

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

QIAGEN Sciences, LLC,
Petitioner,

v.

Tecan Genomics, Inc.,
Patent Owner.

Case IPR2025-00028
Case IPR2025-00029

**EXPERT DECLARATION OF MICHAEL L. METZKER, Ph.D.
IN SUPPORT OF THE PETITIONER'S REPLY REGARDING
THE '012 AND '108 PATENT**

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I. INTRODUCTION

1. I, Michael L. Metzker, prepared a previous Declaration¹ in connection with QIAGEN Sciences, LLC's ("QIAGEN" or "Petitioner") Petition for Inter Partes Review of U.S. Patent No. 10,036,012 ("the '012 Patent") and U.S. Patent No. 10,876,108 ("the '108 Patent").

2. After I submitted my previous Declaration, the Board issued an Institution Decision ("ID") granting review of the '012 Patent and '108 Patent. Subsequently, Tecan Genomics, Inc. ("Tecan" or "Patent Owner") submitted its Patent Owner Response ("POR"), along with a declaration from Dr. Carlos D. Bustamante.² I have been asked by QIAGEN's counsel to opine on the and obviousness arguments set forth in Tecan's POR.

II. EXPERIENCE AND QUALIFICATIONS

3. My experience and qualifications are set out in my previous declaration and have not changed.³

¹ See Ex. 1003, Declaration of Michael L. Metzker, Ph.D.

² See Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D.

³ See Ex. 1003, Declaration of Michael L. Metzker, Ph.D. at § IV.

III. NOTHING IN PATENT OWNER'S RESPONSE CHANGES THE CONCLUSION THAT SHAPERO COMBINED WITH DELSENY RENDERS THE CLAIMS OF THE '012 AND '108 PATENTS OBVIOUS

A. Shapero and the '012/'108 Patents Address the Same Problem and No Evidence of Teaching Away Exists

4. It is my opinion that one of ordinary skill in the art reading Shapero (2004) and the '012/'108 Patents would have understood that each address the same problem: how to prepare a library enriched of certain sequences to be analyzed in a multiplexed reaction that is better than the level of multiplexing that is achieved using traditional forward and reverse target specific primer pairs (*i.e.*, multiplex PCR). As both Shapero (2004) and the '012/'108 Patents recognized, traditional multiplex PCR had limitations on the number of reactions that could be carried out at once, due primarily to primers interacting with each other to create unwanted amplicons. For example, Shapero (2004) explained that (“Traditionally, ***standard multiplex PCR is difficult to design and enable at levels beyond several dozen loci*** per reaction.⁴ The '012/'108 Patents also state that “[t]he simultaneous use of numerous primer pairs ***makes multiplex PCR impractical*** due to high level of non-specific amplification and primer-primer interactions.⁵ Both Shapero (2004) and the

⁴ See Ex. 1006, Shapero (2004) at p. 6. (emphasis added).

⁵ See Ex. 1001, '012 Patent at Col. 1:51-53 (emphasis added) (Ex. 1001 is Ex. 1009 in the IPR2025-0029, all other exhibits cited in this declaration appear in both proceedings).

'012/'108 Patents addressed the solution to the problem in the same way. Shapero (2004) called their enrichment method Multiplexed Anchored Runoff Amplification (MARA). MARA and the '012/'108 Patents method each used a single target specific primer and a common primer, in place of one of the other target specific primer used in traditional PCR, to eliminate the primer-primer interactions. As there were fewer different primer sequences present when the number of reactions go up using the MARA approach, the mathematical chances of primer-primer interactions decreased. This became a significant benefit especially as the number of loci targeted increased beyond a dozen. Shapero (2004) explained that “[i]n standard multiplex PCR, the number of potential PCR products in a reaction of n primer pairs grows as $2n^2 + n$.”⁶ For MARA, the number of primers in a reaction of n are reduced to $n+1$ primers needed.

5. One of ordinary skill in the art would have understood that once the library had been enriched using either MARA or the identical methodology in the '012/'108 Patents, that library could be analyzed for what one was interested in, on various different platforms. This is what one of ordinary skill in the art would have understood when Shapero (2004) stated that:

There are a multitude of genotyping methodologies for SNPS that have been described over the years (5-7). These approaches can vary in terms of how alleles are discriminated, ... Additionally, these

⁶ See Ex. 1006, Shapero (2004) at p. 1. (citation omitted).

approaches can vary in the nature of both the assay formats and labeling and detection strategies. However, many commonly used approaches share a common feature in that there is a requirement for an amplification of the region harboring the polymorphism prior to actual genotyping. ... Thus, one of the continuing challenges in SNP genotyping is the development of molecular methods capable of amplifying locus-specific SNPs in a highly multiplexed manner.”⁷

The '012/'108 Patents make the same recognition that the same library preparation technique can be used to prepare the enriched nucleic acids on different platforms, identifying analysis by various sequencing techniques⁸ and recognizing the enriched library can be analyzed by hybridization techniques using Affymetrix chips.⁹

6. One of ordinary skill in the art would have understood before the priority date that numerous articles (discussed below) taught that NGS, which was being rapidly adopted because of its ability to handle large amounts of sequencing data, identified the value of being able to create enriched libraries for the material one was interested in analyzing, for example by looking for genetic mutations called SNPs, by studying its sequence.

7. I understand that PO and Dr. Bustamante made the argument that despite Shapero (2004) teaching that MARA could be used to prepare enriched

⁷ *Id.* at pp. 1-2. (citations omitted).

⁸ *See* Ex. 1001, '012 Patent at Col. 28:7-65.

⁹ *Id.* at Col. 29:42-62 (*e.g.* citing Liu *et al.*, *Methods for genotyping polymorphisms in humans*, U.S. Patent No. 7,361,468).

libraries that could be analyzed on multiple different platforms, including by sequencing, one of ordinary skill in the art would have understood that next generation sequencing (NGS) required enrichment levels much higher than 50%. For that reason, one of ordinary skill in the art would have been steered away from using Shapero (2004)'s MARA to prepare a library for NGS.¹⁰ I disagree with this claim. There was no minimum level of enrichment that one of ordinary skill in the art before 2012 would have recognized was necessary before sequences could be analyzed on an NGS platform. The assertion does not make any sense. For example, NGS was being used for *de novo* sequencing where little to no information was known about the sequences being analyzed and there was no enrichment for any particular sequences. Yet, that sequence data from those *de novo* experiments proved extremely useful.

8. In any event, I agree with Dr. Bustamante when he ultimately admitted in his deposition that one of ordinary skill in the art would not have understood prior to 2012 that enrichment of sequences to be analyzed must be greater than 50% to be analyzed on an NGS platform.

Q. Alright. Let's go back to Paragraph 16 of the Brooks Declaration.

¹⁰ See, e.g., POR at 67.

Why in 2012 with single primer extension would you have expected only 50 percent of the reads to be target sequenced that you were interested in?

A. I can only speculate as to why Dr. Brooks expected 50 percent.

Q. Do you agree with him?

A. *I'm not sure I would agree to 50 percent.*¹¹

Just like Dr. Bustamante, I too was aware of Green (2010) (Ex. 1102), which reported success in using an NGS platform to sequence and analyze a Neandertal genome that was only a small portion of the material placed on the sequencing platform. Specifically, the Neandertal genome analyzed by Green (2010) started out being only 1-5% of the total material in the sample.¹² It was then enriched to between 4 and 30% and then successfully analyzed/sequenced on an Illumina NGS platform.¹³

9. Another publication, Briggs (2009), enriched mitochondrial DNA (mtDNA) to be analyzed at a slightly higher level of 18-40% before being sequenced on an Illumina NGS platform.¹⁴ Interestingly, the enrichment method used by Briggs (2009) involved as a first step using a single target specific primer that

¹¹ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 279-6-16 (emphasis added).

¹² See Ex. 1102, Green (2010) at 712.

¹³ *Id.*

¹⁴ See Ex. 1116, Briggs (2009) at 319.

extended through a fragment to capture the material the group was interested in.¹⁵ Though the subsequent steps to isolate and further amplify that extended fragment are different than how MARA and the '012/'108 Patents proceeded, the first step is very similar, and would demonstrate to one of ordinary skill in the art that a single target specific primer could provide sufficient on target specificity to allow enrichment at levels that would suffice for NGS sequencing. I would say that one of ordinary skill in the art would have viewed the Briggs (2009) method as a first or second cousin to the MARA and the '012/'108 Patents' methods of enrichment.

10. Multiple other publications demonstrate that one of ordinary skill in the art would have analyzed libraries that had been enriched to levels around the 50% mark on NGS platforms with success. I identified two of these articles in my 2010 review paper.¹⁶ Dr. Bustamante admitted that these publications show enrichment levels around the 50-60% levels before sequencing on an NGS platform.¹⁷

11. One of the articles that PO cites, Garber (2008), even states that because of the rapidly diminishing cost of NGS sequencing, some people performing sequencing of enriched libraries would not even care if they had to throw away half

¹⁵ *Id.* at Figure 1.

¹⁶ See Ex. 1025, Metzker (2010) at 41, namely Albert (2007) and Hodges (2007).

¹⁷ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 322:25-324:3.

of the sequencing reads because they were not sequences they were interested in.¹⁸

I also do not agree with PO's claim, where it relied on the Brooks declaration, that one of ordinary skill in the art before 2012 would expect that a library enriched by the single primer extension ("SPE") method taught in Shapero (2004) and the '012/'108 Patents would only enrich at about the 50% level.¹⁹ Here again I agree with Dr. Bustamante who testified that he did not agree with that 50% enrichment claim.

Q. Alright. Let's go back to Paragraph 16 of the Brooks Declaration.

Why in 2012 with single primer extension would you have expected only 50 percent of the reads to be target sequenced that you were interested in?

B. I can only speculate as to why Dr. Brooks expected 50 percent.

Q. Do you agree with him?

A. *I'm not sure I would agree to 50 percent.*²⁰

As with the general statement about the level of enrichment needed to sequence on an NGS platform, the claim that one of ordinary skill in the art would only expect

¹⁸ See Ex. 2029, Garber (2008) at 1103. ("**[I]f half your reads map someplace else, or aren't directly mappable to your region of interest, maybe you don't care—you just throw them away, . . .**") (emphasis added).

¹⁹ See Ex. 1002, '012 Patent File History at 2013-2017 ("Brooks Declaration" at ¶ 16); see also POR at pp. 56 and 67.

²⁰ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 279-6-16 (emphasis added).

SPE to enrich at a 50% level does not make sense to me. By the time of Shapero (2004)'s work, there were computerized primer design programs like the Primer 3 program²¹ Shapero (2004) identified using.²² When used in conjunction of existing maps of genomes, one of ordinary skill in the art would have been able to design and theoretically test what sequences on the genome the primers would anneal to and extend through based on various hybridization stringency conditions, just like Shapero (2004) did using the July 2003 human reference sequence based on the NCBI Build 34. While Dr. Bustamante claimed that such groundwork might not account for unknown collapsed or repeating regions of the genome, one of ordinary skill in the art would not consider their target specific primer annealing to such regions to be off target. They would be interested to know about those regions. In any event, I see no logical basis for one of ordinary skill in the art to have concluded that SPE would produce high rates of off target events, or those in the range of 50% of which would "confound sequence analysis" on an NGS platform.²³ As described above, the analysis tools on NGS platforms were capable on analyzing data when

²¹ See Ex. 1054, Rozen (2000).

²² See Ex. 1006, Shapero (2004) at p. 2.

²³ See Ex. 1002, '012 Patent File History at 2013-2017 ("Brooks Declaration" at ¶ 14).

there was no enrichment such as in the *de novo* situation or when libraries were enriched down to levels of only 4-5%.²⁴

12. PO claims that one of ordinary skill in the art would have interpreted Shapero (2004)'s MARA in line with SPE in general to produce off-target products at a relatively high rate that would flood the sequencing system and confound analysis.²⁵ I disagree.

13. Dr. Bustamante stated in his declaration that because of the way Shapero (2004) designed his experiments, one of ordinary skill in the art would not be able to tell that Shapero (2004) achieved high on target rates and significantly enriched for the sequences the authors looking for.²⁶ In the first instance, Dr. Bustamante's statement of not being able to tell whether there are high on-target rates in Shapero (2004) is quite different than PO's or Dr. Brooks' argument that one of ordinary skill in the art would expect high off-target rates from SPE enrichment such as MARA.

14. In actuality, one of ordinary skill in the art would have understood that the data presented in Shapero (2004) leads to the conclusion that MARA is achieving high on-target rates, not the opposite. Both Dr. Bustamante and I agree that primer-

²⁴ See Ex. 1102, Green (2010) at 711; see also Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 71:24-73:20, 178:8-25.

²⁵ See POR at p. 56.

²⁶ See Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D at ¶ 229.

primer interactions that create primer-dimers is at least one of the largest contributors to off-target amplification in multiplexed PCR reactions.²⁷ The '012/'108 Patents also state that “[t]he simultaneous use of numerous primer pairs **makes multiplex PCR impractical** due to high level of non-specific amplification and primer-primer interactions.”²⁸ We both agree. Yet, the data that Shapero (2004) presents does not show the presence of primer-dimers in the 250-plex experiment. Based on Shapero (2004)’s primers that are ~25 bases of target-specific sequence at the 3’-end with 23 bases of common sequence at the 5’-end,²⁹ one of ordinary skill in the art would have expected any primer dimers to be ~100 bases or less in length (*i.e.*, 2 x ~48 bases). Shapero (2004) Fig.2a is a gel that shows the sizes of the amplification products that were obtained in the 250-plex experiment. Fig.2a identifies a 100 bp marker on the left-hand side of the gel, where the other molecular weight markers are identified. I see no visible band in the gel of that size if primer dimers existed. The absence of a band indicates to me and would have indicated to one of ordinary skill in the art that no primer dimers are present in Shapero (2004)’s MARA reactions. Dr. Bustamante testified that he also saw no evidence of primer-dimers in the 250-plex experiment because of the absence of any visible bands at the 100

²⁷ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 216:20-218:4; *see also id.* at 350:10-19.

²⁸ See Ex. 1001, '012 Patent at Col. 1:51-53 (emphasis added).

²⁹ See Ex. 1006, Shapero (2004) at p. 2.

bp level or below.³⁰ Dr. Bustamante would not rule out the possibility that there may have been some primer dimers that just ran off the gel.

Q. And in Figure 2(A), which is a gel of run of all the amplicons, do you see a shred of evidence that there are any primer dimers in this?

A. I do not

A. ***I do not see a band.*** As I said, I don't know where exactly they cut off. ***It could be it's runoff. I don't have reason to believe that.***³¹

15. First, I disagree with the possibility that the supposed primer dimer band ran off the gel because if it did, so would have the 100 bp marker. Given the 100 bp marker is present on the gel, then the only conclusion is that no primer dimers were created with the MARA reaction. I also do not agree that one of ordinary skill in the art looking at Fig.2a of Shapero (2004) would have entertained that possibility, or believe it indicated that there might be high levels of off-target events due to primer dimers.

16. One of ordinary skill in the art reading Shapero (2004) would have understood that the 100bp marker was included on the gel for a reason. That reason would have been to simply show that no products of ~100 bp or less formed during the MARA reaction. One of ordinary skill in the art would not have understood that

³⁰ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 228:19-229:13; see also *id.* at 229:14-231:7.

³¹ *Id.* at 229:14-24 (emphases added).

the 100 bp marker was placed there to show that fragments may have run off the gel. Similarly, Fig. 2a of Shapero (2004) is also consistent with the amplicons that were formed being the ones that Shapero (2004) was targeting. In other words, the gel in Fig. 2a also shows data that is consistent with the SPE producing on-target events, not high levels of off- target events. As Shapero (2004) says, the gels showed reproducible amplicons of the expected size.³² If Shapero (2004)'s single target specific primer was annealing to off-target regions, one of ordinary skill in the art would expect it to do so randomly, with a variety of sizes on the gel. But what the gel in Fig. 2a shows is that across nine different patient samples, the fragments are of a consistent size in the expected range. This would have indicated to one of ordinary skill in the art that the single target specific primer in Shapero (2004) are not randomly annealing to and extending to create high-levels of off target events. To the contrary, the gel in Fig. 2a would have indicated to one of ordinary skill in the art that Shapero (2004)'s MARA is producing high levels of on target events. Eventually, Dr. Bustamante also agreed when he testified: "what I said is what they're reporting here [fig.2a gel and Ex. 1006 at p. 4] is a size distribution consistent

³² See Ex. 1006, Shapero (2004) at p. 4. ("Each of the nine genomic DNA samples used in MARA show a reproducible smear from 150 to 550 bp when the 250 locus-specific primers are present. ***This result is consistent with the expected size distribution of products which ranges from 100 to 500 bp, with a mean size of 287 bp.***") (emphasis added).

with what they expected giving a wide range of product lengths between 150 to 500 base pairs consistent with on-target priming. *I'm happy to agree with the experts that this is strong evidence of on-target priming.*"³³

17. I disagree with PO and Dr. Bustamante's claim that the concordance rates reported in Shapero (2004) show that MARA is performing much worse than the prior WGS enrichment system, and this would have steered one of ordinary skill in the art before 2012 away from using the MARA enriched library method on an NGS platform.³⁴ First Shapero (2004) reported for the 250-plex reaction, 99+% SNPs had discrimination scores > 0.08, call rates of 96-97% and concordance rates at ~ 99% with WGS data.³⁵ The differences reported are extremely small and one of ordinary skill in the art would have considered them trivial and not a factor to steer them away from using MARA to enrich a library to be sequenced on an NGS platform. I note that even Shapero (2004)'s lowest level of multiplex, the 250-plex, is just shy of 14 times more multiplexing than the prophetic examples in the '012/'108 Patents that provide no actual data or results,³⁶ Yet the 250-plex data

³³ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 215:13-21 (emphasis added).

³⁴ See POR at p. 26-27; see also Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D at ¶¶ 226-227.

³⁵ See Ex. 1006, Shapero (2004) at p. 5.

³⁶ See Ex. 1001, '012 Patent at Col. 31:35-34:58. In particular, see *id.* at Col. 32:12-15. ("Oligonucleotides representing each of the **18 highly conserved sequence**

showed call rates >96% and concordance of ~99%. Such high levels of concordance would not have led one of ordinary skill in the art to believe that MARA is performing poorly. Rather, the data in Shapero (2004) would have led them to believe that it was performing exceptionally well. Further, one of ordinary skill in the art would have recognized that in MARA, the DS (threshold) was set to a higher level than in the prior enrichment technique (WGSA).³⁷ This gave MARA a higher hurdle in making calls (*i.e.*, fewer SNP called) which would have driven down the concordance rate.

18. At the end of the day, one of ordinary skill in the art reading Shapero (2004) would have recognized that it is a method that produced highly enriched libraries using a single primer extension PCR technique that allowed much higher levels of multiplexing than had previously been achieved with multiplex PCR. That holds true for the 250-plex all the way up to the 4300-plex. While the 4300 plex reported conversion rates of around 75%,³⁸ which is less than the 250-plex, this would not have discouraged one of ordinary skill in the art from using the Shapero (2004) method to prepare a library to be analyzed on an NGS system. Of those 75%

blocks identified across the 16S genomic loci ***are synthesized and mixed in equimolar proportions.***") (emphases added).

³⁷ See Ex. 1006, Shapero (2004) at Table 1. ("***The DS threshold of 0.08 is higher*** than the threshold value of 0.03 used in WGSA as a detection filter to identify weak hybridization signals.") (citation omitted).

³⁸ *Id.* at Abstract.

of SNPs that were identified in the 4300-plex, the concordance rates were still very high, >99% across seven samples when compared to the prior library enrichment method.³⁹

19. One other piece of data that Shapero (2004) presented were negative controls where the samples were only amplified with the common primers (*i.e.*, absent target-specific primers). These data are shown in the gel in Fig. 2b, is also consistent with Shapero (2004) producing high on-target rather than high off-target amplicons. In those experiments, Shapero (2004) reports seeing very few if any amplicons when only the common primers are present in MARA. Shapero (2004) reported that in the case where only the common primers are present, only 6.6 % of the SNPs had a mean call rate with a $DS > 0.08$.⁴⁰ Shapero (2004) concluded that "[t]hese results show that the presence of locus-specific primers allows specific target sequences to be amplified, and in the absence of [target-specific] primers, **only small quantities of non-specific products** are generated [by the common primers]."⁴¹ One of ordinary skill in the art would have understood these results in 2012 or before as further evidence that the common primers are not producing high rates of unwanted amplicons on their own. Dr. Bustamante tries to discount these

³⁹ *Id.* at Abstract, Table 3.

⁴⁰ *Id.* at p. 5.

⁴¹ *Id.* (emphasis added).

results by arguing that the control experiments “underloaded” the array and that is why there were so few SNPs that were called.

Q. Quantitatively if they were off target 50 percent of the time, would there have been enough DNA for 99.4 percent of the time for the chip to light up on the SNPs, as they were supposed to?

A. ***What we know is that when they underloaded the array, they didn't get much data.***

Q. And when did they “underload the array”?

A. ***When they put in less than a hundred nanograms of DNA or about a hundred nanograms of DNA, as I recall.***⁴²

Dr. Bustamante is incorrect about the conditions of the negative control experiment. Shapero (2004) explained that the “[n]egative control reactions were carried out as described above, ***except that no locus-specific primers were used during the primary MARA reaction.***”⁴³ One of ordinary skill in the art reading this passage would have understood that the same amount of DNA was used in both the MARA and negative control, and that the latter only differed because it lacked that target-specific primers. The gel in Fig. 2b shows that the common primers will not produce large amounts of amplification products on their own. Those amplification products would be off-target events. Because those common primers did not produce quantities sufficient for the chip to detect, this demonstrates to one of ordinary skill

⁴² See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 147:21-148:10 (emphases added).

⁴³ See Ex. 1006, Shapero (2004) at p. 3 (emphasis added).

in the art that the common primers are not producing any significant amounts of off-target events.

20. At the end of the day, one of ordinary skill in the art reading Shapero (2004) in 2012 would have understood that the authors are experts in the field and their data and conclusions reported in Shapero (2004) showed high rates of on-target amplification, not high rates of off-target amplification.

21. I disagree with any claim by PO or Dr. Bustamante that prior art relating to PCR in general, or SPE PCR taught away from using Shapero (2004)'s enrichment method with NGS sequencing. Nothing in that art demonstrates to one of ordinary skill in the art that it would have been unproductive or unsuccessful to enrich libraries with Shapero (2004)'s MARA and then analyze those enriched sequences on an NGS platform. I disagree with PO and Dr. Bustamante's claim that it was recognized that SPE produced high off-target results and for that reason the field moved away from using SPE to enrich sequences for analysis by 2012.⁴⁴ Siebert (1995) is a reference that used SPE to enrich regions of interest and prepare them for sequencing.⁴⁵ I agree with Dr. Bustamante that Siebert (1995) taught the same enrichment steps as the '012/'108 Patents, that is ligating adapters onto fragments,

⁴⁴ See POR at pp. 42-43; see also Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D at ¶¶ 60-62.

⁴⁵ See Ex. 1044 (also Ex. 1101), Siebert (1995) at 1088.

performing SPE on those fragments and amplifying the extended fragments with common primers that annealed to the complement of the adaptor sequences that were created in the extension reaction.⁴⁶ Siebert (1995) actually reported high on-target specificity with Siebert's SPE method, not high off-target.⁴⁷ In fact, Siebert (1995) reported "the high specificity of the method described...".⁴⁸ High on-target binding and specificity for SPE were reported a few years later in other Siebert group publications that targeted and then sequenced the enriched fragments, such as '340 Patent (Chenchik),⁴⁹ discussing sequencing the products after SPE, and Fradkov (1998),⁵⁰ discussing SPE with suppression adaptors increases specificity and resulted in no detectable background in sequenced products. These results would not have steered one of ordinary skill in the art away from using SPE to enrich libraries in 2012. Rather, at least these references would have encouraged one of ordinary skill in the art to continue using SPE for enrichment and possibly work to optimize it further.

22. That is exactly what happened. Contrary to PO's claim that references like the '994 PCT Appl. (Sohn) would have steered one of ordinary skill in the art

⁴⁶ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 344:18-345:14.

⁴⁷ *Id.* at 345:15-19.

⁴⁸ See Ex. 1044 (also Ex. 1101), Siebert (1995) at 1088.

⁴⁹ See Ex. 1045, '340 Patent (Chenchik) at 16:30-42; *see also id.* at 16:56-17:29.

⁵⁰ See Ex. 1112, Fradkov (1998) at 138.

away from using SPE,⁵¹ the opposite is true. The '994 PCT Appl. (Sohn) identified and solved two problems they thought existed with SPE used in genome walking: low amplification efficiency and non-specific amplification.⁵² The '994 PCT Appl. (Sohn) solved the low amplification efficiency by increasing the percentage of fragments that successfully had adaptors ligated.⁵³ The '994 PCT Appl. (Sohn) also minimized non-specific amplification by using a 3' OH block on the adaptor.⁵⁴ Given these solutions to the problems that the '994 PCT Appl. (Sohn) encountered with SPE one of ordinary skill in the art in 2012 would not have been discouraged from using it.

23. Notably, Shapero (2004) experienced neither of the problems the '994 PCT Appl. (Sohn) identified. The high level of call rates in Shapero (2004) showed that the ligation of their adaptor was efficient and the common primers were able to produce sufficient amounts of amplification products. Additionally, Shapero (2004) already used a 3'-end blocking group on the adaptors, the solution that the '994 PCT Appl. (Sohn) suggested to prevent the common primers from independently producing amplification products. In fact, Shapero (2004) explained that "[t]he 3'

⁵¹ See POR at p. 42.

⁵² See Ex. 2050, '994 PCT Appl. (Sohn) at ¶ 8.

⁵³ *Id.* at ¶ 10.

⁵⁴ *Id.* at ¶ 63.

amino block helps to prevent background genomic amplification.”⁵⁵ Shapero (2004)’s common primers will not produce amplification products on their own independent of the target specific primer extension through the A2 adapter.⁵⁶ Siebert (1995) had also used an amine based blocking group to prevent the same problem from occurring.⁵⁷

24. With respect to the teaching of the blocking groups to prevent unwanted amplification, the teachings in Siebert (1995), Shapero (2004) and the ’994 PCT Appl. (Sohn), which refer to amine based blocks, are much more detailed than the teaching in the ’012/’108 Patents which merely states that recent advances in adaptor design that use nucleotide analogues and structured oligonucleotides have reduced the occurrence of adaptor dimers.⁵⁸

25. PO and Dr. Bustamante’s citation to Miura (2005) also does not show that one of ordinary skill in the art in 2012 would have been steered away from using SPE to create enriched libraries to be sequenced on an NGS platform. As with the ’994 PCT Appl. (Sohn), quite the opposite is true. What Miura (2005) demonstrates is that one of ordinary skill in the art would have continued to be interested in SPE

⁵⁵ See Ex. 1006, Shapero (2004) at Figure 1 legend; *see also* Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 356:5-16.

⁵⁶ See Ex. 1006, Shapero (2004) at Figure 1.

⁵⁷ See Ex. 1044 (also Ex. 1101), Siebert (1995) at 1087, Figure 1.

⁵⁸ See Ex. 1001, ’012 Patent at Col. 25:57-62.

and worked on optimizing its performance. Miura (2005) solved the problem of off-target events they experienced with SPE by creating an algorithm to improve the specificity of their single target specific primer.⁵⁹ The new algorithm put specific emphasis on the 3'-end of the primer and with the new design they was able to achieve 90% on target events with SPE.⁶⁰ Dr. Bustamante agreed Miura (2005) achieved this level of specificity.⁶¹ Notably, Shapero (2004) also used an algorithm to design their single target specific primer and that algorithm also focused on the 3' end. Shapero (2004) explained that “[l]ocus-specific oligonucleotides were designed using Primer 3 (27)...followed by [approximately] 25 bases of locus-specific sequence at the 3' end.”⁶²

26. PO and Dr. Bustamante's conclusion that “researchers outright dismissed the use of single target specific primer for enrichment,” citing to Bustin (2005), similarly has no basis in fact.⁶³ Bustin (2005) is a review article that describes real-time reverse transcription PCR (“real-time RT-PCR”), not creating enriched libraries for downstream analysis in a separate assay like Shapero (2004)

⁵⁹ See Ex. 2047, Miura (2005) at p. 4363.

⁶⁰ *Id.* at p. 4367.

⁶¹ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 356:17-357:16.

⁶² See Ex. 1006, Shapero (2004) at 2. I note that reference (27) is Ex. 1054, Rozen (2000).

⁶³ See POR at pp. 43-44; see also Ex. 2107, Declaration of Carlos D. Bustamante, Ph.D. at ¶ 94.

and the '012/'108 Patents do.⁶⁴ There is no rejection of using SPE to create enriched libraries as it is not discussed at all.⁶⁵ What Bustin (2005) describes is how to create amplicons and that accurate quantification requires maximum amplification efficiency, not necessarily even in a multiplexed fashion, using traditional primer pairs that have been designed to optimize their specificity.⁶⁶ What Bustin (2005) shows one of ordinary skill in the art is that there continue to be ways to optimize primer design to achieve higher specificity whether one or two target specific primers are being used.

27. One of ordinary skill in the art reading the '012/'108 Patents in 2012 would come away believing that there were no problems with existing SPE forms of enrichment like Shapero (2004) that would have discouraged or prevented one of ordinary skill in the art from analyzing a Shapero (2004) prepared library on an NGS platform because such an enrichment technique produced high rates of off-target products. One of ordinary skill in the art reading the '012/'108 Patents would find no problems with SPE that existed that the '012/'108 Patents purport to solve. In fact, the opposite is true. As mentioned earlier, the '012/'108 Patents teach that there

⁶⁴ See Ex. 2018, Bustin (2005) at Abstract.

⁶⁵ *Id.*

⁶⁶ *Id.* at p. 598.

have been improvements with adaptor design that minimize adaptor dimer issues.⁶⁷ The '012/'108 Patents do not treat adaptor dimers as a problem to be solved by the '012/'108 Patents, but rather one that has already been solved. Similarly, the '012/'108 Patents do not teach any specifics regarding the target specific primer design indicating that there is a problem with creating such primers to produce on-target events. Rather, it speaks in very broad generalities about primer design, noting only what had been known for decades regarding how to design primers that would anneal to specific target sequences and not others. The '012/'108 Patents state that “[i]n some cases, the identity of the investigated target polynucleotide sequence is known and hybridizable sequences specific oligonucleotides or primers can be synthesized precisely according to the antisense sequence of the aforesaid target polynucleotide sequence.”⁶⁸ Shapero (2004), in pointing to Primer 3 program and the length of the 3' end of the target-specific primer, and Miura (2005) describing their new algorithm for the target specific design which also focused on the 3'-end, both discussed previously, were much more informative to one of ordinary skill in the art about how to achieve specificity with SPE than the '012/'108 Patents are. This would have led one of ordinary skill in the art to believe that any problems with

⁶⁷ See Ex. 1001, '012 Patent at Col. 25:55-65.

⁶⁸ *Id.* at Col. 22:3-8.

SPE primer design to achieve specificity had already been solved by the time the '012/'108 Patents were filed.

28. I also do not find that articles mentioning Shapero (2004) would have discourage one of ordinary skill in the art in 2012 from using the MARA method to enrich a library that was later analyzed on an NGS platform. I do not find any of the teachings in the articles that are cited in the Patent Owner Response or singularly in Dr. Bustamante's declaration that would have led one of ordinary skill in the art to believe that it would be unproductive or non-functioning to analyze a MARA enriched library on an NGS platform. At most, some of the articles attempt to promote their own enrichment schemes but not in a way that would have led one of ordinary skill in the art to believe that MARA is a technology that cannot and should not be used to create an enriched library that will be analyzed on an NGS platform.

29. For example, PO cites Liu (2012) as a reference that denigrates the use of MARA.⁶⁹ It does not. What Liu (2012) discusses is that the overall Shapero (2004) system that includes the MARA enrichment method and subsequent custom designed chip used to analyze the MARA enriched libraries to detect mutations is highly efficient for the identification of large numbers of SNPs.⁷⁰ Liu (2012) also discusses other methods may be more practical for identifying low to moderate

⁶⁹ See POR at p. 46.

⁷⁰ See Ex. 2141, Liu (2012) at p. 2.

numbers of SNPS.⁷¹ Liu (2012) never identifies the MARA enrichment technology as being flawed.⁷² Rather, Liu (2012) just says then when you do not need a powerful tool that is capable of detecting many mutations, you can use a more stripped-down overall detection methodology. This would not have steered one of ordinary skill in the art away from using MARA to enrich libraries that are later analyzed on an NGS system. In fact, Liu (2012) teaches that both NGS and array resequencing like Shapero (2004) can be used to identify SNPs.⁷³ This would actually have steered one of ordinary skill in the art into using MARA to prepare an enriched library that could be analyzed for SNPs on an NGS platform.

30. PO's citation to Bui (2009)⁷⁴ would not have steered one of ordinary skill in the art away from using MARA with NGS for reasons similar to those in Liu (2012). Bui (2009) states that overall techniques like Shapero (2004)'s [ref.12] are very powerful for large scale SNP determinations.⁷⁵ Bui (2009) acknowledgement that their own scaled down technology could be more useful in small-scale genotyping⁷⁶ would not have discouraged one of ordinary skill in the art in 2012

⁷¹ *Id.* at p. 6.

⁷² *Id.* at p. 2.

⁷³ *Id.* at p. 2.

⁷⁴ *See* POR at p. 46.

⁷⁵ *See* Ex. 2138, Bui (2012) at p. 2.

⁷⁶ *Id.* at Abstract.

from using the power of MARA to create libraries of many enriched loci that could then be analyzed with the power of NGS.

31. PO's pointing to Palka-Santini (2009) is also not a reference that would steer one of ordinary skill in the art away from combining MARA with NGS. Palka-Santini (2009) actually recognizes that Shapero (2004) (ref.30) allows for highly-multiplexed PCR by replacing a target-specific primer in traditional PCR primer pair with a common primer.⁷⁷ Palka-Santini (2009) does not say that the replacement results in poor on-target results. Rather, Palka-Santini (2009) explains that the Shapero (2004) process is time consuming because it requires multiple steps like digestion, and ligation of adaptors.⁷⁸ But those steps would not have discouraged one of ordinary skill in the art from using MARA. That is especially true as other methods of performing multiplex PCR, especially at the lowest 250-plex levels that Shapero (2004) performed are as time consuming, if not more. For example, hybridization techniques to capture the desired targets would require the preparation of many target specific probes of long length (approximately 100 bp or longer). After capture, there would also be multiple steps to isolate and then further amplify the captured sequence for further analysis. Methodologies like Raindance's rainstorm required special equipment to create droplets that isolated single fragments

⁷⁷ See Ex. 2139, Palka-Santini (2009) at p. 10.

⁷⁸ *Id.* at p. 2.

to be amplified.⁷⁹ I would consider the preparation and execution of that process in 2012 to be as, if not more, time consuming and complex as what Shapero (2004) taught. Moreover, I do not find that Palaka-Santini (2009)'s statement that multistep processes like Shapero (2004) are prone to contamination would have discouraged one of ordinary skill in the art in 2012 from using MARA. All processes dealing with DNA isolation and amplification are prone to some levels of contamination. I see nothing in Shapero (2004) that indicates to one of ordinary skill in the art that MARA is more prone than other processes. Further, Palaka-Santini (2009)'s citation to ref. 25 as suggesting that multi-step processes might be prone to contamination is not one that one of ordinary skill in the art would have read onto Shapero (2004). Ref. 25 was published four years before Shapero (2004) and thus one of ordinary skill in the art would not have understood that it is making any substantiated claims that Shapero (2004) was prone to contamination.

32. PO's pointing to Dahl (2005) also does not indicate to one of ordinary skill in the art that MARA is an unacceptable enrichment methodology that should be avoided as unproductive.⁸⁰ Dahl (2005)'s authors are promoting their padlock probes method of enriching libraries for particular sequences.⁸¹ Dahl (2005) never

⁷⁹ See Ex. 2024, Tewhey (2009).

⁸⁰ See POR at pp. 46-47.

⁸¹ See Ex. 2126, Dahl (2005) at p.1.

states that Shapero (2004) is producing high levels of off-target events or that it is rejecting MARA as PO claims.⁸² In fact, Dahl (2005) never specifically discusses MARA; Shapero (2004) is just included in a string cites to methods that allow subsets of genomes to be amplified using adaptor PCR.⁸³ Dahl (2005) says that the only way to amplify many defined sequences in the same reaction is using primer pairs.⁸⁴ But one of ordinary skill in the art would not have read that sentence to say that Shapero (2004)'s MARA is not capable of multiplexing as PO is attempting to do. Shapero (2004) could not be any clearer that the whole purpose of MARA is to create enriched target specific libraries through multiplexed PCR reactions that use a target specific primer and common primers.⁸⁵ That PO's reading that Dahl (2005) somehow claims Shapero (2004) is not capable of multiplexing is refuted by a subsequent article, Howell (2006), which PO also cites⁸⁶, which was penned by the majority of the Dahl (2005) authors.

33. Howell (2006) states that Shapero (2004) (ref.35) achieved amplification of specific genomic sequences with a single target-specific primer and

⁸² See POR at pp. 46-47.

⁸³ See Ex. 2126, Dahl (2005) at p.1.

⁸⁴ *Id.*

⁸⁵ See Ex. 1006, Shapero (2004) at Abstract.

⁸⁶ See POR at pp. 46-47.

a common primer.⁸⁷ Howell (2006)’s statement that none of the enrichment methods to date have achieved the ideal goal of amplifying large sets of sequences without any undesired amplification products, indicates, in the first instance that Howell (2006) is considering Shapero (2004) to teach multiplexing through his reference to large sets of sequences. Second, the fact that neither Shapero (2004) nor any other methodology achieves zero undesired amplification products would not have steered one of ordinary skill in the art away from using MARA to prepare and enriched library for analysis on an NGS platform. I agree with Dr. Bustamante who recognized that zero undesired amplification products is an aspirational goal, achieved by no methodology, including the ’012/’108 Patents’ “unexpected results” of only 10% undesired products.⁸⁸ In the same vein, Shapero (2004)’s statement that “absolute specificity” is not required in MARA is also not a statement that would indicate to one of ordinary skill in the art that it produced high off-target rates, or that it was not suitable for creating an enriched library that could be analyzed on an NGS platform. No enrichment technology in 2012 or even today can be expected to produce absolute specificity – *i.e.* not even a single undesired event. Because absolute specificity is a goal rather than a requirement in preparing enriched libraries for analysis on subsequent platforms, one of ordinary skill in the art would not have

⁸⁷ See Ex. 2130, Howell (2006) at p. 160.

⁸⁸ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 382:19-385:24.

been discouraged from using MARA by Shapero (2004)'s statement that MARA may not produce zero undesired amplification events.

34. Importantly, Howell (2006) recognizes that while genotyping SNPs in clinical settings has generally focused on known SNPs, the methods they discussed for enrichment, such as Shapero (2004), can be used in combination with, or replaced entirely with, new economical sequencing techniques that can provide the exact nature and position of a genetic mutation, including unknown SNPs.⁸⁹ Thus far from discouraging one of ordinary skill in the art in 2012 to use MARA with an NGS platform, one of ordinary skill in the art reading Howell (2006) would have encouraged the skilled artisan to do so.

35. After ignoring the suggestion in Howell (2006) to combine enrichment techniques like Shapero (2004) with detection techniques like NGS, PO makes the broad statement, relying on a number of publications, that there is no motivation to combine Shapero (2004)'s highly-multiplexed enrichment method, MARA, with NGS detection and no expectation that it would work if combined.⁹⁰ I disagree. None of the references PO cites would have taught away from the combination or indicate the combination would not work.

⁸⁹ See Ex. 2130, Howell (2006) at pp. 160, 163.

⁹⁰ See POR at pp. 16, 46.

First, PO cannot dispute that Shapero (2004) itself teaches that MARA enriched libraries can be analyzed for SNPs not just with an Affymetrix chip, but by sequencing, through Shapero's specific reference to Syvänen (2001) (ref.5).⁹¹ More specifically, Shapero (2004) states that MARA is a highly multiplexed targeted library preparation method that far exceeds typical multiplexed PCR in preparing a targeted library for SNP genotyping.⁹² Shapero (2004) explains that there are numerous genotyping methods, many of which employ DNA polymerases used in mini-sequencing and pyrosequencing:

There are a multitude of genotyping methodologies for SNPs that have been described over the years. (5-7). These approaches can vary in terms of how alleles are discriminated, e.g. the use of nucleic acid hybridization with short oligonucleotide probes alone or in combination with enzymes such as DNA polymerases or DNA ligases. Additionally, these approaches can vary in the nature of both the assay formats and labeling and detection strategies. However, many currently used approaches share a common feature in that there is a requirement for amplification of the region harboring the polymorphism prior to the actual genotyping. PCR significantly increases the copy number of the sequence to be genotyped, thereby effectively reducing the complexity of the target sequences and improving the ability to differentiate correct and incorrect genotype calls.⁹³

That citation is consistent with the Shapero (2004) group's earlier disclosure in '577 Patent Appl. (Jones).

⁹¹ See Ex. 1006, Shapero (2004) at p. 1.

⁹² *Id.* at pp. 1-2.

⁹³ *Id.* at p. 1. I note that reference (5) is Ex. 1012, Syvänen (2001).

The [MARA] amplified sample may be analyzed by any method known in the art, for example MALDI-TOF mass spec, capillary electrophoresis, OLA, dynamic allele specific hybridization (DASH) or TaqMan (Applied Biosystems..). For other methods of genotyping analysis *see* Syvanen, Nature Rev. Gen. 2:930-942 (2001) which is herein incorporated by reference in its entirety...⁹⁴

36. Given the citations in Shapero (2004) and '577 Patent Appl. (Jones), PO cannot dispute that Shapero (2004) would have taught one of ordinary skill in the art before 2012 that their MARA enriched libraries can be analyzed for SNPs, not just with an Affymetrix chip, but with sequencing as well through the reference to Syvänen (2001) which identifies pyrosequencing as one of the specific SNP detection methodologies that exist.⁹⁵

37. PO does not dispute that Delseny (2010) teaches that NGS, the generation of sequencing platforms that came after those described in Syvänen (2001)'s time, can also be used for genotyping.⁹⁶ Dr. Bustamante agreed stating that NGS was used to genotype by 2010.⁹⁷

B. Motivation to Combine and Expectation of Success

38. One of ordinary skill in the art would have understood by 2012 that the same reasons the skill artisan would have analyzed a library enriched by Shapero

⁹⁴ See Ex. 1011, '577 Patent Appl. (Jones) at ¶ 80.

⁹⁵ See Ex. 1012, Syvänen (2001) at p. 934, Table 2.

⁹⁶ See POR at p. 32.

⁹⁷ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 367:18-24.

(2004)'s MARA with earlier sequencing methods would have led them to analyze the same enriched libraries on next generation sequencing platforms. There were no technical hurdles that would have indicated to one of ordinary skill in the art by 2012 that the MARA enriched libraries would function on earlier sequencing platforms but not NGS platforms. Rather, one of ordinary skill in the art would have had an expectation of success when sequencing a MARA enriched library on an NGS platform to obtain useful sequence/genotype information. None of the arguments that PO makes to the contrary detract from my conclusion of a motivation to combine and an expectation of success.

39. PO, nonetheless tries to read Delseny (2010) in such a way as to claim it teaches away from using MARA enriched libraries on an NGS platform. PO claims that Delseny (2010) encourages genotyping by NGS sequencing only when genomes were previously sequenced.⁹⁸ While this would be a preferred approach, even if Delseny (2010) was so limited, it does not constitute a teaching away from the claimed methodology. I see nothing in the claims that would have limited one of ordinary skill in the art to sequencing only unknown sequences. Sequencing and genotyping known sequences fall within the scope of the claims of the '012 and '108 Patents. In fact, the examples in the '012/'108 Patents suggest enriching and then

⁹⁸ See POR at p. 32.

sequencing bacterial 16S loci, which at the time the patent was filed had been well characterized. For instance, examples 1 and 2 of the '012/'108 Patents propose using 16S target specific primers.⁹⁹ And, Delseny (2010) identifies a specific benefit of genotyping already characterized genomes with sequencing as opposed to hybridization techniques like the Affymetrix chip: sequencing will identify InDels and repeats whereas hybridization techniques, such as an Affymetrix chip will not.¹⁰⁰ This benefit would be an additional motivation for one of ordinary skill in the art in 2012 to have analyzed a MARA enriched library by sequencing rather than on an Affymetrix chip.

40. PO next claims that one of ordinary skill in the art would at most have understood Delseny (2010) to at most teach that NGS could be used for whole genome sequencing.¹⁰¹ I do not see how PO could make this claim. Delseny (2010) has an entire section titled, *Targeted Sequencing*.¹⁰² In this section Delseny (2010) explains how targeted sequencing is important for patients in medicine and how targeted sequencing can be used in plant genetics, their area of primary interest.¹⁰³ As to methods of enrichment, Delseny (2010) cites Porreca (2007) [ref 128] as one

⁹⁹ See Ex. 1001, '012 Patent at Col. 31:37-33:67.

¹⁰⁰ See Ex. 1007, Delseny (2010) at § 4.3.

¹⁰¹ See POR at p. 33.

¹⁰² See Ex. 1007, Delseny (2010) at § 4.4.

¹⁰³ *Id.*

example of a targeted enrichment method.¹⁰⁴ Porreca (2007) uses a single target specific probe/primer, which is then amplified to produce the enriched product.

41. Contrary to PO's claim, nothing in Delseny (2010) would have taught one of ordinary skill in the art in 2012 that the skilled artisan should not analyze a MARA enriched library with NGS. The opposite is true. Delseny (2010) does not limit the enrichment methods that could be used with NGS. Delseny (2010) merely points to one example. That example would have encouraged rather than have discouraged one of ordinary skill in the art in 2012 from analyzing a MARA library on an NGS platform.

42. PO next argues that high error rates in NGS would have steered one of ordinary skill in the art in 2012 away from analyzing a MARA enriched library on NGS platforms, and the skilled artisan would have chosen prior generation Sanger sequencing instead.¹⁰⁵ First, this argument ignores Delseny (2010)'s express recommendation to sequence targeted loci by NGS.¹⁰⁶ Second, the argument ignores the teachings of references that the PO identified. Those references, such as Moorthie (2011)¹⁰⁷ teach that while raw sequence error rates were initially higher in

¹⁰⁴ *Id.* I note that reference (128) is Ex. 1049, Porreca (2007).

¹⁰⁵ See POR at pp. 33-34.

¹⁰⁶ See Ex. 1007, Delseny (2010) at § 4.4.

¹⁰⁷ See Ex. 2169, Moorthie (2011) at p. 7.

NGS than in Sanger, the higher read depths present in NGS offset the raw error rates allowing NGS to also detect variants that Sanger could not.¹⁰⁸

43. Tucker (2009) reached the same determination about NGS error rates and read depths. Tucker (2009) concluded, while discussing various enrichment schemes that could be used to prepare a library for NGS sequencing that the accuracy of NGS was high enough that it could replace Sanger sequencing when looking for genetic mutations, even in a clinical diagnostic setting.¹⁰⁹

44. PO also argues that Syvänen (2001) and Shapero (2004)'s reference to it would teach away from using NGS in 2012. PO states this because the PO believes that Syvänen (2001) discourages the use of PCR.¹¹⁰ Syvänen (2001) actually describes numerous methods for detecting SNPs depend on amplification methods.

All methods used for genotyping SNPs in large diploid genomes depend on PCR amplification of the genomic regions that span the SNPs before the actual genotyping reaction. The PCR provides the required sensitivity and specificity for distinguishing between heterozygous and homozygous SNP genotypes in large complex genomes. The difficulty of designing and carrying out multiplex PCR reactions is an important factor that limits the throughput of the current SNP-genotyping assays.¹¹¹

¹⁰⁸ *Id.*

¹⁰⁹ See Ex. 1013, Tucker (2009) at pp. 144-145.

¹¹⁰ See POR at pp. 36-40.

¹¹¹ See Ex. 1012, Syvänen (2001) at p. 4 (emphasis added).

One of ordinary skill in the art reading Syvänen (2001) after Shapero (2004) would have been encouraged, rather than discouraged from genotyping the enriched library prepared by MARA since Shapero (2004) solved what Syvänen (2001) had described three years earlier and as a limitation on high-throughput genotyping: high levels of PCR multiplexing. In particular, Syvänen (2001) disclosed then available sequencing techniques as ways to genotype of SNPs. One of those ways was pyrosequencing, which could sequence up to 50bp.¹¹² Pyrosequencing as practiced at the time was similar to modern day NGS techniques. Again, the reference to pyrosequencing would have encouraged rather than discourage one of ordinary skill in the art in 2012 from using MARA enriched libraries with NGS. In my estimation, NGS, such as practiced on an Illumina sequencer is more efficient to perform than pyrosequencing, which requires one by one addition of unique nucleotides. For example, A's must be added independently of other nucleotides and then checked to see if they produced a signal, G's must be added independently to see if they produce a signal and so on. Pyrosequencing would not have been as accurate as NGS such as Illumina because it could be difficult to distinguish if multiple of the same nucleotide (called homopolymer repeats) had been incorporated indicating a sequence having four A's (*i.e.*, AAAA) versus a sequence with five A's (*i.e.*,

¹¹² *Id.* at p. 934. Table 2 also referring to ref 72,74 in support of pyrosequencing.

AAAAA). Illumina sequencing did not have the same limitation. In any event, while Syvänen (2001) recognized that PCR amplification was the workhorse in many enrichment and detection techniques, she suggested in 2001 that future methods might be best served by avoiding amplification altogether.¹¹³ Syvänen's (2001) suggestion was not followed; by 2010, using PCR amplification to prepare and sequence libraries was dominant and well accepted. For example, Tucker (2009) identifies NGS sequencing based on amplification.¹¹⁴ Mamanova (2010) also identified numerous amplification based target enrichment methods used for NGS and determined that "the specificity of PCR will almost certainly always exceed that of hybrid capture, and its uniformity may never be matched by either hybrid capture or MIPS."¹¹⁵ Consequently, I see nothing in Syvänen (2001) that would have discouraged one of ordinary skill in the art in 2012 from analyzing a Shapero (2004) MARA library on an NGS platform. Shapero (2004) and '577 Patent Appl. (Jones)'s citation to Syvänen (2001) for methods to analyze SNPs and Syvänen (2001)'s identification of sequencing for SNP identification would have actually encouraged one of ordinary skill in the art to analyze MARA libraries with NGS. One of

¹¹³ *Id.* at p. 940.

¹¹⁴ Ex. 1013, Tucker (2009) Syvänen.

¹¹⁵ See Ex. 2011, Mamanova (2010) at p. 117.

ordinary skill in the art reading Arid (2011)¹¹⁶ in 2012 would not be discouraged from using Shapero's MARA to prepare an enriched library on an NGS platform. PO's argument that Arid teaches that the best way to avoid bias during PCR is to avoid amplification altogether, is taken out of context.¹¹⁷ What Arid actually says is that while one can avoid PCR bias by avoiding amplification altogether, that approach would require large amounts of DNA, which is not a practical approach for many sample types.¹¹⁸ Arid also states that amplification free protocols are not amenable to automation and thus would not be applicable to high-throughput sequencing facilities. Last, Arid recognizes that improved PCR conditions, like the ones Arid teaches in the article, "will likely satisfy the vast majority of projects."¹¹⁹

45. PO's claim¹²⁰ that my 2010 review article on NGS "echos" Syvänen (2001)'s concerns and would have steered one of ordinary skill in the art away from analyzing a MARA library on an NGS platform similarly has not basis. PO cites to a portion of my article and crops a quote to support its claim. Put in the proper context, all I said was that if one were to enrich with standard target specific forward and reverse primer pairs, one would need on the order of tens of thousands of primer

¹¹⁶ Ex. 2042, Arid (2011)

¹¹⁷ POR at p. 45.

¹¹⁸ Ex. 2042, Arid (2011) at p. 11.

¹¹⁹ *Id.*

¹²⁰ See POR at p. 40.

pairs to use the entire capacity of an NGS instrument which at that point was not practical.¹²¹ That is merely a restated variation of the problem that Shapero (2004) set out to solve: when you multiplex at high levels with target specific primer pairs, the level of multiplexing is limited to a few dozen loci per reaction.¹²² That statement is not an observation to avoid a MARA prepared library. Rather it is a motivation to pursue it with an expectation of success. Shapero (2004) disclosed that MARA enabled 250, 750, 1,100 and 4,365 loci to be enriched in a single reaction, with all the reported data evidencing high on-target rates.¹²³ Further, Shapero (2004) even suggested that their results demonstrated that MARA might even function at levels of 10,000-plex. For example, Shapero (2004) explained that “[s]ince the efficiency of the primers in MARA did not decrease as the level of multiplexing increased from 1000- to greater than 4000-plex, it is conceivable that MARA could function at the level of 10,000-plex ...”¹²⁴ Such a high level of multiplexing would have been greater than what was available as the state of the art in 2010 and would have motivated one of ordinary skill in the art to use MARA enriched libraries on NGS platforms.

¹²¹ See Ex. 1025, Metzker (2010) at p. 41.

¹²² See Ex. 1006, Shapero (2004) at pp. 1,7.

¹²³ *Id.* at Tables 1, 2, 3, and Figure 3.

¹²⁴ *Id.* at p. 8.

46. More particularly, what I reported in my 2010 review is that Raindance system had the highest level of multiplexing; it reported simultaneous amplification of 3,976 loci using microdroplet PCR.¹²⁵ This is less than the 4,365-plex experiment Shapero (2004) conducted with good results. Additionally, the Raindance methodology required a special instrument to create the individual droplets that act as individual reaction vessels for each PCR reaction amplifying a single locus. MARA avoided that special instrument cost. It also would have been more within the grasp of one of ordinary skill in the art prior to 2012. While multiplexing was a more difficult routine to carry out than single plex PCR reactions, one of ordinary skill in the art in 2012 would have been more comfortable with the techniques used in MARA than in the Raindance system. All the MARA techniques, like fragmentation, ligation of adaptors, design of single target specific primers and common primers targeting the adaptors, would have been familiar to one of ordinary skill in the art in 2012 who had performed PCR reactions in the past.

47. MARA also had advantages over the other enrichment techniques I described in 2010 that would have motivated one of ordinary skill in the art to analyze a MARA library on an NGS platform. Hybrid capture required primers of very long lengths, typically at least 100 bp or longer. These probes/primers were

¹²⁵ See Ex. 1025, Metzker (2010) at p. 41.

harder and more costly to design than the primers MARA used. Hybrid capture required many of these complex probes/primers for multiplexing. Moreover, once a hybrid capture probe/primer captured the sequence of interest, many subsequent steps were necessary to segregate and amplify the targeted sequences for subsequent genotyping use. The other prominent technology for enriching before NGS sequencing was MIPs. As I reported in my 2010 paper, different groups reported coverage problems using this technique and that would have been a motivation for one of ordinary skill in the art in 2012 to try MARA, which was more akin to the traditional PCR they had been practicing for years.

48. Given the results that Shapero (2004) presented that were consistent with high on-target rates, one of ordinary skill in the art in 2012 would have had an expectation of success in analyzing MARA enriched libraries on an NGS platform. I see no technical difficulties that would have impeded the expectation of success. One of ordinary skill in the art using the Illumina platform in 2012 and before were already aware that any sequence fragments would require that Illumina adaptor sequences be attached.¹²⁶ One of ordinary skill in the art would have been very familiar with how to do that by 2012. Thus, to prepare a MARA library for sequencing on an Illumina platform they could either perform an additional step of

¹²⁶ See Ex. 1003, Declaration of Michael L. Metzker, Ph.D at ¶ 66.

ligating the adaptor sequences on after enrichment. Alternatively, and more easily, they would just use the Illumina adaptor sequences as the sequences that were ligated on to the fragments in the first place, which are identified in Shapero (2004) as “A2” and they would use Illumina adaptor sequences in the tails of the target specific primers identified as “A1.”¹²⁷ Since the Illumina adaptor sequences are designed as distinct from naturally occurring genomic sequences, using them as the A1 and A2 sequences would not have posed any technical problems in carrying out the MARA method. Thus, one of ordinary skill in the art would have had an expectation of success in creating a MARA enriched library that could be sequenced on an NGS platform like and Illumina.

49. Additionally, nothing in my 2010 paper where I say it is impractical to use the entire capacity of an NGS platform through multiplexed PCR would have taught away from one of ordinary skill in the art analyzing a MARA enriched library on an NGS platform. Putting aside the fact nothing in the '012/'108 Patents claim requires the use of the entire NGS capacity, by 2012 there were established ways in place that one of ordinary skill in the art would have been using NGS without having to use its entire capacity. The primary way was to pool samples either from the same or different investigators and keep track of them with barcoding. Tucker (2009)

¹²⁷ See Ex. 1006, Shapero (2004) at p. 4, Figure 1.

pointed this out in 2009. “[T]he capacity of current massively parallel sequencing platforms is too great for efficient sequencing of most single genes.... A solution to this problem would be to pool DNA samples from several family members or from unrelated patients that have been prepared by locus-specific PCR and barcoded so that the DNA from each individual can be distinguished.”¹²⁸ Dr. Bustamante confirmed that by 2011, sequencing facilities using NGS pooled samples from different investigators into run and then split the cost of the sequencing run.¹²⁹ Thus, the enormous capacity of NGS systems would not be a deterrent to running small targeted samples on such a platform.

50. I reviewed other articles that PO and Dr. Bustamante cited to claim that the prior art either criticized Shapero (2004) or failed, when citing to him to suggest combining it with NGS. As with the references already discussed allegedly criticizing Shapero (2004), I saw nothing that would have discouraged one of ordinary skill in the art from using a MARA library on an NGS platform. Rather, at most the articles suggest alternatives to MARA enrichment in certain applications where such high throughput is not necessary. I saw nothing in any of those articles that would have led one of ordinary skill in the art in 2012 to believe that MARA produced such poor enrichment that it would have been unproductive to pursue it

¹²⁸ See Ex. 1013, Tucker (2009) at pp. 149.

¹²⁹ See Ex. 1120, October 7, 2025, Carlos D Bustamante, Ph.D. Tr. at 282-284.

further. The opposite is true. For example, Dr. Bustamante on page 183 of his declaration cites to Morin (2007) as follows: “In fact, Morin 2007 cites Shapero as an example where ‘additional modifications will be needed to ... increase throughput to allow for efficient amplification of a larger number of loci in the multiplex PCR.’”¹³⁰ The way that the quote was cropped implies that Shapero (2004) needs to be improved for it to allow higher multiplexing. When the full quote is read without the cropping, it would be apparent to one of ordinary skill in the art that Morin (2007) is stating that their method needs to be improved to obtain high levels of multiplexing while Shapero (2004) is an example of a method that already provides for high levels.¹³¹

51. PO and Dr. Bustamante cite another tranche of articles that reference Shapero (2004) but do not specifically teach that it should be combined with an NGS platform. I do not believe one of ordinary skill in the art reading those articles by 2012 would have been discouraged from analyzing a MARA enriched library on an NGS platform simply because an article identifying Shapero (2004)’s enrichment technique did not specifically suggest analyzing the MARA library on an NGS platform. As I have discussed throughout this declaration, there are many other teachings and reasons one of ordinary skill in the art in 2012 would have been

¹³⁰ See Ex. 2135, Morin (2007) at p. 945.

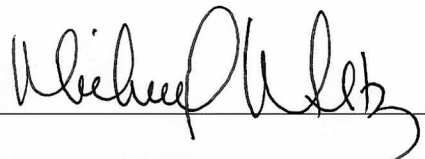
¹³¹ *Id.*

motivated to analyze a MARA enriched library on an NGS platform even if a reference citing to Shapero (2004) did not explicitly suggest that combination. Without truncating what I have discussed previously some of those motivations would have been, the high rate of multiplexing available with MARA, the ease of use and lack of need for specialized equipment when compared to other forms of multiplexing, the advantages that analyzing genomes for SNPs by sequencing provides over hybridization based techniques such as the ability to discover Indels and repeats and the exact location of the mutation.

52. For all these reasons, I continue to believe the claims of the '012 Patent and '108 Patent are obvious over the combination of Shapero (2004) and Delseny (2010) and the other references cited in my opening declaration when viewed from the standpoint of one of ordinary skill in the art in 2012 armed with a knowledge of the prior art.

I declare under penalty of perjury that the foregoing is true and correct. I declare that all statements made herein of my knowledge are true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: November 12, 2025

By: 
Michael L. Metzker, Ph.D.