EUROPEAN PATENT 2,893,040
GUARDANT HEALTH, INC.
RESPONSE TO NOTICES OF OPPOSITION May 2020

1. INTRODUCTION and REQUESTS

1.1. This document provides the patentee's response to the notices of opposition which have been filed against European patent 2893040 by: (O1) Personal Genome Diagnostics, Inc.; (O2) Strawman Limited; (O3) Grünecker Patent- und Rechtsanwälte PartG mbB; and (O4) Foundation Medicine Inc.

1.2. The EPO has already prepared a consolidated document list, going up to D37. The patentee in addition cites D38-D43 (see section 7 on page 21).

1.3. The patentee requests that the patent should be upheld under Article 101(3) EPC on the basis of the enclosed main request (MR), in which claim 1 has been amended to specify that the tagging is achieved using enzymatic ligation. If the opposition division cannot grant this main request, the patent should be upheld in amended form on the basis of the enclosed auxiliary requests (ARs). The claim requests and amendments are described in more detail in section 6 below.

1.4. Prior to any decision which is contrary to the main request, the patentee requests oral proceedings under Article 116 EPC.

2. THE MAIN REQUEST HAS PROPER BASIS IN THE ORIGINAL APPLICATION

2.1. Granted claim 1 finds basis in independent PCT claim 78 combined with its dependent claims 82, 87 & 91, plus two features from [0084] of the description: (i) the 100-100,000 haploid genome equivalent quantity of cell-free DNA ('cfDNA'); and (ii) the requirement for between 2 and 1,000,000 unique identifiers.

The combination of features from the PCT claims and [0084] does not add matter

2.2. The opponents argue that granted claim 1 goes beyond the original disclosure because [0084] of the description relates to an embodiment which is different from claim 78, and thus that the claim is a hybrid of two embodiments which were originally disclosed as separate items. On the contrary, the claim simply combines upstream preparative steps with downstream processing steps, and a skilled reader would clearly and unambiguously derive this combination from the original disclosure.

2.3. PCT claim 78 starts with a step of *"providing at least one set of tagged parent polynucleotides"*, followed by a step of processing and sequencing the tagged parent polynucleotides, but provides no detail about how these tagged parent polynucleotides are obtained. Conversely, [0084] discloses a method of tagging polynucleotides (cfDNA) but does not mention what might be done with the resulting tagged parent polynucleotides. A skilled person would understand the connection between upstream preparative steps and downstream processing steps, and there is no new matter in making this combination.

2.4. In short, [0084] is a clear disclosure of how to prepare a set of tagged polynucleotides, and claim 78 is a clear disclosure of what to do with tagged polynucleotides, so the two passages can naturally be combined. Moreover, it was clear from claim 82 that the method of claim 78 can include upstream preparative steps. This is not a situation where the patentee has used

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the application as a reservoir in order to make an artificial combination of features (*Guidelines* H-V, 3.2.1). All the features of an upstream process ([0084]), have been combined with all the features of a downstream process (claim 78). Granted claim 1 was not the result of picking and choosing different features from various passages, nor was it the result of creating an intermediate generalisation.

2.5. When assessing combinations of features under Article 123(2) EPC, T 296/96 held that *"the relevant question is whether a skilled person would seriously contemplate combining the different features"* (*e.g.* see CLBA, pages 459-60). Combining compatible upstream and downstream steps clearly meets that test, so granted claim 1 did not add matter.

2.6. The main request further requires that tagging is achieved using enzymatic ligation. This feature finds basis in [00238] on page 51 of the description, which clearly and unambiguously explains how tags should be attached to cfDNA molecules (as already required in granted claim 1) prior to sequencing (again, as already required in granted claim 1). Similarly, [0246] discloses the use of ligases for attaching *"a plurality of unique identifier barcodes"*. Ligation of tags prior to PCR is also mentioned in [00240] and [00231]. Furthermore, ligation of tags is also specified in [00243], 3rd sentence, specifically in the context of methods which combine information from the tag with endogenous information from the cfDNA itself (*i.e.* from the beginning region, the end region, and/or its length). Ligation was also used in Example 6 *e.g.* see [00368], in a context where *"initial genetic material is converted into a set of tagged parent polynucleotides" i.e.* precisely as specified in granted claim 1.

2.7. The combination of features in claim 1 therefore has direct and unambiguous basis in the PCT application, and does not provide the skilled person with any technical information which they would not already have derived from the original disclosure.

<u>The deletion of "including a proper subset" does not add matter</u>

2.8. O4 argues that the deletion of claim 78's parenthetical reference to *"including a proper subset"* creates new matter, but this wording was redundant, so its deletion cannot add matter.

2.9. This reference to "*a proper subset*" in claim 78 was merely to ensure that the term *"subset"* would not be given only in its everyday meaning, but would also be given its mathematical meaning. Whereas in everyday usage a *"subset"* excludes the complete set, this is incorrect in a formal mathematical sense (where a *"subset"* can indeed be the complete set). The everyday meaning of the term *"subset"* corresponds to the mathematical term *"proper subset"* (meaning that at least one member of the original set must be absent), so the original claim wording merely ensured that the term "*subset*" was given its broad meaning. Claim 1 still has this meaning – the claims cover embodiments where the *"subset"* is all of the amplified progeny or only part of the amplified progeny, so there has been no change in meaning here. The term "*subset*" still has its original (and correct) meaning, so no matter has been added.

<u>The claims require that cfDNA molecules are tagged</u>

2.10. O2 argues that granted claim 1 includes new matter because, unlike [0084], it allegedly does not require that the tags are applied to the cfDNA polynucleotides in a sample, but could instead be read as permitting tagging of any other genetic material in the sample. This is not the case. Step (a) of claim 1 requires the generation of tagged parent polynucleotides. The only non-tagged polynucleotides referred to in step (a) are the cfDNA polynucleotides. Accordingly, it is clear that the "*tagged parent polynucleotides*" in (a) are tagged parent <u>cfDNA</u> polynucleotides. Therefore, the tagging in the first sentence of [0084] and in step (a) are the

same, as they both tag between 100 and 100,000 haploid human genome equivalents of cfDNA with between 2 and 1,000,000 unique identifiers. There is thus no added matter.

The dependent claims also have proper basis

2.11. O2 objected to granted claim 5, but this claim has direct basis in PCT claim 83, which was dependent on PCT claim 82 (which is incorporated into granted claim 1).

2.12. O3 objected to claim 10 for omitting *"wherein the at least one set is a plurality of sets"* relative to PCT claim 110, but this claim requires *"a plurality of sets"* so the omission does not add matter.

2.13. O1 & O4 objected to claim 17, alleging that there is no basis for combining the recited filtering step with the method of claim 1, but this claim finds basis in [0022] and [00101].

2.14. O1, O2 & O4 objected to claim 18, these arguments are no different from the basic argument made against claim 1 *i.e.* that the tagging method of [0084] cannot be combined with features recited in the original claims. For the same reasons given above, these objections must fail.

2.15. O2 also argued that the ranges in claim 18 result from the selection of multiple lists, the first being the lower boundary and the second being the upper boundary. The relevant passage in [0084] recites:

> *In certain embodiments, the number of unique identifiers is at least 3, at least 5, at least 10, at least 15 or at least 25 and at most 100, at most 1000 or at most 10,000.*

2.16. Each option in claim 17, however, includes at least one end point (10 or 10,000), with each option representing a sub-range. In line with T 2/81, a restriction to such sub-ranges does not represent new subject matter.

3. THE CLAIMS ARE NOVEL

3.1. O1, O3 & O4 (but not O2) have argued that the granted claims lack novelty over D11, D14, and D16. They also argued that the claims are not entitled to any of the priority dates, but D11, D14 and D16 are prior art for novelty regardless of priority entitlement. For the present opposition proceedings the patentee can rely only on the filing date (4th September 2013) and an analysis of priority is therefore unnecessary.

D11 and D16 do not disclose the analysis of cfDNA

3.2. D11 and D16 are very similar, and derive from the same research group, so they will be dealt with together. Neither of these documents describes the analysis of cfDNA and the claims are therefore novel for this reason alone.

3.3. O3 & O4 have argued that there is an implicit disclosure of cfDNA in D11 & D16, citing (i) page 9530, left column, first paragraph of D11 and (ii) paragraph [03] of D16. These two passages contain precisely the same statement within a longer discussion of how DNA detection has been used in the prior art:

> *Detection of donor DNA in the blood of organ transplant patients is an important indicator of graft rejection and detection of fetal DNA in maternal plasma can be used for prenatal diagnosis in a noninvasive fashion (7, 8).*

3.4. This introductory passage does not disclose that the analytical methods disclosed in D11/D16 should be applied to cfDNA. Nor is there any disclosure here that the methods should be used in any of the other settings also mentioned in the same paragraph *e.g.* to assess the rate of human evolution, or to assess drug resistance in AIDS or hepatitis.

3.5. All four opponents cited D11, but O1 & O2 correctly recognised that the passing reference to prior art methods of *"detection of fetal DNA in maternal plasma"* in the document's introduction did not disclose to the skilled person that the D11 method should be used for analysing cfDNA. The claims are novel for this reason alone, so it is not necessary to consider the further distinctions between claim 1 and D11/D16.

D14 does not disclose the generation of a consensus sequence

3.6. O1 has alleged that granted claim 1 lacks novelty over D14, but this document is concerned solely with counting molecules (*e.g.* page 1, lines 5-7) and does not involve the generation of a consensus sequence, as required by step (d) of claim 1.

3.7. In relation to step (d), O1 has referred to various passages which refer to a *"sequence of interest"* or a *"sequence to be determined"* (see page 9 of its arguments), but none of these passages matches step (d). The mere mention of a "*sequence*" in D14 does not mean that a consensus sequence was generated. Indeed, there is no reason why the D14 method would generate a consensus sequence because it is not concerned with error correction, which is the purpose of the consensus step (*cf.* patent: page 10, lines 8-10).

3.8. Furthermore, the only section of D14 which discloses analysis of cfDNA is Example 4, but the methods performed in this example do not match the steps of claim 1 of the main request. O1 imports these features from unrelated parts of the main description of D14, but did not explain why these features should be read into Example 4. For instance, O1 has referred to the second paragraph on page 7 as disclosing the use of 2, 3, 4, *etc.* labels, but it does not explain why the skilled person would import this disclosure into Example 4, rather than the disclosure later in the same paragraph to use 10^7, 10^8, or even up to 10^{18} labels.

3.9. For at least these reasons, the main request is novel over D14.

4. THERE WAS NO OBVIOUS ROUTE FROM THE PRIOR ART TO THE CLAIMS

Claim interpretation

4.1. To argue that claim 1 is obvious, O3 forces a nonsensical interpretation of part (d) of the method of claim 1. This step involves:

> *... collapsing the set of sequencing reads to generate a set of consensus sequences, each consensus sequence corresponding to a unique polynucleotide among the set of tagged parent polynucleotides, wherein the generation of consensus sequences is based on information from the tag and at least one of*
>
> *(i) sequence information at the beginning region of the sequence read*
>
> *(ii) sequence information at the end region of the sequence read, and*
>
> *(iii) the length of the sequence read.*

4.2. Thus step (d) combines the information from the tags (which form the unique identifiers) with endogenous information from the cfDNA itself (from the beginning region, the end region, and/or its length) to group the sequences and collapse them into a consensus sequence, with each consensus sequence corresponding to an individual cfDNA molecule. This approach of using endogenous information, rather than relying solely on the tags, means that the claimed method can achieve molecular tracking using many fewer tags.

4.3. This concept is explained in [0109] of the patent:

plurality. In this example, barcodes may be ligated to individual molecules such that the combination of the bar code and the sequence it may be ligated to creates a unique sequence that may be individually tracked. As described herein, detection of non unique barcodes in combination with sequence data of beginning (start) and end (stop) portions of sequence reads may allow assignment of a unique identity to a particular molecule. The length, or number of base pairs, of an individual sequence read may also be used to assign a unique identity to such a molecule. As described herein, fragments from a single strand of nucleic acid having been assigned a unique identity, may thereby permit subsequent identification of fragments from the parent strand. In this way the polynucleotides in the sample can be uniquely or

4.4. O3 argues, however, that the claim does not require the use of such endogenous information (see 3.2.6.1 of its arguments). Instead, it alleges that the claim extends to embodiments wherein the beginning region, the end region, or the length of the sequencing read does not need to include the endogenous information from the original cfDNA molecule, but instead can relate to the sequence information of the tag itself. This interpretation is illogical and clearly the result of a mind desirous of misunderstanding.

4.5. Step (d) requires that the consensus sequences are generated using *"information from the tag and at least one of"* the endogenous molecule's properties, and O3's interpretation ignores the word "and". Moreover, under Article 69 EPC the skilled person would consult the description to understand step (d), and O3's interpretation is also contrary to the patent's explanation in [0109] (quoted above) and in [0113]-[0116]:

- [0113] outlines the probability of cfDNA polynucleotides from different genomes having the same start and stop positions*:

  [0113] In a sample comprising cell-free DNA (cfDNA) from a plurality of genomes, there is some likelihood that more than one polynucleotide from different genomes will have the same start and stop positions ("duplicates" or "cognates"). The probable number of duplicates beginning at any position is a function of the number of haploid genome equivalents in a sample and the distribution of fragment sizes. For example, cfDNA has a peak of fragments at about 160 nucleotides, and most of the fragments in this peak range from about 140 nucleotides to 180 nucleotides. Accordingly, cfDNA from a genome of about 3 billion bases (e.g., the human genome) may be comprised of almost 20 million ($2x10^7$) polynucleotide fragments. A sample of about 30 ng DNA can contain about 10,000 haploid human genome equivalents. (Similarly, a sample of about 100 ng of DNA can contain about 30,000 haploid human genome equivalents.) A sample containing about 10,000 (10^4) haploid genome equivalents of such DNA can have about 200 billion ($2x10^{11}$) individual polynucleotide molecules. It has been empirically determined that in a sample of about 10,000 haploid genome equivalents of human DNA, there are about 3 duplicate polynucleotides beginning at any given position. Thus, such a collection can contain

- [0114] expands on this and explains the probability of correctly identifying the parent polynucleotide based on the endogenous information in combination with the use of different numbers of tags. With only one tag (*i.e.* no differential tagging) and 1000 haploid genome equivalents the table outlines there is a 96.9643% chance of correctly identifying the molecule as unique. Contrary to O3's interpretation of claim 1, this analysis must include endogenous information, because using sequence information from the tag alone would mean that the probability of correctly identifying the molecule as unique would be zero – all of the molecules contain the same tag.

- [0115] then emphasises that in order to determine which sequence reads are derived from which parent molecules, all that is needed is that the molecules are tagged with *"a sufficient number of unique identifiers (e.g., the tag count) such that there is a likelihood that two duplicate molecules, i.e., molecules having the same start and stop positions, bear different unique identifiers so that sequence reads are traceable back to particular parent molecules."* This approach is contrasted to methods which rely only on the tag sequence, which would require the user to *"uniquely tag every, or nearly every, different parent molecule in the sample"* (*e.g.* as in D3), outlining that this would require a huge number of tags.

- [0116] then outlines various potential disadvantages of this prior art approach.

* The terms "start and stop positions" are used synonymously with the beginning and end regions (e.g. patent: [0011]).

4.6. Accordingly, the claim's use of the word "and", in combination with the patent's description, shows that step (e) generates the consensus sequences using a combination of sequence information from the tag and the endogenous cfDNA. Indeed this is the interpretation taken by O1, O2 and O4, and is the only sensible interpretation.

Context of the invention

4.7. Many of the opponents' objections amount to little more than an argument that it would be obvious to apply various prior art methods to cfDNA. However, their broad brush arguments do not take into account the real-world nature of cfDNA, and specifically the key considerations of the skilled person when working in this field. To provide an insight into the challenges of working with tumor-derived cfDNA, the patentee refers to a declaration from Prof. Jay Shendure (D38). Prof. Shendure is the inventor of D22, and is an expert involved in researching and developing next generation cancer diagnostics techniques using cfDNA (D38: paragraph 17). Unlike the opponents, Prof. Shendure understands the practical challenges of working with cfDNA, and explains why the skilled person would have viewed the main cited prior art as being unsuitable for use with this analyte.

4.8. The invention finds particular utility in analysing DNA from tumors, so that somatic mutations can be detected by sequencing tumor-derived cfDNA rather than needing to sample DNA directly from cells in the tumor. In a cfDNA sample, however, the vast majority of cfDNA will be derived from non-tumor cells because these are much more abundant than tumor cells, and their cfDNA is released as a result of the everyday cellular turnover. Moreover, even within the tumor-derived cfDNA, most of the fragments will not contain mutations (because most of the genome is not mutated). Thus the technical challenge is to identify those few cfDNA molecules which are derived from tumor cells and which contain a mutation, in a population wherein they are massively outnumbered – the proverbial needle in the haystack, which creates a high risk of false negatives.

4.9. To add to this challenge, the error rate in typical sequencing techniques is higher than the mutation rate underlying tumorigenesis, so the level of noise (polymerase errors, *etc.*) will typically be much higher than the level of signal (true mutations). As noted in D3 (p.14510, top-left), *">99.9% of the apparent mutations identified by standard sequencing are erroneous"* (also outlined in [0072] to [0074] of the patent). Thus there is also a very high risk of false positives.

4.10. Accordingly, when analysing cfDNA for true mutations, the assay must be (i) **sensitive** enough to ensure that those few true mutations are not lost in the processing steps, and (ii) **specific** enough to be able to distinguish these true mutations from the errors introduced in the processing steps. To achieve the sensitivity required, the method therefore aims to **reduce data loss** at every available step of the workflow, including in both the wet-lab processing steps and in the bioinformatics analysis; the required specificity is achieved by trackable tags, which are applied to original cfDNA molecules, and these tagged molecules are then amplified and sequenced. The sequence information is then traced back to original cfDNA molecules by using a combination of the tags and endogenous information from the cfDNA molecule itself. This combination of steps permits low-abundance cfDNA molecules to be reliably sequenced, avoiding the noise which arises from amplification and sequencing techniques.

4.11. Furthermore, the claimed invention works in the real-world, and forms the basis of the patentee's Guardant360® assay, which to date has benefitted around 100,000 cancer

patients. This assay achieves 100% analytical sensitivity and 100% analytical specificity in detecting indel mutations at a frequency as low as 0.20% (see Table on page 1 of the Guardant360® specification sheet, submitted as D39). Notably, a study comparing the Guardant360® assay to standard tissue biopsies demonstrated that the claimed method "*rescued 85 patients from a potentially missed genomic diagnosis*" (D40: page 2, third paragraph). Accordingly, the sensitivity and specificity of the claimed method has real-world benefits in the accurate detection of tumor variants in cfDNA from cancer patients.

D3 or D11/D16 could be considered the closest prior art

4.12. The opponents have presented attacks starting from four documents as the closest prior art: D3, D11, D16 and D18. As discussed above, D11 and D16 are essentially the same, so there are in effect three choices of potential starting point: D3, D11/D16, or D18.

4.13. The claimed method involves tagging, amplifying and sequencing of cfDNA, and then collapsing the resulting sequencing reads to generate a set of consensus sequences which each correspond to an original cfDNA molecule. The overall purpose of the method is to permit reliable detection of low-level mutations while avoiding false positives which are introduced due to the relatively high error rate of DNA amplification and sequencing.

4.14. D3 and D11/D16 relate to this same general purpose as they are concerned with the use of unique identifiers/tags for error correction and rare mutation detection (D3: page 14509 and Fig. 1; D11: title and abstract; D16: [0019]).

4.15. In contrast, D18 is directed to a different purpose, namely counting DNA molecules. This point is already clear from its title: *"Counting absolute number of molecules using unique molecular identifiers"*. Thus D18 cannot qualify as the closest prior art, and D3 or D11/D16 would be the most appropriate choice of the closest prior art. D3 was indeed taken as the closest prior art during examination.

4.16. Regardless of the starting point, however, there is no obvious combination of prior art disclosures which would have led the skilled person to a method which uses low-complexity tagging in a method for determining true mutations in cfDNA. Rather, when the prior art was looking at true mutations it typically used high-complexity tags (*e.g.* degenerate 24-mers providing 4^{24} tags), whereas the normal purpose for low-complexity tags was in molecular counting or for sample multiplexing.

4.17. Moreover, the prior art had not applied these techniques to cfDNA, and neither D3 nor D11/D16 had appreciated the challenges which are presented when working with this analyte. Rather, this prior art typically aimed to obtain randomly-sheared DNA fragments which are quite different from cfDNA molecules that arise naturally *in vivo*. cfDNA molecules are generated by enzymatic digestion of nucleosomal structures during cell-death, and this process means that cfDNA fragments do not have a random structure. Rather, their lengths vary periodically (rather than continuously), and the structural differences between cfDNA and randomly-sheared genomic DNA are shown in Figure 4 of D6 – the upper panel shows that cfDNA molecules show peaks & troughs in length, whereas the lower panel shows that randomly-sheared DNA varies continuously, with a central peak. Therefore cfDNA molecules and randomly-sheared DNA fragments are not analogous.

4.18. Thus cfDNA shows a fragmentation pattern which is not seen when random shearing is used, and the end points of cfDNA molecules are not evenly distributed. Compared to randomly-sheared fragments, therefore, cfDNA molecules show lower sequence diversity at

their termini. Furthermore, very few cfDNA molecules are longer than 200 bp (see Figure 3 of D6). This patterned nature and narrow size-range also means that each cfDNA derived from a single haploid genome is essentially unique, because chromosomal sequences typically do not include long repeated sequences, and where such repeats occur their periodicity does not match the periodicity of cfDNA fragmentation. Conversely, two haploid genomes will give rise to very similar cfDNA fragments as each other so, as the number of haploid genome equivalents increases, the chance of seeing duplicate cfDNA molecules also increases (see patent: [0113]).

4.19. A key aspect of the invention was the realisation that the patterns seen in cfDNA can be exploited to assist in molecular tracking. In particular, it means that this goal can be achieved by using low-complexity tagging in combination with endogenous information contained within the natural fragmentation of cfDNA.

The main request would not have been obvious starting from D3

4.20. D3 was already dealt with in detail during examination *e.g.* see the applicant's letter of 20th June 2018 (D34). The opponents have not provided anything which should change the examining division's conclusion *i.e.* that the claims are inventive over D3.

4.21. The method used in D3 involves uniquely tagging sonically-sheared genomic DNA (top of left hand column on p.14510). D3 achieves this by using a number of distinct tags which exceeds the number of DNA fragments in the sample, and it attaches these to the 5' and 3' ends of the fragmented DNA. Thus there is close to zero probability that any two cfDNA molecules will receive the same tag pair, so each molecule is uniquely tagged.

4.22. The tags in D3 include a 12 nt degenerate sequence within adapters which are ligated to both ends of sonicated DNA fragments, thereby providing *"up to 4^{24} = 2.8 x 10^{14} distinct tag sequences"* (p.14512, right-hand side). The 'Supporting Information' for D3 (D5) explains that the method was used with 750 ng of T-tailed DNA fragments, and that these fragments were 200-500 bp long. A single base pair has an average molecular weight of 618 Da, so 750 ng of these fragments represents somewhere between 1.5-3.6 x 10^{12} molecules (depending on whether the length distribution was more towards 200 bp or 500 bp). There are thus around 100 times more unique identifiers (2.8 x 10^{14}) than the number of fragments being tagged (1.5-3.6 x 10^{12} molecules), ultimately resulting in the vast majority fragments being uniquely tagged. This excess of unique identifiers is an essential feature of the D3 method.

4.23. After sequencing the tagged fragments, a consensus sequence is then generated for the original fragments by using the unique pair of tags to group sequence reads into families, where each family corresponds to a single original DNA fragment (see paragraph spanning left and right hand columns of p.14509 in D3).

Claim 1 differs in at least four aspects from D3

4.24. The claim 1 of the MR differs from D3 in at least four aspects:

- it involves analysis of cfDNA;
- it requires the use of $\leq$1 million unique identifiers;
- the number of unique identifiers is lower than the number of analyte DNA fragments; and
- the collapsing step uses a combination of sequence information from the tag and the endogenous information contained within the analyte DNA.

4.25. O2 & O3 have alleged the D3 discloses the analysis of cfDNA, relying on the mention of *"nucleic acid-based serum biomarkers"* in the introduction (p.14508, first paragraph), but this passage merely mentions various contexts in which deep sequencing had been used in the prior art. There is no disclosure that the methodology of D3 should be applied to this sort of DNA sample, and this point is confirmed in the declaration from Prof. Shendure (D38: paragraph 16). The only DNA molecules which are disclosed in D3 are sonically-sheared random fragments of either (i) the 7.2 kbp M13mp2 vector or (ii) the 16.6 kbp human mitochondrial genome.

4.26. O4 refers to the passage on p.14512, which refers to combining the use of the shear points of sheared genomic DNA with the tag sequence, and argues that the reference to *"a shorter tag"* in this passage implies the potential to use only 2 tags. This submission is nonsense, and this passage in D3 discloses only that shorter tags could be used, without implying any particular number of distinct tags.

4.27. Similarly, O1, O2 & O4 have alleged that this passage in D3 discloses a collapsing step which involves combining the exogenous tag sequence with endogenous information, but this is not the case. The cited passage refers only to the potential use of *"information regarding the shear points of DNA"* to permit the use of shorter tags, but the context of this passage is seen in the first sentence of the paragraph, which refers to *"using the randomly sheared ends of the DNA fragments as unique identifiers"*. As noted above, cfDNA does not have *"randomly sheared ends"*, and the end-points of cfDNA fragments are not randomly or evenly distributed. This passage in D3 is therefore not the same as the claimed method's requirement that the consensus sequence is generated *"based on information from the tag and at least one of (i) sequence information at the beginning region of the sequence read (ii) sequence information at the end region of the sequence read, and (iii) the length of the sequence read."*

The technical effect of these differences

4.28. The technical effect arising from these differences is that the claimed method can achieve efficient molecular tracking of sequence reads derived from cfDNA by using many fewer tags than the prior art.

4.29. Looking first at the type of DNA analyte, D3 looked only at fragments artificially derived from short test sequences, namely the M13mp2 vector (7.2 kbp) or the human mitochondrial genome (16.6 kbp). In contrast, the claim looks at cfDNA fragments, and a single haploid human genome (3.3 Gbp) will provide about 20 million cfDNA molecules (see [0113] of patent). The analyte specific in claim 1 is therefore more complex than used in the prior art, and can provide useful real-world information (rather than merely being a test sample).

4.30. The technical effect of the remaining differences can be considered together. The method of claim 1 uses a minimum of 100 haploid genome equivalents of cfDNA, which will provide around 2×10^9 cfDNA fragments (see [0113] of the patent for this conversion factor). With a maximum of 10^6 distinct unique identifiers, the method uses an excess of analyte molecules of at least 10^3-fold. In other words, there will be at least 1,000 analyte molecules per distinct tag sequence, meaning that the vast majority of analyte molecules will be tagged in the same way as another tagged analyte molecule. Compared to D3, therefore, the ratio is not merely reversed (to use an excess of analyte), but differs by at least 10^5-fold.

4.31. Despite this complete reversal in the analyte/tag ratio, the invention out-performs the prior art by using endogenous information in step (d). The use of endogenous information means that the invention can achieve a high probability of correctly identifying an original cfDNA molecule

even when using dramatically fewer tags than D3 (see [0114] of the patent). This is demonstrated by the method of claim 1, which uses a maximum of 1,000,000 unique identifiers, whereas D3 requires 2.8×10^{14} unique identifiers. This reduction in the number of unique identifiers leads to several advantages, including:

- The tag sequences can be much shorter, which means that they (i) are less complex and cheaper to make, (ii) consume less of each sequencing read, and (iii) are inherently more reliable. As noted above, the invention can achieve the same efficiency using 4 nt of tag sequence as the prior art achieves using 23 nt. This means that the invention provides a cost advantage because 1 mole of 4 nt oligos is much cheaper to produce than 1 mole of 23 nt oligos. Generating trillions of different tags is both cumbersome and expensive, as noted in [0115] and [0116] of the patent. In addition, 4 nt of tag sequence consumes a much lower portion of each sequencing read in typical short-read sequencing technologies. Thus the tags take up less overhead in the sequencing step. Even the upper limit of 1,000,000 different tags in claim 1 can be achieved using only 10 nt (4^{10}=1,048,576), which permits an extra 14nt per read. Furthermore, the abstract of D3 reports that next-generation sequencing has an inherent error rate of ~1%, so the 24mer tags used in D3 mean that around ¼ of sequence reads will have an error in the tag, meaning that these molecules will not be grouped into the correct family and thus will be discarded *i.e.* a loss of ~25% of useful data due solely to the length of the tags.
- The use of fewer tags means that they can be designed. D3 relies on degenerate tags in which each nucleotide position is represented by a mixture of A, C, G & T to provide 4^n different tag sequences for a length of n nucleotides. This approach provides maximum diversity for a given length, but the tag sequences can behave with different efficiencies, and they can be easily misidentified if a sequencing error occurs. In contrast, the invention means that tag sequences can be designed. For instance, they can be designed to increase the Hamming distance between the tags, so that they will not be easily confused (and, moreover, that sequencing errors which occur within the tag portion can be corrected, unlike D3). Similarly, the tag sequences can be designed to avoid long stretches of the same nucleotide (*e.g.* to avoid GGGGGG) which could otherwise lead to sequencing mis-reads. Also, sequences can be designed so that they do not interfere with each other (*e.g.* to avoid hybridisation between complementary tags). Furthermore, they can be designed so that they behave in similar ways to each other *e.g.* so that they all display similar efficiencies with any chosen ligase of interest. Overall, therefore, there are several benefits in avoiding the need to use 4^n degenerate tag sequences.

4.32. The **objective technical problem** *vis-à-vis* D3 can therefore be considered as the provision of an improved tag-based method of detecting rare mutations in a bodily sample, in particular by improving accuracy and decreasing both complexity and cost.

4.33. The skilled person seeking a solution to this problem would not have found an obvious route from D3 to the claimed method because, at least because:

- it would not have been obvious to use the D3 method with cfDNA molecules; and
- it would not have been obvious to reverse the excess of distinct unique identifiers relative to the number of analyte molecules, which is required by the D3 method.

The skilled person would not have applied the method of D3 to cfDNA

4.34. Several aspects of the D3 method mean that it would *a priori* be seen as unsuitable for use with cfDNA. Thus, as outlined by Prof. Shendure, the skilled person would not obviously have modified D3 to use cfDNA as the analyte (*e.g.* D38: paragraph 33). Evidence that this view persisted even after the filing date of the application can be found in Perakis *et. al.* 2017 (D41), which outlines the applicability of the method of D3 ("Schmitt") to cfDNA (see middle of page 30):

> erroneously called variants only by approximately 20-fold. Schmitt *et al.* further developed this strategy for the preparation of tagged duplex shotgun libraries in order to correct errors that occur in the first round of amplification and are propagated to subsequent copies [180]. After ligating the adapters harboring degenerate molecular tags, the individually labeled strands are PCR-amplified to create sequence families that share the same tag sequences derived from each of the two single parental strands, leading to a >10,000-fold improvement compared with conventional NGS
>
> [181,182]. Although the same group used this approach for selective enrichment of small genomic regions, the original protocol has not yet been used with ctDNA [181]. Prospects of success are limited since the method is relatively inefficient when limited amounts of input DNA—as it is most likely the case for cfDNA—are used [182]. Newman *et. al.* combined both

4.35. The reasons for this view are clear. The method of D3 prepares DNA analytes by shearing with the *"Covaris AFA system"*, which uses ultrasonication (see *'Sequencing Library Preparation'* on page 1 of D5). Subjecting cfDNA to such acoustic shearing techniques would destroy most of the DNA and further exacerbate the problems caused by having low concentrations of analyte. Sonication is an essential and deliberate step of the D3 method, and it is only with hindsight that the opponents are able to argue that it was obvious to exclude this artificial step, and instead to analyse DNA fragments which arise naturally in the body.

4.36. In addition, after sonication D3 selects a fraction of fragments which would exclude almost all cfDNA. D3 focuses on DNA fragments within *"the optimal range of ~200-500 bp"* (D5, sentence bridging the two columns on page 1). Fragments longer than 500 bp are discarded, and then remaining fragments longer than 200 bp are retained. The peak size of cfDNA, however, is ~160 nucleotides (see [0113] of patent), so the D3 method would discard the large majority of cfDNA. Furthermore, a sonication step would have reduced the size of cfDNA even further, leading to even great loss of original cfDNA molecules. It is only with hindsight that the opponents can argue that it was obvious to analyse molecules which fall well below *"the optimal range"* of the D3 method.

4.37. Furthermore, and as discussed above, the natural cfDNA fragments are not equivalent to randomly-generated DNA fragments. Although p.14512 of D3 refers to *"[c]ombining information regarding the shear points of DNA with the tag sequence"*, the skilled person would realise that the natural termini of cfDNA molecules are not analogous to the *"shear points"* of D3's sonicated fragments (see above). Thus this untested suggestion in D3 would not be obviously transferrable to cfDNA (see D38: paragraphs 32 and 33).

4.38. The importance of the shearing step in this proposed *"hybrid"* method of D3 was outlined by Dr. Stacey Gabriel, who was an expert witness used by O4 in a related *inter partes* review. Dr. Gabriel outlined in her expert report (excerpt submitted as D42) that the proposed "*hybrid*" method in D3 ("Schmitt 2012") *"relies on a combination of barcodes and sheared ends of the*

fragments themselves to uniquely identify the DNA molecules" (D42; paragraph 196). Therefore, according to O4's own expert, the shearing step is a central aspect of D3's proposal. As outlined, above, however, applying such a shearing step to cfDNA would lead to an intolerable loss of analyte.

4.39. Aside from these practical issues, the D3 method would also be seen as unsuitable for use in a 'needle in a haystack' scenario because of its high rate of data loss. As noted in section 4.31 above, the inherent error rate in next-generation sequencing means that the long tags used in D3 lead to around ¼ of sequence reads being discarded. The shearing and selection steps would essentially eliminate any remaining useful data, so the D3 method is effectively useless in analysing cfDNA. This level of data loss can be acceptable for detecting rare mutations in cellular genomic DNA, which is relatively abundant, but the skilled person would understand that this is not tolerable in the context of cfDNA (where a rare mutation is not merely the result of a rare event, but is also rare within the total analyte DNA because of the dominant background of cfDNA from non-mutated cells, see patent: [0240]).

4.40. For all of these reasons it would not have been obvious to apply the D3 method to cfDNA.

It would not have been obvious to reverse D3's excess of distinct adapters

4.41. As outlined above, the D3 method relies on flooding the sample with a large excess of distinct tags relative to the number of analyte molecules so that no two DNA fragments receive the same tags. In contrast, the method of the present patent uses at least 10^3 more analyte molecules relative to distinct unique identifiers, so the situation is reversed. The downstream analytical methods in D3 would fail if multiple fragments receive the same tags (because disparate sequences would be grouped together), so it is essential that the method uses an excess of distinct tags, and it is nonsense to suggest that the skilled person would have found it obvious to abandon this crucial aspect of D3 and make a dramatic change in the ratio of distinct tags to analyte which would prevent the method from working properly.

4.42. The opponents argue that the skilled person would be motivated to reduce the number of distinct tags after reading the right-hand column on p.14512 of D3 which mentions that *"[c]ombining information regarding the shear points of DNA with the tag sequence would allow a shorter tag to be used"*, but they misunderstand and over-stretch this passage. Firstly, the context of this passage is seen in the first sentence of the paragraph, which refers to *"using the randomly sheared ends of the DNA fragments as unique identifiers"*. As noted above, however, cfDNA does not have *"randomly sheared ends"* and so this idea in D3 would already be seen as not compatible with cfDNA analysis. Similarly, the middle of the paragraph cautions that *"this approach will be limited by the finite number of possible shear points that overlap any given DNA position"*, and this warning would apply *a fortiori* with cfDNA where the *"finite number of possible shear points"* is even more limited than in the sonicated DNA used in D3 (due to the non-random periodicity of cfDNA fragmentation – see above). In short, this proposal in D3 requires "randomly sheared ends", but cfDNA cannot provide this (as confirmed by O4's expert, Dr Gabriel; see section 4.38 above).

4.43. Secondly, even if the skilled person would decide to use *"a shorter tag"* and to add *"information regarding the shear points of DNA"*, nothing in D3 suggests that the tags would be shortened so far as to provide as few as 1 million unique identifiers (*e.g.* 4^{10} rather than 4^{24} sequences). D3 is silent about the degree of shortening which would be possible and, even if the skilled person would have modified D3 to use a shorter tag, it is only with hindsight that they would still have shortened them to such an extent that the method would switch from using an excess of distinct tags to an excess of analyte molecules (still less to make the drastic change from a 10^4-fold excess of tags to a 10^3-fold excess of analyte). Indeed, when

D3 suggests using shorter tags it does not state whether the quantity of analyte DNA would be maintained, or would also be reduced – there is no reason to assume that the quantity of DNA fragments should stay the same in these hypothetical embodiments in D3.

4.44. In this regard, several of the opponents note that the right-hand column on page 2 of the 'Supporting Information' (D5) shows 4-mer tags, and argue that D3 thus suggests to reduce the length of tags to this extent. These arguments are a clear misrepresentation of D5's disclosure – the top of this column explicitly states that this passage uses 12-mer tags, but is showing only 4 nucleotides *"for clarity"*:

> **Example: Duplex Sequencing Tag Pairs.** Consider the 4-nucleotide tags below, with flow cell sequences 1 and 2 in the locations marked and dashes representing a ligated DNA fragment. The Duplex Sequencing adapters actually contain 12-nucleotide Duplex Tags. Shorter tags are used here for clarity:
>
> 5′ 1-TAAC————TCCG-2 3′
> 3′ 2-ATTG————AGGC-1 5′.

4.45. Thus there is in fact no suggestion in D3/D5 to use only 4-mer tags. Furthermore, this passage is in no way linked to "*shorter tags*" referred to on p.14512 of D3: it is clear from the preceding section *"Overview of Duplex Sequencing Data Processing"* that the tags alone are being used to group reads (D5: page 2, left hand column, step (vii)), and not by combining information from the shear points.

A combination of D3 with any other document also does not lead to the invention

4.46. As shown above, various aspects of D3 mean that there would have been no practicable way to arrive at the method of the present claims from this starting point – the essentiality of the excess of distinct tags relative to the number of analyte molecules, the reliance on random shearing, the exclusion of DNA fragments <200 bp ("*the optimal range*"), the loss of ¼ of data due to the use of long tags, *etc.*

4.47. The opponents cite various documents which they would combine with D3, but none of these secondary documents can displace these essential parts of D3's disclosure, and none could make the skilled person believe that the method could be sensibly applied to cfDNA. The opponents cite several documents that mention the existence of cfDNA fragments (*e.g.* D6, D9, D10, D17, D27, D35), but this mere disclosure does not provide any reason why any of these documents would be combined with D3 *e.g.* D6 uses tags to detect excess sequence reads from particular chromosomes, in order to detect aneuploidy, so it would make no sense to combine this document with the D3, which aims to detect point mutations.

4.48. Similarly, the opponents cite documents such as D10 to show that it was known to attach barcodes to cfDNA sequences, but this point is irrelevant. The barcodes in D10 were sample identifiers which were included so that that amplicons from multiple samples can be analysed in a single sequencing experiment. Moreover, the barcodes in D10 were attached after PCR amplification was completed (see Fig.1), so they are not at all useful for molecular tracking. This use of multiplex identifiers cannot suggest to use sequence tags in the manner required by claim 1.

4.49. Furthermore, it is not enough for the opponents to merely locate a document which used a number of tags within the 2-1,000,000 range – the opponents must also explain why this document would logically have been combined with D3, and why the skilled person would have focused on this specific aspect of the secondary document despite its *prima facie*

incompatibility with D3's essential requirement for an excess of distinct tags relative to the number of analyte molecules. For instance, O1 cites the use of 960 tag sequences in D7 and D8, but:

- Neither of these documents relates to cfDNA, so a combination of D3 with D7/D8 still does not take the skilled person to a cfDNA assay.
- These two documents both concern molecular counting, and are not concerned with generating a consensus sequence or with eliminating errors which are introduced during amplification or sequencing reactions. Thus there is no clear link between D3 and D7/D8. The D7/D8 method is useful for detecting aneuploidy (*e.g.* Fig. 4 of D8), but not for mutation detection.
- Although D7/D8 disclose the use of 960 tag sequences (which are used in random pairs, to give 921,600 unique identifiers), the cellular DNA analytes were made using *Bam*HI digestion of genomic DNA, which provides around 360,000 fragments per genome equivalent (see [0011], [0113], [0178], [0231], & [0246] of D7; and the abstract and p.9027 of D8), with sizes ranging from 6 bp to 30 Mbp (D7: [0231]). In contrast, cfDNA leads to around 20 million short fragments per genome equivalent. Thus there are 55x more fragments per genome in a cfDNA sample than in D7/D8, so it is pointless to make a simple comparison of the absolute number of tag sequences which would be used in these two situations. The analytes are very different, so the tagging requirements are not comparable.

The same points apply to other documents *e.g.* D23, which uses 12 tags ('DBRs'), but only in the context of molecular counting.

4.50. Therefore none of the opponents' objections provide an obvious route from the method of D3 to the method of claim 1. It would not have been obvious to adapt the D3 method for use with cfDNA analytes; nor would it have been obvious to reduce the number of unique identifiers so that the number of analyte molecules far exceeds the number of distinct unique identifiers, or to incorporate endogenous information from cfDNA fragments into the overall analysis. And still less would it have been obvious that these modifications would lead to any improvements compared to D3.

The main request would not have been obvious starting from D11/D16

4.51. All of the opponents have taken D11/D16 wholesale as the closest prior art, but these two documents each disclose three distinct embodiments. When such distinct embodiments exist within a single document it is necessary to establish which of them is the most likely starting point within the closest prior art document. Importantly, it is not allowable to create an artificial starting point by mixing & matching features from distinct embodiments to create an undisclosed hybrid.

4.52. The three embodiments are as follows:

- The **exogenous UID** embodiment involves the addition of a single unique identifier (UID), which is incorporated into PCR amplicons using two PCR primers, one of which comprises the UID (*e.g.*, D11: Figure 3; D16: [0044]-[0045]; Figure 3).
- The **endogenous UID** embodiment involves the ligation of adaptors (without exogenous UIDs) onto polynucleotides which have been subjected to random shearing (*e.g.*, D11: Figure 2; D16: [0039]-[0043]; Figure 2). No exogenous UID is added in this embodiment.

- The **inverse PCR** embodiment involves an initial step of random shearing, followed by adapter ligation (without exogenous UIDs), circularisation and inverse PCR with gene specific primers. These primers contained 12 different index sequences (D11; page 1 of Supporting Information, left hand column; Figure S1; D16: [0055]-[0056]; Figure 5).

4.53. D11/D16 discuss the suitability of each of the three embodiments for the analysis of clinical samples with relatively few template molecules, outlining that the ligation-based methods (*i.e.* the 'endogenous UID' and 'inverse PCR' embodiments) are <u>not suited</u> to such samples (D11: p.9534, passage spanning left and right hand column; D16: paragraph [33], starting on the last line of page 12, copied below):

> However, this approach can only be performed on a specific instrument. Moreover, for many clinical applications, there are relatively few template molecules in the initial sample and evaluation of nearly all of them is required to obtain the requisite sensitivity. The approach described here with exogenously introduced UIDs (Fig. 3) fulfills this requirement by coupling the UID assignment step with a subsequent amplification in which few molecules are lost. <u>Our endogenous UID approaches</u> (Fig. 2 and Fig. 5) and the one described by Travers *et al.* are <u>not ideally suited</u> for this purpose because of the <u>inevitable losses of template molecules during the ligation</u> and other preparative steps.

4.54. This point had also been acknowledged in prior art which had considered D11/D16. For instance, D35 was concerned with *"ultrasensitive measurement of hotspot mutations in tumor DNA in blood"* (see title of D35) and reports that D11/D16 discloses *"an elegant and powerful error reduction strategy that enables highly sensitive quantitation of DNA variants using massively parallel sequencing … However, this approach <u>not designed</u> to analyse multiple amplicons from <u>samples containing limited DNA</u> and was <u>not tested on clinical specimens</u>"* (p.3497, top-right).

4.55. Accordingly, in pursuit of a method for error correction in the analysis of clinical samples with low input amounts (*e.g.* cfDNA), the most realistic starting point would be the 'exogenous UID' embodiment because D11/D16 specifically teaches that this is the method which is most suitable for this purpose. Put differently, D11/D16 teaches away from using ligation-based methods when there are *"relatively few template molecules in the initial sample"*, and therefore these ligation-based methods cannot be the closest prior art for cfDNA analysis. It is futile to consider inventive step from an embodiment which the closest prior art already states will not be useful for achieving the purpose/effect of the invention.

4.56. The differences between claim 1 and the exogenous UID embodiment of D11/D16 are summarised in the table below:

Claim 1	**D11/D16** – exogenous UID embodiment
Analyses cfDNA	Analyses cellular DNA samples (*e.g.* D11: Supporting information, p. 1, bottom of left hand column; D16: [57])
100-100,000 haploid genome equivalents	DNA from ~100,000 cells = 200,000 haploid equivalents (D11: p.9533 right-hand column; D16: [52])

	Mitochondrial DNA from 1,000 cells (D11: p.9533 right-hand column; D16: Example 6)
Uses 2 to 1,000,000 unique identifiers	Uses 12 or 14 base random sequences, generating 16.8 million and 268 million distinct unique identifiers, respectively (D11: p. 9532, left hand column; D16: [57])
Ligates unique identifiers to analyte polynucleotides	Adds unique identifiers via PCR primer (D11: Fig. 3; D16 Fig. 3)

4.57. The technical effect arising from these differences is that the claimed method provides a much more sensitive method, using fewer unique identifiers and a natural cfDNA analyte. These effects were already explained in sections 4.28-4.31 above in relation to D3, except for the effect of introducing the unique identifiers via ligation rather than PCR. This is probably the most significant effect compared to D11/D16 because the error rate of PCR (even with a high fidelity polymerase) dwarfs the mutation rate which is naturally present in cfDNA.

4.58. Thus, when the unique identifiers are introduced at the start of the D11/D16 method, using two rounds of PCR (see Figure 3), the errors (false positive mutations) in the double-stranded PCR amplicons will already negate the main purpose of the invention. By adding unique identifiers via PCR the D11/D16 method adds more noise to the system than the original observable signal. This limitation was already noted in D3, which analysed D11/D16 and reported that *"the majority of mutations seen in normal human genomic DNA by this method potentially still represent technical artifacts"* (p.14508, right-hand column) and that the method is *"susceptible to the same sort of artifactual, largely damage-mediated, first-round PCR errors we observed"* (p.14512, top-left).

4.59. The technical problem *vis-à-vis* the exogenous UID embodiment of D11/D16 can therefore be considered as the provision of an improved tag-based method of detecting rare mutations in a bodily sample, in particular by improving accuracy and decreasing complexity & cost.

4.60. Nothing in the prior art would have prompted the skilled person to modify the exogenous UID embodiment of D11/D16 to arrive at the method of claim 1. The principal reason is of course that the skilled person would need to abandon a core part of the prior art method, namely the method by which the unique identifiers are introduced *i.e.* the PCR primers. The opponents have not given any cogent reason why a skilled person would consider making this change after reading D11/D16, or why any other document would prompt them to introduce the unique identifiers by ligation.

4.61. Furthermore, the exogenous UID embodiment uses far more unique identifiers than the claimed method. The left-hand column on p.9532 explains that *"there was a stretch of 12–14 random nucleotides between the tail and the sequence-specific nucleotides in the forward primer"* (see also Table S4) which provides for a minimum of 16 million unique identifiers, which is well above the range specified in claim 1. This passage goes on to propose a method which provides 10^8 UIDs, but this is again much higher than specified in claim 1. The prior art gave no good reasons to reduce the number of unique identifiers, as already explained above in relation to D3, still less to do so while additionally including endogenous information when collapsing sequences in step (d).

4.62. During the prosecution of its own patent applications (US 15/811,836), O1 has even argued that D11 does not disclose or suggest a method of using a limited number of unique

identifiers, and emphasised the importance of the use of an excess of distinct UIDs relative to the number of analyte molecules in D11 ("Kinde"). For example, in its response of 9th January 2019 (enclosed as D43), O1 argued:

> Applicants submit that Kinde does not disclose or suggest a method relying on the assignment of a limited number of non-unique barcodes through ligation, nor on the use of a number of non-unique barcodes that is less than the number of nucleic acid fragments to be evaluated, as presently claimed. In fact, Kinde is rather insisting on the importance of the use of a very large amount of distinct UIDs, that exceed the number of original templates. Contrarily to Kinde, and as disclosed in the published application at paragraph [0043] and in claim 17 as originally filed, the present invention requires different nucleic acids to be tagged with a same barcode, directly implying that the number of non-unique barcodes is less than the number of nucleic acid fragments to be evaluated. Moreover, and as previously discussed, Applicants submit

4.63. The opponents have attempted to construct a route from D11/D16 to the claimed method by making a hybrid of the three different embodiments, taking some aspects of the exogenous UID embodiment, some aspects of the endogenous UID embodiment, and some aspects of the inverse PCR embodiment, but this approach is not permissible. It is only with the benefit of hindsight that the opponents can select which parts of each embodiment should be chosen and assembled, in order to get as close to the claimed method as possible.

4.64. Moreover, this impermissible approach must even abandon central aspects of each method. Specifically, the PCR primers used in the initial step of the exogenous UID embodiment would result in amplicons which all start and end at the same position. Accordingly, all diversity in the beginning and end regions, and the length, would be destroyed, so these features would be useless for the purposes of molecular tracking. Thus, starting from the exogenous UID embodiment (as the skilled person is taught– see 4.53), there would be no way to integrate endogenous information without abandoning the PCR-based approach used to introduce the exogenous UIDs. However, D11/D16 teaches that it is this very PCR-based approach which makes the method suitable for samples with relatively few template molecules (D11: p.9534, passage spanning left and right hand column; D16: paragraph [33], starting on the last line of page 12).

4.65. Overall, D11/D16 discloses methods which use an exogenous UID <u>or</u> an endogenous UID, but it does not disclose a method which uses both types of UID simultaneously. Moreover, these two types of UID are introduced using either random acoustic shearing (for endogenous UIDs and inverse PCR) or target-specific PCR (for exogenous UIDs). For the reasons already discussed above, fragments obtained by random shearing are quite different from cfDNA molecules, and PCR-amplified fragments contain more noise than signal.

4.66. Some of the opponents correctly note that the inverse PCR embodiment is based on endogenous UIDs (created by acoustic shearing) but also introduces 12 different index sequences using the forwards PCR primer. The opponents argue that these index sequences provide an exogenous UID which should be used in combination with the endogenous UID, but they have misunderstood the purpose of the index sequences.

4.67. D11 describes the situation in the 'Supporting Information' as follows: *"The resulting DNA fragments contained UIDs composed of three sequences: 2 endogenous ones, represented by the two ends of the original sheared fragments, plus the exogenous sequence introduced during the indexing amplification"* (page 1, left column; see also D16, bottom of p.31). The purpose of the index sequence is not clear from this passage, but is explained in the right-

hand column as follows: *"This index sequence enables the PCR products from multiple different individuals to be simultaneously analyzed in the same flow cell compartment of the sequencer"* (see also D16, top of p.36). Thus the exogenous UID is not used when generating a consensus sequence, but rather is used to permit multiplexing of several different samples in a single sequencing experiment.

4.68. Furthermore, even if the skilled person would consider using the index sequence for collapsing sequences into sets (or *"UID families"* with D11/D16's nomenclature), it remains the case that the inverse PCR embodiment (i) is reported in D11/D16 itself to be *"not ideally suited"* to situations where *"there are relatively few template molecules in the initial sample"* (see section 4.53 above), (ii) relies on random shearing of the fragments (see Fig.S1), and (iii) does not involve tagging with exogenous UIDs by ligation (the index sequences are introduced through primers; D11, Supporting Information, page 1, left hand column, second paragraph). For the reasons already discussed above, therefore, there is still no obvious route from the inverse PCR embodiment to the claimed method.

4.69. The opponents cite various documents which they would combine with D11/D16, but none of them provides an obvious route to the invention. For instance, none of these documents could prompt the skilled person to modify the exogenous UID embodiment to remove the initial PCR step, to reduce the number of unique identifiers, or how to incorporate endogenous information into the analysis. As outlined above, there is no logical method of modifying the exogenous UID embodiment to incorporate endogenous information (see 4.64), without abandoning the essential aspect of the PCR-based approach for introducing the exogenous UIDs.

4.70. Furthermore, if the skilled person would somehow choose to ignore D11/D16's own preferences and warnings, and would start from the endogenous UID or inverse PCR embodiments, none of the secondary documents would prompt the skilled person to remove the requirement for random shearing of the DNA analyte, or to add information from an exogenous unique identifier during step (d). Finally, even if the skilled person would *arguendo* make any of these modifications, they still would not arrive at an assay which uses cfDNA as the analyte. Thus no sensible combination of documents can lead from D11/D16 to the present invention.

4.71. This view is supported by D35. This prior art document acknowledges the earlier disclosure from D11/D16, which it describes as *"an elegant and powerful error reduction strategy that enables highly sensitive quantitation of DNA variants"* (p.3497, top-right). In contrast to D11/D16, however, <u>the analyte in D35 was cfDNA</u>, and the authors describe that the D11/D16 method was *"<u>not designed</u> to analyse multiple amplicons from <u>samples containing limited DNA</u> and was <u>not tested on clinical specimens</u>"* (p.3497, top-right). Moreover, with their goal of *"ultra-sensitive measurement of hotspot mutations in tumor DNA in blood"* (see title), the D35 authors did not follow the D11/D16 approach, but instead developed a completely distinct method which did not use tagging or UIDs but instead relies on sequencing redundancy between the forward and reverse reads from Illumina paired-end sequencing (p.3494, Fig.1). Contemporaneous groups who were aware of D11/D16, but who were specifically interested in cfDNA, therefore did not see that a tagging-based approach would be useful for this analyte.

4.72. Thus none of the opponents' objections provide an obvious route from the method of D11/D16 to the method of claim 1. Even if the skilled person would consider D11/D16 in the context of cfDNA analysis (although it is *"not ideally suited"* to this analyte), they are led directly away

from the invention and towards using solely exogenous UIDs introduced via noisy PCR. Accordingly, there was no obvious reason to modify the method in the manner now claimed, and nor would it have been obvious that these modifications would lead to an improved method.

Conclusion on Article 56 EPC

4.73. The method of claim 1 differs from both D3 and D11/D16 in several ways. From these starting points the opponents' objections at best lead to modified versions of these prior art assays which still differ from the method of claim 1. None of the opponents has provided a credible argument to show how the skilled person would have found an obvious path from D3 or D11/D16 to a method which uses low-complexity tagging in a method for determining true mutations in cfDNA. All of their arguments leave a method which still lacks at least one of these essential features, so the claims meet the requirements of Article 56 EPC.

5. THE INVENTION IS SUFFICIENTLY DISCLOSED

5.1. O2-O4 argue that the invention is insufficiently disclosed, but the patentee agrees with O1 that the skilled person would have no difficulty in putting the method into effect.

5.2. O2 argues that the claim has a more onerous requirement than [0115] of the patent, by requiring that each consensus sequence must represent a unique molecule within the tagged population. This more onerous requirement is said to be out of reach even after reading the patent. They illustrate this point by arguing that the smallest number of tags in the claim (*i.e.* 2) would be unsuitable when a sample contains several duplicate molecules.

5.3. The basic flaw in this argument is O2's view that it must be possible for the skilled person to implement all embodiments of the invention using only 2 tags. For the method to be fruitful, the skilled person will realise that the number of tags must be selected according to the expected number of fragments in the sample. If the nature of the sample means that 2 tags will suffice, then the skilled person can choose to use only 2 tags; but if more complex tagging is needed then they will react accordingly. In the example which O2 gives on p.57 of its arguments, it is clear that a skilled person would choose to use more than 2 tags. They would not face any undue burden in making that choice, and nothing in the patent promises that 2 tags will be suitable for all types of sample.

5.4. Furthermore, the requirement in step (d) that *"each consensus sequence correspond[s] to a unique polynucleotide among the set of tagged parent polynucleotides"* should not be interpreted in an absolute manner, but rather on a statistical basis such that there is a very high chance that all original duplicate molecules which start at any one position will bear unique identifiers, as explained in [0116]. In the statistically rare event that two duplicate molecules receive an identical tag then the practical benefits of the invention can still be enjoyed, albeit with a slight imperfection – the remainder of the sample will be completely and correctly analysed.

5.5. O2 also argues that the invention cannot be put into effect for *"rare variants"*, but this argument is wholly unsubstantiated, and O2 has provided no evidence to explain why the skilled person cannot achieve this goal. This objection does not meet even the basic standards of proof under Article 83 EPC.

5.6. O3 argues in section 9 that *"the claims lack essential technical information to achieve diagnostic usefulness over the whole range claimed"*, but this argument is also completely unsubstantiated. Anyway, [0109] of the patent gives more than enough information for the

skilled person to understand how the combination of tagging and endogenous information permits tracking of individual molecules from the original samples.

5.7. O4 similarly makes various unsupported allegations. For instance, it argues that there is no adequate disclosure of how to make a *"more efficient and cheaper tag-based method of detecting rare mutations in a bodily sample"* (quoting the applicant's letter to the examining division of 20th June 2018 examination), but the absence of any substantiation means that this mere allegation does not require any response from the patentee.

6. CLAIM REQUESTS

6.1. As already discussed in section 2.6 above, the main request is based on the granted claims, with an additional limitation that tagging is achieved using enzymatic ligation. If the opposition division considers that main request is not allowable then the patentee presents auxiliary requests as follows.

6.2. In the first auxiliary request (1AR), the end of step (a) of claim 1 has been amended to clarify that the cfDNA polynucleotides are tagged (*"... and ~~the~~ tagging ~~is~~ <u>the cell-free DNA polynucleotides</u> by enzymatic ligation"*). This amendment responds to section 4.1.3 of O2's opposition, and is based on [0084] of the PCT application. This amendment has been combined with each of the amendments in the subsequent auxiliary requests as the "B" alternative.

6.3. In 2AR, the features from granted claim 13 are incorporated into claim 1, to require an enrichment step of amplified progeny DNA. This provides a further inventive distinction over D3 (which does not include any enrichment step) and D11/D16 (where the exogenous UID embodiments do not include sequence enrichment).

6.4. In 3AR, the features from granted claim 3 are incorporated into claim 1, to require that the method looks at multiple sets of tagged analyte molecules. This provides a further inventive distinction over D11/D16's inverse PCR embodiment which looked only at a single amplicon from the KRAS gene.

6.5. 4AR integrates granted claims 3 and 4 into claim 1, as well as the features of PCT claim 112. As with 3AR, 4AR requires that the method looks at multiple sets of tagged analyte molecules. Furthermore, claim 1 requires an additional step of analysing the consensus sequences to detect the presence of sequence variations compared with the reference sequence. D11/D16's inverse PCR embodiment looks at a single amplicon of the KRAS gene so does not disclose a method of detecting sequence variations in a reference sequence from tagged parent polynucleotides which map to different mappable positions in a reference sequence. 4AR is thus further inventive over D11/D16.

6.6. 5AR incorporates the features of granted claim 9 into claim 1 to require the analysis of multiple sets of tagged parent polynucleotides wherein each set is mappable to the locus of a tumor marker in a reference sequence. The consensus sequences derived from the tagged parent polynucleotides are analysed to detect the tumor marker in the set of consensus sequences. Neither D3 nor D11/D16 analyses multiple sets of tagged parent polynucleotides, wherein each set is mappable to the locus of a tumor marker. 5AR is thus further inventive over D3 and D11/D16.

6.7. 6AR requires a step of mapping sequences onto a reference sequence, with measurement of their frequency. This amendment is based on [0223] of the PCT application. D3 did not include any frequency analysis because it was looking at clonal populations, and D11/D16

did not include any mapping step because it was looking at only one sequence. Thus 6AR provides a further inventive distinction over this prior art.

6.8. Further auxiliary requests are as follows:

- Any of the above requests, with deletion of any dependent claim(s) which are held to be unallowable under Article 123(2) or 83 EPC.
- Any of the individual amendments discussed above, combined to make a further auxiliary request.

6.9. We propose to defer amendment of the description until a specific claim request has been accepted as meeting the requirements of the EPC.

7. NEW CITATIONS

7.1. The patentee additionally relies on the following documents:

D38: Declaration of Prof. Jay Shendure
D39: Guardant360® specification sheet
D40: Fierce Biotech article
D41: Perakis *et. al.* 2017
D42: Excerpt of expert report of Dr. Stacey Gabriel
D43: O1's response of 9th January 2019 for US 15/811,836

MARSHALL Cameron John
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