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Transcript of Michael L. Metzker, Ph.D.

Date: July 31, 2025
Case: Qiagen Sciences, LLC -v- Tecan Genomics, Inc. (PTAB)

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WORLDWIDE COURT REPORTING & LITIGATION TECHNOLOGY

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

QIAGEN SCIENCES, LLC,

Petitioner,

v.

TECAN GENOMICS, INC.,

Patent Owner.

IPR2025-00028

U.S. Patent No. 10,036,012

DEPOSITION OF

MICHAEL L. METZKER, PH.D.

NEW YORK, NEW YORK

JULY 31, 2025

REPORTED BY: KIARA MILLER

FILE NO.: 593985

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Deposition of MICHAEL L. METZGER, PH.D., taken on behalf of PATENT OWNER, at 295 5th Avenue, New York, New York, commencing at 9:34 AM, JULY 31, 2025, before Kiara Miller.

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

ON BEHALF OF PETITIONER:

QUINN EMANUEL URQUHART & SULLIVAN, LLP
50 California Street
22nd Floor
San Francisco, California 94111
BY: DAVID BILSKER, ESQ.

ON BEHALF OF PATENT OWNER:

GOODWIN PROCTER LLP
The New York Times Building
620 Eighth Avenue
New York, New York 10018
BY: LINNEA P. CIPRIANO, ESQ.

ALSO PRESENT:

RICHARD SAMUEL HERRON PHD, GOODWIN PROCTER ADVISOR

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M I C H A E L M E T Z K E R, after having first been duly sworn by a Notary Public of the State of New York, was examined and testified as follows:

EXAMINATION BY

MS. CIPRIANO:

Q Could you please state your name for the record.

A Good morning. My name is Michael Metzker, M-e-t-z-k-e-r.

Q I'm Linnea Cipriano, as I introduced myself a moment before. I'll be taking your deposition today.

Have you been deposed before?

A Yes.

Q How many times?

A Dozens.

Q When was the most recent?

A Let's see, I think it was, we're still in July, I think it was last month.

Q More than likely these ground rules will be similar to those that you've sat through before, but just so we're on the same page,

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I'd like to go through a few before we get started.
I will be asking you questions today and your answers will be under oath as if you were testifying in court.
Do you understand that?
A I do.
Q During the course of the deposition your counsel will likely make objections, but unless you've been instructed not to answer, I expect you to answer my question; is that fair?
A I do understand that.
Q As you probably notice we have a court reporter taking down everything that we say today, that means you and I must speak clearly. I will try not speak over you, I would ask that you try not to speak over me.
Does that the make sense?
A I understand that too.
Q She can't record head nods or um-hmms, so please answer verbally.

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Does that make sense?
A I understand that.
Q We make take breaks from time to time, let me know if you need a break. I'll just ask that you answer any pending question before we break.
Does that make sense?
A I do understand that.
Q If you don't understand a question, please ask me to rephrase it. I'm sure that some of my questions will not be as clear as I intend them to be, but if you answer a question, I will assume you understood it.
Does that make sense?
A That's what I do.
Q Okay. You understand that while I'm asking you questions you're not permitted to discuss the substance of your testimony with your attorneys.
Do you understand that?
A I do.
Q Is there any reason why you will not be able to answer my questions fully and truthfully today?
A No.
Q Did you prepare for today's deposition?
A Yes.
Q Without revealing any privileged communications with your counsel, at a high level what did you do to prepare?
A I met with counsel over the last day and a half.
Q With whom did you speak regarding this deposition?
A Both David Bilsker and Matt Troutman.
Q Did you speak with anyone at Qiagen?
A No.
Q And about how long did you prepare?
A I was here yesterday for about eight hours and Monday was a half day, maybe four and a half, five hours.
Q Did you review any documents to prepare for this deposition?
A Yes.

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Q Do you recall specifically which documents you looked at?
A Yes.
Q What are they?
A They would have been my IPR declaration and references cited in the declaration.
Q So you prepared the declaration as you mentioned regarding Tecan patent ending in '357, do you recall that?
A I do.
Q So I'm going to hand you -- it's my understanding is it's Exhibit 1003 in the IPR2025-00026 proceeding.
(Whereupon, Declaration of Michael L. Metzker, Ph.D., Case IPR2025-00026 was previously marked as Exhibit 1003.)
Q Do you recognize this document?
A I do.
Q And what is it?
A It is my declaration for IPR 2025-00026 regarding the '357 patent.

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Q If we can turn to the last page in that the document, it's page marked 158.
Do you see that?
A I do.
Q Is that your signature?
A It is.
Q At a high level, how did you prepare your declaration?
A Typically I would first have a call with counsel describing what my -- what they're asking me to do, my activities. And then I would start reviewing documents and then there would be a -- I would do most of the technical writing, they would provide a skeleton for me to fill in where I need to fill in to finalize the declaration.
Q And you said typically, is that how it happened in this case?
A Yes.
Q Okay. Did you consult with anyone other than the attorneys that you've mentioned here, in preparation of your declaration?

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A No.
Q Have you read patent owner's preliminary response that was filed after you submitted your declaration?
A I believe so.
Q Is there anything in that paper that would change or make you modify your opinions in your declaration?
A Not that I'm aware of.
Q Have you read the PTAB's institution decision?
A I have.
Q Same question. Anything in that that would make you change or modify your opinions?
A No.
Q Are there any the changes or corrections that you would like to make to your declaration as you sit here today?
A No. Other than if we stumble over a typographical error maybe, but other than that, no.

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Q Understood. If we could take a look at -- we'll come back to your declaration. If we could take a look at the Exhibit 1016.
(Whereupon, Metzker CV was previously marked as Exhibit 1016.)
Q I've handed you what was previously marked as Exhibit 1016, do you recognize this document?
A I do.
Q What is it?
A It's my CV dated February 22nd, 2024.
Q And are there any updates between February 22nd, 2024 and now that are impactful to your experience?
A There might be a couple of updates. I just submitted a new grant, may not have been listed here, just finished a study section with the NIH this month, probably wasn't listed here. I think we may have gotten another patent, so things like that.
Q No changes to your employment?

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A No.
Q Okay. Are you currently employed?
A Yes.
Q What is your current position?
A So as stated here I'm the founder, president and CEO of a biotech company called RedVault Biosciences and I also own a wine company.
Q That's very interesting. We can talk about that off the record.
What do you consider your area of expertise?
A I have a broad area of expertise. I've been in the field for more than 35 years. I've done a lot of technology development, particularly in biotechnology, molecular biology. Genomics. I've had a lot of development work in methods of preparing libraries for sequencing, generating technologies for sequencing itself. Developing methodologies for analyzing sequences, particularly in the area of HIV

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transmission between individuals. I've been involved with the characterizing variation in the genome with respect to certain diseases, such as diabetes. I've done some work in cancer research. Done work in diagnostics. So RedVault is involved in analyzing small RNAs for urine to develop a point of care task for identifying bacterias associated with STDs.

So those are some examples.

I've done instrument work. We've developed an actual fluorescent sequencer for sequencing technologies and things like that.

Q Now, you talked a little bit about RedVault, could you tell me a bit about your work at LaserGen?

A I started LaserGen, it's my first company in 2002. That really was spearheaded from work I did in graduate school in 1994, where I published one of the very first papers on reversible terminator that is currently used in many next generation

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sequencing methods, and I went ahead and finished my Ph.D., then I went to Merck and worked at Merck for a couple years and then in 1999 I came back to Baylor College Medicine where I was a tenured track assistant professor.

During that time I decided I wanted to start a company and build the company based on sequencing technologies. So at that time the National Human Genome Research Institute, the NHGRI, was interested in funding new programs for $100,000 genome and the $1,000 genome. So I was awarded a large grant through Baylor. LaserGen was a subcontractor, and we were chemically synthesizing novel reversible terminators that would be used in the next generation sequencing.

That's what I did for about more than 10 years, we brought in investors, they took the company to the next level, it sold to Agilent and that technology now is kind of

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sidelined, but that's what I did at LaserGen.

Q Was the focus of the LaserGen reversible terminator all the way through 2012, which is where I think you left, at least according to your declaration?

A Right. Well, the reversible terminators was a core chemistry that we then built a platform around and really the goals was to develop a next generation sequencing instrument that utilized the novel reversible terminators for lots of applications for DNA sequencing.

Q Did that platform ever become commercially available?

A It did not. As I mentioned, it was sold to Agilent. Agilent had grand plans to commercialize a clinical instrument for cancer diagnostics. I wasn't involved in that at that time, it's just secondhand news, but at some point, new management came in and they decided to shift gears.

Q Okay. You have been involved in patent

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litigation before, right?

A Yes.

Q And on Page 2 of your CV, Exhibit 1016, you have a list of prior expert experience.

Do you see that?

A I do.

Q Could you, to the extent you remember, would you run down and let me know which of these were patent cases?

A I don't think the 10X Bio-Rad was. It was an ITC case. But the Plexxikon versus Novartis was a patent case. Guardant versus Foundation Medicine was a patent case. Archer versus Qiagen was a patent case. Illumina versus Natera was a patent case. Illumina versus Complete Genomics was a patent case. Complete Genomics versus Illumina was a patent case. Ravgen versus Ariosa Diagnostics was a patent case. The Illumina Cambridge Limited versus MGI was a patent case.

The Guardant versus Natera was not a

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patent case; that was the false advertisements case. DNA Genotek versus Spectrum Solution was a patent case. Twinstrand Bioscience versus Guardant was a patent case. The Trustees of the University of Pennsylvania versus Regenxbio -- and Regenxbio versus Sarepta was a patent case.
Spectrum Solution versus DNA Genotek was an IPR, but it did involve patent, obviously. Invitae versus Natera is a patent case. Natera versus NeoGenomics is a patent case.

Q So almost all of them?

A Almost all of them. I noted the exceptions.

Q Yes, absolutely. Thank you.

And how many of these involved DNA sequencing technologies?

A So going down the list, Guardant Health versus Foundation Medicine is -- involves DNA sequencing technologies. Archer versus Qiagen. Illumina versus Natera. Illumina versus Complete Genomics. Complete Genomics

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versus Illumina. Ravgen versus Ariosa was more about blood collection, but was used for isolating cell-free DNA for sequencing purposes.
Illumina Cambridge versus MGI was sequencing technology. The Guardant Health versus Natera was false advertisement, but it involved MRD tests that involved next generation sequencing. DNA Genotek versus Spectrum was salvia collection tubes that were used for DNA sequencing application. Twinstrand Biosciences versus Guardant involved ways of quantitating variants by DNA sequencing technologies.
Spectrum Solutions versus DNA Genotek was, again, a salvia collection that would be -- that sample would be used for next generation sequencing. Invitae versus Natera involves the analysis of sequencing data. And Natera versus NeoGenomics involves MRD testing, which uses next generation sequencing technologies.

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Q Okay. We can set your CV aside.
So you talk a little bit about some technical background in your declaration. I'd like to the talk through some of the concepts.
PCR is polymerase chain reaction; is that a fair -- we can -- we're on the same page with that language?

A That's what it stands for.

Q Okay. And you explained that PCR can amplify particular regions of genetic material; is that fair?

A Well, PCR is a very broad term, and there's lots of different ways to use PCR. One of those ways, if you use two target-specific primers, you can amplify specific regions within the DNA sample that you have.

Q And that's pretty typical, using two primers to amplify a target section. That's, generally, what we would call a typical PCR reaction; is that fair?

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MR. BILSKER: Objection. Vague and ambiguous. Overbroad.

A As I said, PCR is a very broad term. That's one way to do PCR. There's many ways to do PCR.

Q Okay. If we look at the section of PCR that's on Page 17 and 18 in your declaration, starting at Paragraph 29, you have a figure on Page 18 underneath Paragraph 30.
Do you see that?

A I do.

Q Okay. And this depicts a PCR reaction where two primers are used, right?

A In this figure, two primers are being shown.

Q Okay. And in order for the primers to bind, the DNA must be what's call denatured; is that fair?

MR. BILSKER: Objection. Vague and ambiguous. Overbroad. Foundation.

A Could you repeat the question, please?

Q Sure. And maybe I can clarify a bit.

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So in PCR -- in a PCR reaction, you start with, at least in the figure that is in -- on Page 18 of your declaration, you start with what's called a double stranded piece of DNA, right?

A Right. So this is a figure that comes from a chapter that I wrote. And this is illustrating kind of the basics of how the PCR, as it was described by Kary Mullis in the 1980s, how this method would work.

Q And so you're -- in this figure that you chose to include, you start with a double stranded piece of DNA, right?

A In this illustration, just showing kind of the basic concepts of PCR, we do start with the double stranded -- we start with the double stranded DNA.

Q And there is, in that first step of the figure, there's an arrow that's the labeled "primer extension."

Do you see that?

A Yes.

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Q And the shorter, I guess it's -- shorter lines with five blue ticks, those are the primers, right?

A Well, again, this is kind of a high-level depiction of a primer and those picks are more like base pairs, where, by design, in this example, the primer has been designed to be complementary to a region within the DNA template.

Q As you depict in the figure, one of the typical first steps of a PCR cycle is to separate the strands by denaturing them, right?

MR. BILSKER: Objection. Form.

A Well, again, this is kind of a general figure. There are actually other steps before you actually denature DNA. But I'm just -- I'm trying to describe the three basic steps in the basic PCR method described by Dr. Mullis. And one of those steps is to separate the double strand into single strands.

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Q And what is the purpose of separating a double strand into single strands?

A So in the PCR reaction, there are also primers. And those primers are designed to hybridize to regions that, by design, will create a partially double stranded complex.

Q Primers can't hybridize unless the double stranded DNA has been denatured; is that fair?

MR. BILSKER: Objection. Form.

A Not necessarily.

Q What do you mean by that?

A Well, there's things called triplex DNA, where double stranded DNA can be used, and you can hybridize a third strand of DNA.

Q Is that what typically happens in a PCR reaction?

MR. BILSKER: Objection. Form.

A Again, there's many ways of doing PCR. In the example that I'm showing, which is, again, just kind of the basic PCR steps. In this example, you would denature the DNA, but

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that doesn't necessary mean it's true for all PCR methods.

Q And once, in the figure that you have, once two primers hybridize to the single stranded pieces of DNA, each of the primers, in the reaction, extends.

Can you explain what you mean by "extend" in the figure?

A Sure. So in -- this is all happening in a tube. And in the tube is a solution. And in the solution, in addition to the DNA that we've been talking about and the primers that we've been talking about, there's an enzyme called a DNA polymerase. There are also nucleotides that are in the solution along with buffers that control the PH, magnesium ion, there can be some salts and all of that.

But the idea is once the primer has hybridized to the template to create this partially double stranded complex, that's where the polymerase can then bind and start incorporating those nucleotides in solution

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to create the complement strand of the template strand. And we call that extending.

Q And the top of this figure creates a circle where you go from double stranded DNA down to single stranded DNA with the primers and extension, and then back to a double stranded DNA.

Is that a fair representation of that first level of the figure at the top?

A Well, we call it a cycle, not a circle.

Q Sure.

A And it is depicting the three steps. And in each cycle, if everything works perfectly, the number of DNA molecules that you make double. And over a number of cycles, it become what we call exponential and you can make millions of copies from your starting material.

Q And what's being copied here is the sequence between the two primers, right?

MR. BILSKER: Objection. Form.

A In the example that I'm showing here, the primers define the area that gets copied.

Q Okay. When we were talking about your background, you referred to next generations sequencing.

Do you recall that?

A I do.

Q What is your understanding of what next generation sequencing is?

A It is a broad field of different technologies that replaced what I called first generation sequencing or commonly known as sanger sequencing.

Q And does that broad field of the technologies that replaced sanger sequencing, do they have commonalities, other than they just came next?

MR. BILSKER: Objection. Form.

A Very -- they have a couple general commonalities. For example, they don't require the use of electrophoresis, which was one of the main limitations of sanger sequencing. Most of them start with a single molecule, some of them end up amplifying those molecules, some of them continue to use single molecules.

But typically are not cloned, where in sanger sequencing, all of our DNA templates were cloned into bacteria that we would have to culture and have large freezer farms of glycerol stocks of bacteria.

Q I do remember that in my graduate school days, so understood.

Next generation sequencing is often abbreviated NGS; is that fair?

A That is fair.

Q Okay. And just another vocabulary point, in order to use or perform a next generation sequencing, typically it's understood that you have to prepare what's called a sequencing library; is that accurate?

A I think that's, generally, true for any sequencing technology.

Q Okay. And what is a sequencing library, as you understand it?

A So a library is not a building with books, but in molecular biology, we call a library the collection of DNA fragments, RNA fragments, nucleic acid fragments, for lack of a better word, that were derived from the sample that you want to analyze. Then you can manipulate that library in many different ways.

Q So starting at Paragraph 39 of your declaration, you have a section that's entitled, "Applicable Standards."

Do you see that?

A I do.

Q And the legal standards presented in this section over the next couple of pages, those were provided by counsel, right?

A That's correct.

Q And in your declaration you assumed a particular level of expertise for the person of ordinary skill in art.

Do you recall that?

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A I'm not sure I assumed anything.
Q Okay.
A I was provided some standards of how I should think about what a person of ordinary skill in the art would be relevant to the technology field.
Q Okay. And how did you arrive at the skill set that you applied in this case?
A Well, I considered some of things I was provided by counsel to consider, and then I also based it on my own experience in coming to what I thought the person of ordinary skill in the art -- the level of skill should be.
Q Do you understand the patent owner provided a slightly different level of skill in their papers?
A I believe I understand that.
Q Would your opinions change if you applied patent owner's definition of the person of ordinary skill in the art?
MR. BILSKER: Objection. Form.

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A I don't think it would. I've come to the same conclusions.
Q Okay. If we could look at the claim construction section of the declaration, which starts at, I think, Paragraph 53 -- or 52, excuse me.
Are you there?
A Yes, I'm there.
Q Now, the first sentence of this paragraph: "I understand the petitioner has taken the position that the term 'adapter' means any oligonucleotide that is attached to the target sequence."
Do you see that?
A I do see that sentence.
Q What do you understand "adapter" to mean in your work?
MR. BILSKER: Objection.
Q Let me rephrase that.
How do you use the term "adapter" in your work?
A Well, I pretty much use it the way it's

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described here, where an adapter is an oligonucleotide, and it is something that you will attach typically to your target sequence.
Q In your experience does an adapter serve a function?
A It can.
Q And what functions can an adapter serve?
A It depends on what is included in the adapter.
Q And what kinds of adapters have you used in your research?
A I've used a variety of adapters.
Q Can you give me some examples?
A Sure.
I've used adapters that were helpful for cloning. I've used adapters that were helpful for making sequencing libraries, particularly in next-generation sequencing. Adapters are absolutely required for next-generation sequencing because each platform has its own specific requirements

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that the libraries need to be modified to run on the sequencers.
Q Have you ever used an adapter that serves no purpose?
MR. BILSKER: Objection. Form.
A Not that I would recall. I usually don't use things that have no purpose.
Q You note in your -- in that Paragraph 52 that the patent, the '357 Patent, defines the term "adapter."
Do you see that in the second sentence of Paragraph 52?
A I do. And I actually quote where that comes from in the patent.
Q Is there a reason why you didn't you use the definition provided in the patent in your analysis of the term "adapter"?
MR. BILSKER: Objection. Form.
A Yes.
Q And why is that?
A Well, it's stated in the next sentence that:

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"I'm also aware that during prosecution the examiner reviewed the above definition of 'adapter' and concluded that since any oligonucleotide can perform such function, any oligonucleotide is considered to anticipate this term."
And so based on that, that's my understanding the basis of how the term should be construed.
Q Do you have any understanding of the legal standards that examiners use for claim construction during prosecution?
MR. BILSKER: Objection. Form.
A I'm not an attorney, so I wouldn't know what those standards are.
Q And so do you have any understanding of whether the standard for claim construction that an examiner uses is different than what the PTAB will use?
MR. BILSKER: Objection. Form.
A I don't.

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Q And did you do any further analysis of the specification or the prosecution history other than this -- looking at this statement by the examiner in your construction or proposed construction of the term "adapter"?
A Well, I reviewed the specification, not just this definition, but the entire specification. I also looked at the entire prosecution history, and this is where I came down.
Q Okay. One of the articles that you discuss in your declaration is referred to as "Porreca."
Do you recall that?
A Yes.
Q And if we could -- I'll give it to you to make sure that you have it handy. It's Exhibit 1006.
(Whereupon, US Patent Application Publication No. US 2012/0165202 A1 by Porreca et al. was previously marked as Exhibit 1006.)

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Q I've handed you what's been marked as Exhibit 1006. Is this the publication that you referred to as "Porreca" in your declaration?
A Yes.
Q In general what does Porreca describe?
MR. BILSKER: Objection. Form.
A Well, I mean, I can read the abstract for you. I mean, it really is trying to develop methods and compositions to reduce bias and increase the reproducibility of multiplex analyses of genetic loci. And it describes a number of preparative methods of how that can be accomplished.
Q And do you have an understanding of what is meant by "reduce bias" here?
A Yes.
Q And what does that mean?
A So for example, there are -- I like to think of library preparation, next-generation sequencing, analysis of next-generation

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sequencing as a workflow. And there are various steps within the workflow that can introduce errors, biases, for example, making duplicates, creating errors that affect the overall analyses. And so they're trying to reduce all of those, which they call the reduced bias and increasing the reproducibility of the analysis.
Q And when you say that there are various steps within the workflow that can introduce errors, can you give me some examples of those, please?
MR. BILSKER: Objection. Form.
A Sure.
For example, when starting with genomic DNA, we typically break things up, and then we try to put them back together. In breaking DNA up, sometimes damages the DNA bases themselves. So that when they're analyzed, they may actually look like a different base than what was in the sample. That's one example.

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Q Do you have any other examples?
A Yes.
Q And what are they?
A So PCR can introduce errors. PCR can introduce duplications. Sequencing the actual process of decoding the four letters of the genetic code can incorrectly call a base. Once the reads are generated, the reads can be mapped to the genome and not be placed properly and provide errors. So there's lots of stages within the workflow where bias can occur.
Q And when you say "PCR can introduce duplications," what do you mean by that?
A So just give a brief example of a single molecule, and that's what I start with in my PCR. And when I go through the basic PCR protocol where I have two primers and I denature that single molecule of double-stranded DNA, and then I hybridize my primers, which I've designed to target a very specific region in the genome, and then

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extend that, I make two copies.
But in reality it only started from one copy. And so I mean, that's a simple example of what a duplication can be by PCR if, for example, there's just one molecule.
Q Understood.
And when you said, "PCR can introduce errors," what did you mean by that?
A So in the extension step, we -- I previously described a DNA polymerase. DNA polymerases are enzymes that copy DNA. So they use one strand -- and the strands of DNA and RNA, they have information. The sequence of those letters provide a code. And so the job of the DNA polymerase is to copy one strand into another strand that faithfully reproduces that code. But there are instances where the DNA polymerase will insert or incorporate the wrong nucleotide, and that will be an error.
Q Okay. I'm going to kind of go back and forth between your declaration and the

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Porreca reference, so you can have them handy.
If we can take a look at Paragraph 73 of your declaration.
A Yes. I'm there.
Q And starting at Paragraph 73, you discuss what you've identified as Limitation 1A, and this limitation is the limitation that recites "ligating an adapter to each of a plurality of nucleic acid fragments."
Do you see that?
A I do.
Q And in this section, you point to two disclosures in Porreca for support for your opinion that Porreca discloses this limitation, and I'd like to walk through those.
First, you point to Paragraph 150 of Porreca, and that's quoted in the middle -- or at the end of Paragraph 73.
Do you see that?

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A I do.
Q Okay. And the second piece of information you point to in Porreca for this section of your declaration is Figure 4C, and you've depicted a copy of Figure 4C in the middle of Paragraph 74.
Do you see that?
A I do.
Q And just so I understand these, the ends in Figure 4C, that's the adapter; is that accurate?
A I'd say it's part of the adapter.
Q Okay. And what do you mean by that?
A Well, I think what Porreca is doing here is just illustrating what they call the differentiator tag. We can call it a UMI. But in reality the adapter would include other things in addition to these ends, but I think for simplicity they're just showing a very common method called ligation of an adapter to the end of a fragment.
Q Okay. And Figure 4C is part of a larger

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figure; is that fair? Figure 4 has two other pieces. Do you recall that?

A I do. Figure 4 has A, B and C. And their Figure 4 is just illustrating the number of different well-known ways of putting adapter on -- or appending an adapter on the end of a DNA fragment.

Q And so if you could look at Figure 4 in Porreca. It's on page marked Exhibit 1006.0005.

A I'm there.

Q Okay. In Figure 4A could you explain how the padlock probe incorporates the adapter into a -- onto a target sequence?

A Sure.

So a padlock probe is a linear oligonucleotide that has two targeting ends or arms, as they depict them, which are target-specific to the region of interest. And those two targeting arms are connected by an adapter that contains a number of functions. As illustrated here it includes a

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differentiator tag, PCR priming sites. It may actually contain more functionality, but I'm just describing what I see in Figure 4A.

Q And so in Figure 4A, the molecule of interest is not depicted, right?

A That's correct.

Q So how does a padlock probe incorporate an adapter to -- or let me use the word that you --

If I can rephrase my question.

In Figure 4A, how is the adapter appended to the end of a DNA fragment?

MR. BILSKER: Objection. Form.

A So first, the way the padlock probe is drawn there is a molecule of interest, it's just not shown. Because as I mentioned the padlock probe is a linear oligonucleotide and they're actually showing it as a half circle where the targeting arms appear as vertical lines.

In this example, the adapter is already attached to the targeting arms, it's already

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appended to the targeting arms. And once the targeting arms hybridize to the target, as you can see in Figure 4A, there's a gap, the gap can be just a break in the phosphodiester bond, and a ligation reaction can take place to ligate. Now, the probe containing the adapter around the target sequence or a polymerase if the gap is more than a nucleotide long, a polymerase can extend the targeting arm to the other targeting arm creating a gap, by which again a ligase can then ligate the padlock probe that has the adapter based on hybridization to the target.

Q I'm not sure I understand. In Figure 4A, where is the molecule of interest, where would it be depicted if you were to draw them?

A It would be right below the targeting arms.

Q The targeting arms, is it fair to say those are designed to hybridize with portions of the molecule of interest?

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A They actually wrap around in forming the double helix around the molecule of interest, to which -- (indicating) I'm showing my fingers coming together, which you can't capture, to which there's a gap, whereby the ligase will then ligate the probe arms together around the target of interest.

Q So the targeting arm one is ligated to targeting arm two; is that fair?

A Only when it's wrapped around the target of interest.

Q Okay.

A They can't ligate to themselves in a vacuum.

Q Understood.

A They have to be hybridized and wrapped around the target of interest.

Q Okay. What's the next step, like, what would be the purpose of attaching a padlock probe to a double helix and the molecule of interest?

MR. BILSKER: Objection. Form.

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A What is the purpose? Well, the purpose is to enrich for a sequence of interest that can then be characterized downstream.
Q How would you do that?
A Well, there's lot of ways to do that.
Q With the padlock probe, how would you -- what's the next step after it binds to the molecule of interest, what's the next step in the process?
MR. BILSKER: Objection. Form.
A So the next step would be to introduce an exonuclease into the reaction.
Q What does the exonuclease do?
A The exonuclease will degrade any of the padlock probes that were not formed into a circle.
Q After any of the padlock probes that are not circular are degraded, what happens next?
A There's lots of things that can happen next. I mean, lots of pathways -- different pathways that can occur after the padlock probe has been made into a circle.

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Q If your goal is to enrich for the sequence of interest, what would be the next step?
A A next step could be to amplify.
Q How would you go about amplifying?
A Well, in the Figure 4A, there are two PCR priming sites. So one example would be to isolate the circle and then use PCR to amplify that circle, the circle can be degraded into a linear strand again, and it can be characterized in a different way. There's different ways to handle padlock probe.
Q So at what stage, using a padlock probe, at what stage do you have a molecule of interest appended to the adapter?
A I think you have it here where you form the circle, that's one example.
Q When you form the circle, the targeting arms are attached or hybridized to a molecule of interest, right?
A Well, what I was trying to explain

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though, the way it's drawn here and I think it's described several times, at least in Porreca, that the targeting arms are separated by some region of interest and they will use a polymerase to copy the target, which then gets ligated into a circle. So the targeting arms and then the copied region are all part of the template of interest that gets characterized.
Q If we look at Figure 4B, can you explain to me how the adapter is appended to the molecule of interest in what's depicted in 4B?
A Yes, again 4A, or Figure 4, is just very general ways of putting an adapter onto a target.
In 4B this is using primers, which have what we call five prime tails, and those tails include adapter sequences. As depicted here there would be a differentiator tag or a UMI and then usually just shown as a line, but it can be a priming side for PCR,

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sequencing side for sequencing. There can be restriction enzyme sides for cloning, there's lots of things that would go into any of these adapters.
Q So I don't think that exactly answered my question.
So if we look at Figure 4B, how is the adapter appended to the molecule of interest. We have a primer, you said it's a primer with an adapter, but how do you end up with a molecule of interest that's attached to the adapter?
MR. BILSKER: Objection. Form.
A Oh, I guess I was describing the primer. Once it's hybridized, it's just shown here as the molecule of interest, it would be extended and the copied template, which now has copied the molecule of interest. Because there's a five prime tail, now the adapter has become a part of the molecule of interest.
Q Understood, thank you.

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So if we can look at limitation 1C, which starts at Page 58, Paragraph 77 of your declaration.
A I'm there.
Q So the limitation that you've label as limitation 1C recites "An indexing site unique to a subset of adapters."
Do you see that?
A I do.
Q For this limitation you rely on Paragraph 149 of Porreca, I think that's copied or quoted on the top of Page 59 of your declaration.
Do you see that?
A I mean, it's one cite that I rely on.
Q And this, the portion that is quoted at the top of Page 59, that refers to what's called a bar code.
Do you see that?
A I do.
Q And I guess, do you understand how Porreca uses the term bar code?

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A I do.
Q And how is that?
A So first bar codes are used for lots of things, Porreca is using a bar code as a DNA sequence that can be used for multiplexing samples.
Q And this type of bar code is often called a sample index; is that accurate?
MR. BILSKER: Objection. Form.
A There are lots of names.
I would not say it's a commonly used term, but I wouldn't disagree that it hasn't been referred to that before.
Q Okay. When you used the term multiplex, what did you mean by that?
A Well, that's a good question. In context of bar coding the way Porreca is using it, multiplex would be to tag samples from different individuals or different sources, this is commonly done in next generation sequencing because these machines have so much throughput that to make the

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systems affordable and fast, you can pool samples. And that pooling of samples is called multiplexing, but multiplexing has been used for other descriptions as well, but for this purpose, it's multiplexing is pooling samples.
Q And what other ways has multiplexing been used that you're referring to?
A Well, there's a common method called multiplex PCR, and in that instance, you would amplify in a single tube two or more regions of interest and that can come from a single sample, so that's why I think it's context specific of how you use multiplex.
Q Understood. But you said Porreca uses the -- or contemplates multiplexing in the way that you first used it, the identifying samples from different sources; is that accurate?
MR. BILSKER: Objection. Form.
A Well, I mean it's just what Porreca says and I quote "In addition bar codes may be

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included, for example, to uniquely identify individual samples (e.g. patient samples) that may be mixed together" and that mixing I call pooling, that would run on a single NGS instrument.
Q So if we could look at Paragraph 149 of -- the full Paragraph 149 of Porreca that you quoted from.
A My copy is marked up is that...
Q That is the way that it was filed.
A Okay. I just wanted to let you know.
Q I appreciate it.
MR. BILSKER: Really?
MS. CIPRIANO: Yeah.
MR. BILSKER: Because I have one that's stamped as an exhibit that doesn't have any marking on it.
MS. CIPRIANO: It has highlights?
MR. BILSKER: Mine doesn't have any highlights on it.
THE WITNESS: So mine is marked --
MR. BILSKER: I'm not saying that -- I

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don't remember, but it seems like it would have printed from the same place, right, it's marked the same.
MS. CIPRIANO: Agreed.
MR. BILSKER: It's not a big deal, we're just not going to attribute to one person or another.
MS. CIPRIANO: No, agreed.
MR. BILSKER: We'll agree like if it happens to be yours, it's not a waiver of privilege or anything like that.
MS. CIPRIANO: I appreciate that. I do believe it's what we pulled from the -- at least what was served on us, but we can agree both ways.
Q So if you look at Paragraph 149.
A Yes, I'm there.
Q And 149, refers, the first sentence there it says, "the skilled artisan will appreciate that as a part of a MIP library preparation process, adaptors may be ligated on to the ends of the molecules of interest."

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Do you see that sentence?
A I do.
Q Do you have an understanding of what a MIP library preparation process is?
A Yes.
Q What is it?
A Well, it would be something similar to what we saw in 4A.
Q What do you mean by that?
A So a MIP is, I think it's called a molecule inversion probe and it is a single oligonucleotide that has targeting arms as one example depicted in 4A.
MS. CIPRIANO: So we've been going for about an hour. Maybe it's time for a break.
MR. BILSKER: Okay. That's fine.
(Whereupon, a recess was taken from 10:39 AM until 10:54 AM.)
Q Welcome back.
A Thank you.
Q If we could go back to your declaration.

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I'm going to look at Limitation 1E, and that's around Paragraph 82, I have in my notes.
A Yes. I'm there.
Q And the limitation that you've identified as Limitation 1E, is the limitation that requires amplifying the adapter-ligated fragments into amplicons.
Do you see that?
A Yes.
Q And in Paragraph 82 you point to a portion of Porreca that you quote from, that is Paragraph 150.
Do you see that on Page 62?
A Yes.
Q And then there are two sections highlighted in this, that you've bolded, I guess I will say, in the quoted section. The first is at the top, "For example, nucleic acids comprising differentiator tag sequences may be incorporated by ligation."
Do you see that?

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A I do.
Q And then further down in that paragraph you have another bolded sentence, "Another method is to incorporate the differentiator tag sequence into a PCR primer."
Do you see that?
A I do.
Q And I'd like to talk about the mechanics, the differences of these two-ways to incorporate the differentiator tag sequence.
But is it accurate to say that with ligation the fragments and adapters have blunt ends and there's a ligation reaction that sticks them together?
A I think that can be one way to do it.
Q How else does ligation work?
A Ligation works in a lot of ways, blunt end ligation is one. There's a ligation method called TA ligation, where a single A base is added in a non-template way on the ends of a fragment, and then that is

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hybridized with an adapter that has a T-overhang and a ligation reaction can occur in that way.
Restriction enzymes have been frequently used for creating what we call sticky ends. And sticky ends can both occur on the DNA fragment of interest and on the adapters. And those come together, again, by hybridization, and then the gaps are closed by DNA ligase.
So those are just some examples of how a ligation reaction can take place.
Q You said where a single A base is added in a non-template way.
What do you mean by "non-template way"?
A So earlier I was describing for you the extension reaction where you have a primer that's hybridized to a single strand of template DNA that creates a partially double stranded. The polymerase will then use the single stranded portion of the template to copy the information from the template strand

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into the new strand, and that's called template-dependent DNA polymerase.
An independent addition is where there's no template available for the polymerase to copy from, this is the phenomenon, for example, taq polymerase has been used widely to add a single A base on the end of a fragment in a non-template way.
Q Understood, thank you.
And in this section, in the second bolded sentence in the paragraph that's quoted on Page 62 of your declaration, can you explain to me how you would incorporate the differentiator tag sequence into a PCR primer?
A Yes. So both of these highlighted sections are widely-used methods in molecular biology, they've been used for decades. We've gone over this in Figure 4B, where we put a five prime tail on the end of a target-specific primer, and that tail can incorporate the differentiator tag and other

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adapter-related sequences depending on what your application is for the target of interest.
Q Okay. Thank you.
And just so we're on the same page, the portion of Figure 4, Figure 4C that's reproduced at the bottom of Page 62, is that -- would you understand that to depict a blunt end ligation?
A Yes. This example appears to be a blunt end ligation example.
Q Okay. If we could look at Limitation 1G, that starts at Paragraph 86 of your declaration.
A I'm there.
Q Okay. Limitation 1G is a limitation that recites identifying sequence reads with identical identifier and target sequences as duplicates.
Do you see that?
A I do see that sentence.
Q So it's your opinion that Porreca

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teaches that differentiator tags can be used to identify whether particular nucleic acid molecules are copies of the same molecule, right?
A Could you repeat that question.
Q Sure. Let me rephrase it.
If we look at the last sentence of Paragraph 86 of your declaration, just so we're on the same page, it reads, "Because each nucleic acid is tagged with a unique differentiator tag prior to amplification, molecules having identical nucleic acid sequences and bearing the same differentiator tags are duplicates of one another."
Do you see that?
A I do see that sentence.
Q Does Porreca explain how the differentiator tag and the sequences are used to identify duplicates?
A Yes.
Q Where is that?
A So, for example, if you go to

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Paragraph 85, I show an illustration of Figure 5, where Porreca is showing pretty much the overall workflow of characterizing a library and identifying duplicate sequences. And so on the left-hand side, Porreca is starting with genomic DNA and they've made their library where they've attached their different DNA tags to the ends of DNA fragments. And those DNA fragments are illustrated by vertical lines. And then all those vertical lines represent identical sequences. And then they're highlighting a particular locus that has either a T or an A. That's their starting material, which they then amplify and they're illustrating some of the bias, such as creating duplicate amplicons derived from the same DNA fragment that's been tagged with a differentiator tag. Identifying those duplicates and then collapsing those -- the reads and the differentiator tags into single consensus sequences, based on both the identical target

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sequence and the differentiator tag.

MR. BILSKER: Can I just say one thing. You said vertical lines, I look at those lines --

THE WITNESS: Sorry, horizontal lines.

MR. BILSKER: Okay. Just for the record.

THE WITNESS: Understood.

MR. BILSKER: Or straight lines, I think is probably what you're thinking about, right.

Q The only lines, essentially, in Figure 5 are horizontal lines.

A Horizontal lines.

Q Understood. Let's break that down a little bit.

It's your understanding that the longer, if we look at Figure 5, the longer of the horizontal lines, those all represent identical sequences?

A Yes. That's commonly depicted in sequence alignments.

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Q Does Porreca explain which element that is sorted, which is done first in their steps?

MR. BILSKER: Objection. Form.

A I guess I don't understand the question.

Q Right.

A Because I understand Porreca performs sequencing on its library of fragments, and generates sequence reads. And a single sequence read would have both the target sequence and the identifier sequence together.

Q So there's a world where Porreca, the way they analyze the data, Porreca first sorts by the identifier sequence and then sorts by the sequence; is that fair?

MR. BILSKER: Objection form.

A I'm not sure. Because when I look at Figure 5, it is showing the alignment of different reads that contain both the target sequence and the identifier at the same time.

Q But it's possible that in this arrow of

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collapsing differentiator tag sequences, Porreca first sorts by differentiator tag and then looks at the nucleic acids that it's depicted in the figure; is that accurate?

MR. BILSKER: Objection. Form.

A That's not my understanding of how Porreca does this.

Q Where, in Porreca, does it explain how that collapsing is done?

A Well, it's illustrated here in the figure. I think the figure clearly illustrates that it's looking at both the target sequence and the differentiator tags at the same time, to then collapse the reads into consensus sequences.

So they're removing the duplicates and ending up with the right proportion of target sequences that they started with on the left-hand side of the figure.

Q So based on the analysis that's done in Porreca, how would a person of ordinary skill know that the horizontal lines depicted in

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the figure are identical?
A There are a number of ways to do it. One way is to do sequence alignment. Porreca is showing more than two, so we would call that a multiple sequence alignment. And there are many programs widely available to do such an analysis.
Q Does Porreca explain how it compares sequences to determine if they're identical?
A Well, I think the figure speaks for itself. And this is so common in the field that a person of ordinary skill in the art, just looking at the figure, would know how Porreca is doing this.
Q So Porreca depicts single letters in -- as a kind of break in the horizontal lines that we've been looking at.
Do you have an understanding of why Porreca would have only represented a single nucleotide, here?
A Sure.
Q And why is that?

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A Well, so the region of interest is made up of many nucleotides, but within that region, there may be one variable site. And in this case, it would be a single nucleotide variant, we call it an SNV, and what Porreca is showing that two fragments have one copy, or one variant, T, these are letters that stand for the basis of DNA, and that the other variant is an A.
And so we would call this a single nucleotide variant. And they're just illustrating the variant in these reads in Porreca Figure 5.
Q Let's shift gears a little bit and talk about another reference that you testified about, and that's the Schmitt reference. And I'll hand you Exhibit 1007.
(Whereupon, PCT Application No. WO 2013/142389 A1 was previously marked as Exhibit 1007.)
Q Do you recognize Exhibit 1007?
A I do.

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Q I can point you to, I think it's Paragraph 127 I have in my notes of your declaration.
A Yes. I'm there.
Q So your discussion of Schmitt starts around Paragraph 100.
But let's first start with -- okay.
What is Exhibit 1007?
A Well, it's a PCT patent application by Michael Schmitt, Jesse Salk and Larry Loeb that is titled Methods for Lowering the Error Rate of Massively Parallel DNA Sequencing Using Duplex Consensus Sequencing.
Q And this is the Schmitt application that you rely on in your declaration?
A Yes.
Q So if we could look at your discussion of Limitation 1G, which starts at Paragraph 127 in your declaration. Just for the record, Limitation 1G, again, is identifying sequence reads with identical identifier and target sequences as

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duplicates.
Now, you rely -- I guess let's take a step back. In this section of your declaration, you talk about your opinion that the claims of the '357 Patent are obvious in light of a combination Schmitt and an article by McCloskey.
Does that ring a bell?
A Yes. But McCloskey is also a patent application.
Q Apologies. Thank you for that clarification.
And you rely on Schmitt for certain limitations and McCloskey for certain limitations; is that fair?
MR. BILSKER: Objection. Form.
A McCloskey for one limitation.
Q Okay. So Limitation 1G, you only point to disclosures of Schmitt; is that accurate?
A That's correct. Although that's not to say that those limitations cannot be found in McCloskey, but I only cite to Schmitt here in

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my declarations.
Q Okay. And am I correct that you interpret the SMI tag in Schmitt to be the identifier sites of the claims?
A I do, and I'm just looking at Paragraph 100 of my declaration, which has a table at the bottom of Page 78. And even though it says -- here's one of these typos, "the '241 Patent." It should be "the '357 Patent." And it's showing the identifier site being the equivalent of the single molecule identifier, or SMI, in Schmitt and the barcode in the McCloskey patent application.
Q Okay. Now, on Paragraph 127 you cite to a section of Schmitt, and I'm looking at the middle of Page 98 where you're quoting from -- it looks like Paragraph 85 of Schmitt. This is a section right above Paragraph 128.
Do you see that?
A So are you in the second block quote?

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Q I'm in the second block quote, yes.
A Okay. Yes. Then I do see that.
Q Okay. And at the bottom -- at the end of that section that's quoted at the end of Paragraph 127, you've bolded the statement "True PCR duplicates may be unambiguously identified by virtue of having a common (i.e., the same or identical) SMI sequence."
Do you see that?
A I do.
Q Do you recall any mention in Schmitt where the PCR duplicates are sorted into families by anything other than the SMI tag?
MR. BILSKER: Objection. Form.
A Yes. So for example, if you go to Page 95, and I'm looking in Paragraph 125 at Figure 3, Schmitt is showing that the reads are being sorted and characterized both by the SMI and the target sequence.
And so for example, looking at 3A, the top portion where the Alpha is on the left and the Beta is on the right, four out of

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those five reads have the same SMI and have identical target sequences. So both of those are being evaluated in Schmitt in characterizing true PCR duplicates.
Q So underneath of that figure, you've quoted, according to the footnote, Paragraph 14 of Schmitt. There's a quote, "A-C shows sequence reads brown sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the AB or BA orientation," or Alpha Beta or Beta Alpha orientation.
Do you see that?
A I do see that sentence.
Q Okay. Now, can you point to anything in the description that explains that sequence reads are grouped into paired families by anything other than the SMI tags?
MR. BILSKER: Objection. Asked and answered.
A Again, I would point you to what's being

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depicted in Figure 3. I mean, it's clearly showing the grouping of the SMI tags and the sequences, which are -- in some cases are identical, in some cases they're not identical in the sequence alignments.
Q But that's not what the text says, right?
MR. BILSKER: Objection. Form.
A Well, I think the text is just explaining part of what's in the figure. It's not a comprehensive explanation at all. For example, it refers to "brown," and the figures aren't brown.
But again, looking at the figure, it's very obvious -- it would be obvious to a person of ordinary skill in the art that what's being displayed here are not just the SMI sequences but the actual target sequences themselves in showing where the sequences are identical and where the sequences differ.
MS. CIPRIANO: Okay. If we can take a quick break. I might be almost done.

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THE WITNESS: Okay.
(Whereupon, a recess was taken from 11:22 AM until 11:33 AM.)
MS. CIPRIANO: I have no further questions for you on the UMI.
THE WITNESS: Thank you.
MR. BILSKER: I have no questions.
(Whereupon, this examination was concluded at 11:33 AM.)

_____
MICHAEL L. METZKER PH.D.

Subscribed and sworn to
before me on this ____ day
of _____, _____.

_____
Notary Public

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I N D E X

WITNESS: MICHAEL METZKER

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Exhibit 1016	Metzker CV	11
Exhibit 1006	US Patent Application Publication No. US 2012/0165202 A1 by Porreca et al.	35
Exhibit 1007	PCT Application No. WO 2013/142389 A1	66

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C E R T I F I C A T E

I, KIARA MILLER,
a Shorthand Reporter and Notary Public of the State of New York, do hereby certify:

That the witness whose examination is hereinbefore set forth, was duly sworn or affirmed by me, and the foregoing transcript is a true record of the testimony given by such witness.

I further certify that I am not related to any of the parties to this action by blood or marriage, and that I am in no way interested in the outcome of this matter.

Kiara Miller
_____
KIARA MILLER

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