UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MYRIAD GENETICS, INC., MYRIAD GENETIC LABORATORIES, INC., BIO-RAD LABORATORIES, INC., and RAINDANCE TECHNOLOGIES, INC.

Petitioners

v.

THE JOHNS HOPKINS UNIVERSITY

Patent Owner

U.S. Patent No. 7,824,889

Case No. *To be assigned*

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 7,824,889 UNDER 35 U.S.C. §§ 311-319 AND 37 C.F.R. §§ 42.1-.80, 42.100-.123

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I. STATEMENT OF THE PRECISE RELIEF REQUESTED AND THE REASONS THEREFOR (37 C.F.R. § 42.22(A))

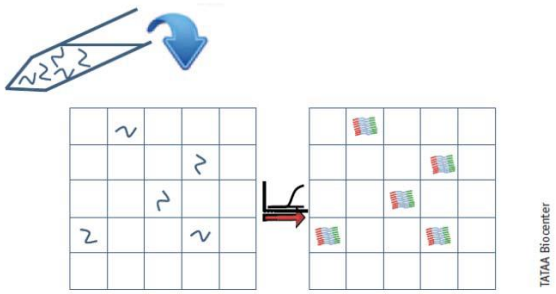
Myriad Genetics, Inc., Myriad Genetic Laboratories, Inc. (collectively, "Myriad"), Bio-Rad Laboratories, Inc., and RainDance Technologies, Inc. (collectively, "Petitioners") respectfully petition for *Inter Partes* Review, and seek cancellation of claims 1, 4-9, 12-22 of USPN 7,824,889 (the "'889 patent") (MYR1001) as unpatentable for anticipation and/or obviousness. The '889 patent is assigned to The Johns Hopkins University (hereinafter "Patent Owner").[1]

II. OVERVIEW

Claims 1, 4-9, and 12-22 of the '889 patent should be canceled as anticipated and/or obvious. MYR1002, ¶¶20-22. Independent claims 1 and 19 recite a method that the Patent Owner calls "digital PCR." MYR1002, ¶¶10-19. The figure below shows the basic steps of the method, which involve distributing a DNA sample into compartments such that each compartment contains, ideally, one or zero molecules of DNA from the sample, carrying out PCR in each compartment, and then

[1] Petitioners note that the Ex Parte Reexamination Certificate for the '889 patent lists the NIH and DHHS as assignees, rather than JHU. Nonetheless, in litigation asserting the '889 patent against Myriad, JHU stated, "The '889 patent is assigned to and owned by JHU." MYR1031, ¶114.

analyzing the resulting amplified DNA molecules, to determine how many compartments contain each different template DNA molecule



MYR1018, 541.

The steps comprising what the Patent Owner calls "digital PCR" were well known in the art before the earliest possible priority date for the '889 patent.[2] MYR1002, ¶11. In the prior art, this method was often called "limiting dilution analysis" or "limiting dilution PCR" ("LDPCR") because the sample is diluted down to the point at which some compartments will be "positive," i.e., contain a

[2] The earliest application to which the '889 patent claims priority is provisional application 60/146,792, filed 8/2/1999. MYR1011. Given that, Petitioners rely almost exclusively on prior art under 35 U.SC. §102(b), they are not aware of any claim to an earlier priority date that would affect any of the arguments set forth herein. Petitioners reserve the right to respond should Patent Owner allege an earlier priority date.

PCR-amplified product, and some will be "negative," i.e., contain no PCR-amplified product. *Id*. For LDPCR, terms such as "assay samples," "replicates," "compartments," "sample chambers," "wells," or "microreactors" all represent the same functional element – a separate space where a diluted single template molecule can undergo PCR without cross-contamination, and produce pure or homogeneous amplified product. *Id*. As discussed in detail below, Patent Owner did nothing more than add a snappy name to the prior art method of LDPCR.

By 1994, Kary Mullis, the Nobel Prize winning inventor of PCR, had edited a book on PCR (MYR1014) that included a chapter on quantitative PCR, the use of PCR to quantitate amounts of nucleic acids in a sample. The Mullis chapter discloses and discusses the work of multiple groups of scientists at the time who were carrying out and publishing work involving LDPCR. MYR1002, ¶15. A common feature of this work is that it involved diluting and distributing nucleic acids down to the single molecule level in assay samples or compartments, amplifying the single molecule templates using PCR, and counting or otherwise analyzing the amplified templates in the assay samples or compartments. As the Mullis chapter disclosed in 1994

> The principle of limiting dilution can also be called on to achieve absolute DNA quantitation. It is based on the use of a qualitative all-or-none endpoint and on the premise that one or more targets in the reaction mixture give rise to a positive endpoint. . . . ***Accurate***

> ***quantitation can be achieved by performing multiple replicates at serial dilutions of the material to be assayed (Simmonds, 1990; Lee et al. 1990; Sykes et al. 1992)***. At the limit of dilution, where some end points are positive and some are negative, the number of targets present can be calculated from the proportion of negative endpoints by using Poisson statistics. . . . This method quantitates the total number of initial DNA targets present in a sample. In this type of quantitative format, it is mandatory that PCR be optimized so that reliable detection of one or a few DNA targets occurs. Therefore, as long as the one copy level still gives a positive signal, ***the quantitation is not dependent on the amplification efficiency. This represents a major advantage of this PCR format***.

MYR1014, 78 (emphases added); MYR1002, ¶15.

As the Mullis chapter discloses, multiple groups of scientists, including Simmonds (MYR1012) and Sykes (MYR1013) – authors of two prior art references discussed in detail below – were carrying out LDPCR and publishing the results of their work prior to the earliest possible priority date for the '889 patent. MYR1002, ¶16.

Some five years after publication of the Mullis chapter, two professors and named co-inventors working for Patent Owner, Vogelstein and Kinzler, published a paper in PNAS (MYR1017), in which they described the steps of what they called "digital PCR." Notably, while much of this paper is reproduced in the

specification of the USPN 6,440,706 patent ("the '706 patent"), which the '889 patent incorporates by reference, there is one important difference. ***The PNAS paper stated that "there are several precedents for the approach described here."*** MYR1017, 9239 (emphasis added). In the applications filed with the USPTO to which the '889 patent claims priority, however, ***Patent Owner abandoned the candor of the PNAS paper and did not include the statement regarding the existence of "several precedents."***

As the 1999 PNAS paper admits, there were "several precedents" to "digital PCR." The 1994 Mullis chapter and cited references confirm the existence of such precedents beyond any reasonable dispute. Every claim of the '889 Patent for which *inter partes* review is sought is invalid as anticipated and/or obvious over these precedents. No secondary considerations or objective indicia can save the challenged claims (§XV). If anything, the secondary consideration of simultaneous invention supports cancellation of all of the challenged claims.

III. THE '889 PATENT DISCLOSURE AND CLAIMS

The '889 patent, titled "Digital Amplification," issued on 2/23/2007, from App. No. 11/709,742, filed on 2/23/2007. MYR1001. The '889 patent claims priority to provisional App. No. 60/146,792 filed 8/2/1999.

The '889 Claims. The '889 patent has 22 claims, 18 of which are challenged here.[2] Claims 1 and 19 are the only independent claims of the '889 patent, both of which are challenged here. Claim 1 is exemplary and provided below, as amended during the *ex parte* reexamination:

> **Claim 1.** A method for determining an allelic imbalance in a biological sample, comprising the steps of:
>
> *distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template molecules are isolated from the biological sample;*
>
> amplifying *the* template molecules within [a] *the* set [comprising a plurality of assay samples] to form a population of amplified molecules in [each of the] *individual* assay samples of the set[, wherein the template molecules are obtained from a biological sample];
>
> analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*;

[2] Petitioners do not concede the validity of any of the unchallenged claims.

comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance in the biological sample.

IV. THE '889 FILE HISTORY AND REEXAMINATION FILE HISTORY

The Mullis chapter and Simmonds were not before the USPTO during initial prosecution of the '889 patent. MYR1005. Sykes was disclosed, but never discussed. *Id*. To overcome a double patenting rejection over the '706 patent, the applicants submitted a terminal disclaimer. MYR1044. Therefore, the prosecution history of the '706 patent is relevant to the '889 patent.

The '706 and '889 patents were the subject of *ex parte* reexamination proceedings, during which multiple claims were amended to overcome rejections over the prior art. MYR1002, ¶¶5-6, 43-44; MYR1008; MYR1009. Although Simmonds and Sykes were nominally before the Patent Office during the *ex parte* reexamination proceedings, the Mullis chapter was not before the Patent Office, and Simmonds and Sykes were never discussed during those proceedings. MYR1002, ¶¶5-6, 43-44. Instead, the proceedings focused on different prior art, which involved the distribution of whole cells, rather than isolated nucleic acids, into compartments. The claims were amended to specify that the method involves "isolated" or "cell-free" nucleic acids rather than whole cells in light of this art. MYR1008, 7/9/2014 Amendment, 2; MYR1036; MYR1009, 7/9/2014 Responsive

Amendment to Final Office Action. While these amendments addressed the prior art discussed during the *ex parte* reexamination, they did nothing to address the Mullis chapter, Simmonds, Sykes or other prior art references discussed in this petition for *inter partes* review.

V. THE PERSON OF ORDINARY SKILL IN THE ART

A person of ordinary skill in the art ("POSA") is a hypothetical person who is presumed to be aware of all pertinent art, thinks along the lines of the conventional wisdom in the art, and is a person of ordinary creativity. As of August 2, 1999, a POSA in the technical field of the '889 Patent – molecular biology – would have had knowledge of the scientific literature concerning methods of DNA manipulation and analysis, including amplification (*e.g.*, PCR), dilution and distribution, including down to the single molecule level and using techniques such as LDPCR, and methods of nucleic acid analysis (*e.g.*, gel electrophoresis, detecting certain sequences using hybridization probes, quantitating specific sequences in a mixture of different nucleic acids, using Poisson statistics for DNA quantitation, or sequencing). MYR1002, ¶27.

As of August 2, 1999, a POSA would typically have had (1) a M.D. degree or a Ph.D. degree in molecular biology, molecular genetics, biology or equivalent discipline, plus at least two years' experience in a laboratory working in the field of molecular biology techniques, including in quantitative amplification techniques,

detection, and analysis; (2) a Master's degree in molecular biology, molecular genetics, biology or equivalent discipline, plus at least five years' experience in the laboratory working in the field of molecular biology techniques, including in quantitative amplification techniques, detection, and analysis. MYR1002, ¶¶27-28.

VI. CLAIM CONSTRUCTION

In accordance with 37 C.F.R. § 42.100(b), the challenged claims must be given their broadest reasonable interpretations (BRI) in light of the specification and prosecution history of the '889 patent. MYR1002, ¶48. Since the '706 and '889 Reexamination Certificates were issued, Patent Owner has asserted the challenged '889 (and '706) patent claims against Myriad's myRisk diagnostic test for hereditary cancer risk. *Esoterix Genetic Laboratories, LLC and The Johns Hopkins University v. Myriad Genetics, Inc. et al.*, Civil Action No. 1:16-cv-01112-WO-JEF (M.D.N.C) (MYR1032). In the Myriad litigation, Patent Owner has accused of infringement the same dilution and distribution steps found in the Mullis chapter and the Simmonds and Sykes prior art references, and is effectively seeking to re-patent the prior art. Given that Patent Owner has adopted a claim construction in the Myriad litigation that requires only dilution and/or distribution, PCR amplification, and any analysis of the PCR products (which construction was not before the USPTO during the *ex parte* reexamination proceedings), it is beyond

reasonable dispute that the challenged claims are invalid over the prior art and should be cancelled by the PTAB. MYR1002, ¶¶45-47.

A. The Preambles Are Not Limiting

Under the BRI of Claims 1 and 19 of the '889 Patent, the preambles *"[a] method for determining an allelic imbalance in a biological sample*" should be construed as non-limiting. These preambles do not recite any structure or step needed to give meaning and life to the claims, or to any dependent claims. *See, e.g., Summit 6, LLC v. Samsung Electronics Co., Ltd.*, 802 F.3d 1283, 1292 (Fed. Cir. 2015) ("[g]enerally, a preamble is not limiting"); *TomTom, Inc. v. Adolph*, 790 F.3d 1315, 1323 (Fed. Cir. 2015). No term in Claims 1 or 19, or in any claim that depends from them, refers back to these preambles, which therefore do not provide any antecedent basis for the body of the claims. Instead, the steps of the claimed method stand independent of these preambles, and the structure they recite is in no way dependent on any preamble language.

A POSA would have understood that these preambles merely recite intended uses of the claimed methods, and therefore do not limit the claims in any way. MYR1002, ¶¶49; *see Summit*, 802 F.3d at 1292; *TomTom*, 790 F.3d at 1323.

B. "allelic imbalance" and "allele"

Under the BRI, a POSA would understand that the term "*allelic imbalance*" should be construed to require a "loss of or an increase in copy number of one

allele relative to the other allele or different allele," wherein "*allele*" refers to "one of various alternative forms of a gene or genomic sequence." MYR1002, ¶¶50. These proposed constructions are consistent with the intrinsic evidence, MYR1001, 1:49-51; 2:64-67; 5:24-29, and were previously agreed to by Patent Owner in the litigation *Esoterix Genetic Laboratories, LLC and The Johns Hopkins University v. Life Technologies Corp.*, et al., Civil Action No. 1:12-cv-01173-CCE-JEP (M.D.N.C.), where Patent Owner asserted the '889 Patent against various defendants (not including Myriad). MYR1032, 2.

VII. IDENTIFICATION OF THE CHALLENGE (37 C.F.R. § 42.104(B))

Petitioners respectfully petition for *inter partes* review of claims 1, 4-9, and 12-22 of the '889 patent based on the unpatentability grounds summarized in the index below. Per 37 C.F.R. § 42.6(c), copies of the cited references accompany this Petition.

Ground	35 U.S.C. § (pre-AIA)	Claims	Reference(s)
1	**§ 102**	1, 5, 8-9, 12-15, 18-22	Simmonds
2	**§ 103**	16-17	Simmonds and Brown
3	**§ 103**	4, 6-7	Simmonds and Heid
4	**§ 102**	1, 5, 8-9, 12-15, 18-22	Sykes
5	**§ 103**	16-17	Sykes and Brown
6	**§ 103**	4, 6-7	Sykes and Heid

The "Simmonds" reference (MYR1012) was published in February 1990 and is prior art to the '889 patent under at least 35 U.S.C. §102(b). The "Sykes" reference (MYR1013) was published in 1992 and is prior art to the '889 patent under at least 35 U.S.C. § 102(b). The "Brown" reference (MYR1015) was filed on 4/17/1997 and issued on 11/7/2000, and is prior art to the '889 patent under at least 35 U.S.C. § 102(e). The Heid reference (MYR1024) was published in 1996, and is prior art to the '889 patent under at least §102(b).

Grounds 1 and 4 are not redundant because Simmonds and Sykes disclose experiments carried out by independent groups applying the LDPCR technique for different purposes. MYR1002, ¶56. For the same reason, Grounds 2 and 5, and 3 and 6 are not redundant. *Id*. Petitioner includes all of these grounds to avoid a repeat of the Patent Owner's conduct in the *ex parte* reexamination, during which it focused on an ancillary point – whole cell distribution versus isolated DNA distribution – to avoid discussing the real issue: the fact that the steps comprising what Patent Owner calls "digital PCR" were well known in the prior art.

This Petition is accompanied by a supporting declaration of Petitioners' technical expert, Dr. Michael L. Metzker. MYR1002.

VIII. THE STATE OF THE ART

POSAs knew long before August 2, 1999 that PCR was a powerful tool for quantitation of DNA. MYR1002, ¶¶30-35. For example, by the late 1980s and

early 1990s, POSAs knew that some of the potential pitfalls of PCR related to its exponential nature – potentially biased amplification of certain sequences over others and artifacts that could arise from using multiple different pairs of primers in a single reaction – could be avoided through the use of parallel, compartmentalized PCR reactions, carried out at limiting dilution. *See* MYR1014, 68; MYR1002, ¶¶32-35. Indeed, as the 1994 Mullis chapter demonstrates, multiple research groups were carrying out and publishing LDPCR methods by the early 1990s. *Id*.

By August 1999, researchers had developed numerous high-throughput technologies to improve limiting dilution analysis, including LDPCR. For example, Brown disclosed a platform to perform a multitude of LDPCR assays in parallel in an efficient manner, and Heid disclosed the use of non-polymorphic markers in the context of quantitative PCR analysis. MYR1002, ¶¶33; 129.

IX. GROUND 1: CLAIMS 1, 5, 8-9, 12-15, AND 18-22 OF THE '889 PATENT ARE ANTICIPATED BY SIMMONDS

As illustrated in the claim charts and discussion below, a POSA would have understood that Simmonds discloses each element of, and therefore anticipates, Claims 1, 5, 8-9, 12-15, and 18-22. MYR1002, ¶¶20, 57-108.

A. Independent Claim 1

Claim	Disclosure in Simmonds (MYR1012)
1. A method of determining an	This non-limiting preamble is nonetheless disclosed:

Claim	Disclosure in Simmonds (MYR1012)
allelic imbalance in a biological sample, comprising the steps of:	"Here we describe a modified PCR method with a sensitivity sufficient to detect a single molecule of target DNA. . . . By combining this double PCR method with a limit dilution approach, both the proportion of infected cells and the number of molecules of HIV provirus per cell can be accurately estimated. . . .The approach described here also provides a way to analyze sequence heterogeneity within populations of HIV provirus which avoids the complications that arise because of the lack of fidelity of the Taq polymerase (33) and other artifacts associated with amplification." (865) "**Separate amplification of individual molecules from a mixture after dilution and distribution**. If the double PCR can detect single DNA molecules, then it should be possible to separate single molecules of two types from a mixture of the two by dilution and distribution and to amplify them separately. In order to test this proposition, a mixture was made of two clones derived from different HIV isolates, pBH10.R3 (HIV-HTLV-IIIB) and lambda HAT 3 (HIV-RF), and the mixture was diluted, distributed, and amplified as before by double PCR. . . . Figure 3, lanes 2 and 32 show the results of amplifying lambda HAT 3 alone, and lanes 3 and 33 show the results of amplifying pBH10.R3 alone. . . . Of 28 reactions, 9 showed amplification of the pBH10.R3 *env* sequence (corrected mean, 0.39 molecules per reaction), while 13 of 28 reactions showed amplification of the lambda HAT 3 *env* sequence (corrected mean, 0.62 molecules per reaction)." (867) "FIG. 3. Separate amplification of single molecules from a mixture. A mixture of pBH10.R3 and lambda HAT 3 was diluted in herring sperm DNA, and the appropriate dilutions were distributed to 28 tubes. Each sample contained (nominally) 6.5 ag of pBH10.R3 (0.5 molecules) and 60 ag of lambda HAT 3 (1.2 molecules)." (867, Figure 3 legend) "Limiting-dilution PCR products from patients 75, 76, and 79, amplified with *gag* primers, were sequenced directly by using

Claim	Disclosure in Simmonds (MYR1012)
	primer 883. . . . These dilutions gave a low frequency of positive reactions (Table 1) and . . . the probability was high that single molecules would be amplified. . . . Five of the seven sequences are unique, with variation both between samples derived from different patients and between parallel amplification reactions originating from the same DNA preparation (Table 4)." (869). Table 1 shows the number of assay samples for Patient 75 (2/8); 76 (4/16), and 79 (4/14), representing a total of 38 assay samples.
(a) *distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template molecules are isolated from the biological sample;*	*See* preamble element above.
(b) amplifying *the* template molecules within [a] *the* set [comprising a plurality of assay samples] to form a population of amplified molecules [each of the] *individual* assay samples of the set [, wherein the template molecules are obtained from a biological	"Figure 3, lanes 2 and 32 show the results of amplifying lambda HAT 3 alone, and lanes 3 and 33 show the results of amplifying pBH10.R3 alone. . . . Of 28 reactions, 9 showed amplification of the pBH10.R3 *env* sequence (corrected mean, 0.39 molecules per reaction), while 13 of 28 reactions showed amplification of the lambda HAT 3 *env* sequence (corrected mean, 0.62 molecules per reaction)." (867) "FIG. 3. Separate amplification of single molecules from a mixture. . . . The first PCR contained the *env* and *gag* outer primers. A single sample of each PCR product was then amplified with radiolabeled inner *env* and *gag* primers." (867, Fig. 3 legend) *See also* preamble element above.

Claim	Disclosure in Simmonds (MYR1012)
sample];	
(c) analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*;	"**Separate amplification of individual molecules from a mixture after dilution and distribution**….After dilution of the mixture of the two sequences (lanes 4 through 31), a clear separation of pBH10.R3 and lambda HAT 3 *env* sequences was seen. Of 28 reactions, 9 showed amplification of the pBH10.R3 *env* sequence (corrected mean, 0.39 molecules per reaction), while 13 of 28 reactions showed amplification of the lambda HAT 3 *env* sequence (corrected mean, 0.62 molecules per reaction)…." (867) "FIG. 3. . . . The products were run on an acrylamide gel, which was exposed to X-ray film. The amplified *env* sequences of lambda HAT 3 (a; 359 bp) and pBH10.R3 (b; 317 bp) are readily distinguishable; c (248 bp) is the amplified *gag* sequence. Lanes: 1, negative control (carrier DNA); 2 and 32, lambda HAT 3 alone; 3 and 33, pBH10.R3 alone; 4 to 31, the 28 samples distributed from the diluted mixture." (p. 867, Fig. 3 legend) "Limiting-dilution PCR products from patients 75, 76, and 79, amplified with *gag* primers, were sequenced directly by using primer 883. In the case of patient 75, 13,000 cell equivalents of DNA were amplified, in the case of patient 76, 25,000 cell equivalents were amplified, and in the case of patient 79, 1,000 and 2,000 cell equivalents were amplified. These dilutions gave a low frequency of positive reactions (Table 1) and hence, if our conclusions are correct, the probability was high that single molecules would be amplified. Each amplification product gave an unambiguous readable sequence of at least 175 bases. Five of the seven sequences are unique, with variation both between samples derived from different patients and between parallel amplification reactions originating from the same DNA preparation (Table 4)." (869) Table 1 shows the number of assay samples for Patient 75 (2/8) (13,000 cell equivalents); 76 (4/16) (25,000 cell equivalents), and 79 (4/14) (1,000 and 2,000 cell equivalents), representing a total of 38 assay samples.

Claim	Disclosure in Simmonds (MYR1012)
(d) comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance in the biological sample.	*See* step (c) above. "If most lymphocytes which contain provirus are transcriptionally silent, it might be because the provirus that they carry is defective. …Using the dilution, distribution, and double PCR method to amplify single sequences, we have determined a 180-bp sequence from the *gag* region of 19 single provirus molecules from six patients who were probably infected with the same virus (19) (7 of these are illustrated in Table 4). Among the sequences, there are 16 variable sites, *i.e.*, sites at which one or more of the sequences differs from the consensus of all of them….Similarly, analysis of a 170-bp sequence from the *env* region of 24 single molecules from four patients revealed 49 variable sites and a minimum of eight independent deletions or insertions…." (870)

Claim 1, preamble. To the extent the preamble is found to be limiting, Simmonds discloses it. MYR1002, ¶¶60-61. Simmonds discloses a method "to analyze sequence heterogeneity within populations of HIV provirus" by comparing two variants of the same viral gene from a biological sample. *See* MYR1012, 865. This method includes "separate amplification of individual molecules from a mixture after dilution and distribution." MYR1012, 867. Simmonds discloses how to determine the ratio of a given HIV provirus variant in a mixed population of proviruses using the method of separate amplification of individual molecules from a mixture after dilution and distribution. MYR1012, 867. Simmonds discloses how to determine that out of 28 assay samples, 9 contained one provirus variant

(*i.e.* genetic sequence) and 13 contained the other provirus variant (*i.e.* different genetic sequence). *Id.* Simmonds showed that these ratios reflected the ratios of the two variants (*i.e.* genetic sequences) in the original sample, namely that one variant was present at about twice the amount of the other, which was in good agreement with how the mixtures were originally created. MYR1012, Fig. 3 legend. Simmonds discloses the use of sequence analysis to determine a ratio of a selected genetic sequence in a population of genetic sequences in a biological sample. MYR1012, 868-869.

Claim 1, step (a). Simmonds discloses diluting and distributing a mixture of isolated provirus template DNA molecules that were isolated from blood samples to form a set of 28 assay samples. MYR1012, 867. Simmonds discloses diluting and distributing a mixture of isolated provirus template DNA molecules that were isolated from three patient blood samples to form a set of 38 assay samples. MYR1012, 868-869.

Claim 1, step (b). Simmonds discloses amplifying the provirus template molecules in the assay samples of the set using PCR to form a population of amplified provirus molecules in the 28 assay samples of the set. MYR1012, 867. Simmonds discloses amplifying the provirus template molecules in the assay samples of the set using PCR to form a population of amplified provirus molecules

in 38 assay samples of the set and 56 assay samples of the set. MYR1012, 868-869.

Claim 1, step (c). Simmonds discloses analyzing the PCR-amplified molecules in each of the 28 assay samples of the set by gel electrophoresis. MYR1012, 867. Gel electrophoresis analysis showed that one provirus variant had been amplified in 9/28 (0.3) of the assay samples, while the other provirus variant had been amplified in 13/28 (0.5) of the assay samples. MYR1012, 867. Therefore, between 0.1 and 0.9 of the assay samples yielded an amplification product.

Simmonds discloses analyzing the PCR-amplified molecules from three patients of the 38 assay samples of the set using a sequencing technique. MYR1012, 869-870. Sequence analysis was performed to determine that 2/8 (0.3) for patient 75 contained one variant, 4/16 (0.3) for patient 76 contained more than one variant, and 4/14 (0.3) for patient 79 contained more than one variant. MYR1012, 868-869. Therefore, between 0.1 and 0.9 of the assay samples yielded an amplification product.

Simmonds discloses that "[i]n order to quantify the amount of provirus, 12 of the DNA samples were assayed by amplification after dilution and distribution and analyzed (Table 1)." MYR1012, 868. Under the dilution of 25,000 cell equivalents of DNA column, there are 28 positive PCR products from a total of 56

assay samples of the set analyzed in the experiment. The fraction of positive reactions detected was 28/56, i.e., 0.5 of the assay samples.

Simmonds determines that human genomic DNA of " . . . each infected cell carried about one copy of the provirus." MYR1012, 865. A POSA would have understood that HIV provirus integrates into human chromosomal DNA. MYR1025; MYR1002, ¶66. As only one provirus would have integrated into only one given chromosome in a more or less random manner, a POSA would have known that each of the sequence variants disclosed in Simmonds are on separate chromosomes. *Id*. Therefore, a POSA would have understood each integrated provirus variant to be either a selected genetic sequence on one chromosome or a reference genetic sequence on a second chromosome. *Id*.

Claim 1, step (d). Simmonds compares the number of assay samples containing each different provirus variant out of the 28 assay samples. MYR1012, 867. Simmonds discloses that 9 samples contain one of the provirus variants, while 13 samples contain the other provirus variant. *Id.* Simmonds compares the number of assay samples containing each different provirus variant out of the 38 assay samples and 56 assay samples. MYR1012, 868-869. Simmonds states that "[f]ive of the seven sequences are unique, with variation both between samples derived from different patients and between parallel amplification reactions originating from the same DNA preparation (Table 4)." MYR1012, 869.

B. Independent Claim 19

Claim	Disclosure in Simmonds (MYR1012)
19. A method of determining an allelic imbalance in a biological sample, comprising the steps of:	*See* Claim 1, preamble element.
(a) distributing *cell-free* nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;	*See* Claim 1, step (a).
(b) amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;	*See* Claim 1, step (b).
(c) analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome;	*See* Claim 1, step (c).
(d) comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first chromosome and the second chromosome in the biological sample.	*See* Claim 1, step (d).

Claim 19, preamble, steps (a), (b), (c), (d). Simmonds discloses these limitations, including any limitations found to be present in the preamble. *See* Claim 1.

C. Dependent Claim 5

Claim	Disclosure in Simmonds (MYR1012)

Claim	Disclosure in Simmonds (MYR1012)
5. The method of claim 1 wherein the biological sample is from blood.	"**Materials**. Seropositive blood samples were donated by members of a cohort of HIV-1-infected hemophiliacs." (865)[3] "PBMC DNA was prepared from 52 blood samples obtained from 28 HIV antibody-positive patients and assayed in the double PCR reaction with *gag*, *pol*, and *env* primers (Fig. 1)." (867)
22. The method of claim 19 wherein the biological sample is blood.	*See* Claim 5.

Simmonds discloses purifying provirus DNA from peripheral blood mononuclear cells (PBMCs) obtained from patient blood samples with hemophilia who were also infected with HIV. MYR1002, ¶69.

D. Dependent Claims 8, 9, 12, 13, 14, 15, 18, 21

Claim	Disclosure in Simmonds (MYR1012)
8. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product *of at least one of the selected and the reference*	Of 28 reactions, 9 showed amplification of the pBH10.R3 *env* sequence (corrected mean, 0.39 molecules per reaction), while 13 of 28 reactions showed amplification of the lambda HAT 3 *env* sequence (corrected mean, 0.62 molecules per reaction)." (867) Table 1 shows the number of assay samples for Patient 75 (2/8); 76 (4/16), and 79 (4/14), representing a total of 38 assay samples.

[3] Each dependent claim discussed herein is invalid for the reasons set forth regarding that claim and regarding the independent claim from which it depends.

Claim	Disclosure in Simmonds (MYR1012)
genetic sequences.	
9. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
12. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield [an] *a homogenous* amplification product [as determined by amplification of the selected genetic sequence] *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
13. The method of claim [1] *19* wherein between 0.1 and 0.6 of the assay samples yield an amplification product [as determined by amplification of the reference	*See* Claim 8.

Claim	Disclosure in Simmonds (MYR1012)
genetic sequence] *of at least one of the selected and the reference genetic sequences*.	
14. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield [an] *a homogenous* amplification product [as determined by amplification of the selected genetic sequence] *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
15. The method of claim [1] *19* wherein between 0.3 and 0.5 of the assay samples yield an amplification product [as determined by amplification of the reference genetic sequence] *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
18. The method of	*See* Claim 8.

Claim	Disclosure in Simmonds (MYR1012)
claim 1 wherein the amplified molecules in each of the assay samples in the first and second numbers of the assay samples are homogeneous such that the first number of assay samples do not contain the reference genetic sequence and the second number of assay samples do not contain the selected genetic sequence.	
20. The method of claim 19 wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
21. The method of claim 20 wherein between 0.1 and 0.9 of the assay samples yield a homogeneous amplification product *of at least*	*See* Claim 8.

Claim	Disclosure in Simmonds (MYR1012)
one of the selected and the reference genetic sequences.	

Simmonds discloses diluting and distributing a mixture of provirus variants into 28 assay samples, and determining that 9/28 contain one variant and 13/28 contain the other variant. *See id*., 867. Specifically, 0.3 of the assay samples yielded one amplified provirus variant, and 0.5 of the assay samples yielded the other amplified provirus variant. Therefore, between 0.1 and 0.6, and between 0.3 and 0.5, of the assay samples yielded an amplification product of at least one of the provirus variants. MYR1002, ¶¶71-84; 95-103.

Simmonds also discloses diluting and distributing a mixture of provirus variants into 38 assay samples derived from three patients, and determining that 2/8 for patient 75 contain one variant, 4/16 for patient 76 contain more than one variant, and 4/14 for patient 79 contain more than one variant. *See* MYR1012, 868-869. Therefore, between 0.1 and 0.6 of the assay samples yielded an amplification product of at least one of the provirus variants. Specifically, 0.3 of the assay samples were derived from patient 75, 0.3 of the assay samples were derived from patient 76, and 0.3 of the assay samples were derived from patient 75. Simmonds discloses that 0.3 of the assay samples derived from patient 75 yielded a

homogeneous amplification product, as confirmed by sequence analysis. MYR1002, ¶¶107-108.

X. GROUND 2: CLAIMS 16-17 WOULD HAVE BEEN OBVIOUS IN VIEW OF SIMMONDS AND BROWN

Claims 16-17 are obvious in view of Simmonds and Brown. *See* U.S. Patent No. 6,143,496 to Brown, filed in April 1997 ("Brown"). MYR1015; MYR1002, ¶¶109-127. To the extent that Simmonds does not disclose any claim limitation related to the number of assay samples, a POSA would have had a reason to combine Simmonds with Brown – to gain the benefit of Brown's increased assay sample numbers, with attendant increased ability to differentiate a variety of DNA template molecule variants, including provirus variants, while maintaining good efficiency and throughput – and would have had a reasonable expectation of success in doing so, as illustrated and explained below. *Id.* In short, there was a reason to increase the number of assay samples as disclosed in Brown, because doing so would increase the power of the LDPCR analytical tool. MYR1015, 10:11-15; MYR1002, ¶109.

Brown, like Simmonds, discusses LDPCR, i.e., methods for "determining the number of template molecules in a sample by conducting replicate nucleic acid sequence amplification reactions on a set of terminally diluted samples and counting the number of positive amplification reactions." MYR1015, Abstract.

Brown states that its invention can detect different nucleic acid sequences in a starting sample and can effectively analyze many assay samples in parallel, in particular, different HIV variants: "it may be desirable to test for the presence of multiple different viruses such as HIV-1, HIV-2, HTLV-1, HBV and HCV in a clinical specimen . . . . " *Id.*, 3:60–4:17. Brown discusses using its methods to "determine simultaneously the presence in a sample of multiple different nucleic acid target molecules." *Id*. 4:41-43.

Brown discloses the use of a variety of methods to perform low volume LDPCR with 10,000 to over 100,000 sample chambers: "Preferred sample chambers according some [*sic*] embodiments of the invention, for nucleic acid amplification methods to detect single target nucleic acid molecules, have volumes of from about 1 microliter to about 1 picoliter or less . . . Photolithographic methods can provide from about 10,000 to over 100,000 sample chambers of about 100 picoliters each on a 1"×3" substrate." *Id*. 16:1-19 (emphasis added).

The claimed methods are merely a combination of known elements with predictable results, given that Simmonds discloses LDPCR, including counting assay samples or compartments containing different templates to obtain template ratios in the starting sample, and Brown discloses LDPCR using 10,000 to over 100,000 sample chambers or compartments. *See, e.g*., MYR1012, 865, 867, 869-70; MYR1015, Abstract, 3:17-24, 7:44-56, 16:1-19. Brown discloses an effective

way to process large numbers of assay samples to generate a powerful data set for use in the LDPCR method of Simmonds. A POSA would have been confident in their ability to scale up Simmonds in the manner disclosed in Brown.

A. Dependent Claims 16 and 17

Claim	Disclosures in Simmonds and Brown (MYR1015)
16. The method of claim 1 wherein the set comprises at least 500 assay samples.	See Claim 1.[4] "Methods of filling miniaturized sample chambers are also provided as are methods for determining the number of template molecules in a sample by conducting replicate nucleic acid sequence amplification reactions on a set of terminally diluted samples and counting the number of positive amplification reactions. The methods can be used to detect a single starting nucleic acid target molecule." (Brown, Abstract) "Preferred sample chambers according some embodiments of the invention, for nucleic acid amplification methods to detect single target nucleic acid molecules, have volumes of from about 1 microliter to about 1 picoliter or less. . . . Photolithographic methods can provide from about 10,000 to over 100,000 sample chambers of about 100 picoliters each on a 1"×3" substrate." (Brown 1997, 16:1-19)
17. The method of claim 1 wherein the set comprises at least 1000 assay	*See* Claim 16.

[4] Each dependent claim discussed herein is rendered anticipated or obvious based on the reasons set forth regarding that claim and regarding the independent claim from which it depends.

Claim	Disclosures in Simmonds and Brown (MYR1015)
samples.	

Brown discloses 10,000 to over 100,000 sample chambers, each for holding an assay sample for LDPCR amplification reactions. *See* MYR1015, 16:1-19.

Reasons to combine. In addition to the discussion above, Brown emphasizes that its disclosures constitute an improvement over the nested primer approach used in Simmonds. MYR1015, 3:60-4:17. A POSA would have had a reason to combine Simmonds with Brown to gain the benefit of Brown's increased assay sample numbers with attendant increased ability to differentiate a variety of DNA template molecule variants, including provirus variants, while maintaining good efficiency and throughput. MYR1015, 16.1-19; MYR1002, ¶120.

Brown's example of testing for the presence of multiple different HIV viruses provided a further reason for a POSA to combine Brown with Simmonds, which is directed to analyzing HIV provirus variants. MYR1015, 3:60–4:17; MYR1002, ¶121. This reason would have been especially strong given the state of HIV research in the relevant time period. *Id*. By the mid-1990s, it was known that the HIV virus replicated rapidly, had a high error rate during replication, and could produce 10 billion virus particles per day in an infected individual. MYR1002, ¶¶121-123; MYR1019; MYR1020; MYR1022, 1582. This diversity was understood to be a major challenge posed by HIV. *Id*.; MYR1021, 483.

High-throughput techniques were needed for HIV research to address the diversity of possible variants. Because Brown met this need, a POSA would have had a strong reason to combine Brown as a high-throughput platform for generating data with Simmonds-type analysis, including LDPCR, and sequencing to count replicates and obtain template ratios. MYR1002, ¶¶121-122.

Furthermore, the claimed methods are a simple and predictable substitution of known elements. LDPCR, including the use of Poisson statistics, was known since at least 1990. MYR1012; MYR1002, ¶123. Simmonds's analytical framework, LDPCR, including counting assay samples or compartments containing different templates to obtain template ratios in the starting sample, is readily applicable to, and can easily be combined with other techniques. *Id*. Using Brown's high-throughput compartmentalization and detection methods would have been a simple substitution for a POSA scaling up the analytical framework of Simmonds. *Id.* Brown expressly discloses an improvement over the nested primers of Simmonds and discloses applications of its high-throughput methods to HIV research. *Id.*

Reasonable expectation of success. A POSA would have had a reasonable expectation of successfully combining Brown with Simmonds. MYR1002, ¶¶125-127. Simmonds discloses, among other things, LDPCR, including counting assay samples or compartments containing different templates to obtain template ratios

in the starting sample. Brown discloses high throughput methods for compartmentalization for LDPCR to determine the presence of multiple different templates in a starting sample. Therefore, Brown's high throughput approach would be a simple substitution for a POSA seeking to use Simmonds's analytical framework, including to analyze HIV provirus variants. *Id*. For all of these reasons, Claims 16 and 17 would have been obvious over the combination of Brown and Simmonds.

XI. GROUND 3: CLAIMS 4, 6, AND 7 WOULD HAVE BEEN OBVIOUS IN VIEW OF SIMMONDS AND HEID

Claims 4, 6 and 7 are obvious in view of the disclosures in Simmonds (MYR1012) and Heid (MYR1024). MYR1002, ¶¶128-147. To the extent that Simmonds does not disclose any limitation of the '889 Patent related to non-polymorphic markers, a POSA would have had a reason to combine Simmonds with Heid, and would have had a reasonable expectation of success, as illustrated and as explained below. MYR1002, ¶¶128-147.

For example, Heid discloses a real time quantitative PCR method for analyzing a sample with a high degree of accuracy and reproducible quantitation of gene copies. *See* MYR1024, abstract. The method measures PCR product accumulation through a dual-labeled fluorogenic probe. MYR1024, abstract. Heid discloses the use of non-polymorphic markers, specifically, human factor VIII and

β-actin, as reference standards for the real time quantitative PCR methods described. *See* MYR1024, 987. These reference standards are used to determine the amount of the gene of interest in the experiments: "Real-time PCR is compatible with . . . quantitative competitive PCR using a normalization gene contained within the sample (i.e., β-actin). . . ." *Id*., 991. Heid describes the state of the art in quantitative gene and genome analysis in terms of cancer and HIV research:

> ***Quantitative gene analysis (DNA) has been used to determine the genome quantity of a particular gene, as in the case of the human HER2 gene***, which is amplified in -30% of breast tumors. ***Gene and genome quantitation (DNA and RNA) also have been used for analysis of human immunodeficiency virus (HIV)*** burden demonstrating changes in the levels of virus throughout the different phases of the disease.

See id., 986 (emphases added, citations omitted). A POSA would have understood that Heid is using these non-polymorphic markers for determining the amounts of sequences of interest present in a biological sample and would have understood that non-polymorphic markers could similarly be used in other PCR techniques to quantitate DNA such as LDPCR. MYR1002, ¶129.

The claimed methods are merely a combination of known elements with predictable results, because (*i*) Simmonds discloses a quantitative DNA analysis

method using PCR, LDPCR, to count assay samples containing different templates and obtain ratios of templates in a starting sample; and (*ii*) Heid discloses a quantitative PCR method in the context of copy number analysis that involves non-polymorphic markers. *See, e.g.*, MYR1012, 865, 867, 869-70; MYR1024, abstract, 986, 987, 992; MYR1002, ¶130.

A. Dependent Claims 4, 6, and 7

Claim	Disclosures in Simmonds (MYR1012) and Heid (MYR1024)
4. The method of claim 1 wherein the selected genetic sequences and the reference genetic sequences are non-polymorphic markers.	"We have developed a novel 'real time' quantitative PCR method. The method measures PCR product accumulation through a dual-labeled fluorogenic probe (i.e., TaqMan Probe)." (Heid Abstract) "Quantitative gene analysis (DNA) has been used to determine the genome quantity of a particular gene, as in the case of the human HER2 gene, which is amplified in -30% of breast tumors. Gene and genome quantitation (DNA and RNA) also have been used for analysis of human immunodeficiency virus (HIV) burden demonstrating changes in the levels of virus throughout the different phases of the disease." (Heid 986) "PCR primers and probes were designed for the human factor VIII sequence and human β-actin gene (as described in Methods)." (Heid 987) "Real-time PCR is compatible with . . . quantitative competitive PCR using a normalization gene contained within the sample (i.e., β-actin). . . ." (Heid, 991)
6. The method of claim 1 wherein the selected genetic sequence is non-polymorphic	*See* Claim 4.

Claim	Disclosures in Simmonds (MYR1012) and Heid (MYR1024)
marker.	
7. The method of claim 1 wherein the reference genetic sequence is non-polymorphic marker.	*See* Claim 4.

Heid discloses that human factor VIII, β-actin, HER2, and HIV sequences are used as non-polymorphic markers for copy number analysis. *See* MYR1024, 987. Moreover, a POSA would have understood that any of these genes could be used as a selected genetic sequence or a reference genetic sequence. *See id*., 986, 992; MYR1002, ¶¶138; 212.

Reasons to combine. In addition to the discussion above, the claimed methods are a simple and predictable substitution of known elements. For example, Heid's disclosures show the benefits of non-polymorphic markers in the context of quantitative DNA analysis methods using PCR, which is the context of Simmonds. *Id*., ¶139.

Heid expressly discloses the benefits of quantitative PCR methods for HIV research: "Gene and genome quantitation (DNA and RNA) also have been used for analysis of human immunodeficiency virus (HIV) burden demonstrating changes in the levels of virus throughout the different phases of the disease." *See id*., 986

(citations omitted). Heid discloses uses of quantitative PCR in gene copy number analysis: "[R]eal time quantitative PCR methodology can be used to develop high-throughput screening assays for a variety of applications [quantitative gene expression (RT-PCR), ***gene copy assays (Her2, HIV, etc.)***, genotyping (knockout mouse analysis), and immuno-PCR]." *See id*. (emphasis added). As discussed above, Simmonds arises in the HIV research context. A POSA would therefore have been motivated to apply Heid's use of such markers in the context of the framework of the techniques and analysis taught in Simmonds. MYR1002, ¶140.

Reasonable expectation of success. A POSA would have had a reasonable expectation of successfully combining Heid with Simmonds to meet the limitations of any claim requiring non-polymorphic markers. MYR1002, ¶141. As discussed above, both references arise in the context of quantitative DNA analysis methods using PCR, and moreover Heid discloses the benefits of its disclosures to the HIV research context, among others. Because a POSA would have understood that Heid discloses the use of non-polymorphic markers as part of its quantitative PCR method, such a person would expect the use of non-polymorphic markers in the context of the Simmonds LDPCR method to be successful. *Id*.

A POSA would have had no reason to believe that use of non-polymorphic markers would have presented any particular problems to LDPCR, an approach that had long been proven to be robust across multiple investigation areas.

MYR1002, ¶141-42. For all of these reasons, a POSA would have had additional confidence in the likely success of combining the disclosures of Simmonds with Heid. MYR1002, ¶143.

For all of these reasons, Claims 4, 6, and 7 of the '889 Patent would have been obvious over the combination of Heid and Simmonds. MYR1002, ¶¶128-147.

XII. GROUND 4: CLAIMS 1, 5, 8-9, 12-15, AND 18-22 ARE ANTICIPATED BY SYKES

As illustrated below, a POSA would have understood that Sykes discloses each element of, and therefore anticipates Claims 1, 5, 8-9, 12-15, and 18-22 of the '889 Patent. MYR1002, ¶¶148-187.

A. Independent Claim 1

Claim	Disclosure in Sykes (MYR1013)
1. A method of determining an allelic imbalance in a biological sample, comprising the steps of:	This non-limiting preamble is nonetheless disclosed: "We describe a general method to quantitate the total number of initial targets present in a sample using limiting dilution, PCR and Poisson statistics. The DNA target for the PCR was the rearranged immunoglobulin heavy chain (IgH) gene derived from a leukemic clone that was quantitated against a background of excess rearranged IgH genes from normal lymphocytes. The PCR was optimized to provide an all-or-none endpoint at very low DNA target numbers. PCR amplification of the *N-ras* gene was used as an internal control to quantitate the number of potentially amplifiable genomes present in a sample and hence to measure the extent of DNA degradation. A two-stage PCR was necessary owing to competition between leukemic and non-leukemic templates. Study of eight leukemic samples showed that approximately

Claim	Disclosure in Sykes (MYR1013)
	two potentially amplifiable leukemic IgH targets could be detected in the presence of 160,000 competing non-leukemic genomes." (Abstract) "The biologic problem in our study was the detection of rare leukemic cells in a large population of normal cells, which in molecular terms became the problem of detection of a rare unique IgH sequence against a background of numerous other IgH sequences. The two-stage PCR system that was developed proved capable of detecting approximately two (1/0.52) potentially amplifiable leukemic IgH sequences against a background of approximately 160,000 total genomes. These genomes would provide a vast excess of sequences that would compete with the leukemic IgH sequences for the PCR primers because they would contain approximately $2.4x10^4$ rearranged IgH genes from normal B lymphocytes and $3x10^5$ germ-line IgH genes, each containing multiple V and J segments." (448) "Nevertheless, as seen in Table 2, in all 8 patients there was an approximately constant ratio between the number of amplifiable IgH targets and the number of amplifiable *N-ras* targets. The data suggest that the number of amplifiable *N-ras* genes, rather than the DNA concentration, is the best indicator of the number of amplifiable genomes present, that virtually all potentially amplifiable leukemic IgH genes are amplified in the absence of competing non-leukemic IgH genes and that approximately half of the leukemic IgH genes are amplified in the presence of competing genes." (448)
(a) *distributing isolated nucleic acid template molecules to form a set comprising a plurality of assay samples, wherein the nucleic acid template*	". . . we have used the principle of limiting dilution, which is based on the use of a qualitative all-or-none end point and on the premise that one or more targets in the reaction mixture give rise to a positive end point. Accurate quantitation is achieved by performing multiple replicate at serial dilutions of the material to be assayed." (444) "PBL1 DNA was obtained from normal blood cells separated . . . to contain predominantly normal lymphocytes, and Ho

Claim	Disclosure in Sykes (MYR1013)
molecules are isolated from the biological sample;	DNA was from a bone marrow sample of a patient . . . . DNA from 7 other patients with ALL was extracted from fresh bone marrow aspirate samples for patients 1, 2, 3 and 7, from frozen Ficoll-Paque separated lymphocytes for patient 4 and from stained, fixed bone marrow slides for patients 5 and 6." (445) Table 1 (showing at a specific dilution that 6/20 samples were positive for N-ras, and 3/20 samples were positive for specific leukemic IgH) (446) "**Quantitation by Limiting Dilution Analysis and Poisson Statistics** Serial dilutions of Ho DNA each involving 10 replicates were analyzed by the optimized, two-round PCR (45 cycles, 10^{-3} dilution, 45 cycles)." (p. 446) "Our usual approach is to . . . perform a detailed experiment, perhaps 40 tubes in all, involving multiple replicate dilutions around this point." (448)
(b) amplifying *the* template molecules within [a] *the* set [comprising a plurality of assay samples] to form a population of amplified molecules [each of the] *individual* assay samples of the set [, wherein the template molecules are obtained from a biological sample];	See Claim 1, step (a). "Serial dilutions of Ho DNA each involving 10 replicates were analyzed by the optimized, two-round PCR (45 cycles, 10^{-3} dilution, 45 cycles). Each tube was scored as positive or negative for amplification (Figure 2)." (pp. 446-447) "We have presented a general method for quantitation of targets by PCR using the principle of limiting dilution and use of Poisson statistics. For this approach, the PCR needs to be optimized so that amplification will take place in an 'all-or-none' fashion, and one or a few starting targets will give a positive result. When the optimal conditions are known, target concentration can be estimated by Poisson statistics applied to the results from replicate tubes taken at the limit of dilution." (447)
(c) analyzing the amplified	*See* Claim 1, step (a).

Claim	Disclosure in Sykes (MYR1013)
molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome, wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*;	"In a particular patient, all leukemic cells will have the same rearranged IgH gene that can act as a genetic marker to distinguish leukemic cells from normal non-lymphoid cells and T lymphocytes, which have not rearranged their IgH genes, and from normal B lymphocytes, which have undergone various and different rearrangements of their IgH genes." (444) Table 1 (showing at a specific dilution that 6/20 samples were positive for N-ras, and 3/20 samples were positive for specific leukemic IgH) (446)((446) "The detailed results of one such experiment are shown in Table 1. In two experiments, 1.7 and 2.6 copies of *N-ras* could be detected, and in three experiments, 3.5, 6.1 and 10 copies of Ho IgH could be detected in the presence of 1 ug of PBL1 DNA. The data in each experiment were consistent with a Poisson distribution." (446-447) "The biologic problem in our study was the detection of rare leukemic cells in a large population of normal cells, which in molecular terms became the problem of detection of a rare unique IgH sequence against a background of numerous other IgH sequences. The two-stage PCR system that was developed proved capable of detecting approximately two (1/0.52) potentially amplifiable leukemic IgH sequences against a background of approximately 160,000 total genomes. These genomes would provide a vast excess of sequences that would compete with the leukemic IgH sequences for the PCR primers because they would contain approximately $2.4x10^4$ rearranged IgH genes from normal B lymphocytes and $3x10^5$ germ-line IgH genes, each containing multiple V and J segments." (448)
(d) comparing the first number of assay samples to the second number of assay samples	*See* Claim 1, preamble and step (c). "We have been using the rearranged immunoglobulin heavy chain (IgH) gene as target DNA in the PCR to study patients with acute lymphoblastic leukemia (ALL) in order to detect

Claim	Disclosure in Sykes (MYR1013)
to ascertain an allelic imbalance in the biological sample.	and quantitate a minor population of leukemic cells within a larger population of normal lymphoid and non-lymphoid cells." (444) Table 1 (446) "Because the number of *N-ras* copies detected depends on both the total number of copies which are present and the proportion which are amplifiable, the ratios of the number of *N-ras* targets detected to the number of IgH targets detected give the proportion of potentially amplifiable IgH targets that were actually amplified." (447) Table 2 (showing "Ratio *N-ras*/IgH") (447) "Nevertheless, as seen in Table 2, in all 8 patients there was an approximately constant ratio between the number of amplifiable IgH targets and the number of amplifiable *N-ras* targets." (448)

Claim 1, preamble. To the extent the preamble is limiting, Sykes discloses a method for the "…detection of rare leukemic cells in a large population of normal cells, which in molecular terms became the problem of detection of a rare unique IgH sequence against a background of numerous other IgH sequences." MYR1013, abstract, 448. Sykes discloses that their method was " . . . capable of detecting approximately two (1/0.52) potentially amplifiable leukemic IgH sequences against a background of approximately 160 000 total genomes." *Id.* Sykes discloses how to determine the ratio of a given leukemic sequence in a mixed population of sequences using the method of limiting dilution, creation of

multiple replicates, and PCR to amplify the template molecules in the replicates. *See generally id*. Sykes discloses how to use this method to calculate the ratio of the number of leukemic templates to the number of non-leukemic templates in various patient samples. *Id.*, abstract, 447-448. Sykes discloses that these ratios reflected the ratios of the two sequences in the original sample, namely that one was present at about twice the amount of the other. *Id.*, 446. Sykes discloses the use of a polyacrylamide gel to determine the ratio of a "minor population" of leukemic rearranged IgH in a "larger population of normal lymphoid and non-lymphoid cells." *See id.*, 444, 446-447, Table 1.

Sykes discloses using "*N-ras*" as an " . . . internal control to quantitate the number of potentially amplifiable genomes present in a sample . . . " – *i.e.*, the number of determined *N-ras* copies corresponds to the total number of leukemic templates and non-leukemic templates in a biological sample. Sykes discloses how to use this method to calculate the ratio of the number of leukemic templates (*i.e.*, first genomic sequence) to the number of non-leukemic templates (*i.e.*, second genomic sequence) in a biological sample. *See id.*, abstract, 447-448. The normal allelic balance of rearranged IgH sequences are characterized by enormous diversity with each specific IgH sequence being represented in extremely low frequency. For a normal sample, the ratio of any specific leukemic rearranged IgH sequence to N-ras is expected to be extremely low. Sykes discloses "detecting

approximately two (1/0.52) potentially amplifiable leukemic IgH sequences against a background of approximately 160 000 total genomes." *See id.,* abstract, 448.

A leukemic sample, on the other hand, will consist predominantly of one specific leukemic rearranged IgH sequence, e.g., the Ho DNA disclosed in Sykes. *See id*., 445. The presence of a single, dominant, rearranged IgH sequence represents an allelic imbalance of the IgH gene region. For such a sample, the ratio of the specific, leukemic IgH sequence to N-ras is expected to be substantially higher, approaching a ratio of one (1) copy of specific IgH sequence to two (2) copies of N-ras. *See id*., Table 2. For leukemia patient samples studied, Sykes disclosed that "…as seen in Table 2, in all 8 patients there was an ***approximately constant ratio between the number of amplifiable IgH targets and the number of amplifiable N -ras targets***." *See id*., 448 (emphasis added). This result, the presence of a dominant leukemic rearranged IgH gene, indicates an allelic imbalance in the sample. *See generally id*.

Claim 1, step (a). Sykes discloses diluting and distributing mixtures of nucleic acids from mixed populations of leukemic and non-leukemic cells to make multiple replicates at the limiting dilutions. *See generally id, e.g.* Tables 1 and 2. Sykes discloses diluting and distributing a mixture of leukemic and non-leukemic templates into 20 replicates, *i.e.* assay samples, and determining that 3/20 contained the leukemic template and 6/20 contained *N-ras*. *See id.,* 446, Table 1.

A POSA would have understood the set of assay samples to comprise 20 assay samples, because each sample of Ho DNA was diluted and distributed into 20 tubes, 10 of which were analyzed for the presence of N-ras, and 10 of which were analyzed for the presence of specific leukemic rearranged IgH. MYR1002, 154.

Claim 1, step (b). Sykes discloses amplifying the leukemic and non-leukemic template molecules in the assay samples of the set using PCR to form populations of amplified molecules in the replicates, *i.e.*, assay samples, created at the limiting dilution. *See id.*, 446-448.

Claim 1, step (c). Sykes discloses analyzing the PCR-amplified molecules in the assay samples of the set by counting the number of positive replicates, *i.e.*, the replicates containing amplified PCR product. *Id*., Table 1. Sykes discloses using Poisson statistics to quantitate the initial numbers of leukemic and non-leukemic templates present. *Id*., abstract, 444, 446-447. Sykes discloses diluting and distributing a mixture of leukemic and non-leukemic templates into 20 replicates, *i.e.* assay samples, and determining that 3/20 (0.15) contained the leukemic templates and 6/20 (0.3) contained *N-ras*. *See id.*, 446, Table 1. Specifically, 0.15 of the assay samples yielded an amplification product of the specific leukemic IgH sequence and 0.3 of the assay samples yielded an amplification product of the *N-ras* sequence.

Claim 1, step (d). Sykes discloses comparing the number of assay samples containing the leukemic templates (IgH) with the number of assay samples containing the total templates, leukemic and non-leukemic (*N-ras*). *See id*., abstract, 444, 447-448 Table 1. The ratio of *N-ras* to rearranged leukemia-specific IgH sequence reflects an allelic imbalance in the biological sample. *See generally* Sykes.

B. Independent Claim 19

Claim	Disclosure in Sykes (MYR1013)
19. A method of determining an allelic imbalance in a biological sample, comprising the steps of:	*See* claim 1, preamble element.
(a) distributing *cell-free* nucleic acid template molecules from a biological sample to form a set comprising a plurality of assay samples;	*See* claim 1, step (a).
(b) amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;	*See* claim 1, step (b).
(c) analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain a selected genetic sequence on a first chromosome and a second number of assay samples which contain a reference genetic sequence on a second chromosome;	*See* claim 1, step (c).
(d) comparing the first number of assay samples to the second number of assay samples to ascertain an allelic imbalance between the first	*See* claim 1, step (d).

Claim	Disclosure in Sykes (MYR1013)
chromosome and the second chromosome in the biological sample.	

Claim 19, preamble, steps (a), (b). *See* Claim 1.

Claim 19, step (c). Sykes discloses distinguishing leukemic cells with a rearranged IgH gene – *i.e.* a selected genetic sequence on a first chromosome - from normal non-lymphoid cells and T lymphocytes, which do not have rearranged IgH genes – *i.e.* a reference genetic sequence on a second chromosome. *See id*., abstract, 444, 446-448. Sykes discloses that their method was "capable of detecting approximately two (1/0.52) potentially amplifiable leukemic IgH sequences against a background of approximately 160 000 total genomes." *See id.*, abstract, 448. *See also* Claim 1, step (c) discussion.

Claim 19, step (d). Sykes discloses comparing the number of assay samples containing the leukemic templates (IgH) with the number of assay samples containing the total templates, leukemic and non-leukemic (*N-ras*). *See id*., abstract, 444, 447-448 Table 1. The ratio of *N-ras* to rearranged leukemia-specific IgH sequence reflects an allelic imbalance in the biological sample. *See generally* Sykes. Sykes therefore discloses comparing the number of assay samples containing leukemic templates (IgH) – *i.e.* a selected genetic sequence on a first chromosome – with the number of assay samples containing normal templates (N-

ras) – i.e. a reference genetic sequence on a second chromosome to determine this allelic imbalance. *See id.* at 444, 448. *See also* Claim 1, step (d) discussion.

C. Dependent Claims 5, 22

Claim	Disclosure in Sykes (MYR1013)
5. The method of claim 1 wherein the biological sample is from blood.	"PBL1 DNA was obtained from normal blood cells separated by Lymphoprep™ (Nycomed Pharma AS. Oslo, Norway) to contain predominantly normal lymphocytes, and Ho DNA was from a bone marrow sample of a patient with ALL obtained at diagnosis." (445) "DNA from 7 other patients with ALL was extracted from fresh bone marrow aspirate samples for patients 1, 2, 3 and 7, from frozen Ficoll-Paque separated lymphocytes for patient 4, and from stained, fixed bone marrow slides for patients 5 and 6." (445)
22. The method of claim 19 wherein the biological sample is blood.	*See* Claim 5.

Sykes discloses purifying DNA from samples taken from the bone marrow of patients with acute lymphoblastic leukemia. *See id.,* 444-445. Sykes discloses purifying PBL1 DNA from normal blood cells. *See id.,* 445.

D. Dependent Claims 8, 9, 12, 13, 14, 15, 18, 21

Claim	Disclosure in Sykes (MYR1013)
8. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield an amplification product *of at least*	See claim 1. Table 1 (showing at a specific dilution that 6/20 samples were positive for N-ras, and 3/20 samples were positive for specific leukemic IgH) (446)

Claim	Disclosure in Sykes (MYR1013)
one of the selected and the reference genetic sequences.	
9. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
12. The method of claim 1 wherein between 0.1 and 0.6 of the assay samples yield [an] *a homogenous* amplification product [as determined by amplification of the selected genetic sequence] *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
13. The method of claim [1] *19* wherein between 0.1 and 0.6 of the assay samples yield an amplification product [as determined by	*See* Claim 8.

Claim	Disclosure in Sykes (MYR1013)
amplification of the reference genetic sequence] *of at least one of the selected and the reference genetic sequences*.	
14. The method of claim 1 wherein between 0.3 and 0.5 of the assay samples yield [an] *a homogenous* amplification product [as determined by amplification of the selected genetic sequence] *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
15. The method of claim [1] *19* wherein between 0.3 and 0.5 of the assay samples yield an amplification product [as determined by amplification of the reference genetic sequence] *of at least one of the selected and the reference*	*See* Claim 8.

Claim	Disclosure in Sykes (MYR1013)
genetic sequences.	
18. The method of claim 1 wherein the amplified molecules in each of the assay samples in the first and second numbers of the assay samples are homogeneous such that the first number of assay samples do not contain the reference genetic sequence and the second number of assay samples do not contain the selected genetic sequence.	*See* Claim 8.
20. The method of claim 19 wherein between 0.1 and 0.9 of the assay samples yield an amplification product *of at least one of the selected and the reference genetic sequences*.	*See* Claim 8.
21. The method of claim 20 wherein between 0.1 and 0.9 of the assay samples yield a homogeneous	*See* Claim 8.

Claim	Disclosure in Sykes (MYR1013)
amplification product *of at least one of the selected and the reference genetic sequences.*	

Sykes discloses diluting and distributing a mixture of leukemic and non-leukemic templates into 20 replicates, *i.e.* assay samples, and determining that 3/20 (0.15) contained the leukemic template and 6/20 (0.3) contained the non-leukemic template. *See* MYR1013, 446, Table 1. Therefore, between 0.1 and 0.6, and between 0.3 and 0.5, of the assay samples yielded an amplification product of at least one of the template molecules. MYR1002, ¶¶161-169; 178-181; 186-187.

XIII. GROUND 5: CLAIMS 16-17 WOULD HAVE BEEN OBVIOUS IN VIEW OF SYKES AND BROWN

Claims 16-17 are obvious in view of the disclosures in Sykes and Brown. MYR1002, ¶¶188-206. If Sykes does not disclose any limitation of the '889 Patent related to the number of assay samples or nucleotide probes, a POSA would have had a reason to combine Sykes with Brown, and would have had a reasonable expectation of success, as illustrated and explained above. *See* Ground 2; MYR1002, ¶¶188-206. A POSA would have had a reason to combine Sykes with Brown to gain the benefit of Brown's increased assay sample numbers with

attendant increased ability to find rarer mutants, while maintaining good efficiency and throughput. *Id*., 188; MYR1015, 10:11-15; MYR1013, 448.

The claimed methods are merely a combination of known elements with predictable results, because: (*i*) Sykes discloses an LDPCR method suitable for detecting a single molecule of target DNA and characterizing the composition of a biological sample by counting genetic sequences; and (*ii*) Brown discloses LDPCR suitable for detecting a single molecule of target DNA and facilitating analysis of many parallel assay samples, including testing for the presence of multiple sequences of interest by means of nucleotide probes. *See, e.g.*, MYR1013, abstract; 444-448; MYR1015, abstract, 1:59–2:5;5 3:17-24; 4:12-17; 7:44-56; 16:1-19; 30:54-57; MYR1002, ¶198.

A. Dependent Claims 16-17

Claim	Disclosures in Sykes (MYR1013)and Brown (MYR1015)
16. The method of claim 1 wherein the set comprises at least 500 assay samples.	See Claim 1 elements above (Sykes). "Methods of filling miniaturized sample chambers are also provided as are methods for determining the number of template molecules in a sample by conducting replicate nucleic acid sequence amplification reactions on a set of terminally diluted samples and counting the number of positive amplification reactions. The methods can be used to detect a single starting nucleic acid target molecule." Brown, Abstract. "Preferred sample chambers according some embodiments of the invention, for nucleic acid amplification methods to detect single target nucleic acid molecules, have volumes of from

Claim	Disclosures in Sykes (MYR1013)and Brown (MYR1015)
	about 1 microliter to about 1 picoliter or less. . . . Photolithographic methods can provide from about 10,000 to over 100,000 sample chambers of about 100 picoliters each on a 1"×3" substrate." (Brown, 16:1-19)
17. The method of claim 1 wherein the set comprises at least 1000 assay samples.	*See* Claim 16.

Brown discloses these limitations, for example through its disclosure of 10,000 to over 100,000 sample chambers, each for holding an assay sample for amplification reactions. *See* MYR1015, 16:1-19.

Reasons to combine. In addition to the discussion above, Brown specifically discloses that " . . . it may be desirable to screen DNA or RNA from a single individual for sequence variants associated with different mutations in the same or different genes, or for sequence variants that serve as 'markers' for the inheritance of different chromosomal segments from a parent." *Id.*, 4:5-9. Sykes discloses diluting and distributing, amplifying, and analyzing leukemic and non-leukemic templates, which is a form of screening for sequence variants associated with different mutations in the same gene. *See, e.g.*, MYR1013, abstract, 444, 446, 448. Sykes discloses the benefits of multiple samples: "More replicates will therefore be required for a given level of precision." MYR1013, 448. As such, a

POSA would have had a reason to combine the highly parallel approach of Brown with the analytical framework taught by Sykes. MYR1002, ¶202.

The claimed methods are a simple and predictable substitution of known elements. LDPCR, including the use of Poisson statistics, was well-established even before Brown and Sykes. MYR1002, ¶203. The analytical framework set forth in Sykes is readily applicable to other techniques which use LDPCR to generate detectable products. The use of Sykes's analytical framework for analyzing multiple target molecules would be especially apparent to a POSA in view of Brown's express disclosure of applicability to screening for mutant genes. *Id*. In this way, a POSA would have understood Brown to disclose a platform for the efficient scale-up of the core techniques and analysis disclosed by Sykes for analyzing mutants against a background of wild-type sequences. *Id*. A POSA would have had a strong reason to apply Sykes's analytical framework to a dataset generated according to the parallel approach of Brown. *Id*.

Reasonable expectation of success. A POSA would have had a reasonable expectation of successfully combining Brown with Sykes. MYR1002, ¶204. Sykes discloses an analytical framework for use on a dataset that is derived from LDPCR that can detect single template molecules and is adapted for use in the context of multiple molecules of interest. Brown discloses a way to create a dataset that is derived from LDPCR that can detect single template molecules and

is adapted for use in the context of multiple molecules of interest. *Id*. A POSA would have known that a dataset created by way of Brown's high-throughput approach would be amenable to the sequence count-based analysis of Sykes, and would have reasonably expected success in combining the references. *Id*. For all of these reasons, Claims 16-17 would have been obvious over the combination of Brown and Sykes. MYR1002, ¶¶200-206.

XIV. GROUND 6 CLAIMS 4, 6, AND 7 WOULD HAVE BEEN OBVIOUS IN VIEW OF SYKES AND HEID

Claims 4, 6 and 7 are obvious in view of the disclosures in Sykes (MYR1013) and Heid (MYR1024). MYR1002, ¶¶207-220. To the extent that Sykes does not disclose any limitation of the '889 Patent related to non-polymorphic markers, a POSA would have had a reason to combine Sykes with Heid, and would have had a reasonable expectation of success, as illustrated and as explained below. *See* Ground 3 above; MYR1002, ¶¶207-220.

Claim	Disclosures in Sykes (MYR1013) and Heid (MYR1024)
4. The method of claim 1 wherein the selected genetic sequences and the reference genetic sequences are non-polymorphic markers.	"We have developed a novel 'real time' quantitative PCR method. The method measures PCR product accumulation through a dual-labeled fluorogenic probe (i.e., TaqMan Probe)." (Abstract) "Quantitative gene analysis (DNA) has been used to determine the genome quantity of a particular gene, as in the case of the human HER2 gene, which is amplified in -30% of breast tumors. Gene and genome quantitation (DNA and RNA) also have been used for analysis of human immunodeficiency virus (HIV) burden demonstrating changes in the levels of virus

Claim	Disclosures in Sykes (MYR1013) and Heid (MYR1024)
	throughout the different phases of the disease." (986) "PCR primers and probes were designed for the human factor VIII sequence and human β-actin gene (as described in Methods)." (987) "Real-time PCR is compatible with . . . quantitative competitive PCR using a normalization gene contained within the sample (i.e., β-actin). . . ." (991).
6. The method of claim 1 wherein the selected genetic sequence is a non-polymorphic marker.	See Claim 4.
7. The method of claim 1 wherein the reference genetic sequence is a non-polymorphic marker.	*See* Claim 4.

As described in Ground 3 above, a POSA would have understood that the non-polymorphic markers, e.g., human factor VIII, β-actin, HER2, or the HIV sequences could be used as a selected genetic sequence or a reference genetic sequence. *See* MYR1024, 986, 992; MYR1002, ¶218.

Reasons to combine. In addition to the discussion above, the claimed methods are a simple and predictable substitution of known elements. For example, Heid's disclosures show the benefits of non-polymorphic markers in the

context of quantitative DNA analysis methods using PCR, which is the context of Sykes. MYR1002, ¶¶210-214.

Heid expressly discloses the benefits of gene and genome quantitation generally, and the Heid approach in particular, to cancer research: "Quantitative gene analysis (DNA) has been used to determine the genome quantity of a particular gene, as in the case of the human HER2 gene, which is amplified in ~30% of breast tumors (Slamon et al. 1987)." MYR1024, 986, 992. As discussed above, Sykes a LDPCR method in the context of cancer research, in particular in analysis of subpopulations of leukemic cells in a background of non-leukemic cells. Because Heid expressly discloses the benefit of non-polymorphic markers in the context of a quantitative method for DNA analysis using PCR, quantitative PCR, a POSA would have had a strong reason to apply Heid's use of such markers in the context of the framework of the techniques and analysis taught in Sykes. MYR1002, ¶214.

Reasonable expectation of success. As discussed above, both references arise in the context of quantitative DNA analysis methods using PCR. Heid discloses the benefits of its disclosures to the cancer research context, among others. Because a POSA would have understood that Heid discloses the use of non-polymorphic markers as part of its method, a POSA would expect success in

the use of non-polymorphic markers in the context of the Sykes LDPCR analysis. MYR1002, ¶215.

A POSA would have had no reason to believe that use of non-polymorphic markers would have presented any particular problems to LDPCR, an approach that had long been proven to be robust across multiple investigation areas. MYR1002, ¶216. For all of these reasons, a POSA would have had additional confidence in the likely success of combining the disclosures of Sykes with Heid. MYR1002, ¶¶207-220.

XV. OBJECTIVE INDICIA DO NOT SUPPORT PATENTABILITY

The challenged claims are all anticipated and/or obvious. Any challenged claim not found anticipated is invalid as obvious, and no objective indicia support a contrary conclusion. MYR1002, ¶¶221-231. To Petitioners' knowledge, Patent Owner only proffered purported objective indicia of non-obviousness during reexamination of the '889 patent, as discussed below. MYR1005 Petitioners are not aware of any cognizable objective evidence of non-obviousness of the challenged claims. *Id.* Accordingly, the challenged claims would have been obvious in view of the analysis above. If Patent Owner relies on any additional purported objective indicia to attempt to support a patentability argument, Petitioners request an opportunity to rebut such evidence. MYR1023, 13.

Patent Owner presented a declaration of its consultant Mr. Stanley N. Lapidus in support of its purported objective indicia.[5] Mr. Lapidus's declaration is directed to the purported benefits of *digital PCR*, but he nowhere defines that term or connects that term to any asserted claim. Mr. Lapidus, however, indicates that he and others of skill in the art were surprised at the success of Drs. Vogelstein's and Kinzler's digital PCR method. Mr. Lapidus provides no evidence of such surprise, does not identify any others who were purportedly surprised, and does not connect the alleged success or surprise to any patent claim. Mr. Lapidus makes conclusory statements regarding digital PCR as constituting an improvement over existing techniques that met an unmet need. Mr. Lapidus, however, fails to explain how digital PCR supposedly improved over the prior art, fails to explain how the prior art did not meet the alleged need, and fails to connect any such improvement to any claim. As demonstrated above, the prior art anticipated and/or rendered obvious each and every claim challenged herein. Therefore, to the extent that Mr.

[5] Mr. Lapidus is President, CEO, and Founder of SynapDx. Laboratory Corporation ("LabCorp") is an investor in SynapDx. Upon information and belief, LabCorp owns Esoterix Genetic Laboratories, the licensee of the '706 patent, a fact Mr. Lapidus did not disclose. As such, Mr. Lapidus's declaration does not provide objective opinions. MYR1002, ¶245.

Lapidus's declaration indicates that any of these claims improved over the prior art and/or satisfied a previous unmet need, his declaration is demonstrably in error. Mr. Lapidus asserts that digital PCR is still in use by a number of companies, but he fails to connect any such success to any patent claim—or even to allege that such products are commercially successful.

Patent Owner also presented a declaration of Dr. Ie-Ming Shih to review and summarize the state of the digital PCR field.[6] Dr. Shih set forth his understanding of the claimed "digital PCR" as involving (*i*) analysis of two different analytes and (*ii*) comparing the number of assay samples containing one of the analytes to the number of assay samples containing the other analyte. As demonstrated above, "digital PCR" within this meaning was well-known and thoroughly described in the prior art, including the Mullis chapter, Simmonds, and Sykes. Therefore, any benefits of, or characteristics related to, *digital PCR* discussed in Dr. Shih's declaration apply equally to the prior art and do not support a finding of non-obviousness.

[6] Dr. Shih is Professor of Pathology at Johns Hopkins University, which is a real-party in interest in this matter. As such, Mr. Shih's declaration does not provide objective opinions.

Dr. Shih stated that many in the field adopted the term *digital PCR*, but use it more broadly. Patent Owner's own declarant thus acknowledges that the digital PCR terminology in industry is broader than what is relevant to the claimed methods. MYR1002, ¶226. There is thus no nexus to the challenged claims and Dr. Shih's declaration is not evidence of non-obviousness with respect to those claims.

Dr. Shih cites a number of publications and review articles in support of his opinion that Dr. Vogelstein and Dr. Kinzler invented digital PCR. But a closer examination of some of those references supports the exact opposite conclusion. For example, Dr. Shih cites to the review article by Baker. MYR1018; MYR1002, ¶248. Contrary to Dr. Shih, Baker states that Sykes (ref. 5) first disclosed the concept of digital PCR and that later it was Dr. Vogelstein and Dr. Kinzler who merely named the technique and applied it in their colorectal cancer research:

> ***The concept behind digital PCR was first described in 1992*** (ref. 5). A few years later, ***Bert Vogelstein and Ken Kinzler at Johns Hopkins University named the technique*** and showed that it could be used to quantify disease-associated mutations in stool from patients with colorectal cancer.

MYR1018, 541 (emphases added); MYR1002, ¶227. Dr. Shih's citation of Baker confirms the invalidity analyses and conclusions set out above. *Id*.

Dr. Shih discusses alleged recognition, advantages, and commercial applications associated with digital PCR, including reference to RainDance and Bio-Rad machines, but he fails to connect any of this to any challenged claim. He fails to explain any differences between the claimed invention and the prior art in terms of any claimed feature. Dr. Shih also fails to allege that the digital PCR products are commercially successful. If anything, Dr. Shih's acknowledgment of the existence of multiple kinds of digital PCR—some of which he acknowledges are not relevant to the purported invention—raises even more questions about which, if any, of his cited examples relate to a challenged claim.

To Petitioners' knowledge, there has never been a showing that any alleged secondary consideration has a nexus to a claimed feature not found in the prior art. LDPCR was known and practiced in the prior art long before the earliest possible priority date for the '889 patent. At a minimum, the use of LDPCR by multiple different groups of scientists for multiple different research projects is evidence of simultaneous invention. MYR1012, MYR1013, MYR1014, MYR1015, MYR1002, ¶230. That LDPCR is still useful and practiced today does not provide any evidence of non-obviousness. If anything, the continued use of prior art LDPCR techniques confirms that the challenged claims do not reflect an improvement over the prior art. *Id*. The challenged claims are all invalid and

cannot be saved with any alleged secondary considerations. *Id.* They should be cancelled. *Id.*

XVI. CONCLUSION

As no less than Nobel Laureate Kary Mullis made clear, by 1994, the steps of "digital PCR" were well known and in frequent use by POSAs. As a result, as explained in detail above, each of the challenged claims is anticipated and/or obvious.

Specifically, Claims 1, 5, 8-9, 12-15, and 18-22 are anticipated by Simmonds.

Claims 16-17 would have been obvious in view of the disclosures in Simmonds and Brown.

Claims 4 and 6-7 would have been obvious in view of the disclosures in Simmonds and Heid.

Claims 1, 5, 8-9, 12-15, and 18-22 are anticipated by Sykes.

Claims 16-17 would have been obvious in view of the disclosures in Sykes and Brown.

Claims 4 and 6-7 would have been obvious in view of the disclosures in Sykes and Heid.

Finally, no objective indicia of non-obviousness support the patentability of the challenged claims of the '889 patent.

XVII. MANDATORY NOTICES (37 C.F.R. § 42.8(A)(1))

A. Real Parties-In-Interest (37 C.F.R. § 42.8(b)(1)) are: Myriad Genetics, Inc., Myriad Genetic Laboratories, Inc. (collectively, "Myriad"), Bio-Rad Laboratories, Inc., and RainDance Technologies, Inc.

B. Related Matters (37 C.F.R. § 42.8(b)(2)) are: the '889 patent is presently the subject of a patent infringement lawsuit brought by the Patent Owner and assignee, JHU, and its licensee, EGL, against Myriad Genetics, Inc. and Myriad Genetic Laboratories, Inc., and captioned *Esoterix Genetic Laboratories, LLC and The Johns Hopkins University v. Myriad Genetics, Inc. and Myriad Genetics Laboratories, Inc*., 1:16-cv-1112-WE-JEP (M.D.N.C.) The '889 patent is also presently the subject of a patent infringement lawsuit brought by the Patent Owner and assignee, JHU, and its licensee, EGL, against Ambry Genetics Corporation, and captioned *Esoterix Genetic Laboratories, LLC and The John Hopkins University v. Ambry Genetics Corporation*, 16-cv-1111-WO-JEP (M.D.N.C.). The two litigations were filed in the U.S. District Court for the Middle District of North Carolina on September 7, 2016, and Myriad was subsequently served on September 12, 2016. Petitioners are concurrently filing petitions for IPR of USPNs 7,915,015 and 8,859,206, as well as the '706 patent, which are also owned by Patent Owner and asserted against Myriad in the Myriad litigation.

C. Designation of Lead and Back-Up Counsel (37 C.F.R. § 42.8(b)(3)):

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D. Notice of Service Information (37 C.F.R. § 42.8(b)(4)): Please direct all correspondence regarding this Petition to counsel at the above addresses. Petitioners consent to service by email at the addresses above.

Respectfully submitted,

Date: March 16, 2017

By: /Anne S. Toker/

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CERTIFICATION THAT THE PATENT MAY BE CONTESTED VIA INTER PARTES REVIEW BY THE PETITIONERS AND STANDING (37 C.F.R. § 42.104(A))

Petitioners certify that (1) the '889 patent is available for IPR and (2) Petitioners are not barred or estopped from requesting IPR of any claim of the '889 patent. This Petition is filed in accordance with 37 C.F.R. § 42.106(a). Concurrently filed herewith are a Power of Attorney and Exhibit List under 37 C.F.R. § 42.10(b) and § 42.63(e), respectively. The required fee is paid through Deposit Acct. No. 505708. The Office is authorized to charge any fee deficiency, or credit any overpayment, to Deposit Acct. No. 505708.

Date: March 16, 2017

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CERTIFICATION OF WORD COUNT UNDER 37 C.F.R. § 42.24(a)

I, the undersigned, do hereby certify that the attached Petition, including footnotes, contain 13,835 words, as measured by the Word Count function of Word 2013. This is less than the limit of 14,000 words as specified by 37 C.F.R. § 42.24(a)(i).

Date: March 16, 2017

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CERTIFICATION OF SERVICE (37 C.F.R. §§ 42.6(E), 42.105(A))

The undersigned hereby certifies that the above-captioned "Petition for *Inter Partes* Review of U.S. Patent No. 7,824,889 under 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42.1-.80, 42.100-.123" including its supporting evidence Exhibits 1001 – 1047 were served in their entirety on March 16, 2017 upon the following parties via Electronic Mail based on prior agreement of the parties.

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