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Transcript of Michael L. Metzker, Ph.D.

Date: August 1, 2025

Case: Qiagen Sciences, LLC -v- Tecan Genomics, Inc. (PTAB)

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WORLDWIDE COURT REPORTING & LITIGATION TECHNOLOGY

Conducted on August 1, 2025

<p>1 UNITED STATES PATENT AND TRADEMARK OFFICE</p> <p>2 -----</p> <p>3 BEFORE THE PATENT TRIAL AND APPEAL BOARD</p> <p>4 -----</p> <p>5 QIAGEN SCIENCES, LLC,</p> <p>6 Petitioner,</p> <p>7 v.</p> <p>8 TECAN GENOMICS, INC.,</p> <p>9 Patent Owner.</p> <p>10 -----</p> <p>11 IPR2025-00028 & IPR2025-00029</p> <p>12 U.S. Patent Nos. 10,036,012 & 10,876,108</p> <p>13 -----</p> <p>14 DEPOSITION OF</p> <p>15 MICHAEL L. METZKER, PH.D.</p> <p>16 NEW YORK, NEW YORK</p> <p>17 AUGUST 1, 2025</p> <p>18</p> <p>19</p> <p>20</p> <p>21 REPORTED BY: KIARA MILLER</p> <p>22 FILE NO.: 593987</p>	<p>3</p> <p>1 A P P E A R A N C E S:</p> <p>2</p> <p>3 ON BEHALF OF PETITIONER:</p> <p>4 QUINN EMANUEL URQUHART & SULLIVAN, LLP</p> <p>5 50 California Street</p> <p>6 22nd Floor</p> <p>7 San Francisco, California 94111</p> <p>8 BY: DAVID BILSKER, ESQ.</p> <p>9</p> <p>10 ON BEHALF OF PATENT OWNER:</p> <p>11 GOODWIN PROCTER LLP</p> <p>12 The New York Times Building</p> <p>13 620 Eighth Avenue</p> <p>14 New York, New York 10018</p> <p>15 BY: LINNEA P. CIPRIANO, ESQ.</p> <p>16</p> <p>17 ALSO PRESENT:</p> <p>18 RICHARD SAMUEL HERRON PHD, GOODWIN PROCTER ADVISOR</p> <p>19</p> <p>20</p> <p>21</p> <p>22</p>
<p>2</p> <p>1 Deposition of MICHAEL L. METZGER, PH.D., taken</p> <p>2 on behalf of PATENT OWNER, at 295 5th Avenue,</p> <p>3 New York, New York, commencing at 9:33 AM,</p> <p>4 AUGUST 1, 2025, before Kiara Miller.</p> <p>5</p> <p>6</p> <p>7</p> <p>8</p> <p>9</p> <p>10</p> <p>11</p> <p>12</p> <p>13</p> <p>14</p> <p>15</p> <p>16</p> <p>17</p> <p>18</p> <p>19</p> <p>20</p> <p>21</p> <p>22</p>	<p>4</p> <p>1 MICHAEL METZKER, after having first</p> <p>2 been duly sworn by a Notary Public of the State of</p> <p>3 New York, was examined and testified as follows:</p> <p>4 EXAMINATION BY</p> <p>5 MS. CIPRIANO:</p> <p>6 Q Good morning. Welcome back. I am not</p> <p>7 going to go through the basics here. I hope</p> <p>8 you -- we can agree that the ground rules are</p> <p>9 going to be applied the same as they did</p> <p>10 yesterday. Does that make sense?</p> <p>11 A We can agree to that.</p> <p>12 Q Okay. You understand you're under oath</p> <p>13 today?</p> <p>14 A I do.</p> <p>15 Q Okay. Any reason you can't answer any</p> <p>16 questions fully and truthfully today?</p> <p>17 A No.</p> <p>18 Q Okay. Now, I've handed you the exhibits</p> <p>19 that have been marked 1003.</p> <p>20 (Whereupon, Metzker '357 Patent</p> <p>21 declaration was previously</p> <p>22 marked as Exhibit 1003.)</p>

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<p>1 MR. BILSKER: Yes, well, one of them 2 is. 3 Q So you can -- I think it was resubmitted 4 as 1003 in the '012 proceedings. So in front 5 of you, you have two exhibits marked 6 Exhibit 1003 in the proceeding; one relates 7 to the '012 patent, and that's for 8 IPR 202500028. 9 Do you see that? 10 A I do, though my copy is not marked 11 Exhibit 1003. 12 Q Okay. Understood. And then you have a 13 declaration that is Exhibit 1003 in -- 14 related to the '108 Patent that is for IPR 15 202500029. 16 Do you have that? 17 A I do. 18 Q Okay. So if we can turn to the last 19 page of the declaration that's marked -- 20 declaration that's for the '012 Patent, if 21 you can turn to the last page of that. 22 Is that your signature?</p>	<p>1 Q Have you read the patent owner's 2 preliminary response in these proceedings? 3 A Yes. 4 Q Is there anything in that paper that 5 would change your opinions or cause you to 6 make edits to your declaration? 7 A No. 8 Q Did you lead Dr. Bustamante's 9 declaration that was the submitted in these 10 proceedings? 11 A Yes. 12 Q Do you know Dr. Bustamante? 13 A Yes. 14 Q Do you have an opinion of 15 Dr. Bustamante? 16 A No. 17 Q Have you ever worked with 18 Dr. Bustamante? 19 A No. 20 Q Okay. How do you know him? 21 A I have seen him as an expert in another 22 case where he was the opposing expert.</p>
<p>1 A It is. 2 Q Okay. And if we can look at the 3 declaration that's for the '108 Patent on 4 Page 150. 5 Is that your signature? 6 A It is. 7 (Whereupon, Metzker '012 Patent 8 declaration was previously 9 marked as Exhibit 1003.) 10 Q And at a high level, how did you prepare 11 these declarations? 12 A I would have done them the same way that 13 I described yesterday. 14 Q As a part of drafting these 15 declarations, did you consult with anyone 16 other than the attorneys that you worked with 17 on these matters? 18 A No. 19 Q Did you have any communications with 20 Qiagen employees at part of drafting these 21 declarations? 22 A No.</p>	<p>1 Q Do you know him professionally? 2 A I know of him professionally. 3 Q Okay. Did you read the institution 4 decisions that were issued in these 5 proceedings? 6 A Yes. 7 Q Anything in that -- those papers that 8 would change your opinions or cause you to 9 revise your declaration? 10 A No. 11 Q Any other corrections that you would 12 like to make to your declarations at this 13 time? 14 A No, other than if we stumble across a 15 typographical error, we might make a 16 correction there, otherwise no. 17 Q Understood. Would you agree that there 18 is overlap in substance between these two 19 declarations? 20 A Generally, yes, because I think there's 21 overlap in the two patents that have been 22 asserted in this case.</p>

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<p>9</p> <p>1 Q Understood. Just so we're on the same 2 page, I'm probably going to focus on the '012 3 Declaration, but I'd like to go through and 4 get confirmation of the sections or the 5 paragraphs that are the same between the two. 6 So if we can take a couple of minutes to go 7 through them. 8 So if we look at paragraphs, the section 9 starting with Paragraph 8 of the two 10 declarations. 11 A You mean the background stuff? 12 Q Yeah. 13 A Yes. I'm there. 14 Q Okay. Now, would you agree that the 15 background and qualifications sections in the 16 two declarations are identical? 17 A Yeah, I see no reason why they wouldn't 18 be, given I'm the same expert in both 19 declarations. 20 Q Understood. Okay. So that's 21 Paragraphs 8 through 24. There's a section 22 entitled, Materials and information</p>	<p>11</p> <p>1 A Most likely, yes, given they're -- 2 again, these patents have the same 3 specifications. 4 Q Okay. Now, if we skip over the claim 5 construction sections, which are slightly 6 different. If we look at the technical 7 background starting at Paragraph 42 in the 8 two declarations, is it your understanding 9 that the technical background in these two 10 declarations is the same? 11 A Yeah, again, they're the same technical 12 field, I have no reason to doubt that they're 13 not very similar. I haven't compared them 14 word for word, though. 15 Q Okay. And then the last section I 16 wanted you to take a look at and compare is 17 in the '012 declaration, it starts at 18 Paragraph 95. There's a section that starts 19 with "overview of the prior art." 20 A Yes, I'm there. 21 Q And then it starts at Paragraph 93 of 22 the '108 Declaration.</p>
<p>10</p> <p>1 considered, that's Paragraphs 25 and 26. 2 Those are also identical between the two 3 declarations, right? 4 A Well, except when referring to each 5 specific patent and the file history for each 6 specific patent, but I think otherwise 7 they're probably close. 8 Q Understood. And if we look at 9 Paragraphs 27 through 38, there's a section 10 called, Understanding of the Law. 11 A Yes, I'm there. 12 Q Do you see that? 13 That section is also identical between 14 the two declarations? 15 A I mean, I haven't compared them word for 16 word, but they are legal standards provided 17 by counsel; and I have no reason to think 18 that they would be different. 19 Q Okay. And then the qualifications and 20 knowledge of one of ordinary skill in the 21 art, Paragraph 39, other than the reference 22 to the specific patent, those are identical?</p>	<p>12</p> <p>1 A Yes. I'm there too. 2 Q So paragraphs in the '108 Patent, it is 3 Paragraphs 93 through 127. And in the '012 4 Patent, it is 95 through 129. 5 Can you confirm that those are the same? 6 A Well, I haven't compared them word for 7 word, flipping the pages side by side they 8 look very similar. 9 Q Okay. As I said, as we work through 10 the -- my questions today, I'll focus on the 11 '012 declaration. I'll let you know what 12 paragraph it is in the '108, if you think 13 your answer would be different depending on 14 the patent, can you let me know that? 15 A I'll do my best. 16 Q Okay. Great, thank you. 17 Okay. So if we could turn to 18 Paragraph 54 of the declaration for the '012 19 Patent. 20 A Yes. I'm there. 21 Q At the part of Paragraph 54 that is at 22 the top of Page 33. There's a sentence that</p>

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<p>1 reads --</p> <p>2 A I'm sorry, 53?</p> <p>3 Q 33.</p> <p>4 A Okay. I'm there.</p> <p>5 Q There's a sentence at the top of the</p> <p>6 page, it says, "It is important not only to</p> <p>7 amplify or capture the target nucleic acid</p> <p>8 sequence, but to increase the portion of the</p> <p>9 target nucleic acid above unwanted sequences</p> <p>10 to reduce noise in the sequencing reactions."</p> <p>11 Do you see that?</p> <p>12 A I do. Can I have a moment to review the</p> <p>13 whole paragraph.</p> <p>14 Q Absolutely.</p> <p>15 A Okay.</p> <p>16 Q Why do you say it is important?</p> <p>17 A Well, it's important with respect to</p> <p>18 progress of the target enrichment. So if</p> <p>19 you're interested in -- for example, let's</p> <p>20 just use the human genome, which is the</p> <p>21 haplotype is 3 billion base pairs big, it's</p> <p>22 huge. And if you're only interested in</p>	<p>1 Which would be more expensive than focusing</p> <p>2 and sequencing just a 200 base pair fragment.</p> <p>3 Q Anything else?</p> <p>4 A Yes. So next generation sequencing</p> <p>5 technologies produce an enormous amount of</p> <p>6 data. Sometimes if you're only interested in</p> <p>7 a 200 base pair fragment to make the process</p> <p>8 economical, not just focusing on the region</p> <p>9 of the interest, but you may want to -- we</p> <p>10 talked about this yesterday, you may want to</p> <p>11 multiplex or pool samples together. So</p> <p>12 you're now simultaneously characterizing that</p> <p>13 200 base pairs region across lots of</p> <p>14 individuals at the same time, so it can be a</p> <p>15 process of efficiency.</p> <p>16 Q Anything else?</p> <p>17 A Anything else.</p> <p>18 So I've given you focus of region. I've</p> <p>19 given you cost. I've given you efficiency.</p> <p>20 There may be others but not that come to the</p> <p>21 top of my head right now.</p> <p>22 Q Understood. In this process, how would</p>
<p>1 something that has a region of 200 base</p> <p>2 pairs, you would use a method like target</p> <p>3 enrichment. And you would want to select</p> <p>4 just that 200 base pair fragment. That's why</p> <p>5 it's important.</p> <p>6 Q What's the benefit of focusing on a</p> <p>7 region of 200 base pairs in that scenario?</p> <p>8 A There could be a number of reasons.</p> <p>9 Q And what are they?</p> <p>10 A So one reason is you're only interested</p> <p>11 in a region of 200 base pairs. You really</p> <p>12 are not interested in anything else, so you</p> <p>13 want to focus your time and efforts on</p> <p>14 sequences that would be within that 200 base</p> <p>15 pair region.</p> <p>16 Q And are there other reasons?</p> <p>17 A Yes.</p> <p>18 Q And what are they?</p> <p>19 A Sequencing can be a very expensive</p> <p>20 endeavor and sequencing a whole genome would</p> <p>21 be tremendously more expensive than</p> <p>22 sequencing -- which is 3 million base pairs.</p>	<p>1 a scientist know that they have successfully</p> <p>2 increased the proportion of the target</p> <p>3 nucleic acid above what you call unwanted</p> <p>4 sequences?</p> <p>5 MR. BILSKER: Objection. Form.</p> <p>6 A There are lots of ways to do that. A</p> <p>7 very standard way and yesterday we talked</p> <p>8 about PCR having lots of different methods</p> <p>9 that can be performed, but if we look at the</p> <p>10 very basic method of PCR, which is the figure</p> <p>11 from yesterday's deposition and let's say we</p> <p>12 have two primers and we're targeting a region</p> <p>13 that has 200 base pairs long and we perform a</p> <p>14 standard PCR reaction, which is described in</p> <p>15 that cycle in the figure that we talked about</p> <p>16 yesterday, you can run it on agarose gel and</p> <p>17 you can do agarose gel electrophoresis, and</p> <p>18 when you stain that product and compare it to</p> <p>19 a molecular weight marker, if you get a</p> <p>20 single band that is 200 base pairs in size</p> <p>21 you would have very good evidence that you</p> <p>22 increased the amount of that particular</p>

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<p>17</p> <p>1 region over everything else.</p> <p>2 Q And the comparator there would be some</p> <p>3 sample before the target enrichment, right,</p> <p>4 you'd have to know where you started from in</p> <p>5 order to know that you enriched for a target?</p> <p>6 MR. BILSKER: Objection. Form.</p> <p>7 A Not necessarily at all. The example I</p> <p>8 provided is what we call a molecular weight</p> <p>9 marker. You can buy these commercially from</p> <p>10 lots of vendors, they have known sizes that</p> <p>11 have been well characterized. You can run it</p> <p>12 on a gel alongside the 200 base pair amplicon</p> <p>13 that you've generated by enriching that</p> <p>14 region over everything else. And if you did</p> <p>15 achieve enrichment, you expect to see a</p> <p>16 single band that lights up very brightly that</p> <p>17 corresponds with the 200 base pair marker.</p> <p>18 That would be the evidence that you enriched</p> <p>19 that region.</p> <p>20 Q What would you expect if you hadn't</p> <p>21 enriched for that region?</p> <p>22 A There are a number of things you can see</p>	<p>19</p> <p>1 you can -- and we're talking just standard</p> <p>2 PCR, which I talked about yesterday in the</p> <p>3 figure. Where each target will have two</p> <p>4 primers that are specific to a region of</p> <p>5 interest, and you can do that all in one</p> <p>6 tube. So one advantage is you save on</p> <p>7 reagents. Reagents are expensive. And that</p> <p>8 has some advantages when you want to analyze</p> <p>9 lots of samples.</p> <p>10 Q And you said there are many reasons,</p> <p>11 what other reasons are there?</p> <p>12 A Another reason is, if you have limited</p> <p>13 quantities of the material that you're</p> <p>14 interested in amplifying. If you did five</p> <p>15 singleplex PCRs, you would have to split that</p> <p>16 sample across five different tubes, which if</p> <p>17 you're looking for a rare variant you may</p> <p>18 miss the right primer pair in the right tube</p> <p>19 if the variant is in one of five copies and</p> <p>20 only one of the five tubes get it. Multiplex</p> <p>21 PCR allows you to amplify all five targets</p> <p>22 with limited quantities of starting material,</p>
<p>18</p> <p>1 on a gel.</p> <p>2 Q What are they?</p> <p>3 A You could see no product. So you could</p> <p>4 see just a blank lane. You can see what we</p> <p>5 call a smear, so you may see a lot of</p> <p>6 products that are all spread out over a</p> <p>7 variety of sizes. Or you can see a single</p> <p>8 band, but it doesn't appear at the right</p> <p>9 size. And a competent scientist would be</p> <p>10 alerted that they're not actually enriching</p> <p>11 for the region of interest.</p> <p>12 Q In Paragraph 56, of your declaration for</p> <p>13 the '012 Patent, you discuss multiplex PCR</p> <p>14 and singleplex PCR.</p> <p>15 Do you see that?</p> <p>16 A Yes.</p> <p>17 Q Why might a researcher choose multiplex</p> <p>18 PCR over a singleplex PCR?</p> <p>19 A There are numerous reasons for that.</p> <p>20 Q And what are they?</p> <p>21 A So one, for multiplex PCR you can and</p> <p>22 let's just use an example of five targets, so</p>	<p>20</p> <p>1 which increases your chance of identifying</p> <p>2 the thing you're interested in.</p> <p>3 Q Are there other reasons?</p> <p>4 A Yes. It also saves on technician time</p> <p>5 and setting up five reactions just takes more</p> <p>6 time than setting up one reaction. So it's</p> <p>7 more efficient in a laboratory to set up a</p> <p>8 multiplex PCR.</p> <p>9 Q Anything else you can think of?</p> <p>10 A Not off the top of my head, but I'm sure</p> <p>11 there could probably be a couple more.</p> <p>12 Q Are there instances where a researcher</p> <p>13 would choose singleplex PCR over multiplex</p> <p>14 PCR?</p> <p>15 A Yes.</p> <p>16 Q What are they?</p> <p>17 A They may only be interested in one</p> <p>18 target.</p> <p>19 Q Anything else?</p> <p>20 A It would depend on -- it would depend on</p> <p>21 what is trying to be accomplished. Multiplex</p> <p>22 PCR can have disadvantages such as there may</p>

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<p>21</p> <p>1 be the creation of what we call primer 2 dimers. The more primer pairs you add into 3 the mixture, the higher the risk of 4 amplifying just the primers themselves and 5 not the target. 6 So it may be a situation if the 7 multiplex PCR just cannot be optimized for 8 any given reason, a researcher may want to go 9 to a singleplex where they don't have that 10 type of complication in the laboratory. 11 Q You mentioned primer dimers, just so 12 we're on the same page, what are primer 13 dimers? 14 A Again, going back to the basic PCR 15 model, where we have two target specific 16 primers. Primer dimers are when the primers 17 themselves can hybridize to one another and 18 be extended and make amplicons that are 19 essentially the combination of two primer 20 sequences, and we call those primer dimers. 21 Q So I'm looking at Paragraph 58, where 22 you specifically talk about primer dimers and</p>	<p>23</p> <p>1 talking about primer dimers, the 2 presence of primer dimers. 3 Q Okay. 4 A There's lots of ways to avoid primer 5 dimers, but if there is a reaction that has 6 substantial primer dimers, it can create some 7 non-specific background noise. 8 Q My questions is getting at, you say 9 background noise is a problem, why is it a 10 problem? 11 MR. BILSKER: Objection. Form. 12 A Well, if it's not addressed it can 13 appear in all kinds of analyses depending on 14 what analyses that you do. 15 So researchers are always talking about 16 this and then ways to remove or avoid or 17 minimize the amount of primer dimer, for 18 example, is background noise. 19 Q Are there other issues in sequencing 20 that cause background noise? 21 MR. BILSKER: Objection. Form. 22 A Yeah, there are.</p>
<p>22</p> <p>1 you talk about non-specific background noise. 2 How does the non-specific background noise 3 impact the ability to sequence a sample? 4 MR. BILSKER: Objection. Assumes 5 facts. 6 A How does – well, can you define what 7 background noise is, because there's lots of 8 sources in the background noise. 9 Q So that's actually my question. I'm 10 trying to understand what you mean by 11 background noise when you use it in your 12 declaration? 13 A So the sentence that I think it appears 14 is – I'll just read it. 15 "The presence of multiple primers 16 in multiplex PCR tends to increase the 17 propensity of primer dimer formation, 18 which has the potential to obscure 19 downstream sequencing by increasing the 20 amount of non-specific background 21 noise." 22 So in that context, I'm just</p>	<p>24</p> <p>1 Q And what are some examples? 2 A So for example, in the Illumina system, 3 and again, Illumina has many, many ways of 4 making a library and we talked about what a 5 library is. But let's just take the standard 6 protocol of taking the genome and fragmenting 7 it to base pairs between 200 and 500 base 8 pairs and I talk about it in my declaration. 9 Then you ligate on adapters, which are 10 compatible with the Illumina system, and then 11 you would perform a universal PCR amplify 12 those. In that universal PCR you're 13 attaching now P5 and P7 sequences and those 14 can also form primer dimers where the 15 adapters actually. Now, if those were to get 16 into the sequencer, they can form clusters on 17 the flow cell and they can actually be 18 sequenced. Essentially all you're ending up 19 doing is sequencing P5 and P7, that's a 20 source of background noise. 21 So there are protocols that we employ, 22 such as bead clean ups, so we use these SPRI,</p>

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<p>25</p> <p>1 it's an acronym, S-P-R-I, all caps, beads and 2 we use various ratios of beads to sample, to 3 remove a lot of that background noise that 4 otherwise would interfere with some of the 5 sequencing reads that are generated. 6 Q Now, other than the primer dimers from 7 the adapters are there other aspects -- let's 8 focus on the Illumina system, what other 9 steps can cause background noise? 10 A So the Illumina system -- I think I 11 talked about this yesterday -- is one of the 12 advantages of next-generation sequencing over 13 Sanger sequencing is we deal with single 14 molecules. And these are -- they came from 15 our library. They've been modified, so they 16 have all of the adapters necessary to go onto 17 the flow cell to prevent -- or to start ridge 18 amplification. 19 You can add too much of the library to 20 the flow cell. And these fragments are 21 dispersed -- well, the newer systems, they're 22 in patterned arrays, but in the 2012 time</p>	<p>27</p> <p>1 handling multiple sample that are not 2 bar-coded. And one sample gets into another 3 sample, and they run it on a sequencer. And 4 if it's the same species, it's even more 5 problematic because you can't figure out 6 where the contamination is although there's 7 not one person there. 8 But if it's -- you know, we've done a 9 lot of bacterial and yeast and arabidopsis, 10 which is a flowering plant. And if that gets 11 into a human sample, you would have a lot of 12 garbage because it's not specific to the 13 subject you're trying to analyze. There's 14 lots of ways this can happen. 15 Q And we talked a little bit yesterday 16 about library preparation. Are there aspects 17 of library preparation that can cause 18 background noise? 19 MR. BILSKER: Objection. Form. 20 A Yeah, and I just gave you one. So if 21 you have two samples that you've 22 inadvertently mixed together, one</p>
<p>26</p> <p>1 frame, which is what we're talking about, 2 they were still introduced into the flow cell 3 in a random array. And the goal there is 4 that one molecule lands in a certain region, 5 and then you can make lots of copies. 6 You add too much, then you have multiple 7 molecules of different sequences all landing 8 in the same place, and you grow them up. And 9 it gives a lot of background noise that is 10 uninterpretable sequence. You can also add 11 too little of that library to the flow cell 12 and essentially have a blank flow cell that 13 doesn't produce very much sequencing data as 14 well. 15 So those are two examples of noise in 16 the sequencing system. 17 Q Can you think of any others? 18 A Sure. There's plenty of noise. 19 You can have sample contamination. So 20 in the laboratory you may have -- I used to 21 work at a genome center, and we did our very 22 best, but sometimes you have a person</p>	<p>28</p> <p>1 contaminated another one, and it goes into 2 the library, that would create problems in 3 the library. 4 Q Okay. Is there anything other than 5 contamination in the library preparation 6 steps that would cause background noise? 7 MR. BILSKER: Objection. Form. 8 A Yes. So again, I think I talked about 9 the NGS as a workflow. There's lots of steps 10 in the workflow. If steps are omitted or a 11 technician adds the wrong enzyme when they 12 think they're ligating and they're actually 13 adding an exonuclease, something -- I mean, 14 there's lots of steps that -- individuals can 15 make mistakes -- that create inefficient or 16 inferior libraries that may go on to the 17 sequencer and create all kinds of 18 uninterpretable data that frustrates the 19 managers. 20 Q Understood. 21 And I guess, is there anything about the 22 library preparation steps other than human</p>

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<p>29</p> <p>1 error that is known to cause background 2 noise?</p> <p>3 MR. BILSKER: Objection. Form.</p> <p>4 A Well, again, it depends on what you're 5 calling -- I'm trying to define what I think 6 background noise is.</p> <p>7 Q Okay.</p> <p>8 A To generally just try to speculate what 9 background noise you're asking about, I can't 10 do that. But if you want to ask me a 11 specific type of background noise, I can try 12 and answer how things in the library may 13 contribute to that.</p> <p>14 Q Okay. So you're saying background noise 15 doesn't have a single definition to you?</p> <p>16 A Well, it doesn't because I've given at 17 least two definitions, one where -- well, 18 actually three. If there was a primer dimer 19 that got through, that could -- and it wasn't 20 removed by cleanup -- and there are standard 21 ways to remove that. If that got through, 22 that could be a source of background noise</p>	<p>31</p> <p>1 A Right. So I think I describe that in my 2 declaration.</p> <p>3 Right. So I'm looking at Paragraph 57. 4 And in the second sentence it says: 5 "Rather than having only one set 6 of primers (a forward primer and a 7 reverse primer) in a reaction as a 8 singleplex PCR, however multiplex PCR 9 requires at least two or more sets of 10 target-specific primers and include 11 dozens of different primers in the same 12 reaction."</p> <p>13 Now beyond that, this is where 14 traditional multiplex PCR tends to fail 15 because getting above a couple dozen primer 16 pair sets in a traditional multiplex PCR 17 increases primer dimers, and that can -- if 18 it's not removed by the cleanup, for example, 19 that can reduce the number of reads that you 20 would see in the sequencer.</p> <p>21 Q And when you say the "traditional 22 multiplex PCR," what are you contrasting that</p>
<p>30</p> <p>1 where you're just sequencing PCR primers that 2 created a primer dimer.</p> <p>3 I've given you an example of the 4 Illumina adapters creating dimers that could 5 get into the sequencer. And again, there are 6 steps that can clean that up, but if somebody 7 forgets it, it becomes a problem. I've given 8 you background noise where there's 9 contamination where there's things that you 10 did not expect to be there, but because of 11 some cross contamination -- and it happens -- 12 that can create background noise.</p> <p>13 Q So going back to primer dimers, the 14 background noise created by primer dimers, is 15 it fair to say that limits the number of 16 primers that can be used in a single 17 reaction?</p> <p>18 MR. BILSKER: Objection. Form.</p> <p>19 A So are we talking about multiplex PCRs? 20 Which type of PCR are we talking about?</p> <p>21 Q Well, I guess we can start with 22 multiplex.</p>	<p>32</p> <p>1 with? What is nontraditional multiplex PCR?</p> <p>2 A Yeah, that's a great question because 3 there's lots of multiplex PCR methods. I'll 4 give you the traditional one first, and then 5 I'll try to answer the other question.</p> <p>6 But the traditional multiplex PCR is 7 where each target is defined by two 8 target-specific primers. We call it forward 9 and a reverse. And if I have ten targets, 10 then I would have ten primer pair sets. And 11 if I had 50 targets, I would have 50 primer 12 pair sets. That would be a total of a 13 hundred primers, right. That's what I'm 14 calling traditional multiplex PCR.</p> <p>15 Now, there are ways of using adapters to 16 do multiplex PCR, which was well-known in the 17 art. I'm thinking of a patent application by 18 Rabinowitz that describes many ways of doing 19 multiplex PCR. They call it mini-multiplex 20 PCR, hemi-nested multiplex PCR, nested 21 multiplex PCR. And it is taking advantage of 22 using an adapter, taking a genome -- and</p>

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<p>33</p> <p>1 let's just say it's a human genome and it's 2 cellular DNA; we have to fragment it. 3 And then you can ligate adapters onto 4 the ends of those fragments. And then they 5 want to target, let's say, five regions. So 6 instead of having primer pairs, they'll have 7 a single target-specific primer, and the 8 other primer will hybridize to the adapter. 9 And if you do that in one tube with five 10 targets, that would be a mini-multiplex PCR, 11 and that's well described in the literature. 12 And the advantage there is now you only 13 use 5 primers, plus 1 universal instead of 14 trying to use 10. When you're talking about 15 50 targets, now you're only using 50 primers 16 versus a hundred primers. So the advantage 17 is fewer primers, and that really helps solve 18 the primer dimer issue that traditional 19 multiplex PCR faces. 20 Q You mentioned a patent application by 21 Rabinowitz -- 22 A Yes.</p>	<p>35</p> <p>1 and cost-effective feature also facilitated 2 the use of NGS-based approaches over 3 traditional microarrays." 4 Do you see that first part of the 5 sentence? 6 A I do. 7 Q Okay. What do you mean by "traditional 8 microarrays"? 9 A So microarrays have been around since -- 10 actually, I think the first publication came 11 out in the late '80s. And then these -- what 12 we think of, the traditional microarrays 13 these days that involve either synthesizing 14 DNA directly onto a chip, like Affymetrix 15 does, or synthesizing DNA in a tube, 16 attaching the DNA to beads and then putting 17 them on a bead chip like Illumina does, have 18 been used for lots of big science projects. 19 Those are the ones I'm thinking of, for 20 example, the HapMap Project, so H-A-P-M-A-P, 21 where the NIH was soliciting big companies 22 and academic groups to identify all the</p>
<p>34</p> <p>1 Q -- right? 2 A Yes. 3 Q Do you recall approximately when that 4 came out or when you were aware of that? 5 A I want to say 2010 or 2011. 6 Q And is that an exhibit to your 7 declaration or exhibit -- 8 A I don't think so. There's also one by 9 Zimmerman too, and I may be thinking of 10 Zimmerman as well. 11 Q Okay. 12 MR. BILSKER: There's Qiagen 1055. 13 Qiagen Exhibit 1055, I'm sorry. 14 MS. CIPRIANO: Yeah, I understand. 15 16 Q Okay. If we could skip ahead to 17 Paragraph 95 of your '012 declaration. This 18 is -- if you want to refer to the '108 19 declaration, it's Paragraph 93. 20 A Okay. I'm there. 21 Q Near the end of Paragraph 95, you have a 22 sentence that reads, "This high throughput</p>	<p>36</p> <p>1 common variation in the human genome. These 2 microarrays would be used for genotyping 3 applications. But also for gene expression, 4 these microarrays were used for understanding 5 the abundance of certain genes in certain 6 tissues, both in a normal setting and a 7 disease setting. And all of these were 8 traditionally put into databases that 9 researchers can have access to. 10 Q And you contrast the NGS-based 11 approaches versus traditional microarrays, 12 right? Am I reading that sentence correct? 13 A That's correct. 14 Q And how did traditional microarrays 15 differ from the NGS-based approaches you're 16 talking about here? 17 A So we can break it down because I do 18 talk about the gene expression and 19 genotyping. Let's talk about genotyping, 20 first. 21 So genotyping in the traditional 22 microarrays setting is where you have known</p>

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<p>37</p> <p>1 variation. You've already figured out --</p> <p>2 let's say, in gene X, there is a single</p> <p>3 nucleotide variant -- we talked about that</p> <p>4 yesterday in the Porreca patent application</p> <p>5 where at one particular loci in the genome,</p> <p>6 there may either be an A or a T. And</p> <p>7 traditional microarrays were very good at</p> <p>8 determining whether A or T was at that</p> <p>9 position across lots of samples because they</p> <p>10 knew what they were looking for.</p> <p>11 And so microarrays were built where a</p> <p>12 chip, a microarray chip, may have a million</p> <p>13 different SNPs or SNVs, that can be typed all</p> <p>14 in a single experiment, but you knew what you</p> <p>15 were looking for. Contrast that with NGS,</p> <p>16 NGS discovers things. It doesn't need to</p> <p>17 know a priori, whether a known variation is</p> <p>18 present or not.</p> <p>19 And so it has a lot more flexibility in</p> <p>20 looking for variants, and it can discover</p> <p>21 things that you never knew were present. And</p> <p>22 those are tremendous advantages, particularly</p>	<p>39</p> <p>1 just measuring how much is there, but it</p> <p>2 can't count how many molecules were present</p> <p>3 in the sample.</p> <p>4 So, I mean, this really turned the whole</p> <p>5 biomedical field on its face when these new</p> <p>6 platforms came out. And there was immediate</p> <p>7 transition away from microarrays.</p> <p>8 Q And help me understand; the traditional</p> <p>9 microarray, it's on a chip, right, or some</p> <p>10 version of those are on a chip?</p> <p>11 A Yeah, again, their different formats,</p> <p>12 but they typically have a solid support.</p> <p>13 Q Okay. And attached to that solid</p> <p>14 support are target-specific probes, is that</p> <p>15 an okay term?</p> <p>16 A There can be, yes.</p> <p>17 Q Okay. I guess alternatively, what else</p> <p>18 would be on the chip or the solid support?</p> <p>19 A So, for example, the Illumina system</p> <p>20 that used beads, they would -- if they were</p> <p>21 genotyping with, for example, their Infinium</p> <p>22 platform, they would actually capture the</p>
<p>38</p> <p>1 when NGS produces so much data. You can</p> <p>2 genotype beyond a million and you can</p> <p>3 actually genotype entire whole genomes now,</p> <p>4 where microarrays suffer on that throughput,</p> <p>5 and the flexibility of finding things you</p> <p>6 don't know about.</p> <p>7 Now, on gene expression, NGS was also</p> <p>8 able to find things that weren't on the</p> <p>9 microarrays chip. So, again, the microarray</p> <p>10 is looking for certain genes and looking for</p> <p>11 ups and downs in their expression patterns,</p> <p>12 whereas NGS could find alternative</p> <p>13 transcripts or genes that were not on the</p> <p>14 chip and an advantage of next generation</p> <p>15 sequencing is it's digital.</p> <p>16 And we talked about single molecules</p> <p>17 being introduced onto the flow cell to go</p> <p>18 under bridge amplification. Each of those</p> <p>19 single molecules, if they represent a unique</p> <p>20 transcript, can actually be counted, which is</p> <p>21 far better in accuracy than gene expression,</p> <p>22 which is more of an analog-type system that's</p>	<p>40</p> <p>1 target nucleic acid and then perform a single</p> <p>2 nucleotide extension to identify whether an</p> <p>3 "A" or a "T" was present.</p> <p>4 On the alpha metric system, they use</p> <p>5 more traditional probe-type arrays, where a</p> <p>6 very short oligonucleotide would be specific</p> <p>7 to something they wanted to measure up.</p> <p>8 Either a gene transcript or whether they were</p> <p>9 doing allelic discrimination. Which means do</p> <p>10 I have an A present or do I have T present.</p> <p>11 Q Okay. The alpha metrics that is what you</p> <p>12 would consider a traditional microarray?</p> <p>13 A No.</p> <p>14 Q Okay.</p> <p>15 A So an Illumina would be a traditional</p> <p>16 microarray. There were groups printing cDNAs</p> <p>17 onto solid, to make microarrays. There were</p> <p>18 groups printing BAC, it's a capital, B-A-C,</p> <p>19 clones which are sizes of 100,000</p> <p>20 nucleotides. And that could be used for</p> <p>21 measuring copy number variation. So there's</p> <p>22 lots of different formats for microarrays.</p>

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<p>41</p> <p>1 Q So can you help me understand the -- we 2 just talked about, in Paragraph 95, you're 3 contrasting the NGS-based approach with the 4 traditional microarrays. Illumina is an NGS 5 system? 6 A It's one system, yes. 7 Q So would you -- what's the difference 8 between Illumina, that uses a microarray 9 versus what you're calling traditional 10 microarrays and kind of putting them in 11 different buckets? 12 A Well, I would consider Illumina -- so 13 Illumina makes multiple products. They make 14 a microarray product and they make an NGS 15 product. 16 Q Oh, understood. 17 A They're very different technologies. 18 Q Thank you. 19 A Sure. 20 Q So for clarification, just so we're on 21 the same page; the Illumina NGS product is 22 not a traditional microarray?</p>	<p>43</p> <p>1 with one of them, so we don't... 2 MS. CIPRIANO: If we can agree that 3 they're applicable to both 4 proceedings, I'm happy to do that. 5 MR. BILSKER: Yeah. 6 Q If we look at Paragraph 96 of your 7 declaration, near the end of that paragraph 8 at the bottom, the sentence that starts at 9 the bottom of the paragraph on Page 62, 10 starting early techniques. 11 Do you see that? 12 A Yes. 13 Q There you list, if I'm counting 14 correctly, five early techniques for creating 15 enriched DNA libraries. 16 Do you see that? 17 A Yes. 18 Q The first is PCR. 19 A Yes. 20 Q And what are you referring to when you 21 just say, "PCR," here? 22 A Well, again, there are lots of ways of</p>
<p>42</p> <p>1 A That's correct. It's an NGS platform. 2 Q Okay. So one of the concepts that I 3 think is talked about in your declaration is 4 called a hybridization array. 5 Do you recognize that term? 6 A Yes. Can you point me to the paragraph 7 you're referring to? 8 Q Well, I think we can look at 9 Paragraph 96 and maybe that will help 10 clarify. 11 A Can I have just a moment? 12 Q Yeah, take your time. 13 A Okay. I've read the paragraph. 14 Q So just so we're on the same page, this 15 is Paragraph 93 of the '108 Declaration. If 16 you want to cross-reference. 17 A I thought that was 95? 18 I thought 95 was '102. And 93 was '108. 19 You were talking about 96. 20 Q Yeah. Oh yeah, sorry. 21 A So that's 94. 22 MR. BILSKER: Why don't we just stick</p>	<p>44</p> <p>1 performing a PCR. An example can be just the 2 traditional PCR that we've talked about 3 before referencing, again, the figure from 4 yesterday. Where you have a single primer 5 pair and you're interested in enriching one 6 target in the sample that you're 7 characterizing. That would be an example of 8 PCR. 9 Q And this -- your reference to PCR here 10 would also encompass nontraditional PCR, the 11 various way that you talked about doing PCR 12 earlier? 13 A You can use traditional multiplex PCR. 14 You can use other types of PCR. Again, there 15 are lots of ways of doing PCR. 16 Q Okay. And then the next in the list is 17 genome walking. 18 Do you see that? 19 A I do. 20 Q Can you explain what the genome walking 21 is? 22 A Yes. So genome walking -- it's</p>

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<p>45</p> <p>1 important because this time frame where these 2 papers first came out, were before the human 3 genome draft was done. I actually started at 4 Baylor in 1991, as part of the genome center. 5 And we actually did Chromosome 3, 12, and 6 part of X. But in the 1991 to 1997 time 7 frame, our databases weren't full of 8 reference genomes that we can go and search 9 for things. So there were lots of 10 individuals that were interested in 11 characterizing certain gene regions, or 12 certain transcripts. Particularly trying to 13 get the full length. Only part of it would 14 be available, but they wanted the full 15 length. 16 So a genome walking strategy was to 17 characterize a sequence in a region where 18 there was no sequence available. And the way 19 that's done, for example, human genome 20 fragmented adapters were ligated onto the 21 ends of fragments, and they knew part of the 22 sequence. And they wanted to get into the</p>	<p>47</p> <p>1 is actually a patent application. 2 Exhibit 1044, which is Siebert, which 3 described an improved PCR method for walking 4 into uncloned genomic DNA. Then there's a 5 method by Chenchik, which is Exhibit 1045, 6 which is an improvement on a RACE method, 7 which was more in line with finding the five 8 prime ends or three prime ends of 9 transcripts. You can see that Seibert was 10 published in 1995. Chenchik was published in 11 1996 and the Markham patent application 12 published in 1990. 13 Q So the next in your list of early 14 techniques for creating enriched DNA 15 libraries is hybrid capture. 16 Do you see that? 17 A I do. 18 Q What is hybrid capture? 19 A Hybrid capture is for NGS and it is a 20 way of enriching regions of interest. So for 21 example, our human genome is big, 3 billion 22 letters long. But the genes that get</p>
<p>46</p> <p>1 unknown part of the sequence, so they would 2 use a target-specific primer that would 3 extend across to where the adapter was and 4 then they could amplify that region and then 5 sequence it. And find new sequences that 6 they didn't have before. 7 So that was done for genomes and it was 8 actually done for a technique called RACE, 9 which stands for Rapid Amplification of cDNA 10 Ends. And those are classical genome walking 11 strategies. And I list a couple of 12 references that describe efforts that 13 scientists were doing at the time and the 14 results. And how they observed problems and 15 how those genome walking strategies solved 16 those problem. 17 Q Okay. And genome walking was known in 18 the -- you said, no. Early 90s -- 19 A Well, so, for example, I have three 20 references here -- sorry, I didn't mean to 21 talk over you. Three references here, 22 there's Exhibit 1043, which is Markham, which</p>	<p>48</p> <p>1 expressed out of our genome are maybe one or 2 2 percent of the genome. Most of the genome 3 isn't involved in the sequences that make 4 proteins. So hybrid capture was developed to 5 capture what we call the Exome, E-x-o-m-e, 6 which is all the exons that create proteins 7 in the cell. There are anywhere from 25 to 8 30,000 different genes and each gene has 9 multiple exons, so that is a lot of sequences 10 to capture. 11 So groups would create oligonucleotides 12 that were complementary to the exons of 13 interest. And there are different ways of 14 doing that, there's a group called NimbleGen 15 that was purchased by Roche. This is the 16 Albert paper where the oligonucleotides were 17 on solid supports and you can hybridize your 18 genome of interest on the solid support. 19 Anything that did not hybridize was washed 20 away. The enriched exonic regions were then 21 eluted and made into NGS libraries and then 22 sequenced. And then there's Gnirke,</p>

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<p>49</p> <p>1 Exhibit 1048, that used a solution-based 2 hybridization approach where oligonucleotides 3 can be synthesized and they would have a 4 biotin group attached to that 5 oligonucleotide, again, designed to target 6 the exons. You would hybridize the 7 oligonucleotides to the exons of interest. 8 Then you could use, they called those 9 baits. You could use streptavidin coated 10 magnetic beads to then capture all of those 11 hybrids that had biotin groups attached to 12 them. Wash away all the stuff that didn't 13 hybridize and then also make that in NGS 14 libraries. 15 Now, you can do that for exomes, you 16 could target arms of a chromosome or you can 17 target other regions of interest. So there 18 were different applications of the capture 19 method. 20 Q All right. The next on your list is 21 molecular inversion probes, those are the 22 MIPs that we talked about yesterday?</p>	<p>51</p> <p>1 This is an example of how technology 2 evolves from microarrays to NGS very fluidly 3 just because of the power of NGS. 4 Q And then the last in your list is 5 microdroplet technologies. What are 6 microdroplet technologies? 7 A The one that I'm referencing here, and I 8 actually talk about in my review paper, which 9 is Exhibit 1025, is from a company called 10 Raindance. And Raindance created this 11 microfluidic system where they could create 12 these little aqueous droplets and otherwise 13 oil solvent, and they could merge droplets 14 where in one droplet you could have your 15 target of interest and the other droplet 16 could have unique primer pairs. So they're 17 doing Singleton PCR in microdroplets. But 18 each of the droplets have a different primer 19 pair set. So they could enrich multiple 20 targets by having different droplets having 21 different primer pair sets to enrich for a 22 target of interest.</p>
<p>50</p> <p>1 A Those are MIPs. 2 Q And how are Mips used to create enriched 3 DNA libraries? 4 A So MIPs actually started from a 5 technology that came out of Mats Nilsson's 6 lab in the mid-1990s, I think it came out in 7 '94, where he identified what he calls 8 padlock probes. And a padlock probe we saw 9 yesterday in Porreca where it has two 10 targeting arms and you can then capture the 11 target of interest and ligate those to make 12 circles. 13 Molecular inversion probes were kind of 14 a spin off of a padlock probe and really 15 first came out in the HapMap project by a guy 16 named Paul Hardenbol, I think. They were 17 using molecular inversion probes for 18 genotyping that would end up on a microarray. 19 More recently, I think some like Porreca, 20 2007, used MIPs to capture regions of 21 interest that can then be made into a NGS 22 library that would then be sequenced.</p>	<p>52</p> <p>1 That's what microdroplet technologies 2 are. And microdroplet technologies have been 3 used for digital PCR, single cell analysis, 4 all types of other things. Again, mostly for 5 NGS applications. 6 Q When you say digital PCR, what do you 7 mean by that? 8 A So again, another flavor of the PCR. 9 Digital PCR was first – well, it was 10 actually published in the early 1990s, but it 11 was resurfaced out of Bert Vogelstein's Lab 12 in 1999. Where you would transform PCR from 13 an analog technology to a digital technology 14 and you do that by diluting the amount of 15 template into your reaction. 16 So let's say you have a 96 well plate, 17 so it has eight rows and 12 columns, 12 18 columns and eight rows, I don't know. And 19 you can dilute your template sufficiently 20 that when you add template to every well, one 21 will have one molecule of DNA template and 22 one molecule will not, it will be blank.</p>

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<p>53</p> <p>1 You're trying to get into the Poisson 2 distribution and then you will add primer 3 pairs to each of those wells and amplify 4 them. If you see a signal you would say I 5 have one molecule there. And if you don't 6 see a signal, you would say there is no 7 molecule there. 8 And that is the basis of digital PCR 9 where it now becomes a counting method for 10 accurately figuring out how many copies of a 11 certain sequence are in your sample. 12 You can do that in droplet form, too, 13 which is more high throughput than doing it 14 in a 96 well plate. 15 MR. BILSKER: Good place to break? 16 MS. CIPRIANO: I was going to say the 17 same thing. 18 (Whereupon, a recess was taken 19 from 10:37 AM until 10:59 AM.) 20 MS. CIPRIANO: So just so we're clear, 21 the document that we've been looking 22 at, the declaration that's on the</p>	<p>55</p> <p>1 Q Let's take a look at what's been marked 2 as Exhibit 1025. 3 (Whereupon, 2010 Metzker review 4 article was previously marked as 5 Exhibit 1025.) 6 Q Do you recognize Exhibit 1025? 7 A I do. 8 Q What is it? 9 A It's a review paper that I wrote that 10 was published in 2010 titled Sequencing 11 Technologies, the Next Generation. 12 Q And it's a review article? 13 A It is. 14 Q What is on your understand of what a 15 review article, what's the purpose of a 16 review article? 17 A Well, I'm sure there are multiple 18 purposes. I was asked by Nature Review 19 Genetics to provide a review of the NGS 20 field, both from the state of the technology 21 and how NGS has been applied to several 22 applications.</p>
<p>54</p> <p>1 title page has reference to 2 IPR 202500028 regarding the '012 3 Patent. The version you're looking at 4 right now does not have an exhibit 5 stamp, but it's our understanding that 6 the same exhibit was submitted with an 7 exhibit stamp as Exhibit 1003-1. 8 THE WITNESS: Understood. 9 BY MS. CIPRIANO: 10 Q Welcome back. 11 A Thank you. 12 Q Going to take a look at Paragraph 99 of 13 your '012 declaration. 14 So here in that first sentence of 15 Paragraph 99, you say, "Prior to the claimed 16 invention of the '012 Patent, nucleic acid 17 enrichment for NGS was well understood and 18 widely practiced." 19 Do you see that? 20 A I do. 21 Q You cite your 2010 paper, right? 22 A That's right, I do.</p>	<p>56</p> <p>1 Q And do you feel like your article 2 accomplished that goal? 3 A Yes. It's been well-cited. I think 4 it's been cited more than 10,000 times, now. 5 Q Would you agree that this review article 6 reflects what a person of ordinary skill in 7 the art would have understood regarding what 8 was thought to be possible with NGS 9 technologies? 10 MR. BILSKER: Objection. Form. 11 A Like I said, I described several 12 applications. There would be other 13 applications, and in some cases, I kind of -- 14 it's been a long time since I wrote this 15 article, but for example, RNA-Seq, I don't 16 talk about, but I do say that researchers 17 were interested in it. And there were other 18 review articles that readers could go look 19 for. Nanopore sequencing would be another 20 example of where I pointed someone off to go 21 look somewhere else, it's not going to be in 22 my review.</p>

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<p>57</p> <p>1 Q Understood. And your review article</p> <p>2 also reflects what a person of ordinary skill</p> <p>3 in the art would have understood as some of</p> <p>4 the limitations of NGS technologies at the</p> <p>5 time, right.</p> <p>6 MR. BILSKER: Objection. Form.</p> <p>7 A Well, I think the person of ordinary</p> <p>8 skill in the art, I've stated was a little</p> <p>9 bit later than what I had in my review</p> <p>10 article, my review article is 2010. The</p> <p>11 priority date is 2012.</p> <p>12 The person of ordinary skill in the art</p> <p>13 has the distinct advantage of being aware of</p> <p>14 all of the art, where I'm not citing all of</p> <p>15 the art, so if it's not cited here, it</p> <p>16 wouldn't necessarily mean that a person of</p> <p>17 ordinary skill in the art wasn't aware of the</p> <p>18 art.</p> <p>19 Q Understood. But you've identified a few</p> <p>20 limitations of NGS technologies at the time</p> <p>21 in January 2010; is that accurate?</p> <p>22 MR. BILSKER: Objection. Form.</p>	<p>59</p> <p>1 A I do.</p> <p>2 Q And what is it?</p> <p>3 A It's a paper by Michael Shapero and</p> <p>4 other colleagues titled MARA, M-A-R-A, all</p> <p>5 caps, a novel approach for highly multiplexed</p> <p>6 locus-specific SNP genotyping using</p> <p>7 high-density DNA oligonucleotide arrays.</p> <p>8 Q And this is the Shapero paper that you</p> <p>9 discuss in your declaration?</p> <p>10 A It is.</p> <p>11 Q And for reference I'm going to -- I</p> <p>12 think it's starting at Paragraph 105 of your</p> <p>13 declaration if you want to refer to that.</p> <p>14 A Yes. I'm there.</p> <p>15 Q Now, the Shapero article does not use</p> <p>16 NGS technology, right?</p> <p>17 A You are right. Shapero is published</p> <p>18 in 2004, and the first platforms for NGS did</p> <p>19 not even hit the market until at least 2005.</p> <p>20 Q And the technology that Shapero relied</p> <p>21 on, was a traditional microarray?</p> <p>22 MR. BILSKER: Objection. Form.</p>
<p>58</p> <p>1 A I wouldn't say that's accurate. I</p> <p>2 actually tried to provide perspectives of</p> <p>3 both the advantages and disadvantage of the</p> <p>4 state of art at the time.</p> <p>5 Q But you would agree that it includes</p> <p>6 both advantages and disadvantages, right?</p> <p>7 A It does, and I go into specific details</p> <p>8 of what those are.</p> <p>9 Q Okay. We can put that aside.</p> <p>10 Okay. We're going to turn to Shapero.</p> <p>11 Shapero is one of the patent application,</p> <p>12 article?</p> <p>13 A It's a paper.</p> <p>14 Q It's a paper.</p> <p>15 One of the articles that you discuss in</p> <p>16 your declaration.</p> <p>17 (Whereupon, Shapero article was</p> <p>18 previously marked as Exhibit</p> <p>19 1006.)</p> <p>20 MS. CIPRIANO: I've handed you what's</p> <p>21 been marked as Exhibit 1006.</p> <p>22 Q Do you recognize this document?</p>	<p>60</p> <p>1 A Well, the authors of Shapero all are</p> <p>2 employees of Affymetrix, and they were</p> <p>3 describing a new enrichment method that used</p> <p>4 the Affymetrix platform because they're</p> <p>5 trying to make products for Affymetrix.</p> <p>6 Q And Affymetrix sold a traditional</p> <p>7 microarray, right?</p> <p>8 A It was one of many companies that I</p> <p>9 would consider a -- well, I don't know. Even</p> <p>10 if it's traditional microarray, but if you</p> <p>11 are talking with respect to my NGS, yes, I</p> <p>12 would think Affy was, by 2010, a traditional</p> <p>13 microarray. I don't know in 2004 whether I</p> <p>14 would have considered it a traditional</p> <p>15 microarray.</p> <p>16 Q And what is the distinction you're</p> <p>17 making there?</p> <p>18 A Affymetrix was still developing its</p> <p>19 product. Affymetrix was trying to expand its</p> <p>20 capacity to do multiplex snip analysis for</p> <p>21 the HapMap project. So the HapMap project</p> <p>22 only started in 2003, and Affymetrix was</p>

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<p>61</p> <p>1 developing new assays that would work on 2 their platform. So I don't know if I would 3 consider it traditional back in that time 4 period. 5 Q Okay. So if you're looking at the 6 Shapero paper and the Affymetrix technology 7 they use in the Shapero paper as of 2012, 8 that falls into the bucket that we were 9 talking about of traditional microarrays; is 10 that accurate? 11 A Yeah, I would consider in 2012 this 12 would be more of a traditional type, but 13 there are, again, very -- Affy had multiple 14 products, and there were different ways of 15 using the Affy chip for either genotyping or 16 gene expression. 17 Q Okay. And the method that is referred 18 to in the title, abbreviated MARA, do you 19 have an understanding of what "MARA" means? 20 A Yes. 21 Q And what is it? 22 A Again, it's an acronym, and they</p>	<p>63</p> <p>1 and then a 4,000-plus plex, where each 2 target-specific primer would have a tail, and 3 they would do a primer extension. So we're 4 not in the PCR realm yet. So they've done 5 all of that. 6 And the idea is -- and this is part of 7 the genome walking strategies that Siebert 8 published and Chenchik published -- that when 9 the primer extends across the target region 10 of interest and makes the complement of the 11 adapter that's been ligated on the end of the 12 fragment, then a universal PCR reaction can 13 take place. And MARA uses the terms A1 and 14 A2, and then they perform a PCR to enrich for 15 those fragments that were designed by the 16 target-specific primers in the primer 17 extension reaction. 18 And then that was characterized. That 19 enriched library was characterized. And 20 their approach was to use a chip to do allele 21 discrimination because they're trying to 22 figure out whether there's an A or a T</p>
<p>62</p> <p>1 actually describe it in the abstract. It's 2 Multiplexed Anchored Runoff Amplification, 3 M-A-R-A. And I should just note that even 4 Affymetrix with their chips were used for 5 sequencing purposes. So they actually built 6 chips to sequence DNA in addition to 7 genotyping or gene expression. 8 Q And the MARA method used in Shapero 9 relies on a PCR to amplify the target 10 sequence; is that accurate? 11 A Not really. 12 Q Okay. Explain what you mean by "not 13 really"? 14 A So the MARA diagram of how the workflow 15 works, you can find in Figure 1. And it 16 first starts by digesting genomic DNA with a 17 restriction enzyme, and then it ligates on 18 partially duplex adapters onto each end of 19 those fragments. 20 And then by design, the MARA scientist 21 at Affymetrix designed target-specific 22 primers, and they did a 250 plex, a 750 plex,</p>	<p>64</p> <p>1 present in the target loci. 2 Q So in Figure 1 of Shapero, Exhibit 1006, 3 the middle of the figure -- it's Step 4, it 4 looks like, 4 and 5, I guess -- that's a PCR 5 reaction, right? 6 A Well, Step 4 is just illustrating 7 various extension products, and there are 8 four listed. There's one where the top one, 9 the target-specific loci or the 10 target-specific primer that contains the A1 11 tail hybridized and extended across the SNP, 12 which is the green dot, and also extended 13 through the A2 adapter sequence, making the 14 complement of the A2 sequence. 15 The second product is where an extension 16 product occurred but maybe the fragment did 17 not have the A2 adapter ligated to the end of 18 it. So you can see that it's short on the 19 right-hand side. 20 And then the third product is even -- 21 looks like an incomplete extension where the 22 primer that's hybridized does not extend</p>

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<p>65</p> <p>1 completely and falls short of even the SNP 2 spot, which is designated by the green dot. 3 And then the fourth product looks like 4 just the primer itself. So there's four 5 products. 6 And what Shapero is then showing going 7 into Step 5 is a universal PCR step that 8 includes universal A1 and A2 primers, with 9 the goal that only the top product in Step 4 10 would be amplified, and the other three 11 products that are present are not amplified. 12 Q Okay. So you agree that Shapero uses 13 PCR to amplify its products. You disagreed 14 with that -- with me on that before, but I'm 15 not understanding why, I guess. 16 A Well, so they're not doing a traditional 17 PCR. We talked about traditional PCR, where 18 you may use two target-specific primers and 19 you may amplify a single target. 20 What Shapero is doing, because of the 21 primer extension reaction, they can use what 22 is called universal PCR, where they use a</p>	<p>67</p> <p>1 because only that construct can then be 2 amplified in the universal PCR step because 3 of the A2 primer. 4 So that is not traditional PCR. 5 Q Okay. And I guess what I'm trying to 6 get at is other than the primer design, the 7 steps of the PCR methods are the same between 8 universal PCR and traditional PCR, right? 9 A Sorry. Which steps are we talking 10 about? 11 Q The -- that you put in a polymerase and 12 you hybridize primers and you -- the 13 amplification process of PCR is the same in 14 traditional PCR versus universal PCR except 15 for the primer design, right? 16 A Well, again, I disagree with that. I 17 can agree it'll use a polymerase and it'll 18 use nucleotides and it can heat to denature 19 strands and it can anneal primers. But 20 that's a very general way of thinking about 21 PCR, which a person of ordinary skill in the 22 art wouldn't think of. They would want to</p>
<p>66</p> <p>1 common set of primers that can amplify 2 multiple target regions in the same reaction. 3 Q It's still PCR, right? Both traditional 4 and, apologies, nontraditional PCR, there 5 still kinds of PCR, right? 6 A So again, globally PCR can be a bunch of 7 things and the context is actually really 8 important. 9 Q Okay. 10 A Because, again, traditional PCR, if I 11 were to be interested in amplifying gene X on 12 Chromosome 3, I could not use A1 and A2 13 primers. If I put A1 and A2 primers in my 14 reaction that had genomic DNA, I'd get 15 nothing, or I'd probably get something, but 16 it wouldn't be what I'm looking for. Because 17 those primers are not specific for the thing 18 I'm trying to amplify. 19 MARA was designed very particularly to 20 have a primary extension reaction that would 21 extend through an adapter sequence to create 22 the complement. That's super important</p>	<p>68</p> <p>1 understand the context of the PCR. 2 And it's by design that multiple targets 3 can be amplified with a single primer set, 4 which you can't do with traditional PCR. You 5 can't do it that way. 6 Q Now, in your paper, Exhibit 1025. Do 7 you use the term "traditional PCR"? 8 A So I'm looking at Page 41, and it's 9 under the section titled "Genome Enrichment," 10 which for purposes here could also be 11 considered target enrichment. And about, I 12 don't know, seven, eight lines down, I say: 13 "The concept of targeting 14 specific regions of the genome is well 15 established, with PCR being the most 16 widely used method, albeit on a small 17 scale. PCR coupled with Sanger 18 sequencing is suitably matched for 19 analyzing a handful of candidate genes. 20 Coupling PCR with high throughput NGS 21 platforms for targeting strategies is 22 not practical, as sample preparation</p>

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<p>69</p> <p>1 would require handling tens of 2 thousands of primers individually or in 3 large multiplex groups to meet the 4 needs of a single instrument run." 5 So I would argue that when I talk about 6 the concept of targeting specific regions, 7 that is traditional PCR right there. 8 Q But you don't use the term traditional 9 PCR -- in 2010 you didn't use that term? 10 A Obviously, I didn't read that section. 11 But I'm explaining to anyone reading this, a 12 person of ordinary skill reading this would 13 understand what I was referring to was 14 traditional PCR. Where, again, we're talking 15 about the two target-specific primers 16 amplifying a single region of interest. 17 Q Okay. If we look on Page 32 of this 18 article. 19 A Yes. I'm there. 20 Q At the bottom of the left-hand column. 21 A Yes. 22 Q There's a clonally amplified templates</p>	<p>71</p> <p>1 Paragraph 110 and 111 of your declaration. 2 A Can I have a moment. 3 MS. CIPRIANO: I think those are 4 wrong. 5 A I was in the wrong declaration. 6 Q Please look at Paragraphs 110 and 111 of 7 your '012 Declaration. 8 A Okay. 9 Okay. Thank you. 10 Q You talk about -- in these paragraphs, 11 you talk about a discrimination score. 12 Do you recall that, or a DS? 13 A Well, I'm describing what Shapero 14 describes as a discrimination score. 15 Q Okay. Do you have an understanding of 16 what a discrimination score is? 17 A Yes. 18 Q And what is it? 19 A Well, it's defined here as the block 20 quote, it looks like starting at the third 21 line down it says, "DS" -- which is 22 discrimination score -- "of a probe pair is a</p>
<p>70</p> <p>1 section. 2 Do you see that? 3 A I do. 4 Q There, you referred to the two most 5 common methods are emulsion PCR, EM PCR and 6 solid-face amplification. 7 Do you see that? 8 A I do. 9 Q You don't distinguish whether this 10 emulsion PCR is traditional or universal, 11 right? 12 A Well, I actually give them specific 13 names. So I would not consider emulsion PCR 14 traditional PCR, and I would not consider 15 solid face, or it's also known as bridge 16 amplification, as traditional PCR. Again, 17 these methods can amplify multiple targets, 18 and here they're amplifying single individual 19 molecules in the compartments or specific 20 locations, that can produce lots of template 21 sequences with one or two primers. 22 Q Okay. If we could take a look at</p>	<p>72</p> <p>1 ratio of" -- and then there's an equation in 2 parenthesis, PM-MM divided by PM plus MM, 3 where PM is perfect match cell intensity, and 4 MM is mismatch cell intensity. And thus 5 serves as a measure of detection of DNA 6 target sequences. 7 Q And what is meant by the perfect match 8 cell intensity? 9 A So on the Affymetrix chip array, they 10 use probes, oligonucleotides, that are 25 11 base pairs in length. And this is used for 12 allele discriminations. So, again, they're 13 trying to figure out whether there's an A or 14 a T in the locus of interest. 15 So they'll have a perfect match for the 16 A allele. Then they'll mismatch that A with 17 some other nucleotide, it could be a C or a 18 G. 19 And then for the T allele, they'll have 20 a perfect match. And usually these 21 interrogation positions for looking for the 22 perfect match or the mismatch will be at Base</p>

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<p>73</p> <p>1 13, so it'll be right in the middle of the</p> <p>2 25 base pair probe sequence. And then</p> <p>3 they'll have perfect match for A, and then</p> <p>4 they'll have mismatches.</p> <p>5 In addition to that, they essentially</p> <p>6 slide the sequence window around a little</p> <p>7 bit, where the mismatch may not be at 13, it</p> <p>8 may be at 9 or may be at Base 17 of that</p> <p>9 probe. They almost take a series of 20</p> <p>10 different probe intensities in doing this</p> <p>11 type of calculation, for each S and D that</p> <p>12 they're interrogating.</p> <p>13 Q And you say "probe intensities." What</p> <p>14 do you mean by probe intensities?</p> <p>15 A So if we go back, I guess they don't</p> <p>16 show it. But after they've done their MARA</p> <p>17 method, they need to attach a label to the</p> <p>18 amplicon. And there's a way of -- and a</p> <p>19 label is usually a fluorescent signal. And</p> <p>20 once the amplicon has this fluorescent</p> <p>21 signal, it's then hybridized to the chip.</p> <p>22 And based on the position, they would know</p>	<p>75</p> <p>1 already know the answer. And they now</p> <p>2 perform the MARA test, and the question is</p> <p>3 how good did the MARA test actually call the</p> <p>4 SNPs given the known answer?</p> <p>5 Q Understood. So in Paragraph 117 of your</p> <p>6 declaration, you mention that Shapero cites</p> <p>7 an article by Syv nen.</p> <p>8 Do you see that?</p> <p>9 A I do.</p> <p>10 Q And do you recall what was disclosed in</p> <p>11 Syv nen?</p> <p>12 A Yes.</p> <p>13 Q Okay. Syv nen describes a method called</p> <p>14 minisequencing; is that accurate?</p> <p>15 A One of many techniques, it's a review</p> <p>16 article as well.</p> <p>17 Q Okay. You specifically say that Shapero</p> <p>18 cites a section of Syv nen that explains</p> <p>19 minisequencing, right?</p> <p>20 A Well, no, I think what I'm saying is one</p> <p>21 of the references Shapero 2004 cites above is</p> <p>22 Reference 5, which is Syv nen, or I'm not</p>
<p>74</p> <p>1 whether that's a perfect matching probe or a</p> <p>2 mismatching probe, and -- or it's looking for</p> <p>3 the allele or it's like looking for the T</p> <p>4 allele.</p> <p>5 They use a fluorescent scanner, a</p> <p>6 full-color scanner that will scan the chip</p> <p>7 and measure the intensities coming from each</p> <p>8 location on the chip. And that's what the</p> <p>9 cell intensity that they're talking about</p> <p>10 are.</p> <p>11 Q And another -- something else that</p> <p>12 Shapero looked at was call rate.</p> <p>13 Do you recall that?</p> <p>14 A Yes.</p> <p>15 Q What is the call rate?</p> <p>16 A So the call rate is basically how good</p> <p>17 was the MARA method of determining whether it</p> <p>18 was an A or a T. And they compared that to</p> <p>19 another assay that Affymetrix was developing</p> <p>20 at the time.</p> <p>21 Again, these are known and the samples</p> <p>22 that they're characterizing are known. They</p>	<p>76</p> <p>1 sure how the say it as well, which explains</p> <p>2 that genotyping is a form of sequencing</p> <p>3 called minisequencing. I was just pointing</p> <p>4 out this one thing, Shapero didn't point this</p> <p>5 out, I'm pointing this out.</p> <p>6 Q Okay. You're familiar with the</p> <p>7 technique of minisequencing?</p> <p>8 A Very familiar.</p> <p>9 Q Minisequencing is not what you would</p> <p>10 consider next generation sequencing, right?</p> <p>11 A It is not. So this review article came</p> <p>12 out in 2001, which is four years before the</p> <p>13 first commercial platform hit the market.</p> <p>14 Q And then another reference you talk</p> <p>15 about is the Delseny reference. This was</p> <p>16 marked Exhibit 1007.</p> <p>17 (Whereupon, PCT Application No.</p> <p>18 WO 2013/142389 A1 was previously</p> <p>19 marked as Exhibit 1007.)</p> <p>20 (A discussion was held off the record.)</p> <p>21 Q I've handed -- you're looking at an</p> <p>22 Exhibit that -- that's been marked</p>

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<p>1 Exhibit 1007.</p> <p>2 Do you recognize this document?</p> <p>3 A Yes.</p> <p>4 Q What is it?</p> <p>5 A It's a review article by Delseny, and</p> <p>6 colleagues, titled, "High-throughput DNA</p> <p>7 sequencing, the next sequencing revolution."</p> <p>8 Q And this is the Delseny paper you talk</p> <p>9 about in your declaration?</p> <p>10 A Yes. It's another review paper.</p> <p>11 Q And I'm looking at Paragraph 125 of your</p> <p>12 declaration, that reproduces Figure 1 from</p> <p>13 the Delseny paper.</p> <p>14 So if you want to look at in your</p> <p>15 declaration or in the Delseny paper, it's</p> <p>16 your preference.</p> <p>17 A Okay. Yes, I'm there.</p> <p>18 Q Okay. Now, you discuss in detail the</p> <p>19 column that is the third from the left, that</p> <p>20 starts "Sequencing by synthesis."</p> <p>21 Do you see that?</p> <p>22 A Yes.</p>	<p>1 Helicos and PacBio in the next generation.</p> <p>2 People can sometimes split them up, sometimes</p> <p>3 not. But I all bucket them into first</p> <p>4 generation or next generation.</p> <p>5 Q All the way in the left, the box that's</p> <p>6 in the left at the top of Figure 1,</p> <p>7 "classical Sanger ABI."</p> <p>8 Do you see that?</p> <p>9 A I do.</p> <p>10 Q That's not next generation sequencing?</p> <p>11 A No, I characterize that as first</p> <p>12 generation sequencing, and it's in my report.</p> <p>13 Q And then sequences by hybridization</p> <p>14 Affymetrix/Paragon in the next box, those</p> <p>15 technologies are not what would you consider</p> <p>16 next generation sequencing, right?</p> <p>17 MR. BILSKER: Objection. Form.</p> <p>18 A So it should be Perlegen. That must be</p> <p>19 a typo. He's only listing two there. So</p> <p>20 there was actually a high throughput</p> <p>21 sequencing platform that used sequencing by</p> <p>22 hybridization.</p>
<p>1 Q And would you agree that this column</p> <p>2 refers to next generation platforms?</p> <p>3 A It refers to some of them. You know,</p> <p>4 for example, 454 was the first next</p> <p>5 generation sequencing. It used a technique</p> <p>6 called pyrosequencing. And it's actually a</p> <p>7 technique that was developed in the late</p> <p>8 1990s by Mathias Uhlen and Mostafa Ronaghi.</p> <p>9 I think even Syv nen in 2001 talks about</p> <p>10 pyrosequencing as a sequencing technology.</p> <p>11 Q Okay. But the 454 and Illumina that are</p> <p>12 referenced in that column, they're both</p> <p>13 considered NGS technologies, right?</p> <p>14 A Yes, they are.</p> <p>15 Q And the other columns here are all</p> <p>16 referring to methods that are nonspecific --</p> <p>17 what we would consider not NGS; right?</p> <p>18 MR. BILSKER: Objection.</p> <p>19 Mischaracterization.</p> <p>20 A I'm not sure that I would say that. For</p> <p>21 example, we talked about my review article,</p> <p>22 which is next generation. And I talk about</p>	<p>1 Some published as early as late 1980s</p> <p>2 and more recently even 2010, and '11, which</p> <p>3 is, I think is bucketed under next generation</p> <p>4 sequencing. So I think sequencing by</p> <p>5 hybridization has been used through decades</p> <p>6 for different purposes. But I can remember</p> <p>7 there was an investigator, Dr. Drmanac, who,</p> <p>8 in like 1993 was proposing doing the whole</p> <p>9 genome on -- sequencing by hybridization</p> <p>10 strategy. And he made a company and was</p> <p>11 really making a solid effort after it. So I</p> <p>12 don't know sequencing by hybridization, it</p> <p>13 depends on the context of whether you think</p> <p>14 of it as NGS. Because it's not Sanger and</p> <p>15 it's not sequencing by synthesis. So it is a</p> <p>16 different strategy, but you know, it can be</p> <p>17 argued whether, at least parts of it weren't</p> <p>18 also considered NGS.</p> <p>19 Q How do you define what is NGS and what</p> <p>20 isn't NGS?</p> <p>21 A Well, NGS is traditionally thought of</p> <p>22 not Sanger sequencing and doesn't involve</p>

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<p>81</p> <p>1 electrophoresis. We kind of talked about 2 this yesterday, what are the defining things. 3 And throughput. You know, I think a platform 4 that can do the whole chromosome a whole 5 genome, can be considered an NGS platform. 6 Q Okay. So we saw this Delseny article, 7 right? 8 A Yes. 9 Q And specifically it's a review article 10 of the reports on improvements in genetic 11 sequencing with a focus on their impact on 12 plant genomics, right? 13 A You know, I think they are plant 14 biologists, so I think they would have 15 thought about in the context of plant 16 biology, but it doesn't necessarily limit it 17 to plant biology. 18 Q Sure. And Delseny talks about a few and 19 you -- as we talked about, talked about a few 20 different NGS technologies. 21 Well, even in the figure that we've 22 talked about. But Delseny doesn't report on</p>	<p>83</p> <p>1 acquired Solexa, I think was early 2007. By 2 2007, there were investigators already 3 publishing on what the first Illumina 4 sequencer was called, the GA or the genome 5 analyzer. In January of 2008, Illumina 6 published its first paper of sequencing a 7 human genome. 8 And after the GA was improved into the 9 GA2, by 2010 HiSeq became the new platform 10 that could sequence a human genome in a 11 single run. And from there they've continued 12 to release MiSeq and MiniSeq and NextSeq and 13 NovaSeq and all kinds of different platforms. 14 Q Understood. 15 A All from Illumina. 16 MS. CIPRIANO: Why don't we take a 17 break. 18 (Whereupon, a recess was taken 19 from 11:41 AM until 12:04 PM.) 20 MR. BILSKER: I'm going the wrap it 21 up. I have no further questions. 22 THE WITNESS: Oh, great.</p>
<p>82</p> <p>1 any new -- they're not discovering or 2 developing any new NGS technology, right? 3 A I don't think I understand your 4 question. 5 Q Okay. So the -- Delseny was published 6 in 2010? 7 A Yes. 8 Q Right? The sequencing by synthesis, the 9 454, Illumina and solid sequencing by 10 synthesis methods that are discussed in 11 Figure 1, those were known well prior 12 to 2010, right? 13 A Yes. 14 Q Do you recall when, like, the Illumina 15 became popular in the field? 16 A Yes. 17 Q When was that? 18 A So Illumina actually purchased a company 19 call Solexa. Solexa had been in the field 20 since the early 2000s. And I believe Solexa 21 had a couple of beta instruments in the field 22 by 2006. The transaction where Illumina</p>	<p>84</p> <p>1 MR. BILSKER: I don't have any 2 questions. 3 (Whereupon, this examination was 4 concluded at 12:04 PM) 5 6 7 8 MICHAEL L. METZKER, PH.D. 9 10 11 Subscribed and sworn to 12 before me on this ____ day 13 of _____, _____. 14 15 _____ 16 Notary Public 17 18 19 20 21 22</p>

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5		
6		
7	PREVIOUSLY	DESCRIPTION PAGE
8	MARKED	
9	Exhibit 1003	Metzker '357 Patent 5
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2		
3	I, KIARA MILLER,	
4	a Shorthand Reporter and Notary Public of the	
5	State of New York, do hereby certify:	
6		
7	That the witness whose examination is	
8	hereinbefore set forth, was duly sworn or	
9	affirmed by me, and the foregoing transcript is	
10	a true record of the testimony given by such	
11	witness.	
12		
13	I further certify that I am not related to any	
14	of the parties to this action by blood or	
15	marriage, and that I am in no way interested in	
16	the outcome of this matter.	
17		
18	<i>Kiara Miller</i>	
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