

## **REMARKS**

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and the reasons that follow.

### **Statement of the Substance of an Interview**

Applicant thanks Examiner Brusca for the courtesy extended in conducting an interview on July 22, 2022. During the interview, the outstanding rejections and proposed claim amendments to overcome the rejections were discussed. The official interview summary dated July 28, 2022 accurately reflects the contents of this discussion. If the Examiner believes that more review in the record is needed about this interview, he can notify the undersigned.

### **Status of Claims**

Claims 1-15 were pending in the subject case. With this submission, claims 1, 11, 12, and 15 are amended to more clearly and particularly describe the claimed invention; claim 2 is cancelled without prejudice or disclaimer thereof; claims 16-29 are added. Support for the amendments to claim 1 can be found in claim 2 as well as paragraphs 0453, 0457 and 0761 of the as-filed specification; support for the amendments to claim 15 can be found in claim 1; support for new claims 16-29 can be found in claims 2-14 as well as paragraphs 0438 and 0458 of the as-filed specification. No new matter has been introduced.

After amending the claims as set forth above, claims 1 and 3-29 will be pending in this application.

### **Claim Objection**

Claim 1 is objected to for grammatical informalities. For the sole purpose of expediting prosecution, Applicant has amended claim 1 pursuant to the Examiner's suggestion on page 2 of the Office Action, thereby resolving the issue.

### **Claim Rejections Under 35 U.S.C. § 112**

Claims 1-15 are rejected under 35 U.S.C. § 112(b) for alleged indefiniteness in reciting “cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom.” Applicant respectfully traverses this rejection. As described throughout the as-filed specification, the isolated cell-free DNA can be subject to optional library preparation steps prior to performing targeted multiplex amplification. See, e.g., Example 1 (“*Universal ligation adapters were appended to the end of each molecule of 40 uL of purified plasma DNA and libraries were amplified for 9 cycles using adaptor specific primers.*”). Thus, a person skilled in the art would understand that the targeted multiplex amplification step can be performed on the isolated cell-free DNA or on DNA derived from the cell-free DNA.

Claims 1, 2 and 12 are rejected under 35 U.S.C. § 112(b) for alleged indefiniteness in reciting “high-throughput sequencing.” Applicant respectfully traverses this rejection. For the sole purpose of expediting prosecution and without acquiescing to the rejection, Applicant has amended claims 1 and 12 to recite “sequencing” instead, thereby resolving the issue.

Claim 11 is rejected under 35 U.S.C. § 112(b) for alleged indefiniteness in reciting “patient-specific PCR primers.” Applicant respectfully traverses this rejection. For the sole purpose of expediting prosecution and without acquiescing to the rejection, Applicant has amended claim 11 to recite “PCR primers...targeting the plurality of SNV mutations identified in the tumor sample,” thereby resolving the issue.

For at least these reasons, Applicant respectfully requests reconsideration and withdrawal of the subject rejections.

### **Claim Rejections Under 35 U.S.C. § 103**

Claim 1, 3, 7, 9 and 11-14 are rejected under 35 U.S.C. § 103 for allegedly being unpatentable over Forshaw *et al.* 2012 (“Forshaw”) in view of Benesova *et al.* 2013 (“Benesova”). Claims 4-6 and 8 are rejected under 35 U.S.C. § 103 for allegedly being unpatentable over Forshaw in view of Benesova, and further in view of Marusky *et al.* 2010 (“Marusky”). Claim 10 is rejected under 35 U.S.C. § 103 for allegedly being unpatentable over Forshaw in view of Benesova, and further in view of WO2004/051218 (“Anderson”). Claims 14 and 15 are rejected under 35 U.S.C. § 103 for allegedly being unpatentable over Forshaw in view

of Benesova, and further in view of Diehl *et al.* 2005 (“Diehl”). Applicant respectfully traverses these rejections.

Claim 2 stands free of the subject rejections. For the sole purpose of expediting prosecution and without acquiescing to the rejection, Applicant has amended independent claim 1 to incorporate the limitation of previous claim 2, thereby rendering the rejections moot. None of the cited references teaches or suggests sequencing each of the target loci with a depth of read of at least 50,000 per target locus. For at least these reasons, Applicant respectfully request withdrawal of the subject rejections against claims 1 and 3-14.

Applicant further submits that amended claim 15 and claims dependent therefrom are also unobvious over the cited references. As amended, claim 15 recites:

15. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:  
 performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;  
 performing targeted multiplex amplification to amplify 10 to 200 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and  
 sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.

In contrast to the presently claimed method which is capable of “detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus”, Forshew’s TAm-Seq workflow was only able to achieve a detection limit of 2% (see abstract). Forshew also expressly acknowledged that “[a] current limitation of TAm-Seq is the detection limit compared to assays that target individual loci, which have been shown to detect two to three orders of magnitude lower frequencies” (pg. 10). There was no teaching or

suggestion in Forsheew about detecting an SNV mutation having a variant allele frequency of less than or equal to 0.015% in the cell-free DNA sample, and there was no teaching or suggestion in Forsheew that such detection limit could be achieved by the TAM-Seq method disclosed therein.

The Office cites Diehl for allegedly showing a sensitivity of as low as 0.001% in detection of colorectal cancer DNA. Diehl, however, performs neither targeted multiplex amplification of 10 to 200 target loci in the same reaction nor high-throughput sequencing of the amplicons. Instead, Diehl relies on bead-based digital PCR in emulsions (BEAMing) to amplify in a single target locus to detect adenomatous polyposis coli (APC) mutations. Because the workflow of BEAMing is completely different from the workflow of TAM-Seq, the sensitivity of BEAMing reported by Diehl is not applicable to the Forsheew's TAM-Seq method.

Moreover, Forsheew already considered Diehl's BEAMing method and pointed out its limitation in detecting cancer mutations outside of well-defined hotspot regions (see Forsheew, p.9). There is no teaching or suggestion in the cited references about whether or how the detection limit of Forsheew's TAM-Seq method could be improved by Diehl's BEAMing method.

Furthermore, Forsheew's TAM-Seq process was rationally and deliberately designed to rely on single-plex, "*individual amplification to purify and select for intended targets*" (pg. 3). In fact, Forsheew had considered but dismissed multiplex targeted amplification – "*Multiplex amplification using a large set of primers could result in nonspecific amplification products and biased coverage*" (Fig. 1B, pg. 3). Forsheew deliberately resorted to parallel single-plex amplification on Access Array, which physically separate individual primer pairs and individual reaction volumes to avoid issues such as nonspecific amplification and primer dimer formation. There was no reason or motivation for one of ordinary skill in the art to alter Forsheew's TAM-Seq process by substituting a step rationally and deliberately designed by Forsheew for purpose of addressing the issue of nonspecific amplification, for a step already considered but dismissed by Forsheew.

Benesova is cited for allegedly teaching identification of a tumor-specific mutation in a primary tumor. Marusky is cited for allegedly teaching analysis of clonal heterogeneity. Anderson is cited for allegedly teaching a 95-plex amplification using low primer concentrations. None of Benesova, Marusky, and Anderson could cure the aforementioned deficiencies of

Forsheew and Diehl. Accordingly, so far as the combined disclosure of the cited references still fails to meet each and every limitation of the claimed invention, the *prima facie* case of obviousness cannot be sustained. Reconsideration and withdrawal of the subject rejection are therefore respectfully requested.

### **Double Patenting**

Claim 1 is rejected on the ground of nonstatutory double patenting for allegedly being unpatentable over claims of U.S. Pat. No. 11,319,596. Claim 1 is rejected on the ground of nonstatutory double patenting for allegedly being unpatentable over claims of U.S. Pat. No. 11,371,100. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting for allegedly being unpatentable over claims of U.S. Appl. No. 16/288,416. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting for allegedly being unpatentable over claims of U.S. Appl. No. 16/359,917.

Applicant respectfully traverses these rejections. For the sole purpose of expediting prosecution and without acquiescing to the rejections, Applicant submits herewith a terminal disclaimer over U.S. Pat. Nos. 11,319,596 and 11,371,100, and U.S. Appl. Nos. 16/288,416 and 16/359,917, to overcome the subject rejections.

Claim 1 is also provisionally rejected on the ground of nonstatutory double patenting for allegedly being unpatentable over claims of U.S. Appl. No. 17/018,966. Applicant respectfully traverses this rejection. For the sole purpose of expediting prosecution and without acquiescing to the rejections, Applicant has amended the claims of U.S. Appl. No. 17/018,966 to be patentably distinct from the pending claims of the instant application, thereby resolving the issue.

Claims 1-15 are provisionally rejected on the ground of statutory double patenting for allegedly claiming the same invention as claims 1-15 of U.S. Appl. No. 17/692,469. Applicant respectfully traverses this rejection. For the sole purpose of expediting prosecution and without acquiescing to the rejections, Applicant has amended the claims of U.S. Appl. No. 17/692,469 to claim a different invention. In addition, Applicant submits herewith a terminal disclaimer over U.S. Appl. No. 17/692,469 to expedite prosecution.

For at least these reasons, Applicant respectfully requests reconsideration and withdrawal of the subject rejections.

**Concluding Remarks**

Applicant believes that the application is in condition for allowance. Favorable reconsideration is respectfully requested. The Examiner is invited to contact the undersigned to advance prosecution.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-6075. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extension fees to Deposit Account No. 50-6075.

Respectfully submitted,

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