leave behind the rare transcripts. So you can increase concentrations or you can decrease concentrations.

Q. And when you say the early days, what period are you referring to?

A. I was working on cDNA sequencing involving normalized libraries in the 1990s and early 2000s. So those techniques have been well known for a long period of time.

Q. And in terms of increasing

Personalis EX2009.150

concentrations, how would you do that?

A. Again, there are a wide variety of ways you could do that. If you wanted to detect rare transcripts -- right? -- what one could do is one could design, say, oligonucleotide probes that were specific --

Well, it's actually harder to increase concentrations if you don't know what's there, so the easiest way is actually to decrease concentrations of things that are known. If you don't know what's there, you want to discover it, the easiest way to do it is to decrease concentration. So most of the normalization techniques used early on were actually subtractive techniques.

Q. And how do you subtract the transcripts that you don't want to be in your library?

A. There are lots of different ways you can do this. Okay?

Some early techniques involved arraying cDNA clones in 96-well plates. You would begin to sequence -- you would take those clones in 96-well plates, so a clone is DNA which is induced to grow in bacteria. You would extract DNA from those 96-well plates. You would stamp them down on a membrane. You would make many membranes with thousands of those.

Personalis EX2009.151

And then you would start sequencing. You'd take the first plate and sequence those, and you sequence 10 plates, and then you'd look at the repertoire of sequences. You would design probes to those sequences.

In the early days you would label those with radioactive labels. You would hybridize them to the membranes. You would detect the hybridization positions and then you would exclude those from further sequencing. Right? So it's subtraction by picking.

Other approaches involved taking cDNAs and making probes to those particular genes you had already sequenced and then doing a subtractive hybridization, where you had a solution with a library and you'd pull out the things you had already seen.

The efficiency was never a hundred percent, but you'd reduce the relative concentration.

Those are just two examples of a wide array of different techniques people have used.

Q. In the context of using solution-based hybridization to pull out the things you've already seen, as you've described, how is that done to preserve what you don't know is in there? Is that through washing? What is the process that's actually

Personalis EX2009.152

used?

A. So if I were to pull things out of solution, then whatever remained behind in solution would be hopefully enriched with relative concentrations for the things I had not yet seen.

Q. And you say "hopefully." Are there risks as to that particular method of losing things in your solution?

A. In any hybridization assay there are risks of mishybridization, partial hybridization, you know -- again, like in all of molecular biology, everything depends on the specifics of the assay you're using.

If you run this at low temperatures, you can get a lot of formation of DNA complexes and pull things out of the solution you don't want to pull out. You risk -- if you don't know the sequences that you're looking for, you risk having nonspecific hybridization and pulling out gene family members that may be expressed at low levels.

So there are a lot of factors that go into designing a high-quality assay to get what you're looking for.

Q. I want to turn to one of your other declarations in this IPR. It's the 14 -- 1447.

Personalis EX2009.153

Do you have that in front of you?

A. Yes.

Q. And that's the one that's labeled "Declaration of Dr. John Quackenbush (Claims 1, 2, 8, 9, 11-14, 16, 48-53, and 62-64)"; is that right?

A. Yes.

Q. Okay.

Can you turn to Page 82 of your declaration, please.

Are you there?

A. Yes.

Q. And starting on Page 82 and going into Page 84, there's a table that you've presented in your declaration; is that correct?

A. Yes.

Q. And the header for that table appears to be "Claim 8. The method of claim, 1 wherein the bait sets are chosen from two or more of." Do you see that?

A. Yes.

Q. And in this table it appears that you have put the claim limitations of Claim 8 in the left column and a number of citations and text in the right column. Do you see that?

A. Yes, I do.

Personalis EX2009.154

Q. Okay.

So according to this chart that you've put in your declaration, you do not point to Levin as teaching claim -- Set B, "a bait set that selects an in-frame deletion of 1 or more codons from a reference nucleotide sequence"; is that correct?

A. There's no reference to Levin here.

Q. Okay.

And likewise for Set C, "a bait set that selects an intragenic deletion," you do not cite Levin there either, correct?

A. No.

Q. And for Section D, "a bait set that selects an intragenic insertion," you also do not cite Levin for that, correct?

A. No.

Q. And for E, "a bait set that selects the deletion of a full gene," Levin does not teach that, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I wouldn't say Levin doesn't teach it. I simply didn't cite it.

Q. BY MS. WANG: You don't cite to Levin as teaching that limitation, correct?

A. I don't cite to Levin. I'm not sure

Personalis EX2009.155

whether or not Levin actually refers to missing genes in his paper.

Q. But if Levin, in fact, did teach a bait set that selects an intragenic insertion or the deletion of a full gene or an intragenic deletion, you would have cited it in your report regarding Levin; isn't that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: One skilled in the art would understand that any of these are simply variations on a theme, and that what's taught in Levin could easily be applied to detecting these individual genetic alterations, genomic alterations. So simply the fact that Levin didn't explicitly describe these doesn't mean that the method described in Levin couldn't be used to perform any of these and all of these. Anyone skilled in the art, particularly anyone who works in cancer, would understand that these are the types of genomic alterations one would like to look at.

Levin was demonstrating a technology using cDNA sequencing, but, again, one skilled in the art would understand that all of these things could easily be found by applying the techniques in Levin to different types of DNA samples.

Personalis EX2009.156

Q. BY MS. WANG: But you don't cite Levin as teaching this bait set that selects a deletion of a full gene, correct?

MR. ROSATO: Objection. Form. Asked and answered.

THE WITNESS: As I mentioned, you know, Levin talks about general technologies for identifying different types of DNA alterations.

In the sample that Levin analyzed, there's no guarantee that all of these different alterations didn't -- that they did occur, and he may not have reported them simply because in that sample they didn't occur.

But, again, one skilled in the art would understand very simply that you could identify these types of alterations. And, in fact, I cite to Shah because Shah details all of these different types of alterations that they were able to find.

Q. BY MS. WANG: Dr. Quackenbush, this is a chart that you included in your declaration, and in this chart and in your declaration you do not cite to Levin as teaching a bait set that selects a deletion of a full gene, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: One skilled in the art

Personalis EX2009.157

would understand that Levin provides enough teaching that anyone could have detected any of these. The fact that Levin didn't find them is the only -- that I do not recall Levin having found them is the reason they're not cited to.

Q. BY MS. WANG: So in your view, the fact that Levin is missing from your chart is because you do not recall whether Levin found them at all; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: The reason they're not here is because there are better examples in the literature where, for example, Shah and his colleagues explicitly found these different types of alterations. Right?

Again, one skilled in the art would understand that one could take what's applied in Levin and apply it to genomic DNA and to find any of these.

Q. BY MS. WANG: Sure. But I'm again asking, in this chart you yourself do not cite Levin as teaching a bait set that selects a deletion of a full gene. You did not include a citation to Levin in this chart; true or false?

MR. ROSATO: Objection. Form.

THE WITNESS: I did not cite Levin in this chart. It wasn't necessary to cite Levin in this

Personalis EX2009.158

chart. But I provided other references that demonstrate that all of these things could be found.

Q. BY MS. WANG: And for letter F, "a bait set that selects an inversion," you did not cite Levin in this chart as teaching a bait set that selects an inversion; is that correct?

A. There's no citation to Levin in element F in the chart, and, again, that's not necessary.

And remember, the claim language only requires that bait sets are chosen from two or more of this entire long list. So I cite to Levin in two or more, and, again, anyone who knows anything about this technology would understand that you could detect all of these things using the methods described in Levin.

Q. I understand what you're saying. Again, I would like to point to what you actually put in your declaration, which is for the Section F, "a bait set that selects an inversion," you did not cite -- you, Dr. Quackenbush, in your declaration, did not cite Levin as teaching this particular limitation, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: I would not say Levin doesn't teach this. Levin didn't find it. And there's a difference. Right?

Personalis EX2009.159

If I'm going to teach you how to perform an assay -- right? -- I can teach you how to use a telescope. And you could look in that telescope, and there's no guarantee that, like Galileo, you would find the moons of Jupiter or that you would discover a supernova. Right?

So the fact that Levin didn't find it doesn't mean that Levin didn't teach it. Right? There's a distinction.

And in interpreting these claims, one has to ask: Well, would one skilled in the art be able to take that method and practice it?

Sure, you can practice it.

Levin didn't find those things, so I provide other citations showing that other people, using similar methods, were able to identify these. And, in fact, Shah was able to quantify the relative frequencies of many mutations and many different genomic alterations.

Q. BY MS. WANG: And you know that Levin did not find this particular bait set that selects an inversion because it's not stated in Levin; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: It's not that Levin didn't

Personalis EX2009.160

find a bait set that found an inversion. Levin used bait sets that didn't detect those inversions. Right?

And, again, there's a difference. Levin wasn't searching for bait sets. Levin was demonstrating that one could use bait sets to characterize a whole host of genomic alterations.

Q. BY MS. WANG: But you do not cite Levin as stating or finding a bait set that selects an inversion, what the limitation says, correct?

A. Incorrect.

MR. ROSATO: Objection. Form, foundation, asked and answered.

Q. BY MS. WANG: You do cite Levin in this chart?

A. If you let me finish.

Your answer -- the question said -- you said Levin didn't find a bait set, and I think the proper way to phrase that question is you don't cite Levin because Levin and his bait sets didn't find this alteration. It doesn't mean that using a different sample, he couldn't have found that alteration. And it doesn't mean that someone skilled in the art couldn't take what's described in Levin and apply it directly to identifying those types of alterations.

So the purpose of this chart is to show

Personalis EX2009.161

examples where people have actually identified all of these different types of alterations in their assays, to show that anyone skilled in the art could have easily designed bait sets to do that.

Q. Levin did not find these alterations; is that correct?

A. The bait sets Levin used and the DNA sample that he used did not find those alterations, that I recall, that were reported in the paper; therefore, there isn't a citation to those.

Q. The bait sets that Levin used were not designed to find these inversions, were they?

A. I can't say that they weren't designed to find those inversions, because those inversions often exist in regions of genes. Simply because Levin didn't find them doesn't mean they weren't designed to find them, doesn't mean they couldn't have been found. Right? It simply means that in the sample he looked at, they weren't there.

Q. But you're not aware of any evidence in Levin that he, in fact, designed these bait sets to select these particular inversions; is that correct?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: Anyone skilled in the art

Personalis EX2009.162

could have easily designed bait sets using the methods described in Levin to identify any of these.

Q. BY MS. WANG: I'm not asking about what anyone skilled in the art could have done or could not have done. I'm asking you whether in Levin you can point me to any specific design of any bait to select an inversion.

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: Levin describes the principles he used to design the bait sets and the methods used. I don't know what his motivation was, and I don't know whether he designed bait sets to look for these.

What I do he know is he didn't find these. Because he didn't find these, they weren't cited in the chart.

Q. BY MS. WANG: So you don't have any evidence or can't point to me -- can't point me to any evidence in Levin that he designed his baits to look for these inversions or not?

MR. ROSATO: Objection. Form.

THE WITNESS: Levin wouldn't have to tell me that he designed bait sets to find these inversions. Right?

Personalis EX2009.163

I work in cancer biology; I know all these different types of alterations are important. When I design an assay, I'm going to design assays that give me a broad net that I can cast to find many things.

Anyone else who works in cancer would know that my assay, if it's well designed, would capture a whole host of things other than those I simply report. Right?

Levin is really reporting the use of this technology. The fact that he didn't detail everything doesn't mean he couldn't have found them. And I don't know what he and his colleagues used in discussing their design criteria, so I can't point you to that, but I don't think I need to. Right?

If we think about how one approaches cancer biology and the detection of genomic alterations, typically one would like to cast a broad net.

Q. BY MS. WANG: Is it your opinion, Dr. Quackenbush, that if one designed a broad net that captures a wide variety of targets, that one can be said to have designed an assay to target each of those that it happens to catch?

MR. ROSATO: Objection. Form.

Personalis EX2009.164

THE WITNESS: Again, any particular experiment is going to have its own design criteria, its own approach. I can't comment on each and every particular type of assay people are going to run.

I can tell you that I -- you know, I've seen Levin, I've seen his work, I know a lot of the other people who work in the field, and that any of these things would be pretty obvious to someone who works in the field, particularly in the field of cancer biology, as things one would like to identify in these assays. They're not cited here in Levin because Levin didn't report them.

Q. BY MS. WANG: So for -- back to your chart.

A. Huh-uh.

Q. For letter H, "a bait set that selects a tandem duplication," you also don't cite Levin here because Levin does not report that he found a tandem duplication; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So Levin didn't find a tandem duplication that he reported. Whether or not he found it, I don't know.

But, again, I cite to Shah because Shah reports tandem duplications in their dataset, and,

Personalis EX2009.165

again, one skilled in the art would understand that one could apply what one learned in Levin to do this with genomic DNA.

Q. BY MS. WANG: So I think I -- we're on the same page here. You do not cite Levin for subset H, "a bait set that selects a tandem duplication," because Levin does not report that he found a tandem duplication; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: To the best of my knowledge, Levin doesn't report that he found a tandem duplication.

Q. BY MS. WANG: And in Section I, "a bait set that selects a nucleotide sequence of interest flanked by adjacent non-repetitive sequences," you don't cite anything there, correct?

A. I don't cite anything there because either of the two papers I cited in providing evidence that these things could easily be found by one with skilled in the art -- neither of those two references reported these, but, again, discovering these things would be relatively easy using the methods laid out in Levin and Shah and a variety of other publications, and using commonly available tools and techniques to anyone working in molecular biology and molecular

Personalis EX2009.166

cancer biology.

Q. Is it your view, Dr. Quackenbush, that if Levin reports that it found a particular alteration, that Levin designed its baits in order to target that particular alteration?

A. I don't know what Levin uses as his design criteria, so I couldn't confirm nor deny how he designed his baits.

Q. Let's now actually turn to Levin, which I believe is Exhibit 1010.

A. Uh-huh.

Extra copy for you.

MR. ROSATO: Thank you.

MS. WANG: Thank you.

Q. And Exhibit 1010, this is the Levin paper you were referencing, correct?

A. Yes, it is.

Q. And this one was published in 2009?

A. Yes, in Genome Biology.

Q. And in the abstract of this paper, it states that, "Targeted RNA-Seq combines next-generation sequencing with capture of sequences from a relevant subset of a transcriptome," correct?

A. That's what it says, yes.

Q. And so Levin is dealing with RNA-Seq; is

Personalis EX2009.167

that correct?

A. He is describing an assay for targeted RNA sequencing, so, again, it's reducing the representation or eliminating some genes in reaching the representation of others.

It describes RNA-Seq, which uses complementary DNA, and the techniques that are used with RNA-Seq can be used with genomic DNA as well.

Q. But the reason that Levin is specifically looking at RNA is because, according to Levin, there are more sequences in RNA that are likely to have functional consequences in cancer than in DNA; is that correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: Can you point me to that, please?

Q. BY MS. WANG: Sure. If you go on that first page to the right-hand column, it's the first full sentence starts with "Although." Do you see that?

A. No.

Oh. Yes, I do.

Q. It states, "Although chromosomal rearrangements can be detected by next-generation

Personalis EX2009.168

sequencing of genomic DNA, RNA-Seq is a powerful tool to identify those rearrangements that lead to chimeric transcripts and are more likely to have functional consequences in cancer."

Do you see that?

A. I see where it says that, yes.

Q. And that was what Levin was looking for; is that correct?

A. That's not the only thing Levin was looking for. You know, Levin describes the discovery of lots of different types of alterations. One of the things Levin was -- things Levin was looking for were chimeric transcripts, so gene fusions, and the reason they cite to these gene fusions is that if an RNA appears in the cell, that gene, in this case a fusion gene, which is the result of a chromosomal translocation, is likely to be expressed and may have a functional consequence.

But it doesn't say it's the only way to find them. It says it's a way to find those which may be functional. Right?

So it doesn't eliminate the possibility of finding these in other ways. And, again, the techniques used to find them can be easily applied to genomic DNA.

This is at the tail end of this paragraph, and if I read the entire paragraph, it's very likely that we talk about many different type -- that they talk about many different types of alterations that this assay was designed to detect, and the proof of the ability of this assay to detect many types of alterations is reflected in what's reported in the paper.

Q. If you go to the second page of Levin.

A. Sure.

Q. Left-hand column, at the bottom of the first full paragraph.

A. Okay.

Q. Do you see where it says, "To date, however, hybridization-based capture approaches have been applied primarily to genomic DNA, typically for the purpose of enriching exonic DNA of interest"? Do you see that?

A. Yes, I do.

Q. And then it says, "Although targeted sequencing of genomic DNA facilitates mutation-discovery/profiling, it is unable to interrogate the myriad additional genomic alterations affecting DNA and mRNA that are critical to tumor biology and therapeutic development."

Personalis EX2009.170

Do you see that?

A. I see that, yes.

Q. And in the next following sentence, in the next paragraph, it states, "In this study, we explore the feasibility and power of 'targeted RNA-Seq,' the application of hybridization capture methods to transcriptome analysis"; is that correct?

A. That's what it says, yes.

Q. So Levin is looking at RNA, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: What Levin is describing is the fact that well-known techniques which are used for DNA can be generalized to RNA.

Q. BY MS. WANG: And if you go back to the paragraph above, where we were just looking, Levin describes that there are targeted next-generation sequencing -- there are methods that allow for targeted next-generation sequencing. Do you see that?

A. Sorry. Where?

Q. At the top of the page.

A. The "Although"?

The top of the page?

Q. "Cost and efficiency considerations." Do you see that?

A. "Cost and efficiency" -- okay. So I see

Personalis EX2009.171

the sentence, the first sentence, that begins "Cost and efficiency considerations."

Could you repeat your question, please?

Q. Sure.

So Levin states that, "Cost and efficiency considerations have prompted the emergence of methods that allow 'targeted' next-generation sequencing."

Do you see that?

A. Yes.

Q. And the next line says, "Two suitably high-throughput approaches to enrich specific sequences from genomic DNA have been developed: multiplexed molecular inversion probes (MIPs) and capture by hybridization to oligonucleotide probes on microarrays or in solution."

Do you see that?

A. Yes.

Q. And the hybridization to oligonucleotide probes in solution is the same thing as the solution-phase hybridization that we've been discussing this morning; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, I don't know. I wouldn't say solution-phase hybridization that we have

Personalis EX2009.172

been discussing. I would say that this alludes to an example of solution-phase hybridization.

Q. BY MS. WANG: And that --

A. It cites --

Excuse me.

It cites a particular reference, which is Reference 20.

Q. What is Reference 20, Dr. Quackenbush?

MR. ROSATO: I think he's -- can we let him finish his answers?

THE WITNESS: It's a paper that was -- I believe it's the Gnirke paper that was published in Nature Biotechnology.

Q. BY MS. WANG: And that is the paper we were discussing this morning, correct?

A. It's a -- one of the things we discussed this morning, yes.

Q. So Levin is referring to the solution-phase hybridization technique in Gnirke, correct?

A. He's referring to solution -- to Gnirke as an example of solution-phase hybridization. The fact that he cites it means he's using it as an example; it doesn't mean to limit it to only that.

Q. He doesn't cite to any other papers

Personalis EX2009.173

referencing solution-phase hybridization in this paragraph, correct?

A. He doesn't cite to any others, but you wouldn't necessarily expect him to, either.

Q. The next sentence right after that, after the citation to Gnirke, states, "MIPs are similar to PCR primers in that they enrich loci defined by two flanking specific sequences."

Do you see that?

A. Yes.

Q. "Thus, they are not appropriate for the discovery of novel chromosomal rearrangement such as translocations."

Do you see that?

A. I see where it says that, yes.

Q. So Levin is stating that MIPs and PCR is not appropriate for the discovery of novel chromosomal rearrangements such as translocations, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: No. The sentence says MIPs are similar to PCR primers and describes why they're similar, but also describes why MIPs wouldn't be appropriate.

One could use PCR techniques and, in fact, people have used PCR techniques to discover

Personalis EX2009.174

things like --

What does it say?

I'll read this again.

PCR has been broadly used in lots of variations of -- PCR has been used.

So MIPs themselves cannot be used for the discovery of novel chromosomal rearrangement such as translocations. And that has to do in large part with the technology used in MIPs, where you have two oligonucleotides which are specifically designed -- they're linked together by another synthetic piece of DNA. And they're typically used with an assay known as rolling circle amplification.

So if I wanted to discover a translocation -- right? -- I could actually use multiple pairs of primers scattered throughout the genome and look for amplification products that come that, when I sequence them, reveal the fact that there are translocations that occur.

So, you know, he's referring specifically to MIPs, not to other types of -- not to all versions of PCR, not specifically to PCR. MIPs have a very -- the molecular inversion probes have a very specific structure, which would make discovering chromosomal inversions difficult.

Personalis EX2009.175

Discovering them, not detecting them if you know that they're there.

Q. BY MS. WANG: He's comparing MIPs to PCR primers, correct?

A. He's saying they're similar to PCR primers, but he doesn't say they're the same as PCR primers.

Q. I'm not asking if they're the same. I'm saying Levin is comparing MIPs to PCR primers, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: The sentence says, "MIPs are similar to PCR primers in that they enrich loci defined by flanking sequences. Thus, they" -- MIPs, not PCR, not all variations of PCR -- "are appropriate for the discovery of novel chromosomal rearrangements such as translocations."

So Levin is pointing out similarities between MIPs, but when he refers to the limitations on MIPs, he's not referring to the limitations on PCR.

Q. BY MS. WANG: So in your view the similarity between MIPs and PCR primers, in that they both enrich loci defined by two flanking specific sequences, has nothing to do with whether it's appropriate for the discovery of novel chromosomal

Personalis EX2009.176

rearrangements?

MR. ROSATO: Objection. Form.

THE WITNESS: Here's the difference. Right? I can design oligonucleotides that will hybridize anywhere in the genome. Right?

I can use variations of PCR, like random PCR, to amplify the entire genome, sequence that genome, and discover novel translocation events.

However, if I have two primers which are physically constrained not to span large regions of the genome but only designed to span small regions of the genome, then, yes, those MIPs probes are not appropriate for discovering translocations. But it has to do with the physical construction of the MIPs probes which limits their application.

Q. BY MS. WANG: So in your view this statement, referring to similar to PCR primers and that they enrich loci defined by two flanking specific sequences, has nothing to do with Levin's statement that the discovery of novel chromosomal rearrangements is difficult using this particular approach?

A. Using --

MR. ROSATO: Objection. Form.

THE WITNESS: If you read the language stated in Levin, Levin is saying that discovering

Personalis EX2009.177

chromosomal rearrangements that had not previously been reported using MIPs would be difficult. He doesn't limit PCR.

Q. BY MS. WANG: If you could look to Page R115.6 of Levin, in the top right-hand corner.

A. Okay.

Q. Do you see the paragraph under "Bait design and synthesis"?

A. Yes.

Q. This section describes how the baits were designed in Levin, correct?

A. It gives a general overview. It doesn't give complete details of the entire protocol, but it talks about the -- the general characteristics of the baits that were designed.

Q. Are you aware of any other place in Levin where it gives you the complete protocol of how he designed his baits?

A. Well, let's see.

So I know there are a number of online supplements. I don't know if there's any additional detail in the online supplements. Journals typically limit what you can report, and so, unfortunately, the methods section is always the one that gets trimmed the most.

Personalis EX2009.178

There may be additional information in the supplements I just don't recall at this moment.

Q. If you look at this paragraph that's reported in the main article, under "Materials and methods" here --

A. Uh-huh.

Q. -- Levin states that, "We designed 11,566 bait sequences"; is that correct?

A. Yes, that's what it says.

Q. And all of these bait sequences are targeting the coding sequence of 887 transcripts of 467 genes; is that correct?

A. So the 11,566 bait sequences, they tell us in -- I believe those are all reported -- all 11,566 are reported in supplemental -- in Table S5, which is in the supplement. That may have information about the design programs they use and the criteria; I'm not sure. "Targeting the coding sequences of 877 transcripts of 467 genes," which were identified in NCBI's RefSeq database.

Q. So based on this sentence, 11,566 --

(Interruption.)

MS. WANG: Apologies for that interruption.

Q. So based on this sentence, the 11,566

Personalis EX2009.179

bait sequences of Levin all target the coding sequence of 887 transcripts of 467 genes that were described in this NCBI database, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So the 11,566 baits target collectively the coding sequences of these 887 transcripts, which represent 467 genes. Right? So they're gene variants. And those 11,566 baits together as a collective target those.

Q. BY MS. WANG: There's no indication here that those 11,566 baits target any other transcripts other than the 887 transcripts of the 467 genes reported here, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: These baits were designed to enrich, from the cDNA library, specific sequences they were interested in, but they could have easily added or subtracted other sequences. Right? The basic principles are the same.

So as a demonstration, they chose to look at 467 individual genes.

Q. BY MS. WANG: I'm not sure if you understood my question.

There's no indication in this paragraph that the baits were designed to target anything other

Personalis EX2009.180

than the 887 transcripts of the 467 genes, correct?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: I don't know what you mean by "other than." Right? These baits were designed for a specific purpose. They were designed to demonstrate enrichment technologies. So as an example, he chose -- or they chose 467 genes.

It's very interesting that you demonstrate a technology by showing you pick up different splice forms, different variants, and they designed 11,566 baits.

Q. BY MS. WANG: They designed 11,566 baits for a specific purpose; is that correct? That's what you just said?

MR. ROSATO: Objection. Form.

THE WITNESS: They designed 11,566 baits, and they were demonstrating that they could sequence these 467 genes and their variants.

Q. BY MS. WANG: So in your view, these 11,566 baits were designed to be able to target the 887 transcripts of these 467 genes, correct?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: The language here is very

Personalis EX2009.181

clear. They designed 11,566 baits. Those were designed to 887 different transcripts that reflect sequences -- or reflect collectively 467 genes.

Q. BY MS. WANG: And the next sentence below that says -- actually, the sentence below that says, "Each bait was composed of 170 bases matching the transcript sequence it was intended to enrich."

Do you see that?

A. Yes, I do.

Q. And that means all 11,566 baits were each comprised of 170 bases, correct?

A. I don't know if that's absolutely true. I'd have to look in Table S5. But because he said this, I would assume that that's probably correct, that the bait sequences were 170 bases in length.

Q. Okay.

And then the next sentence says, "Baits were tiled across the coding region of each transcript, from 5' to 3', such that all coding bases were covered by at least one bait and that overlap between baits was minimized and distributed evenly among all baits."

Do you see that?

A. Yes.

Q. And so he's saying that each of the 1 --

Personalis EX2009.182

even of the 11,566 baits were tiled across the coding region of each transcript that they were targeting from 5' to 3'; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So the baits -- this is talking about bait design. How they actually hybridize may or may not reflect this. But they downloaded from the NCBI RefSeq database reference sequences for 467 genes. They downloaded variations on those genes. Right? These are the transcript isoforms, where they -- 4 -- 887 transcripts of these genes.

So when we refer to transcript isoforms, we're talking about different variations, different forms of these genes as they're converted into RNA. Right? And they, in their design parameters, designed baits that are 170 bases long and which tile across the coding regions of those transcripts.

The reason they focused on coding regions is because they were going to demonstrate this genomic technology with cDNAs, which typically -- although not always, because not all mRNAs are fully processed, but typically do not contain intronic sequences.

So as a demonstration of the technology, they wanted to optimize it for enrichment of these

Personalis EX2009.183

coding sequences and full coding sequences from the cDNA libraries. And so they designed the baits with a specific overlap, where the overlap was minimized, but they wanted to assure that each base was covered at least once.

Q. BY MS. WANG: For all 11,566 baits, correct?

A. I believe that's how they designed them.

MS. WANG: Let's go off the record because he has to change the tape.

THE VIDEOGRAPHER: Here marks the end of Media Unit No. 2 in the deposition of John Quackenbush. We are going off the record, and the time is 2:05 p.m.

(Discussion off the record.)

(Short recess.)

THE VIDEOGRAPHER: Here marks the beginning of Media Unit No. 3 in the deposition of John Quackenbush. We are back on the record, and the time is 2:16 p.m.

Q. BY MS. WANG: Dr. Quackenbush, earlier today we talked about somatic mutation frequency. Do you remember that?

A. Yes.

Q. And we talked about it both in the

Personalis EX2009.184

context of population frequency as well as frequency within a tumor; is that correct?

A. Yes.

Q. Do you agree that a mutation in DNA may not occur at the same frequency as a corresponding RNA transcript?

A. You may see differences in the relative frequencies in RNA transcripts and in DNA, yes, because the RNA is expressed.

Q. And, in fact, it's possible that you may not even see a mutation in an RNA transcript, even though it exists in DNA, correct?

A. You may not see it if it only exists in one copy of the gene and for some reason that one copy is not expressed.

Q. So just because I had a mutation frequency of, say, five percent in DNA, that doesn't mean that the RNA transcript has a five percent mutation frequency as well, correct?

A. It could be higher, it could be lower.

Q. Let's go back to Levin --

A. Sure.

Q. -- which I think is in front of you.

And we were looking at the section about bait design, correct?

Personalis EX2009.185

A. That's where we were last looking, yes.

Q. Okay.

Now, Dr. Quackenbush, your opinion in these IPRs is that Levin teaches two or more bait sets, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So if we look at the definition of bait sets, which is laid out in the patent itself -- I believe it's Column 28, Line 60. "'Bait set,' as used herein, refers to one or the plurality of bait molecules." So if we apply this definition of "bait sets" to what's described in Levin on Page 6, in the section "Bait set" -- "Bait design and synthesis," there are at least 11,566 sequences, which I assume are then synthesized into different molecules. So applying the definition of "bait set" laid out in the patent, one would say there are 11,566 bait sets.

Q. BY MS. WANG: Let's go back to your declaration.

A. Sure.

The very first one or the second one?

Q. Good question.

Let's go back to the first one, which is

Personalis EX2009.186

the 01170.

A. Okay.

Q. So if you go to Paragraph 79 of your declaration, which is on Page 34.

A. Yes.

Q. And you state in the first sentence, "As far as selecting baits, Levin discloses designing and using a plurality of different bait sets targeting genes known to be somatically mutated in cancer."

Do you see that?

A. Yes.

Q. And so based upon what you just told me, your view is that that plurality of different bait sets is 11,566 different bait sets, correct?

A. If we look at the definition in the patent, the patent -- the bait set is one or more molecules. Right? And they designed 11,566 different molecules through sequence.

So applying that definition, one could argue there are 11,566 bait sets.

Q. And that's your opinion as you've expressed it in Paragraph 79; is that correct?

A. Paragraph 69 doesn't talk about the absolute number of bait sets. Right? It says, "As far as selecting bait sets, Levin discloses designing

Personalis EX2009.187

and using a plurality of different bait sets targeting genes known to be somatically mutated in cancer." Right?

And we talked about the sentence that says they targeted 467 genes in total, 877 different transcripts. Right?

So the simplest reading of the language in the claim is that there are 11,566. People could argue different numbers, depending on how you define a bait set. Probably the most strict version would be that there are 467 for the genes, but, in fact, the transcripts are different. So there are at least 887, but, you know, the 11,566 meets the definition laid out.

So, you know, depending on what definition you want to apply, we have potentially different numbers, but they're all large numbers.

Q. When you say in your declaration that Levin discloses at least two bait sets, which definition are you applying?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So the fact that Levin discloses two bait sets could reflect the fact that there are 11,566. That's at least two. Could reflect

the fact that he's looking at 467 genes. That's at least two. Could reflect the fact that he's looking at 887 distinct transcripts. That's more than two.

If we actually look at the Levin reference, what Levin describes is identifying a whole different set of classes of genomic alterations. Right? They include sequence variants, so -- I believe they refer to them as SNVs. They identify splice isoform variants. They identify fusion transcripts. And they identify other sequence alterations, novel SNPs.

So they have many, many different bait sets, which identify many, many different things in the sequence.

Q. BY MS. WANG: So you've given me a lot of options, and my question for you is: Applying the definition that you put forth from the patent, which is one or more, correct?

A. Mm-hmm.

Q. What to you are the different bait sets of Levin?

MR. ROSATO: Objection. Form.

THE WITNESS: One could look at Levin, and I think the simplest definition is there are 11,566 bait sets, but however you want to limit it,

Personalis EX2009.189

based on characteristics, based on larger targets, there are definitely more than two.

Q. BY MS. WANG: Are you using the 11,566 different bait sets definition in your opinions in this IPR?

MR. ROSATO: Objection. Form.

THE WITNESS: Any one of those different definitions, in fact, at different places would be relevant. So if you want to talk about specific uses, I'd be happy to talk about them in context.

Q. BY MS. WANG: But you don't have one general definition that you're applying across all your opinions in this IPR?

MR. ROSATO: Objection. Form.

THE WITNESS: Everything depends on context, and if you want to look at things in context, I'd be happy to do that and explain how some or all of the definitions apply in each situation.

Q. BY MS. WANG: The definition from the patent that you read to me is also one or more; is that correct?

I believe you were citing Column 28 of the patent.

A. Column --

MR. ROSATO: Objection. Form.

Personalis EX2009.190

THE WITNESS: Column 28 Line 60 says "'Bait set," as used herein, refers to one or the plurality of" -- "one or a plurality of bait molecules."

Q. BY MS. WANG: So isn't it possible that Levin also discloses one bait set of 11,566 bait molecules?

MR. ROSATO: Objection. Form.

THE WITNESS: I wouldn't characterize it that way at all, since all of these bait molecules are different, all of these bait molecules have different characteristics, all of these bait molecules are going to have different efficiencies in terms of their ability to generate DNA sequence.

And, in fact, Levin talks about, in -- on Page 6, the same page that we were looking at, Levin describes --

Oops. Wrong one.

I'm grabbing the wrong page.

Here we go.

On Page 6, in the column adjacent to the one which says "Bait design and synthesis" -- in fact, in the paragraph immediately next to it, Levin talks about the fact that one could adjust the design parameters. So "If coverage of lower abundance

Personalis EX2009.191

transcripts is a priority in a given experiment, information about transcript abundance can be used during bait design to focus on those transcripts with targeted RNA-Seq." Right?

So, again, all of -- if we define the baits by different levels of coverage, Levin actually tells us that we can design those baits to achieve different levels of coverage. Right?

The reason, you know, I'm using different numbers is just to illustrate that there are over 11,000 bait molecules, each of which is designed -- each of which was designed with a set of parameters. I'm not sure what those parameters are, since they aren't disclosed here, but Levin tells us you can adjust those parameters to adjust their efficiency.

So if we want to characterize baits based on numbers, we can do that. If we want to characterize bait sets based on efficiencies, we can do that. But Levin clearly teaches that what one can do is design bait sets that address a wide range of different genomic alterations and levels of coverage.

Q. BY MS. WANG: We'll get to what your opinions are with respect to what Levin teaches. I'm asking about the 11,566 baits that Levin actually synthesized.

Personalis EX2009.192

A. Yes.

Q. With respect to the 11,566 baits that Levin actually synthesized, each bait was comprised of 170 bases, correct?

A. I believe that's what it says, yes.

Q. And each of the baits were tiled so that at a minimum the overlap between the baits was minimized and distributed evenly among all baits. That's what it says, correct?

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

Q. Okay.

So my question to you is: Under the definition described in the patent that you read, which is "one or a plurality of bait molecules," isn't it possible that Levin's 11,566 baits comprises of one bait set?

A. I wouldn't --

MR. ROSATO: Objection. Form.

THE WITNESS: I wouldn't characterize it that way at all. Right?

These baits have design parameters which make them similar to each other, but they're going to attack different types of alterations and they're going to have different efficiencies. They have different sequences, they have different overlaps,

Personalis EX2009.193

they have different base contents, they have different secondary structures. Right?

So, you know, I would be hesitant to characterize this as a single bait set, since the definition of "bait set" has to really be used in context, and in the context of this patent, bait sets are molecules with different characteristics, and one of the primary ways it's used in the patent is in terms of different efficiencies. Right?

Q. BY MS. WANG: So if you look to Paragraph 28 and -- sorry -- Column 28 of the patent that we were looking at --

A. Sure.

Q. The patent definition of "'bait set,' as used herein, refers to one or a plurality of bait molecules." Do you see that?

A. Yes.

Q. There's no mention there of any other parameters distinguishing these bait molecules from each other, correct?

A. In that particular location, no, but if we want to interpret bait sets in the context of the patent and what the patent is describing, it's helpful to look at the claim language. Right?

And what the claim language actually

Personalis EX2009.194

tells us -- and we can look at Claim 1 or Claim 65. Right? So if we look at Claim 1, it says in Claim 1 Step (b), "contacting the library with at least two bait sets to provide selected tumor members, wherein said bait sets hybridize with the tumor members, thereby providing a library catch."

So the bait sets are hybridized. Right?

And in Levin there are 11,566 distinct molecules which are hybridized.

So "wherein the at least two bait sets of step (b) are chosen from two of the following bait sets." So this is following (e). And there are five different categories of bait sets that are listed here. Right? "A first bait set that selects a high-level target chosen from one or more tumor nucleic acid molecules that comprise a subgenomic interval comprising a somatic mutation that appears at a frequency of about 5% or less of the cells from the tumor sample." All right?

So we're characterizing here bait sets based on mutations and mutation frequencies.

And then we list another type. Right? Those that have somatic mutations that appear with a frequency of about 10 percent or higher of the cells, "a third bait set that selects a low-level target

chosen from one or more nucleic acid molecules." All right? And then lists, again, a variety of different classes that these fall into. "A fourth bait set that selects a nucleic acid molecule that comprises a intron sequence that detects a structural break point; or a fifth bait set that selects one or more" -- "selects a one-copy deletion of several terminal exons." Right?

So you can classify bait sets different ways. There are at least 1,000 -- 11,566 baits, if you think of individual molecules based on 28 as baits. That's how many bait sets there are. If you want to try to partition them into other classes based on the claim language, then we can see examples of at least two of these -- right? -- being detected using the technique -- technology laid out in Levin's paper.

Q. So is it your opinion that the 11,566 baits in Levin cannot be one bait set because in your opinion the bait sets described in this patent have other distinguishing features? Is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I wouldn't call them one bait set. I mean, they clearly have independent features which would distinguish them from each other.

Q. BY MS. WANG: But the definition in the

Personalis EX2009.196

patent in Column 28 that we talked about merely describes "bait set" as one or a plurality of bait molecules, correct?

A. What it does is it says that a bait set is -- I believe it says "one or a plurality of molecules," yes.

Q. Okay.

So my question you to is: Why is it the case that the 11,566 bait molecules, which is a plurality of bait molecules, is not one bait set according to the definition of the patent?

A. Because those bait sets detect different things, different alterations in the genome.

If you wanted to try to limit it, there are different genes. So if you really want to try to push me to limit this, I would say, well, yeah, there are I believe 467 genes. But I would be uncomfortable saying that because there are 800-and-some-odd transcripts.

So it's not one bait set. Right? It's many bait sets. And there are at least two or more. Right?

So given the fact that there are at least two, I think, you know, whatever the numbers we settle on doesn't matter so much. There are more than two

Personalis EX2009.197

bait sets.

Q. If you look at the claim that you were just looking at --

A. Mm-hmm.

Q. And the very last -- we actually go to Claim 65, if you don't mind.

A. All right, Claim 65.

Q. Which I believe is similar, but let's take a look at that.

A. All right.

Q. And you'll agree with me that Claim 65 also has the section where it says "wherein the at least two bait sets are chosen from two of the following," correct?

A. "From at least two of the following."

Q. "From at least two of the following."

And it lays out again five different types of bait sets; is that correct?

A. It has the verbatim language that was in Claim 1.

Q. Okay.

And at the very end there is another wherein clause which says that "each bait set of said plurality has a unique preselected efficiency for selection for its target as compared with the other

Personalis EX2009.198

bait sets in the plurality."

Do you see that?

A. I see where it says that, yes.

Q. Is it your opinion that the 11,566 baits which you describe in their simplistic form are 11,566 bait sets, each have different or unique predesigned selection -- selection -- sorry -- efficiencies for selection?

MR. ROSATO: Objection. Form.

THE WITNESS: So let's look at what Levin is actually teaching us how to do. Levin is teaching -- what Levin is -- not how but what Levin is teaching us to do. Levin is teaching us to design -- or to use bait sets to enrich targets for a subset of genes. Right? So he didn't want to sequence all the genes, he wanted to sequence a subset of those genes. Right? As a demonstration that you can do enrichment. Right?

In doing that, he discovers a number of different types of genomic alterations. Right?

And he also tells us that if we wanted to detect lower representative transcripts that we might have missed, or lower representative sequences, we can alter our design parameters.

So Levin is designing a large number of

Personalis EX2009.199

baits which have unique characteristics, and also tells us that we can alter the parameters.

What we need to know, if we wanted to do what's described here and look at different mutation frequencies, is we'd want to know those mutation frequencies ahead of time. How would you do that?

Well, the reason in my report I included Shah is that Shah provides a demonstration of how one can determine what those mutation frequencies are. Right?

So we can use the method described in Shah, we can determine the mutation frequencies, and then, as Levin teaches us, what we can do is alter our design of those bait sets. And whether it's one bait or 11,566 -- each individual 11,566 baits or subsets representing individual genes or alterations. Right? Levin tells us, using methods that were well known to anyone practicing the art then, that one could alter parameters of the reaction, alter parameters of the design, to detect those mutation frequencies. Right?

We could enhance some, decrease others. We talked about turning dials before.

So Levin tells us we can do that, if we want to target things with different frequencies. And we can preselect those efficiencies based on

Personalis EX2009.200

well-known design and optimization parameters. Right?

All this patent is telling us is if you know the mutation frequencies, turn the dials for different subsets of baits, and create baits that are going to allow you to capture things with different efficiencies, which means different sequencing depths, as we talked about earlier. Right? So you can turn those dials.

Shah teaches us one way to determine those frequencies. We can also look at databases like COSMIC. We can look at hundreds if not thousands of papers in the literature that report different mutation frequencies.

So, you know, Levin by itself doesn't teach us everything, but Levin and Shah, yeah, do a pretty good job of teaching us how to design different bait sets and adjust the relative efficiencies of those baits.

Q. BY MS. WANG: I appreciate that you just stated your entire position on the record, but I assure you that we will be here much later than you expect to be if you don't answer my questions that I'm asking.

What I'm asking you is whether or not the 11,566 baits of Levin, which you described to be

Personalis EX2009.201

11,566 individual bait sets --

A. Yes.

Q. -- whether --

MR. ROSATO: Objection. Form.

Q. BY MS. WANG: -- each of those bait sets, in fact, have a preselected unique efficiency for selection.

MR. ROSATO: Objection. Form.

THE WITNESS: I don't know exactly how Levin defined them. He doesn't use the term "unique preselected efficiency." Right? He describes the general characteristics and tells us that we can adjust parameters to adjust the efficiency.

So although he doesn't use the words "preselected efficiency" in his description, my assumption is, based on the fact that he's telling us that you can adjust efficiencies, that that's one of the things he did.

Q. BY MS. WANG: But you have no indication that he, in fact, created or adjusted a different efficiency for selection for each of the 11,566 bait sets, as you describe them?

MR. ROSATO: Objection. Form.

THE WITNESS: He doesn't tell us explicitly that he did that, but he does tell us that

Personalis EX2009.202

you can do that, and anyone skilled in the art in 2009-2010 would have known ways in which you could do that.

Q. BY MS. WANG: What does a low-abundance transcript mean to you?

A. So if we want to --

Are you talking about the call-out in Levin?

Q. No, I'm just asking what does the term "low-abundance transcript" mean?

A. It's a relative term. Right?

Q. You have no general understanding of what that term means?

A. I do have a general understanding.

MR. ROSATO: Objection form.

THE WITNESS: That's why I'm telling you it's a relative term.

Q. BY MS. WANG: What is the general understanding of that term, as you understand it?

A. Low abundance would mean lower than other transcripts.

Q. And --

A. Right?

Fewer -- probably fewer in number, since we're talking about abundance.

Personalis EX2009.203

Q. So that was my question. Previously we talked about abundance as in a number; is that correct?

A. I -- in context, I don't know. I'm assuming we might have.

Q. Okay.

Does the abundance of a particular transcript give you any indication as to the frequency of the mutation?

A. It can. Right? It depends on the context of the assay that you're running. It depends on the system that you're looking at. Right?

And the thing about it is, if we have a low representation of a particular genetic variant in a cDNA library, whether or not that affects the genomic variation -- right? -- it indicates that we can use approaches which are very similar to what's described to adjust our parameters to detect those low abundance -- right? -- genetic or genomic variations.

Q. It's possible, isn't it, that the transcript is present in a low abundance in a particular sample but has a high frequency of mutation?

A. It could very well be, yes, that it's high-frequency mutation in the population of cells

Personalis EX2009.204

that we're looking at. Could be high frequency in a population of individuals and simply not exist in that particular individual. So when you say frequency, we have to talk about population versus individual --

Q. Sure.

A. -- yeah.

Q. It's possible that if a transcript is present in a low abundance in a particular sample, that, in fact, it could have a high frequency of mutation in the cells that we're looking at?

A. Could you repeat that?

Q. It's possible that if a transcript is present in a low abundance in a sample, that, in fact, it also has a high frequency of mutation in the cells that we're looking at?

A. The transcript -- I wouldn't say the transcript has a high frequency of mutation. Right? The gene itself might be mutated at a relatively high frequency, and there may be other mutations which affect, for example, the ability of a transcription factor to bind and activate transcription. So there may be a mismatch between the frequency of observed mutations in RNA sequencing and the number of observed mutations in the genome, but the general principles for detecting them are exactly the same.

Personalis EX2009.205

Q. And it's true, isn't it, that the abundance of a particular transcript can vary from sample to sample?

A. The abundance of almost anything can vary from sample to sample.

Q. So when you're referring to a low-abundance transcript in a particular sample, that may not be the same transcript in another sample; is that correct?

A. If I took two biopsies from a tumor -- and there have been very nice papers described -- published describing this. Tumors themselves exhibit an extraordinary amount of molecular heterogeneity, so if I were to take -- if -- there's a nice paper published I believe with a kidney tumor, where they took samples from the left- and right-hand side, and showed that mutational profiles of those cells were different. The RNA expression levels are going to be different. Every single cell is going to have potentially its own unique mutational profile and its own unique expression levels. So biology is heterogeneous.

Q. So it's possible that in a particular sample you have a relative low abundance of a particular transcript that corresponds to a gene that,

Personalis EX2009.206

in fact, has a high frequency of mutation?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: So the RNA transcript may appear in a large number of cells but may -- may be expressed in those cells at a low level. But the general approach to detecting these is going to be identical. We're talking about sequencing DNA. Right?

Q. BY MS. WANG: Nowhere does Levin actually give us the frequency of what it calls the low-abundance transcripts, correct?

A. I'll take your word for it. I don't recall him actually defining low-abundance transcripts explicitly.

I would assume that those are transcripts which are difficult to detect. Right?

Q. And you're aware that Levin also uses the term high-interest transcripts?

A. I'll take your word for it. I assume he does.

Q. Okay.

But are you aware of any indication that he provided the mutation frequency for these high-interest transcripts?

Personalis EX2009.207

A. So the best indication that we have in Levin of any kind of frequencies that was observed -- that were observed in the data, in fact, come from Table 2, where there's a discussion about fusion transcripts. That's a type of mutation. Right? And those are detected with different frequencies.

They may be -- that may be, as you alluded to earlier, because the genes are expressed at different levels. It may reflect heterogeneity in the samples.

But, again, Levin didn't start with a pre-identified frequency of mutations in designing his baits. Right?

But if he wanted to do that, he tells us that he could rescue low-frequency things by adjusting parameters of design, and if he wanted to find those -- the frequencies to mutations, he could look to COSMIC or he could look to Shah.

Q. Well, he said he could rescue low-abundance transcripts, correct? There's no mention of frequency.

MR. ROSATO: Objection. Form.

THE WITNESS: Anyone skilled in the art would understand that detecting a low-abundance transcript in a cDNA library uses the same general

Personalis EX2009.208

principles one would use to detect a low-frequency mutation in a population.

How do you do that? You enrich for that sequence and you enrich the number of reads, you increase the number of sequence reads.

So Levin actually instructs us that if you want to find something that's rare -- right? And different definitions of "rare" might apply -- that there are well-known molecular biology techniques that you can apply to optimize the detection of those rare events.

Q. BY MS. WANG: But he doesn't talk about rare frequencies, correct? He talks about rare abundance.

MR. ROSATO: Objection. Form.

THE WITNESS: Levin talks about low-abundance transcripts and adjusting parameters in design to be able to more reliably detect those low-abundance transcripts. But anyone skilled in the art would understand that's exactly the same set of techniques you'd use to identify a low-frequency mutation.

Q. BY MS. WANG: You mentioned Table 2 of Levin. Can we actually take a look at that?

A. Sure.

Personalis EX2009.209

Q. And this is the table that you referred to as the one example from Levin that mentions frequencies; is that correct?

A. I don't think it actually mentions frequencies, but it talks about the number of reads before and after enrichment.

Q. Do you see the caption underneath this table?

A. Yes.

Q. And do you see -- there's a little a, a little b, and a little c, and a little d in that caption. Do you see that?

A. Yes.

Q. And under little c it says, "Not included in hybrid selection bait genes." Do you see that?

A. I see where it says that, yes.

Q. And that's referring to, I believe, one -- or two of the fusion transcripts that are listed in this table; is that correct?

A. Yes, there are two that are listed here.

Q. Okay.

So these gene fusions that are marked with the c were detected despite the fact that Levin didn't actually use hybrid selection baits to go find them; is that correct?

Personalis EX2009.210

A. So according to what it says here, yes, they weren't included in the hybrid selection bait gene set. Right?

And that reflects the fact that there can be nonspecific hybridization that may capture these.

To really understand why, one would have to look at the gene sequences, one would have to look at the reaction conditions. But, yes, you can capture things which are not necessarily represented in your bait set.

Q. Okay.

If you can go to Page -- Figure 2 now, which is back a couple pages, on Page R115.3.

A. Yes.

Q. And you see Figure 2, it states in the caption, "Preservation of expression levels of target transcripts in hybrid selection. For each target transcript, the sequence coverage of the coding region is plotted before and after hybrid selection."

Do you see that?

A. Yes.

Q. Okay.

And so you agree with me that in Figure 2 it looks like the differences in transcript abundance were measured in terms of sequence coverage; is that

Personalis EX2009.211

correct?

A. I'm sorry. Can you rephrase the question?

Q. The differences in transcript abundance as shown in Figure 2 were measured in terms of sequence coverage?

A. I want to take a look at the figure and understand the caption again.

Okay. So can you ask your question again?

Q. Sure.

You would agree with me that Figure 2 shows that the differences in transcript abundance was measured in terms of sequence coverage?

MR. ROSATO: Objection. Form.

THE WITNESS: So what's plotted here is the CDS, the coding sequence, coverage before on the horizontal axis and after on the vertical axis of hybrid selection. Right?

So what this shows is that for different transcripts -- right? -- for these target transcripts, there's approximately a linear relationship in the sequence coverage for those individual target genes. Right?

So you're enriching the representation of

Personalis EX2009.212

those target genes.

Q. BY MS. WANG: So you're, in fact, preserving the transcript abundance of those particular target genes before and after hybridization; is that correct?

A. Well, if you look, there's a lot of spread. Right? So they're not exactly preserved. Some are better enriched than others. Right?

And, as Levin tells us, if there's something at the low end that we'd want to see -- right? -- more accurately, we could change our design parameters to change these.

Q. Sure, but based on Figure 2, which appears to be what Levin actually did, you'll agree with me that this shows that there was a preservation of the expression levels of the target transcripts before and after hybrid selection, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: There's a correlation between the two. I wouldn't say they're preserved. Right? You've enriched the number of reads.

And so what Levin shows us is we have a technology that we can use to enrich representation of target sequences. Right? And in this case his design parameters might have been to preserve these linear --

Personalis EX2009.213

these linear associations.

But, again, he tells us we can do a variety of different things to represent or to enhance different levels.

So the fact that I can take low-abundance things and increase them and high-abundance things and increase them means that I could have easily adjusted the parameters to increase even more the low abundance or to diminish the increase in the high abundance.

Q. BY MS. WANG: But in Levin, according to this figure, what he did was take low abundance and high abundance and middle abundance and increase them all according to that linear relationship, correct?

A. They approximately increased with a linear relationship, yes. The efficiencies weren't identical for all of them, but there's a general linear relationship between the number of sequence reads mapping to a transcript before and after enrichment.

MS. WANG: Let's take a quick break.

THE WITNESS: Sure.

THE VIDEOGRAPHER: Going off the record. The time is 2:51 p.m.

(Short recess.)

THE VIDEOGRAPHER: We are back on the

Personalis EX2009.214

record. The time is 3:01 p.m.

Q. BY MS. WANG: Dr. Quackenbush, just a couple more questions on Levin.

A. Sure.

Q. Do you recall whether Levin actually ever mentions the word "pharmacogenomic"?

A. I'm not sure. I'd have to look.

Q. Do you have any memory of whether the term PGx was used in Levin?

A. Again, I'm not sure. I'd have to look.

Q. Do you recall any characterization of -- in Levin of any SNP as a pharmacogenomic or PGx SNP?

A. I don't recall that he characterizes SNPs those ways, but anyone skilled in the art would know that if you determine one SNP you could determine SNPs which are in -- of pharmacogenomic relevance.

To actually know whether he sequenced any genes that are of pharmacogenetic relevance, I'd have to look at the entire gene list of all 467 genes. So I'm not sure what's in there.

Q. Do you recall if Levin talks about designing any baits to target SNPs that are pharmacogenomic?

MR. ROSATO: Objection. Form, asked and answered.

Personalis EX2009.215

THE WITNESS: Again, I don't recall Levin talking about designing those specific types of baits, but one could easily design bait sets given the principles in Levin to look at individual genes. Right?

Levin chose a subset of genes. He could have easily chosen genes that are of pharmacogenomic significance.

Q. BY MS. WANG: And we talked about a little bit how Levin is describing targeted RNA-Seq, correct?

A. Yes. I believe that's how Levin characterizes the paper.

Q. So Levin is not detecting any mutations in introns; is that correct?

A. The principles for detecting mutations in introns are the same as in exons. I don't actually know if he -- I don't think he's reported any.

It's not clear whether or not he detected any, because remember that when messenger RNA is made, sometimes those RNAs are not fully spliced, so they may or may not have been able to detect mutations in introns. But the principle is exactly the same to what he described.

Q. But what he's described or reported does

Personalis EX2009.216

not include designing of bait sets to target mutations in introns, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: He designed -- he describes designing bait sets to target a particular subset of genes.

And, again, it's a demonstration that the technology, which was widely used in genomic sequencing, could be applied to cDNA sequencing. Right?

And anyone would recognize that if you detect mutations in coding regions from cDNA libraries, you could detect mutations in introns or in coding regions or in intragenic regions from genomic DNA.

Q. BY MS. WANG: But Levin does not specifically describe or report that he designed bait sets to target mutations in introns, correct?

MR. ROSATO: Objection. Form, asked and answered.

THE WITNESS: Levin describes application of a well-known technology for enrichment and sequencing of cDNA libraries. There are unlikely to be a lot of introns in those cDNA libraries.

So whether he detected them or not, I

Personalis EX2009.217

don't know. He could have easily applied the design principles he used to detect those, though, in genomic DNA.

Q. BY MS. WANG: But you don't know if he actually designed baits to target introns, correct?

MR. ROSATO: Same objection.

THE WITNESS: I don't know whether or not he did. I don't suspect that he designed bait sets to target introns. The design principles for the baits -- the bait sets tell us that they tile across the CDS, the coding sequences, so it's unlikely that they were specifically designed for those.

But, again, the design principles are exactly the same. If you look at, you know, 467 genes, it's a large stretch of DNA, and if I can capture DNA I can capture DNA, if I can sequence DNA I can sequence DNA, if I can detect mutations I can detect mutations, and as he tells us, if we want to find rare things we can adjust the parameters to allows us to do that.

Q. BY MS. WANG: Levin also doesn't report any copy number alterations that he detected, correct?

A. I don't believe he reported that, no.

Q. And Levin also does not report that he's detected any one-copy deletions of terminal exons; is

Personalis EX2009.218

that correct?

A. Well, it's interesting. Right? What Levin actually talks about are detection of fusion transcripts and splice isoform identification. So I don't know whether or not because I haven't looked and analyzed each and every one of those genes, but a splice isoform can include deletion of terminal exons. Right?

And a fusion transcript means two genes are fused together, so in a sense -- right? -- the terminal exons of one gene are deleted and connected to another gene.

So while I don't know if he uses that exact phrase, he clearly describes detections of things which are relevant to that, and, in fact, if you look at what he's detected, he's detected two or more different classes of genetic variation and alteration.

Q. But Levin doesn't report that he designed baits specifically to target one-copy deletions of terminal exons, does he?

MR. ROSATO: Objection. Form.

THE WITNESS: The way Levin describes his bait design does not preclude the detection of those. Whether or not he specifically designed them or not, I

Personalis EX2009.219

can't say. I can only tell you what it says here in the protocol. But the principles are exactly the same.

Q. BY MS. WANG: Let's go to Exhibit 1005 in your binder, which is Shah.

MR. ROSATO: Thank you.

Q. BY MS. WANG: Are you there?

A. Yes.

Q. And this is the Shah reference that you talked about earlier, correct?

A. Yes.

Q. Had you read Shah when it was published in 2009?

A. I actually think I did. I know Sohrab pretty well, I know Marco Marra, and a lot of the people involved here. So I think I read this when it was published.

I may have actually reviewed it before it was published, but I -- I'm not completely sure.

Q. Shah is talking about at a broad level a breast tumor that they profiled over a course of about nine years; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I'd have to look at this. I believe they looked initially at a -- an archival

Personalis EX2009.220

breast sample that was preserved as a formalin-fixed paraffin-embedded sample, and a more recent biopsy, and I also believe they then validated their methods by looking at a larger population of samples.

Q. BY MS. WANG: Nowhere in Shah is the word "bait" used, correct?

A. I don't know. I'll take your word for it.

Q. And nowhere in Shah is the phrase "bait set" used?

A. Again, I don't recall, but I'll take your word for it.

Q. Now, Shah does not say that it designed a bait set for selecting somatic mutations at a frequency of about five percent or less, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Actually, what Shah does, and -- what Shah does is he demonstrates a technology that can be used to determine relative frequencies of mutations. And if you look at Table -- I believe it's Table --

Let me see which table it is.

In my report it appears in Paragraph 125, and -- yeah, it's Table 3 in the paper as well.

So if we look at Paragraph 5, the

Personalis EX2009.221

frequency of different genetic variants -- this time it's in genomic DNA -- some of those variants appear at frequencies below five percent. So Shah demonstrates that you can determine the relative frequencies of mutations at a -- at a wide range of abundances and samples.

Q. BY MS. WANG: And all of the frequencies I think you mentioned in Table 3 are of genomic DNA; is that correct?

A. I believe this is all done in genomic DNA, yes.

Q. Shah does not describe designing a particular bait set to target a somatic mutation that appears at a frequency of less than five percent, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Shah describes a technology that one can use to assay different frequencies of mutations, and then if one looks at what Levin does, Levin describes how one designs bait sets, and can adjust those bait sets to determine low- and high-abundance elements within the DNA sample being profiled.

Q. BY MS. WANG: Right, but I'm talking about Shah, not Levin, now. Shah itself does not

describe designing a bait set to target somatic mutations with a frequency of five percent or less, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So one would have to really look at this. Right?

What -- what Shah does is he describes, I believe, a PCR-based protocol to amplify different genomic regions. Those -- and then sequence them. Those genomic regions include low, high and middle frequency mutations. They include lots of different mutations and different mutation types.

So what his motivation was in designing his set of reagents for this reaction, I'm not sure, but he clearly demonstrates that one can detect those using conventional methods.

Q. BY MS. WANG: And you're relying on the fact that he used PCR to amplify particular sequences that have these mutations; is that correct?

A. I don't know what you mean by "relying on."

MR. ROSATO: Yeah. Objection. Form, foundation.

THE WITNESS: But I could read the methods. I believe most of the methods for his

Personalis EX2009.223

library construction are in a supplemental -- in one of the supplemental materials. And I don't have those handy.

If you can provide them to me, I'd be happy to take a look at them and tell you exactly what methodology he used.

But clearly what he demonstrated is that one skilled in the art could identify these, could measure mutation frequencies, and then if we think about what Levin tells us, one can design bait sets to detect them.

Q. BY MS. WANG: Sure. But, again, I'm not talking about Levin, I'm talking about what Shah itself describes, and Shah, I think I asked you previously, does not describe designing bait sets to target somatic mutations at a frequency of five percent or less, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: I would like to look at the supplemental material to know what's described there. I can't speak to his motivation, but clearly what he demonstrates is one can design a set of assays that can determine low-frequency variants in a DNA sample.

Q. BY MS. WANG: Do you have any

Personalis EX2009.224

understanding of whether or not Shah ever mentions the word "pharmacogenomic"?

A. I don't believe Shah reports any detection of pharmacogenomic SNPs or genetic variants or describes mutations in pharmacogenomic genes, but the principles are exactly the same as detecting variants in other genes.

Q. Let's go to Exhibit 1006 in your binder, which I believe is Shah Supplement 1.

A. So I actually don't have that.

Q. Oh, you don't have a copy of that in your binder. Excellent. Then I will give you a copy here.

A. Do you want to mark this?

Q. It's already marked. It's --

A. Okay.

Q. And have you seen this before?

A. Yes, I have.

Q. Okay.

And this is Shah Supplement 1, Guardant Exhibit 1006, correct?

A. Guardant Exhibit 1006. I think it's Supplementary Information 1. Table 1.

Q. Now, if you can flip through to Page 8.

A. All right.

Q. And do you see that there is a subheading

Personalis EX2009.225

here that are -- well, actually, there are two subheadings. One is "Mutation revalidation" and the other one says "Frequency analysis"?

A. Sorry. I was on the wrong page.

Yes.

Q. And does this describe to you what Shah did, in terms of looking at frequency?

MR. ROSATO: Objection. Form.

THE WITNESS: So I've been rereading this to try to understand specifically what this paragraph refers to. The good news is, looking back on Page 4, there are actually two genes on the bottom which are pharmacogenomic genes, ABCB11 and SLC24A4. They're multipass membrane solute transporters, which are involved in the transport of drugs, where they've actually discovered some novel mutations.

So if you want to talk about pharmacogenomics, those are there.

In the entire protocol they talk about multisteps of the analysis, and this frequency analysis appears to describe a protocol where they've identified mutations and then gone in and performed targeted assays to identify the relative frequencies.

So your question about this was?

Q. BY MS. WANG: So my question is -- so let

Personalis EX2009.226

me take it in parts, based on what you just said.

A. Okay.

Q. Based upon this protocol, the mutations are previously detected using sequencing, correct?

A. They were -- yes, they appear to be detected using sequencing of these samples.

Q. And then they were validated by using PCR with Sanger sequencing, according to this?

A. So they were validated initially by Sanger sequencing. Right? So they took the assays that came from these next-generation -- from the initial sequencing using next-generation sequencing. They then performed an additional validation using an older technology. So I'm not sure exactly how they prepared the sequencing libraries here, but clearly they used Sanger sequencing to verify that those mutations were actually there using an independent technology. They then used another protocol to actually measure their frequencies.

Q. And that protocol is described under "Frequency analysis"; is that correct?

A. It's described in part there, yes.

Q. And when you say "in part," where else is it described?

A. It's -- I don't know where else it might

Personalis EX2009.227

be described. I mean, they clearly don't provide all of the details for what they did. But they give us a general outline of how they validated the frequency of these -- or measured the frequency of these mutations.

Q. And what they were using are PCR amplicons to measure the frequency; is that correct?

A. They -- I wouldn't say they used PCR amplicons to measure the frequency. They used sequencing to measure the frequency. They used PCR to amplify subgenomic regions, that they then sequenced to determine the frequency of these mutations.

Q. And those subgenomic regions are the regions where mutations had been confirmed via sequencing previously to be located; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So a quick read of this suggests that they identified mutations, they validated those mutations -- the presence of those mutations using Sanger sequencing, and then they used this additional protocol to measure their frequency.

Q. BY MS. WANG: Right. I'm referring you to the sentence in the middle -- perhaps this will be helpful -- where it says, "Amplicons were therefore designed to span the target mutation positions with short amplicons (approximately a hundred base pair)

Personalis EX2009.228

such that the mutation position could be reached with 75 base pair Illumina reads."

Do you see that?

A. Yes.

Q. So the target mutation positions were those described by the mutation sequencing, the sequencing described earlier?

A. I'm assuming those are mutations they identified in these samples, yes.

Q. Now, nowhere in this description of frequency analysis is there any discussion of designing baits; is that right?

A. Well --

MR. ROSATO: Objection. Form.

THE WITNESS: Again, the PCR primers -- the principles we use to design those primers are essentially identical to those which are used to design baits. Right? They're synthetic oligonucleotides.

Nevertheless, whether or not they describe baits here doesn't matter. What they tell us they can do is they have a protocol for measuring frequencies. Once we know those frequencies, we could, as Levin teaches us, design baits to capture them, and we can adjust the relative concentrations.

Personalis EX2009.229

That's what anyone working in cancer would do.

Q. BY MS. WANG: But you'll agree with me that in Shah there's no description of any bait design, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: They clearly design amplification primers, and the approach you would use to design any synthetic oligonucleotide DNA or RNA follows the same basic principles. So they're designing oligonucleotides; they don't refer to them as baits.

Q. BY MS. WANG: Is it your opinion, based upon your experience in designing -- I think you mentioned this morning millions of primers -- that you would use the exact same design principles to design those millions of primers as you would baits for purposes of solution-phase hybridization?

MR. ROSATO: Objection. Form.

THE WITNESS: The thermodynamics and kinetics of oligonucleotides are fairly universal. Would I use the same parameters to design oligonucleotides for one application or another? No. It's going to depend on the context. But the principles are the same. Right?

Personalis EX2009.230

We can talk about principles in science anywhere. The same principles that describe how a paper airplane flies describe how a 747 flies, but, you know, would I design a 747 -- now they're retired, but, you know, a jet airliner with the same exact design parameters you use for a paper airplane? No.

Q. BY MS. WANG: So you would agree that there are different design parameters for baits as they would be for primers; is that correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: The design principles are universal. For each application I'm going to choose parameters that are relevant for my goals in that particular experiment.

So I may call them baits and choose one set of parameters; I may call them primers and choose exactly the same set of parameters. It depends on how I'm going to use them and what I'm going to do.

Oligonucleotides bind through complementary binding of DNA bases across strands, and that is a universal fact.

Q. BY MS. WANG: Now, just as we talked about in Shah specifically, does -- Shah does not describe designing a bait set for detecting copy

Personalis EX2009.231

number alterations, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So I'm not sure if Shah actually detects copy number alterations. I'm not sure he reports copy number alterations. But the design principles for detecting copy number alterations are identical to those to detecting other types of alterations.

I published a paper a few years ago describing a method called ExomeCNV, that uses exome sequencing to detect copy number variation, and what that method describes is an approach which simply assays genomic regions -- in this case exons -- which are close to each other to look for a consistent pattern of overabundance in a particular region.

So whether or not he describes it, one skilled in the art would know how to do it based on what Shah describes.

Q. BY MS. WANG: But Shah does not specifically report that he designed any baits to target these particular copy number alterations, correct?

MR. ROSATO: Objection. Form, foundation.

Personalis EX2009.232

THE WITNESS: The method described in Shah could be easily used to detect copy number variations. The fact that he didn't detect them in these samples doesn't -- doesn't mean that he could not have detected them. And, in fact, it may simply be that those copy number alterations didn't exist in the samples he analyzed.

Q. BY MS. WANG: I'm asking you as to what Shah reported he actually did. And my question is: Does Shah report that he actually designed baits to detect copy number variations for alterations?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: I don't know all the design principles Shah was thinking about when he put together the protocols he used to measure genomic variants. I can tell you that in the sequencing protocols he describes, one could detect them.

So I don't know his motivation, but I'm telling you one skilled in the art would have no reason not to use this kind of approach in exactly the way it's described to try to assay the relative frequency of copy number variations.

One skilled in the art would also know one could use the methods described in Levin to assay

Personalis EX2009.233

those copy number variations.

Q. BY MS. WANG: But based upon what Shah actually states, you don't know whether Shah designed his baits or his primers or anything to detect copy number alterations, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: I have no idea what was in Shah's mind when he put together the protocol. I can tell you what he reported, and he didn't report having identified those copy number variations.

If I don't find something in a paper, I may not report it simply because for the samples I analyzed it wasn't relevant. But anyone skilled in the art would understand that these basic protocols could be easily adapted to simple optimization and design paradigm to be used to detect CNVs.

Q. BY MS. WANG: Shah also does not report that they, in fact, designed baits to detect structural break points in introns, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: Could you repeat your question, please?

Q. BY MS. WANG: Sure.

Shah also does not report that they, in

Personalis EX2009.234

fact, designed baits to detect structural break points in introns, correct?

MR. ROSATO: Same objection.

THE WITNESS: So if we look at what Shah describes, the patent actually discusses having -- identifying genomic alterations that fall in a variety of different classes. Right? And asks to identify two or more. And Shah clearly identifies at its face, from the table I referred to earlier, genetic variants that occur at different frequencies of mutations.

Whether or not he identified alterations in a particular intron, I don't recall that he reported that. Whether or not he designed any of his assays to detect that, I don't know; he didn't report it.

But in my report I refer to a paper by Chmielecki, who uses baits to identify break points in introns and exons. And the whole point is that these technologies can be broadly applied, and anyone skilled in the art would recognize that if I can detect a copy number variation or a deletion or a mutation, I can also detect break points in introns.

Q. BY MS. WANG: You would agree with me that Shah does not describe designing any bait sets to determine structural break points in introns with a

Personalis EX2009.235

unique preselected efficiency for selection, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: I don't -- I don't know exactly how Shah -- how Shah designed his assays. I can know he -- I know he didn't detect them and report them. He didn't detect those kind of break points.

But others at the same time reported applications that detected those, and, again, the design principles are the same. Right?

Anyone skilled in the art could have easily taken what's described in Shah and detected break points, could have taken that -- and we can argue whether PCR primers are baits or not, but taken Levin and designed baits to detect those. And could refer to Paragraph -- to Page 6 in Levin where Levin tells us you can adjust the parameters to be able to detect even rare things. Right?

This is all a simple optimization problem based on applying known technologies. Right?

Anyone skilled in the art would recognize there are a wide range of things that you could detect. Those aren't necessarily all detected in each individual cancer patient. That's really what's important, is we design these broad assays that are

Personalis EX2009.236

generally applicable so we can detect in every patient the spectrum of what we believe are the most relevant mutations. That's the goal of precision medicine. And that's been what we've all been working toward.

Q. BY MS. WANG: So I just want to go back again with respect to Shah itself.

A. Yes.

Q. You would agree with me that Shah does not describe designing any bait sets with any particular unique preselected efficiencies for selection?

MR. ROSATO: Objection. Form, foundation.

Q. BY MS. WANG: Correct?

A. I don't know exactly all the parameters that were used by Shah to design his assays, so I can't answer that.

Whether or not he does, Levin teaches us how to do that.

Q. I'm going to go back. You said you can't answer that, but the answer is you don't know if he, in fact, designed any bait sets whatsoever with unique preselected efficiencies of selection; is that correct?

MR. ROSATO: Objection. Form,

Personalis EX2009.237

foundation.

THE WITNESS: I can't read Shah's mind. I don't know what he did.

I can -- I know what he describes. He doesn't describe specifically that, but anyone skilled in the art would recognize that what he described could be -- could be adapted to detect a wide range of different variants and could be adapted to do that with a wide range of different efficiencies.

Q. BY MS. WANG: Sorry. It seems as if we're still going in circles here.

You don't know whether Shah, based upon the disclosure of Shah -- whether Shah, in fact, designed bait sets with any unique preselected efficiencies for selection, correct?

MR. ROSATO: Objection.

Q. BY MS. WANG: You don't know?

MR. ROSATO: Form, foundation, asked and answered.

THE WITNESS: Anyone designing any kind of oligonucleotides for any kind of assay is going to have design parameters that are going to reflect the efficiencies of the experiment. Right?

So, you know, if we want to craft exact language, does Shah use that language? No. Was that

Personalis EX2009.238

a factor in his design? I would say almost certainly that he took efficiency of design of oligonucleotides into account when doing this. Right?

What was in his mind, I don't know. I can report that he didn't -- I can confirm that he didn't report finding certain things, but I don't know exactly what he was thinking about when he designed these assays.

Q. BY MS. WANG: We're not talking about efficiency of design; we're talking about efficiency of -- efficiency of selection. Okay? So I want to -- I just want to make sure we're talking about the same thing here.

Do you know whether Shah designed baits with unique preselected efficiencies for selection? Yes or no?

MR. ROSATO: Objection.

Q. BY MS. WANG: Do you know or not?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: The -- the assays that Shah designed would have different efficiencies, and I'm sure he took those into account in his design. He doesn't report that, but anyone skilled in the art would do that.

Personalis EX2009.239

Q. BY MS. WANG: So you assume that he designed them with unique preselected efficiencies for selection, but you don't know whether he did or not; is that correct?

A. Anyone --

MR. ROSATO: Objection. Form.

THE WITNESS: Anyone skilled in the art would take a wide variety of factors into account. Sohrab Shah is an outstanding scientist, someone I've known for a long time. I don't know what his thought process was, but I can be pretty confident that other people skilled in the art would take into account efficiencies when designing assays.

Q. BY MS. WANG: We can go round and round on the same question over and over for the next three hours. My next question, again, is: Do you know, yes or no, whether Shah designed bait sets with unique preselected efficiencies for selections?

MR. ROSATO: Actually, we can't go around. So let's --

MS. WANG: If you have an objection --

MR. ROSATO: -- move on.

MS. WANG: -- state your objection. Otherwise please --

MR. ROSATO: Objection. Asked and

Personalis EX2009.240

answered.

MS. WANG: -- let your witness answer the question.

MR. ROSATO: Form.

MS. WANG: He has not answered the question.

MR. ROSATO: He's answered. This is getting to be badgering at this point.

MS. WANG: You're welcome to take that up with the Board if you would like. He has not answered my question.

I'm letting him speak at length about the topics of which he was prepared for. I'm asking him this question, which is: Do you know, Dr. Quackenbush, whether Shah designed bait sets that have a unique preselected efficiency for selection?

MR. ROSATO: Objection. Asked and answered.

THE WITNESS: Sohrab Shah obviously took into account a wide variety of factors when designing these assays. I cannot speak to what was in the mind of Dr. Shah when he designed these assays, but anyone skilled in the art would take into account the efficiency of the entire reaction, including the binding of oligonucleotide primers to targets, when

Personalis EX2009.241

designing assays. That's what one does in molecular biology.

So whether he reported it or not, it's relatively safe to assume that he probably considered those things. Whether he reports it or not, that's what someone skilled in the art would do, and Dr. Shah is clearly skilled in the art.

Q. BY MS. WANG: Still not an answer to my question. I'm not talking about the efficiency of the entire reaction, Dr. Quackenbush.

You understand the term "preselected efficiency for selection" is a term that is described in the '830 patent, correct?

A. It's a term --

MR. ROSATO: Objection. Form.

THE WITNESS: -- which is used in the '830 patent.

Q. BY MS. WANG: And you yourself have opined on what that term means, correct?

A. I have provided some interpretation of that term, and if we look at that term, we understand that that term is actually construed in part of a large process, which includes things like DNA sequencing. So it doesn't stand on its own. Right?

It's part of a larger protocol. And one

Personalis EX2009.242

has to interpret it in that context -- right? -- as one skilled in the art would interpret it at the time the patent was filed.

Q. Dr. Quackenbush, if you turn back to the exhibit that was marked as your expert declaration in the litigation. I believe that was Exhibit -- I think 3 or 4.

A. This one?

Q. What number is that marked as?

A. Exhibit 4.

Q. Yes, Exhibit 4, please.

Can you please turn to Page 25, Paragraph 63 of your exhibit.

MR. ROSATO: I'm sorry. Just so I'm -- are you talking about the one from 10x? Mine wasn't marked.

MS. WANG: No. The one from the declaration.

MR. ROSATO: Okay. Thank you.

I think we had standing objections on this on any questions, so we'll just remind that we're maintaining those.

THE WITNESS: So can you tell me which page you're referring to, please?

Q. BY MS. WANG: Page 25, Paragraph 63,

Personalis EX2009.243

please.

Do you see Paragraph 63?

A. I see it.

Q. And this is in your declaration, which you submitted in the District Court litigation regarding the same patent, correct?

A. I believe so, yes.

Q. And you state in Paragraph 63, "As explained above, the claims recite choosing a plurality of bait sets (at least two), each having its own 'unique preselected efficiency for selection' for its targeted subgenomic interval." You then state, "As defined in the specification, 'efficiency of selection' refers to the level or depth of sequencing coverage as it is adjusted according to a target subgenomic interval(s).'"

Do you see that?

A. I see where it says that, yes.

Q. And then you state, "Therefore, bait sets are chosen based on the amount of enrichment necessary to achieve the respectively unique preselected depths of coverage."

Do you see that?

A. Yes, I do.

Q. So my question to you, sir, is: Based

Personalis EX2009.244

upon what is stated in Shah, does Shah discuss or describe designing bait sets that are chosen based on the amount of enrichment necessary to achieve the respectively unique preselected depths of coverage as stated in Paragraph 63?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: So let's take a look at this paragraph, because I explained this earlier. Right? "As explained above, the claims recite choosing a plurality of bait sets," and we'll -- at least two, right? -- "having" -- and those two refer to large classes of different variants that one would detect -- "each having its own 'unique preselected effic" -- "preselected efficiency for selection' for its targeted subgenomic interval." Right?

So the bait sets in the '430 patent are designed to hybridize to DNA. Right?

If I hybridize to that DNA, in solution and attach to beads, I know nothing. Right? Nothing at all.

If I just design baits and let them hybridize, that predetermined efficiency can't be known, can't be measured, can't be determined. Right?

Personalis EX2009.245

What one has to do is -- "As defined in the specifications, the 'efficiency of selection' refers to the level or depth of sequence coverage as it is adjusted according to a target subgenomic interval or intervals." Right?

So if I design a set of oligonucleotides and let them attach to stuff, until I perform a series of additional steps, I can't measure their efficiency. Right?

I only measure their efficiency after I carry out a series of other reactions. Right? Including, in this case, DNA sequencing. So it's part of a larger, more complex process.

Shah is measuring mutations. He sees low frequencies. Do I know exactly whether or not he altered parameters between a relatively -- what appears to be relatively high-frequency or common mutation and a low-frequency mutation? I don't know.

I would tell you that one skilled in the art would probably do that, to try to assure that you actually see the things that you're looking at. And if I have a low-frequency mutation, I'd want to generate lots of reads, because it's harder to detect. It's a counting experiment.

So I don't know how Shah adjusted the

Personalis EX2009.246

parameters. One would assume that he's smart enough that if something appears to be rare, he would probably adjust the parameters appropriately.

Does he describe that? No. Would I fault him for it? No. Because anyone would know to do that.

Q. BY MS. WANG: I'm sure that was the answer to some question. My question is still: Based upon the language "preselected" -- or "unique preselected efficiency for selection" -- that's the language we're talking about.

A. Mm-hmm.

Q. Shah does not describe or report that he designed any bait sets with unique preselected efficiencies for selection, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: If we look at Table 2 -- right? We can just focus in Shah on Table 2.

Sorry. Table 3.

Table 3 reports detection of mutations occurring at a wide range of different frequencies. I would assume that Dr. Shah, in designing the assays to measure these, took into account what was observed in the previous rounds of experiments. Right?

Personalis EX2009.247

Do I know exactly how he did it? No. Do I know how he adjusted parameters? No. But it's one of those things that would be pretty obvious to someone who works in this field that if you want to really measure accurately something which is rare, you would try to sequence it at greater depth.

Q. BY MS. WANG: I'm not asking about trying to sequence at a greater depth; I'm asking about designing baits -- specifically designing bait sets with a unique preselected efficiency for selection.

Shah does not describe in Table 3 or otherwise designing bait sets with a unique preselected efficiency for selection, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: So if we turn back to Exhibit 4, which you referred me to earlier -- right?

If we look at Paragraph 63, how do we determine the efficiency of selection? We determine the efficiency of selection by DNA sequencing. Right? Do we agree on that?

Q. BY MS. WANG: I -- I'm not answering your questions, Dr. Quackenbush. Please --

A. Well, you keep on --

Q. What I'm asking --

Personalis EX2009.248

A. You keep on asking me the same question over and over again, and I've explained to you that efficiency of selection -- right? -- refers to the level or depth of sequence coverage. So we can't decouple sequencing from efficiency. Right?

Efficiency of selection in the patent itself is -- refers to the level or depth of sequence coverage.

You've asked me the same question multiple times, and I've told you the same -- I'm giving you variations on the same answer, that in order to determine the efficiency, we have to know the depth of sequence coverage. Right?

And so I can't decouple those two in answering those questions. You're asking me about efficiency. Efficiency refers to sequencing.

If I think about sequencing, if I have a rare event, I'm going to sequence it at greater depth.

Shah obviously knew that. Levin tells us how to do it. Right? To adjust our design parameters.

Shah I'm sure knew how to adjust design parameters. Whether or not he did it or was in the back of his mind, I don't know. I can assume he did that, particularly since he detected these variants at

Personalis EX2009.249

low frequencies.

In fact, if we look at Table 3, one of the columns says "Primary depth," and you'll notice there's a wide range of depth of sequence.

Now, did he design for those explicitly? I don't know. But clearly there are different efficiencies in his assay that are reflected in the depth of sequence coverage. Right?

In fact, there's no number which is repeated.

Now, I don't know what was in his mind when he set out to design this, but clearly he was capable of designing assays which had different depths of sequence coverage, which we would read back on being an efficiency of selection. Right?

Did he design for that ahead of time? I don't know. But I'm assuming if he wanted to detect rare events -- right? -- these very low-frequency mutations, he would probably sequence them at greater depth, since that's what one skilled in the art would do.

MS. WANG: Let's take a break.

THE VIDEOGRAPHER: Here marks the end of Media Unit No. 3 in the deposition of John Quackenbush. We are going off the record, and the

Personalis EX2009.250

time is 3:49 p.m.

(Short recess, at which time Mr. Smith excused himself from the proceedings.)

THE VIDEOGRAPHER: Here marks the beginning of Media Unit No. 4 in the deposition of John Quackenbush. We are back on the record, and the time is 4:09 p.m.

Q. BY MS. WANG: Dr. Quackenbush, previously we talked about how tumors, I believe you said, were heterogeneous; is that correct?

A. I probably said that, yes. Tumors are heterogeneous.

Q. And so if you -- in an example I think you used previously, if you had two biopsies of the same tumor, you may have different frequencies of mutations in the cells from each of those biopsies; is that correct?

A. Different frequencies, different spectrum.

Q. Okay.

A. You may have mutations in one that don't occur in the other.

Q. So in order to have a reliable sample size, you would need multiple biopsies, is that

Personalis EX2009.251

correct, to establish a common frequency of mutation?

MR. ROSATO: Objection. Form.

THE WITNESS: It -- I mean, it depends on the assay and what you want to do. Right?

You could look at multiple biopsies and look at the frequency mutation in that tumor, you can look at a population. It really depends on what you want to do.

You could look at one tumor and simply assay the frequency there. So -- or one site.

So it depends on your application, how you're going to use the data, what you want to discover.

Q. BY MS. WANG: If you wanted to understand or have an accurate measurement of a mutation frequency within a particular tumor, would you expect to want multiple biopsies and then average the data?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, what you would do would depend on the assay. If I had a small tumor I, might sequence the entire tumor. Right?

It -- it really depends.

The example I was talking about was a fairly large kidney tumor, where people looked at the heterogeneity. Part of the reason they looked at such

Personalis EX2009.252

a large tumor is one would expect over the course of its growth and development, progression of the disease, it would pick up lots of mutations. Some of those mutations may be clinically relevant, some of them may not.

So what you want to use to estimate frequency is going to depend on how you're going to use the data and information.

Q. BY MS. WANG: Would you generally agree with me that the bigger the sample size the more reliable your data is going to be?

A. When we talk about sample size, again, we can talk about lots of different things. Right?

So if I'm going to sample population, I'm going to give a survey. Voter preference. If I ask everybody in the country, I get a much better estimate than if I pick a thousand people. If I pick those thousand people, you know, using good design criteria, then I can get a fairly accurate representation of the entire population.

So design of the experiment in the survey is going to influence the accuracy, the reliability of the measurements you make. Right?

If I'm going to look at a population frequency of mutations or genetic variants, if I look

Personalis EX2009.253

at a larger population, I'm going to get a better estimate of the frequencies, I'm going to be more likely to detect rare variants.

But, you know, if I'm sequencing a tumor and I want to find rare variants, I can sequence them in greater depth. If I sequence lots of different biopsies at very low depth, I may not discover anything meaningful.

So, you know, how you optimize the design of your experiment is going to really be influenced by the questions you want to ask, how you want to use the data, and you want to choose smart design.

Q. But in terms of -- I think you just said if you're looking at a larger population you'll get a better sense of -- or a better estimate of the frequencies. With respect to tumor samples, with a larger amount of tumor samples, would you have a better estimate as to the mutation frequencies within the tumor?

MR. ROSATO: Objection. Form.

THE WITNESS: It would depend on other parameters in the experiment. If I took a thousand biopsies from a tumor and sequenced them at very low depth, I might not discover anything. If I use targeted sequencing and sequence them at very great

Personalis EX2009.254

depth, then I might discover things. Right?

What you discover depends on more things than just counting how many biopsies you take.

Q. BY MS. WANG: So suffice it to say there's not a uniform way of determining mutation frequencies within a tumor; is that correct?

A. There are lots of different ways that one can use to determine mutation frequencies. Right?

This is molecular biology. And so, yes, there's not a way. There are many ways.

And, you know, again, in designing an experiment, one would optimize the design of that experiment for a particular endpoint one hoped to achieve or a particular measurement one might like to make.

Q. Do you have Exhibit 1008 in your binder?

A. I don't think I do.

Q. Okay.

MR. ROSATO: Thanks.

Q. BY MS. WANG: I'm going to show you what is actually an excerpted copy of Exhibit 1008, and you can see that on the bottom of the document what the pages that are excerpted. Do you see that?

A. No, I don't.

Q. So at the bottom of your document it says

Personalis EX2009.255

"Guardant - Exhibit 1008," and then there's 0001. Do you see that?

A. Yes.

Q. And then if you go to the next page, it says "Table 9," and the bottom of that says 0798. Do you see that?

A. Yes.

Q. And then if you flip through the rest, it goes from 0799 all the way through to 0819.

A. Okay.

MR. ROSATO: Counsel, just so I'm clear, is this just less than all of the pages to the exhibit?

MS. WANG: Yes. There are over 900 pages, so I'm representing to you that this is an excerpt of Pages 0798 through 0819 of Exhibit 1008.

Q. And you've confirmed, Dr. Quackenbush, that those page numbers do appear in the document that you're looking at?

A. Those numbers are in the document, yes.

Q. Okay.

And you see the first page of this document states that this is Shah, and then Supplementary Tables S1 through S12? Do you see that?

A. Yes.

Personalis EX2009.256

Q. And have you looked at this before?
A. So the entire document is S1 through S12?
Q. Not the entire document. This is an excerpt of S1 through --
A. Right. The original file -- the online file --
Q. Yes.
A. -- is all -- okay. So these are all 12 tables, and you're telling me this is only Table 9 from those 12?
Q. Correct.
A. Okay.
Q. Have you looked at Table 9 before?
A. I've probably looked through it, yes. It's one of the supplemental -- it's part of one of the supplemental files for the Shah paper.
Q. Okay.
Then I'm also going to give you another excerpt from the same list of tables, and you'll see that this one starts on Page 0981 and it says "Table S3" --
A. Okay.
Q. -- and ends at 0983. Do you see that?
A. Yes.
Q. Okay.

Personalis EX2009.257

And I'll represent to you that this is a copy of Table S3 from this larger document.

A. Okay.

Q. And have you seen this table before?

A. Yes.

Q. Okay.

And this table -- the one that we're looking at right now is S3. This table lists germline mutations; is that correct?

A. I don't have the table legend. What this says is that one of the table headings is "confirmed germline indel." So an indel is an insertion or deletion. It's one of a subset of mutations but it's a type of genetic mutation. It's not inclusive of all types of genetic mutations.

Q. But this doesn't list in any header or anything any somatic mutations; is that correct?

A. I don't know if these are somatic or germline mutations. I would assume that these are --

Well, I don't have the table headings or the table legend, so I don't recall whether or not this is a somatic mutation or germline mutation.

I would guess that it's probably a germline mutation -- or sorry. I would guess it's probably a somatic mutation, but, again, I have -- I

Personalis EX2009.258

don't have the context right now to tell you exactly what's here.

Would it be possible to get the entire supplement?

Q. I don't have the entire supplement but I just want to ask you a couple questions about this and then we'll -- we'll get to the next part of this.

With respect to the confirmed germline indel, is it your understanding that that does not tell you that this is dealing with germline mutations?

A. Oh. It does say "germline indel." Sorry. I didn't see that earlier.

Q. Yeah.

A. Again, it's hard to take this out of context.

So --

What was your question?

Q. My question is simply: Does this table seem to report on germline mutations as opposed to somatic mutations?

A. Again, out of context, it's my best guess.

And I don't know if these also occur in the tumor or not, but they appear to be germline insertions or deletions.

Personalis EX2009.259

Q. Okay.

A. So these are insertions and deletions relative to a reference sequence.

Q. And you said that indel -- just to be clear, indels, you said, are a type of insertion or deletion? Is that what you said?

A. Indel means insertion or deletion, so what this indicates is a set of bases that are -- that appear to be inserted in the DNA sequence or deleted from the DNA sequence.

And there's a little reference here. INS I would assume means insertion. DEL I believe means deletion. INS -- or sorry. DUP is probably a duplication and a repeated sequence.

Q. And this table does not characterize any of these indels as in-frame deletions; is that correct?

A. There's -- there's no characterization at all here. So, again, you know, out of context, I don't know exactly what this table is referring to.

The supplement's online. If you'd, you know, like to call it up, we can look at it in more detail.

Q. So I'm going to hand you -- I don't know if you have this in your binder. Exhibit 1007.

Personalis EX2009.260

A. So that's another one of the supplements --

Q. That is correct.

A. And I don't believe I have that.

Q. So that is Guardant Exhibit 1007.

And this, I will represent to you, is Shah Supplement 2.

And have you seen this before?

A. Yes.

Q. Now, if you go to Page 10 of this document. And you see that there is a legend for Table S3 on Page 10?

A. Okay.

Q. And do you see that for Table S3 it says, "Confirmed germline insertions and deletions validated using PCR cloning and Sanger sequencing"; is that correct?

A. Yes.

Q. Okay.

A. It says that.

Q. And so, in other words, next-generation sequencing was not used in sequencing the indels that are in Table S3; is that correct?

A. That's not what it says. It says, "Confirmed germline insertions and deletions validated

Personalis EX2009.261

using PCR cloning and Sanger sequencing."

So my assumption is that since they went through the effort to confirm them, they probably discovered them using next-generation sequencing.

Q. But there's no evidence in this document of that; is that correct?

A. If you'd like to go back and look at the description of how this was found, the fact that it's confirmed means that they had discovered it previously.

If we look back at the protocol as described previously, I believe that they describe using next-generation sequencing. So whether or not it says how it was discovered isn't reflected -- or how it was discovered isn't reflected in what's described in the table label. This is the result of this validation experiment.

Q. Okay.

Dr. Quackenbush, let's go back to your declaration, but the one 1447.

A. All right.

Q. And let's go back to Page 82.

A. Okay.

Q. We're back to the same chart that we talked about previously with respect to Claim 8; is

Personalis EX2009.262

that correct?

A. Yes.

Q. And in this chart, as we talked about before, in some boxes you cite Levin and in other boxes you cite Shah, and in one box you cite -- or two boxes you cite nothing; is that correct?

A. I believe that's correct, yes.

Q. So the first box that I want you to look at is B, "a bait set that selects an in-frame deletion of 1 or more codons from a reference nucleotide sequence." Do you see that?

A. Yes.

Q. And you cite to Shah Supplement 3, "Listing 66 confirmed insertions and deletions identified in patient's germline," correct?

A. Yes, that's what it refers to.

Q. But nowhere in Supplement 3 or the rest of Shah is there any discussion of a design of a bait set to find these 66 insertions and deletions, correct?

A. So the fact that this is a validation experiment, as detailed in the legend for the table, means they specifically designed the assay to detect these. Right?

They found them initially and then

Personalis EX2009.263

designed an assay to detect and confirm them.

Q. So in your view, the fact that it's a validation experiment means that Shah designed bait sets to target these specific insertions and deletions; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Look, they designed assays to determine these. They could have used bait sets. What they tell us is they used PCR cloning and Sanger sequencing.

One skilled in the art would have understood that one could design bait sets as easily as one could design PCR primers. Shah made one choice, others make other choices. But these can be determined, identified, confirmed, and validated using well-known sequencing techniques.

Q. BY MS. WANG: And with respect to the sublimitation C, "a bait set that selects an intragenic deletion," is it also your view that the fact that this data is validated means that Shah designed baits to look for 66 confirmed insertions and deletions?

MR. ROSATO: Objection. Form.

THE WITNESS: So, as I explained earlier, Shah designed assays to identify and confirm these

Personalis EX2009.264

deletions. Confirming a deletion in an intragenic region, in a coding region, anywhere in DNA, is going to be exactly the same.

They tell us they use PCR, which was the choice that they used, but one skilled in the art could have designed other experiments.

The important thing about Shah is they show that with standard laboratory techniques you can identify a wide range of genomic variants.

Q. BY MS. WANG: And that's true with respect to Subsections D, E, F, H -- and H going down, correct? That Shah chose to use PCR and Sanger in order to detect these particular insertions and deletions?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: Well, these are referring to Supplemental Table 3, and Supplemental table 3, the legend tells us that these were validated using PCR cloning and Sanger sequencing. So that was the choice that Shah made.

But, again, Shah could have chosen a wide variety of other techniques.

Q. BY MS. WANG: Let's go back to Shah's actual document, the Shah paper itself.

Personalis EX2009.265

And you were looking previously at Table 3 of Shah; is that correct?

A. I was looking at many tables, but, yes, I was looking at Table 3 earlier.

Q. For frequency data.

A. Yes.

Q. Okay.

Now, in Table 3, this table does not list any structural break points; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So among the many different types of genomic alterations listed here, I don't believe they identified any sample structural break points. But, again, one skilled in the art would know that the techniques used to identify these could be used to identify break points.

Q. BY MS. WANG: And this table also does not list any rearrangements, does it?

MR. ROSATO: Objection. Form.

THE WITNESS: Well, again, there are alterations in copy number. That can be considered a rearrangement. But no specific high-level genomic rearrangements are listed explicitly in this table.

But, again, the techniques used here could easily be used to identify those kinds of

Personalis EX2009.266

rearrangements, and there are at least two different types -- in fact, there are many different types of genomic alterations which are listed here.

Q. BY MS. WANG: It also does not specifically list any translocations; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So in quickly reading this table, it does not appear that they listed -- or called out any specific translocations, but, again, the techniques used to identify all these variants could easily be adapted to identify translocations.

Q. BY MS. WANG: And it does not specifically list any inversions; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Among the many different types of genomic alterations listed here, I do not believe they call it inversions, but, again, one skilled in the art would understand that one could identify inversions using exactly the same techniques listed here. The fact that they don't occur in the samples doesn't limit the applicability of the methods described by Shah.

Q. BY MS. WANG: This table also does not specifically list tandem duplication; is that correct?

MR. ROSATO: Objection. Form.

Personalis EX2009.267

THE WITNESS: They don't use exactly that language, but there are descriptions of amplifications, and some of those may or may not be tandem applications or duplications. So it's hard for me to tell exactly whether or not they found those based on what's in this table, but they don't use the word "tandem duplication."

Again, there are a large number of different types of variants that are listed here, and those variants can be used -- or the methods used to detect those can be used to detect tandem duplications.

Q. BY MS. WANG: And Table 3 does not specifically list a gene fusion, either, does it?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, Table 3 does not explicitly call out the name "gene fusion," but the techniques used to identify the many types of genomic variants here could easily be applied to fusions. That's very clear from other references, including the reference to Levin.

Q. BY MS. WANG: And this table does not list the deletion of the full gene, either, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Again, in this table I

Personalis EX2009.268

don't believe they list any full gene deletions. I would want to look at this more carefully to assure myself that they didn't find any complete gene deletions.

The fact that it's not listed in this table doesn't mean it's not listed elsewhere or described elsewhere in all the supplementary data. And, again, the same techniques could be used. If you can find an amplification, you can find a deletion.

So they may not have been in the set of samples that Shah chose to analyze.

Q. BY MS. WANG: If you look at this table, there is, in fact, only one deletion listed, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: Yes, I believe there's one genomic alteration, which is classified as deletion.

Q. BY MS. WANG: And that deletion, the one that I'm looking at, is fifth from the bottom, and it's associated with a germline mutation, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So the indication here is that this is a germline deletion, which indicates that it was found in both the germline and the tumor itself.

Q. BY MS. WANG: But this is not a somatic

Personalis EX2009.269

deletion; is that correct?

A. The techniques used to find germline mutations are the same as those which are used to find somatic mutations, so if we can classify germline mutations, we can look for somatic mutations.

The fact that it didn't occur in this sample doesn't reflect an inability of Shah to detect those, and, in fact, what Shah demonstrates is that at least two or more different classes of genetic variation can be found and have been found using the methods described here.

Q. Sure, but I'm talking about this specific deletion that's listed in Table 3, that's denoted with a G for germline mutation. That is not a somatic mutation; is that correct?

MR. ROSATO: Objection. Form.

THE WITNESS: The fact that it's listed as G, the legend tells us that G means that this is a germline variant, germline mutation, so it probably also appears in the tumor itself, but the evidence indicates it's likely not germline -- sorry -- likely not somatic that it occurred in the germline genome.

But, again, the techniques used to detect these can easily be applied to identify germline somatic -- excuse me -- to identify somatic mutations.

Personalis EX2009.270

The fact that they found a somatic -- germline mutation means that if one existed in the somatic genome, they could have easily found that as well.

Q. BY MS. WANG: But they did not find it, at least according to this table, correct?

A. According to this table, they didn't find one, but what this really indicates very clearly is the technology they used could have been used to find somatic deletions if they existed in the samples they analyzed.

Q. Dr. Quackenbush, what is an amplicon?

A. There are a lots of different definitions of amplicons, and it depends on the context, so how would you like to discuss amplicons? In what context?

Q. In the context of amplification.

A. There are lots of different amplification protocols, and there are lots of different potential definitions of amplicon.

Q. What different definitions of amplicons are there?

A. People talk about amplification products that come from Kary Mullis' PCR, right? Sort of standard PCR. Those amplicons would be the segments of DNA that fall between two specific DNA primers.

Personalis EX2009.271

One can apply a very different amplification protocol, like rolling circle amplification, in which case the amplicon, if we would call it that, would be a long circular -- a long piece of DNA, which has many copies of whatever region was in -- captured in that rolling circle.

One can create amplicons using MIPs probes, and, again, there are a variety of ways of doing that.

So the sort of generic definition of an amplicon would be an amplification product, but there are lots of variations on amplicons, again, depending on what protocol you're going to use, what technique you're going to use.

Q. You would agree with me that Shah generated PCR amplicons, correct?

A. As part of the assay that Shah used, Shah used PCR. His validation experiments he describes as being the result of PCR cloning and Sanger sequencing, and I believe that other places he referred to PCR.

So if he's using PCR -- and, again, I'd want to look at the exact protocol to define what amplicon is, but if he uses PCR and we say the general product of that PCR is an amplicon, then one could construe what he used as PCR amplicons as part of but

Personalis EX2009.272

not the entire process.

Q. If you go back to Shah Supplement 1, which is Exhibit 1006.

A. Here we go.

Q. Go to Page 8 of this document.

A. Okay.

Q. And if you go under the subheading of "Mutation revalidation."

I think we talked about this earlier, that the "Mutations were validated initially by Sanger amplicon sequencing on both strands, in the metastatic tumor and germline DNA," and then it states that "Confirmed somatic mutations were subsequently revalidated in at least 2 additional independent PCR amplicon reactions."

Do you see that?

A. Yes, I do.

Q. And when they say "at least 2 independent PCR amplicon reactions," is that two cycles of PCR, as we talked about earlier?

A. So --

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: So if I read this, they identified mutations using next-generation sequencing.

Personalis EX2009.273

They then went and validated those using Sanger sequencing on both strands. And they then subsequently validated them again in two additional independent PCR amplicon reactions. So my interpretation of this is that they took DNA from their samples, set up two independent tubes, and ran two separate reactions.

Q. BY MS. WANG: There's no indication that Shah multiplexed the PCR reactions; is that correct?

A. There's no indication that they didn't, so I don't know how they conducted their PCR.

Q. But the fact that there was amplification means that multiple copies were created; is that correct?

A. The term "amplification" or "amplicon" generally indicates that multiple copies were created.

Q. Okay.

A. So they could adjust the number of copies depending on the depth of sequence coverage they hoped to achieve.

Q. Okay.

Let's take a break.

THE VIDEOGRAPHER: Going off the record. The time is 4:39 p.m.

(Short recess.)

Personalis EX2009.274

THE VIDEOGRAPHER: We are back on the record. The time is 5:06 p.m.

Q. BY MS.WANG: Dr. Quackenbush, Shah discusses analyzing the tumor of just one patient; is that correct?

A. So I believe you're right. Shah describes sequencing of tumor and metastasis samples from the same individual patient.

Q. And in Shah, the metastatic tumor was analyzed by whole genome and whole transcriptome sequencing; is that correct?

A. So just rereading the description of the assay. Could you repeat the question, please?

Q. Sure.

Would you agree with me that the metastatic tumor in Shah was analyzed by whole genome and whole transcriptome sequencing?

A. So, yes, they analyzed DNA from the metastatic -- well --

You said the metastasis or the metastatic sample?

Q. The metastatic sample.

A. So, yes, I believe DNA and RNA sequencing were used on the metastatic sample.

Q. And that was whole genome and whole

Personalis EX2009.275

transcriptome sequencing, correct?

A. I believe that's what they state, yes.

Q. And if you look at -- in Shah, on the left-hand column of the first page, near the bottom, it says that, "The aligned (hg18) reads were used to identify (Supplementary Figure 2) the presence of genomic aberrations, including SNVs (Supplementary Table 2), insertions/deletions (indels), gene fusions, translocations, inversions and copying number alterations (Supplementary Methods)."

Do you see that?

A. Yes.

Q. So at least as reported in Shah, that information was derived from the whole genome or whole transcriptome sequencing data, correct?

A. It was derived from looking at the combined data. I'm not sure exactly in what way it was used.

But, yes, from looking at those, aligning those sequence reads to a reference genome -- in this case hg18 is a release of the human genome -- and looking for these types of alterations.

Q. And none of these alterations as described at least in this paragraph of Shah were originally detected using any targeted enrichment

Personalis EX2009.276

methods, correct?

A. They weren't detected using targeted enrichment methods, but they were verified using targeted methods, and, again, once one knows the mutation frequencies, one can design targeted methods such as Levin to look for those types of genomic alterations.

Q. I want to go back to your declaration, the first one, 01170.

A. All right.

Q. And if you could please go to Page 46 of that declaration.

And I believe it's part of Paragraph 104. I think it's continued on to Page 46. Is that correct?

A. It starts at 45, goes to 46.

Q. Okay.

If you're on Page 46, and about the middle of the paragraph, you state, "For example, Shah discloses contacting a tumor library with 28 pairs of PCR primers that flank the target mutation positions."

Do you see that?

A. I see where it says that, yes.

Q. And you cite to the Shah Supplement 1 at Page 8, "Frequency analysis," which we have looked at

Personalis EX2009.277

earlier, correct?

A. Yes, we looked at that.

Q. Okay.

Of the 28 pairs of PCR primers, which pairs of PCR primers are you alleging are the two or more bait sets of the claims?

MR. ROSATO: Objection. Form.

THE WITNESS: So I never said earlier that Shah, in fact, had to identify two or more bait sets and use two or more bait sets. So I don't know off the top of my head -- I can go back and look through the materials -- what the spectrum of different mutations potentially represents.

What this paragraph is designed to show is -- and what the use of the Shah reference was designed to illustrate was that one could detect, classify, and quantitate relative frequencies of a large variety of different genomic alterations.

One of the simplest examples was the example we looked at, which appeared in Table 3, where we see a wide variety of mutational frequencies, including those that appear at less than five percent and greater than five percent.

In addition, there are a number of other alterations, so there are two or more classes of

Personalis EX2009.278

alterations as defined by the bait set definitions in the '830 patent that Shah was able to quantitate. And what that shows is that one skilled in the art could measure those frequencies.

If one measures those frequencies, then, as Levin teaches us, one could design an assay using baits to detect those types of alterations. And one skilled in the art would know that there are a wide variety of alterations one would want to identify, particularly in cancer, and, as Levin tells us, one could adjust the parameters appropriately to enhance or to diminish the relative representation of different potential targets.

So the purpose in using Shah was really to illustrate the fact that one could identify a large number of mutations and that one could quantitate those, and in particular in looking at Paragraph 26, "As discussed in the claim construction section, 'bait' is a nucleic acid molecule capable of hybridizing with and capture of a target nucleic acid and as such, encompasses PCR primers." Right?

Because, as we discussed earlier, PCR primers are synthetic oligonucleotides that we use in assays.

And what Shah describes, which is called

Personalis EX2009.279

out here, discloses contacting a tumor library with 28 pairs of primers that flank mutation positions. Right?

So he's analyzing frequencies of 28 individual mutations. Anyone skilled in the art would realize that there are lots of different classes you could use -- lots of different classes of genetic variants that one could detect and quantify using this kind of technology.

So -- whether it's one or more, I don't know, because I don't know the relative frequencies of the different mutations referred to explicitly in this call-out.

I would assume that, based on Table 3, which I'm -- again, if I recall correctly, are the different types of mutations that Shah was able to characterize the frequency of, at least mutations appearing at low and higher frequencies were identified and quantitated using his method.

Q. BY MS.WANG: I'm not even sure what question you were answering at that point.

My question you to was: With respect to this paragraph, where you specifically identified the 28 pairs of PCR primers, is it your opinion that those 28 pairs of PCR primers are bait sets as defined by

Personalis EX2009.280

the patent?

MR. ROSATO: Objection. Form.

THE WITNESS: So if you read what I wrote here, and what I just told you, what Shah demonstrates in the paper very clearly is that one can use a technique to quantify the relative frequencies of individual mutations. One could also use a database like COSMIC to identify those mutations, as discussed in the patent itself.

Q. BY MS.WANG: I'm not asking about mutations, again, or mutation frequencies. I'm asking about bait sets. We talked about this at length earlier, that the definition, according to the patent, which you pointed me to, is a bait set is one or a plurality of bait molecules. We can agree on that, correct?

A. I would --

MR. ROSATO: Objection. Form.

THE WITNESS: I will agree that what the patent says is that a bait set --

Sorry. I want to look at the language, and I want to make sure --

Q. BY MS.WANG: Sure. Column 28 of the patent, as you directed to me earlier.

A. Yes.

Personalis EX2009.281

So a "'bait set,' as used herein, refers to a plurality of bait" -- "one or more" -- sorry -- "one or a plurality of bait molecules."

Q. Correct.

A. Right?

Q. And what I'm asking you is that in Paragraph 104 you state -- the first sentence of Paragraph 104 states, "Shah discloses contacting the library with at least two bait sets to provide selected tumor members."

And I'm asking you, is it your opinion that the 28 pairs of PCR primers in Shah are the at least two bait sets of the patent?

A. So if we look at the patent -- right? -- what the patent tells us is that the claimed invention involves at least two bait sets of Step (b), so bait sets are baits which are -- sorry -- "contact the library with at least two bait sets to provide selected tumor members, wherein said bait sets hybridize with the tumor members, thereby providing a library catch."

So "the at least two bait sets of step (b) are chosen from two of the following bait set classes," and we describe in Romanette (i) mutations that appear at a frequency of about 5% or less,

Personalis EX2009.282

Romanette (ii), mutations that appear at a frequency of about 10% or higher, and then there are other classes of bait sets.

So if we look at Shah and the line that you referred to -- right? -- in the frequency analysis, where we have 28 individual regions, those regions are contacted with oligonucleotides. Right?

So as I wrote here, "As discussed in the claim construction section, 'bait' is a nucleic acid molecule capable of hybridization." PCR primers are clearly nucleic acid molecules capable of hybridization. Right? "And with capture of target nucleic acid sequences and as such, encompasses PCR primers," which these are.

"For example, Shah discloses contacting a tumor library with 28 pairs of primers that flank the target mutations." Right?

So those target mutations appear at different frequencies. Right?

At least we know that. And I'm not sure what all those different mutations are off the top of my head, but at least two different relative frequencies of mutations are classified using the method described in Shah.

Q. Dr. Quackenbush, when we talked about

Personalis EX2009.283

Levin, you told me that in your opinion the simplest approach to the 11,566 bait molecules of Levin was that they were 11,566 separate bait sets, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: I don't know if I said exactly that, but I said one interpretation of the 11,566 baits that --

See the language used in Levin.

So Levin tells us they designed 11,566 bait sequences. The patent tells us in the specification that a bait sequence in Column 28 -- or bait set, excuse me, refers to one or more -- or a plurality of bait molecules.

Those bait molecules are designed, according to Claim 1 and Claim 65 in the Romanettes, and (b) in the wherein clause, at least two of a wide variety of different classes of mutations, including those distinguished by frequency.

So clearly we can have DNA molecules, which we hybridize and detect different frequencies of mutations.

Q. BY MS.WANG: So I'm going to go back again. You had told me, and I just want to make sure that we're on the same page here, that it was not true

Personalis EX2009.284

that Levin discloses one bait set comprised of 11,566 bait molecules, correct?

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: I don't remember exactly what we said.

Q. BY MS.WANG: You disagree with that premise, correct?

MR. ROSATO: Let him finish his answer.

THE WITNESS: I don't remember exactly what I said. If you can show me that in the transcript, I'd be happy to read it and talk about it.

If you want to talk about the bait sets, what the -- what Levin, in fact, describes are 1,566 bait sequences, and if we look at what Levin actually discloses in the paper -- right? These bait sets actually have different frequencies or different enrichment capabilities. Right?

And the easiest way to see that is to look at Figure 1 in Levin, and what Figure 1 in Levin shows is mean sequence coverage by region. Right?

So we can classify these bait sets in a lot of different ways. Efficiency of coverage, which means number of sequence reads. And clearly each one of those molecules is going to capture a different

Personalis EX2009.285

number of sequence reads.

What's very clear from Levin is that there are more than two, and we can discuss whether or not there are 11,566 or some smaller number, but if we simply look at the sequence coverage that comes out of Levin, it's very clear there are different levels of efficiency and different levels of coverage, which is what we're told in the '830 patent we want to design for.

What Shah tells us is we can actually predetermine what those mutation frequencies are, and what Levin tells us is we can adjust the efficiency.

So if we take Shah and Levin together, it's very clear to anyone skilled in the art that you can perform simple optimization and do exactly what this patent claims to invent.

Q. BY MS.WANG: I'm going to again say that that may have been the answer to some question but is not the answer to my question, which is, again --

MR. ROSATO: Objection.

Q. BY MS.WANG: Do you agree that Levin's 11,566 bait molecules are one bait set? Do you agree with that principle?

A. I would not say --

MR. ROSATO: Objection. Form,

Personalis EX2009.286

foundation, asked and answered.

THE WITNESS: I would not say that Levin's 11,566 bait molecules represent one bait set, because they are distinct molecules with distinct properties, distinct levels of efficiency, and they detect different types of mutations.

Q. BY MS.WANG: Would you say that Levin's 11,566 bait molecules represent 11,566 bait sets?

MR. ROSATO: Objection. Asked and answered.

THE WITNESS: One could say that the -- I would say clearly there are two or more distinct bait sets capturing two or more distinct classes of genomic alterations with different sequence efficiencies, so there are between two and 11,566 bait sets.

Q. BY MS.WANG: And you cannot identify for me which -- between two and 11,566 bait sets, which definition you're relying on in your declaration --

MR. ROSATO: Objection.

Q. BY MS.WANG: -- is that correct?

MR. ROSATO: Form, foundation, asked and answered.

THE WITNESS: The patent requires two or more -- right? -- from a set of classes which are outlined. If we look in Levin, it's what's detected.

Personalis EX2009.287

Right?

We can detect sequence variants. Right? And that's on Levin Page 7. Right? Sequence variant identification. One type of mutation. Splice isoform identification. Fusion transcript identification. Other sequence alterations. Right?

So there are a variety of different classes of variants that Levin clearly discloses he can detect.

Q. BY MS.WANG: I'm not asking about the variants that Levin can detect; I'm talking about the bait sets of Levin that you claim are two or more. And I want to understand, out of the 11,566 bait molecules -- and I don't know how to make this more precise for you -- what are you talking about when you talk about a bait set? Is it one set of 200 molecules? Is it five sets of 400 molecules? What is the definition of a bait set as applied to Levin that you are using in your declaration?

A. So --

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: One of the challenges --

MR. ROSATO: Hold it.

Multiple of those questions that were

Personalis EX2009.288

posed were asked and answered.

MS.WANG: Again, those are not actual objections, they're speaking objections, and I'm going to let him actually answer my question, so please let him --

MR. ROSATO: You asked him five or six questions in one paragraph, so I'm noting my objection in part applies to, you know, a limited subset of the multiple questions.

Q. BY MS.WANG: Dr. Quackenbush, we have about an hour and a half, two hours to go, and I can spend the entire time on this, because you have not answered my question. If you don't have an answer, you can tell me you don't have an answer.

According to your declaration, Levin --

MR. ROSATO: Objection.

Q. BY MS.WANG: -- discloses at least two or more bait sets, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: If you can point me to the line in the declaration, I'd be happy to verify that for you.

Q. BY MS.WANG: Sure. Go to Paragraph 103 of your declaration.

Personalis EX2009.289

A. All right.

MR. ROSATO: What declaration are we on, just --

MS.WANG: The same one we've been on. It's 1170.

MR. ROSATO: Okay.

THE WITNESS: All right.

Q. BY MS.WANG: Okay.

And you state in Paragraph 103, "Levin discloses contacting the library with at least two bait sets to provide selected tumor members."

Do you see that?

A. I see where it says that, yes.

Q. And you cite to, as shown in this block quote, a quote from Levin, which says, "We designed 11,566 bait sequences (Table S5 in Additional data 5" -- "data file 5) targeting the coding sequence of 887 transcripts of 467 genes described in the NCBI RefSeq database."

Do you see that?

A. "RefSeq database." Yes.

Q. Okay.

All I'm asking -- and I don't think it's a hard question so I'm just trying to understand. What out of those 11,566 bait sequences -- where are

Personalis EX2009.290

the bait sets? What are you describing as bait sets?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: If we look at the patent language, it's somewhat imprecise in the way they define bait sets. And in Paragraph -- or sorry -- in Column 28, Line 60, "'Bait set,' as used herein, refers to one or a plurality of bait molecules." Right? Could be one, it could be many.

Then if we turn to the language of one of the claims -- and I'll talk about Claim 1 unless you prefer I talk about Claim 65.

Q. BY MS.WANG: I don't want you to talk about the claim at all. I'm referring to the definition that you're applying to Levin.

You just described for me the definition from the patent, which is one or a plurality of bait molecules.

A. Mm-hmm.

Q. Levin, we can agree, discloses 11,566 bait molecules, correct?

A. Yes. Levin talks about 11,566 baits.

Q. So in your opinion is that one bait set of a plurality of 11,566 baits, or is it something else?

Personalis EX2009.291

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: If we look at the definition of "bait set," it's somewhat imprecise, so it's hard to partition those 11,566 individual molecules, because the patent tells us it can be one or a plurality, so one or many, and -- right? It tells us that we have to contact a library, DNA, with at least two bait sets.

So my question, when I look at Levin, is: Are there at least two bait sets, wherein said bait sets hybridize with tumor members, providing a library catch. Right?

Levin describes hybridizing those baits -- right? -- to the 11,566, to bait set -- sorry -- to a library. Right?

And then we talk about some sequencing in Claim 1, which I'm going to leave aside, "wherein the at least two bait sets of step (b) are chosen from two of the following bait sets." Right?

Romanette (i):

"a first bait set that selects a high-level target chosen from one or more tumor nucleic acid molecules that comprise a subgenomic interval comprising a somatic mutation that appears at

Personalis EX2009.292

a frequency of about 5% or less of the cells from the tumor sample." Okay?

Romanette (ii):

"a second bait set that selects a mid-level target chosen from one or more tumor nucleic acid molecules that comprise a subgenomic interval comprising a somatic mutation that appears at a frequency of about 10% or higher of the cells from the tumor sample;

"(iii) a third bait set that selects a low-level target chosen from one or more nucleic acid molecules that comprise a subgenomic interval chosen from one or more of:

"a) a pharmacogenomic single nucleotide polymorphism that distinguishes the ability of a patient to metabolize different drugs,

"b) a plurality of genomic SNPs that uniquely identify or fingerprint a patient,

"c) a genomic SNP or locus that is used to assay copy number gains or losses of hetero" -- "of heterozygosity (LOH);

"iv) a fourth bait set that selects a nucleic acid molecule that comprises an intron sequence that detects a structural break point; or

"v) a fifth bait set that selects

Personalis EX2009.293

one-copy" -- "a one-copy deletion of several terminal exons, wherein each bait set of said plurality has a unique predetermined efficiency for selection of target compared to the other bait sets in that plurality."

Right?

So the definition of "bait sets" is pretty convoluted in here, but my interpretation of this language is we have to detect different types of alterations to classify a bait set, but whether or not even A represents one bait set or many bait sets is not clear.

I would say that what we have to do if we're going to look at this patent and the language is have different molecules which are capable of detecting different types of alterations, and that they have to have different efficiencies.

So I can't tell you, based on this convoluted definition, how many bait sets there are, but I can tell you that those 11,566 molecules have different efficiencies, that one can adjust those efficiencies using a variety of different protocols, even something as simple as adjusting the concentration, and that Levin clearly demonstrates he can identify a variety of different mutations and

Personalis EX2009.294

mutation types.

And if you'd like, I'd be happy to step through each one of those different subtypes and talk about the evidence in Levin for identifying those.

Can I take those 11,566 and partition them into these different buckets? Off the top of my head, no.

If you'd like, we can sit down and analyze all of them one by one and come up with a number.

But are there two or more? Absolutely.

Q. BY MS.WANG: But you did not identify in your declaration analyzing each of those 11,566 baits and identify bait sets by number, correct? That's not in your declaration.

MR. ROSATO: Objection. Form.

THE WITNESS: There's no need to identify the absolute number. You have to have two or more, and Levin clearly has two or more different bait sets.

Q. BY MS.WANG: But your declaration does not point me to a specific bait set from Levin with a specific set of baits within that bait set, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: If you'd like, we can look through each one of the bait classes --

Personalis EX2009.295

Q. BY MS.WANG: Dr. Quackenbush, I'm asking you --

MR. ROSATO: You've got to let him finish his answers.

MS.WANG: He's not answering my questions.

MR. ROSATO: It doesn't -- you have to let him finish his answer. You can't cut him off like that.

Go ahead and finish your answer.

THE WITNESS: If you'd like, we can step through each of the different types of bait sets, of which we have to have two or more, and we can look at what Levin discloses that he discovered using the baits -- the 11,566 baits that he had designed, and talk about those.

Can I assign individual baits to each one of the classes? Sitting here today, I can't.

If you'd like, we can sit down and map each and every one of those baits to its position in the genome and the transcriptome and the sequences, map each and every one of the alterations, and put them into those classes.

Could I -- can I put absolute numbers in each class? No. Can I tell you --

Personalis EX2009.296

Q. BY MS.WANG: I do not want that today, Dr. Quackenbush.

A. Can I tell you that there are two or more? Absolutely.

Q. I do not want that, and I do not want you to go into that.

My question is simply this. Did you include that in this declaration?

A. You keep on --

MR. ROSATO: Objection. Form, foundation.

THE WITNESS: You keep on asking me for absolute numbers.

Q. BY MS.WANG: Did you include absolute numbers in your declaration? That's all I'm asking.

Other than 11,566, as reported in Levin, did you include absolute numbers in your declaration as to what a bait set of Levin comprises?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: If you read the paragraph you referred me to earlier, and I don't recall which paragraph it was, there are two or more. And so there are two or more. What the absolute number is, we'd have to look at it.

Personalis EX2009.297

I would say a simple definition, given the fact that each bait is going to have a different capture efficiency and sequencing efficiency, is that there are 11,566. Right? Based simply on the sequencing efficiency.

If you want to talk about classes of mutations, there's some other number. But there are at least two, because Levin discloses 11,566 baits with different efficiencies, and discloses detection of at least two different classes of mutations, and tells us that if we want to find low-frequency events, and if we know that ahead of time, which Shah tells us how to do, we can alter our design parameters to detect those.

So if you want to take a look and classify these, I'd be happy to. But the absolute number I provided is two or more, and it's very clear there are two or more.

Q. BY MS.WANG: Maybe we're talking past each other. When I say "absolute number," I'm referring to of those two or more that you were describing, did you include in your declaration how many baits -- individual bait molecules comprise one of those two or more bait sets?

MR. ROSATO: Objection. Form,

Personalis EX2009.298

foundation, asked and answered.

THE WITNESS: So let's take a look at each one of the individual types of molecules -- if you like, we can do that. We can look at each one of the individual types of baits that are described in the '830 patent.

Q. BY MS.WANG: That's not what I would want to do, Dr. Quackenbush. I'm asking you a pretty simple question here.

In your declaration, in this document, do you have a number assigned to a bait set of how many bait molecules are in that bait set?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: Depending on your definition, there are more than two and less than or equal to 11,566 bait sets.

The reason is that there are multiple types of mutations and genomic alterations that are identified, and there are 11,566 bait molecules that have different amplification effici -- efficiencies -- I can get the exact language in the patent. But different efficiencies for capture and detection based on our description of efficiencies, representing the number of sequence reads that come from that capture.

Personalis EX2009.299

Q. BY MS.WANG: So the answer is the only numbers that you have definitively included in your declaration is 11,566 bait sequences, which you say could represent 11,566 bait sets, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: That's not true. Right?

There are 11,566 baits that were used. Those baits have different characteristics, so they could be characterized as different bait sets.

If we want to then further constrain them based on the language in the '830 patent, which requires bait sets that detect different types of alterations, listed in Romanette (i) through (v), those baits detect two or more of those.

If we want to say a bait set represents an individual gene or an individual transcript, we can come up with a different number. Right?

But the simplest -- because there's a lack of clarity in the patent itself, it's hard to understand how to exactly map this. So I'm giving you a range. Right?

I'm trying to be as honest as possible, given the vagaries of the patent itself. Right?

The bait set is a single molecule, and

Personalis EX2009.300

molecules have different efficiencies. There are 11,566.

If we want to throw them into buckets based on different classes of mutations that have been detected by Levin, we can do that. If we want to throw into buckets based on what could be detected, we could do that. Right?

Those may not have existed in this particular sequence, but one skilled in the art would clearly understand that one could do that.

One skilled in the art would clearly understand that in cancer there are a wide variety of different mutations one would like to detect. And so whether Levin detected them or not, one skilled in the art would understand that one could design to, detect, and characterize a wide range of mutations.

Q. BY MS.WANG: You did not divide, in your declaration, the 11,566 baits into buckets with specific numbers of baits in each bucket, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: There are two or more bait sets based on the most stringent classification that they detect -- different types of alterations. There are many more based on the individual efficiency of

Personalis EX2009.301

capturing sequencing. I don't have access to the raw sequencing data, so it would be difficult for me to tell you exactly whether or not any of those numbers happen to match.

But I can tell you, based on my more than 25 years of experience in working in molecular biology, that each one of those capture probes is going to produce a different number of sequence reads. So if we define bait sets based on absolute sequence reads, there are 11,566. If we define bait sets based on classes of mutations that were detected, there are more than two. If we define bait sets based on classes of mutations that could have been detected, there are clearly more than two. Right?

Levin didn't design his bait sets to capture all the molecules, all the genes in the genome. He could have done that. He could have done that, he could have focused on a different set of genes. Right?

This is a simple optimization problem. The patent asks, well, is it invention to identify -- or to set -- find bait sets that find two or more classes of mutations in its simplest read?

Levin finds those.

So there are more than two, less than --

Personalis EX2009.302

less than or equal to 11,566.

Q. BY MS.WANG: And you do not specify in the more than two -- let's say there's just two. You do not specify how many bait molecules out of the 11,566 baits in Levin fall into each of the two bait sets that you assert are disclosed, correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

Counsel, we've got to find a solution to this.

MS.WANG: He's not answering my question.

MR. ROSATO: He has answered it many times. This sequence -- you also went through these same questions earlier, and he gave you very specific answers.

What I would suggest is maybe go back through the transcript, because I actually remember -- the problem is --

Well, we can have a discussion off record, but I think there's also stuff earlier today that addressed this and pretty specifically answered your question.

MS.WANG: I disagree, and you are not testifying for him, and he still has not answered my question, which is whether or not his declaration --

Personalis EX2009.303

and I can sit here and ask him to read his whole declaration, and we can spend that entire time doing that, and he can spend the full seven hours, and then we'll call him back if he's not going to answer this question.

Q. Does your declaration -- I'm just trying to understand. You say there are two or more bait sets, and all I'm trying to understand is whether those two or more bait sets that you described from Levin -- how many bait molecules are in each of those bait sets?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: So how many bait molecules are in each bait set? Probably millions or tens of millions. Right?

If you design a bait set, you design a single oligonucleotide, and you make millions of copies so you can capture DNA.

So how many bait molecules are in each one? Millions, right?

How many distinct bait sets are there? More than two. Right?

And using one definition -- using the broadest definition cast out in the patent in

Column 28, if a bait set is one or more molecules -- right? And I'm now restricting it from the millions to distinct molecules with distinct efficiencies. There are 11,566.

If you would like -- and I'd be happy to do this, since you invited me to read my report. I'd be happy to start with the description of the different bait sets with Romanette No. (i) through Romanette No. (v), and talk about the different classes of mutations that Levin was able to discover using those baits.

Q. BY MS.WANG: I'm going to try one more time here, Dr. Quackenbush. We have talked about this 11,566 baits of Levin, correct?

A. Levin discloses -- or describes designing and synthesizing 11,566. I think the term is --

We've talked about this so many times. I'm forgetting the exact language which is used, but -- "We designed 11,566 bait sequences," which are detailed in Table S5 in additional dataset -- additional data file 5.

Q. And sitting here today, having submitted your opinions in these declarations, three of them --

A. Mm-hmm.

Q. -- you have not pointed to a specific

Personalis EX2009.305

bait set within Levin that contains a specific number of baits other than the 11,566 baits; is that correct?

MR. ROSATO: Objection. Form, foundation, asked and answered.

THE WITNESS: So if we take the definition in Column 28, which says a bait set is one or more --

Let me get the exact language.

Since you keep asking me the same question, I want to make sure that I answer it as precisely as I can. Right?

Column 28, Line 60. "'Bait set,' as used herein, refers to one or a plurality of bait molecules."

So there are one or more bait molecules.

Now, I'm going to assume that we're talking about molecules with different sequences. Right? So baits that can be one molecule with a particular sequence, it can be that molecule with a million copies of it. Right?

Or it can be more. It can be two different sequences and millions of copies of each which are used.

And if we really look at the definition of bait sets -- right? Claim 1(b), "contacting the

Personalis EX2009.306

library with at least two bait sets providing" -- "that provide selected tumor members, wherein said bait sets hybridize with tumor members, thereby providing a library catch."

So we contact them with bait sets. So one or more molecules --

Q. BY MS.WANG: Professor Quackenbush, I'm not asking about the patent Claim 1, and I understand you're very good at reading that claim in its entirety. What I am asking you about is your opinion about Levin.

You have stated that Levin teaches contacting a library with at least two bait sets, and all I'm asking you is: In the entirety of your 100-plus-page declaration, do you identify with specificity any specific number of bait sequences or bait molecules within any such bait set?

MR. ROSATO: Objection. Form, foundation, asked and answered.

I'm just going to say, I think we might want to take a break and try to come up with a solution to this. You have a question pending, so he can answer that, but I think we might be able to figure out a way to make this work.

MS.WANG: Okay.

Personalis EX2009.307

Q. Can you answer my question? That's my question.

Can you answer my question, whether or not you, in fact, have pointed to any specific number of baits within a single bait set in Levin?

MR. ROSATO: Same objection.

THE WITNESS: So I've answered this question I don't know how many times now.

If you look at my first report, the one we were referring to earlier, on Page 44, Paragraph 103, "Levin discloses contacting the library with at least two bait sets to provide selected tumor members. For example, Levin discloses the design and synthesis of biotinylated solution-phase hybridization bait sets."

I quote from the article. "We designed 11,566 bait sequences," which are in Table S5 in additional dataset 5, "targeting the coding sequence of 887 transcripts of 467 genes described in the NCBI reference database. The RefSeq file used contains 45,376 transcript sequences" -- right? -- from different classes of NCBI identifiers, prefixes to the identifiers.

So they chose, out of the 45,000-and-some-odd transcript sequences in that

Personalis EX2009.308

database, RefSeq, a subset, and they designed oligonucleotide probes -- right? -- what we've been referring to as baits -- right? -- different bait sequences, and hybridized those. Right?

"Levin further discloses wherein said bait sets hybridize with tumor members" -- right? That's what bait sets have to do -- "providing a library catch."

And then, "Levin discloses that the bait sets are contacted with the cDNA library and the targeted tumor members are captured to provide a library catch." That's what the patent language tells us.

And then Levin describes identifying a range of different mutations. The patent language requires that we have two or --

Sorry. Again, let's get the exact language so there's no confusion. "Wherein the at least two bait sets of step (b) are chosen from two of the following bait sets."

And without reading the entire language here, the bait sets are classified by different relative -- sorry -- by different types of genomic alterations, including those that are differentiated by the relative frequency, and there's additional

Personalis EX2009.309

language that says, "wherein each bait set of said plurality has a unique preselected efficiency for selection for its target as compared with the other bait sets in the plurality."

So if we apply the definition that bait sets have different efficiencies, I would posit that the 11,566 individual molecules have each an individual efficiency that differs from all of the others. Right?

So using the definition in Column 28 and a restriction that we have different efficiencies, there are 11,566.

If we start to classify them, then, and try to reduce that number, there are a variety of these different classes that Levin was able to detect. Levin could have detected others -- he could have selected different genes and detected others.

What the technology in Levin demonstrates is that anyone skilled in the art could have taken the same kind of approach that Levin used, could have used information provided by Shah or COSMIC or any other assay that told you about relative frequencies, adjusted the relative efficiencies as Levin describes on Page 6 of the application, and identifies classes of mutations.

Personalis EX2009.310

If you want to talk about the classes of mutations, there are at least two, and I believe there are three or four. I'd have to go back and actually look at the report to refresh my memory about exactly how many. But there are at least two different classes of genetic variants, genomic variants, that Levin was able to provide.

So there are more than two using one definition that's provided here, and if we just restrict ourselves to different efficiencies, I would say there are 11,566, but there are at least two.

MR. ROSATO: Do you want to have a meet and confer?

MS.WANG: Let's -- yeah, let's go off the record.

MR. ROSATO: Well, we can stay on the record, but you should leave the room.

THE WITNESS: All right.

MR. ROSATO: Thank you.

(At this time Dr. Quackenbush excused himself from the deposition room.)

MR. ROSATO: Okay. The witness has now left the room.

So my suggestion was going to be I do

Personalis EX2009.311

think he's answered your question. I know you disagree. But I do recall earlier in the day him specifically saying that -- taking the broadest interpretation as the term is defined in the patent, he's interpreting 11,566. Then he further explained that you could look at -- if you were to look at this in more narrow terms and define it by transcript, there's 887 transcripts.

He also said if you're going to define it or look at it in a narrow way and look at it by gene, there are 467 different genes looked at. This is all according to Levin.

And his conclusion was that's at least -- no matter which of those you pick, that's at least two.

I mean, I recall him giving that answer, and that seems pretty on point to what you're asking. I do think now he's -- the witness is just confused and he's stating as much.

So I -- I'm a little worried that this is not really going to go anywhere, and I do think you got your answer.

MS.WANG: So I disagree, obviously. I do recall that he stated those previously. What I'm trying to get from him and what I've been asking

Personalis EX2009.312

repeatedly is -- he continuously says there are at least two bait sets, and I want to understand, of the two that he's identifying -- because in his mind he has stated that there's at least two, there may be three or four, based upon the classifications according to the claim that he's read now a half a dozen times. He keeps saying that there are at least two bait sets, and all I'm trying to get from him is whether or not he's ever looked at how many baits out of the 11,566 bait sequences fall into those two that he stated.

MR. ROSATO: But I think he's answered that. He's pointing to at least two because that's the claim language, and the point of his declaration that we're pointing to is mapping the disclosure of Levin to the at least two part. So I think that's what he's trying to say. He's trying to say the claim requires at least two, and he's found at least two.

Where has he found that? He's found that in 11,566, alternatively read at 887, or even if it's viewed as 467. Any one of those three options give you at least two.

So I think he's just trying to link it up to the claim. I don't think he's trying to cause confusion here.

Personalis EX2009.313

MS. WANG: If that's his answer, then I think if he can say that on the record, that's his answer.

What I'm troubled by and, again, what I've been trying to understand is he keeps going back to these classifications based on the claim language, and what I'm trying to understand is in those classifications, which he says would demonstrate at least two, whether or not he's ever associated any particular bait -- numbers of baits with each of those classes. That's all I'm trying to get from him.

And I believe the answer is no, that he does not have a specific number of baits with respect to each class.

(Interruption.)

MS.WANG: But that is what I'm trying to get from him. And my understanding is that he's refusing to answer the question as to whether he's ever specifically figured out the number of bait molecules that correspond to each of these classes of at least two bait sets that he's talking about. I don't believe he's ever said that he, in fact, has done it or hasn't done it or acknowledged that this is something that's not in his declaration.

MR. ROSATO: Okay. I think I'm hearing a

Personalis EX2009.314

different question than what was being posed. My interpretation of what was being posed is more along the lines of you're asking what he considers the at least two or you're asking for a specific number, and I think that's why he keeps going to 11,566, and then also coming back and saying, "And my task was to map to at least two in the claim, and that meets it."

So I don't think he's hearing the question of, you know, how -- the slightly different question that you just posed to me.

I mean, that's -- do you have a different take on that?

MS. GERRARD: No, I don't.

MR. ROSATO: You're confused too?

MS. GERRARD: Yes.

MR. ROSATO: Okay.

MS.WANG: So I think my -- what it will come back to is he seems to have a different interpretation of the limitations of the claim as to which one applies in each instance, because he keeps saying if you're just limiting it to efficiency of selection, the answer is 11,566, but if you go to the classes, there's some different answer. And all I'm trying to get from him is that he said that if you limit it to the other classes, there's some different

Personalis EX2009.315

answer. He has not stated what that answer is.

MR. ROSATO: I think the witness is confused. I think he's trying to look at the definition in the patent and then look to where he was mapping it to the claim, the "at least two" in the wherein clause, and explain that he's finding at least two in Levin by virtue of these three numbers we keep referring to. And I think he -- in combination with that, he just seems confused, so he's just trying to talk and give something that is satisfactory.

But I think everybody's confused at this point.

MS.WANG: Okay.

MR. ROSATO: Do you want to take a five-minute break and think about how we want to approach this?

MS.WANG: I can take a five-minute break, yes.

I do think I would like to continue asking him these questions. I will think of a way -- if there's a better way to get him to discuss what I think is a pretty clear answer based upon what's in his declaration, and so we can do that off the record and then we'll come back.

MR. ROSATO: Which part -- Paragraph 103?

Personalis EX2009.316

Is that the part you're referring to?

MS.WANG: Yes.

MR. ROSATO: Okay.

And I think that's what he explained. There's three numbers there, and he says saying that -- whether you view it as 8 -- I'm sorry -- 11,566, which would be the broad interpretation, or if it's -- if he's confined to a more narrow perspective, under that more narrow perspective you still have at least 887 different transcripts being targeted. If that's considered a set, that's more than two. If the 467 different genes are each considered a set, then that's more than two. I think that's what he's saying.

MS.WANG: Okay.

My question is still with respect to the rest of the limitations and the five classes that he keeps talking about, and how you have to pick a bait set that's -- at least two bait sets that select from those five classes, correct?

MR. ROSATO: Well, those are mapped elsewhere in the declaration. I mean, the Paragraph 103 is addressing the Part (b), which is the at least two, if I'm reading this --

MS.WANG: Okay. So perhaps -- so perhaps

Personalis EX2009.317

I should direct him to other parts of the declaration, and we'll see if that fixes the problem when we get back.

MR. ROSATO: Okay.

MS.WANG: Okay.

MR. ROSATO: We can go off.

THE VIDEOGRAPHER: Here marks the end of Media Unit No. 4 in the deposition of John Quackenbush. We're going off the record. The time is 6:03 p.m.

(Short recess, at which time Dr. Quackenbush reentered the deposition room.)

THE VIDEOGRAPHER: Here marks the beginning of Media Unit No. 5 in the deposition of John Quackenbush. We are back on the record, and the time is 6:14 p.m.

Q. BY MS.WANG: Dr. Quackenbush, I'm going to try to sort of set the context a little more, so I think we're talking past each other.

A. Okay.

Q. When we were looking at Paragraph 103 of your declaration, which we can turn back to, my understanding of your opinion is that in the broadest context your opinion is that there are 11,566 bait

Personalis EX2009.318

sets, comprised of one bait sequence each, correct?

A. That would be one way to describe it, yes. Sort of a good characterization of what we were talking about earlier.

Q. And you testified earlier that in another way of describing it, you could look at Levin as 887 bait sets, comprised of some number of bait molecules each, that are targeting these 887 transcripts, correct?

A. One could characterize it that way, yes.

Q. And yet another way of characterizing it, that you testified previously, is that Levin could disclose 467 different bait sets, comprised of some number of bait sequences each, that are matching the 467 genes as described in Levin, correct?

A. Yes.

Q. Okay.

You then in your declaration, in Paragraph 116 on Page 51, through Paragraph 146 on Page 69, walk through each of the five classes of bait sets that are described in Claim 65, correct?

A. I think that's a reasonable characterization, yes.

Q. And in each of those five classes of bait sets, you describe why you believe Levin or Shah

Personalis EX2009.319

teaches that particular bait set, correct?

A. I wouldn't characterize it exactly that way. I would say that Levin teaches a technique that could detect this type of bait -- this type of -- sorry -- this broad class of bait set. The bait set is defined, for example, 1, by a frequency of mutation, and Shah or COSMIC would teach us ways to determine that frequency. Right? Since part of this requires designing bait sets ahead of time for particular preselected efficiencies. Right?

So if I had a bait set which targeted -- and that could be one bait or many baits -- targeted a high-level target with five percent or less of the cells harboring that -- if I knew that ahead of time, I could use the techniques described in Levin to select this type of mutation.

And for each of the other classes, I lay out a very similar argument. That if we look at Shah, Shah teaches us a way to determine frequencies of mutations, and then one could apply what's described in Levin, including in Par -- Page 6, where he tells us you can adjust the frequency of -- the efficiency of selection to detect what I would characterize, without quoting Levin, less frequent or rare targets. That one could detect all of these.

Personalis EX2009.320

Q. But going back to sort of what we were talking about in terms of how many bait sets with how many bait sequences contained in each bait set, you will agree with me that when you walked through each these five classes of bait sets, you do not describe a specific number of bait molecules corresponding to each class, correct?

MR. ROSATO: Objection. Form.

THE WITNESS: So in the declaration, if we read the declaration -- and I'm not going to go through and read the entire declaration, but it doesn't describe -- it doesn't take each one of the 11,566 individual bait molecules and assign them to Class A or Class B or Class C. Right?

One skilled in the art would understand that if one knew the frequency of mutation ahead of time, which Shah teaches us how to find -- if we knew that, and if we wanted to detect these, as Levin teaches us, we could design bait sets, and we could adjust the parameters of that bait design and use including things like concentration to detect these different types of variants.

So individuals aren't assigned here. In fact, part of the challenge in doing that is that a molecule -- or a mutation which is at five percent in

Personalis EX2009.321

one tumor may be in greater than 10 percent in another tumor. Right? The vagaries of how this is defined makes it difficult to make that type of assignment.

So one skilled in the art would understand that simple optimization of very well understood laboratory protocols would allow you to detect two or more of these classes defined in Romanettes (i) through (v).

Q. BY MS.WANG: And if you go back to Paragraph 104 of your declaration, which we had looked at earlier.

A. Sure.

Q. And that was the paragraph that started with, "Shah discloses contacting the library with at least two bait sets to provide selected tumor members."

Do you see that?

A. I see that, yes.

Q. And the paragraph that you're quoting from Shah, the only numbers that I see there are "9 years earlier" and then "Twenty-eight of the 32 mutations yielded amplicons compatible with Illumina sequencing."

Do you see that?

A. I see where it says that, yes.

Personalis EX2009.322

Q. Okay.

And then we talked a little bit about the sentence a couple of sentences down, where you said, "For example, Shah discloses contacting a tumor library with 28 pairs of PCR primers that flank the target mutation positions."

Do you see that?

A. I see where it says that, yes.

Q. So just like we talked about with Levin with respect to Paragraph 103, where we talked about how many baits there are and how many different interpretations of how many bait sets with how many bait molecules there are, what I'm trying to understand is with respect to Shah. Do you have an opinion as to how many bait molecules there are or molecules that you were describing as baits in Shah that comprise the two or more bait sets? Or do you not have that information in here?

MR. ROSATO: Objection. Form.

THE WITNESS: So if we look at what Shah teaches us, Shah teaches us a way to determine frequencies. Right? That's what Shah reports. So Shah teaches us a way to determine those frequencies.

One can then design PCR primers or other types of baits -- right? -- the kind of bait sets

which Levin describes, and adjust the parameters to capture those. Right?

Q. BY MS.WANG: Does Shah actually report a bait set --

A. So --

Q. -- comprised of a number of bait molecules? That's all I'm asking.

MR. ROSATO: Objection. Form.

THE WITNESS: If we look at what -- if we look at the Shah paper --

Q. BY MS.WANG: I'm actually not looking at Shah. I want to talk about your declaration.

Do you state that Shah, in fact, reports a bait set or two bait sets with a specific number of bait molecules?

A. So you're asking me about Shah?

If we're going to discuss Shah, I think it's important for us to refer back to Shah.

Q. I'm actually asking about your opinion. I want to understand your opinion as stated in your declaration.

A. My opinion as stated in my declaration refers back to Shah, and if you're asking me -- if you'd like, I can read the declaration, or if you'd like more context, I can discuss Shah. So would you

Personalis EX2009.324

like me to read the declaration or would you like me to provide more context, as it seems you're asking me to do?

Q. I'm not asking you to provide context. I'm trying to understand your opinion as you stated in this declaration in Paragraph 104.

A. Okay.

Q. And all I'm asking, as we talked about for Levin, is: Have you identified in Paragraph 104, or anywhere else in your declaration, a specific number of molecules in Shah that you assert comprise a bait set or more than one bait set?

A. Sure.

MR. ROSATO: Objection. Form.

THE WITNESS: All right. So it states here very clearly, "Shah further discloses said bait sets hybridize with tumor members thereby providing a library catch. As discussed in the claim construction section" -- we can refer back to that --" 'bait' is a nucleic acid molecule capable of hybridization with and capture of a target nucleic acid and as such, encompasses PCR primers." Right?

"For example, Shah discloses contacting a tumor library with 28 pairs of PCR primers that flank the target mutation positions. All" right?

Personalis EX2009.325

And then, quote, "Frequency analysis," which you had referred to earlier and we discussed, "Amplicons were therefore designed to span the target mutation positions with short" -- "with short amplicons (about 100 base pairs) such that the mutation position could be reached with 75 base pair Illumina reads. The same amplicon primers and batch were used for all amplifications. To control the amplification from the primary and metastatic samples, 28 randomly selected heterozygous germline amplicons were also amplified and sequenced together with the mutation positions."

So we have 28 somatic genetic variants and 28 randomly selected SNPs. Right?

So if we look at the definitions of different bait types -- right? If we take the broadest definition, we have 28 and 28 pairs of primers, so 56 -- sorry. Each one of those individual DNA fragments could be considered a bait, so we can have two times 56, which is 112 individual baits, using that definition.

If we're going to use them to capture and catch different fragments -- right? -- by amplifying them, as in PCR, there are 28 plus 28, which are 56.

If we further divide those into different

Personalis EX2009.326

representations -- right? -- we have single-nucleotide polymorphisms, which anyone skilled in the art would recognize can capture any type of variant you'd want to look at, and we have 28 different pairs of PCR primers. Those allow the amplification and detection of different types of genomic alterations, including alterations that appear at frequencies lower than five percent and greater than 10 percent.

So there are, depending on your definition, any variable number of baits, but there are clearly two or more.

Q. BY MS.WANG: So I just -- I think you're answering my question so I just want to make sure I understand, because I think you weren't quite clear about what those numbers represented.

The first -- the broadest one that you discussed is 56 plus 56 equals 112 individual baits; is that correct?

A. There are 112 different primers. Right? And a bait molecule can incorporate -- or can encompass PCR primers. Right? Because they can hybridize and they can provide -- help provide a library catch. So the broadest possible definition could be 112.

Q. And those are 112 baits within a single

Personalis EX2009.327

bait set, or 112 baits divided among some number of other bait sets?

A. They are going to have different hybridization efficiencies, so, again, the definition of "bait set" is fairly vague. If we apply the Column 28 definition -- right? -- it's one or more -- one or a plurality of molecules. So there could be 112 using that broad definition.

If we look at what -- how this is further defined in Claim 1 and Claim 65, there would be a smaller number, but there are at least two. So there are two or more.

Q. Sure. And, again, I'm not fighting you on that at all. I'm just trying to understand the numbers.

Under the broadest definition, which we've been talking about, your view is that Shah discloses 112 bait sets, correct?

A. There are 112 individual molecules, and if we wanted to classify them as molecules which are capable of hybridizing, there are 112.

If we want to characterize them as a set of molecules which we can use to produce amplicons and enrich a library, I would say there are 28 plus 28, which would be 56.

If we want to further characterize them based on the types of alterations they can classify, I can look at Shah in the table and look at the different types of alterations, but I can also say that anyone skilled in the art would understand that if you could do this, you could, in fact, use a protocol like this to capture all the different classes.

So what Shah captured represents two or more, and how one could use this would allow you to capture two or more.

Q. And, again, I'm not talking about how one can use this; I'm specifically just talking about the numbers that are reported in Shah.

A. Mm-hmm.

Q. And I think we're still not on the quite same page with respect to terminology.

When we discussed Levin, we talked about how there were 11,566 bait sequences, and under the broadest definition you agreed with me that that could be 11,566 individual bait sets comprised of one bait sequence each, correct?

A. Yes. I believe that's correct.

Q. So what I'm asking is: With respect to Shah, is it your opinion that under the broadest

Personalis EX2009.329

definition, there are 112 bait sets comprised of one primer or bait sequence each? Is that correct?

A. So I want to refer back to -- just to make sure that I'm characterizing this the correct way and using exactly the definition.

So "'Bait set,' as used herein, reflects one or a plurality of bait molecules."

I'm going to go up and I'm going to refer back to the definition of "bait."

"'Bait,' as used herein, is a type of hybridization capture reagent. A bait molecule can be a nucleic acid," and details a lot of different approaches and different types of molecules.

So if a bait is a type of molecule -- well --

These definitions get very convoluted.

Q. I think I'm -- all I'm trying to do is -- and, again, I'm not trying to stop you here, but I want to just point us because I think we're getting too far into the weeds. You mentioned that there were 28 pairs of PCR primers that are used in Shah, correct?

A. There are actually 56 pairs of PCR primers that Shah describes in that paragraph that we had just discussed.

Personalis EX2009.330

Q. Okay.

And it is your opinion, based upon the paragraph we just discussed, that the definition of "bait" in the patent covers PCR primers. That's your opinion, correct?

A. Yes. I believe one could easily say that a bait -- "As discussed in the claim construction section, 'bait' is a nucleic acid molecule capable of hybridization with and capture of a target nucleic acid sequence and as such, encompasses PCR primers."

Q. Okay.

So you agree with me that your opinion is that the definition of "bait," according to the '830 patent, includes PCR primers, correct?

A. The definition of "bait" in the '830 patent is vague, and it could include PCR primers. Right?

One skilled in the art would understand that PCR primers are designed with exactly the same design principles and considerations one would use in designing any kind of synthetic oligonucleotide.

Q. And I am not asking about the -- I am not asking you to describe your opinion again. All I'm trying to get at is this one sentence here --

A. Yes.

Personalis EX2009.331

Q. -- where you are stating under your -- in your opinion "bait" encompasses PCR primers, correct?

A. Point me to the --

Q. Page 46.

A. Okay.

Q. Top of the paragraph it says "bait" -- as discussed in the claim construction section of your declaration, "'bait' is a nucleic acid molecule capable of hybridization with and capture of a target nucleic acid and as such, encompasses PCR primers," correct? That's -- I read that correctly?

A. You read it correctly, yes.

Q. And that is your opinion, correct?

A. That is my opinion stated in this report.

Q. And then you follow along to say that, "Shah discloses contacting a library tumor with 28 pairs of PCR primers."

Do you see that?

A. Yes, I do.

Q. Okay.

So is it your opinion that those 28 pairs, meaning 56 total PCR primers disclosed there, are, in fact, 56 bait molecules, correct?

A. Using the definition of "baits," yes, one skilled in the art would understand that those PCR

Personalis EX2009.332

primers could easily be, for example, biotinylated and used to amplify and then capture different regions.

Q. I'm just referring to the 28 pairs of PCR primers that you state here that are disclosed in Shah. Okay?

And all I'm trying to understand is now under your definition of "bait" and "primer" -- and which I'm not getting into the reasons why, but under your definition, is that one bait set of 56 primers or baits, or is that multiple bait sets comprised of 28 primers, 10 primers? Do you have any specific number of primers that comprise a single bait set?

A. Again, the definition of "bait set" in the patent is somewhat vague. Right? Are -- do we have 56 -- sorry -- 112 distinct DNA molecules, which are capable of hybridizing? Yes. Do they represent different classes of mutations? Some of them do, some of them don't. Right?

What's very clear here is that Shah describes a technology that can be used to characterize mutation frequencies in other types of genomic variation. Right?

SNPs. Some are rare in a population, some are common in a population.

Taking this and trying to partition it

Personalis EX2009.333

into a bunch of vague classes is very, very difficult, but if we look at Table 3 in Shah, what Shah clearly demonstrates is one can determine a wide range of frequencies of mutation in an individual sample, and so applying the simplest set of constraints, not talking about all the other mutations they found, including pharmacogenomic mutations and others -- right? If we only look at Class 1 and Class 2, laid out in Romanette No. (i) and Romanette No. (ii), frequencies of mutation less than five percent and greater than 10 percent, Shah describes a way of using oligonucleotides to detect and quantify those mutations.

Levin tells us that we can use baits to capture a wide variety of different mutations, and also tells us that we can adjust parameters to change the different representations.

So it's difficult for me to say there are two different baits or five different baits or 28 different baits or 112 different baits. The definition is so vague.

But do we capture different classes and different frequencies of mutations and different types of alterations? Yes. We do.

Can I tell you how many, of those 28, the

Personalis EX2009.334

different versions are? I'd have to go and look at each one individually.

There's a lot of different mutations that have been described, but one skilled in the art would understand that what's disclosed here would allow you to quantify mutations, would allow you to capture, amplify, and sequence mutations of different classes and different frequencies.

Q. And in your declaration you have not yet gone and actually tried to put those 28 primers into different classes, correct?

A. I don't believe I did that because there's no need to. Right?

All we have to do is say that there are two or more.

Q. Okay.

A. And we -- and, clearly, Shah describes two or more of the different classes which are outlined in the '830 patent.

Q. Okay.

Let's take a break, and we'll see if there's any wrap-up.

MR. ROSATO: Okay.

THE VIDEOGRAPHER: We are going off the record. The time is 6:36 p.m.

Personalis EX2009.335

(Short recess.)

THE VIDEOGRAPHER: We are back on the record. The time is 6:41 p.m.

MS. WANG: So thank you, Dr. Quackenbush. At this time I have no further questions.

I will just state on the record that Foundation reserves the usual reservations, including the right to call you back if, upon review of your transcript, we believe your testimony was insufficient.

THE WITNESS: Thank you.

MS. WANG: Anything else from you, Counsel?

MR. ROSATO: Well, we'll probably take a two-minute break and figure out -- I mean, as far as reserving right to call back, we would view this as concluding, so I don't think there is any reservation to be made, but, you know, we don't have to get into that today.

But let's take a two-minute break and we'll figure out if we want to do any redirect.

THE VIDEOGRAPHER: We're going off the record at 6:42 p.m.

(Short recess.)

THE VIDEOGRAPHER: Back on the record.

Personalis EX2009.336

The time is 6:43 p.m.

MR. ROSATO: Thank you, Dr. Quackenbush. Thank you, Counsel. We have no further questions, either.

MS. WANG: Thank you.

THE VIDEOGRAPHER: We are -- we are off the video record at 6:43 p.m., ending Media Unit No. 5 of 5 in the deposition of John Quackenbush.

Thank you and good night.

(Ending time: 6:43 p.m.)

Personalis EX2009.337

DECLARATION UNDER PENALTY OF PERJURY

I, the undersigned, declare under penalty of perjury that I have read the entire foregoing transcript of my deposition or the same has been read to me, and the same is true and accurate, save and except for changes, corrections, additions or deletions indicated by me on the DEPOSITION ERRATA SHEET hereof, with the understanding that I offer these changes as if still under oath.

Signed on the _____ day of _____, 20____, at _____, _____.
(City) (State)

_____
JOHN QUACKENBUSH, PH.D.

Personalis EX2009.338

I, RONALD L. COOK, California CSR No. 13928, Washington CCR No. 2523, RDR, CCRR, CRR, CCP, FAPR, certify: that the foregoing proceedings were taken before me at the time and place herein set forth; at which time the witness was duly sworn; and that the transcript is a true record of the testimony so given.

I further certify that the adverse party, GUARDANT HEALTH, INC., was represented by counsel at the deposition.

Witness review, correction and signature was (x) by code. (x) requested.

I further certify that the depositon of JOHN QUACKENBUSH, PH.D., occurred at the offices of Wilson Sonsini, 701 Fifth Avenue, Suite 5100, Seattle, Washington, on Wednesday, January 31, 2018 commencing at 9:15 a.m. to 6:43 p.m.

I further certify that I am not financially interested in the action, and I am not a relative or employee of any attorney of the parties, nor of any of the parties.

Dated this 5th day of February, 2018.

RONALD L. COOK

Personalis EX2009.339

February 5, 2018

John Quackenbush, Ph.D.
c/o
Michael T. Rosato, Esq.
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701 5th Avenue
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Seattle, WA 98104-7036

Re: Guardant Health v Foundation Medicine
Deposition Of: John Quackenbush, Ph.D.
Taken On: 01/31/2018 Chase File No. 24426

Please be advised that the transcript in the above-referenced matter is available for reading and signature. Unless instructed otherwise, the witness should complete the following steps within 35 days of the date of this letter:

[X] Please contact our office to schedule the review of your printed original deposition in person or to schedule **the review of an electronic certified transcript online**.

[X] If you would like to review your attorney's certified copy of the transcript (either printed or electronic), we have enclosed an errata sheet for that purpose.

[ ] Enclosed you will find the original deposition transcript.

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If you should have any questions relating to this matter, please do not hesitate to contact our office.

Sincerely,
Ronald L. Cook, Deposition Officer

Enc.
cc: Sophie F. Wang, Esq.

DEPOSITION ERRATA SHEET

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WITNESS: John Quackenbush, Ph.D.
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I declare under penalty of perjury that I have read the foregoing transcript of my deposition testimony taken in the matter and on the date above, and that, with the following exceptions, the same is a true record of the testimony given by me under oath or affirmation.

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Witness Signature: _____Date: _____
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